

## Supporting Information

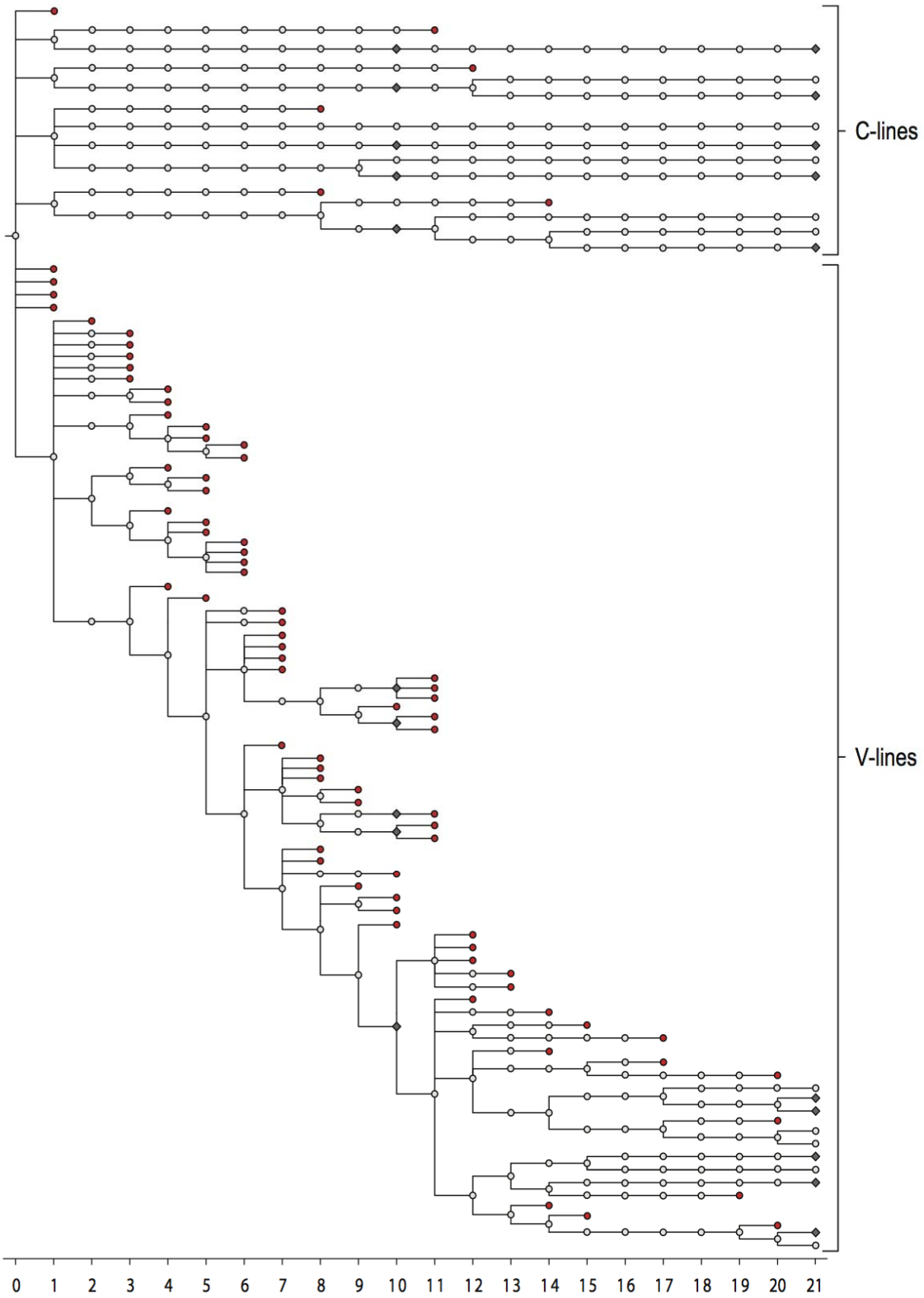
Barclay et al. (2012) The Evolutionary Consequences of Blood-Stage Vaccination on the Rodent *Malaria Plasmodium chabaudi*. PLoS Biol 10(7): e1001368. doi:10.1371/journal.pbio.1001368.

### Fig S1

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CTTGGGTAATTGTTCCGAAGGTACAGATAAAAATTATTTTCAGAAAATGGTGACGTTAA
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**Fig S1 Caption** Nucleotide sequence of *P. chabaudi ama-1*. Consensus *P. c. adami ama-1* nucleotide sequence between the derived virulent parasites from used in “evaluation” experiment 1, the V-lines and C-lines used in “evaluation” experiments 3 and 4 (21 serial passages), and the ancestral lineages from which all lines were derived and all compared to the published *P. c. adami* DK *ama-1* (genebank accession number U49745). There was 100% *ama-1* sequence identity among and between all of the derived lines and with their ancestral lineages and to the published genebank sequence (shaded in grey). The outer forward and inner reverse primers used for amplification and sequencing are highlighted in bold and the inner forward and outer reverse primers are shown in lowercase lettering. All traces were examined by eye for multiple peaks, and none were observed. If parasites with base-pair changes were present in sequenced samples, they must have been there at frequencies less than about 20%.

Fig S2

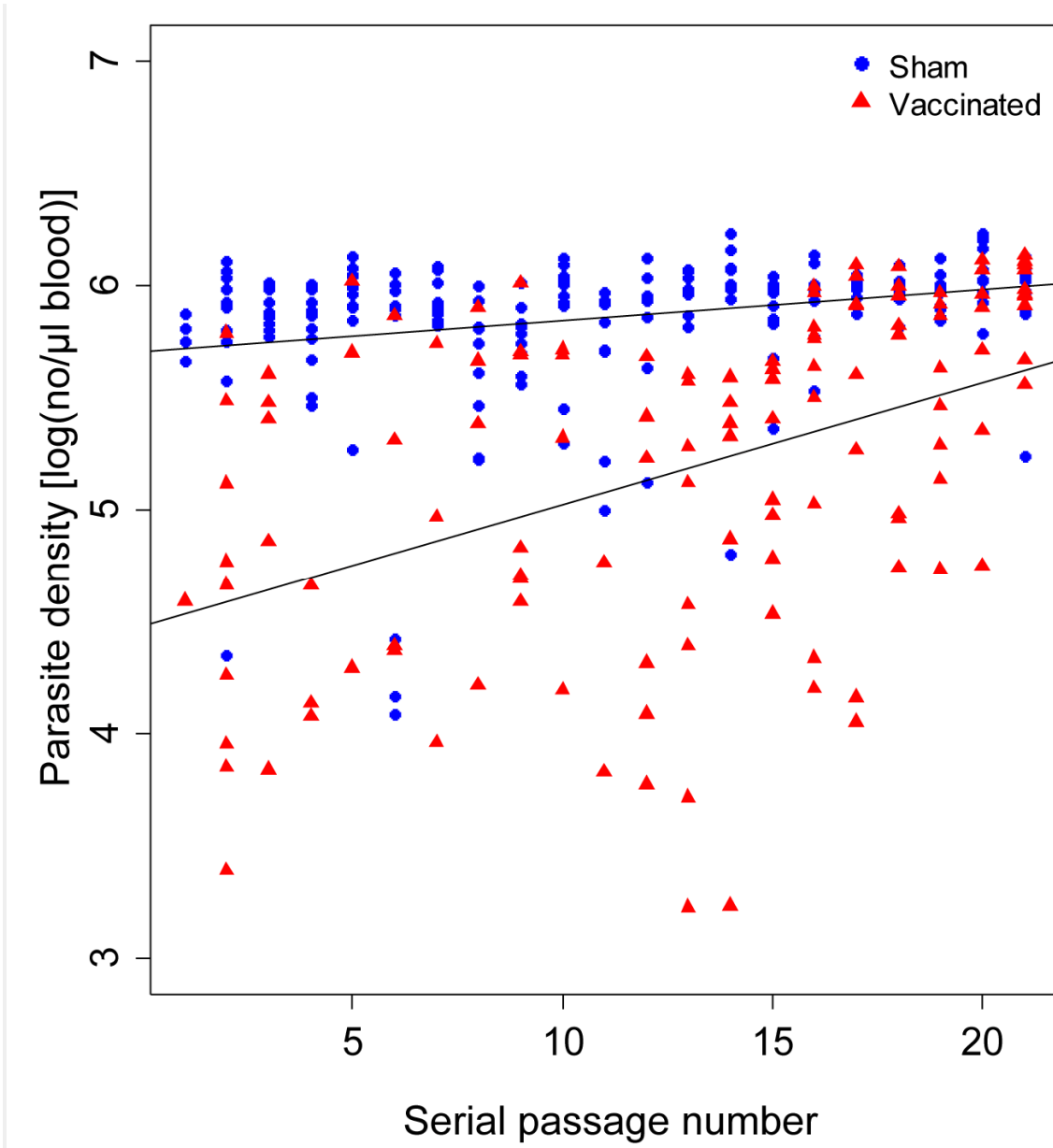


**Fig S2 Caption:** Experimental evolution (serial passage B) in sham- and AMA-1-vaccinated animals. Schematic genealogy illustrating passage history of the C-lines and V-lines from the ancestral lineage. Nodes represent mice.

To start, five mice that had been previously immunized with the AMA-1 vaccine or a sham vaccine were infected with *P. c. adami* genotype DK<sub>247</sub> (passage 1) to initiate the V-lines and C-lines, respectively. Parasites from each one of the five mice at passage 1 were then used to infect at least two mice at passage 2 (forming a total of 10 sublines per treatment). From passage 2 to 21 parasites from each mouse within a selection treatment were used to infect a fresh mouse in the next passage. Where parasite lines were lost (filled red circles) blood from a mouse in another line within that treatment group was used to infect at least two other mice in the next generation. Lines were lost when parasite densities were below transmissible frequencies on day 7 PI either because of vaccine-induced immunity (V-lines) or errors in dose delivered to mice (C-lines). Diamonds represent parasite lines used in the different evaluation experiments.

doi:10.1371/journal.pbio.1001368.s002

Fig S3



**Fig S3 Caption:** Parasite densities of each mouse during serial passage B in sham- and AMA-1- vaccinated animals. Each data point represents the log parasite density of each mouse in the C-lines (blue circles) or V-lines (red triangles) from passage 1 to 21. Solid black lines represent the log linear regression change in parasite density per selection treatment over time.

doi:10.1371/journal.pbio.1001368.s003

**Table S1**

<b>Evaluation exp</b>	<b>Immune status</b>	<b>Parasites</b>	<b>Subline</b>	<b>No. mice</b>
1	Sham-vaccine	Ancestral	Ancestral (DK <sub>122</sub> )	4
	Sham-vaccine	Derived	Derived (DK <sub>294</sub> )	4
	AMA-1 vaccine	Ancestral	Ancestral (DK <sub>122</sub> )	4
	AMA-1 vaccine	Derived	Derived (DK <sub>294</sub> )	4
2	Naïve	Passage 10	C2	3
	Naïve		C4	3
	Naïve		C6	3
	Naïve		C8	3
	Naïve		C10	3
	Naïve	Passage 10	V1	3
	Naïve		V3	3
	Naïve		V4	3
	Naïve		V5	3
	Naïve		V10	3
3	Naïve	Passage 21	Ancestral (DK <sub>247</sub> )	9
	Naïve		C1	3
	Naïve		C3	3
	Naïve		C5	3
	Naïve		C7	3
	Naïve		C10	3
	Naïve	Passage 21	V2	3
	Naïve		V3	3
	Naïve		V6	3
	Naïve		V8	3
	Naïve		V9	3
4	Sham-vaccine	Passage 21	C1	3
	Sham-vaccine		C3	3

Sham-vaccine	C5	3
Sham-vaccine	C7	3
Sham-vaccine	C10	3
AMA-1 vaccine	C1	3
AMA-1 vaccine	C3	3
AMA-1 vaccine	C5	3
AMA-1 vaccine	C7	3
AMA-1 vaccine	C10	3
Sham-vaccine	V2	3
Sham-vaccine	V3	3
Sham-vaccine	V6	3
Sham-vaccine	V8	3
Sham-vaccine	V9	3
AMA-1 vaccine	V2	3
AMA-1 vaccine	V3	3
AMA-1 vaccine	V6	3
AMA-1 vaccine	V8	3
AMA-1 vaccine	V9	3

**Table S1 Caption.** Description of evaluation experiments 1 to 4. V and C, V-or C-lines. Numbers indicate subline used. DK<sub>122</sub>, DK<sub>247</sub>, and DK<sub>294</sub>, DK ancestral genotypes with subscript codes used to identify their position in clonal history.

doi:10.1371/journal.pbio.1001368.s004

**Table S2**

<b>Evaluation Exp.</b>	<b>Linear mixed effects test (line as random effect)</b>	<b>Result</b>
<b>2</b>	Anaemia: V-lines versus C-lines in naïve mice	$F_{1,8} = 8.3, p = 0.02$
	Total parasites: V-lines versus C-lines in naïve mice	$F_{1,8} = 11.5, p = 0.009$
	Parasites on day 7: V-lines versus C-lines in naïve mice	$F_{1,8} = 3.6, p = 0.08$
<b>3</b>	Anaemia: V-lines versus C-lines in naïve mice	$F_{1,10} = 6.2, p = 0.03$
	Anaemia: V-lines versus ancestral parasites in naïve mice	$F_{1,10} = 9.7, p = 0.01$
	Total parasites: V-lines versus C-lines in naïve mice	$F_{1,10} = 1.1, p = 0.3$
	Total parasites: V-lines versus ancestral parasites in naïve mice	$F_{1,10} = 7.3, p = 0.02$
<b>4</b>	Anaemia: V-lines versus C-lines in sham-vaccinated mice	$F_{1,8} = 4.0, p = 0.08$
	Anaemia: V-lines versus C-lines in AMA-1 vaccinated mice	$F_{1,8} = 2.2, p = 0.1$
	Anaemia interaction = Line*Vaccination	$F_{1,16} = 0.5, p = 0.4$
	Total parasites: V-lines versus C-lines in sham-vaccinated mice	$F_{1,8} = 2.5, p = 0.1$
	Total parasites: V-lines versus C-lines in AMA-1 vaccinated mice	$F_{1,8} = 4.0, p = 0.07$
	Parasite interaction = Line*Vaccination	$F_{1,16} = 2.6, p = 0.1$

**Table S2 Caption.** Most conservative statistical analysis of evaluation experiments 2 to 4.  
doi:10.1371/journal.pbio.1001368.s005