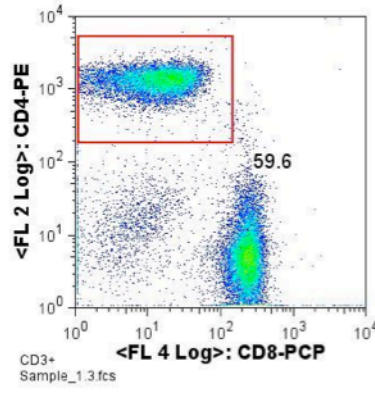
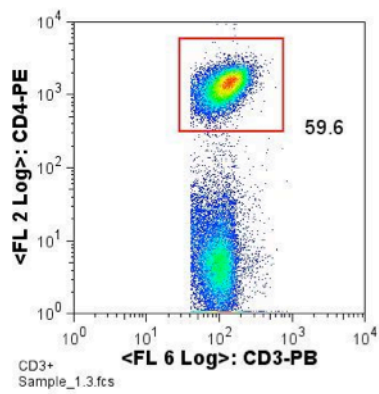


Majority	MKEI YYI VI LCSLYLI NLGNCS EGT DNI I S ENGDVVKF DLI PKENT ERS HKLI NPWEKFMKEYDI EKVHGS GI RVDLGEDA	
	10 20 30 40 50 60 70 80	
<i>P. chabaudi chabaudi</i> AS	MKEI YYI VI LCSLYLI NLGNCS EGT DNI I S ENGDVVKF DLI PKENT ERS HKLI NPWEKFMKEYDI EKVHGS GI RVDLGEDA	80
<i>P. chabaudi adami</i> DS	MKEI YYI VI LCSLYLI NLGNCS EGT DNI I S ENGDVVKF DLI PKENT ERS HKLI NPWEKFMKEYDI EKVHGS GI RVDLGEDA	80
Majority	RVENQDYRI PSGKCPVMGKGI TI QX SXX SFLXXVATGX QKVREGGLAF PXXDVNI SPXXI XNLXXMYKXHXEI XALNDMS	
	90 100 110 120 130 140 150 160	
<i>P. chabaudi chabaudi</i> AS	RVENQDYRI PSGKCPVMGKGI TI QSSKVSFLVATGQKVREGGLAF PXXDVNI SPXXI XNLXXMYKXHXEI XALNDMS	160
<i>P. chabaudi adami</i> DS	RVENQDYRI PSGKCPVMGKGI TI QKSTKSF LDPVATGQKVREGGLAF PKADVNI SPVLI QNLREMYKEHPEI IALNDMS	160
Majority	LCAKHASFXVPGXNXNAYRHPAVYDKNXTCYI LYVAAQENMGPRYCSNEEXNENQPF CFTPEKKDEYKNLSYLTKNLR	
	170 180 190 200 210 220 230 240	
<i>P. chabaudi chabaudi</i> AS	LCAKHASFXVPGXNAYRHPAVYDKNXTCYI LYVAAQENMGPRYCSNEEXNENQPF CFTPEKKDEYKNLSYLTKNLR	240
<i>P. chabaudi adami</i> DS	LCAKHASFXVPGNNANAYRHPAVYDKHNKTCYI LYVAAQENMGPRYCSNEENNENQPF CFTPEKKDEYKNLSYLTKNLR	240
Majority	EDWET SCPNKS I QNAKF GVMMDGYCSEYQKKEVDSXSL SXCXI VFDESASDQP KQYEKHL EDTXKRRGI VDRNGKLI	
	250 260 270 280 290 300 310 320	
<i>P. chabaudi chabaudi</i> AS	EDWET SCPNKS I QNAKF GVMMDGYCSEYQKKEVDSXSL SXCXI VFDESASDQP KQYEKHL EDTXKRRGI VDRNGKLI	320
<i>P. chabaudi adami</i> DS	EDWET SCPNKS I QNAKF GVMMDGYCSEYQKKEVHDSKSL SECNRI VFDESASDQP KQYEKHL EDTTKFRRGI VDRNGKLI	320
Majority	GEALLPI GSYRADQVKS KGKGYNWMANYDKKEKKCYI FNKKPT CLI NDKNFVATT ALS SLEEAQESFP CDI YKKKI AEEI	
	330 340 350 360 370 380 390 400	
<i>P. chabaudi chabaudi</i> AS	GEALLPI GSYRADQVKS KGKGYNWMANYDKKEKKCYI FNKKPT CLI NDKNFVATT ALS SLEEAQESFP CDI YKKKI AEEI	400
<i>P. chabaudi adami</i> DS	GEALLPI GSYRADQVKS KGKGYNWMANYDKKEKKCYI FNKKPT CLI NDKNFVATT ALS SLEEAPQESFP CDI YKKKI AEEI	400
Majority	KVMNVNRNNGNX TI XFPRI FISDDKESL KCPCEPTQLT QSSCNFVFCNCVEKRFI SENNEVEI KXEFKSEYESPI NQR	
	410 420 430 440 450 460 470 480	
<i>P. chabaudi chabaudi</i> AS	KVMNVNRNNGNXTI XFPRI FISDDKESL KCPCEPTQLT QSSCNFVFCNCVEKRFI SENNEVEI KXEFKSEYESPI NQR	480
<i>P. chabaudi adami</i> DS	KVMNVNRNNGNDTI XFPRI FISDDKESL KCPCEPTQLT QSSCNFVFCNCVEKRFI SENNEVEI KDEFKSEYESPI NQR	480
Majority	MLIIII LI ATGAI LASLLI FYFFKSNKP GDDYDKMGQADT YGKAQSRKDEMLDPEVSWGEGDKRASHTT PVLMEKPY	
	490 500 510 520 530 540 550	
<i>P. chabaudi chabaudi</i> AS	MLIIII LI ATGAI LASLLI FYFFKSNKP GDDYDKMGQADT YGKAQSRKDEMLDPEVSWGEGDKRASHTT PVLMEKPY	558
<i>P. chabaudi adami</i> DS	MLIIII LI ATGAI LASLLI FYFFKSNKP GDDYDKMGQADT YGKAQSRKDEMLDPEVSWGEGDKRASHTT PVLMEKPY	558

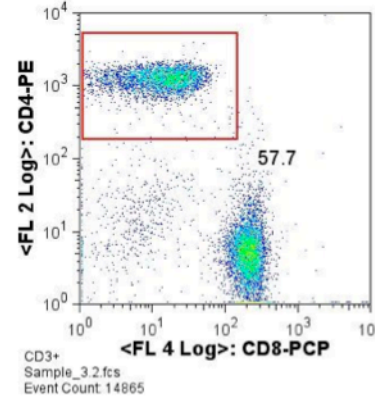
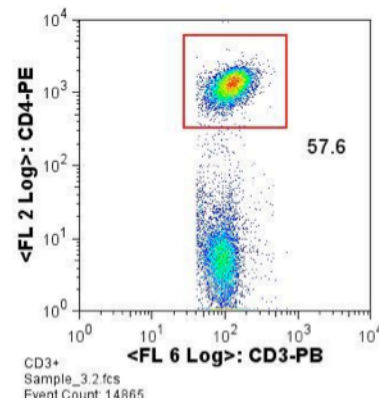
### Supplemental Figure 1: Sequence comparison of PccAS AMA1

The sequence of PccAS AMA1 was aligned (by Clustal W method) to that of related parasite *P. chabaudi adami* DS. The sequence differs by 34  $\alpha$  (shown in grey).

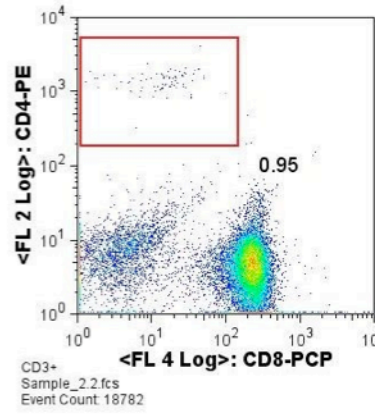
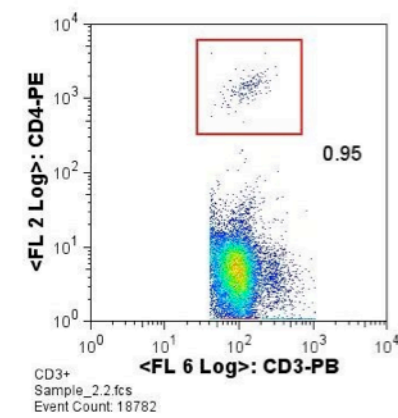
**A**



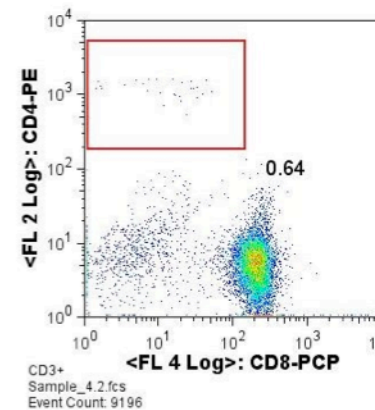
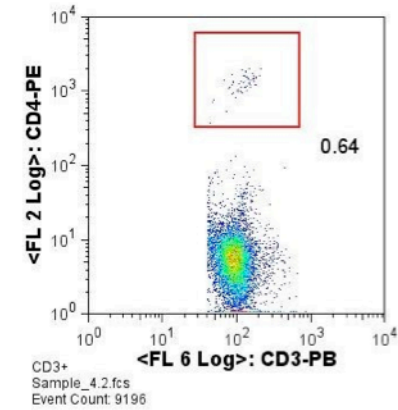
**B**



**C**



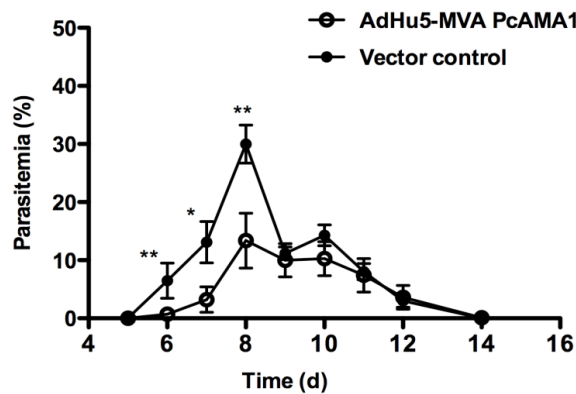
**D**



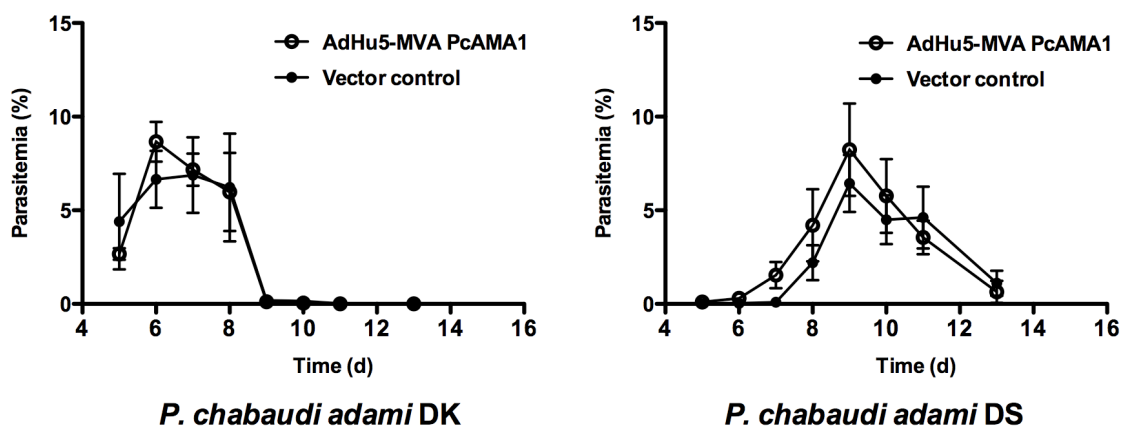
**Supplemental Figure 2: *In vivo* CD4<sup>+</sup> T cell depletion using anti-CD4 GK1.5 mAb.**

Two groups of BALB/c mice ( $n=6$ ) were immunised with  $5 \times 10^{10}$  vp AdHu5-PcAMA1 and boosted 8 weeks later with  $1 \times 10^7$  pfu MVA-PcAMA1. CD4<sup>+</sup> T cells were depleted in one group of naïve and one group of vaccinated mice with anti-CD4 GK1.5 mAb. The mice received 200µg of depleting anti-CD4 mAb intraperitoneally (i.p.) on days -2, -1 and day of challenge (day 0). Seven days after challenge the PMBCs were isolated from a blood sample and surface stained for CD3 (clone 145-2C11), CD4 (clone LT34) and CD8α (clone 53-6.7). Cells were analyzed by flow cytometry for % CD3<sup>+</sup> CD4<sup>+</sup> T cells (left panel) in the depleted and non-depleted samples, and the CD4<sup>+</sup> and CD8<sup>+</sup> T cells were plotted against each other (right panel). The figure shows representative flow plots for vaccinated non-depleted (A), naïve non-depleted (B), vaccinated depleted (C) and naïve depleted (D). There was still > 98% depletion at day 7 post challenge.

A



B



**Supplemental Figure 3: (A) Comparison of efficacy after immunization of BALB/c mice with control viral vaccines and those recombinant for PcAMA1 (B) Efficacy of the AdHu5-MVA PcAMA1 vaccination regime against challenge with heterologous parasite strains.**

Mice were primed with  $1 \times 10^{10}$  vp AdHu5-PcAMA1 or non-recombinant AdHu5 control and boosted 8 weeks later with  $1 \times 10^7$  pfu MVA-PcAMA1 or MVA expressing GFP. Two weeks after the boost mice were challenged i.v. with (A)  $10^5$  PccAS pRBC or (B) *P. chabaudi adami* DK or *P. chabaudi adami* DS). Parasitemia was monitored from day 5 by Giemsa-stained thin blood smears and results are expressed as the % infected RBCs (mean  $\pm$  SEM). The difference in parasitemia between the groups was taken as the measure of vaccine efficacy. As seen with naïve mice, there was a significant difference in parasitemia between the control and PcAMA1 immunized mice by Mann-Whitney test (\*  $P \leq 0.05$  and \*\*  $P \leq 0.01$ ) (A). There was no significant difference in parasitemia between the vector control and PcAMA1 immunized mice when challenged with heterologous *P. chabaudi* parasites (B).

<b>POOL</b>	<b>PEPTIDE #</b>	<b><math>\alpha\alpha</math> SEQUENCE</b>	<b><math>\alpha\alpha</math> POSITION</b>
<b>Pool 7</b>	Pcc 1	CSEGTDNIISENGDV	21-35
	Pcc 2	DNIISENGDVKFDLI	26-40
	Pcc 3	ENGDVKFDLIPKENT	31-45
	Pcc 4	KFDLIPKENTERSHK	36-50
	Pcc 5	PKENTERSHKLINPW	41-55
	Pcc 6	ERSHKLINPWEKFME	46-60
	Pcc 17	ITIQNSKVSFLTRVA	101-115
	Pcc 18	SKVSFLTRVATGNQK	106-120
	Pcc 19	LTRVATGNQKVREGG	111-125
<b>Pool 8</b>	Pcc 20	TGNQKVREGGLAFPQ	116-130
	Pcc 21	VREGGLAFPQTDVNI	121-135
	Pcc 22	LAFPQTDVNIAPITI	126-141
	Pcc 23	TDVNIAPITIANLKL	131-145
	Pcc 24	APITIANLKLMYKDH	136-150
	Pcc 27	KEILALNDMSLCAKH	151-165
	Pcc 28	LNDMSLCAKHASFYV	156-170
	Pcc 31	PGTNVNTAYRHPAVY	171-185
	Pcc 32	NTAYRHPAVYDKSNQ	176-190
	Pcc 33	HPAVYDKSNQACYIL	181-195
	Pcc 34	DKSNQACYILYVAAQ	186-200
<b>Pool 9</b>	Pcc 35	ACYILYVAAQENMGP	191-205
	Pcc 36	YVAAQENMGPARYCSN	196-210
	Pcc 37	ENMGPARYCSNEEDNE	201-215
	Pcc 38	RYCSNEEDNENQPFQ	206-220
	Pcc 39	EEDNENQPFQFTPEK	211-225
	Pcc 40	NQPFQFTPEKKDEYK	216-230
	Pcc 41	FTPEKKDEYKNLAYL	221-235
	Pcc 42	KDEYKNLAYLTKNLR	226-240
	Pcc 43	NLAYLTKNLRDREWET	231-245
	Pcc 44	TKNLRDREWETSCPNK	236-250
<b>Pool 10</b>	Pcc 45	EDREWETSCPNKAIQNA	241-255
	Pcc 46	SCPNKAIQNAKFGVW	246-260
	Pcc 47	AIQNAKFGVWVDGYC	251-265
	Pcc 48	KFGVWVDGYCSEYQK	256-270
	Pcc 49	VDGYCSEYQKKEVRD	261-275
	Pcc 50	SEYQKKEVRDSENSLS	266-280
	Pcc 51	KEVRDSENSLSDCSKI	271-285
	Pcc 52	SNSLSDCSKIVFDES	276-290
	Pcc 53	DCSKIVFDESASDQP	281-295
	Pcc 54	VFDESASDQPKQYEK	286-300
<b>Pool 11</b>	Pcc 55	ASDQPKQYEKHLEDT	301-315
	Pcc 56	KQYEKHLEDTAKIRR	306-320
	Pcc 57	HLEDTAKIRRGIVDR	311-325
	Pcc 58	AKIRRGIVDRNGKLI	316-330
	Pcc 59	GIVDRNGKLIGEALL	321-335
	Pcc 60	NGKLIGEALLPIGSY	326-340
	Pcc 61	GEALLPIGSYRADQV	331-345
	Pcc 62	PIGSYRADQVKSCKGK	336-350
	Pcc 63	RADQVKSCKGKGYNWA	341-355
Pcc 64	KSKGKGYNWANYDKK	346-360	
<b>Pool 12</b>	Pcc 65	GYNWANYDKKEKKCY	351-365
	Pcc 66	NYDKKEKKCYIFNKK	356-370

	Pcc 67	EKKCYIFNKKPTCLI	361-375
	Pcc 68	IFNKKPTCLINDKNF	366-380
	Pcc 69	PTCLINDKNFVATTA	371-385
	Pcc 70	NDKNFVATTALSSLE	376-390
	Pcc 71	VATTALSSLEEASQE	381-395
	Pcc 72	LSSLEEASQESFPCD	386-400
	Pcc 73	EASQESFPCDIYKKK	401-415
	Pcc 76	IAEEIKVMNVNRNNN	406-420
<b>Pool 13</b>	Pcc 77	KVMNVNRNNNGNGTI	401-415
	Pcc 78	NRNNNGNGTIQFPRI	406-420
	Pcc 81	FISDDKESLKPCPEP	421-435
	Pcc 82	KESLKPCPEPTQLTQ	426-440
	Pcc 83	CPCEPTQLTQSSCNF	431-445
	Pcc 84	TQLTQSSCNFFVCNC	436-450
	Pcc 87	VEKRQFISENNEVEI	451-465
	Pcc 88	FISENNEVEIKEEFK	456-470
	Pcc 89	NEVEIKEEFKSEYES	461-470
	Pcc 90	KEEFKSEYESPINQ	466-480

**Supplemental Table 1: PccAS peptide pools P7 to P13 covering the entire ectodomain of PccAS AMA1.**

The table shows the seven peptide pools tested by ICS (Figure 3) and the  $\alpha$  sequence of each peptide. Each pool contains 10 peptides (each 15mers) and covers the remainder of the entire ectodomain of PccAS AMA1 (not covered by peptides shown in Table 1).