Supporting Information

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SI Discussion

The Impact of pABA Limitation on Susceptible Parasites in Experiment 1. Limitation of pABA prevented the rebound of susceptible parasites, in addition to the emergence of resistance (Fig. 1). There are several possible explanations for this. First, susceptible parasites did not require as much pABA as resistant parasites, but they do require pABA for optimal growth, as evidenced by the fact that pABA limitation reduced the total size of single susceptible parasite infections in experiment 2 (Fig. S2). During the posttreatment period, in the face of excess mortality caused by drugs and immunity, a small difference in replication rate caused by pABA limitation could mean the difference between a rebound and none (see also Fig. S4, bottom two rows). Second, pABA limitation could potentiate the activity of pyrimethamine, as it does in other species of malaria parasites (1–3). A third possibility is that rebounding parasites in the pABA-supplemented treatment are not, in fact, susceptible to pyrimethamine. Our phenotypic test of resistance is very conservative, so that only a very high level of resistance is detected. Our genetic test of resistance is also not a catch-all. The S106N is characteristic of pyrimethamine resistance in this system (as is its homolog S108N in *Plasmodium falciparum*), but it is not the only genetic route to pyrimethamine resistance (e.g., refs. 4, 5). Mutants carrying mutations other than S106N would not be deemed resistant in our assay.

The Impact of pABA Limitation on Pyrimethamine-Resistant Parasites in the Absence of Competition. While resistant parasites grew relatively unabated in single infections in the absence of the drugs in both pABA treatments (Fig. S2*A*) and when drugs are administered in the pABA-supplemented treatment (Fig. 2*C*), the growth of resistant parasites stalls during drug treatment in the pABA-limited treatment. This observation suggests there is an interaction between the activity of pyrimethamine and pABA limitation, as has been observed in in vitro cultures of *P. falciparum* (3, 6). A plausible explanation for the interaction is suggested by work on *P. falciparum*. Parasites acquire tetrahydrofolate endogenously by producing it from pABA via the folate pathway and exogenously by acquiring preformed folates from the host environment (2, 7–9). Pyrimethamine-resistant parasites carrying a single resistance mutation in the DHFR gene have a low capacity to acquire folate exogenously, which likely causes their higher requirements for pABA (10). Moreover, pyrimethamine inhibits the ability of pyrimethamine-resistant parasites to acquire folate via the exogenous route (11). Thus, the simultaneous application of pABA limitation and pyrimethamine treatment might retard the ability of resistant parasites to replicate. There being an interaction between pABA limitation and the activity of pyrimethamine does not, however, account for the impact of resource limitations on resistance emergence, since resistant parasites do flourish in the period after drug treatment. To what extent the "stalling" effect of pABA limitation alters the dynamics of competition, and hence the efficacy of resource limitation as a resistance management strategy, is an empirical question that warrants further research.

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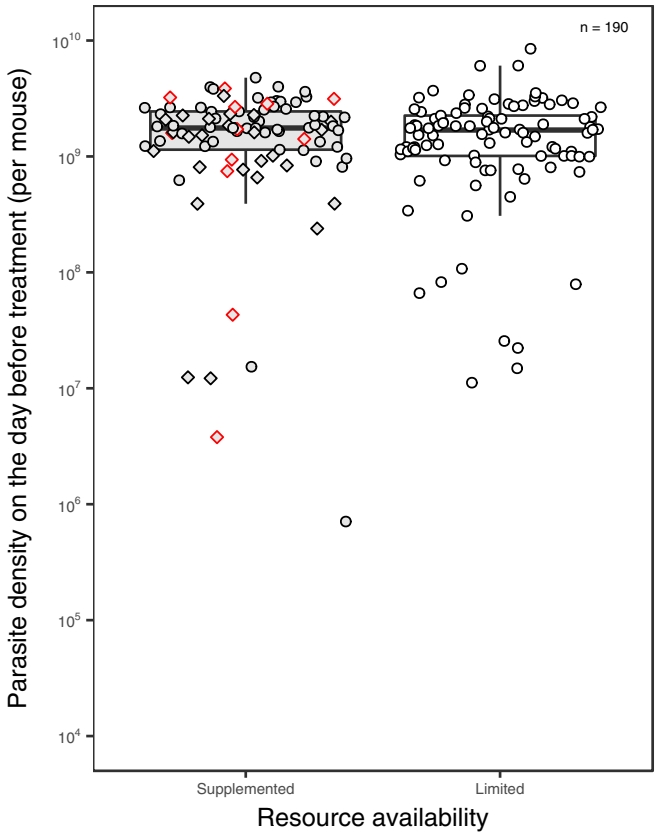

Fig. S1. Resource limitation does not impact the population size of parasites before drug treatment. The density of parasites on the day before the onset of pyrimethamine treatment in experiment 1 (resource treatment: $t_{178} = -0.1$; $P = 0.9$, Welch's two-sided t test; $n = 190$). Each point represents the density of parasites in a single mouse. Black-outlined diamonds indicate that parasites rebounded posttreatment, and red-outlined diamonds indicate that rebounding parasites were confirmed to be either genotypically or phenotypically resistant. *n*, number of mice. The box shows the median (central line), 25% (bottom edge), and 75% (upper edge) percentiles; whiskers indicate the highest and lowest values that lie within 1.5-fold of the interquartile range of the upper and lower edges of the box.

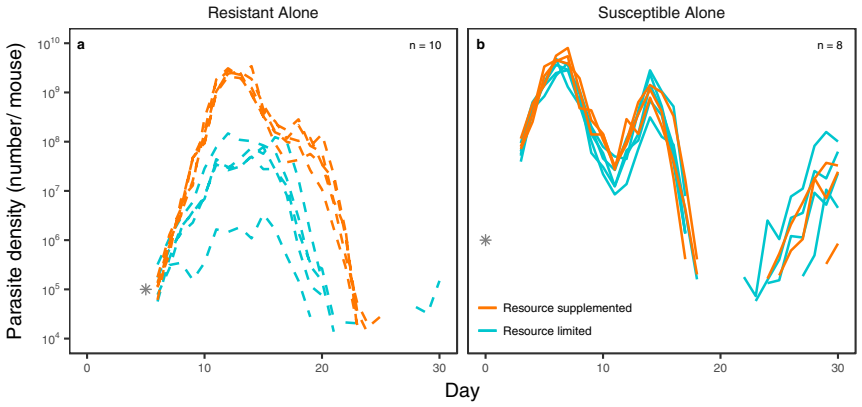

Fig. S2. Resource limitation disproportionately impacts the population growth of resistant parasites. Infection dynamics of mice infected with pyrimethamine-resistant (*A*; dashed lines) and pyrimethamine-susceptible (*B*; solid lines) parasites in resource-supplemented (0.05% pABA, orange) or resource-limited (0% pABA, turquoise) treatments are shown. Gray stars represent the number of parasites inoculated and the time at which they were administered. Resource limitation reduced the size of resistant infections significantly more than susceptible infections (total parasite density, resource treatment * strain: $\chi^2 = 10.2$; $P < 0.01$, generalized least squares regression). *n*, number of mice.

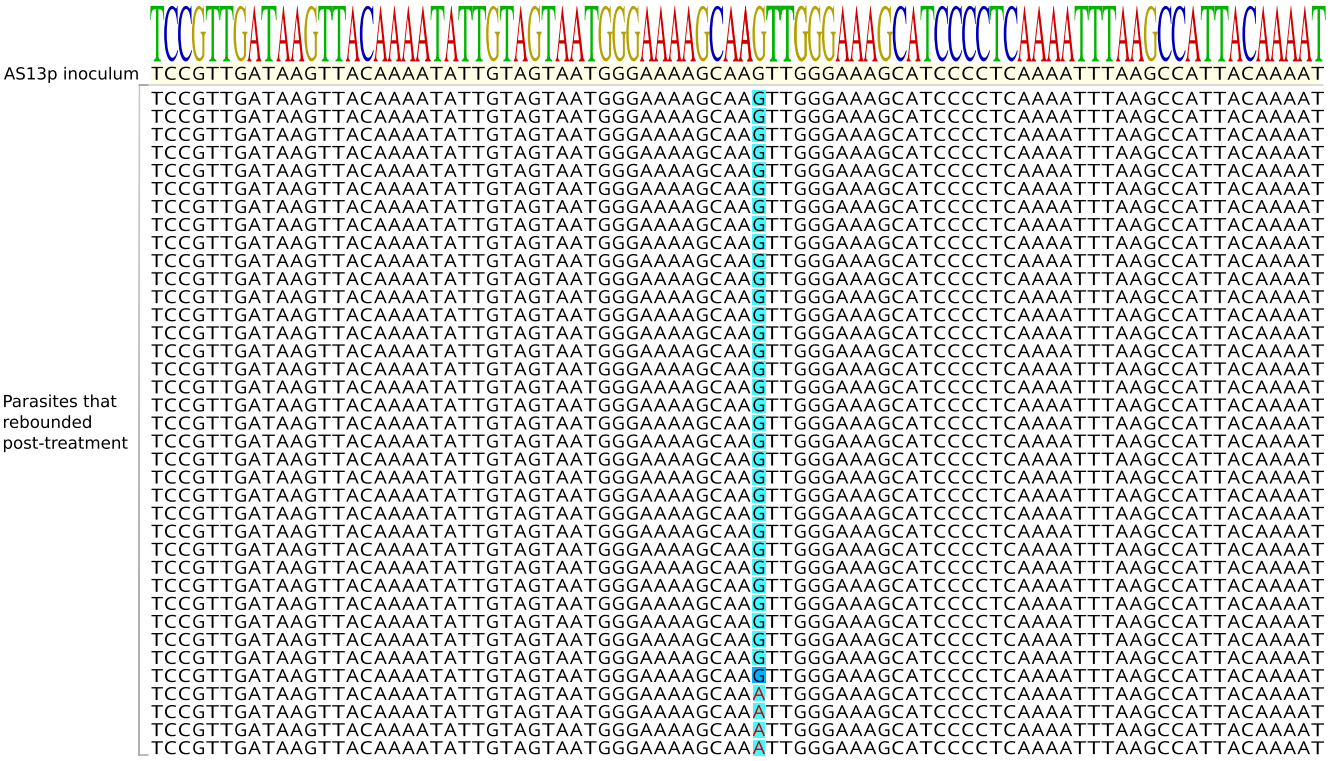

Fig. S3. DHFR genotype of parasites that rebounded following pyrimethamine treatment in experiment 1. The partial DHFR sequence of parasites that rebounded following drug treatment is shown; each sequence below the gray line is derived from a different mouse in the resource-supplemented treatment. When a base is identical to that of the reference sequence (GenBank accession no. L28120.1, large colored letters), the base is written in black. A mutation from G to A at the shaded position is associated with pyrimethamine resistance in *Plasmodium chabaudi*. The shade of blue indicates the confidence with which the base was called: The probability of an incorrect base call was less than 1 in 10^4 at the majority of bases (turquoise shading). In one case (blue shading), the probability of an incorrect base call was between 1 in 10^2 and 1 in 10^4; this base was called as "mixed" (Fig. 1*A*). The sequence of the drug-susceptible AS13p strain with which mice were inoculated is also shown (yellow shading).

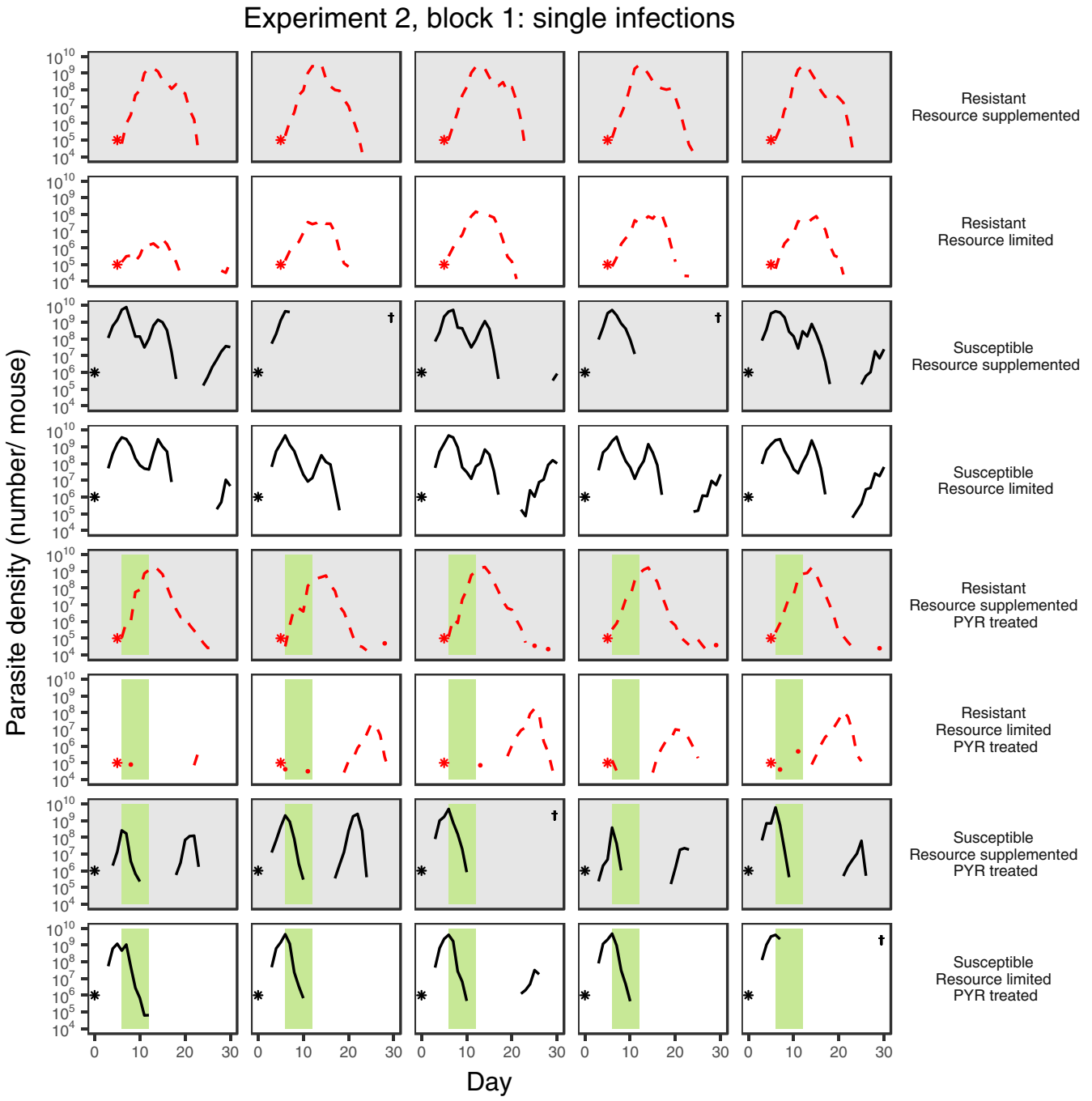

Fig. S4. Dynamics of single infections of individual mice in block 1 of experiment 2. Dynamics of susceptible (S_{AJ}; black, solid lines) and pyrimethamine-resistant (R_{AS}; red, dashed lines) parasites in single infections of mice in resource-supplemented (gray background) and resource-limited (white background) treatments are shown. Each subplot shows the infection dynamics of an individual mouse. Stars represent the number of parasites inoculated and the time at which they were administered. Dots represent the density of parasites detected on a particular day in instances where parasites were not detected the day before or after. The green bar indicates the duration and timing of pyrimethamine treatment. Daggers indicate mice that died during the experiment; mice died within a day of where the trajectory stops. Mice that died were excluded from the analysis. PYR, pyrimethamine.

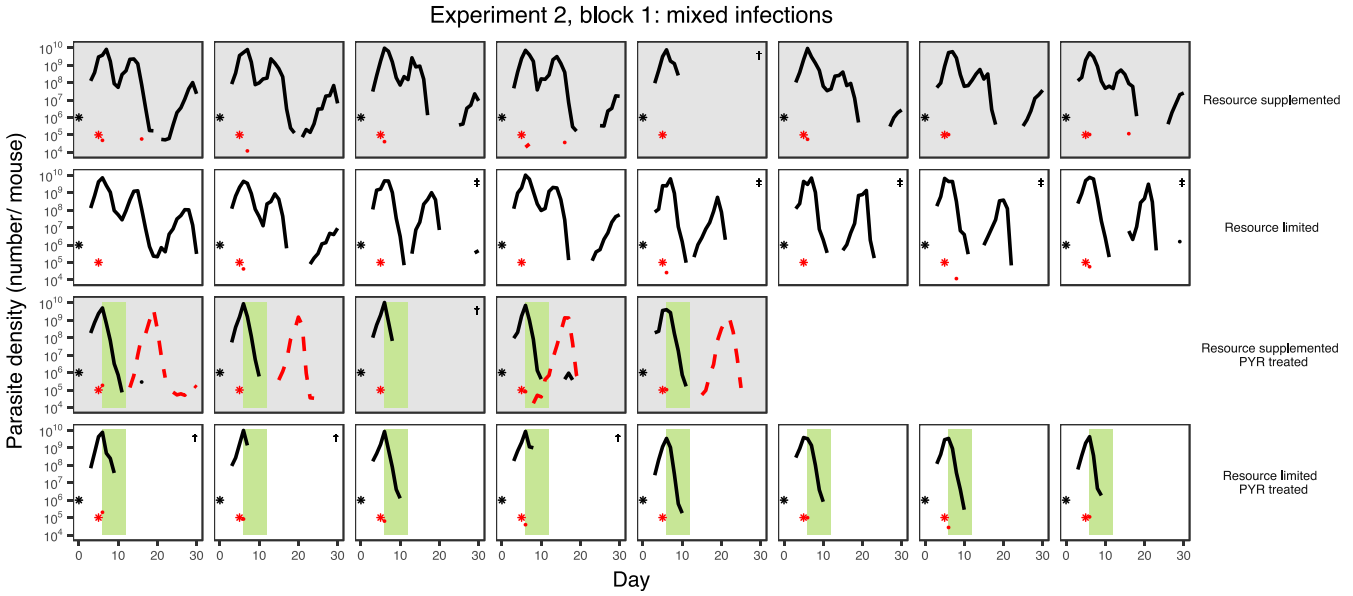

Fig. S5. Dynamics of mixed infections of individual mice in block 1 of experiment 2. Dynamics of susceptible (S_{AJ}; black, solid line) and pyrimethamine-resistant (R_{AS}; red, dashed lines) parasites in mixed infections of mice in resource-supplemented (gray background) and resource-limited (white background) treatments are shown. Each subplot shows the infection dynamics of an individual mouse. Stars represent the number of parasites inoculated and the time at which they were administered. Dots represent the density of parasites detected on a particular day in instances where parasites were not detected the day before or after. The green bar indicates the duration and timing of pyrimethamine treatment. Daggers indicate mice that died during the experiment; mice died within a day of where the trajectory stops. Double daggers indicate that mice were inadvertently given pyrimethamine treatment. Both groups were excluded from the analysis. PYR, pyrimethamine.

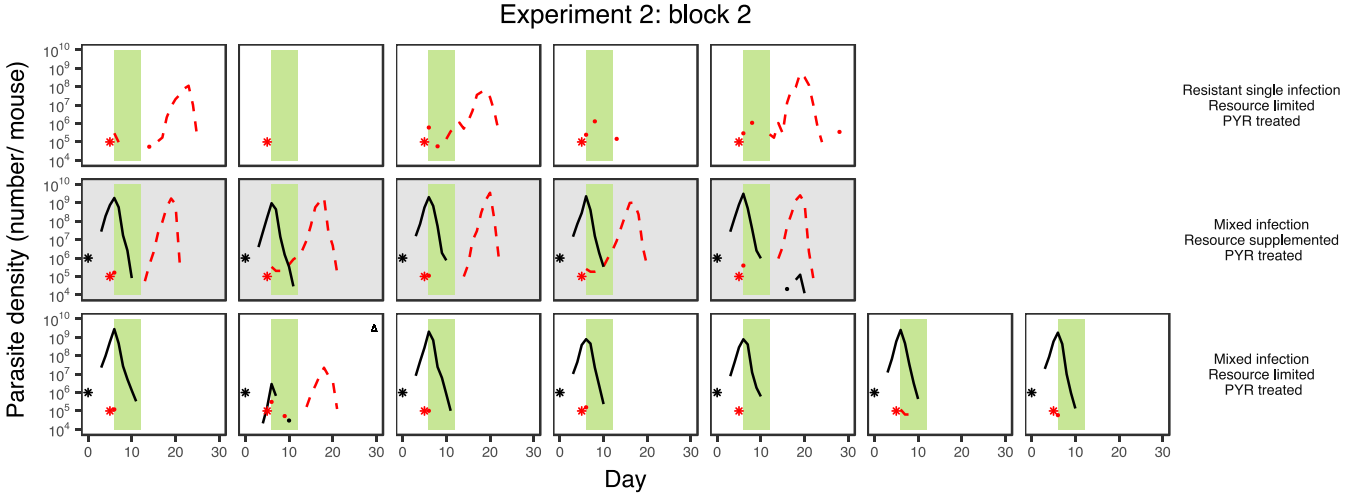

Fig. S6. Dynamics of infections of individual mice in block 2 of experiment 2. Dynamics of susceptible (S_{AJ}; black, solid lines) and pyrimethamine-resistant (R_{AS}; red, dashed lines) parasites in single (*Top*) and mixed (*Middle* and *Bottom*) infections of mice in resource-supplemented (gray background) and resource-limited (white background) treatments are shown. Each subplot shows the infection dynamics of an individual mouse. Stars represent the number of parasites inoculated and the time at which they were administered. Dots represent the density of parasites detected on a particular day in instances where parasites were not detected the day before or after. The green bar indicates the duration and timing of pyrimethamine treatment. The open triangle indicates that the mouse was inoculated with fewer susceptible parasites than intended and was excluded from the analyses. PYR, pyrimethamine.

Fig. S7. DHFR genotype of parasites used in experiments 2, 3, and 4. Partial DHFR sequences of susceptible (labeled in black) and pyrimethamine-resistant (labeled in red) parasite strains used in experiments 2–4 are shown. Differences from the pyrimethamine-susceptible reference sequence (accession no. L28120.1; top, large colored letters) are indicated by colored bases. The mutation from G to A present in the resistant parasites confers pyrimethamine resistance in *P. chabaudi*. Note that the mutation from C to T in the AT2p sequence is a synonymous mutation.

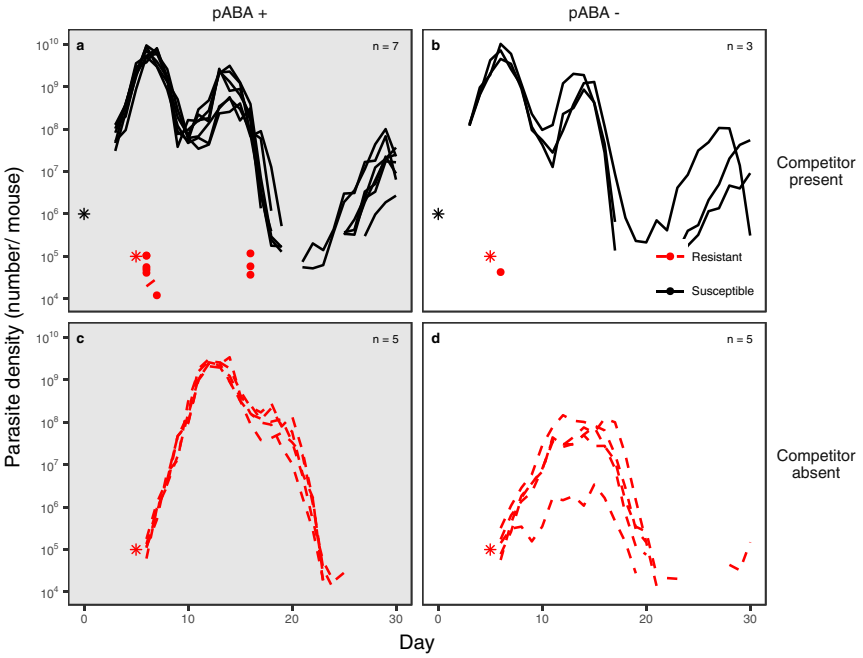

Fig. S8. Resistant parasites are competitively suppressed by susceptible parasites in untreated mice. Parasite dynamics of individual mice infected with both susceptible (black, solid lines) and resistant (red, dashed lines) parasites (*A* and *B*) and only resistant parasites (*C* and *D*) in block 1 of experiment 2 are shown. Stars represent the number of parasites inoculated and the time at which they were administered. Dots indicate the density of parasites detected on a particular day in instances where parasites were not detected the day before or after. n, number of mice.

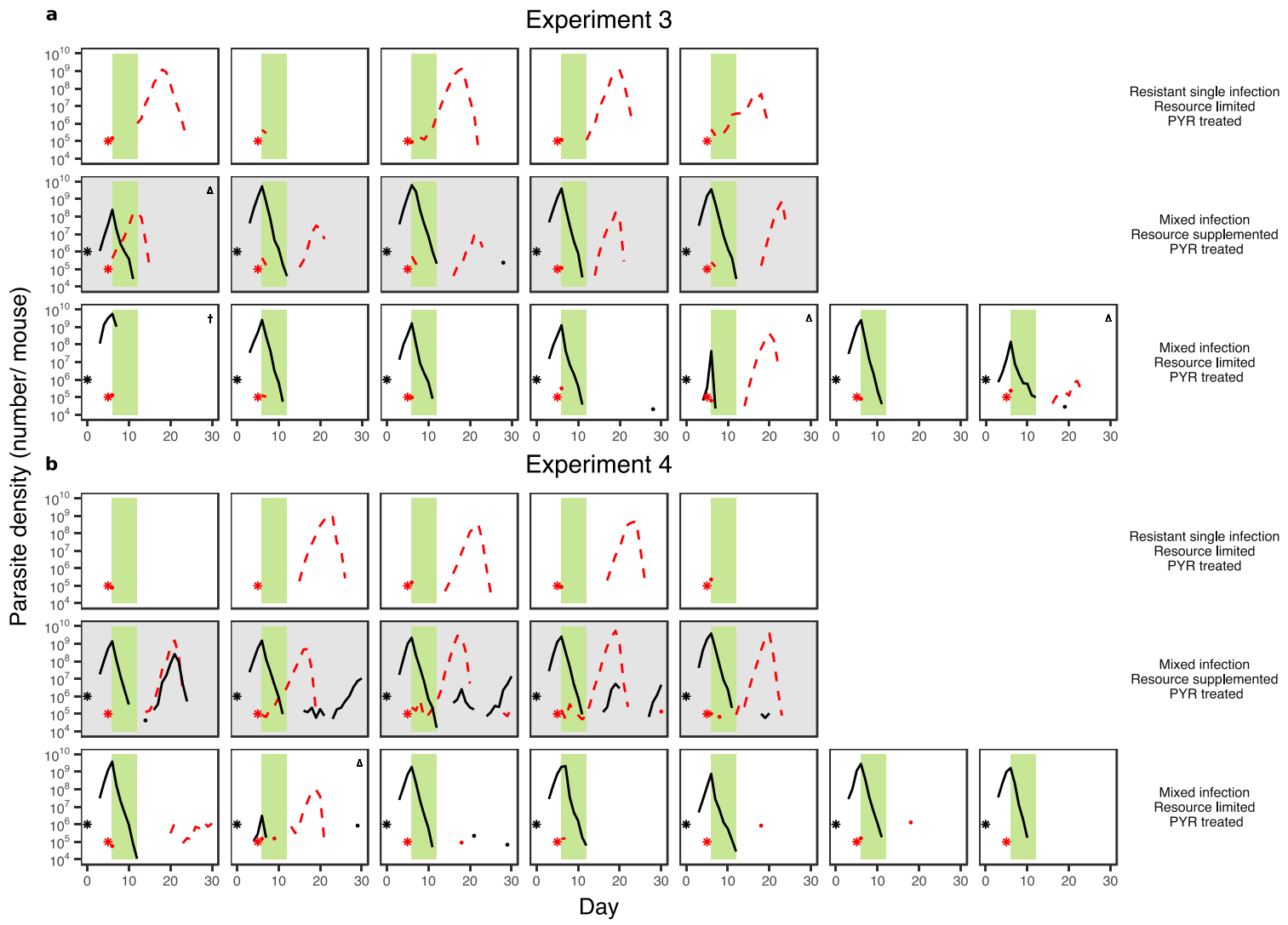

Fig. S9. Dynamics of infections of individual mice in experiments 3 and 4. Dynamics of susceptible (black, solid lines) and pyrimethamine-resistant (red, dashed lines) parasites in single (*A* and *B*, *Top*) or mixed (*A* and *B*, *Middle* and *Bottom*) infections of mice in resource-supplemented (gray background) and resource-limited (white background) treatments are shown. All mice in experiment 3 were infected with R_{AJ}, and those in mixed-infection treatments were also infected with S_{AS}. In experiment 4, mice were infected with R_{CW}, and those in the mixed-infection treatments were also infected with S_{AT}. Each subplot shows the infection dynamics of an individual mouse. Stars represent the number of parasites inoculated and the time at which they were administered. Dots represent the density of parasites detected on a particular day in instances where parasites were not detected the day before or after. The green bar indicates the duration and timing of pyrimethamine treatment. The open triangles indicate that mice were inoculated with fewer susceptible parasites than intended and were excluded from all analyses. PYR, pyrimethamine.

Table S1. Rates of resistance emergence and number of mice in each treatment of the competition experiments

	Experiment 2 (S_{AJ}, R_{AS})						Experiment 3 (S_{AS}, R_{AJ})		Experiment 4 (S_{AT}, R_{CW})	
	Block 1				Block 2					
	pABA$^+$		pABA$^-$		pABA$^+$	pABA$^-$	pABA$^+$	pABA$^-$	pABA$^+$	pABA$^-$
Infection composition	Drug-treated	Untreated	Drug-treated	Untreated	Drug-treated		Drug-treated			
Resistant + susceptible	**4/4** 5 (1*)	8 (1*)	**0/5** 8 (3*)	8 (5‡)	**5/5** 5	**0/6** 7 (1†)	**4/4** 5 (1†)	**0/4** 7 (2†, 1*)	**5/5** 5	**0/6** 7 (1†)
Resistant alone	**5/5** 5	5	**5/5** 5	5		**3/5** 5		**4/5** 5		**3/5** 5
Susceptible alone	**NA** 5 (1*)	5 (2*)	**NA** 5 (1*)	5						

The frequency of resistance emergence (number of mice in which resistant parasites grew continuously posttreatment/number of mice included in the analysis) in drug-treated treatments is indicated in bold in the top row of each cell. The initial number of mice in each treatment is indicated in bold in the bottom row. The genetic background of susceptible (S) and resistant (R) strains used in each experiment is indicated by the subscript (*Materials and Methods*). NA, not applicable.

*Number of mice that were removed from the analysis because they died.

†Number of mice that were inoculated with fewer parasites than intended.

‡Number of mice that accidentally received drug treatment.