

Toxoplasma gondii chromatin remodeler SWI/SNF controls parasite division and gene expression

Corresponding Author: Professor Xingju Song

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This manuscript presents an in-depth analysis of the SWI/SNF chromatin remodeling complex, specifically its ATPase subunits TgSNF2a and TgSNF2b, and their role in *Toxoplasma gondii* development and division. The authors provide extensive experimental evidence supporting the involvement of SWI/SNF in chromatin accessibility, transcriptional shifts, and alterations in the parasite's division cycle. However, there are substantial conceptual flaws and overstatements regarding the connection between SWI/SNF depletion and *T. gondii* stage conversion, particularly the assertion that this work demonstrates a transition to schizogony.

Major Concerns and Required Revisions:

1. Misrepresentation of Schizogony: No Convincing Evidence for True Stage Transition

The central claim of this manuscript—that depletion of TgSNF2a and TgSNF2b promotes schizogony—is not fully supported by the data. Instead, the observations point to an altered division process, best described as incomplete endopolygony. True schizogony in *T. gondii* involves multiple nuclear divisions followed by segmentation into distinct merozoites, which is not convincingly demonstrated in this study.

Figure 2a-d presents immunofluorescence staining showing multinucleated cells following SWI/SNF depletion. However, while this suggests a departure from strict endodyogeny, it does not constitute full schizogony. The division process remains disorganized, lacking clear evidence of synchronous nuclear division, segmentation, or merozoite formation.

The transmission electron microscopy (TEM) images in Figure 2i-k do show an increase in cell size, multiple nuclei, and some evidence of budding, but the images do not depict the classic organization of schizont-stage parasites. The absence of well-defined merozoites within vacuoles is a critical gap in the interpretation.

Figure 3a-f further supports this incomplete transition, as centrosome replication and apicoplast division appear uncoordinated rather than progressing in a controlled schizogonous cycle.

Thus, the claim that SWI/SNF knockdown drives *T. gondii* toward a schizogony-like division is not fully substantiated.

Instead, the observed phenotype is an aberrant and asynchronous division mode, which should be carefully distinguished from true schizogony.

2. Inappropriate Use of Tachyzoites for Sexual Conversion Studies

One of the most significant methodological flaws in this study is the exclusive use of tachyzoites to model pre-sexual or sexual differentiation. It is well established that *T. gondii* undergoes sexual differentiation exclusively in felines, with schizogony initiating from bradyzoites within the cat gut. The decision to analyze stage conversion from tachyzoites rather than bradyzoites overlooks this crucial biological context.

The manuscript does not provide any justification for studying stage conversion in tachyzoites when previous studies have clearly demonstrated that sexual differentiation in *T. gondii* begins in the cat gut from bradyzoites.

Figure 4a-d shows transcriptional shifts in response to SWI/SNF knockdown, with an upregulation of pre-sexual and early gametocyte genes. However, without a bradyzoite model or cat intestinal conditions, these changes do not confirm a true commitment to sexual development.

The upregulation of schizont-associated genes such as TgGRA80, TgGRA82, and TgGRA11b (Figure 4d) is interesting but does not equate to a functional stage transition. These genes are also induced in other conditions that do not necessarily lead to viable merozoite formation.

The lack of a mature schizont structure (Figures 2i-k) further weakens the claim that SWI/SNF depletion initiates true pre-sexual development.

To improve the study's accuracy, the authors must acknowledge this limitation and clearly state that their findings reflect changes in transcriptional programs rather than full sexual conversion.

3. Failure to Contextualize Findings Within the Established Literature on *T. gondii* Sexual Development

The manuscript does not sufficiently integrate existing knowledge on *T. gondii* schizogony and sexual development. Several critical aspects are missing:

Prior research has established the requirement of specific host intestinal factors for sexual differentiation. The manuscript does not address how SWI/SNF interacts with these known pathways.

The MORC-HDAC3-AP2XII-1-AP2XI-2 complex has been shown to be a major regulator of *T. gondii* pre-sexual development. While the authors acknowledge some overlap between SWI/SNF depletion and MORC-related changes (Figure 4e), they do not adequately discuss how these pathways interact or whether SWI/SNF acts independently.

The study also overlooks the well-characterized role of TgAP2XII-2 in pre-sexual differentiation, which would be important for comparison.

Figure 4g suggests that SWI/SNF knockdown induces genes characteristic of enteric stages, but the authors fail to discuss why this shift does not produce viable merozoites, further emphasizing the incomplete nature of the transition.

A clearer comparison with these known regulatory pathways is essential to strengthen the manuscript's conclusions.

Suggested Revisions:

Reframe the claims about schizogony. The data support incomplete endopolygeny, but do not confirm schizogony. The authors should revise the text to acknowledge that while SWI/SNF influences cell division, a complete stage conversion was not observed.

Explicitly state the limitations of using tachyzoites. Since true schizogony occurs from bradyzoites in feline intestines, the study's findings must be presented with appropriate caveats.

Incorporate a broader discussion of *T. gondii* sexual development. The manuscript should better integrate prior work on the MORC-HDAC3 complex, AP2 transcription factors, and enteric factors that regulate stage conversion.

Clarify that the study focuses on SWI/SNF function rather than sexual differentiation. If the main goal is to investigate chromatin remodeling, the study should be framed as an analysis of SWI/SNF's role in gene regulation rather than a paper on sexual conversion.

While this manuscript provides interesting insights into the SWI/SNF complex and its role in gene expression and cell division in *T. gondii*, its conclusions regarding stage conversion and schizogony are overstated. The data demonstrate an incomplete transition characterized by endopolygeny but lack convincing evidence of full schizogony or pre-sexual commitment. Furthermore, using tachyzoites instead of bradyzoites is a major limitation, given what is known about *T. gondii* sexual development in felines.

To improve the manuscript, the authors should temper their conclusions, provide a clearer discussion of existing literature, and refine their claims to more accurately reflect their findings.

Reviewer #2

(Remarks to the Author)

In this manuscript Hu and colleagues present a thorough and detailed analysis of the contributions of two chromatin remodelers (TgSNF2a and TgSNF2b) to viability, development and chromatin structure in the protozoan parasite *Toxoplasma gondii*. Using inducible knockdown lines, they confirm that they are required for parasite proliferation and identify a previously unknown role in regulation of parasite development from asexual to sexual stages. Transcriptomics and epigenomic profiling during knockdown has determined that both TgSnf2a and TgSnf2b likely regulate chromatin accessibility at parasite promoters and loss of these proteins leads to disruption of chromatin structure and dysfunctional gene expression. Proteomic analysis of the interacting proteins of both TgSnf2a and TgSnf2b suggests they both interact within the same functional complex with some other regulatory factors that are known to influence parasite development. The study is detailed and will be of interest to the parasitology community as well as provide important insight into epigenetics mechanisms of an early branching eukaryote.

Major comments

The proteomics data presented in Supp Tables 2 and 3 appear to contain a lot of non-specific hits, reducing confidence that the data is representative of the true interactome of Snf2a and Snf2b. This reviewer appreciates the validation approach in Fig 2c-f to confirm interaction between a couple of these proteins, but it would help if the authors provided some more insight into why there are so many proteins detected. Please also explain the metric in Columns B-E in Supp Table 2 and 3. What does this signify?

It appears that the authors have tried to highlight some interesting hits in red text in the tables, but it is hard to determine why this is a relevant hit over another protein that has a similar statistical significance, but did not fit with the expected model.

Can the author review this data using a more stringent analysis to try and reduce the apparent high background in the current datasets. Perhaps an analysis such as SAINT would be helpful in this context.

Line 245. The authors should explain what different structures TgIMC1 and TgGAP45 stain and how it is informative to compare both in the knockdown parasites. References should also be provided for each of the antibodies that have been used in this study so the reader can compare staining here to previously published images.

Line 501/Figure 6.

I do not think the authors can confidently state that the Snf2 complex is recruited specifically to the GCATGCA motif. There is no direct evidence that this complex contains a DNA binding protein that binds this motif. The authors refer to a recent study that claims GCATGCA is the target for AP2XII-8, but there is no direct biochemical data that demonstrates that it binds this sequence. The GCATGCA motif is highly abundant in Toxoplasma promoter regions and so is regularly identified with high statistical significance as an enriched motif from any epigenomic profiling experiment of a protein that binds parasite promoters. I would be very cautious in concluding that the Snf2a/b complex is recruited to this motif without direct in vitro binding assay data.

Minor comments

The immunofluorescent images presented in Figs 1, 2 and 3 are beautiful and of high quality, but it is a little challenging to see the exact phenotypes that are described in the text. The authors should either annotate these images to identify the morphological phenotypes they see, or alternatively draw the readers' attention to particular morphology defects with inset boxes that zoom in on particular parasites in the image.

There are certain sections of the methods that need more detail. More information on the exact sequences used in building the transfection plasmids is required. For examples line 810: which existing plasmids does this refer to?

Line 803: Which Nucleofector was used for electroporation? Nucleofector II or 4d systems? Which transfection buffer was used?

Reviewer #3

(Remarks to the Author)

Summary

In this manuscript it is shown that Toxoplasma Snf2a and Snf2b are part of the same Swi/Snf complex, with Snf2a a more peripheral component than Snf2b. Depletion of Snf2b, and milder upon Snf2a depletion results in a convincingly documented cell division switch from endodyogeny to endopolygeny. Besides the AP2XII-1/XI-2 complex and FXBOL2 similarities, an additional relevant comparison can be made to the MAPK-L1 inhibition phenotypes (PMID: 24533298). However, the Snf2a/b depleted transcriptome shows that many tachyzoite features are still maintained, and it is therefore concluded that stage transition is incomplete. Furthermore, transcriptomics, CUT&Tag & ATAC show that the SWI/SNF complex is critical for maintaining chromatin accessibility at promoters (creating nucleosome free regions) of key tachyzoite-specific genes. On the other hand, changes in non-tachyzoite gene expression do not directly correlate with Snf2a/b on these promoters and upregulation of such genes is indirectly regulated by SNF2a/b, potentially through MORC and AP2 transcription factors for which significant evidence is provided. The discussion nicely puts all findings in perspective of both the Apicomplexa and general Swi/Snf biology, and the parts fit together very well, and provide key novel insights in control of stage specific gene expression. Some sections in the methods and data presentation should be clarified, for example what phenotypic features exactly are quantified, why are there 6 expression clusters defined, and eliminating/considering cell cycle effects from transcriptomic data interpretation, as well as giving a bit more perspective regarding primary and secondary phenotypic defects and use of terminology (there is no schizogony in Toxoplasma).

Specific points

1. Fig 2a-d. It is not clear how a "large nucleus" is defined. The IFA panels show many nuclei that appear much larger than 2N, yet in the quantification only ~5% of nuclei are scored as large nucleus. The Results section mentions up to 14% has both more than 2 and large nuclei, which must mean that the same nucleus is scored in two different categories, yet the large nuclei bar at no point seems to rise much above 1-2%. This does not add up and should be clarified.
2. It looks like mitosis very frequently does not complete (the final karyokinesis step). Skipping karyokinesis is seen in Plasmodium schizogony in the liver cell at a somewhat comparable sequence as seen here, as well as in Sarcocystis neurona endopolygeny. Furthermore, asynchronous development is the rule in endopolygeny with multiple nuclei, e.g. Cystoisospora suis). Hence, all of this is within the capacity range of how Apicomplexa can vary their nuclear cycle. And the Snf2b phenotype shows many of the right features. Reading further, looks like the large nuclei are a defect as they do not get engulfed by IMC buds (comments below).
3. Fig 3a. The co-stain with Centrin 1 shows that centrosomes lose nucleus association. That might be a defect, as that loss precludes successful assembly of zoites with a nucleus at the budding step. This loss of centrosome-nucleus association is never seen in schizogony or any variation of endopolygeny. If association is lost, additional cell cycles result in the clustered centrosomes as seen in Fig 3a as they cannot split in association with the mitotic spindle, which therefore is a secondary defect. Hence, the polyploid nuclei might be the result of this defect, and not variations on normal nuclear cycles seen in Apicomplexa (as pointed out above). In this light, the polyploid nuclei are often not associated with IMC1 in Fig 2e and 5a, c. So the phenotype is a mix of endopolygeny and (more minor) stochastic defects in centrosome-nuclear association loss. A co-stain of Centrin and IMC could more conclusively resolve these scenarios.
4. Fig 3. I suspect panels 3b,d quantifications do not seem to match the shown IFAs as I see multiple plastids upon both Snf2a and b depletion, in all vacuoles shown. Probably it should be better defined what is quantified by pointing out "normal" and "multiple" apicoplast vacuoles in the IFA panels with arrows/asterisks.
5. Line 315/316. Related to an above comment (loss of centrosome – nucleus association), the comparison to Cystoisospora suis is not entirely correct, as nuclear division (karyokinesis) is completed after every round, as seen by clear centrosome splitting and segregation: that is not seen unanimously for Snf2b, and to a milder degree for the Snf2a phenotype. Furthermore, this is not schizogony, which never occurs in T. gondii (see comment above), but rather the phenotypes display

feature of endopolygeny.

6. Line 331, Fig 4d. The text refers to merozoite specific genes, but these are not separately shown in Fig 4d (there is a label like that in 6b grouped with EES, but not explained where it comes from). Also, in this section it would be helpful to explain what EES is and represents (a mix of sexual stages from merogony, gametocytogenesis, to zygote formation?).

7. Figure 4g. How did the data get split up in 6 clusters, what were the criteria and/or clustering settings? More details are needed to understand how this came about.

8. Line 346: "...73 gametocyte-specific genes..." how was this list assembled, based on what criteria? If this was a previously assembled list, please provide a reference (the legend seems to suggest ToxoDB searches, but details are needed to understand the choices and logic).

9. Line 389/390. "Merozoites express a distinct and much more limited repertoire of microneme, rhoptry, and dense granule proteins compared to tachyzoites". Although this statement is true, it is not the complete truth, and it does not completely apply to this situation. As panel 4h already showed there is not much daughter scaffold assembly going on (IMC genes), it is not surprising that none of the secretory organelles are being assembled (with exception of at least a sub-set of GRAs). The timing of transcriptome analysis is key here (please clearly define). Please revise this statement, or include all MIC, ROP and GRA proteins (it is unclear why only a subset of MICs and GRAs was selected in these heatmaps) to show unique signatures are expressed upon Snf2a/b depletion. In addition, it should be strongly considered to correct for cell cycle specific gene expression patterns, notably the budding cycle, as what we see here might simply be the absence of any, or very limited budding going on in the mutants.

10. Regarding differential gene expression, standard methods and stringent cut-offs for DEGs (DeSeq2) were used, but there are several instances (lines 332, 339, 360, 515, 518, 632) where percentages/proportions of genes of a particular class are given rather than a Fisher's test for enrichment (e.g. the percentage of genes overlapping with known enteroepithelial or merozoite specific-genes lack statistical support). Please apply Fisher's test throughout these cases.

11. For the CUT&Tag and ATAC analysis, please provide the specific parameters used in MACS2 for peak calling, such as the p-value cut-offs and the fold enrichment cut-off.

Minor points

1. The Introduction needs a thorough read through for English grammar and spelling (the remainder of the manuscript is much better). The following are just from lines 40-50:

- Line 41. "medial" should be "medical"
- Line 43/44. "...and life-threatening for the immunocompromised..." should be "...and is and life-threatening for immunocompromised..."
- Line 46. "intermediated" should be "intermediate"
- Line 47. "happened" should be "happens"
- Line 47/48. "...animals, including tachyzoite and bradyzoite." should be "...animals, comprising tachyzoite and bradyzoite stage."
- Line 48: "divided" should be "divide"
- Line 50: "differentiated" should be "differentiate"

2. Line 53. Toxoplasma never divides by "schizogony"; it divides by "endopolygeny" in the pre-sexual stages in the cat intestine. Please note these are principally different division modes, though both have "schizont" intermediate stages, defined a cells with more than two nuclei.

3. First results section. Please write out all the abbreviations as there are a lot of them, and it is not immediately clear what they mean or refer to, e.g. RAD, BDP, HNH, SANT, SLIDE, HAS

4. The font/scale in Fig 1a is much too small to read; same comment to Fig S1a,d,f,i,l, and other panels as well. I suspect these font sizes are below the minimal font sizes in the journal's policy

5. Line 215/216. Instead of "non-binary exponential" I would recommend using "non-geometric"

6. Fig 2a has an asterisk in the 3rd from the top panel on the right. This should be explained in the legend.

7. Fig 3a. The shade of green in these panels is different from the green color used in the other panels

8. Fig 4c. It is unclear what the colors of the points are

9. Line 421 should refer to figure 5f-h (not 1f-h)

10. Line 611 should refer to figure 7e-f (not 8e-f)

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Auxin-inducible depletion of the two SWI/SNF ATPases (TgSNF2a, TgSNF2b) is reported to (i) arrest tachyzoite growth, (ii) generate multinucleated "endopolygeny-like" parasites, and (iii) unlock transcripts normally confined to feline enteric (EES/merozoite) stages. The authors argue that SWI/SNF licences tachyzoite promoters and, when lost, triggers an

“incomplete endopolygeny” rather than true schizogony. The topic is timely, but overinterpretation and data presentation issues weaken the central claims.

Major concerns (M)

M1 Stage-conversion claim is overstated. No Dolichos lectin (DBL)–positive cyst walls appear after SWI/SNF knock-down (Fig 5b); the schizont marker TgGRA82 rises but every parasite remains TgSAG1-positive (Fig 5e–f); parasites never acquire the elongated merozoite shape (p 22/69 457–460). Re-title and re-write to focus on cell-cycle dysregulation, not stage conversion.

M2 Aberrant-nucleus counts are inconsistent. The rebuttal admits uncertainty in scoring “large nucleus” vs. “multinucleated” categories, yet Fig 2b/d statistics rely on them. Define thresholds, provide blinded recounts, and share raw spreadsheets.

M3 Centrosome–nucleus uncoupling persists. Reviewer #2 already noted Centrin-positive centrosomes detached from nuclei; single-plane IFAs in Fig 3a cannot refute this. Supply 3-D stacks demonstrating 1:1 nucleus–centrosome–centrocone linkage in ≥ 30 cells.

M4 Interactome under-filtered. Hundreds of low-confidence prey survive a 1.5-fold / $P < 0.05$ cut-off. Apply SAINTexpress/MiST (> 0.9) and show replicate volcano plots.

M5 CUT&Tag/ATAC statistics absent. No replicate correlation, IDR, or spike-in details accompany Fig. 6. Provide Pearson/IDR scores and DESeq2-style differential accessibility with adj. $P < 0.05$.

M6 Tachyzoite-only system limits biological relevance. The Discussion admits that schizonts arise from bradyzoites, yet still assigns SWI/SNF a direct role in “pre-sexual” genes. Frame conclusions around tachyzoite cell-cycle control and propose bradyzoite or feline-organoid follow-ups.

M7 Eight-day auxin exposure may confound phenotypes. High IAA perturbs host cells and parasite auxotrophy. Include 24 h wash-out rescue or Tet-OFF controls.

M8 Linoleic-acid / SC-26196 experiment is undocumented and logically flawed. The authors cite prior work showing that linoleic acid + $\Delta 6$ -desaturase inhibition promotes bradyzoite-to-schizont conversion in feline organoids. They then state that tachyzoites depleted for SWI/SNF and grown for 8 days under the same cocktail “gradually disintegrated” and “died (data not shown). No images or quantification are provided, and the design ignores the acknowledged need for a bradyzoite intermediate and the use of feline cells infected with bradyzoites from tissue cysts. Where is the complete data in a bradyzoite-appropriate model (with early-, mid-, and late-stage markers plus viability controls)?

Selected minor issues

Page / Line Comment

12/69 231–233 “H3K4me3 (severed as an additional nuclear marker)” – spelling/grammar.

Fig 2e legend n and error-bar definition missing.

14/69 270–276 Category “large nucleus without budding” appears in graph key but not defined in text.

24/69 491–499 Misuses “merozoite” and “schizont”; keep terminology consistent.

Figures 2 & 3 Several TEM/IFA panels lack scale bars.

Statistics Asterisks appear without specifying tests or α (e.g., Fig 4i, Supp Fig 1).

Data access GEO/PRIDE accession numbers for RNA-seq, CUT&Tag and proteomics are missing.

Recommendations

Re-focus narrative on SWI/SNF’s role in tachyzoite cell-cycle regulation. The merozoite claims are overstated and not supported.

Strengthen quantitation – blinded counts, replicate concordance, transparent statistics.

Apply stringent interactome filters and validate top hits reciprocally.

Control for auxin artefacts via wash-out or orthogonal depletion.

Provide or delete the linoleic-acid experiment—current text (“data not shown”) is uninformative and biologically mismatched.

Improve figure clarity (scale bars, symbol definitions) and deposit raw data with public accessions.

Addressing these points will greatly enhance the manuscript’s rigor and impact.

Reviewer #2

(Remarks to the Author)

The revised manuscript is much improved with clarified and detailed methods. The authors have edited the text to remove their initial conclusions that knockdown of the SNF2 homologues triggered stage conversion to sexual stages, as appropriate.

No comments for major revisions.

Minor revisions

1. The manuscript refers to “epistatic” control in a couple of instances (lines 185 and 766). These are inaccurate

comparisons to epistasis which is more appropriate to describe interactions of different alleles in diploid organisms.

For example line 185, the SWI/SNF complex directly regulates sexual development in *P. falciparum* through epigenetic mechanisms. Similarly, line 766, the appropriate conclusion here is that SNF2h somehow regulates MORC levels, either directly, or indirectly through other transcriptional mechanisms, not epistasis.

2. Line 93-94. The text specifies studies on chromatin remodeling complexes in Apicomplexan parasites, but a couple of the references cited discuss Trypanosomes, that are single celled parasites, but not apicomplexans. The following sentence details one of these studies, an SWR1-like complex that regulates H2A.Z incorporation – this is also describing Trypanosome biology.

3. Careful rereading of the text and figure legends should be done to catch errors of word usage. For example, Figure 1 legend, second-last line "...indicated strains incubation with IAA or vector...", the word vector should be replaced with vehicle.

Reviewer #3

(Remarks to the Author)

Summary

In this manuscript the authors have been very responsive to the review and many several manuscript improving changes and included additional experiments. Notably, the added evaluation of CEP250L (inner core) co-stained with Centrin 1 (outer core) in Fig S4C does demonstrate the centrosome structure is indeed intact in the vast majority of mutants and does effectively reject the alternative interpretation I raised. One point I missed before is the overexpression strains, which are not well embedded in the manuscript (specific point below).

Specific points

1. The rationale for studying a SNF2a-OE strain is not given, and it is also not described how these strains were generated in the M&M, notably what the promoter is and how/where they are inserted in the genome. Please provide rationale and M&M details.
2. I do disagree with Reviewer #1 comment 2. So if it was up to me, I would remove or streamline that in the discussion, as the goal of this study was not to generate a merozoite model to accurately reflect that biology. (e.g. I think this statement is not necessary: "We therefore acknowledge that a bradyzoite-based system and/or an organoid model would more faithfully recapitulate the natural sexual development of *T. gondii* in felines"). I leave it up to authors and editor to resolve this.

Minor points

1. Introduction. I was somewhat surprised to read that merozoites can form directly from sporozoite infections of the cat. It is not what I see in the typical life cycle schematics, but I am also not sure which one is correct. I suggest the authors double check the literature.
2. The added statement regarding IMC1 and GAP45 is not complete as IMC1 outlines both mother and budding daughters (not just the mother as mentioned in the text), whereas GAP45 indeed labels both the cortex/plasma membrane of the mother and emerging daughters. Please adjust this statement.
3. Fig 7a, b. The "SNF2a-OE vs HXGPRT" labels are confusing when compared to the "SNF2a-Ty vs ME49" labels. Does "HXGPRT" refer to the RH strain in these plots? Please clarify and label accordingly?

Reviewer #4

(Remarks to the Author)

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Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The reviews are adequate, and I have no further comment.

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POINT-BY-POINT RESPONSE TO REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

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Response: Thank you very much for reviewing our manuscript and for your valuable comments and suggestions. We have carefully addressed each of your comments point by point and revised the manuscript accordingly. Our detailed responses are as follows:

Major Concerns and Required Revisions:

1. Misrepresentation of Schizogony: No Convincing Evidence for True Stage Transition.

The central claim of this manuscript—that depletion of TgSNF2a and TgSNF2b promotes schizogony—is not fully supported by the data. Instead, the observations point to an altered division process, best described as incomplete endopolygony. True schizogony in *T. gondii* involves multiple nuclear divisions followed by segmentation into distinct merozoites, which is not convincingly demonstrated in this study.

Figure 2a-d presents immunofluorescence staining showing multinucleated cells following SWI/SNF depletion. However, while this suggests a departure from strict endodyogony, it does not constitute full schizogony. The division process remains disorganized, lacking clear evidence of synchronous nuclear division, segmentation, or merozoite formation.

The transmission electron microscopy (TEM) images in Figure 2i-k do show an increase in cell size, multiple nuclei, and some evidence of budding, but the images do not depict the classic organization of schizont-stage parasites. The absence of well-defined merozoites within vacuoles is a critical gap in the interpretation.

Figure 3a-f further supports this incomplete transition, as centrosome replication and apicoplast division appear uncoordinated rather than progressing in a controlled schizogonous cycle.

Thus, the claim that SWI/SNF knockdown drives *T. gondii* toward a schizogony-like division is not fully substantiated. Instead, the observed phenotype is an aberrant and asynchronous division mode, which should be carefully distinguished from true

schizogony.

Response: We fully agree with the opinion of reviewer #1. Knockdown of TgSNF2a or TgSNF2b caused a pronounced alteration in parasite replication, yielding numerous multinucleated and multi-budded progeny. Transcriptomic profiling revealed up-regulation of only a subset of EES genes, and immunofluorescence assays demonstrated simultaneous expression of the schizont-stage marker GRA80 and the tachyzoite marker SAG1 in the TgSNF2b mutant. Crucially, we did not observe synchronous nuclear division or fully mature schizonts, as confirmed by IMC7 staining.

Taken together, and in line with the reviewer's observation, our results indicate that loss of TgSNF2a or TgSNF2b redirects division toward an endopolygeny-like or incomplete endopolygeny pattern, rather than true schizogony or canonical endodyogeny. Accordingly, we have revised the whole manuscript to describe this phenotype explicitly as a cell-division defect as "endopolygeny-like division" or "incomplete endopolygeny". We also modified about the terminology about schizogony, which does not occur in *T. gondii*. These changes refocus the paper on the role of the SWI/SNF complex in regulating cell division, rather than on sexual differentiation. Additionally, we performed further immunofluorescent experiments examining centrosome positioning during parasite division, which confirmed that the nucleus-centrocone-centrosome-daughter bud axis remained intact and did not become uncoupled.

2. Inappropriate Use of Tachyzoites for Sexual Conversion Studies

One of the most significant methodological flaws in this study is the exclusive use of tachyzoites to model pre-sexual or sexual differentiation. It is well established that *T. gondii* undergoes sexual differentiation exclusively in felines, with schizogony initiating from bradyzoites within the cat gut. The decision to analyze stage conversion from tachyzoites rather than bradyzoites overlooks this crucial biological context.

The manuscript does not provide any justification for studying stage conversion in tachyzoites when previous studies have clearly demonstrated that sexual differentiation in *T. gondii* begins in the cat gut from bradyzoites. Figure 4a-d shows transcriptional shifts in response to SWI/SNF knockdown, with an upregulation of pre-sexual and early gametocyte genes. However, without a bradyzoite model or cat intestinal conditions, these changes do not confirm a true commitment to sexual development.

The upregulation of schizont-associated genes such as TgGRA80, TgGRA82, and TgGRA11b (Figure 4d) is interesting but does not equate to a functional stage transition. These genes are also induced in other conditions that do not necessarily lead to viable merozoite formation.

The lack of a mature schizont structure (Figures 2i-k) further weakens the claim that SWI/SNF depletion initiates true pre-sexual development.

To improve the study's accuracy, the authors must acknowledge this limitation and clearly state that their findings reflect changes in transcriptional programs rather than

84 full sexual conversion.

85 **Response:** We thank the reviewer for emphasizing the biological context of *T.*
86 *gondii* sexual differentiation. As noted, our revised study now focused on the role of
87 the SWI/SNF complex in regulating tachyzoite division, and we observed only partial
88 upregulation of schizont-specific genes and an incomplete endopolygeny phenotype—
89 not full sexual conversion or commitment. This contrasts with the MORC-HDAC3-
90 AP2XII-1-AP2XI-2 complex, whose simultaneous depletion of AP2XII-1 and
91 AP2XI-2 drives ~98% of parasites from tachyzoites directly into merozoites without a
92 bradyzoite intermediate.

93 We acknowledge that true stage conversion requires extensive reprogramming of
94 gene expression and biological processes, and that bypassing the bradyzoite stage
95 presents a significant barrier. Accordingly, we have revised the Introduction to clarify
96 that tachyzoite-to-schizont conversion naturally proceeds *via* a bradyzoite
97 intermediate, and we have also added explicit statements in the Results and
98 Discussion to acknowledge the limitations of using a tachyzoite-only model.

99 At the same time, we emphasize in our revised manuscript that our tachyzoite
100 model provides an experimentally tractable system to dissect how SWI/SNF controls
101 transcriptional programs and division modes in *T. gondii*. We have revised the Results
102 and Discussion sections to state clearly that our findings reflect transcriptional
103 reprogramming and altered division mode, rather than *bona fide* full stage transition
104 or sexual conversion.

107 3. Failure to Contextualize Findings Within the Established Literature on *T. gondii* 108 Sexual Development.

109 The manuscript does not sufficiently integrate existing knowledge on *T. gondii*
110 schizogony and sexual development. Several critical aspects are missing:
111 Prior research has established the requirement of specific host intestinal factors for
112 sexual differentiation. The manuscript does not address how SWI/SNF interacts with
113 these known pathways.

114 **Response:** This comment is actually divided into several parts, which we will
115 address one by one. Firstly, we thank the reviewer for highlighting the role of host
116 intestinal cues in driving *T. gondii* sexual development. Prior work by Martorelli Di
117 Genova et al., (2019, PLOS Bio) demonstrated that supplementation of linoleic acid
118 and SC-26196 (a $\Delta 6$ -desaturase inhibitor) in feline intestinal organoid cultures
119 induces bradyzoite-to-schizont conversion *in vitro*, and that oral administration of
120 these compounds to mice triggers oocyst shedding (also consistent with the
121 requirement that merozoite derives from bradyzoite under specific enteric conditions.
122 We have now cited this study in the Discussion to underscore the biological necessity
123 of a bradyzoite intermediate).

124 We also tried to cultured our dKD parasites in HFF cells with combined IAA,
125 linoleic acid, and SC-26196 treatment for up to 5 days. Under these conditions, we
126 observed neither expression of the gamete-specific marker HAP2 nor oocyst
127 formation. Consequently, it remains unclear whether SWI/SNF activity is directly

influenced by these host intestinal factors.

The MORC-HDAC3-AP2XII-1-AP2XI-2 complex has been shown to be a major regulator of *T. gondii* pre-sexual development. While the authors acknowledge some overlap between SWI/SNF depletion and MORC-related changes (Figure 4e), they do not adequately discuss how these pathways interact or whether SWI/SNF acts independently.

Response: To investigate how SWI/SNF relates to the established MORC-HDAC3-AP2XII-1-AP2XI-2 complex, we performed chromatin and interaction studies. CUT&Tag analyses revealed that both TgSNF2a and TgSNF2b occupy the promoters of key schizont regulators (including MORC, HDAC3, AP2XI-2, and AP2XII-5), while transcriptional profiling demonstrated that only AP2XII-5 is significantly downregulated 72 h after TgSNF2b depletion. This indicates that SWI/SNF does not indirectly control EES gene expression by altering MORC complex abundance.

Crucially, Co-IP experiments revealed a direct physical interaction between TgSNF2b and MORC/HDAC3 but not AP2XII-1, suggesting that SWI/SNF can regulate a subset of EES genes *via* the MORC remodeler. Together, these data support a model in which SWI/SNF and MORC complexes cooperate in tachyzoites to promote tachyzoite-specific transcriptional programs while repressing EES genes. Although the precise molecular mechanism of their cooperation remains to be elucidated, it is clear that SWI/SNF depletion alone does not trigger a *bona fide* stage conversion, as seen with loss of the MORC-HDAC3-AP2XII-1-AP2XI-2 complex, but rather selectively alters gene expression and parasite division mode.

The study also overlooks the well-characterized role of TgAP2XII-2 in pre-sexual differentiation, which would be important for comparison.

Response: The S/M-phase transcription factor TgAP2XII-2 plays a crucial role in parasite growth and development, with its loss resulting in slower tachyzoite proliferation due to delayed S-phase progression. Importantly, TgAP2XII-2 knockout parasites also exhibit upregulation of certain schizont-specific genes, including the sexual stage-specific AP2 transcription factor (AP2X-10) and the oocyst maturation-associated gene (AAH1), although these transcriptional changes are less pronounced compared to those following disruption of the MORC/HDAC3 complex.

In the revised manuscript, we briefly introduced the TgAP2XII-2 phenotype in the Introduction and monitored its transcriptional changes following SWI/SNF depletion in our Results section. Furthermore, we discussed additional genes previously associated with endopolygeny-like phenotypes in *T. gondii*. In this revised version, we have expanded our comparative analysis by including discussions on TgMAPK-L1, TgOTUD3A and ISWI ATPases, aiming for a more comprehensive dissection of the SWI/SNF-depletion phenotype.

Figure 4g suggests that SWI/SNF knockdown induces genes characteristic of enteric stages, but the authors fail to discuss why this shift does not produce viable

merozoites, further emphasizing the incomplete nature of the transition.

Response: Following SWI/SNF knockdown, we did not observe the formation of mature schizonts as seen with AP2XI-2/AP2XII-1 depletion. Instead, nuclear division frequently appeared aberrant, distinct from the coordinated division observed in both endopolygeny and schizogony. We interpret this as a defective replication phenotype rather than a true switch to either division mode.

At the transcriptional level, only a subset of pre-sexual genes was upregulated, and in some cases, tachyzoite- and schizont-specific genes were co-expressed, indicating an incomplete transcriptional transition. These defects in both nuclear division and gene expression programs likely underlie the parasite's inability to produce viable merozoites. We have included this interpretation in the revised manuscript.

A clearer comparison with these known regulatory pathways is essential to strengthen the manuscript's conclusions.

Response: We sincerely thank the reviewer for raising these important points. In the revised manuscript, we have expanded the discussion and incorporated additional comparisons related to *T. gondii* sexual development, with the aim of strengthening the overall conclusions of the study.

Suggested Revisions:

Reframe the claims about schizogony. The data support incomplete endopolygeny, but do not confirm schizogony. The authors should revise the text to acknowledge that while SWI/SNF influences cell division, a complete stage conversion was not observed.

Response: Thank you very much for your valuable suggestion. We have thoroughly revised the manuscript and now clearly state that SWI/SNF knockdown disrupts parasite division, resulting in incomplete endopolygeny rather than driving a bona fide stage conversion.

Explicitly state the limitations of using tachyzoites. Since true schizogony occurs from bradyzoites in feline intestines, the study's findings must be presented with appropriate caveats.

Response: As described above, we have clarified in the Introduction that tachyzoite-to-schizont conversion naturally proceeds via a bradyzoite intermediate, and in the Discussion we explicitly acknowledge the limitations of using a tachyzoite-only model for studying stage conversion.

Incorporate a broader discussion of *T. gondii* sexual development. The manuscript should better integrate prior work on the MORC-HDAC3 complex, AP2 transcription factors, and enteric factors that regulate stage conversion.

Response: In the revised manuscript, in addition to expanding our discussion of MORC-HDAC3, AP2XII-1, AP2XI-2, and TgAP2XII-2, we have also introduced comparisons to TgMAPK-L1, TgOTUD3A as well as TgSNF2L, which exhibit

similar division phenotypes. Furthermore, we added consideration of host enteric factors influencing parasite development in the Discussion. Finally, we performed detailed analyses of the nucleus-centrocone-centrosome-daughter bud axis following SWI/SNF disruption. We hope that these enhancements could provide a more comprehensive dissection of the SWI/SNF-depletion phenotype and deepen our understanding of its role in *T. gondii* developmental regulation.

Clarify that the study focuses on SWI/SNF function rather than sexual differentiation. If the main goal is to investigate chromatin remodeling, the study should be framed as an analysis of SWI/SNF's role in gene regulation rather than a paper on sexual conversion.

Response: In the revised manuscript, we have extensively reorganized the text and emphasized explicitly that this study centers on the function of the SWI/SNF complex, rather than on sexual differentiation.

While this manuscript provides interesting insights into the SWI/SNF complex and its role in gene expression and cell division in *T. gondii*, its conclusions regarding stage conversion and schizogony are overstated. The data demonstrate an incomplete transition characterized by endopolygeny but lack convincing evidence of full schizogony or pre-sexual commitment. Furthermore, using tachyzoites instead of bradyzoites is a major limitation, given what is known about *T. gondii* sexual development in felines.

To improve the manuscript, the authors should temper their conclusions, provide a clearer discussion of existing literature, and refine their claims to more accurately reflect their findings.

Response: We thank the reviewer again for their critical comments and suggestions. We have conducted a thorough revision of the entire manuscript in accordance with the recommendations, refined and focused the conclusions, and supplemented additional experimental data where appropriate to enhance the scientific rigor of the study.

Reviewer #2 (Remarks to the Author):

In this manuscript Hu and colleagues present a thorough and detailed analysis of the contributions of two chromatin remodelers (TgSNF2a and TgSNF2b) to viability, development and chromatin structure in the protozoan parasite *Toxoplasma gondii*. Using inducible knockdown lines, they confirm that they are required for parasite proliferation and identify a previously unknown role in regulation of parasite development from asexual to sexual stages. Transcriptomics and epigenomic profiling during knockdown has determined that both TgSnf2a and TgSnf2b likely regulate chromatin accessibility at parasite promoters and loss of these proteins leads to disruption of chromatin structure and dysfunctional gene expression. Proteomic

analysis of the interacting proteins of both TgSnf2a and TgSnf2b suggests they both interact within the same functional complex with some other regulatory factors that are known to influence parasite development. The study is detailed and will be of interest to the parasitology community as well as provide important insight into epigenetics mechanisms of an early branching eukaryote.

Response: We sincerely thank the reviewer for their thoughtful and encouraging assessment of our work. We greatly appreciate your positive feedback and have carefully revised the manuscript in accordance with your suggestions.

Major comments

The proteomics data presented in Supp Tables 2 and 3 appear to contain a lot of non-specific hits, reducing confidence that the data is representative of the true interactome of Snf2a and Snf2b. This reviewer appreciates the validation approach in Fig 2c-f to confirm interaction between a couple of these proteins, but it would help if the authors provided some more insight into why there are so many proteins detected. Please also explain the metric in Columns B-E in Supp Table 2 and 3. What does this signify?

Response: Because we employed Astral DIA quantitative proteomics for analyzing IP products, a method significantly more sensitive than traditional qualitative proteomics, we detected a greater number of lower-abundance proteins and consequently more nonspecific peptides. Columns B–E in Supplementary Tables 2 and 3 represent relative quantitative values (signal peak areas) of these proteins in their respective samples. To further optimize our interactome results and minimize nonspecific background, we implemented an additional strategy (described as followed), and replaced these two supplementary tables.

It appears that the authors have tried to highlight some interesting hits in red text in the tables, but it is hard to determine why this is a relevant hit over another protein that has a similar statistical significance, but did not fit with the expected model. Can the author review this data using a more stringent analysis to try and reduce the apparent high background in the current datasets. Perhaps an analysis such as SAINT would be helpful in this context.

Response: Our initial IP-MS strategy involved replacing endogenous promoters of TgSnf2a and TgSnf2b with a strong promoter (Tubulin) and performing N-terminal tagging with a 6× Ty epitope, followed by IP with anti-Ty antibody and subsequent Astral DIA quantitative proteomics. To reduce background noise and improve confidence in identifying interacting proteins, we have now employed TgSNF2a-mAID-6HA and TgSNF2b-mAID-6HA endogenously tagged parasite lines, using anti-HA IP followed by mass spectrometry and MSstats analysis. Integrative analysis of results from both IP strategies (targeting TgSnf2a and TgSnf2b) identified 10 consistently enriched proteins, of which 8 localized to nuclear chromatin, including several homologues of known SWI/SNF subunits from other species. These combined strategies allowed us to significantly reduce nonspecific background and increase the

reliability of predicted SWI/SNF complex composition in *T. gondii*.

Line 245. The authors should explain what different structures TgIMC1 and TgGAP45 stain and how it is informative to compare both in the knockdown parasites. References should also be provided for each of the antibodies that have been used in this study so the reader can compare staining here to previously published images.

Response: TgGAP45 is a glideosome-associated protein localized exclusively between the mother parasite's plasma membrane and inner membrane complex, and thus serves to delineate the maternal cell outline. In contrast, TgIMC1 is an inner membrane complex protein present on both the mother and emerging daughter cell membranes. These two antibodies are routinely used in combination to monitor daughter cell budding. We have added this explanation and relevant references in the revised manuscript.

Line 501/Figure 6.

I do not think the authors can confidently state that the Snf2 complex is recruited specifically to the GCATGCA motif. There is no direct evidence that this complex contains a DNA binding protein that binds this motif. The authors refer to a recent study that claims GCATGCA is the target for AP2XII-8, but there is no direct biochemical data that demonstrates that it binds this sequence. The GCATGCA motif is highly abundant in Toxoplasma promoter regions and so is regularly identified with high statistical significance as an enriched motif from any epigenomic profiling experiment of a protein that binds parasite promoters. I would be very cautious in concluding that the Snf2a/b complex is recruited to this motif without direct in vitro binding assay data.

Response: We thank the reviewer for this important caution. While structural studies in yeast and mammals have shown that SWI/SNF complexes engage nucleosomal DNA at superhelical locations and interact with DNA via subunits containing DNA-binding domains (e.g., the ARID domain in TgARID and the SANT/Myb domain in TgSwi3) (; Bieluszewski *et al.*, 2023, *Annu Rev Plant Bio.*), we agree that the pervasive "GCATGCA" motif in the *T. gondii* genome can lead to spurious statistical enrichment. Consequently, we have removed all claims regarding specific recruitment of the SWI/SNF complex to this motif from the revised manuscript.

Minor comments

The immunofluorescent images presented in Figs 1, 2 and 3 are beautiful and of high quality, but it is a little challenging to see the exact phenotypes that are described in the text. The authors should either annotate these images to identify the morphological phenotypes they see, or alternatively draw the readers' attention to particular morphology defects with inset boxes that zoom in on particular parasites in the image.

Response: Based on the suggestions from Reviewer 2 and Reviewer 3, we have added the requested annotations to the immunofluorescence images.

There are certain sections of the methods that need more detail. More information on the exact sequences used in building the transfection plasmids is required. For examples line 810: which existing plasmids does this refer to?

Line 803: Which Nucleofector was used for electroportation? Nucleofector II or 4d systems? Which transfection buffer was used?

Response: We have expanded the Materials and Methods to include detailed information on plasmid construction and transfection procedures, and have supplemented other relevant methodological details. Thank you again for your positive evaluation and valuable suggestions.

Reviewer #3 (Remarks to the Author):

Summary

In this manuscript it is shown that *Toxoplasma* Snf2a and Snf2b are part of the same Swi/Snf complex, with Snf2a a more peripheral component than Snf2b. Depletion of Snf2b, and milder upon Snf2a depletion results in a convincingly documented cell division switch from endodyogeny to endopolygeny. Besides the AP2XII-1/XI-2 complex and FXBOL2 similarities, an additional relevant comparison can be made to the MAPK-L1 inhibition phenotypes (PMID: 24533298). However, the Snf2a/b depleted transcriptome shows that many tachyzoite features are still maintained, and it is therefore concluded that stage transition is incomplete. Furthermore, transcriptomics, CUT&Tag & ATAC show that the SWI/SNF complex is critical for maintaining chromatin accessibility at promoters (creating nucleosome free regions) of key tachyzoite-specific genes. On the other hand, changes in non-tachyzoite gene expression do not directly correlate with Snf2a/b on these promoters and upregulation of such genes is indirectly regulated by SNF2a/b, potentially through MORC and AP2 transcription factors for which significant evidence is provided. The discussion nicely puts all findings in perspective of both the Apicomplexa and general Swi/Snf biology, and the parts fit together very well, and provide key novel insights in control of stage specific gene expression. Some sections in the methods and data presentation should be clarified, for example what phenotypic features exactly are quantified, why are there 6 expression clusters defined, and eliminating/considering cell cycle effects from transcriptomic data interpretation, as well as giving a bit more perspective regarding primary and secondary phenotypic defects and use of terminology (there is no schizogony in *Toxoplasma*).

Response: We sincerely thank the reviewer for the positive evaluation of our work and for the valuable and insightful comments and suggestions. In response, we have included additional experimental data and thoroughly revised the manuscript.

Below, we provide point-by-point responses to each of the reviewer's comments.

Specific points

1. Fig 2a-d. It is not clear how a "large nucleus" is defined. The IFA panels show many nuclei that appear much larger than 2N, yet in the quantification only ~5% of nuclei are scored as large nucleus. The Results section mentions up to 14% has both more than 2 and large nuclei, which must mean that the same nucleus is scored in two different categories, yet the large nuclei bar at no point seems to rise much above 1-2%. This does not add up and should be clarified.

Response: In our study, the classification criterion for a "large nucleus" was defined as a parasite nucleus significantly larger than the typical 2N size and lacking clear evidence of division. Conversely, nuclei that exhibited partial but incomplete separation, indicating an ongoing but unfinished division process, were categorized strictly as "multinucleated," due to uncertainty regarding whether these nuclei would eventually separate fully upon extended culture.

We apologize for the previous ambiguity in our results description. Since multinucleation represented the primary abnormal phenotype observed, to avoid counting the same nucleus in multiple categories and subsequent statistical confusion, we adopted the following strategy in Figures 2b and 2d: the "large nucleus" category specifically included only parasites containing a single enlarged nucleus without multinucleation. Meanwhile, the "multinucleated" category encompassed parasites with multiple nuclei and parasites with multiple enlarged nuclei. Given this approach, we separately presented the specific subset ("multinucleated parasites with enlarged nuclei") as a detailed breakdown in Supplementary Figure 2.

Thus, each nucleus was counted only once, and the separate supplementary figure 2 was provided to clearly illustrate the proportion of parasites showing both multinucleation and enlarged nuclei. Overall, parasites were classified into four distinct categories during counting: normal parasites, parasites with a large nucleus (single enlarged nucleus), multinucleated parasites (including those with and without enlarged nuclei), and separately, the subset of multinucleated parasites with enlarged nuclei (presented in supplementary figure 2). Since multinucleated parasites frequently exhibited enlarged nuclei, this phenotype reached approximately 14% (Supplementary Figure 2), whereas parasites containing only a single enlarged nucleus without multinucleation were relatively rare (1–2%).

2. It looks like mitosis very frequently does not complete (the final karyokinesis step). Skipping karyokinesis is seen in *Plasmodium* schizogony in the liver cell at a somewhat comparable sequence as seen here, as well as in *Sarcocystis* neurona endopolygeny. Furthermore, asynchronous development is the rule in endopolygeny with multiple nuclei, e.g. *Cystoisospora* suis). Hence, all of this is within the capacity range of how Apicomplexa can vary their nuclear cycle. And the Snf2b phenotype shows many of the right features. Reading further, looks like the large nuclei are a defect as they do not get engulfed by IMC buds (comments below).

Response: We thank the reviewer for this insightful comparison. Indeed,

TgSNF2b depletion produces a subset of parasites with enlarged nuclei exceeding the 2N state, reflecting a mitotic defect. Importantly, we also observe parasites that exhibiting multinucleation with or without daughter buds. In these endopolygeny-like forms, the number of buds typically corresponded to the number of nuclei (1:1 or 1:2), indicating that many nuclei completed mitosis successfully. Thus, SWI/SNF disruption produces a mixed phenotype that combines defective karyokinesis with division patterns partially resembling the endopolygeny reported for *C. suis*.

3. Fig 3a. The co-stain with Centrin 1 shows that centrosomes lose nucleus association. That might be a defect, as that loss precludes successful assembly of zoites with a nucleus at the budding step. This loss of centrosome-nucleus association is never seen in schizogony or any variation of endopolygeny. If association is lost, additional cell cycles result in the clustered centrosomes as seen in Fig 3a as they cannot split in association with the mitotic spindle, which therefore is a secondary defect. Hence, the polyploid nuclei might be the result of this defect, and not variations on normal nuclear cycles seen in Apicomplexa (as pointed out above). In this light, the polyploid nuclei are often not associated with IMC1 in Fig 2e and 5a, c. So the phenotype is a mix of endopolygeny and (more minor) stochastic defects in centrosome-nuclear association loss. A co-stain of Centrin and IMC could more conclusively resolve these scenarios.

Response: We appreciate the reviewer's insightful hypothesis regarding the potential loss of centrosome-nucleus association. However, we respectfully suggest there might be some misinterpretation regarding the images. First, the centrosomes shown in Fig. 3a predominantly align closely with individual nuclei, although their clustered arrangement among multiple nuclei may have created an impression of dissociation. Second, contrary to the concern raised, polyploid nuclei were generally associated with IMC1-positive daughter buds. The scenario depicted in Fig. 2e and Fig. 5a,c represents parasites actively undergoing budding during the S/M phases, where daughter buds (IMC1 staining) form prior to complete nuclear segregation. Third, our electron microscopy analyses also demonstrated clear alignment among nuclei, IMC structures, and parasite apices in SWI/SNF-disrupted parasites, indicating maintained spatial coordination.

To rigorously test the reviewer's hypothesis, we generated endogenous MORN1 (centrocone) and CEP250L1 (centrosome inner core) tagged strains in the SNF2b-mAID parasite background. Immunofluorescence analysis with specific antibodies revealed that both the centrocone and centrosomes (inner and outer cores) consistently maintained spatial correspondence with nuclei and developing daughter buds. Thus, we conclude that loss of SWI/SNF function does not cause centrosome-nucleus dissociation in *T. gondii*.

4. Fig 3. I suspect panels 3b,d quantifications do not seem to match the shown IFAs as I see multiple plastids upon both Snf2a and b depletion, in all vacuoles shown. Probably it should be better defined what is quantified by pointing out "normal" and

“multiple” apicoplast vacuoles in the IFA panels with arrows/asterisks.

Response: The frequency of parasites with multiple plastids varies with the duration of IAA treatment; the IFA images shown are representative examples. For quantification, a vacuole was scored as “multiple” whenever at least one parasite within it contained more than two plastids. In the revised manuscript we have added arrows to the IFA panels to indicate vacuoles with normal versus multiple plastids.

We also apologize for a layout error in the original submission and have corrected the bar graphs in Figures 3b and 3f accordingly.】

5. Line 315/316. Related to an above comment (loss of centrosome – nucleus association), the comparison to *Cystoisospora suis* is not enterally correct, as nuclear division (karyokinesis) is completed after every round, as seen by clear centrosome splitting and segregation: that is not seen unanimously for *Snf2b*, and to a milder degree for the *Snf2a* phenotype. Furthermore, this is not schizogony, which never occurs in *T. gondii* (see comment above), but rather the phenotypes display feature of endopolygony.

Response: We fully agree with the reviewer. The enlarged and multinucleated nuclei seen after *SNF2a/b* knockdown represent a defective phenotype that does not fully match the division modes of *C. suis* or *S. neurona*. In addition, we inadvertently used the term “schizogony” when referring to the endopolygony division of *T. gondii* in feline intestine. This has been corrected in the revised manuscript.

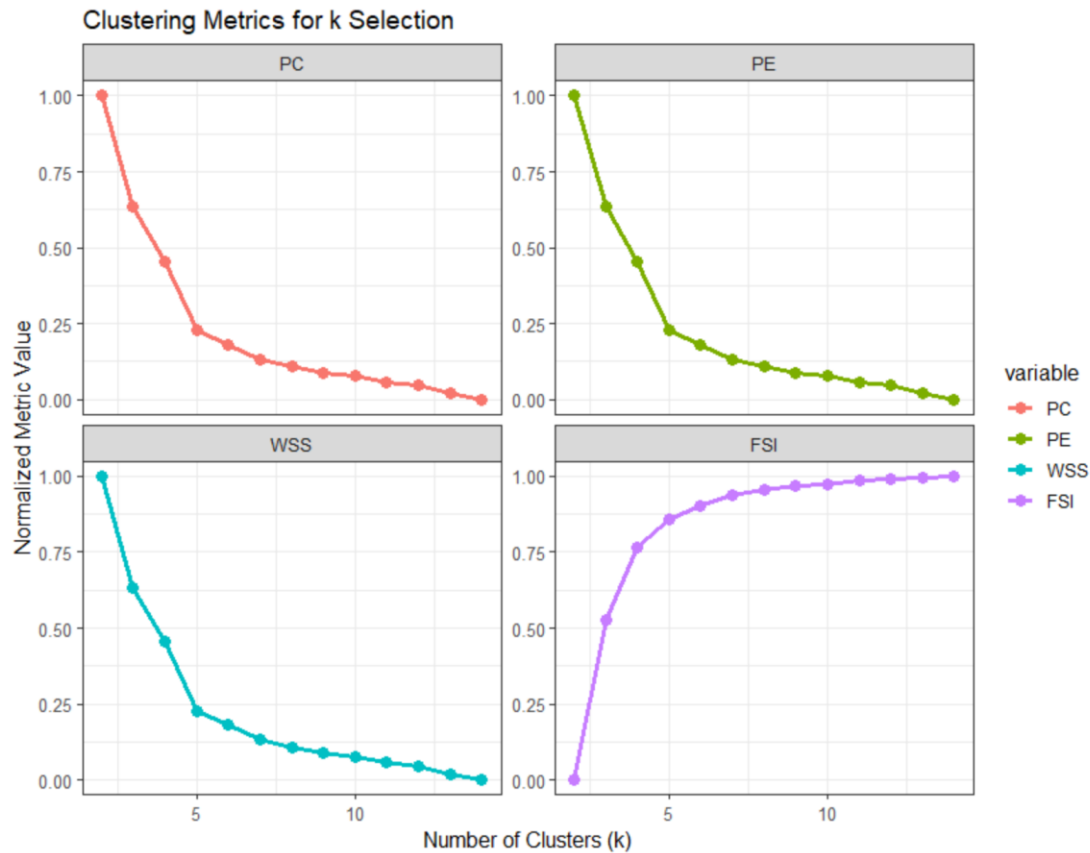
6. Line 331, Fig 4d. The text refers to merozoite specific genes, but these are not separately shown in Fig 4d (there is a label like that in 6b grouped with EES, but not explained where it comes from). Also, in this section it would be helpful to explain what EES is and represents (a mix of sexual stages from merogony, gametocytogenesis, to zygote formation?).

Response: We have re-curated the stage-specific gene sets, with EES genes compiled by integrating feline enteric transcriptomes from Ramakrishnan et al. (2019) and merozoite-specific genes from Behnke et al. (2014), then de-duplicated all lists prior to analysis. Using these updated gene definitions, we reconstructed the Venn diagrams in Figures 4d and 6b and revised the corresponding statistical results in the text. Additionally, we have clarified that “EES” represents a continuum of stages, including early merogony, gametocytogenesis, and late sexual forms.

7. Figure 4g. How did the data get split up in 6 clusters, what were the criteria and/or clustering settings? More details are needed to understand how this came about.

Response: The optimal number of clusters (k) was determined by integrating fuzzy clustering evaluation metrics and biological relevance. Statistical indicators—Partition Coefficient (PC, maximized), Partition Entropy (PE, minimized), Fuzzy Silhouette Index (FSI, maximized), and Within-Cluster Sum of Squares (WSS, elbow point analysis)—were analyzed across k -values (2–14, see the figures below). Although no distinct WSS elbow emerged beyond $k=6$, the rate of improvement in all metrics diminished significantly. K-means pre-clustering supported six dominant

clusters, with minor clusters (low gene counts) excluded. Biologically, six distinct expression phases correspond to developmental stages (tachyzoite, cyst, EESs, USO, sporulating oocyst and SO). Thus, $k = 6$ was selected as the optimal balance between statistical robustness and biological interpretability.



8. Line 346: “...73 gametocyte-specific genes...” how was this list assembled, based on what criteria? If this was a previously assembled list, please provide a reference (the legend seems to suggest ToxoDB searches, but details are needed to understand the choices and logic).

Response: We regret that no formally curated gametocyte-specific gene sets exist for *T. gondii*. Consequently, we manually assembled our “gametocyte” list following the solution of Farhat *et al.* (2020, *Nat. Microbiol.*), selecting genes annotated for male gamete flagellar axoneme components (e.g., intraflagellar transport proteins, cilia- and flagella-associated proteins) and motor proteins such as kinesins and dyneins based on domain architecture and functional annotation. We have now included this in the revised manuscript.

9. Line 389/390. “Merozoites express a distinct and much more limited repertoire of microneme, rhoptry, and dense granule proteins compared to tachyzoites”. Although this statement is true, it is not the complete truth, and it does not completely apply to this situation. As panel 4h already showed there is not much daughter scaffold assembly going on (IMC genes), it is not surprising that none of the secretory

organelles are being assembled (with exception of at least a sub-set of GRAs). The timing of transcriptome analysis is key here (please clearly define). Please revise this statement, or include all MIC, ROP and GRA proteins (it is unclear why only a subset of MICs and GRAs was selected in these heatmaps) to show unique signatures are expressed upon Snf2a/b depletion. In addition, it should be strongly considered to correct for cell cycle specific gene expression patterns, notably the budding cycle, as what we see here might simply be the absence of any, or very limited budding going on in the mutants.

Response: Our transcriptomic analyses included multiple time points and conditions (24 h, 72 h, and double-knockdown), which consistently showed similar expression trends without further expansion in the downregulated IMC-related organellar genes over time. Moreover, phenotypic analyses clearly demonstrated the formation of daughter buds (IMC1 staining) and the presence of organelles such as dense granules, micronemes, mitochondria, and rhoptries within multi-budded parasites, as verified by electron microscopy (Fig. 2, Fig. 5a, c). These findings indicate that despite the downregulation of certain daughter assembly-related IMC genes following SWI/SNF knockdown, daughter buds and associated organelles were still successfully formed, albeit in an altered mode of parasite division. To avoid ambiguity, we have therefore removed this specific statement from the revised manuscript.

Moreover, in our previous analysis, we had listed only selected MIC, ROP, and GRA genes commonly downregulated following Snf2a/b knockdown. To provide a more comprehensive picture, we have now generated heatmaps that include all commonly differentially expressed MIC, ROP, and GRA genes identified based on the hyperLOPIT organellar proteomics dataset (Barylyuk et al., 2020, *Cell Host & Microbe*). The trends observed in this expanded analysis remain consistent with our original findings.

10. Regarding differential gene expression, standard methods and stringent cut-offs for DEGs (DeSeq2) were used, but there are several instances (lines 332, 339, 360, 515, 518, 632) where percentages/proportions of genes of a particular class are given rather than a Fisher's test for enrichment (e.g. the percentage of genes overlapping with known enteroepithelial or merozoite specific-genes lack statistical support). Please apply Fisher's test throughout these cases.

Response: We thank the reviewer for this suggestion. In the revised manuscript, we applied Fisher's exact test to assess the enrichment of stage-specific genes, organelle-specific gene sets (e.g., ROP, MIC, GRA, IMC), and protein-family clusters (Families A-E, SRS). The resulting *P* values were adjusted by the Benjamini-Hochberg method and are indicated in the text.

11. For the CUT&Tag and ATAC analysis, please provide the specific parameters used in MACS2 for peak calling, such as the p-value cut-offs and the fold enrichment cut-off.

Response: We have updated the Methods to include the key parameters and cut-

off criteria used during mapping of CUT&Tag and ATAC-seq reads to the reference genome, as well as the settings applied for peak calling.

Minor points

1. The Introduction needs a thorough read through for English grammar and spelling (the remainder of the manuscript is much better). The following are just from lines 40-50:

- Line 41. “medial” should be “medical”
- Line 43/44. “...and life-threatening for the immunocompromised...” should be “...and is and life-threatening for immunocompromised...”
- Line 46. “intermediated” should be “intermediate”
- Line 47. “happened” should be “happens”
- Line 47/48. “...animals, including tachyzoite and bradyzoite.” should be “...animals, comprising tachyzoite and bradyzoite stage.”
- Line 48: “divided” should be “divide”
- Line 50: “differentiated” should be “differentiate”

Response: We thank the reviewer for these constructive suggestions. All noted grammatical errors have been corrected, and, following Reviewer 1’s recommendation, we have restructured the Introduction to emphasize that the central focus of this study is the function of the SWI/SNF complex rather than sexual differentiation.

2. Line 53. Toxoplasma never divides by “schizogony”; it divides by “endopolygony” in the pre-sexual stages in the cat intestine. Please note these are principally different division modes, though both have “schizont” intermediate stages, defined a cells with more than two nuclei.

Response: We apologize for this error and have corrected it in the revised manuscript.

3. First results section. Please write out all the abbreviations as there are a lot of them, and it is not immediately clear what they mean or refer to, e.g. RAD, BDP, HNH, SANT, SLIDE, HAS.

Response: We have added the full definitions of these abbreviations at their first occurrence in the revised manuscript.]

4. The font/scale in Fig 1a is much too small to read; same comment to Fig S1a,d,f,i,l, and other panels as well. I suspect these font sizes are below the minimal font sizes in the journal’s policy

Response: We have tried to adjust the font sizes in the relevant figures to meet

the journal's requirements.

5. Line 215/216. Instead of “non-binary exponential” I would recommend using “non-geometric”

Response: We have corrected this in the revised manuscript.

6. Fig 2a has an asterisk in the 3rd from the top panel on the right. This should be explained in the legend.

Response: We have added arrows and asterisks in Fig 2 and 3 to highlight the phenotypes, and have defined their meanings in the updated figure legends.】

7. Fig 3a. The shade of green in these panels is different from the green color used in the other panels.

Response: We apologize for the inconsistency in the fluorescence colors, which arose from using different confocal microscopes and their respective image-processing software. We have now applied a uniform, global color calibration to all affected panels. Additionally, as requested by Reviewer 2, we have replaced the lower-magnification images and added annotations to indicate the relevant phenotypes.

8. Fig 4c. It is unclear what the colors of the points are.

Response: Green dots represent genes significantly expressed in both SNF2a and SNF2b knockdown parasites; pink dots represent genes significantly expressed only in SNF2a knockdown parasites; purple dots represent genes significantly expressed only in SNF2b knockdown parasites; grey dots represent non-significant genes. And we have add these explanations to the revised figure legend.

9. Line 421 should refer to figure 5f-h (not 1f-h).

Response: In fact, we intended to reference the strain construction and validation data of the TgIMC7- and TgGRA82-endogenously tagged strains (now corrected to supplementary figure 3d-f), and their immunofluorescence staining images are cited in the corresponding Results section.

10. Line 611 should refer to figure 7e-f (not 8e-f).

Response: We have corrected this in the revised manuscript.

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667 **Reviewer #4 (Remarks to the Author):**

668 I co-reviewed this manuscript with one of the reviewers who provided the listed
669 reports. This is part of the Nature Communications initiative to facilitate training in
670 peer review and to provide appropriate recognition for Early Career Researchers who
671 co-review manuscripts.

672 **Response:** We sincerely appreciate the reviewer's efforts in co-reviewing this
673 manuscript and providing thoughtful feedback. Your contribution to the peer review
674 process has been invaluable in strengthening our study.

POINT-BY-POINT RESPONSE TO REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

Auxin-inducible depletion of the two SWI/SNF ATPases (TgSNF2a, TgSNF2b) is reported to (i) arrest tachyzoite growth, (ii) generate multinucleated “endopolygony-like” parasites, and (iii) unlock transcripts normally confined to feline enteric (EES/merozoite) stages. The authors argue that SWI/SNF licences tachyzoite promoters and, when lost, triggers an “incomplete endopolygony” rather than true schizogony. The topic is timely, but overinterpretation and data presentation issues weaken the central claims.

Response: Once again, we are deeply grateful for your constructive suggestions and generous support, which have greatly helped us to refine and strengthen this study. In accordance with your and the other reviewers’ comments, we have carefully revised the manuscript in detail. A point-by-point response to each of your concerns is provided below.

Major concerns (M)

M1 Stage-conversion claim is overstated. No Dolichos lectin (DBL)–positive cyst walls appear after SWI/SNF knock-down (Fig 5b); the schizont marker TgGRA82 rises but every parasite remains TgSAG1-positive (Fig 5e–f); parasites never acquire the elongated merozoite shape (p 22/69 457-460). Re-title and re-write to focus on cell-cycle dysregulation, not stage conversion.

Response: We have further revised the manuscript in response to this concern. In particular, we have removed or greatly reduced unnecessary discussions related to stage conversion and regulation of merozoite-specific genes. The focus of the revised manuscript has been narrowed to the functional characterization of the SWI/SNF complex and its impact on parasite cell-cycle regulation and division mode.

M2 Aberrant-nucleus counts are inconsistent. The rebuttal admits uncertainty in scoring “large nucleus” vs. “multinucleated” categories, yet Fig 2b/d statistics rely on them. Define thresholds, provide blinded recounts, and share raw spreadsheets.

Response: We apologize for not clearly defining the categories of “large nucleus” and “multinucleated,” which led to confusion. In fact, during our analysis we distinguished four phenotypic categories: “normal,” “large nucleus only,” “multinucleated only,” and “large nucleus with multinucleation.” However, in the previous version we merged the latter two into a single “multinucleated” group, while presenting the “large nucleus with multinucleation” subset separately in the Supplementary Information.

To resolve this issue, we have now presented all four categories together in the same figure, clearly labeled as “normal,” “large nuc,” “>two nuc,” and “>two nuc & large nuc.” We have also added explicit descriptions in the main text and provided representative images and schematic diagrams to facilitate reader understanding.

M3 Centrosome–nucleus uncoupling persists. Reviewer #2 already noted Centrin-positive centrosomes detached from nuclei; single-plane IFAs in Fig 3a cannot refute this. Supply 3-D stacks demonstrating 1:1 nucleus–centrosome–centrocone linkage in ≥ 30 cells.

Response: We sincerely thank the reviewer for highlighting this important point. In our previous report, we described the presence of multinucleated parasites without daughter buds following TgSNF2b knockdown, but we did not fully recognize that this phenotype itself represents direct evidence of karyokinesis–cytokinesis uncoupling (since in normal *T. gondii* division, daughter budding initiates before nuclear division is completed).

During the first round of revision, our observations were mainly focused on multinucleated parasites with multiple buds (prevalent in the mixed population), in

which the centrosomes, centrocones, and daughter buds aligned properly. As a result, we overlooked the less frequent but critical phenotype of enlarged and incompletely divided nuclei, in which centrosomes and centrocones undergo excessive duplication. As shown in Fig 3a and described in the original text, the supernumerary centrosomes also originate from nuclei that had not fully separated.

In this revision, we have carefully reanalyzed organelle division in TgSNF2b-depleted parasites, clearly documenting the phenomenon of centrosome and centrocone overduplication, and we propose that this defect may underlie the emergence of the endopolygeny-like division phenotype.

M4 Interactome under-filtered. Hundreds of low-confidence prey survive a 1.5-fold / $P < 0.05$ cut-off. Apply SAINTexpress/MiST (> 0.9) and show replicate volcano plots.

Response: We have re-analysed the IP-MS dataset to address background arising from low-frequency noise. Specifically, all proteins supported by < 2 unique peptides were removed, which partially reduced nonspecific signals. We additionally processed the same dataset with SAINTexpress. However, for this particular experiment, SAINTexpress provided less effective noise suppression than our MSstats workflow. We therefore retained the MSstats-based analysis, now coupled with the revised peptide-count filter, to generate the updated interactome.

M5 CUT&Tag/ATAC statistics absent. No replicate correlation, IDR, or spike-in details accompany Fig. 6. Provide Pearson/IDR scores and DESeq2-style differential accessibility with adj. $P < 0.05$.

Response: We added both genome-bin-based and peak-based Pearson correlation analyses to the revised manuscript. CUT&Tag and ATAC-seq biological replicates show high concordance (Pearson $r > 0.90$). IDR output tables and the annotated regions with differential accessibility were provided in Supplementary Table 1.

M6 Tachyzoite-only system limits biological relevance. The Discussion admits that schizonts arise from bradyzoites, yet still assigns SWI/SNF a direct role in “pre-sexual” genes. Frame conclusions around tachyzoite cell-cycle control and propose bradyzoite or feline-organoid follow-ups.

Response: In accordance with your guidance and Reviewer #3’s opinion, we have streamlined the manuscript to focus on the role of the SWI/SNF complex and its impact on the *T. gondii* cell-division cycle. Extended discussions of stage conversion, regulation of merozoite-specific genes, and bradyzoite/organoid-related topics have been removed or reduced to avoid distracting from the central narrative.

M7 Eight-day auxin exposure may confound phenotypes. High IAA perturbs host cells and parasite auxotrophy. Include 24 h wash-out rescue or Tet-OFF controls.

Response: Thank you for this excellent suggestion. To exclude potential artifacts caused by prolonged IAA exposure, we performed wash-out rescue experiments using both TgSNF2a-mAID and TgSNF2b-mAID strains. After 24 h of IAA treatment followed by IAA removal and an additional 24 h of culture, expression of the depleted proteins was restored and parasite intracellular replication also recovered. Furthermore, plaque assays conducted after additional 9 days of continuous culture confirmed that growth capacity was rescued. In addition, when TgSNF2b-mAID parasites were treated with IAA for 24 h and then cultured for 3 days without IAA, the endopolygeny-like phenotype was almost abolished. As an additional control, we treated wild-type parasites with IAA for 7 days and observed no endopolygeny-like phenotype. Together, these results demonstrate that the observed defects are attributable to targeted gene depletion rather than prolonged IAA exposure.

M8 Linoleic-acid / SC-26196 experiment is undocumented and logically flawed.

The authors cite prior work showing that linoleic acid + $\Delta 6$ -desaturase inhibition promotes bradyzoite-to-schizont conversion in feline organoids. They then state that tachyzoites depleted for SWI/SNF and grown for 8 days under the same cocktail “gradually disintegrated” and “died (data not shown). No images or quantification are provided, and the design ignores the acknowledged need for a bradyzoite intermediate and the use of feline cells infected with bradyzoites from tissue cysts. Where is the complete data in a bradyzoite-appropriate model (with early-, mid-, and late-stage markers plus viability controls)?

Response: Following your advice, and in order to more thoroughly remove discussions related to merozoite conversion, we have deleted the description of these experiments.

Selected minor issues

Page / Line Comment

12/69 231-233 “H3K4me3 (severed as an additional nuclear maker)” – spelling/grammar.

Response: This point has been corrected in the revised manuscript.

Fig 2e legend n and error-bar definition missing.

Response: We have clarified these issues in the figure legend.

14/69 270-276 Category “large nucleus without budding” appears in graph key but not defined in text.

Response: We have carefully revised the Results section to include a clear description and definition of all abnormal phenotypes.

24/69 491-499 Misuses “merozoite” and “schizont”; keep terminology consistent.

Response: We have carefully checked the manuscript to ensure that this terminology is used consistently.

Figures 2 & 3 Several TEM/IFA panels lack scale bars.

Response: Thank you for pointing this out. Scale bars were present in all IFA and TEM images, but the lines may be too thin to be easily observed. We have now increased the line thickness to improve visibility.

Statistics Asterisks appear without specifying tests or α (e.g., Fig 4i, Supp Fig 1).

Response: We have specified the statistical analyses used in the *Materials and Methods* section. In the revised manuscript, the statistical methods are now described in detail in each figure legend.

Data access GEO/PRIDE accession numbers for RNA-seq, CUT&Tag and proteomics are missing.

Response: All sequencing and proteomics data have been deposited in NCBI GEO (GSE287537) and ProteomeXchange (PXD060237 and PXD064041), as stated in the Data availability section.

Recommendations

Re-focus narrative on SWI/SNF's role in tachyzoite cell-cycle regulation. The merozoite claims are overstated and not supported.

Response: Thank you very much for these valuable suggestions. We have thoroughly revised the manuscript and refocused the narrative on elucidating the role of the SWI/SNF complex in tachyzoite cell-cycle regulation.

Strengthen quantitation – blinded counts, replicate concordance, transparent statistics.

Response: We have added definitions for all phenotypes in the manuscript and clarified the statistical methods used. In addition, we have uploaded the raw statistical data to ensure transparency, professionalism, and scientific rigor.

Apply stringent interactome filters and validate top hits reciprocally.

Response: We have re-filtered and re-analyzed the IP-MS results to minimize nonspecific signals, and we have also validated the interactions of several candidate proteins, thereby reinforcing the robustness of our conclusions.

Control for auxin artefacts via wash-out or orthogonal depletion.

Response: In the revised manuscript, we have included a 24h wash-out experiment, which eliminated concerns about potential artifacts associated with extended IAA exposure.

Provide or delete the linoleic-acid experiment—current text (“data not shown”) is uninformative and biologically mismatched.

Response: In line with your advice, we have removed this section to improve the focus and clarity of the manuscript.

Improve figure clarity (scale bars, symbol definitions) and deposit raw data with public accessions.

Response: In the revised manuscript, we have improved figure clarity by adding scale bars and symbol definitions, and we have deposited all raw data with the appropriate public accessions.

Addressing these points will greatly enhance the manuscript’s rigor and impact.

Response: We are deeply grateful to the reviewer for these valuable suggestions, which have been instrumental in refining our study and improving the overall quality of the manuscript.

Reviewer #2 (Remarks to the Author):

The revised manuscript is much improved with clarified and detailed methods. The authors have edited the text to remove their initial conclusions that knockdown of the SNF2 homologues triggered stage conversion to sexual stages, as appropriate.

Response: We sincerely thank you once again for your insightful comments and kind support, which greatly contribute to improve the quality of our work.

No comments for major revisions.

Minor revisions

1. The manuscript refers to “epistatic” control in a couple of instances (lines 185 and 766). These are inaccurate comparisons to epistasis which is more appropriate to describe interactions of different alleles in diploid organisms.

For example line 185, the SWI/SNF complex directly regulates sexual development in *P. falciparum* through epigenetic mechanisms. Similarly, line 766, the appropriate conclusion here is that SNF2h somehow regulates MORC levels, either directly, or indirectly through other transcriptional mechanisms, not epistasis.

Response: We thank the reviewer for pointing out the inappropriate use of this term. We fully agree with your comment, and have revised the manuscript by replacing the word “epistatic” accordingly.

2. Line 93-94. The text specifies studies on chromatin remodeling complexes in Apicomplexan parasites, but a couple of the references cited discuss Trypanosomes, that are single celled parasites, but not apicomplexans. The following sentence details one of these studies, an SWR1-like complex that regulates H2A.Z incorporation – this

is also describing Trypanosome biology.

Response: We have revised the text by replacing “Apicomplexan parasites” with “single celled parasites,” as suggested.

3. Careful rereading of the text and figure legends should be done to catch errors of word usage. For example, Figure 1 legend, second-last line “...indicated strains incubation with IAA or vector...”, the word vector should be replaced with vehicle.

Response: We sincerely thank you for this reminder. We have thoroughly re-checked the entire manuscript and revised it accordingly.

Reviewer #3 (Remarks to the Author):

Summary

In this manuscript the authors have been very responsive to the review and many several manuscript improving changes and included additional experiments. Notably, the added evaluation of CEP250L (inner core) co-stained with Centrin 1 (outer core) in Fig S4C does demonstrate the centrosome structure is indeed intact in the vast majority of mutants and does effectively reject the alternative interpretation I raised. One point I missed before is the overexpression strains, which are not well embedded in the manuscript (specific point below).

Response: We sincerely thank the reviewer for the valuable guidance. We would like to point out that we initially overlooked one of the most direct pieces of evidence

supporting karyokinesis-cytokinesis uncoupling, that is, the presence of multinucleated parasites without daughter buds. This phenotype itself represents a clear indication that nuclear division can proceed without the initiation of budding.

By contrast, the more prevalent multinucleated parasites with multiple buds indicate that once nuclear division is completed, centrosomes can still divide and properly coordinate with daughter budding. In these parasites, the nucleus-centrocone-centrosome-daughter bud axis remains aligned, as we noted in our first revision.

In addition, by re-examining the less frequent enlarged parasites with incompletely divided nuclei (previously overlooked due to their low proportion), we identified excessive duplication of centrosomes and centrocones. We propose that this overduplication may be a key factor contributing to the emergence of the endopolygeny-like division phenotype. This point has now been explicitly stated in the revised manuscript.

Specific points

1. The rationale for studying a SNF2a-OE strain is not given, and it is also not described how these strains were generated in the M&M, notably what the promoter is and how/where they are inserted in the genome. Please provide rationale and M&M details.

Response: We appreciate the reviewer's comment regarding the lack of clarity in the description of the overexpression strains. While the construction strategy was briefly described in the *M&M* section of the original submission, it was not explicitly annotated or clearly presented. In the revised version, we have now added specific annotations for the TgSNF2b-OE and TgSNF2a-OE strains in the text.

As detailed in the revised *M&M* section, the overexpression strains TgSNF2b-OE and TgSNF2a-OE were generated by replacing the endogenous promoters with the β -tubulin promoter. Specifically, the RH Δ HXGPRT strain was co-transfected with a Cas9-expressing plasmid targeting the upstream region of the gene of interest and a

donor DNA fragment containing a *pTub*-6×Ty epitope tag and HXGPRT selection cassette flanked by 39-bp homology arms (supplementary figure 5 g-i).

2. I do disagree with Reviewer #1 comment 2. So if it was up to me, I would remove or streamline that in the discussion, as the goal of this study was not to generate a merozoite model to accurately reflect that biology. (e.g. I think this statement is not necessary: “We therefore acknowledge that a bradyzoite-based system and/or an organoid model would more faithfully recapitulate the natural sexual development of *T. gondii* in felines”). I leave it up to authors and editor to resolve this.

Response: We sincerely thank you for raising this important point. After carefully considering both your comments and those of Reviewer #1, we have refocused the manuscript on the function of the SWI/SNF complex and its role in regulating parasite division. To avoid distracting readers from this central theme, we have removed sections that were not directly relevant, including discussions on stage conversion, pre-sexual gene regulation, and, as you suggested, references to bradyzoite- or organoid-based models. We hope that these revisions address your concerns and improve the clarity and focus of the manuscript.

Minor points

1. Introduction. I was somewhat surprised to read that merozoites can form directly from sporozoite infections of the cat. It is not what I see in the typical life cycle schematics, but I am also not sure which one is correct. I suggest the authors double check the literature.

Response: We thank the reviewer for this important observation. We have carefully re-examined the literature and clarified this point in the revised manuscript. Cats can shed oocysts after ingesting any of the three infectious stages of *T. gondii*—tachyzoites, bradyzoites, or sporozoites (Frenkel et al., 1970; PMID: 4903651; Dubey, 1998 and

2009; PMID: 9724872, PMID: 19630138). Similar to other coccidia such as *Eimeria*, *T. gondii* follows a coccidia-like life cycle: after ingestion of sporulated oocysts, sporozoites excyst, invade the intestinal epithelium of the definitive host, and undergo merogony and gametogony before developing into oocysts.

2. The added statement regarding IMC1 and GAP45 is not complete as IMC1 outlines both mother and budding daughters (not just the mother as mentioned in the text), whereas GAP45 indeed labels both the cortex/plasma membrane of the mother and emerging daughters. Please adjust this statement.

Response: We apologize for having inadvertently reversed the description. This has now been corrected in the revised manuscript.

3. Fig 7a, b. The “SNF2a-OE vs HXGPRT” labels are confusing when compared to the “SNF2a-Ty vs ME49” labels. Does “HXGPRT” refer to the RH strain in these plots? Please clarify and label accordingly?

Response: We thank the reviewer for pointing out this confusing labeling. In the figures, “HXGPRT” refers to the parental *RH* Δ *HXGPRT* strain that was used to generate the overexpression lines, whereas “ME49” indicates the ME49 Δ Ku80 parasite for the endogenous tagging lines. We have now revised the labels in Fig. 7a,b and clarified the strain backgrounds in the figure legends to avoid confusion.

Additionally, we would like to express our gratitude once again for your constructive comments and suggestions, which have greatly helped us to improve the clarity and focus of the manuscript.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: We sincerely thank you for your time and thoughtful comments, which have been invaluable in strengthening our study.