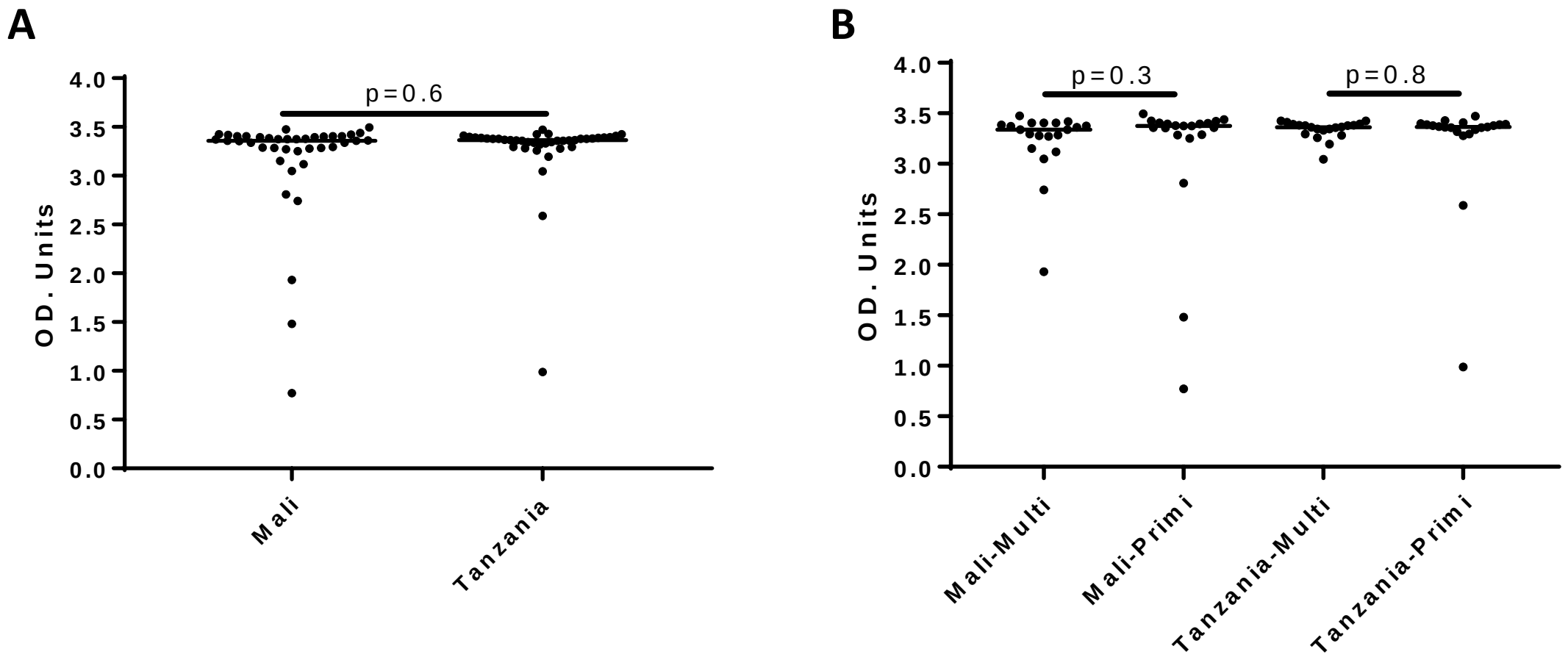
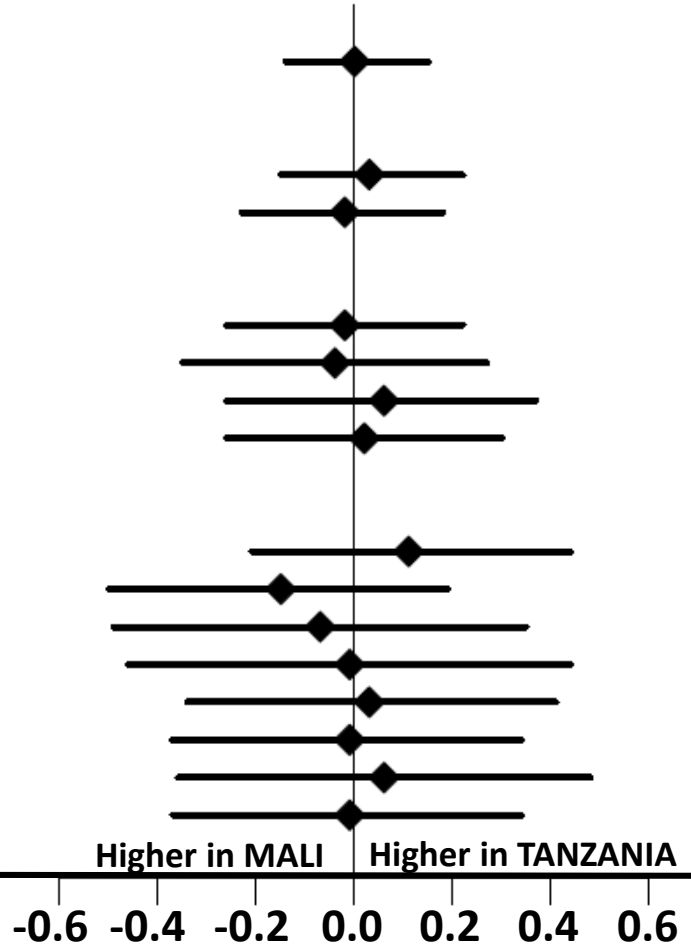
Supplemental Figure 1



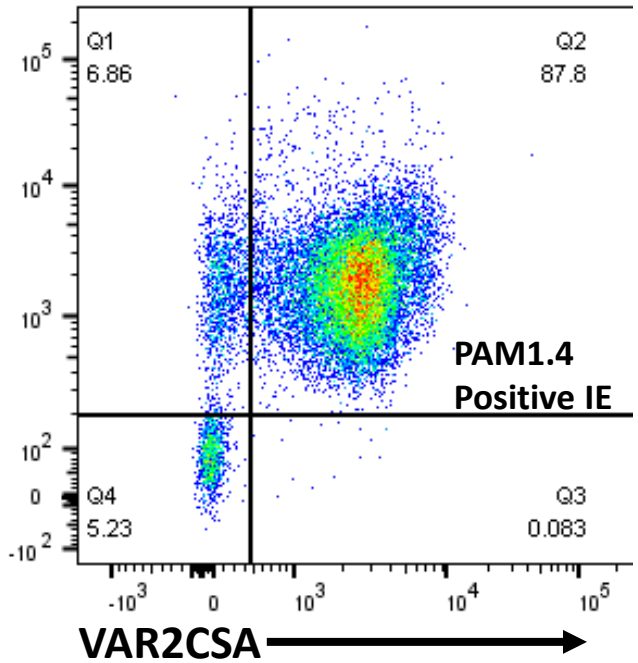
Supplemental Figure 2

	MALI N, Mean (SD)	TANZANIA N, Mean (SD)	Mean Difference (95% CI)	P-value
All VAR2CSA Data				
	160, 0.78 (0.59)	160, 0.79 (0.69)	0 (-0.14, 0.15)	0.9
Stratify by Parity				
Primigravid	80, 0.59 (0.52)	80, 0.63 (0.66)	0.03 (-0.15, 0.22)	0.7
Multigravid	80, 0.97 (0.61)	80, 0.95 (0.69)	-0.02 (-0.23, 0.18)	0.8
Stratify by Domain				
ID1-ID2a	40, 0.64 (0.49)	40, 0.62 (0.57)	-0.02 (-0.26, 0.22)	0.9
DBL3	40, 0.88 (0.64)	40, 0.84 (0.76)	-0.04 (-0.35, 0.27)	0.8
DBL4	40, 0.80 (0.64)	40, 0.86 (0.77)	0.06 (-0.26, 0.37)	0.7
DBL5	40, 0.81 (0.59)	40, 0.83 (0.66)	0.02 (-0.26, 0.30)	0.9
By Domain and Parity				
ID1-ID2a Primi	20, 0.45 (0.22)	20, 0.57 (0.67)	0.11 (-0.21, 0.44)	0.5
ID1-ID2a Multi	20, 0.82 (0.61)	20, 0.67 (0.46)	-0.15 (-0.50, 0.19)	0.4
DBL3 Primi	20, 0.68 (0.63)	20, 0.62 (0.67)	-0.07 (-0.49, 0.35)	0.7
DBL3 Multi	20, 1.08 (0.60)	20, 1.07 (0.78)	-0.01 (-0.46, 0.44)	0.9
DBL4 Primi	20, 0.61 (0.49)	20, 0.64 (0.66)	0.03 (-0.34, 0.41)	0.9
DBL4 Multi	20, 0.99 (0.49)	20, 0.98 (0.61)	-0.01 (-0.37, 0.34)	0.9
DBL5 Primi	20, 0.63 (0.63)	20, 0.69 (0.69)	0.06 (-0.36, 0.48)	0.8
DBL5 Multi	20, 0.99 (0.49)	20, 0.98 (0.61)	-0.01 (-0.37, 0.34)	0.9



Supplemental Figure 3

A



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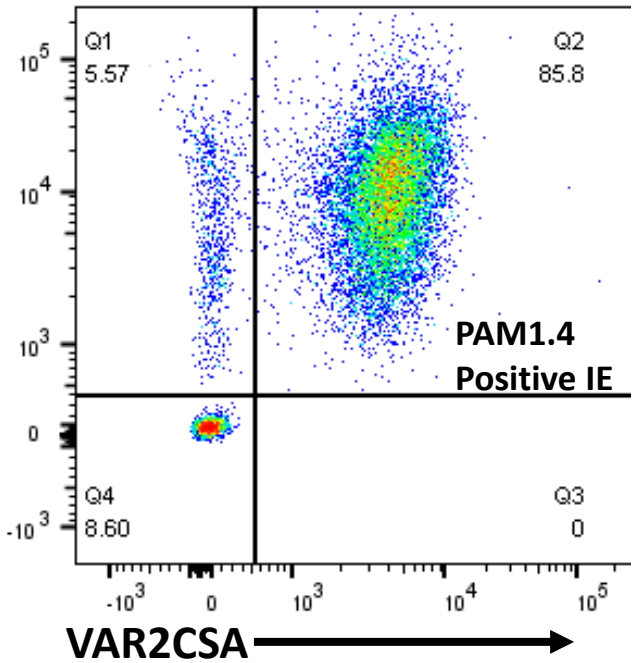
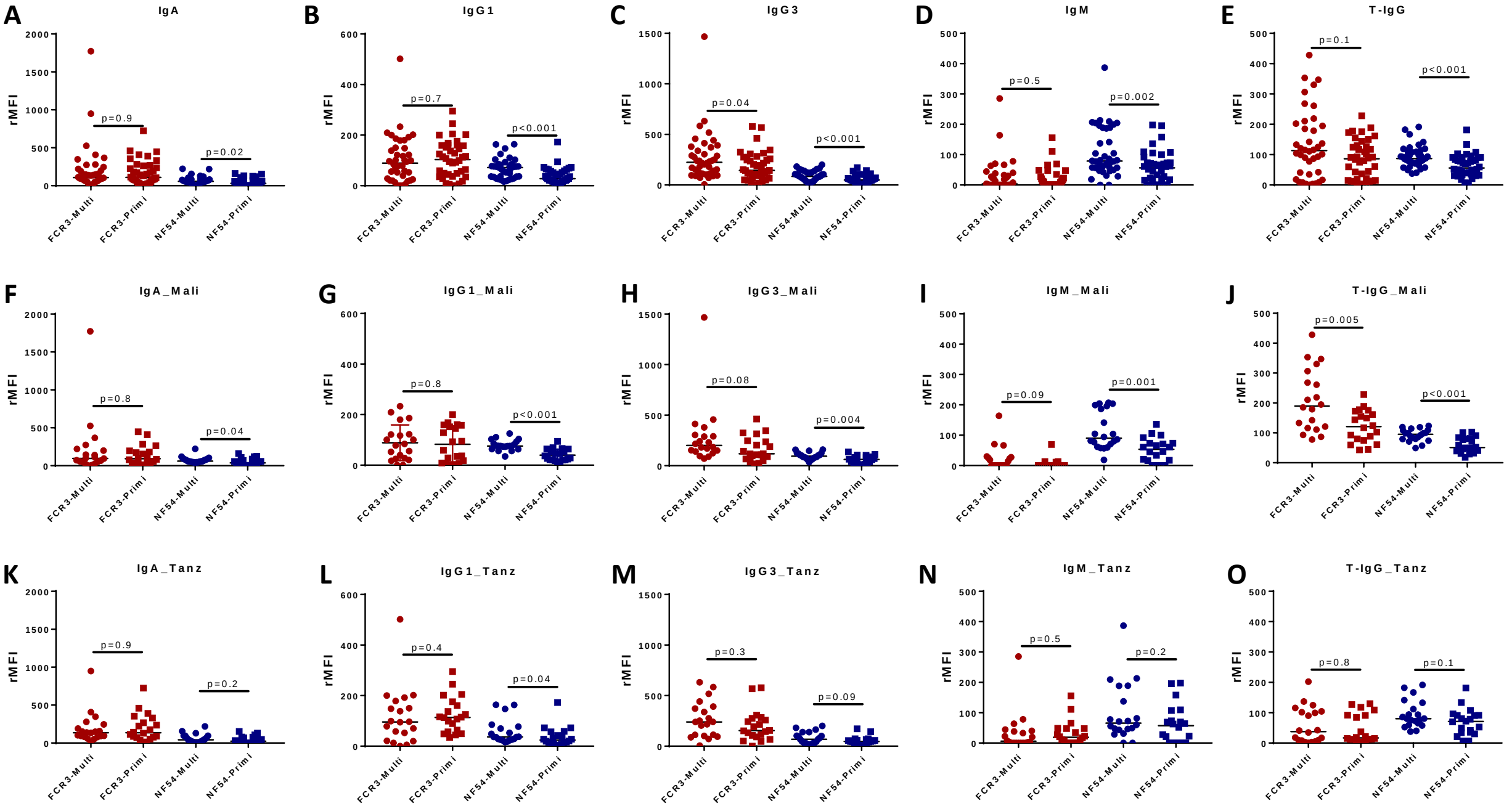
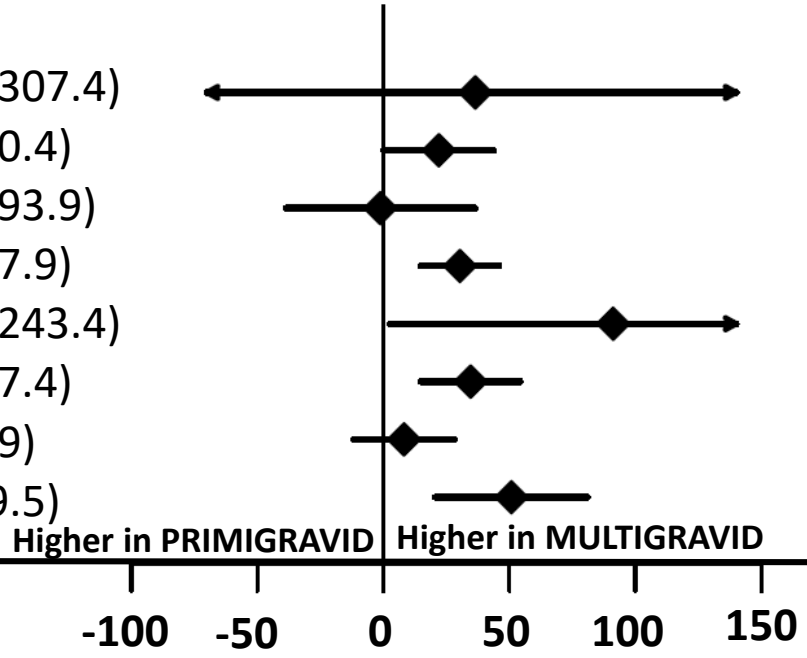


Supplemental Figure 4



Supplemental Figure 5

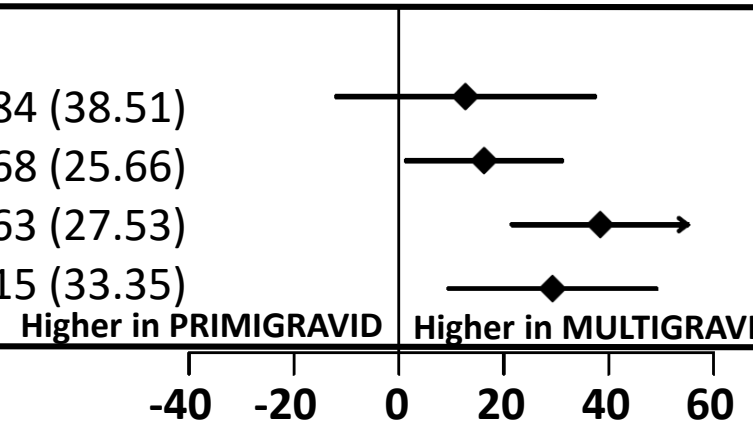
	PRIMIGRAVID N, Mean (SD)	MULTIGRAVID N, Mean (SD)	Mean Difference (95% CI)	P-value
Ig Type & Parasite				
IgA FCR3	40, 166.6 (158.9)	40, 203.1 (307.4)	36.5 (-73, 146)	0.5
IgA NF54	40, 45.3 (47.1)	40, 67.3 (50.4)	22.0 (0.2, 43.7)	0.05
IgG1 FCR3	40, 103.5 (72.8)	40, 102.4 (93.9)	-1.2 (-38.6, 36.3)	0.9
IgG1 NF54	40, 37.7 (31.7)	40, 68.1 (37.9)	30.4 (14.8, 45.9)	<0.001
IgG3 FCR3	40, 182.3 (138.5)	40, 273.5 (243.4)	91.2 (2.7, 179.8)	0.04
IgG3 NF54	40, 54.9 (40.7)	40, 89.5 (47.4)	34.6 (14.9, 54.2)	0.001
IgM FCR3	40, 18.8 (33.5)	40, 27 (52.9)	8.2 (-11.7, 28.0)	0.4
IgM NF54	40, 56.2 (52.9)	40, 107 (79.5)	50.8 (20.7, 80.9)	0.001



Supplemental Figure 6

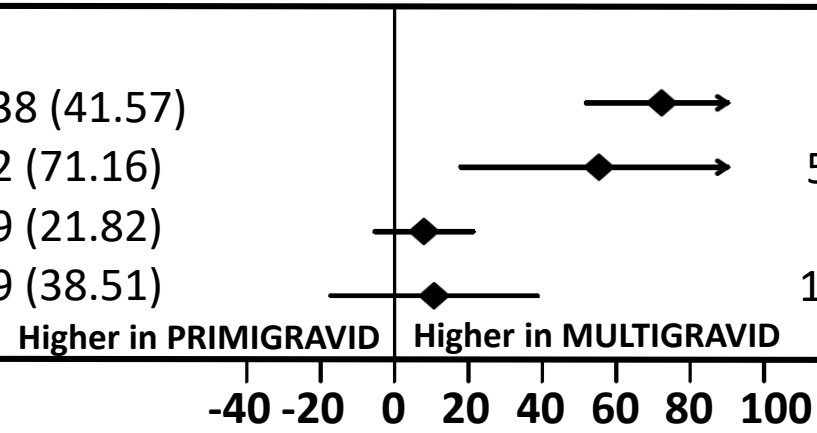
A. Comparison of Binding Inhibition: by Parity

	PRIMIGRAVID N, Mean (SD)	MULTIGRAVID N, Mean (SD)	Mean Difference (95% CI)	P-value
By Site and Parasite				
Mali FCR3	20, 49.14 (38.28)	20, 61.84 (38.51)	12.7 (-11.88, 37.28)	0.3
TZ FCR3	20, 12.43 (20.06)	20, 28.68 (25.66)	16.25 (1.47, 31.02)	0.03
Mali NF54	20, 30.23 (24.67)	20, 68.63 (27.53)	38.4 (21.66, 55.14)	< 0.001
TZ NF54	20, 21.85 (27.83)	20, 51.15 (33.35)	29.3 (9.62, 48.98)	0.005

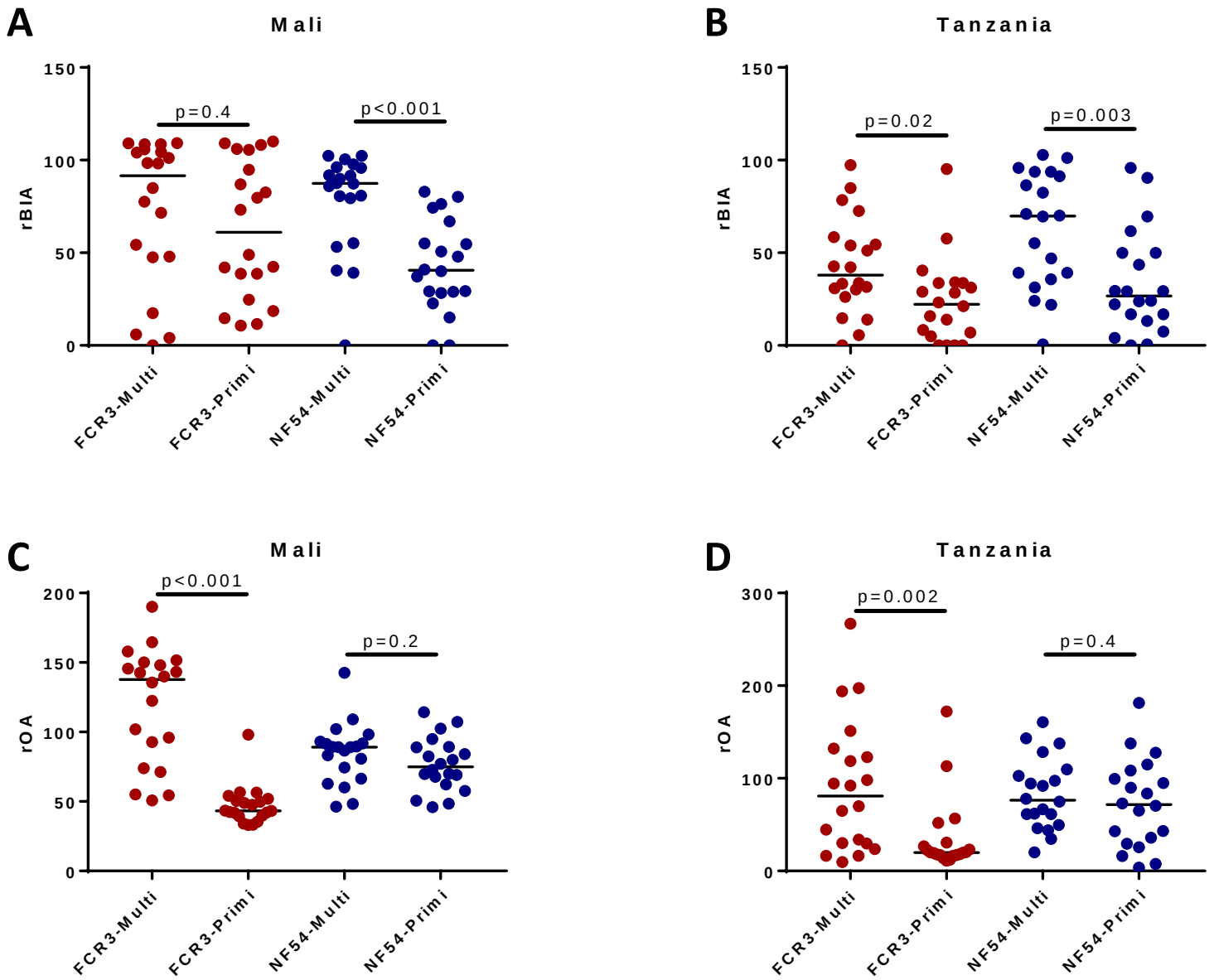


B. Comparison of Opsonizing Activity: by Parity

	PRIMIGRAVID N, Mean (SD)	MULTIGRAVID N, Mean (SD)	Mean Difference (95% CI)	P-value
By Site and Parasite				
Mali FCR3	20, 47.08 (14.09)	20, 119.38 (41.57)	72.3 (52.02, 92.59)	< 0.001
TZ FCR3	20, 35.01 (39.79)	20, 90.32 (71.16)	55.31 (18.07, 92.56)	0.005
Mali NF54	20, 76.74 (19.2)	20, 84.69 (21.82)	7.95 (-5.21, 21.11)	0.2
TZ NF54	20, 72.5 (47.68)	20, 83.19 (38.51)	10.69 (-17.09, 38.48)	0.4

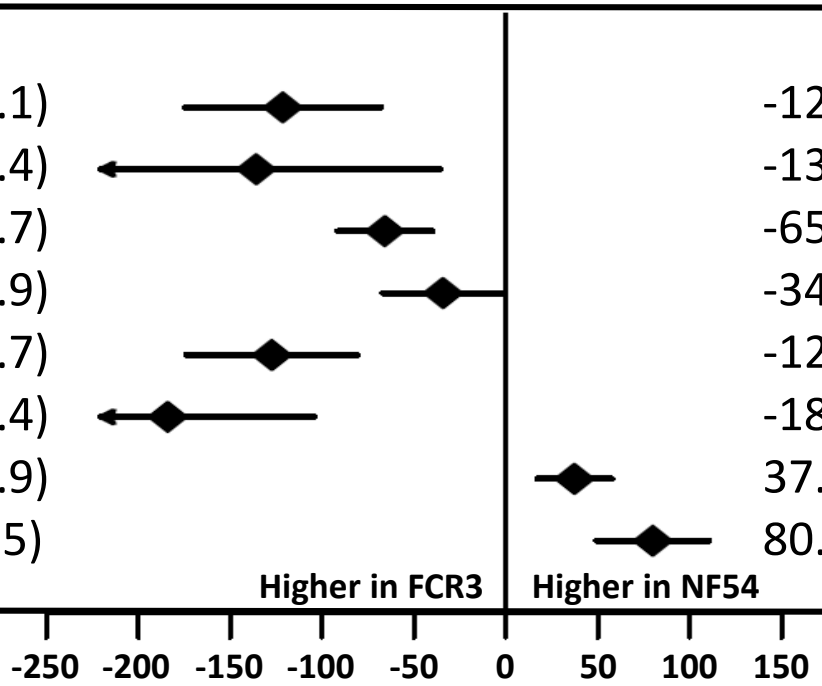


Supplemental Figure 7



Supplemental Figure 8

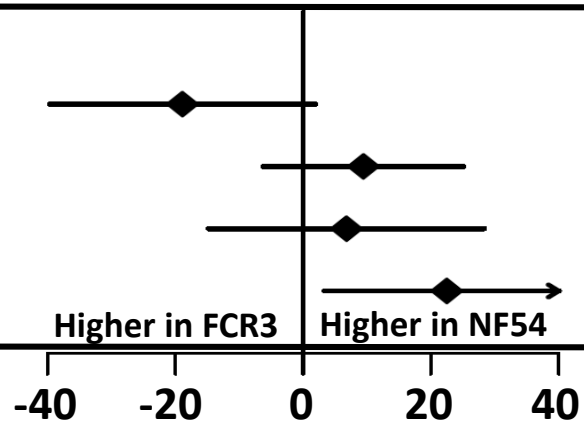
	FCR3 N, Mean (SD)	NF54 N, Mean (SD)	Mean Difference (95% CI)	P-value
By Ig Type & Parity				
IgA Primi	40, 166.6 (158.9)	40, 45.3 (47.1)	-121.3 (-174, -68.5)	< 0.0001
IgA Multi	40, 203.1 (307.4)	40, 67.3 (50.4)	-135.8 (-235.3, -36.4)	0.009
IgG1 Primi	40, 103.5 (72.8)	40, 37.7 (31.7)	-65.8 (-90.9, -40.6)	< 0.0001
IgG1 Multi	40, 102.4 (93.9)	40, 68.1 (37.9)	-34.2 (-66.4, -2.1)	0.04
IgG3 Primi	40, 182.3 (138.5)	40, 54.9 (40.7)	-127.3 (-173.3, -81.3)	< 0.0001
IgG3 Multi	40, 273.5 (243.4)	40, 89.5 (47.4)	-184 (-263.1, -104.9)	< 0.0001
IgM Primi	40, 18.8 (33.5)	40, 56.2 (52.9)	37.4 (17.6, 57.2)	0.0004
IgM Multi	40, 27 (52.9)	40, 107 (79.5)	80.0 (49.9, 110.1)	< 0.0001



Supplemental Figure 9

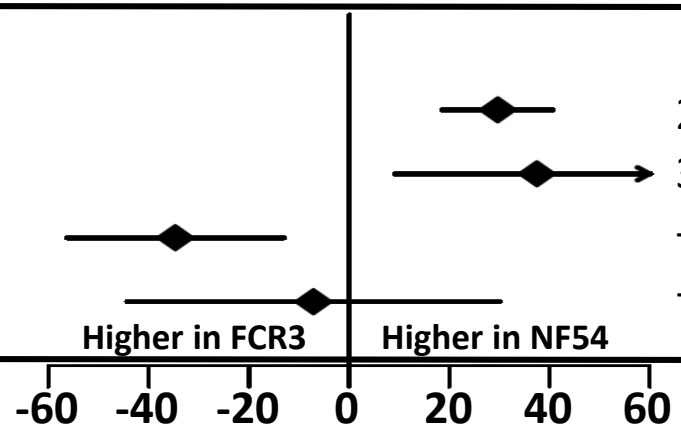
A. Comparison of Binding Inhibition

	FCR3 N, Mean (SD)	NF54 N, Mean (SD)	Mean Difference (95% CI)	P-value
By Site and Parity				
Mali Primi	20, 49.14 (38.28)	20, 30.23 (24.67)	-18.91 (-39.64, 1.82)	0.07
TZ Primi	20, 12.43 (20.06)	20, 21.85 (27.83)	9.42 (-6.16, 25.00)	0.2
Mali Multi	20, 61.84 (38.51)	20, 68.63 (27.53)	6.78 (-14.72, 28.29)	0.5
TZ Multi	20, 28.68 (25.66)	20, 51.15 (33.35)	22.48 (3.39, 41.57)	0.02

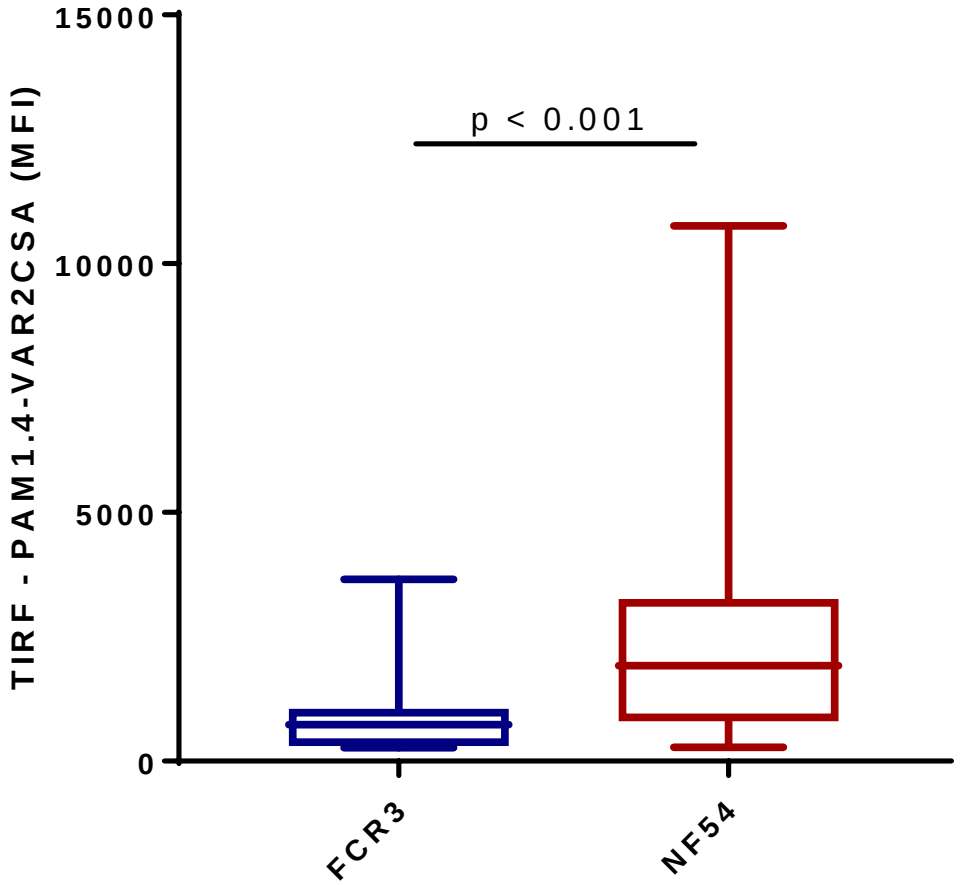


B. Comparison of Opsonizing Activity

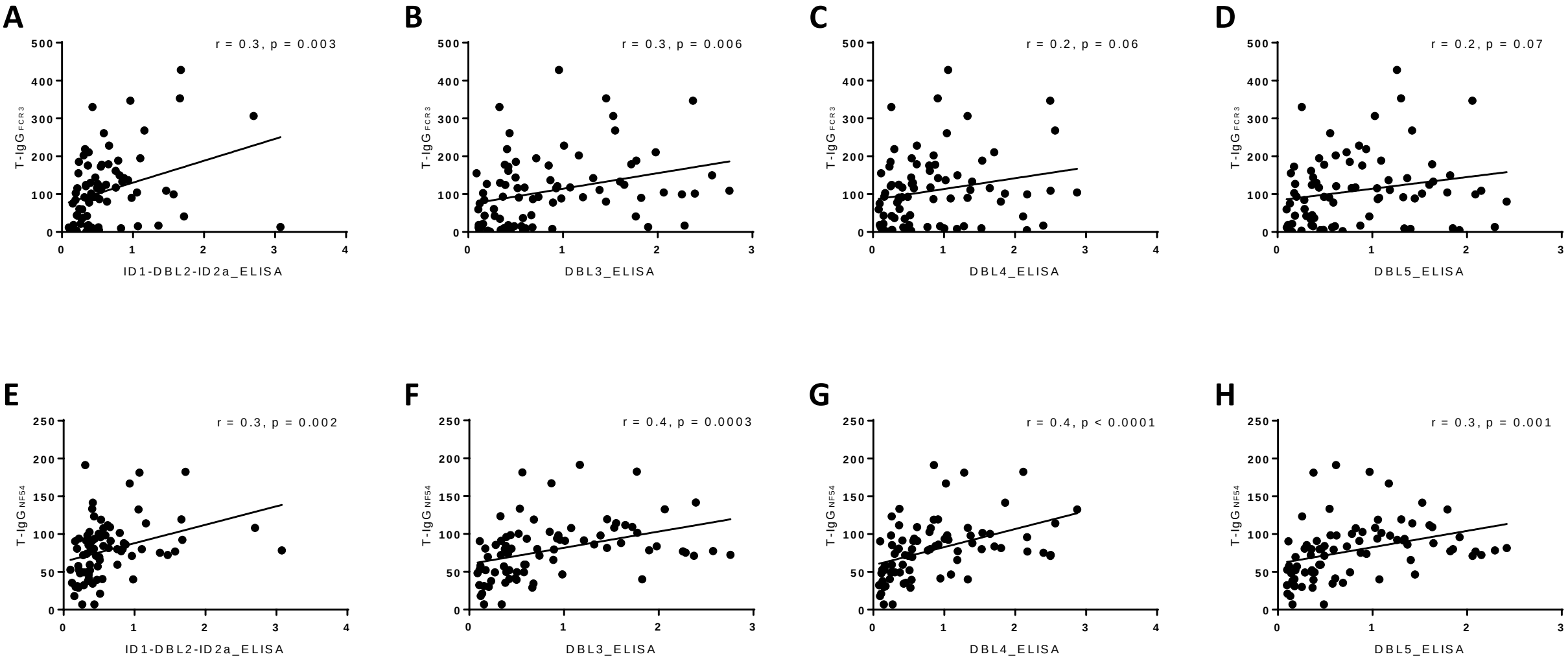
	FCR3 N, Mean (SD)	NF54 N, Mean (SD)	Mean Difference (95% CI)	P-value
By Site and Parity				
Mali Primi	20, 47.08 (14.09)	20, 76.74 (19.2)	29.66 (18.85,40.47)	< 0.0001
TZ Primi	20, 35.01 (39.79)	20, 72.5 (47.68)	37.49 (9.35,65.63)	0.01
Mali Multi	20, 119.38 (41.57)	20, 84.69 (21.82)	-34.69 (-56.17,-13.21)	0.003
TZ Multi	20, 90.32 (71.16)	20, 83.19 (38.51)	-7.13 (-44.12,29.86)	0.7



Supplemental Figure 10



Supplemental Figure 11



Supplemental Figure Legends

Supplemental Figure 1. ELISA reactivity of plasma from Malian and Tanzanian pregnant women to recombinant AMA-1. (A) Site comparison of anti-AMA-1 antibody titer as optical density (OD) units and a stratification by parity (B) are shown. Mann-Whitney test was performed for significance and p-values are reported. Primi = Primigravid women; Multi = multigravid women. Bars indicates the median.

Supplemental Figure 2. Forest plot of mean difference for IgG reactivity to VAR2CSA recombinants between pregnant women from Mali and Tanzania. Mean difference with 95% confidence interval (CI) of IgG reactivity to VAR2CSA in Mali and Tanzania is plotted. Primi = Primigravid women; Multi = multigravid women; N = number of samples; SD = standard deviation.

Supplemental Figure 3. Surface expression of VAR2CSA by FCR3 and NF54 isolates. Magnetically enriched trophozoites/schizonts *P. falciparum* infected erythrocytes (IE) of (A) FCR3 and (B) NF54 isolates were analysed by Flow cytometry for VAR2CSA expression on the surface of the IE using PAM1.4 monoclonal antibody. Approximatively 93% and 94% respectively of FCR3 and NF54 IE expressed VAR2CSA as shown by proportion of PAM1.4 stained cells.

Supplemental Figure 4. Plasma immunoglobulins reactivity on FCR3 and NF54 isolates. In multigravidae (multi) and primigravidae (primi), levels of immunoglobulins binding to both parasites were analysed by Flow cytometry and compared. The comparisons of each immunoglobulin type irrespective to the origin of the samples (panels **A – E**) and the stratified

analysis for Mali (panels **D – J**) and Tanzania (Tanz) (panels **H – O**) are shown. Mann-Whitney test was performed for significance and p-values are reported. Bars indicates the median.

Supplemental Figure 5. Forest plot of mean difference for reactivity of plasma antibodies by parity, and stratified by immunoglobulin type and parasite variant. Mean difference with 95% confidence interval (CI) of different type immunoglobulin (Ig) reactivity to FCR3 and NF54 in primigravid and multigravid women analysed by Flow cytometry, is plotted. N = number of samples; SD = standard deviation.

Supplemental Figure 6. Forest plot of mean difference for function of plasma antibodies by parity, and stratified by site and parasite variant. Mean difference with 95% confidence interval (CI) of primigravid and multigravid women plasma blocking **(A)** and opsonizing **(B)** activities against FCR3 and NF54 parasites in Mali and Tanzania (Tanz) is plotted. N = number of samples; SD = standard deviation.

Supplemental Figure 7. Function of plasma antibodies by parity and study sites. The levels of Binding inhibition (rBIA) and opsonizing (rOA) activities of the plasma against the both parasites were assessed. Levels of rBIA in multigravidae (multi) and primigravidae (primi) from **(A)** Mali and **(B)** Tanzania as well as rOA of plasma from **(C)** Mali and **(D)** Tanzania are shown. Mann-Whitney test was performed for significance and p-values are reported. Bars indicates the median.

Supplemental Figure 8. Forest plot of mean difference for reactivity of plasma antibodies by parasite variant, and stratified by immunoglobulin type and parity. Mean difference with 95% confidence interval (CI) of different type immunoglobulin (Ig) reactivity to FCR3 and NF54 parasites is plotted. Primi = Primigravid women; Multi = multigravid women; N = number of samples; SD = standard deviation.

Supplemental Figure 9. Forest plot of mean difference for function of plasma antibodies by parasite variant, and stratified by site and parity. Mean difference with 95% confidence interval (CI) of plasma blocking **(A)** and opsonizing **(B)** activities against FCR3 and NF54 parasites in Mali and Tanzania (Tanz) is plotted. Primi = Primigravid women; Multi = multigravid women; N = number of samples; SD = standard deviation.

Supplemental Figure 10. Surface expression of VAR2CSA measured by TIRF. Horizontal lines indicate medians, boxes indicate interquartile ranges, and error bars indicate ranges. Mann-Whitney test was performed for significance and p-values are reported.

Supplemental Figure 11. Correlation between antibodies binding to recombinant and native VAR2CSA antigens. Relationships between the levels of IgG binding to surface antigens of CSA-binding FCR3 (**A – D**) and NF54 (**E – H**) IE measured by Flow cytometry and the level of IgG reactive to ID1-DBL2-ID2a, DBL3, DBL4 and DBL5 recombinants of $VAR2CSA_{FCR3}$ measured by ELISA are shown. Pearson's (r) coefficient of correlation and p-values are reported for each comparison.

Supplemental Table 1: Power estimates for opsonizing and binding-inhibition assays

	Differences by Parity						Differences by parasite variant							
	Opsonizing Assay			Binding-Inhibition assay			Opsonizing Assay				Binding-Inhibition assay			
Number per group	40	80	120	40	80	120	40	80	120	851	40	80	120	1254
Mean Difference	36.57	36.57	36.57	23.26	23.26	23.26	-6.33	-6.33	-6.33	-6.33	-3.87	-3.87	-3.87	-3.87
SD	46.59	46.59	46.59	34.54	34.54	34.54	46.59	46.59	46.59	46.59	34.54	34.54	34.54	34.54
Alpha	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Power	0.934	0.999	>.999	0.845	0.989	>.999	0.092	0.137	0.182	0.8	0.078	0.108	0.139	0.8

Footnote: For each marker, the power for observing a significant difference between primigravid and multigravid samples as well as the difference of those samples in assays comparing FCR3 vs. NF54 parasites, were calculated. This was run for the sample size in the current study, and the needed sample sizes to detect a meaningful difference (Power ≥ 0.8). The power is displayed at 40, 80, and 120 people per arm. If a marker did not achieve significance at 120 samples per arm, a row was added to calculate the sample size needed to achieve 0.80 power. SD = Standard Deviation