



Article scientifique

Article

2024

Accepted version

Public access

This is an author manuscript post-peer-reviewing (accepted version) of the original publication. The layout of the published version may differ .

γ -tubulin complex controls the nucleation of tubulin-based structures in Apicomplexa

Haase, Romuald; Puthenpurackal, Annet; Maco, Bohumil; Guérin, Amandine; Soldati-Favre, Dominique

How to cite

HAASE, Romuald et al. γ -tubulin complex controls the nucleation of tubulin-based structures in Apicomplexa. In: Molecular biology of the cell, 2024, p. mbc.E24-03-0100. doi: 10.1091/mbc.E24-03-0100

This publication URL: <https://archive-ouverte.unige.ch/unige:179126>

Publication DOI: [10.1091/mbc.E24-03-0100](https://doi.org/10.1091/mbc.E24-03-0100)

© The author(s). This work is licensed under a Creative Commons Attribution (CC BY)

<https://creativecommons.org/licenses/by/4.0>

Last update in Archive ouverte UNIGE on 05.08.2024 08:39

γ -tubulin complex controls the nucleation of tubulin-based structures in Apicomplexa

Romuald Haase, Annet Puthenpurackal, Bohumil Maco, Amandine Guérin* and Dominique Soldati-Favre*

Affiliation: Department of Microbiology and Molecular Medicine, Faculty of Medicine, University of Geneva, CH-1221 Geneva 4, Switzerland

Corresponding authors: dominique.soldati-favre@unige.ch and amandine.guerin@unige.ch

Running Head: **Microtubules nucleation in Apicomplexa**

Abbreviations: SPMTs, IMC, U-ExM, GCPs, ICMTs

Keywords

Apicomplexa, *Toxoplasma gondii*, *Cryptosporidium parvum*, Microtubules, Centrosome, Centrin, Endodyogeny, Schizogony, Microtubule nucleation, Spindle microtubules, Subpellicular microtubules, Conoid, Nucleus, Expansion microscopy

Abstract 200 words or less.

Apicomplexan parasites rely on tubulin structures throughout their cell and life cycles, particularly in the polymerization of spindle microtubules to separate the replicated nucleus into daughter cells. Additionally, tubulin structures, including conoid and subpellicular microtubules, provide the necessary rigidity and structure for dissemination and host cell invasion. However, it is unclear whether these tubulin structures are nucleated via a highly conserved γ -tubulin complex or through a specific process unique to apicomplexans. This study demonstrates that *Toxoplasma* γ -tubulin is responsible for nucleating spindle microtubules, akin to higher eukaryotes, facilitating nucleus division in newly formed parasites. Interestingly, γ -tubulin colocalizes with nascent conoid and subpellicular microtubules during division, potentially nucleating these structures as well. Loss of γ -tubulin results in significant morphological defects due to impaired nucleus scission and the loss of conoid and subpellicular microtubule nucleation, crucial for parasite shape and rigidity. Additionally, the nucleation process of tubulin structures involves a concerted action of γ -tubulin and Gamma Tubulin Complex proteins (GCPs), recapitulating the localization and phenotype of γ -tubulin. This study also introduces new molecular markers for cytoskeletal structures and applies iterative expansion microscopy to reveal microtubule-based architecture in *Cryptosporidium parvum* sporozoites, further demonstrating the conserved localization and probable function of γ -tubulin in *Cryptosporidium*.

43 **Introduction**

44 The phylum of Apicomplexa encompasses thousands of species ranging from free-living to
45 intracellular parasites, including pathogens responsible for human diseases such as
46 *Cryptosporidium* (cryptosporidiosis), *Plasmodium* (malaria) and *Toxoplasma gondii*
47 (toxoplasmosis). To invade host cells, these obligate intracellular parasites rely on a gliding
48 motility machinery powered by its actomyosin system and the secretion of specialized
49 organelles termed rhoptries and micronemes (Opitz and Soldati 2002; Green et al. 2017;
50 Dubois and Soldati-Favre 2019; Dos Santos Pacheco et al. 2022; Ben Chaabene, Lentini, and
51 Soldati-Favre 2021; Guérin et al. 2021; Martinez et al. 2023).

52 *T. gondii* exhibits a remarkable ability to invade virtually all types of nucleated cells in warm
53 blooded animals. Upon invasion, the parasite resides within a parasitophorous vacuole (PV)
54 and undergoes division through endodyogeny, a process where two daughter cells form within
55 the mother cell (Gubbels et al. 2020). The centrosomes serve as a hub to nucleate the spindle
56 microtubules during mitosis and orchestrate the assembly of daughter cell components
57 including the cortical cytoskeleton (Morlon-Guyot et al. 2017; Suvorova et al. 2015).

58 In contrast *Cryptosporidium* parasite replicates by schizogony where three successive rounds
59 of highly coordinated and synchronous nuclear divisions give rise to eight nuclei, followed by
60 segmentation (English et al. 2022). Despite the importance of this process, the biogenesis of
61 tubulin-based structures during *Cryptosporidium* replication remains poorly understood (Wang
62 et al. 2021).

63 Gamma tubulin (γ -tubulin) is a highly conserved and essential protein in eukaryote, required
64 for microtubule nucleation (Oakley, Paolillo, and Zheng 2015). A dimer of γ -tubulin assembles
65 in the centrosome with additional Gamma Tubulin Complex proteins (GCPs) to form γ -TuSCs
66 (γ -tubulin small complexes) (Zheng et al. 1995). Following this association γ -TuSCs and
67 additional GCPs assemble into large γ -TuRCs (γ -tubulin ring complexes) providing the crucial

structure needed for microtubules nucleation (Thawani and Petry 2021; Aher et al. 2023). The conservation of γ -tubulin in eukaryotic organisms underscores its significance in cell division. Both *Toxoplasma* and *Cryptosporidium* possess essential tubulin-based structures of unclear origin, including centrioles, conoid fibers, subpellicular microtubules (SPMTs), and intraconoidal microtubules (ICMTs, in *T. gondii*) (Dos Santos Pacheco et al. 2020). In this study, we elucidate the dynamic localization of γ -tubulin during cell cycle in *T. gondii*. Using U-ExM, we uncover the transient association of γ -tubulin with the nascent apical complex providing evidence of tubulin structure nucleation. Moreover, γ -tubulin depleted parasites die due to a failure of nuclei separation and cytoskeleton formation. In addition, the absence of γ -tubulin results in the formation of abnormally long microtubules emerging from collapsing centrosomes, which exhibit SPMT-like properties such as polyglutamylation and IMC budding. Our findings establish that the nucleation activity relies on the coordinated action of γ -tubulin and GCP proteins as evidenced by the localization and phenotype of GCP2 and 3 depleted parasites mirroring those of γ -tubulin-depleted ones. Significantly, we show that the dynamic localization of γ -tubulin and spindle microtubule formation follow a similar pattern in *Cryptosporidium parvum* which divide in a different process compared *T. gondii*, underlaying the crucial function of γ -tubulin in apicomplexan. Furthermore, through iterative expansion microscopy coupled with new available molecular markers we reveal the entire tubulin cytoskeleton of *C. parvum* sporozoites.

Results

Toxoplasma gondii γ -tubulin transiently associates with the nascent apical complex

T. gondii tachyzoites divide by a process known as endodyogeny characterized by the formation of two daughter cells within the mother cell. The initiation of endodyogeny involves the duplication of the centrioles, pivotal components of the centrosome (**Fig1**). The duplicated centrioles connect to the centrocone, a conical structure linking the replicated nucleus to the centrosomes via the mitotic spindle (**Fig1**). Concurrently, the nascent apical complex consisting of the conoid, ICMTs and SPMTs begins to emerge near the duplicated centrioles in a synchronous and circular manner (Padilla, Murray, and Hu 2024). As the division proceeds, daughter cell components including the secretory organelles and the inner membrane complex (IMC) are synthesized and accumulate adjacent to the growing tubulin cytoskeleton. Finally, constriction occurs, leading to formed daughter cells, each that encapsulating their nucleus and organelles within the cell body (**Fig1**).

To investigate the localization of γ -tubulin throughout the cell cycle, we employed an endogenous tagging approach by inserting a mini-auxin inducible degron sequence (mAiD) alongside a 3HA-epitope tag in the 3'UTR (**Supplemental Fig. 1A**). Immunostaining of γ -tubulin displays a highly dynamic pattern throughout the cell cycle. In intracellular non-dividing parasite, γ -tubulin signal appears very weak and almost undetectable (**Fig2A**). As parasites proceed to divide, first a single punctum of γ -tubulin is detected. Subsequently, two close puncta can be observed with a continuous staining between them, presumably reflecting centrosome duplication (**Fig2A**). The two puncta gradually separate until the budding of the inner membrane complex (IMC) eventually starts at which point the γ -tubulin signal becomes faint and disperses throughout the cytosol (**Fig2A**). Notably, in extracellular parasites approximately 40% of tachyzoites retained a punctate staining while the others show a dispersed cytosolic staining pattern (**Fig2B**) (**Supplemental Fig. 2A**). We compared the

staining of γ -tubulin to another early daughter marker, Pcr4, in extracellular Pcr4-mAID-HA parasites, however the absence of signal indicated no active daughter cell formation (**Supplemental Fig. 2B**).

In *T. gondii*, the centrosome architecture consists of an outer core (distal) and inner core (proximal) (Suvorova et al. 2015). The centrioles, along with centrin1 protein, are part of the outer core. Dual labelling of γ -tubulin and centrin1 suggest that both proteins localize to the outer core of the centrosome as previously reported (Suvorova et al. 2015) (**Supplemental Fig. 2C**). To increase the resolution of the molecular organization of the centrosome, we applied ultrastructure expansion microscopy (U-ExM) applied to intracellular and extracellular parasites, (**Fig2B**). In extracellular, a punctum of γ -tubulin was observed between the two centrioles while the centrin1 “fiber like” structure was bridging the two centrioles (**Fig2B**). The same organization was observed for intracellular parasite prior to centrosome duplication (**Fig2B**). However, after centrosome duplication, centrin1 still bridged the two centrioles, while γ -tubulin localized on both sides of the nascent spindle microtubules as well as surrounding the centrioles (**Fig2B**) Interestingly, despite the dense proteinaceous nature of the centrosome, γ -tubulin-mAiD-HA protein was found soluble in PBS as well as in RIPA and Cytoskeletal buffers (**Supplemental Fig. 2D**).

Remarkably, as the endodyogeny continues, γ -tubulin associated with the nascent apical complex (conoid, ICMTs, SPMTs) as well as close to the centriole and on both sides of the mitotic spindle as indicated by the acetylated tubulin staining (**Fig2C**). Importantly, during the forming apical complex γ -tubulin staining was observed on the opposite side of the already formed part, highlighting its crucial role in the nucleation of these tubulin-based structures (**Fig2D**).

Loss of *T. gondii* γ -tubulin leads to major morphological abnormalities due to defects in nucleus scission and cytoskeleton formation

To assess the γ -tubulin function in *T. gondii* life cycle, we employed the mAiD system for conditional downregulation at the protein level. Parasites depleted of γ -tubulin for seven days failed to form plaque in the fibroblast monolayers, revealing the essential role of the gene for the parasite lytic cycle (**Fig3A**). The γ -tubulin protein depletion exhibited fast regulation within one hour (**Fig3B**).

To dissect the role of γ -tubulin in the cell cycle, parasites were allowed to replicate for 12 to 14h in presence or absence of auxin. *T. gondii* parasites depleted for γ -tubulin display significant morphological defect, adopting a “bulbous” shape, defined by the IMC1 signal (**Fig3C**). Although the production of new microtubules represented by the acetylated tubulin staining appears highly reduced, some signal could still be detected. Strikingly, parasites continue to produce new daughter cell material such as IMC and secretory organelles (micronemes and rhoptries), which became mis-localized and dispersed (**Fig3C**). Additionally, γ -tubulin depletion results in the failure of apicoplast scission that appears fragmented as well as the centrosome duplication with an odd number of centrin1 puncta (**Fig3C**). Moreover, the enlarged replicated nucleus is unable to divide likely due to the lack of spindle microtubules formation (**Fig3C**).

To investigate the cause of the bulbous shape, intracellular γ -tubulin depleted parasites were examined by U-ExM. Short-time treated parasites showed no centriole duplication while sparks of microtubules emerged from these unduplicated centrioles (white arrows) (**Fig3D**). With prolonged auxin treatment, these sparks of microtubules, still connected to the centrioles, elongated to an abnormally long filaments causing the parasite’s tubulin cytoskeleton to lose its typical polarized shape (**Fig3D**). Eventually, the classical cytoskeleton architecture of *T. gondii* becomes unrecognizable and extremely long microtubules are observed twirling around.

Interestingly, while centriole duplication is blocked, the 2 original centrioles separate from each other leading to a fragmented centrin1 staining that appears with multiple dots in these defective parasites. **(Supplemental Fig. 2E)**

To gain further information we employed electron microscopy to image the cellular abnormalities of the γ -tubulin depleted parasites. Layers of IMC budding were observed appearing in the cytosol in unconventional places such as basal pole, possibly attaching to the sparks microtubules **(Fig3E)**.

Sparks microtubules display SPMTs like properties

In absence of γ -tubulin, the formation of SPMTs, ICMTs, spindle microtubules and centrioles were abolished and the origin of the abnormally formed long microtubules is unclear. In wild-type parasites, the alveolin network attaches to the growing SPMTs (Mann and Beckers 2001; Gould et al. 2008; Anderson-White et al. 2011). Interestingly, under U-ExM, budding of the alveolin network denoted by the IMC1 staining was observed on these sparks microtubules suggesting shared properties with the SPMTs **(Fig4A)**. A hallmark of SPMTs is the appearance of poly-glutamylation in a gradient, with the highest intensity at the apical side (Delgado et al. 2024). Similarly, the sparks microtubules emerging from unduplicated centrioles were observed to be gradually poly-glutamylated **(Fig4A)**.

To gain further insight about the molecular identity of these sparks microtubules, we tagged into the γ -tubulin-mAID-HA background four microtubules associated proteins namely DCX for conoid fibers, ICMAP2 for ICMTs, SPM1 for SPMTs and EB1 for spindle microtubules **(Fig4B)**. All four proteins were correctly localized in their respective compartments and their correct expression was verified by western-blot **(Fig4B) (Supplemental Fig. 2F)**. In absence of γ -tubulin, none of the four selected candidates were seen colocalizing with the sparks

microtubules defined by the acetylated tubulin and PolyE staining, suggesting that these sparks
microtubules are molecularly divergent microtubules (**Fig4B**).

The γ -tubulin, in conjunction with GCP proteins, shares localization and phenotypic characteristics

The nucleation of microtubules is a multifactorial process in which γ -tubulin associates with additional proteins called GCPs (Gamma tubulin Complex Proteins) to provide the molecular scaffold for microtubule nucleation (Moritz et al. 2000; Farache et al. 2016). *T. gondii* possesses genes encoding GCPs albeit a reduced number compared to other organisms, with GCP2, GCP3, and GCP4 being identified (Morlon-Guyot et al. 2017).

To investigate their localization and function, we endogenously tagged the three genes by inserting a mini-auxin inducible degron sequence (mAiD) alongside a 3HA-epitope tag in the 3'UTR. We also endogenously Ty-tagged the GCPs in the background of γ -tubulin-mAiD-HA to explore the interactions with γ -tubulin. While we obtained epitope tagged GCP2 and GCP3, recovery of parasites with tagged GCP4 was unsuccessful (**Supplemental Fig. 1A**).

Parasites depleted of GCP2 or GCP3 for seven days were unable to form plaque in the fibroblast monolayers, underscoring the essential role of these genes (**Fig5A**). Immunofluorescence analysis revealed colocalization of GCP2 and GCP3 with the γ -tubulin signal (**Fig5B**). Interestingly, depletion of γ -tubulin led to no detectable signal of GCP2 and GCP3 in immunofluorescence along with reduced protein levels by western-blot (**Fig5C-D**). Conditional depletion of GCP2 or GCP3 led to a phenotype resembling that observed for γ -tubulin, characterized by a major morphological defect and adoption of a “bulbous” structure (**Supplemental Fig. 2G**). U-ExM revealed the association of both GCP2 and GCP3 with the forming apical complex during parasite division (**Fig5E**). Moreover, similar to γ -tubulin, the signals for GCP2 and GCP3 were consistently observed on the side of the apical complex that

was not yet formed, suggesting their role in the very early stages of this structure's formation (Fig5E).

***Cryptosporidium parvum* γ -tubulin adopts a dynamic localization throughout the cell cycle**

We sought to investigate the conservation of microtubule nucleation in another apicomplexan parasite, *Cryptosporidium parvum*, one of the major cause of children severe diarrheal disease (Khalil et al. 2018). Despite its reduced genome size, *C. parvum* harbors multiples genes related to microtubules formation suggesting a conservation (Fig6A). This parasite exhibits a polarized morphology that comprises cytoskeletal elements including the apical complex (conoid, preconoidal rings, apical polar ring) and subpellicular microtubules (Wang et al. 2021; Mageswaran, Guerin, et al. 2021) (Fig6B).

Commercially available antibodies known to label *T. gondii* tubulin structures were tested on *C. parvum* sporozoites. While anti-acetylated tubulin and polyglutamine (polyE) antibodies showed no cross-reactivity, the alpha/beta-tubulin antibodies provided informative staining on *Cryptosporidium* sporozoite. We obtained a similar pattern using the alpha-tubulin or a combination of alpha and beta that resembles the staining with the antibody generated against the *Cryptosporidium* beta-tubulin (Wang et al. 2021; Uni et al. 1987). These antibodies label long and short tubulin filaments with a connection observed between them likely corresponding to SPMTs and the conoid (Fig6C). U-ExM on extracellular sporozoites revealed additional apical staining as well as staining close to the nucleus (Fig6C) which is distinct from centrin1 staining, suggesting a unique arrangement of tubulin structures (Fig6C). Iterative expansion microscopy (iUEXm) (Louvel et al. 2023) revealed multiple shorter SPMTs in addition to the short and long SPMT (Fig6D). These observations mirror previous images of *Cryptosporidium* apical pole acquired by cryo-electron tomography (CryoET) (Martinez et al. 2023), where a

long SPMT, a short and multiple shorter SPMTs are linked to the apical polar ring (APR) (blue), follow by the conoid (purple) and the preconoidal rings PCRs (green) (**Fig6D**) (**Supplemental Fig3A**).

Despite sharing cytoskeletal morphology with *T. gondii*, *C. parvum* parasites divide by schizogony (English et al. 2022) that involve the formation of 8 nuclei prior cytokinesis (**Fig6E**). To assess the role of γ -tubulin in *C. parvum*, the gene was endogenously epitope-tagged (3HA) allowing its localization within the cell cycle (**Supplemental Fig1B**). Interestingly, γ -tubulin protein is undetectable in extracellular sporozoites and in intracellular parasites harboring 1 nucleus contradicting a previous report using anti- γ -tubulin antibody (Wang et al. 2024)(**Fig6F**) (**Supplemental Fig3B**). However, upon nuclear scission 2 dots of γ -tubulin were detected on each side of the nuclei, near the centrin1 staining presumably in the centrosomal region (**Fig6F**). U-ExM on intracellular dividing parasites revealed γ -tubulin accumulation on both side of the spindle microtubules preceding their detection (**Fig6G**) (**Supplemental Fig3C**) and without accumulation observed after the budding phase once the cytoskeleton of the merozoites were formed (**Fig6G**).

Discussion

Apicomplexan parasites rely on their tubulin cytoskeleton in many aspects of their cell and life cycles. While it has been suggested that γ -tubulin could play the same role in nucleating the spindle microtubules, functional analyses supporting this hypothesis were lacking (Oakley, Paolillo, and Zheng 2015). Additionally, the nucleation of the cortical cytoskeleton such as the SPMTs, conoid, ICMTs remained elusive with some proposing a γ -tubulin-independent process due to its observed localization exclusively to the centrosomal region (Suvorova et al. 2015; Padilla, Murray, and Hu 2024). We present evidence for the conserved localization of the γ -tubulin in two apicomplexan parasites *T. gondii* and *C. parvum* in the formation on

spindle microtubules, during nucleus scission, Applying U-ExM allowed to explore the early formation of the apical complex and cortical cytoskeleton, revealing the transient localization of γ -tubulin to these structures, presumably to control their nucleation process. The association of γ -tubulin with the forming apical complex appears to be very transient, always on the opposite side as the tubulin staining suggesting a role in the initiation but not for elongation of microtubules. Importantly, we found that the reduced set of γ -tubulin complex proteins (GCPs) originally identified in a in silico analysis recapitulated the same localization and phenotype as γ -tubulin, emphasizing their functional conservation (Morlon-Guyot et al. 2017). Depletion of γ -tubulin in parasites resulted in dramatic morphological impairments, primarily due to the lack of nuclear scission and cortical/conoid cytoskeleton formation. However, residual microtubules could still be observed in these defective parasites. In absence of γ -tubulin, microtubules emerge from unduplicated centrioles and as the cell cycle progresses these microtubules end elongate to an extreme length, forming abnormal structures that twirled around the misshaped parasite. These microtubules are associated alveolin network budding and exhibit gradual poly-glutamylolation. However, none of the four microtubule-associated proteins tagged in the γ -tubulin-mAID-HA background localized on these microtubules, suggesting a divergent composition with SPMTs-like properties. The origin and nucleation process of these abnormal microtubules, which appeared to be γ -tubulin-independent, remain open questions, warranting further investigation.

In our investigation of *C. parvum*, we aimed to enhance our understanding of its cytoskeleton organization. Recent advances in Cryo-ET have filled considerably the gap of knowledge on the cytoskeleton of *C. parvum* (Wang et al. 2021; Mageswaran, Guérin, et al. 2021; Martinez et al. 2023), although some aspects remain contentious in the literature. Indeed, *Cryptosporidium* sporozoite has been first suggested to possess numerous long SPMTs by EM analyses (Baba et al. 2008; Uni et al. 1987), however, the IMC surface filaments were likely

misinterpreted as SPMTs (Martinez et al. 2023). Additionally, some studies reported the presence of two central microtubules, which are not consistently identified as SPMTs (Uni et al. 1987; Wang et al. 2021). To clarify these discrepancies and provide a detailed view of the tubulin cytoskeleton in *Cryptosporidium* sporozoites, we employed U-ExM and iU-ExM. We observe two long SPMTs with one longer than the other and attached to the APR. We also observe architecture of the sporozoites centrosome with a conserved localization of the centrin1 as well as numerous short SPMTs below the APR and the extruded conoid consistent with previous EM and Cryo-ET analysis (Martinez et al. 2023; Uni et al. 1987).

Given that nucleation of the tubulin cytoskeleton of *C. parvum* remains an open question, we investigated the role of γ -tubulin. Contrary to previous observations using a commercial anti- γ -tubulin antibody, endogenous tagging of the gene shows no accumulation of γ -tubulin at the apical pole of sporozoites (Wang et al. 2024). This observation aligns with the absence of microtubule nucleation at this stage. Mass spectrometry analysis further supported the absence of γ -tubulin complex proteins in sporozoites, suggesting no active nucleation in these parasites (Guérin et al. 2023). Additionally, γ -tubulin was observed to accumulate in intracellular parasite during merogony before the formation of the spindle microtubules presumably to nucleate them for nuclear division. As soon as the 8 nuclei were separated the γ -tubulin signal disappeared. However, in contrast to *T. gondii*, we were not able to detect γ -tubulin in the forming cytoskeleton of merozoites. This discrepancy could be attributed to the challenge of catching merozoites cytoskeleton formation or that their nucleation is guided by a γ -tubulin independent mechanism.

Recent advances such as the implementation of conditional knock down system combined to advanced microscopy techniques should help to unravel the formation of *C. parvum* tubulin cytoskeleton (Choudhary et al. 2020; Tandel et al. 2023; Xu et al. 2024).

Materials and Methods

312 ***T. gondii* maintenance in tissue culture**

313 *Toxoplasma gondii* tachyzoites were amplified in HFFs (ATCC) in Dulbecco's Modified
314 Eagle's Medium (DMEM, Gibco) supplemented with 5% of fetal calf serum (FCS, Gibco),
315 2 mM glutamine and 25 µg/ml gentamicin (Gibco). Parasites and HFFs were maintained at
316 37°C with 5% CO₂.

317

318 ***C. parvum* strain lineage, host cell culture and mouse model**

319 *C. parvum* oocysts (Iowa II strain) used to make the transgenic line were purchased from the
320 Bunchgrass Farms Deary, ID.

321 Cell lines used for this study were HCT8 (ATCC: CCL-244) purchased from ATCC. The cells
322 were cultured in DMEM supplemented with 10% bovine serum.

323 Ifng ^{-/-} mice with C57BL/6J background were bought from Charles River Laboratories and
324 were bred at the University of Geneva. 4 male mice at the age of 6 weeks were used to infect
325 with the *C. parvum* transgenic line. Feces were collected from day 5 to day 21. All the animal
326 studies performed at the University of Geneva are complied with the Swiss National
327 Institutional Guidelines on Animal Experimentation and were approved by the respective
328 Swiss Cantonal Veterinary Office Committees for Animal Experimentation (GE276A).

329

330 **Generation of transgenic *T. gondii* lines**

331 Freshly egressed tachyzoites were transfected by electroporation (Soldati and Boothroyd
332 1993).

333 All the strains used in this study were constructed using CRISPR-mediated homology directed
334 repair. For construction of 3'-insertional epitope tagging, 40 µg pU6-Universal (pU6-Universal
335 was a gift from Sebastian Lourido, Addgene plasmid # 52694) bearing the gRNA to target the
336 3'UTR of gene of interest (GOI) was transfected together with PCR product containing

homology regions for the selected gene. To generate the knock-out lines, a plasmid bearing dual gRNA targeting at 5'- and 3'-locus of GOI was co-transfected with PCR product encoding DHFR resistance cassette. For enrichment of transfected population, parasites carrying an HXGPRT cassette were selected with 25 mg/ml of mycophenolic acid (MPA) and 50 mg/ml of xanthine. Parasites carrying a DHFR cassette were selected with 1 µg/ml of pyrimethamine. All the assays involving AID-based conditional knockdown system, the protein depletion was achieved by adding 500µM of auxin (IAA) (Brown, Long, and Sibley 2018)

Generation of transgenic *C. parvum* transformants

20 million *C. parvum* oocysts were excysted and electroporated as previously described (Guérin et al. 2021). Briefly, a guide RNA corresponding to the 3'UTR of *cgd7_1980* (AGAAAGGAGAAGTACGCGGA) was inserted by ligation in the Cas9 vector. 50ug of the corresponding vector was precipitated with 200ul of repair template PCR containing 30bp homology with *cgd7_1980*, the triple hemagglutinin tag, a generic 3' UTR and the nanoluciferase-neomycin cassette under the enolase promoter (**Supplemental Table1**). Transfected parasites were gavaged into ifng -/- mice and paromomycin in the mice drinking water was used to select resistant transformants. Shedded oocysts from the feces were collected and purified using sucrose floatation and cesium chloride gradient. They were stored in PBS at 4°C until further use.

Genomic PCR analysis to confirm correct integration in the genome

For both *T. gondii* and *C. parvum* genomic DNA of the parasites was extracted using Wizard SV genomic DNA purification system ki (*T. gondii*) or Quick-DNA Fecal/Soil Microbe Microprep kit (*C. parvum*). For *T. gondii* PCR primers were designed to bind outside the 30 bp homology arms in 5' region as well as to the mAID cassette or DHFR resistance cassette. For

C. parvum, PCR primers were designed to bind outside the 30 bp homology arms in 5' and 3' region as well as to the triple HA tag and the Neomycin resistance cassette inserted. For both parasites an independent strain was used as a PCR control (**Supplemental Fig1**).

Indirect immunofluorescence Assay (*T. gondii*)

Parasites were fixed using a mix 4% paraformaldehyde; 0,05% Glutaraldehyde during 10min at room temperature. Following fixation, they were permeabilized by 20min incubation with PBS-Tx100 0,2% at room temperature. Parasites were blocked during 20 min by PBS-BSA (bovine serum albumin) 5% followed by incubation with the primary antibodies during 1h in PBS-BSA 2%. Coverslips were washed 3 times during 5min by PBS-Tx100 0,2% and then incubated with secondary antibodies diluted in PBS-BSA 2% during 1h at room temperature. Coverslips were washed 3 times during 5min by PBS-Tx100 0,2% and then mounted using Fluoromount G on microscope slides.

Indirect immunofluorescence Assay (*C. parvum*)

Oocysts were bleached using 2.7% bleach and were excysted with 0.8% sodium taurocholate for 10 min. To image sporozoites, excysted oocysts were incubated for 1h at 37°C in PBS. Following incubation, they were allowed to settle onto poly-L-lysine coated coverslips for 30 min. To investigate intracellular parasites, they were allowed to infect human adeno carcinoma HCT-8 cells for various times mentioned in individual experiments. These were then fixed with 4% paraformaldehyde for 15 min or with methanol for 7 min at -20°C and permeabilized with 0.5% Triton X-100 for 15 min. Samples were blocked with 4% bovine serum albumin (BSA) for 1 hour at room temperature, following by 1h of primary antibodies diluted in 1% BSA. After being washed 3 times with PBS, secondary antibodies were diluted in 1% BSA for 1 hour at room temperature (Dilution of antibodies and dyes used in supplementary table). The cells

were stained with the DNA dye Hoechst for 5 min which was diluted in PBS. A 3 times wash with PBS is followed and the coverslips were mounted on glass slides with Fluoromount. The cells were imaged using the Leica TCS SP8 microscopy with the lens HC PL Apo 100x/1.40 Oil CS2.

Expansion microscopy (*T. gondii*)

The expansion microscopy protocol applied to *Toxoplasma gondii* tachyzoites was followed as described in this study (Dos Santos Pacheco and Soldati-Favre 2021). Coverslips containing intracellular dividing parasites were incubated in PBS containing 0.7% formaldehyde and 1% acrylamide for 3h at 37°C. Polymerization of expansion gel were performed on ice containing monomer solution (19% sodium acrylate/10% acrylamide/0.1% (1,2-Dihydroxyethylene) bisacrylamide), 0.5% ammonium persulfate (APS) and 0.5% tetramethyl ethylenediamine (TEMED) as described in this study (Louvel et al. 2023). Fully polymerized gels were denaturated at 85°C for 90 min in the denaturation buffer (200 mM SDS, 200 mM NaCl, 50mM Tris, pH = 6.8) and expanded in pure H₂O overnight.

On the next day, the expansion ratio of fully expanded gels was determined by measuring the diameter of gels. Well expanded gels were shrunk in PBS and stained with primary and secondary antibodies diluted in freshly prepared PBS/BSA 2% at 37°C for 2h. Three washes with PBS/0.1% Tween for 10min were performed after primary and secondary antibody staining. Stained gels were expanded again in pure H₂O overnight for further imaging. All U-ExM images used in this study were acquired using Leica TCS SP8 microscopy with the lens HC PL Apo 100x/1.40 Oil CS2. Images were taken with Z-stack and deconvolved with the built-in setting of Leica LAS X or with Huygens software. Final images were processed with ImageJ and the maximum projected images were presented in this study.

Expansion microscopy (*C. parvum*)

Ultrastructure expansion microscopy was applied as described for *Toxoplasma*. Parasites were excysted and fixed the same way as mentioned for IFA. Samples were embedded overnight in a mixture of acrylamide and formaldehyde which adds a molecular anchor to each protein and thus, crosslinking of protein groups was avoided. The formed gel was transferred into denaturation buffer and denatured at 95 °C. Following denaturation, the gels were then placed in water to expand around 4 times its size. The gels were shrunk with PBS before incubation for 3h with primary antibodies, 3 washes with PBS-Tween 0.5% and incubation for 3h with secondary antibodies containing Hoechst DNA dye. The gels were bathed in water for expansion. All U-ExM images used in this study were acquired using Leica TCS SP8 microscopy with the lens HC PL Apo 100x/1.40 Oil CS2. Images were taken with Z-stack and deconvolved with the build-in setting of Leica LAS X or with Huygens software. Final images were processed with ImageJ and the maximum projected images were presented in this study.

Iterative Ultrastructure Expansion Microscopy (iU-ExM)

Iterative ultrastructure expansion microscopy was applied as described (Louvel et al. 2023). Parasites were excysted, fixed and anchored the same way as mentioned for U-ExM. The first monomer solution was added to fill in the space of the gelation chamber. Following an incubation of 15 min on ice, an incubation at 37 °C for 45 min was completed. The formed gels were transferred into the denaturation buffer and incubated at 85 °C for 90min. The gels were placed in water to expand 5-6 times their size and were shrunk with PBS to apply primary and secondary antibodies. The gels were bathed in water again and were cut into pieces that were incubated 3 times for 10 min on an agitating platform with activated neutral gel. The gels were dried by sliding it onto the microscope slide. A coverslip was placed on top and the chamber was incubated at 37 °C for 1h. The gel was bathed in the combination of acrylamide and formaldehyde for overnight at 37 °C under agitation. The following day, the gel was washed

twice with 1x PBS for 30 min before applying the 3rd monomer solution to achieve an expansion factor of approximately 16x. The gel was washed 3 times for 10 min under agitation on ice and was dried on the microscope slide. A coverslip was placed on top within a chamber and incubated for 1h at 37 °C. The gel was bathed in 200 mM NaOH solution for 1h under agitation at RT and was washed in 1x PBS for 20 min until the pH reaches 7. The last round of expansion was carried out with water and cells were imaged using Leica TCS SP8 microscopy with the lens HC PL Apo 100x/1.40 Oil CS2. Images were taken with Z-stack and deconvolved with the built-in setting of Leica LAS X or with Huygens software. Final images were processed with ImageJ and the maximum projected images were presented in this study.

Plaque assay

HFF monolayers were infected with a serial dilution of *T. gondii* tachyzoites and grown for seven days at 37 °C. Cells were fixed with using paraformaldehyde-glutaraldehyde for 10 min followed by a neutralization by PBS/Glycine 0.1M. The fixed monolayer was then stained with crystal violet for 2 h and then washed three times with PBS.

Western blot analysis for monitoring auxin-induced protein degradation

HFF monolayers seeded in 6 cm petri dishes were infected with freshly egressed parasites and grown for a defined time (1h to 10h) in absence or presence of IAA (500 µM). Cells containing the parasites were scrapped and pelleted at 1200 rpm and resuspend in protein loading buffer containing 2% SDS and boiled for 15min at 95°C. Protein depletion was assessed by Western-Blot using anti-HA antibody against the protein of interest and anti-MIC2 as a loading control.

Acknowledgments

We thank for their technical assistance and imaging analysis, the team at the Bioimaging Core Facility, François Prodon, Olivier Brun and Nicolas Liaudet.

We generously thank Yi-Wei Chang for allowing us to use a tomogram image of *Cryptosporidium parvum*. The project is funded by the Swiss National Science Foundation to DSF (310030_215445) and to A.G (PR00P3_208568).

Author contributions

D.S-F, R.H, A.G conceptualized the project. D.S-F, R.H, A.G conceptualized the methodology and R.H, A.G, B.M and A.P performed the investigations. R.H, A.G and A.P did the formal analysis and R.H and A.P wrote the original draft. D.S-F, A.G and A.P reviewed and edited the manuscript. D.S-F and A.G did the funding acquisition and obtained resources. All authors contributed to this article and approved the submitted version.

Competing interests

The authors declare no competing interests.

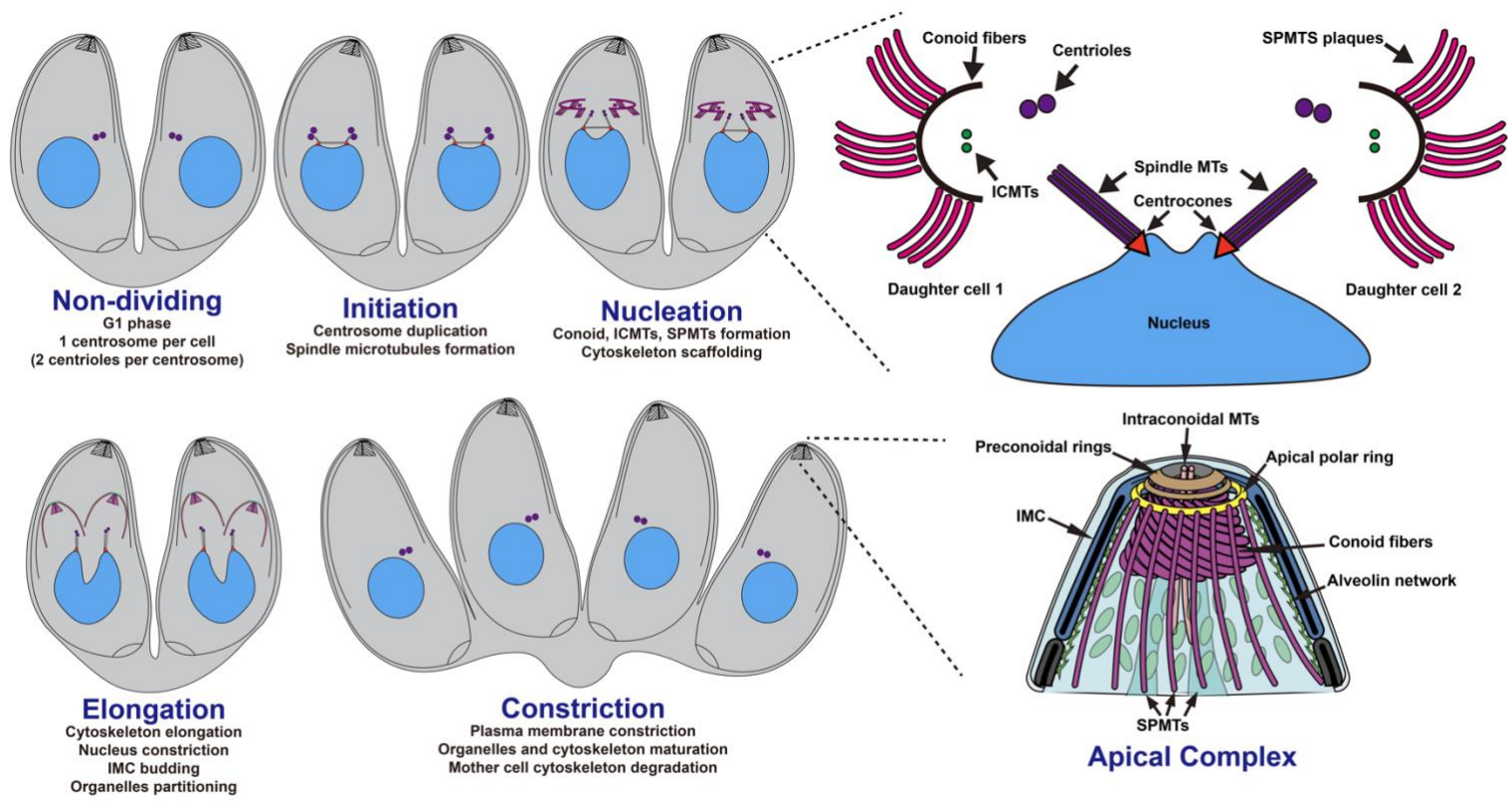
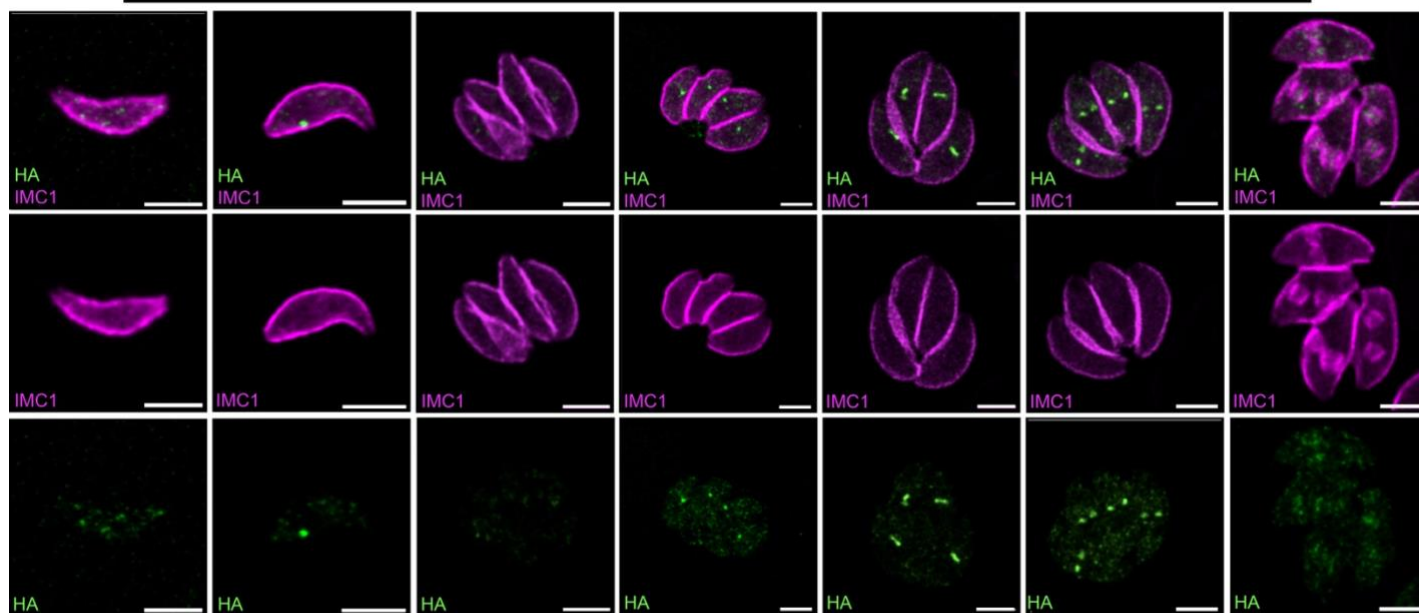


Figure 1: *Toxoplasma gondii* cytoskeletal organization during endodyogeny

Schematic representation of the endodyogeny process of *T. gondii*. Upper right corner: zoom on the nucleation with focus on the circular formation of the apical complex. Lower right corner: zoom on the fully developed apical complex.

A γ -tubulin-mAiD-HA

extracellular

intracellular

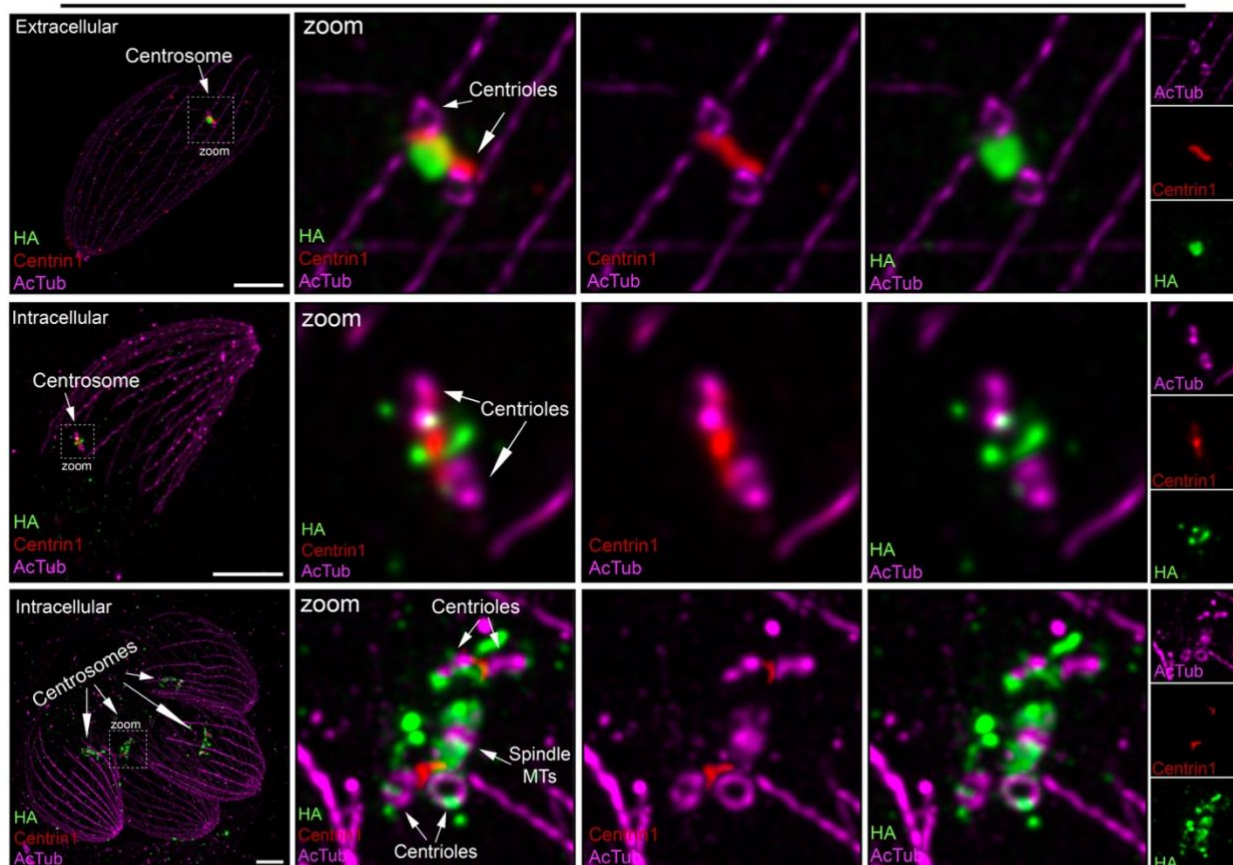
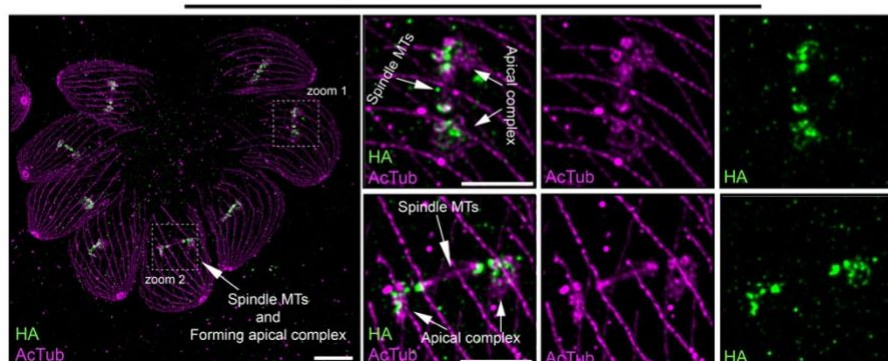
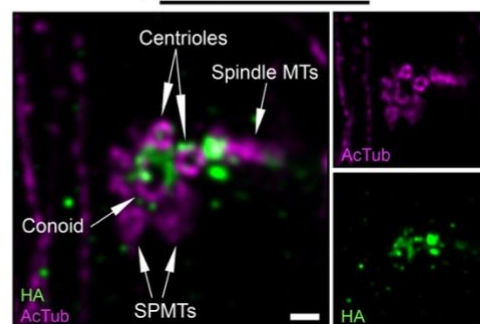
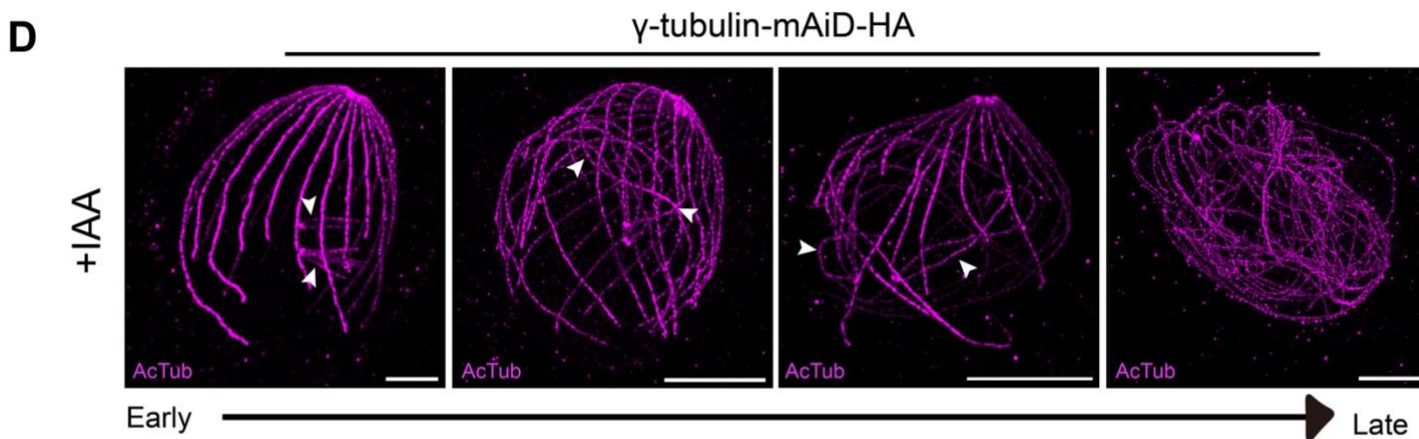
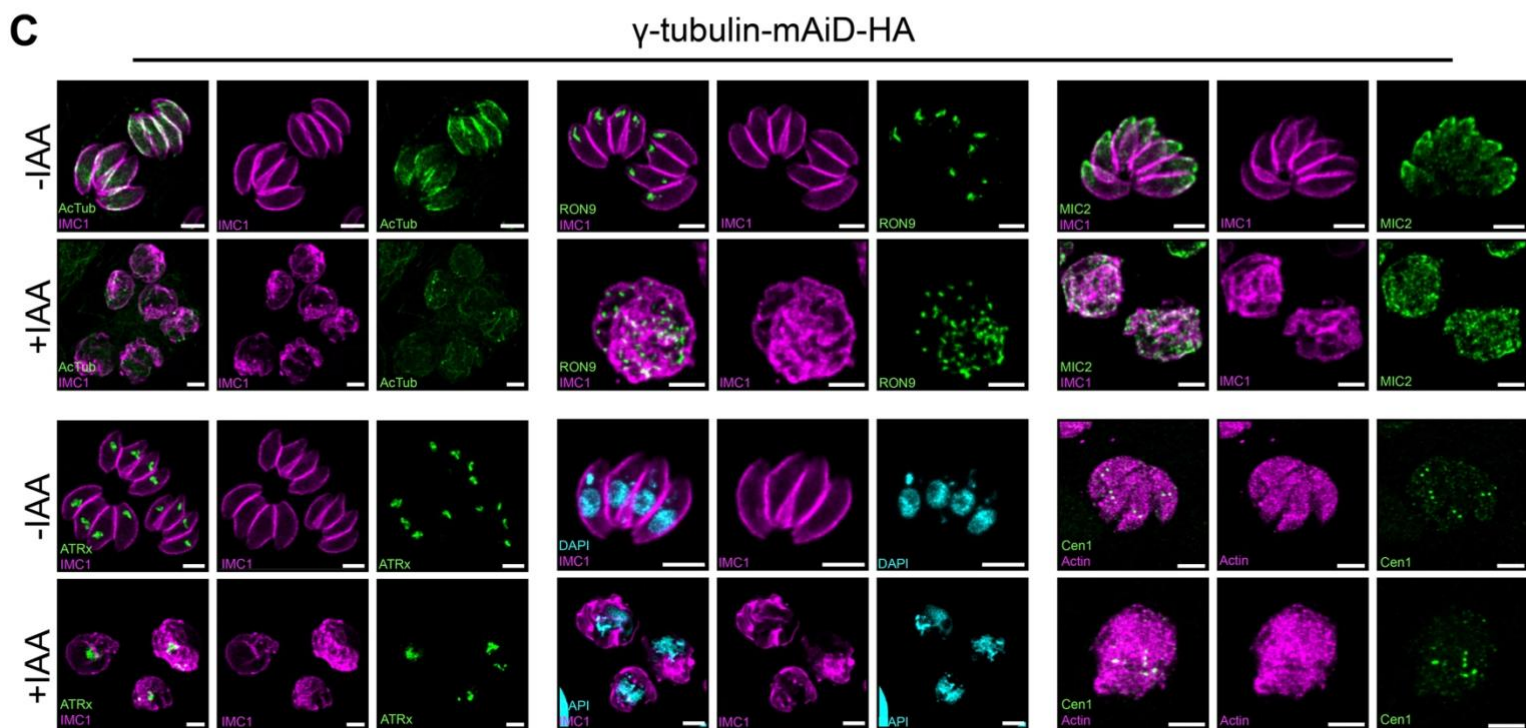
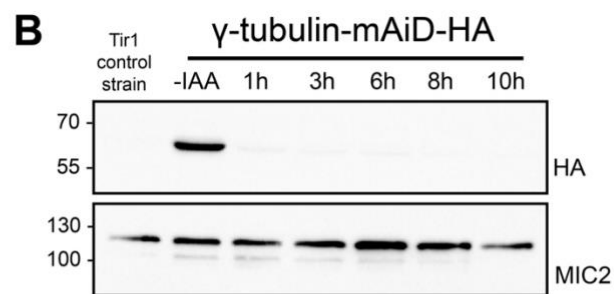
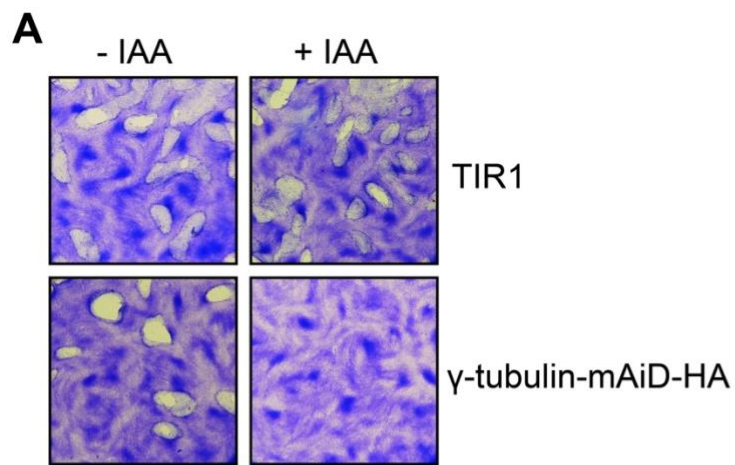
B γ -tubulin-mAiD-HA**C** γ -tubulin-mAiD-HA**D** γ -tubulin-mAiD-HA

Figure 2: Localization of γ -tubulin within *Toxoplasma gondii* cell cycle

(A): Immuno-fluorescence (IFA) images of γ -tubulin localization in extracellular and intracellular parasites. Scale bar = 3 μ m. **(B):** Ultrastructure Expansion Microscopy (U-ExM) images of the centrosomal organization in regards to centrioles (acetylated tubulin), γ -tubulin and centrin1 protein in extracellular and intracellular parasite. Scale bar = 5 μ m. **(C):** U-ExM images of intracellular dividing parasites showing the γ -tubulin localization during daughter cell cytoskeleton formation. Arrows = Forming conoid. Scale bar = 10 μ m. Scale bar zoom regions = 5 μ m **(D):** U-ExM images showing the localization of γ -tubulin on a forming conoid. Scale bar = 1 μ m.



E

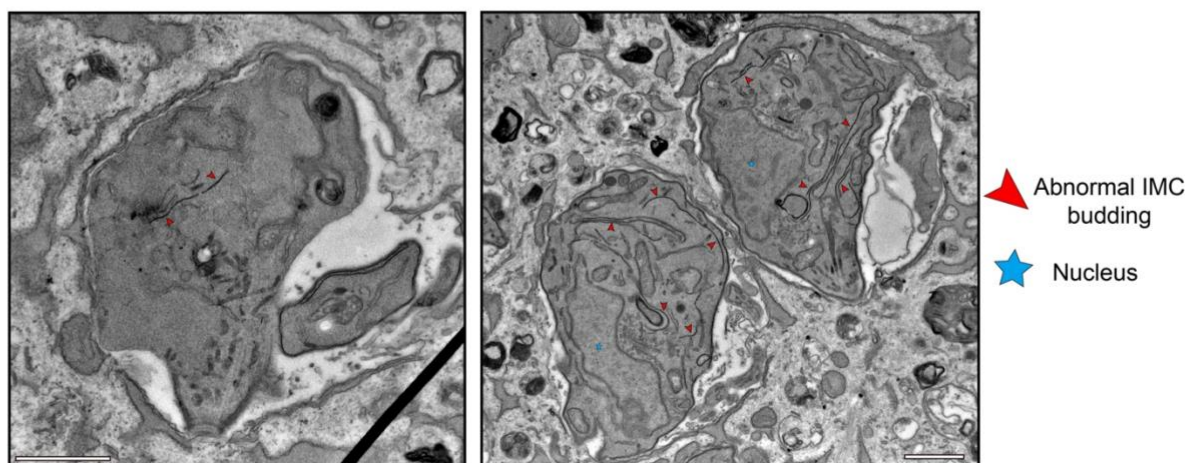


Figure 3: γ -tubulin depletion leads to dramatic morphological defects

(A): Images of plaque assay of the γ -tubulin-mAiD-HA and TIR (parental) strain in absence or presence of auxin (IAA). **(B):** Western-blot analysis of time point protein depletion using anti-HA antibody. MIC2 = loading control. **(C):** IFA of different cellular structures of *T. gondii* in presence or absence of auxin (IAA). Acetylated Tubulin = AcTub as cytoskeletal marker. Rhoptry Neck protein 9 = RON9 as rhoptry marker. Micronemal protein 2 = MIC2 as microneme marker. Apicoplast-associated thioredoxin family protein = ATRx as apicoplast marker. DAPI as nuclear marker. Centrin1 as centrosomal marker. Scale bar = 3 μ m **(D):** U-ExM of intracellular dividing parasites depleted of γ -tubulin for 12h. Scale bar = 10 μ m. **(E):** Electron microscopy images on intracellular dividing parasites. Scale bar = 1 μ m

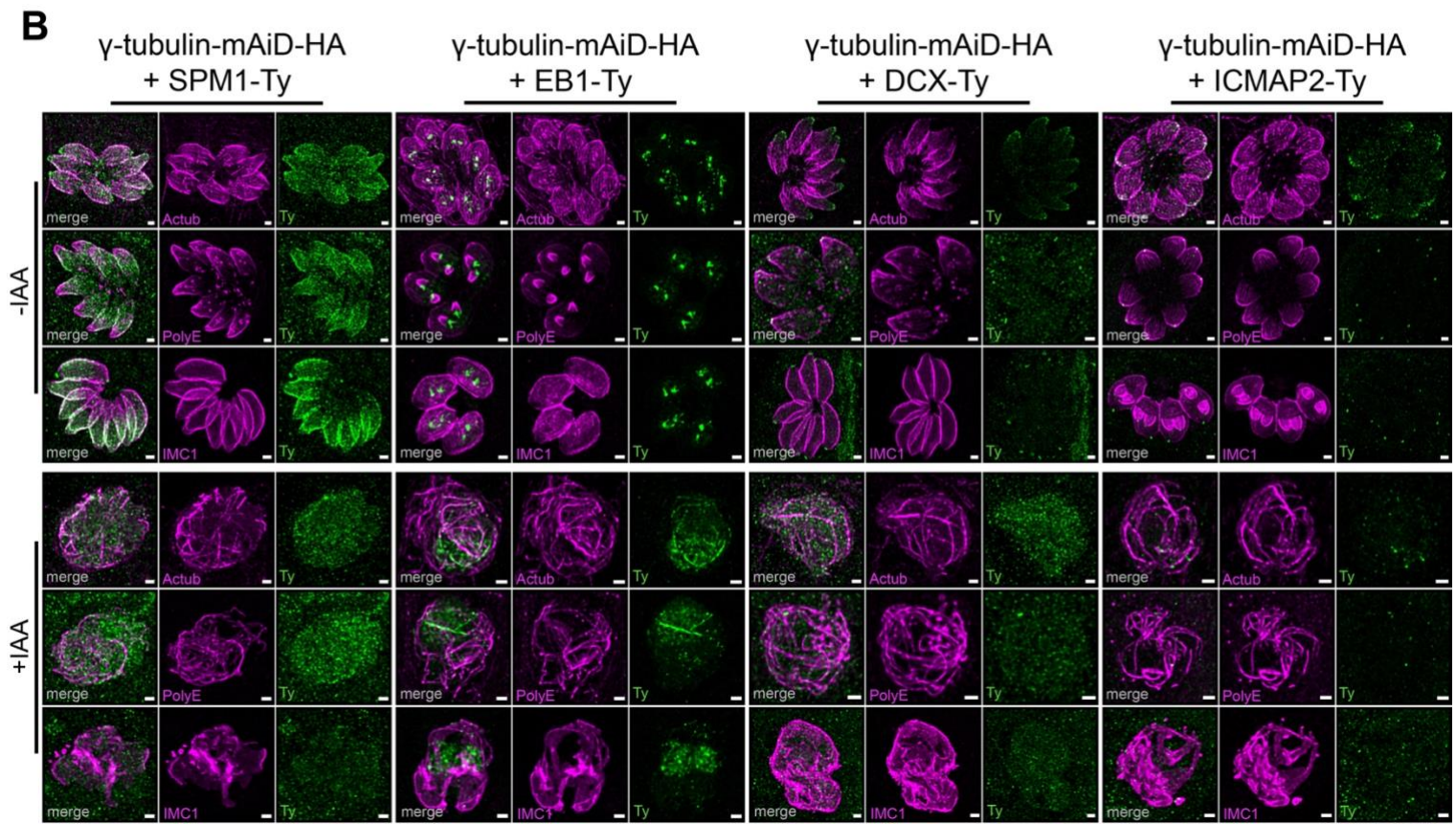
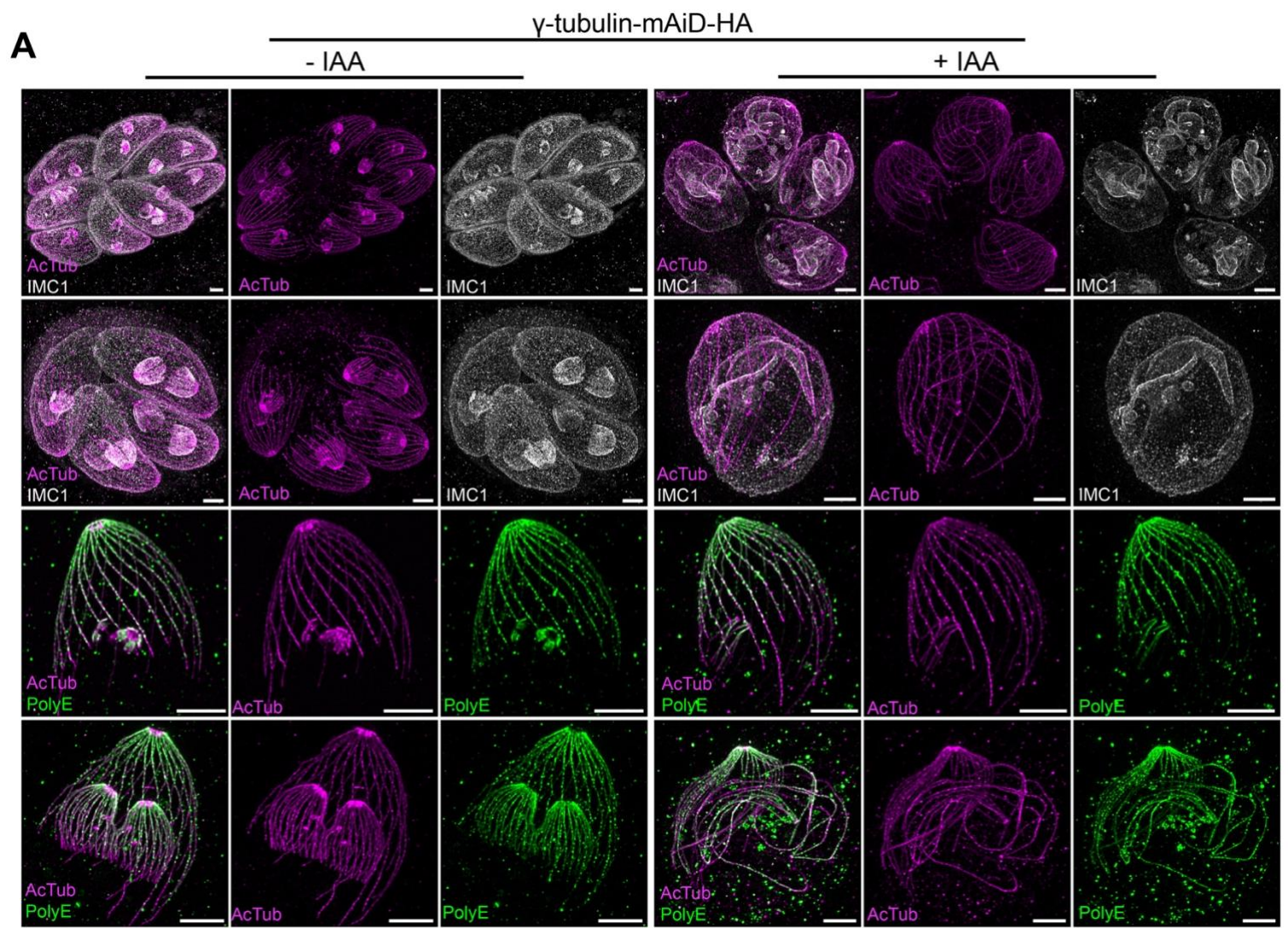


Figure 4: Sparks microtubules are divergent microtubules with SPMTs like properties

(A): Ultrastructure Expansion Microscopy (U-ExM) images of intracellular dividing parasites in presence or absence of γ -tubulin. Upper part: IMC1 staining as a marker of alveolin network budding. Lower part: PolyE staining as a marker of specific posttranslational modification of SPMTs. Scale bar = 5 μ m. **(B):** IFA showing the localization of four microtubules associated protein in presence or absence of γ -tubulin in respect to three cellular markers : Acetylated Tubulin, PolyE and IMC1. In +IAA panels, parasites were treated with auxin during 12h. Scale bar = 1 μ m.

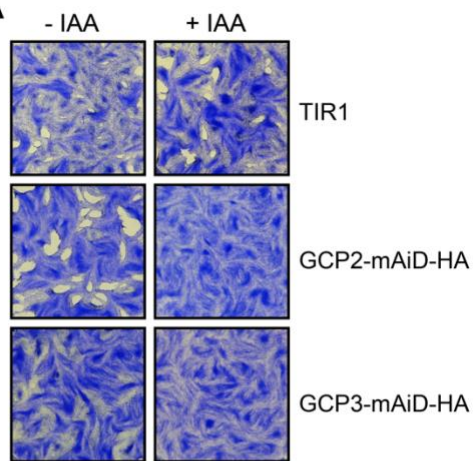
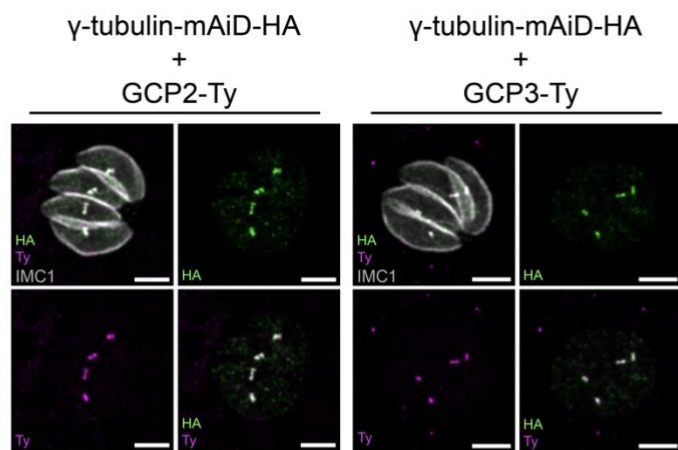
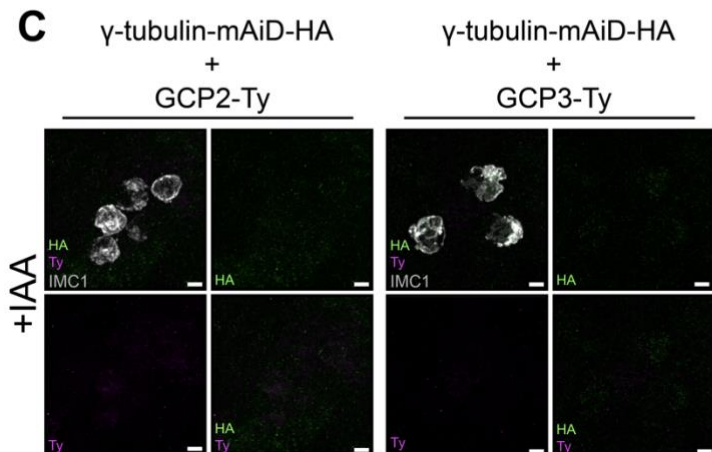
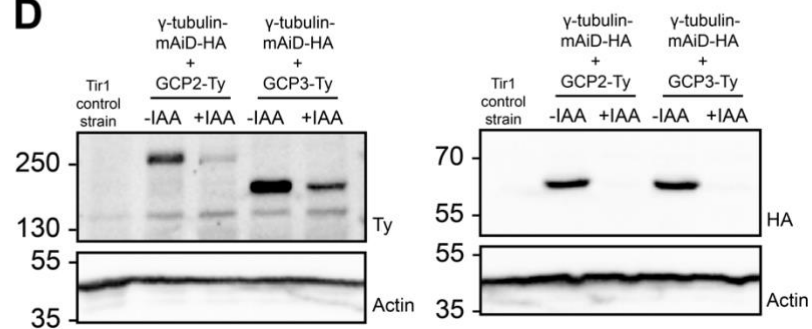
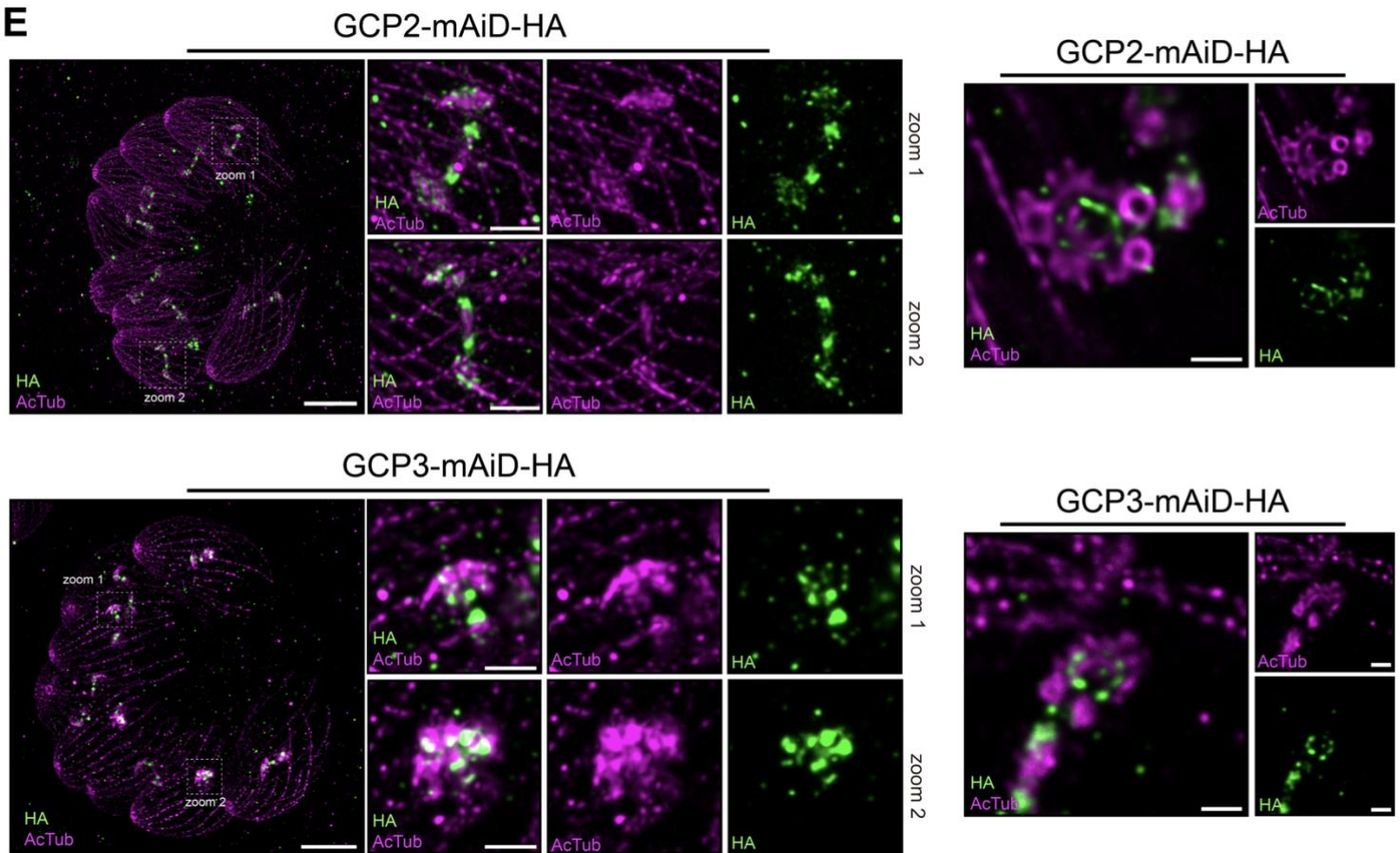
A**B****C****D****E**

Figure 5: Nucleation of microtubules involve a concerted action of γ -tubulin and GCP proteins

(A): Images of plaque assay of the GCP2-mAiD-HA, GCP3-mAiD-HA and TIR (parental) strain in absence or presence of auxin (IAA). **(B):** IFA images of the colocalization of γ -tubulin with GCP2 or GCP3 protein in intracellular dividing parasite. Scale bar = 3 μ m. **(C):** IFA images of the intracellular dividing parasites depleted for γ -tubulin showing the loss of GCPs signal. **(D):** Western-blot analysis of protein depletion using Ty-antibody (left panel) and anti-HA antibody (right panel). Actin = loading control. IAA treatment for 12h. **(E):** Expansion microscopy images of intracellular dividing parasites showing the localization of GCP2 and GCP3 during the formation of the daughter cytoskeleton (left panel) and on the forming conoid (right panel) Scale bar = 10 μ m. Scale bar zoom regions = 2 μ m

A

Name	ID ToxoDB	ID CryptoDB	Evalue
Alpha tubulin	316400	cgd4_2860	0
Beta tubulin	266960	cgd6_4760	0
Gamma tubulin	226870	cgd7_1980	0
GCP2	263510	cgd7_3060	2e-05
GCP3	310130	cgd6_3410	8e-10
Centrin1	247230	cgd3_1270	1e-74

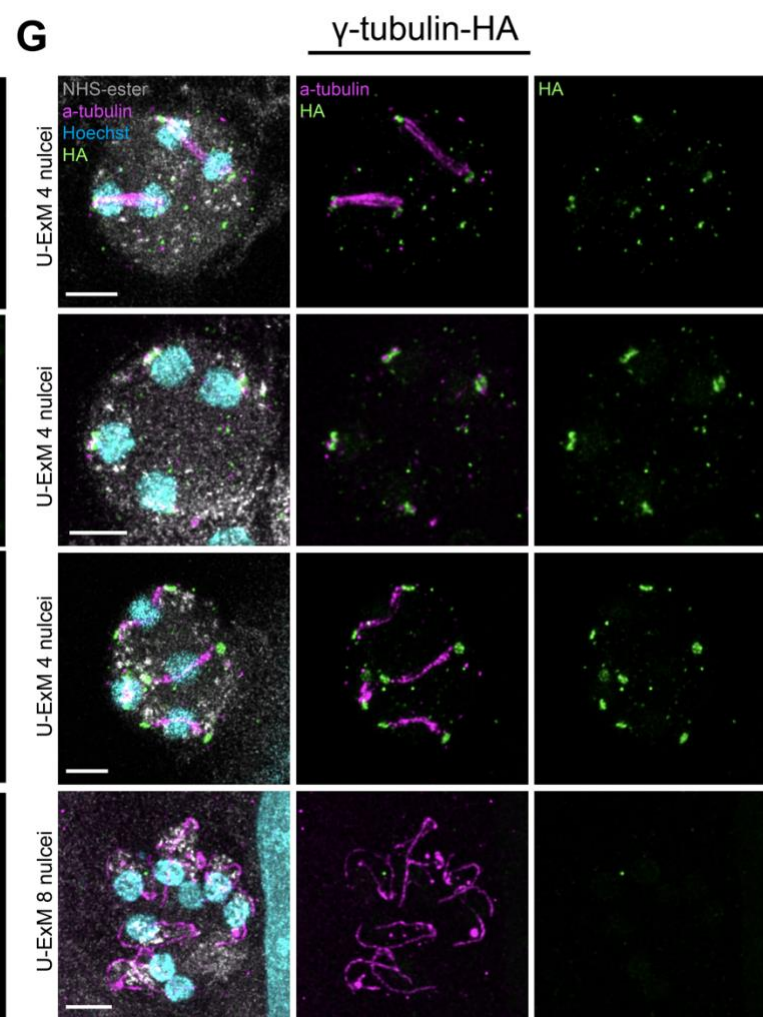
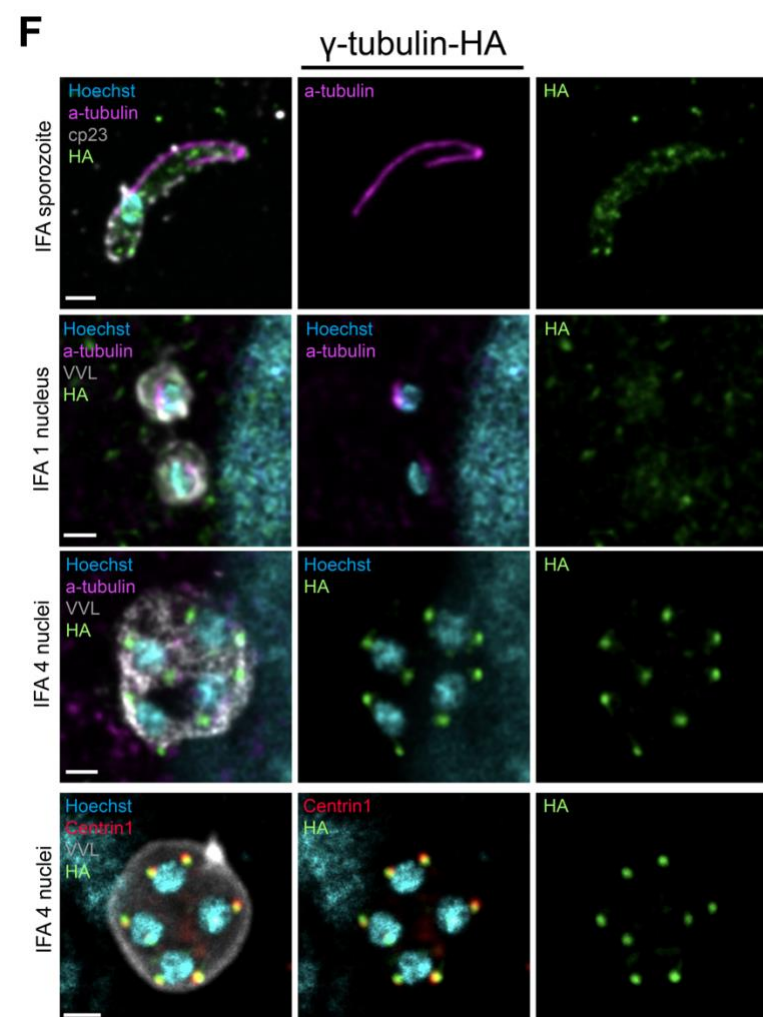
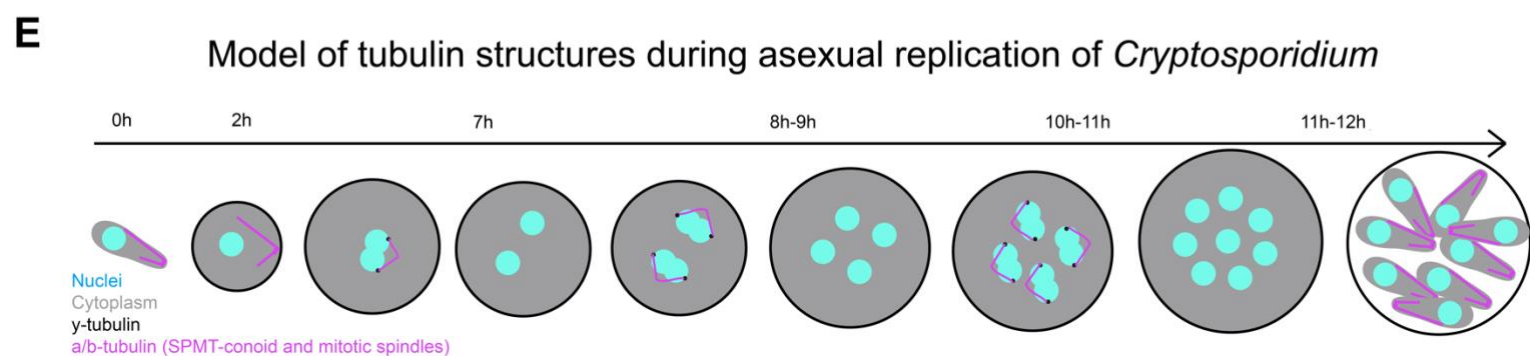
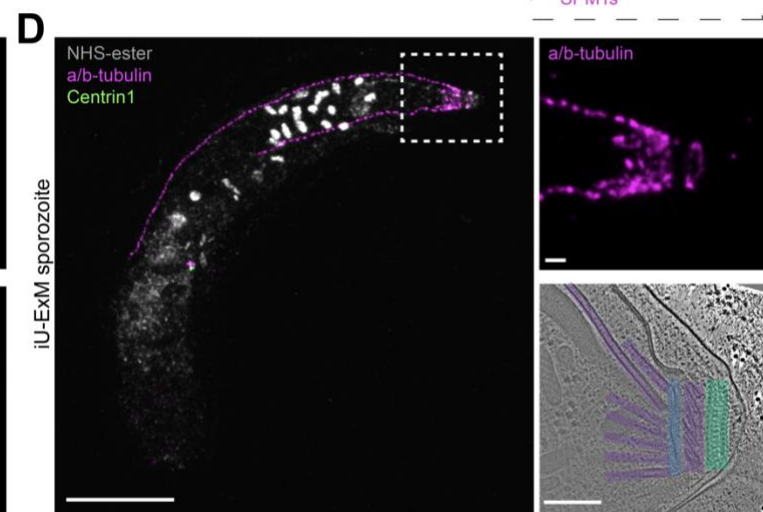
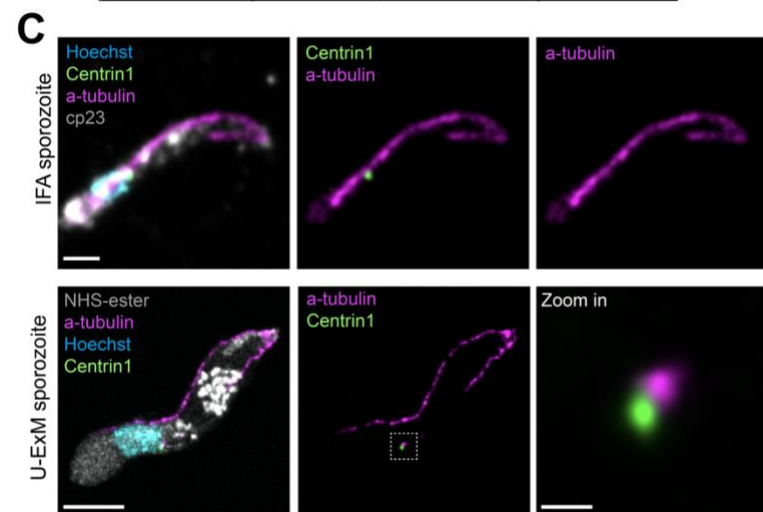
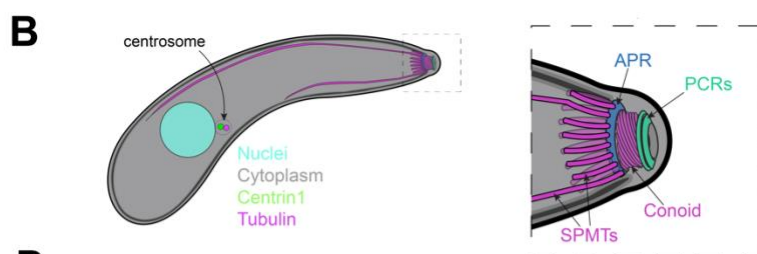
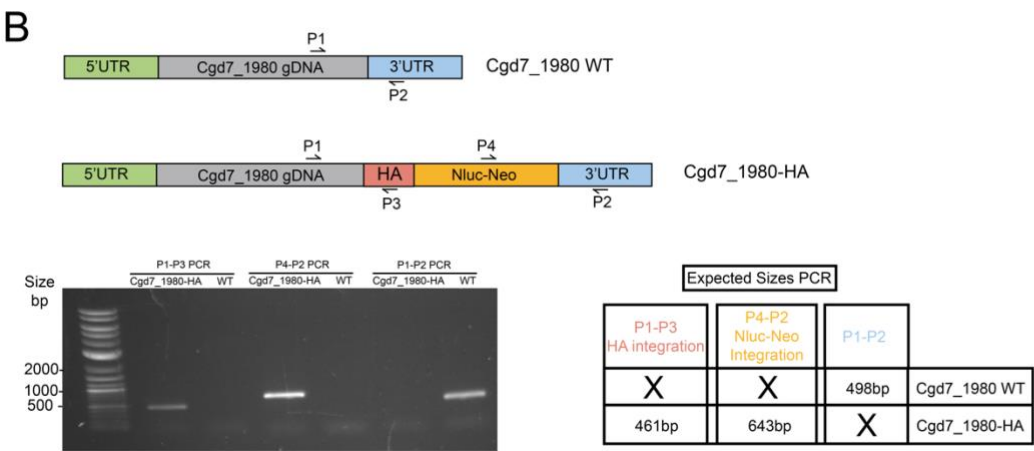
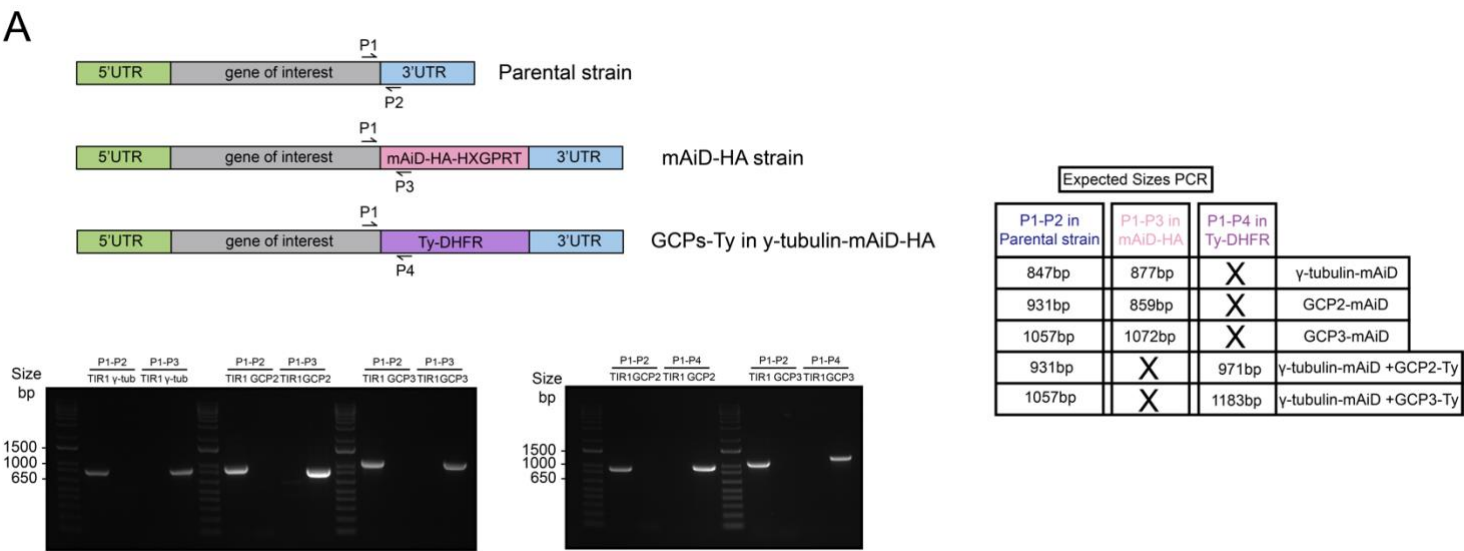


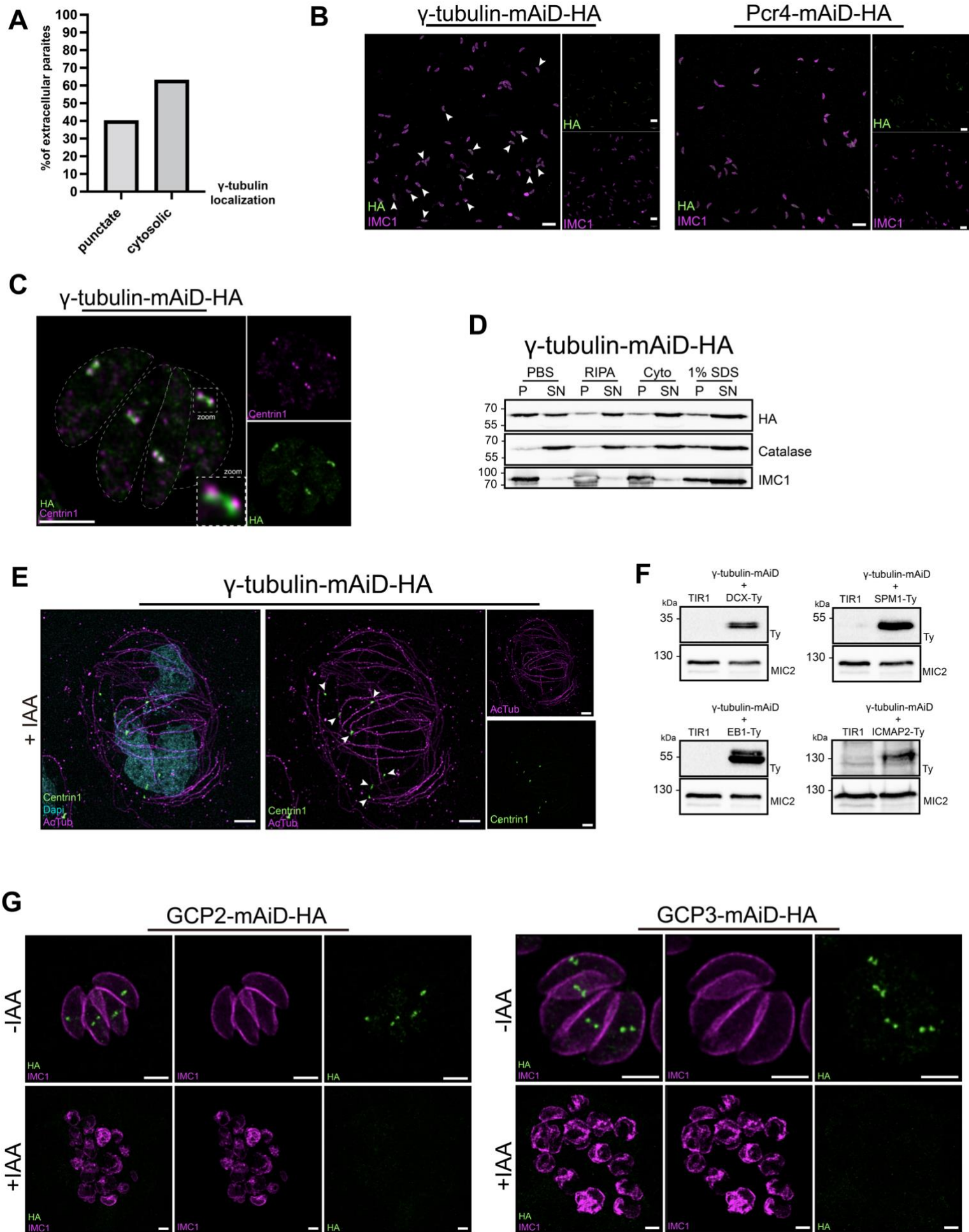
Figure 6: Analysis of *Cryptosporidium parvum* cytoskeleton and γ -tubulin dynamic during merogony

(A): Table of conservation of proteins involved in cytoskeleton formation in *T. gondii* and *C. parvum*. **(B):** Schematic representation of *C. parvum* sporozoite cytoskeletal architecture. Right panel = zoom in the apical region. **(C):** Upper panel = IFA image of *C. parvum* sporozoite. Scale bar = 1 μ m. Lower panel = U-ExM image of *C. parvum* sporozoite. Scale bar = 5 μ m. Zoom in scale bar = 0.5 μ m. **(D):** Iterative Ultrastructure Expansion Microscopy image of *C. parvum* sporozoite. Scale bar = 15 μ m. Zoom in scale bar = 1 μ m. Lower right panel = Cryo-electron microscopy tomogram slice of the apical end of *C. parvum* sporozoite. Scale bar = 0.2 μ m **(E):** Schematic representation of *C. parvum* asexual replication at different time points. **(F):** IFA images showing the γ -tubulin-HA localization in extracellular and intracellular parasites. Scale bar = 1 μ m. VVL: *Vicia villosa* lectin used as a parasitophorous vacuole marker. **(G):** U-ExM images of the γ -tubulin-HA localization in intracellular parasites at different points of the asexual replication. Scale bar = 5 μ m.



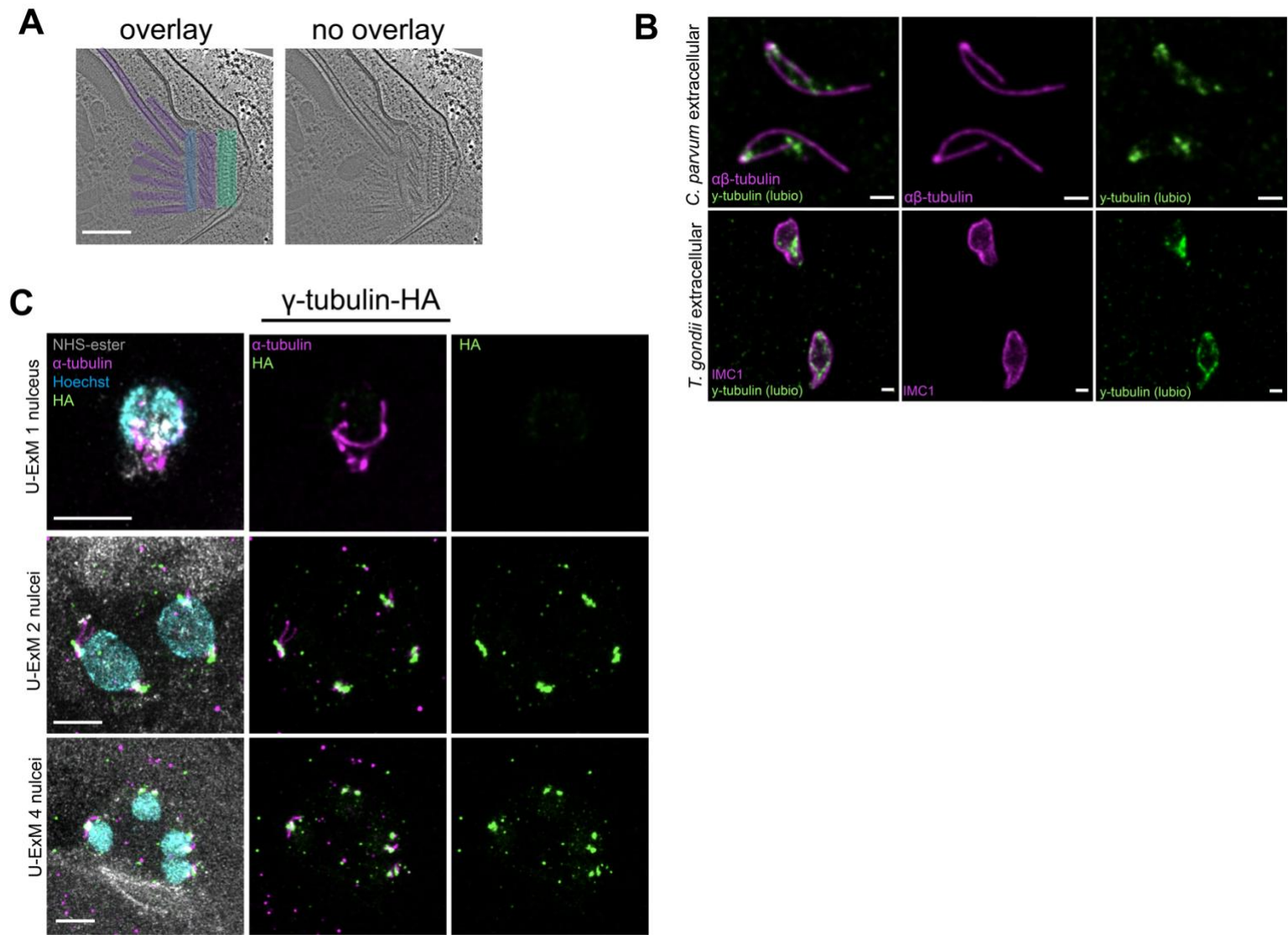
Supplemental Figure 1

(A): Schematic representation of the genomic construction for *T. gondii* strains used in this study. Verification by genomic PCR of the correct integration of the constructs. **(B):** Schematic representation of the genomic construction for *C. parvum* strains used in this study. Verification by genomic PCR of the correct integration of the constructs.



Supplemental Figure 2

(A): Proportion analysis of the pattern staining of γ -tubulin-mAiD-HA in extracellular *T. gondii* parasites. N = 100 parasites. **(B):** IFA images of extracellular parasites of γ -tubulin-mAiD-HA and Pcr4-mAiD-HA strains. Arrows showing punctate staining for γ -tubulin protein. Scale bar = 10 μ m. **(C):** IFA images of colocalization of γ -tubulin and centrin1 protein in intracellular dividing parasites. Scale bar = 3 μ m. **(D):** Western-Blot analysis of the solubility of γ -tubulin on extracellular parasites extract. Catalase = PBS soluble protein control. IMC1 = SDS1% soluble protein control. **(E):** U-ExM of intracellular dividing parasites depleted of γ -tubulin representing the fragmented centrin1 staining. Parasites were treated with auxin during 12h. Scale bar = 5 μ m. **(F):** Western-Blot analysis of the protein expression of the four microtubules associated proteins tagged into the γ -tubulin-mAiD-HA background. MIC2 protein used as a loading control. **(G):** Immuno-fluorescence representing the phenotype and localization of GCP2-mAiD-HA and GCP3-mAiD-HA in intracellular dividing parasite in absence or presence of auxin (IAA) Scale bar = 3 μ m. In +IAA panels, parasites were treated with auxin during 12h.



Supplemental Figure 3

(A): Cryo-electron microscopy tomogram slice of the apical end of *C. parvum* sporozoite with or without overlay annotation. **(B):** IFA images representing the staining of a commercially available anti- γ -tubulin antibody in *T. gondii* tachyzoites and *C. parvum* sporozoites Scale bar = 1 μ m. **(C):** Additional U-ExM images of γ -tubulin-HA localization during *C. parvum* merogony. Scale bar = 5 μ m.

678 References

- 679 Aher, Amol, Linas Urnavicius, Allen Xue, Kasahun Neselu, and Tarun M. Kapoor.
680 2023. 'Structure of the γ -tubulin ring complex-capped microtubule', *bioRxiv*:
681 2023.11.20.567916.
- 682 Anderson-White, Brooke R., F. Douglas Ivey, Katherine Cheng, Tomasz Szatanek,
683 Alexander Lorestani, Con J. Beckers, David J. P. Ferguson, Nivedita Sahoo,
684 and Marc-Jan Gubbels. 2011. 'A family of intermediate filament-like proteins is
685 sequentially assembled into the cytoskeleton of *Toxoplasma gondii*', *Cellular*
686 *Microbiology*, 13: 18-31.
- 687 Baba, E., I. Kimata, M. Matsubayashi, H. Nakagawa, K. Sasai, H. Takase, and H.
688 Tani. 2008. 'Electron microscopic observation of cytoskeletal frame structures
689 and detection of tubulin on the apical region of *Cryptosporidium parvum*
690 sporozoites', *Parasitology*, 135: 295-301.
- 691 Ben Chaabene, Rouaa, Gaëlle Lentini, and Dominique Soldati-Favre. 2021.
692 'Biogenesis and discharge of the rhoptries: Key organelles for entry and hijack
693 of host cells by the Apicomplexa', *Molecular Microbiology*, 115: 453-65.
- 694 Brown, Kevin M., Shaojun Long, and L. David Sibley. 2018. 'Conditional Knockdown
695 of Proteins Using Auxin-inducible Degron (AID) Fusions in *Toxoplasma*
696 *gondii*', *Bio-protocol*, 8: e2728.
- 697 Choudhary, Hadi H., Maria G. Nava, Brina E. Gartlan, Savannah Rose, and Sumiti
698 Vinayak. 2020. 'A Conditional Protein Degradation System To Study Essential
699 Gene Function in *Cryptosporidium parvum*', *mBio*, 11: 10.1128/mbio.01231-
700 20.
- 701 Delgado, Inês L. S., João Gonçalves, Rita Fernandes, Sara Zúquete, Afonso P.
702 Basto, Alexandre Leitão, Helena Soares, and Sofia Nolasco. 2024. 'Balancing
703 Act: Tubulin Glutamylation and Microtubule Dynamics in *Toxoplasma gondii*',
704 *Microorganisms*, 12: 488.
- 705 Dos Santos Pacheco, N., N. Tosetti, L. Koreny, R. F. Waller, and D. Soldati-Favre.
706 2020. 'Evolution, Composition, Assembly, and Function of the Conoid in
707 Apicomplexa', *Trends Parasitol*, 36: 688-704.
- 708 Dos Santos Pacheco, Nicolas, Lorenzo Brusini, Romuald Haase, Nicolò Tosetti,
709 Bohumil Maco, Mathieu Brochet, Oscar Vadas, and Dominique Soldati-Favre.
710 2022. 'Conoid extrusion regulates glideosome assembly to control motility and
711 invasion in Apicomplexa', *Nature Microbiology*, 7: 1777-90.
- 712 Dos Santos Pacheco, Nicolas, and Dominique Soldati-Favre. 2021. 'Coupling Auxin-
713 Inducible Degron System with Ultrastructure Expansion Microscopy to
714 Accelerate the Discovery of Gene Function in *Toxoplasma gondii*.' in, *Parasite*
715 *Genomics: Methods and Protocols pages 121-137* (Springer US: New York,
716 NY).
- 717 Dubois, D. J., and D. Soldati-Favre. 2019. 'Biogenesis and secretion of micronemes
718 in *Toxoplasma gondii*', *Cell Microbiol*, 21: e13018.
- 719 English, Elizabeth D., Amandine Guérin, Jayesh Tandel, and Boris Striepen. 2022.
720 'Live imaging of the *Cryptosporidium parvum* life cycle reveals direct
721 development of male and female gametes from type I meronts', *PLOS*
722 *Biology*, 20: e3001604.
- 723 Farache, D., A. Jauneau, C. Chemin, M. Chartrain, M. H. Rémy, A. Merdes, and L.
724 Haren. 2016. 'Functional Analysis of γ -Tubulin Complex Proteins Indicates
725 Specific Lateral Association via Their N-terminal Domains', *J Biol Chem*, 291:
726 23112-25.

- Gould, Sven B., Wai-Hong Tham, Alan F. Cowman, Geoffrey I. McFadden, and Ross F. Waller. 2008. 'Alveolins, a New Family of Cortical Proteins that Define the Protist Infrakingdom Alveolata', *Molecular Biology and Evolution*, 25: 1219-30.
- Green, J. L., R. J. Wall, J. Vahokoski, N. A. Yusuf, M. A. M. Ridzuan, R. R. Stanway, J. Stock, E. Knuepfer, D. Brady, S. R. Martin, S. A. Howell, I. P. Pires, R. W. Moon, J. E. Molloy, I. Kursula, R. Tewari, and A. A. Holder. 2017. 'Compositional and expression analyses of the glideosome during the Plasmodium life cycle reveal an additional myosin light chain required for maximum motility', *J Biol Chem*, 292: 17857-75.
- Gubbels, M. J., C. D. Keroack, S. Dangoudoubiyam, H. L. Worliczek, A. S. Paul, C. Bauwens, B. Elsworth, K. Engelberg, D. K. Howe, I. Coppens, and M. T. Duraisingh. 2020. 'Fussing About Fission: Defining Variety Among Mainstream and Exotic Apicomplexan Cell Division Modes', *Front Cell Infect Microbiol*, 10: 269.
- Guérin, Amandine, Nathan H. Roy, Emily M. Kugler, Laurence Berry, Janis K. Burkhardt, Jung-Bum Shin, and Boris Striepen. 2021. 'Cryptosporidium rhoptry effector protein ROP1 injected during invasion targets the host cytoskeletal modulator LMO7', *Cell Host & Microbe*, 29: 1407-20.e5.
- Guérin, Amandine, Katherine M. Strelau, Konstantin Barylyuk, Bethan A. Wallbank, Laurence Berry, Oliver M. Crook, Kathryn S. Lilley, Ross F. Waller, and Boris Striepen. 2023. 'Cryptosporidium uses multiple distinct secretory organelles to interact with and modify its host cell', *Cell Host & Microbe*, 31: 650-64.e6.
- Khalil, Ibrahim A., Christopher Troeger, Puja C. Rao, Brigitte F. Blacker, Alexandria Brown, Thomas G. Brewer, Danny V. Colombara, Eugenio L. De Hostos, Cyril Engmann, Richard L. Guerrant, Rashidul Haque, Eric R. Houpt, Gagandeep Kang, Poonum S. Korpe, Karen L. Kotloff, Aldo A. M. Lima, William A. Petri, James A. Platts-Mills, David A. Shultz, Mohammed H. Forouzanfar, Simon I. Hay, Robert C. Reiner, and Ali H. Mokdad. 2018. 'Morbidity, mortality, and long-term consequences associated with diarrhoea from Cryptosporidium infection in children younger than 5 years: a meta-analyses study', *The Lancet Global Health*, 6: e758-e68.
- Louvel, Vincent, Romuald Haase, Olivier Mercey, Marine H. Laporte, Thibaut Eloy, Étienne Baudrier, Denis Fortun, Dominique Soldati-Favre, Virginie Hamel, and Paul Guichard. 2023. 'iU-ExM: nanoscopy of organelles and tissues with iterative ultrastructure expansion microscopy', *Nature Communications*, 14: 7893.
- Mageswaran, S. K., A. Guerin, L. M. Theveny, W. D. Chen, M. Martinez, M. Lebrun, B. Striepen, and Y. W. Chang. 2021. 'In situ ultrastructures of two evolutionarily distant apicomplexan rhoptry secretion systems', *Nat Commun*, 12: 4983.
- Mageswaran, Shrawan Kumar, Amandine Guérin, Liam M. Theveny, William David Chen, Matthew Martinez, Maryse Lebrun, Boris Striepen, and Yi-Wei Chang. 2021. 'In situ ultrastructures of two evolutionarily distant apicomplexan rhoptry secretion systems', *Nature Communications*, 12: 4983.
- Mann, Tara, and Con Beckers. 2001. 'Characterization of the subpellicular network, a filamentous membrane skeletal component in the parasite Toxoplasma gondii', *Molecular and Biochemical Parasitology*, 115: 257-68.
- Martinez, M., S. K. Mageswaran, A. Guérin, W. D. Chen, C. P. Thompson, S. Chavin, D. Soldati-Favre, B. Striepen, and Y. W. Chang. 2023. 'Origin and

arrangement of actin filaments for gliding motility in apicomplexan parasites revealed by cryo-electron tomography', *Nat Commun*, 14: 4800.

Moritz, Michelle, Michael B. Braunfeld, Vincent Guénebaut, John Heuser, and David A. Agard. 2000. 'Structure of the γ -tubulin ring complex: a template for microtubule nucleation', *Nature Cell Biology*, 2: 365-70.

Morlon-Guyot, Juliette, Maria E. Francia, Jean-François Dubremetz, and Wassim Daher. 2017. 'Towards a molecular architecture of the centrosome in *Toxoplasma gondii*', *Cytoskeleton*, 74: 55-71.

Oakley, Berl R., Vitoria Paolillo, and Yixian Zheng. 2015. ' γ -Tubulin complexes in microtubule nucleation and beyond', *Molecular Biology of the Cell*, 26: 2957-62.

Opitz, Corinna, and Dominique Soldati. 2002. 'The glideosome': a dynamic complex powering gliding motion and host cell invasion by *Toxoplasma gondii*', *Molecular Microbiology*, 45: 597-604.

Padilla, Luisa F. Arias, John M. Murray, and Ke Hu. 2024. 'The initiation and early development of the tubulin-containing cytoskeleton in the human parasite *Toxoplasma gondii*', *Molecular Biology of the Cell*, 35: ar37.

Soldati, Dominique, and John C. Boothroyd. 1993. 'Transient Transfection and Expression in the Obligate Intracellular Parasite *Toxoplasma gondii*', *Science*, 260: 349-52.

Suvorova, Elena S., Maria Francia, Boris Striepen, and Michael W. White. 2015. 'A Novel Bipartite Centrosome Coordinates the Apicomplexan Cell Cycle', *PLOS Biology*, 13: e1002093.

Tandel, Jayesh, A. Walzer Katelyn, H. Byerly Jessica, Brittain Pinkston, P. Beiting Daniel, and Boris Striepen. 2023. 'Genetic Ablation of a Female-Specific Apetala 2 Transcription Factor Blocks Oocyst Shedding in *Cryptosporidium parvum*', *mBio*, 14: e03261-22.

Thawani, Akanksha, and Sabine Petry. 2021. 'Molecular insight into how γ -TuRC makes microtubules', *Journal of Cell Science*, 134: jcs245464.

Uni, S., M. Iseki, T. Maekawa, K. Moriya, and S. Takada. 1987. 'Ultrastructure of *Cryptosporidium muris* (strain RN 66) parasitizing the murine stomach', *Parasitol Res*, 74: 123-32.

Wang, C., D. Wang, J. Nie, X. Gao, J. Yin, and G. Zhu. 2021. 'Unique Tubulin-Based Structures in the Zoonotic Apicomplexan Parasite *Cryptosporidium parvum*', *Microorganisms*, 9.

Wang, Dongqiang, Peng Jiang, Xiaodong Wu, Ying Zhang, Chenchen Wang, Meng Li, Mingxiao Liu, Jigang Yin, and Guan Zhu. 2024. 'Requirement of microtubules for secretion of a micronemal protein CpTSP4 in the invasive stage of the apicomplexan *Cryptosporidium parvum*', *mBio*, 15: e03158-23.

Xu, Rui, Wandy L. Beatty, Valentin Greigert, William H. Witola, and L. David Sibley. 2024. 'Multiple pathways for glucose phosphate transport and utilization support growth of *Cryptosporidium parvum*', *Nature Communications*, 15: 380.

Zheng, Yixian, Mei Lie Wong, Bruce Alberts, and Tim Mitchison. 1995. 'Nucleation of microtubule assembly by a γ -tubulin-containing ring complex', *Nature*, 378: 578-83.