

## **SUPPLEMENTAL TABLES**

**Supplemental Table 1. Constructs and primers used in this study.**

<b>AP2IX-4<sup>HA</sup></b>				
<b>Amplicon</b>	<b>Primer</b>	<b>Direction</b>	<b>Sequence</b>	<b>Cloning Notes</b>
AP2IX-4 (CDS)	Ap2ix4Ha3-F	FWD	<u>TACTTCCAATCCAATTTAATGC</u> AGCAGCATCCAACCTCGCCCT	Insertion into pLIC-3xHA via PacI linearization; underlined portion contains PacI homology
AP2IX-4 (CDS)	Ap2ix4Ha3-R	REV	<u>TCCTCCACTTCCAATTTTAGC</u> TGCGAGGACGGGGACGCTG	Insertion into pLIC-3xHA via PacI linearization; underlined portion contains PacI homology

<b>Δap2IX-4</b>				
<b>Amplicon</b>	<b>Primer</b>	<b>Direction</b>	<b>Sequence</b>	<b>Cloning Notes</b>
AP2IX-4 upstream genomic	Ap2ix4B4-F	FWD	<u>GGGGACAACCTTTGTATAGAAAAGTTG</u> CTCGCAACAGCCACTCCCCG	underlined portion contains attB4 for integration into pDONR P4-P1R
AP2IX-4 upstream genomic	Ap2ixB1r-R	REV	<u>GGGGACTGCTTTTTTGTACAAACTTG</u> CCAACAACGCAAGCAGCCCC	underlined portion contains attB1r for integration into pDONR P4-P1R
AP2IX-4 downstream genomic	Ap2ix4B2r-F	FWD	<u>GGGGACAGCTTTCTTGTACAAAGTGG</u> TCAACACGGCGTCCAGCGTC	underlined portion contains attB2r for integration into pDONR P2R-P3
AP2IX-4 downstream genomic	Ap2ix4B3-R	REV	<u>GGGGACAACCTTTGTATAATAAAGTTG</u> GCCGATCAGCCACTCCCTG	underlined portion contains attB3 for integration into pDONR P2R-P3
DHFR <sup>+</sup> -TS minigene	DHFR1st-F	FWD	<u>GGGGACAGCTTTCTTGTACAAAGTGGCT</u> CAGGAAAGACAGGCTCCGGA	amplicon spans promoter and coding sequence; underlined portion contains attB1 for integration into pDONR221
DHFR <sup>+</sup> -TS minigene	DHFR1st-R	REV	GGGGACAACCTTTGTATAATAAAGTTGCTGACCGCTGATACATGATGCA	amplicon spans promoter and coding sequence
DHFR <sup>+</sup> -TS minigene	DHFR2nd-F	FWD	TATCTAGAGACCCCAAGAGGGGCATC	amplicon is coding sequence with overlapping homology to DHFR1st-F and DHFR-1st R amplicon
DHFR <sup>+</sup> -TS minigene	DHFR2nd-R	REV	<u>TAGCGGCCGCGGGGACCACCTTTGTACAAGAAAGCTGGGT</u> AGGATCGATCCCCCGTTTG	amplicon is coding sequence with overlapping homology to DHFR1st-F and DHFR-1st R amplicon; underlined portion contains attB2 for integration into pDONR221

<b>Δap2IX-4::AP2IX-4<sup>HA</sup></b>				
<b>Amplicon</b>	<b>Primer</b>	<b>Direction</b>	<b>Sequence</b>	<b>Cloning Notes</b>
AP2IX-4 upstream genomic	IX-4p-F	FWD	<u>TTCCAATCCAATTTAGC</u> CACCGCTACCATGACTGGGGA	Insertion into pLIC-3xHA via PacI linearization; underlined portion contains PacI homology
AP2IX-4 upstream genomic	IX-4p-R	REV	CCCCCGTGTCTACCGACAT	Insertion into pLIC-3xHA
AP2IX-4 (CDS)	IX-4cDNA-F1	RWD	ATGTCGGTAGACACGGGGGG	Insertion into pLIC-3xHA
AP2IX-4 (CDS)	IX-4cDNA-R1	REV	TGCAGATACTTGGGGACGCA	Insertion into pLIC-3xHA
AP2IX-4 (CDS)	IX-4cDNA-F2	FWD	TGCGTCCCCAAGTATCTCGCA	Insertion into pLIC-3xHA
AP2IX-4 (CDS)	IX-4cDNA-R2	REV	<u>CCACTTCCAATTTTAGC</u> TGCGAGGACGGGGACGCT	Insertion into pLIC-3xHA via PacI linearization; underlined portion contains PacI homology

**Confirmation of gene deletion and correct construct integration in  $\Delta ap2IX-4$  and in  $\Delta ap2IX-4::AP2IX-4^{HA}$** 

Primer	Direction	Sequence
A	FWD	TCCCTGTGCCACGCCGAAAT
B	FWD	TCAGCACGCAAACCACCGCA
C	FWD	TAGGGACAGTCTCCGCTCCCA
D	REV	AGGACACACCACTGTCACCCC
E	REV	TAGCTGATGCAAGCGCCCGA
F	FWD	TCTGCCCACGACAGCAGACA
G	REV	GCCATTGCGGTGTCGTGGAT
H	FWD	CTGCGGCTGTTGATTCTCC
I	FWD	CCTAGGTACCCGTACGACGT
J	REV	TACCGGCACCACTTCTCGTACT

**RT-PCR confirmation of gene deletion; GAPDH control**

Primer	Direction	Sequence
B	FWD	TCAGCACGCAAACCACCGCA
E	REV	TAGCTGATGCAAGCGCCCGA
qPCR_GAPDH_Fw	FWD	GGTGTTCCTGCTGCGAT
qPCR_GAPDH_Rv	REV	GCCTTTCCGCCGACAAT

**Supplemental Table 2. Differentially expressed genes (FC $\geq$ 2) under tachyzoite conditions in PruQ $\Delta$ *ap2IX-4* compared to parental PruQ parasites.**

Probe Set ID	Gene	Annotation	FC of PruQ $\Delta$ ap2IX-4 vs. PruQ Parental
80.m02181_at	TGME49_288950	AP2IX-4	-32.9
611.m00052_at	TGME49_317705	enoyl-CoA hydratase/isomerase family protein	-5.7
55.m00162_at	TGME49_261650	hypothetical protein	-4.7
83.m01216_at	TGME49_293480	MoeA N-terminal region (domain I and II) domain-containing protein	-4.3
25.m01822_at	TGME49_208730	microneme protein, putative; MCP4	-3.9
551.m00220_at	TGME49_307760	tubulin-tyrosine ligase family protein	-3.3
31.m00936_at	TGME49_212940	hypothetical protein	-3.2
20.m00337_at	TGME49_203720	vitamin K epoxide reductase complex subunit 1	-3.2
37.m00739_at	TGME49_217400	hypothetical protein	-3.2
641.m00181_at	TGME49_320740	hypothetical protein	-3.0
23.m00155_at	TGME49_207210	hypothetical protein	-3.0
72.m00004_at	TGME49_280570	SRS35A bradyzoite surface antigen (SAG4.2, p18)	-2.8
541.m02517_at	TGME49_304930	hypothetical protein	-2.7
25.m00212_at	TGME49_208740	microneme protein, putative	-2.5
50.m03090_at	TGME49_245770	hypothetical protein	-2.5
80.m02369_s_at	TGME49_292375	KRUF family protein	-2.4
94.m00031_s_at	TGME49_296340	hypothetical protein	-2.2
76.m01568_s_at	TGME49_284420	hypothetical protein	-2.2
41.m01360_at	TGME49_222370	SAG-related sequence SRS13	-2.2
55.m04865_at	TGME49_260190	microneme protein MIC13	-2.2
49.m03275_at	TGME49_242110	Rhoptry kinase family protein ROP38	-2.2
55.m00276_at	TGME49_255210	ATPase, AAA family protein	-2.1
52.m00020_at	TGME49_253710	hypothetical protein	-2.1
49.m03157_at	TGME49_239580	hypothetical protein	4.6
583.m00678_at; 5f	TGME49_312905	hypothetical protein	3.75; 2.3

**Supplemental Table 3. Differentially expressed genes (FC $\geq$ 2) under bradyzoite conditions in PruQ $\Delta$ *ap2IX-4* compared to parental PruQ and  $\Delta$ *ap2IX-4::AP2IX-4*<sup>HA</sup> parasites.**

Probe Set ID	Gene	Annotation	FC of PruQ $\Delta$ ap2IX-4 vs. PruQ Parental	FC of $\Delta$ ap2IX-4::AP2IX-4 <sup>HA</sup> vs PruQ $\Delta$ ap2IX-4
80.m02181_at	TGME49_288950	AP2IX-4	-38.1	22.0
74.m00761_at	TGME49_281590	hypothetical protein	-9.8	ND
641.m01540_at	TGME49_319890	hypothetical protein	-7.6	1.8
		MoeA N-terminal region (domain I and II)		
83.m01216_at	TGME49_293480	domain-containing protein	-7.4	3.4
49.m03376_at	TGME49_243940	hypothetical protein	-7.0	1.8
42.m05851_at	TGME49_224760	SAG-related sequence SRS40E (SRS4)	-6.5	ND
20.m00387_at	TGME49_204050	subtilisin SUB1 (SUB1)	-4.9	1.7
641.m01563_at	TGME49_320200	SAG-related sequence SRS16A (SRS16A)	-3.9	1.0
611.m00052_at	TGME49_317705	enoyl coA hydratase/isomerase	-3.8	2.2
80.m05017_at	TGME49_288685	Fe-S protein assembly co-chaperone HscB prote	-3.7	ND
113.m01573_at	TGME49_297910	hypothetical protein	-3.7	2.1
55.m04651_at	TGME49_256792	hypothetical protein	-3.6	2.1
65.m01112_at	TGME49_277490	hypothetical protein	-3.6	ND
52.m01621_at	TGME49_253930	GCC2 and GCC3 domain-containing protein	-3.6	1.3
37.m00739_at	TGME49_217400	hypothetical protein	-3.5	3.2
33.m01360_at	TGME49_215360	hypothetical protein	-3.3	ND
1.m00014_at	TGME49_200010	hypothetical protein	-3.3	1.2
55.m04769_at	TGME49_258550	SAG-related sequence SRS28 (SRS28)	-3.3	ND
65.m01169_at	TGME49_278390	Toxoplasma gondii family A protein	-3.2	ND
20.m00368_at	TGME49_005680	hypothetical protein	-3.2	1.7
76.m01687_at	TGME49_287040	hypothetical protein	-3.1	1.8
541.m02054_at	TGME49_306270	hypothetical protein	-3.0	1.7
83.m00011_at	TGME49_294980	hypothetical protein	-3.0	1.1
49.m03110_s_at	TGME49_038630	SAG-related sequence protein SRS22I	-2.9	ND
65.m01167_at	TGME49_278370	Toxoplasma gondii family A protein inorganic anion transporter,	-2.7	1.2
		sulfate permease (SulP) family protein	-2.6	2.2
72.m00387_at	TGME49_280500	hypothetical protein	-2.6	1.1
59.m03715_at	TGME49_273980	hypothetical protein	-2.6	1.1
55.m04926_at	TGME49_261250	histone H2A1	-2.6	1.7
83.m01260_at	TGME49_294330	EGF-like domain-containing protein	-2.6	ND
644.m00075_at	TGME49_321170	Toxoplasma gondii family C protein	-2.5	1.1
49.m05665_at	TGME49_240090	rhoptyr kinase family protein ROP34, putative	-2.5	1.6
55.m04942_at	TGME49_261580	histone H2AX	-2.5	-1.0
107.m00007_s_at	TGME49_207010	SAG-related sequence SRS48K (SRS48K)	-2.5	ND
145.m00602_at	TGME49_300220	hypothetical protein	-2.5	ND
49.m03102_at	TGME49_238440	SAG-related sequence SRS22A (SRS22A)	-2.5	-1.2
46.m02899_at	TGME49_235500	hypothetical protein	-2.4	1.4
55.m00103_at	TGME49_258870	hypothetical protein	-2.4	-1.0
57.m01872_s_at	TGME49_267680	microneme protein MIC12	-2.3	1.1
72.m00383_at	TGME49_280460	AP2 domain transcription factor AP2VIIa-2 (AP2)	-2.3	1.3
25.m01903_s_at	TGME49_209985	cAMP-dependent protein kinase	-2.3	1.4
33.m01267_at	TGME49_214190	SRS46	-2.3	1.6
145.m00583_at	TGME49_299780	hypothetical protein	-2.3	1.4
37.m00779_at	TGME49_217860	hypothetical protein	-2.2	-1.1
49.m03134_at	TGME49_239260	histone H4	-2.2	1.7
59.m03529_at	TGME49_270700	hypothetical protein	-2.2	1.3
25.m00004_at	TGME49_208850	SAG-related sequence SRS11 (SAG2B)	-2.2	1.0
65.m01111_s_at	TGME49_277270	NTPase II	-2.2	-1.1
35.m00905_at	TGME49_216720	hypothetical protein	-2.2	-1.1
63.m00155_at	TGME49_275460	hypothetical protein	-2.1	1.4
65.m02534_at	TGME49_278460	hypothetical protein	-2.1	1.1
59.m06125_at	TGME49_268980	hypothetical protein	-2.1	-1.2
55.m00013_at	TGME49_261240	histone H3	-2.1	2.4
		ICE family protease (caspase)		
65.m02538_at	TGME49_278975	p20 domain-containing protein	-2.1	1.0
55.m08192_at	Not annotated	N/A	-2.1	ND
44.m00027_at	TGME49_230180	hypothetical protein (GRA24 )	-2.1	-1.0
551.m00227_at	TGME49_307830	hypothetical protein	-2.1	1.0
59.m06089_at	TGME49_273650	hypothetical protein	-2.1	1.3
65.m01152_at	TGME49_278130	hypothetical protein	-2.1	-1.2
44.m02688_at	TGME49_231960	omega secalin, putative	-2.0	1.3
541.m02039_at	TGME49_305160	histone H2Ba	-2.0	2.9
37.m00011_at	TGME49_217610	hypothetical protein	-2.0	1.4
583.m05572_at	TGME49_313680	hypothetical protein	-2.0	-1.0

79.m00015_at	TGME49_245432	hypothetical protein	-2.0	1.1
57.m01786_s_at	TGME49_266340	Toxoplasma gondii family A protein cytochrome b(N-terminal)/b6/ petB subfamily protein	-2.0	1.0
54.m00004_x_at	TGME49_255060		-2.0	3.9

ND = Not detected



Probe Set ID	Gene	Annotation	FC of PruQ $\Delta$ ap2IX-4 vs. PruQ Parental	FC of $\Delta$ ap2IX-4::AP2IX-4 <sup>HA</sup> vs PruQ $\Delta$ ap2IX-4
59.m03411_at	TGME49_268860	ENO1	10.2	-2.3
100665_at; 583.m006	TGME49_312905	hypothetical protein	9.7; 2.4	-7.3
25.m01888_at	TGME49_209755	hypothetical protein	7.0	-2.5
55.m00009_at	TGME49_259020	bradyzoite antigen BAG1 (BAG1)	5.7	-1.6
20.m03909_at	TGME49_205430	isovaleryl-CoA dehydrogenase	5.4	-1.4
83.m01239_at	TGME49_293790	hypothetical protein	4.9	-2.1
65.m01148_at	TGME49_278080	Toxoplasma gondii family A protein	4.9	-1.7
80.m03985_at	TGME49_288630	nucleosome assembly protein (NAP), putative	4.5	ND
35.m00032_at	TGME49_216140	tetratricopeptide repeat-containing protein	4.4	-1.8
52.m01553_at	TGME49_252640	P-type ATPase PMA1	4.4	-1.7
57.m00029_at	TGME49_266860	BTB/POZ domain-containing protein	4.3	-1.2
		GDA1/CD39 (nucleoside phosphatase)		
42.m03416_at	TGME49_225290	family protein (B-NTPase)	4.1	-2.1
		ATP-binding cassette G family transporter		
583.m05692_at	TGME49_315560	ABCG77 (ABCG77)	4.0	-1.5
80.m00010_at	TGME49_291040	LDH2	4.0	-1.6
20.m00351_at	TGME49_202020	DnAK-TPR	3.9	-1.8
59.m06103_at	TGME49_271320	hypothetical protein	3.9	-1.4
41.m01304_at	TGME49_221550	se, MAPK family, MEK kinase-related (incomplete cat	3.7	1.9
80.m02361_at	TGME49_292260	SAG-related sequence SRS36B (SAG5D )	3.6	-1.9
57.m01804_at	TGME49_266700	hypothetical protein	3.5	-1.9
25.m01805_at	TGME49_208370	myosin heavy chain, putative	3.3	-1.2
49.m03214_at	TGME49_240470	hypothetical protein	3.2	-1.7
23.m00152_at	TGME49_207160	SAG-related sequence SRS49D (SAG2C)	3.1	-2.2
		cold-shock DNA-binding		
57.m01861_at	TGME49_267470	domain-containing protein	3.1	-1.7
52.m02726_at	TGME49_253680	hypothetical protein	3.0	-1.5
25.m01822_at	TGME49_208730	MCP4	3.0	-1.6
641.m01512_at	TGME49_319340	hypothetical protein	2.9	-1.3
23.m00015_s_at	TGME49_207150	SAG-related sequence SRS49C (SAG2D)	2.9	-1.9
49.m07181_at	TGME49_244335	hypothetical protein	2.9	-1.3
80.m02121_at	TGME49_287960	hypothetical protein	2.7	-1.6
52.m01629_at	TGME49_254020	dynein intermediate chain, putative	2.7	-1.3
38.m02376_at	TGME49_218470	protein disulfide-isomerase	2.6	-1.9
52.m00020_at	TGME49_253710	hypothetical protein	2.6	-1.7
80.m05025_at	TGME49_288475	hypothetical protein	2.6	-1.2
49.m07182_at	TGME49_244335	hypothetical protein	2.6	-1.3
38.m01099_at	TGME49_218930	BTB/POZ domain-containing protein	2.6	-1.2
23.m00155_at	TGME49_207210	hypothetical protein	2.5	-1.3
		dynein gamma chain,		
541.m01230_at	TGME49_306338	flagellar outer arm, putative	2.5	-1.9
		peroxisomal biogenesis factor		
49.m05712_at	TGME49_243720	PEX11 (PEX11)	2.5	-1.9
583.m05655_at	TGME49_315100	hypothetical protein	2.5	-1.3
55.m04853_at	TGME49_259960	Nucleoside-diphosphatase	2.4	-1.5
583.m00678_at	TGME49_312905	hypothetical protein	2.4	-2.0
583.m09133_at	TGME49_314250	bradyzoite rhopty protein BRP1 (BRP1)	2.4	-1.8
52.m01578_at	TGME49_253330	kinase family protein, truncated (incomplete catalytic tri	2.3	-1.5
46.m01670_at	TGME49_235860	subtilisin SUB11 (SUB11)	2.2	-1.0
		NAD(P) transhydrogenase		
162.m00002_at	TGME49_301210	subunit beta, putative	2.2	-1.4
83.m02719_at	TGME49_295015	patched family protein	2.2	1.0
42.m03382_at	TGME49_224830	hypothetical protein	2.2	1.5
41.m01322_at	TGME49_221840	hypothetical protein	2.2	-1.5
588.m00009_at	TGME49_220720	hypothetical protein	2.2	-1.4
541.m00143_at	TGME49_305460	methionine aminopeptidase 2, putative	2.1	-1.3
57.m01692_at	TGME49_264650	phosphoacetylglucosamine mutase	2.1	-1.4
		methylmalonate-semialdehyde		
583.m05427_at	TGME49_311370	dehydrogenase [acylating], putative	2.1	-2.0
44.m00019_at	TGME49_230130	hypothetical protein	2.0	-1.4
31.m01831_at	TGME49_213460	hypothetical protein	2.0	-1.7
57.m01820_at	TGME49_266900	cyclin, N-terminal domain-containing protein	2.0	-1.6