

S3 Table Primers and plasmids used in this study

Primer Category	Primer Name	Primer Sequence	Use	Used for plasmid	Source	
<u>AAH2 Knockout, Cleanup, Complementation</u>	222 TgAaaH2 KO fwd cassette fwd primer attB4	5'GGGGACAACCTTTGTATAGAAAAGTTGCAATTGTGCTACGCAAGCTCGGC3'	AAH2 Knockout	<i>pAah2::HXG</i>	Wang et al. 2014	
	223 TgAaaH2 KO fwd cassette rev primer attB1r	5'GGGGACTGCTTTTTGTACAAACTTGCAAAAGCATTAGGCAAGGCTCGAG3'	AAH2 Knockout	<i>pAah2::HXG</i>	Wang et al. 2014	
	224 TgAaaH2 KO rev cassette fwd primer attB2r	5'GGGGACAAGCTTTCTGTACAAAAGTGGAAAGTCGTGCTCCCTCGGATTCG3'	AAH2 Knockout	<i>pAah2::HXG</i>	Wang et al. 2014	
	225 TgAaaH2 KO rev cassette rev primer attB3	5'GGGGACAACCTTTGTATAAATAAAGTTGGGTGAACATCGCGAGATGAAAGACCC3'	AAH2 Knockout	<i>pAah2::HXG</i>	Wang et al. 2014	
	222 TgAaaH2 KO fwd cassette fwd primer attB4	5'GGGGACAACCTTTGTATAGAAAAGTTGCAATTGTGCTTACGCAAGCTCGGC3'	AAH2 Cleanup	<i>pAah2</i>	Wang et al. 2014	
	269 TgAaaH2 Clean KO pri1 rev	5'CGAATCGCAGGGGACGACGACTTCAAAGCATTAGGCAAGGCTCGAGG3'	AAH2 Cleanup	<i>pAah3</i>	Wang et al. 2014	
	270 TgAaaH2 Clean KO pri2 fwd	5'AAAGTCGTGCTCCCTCGGATTCG3'	AAH2 Cleanup	<i>pAah4</i>	Wang et al. 2014	
	271 TgAaaH2 Clean KO pri2 rev attB1r	5'GGGGACTGCTTTTTGTACAAACTTGGTGAACATCGGAGATGAAAGACCC3'	AAH2 Cleanup	<i>pAah5</i>	Wang et al. 2014	
	260 TgAaaH2 Complement pri1 fwd attB4	5'GGGGACAACCTTTGTATAGAAAAGTTGTGCGAGCAGTATCAAATCTGG3'	AAH2 Complement	<i>pAAH2</i>	Wang et al. 2014	
	272 TgAaaH2 Complement pri1 rev	5'GCACGACGCGGGTGTAGAGACATCGATTAGAATGGTTCAGGTGATCGGAG3'	AAH2 Complement	<i>pAAH2</i>	Wang et al. 2014	
	266 TgAAH1/2 cDNA Fwd	5'ATGTCCTATCCACCGCTCGC3'	AAH2 Complement	<i>pAAH2</i>	Wang et al. 2014	
	273 TgAAH2 Complement pri3 fwd	5'GCCTCTGCTCCCTCAAGATCTAGACAGACACTGTATCTCTACTTGTGATTGG3'	AAH2 Complement	<i>pAAH2</i>	Wang et al. 2014	
	265 TgAAH2 Complement pri3 rev attB1r	5'GGGGACTGCTTTTTGTACAAACTTGCCTCGCTGACTGTATCTGCTTCG3'	AAH2 Complement	<i>pAAH2</i>	Wang et al. 2014	
	A14 HXGPRT 5' Rev screening primer	5'CATACTAGTTTCAATGATCCCCCTCACCCGG3'	HXGPRT Screening		Wang et al. 2014	
	A26 HXGPRT 3' Fwd screening primer	5'GATGAATTCAGCAGCAAACTTTCATTTAAAC3'	HXGPRT Screening		Wang et al. 2014	
	228 AAH1/2 internal 5' primer	5'GGGGACAACCTTTGTATAGAAAAGTTGCAGGATTCCTCTCACCAGGGG3'	<i>Δaah2</i> Screening		Wang et al. 2014	
	268 AAH2 3' rev	5'CTAGATCTTGAGGGAGACAGGAGGCATATG3'	<i>Δaah2</i> Screening		Wang et al. 2014	
	<u>AAH1 Knockout & Complementation</u>	X67 AAH1 Knockout 5' fwd pUC19 homology	5'TTGTAAAACGACGGCCAGTGGCATGTTTCATGCAAGTTGGTGTCTC3'	AAH1 Knockout	<i>pAah1::DHFR-Ts</i>	This paper
		X68 AAH1 Knockout 5' rev DHFR homology	5'GCTATACGAAGTTATGGATCCATCGAGGGACAGACTCGCGTACATCG3'	AAH1 Knockout	<i>pAah1::DHFR-Ts</i>	This paper
		X69 AAH1 Knockout 3' fwd DHFR homology	5'ATTATACGAAGTTATCGATGCTGAGTCTGGTGTCTCCCACTATCTCTGC3'	AAH1 Knockout	<i>pAah1::DHFR-Ts</i>	This paper
		X70 AAH1 Knockout 3' rev pUC19 homology	5'GATTACGCCAAGCTTGCATGAGACACTCCGACAGTCAGGAATCC3'	AAH1 Knockout	<i>pAah1::DHFR-Ts</i>	This paper
		E06 DHFR-Ts Construct 5'	5'GATGGATCCATAACTCGTATAGCATACATTATACGAAGTTATCAGCAGCAAACTTGCATTCAAACCCGCAAH1 Knockout	AAH1 Knockout	<i>pAah1::DHFR-Ts</i>	This paper
		E07 DHFR-Ts Construct 3'	5'CTAGCATGATAACTCGTATAATGTATGCTATCAAGATTATATCTGCAAGTGCATAGAAAGAAAGTT(AAH1 Knockout)	AAH1 Knockout	<i>pAah1::DHFR-Ts</i>	This paper
		X44 UPRT 5' UTR fwd pUC19 homology	5'TTGTAAAACGACGGCCAGTGGGAAACCATATTTGATTGACAGCTTG3'	AAH1 Knockout	<i>pDuprt::AAH1</i>	This paper
		X48 UPRT 5' UTR rev +loxP X25 homology	5'GACCTCAATTTTGGCGAATAACTCGTATAATGTATGCTATACGAAGTTATTCAGAGATAACGGTTC(AAH1 Knockout)	AAH1 Knockout	<i>pDuprt::AAH1</i>	This paper
		X25 AAH1 5' UTR fwd	5'ATCCGACAAAATGGAAGTCCGAGAGAAAGAAACAACTCCGTC3'	AAH1 Knockout	<i>pDuprt::AAH1</i>	This paper
283 AAH1 5' UTR rev		5'CGCAGCGGTTGGATAGAGACATCGATTTAGAAATACACGGTTACCCGAGAC3'	AAH1 Knockout	<i>pDuprt::AAH1</i>	This paper	
266 AAH1/AAH2 cDNA 5' fwd		5'ATGTCCTATCCACCGCTCGC3'	AAH1 Knockout	<i>pDuprt::AAH1</i>	Wang et al. 2014	
267 AAH1 cDNA 3' rev		5'CTGAACCTGAGGGAAACGGGACAGACTCG3'	AAH1 Knockout	<i>pDuprt::AAH1</i>	Wang et al. 2014	
284 AAH1 3' UTR fwd		5'CGAGCTCGCCGTTTCCCTCAGGTTCTAGGTTACAGACCAGCTACTCATTTCCAGTTG3'	AAH1 Knockout	<i>pDuprt::AAH1</i>	This paper	
X19 AAH1 3' UTR rev		5'GTTTCTGCTGGAATTCATCGATGGCAACGAACAGTATGTTGC3'	AAH1 Knockout	<i>pDuprt::AAH1</i>	This paper	
X49 UPRT 3' UTR fwd loxP X19 homology		5'GATGAATCCAGCACGAACAATAAATCGTATAGCATACATTATACGAAGTTATGTTGATATCTTTGTCGCAAH1 Knockout	AAH1 Knockout	<i>pDuprt::AAH1</i>	This paper	
X47 UPRT 3' UTR rev pUC19 homology		5'GATTACGCCAAGCTTGCATGTGACCGCAGAAAGCACTGCTATCC3'	AAH1 Knockout	<i>pDuprt::AAH1</i>	This paper	
X58 HXG insertion into pDuprt::AAH1 fwd		5'GAAGAGAAGAAAAAATGGATGAATCCAGCACGAACCTTCG3'	AAH1 Knockout	<i>pDuprt::AAH1::HXG</i>	This paper	
X59 HXG insertion into pDuprt::AAH1 rev		5'TTCTCGCGGGTGGCCTTGCATACTAGTTTCAATGATCCCCCTC3'	AAH1 Knockout	<i>pDuprt::AAH1::HXG</i>	This paper	
<u>AAH1/AAH2 Knockout Screening</u>		Y46 TgTubulin fwd	5'GGGGACAGCTTTCTGTACAAAAGTGGAAAGCGTAACCTCAGGACGCTTG3'	Screening		This paper
	Y47 TgTubulin rev	5'CGCAGCGGTTGGATAGAGACATAAAAGGGAATTCAGAAAAATGCCAACGAGTAG3'	Screening		This paper	
	228 AAH1/AAH2 fwd (exon)	5'GGGGACAACCTTTGTATAGAAAAGTTGCAGGATTCCTCTCACCAGGGG3'	Screening		Wang et al. 2014	
	267 AAH1 3' rev	5'CTGAACCTGAGGAAACGGGACAGCTCG3'	Screening		Wang et al. 2014	
	268 AAH2 3' rev	5'CTAGATCTTGAGGGAGACAGGAGGCATATG3'	Screening		Wang et al. 2014	
<u>AAH1/AAH2 Locus Screening</u>	228 AAH1/AAH2 fwd (exon)	5'GGGGACAACCTTTGTATAGAAAAGTTGCAGGATTCCTCTCACCAGGGG3'	PCR Amplification		Wang et al. 2014	
	265 AAH1/AAH2 rev (3' UTR homologous region)	5'GGGGACTGCTTTTTGTACAAACTTGCCTTCCTGACTGTATCTGCTTCG3'	PCR Amplification		Wang et al. 2014	
	228 AAH1/AAH2 fwd (exon)	5'GGGGACAACCTTTGTATAGAAAAGTTGCAGGATTCCTCTCACCAGGGG3'	Sanger Sequencing		Wang et al. 2014	
<u>CRISPR Plasmid Mutagenesis</u>	X13 CRISPR HXGPRT Targeting Site 1	5'CACATCATTTGCATCCTGAAGTTTTAGAGCTAGAAATAGC3'	5' sgRNA for Q5 mutagenesis	<i>pSAG1::CAS9,U6:dgAAH1</i>	This Paper	
	X14 CRISPR AAH1 Targeting Site 1 (5' UTR)	5'TCCCTATTGCTTCTGTACGTTTTAGAGCTAGAAATAGC3'	5' sgRNA for Q5 mutagenesis	<i>pSAG1::CAS9,U6:dgAAH1</i>	This Paper	
	X15 CRISPR AAH1 Targeting Site 2 (3' UTR)	5'TATCAGAGAGCACAGCAACGGTTTTAGAGCTAGAAATAGC3'	5' sgRNA for Q5 mutagenesis	<i>pSAG1::CAS9,U6:dgAAH1</i>	This Paper	
	X16 CRISPR AAH2 Targeting Site 1 (5' UTR)	5'AATTCTCAATTGCAGCACAGTTTTAGAGCTAGAAATAGC3'	5' sgRNA for Q5 mutagenesis	<i>pSAG1::CAS9,U6:dgAAH2</i>	This Paper	
	X17 CRISPR AAH2 Targeting Site 2 (3' UTR)	5'GTTACGTTCTTCTCGTGTTTAGAGCTAGAAATAGC3'	5' sgRNA for Q5 mutagenesis	<i>pSAG1::CAS9,U6:dgAAH2</i>	This Paper	
	X50 CRISPR HXGPRT Targeting Site 2	5'TGTTGTGCACTCTCAATGAGTTTTAGAGCTAGAAATAGC3'	5' sgRNA for Q5 mutagenesis	<i>pSAG1::CAS9,U6:dgHXGPRT</i>	This Paper	
	3'-Rop18-gRNA rev	5' AACTTGACATCCCAATTAAC3'	3' Primer for Q5 mutagenesis	all CAS9 plasmids	This Paper	
	sgRNA cassette KpnI fwd	5'CGAATGGGTACCCCAAGTAAAGCAGAACGACCTCG3'	Amplifying 2ndary sgRNA	all CAS9 doublecutter plasmids	Behnke et al. 2015*	
	sgRNA cassette XhoI rev	5'TGCACCTCGAGAATTAACCTCACTAAAGG3'	Amplifying 2ndary sgRNA	all CAS9 doublecutter plasmids	Behnke et al. 2015*	

* Behnke MS, Khan A, Lauron EJ, Jimah JR, Wang Q, et al. (2015) Rhostry Proteins ROP5 and ROP18 Are Major Murine Virulence Factors in Genetically Divergent South American Strains of Toxoplasma gondii. PLOS Genetics 11(8): e1005434. doi: 10.1371/journal.pgen.1005434