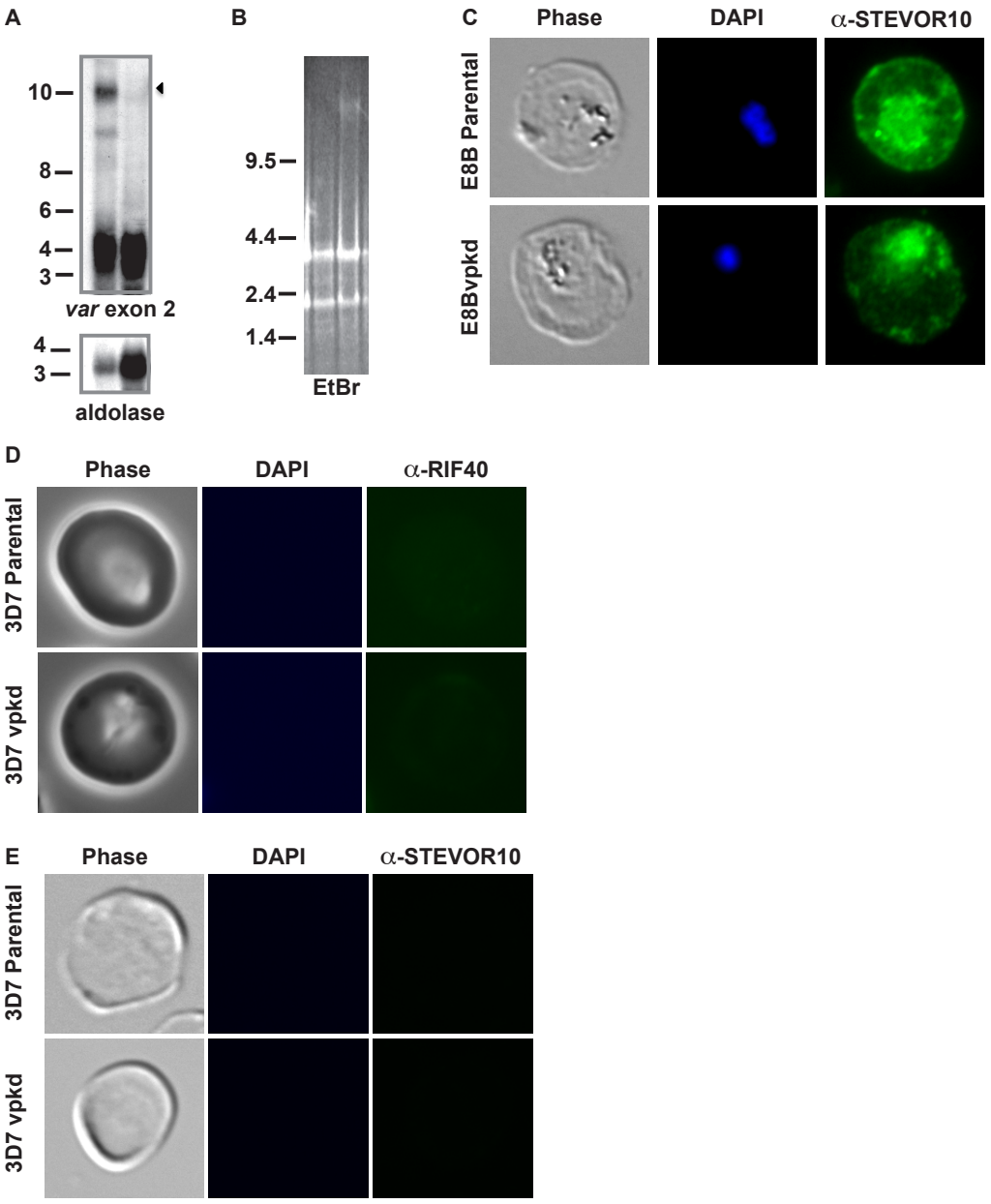
J Chan et al 2012
Supplementary Materials

Supplementary Figure S1



A. Northern blot analysis of *var* gene transcription by hybridization with a specific *var* exon 2 sequence. The position of molecular weight standards (kb) are indicated on the left and the arrow represents *var* transcripts. Compared to 3D7 parental, *var* transcripts

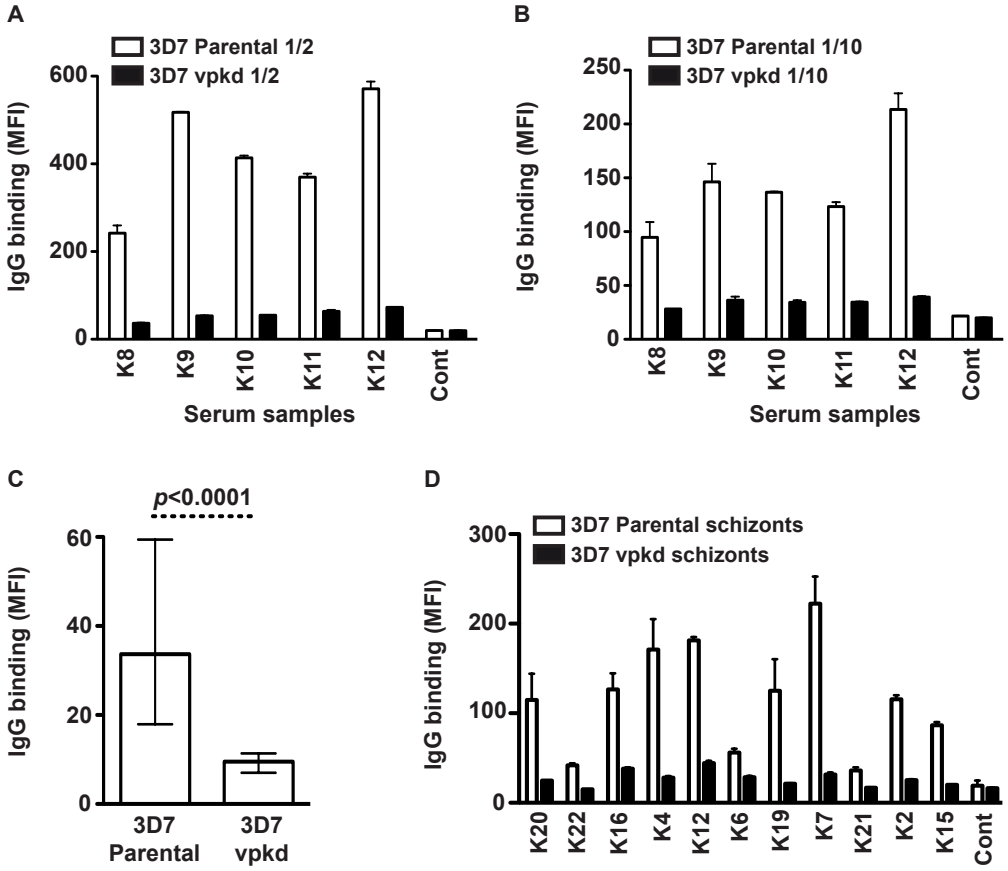
are markedly reduced or absent in 3D7vpkd parasites. RNA was extracted from highly synchronous ring-staged parasite cultures at 10 hours post-invasion. Aldolase-specific cDNA were used as loading controls (bottom panel), which suggests greater loading of RNA from the 3D7vpkd IEs compared to 3D7 parental.

B. Ethidium bromide stained gel prior to blotting served as a loading control for Northern blot in Figure 1A.

C. Immunofluorescence assays demonstrate the expression of STEVOR proteins by mature trophozoite stage parasites (green). Despite the lack of PfEMP1 expression, STEVOR proteins were detected in the transfected E8Bvpkd parasites (lower panel), similar to E8B parental parasites (upper panel). Cells were fixed with a mixture of acetone (90%) and methanol (10%); images were taken at equal exposure.

Reflecting the specificity of antibodies, the anti-RIF40 (**D**) and anti-STEVOR10 (**E**) antibodies did not show any specific labeling of uninfected erythrocytes by immunofluorescence microscopy.

Supplementary Figure S2



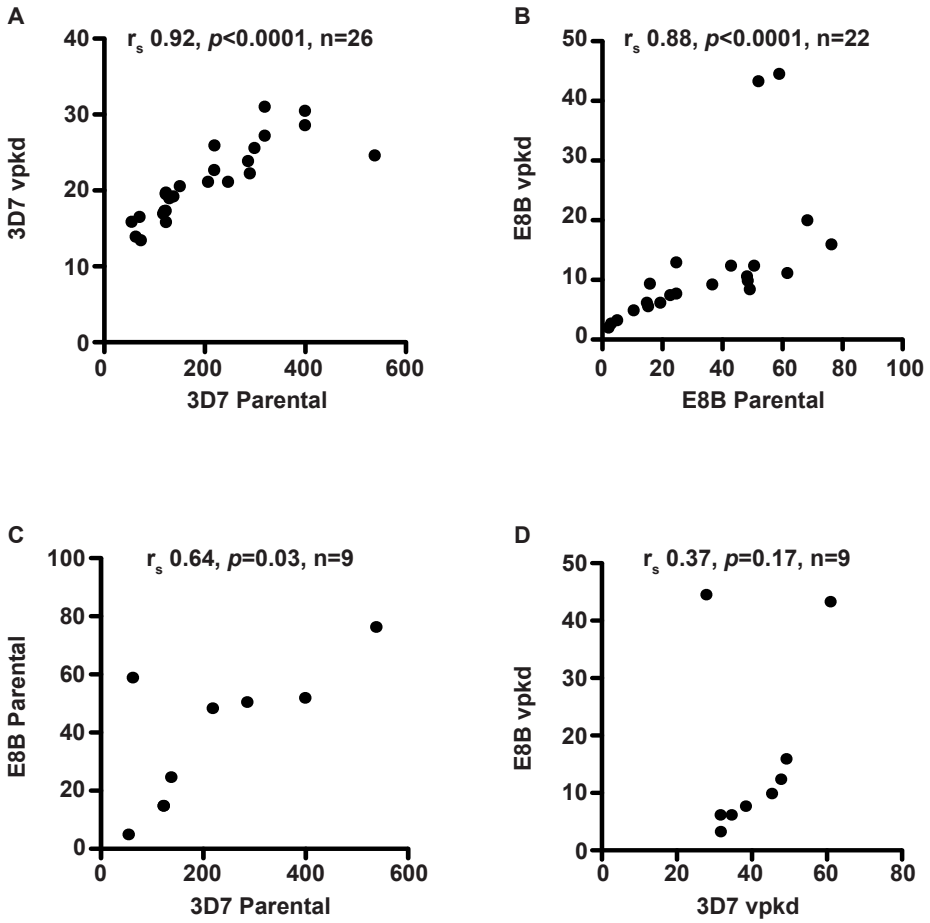
A, B. A representative selection of sera samples tested at varying serum dilutions (1/2 and 1/10) is shown. Samples tested were from malaria-exposed Kenyan adults (K8-12) and non-exposed Melbourne residents (Cont). The pattern of IgG binding of 3D7 parental and 3D7vpkd parasites remained similar regardless of sera dilution. Assay was performed once; bars represent mean and range of samples tested in duplicate. IgG levels are expressed as geometric mean fluorescence intensity (MFI) for all graphs.

C. IgG binding to the surface of erythrocytes infected with 3D7 schizont stage IEs were significantly reduced in 3D7vpkd parasites compared to 3D7 parental, similar to results obtained with mature trophozoite IEs (Figures 3 and 4). Assays were performed thrice

independently; bars represent median and interquartile ranges (n=20); *p* value was calculated using a paired Wilcoxon signed rank test.

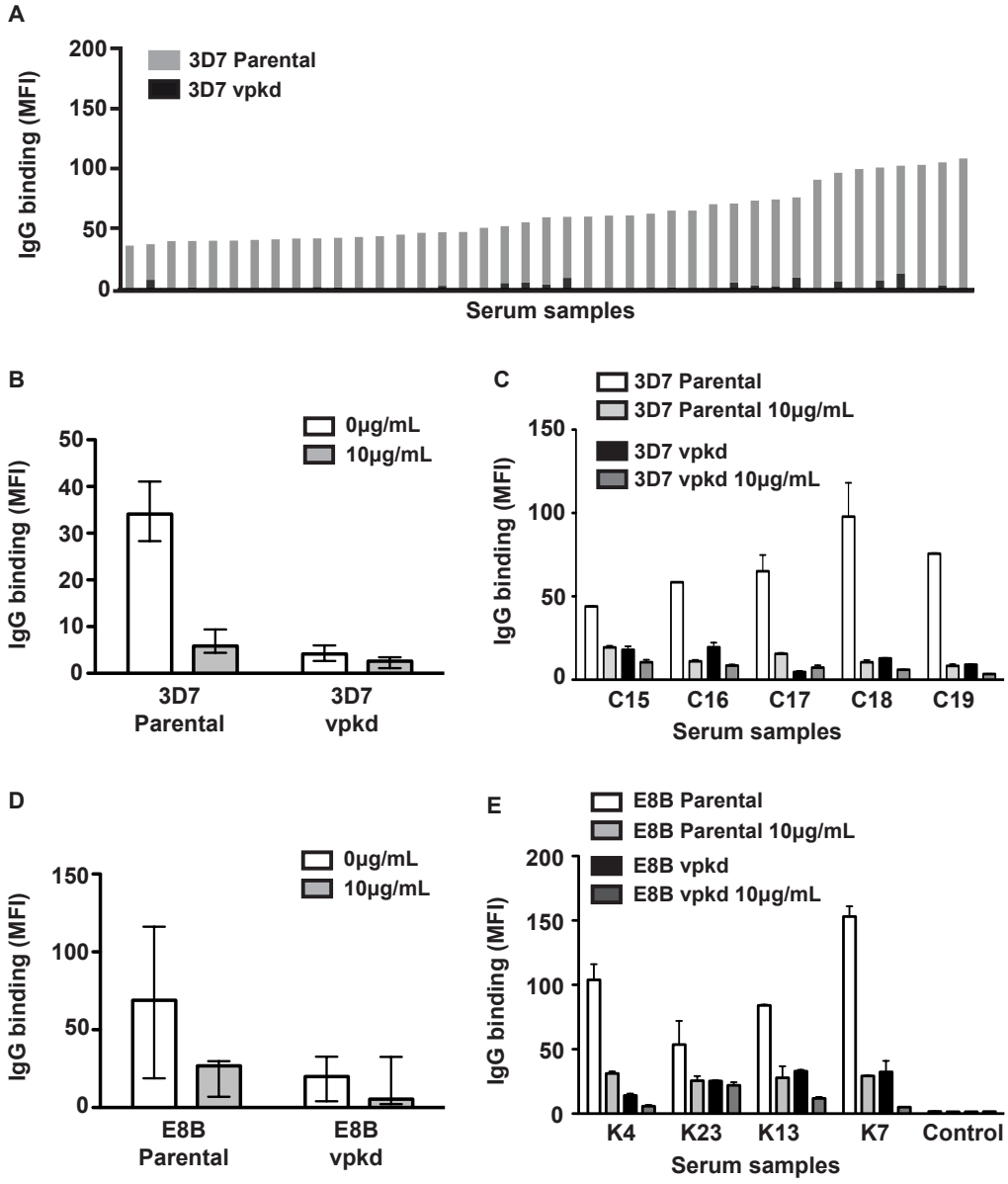
D. A representative selection of serum samples tested for antibodies to the surface of erythrocytes infected with 3D7 schizont stage IEs. Samples tested were from malaria-exposed Kenyan residents (K2-K22; same samples as in C). Assays were performed thrice independently; bars represent mean and range for samples tested in duplicate.

Supplementary Figure S3



The correlation between antibody levels measured IgG binding by flow cytometry to parental and vpkd parasites of 3D7 (**A**) and E8B (**B**) isolates are shown. The correlation between antibody levels measured to E8B and 3D7 isolates are shown for parental (**C**) and vpkd parasites (**D**).

Supplementary Figure S4



A. A representative selection of high IgG responders from the Chonyi cohort plotted to show reactivity to PfEMP1 (defined as IgG to 3D7 parental minus 3D7vpkd).

B. IgG binding to the surface of erythrocytes infected with 3D7 parental and 3D7vpkd parasites were sensitive to trypsin treatment. Trypsin concentrations are represented in µg/mL and IgG binding levels are expressed as geometric mean florescence intensity

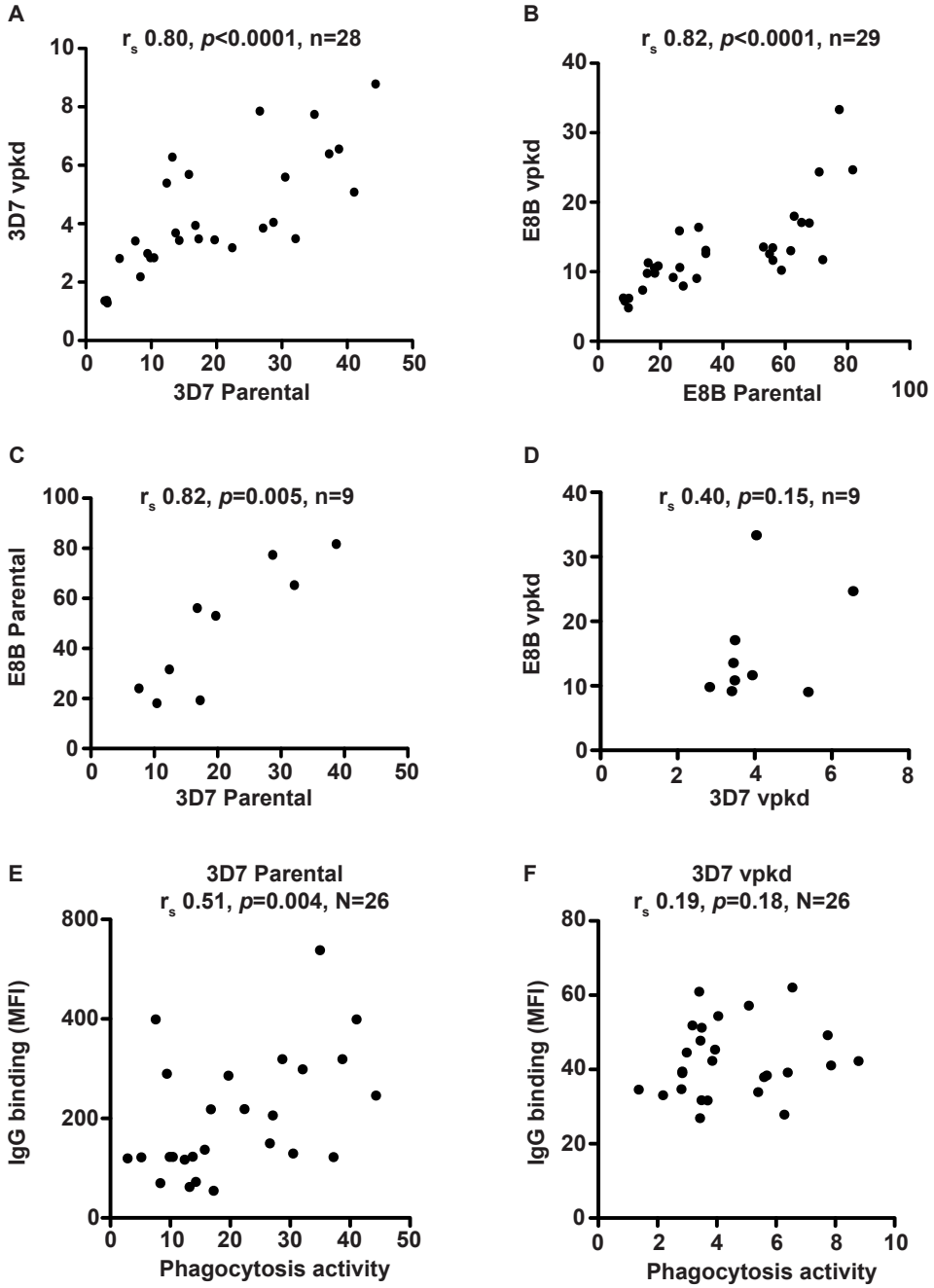
(MFI) for all graphs. Assays were performed twice independently; bars represent median and interquartile ranges.

C. A representative selection of serum samples tested for antibody binding to trypsin-treated 3D7 parental and 3D7vpkd IEs. Samples were from malaria-exposed individuals from the Chonyi cohort (C15-C19). IgG binding to 3D7 parental was highly trypsin sensitive, whereas IgG binding to 3D7vpkd parasites were trypsin resistant with some samples. Assays were performed twice independently; bars represent mean and range of samples tested in duplicate.

D. IgG binding to the surface of erythrocytes infected with E8B parental parasites were highly trypsin sensitive whereas IgG binding to E8Bvpkd appeared to be less sensitive to trypsin. Assays were performed twice independently; bars represent median and interquartile ranges.

E. A representative selection of serum samples tested for antibody binding to trypsin-treated E8B parental and E8Bvpkd IEs. Samples were from malaria-exposed Kilifi adults (K4-K23) and non-exposed Melbourne residents (Cont). IgG binding to E8B parental was trypsin sensitive, and while IgG binding to E8Bvpkd was also trypsin sensitive, some samples appeared trypsin resistant. Assays were performed twice independently; bars represent mean and range of samples tested in duplicate.

Supplementary Figure S5



The correlation between antibody levels measured opsonic phagocytosis activity to parental and vpkd parasites of 3D7 (**A**) and E8B (**B**) isolates are shown. The correlation between antibody levels measured to E8B and 3D7 isolates are shown for parental (**C**) and vpkd parasites (**D**). The correlation between antibody levels measured as IgG binding

and as opsonic phagocytosis activity is shown for 3D7 parental parasites (**E**) and 3D7vpkd parasites (**F**).