Cooke et al., http://www.jcb.org/cgi/content/full/jcb.200509122/DC1

FRET was performed using the acceptor photobleaching method (Kenworthy, 2001) as previously described for malaria parasite proteins (Topolska et al., 2004) using AlexaFluor488– and -568–conjugated secondary antibodies. FRET was determined as a function of increased fluorescence intensity of the donor fluorophore after photobleaching of the acceptor using a confocal microscope (model TCS NT; Leica) and confocal software (Leica). Several different Maurer's clefts in multiple IRBCs were analyzed. Typical fluorescence/time curves for PfEMP1 and SBP1 were obtained during acceptor photobleaching at 568 nm.

For immunoprecipitation experiments, soluble parasite lysate was immunoprecipitated with rabbit polyclonal antibody raised against the NH_2 terminus of SBP1 for 2 h at 4°C, and the resulting immune complexes were captured by incubating with GammaBind G–Sepharose beads (GE Healthcare) for 2 h at 4°C. After four washes with lysis buffer, beads were dissolved in Laemmli buffer, boiled for 5 min, and proteins were separated by SDS gel electrophoresis and probed with either a mouse polyclonal antibody raised against the NH_2 terminus of SBP1 or mouse monoclonal anti-VARC antibody to detect PfEMP1.

References

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