Tables

Table S1. Primers used in this study

Primer name	Sequence	product sizes (bp)
P1	CGTCTGTCTTCAACCAGATCTATGACGAAGCCTCAAGAGACAC	1416
P2	AGCTCCACCGCGGTGGCGGCCGCGCATGCCATTACTACACATCAGA	
P3	TCTCGTTTCGCTCAAAGTGTG	P3/P4 : 1775 P3/P5 : 1715 P6/P4 : 1744
P4	CCTTCCTTTGGTGGATCAGTC	
P5	CTGCAAGGCGATTAAGTTGG	
P6	GAAAACATCGTGAGGCTGGTAC	
P7	GACCTGGCCATGGCCATGCATTACCCGTACGACGTCCCGGACTACGCTTAT CCCTATGATGTGCCCGATTATGCGTATCCTTACGATGTTCCAGATGCTGCAG CAATGACGAAGCCTCAAGAGACACC	1281
P8	TGAGCACAACGGTGATTAATTAACTACTTCTCGTTTTTTGTACTGCGG	
P9	GGCGAATTGGGTACCGGGCCCGTGCCTAGTATCGAAAGCTGTAG	971
P10	GACGTCGTACGGGTAATGCATTTTAGAAGCCCTGTGGACAG	
P11	AAAAACGAGAAGTAGTTAATTAAGTCTCTAGTTTTTTTGACAGACCG	889
P12	AGTGACACCGCGGTGGCGGCCGCAGAGAAGTTGTGTGCTAGAGGTTC	
P13	GTGCCTAGTATCGAAAGCTGTAG	3173
P14	AGAGAAGTTGTGTGCTAGAGGTTC	
P15	GCGATTCCCGTATTGGTC	1492
P16	GTTCCTGCCGATGGTGAG	
P17	CTGCTTTCGTCTGTCTTC	P17/P18 : 1554 P19/P18 : 1556 P19/P20 : 1565
P18	CAAATGGCTATGTTCGCC	
P19	CCAAACCAGATATTCGCC	
P20	TACGACTCACTATAGGGC	
P21	GTATCCCAACGAAATTCCGGTCATCTGC	9681
P22	TTTGCCCGAATGCGGTGT	
P23	AGTTTCACCTCTGCCCCGA	124
P24	CTACGCCTTTCCACTGCCA	
P25	CATCTGCGAAAAAGCTCCCC	195
P26	GCCTTCGGTGCCGTATTTTC	
P27	ATGTTCCGTGGTCGCATGT	104
P28	TTCATGTTGTTGGGAATCCAC	

Table S2. The antibodies used throughout this study are indicated.

Antibodies	Dilutions	Sources
Rabbit anti-TgAtg8	1:500 for WB and IFA	Laboratory-prepared
Rabbit anti-TgAtg3	1:1000 for WB; 1:200 for IFA	Laboratory-prepared
Mouse anti-TgβTubulin	1:1000 for WB	Laboratory-prepared
Mouse anti-TgCpn60	1:200 for IFA	Laboratory-prepared
Mouse anti-TgSAG1	1:2000 for WB and IFA	Abcam, ab8313
Rabbit anti-HA	1:2000 for WB; 1:1000 for IFA	Cell Signalling Technology, 3724S
Goat anti-rabbit IgG (H+L), HRP-Labelled	1:5000 for WB	Biosharp, BL003A
Goat anti-mouse IgG (H+L), HRP-Labelled	1:5000 for WB	Biosharp, BL001A
Goat anti-mouse Alexa Fluor 488	1:1000 for IFA	YEAST, 33106ES60
Goat anti-rabbit Alexa Fluor 546	1:1000 for IFA	YEAST, 33112ES60

Figures

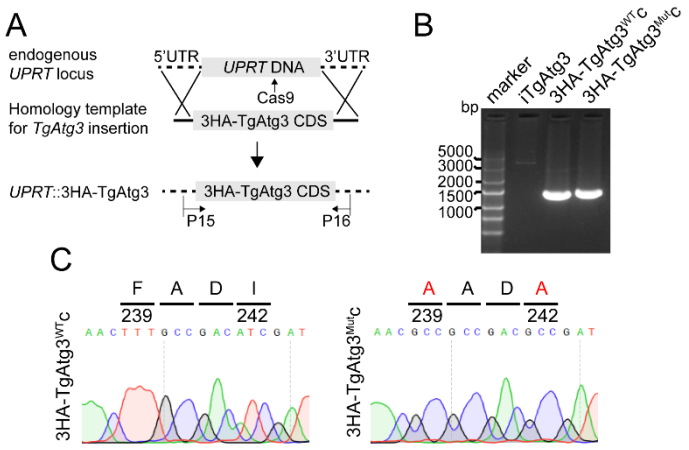

FIG S1 Validation of two TgAtg3 complemented lines. (A) Schematic representation of generation of the 3HA-TgAtg3^{WT}c and 3HA-TgAtg3^{Mut}c complemented cell lines by replacement of the endogenous *UPRT* locus. P15 and P16 represent the primer binding sites in the complemented lines (B) Clone derived from the stable FUDR-resistant population was detected for the recombined loci by using diagnostic PCR. iTgAtg3 line is used as control. (C) Sequencing validation of the PCR products from panel A.

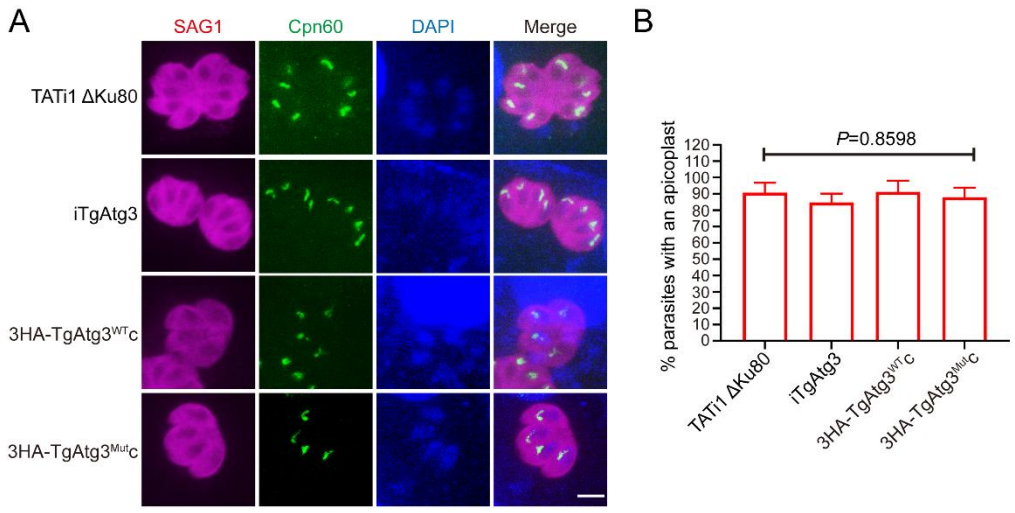

FIG S2 TgAtg8-TgAtg3 interaction regulates apicoplast inheritance. (A) IFA assay for detection of the apicoplast marker TgCpn60 in HFFs infected with each line in the absence of ATc for 48 h. Scale = 5μm. (B) Quantification of the percentages of tachyzoites with an apicoplast in the absence of ATc for 48 h. Data are means ± SEM from three biologic replicates, each with at least 200 parasites per line counted. Statistical analysis was done using one–way ANOVA.

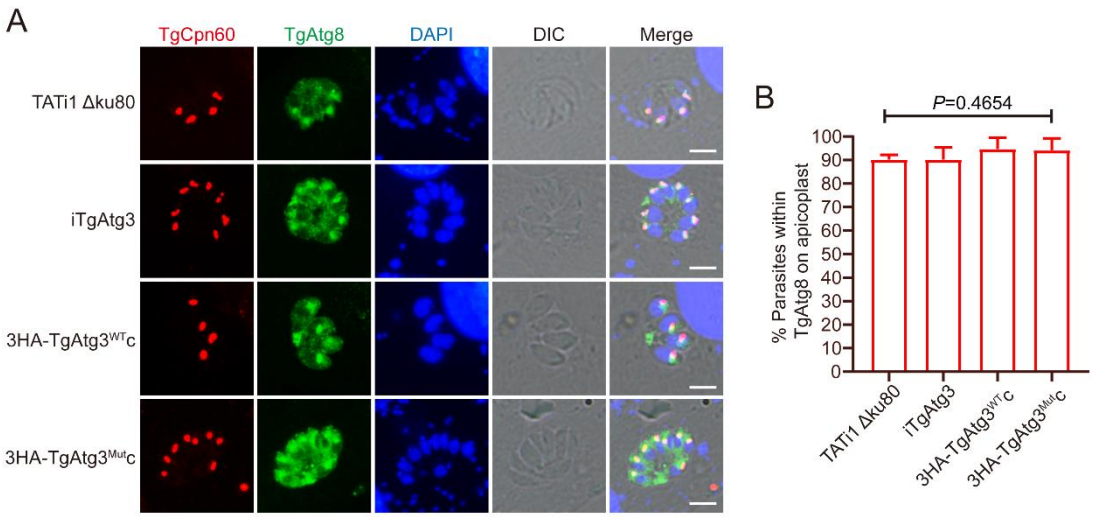

FIG S3 TgAtg8-TgAtg3 interaction regulates TgAtg8 apicoplast localization. (A) IFA was performed to observe TgAtg8 localization in the absence of ATc for 48 hours. Scale = 5μm. (B) The number of apicoplast-bearing parasites that displayed the presence of TgAtg8 on apicoplast. Data are means ± SEM from two biologic replicates. Statistical analysis was done using one–way ANOVA.

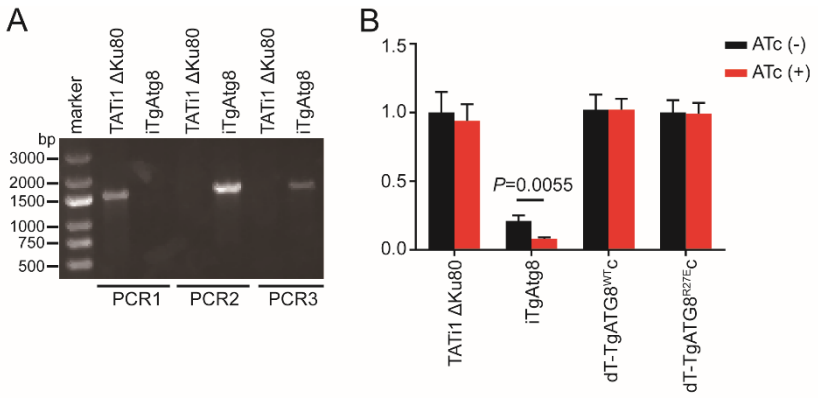

FIG S4 Generation of iTgAtg8 and complemented lines. (A) Clone derived from a stable pyrimethamine-resistant population was detected for the endogenous and recombined loci by using diagnostic PCR. TATi1 Δ*Ku80* line is used as control. (B) RT-qPCR analyses of *TgAtg8* transcriptional level in each line preceded or not by 48 hours of induction with ATc to regulate expression.