Supplemental Data

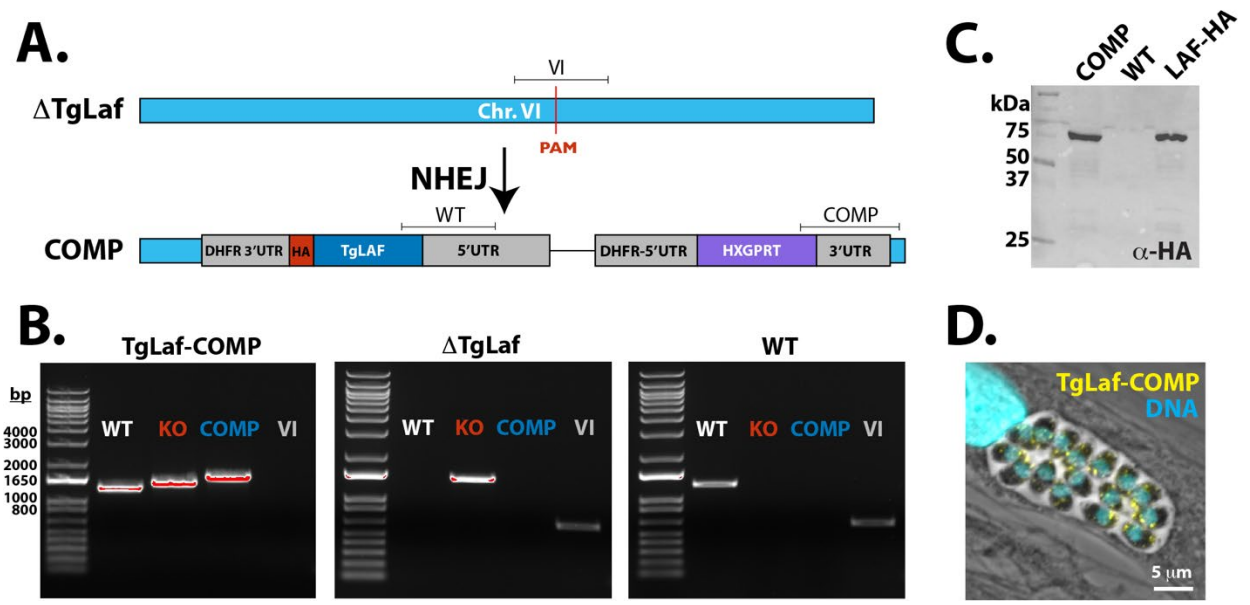

Figure S1. Schematic of TgLaforin complementation strategy and confirmation of successful expression of TgLaforin. **A,** Schematic of TgLaforin complementation into ΔTgLaf parasites in which a PAM site was chosen at a neutral locus previously identified in chromosome VI (1) to insert TgLaforin cDNA under its endogenous promoter. The TgLaforin construct was connected to the HXGPRT selectable drug marker and inserted using NHEJ. **B,** PCR confirmation of integration of TgLaforin construct into chromosome VI. Primer sets are indicated above amplicons. WT primers amplify the same locus as in Figure 3 ("PCR 1"), also present in the COMP line. KO primers amplify the chimeric locus depicted in Figure 3 ("PCR 2"). Presence of the KO amplicon confirms that KO locus remains intact in COMP line. COMP primers amplify the chimeric locus generated upon insertion of the complementation construct. VI primers amplify the native chromosome VI locus, which is lost only in the COMP line. **C,** Western blot confirms expression of TgLaforin-HA in complemented parasites. Tagged LAF-HA parasites serve as a comparison to confirm the correct MW (62 kDa) and expression level. **D,** IFA demonstrates restoration of cytoplasmic, punctate localization of TgLaforin. Scale bar = 5 μm.

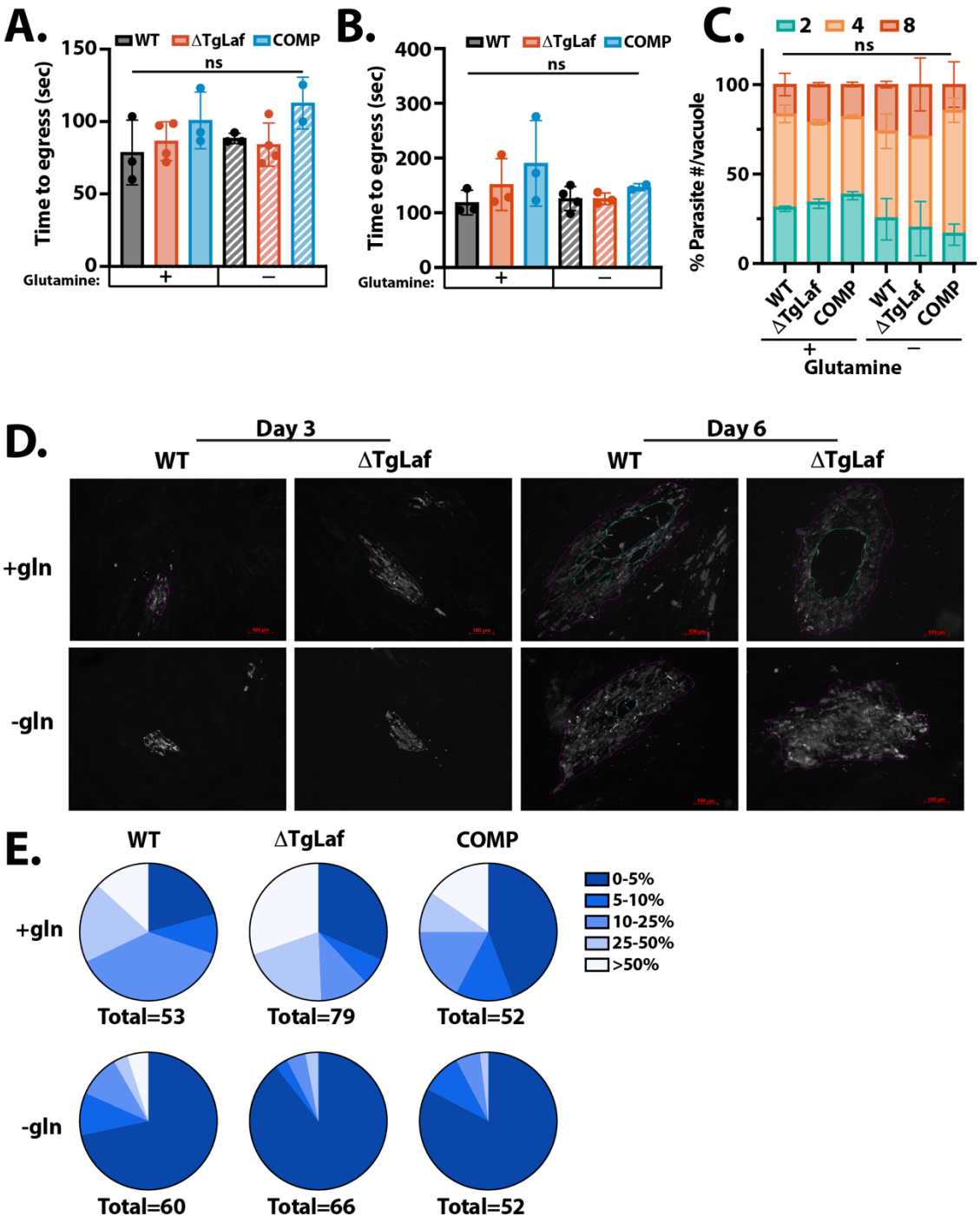

Figure S2. Loss of TgLaforin results in cumulative defects that cannot be pinpointed to one aspect of lytic cycle. **A,** Calcium ionophore-stimulated egress assay in which parasites were pre-starved of glutamine for 48 hours, seeded onto HFFs and allowed to grow for 48 hours to produce vacuoles containing >16 parasites, and stimulated with 3 μM A23187. Egress was monitored by video microscopy, and time to egress was monitored as described in Materials and Methods. Data is the average of 3 biological replicates that each consist of 4-5 technical replicates. COMP experiments measured 2

biological replicates. **B,** Zaprinast stimulated egress assay performed as described for ionophore, however 500-μM zaprinast was used to stimulate egress. Data is the average of 3 biological replicates that each consist of 2-5 technical replicates. COMP experiments measured 2 biological replicates. **C,** Replication assay in which parasites were pre-starved of glutamine for 48 hours, re-seeded into HFFs, and counted after 24 hours of growth. Numbers (2, 4, or 8) indicate the number of tachyzoites counted per vacuole. Data is the average of 3 biological replicates with at least 70 vacuoles counted per replicate. **D,** Representative images of plaque formation at days 3 and 6, +/- glutamine. Images were taken at 10X magnification using a SAG1 antibody to visualize plaque size. Inner clearing area is circled. **E,** Percent of plaques cleared was measured at both days 3 and 6 by dividing the area of the clearing by total plaque size. Statistical comparisons were done using an ordinary one-way ANOVA using Tukey's post-hoc test to correct for multiple comparisons. Error bars depict SD from the mean. Statistical significance is indicated as follows: ns=$p>0.05$.

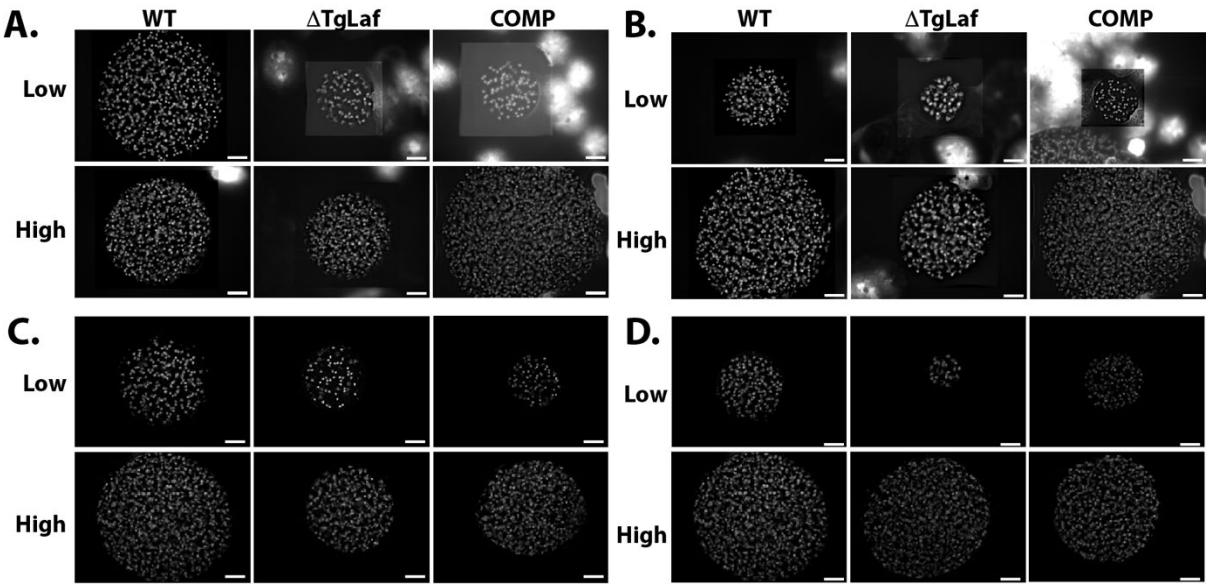

Figure S3. Representative *in vivo* cyst profiles of: **A,** Lowest and highest packing density from each line at week 4. **B,** Lowest and highest diameters from each line from week 4. **C,** Lowest and highest packing density from each line at week 6. **D,** Lowest and highest diameters from each line at week 6. All scale bars = 10 μm.

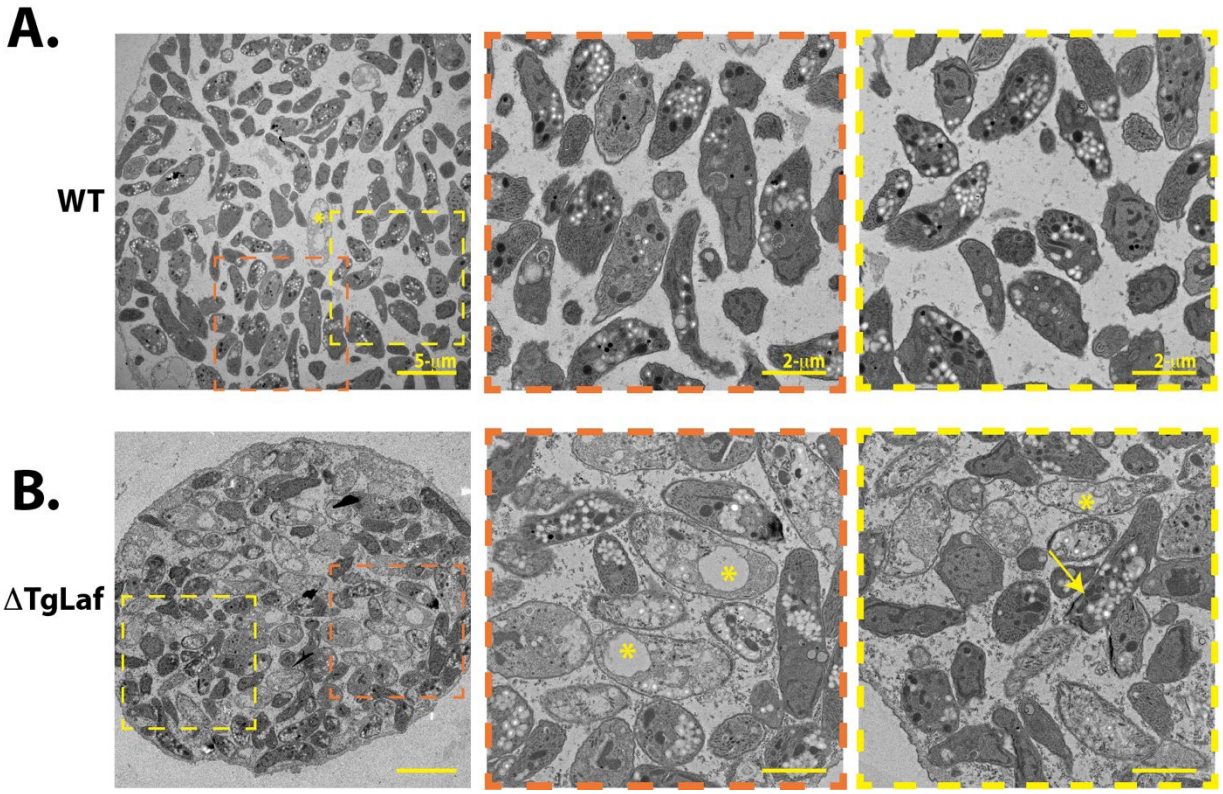

Figure S4. Additional images of *in* vivo tissue cysts. **A,** WT tissue cyst (too large to fit in camera view) with two zooms depicted at right. **B,** ΔTgLaf tissue cyst also with two zooms depicted at right. Asterisks highlight empty nuclei and the arrow shows an example of rhoptry displacement by AGs. Left column scale bar = 5 µm; right column zoom scale bars = 2 µm.

Supplemental Table 1. sgRNA sequences used in this study.

Target	Use	Sequence	Strand
TgLaforin 3'UTR	TgLaforin epitope tag	GCTTAGCGTGTGAACA GCAG	+
TgLaforin (exon 1)	TgLaforin KO	GAAGTCCCGATAACCTA CGC	+
Chromosome VI	TgLaforin complementation	GCCGTTCTGTCTCACG ATGC	+

Supplemental Table 2. Plasmids used in this study.

Name	Use	Source
pSAG1::CAS9-U6::sgUPRT	Genetic modifications in *T. gondii*	David Sibley, Washington University
pJET-NcGra7_DHFR	TgLaforin knockout via DHFR-TS* knock in	Peter Bradley, UCLA
pHA3x-LIC	Tagging and complementation of TgLaforin	Peter Bradley, UCLA
TgLaforin-HA3x-LIC	Tagging and complementation, derived from pHA3x-LIC	GenScript

Supplemental Table 3. Primers used in this study.
Primers are presented in uppercase where the primer binds directly to the template, and in lowercase-bold where new sequence is being introduced. F, forward sequence; R, reverse sequence.

Name	Use	Sequence (5' to 3')
TgLaf_3'UTR_sgRNA_F	sgRNA mutagenesis for TgLaf-HA epitope tagging	**tgaacagcag**GTTTTAGAGCTAGAAATAGC
TgLaf_3'UTR_sgRNA_R		**cacgctaagc**AACTTGACATCCCCATTTAC
TgLaf_exon5_homology_F	Generation of TgLaf-HA tagging construct	AGAGGAGGCGGAGGAGAG
TgLaf_3'UTR_HX+homology_R		TCCGTATCGCCCCCTCTCGTCTGACACGCCCTCTTTCCTCCAGCACGAAACCTTGCATTC
TgLaforin_sgRNA_E1_F	sgRNA for TgLaf-KO	**taacctacgc**GTTTTAGAGCTAGAAATAGC
TgLaforin_sgRNA_E1_R		**tcgggacttc**AACTTGACATCCCCATTTAC
NcGra7-DHFR*_Fwd-TgLaf_homology	Amplification of NcGra7-DHFR* flanked with 40-nt TgLaforin	**gtctcttttctgcgcgtctccctgctcgtgcgtagaagg**CCACTCCATGGAACCTGACTG
NcGra7-DHFR*_Rev-TgLaf_homology		**gctctttcccccatttttcttttctctctcacgggttccg**CCTGCAAGTGCATAGAAGGAA
TgME49_ChrVI_sgRNA_F	sgRNA for ChrVI used in TgLaf complementation	**ctcacgatgc**GTTTTAGAGCTAGAAATAGC
TgME49_ChrVI_sgRNA_R		**acagaacggc**AACTTGACATCCCCATTTAC
TgLAF_WT_F	"PCR1": Figure 3A. Amplifies WT TgLaforin	TCCTACATTCTGGAGCGAAG
TgLAF_WT_R		AAAGCCACTTTCTCCAGGAG
DHFR*_R	"PCR2": Figure 3A. Amplifies ΔTgLaf chimeric locus in conjunction with TgLAF_WT_F.	GCATTATGAGGAAAGCCCAC
TgChrVI_WT_F	Amplification of WT Chr VI locus: ("VI": Figure	CAGGAAATATGCTGCGAGGA
TgChrVI_WT_R		TGTGTCTGCTCTTGAAGGTG
TgLaf_COMP_F	Binds to HXGPRT cassette within complementation construct (derived from pHA3x_LICs	TGCAAGCCCTACATTGACAA