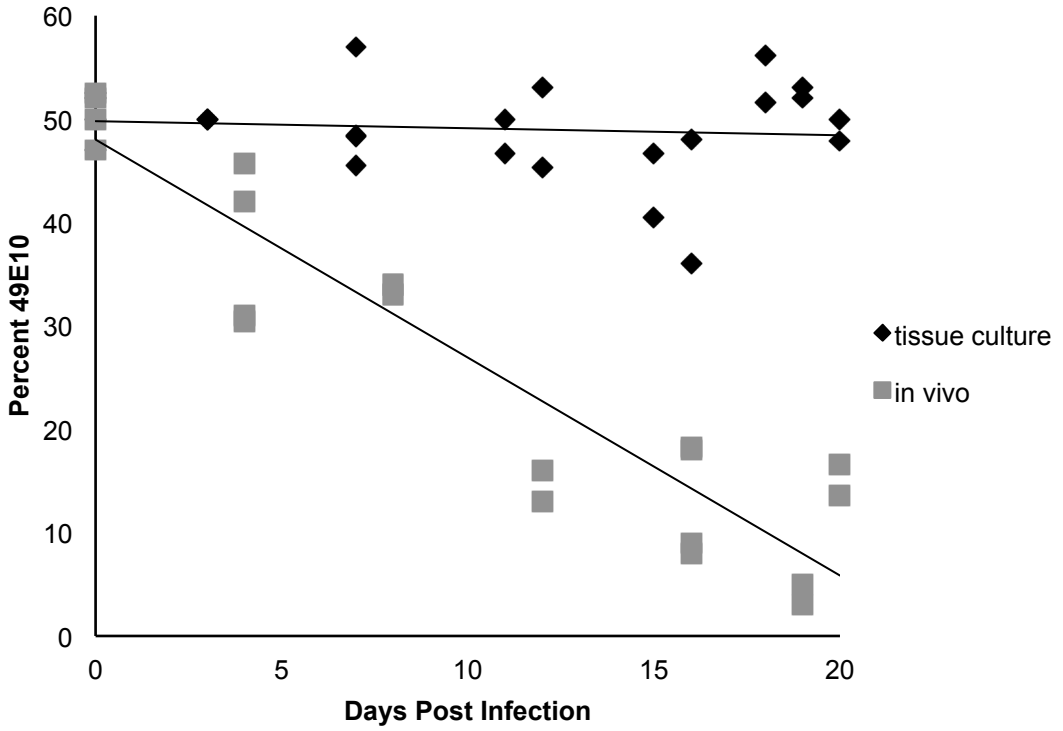

Fig. S1. The E10 outcompetes the 49E10 mutant in vivo but not in vitro

Competitive fitness assay show that the 49E10 mutant grows equally well as the parental E10 strain in standard tissue culture conditions (black diamonds), but not *in vivo* if the mice are infected with 10^6 mixed parasites (gray squares). Each of the four data points per time point represents an independent tissue culture flask or mouse that was serially passaged. R-squared value for the *in vivo* experiment is 0.8702.

Fig. S2. ClustalW alignment of TGME49_023970 and EFG from E. coli

TGME49_023970 from *T. gondii* is marked as “Tg” and *fusA* (EFG) from *E. coli* is marked as “Ec.” Identical residues are marked with a “*”. Highly conserved residues are marked with a “:”. Weakly conserved residues are marked with a “.”.

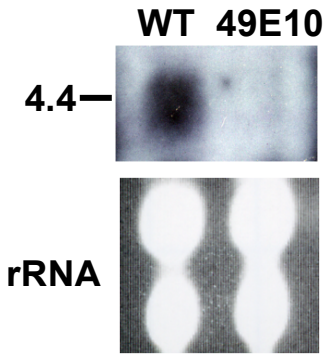

Fig. S3. TGME49_023970 is disrupted in 49E10

RNA from PruΔHPT (WT) and 49E10 parasites were analyzed by northern blot analysis using a probe downstream of the insertion site of TGME49_023970 in 49E10. Ethidium bromide staining of the rRNA is shown beneath as a loading control.

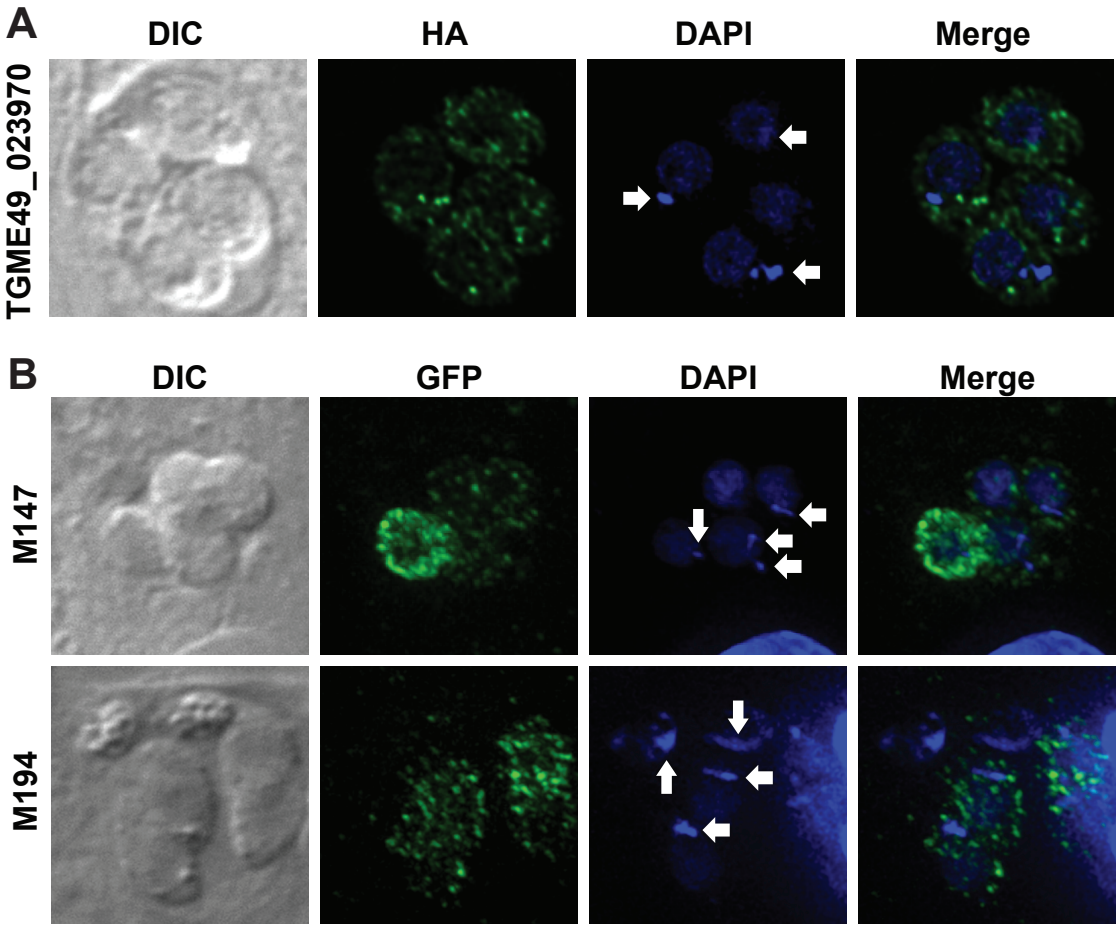

Fig. S4. TgEFG does not target to the apicoplast when translated from M147, M194 or M262.

A. An HA-tagged version of TGME49_023970 was stably expressed in WT parasites using a heterologous promoter and UTRs. The IFA used an anti-HA antibody to visualize the HA-tagged protein (green) and DAPI to visualize nucleic acid (blue). White arrows indicate the apicoplasts in the DAPI panel. The prediction TGME49_023970 starts at M262 of the *TgEFG* mRNA.

B. *T. gondii* expressing various TgEFG-GFP fusion constructs were examined by IFA using an anti-GFP antibody to visualize the TgEFG-GFP fusion (green) and DAPI to visualize nucleic acid (blue). The rows are labeled according to the ATG codon used to create the TgEFG-GFP fusions with M147 (top row) corresponding to the second in-frame ATG codon, M194 (bottom row) corresponding to the third in-frame ATG codon. White arrows indicate the apicoplasts in the DAPI panel and elongated apicoplasts are in the process of dividing.

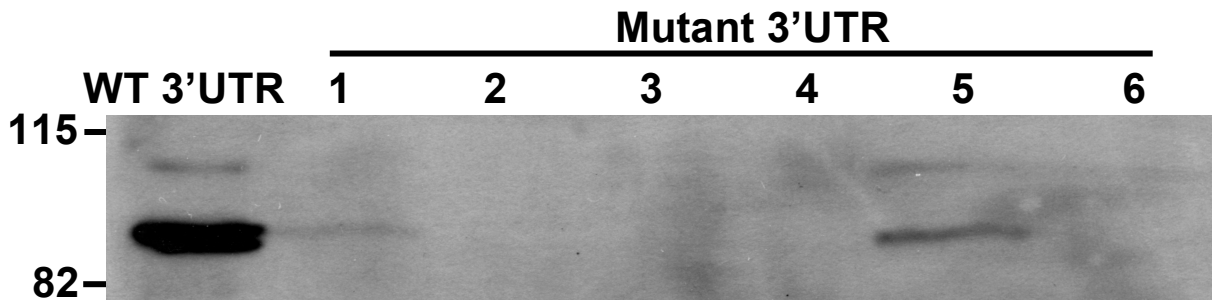

Fig. S5. Insertion into the 3'UTR of TgEFG affects protein expression

One clone of TgEFG expressed with its endogenous 3'UTR (WT 3'UTR) and six different clones of TgEFG expressed with the mutant 3'UTR found in 49E10 (Mutant 3'UTR #1-6) were analyzed by western immunoblot. $1x10^7$ tachyzoites were loaded per lane and protein was visualized using a mouse anti-HA monoclonal antibody to detect the HA epitope tagged TgEFG. Size markers are shown to the left in kilodaltons.

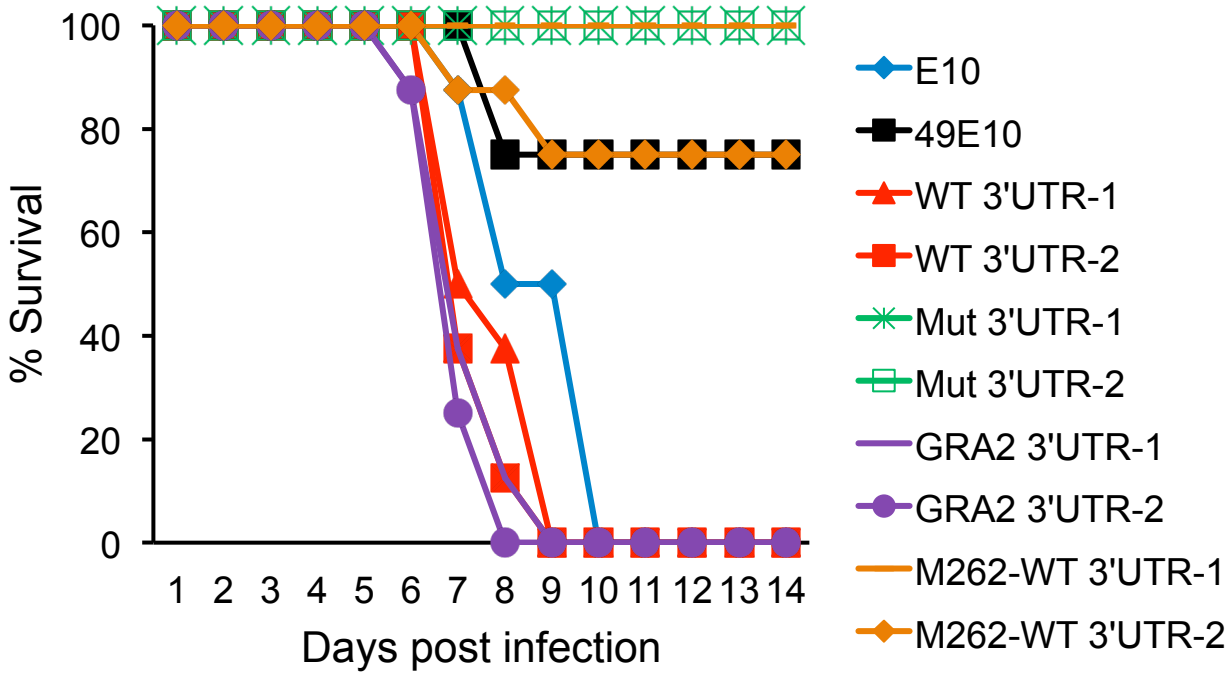

Fig. S6. TgEFG protein but not the endogenous 3'UTR itself restores virulence to 49E10. The virulence of E10 (blue diamond) and 49E10 (black square) parasites was compared to two independent clones of 49E10 complemented with TgEFG expressed with its endogeneous 3'UTR (WT 3'UTR, red), the mutant 3'UTR found in 49E10 (Mut 3'UTR, green), the GRA2 3'UTR (purple) or the shortened M262 form of TgEFG with an endogenous 3'UTR (M262-WT 3'UTR, orange). For this acute mouse model, $1.5x10^6$ tachyzoites were i.p. injected into four mice per strain. Two independent experiments were combined for a total of eight mice per strain.