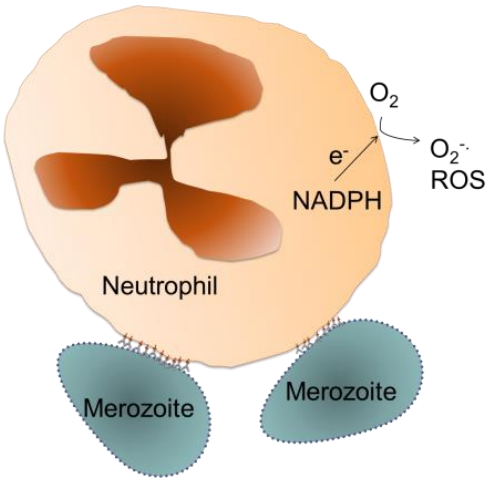
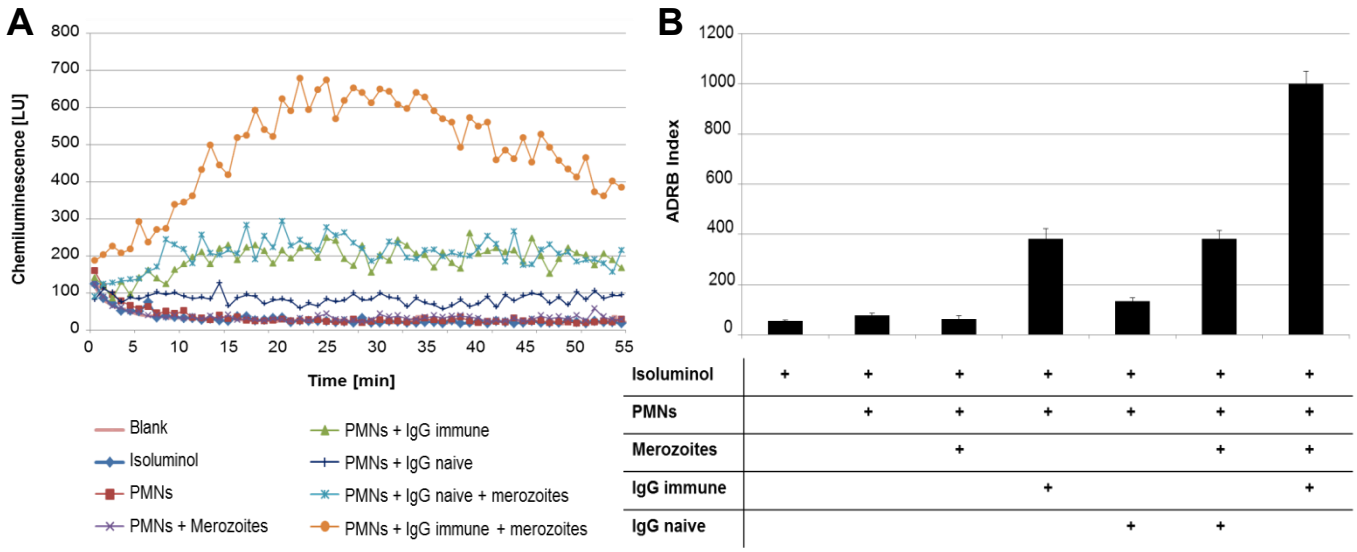
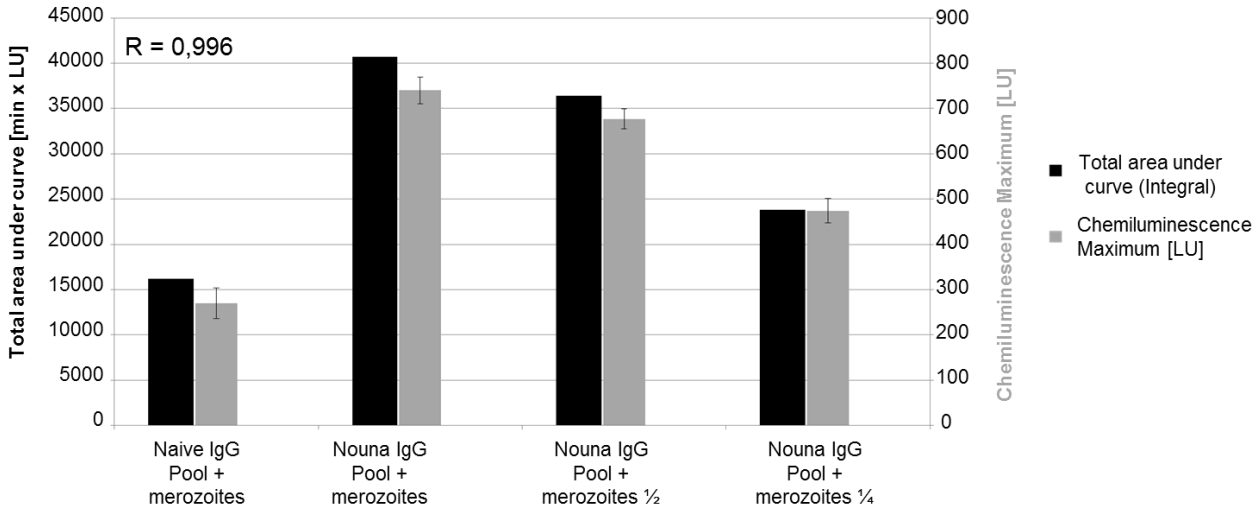
Supplemental Material



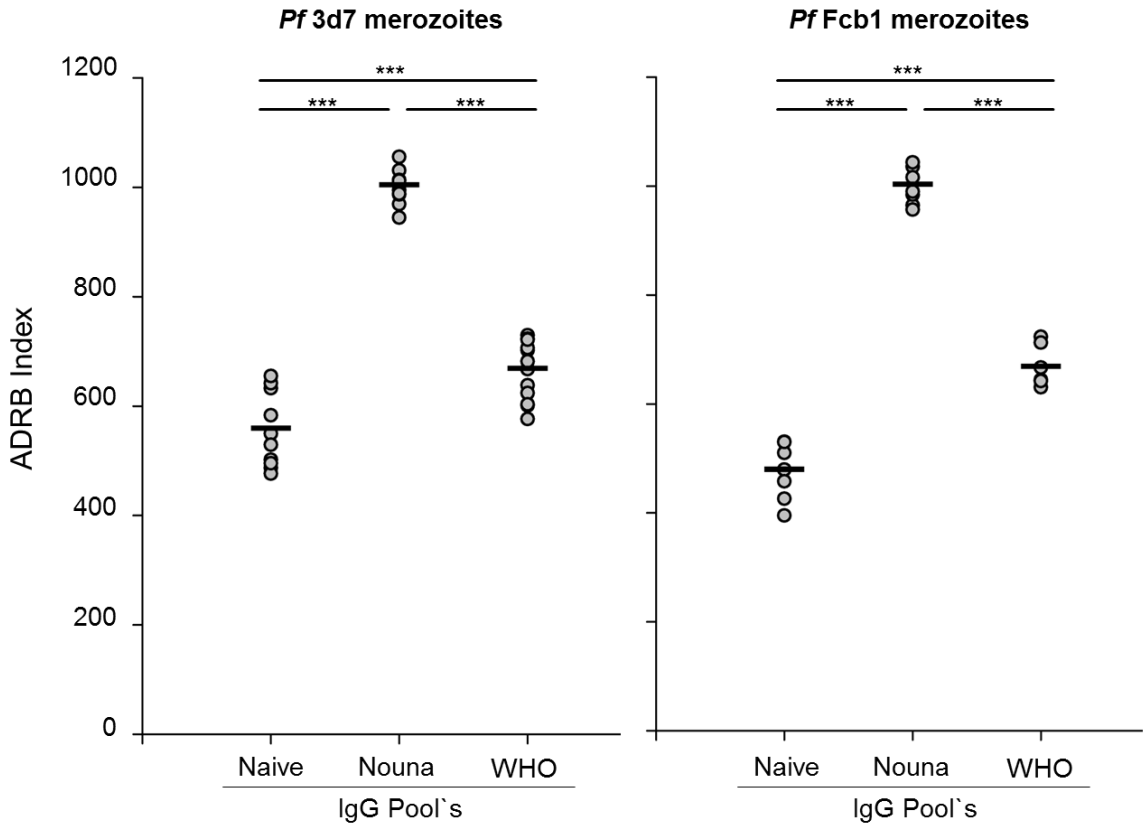
Supplementary figure 1: Mechanism of the Antibody-dependent Respiratory Burst (ADRB). Antibodies opsonize merozoites, bind to the Fc receptors of neutrophils and cross-link them. Thereby, the neutrophil gets activated and its NADPH oxidase produces reactive oxygen species (ROS) by electron transfer to molecular oxygen. ROS can be measured *via* chemiluminescence.



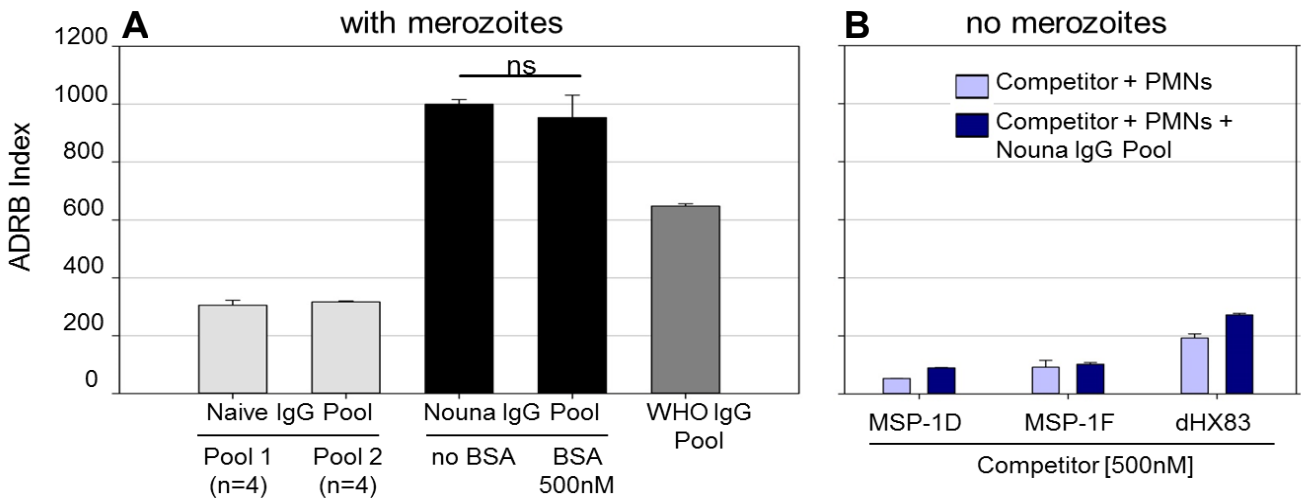
Supplementary figure 2: Controls for the ADRB assay. (A) Chemiluminescence raw data of a semi-immune IgG pool opsonizing *P. falciparum* 3d7 merozoites (orange line) and different controls. Each dot represents the mean of duplicate measurements. (**B**) Calculation of the ADRB Index for all samples shown in A using the maximum value of the chemiluminescence curve within the first 30 min. ADRB Index = LU maximum sample / LU maximum semi-immune IgG pool x 1000. Shown is the mean with SEM. LU: Light units; PMNs: Polymorphonuclear neutrophils; IgG naïve: Prot-G purified IgG from a malaria-naïve pool (n=4); IgG immune: Prot-G purified IgG from semi-immune individuals from Nouna, Burkina Faso (n=11).



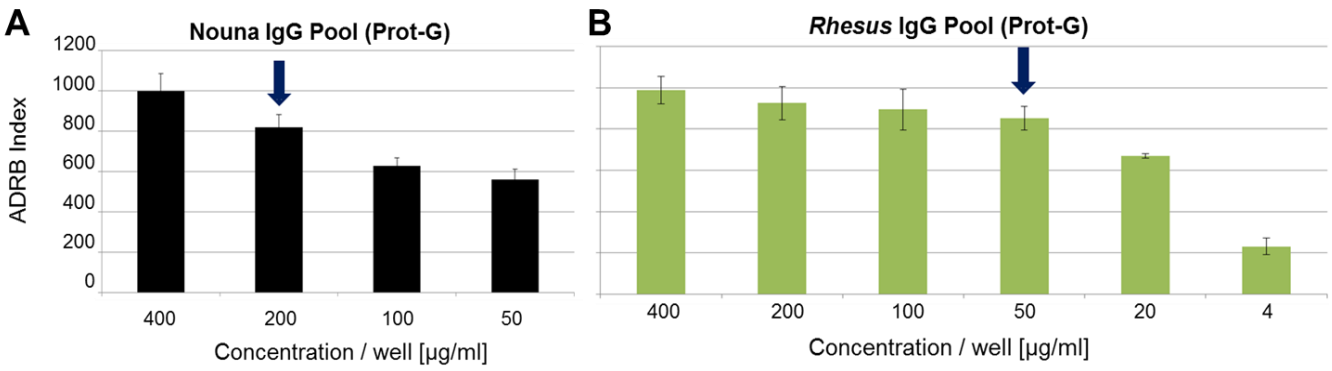
Supplementary figure 3: Comparison of possible readouts of ADRB data. ADRB raw data are analyzed by either calculating the total area under curve (isoluminol as baseline, 0-60min) or by using the chemiluminescence maximum value (0-30min). As an example, the ADRB activity of a malaria-naïve IgG Pool (n=4) and a semi-immune IgG Pool (n=11, Nouna Pool) against different concentrations of *P. falciparum* 3d7 merozoites was analyzed using both methods; these show a very good correlation (Pearson correlation coefficient R = 0,996).



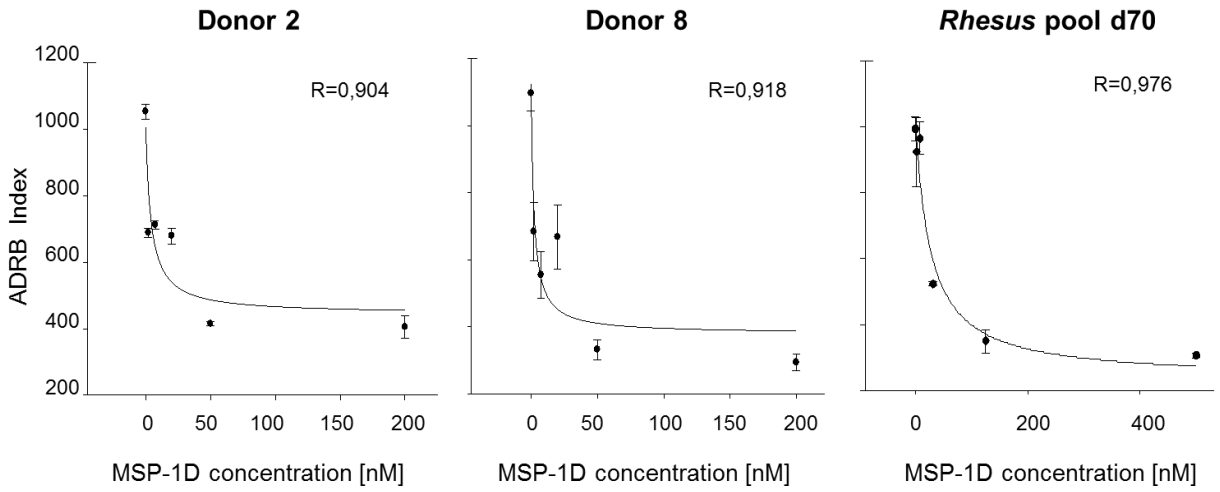
Supplementary figure 4: Precision of the ADRB assay. ADRB activity against *P. falciparum* 3d7 and FCB1 merozoites was determined in 5 (3d7) or 4 (FCB1) independent experiments with two different analysts. Intra-assay CV was < 6 %, inter-assay CV was < 12%. Statistical differences were assessed by One Way Anova. Naïve: Prot-G purified IgG from malaria-naïve individuals (n=4); Nouna: Prot-G purified IgG from semi-immune individuals (n=11) from Burkina Faso; WHO: Prot-G purified IgG from malaria-exposed Kenyan adults (NIBSC code: 10/198; (69)).



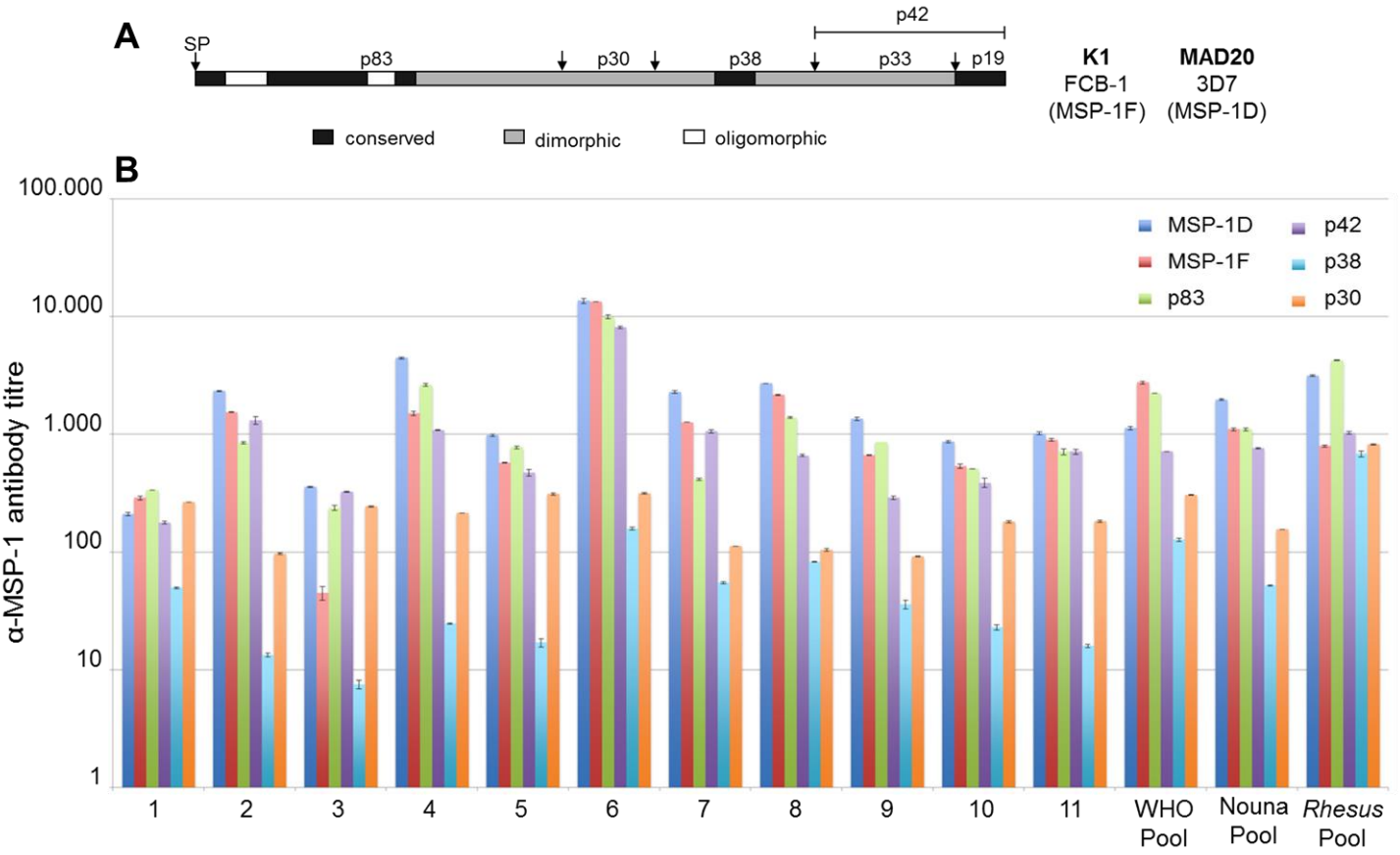
Supplementary figure 5: Controls for Antigen-Reversal ADRB. (**A**) ADRB activity of IgG from 2 malaria-naïve European pools (each n=4) in comparison to malaria-exposed donors from Burkina Faso (Nouna Pool, n=11) and from Kenya (WHO pool, NIBSC code: 10/198; (69)) was determined against *P. falciparum* FCB1 merozoites. Presence of 500nM BSA does not reduce ADRB activity of semi-immune donors (Nouna IgG Pool, black bars). (**B**) ADRB activity of the indicated competitor antigens (500nM) alone and in combination with purified IgG from 11 semi-immune donors (Nouna pool) but in absence of merozoites was assessed; only background activity was detected. Mean values of duplicate measurements are shown; statistical differences were calculated using paired t-test.



Supplementary figure 6: Finding an appropriate IgG concentration for Antigen-Reversal ADRB. ADRB activity of Prot-G purified IgG from 11 semi-immune individuals from Burkina Faso (**A**) and from *rhesus* monkeys (n=5) immunized 3x with rMSP-1D and adjuvant (d70) (**B**) was analyzed against *P. falciparum* 3d7 merozoites using different IgG concentrations. For Antigen-Reversal ADRB we will use the following IgG concentrations per well: 200 µg/ml for human IgG and 50 µg/ml for α-MSP-1D *rhesus* IgG.



Supplementary figure 7: Antigen-Reversal ADRB with increasing concentrations of MSP-1D. Prot-G purified IgG from two semi-immune donors and *rhesus* α-MSP-1D antibodies (d70) were tested against *P. falciparum* 3d7 merozoites in presence of increasing concentrations of MSP-1D. ADRB activity strongly decreases even at low MSP-1D concentrations. The data points fit well ($R > 0.9$) to a hyperbolic decay curve with 3 parameters.



Supplementary figure 8: MSP-1 structure and its immunogenicity in individuals with naturally acquired immunity. (**A**) Schematic outline of MSP-1. The 190kDa precursor protein consists of 1720 amino acids and is cleaved during merozoite maturation into the four subunits p83, p30, p38 and p42. During invasion, p42 is further processed into the p33 and p19 fragment. Black, grey and white boxes represent conserved, dimorphic and oligomorphic regions, respectively. (**B**) MSP-1 antibody titers to MSP-1D/F and the four MSP-1 subunits were determined *via* ELISA in malaria-exposed individuals from Burkina Faso (n=11, Nouna Pool and single donors 1-11) and from Kenya (WHO Pool, NIBSC code: 10/198; (69)). Additionally, the MSP-1 antibody titers of *rhesus* monkeys (n=5, *rhesus* pool, sample from d70) immunized 3x with recombinant MSP-1D are shown.

ELISA	Growth inhibition(3d7) r	P	ADRB activity (3d7) r	P
α-SZL (3d7)	0,755	0,00336 (**)	0,767	0,000695 (***)
α-Merozoites (3d7)	0,720	0,00707 (**)	0,855	0,0000002 (***)
α-MSP-1D	0,769	0,00228 (**)	0,758	0,00102 (**)
α-MSP-1F	0,791	0,00211 (**)	0,615	0,0237 (*)

Table 1: Spearman`s rank correlation coefficients between antibody levels to MSP-1 or *P. falciparum* blood stages and GIA or ADRB activity. Antibody levels to *P. falciparum* 3d7 schizont lysate or merozoites or the recombinant proteins MSP-1D/F were determined *via* ELISA in 11 semi-immune individuals from Burkina Faso and correlated to their GIA and ADRB activity against *P. falciparum* 3d7**.** All correlations are significant, the P value is indicated.