SUPPLEMENTAL MATERIAL

Demarta-Gatsi et al., http://www.jem.org/cgi/content/full/jem.20151976/DC1

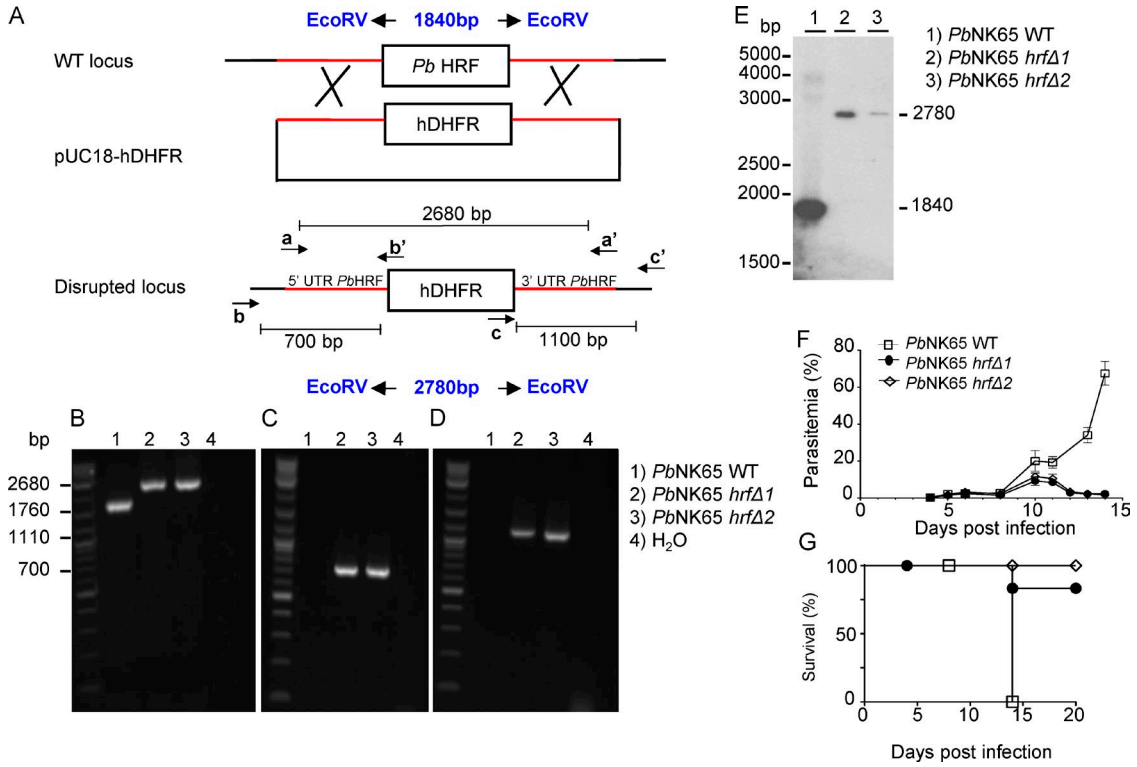


Figure S1. **Disruption of the *pbhrf* gene in *Pb*NK65 parasites.** (A) Schematic representation of the strategy used to delete the *pbhrf* open reading frame in *Pb*NK65 parasites using double-crossover homologous recombination. Red lines represent regions of homology. Successful recombination disrupts the HRF-coding sequence and replaces it with the drug resistance marker *hDHFR*. (B–D) Specific PCR primers (Table S2) were used to assess genomic integration of *hDHFR* in *Pb*NK65-*hrf*Δ clones. The primers used for PCR analysis include a + a' (B), b + b' (C), and c + c' (D), with gDNA from the following: lane 1, WT parasites; lane 2, *hrf*Δ clone 1; lane 3, *hrf*Δ clone 2; and lane 4, H_2O. (E) Southern blot analysis of the *pbhrf* locus in *Pb*NK65 WT, *Pb*NK65 *hrf*Δ1, and *Pb*NK65 *hrf*Δ2 mutant locus in *Pb*NK65 parasites. WT locus = 1,840 bp, whereas *hDHFR* insertion = 2,780 bp. (F and G) C57BL/6 mice were inoculated with either 10^5 GFP-expressing WT, *Pb*NK65-*hrf*Δ1, or *Pb*NK65-*hrf*Δ2 iRBCs, and parasitemia (F) or survival (Kaplan-Meier survival plots: log-rank test; $n = 11$; $P = 0.007$; G) was followed over time. Error bars, SEM. Experiments were replicated three times.

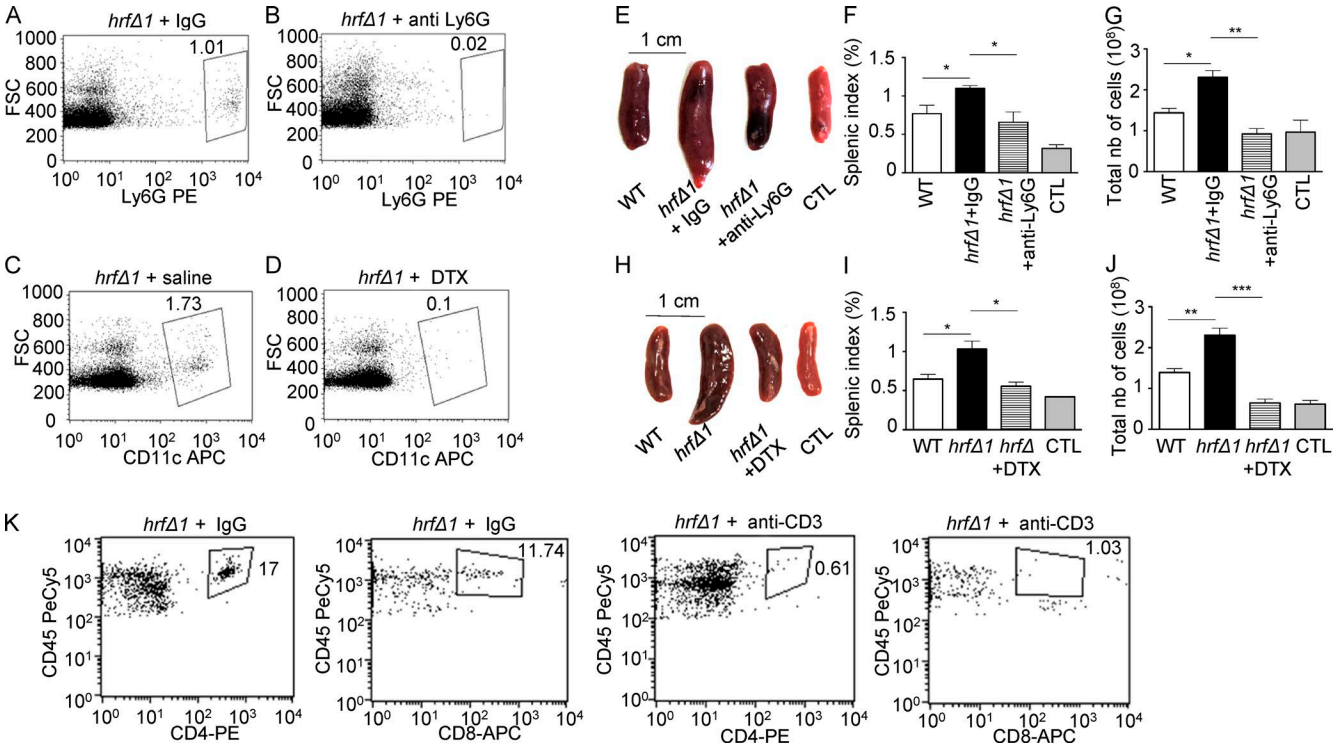
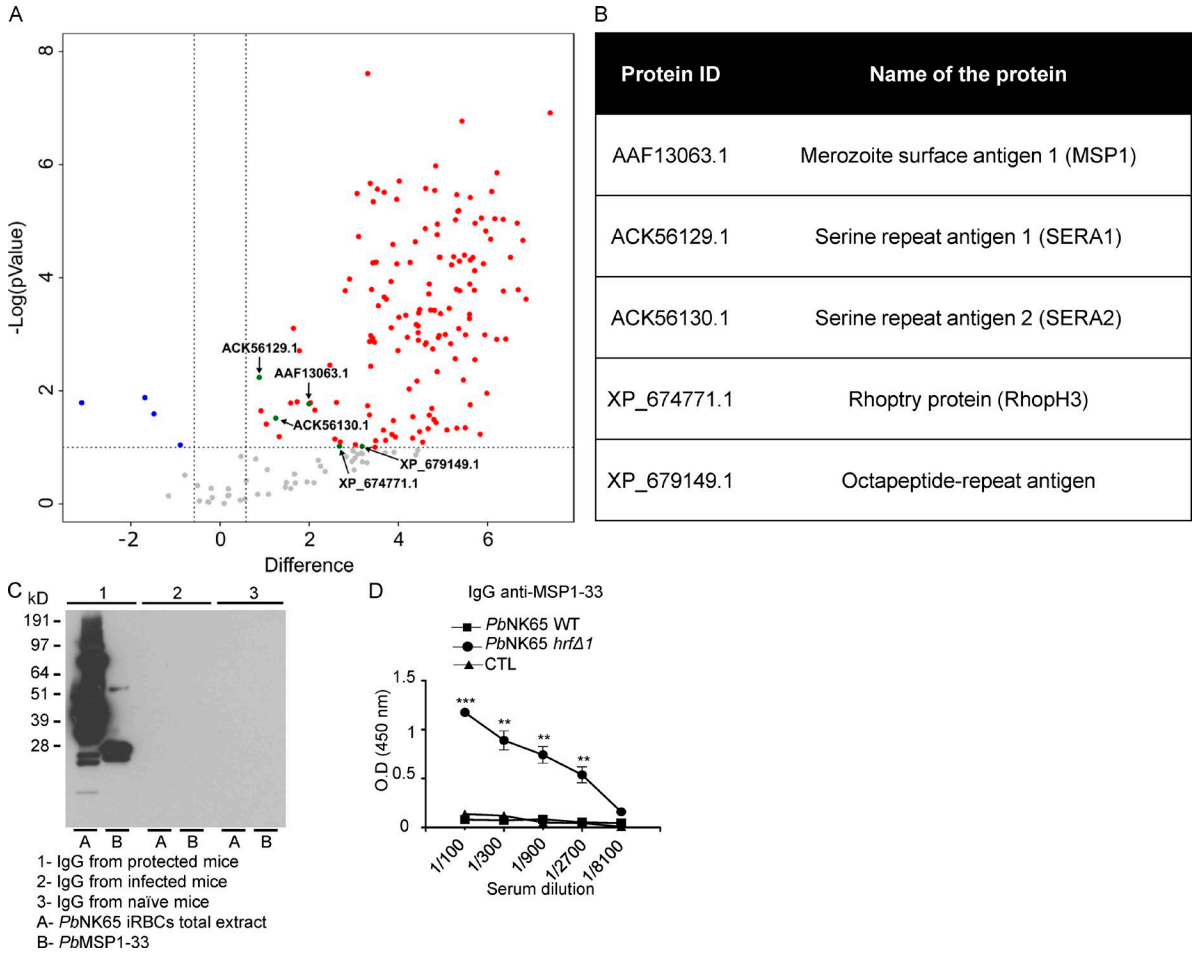


Figure S2. **Assessment of leukocyte depletion and role of neutrophils and DCs in the occurrence of splenomegaly.** (A and B) In vivo depletion of neutrophils using antineutrophil antibody (B) or normal IgG (A) was assessed by measuring at day 6 p.i. the percentage of residual Ly6G$^+$ neutrophils in spleens by FACS analysis. (C and D) Depletion of DCs was performed by injection of diphtheria toxin (DTX; D) or saline (C) into CD11c-DTR-GFP mice, and at day 6 p.i., the percentage of CD11c$^+$ cells was determined in spleens by FACS analysis. (E–J) Comparison at day 6 p.i. of splenic indexes and total number of leukocytes in WT and *Pb*NK65-*hrf*Δ1–infected mice untreated or depleted of neutrophils (E–G) or DCs (H–J). (K) Control of T cell depletion (Fig. 2 G): protected mice received anti-CD3–depleting antibody 1 d before a challenge with WT parasites followed by two booster injections of anti-CD3 at days 1 and 3 after challenge with *Pb*NK65 WT parasites. T cell depletion efficiency was assessed by FACS analysis using anti-CD4–PE or anti-CD8–APC in blood samples from protected mice that were challenged at day 15 p.i. with *Pb*NK65 WT parasites. Analysis was performed 10 d after challenge. Error bars, SEM. Data are representative of two independent experiments with five to six mice per group. *, P = 0.028; **, P = 0.015; ***, P = 0.009; Mann-Whitney test. CTL, control; FSC, forward side scatter; nb, number.



Protein ID	Name of the protein
AAF13063.1	Merozoite surface antigen 1 (MSP1)
ACK56129.1	Serine repeat antigen 1 (SERA1)
ACK56130.1	Serine repeat antigen 2 (SERA2)
XP_674771.1	Rhoptry protein (RhopH3)
XP_679149.1	Octapeptide-repeat antigen

Figure S3. **Identification of immune sera–derived immunoprecipitated proteins.** (A–D) *P. berghei* antigens recognized specifically by IgGs from *Pb*NK65-*hrf*Δ1–protected mice serum were identified by mass spectrometry. (A) Volcano plot representing results of the immunoprecipitated proteins of *Pb*NK65 parasite extract. This plot is colored such that those points having a fold change <1.5 are shown in gray, points >1.5 are in red, and points <1.5 are in blue. Green and red dots display both large-magnitude fold changes (x axis) as well as high statistical significance (−log10 of p-value, y axis). The dashed black line shows where P = 0.05, with points above the line having P < 0.05 and points below the line having P > 0.05. Statistical analysis was performed on triplicate samples. (B) Selected proteins for futher validation are in green in A, and they are reported in the table. (C and D) Evidence that protected sera actually recognize the green dot, GenBank accession no. AAF13063.1 identified as MSP1 protein was assessed by using a recombinant *Pb*MSP1-33 protein by immunoblotting (C) and by ELISA (D). Error bars, SEM. Experiments were replicated three times (six mice per group). **, 0.02 < P < 0.028; ***, P = 0.0002; Mann-Whitney test. CTL, control.

Table S1. **List of oligonucleotides used for RT-qPCR analyses**

Primer	Forward / Reverse	Sequence (5′–3′)
Pb 18S	Forward	ATTAATCTTGAACGAGGAATGGCT
	Reverse	TCAATCGGTAGGAGCGACG
Pb LSP2	Forward	GCCAAATGCTAAACCTAATG
	Reverse	TGGGTTTGTATTGTATGCAC
Pb HSP70	Forward	TGCAGCTAATCAAACTC
	Reverse	ACTTCAATTTGTGGAACACC
mu IL-23	Forward	CCACCAGGACTCAAGGACAACA
	Reverse	GCAGGCTCCCCTTTGAAGA
mu EBI3	Forward	CAGAGTGCAATGCCATGCTCC
	Reverse	GCCACACCGAGCCTGTAAGT
mu IL-12p35	Forward	TACTAGAGAGACTTCTTCCACAACAAGAG
	Reverse	GATTCTGAAGTGCTGCGTTGAT
mu IL-12p40	Forward	GGAAGCACGGCAGCAGAATA
	Reverse	AACTTGAGGGAGAAGTAGGAATGG
mu IFN-γ	Forward	AAAGGATGCATTCATGAGTATTGC
	Reverse	CGCTTCCTGAGGCTGGATT
mu IL-6	Forward	AAAGAAATGATGGATGCTACCAAAC
	Reverse	CTTGTTATCTTTTAAGTTGTTCTTCAT GTACTC
mu IL-10	Forward	GGCGCTGTCATCGATTTCTC
	Reverse	GACACCTTGGTCTTGGAGCTTATTAA
mu HPRT	Forward	CTGGTGAAAAGGACCTCTCG
	Reverse	TGAAGTACTCATTATAGTCAAGGGCA

Table S2. **List of oligonucleotides used for PCR of WT and recombinant parasites**

Oligonucleotide	Sequence (5′–3′)
ApaI-5′UTR-*PbHRF*-F (**a**)	CGCGGGCCCGCGCATTATTACCGTTGTCA
PstI-5′UTR-*PbHRF*-R	CGCCTGCAGGGCTTATGCAAGTATCGAACAA
KpnI-3′UTR-*PbHRF*-F	CGCGGTACCTTGCTACATGACGCATAAACC
EcoRI-3′UTR-*PbHRF*-R (**a′**)	CGCGAATTCTGTGAAATCGACAATGTTTTGG
*HRF*5′-F (**b**)	GCGATACAAACAAATTTATTCAGC
*HRF*3′-R (**c′**)	CGCAAGATATCAGAGCTTTTCA
h*DHFR*3′-F (**c**)	TGTTGTCTCTTCAATGATTCATAAATAGTTGG
h*DHFR*5′-R (**b′**)	TGCTTTGAGGGGTGAGCATTTAAAGC
PbHRF-5′orf-F	CCATTTGGAAATGCGGAAT
PbHRF-3′orf-R	TTTTTCTTCAAATAAACCATCTGA

Bold letters refer to the oligonucleotide's position in Fig. S1 (A and B).