Table S1. A compilation of non-synonymous SNP sites in RALP1 of different strains deposited in PlasmoDB[a]

Amino acid position[b]	422	428	452	455	458	461	464	467	580
3D7	G	E	E	H	G	N	G	H	L
7G8	—[c]	—	—	—	—	—	—	—	n.d.[d]
D10	—	G	—	—	—	—	—	—	n.d.
D6	n.d.	n.d.	G	N	—	H	—	N	—
Dd2	—	—	G	N	—	—	—	—	V
FCC-2	n.d.	—	n.d.	n.d.	—	—	—	—	—
FCR3	—	—	—	—	—	—	—	—	I
GHANA1	n.d.	n.d.	—	—	—	—	—	—	—
HB3	—	—	—	—	E	H	E	—	—
K1	E	n.d.	—	—	—	—	—	—	—
RO-33	—	—	—	—	—	—	—	—	—
SantaLucia	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	—	n.d.	—
Senegal3404	—	n.d.	—	—	—	n.d.	—	n.d.	—
V1_S	—	—	—	—	—	—	—	—	I

[a] The data were obtained from PlasmoDB version 9.3 (http://plasmodb.org)
[b] Amino acid position represent the position of amino acids in RALP1 of the reference strain 3D7
[c] —, same amino acid residue as in the 3D7 sequence.
[d] n.d., no data

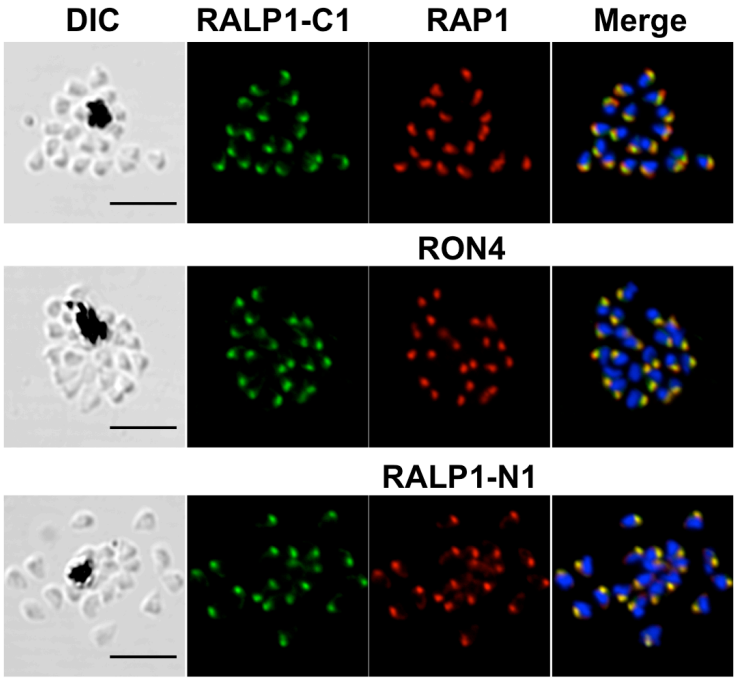

Fig. S1. Localization of RALP1 in *Plasmodium falciparum* merozoite. RALP1 localization using an immunofluorescence assay. Paraformaldehyde-fixed mature schizonts were probed with rabbit anti-RALP1-C1 (green) and mouse anti-RAP1 (rhoptry bulb marker) (top panel) or anti-RON4 (rhoptry neck marker) (middle panel) or anti-RALP1-N1 (bottom panel) (red). Parasite nuclei were stained with DAPI (blue). Scale bars represent 5 μm.

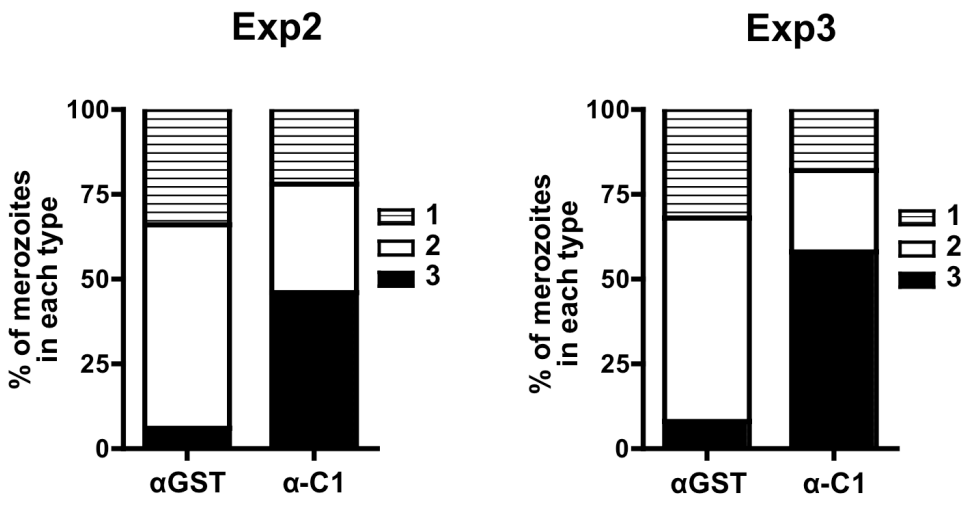

Fig. S2. Anti-RALP1 antibodies cause merozoites to aberrantly release RAP1 in invasion inhibition assay. Second experiment (Exp2) and third experiment (Exp2) refer to two independent experiments conducted to observe the effect of anti-RALP1 antibodies on merozoite invasion: Stacked bar graph (FigS2. Exp2, 1-3) compares the number of merozoites seen attached to erythrocytes in the presence of anti-GST (αGST) or anti-RALP1-C1 (α-C1) antibody. The proportion of RAP1 unreleased parasites [FigS2. Exp2(1)] observed in the presence of anti-RALP1-C1 antibody was not significantly different (Fisher's exact test adjusted $P = 0.42$) from that observed in the presence anti-GST. However, the proportion of invaded merozoites (measured by counting properly secreted RAP1 staining), [FigS2. Exp2(2)] was significantly reduced by anti-RALP1-C1 antibody as compared to anti-GST antibody (Fisher's exact test adjusted $P = 0.0018$). The

proportion of merozoites that released RAP1 aberrantly [FigS2. Exp2(3)] was significantly higher in the presence of anti-RALP1-C1 antibody than in the presence of control anti-GST antibody (Fisher's exact test adjusted $P < 0.001$) . Stacked bar graph (FigS2. Exp3, 1-3) compares the number of merozoites seen attached to erythrocytes in the presence of anti-GST (αGST) or anti-RALP1-C1 (α-C1) antibody. The proportion of RAP1 unreleased parasites [FigS2. Exp3(1)] observed in the presence of anti-RALP1-C1 antibody was not significantly different (Fisher's exact test adjusted $P = 0.12$) from that observed in the presence anti-GST. However, the proportion of invaded merozoites (measured by counting properly secreted RAP1 staining), [FigS2. Exp3(2)] was significantly reduced by anti-RALP1-C1 antibody as compared to anti-GST antibody (Fisher's exact test adjusted $P < 0.001$). The proportion of merozoites that released RAP1 aberrantly [FigS2. Exp3(3)] was significantly higher in the presence of anti-RALP1-C1 antibody than in the presence of control anti-GST antibody (Fisher's exact test adjusted $P < 0.001$) .