Supplemental Data
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Erythrocyte Binding Protein PfRH5 Polymorphisms Determine Species-Specific Pathways of *Plasmodium falciparum* Invasion

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Supplemental Experimental Procedures and Results

Secondary QTL Scan

To search for additional QTL, a secondary scan was performed after removing the effects of the primary QTL on chromosome 4, as described by (Ferdig et al., 2004).

Microarray Analysis

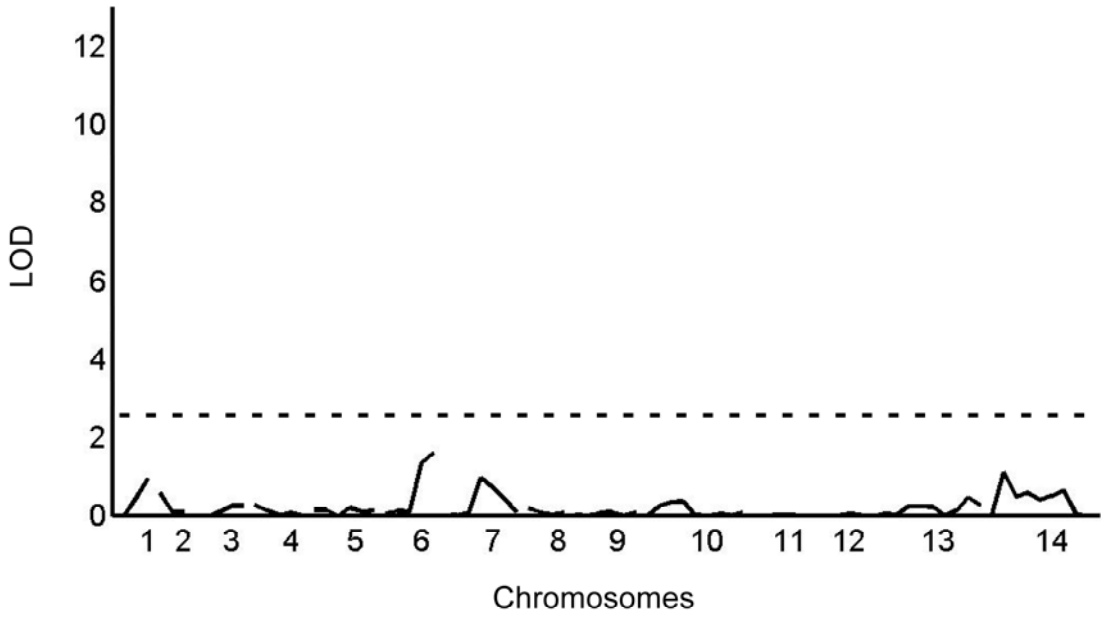
Total RNA was extracted from highly synchronized saponin-treated schizont pellets from 7G8 and GB4 using Trizol (Invitrogen). cDNA was directly labeled with dUTP-Cy3 or dUTP-Cy5 from 30 µg of total RNA with oligo and random hexamer as described previously (Gaur et al., 2005). The *P. falciparum* oligonucleotide array set (Operon) (Bozdech et al., 2003) was printed onto polylysine-coated glass slides using an Omnigrid robotic arrayer (Genomic Solutions) and the hybridizations were done at the NIAID Microarray Research Facility, National Institutes of Health, Bethesda, MD. Hybridizations were performed in cDNA hybridization solution (5xSSC, 0.2% SDS, 25% formamide with 10 µg salmon sperm DNA) (Invitrogen) using the MicroArray User Interface (MAUI) hybridization system (BioMicro Systems, Salt Lake City, UT). After incubation at 45°C overnight, the slides were washed twice with 0.05% SDS in 1xSSC followed by two washes of 0.1xSSC and scanned with a GenePix 4000B array scanner (Axon Instruments, Union City, CA). Microarray signals from each array spot were normalized so that the median Cy5/Cy3 ratio was set as 1.0. Data from repeat

experiments were filtered and analyzed with GenePix Pro 4.0 (Axon Instruments) and web based microarray tools (mAdb) developed by the National Cancer Institute (http://nciarray.nci.nih.gov/)

Erythrocyte Invasion Assays with Chimpanzee and Neuraminidase-treated Erythrocytes

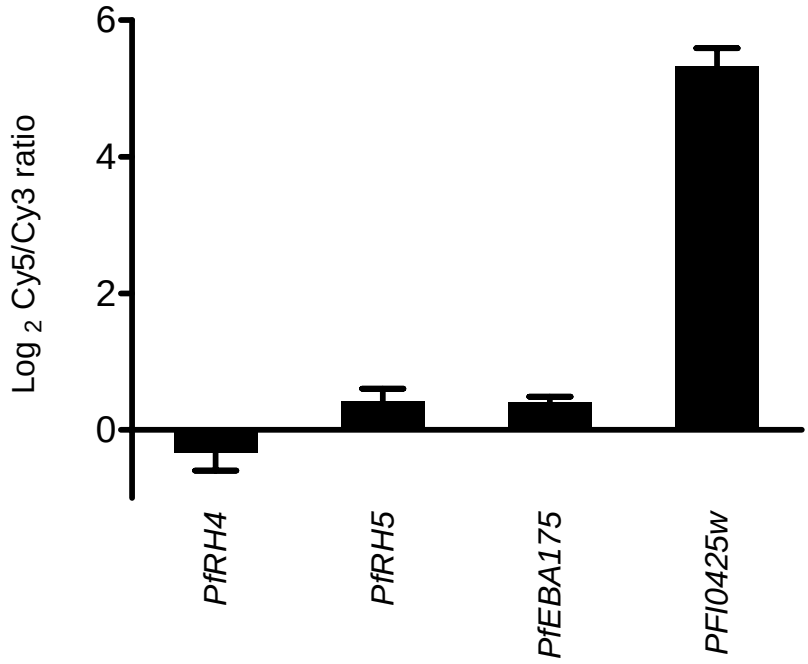
Chimpanzee erythrocytes were obtained from Bioqual, Inc, Rockville. For neuraminidase treatment of human, *A. nancymaae* and chimpanzee erythrocytes, 1 x 10^8 cells/ml were treated with 0.025 U/ml of neuraminidase in RPMI (*Vibrio cholerae*, Calbiochem) at 37°C for 1 hr and washed three times before use.

FIGURE S1



Secondary genome-wide scans did not detect significant additional QTL associated with *A. nancymaae* erythrocyte invasion. A dashed line represents the significance threshold (0.05) from 1000 permutations (Churchill and Doerge, 1994).

FIGURE S2



Microarray analysis shows levels of *PfRH4, PfRH5* and *PfEBA175* expression in GB4 are not significantly different from those in 7G8. Log_2 of GB4/7G8 expression ratios for *PfRH4, PfRH5, PfEBA175* and for comparison, an upregulated gene *PFI0425w*, are shown, ± SEM.

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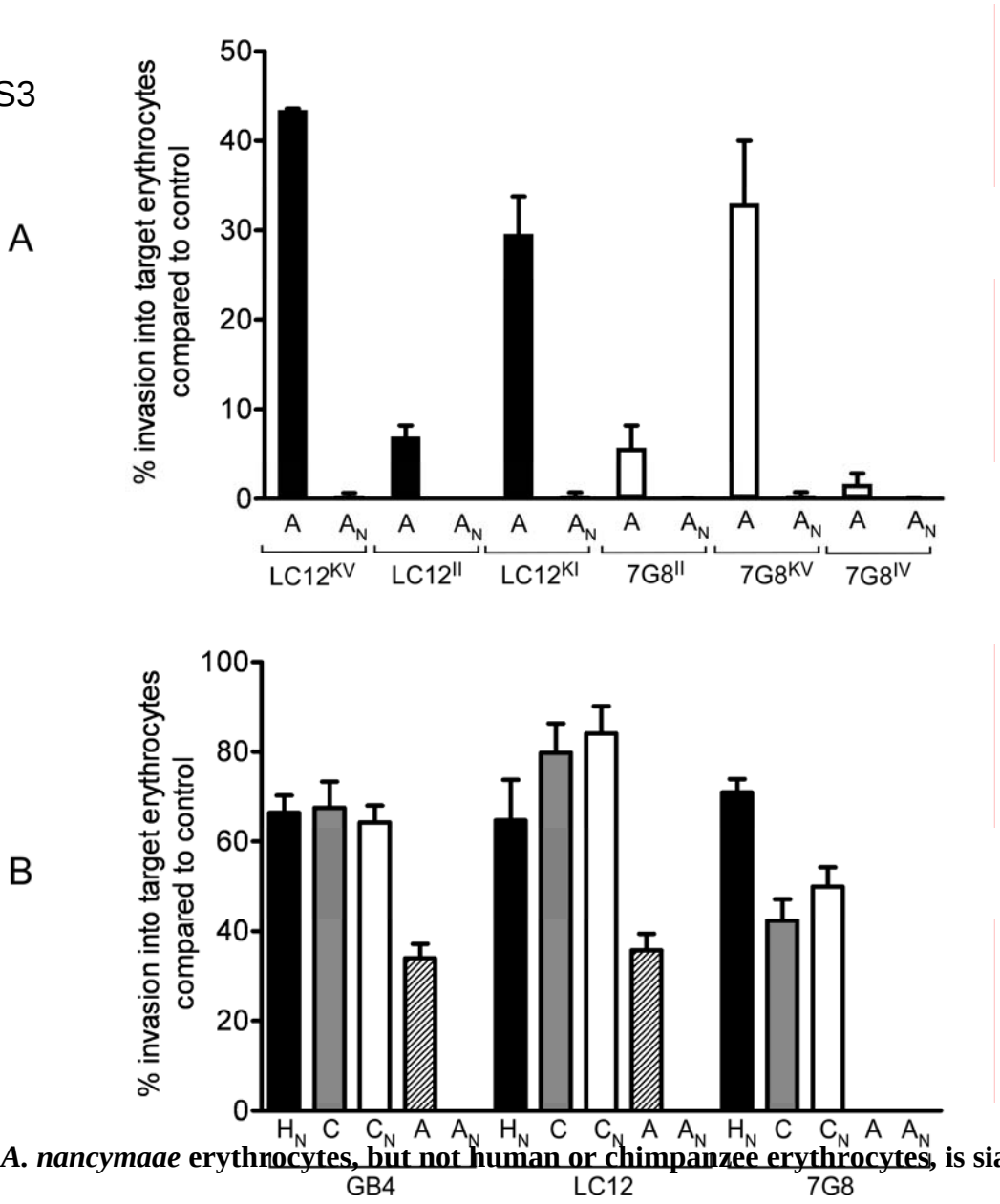
FIGURE S3



Invasion of *A. nancymaae* erythrocytes, but not human or chimpanzee erythrocytes, is sialic acid-dependent. A). Neuraminidase-treated *A. nancymaae* erythrocytes are fully refractory to invasion. A, *A. nancymaae* erythrocytes; A_N, neuraminidase-treated *A. nancymaae* erythrocytes. B). Comparison of the invasion rates of GB4, LC12 and 7G8 into neuraminidase-treated human erythrocytes (H_N, black bars), chimpanzee erythrocytes (C, grey bars), neuraminidase-treated chimpanzee erythrocytes (C_N, open bars), *A. nancymaae* erythrocytes (A, hatched bars) and neuraminidase-treated *A. nancymaae* erythrocytes (A_N). Percentage values are relative to rates of invasion into human control erythrocytes ± SEM.

Supplemental References

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