17th International Congress on Toxoplasmosis

Berlin, Germany | May 26th–29th 2024

Programme

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WELCOME NOTE

Dear colleagues, dear friends,

welcome to the 17th International Congress on Toxoplasmosis in Berlin. We are delighted that you will join us for 4 days of great science! Since the first *Toxoplasma* meeting in 1990 with 26 (!) attendees the interest in *Toxoplasma gondii* as an important zoonotic pathogen and exciting model organism to study fundamental principles of biology has continuously grown. In Berlin, we welcome more than 210 participants from around the world, many of whom will have the opportunity to present their latest work in talks (73), turbo talks (30) or posters (123)!

The International Congress on Toxoplasmosis is an integral event for our field as it unites the global community of students, young researchers and established leaders. In line with tradition, this conference will cover all aspects of *Toxoplasma* research, ranging from molecular biology to clinical disease and epidemiology – and thus we hope this will spark interdisciplinary interactions.

Apart from scientific presentations, we look forward to many stimulating discussions and networking opportunities. As in previous years, ToxoXVII is preceded by two workshops from special interest groups – those keeping an eye on the epidemiologically important oocyst stage (3rd Environmental Toxoplasmosis Workshop), and the big data scientists participating in and running the ToxoDB workshop (who are an invaluable asset to our community!).

This is also the place to say THANK YOU to a great number of people who helped to make this conference happening. We thank all the funding institutions and private and industrial sponsors who did support the conference despite difficult political and financial times. We are grateful to the Federation of European Microbiological Societies (FEMS) and ToxoInc who allowed us to sponsor 30 young scientists with travel grants. Without everyone's support the meeting would be less enjoyable. We also thank the team at Conventus, in particular Beatrisa Mamiewa, for the professional handling of all organizational issues, and the staff at the Harnack Haus for their service. We are also grateful to the many members of the International Scientific Committee for their efforts in reviewing all the abstracts and to those who were willing to serve as session chairs.

Berlin is a terrific place to come together, discuss great science – and then party (after the conference finished, of course!). We have made every effort to make it affordable and pleasant at the same time. Let's enjoy it in every possible way!

Frank Seeber (Berlin)
Martin Blume (Berlin)
Ildiko Rita Dunay (Magdeburg)
Carsten Lüder (Göttingen)
Markus Meissner (München)
Lena Pernas (Köln/Los Angeles/LA, US)
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The ToxoXVII Organizing Committee

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Experimentelle Parasitologie, Ludwig-Maximilians-Universität München

Federation of European Microbiological Societies (FEMS)

FEMS Microbes

Heyl Chemisch-pharmazeutische Fabrik GmbH & Co. KG

Max-Planck Gesellschaft (MPG)

Robert-Koch-Institut (RKI)

Springer

Toxo Inc.

Thermo Fisher Scientific

Zymo Research Europe GmbH

State at printing

Venue
Harnack-Haus
Ihnestraße 16–20
14195 Berlin

Date
26th–29th May 2024

Congress website
www.toxocongress2024.org

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Design/layout

Layout	Conventus Congressmanagement & Marketing GmbH
Front cover	David Warschkau/Frank Seeber/Conventus Congressmanagement & Marketing GmbH
Last page	Frank Seeber & all previous organizers of Toxo conferences (1996–2022)
Print	printworld.com GmbH | Messering 5 | 01067 Dresden
Circulation	230
Editorial deadline	8 May 2024

PROGRAMME OVERVIEW

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Selected Talks
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20:00–21:30
Get Together with dinner buffet
(Restaurant area, Laue-Saal, Einstein-Lounge)
S. 8

Planck-Lobby

15:30–17:30
Arrival & Registration
S. 8

17:30–18:00
Meet and Greet

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Parasite-Host Interactions & Signalling I
S. 9

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13:30–15:00
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Cell Biology I
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Turbo Talks for PS I

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Meitner-Saal I+II

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odd poster ID´s
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odd poster ID´s
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PROGRAMME OVERVIEW

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15:30–16:00
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Meitner-Saal I+II

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Poster Session II
even poster ID´s
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even poster ID´s
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Lynen-Zimmer

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SCIENTIFIC PROGRAMME • 26 MAY

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17.30–18.00 Meet and Greet (Planck-Lobby)

18.00–18.30 Conference Opening
Goethe-Saal & Galerie

18.30–19.00 Plenary Talk I
Goethe-Saal & Galerie

18.30 T01 The endless enigma of *Toxoplasma*: forty years of hard work by many and yet we still don't really understand …
John Boothroyd (Standford, CA/US)

19.00–20.00 Selected Talks
Goethe-Saal & Galerie

19.00 T02 Is *Toxoplasma gondii* an under diagnosed cause of morbidity and mortality in humans? – my personal experience
David Ferguson (Oxford/GB)

19.15 T03 Uncovering the minimalist adhesive strategy of the *Toxoplasma* parasite for high-speed motility
Isabelle Tardieux (Grenoble/FR)

19.30 T04 The *Toxoplasma gondii* mitoribosome reveals novel features of ribosome evolution
Shikha Shikha (Glasgow/GB)

19.45 T05 Evolutionary Insights into the Composition and Assembly of Mitoribosomes in Alveolates
Yonggen Jia (Beijing/CN)

20.00–21.30 Get Together with dinner buffet (Restaurant Area)

08.30–09.00 Plenary Talk II
Goethe-Saal & Galerie

08.30 T06 Ocular Toxoplasmosis: Myths and Realities
Alejandra de la Torre (Bogotá/CO)

09.00–10.30 Session I: Genomics
Goethe-Saal & Galerie

09.00 T07 An ISWI-related chromatin remodeler tunes MORC DNA binding and insulates gene expression in a densely packed genome
Belen Pachano (La Tronche/FR)

09.15 T08 Forward genetic screen identifies SRS15 and IFT88 as critical determinants controlling *Toxoplasma* oocyst shedding in cats
Michael E. Grigg (Bethesda, MD/US)

09.30 T09 mRNA cap-binding protein eIF4E1 is a novel regulator of *Toxoplasma gondii* latency
Michael Holmes (Indianapolis, IN/US)

09.45 T10 Integrative Analysis of AP2 Transcription Factor Dynamics and Binding Motifs in *Toxoplasma gondii*
Forouzandeh Farsaei (Tampa, FL/US)

10.00 T11 ToxoDB: Tools for Genomic-Scale Data Exploration, Analysis, Integration and Discovery
Omar Harb (Philadelphia, PA/US), Ulrike Böhme (Liverpool/GB), David Roos (Philadelphia, PA/US)

10.15 T12 Understanding the History of the Domestic Cycle of *Toxoplasma gondii* in South America
Lokman Galal (Limoges/FR)

10.30–11.00 Coffee Break & Industrial Exhibition
Meitner-Saal I+II & Planck-Lobby

11.00–12.30 Session II: Parasite-Host Interactions & Signalling I
Goethe-Saal & Galerie

11.00 T13 Identification of proteins mediating ER-*Toxoplasma* contact sites
Chahat Mehra (Köln/DE)

11.15 T14 Formation of mammalian ER membrane contact sites, via host VAPA and VAPB, with the *Toxoplasma* PV
Julia Romano (Baltimore, MD/US)

11.30 T15 The role of the GPI in *Toxoplasma gondii* pathogenesis and mRNA vaccines
Kirk D. C. Jensen (Merced, CA/US

11.45 T16 A new *Toxoplasma* rhoptry protein is a major virulence factor that prevents inflammatory host cell death
Diana Penarete Vargas (Montpellier/FR)

12.00 T17 A high-throughput screen to elucidate the function of *Toxoplasma gondii* rhoptry proteins within human host cells
Grant Stevens (Toronto/CA)

12.15 T18 Rhoptry-associated perforation of the host cell plasma membrane during *Toxoplasma gondii* invasion
Frances Male (Burlington, VT/US)

12.30–13.30 Lunch Break (Restaurant area) & Industrial Exhibition

13.30–15.00 Session III: Cell Biology I
Goethe-Saal & Galerie

13.30 T19 — A Kinase/Phosphatase Negative Feedback Loop Controls *Toxoplasma* Egress
David Sibley (St. Louis, MO/US)

13.45 T20 — Exploring the role an Apical Polar Ring Protein in controlling F-actin translocation and gliding motility in *Toxoplasma gondii*
Bingjian Ren (Genève/CH)

14.00 T21 — Revealing cellular and molecular organization in *Toxoplasma's* invasion machinery after ionophore stimulation
Li-av Segev-Zarko (Stanford, CA/US)

14.15 T22 — Unveiling the role of conoid gliding protein in *Toxoplasma gondii* invasion and egress: insights from cryogenic correlative light and electron microscopy (cryo-CLEM)
Oliwia Koczy (Heidelberg/DE)

14.30 T23 — Bumped Kinase Inhibitors (BKI) inhibit *Toxoplasma gondii* MAPKL-1 and CDPK1
Jemma Montgomery (Portland, OR/US)

14.45 T24 — Myosin F controls actin organization and dynamics in *Toxoplasma gondii*
Jacob Kellermeier (Storrs, CT/US)

15.00–15.30 Turbo Talks for Poster Session I (odd poster IDs, 001–029)
Goethe-Saal & Galerie

15.30–16.00 Coffee Break & Industrial Exhibition
Meitner-Saal I+II & Planck-Lobby

16.00–17.30 Session IV: Immunology
Goethe-Saal & Galerie

16.00 T25 — Inducible nitric oxide synthase (iNOS) is necessary for GBP-mediated *Toxoplasma gondii* restriction in murine macrophages via vacuole nitration and intravacuolar network collapse
Sarah Ewald (Charlottesville, VA/US)

16.15 T26 — Caspase-8-mediated cell death of $CD8^+$ T cells is required for control of *Toxoplasma gondii* in the brain
Tajie Harris (Charlottesville, VA/US)

16.30 T27 — CD8 T memory subset response in encephalitis model of *Toxoplasma gondii* infection.
Imtiaz Khan (Washington DC/US)

16.45 T28 — Chronic IL-1-induced DNA double-strand break response in hippocampal neurons drives cognitive deficits upon latent *Toxoplasma gondii* infection
Nicolas Blanchard (Toulouse/FR)

17.00 T29 — Dense Granule Protein 3 of *Toxoplasma gondii* Plays a Crucial Role in the Capability of the Tissue Cysts of the Parasite to Persist under the Presence of Anti-Cyst CD8+ T Cells during the Chronic Stage of Infection
Rajesh Mani (Lexington, VT/US)

17.15 T30 — NLRP3 inflammasome assembly and activation are mediated by dynein-dependent microtubule transport during *Toxoplasma gondii* infection of human immune cells
Melissa B. Lodoen (Irvine, CA/US)

17.30–19.00 Poster Session I odd poster IDs (001–123)
Meitner-Saal I+II & Planck-Lobby

P001 A Golgin-Like Protein is Required for Golgi Structural Maintenance and Function in *Toxoplasma gondii's* Endomembrane System
Camille Pearce (Storrs, CT/US)

P003 The Role of Purine Auxotrophy in *Toxoplasma*-Host Interaction
Bruno Martorelli Di Genova (Burlington, VT/US)

P005 In silico, transcriptomic and proteomic comparative analysis of Dense Granule Proteins orthologues in *Toxoplasma gondii* and *Neospora caninum*
Tiago Mineo (Uberlândia/BR)

P007 Uncovering the mechanisms employed by latent *Toxoplasma gondii* within neurons to evade immune surveillance
Renzo Gutierrez-Loli (Toulouse/FR)

P009 Bridging Molecules: Exploring the Structural and Biochemical Interface between the Glideosome-Associated Connector and F-actin
Gloria Meng-Hsuan Lin (Geneva/CH)

P011 *Toxoplasma gondii* DNA in tissues of Arctic charr, Salvelinus alpinus, collected from Nunavik, Québec, Canada
Brent Dixon (Ottawa/CA)

P013 A cytoplasmic dynein complex involved in host cell invasion by *Toxoplasma gondii*
Karine Frénal (Bordeaux/FR)

P015 Deciphering the transport of heme intermediates in *Toxoplasma gondii*: Insights from comparative genome wide CRISPR screens
Estefania Delgado Betancourt (Geneva/CH)

P017 Human toxoplasmosis in the Czech Republic 1923-2023: From the first case through a massive outbreak to a long-term decline
Petr Kodym (Prague/CZ)

P019 T Lymphocyte-Dependent IL-10 Protects Against a Lethal IL-12 Driven Cytokine Storm Initiated by *Toxoplasma gondii* GRA24
Claire Doherty (Albuquerque, NM/US)

P021 CellFlow: A comprehensive toolbox for the analysis of apicomplexan gene expression and chromatin dynamics using single-cell technologies
Argenis Arriojas (Boston, PA/US)

P023 The *Toxoplasma gondii* mitoribosome - build from tiny rRNA fragments expressed from mitochondrial genome recombination sites
Sabrina Tetzlaff (Berlin/DE)

P025 Autophagy in $CD11c^{+}$ cells is required for the development of protective immunity to *Toxoplasma gondii* infection
David Christian (Philadelphia, PA/US)

P027 Does T cell Exhaustion occur in pregnant women with chronic *Toxoplasma gondii* infection?
Maureen Groer (Knoxville, TN/US)

P029 Determination of *Toxoplasma gondii*'s genetic variability in cases of maternal seroconversion during pregnancy from Uruguay
Alejandra Valentín-Decuadro (Montevideo/UY)

P031 Identification and function analysis of novel anti-host immunity factors of *Toxoplasma*
Emi Hashizaki (Suita, Osaka/JP)

P033 Cloning and expression of the rop18 from *Toxoplasma gondii* in plasmid vectors
João Luís Garcia (Londrina/BR)

P035 Evaluation of the immune response against the formation of tissue cyst of *Toxoplasma gondii* IN pigs immunized with DNA+sag1+rop18 vaccine
João Luís Garcia (Londrina/BR)

P037 How the diagnostic and epidemiological management of selected infectious diseases is organized in Germany – the German Consulting Laboratory for *Toxoplasma* as an example for national network activities
Uwe Groß (Göttingen/DE)

P039 Role of the hypothetical protein, TGME49_207210 in bradyzoite differentiation
Chandrasekaran Sambamurthy (Tucson, AZ/US)

P041 Assessing the impact of preventative measures to limit the spread of *Toxoplasma gondii* in wild carnivores of Madagascar: A modeling study
Fidisoa Rasambainarivo (Greenville, MS/US)

P043 To Per-Cyst or Not: Unravelling the Secrets Behind an Attenuated *Toxoplasma* Strain
Saniya Crouch (Edinburgh/GB)

P045 Exploring infection dynamics of virulent and non-virulent strains of *Toxoplasma gondii*
Xuhang Wu (Edinburgh/GB)

P047 Treatment with BKI-1748 after *Toxoplasma gondii* systemic dissemination in experimentally infected Sheep significantly improves clinical outcome and prevents congenital infection
Roberto Sánchez Sánchez (Madrid/ES)

P049 *In vitro* models using target ovine cell lines to evaluate the *Toxoplasma gondii* phenotype
Yanina Hecker (Madrid/ES)

P051 The Mitochondrial Iron Transporter is important for *Toxoplasma gondii*
Lucas Pagura (Glasgow/GB)

P053 Human-derived cerebral organoids as a model for chronic *Toxoplasma gondii* infection
Tina Mutka (Tampa, FL/US)

P055 Genotyping of European *Toxoplasma gondii* strains by a new high-resolution next-generation sequencing-based method
Gereon Schares (Greifswald-Insel Riems/DE)

P057 Effects of chronic infection by *Toxoplasma gondii* on spatial memory, olfactory sensibility, and social behavior in male mice
Liliana Montserrat Molina-Lopez (Huixquilucan/MX)

P059 Sending out an SOS: novel insights into a *Toxoplasma* effector protein
Ana Matias (Oeiras/PT)

P061 Anti-*Toxoplasma gondii* antibodies in Neotropical primates from captivity and the wild: low concordance between serological tests
Helio Langoni Langoni (Botucatu/BR)

19.00–20.00 Buffet Dinner (Restaurant area)

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P063 Serological and molecular survey of *Toxoplasma gondii* infection in wild carnivores of the Iberian Peninsula
Martha Ynés Salas-Fajardo (Madrid/ES)

P065 Persistence of viable *Toxoplasma gondii* oocysts in Pacific oysters (Crassostrea gigas)
Lezlie Rueda (Davis, CA/US)

P067 FACS-based CRISPR knockout screen identifies interferon stimulated genes that control *Toxoplasma* in pig cells
Marzuq Ungogo (Edinburgh/GB)

P069 Commercial kitten moist food inactivated *Toxoplasma gondii* tissue cysts instantaneously
Andreas Lazaros Chryssafidis (Lages-SC/BR)

P071 Investigating contamination of ready-to-eat salads with *Toxoplasma gondii* oocysts in a European-wide multicenter survey
Rafael Calero-Bernal (Madrid/ES)

P073 MyoJ-BCC1-Cen2: is there a contractile ring at the basal pole of *Toxoplasma gondii*?
Chloé Roumégous (Bordeaux/FR)

P075 Latent cerebral *Toxoplasma gondii* infection exacerbates Tauopathy in a mouse model of Alzheimer‘s disease
Sabrina Marion (Lille/FR)

P077 Cooperation or Competition? Unraveling the interplay between transcription and epigenetic factors in *Toxoplasma gondii* gene expression
Marc-Jan Gubbels (Brighton, MA/US)

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Danilo Donizete da Silva (São José do Rio Preto/BR)

P081 Redefining the role of latency during chronic *Toxoplasma gondii* infection in the central Nervous system
Julia Eberhard (Philadelphia, PA/US)

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Rémi Mascarau (Lille/FR)

P085 CRISPR/Cas9 screens for the identification of essential host factors for *Toxoplasma gondii* infection
Andrea Gaspare Valenti (Bern/CH)

P087 First data of *Toxoplasma gondii* genotyping from animals in Ukraine
Maryna Galat (Bern/CH)

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Rajesh Mani (Lexington, VT/US)

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Swaroop Peddiraju (Hyderabad/IN)

P093 Investigating the response to iron deprivation in *Toxoplasma*
Jack Hanna (Glasgow/GB)

P095 *Toxoplasma* CRMPs: microneme sensors specifically devoted to rhoptry secretion
Daniela Sparvoli (Montpellier/FR)

P097 2,3-dihydro-1,4-benzodioxins are potent inhibitors of *Toxoplasma gondii* tachyzoite regrowth *in vitro*
P. Holland Alday (Portland, OR/US)

P099 Biochemical and Molecular Elucidation of Hydroquinine Mechanism(s) of Action against *Toxoplasma gondii*
Daniel A. Abugri (Montgomery, AL/US)

P101 RNA triphosphatase-mediated mRNA capping is essential for maintaining transcriptome homeostasis and the survival of *Toxoplasma gondii*
Kalyani Aswale (Hyderabad/IN)

P103 Recurrent ocular toxoplasmosis after SARS-CoV-2 infection: three case reports
Olivera Lijeskić (Belgrade/RS)

P105 Molecular evidence of *Toxoplasma gondii* infection, neuropathology and immunofluorescent detection in the amygdala, hippocampus, pre-frontal cortex, and occipital areas from forensic humanbrain tissue from suicide, traffic accidents, and homicide victims
Jennifer Nessim (Cali/CO)

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Chandra Ramakrishnan (Zurich/CH)

P109 GET-TOXO Project (Spanish Working Group on Toxoplasmosis): a multidisciplinary “One health” approach for controlling toxoplasmosis in human and animals in Spain
Ana Montoya Matute (Madrid/ES)

P111 Humoral immunity to *Toxoplasma gondii* is mediated by the expression of Nfkbid in T cells
Juan Sanchez-Arcila (Merced, CA/US)

P113 Chronic *Toxoplasma gondii* Infection Results in Elevated Matrix Metalloproteinase-9 and Reduced Cerebellar Perineuronal Nets
Jianchun Xiao (Baltimore, MD/US)

P115 From TgO/GABA-AT, GABA, and T-263 Mutant to Conception of *Toxoplasma*
Rima McLeod (Chicago, IL/US)

P117 Dissecting the mechanism by which *Toxoplasma gondii*-specific CD8+ T cells differentiate to become IFNγ producers
Laura Lopez (Merced, CA/US)

P119 Unraveling the biochemical interactions that regulate *Toxoplasma* GRA trafficking
Elisia Paiz (Dallas, TX/US)

P121 *In Vitro* Activity of Fractionated Methanoic Blueberry Extract against *Toxoplasma gondii* growth
Japhet Senyo Kamasah (Montgomery, AL/US)

P123 Populations of inflammation driven neuroprotective lipocalin-2 reactive mouse astrocytes resolve following chronic *Toxoplasma* infection
Emma Wilson (Riverside, CA/US)

08.30–09.00 Plenary Talk III
Goethe-Saal & Galerie

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Jonathan C. Howard (Oeiras/PT)

09.00–10.30 Session: Session V: Metabolism, Biochemistry & Drug Development
Goethe-Saal & Galerie

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Eléa Renaud (Montpellier/FR)

09.15 T33 Exploring redundancies in central carbon metabolism in *Toxoplasma* parasites
Capella S Maguire (Canberra/AU)

09.30 T34 The regulation of the constant flux of fatty-acids relies on key acyltransferases regulating the nutrient flexibility in *Toxoplasma gondii*
Sarah Charital (La Tronche/FR)

09.45 T35 Genetic screens in physiological contexts nominate metabolic pathways for deep learning-assisted drug discovery
Christopher J. Giuliano (Cambridge, MA/US)

10.00 T36 Genetic deletion of an essential lipid storage enzyme attenuates the virulence of *Toxoplasma gondii*
Shahbaz Khan (Baltimore, MD/US)

10.15 T37 A drug repositioning strategy identifies a compound inhibiting GSK3 activity in *Toxoplasma gondii*
Silvia Diaz-Martin (Grenoble/FR)

10.30–11.00 Coffee Break & Industrial Exhibition
Meitner-Saal I+II & Planck-Lobby

11.00–12.30 Session VI: Stage Conversion
Goethe-Saal & Galerie

11.00 T38 Single-cell RNA sequencing and immunohistochemical analysis of reactivating bradyzoites in vivo
Robyn Kent (Oklahoma City, OK/US)

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Chris Tonkin (Melbourne/AU)

11.30 T40 Translation initiation factor eIF1.2 is a crucial early regulator of *Toxoplasma* bradyzoite cyst formation
Fengrong Wang (Ann Arbor, MI/US)

11.45 T41 *In vitro* reactivation of chronic-stage *Toxoplasma gondii* parasites
Dylan McCormick (Cambridge, MA/US)

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Hisham Alrubaye (Pittsburgh, PA/US)

12.15 T43 Characterizing the impact of mRNA regulatory features on translation efficiency in *Toxoplasma gondii*
Michelle Peters (Cambridge, MA/US)

12.30–13.30 Industrial Exhibition
Meitner-Saal I+II & Planck-Lobby

12.30–13.30 Lunch Break (Restaurant area)

13.30–15.00 Session VII: Epidemiology, Public Health & Clinical Aspects
Goethe-Saal & Galerie

13.30 T44 The Role of Age in Ocular Toxoplasmosis: Clinical Signs of Immunosenescence and Inflammageing – a perspective study
Armin Taghavi Eraghi (Berlin/DE)

13.45 T45 Chronic latent *Toxoplasma gondii* infection precipitates cognitive decline in an inducible Alzheimer's disease model
Elsa Suberbielle (Toulouse Cedex 3/FR)

14.00 T46 Chronic *Toxoplasma gondii*, Diabetes, and Retinopathy in Pregnancy
Maureen Groer (Knoxville, TN/US)

14.15 T47 Serological Responses to *Toxoplasma gondii* and Matrix Antigen 1 Predict the Risk of Subsequent Toxoplasmic Encephalitis in People Living with HIV
Jianchun Xiao (Baltimore, MD/US)

14.30 T48 An *in-vitro* method and predictive model to quantify *Toxoplasma gondii* inactivation by salting
Marieke Opsteegh (Bilthoven/NL)

14.45 T49 Usefulness of serotyping tools in *Toxoplasma gondii* infections in sheep and pigs
David Arranz-Solís (Madrid/ES)

15.00–15.30 Turbo Talks for Poster Session II (even poster IDs, 002-030)
Goethe-Saal & Galerie

15.30–16.00 Coffee Break & Industrial Exhibition)
Meitner-Saal I+II & Planck-Lobby

16.00–17.30 Session VIII: Intracellular Replication & Survival
Goethe-Saal & Galerie

16.00 T50 Exploring the ultrastructure of cell division coordination in *Toxoplasma gondii* through expansion microscopy
María Eugenia Francia (Montevideo/UY)

16.15 T51 FIT plays a role in iron acquisition in *Toxoplasma gondii*
Dana Aghabi (Glasgow/GB)

16.30 T52 Functional dissection of NudCL1 protein in *Toxoplasma gondii* cell division.
Adeline Ribeiro E Silva (Chestnut Hill, MA/US)

16.45 T53 AP2XII-9 is a crucial factor regulating the cell cycle of *Toxoplasma gondii* tachyzoites.
Maanasa Bhaskaran (Lille/FR)

17.00 T54 Defining the Role of the Kinase TgGSK in *Toxoplasma gondii*
Amanda Krueger (Indianapolis, IN/US)

17.15 T55 A pair of large centrosomal proteins are crucial for daughter cell formation in *Toxoplasma gondii*.
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Kseniia Bondarenko (Edinburgh/GB)

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Min Chen (Genève/CH)

14.00 T70 — A multitude of small mitochondrial RNAs are used in the mitoribosome of *Toxoplasma gondii*
Christian Schmitz-Linneweber (Berlin/DE)

14.15 T71 — TgORP2, an Oxysterol binding protein Related Protein putatively involved in Endoplasmic reticulum and Mitochondrion Membrane Contact Site formation and lipid exchange in *Toxoplasma gondii*
Nyamekye Quansah (La Tronche/FR)

14.30 T72 — To inhibit or not to inhibit: characterization of the *Toxoplasma gondii* homolog of ATPase inhibitory factor 1 (IF1)
Madelaine Usey (Athens, GA/US)

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Lynen-Zimmer

19.00–22.00 Social evening & Conference dinner at Event Location Wasserwerk Berlin

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GENERAL INFORMATION

Certificate of attendance

Certificates of attendance will be sent by e-mail after the congress.

Catering

Catering with snacks and drinks will be provided during the breaks.

Name badge

Please wear your name badge during all congress events, including the networking activities. Participants will receive their name badge at the check-in.

For speakers only: Media check-in

The media check-in for uploading your presentation is located in the lecture hall “Goethe-Saal”. For submission, please use a USB flash drive. Technical staff and equipment will be available for you to arrange and preview your presentation.

Poster session/exhibition

All posters will be displayed on poster boards, marked with your individual abstract ID. Poster authors can find their abstract ID (from P001 etc.) in the programme part of this book or in our previous mailings. Please be present at your poster during your poster session:

- Poster session I: odd abstract ID´s on Monday, 27 May
- Assembly time: from 11.00
- Disassembly time: after poster session I at 21.30 (same day!)
- Poster session II: even abstract ID´s on Tuesday, 28 May
- Assembly time: from 8.00 am
- Disassembly time: after poster session II at 21.30 (same day!)

For each poster session each participant receives 2 drink vouchers for alcoholic and non-alcoholic drinks, valid from 20–21.30 hrs. Please ask for them at the check-in. Additional drinks can be purchased on a self-pay basis.

Social evening & Conference dinner

Day: Wednesday, 29 May
Time: 19–22.00
Location: Wasserwerk, Hohenzollerndamm 208 a, 10717 Berlin

How to get there via underground railway:

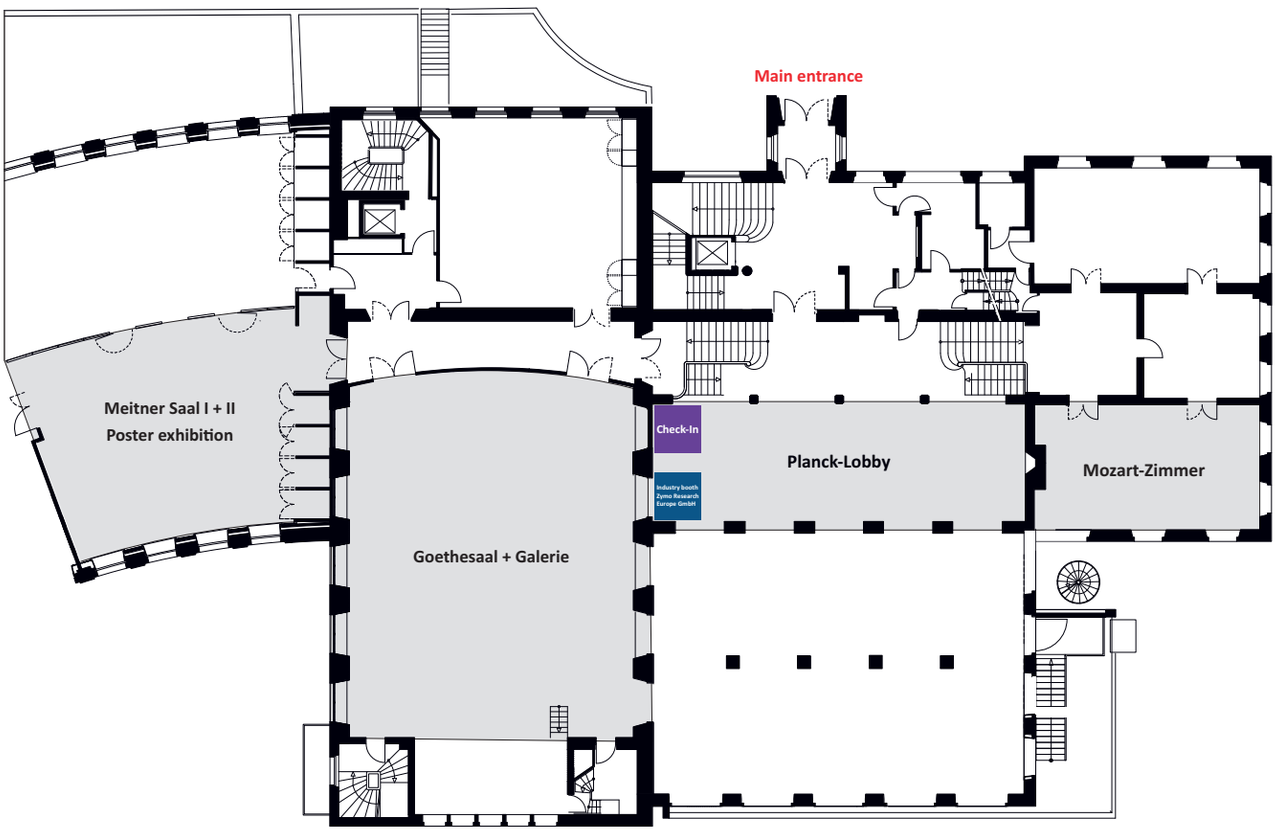
- Walk from the Harnack Haus to the underground station “Freie Universität” (5 min walk)
- Take the U3 in the direction of “Warschauer Straße” and get off at “Hohenzollernplatz” (10 min drive). A ticket was given to you at the Check-in, please don’t forget to stamp it BEFORE you board the subway.
- Walk from “Hohenzollernplatz” to the location “Wasserwerk Berlin” (5 min walk)

How to get there by taxi:
Taxi service Rainer Gau:
Phone 030 831 2754 or

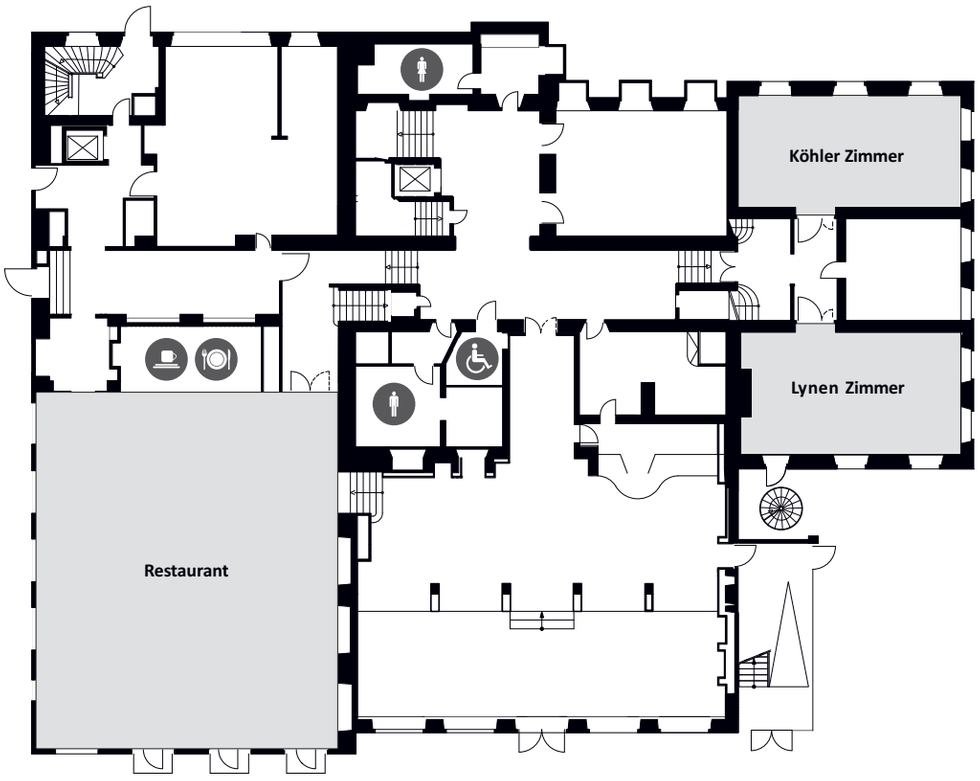
Taxifunk Berlin:
Phone 030 44 33 22.

More taxi providers can be found on the internet. Please note that participants are responsible for organizing a taxi.

Ground floor



Basement



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17th International Congress on Toxoplasmosis

Berlin, Germany | May 26th–29th 2024

Abstracts

T02

Is *Toxoplasma gondii* an under diagnosed cause of morbidity and mortality in humans?: My personal experience

*D. Ferguson[1]

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Toxoplasma is known to infect ~20% of the human population in the UK but is not considered a major cause of disease. However, there is an old saying "you will not find something unless you look for it". This presentation reflects my experience in a teaching hospital in Oxford, UK where pathologists knew of my interest in *Toxoplasma* (unrelated to my role in providing an ultrastructural diagnostic service). I had access to anti-*Toxoplasma* stage specific antibodies (tackyzoite-SAG1 and bradyzoite-Bag1). In the UK, where clinicians know of *Toxoplasma* in theory, it is rarely considered in differential diagnosis, except in cases of pregnancy. In other difficult cases, I was asked to examine pathology sections using immuno-cytochemistry to identify the presence or absence of *Toxoplasma*. In a period of 10 years, 10 cases were diagnosed at biopsy or autopsy plus two cases of congenital infection. These ranged from a middle-age woman (with unexplained headaches) to transplant patient (with negative serology). All the cases, except for the congenital cases, appeared to be associated with recrudescence rather than primary infection. In all cases, host tissue destruction is due to the uncontrolled proliferation of the tachyzoite stage. The devastating effect of uncontrolled proliferation will be illustrated. On review, most of these cases were subsequently shown to be associated with patients with a possibly impaired immune system. It is possible that earlier diagnosis and treatment could have prevented death but still cause some morbidity. In the congenital cases (diagnosed at 20 and 32 weeks scans due to foetal abnormalities), when the blood samples, taken at 12 weeks, was examined they were positive for *Toxoplasma* antibodies consistent with a recent primary infection. However, Toxoplasmosis is not routinely checked for in pregnant women in the UK. Could treatment at 12 weeks have protected the foetus? In adult cases, without the autopsy examination these deaths would have attributed to unknown infectious agent. Two changes could reduce the mortality: 1. Test the blood sample taken from pregnant women at 12 weeks for evidence of recent infection (IgM positive) and treat. 2. Check the immune status of patients with infections of unknown cause and, if immuno-compromised, consider *Toxoplasma* infection. From this local experience it could be suggested that *Toxoplasma* is an under diagnosed cause of mortality in the UK and probably many other countries.

T03

Uncovering the minimalist adhesive strategy of the Toxoplasma parasite for high-speed motility

*I. Tardieux[1], B. Touquet[1], L. Vigetti[1], D. Debarre[2], T. Rose[3], L. Bureau[2], D. Abdallah[1], G. Dubacheva[4]

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Toxoplasma gondii is a intracellular protozoan and the etiological agent of toxoplasmosis, a set of chronic diseases. The *T. gondii* tachyzoite morphotype serves as model to study cell motility in simple living non swimming systems, since with a permanent apicobasal polarity, it bypasses the cell symmetry breaking needed for motility. Its moves by gliding front first between cells or into and out from host cells with a repetitive helical trajectory. Gliding is thought to proceed through a series of sub-membranous myosin motors which direct retrograde translocation of actin filaments along the parasite length, and power continuous sub-membranous forces. In this scenario, the tachyzoite secretes adhesins that, once exposed at the cell apex, engage with ECMs ligands, and flow backward with the newly formed actin filaments. We recently uncover a critical specific anchorage site for the generation and transmission of a periodic traction force during gliding (Pavlou et al, 2020). In this new study, we have interrogated the minimal spatial and molecular requirements for productive adhesion and force transmission beneath efficient gliding. To this end, we have combined submicron resolution micropatterning with live, quantitative reflection interference contrast microscopy and expansion microscopy. Using 4D image modeling, we bring first nanoscale evidence that the tachyzoite needs to build only one apical anchoring contact with the substrate, which spatially defines a minimal force transmission platform over which it slides. We clarify the relationship between surface flow and force generation, by monitoring microbead flow at 200 Hz and uncover that the apicobasal driven surface flow is set up independently from adhesin release and adhesion, prior to motile activity. Finally, to screen for the essential adhesion requirements for helical gliding at the single molecular species level in absence of any absorption of molecular components from the environment, we developed biochemical and biophysical quantitative assays using tunable surface chemistry and quartz crystal microbalance with dissipation monitoring. These approaches uncover the sufficiency of glycosaminoglycan (GAG)-parasite interaction to promote a productive contact for helical gliding and offer a new versatile platform to dissect the structure and density of the molecules functionally involved in the *T. gondii* gliding force

Pavlou et al., ACS Nano 2020. doi: 10.1021/acsnano.0c01893

T04

The *Toxoplasma gondii* mitoribosome reveals novel features of ribosome evolution

*S. Shikha[1], V. Tobiasson[2], M. Silva[1], J. Ovciarikova[1], D. Beraldi[1], A. Muehleip[3], L. Sheiner[1]

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[3]*Institute of Biotechnology, Helsinki Institute of Life Science HiLIFE, University of Helsinki, Helsinki, Finland*

Mitochondrial ribosomes (mitoribosomes) are fundamental, and their function of synthesising mitochondrial proteins is universal, including in parasites. The apicomplexan mitochondrion is essential for parasite survival, virulence, and dissemination, and the same is thus expected for its mitoribosome. In agreement with this prediction, evidence of the essentiality of conserved mitoribosomal proteins in apicomplexan, accumulate, including from our own work. In addition to being essential, indirect observations further suggest that this mitoribosome is highly divergent from its human parallel. Divergence is expected from the prediction of rRNA fragmentation, based on different apicomplexan mitochondrial genome sequences, as well as on the sensitivity profile of some apicomplexan parasites to mitoribosome inhibitors.

Despite its essentiality and divergence, the biology of the apicomplexan mitoribosome is poorly studied. Here, using *Toxoplasma gondii* as a model organism, we employed a combination of complementary approaches to expand our understanding of the apicomplexan mitoribosome function and assembly. We discovered an rRNA fragmentation that is much more extensive than predicted according to the parasite mitochondrial genome, and revealed several novel features that enable this highly divergent ribosome to still perform its critical function.

One example of an apicomplexan, and likely myzozoan, mitoribosome signature feature we discovered is the repurposing of several transcription factors as new mitoribosomal proteins, which we believe compensate for rRNA remodelling and we postulate that they effectively replace conserved and critical ribosomal domains. On top of addressing the fundamental question of how divergent ribosomes function, our work has further potential to inform apicomplexan drug discovery, which we demonstrate by revisiting a previously proposed resistance mechanism of apicomplexan to a known mitoribosome inhibitor.

T05

Evolutionary Insights into the Composition and Assembly of Mitoribosomes in Alveolates

*Y. Jia[1], C. Wang[2], S. Kassem[3], P. Sun[2], A. Bonavoglia[3], J. Kloehn[3], X. Liu[2], X. Suo[2], D. Soldati-Favre[3]

[1]*Beijing Friendship Hospital, Capital Medical University, Beijing Institute of Tropical Medicine, Beijing, China*

[2]*China Agricultural University, College of Veterinary Medicine, Beijing, China*

[3]*University of Geneva, Department of Microbiology and Molecular Medicine, Geneva, Switzerland*

The Myzozoa, a grouping of phyla within the Alveolates, emerged through endosymbiosis with a red alga, resulting in the acquisition of a plastid. This cluster includes Apicomplexa, Chromerida, Perkinsozoa, and Dinoflagellates, all sharing a notably reduced mitochondrial genome that encodes only three proteins for the mitochondrial electron transport chain, cytochrome c oxidase subunits 1 and 3 and cytochrome b. Intriguingly, the mitoribosomal rRNAs in these phyla exhibit high segmentation, prompting questions about the process of mitoribosome assembly.

Furthermore, these phyla possess four conserved proteins with domains resembling the plant Apetala2/ethylene response factor (AP2/ERF) integrase DNA binding domain. In *Toxoplasma gondii*, these four AP2 proteins localize within the mitochondrion and play a crucial role in oxidative phosphorylation and parasite viability. Surprisingly, instead of acting as transcription factors, these mtAP2s form tight interactions with the small and large mitoribosomal subunits. These genes are highly fitness-conferring and prove critical for mitoribosome assembly and integrity.

Applying mass spectrometry and small RNA sequencing to the purified T. gondii mitoribosome revealed its distinctive protein-rich composition, abundance of fragmented rRNAs, and assembly factors. Besides mtAP2s, other contributors, such as the RAP (RNA binding domain abundant in apicomplexans) proteins, and HPR (heptatricopeptide repeat) proteins, contribute to molecular diversity and evolutionary adaptations. Together, our results provide insight into the composition, biogenesis and evolution of the ribosomal machinery in Alveolates.

T07

An ISWI-related chromatin remodeler tunes MORC DNA binding and insulates gene expression in a densely packed genome

*B. Pachano[1], D. C. Farhat[1], C. Corrao[1], M. Shahinas[1], L. Belmudes[2], Y. Couté[2], A. Bougdour[1], C. Swale[1], M. A. Hakimi[1]

[1]*INSERM, Universite Grenoble Alpes, Grenoble, France*

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ATP-dependent nucleosome remodeling complexes, also known as chromatin remodelers, are specialized multiprotein machines involved in genome organization and accessibility. They alter the structure, composition and positioning of nucleosomes by histone displacement, rejection or incorporation of histone variants. These actions control the accessibility of regulatory regions such as promoters and enhancers for transcription factors, RNA and DNA polymerases as well as coactivators or repressors. Phylogenetic studies have shown that Toxoplasma has evolved two divergent ATP-dependent remodeler proteins of the ISWI family, TgSNF2h and TgSNF2L. These proteins differ not only in their internal structure, but also in their partnerships. They associate with different AP2 transcription factors and unique scaffold proteins, each characterized by specific folded domains. ChIP-seq data show significant colocalization of TgSNF2h and MORC throughout the genome. Depletion of TgSNF2h leads to a marked decrease in the binding of MORC to chromatin, but does not affect its binding to telomeres. When TgSNF2h is released from chromatin at highly active genes (approximately 572 genes), there is a corresponding decrease in chromatin accessibility near the transcription start site (TSS), as shown by ATAC-seq, which in turn triggers a marked drop in mRNA levels of these genes, many of which are essential for the tachyzoite lytic cycle (e.g., AMA1, MIC1, SAG1, and GRA15). In TgSNF2h-depleted zoites, release of MORC from chromatin leads to activation of the 1,017 genes associated with the sexual stages regulated by MORC. In this context, TgSNF2h functions as a chromatin remodeler that insulates neighboring genes by potentially blocking the influences of transcriptional activators and repressors. At highly transcribed loci, TgSNF2h and MORC appear to act as barriers to demarcate active chromatin regions typified by unique levels of acetylation or to polarize promoter regions. This action likely sets the stage for the establishment and maintenance of specific gene expression patterns. Together, TgSNF2h and MORC serve as a safeguard against the unintended spreading of transcriptionally active euchromatin from a gene active in tachyzoites to a neighboring developmental gene that is silenced. Thus, TgSNF2h, having an epistatic effect over MORC, plays a critical role in the partitioning of developmental genes in *Toxoplasma*.

T08

Forward genetic screen identifies SRS15 and IFT88 as critical determinants controlling *Toxoplasma* oocyst shedding in cats

A. Sardinha-Silva[1], V. Pszenny[1], E. V. C. Alves-Ferreira[1], D. Stewart[1], E. Thin[1], M. Quinones[1], Z. Vavrušková[2], B. Sak[2], J. P. Dubey[3], J. Lukeš[2], *M. E. Grigg[1]

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Transmission of *Toxoplasma gondii* in nature is highly dependent on the parasite"s sexual cycle, which occurs exclusively in felids. Infected cats shed high numbers of infectious oocysts, which are capable of causing toxoplasmosis outbreaks in both animals and people. The molecular pathways promoting sexual stage development in *T. gondii* are largely unknown. To identify essential genes that regulate oocyst formation we undertook a forward genetic signature-tag mutagenesis screen, using CRISPR/Cas9 to generate a library of *T. gondii* strains that each possess a unique barcode and are each deficient in a single gene predicted to be highly expressed in the parasite"s sexual stages. Specifically, we used previous RNA-Seq datasets to identify 192 sexual stage-specific transcripts to test for their role promoting sexual competency. These included surface antigen genes related to 6-CYS proteins in *Plasmodium*, genes required to form flagella, a family of transcription factors collectively referred to as ApiAP2s, and a large family of secreted proteins expressed exclusively in merozoites (Families A-D). To identify which genes impacted oocyst formation, we pooled the 192 *T. gondii* knock-out (KO) strains, infected mice, then challenged cats with brain cysts to perform an input/output screen that identified sexual stage-specific genes critical for oocyst formation. Mouse brains were harvested 50 d.p.i. and split into an input sample and a cat inoculation sample. Mi-Seq analysis identified 2 genes, referred to as IFT88-, SRS15-KO that had reduced, or failed to produce oocysts in cats, respectively. When assayed individually through cats, IFT88- and SRS15-KO strains did not produce oocysts. Complementation of the IFT88 gene restored oocyst production. Cats vaccinated with either IFT88- or SRS15-KO strains were immune to homologous rechallenge, heterologous

rechallenge experiments are ongoing and results will be presented. Our data demonstrate the potential efficacy of an attenuated oral vaccine to block oocyst shedding and reduce the transmission of *Toxoplasma* in food and water sources destined for human and animal consumption.

T09

mRNA cap-binding protein eIF4E1 is a novel regulator of *Toxoplasma gondii* latency

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The protozoan parasite *Toxoplasma gondii* causes serious opportunistic disease due to its ability to persist in patients as latent tissue cysts. The molecular mechanisms coordinating conversion between proliferative parasites (tachyzoites) and dormant cysts (bradyzoites) are not fully understood. We previously showed that phosphorylation of eIF2α accompanies bradyzoite formation, suggesting that this clinically relevant process involves regulation of mRNA translation. In this study, we investigated the composition and role of eIF4F multi-subunit complexes in translational control. Using CLIPseq, we find that the cap-binding subunit, eIF4E1, localizes to the 5”-end of all tachyzoite mRNAs, many of which show evidence of stemming from heterogenous transcriptional start sites. We further show that eIF4E1 operates as the predominant cap-binding protein in two distinct eIF4F complexes. Using genetic and pharmacological approaches, we found that eIF4E1 deficiency triggers efficient spontaneous formation of bradyzoites without stress induction. Consistent with this result, we also show that stress-induced bradyzoites exhibit reduced eIF4E1 expression. Overall, our findings establish a novel role for eIF4F in translational control required for parasite latency and microbial persistence.

T10

Integrative Analysis of AP2 Transcription Factor Dynamics and Binding Motifs in *Toxoplasma gondii*

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The complex life cycle of *Toxoplasma gondii* encompasses two infectious phases in intermediate hosts: the rapidly replicating tachyzoite and the dormant bradyzoite, responsible for acute and chronic infections, respectively. The ability of the parasite to form tissue cysts, predominantly in the brain, eyes, heart, and kidneys, and to reactivate to cause acute disease underlies its significant pathogenicity, particularly in immunocompromised individuals such as HIV patients. Despite its prevalence, effective treatments targeting cyst formation and reactivation are lacking. Our study aims to unravel the mechanisms driving the formation and reactivation of these tissue cysts, focusing on the role of AP2 transcription factors (TFs), the major family of transcription factors in Apicomplexa. AP2 TFs are implicated in controlling the gene expression necessary for the parasite’s lifecycle, pathogenicity, and developmental transitions. Therefore, interaction between AP2 TFs and their DNA binding sites is very important. We constructed an integrated single-cell RNA sequencing (scRNA-seq) atlas using published data from *T. gondii* Type II strains, ME49 and Pru that are typically used for bradyzoite models. This atlas delineates gene expression profiles across different cell cycle phases and environmental conditions, offering a detailed map of transcriptional changes associated with the parasite’s development, and highlighting the functionality of specific genes and pathways. We then investigated this atlas using the binding dynamics of AP2 TFs. Utilizing Protein Binding Microarray (PBM) data, we performed motif scanning to align AP2 binding motifs with scRNA-seq data using the MAST algorithm from MEME. We focused on mapping the expression profiles of AP2 genes and their targets within the single-cell dataset. These data implicate AP2 in both gene activation and gene repression during regulation of cell cycle progression and tachyzoite to bradyzoite differentiation. Our research provides insights into the transcriptional control in *T. gondii*, emphasizing the critical role of AP2 TF motifs in its adaptive life cycle. The integrative approach of mapping AP2 factor dynamics and gene expression profiles opens new avenues for therapeutic interventions, targeting the developmental transitions of *T. gondii*, and potentially leading to more effective treatments for toxoplasmosis.

T11

ToxoDB: Tools for Genomic-Scale Data Exploration, Analysis, Integration and Discovery

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Biomedical research is increasingly driven by Big Data: genome sequences & diversity data, all manner of multi-Omics datasets, *etc*. How can we effectively collect, store, maintain and integrate this information to ensure FAIR (**F**indable,**A**ccessible, **I**nteroperable, **R**eusable) data access, advancing biological understanding and defining targets for further study in the lab, field & clinic? The Eukaryotic Pathogen & Vector Genomics Resource (VEuPathDB.org) – including ToxoDB.org– provides a robust, sustainable data-mining resource, accessed by 1000s of researchers daily, and demonstrably expediting discovery & translational research on diverse eukaryotic microbes (fungi & protists). We will be available at Toxo XVII to demonstrate database functionality and discuss topics of community interest, *e.g.:*

- accessing & interpreting information on genes, genomes, population diversity, comparative genomics, DNA/protein motifs, protein structures, interactomes, epigenetics, transcriptomes, proteomes, metabolomes, pathways, subcellular localization, phenotypic characterization, orthology-based functional inference, automated & curated annotation, *etc*
- strategies for integrating & interrogating diverse datasets … and analyzing & sharing the results obtained
- assessing & improving the quality and accuracy of available annotation … capturing expert knowledge from the community
- analyzing *your own* (or any public) datasets via the free, private, easy-to-use, cloud-based VEuPathDB Galaxy instance and integrating/querying results in the context of other data in FungiDB
- identification and prioritization of new *Toxoplasma* datasets for integration, and development of new database features; prospects for sustainable coordination with other resources
- new datasets & functionalities currently under development at VEuPathDB/FungiDB … what to expect over the coming years
- additional help & assistance with database mining & FAIR data access/sharing

T12

Understanding the History of the Domestic Cycle of *Toxoplasma gondii* in South America

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A growing body of evidence supports the significant role of Toxoplasma gondii genotype in determining disease severity. However, isolating and characterizing T. gondii strains infecting humans remains challenging. Clinical severity of toxoplasmosis varies geographically, with South America experiencing the highest burden, coinciding with the continent's remarkable T. gondii genetic diversity. In our study, we analyzed 156 T. gondii genomes and we provide the first direct estimates of T. gondii's mutation rate and generation time. Our findings offer insights into the epidemiology, ecology, and evolutionary history of South American T. gondii populations. We identified three distinct categories: (1) wild populations, transmitted through the sylvatic cycle involving wild felids and likely poorly adapted to domestic cats. This includes the "Amazonian" population, linked to various clinical forms in immunocompetent individuals, and the "Pan-American" population, isolated from wildlife and not yet associated with severe disease forms, (2) domestic intercontinental clonal lineages (types I, II, III, and Africa 1), introduced to the continent with the domestic cat and house mouse by European sailors. (3) hybrids of the first two categories, probably well adapted to domestic cats, and representing the majority of strains found in South America's domestic environment. We discuss how hybridization events and strong selection for a unique haplotype comprising only 0.16% of the T. gondii genome on chromosome 1a are likely driving the public health challenge associated with toxoplasmosis in South America.

T13

Identification of proteins mediating ER-Toxoplasma contact sites

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The formation of membrane contact sites (MCSs) between intracellular pathogens and host organelles is a common consequence of infection. We previously found that the human parasite Toxoplasma gondii forms contact sites with host mitochondria and drives the shedding of their outer membrane. Here, we investigate the molecules that mediate the MCSs between Toxoplasma and host endoplasmic reticulum (ER). Although these MCSs were first described in 1968, the proteins mediating this contact remain unknown. To this end, we developed a split-GFP based sensor wherein GFP reconstitution indicates successful formation of contact sites between the pathogen and host organelles. To validate our sensor for FACS based CRISPR-Cas9 screening, we first applied it to monitor Toxoplasma-mitochondria MCSs and as expected found that our sensor reconstituted GFP at these contact sites, but failed to do so in the absence of the mitochondrial tether TOM70 or its Toxoplasma counterpart MAF1. To identify the proteins that tether the ER to the parasite vacuole in an unbiased manner, we adapted our sensor to Toxoplasma-ER MCSs and performed a genome-wide loss of function CRISPR screen. Our candidate ER tether suggests a conserved mechanism by which pathogens contact diverse host organelles. Defining the molecules that mediate pathogen-organelle MCSs will enable us to determine their function during infection and enhance our understanding of contact site biology in general.

T14

Formation of mammalian ER membrane contact sites, via host VAPA and VAPB, with the *Toxoplasma* PV

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The *Toxoplasma* parasitophorous vacuole membrane (PVM) forms a barrier protecting the parasite from cellular assaults and serves as a platform for nutrient retrieval from the host cell. The PVM physically associates with many host organelles (e.g., ER and mitochondria), at distances reminiscent of membrane contact sites (MCS < 15 nm). We hypothesize that the parasite establishes functional MCS between the PVM and selected host organelles. In support, our RNAseq data show altered expression of several host lipid transfer proteins located at MCS (e.g., StAR, OSBP2, NPC1) in *Toxoplasma*-infected cells, suggesting hijacking of nonvesicular lipid transport pathways. Our fluorescence microscopy observations illustrate the recruitment at the PV of the host ER proteins VAPA and VAPB that both function as mammalian MCS tethering factors that bind to proteins with FFAT motifs. Our EM data show no host ER at PV in VAPA- and VAPB-deficient HeLa cells (DKO VAP, gift from P. De Camilli) infected up to 6h, suggesting *Toxoplasma* recognition of VAPA/VAPB for ER recruitment.However, 24h p.i. in DKO VAP cells, host ER was observed at the PV (27% coverage) though less than in WT HeLa (43%), suggesting that VAPA/VAPB are not critical for recruitment later in infection. We identified a PVM-localized *Toxoplasma* protein (we named TgVIP) with two putative FFAT motifs, as a potential host VAP interactor; a knock-out *vip* mutant exhibits a growth delay. Our EM data show TgVIP KO still recruits host ER, suggesting TgVIP is not involved in host ER-PVM attachment, but it may bind to host VAPs following host ER attachment to the PVM; we are assessing TgVIP and VAP interaction in situ. Previously, we demonstrated that *Toxoplasma* retrieves nutrients from host organelles (e.g., endolysosomes, lipid droplets, Rab vesicles) trapped in tubules of the intravacuolar network (IVN). The IVN-deficient parasite (Δgra2Δgra6), impaired in intra-PV host organelle sequestration, forms an extensive network of PVM projections (PVMP) extending into the host cell. As this mutant retains virulence, we hypothesize that its survival correlates with increased PVM-host organelle MCS formation. Indeed, host VAPA and a related host ER MCS protein MOSPD2 localize to Δgra2Δgra6 PVMP. In DKO VAP cells at 24h pi, less host ER is recruited to Δgra2Δgra6 PV and PV-associated host mitochondria show aberrant morphology. These observations point to a physiological relevance for MCS formation to support parasite infectivity.

T15

The role of the GPI in *Toxoplasma gondii* pathogenesis and mRNA vaccines

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A fully protective vaccine to prevent human parasitic disease has remained elusive. Our investigation of acquired immunity to *Toxoplasma gondii* that follow either natural infection or vaccination has led us reconsider the role of glycosylphosphatidylinositols (GPIs) in parasite pathogenesis and vaccination. GPIs are highly conserved anchors for eukaryotic cell surface proteins. *T. gondii"s* plasma membrane is covered with GPI-anchored proteins, and free GPIs called GIPLs. While the glycan portion is conserved, species differ in sidechains added to the triple mannose core. The functional significance of the Glcα1,4GalNAcβ1- sidechain reported in *T. gondii* has remained largely unknown without an understanding of its biosynthesis. We became curious of the gene *Tg_207750* as it was one of the most differentially expressed genes between type I strains that either evade immunological memory (GT1) or are controlled by it (RH) and is a predicted glycosyltransferase that adds hexoses to mannose chains. Here we report the identification and disruption of *Tg_207750* ("PIGJ") and *Tg_266320* ("PIGE") GPI sidechain glycosyltransferase genes and confirm their respective roles by serology and mass spectrometry. Parasites lacking the sidechain on account of deletion of the first glycosyltransferase, PIGJ, exhibit increased virulence during primary and secondary infections, suggesting it is an important pathogenesis factor. Cytokine responses, antibody recognition of GPI-anchored SAGs, and complement binding to PIGJ mutants are intact. Parasite numbers are not affected by *Δpigj* early in the infection, suggesting a breakdown of infectious tolerance, which is dependent on host galectin-3 but not TLR2/4 GPI interacting partners. In contrast, humoral recognition of GIPL is entirely dependent on the terminal Glucose as inferred from PIGE mutants. Furthermore, we present evidence that the GPI of type I RH but not GT1 strains lacks sidechains, further implicating this pathway in secondary infection immunity. To directly investigate the role of the GPI in humoral responses, we have tested SAG1 immunization with and without GPI attachment through mRNA vaccination, which revealed a fundamental requirement for the GPI in eliciting humoral responses to GPI-anchored proteins. Ongoing results will be discussed.

T16

A new *Toxoplasma* rhoptry protein is a major virulence factor that prevents inflammatory host cell death

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Programmed-cell death is an antimicrobial mechanism of defense that promotes rapid clearance of intracellular pathogens. *Toxoplasma* counteracts host immune defenses by secreting effector proteins into host cells; however, how the parasite evades inflammatory cell death and the effectors involved remain poorly characterized. We identified a new *Toxoplasma* virulence factor that ensures parasite survival by blocking host cell death. RNA-Seq analysis revealed that this rhoptry protein acts as a repressor of host pro-inflammatory responses. THP1 human monocytes infected with a mutant *Toxoplasma* strain, showed increased nuclear translocation of NF-kB p65, IL-1β and LDH release, compared to infection with wild type or complemented parasites. Moreover, mutant parasites were dramatically impaired in virulence in mice also preventing NF-κB signalling and lytic cell death in BMDM. These findings unravel the role of a major virulence factor of *Toxoplasma*, supressing inflammatory cell death as a strategy to evade parasite clearance.

T17

A high-throughput screen to elucidate the function of *Toxoplasma gondii* rhoptry proteins within human host cells

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Toxoplasma gondii contain apicomplexan-specific rhoptry organelles whose proteins are essential in mediating invasion of host cells and host-pathogen interactions at early intracellular timepoints. Rhoptry proteins (ROPs) are deposited within the host prior to invasion and localize to various host compartments to rewire the host cell for the parasite"s survival. While some of these proteins have been characterized, the function of the majority of ROPs remains unknown. Here we systematically screen the function of ROPs within host cells utilizing a BioID approach, where ROP baits fused to miniTurboID, are expressed in HEK293 cells and biotinylated proximal prey are identified by mass spectrometry. We have generated a partial dataset containing 23 rhoptry baits and 1472 prey. These data recapitulate known features of rhoptry host biology. Toxofilin prey are significantly enriched in actin cytoskeleton localizing proteins, including the known interactor actin. ROP18 prey are significantly enriched in endoplasmic reticulum proteins, including the known substrate RPN1. Furthermore, ROPs that localize to host compartments do so in this experimental system: ROP47 and PP2C-hn prey are significantly enriched in nucleoplasm proteins and ROP39 prey are significantly enriched in mitochondrial proteins. Novel insights include an enrichment of microtubule proteins in ROP19A prey and peroxisomal proteins in ROP13 prey. Together these results establish the efficacy of this approach and highlight the potential for novel discoveries regarding the function of rhoptry proteins in human host cells.

T18

Rhoptry-associated perforation of the host cell plasma membrane during *Toxoplasma gondii* invasion

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The exocytosis and delivery of rhoptry proteins into the host cell is essential for *Toxoplasma gondii* invasion and virulence. The rhoptry secretory apparatus (RSA) has become increasingly well-defined and shows conservation among Apicomplexans. While we now know a great deal about both the RSA structure and the individual rhoptry effector proteins that are released, comparatively little is known about how those proteins are delivered into the host cell. Previous electrophysiology experiments revealed that invasion by *T. gondii* is preceded by a transient increase in host cell plasma membrane conductance. We hypothesize that the conductance transient represents a perforation in the host cell plasma membrane through which rhoptry proteins are delivered into the host cell. To further investigate this event, we have developed a higher throughput assay that utilizes high-speed, multi-wavelength, fluorescence imaging to simultaneously visualize the host cell perforation and invasion of the parasite. Using this assay, we have interrogated a panel of mutant parasites conditionally depleted of key invasion-related proteins. Parasites lacking the rhoptry effector RON2 generate the perforation, suggesting that perforation is upstream of effector injection and moving junction formation. In contrast, parasites lacking components of the rhoptry exocytosis pathway (*e.g.*, RASP2, Nd9) or proteins that regulate rhoptry secretion (*e.g.*, CLAMP), are largely unable to induce the perforation. These data, which have been corroborated by electrophysiology studies, demonstrate that rhoptry exocytosis is required for formation of the perforation. Our results are consistent with a model in which rhoptry exocytosis results in transient perforation of the host cell membrane, enabling the subsequent delivery of rhoptry effector proteins into the host cell.

T19

A Kinase / Phosphatase Negative Feedback Loop Controls *Toxoplasma* Egress

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cAMP-dependent protein kinase A (PKAc1) plays a critical role as a negative regulator of egress by rapidly suppressing calcium signaling after invasion. The activity of protein kinase A (PKAc1) is governed by adenylate cyclases and phosphodiesterases that serve to produce and hydrolyze cAMP, respectively. Herein, we investigated the phosphodiesterase PDE2, which one of two essential isoforms out of a total 18 PDE genes in *T. gondii*. Immunoprecipitated PDE2 preferentially hydrolyzed cAMP *in vitro* and knockdown of PDE2 led to elevated levels of cytosolic cAMP *in vivo*, consistent with its role as a cAMP-specific PDE. Knockdown of PKAc1 also resulted in higher cytosolic cAMP levels suggesting that PKAc1 is likely required for PDE2 activation and cAMP turnover. PKAc1 phosphorylation of PDE2 was confirmed *in vitro* and differentially phosphorylated residues were identified using a conditional PKAc1 knockdown line expressing epitope tagged PDE2. Phospho-mimetic and -ablative forms of PDE2 were purified and tested for activity. Select phospho-mimetic mutations in PDE2 retained activity whereas the corresponding phospho-ablative mutations lost activity, confirming that PKAc1 phosphorylation activates PDE2, while dephosphorylation inhibits activity. Finally, to examine the impact of PDE2 on PKAc1 function, PDE2 depleted parasites were stimulated with zaprinast or ionomycin and then assessed for egress. Zaprinast, but not ionomycin, failed to induce Ca2+ mobilization and egress in PDE2 depleted parasites. These defects were partially rescued when PKAc1 was inhibited by H89 confirming that PKAc1 is primarily responsible inhibiting egress. These data support a model in which PKAc1 targets a process downstream of cGMP and upstream of Ca2+ release to prevent egress, and this block is relieved when PKAc1 phosphorylates PDE2 leading to consumption of cAMP and inhibition of PKAc1. The resulting negative feedback loop counteracts the PKG pathway that drives motility, invasion and egress, thus allowing equilibrium conditions during intracellular development.

T20

Exploring the role an Apical Polar Ring Protein in controlling F-actin translocation and gliding motility in *Toxoplasma gondii*

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In *Toxoplasma gondii*, the conoid comprises a cone with spiraling tubulin fibers, preconoidal rings (PCRs), and intraconoidal microtubules (ICMTs). This dynamic organelle undergoes extension and retraction through the apical polar ring (APR) during egress, gliding, and invasion processes. The forces involved in conoid extrusion start to be better understood and its role in directing the F-actin flux to the pellicular space, therefore controlling parasite motility, has been proposed[1]. However, the contribution of the APR and its interactions with the conoid remain unclear. To advance our comprehension of the APR's architecture, Ultrastructure Expansion Microscopy (U-ExM) was applied to pinpoint known and newly identified APR proteins (APR2-APR7). Our results indicate that the APR forms a fixed multilayered structure. Intriguingly, the conditional depletion of APR7 resulted in significant impairments in both motility and invasion. Electron microscopy revealed a disrupted upper layer of the APR in the absence of APR7, and the use of F-actin binding chromobodies uncovered abnormal flux of F-actin. In summary, this study provides valuable insights into APR assembly and help elucidating how its structure regulates parasite motility.

1. Haase, R et al. Nanoscale imaging of the conoid and functional dissection of its dynamics in Apicomplexa. Curr Opin Microbiol. 2022 Dec:70:102226.

T21

Revealing cellular and molecular organization in *Toxoplasma's* invasion machinery after ionophore stimulation

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Host cell invasion by *Toxoplasma gondii* and other important species within the phylum Apicomplexa, is an active process involving the coordinated action of a remarkable machine at their anterior end known as the apical complex (AC), for which the phylum is named. To date, fundamental details of the AC and capturing how the various apical organelles interact during invasion have been relatively elusive due, in part, to the fact that the entire complex is approximately a single diffraction-limited volume in size (in the visible). The conoid, a dynamic structure within the AC of *Toxoplasma gondii* and related coccidia, comprises a barrel of uniquely arranged tubulin-based filaments that protrudes during invasion. Numerous proteins have been shown to localize to the conoid, but many of their functions and precise locations have yet to be determined. Using cryogenic electron tomography, a powerful tool to study cellular architecture *in situ*, we visualize the parasite's AC in a near-native state. By averaging subvolumes of interest, we generated a density map that allowed us to accurately assign tubulins in the conoid fibrils and reveal the organization of tubulin-associated proteins. Using single-molecule, super-resolution fluorescence microscopy, we have determined with high resolution the molecular organization of Myosin H (Myo-H), a motor protein that associates with the conoid and is indispensable for parasite invasion. We similarly imaged tubulin in the parasites to provide context at the spatial scale necessary to determine how the Myo-H distribution organizes with respect to the AC as a whole. By imaging conoid-protruded and -retracted parasites, we monitor Myo-H reorganization in preparation for host cell invasion, bringing greater detail to this critical biological process.

T22

Unveiling the role of conoid gliding protein in *Toxoplasma gondii* invasion and egress: insights from cryogenic correlative light and electron microscopy (cryo-CLEM)

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*Toxoplasma gondi*i exhibits numerous cellular adaptations facilitating the invasion of host cells and the subsequent egress from them after multiplication inside of parasitophorous vacuoles. The apical complex comprised of cytoskeletal machinery and secretory organelles is pivotal in these processes. While the overall morphology of the parasites and the apical complex have been extensively studied using various techniques [1-3], the mechanistic understanding and structural composition of these components remain elusive. In a previous study, we identified novel genes implicated in invasion and egress, notably *cgp*, which encodes the conoid gliding protein. This protein colocalizes with RNG2, an integral component of Toxoplasma"s conoid. Conditional knockout of the *cgp* gene resulted in significantly reduced egress rates compared to wild-type parasites. To elucidate the impact of *cgp* depletion on the structure of the conoid and to gain insight into the underlying molecular mechanisms, we developed cryogenic correlative light and electron microscopy (cryo-CLEM) workflows to target and visualize the molecular architecture of the conoid within intact, cryo-preserved parasites. Our *in situ* cryo-electron tomography analyses revealed a loss of PCR structure in *cgp* knockout parasites compared with the wild-type phenotype, while other components of the conoid remained unaffected. Our results show the structural role of cgp protein for proper PCR formation and demonstrate the potential of cryo-CLEM targeted *in situ* tomography for studying the architecture and life cycle of native apicomplexa at unprecedented detail.

1) Tosetti et al., *Essential function of the alveolin network in the subpellicular microtubules and conoid assembly in Toxoplasma gondii*, eLife, 2020

2) Koreny et al., *Molecular characterization of the conoid complex in Toxoplasma reveals its conservation in all apicomplexans, including Plasmodium species*, PLoSBiol, 2021

3) Gui et al., *Cryo-tomography reveals rigid-body motion and organization of apicomplexan invasion machinery*, Nature Communications, 2023

T23

Bumped Kinase Inhibitors (BKI) inhibit Toxoplasma gondii MAPKL-1 and CDPK1

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Bumped Kinase Inhibitors (BKIs) based on a 5aminopyrazole-4-carboxamide (AC) core are a series of compounds that can be optimized to inhibit the calcium-dependent protein kinase 1 (CDPK1) of Toxoplasma gondii, Cryptosporidium species, and other apicomplexan pathogens. Extensive preclinical development of BKIs has identified AC-BKI-1748 as a promising lead compound for toxoplasmosis that is highly effective and non-toxic in animal models. Phenotypic and therapeutic effects of BKIs and previous experiments have suggested other potential inhibitory mechanisms of action in addition to TgCDPK1. The mechanism of BKI-1748 was therefore investigated using a forward genetic screen of chemical mutagenesis, selection of BKI-1748 resistant clones, and whole genome sequencing. Three clones were isolated with unique single nucleotide changes in the ATP binding site of T. gondii mitogen-activated protein kinase-like 1 (MAPKL1) gene in BKI-1748 resistant clones. One of these mutations, Leu162Gln, was introduced into a wild-type strain, resulting in 2- to 9-fold resistance to BKI-1748 and other BKIs including 1-NM-PP1. Because these compounds also were potent inhibitors of the isolated TgCDPK1 enzyme, we introduced a Gly128Met substitution into the TgCDPK1 gatekeeper position and found 2- to 8-fold resistance across BKIs. To further investigate the significance of these targets, we created a clone with both the above substitutions, which demonstrated a 14-fold to 153-fold resistance to BKIs. The identification of TgMAPKL-1 and TgCDPK1 as dual targets of multiple BKIs provides a greater understanding of the BKI mechanism of action and suggests a high genetic barrier to meaningful drug resistance for this promising class of compounds.

T24

Myosin F controls actin organization and dynamics in *Toxoplasma gondii*

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In eukaryotes, intracellular cargo transport typically involves molecular motors associating with the surface of membrane-bound cargo and transporting it along microtubule and actin tracks. In *Toxoplasma gondii* (*T. gondii*), intracellular cargo transport relies on actin and the unconventional myosin, myosin F (MyoF). Loss of MyoF disrupts vesicle transport, endomembrane organelle positioning, and apicoplast inheritance, yet the mechanism by which this actomyosin system's facilitates this process remains unclear. Using live-cell imaging, we determined that MyoF-EmFP has a dynamic and filamentous-like localization in the parasite cytosol, reminiscent of cytosolic actin filament dynamics. MyoF was not associated with Golgi, apicoplast or dense granule surfaces, suggesting that MyoF does not function using the canonical cargo transport mechanism. Instead, we find that loss of MyoF results in a dramatic rearrangement of the actin cytoskeleton in interphase parasites accompanied by significantly reduced actin dynamics, while actin organization during replication and motility were unaffected. Thus, these findings reveal that MyoF is an actin-organizing protein in *T. gondii* which facilitates cargo movement using an unconventional transport mechanism.

T25

Inducible nitric oxide synthase (iNOS) is necessary for GBP-mediated *T. gondii* restriction in murine macrophages via vacuole nitration and intravacuolar network collapse

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Toxoplasma gondii is an obligate intracellular parasite of rodents and humans. Interferon-inducible guanylate binding proteins (GBPs) are mediators of *T. gondii* clearance, however, the precise mechanism of *T. gondii* restriction is not known. Using automated spatially targeted optical micro proteomics we determined that inducible nitric oxide synthetase (iNOS) was highly enriched at GBP-positive PV in murine macrophages. iNOS expression in myeloid cells was necessary to control *T. gondii* growth in vivo and in vitro. iNOS activity was dispensable for GBP2 recruitment and PV membrane

ruffling, however, these vacuoles contained dividing parasites. *T. gondii* restriction by iNOS required nitric oxide, nitration of the PV and collapse of the intravacuolar network of membranes in a chromosome 3 GBP-dependent manner. We conclude that reactive nitrogen species generated by iNOS cooperate with the chromosome 3 GBPs to target distinct biology of the PV that are necessary for optimal parasite clearance in murine myeloid cells.

T26

Caspase-8-mediated cell death of CD8^{+} T cells is required for control of *Toxoplasma gondii* in the brain

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Host cell death represents a major mechanism of limiting intracellular pathogens. *Toxoplasma gondii* is an intracellular protozoan parasite that establishes a chronic infection of the brain and other immune-privileged tissues. We sought to determine whether cell death is a mediator of parasite control in the brain. Numerous types of programmed cell death have been defined and are regulated by distinct molecular pathways. We focused on the role of caspase-8, which is associated with the extrinsic pathway of apoptosis. The deletion of *Casp8* alone in mice results in embryonic lethality associated with widespread necrosis. The additional deletion of *Ripk3* results in mice that are viable. We began by infecting mice that lacked *Casp8* and *Ripk3*, *Ripk3* alone, and WT C57BL/6 controls with 10 cysts of Me49 parasites. We found that *Casp8*$^{-/-Ripk3-/-}$ mice were susceptible to *T. gondii* infection and survived approximately 50 days with overwhelming parasite burdens in the brain. We found that mice lacking *Ripk3* and the ability to trigger necroptosis were resistant to infection and had parasite burdens comparable to WT mice. Intriguingly, *Casp8*$^{-/-Ripk3-/-}$ mice had equivalent or stronger immune responses to *T. gondii* in the brain with the recruitment of IFN-γ-producing T cells and iNOS-producing macrophages. These results suggested that specific cell types in the brain may be particularly susceptible to *T. gondii* infection in the absence of *Casp8*. To this end, we infected WT and *Casp8*$^{-/-Ripk3-/-}$ mice on a cre-reporter background (flox-stop-flox ZsGreen) with a cre-secreting parasite (Pru-cre-mCherry). As previously reported, we found that primarily neurons are infected in wildtype mice. In *Casp8*$^{-/-Ripk3-/-}$ mice we found that many more neurons, astrocytes, and surprisingly CD8^{+} T cells had interacted with parasite. Infection of CD8^{+} T cells by *T. gondii* in WT mice has been previously reported by several groups. Given that CD8^{+} T cells are highly migratory in the brain, cell death may be required to restrict pathogen spread throughout the brain. To test the role of *Casp8* specifically in CD8^{+} T cells, we generated CD8a-cre x Casp8$^{fl/fl}$ x *Ripk3*$^{-/-}$ x Ai6 mice and Casp8$^{fl/fl}$ x *Ripk3*$^{-/-}$ x Ai6 controls. We find that mice with *Casp8*-deficient CD8^{+} T cells have elevated levels of parasite and CD8^{+} T cells in the brain at six weeks post-infection. These data suggest that cell death is an additional mechanism by which CD8+ T cells restrict parasite in the brain.

T27

CD8 T memory subset response in encephalitis model of Toxoplasma gondii infection.

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CD8 T cells play an important role in protection against *Toxoplasma gondii*, especially during the chronic phase of infection. Due to the ability of these cells to exhibit polyfunctional response including IFNg production and cytotoxic effect, the parasitic replication is kept under control. However, in an encephalitis model of infection, it has been demonstrated that memory CD8 T cells become exhausted due to an increased expression of inhibitory receptors like PD-1 that leads to reactivation of infection and host mortality. While studies performed with chronic viral infections and tumor models have reported that CD8 T cell memory is comprised of a heterogeneous population, in-depth information related to this response against T. gondii infection is scarce. We observed that after T. gondii infection, memory CD8 T cells do not fit into classical effector and memory phenotype but can be distributed into four subsets based on KLRG1 and CD62L expression. KLRG1+CD62L- CD8 T cell subset (**pop1**) displays the classical attribute of effector T cells while the 3 remaining subsets KLRG1-CD62L– (**pop2**), KLRG1-CD62L+ (**pop3**) and KLRG1+CD62L+ (**pop4**) exhibit higher CD127 expression, a characteristic of memory population signature. Interestingly, pop3 and 4 exhibit increased levels of multiple inhibitory receptors. Adoptive transfer studies suggest that the effector population (pop1), needed for controlling infection, is derived from pop3 that displays stem-like characteristics, with pop4 most likely acting as the intermediate population. These observations suggest that pop3 needs to be efficiently maintained to generate highly polyfunctional pop1 effectors to keep the chronic infection under control.

T28

Chronic IL-1-induced DNA double-strand break response in hippocampal neurons drives cognitive deficits upon latent *T. gondii* infection

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Chronic inflammation characterized by increased cytokine levels, such as interleukin-1 (IL-1), accompanies many neurological diseases but little is known about IL-1 contribution to cognitive impairment and its interplay with epigenetic processes, including the DNA double-strand break (DSB) response.
Here, we demonstrate that H2A.X-dependent DSB signaling in hippocampal neurons drives cognitive deficits upon chronically elevated IL-1. Mice persistently and latently infected with *Toxoplasma gondii* display impaired spatial memory consolidation along with elevated IL-1β in the hippocampus. We find that neuronal IL-1 signaling in excitatory neurons is required for the spatial memory deficits caused by *T. gondii* infection and by chronic systemic infusion of IL-1β. In both cases, the deficit in spatial memory was prevented by the abrogation of neuronal H2A.X-dependent signaling. Our results highlight the instrumental role of cytokine-induced DSB-dependent signaling in spatial memory defects. This novel pathological mechanism in inflammation control of neuronal function may extend to several neurological diseases.

T29

Dense Granule Protein 3 of *Toxoplasma gondii* Plays a Crucial Role in the Capability of the Tissue Cysts of the Parasite to Persist under the Presence of Anti-Cyst CD8+ T Cells during the Chronic Stage of Infection

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Toxoplasma gondii establishes chronic infection by forming tissue cysts, and this chronic infection is one of the most common parasitic infections in humans. Our recent studies revealed that whereas CD8+ T cells of genetically resistant BALB/c mice have the capability to remove the tissue cysts of the parasite through their perforin-mediated activities, small portions of the cysts are able to persist under the presence of the anti-cyst CD8+ T cells. It is currently unknown how those small portions of the cysts resist or escape the T cell immunity and persist in the hosts. In the present study, we discovered that the cysts, which persisted under the presence of the perforin-mediated CD8+ T cell immunity, have significantly greater mRNA levels for four dense granule proteins, GRA1, GRA2, GRA3, and GRA7, and one rhoptry protein, ROP35, than the total population of the cysts present in the absence of the T cells. In addition, increased levels of mRNA for GRA1, GRA3, and ROP35 in the cysts significantly correlated with their successful persistence through the condition in which greater degrees of reduction of the cyst burden occurred through anti-cyst CD8+ T cells. In addition, GRA3-deficient *T. gondii* displayed significantly enhanced elimination of the cysts by anti-cyst CD8+ T cells when compared to the wild-type parasite. These results indicate that GRA3 is a key molecule that mediates the capability of *T. gondii* cysts to persist by resisting or evading the anti-cyst activity of CD8+ T cells during the later stage of infection.

T30

NLRP3 inflammasome assembly and activation are mediated by dynein-dependent microtubule transport during *Toxoplasma gondii* infection of human immune cells

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IL-1β is a potent pro-inflammatory cytokine that plays a key role in innate immunity against infection. The NLRP3 inflammasome is a multi-protein complex that mediates the processing of pro-IL-1β into mature bioactive IL-1β during *T. gondii* infection of human peripheral blood monocytes. However, the cellular mechanisms that facilitate the assembly of NLRP3 inflammasome components into a complex in *T. gondii*-infected cells remain unknown. We utilized super-resolution imaging to visualize the transport of inflammasome components during *T. gondii* infection of human monocytic cells. NLRP3 co-localized with host cell microtubules and the microtubule-organizing center (MTOC), specifically in infected cells, and proximity-ligation assays (PLA) revealed close interaction between NLRP3 and α-tubulin during infection. Treatment of cells with an inhibitor of cytoplasmic dynein motor proteins to prevent dynein-dependent microtubule transport

resulted in reduced NLRP3 interaction with α-tubulin, decreased caspase-1 cleavage, and reduced IL-1β processing and release, suggesting a key role for dynein-mediated transport of NLRP3 on microtubules for assembly and activation of the NLRP3 inflammasome. After inflammasome-mediated cleavage, bioactive IL-1β was released from *T. gondii*-infected human monocytes in a gasdermin D- and E-independent manner and in the absence of pore formation. To visualize and track IL-1β processing and release during infection, we expressed an IL-1β fluorescence resonance energy transfer (FRET) construct in human monocytic cells, in which mTurquoise and mNeonGreen were fused to the N- and C-termini of pro-IL-1β, respectively. Intact pro-IL-1β exhibited FRET signal, whereas cleavage of pro-IL-1β was detected through the loss of FRET. These studies revealed that *T. gondii* infection increased processing of IL-1β, which localized proximal to the plasma membrane and in plasma membrane-coated vesicles and protrusions. Collectively, these findings expand our understanding of the mechanisms of NLRP3 inflammasome assembly and IL-1β processing and release during *T. gondii* infection of human immune cells.

T32

A novel component of the cytosolic iron-sulfur biogenesis pathway, TgHCF101, as a potential drug target in *Toxoplasma gondii*

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Toxoplasma gondii is a unicellular eukaryotic parasite responsible for causing toxoplasmosis in Humans. The disease, asymptomatic in most instances, can be threatening to immunocompromised individuals. The parasite infects about a third of the global population, mainly through undercooked meat consumption, utilizing cysts as a form of persistence. *T. gondii*, as an obligate intracellular pathogen, scavenges essential nutrients, including iron, crucial to its growth, from its host cell. Both the host and the parasite are using iron; as it is a component of protein cofactors like heme or iron-sulfur (Fe-S) clusters, vital to the parasite. Fe-S clusters represent ancient and indispensable molecular structures formed by specific arrangements of coordinated iron and sulfur atoms. Their biogenesis and specific insertion into target proteins rely on multiprotein complexes fairly conserved across species. Proteins containing Fe-S clusters play crucial roles in cellular functions such as genome maintenance, protein translation, energy conversion and various enzymatic activities implying electron transfer mechanisms. Within *T. gondii*, three metabolic pathways for Fe-S clusters biogenesis co-exist, located within the mitochondrion, in the plastid of the parasite, or in the cytoplasm. We have characterized the *T. gondii* homologue of a protein called HCF101, absent from mammals but playing a role in transferring Fe-S clusters to client proteins in the chloroplasts of land plants. Our findings demonstrate that TgHCF101 is essential for parasite viability. However, in contrast to plants, we have shown that it is not associated with any plastid-related function in *T. gondii*; instead, it is part of the CIA (Cytosolic Iron-Sulfur cluster Assembly) pathway. Even in pan-eukaryotic pathways like the CIA pathway, the involvement of an essential lineage-specific protein like TgHCF101 provides the perspective of discovering parasite-specific features that may be exploited for therapeutic intervention.

T33

Exploring redundancies in central carbon metabolism in *Toxoplasma* parasites

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Toxoplasma gondii is an apicomplexan parasite that causes severe disease in immunocompromised individuals, newborns, and livestock. This ubiquitous parasite uses central carbon metabolism pathways to generate energy and macromolecules for its proliferation and survival. In other eukaryotes, the mitochondrial tricarboxylic acid (TCA) cycle plays a key role in energy generation and the provision of biosynthetic intermediates. *T. gondii* harbours a complete functional mitochondrial TCA cycle, but the enzymes that catalyse the individual metabolic reactions remain understudied, and the overall importance of the TCA cycle is still uncertain. To address this knowledge gap, we investigated the importance and role of the TCA cycle in *T. gondii*, using a combination of forward and reverse genetics as well as physiological and metabolomic analyses. We found that the loss of some TCA cycle reactions led to severe defects in parasite proliferation and mitochondrial oxygen consumption *in vitro*, whereas loss of others had minimal impact. Using CRISPR/Cas9 genome-wide screening of TCA cycle mutants we discovered the presence of several functional redundancies. One notable redundancy was in the reactions catalysed by the mitochondrial TCA cycle enzyme malate:quinone oxidoreductase

(MQO) and the cytosolic enzyme malate dehydrogenase (MDH), both of which mediate malate oxidation but in different subcellular compartments. Parasites which simultaneously lack both MQO and MDH exhibited a defective TCA cycle, impaired pyrimidine biosynthesis and accumulation of fumarate. We are currently investigating the essential metabolic process(es) that require malate oxidation in the parasite. Overall, our findings enhance the understanding of a key metabolic pathway and the flexible nature of central carbon metabolism in *T. gondii*.

T34

The regulation of the constant flux of fatty-acids relies on key acyltransferases regulating the nutrient flexibility in *Toxoplasma gondii.*

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To ensure a rapid propagation and pathogenicity within its human host, *Toxoplasma gondii* require large amount of lipids, through massive scavenging of host resources and *de novo* fatty acid (FA) synthesis [1, 2]. FAs coming from both sources are remodeled and channeled towards lipid storage, i.e. lipid droplet (LD), avoiding an accumulation of potentially lipotoxic FAs, and being mobilized for parasite division [3]. Incorporation and remodeling of FA in glycerolipids (major membrane lipids) is conducted by a group of protein called acyltransferase. Here, we tried to understand how the vital FA fluxes of the glycerolipid pathway are regulated: we identified two key acyltransferases, *Tg*GPAT (glycerol-3-phosphate acyltransferase) and *Tg*DGAT2 (diacylglycerol acyltransferase). *Tg*GPAT allows bulk membrane biogenesis and parasite formation by generating the key lipid precursor LPA. Metabolic labelling combined to lipidomics reveal major changes on FA substrate (host *vs* de novo) depending on the nutrient context (rich vs poor). On the other hand, *Tg*DGAT is crucial for LD biogenesis only when the parasite lacks host nutrient. This study reveals that both proteins are essential for glycerolipid metabolism and act in a regulatory manner in different nutrient conditions. The loss of these proteins results in an accumulation of FFAs that cannot be “detoxified” by incorporating them into LDs, leading to parasite death. The nutrient-dependency of these proteins provides insight into the parasite”s metabolic flexibility: they can adapt to different environmental conditions by setting up a specific metabolic program accordingly, rendering proteins non-essential, essential, or vice-versa. Understanding FA remodelling and how these fluxes are regulated in Apicomplexa parasites will be crucial in conceptualizing a novel therapeutic strategy against the parasites.

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T35

Genetic screens in physiological contexts nominate metabolic pathways for deep learning-assisted drug discovery

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Acquisition of host metabolites is a defining feature of parasitism, yet culture systems are typically replete with excess nutrients that may obscure metabolic relationships between parasites and hosts. We performed two genetic screens to identify metabolic restrictions that occur during infection. First, we conducted a genome-wide screen of *Toxoplasma* in mice, revealing dozens of metabolic pathways that are required for animal infection. We next assessed which of these pathways could be studied in culture by performing a targeted screen of parasites grown under physiological media conditions. Among the top hits was GTP Cyclohydrolase I (GCH), an enzyme responsible for the first step in the biosynthesis of two metabolites, biopterin and folate. In addition to being synthesized by parasites, these metabolites can be salvaged from the host. We demonstrate that parasites adaptively rely on salvage when host folate levels are artificially elevated by standard culture conditions. As the target of anti-parasitic drugs like pyrimethamine, folate metabolism has

long been appreciated as critical for parasite replication. However, anti-folate drugs have traditionally targeted enzymes downstream of the convergence of folate biosynthesis and salvage. Our work demonstrates that targeting biosynthesis alone is sufficient to restrict parasite growth under physiological conditions, nominating new enzymes as putative anti-parasitic targets. As a proof-of-concept, we demonstrate that an inhibitor of mammalian GCH can be repurposed to inhibit parasite growth. We have additionally devised a screening platform to identify compounds that more potently inhibit GCH. Leveraging conservation in bacteria, we created *E. coli* strains in which bacterial GCH is replaced with parasite or human homologs, enabling rapid screening of large chemical libraries through simple measurements of bacterial growth. An initial screen of 2500 compounds revealed dozens of molecules that selectively inhibit parasite GCH. In ongoing work, we are combining the graph neural network Chemprop and large language models (LLM) to learn the molecular features that enable preferential binding of a compound to parasite GCH, while reducing inhibition of the human homolog. In total, this work demonstrates how examining parasite metabolism in a physiological context can reveal previously overlooked druggable targets, and suggests a new methodology for high-throughput drug screening via bacterial complementation.

T36

Genetic deletion of an essential lipid storage enzyme attenuates the virulence of *Toxoplasma gondii*

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The ability of *Toxoplasma gondii* to infect a wide range of hosts is greatly influenced by its capability to salvage essential nutrients from the host cell and its environment. *Toxoplasma* relies on lipids for its critical functions, acquiring fatty acids from the host cell, in addition to *de novo* synthesis, to support its growth and ensure survival. Fatty acids are important components for *Toxoplasma'*s membrane biogenesis, energy generation, and other metabolic processes. However, excessive free fatty acids (FFA) are toxic to the parasite. To evade lipotoxicity, *Toxoplasma* expresses lipid-esterifying enzymes to divert surplus FFA into major storage neutral lipids, such as triacylglycerol (TAG) and cholesteryl ester (CE), stored within intracellular lipid droplets (LD). One such enzyme, the acyl-CoA:diacylglycerol acyltransferase (TgDGAT1) catalyzes the final step in the acyl-CoA-dependent synthesis of TAG. TgDGAT1, a member of the membrane-bound *O*-acyltransferase (MBOAT) superfamily, shares structural and functional features with enzymes in the DGAT1 family. Interestingly, the DGAT1 inhibitor T863 arrests the growth and multiplication of *Toxoplasma* in cultured mammalian cells, suggesting an important role for this enzyme in storing energy-rich FAs as TAGs and protecting the parasite from FFA-induced toxicity. However, the direct effect of loss of DGAT1 enzyme activity in the parasite remains unexplored. In this study, we generated a genetically modified *Toxoplasma* type I strain devoid of the TgDGAT1 gene, rendering it incapable of storing FFAs as TAGs within LDs. Of interest, we note that RH ΔDGAT1 parasites can grow and multiply *in vitro* under lipid-depleted conditions, showing a moderate growth defect. However, in standard growth media, the mutant's inability to store fatty acids severely impairs its ability to thrive within host mammalian cells. Additionally, EM studies reveal gross morphological abnormalities, with skinny parasites disorganized in the parasitophorous vacuole. We further show that the RH ΔDGAT1 strain is avirulent in mice, even at higher infection doses. Active immunization with these genetically attenuated whole-cell tachyzoites boosts the host immune response, providing protective immunity against subsequent acute lethal challenges by wild-type parasites. Overall, our findings suggest that unraveling the mechanisms of fatty acid storage in *Toxoplasma* is crucial for developing strategies to disrupt the parasite's survival mechanisms.

T37

A drug repositioning strategy identifies a compound inhibiting GSK3 activity in *Toxoplasma gondii*

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The phylum Apicomplexa encompasses many of the world"s pre-eminent protozoan pathogens, including *Toxoplasma gondii* causing toxoplasmosis, a highly prevalent foodborne parasitic disease. Despite its widespread impact, existing therapies exhibit limited effectiveness, highlighting the urgent need for potent drugs targeting both *T. gondii* and related pathogens within the phylum. Here, compound 209 (C209) was identified in a phenotypic screen using a repurposed drug library aiming to uncover novel antiparasitic compounds and their corresponding targets. Previously assessed in clinical

trials for cancer therapy, C209 demonstrated significant parasiticidal activity by disrupting the intracellular development of tachyzoites in the nanomolar range. Using a forward genetic approach, we identified the essential cell cycle-associated kinase *Tg*GSK3 as the primary target of C209, identifying three single nucleotides substitutions conferring resistance to the drug. Through high-resolution crystallization and structure determination of the GSK3/C209 complex, we elucidated the compound's unique binding mode which displays a remarkable fold of its own and we could rationalize the interfering effect of the three amino acid mutations within the compound binding cavity. Biochemical analysis confirmed this inhibition, revealing a 2- to 18-fold loss of compound binding affinity in mutated proteins and a 2- to 5-fold decrease in kinase activity compared to the native protein. The conservation of *GSK3* in *Cryptosporidium* has triggered interest in its potential as a new target offering an avenue for the development of broader anti-parasitic drugs.

T38

Single-cell RNA sequencing and immunohistochemical analysis of reactivating bradyzoites *in vivo*.

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Reactivation of chronic *Toxoplasma* infection is a critical element in disease pathology and is commonly associated with HIV comorbidity and the occurrence of toxoplasmic encephalitis. Reactivation of latent infection during pregnancy can result in congenital transmission and life-threatening disease in the neonate, whereas reactivation in the eye can cause severe ocular disease. Although a clinically relevant and critical component of the *Toxoplasma* life cycle, very little is known about the reactivation process.

To map the process *in situ*, we synchronously dysregulated the immune system of chronically infected mice and analyzed parasite transcriptional changes using single-cell RNA sequencing. We also performed immunohistochemistry on the brains of these same mice to correlate morphological changes in the cysts with changes in gene expression at different time points.

Analysis of gene expression in individual parasites before reactivation revealed significant heterogeneity in cell cycle progression and the expression of canonical bradyzoite-specific genes. This heterogeneity was unexpected based on what has been previously captured using *in vitro* models. Immunohistochemistry and transcriptional profiling of encysted parasites during the first five days after stimulating reactivation show that the transition from bradyzoites-to-tachyzoites occurs mainly after significant structural changes to cysts, demonstrating that within-cyst transcriptional changes are likely only the beginning of the bradyzoite-to-tachyzoite transition.

This work represents the first step in mapping the reactivation process *in vivo*. It suggests that the process may be more dynamic and complex than previously recognized, involving heterogeneous starting populations of encysted parasites and intermediate transitional stages

T39

A Genetic Screen Identifies Factors Required for *Toxoplasma* Differentiation into Latent Forms

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Toxoplasma differentiates from acute stage tachyzoites into latent bradyzoite forms in the muscle and central nervous system. How *Toxoplasma* senses the tissue environment and undergoes differentiation, at the molecular level, in poorly understood. We undertook a whole genome CRISPR screen in RH strain to determine genes required for utilization of different carbon sources and serendipitously discovered that switching to glutamine, from glucose, triggers a differentiation-like program, highlighting 60 genes involved in this process. We show that glutamine, as the sole carbon source, also triggers differentiation in cytogenic strains and relies on the transcription factor BFD1. We then generated a CRISPR sub-library of these 60 genes and tested their role in differentiation in cystogenic strains in vitro and in mice. Individual mutants show that several genes involved in central carbon metabolism are required for differentiation in vitro and in vivo as well as genes involved in regulation of RNA. We also identified orthologs of the "GID" E3 ubiquitin

ligase complex, which regulates translational repression in during embryogenesis in Drosophila. By genetic tagging and immunoprecipitation, we show that *Toxoplasma* also has a GID complex, whilst gene knockouts of GID components show impaired differentiation. Current work is centered around identifying TgGID ubiquitinated substrates. This work, therefore, identifies new molecular players and biological processes, including ubiquitination, required for *Toxoplasma* to differentiate into latent forms

T40

Translation initiation factor eIF1.2 is a crucial early regulator of *Toxoplasma* bradyzoite cyst formation

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The bradyzoite form of Toxoplasma gondii poses a therapeutic challenge since there is no available effective drug or vaccine for humans. Although differentiation of *T. gondii* tachyzoites to bradyzoites is a pivotal event during infection, our understanding of this process remains limited to a few differentiation factors that are sparsely linked. Using a chemical mutagenesis screen, we identified the translation initiation factor eIF1.2 as a critical player in *T. gondii* differentiation. A F97L mutation in eIF1.2 or complete knockout of eIF1.2 (Δ*eIF1.2*) markedly impaired bradyzoite cyst formation *in vitro* and *in vivo*. Utilizing single-molecule scanning technology, we demonstrated that the *T. gondii* eIF1.2 F97L mutation alters the scanning process of the yeast ribosome preinitiation complex on a model yeast mRNA. RNA sequencing and ribosome profiling experiments unveiled that Δ*eIF1.2* parasites exhibit defects in upregulating differentiation markers, such as BAG1, LDH2, and regulators, including BFD1 and BFD2/ROCY1, during stress-induced differentiation. Forced expression of BFD1 or BFD2 effectively restored differentiation in Δ*eIF1.2* parasites. Our findings indicate that eIF1.2 regulates the translation of key differentiation factors that are essential for establishing chronic infection. Notably, non-tissue cyst-forming apicomplexan parasites only possess one eIF1, whereas most tissue cyst-forming apicomplexans like *T. gondii* have two eIF1 paralogs, eIF1.1 and eIF1.2. This work sets the stage for further elucidating the specific regulatory role of eIF1.2 in the expression of differentiation-related genes.

T41

In vitro reactivation of chronic-stage *Toxoplasma gondii* parasites

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In the intracellular apicomplexan parasite *Toxoplasma gondii*, differentiation from proliferative to chronic, cyst-forming life stages facilitates stress tolerance, immune evasion, and environmental persistence. Differentiation is accompanied by global changes in parasite gene expression and regulation, but tissue cysts retain the capacity to reactivate and resume rapid growth in immunocompromised hosts, which largely drives the morbidity associated with *Toxoplasma* infection. Our lab previously demonstrated that BFD1, a Myb-like transcription factor required for differentiation, is upregulated under stress via a positive feedback loop involving one of its targets, the RNA-binding protein BFD2. However, the molecular pathways underlying reactivation, including the role of the BFD1-BFD2 circuit, remain poorly understood. We established a genetic model to study *Toxoplasma* reactivation in vitro by introducing an additional, conditionally stabilizable copy of BFD2 (DD-BFD2) into differentiation-competent ME49 parasites. Similar to conditional BFD1 stabilization (DD-BFD1) in Δ*bfd1* parasites, this new strain reliably differentiates and expresses canonical markers of early and late bradyzoite development. Using stage-specific fluorescent reporters, we demonstrate that DD-BFD2 stabilization induces bradyzoite reporter expression to a higher magnitude and with greater heterogeneity compared to DD-BFD1 stabilization, suggesting that DD-BFD2 parasites explore a broader range of cellular states during differentiation. Critically, DD-BFD2 destabilization in chronic-stage parasites produces a subpopulation of cysts that remain differentiated over time, consistent with long-term maintenance of the endogenous BFD1-BFD2 positive feedback loop. Under these conditions, we simultaneously observe the emergence of a rapidly growing tachyzoite population distinct from mature cysts. Conversely, DD-BFD1 destabilization in Δ*bfd1* cysts leads to a rapid switch in stage-specific reporter expression. These observations highlight the role of heterogeneity in bradyzoite development and imply that reactivating parasites may originate from an as-yet-undefined parasite subpopulation.

T42

Single-cell analysis of *Toxoplasma* cat stages identifies multiple developmental trajectories.

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Toxoplasma gondii"s sexual development is limited to the epithelial cells of the small intestine of felids. Sexual development is a key to genetic diversity and this process is crucial to dissemination, as a single felid can shed millions of environmentally stable oocysts. Due to challenges associated with cultivating presexual and ultimately sexual stages, there is still much to learn about how this developmental program is triggered and executed by the parasite in response to infection of the feline host. Prior studies (Hehl *et al*., 2015 and Behnke *et al*,. 2014) demonstrated clear differences in the transcriptomes of asexual and sexual stages using bulk transcriptomics, and metabolic differences in the feline host play a key role in triggering the transition into sexual stages (Martorelli di Genova *et al.*, 2019). Important recent work (Artunas *et al.*, 2024, among others) has identified multiple parasite transcription factors as critical suppressors of presexual development, permitting the study of presexual stages and opening ways to using genetics to drive the full sexual cycle *in vitro*. To further investigate the sexual developmental phase of *Toxoplasma* and identify previously unknown regulators of the distinct phases of sexual development, we have conducted multiple single-cell transcriptomics analyses of *Toxoplasma* parasites isolated from the small intestines of infected cats. To do this we used flow cytometry to sort fluorescent reporter strains of *T. gondii* VEG from scraped small intestine samples and obtained single-cell transcriptomic data from 16,966 cells across two experiments. The data made it possible to classify, characterize, and parse each cell at the transcriptome level, which led to the identification of rare cell populations, including some that we hypothesize are macrogametes and microgametes. Candidate genes emerging from this study have now been targeted in a CRISPR-CAS9 screen and we are using Perturb-seq and loss of function assays on feline enteroepithelial stages to determine which factors are required for sexual development and/or the regulation of sexual stage gene networks. We aim to identify previously unknown drivers of differentiation, especially those that mediate gametogenesis. Our single-cell data also extends what is known about gene expression changes throughout sexual development and should be useful to those in the field working towards inducing gametogenesis, mating, and oocyst formation in feline-free conditions.

T43

Characterizing the impact of mRNA regulatory features on translation efficiency in *Toxoplasma gondii*

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Protein expression can be regulated through *cis* and *trans* factors acting on mRNA and translation machinery. This regulation allows cells to fine-tune expression of specific factors in response to environmental cues. The transcriptome of *Toxoplasma gondii* represents a challenge to canonical models of translational regulation in eukaryotes as its 5' UTRs are several times longer than most other characterized species and the vast majority of 5' UTRs harbor at least one, if not several, upstream AUGs. We hypothesize that these unusual features may be maintained in *Toxoplasma* because they confer regulatory information important for gene expression across the parasite life cycle. To understand how the parasite regulates translation across both its tachyzoite and bradyzoite stages, we performed high-resolution ribosome profiling on *Toxoplasma* and human host cells. Bioinformatic analyses of these data have characterized general transcript features that contribute to translation efficiency differently in *Toxoplasma* and human cells. Additionally, we identified a cohort of translationally-regulated factors whose expression changes between tachyzoites and bradyzoites. Based on these data, we aim to dissect the relative contributions of mRNA regulatory features to translation efficiency in the parasite. Using quantitative reporter systems, we are examining the sufficiency of endogenous 5' UTRs to regulate both steady-state protein expression and changes under stress. Mutational analyses will reveal how specific sequence features, such as upstream open reading frames, impact translation efficiency. Together, this work expands our molecular framework for gene regulation in *Toxoplasma*.

T44

The Role of Age in Ocular Toxoplasmosis: Clinical Signs of Immunosenescence and Inflammageing – a perspective study

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Purpose: To investigate the association between age, immune response, and the clinical presentation in ocular toxoplasmosis (OT).
Design: Retrospective, observational cohort study.
Methods: A review of medical records of patients with active OT at the Uveitis Center, Charité Universitätsmedizin was conducted. Baseline parameters included age at presentation, primary manifestation or recurrence, visual acuity, intraocular pressure (IOP), size and location of active lesions, inflammatory activity, antibody index (AI), and complications of intraocular inflammation. The data are presented as mean ± standard deviation (SD).
Results: Between 1998 and 2019, 290 patients with active OT were diagnosed at our tertiary reference center. The mean age was 37.7 ± 17.1 years, 53.8% were females, and 195 patients (70.9%) showed recurrent disease. Older age was associated with lower-baseline visual acuity ($p = 0.043$), poor visual outcome ($p = 0.019$), increased inflammatory activity ($p < 0.005$), and larger retinal lesions ($p < 0.005$). Older patients presented a lower AI (<35 years: 45.1 ± 82.7, median: 12.1; ≥35 years: 18.6 ± 50.5, median: 5.8; $p = 0.046$), confirmed by a decrease of AI with increasing age ($R^2 = 0.045$; $p = 0.024$). Complications were observed more frequently in patients with primary ocular toxoplasmosis ($p = 0.046$) and were mostly accompanied by panuveitis ($p = 0.018$) and a longer duration of illness ($p = 0.037$). Furthermore, panuveitis ($p = 0.025$), ocular hypertension ($p = 0.037$), and improved visual acuity development ($p = 0.035$) were associated with the presence of primary ocular toxoplasmosis. Macular involvement (24.3% of patients) was positively correlated with complications (macular/peripapillary edema and retinal detachment, $p < 0.005$) and poor visual outcome ($p < 0.005$) and was negatively correlated with inflammatory activity ($p < 0.005$).
Conclusions: We found a strong and clinically-relevant impact of age on the clinical presentation and course of OT. While an unspecific inflammatory response increased with age, the specific, local humoral immune response declined. These findings are well in line with the concept of immunosenescence and inflammageing in uveitis. In addition, alongside patient age, the primary manifestation and the retinal lesion location were identified factors contributing to the severity of intraocular inflammation and the occurrence of ocular complications.

T45

CHRONIC LATENT TOXOPLASMA GONDII INFECTION PRECIPITATES COGNITIVE DECLINE IN AN INDUCIBLE ALZHEIMER'S DISEASE MODEL

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Neuroimmune dysregulations strongly contribute to the pathophysiology of Alzheimer's Disease (AD), but the etiology of AD remains ill-defined and is certainly diverse. Because pathogens shape the immune system over lifetime, their association with AD have been suggested. Increasing evidence supports a role for the prevalent, brain-persisting parasite *Toxoplasma gondii* (*Tg*) in chronic neurological diseases. Yet, overall, our current knowledge about the impact of chronic *Tg* infection on AD pathology and clinical progression remains sparse. There have been limited attempts to describe the impact of *Tg* infection on neuropathological lesions in AD mouse models. Up to now, the consequences of latent *Tg* infection on the severity and progression of cognitive decline and on the neuroimmune landscape, as well as the underlying molecular mechanisms, remain ill-defined.
Considering the temporal relationship between natural *Tg* infection and AD occurrence, it seems legitimate to evaluate the effects of a strain of *Tg* which is effectively controlled and result in latent infection, on the later development of AD. To tackle this question, we combined infection by transgenic *Tg* expressing a model antigen (*Tg*.GRA6-OVA) that is efficiently presented by MHC I in C57BL/6 mice and results in a CD8+ T cell-controlled persisting and latent infection, with an inducible mouse model of AD amyloidosis, the TetO-APPSwelnd mouse (ihAPP), in which amyloid-β peptide production and deposition, can be induced upon removal of doxycycline treatment. Mice were kept on doxycycline-dosed chow until chronic latent *Tg* infection is established. Behavioral, immune and biochemical analysis were performed after doxycycline withdrawal.

We found that *Tg*-infected ihAPP mice (*Tg*-ihAPP), displayed cognitive deficits earlier than non-infected AD mice (NI-AD), including impaired object recognition memory and spatial learning and memory deficits. NI-ihAPP mice are impaired in these tasks only 2 months later. Interestingly, *Tg*-ihAPP mice had less β-amyloid plaques in the cortex and hippocampus compared to NI-AD, confirming previous findings. Facs analyses of brain-isolated cells revealed unique changes in monocytes and CD8+ T cells in *Tg*-ihAPP mice compared to the other groups.
Together, our data indicate that preexistent and in appearance silent chronic *Tg* infection may hasten the progression of cognitive decline in AD while contributing to Aβ plaques nibbling and involving neuroimmune mechanisms.

T46

Chronic *Toxoplasma gondii*, Diabetes, and Retinopathy in Pregnancy

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We carried out a prospective study of 690 Hispanic pregnant women (158 *T.gondii* (TG) positive, 532 TG negative) enrolled at an initial early prenatal visit and then followed for adverse events across the course of their pregnancies. After the initial study visit data were collected from the electronic health record (EHR). From the EHR, pre-pregnancy type 2 diabetes (T2D), and the occurrences of gestational diabetes (GDM) were significantly higher in the *T. gondii* positive women. GDM was diagnosed in 20% of the *T. gondii* positive women ($\chi2=4.4$,df=1, $p=0.04$). compared to 11% in the *T. gondii* negative women ($\chi2=7.3$, df=1, $p=0.007$). The frequency of an abnormal insulin glucose tolerance test (IGTT) without diagnosis of GDM was the same in both groups (about 16%).
Additionally, from the initial enrollment, 285 women were recruited into a prospective study with 5 study visits in their prenatal clinics. There were 158 TG positive women who had a chronic, high avidity chronic infection and 127 *T.gondii* negative "controls". Blood samples, health exams and retinal scannings were done at the clinic study visits. Serial retinal scanning was done only in the *T. gondii* positive women, as the aim of this analysis was to explore lesions and scars characteristic of toxoplasma disease. Only one scar was found in the population. Retinal images were scanned and graded by an independent retinal scanning service. We found diabetic retinopathy, mostly mild, non-proliferative in type, in 55 of the 130 women who had retinal images done, with 30 of these women having either GDM, positive IGTT, or T2D. The remaining 25 had no other related pathology.
The relationship of chronic TG infection with retinopathy is unknown at this time. The increased prevalence of GDM and T2D in TG positive pregnant women may be important factors, as we have found differences in immune function in pregnant TG positive women (Prescott S, et al.,2023). Plasma levels of IFN- γ and IL-10 were significantly lower in GDM and IL-17 was lower in T2D. A comparison group of TG negative pregnant Hispanic women is necessary to determine this relationship.
Prescott et al., Am J Reprod Immunol. 2023 Sep;90(3):e1376.

T47

Serological Responses to *Toxoplasma gondii* and Matrix Antigen 1 Predict the Risk of Subsequent Toxoplasmic Encephalitis in People Living with HIV

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Background: There is a lack of clinically useful predictors for fatal toxoplasmosis. We investigated the value of serological assays for antibodies to whole Toxoplasma antigens and to peptide antigens of the Toxoplasma cyst protein MAG1 for predicting incident toxoplasmic encephalitis (TE) in people living with HIV (PLWH).
Methods: We performed a nested case-control study, conducted within the Multicenter AIDS Cohort Study (MACS), using serum samples obtained two years prior to diagnosis of TE from 28 cases and 37 HIV disease-matched Toxoplasma seropositive controls at matched time points. Sera were tested for Toxoplasma antibodies using a commercial assay and for antibodies to MAG1_4.2 and MAG1_5.2 peptides in ELISA.
Results: Two years before clinical diagnosis, 68% of TE cases were MAG1_4.2 seropositive, as compared to 16% of controls (OR 25.0, 95% CI 3.14-199.18). The results for MAG1_5.2 seropositivity were 36% and 14% (OR 3.6, 95%

CI 0.95-13.42). Higher levels of antibody to MAG1_4.2 (OR 18.5 per doubling of the OD value, 95% CI 1.41-242) and Toxoplasma (OR 2.91 for each OD unit increase, 95% CI 1.48-5.72) were also associated with the risk of TE. When seropositivity was defined as the presence of MAG1 antibody or relatively high levels of Toxoplasma antibody, the sensitivity was 89%, and specificity was 68% for subsequent TE.
Conclusions: The presence of antibodies to MAG1 in PLWH can predict the occurrence of TE. The predictive performance of these antibodies is further improved by adding the levels of Toxoplasma antibodies. These measures could be clinically helpful in predicting subsequent diseases in multiple at-risk populations.

T48

An *in-vitro* method and predictive model to quantify *Toxoplasma gondii* inactivation by salting

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Consumption of raw meat products is considered an important source of *T. gondii* infections. These products are usually processed with NaCl and other additives. Such additives affect the viability of *T. gondii*, but results described in literature are variable. Mouse bioassay is currently the standard method for *T. gondii* inactivation experiments, but ethically undesirable, costly and time consuming. Moreover, mouse bioassays provide very limited quantitative information on actual reductions in the concentration of viable parasites in tested meat products. Therefore it was our aim to validate and compare an *in-vitro* method as a quantitative alternative to the mouse bioassay for determining the effect of salting on *T. gondii* viability.
Muscle tissue of sheep experimentally infected with *T. gondii* was used to prepare 50 g portions of minced meat supplemented with 0.6 - 2.7% NaCl and sodium lactate or sodium acetate. Portions were stored overnight at 4°C. Pepsin-HCl digestion was performed and digests were inoculated onto RK13 cell monolayers or into IFNγ-KO mice, to evaluate the presence of infective parasites. In addition, a four-fold dilution series ranging from 4 to 256 bradyzoites per ml of tissue digest or culture medium was tested to evaluate and compare the detection limit of these methods.
Both, the *in-vitro* and the *in-vivo* method gave positive results for samples spiked with 4 or 16 bradyzoites and were consistently positive when a minimum of 64 bradyzoites was added. Viable parasites were detected in portions of experimental sheep tissue supplemented with up to 1.5% NaCl, but not in samples supplemented with both 1.2% NaCl and 1.4% sodium lactate. A statistical model was fitted, using actual and previous results with cell culture and, as an example, indicates a 5-log reduction in infective parasites after supplementation with 2.5% NaCl.
In conclusion, the *in-vitro* method was successfully used to obtain data on *T. gondii* inactivation by salting. The model provides more insight in the possibilities to use salt for controlling the risk of *T. gondii* in raw meat products. Replacement of the mouse bioassay opens up possibilities to investigate other potential inactivation methods. In the future, we hope to also overcome the need for experimental infection to obtain positive muscle tissue and we look forward to discus possibilities with *in-vitro* cultivated tissue cysts.

T49

Usefulness of serotyping tools in *Toxoplasma gondii* infections in sheep and pigs

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Toxoplasma gondii is the causative agent of toxoplasmosis, one of the most relevant food-borne zoonotic diseases worldwide that causes important economic losses in the livestock sector related to reproductive failure, mainly in sheep and goats. To better understand the epidemiology of toxoplasmosis, predict the clinical consequences and design appropriate control strategies, it is crucial to determine the strain causing the infection. In this sense, serotyping methods offer a cost-effective, rapid, sensitive, and non-invasive alternative to DNA-dependent genotyping techniques, allowing the analysis of subclinical cases. This technique is based on the detection of strain-specific antibodies that react against segments of antigenic proteins presenting polymorphic variations. However, the prediction capability of promising peptides needs to be determined in each animal species, as differences in the strain-specific reactivity patterns have been described. In the present study we sought to evaluate the applicability in sheep and pigs of a panel of peptides previously characterized in mice and humans. To this end, we used 51 sheep serum samples obtained in previous experimental infections (32 type II and 19 type III), 20 sheep samples from naturally infected sheep where the causative strain was genotyped (18 type II and 2 type III), and 40 serum samples from experimentally infected pigs (22 type I and 18 type III). Our ELISA test results show that a combination of GRA3, 5, 6 and 7 peptide homologous pairs can discriminate infections caused by type II and III strains in sheep and pigs. Namely, the best peptide pairs to discriminate infections in these two livestock species were GRA6-I/III-213 *vs*. GRA6-II-214 and GRA6-II-44 *vs*. GRA6-III-44, together with GRA3-I/III-38 *vs*. GRA3-II-38 in sheep and GRA7-II-224 *vs*. GRA7-III-224 in pigs. Notably, the GRA6-44 peptide pair, which were previously described to be inefficient in mice and humans, showed a high prediction capacity, especially in sheep, while GRA5-38 peptide versions failed to correctly predict the strain type in most sheep and pig samples, underpinning the notion that individual standardization is needed for each animal species. These results lay the grounds for future large-scale serotyping studies in sheep and pigs, as well as its potential extrapolation to other animal species in future studies.

T50

Exploring the ultrastructure of cell division coordination in Toxoplasma gondii through expansion microscopy

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The ability of protozoan parasites to rapidly proliferate within their host is at the core of their mechanisms of pathogenesis. *Toxoplasma gondii*, resorts to flexible cell division modes, resulting in variable outputs. It can proliferate by means of endodyogeny, endopolygeny and schizogony. These modes of division share mechanistic features such as lack of chromatin condensation, nuclear fission by semi-closed mitosis and de novo daughter cell assembly. However, the underlying mechanisms of this flexibility are only partially understood. The centrosome has long been staged at the center of regulation. However, its biogenesis and homeostasis are poorly understood. Here, we have dissected the contribution of different centrosomal components in *T. gondii*, using ultrastructure expansion microscopy. We analyze the phenotypes displayed by conditional mutants of centrosomal proteins TgSAS6 and TgCEP250L1 during asexual cell division and delve into pre-sexual division leading up to sexual differentiation. This work uncovers unexpected features of microtubule nucleation and centriole biogenesis. Overall, our work proposes a model for the modular organization of compartmentalized functional domains which may ultimately underly cell division flexibility allowing these parasites to adapt to different niches and proliferate accordingly.

T51

FIT plays a role in iron acquisition in *Toxoplasma gondii*

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The obligate intracellular apicomplexan parasite *Toxoplasma gondii* requires iron as a cofactor for essential metabolic proteins. Due to its importance to almost all life, iron uptake and regulation have been well studied across model eukaryotes. Iron acquisition has been studied in some detail in parasitic kinetoplastids like *Leishmania*. However, despite its importance, little is known about how *Toxoplasma gondii* acquires iron. Many organisms make use of specific transporters, named Zinc and Iron Permeases (ZIPs) to take up iron from their environments. *T. gondii* encodes four ZIP-domain containing proteins, one of which, named here as FIT, is predicted to localize to the plasma membrane and is essential for parasite growth. We endogenously tagged FIT with 3HA tags and show that its localization is dynamic, localizing initially peripherally, before moving basal during parasite replication. Overexpression of FIT provides a protective effect when iron is depleted, but cells are hypersensitive to excess iron, when compared to wildtype parasites. Interestingly, ICP-MS experiments show that FIT overexpression leads to Zn accumulation in the parasite, however no change was seen in parasite associated iron. To determine function, we conditionally depleted of FIT, which led to a severe growth defect *in vitro*, suggesting it is essential for parasite survival. X-ray fluorescence microscopy (XFM) data show that FIT knockdown leads to reduced parasite iron. Moreover, ICP-MS data show that knockdown of FIT results in a decrease in Zn in the parasites. Finally, we show that knocking down FIT leads to an increased expression of bradyzoite markers including BAG1 and DBL, suggesting that knockdown of FIT may lead to the conversion/differentiation of tachyzoite parasites to the cyst-forming bradyzoite form of the parasite. Interestingly, depletion of iron by chelation also promotes differentiation, suggesting a similar mechanism and a role for iron in triggering differentiation. Together, we show that FIT has a role in iron uptake, and possibly zinc uptake in *Toxoplasma gondii*, and that this is important for parasite survival *in vitro.*

T52

Functional dissection of NudCL1 protein in *Toxoplasma gondii* cell division.

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Nuclear distribution protein C (NudC) is a highly conserved eukaryotic protein initially identified in *Aspergillus nidulans* as a dynein-mediated nuclear migration factor. However, vertebrates typically contain four paralogs sharing a common p23 domain, which engages the cochaperone HSP90 involved in protein folding and interactions. Additionally, a conserved NudC-N-terminal domain is present in two vertebrate NudC proteins. The annotated genome of *Toxoplasma gondii* encodes four NudC proteins based on the conservation of the p23 domain. So far, NudC proteins have not been characterized in apicomplexan parasites. We studied all four TgNudC family genes, which are all essential. Here, we focus on NudC-like 1 protein (NudC-L1), which possesses both conserved NudC and p23 domains. Surprisingly, despite its predicted fitness score of -0.47, we demonstrated that NudC-L1 is essential for the parasite lytic cycle. Depleting NudC-L1 inhibits parasite replication and leads to the development of multinucleated, unindividualized parasites, associated to elongated intercellular bridges between daughter cells. Pan Expansion Microscopy was used to assess parasite ultrastructure, revealing that NudC-L1 knockdown induces a Russian doll-like, “bud-in-bud” parasite development, with no observed defects in nuclear division. In humans, NudC protein regulates cytokinesis. In fact, Aurora B, a kinase essential for cell abscission, phosphorylates NudC at a conserved Threonine 40 residue. Remarkably, we found that TgNudC-L1 possesses this conserved Threonine residue. Moreover, previous work has demonstrated a similar Russian doll phenotype upon depletion of *T. gondii* Aurora kinase 1 (Ark1). This suggests that TgArk1 directly phosphorylates NudC-L1, which implies a putative role for the spindle midbody in promoting cytokinesis. This study represents the first comprehensive characterization of NudC proteins in *T. gondii* and has uncovered a putative connection between Ark1 and NudC-L1 in cytokinesis.

T53

AP2XII-9 is a crucial factor regulating the cell cycle of *Toxoplasma gondii* tachyzoites.

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A rapid and efficient division is key for the ability of apicomplexan parasites to proliferate and therefore is crucial for their pathogenesis. During the *Toxoplasma gond*ii tachyzoite cell cycle, division and segregation of organelles is a highly coordinated process that ensures that each daughter parasite is properly complemented with all organelles. This highly regulated mechanism implies a tight regulation of gene expression that is crucial for the production of daughter cells in this parasite. However, which proteins are key actors in this regulation remain to be discovered. We discovered that TgAP2IX-5 is a cell-cycle-dependent ApiAP2 transcription factor (TF) bearing a crucial regulatory role during the tachyzoite cell cycle.TgAP2IX-5 also acts as a master regulator that controls the expression of other TFs that may be essential for the completion of the cell cycle. TgAP2IX-5 directly controls the expression of TgAP2XII-9 (Gissot M, Nat Comm. 2021). Nevertheless, the putative TFs' role in the biology of *T. gondii* has not been studied. To investigate the role of TgAP2XII-9, we produced conditional knock-down strain and characterized its phenotypes. TgAP2XII-9 is essential for the growth of parasites indicating that it may exert essential roles during the cell cycle. We have shown that TgAP2XII-9 localizes to the nucleus and is expressed during the S/M phase in line with its transcript expression profile. Depletion of TgAP2XII-9 causes significant defects in the formation of the daughter cells inner membrane complex therefore producing a significant number of multinucleated parasites. Our CUT&Tag and RNA-seq data unveiled AP2XII-9's direct control over genes including other AP2 TFs, IMC coding genes, and notably, microneme and rhoptry coding genes. There is a strong correlation in these results with the phenotypes observed by investigating cell-cycle markers by IFA with clear IMC and daughter cell formation defects. Surprisingly, most of the genes that are found in both the RNA-seq and CUT&Tag datasets are upregulated suggesting that AP2XII-9 is a repressor of gene expression that controls the cell cycle of *T. gondii.* To our knowledge, this is the first description of a transcriptional repressor with a direct role in the tachyzoite cell cycle.

T54

Defining the Role of the Kinase TgGSK in *Toxoplasma gondii*

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TgGSK is a serine/threonine kinase in *T. gondii* that is most closely related to the glycogen synthase kinase BIN2 in plants. In plants, BIN2 is a negative regulator of brassinosteroid signaling, which plays a role in plant growth and development and stress responses. Previously, we have shown that another *T. gondii* brassinosteroid signaling homolog, PPKL, is essential for the regulation of proper parasite division. Therefore, we wanted to investigate if TgGSK plays a similar role. We have established a conditional knockdown strain and determined that TgGSK is essential for parasite propagation. Using immunofluorescence, we have determined that TgGSK has dynamic localization, which is concurrent with the parasite"s division cycle. Moreover, based on expansion microscopy we observe TgGSK in the centrosomes, corroborating a possible role in parasite division. Partial knockdown of TgGSK causes abnormal division phenotypes such as asynchronous division, uneven segregation of nuclear material, and abnormal amounts of centrosomes in each parasite as shown by both immunofluorescence and expansion microscopy. Immunoprecipitation has shown that TgGSK interacts with the GCN5b lysine acetyltransferase complex. This complex acetylates histones to open up chromatin for gene transcription and has been shown to interact with ~40% of *T. gondii* genes. While global transcriptomics did not show a clear pattern of differential gene transcription after TgGSK knockdown, we are currently performing global phosphoproteome analysis to determine differences in phosphorylation. These experiments will begin to elucidate a unique signaling pathway that is essential for T. gondii survival and replication.

T55

A pair of large centrosomal proteins are crucial for daughter cell formation in *Toxoplasma gondii*.

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Toxoplasma gondii fast replication is key to its pathogenesis in intermediate hosts such as humans. The centrosome is central to the organization and coordination of the cell cycle and division of these parasites. One of the main roles of the centrosome is to ensure proper positioning and production of the daughter cell scaffold. Daughter cell assembly is a complex process that is regulated at distance by the centrosome through the nucleation of a fiber that is visible by electron microscopy. However, the centrosome components involved are poorly described. To gain novel insights into the biology and the composition of the *T. gondii* centrosome, we identified and characterized TgCep404 and TgCep359, two proteins associated with the centrosome, which dynamically elongate towards the apical pole of the forming daughter cells. TgCep404 and TgCep359 interact with each other, playing an essential role in the survival of the parasite. Combined depletion of these proteins leads to the disorganization of the daughter cell inner membrane complex and disruption of the normal cell cycle. It also affects the division and segregation of the centrosome. We propose that TgCep404 and TgCep359 could form a structure that positions the apical plaque of the IMC in the budding daughter cells, hence explaining its essential role in the construction of the daughter cell IMC.

T56

Deciphering the core complex and regulatory elements of the rhoptry secretory machinery

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Rhoptries are specialized secretory organelles at the heart of Apicomplexa pathogenesis. They inject parasite proteins directly into the cytoplasm of host cells not only for invasion but also for hijacking host functions crucial to establishing and sustaining infection. Rhoptries are connected to an apical vesicle (AV), which is docked to the parasite plasma membrane (PPM) by a complex machinery called the rhoptry secretory apparatus (RSA). This arrangement suggests the requirement for two membrane-fusion events during rhoptry secretion, yet little is known about the proteins that regulate them. Nd complex members (Nd6, Nd9, NdP1, NdP2, and Fer2) are essential for rhoptry exocytosis. Nd9, NdP1, and NdP2 are required to build the RSA, and Nd6 is linked to the exocytic site at the PPM/AV in *Toxoplasma*. These observations suggest a role for the Nd complex in the fusion event between the AV and the PPM. Here, we performed immunoprecipitation coupled with mass spectrometry to obtain a more comprehensive inventory of the rhoptry secretion machinery. We identified 9 additional NdPs, of which almost all are required for invasion of *T. gondii in vitro*, yet they are not essential. When tested for virulence in mice, the mutants appear highly attenuated, even the ones with no or mild *in vitro* defect. Like Nd6, most of these proteins are present at the exocytic site. A better visualization of their spatial organization is being achieved by iterative U-ExM. Two of the NdPs are predicted to be GTPases. One is an Arf-like GTPase and directly interacts with the predicted GAP-domain of Nd6. The other one, named NdP-GTPase, is a direct partner of Nd9. Phylogenetic analyses revealed that NdP-GTPase is part of an *Alveolata*-specific distinct clade that does not fall into any of the classical small GTPase sub-families. Further characterization of both *Toxoplasma* and *Tetrahymena* NdP-GTPases showed that they are present at the exocytic site, and their mutants are defective in rhoptry and mucocysts exocytosis, respectively. A GDP-locked mutant in *Tetrahymena* revealed the essentiality of GDP to GTP switch for mucocysts release upon stimulation. Similar experiments in *Toxoplasma* are currently ongoing. Collectively, these findings support the notion that rhoptry secretion is governed by GTPase activities, and certain Nd/NdP proteins may act as regulators or effectors of GTPase activity.

T57

Untangling the functional hierarchal architecture of the *Toxoplasma gondii* apical annuli proteins

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The *Toxoplasma gondii* cytoskeleton contains a network of intermediate filaments, supporting a quilt of alveolar membrane compartments, together known as the inner membrane complex (IMC). Embedded within the IMC are the apical annuli: 5-6 donut shaped pores that facilitate secretion of dense granules. Annuli component LMBD3 spans from IMC to plasma membrane, as do several annuli localized SNARE proteins (Stx1, Stx20, Stx21). Here we describe a novel apical annuli protein, AAP7, which we functionally dissected by conditional knock-down (AAP7-cKD). AAP7 localizes to the annuli of mature parasites only, appearing when new daughters emerge. AAP7 is present in the second largest donut with an average diameter of 300 nm. However, in other parasites AAP7 is present in smaller diameter donuts, which hints at two distinct annuli pore states. Depletion of AAP7 causes a severe fitness defect but ~10% of parasites survive and develop a compensatory phenotype. To follow up on this resistance mechanism, we performed scRNA-seq to reveal any gene candidates that could be associated with overcoming the loss of AAP7. To further probe apical annuli function, we created a lethal double annuli deletion (DAD) strain, combining AAP7-cKD with a direct knock-out of AAP4 (AAP4-KO), the largest and most conserved annuli protein. DAD parasites have a slow death phenotype, and we are currently deciphering how the DAD parasites die. Dense granule secretion is reduced in the absence of AAP7, AAP4, and in the DAD parasites. In the absence of AAP4, annuli component Centrin2 is not recruited to the apical annuli. AAP7 is mis-localized in the AAP4-KO background, demonstrating AAP4 recruitment of AAP7. AAP2 is unaffected by the loss of AAP4, indicating independent recruitment. In the absence of AAP7, Centrin2 and AAP4 localize normally. Overall, our results demonstrate that Centrin2 and AAP7 recruitment to the annuli require AAP4, which is not essential to parasite viability. Both AAP4 and AAP7 are needed for the essential function of the apical annuli. Hence, we have provided insights into the functional hierarchy of apical annuli architecture and established the tool needed to probe the fitness-conferring mechanism of the *T. gondii* apical annuli.

T58

The initiation and early development of the tubulin-containing cytoskeleton in *Toxoplasma gondii*

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The tubulin-containing cytoskeleton of *Toxoplasma gondii* includes several distinct structures: the conoid, formed of 14 ribbon-like tubulin polymers, and the array of 22 cortical/subpellicular microtubules (MTs) rooted in the apical polar ring. Here we analyze the structure of developing daughter parasites using both 3D-SIM and expansion microscopy. Cortical MTs and the conoid start to develop almost simultaneously, but from distinct precursors near the centrioles. Cortical MTs are initiated in a fixed sequence, starting around the periphery of a short arc that extends to become a complete circle. The conoid also develops from an open arc into a full circle, with a fixed spatial relationship to the centrioles. The patterning of the MT array starts from a “blueprint” with ~ 5-fold symmetry, switching to 22-fold rotational symmetry in the final product, revealing a major structural rearrangement during daughter growth. The number of MT is essentially invariant in the wild-type array, but is perturbed by the loss of some structural components of the apical polar ring. This study provides structural insights into the development of tubulin-containing structures that diverge from conventional models, insights that are critical for understanding the evolutionary paths leading to construction and divergence of cytoskeletal frameworks.

T59

From the plasma membrane to the plasma membrane, following recycling and flow of *Toxoplasma gondii* membrane proteins.

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The plasma membrane (PM) plays a critical role in numerous functions, from motility to cell communication, and serves as a gateway between intracellular content and the extracellular environment[1,2]. This is particularly true for intracellular parasites, which switch between drastically different environments during their life cycles[3]. Endocytosis is an important mechanism involved in the regulation of plasma membrane surface proteins and uptake of environmental molecules. We recently demonstrated endocytosis in *Toxoplasma gondii* (*T. gondii*) by labelling endogenous Halo-tagged SAG1 with a non-permeable dye[4]. This uptake occurring at the micropore is important for intracellular development, as knockdown of the protein composing the micropore impairs endocytosis and disturbs plasma membrane dynamics, leading to membrane accumulation and death of the parasite[5,6]. However, little is known about the normal behaviour of PM in *T. gondii*. Here, using SAG1-Halo as a proxy, we followed the dynamics of the PM using a dual (spatial and/or temporal) labelling strategy, allowing the differentiation between internal and surface proteins, but also between the mother and de novo synthesized material during replication. Using this strategy, we showed the presence of a complete cycle of endo-exocytosis in *Toxoplasma gondii*, regulating PM dynamics during the parasite lytic cycle.

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T60

Intrinsically low arachidonic acid synthesis in cat cells, its potential role in ferroptosis resilience and its relevance for *Toxoplasma gondii*

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Recent studies have shown that *Toxoplasma gondii* requires iron for growth, but that it is also sensitive to iron overload. Felines, the definitive hosts of *T. gondii*, are strict carnivores. Their heme-iron-rich diet may pose a particular challenge, as it could cause iron-dependent lipid peroxidation, leading to intestinal cell death by ferroptosis. In other cellular systems, sensitivity to ferroptosis was dependent on the level of fatty acid desaturase 2 (FADS2) expression, due to the enzyme"s involvement in arachidonic acid (AA) synthesis from linoleic acid. Although most studies have suggested minimal or absent FADS2 activity in cats and other felids, others have provided contrasting results. Strikingly, *FADS2* transcripts are annotated in the published genomes of felids.

Given the potential importance of host cell longevity on the sexual development of *T. gondii* in the cat intestine, we have started to study ferroptosis in a cat *in vitro* system using stem cell-derived intestinal organoids (IO) and cell lines. We generated continuous, long-term IO cultures from cats, which will enable us to address the following questions: (i) are cat IOs deficient in FADS2 activity; (ii) are they sensitive to ferroptosis, and (iii) how do parasites react to ferroptosis induction in this cellular system? Indeed, we observed *FADS2* transcription in all cat cell systems tested. Despite this, metabolic labeling showed that feline cells have very limited, but not lacking, FADS2 activity, resulting in only very little AA synthesis. Intriguingly, we found that cat IOs are surprisingly resistant to usual ferroptosis inducers. The observed low amounts of AA due to FADS2 deficiency could thus play a role in ferroptosis resilience. Ongoing studies are examining the interplay of the parasite and feline intestinal cells in the context of ferroptosis. Overall, this study provides new perspectives on the molecular factors determining *T. gondii*"s definitive host specificity.

T61

Stuck Together: Investigating Interorganellar Communication in *Toxoplasma gondii*

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In conventional model organisms, it has been shown that organellar interactions are essential for metabolite and lipid exchange, organelle dynamics, cellular homeostasis, and cell fate. While organelles allow for specialized biochemical processes to occur, this necessitates communication between organelles to share signals and metabolites. Originally, it was thought this occurred simply through cytoplasmic diffusion or vesicular trafficking before recent work highlighted the importance of direct contact between organelles. These interactions are supported by areas of close proximity between the two organelles, maintained by protein tethers, and referred to as membrane contact sites. Currently, the majority of our knowledge on membrane contact sites is limited to yeast and mammals, which are part of the Opisthokont clade. Thanks to its genetic amenability, *Toxoplasma gondii* provides the unique opportunity to investigate membrane contact sites in a divergent model eukaryote. Additionally, *T. gondii* possesses a phylum-specific organelle, the apicoplast, which has long been observed in close proximity to the mitochondrion. The two organelles have co-evolved in Apicomplexans, resulting in shared biosynthetic and biochemical pathways. We hypothesize that phylum-specific contacts mediate the proximity of these organelles and promote the exchange of materials. To identify membrane contact site candidates in *T. gondii*, we have taken an unbiased proximity biotinylation-based approach by generating localization handles to anchor a biotin ligase to the surface of the apicoplast, mitochondrion, and also the ER. Preliminary biotinylation experiments followed by mass spectrometry analysis uncovered membrane contact site protein candidates. Our results include several proteins with expected membrane contact site functions, such as lipid-transfer proteins and small GTPases. We also found apicomplexan-specific proteins with no predictable functions or domains that may serve as potential drug targets. Finally, we will also use our data to generate surface proteomes for the ER, mitochondrion, and apicoplast. The results of this work will both expand our knowledge of membrane contact sites across the evolutionary tree, potentially uncover apicomplexan-specific ones, and provide an additional protein localization resource for the community.

T62

The *Toxoplasma* secreted effector *Tg*WIP modulates dendritic cell motility by activating host tyrosine phosphatases Shp1 and Shp2

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The obligate intracellular parasite *Toxoplasma gondii* causes life-threatening toxoplasmosis to immunocompromised individuals. The pathogenesis of *Toxoplasma* relies on its swift dissemination to the central nervous system through a “Trojan Horse” mechanism using infected leukocytes as carriers. Previous work found *Tg*WIP, a protein secreted from *Toxoplasma*, played a role in altering the actin cytoskeleton and promoting cell migration in infected dendritic cells (DCs). However, the mechanism behind these changes was unknown. Our results show that *Tg*WIP harbors two SH2-binding motifs that interact with tyrosine phosphatases Shp1 and Shp2, leading to phosphatase activation. DCs infected with *Toxoplasma* exhibited hypermigration, accompanying enhanced F-actin stress fibers and increased membrane protrusions such as filopodia and pseudopodia. By contrast, these phenotypes were abrogated in DCs infected with *Toxoplasma* expressing a mutant *Tg*WIP lacking the SH2-binding motifs. We further showed that wildtype infected DCs treated with a Shp1/2 inhibitor no longer induced the F-actin stress fiber phenotype and hypermigration. Finally, we demonstrated that the Rho-associated kinase (Rock) is involved in the induction of these phenotypes, in a *Tg*WIP-Shp1/2 dependent manner. Collectively, the data uncover a molecular mechanism by which *Tg*WIP modulates the migration dynamics of infected DCs *in vitro*.

T63

Mechanism of *Toxoplasma gondii* dissemination using immune cells as a shuttle carriers

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Toxoplasma gondii has developed efficient mechanisms to disseminate within the host, reaching virtually any tissue including immunoprivileged sites like the eye and the brain. This wide distribution is achieved by using migratory immune cells as shuttle carriers. Migratory immune cells are specialised in navigation, travelling long distances and crossing complex biological barriers, to reach any cell in the host. However, using immune cells as dissemination mechanism involves a complex balance between maintaining parasite replication and migratory fitness of the infected immune cell, which is bearing an increasing large cargo. Although molecular biological data on Toxoplasma -cell hijacking are well studied, the biophysical mechanisms underneath the trojan-horse migration are unresolved. Here, using advance microscopy techniques, custom-designed microchannels, 3D matrices and parasite and immune cell mutants we investigate the biophysical principles of locomotion of immune cells with parasite cargo. Our results show that infected immune cells are able to maintain migration, path finding and navigation properties through extraordinarily tight constrictions. Under extreme cell deformation, caused by crossing narrow and rigid constrictions, host-Toxoplasma adaptations show a dynamic actin network surrounded by a cage-like host microtubule network that ensure optimal balance between host migratory properties and mechanical resilience of the parasitophorous vacuole.

T64

Targeted *in vivo* screens identify GRA12 as a strain and mouse-transcendent secreted virulence factor of *Toxoplasma gondii*

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Toxoplasma survives in a remarkably vast repertoire of host cell types and species. This ability is granted by over 200 rhoptry and dense granule proteins secreted by the parasite during or after invasion. Most effectors have been shown to be either host- and/or parasite strain-specific, such as the ROP18 kinase which is a key virulence factor only in the context of virulent parasites infecting laboratory mouse strains. However, effector proteins required to colonise more resistant mice such as the *M. m. castaneus* and the *M. m. musculus* subspecies, or virulence factors that are shared across *Toxoplasma* lineages are still unknown.

Here, we performed CRISPR-Cas9 *in vivo* screens of the *Toxoplasma* secretome in type I, II, III and the South American VAND isolate in mouse strains that differ in their susceptibility to infection. We identified the dense granule protein 12 (GRA12) as a prominent strain-transcendent virulence factor during acute infection in resistant and susceptible mice. A CRISPR screen in IFNγ-stimulated macrophages *in vitro* confirmed the importance of GRA12 for *Toxoplasma* survival and suggests the protein protects the parasite from cell-autonomous immune responses.

We established GRA12 KO and complemented strains in the RH ΔKU80 background and in a newly created VAND ΔKU80 strain and confirmed GRA12 role as a virulence factor in resistant murine subspecies *in vitro* and *in vivo*. Lack of GRA12 results in loss of vacuolar space and ruffling of the parasitophorous vacuole membrane as observed by transmission electron microscopy. Macrophages infected with GRA12-KO parasites, but not with complemented or parental strains, undergo a IFNγ-dependent cell death with features of necrosis. GRA12 homologs of the closely related species *Neospora caninum* and *Hammondia hammondi* rescue the restriction of GRA12-KO parasites, suggesting a conserved function beyond *Toxoplasma*.

In summary, we identified the first *Toxoplasma* virulence factor to date that is shared across parasite and host murine strains, and which is critical for cell-autonomous survival. Further investigation on GRA12 will elucidate a *Toxoplasma* core mechanism to evade the host-mediated clearance.

T65

In vivo CRISPR screen identifies a novel microneme protein essential for *Toxoplasma* egress and virulence

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In the lytic cycle of Toxoplasma gondii, following successful invasion and replication, parasites eventually egress from the host cell. Parasite egress is not a passive process but a strictly orchestrated event driven by the interaction of factors derived from the host and parasites. The phylum Apicomplexa possesses secretory organelles called micronemes that release their contents: microneme proteins (MICs). MICs comprise various types of proteins, such as adhesins, proteases, and perforins, that are crucial for motility, invasion, and egress. In particular, perforin-like protein 1 (PLP1) is the single known MIC essential for both egress and virulence. However, it is incompletely understood whether other MICs also play pivotal roles during infection *in vivo*. In this study, we have generated a small-scaled gRNA library targeting approximately 300 *T. gondii* genes encoding proteins predicted to be localized at the apical complex (conoid and micronemes) and pellicles (IMC and plasma membrane) by hyperLOPIT. *In vivo* CRISPR screen analysis revealed various apical complex/pellicle-related genes that are important for parasite virulence. Among the top hits, we discovered that a novel microneme protein, whose function had been previously unknown, plays an essential role in the induction of egress by permeabilizing and disrupting both parasitophorous vacuole membrane and host plasma membrane. Moreover, we found that PLP1 alone is insufficient for parasite egress and that this novel MIC is also required. Collectively, our *in vivo* CRISPR screen provides a crucial clue to understanding the molecular mechanism of how MICs orchestrate parasite egress that contributes to the parasite *in vivo* adaptation and virulence.

T66

Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes and Neurons as Models for *Toxoplasma gondii* Infection and Persistence

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Toxoplasma gondii infection may be asymptomatic in healthy people but results in the lifelong persistence of cysts containing bradyzoites in brain and muscle. We used human induced pluripotent stem cell-derived cardiomyocytes (iCMs), cardiac fibroblasts (iCFs) and neurons (iNEs) from a single human donor to study the interactions of *T. gondii* with human cells *in vitro*. iCMs, iCFs and iNEs infected with the *T. gondii* TypeI/III EGS reporter strain expressing *SAG1*-mCherry (tachyzoite) and *LDH2*-sfGFP (bradyzoite) displayed higher rates of bradyzoite formation in iCMs and iNEs, compared to iCFs from the same donor at 72 hours post-infection by fluorescence microscopy of live cells and flow cytometry. Bradyzoite induction occurred spontaneously in normal media.

Using RNA-seq we compared uninfected iCMs, iCFs and iNEs to cells infected for 24 hours and 72 hours. Both iCFs and iCMs showed thousands of differentially expressed genes (DEGs) at both timepoints. In contrast, iNEs infected for 24 hours showed 190 upregulated and 321 downregulated DEGs whereas iNEs infected for 72 hours showed 244 upregulated and 91 downregulated DEGs. Enrichment analysis of gene sets (GSEA) regulated by infection was performed using the hallmark gene set collection from the Molecular Signatures Database. To correlate the higher rate of bradyzoite formation in iCMs and iNEs to the relevance of each GSEA hallmark pathway, we identified hallmark pathways having enriched DEGs in a similar manner between iCMs and iNEs but different from iCFs. At 24 hours post-infection, fatty acid metabolism and oxidative phosphorylation pathways were upregulated in iCFs but unchanged in iCMs or iNEs, whereas MYC targets pathway was downregulated in iCMs or iNEs but unchanged in iCFs. At 72 hours post-infection, glycolysis pathway was downregulated in iCMs or iNEs but unchanged in iCFs, whereas myogenesis and protein secretion pathways were upregulated in iCFs but were unchanged in iCMs or iNEs.

Our *in vitro* generated human iCMs, iCFs and iNEs provide a new model to understand the impact of host cell type and host-specific factors upon *Toxoplasma* infection. We anticipate this model can be used to develop cell-based assays for drug screening of potential bradyzoite therapeutic targets and to explore the role of host genetics in *T. gondii* pathogenesis and persistence.

T67

Toxoplasma gondii restricts the developmental trajectory of placental trophoblasts from an infection-resistant cell fate

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Pregnancy is one of the most critical points of vulnerability to *Toxoplasma* infection. Despite this, no specific mechanisms used by the parasite to cross the placenta have been discovered. Here, we investigate an entirely new phenomenon where *Toxoplasma* is able to coopt canonical host cell development to propagate its own transmission through the placenta. Using a bipotent stem cell system, we can grow cells that recapitulate placental trophoblast progenitors, called cytotrophoblasts (CYT), in addition to both terminally differentiated lineages, extravillous trophoblasts (EVT) and syncytiotrophoblasts (SYN). These cells vary in their susceptibility to *T. gondii* infection, with SYN being unusually resistant and EVT being comparably permissive. Importantly, the high proportion of infection-resistant SYN at the surface of the maternofetal interface prevents the vast majority of parasite invasion to deeper cell layers. Using scRNA and scATAC-seq datasets of 50,000 cells, we have convincingly determined that *T. gondii*-infected progenitor CYT undergo global transcriptional changes consistent with a departure from their stem state and activation of host cell differentiation. Moreover, these data indicate that this parasite-induced host cell differentiation is specifically antagonistic to the infection-resistant SYN cell lineage. We see simultaneous reduction of transcripts and proteins governing SYN development and induction of transcripts governing EVT development. We also demonstrate that parasite infection of 3D primary trophoblast organoids (a system that best recapitulates inter-cell type signaling) for just 24 hours is sufficient to elicit CYT differentiation to EVT-like cells that, ordinarily, must be chemically induced for multiple days in culture. Finally, we use a novel functional assay to show that *Toxoplasma* infection results in such severe lineage restriction that host cells almost completely lose their capacity to fuse with other SYN. Taken together, these results indicate that *Toxoplasma* infection dysregulates progenitor trophoblast cells at both the levels of cell fate and cell function, meaning, infection of progenitor cells specifically promotes one cell lineage at the cost of another. This significant shift of CYT away from SYN and towards EVT could alter the balance of resistant and susceptible cells at the maternofetal interface, potentiating robust *T. gondii* dissemination through placental tissue and, ultimately, into the fetal compartment.

T68

A Novel Pyruvate Transporter in the Apicoplast of Apicomplexan Parasites

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Pyruvate lies at a pivotal node of carbon metabolism in eukaryotes. It is involved in diverse metabolic pathways in multiple organelles, and its inter-organelle shuttling is crucial for cell fitness. Many Apicomplexan parasites harbor a unique organelle called the apicoplast that houses metabolic pathways like fatty acid and isoprenoid precursor biosyntheses, requiring pyruvate as a substrate. However, how pyruvate is supplied in the apicoplast remains enigmatic. Here, deploying the zoonotic parasite *Toxoplasma gondii* as a model apicomplexan, we discovered two proteins residing in the apicoplast membranes that together constitute a functional apicoplast pyruvate carrier (APC) to mediate the import of cytosolic pyruvate. Depletion of APC results in reduced activities of metabolic pathways in the apicoplast and impaired integrity of this organelle, leading to parasite growth arrest. APC is a novel pyruvate transporter in diverse apicomplexan parasites, suggesting a common strategy for pyruvate acquisition by the apicoplast in these clinically-relevant intracellular pathogens.

T69

Exploring Apicoplast Functions through *Toxoplasma gondii* lacking the apicoplast

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Apicomplexan parasites, notorious for causing severe diseases like malaria (*Plasmodium* spp.) and toxoplasmosis (*Toxoplasma gondii*), harbor a distinctive organelle known as the apicoplast. The apicoplast is derived from secondary endosymbiosis and, while no longer photosynthetic, plays a pivotal role in housing metabolic functions critical for the survival of *T. gondii*. In this study, we aimed to dissect apicoplast functions and identify potential drug targets by bypassing the four metabolic pathways generating essential output for the parasite, namely *de novo* fatty acid (FA) synthesis via a prokaryotic fatty acid synthase II (FASII), synthesis of the lipid precursor lysophosphatidic acid (LPA), parts of the heme synthesis and synthesis of isopentenyl pyrophosphate (IPP) via a 2-*C*-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate (MEP/DOXP) pathway. To achieve this, we supplemented the culture medium with palmitate, myristate, LPA, 5-aminolevulinic acid, and mevalolactone, after genetically engineering parasites to express an MVA cassette[1,2], enabling parasites to synthesize IPP from mevalonolactone independent of their endogenous pathway. Under these apicoplast-bypassing conditions, pharmacological disruption of apicoplast-resident protease FtsH1 with actinonin, led to a rapid loss of the organelle but did not kill parasites. The continuous culture of *T. gondii* devoid of its plastid organelle serves as a unique platform to uncover novel apicoplast functions. Comparative proteomic analysis between wildtype and apicoplast-less parasites revealed a set of downregulated proteins residing in the apicoplast. Intriguingly, some of these proteins were identified as fitness-conferring critical components involved in the biology of the organelle. In conclusion, this study sheds light on the adaptability of *T. gondii* to survive in the absence of the apicoplast and uncovers novel functions associated with the organelle.

1 Swift R, et al. A mevalonate bypass system facilitates elucidation of plastid biology in malaria parasites PLoS Pathog. 2020 Feb 14;16(2): e1008316.
2 Niu Z, et al. Two apicoplast dwelling glycolytic enzymes provide key substrates for metabolic pathways in the apicoplast and are critical for Toxoplasma growth. PLoS Pathog. 2022 Nov 30;18(11):e1011009.

T70

A multitude of small mitochondrial RNAs are used in the mitoribosome of *T. gondii*

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The mitochondrial genome of T. gondii is made up of 21 sequence blocks that are found in various combinations. It comprises merely three protein-coding genes, alongside a set of thirty to forty genes encoding small RNAs (sRNAs), many of which exhibit homologies to rRNA from *E. coli*. The expression status and integration of these short RNAs into ribosomes remains unclear and direct evidence for active ribosomes within apicomplexan mitochondria is still lacking. We conducted small RNA sequencing on the apicomplexan *Toxoplasma gondii* to investigate the occurrence and function of mitochondrial sRNAs. We find that many small RNAs originated from the junction sites between sequence blocks in the mitochondrial genome. Surprisingly, such block border sRNAs were incorporated into polysomes together with canonical rRNA fragments and mRNAs. In conclusion, apicomplexan ribosomes are active within polysomes and are indeed assembled through the integration of sRNAs, including previously undetected sRNAs with merged mRNA-rRNA sequences. Our findings lead to the hypothesis that *T. gondii's* block-based genome organization enables the dual utilization of mitochondrial sequences as both messenger RNAs and ribosomal RNAs, potentially establishing a link between the regulation of rRNA and mRNA expression.

T71

TgORP2, an Oxysterol binding protein Related Protein putatively involved in Endoplasmic reticulum and Mitochondrion Membrane Contact Site formation and lipid exchange in *Toxoplasma gondii*.

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Apicomplexa are obligate intracellular parasites responsible for major human diseases such as toxoplasmosis, caused by Toxoplasma gondii (*Tg.*). Its intracellular survival is closely linked to fatty acids (FAs) acquisition and usage to produce essential complex lipids for membrane biogenesis, signal transduction, membrane stability and energy storage (1). To fulfil its needs in FAs, the parasite is able to produce some FAs *de novo* through the apicoplast FASII pathway as well as massively scavenging FAs from its host (1). To maintain this critical need for FA to maintain parasite propagation and to avoid their toxic accumulation, the trafficking of lipids is regulated by key proteins that act during their synthesis/import, their storage, and their transport between organelles (2). Endoplasmic reticulum (ER), Mitochondria (Mito) and Lipid droplets (LD) are key organelles for lipid trafficking and homeostasis. Recent work show that there is intense lipid exchanges happening between these organelles through membrane contact sites (MCSs) and specific lipid transfer proteins. In eukaryotic cells, ER MCSs involvement in lipid homeostasis have been shown to be pivotal for survival (3,4), these include ER-LD and ER-Mito. We have identified such pivotal LTPs, and we characterized the members of the Oxysterol Binding Protein Related Protein (ORP) in Toxoplasma. Among them proteins, we found that TgORP2 was located at the ER-Mito MCS and is responsible for lipid exchange between the two organelles. Here, I will be presenting my PhD project data deciphering on the key function of TgORP2 to maintain lipid trafficking and homeostasis, allowing parasite propagation during tachyzoite life stage.

1. Amiar, S. et al. Cell Rep 30, 3778-3792.e9 (2020). 2. Dass, S. et al. Nat Commun 12, 2813 (2021). 3. Wu, H. et al. Science (1979) 361, (2018). 4. Rakotonirina-Ricquebourg, R. et al. Prog Lipid Res 85, 101141 (2022).

T72

To inhibit or not to inhibit: characterization of the *Toxoplasma gondii* homolog of ATPase inhibitory factor 1 (IF1)

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In many eukaryotes, mitochondria play a key role in the generation of cellular energy as the so-called powerhouses of the cell. Within the powerhouse, the ATP synthase is the true power generator; it harvests the proton gradient created by the complexes of the electron transport chain to produce ATP through oxidative phosphorylation. In mammals and yeast, the activity of the ATP synthase is tightly controlled via many mechanisms, including by the binding of interactor proteins. One such interactor is ATPase inhibitory factor 1 (IF1), which can inhibit the ATP synthase and mediate the induction of cytoprotective gene expression pathways through a process known as mitohormesis. As we know little about how *Toxoplasma gondii* ATP synthase activity is regulated, we wanted to investigate whether the *T. gondii* homolog of IF1 (TgIF1) played similar roles to the IF1 found in other organisms. Using CRISPR/Cas9, we tagged TgIF1 and generated TgIF1 knockout and overexpression parasite strains. With these strains, we found that TgIF1 knockout and overexpression had no observable impact on metabolism, mitochondrial membrane potential, or ATP synthase dimerization. We did, however, find that TgIF1 overexpression appeared to increase higher order oligomerization of the ATP synthase and that knockout significantly reduced cristae density. Although TgIF1 levels had no impact on parasite growth under normal culture conditions, we found that TgIF1 knockout and overexpression both negatively impacted growth under hypoxia. Further, we illustrated that TgIF1 overexpression improved growth when parasites were exposed to oxidative stress, suggesting a mitohormetic role of TgIF1. This phenotypic observation was corroborated by bulk RNAseq data which showed that the main group of genes impacted by TgIF1 overexpression is involved in gene expression regulation and RT-qPCR data indicating that TgIF1 levels correlate with expression levels of several antioxidants. In summary, it appears that while TgIF1 does not have significant effects on parasite metabolism and growth under normal conditions, it plays a key role in mitochondrial cristae biogenesis and is important for mediating the response to various stressors.

T73

Fragmented and on the move - the enigmatic mitochondrial genome sequences of *Toxoplasma* and *Sarcocystis*

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Recently we have reported on two remarkable aspects of the *Toxoplasma gondii* mitochondrial genome. First it exists as a redundant and highly fragmented sequence of unknown topology[1]. Second, ~10,000 relatively small pieces of the mitochondrial genome (NUMTs) have been transferred to the nuclear genome[2]. These transferred fragments account for an astonishing 1.4% of the nuclear genome sequence and appear to have contributed to evolutionary innovation. We find very similar mtDNA genome sequence novelties in *Hammondia* and *Neospora*, including very large numbers of NUMTs[1]. Thus, we decided to examine the more distantly related *Sarcocystis neurona* SN3. We assembled a new *S. neurona* SN3 genome sequence using ONT, Illumina and Hi-C. It is 127 Mb and we were able to observe at least 10 putative chromosomes, the largest of which is 24 Mb. The assembly has been annotated using orthology, as well as RNA-seq and ISO-seq from different lifecycle stages. We used this new assembly to study the distribution of NUMTs and characterize the mtDNA. We find a markedly reduced number of mtDNA insertions, 1077 or about 0.06% of the nuclear genome content. Interestingly, we find a redundant, fragmented mtDNA that cannot be assembled, as was the case in *T. gondii*. However, the fragments in *S. neurona* are quite different from those observed in other related tissue coccidians. This is significant, because as reported by Tetzlaff et al.,[3] the *T. gondii* mtDNA breakpoints are associated with transcription initiation for the generation of small ribosomal RNAs in *Toxoplasma*.

[1]Namasivayam et al., doi: 10.1101/gr.266403.120

[2]Namasivayam et al., doi: 10.1101/2023.05.22.539837

[3]Tetzlaff et al., doi: 10.1101/2023.09.02.556019

P001

A Golgin-Like Protein is Required for Golgi Structural Maintenance and Function in *Toxoplasma gondii*s Endomembrane System

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The Golgi complex is an essential organelle in all eukaryotic cells with important roles in secretory protein processing and trafficking. In *Toxoplasma gondii,* the Golgi is composed of a single stack of 4-8 flattened cisternae localized at the apical side of the nucleus. It is an essential part of the parasite"s endomembrane system, but few structural proteins have been identified with roles in Golgi architectural maintenance, positioning, or function. We recently identified an uncharacterized protein (TGME49_213392) that localizes to the Golgi. This 253kDa protein contains an N-terminal intrinsically disordered domain and C-terminal 1000 amino acid coiled-coil domain. It has therefore been named "GLCC" for Golgi-localized coiled-coil domain containing protein. To investigate the role of this protein, GLCC was endogenously tagged with a 3xTy epitope and mAID to facilitate protein depletion using the auxin inducible degron system. GLCC was found to be essential for parasite survival by plaque assay. 15 hours after GLCC depletion, Golgi length was reduced by 56.5% from 0.95±0.02µm in control parasites to 0.53±0.01µm in GLCC knockdown parasites. Transmission electron microscopy showed disorganization of the Golgi cisternae. After 48 hours of auxin treatment, GRASP had a diffuse cytosolic localization in ~50% of parasites, indicating that GLCC is essential for maintaining Golgi structure. GLCC depleted parasites showed a decrease in growth rate starting just 18 hours post GLCC knockdown and asynchronous replication of daughter parasites. 44% of knockdown parasites had an aberrant morphology having lost their canonical crescent shape. GLCC depleted parasites failed to egress naturally, even after 60 hours of growth in their host cells. Interestingly, micronemes and rhoptries, organelles thought to be formed in a Golgi dependent manner, appeared unaffected by the loss of GLCC even at late time points when Golgi integrity was compromised. GLCC knockdown did not affect calcium ionophore induced egress. This suggests there is a Golgi-independent pathway for organelle recycling and/or formation. Collectively, this study identified a protein essential for Golgi structural maintenance and the GLCC knockdown parasite line provides a tool for an in-depth characterization of Golgi function within the parasite endomembrane pathway.

P002

Immunization with the amino-terminus region of dense granule protein 6 (GRA6) of *Toxoplasma gondii* activates CD8+ cytotoxic T cells capable of removing tissue cysts of the parasite through antigen presentation by human HLA-A2.1

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Our previous studies identified that the amino-terminus region (amino acids 41-152) of dense granule protein 6 (GRA6Nt) of *Toxoplasma gondii* potently activate CD8+ T cells capable of removing tissue cysts in BALB/c mice genetically resistant to this infection. In the present study, we examined whether GRA6Nt is able to activate anti-cyst CD8+ T cells through antigen presentation by the HLA-A2.1, one of the three most common MHC class I molecules in humans. HLA-A2.1-transgenic and wild-type (WT) control mice were immunized intraperitoneally with 50 mg of recombinant GRA6Nt (rGRA6Nt) twice with a four-week interval. Two weeks after the 2nd immunization, CD8+ cells from the immunized HLA-A2.1-transgenic mice, but not WT mice, secreted large amounts of perforin and granzyme B in response to GRA6Nt through antigen presentation by HLA-A2.1 *in vitro*. When those CD8+ T cells were transferred into chronically infected HLA-A2.1-expressing NSG mice deficient in T cells, cerebral cyst burden of the recipients of HLA-A2.1-transgenic T cells, but not of WT T cells, became significantly less than that of control mice with no cell transfer. Furthermore, the significant reduction of the cyst burden by a transfer of the HLA-A2.1-transgenic CD8+ immune T cells required an expression of HLA-A2.1 in the recipient NSG mice. Thus, antigen presentation of GRA6Nt by human HLA-A2.1 is able to activate anti-cyst CD8+ T cells that eliminate *T. gondii*cysts through antigen presentation by the HLA-A2.1.

P003

The Role of Purine Auxotrophy in Toxoplasma-Host Interaction

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Studying cellular survival strategies in resource-scarce environments is vital for revealing novel starvation responses across diverse life forms. Substantial advancements have been achieved in understanding purine metabolism in higher eukaryotes. Nevertheless, our understanding of these processes in protozoans is incomplete. This study presents a comprehensive analysis of Toxoplasma's purine dependency due to the absence of purine synthesis pathways 1,2.
We developed a model to study purine starvation in Toxoplasma, revealing significant transcriptional changes in response to purine scarcity. This includes the upregulation of histone modifiers during starvation and the downregulation of rhoptry transcripts, which are crucial for host cell invasion 3. Additionally, the study delineates the stage-specific roles of TgENTs, notably TgENT1 and TgENT2, in the parasite lifecycle. Notably, the deletion of TgENT1 led to a reduced number of tissue cysts in infected mice, underscoring its importance in chronic infection. Furthermore, this study investigated the essential nature of TgENT3, whose deletion results in complete growth defects.
The outcomes of this project are anticipated to significantly enhance our understanding of purine acquisition and metabolism in intracellular parasites, shedding light on alternative evolutionary pathways that lead to distinct purine-sensing and scavenging strategies. This has broader implications for understanding metabolic adaptations in other intracellular parasites.

1. Kloehn, J., Hammoudi, P.-M. & Soldati-Favre, D. Metabolite salvage and restriction during infection — a tug of war between Toxoplasma gondii and its host. *Syst. Biol. Nanobiotechnology* 68, 104–114 (2021).
2. Olson, W. J. *et al.* Dual metabolomic profiling uncovers Toxoplasma manipulation of the host metabolome and the discovery of a novel parasite metabolic capability. *PLOS Pathog.* 16, e1008432 (2020).
3. Dubremetz, J. F. Rhoptries are major players in Toxoplasma gondii invasion and host cell interaction. *Cell. Microbiol.* 9, 841–848 (2007).

P004

TOXOPLASMA OXYGEN SENSING: PHDs HARD AT WORK

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Toxoplasma gondii is an obligate intracellular parasite that can infect any nucleated cell in any tissue of warm-blooded animals. How does the parasite have such a wide host cell and tissue tropism? One mechanism is by the parasite expressing its own host cell receptor complex that it injects into the host cell. But as the parasite disseminates through its host, it encounters diverse environments that it that it must adapt to so that it can replicate, grow, and cause disease. Oxygen is one environmental factor whose levels change dramatically in tissues and all cells must detect and respond to changes in oxygen availability. Prolyl hydroxylases are dioxygenases that function as key cellular oxygen sensing proteins. In mammals, the transcription factor Hypoxia-Inducible Factor-1a is the best recognized prolyl hydroxylase substrate and our earlier work revealed that parasite growth at low oxygen requires HIF-1 activation. These studies led us to study how *Toxoplasma* itself senses oxygen? To address this question, we used BLAST analysis and identified 2 *Toxoplasma*-encoded prolyl hydroxylase homologs that we named PHYa and PHYb. PHYa prolyl hydroxylates SKP1, which is an adaptor in the Skp1/Cullin-1/F-box protein (SCF)-E3 ubiquitin ligase complex. SKP1 prolyl hydroxylation alters its binding preferences for F-Box proteins leading to changes in which proteins are ubiquitinated by the SCF-E3. PHYa is required for growth at low oxygen and is required for virulence as it is critically required to resist IFNγ-dependent killing. PHYb, on the other hand, is required for growth and survival of parasites exposed to stress at elevated oxygen levels and does so by inhibiting phosphorylation of elongation factor 2 (eEF2). eEF2 phosphorylation inhibits it from catalyzing the translocation of the peptidyl-tRNA complex from the A-site to the P-site of the ribosome. We have shown that PHYb promotes protein synthesis to ensure that the parasite proteins are available to ensure that the stress response is properly executed. In this talk, we will discuss new data regarding how PHYa and PHYb function. Taken together, these data indicate that the two prolyl hydroxylases work together as an oxygen sensing rheostat in regulating protein synthesis and degradation to ensure that the parasite proteome is programmed to enable *Toxoplasma* to grow, survive, and cause disease at whatever oxygen tension it faces.

P005

In silico, transcriptomic and proteomic comparative analysis of Dense Granule Proteins orthologues in *Toxoplasma gondii* and *Neospora caninum*

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Apicomplexa parasites require a parasitophorous vacuole (PV) for replication and infection maintenance, which is formed through protein secretion from organelles such as dense granules. Thus, Dense granule proteins (GRAs) are important for the intracellular survival of these parasites, as they influence host-parasite interactions, the acquisition of nutrients and the modulation of the host's immune responses. Research has focused on GRAs of *Toxoplasma gondii*, but little is known about related *Neospora caninum*. Thus, the aim of this study is to fill this gap by carrying out a bioinformatic, transcriptomic and proteomic analysis, providing information on the conservation and divergence of GRAs in these parasites. For that purpose, we generated a list of predicted GRAs obtained at the Toxoplasma Informatics Resources Database (ToxoDB) and the literature, and checked for *N. caninum* orthologues, in order to initially analyze the genes for synteny, identity, conserved or unique motifs and to compare their predicted conformational structure. We found a high level of synteny and a reasonable ammino acid similarity. Differences in transmembrane domains and signal peptides indicate possible disparities in subcellular localization and functionality between GRAs, whereas little divergence was found in the presence of other conserved domains and predicted 3D structures. On the other hand, gene expression of the GRAs in *T. gondii* and *N. caninum* presented a notable distinction in profile for part of the genes, indicating possible adaptations in the host-parasite interactions. In order to confirm the presence of those proteins in the host-*N. caninum* interface, we used proximity biotinylation to identify the predicted targets in the PV. We have also generated knockout parasites of selected genes and submitted those to in vitro and in vivo assays to assess their role in host resistance to the infections. In conclusion, we believe our work contributes to a deeper understanding of the molecular aspects, functional diversity and evolutionary history of TgGRAs and NcGRAs, and may have far-reaching implications for the development of interventions and therapies for toxoplasmosis and neosporosis.

P006

Preliminary data of a time-controlled splitCas9 genome wide screen in *Toxoplasma gondii*

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CRISPR/Cas9 technology has revolutionized genome editing and gene knockout studies across various organisms. Its successful application in *Toxoplasma gondii* has provided crucial insights into this parasite"s biology. Previously, we utilized splitCas9, an inducible Cas9 system relying on rapamycin-induced dimerization of FRB and FKBP domains fused to either the N- or C-terminal domain of Cas9, for targeted gene knockout in *T. gondii*. This approach facilitated a time-controlled induction of gene knockouts through continuous co-expression of suitable guide RNAs (gRNAs), leading to the identification of essential egress genes in a small-scale phenotypic screen.
Here, we present a genome-wide screen employing the splitCas9 system, targeting all annotated genes of *T. gondii*. After integrating a genome-wide gRNA library into a strain constitutively expressing splitCas9, we induced dimerization of the N- and C-terminal Cas9 domains. We collected samples at various time points post-induction, allowing for the assignment of genes to their respective "dropout times" and providing first insights into their potential roles in the cell cycle. Specifically, we anticipated dropout of genes responsible for replication processes at 24 hours post-induction, followed by a reduction in essential egress, motility, and invasion genes at later time points such as 48 and 96 hours.

P007

Uncovering the mechanisms employed by latent Toxoplasma gondii within neurons to evade immune surveillance

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IFN-γ-induced anti-microbial mechanisms are instrumental for parasite control and host protection during *T. gondii* (*Tg*) infection. Upon brain invasion by *Tg*, neurons upregulate surface MHC class I molecules, allowing them to present tachyzoite antigens to CD8+ T cells, which is key to avoid excessive parasite burden (Salvioni, Cell Rep 2019 PMID: 31189109). Neurons also restrict parasite growth in response to IFN-γ, although neurons may be less able to do so than other cells. *Tg* eventually persists in the brain throughout the latent phase of infection as bradyzoites within intra-neuronal cysts. The mechanisms by which *Tg* bradyzoite-infected neurons evade CD8+ T cell recognition, remain unclear. Here we investigate to which extent *Tg* effectors that hijack IFN-γ responses in various cell types, also modulate the responses of infected neurons to IFN-γ. Using primary cultures of hippocampal neurons stimulated or not with IFN-γ, we assessed the nuclear translocation of IRF1 transcription factor and MHC I surface expression in neurons containing mostly tachyzoites (24 hpi) or bradyzoites (72 hpi). IRF1 was translocated in the host cell nucleus and surface expression of MHC I was upregulated after IFN-γ stimulation. Neurons stimulated by IFN-γ and infected by WT tachyzoites or bradyzoites failed to accumulate IRF1 in their nuclei. Conversely, neurons infected with TgIST-deficient parasites showed higher nuclear IRF1 intensity. Strikingly, neurons infected by tachyzoites upregulated surface MHC I similarly as uninfected ones, but neurons infected by bradyzoites showed a clear TgIST-dependent inhibition of surface MHC I upregulation. These results suggest that the impairment of neuronal MHC I is TgIST-dependent but is delayed compared to the subversion of IRF1, suggesting that MHC I subversion may occur after or upon bradyzoite differentiation. Experiments are underway to explore the impact of bradyzoite-derived TgIST and other parasite effectors on the neuronal responses to IFN-γ, and ultimately, on the ability of CD8+ T cells to detect infected neurons and control the parasite *in vivo*.

P008

Stage-specific gene expression and signaling through chromatin: the paradigmatic interplay of *Toxoplasma* GCN5b and HDAC3

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GCN5 (General Control Non-repressed protein 5) plays a central role in the regulation of gene expression through its histone acetyltransferase (HAT) activity. Within the SAGA complex (Spt-Ada-Gcn5 acetyltransferase), GCN5 regulates transcription by modifying the chromatin structure and thus influencing the accessibility of gene promoters. Its conservation in all eukaryotes underscores its essential role in cell biology. Remarkably, among invertebrates, *Toxoplasma* possesses two HAT variants of the GCN5 family. GCN5a plays an important role in parasite gene expression under alkaline conditions, but is not critical for growth. In contrast, GCN5b is essential for parasite survival. Previous pioneering studies have begun to elucidate the unique molecular properties of GCN5b HAT activity, but also its partnership. However, these studies were performed on zoites still expressing the native GCN5b gene, which may lead to potentially misleading or incomplete conclusions. Here, we re-examine the unique interactome associated with the native GCN5b protein and we investigate the transcriptional consequences of depleting the protein using auxin-inducible degron technology. We are also investigating the interplay between GCN5b and its counterpart HDAC3 at the chromatin level as the parasite transitions from the tachyzoite to the merozoite stage. We will analyze their selective engagement as activator, repressor or chromatin insulator using ChIP-seq and try to decipher the “histone code” that ensures precise spatial and temporal regulation of stage-specific genes. We are also comparing the dynamics of the Toxoplasma SAGA complex between the two different zoite populations, focusing on the DNA-targeting AP2 transcription factors, but also on the PHD-containing histone PTM reader identified in the interactome of GCN5b.

P009

Bridging Molecules: Exploring the Structural and Biochemical Interface between the Glideosome-Associated Connector and F-actin

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Toxoplasma gondii relies on gliding motility for crucial processes such as invasion, egress, and traversing biological barriers. This motility is orchestrated by the glideosome, a molecular machine unique to apicomplexans. Gliding motility involves conoid extrusion and microneme secretion and is powered by the actomyosin system, including myosin H, myosin A, and filamentous actin produced by formin1 (FRM1). The glideosome-associated connector (GAC) plays a pivotal role in bridging the cytosolic tail of MIC2 to F-actin. GAC is a large protein conserved across Apicomplexa, composed of numerous armadillo repeats and a PH domain that binds to phosphatidic acid. Positioned initially at preconoidal rings, GAC translocates to the basal pole via the flux of F-actin during motility1.
The coordinated interactions of GAC with F-actin, adhesins, and membranes are essential for transmitting the power generated by motors and propelling the parasite forward. Determination of GAC structure by X-ray crystallography highlights a model composed of 53 armadillo repeats forming a large arch that folds back onto itself, making intramolecular interactions between N- and C-terminal ends2.In solution, GAC exhibits dynamic properties, adopting various conformations from closed to open and extended. Insights into interaction of GAC with F-actin were obtained using hydrogen/deuterium exchange coupled with mass spectrometry (HDX-MS). Results identified several F-actin binding sites that localise at the intramolecular contact site of GAC, predicting that a conformational change facilitating F-actin binding is required. Several mutants have been designed and are poised for testing in the parasite to unravel the contribution of critical GAC residues to

1 Tosetti, N. et al, Elife. 2019 Feb 12;8:e42669.
2 Kumar, A. et al, Elife, 2023 Apr 4:12:e86049

P010

Anti-Apicomplexa drug discovery: Chemotherapeutic drug screening and target deconvolution in *Toxoplasma gondii*.

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Whole-cell phenotypic screening is proving to be an effective approach to drug discovery, leading to the identification of numerous lead compounds with antimicrobial properties. However, uncovering the targets and understanding the mode of action remains a challenging and elusive aspect of this approach. In this project, we focused on identifying novel drug candidates against apicomplexan parasites using a comprehensive approach that combines small compound library screening and target deconvolution based on forward genetics with transcriptome sequencing and computational mutation discovery, using *Toxoplasma gondii* as a relevant model for *Apicomplexa*. Phenotypic screening included the monitoring of intracellular parasite growth and the cytotoxicity of the drug towards the host cell.

The target deconvolution process employed a forward genetics strategy involving Ethyl Methanesulfonate (EMS) mutagenesis and mRNA sequencing. This approach facilitated the identification of point mutations in genes conferring drug resistance and provided valuable insights into the genetic basis of drug response in apicomplexan parasites. Several novel drug-target pairs were successfully identified through this strategy, identifying new potential therapeutics.

Since certain drug targets are conserved in different apicomplexan parasites, the effectiveness of the identified compounds was systematically assessed across *Plasmodium* and *Cryptosporidium* species. This cross-species evaluation reinforces the translational potential of the drug candidates identified, and offers the prospects of a broad-spectrum antiparasitic therapeutics.

The integration of phenotypic screening with forward genetic approaches represents a powerful workflow in the search for effective antiparasitic drugs. The findings from this study significantly contribute to the field of drug discovery, providing a framework for the identification, validation, and assessment of novel drug-target pairs across diverse apicomplexan parasites, addressing the urgent need for innovative solutions against malaria and cryptosporidiosis.

P011

Toxoplasma gondii DNA in tissues of Arctic charr, *Salvelinus alpinus*, collected from Nunavik, Québec, Canada

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Toxoplasma gondii is a very common zoonotic parasite in humans and animals worldwide. Human seroprevalence is very high in some regions of Canada"s North and is thought to be associated with the consumption of traditionally prepared country foods, such as caribou, walrus, ringed seal and beluga. While numerous studies have reported on the prevalence of *T. gondii* in these animals, in the general absence of felid definitive hosts in the North there has been considerable debate regarding the source of infection, particularly in marine mammals. It has been proposed that fish could be involved in this transmission, and a few studies have, in fact, detected *T. gondii* in various fish species, or have demonstrated the possibility of experimental infection. The objectives of the present study were to perform a targeted survey to determine the prevalence of *T. gondii* DNA in various tissues of Arctic charr sampled in the Nunavik Region of northern Québec, and to investigate the possible role of this commonly consumed anadromous fish in the transmission of infection to humans and marine mammals in Canada"s North. Overall, 12 out of 126 (9.5%) Arctic charr tested in the present study were PCR-positive for *T. gondii*, as confirmed by DNA sequencing. Brain tissue was most commonly found to be positive, followed by heart tissue, while none of the dorsal muscle samples tested were positive. It is likely that these fish are becoming infected with *T. gondii* through the ingestion of oocysts transported by surface water and ocean currents from more southerly regions where the definitive felid hosts are more abundant. The majority of PCR-positive Arctic charr were sampled on the Hudson Bay side of Nunavik, with just a single positive fish sampled in the Ungava Bay region, as might be expected due to the strong northward circulation of water from James Bay to the Hudson Strait. Arctic charr may, therefore, play an important role in the transmission of toxoplasmosis to Inuit, either directly through the consumption of raw fish or indirectly through the infection of fish-eating marine mammals harvested as country foods.

P012

Structural insight into the apicomplexan drug target cytochrome *bc*1

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The mitochondrial electron transport chain (mETC) and F1Fo-ATP synthase are of central importance for energy and metabolism in eukaryotic cells. The Apicomplexa, important pathogens of humans causing diseases such as toxoplasmosis and malaria, depend on their mETC in every known stage of their complicated life cycles. Complex III, also known as the cytochrome *bc*1 complex, is the target of clinically used drugs such as atovaquone. The apicomplexan mETC is highly divergent from the mammalian system. Our previous proteomic work uncovered the composition of the *Toxoplasma* mETC complexes and F1Fo-ATP synthase identifying 70 proteins, including 20 newly discovered protein subunits, highlighting their divergence from mammals.
To understand how these divergent complexes work and elucidate the mechanism of action of drug binding we used Cryo-EM to determine the structure of complex III and IV. Using native purification approaches we solved the structure of the respiratory supercomplex of complex III-IV from *Toxoplasma*, identifying new subunits and parasite-specific domains, as well as unique supercomplex architecture. Using a combination of native and immunoprecipitation approaches we were able to determine high resolution structures of the drug target *Toxoplasma* complex III in *Toxoplasma* with the inhibitors atovaquone or ELQ-300 bound. This gave us a detailed understanding of the mechanism of inhibitor binding and species specificity of drug action. This includes insight into why atovaquone displays much higher potency against apicomplexan complex III, compared to host, as well as showing that ELQ-300 has a different binding mode in apicomplexans compared to mammals. Insights from structural work opens the way for future drug design in both *Toxoplasma* and *Plasmodium*.

P013

A cytoplasmic dynein complex involved in host cell invasion by *Toxoplasma gondii*

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In all eukaryotes, intracellular cargo transport contributes to diverse cellular processes that are essential for the survival of the cells. This includes molecular motors, such as myosins that transport cargo along actin tracks and kinesins and dyneins that move along microtubules. We are particularly interested in the cytoplasmic dynein complex that, in mammalian cells, transports a vast diversity of cargoes. We identified homologs of most of the components of this complex in *Toxoplasma gondii* and undertook its functional characterization. According to the polarity of the subpellicular microtubules, this complex should move cargos towards the apical end of the parasites. We determined that the dynein heavy chain (DHC1) and the dynein light intermediate chain (DLIC) are both individually critical for the lytic cycle of the tachyzoite. Parasites depleted in either of these proteins are defective mostly in the invasion step. A deeper dissection of this step showed that microneme secretion was impacted in these parasites as well as the positioning of the rhoptries and their secretion. These phenotypes are reminiscent of the one described for the dynein light chain DLC8a (PMID: 31206964) which might be part of the same complex or the HOOK-FTS-HIP complex recently described (PMID: 37933960 and PMID: 37093045). By co-immunoprecipitation experiments, we are investigating the composition of the complex and aim to identify new adaptors and cargo proteins.

P014

Establishment of an *E. coli* model for studying the interaction and flexibility of apicoplast resident ferredoxin and bacterial flavodoxin redox systems towards IspH of the isoprenoid pathway

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The apicoplast ferredoxin (Fd) redox system of Apicomplexa and flavodoxin (fldA) redox system of bacteria serves as electron donors to aid the catalytic activity of enzymes of various metabolic pathways. One of the enzymes that benefit from the redox activity of both proteins in the respective organisms is the 4Fe-4S containing IspH, involved in the last

step of the MEP pathway for isoprenoid precursor synthesis, an essential pathway for survival in both, *Escherichia coli* and apicomplexan parasites harboring an apicoplast. We are interested in knowing whether these two structurally very different redox proteins can replace each other and if it would allow us to use *E. coli* as a surrogate for the apicoplast for drug screening purposes.
We report the complementation of *EcDIspH* by *Plasmodium falciparum* and *Toxoplasma gondii* IspH (*PfIspH* and *TgIspH*). Using homologous recombination, we deleted the essential *EcfldA* and introduced the mevalonate by-pass pathway to make the *E. coli* double mutant strain entirely dependent on mevalonate. Removing mevalonate in a simple growth assay allowed us to further use this strain as a model to show that whereas *Cyanobacteria (Nostoc)* flavodoxin complements EcMP2*, Chromera velia* flavodoxin does not, raising interesting evolutionary questions. Strain EcMP2 is entirely dependent on the simultaneous expression of plasmid-encoded PfFd, PfFNR, PfIspH, which shows that the three parasite proteins are active and required to replace the respective endogenous *E. coli* proteins. We have also used the model to test for likely residues at the interaction interface between *Pf*Fd and *Pf*IspH based on AlphaFold2 predictions and verified their importance for growth. Metabolomic studies are on the way to unravel the impact of the various complementation results on the MEP pathway. Altogether, we describe an engineered *E. coli* strain useful for functional and drug screening purposes for inhibitors of the essential MEP pathway. It will be very useful as a first screening platform for functional mutations or drug effects before more laborious and time-consuming assays in the parasite are performed.

P015
Deciphering the transport of heme intermediates in *Toxoplasma gondii:* Insights from comparative genome wide CRISPR screens

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Heme serves as a crucial cofactor for numerous enzymes, particularly those in the mitochondrial respiratory chain. *Toxoplasma gondii* can synthesize heme de novo through a biosynthesis pathway spanning the mitochondrion, apicoplast, and cytosol. Interestingly, the parasite can bypass heme synthesis by salvaging host cell heme intermediates, such as protoporphyrin IX (PPIX), which are elevated with excess 5-aminolevulinic acid (5-ALA)[1]. However, the mechanisms and transporters facilitating the parasite's access to PPIX or other heme intermediates remain elusive.
To identify these transporters, we conducted genome-wide CRISPR-based forward genetic screens, comparing wildtype and mutant parasites with defects in the cytosolic enzyme coproporphyrinogen III oxidase (CPO). Mutants were rescued by the addition of 5-ALA, facilitating the identification of transporters involved in heme intermediate salvage. While these transporters are non-essential in wildtype parasites, they become indispensable in heme synthesis-deficient mutants. Three ABC transporters identified from the screen are predicted to localize to the plasma membrane and mitochondrion. Our current efforts are focused on characterizing these candidate transporters to validate their localization and function. Given the limited understanding of metabolic needs during the chronic stage, we plan to employ an in vitro model system based on human myotubes capable of harboring mature cysts[2]. Through this model, we aim to evaluate the significance and function of the newly identified transporters during the bradyzoite stage.
[1]Krishnan A et al, Cell Host Microbe. 2020 Feb 12;27(2):290-306.e11.
[2]Christiansen C et al, Nat Commun. 2022 Mar 4;13(1):1168.

P016
Biochemical mutagenesis signatures systematically identify functional palmitoylation post-translational modifications in *Toxoplasma gondii*

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Apicomplexan parasites are obligatorily intracellular, with motility and invasion of host cells necessitating the discharge of a multitude of conserved proteins from the parasite"s apical organelles. Post-translational modifications (PTM) such as palmitoylation regulate the function of apical organelle-associated proteins. Palmitoylation has been shown to be critical for the parasite"s lytic cycle with inhibition of its addition and removal found to disrupt parasite motility and invasion1,2. Additionally, mutation of palmitoylated cysteines in proteins such as the *Toxoplasma* protein TgARO resulted in the disruption of rhoptry organisation and secretion3,4. Despite this knowledge, the full extent to which palmitoylation regulates organelle secretion is not fully understood. While the writers and erasers for this PTM are known and

palmitoylated proteins globally profiled, these datasets do not indicate which cysteines are palmitoylated5, and more importantly, which contribute to protein function.
We hypothesised that 1) palmitoylation is an important and general regulator apical organelle secretion, and 2) since enzymatic addition of palmitate onto cysteine thiols is chemoselective, should a palmitoylation event be functionally important, the target cysteine should be intolerant to mutagenesis.
We used CORe6 to generate saturated mutagenesis profiles, revealing biochemical signatures of functional palmitoylation modifications. We have tested the function of 38 predicted cysteine targets of palmitoylation distributed across 23 palmitoylated proteins, profiling a total of 798 individual amino acid substitutions in a single experiment, in live parasites, and in high throughput. These data have allowed us to identify candidate cysteine residues on apical organelle secretion-associated proteins that are tolerant and intolerant to substitution, confirming our hypothesis that biochemical mutagenesis profiles serve as signatures for functional PTMs.

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P017

Human toxoplasmosis in the Czech Republic 1923-2023: From the first case through a massive outbreak to a long-term decline

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When the Prague ophthalmologist Josef Janků in March 1922 noted and a year later described strange symptoms in a deceased newborn, he did not know that this would be the first documented case of toxoplasmosis in humans, moreover with congenital transmission and eye damage. Only the improvement of serological diagnostics revealed that toxoplasmosis was one of the most common serious parasitoses in the Czech Republic. The annual number of reported cases of clinical toxoplasmosis increased from one hundred in the early 1970s to 513-860 between 1983 and 1993. In 1994, the number of cases of clinical toxoplasmosis rose transiently to a total of 2056 cases as a result of an unusually widespread epidemic. Since then, the numbers have been steadily declining and have returned to one hundred cases in recent years.
The reasons for the decrease in the incidence of toxoplasmosis in the Czech Republic could be the gradual improvement of social and hygiene standards and education, control and supervision of food safety. The process of urbanisation has resulted in fewer outdoor activities. On the other hand, the growing popularity of organic food, organic farming and unconventional cooking practices could theoretically contribute to the spread of toxoplasmosis, as well as travelling to southern countries rich in stray cats.
However, seroprevalence data do not match such a significant decrease in toxoplasmosis. According to 37 studies performed on different groups of persons in the years 2002-2018, seropositivity in men can be found between 7.9-40.2% (average: 23,9%) and between 15.9-45,9% (average 28,9%) in women. This is not much less than the 26.3% of positive men and 34.1% of positive women found in surveys from 1971 to 1996. The declining reporting discipline associated with the skyrocketing administration can also be a problem.
Clinical toxoplasmosis in the past and present is reported twice as often in women as in men. The younger age categories predominate - men 10-24 years, women 20-34 years. The most common clinical forms are lymphadenitis (72.8%), ocular toxoplasmosis (6.0%), primary infection during pregnancy (5.4%), and congenital infection accounts for 0.6%.
The incidence is highly locally variable. The highest is in some districts of West Bohemia and Moravia, while the lowest number of cases is reported from Prague and the surrounding area.
Supported by MH CZ - DRO (The National Institute of Public Health – NIPH, 75010330)

P018

Exploring the Seroprevalence and Molecular Epidemiology of *Toxoplasma gondii* in Sheep Populations of Uruguay

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Toxoplasmosis poses a substantial risk as a globally prevalent foodborne zoonotic disease. This disease does not only impact public health, but also results in noteworthy economic losses for countries dependent on rural activities, particularly those associated to the livestock industry, particularly pronounced in sheep and goats, where reproductive failure is a prevailing consequence. With a history deeply rooted in sheep farming, Uruguay ranks as the fifth-largest global exporter of sheep meat and the third-largest exporter of combed wool. However, the reproductive performance of sheep flocks remains suboptimal in the country. Our previous studies unveiled a concerning proportion of abortions attributable to *Toxoplasma gondii* 27% among aborted fetuses/placentas subjected to laboratory diagnostic investigations to assess for abortion causality. However, the overall prevalence of the parasite and the genotypes infecting Uruguayan sheep flocks have not been investigated. In this study, we aimed to assess *T. gondii* seroprevalence in sheep using an in-house ELISA assay, elucidate the prevailing genotypes in sheep employing molecular and serological typing, and characterize the impact of genetic diversity on parasite phenotype. Our findings indicate that around 17% of sheep in Uruguay are seropositive for T. gondii, and there is notable genetic variability in T. gondii detected in aborted ovine fetuses. In addition, we detected a predominance of type III strains, along with atypical and previously unreported genetic types. Furthermore, isolation and characterization of two Uruguayan strains obtained from aborted sheep, named TgUru1 and TgUru2, revealed diversity in terms of virulence and the ability to cause chronic/persistent infections. Thorough genetic and phenotypic analysis of these strains, encompassing in vivo and in vitro assessments revealed that both exhibit markedly distinct phenotypes, with TgUru1 displaying high virulence and rapid growth, and TgUru2 exhibiting slow growth and high rates of spontaneous cystogenesis. These differences extend to in vivo studies and gene-specific developmental stage expression. We are currently delving into the genetic and transcriptomic underpinnings of these variations, aiming to comprehend their potential impact on disease outcome. Additionally, our research extends to exploring the correlation between our animal findings and the human population in Uruguay concerning the prevalent strain's genetic background.

P019

T Lymphocyte-Dependent IL-10 Protects Against a Lethal IL-12 Driven Cytokine Storm Initiated by *Toxoplasma gondii* GRA24

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A model organism in the study of immunity to infection, *Toxoplasma gondii* has been instrumental in establishing many key principles of host anti-microbial defense and its regulation. Here, we employed an attenuated uracil auxotroph strain of Type I *Toxoplasma* designated OMP to further untangle the early immune response to this parasitic pathogen. Experiments using T cell-deficient TCRβ$^{-/-}$ mice unexpectedly revealed that an intact αβ T lymphocyte compartment was essential to survive infection with this uracil auxotroph. Subsequent antibody depletion and knockout mouse experiments demonstrated contributions from CD4^{+} T cells and most predominantly CD8^{+} T cells in resistance to OMP. Using transgenic knockout mice, we found only a partial requirement for IFN-γ, and a lack of requirement for Toll-like receptor (TLR) adaptor MyD88 in resistance. In stark contrast to other studies on *Toxoplasma*, resistance to OMP did not require IL-12p40. Surprisingly, given the attenuated nature of OMP, T cell-dependent IL-10 was found to be critical for survival and deficiency of this cytokine triggered an abnormally high systemic inflammatory response. We also found that parasite molecule GRA24, a dense granule protein that triggers TLR-independent IL-12 production, acts as a virulence factor contributing to death of OMP-infected TCRβ$^{-/-}$ mice. Further, resistance against OMP was restored in TCRβ$^{-/-}$ mice via monoclonal depletion of IL-12p40, suggesting that GRA24-induced IL-12 underlies the fatal immunopathology observed. Collectively, our studies provide insight into a novel and rapidly arising T lymphocyte-dependent anti-inflammatory response to *T. gondii* which operates independently of MyD88 and IL-12, and that provides protection against the proinflammatory effects of GRA24.

P020

Toxoplasma gondii Harbors a Pathway to Synthesize Aspartate-family Amino Acids Otherwise Essential for its Human Host Cells

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The aspartate-family amino acids (threonine, methionine, isoleucine, lysine) are synthesized *via* five sequential enzymatic steps in plants, bacteria, and fungi but essential for mammalian host cells of *Toxoplasma gondii*. How the parasite satisfies its metabolic requirement for these amino acids is not understood. Herein, we report the occurrence of four proteins of this pathway in *T. gondii*, namely aspartate kinase (AK), aspartate semialdehyde dehydrogenase (ASDH), homoserine kinase (HK) and threonine synthase (TS). We show that ectopically expressed epitope-tagged AK, ASDH, HSK and TS localize in the parasite cytosol. We also show that the acutely infectious tachyzoite stage transcribes all mRNAs except for TS, but the respective endogenous proteins are barely detectable indicating a post-transcriptional regulation of the pathway. We demonstrated that TS is dispensable for the lytic cycle; however ectopic overexpression of TS enables the parasite growth without threonine, indicating its functional reconstitution in tachyzoites otherwise dependent on the host supply. Likewise, aspartate can rescue the TS-overexpressing strain in a threonine-depleted medium, implying its import from the milieu and functional restoration of the entire pathway in tachyzoites. Moreover, the yeast mutants auxotrophic for threonine can be physiologically complemented by ASDH and HSK. Finally, our extended work demonstrates that external supply of methionine, isoleucine and lysine is vital for parasite survival in human cells, suggesting the auxotrophy of *T. gondii* for these amino acids. In a nutshell, we discover the presence of a novel pathway in *T. gondii* to produce metabolites essential for its host cells and underline the metabolic plasticity of tachyzoites to survive in varied nutritional milieus.

P021

CellFlow: A comprehensive toolbox for the analysis of apicomplexan gene expression and chromatin dynamics using single-cell technologies

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The cell division cycle of *T. gondii* tachyzoites shows remarkable differences compared to higher eukaryotes. Most notably, the G2 phase is absent, and the S-phase and mitosis occur concurrently with the assembly of the new daughters. Moreover, gene expression in *T. gondii* tachyzoites is periodic and follows a “just-in-time” principle, such that the timing of mRNA expression is a proxy for the cell biological events - a typical feature in Apicomplexa. This periodicity imposes topological constraints on the gene expression data manifold, which is observable in low-dimensional PCA/UMAP projections as a twisted torus. To study cell cycle progression, trajectory analysis is typically performed on the data manifold. However, available tools for trajectory analysis do not account for periodicity, resulting in incorrect inference of trajectory flows. We have developed a Fourier Transform based approach for analyzing periodic trajectories, allowing us to effectively capture the flow of replicating cells. Moreover, we present a stochastic traffic model to study the cell cycle progression flow that enables the identification of phase transition points and cell cycle checkpoints. Together, these tools enable the reconstruction of time-series of pseudo-synchronized parasites in a pseudo-time scale, which can be transformed into a real-time scale. Our algorithms are packaged into a toolbox which is available for download as a Python library from GitHub, Pypi, and Bioconda. The library features interactive functionality for seamless processing and analysis. We demonstrate the utility of our tool by studying the cell asexual replication cycle of *T. gondii* using single-cell multi-omics data. The analysis identified previously known and potentially new transition points and revealed that chromatin accessibility and transcriptional activity display variation in peak timing, hinting at gene classes that are activated/repressed by potentially similar mechanisms.

P022

Cross linking mass spectrometry: A new tool for studies on the *Toxoplasma gondii* protein interactome

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Protein complexes within cells are fundamental to biological understanding, yet cataloging protein-protein interactions at the proteome level remains a significant challenge due to technical limitations of standard methodologies. This study evaluates the efficacy of crosslinking mass spectrometry (XL-MS) for large-scale identification of protein-protein interactions within *Toxoplasma gondii*. Utilizing the soluble fraction of tachyzoite lysates, we used a novel, MS-cleavable, and click-enrichable crosslinker, azide-A-DSBSO, alongside mass spectrometry optimization techniques including stepped-HCD coupled with FAIMS. This approach significantly enhanced the detection of crosslinks, yielding over 11,000 crosslink spectra, 3,000 unique residue-to-residue crosslinks, and 300 protein-protein interactions. Remarkably, the majority of these interactions corroborate with previously established hyperLOPIT data. Further analysis focused on the GRA1 protein, revealing a detailed interactome through immunoprecipitation combined with XL-MS. Crosslink mapping to existing crystal, cryo-EM, and AlphaFold structures confirmed that the distances between crosslinks predominantly align with theoretical expectations. Our findings underscore the utility of XL-MS as a powerful tool for elucidating the proteome-level interactome and extracting structural insights into proteins. This technique holds promise for investigating complex and elusive structures, such as cyst walls and dense granules, and for comprehensive interactome cataloging through the integration of hyperLOPIT and XL-MS.

[Funding: NIHAI134753]

P023

The *T. gondii* mitoribosome - build from tiny rRNA fragments expressed from mitochondrial genome recombination sites.

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Apicomplexan mitochondrial genomes are highly reduced comprising only three open readings frames (ORFs) encoding essential subunits of the oxidative phosphorylation machinery along with genes for ribosomal RNAs (rRNA). Intriguingly, the rRNA genes are not organized as continuous long units, as observed in most other organisms, rather, they are scattered as multiple small fragments throughout the mitochondrial genome. The organization of the *Toxoplasma gondii* mitochondrial genome is even more striking consisting of 23 sequence blocks that undergo non-random recombination with each other. Recent studies in our lab have unveiled the expression of 34 small RNAs in *T. gondii* mitochondria with majority of them having homology to rRNA fragments previously described in other Apicomplexans. Surprisingly, several of these small RNAs are encoded at genomic recombination sites leading to the intriguing situation that the sequence at block borders is dually used for different RNAs. We found several instances where small RNAs partially share sequence. In some even more remarkable cases sequence is dually used for a small RNAs and a messenger RNA. We wondered whether these peculiar small RNAs could function as ribosomal RNA, being integrated into mitochondrial ribosomes together with canonical rRNA fragments and ribosomal proteins. Thus, we established a density-based centrifugation approach to study translation in *T. gondii* mitochondria. For several mitochondrial small RNAs we observed migration into deep fractions of sucrose density gradients. Additionally, putative mitoribosomal proteins of the large and small ribosomal subunit as well as the three mitochondrial mRNAs have shown comparable migration patterns providing the first strong evidence for the presence of polysomes in *T. gondii* mitochondria. Among the small RNAs found in polysomal fractions are several RNAs that are encoded at genome recombination sites. Outstanding examples are RNA3 and RNA19 which partially share sequence with *coxI* and *coxIII* mRNA, respectively. Whether the dual sequence usage for rRNA fragments and mRNAs could be relevant for regulatory processes remains unclear and will be addressed in future experiments. Moreover, our density gradient centrifugation protocol offers a powerful tool for comprehensive studies on the composition and assembly of the *T. gondii* mitoribosome.

P024

Identifying novel proteins that function in the regulation of arginine uptake in *Toxoplasma gondii*

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Arginine is an essential nutrient for the proliferation of *Toxoplasma gondii* parasites. We have shown previously that parasites encounter different arginine concentrations as they infect different organs of their host, and that parasites respond by tightly controlling arginine uptake. They do this through regulating the expression of the major plasma membrane-localised arginine transporter, *Tg*ApiAT1. In arginine-replete conditions, *Tg*ApiAT1 expression is decreased, whereas *Tg*ApiAT1 abundance increases when arginine concentrations are lowered. Arginine-dependent *Tg*ApiAT1 regulation is mediated post-transcriptionally by an upstream open reading frame in the *Tg*ApiAT1 transcript. To better understand how parasites sense and respond to arginine availability in their environment, we have developed an arginine-sensitive fluorescence-based reporter strain of *T. gondii*. Using this strain, we have undertaken a flow cytometry-based, genome-wide positive selection screen to identify genes important for arginine-dependent regulation of *Tg*ApiAT1. This screen identified a candidate nucleolar RNA methyltransferase as being important for repressing *Tg*ApiAT1 expression when arginine is abundant. The screen also identified a putative RNA-binding protein and protein that may prevent ribosome stalling as being important for upregulation *Tg*ApiAT1 in arginine-limiting conditions. We are now developing approaches to understand the molecular mechanisms of how these proteins function in mediating arginine-dependent expression of *Tg*ApiAT1. Overall, our findings enhances our understanding of how a model intracellular pathogen responds to nutrient availability in its environment.

P025

Autophagy in CD11c^{+} cells is required for the development of protective immunity to *T. gondii* infection

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Autophagy is a lysosomal degradation pathway that maintains cellular homeostasis by sequestering cytosolic materials and targeting them to lysosomes. Proteins in this classical autophagy pathway are also important for many other cellular functions including phagocytosis, inflammatory signaling, control of intracellular pathogens, and antigen presentation. One cassette of these autophagy proteins, which includes the ATG5-ATG12-ATG16L1 complex, has been shown to be critical in targeting IFN-γ mediated effectors to the *T. gondii* parasitophorous vacuole (PV) to disrupt the PV and kill the parasite. Conditional deletion of any of the genes from this ATG5-ATG12-ATG16L1 complex in myeloid cells using LysM-Cre mice (LysM x ATG-flox) has shown this protein complex is required for infected macrophages and monocytes to kill the parasite and control acute *T. gondii* infection *in vivo*. To investigate the role of this autophagy protein complex in conventional dendritic cells (cDC) during *T. gondii* infection, ATG5-flox and ATG16L1-flox mice were bred with CD11c-Cre mice. Surprisingly, deletion of autophagy proteins in CD11c-Cre expressing cells also resulted in increased host susceptibility during the acute phase of *T. gondii* infection. Lineage tracing of CD11c-Cre expression showed that Cre expression is not isolated to cDCs and occurs in a subpopulation of myeloid precursors as well as in all inflammatory monocytes and macrophages. Deletion of the ATG16L1 protein was equivalent in both LysM-ATG16L1 and CD11c-ATG16L1 bone marrow-derived macrophages. Functionally, CD11c-ATG16L1 macrophages failed to localize the IFN-γ mediated effectors to the PV, which resulted in uncontrolled parasite replication. Additionally, ATG16L1 protein expression in CD11c^{+} cells is required for the development of a protective CD8^{+} T cell response following vaccination with the non-replicating *CPS* strain of *T. gondii*. While LysM-ATG16L1 and CD11c-ATG16L1 mice generate an impaired T cell response to *CPS* immunization, only CD11c-ATG16L1 mice succumb to rechallenge with the virulent RH strain of the parasite. Ongoing work is focusing on the mechanistic difference in the response to immunization between LysM-ATG16L1 and CD11c-ATG16L1 mice.

P026

Critical roles of an oxysterol-binding protein in lipid synthesis and trafficking in *Toxoplasma gondii*

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Lipids are essential components in all living cells with diverse roles in membrane structure maintenance, signaling, energy storage and many other fundamental cellular processes. *Toxoplasma gondii*, a ubiquitous zoonotic parasite infecting one third of the world"s human population, has complex pathways to synthesize variety of lipids. The enzymes involved in lipid

synthesis are localized in many different organelles that include the apicoplast, endoplasmic reticulum, mitochondrion, Golgi, and nucleus. The compartmentalization of the lipid synthesis pathways requires the transport of lipid precursors among different organelles and the delivery of the final lipid products to the correct destination. However, the underlying mechanisms are largely unknown. In cells, lipid transport may occur mainly through vesicular and non-vesicular mechanisms. Non-vesicular lipid transport requires lipid transfer proteins. Oxysterol-binding proteins (OSBPs) and their related proteins (ORPs) form a large family of lipid binding/transfer proteins. Some of the OSBP and ORP proteins have important roles in mobilizing lipids between different organelles. In this study, we have identified two OSBP proteins in *T. gondii*. Using genetic and cell biology approaches, we found that one of the OSBP protein (OSBP1) is critical for the asexual reproduction of tachyzoites while the other one is dispensable. Further analyses showed that OSBP1 is located at the membrane contact sites between the endoplasmic reticulum and the Golgi, and it played important roles in the synthesis and proper cellular distribution of a number of phospholipids, including the abundant phosphatidylinositol.

P027

Does T cell Exhaustion occur in pregnant women with chronic *Toxoplasma gondii* infection?

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We have recently reported finding significantly suppressed levels of inflammatory cytokines, IFN- γ , IL-6, IL-17, and IL-12 across pregnancy in women with *T.gondii* (TG) chronic infection (n=158) (Prescott S, et al. (2023). A mechanism that may be related to the reduced cytokines in latent infection is T cell exhaustion due to lifelong exposure of the immune system to parasite antigens. Latent, persistent, infections may continuously provide antigen exposure to T cell receptors (TCRs), leading to exhaustion of TCRs on antigen specific T cells. Pregnancy related fetal antigen exposure at the placenta also can produce T cell exhaustion. T cell exhaustion is marked by increased expression of inhibitory receptors on CD8 cells, loss of the ability to secrete cytokines, and development of different transcriptional programs. Exhaustion progresses over the time of continuous antigen exposure and is marked by cell signature immune checkpoint molecules. The cells most affected are the CD8 effector cells, which, in the case of TG, produce the proinflammatory milieu suppressing bradyzoite cysts. A loss of these CD8 effectors allows for the loss of memory responses to the antigens. Our data also suggested TG associated significant suppressed activation of the indoleamine 2,3-dioxygenase (IDO) enzyme, which is activated by inflammatory cytokines, and acts to reduce tryptophan availability to cysts. Both the immune and tryptophan/kynurenine effects could potentially cause low level parasitemia. Consistently lower levels of peripheral circulating inflammatory cytokines in the latently infected pregnant women suggest a global immunosuppression related to *T.gondii* and pregnancy induced T cell exhaustion. We are planning immunophenotyping and flow cytometry studies to determine if pregnancy associated TG latent infection produces T cell exhaustion.

Prescott, S., Mutka, T., Baumgartel, K., Yoo, J. Y., Morgan, H., Postolache, T. T., Seyfang, A., Gostner, J. M., Fuchs, D., Kim, K., & Groer, M. E. (2023). Tryptophan metabolism and immune alterations in pregnant Hispanic women with chronic *Toxoplasma gondii* infection. *American journal of reproductive immunology 90*(3), e13768.

P028

Insights into the formation of the *Toxoplasma* plant-like vacuolar compartment

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During intracellular replication, *Toxoplasma* ingests and digests proteins derived from the cytosol of infected host cells. These ingested host proteins are transported to a specialized lysosome-like organelle known as the plant-like vacuolar compartment (PLVAC). Disrupting protein degradation within the PLVAC or the export of degradative products significantly attenuates both the acute and chronic stages of *Toxoplasma* infection. The PLVAC is strikingly dynamic. Whereas the it is usually a single entity in extracellular or newly invaded parasites, PLVAC undergoes sequential rounds of fission (fragmentation) and fusion (reformation) during intracellular replication. However, molecular mechanisms underlying this dynamic behavior remain unknown. To address this, we isolated the PLVAC by organellar pulldown and identified

a Qbc-SNARE called TgSNAP29. Although TgSNAP29 was previously implicated in trafficking to the apicoplast, we found that TgSNAP29 predominantly localizes with the PLVAC and is essential for both the biogenesis of the PLVAC and parasite viability. Additionally, IP-MS/MS analysis of TgSNAP29 identified an R-SNARE protein, TgVAMP7, which also localizes to the PLVAC. Interestingly, although the PLVAC appeared normal in parasites lacking TgVAMP7, we found that these KO parasites markedly upregulated a closely related R-SNARE, TgVAMP8, suggesting a compensatory effect. Together these studies have opened a new avenue toward understanding how the PLVAC is formed and dynamically reformed during the parasite cell cycle. Future work will identify other novel players that are critical for PLVAC dynamics and biogenesis along with assessing the role of such processes during the chronic stage of infection.

P029

Determination of *Toxoplasma gondii*"s genetic variability in cases of maternal seroconversion during pregnancy from Uruguay

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The paradigm for transmission and manifestations of human toxoplasmosis arose based on clinical data and experimentation in mice using strains of *Toxoplasma gondii* circulating in Europe and North America known as "typical." However, we now understand that the severity of human toxoplasmosis depends on the gestational period upon which the infection is acquired, the parasitic load and genotype of the parasite, which is closely linked to its geographic origin. Worldwide genotyping studies describe a higher incidence of atypical strains in South America, but this concept is mainly based on data obtained in Brazil, Colombia, and Argentina. Pioneering work in Uruguay described the existence of "atypical" serotypes in patients and the isolation of a genetically atypical strain of Uruguayan origin. We hypothesize that there is ample genetic variability of circulating *T. gondii* strains in humans in our country. To address this, we genotyped *T. gondii* strains in women who acquired the infection during pregnancy, as determined by the patient's seroconversion, detectable during routine pregnancy checkups. We amplified parasite DNA from peripheral blood, placental tissue, umbilical cord blood and newborn babies peripheral blood. T. gondii DNA was detectable in 17 of 32 patients. Positive samples were genotypes using in silico PCR-RFLP and sequence analyses, of nine polymorphic genetic markers. The presence of "atypical" strains was evidenced in 7 samples. We are currently advancing in their genetic characterization and pursuing the isolation of circulating strains with the aim of correlating genotype with phenotype. Preliminary data suggests that the severity of ocular toxoplasmosis is linked to the genotype. In addition, we updated the epidemiological data on *T. gondii* prevalence for the general population in the country, extrapolating from serological data obtained from pregnant women, and determined the seroconversion rates during pregnancy for the studied population.

P030

The role of NETosis and ROS production in human neutrophil-mediated host defense against *Toxoplasma gondii* infection

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Toxoplasma gondii has the remarkable ability to infect and replicate in neutrophils. These innate immune cells are rapidly recruited to sites of *T. gondii* infection and, at first glance, would appear to be an inhospitable option for an intracellular parasite since neutrophils have several antimicrobial effector mechanisms. Nevertheless, studies have shown that, in *T. gondii*-infected hosts, a high proportion of neutrophils in the intestine and lymph nodes contain replicating parasites. Despite evidence of neutrophil contributions to immunity against *T. gondii*, the interactions of *T. gondii* with neutrophils are still poorly understood and have been primarily studied in the mouse. Here, we differentiated the human promyelocytic cell line HL-60 into neutrophil-like cells (NLCs) and focused on characterizing two neutrophil effector functions during acute *T. gondii* infection: the release of neutrophil extracellular traps (NETs) and the production of reactive oxygen species (ROS). NETs are web-like chromatin structures that can trap and kill pathogens in the extracellular space. We first observed

the presence of extracellular DNA in *T. gondii*-infected neutrophil cultures by using Sytox Orange, a cell-impermeable dye. Next, we confirmed that the increase in Sytox fluorescence signal was due to NETs, as opposed to merely cell death, by staining the cells with an anti-citrullinated histone 3 antibody. We also demonstrated that neutrophils remain viable after undergoing NETosis, as assessed by staining the cells with a live dye and performing a cytotoxicity assay measuring lactate dehydrogenase (LDH) activity. Our current experiments are characterizing the release of DNA through vesicles, as opposed to membrane lysing, and the effectiveness of NETosis in killing *T. gondii* parasites. In parallel, we were able to observe the production and release of ROS from *T. gondii*-infected NLCs. Analysis of assembling and activation of the NADPH oxidase suggested that *T. gondii*-induced ROS in neutrophils is mainly dependent on NOX2 activity. Our current experiments are focused on characterizing the cellular events that lead to ROS production, as well as the contributions of it to other effector functions. Together, these findings expand our understanding of the neutrophil-mediated host defense against *T. gondii* and contribute to characterizing the very early immune events that affect the progression of the host response and the disease.

P031

Identification and function analysis of novel anti-host immunity factors of Toxoplasma

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Toxoplasma gondii forms a parasitophorous vacuole (PV) upon invading a cell. The host adopts a cell-autonomous response driven by interferon-gamma (IFN-γ), in which interferon-inducible proteins lead to parasite growth inhibition and clearance within the vacuole. Highly virulent strains of *T. gondii* are known to evade this anti-*T. gondii* IFN-γ-dependent host defense; however, the molecular mechanisms are incompletely understood. Through *in vivo* CRISPR/Cas9 screening, we identified candidate genes associated with *T. gondii* virulence and resistance to the IFN-γ-dependent host defense. Among these candidates, we selected genes encoding non-secreted proteins linked to fundamental cellular processes; apart from rhoptry and dense granule proteins, to assess their influence on virulence. Notably, parasites lacking the deubiquitinase txDUB1 or DNA repair protein 1(txDRP1) exhibited significantly lower virulence than wild-type parasites infecting in wild-type mice, while being comparable to wild-type parasites infecting in mice lacking the IFN-γ receptor. Collectively, these data suggest that txDUB1 and txDRP1 are important for resistance to IFN-γ-dependent anti-*T. gondii* host defense. We aim to further explore their molecular functions in detail.

P032

First report of isolation of *Toxoplasma gondii* from Franciscana dolphin (*Pontoporia blainvillei*) in the South Coast of State of Santa Catarina, Brazil

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Toxoplasma gondii infects several species of marine mammals, including wild and captive ones, in countries of America, Europe, and Asia. The infection of these animals, which act as sentinels for the presence of *T. gondii* and other agents, indicates contamination of the marine environment by oocysts. This study aims to report the isolation of *Toxoplasma gondii* in a “Porpoise” (*Pontoporia blainvillei*) collected after death in the municipality of Laguna, South Coast of State of Santa Catarina, Brazil. A necropsy was performed on a juvenile, sex undetermined (possibly due to the action of scavenger birds), by the Santos Basin Beach Monitoring Project (section 1). Heart, lung, brain, and liver samples were processed in a pool for enzymatic digestion and use in the bioassay in mice. The digestion product was subcutaneously inoculated into three mice (1 mL each), which were observed daily for the evaluation of clinical signs compatible with *T. gondii* infection and then euthanized. Serum, organs (brain, heart, and lung), and peritoneal lavage were collected from euthanized mice to detect antibodies (Indirect Immunofluorescence Reaction – IFAT; cut-off 1:16), cysts and tachyzoites, respectively. Brain fragments were analyzed (squash) for the search for cysts and lung imprint and peritoneal lavage searching for tachyzoites. The three mice died at 21 days after inoculation and presented tachyzoites in the peritoneal lavage and lung imprint. Two of the three mice had antibodies against *T. gondii* with titers of 1:64 and 1:256. To the authors’ knowledge, this is the first report of isolation of *T. gondii* in *P. blainvillei*. T.he diet of *Pontoporia blainvillei* is based on eating fish such as anchovies and sardines, which have the characteristic of bioaccumulating molecules, among them, oocysts. In addition, these species of fish are also used in human food. This fact highlights the importance of determining *T. gondii* infection in marine mammals, which can act as indicators for the risk of infection in humans.

P033

Cloning and expression of the *rop18* from *Toxoplasma gondii* in plasmid vectors

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Toxoplasma gondii is an obligate intracellular protozoan parasite that infects mammals and birds, including humans. The ROP18 protein was described as an important virulence factor of the parasite, which may make it a potential candidate for protection against this parasite. The present study aimed to clone, sequence, and express the ROP18 gene in plasmid vectors. The partial fragment of *rop18* was amplified by PCR, and the DNA was cloned into vectors pcDNA 3.1-TOPO and pTrcHis-TOPO. Subsequently, they were transformed into *Escherichia coli* TOP10 cells. The confirmation of the reading frame of the gene in the plasmids was performed by sequencing. The obtained vectors pcDNA-rop18+were transfected into VERO cells, which were fixed on slides and submitted to the technique of indirect fluorescent antibody test (IFAT) with anti-histidine, and pTrcHis-rop18+ were transformed in *E. coli* BL21 to evaluate the expression of the protein. After expression in *E. coli* BL21, the recombinant rROP18 proteins were purified on a Ni-NTA column under denaturing conditions and visualized on an SDS polyacrylamide gel (42 kDa) with a concentration of 230 µg/ml. A reaction with anti-histidine was observed in Vero cells transfected with pcDNA-rop18+. In conclusion, the rop18 partial gene was successfully cloned and expressed in Vero cells and in *E. coli,* producing a protein under insoluble conditions, and the characteristics of the hydrophilic antigen suggest its use for studies of vaccine development of recombinant proteins or DNA and tests for diagnosis.

P034

DNA VACCINE ENCODING THE ROP18 GENE OF *Toxoplasma gondii* AGAINST OOCYSTS SHEDDING IN DOMESTIC CATS

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The present study aimed to evaluate the immunogenicity of a DNA vaccine encoding rop18-*Toxoplasma gondii* gene against oocyst shedding in domestic cats. The pcDNA-rop18+ vector under the control of cytomegalovirus enhancer/promoter and intron A was constructed by molecular cloning technique. The antigen expression and immune response were evaluated by inoculation of these recombinant plasmids in cats. Five domestic cats (Felis catus) were divided into two groups G1 (n=3), and G2 (n=2), animals from G1 received 25 µg of pcDNA 3.1+rop18, and G2 received 25 µg of pcDNA 3.1 TOPO (control group), both immunizations were performed by intramuscular route with four doses every 3 weeks plus levamisole(1%). The challenge was performed in all animals on day 93 with 300 tissue cysts of ME49 *T. gondii* strain by a gastric tube. Animals in all groups had their feces examined and the number of oocysts was determined for 20 days after the challenge. Animals from G1 shed fewer oocysts (≈40% less) than control groups. Elisa was used to detect anti-rROP18 IgG before and after the challenge, despite the statistical difference in antibody response of groups before the challenge, there was no correlation between oocysts shed and antibody levels. In conclusion, there was a perspective to use ROP18 as a vaccine candidate for *T. gondii* in cats.

P035

EVALUATION OF THE IMMUNE RESPONSE AGAINST THE FORMATION OF TISSUE CYST OF *Toxoplasma gondii* IN PIGS IMMUNIZED WITH DNA+sag1+rop18 VACCINE.

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Toxoplasma gondii is an obligatory intracellular protozoan that affects all homeothermic animals and humans. Pork-containing cysts are considered one of the most important routes of transmission to humans. Vaccination of domestic animals, aiming to reduce the number of tissue cysts, may be one of the strategies for controlling *T. gondii*. The objective of the work was to evaluate the animals' immune response and protection against the formation of tissue cysts in pigs immunized with a DNA vaccine encoding sag1 and rop18. Twelve crossbreed pigs were used, divided into three

groups (n=4), animals from G1 received pcDNA 3.1 + sag1+ rop18 (300µg) intramuscularly, while animals from G2 (n=4) received pcDNA 3.1 + sag1+ rop18 (300µg) via the intradermal route, and the G3 animals received only pcDNA 3.1 (300µg, two animals via the intramuscular route and two via the intradermal route). Four doses were made every 14 days, and blood samples were collected for Enzyme Immunosorbent Assay (ELISA) at the same dose interval, 15 days after the challenge and on the day of euthanasia. Fourteen days after the last dose, the challenge was carried out with 3x103 oocysts of strain ME49 orally. Thirty days after the challenge, the animals were euthanized and a pool of muscles (masseter, tongue, heart, and diaphragm) were used for bioassay in mice. One animal from G2 died before the challenge due to other causes. The bioassay showed that 6/12 (50%), 6/9 (66.6%), and 10/12 (83.3%) of the mice were positive for groups G1, G2, and G3, respectively, and the mean number of brain cysts was 513; 586; 634; for G1, G2, and G3, respectively. There was no statistical difference between G1 x G2 (p=0.34), G1 x G3 (p=0.22), and G2 x G3 (p=0.37). Although there are no statistical differences, effectiveness in reducing the number of tissue cysts by 38% in G1 and 20% in G2 was observed. The ELISA demonstrated an increase in the humoral immune response before the challenge for IgG and IgM, with a better response for the group immunized via the intramuscular route. These data show the importance of studying possible *T. gondii* proteins as antigens to achieve adequate protection against the formation of tissue cysts in pigs, one of the main routes of transmission of this zoonosis to humans.

P036

Activity and efficacy of the bumped kinase inhibitor BKI-1708 *in vitro* and in non-pregnant and pregnant toxoplasmosis and neosporosis mouse models

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Toxoplasma gondii and *Neospora caninum* are major worldwide morbidity-causing pathogens. Among the compound classes being currently developed against apicomplexan parasites, bumped kinase inhibitors (BKIs) – optimized to target the apicomplexan calcium-dependent protein kinase 1 (CDPK1) – proved to be safe and highly active *in vitro* and *in vivo*. The structure of BKI-1708 is based on the same central scaffold as BKI-1748, which showed proven *in vitro* activity and *in vivo* efficacy against both *N. caninum* and *T. gondii*. When applied *in vitro* concomitantly to infection, BKI-1708 exhibits IC50 values of 120nM for *T. gondii* and 480nM for *N. caninum* and does not affect HFF at concentrations up to 20µM. However, electron microscopy and immunofluorescence established that exposure of tachyzoite-infected cells to 2.5µM BKI-1708 *in vitro* induces the formation of multinucleated schizont-like complexes (MNC) - characterized by continued nuclear division and enclosing intracellular zoites lacking the outer plasma membrane. These zoites are unable to finalize cytokinesis. Treatments of zebrafish (*Danio rerio*) embryos during the first 96h following egg hatching showed that BKI-1708 did not affect early embryo development at concentrations up to 2µM. Treatments of mice with BKI-1708 at 20mg/kg/day during five consecutive days resulted in plasma levels ranging from 0.14 to 4.95µM, establishing an average threshold bellow 2µM. *In vivo* efficacy was evaluated by applying the compound at 20mg/kg/day from day 9-13 of pregnancy in mice experimentally infected with *N. caninum* (NcSpain-7) tachyzoites or *T. gondii* (TgShSp1) oocysts. This resulted in significantly decreased cerebral parasite loads and reduced vertical transmission in both models without drug-induced pregnancy interference. Thus, BKI-1708 is highly efficacious and pregnancy-safe in these mouse models, and certainly suitable for further trials.

P037

How the diagnostic and epidemiological management of selected infectious diseases is organized in Germany – the German Consulting Laboratory for Toxoplasma as an example for national network activities

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In Germany, National Reference Centers (NRC) and so-called Consulting Laboratories (CL) are appointed by the Robert Koch Institute (RKI) for defined infectious diseases or pathogens. The catalog of tasks focuses on advisory skills, epidemiological investigations and diagnostic services that go beyond routine.
The German Consulting Laboratory for Toxoplasma (CL-Toxo) answers an annual number of 250-300 medical requests and provides specialized diagnosis for 800-900 clinical samples. Requests and clinical samples come from all regions of Germany. Questions regarding the management of pregnancy-relevant *Toxoplasma* infections are currently in the focus of medical requests. They have become the basis for the creation of corresponding recommendations and guidelines established by the CL-Toxo together with the RKI and the Working Group for Obstetrics and Prenatal Diagnostics.
Surveys and epidemiological studies performed by the CL-Toxo together with partners (e.g. RKI, ToxoNet, DGHM) show that (i) seroprevalence rates are higher in East-German states, (ii) genotype II of *Toxoplasma gondii* predominates, and (iii) congenital infections are rare with fewer than 20 cases discovered annually in Germany. In addition, therapy initiated early can significantly reduce transmission from the mother to the child and symptomatic progression (<20% versus 70%). Amniocentesis and PCR detection of *T. gondii* only play a minor role in Germany.
In order to improve diagnostics, the CL-Toxo has developed, for example, the CGMC test to improve the detection of prenatally acquired infections, as well as recombinant antigens (e.g. SUB1) for general serological diagnosis.
In keeping with the network idea, besides the above-mentioned partners the CL-Toxo is currently involved in activities of the German Maternity Protection Committee and the Toxoplasma working group of the Paul-Ehrlich-Society.

P038

TgGloL is an atypical glyoxalase domain-containing apicoplast protein that is important for the growth of Toxoplasma

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Glycolysis is a conserved metabolic pathway that typically converts glucose into pyruvate in the cytosol, generating ATP and the reducing agent NADH. Like in plants, in *Toxoplasma gondii* and several other apicomplexan parasites some cytosolic glycolytic enzymes also have isoforms which are located in their plastid (called the apicoplast in apicomplexans). Noticeably, the glycolytic intermediates glyceraldehyde 3-phosphate (GA3P) and dihydroxy acetone phosphate (DHAP) are imported from the cytosol and metabolized further by these glycolytic enzymes in the apicoplast, presumably to provide ATP and reducing power, but also to generate precursors for anabolic pathways like isoprenoid synthesis. However, GA3P and DHAP can spontaneously convert into methylglyoxal, a highly reactive and very cytotoxic by-product. Methylglyoxal is detoxified principally via the glyoxalase system which consists of the enzymes Glyoxalase-1 (Glo-1) and Glyoxalase-2 (Glo-2). In addition to the canonical Glo-1 and Glo-2 present in the cytosol of *T. gondii*, we have identified an atypical protein that contains a Glo-1-like motif but has a very limited overall homology with typical Glo enzymes. We have shown that this protein localizes to the apicoplast and by generating a conditional knock-down cell line, we have demonstrated that it is important for optimal parasite growth. These results suggest that there might be a specific system for detoxifying derivatives of glycolytic intermediates in the apicoplast, and that this system is important for maintaining parasite fitness.

P039

Role of the hypothetical protein, TGME49_207210 in bradyzoite differentiation

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Toxoplasma gondii naturally causes a lifelong, asymptomatic infection of the central nervous system (CNS) in immunocompetent humans and rodents. In the CNS, *T. gondii* establishes a chronic infection by converting to slow-growing

bradyzoites that encyst. Consistent with prior studies, we have observed strain-specific differences in encystment, with Pru (type II) parasites encysting faster and in higher numbers than CEP (type III) parasites in pH-stressed fibroblasts, in human and murine neurons, and *in vivo*. To use these strain-specific differences in the encystment to identify bradyzoite-specific genes, we performed RNAseq on murine neurons infected with Pru or CEP parasites. We identified 502 *T. gondii* genes that showed more than 2-fold upregulation in Pru compared to CEP. We focused on defining the role of TGME49_207210 (TLO) in encystment because it was one of the top 10 upregulated genes in Pru, had been identified in other studies of bradyzoite genes, had a fitness score of 0, and was predicted to have BFD1 binding sites. We generated a CRISPR-mediated deletion of TGME49_207210 (TLO) in Pru and confirmed that PruΔ*207210* had no plaque or growth defect in human foreskin fibroblasts. Conversely, in primary murine neurons, PruΔ*207210* had a 50+% reduction in encystment even at 3 days post-infection; complementing the gene back rescued the encystment defect to wild-type levels. *In vivo*, PruΔ*207210* showed no differences in the CNS parasite burden (Q-PCR for B1), cyst size, or cyst numbers at 3 weeks post-infection (wpi) but all three measures were decreased at 8 wpi compared with wild-type Pru. Overall, these data highlight how strain-specific differences can be used to gain insights into chronic infections. Our current work is focused on generating tagged-complemented strains so that we can mechanistically define the cellular and molecular function for this gene.

P040

Exploiting Host Endoplasmic Reticulum: *Toxoplasma gondii*'s Strategy for Amino Acid Acquisition and Brain Persistence

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Toxoplasma gondii, a widespread parasite, has the ability to infect nearly any nucleated cell in warm-blooded vertebrates. Approximately 2 billion people worldwide are estimated to have been infected by this pathogen. Although most healthy individuals can effectively control parasite replication, certain parasites may evade the immune response, establishing cysts in the brain that resist both immune reactions and available drugs. For its chronic persistence in the brain, the parasite relies on host cells' nutrients, particularly amino acids and lipids. The current recommended therapy for toxoplasmosis has severe adverse effects and targets only acute forms of the parasite. Therefore, understanding how latent parasites persist in the brain is crucial for identifying potential drug targets against chronic forms. While shielded within parasitophorous vacuoles (PVs) or cysts, *Toxoplasma* has developed mechanisms to exploit the host endoplasmic reticulum (ER) metabolism, acquiring amino acids to sustain brain persistence, resulting in host neurological alterations. In this study, we demonstrate that *Toxoplasma gondii* disrupts host ER homeostasis, leading to unfolded protein accumulation in the host ER lumen. In response to this stress, the host activates an autophagic pathway called ER-phagy to alleviate the stress. Remarkably, by restricting amino acids in cell culture or during latent infection in mice, we successfully decreased the persistence of latent forms and restored behavioral changes in mice caused by the infection. Our findings unveil the underlying mechanisms employed by *Toxoplasma gondii* to exploit host ER and lysosomal pathways, enhancing nutrient levels during infection. These insights provide new strategies for the treatment of toxoplasmosis.

P041

Assessing the impact of preventative measures to limit the spread of *Toxoplasma gondii* in wild carnivores of Madagascar: A modeling study

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Novel multi-host pathogens can threaten endangered wildlife species alongside impacts on human and domestic animals. The zoonotic protozoan parasite *Toxoplasma gondii* is transmitted by members of the Felidae family and can infect a large number of animal species, including humans. This parasite can cause significant health consequences to infected intermediate hosts and has the potential to further endanger the wild carnivoran populations of Madagascar. Building on an empirical characterization of the prevalence of the pathogen in local mammals, we use mathematical models of pathogen transmission in a multi-host community to compare different preventative measures that aim to limit the spread of this parasite in the wild carnivoran population. Specifically, we study the effect of hypothetical cat vaccination

and population control campaigns on reducing the risk of infection by *Toxoplasma gondii* in wild Eupleridae. Our model predicts that the prevalence of exposure to *Toxoplasma gondii* in cats would be around 72%; while in rodents and wild carnivores, seroprevalence would reach 2% and 43% respectively. Reducing the rodent population in the landscape by half may only decrease the prevalence of *T. gondii* in carnivores by 10%. Similarly, cat vaccination and reducing the population of definitive hosts have limited impact on the prevalence of *T. gondii* in wild carnivorans of Madagascar and a significant reduction in prevalence would require extremely high vaccination and/or low turnover in cat population. Our study provides insights on potential control methods of *T. gondii* in endangered Eupleridae while highlighting the challenges of eliminating this threat entirely.

P042

Isolation and analysis of *Toxoplasma gondii* from a Japanese patient with congenital toxoplasmosis

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We have isolated, whole genome-sequenced, and compared *Toxoplasma gondii* strains from Japan and found that *T. gondii* in Japan has evolved in a unique manner (Fukumoto et al. (2020), Matsuzaki et al. *in preparation*). In this study, we have succeeded in isolating a Japanese strain of *T. gondii* from congenital toxoplasmosis and report the results we have been able to analyze to date.

Isolation was attempted using spinal fluid collected from a 9-day-old patient child. Anti-*T. gondii* antibody was observed during the mother's pregnancy, and the child had intracranial calcification, enlarged ventricles, bilateral choroiditis, and was positive for anti-*T. gondii* IgM. There was no history of international travel by the mother since one year prior to pregnancy. PCR testing was performed on the collected specimens to confirm positivity, and the remaining specimens were inoculated into mice previously treated with dexamethasone as part of the bioassay. Two weeks after inoculation, abdominal lavage fluid was collected and added to human foreskin fibroblast cultures, resulting in the successful establishment of a parasite strain. During this period, the same parasite strain was maintained in mice, but its virulence to mice seemed to be weak. Genomic DNA was purified from the obtained strain and the entire genome sequence was determined using Ilumina Hiseq. Comparison of the obtained sequence with known genomes of isolates from all over the world suggests that this isolate is not a unique genotype found in Japan, but is identical to Type II, which is widely recognized in the United States and Europe. We are currently conducting more detailed analyses of the whole genome sequence and pathogenicity against mouse.

P043

To Per-Cyst or Not: Unravelling the Secrets Behind an Attenuated *Toxoplasma* Strain

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The only commercial vaccine against *Toxoplasma gondii* is the attenuated S48 strain (Toxovax®) used to protect pregnant ewes from congenital toxoplasmosis. Our research aims to understand the genotypic and phenotypic mechanisms governing S48›s attenuation, with potential implications for identifying novel genes crucial to parasite persistence and uncovering new therapeutic targets. Furthermore, understanding the genetic basis of *T. gondii* attenuation can inform the development of *Toxoplasma* as a protein delivery vehicle, in both humans and animals.

We show S48 has an incomplete differentiation phenotype *in vitro*, where it is not able to fully differentiate into the persistent bradyzoite form. This informed our search for identifying genetic factors responsible for its attenuation. We assembled a high-quality genome of the S48 strain using Oxford Nanopore long reads and DNBseq short reads. SNP calling revealed over 500,000 total mutations, from which we identified nine key predicted loss-of-gene-function mutations unique to S48 when compared to five other normally differentiating Toxoplasma strains.

From these nine genes, we selected three genes with unknown functions to knock out and tag in the cystogenic ME49 strain. We aim to investigate the roles these genes play in bradyzoite differentiation and elucidate the functions of these

previously uncharacterized genes. Among the nine genes, two have been shown to have a significant involvement in bradyzoite differentiation. We plan to use Cosmids to restore the function of these two genes in S48 and evaluate their impact on both bradyzoite differentiation and viability. The initial characterization of all genetically modified strains will primarily focus on discerning the effects of gene knockouts on parasite differentiation, including cyst formation and the expression of bradyzoite genes.
Simultaneously, we aim to use RNA sequencing to understand the stage conversion progression from tachyzoites to bradyzoites in S48, compared to ME49.

P044

The *Toxoplasma* PQ-loop protein TgMPDU1 is essential for efficient parasite C-mannosylation

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N-glycosylation, GPI anchoring, and C-mannosylation are essential post-translational protein modifications that all contain mannose residues transferred from the donor Dolichol-phosphate-mannose (DPM) within the endoplasmic reticulum (ER). DPM is initially generated on the cytosolic side of the ER by transferring mannose from GDP-mannose to the lipid dolichol-phosphate, and then flipped to the ER lumen. Despite more than three decades of research, the mechanism responsible for the luminal translocation of DPM has not been uncovered, although some evidence suggests that a lipid scramblase may be the basis of this process. MPDU1, a member of the PQ-loop protein family resident in the ER, is potentially involved in the translocation reaction of DPM, perhaps being the elusive scramblase, but definitive and convincing evidence supporting this role is lacking. Human MPDU1 loss of function leads to Congenital Disorders of Glycosylation type I f (CDG-If) due to defective biosynthesis of N-glycosyl moieties and GPI anchors and loss of tryptophan C-mannosylation. Understanding the precise role of MPDU1 may open new avenues for developing therapies to control CDG-If. In this study, we identified a putative MPDU1 homologue in *T. gondii*, named TgMPDU1, which localizes to the ER. Ablation of TgMPDU1 resulted in the loss of C-mannosylation and a decrease in parasite fitness. N-glycosylation was not altered in the TgMPDU1 knock-out strain (ΔMPDU1) because, unlike mammals, N-glycosylation in *Toxoplasma* does not require mannose in the ER lumen. GPI anchor formation was defective only when ΔTgMPDU1 parasites were grown in conditions at reduce levels of DPM. However, C-mannosylation seemed to be more dependent on MPDU1 activity, as it was defective even when ΔTgMPDU1 parasites were cultured under normal conditions. Overall, these results shed new light on the function of MPDU1, suggesting that this protein might play a role in increasing the efficiency of using DPM as a mannose donor rather than being the scramblase responsible for flipping DPM from the ER cytosol side to the lumen.

P045

Exploring infection dynamics of virulent and non-virulent strains of *Toxoplasma gondii*

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Objective: *Toxoplasma gondii* is a highly successful parasite of veterinary and medical importance. Disease outcome can depend on many factors, including host background and virulence of the infecting strain. This work aimed to compare infection dynamics between mouse virulent and non-virulent strains of *T. gondii* in different host cells, and examine host and parasite responses during infection using transcriptomics.
Methods: Intestinal epithelial cells (IEC) from mice and humans were infected with 2×10^5 tachyzoites from 5 different *T. gondii* strains of differing mouse virulence (virulent TgCatBr71 and TgCkStK9; non-virulent TgCatBr60, TgWildBrMG6 and TgRhHmBr1), and cells were either fixed at 24 hpi for microscopy (invasion assay) or DNA was extracted for parasite quantification at 24, 48, 72 and 96 hpi (proliferation assay). Dual RNA-Seq of *T. gondii*-infected mouse and human IECs were sequenced by DNBSeq (BGI) at 24 and 72 hpi. DNA was extracted from TgCkStK9, TgRhHmBr1, and TgWildBrMG6

for short-read WGS by DNBSeq, and long-read WGS by Pacbio HiFi sequencing.
Results: The invasion assay demonstrated a statistically significant interaction between parasite strain and host (p=0.005), with the highest number of infection events being recorded for TgCkStK9. The level of parasite invasion for each strain was comparable between mouse and human hosts, with the exception of TgCkStK9, which showed statistically significantly higher invasion of human cells (p=0.033). The proliferation assay demonstrated statistically significant effects of strain and host (p< 0.001), with TgRhHmBr1 estimated to replicate at the fastest rate compared to the other strains when averaging across human and mouse cells. RNA-Seq analysis revealed that hundreds of genes were differentially expressed (DEGs) between strains. Long-read sequences of TgCkStK9, TgRhHmBr1, and TgWildBrMG6 were assembled and polished. The comparison revealed that while TgCkStK9 and TgWildBrMG6 exhibited no structural variants compared to the RH88 reference genome, TgRhHmBr1 displayed a structural variant.
Conclusions: This study highlights the role of host background in parasite virulence and indicates that the virulence of *T. gondii* in mice may not correlate with that in humans. RNA-Seq revealed DEGs between strains. High-quality precision genome assemblies were produced to aid in future research, which includes screening for potential virulent markers and conducting a comparative genome analysis.

P046

In vitro evaluation of natural compounds extracts from *Tabebuia* against *Toxoplasma gondii* proliferation

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Tabebuia is a large flowering tree genus widely used in traditional medicine in Colombia. We obtained methanol, n-hexane, chloroform, ethyl acetate, n-butanol, and water extracts from the leaves and stems of *Tabebuia rosea* and *Tabebuia chrysantha* collected on the Campus of the Universidad Tecnológica de Pereira in May 2021, under permission number 1133/2014, issued by the National Environmental Licensing Authority 431 (Autoridad Nacional de Licencias Ambientales - ANLA) in Colombia. The *in vitro* anti-*Toxoplasma* activity of triplicates of increasing concentrations (6.25, 12.5, 25, 50 and 100 mg/ml) from 17 extracts was evaluated using the β-galactosidase colorimetric assay to obtain half-maximal inhibitory concentration (IC50). The cytotoxicity for human cells was assayed by an Alamar Blue assay with 0.5 mM resazurin in non-cancer human foreskin fibroblast cell culture –HFF (ATCC SCRC-1041) in T-25 flasks until 100% of cell confluence. Host cell viability was determined by comparing treatments with no compound treatment at 100% viability. The median toxicity dose (TD50) on HFF cells was obtained, therapeutic index (TI) was calculated by TD50/IC50. We found four extracts that showed significant inhibitory effects on the proliferation of *T. gondii*: one from the leaves of *T. rosea* (IC50:6,8, TI: 7,3) and three from *T. chrysantha*, two from the inner bark, one extracted with chloroform (IC50:3,4; TI: 41,7) and one with hexane (IC50:74,8; TI: 54), and one from leaves after extraction with chloroform (IC50:5,6; TI: 4,4). Our study successfully identified components from native *Tabebuia* species with promising anti-*Toxoplasma* activity.
Funding: Sistema General de Regalías de Colombia (BPIN 2020000100077).

P047

Treatment with BKI-1748 after *Toxoplasma gondii* systemic dissemination in experimentally infected sheep significantly improves clinical outcome and prevents congenital infection

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Drug development for congenital toxoplasmosis is challenging since first line antifolate therapy has a high rate of adverse effects. Besides, several studies in humans highlight the importance of drug application early after seroconversion to reduce fetal damage. Calcium-dependent protein kinase 1 (CDPK1) of apicomplexan parasites represents a promising drug target and bumped kinase inhibitors (BKIs) inhibit the activity of CDPK1 and perhaps other targets in *Toxoplasma gondii*. BKI-1748 displayed an excellent safety profile at the therapeutic level and efficacy features *in vitro* (parasitostatic effect) and in *T. gondii* infected mice. For ovine toxoplasmosis, full protection against abortion and congenital infection has been demonstrated with BKI-1748 treatment beginning already at 2 days post-infection (pi). However, the efficacy of the compound applied later after infection (when signs of infection are recognized) has not been investigated so far and it may better reflect real-world use of a therapeutic. Nineteen pregnant sheep were distributed in three experimental groups. Group 1 (G1, n=8) and group 2 (G2, n=8) were dosed orally with 10 TgShSp1 sporulated oocysts at 90 days of gestation (dg). Animals from group 3 (G3, n=3) were mock dosed with PBS at 90 dg. In G1, ten doses at 15 mg/kg of BKI-1748 was administered every other day from day 7 pi (fever peak) onwards. Treatment reduced parasite proliferation in G1 compared to G2 since treated animals exhibited significantly lower rectal temperatures (on days 8 and 9 pi, $p<0.0001$), serum IFN-gamma levels (on day 10 pi, $p<0.05$) and IgG levels (none of the treated animals seroconverted). Regarding survival of the lambs, significantly higher percentages of healthy lambs were found in G1 (73.3%, 11/15) and G3 (80%, 4/5) compared to G2 (31.3%, 5/16, $p<0.05$). Similarly, lambs from G1 (4.2 ± 0.8 kg) and G3 (4.5 ± 0.7 kg, $p<0.01$) were significantly heavier than those in G2 (3.2 ± 0.7 kg, $p<0.01$). Looking at the congenital infection, parasite DNA was not detected in cotyledons nor target tissues from the lambs in G1 and G3. By contrast, parasite DNA was detected in all cotyledons and lambs from G2, except those from one sheep that aborted on day 13 pi. Therefore, results obtained here demonstrate that BKI-1748 applied one week after infection, when infection is systemic and fever is maximal, confers protection against congenital toxoplasmosis in sheep.

Funded by United States Department of Agriculture (USDA) (grant number 2020–67015–30881), US National Institutes of Health (NIH) (grant number R01 HD102487) and the Swiss National Science Foundation (grant numbers 310030_214897).

P048

Unraveling Morphological and Biomechanical Responses to Harsh Environmental Conditions in Coccidian Oocysts

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Coccidia are environmentally resistant parasites with significant implications for global health and economy due to severe tissue infections they can cause in humans and/or animals. Understanding the transmission and host infection by coccidia requires a close examination of the biomechanical responses of their oocyst form to environmental factors and disinfectant treatments.

Our study delved into the effects of a chlorinated household disinfectant and thermal treatment on the oocyst wall, with a focus on *Eimeria* species as a model of coccidia. For this, we employed fluorescence microscopy to quantify the

morphological characteristics and autofluorescence of oocysts. To improve automated data processing, a StarDist model was trained to segment and classify oocysts. Complementing our microscopy-based investigation, we used microindentation techniques with a flexible micropipette as a spring to evaluate the rupture force of the oocyst wall under various treatment conditions.
Our microscopy findings show a significant reduction in autofluorescence and oocyst apparent area for both *Eimeria acervulina* and *E. tenella,* with the most dramatic effects found with combined disinfectant and thermal treatments, indicating impaired wall structure. Our innovative micromanipulation system quantified, for the first time to our knowledge, the rupture force of the oocyst wall, which revealed significant variations in *E. acervulina* in response to the same treatments, while *E. tenella* displayed less discernible differences.
This work sheds light on the varied effects of chemical and physical factors on coccidian oocyst features, providing important information about potential changes in responses of such parasites to environmental conditions. Future investigations will be performed to study the correlation between their infectivity and biomechanics.

P049

In vitro models using target ovine cell lines to evaluate the *Toxoplasma gondii* phenotype

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Toxoplasma gondii possesses a significant genetic and phenotypic diversity and in most of the studies, its virulence has been defined in laboratory mice using parasite strains adapted to laboratory conditions. *T. gondii* clonal types II and III are the predominant strains in Europe and have been described as low or non-virulent. Although a relative correspondence between the virulence of *T. gondii* in mice and other species has been traditionally assumed, latest studies evidenced that the virulence in mice was not a reliable predictor of virulence in other target species such as pregnant sheep. In addition, different degrees of virulence among type II and type III isolates in *in vitro* and *in vivo* models have been reported. Therefore, to learn more about *T. gondii* virulence mechanisms is important to investigate the parasite phenotypic traits in target cell lines from a relevant livestock species such as sheep and using parasite isolates not adapted to the laboratory. For this, we have evaluated the interaction of a low *in vitro* passage of the isolate TgShSp1 with two ovine target cells of *T. gondii* infection: placental trophoblast cell line (AH-1) and monocyte-derived macrophages. Preliminary results in AH-1 cells, which is a target cell during *Toxoplasma* transplacental transmission, were similar to those obtained with the TgShSp1 isolate in other established cell lines, showing that this isolate had low invasion rate and a poor tachyzoite yield. In case of monocyte-derived macrophages, one of the most important immune target cells during *T. gondii* infection, preliminary results of the TgShSp1 proliferation kinetics showed that there was a notable exponential growth at 48 hpi. Further characterizations in both cells are necessary to finish the TgShSp1 phenotypic characterization. In addition, we are starting the *in vitro* characterization of other recently obtained isolates in both cell types to deepen in how the genetic background of the different isolates impacts in an isolate-specific manner in these ovine target cells. We believe that the standardization of an *in vitro* methodological approach using target cells for *T. gondii* studies could be an alternative to animal experimentation following the 3R principle. Moreover, the expected results will be extremely relevant for the One Health strategy, considering that *T. gondii* is an important cause of reproductive failure in sheep and humans.
*Funded by the Seal of Excellence ISCIII Health (IHMC22/00014).

P050

Toxoplasma resistance to mucin 2 and human cathelicidin LL-37

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As an enteric pathogen, *Toxoplasma gondii* must cross the intestinal mucosal barrier (IMB) to reach the intestinal epithelium to invade host cells. The IMB is a physicochemical barrier against invading pathogens and noxious substances. It is comprised of a mucus layer produced and secreted by goblet cells, an adherent glycocalyx on the surface of enterocytes, and epithelial tight junctions. Specifically, the mucus layer is an adhesive structure made up of large glycoproteins called mucins. Mucin 2 (MUC2) is the major gel-forming mucin in intestinal mucus. Additionally, the mucus layer is replete with antimicrobial peptides (AMPs), including cathelicidins such as LL-37. While *Toxoplasma* bradyzoites are major mediators of intestinal infection in a new host, their interactions with the IMB are unknown. Here, using immunofluorescence assays (IFA), we show that, unlike tachyzoites that remained trapped in the mucus layer secreted by human goblet cells HT29-MTX-E12

6 hours post-infection, bradyzoites were able to cross this barrier and invade these cells within one hour. By 24h, some of these parasites have divided and/or differentiated into tachyzoites. Furthermore, MUC2 and LL-37 show no antiparasitic effects on bradyzoites, rather parasites pre-treated with these compounds exhibit increased infectivity, as determined by IFA and plaque assays. Together, our findings indicate that bradyzoites possess unique factors that promote their survival in the intestinal mucus layer and subsequent invasion of intestinal epithelial cells. Such molecules may serve as novel therapeutic and diagnostic targets in the fight against toxoplasmosis.

P051

The Mitochondrial Iron Transporter is important for *Toxoplasma gondii*

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Toxoplasma gondii is an obligate intracellular apicomplexan parasite that, as many organisms, require iron as a cofactor to be used in many essential proteins. Excess iron can be toxic for the cells, meaning incorporation and storage are tightly regulated. The full mechanisms by which *T. gondii* responds to iron availability through infections and how iron is incorporated and driven into the mitochondria, where heme and Fe-S clusters are synthesized remain unknown. However, previous results have implicated the homolog of the yeast mitochondrial iron transporter Mrs3/4, named mitochondrial iron transporter (MIT)[1]. MIT levels were seen to increase in the absence of the iron storage transporter VIT, suggesting a role for MIT in iron detoxification. Here we assessed the importance of the putative mitochondrial iron transporter (MIT) in *T. gondii* biology. Our preliminary results indicate that MIT transcript and protein level increases upon iron deprivation, suggesting that it may play a role on iron uptake by the mitochondria. Despite the prediction of essentiality, we were able to generate a knock-out line for MIT and started its characterization. We find that deletion of MIT causes significant growth defect *in vitro* when compared to WT parasites. This was seen both by plaque assay and in competition assays. Interestingly, excess iron fails to overcome this growth phenotype, demonstrating that MIT is not the primary route of iron detoxification in the parasite. Currently, we continue characterizing MIT-KO by analysing mitochondrial functions that may be compromised. Together, we show for the first time that MIT is important for *T. gondii* survival and iron homeostasis.

1- Aghabi, D., Sloan, M., Gill, G. *et al.* The vacuolar iron transporter mediates iron detoxification in *Toxoplasma gondii*. *Nat Commun* 14, 3659 (2023). https://doi.org/10.1038/s41467-023-39436-

P052

Susceptibility of eight Brazilian human isolates of *Toxoplasma gondii* to Sulfadiazine and Pyrimethamine

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Different from strains circulating in Europe and North America, Brazil has a great diversity of recombinant or unusual strains of *Toxoplasma gondii* (atypical strains), and high genetic variability is observed in this country (de Lima-Bessa et. al., 2021). Low response to treatment in the clinics has also been reported, especially in Brazil. Previous studies, including ours, have shown a different pattern of susceptibility to drugs from Brazilian isolates of *T. gondii* (Silva et. al., 2017 and 2019; de Lima Bessa et. al., 2023). However, the molecular basis of the reduced susceptibility observed for these strains is not known yet. To amplify the knowledge regarding the susceptibility of different Brazilian strains to the conventional therapy of toxoplasmosis, the objective of this study was to evaluate the effectiveness of the drugs pyrimethamine and sulfadiazine to eight Brazilian human isolates of *T. gondii* (CTBr11, CTBr14, CTBr15, CTBr18, CTBr21, CTBr22, CTBr26, and CTBr27) in a murine model of acute infection. Infected mice were treated for ten days with different doses of sulfadiazine (SDZ), pyrimethamine (PYR), and a combination of SDZ + PYR. While groups of mice infected with CTBr26 and CTBr27 responded well to all doses SDZ, PYR and SDZ+PYR, mice infected with CTBr11 and CTBr14 responded moderately to PYR, and CTBr15, CTBr18, CTBr21, and CTBr22 showed high mortality even when treated with the highest doses of PYR (50mg/kg/day), SDZ (160 mg/kg/day) and their combination (6.2 mg/kg/day PYR and 20 mg/kg/day SDZ). Interestingly, CTBr11, CTBr14, and CTBr27 share the same genotype 11. Other works of drug susceptibility with strains of the same genotype also showed similar results, suggesting that the variability to treatment could be genotype-dependent and not only to a reduced effect on the drugs.

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P053

Human-derived cerebral organoids as a model for chronic *Toxoplasma gondii* infection

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Self-organizing, three-dimensional cerebral organoids differentiated from human induced pluripotent stem cells (hiPSC) have emerged as a new *in vitro* model to investigate pathogenesis of infectious diseases. Cerebral organoids are beneficial over animal models because they allow for the investigation of infectious disease pathogenesis in a human context. The cellular components and structural configuration of mature cerebral organoids resemble the developing human brain. *Toxoplasma gondii* displays a tropism for brain tissue after infecting humans and other warm-blooded animals. Within brain tissue, *T. gondii* tachyzoites transform into bradyzoites and encyst, leading to chronic, latent infection. Our goal is to develop a reliable model of chronic *T. gondii* infection in brain tissue using human-derived cerebral organoids to further our understanding of chronic infection and host-parasite interactions.

A commercially available kit (STEMdiff Cerebral Organoid kit, STEMCELL Technologies) was used to generate cerebral organoids using hiPSCs from a single human donor (ATCC-ACS-1026). Cerebral organoids matured for 4 months before infection with *T. gondii*. To investigate brain tissue tropism and bradyzoite transformation, we infected cerebral organoids with a *T. gondii* Type I/III EGS reporter strain that expresses *SAG1*-mCherry (tachyzoite stage) and *LDH2*-sfGFP (bradyzoite stage). Each cerebral organoid was exposed to 1,000 *T. gondii* tachyzoites for 4-hours then organoids were washed and re-incubated. Fluorescent microscopy was used to visualize tachyzoite proliferation and bradyzoite transformation in the cerebral organoids. After 17 days incubation cerebral organoids were fixed for cryosectioning. A cryotome was used to make 5 uM cryosections for fluorescent microscopy. Cryosections of cerebral organoids were stained using the following antibodies to verify cerebral organoid structural components: anti-SOX2 (radial glial cell, neuronal precursor marker) and anti-tubulin β III isoform (neuronal differentiation marker). DAPI DNA stain was used to visualize cell nuclei. Fluorescent microscopy verified *T. gondii* infection, tachyzoite to bradyzoite transformation, and the presence of cerebral organoid structural components. Additional fluorescent microscopy is underway to investigate *T. gondii* cyst formation and to verify presence of additional cerebral organoid structural components (astrocytes and oligodendrocytes).

P054

Host cell traversal by *Toxoplasma gondii* sporozoites: evidence of an unprecedented force-producing mechanism

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Toxoplasma gondii systemic dissemination to the tissues of intermediate hosts (IHs) is key to parasite transmission to cat and other felids through predation. The spreading of infection is operated by the tachyzoites, whose journey to all IHs" tissues initiates from the vascularized lamina propria underlying the intestinal epithelium. To establish themselves in this strategic anatomical niche, tachyzoites rely on the capability of *T. gondii* transmission stages, sporozoites and bradyzoites, to exit the invaded enterocytes and reach the lamina propria, where their differentiation into fast replicating tachyzoites takes place. Sporozoites and bradyzoites migrate from the cytoplasm of IH"s enterocytes to the lamina propria as soon as 2 h post-feeding of mice with *T. gondii* oocysts or tissue cysts, as originally shown by transmission electron microscopy (TEM) studies [1,2]. Using an in vitro model of fibroblast infection, we showed by immunofluorescence (IF) that after host cell (HC) invasion by a moving junction-dependent mechanism, *T. gondii* sporozoites posteriorly release long membranous tails, named sporozoite-specific trails (SSTs), that define the trajectories followed by sporozoites during HC traversal [3]. SST production was also demostrated in the closely related coccidian *Hammondia heydorni*. The SSTs contain several dense granule proteins generally localized to the parasitophorous vacuole (PV) lumen (GRA1), the intravacuolar network (IVN; GRA2, GRA4, GRA6) or the PV membrane (GRA5, GRA7). Surprisingly, TEM studies demonstrated that SST-associated sporozoites reside in the PV and that this structure is retained during parasite exit from the HC and cell-to-cell passage. To investigate the role of IVN in SST formation, we generated *T. gondii* sporozoites lacking GRA2, which plays a central role in membrane tubulation and IVN establishment [4]. TEM and IF analysis showed that GRA2 depletion not only altered the IVN ultrastructure but also the release pattern of SSTs" constituents. Collectively, our data suggest that HC traversal by *T. gondii* sporozoites exploits a novel force-producing mechanism,

based on the massive extrusion at the posterior pole of intravacuolar parasites of GRA-associated membranous material derived from the same pool of membranes and nanotubules that constitute the IVN.
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P055

Genotyping of European *Toxoplasma gondii* strains by a new high-resolution next-generation sequencing-based method

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A new high-resolution next-generation sequencing (NGS)-based method was established to type and genetically-differentiate (i.e. subtype) closely related European type II *Toxoplasma gondii* strains.
T. gondii field isolates were collected from different parts of Europe and assessed by whole-genome sequencing (WGS). In comparison to ME49 (a type II reference strain), highly polymorphic regions (HPRs) were identified, showing a considerable number of single nucleotide polymorphisms (SNPs). After confirmation by Sanger sequencing, 18 HPRs were used to design a primer panel for multiplex PCR to establish a multilocus Ion AmpliSeq typing method. *T. gondii* isolates and *T. gondii* DNA present in clinical samples were typed with the new method. The sensitivity of the method was tested with serially diluted reference DNA samples.
Among type II specimens, the method could differentiate the same number of haplotypes as the reference standard, microsatellite (MS) typing. Passages of the same isolates and specimens originating from abortion outbreaks were identified as identical. In addition, seven different genotypes, two non-archetypal and two recombinant specimens were clearly distinguished from each other by the method. Furthermore, almost all SNPs detected by the Ion AmpliSeq method corresponded to those expected based on WGS. By testing serially diluted DNA samples, the method exhibited similar analytical sensitivity as MS typing.
The new method can distinguish different *T. gondii* genotypes and detect intra-genotype variability among clonal European type II *T. gondii* strains. Furthermore, with available WGS data, additional target regions can be added to the method to potentially increase typing resolution.
Acknowledgements: This research was part of TOXOSOURCES, supported by funding from the European Union"s Horizon 2020 Research and Innovation program under grant agreement No. 773830: One Health European Joint Programme

P056

Comparative proteomic analysis of *Toxoplasma gondii Me49*-derived strains adapted to *in vitro* treatments with thiosemicarbazones

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Thiosemicarbazones and their metal complexes have been studied widely for their diverse biological activities, including against bacteria, cancer cells and protozoan parasites [1,2]. We have identified one gold (III) complex (C3) and its respective ligand (C4) that profoundly inhibit the proliferation of *T. gondii* tachyzoites *in vitro* (IC50 = 0.103 µM and 0.030 µM, respectively). Proteins potentially interacting with these two compounds were identified using differential affinity chromatography coupled with mass spectrometry (DAC-MS). Moreover, long-term *in vitro* treatment with C3 and C4 was also

performed to investigate whether the compounds have parasitostatic or parasiticidal activity.
DAC-MS showed that among the most abundant *T. gondii* proteins binding both compounds were 18 ribosomal proteins, suggesting that they interfere in protein synthesis. The study also revealed that prolonged exposure to these compounds resulted in parasitostatic activity, and that the parasites adapted to the treatment within 5-6 days. Thus, we explored the underlying mechanisms of adaptation using comparative shotgun proteomics of 3 clones of adapted parasites for each compound comparatively to the respective wild-type (WT) *Tg Me49*.
Whole-cell shotgun analysis in C3 and C4 adapted clones identified a total of 3860 proteins, with only 31 proteins being differentially expressed. Of these, 23 were found in clones adapted to the gold compound C3, and 8 in clones adapted to compound C4. Clone P1E5 (adapted to C3) exhibited the highest number of differentially expressed proteins (19), followed by clone P2F3 (adapted to compound C4) (6). Notably, two proteins, a transporter encoded by TGME49_258700 and a putative transmembrane protein encoded by TGME4_248140, were upregulated in clones adapted to both C3 and C4. These results suggest that parasites become less susceptible to these drugs by potentially reducing the intake or increasing the excretion of the toxic compounds. The next step will be to calculate the IC_{50} of the compounds on the single clones of adapted parasites, to confirm (or not) resistance.

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P057

Effects of chronic infection by *Toxoplasma gondii* on spatial memory, olfactory sensibility, and social behavior in male mice

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Introduction

It has been reported that *Toxoplasma gondii* causes spatial memory, sensitivity, and social behavior impairments. However, the strategy used by the animal to solve the spatial maze has not been studied. On the other hand, olfactory route has not been dissected from the other senses in the social behavior tests, by performing them in total darkness.

Objective

To evaluate spatial memory maze solving strategy, social behavior and olfactory sensitivity in chronic *T. gondii* infection

Methods

Twenty four BALB/c 10-12 weeks old male mice were used. The control groups were administrated with PBS via i.p. (n=10) while the experimental groups were infected with $1x10^4$ tachyzoites of ME49 strain. At 63-66 days post-infection, the behavioral tests started. Six controls and nine infected mice were tested in the Barnes test for spatial memory, while four control and five infected animals were evaluated in the open field (for "curiosity"), in the Crawley test for social discrimination, and female urine detection for olfactory sensitivity. The last three tests were performed in a red-light room, restricting perception to smell only. Serum specific antibodies against *T. gondii* were tested by ELISA. Two-factor repeated measures ANOVA with Tukey post hoc analysis, was used in the Barnes maze. For the remaining tests medians and percentiles were compared between controls and experimental animals, by Mann Whitney U test, and Friedman test for repeated measurements.

Results

Five of nine mice of the group tested for memory had α-*T. gondii* specific antibodies, which also had a longer latency to escape during learning, explored less, and used the direct strategy until the third day, while the four seronegative mice presented a longer latency to escape in the second day, but they improved learning in the third day. All the sociability/sensitivity group mice were negative to antibodies; however, they remained longer time in the center of the open field and showed more curiosity than non-exposed animals. In the Crawley test these animals did not discriminate between new and familiar subjects and showed a deficit in olfactory sensitivity, compared to the controls.

Conclusions
Toxoplasma gondii chronic infection changes Barnes mazeresolution strategy, and was different between seronegative and seropositive animals. The social/olfactory deficit caused by *T. gondii* could be attributed to a decrease in olfactory sensitivity, but not to an innate curiosity loss.

P058
Fatal systemic toxoplasmosis with atypical steatitis in southern sea otters (*Enhydra lutris nereis*) associated with the COUG (TgCgCa1) strain

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Between 2020 and 2022, four southern sea otters (*Enhydra lutris nereis*) stranded and died in California, USA. The most striking gross lesion in all four otters was marked discoloration and an unusual, granular texture of the subcutaneous and internal fat tissues. Histopathologic evaluation revealed that systemic toxoplasmosis was the primary cause of death for all four otters which was confirmed via immunohistochemistry and PCR. High parasite burdens were seen microscopically in many tissues, including fat tissues which were also markedly inflamed (steatitis). Protozoal-associated steatitis is highly unusual and has never before been documented in sea otters with systemic protozoal infections. Multilocus sequence typing across 13 loci revealed that all four otters were infected with the COUG (TgCgCa1) strain of *Toxoplasma gondii*, an atypical and rare North American strain that has never been reported in southern sea otters nor any other aquatic intermediate host. The COUG strain was first isolated from two mountain lions in British Columbia, Canada during an investigation into a waterborne outbreak of toxoplasmosis in humans in 1995. Similar to other *T. gondii* strains, COUG strain oocysts presumably travel from terrestrial felid feces via surface water runoff to contaminate the coastal nearshore environment where sea otters become infected, but the felid population shedding COUG oocysts in California remains unknown. This genotype appears to have been recently introduced to this environment given that it has not previously been detected in more than two decades of *T. gondii* surveillance in the southern sea otter population. The discovery of this highly virulent strain is concerning for threatened southern sea otters, whose population is already under pressure from other stressors including infectious agents, predation, anthropogenic activities, and resource limitations. Although no reports of the COUG strain infecting humans exist to-date, the detection of this virulent strain in sea otters also raises public health concerns for humans that share the same marine environment and food source (e.g. shellfish) as sea otters. This presentation will discuss our findings from the initial case series of four sea otters, as well as new insight from additional sea otters infected with the COUG strain that have been identified since 2022.

P059
Sending out an SOS: novel insights into a *Toxoplasma* effector protein

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To survive within the host cell *Toxoplasma* secretes proteins from its specialized secretory organelles that interfere with host cell immune functions. A *Toxoplasma* rhoptry kinase named ROP16 promotes the phosphorylation of STAT6 (P-STAT6), a host transcriptional factor, leading to its activation and ultimately inducing M2-like polarization of macrophages. In addition, ROP16 was shown to be involved in enhanced cyst development.
We have previously shown that SOS1 knockout (KO) parasites induce a host transcriptional profile similar to that of ROP16 KO infected cells. SOS1 KO parasites were unable to sustain the P-STAT6 signal 24 h post-infection (hpi), revealing that, although SOS1 is not required to initially phosphorylate STAT6, it is necessary to sustain it. Similarly, SOS1 seems to be involved in maintaining cyst formation *in vitro*.
In the current study, we observe that maintenance of the P-STAT6 signal requires parasite invasion, and that co-infection with ROP16 KO and SOS1 KO parasites does not restore the P-STAT6 phenotype. This suggestes that invasion is required for signal maintenance and that both effectors must be present in the same parasite cell. Furthermore, increasing the parasite infection dose leads to a higher P-STAT6 signal at 24 hpi, indicating a dose-dependent effect.
Interestingly, SOS1 is conserved not only across *Toxoplasma* strains but also in other Apicomplexa species, such as *Plasmodium falciparum* and *Cryptosporidium parvum*, leading us to hypothesize that it may have a broader role in parasite biology. Ongoing work focuses on high-resolution spatial imaging of SOS1 KO parasites to unravel potential

defects in rhoptry morphology and transcriptomic analysis of SOS1 KO and ROP16 KO infected cells to understand the wider function of SOS1. Future work also aims to investigate the role of SOS1 homologs in other Apicomplexa. This work will deepen our understanding of *Toxoplasma'*s mechanisms of immune system evasion while simultaneously providing insights into the overall biology of the parasite.

P060

The Impact of Host Arginine Metabolism on *Toxoplasma gondii* Infection and Growth Dynamics.

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Arginase enzymes play a crucial role in cellular homeostasis across all Domains of life. In vertebrates, arginase isoform I (Arg1) drives the urea cycle in the liver, and arginase isoform II (Arg2) systemically contributes to the biosynthesis of essential molecules such as polyamines. While these isoenzymes share the same function, their distinguishing roles in various metabolic and immunologic pathways remain undefined. Arg1 is well-characterized as an essential enzyme in mammalian homeostasis. Literature suggests that Arg2, though non-essential, may modulate the host response to infection via unknown mechanisms. *Toxoplasma gondii* is an intracellular eukaryotic parasite that uniquely lacks both arginases and other essential enzymes in arginine metabolism. Consequently, *T. gondii* relies on siphoning arginine and downstream metabolites, including polyamines, from the host for its survival and replication. In investigating the role of arginase 2 in *T. gondii* infection, we discovered a significant growth defect of *T. gondii* in tissue culture fibroblasts treated with the arginase inhibitor, norNOHA. Interestingly, when attempting to rescue the growth of the parasite by supplementing polyamines, a compounding defect in *T. gondii* growth is observed. Finally, Arg2 knockout mice are more likely to survive *T. gondii* infection compared to wildtype mice. These results reinforce our hypothesis suggesting that Arg2 contributes to *T. gondii* infection both in vitro and in vivo. Future studies will utilize metabolomics and transcriptomics to define the role of Arg2 in *T. gondii* infection to offer a broader context in understanding complex interactions for both the host and parasite. The aim of this study is to identify potential drug targets in the early stages of *T. gondii* infection.

P061

Anti-*Toxoplasma gondii* antibodies in Neotropical primates from captivity and the wild: low concordance between serological tests

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Neotropical primates are extremely sensitive to *Toxoplasma gondii* infection, with a high lethality rate. Infection and disease development occur most frequently through contact and ingestion of oocysts shed by felines in the soil. The aim of this study was to assess the frequency of anti-*T. gondii* antibodies in serum samples from 77 primates treated at the Center for Research and Medicine of Wild Animals (CEMPAS) at the School of Veterinary Medicine and Animal Science, UNESP, Botucatu, SP, Brazil, as well as free-living primates from Vitória do Xingu, PA, in brazilian Amazonia, using the indirect fluorescent antibody test (IFAT) and modified agglutination test (MAT). Considering the MAT results, frequency of anti-*T. gondii* antibodies was higher in *Alouatta guariba* (14/24; 58.33%; 95% CI: 38.66-75.60), followed by *Sapajus nigritus* (3/22; 13.64%; 95% CI: 4.95-33.59) and *Alouatta belzebul* (2/30; 6.67%; 95% CI: 2.04-21.42). *Cebus olivaceous* had only one animal evaluated, which reacted to MAT. Frequency of reactive animals was higher in those from captivity (33.33%; 95% CI: 20.21-49.79) in contrast to those in the wild (19.51%; 95% CI: 10.30-34.11), without significance ($P > 0.20$). Regarding IFAT, three samples were positive, revealing a low Kappa association index (0.21) compared to MAD, with a positive agreement of 26.09%. Based on the results, the use of MAD are recommended for evaluating serum samples from Neotropical primates due to its ease of execution and absence of the need for species-specific reagents or specialized equipment.

P062

CD8+ T cell recognition of cyst-derived antigen during chronic CNS infection with *Toxoplasma gondii*

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Toxoplasma gondii establishes persistent infection within neurons by forming cysts that harbor bradyzoites and it was previously thought that cysts were protected from immune recognition. However, current evidence suggests that CD8+ T cells can both recognize cyst-derived antigens and potentially participate in cyst control – though it remains unclear how cyst antigens would become available to enter the MHC-I pathway for presentation to CD8+ T cells. To explore if cyst-derived antigens can be transported beyond the cyst wall for direct presentation by infected neurons, we generated a BAG1-GRA6-SIINFEKL (BAG1-GRA6-SIIN) parasite line in which there is bradyzoite-specific expression of the SIINFEKL CD8+ T cell epitope linked to GRA6, a secreted dense granule protein hypothesized to be transported across the cyst wall into the host cell cytoplasm. In C57BL/6 mice infected with BAG1-GRA6-SIIN, SIINFEKL-specific CD8+ T cell responses were generated yet only detectable primarily in the brain during the chronic phase, confirming both the stage-specific expression of the construct and its availability to enter the MHC-I pathway. To assess if SIINFEKL-specific CD8+ T cells encounter their cognate antigen in the CNS, TCR reporter Nur77-GFP-OT-Is were transferred into and then recovered from the brains of BAG1-GRA6-SIIN-infected mice. A proportion of CNS-derived OT-Is were found to express Nur77-GFP, indicating that these T cells had recently encountered cyst-derived antigen. The use of bone marrow chimeras to restrict the expression of MHC-I expression suggested that CNS-resident cells (including neurons) were not required for local antigen presentation of cyst-derived antigens. Future studies will identify which cells present cyst-derived antigen in the CNS and determine if antigen can be transported beyond the cyst wall for direct presentation by neurons.

P063

Serological and molecular survey of *Toxoplasma gondii* infection in wild carnivores of the Iberian Peninsula

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Within the One Health framework, knowledge gaps regarding the circulation of *Toxoplasma gondii* in wild animals still need to be uncovered. Due to the lack of validated diagnostic techniques in such host species, the use of different serological and molecular assays could hinder the reliable comparison of epidemiological data regarding *T. gondii*. Herein, we propose a workflow to investigate parasite exposure and circulating genotypes in the Iberian Peninsula, with a focus on two species of wild carnivores (red fox -*Vulpes vulpes*- and Iberian lynx -*Lynx pardinus*-). A total of 377 serum samples from lynxes and 211 from foxes were analyzed by two serological techniques (lynx: modified agglutination test -MAT- and western blot -WB-; fox: enzyme-linked immunoassay -ELISA- and WB). For molecular assays and genotyping, we followed the protocols described by Calero-Bernal *et al.* (2022) and Joeres *et al.* (2023), respectively. Briefly, pools of artificially digested tissues from 123 foxes and 143 lynxes were used. After DNA extraction using an automated system, a nested PCR targeting ITS-1 was performed, and those testing positives were subjected to quantitative PCR targeting 529 RE fragment. An attempt to genotype positive samples with a high quantity of parasite DNA (Ct values ≤32) was performed using microsatellite (MS) fragments analysis to discriminate the parasite strain. If Ct>32, GRA6 and SAG3 genotyping markers were analyzed by Sanger sequencing. Considering the coincident positive results in both serological tests, the prevalence of *T. gondii* antibodies was 28.9% (61/211) in foxes and 44.3% (167/377) in lynxes. *Toxoplasma*

gondii DNA was detected in 7/123 (5.7%) foxes and 27/143 (18.9%) lynxes. Only a complete genotyping profile by MS was achieved for 3/5 lynxes samples with Ct T. gondii sylvatic life cycle. We propose the use of this exhaustive workflow (involving several serological techniques, PCR-based procedures and DNA sequencing confirmation) to provide reliable data for epidemiological and genotyping studies of *T. gondii* infection in wildlife.

References

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P064

Innovative Assay Development and Targeted Screening Unveil Compounds Against *Toxoplasma gondii* Bradyzoite and Tachyzoite Forms

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Toxoplasma gondii, a pathogen of global concern, and its ability to interconvert between bradyzoite and tachyzoite forms necessitates innovative approaches for therapeutic discovery. This study centers on the pioneering development of a high-throughput assay and subsequent screening of the Library of Pharmacologically Active Compounds (LOPAC) against both tachyzoite and bradyzoite forms of *T. gondii*.

We used a unique isolate from a patient with congenital toxoplasmosis, called *Tg*68, which exhibits robust differentiation into mature bradyzoites under stress conditions *in vitro*. Comparative analyses highlighted *Tg*68's slower replication and enhanced bradyzoite differentiation relative to ME49. Notably, *Tg*68 maintains high rates of bradyzoite formation at high MOI under stress conditions, presenting a valuable model for identifying compounds effective against the bradyzoite stage. In this study, *Tg*68 parasites were engineered to express nano luciferase under pBAG1 for investigating bradyzoite stage inhibitors. Additionally, *Tg*68 parasites expressing firefly luciferase constitutively under the pTub1 promoter were used to study tachyzoite stage inhibitors. A 384-well format assay was developed using automated liquid handlers. Screening the LOPAC library revealed 44 compounds with >50% inhibitory effects against *Tg*68 bradyzoites. Subsequent characterization included TgEC50, HepG2 CC50, and *in vitro* EC50 assessment against bradyzoites. Now we are expanding the screen to include the ReFRAME (Repurposing, Focused Rescue, and Accelerated Medchem) library comprising ~15,000 FDA-approved and late clinical leads in collaboration with Scripps. Our approach prioritizes compounds based on efficacy against *Tg*68, specifically targeting bradyzoites, while ensuring safety profiles. These findings not only unveil a promising set of compounds for further preclinical development against *T. gondii* including bradyzoites that are responsible for chronic infection.

P065

Persistence of viable Toxoplasma gondii oocysts in Pacific oysters (Crassostrea gigas)

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The presence of Toxoplasma gondii in shellfish has been reported across diverse geographical regions worldwide. While attention to shellfish-borne disease has largely focused on bacterial and viral pathogens, protozoan parasites including T. gondii have been recognized as foodborne pathogens that are likely underestimated as causes of illness through shellfish consumption. Furthermore, T. gondii can infect essentially all warm-blooded animals, which can result in severe morbidity and mortality in marine mammals worldwide. To date, detection methods have focused on parasite DNA, which does not inform public health and veterinary professionals about the true risk to susceptible hosts including people and wildlife. In this study, the persistence of viable T. gondii oocysts contaminating Pacific oysters (Crassostrea gigas) was assessed by quantifying the concentrations of mRNA in oysters following systematic spiking and a subsequent depuration period. To determine parasite persistence, oysters were exposed to viable T. gondii oocysts for 24 hours and then transferred into clean depuration beakers. Oysters (whole tissue) and feces/pseudofeces in water were collected at specific time points for up to 70 days. Samples were analyzed using multiplex nested polymerase chain reaction (PCR) for DNA detection and reverse transcription quantitative PCR (RT qPCR) for mRNA quantification. Our results suggest that DNA can be detected after viable parasites, as measured by mRNA, are no longer present in shellfish, providing important implications for surveillance approaches in shellfish that serve as an important seafood commodity to people as well as prey for marine wildlife.

P066

Imiquimod Treatment targets Chronic Toxoplasmosis and its Associated Neurological Complications in a Rat Model

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Toxoplasma gondii (T.gondii) is the etiologic agent of toxoplasmosis, a prevalent parasitosis infecting one-third of the human population worldwide. Under the host immune control, tachyzoites responsible for acute toxoplasmosis (AT), transform into latent bradyzoites responsible for chronic toxoplasmosis (CT). In immunocompromised patients, CT may reactivate leading to a potentially life-threatening condition. Several associations between CT and behavioral neurological complications were highlighted. Yet, a direct pre-clinical or clinical implication of toxoplasmosis with most of these diseases remains elusive. Moreover, an approved and efficient treatment targeting CT is still lacking despite its high prevalence. We previously demonstrated the high potency of an immunomodulatory drug, Imiquimod, against AT and CT, and unraveled its molecular mechanism of action. Briefly, Imiquimod induces interconversion from bradyzoites to tachyzoites, upregulated Toll-like receptors, leading to the consequent activation of the MyD88 pathway, triggering the host immune response and the control of reactivated *Toxoplasma* foci. Here, we investigated the effects of Imiquimod or the first-line therapy (Sulfadiazine (S) and Pyrimethamine (P)) followed by Imiquimod on CT-associated behavioral complications, namely anxiety-like behavior and hippocampal cognitive functions, in a CT rat model. Consistent with our previous results in mouse models, Imiquimod decreased the number of cysts in the brain of chronically infected rats and induced the reactivation of bradyzoites into tachyzoites. Strikingly a higher potency was observed when chronically infected rats were treated with Imiquimod followed by the combination S+P. Moreover, an upregulation of proinflammatory cytokines in the brains of chronically infected rats was observed upon treatment with Imiquimod alone or followed by S+P. This upregulation triggered a concurrent anti-inflammatory immune response to lessen the proinflammatory immune-pathological response. Importantly, in chronically infected rats with *T. gondii,* Imiquimod and more potently Imiquimod followed by S+P alleviated the anxiety-like behavior and reversed *Toxoplasma*-induced the learning deficits observed in untreated infected rats. Collectively, our results enhance our knowledge on the implications of toxoplasmosis on behavioral aberrancies, and open perspectives towards new therapeutic approaches targeting CT and its associated diseases

P067

FACS-based CRISPR knockout screen identifies interferon stimulated genes that control *Toxoplasma* in pig cells

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Acute *Toxoplasma* infection in pigs can cause severe morbidity and mortality, while chronic *Toxoplasma* infection suppresses immunity and presents significant risk to human foodborne infection. In virtually all vertebrate hosts, interferons (IFNs), through the induction of hundreds of interferon-stimulated genes (ISGs), control *Toxoplasma* pathogenesis. Compared to mice and humans, very little is known about the ISGs that control Toxoplasma in pigs.
To address this knowledge gap, we developed the first CRISPR knockout library exclusively targeting over 1000 canonical porcine ISGs (pISG-knockout library) and used it to screen for ISGs that control a type II Toxoplasma strain in Neonatal Swine Kidney (NSK) cells. Known ISGs such as GBP1, STAT1, IRF1 and IFNGR1 were among the top 10 hits from the screen, confirming the potential of the pISG-knockout library to identify ISGs controlling Toxoplasma in pigs. We are currently working on the functional validation and mechanistic characterization of the candidate genes arising from the screen.

P068

Evaluation of the Trithiolato-Bridged Arene Ruthenium Complex Conjugated to 9-(2-hydroxyethyl)-Adenine (OD62-18) as a Potential Treatment for *Toxoplasma gondii* Infection.

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The apicomplexan parasite *Toxoplasma gondii* is a major food-borne pathogen, causing toxoplasmosis. Current treatment options are limited due to suboptimal efficacy and adverse side effects. From a library of > 300 organometallic drugs we explored the therapeutic potential of a trithiolato-bridged arene ruthenium complex conjugated to 9-(2-hydroxyethyl)-adenine (OD62-18*).*

OD62-18 inhibited parasite proliferation *in vitro* with an IC50 < 60nM, induced significant alterations in the mitochondrial matrix and the disappearance of cristae, and had a negative impact on the mitochondrial membrane potential, while the overall morphology and secretory organelles were not notably affected. Differential affinity chromatography and mass spectrometry identified the YOU2 family C2C2 zinc finger protein, a Tim10 homologue, as the primary OD62-18 binding protein. However, knockout parasites lacking *Tg*Tim10 displayed no discernible differences in growth, proliferation, or plaque formation, and maintained a similar IC50 under OD62-18 treatment. OD62-18 treatment of *T. gondii* oocyst infected mice resulted in only negligible effects on parasite load in various tissues, and inductively coupled plasma mass spectrometry (ICP-MS) analysis revealed predominant drug excretion after 24 hours, with no penetration into the brain. While OD62-18 demonstrates notable *in vitro* efficacy against *T. gondii*, further investigations are necessary to address the observed *in vivo* limitations and optimize its therapeutic potential.

P069

Commercial kitten moist food inactivated *Toxoplasma gondii* tissue cysts instantaneously

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Toxoplasmosis is an important parasitic disease for human and animal health. Felines are the definitive hosts, with great importance in the epidemiology of this parasitosis. The present report is part of an ongoing project for the development of a new product for controlling feline toxoplasmosis. Swiss albino mice have been inoculated subcutaneously with 25 ME49 tissue cysts, obtained from the brain of previously inoculated mice. They were euthanized with isoflurane, 42 days post inoculation (dpi), and approximately 22,400 bradyzoite cysts were harvested from their brains. Aliquots of 800 cysts were distributed in 12 labelled microtubes, with the volume adjusted to 500 µl with sterile saline. All the infective material was kept at 4 °C. Twelve seronegative kittens were experimentally infected by oral route, with those 800 bradyzoite cysts of *T. gondii* ME49 strain, on Study Day 0. The inoculum doses were placed over a small portion (one spoon) of moist cat food (Kelcat Alimento Úmido Lata, Peixe com Ervilha e Cenoura 280g, Kelco). All kittens ingested 100% of the material. This procedure was chosen based on animal welfare. After inoculation, fecal samples from each kitten were collected and examined daily, for 28 days, by a coproparasitological fluctuation test in sucrose solution (S.G. ≥ 1.27). Blood samples were collected from the kittens at 7, 14, 28 and 35 dpi. Serum samples were tested by immunofluorescence antibody test (IFAT). None of the kittens shed oocysts, neither seroconverted with this initial inoculation. On 35 dpi, the remaining material collected from the mice brain was used to produce aliquots containing 200 tissue cysts, with the volume adjusted to 500 µl with sterile saline. The kittens were then inoculated by intranasal (IN) route, with a nasogastric tube no 4, on the same day. All samplings and tests were repeated as before. The kittens began to shed oocysts on 41 dpi (6 days after IN inoculation (daii)), and were shedding up to 47 dpi (12 daii). All kittens were seropositive by 49 dpi (14 daii). These results demonstrated that the kitten moist food was able to inactivate the tissue cysts instantaneously, even without mixing or any other preparation with the inoculum. Further experiments will be carried out with distinct components of the commercial food, in order to identify the specific substances that inactivated the *T. gondii* cysts with that single contact.

P070

The IRE1 pathway of the UPR regulates cDC1 function during acute T. gondii infection

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Induction of the unfolded protein response (UPR) has emerged as a key regulatory mechanism that controls inflammatory processes, notably in response to infections. We previously demonstrated that induction of the UPR sensor IRE1 in Bone-Marrow derived Dendritic Cells (BMDCs) infected by Toxoplasma gondii promotes inflammatory cytokine production and MHC-I presentation of secreted parasite antigens. In addition, in vivo, specific deletion of IRE1 in DCs correlated with impaired cDC1 expansion and altered T cell mediated protective responses resulting in uncontrolled parasite dissemination and high susceptibility to infection. In the current study, we explored these findings further and aimed to dissect how loss of IRE1 affects DC function in response to T. gondii infection. We showed that IRE1 is specifically needed at an early step in the DC maturation process, at the moment that DCs are maximally engulfing antigens, associated with increased lipid influx. IRE1 appears to regulate key signalling pathways involved in lipid homeostasis but also in the induction of type I interferon response, known to be involved in cDC1 maturation and immunogenic functions. Targeting the UPR may open new strategies to boost antigen presentation by cDC during an infection and ameliorate vaccine efficacy.

P071

Investigating contamination of ready-to-eat salads with *Toxoplasma gondii* oocysts in a European-wide multi-center survey

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The majority of horizontal *Toxoplasma gondii* infections in humans are assumed to be foodborne. The relative contribution of the meat-borne (tissue cysts) *versus* environmental (oocyst-driven) routes of infections is largely unknown. The consumption of unwashed raw fruits or vegetables contaminated with *T. gondii* oocysts has been identified as a putative risk factor. Important methodological inconsistencies and limitations have been identified in the surveys carried out worldwide in fresh produce. The present study aimed to unravel the occurrence of *T. gondii* oocysts in commercially available ready-to-eat (RTE) salad mixes in ten European countries. For that purpose, the detection methodology was first validated in an interlaboratory ring trial. Next, a harmonized sampling strategy and the validated methodology were implemented by all participants.

In this multicentre study, weekly sampling (from October 2021 to October 2022) was set to detect seasonal variations. Two categories of RTE salad mixes (baby leaves and cut leaves mixes) were sampled to explore potential associations between oocyst contamination and cultivation and growth conditions. In the study period, 3,329 RTE salad samples were tested using a standard operating procedure (SOP) that allows molecular detection of *T. gondii* oocysts in such matrices with a detection limit of 10 oocysts per 30 g of salad. Robustness and sensitivity of this SOP as well as the comparability of results among the laboratories had been confirmed in the ring trial before the start of the study. The key analytical steps include oocysts recovery by sample washing followed by pelleting of the eluate by centrifugation, DNA extraction and DNA detection by triplex qPCR, targeting two multicopy fragments (529RE, *B1* gene) and an internal amplification control. Positive samples were confirmed by ITS-1 single tube nested PCR followed by Sanger sequencing. This is the first European-wide study to estimate the prevalence of *T. gondii* oocysts in RTE leafy green salads using a validated and standardized procedure to assess the associated potential risk for human infections.

This work was done as part of TOXOSOURCES project, EU Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme.

P072

PDL1 in ocular toxoplasmosis: Bridging inflammation and immune regulation

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The particularities of the ocular immune environment and its barrier protection in the context of infection are not well elucidated. *Toxoplasma gondii* is one of the pathogens successfully crossing this barrier and establishing chronic infection in retinal cells. The establishment of this infection requires the modulation of the host ocular immune privilege in order to allow pathogen control while avoiding exacerbated inflammation. The ligand PD-L1 (CD274) is an immune checkpoint regulator and a key actor in the immune privilege by limiting T cell activity. Furthermore, IFN-β and IFN-λ play important roles in the outer blood-retinal barrier permeability and could also modulate the ocular immunomodulatory landscape, in addition to IFN-γ. To address these questions, we use confocal microscopy and transcriptomic approaches on a human *in vitro* model and a mouse model for the acute phase of ocular toxoplasmosis. Our first results show that *T. gondii* infection, but also secreted/excreted factors alter PD-L1 expression in human retinal pigment epithelial cells. We then confront these results with those obtained in our mouse model of PD-L1 expression and localization after types I and III interferon neutralization. These results will give us a better understanding of the interplay between *T. gondii* and the ocular immune privilege in order to find more targeted treatments for ocular toxoplasmosis.

P073

MyoJ-BCC1-Cen2: is there a contractile ring at the basal pole of *Toxoplasma gondii*?

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Toxoplasma gondii basal complex is essential for parasite motility, as it constitutes a force-transmission platform together with the apical complex, and also for division. The basal complex is assembled early during endodyogeny, close to the centrosomes and the apical complex. The polarization of the daughter cells is driven by the extension of each pole to an opposite side. Daughter cells are then individualized by the constriction of the basal pole, which involves the myosin heavy chain J (MyoJ). To identified new components of the basal complex, we performed a proximity labeling experiment using MyoJ as a bait. In this screen, we identified the basal complex component 1 (BCC1), which colocalizes with MyoJ at the posterior cup and is also involved in basal pole constriction and motility. The conditional knock-down of BCC1 impacts the localization of MyoJ at the basal pole and conversely, the absence of MyoJ impacts BCC1 localization. In addition, Centrin 2 (CEN2), a calcium-binding protein, was shown to localize at the posterior cup, and its depletion also leads to a defect in basal complex constriction. This draws the hypothesis of a contractile ring at the basal pole, consisting of the motor MyoJ, Cen2 as the light chain and BCC1, the cargo.
To characterize the interactions taking place within this complex, we set up complementation experiments in parasites depleted for MyoJ or knock-out for BCC1. In these strains, we follow the localization of BCC1 and MyoJ respectively, while adding different constructs of MyoJ and BCC1. By doing so, we found that the motor domain of MyoJ is necessary to drive the constriction of the basal complex while the neck and tail region is necessary for the localization of BCC1. For BCC1, we showed that the coiled-coil domain is essential for the localization of MyoJ but not the constriction. In parallel, we undertook yeast-two-hybrid experiments to test the direct interaction between the three components of the putative contractile ring. These experiments should allow us to decipher the interacting domains involved and get a better understanding of the function of this complex, which seems different from other eukaryotic organisms.

P074

MQ-1: A Promising Marinoquinoline Derivative Demonstrating Effectiveness in Treating Acute and Chronic Toxoplasmosis in Murine Models

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Natural products have yielded a diverse array of bioactive compounds, laying the foundation for clinically utilized derivatives. The exploration of natural products derived from marine bacteria has revealed marinoquinolines (MQs), distinguished by a pyrroloquinoline core, showcasing notable *in vitro* and *in vivo* anti-*Plasmodium* activity. Recognizing the imperative to explore novel therapeutic options for toxoplasmosis chemotherapy, this study investigates the potential of MQ derivatives against *T. gondii*. The study involves the synthesis and characterization of six MQ derivatives, followed by *in vitro* evaluations on human cells and *T. gondii* tachyzoites. MQ-1 emerges as the most promising derivative, exhibiting a half-maximal effective concentration (EC_{50}) of 1.46 µM (± 0.45) and a half-maximal cytotoxic concentration (CC_{50}) of 30.51 µM (± 1.80), resulting in a selectivity index (SI) of 20.85. *In vivo* studies using murine models of acute and chronic toxoplasmosis demonstrate the effectiveness of MQ-1 in reducing parasite burden without observable toxicity. Additionally, the study compares MQ-1 with the standard drug pyrimethamine, showing comparable efficacy in both acute and chronic scenarios. The findings suggest that MQ-1 holds promise as a novel anti-*Toxoplasma* agent, with potential applications in the treatment of acute and chronic infections. Further research is warranted to explore the full therapeutic potential of MQs and assess their safety profile for future clinical applications. This study provides valuable insights in the quest for new treatment options for toxoplasmosis.

P075

Latent cerebral *T. gondii* infection exacerbates Tauopathy in a mouse model of Alzheimers disease.

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Brain infections by pathogenic micro-organisms are of growing interest for their role in triggering a damaging inflammation cascade that may exacerbate neurodegenerative processes. Toxoplasmosis is one of the most common infections caused by protozoan parasites, with a world seroprevalence of about 30%. Infection of immuno-competent individuals is characterized by bradyzoite-containing cyst development in the brain, leading to life-long persistent infections. Control of cerebral toxoplasmosis is correlated with the establishment of a sustained low-grade neuroinflammation, characterized by the activation of resident immune cells. Using a mouse model of Alzheimer disease (AD), the Tau22 mouse model of progressive tauopathy, our preliminary results indicate that long-term cerebral *T. gondii* infection exacerbates hippocampal Tau pathology. We also found that *T. gondii* infection and the Tau22 background synergizes and potentiates immune responses, notably unique microglia signatures known to be detrimental for neuronal functions. In line, our data suggest that cerebral infection impacts on synapse components in Tau22 mice. Thus, long-term cerebral *T. gondii* infection precipitates the course of Tau pathology in mice, an aspect that we also currently examine in *T. gondii* seropositive AD patients.

P076

Identifying genes associated with acute lethality in *Toxoplasma*

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The development of effective antiparasitic interventions relies on the identification of protein targets whose inhibition is lethal to the parasite to enhance the potential for rapid and sustained clinical impact. The use of genetic disruption to categorize genes as essential does not allow us to distinguish between two sources of fitness defects: genes whose disruption causes cell death (cidal), versus genes whose disruption simply halts or slows growth (static), diluting the mutant in the population without causing cell death. Recent recombination-based, screening modalities afford new opportunities to investigate gene function without causing complete gene disruption. By utilizing a high-throughput

tagging (HiT) approach we can label each fitness-conferring protein with a synthetic sequence encoding the minimal auxin-inducible degron (mAID) and induce transient protein depletion to distinguish between perturbations that kill the parasite, versus ones that allow recovery and subsequent replication once the inhibition period concludes. Towards the goal of highlighting proteins with cidal effects and contributing to the development of selective therapies, we have curated a list of 700 fitness-conferring genes that are shared across apicomplexans but absent in vertebrates. To efficiently degrade mAID-tagged proteins and reduce non-induced protein degradation, we have improved the degron system by introducing only one copy of the auxin receptor TIR1 into the parasite genome. Lastly, to gain functional insights for each fitness-conferring protein, we are developing an *in-situ* sequencing approach to enable linkage between each cell's morphology and the mutation it carries, in a pooled library of genetic perturbations. The next steps include performing the transient inhibition screen and expanding the *in-situ* sequencing approach to perform optical pooled screens in *T. gondii*. As most of these fitness-conferring genes have never been functionally annotated, this scheme will not only reveal proteins with parasite-killing potential but also provide expansive functional insight into uncharted territories of apicomplexan biology.

P077

Cooperation or Competition? Unraveling the interplay between transcription and epigenetic factors in *Toxoplasma gondii* gene expression

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Regulation of gene expression in *Toxoplasma gondii* is mediated by a complex interplay between ApiAP2 transcription factors (TFs), chromatin remodelers, and protein complexes. Although this interplay has been experimentally interrogated (e.g., MORC/HDAC3 complex), the degree of these interactions and the universality of this mechanism has not been examined. Here, we present a comprehensive dissection of all publicly available as well as newly generated chromatin immunoprecipitation data (ChIP-chip, ChIP-seq, CUT&RUN, and CUT&TAG) performed on multiple TFs and epigenetic factors (EFs) in *Toxoplasma gondii.* Analysis of the data revealed that several TFs and EFs have overlapping binding loci and common motifs across the genome, hinting at the existence of core complex(es) that operate cooperatively or competitively to regulate gene expression and developmental programs. We developed a Hidden Markov Model (HMM) that segments the genome based on chromatin engagement signatures by various factors into distinct states that correlate with gene expression patterns. Overall, our analysis reveals that there may be universal mechanisms involving core complexes of TFs and EFs that regulate gene expression in *T. gondii*.

P078

Elucidating the role of a mitochondrial superoxide dismutase in *Toxoplasma gondii*

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Toxoplasma gondii is the only apicomplexan parasite that possesses a well-developed reactive oxygen species (ROS) scavenging system, which includes an endogenous catalase and complete glutathione and thioredoxin pathways. Our previous studies generating auranofin-resistant *T. gondii* parasites led to mutations in *T. gondii"s* mitochondrial superoxide dismutase (TgSOD2). These parasites showed reduced accumulation of ROS upon exposure to auranofin. TgSOD2 is an essential gene, as evidenced by its low CRISPR-Cas phenotype index and non-viability of constitutive knockouts. During stages of extracellular stress, TgSOD2 is upregulated as the parasite attempts to survive. To understand the essential role of TgSOD2 in *T. gondii*, we generated knockdown mutants using an auxin-inducible degron system that allowed us to explore the role of TgSOD2 in parasite replication and replication fitness. Its depletion leads to a reduction in replication fitness as these parasites showed decreased plaque formation when compared to their parental line. TgSOD2 depletion led to a reduced number of parasites per parasitophorous vacuole. At the mitochondrial level, depletion of TgSOD2 results in aberrancies in the parasite"s mitochondrion as well as reduction of the parasite"s mitochondrial membrane potential. Through a proximal biotinylation approach, we found that TgSOD2 localizes adjacent to complexes IV and V of the *T. gondii* mitochondrial electron transport chain, suggesting that these may be sites of ROS generation or ROS-sensitive enzyme subunits. Our studies are the first ones to demonstrate the role of TgSOD2 in parasite life and mitochondrion maintenance.

P079

Relationships of MicroRNA 511_5p and ocular clinical characteristics in patients with ocular toxoplasmosis

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Introduction: Ocular toxoplasmosis (OT), one of the common clinical manifestations of *Toxoplasma gondii* infection, is characterized by an intraocular inflammatory process (retinochoroiditis). Affected patients may have increased intraocular pressure (IOP). Variations in the expression of microRNAs (miRNAs) can result in specific immunological alterations in infected ocular tissues.
Objectives: This study correlated the expression of miR_511_5p with IOP in patients with and without OT.
Materials and methods: Patients were grouped into G1: With active ocular lesions and seropositive (n=33); G2: With ocular scars and seropositive (n=16); G3: Without ocular lesions and scars and seropositive (n=21) and G4: Without lesions or scars and seronegative (n=24). The presence of anti-*T. gondii* IgM and IgG antibodies were determined using the ELFA test. MiRNA was extracted from peripheral blood, and miRNA 511_5p was amplified by real-time PCR. Ocular characteristics were obtained from medical records and organized into right eye (RE) and left eye (LE). Non-parametric Kruskal-Wallis and ANOVA tests were used to compare the variables; Pearson's test was used to estimate the correlation between the mean expression of miRNA 511_5p and IOP values, adopting a significance level of 5%.
Results and Discussion: In G1 the mean age was 39.48±14.43 (range:19-67); in G2 the mean age was 43.31±14.59 (range: 22-65); in G3 it was 58.29±13.33 (range: 24-78); and in G4 it was 66.08±7.98 (range:53-83). There was a statistically significant difference in relation to the mean age (G1xG3= $p<0.001$, $t=4.8$, $df=52$; G1xG4= $p<0.001$, $t=8.15$, $df=55$; G2xG3= $p<0.05$, $t=3.5$, $df=35$ and G2xG4= $p<0.001$, $t=6.37$, $df=38$). The mean expression of miR 511-5p in G1 was 21.67 ± 13.67 and statistically higher than in the other groups ($p<0.001$). In G1, the expression of miR 511_5p was related to a higher mean IOP value in the LE ($p=0.04$; CI=0.014-0.66). These results demonstrate that the expression of miRNA 511_5p is elevated in patients with active ocular damage and reactive serology for *T. gondii*, and correlates with IOP. It is possible that the 511_5p miRNA influences the clinical evolution of OT. These observations may contribute to the creation of new therapeutic strategies.
Conclusion: The microRNA 511_5p can act as a biological marker for the prognosis of OT and IOP.
Keywords: microRNA, ocular toxoplasmosis, intraocular pressure, ophthalmology.

P080

Short-term *in vitro* culture adaptation of *Toxoplasma gondii* archetypal II and III field isolates induces relevant changes in the transcriptomic profile

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Type II and III archetypal strains predominate in Europe and usually show intermediate to low virulence in mice. Recently, short-term laboratory adaptation has been demonstrated in a set of 6 recently isolated canonical strains, where their phenotypic traits, in terms of parasite proliferation, cystogenesis and virulence in outbred mice, suffered from modifications after 40 passages *in vitro* (Colos-Arango et al., 2023). Aiming to investigating the molecular basis underlying such phenotypic changes, a comparative transcriptomic analysis of parasites harvested at low (10-15) and high (50-55) passage numbers, was carried out. Principal Component Analysis, showed that the transcriptional changes are mainly determined by the culture passage number and the clonal genetic type of the parasites. Passage conditions are presumably determined by specific genes related to protein synthesis, cytoskeleton and secretory pathogenesis. Enrichment analyses demonstrated that genes related to host-parasite interaction, protein synthesis, and metabolism-related mechanisms are overexpressed in Type II strains, while those related to gene expression regulation are predominant within Type III isolates. In addition, we found that genes involved in cyst formation and chronic infection in mice, were overexpressed at the low passage condition. Finally, Weighted Gene Co-expression Network Analysis was able to correlate phenotypic data obtained *in vitro* (e.g. tachyzoite yield and tachyzoite-to-bradyzoite conversion) and *in vivo* (e.g. morbidity and parasite loads in lung) with differential expression of gene modules. In conclusion, profound changes are

observed in the transcriptomic profile after short term passage *in vitro* of recently obtained *T. gondii* isolates, warranting future experiments aimed at unraveling gene expression regulation (e.g., epigenetic) in these isolates.
Funded by Spanish Research Agency through the grant PID2022-138673OB-C21
Reference
Colos-Arango et al. (2023). Short-term culture adaptation of *Toxoplasma gondii* archetypal II and III field isolates affects cystogenic capabilities and modifies virulence in mice. International Journal for Parasitology, 53(9), 491–504.

P081
Redefining the role of latency during chronic *Toxoplasma gondii* infection in the central nervous system

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Latency is a conserved microbial strategy for establishing chronic infection, and the ability of *Toxoplasma gondii* to form long-lived cysts in neurons was thought to enable parasite persistence through immune evasion. To explore the role of latency in evasion and persistence, we first developed a system of ordinary differential equations (ODE) to model immune pressure and its impacts on parasite dynamics in the central nervous system (CNS). Our model recapitulated the natural dynamics of chronic *T. gondii* infection only with the incorporation of immune pressure on both tachyzoites and latent bradyzoite cysts. The role of anti-cyst immunity outlined in the model was further supported by *in vivo* experimental data. The use of transgenic parasites with cyst-restricted model antigen expression (bag1-OVA) demonstrated that cyst-derived antigens are recognized by OT-I CD8+ T cells. OT-I T cells responding to cyst-derived antigen exhibit distinct phenotypes and cytokine production when compared to T cells responding to antigen expressed by both tachyzoites and bradyzoites (tub1-OVA). Infection in mice lacking neuronal STAT1 (*Stat1*$^{\Delta NEU}$) further demonstrated the presence of anti-cyst immunity, as *Stat1*$^{\Delta NEU}$ mice showed an increase in cyst number and size compared to WT mice. This suggests a noncytopathic mechanism of cyst control mediated by IFN-γ. However, the cellular source of IFN-γ to control neuronal cysts, responsiveness of cyst infected cells to IFN-γ, and regulation of IFN-γ production in the CNS remain active areas of investigation. To test the importance of latency in persistence, we used a mutant strain of *T. gondii* with a defect in cyst conversion (*Δbfd1*). Surprisingly, infection with *Δbfd1* parasites revealed that the latent stage is not required for long term parasite persistence. Instead of parasite clearance, the absence of cyst formation resulted in increased parasite replication in the CNS, associated tissue necrosis, and decreased host survival. Together, our datasets reveal that the latent form of *T. gondii* is under immune pressure in the CNS but promotes persistence by mitigating infection-induced damage and preventing host mortality.

P082
Uncovering the role of DEAD-box RNA helicase *Tg*mRHel1 in mitochondrial gene expression

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Apicomplexan parasites have a unique mitochondrion with a highly reduced genome. The genome consists of fragmented rRNA genes and only three protein-coding genes: *cob* (cytochrome b), *coxI* (cytochrome oxidase subunit I), and *coxIII* (cytochrome oxidase subunit III). These genes are crucial for the survival of the parasite as they encode components of the electron transport chain at the heart of oxidative phosphorylation. In *Toxoplasma gondii* the mitochondrial genome is particularly intriguing. It is composed of 23 sequence blocks that occur in non-random combinations and display an even higher degree of fragmentation. Previous work in our group has shown that genome blocks and their combinations are actively transcribed, identifying a set of 34 mitochondrial small RNAs. It is assumed that in *T. gondii* similarly to the other

Apicomplexa, mRNAs and rRNA fragments are transcribed polycistronically and processed into monocistronic forms by nuclear-encoded factors. However, the processes of mitochondrial RNA processing and translation, including their regulation, remain poorly understood. We identified a putative DEAD-box RNA helicase which we named *Tg*mRHel1. *Tg*mRHel1 localises to the *T. gondii* mitochondrion and we demonstrated it is required for parasite growth and that its absence severely impacts mitochondrial respiration. Further analysis of the mutant revealed depletion in ETC complexes III and IV. Given the compact nature of the *T. gondii* mitochondrial genome and the extensive fragmentation of rRNAs, we hypothesize that the observed defects are due to *Tg*mRHel1 playing a role in small RNA processing and mitoribosome assembly or in the processing of mRNA to form mature *coxI*, *coxIII*, or *cob* transcripts. To investigate this, we performed sucrose density centrifugation analysis and shown that in the absence of *Tg*mRHel1 mitoribosomes cannot properly assemble. We further examined the effects on mitochondrial RNAs by RT-qPCR and small RNA sequencing. Our work will present insights and first characterisation of the RNA helicase involved in mitochondrial gene expression in *T. gondii.*

P083

Cross-talk between lipid metabolism, stress response and *Toxoplasma gondii* infection in dendritic cells.

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Toxoplasmosis is a disease caused by the apicomplexan parasite *Toxoplasma gondii*. Although, the parasite is well controlled in immunocompetent people, toxoplasmosis is still a major issue for pregnant women and immunodeficient individuals. Thus, it is important to understand how *T. gondii* interacts with the immune system. The control of *T. gondii* relies on cell-autonomous immunity and the establishment of a Th1 response. Here, we aim at understanding how infection of dendritic cells (DCs) impacts their activation, metabolism, and ability to trigger adaptive immune responses. Indeed, DCs are key players in the detection of invading pathogens, and activation of specific T cells through antigen presentation. Importantly, immune signalling tightly interacts with metabolic pathways, which are known to be modulated by intracellular pathogens like *T. gondii*. We previously showed that the Unfolded Protein Response (UPR), a key sensor of lipid metabolic and oxidative stresses, is critical for pro-inflammatory cytokine production and parasite-derived MHC-I antigen presentation in infected Bone-Marrow derived DCs. In this project, using transcriptomic and lipidomic analysis, we evaluate the differential modulation of immune and metabolic pathways in type I *versus* type II parasite infected DCs. We aim at elucidating how these changes may impact on parasite vacuole membrane integrity and MHC-I antigen presentation. We also investigate the role of the IRE1 pathway of the UPR in these processes. On a larger scale, our study aims to shed light on the pivotal role of the UPR response and lipid metabolism in the regulation of cellular defenses triggered against intracellular pathogens.

P084

IFN-gamma production by brain-resident cells activates cerebral mRNA expression of a wide spectrum of molecules critical for both innate and T cell-mediated protective immunity to control reactivation of chronic infection with *Toxoplasma gondii*

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We previously demonstrated that brain-resident cells including microglia produce IFN-g in response to reactivation of cerebral *Toxoplasma gondii* infection, and that their IFN-g production is critical for preventing reactivation of the infection. To obtain an overall landscape view of the effects of IFN-g from brain-resident cells on the cerebral protective immunity, in the present study we employed NanoString nCounter assay and quantified mRNA levels for 734 genes in myeloid immunity in the brains of T and B cell-deficient, bone marrow chimeric mice with and without IFN-g production by brain-resident cells in response to reactivation of cerebral *T. gondii* infection. Our study revealed that IFN-g produced by brain-resident cells amplified mRNA expression for the molecules to activate the protective innate immunity including 1) chemokines for recruitment of microglia and macrophages (CCL8 and CXCL12) and 2) the molecules for activating those phagocytes (IL-18, CD180, and NOD1). Importantly, IFN-g produced by brain-resident cells also upregulated cerebral expression of molecules for facilitating the protective T cell immunity, which include the molecules for 1) recruiting effector T cells (CXCL9, CXCL10, and CXCL11), 2) antigen processing (PA28ab, LMP2, and LMP7), transporting the processed peptides (TAP1 and TAP2), assembling the transported peptides to the MHC class I molecules (Tapasin),

and the MHC class I (H2-K1 and H2-D1) and Ib molecules (H2-Q1, H-2Q2, and H2-M3) for presenting antigens to activate the recruited CD8+ T cells, 3) MHC class II molecules (H2-Aa, H2-Ab1, H2-Eb1, H2-Ea-ps, H2-DMa, H2-Ob, and CD74) to present antigens for CD4+T cell activation, 4) co-stimulatory molecules (ICOSL) for T cell activation, and 5) cytokines (IL-12, IL-15, and IL-18) facilitating IFN-g production by NK and T cells. Notably, the present study also revealed that IFN-g production by brain-resident cells also upregulates cerebral expressions of mRNA for the down-regulatory molecules (IL-10, STAT3, SOCS1, CD274 [PD-L1], IL-27, and CD36), which can prevent overly stimulated IFN-g-mediated pro-inflammatory responses and tissue damages. Thus, the present study uncovered the previously unrecognized the capability of IFN-g production by brain-resident cells to upregulate expressions of a wide spectrum of molecules for coordinating both innate and T cell-mediated protective immunity with a fine-tuning regulation system to effectively control cerebral infection with *T. gondii*.

P085

CRISPR/Cas9 screens for the identification of essential host factors for *Toxoplasma gondii* infection

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Toxoplasma gondii is an apicomplexan parasite infecting virtually all warm-blooded animals, including birds. Currently, none of the treatments available are sufficient to eliminate quiescent tissue cysts and eradicate the parasite from its host. Notably, obligate intracellular parasites such as *Toxoplasma* heavily rely on the host cell, *e.g.* for the acquisition of crucial metabolites and the disposal of toxic waste products. Still, to date little is known regarding the host factors that this pathogen requires for intracellular development and egress, as much of the existing research in the field of host-parasite interaction predominantly focuses on the parasite itself rather than the host. Here, we performed unbiased and fluorescence-activated cell sorting based genome-wide CRISPR/Cas9 knockout screens with the aim of identifying host factors that potentially contribute to initiation and progression of the lytic cycle in human cells. Based on our initial results, we designed a custom library of sgRNAs targeting 3549 enriched genes and we performed secondary small-scale pooled CRISPR screens at a higher coverage to increase confidence. These screens will be validated by in-depth phenotypic characterization of the most promising hits using single-gene KO and microscopy-based arrayed approaches to shed new light on *Toxoplasma* host-dependency. Some of the metabolic factors identified may also be required by other parasites of the phylum Apicomplexa, constituting shared unifying fingerprints across different species. If successful, our results would broaden existing knowledge on host-pathogen interactions and offer new promising avenues for the treatment of toxoplasmosis by specifically targeting the host instead of the parasite.

P086

Unraveling the role of IL-36γ in host susceptibility during *Toxoplasma gondii* infection

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The IL-36 cytokines (IL-36α, IL-36β, IL-36γ) are an emerging family of interest that play a multifaceted immune regulatory role in various diseases and infections. Each of the three cytokines bind to the same IL-36R receptor and are therefore thought to have similar biological function. In some contexts, IL-36 plays a proinflammatory role, while in others it promotes an anti-inflammatory effect. Here, we aim to define how IL-36 influences susceptibility and establishment of immunity in the small intestine and mesenteric lymph nodes following oral infection with ME49 *Toxoplasma* cysts. We first examined expression of these cytokines in intestinal epithelial cells and intestinal epithelial organoids where we found strong upregulation of IL-36γ, but not IL-36α or IL-36β, in response to *T. gondii* infection. Accordingly, we focused our attention on IL-36γ for subsequent investigation. Survival studies using IL-36γ$^{+/+}$ and IL-36γ$^{-/-}$ mice indicated that the knockout strain displays greater susceptibility, as measured by elevated parasite burden in the small intestine ileum and decreased survival following oral inoculation. As a surrogate indicator of inflammation, we measured lipocalin-2 levels in fecal pellets, ileum contents, and in supernatants of cultured lamina propria cells. We found that IL-36γ$^{-/-}$ mice have higher levels of lipocalin at steady state than IL-36γ$^{+/+}$, and levels were further heightened after cyst inoculation. During acute infection, increased IFN-γ production was found in both the lamina propria and mesenteric lymph nodes of IL-36γ$^{-/-}$ relative to wild-type mice, with no marked difference in IL-12 production between the two strains. Thus, we

are now investigating sources of IFN-γ in both compartments. IL-36γ$^{-/-}$ mice display no difference in frequency or IFN-γ production by T cells in either compartment. While innate lymphoid cell frequency is increased in the lamina propria of IL-36γ$^{-/-}$ mice, IFN-γ expression appears to be independent of IL-36γ. Ongoing investigations are assessing other potential IFN-γ sources including natural killer cells and neutrophils. Collectively we report that IL-36γ influences host susceptibility to *Toxoplasma* infection.

P087

First data of *Toxoplasma gondii* genotyping from animals in Ukraine

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Toxoplasma gondii is a protozoan parasite that can cause disease in many animal species as well as in humans. Isolation and genotyping of *T. gondii* provide useful information on distribution of different genotypes in a particular area, as well as the potential virulence and infection sources. No information is available on the parasite genotypes infecting animals or humans in Ukraine.

According to our previous studies, the seroprevalence of *T. gondii* in Ukraine among sheep was 26.1% (57/218). At the next stage of our research, DNA of *T. gondii* was detected in the heart apex muscles using real time PCR in four sheep slaughtered for human consumption in Ukraine out of 21 (randomly selected) investigated (19.0%). DNA of the parasite was genotyped using nested PCR-sequencing and in silico RFLP analysis of 10 genetic markers and microsatellite (MS) typing by sequence length polymorphism of 15 MS. The partial typing of two samples revealed that the parasites are type II-like, which is one of the most common in Europe.

Out of 43 serum samples from free-range chickens, 6 were seropositive (14.0%). When examining the brain tissue and neck muscles of these chickens, *T. gondii* DNA was detected by real time PCR in only one brain sample, which is planned to be genotyped in the near future.

This is the first time *T. gondii* DNA isolates from Ukraine were genotyped. Further studies will be conducted to increase the number of genotyped isolates, also from further host animal species. Such data is necessary to increase our knowledge on the population structure of *T. gondii.*

P088

Estimating *Toxoplasma gondii* seroprevalence in wild boars (*Sus scrofa*) hunted in Ukraine, using three serological methods

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Toxoplasma gondii is an important zoonotic parasite worldwide. Wild boars are considered a good indicator host species for presence and spread of *T. gondii*, and they can also constitute a source of infection to other hosts. We tested samples from wild boars hunted in Ukraine using three methods: locally available ELISA, commercial multispecies ELISA, and IFAT. With the locally available ELISA, 35 of 452 wild boars tested seropositive, yielding a seroprevalence estimate of 7.7% (95% confidence interval 5.5-10.5). Using the majority criteria, 10/92 samples that were tested using all three tests were considered positive, yielding an estimated seroprevalence of 10.9% within the subset of samples.

Reference: Galat, M., Moré, G., Frey C.F., Kovalenko G., Maliuk, I., Halka, I., Sytiuk, M., Bezymennyi, M., Galat, V., Jokelainen, P., 2024. Seroprevalence of *Toxoplasma gondii* in wild boars (*Sus scrofa*) hunted in Ukraine. Int. J. Parasitol. Parasites Wildl. 23, 100901. https://doi.org/10.1016/j.ijppaw.2023.100901.

P089

Human MHC Class I Molecule, HLA-A2.1, Medicates Activation of CD8+ T cell IFN-g Production and the T cell-Dependent Protection against Reactivation of Cerebral *Toxoplasma* Infection

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HLA-A2.1 is one of the most common MHC class I molecules in humans. To examine whether antigen presentation by the HLA-A2.1 activates the protective immunity against reactivation of cerebral infection with *Toxoplasma gondii*, C57BL/6-background HLA-A2.1-transgenic and wild-type (WT) mice were infected and treated with sulfadiazine beginning at 7 days after infection for 10 days to control tachyzoite proliferation and establish chronic infection in their brains. Due to the genetic susceptibility of C57BL/6 mice to chronic *T. gondii* infection, these mice develop reactivation of the infection after discontinuation of sulfadiazine treatment. One month after discontinuation of sulfadiazine, mRNA levels for tachyzoite-specific SAG1 and numbers of the foci associated tachyzoites were significantly less in the brains of the HLA-A2.1-transgenic than WT mice. Greater numbers of IFN-g-producing CD8+ T cells were detected in the spleens of infected transgenic than WT mice, and CD8+ T cells from the former produced markedly greater amounts of IFN-gthan the T cells from the latter in response to tachyzoite antigens *in vitro*. When their CD8+ T cells were systemically transferred to infected immunodeficient NSG mice expressing the HLA-A2.1, the CD8+ T cells from HLA-A2.1-transgenic mice inhibited reactivation of the cerebral infection in the recipients more efficiently than did the WT T cells. Furthermore, the inhibition of reactivation of the infection by CD8+ T cells from the transgenic mice was associated with increased cerebral expression of IFN-g and effector molecules against tachyzoites in the recipients when compared to the WT CD8+ T cell recipients. Thus, the human HLA-A2.1 is able to effectively activate IFN-g production of CD8+ T cells against *T. gondii* tachyzoites and confer a potent protection against reactivation of cerebral infection with this parasite through the CD8+ T cells activation.

P090

In vitro production of cat-restricted *Toxoplasma* pre-sexual stages by epigenetic reprogramming

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Sexual reproduction of *Toxoplasma gondii*, confined to the felid gut, remains largely uncharted owing to ethical concerns regarding the use of cats as model organisms. Chromatin modifiers dictate the developmental fate of the parasite during its multistage life cycle, but their targeting to stage-specific cistromes is poorly described. Here we found that the transcription factors AP2XII-1 and AP2XI-2 operate during the tachyzoite stage, a hallmark of acute toxoplasmosis, to silence genes necessary for merozoites, a developmental stage critical for subsequent sexual commitment and transmission to the next host, including humans. Their conditional and simultaneous depletion leads to a marked change in the transcriptional program, promoting a full transition from tachyzoites to merozoites. These in vitro-cultured pre-gametes have unique protein markers and undergo typical asexual endopolygenic division cycles. In tachyzoites, AP2XII-1 and AP2XI-2 bind DNA as heterodimers at merozoite promoters and recruit MORC and HDAC3 , thereby limiting chromatin accessibility and transcription. Consequently, the commitment to merogony stems from a profound epigenetic rewiring orchestrated by AP2XII-1 and AP2XI-2. Successful production of merozoites in vitro paves the way for future studies on *Toxoplasma* sexual development without the need for cat infections and holds promise for the development of therapies to prevent parasite transmission.

P091

Characterization of a Lanthionine-Synthetase C in *Toxoplasma*

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Toxoplasma gondii, adapted to obligate intracellular parasitism, shows a remarkable capacity for nutrient sensing and metabolic rewiring during asexual reproduction in mammalian host cells. Cyclic nucleotides- and AMPK-mediated signaling are suggested to sense the parasite milieu, thereby controlling the extracellular-infective and intracellular-replicative transition. We found a lanthionine synthetase C family protein (LanCL) in *T. gondii*, which is potentially involved in ligand sensing and redox balance. *Tg*LanCL is structurally similar to *Hs*LanCL2, indicating a functional convergence in substrate/ligand binding. 3"-genomic tagging of *Tg*LancL revealed its localization in the parasite cytosol. We are now investigating the physiological relevance and interaction partners of *Tg*LanCL in tachyzoites.

P092

Serological determination of *Toxoplasma gondii* infection in sheep and molecular detection of *Toxoplasma gondii* in ovine aborted fetus in Gilan province, North of Iran.

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Toxoplasma gondii is one of the most common meat-born protozoan parasites that cause congenital infection, abortion, and stillbirth in humans and animals. sheep are one of the important sources of meat production in Gilan Province. Toxoplasmosis in patients with intact immune systems is usually asymptomatic, but can be life-threatening in patients with a weak immune system. Gilan is divided into three regions of plains with moderate climatic conditions, hillsides with semi-humid climates, and heights with cold mountainous weather. Climate situations are involved in the prevalence of toxoplasmosis. According to the geographical location and age of the animals, the samples were divided into different groups.

The present study aimed to investigate the seroprevalence of *T. gondii* infection in sheep and molecular detection to determine the genetic diversity of *T. gondii* in ovine aborted fetus in Gilan Province, North of Iran.

In a current cross-sectional study, 400 sheep sera samples were tested for *T. gondii*-IgG antibody by the enzyme-linked immunosorbent assay technique (ELISA). In addition, molecular detection was performed on 44 brain samples of ovine aborted fetuses by nested pcr method using GRA6 gene . Also, genotyping was conducted with GRA6 gene on positive isolates by PCR RFLP method.

The results showed that *T. gondii*-IgG antibody was detected in 166 sera samples of sheep (41.5%). The highest frequency (72.7%, n=56) was observed in the age group of >4 years, which the difference was statistically significant (P = 0.0001) in comparison with other groups. Also, the seroprevalence of *T. gondii* was significantly higher in plains (53.9%) than those of hillsides and heights (P = 0.0001).Moreover , molecular detection showed that the GRA6 gene was detected in 30 brain samples of ovine aborted fetuses (68.18%). PCR RFLP showed that all of the isolates were belonging to the type II T.gondii genotype.

consequently The prevalence of *T. gondii* infection in Gilan Province is high, indicating a significant relationship with geographical location and age of animals.

Keywords: Toxoplasmosis, Enzyme-Linked Immunosorbent Assay, Genotyping, Abortion, Sheep.

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P093

Investigating the response to iron deprivation in *Toxoplasma*

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Iron is essential for almost all life due to its role as a cofactor in numerous biological processes. Excess iron is however toxic, meaning regulation of cellular iron abundance is critical. For *Toxoplasma gondii* iron must be obtained from host cells, however its host cell promiscuity means it must contend with diverse and dynamic levels of accessible iron. To investigate how Toxoplasma regulate and respond to changing iron availability, we used quantitative proteomics to determine the impact of iron deprivation on the *Toxoplasma* proteome. We analysed changes to 4742 proteins from *Toxoplasma* tachyzoites and found that 1373 proteins had significant changes in their abundance. Of these significant changes, 835 proteins were downregulated, suggesting a general decrease in protein abundance in response to iron deprivation. Low iron predictably induced downregulation of proteins associated with DNA replication, supporting previous observations of reduced growth under these conditions. Iron deprived parasites also exhibited a downregulation of translation machinery and an accompanying reduction to translation measured by puromycin incorporation. Despite this reduction to translation, proteins related to glycolysis are upregulated in these iron deprived parasites. Metabolic analysis determined that these parasites also exhibit a reduction in mitochondrial oxygen consumption. These data highlight the importance of iron to diverse processes and indicate that while downregulating protein synthesis Toxoplasma also rewire their metabolism in response to iron deprivation.

P094

Exploring the Role of PCKMT in the Gliding Initiation Complex: Implications for *Toxoplasma gondii* Infectivity and Motility

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Toxoplasma gondii's ability to move is crucial for its pathogenesis. Our recent research has discovered that the initiation of motility depends on the presence of the gliding initiation complex (GIC), which consists of key components such as the structural protein conoidal gliding protein (CGP), a putative lysine methyltransferase (PCKMT), and the actin nucleator Formin 1 (FRM1).The stability of the preconoidal rings (PCRs), the primary location for these three components, heavily relies on the presence of CGP. However, our data suggest that the initiation of motility and the recruitment of FRM1 to intact PCRs relies on the activity of PCKMT.

Proximity labelling assays have revealed a group of proteins that may interact within the GIC complex, and their proximity to other known proteins involved in initiating motility and transmitting force, such as the apical methyltransferase (AKMT) and the glideosome connector (GAC).

Our study aims to analyse the potential implication of these GIC interactors in motility, as well as the activity of the methylase domain of PCKMT and its role in actin dynamics and motility.

P095

Toxoplasma CRMPs: microneme sensors specifically devoted to rhoptry secretion

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Toxoplasma gondii owes its unparalleled success as pathogen to a sophisticated arsenal of secretory organelles filled with virulence factors, concentrated at the parasite apex. The content of these micro-weapons, called micronemes and rhoptries, is used by the parasite to invade and take control of the host cell and subsequently, upon replicating, to escape from the host and find a new prey. Micronemes and rhoptries are secreted sequentially to fulfill distinct functions: microneme proteins ensure parasite egress, motility and host recognition, while rhoptry proteins are key for vacuole formation and subversion of host immune response. In addition, specific microneme and rhoptry proteins interact to form a tight junction at the host-parasite interface, supporting parasite entry into the host. Rhoptry discharge appears to rely on microneme secretion since the microneme protein MIC8 and the CLIP/CLAMP/SPATR microneme complex have been shown to have an exclusive role in regulating rhoptry release. We recently described *Toxoplasma* cysteine-repeat

modular proteins CRMPa and CRMPb, which are part of a complex that comprises four essential mediators of rhoptry secretion. TgCRMPs and partners contain host cell binding domains and are translocated to the apical end of extracellular parasites at the onset of invasion, suggesting that they activate rhoptry discharge upon sensing the host. Here, we further characterize *Toxoplasma* CRMPs and investigate their possible association to micronemes. Using ultrastructure expansion microscopy (U-ExM), we confirm with a higher resolution that TgCRMPs show partial overlap with microneme markers. Their accumulation at the apical tip of extracellular parasites during invasion requires functional micronemes exocytosis, since it is abolished in parasites depleted of the TFP1 transporter that are defective in both biogenesis and secretion of micronemes. In addition, we show that TgCRMPs undergo proteolytic processing by the ASP3 protease, an enzyme responsible for the maturation of microneme and rhoptry factors. Altogether these data support a scenario where CRMPs traffic towards the parasite apex through micronemes and are then translocated in proximity of the rhoptry exocytic site upon microneme discharge.

P096

Phospholipid-mediated regulation of calcium homeostasis and lytic cycle in Toxoplasma

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Glycerophospholipids have emerged as a significant contributor to the intracellular growth of Toxoplasma gondii. Phosphatidylserine (PtdSer) and phosphatidylthreonine (PtdThr) are two such lipids attributed to calcium-regulated gliding locomotion and motility-dependent invasion and egress events in T. gondii. However, endogenous synthesis of PtdSer and importance of de novo pathway in tachyzoites remain poorly understood. Besides, it is not clear how these lipids contribute to the regulation of calcium homeostasis in the parasite. Here, we show that conditional depletion of PtdSer synthase (PSS) and sarcoendoplasmic reticulum calcium- ATPase (SERCA) in the endoplasmic reticulum of tachyzoites abrogates the lytic cycle due to impaired cell division and gliding motility. The base-exchange-type PSS enzyme produces PtdSer, which is rapidly converted to phosphatidylethanolamine (PtdEtn). The PSS-depleted mutant exhibits a lower abundance of the major ester-linked PtdEtn species with concurrent accrual of host-derived ether-PtdEtn species. Most phosphatidylthreonine (PtdThr) species – a rare natural analog of PtdSer, also made in the endoplasmic reticulum – were repressed, while the amount of PtdSer remained largely unaltered, likely driven by the serine-exchange reaction of the PtdThr synthase (PTS) in favor of PtdSer production upon PSS depletion. On the other hand, the PTS-knockout mutant lacks PtdThr but displays a proportionally higher content of PtdSer, further asserting a functional relation between the two lipids. Furthermore, PTS mutant is impaired in calcium homeostasis and gliding motility but ectopic expression of SERCA restored its lytic cycle, suggesting a physiological dependence of the calcium pump on endoplasmic reticulum"s lipid milieu. Our extended work on structure modeling of SERCA and ligand docking with an in-house library comprising >5000 chemicals identified "drug-like" analogs (RB-15, NR-301) inhibiting the parasite growth. In a nutshell, we reveal SERCA as a druggable lipid-assisted calcium pump, and highlight a physiologically-vital repurposing of PtdSer and PtdThr in a clinically-relevant intracellular pathogen.

P097

2,3-dihydro-1,4-benzodioxins are potent inhibitors of *T. gondii* tachyzoite regrowth *in vitro*

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Existing therapies for toxoplasmosis have numerous deficiencies including frequent toxic side effects and the inability to completely eradicate infection. We have screened the Global Health Chemical Diversity Library (GHCDL) for activity against *T. gondii* tachyzoites. The GHCDL is a 68,000-member library of diverse molecules selected for physicochemical characteristics that predict excellent oral absorption and widespread distribution into diverse tissues. In this screen, the largest ever conducted against *T. gondii*, we identified a novel molecule with a 2,3-dihydro-1,4-benzodioxin chemotype that inhibits the growth of *T. gondii* tachyzoites *in vitro* with an EC50 of 275 nM. This compound and related derivatives also prevent the regrowth of *T. gondii* tachyzoites *in vitro* at concentrations that are a half log (3.16x) above their EC50s.

While current drugs in clinical use and compounds in preclinical development can prevent the *in vitro* regrowth of *T. gondii*, typically concentrations that are hundreds or thousands of multiples of the EC50 are needed. This finding suggests that the 2,3-dihydro-1,4-benzodioxins interrupt a vital process in *T. gondii*, one that may allow for a therapy capable of eradicating infection. We present data regarding the structure-activity relationships of the 2,3-dihydro-1,4-benzodioxin chemotype as well as genetic evidence which supports a novel mode of action. Future efforts will focus on creating derivatives with enhanced potency and metabolic stability.

P098

Elucidating the Role of CST in Cyst Wall Structure and Integrity

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The cyst wall, acting as the interface between dormant cysts and the host cell, is thought to offer *Toxoplasma gondii* protection from the host environment. However, the specifics of cyst wall formation and the functional roles of CST1 are not well understood. Our study seeks to elucidate the role of CST1, a major cyst wall protein, in constructing the cyst wall layer through the formation of a filament network mediated by its distinct domains. To this end, we engineered several CST1 truncation mutants and assessed their impact on cyst wall integrity and the localization of other cyst wall proteins such as MCP4. Our findings reveal that the cysteine-rich domain of CST1 is pivotal for polymerization, facilitating the formation of inter-molecular disulfide bonds. The mucin and SRS domains are crucial for maintaining the integrity of the cyst wall and retaining various cyst wall proteins, including MAG1. Utilizing a crosslinking mass spectrometry approach (XL-MS) we are constructing an interactome of the cyst wall, delineating the interactions between proteins, and investigation the structure of CST1. Our findings led to the development of a model for the cyst wall filament network, in which the CST1 homopolymers form the spongy inner layer, acting as a scaffold for other cyst wall proteins to establish the structure. These insights into the function of CST1 provide a deeper understanding of cyst wall architecture and its critical role in parasite survival.

[Funding: NIHAI134753]

P099

Biochemical and Molecular Elucidation of Hydroquinine Mechanism(s) of Action against *T. gondii*

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Hydroquinine also known as dihydroquinine (DHQ), is a quinine-derived compound. DHQ has a history of inhibiting *Plasmodium falciparum*, and *Plasmodium berghei*, and possesses anti-arrhythmia properties. Though, previous studies in *Plasmodium* spp have shown its target to be nucleic acids and protein synthesis. Our team recently showed that it disrupts mitochondria membrane potential, upregulates ROS production, and depletes ATP production in *T. gondii*. To unify these observations with previously identified targets, we tested for the first time the effect of DHQ on *T. gondii* tachyzoites metabolites and lipid production in a concentration dependent manner. Interestingly, the multi-omics (metabolomics and lipidomics) studies showed that DHQ affects certain lipid classes, nucleic acid precursors, and amino acid synthesis in a concentration-dependent manner. Also, for the first time, *in silico* analysis showed that DHQ binds strongly to DNA gyrase, Calcium Dependent Protein Kinase 1 (CDPK 1), and prolyl tRNA synthetase and thus could affect DNA replication and translational activities in *T. gondii*. In summary, our findings indicate that DHQ will be an effective anti-*T. gondii* agent and could be further developed for clinical use.

Keywords: Dihydroquinine, metabolomics, lipidomic, DNA Gyrase, tRNA Synthetase, Calcium Dependent Protein Kinases, *T. gondii*

P100

Deciphering the host defence mechanisms targeting *Toxoplasma* in retinal epithelial cells

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Toxoplasma strains are restricted to a few clonal variants in Europe and North America and infections are usually asymptomatic unless individuals are immunosuppressed. This contrasts with the occurrence of multiple diverse *Toxoplasma* strains in South America where clinical disease manifests as ocular and congenital toxoplasmosis even in immunocompetent individuals, leading to high morbidity and mortality.
We have previously shown that *Toxoplasma* strains actively invade and proliferate within endothelial cells, with a proportion of parasites being eliminated in an acidified vacuole after immune stimulation of the cells by interferon gamma (IFNg)[1]. In macrophages, however, we have demonstrated that parasites are killed by a mechanism involving GBP1-mediated vacuole breakage and Caspase 8-dependent apoptotic host cell death[2].
Our study explores IFNg-driven control of diverse *Toxoplasma* strains in retinal pigment epithelial cells (ARPE-19), where the mechanism of host defence and parasite elimination is not well understood. We show that IFNg-stimulation of these non-immune cells initiates cell death after infection with type I RH *Toxoplasma* and a variety of South American strains. Host defence molecules are also recruited to parasite vacuoles in an IFNg-dependent manner. We are delineating the pathways that cause infection-driven host cell death and the host proteins driving this at the vacuolar membrane in order to better understand the drivers of ocular toxoplasmosis.

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P101

RNA triphosphatase-mediated mRNA capping is essential for maintaining transcriptome homeostasis and the survival of *Toxoplasma gondii*

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RNA processing is necessary for the protozoan parasite *Toxoplasma gondii* to accomplish differential gene expression required during life cycle stage transitions. Here, we show how RNA capping, the first major pre-mRNA processing event, safeguards transcriptome integrity in Toxoplasma. A functional RNA capping system of Toxoplasma consists of separate RNA triphosphatase (TgRT), guanylyltransferase (TgGT), and guanine-N7-methyltransferase (TgGMT) enzymes, which together add 5” 7-methylguanosine (m^7G) cap to RNA. In vitro, these capped RNAs bind to the Toxoplasma translation initiator factor, eIF4E, and are translated to protein upon transfection in the parasite. Biochemical and genetic characterization reveal that among three capping enzymes, triphosphatase (TgRT) is unique and a member of the tunnel family of metal-dependent phosphohydrolases, structurally and mechanistically unrelated to the human cysteine-phosphatase-type RNA triphosphatases. Using cap sequencing, we demonstrate that the knockdown of TgRT diminished global m^7G-capped transcripts of genes primarily related to DNA packaging and cell membranes, which resulted in a complete arrest of parasite replication in culture and in vivo protected mice from lethal infection. Overall, this study shows the critical role of TgRT in gene expression and Toxoplasma survival, which may be conserved in the related parasites, thereby presenting RNA triphosphatase as an attractive target for protozoan infections.

P102

Expansion of *Toxoplasma gondii* cysts *in vitro* using enzymatically enhanced ultrastructure expansion microscopy

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Expansion microscopy (ExM) is an innovative approach to achieve super-resolution images without using super-resolution microscopes, based on the physical expansion of the sample. The advent of ExM has unlocked super-resolution imaging for a broader scientific circle, lowering the cost and entry skill requirements to the field. One of its branches, ultrastructure ExM (U-ExM), has become popular among research groups studying Apicomplexan parasites, including the acute stage of *Toxoplasma gondii* infection. The chronic cyst-forming stage of *Toxoplasma*, however, resists U-ExM expansion, impeding precise protein localisation. Here, we solve the cyst"s resistance to denaturation required for successful U-ExM of the encapsulated parasites. As the cyst's main structural protein CST1 contains a mucin domain, we added an enzymatic digestion step using pan-mucinase StcE prior to the expansion protocol. This allowed full expansion of the cysts in fibroblasts and primary neuronal culture without interference with the epitopes of the cyst-wall associated proteins. Using StcE-enhanced U-ExM, we clarified the shape and location of the GRA2 protein important for establishing a normal cyst. Expanded cysts revealed GRA2 granules spanning across the cyst wall, with a notable presence observed outside on both sides of the CST1-positive layer.

P103

Recurrent ocular toxoplasmosis after SARS-CoV-2 infection: three case reports

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Ocular toxoplasmosis (OT) is the main cause of infectious posterior uveitis worldwide, that can lead up to complete loss of vision. Since tissue cysts of *Toxoplasma gondii* persist in the retina following infection, patients with OT have a lifelong risk of reactivation. As SARS-CoV-2 alters the host's immune response during COVID-19, modulating cytokines known to regulate the chronic *T. gondii* infection, it could potentially trigger the recurrence of OT. In our laboratory, three cases of OT after SARS-CoV-2 infection have been documented over the period of four years (2020-2023), in otherwise immunocompetent adults. The first case of recurrent OT after symptomatic SARS-CoV-2 infection occurred during Delta, and the other two, both asymptomatic, during the peak of the Omicron wave in Serbia. All cases of OT were recurrent, as patients had chorioretinal scarring noted before. *T. gondii*-specific IgG antibodies of high avidity were detected in all cases, in an absence of specific IgM antibodies. In first case, a 20-year-old female developed symptoms of COVID-19, confirmed by a positive rapid antigen test, five days after the first dose of an mRNA vaccine (BNT162b2). A month later, she experienced blurred vision and photophobia in her left eye, which was discovered to be caused by an active, peripheral chorioretinal lesion. In second case, a 26-year-old female reported having blurred vision and photophobia in the right eye, 1.5 months after a positive rapid antigen SARS-CoV-2 test and asymptomatic COVID-19. Upon examination, an active paramacular lesion was noted in the right eye. In third case, a 26-year-old female developed blurred vision in the right eye, following recurrent focal chorioretinitis. Patient was not vaccinated against SARS-CoV-2 and denied having COVID-19. However, IgG antibodies specific for the receptor-binding domain of SARS-CoV-2 S protein (47.78 BAU/ml) were detected in this sample, indicating a possible recent asymptomatic infection. Having in mind profound consequences of OT, an ophthalmology exam is strongly advised in cases with positive SARS-CoV-2 diagnostic test and known history of OT, regardless of the presence of COVID-19 symptoms.

P104

Reinvention of the diagnostic algorithm and treatment options for reactivated toxoplasmosis – ToxoReTREAT project

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Toxoplasma gondii is a protozoan parasite infecting nearly one third of the global population. Severe immunosuppression in chronically infected individuals results in reactivated toxoplasmosis (RT), a devastating complication following hematopoietic stem cell transplantation (HSCT), particularly in allogeneic (allo-) recipients. Despite the staggering annual rise in the number of HSCT across Europe, there are still no standardized guidelines on the monitoring and treatment of RT. Moreover, limited treatment options for RT are inadequate for HSCT patients due to side myelotoxicity and ineffective since they act only on circulating parasites. Our project entitled "Reinvention of the diagnostic algorithm and treatment options for reactivated toxoplasmosis" (acronym ToxoReTREAT) funded for the next 3 years by the Science Fund of the Republic of Serbia (as of December 1st, 2023), is using multidisciplinary approach to tackle this challenge.
The aim of ToxoReTREAT is to resolve the problem of RT in allo-HSCT recipients combining medicine, pharmacy and computer science to provide efficient novel treatment options and cost-benefit improvements in the management of RT. The results of molecular monitoring of RT (18 allo-HSCT recipients already recruited), *T. gondii* isolation and characterization, identification of host-related and treatment-related risk factors, will lead to improved diagnostic algorithm and eventually the up-to-date National guidelines. Acridine and acridone derivatives, newly synthetized and modified throughout this project (20 acridines already synthetized), will be tested in both *in vitro* and *in vivo* experimental models of RT, using *T. gondii* strains isolated from allo-HSCT recipients. ToxoReTREAT will provide at least one drug candidate for RT with reduced toxicity and increased overall efficacy on *T. gondii* tachyzoites and bradyzoites, in comparison to standard treatment. This research should instigate an increased interest of the pharmaceutical industry to engage in development of better new drugs for the treatment of toxoplasmosis. Application of a freely available ImageJ software to advance automated image scoring for chemotherapy results (we"ve obtained 15 high-resolution images of in vitro experimental results so far), both straightforward and cost-effective mathematically quantifiable approach, will contribute to more feasible transfer of knowledge gained through chemotherapy experiments.

P105

Molecular evidence of *Toxoplasma gondii* infection, neuropathology and immunofluorescent detection in the amygdala, hippocampus, pre-frontal cortex, and occipital areas from forensic human brain tissue from suicide, traffic accidents, and homicide victims.

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Several studies have linked to human toxoplasmosis with neuropsychiatric disorders, (Contopoulus-loannidis, Gianniki, Ai-Nhi Truong, & Montoya, 2022) (Milne, Webster, & Walker, 2020) (Sutterland, y otros, 2015) suicide behavior (Soleymani, Faizi, Heidarimonghadam, Davoodi, & Mohammadi, 2020) and traffic accidents (Sutterland, y otros, 2019; Gohardehi, y otros, 2018). Few studies in forensic samples are looking for the correlation between positive results using PCR or Immunohistochemistry to detect *T. gondii* presence in brain tissue from mental disease (Alvarado-Esquivel, y Otros, 2021) (Conejero-Goldberg, Torrey, & Yolken, 2003), traffic accidents (Samojlowicz, y otros, 2019) or suicide behavior (Mendoza-Larios, y otros, 2023). All of these report no statistical association and low rates of prevalence. new questions are made around the findings in the neurological areas associated with suicide behavior (Abou Chahla, y Otros, 2023) of people with and without mental disease (Kékesi, y Otros, 2012) and in relationship to infection associated with behavior changes (Boillat, y otros, 2020; Berenreiterová, Flegr, Kubëna, & Nëmec, 2011), to human brain architecture change (Halonen, Lyman, & Chiu, 1996) and neurotransmitters change from tissue infected with strains type I (Xiao, Ye, Jones-Brando, & Yolken, 2013). Methodology: prospective case-control study where the cases correspond to suicide victims and controls were assigned to two groups: pedestrian and homicide victims from the coffee region of Colombia. Samples correspond to ~4mg of brain tissue from the amygdala, hippocampus, occipital, and prefrontal areas

and ~2mg for indirect immunofluorescence antibody test (IFAT) and histopathological analyses. Inclusion criteria: only habitants belong to this region with i) consent informed signed, ii) 8 brain samples, iii) one serum sample iv) samples labeled with high quality, and v) three days *postmortem* maximum. Of the 57 forensic events, 29,8% (17/57) of the cases were positive by real-time PCR: the suicide group (26.9%) and (32.2%) in the control group (6/17 in homicide and 4/14 in traffic accidents). There were individuals with three positive areas in suicide, the neurological area with the highest positivity by real-time PCR was the amygdala (p=0.03). IFAT with anti-BAG1 antibody was positive in six of 21 cases (28.5%), Cysts ~30 µm were found with an approximate density of one cyst per 20 fields. In 42 forensic serum samples, (45.2%) IgG titers

P106

Single-RNA sequencing analysis of merozoite stages of Toxoplasma gondii in the intestine of cats

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Toxoplasma gondii, a distinctive intracellular parasite, is capable of infecting almost all warm-blooded animals. Its merozoite stage, restricted to epithelial cells of the intestinal of final hosts (felines), is categorized into five distinct types— —types A through E, but the kinetic development processes through A to E and the variation in gene expression levels among these five types have not been extensively explored.

To elucidate the transcriptional patterns of single individuals of the five types, we harvested merozoites 35 hours and 7 days post tissue cyst infection and conducted single-cell RNA sequencing. Through this process, we were able to effectively get 6,762 parasites and categorize them into 7 clusters after clustering analysis. Cluster 4, consisting of 467 parasites, was identified as tachyzoites due to their specific expression of *Sag1*. Cluster 0, with 2,635 parasites, exhibited specific expression of both *Sag2A* and *LDH2* (but not *Gra11b*), such being defined as tachyzoite-bradyzoite intermediates. Other five clusters all expressed a merozoite marker gene, *GRA80*, with Cluster 1, 2, 3, and 6 also expressing *Gra11b*. Additionally, Cluster 1 highly expressed *SRS22E* (1,184 parasites), Cluster 2 expressed AT1 but not *TGME49_287040* (1,055 parasites), Cluster 3, expressed *TGME49_231880* without *LDH2* (993 parasites), Cluster 5, specifically expressed *SRS22b* (330 parasites), and Cluster 6, specifically expressed *TGME49_278410* (124 parasites).

To correlate our transcriptional findings with Types A to E, we constructed a reporter strain employing *Eno2*, *Bag1*, and *SRS22b* as promoters, which resulted in Eno2-induced green fluorescence in the nucleus, *LDH2*-induced red fluorescence in the cytoplasm, and *SRS22b*-induced green fluorescence on the parasite membrane or cytoplasm. Intestinal smears from cats 7 days post infection of this strain showed that *Eno2* was present in B through E, with Type D also expressing *SRS22b*.

Furthermore, one reporter strain with promoters for *Sag2A*, *LDH2*, and *Gra81*, and another with promoters for *Sag1*, *Gra11b*, and *Gra80* are also constructed to pinpoint distinct biomarkers corresponding to Type A through E. Ongoing single-cell RNA sequencing investigations will be conducted to investigate earlier stages of presexual development, aiming to provide valuable insights into merozoite marker typing and the presexual evolution of *T. gondii* within feline intestines.

P107

AP2 transcription factors involved in the stage progression of *Toxoplasma gondii* in the cat intestine

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In *Toxoplasma gondii*, the expression of stage-specific genes is regulated by Apetala-2 (AP2) transcription factors. Recently, two TgAP2 transcription factors, restricted to tachyzoites, were shown to inhibit the induction of merogony: conditional depletion of these TgAP2s in tachyzoites induced the expression of merozoite-specific mRNAs and resulted in the emergence of merozoite phenotypes *in vitro*. Taking a different approach, we asked whether inducible ectopic expression of enteroepithelial stage (EES)-specific TgAP2s in tachyzoites, which are restricted to EESs, would result in the upregulation of substrate gene mRNAs of stage-regulated factors linked to merozoites, gamonts, and oocysts, with maximal mRNA levels in these developmental stages. In connection with experiments addressing this question, we are also exploring the role of epigenetic blocks by the MORC-HDAC complex, which restrict access of induced TgAP2s to binding sites of EES-specific substrate genes in tachyzoites. Therefore, we have re-designed the original experimental strategy to include the inhibition of histone deacetylase 3 (HDAC3). We will present RNA-Seq data showing the initial substrate gene pools of EES-specific TgAP2s, preliminary results of ChIP-Seq experiments as well as computational

strategies for validating target sequences.
In summary, we present a refined model system to study gene regulation in cat enteroepithelial stages of *T. gondii* using a simple standard culturing method, eliminating the need for either experimental cat infections or specialized and labour-intensive cell culture systems such as enteroid cultures, and yielding robust and reproducible outputs.

P108
Searching for a vaccine against toxoplasmosis

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Around 1/3 of the world"s population is infected by *Toxoplasma gondii*, an obligatory intracellular parasite that causes toxoplasmosis (1). This infection can lead to encephalitis and death of immunocompromised individuals. In pregnant women, primo infection or re-infection with highly virulent strains can lead to abortion or congenital toxoplasmosis (1). To date, there is no effective human vaccine, and recent studies using nanotechnology in vaccine development showed promising results (2).
The goal of this work was to optimize a poly lactic-co-glycolic acid (PLGA) delivery nano system containing an antigen extract of *T. gondii* for intranasal administration inducing an effective immune response and protection against infection. Single emulsion method was used for the synthesis of the formulation and its physical chemical parameters were evaluated by nanoparticle tracking analysis and dynamic light scattering. Lowry assay and UV-Vis spectroscopy were used to determine the encapsulation efficiency. To access biocompatibility of the nanoformulation, cell viability assay was performed. To infer about the permeation of the nasal epithelium and delivery to target cells, cell permeability and cell internalization assays were performed using human nasal epithelial cells (RPMI) and mouse bone marrow-derived dendritic cells (BMDCs), respectively.
The nanoparticles obtained presented a size of 207 nm, a polydispersity index of 0.227, a zeta potential of -14.15 mV, which are adequate physical-chemical properties for induction of immune response, and an encapsulation efficiency of 55%. After 24h of exposure there was no cytotoxicity for a nanoparticle concentration of 7.5 mg/ml in L929 and RPMI cell lines, and 0.25 mg/ml in BMDCs. At the same timepoint, nanoparticles reached a 66.4% of permeability across a nasal epithelial monolayer. There was also a time-dependent increase in the nanoparticle uptake by BMDCs.
These data are determinant to advance in the development of an effective vaccine preventing congenital toxoplasmosis.

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P109

GET-TOXO Project (Spanish Working Group on Toxoplasmosis): a multidisciplinary "One health" approach for controlling toxoplasmosis in human and animals in Spain

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Introduction: A third of the world's population is affected by toxoplasmosis, a zoonosis with significant impact. Congenital toxoplasmosis (CT) occurs after a primary infection during pregnancy, potentially leading to severe neurological and systemic symptoms. In Spain there is a lack of updated information.
Objective: To understand the situation of congenital toxoplasmosis in Spain from a "One Health" perspective and apply this knowledge to the healthcare field to enhance detection, treatment, and control.
Materials and Methods: A multidisciplinary approach study has been designed, involving researchers from various healthcare fields (medical, parasitological, veterinary, and public health) organized through the Spanish Working Group on Toxoplasmosis (GET-TOXO). It applies to epidemiological knowledge in both human and animal environments, focusing on improving diagnosis, prevention, and control. The study aims to identify the incidence of CT and its clinical, epidemiological, and molecular characteristics. This will be achieved by analyzing seroprevalence and incidence in pregnant women and animals of different species. Immunological and molecular techniques will be developed to enhance diagnostic capabilities. Molecular characterization of *T. gondii* isolates from humans and animals will be conducted using an 11-marker analysis with RFLP-PCR, aiming to determine the potential relationship between genotypes and pathology, as well as their implications in epidemiology and control.
Results: Preliminary results are presented. CT has been a notifiable disease since 2015, but there is an underreporting to the National Epidemiological Surveillance Network (RENAVE). Only 17 cases of CT were reported to RENAVE from 2010-20, while analysis of the Specialized Primary Care Attention Registry (RAE-CMBD) identified 547 hospital discharges with this diagnosis in the same period (3% of reported cases). Molecular characterization of *T. gondii* isolates analyzed so far showed genotype II as the most prevalent, along with types I and III and atypical genotypes.
Conclusions: The lack of information on CT in Spain requires a thorough analysis, active case review and ongoing epidemiological studies in human and animal settings within the "One Health" approach. Establishing the required detection and control measures is essential, mainly in pregnant women.
Funding: FIS-AESI PI 21CIII/0031 Project, Health Research Fund, ISCIII, Ministry of Science and Innovation.

P110

Expression of the pro-apoptotic proteins Bak and Bax during in-vitro infection of murine cells with Toxoplasma gondii Tachyzoites

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Parasite survival depends greatly on how well they can exploit their host. *Toxplasma gondii* is no exception to this and its exploitation game starts very early in its host-invasion process. More specifically, right after the parasite infects the cell. *T. gondii* can not only manipulate the arrangement of certain organeles, but it is also can regulate the cell apoptotic mechanisms by delaying them. With this, the parasite can comfortably replicate within the cell and leave it on its own terms. The understanding of how *T. gondii* delays apoptosis has not been completly elucidated. The objective of this study is to evaluate the expression of Bak and Bax: two pro-apoptotic proteins from the BCL-2 family in murine neurons.

These proteins normally work together to produce permeabilization of the mitochondrial outer membrane (MOMP) For this, we used two different *T. gondii* strains: RH (Type I) and Pru (Type II). We separately infected confluent neuroblastoma-derived cell (cell line Na 42/13) monolayers with each parasite strain and 2 infection groups: MOI=1 and MOI =2 for 3, 24, and 24 hours (h). A negative control of non-infected na 42/13 monolayers was added to the study. Afterwards, we purified RNA from the cells with a commercial kit and generated a complementary DNA strain through a reverese transcriptase-regulared process. Afterwards, we amplify the mRNA region of both BAK and Bax using quantitative PCR. At 3h post infection (hpi), downregulation of Bax was already shown in all infection groups. The major difference was shown at 24 hpi with Bax expression levels significantly low in both RH strain infection groups. Cells infected with T. gondii Pru strain show Bax expression levels mildly higher than the negative control at a MOI =1 and mildly lower than the negative control at a MOI = 2. Bak levels were always higher than Bax at 3hpi and 24hpi. At 48hpi all expression levels of both Bak and Bax were lower than the negative control.
It is common to use Bak/Bax as a unit in the apoptotic reaction. However, in the case of neurons, it is considered that Bax is the main excecutor of MOMP (toghether with other apoptotic proteins). In this study we demostrate that Toxoplasma specifically targets Bax downregulation, particularly at 24 hpi and more importantly, such singling out do not seem to be the same in all strains. How well T. gondii can play the apoptotic game would need to be further investigated.

P111

Humoral immunity to *Toxoplasma gondii* is mediated by the expression of *Nfkbid* in T cells

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The immunological mechanisms underlying the development of a protective immune response against parasitic diseases through vaccination remain unclear. *Toxoplasma gondii* is a parasite that excels in evading vaccine-induced immunity. Strong $CD8^+$ T cell responses with IFNγ production are required to generate a protective immune response. However, the contribution of B cell responses to *T. gondii* immunity is less understood. Through genetic mapping, we recently reported that *Nfkbid*, a gene that encodes IκBNS, a member of the atypical family of NF-κB regulators, was required for humoral immunity to *T. gondii*, with contributions from both conventional B-2 and innate-like B-1 B cells. However, it is unclear which cell populations must express *Nfkbid* to elicit an effective humoral response to *T. gondii*. To test the contribution of the *Nfkbid* expression by T cells, we created a conditional knockout that deletes *Nfkbid* exclusively in T cells using the Cre-LoxP system. We infected B6.CD4^{Cre}*Nfkbid*$^{-/-}$ and B6.CD4*wt*Nfkbid$^{fl/fl}$ with the low virulence type III CEP strain, and after 35 days, during the chronic phase of the infection, we challenged the mice with the type I RH strain. We observed that 100% of B6.CD4^{Cre}*Nfkbid*$^{-/-}$ (n=6) succumbed to the virulent challenge while all B6.CD4^{wt}*Nfkbid*$^{fl/fl}$ (n=5) survived. While no differences were observed in T cell memory subpopulations, we observed a reduction in Tfh and B-1 cells, accompanied by a strong reduction of parasite-specific IgG isotypes and IgM in B6.CD4*Nfkbid*$^{-/-}$, phenocopying our previous observations in *Nfkbid*$^{-/-}$ mice. Altogether, these results suggest that in the context of *T. gondii*, *Nfkbid* modulates the profound interplay between $CD4^+$ and B cells, directly affecting humoral responses to *T. gondii*. Because follicular helper T cell (Tfh) differentiation promotes IgG responses to model antigens by their CXCR5-dependent entry into the germinal center, we hypothesize that *Nfkbid* deletion will mainly regulate Tfh cells, resulting in a loss of anti-*T. gondii* antibody response by B cells, an effect currently being investigated in our laboratory.

P112

Compounds From the MMV Pathogen Box Target *Toxoplasma gondii* Tachyzoites and Bradyzoites: Insights into Metabolic Responses and the Role of the bc_1-Complex in Bradyzoites

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Toxoplasma gondii forms enduring bradyzoites within cysts in brain and muscle tissues that are crucial for the transmission and remission. Current medical treatments effectively target the pathogenic tachyzoites, however they fail to eradicate the chronic *T. gondii* infections. It is currently not well understood why treatments fail and how drugs impact bradyzoites metabolism.

To address these shortcomings, we screened 400 compounds from the MMV Pathogen Box against in vitro tachyzoites and bradyzoites. Our screening identified 16 compounds that simultaneously inhibit tachyzoite growth and act cidal against bradyzoites. Notably, the known bc_1-complex inhibitors ELQ-400 and buparvaquone were among the identified hits. To characterize parasite responses to inhibitors and delineate their modes of actions we used HILIC-UHPLC-MS to profile and trace stable-isotope incorporation into metabolites of treated parasites. The impact of a non-metabolic control inhibitor (BKI 1294) revealed a general stress response marked by elevated oxidized glutathione and AMP and a depletion of mitochondrial and glycolytic metabolites. We also identified a bc_1-complex inhibitor by comparing its metabolic fingerprint to known inhibitors. The metabolic impact of MMV1028806 mirrored that of buparvaquone and atovaquone in both tachyzoites and bradyzoites. While treated tachyzoites exhibited many metabolic changes including dysregulation of the TCA cycle and pyrimidine synthesis pathway, bradyzoites showed a more conservative response consistent with an energy shortage. Direct measurements of mitochondrially produced ATP demonstrated very low ATP levels in bradyzoites that can be further depleted by atovaquone but not by a non-bradyzocidal coenzyme Q analog. Our findings further challenge the notion of metabolically inactive bradyzoites. Our data also suggest that a functional bc_1-complex plays a crucial role in ATP production and bradyzoite survival, both of which can be disrupted by approved bc_1-complex targeting drugs in vitro.

P113

Chronic *Toxoplasma gondii* Infection Results in Elevated Matrix Metalloproteinase-9 and Reduced Cerebellar Perineuronal Nets

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Matrix Metalloproteinase-9 (MMP-9) is essential for regulating and organizing perineuronal nets (PNN), which are specialized extracellular matrix (ECM) structures, by cleaving and degrading ECM components. PNNs have neuroprotective capacities and participate in signal transduction, controlling neuronal activity and plasticity. Synaptic plasticity is considered to be the basis for learning and memory. Brain infection by the parasite *Toxoplasma gondii* impairs learning and memory, although the underlying mechanisms remain largely unknown. We examined the roles of MMP-9 and PNN in a mouse model with chronic infection. In mice with a high parasite burden of chronic infection, we found that MMP-9 expression was increased in the peripheral circulation and the brain. A correlation was found between the serum levels of MMP-9 and antibodies to the *Toxoplasma* matrix antigen MAG1, a surrogate marker for *Toxoplasma* tissue cysts in the brain. MMP-9 elevation was accompanied by increased expression of its endogenous regulators, TIMP-1 and NGAL. MMP-9 expression was notably associated with the loss of PNNs. There was a trend toward a negative correlation between MMP-9 and aggrecan expression, a critical component of PNN. A significant increase in the synaptic vesicle protein synaptophysin was observed, consistent with the role of PNN reduction, which decreases inhibitory activity. Our findings suggest that tissue cysts are crucial for the elevation of MMP-9 expression, leading to the degradation of PNN essential for synaptic plasticity. These findings provide a possible mechanism for *Toxoplasma*-associated deficits in learning and memory.

P114

The myxobacterial compound Argyrin D inhibits protein translation in the mitochondria of *Toxoplasma gondii* and arrests tachyzoites and bradyzoites.

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Treatments that eradicate tachyzoites and bradyzoites *Toxoplasma gondii* (*T. gondii*) remain non-existent and essential and druggable processes in bradyzoites remain hard to identify. Using a new *in vitro* model for pan-drug tolerant bradyzoites[1], we found Argyrin D (ArgD) among 253 myxobacterial compounds to inhibit tachyzoite growth while also acting on bradyzoites at half maximal concentrations of 3 and 60 nM, respectively. In other eukaryotes and bacterial pathogens argyrin compounds have been shown to disrupt translation by binding to the elongation factor G (EFG).[2] *T. gondii* harbors three isoforms of EFG that are expressed in its cytosol, apicoplast and mitochondrion. Surprisingly, fluorescent streptavidin staining of treated tachyzoites demonstrates absence of a delayed death phenotype. Instead, parasites that express GFP in their mitochondria revealed a defect in the mitochondrial morphology. We used a SILAC approach to monitor the change of global protein synthesis in ArgD-treated parasites by incorporation of stable-isotope labelled amino acids. In total the synthesis of 119 proteins was significantly attenuated over the course of three days.

After 24 hours of treatment we identified 41 proteins that were enriched in mitochondrial proteins and included the mitochondrially encoded cytochrome b and cytochrome c oxidase III. After 48 and 72 h the synthesis of proteins from all cellular compartments was impacted. In summary, we identified an inhibitor of mitochondrial translation that will be a useful tool to identify and study essential mitochondrial processes in both parasite stages.
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P115
From TgO/GABA-AT, GABA, and T-263 Mutant to Conception of Toxoplasma

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Toxoplasma gondii causes morbidity, mortality, and disseminates widely via cat sexual stages. Here, we find T. gondii ornithine aminotransferase (OAT) is conserved across phyla. We solve TgO/GABA-AT structures with bound inactivators at 1.55A°and identify an inactivator selective for TgO/GABA-AT over human OAT and GABA-AT. However, abrogating TgO/GABA-AT genetically does not diminish replication, virulence, cyst-formation, or eliminate cat"s oocyst shedding. Increasedsporozoite/merozoite TgO/GABAAT expression led to our study of a mutagenized clone with oocyst formation blocked, arresting after forming male and female gametes, with ""Rosetta stone""-like mutations in genes expressed in merozoites. Mutations are similar to those in organisms from plants to mammals, causing defects in conception and zygote formation, affecting merozoite capacitation, pH/ionicity/sodium-GABA concentrations, drawing attention to cyclic AMP/PKA, and genes enhancing energy or substrate formation in TgO/GABA-AT related- pathways. These candidates potentially influence merozoite"s capacity to make gametes that fuse to become zygotes, thereby contaminating environments and causing disease.
Origonally published in iScience 27, 108477, January 19, 2024 ª 2023 The Authors.

P116
Differential Gene Expression of Toxoplasma gondii ME49 and RH Strains during ELQ-316 Inhibition

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Endochin-like quinolones (ELQ) are highly potent inhibitors of the Toxoplasma gondii cytochrome bc1 complex, the third complex of the mitochondrial electron transport chain. We evaluated differential gene expression of type 1 (RH) and type 2 (ME49) T. gondii treated with ELQ-316 using RNA sequencing at multiple time points over 4 and 10 days, respectively. The transcriptional response to ELQ-316 substantially differed over time and across the two strains. Between 24 hours and 96 hours of ELQ-316, ME49 undergoes a marked shift in gene expression compared to RH. Much of the difference

in strain response appears to be attributable to in vitro bradyzoite differentiation and cyst formation in ME49. At 96 hours, 439 genes in ME49 were upregulated more than 4-fold, compared to 373 genes in RH. Of the upregulated genes, only 165 were common between the two strains, demonstrating unique gene expression responses to ELQ-treatment between strains. In ME49, 107 of 190 bradyzoite-associated genes (upregulated in multiple other bradyzoite RNAseq datasets) were upregulated at 96 hours, including BAG1, Enolase 1, LDH2, and AP2IX-9. In RH, 49 bradyzoite-associated genes were upregulated, and typical bradyzoite signatures were not. All changes in transcription in ME49 were not explained by bradyzoite transformation. In ME49 and RH, 351 and 324 genes non-bradyzoite-associated genes, respectively, were upregulated under ELQ-treatment: less than half of these genes were common between strains. Analysis of metabolic pathways and transcriptional regulation in response to cytochrome bc1 inhibition provides insight into mechanisms of T. gondii adaptation and key differences between strains.

P117

Dissecting the mechanism by which *Toxoplasma gondii*-specific CD8+ T cells differentiate to become IFNγ producers

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Innate immunity is the first protection mechanism against invading pathogens and is critical for initiating adaptive immune responses to clear infections. As an obligate intracellular parasite, the immune protection against *Toxoplasma gondii* is governed by a robust secretion of interferon-gamma (IFNγ) by CD8+ T cells. However, the differentiation pathway that promotes IFNγ by the naïve CD8+ T cells is unclear. Our previous studies using naïve transnuclear CD8+ T cells (T57) to the endogenous *T. gondii* vacuolar resident antigen, TGD057, identified a novel pathway by which the microbial intracellular sensor NLR Family Pyrin Domain Containing 3 (NLRP3) is required for CD8+ T cells IFNγ responses in an inflammasome cascade-independent manner. Here we report a new requirement for eliciting this response through the activation of the cytosolic DNA sensor, STING, in *T.gondii-infected* cells. STING is a signal transducer for the presence of cyclic GMP-AMP (cGAMP) generated by the cytosolic DNA sensor cGAMP synthase (cGAS). However, we found only partial requirement for cGAS and IFNβ in this pathway, suggesting a novel mechanism by which STING promotes CD8+ T cell IFNγ differentiation. Our working model proposes that following *T. gondii* infection, NLRP3 and STING work independently of their conventional roles in the inflammasome and type I IFN response, which in turn promotes the IFNγ differentiation of naïve parasite-specific CD8+ T cells. Dissecting these less understood mechanisms for NLRP3 and STING could have important implications for vaccine design and clearance of protozoan pathogens.

P118

The Role of Polyphosphates in *Toxoplasma gondii*

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Polyphosphate (PolyP) is a linear polymer of tens to hundreds of phosphate residues that can reach millimolar levels in protozoan parasites while the concentration in host cells is at the micromolar level. In bacteria, PolyP functions as phosphate reservoir, energy source, chelator of metal ions, regulator of metabolism, stress response, differentiation, and gene expression. In eukaryotes, PolyP plays an equally large number of diverse roles. This catalog of apparently unrelated functions may be the result of PolyP"s ability to function as a protein-stabilizing scaffold. *T. gondii* stores PolyP at molar levels in acidocalcisomes which are hydrolyzed upon alkalinization followed by release of calcium into the cytosol. Nothing is known about the function of acidocalcisome PolyP in the *T. gondii* infection cycle, virulence, or the establishment of the chronic infection. In *Saccharomyces cerevisiae*, a vacuolar transporter chaperone complex (VTC complex) synthesizes PolyP, in expense of Pi and ATP, and translocates PolyP to the yeast vacuole and acidocalcisomes. The VTC complex is composed of 5 subunits (VTC1-5), in which VTC4 acts as the catalytic subunit. ToxoDB supports the presence of two homologous genes: TGGT1_298630 (TgVTC2) and TGGT1_299080 (TgVTC4). We created mutants for both genes and found that TgVTC2 and TgVCT4 are essential for the *T. gondii* lytic cycle, and that TgVTC2 and TgVTC4 co-localize with the plant-like vacuolar compartment (PLVAC) markers VP1 and CPL, and with the Zinc transporter previously localized to the PLVAC and acidocalcisomes. Both mutants showed a significant decrease in PolyP content. We are characterizing the role of polyP as a protein-like chaperone and its role in acidic calcium storage.

P119

Unraveling the biochemical interactions that regulate Toxoplasma GRA trafficking

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Toxoplasma gondii owes much of its success to the battery of secreted effectors that allow it to develop its replicative niche, the parasitophorous vacuole (PV), and evade host immune clearance. *Toxoplasma*"s dense granule proteins (GRAs) are a class of effectors that carry out these functions in the PV, at the PV membrane (PVM), and in the host cytosol. In general, GRA proteins remain largely under described as they are often dispensable in tissue culture despite being critical to parasite pathogenesis. For example, a small subset of GRAs are responsible for reshaping the PVM into the intravacuolar network (IVN), a tubulovesicular network that is necessary for parasite virulence. While the exact role of the IVN is undefined, genetic phenotypes have implicated that it mediates diverse processes including nutrient uptake and effector export across the PVM. Interestingly, despite having typical traits of integral membrane proteins, the GRAs that build and decorate the IVN are trafficked through the parasite secretory system as soluble, heteromultimeric complexes. This regulation of membrane insertion and switch in solubility suggest that there are chaperoning interactions that keep these GRAs out of surrounding membranes, such as those of the endomembrane system or the parasite plasma membrane, until they reach the IVN. Outside of these observations, how these GRAs contribute to IVN biogenesis, how they are regulated during secretion and trafficking, and what proteins are critical at each of these trafficking steps remains unknown.

To identify regulators of trafficking, I will use immunoprecipitation of soluble versus membrane bound GRAs coupled with mass spectrometry. Taken together, the data from these experiments will provide mechanistic details on *Toxoplasma* dense granule trafficking in the parasite and PV as well as on IVN biogenesis. Using this information, I aim to develop an *in vitro* system that reconstitutes the release of chaperoning and insertion into the membrane for the IVN GRAs. I will further use this system to determine structures of both the chaperoned complexes and the GRA oligomers that form the IVN tubules. Finally, I am using structural biology to determine the structures of the folded GRAs to determine how they contribute to successful GRA trafficking and parasite survival.

P120

Investigating the role of the residual body in cytoskeletal turnover during *Toxoplasma* replication

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Accurate cellular replication balances the biogenesis and turnover of complex structures. In the apicomplexan parasite *Toxoplasma gondii*, daughter cells form within an intact mother cell, creating additional challenges to ensuring fidelity of division. The cells must distinguish between materials intended to be packaged into daughter buds, and maternal cell material destined for turnover. We have previously shown that depletion of ERK7 interupts this balance, causing the Ubiquitin E3 ligase, CSAR1, to improperly localize from the residual body to the apical complex of daughter buds. This mislocalization of an active E3 ligase causes the improper turnover of daughter cytoskeletal components and results in the loss of the conoid. We hypothesize that the residual body is used to compartmentalize the turnover of cell components during cytokinesis. We aim to determine the mechanisms of regulation of this process, using CSAR1 as an entry point. We are combining multiple cell biological and biochemical methods to identify CSAR1 substrates and regulators, including proximity biotinylation, ubiquitin proteomics, and *in vitro* reconstitution. This multi-faceted approach will allow us to decipher the components of the ERK7 regulated CSAR1 ubiquitin-proteosome pathway and its role in cytoskeletal turnover.

P121

In Vitro Activity of Fractionated Methanoic Blueberry Extract against *Toxoplasma gondii* growth

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Toxoplasma gondii (*T. gondii*) is the causative agent of congenital and ocular toxoplasmosis in humans. Fewer drugs are available for the treatment of parasite infection; however, they have serious drawbacks making their use limited. Thus, necessitated the search for new compounds that can inhibit the parasite *in vitro* and *in vivo*. Here, we report the anti-*T. gondii* activity of different solvent fractionates obtained from blueberry in vitro. Interestingly, all fractions exhibited optimal *T. gondii* growth inhibition at 72 hours of interaction. The minimum inhibitory concentrations (IC50s) for fractions obtained using the combination of Chloroform, Methanol and water, Hexane and Diethyl ether, Ethyl acetate, Dichloromethane and 1-propanol, Ethyl acetate and Hexane, and Chloroform only were 3.29, 41.45, 7.11, 24.91, and 0.34 µg/mL respectively. These fractions' IC_{50}s for parasites were not toxic to host cells. We therefore believed that the fraction contained bioactive compounds that could be separated for future *in vitro* and *in vivo* studies for the development of anti-toxoplasma agents.

P122

CD4+ T Cell Responses to Toxoplasma gondii Are a Double-Edged Sword

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CD4+ T cells have been found to play critical roles in the control of both acute and chronic Toxoplasma infection. Previous studies identified a protective role for the Toxoplasma CD4+ T celleliciting peptide AS15 (AVEIHRPVPGTAPPS) in C57BL/6J mice. Herein, we found that immunizingmice with AS15 combined with GLA-SE, a TLR-4 agonist in emulsion adjuvant, can be either helpful in protecting male and female mice at early stages against Type I and Type II Toxoplasma parasites or harmful (lethal with intestinal, hepatic, and spleen pathology associated with a storm of IL6). Introducing the universal CD4+ T cell epitope PADRE abrogates the harmful phenotype of AS15. Our findings demonstrate quantitative and qualitative features of an effective Toxoplasma-specific CD4+ Tcell response that should be considered in testing next-generation vaccines against toxoplasmosis. Our results also are cautionary that individual vaccine constituents can cause severe harm depending on the company they keep.

Published as:
El Bissati, K.; Krishack, P.A.; Zhou, Y.;Weber, C.R.; Lykins, J.; Jankovic, D.; Edelblum, K.L.; Fraczek, L.; Grover, H.; Chentoufi, A.A.; . P. Dubey , Steve Reed , Jeff Alexander , John Sidney , Alessandro Sette , Nilabh Shastri and Rima McLeod .CD4+ T Cell Responses to Toxoplasma gondii Are a Double-Edged Sword. Vaccines 2023, 11, 1485. https://doi.org/10.3390/vaccines11091485

P123

Populations of inflammation driven neuroprotective lipocalin-2 reactive mouse astrocytes resolve following chronic Toxoplasma infection.

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Astrocytes are known to provide physical and metabolic support for neurons, control the blood brain barrier, and regulate crucial neurotransmitters. When CNS homeostatic levels are disrupted, such as during injury or infection, astrocytes become reactive. It is unknown if reactive astrocytes are beneficial or detrimental to the system. It has long been known that following *Toxoplasma gondii* infection, astrocytes upregulate the astrocyte-specific protein GFAP, produce cytokines and chemokines and are able to kill replicating parasites. However, whether all astrocytes undergo these inflammatory transformations and if distinct subsets are involved in these protective processes is unknown. Using previously published flow cytometry phenotyping, we determined there is inflammation-induced astrocyte heterogeneity over the course of Toxoplasma infection, based on astrocytic expression of CD51, CD63, and CD71. Furthermore, we characterized previously undescribed subsets of astrocytes using this technique. Analysis of single cell RNA sequencing revealed a diverse clustering of astrocytes throughout infection, and identified specific inflammatory responsive populations. In addition to immune regulation, a specific set of lipocalin-2 astrocytes expressing transthyretin were found to correlate with neuroprotective functions such as vasculature regulation and angiogenesis. Finally, the use of a novel inducible Lcn2 reporter mouse allowed the characterization and tracking of astrocyte reactivation over time and revealed populations of labeled astrocytes resolve even in the context of ongoing inflammation.

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