

Article

The Centrocone Protein SMC_N1 Is Important for the Proliferation of *Toxoplasma gondii* Tachyzoites

Chuan Li , Jin Gao *, Xiao-Jing Wu , Shi-Chen Xie , Hai-Sheng Zhang and Xing-Quan Zhu * 

Shanxi Key Laboratory of Animal Disease Research, Prevention and Control, College of Veterinary Medicine, Shanxi Agricultural University, Jinzhong 030801, China; 15796845248@163.com (C.L.); wuxiaojing2017@163.com (X.-J.W.); xieshichen221@163.com (S.-C.X.); zhs15095372330@126.com (H.-S.Z.)

* Correspondence: jingao2022@163.com (J.G.); xingquanzhu1@hotmail.com (X.-Q.Z.)

Simple Summary

Toxoplasma gondii, an apicomplexan parasite with a broad host range, undergoes efficient proliferation through the unique endodyogeny during its tachyzoite stage, which is critical for triggering acute infections. As a specialized structure involved in *T. gondii* cell division, the centrocone and the regulatory mechanisms of its associated proteins remain incompletely elucidated. This study examined the functions of the centrocone protein SMC_N1 by constructing a conditional knockdown strain using CRISPR-Cas9 combined with the mini auxin-inducible degron (mAID) system. Immunofluorescence analysis (IFA) demonstrated that SMC_N1 exhibited periodic expression, with peak levels during the S phase and undetectable expression in the G1 phase. Depletion of SMC_N1 disrupted the integrity of inner membrane complex (IMC) assembly, the process of endodyogeny, and nuclear division. Additionally, SMC_N1 was involved in the stable inheritance of centrosomes and apicoplasts. In conclusion, this study revealed that the centrocone protein SMC_N1 is indispensable for the proliferation of *T. gondii* tachyzoites. Its unique expression pattern and functional specificity not only expand our understanding of the regulatory mechanisms underlying *T. gondii* cell division, but also provide a potential target for the development of novel anti-*Toxoplasma* drugs targeting parasite proliferation.

Abstract

The highly efficient endodyogeny of tachyzoites is a key process driving acute infection by *Toxoplasma gondii*. The centrocone is a specialized and critical structure for parasite cell division, but the regulatory mechanisms of centrocone proteins in *T. gondii* remain poorly understood. In this study, we characterized the centrocone protein SMC_N1, which exhibited periodic expression in tachyzoites, peaking during the synthesis phase. Conditional depletion of SMC_N1 was achieved in the type I RH strain and type II cyst-forming PRU strain using the mAID system combined with CRISPR-Cas9. Depletion of SMC_N1 disrupted IMC assembly, endodyogeny and nuclear division, as well as the stable inheritance of the apicoplast and centrosome, resulting in severe defects in intracellular replication and impaired tachyzoite growth. Collectively, these results indicate that SMC_N1 regulates cell division by coordinating organelle inheritance and cytoskeletal dynamics, ensuring proper replication of *T. gondii* tachyzoites and provide insights into mechanisms controlling parasite proliferation.

Keywords: *Toxoplasma gondii*; endodyogeny; centrocone; cell cycle; growth



Academic Editors: Guangwen Yin and Sixin Zhang

Received: 18 December 2025

Revised: 12 February 2026

Accepted: 13 February 2026

Published: 18 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Toxoplasma gondii (Nicolle and Manceaux, 1908), an apicomplexan parasite with a broad host range, can infect nearly all warm-blooded animals and humans, and approximately one-third of the world population is chronically infected [1,2]. The pathogenic potential of this parasite largely depends on the efficient asexual reproduction of the tachyzoite stage, which proliferates rapidly through a unique mode of endodyogeny, leading to host tissue damage and disease progression [3,4]. The chronic phase of toxoplasmosis is characterized by the formation of tissue cysts by the parasite. Existing therapeutic agents have limited ability to penetrate these cysts to exert their efficacy, resulting in a lack of specific therapeutic drugs for this phase. Furthermore, drug resistance associated with prolonged medication further compromises treatment outcomes [5]. Clinically, current interventions are largely restricted to symptomatic and supportive care, which fail to completely eradicate the parasites [6]. Consequently, this therapeutic gap represents a major challenge in the prevention and control of toxoplasmosis, highlighting the urgent need for the development of targeted therapies.

T. gondii is capable of invading the nucleated cells of all warm-blooded animals [7]. Due to its incomplete metabolic machinery, the parasite is unable to replicate independently in the extracellular environment and instead relies on host-derived nutrients, energy, and organelles for proliferation [8]. Numerous organelles of *T. gondii* are indispensable for host cell invasion and proliferation. Among these, micronemes secrete adhesion and motility-related proteins, such as MIC2, which mediates the initial adhesion and gliding motility between the parasite and host cells [9]. Rhoptries release ROP family proteins that facilitate penetration of the host cell membrane and contribute to the biogenesis of the parasitophorous vacuole (PV) [10]. Dense granules secrete GRA proteins that continuously modify the PV membrane to evade host immune recognition signals [11]. The apicoplast is involved in the synthesis of essential metabolites, including fatty acids, and its accurate inheritance is required to maintain the normal proliferation cycle of the parasite [12]. The inner membrane complex (IMC), as the core of the cytoskeleton, maintains parasite morphology and coordinates cell division [13]. The centrosome functions as the central regulatory organelle for daughter cell division; its migration from the apical to the basal side of the nuclear envelope marks the initiation of endodyogeny, and upon maturation, it coordinates daughter cell budding [14]. Furthermore, the centrosome cooperates with the apicoplast to ensure the synchrony of parasite division [15]. Through their coordinated functions, these organelles constitute the fundamental biological basis for the efficient invasion and intracellular proliferation of *T. gondii*. The basis of endodyogeny in *T. gondii* is a unique internal budding process generating two daughter cells *de novo* [16]. This process initiates with the activation of the cell cycle, encompassing growth during the G1 phase, DNA synthesis in the S phase, mitosis in the M phase and cytokinesis in the C phase [17,18]. Cell cycle regulation involves multiple molecular networks, focusing on centrosome function control, the apicomplexan-specific kinases MAPKL1 and MAPK2 govern the normal progression of centrosome duplication as well as cell growth and division [19,20] and the cyclin-dependent kinase (CDK) family regulates cell cycle checkpoints, strictly controlling the transition timing between various stages of the cell cycle [21,22]. Furthermore, Aurora kinases and NIMA-related kinases are responsible for regulating mitotic progression [23,24]. As a spindle organizing center and a key hub connecting nuclear–cytoplasmic mitotic processes, the centrocone also serves as a critical site for the integration of cell cycle regulatory signals [25].

As a membrane sac structure unique to apicomplexan parasites, the centrocone acts as a key mediator of the physical connection between nuclear and cytoplasmic mitotic processes and persists throughout the entire division process of apicomplexan parasites [21,25].

Although the structure and localization of several centrocone-associated proteins have been reported, their molecular mechanisms in regulating cell cycle progression remain poorly understood [26].

A previous study revealed that TGME49_270810, which we designated as SMC_N1 based on its functionally dominant domain, localizes to the centrocone [27], suggesting a potential role in *T. gondii* replication and growth. To investigate its function, conditional knockout strains were generated in type I RH and type II PRU backgrounds using the CRISPR-Cas9 system, and the impact of SMC_N1 depletion on parasite pathogenicity was assessed through a series of phenotypic assays. Depletion of SMC_N1 was found to severely impair tachyzoite replication and growth in vitro, causing IMC assembly defects and tachyzoite division abnormalities, while bradyzoite differentiation remained unaffected, indicating that SMC_N1 is required for efficient parasite replication.

2. Materials and Methods

2.1. Cell Culture and Parasite Strains

All tachyzoites used in this experiment, including RH $\Delta ku80\Delta hxprrt::TIR1$ (referred as RH::TIR1), PRU $\Delta ku80\Delta hxprrt::TIR1$ (referred as PRU::TIR1), RH $\Delta ku80\Delta hxprrt::TIR1$ -SMC_N1-mAID-6HA (referred as RH::TIR1-SMC_N1-mAID) and PRU $\Delta ku80\Delta hxprrt::TIR1$ -SMC_N1-mAID-6HA (referred as PRU::TIR1-SMC_N1-mAID), were cultured in monolayers of human foreskin fibroblasts (HFFs, American Type Culture Collection), as previously described [28]. For the cultivation of HFFs, Dulbecco's Modified Eagle Medium (DMEM) high-glucose medium (Thermo Fisher Scientific, Waltham, MA, USA) was supplemented with 10% fetal bovine serum (FBS, GIBCO, Waltham, MA, USA), 10 mM HEPES (pH 7.2, Solarbio, Beijing, China), 100 μ g/mL streptomycin and 100 U/mL penicillin (Solarbio, Beijing, China), with the culture environment maintained at 37 °C and 5% CO₂. The required tachyzoites were harvested from heavily infected HFFs using a 27-gauge needle (BD Medical, Franklin Lakes, NJ, USA).

2.2. Construction and Transfection of Epitope-Tagged Strains

The conditional knockout strains generated in this study were constructed using CRISPR-Cas9 technology in combination with the mAID system, as previously described [29]. For C-terminal endogenous tagging, CRISPR-Cas9 plasmids targeting the 3'-untranslated region (3'-UTR) of the gene of interest (GOI) near the stop codon were engineered. Meanwhile, amplicons containing the mAID-6HA tag with hemagglutinin (HA) and the dihydrofolate reductase (DHFR) marker were prepared, with short homologous regions flanking these amplicons. The plasmids and amplicons were co-transfected into RH::TIR1 and PRU::TIR1 strains. Individual clones were isolated by limiting dilution screening with 3 μ M pyrimethamine, and the successful insertion of the mAID-6HA tag into the target site of the GOI was confirmed by PCR and sequencing. For conditional knockdown experiments, parasites were treated with 500 μ M 3-indoleacetic acid (IAA), while control treatments were performed with 0.1% ethanol.

2.3. Indirect Immunofluorescence Analysis (IFA)

HFF monolayers infected with tachyzoites were fixed with 4% paraformaldehyde (PFA) for 30 min, as previously described [30], followed by permeabilization in phosphate-buffered saline (PBS) containing 0.1% Triton X-100 for 20 min. Samples were then blocked with 5% bovine serum albumin (BSA) for 2 h and incubated with primary antibodies diluted in PBS at 37 °C for 2 h. After washing, secondary antibodies diluted in PBS were added and incubated at 37 °C in the dark for 1 h. Nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI, 1:1000, Thermo Fisher Scientific, Waltham, MA, USA)

in the dark. Images were acquired using a Leica TCS SP8 confocal microscope (Leica, Munich, Germany). After each step, samples were washed with PBS five times to remove residual reagents.

The primary antibodies used in this study included rabbit anti-ISP1, rabbit anti-CPN60, rabbit anti-GAP45, rabbit anti-Centrin1, mouse anti-IMC1 (1:500, all the above antibodies were preserved in our laboratory), rabbit anti-IMC1, mouse anti-HA (1:500, Thermo Fisher Scientific, Waltham, MA, USA), mouse anti-Centrin1 (1:500, Sigma, Temecula, CA, USA) and rabbit anti-HA (1:500, CST, Danvers, MA, USA). The secondary antibodies were Alexa Fluor 488 Goat anti-Rabbit IgG (H + L), Alexa Fluor 594 Goat anti-Rabbit IgG (H + L), Alexa Fluor 488 Goat anti-Mouse IgG (H + L), Alexa Fluor 594 Goat anti-Mouse IgG (H + L) (1:500, Thermo Fisher Scientific, Waltham, MA, USA) and FITC-conjugated Dolichos biflorus lectin (DBL, 1:500, Vector Laboratories, Newark, CA, USA).

2.4. Western Blotting

Heavily infected HFFs were mechanically disrupted using a 27-gauge syringe, and the liberated tachyzoites were filtered and collected by centrifugation at 4 °C. The parasite pellets were washed twice with PBS, followed by resuspension in RIPA lysis buffer supplemented with EDTA and a protease inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA, USA). The lysates were incubated on ice for 1 h and clarified by centrifugation. Total protein samples were separated by SDS-PAGE using 5% stacking gel and 12% resolving gel. Electrophoresis was performed at 80 V during the stacking phase, followed by 120 V during the resolving phase. Subsequently, the separated proteins were transferred onto polyvinylidene fluoride (PVDF) membranes. Membranes were blocked with 5% non-fat milk in TBST (TBS containing 0.2% Tween-20) for 2 h, incubated with primary antibodies at 37 °C for 2 h or overnight at 4 °C and then washed three times in TBST. After incubation with HRP-conjugated secondary antibodies for 1 h at room temperature, membranes were washed again and developed using ECL substrate. Signals were detected with a ChemiDoc XRS+ imaging system (Bio-Rad, Hercules, CA, USA).

The primary antibodies used in this experiment were rabbit anti-aldolase antibody (ALD, 1:500, preserved in our laboratory), rabbit anti-HA antibody (1:1000, CST, Danvers, MA, USA), and the secondary antibody was goat anti-rabbit HRP-conjugated antibody (1:1000, Biodragon, Jiangsu, China).

2.5. Plaque Assay

Plaque assays were performed to assess parasite growth. HFF monolayers were seeded in 12-well plates and allowed to reach full confluence. Tachyzoites were liberated from heavily infected T25 flasks by mechanical disruption and filtration. Approximately 400 freshly isolated SMC_N1-mAID parasites were inoculated onto confluent HFF monolayers in the presence or absence of IAA. Cultures were maintained at 37 °C with 5% CO₂ for 7 or 8 days. Following incubation, the culture medium was carefully aspirated and discarded into a biohazardous waste container for subsequent decontamination, and HFF monolayers were fixed with 4% PFA for 30 min, stained with 0.5% crystal violet for 30 min. Following staining, residual dye was thoroughly washed off with PBS, and the plates were air-dried. Plates were then photographed vertically on a non-reflective surface under uniform lighting. Images were analyzed using ImageJ 1.8.0 to quantify plaque number and area. Statistical analyses were performed to compare differences between strains and assess *T. gondii* growth, as previously described [31,32].

2.6. Invasion Assay

For the invasion assay, tachyzoites were pretreated with or without IAA for 24 h. Following heavy infection of HFFs, the infected cells were scraped off with a cell scraper and

passed through a 27-gauge needle to disrupt the host cells and release tachyzoites, yielding freshly egressed, highly viable tachyzoites. Subsequently, approximately 1×10^6 parasites, quantified by hemocytometer counting, were seeded on 12-well plates confluent with HFF monolayers. The cultures were incubated at 37 °C with 5% CO₂ for 30 min in the continued presence or absence of IAA, and then fixed with 4% PFA for 30 min. Extracellular parasites were first labeled with mouse anti-SAG1 antibody (1:500, Invitrogen, Carlsbad, CA, USA) at 37 °C for 2 h, followed by three gentle washes with PBS and incubation with the corresponding Alexa Fluor-conjugated secondary antibody for 1 h. Afterward, cells were permeabilized with PBS containing 0.1% Triton X-100 and incubated with rabbit anti-GAP45 antibody at 37 °C for 2 h, followed by incubation with the appropriate secondary antibody for 1 h. The invasion rate was calculated as the proportion of intracellular parasites relative to the total number of parasites. At least 100 PVs were counted per experiment, and all experiments were performed independently three times.

2.7. Replication Assay

For the replication assay, $\sim 10^6$ parasites were inoculated onto HFF monolayers in 12-well plates and incubated for 1 h. After washing, the infected cultures were incubated for 24 h (RH::TIR1-SMC_N1-mAID) or 28 h (PRU::TIR1-SMC_N1-mAID) in the presence or absence of IAA. After incubation, samples were fixed with 4% PFA, permeabilized with 0.1% Triton X-100, and stained with rabbit anti-IMC1 antibody. The number of parasites per PV was quantified by fluorescence microscopy. At least 100 vacuoles were analyzed across three independent experiments to evaluate replication efficiency.

2.8. Egress Assay

The ionophore-induced egress assay was used to assess tachyzoite escape from host cells. Approximately 10^6 freshly harvested tachyzoites were added onto confluent HFF monolayers in 12-well plates, and allowed to develop for 36–60 h in medium supplemented with or without IAA until PVs reached comparable sizes. The infected HFF monolayers were stimulated with 3 μ M calcium ionophore A23187 (Sigma, Darmstadt, Germany) at 37 °C for 2 min and egress efficiency was evaluated with reference to at least 100 PVs. The assay was performed in biological triplicate.

2.9. In Vitro Bradyzoite Differentiation

In vitro conversion of tachyzoites into bradyzoites was assessed, as previously described [33,34]. Freshly purified tachyzoites were inoculated onto confluent HFF monolayers, under normal conditions, parasites were cultured for 2.5 days at 37 °C with 5% CO₂. For alkaline induction, tachyzoites were allowed to invade HFFs for 4 h in DMEM (pH 7.4, 1% FBS), after which the medium was replaced with alkaline RPMI-HEPES (pH 8.2, 2% FBS). Cultures were maintained at 37 °C under ambient CO₂ ($\sim 0.03\%$), and the alkaline medium was renewed daily to sustain bradyzoite induction. Cyst wall formation was assessed by staining with FITC-Dolichos biflorus lectin (DBL), while parasites were detected using a panel of anti-*T. gondii* antibodies. The extent of bradyzoite differentiation was quantified by calculating the proportion of DBL-positive PVs, with at least 100 PVs examined in each of three independent experiments. The assay was performed in biological triplicate.

2.10. Statistical Analyses

All statistical analyses were performed using GraphPad Prism 10.0 software. Statistical comparison of experimental data between two groups was conducted by two-tailed unpaired *t*-test, while comparisons among three or more groups were performed using one-way analysis of variance (ANOVA). Experimental data are presented as the

mean $\pm$ standard deviation (SD) and were obtained from at least three independent biological replicates. p value < 0.05 was considered statistically significant.

3. Results

3.1. SMC_N1 Is Dynamically Regulated by the Cell Cycle and Exhibits the Highest Expression Level in the S Stage

SMC_N1 is composed of 567 amino acids and contains two conserved domains, PRK12323 and SMC_N (Figure 1A), with the former associated with DNA synthesis and replication fidelity, and the latter linked to chromosome maintenance and DNA repair [35–37]. Genome-wide CRISPR screening of *T. gondii* revealed a phenotype score of -5 for SMC_N1, suggesting that it is essential for parasite survival, and that complete knockout may not be possible [38]. To investigate the function of SMC_N1, a mAID system combined with CRISPR-Cas9 technology was applied (Figure 1B) [29]. The successful insertion of the mAID-6HA tag into the SMC_N1 locus in type I RH strain was confirmed by PCR1 and PCR2, while IFA and Western blot analyses further validated the correct construction of the RH::TIR1-SMC_N1-mAID strain (Figure S1A and Figure 1C,D). IFA results showed that SMC_N1 exhibited periodic nuclear expression, peaking during S phase and disappearing in G1 parasites, and after 24 h of IAA, the HA signal was completely lost (Figure 1C). Western blotting showed a SMC_N1 band at the expected ~ 100 kDa in the absence of IAA treatment, whereas no signal was detected after 24 h of IAA treatment (Figure 1D). To further clarify the subcellular localization of SMC_N1, an IFA was performed using the outer centrosome marker Centrin1. The SMC_N1 signal did not co-localize with Centrin1; instead, SMC_N1 was enriched near the inner centrosome core. These results suggest that SMC_N1 is a centrocone-associated protein localized to the inner centrosome core, warranting further investigation (Figure 1E).

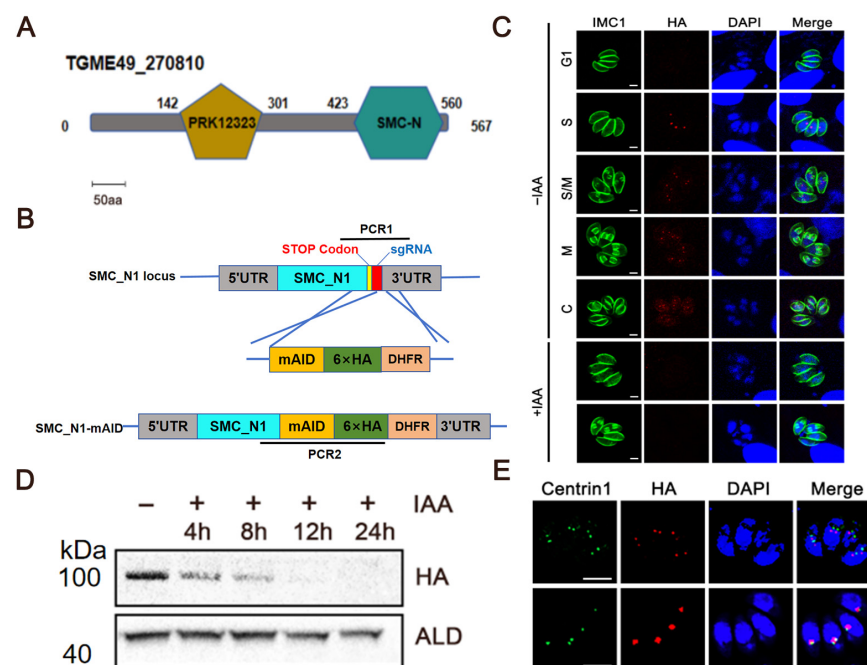


Figure 1. Construction of the *T. gondii* SMC_N1-mAID strain and analysis of SMC_N1 expression. (A) Domain analysis of SMC_N1 using the NCBI Conserved Domain Database (CDD, <https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml>) (accessed on 2 September 2025) identified that SMC_N1 contains two conserved domains, PRK12323 (amino acid positions 142 aa–301 aa) and SMC_N (amino acid positions 423 aa–560 aa). Scale bar = 50 aa (B) Schematic diagram of the construction of the

SMC_N1-mAID strain via C-terminal tagging using the mAID system combined with CRISPR-Cas9 technology. (C) Immunofluorescence assays using rabbit anti-IMC1 (green), mouse anti-HA (red), and DAPI (blue) were performed to label the IMC, SMC_N1-6×HA, and nuclei, respectively. The IFA results showed that SMC_N1 is expressed in a periodic manner, with peak abundance during the S phase and low or undetectable levels during G1. Consistent with this pattern, IAA treatment of the SMC_N1-mAID strain resulted in complete degradation of SMC_N1 after 24 h. Scale bar = 2 μ m. (D) Western blot analysis confirmed the expression of the SMC_N1-6×HA fusion protein, with ALD used as a loading control. The SMC_N1-6×HA signal became undetectable after 24 h of IAA treatment. (E) IFA revealed that SMC_N1 does not co-localize with the centrosome. Parasites were stained with mouse anti-Centrin1 antibody (green), rabbit anti-HA antibody (red), and DAPI (blue). Scale bar = 5 μ m.

3.2. SMC_N1 Is Critical for the Growth and Replication of Type I Strain

To evaluate whether SMC_N1 is required for the growth of type I strains, plaque assays were performed on the parental and SMC_N1-mAID strains in the presence or absence of IAA treatment. Consistent with the previously known low phenotype score, SMC_N1 depletion led to a complete loss of plaque formation (Figure 2A–C), indicating that SMC_N1 is essential for the in vitro lytic cycle of the type I RH strain.

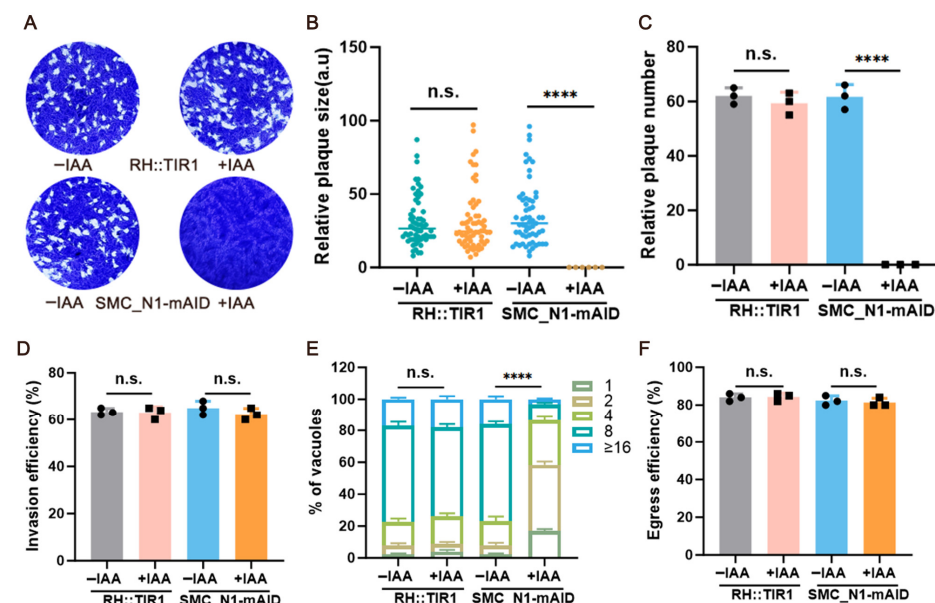


Figure 2. Depletion of SMC_N1 severely impairs the growth and replication of Type I strain. (A) Representative plaque images of the RH parental strain and the SMC_N1-mAID strain cultured with or without IAA for 7 days under standard conditions. SMC_N1 depletion almost completely abolished plaque formation. (B) Quantification of relative plaque areas for each strain. Statistical analysis was performed using a two-tailed, unpaired *t*-test, **** $p < 0.0001$; n.s., no statistical significance. (C) Quantification of plaque numbers generated by each strain, analyzed using a two-tailed, unpaired *t*-test, **** $p < 0.0001$; n.s., no statistical significance. (D) Parasites pretreated with or without IAA for 24 h were allowed to invade host cells for 30 min. The proportion of intracellular tachyzoites relative to total tachyzoites was analyzed using a two-tailed, unpaired *t*-test, and at least 100 PVs were counted and analyzed per sample, n.s., no statistical significance. (E) Parasites were allowed to invade host cells for 1 h and subsequently cultured with or without IAA for 24 h. Replication capacity was analyzed using two-way ANOVA followed by Tukey's multiple-comparison test, and at least 100 PVs were counted and analyzed per sample, **** $p < 0.0001$; n.s., no statistical significance. (F) Egress efficiency, measured 36 h post infection, was analyzed using a two-tailed, unpaired *t*-test, and at least 100 PVs were counted and analyzed per sample, n.s., no statistical significance. All data are presented as the mean $\pm$ standard deviation of three independent experiments.

To determine at which stage of the lytic cycle SMC_N1 functions, invasion, intracellular replication and egress assays were conducted. Parasites pretreated with or without IAA for 24 h were used to infect HFF monolayers for 30 min. The results demonstrated that no significant differences in invasion efficiency were detected between IAA-treated and untreated parasites (Figure 2D). For the replication assay, parasites were allowed to invade HFFs for 1 h in the absence of IAA, followed by 24 h incubation with or without IAA. Compared with the untreated group, SMC_N1-depleted parasites exhibited a marked reduction in replication, as evidenced by an increased proportion of vacuoles containing ≤ 4 parasites, and a decreased proportion of vacuoles containing ≥ 8 parasites (Figure 2E). Finally, for the egress assay, parasites were incubated with or without IAA for 48–60 h and egress efficiency was assessed by induction with 3 μM calcium ionophore A23187. The results revealed that no significant differences in egress efficiency was observed between IAA-treated parasites and controls (Figure 2F). These results indicate that SMC_N1 is required for efficient intracellular replication and sustained growth of the type I RH strain in vitro.

3.3. SMC_N1 Is Required for Normal Morphology, Endodyogeny, and Nuclear Segregation in Type I Strain

Given the critical role of SMC_N1 in *T. gondii* replication, its involvement in parasite division was further examined using IFA. IMC1 staining of the SMC_N1-mAID strain following 24 h of IAA treatment revealed pronounced structural and morphological defects, including abnormal tachyzoite rosette formation (Figure 3(Ai),B, $86.67 \pm 1.56\%$) and impaired IMC assembly (Figure 3(Aii),B, $29.33 \pm 1.76\%$). In addition, a significant increase in aberrant daughter cell division within the same vacuole was also observed, characterized by asynchronous division and endopolygeny (Figure 3(Aiii,iv),B, $17 \pm 1.89\%$). Collectively, these findings demonstrate that SMC_N1 is essential for efficient tachyzoite replication by preserving parasite morphology and ensuring proper coordination of endodyogeny.

To examine the effects of SMC_N1 depletion on parasite membrane organization, the marker proteins TgISP1 and TgGAP45 were analyzed by IFA. TgISP1, localized to the apical cap, reflects early IMC subcompartment organization [39,40], whereas TgGAP45, positioned between the plasma membrane and the IMC, reports on membrane integrity and the glideosome complex [41]. SMC_N1 depletion did not alter TgISP1 distribution, indicating that early IMC subcompartment formation remained largely intact (Figure 3C). In contrast, abnormal GAP45 staining ($34 \pm 2.06\%$) was observed in a subset of SMC_N1-depleted parasites, consistent with disrupted membrane structure and impaired IMC–plasma membrane organization (Figure 3D,E).

Moreover, SMC_N1 depletion parasites exhibited abnormal nuclear division, characterized by unequal segregation or failure to divide ($19.67 \pm 1.49\%$) (Figure 3F,G). These findings demonstrate that SMC_N1 depletion perturbs parasite morphology, daughter cell division, and nuclear segregation in the type I RH strain.

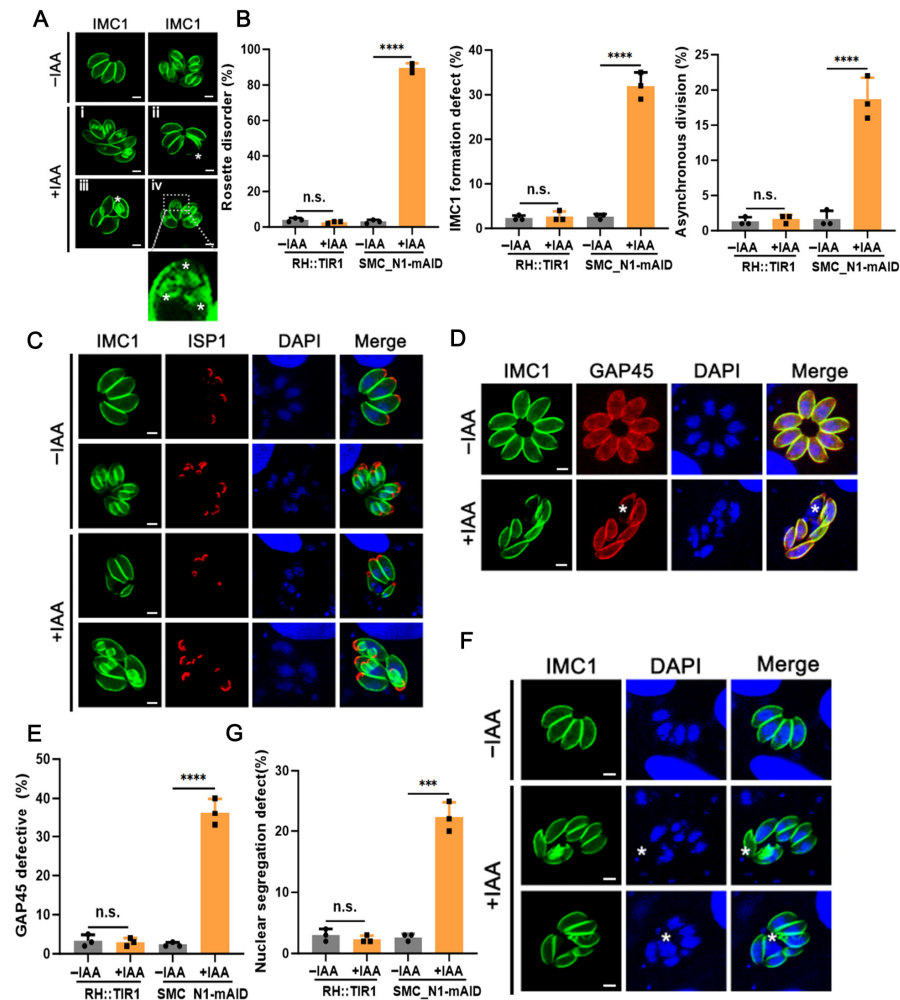


Figure 3. SMC_N1 plays a critical role in maintaining normal parasite morphology, daughter bud formation and nuclear segregation. (A) IMC1 was labeled with a rabbit anti-IMC1 antibody (green). Immunofluorescence analysis showed that SMC_N1 depletion markedly increased the frequency of morphological and endodyogeny defects, including disruption of the rosette pattern (i), loss of IMC1 integrity (ii), asynchronous division (iii) and endopolygeny (iv). All aberrant structures except (i) are indicated by white asterisks. Scale bar = 2 μ m. (B) Quantification of IMC1-associated defects in the RH::TIR1–SMC_N1-mAID strain cultured with or without IAA for 24 h. Data are presented as mean $\pm$ SD from three independent experiments. Statistical differences were analyzed using a two-tailed, unpaired *t*-test, **** $p < 0.0001$; n.s., no statistical significance. (C) The ISP1 structure remained intact in the SMC_N1-mAID strain following 24 h of IAA treatment. Scale bar = 2 μ m. (D) SMC_N1 depletion compromised the integrity of the membrane structure in a subset of maternal parasites. GAP45 was labeled with a rabbit anti-GAP45 antibody (red), and white asterisks denote regions exhibiting GAP45 staining defects. Scale bar = 2 μ m. (E) Quantification of GAP45-associated defects in the RH::TIR1–SMC_N1-mAID strain with or without 24 h of IAA treatment. Data represent mean $\pm$ SD from three biological replicates, and were analyzed using a two-tailed, unpaired *t*-test, and at least 100 PVs were counted and analyzed per sample, **** $p < 0.0001$; n.s., no statistical significance. (F) Nuclear division defects were observed in the SMC_N1-mAID strain after 24 h of IAA treatment. Nuclei were stained with DAPI (blue), and white asterisks indicate nuclear loss or uneven nuclear segregation. Scale bar = 2 μ m. (G) Quantitative data are presented as mean $\pm$ SD from three independent experiments. Statistical differences were assessed using a two-tailed, unpaired *t*-test, and at least 100 PVs were counted and analyzed per sample, *** $p < 0.001$; n.s., no statistical significance.

3.4. SMC_N1 Depletion Causes Centrosome and Apicoplast Defects in Type I RH Strain

The replication of tachyzoites requires strict coordination among subcellular organelles [42,43]. Given that SMC_N1 depletion caused defects in daughter cell division and nuclear segregation, the replication status of centrosomes and apicoplasts was further examined. SMC_N1-mAID tachyzoites treated with IAA for 24 h were stained with the centrosome marker Centrin1. SMC_N1 depletion leads to the loss of centrosomes ($12.33 \pm 0.88\%$) (Figure 4A,B).

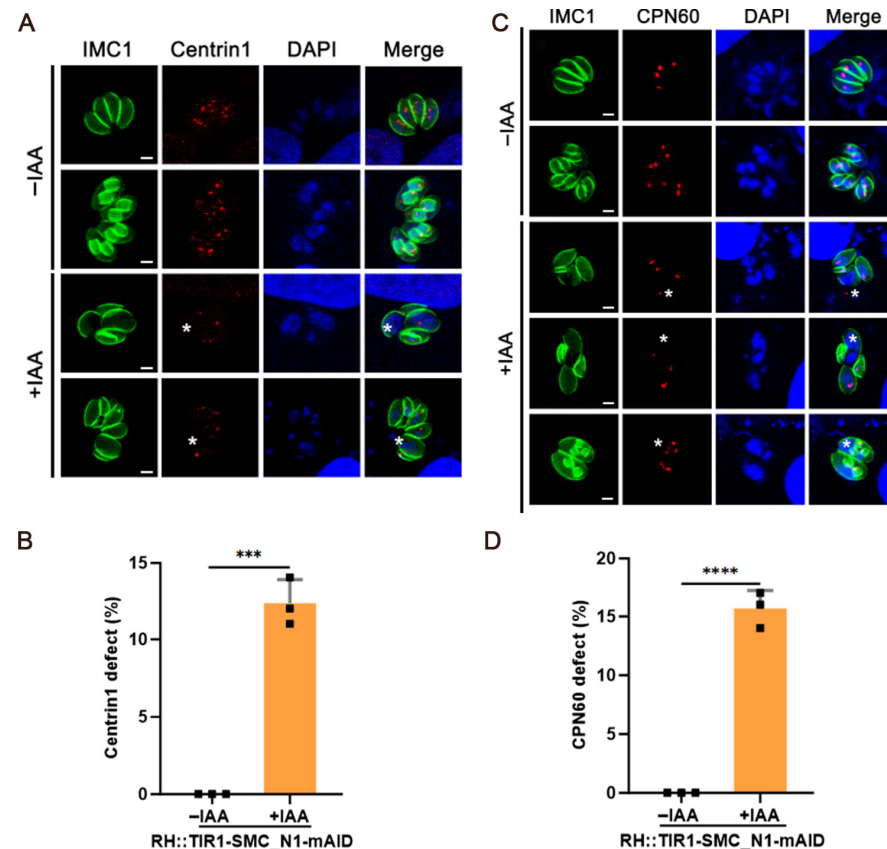


Figure 4. Loss of SMC_N1 impairs proper apicoplast inheritance and causes centrosome loss. (A) Immunofluorescence analysis demonstrated that SMC_N1 depletion for 24 h led to centrosome loss. Parasites were stained with a rabbit anti-IMC1 antibody (green) and a rabbit anti-Centrin1 antibody (red), and white asterisks indicate normally duplicated centrosomes as well as centrosome loss in parasites exhibiting asynchronous budding or endopolygeny. Scale bar = 2 μ m. (B) Quantification of centrosome loss after SMC_N1 depletion. Data are shown as mean $\pm$ SD from three biological replicates, and were analyzed using a two-tailed, unpaired *t*-test, and at least 100 PVs were counted and analyzed per sample, *** *p* < 0.001. (C) Immunofluorescence staining showed that 24 h of IAA treatment in the SMC_N1-mAID strain resulted in apicoplast missegregation, loss and over-replication. Parasites were labeled with a rabbit anti-IMC1 antibody (green) and a rabbit anti-CPN60 antibody (red), and white asterisks denote apicoplast abnormalities. Scale bar = 2 μ m. (D) Quantification of apicoplast inheritance defects following SMC_N1 depletion. Data represent mean $\pm$ SD from three independent experiments, and were analyzed using a two-tailed, unpaired *t*-test, and at least 100 PVs were counted and analyzed per sample, **** *p* < 0.0001.

Given that centrosomes mispositioning disrupts apicoplast-centrosome association, leading to apicoplast loss or failed inheritance and ultimately causing severe growth arrest or parasite death [14]. To assess apicoplast integrity, IAA-treated SMC_N1-mAID tachyzoites were stained with the apicoplast marker CPN60. Consistent with centrosome defects, SMC_N1 depletion caused pronounced apicoplast abnormalities, including aber-

rant replication, loss and mislocalization ($15.67 \pm 0.88\%$) (Figure 4C,D). Collectively, these data indicate that SMC_N1 may slightly influence the stable inheritance of centrosomes and apicoplasts.

3.5. SMC_N1 Depletion Does Not Impair Bradyzoite Differentiation in Type II PRU Strain

To evaluate the function of SMC_N1 in the type II cyst-forming PRU strain, a conditional knockdown line was generated using the mAID system in combination with CRISPR–Cas9, and correct insertion of the mAID-6HA in type II PRU strain was confirmed by PCR1 and PCR2 (Figure S1B). Phenotypic analyses revealed that IAA-treated SMC_N1-mAID parasites failed to form visible plaques, showed no detectable defects in invasion or egress, and exhibited markedly impaired replication capacity, consistent with the phenotype observed in the type I strain (Figure 5A–F).

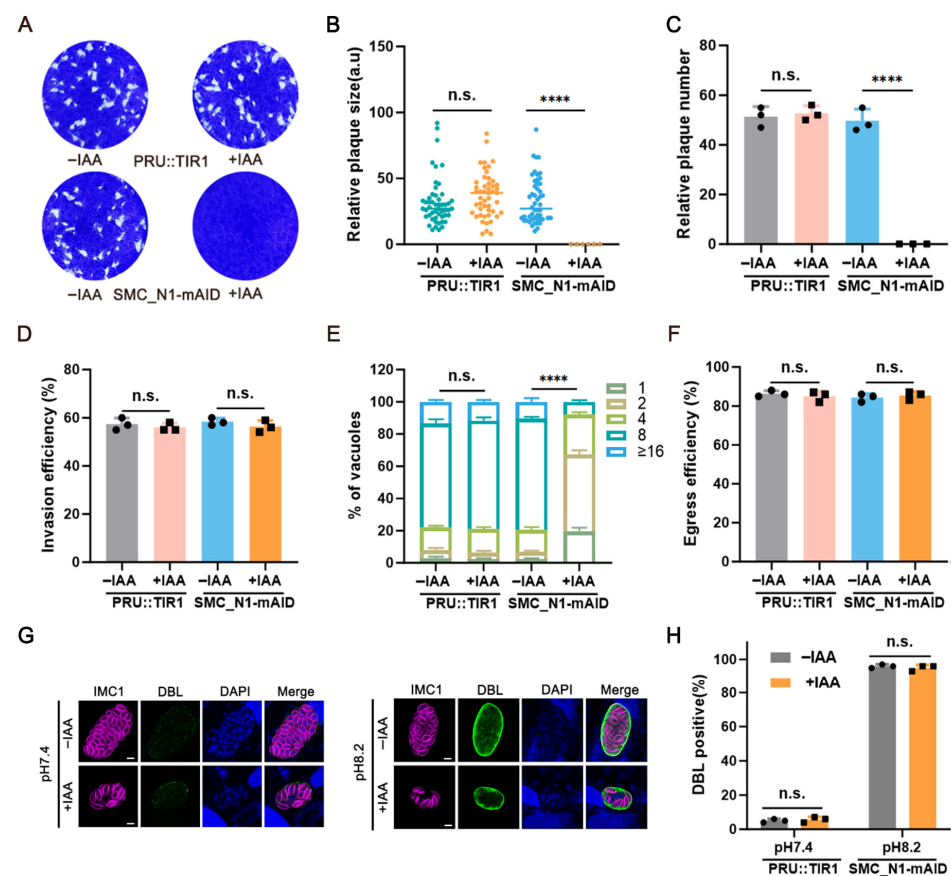


Figure 5. Loss of SMC_N1 further impairs the replication efficiency of PRU tachyzoites but has no effect on their differentiation into bradyzoites. (A) Representative images of plaques formed by PRU strain and SMC_N1-mAID strain with or without 24 h IAA treatment under normal culture conditions for 8 days. The results indicate that the plaque-forming ability of the SMC_N1-deficient strain was almost completely inhibited. (B) The relative plaque size formed by each strain on the host cell monolayer was analyzed using a two-tailed, unpaired *t*-test, **** $p < 0.0001$; n.s., no statistical significance. (C) The number of plaques formed by each strain on the host cell monolayer was analyzed using a two-tailed, unpaired *t*-test, **** $p < 0.0001$; n.s., no statistical significance. (D) After 24 h IAA treatment or no treatment, the indicated strains infected host cells for 30 min. The invasion rate of intracellular tachyzoites relative to total tachyzoites was analyzed using a two-tailed, unpaired *t*-test, and at least 100 PVs were counted and analyzed per sample, n.s., no statistical significance. (E) After the indicated strains infected host cells for 1 h, they were cultured for an additional 28 h in the presence or absence of IAA. Their replication ability was analyzed using two-way ANOVA combined

with Tukey's multiple-comparison test, and at least 100 PVs were counted and analyzed per sample, *** $p < 0.0001$; n.s., no statistical significance. (F) The egress efficiency of each strain was determined 36 h post infection of HFF monolayers, and analyzed using a two-tailed, unpaired t -test, and at least 100 PVs were counted and analyzed per sample, n.s., no statistical significance. All data are presented as the mean $\pm$ standard deviation from three independent experiments. (G) Under normal culture conditions, PRU::TIR1-SMC_N1-mAID strain-infected HFFs were stained with rabbit anti-IMC1 antibody (magenta), DBL (green), and DAPI (blue) at 3 days post infection. Scale bar = 2 μ m. (H) Quantitative analysis of the bradyzoite conversion rate of the PRU::TIR1-SMC_N1-mAID strain with or without 3-day IAA treatment under normal culture conditions. Data are presented as the mean $\pm$ standard deviation from three independent experiments. Statistical differences were analyzed using a two-tailed, unpaired t -test, and at least 100 PVs were counted and analyzed per sample, n.s., no statistical significance.

To determine whether SMC_N1 is required for tachyzoite-to-bradyzoite differentiation, HFF monolayers were infected with IAA-treated or untreated SMC_N1-mAID parasites, and bradyzoite development was induced under standard or alkaline (pH 8.2) conditions. After 3 days, cyst wall formation was assessed by immunofluorescence staining. No significant differences in bradyzoite differentiation efficiency was detected between IAA-treated and untreated SMC_N1-mAID parasites (Figure 5G,H). Collectively, these results indicate that SMC_N1 is dispensable for the tachyzoite-to-bradyzoite conversion process in vitro.

4. Discussion

In this study, we show that the centrocone protein SMC_N1 is essential for *T. gondii* tachyzoite proliferation. Rather than acting as a single-pathway regulator, SMC_N1 functions as a cell cycle-coupled coordinator, as indicated by its S-phase-restricted expression. Its depletion causes broad defects in nuclear division, IMC organization, and organelle inheritance, resulting in severe growth impairment. These findings support a model in which SMC_N1 links genome replication to cytoskeletal and membrane remodeling during endodyogeny.

SMC_N1 contains two conserved domains, PRK12323 and SMC_N. Among these, the PRK12323 domain is associated with the gamma/tau subunits of DNA polymerase III, playing an important role in DNA synthesis and replication fidelity [44]. The SMC_N domain, as the N-terminal domain of RecF/RecN/SMC proteins, is primarily involved in processes such as chromosome structure maintenance, DNA damage repair and homologous recombination [45]. SMC_N1 displays dynamic, periodic expression throughout the tachyzoite cell cycle; its expression peaks during S phase, decreases during daughter cell budding and becomes undetectable in G1 phase. This expression profile closely mirrors that of key cell cycle regulatory proteins. Among the known centrocone-localized proteins, TgCrk5 also shares a similar cell cycle-dependent expression pattern, as a CDK-related kinase, it forms a complex with ECR1 to jointly regulate chromosome replication and mitosis [25]. Notably, while SMC_N1 shares partial functional overlap with other centrocone-localized regulatory factors such as TgCrk5, the depletion phenotype of SMC_N1 indicates that these proteins possess non-redundant functions. They likely act at distinct yet interconnected regulatory nodes during cell division. TgCrk5 primarily functions through kinase-mediated cell cycle control, whereas SMC_N1 probably provides structural or scaffolding support for chromosome dynamics. Thus, SMC_N1 may ensure the spatial organization required for precise signaling during nuclear division and daughter cell assembly.

To investigate the biological role of SMC_N1, we generated conditional knockout lines in both the type I RH and type II PRU strains using the mAID system in combination with CRISPR-Cas9. Phenotypic characterization demonstrated that depletion of SMC_N1

resulted in severe defects in tachyzoite proliferation, intracellular replication and IMC architecture. During endodyogeny, parasites assemble daughter cells through a highly ordered sequence of membrane remodeling events [46]. As a major IMC-associated component, the disrupted IMC1 staining observed in parasites with SMC_N1 depletion indicates that IMC disruption is a downstream consequence of nuclear-cytoskeleton uncoupling, highlighting that SMC_N1 is essential for preserving the structural integrity and organizational order of the IMC [47,48]. In addition, the IMC provides the platform for anchoring the actin–myosin motor complex, which drives parasite motility and host cell invasion. This molecular machinery consists of the unconventional myosin MyoA, its regulatory light chains (MLC1, ELC1), and glideosome-associated proteins (GAP40, GAP45, GAP50) [49,50]. The compromised GAP45 signal observed in SMC_N1-deficient parasites further supports the notion that loss of SMC_N1 destabilizes the coordination between membrane scaffolds and motor complexes, thereby impairing the mechanical linkage between the IMC and the plasma membrane and ultimately undermining the mechanical integrity required for proper daughter cell assembly. Future studies are required to elucidate the precise molecular mechanism underlying the regulatory role of SMC_N1 in IMC–plasma membrane linkage.

As a core regulatory hub for nuclear division and budding in *T. gondii*, the centrosome, with its bipartite structure, is key to enabling efficient multinuclear replication and rapid cell division in apicomplexan parasites [51,52]. Previous studies have demonstrated that the physical connection between the centrosome and centrocone, as well as microtubule-mediated signal transduction, is required for the accurate segregation of nuclear materials. Furthermore, the differential regulation of distinct microtubule populations can uncouple the processes of nuclear division and budding [53,54]. While depletion of SMC_N1 leads to reduced parasite proliferation and abnormal nuclear division, the observed lower proportion of centrosome defects in vacuoles in the present study indicates that loss of SMC_N1 did not completely abrogate centrosome function. Furthermore, as an essential organelle of *T. gondii*, the apicoplast is involved in the synthesis of key substances such as fatty acids and isoprenoids, and disruption of its structure or inheritance can lead to severe metabolic dysfunction [12]. However, the present study reveals that only mild defects in apicoplast inheritance were observed in SMC_N1-deficient parasites. Thus, the marked reduction in tachyzoite replication capacity is more likely a consequence of other cellular disorganization rather than a primary defect in apicoplast segregation itself.

In summary, this study reveals that SMC_N1 is an important regulatory factor in the endodyogeny of *T. gondii* tachyzoites. By coordinating the assembly of membrane structures, synchronizing nuclear division, and ensuring stable inheritance of essential organelles, SMC_N1 supports efficient tachyzoite proliferation. Notably, SMC_N1 shows no obvious role in bradyzoite differentiation, highlighting its function in rapid tachyzoite-specific replication. However, bradyzoites within cysts exhibit hollow morphological defects, likely arising from reduced replication capacity. While DBL staining showed no significant difference, these observations suggest that SMC_N1 may have a subtle influence on cyst morphology or maintenance, and examining cyst reactivation may reveal whether tachyzoite replication defects also affect bradyzoite infectivity. Its unique expression pattern, centrocone localization, and domain architecture not only expand our understanding of *T. gondii* cell division regulation but also highlight SMC_N1 as a potential target for anti-*Toxoplasma* therapies. Although this study reveals that SMC_N1 is a multifunctional regulator of *T. gondii* proliferation, its interaction with cell cycle and endodyogeny regulatory proteins, as well as the specific regulatory pathways underlying tachyzoite proliferation, remain to be further elucidated.

5. Conclusions

This study clearly identifies the centrocone protein SMC_N1 as an important regulatory factor for the proliferation of *T. gondii* tachyzoites. Containing conserved PRK12323 and SMC_N domains, this protein exhibits cell cycle-dependent expression, with peak levels during the S phase, and is essential for parasite survival. Analysis of the conditional knockdown strain constructed via the CRISPR-Cas9 system combined with the mAID system reveals that depletion of SMC_N1 does not affect tachyzoite invasion or egress, but disrupts IMC assembly, nuclear division synchronization, and the stable inheritance of centrosomes and apicoplasts. This severely impairs intracellular replication in both type I RH and type II PRU strains. Notably, depletion of SMC_N1 has no significant impact on the differentiation of PRU strain tachyzoites into bradyzoites, indicating that its functional specificity is concentrated in the acute infection stage. These results demonstrate that SMC_N1 ensures the efficient proliferation of *T. gondii* tachyzoites by coordinating membrane structure assembly, nuclear division, and organelle inheritance. These findings enrich our understanding of the regulatory network governing *T. gondii* cell division and provide a potential target for the development of anti-*Toxoplasma* drugs targeting the acute proliferation of the parasite. Future studies are required to further explore its interaction mechanisms with other cell cycle regulatory proteins.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ani16040653/s1>. Figure S1: Successful construction of epitope tagging strains (RH::TIR1-SMC_N1-mAID and PRU::TIR1-SMC_N1-mAID) was verified by PCRs.

Author Contributions: Conceptualization, X.-Q.Z., J.G. and C.L.; Formal analysis, C.L., J.G., X.-J.W. and S.-C.X.; Funding acquisition, X.-Q.Z.; Investigation, C.L., J.G., X.-J.W., S.-C.X. and H.-S.Z.; Methodology, J.G., X.-J.W. and S.-C.X.; Project administration, X.-Q.Z.; Resources, X.-Q.Z.; Supervision, J.G. and X.-Q.Z.; Writing—original draft, C.L. and J.G.; Writing—review and editing, J.G., C.L. and X.-Q.Z.; All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the NSFC-Yunnan Joint Fund (Grant No. U2202201), the Research Fund of Shanxi Province for Introduced High-level Leading Talents (Grant No. RFSX-IHLT202101), and the Special Research Fund of Shanxi Agricultural University for High-level Talents (Grant No. 2021XG001). The funders had no role in the study design, data analysis, data interpretation, and the writing of this manuscript. All authors have full access to the study data and take responsibility for the decision to submit the manuscript for publication.

Institutional Review Board Statement: This study was approved by the Institutional Animal Care and Use Committee of Shanxi Agricultural University (Approval No.: SXAU-EAW-2023ME8.ZZ.012016327). The study was conducted in accordance with local legislation and institutional ethical regulations.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data generated or analyzed during this study are included in this published article and its Supplementary Materials. For additional information, please contact the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Smith, N.C.; Goulart, C.; Hayward, J.A.; Kupz, A.; Miller, C.M.; van Dooren, G.G. Control of human toxoplasmosis. *Int. J. Parasitol.* **2021**, *51*, 95–121. [\[CrossRef\]](#)
- Liu, S.; Wu, M.; Hua, Q.; Lu, D.; Tian, Y.; Yu, H.; Cheng, L.; Chen, Y.; Cao, J.; Hu, X.; et al. Two old drugs, NVP-AEW541 and GSK-J4, repurposed against the *Toxoplasma gondii* RH strain. *Parasit. Vectors* **2020**, *13*, 242. [\[CrossRef\]](#) [\[PubMed\]](#)
- Prandovszky, E.; Severance, E.G.; Splan, V.W.; Liu, H.; Xiao, J.; Yolken, R.H. *Toxoplasma*-induced behavior changes—Is microbial dysbiosis the missing link? *Front. Cell Infect. Microbiol.* **2024**, *14*, 1415079. [\[CrossRef\]](#) [\[PubMed\]](#)
- Elsheikha, H.M.; Marra, C.M.; Zhu, X.Q. Epidemiology, pathophysiology, diagnosis, and management of cerebral toxoplasmosis. *Clin. Microbiol. Rev.* **2021**, *34*, e00115–19. [\[CrossRef\]](#) [\[PubMed\]](#)
- Nayeri, T.; Sarvi, S.; Daryani, A. Effective factors in the pathogenesis of *Toxoplasma gondii*. *Heliyon* **2024**, *10*, e31558. [\[CrossRef\]](#)
- Dunay, I.R.; Gajurel, K.; Dhakal, R.; Liesenfeld, O.; Montoya, J.G. Treatment of toxoplasmosis: Historical perspective, animal models, and current clinical practice. *Clin. Microbiol. Rev.* **2018**, *31*, e00057–17. [\[CrossRef\]](#)
- Francia, M.E.; Striepen, B. Cell division in apicomplexan parasites. *Nat. Rev. Microbiol.* **2014**, *12*, 125–136. [\[CrossRef\]](#)
- Yoneda, S.; Castellani, B.R.; Uemura, K.; Radtke, B.M. Autoradiographic study on the pyrimidine metabolism of *Toxoplasma gondii*, in its extracellular forms. *Rev. Bras. Biol.* **1979**, *39*, 653–655.
- Zhang, S.; Wang, F.; Zhang, D.; Liu, D.; Ding, W.; Springer, T.A.; Song, G. Structural insights into MIC2 recognition by MIC2-associated protein in *Toxoplasma gondii*. *Commun. Biol.* **2023**, *6*, 895. [\[CrossRef\]](#)
- Fukumoto, J.; Sakura, T.; Matsubara, R.; Tahara, M.; Matsuzaki, M.; Nagamune, K. Rhoptry kinase protein 39 (ROP39) is a novel factor that recruits host mitochondria to the parasitophorous vacuole of *Toxoplasma gondii*. *Biol. Open* **2021**, *10*, bio058988. [\[CrossRef\]](#)
- Wang, X.; Qu, L.; Chen, J.; Jin, Y.; Hu, K.; Zhou, Z.; Zhang, J.; An, Y.; Zheng, J. *Toxoplasma* rhoptry proteins that affect encephalitis outcome. *Cell Death Discov.* **2023**, *9*, 439. [\[CrossRef\]](#) [\[PubMed\]](#)
- Charron, A.J.; Sibley, L.D. Host cells: Mobilizable lipid resources for the intracellular parasite *Toxoplasma gondii*. *J. Cell Sci.* **2002**, *115*, 3049–3059. [\[CrossRef\]](#) [\[PubMed\]](#)
- Anderson-White, B.; Beck, J.R.; Chen, C.T.; Meissner, M.; Bradley, P.J.; Gubbels, M.J. Cytoskeleton assembly in *Toxoplasma gondii* cell division. *Int. Rev. Cell Mol. Biol.* **2012**, *298*, 1–31. [\[PubMed\]](#)
- Krueger, A.; Horjales, S.; Yang, C.; Blakely, W.J.; Francia, M.E.; Arrizabalaga, G. The essential kinase TgGSK regulates centrosome segregation and endodyogeny in *Toxoplasma gondii*. *mSphere* **2025**, *10*, e0011125. [\[CrossRef\]](#)
- Devarakonda, P.M.; Sarmiento, V.; Heaslip, A.T. F-actin and myosin F control apicoplast elongation dynamics which drive apicoplast-centrosome association in *Toxoplasma gondii*. *mBio* **2023**, *14*, e0164023. [\[CrossRef\]](#)
- Delbac, F.; Sanger, A.; Neuhaus, E.M.; Stratmann, R.; Ajioka, J.W.; Toursel, C.; Herm-Gotz, A.; Tomavo, S.; Soldati, T.; Soldati, D. *Toxoplasma gondii* myosins B/C: One gene, two tails, two localizations, and a role in parasite division. *J. Cell Biol.* **2001**, *155*, 613–623. [\[CrossRef\]](#)
- White, M.W.; Suvorova, E.S. Apicomplexa cell cycles: Something old, borrowed, lost, and new. *Trends Parasitol.* **2018**, *34*, 759–771. [\[CrossRef\]](#)
- Alvarez, C.A.; Suvorova, E.S. Checkpoints of apicomplexan cell division identified in *Toxoplasma gondii*. *PLoS Pathog.* **2017**, *13*, e1006483. [\[CrossRef\]](#)
- Hu, X.; O’Shaughnessy, W.J.; Beraki, T.G.; Reese, M.L. Loss of the conserved alveolate kinase MAPK2 decouples *Toxoplasma* cell growth from cell division. *mBio* **2020**, *11*, e02517–20. [\[CrossRef\]](#)
- Tomasina, R.; Gonzalez, F.C.; Francia, M.E. Structural and functional insights into the microtubule organizing centers of *Toxoplasma gondii* and *Plasmodium* spp. *Microorganisms* **2021**, *9*, 2503. [\[CrossRef\]](#)
- Hawkins, L.M.; Naumov, A.V.; Batra, M.; Wang, C.; Chaput, D.; Suvorova, E.S. Novel CRK-Cyclin complex controls spindle assembly checkpoint in *Toxoplasma* endodyogeny. *mBio* **2021**, *13*, e0356121. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sinai, A.P.; Suvorova, E.S. The RESTRICTION checkpoint: A window of opportunity governing developmental transitions in *Toxoplasma gondii*. *Curr. Opin. Microbiol.* **2020**, *58*, 99–105. [\[CrossRef\]](#) [\[PubMed\]](#)
- Chen, C.T.; Gubbels, M.J. The *Toxoplasma gondii* centrosome is the platform for internal daughter budding as revealed by a Nek1 kinase mutant. *J. Cell Sci.* **2013**, *126*, 3344–3355. [\[CrossRef\]](#) [\[PubMed\]](#)
- Reininger, L.; Wilkes, J.M.; Bourgade, H.; Miranda-Saavedra, D.; Doerig, C. An essential aurora-related kinase transiently associates with spindle pole bodies during *Plasmodium falciparum* erythrocytic schizogony. *Mol. Microbiol.* **2011**, *79*, 205–221. [\[CrossRef\]](#)
- Naumov, A.; Kratzer, S.; Ting, L.M.; Kim, K.; Suvorova, E.S.; White, M.W. The *Toxoplasma* centrocone houses cell cycle regulatory factors. *mBio* **2017**, *8*, e00579–17. [\[CrossRef\]](#)
- Butler, C.L.; Lucas, O.; Wuchty, S.; Xue, B.; Uversky, V.N.; White, M. Identifying novel cell cycle proteins in Apicomplexa parasites through co-expression decision analysis. *PLoS ONE* **2014**, *9*, e97625. [\[CrossRef\]](#)
- Engelberg, K.; Bechtel, T.; Michaud, C.; Weerapana, E.; Gubbels, M.J. Proteomic characterization of the *Toxoplasma gondii* cytokinesis machinery portrays an expanded hierarchy of its assembly and function. *Nat. Commun.* **2022**, *13*, 4644. [\[CrossRef\]](#)

28. Wang, J.L.; Li, T.T.; Elsheikha, H.M.; Liang, Q.L.; Zhang, Z.W.; Wang, M.; Sibley, L.D.; Zhu, X.Q. The protein phosphatase 2A holoenzyme is a key regulator of starch metabolism and bradyzoite differentiation in *Toxoplasma gondii*. *Nat. Commun.* **2022**, *13*, 7560. [\[CrossRef\]](#)
29. Brown, K.M.; Long, S.; Sibley, L.D. Conditional knockdown of proteins using auxin-inducible degron (AID) fusions in *Toxoplasma gondii*. *Bio Protoc.* **2018**, *8*, e2728. [\[CrossRef\]](#)
30. Li, D.; Zhang, Y.; Li, S.; Zheng, B. A novel *Toxoplasma gondii* TGGT1_316290 mRNA-LNP vaccine elicits protective immune response against toxoplasmosis in mice. *Front. Microbiol.* **2023**, *14*, 1145114. [\[CrossRef\]](#)
31. Yang, C.; Broncel, M.; Dominicus, C.; Sampson, E.; Blakely, W.J.; Treeck, M.; Arrizabalaga, G. A plasma membrane localized protein phosphatase in *Toxoplasma gondii*, PPM5C, regulates attachment to host cells. *Sci. Rep.* **2019**, *9*, 5924. [\[CrossRef\]](#)
32. Schneider, C.A.; Rasband, W.S.; Eliceiri, K.W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671–675. [\[CrossRef\]](#)
33. Zheng, X.N.; Sun, L.X.; Elsheikha, H.M.; Li, T.T.; Gao, J.; Wu, X.J.; Zhang, Z.W.; Wang, M.; Fu, B.Q.; Zhu, X.Q.; et al. A newly characterized dense granule protein (GRA76) is important for the growth and virulence of *Toxoplasma gondii*. *Int. J. Parasitol.* **2024**, *54*, 109–121. [\[CrossRef\]](#)
34. Wu, X.J.; Wang, M.; Zhang, N.Z.; Li, T.T.; Gao, J.; Sun, L.X.; Zhu, X.Q.; Wang, J.L. AP2XII-9 is essential for parasite growth and suppresses bradyzoite differentiation in *Toxoplasma gondii*. *FASEB J.* **2025**, *39*, e70476. [\[CrossRef\]](#)
35. Dosemeci, A.; Loo, H.K.; Toy, D.; Winters, C.A.; Reese, T.S.; Tao-Cheng, J.H. FAM81A protein, a novel component of the postsynaptic density in adult brain. *Neurosci. Lett.* **2019**, *699*, 122–126. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Yatskevich, S.; Rhodes, J.; Nasmyth, K. Organization of chromosomal DNA by SMC Complexes. *Annu. Rev. Genet.* **2019**, *53*, 445–482. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Löwe, J.; Cordell, S.C.; van den Ent, F. Crystal structure of the SMC head domain: An ABC ATPase with 900 residues antiparallel coiled-coil inserted. *J. Mol. Biol.* **2001**, *306*, 25–35. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Sidik, S.M.; Huet, D.; Ganesan, S.M.; Huynh, M.H.; Wang, T.; Nasamu, A.S.; Thiru, P.; Saeij, J.P.J.; Carruthers, V.B.; Niles, J.C.; et al. A genome-wide CRISPR screen in *Toxoplasma* identifies essential apicomplexan genes. *Cell* **2016**, *166*, 1423–1435.e12. [\[CrossRef\]](#)
39. Tonkin, M.L.; Beck, J.R.; Bradley, P.J.; Boulanger, M.J. The inner membrane complex sub-compartment proteins critical for replication of the apicomplexan parasite *Toxoplasma gondii* adopt a pleckstrin homology fold. *J. Biol. Chem.* **2014**, *289*, 13962–13973. [\[CrossRef\]](#)
40. Beck, J.R.; Rodriguez-Fernandez, I.A.; de Leon, J.C.; Huynh, M.H.; Carruthers, V.B.; Morrisette, N.S.; Bradley, P.J. A novel family of *Toxoplasma* IMC proteins displays a hierarchical organization and functions in coordinating parasite division. *PLoS Pathog.* **2010**, *6*, e1001094. [\[CrossRef\]](#)
41. Frénal, K.; Polonais, V.; Marq, J.B.; Stratmann, R.; Limenitakis, J.; Soldati-Favre, D. Functional dissection of the apicomplexan glideosome molecular architecture. *Cell Host Microbe* **2010**, *8*, 343–357. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Nishi, M.; Hu, K.; Murray, J.M.; Roos, D.S. Organellar dynamics during the cell cycle of *Toxoplasma gondii*. *J. Cell Sci.* **2008**, *121*, 1559–1568. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Behnke, M.S.; Wootton, J.C.; Lehmann, M.M.; Radke, J.B.; Lucas, O.; Nawas, J.; Sibley, L.D.; White, M.W. Coordinated progression through two subtranscriptomes underlies the tachyzoite cycle of *Toxoplasma gondii*. *PLoS ONE* **2010**, *5*, e12354. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Walker, J.R.; Hervas, C.; Ross, J.D.; Blinkova, A.; Walbridge, M.J.; Pumarega, E.J.; Park, M.O.; Neely, H.R. Escherichia coli DNA polymerase III tau- and gamma-subunit conserved residues required for activity in vivo and in vitro. *J. Bacteriol.* **2000**, *182*, 6106–6113. [\[CrossRef\]](#)
45. Bickel, J.S.; Chen, L.; Hayward, J.; Yeap, S.L.; Alkers, A.E.; Chan, R.C. Structural maintenance of chromosomes (SMC) proteins promote homolog-independent recombination repair in meiosis crucial for germ cell genomic stability. *PLoS Genet.* **2010**, *6*, e1001028. [\[CrossRef\]](#)
46. Ouologuem, D.T.; Roos, D.S. Dynamics of the *Toxoplasma gondii* inner membrane complex. *J. Cell Sci.* **2014**, *127*, 3320–3330.
47. Hu, K.; Johnson, J.; Florens, L.; Fraunholz, M.; Suravajjala, S.; DiLullo, C.; Yates, J.; Roos, D.S.; Murray, J.M. Cytoskeletal components of an invasion machine—the apical complex of *Toxoplasma gondii*. *PLoS Pathog.* **2006**, *2*, e13. [\[CrossRef\]](#)
48. Sun, M.; Tang, T.; He, K.; Long, S. TBC9, an essential TBC-domain protein, regulates early vesicular transport and IMC formation in *Toxoplasma gondii*. *Commun. Biol.* **2024**, *7*, 596. [\[CrossRef\]](#)
49. Egarter, S.; Andenmatten, N.; Jackson, A.J.; Whitelaw, J.A.; Pall, G.; Black, J.A.; Ferguson, D.J.; Tardieux, I.; Mogilner, A.; Meissner, M. The *Toxoplasma* Acto-MyoA motor complex is important but not essential for gliding motility and host cell invasion. *PLoS ONE* **2014**, *9*, e91819. [\[CrossRef\]](#)
50. Williams, M.J.; Alonso, H.; Enciso, M.; Egarter, S.; Sheiner, L.; Meissner, M.; Striepen, B.; Smith, B.J.; Tonkin, C.J. Two essential light chains regulate the MyoA lever arm to promote *Toxoplasma* gliding motility. *mBio* **2015**, *6*, e00845–15. [\[CrossRef\]](#)
51. Suvorova, E.S.; Francia, M.; Striepen, B.; White, M.W. A novel bipartite centrosome coordinates the apicomplexan cell cycle. *PLoS Biol.* **2015**, *13*, e1002093. [\[CrossRef\]](#)

52. Chen, C.T.; Gubbels, M.J. Apicomplexan cell cycle flexibility: Centrosome controls the clutch. *Trends Parasitol.* **2015**, *31*, 229–230. [[CrossRef](#)]
53. Brooks, C.F.; Francia, M.E.; Gissot, M.; Croken, M.M.; Kim, K.; Striepen, B. *Toxoplasma gondii* sequesters centromeres to a specific nuclear region throughout the cell cycle. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 3767–3772. [[CrossRef](#)]
54. Tomasina, R.; Gonzalez, F.C.; Martins-Duarte, É.S.; Bastin, P.; Gissot, M.; Francia, M.E. Separate to operate: The centriole-free inner core of the centrosome regulates the assembly of the intranuclear spindle in *Toxoplasma gondii*. *mBio* **2022**, *13*, e0185922. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.