Intracellular *Toxoplasma gondii* parasites harbors three druggable FNT-type formate and L-lactate transporters in the plasma membrane

Holger Erler, Bingjian Ren, Nishith Gupta and Eric Beitz

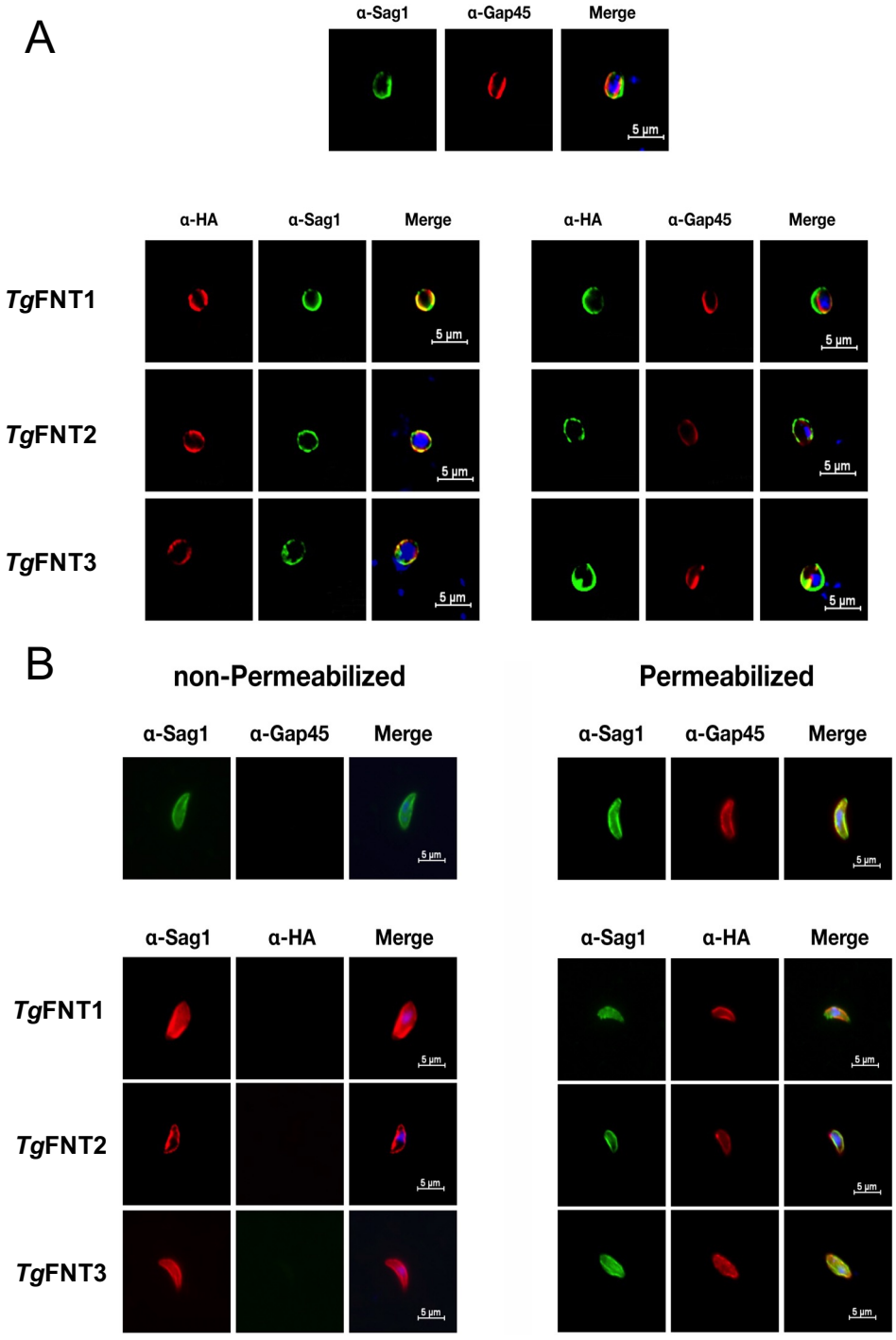

Figure S1. *Tg*FNT1-3 localize in the plasma membrane with their C-termini facing intracellularly in *T. gondii* tachyzoites. (A-B) Immunostaining of *Tg*FNT1-3 constructs with a C-terminal HA epitope in extracellular tachyzoites. (A) Co-staining of the HA-tag with markers of the plasma membrane (α-Sag1) and the inner membrane complex (α-Gap45) following treatment of free parasites with a bacterial toxin (see *methods*). Biochemical separation of both organelles was verified by 'split' immunostaining of Sag1 and Gap45 (see top row). (B) Immunofluorescence of transgenic tachyzoites, performed prior to or after detergent permeabilization. Yellow color indicates co-localization of the HA epitope with Sag1 or Gap45 proteins. Nuclei were stained using DAPI (blue). Note that Gap45 is visible only after detergent permeabilization, whereas Sag1 appears under both conditions, as expected.

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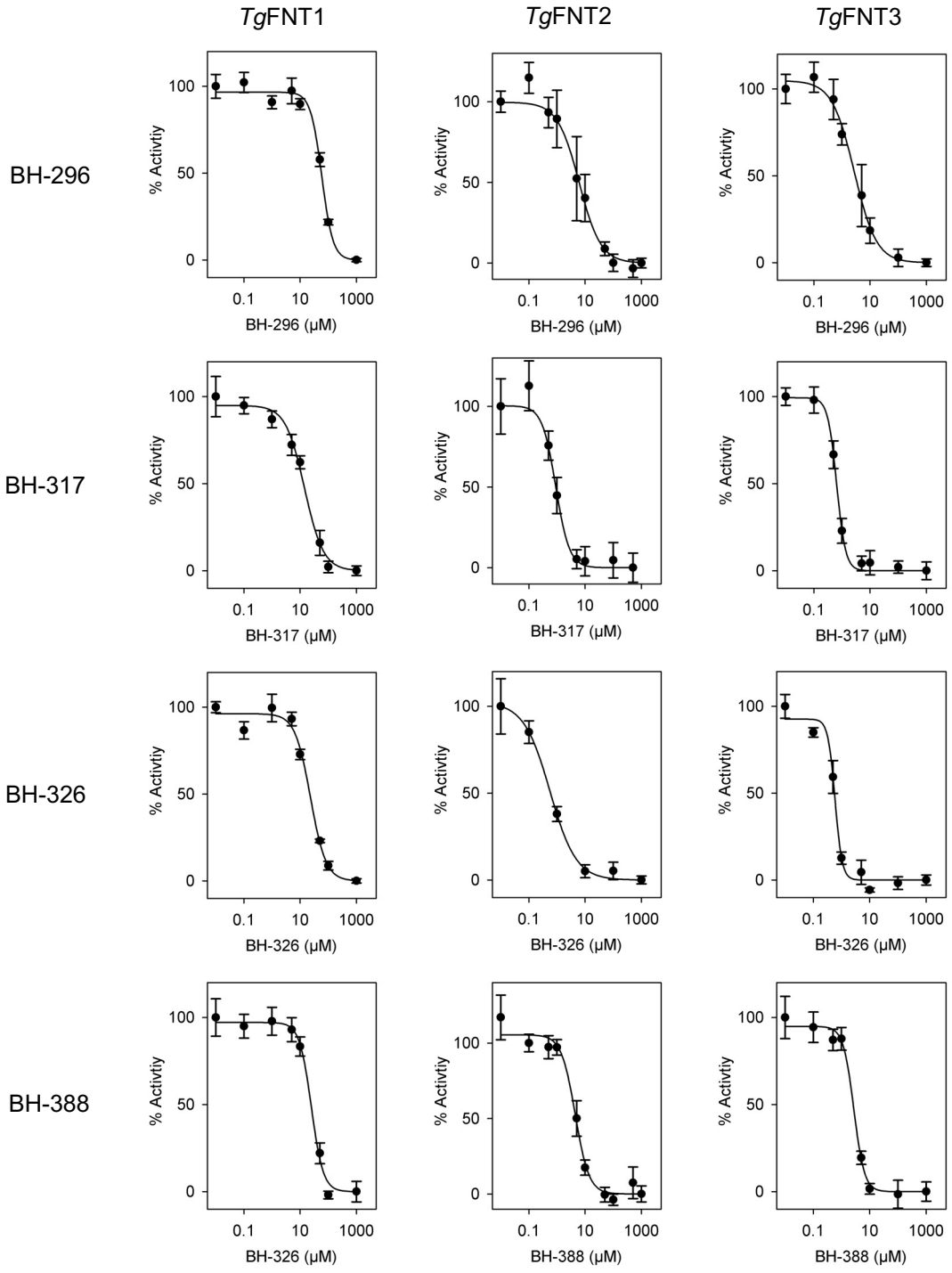

Figure S2. Dose-dependent inhibition of *Tg*FNT1-3 expressed in yeast (pH 6.8, 1 mM inward L-lactate gradient). The experiments were carried out in triplicate; the error bars denote S.E.M.

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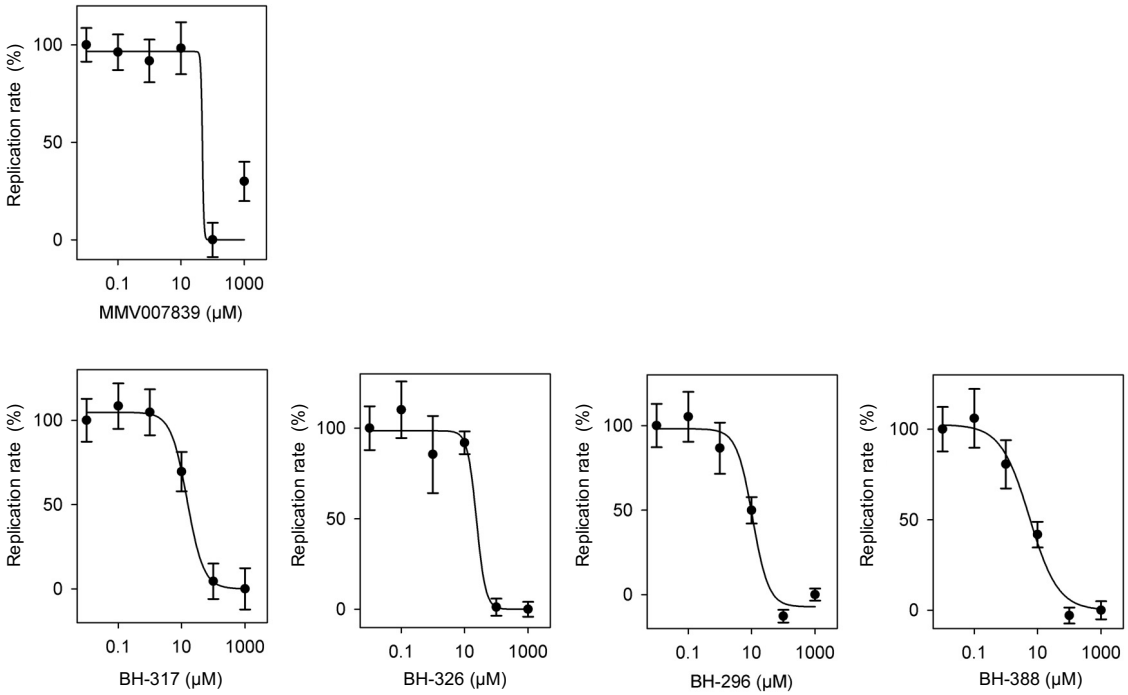

Figure S3. Dose-dependent inhibition of *T. gondii* tachyzoite replication by *Tg*FNT inhibitors. The experiments were carried out in triplicate; the error bars denote S.E.M.

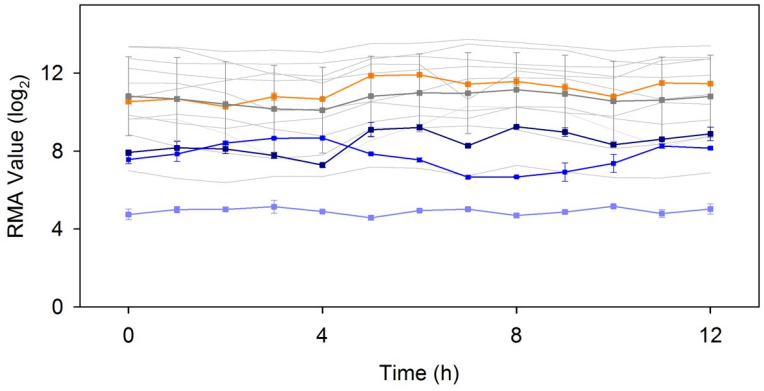

Figure S4. Expression of *Tg*FNT1-3 correlates with the glucose transporter and glycolytic enzymes in *T. gondii* tachyzoites. Transcript profiles of *Tg*FNT1 (dark blue), *Tg*FNT2 (blue) and *Tg*FNT3 (light blue) are shown next to the glucose transporter *Tg*GT1 (TGGT1_214320) (orange) and glycolytic enzymes (gray). Thin gray traces show expression of lactate dehydrogenase 1 (TGGT1_232350), hexokinase (TGGT1_265450), glucose-6-phosphate isomerase (TGGT1_283780), 6-phosphofructokinase (TGGT1_240890), fructose-1,6-bisphosphate aldolase (TGGT1_236040), triose-phosphate isomerase (TGGT1_233500), glyceraldehyde-3-phosphate dehydrogenase (TGGT1_289690), phosphoglycerate kinase (TGGT1_222020), phosphoglycerate mutase (TGGT1_297060), enolase 2 (TGGT1_268850) and pyruvate kinase (TGGT1_299070). The gray squares indicate the average of the traces ± SD. The expression profiles were obtained from ToxoDB, and transcript levels are given as robust multi-array average (RMA) normalized values (log base 2).

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Table S1: Primers for cloning and mutagenesis of *Tg*FNT1-3

Name	Sequence (restriction sites underlined)	Purpose
*Tg*FNT1-F	CTCATCCCTGCAGGATGGTCGTGACAGCGAGC	*Tg*FNT1 ORF in *pGRA1-UPKO* using *Nsi* I-*Sbf* I (F) and *Pac* I (R)
*Tg*FNT1-R	CTCATCTTAATTAATCACGCGTAGTCCGGGACGTCGTACGGGTAACTGGTCGCGGTAGG	
*Tg*FNT2-F	CTCATCATGCATATGTGTTCTATCCCACCATTGAG	*Tg*FNT2 ORF in *pGRA1-UPKO* using *Nsi* I (F) and *Pac* I (R)
*Tg*FNT2-R	CTCATCTTAATTAATCACGCGTAGTCCGGGACGTCGTACGGGTAAACTTGTTGAGCAACAGATTC	
*Tg*FNT3-F	CTCATCATGCATATGGTTTTGGCTGCTTCTCC	*Tg*FNT3 ORF in *pGRA1-UPKO* using *Nsi* I (F) and *Pac* I (R)
*Tg*FNT3-R	CTCATCTTAATTAATCACGCGTAGTCCGGGACGTCGTACGGGTAGATAGTAGAAGCTGGGTGTTCT	
*Tg*FNT1 ΔC277-ter F	ATTGCTAGCAAAGTATCGTATGGCTGGGGCAGCTGC	*Tg*FNT1 C-term. truncation in pDR196 using *Nhe* I (F) and *Sal* I (R)
*Tg*FNT1 ΔC277-ter R	ATTGTCGACAATGCTGCCTTCCGCGGCGTCA	
*Tg*FNT2 ΔN1-73 ΔC350-ter F	ATTACTAGTATGGTTGTTACTGCTGGTGCTGACGC	*Tg*FNT2 N-term. and C-term. truncation in pDR196 using *Spe* I (F) and *Xho* I (R)
*Tg*FNT2 ΔN1-73 ΔC350-ter R	GTACTCGAGAACTTGTTGAGCAACAGATTCAGCAG	
*Tg*FNT3 ΔC277-ter F	GCGCTGCAGTGTTTCTGTTTGGCTGTTGGTTGTAAC	*Tg*FNT3 C-term. truncation in pDR196 using *Pst* I (F) and *Xho* I (R)
*Tg*FNT3 ΔC277-ter R	GCACTCGAGTGGCAATGGTTGTCTAGCTTCTTGAAC	
*Tg*FNT1 G93S F	GCAGAGCTGTTTACAaGtAATACAATGACT	Site-directed mutagenesis of *Tg*FNT1; the mutating nucleotides are in lower case
*Tg*FNT1 G93S R	TGTAAACAGCTCTGCACCGGTCATGATAAT	
*Tg*FNT2 G166S F	GGTGCTGAATTGTTCACTaGTAACACTATGAC	Site-directed mutagenesis of *Tg*FNT2; the mutating nucleotides are in lower case
*Tg*FNT2 G166S R	AGTGAACAATTCAGCACCAGTCAAGATGAT	
*Tg*FNT3 G93S F	GCTGAATTGTTCACTtcTAACACTATGACT	Site-directed mutagenesis of *Tg*FNT3; the mutating nucleotides are in lower case
*Tg*FNT3 G93S R	AGTGAACAATTCAGCACCAGTGAAGATGAT	