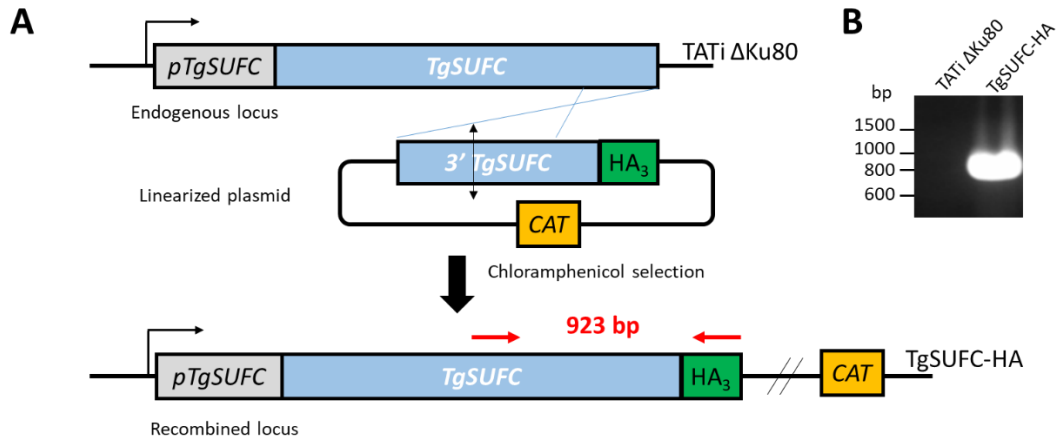

Figure S1. Generation of an HA-tagged TgSUFC cell line. A) Schematic representation of the strategy for expressing HA-tagged versions of TgSUFC by homologous recombination at the native *TgSUFC* locus. Chloramphenicol was used to select transgenic parasites based on their expression of the Chloramphenicol acetyltransferase (CAT). B) Diagnostic PCR for verifying correct integration of the construct. The amplified fragments correspond to the red arrows in A), and specific primers used were ML3984/ML1476.

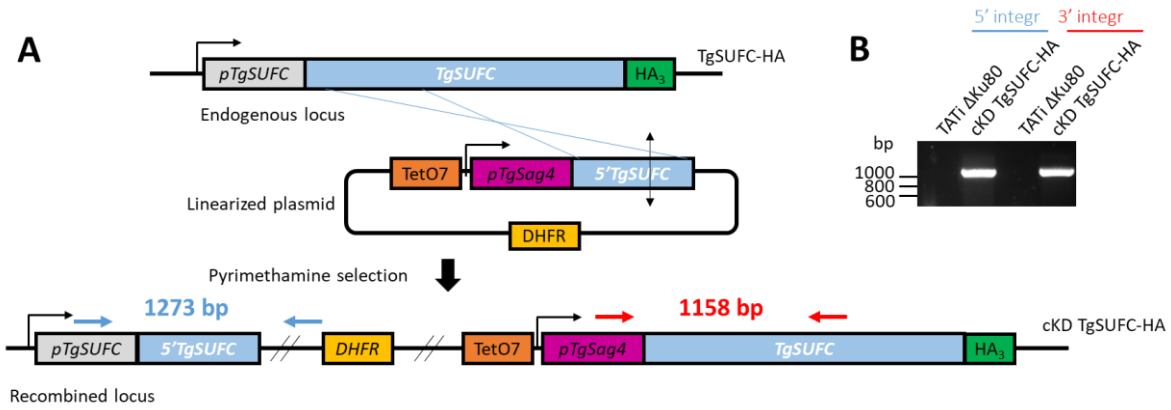

Figure S2. Generation of a TgSUFC conditional mutant. A) Schematic representation of the strategy for generating a TgSUFC conditional knock-down cell line by homologous recombination at the native locus. Pyrimethamine was used to select transgenic parasites based on their expression of Dihydrofolate reductase (DHFR). B) Diagnostic PCR for verifying correct integration of the construct. The amplified fragments confirming 5' and 3' integration correspond to the blue and red arrows displayed in A), respectively, and specific primers used were: ML4111/ML687 (5' integration), and ML1041/ML4112 (3' integration).

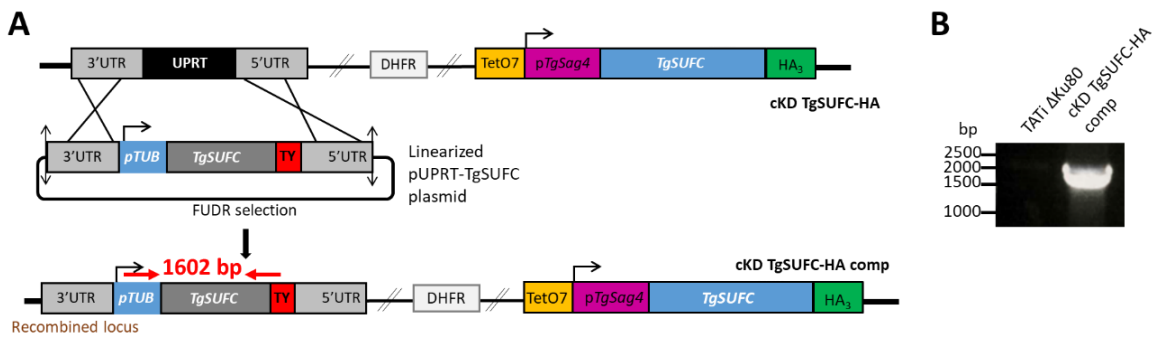

Figure S3. Generation of a TgSUFC complemented cell line. A) Schematic representation of the strategy for generating a TgSUFC complemented cell line by integrating an extra copy of the gene of interest by double homologous recombination at the *Uracil Phosphoribosyltransferase* (*UPRT*) locus. Negative selection with 5-fluorodeoxyuridine (FUDR) was used to select transgenic parasites based on their absence of UPRT expression. B) Diagnostic PCR for verifying correct integration of the construct. The amplified fragment confirming integration correspond to the red arrows displayed in A), and specific primers used were ML801/ML4816.

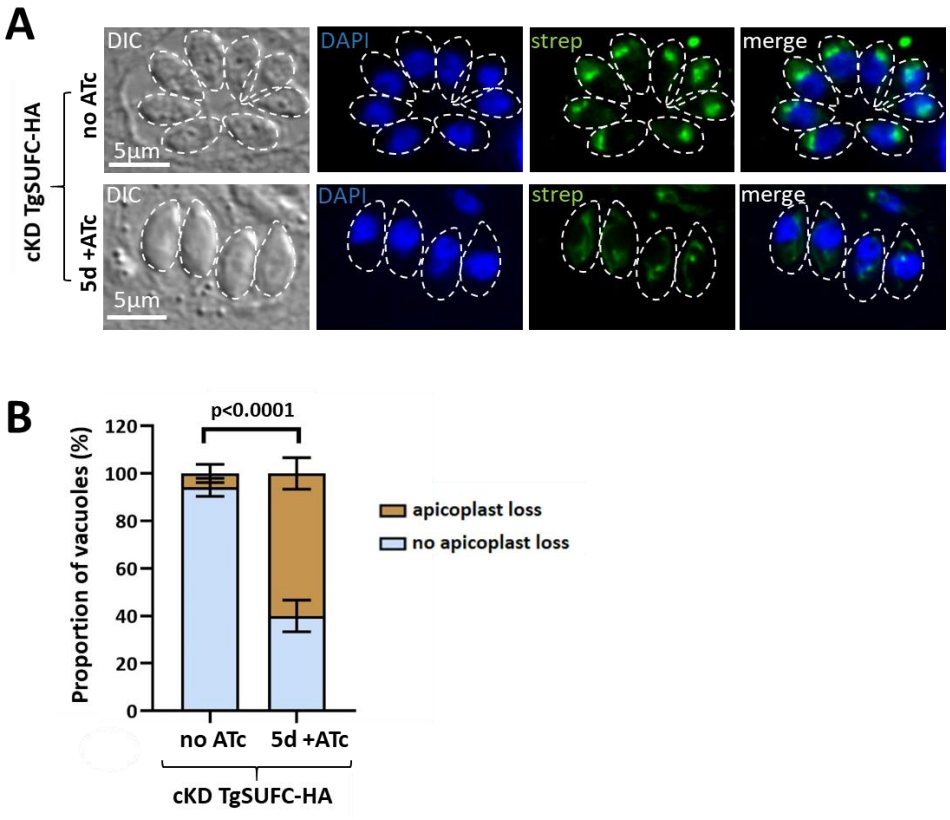

Figure S4. Apicoplast loss in upon depletion of TgSUFC monitored with streptavidin. A) Typical streptavidin-Fluorescein Isothiocyanate labeling of the apicoplast (green) vacuoles containing cKD TgSUFC-HA parasites (outlined) grown in the presence or absence of ATc for 5 days; DNA was stained with DAPI (blue); DIC: differential interference contrast. B) Percentage of cKD TgSUFC-HA parasites-containing vacuoles displaying at least a partial loss of apicoplast signal when labelled with streptavidin-Fluorescein Isothiocyanate after culture in the presence or absence of ATc for 5 days. Data are mean values from *n* = 3 independent experiments ±SD. *p*-value from two-tailed Student's *t*-test is indicated.

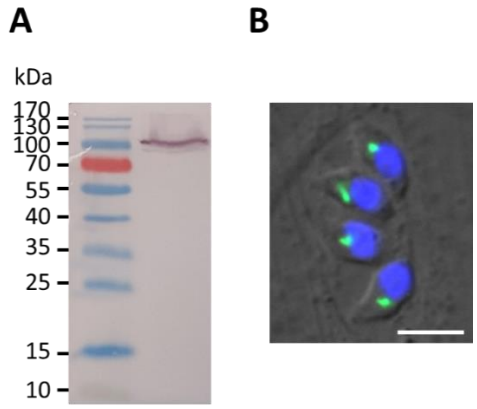

Figure S5. Specificity of the antibody raised in rabbit against the E2 subunit of the pyruvate dehydrogenase. A) Immunoblot analysis of total protein extracts from TATi ΔKu80 tachyzoites using the anti-PDH E2-specific peptide reveals a single main product at around the expected molecular mass (97 kDa). B) Immunofluorescence analysis shows the antibody specifically labels the apicoplast. Displayed is a merged image with anti-PDH E2 (green) together with DAPI-stained DNA (blue) and differential interference contrast (grey). Scale bar is 5 µm.

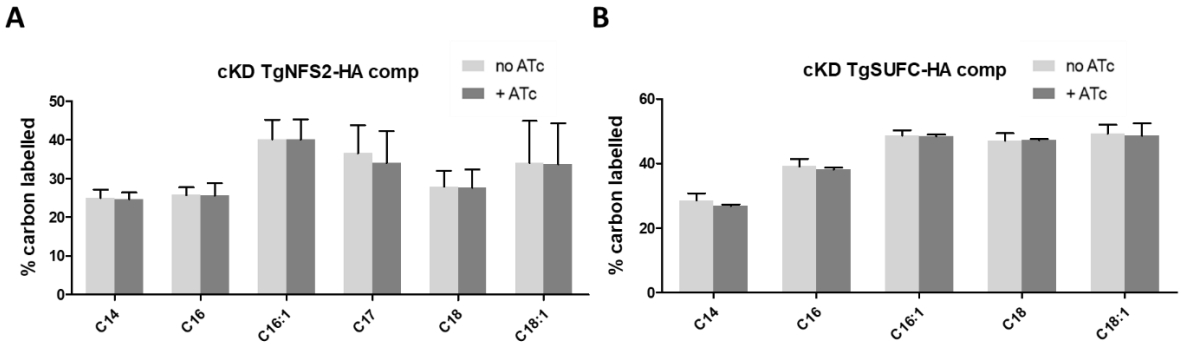

Figure S6. No major change in host-derived FA uptake in the complemented SUF mutant cell lines. Host-scavenged lipid flux analyses by stable isotope labeling combined to gas chromatography/mass spectrometry analyses on the complemented TgNFS2-HA (A) and TgSUFC-HA mutant cell lines (B). Data are mean from *n*=3 independent experiments ±SD.

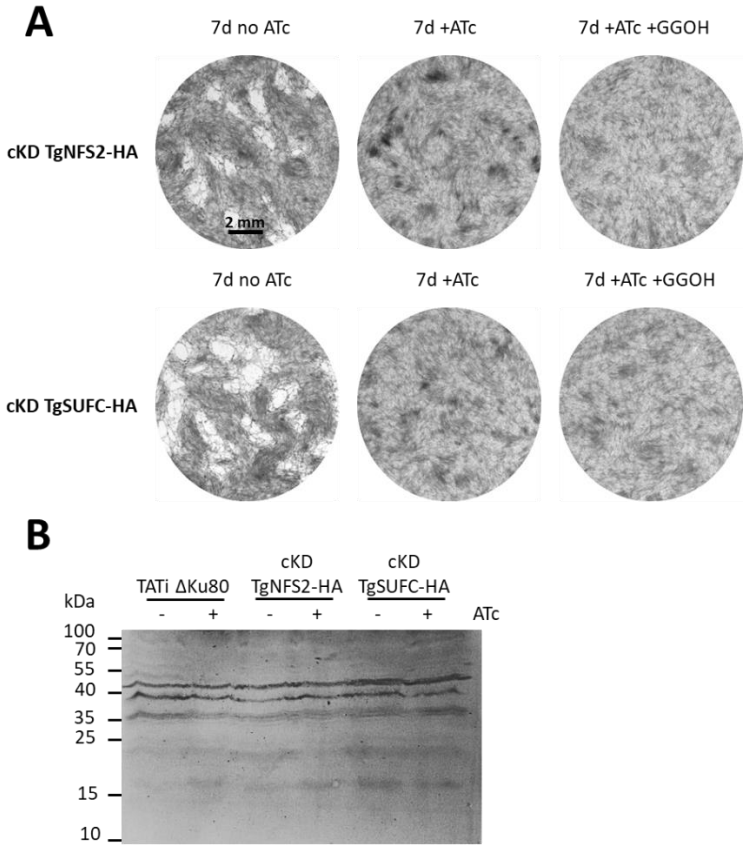

Figure S7. Depletion of either TgNFS2 or TgSUFC has no strong visible impact on protein farnesylation or prenylation. A) Plaque assays were performed in the presence or absence of ATc or of 20 µM geranylgeraniol (GGOH). GGOH supplementation did not restore parasite growth in parasites depleted of TgNFS2 or TgSUFC. B) Depletion of TgNFS2 or TgSUFC upon incubation of cKD parasites with ATc for three days has no obvious impact on the global *T. gondii* prenylation profile, as analyzed by mmunoblot probed with an anti-farnesyl antibody. Specific prenylated species are currently unidentified in *T. gondii*.

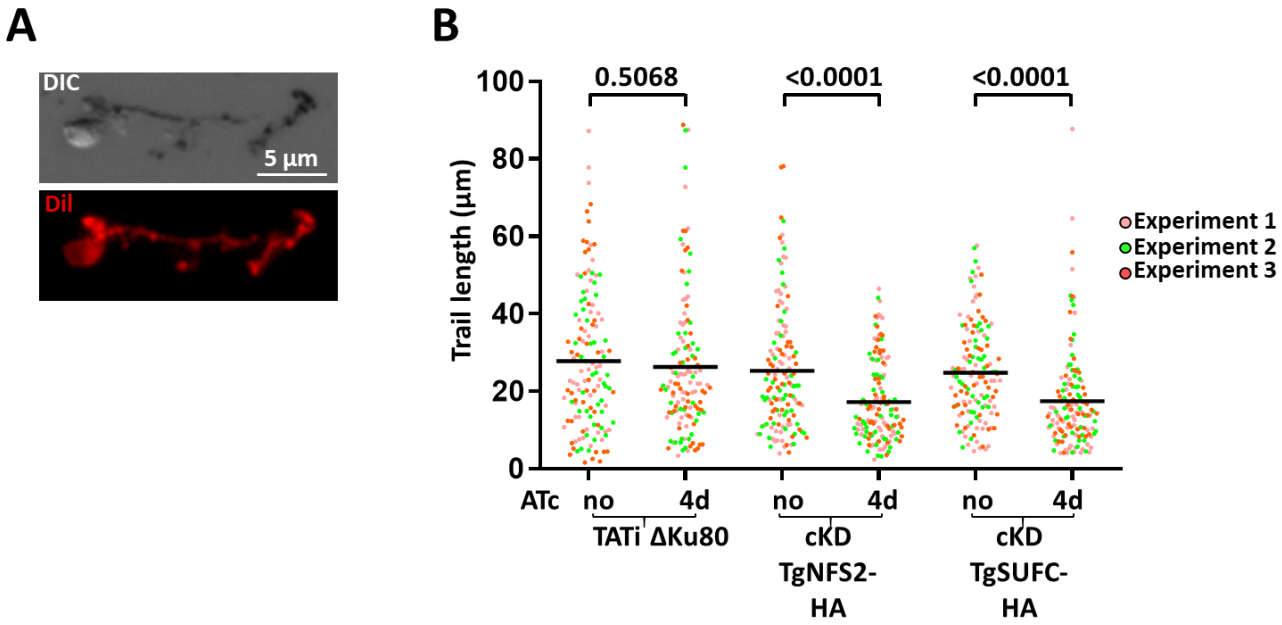

Figure S8. Trails measured by assessing lipid deposition confirm that depletion of TgNFS2 or TgSUFC impacts gliding motility. A) The lipophilic membrane stain DiI can be used to label trails deposited by gliding motility of tachyzoites. DIC: differential interference contrast. B) Individual measurements of DiI trail lengths. Horizontal lines represent mean values from *n* = 3 independent experiments. At least 50 trails were measured for each dataset. Two-tailed Student's *t*-test p-values are indicated.

Primer name	Primer sequence
ML687	GTTTGAATGCAAGGTTTCGTGCTGTCG
ML801	GAAGACATCCACCAAACGG
ML1041	CGGATCATTTGAAAACATCGTGAGGCTGG
ML1476	CAGCGTAGTCCGGGACGTCGTAC
ML2087	AAGTGCTCCCACGTCCCTCACCAT
ML2088	AAAAATGGTGAGGGACGTGGGAGC
ML3445	AAGTCAGGGCTTCTAAAATGGCGC
ML3446	AAAAGCGCCATTTTAGAAGCCCTG
ML3952	AAGTGTTGAGGTACGATGTGAACAT
ML3953	AAAAATGTTCACATCGTACCTCAAC
ML3980	GCGGCAGACGCAATCTAGACCGCCTGCTCTACCCGTACGACGTCC
ML3981	GGTTTGGCATGAAATTACTGTTACACCGCTCTAGAACTAGTGGATCCCC
ML3984	CGATCTGAGTGCACAGCGAGCC
ML4010	GCCGGATCCTTAGAGCAGGCGGTCTAG
ML4107	TCTGTCTTCCGCGAACCTGAGTTCCGCTTGAAGCTTCGCCAGGCTGTA
ML4108	CTCAACAAGGAACACTGGTAGCGACGCCATAGATCTGGTTGAAGACAGAC
ML4109	AAGTGTGCCACGCCTTCTCATCAAG
ML4110	AAAACTTGATGAGAAGGCGTGGCAC
ML4111	GTCGGACACATCGCACACAGCG
ML4112	TCTGTCTGTGTTTCGTTAGAGCC
ML4200	GCCAAGCTTCATGCCGCTCCTCGAGATCAAAG
ML4815	TTTAGATCTATGGCGTCGCTACCAGTG
ML4816	TTTCCTAGGGAGCAGGCGGTCTAGATTG
ML4816	TTTCCTAGGGAGCAGGCGGTCTAGATTG

Table S1. Primers used in this study.