

Neospora caninum SBE1
Toxoplasma gondii SBE1
Besnoitia besnoiti SBE1
Eimeria tenella SBE1
Vitrella brassicaformis GlgB
Cryptosporidium parvum SBE1
Gregarina niphandrodes SBE1
Paludisphaera borealis GlgB
Aquisphaera giovannonii GlgB
Isoosphaera pallida GlgB
Thiohalomonas denitrificans GlgB
Solemya velesiana GlgB
Nitrosomonas communis GlgB
Candidatus Thiomargarita
Homo sapiens GBEL
Mus musculus GBEL
Arabidopsis thaliana SBE2

-----EGTAAAEETAAASSKGDTEQAKVYVPLLEHLPQSTLSEHDVHLLHKAHGGDPFVGLGCHEVKGQKQEK-IVVVRWIRNAKSIIRLR-ASGRSECVLIT-EEFVPMKEK---ADFLFQKAF 117
 -----KGSDEVREAGAPAKGEGRRVYVYVPLKDLPGSSLSEHDVHLLHKAHGGDPFVGLGCHEVKGQKQEK-ILIVRAWIRNAKTIIRLR-ALECECVVLA-EEFVPMKEK---ADFLFQKAF 117
 -----RKENGDCGGTAVKREGTGDPPAVNFVLPDLSLPAGASLSDYVHLLHKAHGGDPFVGLGCHEVKGQKQEK-ILIVRAWIRNAQSLQLR-ASGRSECVLA-AAITVGMKEK---AEFLFQRAF 120
 -----KEQPYTNMNDVAKVDHVEEAQRHDQTHFMAVPIVLPNADIPYDMHLLNLAHADPPRMLGCHKVECADSKRIVVVRVWLNKVDHVELRPRAGVSDWRLPPPLESGLERK---GELLPHKAS 123
 -----HEAEGSNELDAAPVTYEPFVPGEP--SYFVETLDSVPGGAHISDHDHLLHKAHGGDPFVGLGCHPVDHGDGAK-YFLVRAWIKDARDARLR-CVPGSAVQLVNDNEPVWLHKK---GEWMPQMFV 119
 -----TAANSPTSYSSSMASIPDERGDIYEALDRVPDDGKLLDHLHLLNKAHPEPFVGLVQKFG-DDGQYEVYVVRWVRNARRIQK-AIDYFSSIGTNNTPVEMEQRIIDGNPSWMPKCAF 120
 -----LHSAHADPFAVLGPHAYGGQDRAVVIRAWIKDAAEVELR-FTGSLATVPQDLTEAPASESEADHLQGERLQONHOGGHEPDQAESPASCRLOASGGVWPKLHRR---ADWLFGCAF 120
 -----MALMTRQQSIVSQADLDLITHASHWNPFITLGPSPSTKEGS-KGWTIRAFLEARRASV--DLRGEPEGK---LYPMEKTH---ADGFFBAS 86
 -----MFLVNVNADHWNPFVVLGPHMEGQNGRKSQVIRAFLEARRASLV--DLAGEPEGK---LYPMEKTH---ADGFFBAS 72
 -----SDFVASSTSSVSPSPSPSLRVDSQAASGSGSSRPSWIAEADVQRLIHADDDPFAVLGPHAEHLGVP--GWVVRAFLEPTAREATV--LITSPAPYQGGPFFMTKRVH---PDGVVEVAF 119
 -----MISLISLNEAHVKNLSEGEQKILEARHDDPFEVLGRHPDGD--GIVIRAFLEPTAREAAIE--SRDRP---LPMDRIP---NTDLFEWRG 79
 -----MDSKLEELPKRLADARHDDPFEVLGRHVDG--HAMIRAVLPQAQQARTV--GNP---LPLERIP---GTDFFPEWG 68
 -----MP-LNNDIAKIIAARHDDPFTVLGRHKEG--MIVRAFPHAKSVTIA--ENG---LTMORVG---GTDLFEWQG 65
 -----MKIPKLDLQKIVDACHDDPFSVLGKHTVRC--QLVVRVYIPYAEQVTTA--EGN---LSMRRIE---GTDLFEWQG 68
 -----MAAPMTPAAR-----PEDYEAALNAALADVPELA----- 29
 -----MAAPAAPAAG-----ETGPEARLEAALADVPELA----- 29
 MVTYISGVRFPPLPSIKKKNSLHFNEDLRRSNAVSLRKRDSRSGKVFARKPSYSDSSSLATLASEKLRHQSDSSSSASDQVQSRDTSVDDTQVLGNVDVQKLEEAQETEILDQISALSTGSGISYKEDFAK---MSHVDQEV 146
 1.....10.....20.....30.....40.....50.....60.....70.....80.....90.....100.....110.....120.....130.....140.....150



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AVHR-----VAS-EETASSP-----KRTEPVKPEALRE 146
 VVHR-----LASEKETASSP-----CKATQSKPGEILESD 147
 TVHR-----IAGDASAVNSTD-----ARLETAEPGEAVASD 151
 ANAR-----AASAFEMVTTD-----PSASSRGCVPKSS 151
 KVVR-----AAEGKPLPTKD-----S 135
 KTLKEKIPHCRTHFCGYDENLENSNIENKRIQNSNKKDDYSTDKFSDVGSSEANNKVNKNTIVSKKKEITADCNISIVEKTYSNVIVNTLNKSKTNLVPNKESHKGNEMPKLELNQSNLMSNIIPRNKNIRESACSSDEACNICYVHDNINIQ 270
 NNLPL-----ITIEAAEATD-----L 135
 PDRS----- 90
 ADDR----- 76
 AGLE----- 123
 KPDA----- 83
 TPDQ----- 72
 DGSN----- 69
 KHK----- 71
 ----- 29
 ----- 29
 GQRK----- 150
160.....170.....180.....190.....200.....210.....220.....230.....240.....250.....260.....270.....280.....290.....300



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DSPVAFKVDLLCVYEGDETNEERVADDTYFQVLLPRFDLELFQSSGCSWHDVNLGSHHILTVEGIRGVRFVAVWAPSACFVSVVGDWNGWDGRAHPMRRRIEFGVWELFVDPDIG-AGEKYGYRIHTR-GGLDIIKIDPVAQEFEPKPTAS 294
 DSPVTFKYELLCVYEGDETNEERVAHDYFQVLLPRFDLELFQSSGCSWHDVNLGSHMLTVEGIRGVRFVAVWAPSACFVSVVGDWNAWDGRAHPMRRRVEFGVWELFVDPDIG-AGEKYGYRIHTR-GGLDVIKIDPVAQEFEPKPTAS 295
 APPVTFKYEMLCIYEGDETQRERVIQDTYFQVLLPRFDLELFQSSGCSWHDVNLGSHIMTVDGQGVRFVAVWAPSACFVSVVGDWNGWDGRVHPMRRRIEFGVWELFVDPDIG-AGEKYGYRIHTR-GGTDIIKIDPVAQEFEPKPTAS 299
 DPYVPLDYELLVREGGEETP--RVVYDYSFGMTVPQNLLELFQSSGCSWHDVNLGSHIITVDGVTGVRFVAVWAPNCICVSVVGDWNGWDGRAHPMRRRVEFGVWELFVDPDIG-AGEKYGYRIHTR-NGTDFIKIDPVAQEFEPKPTAS 297
 HDLPPFLVELIVREGDGSNTEPVKRDANFSGCLLPDFDLNLFQSSGCSWHDVCLLGGHVVEVDAAGVRFVAVWAPNAVFSVVDWNGWDGRAHPMRRRHHYGVWELFIADVA-PGMKYGYRIHTR-NGMDFVKIDPVAQEFENPPAYAS 283
 CVRKLYVELLVEVNGDSDGKVAIRDYFSGLLLSNGDMELFQSSGCSWHDVNLGSHIIEVNGVGVRFVAVWAPNAQFLSVVGFNQWDGRAHPMRRRHHYGVWELFVDPDIPRVGHRVMYKIHTR-RGTDIMKIDPVAQEFENPPAHAS 418
 LAVPVEVELRVKICGDASNQSILKEDANRFCSLGEDQLRFLQSSGCSWHDVNLGSHIFVEVGGVRFVAVWAPNAQFLSVVGFNQWDGRAHPMRRRHHYGVWELFVDPDIPRVGHRVMYKIHTR-RGTDIMKIDPVAQEFENPPAHAS 284
 ---EAFVRLKIE--NHEGHSWELVDFVAFEPVLTDFDLHLLGEGTHLHNYERLGAHLRMHEGFRGVHFAVWAPNAQRVSVVGNFNHWDGRRHQLNRGAAGIWEIFIPDIT-AGEVYKFEIKSRHSDYLVQKSDPYGFAEFRPKTAS 233
 ---AAPRVRFRIE--NFEGHSWEQVDFVQFQVPLTDFDLHLLGEGTHLHNYERLGAHLRMHEGFRGVHFAVWAPNAQRVSVVGNFNHWDGRRHQLNRGAAGIWEIFIPDIT-AGEVYKFEIKSRHSDYLVQKSDPYGFAEFRPKTAS 219
 ---GRPRVIRAT--DAEGRSWSQVDFVQFGRVLTDFDLHLLGEGTHYKNEYKMGAHIMEHEGVGHFAVWAPNARRVSVVGDWNGWDGRRLPMMRCGPHGIWEIFVPGVLV-QGDLVKKFETQSGFHGYLVEKSDPYGFAEFRPKTAS 266
 ---VPERVALVLT--DHHGRTRIRHDDYAFPPRLTDFDLHLLGEGTHYKNEYKMGAHIMEHEGVGHFAVWAPNARRVSVVGDWNGWDGRRLPMMRCGPHGIWEIFVPGVLV-QGDLVKKFELRDR-NGGTHLKTDPYGRFELRPSHAA 225
 ---VPERVQIAWT--DNYGHSARVDFVCFPPQLDFDVLHLLGEGTHYKNEYKMGAHIMEHEGVGHFAVWAPNARRVSVVGDWNGWDGRRLPMMRCGPHGIWEIFVPGVLV-QGDLVKKFELRDR-NGGTHLKTDPYGRFELRPSHAA 214
 ---LPQHYRFIWH--DYDNYEIHADDPYVLPQLGDFDLHLLFAEGKHWAYRFLGAHPHEVNVNRCGLFSVWAPGAERVSIVGDFNRWDGRRHMPMRVQGYGVWELFIPDVH-PGMLYKFEIRNKEYGTIHSKIDPYGQEFELRPRNAS 212
 ---LPDRYRLIWR--DKEHRTIHTDPCFPAQLSDFDMLHLLFAEGKHWAYRFLGAHPHEVNVNRCGLFSVWAPGAERVSIVGDFNRWDGRRHMPMRVQGYGVWELFIPDVH-PGMLYKFEIRNKEYGTIHSKIDPYGQEFELRPRNAS 214
 ---RLEIDPYLKPVAVDQRRYKQFQILKNIGENEGGIDKFSRGEYFVGHRCADGGLYCKEAWPAGVFLGDFGNWNPFSYPYK-KLDYKWEIYIPPKQKSVLPHGSKL---VVITSKSGEILYRISPAWKY 163
 ---RLEIDPYLKPVAVDQRRYKQFQILKNIGENEGGIDKFSRGEYFVGHRCADGGLYCKEAWPAGVFLGDFGNWNPFSYPYK-KLDYKWEIYIPPKQKSVLPHGSKL---VVITSKSGEILYRISPAWKY 163
 ---LPPPGDGKRIYDIDPMLNSRNHLDYRYQYRKLREIDKNEGGLEAFSRGEYFVGHRCADGGLYCKEAWPAGVFLGDFGNWNPFSYPYK-KLDYKWEIYIPPKQKSVLPHGSKL---VVITSKSGEILYRISPAWKY 290
310.....320.....330.....340.....350.....360.....370.....380.....390.....400.....410.....420.....430.....440.....450



Neospora_caninum_SBE1 IISACDDAFRKP... 441
Toxoplasma_gondii_SBE1 IISACDDAFRKP... 442
Besnoitia_besnoiti_SBE1 IISACDDAFRKP... 446
Eimeria_tenella_SBE1 IISACDDAFRKP... 444
Vitrella_brassicaformis_GlgB IVSGCDDAYR... 430
Cryptosporidium_parvum_SBE1 IISACDDAYR... 565
Gregarina_niphandrodes_SBE1 VISGDDAYR... 429
Paludisphaera_borealis_GlgB VVV-----... 370
Aquisphaera_giovannonii_GlgB VVV-----... 356
Isosphaera_pallida_GlgB VVV-----... 403
Thiohalomonas_denitrificans_GlgB IVE-----... 360
Solemya_velesiana_GlgB IIT-----... 349
Nitrosomonas_communis_GlgB IIA-----... 347
Candidatus_Thiomargarita IVI-----... 349
Homo_sapiens_GBE1 VVREGDN... 302
Mus_musculus_GBE1 VVRENNN... 302
Arabidopsis_thaliana_SBE2 SVQPPGE... 431



Neospora_caninum_SBE1 YDGEPCY... 571
Toxoplasma_gondii_SBE1 YDGEPCY... 572
Besnoitia_besnoiti_SBE1 YDGEPCY... 576
Eimeria_tenella_SBE1 YDGEPCY... 592
Vitrella_brassicaformis_GlgB YDGVPL... 560
Cryptosporidium_parvum_SBE1 YDGTPT... 695
Gregarina_niphandrodes_SBE1 FDGEP... 575
Paludisphaera_borealis_GlgB FDGTH... 502
Aquisphaera_giovannonii_GlgB FDGTH... 488
Isosphaera_pallida_GlgB FDGTH... 535
Thiohalomonas_denitrificans_GlgB FDGTP... 492
Solemya_velesiana_GlgB FDGTAL... 481
Nitrosomonas_communis_GlgB FDGTP... 477
Candidatus_Thiomargarita FDGTP... 479
Homo_sapiens_GBE1 FDGTD... 436
Mus_musculus_GBE1 FDGTD... 436
Arabidopsis_thaliana_SBE2 FDGTD... 565



Neospora_caninum_SBE1 LGWMD... 698
Toxoplasma_gondii_SBE1 LGWMD... 699
Besnoitia_besnoiti_SBE1 LGWMD... 703
Eimeria_tenella_SBE1 LGWMD... 717
Vitrella_brassicaformis_GlgB LGWMD... 679
Cryptosporidium_parvum_SBE1 LGWMD... 820
Gregarina_niphandrodes_SBE1 LGWMD... 682
Paludisphaera_borealis_GlgB MGWMD... 624
Aquisphaera_giovannonii_GlgB MGWMD... 610
Isosphaera_pallida_GlgB MGWMD... 657
Thiohalomonas_denitrificans_GlgB MGWMD... 614
Solemya_velesiana_GlgB MGWMD... 603
Nitrosomonas_communis_GlgB MGWMD... 599
Candidatus_Thiomargarita MGWMD... 601
Homo_sapiens_GBE1 MAIPDK... 574
Mus_musculus_GBE1 MAIPDK... 574
Arabidopsis_thaliana_SBE2 MAIAD... 713



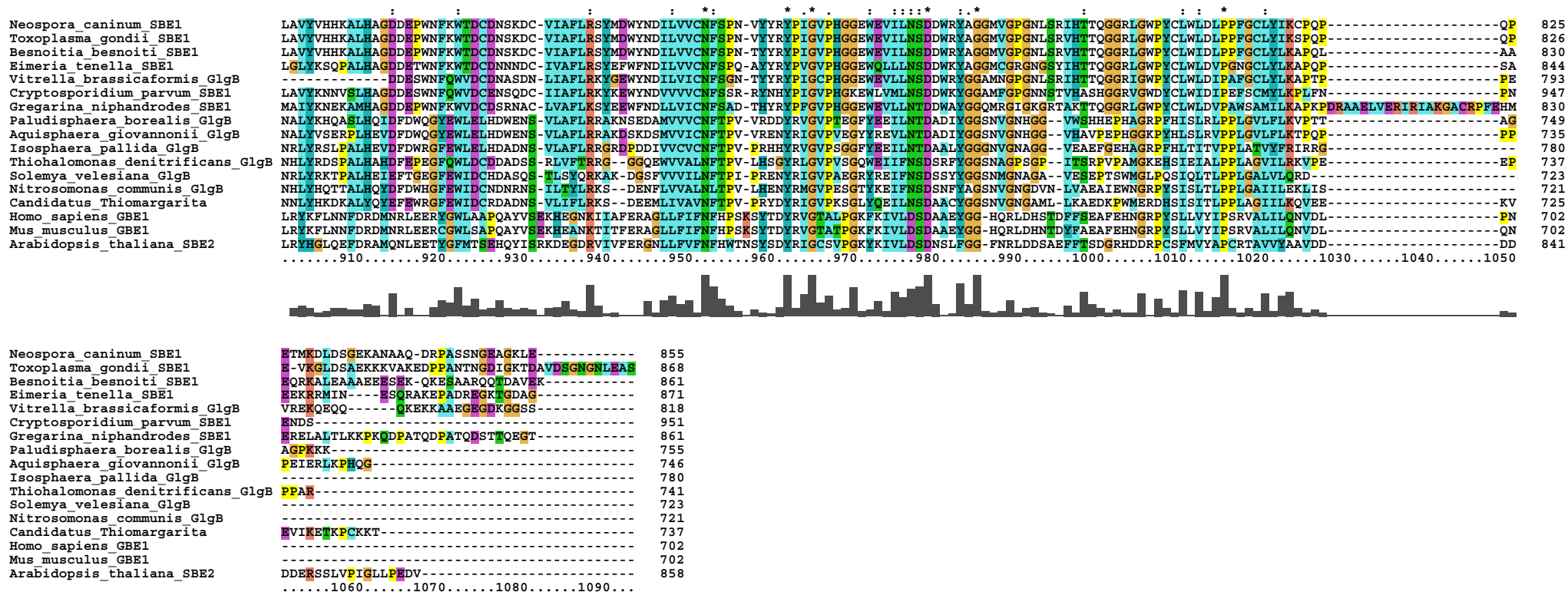
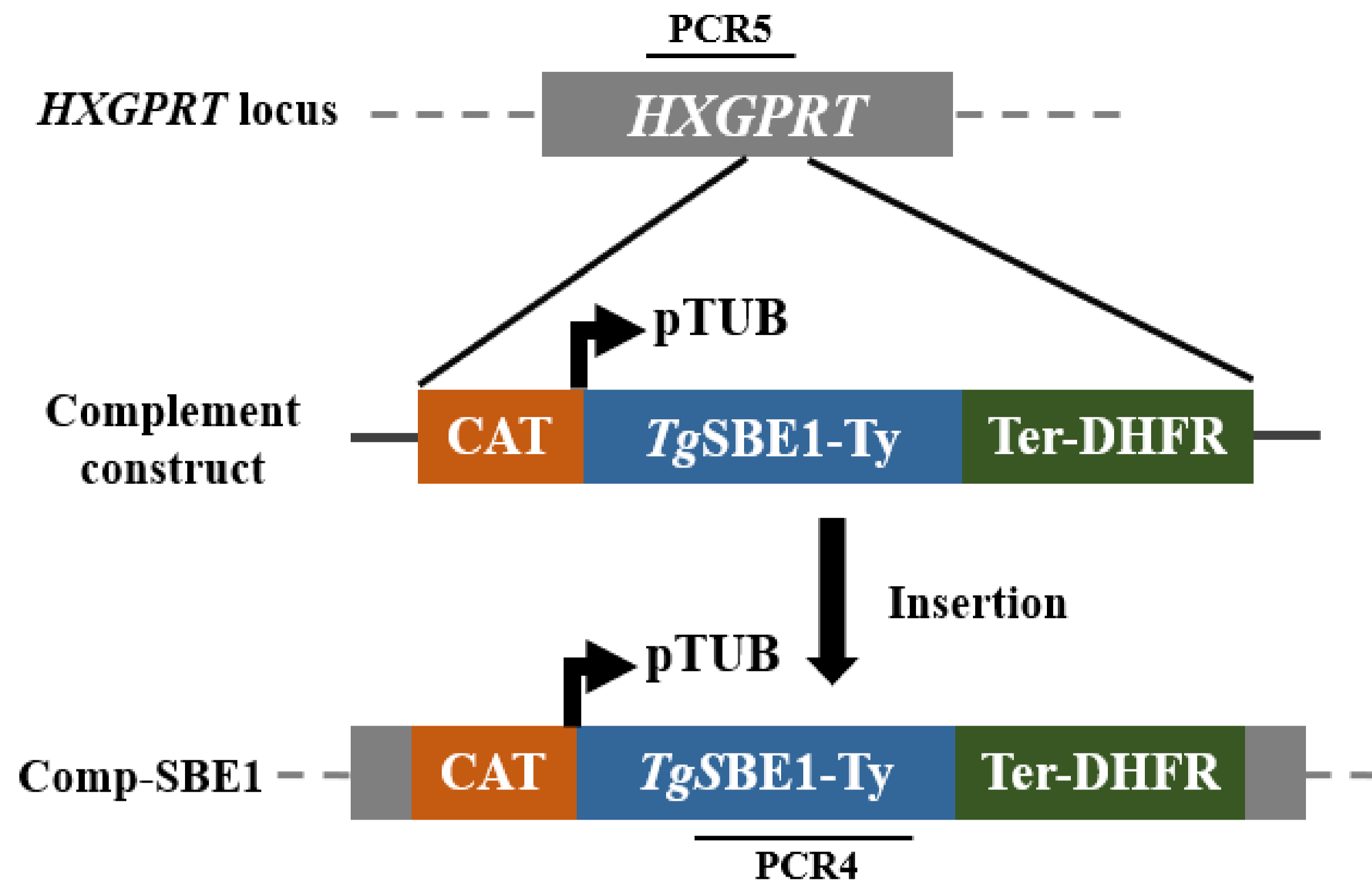
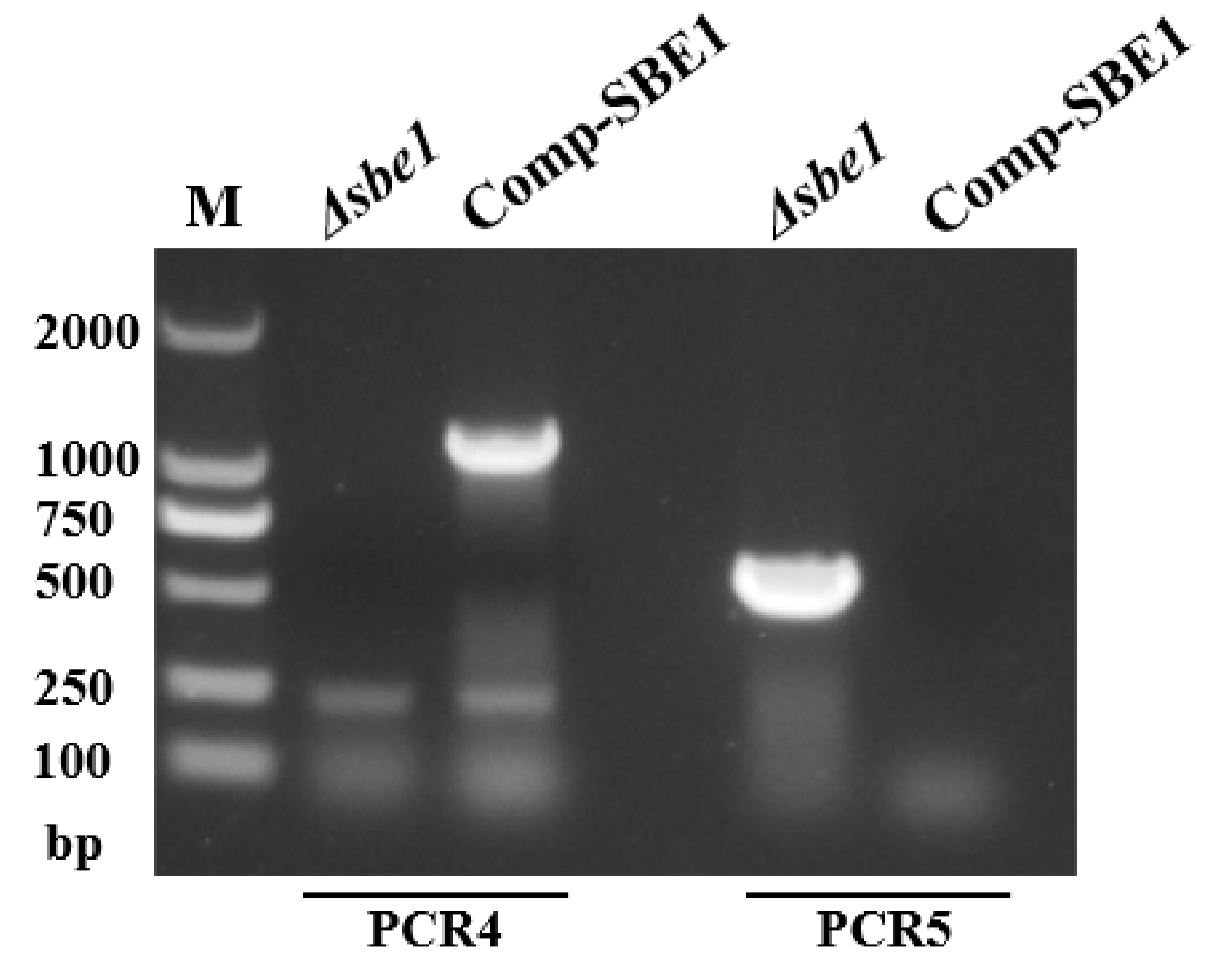


Fig. S1. Alignment of amino acid sequence and phylogenetic relationships analysis of starch branching enzyme in *T. gondii* and selected species.

A



B



C

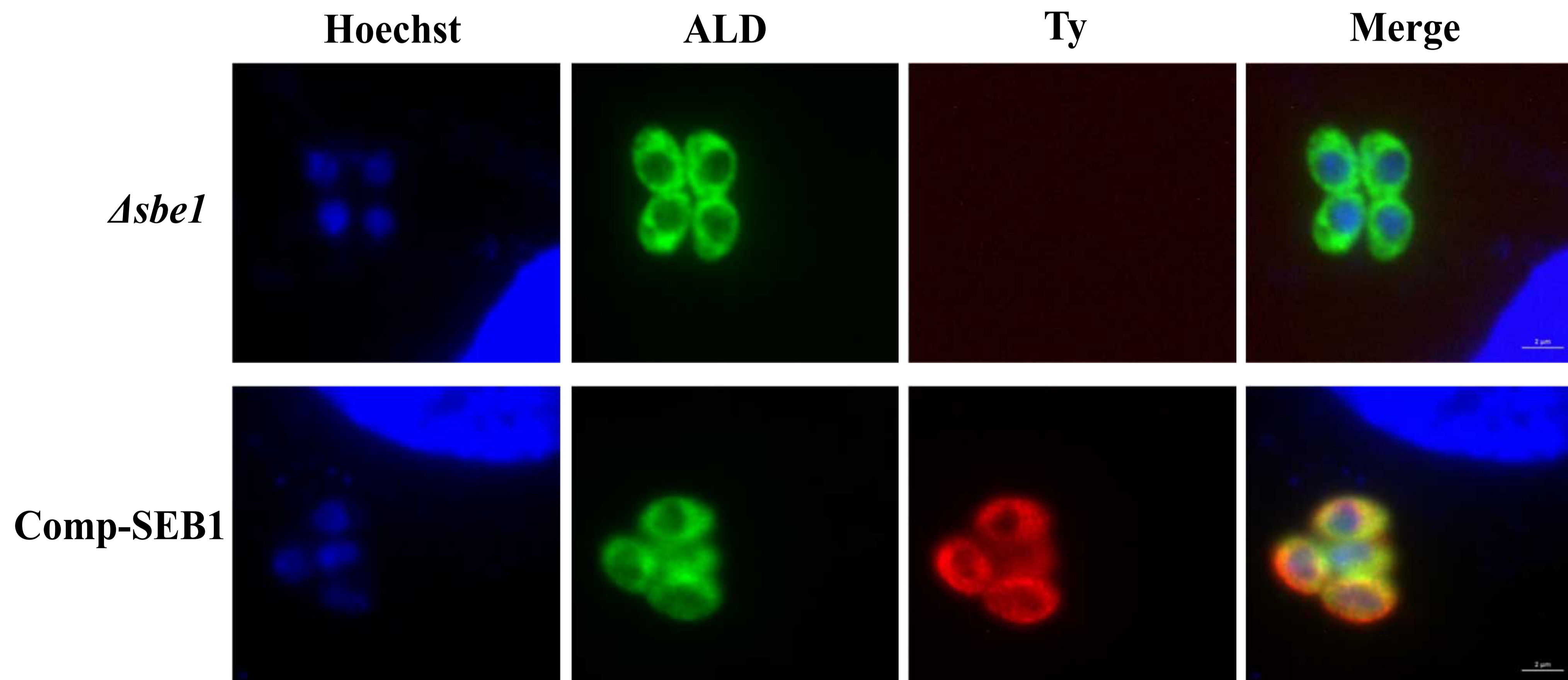


Fig. S2. Construction of SBE1 complementation strains.

A. Schematic illustration of generation of the Comp-SBE1 strain via CRISPR/CAS9-mediated technique.

B. Diagnostic PCRs on a Comp-SBE1 clone. C. The presence of SBE1 was confirmed by IFA. Samples were stained with mouse anti-Ty and rabbit anti-*T. gondii* ALD.

Table S1: Primers used in this study.

Primer	Sequence (5'-3')	Used for
gRNA- <i>SBE1</i> -Fw	GGGTCTGCATATTCATAGCAGTTTTAGAGCTAGAAAT AGC TAGAAATAGC	Construction of gRNA plasmid for <i>SBE1</i>
gRNA-Rv	AACTTGACATCCCCATTTAC	knockout
pUC19-Fw	GGCGTAATCATGGTCATAGC	Amplification of pUC19
pUC19-Rv	ACTGGCCGTCGTTTTACAAC	vector
loxp-DHFR*-loxp-Fw	CAACCCGCGCAGAAGACATC	Amplification of loxp-
loxp-DHFR*-loxp-Rv	GGACACGCTGAACTTGTGGC	DHFR*-loxp fragment
U5- <i>SBE1</i> -Fw	GTTGTAAAACGACGGCCAGTCTCTCATGCACTCAAC ATC	Amplification of the 5' homologous arm of
U5- <i>SBE1</i> -Rv	GATGTCTTCTGCGCGGGTTGCTTCTCCTGACTGAGG TGAC	<i>SBE1</i>
U3- <i>SBE1</i> -Fw	GCCACAAGTTCAGCGTGTCCGTGAAGGCGCGTGAA AACCC	Amplification of the 3' homologous arm of
U3- <i>SBE1</i> -Rv	GCTATGACCATGATTACGCCCGTACTCCTCCATCTTG GC	<i>SBE1</i>
5'-UpU5- <i>SBE1</i>	GGTAGCCCGATATTCACATGCAG	Verification of
3'-In DHFR*	GATTTGTGAGGACGACTCAC	<i>SBE1</i> deletion by PCR1
5'-In DHFR*	CACGACAGCAGACAACCTTC	Verification of <i>SBE1</i>
3'-DnU3- <i>SBE1</i>	GTGCCGTGCGAAAAACACGGAAC	deletion by PCR2
In- <i>SBE1</i> -Fw	GTCACAGGTTTATACGCGCC	Verification of <i>SBE1</i>

In- <i>SBE1</i> -Rv	GCTCTTGGAGGAGAGACATG	deletion by PCR3
Vec-Fw	GAGGTCCACACGAACCAGGAC	Amplification of the
Vec-Rv	CTTGCTCACCATTTTAGATC	complementation vector
<i>SBE1</i> -CDS-Fw	GATCTAAAATGGTGAGCAAGATGGGAGATCTTCGAG CATC	Amplification of <i>SBE1</i>
<i>SBE1</i> -CDS-Rv	GTCCTGGTTCGTGTGGACCTCCTCCTGGCGCTCGTA AACAG	CDS
gRNA- <i>HXGPRT</i> -Fw	GCAGAACTTACTTCGGCGAGGGTTTTAGAGCTAGAA ATAGC	Construction of gRNA plasmid for <i>HXGPRT</i> knockout
PCR4- <i>SBE1</i> -Fw	GTGGACATGGCCGCTGCATG	Verification of <i>SBE1</i>
PCR4- <i>SBE1</i> -Rv	GTTGTTGTCGCCACGCATCCAG	complementation by PCR4
In- <i>HXGPRT</i> -Fw	CAAGGGCCGTATTGAGCCCATG	Verification of <i>HXGPRT</i>
In- <i>HXGPRT</i> -Rv	CACACGCCTTCCAAATGTCGCG	deletion by PCR5
CAT-Fw	CGTGTTTACTCGGATACTGACGAGGTCGACGGTATC GATAAGC	Amplification of CAT
CAT-Rv	CCCTGCCACTGTTTATTCGCGCTCTAGAACTAGTGGA TCG	fragment
p- <i>SBE1</i> -CAT-U5-Fw	GATGCTACACATTTCTCACC	Amplification of the 5'
p- <i>SBE1</i> -CAT-U5-Rv	GTCAGTATCCGAGTAAACACG	homologous arm of <i>SBE1</i> based on CAT tag

p- <i>SBE1</i> -CAT-U3-Fw	CGAATAAACAGTGGCAGGG	Amplification of the 3'
p- <i>SBE1</i> -CAT-U3-Rv	AGTTGCTAGCTCTACTTCGC	homologous arm of <i>SBE1</i> based on CAT tag
3'-In CAT	GCACTGTGAGCACTGATAGG	Verification of <i>SBE1</i> deletion by PCR1
5'-In CAT	GGAGTGTTTCGCAGCAAGC	Verification of <i>SBE1</i> deletion by PCR2
