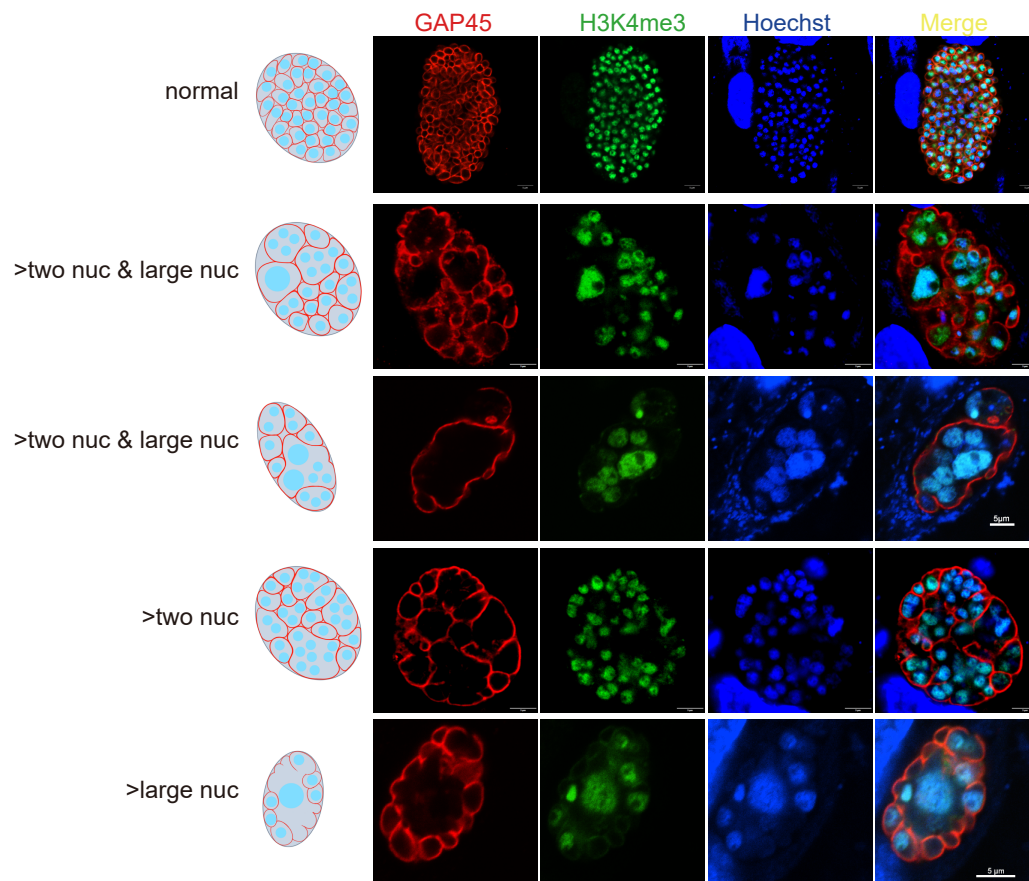
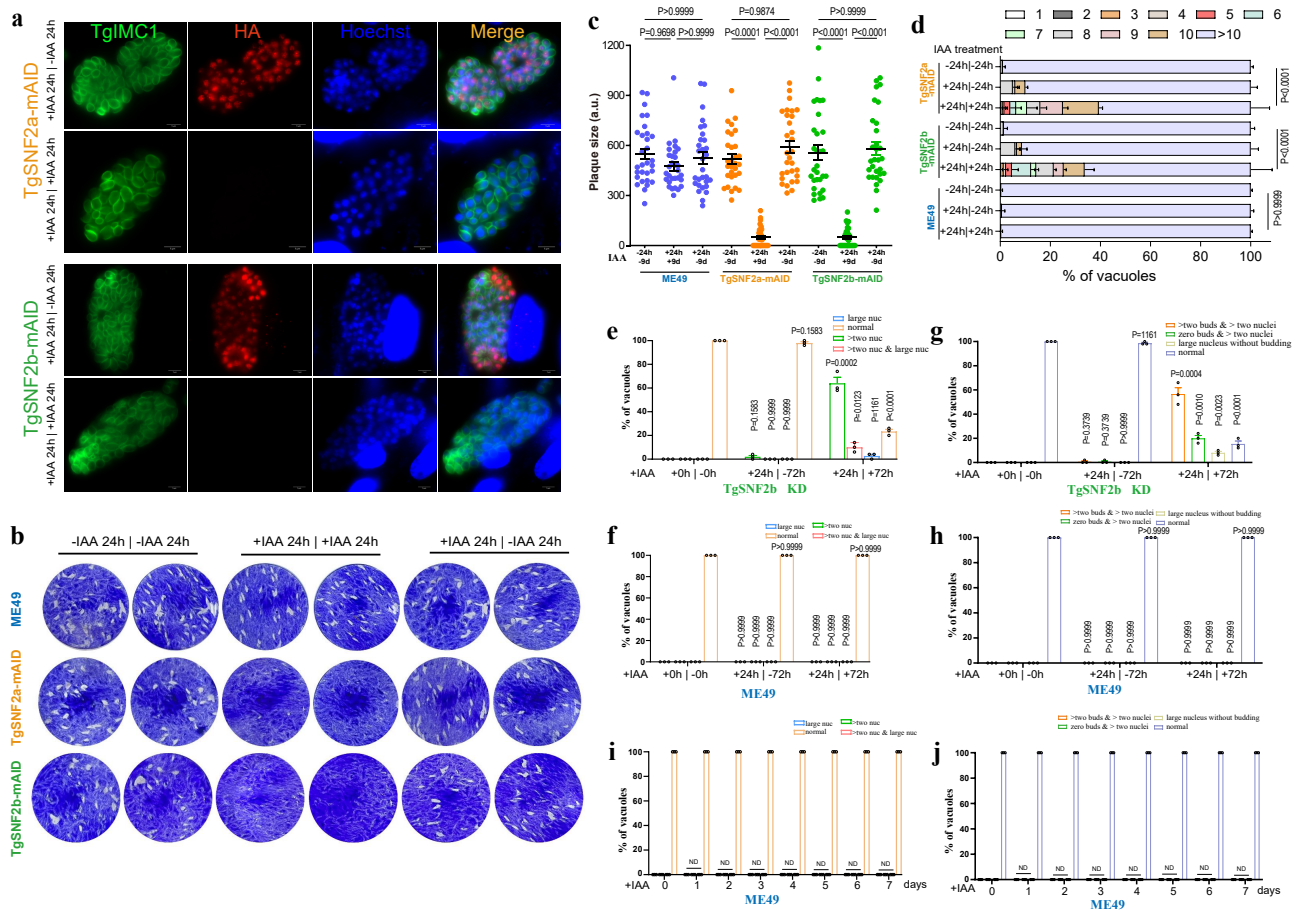
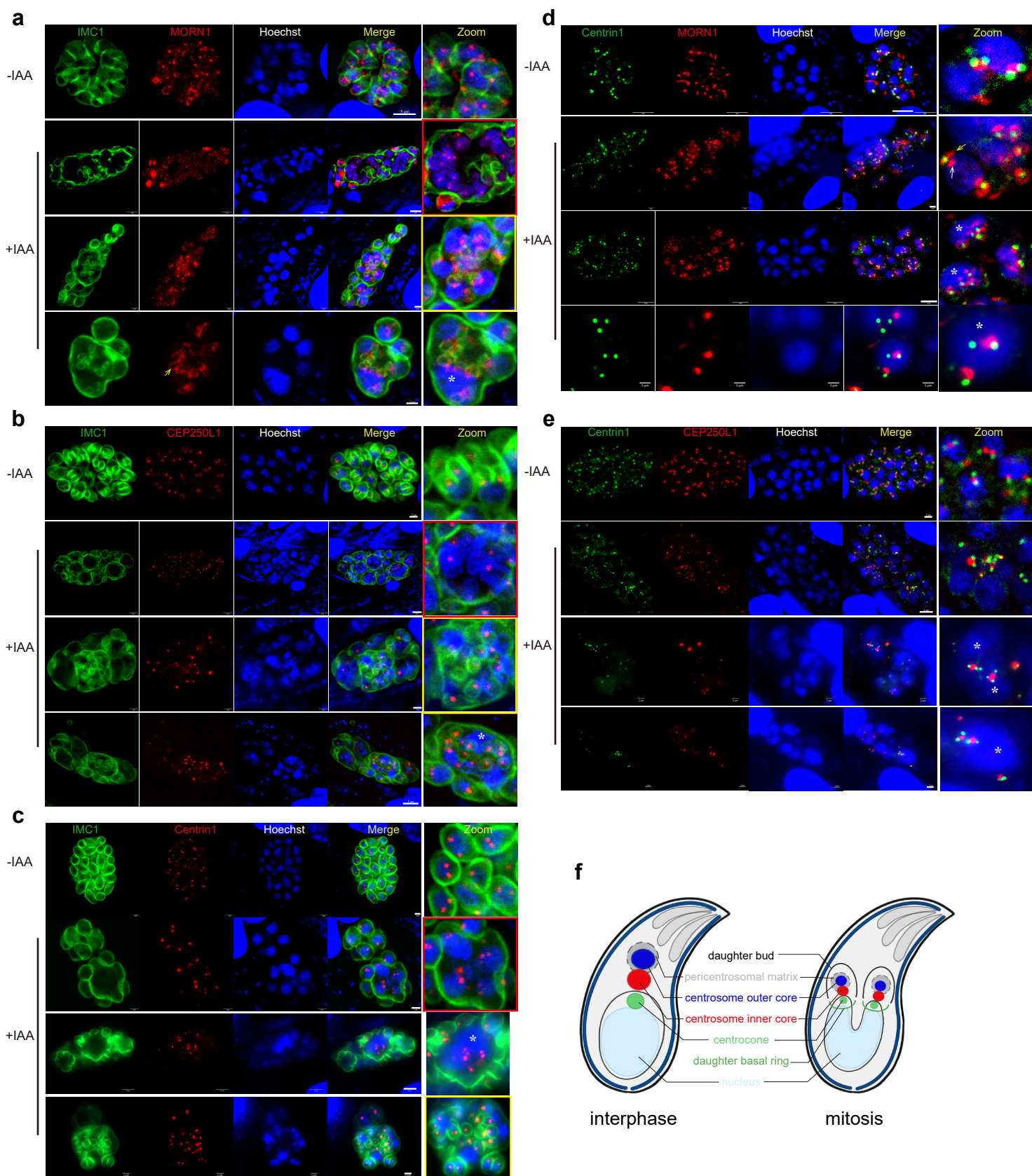




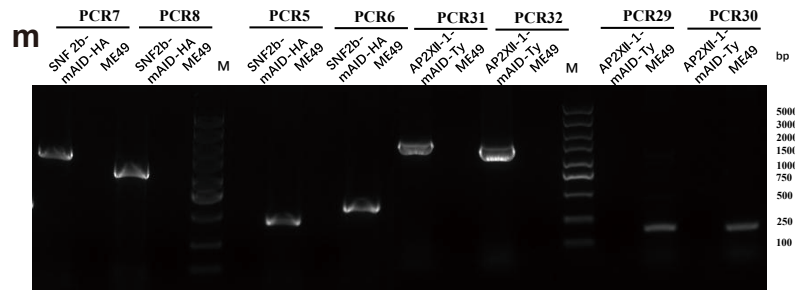
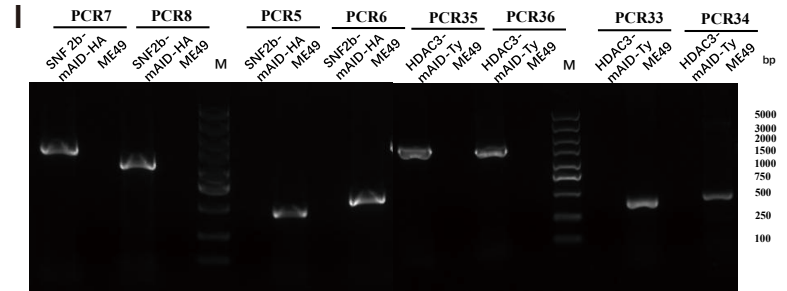
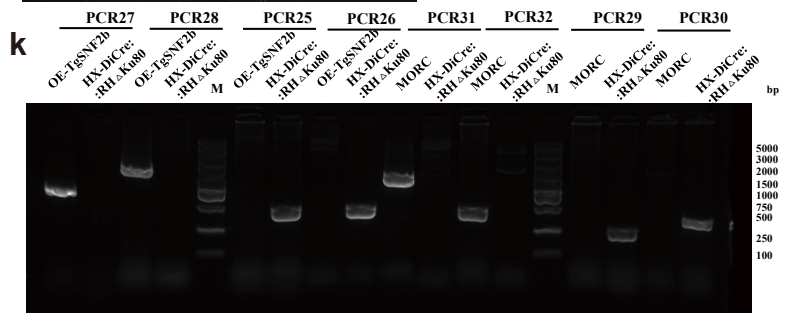
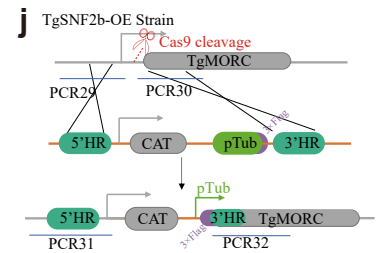
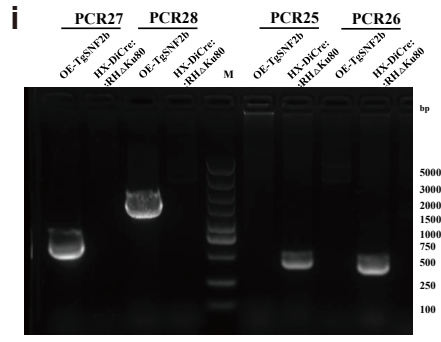
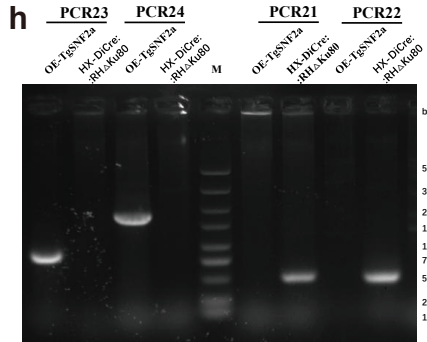
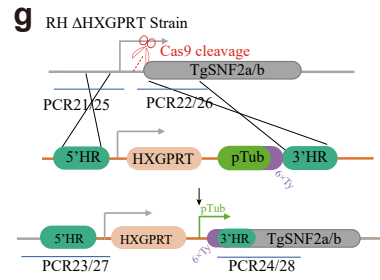
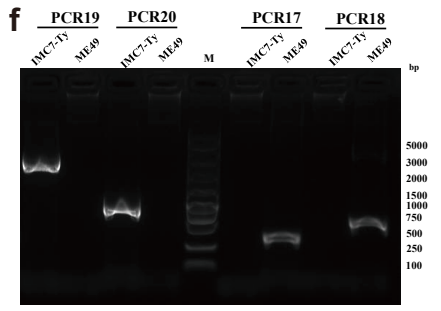
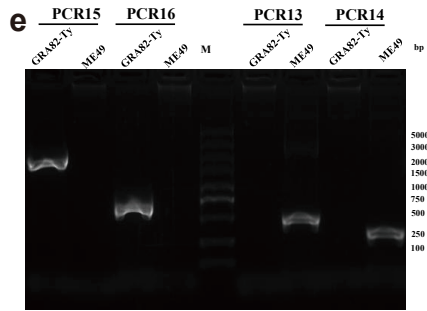
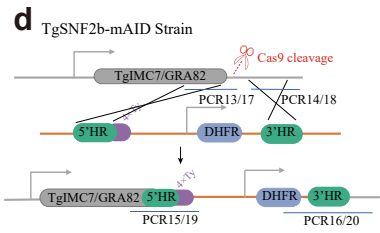
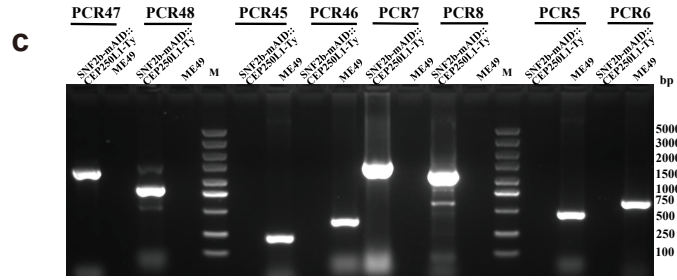
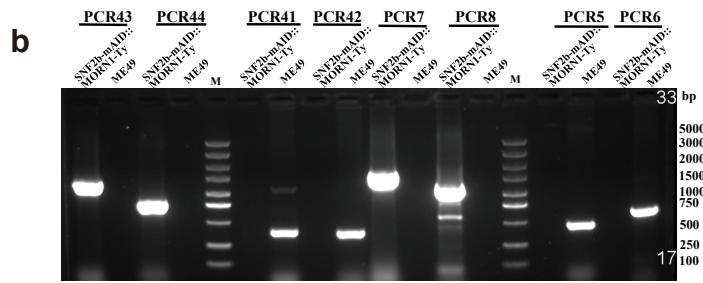
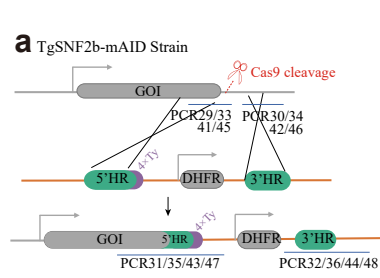
Supplementary figure 2: Immunofluorescence assays and definition of nuclear phenotypes following TgSNF2b knockdown. Intracellular parasites were stained with anti-TgGAP45 and anti-H3K4me3 antibodies. Representative diagrams illustrate the different nuclear phenotypes observed.



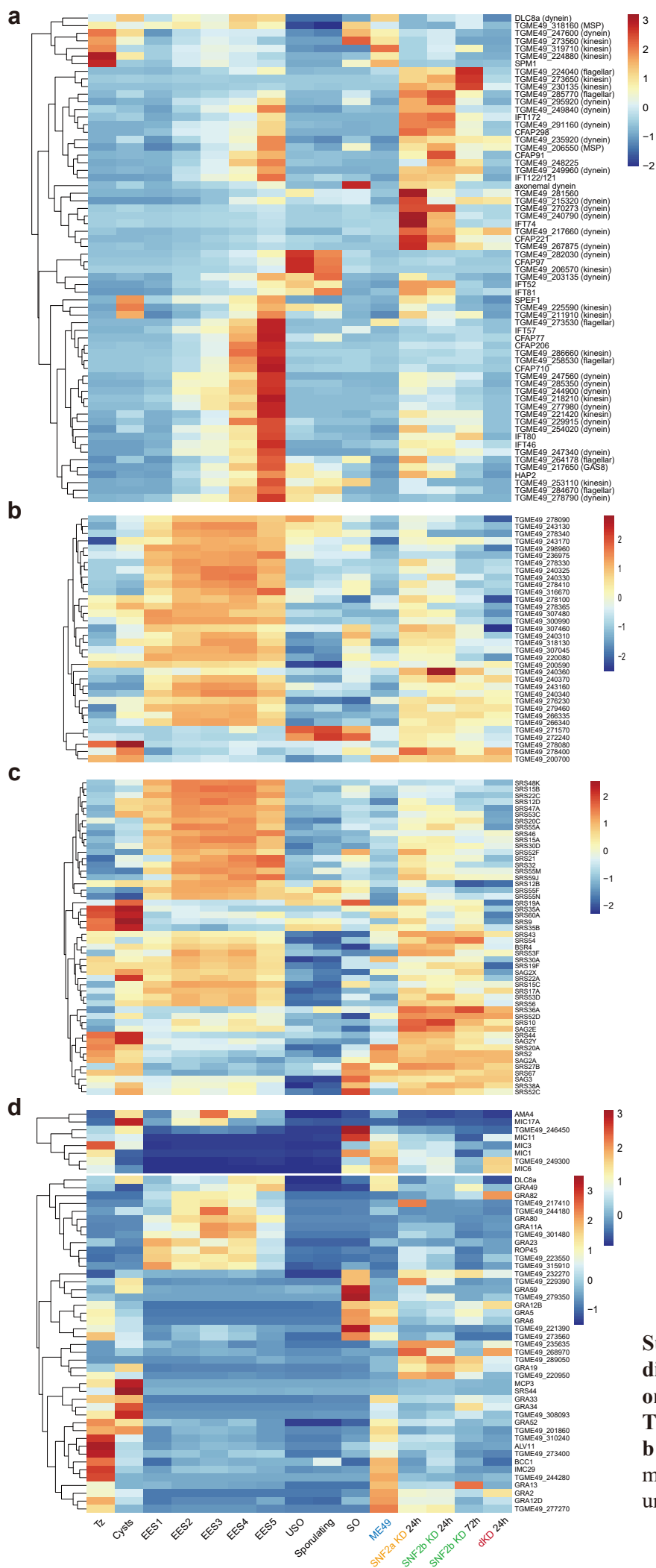
Supplementary figure 3: IAA wash-out rescues parasite growth. **a** Restoration of TgSNF2a and TgSNF2b expression following IAA removal. TgSNF2a-mAID and TgSNF2b-mAID parasites were treated with IAA for 24 h, followed by a 24h wash-out, and stained with anti-TgIMC1 and anti-HA antibodies. **b, c** Plaque assays of TgSNF2a-mAID and TgSNF2b-mAID parasites grown on HFFs. Parasites were treated with IAA for 24 h, then cultured for an additional 9 days with/without IAA. Plaque areas were measured and counted by Photoshop C6S software, and statistical analysis was performed by unpaired Student's *t*-test (two-tailed). Representative data are presented as mean $\pm$ SEM from 30 randomly selected plaque areas ($n = 30$). **d** Intracellular replication assay. TgSNF2a-mAID, TgSNF2b-mAID, and parental strains were treated with IAA or vehicle as indicated, and tachyzoites per PV were counted and two-way ANOVA was used for statistics. **e, f** Quantitative analysis of nuclear phenotypes by immunofluorescence staining. TgSNF2b-mAID and parental strains were treated with IAA or vehicle as indicated, and stained with anti-TgGAP45, anti-H3K4me3, and Hoechst. **g, h** Quantitative analysis of budding phenotypes by immunofluorescence staining. TgSNF2b-mAID and parental strains were treated with IAA or vehicle as indicated, and stained with anti-TgGAP45, anti-TgIMC1, and Hoechst. **i, j** Immunofluorescent and statistical assays of the parental ME49 strain. Intracellular parasites were treated with or without IAA for indicated times, and were then stained with anti-TgGAP45+anti-H3K4me3 (i) and anti-TgGAP45+anti-TgIMC1 antibodies (j). Data in d-j represents the mean $\pm$ SEM of three independent experiments, each counting at least 100 PVs to determine the indicated % of mutants. Unpaired student's *t*-test (two-tailed) was used for statistical analysis of e-j; ND, not detected.



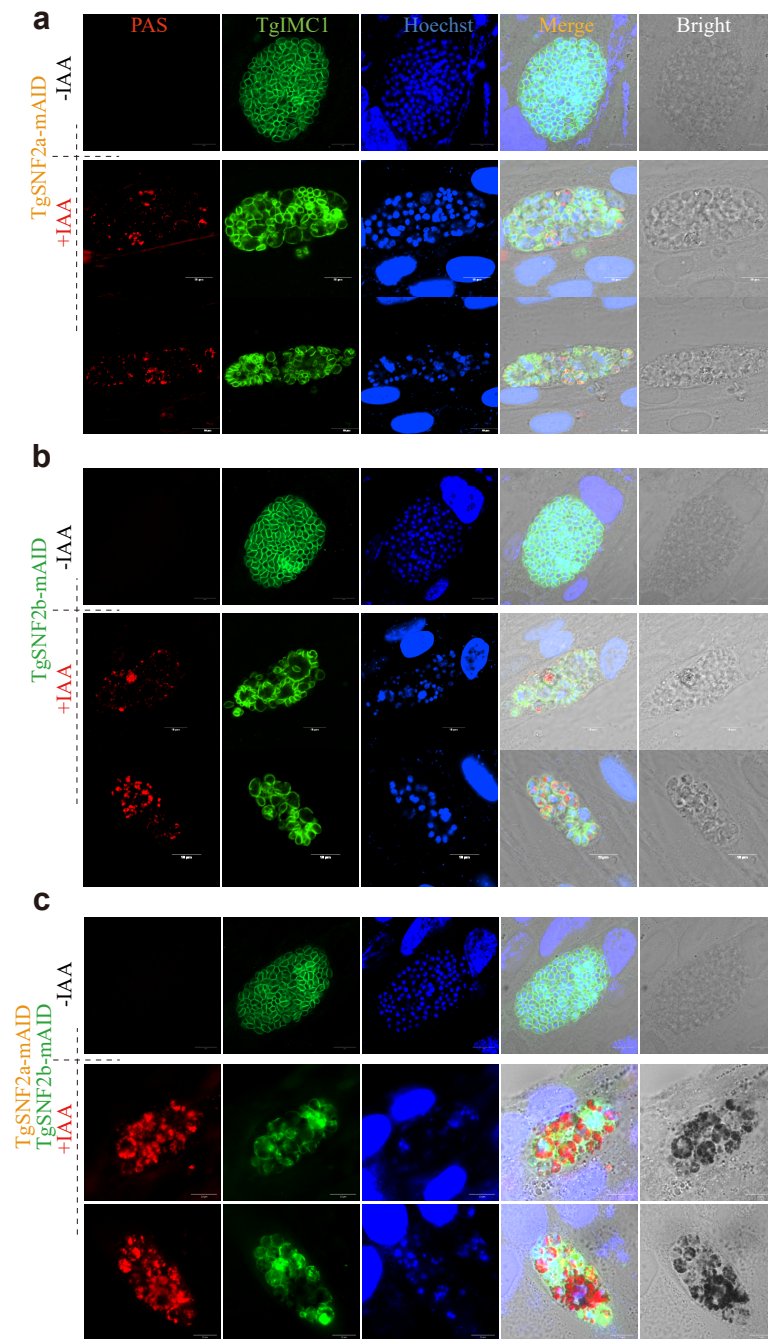
Supplementary figure 4: Centrosome over duplication after SWI/SNF disruption. **a** IFA of TgSNF2b-mAID::MORN1-Ty parasites treated for 3 days with IAA or vehicle, stained with anti-TgIMC1 and anti-Ty antibodies. **b** IFA of TgSNF2b-mAID::CEP250L1-Ty parasites after 3 days of IAA or vehicle; samples were co-stained with anti-Ty and anti-TgIMC1. **c** IFA of TgSNF2b-mAID parasites after 3 days of IAA or vehicle; samples were co-stained with anti-TgCentrin1 and anti-TgIMC1. **d** IFA of TgSNF2b-mAID::MORN1-Ty parasites treated as above, co-stained with anti-Ty and anti-TgCentrin1. **e** IFA of TgSNF2b-mAID::CEP250L1-Ty parasites after 3 days of IAA or vehicle; samples were co-stained with anti-Ty and anti-TgCentrin1. Red box: multinucleated parasite without buds; yellow box: multinucleated parasite with buds; asterisk: enlarged nucleus; white arrow: centrocone; yellow arrow: daughter basal ring. **f** Diagrams illustrate the organelle distribution of *T. gondii* tachyzoite during interphase and mitosis.



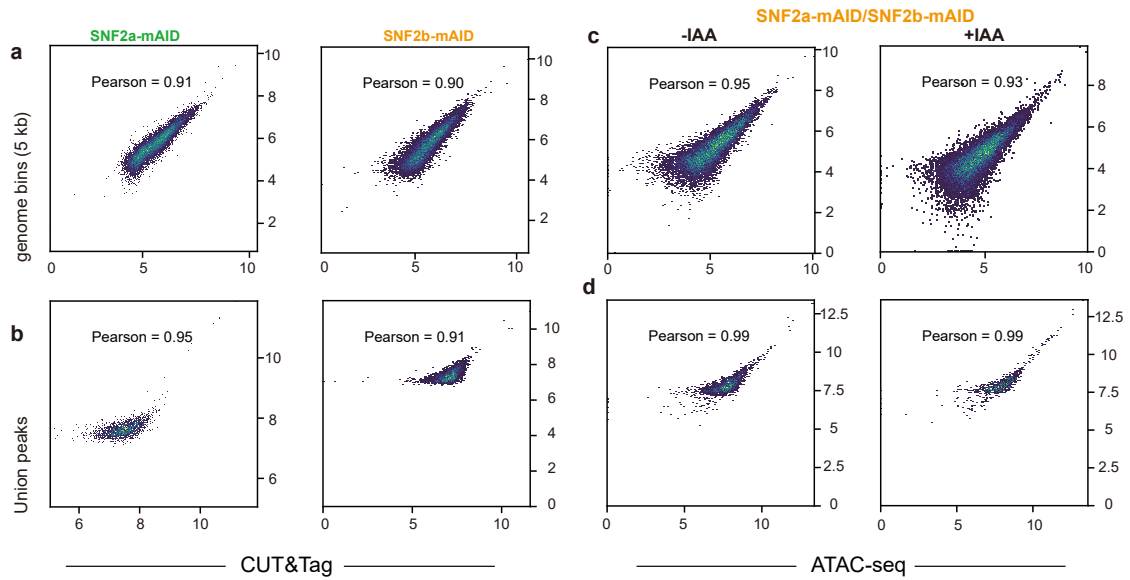
Supplementary figure 5: Plasmid construction and PCR verification of endogenously tagged parasite lines. **a** Diagram shows the construction of TgHDAC3, TgAP2XII-1, TgMORN1, or TgCEP250L1 endogenous tagging strains based on TgSNF2b-mAID strain. **b, c** PCR identifications of the TgMORN1, and TgCEP250L1 endogenous tagging strains, respectively. **d** Diagram shows the construction of TgIMC7 or TgGRA82 endogenous tagging strains based on TgSNF2b-mAID strain. **e, f** PCR identifications of the TgIMC7 and TgGRA82 endogenous tagging strains, respectively. **g** Diagram shows the construction of TgSNF2a and TgSNF2b overexpressing strains based on RH HXGPRT knockout strain. **h, i** PCR identifications of the TgSNF2a and TgSNF2b overexpressing strains, respectively. **j** Diagram shows the construction of MORC N-terminal tagging strain based on TgSNF2b overexpressing strain. **k** PCR identifications of the MORC N-terminal tagging strain. **l, m** PCR identifications of the TgHDAC3 and TgAP2XII-1 endogenous tagging strains, respectively. Detailed primers used were listed in **Supplementary data 3**.



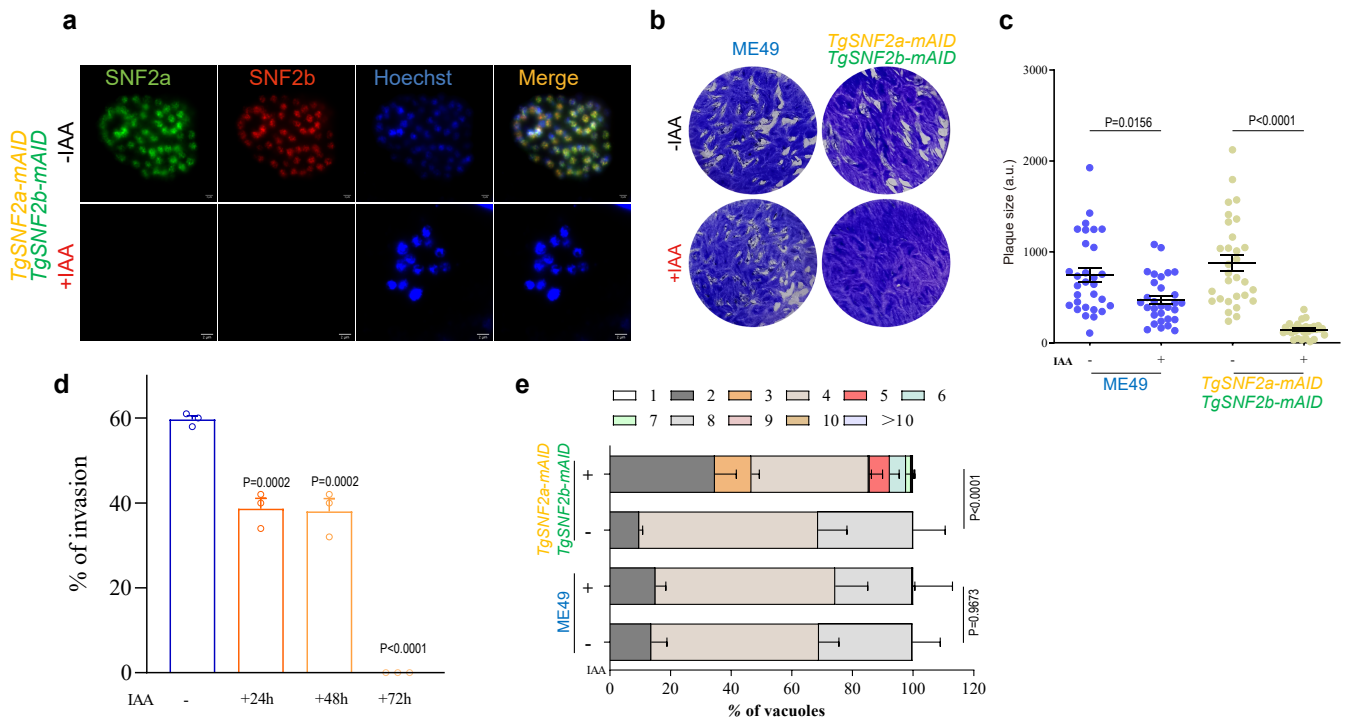
Supplementary figure 6: Heatmap showing different stage expressions of the co-upregulated or co-downregulated genes after TgSNF2a and TgSNF2b knockdown. a gametocyte related genes, **b** *T. gondii* protein A to E families, **c** srs genes, **d** microneme and dense granule protein genes. USO, unsporulated oocyst; SO, sporulated oocyst.



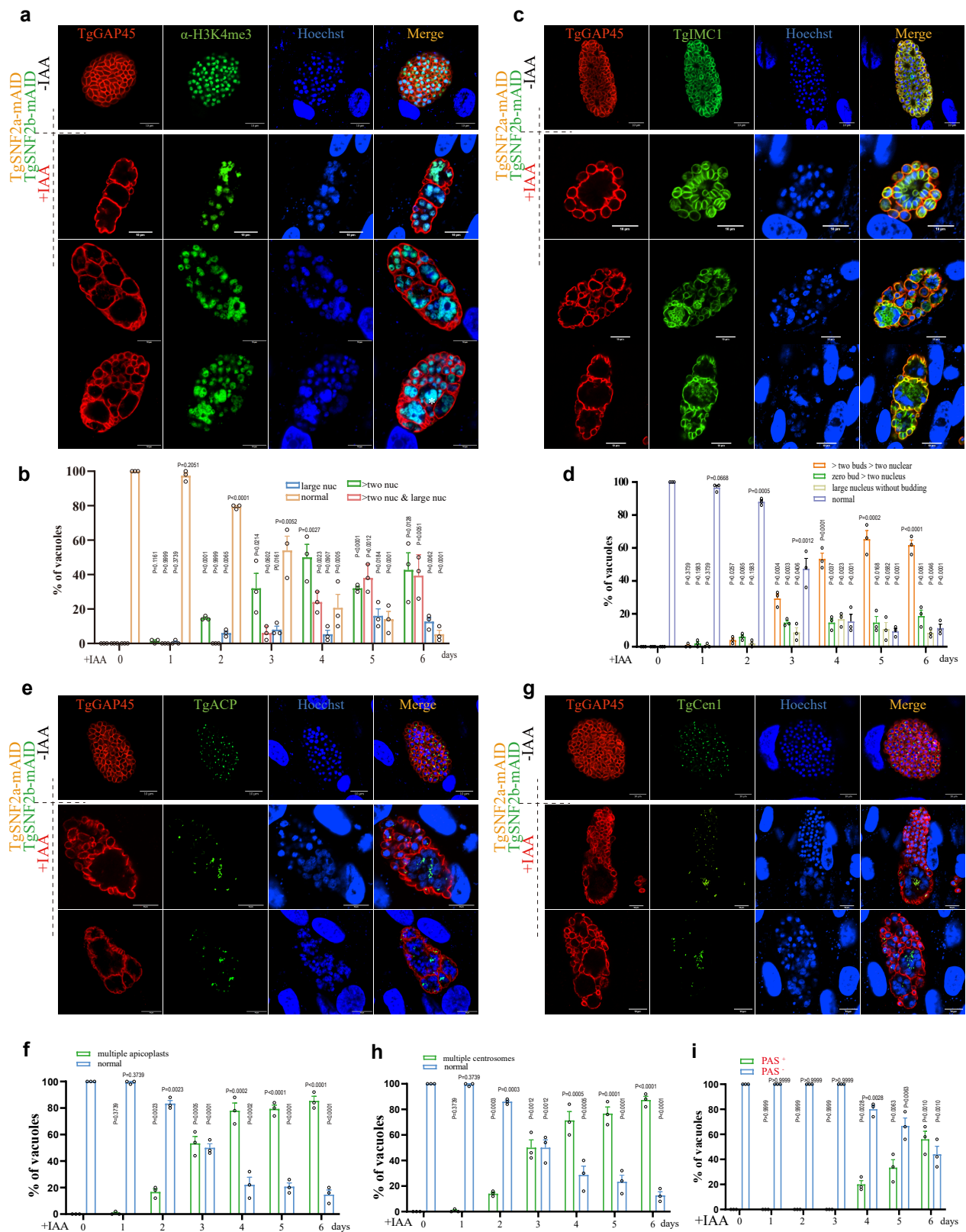
Supplementary figure 7: PAS staining of TgSNF2a and TgSNF2b knockdown parasites. TgSNF2a-mAID (a), TgSNF2b-mAID (b), and the dKD TgSNF2a-mAID/TgSNF2b-mAID (c) parasite strains were treated with or without IAA for 4 days and were then stained with PAS and anti-TgIMC1.



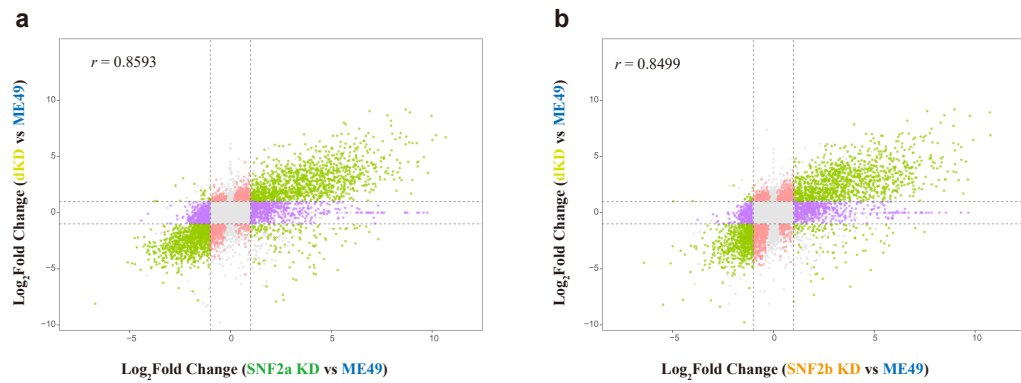
Supplementary figure 8: Genome-wide concordance between CUT&Tag and ATAC-seq biological replicates. The RPKM normalized bigwig files from two biological replicates of each protein were first averaged per protein using deepTools bigwigCompare. Correlations were then evaluated at two levels: **(a, c)** genome-wide, by partitioning the genome into non-overlapping 5kb bins and extracting log-transformed read densities ($\ln[\text{RPKM}+1]$) with multiBigwigSummary, followed by computation of Pearson correlation coefficients; and **(b, d)** peak-based, by generating a union peak set from high-confidence peaks ($\text{IDR} \leq 0.05$) of both proteins, summarizing signals across these regions with multiBigwigSummary, and computing pairwise Pearson correlation coefficients. Correlation matrices and scatterplots were visualized using plotCorrelation.



Supplementary figure 9: Phenotyping of TgSNF2a and TgSNF2b double knockdown strain. **a** IFA shows the efficient knockdown of TgSNF2b and TgSNF2a. dKD strain parasites were treated with or without 500 mM IAA for 24 h, and parasites were stained with anti-Ty (red) and anti-HA (green) antibody. The nuclei were stained by Hoechst. **(b, c)** Plaque assay of dKD strain parasites grown on IAA- or vehicle-treated HFF cells for 12 days. Plaque areas were measured and counted by Photoshop C6S software, and analyzed with unpaired student's *t*-test. Representative data are presented as mean $\pm$ SEM from 30 randomly selected plaque areas ($n = 30$). **d** Invasion efficiency of dKD strain that was pretreated with IAA for 0, 24, 48 and 72 h. After pretreatment, parasites were inoculated to HFF cells for 1 h at 37°C, and the invasion efficiency was counted based on immunofluorescence assay with counting 15 fields per strain, and analyzed with unpaired student's *t*-test. **e** Intracellular parasites replication of the dKD strain incubation with IAA or vehicle for 24 h. Data represents the mean $\pm$ SEM of three independent experiments, each counting at least 100 PVs to determine the indicated % of mutants. Two-way ANOVA was used for statistics.



Supplementary figure 10: Double knockdown of TgSNF2a and TgSNF2b phenocopies TgSNF2b single knockdown. Immunofluorescent and statistical assays of dKD strain after treatment with IAA for different times. (a, b) parasites stained with anti-TgGAP45 and anti-H3K4me3 antibodies, with emphasis on nuclei multiplication. Asterisk indicates enlarged nucleus. (c, d) Parasites were stained with anti-TgGAP45 and anti-TgIMC1 antibodies with emphasis on multiple budding. (e, f) parasites stained with anti-TgGAP45 and anti-TgAPC1 antibodies, with emphasis on apicoplast multiplication. (g, h) parasites stained with anti-TgGAP45 and anti-TgCentrin1 antibodies, with emphasis on centrosome multiplication. i statistical analysis for PAS staining after different times of IAA treatment. Data in b, d, f, h, and i represents the mean $\pm$ SEM of three independent experiments, each counting at least 100 PVs to determine the indicated % of mutants. Unpaired student's t-test (two-tailed) was used for statistical analysis.



Supplementary figure 11: Pearson correlation of gene expression between double knockdown and single knockdown of TgSNF2a and TgSNF2b. Person correlation analysis of gene expression after dKD and knockdown of TgSNF2a (**a**) or TgSNF2b (**b**). r : Pearson correlation coefficients.