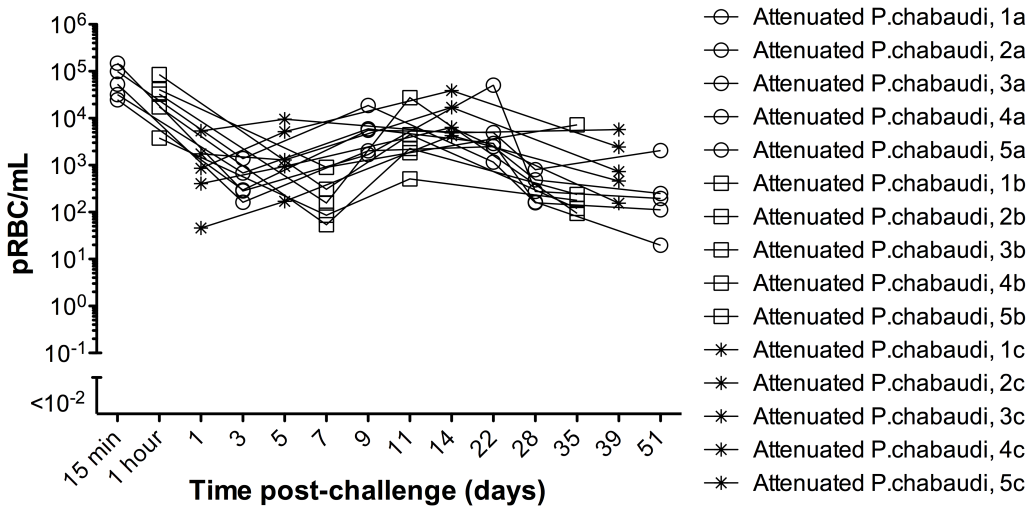
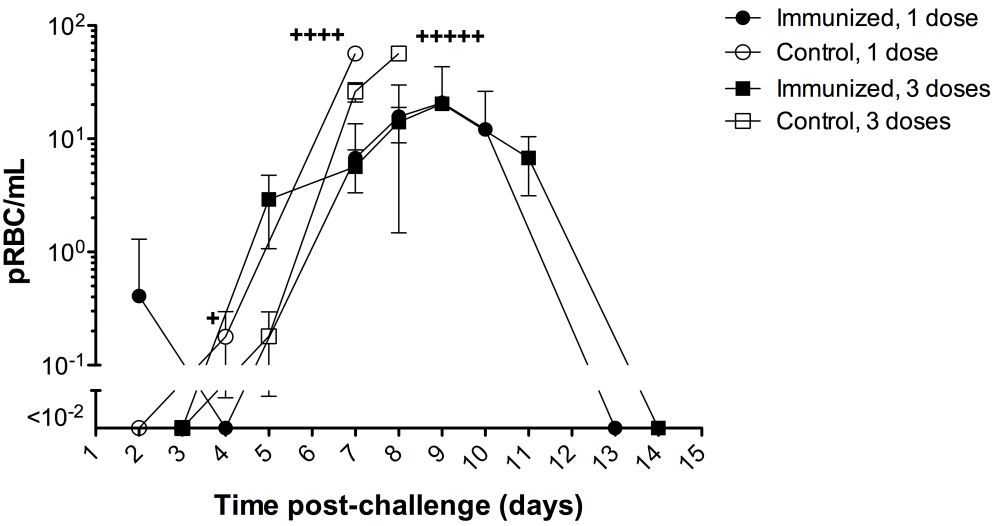
Supp. Fig 1


pRBC/mL
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
<10^{-2}
15 min
1 hour
1
3
5
7
9
11
14
22
28
35
39
51
Time post-challenge (days)
Attenuated P.chabaudi, 1a
Attenuated P.chabaudi, 2a
Attenuated P.chabaudi, 3a
Attenuated P.chabaudi, 4a
Attenuated P.chabaudi, 5a
Attenuated P.chabaudi, 1b
Attenuated P.chabaudi, 2b
Attenuated P.chabaudi, 3b
Attenuated P.chabaudi, 4b
Attenuated P.chabaudi, 5b
Attenuated P.chabaudi, 1c
Attenuated P.chabaudi, 2c
Attenuated P.chabaudi, 3c
Attenuated P.chabaudi, 4c
Attenuated P.chabaudi, 5c

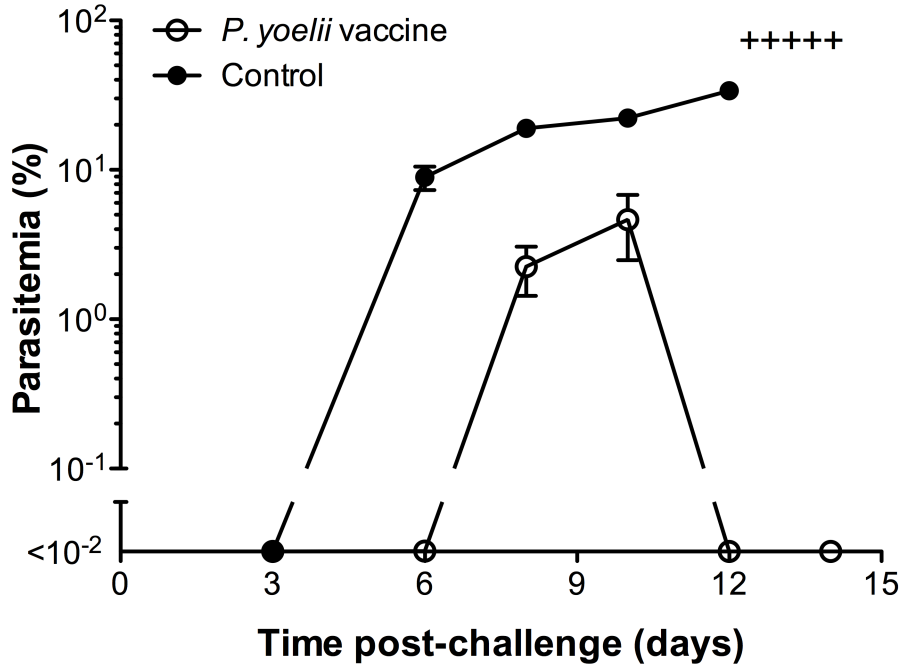
Supp. Figure 1. *Monitoring attenuated parasites by qPCR*. (a) 15 immunodeficient SCID mice were administered 10^6 *P. chabaudi* pRBC attenuated with 2 μM centanamycin. 50ul of blood was collected from groups of 5 mice at each time point and qPCR performed to estimate parasite density. The 3 groups were bled every third timepoint to prevent anemia from repeated bleeding. Y-axes show the estimated parasite density.

Supp. Fig 2


Immunized, 1 dose
Control, 1 dose
Immunized, 3 doses
Control, 3 doses
pRBC/mL
10^2
10^1
10^0
10^{-1}
$<10^{-2}$
++++
+++++
+
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Time post-challenge (days)

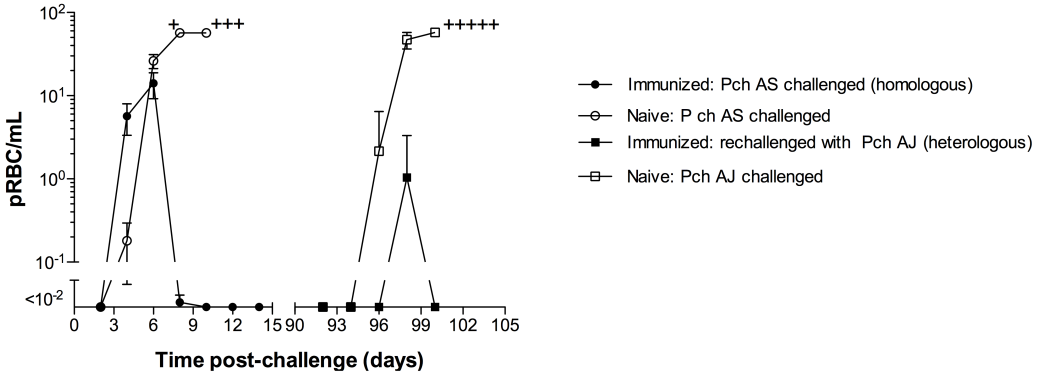
Supp. Figure 2. *Comparison of 1 dose vs 3 doses of vaccine*. (a) Cohorts of five A/J mice were vaccinated with a single dose or three doses of 10^6 *P. chabaudi* pRBC attenuated with centanamycin. All mice were challenged 5 weeks post the first dose (two weeks post 3rd dose in thrice immunized mice). '+' indicates that a mouse succumbed to the infection.

Supp. Fig 3


P. yoelii vaccine
Control
+++++
Parasitemia (%)
10^2
10^1
10^0
10^-1
<10^-2
0
3
6
9
12
15
Time post-challenge (days)

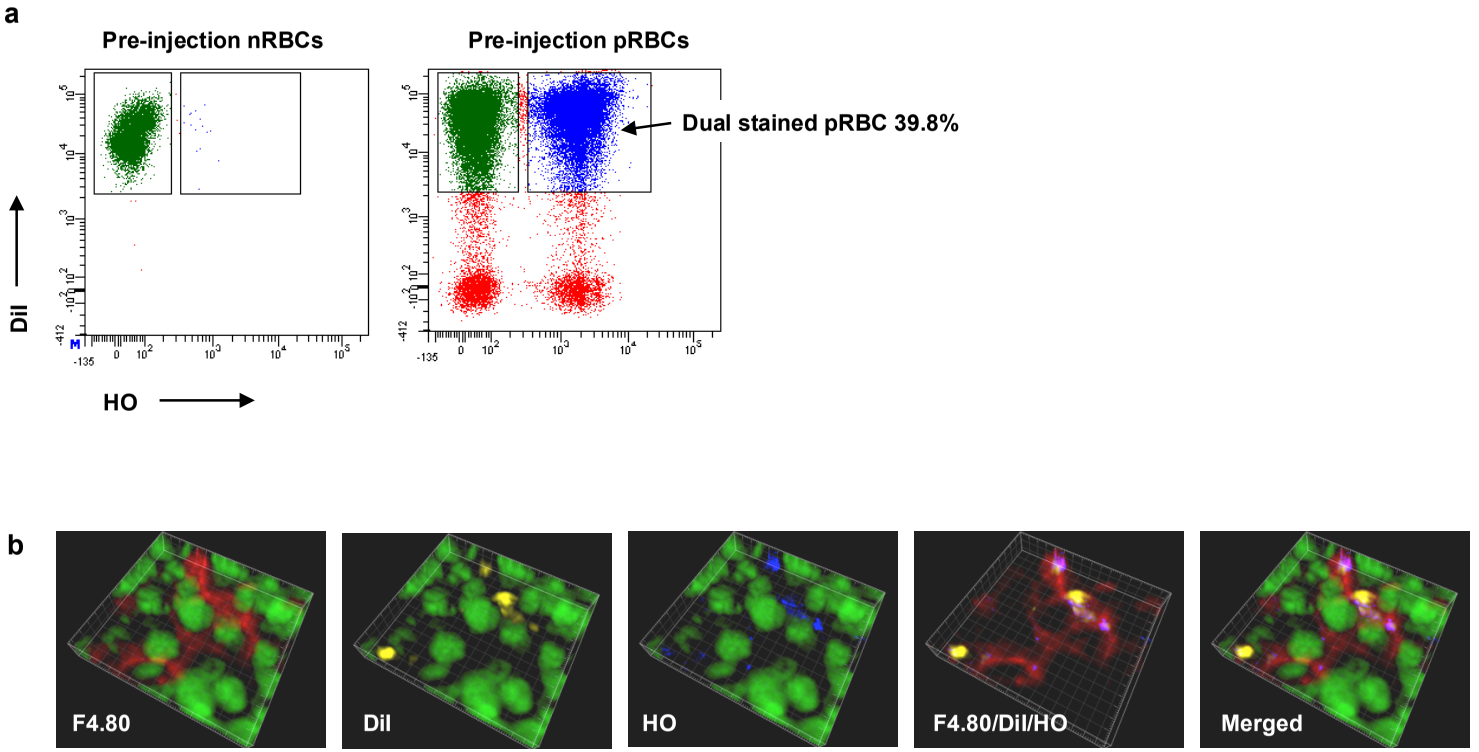
Supp. Figure 3. *Vaccine immunity is long-lived*. (a) Five A/J mice were vaccinated with a single dose of 10^6 *P. yoelii* pRBC attenuated with centanamycin or left naive. All mice were challenged 6 months post vaccination. '+' indicates that a mouse succumbed to the infection.

Supp. Fig 4


pRBC/mL
10^2
10^1
10^0
10^{-1}
$<10^{-2}$
+
+++
+++++
0
3
6
9
12
15
90
93
96
99
102
105
Time post-challenge (days)
Immunized: Pch AS challenged (homologous)
Naive: P ch AS challenged
Immunized: rechallenged with Pch AJ (heterologous)
Naive: Pch AJ challenged

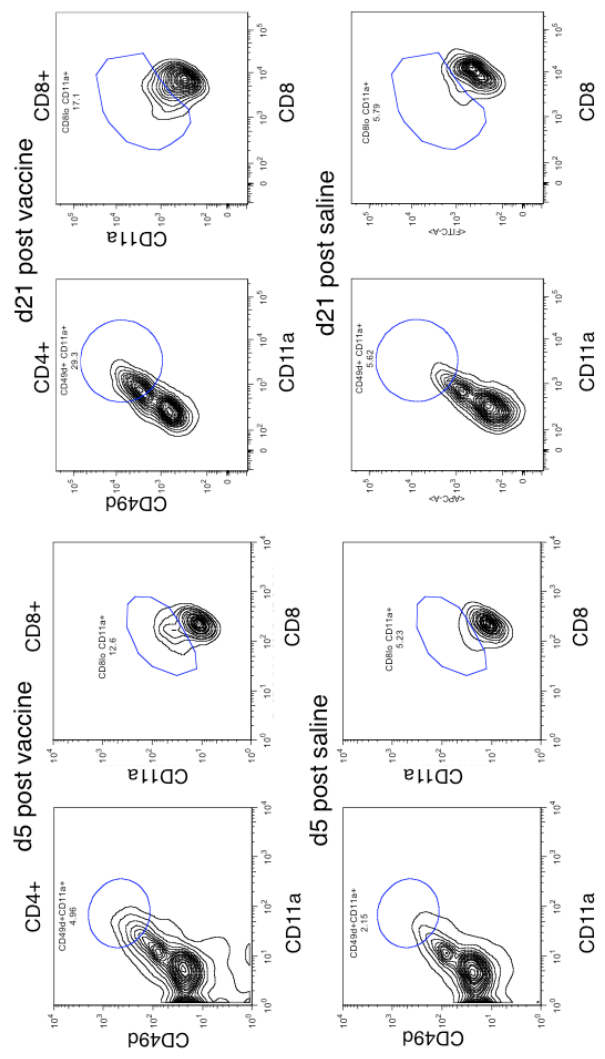
Supp. Figure 4. *Vaccine immunity protects against homologous and heterologous parasites*. (a) A/J mice were vaccinated with a single dose of 10^6 *P. chabaudi AS* pRBC attenuated with centanamycin (5 mice) or left naïve (4 mice). Mice were challenged with homologous parasite, *P. chabaudi AS*. Three months later immunized mice were re-challenged with heterologous strain *P. chabaudi AJ* and naïve mice also challenged with *P. chabaudi AJ.* '+' indicates that a mouse succumbed to the infection.

Supp. Fig 5


a
Pre-injection nRBCs
Pre-injection pRBCs
Dual stained pRBC 39.8%
DiI
HO
b
F4.80
DiI
HO
F4.80/DiI/HO
Merged

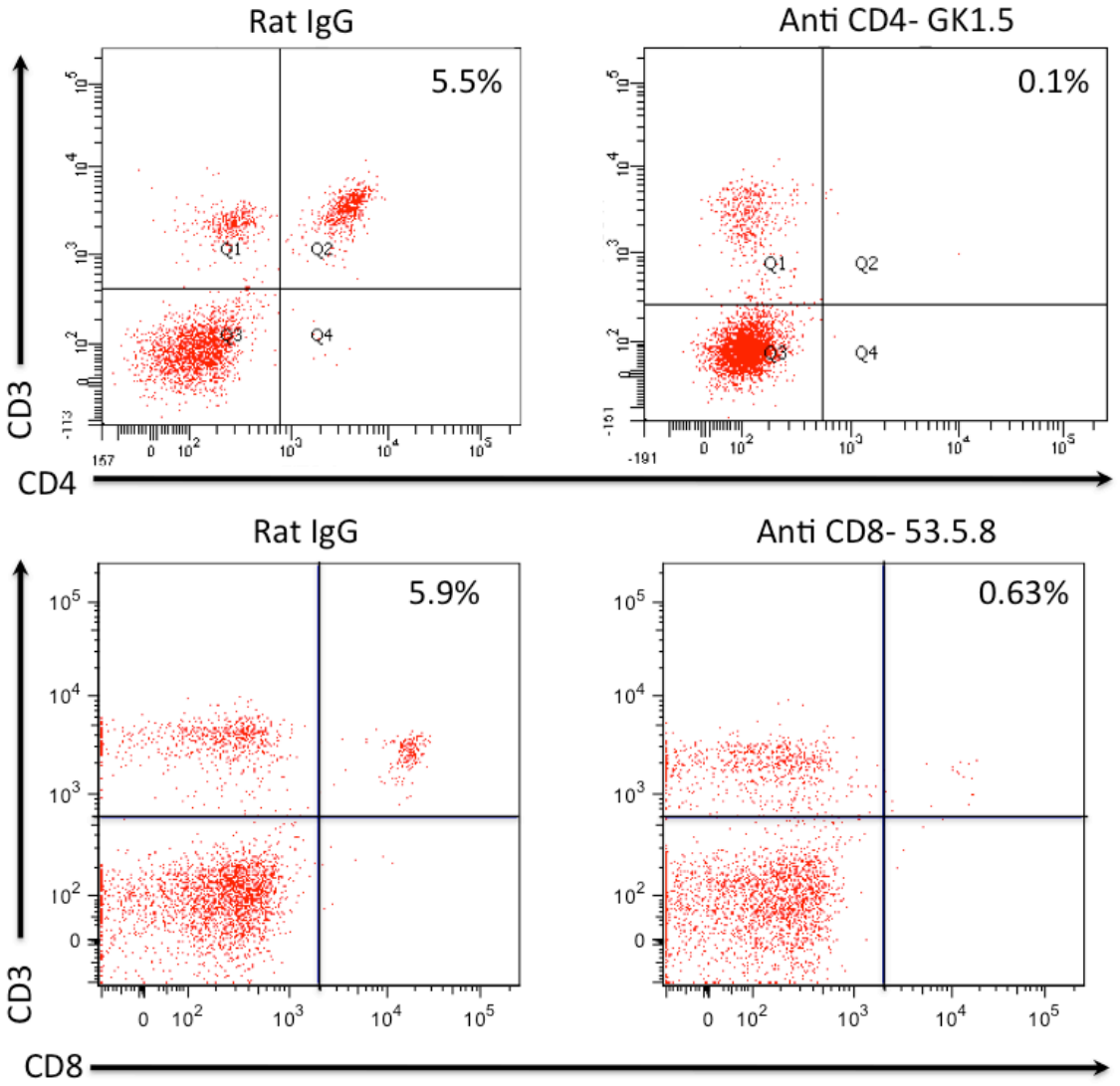
Supp. Figure 5. *Visualization of attenuated parasites in spleen*. (a) RBCs from *P. chabaudi*–infected mice (39.8% parasitemia) were treated with 2μM centanamycin. RBC membranes and parasite DNA were stained with DiI and HO, respectively. Membrane-stained normal RBCs from naïve A/J mice and dual stained attenuated parasites were analysed by flow cytometry prior to injection into naïve A/J mice. (b) Spleen sections from recipient mice were prepared at 24hr and stained with APC anti-mouse F4/80 IgG and nuclei counter-stained with SYTOX Green**.** The slides were analysed by confocal microscopy. The colours indicated are: F4/80 (red); DiI (yellow); HO (blue); and nuclei (green).

Supp. Fig 6


CD4+
d5 post vaccine
CD8+
CD49d
CD11a
CD11a
CD8
CD4+
d21 post vaccine
CD8+
CD49d
CD11a
CD11a
CD8
d5 post saline
CD49d
CD11a
CD11a
CD8
d21 post saline
CD11a
CD8

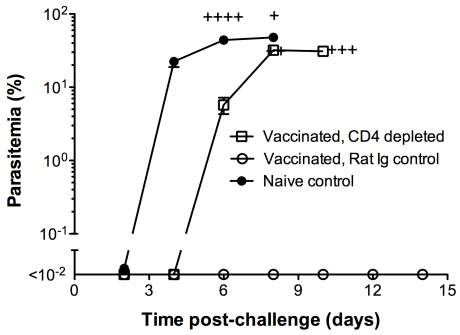
Supp. Figure 6. *Flow cytometry plots of $CD4^+CD49d^+$ $CD11a^+$ and $CD8^{lo}$ $CD11a^+$.* Here are representative plots showing *$CD4^+CD49d^+$ $CD11a^+$ and $CD8^{lo}$ $CD11a^+$* populations in peripheral blood of saline and vaccine immunized mice at days 5 and days 21 post injection. Inset numbers indicate the percentage of cells found within their respective gates. The gates chosen for the saline controls and vaccine groups were identical for each time point and chosen to include only the brightest CD49d and CD11a cells.

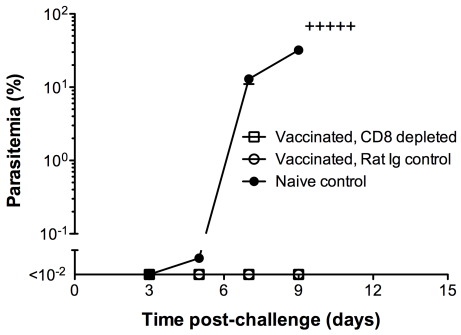
Supp. Fig 7


Rat IgG
5.5%
Anti CD4- GK1.5
0.1%
Q1
Q2
Q3
Q4
CD3
CD4
Rat IgG
5.9%
Anti CD8- 53.5.8
0.63%
CD3
CD8

Supp. Figure 7. *Depletion of CD4 and CD8 T cells in vivo*. Mice were treated with 500ug of anti-CD4 (GK1.5), anti-CD8 (53.5.8) or rat IgG i.p. on days -4, -3,-2,-1,0,+1 relative to challenge. Here are representative plots showing CD4 and CD8 in spleens of control and depleted mice.

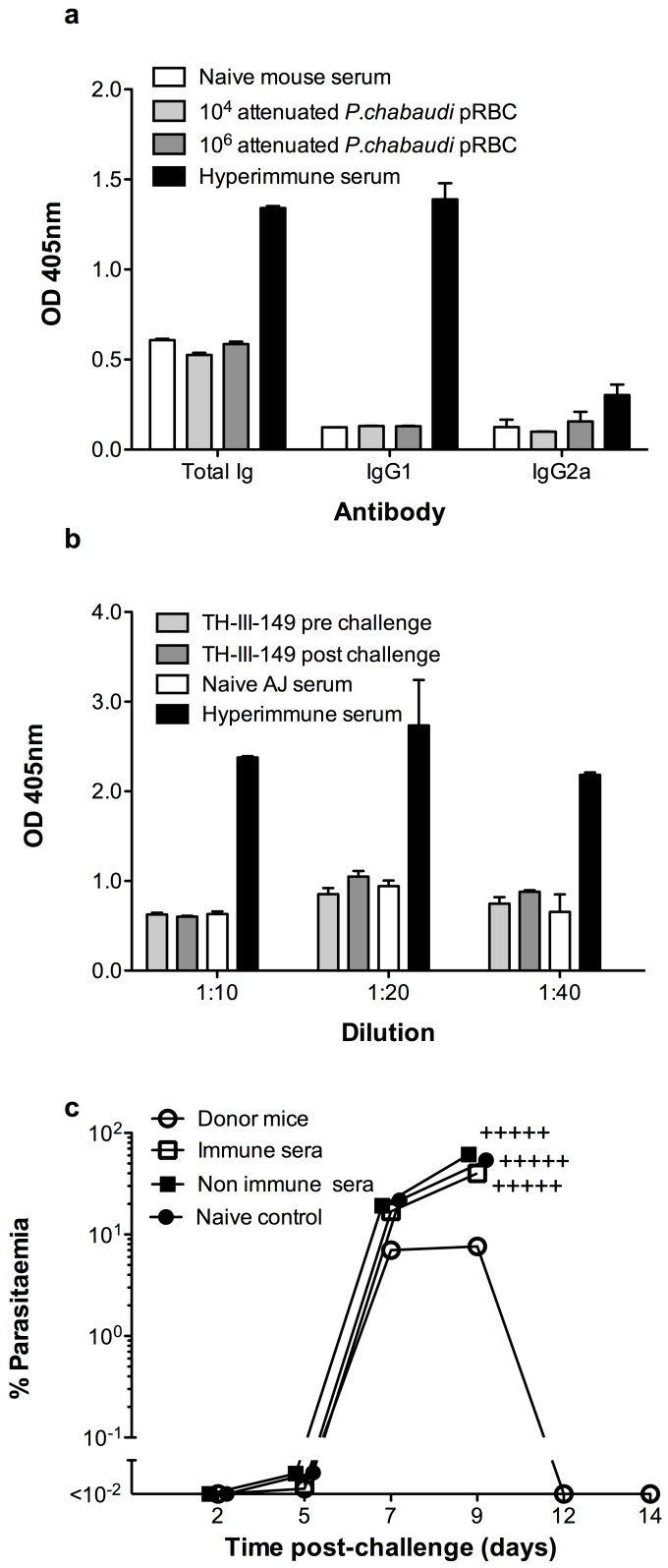
Supp. Fig 8


Parasitemia (%)
10^2
10^1
10^0
10^{-1}
$<10^{-2}$
++++
+
+++
Vaccinated, CD4 depleted
Vaccinated, Rat Ig control
Naive control
0
3
6
9
12
15
Time post-challenge (days)


Parasitemia (%)
10^2
10^1
10^0
10^{-1}
$<10^{-2}$
+++++
Vaccinated, CD8 depleted
Vaccinated, Rat Ig control
Naive control
0
3
6
9
12
15
Time post-challenge (days)

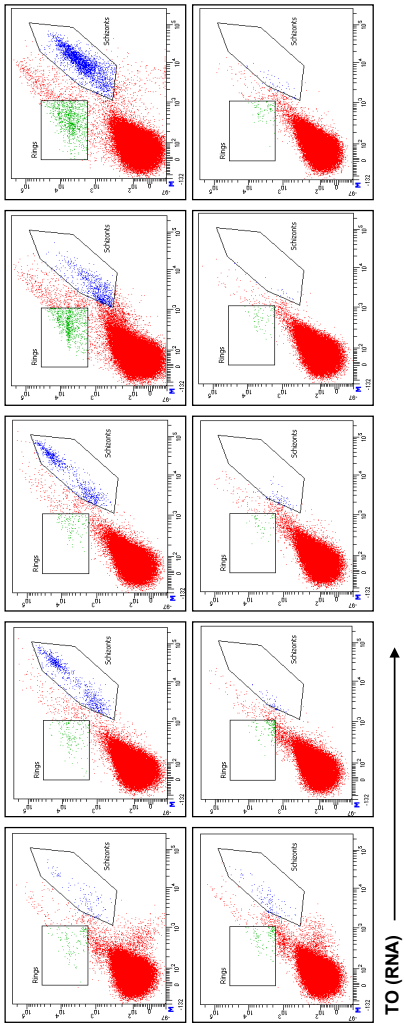
Supp. Figure 8. *CD4 T cells contribute to vaccine mediated protection.* To assess the role of T cell subsets in immunity, A/J mice were immunized with three doses of 10^6 *P. chabaudi* pRBC attenuated with centanamycin. Mice were immunodepleted as indicated eight weeks after immunization, then challenged with 10^5 *P. chabaudi* pRBC i.v. and parasitemia monitored. '+' indicates that a mouse succumbed to the infection..

Supp. Fig 9


a
Naive mouse serum
10^4 attenuated P.chabaudi pRBC
10^6 attenuated P.chabaudi pRBC
Hyperimmune serum
OD 405nm
2.0
1.5
1.0
0.5
0.0
Total Ig
IgG1
IgG2a
Antibody
b
TH-III-149 pre challenge
TH-III-149 post challenge
Naive AJ serum
Hyperimmune serum
OD 405nm
4.0
3.0
2.0
1.0
0.0
1:10
1:20
1:40
Dilution
c
Donor mice
Immune sera
Non immune sera
Naive control
+++++
+++++
+++++
% Parasitaemia
10^2
10^1
10^0
10^-1
<10^-2
2
5
7
9
12
14
Time post-challenge (days)

Supp. Figure 9. *Vaccine does not elicit elevated parasite specific antibodies and serum from immunized mice does not confer protection.* (a) Parasite-specific antibody titers were assessed by ELISA eight weeks after immunization with three doses of 10^4 or 10^6 centanamycin-attenuated *P. chabaudi* pRBC. Antigen was prepared from *P. chabaudi* blood stages as described in methods. Hyperimmune serum from a mouse that had received multiple patent infections. (b) Parasite-specific antibody titers were assessed by ELISA from samples collected after immunization with three doses of 10^6 TH-III-149 attenuated *P. chabaudi* pRBC (pre-challenge) and then again on d38 post challenge with 10^5 *P. chabaudi* pRBC. Error bars represent S.E.M. for triplicate ELISA samples. (c) Sera from 10 A/J mice immunized with 3 doses of *P chabaudi AS* centanamycin attenauated or 10 naïve A/J mice was transferred to groups of 5 naïve A/J recipients on days -1, 0, and 1 relative to challenge on day 0 with 10^5 *P chabaudi AS.* Donor immunized A/J mice were challenged and protected. '+' indicates that a mouse succumbed to the infection.

Supp. Fig 10


0 hr
16 hr
32 hr
48 hr
64 hr
WT
Centanamycin treated
HO (DNA)
TO (RNA)
Rings
Schizonts

Supp. Figure 10. *Attenuation of P. falciparum by centanamycin*. Parasites were synchronized to ring stages (rectangles) and either treated with centanamycin (2 μM) or left untreated before culturing. At the given time points, samples were stained with Hoechst (HO) and Thiazole Orange (TO) and analyzed by flow cytometry.