Supplement

Table S1 Plasmids used in this study

Plasmid	Description	Origin
pSAG1-CAS9-U6gRNA (UPRT)	Vector expressing CRISPR/Cas9 fused to GFP. Used in mutagenesis to create NTPase I and NTPase II targeted CRISPR cutting constructs.	This study
pHTN HALOTAG CMV- neo plasmid	Vector for expression of GLUC used as backbone to clone in 5× GAS site sequence upstream of a mCMV promoter.	Promega

Table S2 Primers used for QTL mapping analysis

Primer	Direction	Sequence (5'-3')	Marker
M163-1F*	forward	GCTTTTGACGTCCACTTCG	M163
M163-2R*	reverse	CCTCCTTCTCAAAGGTCAAGC	M163
BM17053-F*	forward	CGACAAGTCCATGCGAACTA	BM1705
BM17053-R*	reverse	GGCAACAGGAGGTAGAGGAG	BM1705
4B-fw	forward	ACGCATGCAGATCCGTCCTC	4B
4B-rev	reverse	GAGCTTCTCTTCCGAGCAACG	4B
4C-fw	forward	AGCTCGCATCATATCGCCGTC	4C
4C-rev	reverse	TAGAAACGAGTGAGGGACACTG	4C
5-fw	forward	GATACTCCAAACTGCGGAGGC	5
5-rev	reverse	TGGAATGCATGTGCCGAGTTCG	5
MIC5-F*	forward	GCGGTGGTCAGATTCCTCTA	MIC5
MIC5-R*	reverse	GCCCAGTGTCGATAGCAAAT	MIC5
9-fw	forward	TCCTGATGGATAAACACTACGAG	9
9-rev	reverse	TCTTCATCGCCTTCAAGTCGAG	9

*Su C1, Howe DK, Dubey JP, Ajioka JW, Sibley LD. 2002. Identification of quantitative trait loci controlling acute virulence in *Toxoplasma gondii*. Proc Natl Acad Sci U S A. 99:10753-8.

Table S3 Primes used for generating NTPase knockouts

Experiment	Primer	Direction	Sequence	Plasmid or purpose
NTPase I CRISPR	A44	forward	GGCGCTGGCAAGCGGTTCGCGTTTTAGAGCTAGAAATAGC	pSAG1-CAS9-U6gRNA (NTPase I)
NTPase II CRISPR	A45	forward	CGCGAGGGTAAACGGTTCACGTTTTAGAGCTAGAAATAGC	pSAG1-CAS9-U6gRNA (NTPase II)
NTPase I and II CRISPR	F4	reverse	AACTTGACATCCCCATTTAC	
Diagnostic PCR				
	A34	forward	ATGTGCCTCTCTTGCTGGTGT	5'-DHFR drug marker integration
	A35	reverse	GCACGGCAGTCAGATAACAG	5'-DHFR drug marker integration
	A38	forward	TGCATCATGTATCAGCGGTCG	3'-DHFR drug marker integration
	A39	reverse	CGTGTCTTCTTCCAAGAGTTGC	3'-DHFR drug marker integration
	A47	forward	ATGCAGGCAGTAGTTCGACGAG	NTPase II integrity
	A48	reverse	ATCGCCGCTAGCATACCTCCG	Pseudogene integrity
	A49	reverse	GCAAGCATCTCTCGTCCCGTTATA	NTPase II integrity
	A52	forward	ATGACGTCCTACCGCTAGTTGC	Pseudogene integrity
	A66	forward	AAGTCCTACCAGAACGACAACAGC	3'-HXGPRT drug marker integration
	A70	reverse	GCTGTTGTCGTTCTGGTAGGACTT	5'-HXGPRT drug marker integration