

Supplementary Materials.

Figure S1: AP2IV-4 and AP2VI-1 expression during daughter budding.

Figure S2: Genetic knockout strategy for AP2IV-4.

Figure S3: Deletion of the AP2IV-4 gene in the PruQ strain leads to de-repression of bradyzoite surface and cyst wall mRNAs and proteins in the tachyzoite stage.

Dataset S1

Complete microarray results for all gene expression altered by knockout of AP2IV-4 in PruQ and RHCRe strains.

Dataset S2

Comparison of genes controlled by AP2IV-3, AP2IV-4, AP2IX-9, and AP2XI-4.

Dataset S3

Full list of transgenic strains and oligonucleotides used in this study.

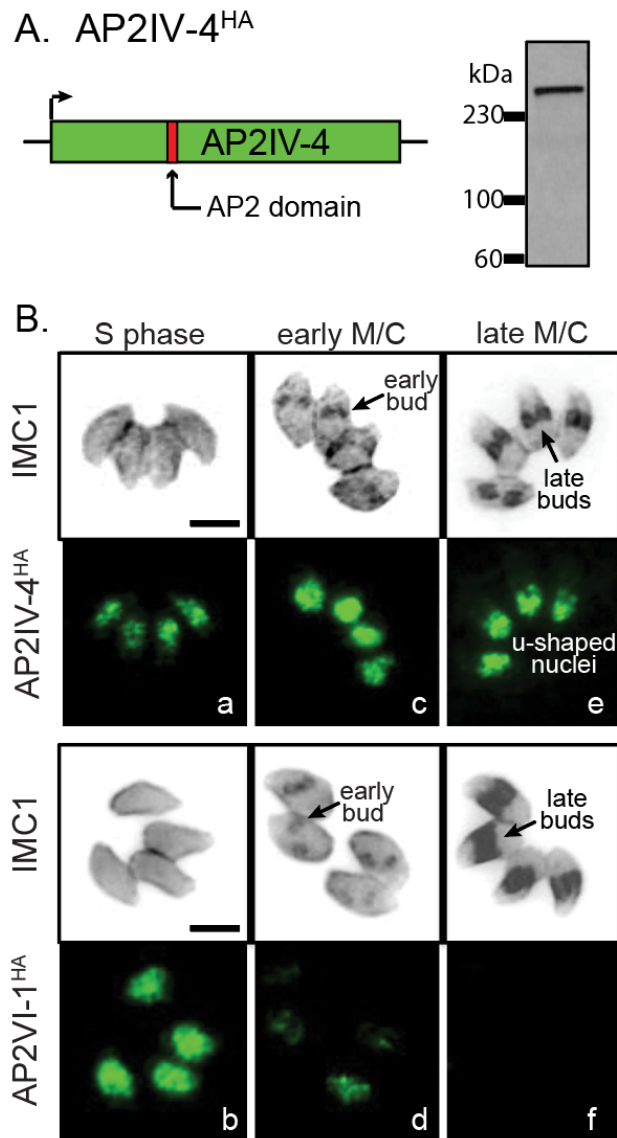
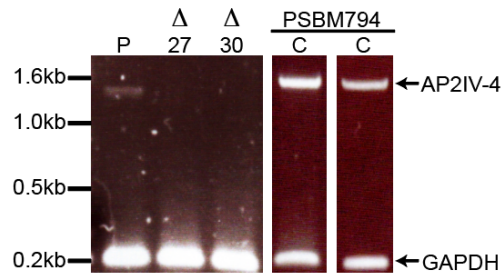


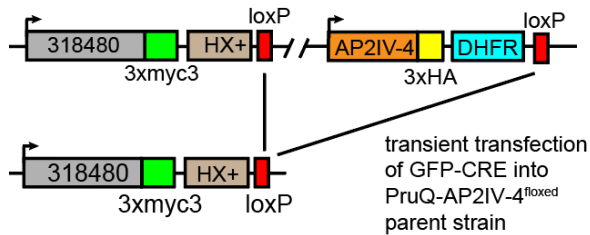
Figure S1: AP2IV-4 and AP2VI-1 expression during daughter budding.

(A.) The AP2IV-4 single exon and AP2 domain gene is predicted to encode a ~250kDa protein (http://toxodb.org/toxo/app/record/gene/TGME49_318470), which was confirmed by Western analysis (α -HA) of AP2IV-4^{HA} parasites (plus 3xHA, actual protein size is ~254kDa). Molecular mass standards indicated on the left. **(B.)** AP2IV-4 and AP2VI-1 were C-terminally epitope tagged in RHQ strain parasites with 3xHA at the endogenous loci by genetic knock-in. IFA analysis of the resulting transgenic parasites grown in HFF monolayers utilized co-stains α -HA (green, AP2IV-4^{HA} or AP2VI-1^{HA} expression) and α -IMC1 (black and white panels). To improve visualization of internal bud structures, IMC1 images were decolorized and then inverted. AP2IV-4^{HA} and AP2VI-1^{HA} are exclusively localized to the nucleus and the relative cell cycle expression profile predicted by the cyclical mRNA patterns (Fig. 1A, green and red curves) was confirmed for each factor. Black scale bar=5 μ m

A. Rapamycin-induced AP2IV-4 KO in RHC*Cre*-AP2IV-4^{flxed} parent strain.



B. Strategy for AP2IV-4 KO in PruQ strain



C. AP2IV-4 KO in PruQ-AP2IV-4^{flxed} parent strain.

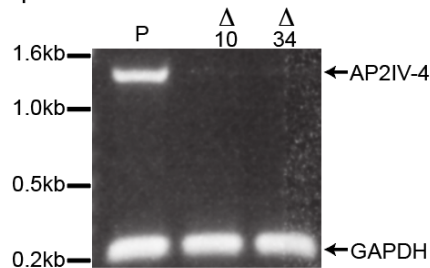


Figure S2: Genetic knockout strategy for AP2IV-4.

(A.) Verification of AP2IV-4 knockout by RT-PCR in the RHC*Cre*- Δ *ap2IV-4* transgenic clones. AP2IV-4 mRNA is detected in lane P (parent strain, RHC*Cre*-AP2IV-4^{flxed}), but absent from both AP2IV-4 knockout clones evaluated (RHC*Cre*- Δ *ap2IV-4* clones 27, and 30). For a diagram of the RHC*Cre* knockout strategy see Figure 3A. Complementation for the loss of AP2IV-4 in RHC*Cre*- Δ *ap2IV-4* parasites was accomplished using cosmid PSBM794, which reintroduces a full copy of the AP2IV-4 gene and restores AP2IV-4 mRNA expression (C lanes). **(B.)** Diagram of the AP2IV-4 knockout strategy in Type II PruQ parasites, which is similar to the knockout of AP2IV-4 in the RHC*Cre* strain (see Fig. 3A). Here transient transfection of pMIN-CRE-eGFP plasmid [76] into the PruQ-AP2IV-4^{flxed} strain was required to introduce active Cre recombinase. **(C.)** Parent strain PruQ-AP2IV-4^{flxed} (lane P) expresses the expected AP2IV-4 transcript, whereas PruQ- Δ *ap2IV-4* clones 10 and 34 (lanes Δ 10 and Δ 34) lack the AP2IV-4 mRNA. AP2IV-4 cDNA primers amplify a 1367bp fragment of the single exon gene (7221bp full length). For loading and template quality control, GAPDH primers were designed around a 432bp intron, allowing detection of both mRNA (202bp, intron spliced out) and contaminating genomic DNA (634 bp). The absence of a detectable 634bp amplicon in all samples indicates the RNA templates were free of genomic DNA contamination. See Dataset S3 for all primer designs.

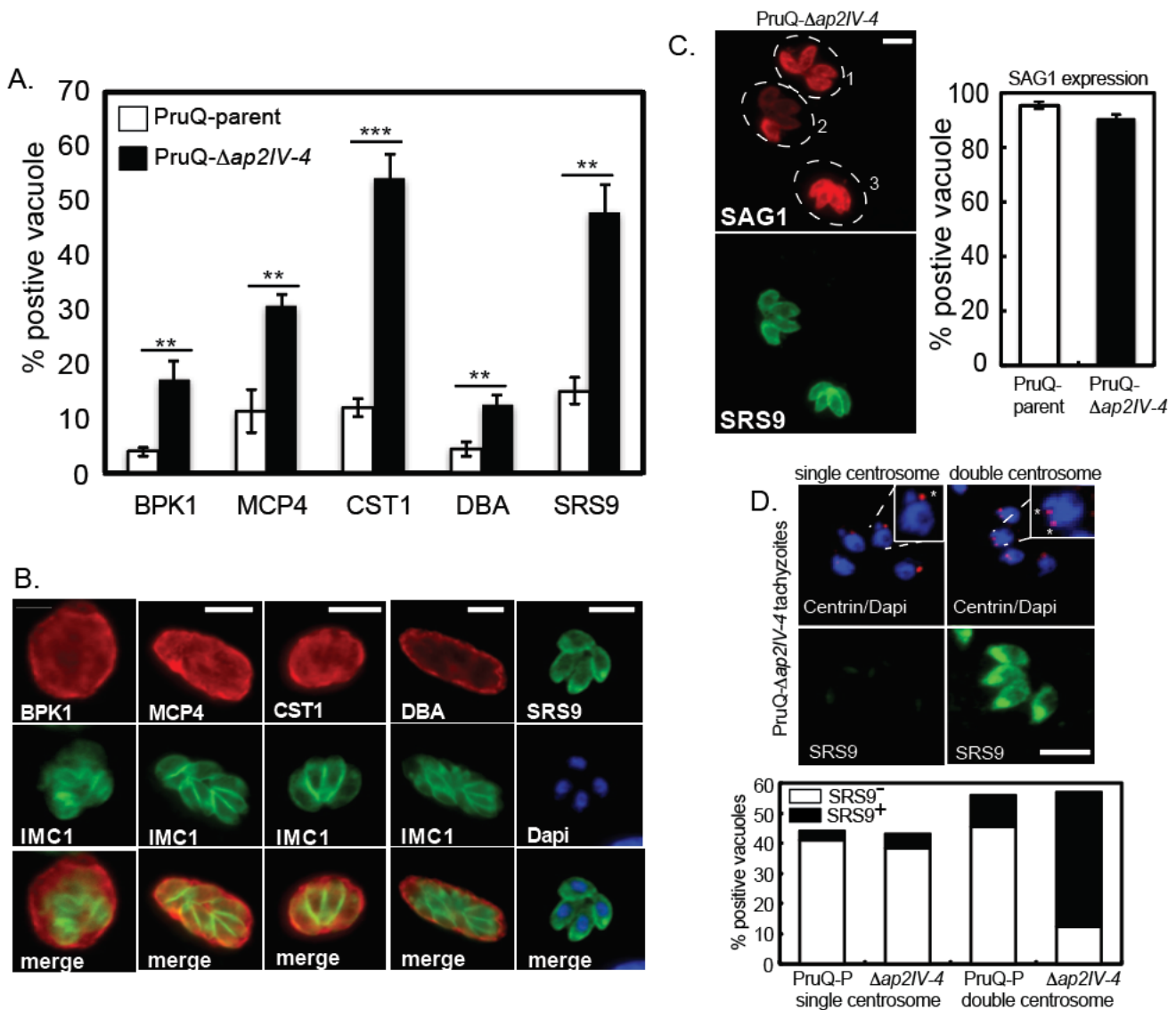


Figure S3: Deletion of the AP2IV-4 gene in the PruQ strain leads to de-repression of bradyzoite surface and cyst wall mRNAs and proteins in the tachyzoite stage.

(A.) Average fraction of vacuoles forming tissue cysts (DBA+) and misexpressing bradyzoite cyst wall proteins BPK1, MPC4, CST1 and the bradyzoite-specific surface antigen SRS9 in PruQ- $\Delta ap2IV-4$ versus the PruQ-AP2IV-4^{flxed} parent strain with representative IFA images in (B.) Statistical significance of pairwise parent versus knockout results in (A.) is indicated (**, p < 0.01; ***, p < 0.001). Positive staining for each antigen and DBA+ vacuoles were quantified in triplicate by counting 100 vacuoles from randomly selected microscopic fields. Note also the proper localization of BPK1, CST1, and MCP4 in cyst walls also stained by DBA and the parasite surface localization of SRS9. Scale bar=5 μm . (C.) The tachyzoite nature of SRS9+ PruQ- $\Delta ap2IV-4$ parasites was confirmed by IFA analysis with α SRS9 and α SAG1 antibodies. The expression of SRS9 in these populations was nearly ~50% (as shown in A.), and thus, about half the population was SAG1+/SRS9- (#1 vacuole of four parasites circled). Nearly all PruQ- $\Delta ap2IV-4$ parasites expressing SRS9+ were also positive for SAG1+ surface expression (representative vacuole #3 circled). The fraction of SAG1+ positive vacuoles in PruQ-parent and PruQ- $\Delta ap2IV-4$ (SRS9- and SRS9+) infected HFF cell cultures were quantified (see graph). In rare examples, PruQ- $\Delta ap2IV-4$ parasites that were SRS9+ also showed diminished SAG1 expression (#2 vacuole circled). Scale bar=5 μm (D.) The cell cycle distribution of SRS9+ parasites in PruQ- $\Delta ap2IV-4$ versus the PruQ-AP2IV-4^{flxed} parent strains was determined by IFA analysis with α SRS9 and α Centrin

antibodies. Representative PruQ- $\Delta ap2IV-4$ co-stained images demonstrate the association of SRS9 expression with parasites containing duplicated centrosomes (S/M phases) that was quantified and graphed for PruQ- $\Delta ap2IV-4$ and the PruQ-AP2IV-4^{floxed} parent strains. All parasite counts were determined in triplicate from 100 vacuoles selected at random. The association of SRS9+ expression in S/M phase PruQ- $\Delta ap2IV-4$ parasites (duplicated centrosome) versus G1 phase (single centrosome) parasites was statistically significant ($p < 0.0001$). Scale bar=5 μ m.

Dataset S1: Combined lists of mRNAs altered >2 fold in RHC*Cre-Δap2IV-4* or PruQ-*Δap2IV-4* parasites grown as tachyzoites in normal pH 7.0 media (GSE93531). Yellow highlighted genes are changed >2 fold in the same direction in both knockout parasites, Grey highlighted genes are >2 fold changed in RHC*Cre-Δap2IV-4* parasites, and white highlighted genes are >2-fold changed in PruQ-*Δap2IV-4* parasites. In general, mRNAs increased >2-fold in RHC*Cre-Δap2IV-4* parasites show higher baseline expression in Pru parental parasites leading to lower fold changes in PruQ-*Δap2IV-4* parasites. However, >90% of mRNAs >2-fold in RHC*Cre-Δap2IV-4* parasites also show expression changes in PruQ-*Δap2IV-4* parasites in the same direction either up or down regulated. Values for baseline mRNA expression in Type III CTG parasites (see GEO accession GSE89469) was included for those genes increased >2-fold in RHC*Cre-Δap2IV-4* parasites to show that higher baseline mRNA expression is a characteristic of strains that have a greater capacity to develop into the tissue cyst; nearly 70% of CTG mRNAs showed higher baseline expression than in RH parental parasites. GAPDH2 expression was included here as an internal reference for constitutive mRNA expression across all datasets; there is a 1.5 fold difference in GAPDH2 expression across all the datasets.

ToxoDB ID	Parent RHC <i>Cre-AP2IV-4</i> ^{flxed}	RHC <i>Cre-Δap2IV-4</i>	Fold Change <i>Δap2IV-4</i> vs parent	RHC <i>Cre-Δap2IV-4</i> comp.	Fold Change complemented vs parent	Description	PruQ- <i>AP2IV-4</i> ^{HA}	PruQ- <i>Δap2IV-4</i>	Fold Change	CTG
TGME49_261650	30.1	517.4	17.2	48.2	1.6	hypothetical protein	144.3	430.2	3.0	261.8
TGME49_207150	19.8	320.8	16.2	36.6	1.8	SRS49C (= SAG2D)	224.6	401.8	1.8	474.1
TGME49_216140	20.9	323.7	15.5	58.5	2.8	tetratricopeptide repeat-containing protein	36.4	301.8	8.3	1742.4
TGME49_280580	59.6	709.7	11.9	116.3	2.0	SAG4 homolog (SAG4.2)	1046.2	1899.6	1.8	4454.9
TGME49_301890	33.3	394.2	11.8	153.4	4.6	Toxoplasma gondii family B protein	473.2	541.9	1.1	390.4
TGME49_253330	42.4	455.8	10.7	29.3	0.7	BPK1	835.1	2315.6	2.8	1018.1
TGME49_207210	8.5	67.7	8.0	26.5	3.1	hypothetical protein	209.5	903.5	4.3	233.8
TGME49_320190	23.9	183.6	7.7	56.5	2.4	SRS16B (= SRS9)	28.0	256.7	9.2	17.1
TGME49_207130	49.0	372.9	7.6	54.0	1.1	SRS49A (= SAG2Y)	171.2	833.7	4.9	2151.8
TGME49_208730	88.8	588.8	6.6	97.6	1.1	MCP4	249.0	887.6	3.6	141.5
TGME49_225290	8.3	38.5	4.6	11.5	1.4	bradyzoite-NTPase	24.9	102.4	4.1	428.6
TGME49_208740	654.5	2872.4	4.4	721.7	1.1	microneme protein	3657.3	7389.4	2.0	226.5
TGME49_237170	64.1	242.2	3.8	73.4	1.1	hypothetical protein	934.6	733.3	-1.3	292.1
TGME49_290970	59.9	218.8	3.7	112.5	1.9	8-amino-7-oxononanoate synthase	376.1	1610.5	4.3	475.3
TGME49_273110	49.8	163.0	3.3	44.9	0.9	SRS30D	13.3	13.4	1.0	19.1
TGME49_215500	35.0	108.1	3.1	30.3	0.9	TPR repeat containing protein	43.9	69.9	1.6	28.9
TGME49_250930	58.4	160.5	2.7	130.1	2.2	hypothetical protein	49.7	43.3	-1.1	52.3
TGME49_235700	121.1	316.9	2.6	281.1	2.3	sedoheptulose-1,7 bisphosphatase	689.5	681.0	-1.0	435.9
TGME49_207140	241.8	619.8	2.6	268.9	1.1	SRS49B (= SAG2X)	169.1	496.3	2.9	197.0
TGME49_292260	24.9	62.0	2.5	23.9	1.0	SRS36B (= SAG5D)	20.7	100.4	4.8	1364.3

TGME49_250940	185.2	453.5	2.4	484.2	2.6	hypothetical protein	384.3	355.6	-1.1	177.7
TGME49_301210	98.7	236.0	2.4	91.8	0.9	NAD(P) transhydrogenase	254.1	523.7	2.1	610.5
TGME49_236010	446.7	1066.0	2.4	477.7	1.1	prenylcysteine oxidase	1220.6	1893.2	1.6	1239.2
TGME49_234440	203.7	444.3	2.2	172.3	0.8	aminotransferase	189.0	639.8	3.4	144.4
TGME49_246070	40.6	87.0	2.1	41.3	1.0	SRS56A	22.2	24.5	1.1	32.5
TGME49_213072	156.3	325.4	2.1	141.6	0.9	Sec1 domain containing family	182.1	176.8	-1.0	746.0
TGME49_227630	19.9	33.7	1.7	21.0	1.1	hypothetical protein	181.3	737.4	4.1	
TGME49_301170	198.6	319.2	1.6	142.3	0.7	SRS19D	50.5	666.1	13.2	
TGME49_301180	385.4	570.3	1.5	302.2	0.8	SRS19F	222.6	628.1	2.8	
TGME49_224770	19.5	27.4	1.4	21.5	1.1	SRS40D	41.3	164.1	4.0	
TGME49_321480	24.9	34.0	1.4	22.7	0.9	SRS12B	109.8	328.6	3.0	
TGME49_237090	6.9	8.9	1.3	9.1	1.3	AP2X-5	232.7	104.4	-2.2	
TGME49_293840	119.4	152.0	1.3	154.7	1.3	hypothetical protein	20.1	109.0	5.4	
TGME49_224710	30.2	37.2	1.2	27.0	0.9	vacuolar sorting receptor protein	116.7	263.1	2.3	
TGME49_256800	6.9	8.1	1.2	8.6	1.3	hypothetical protein	60.1	126.1	2.1	
TGME49_301160	144.8	167.5	1.2	132.1	0.9	SRS19C (= SRS7)	161.0	395.3	2.5	
TGME49_304930	22.1	22.7	1.0	29.0	1.3	hypothetical protein	58.1	153.2	2.6	
TGME49_255210	396.9	381.9	-1.0	367.0	0.9	ATPase, AAA family protein	68.7	400.7	5.8	
TGME49_231960	645.5	600.3	-1.1	537.7	0.8	omega secalin, putative	755.6	320.8	-2.4	
TGME49_251170	35.8	32.8	-1.1	26.9	0.8	KRUF family protein	341.8	107.8	-3.2	
TGME49_295100	139.5	127.4	-1.1	129.2	0.9	hypothetical protein	234.2	114.4	-2.0	
TGME49_240060	598.8	538.4	-1.1	461.0	0.8	hypothetical protein	1304.6	542.9	-2.4	
TGME49_315290	206.0	185.0	-1.1	153.1	0.7	hypothetical protein	359.0	170.9	-2.1	
TGME49_265150	106.0	90.2	-1.2	98.9	0.9	hypothetical protein	197.5	489.7	2.5	
TGME49_246710	41.5	34.9	-1.2	35.4	0.9	hypothetical protein	118.7	51.7	-2.3	
TGME49_205370	85.8	70.0	-1.2	47.6	0.6	hypothetical protein	114.9	51.8	-2.2	
TGME49_268210	54.9	44.8	-1.2	57.4	1.0	AGC kinase	76.8	189.5	2.5	
TGME49_264870	93.7	71.9	-1.3	61.7	0.7	Sodium:neurotransmitter symporter family protein	337.3	145.9	-2.3	
TGME49_229220	187.1	132.7	-1.4	198.0	1.1	hypothetical protein	496.2	213.1	-2.3	
TGME49_286150	35.3	25.0	-1.4	26.5	0.7	PAN/Apple domain-containing protein	156.9	347.6	2.2	
TGME49_277490	47.3	33.3	-1.4	45.6	1.0	hypothetical protein	108.3	50.0	-2.2	
TGME49_262050	1286.2	899.1	-1.4	1173.3	0.9	Rhoptry kinase family protein ROP39	1748.8	694.8	-2.5	
TGME49_271770	26.7	18.0	-1.5	23.8	0.9	hypothetical protein	150.0	74.2	-2.0	
TGME49_239600	211.8	131.4	-1.6	225.1	1.1	ROP23 (incomplete catalytic triad)	1136.4	344.4	-3.3	

TGME49_218550	160.2	99.0	-1.6	116.5	0.7	PIK3R4 kinase-related protein	281.2	95.5	-2.9
TGME49_250820	113.4	69.9	-1.6	79.3	0.7	hypothetical protein	171.0	77.3	-2.2
TGME49_297180	131.3	78.4	-1.7	100.7	0.8	hypothetical protein	277.1	126.0	-2.2
TGME49_255190	670.2	378.3	-1.8	539.1	0.8	myosin C	843.6	350.3	-2.4
TGME49_224530	367.6	206.5	-1.8	290.8	0.8	IMC5 (ALV11)	491.6	232.3	-2.1
TGME49_272520	675.2	371.4	-1.8	739.3	1.1	hypothetical protein	688.6	247.1	-2.8
TGME49_226290	180.6	96.0	-1.9	136.2	0.8	hypothetical protein	220.9	110.3	-2.0
TGME49_289600	1485.4	705.6	-2.1	1545.8	1.0	HSP29	2599.6	967.2	-2.7
TGME49_200230	734.5	335.5	-2.2	788.3	1.1	MIC17C	2599.0	825.9	-3.1
TGME49_232280	394.4	178.8	-2.2	400.6	1.0	hypothetical protein	551.7	243.7	-2.3
TGME49_289100	111.9	52.0	-2.2	91.8	0.8	hypothetical protein	188.8	104.9	-1.8
TGME49_306270	1059.4	428.6	-2.2	1203.8	1.1	hypothetical protein	624.3	259.8	-2.4
TGME49_232410	3042.2	1129.2	-2.7	3393.0	1.1	PDI family protein	6234.5	2218.5	-2.8
TGME49_229930	1041.2	373.8	-2.8	1046.5	1.0	p25-alpha domain-containing protein	1460.2	463.0	-3.2
TGME49_292110	82.5	30.0	-2.8	96.8	1.2	formate/nitrite transporter	279.8	159.0	-1.8
TGME49_221630	123.9	40.7	-3.0	101.7	0.8	hypothetical protein	204.7	108.2	-1.9
TGME49_233300	89.1	25.6	-3.5	69.4	0.8	rhoGAP protein	124.9	41.2	-3.0
TGME49_276930	465.7	129.4	-3.6	502.2	1.1	hypothetical protein	1461.8	451.6	-3.2
TGME49_225790	346.0	76.9	-4.5	316.1	0.9	PDI family protein	499.9	118.7	-4.2
TGME49_226310	195.8	12.5	-15.7	67.8	0.3	zinc finger (CCCH type)	59.5	11.9	-5.0
TGME49_318470	191.6	6.4	-29.9	36.1	0.2	AP2IV-4	195.6	11.6	-16.8
TGME49_289690	6743.8	5795.6	-1.2	7341.5	1.1	GAPDH2	8915.0	8402.1	-1.1

Dataset S2 Common and specific gene targets of four ApiAP2 transcription factors (two prepressors, AP2IV-4 & AP2IX-9, and two activators, AP2XI-4 & AP2IV-3). Column G indicates genes included in Figure 6 of the main paper.

Gene ID	AP2IV-3	AP2XI-4	AP2IV-4	AP2IX-9	Product Name	Summary Fig
TGME49_207210	x	x	x	x	hypothetical protein (UNK)	
TGME49_202020		x	x	x	DnaK-TPR	
TGME49_207130		x	x	x	SRS49A (= SAG2Y)	
TGME49_208740	x		x	x	microneme protein (MIC UNK)	
TGME49_216140		x	x	x	tetratricopeptide repeat-containing protein	
TGME49_225290		x	x	x	bradyzoite-NTPase (B-NTPase)	x
TGME49_253330		x	x	x	BPK1	x
TGME49_252640	x	x		x	TgPMA1	
TGME49_259020	x	x		x	bradyzoite antigen (BAG1)	x
TGME49_261650		x	x	x	hypothetical protein	
TGME49_280580		x	x	x	SAG-related sequence SRS35B (SAG4.2)	
TGME49_291040	x	x		x	lactate dehydrogenase (LDH2)	x
TGME49_207140			x	x	SRS49B (= SAG2X)	
TGME49_207150		x		x	SAG-related sequence SRS49C	
TGME49_208730			x	x	MCP4	x
TGME49_240470	x	x			hypothetical protein	
TGME49_268860		x		x	enolase 1 (ENO1)	x
TGME49_280570		x		x	SAG-related sequence SRS35A	
TGME49_267670	x			x	hypothetical protein	
TGME49_290970			x	x	8-amino-7-oxononanoate synthase	
TGME49_320190		x	x		SRS16B (= SRS9)	x
TGME49_203290				x	hypothetical protein	
TGME49_207150			x		SRS49C (= SAG2D)	x
TGME49_209755				x	hypothetical protein	
TGME49_209985				x	cAMP-dependent protein kinase	
TGME49_213072			x		Sec1 domain containing family	
TGME49_215500			x		TPR repeat containing protein	

TGME49_215775	x				Rhoptry kinase family protein ROP8	x
TGME49_218910				x	hypothetical protein	
TGME49_221550		x			CMGC kinase, MAPK family, MEK kinase-related	
TGME49_224170		x			SRS domain-containing protein	
TGME49_227430	x				hypothetical protein	
TGME49_232400				x	PAN domain-containing protein	
TGME49_234440			x		aminotransferase	
TGME49_235700			x		sedoheptulose-1,7 bisphosphatase	
TGME49_236010			x		prenylcysteine oxidase	
TGME49_237170			x		hypothetical protein	
TGME49_238440	x				SRS22A	
TGME49_240930				x	MoaC family protein	
TGME49_246070			x		SRS56A	
TGME49_250930			x		hypothetical protein	
TGME49_250940			x		hypothetical protein	
TGME49_253340		x			hypothetical protein	
TGME49_255210				x	ATPase, AAA family protein	
TGME49_264150	x				hypothetical protein	
TGME49_268210				x	AGC kinase	
TGME49_268220				x	hypothetical protein	
TGME49_273110			x		SRS30D	
TGME49_278080		x			hypothetical protein	
TGME49_286000	x				hypothetical protein	
TGME49_289920				x	hypothetical protein	
TGME49_290000				x	hypothetical protein	
TGME49_291910				x	hypothetical protein	
TGME49_292260			x		SRS36B (= SAG5D)	
TGME49_293790				x	hypothetical protein	
TGME49_301210			x		NAD(P) transhydrogenase	
TGME49_301890			x		Toxoplasma gondii family B protein	

TGME49_306450				x	short chain dehydrogenase family protein, putative	
TGME49_311370		x			Methylmalonate-semialdehyde dehydrogenase	
TGME49_314250	x				bradyzoite rhoptry protein BRP1	x
TGME49_319340				x	hypothetical protein	

Dataset S3 Oligonucleotides used in this study.

Oligonucleotide function	Gene/locus	Orientation	Oligonucleotides (5'->3')
Tagging of endogenous gene loci with 3xhemagglutinin (HA) or 3x MYC epitope tags			
LIC 5' primer for AP2IV-4	AP2IV-4	Forward	TACTTCCAATCCAATTTAATGCGACTCTCTCTGCCATTGGCCCG
LIC 3' primr for AP2IV-4	AP2IV-4	Reverse	TCCTCCACTTCCAATTTTAGCGGCGAGCATGATGGTCTGCTTG ACGTC
LIC AP2IV-4 5' primer to check for recombination	AP2IV-4	Forward	CAGGAGCTGTGTGAGCACGAGC
LIC-HA 3' primer to check for 3' recombination	N/A	Reverse	CGCATATCGGGCACATCATAGGGAT
LIC 5' primer for TGGT1_318480	TGGT1_318480	Forward	TACTTCCAATCCAATTTAATGCTGGTCCCGAGAAGAACCATGAG
LIC 3' primr for TGGT1_318480	TGGT1_318480	Reverse	TCCTCCACTTCCAATTTTAGCTACAGGTGCGTCCTGTAAGGAAC TGCT
LIC TGGT1_318480 5' primer to check for recombination	TGGT1_318480	Forward	GGAACGTGAGGCCGATGATGG
LIC-MYC 3' primer to check for 3' recombination	N/A	Reverse	CTGTGTGAAATGCCACATGAAGAGTC
LIC 5' primer for AP2VI-1	AP2VI-1	Forward	TACTTCCAATCCAATTTAATGCGAACAGACATTACATCGGACTT ATCTCCAAC
LIC 3' primr for AP2VI-1	AP2VI-1	Reverse	TCCTCCACTTCCAATTTTAGCCGCGTGGGCCGCTGATACTTCA GC
LIC AP2VI-1 5' primer to check for recombination	AP2VI-1	Forward	GAG AAG CAA GAG GCC ACA GAG GCC
LIC-HA 3' primer to check for 3' recombination	N/A	Reverse	CGCATATCGGGCACATCATAGGGAT
Introduction of loxP sites into pLIC vectors			
insertion of loxP site into pLIC vectors	N/A	Forward	attgggcccATAACTTCGTATAGCATAACATTATACGAAGTTATgctggcgt aatagcgaagagg
insertion of loxP site into pLIC vectors	N/A	Reverse	attgggcccgcgattaagttgggtaacgccag

AP2IV-4 3 fragment knockout constructs			
attB4 AP2IV-4	5' UTR AP2IV-4	Forward	GGGGACAACCTTTGTATAGAAAAGTTGAAGACGGTGACGGTGGTACGGTTG
attB1r AP2IV-4	5' UTR AP2IV-4	Reverse	GGGGACTGCTTTTTTGTACAACTTGCCCTGTGGAAGCCACTGCCTTG
attB2r AP2IV-4	3' UTR AP2IV-4	Forward	GGGGACAGCTTTTCTTGTACAAAGTGGAACGCAAGGCTGCCATGGAAGCG
attB3 AP2IV-4	3' UTR AP2IV-4	Reverse	GGGGACAACCTTTGTATAATAAAGTTGCCGACACTGAGGTGTGTGCGCG
AP2IV-4 3 Frag KO verify	5' UTR AP2IV-4	Forward	GAGCCTGCCGTCGGACACTGG
AP2IV-4 3 Frag KO verify	3' UTR AP2IV-4	Reverse	CTTCGCGTTCACCGAGGCGCC
AP2IV-4 conditional expression construct			
cloning AP2IV-4 CDS into pCTDDHA3 (MfeI)	AP2IV-4 CDS	Forward	atacaattgGCCGCACCCGCACCGAGCGCG
cloning AP2IV-4 CDS into pCTDDHA3 (EcoRV)	AP2IV-4 CDS	Reverse	tatgataTCAGGCGAGCATGATGGTCTGCTTGACGTCGCCAGAA GGAGAGAGAAAAGACGCAATGTCTCCTGTCTCGCCTCCTTCC GTCCCCTCTGTCGG
Recombinant protein construct			
pGEX4T3	AP2IV-4 recombinant protein-PBM	Forward	TATGGATCCGTACCCTTGAGAGCAGACGTGAC
pGEX4T3	AP2IV-4 recombinant protein-PBM	Reverse	ATAGCGGCCGCGTGCGAGAGGCACAGTTTGCCTGA
Semi quantitative RT-PCR			
RT-PCR	AP2IV-4 +5952 (from start)	Forward	GTCGCGTGATCCGCGATCAGG
RT-PCR	AP2IV-4 @ stop codon	Reverse	GGCGAGCATGATGGTCTGCTTGACG
RT-PCR	GAPDH	Forward	GTCAATCATTCTGTCAATTCGA
RT-PCR	GAPDH	Reverse	ACACCATGTAGTCCAGGGACATG
RT-PCR	CST1	Forward	CCTGTGGTTTTTCGAGCTTCC
RT-PCR	CST1	Reverse	TCGAAGAGTGACGTAGCAGG

DNA probes for EMSA			
EMSA	PBM motif validation	Forward	TTTGTAAATATACGCTCCACCGCGTGCACCCCCCTCGACATGCC CCCGTATTGCTGATCG
EMSA	PBM motif validation	Reverse	CGATCAGCAATACGGGGGCATGTCGAGGGGGGTGCACGCGG TGGAGCGTATATTACAAA
EMSA	PBM scrambled cold	Forward	TACCCTGCCTCTTGTAGTAGTAACGTAAAAGAGGTAACGTGGT CAAATGCGATCAGAGAT
EMSA	PBM scrambled cold	Reverse	ATCTCTGATCGCATTGACCACGTTACCTCTTTTACGTTACTACT ACAAGAGGCAGGGTA
Target regions for CHIP-qPCR			
ChIP-qPCR	SRS9 region 1	Forward	GGACTTCTTGGATCCATTTTC
ChIP-qPCR	SRS9 region 1	Reverse	AGACTCTGACACAGGGA
ChIP-qPCR	SRS9 region 2	Forward	TGCCAGAAATCCCACAA
ChIP-qPCR	SRS9 region 2	Reverse	TAGTGTCGGAATCAAAGAGG
ChIP-qPCR	SRS9 region 3	Forward	CGGACTATCTGTGGCTTT
ChIP-qPCR	SRS9 region 3	Reverse	CACGAAAGCAAGGAACAC
ChIP-qPCR	SRS9 region 4	Forward	AGGTCTTCGACACAATCC
ChIP-qPCR	SRS9 region 4	Reverse	GCTGATTCACGTTGCTTC
ChIP-qPCR	SRS9 region 5	Forward	AAAGAGGAGAGCGGCTA
ChIP-qPCR	SRS9 region 5	Reverse	CACGGGTTTGCTTAAACG
ChIP-qPCR	SRS9 region 6	Forward	GAGCAGTCAGCTCTAGTAAT
ChIP-qPCR	SRS9 region 6	Reverse	GGACCCAATGATACAACATC
ChIP-qPCR	SRS9 region 7	Forward	TGACCTGATGTTTAGCAGTC
ChIP-qPCR	SRS9 region 7	Reverse	GACGATTGCTGCGAAGA
ChIP-qPCR	SRS9 region 8	Forward	GGCGTTTGTGACTGTAGA
ChIP-qPCR	SRS9 region 8	Reverse	GGTTCCTTGAGTTGTTGAAG
ChIP-qPCR	BAG1 region 1	Forward	GCCAGTCGAAAGCCATTA
ChIP-qPCR	BAG1 region 1	Reverse	TCACGCGTTCAGTCTACTC
ChIP-qPCR	BAG1 region 2	Forward	CGTCCTCTCTGTCCACGAAC

ChIP-qPCR	BAG1 region 2	Reverse	ATGGGGAAGTGGAGGAAGAT
ChIP-qPCR	BAG1 region 3	Forward	AGGTCGCCCCTCTTATTCTT
ChIP-qPCR	BAG1 region 3	Reverse	ACCCAAACATGGAGTTCAGG
ChIP-qPCR	BAG1 region 4	Forward	GACGAGTCCCGTTTTTCCT
ChIP-qPCR	BAG1 region 4	Reverse	ACAGAGTCGCTGCGTAGAC
ChIP-qPCR	BAG1 region 5	Forward	CTTTTGCCAAAGGAGACCTG
ChIP-qPCR	BAG1 region 5	Reverse	ATGCCACTGAGCTACACAG
ChIP-qPCR	BAG1 region 6	Forward	GTCGTCTGTGAGAGCGTCAA
ChIP-qPCR	BAG1 region 6	Reverse	GACCTGGGCTTTGCAGATAA

Database S3 Transgenic strains produced for this study

Name in this study	experimental use	Parent strain	Transgene/selectable marker	Drug Selection	Clone #
AP2IV-4 ^{HA}	endogenous expression of AP2IV-4	RHΔ <i>hxgprtΔku80</i>	endogenous promoter::AP2IV-4(3xHA)/DHFR-TS	pyrimethamine	A2, C9
RH- ^{DD} AP2IV-4	conditional expression of AP2IV-4	RHΔ <i>hxgprt</i>	tubulin promoter:(DD-3xHA)-AP2IV-4/sagCAT	chloramphenicol	B5
RHCre-318480 ^{MYC}	endogenous expression of 318480	RHΔ <i>hxgprtΔku80</i> diCRE	endogenous promoter::318480(3xMYC)/HXGPRT/loxP	pyrimethamine	IB9
RHCre-AP2IV-4 ^{floxed}	endogenous expression of 318480 endogenous expression of AP2IV-4	RHΔ <i>hxgprtΔku80</i> diCRE	endogenous promoter::318480(3xMYC)/HXGPRT/loxP endogenous promoter::AP2VI-1(3xHA)/DHFR/loxP	pyrimethamine/HXGPRT	ID6
RHCre-Δ <i>ap2IV-4</i>	knockout of AP2IV-4	RHΔ <i>hxgprtΔku80</i> diCRE	endogenous promoter::318480(3xMYC)/DHFR-TS/loxP	HXGPRT	27, 30
RHCre-Δ <i>ap2IV-4</i> ::AP2IV-4	complemented AP2IV-4 knockout	RHCre-Δ <i>ap2IV-4</i>	endogenous promoter::318480(3xMYC)/DHFR-TS/loxP cosmid PSBM794	HXGPRT, Phleomycin	n/a
PruQ-318480 ^{MYC}	endogenous expression of 318480	PruΔ <i>hxgprtΔku80</i>	endogenous promoter::318480(3xMYC)/HXGPRT/loxP	HXGPRT	B8
PruQ-AP2IV-4 ^{floxed}	endogenous expression of 318480 endogenous expression of AP2IV-4	PruΔ <i>hxgprtΔku80</i>	endogenous promoter::318480(3xMYC)/HXGPRT/loxP endogenous promoter::AP2IV-4(3xHA)/DHFR/loxP	pyrimethamine/HXGPRT	C3
PruQ-Δ <i>ap2IV-4</i>	knockout of AP2IV-4	PruΔ <i>hxgprtΔku80</i>	endogenous promoter::318480(3xMYC)/DHFR-TS/loxP	HXGPRT	10, 34
AP2VI-1 ^{HA}	endogenous expression of AP2VI-1	RHΔ <i>hxgprtΔku80</i>	endogenous promoter::AP2VI-1(3xHA)/DHFR-TS	pyrimethamine	A2