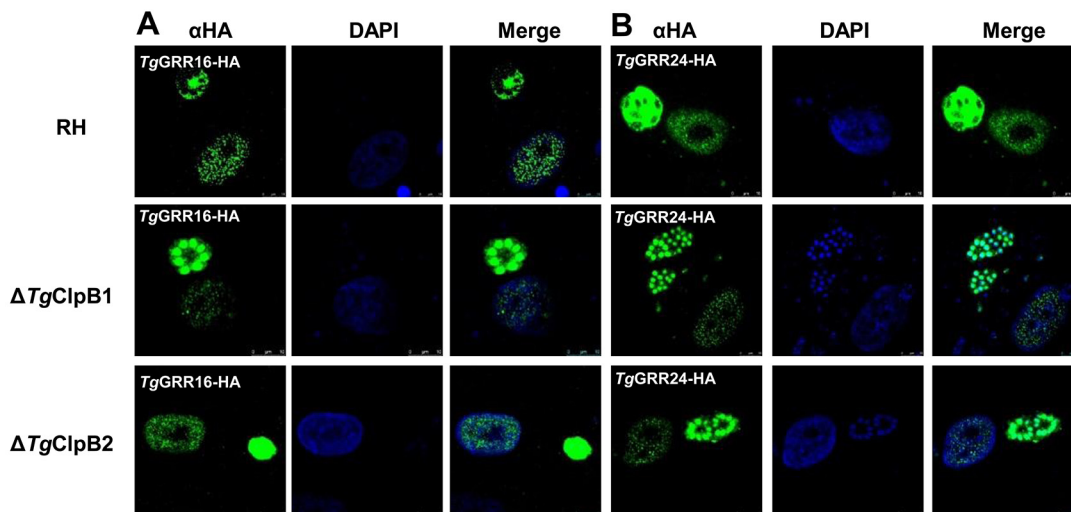


Toxoplasma gondii Clp family protein: TgClpB1 plays a crucial role in thermotolerance

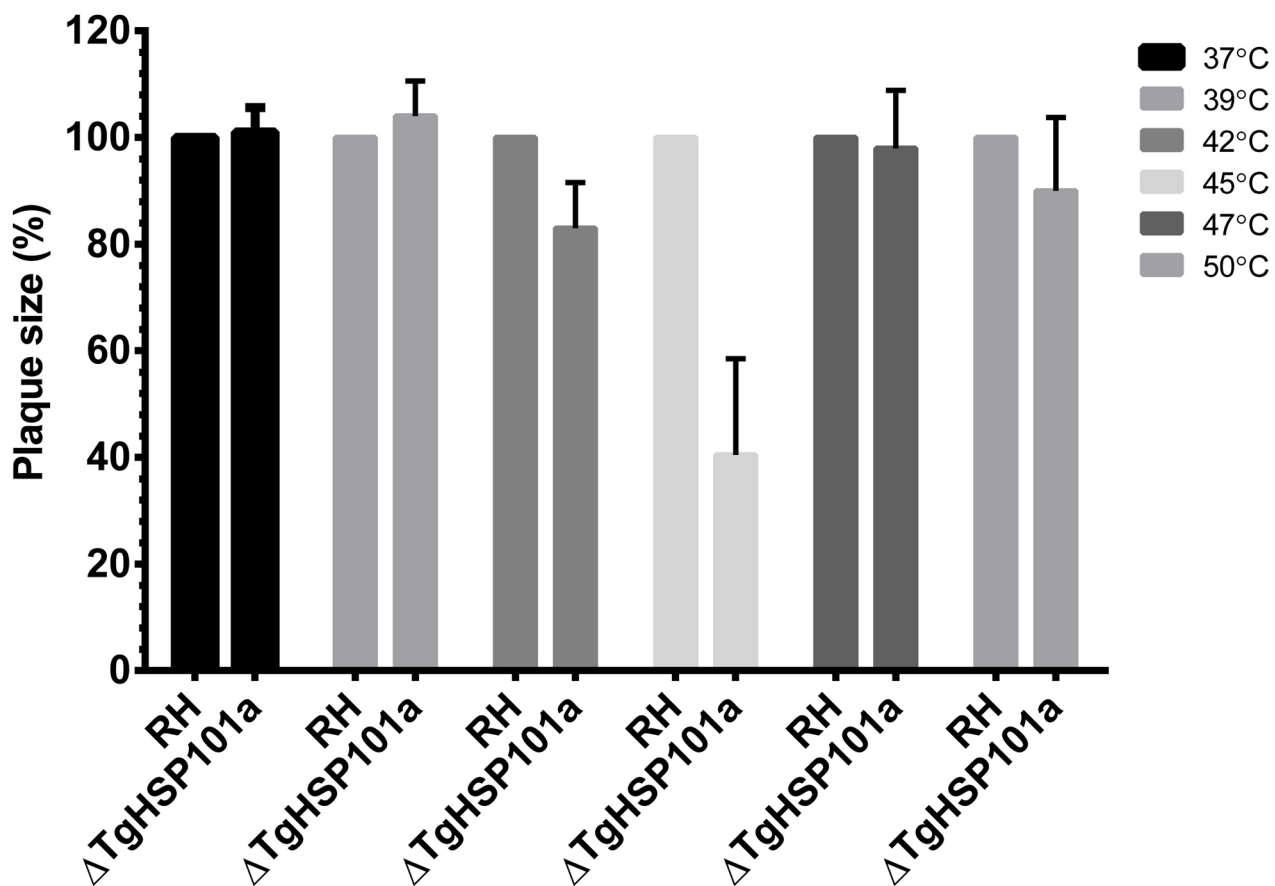
SUPPLEMENTARY MATERIALS

<i>PfClp2</i> MTRRYLKYIYFVILLFFVQVNNVLCAPDNKQEQKYLNRITINILNAGKNIKSYGHNKLK. PIHLSALAKS. . DVGSTLFKE	81
<i>TgClp1</i> mdadswtlvkkaflAGvecrerknpgLd. PIHLLeAmldqkqsfatqvlsq	52
<i>TgClp2</i>	mLrqgcrclpLgpkavafvapearsvsnrsgcslsvlrsfstgrslifsgdfssryrgrfLgysdlLgferRprealdhprfmqascfsaaanPgfsLkgddYtdRaleaLagmslAQeYspttLelLlSLNqgedglftmimKq	160
Consensus 1 1	
<i>PfClp2</i>	NNVNAANLKEYIDIALEQTRAGAPLDNKSKIVNSAEVKETLALAEAAAANKYSPKVDVHLLSGLSND. . . LVNEIFNEVYLLDEAIKAILKRRKFEKTKKDKGKTGTLVLEQFGSMNNEKVRNGKLQGIYGRDEEIRAIIESLLRYNKNNSPVLVGNPG	238
<i>TgClp1</i>	cpqdfeqLKEEdvhIavlkfpqghPpdpf. . spnhsImavLrhAkdiqkMmndslmsadLsLiQeKeg. . irshltaagfmmkqieekavsvrgsKkiassDddanfealkkyGtdfdLaeKGLDpviGRedEIRvIriLcRrtKnnPvLiGePG	208
<i>TgClp2</i>	agVldkLrsgvdfvErhrpvtgdnstrt. . . lgpvlqkvlssAnsrlrIqhdeyisVEHLaaLadedrsrLvrflkesklTandirAlkairgtrrvntktpevsyqslkkyGrdlTeaamanelDpviGRdKvRvrlqLlSLrtrtKnnPiiIGePG	317
Consensus	ll a 1 1 g g e r i l r kn p g pg	
<i>PfClp2</i>	TGKTTIVEGLVYRIEKGdVpKELQGYTVISLNFRRKFTSGTSYRGEFETRMKNIKELKNKNKIILFVDEIHLLLGAGKA. EGGTDAANLLKPVLSKGEIKLIGATTIAEYRKFIESCSAFERRFEKILVEPPSVDMTVKILRSKSKYENFYGINITDK	397
<i>TgClp1</i>	vKsAVVEGLarIvvhDvPsnLr. crivSLdvgsliadgkFRGEFEeRltaVlqEvKdaagKILFIDEIHVLLGAGKt. EoaLDAANLLKPVLSKGEIKLIGATTIAEYRKYvEkdaAFERRFqqvhvRePSVqgTisILRqLAdrYashhcvrTlDs	366
<i>TgClp2</i>	vgkTalaEGLAqRlvsGdPdtLaGrqLISLdlgallaCaklRGEFEeRlKsvlrEvqessgqLILFIDEIHmvvGAGsAgEsmGadAgNlKPLarGElrcIGATTIAEYRKYIEKdkALERRFvvlVdePrVedalsILRqLkerYEmhhGvsIrdS	477
Consensus	gk egl ri dvp l sl g rgefe r e iilf deih gag e da n lkp l ge igatt eyrk e a errf v p v ilr lk y g id	
<i>PfClp2</i>	ALVAAAKISDRIFKDRVLPDKAIDLLNKACSLFQVLSGKPRIDVTERIERLVEIISTLEKDV. . . DKVSKKYNKLIKFEFEKKEQLKYYEYVITGERLKRKKEIEKKLNDLKELTQNYVYS. NKEPPIELQNSLKEAQOQKYLELYKETVAAYVE	552
<i>TgClp1</i>	ALVeAqlaDRyItsRFLPDKAIDLmdeAcaiarVqdsKPeavDVIERqkvqLevElialLEKek. . . DpaSqKrlaevkehlGvadaLrplYlYqgekaRidelgkLaqQdeLkakiaraqrvgdldlvaELrfdalpgvearfklqEeoeqE	522
<i>TgClp2</i>	ALVAAcvsRyIqDRFLPDKAIDLideAaSkikievtsKPtrlDeidRkLmqLemEkisivsDmkggQdaeqqrLqLteKkmdAKEEqsrInaiveqeraeiekadlkQeideaKveqQkaereyNlnkaaqirygkipelqtqLatleaaakre	637
Consensus	alv a r i r lpdkaidl a kp d r l e d	
<i>PfClp2</i>	AKTHNAMNDVAVYQEHVSYYLDRSGMPLGSLSPESKGAALKLYNSLSKSIIGNEDIIKSLSDAVVKAATGMKDPKPIGTFLFLGPTGVGKTELAKTLAIELFNSKNDLIRVNMSEFTEAHSVSKITGSPPGYVGFSDGQLTEAVREKPHSVVLFDEL	712
<i>TgClp1</i>	r. THkplltevVgpEqiadvvhRwtniPvqkLqteterfLTLgkSLaeqvIGapqavevtqAilrsAaGlsrnrPIGSLFLGPTGVGKTELCkrvAesLpdsKerLvrfdMSEymEqHSVsrliGaPPGYVghdegGQLTeiRrnPsvVLFDEv	681
<i>TgClp2</i>	l. sssrllrDtVtaEdiaqvswtGivPsrLvegerekLLgkLndrvIGqEegvrSvaeAiqrsraGlcDPnrPiaslvFLGPTGVGKTELCkALArqLPdteeaLlrfdMSEymEqHStarliGaPPGYVgfdkgGQLTEAVRrnPsvVLFDEv	796
Consensus	v e p l l l l ig a g pi flgptgvgtel k a lf l r mse e hs g ppy g gqte r p sv lfde	
<i>PfClp2</i>	EKAHADVFKVLLQILGDGYINDNRRNIDFSNTIIIMTSLGAELFKKLLFPDADNSGTEPYKRVMEDEVRLSLIKKCKVKVFPKFPVNRIDKIGVPEFLNKNLHKIIVALRFFKLEKRLKRNQVSVSEKAIDYIIDQSYDPELGAQRTLIFIESVIMTK	872
<i>TgClp1</i>	EKAHsqVwnVLLQvLdGrItDsqgrtvDFSNTHIIITSNLGA. . . flleaAqrVdpvEpraeeaaatvmmevrKFFrPELlNRIDdIvIFkaLdvNlRqvmkLmqevreRLaEkrIeItmtrnAaDhIvkearDPayGARPlkrFIerhvsvd	837
<i>TgClp2</i>	EKAHpeVniFLQILdGiltDshgntvsFkNcIIITSNMgsd. . . lllqAsgtkdrE. eVtqaLmeivrrhrIFELVNRmDfVvPnPLsealNfgIfdLevaLqaRLtdrrrltISVchrAkadIvaqaDfnFGARPlkrFvrravqhteltp	945
Consensus	ekah v lq l dg d f n i i i tsn g l a e pe nr d f i nl l ri a i dp garp	
<i>PfClp2</i>	FAIMYLKELVDDMDVFDVYNSKAKNLVNLskT.	906
<i>TgClp1</i>	lsklkGEiFadshVvcDwdeKrrgwVtttSalevppsagrVpghveadmdesltpdsrslslgsrtdsyrnsamhetnkvkfrY	927
<i>TgClp2</i>	lsrlllsgsinsgrrrVgVas. . . KqeelPvssekiedskipenlqfflypqeqtaqhmLagsdtgaskdtgetaafs.	1020
Consensus	l v k	

Supplementary Figure 1: Polypeptide alignments of the full open reading frame of PfClp2, TgClp1 and TgClp2.



Supplementary Figure 2: Neither TgClpB1 nor TgClpB2 is not required for the translocation of TgGRA16 and TgGRA24 to the host nucleus. HFFs were infected with RH-WT, ΔTgClpB1 or ΔTgClpB2 strains expressing HA-tagged TgGRA16 (A) and TgGRA24 (B), respectively. After 24 h, the cultures were washed, fixed, and stained with antibodies to the HA tag.



Supplementary Figure 3: Growth analysis of the indicated parasites grown on HFF cells for 7 days. When parasites were grown in HFF cells after the released parasites treated at 37, 39, 42, 45, 47, 50°C, plaque sizes of Δ TgClpB1 was determined and compared with RH-WT.

Supplementary Table 1: Primer sequences used to amplify and sequencing

Primer name	Sequence
TgClpB1_Ex_F	AATGGGTCGCGGATCCAGCCGAACCACTCGCTCAT
TgClpB1_Ex_R	GGTGGTGGTGCTCGAGCACCGCAGTCAATCGCTCC
TgClpB2_Ex_F	AATGGGTCGCGGATCCGGTCTGCTTTGGTCTGTCTG
TgClpB2_Ex_R	GGTGGTGGTGCTCGAGGATGATGGGATTGTTCTTA
TgClpB3_Ex_F	AATGGGTCGCGGATCCATTGAGAAGGACAAGGCACT
TgClpB3_Ex_R	GGTGGTGGTGCTCGAGTTCGGCAGCTCTGTTTAGG
TgHSPClpB1_gRNA_1S	CCACTAGTTCTAGAGCGGCCGTTTAAACGAGCTCCAAGTAAGCAGAAG
TgHSPClpB1_gRNA_1aS	CGGAGCAGGAAAGACCGAAGAAGTACATCCCCATTTAC
TgHSPClpB1_gRNA_2S	CTTCGGTCTTTCCTGCTCCGGTTTTAGAGCTAGAAATAGC
TgHSPClpB1_gRNA_2aS	CCGTATTACCGCCTTTGAGTGTTTAAACGAGCTGATACCGCTCGCC
TgHSPClpB2_gRNA_1S	CCACTAGTTCTAGAGCGGCCGTTTAAACGAGCTCCAAGTAAGCAGAAG
TgHSPClpB2_gRNA_1aS	CGGTACCTACTGCTGAAATCAACTTGACATCCCCATTTAC
TgHSPClpB2_gRNA_2S	GATTCAGCAGTAGGTACCGTTTTAGAGCTAGAAATAGC
TgHSPClpB2_gRNA_2aS	CCGTATTACCGCCTTTGAGTGTTTAAACGAGCTGATACCGCTCGCC
TgHSPClpB3_gRNA_1S	CCACTAGTTCTAGAGCGGCCGTTTAAACGAGCTCCAAGTAAGCAGAAG
TgHSPClpB3_gRNA_1aS	GCGGGGAGTTAAGCGCCGACAAGTACATCCCCATTTAC
TgHSPClpB3_gRNA_2S	GTCGGCGCTTAACTCCCCGCTTTTAGAGCTAGAAATAGC
TgHSPClpB3_gRNA_2aS	CCGTATTACCGCCTTTGAGTGTTTAAACGAGCTGATACCGCTCGCC
TgClpB1_C_1F	CCGCTCTAGACTAGTGGATCCATGGACGCGGACTCCTGGACGAC
TgClpB1_C_1R	CTTCTGCACCGAGTATGACGTGTA
TgClpB1_C_2F	TACACGTCATACTCGGTGCAGGAAAG
TgClpB1_C_2R	GGTACCGGGCCCCCTCGAGTCAGTATCTGAATTTCTTGACATTC
TgClpB2_C_1F	CCGCTCTAGACTAGTGGATCCATGCTGCGCCAGGGTTGT
TgClpB2_C_1R	GACAGACCAAAGCGACCTCTATATCGTGAGCTGAAATCC
TgClpB2_C_2F	GGATTCAGCTCACGATATAGAGGTGCTTTGGTCTGTC
TgClpB2_C_2R	GGTACCGGGCCCCCTCGAGTCAGTATCTGATCCTTGTAAATCCGAGAACCGAGCCGTTTCC
TgGRA16_HX_F	AAGAAGCTTGATGGGGATATCATGTATCGAAACCACTCAGG
TgGRA16_HX_R	GTCGTACGGATACATGATATCTCAAGCGTAGTCTGGGACGTCGTATGGGTACATCTGATCAATTTTCC
TgGRA24_HX_F	AAGAAGCTTGATGGGGATATCATGCTCCAGATGGCAGATATAC
TgGRA24_HX_R	GTCGTACGGATACATGATATCTTAAAGCGTAGTCTGGGACGTCGTATGGGTAATTACCCTTAGTGGGTGGTTAAC
TgPRX3_DHFR_F	AAGAAGCTTGATGGGGATATCATGGCGGCTTGCCCTTCGAGCGGCCGTTTGTCTCTCAGACAAATGGAAGGCTTGATA
TgPRX3_DHFR_R	GTCGTACGGATACATGATATCTTAAAGCATAATCTGGAACATCATATGGATAGTTTTTCAGTTGTCCAAGGTAG
TgHSPClpB1_SEQ_F	ATCGCCGGCGCCAAGTTCC
TgHSPClpB1_SEQ_R	GGTAAAGCGCCTCATCTC
TgHSPClpB2_SEQ_F1	AGGATGCTCCTCCTTAGTTT
TgHSPClpB2_SEQ_R1	GCACGAGAAGTTCGGTTT
TgHSPClpB2_SEQ_F2	AGCCCGAACTGGTAATGC
TgHSPClpB2_SEQ_R2	AGTGCGGCGACTGAGGAT

(Continued)

Primer name	Sequence
TgGRA16_SEQ_F	AACCACTCAGGGATACGGC
TgGRA16_SEQ_R	CGGACTTCGCTTTGAACACTT
TgCAS9_SEQ_1S	CCACTAGTTCTAGAGCGGCCGTTTAAACGAGCTCCAAGTAAGCAGAAG
TgCAS9_SEQ_2AS	CCGTATTACCGCCTTTGAGTGTTTAAACGAGCTGATACCGCTCGCC
TgLucb-DHFR-1F	AAGAAGCTTGATGGGGATATCATGCTGCGCCAGGGTTGT
TgLucb-DHFR-1R	GTTTTTGGCGTCTTCCATGTAGTCATCTCCTTTCAGAG
TgLucb-DHFR-2F	CTCTGAAAGGAGATGACTACATGGAAGACGCCAAAAAC
TgLucb-DHFR-2R	GTCGTACGGATACATGATATCTTAAGCATAATCTGGAACATCATATGGATACAATTTGGACTTTCCG