Supplementary Material

The *Plasmodium falciparum* blood stages acquire factor H family proteins to evade destruction by human complement

Thiago F. A. Rosa[1#], Ansgar Flammersfeld[1#], Che J. Ngwa[1], Meike Kiesow[1], Rainer Fischer[2], Peter F. Zipfel[3], Christine Skerka[3], and Gabriele Pradel[1,2*]

[1]*Division of Cellular and Applied Infection Biology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany*

[2]*Fraunhofer Institute for Molecular Biology and Applied Ecology, Forckenbeckstr. 6, 52074 Aachen, Germany*

[3]*Department of Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Beutenbergstr. 11a, 07745 Jena, Germany*

Running title: Complement evasion by the *P. falciparum* blood stages

[#]These authors contributed equally.

*For correspondence: E-mail: pradel@bio2.rwth-aachen.de, Tel.: (+49) 241 80-20848, Fax: (+49) 241 80-22146.

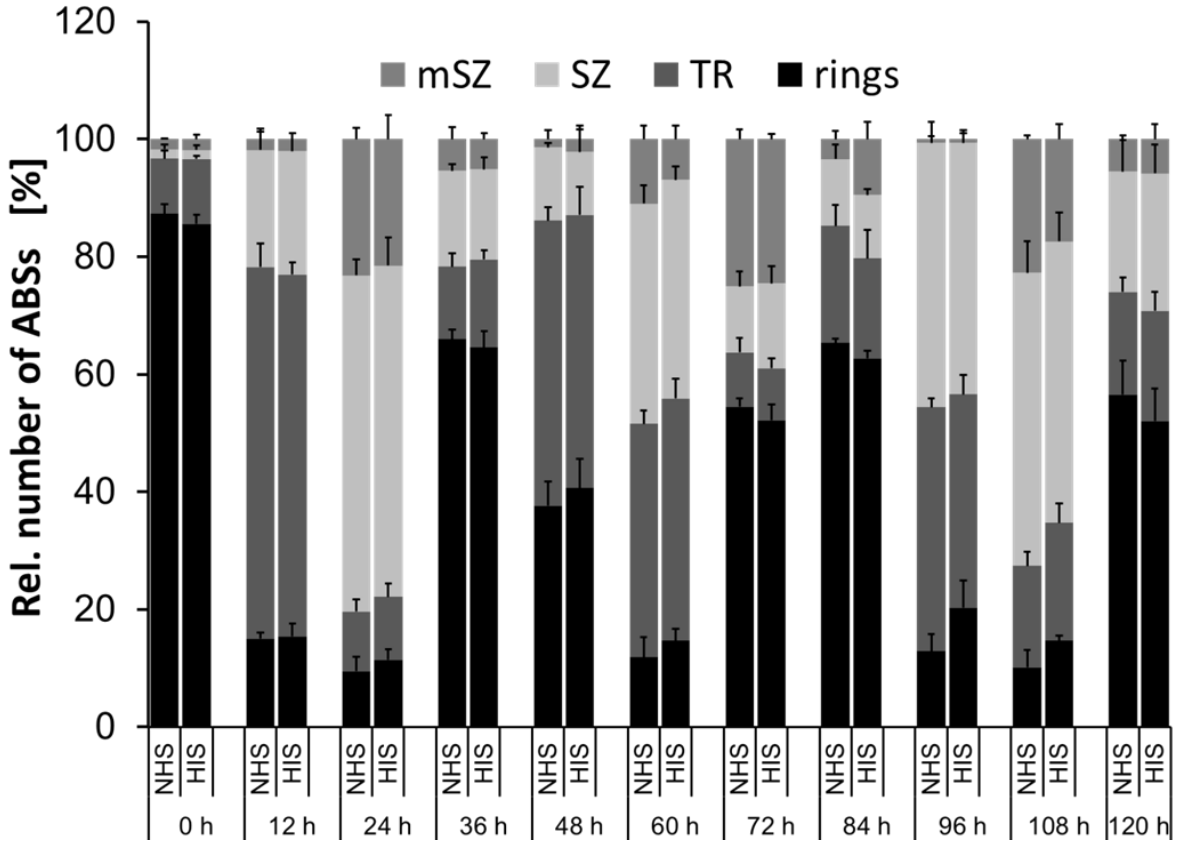

Figure S1: Effect of human complement on ABS differentiation.

Ring stage cultures of strain NF54 with a starting parasitemia of 0.5% were maintained in cell culture medium supplemented with either 10 vol% NHS or HIS at 37°C over a period of 0-120 h. The different developmental stages, ring stages, trophozoites (TR), immature schizonts (SZ) and mature schizonts (mSZ), were determined in a total of number of 100 iRBCs every 12 h via Giemsa smears. The experiment was performed in triplicate (mean ± SD); the data are representative for one of two independent experiments.

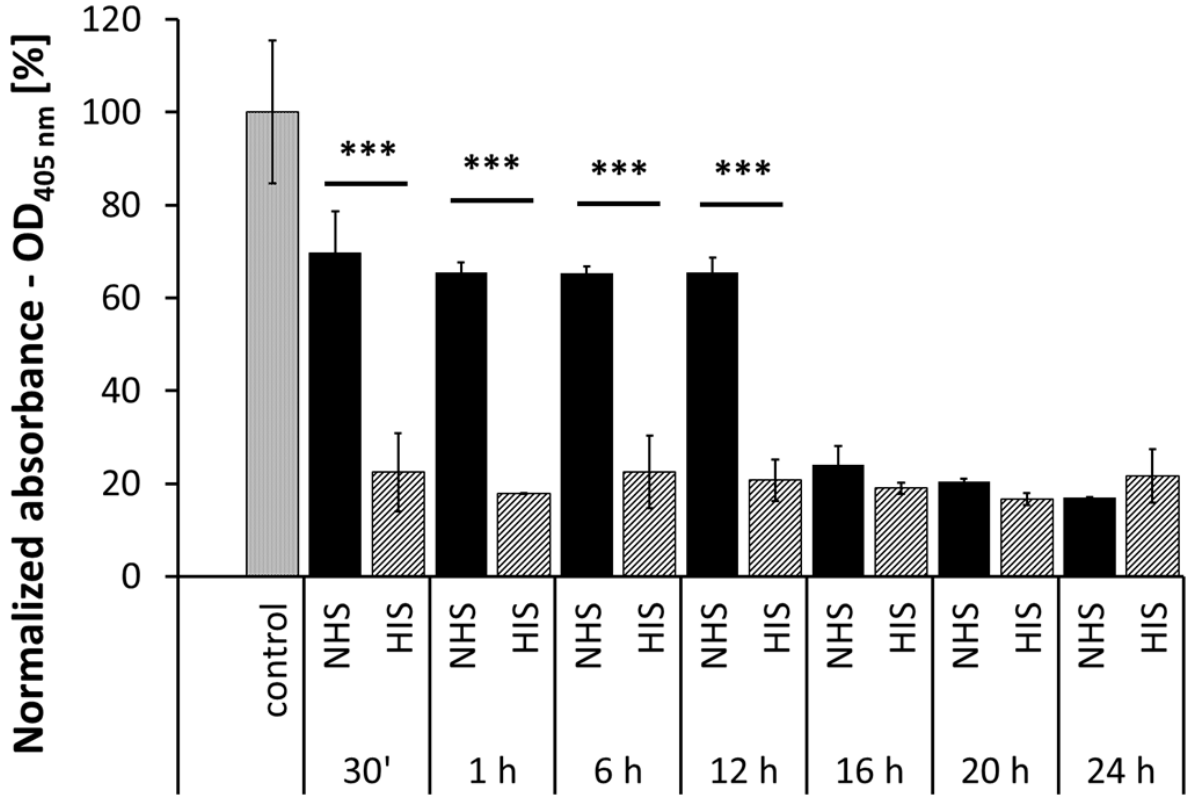

Figure S2: Pig RBC lytic activity of NHS.

Frozen pooled NHS or HIS samples were thawed and added to cell culture medium at 20 vol%. The medium was then kept at 37°C for 30 min to 24 h before being incubated with pig erythrocytes at 37°C for 1 h. The absorbance of the supernatant was subsequently measured at $OD_{405\ nm}$. Pig erythrocytes lysed by distilled water were used as a positive control (absorbance set to 100%). Significant differences in absorbance are indicated ($^{***}p < 0.001$; Bonferroni post-test, two-way ANOVA). The experiment was performed in triplicate (mean ± SD); the data are representative for one of three independent experiments.

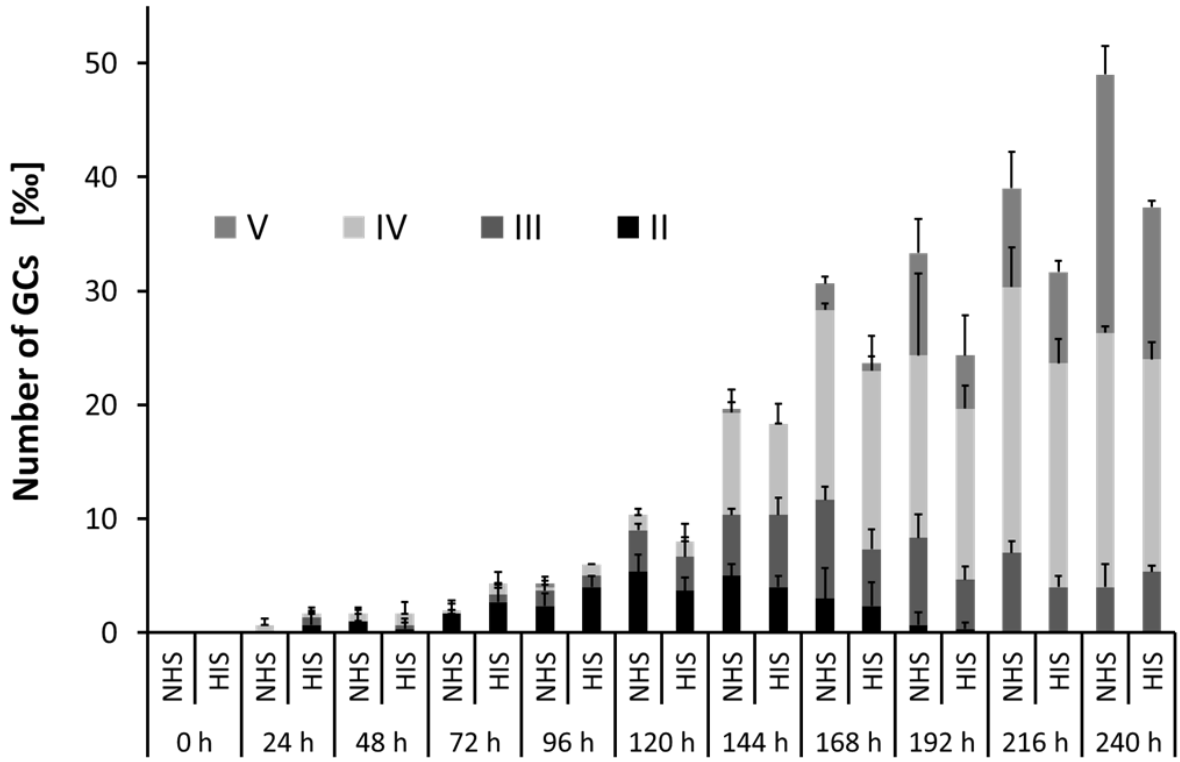

Figure S3: Effect of human complement on gametocyte differentiation.

Ring stage cultures of strain NF54 with a starting parasitemia of 5% were maintained in cell culture medium supplemented with either 10 vol% NHS or HIS at 37°C over a period of 0-240 h. The numbers of gametocyte stages II-V were determined every 24 h via Giemsa smears. The experiment was performed in triplicate (mean ± SD); the data are representative for one of two independent experiments.

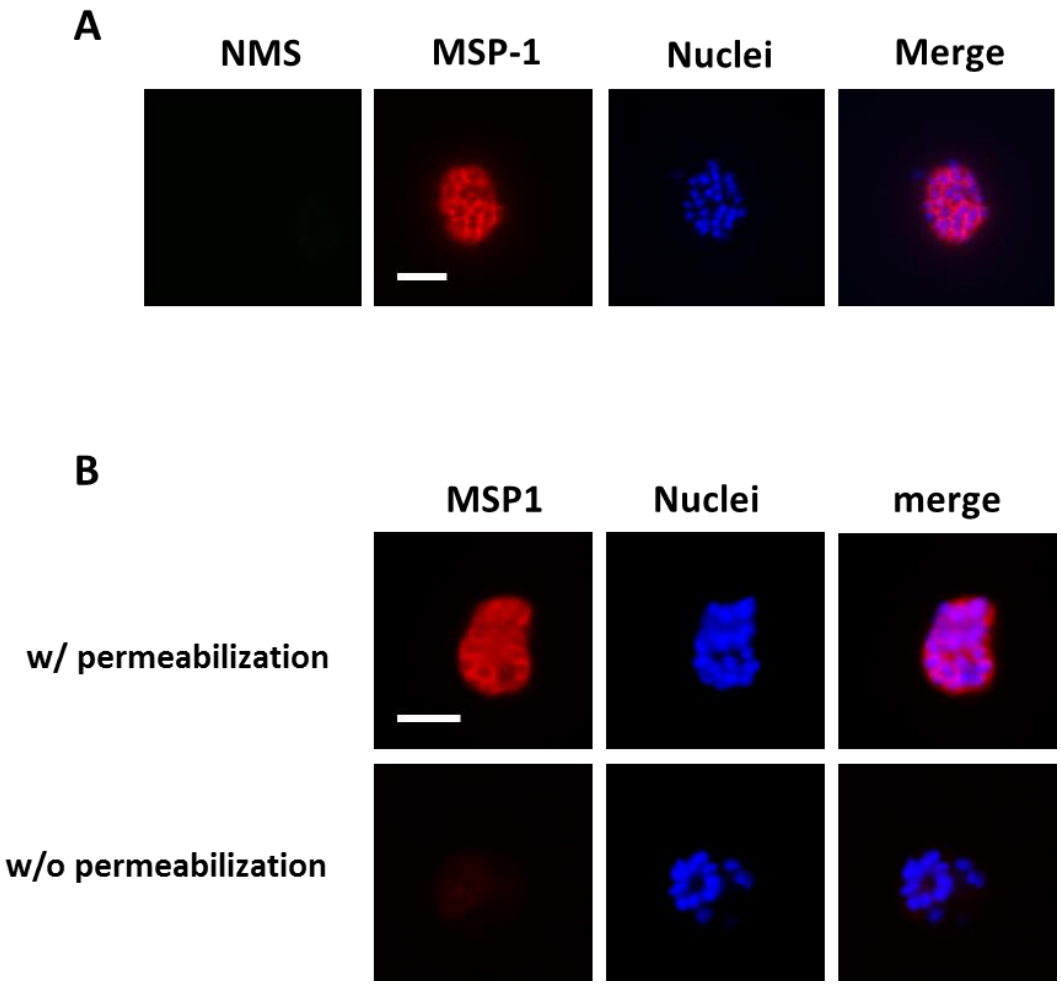

Figure S4: IFAs of NMS and saponin-permeabilization controls.

A. Immunolabeling of schizonts with NMS. Schizonts were incubated with 20 vol% NHS for 1 h and immunolabelling with NMS (green). ABS parasites were detected by labelling with anti-MSP1 antibody (red).

B. Accessibility of merozoites for molecules following saponin-permeabilization of schizonts**.** Live schizonts were permeabilized with 0.05% saponin prior to incubation with 20 vol% NHS for 1 h and fixation with glutaraldehyde in suspension. Schizonts not permeabilized by saponin were used for negative control. The fixed samples were immunolabelled with anti-MSP1 antibody (red). Nuclei were counterstained with Hoechst stain (blue). The data are representative for one of two independent experiments. In the IFAs, strain F12 was used, similar results were observed for strain NF54. Bar, 5 µm.

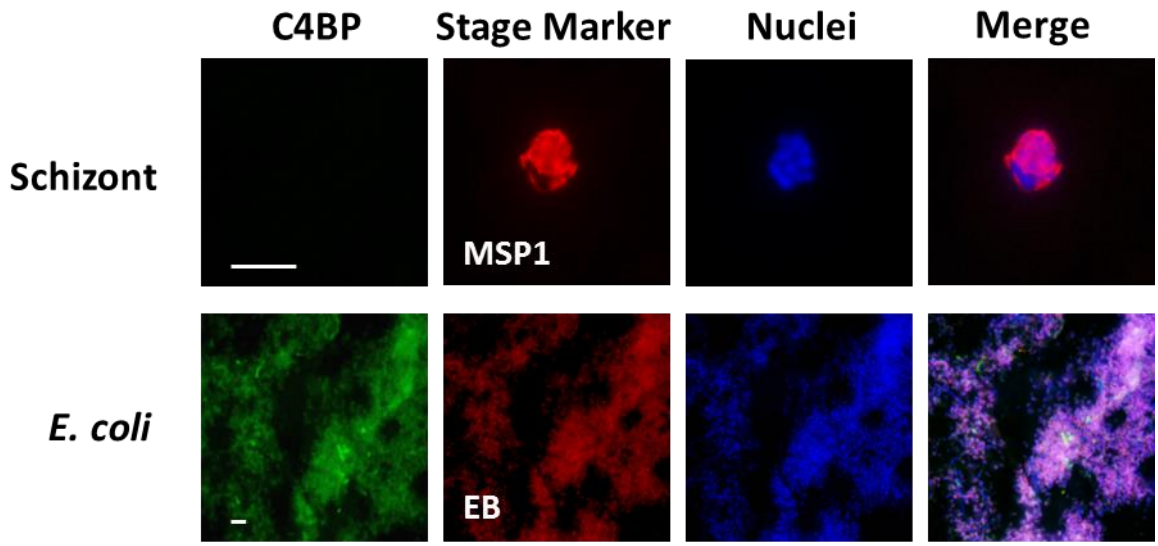

Figure S5: Binding of C4BP to the surface of pathogens.

Schizonts of strain NF54 or *Escherichia coli* (*E. coli*) were incubated with 20 vol% NHS for 1 h and subjected to IFAs. C4BP binding was detected using anti-C4BP antibody (green); ABSs were counter-labelled with anti-MSP1 antibody; *E. coli* was stained with Evans Blue (EB) staining (red). The nuclei were counterstained with Hoechst stain (blue). Bar, 5 µm.

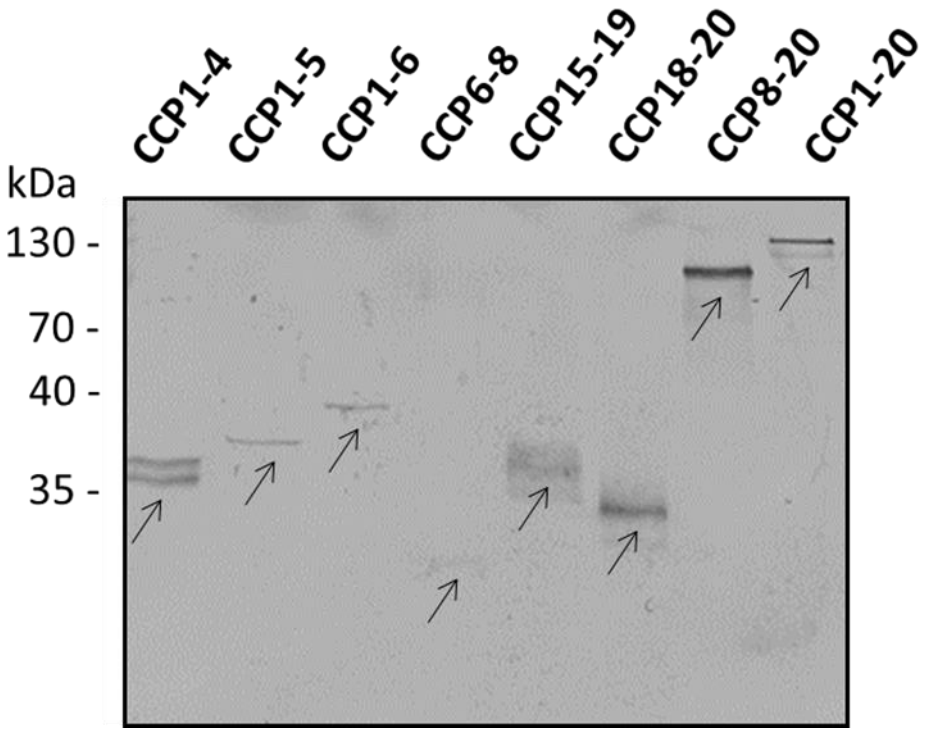

Figure S6: Determination of purity of FH deletion mutant peptides.

The purified recombinant FH deletion mutants CCP1-4, CCP1-5, CCP1-6, CCP6-8, CCP15-15, CCP18-20, CCP8-20 and CCP1-20 (FH) were separated by non-reducing gel electrophoresis followed by Western blotting using polyclonal anti-CCP1-20 antibody. Arrows indicate the peptide bands. The data are representative for one of two independent experiments.

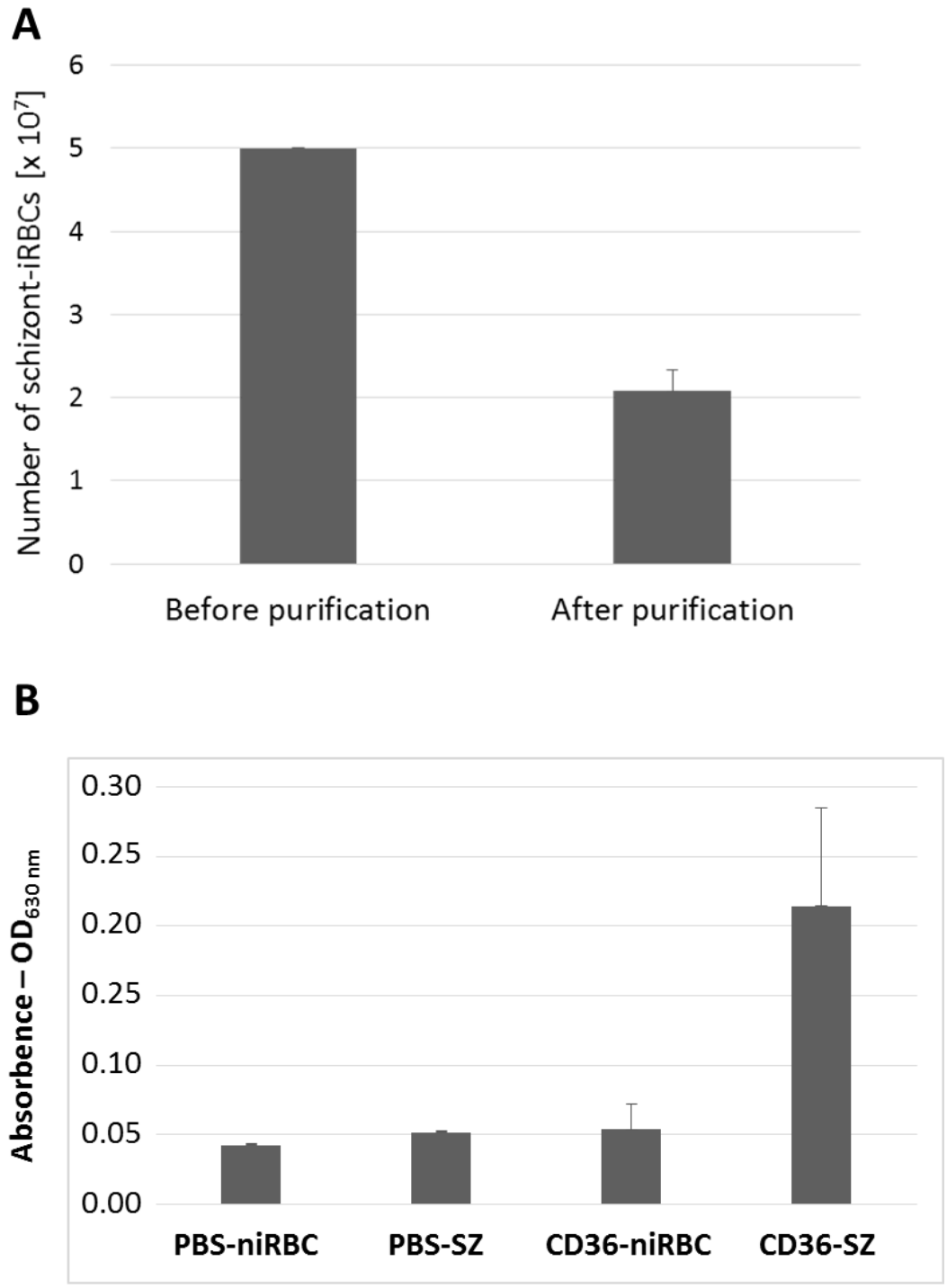

Figure S7: Evaluation of knob formation in *P. falciparum* strain F12.

A. Gelatin flotation behavior of schizonts. A total number of 5 x 10^7 schizonts of strain F12 were incubated in 0.7% gelatin for 1 h and subsequently the numbers of cells floating in the supernatant was determined for five independent experiments (mean ± SD).

B. Binding of schizonts to CD36. A total number of 1 x 10^7 schizont-iRBCs of strain F12 or niRBC used for control were plated per CD36-coated well and incubated for 2 h. Uncoated wells, treated with PBS, were used for negative control. Following washing, the relative numbers of bound parasites were compared via Malstat assay by measuring the absorbance at $OD_{630\ nm}$. The experiment was performed in triplicate (mean ± SD); the data are representative for one of five independent experiments.

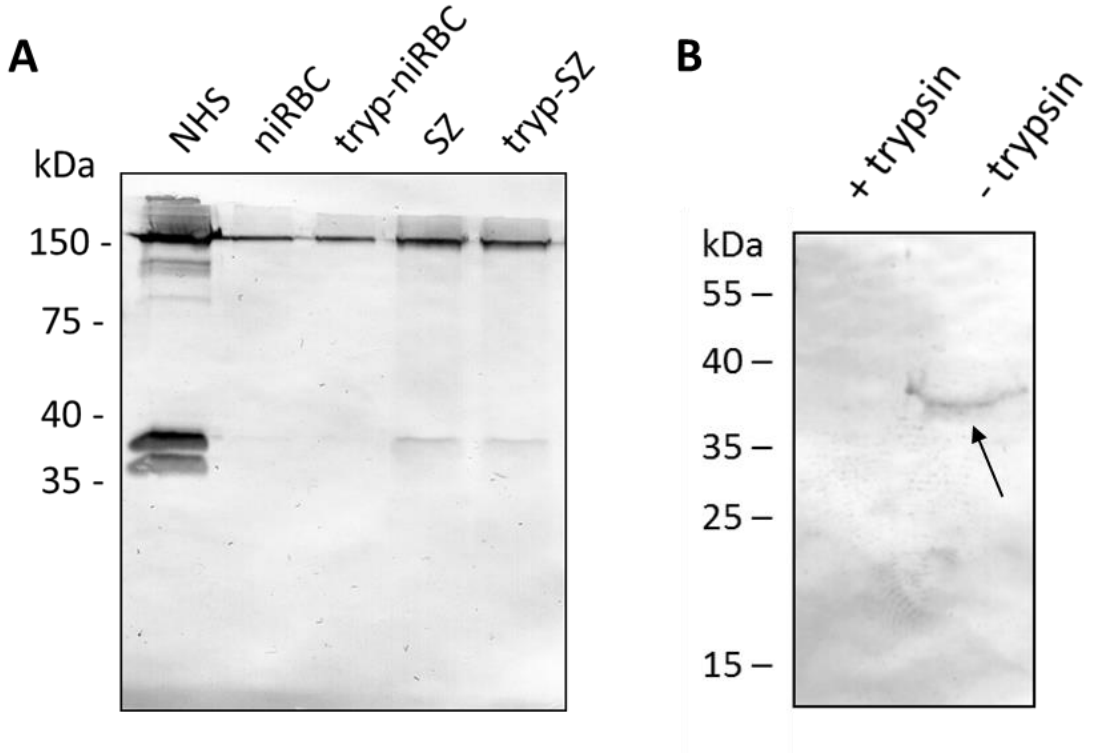

Figure S8: Binding of FH by ABS parasites following trypsin-treatment.

A. Binding of FH to the ABS surface with and without prior trypsin-treatment. Schizonts of strain F12 were treated with 100 µg/ml trypsin for 30 min prior to incubation with 20 vol% NHS for 1 h. Lysates of schizonts or niRBCs for control with and without trypsin-treatment were prepared and subjected to non-reducing gel electrophoresis followed by Western blotting using anti-CCP1-20 antibody. NHS was loaded for positive control. The data are representative for one of five independent experiments.

B. Effect of trypsin on the cleavage of RBC glycophorin A. Schizonts were treated with trypsin as described above and lysates of schizonts with and without trypsin-treatment were subjected to Western blot analysis using anti-glycophorin A antibody to detect the glycoprotein (~38 kDa; arrow).