Supplemental Figure Legends

Figure S1. Pre-vaccine HI and MN titers in pregnant women, stratified by self-reported influenza vaccination history. A) pH1N1, B) H3N2/Victoria, C) B/Wisconsin. Groups were compared using a Mann-Whitney U test, * $p < 0.05$.

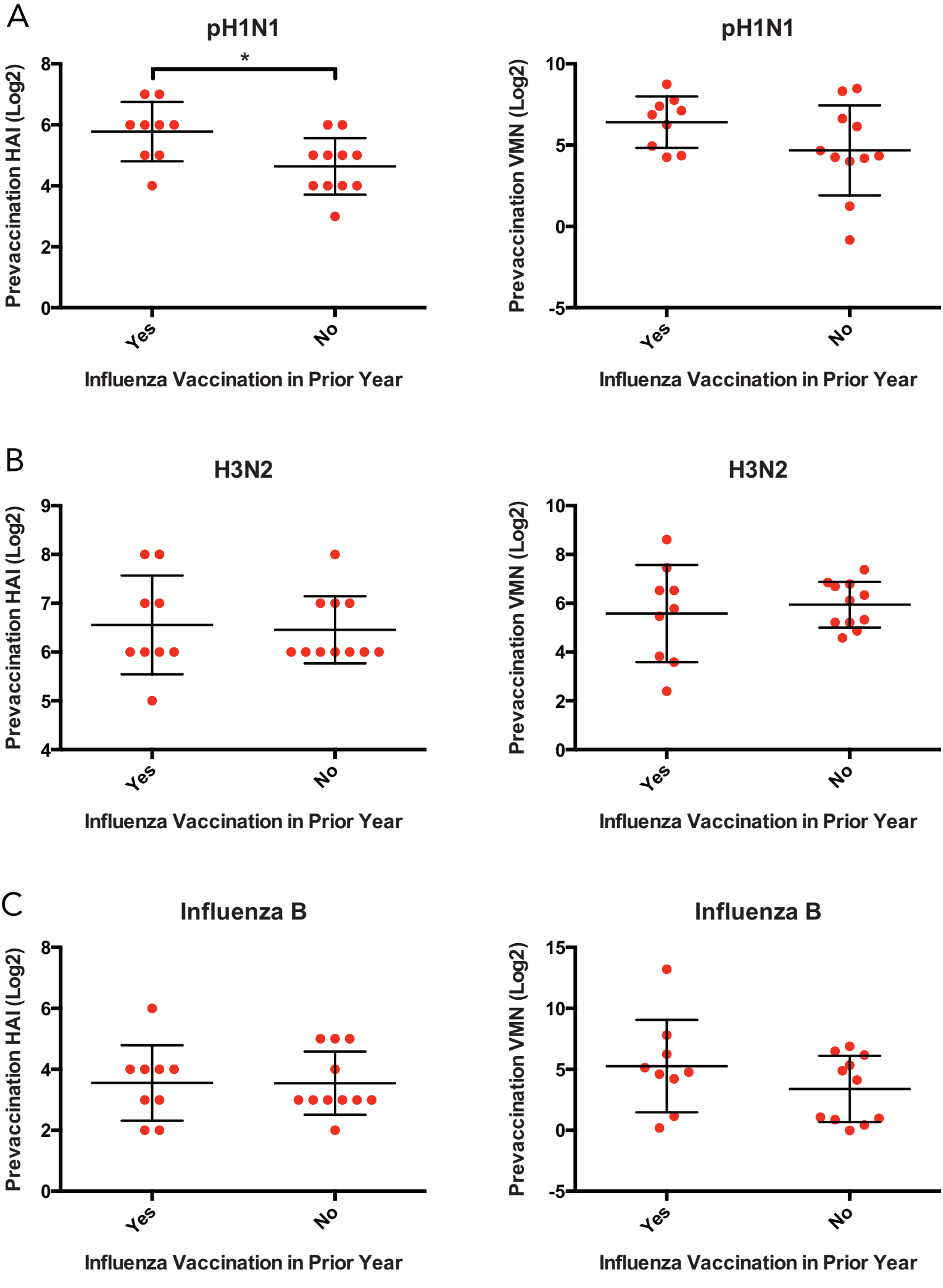
Figure S2. Graphical display of ANCOVA model used to analyze A) HI and B) MN data. ANCOVA was used to determine the relative contributions of baseline titer and pregnancy status on titer fold-change following IIV. Data is shown on a log2 scale, and 95% confidence intervals were determined by bootstrapping. P-values represent a test of the effect of pregnancy on GMR while controlling for baseline GMT, n.s. = not significant.

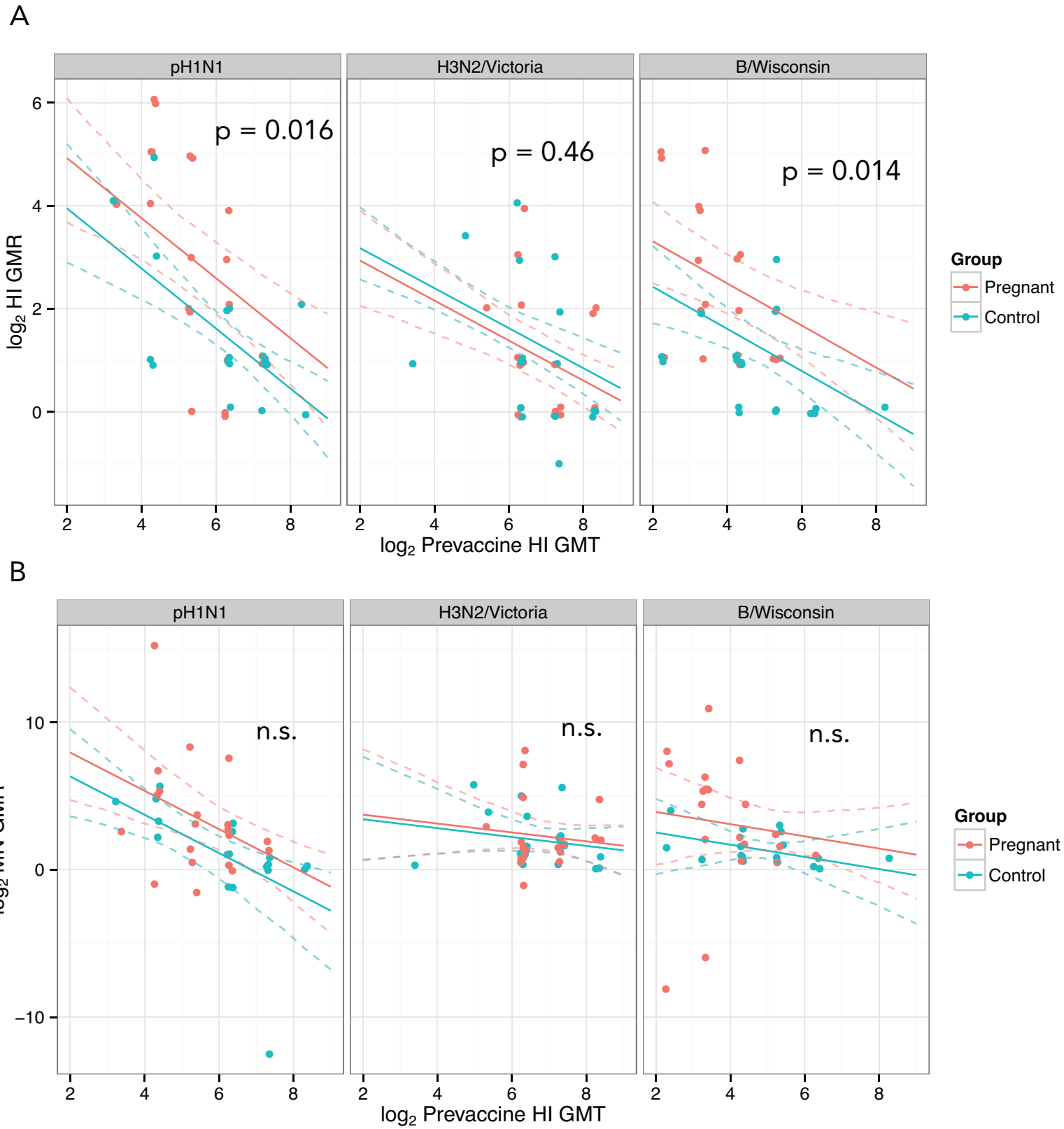
Figure S3. HI and MN titers correlate in pregnant and control women. HI and MN fold changes (post-vaccination titer/pre-vaccination titer) for A) A/H1N1/California/2009 (pH1N1), B) A/H3N2/Victoria/2011 and C) B/Wisconsin were compared using Spearman correlation testing in control (blue) and pregnant (red) women.

Figure S4. Total serum IgG by week of pregnancy. Correlation analysis revealed a trend towards lower IgG concentrations as pregnancy progresses in pre-vaccine samples. However, this effect was absent in the post-vaccination samples. P-values represent a t-test for a non-zero slope, and r is the Pearson correlation coefficient.

Figure S5. Gating strategy to define B cell and plasmablast populations using mass cytometry.

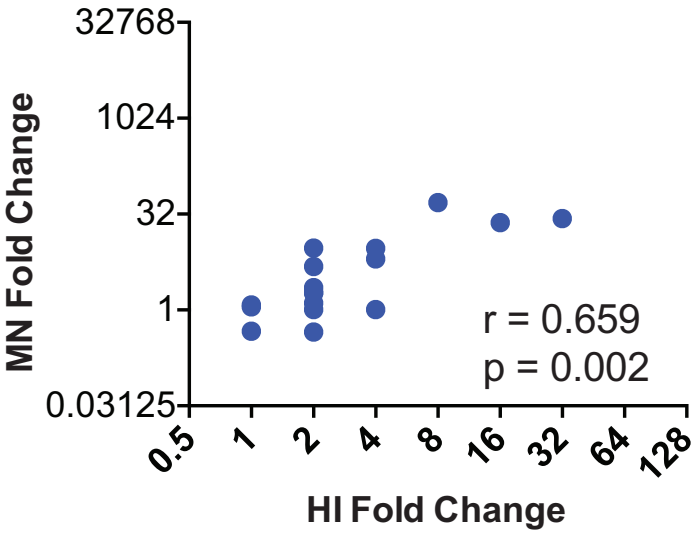
Figure S6. Plasmablast and HI correlations. Spearman correlations in control (blue) and pregnant (red) women comparing plasmablast fold change and average HI fold change for pH1N1, H3N2 and Influenza B were not significantly different.


A
pH1N1
*
Prevaccination HAI (Log2)
8
6
4
2
0
Yes
No
Influenza Vaccination in Prior Year
pH1N1
Prevaccination VMN (Log2)
10
5
0
-5
Yes
No
Influenza Vaccination in Prior Year
B
H3N2
Prevaccination HAI (Log2)
9
8
7
6
5
4
Yes
No
Influenza Vaccination in Prior Year
H3N2
Prevaccination VMN (Log2)
10
8
6
4
2
0
Yes
No
Influenza Vaccination in Prior Year
C
Influenza B
Prevaccination HAI (Log2)
8
6
4
2
0
Yes
No
Influenza Vaccination in Prior Year
Influenza B
Prevaccination VMN (Log2)
15
10
5
0
-5
Yes
No
Influenza Vaccination in Prior Year

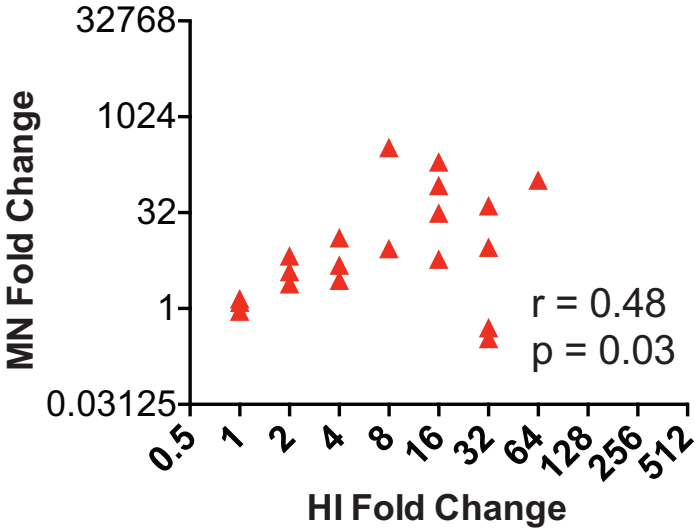

A
pH1N1
H3N2/Victoria
B/Wisconsin
p = 0.016
p = 0.46
p = 0.014
log2 HI GMR
log2 Prevaccine HI GMT
Group
Pregnant
Control
B
n.s.
n.s.
n.s.
log2 MN GMR
log2 Prevaccine HI GMT
Group
Pregnant
Control

A

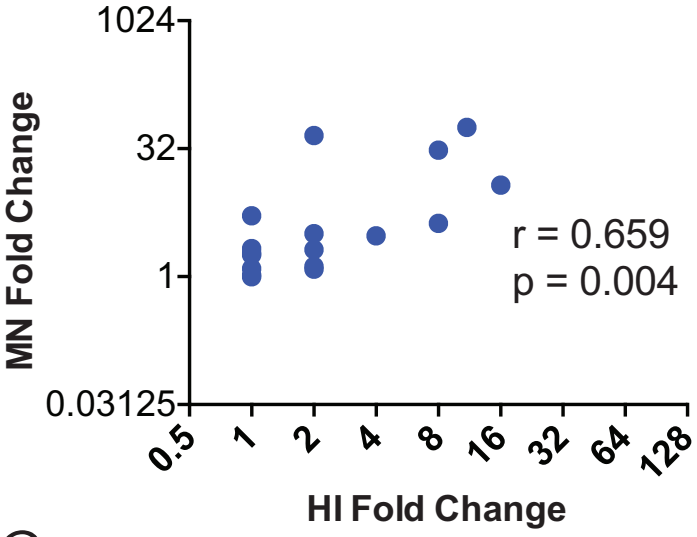
Control

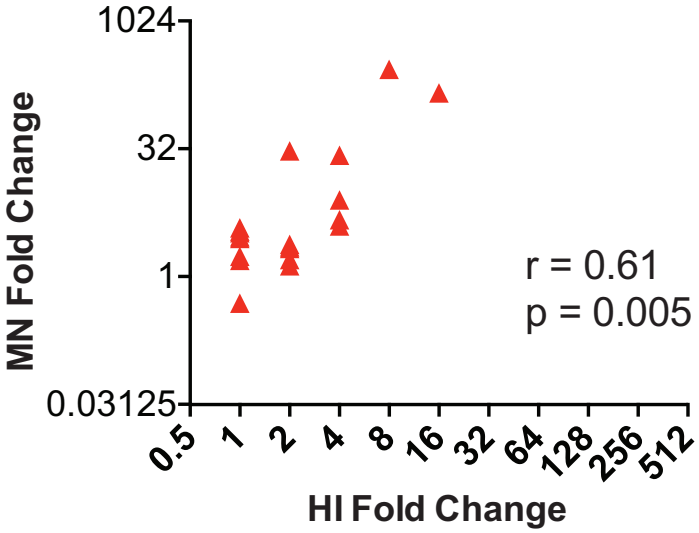


Pregnant

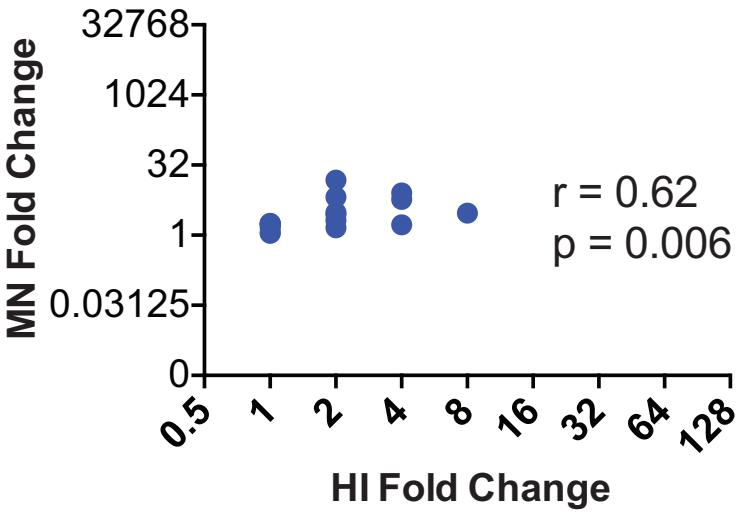


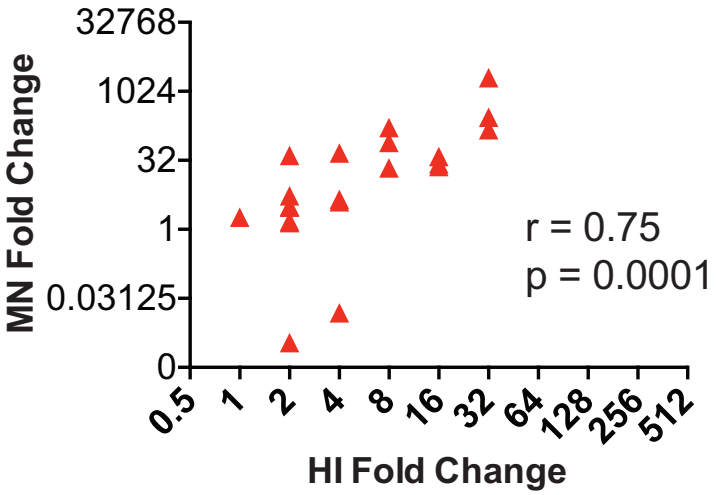
B

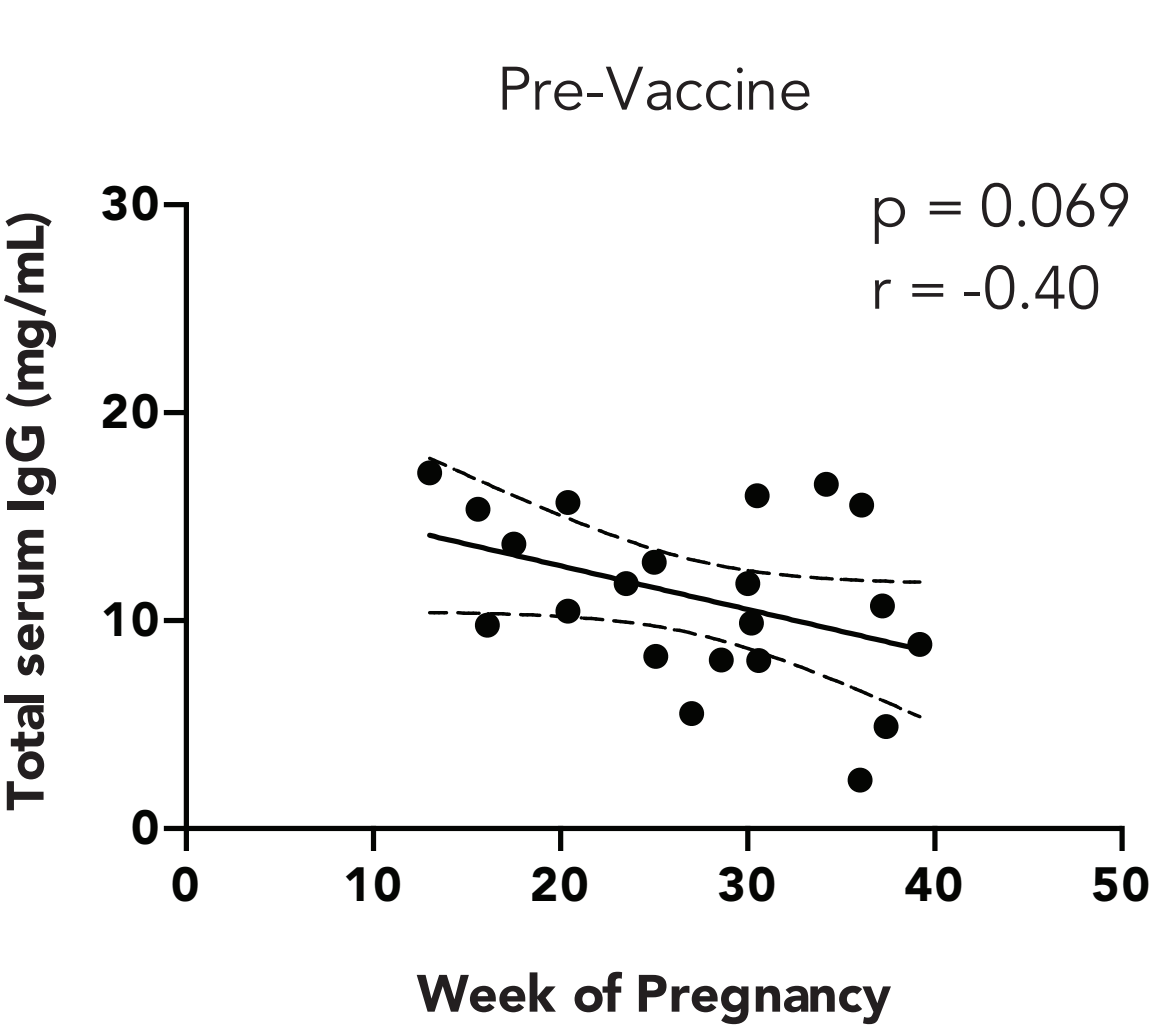


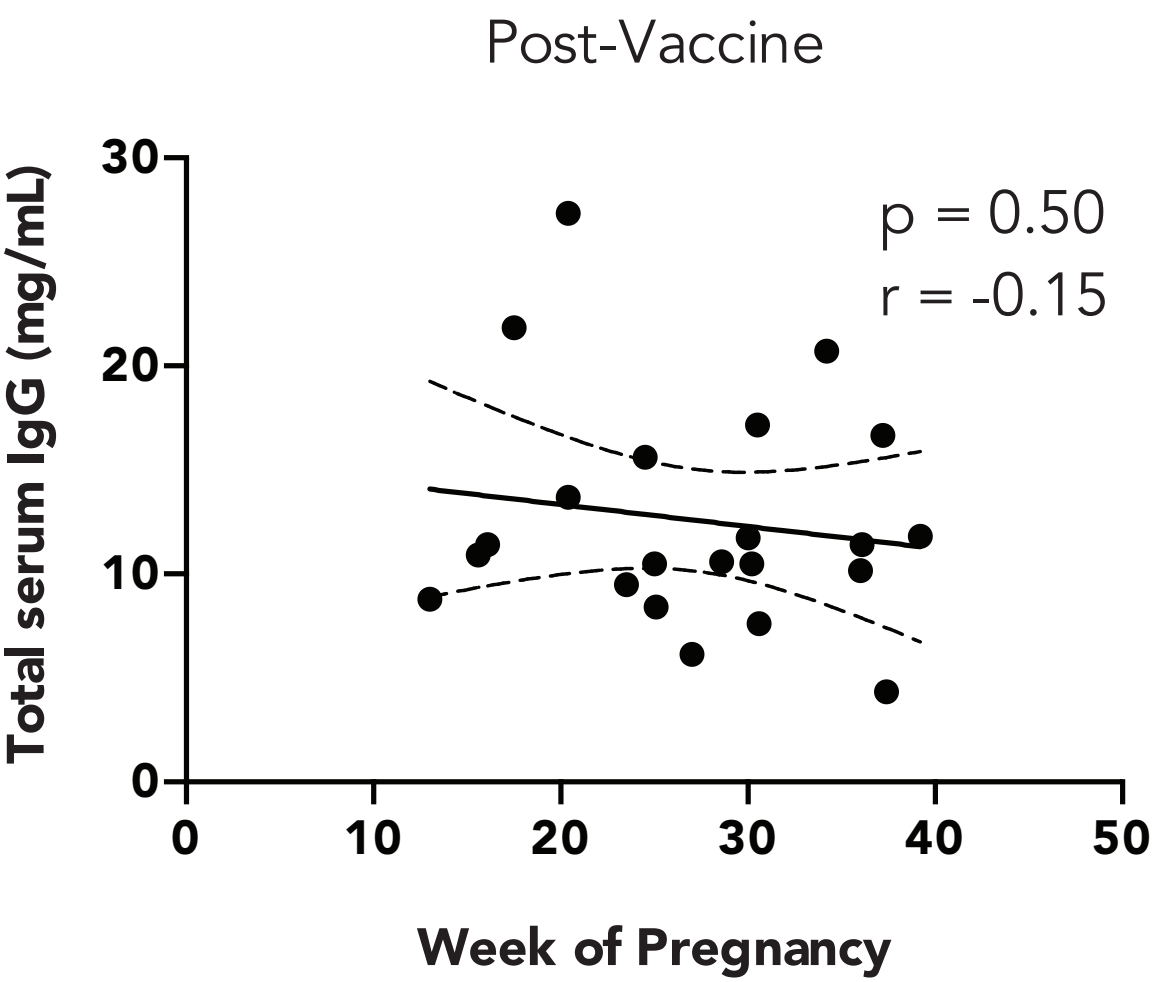


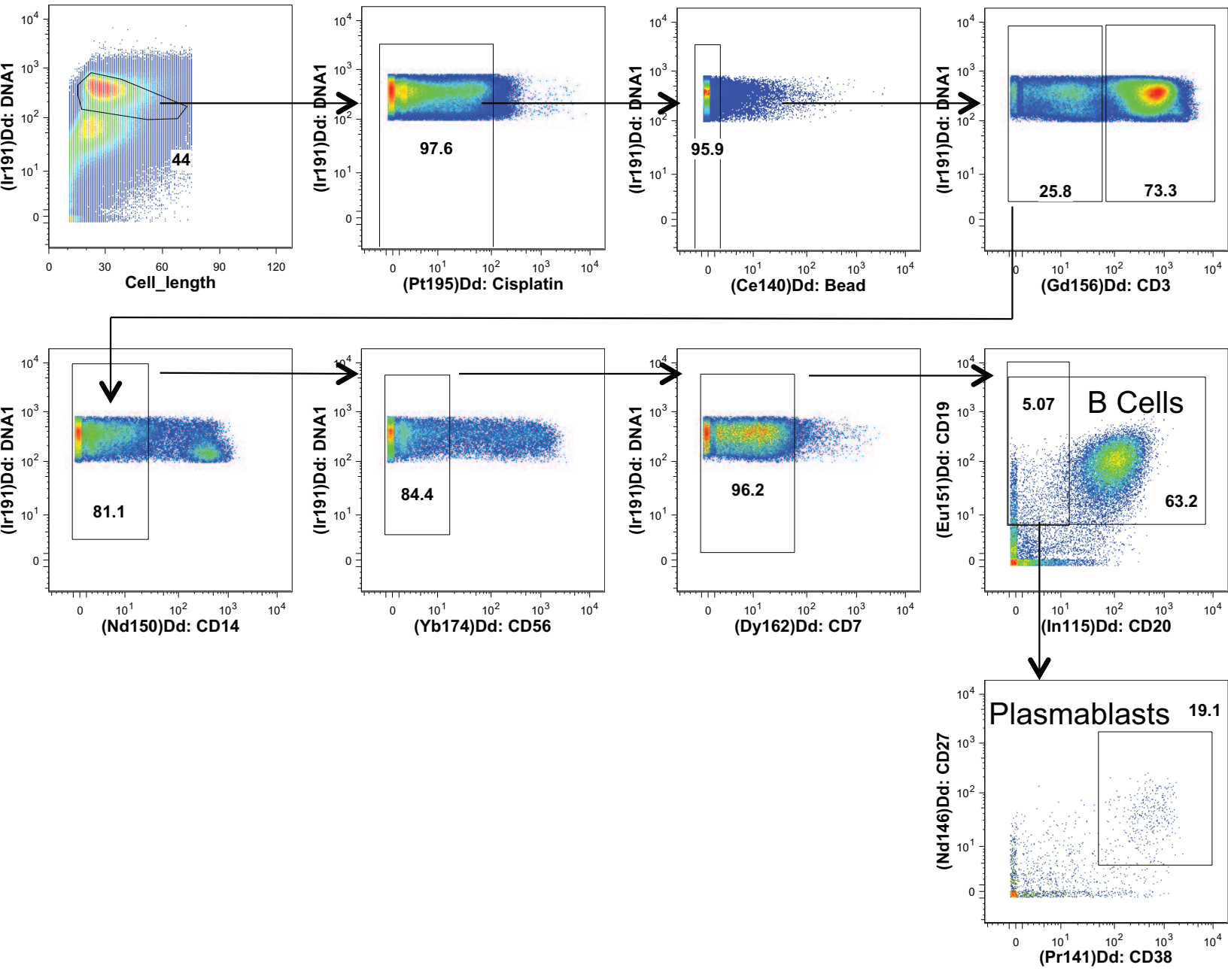
C

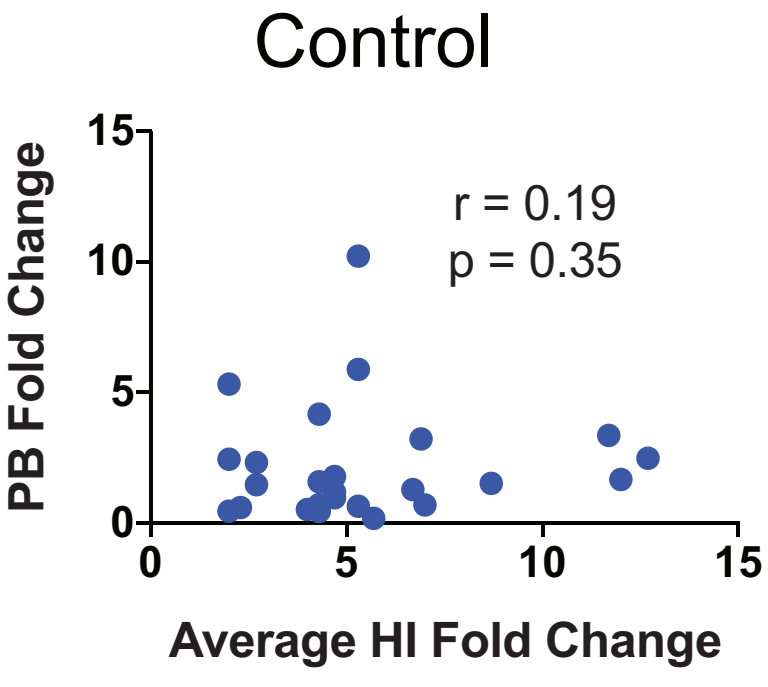





Pre-Vaccine
p = 0.069
r = -0.40
Total serum IgG (mg/mL)
0
10
20
30
0
10
20
30
40
50
Week of Pregnancy


Post-Vaccine
p = 0.50
r = -0.15
Total serum IgG (mg/mL)
0
10
20
30
0
10
20
30
40
50
Week of Pregnancy


(Ir191)Dd: DNA1
Cell_length
44
(Pt195)Dd: Cisplatin
97.6
(Ce140)Dd: Bead
95.9
(Gd156)Dd: CD3
25.8
73.3
(Nd150)Dd: CD14
81.1
(Yb174)Dd: CD56
84.4
(Dy162)Dd: CD7
96.2
(Eu151)Dd: CD19
(In115)Dd: CD20
5.07
B Cells
63.2
(Nd146)Dd: CD27
(Pr141)Dd: CD38
Plasmablasts
19.1


Control
PB Fold Change
Average HI Fold Change
r = 0.19
p = 0.35
0
5
10
15

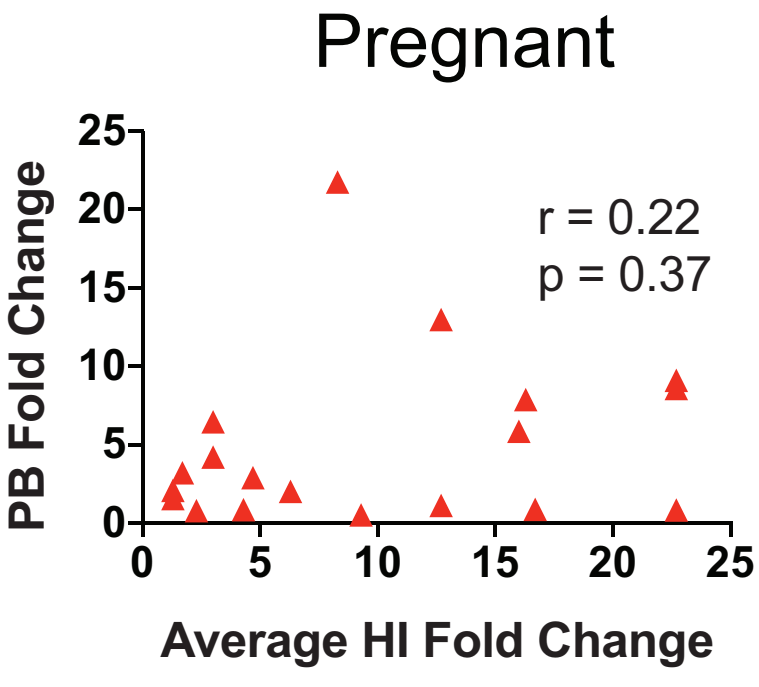

Pregnant
PB Fold Change
Average HI Fold Change
r = 0.22
p = 0.37
0
5
10
15
20
25

Table S1. Mass Cytometry antibody and isotope information for plasmablast identification

Isotope	Isotope Source	Antibody	Antibody Clone	Antibody Source
115 Ln	DVS Sciences	CD20	2H7	Biolegend
141 Pr	DVS Sciences	CD38	HIT2	Biolegend
146 Nd	DVS Sciences	CD27	O323	Biolegend
150 Nd	DVS Sciences	CD14	M5E2	Biolegend
151 Eu	DVS Sciences	CD19	HIB19	Biolegend
156 Gd	DVS Sciences	CD3	UCHT1	Biolegend
162 Dy	DVS Sciences	CD7	6B7	Biolegend
174 Yb	DVS Sciences	CD56	NCAM16.2	BD Biosciences