

# Antibody mediated activation of Natural Killer cells in malaria exposed pregnant women

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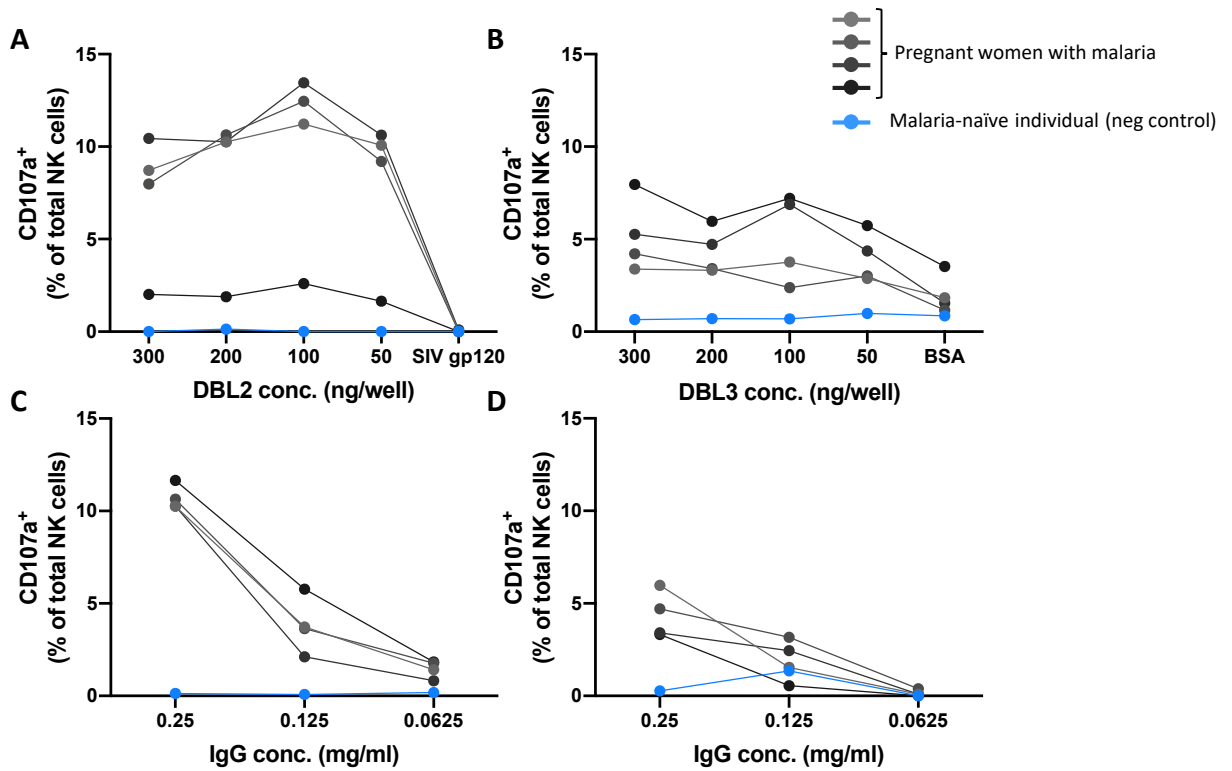
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Amy W. Chung

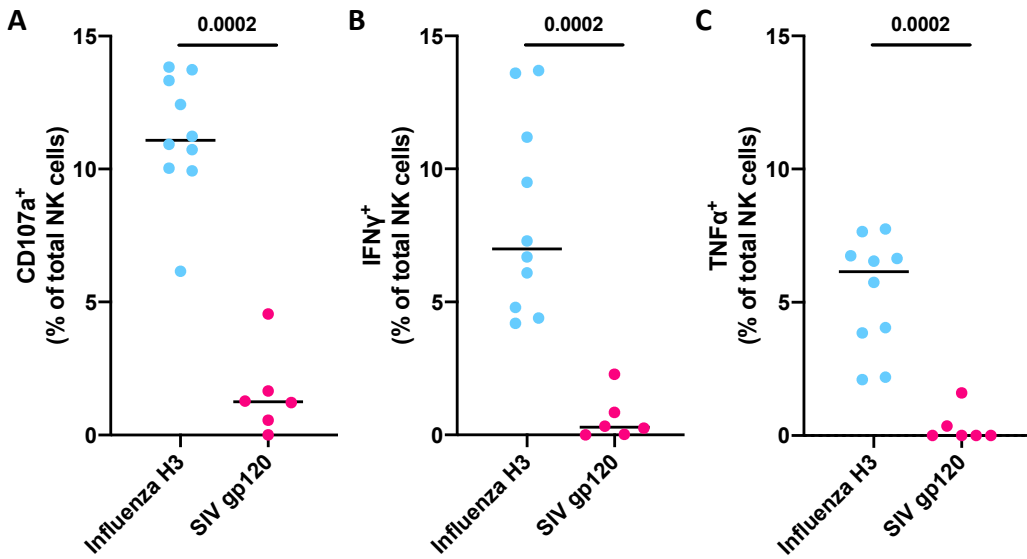
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# Figure S1



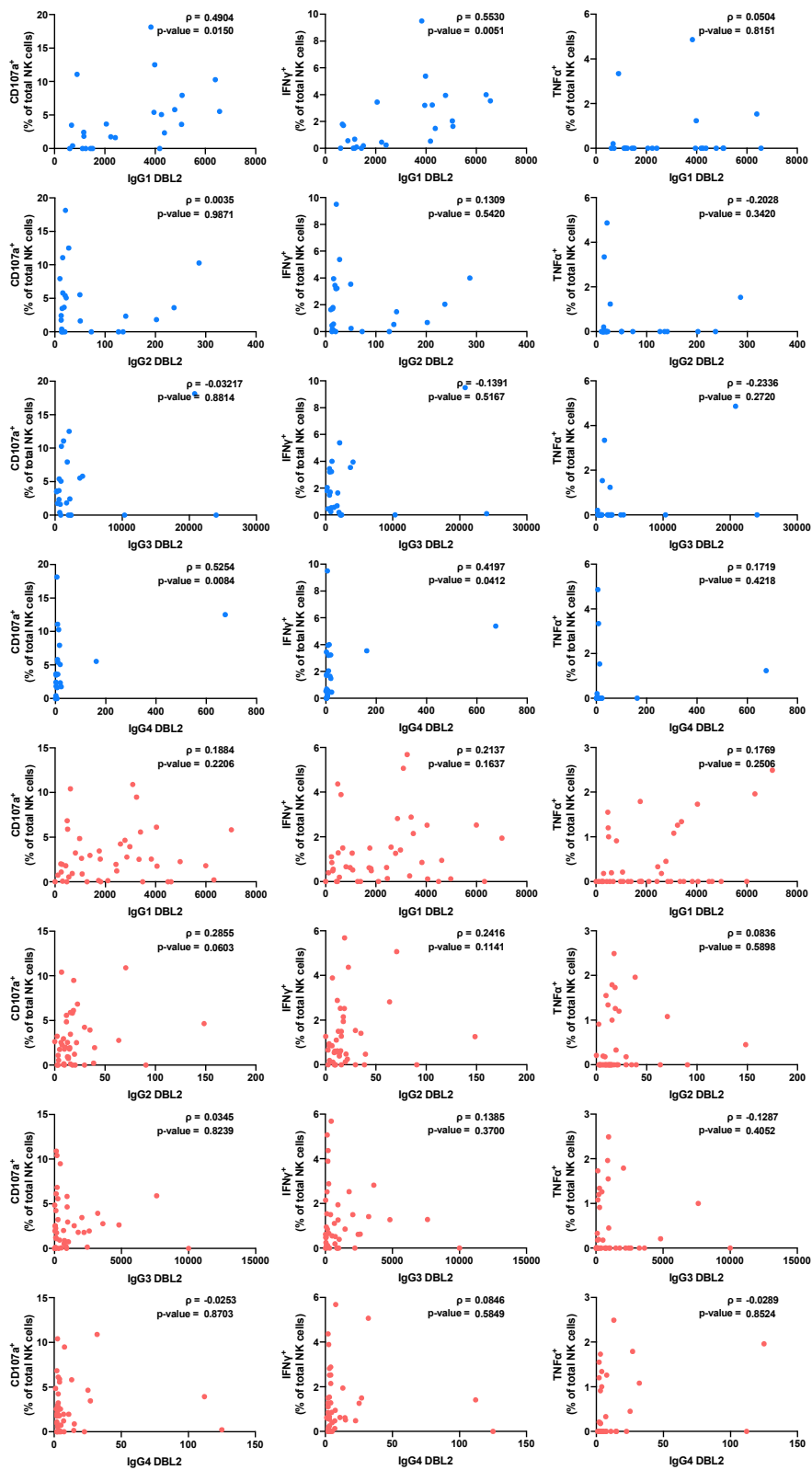
**Figure S1. Optimization of Ab-dependent plate-bound NK cell activation assays for use of DBL domains.** Optimal antigen (A-B) and IgG concentrations (C-D) were tested for Ab-dependent plate-bound NK cell activation assays. For comparison (A) & (C) are normalized to same condition (200ng DBL2 and 0.25mg/ml IgG) and (B) & (D) are normalized to same condition (200ng DBL3 and 0.25mg/ml IgG). IgG from four pregnant women with malaria (shades of grey) and a malaria-naïve individual (blue) are shown.

## Figure S2



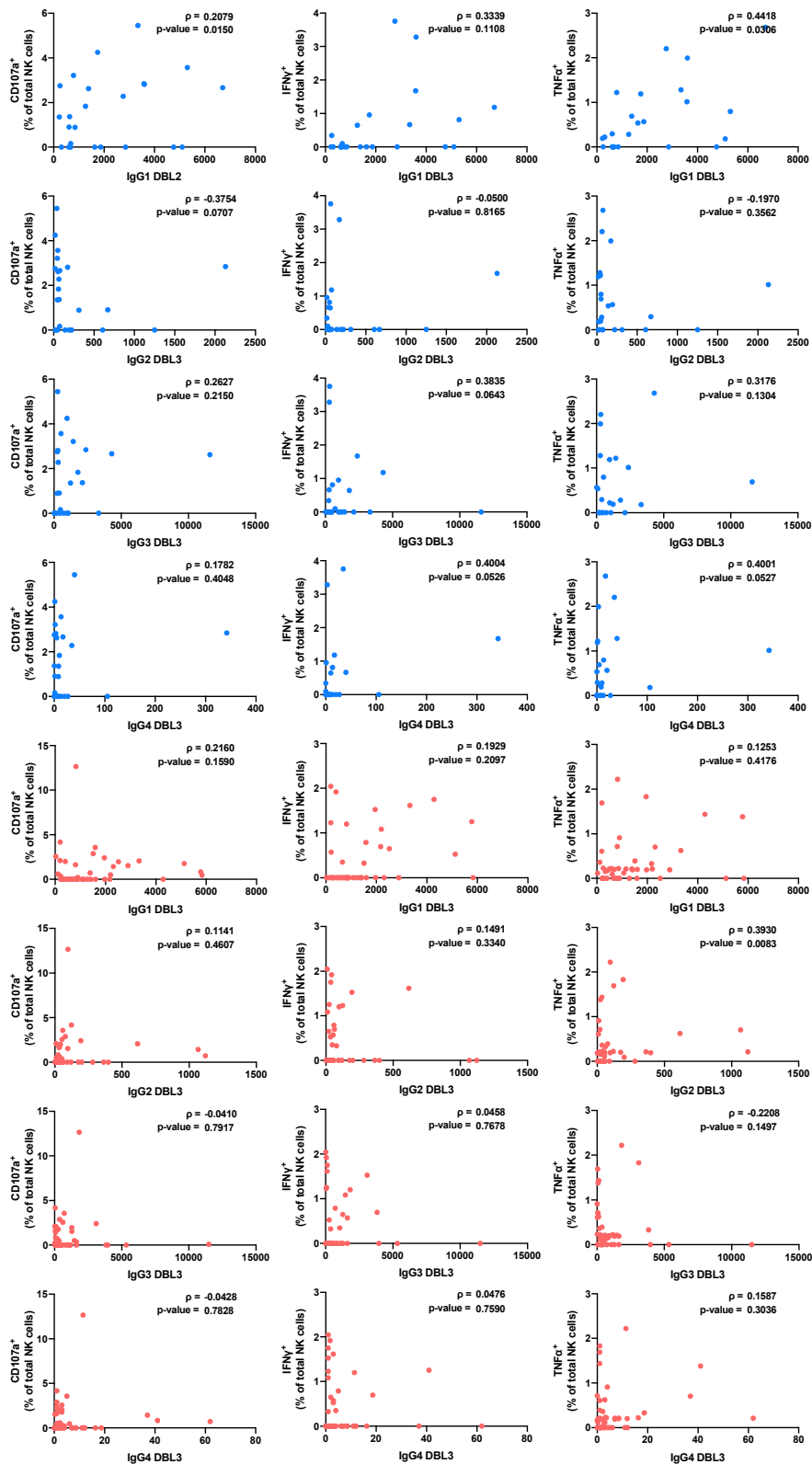
**Figure S2. Validation of Ab-dependent plate-bound NK cell activation assays using control antigens.** NK cell activation assays were validated using IgG Abs from malaria-naïve individuals in the presence of influenza hemagglutinin (H3) (positive control; light blue) and SIV gp120 (negative control; pink) proteins (200ng and 0.25mg/ml IgG). NK cells were assessed for surface **(A)** CD107a expression and intracellular **(B)** IFN $\gamma$  and **(C)** TNF $\alpha$  production. Significance between groups was analyzed using Mann-Whitney U tests (p-values are shown on graphs).

# Figure S3



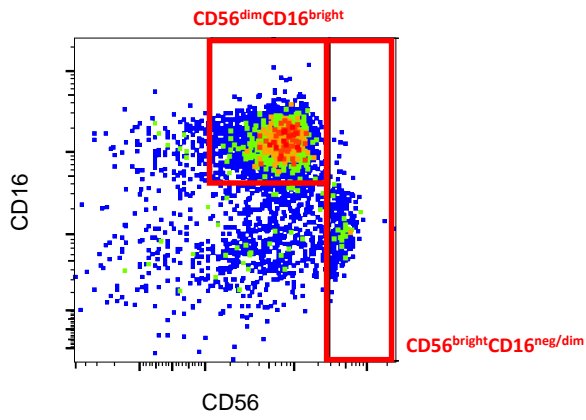
**Figure S3. Correlations between DBL2-binding capacity of IgG Abs and expression of activation markers by NK cells.** DBL2-binding capacity of IgG1, IgG2, IgG3 and IgG4 Abs from pregnant women with non-placental malaria (N=27; blue) and placental (N=50; red) was measured in multiplex assays. Spearman's rank correlation coefficients ( $\rho$  values and p-values) between antigen binding and expression of CD107a, IFN $\gamma$  and TNF $\alpha$  by Ab-activated NK cells are shown.

# Figure S4



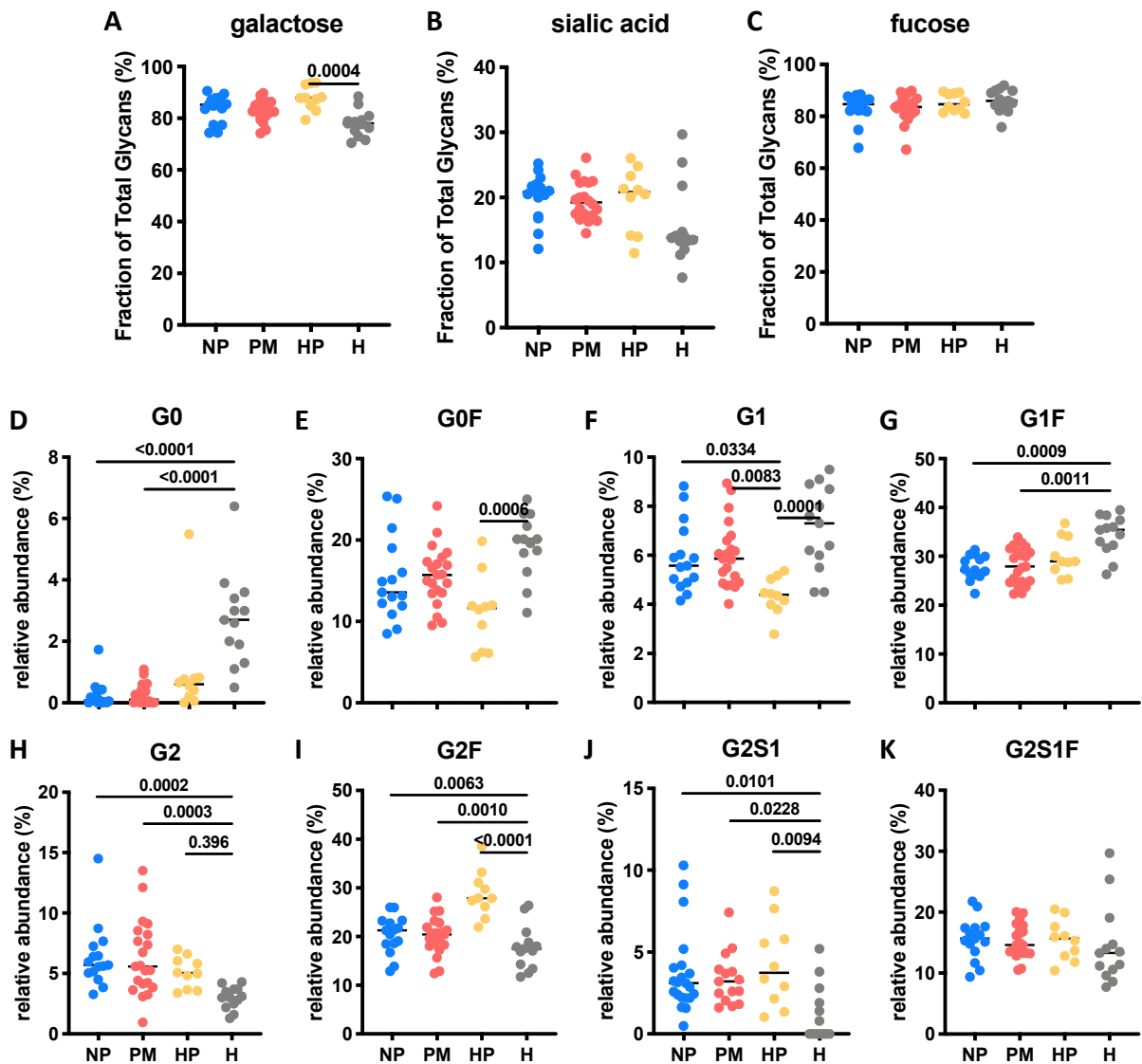
**Figure S4. Correlations between DBL3-binding capacity of IgG Abs and expression of activation markers by NK cells.** DBL3-binding capacity of IgG1, IgG2, IgG3 and IgG4 Abs from pregnant women with non-placental malaria (N=27; blue) and placental (N=50; red) was measured in multiplex assays. Spearman's rank correlation coefficients ( $\rho$  values and p-values) between antigen binding and expression of CD107a, IFN $\gamma$  and TNF $\alpha$  by Ab-activated NK cells are shown.

## Figure S5



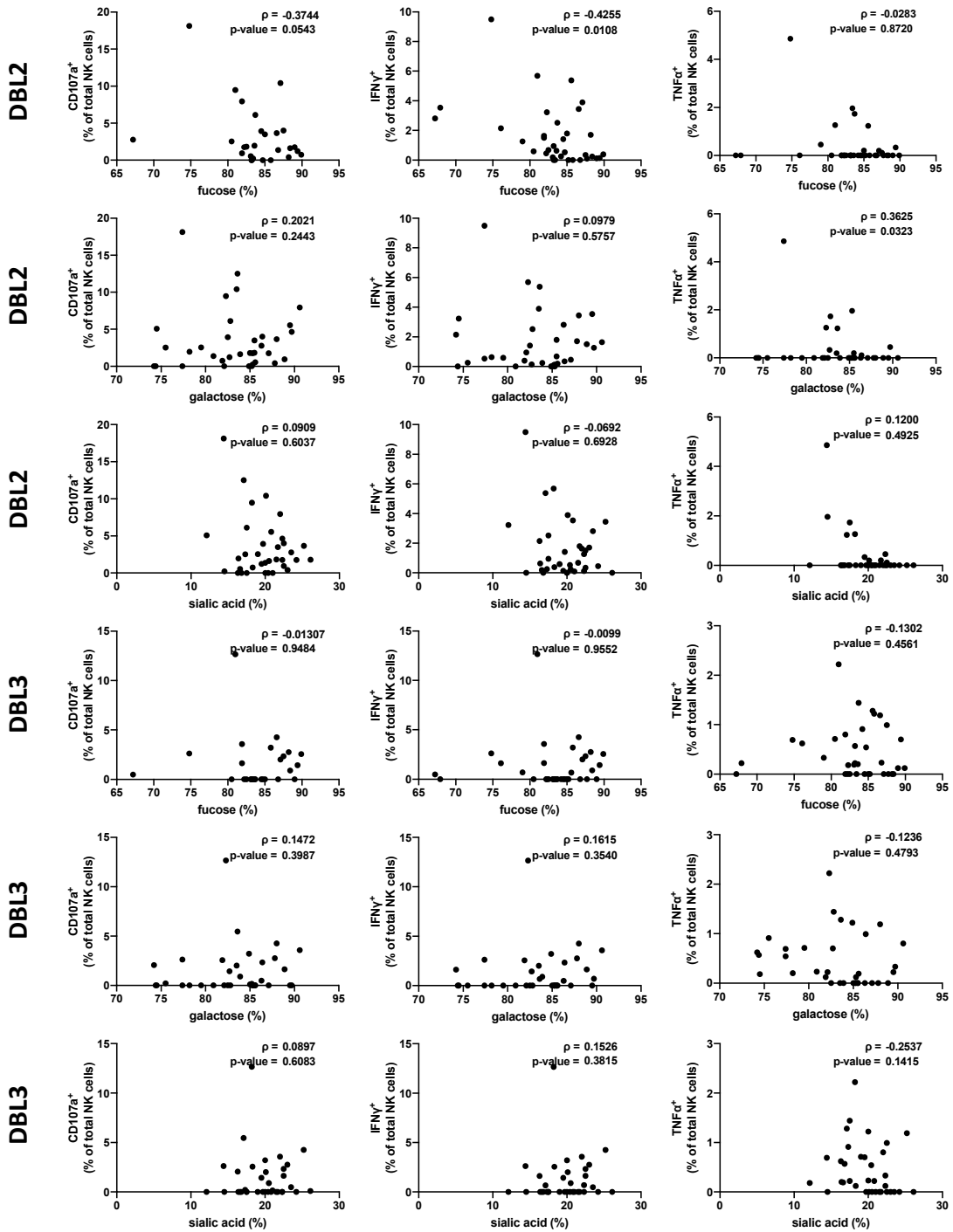
**Figure S5.** Subpopulations of NK cells selected for polyfunctional analysis based on the relative expression of CD56 and CD16.  $CD56^{dim}CD16^{bright}$  were gated;  $CD56^{bright}CD16^{neg}$  and  $CD56^{bright}CD16^{dim}$  were combined due to low cell numbers.

## Figure S6



**Figure S6. Glycan profiles of IgG Abs in pregnant women with malaria.** Specific types of *N*-linked glycan structures of purified IgG Abs from pregnant women were profiled based on clinical outcome: non-placental malaria (NP; N=21; blue) and placental malaria (PM; N=15; red) in comparison to malaria-naïve healthy pregnant women (HP; N=10; yellow) and uninfected healthy non-pregnant women (H; N=13; grey). **(A-C)** Fraction of **(A)** galactose, **(B)** sialic acid and **(C)** fucose of total glycans are shown. **(D-K)** The relative prevalences of several major glycan patterns are graphed (G0, agalactosylated; G1, monogalactosylated; G2, digalactosylated; F, fucosylated; S1, sialylated). Statistical comparison between groups was performed using a Kruskal-Wallis test corrected for multiple comparisons using Dunn's multiple comparison method (p-values are shown on graphs).

# Figure S6



**Figure S6. Correlations between DBL2- and DBL3-specific Ab-mediated NK cell activation and IgG N-linked glycosylation patterns of malaria-exposed pregnant women.** Spearman's rank correlation coefficients ( $\rho$  values and p-values) of DBL2- and DBL3-specific Ab-mediated expression of activation markers (CD107a, IFN $\gamma$  and TNF $\alpha$ ) and IgG N-linked glycosylation patterns (fucose, galactose and sialic acid) from pregnant women exposed to malaria are shown.