Supplemental Acknowledgements

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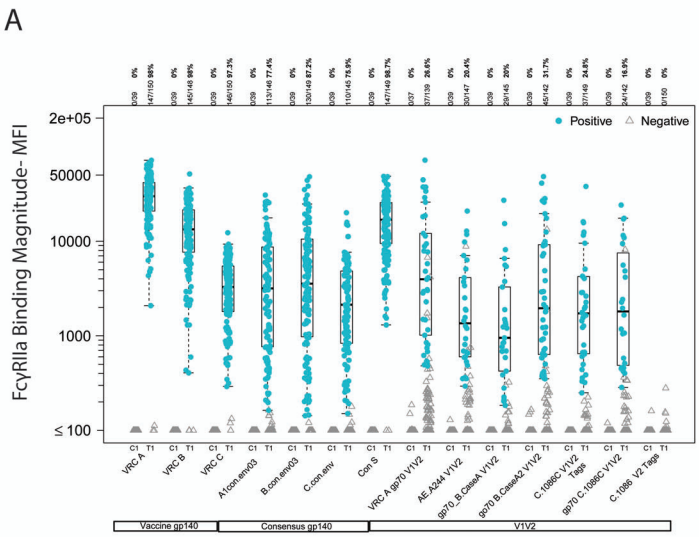
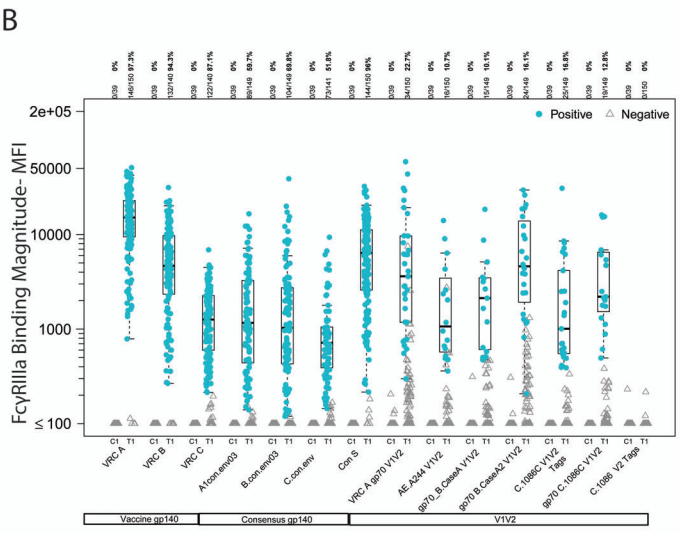
SOM Figure. 1 Distinct response rates and magnitude of antibody FcγRIIa and FcγRIIIa binding to gp140, gp120 and V1V2 proteins. Antibody FcγRIIa (**A, C, E**) and FcγRIIIa (**B, D, F**) binding to an array of envelope glycoprotein gp140 and V1V2 proteins, gp41, gp120, V3 and p24 Gag proteins. Positivity was defined as exceeding the 95th percentile of baseline, MFI>100, and participant-specific three-fold increase from baseline.

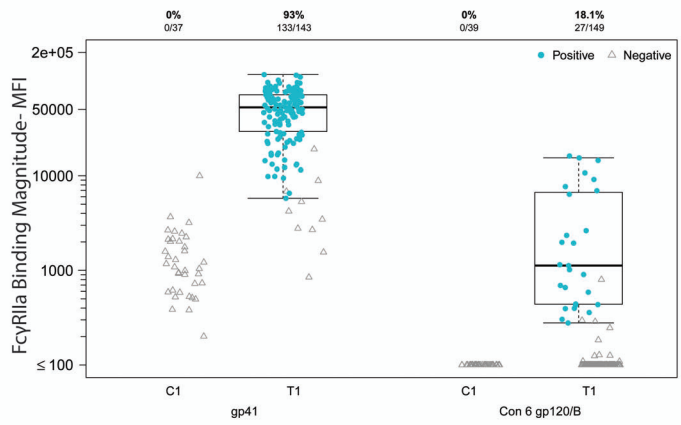
SOM Figure 2. IgG3 response magnitude and rate. Boxplots of MFI are displayed for antigens including vaccine matched gp140s, suptype consensus gp140s, Consensus gp140 and gp120 (A), as well as V1V2 conformational and sequence motifs, gp41, and p24 Gag (B). The mid-line of the box denotes the median and ends of the box denote the 25th and 75th percentiles, with whiskers extending to the most extreme data points. Positivity was defined as exceeding the 95th percentile of baseline, MFI>100, and participant-specific three-fold increase from baseline.

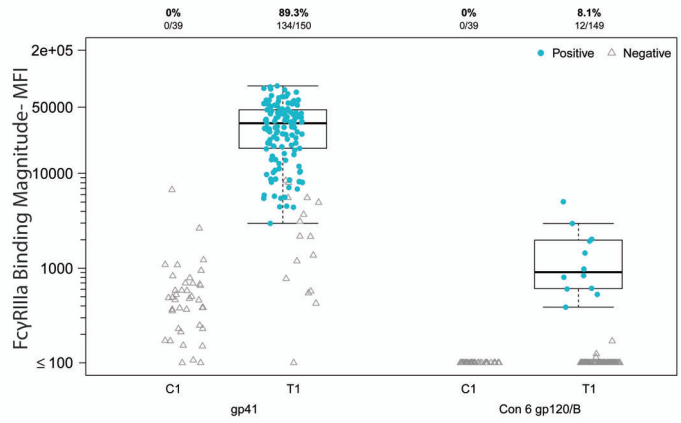
SOM Figure 3. Sieve signatures based on distances of breakthrough viruses to Con S gp140 and B Con gp140. Boxplots of hamming distances of *mindist* sequences to ConS gp 140 **(A,C,E)** or B.con.env03 gp140 **(B, D,F)** by treatment group for three sets of Env-gp120 sites: **(A,B)** all Env-gp120 sites that could be aligned with confidence (n = 432); **(C,D)** the 93 CD4bs antibody contact sites; and **(E,F)** 54 sites corresponding to the 4 k-mers overlapping the CD4bs where significant sieve effects were found (4). The mid-line of the box denotes the median and the ends of the box denote the 25th and 75th percentiles. The whiskers that extend from the top and bottom of the box extend to the most extreme data points that are no more than 1.5 times the interquartile range or if no value meets this criterion, to the data extremes. The dashed horizontal lines show hamming distances of the subtype B vaccine strain to the gp140 antigen for the specified Env-gp120 site set. P-value reports the result of a 2-sided Wilcoxon Test for a difference in distribution of the distances by treatment group.

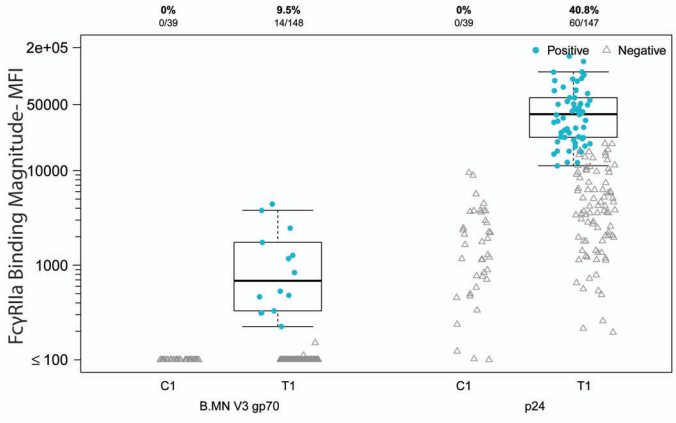
SOM Figure 1

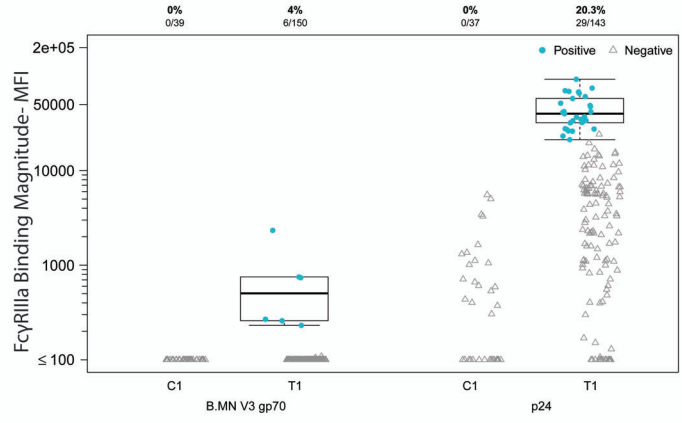






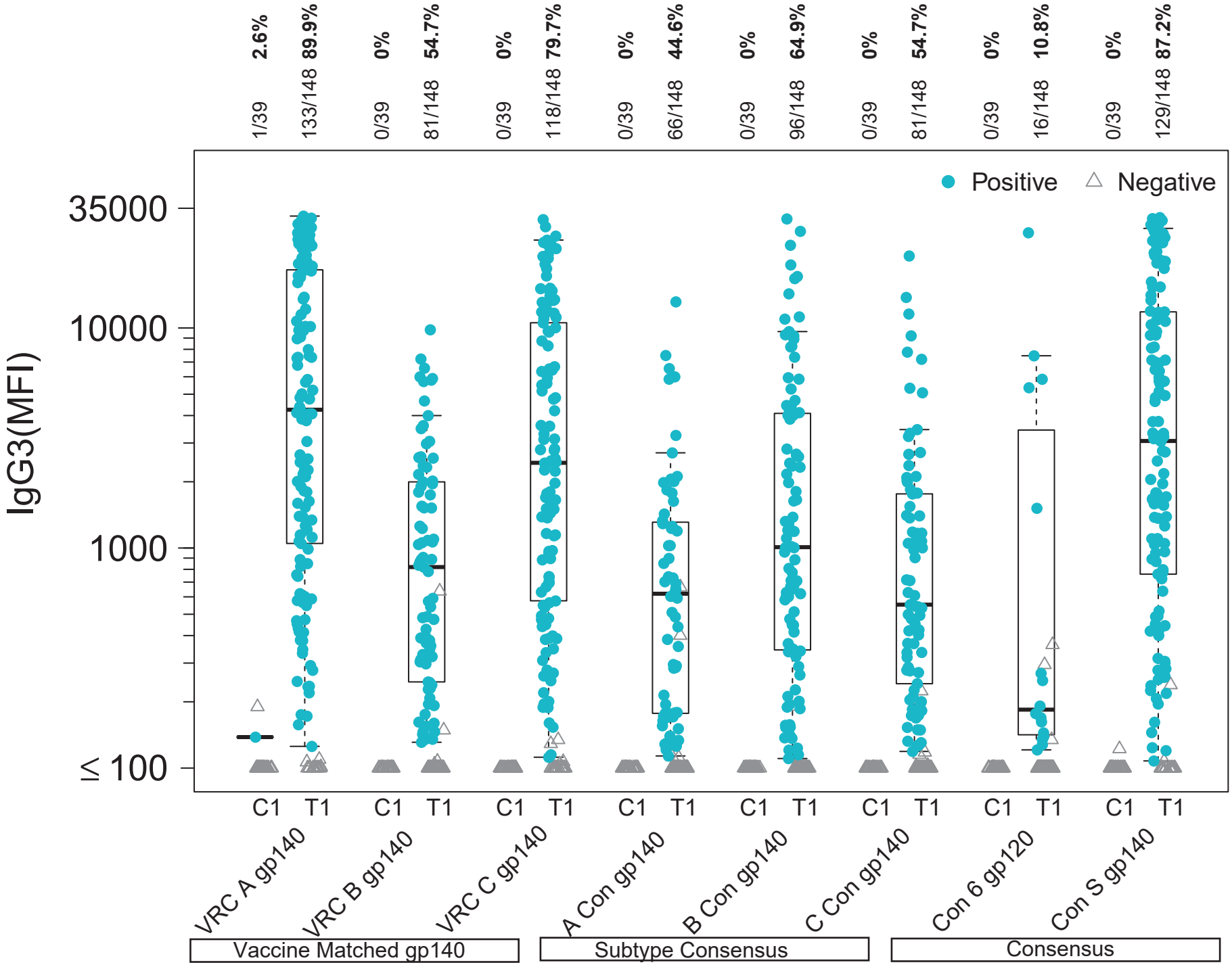




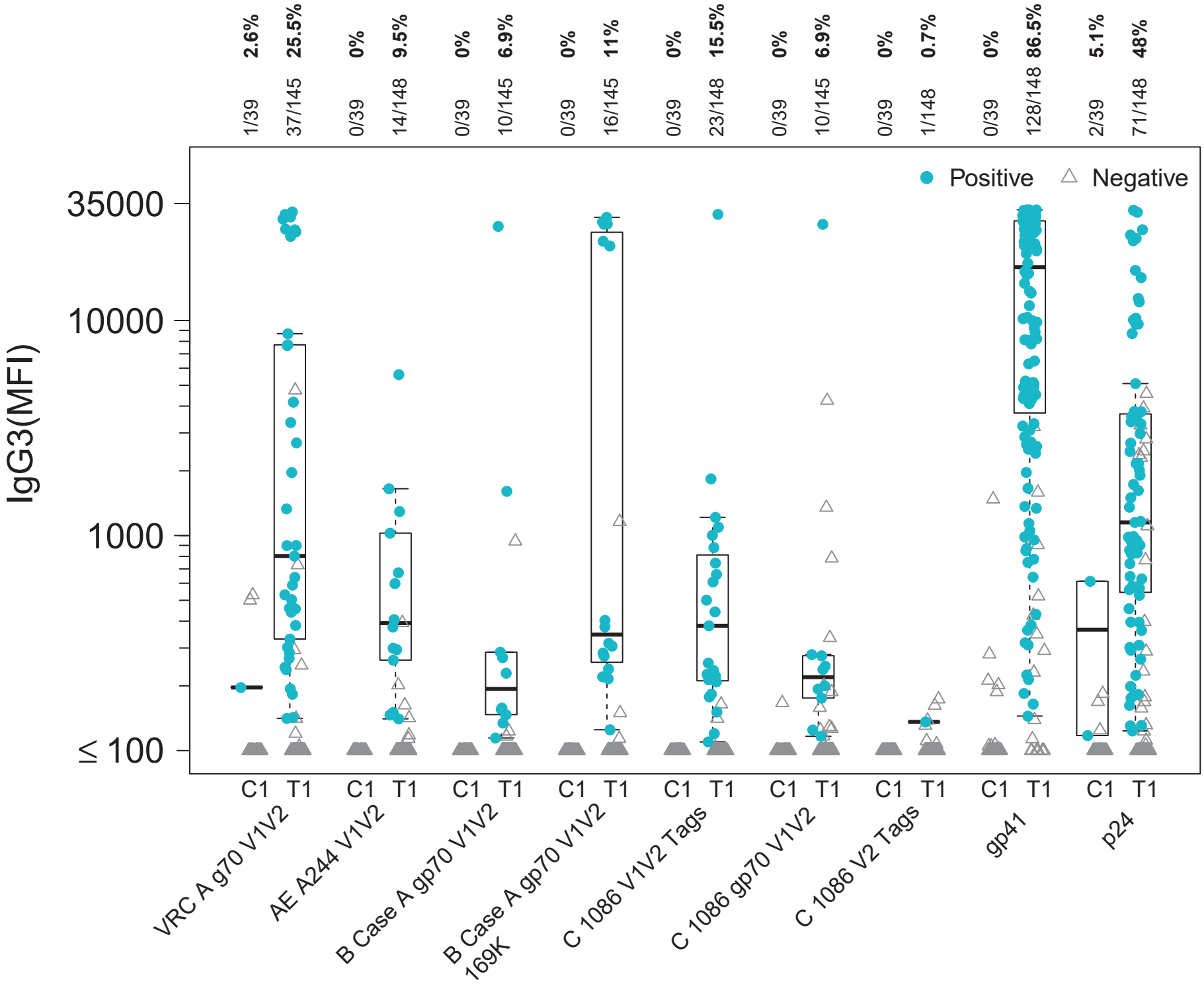


C1: Placebo
T1: Vaccinee

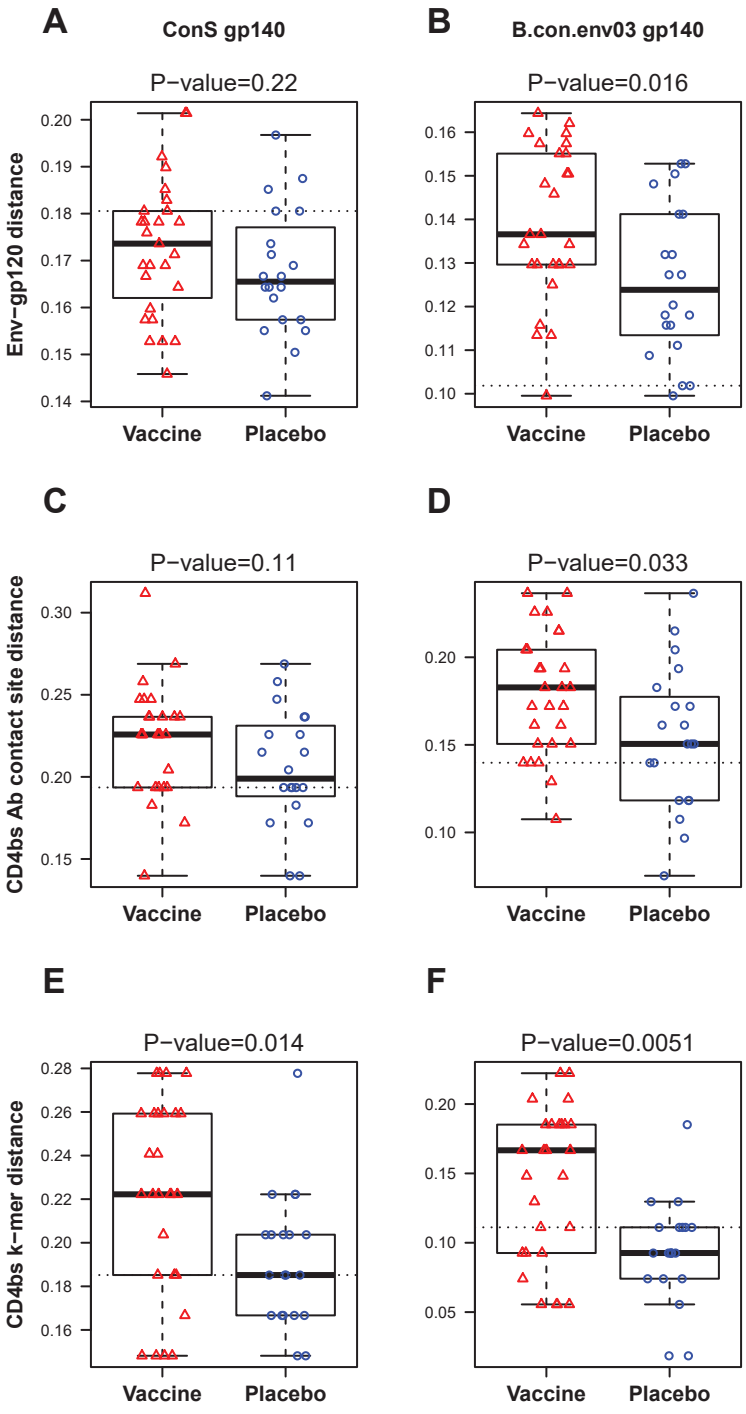
SOM Figure 2


1/39 2.6%
133/148 89.9%
0/39 0%
81/148 54.7%
0/39 0%
118/148 79.7%
0/39 0%
66/148 44.6%
0/39 0%
96/148 64.9%
0/39 0%
81/148 54.7%
0/39 0%
16/148 10.8%
0/39 0%
129/148 87.2%
Positive
Negative
IgG3(MFI)
35000
10000
1000
≤ 100
C1 T1 C1 T1 C1 T1 C1 T1 C1 T1 C1 T1 C1 T1 C1 T1
VRC A gp140
VRC B gp140
VRC C gp140
A Con gp140
B Con gp140
C Con gp140
Con 6 gp120
Con S gp140
Vaccine Matched gp140
Subtype Consensus
Consensus

SOM Figure 2B



SOM Figure 3


A
ConS gp140
P−value=0.22
Env−gp120 distance
0.20
0.19
0.18
0.17
0.16
0.15
0.14
Vaccine
Placebo
B
B.con.env03 gp140
P−value=0.016
0.16
0.15
0.14
0.13
0.12
0.11
0.10
Vaccine
Placebo
C
P−value=0.11
CD4bs Ab contact site distance
0.30
0.25
0.20
0.15
Vaccine
Placebo
D
P−value=0.033
0.20
0.15
0.10
Vaccine
Placebo
E
P−value=0.014
CD4bs k−mer distance
0.28
0.26
0.24
0.22
0.20
0.18
0.16
Vaccine
Placebo
F
P−value=0.0051
0.20
0.15
0.10
0.05
Vaccine
Placebo

SOM Table 1A. FcγRIIa (H131) Antigen Specific Response Rates

Antigen	Treatment Arm	Response Rate	95% CI
A1.con.env 03 gp140CF	Vaccine	113/146 = 77.4%	(70.0%, 83.4%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
AE.A244 V1V2 Tags	Vaccine	30/147 = 20.4%	(14.7%, 27.6%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
B.MN V3 gp70	Vaccine	14/148 = 9.5%	(5.7%, 15.3%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
B.con.env03 gp140 CF	Vaccine	130/149 = 87.2%	(80.9%, 91.7%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
C.1086 V2 tags	Vaccine	0/150 = 0.0%	(0.0%, 2.5%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
C.1086C V1V2 Tags	Vaccine	37/149 = 24.8%	(18.6%, 32.3%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
C.con.env03 gp140 CF	Vaccine	110/145 = 75.9%	(68.3%, 82.1%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
Con 6 gp120/B	Vaccine	27/149 = 18.1%	(12.8%, 25.1%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
Con S gp140 CFI	Vaccine	147/149 = 98.7%	(95.2%, 99.6%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
VRC A gp140	Vaccine	147/150 = 98.0%	(94.3%, 99.3%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
VRC B gp140	Vaccine	145/148 = 98.0%	(94.2%, 99.3%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)

Antigen	Treatment Arm	Response Rate	95% CI
VRC C_gp140	Vaccine	146/150 = 97.3%	(93.3%, 99.0%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
VRC_A gp70V1V2	Vaccine	37/139 = 26.6%	(20.0%, 34.5%)
	Placebo	0/ 37 = 0.0%	(0.0%, 9.4%)
gp41	Vaccine	133/143 = 93.0%	(87.6%, 96.2%)
	Placebo	0/ 37 = 0.0%	(0.0%, 9.4%)
gp70_B. CaseA2 V1V2/169K	Vaccine	45/142 = 31.7%	(24.6%, 39.7%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
gp70_B. CaseA_ V1V2	Vaccine	29/145 = 20.0%	(14.3%, 27.2%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
gp70_C.1086 V1V2	Vaccine	24/142 = 16.9%	(11.6%, 23.9%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
p24 Gag	Vaccine	60/147 = 40.8%	(33.2%, 48.9%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)

SOM Table 1B. FcγRIIIa (F158) Antigen Specific Response Rates

Antigen	Treatment Arm	Response Rate	95% CI
A1.con.env03 gp140CF	Vaccine	89/149 = 59.7%	(51.7%, 67.3%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
AE.A244 V1V2 Tags	Vaccine	16/150 = 10.7%	(6.7%, 16.6%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
B.MN V3 gp70	Vaccine	6/150 = 4.0%	(1.8%, 8.5%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
B.con.env03 140 CF	Vaccine	104/149 = 69.8%	(62.0%, 76.6%)

	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
C.1086 V2 tags	Vaccine	0/150 = 0.0%	(0.0%, 2.5%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
C.1086C V1V2 Tags	Vaccine	25/149 = 16.8%	(11.6%, 23.6%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
C.con.env03 gp140CF	Vaccine	73/141 = 51.8%	(43.6%, 59.9%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
Con 6 gp120	Vaccine	12/149 = 8.1%	(4.7%, 13.5%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
Con S gp140 CFI	Vaccine	144/150 = 96.0%	(91.5%, 98.2%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
VRC A gp140	Vaccine	146/150 = 97.3%	(93.3%, 99.0%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
VRC B gp140	Vaccine	132/140 = 94.3%	(89.1%, 97.1%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
VRC C gp140	Vaccine	122/140 = 87.1%	(80.6%, 91.7%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
VRC_A gp70 V1V2	Vaccine	34/150 = 22.7%	(16.7%, 30.0%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
gp41	Vaccine	134/150 = 89.3%	(83.4%, 93.3%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
gp70_B. CaseA2 V1V2/169K	Vaccine	24/149 = 16.1%	(11.1%, 22.8%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
gp70_B. CaseAV1V2	Vaccine	15/149 = 10.1%	(6.2%, 15.9%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)

gp70_C.1086 V1/V2	Vaccine	19/149 = 12.8%	(8.3%, 19.1%)
	Placebo	0/ 39 = 0.0%	(0.0%, 9.0%)
p24 Gag	Vaccine	29/143 = 20.3%	(14.5%, 27.6%)
	Placebo	0/ 37 = 0.0%	(0.0%, 9.4%)

SOM Table 2. Weights of summary of immune response markers when calculating the first (PC1) and second (PC2) principal component, otherwise known as loadings. The markers that most substantially contribute to each principal component are in bold.

	PC1	PC2
Cd8.env.poly	0.224	**0.566**
IgG env	**0.450**	-0.006
IgG V2	0.301	-0.040
IgG gp41	**0.411**	-0.083
ADCP1	**0.401**	0.038
FcγRIIa	**0.428**	-0.056
IgA env	0.173	**-0.792**
IgG3 Env	**0.337**	**0.200**

SOM Table 3. Weights of individual immune response markers when calculating the first (PC1) and second (PC2) principal component.

	Target	PC1	PC2
ADCP	Phagocytosis of Con S gp140	0.177	0.127
CD8	Polyfunctionality	0.090	0.004
IgA	AE A244 V1V2 Tags	-0.009	-0.014
IgG	ABDM HVTN505 gp41 ID	0.096	-0.011
	AE A244 V1V2 Tags	0.082	-0.035
	Bio V3 B	0.128	-0.051
	C HVTN505 gp41 ID	0.106	-0.032
	C Con gp140	0.180	-0.118
	Con S gp140	0.185	-0.175
	gp41	0.177	-0.098
	VRC A gp140	0.195	-0.184
	VRC B gp140	0.164	-0.162
	VRC C gp140	0.197	-0.151
IgG3	A1 Con gp140	0.165	0.275
	B Con gp140	0.168	0.316
	C Con gp140	0.158	0.329
	Con 6 gp140	0.083	0.141
	Con S gp140	0.160	0.334
	gp41	0.174	0.190
	VRC A gp140	0.162	0.259
	VRC B gp140	0.160	0.300
	VRC C gp140	0.139	0.323
FcγRIIa	A1 Con gp140	0.202	-0.116
	B Con gp140	0.212	-0.094
	C Con gp140	0.207	-0.074
	gp41	0.189	-0.084
	p24	0.041	-0.028
	VRC_A_gp70V1V2_avi	0.101	-0.005
	VRC A gp140	0.187	-0.168
	VRC B gp140	0.202	-0.157
	VRC C gp140	0.203	-0.123
FcγRIIIa	A1 Con gp140	0.193	-0.076
	B Con gp140	0.206	-0.044
	C Con gp140	0.194	-0.025
	Con S gp140	0.214	-0.065
	p24	0.039	-0.028
	VRCBgp140	0.209	-0.108

Statistical Analysis Plan for Superlearner CoR Analysis of Month 7 Markers in HVTN 505

April 19, 2019

Analysis Notes Specific to Superlearner Supervised Learning

- Keep for CoR analysis individual variables that are matched to vaccine antigens. For vaccine-mismatched biomarkers, if there is only one vaccine-mismatched variable in a set, use it, otherwise use the scores PC1 and/or mdw. If PC1 and mdw have Spearman rank r > 0.9, only keep PC1. The HVTN505: var.super object contains this set of variables. For every variable in this list, there is also a dichotomized version, whose name has a suffix _bin at the end.
- Pre-scale each variable to have mean 0 and sd 1 in the vaccine arm (both quantitative and binary variables)
- Given there are only 25 total HIV infected cases, only allow learning algorithms to have a maximum of 4 immune response variables. Also use leave-one-out cross-validation and negative log-likelihood loss, which some studies have shown tend to perform well in small sample size settings.
- Include learning algorithms with and without lasso pre-screening (with default tuning parameter selection), and perhaps with and without logistic regression univariate 2-sided p-value screening (at levels $p < 0.01, < 0.05, < 0.10$)
- Another screen to use with Superlearner would be to only include markers with a certain 'dynamic range,' knowing that biologically-relevant inter-vaccinee variability majorly impacts power. Exclude variables such that the 20^{th} percentile of the readout equals the 80^{th} percentile of the readout in vaccine recipients. In addition, for each variable in a set (i.e., vaccine-matched, PC1, MDWS), compute the 'dynamic range' score as the standard deviation of the marker in vaccinees divided by the standard deviation of the marker in placebo recipients. One screen that is used only includes variables in the top half of dynamic range scores.
- Do high-correlation screening, not allowing any pair of immune response variables to have r > 0.9
- Include easy-to-interpret models in Superlearner and include models that study pairwise interactions of variables
- Run the Superlearner averaging over 10 random seeds
- The output may include CV-AUC forest plots similar to Ted Westling's tutorial (http://faculty.washington.edu/peterg/SanofiPasteurCorrelatesRTraining.2018.html) and Magaret et al. 2019. An important aspect of the results to communicate is the quality of classification accuracy obtained via different variable sets. Therefore, the forest plots include best models based on different combinations of assays, assuming there are too many (assay x antigen) combinations to adequately display. This has important upstream effects of how to set up the library of learners. Looking at Ted's example, there are models presented for "IgA, IgG, Both, Neither." Conceptually, we want the same thing, but there are more assay categories. Note that models with IgG and IgA also include log IgA/IgG ratio variables. The classes to use (with legend labels) are as follows:
    1. None (No Month 7 markers − only the baseline variables in every model – BMI, age, risk score)

2. IgG+IgA (considering all antigens)
3. IgG3 (IgG subclass associated with functional responses)
4. T cells (ICS CD4 and CD8 T cells) (considering all antigens)
5. Fx Ab (Functional Ab variables “phago”, “fcrR2a”, “fcrR3a”, “Tier 1 nAb”) (considering all antigens)
6. 1+2+3 (IgG+IgG3+IgA)
7. 1+2+4 (IgG+IgA+T cells)
8. 1+2+3+4 (IgG+IgG3+IgA+T cells)
9. 1+2+3+5 (IgG+IgG3+IgA+Fx Ab)
10. 1+4+5 (T cells+Fx Ab)
11. All (IgG+IgG3+IgA+T cells+Fx Ab)

Technically, only phago is a purely functional readout. We consider FcR a function but it is still a biophysical measurement, IgG3 is binding but is associated with function in a number of studies in the literature.

In terms of display, if results are shown for each algorithm type and screen, then there would be too many results to display. Only the SL and the top few performing models will be shown for each.

- Apply the usual nested cross-validation to be able to estimate cross-validated risk to appropriately characterize the classification accuracy (free of overfitting) of the Superlearner model and the other models.
- The output may include a plot of the distribution of estimated risk of HIV-1 infection (fitted values), and overlay on the plot an estimate of the overall HIV-1 infection risk of placebo recipients between Month 7 and 24.