Supplemental Data.

Supplementary Figure S1. Western blot of protein extracts from schizonts of W2mef-wt (lane 1) and W2mefSelNm (lane 2). The top part of the blot shows results from probing with rabbit antibodies against EBA175 (W2mef allele) and the bottom shows results from probing with rabbit antibodies against HSP70 as a loading control. Binding of rabbit antibodies was detected using HRP-conjugated anti-rabbit IgG and chemiluminescence. One μg of protein was loaded in each well. Molecular mass markers are shown to the left.

Supplementary Figure S2. Examples of invasion inhibition of 3D7 parasite lines by individual samples and a schematic representation of interactions between merozoites ligands and erythrocytes. **A, B and C** show the results from invasion inhibition assays for 3 different Kenyan serum samples. Green bars show invasion for 3D7-wt parasites, blue are for 3D7ΔEBA175 parasites cultured with normal erythrocytes (RBCs), and blue striped bars show 3D7ΔEBA175 parasites cultured with neuraminidase (Neuram)-treated erythrocytes. In **A**, serum antibodies against SA-dependent antigens such as EBA140, EBA181 and Rh1 could explain the differences seen. EBA175 does not appear to be a major target of antibodies because there was little difference between 3D7-wt and 3D7ΔEBA175 cultured with normal erythrocytes. In **B**, serum antibodies against EBA175 could explain the 37% difference in invasion seen between 3D7-wt and 3D7ΔEBA175. In **C**, antibodies against ligands of SA-independent invasion, such as PfRh4 and PfRh2b, could account for the greater inhibition of 3D7ΔEBA175 compared to wild-type, particularly when 3D7ΔEBA175 is cultured with neuraminidase-treated erythrocytes.

D, E and F show schematic representations of the interactions between the EBA and PfRh merozoite proteins and the erythrocyte surface in 3D7-wt and 3D7ΔEBA175. SA-dependent interactions are coloured orange and SA-independent interactions are purple. Unknown receptors on the erythrocyte are marked with the symbol '?'. In **D**, all EBA and PfRh interactions are thought to be functional. A cross (in **E** and **F**) over ligand(s) indicates that the ligand(s) are not functioning, either because of targeted disruption by transfection (EBA175 in **E**) or because the erythrocyte receptors have been removed by neuraminidase-treatment of the erythrocyte (**F**).

Supplementary Figure S3. Antibodies among Kenyan donors (n=150) against recombinant EBA and PfRh proteins measured by ELISA. Results are grouped by age, and samples from non-exposed donors (Contr.) are also shown. **A.** The proportion of individuals classified as positive for antibodies to each antigen. The cut-off for classification as positive was defined as an OD value greater than the mean + 3SD of the non-exposed controls. $P<0.001$ for EBA140 and EBA175(W2mef allele); $p=0.06$ for PfRh2 and PfRh4; $p>0.1$ for EBA175(3D7) and EBA181. **B.** The proportion of individuals with an OD value greater than the 75th centile for each age group. $P<0.001$ for all antigens except PfRh2 ($p=0.06$).

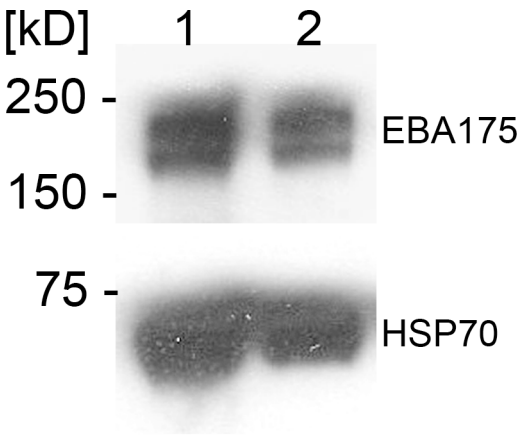

Figure S1

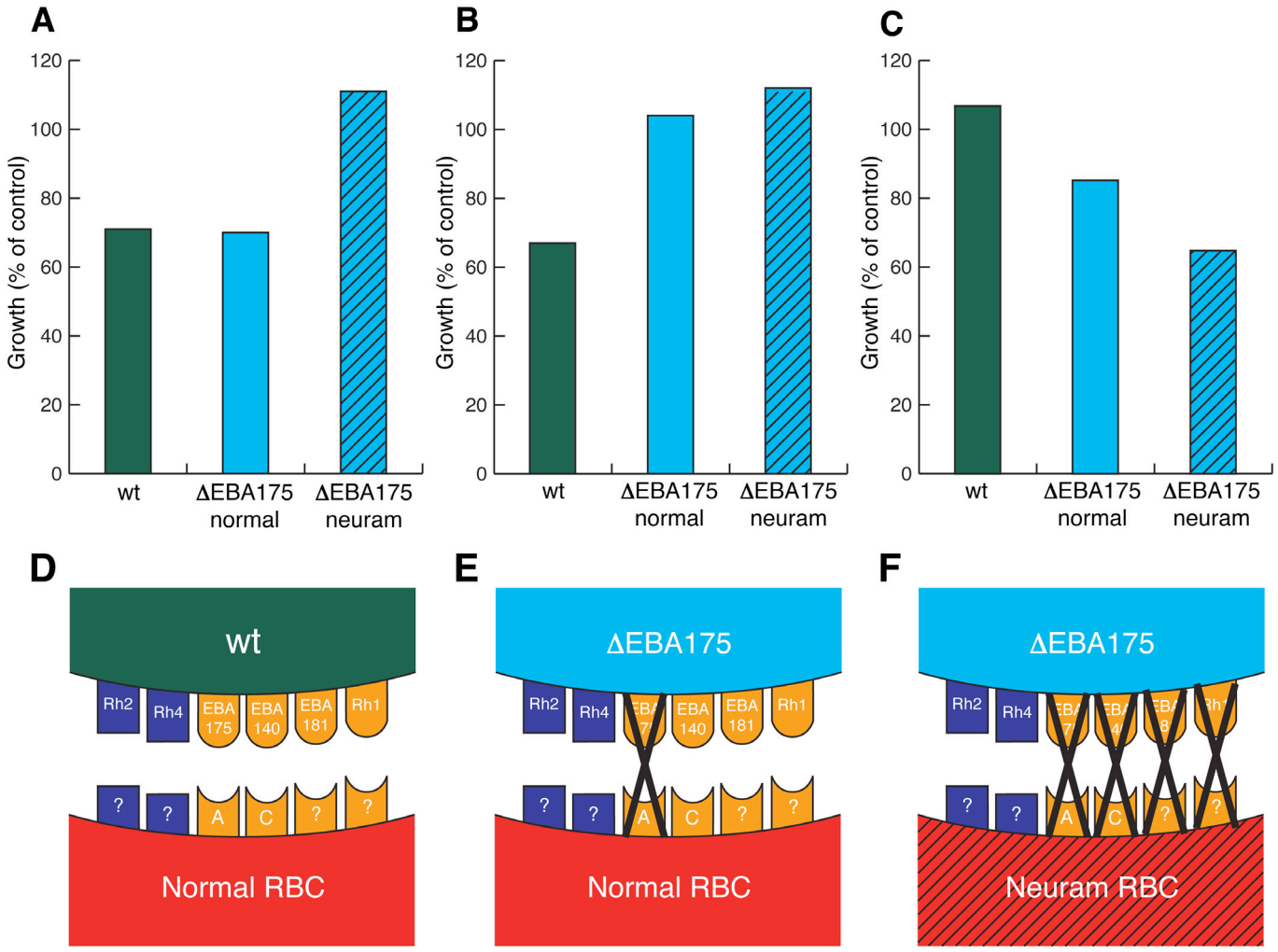
A
B
C
Growth (% of control)
120
100
80
60
40
20
0
wt
ΔEBA175 normal
ΔEBA175 neuram
D
E
F
wt
ΔEBA175
Rh2
Rh4
EBA 175
EBA 140
EBA 181
Rh1
?
A
C
Normal RBC
Neuram RBC

Figure S2

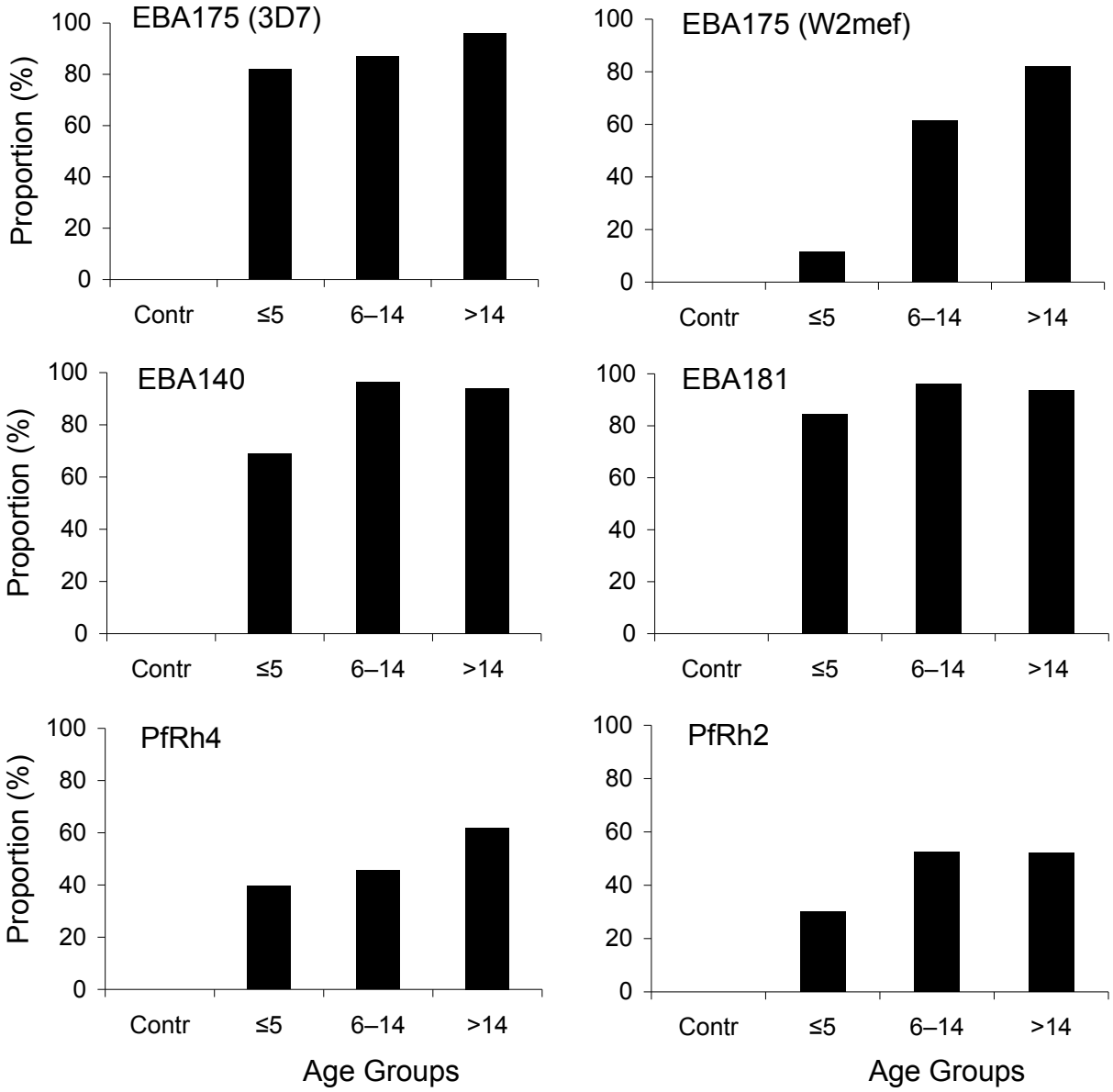
EBA175 (3D7)
EBA175 (W2mef)
EBA140
EBA181
PfRh4
PfRh2
Proportion (%)
100
80
60
40
20
0
Contr
≤5
6–14
>14
Age Groups

Figure S3. A

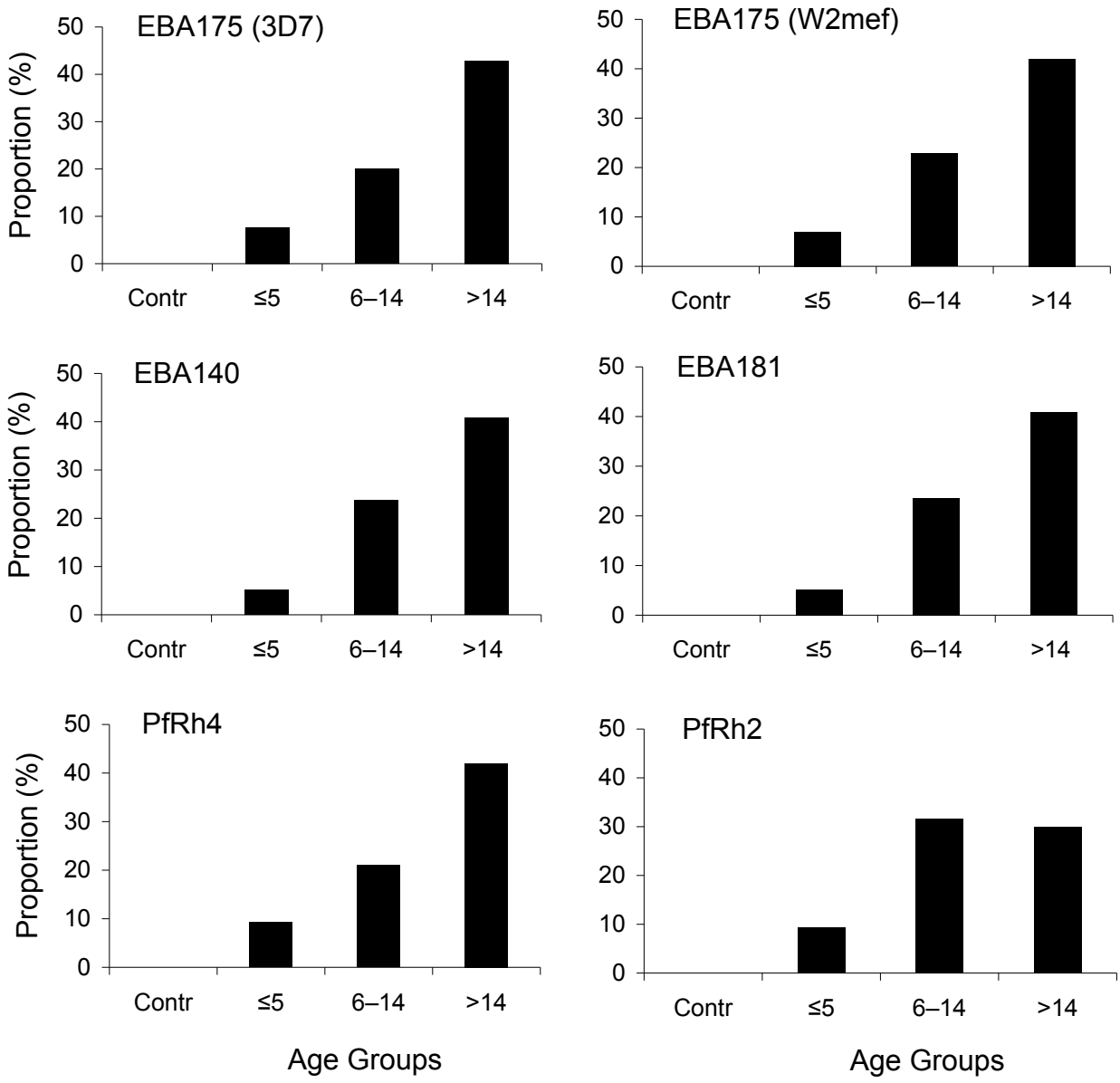
EBA175 (3D7)
EBA175 (W2mef)
EBA140
EBA181
PfRh4
PfRh2
Proportion (%)
50
40
30
20
10
0
Contr
≤5
6–14
>14
Age Groups

Figure S3. B