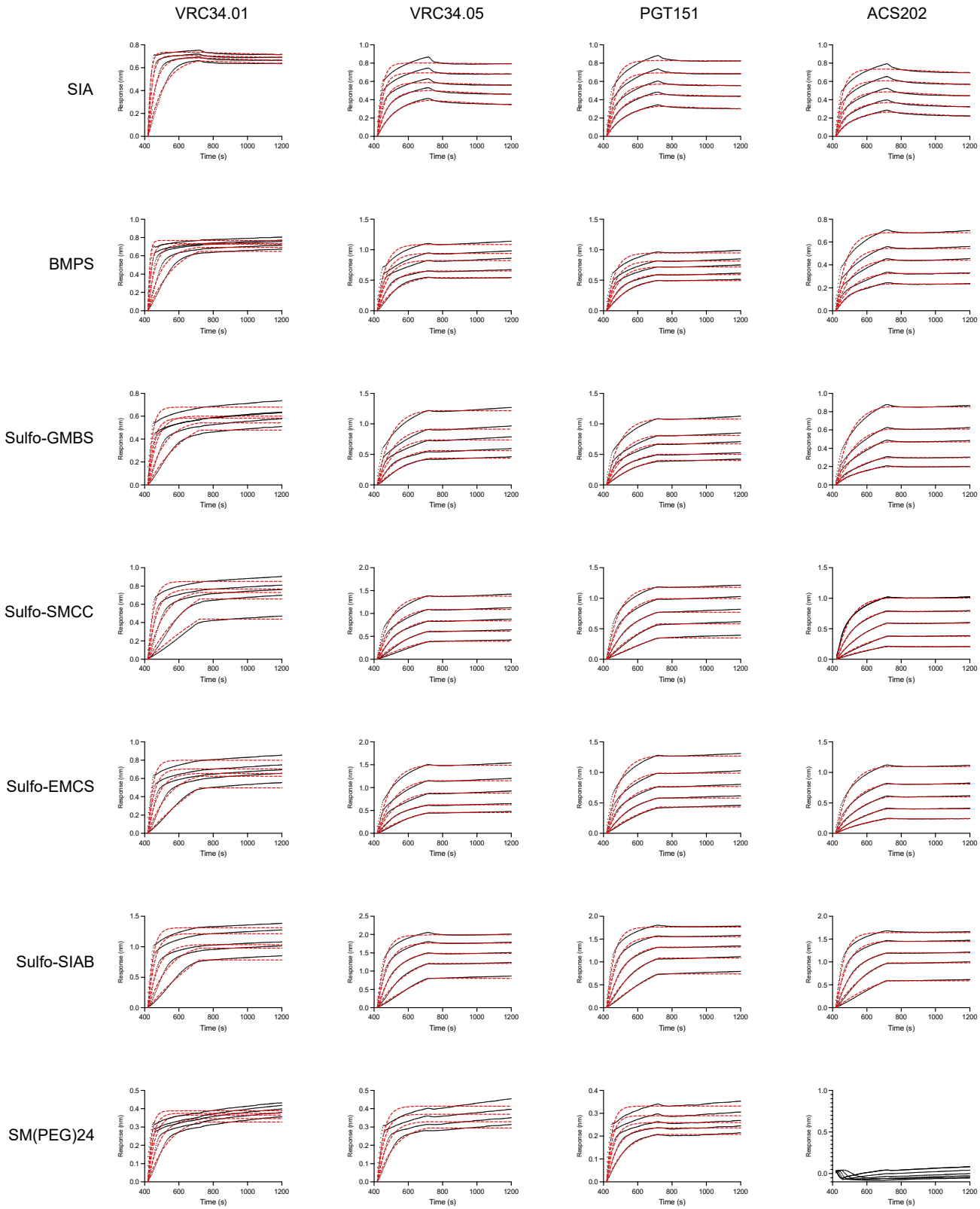



**Figure S1. BLI binding curves for antigenicity measurements of the conjugates.**
Binding curves are shown as black lines, while red lines indicate the global fits generated by using a 1:1 Langmuir binding model.

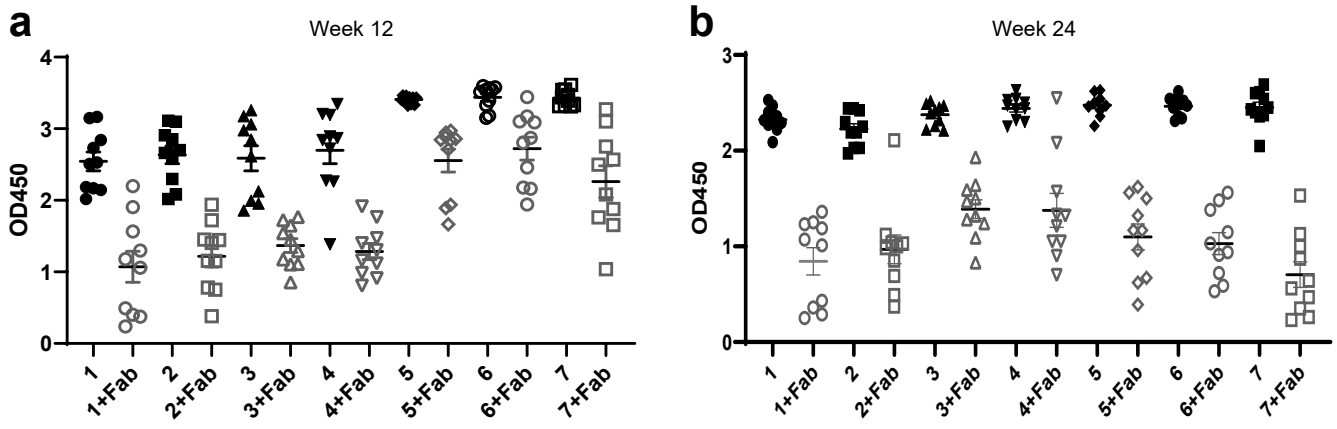



**Figure S2. Env trimer anti-base responses.**
Anti-base responses were measured using the antigen-binding fragment (Fab) of base-directed antibody R19R in a competition assay at wk12 (**a**) and at wk24 (**b**). Solid and empty symbols represent OD450 values of serum at 1:500 dilution without and with R19R competitor with BG505 DS-SOSIP trimer.

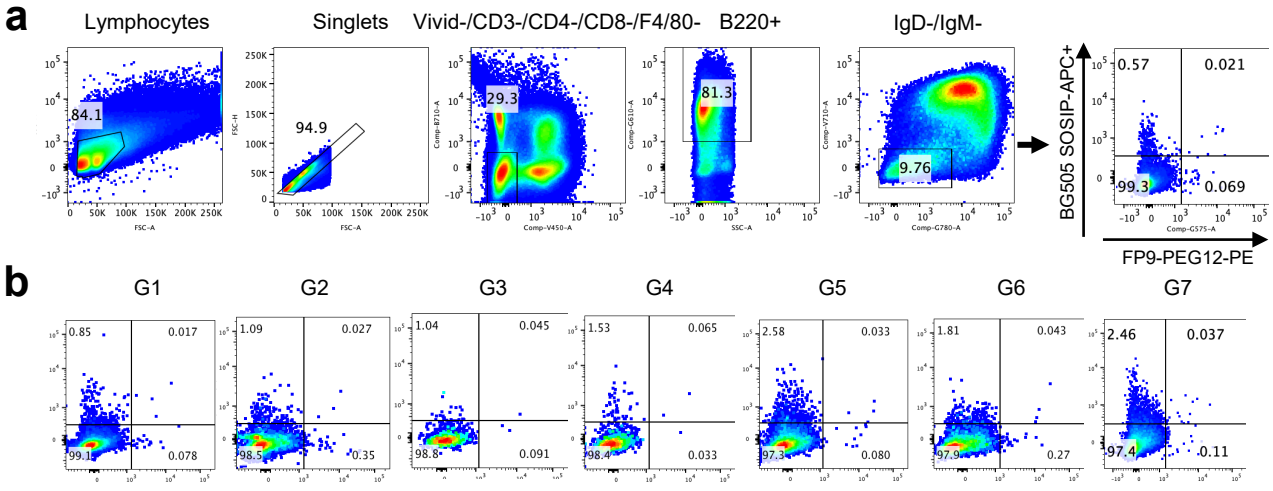
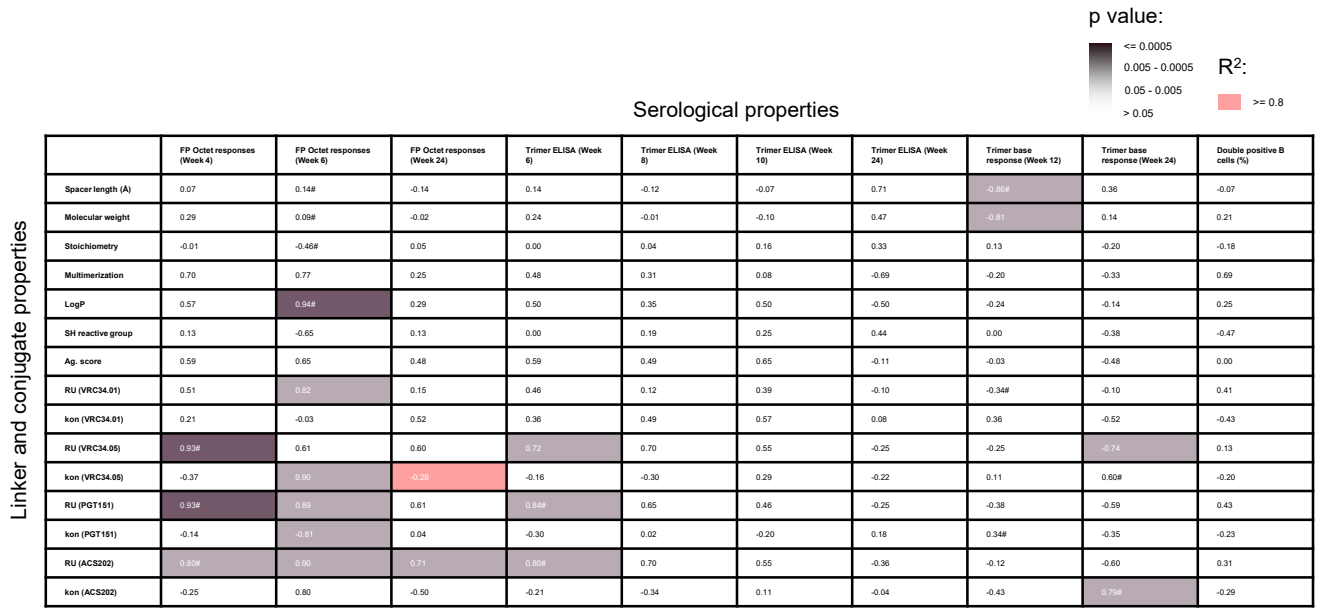



**Figure S3. Double positive B cell quantification.**
(**a**) Gating strategy for antigen-specific B cell FACS analysis. Splenocytes from a naïve mouse were used as a control to set up gating for the analysis of splenocytes from immunized mice. (**b**) Representatives with median values of FP+BG505+ B cell frequency for each group are shown for 7 groups.




| | FP Octet responses (Week 4) | FP Octet responses (Week 6) | FP Octet responses (Week 24) | Trimer ELISA (Week 6) | Trimer ELISA (Week 8) | Trimer ELISA (Week 10) | Trimer ELISA (Week 24) | Trimer base response (Week 12) | Trimer base response (Week 24) | Double positive B cells (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Spacer length (Å) | 0.07 | 0.14# | -0.14 | 0.14 | -0.12 | -0.07 | 0.71 | -0.86# | 0.36 | -0.07 |
| Molecular weight | 0.29 | 0.09# | -0.02 | 0.24 | -0.01 | -0.10 | 0.47 | -0.81 | 0.14 | 0.21 |
| Stoichiometry | -0.01 | -0.46# | 0.05 | 0.00 | 0.04 | 0.16 | 0.33 | 0.13 | -0.20 | -0.18 |
| Multimerization | 0.70 | 0.77 | 0.25 | 0.48 | 0.31 | 0.08 | -0.69 | -0.20 | -0.33 | 0.69 |
| LogP | 0.57 | 0.94# | 0.29 | 0.50 | 0.35 | 0.50 | -0.50 | -0.24 | -0.14 | 0.25 |
| SH reactive group | 0.13 | -0.65 | 0.13 | 0.00 | 0.19 | 0.25 | 0.44 | 0.00 | -0.38 | -0.47 |
| Ag. score | 0.59 | 0.65 | 0.48 | 0.59 | 0.49 | 0.65 | -0.11 | -0.03 | -0.48 | 0.00 |
| RU (VRC34.01) | 0.51 | 0.82 | 0.15 | 0.46 | 0.12 | 0.39 | -0.10 | -0.34# | -0.10 | 0.41 |
| kon (VRC34.01) | 0.21 | -0.03 | 0.52 | 0.36 | 0.49 | 0.57 | 0.08 | 0.36 | -0.52 | -0.43 |
| RU (VRC34.05) | 0.93# | 0.61 | 0.60 | 0.72 | 0.70 | 0.55 | -0.25 | -0.25 | -0.74 | 0.13 |
| kon (VRC34.05) | -0.37 | 0.90 | -0.28 | -0.16 | -0.30 | 0.29 | -0.22 | 0.11 | 0.60# | -0.20 |
| RU (PGT151) | 0.93# | 0.89 | 0.61 | 0.84# | 0.65 | 0.46 | -0.25 | -0.38 | -0.59 | 0.43 |
| kon (PGT151) | -0.14 | -0.81 | 0.04 | -0.30 | 0.02 | -0.20 | 0.18 | 0.34# | -0.35 | -0.23 |
| RU (ACS202) | 0.80# | 0.90 | 0.71 | 0.80# | 0.70 | 0.55 | -0.36 | -0.12 | -0.60 | 0.31 |
| kon (ACS202) | -0.25 | 0.80 | -0.50 | -0.21 | -0.34 | 0.11 | -0.04 | -0.43 | 0.79# | -0.29 |

**Figure S4. Correlations between linker and serological properties.**
Spearman rho values are shown. Correlations with high Spearman significance (p <= 0.05) are highlighted in purple (scale shown). Correlations which maintained Spearman significance when outlier SM(PEG)24 was excluded are denoted with a #. Correlations which failed to meet requirements for Spearman significance and Pearson correlation assumptions but fit to a linear (or log-transformed linear) model ($R^2$ >= 0.8) are highlighted in red. For serological properties, the geometric mean was taken over each participant group, with 0 values converted to 1. For the SH reactive linker property, Iodoacetyl was coded as 0 and Maleimide was coded as 1.

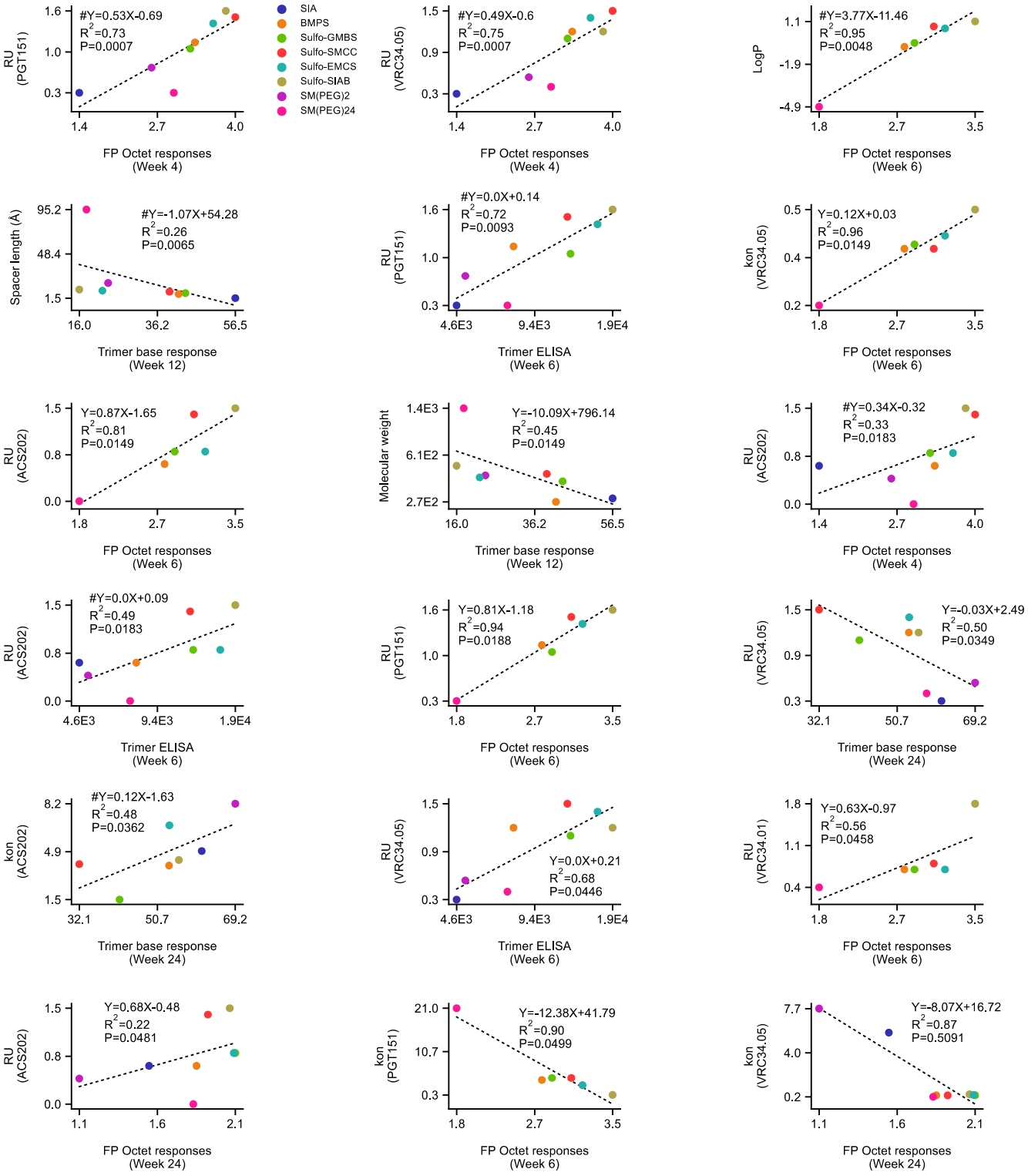



**Figure S5. Visualizing correlations between linker and serological properties.**
Correlation plots which 1) have high Spearman significance (p <= 0.05) or 2) fit to a linear (or log-transformed linear) model ($R^2$ >= 0.8) are shown, ordered left to right and top to bottom in terms of significance. Correlations which maintained Spearman significance when outlier SM(PEG)24 was excluded are denoted with a #. The linear (or log-transformed linear) regression model is also shown. Participant groups are colored according to linker, legend is shown.

| Virus | | BG505.W6M.C2.SG3 | | BG505.W6M.C2.N611Q.SG3 | | MW965 | SIVmac251.30.SG3 | |
|---|---|---|---|---|---|---|---|---|
| Immunogen | Animal ID | week 10 | week24 | week 10 | week24 | week24 | week 10 | week24 |
| Group1 SIA_FP8v1-rTTHC | N01 | -43 | 35 | -9 | 96 | -15 | -70 | 25 |
| | N02 | -24 | 31 | 91 | 98 | 30 | -45 | 17 |
| | N03 | -30 | 3 | 57 | 85 | 2 | -58 | 1 |
| | N04 | 7 | 38 | 88 | 100 | -3 | -48 | 7 |
| | N05 | -3 | 35 | 42 | 78 | -39 | -25 | 14 |
| | N06 | -33 | 11 | -7 | 83 | -8 | -35 | -17 |
| | N07 | -14 | 36 | 22 | 68 | -56 | -54 | -4 |
| | N08 | -13 | 56 | -17 | 55 | 34 | -41 | -13 |
| | N09 | -2 | 37 | 74 | 75 | 14 | -19 | -5 |
| | N10 | 1 | 50 | 85 | 77 | -19 | -50 | -8 |
| Group2 BMPS_FP8v1-rTTHC | N11 | -37 | 69 | 29 | 98 | 43 | -61 | -15 |
| | N12 | -30 | 48 | 26 | 98 | -18 | -34 | -2 |
| | N13 | -2 | 49 | 85 | 92 | -4 | -20 | 3 |
| | N14 | 5 | 57 | 18 | 61 | -42 | -28 | 6 |
| | N15 | -30 | -6 | 80 | 92 | -18 | -38 | -13 |
| | N16 | -31 | 36 | 53 | 53 | -2 | -14 | 0 |
| | N17 | -53 | 27 | 95 | 98 | -42 | -43 | -23 |
| | N18 | -48 | 44 | -10 | 53 | -57 | -44 | 7 |
| | N19 | -32 | 13 | 37 | 55 | -58 | -26 | -9 |
| | N20 | 2 | 41 | 95 | 57 | -4 | 2 | 7 |
| Group3 Sulfo-GMBS_FP8v1-rTTHC | N21 | -27 | 37 | 37 | 97 | -43 | -30 | 1 |
| | N22 | -33 | 25 | 20 | 85 | -46 | -40 | -9 |
| | N23 | -21 | 93 | 71 | 97 | -34 | -23 | 8 |
| | N24 | -17 | 22 | 30 | 51 | -57 | -12 | -5 |
| | N25 | -22 | 37 | 47 | 83 | -45 | -43 | -3 |
| | N26 | -30 | 40 | 86 | 92 | -9 | -44 | -5 |
| | N27 | -25 | 46 | 45 | 61 | -67 | -44 | 1 |
| | N28 | -30 | 83 | 60 | 99 | -35 | -18 | 7 |
| | N29 | -15 | 28 | 84 | 95 | -27 | -11 | -4 |
| | N30 | -34 | 17 | 62 | 78 | -62 | -20 | -13 |
| Group4 Sulfo-SMCC_FP8v1-rTTHC | N31 | -32 | 52 | 76 | 96 | -51 | -28 | -9 |
| | N32 | -38 | 25 | 37 | 84 | 78 | -23 | -6 |
| | N33 | -41 | 43 | 70 | 79 | -32 | -43 | -1 |
| | N34 | -49 | 64 | 16 | 95 | -16 | -33 | 0 |
| | N35 | -30 | 61 | 69 | 80 | 0 | -47 | -8 |
| | N36 | -19 | 55 | 27 | 90 | 8 | -82 | -6 |
| | N37 | -21 | 0 | 90 | 94 | -15 | -37 | -5 |
| | N38 | -24 | -4 | 9 | 78 | -20 | -64 | -12 |
| | N39 | -22 | 18 | 30 | 92 | -4 | -43 | -11 |
| | N40 | -19 | 51 | 68 | 64 | -35 | -52 | -23 |
| Group5 Sulfo-EMCS_FP8v1-rTTHC | N41 | -2 | 37 | 90 | 97 | -33 | -71 | -16 |
| | N42 | -33 | 18 | 50 | 88 | 17 | -71 | -24 |
| | N43 | -48 | 45 | 33 | 92 | 27 | -67 | -28 |
| | N44 | -23 | 32 | 84 | 96 | -12 | -66 | -5 |
| | N45 | 3 | 13 | 100 | 98 | -12 | -39 | -47 |
| | N46 | -16 | 53 | 85 | 87 | -13 | -57 | -9 |
| | N47 | -7 | 4 | -4 | 48 | -72 | -51 | -6 |
| | N48 | -15 | 49 | 72 | 96 | 24 | -76 | 8 |
| | N49 | -23 | 22 | 35 | 95 | -18 | -72 | 0 |
| | N50 | -8 | 9 | 72 | 86 | -51 | -45 | -1 |
| Group6 Sulfo-SIAB_FP8v1-rTTHC | N51 | -20 | 3 | 58 | 86 | -74 | -68 | -24 |
| | N52 | -2 | 35 | 68 | 89 | -43 | -41 | -9 |
| | N53 | 10 | 43 | 90 | 80 | -52 | -37 | -5 |
| | N54 | 10 | 24 | 59 | 71 | -26 | -18 | 1 |
| | N55 | 5 | 23 | 88 | 93 | 14 | -48 | -31 |
| | N56 | -18 | -3 | 28 | 79 | -84 | -35 | -21 |
| | N57 | -24 | 8 | 94 | 69 | -58 | -52 | -21 |
| | N58 | -21 | 31 | 92 | 96 | 3 | -62 | -15 |
| | N59 | -13 | 10 | 17 | 68 | -23 | -44 | 5 |
| | N60 | -18 | 42 | 61 | 87 | -4 | -48 | 4 |
| Group7 SM[PEG]24_FP8v1-rTTHC | N61 | -5 | 53 | -24 | 73 | -23 | -36 | 8 |
| | N62 | -12 | 7 | 84 | 87 | -36 | -47 | -1 |
| | N63 | -1 | 5 | -5 | 14 | -36 | -38 | -25 |
| | N64 | 8 | 48 | 1 | 53 | -37 | -30 | 6 |
| | N65 | -14 | 16 | 5 | 58 | 10 | -62 | -1 |
| | N66 | -33 | -5 | -52 | 6 | -50 | -35 | 1 |
| | N67 | -23 | 40 | 16 | 88 | -39 | -40 | 13 |
| | N68 | -50 | 10 | -62 | 57 | -32 | -55 | -4 |
| | N69 | -5 | 65 | -1 | 88 | -16 | -35 | -18 |
| | N70 | -22 | 17 | -31 | 60 | -35 | -47 | 11 |
| | VRC01 | 100 | | 100 | | 100 | | |


**Figure S6. Week 10 and 24 serum neutralization.**
Virus neutralization assays with the immune sera at week 10 and 24. Sera at 1:50 dilution were used to neutralize the BG505 and its Δ611 virus.

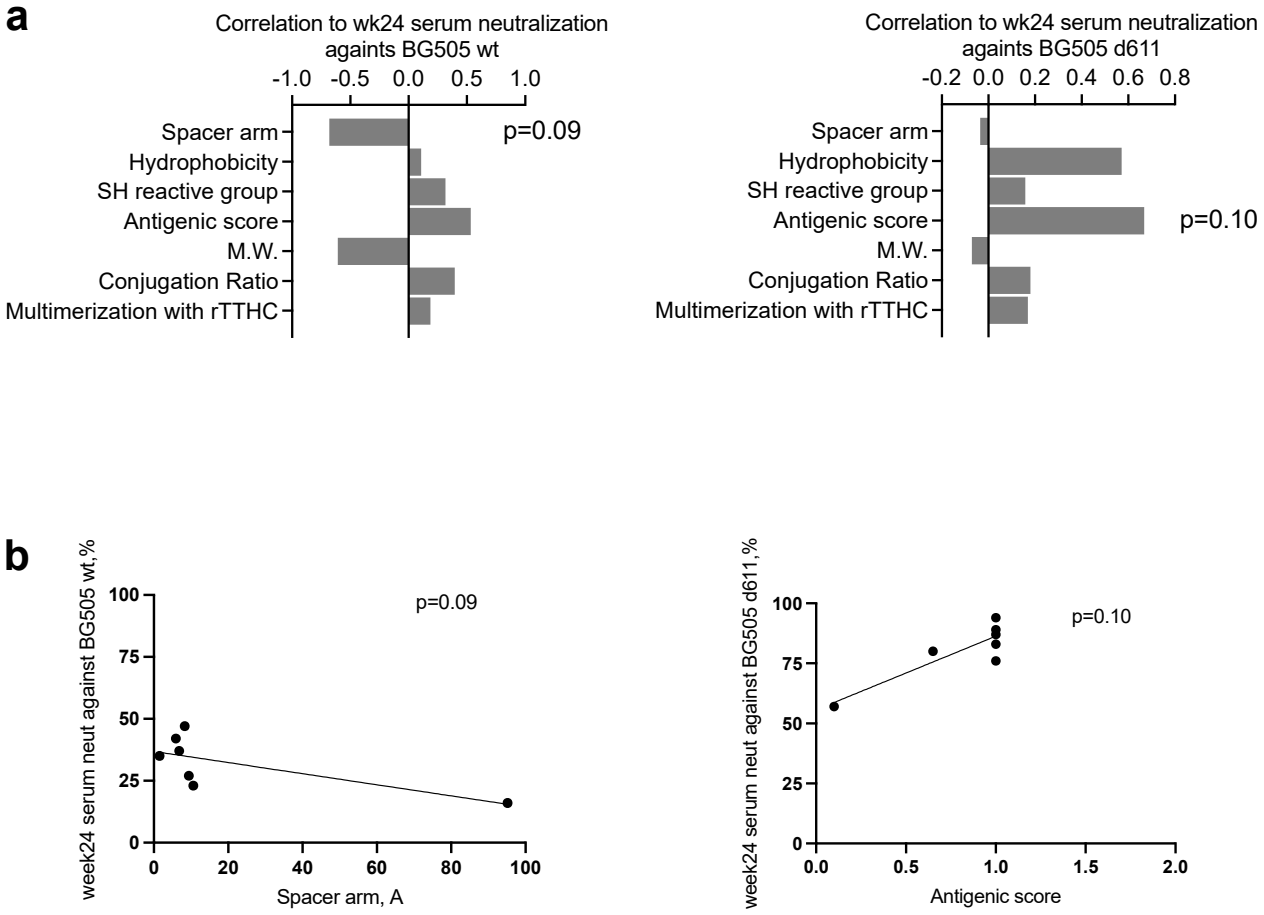



**Figure S7. Correlation analyses between serum neutralization data and physical parameters of immunization.**
(**a**) Virus neutralization assays with the immune sera at week 24. Sera at 1:50 dilution were used to neutralize the BG505 Δ611 virus. *P* values were calculated with Spearman correlation. (**b**) Plots for the largest correlations in panel a are shown, these correlation are not statistically significant, and correlation relative to the spacer arm are defined by a single outlier.

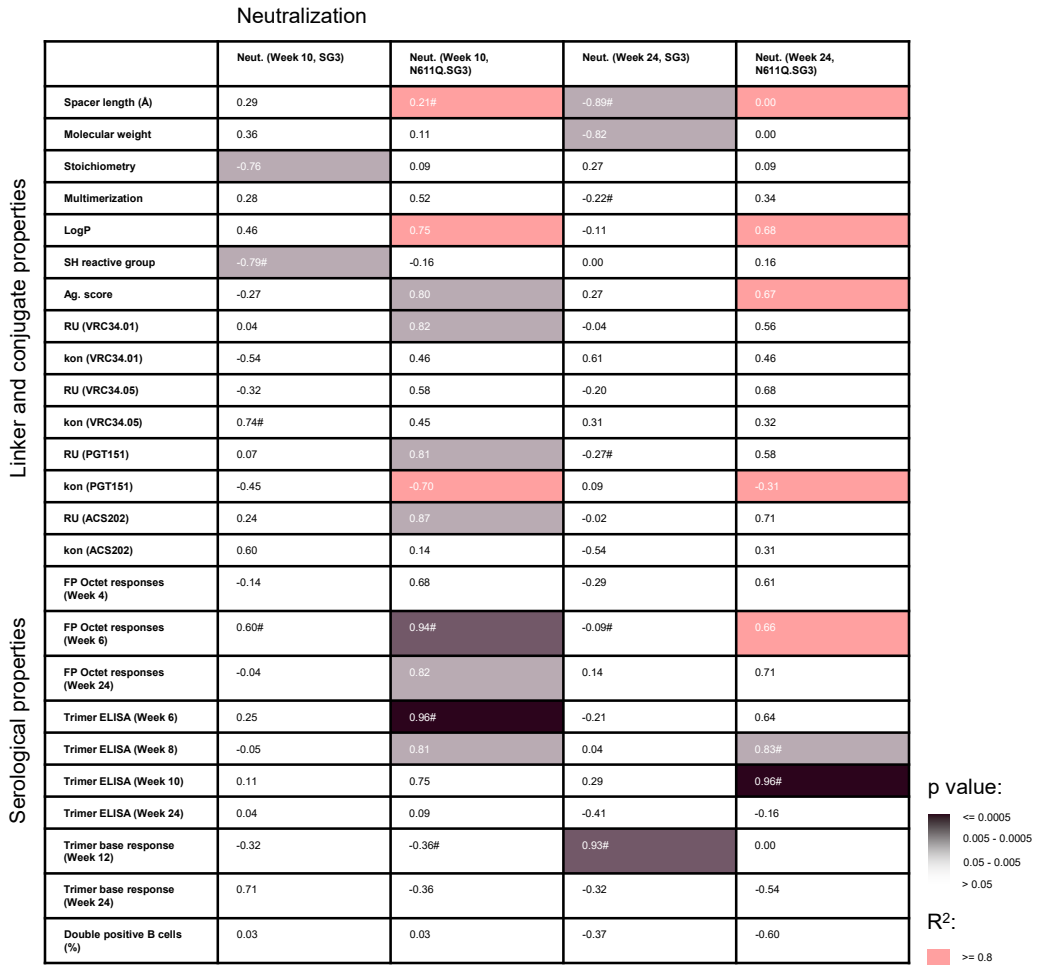



**Figure S8. Correlations between neutralization, linker, and serological properties.**
Spearman rho values are shown. Correlations with high Spearman significance ($p <= 0.05$) are highlighted in purple (scale shown). Correlations which maintained Spearman significance when outlier SM(PEG)24 was excluded are denoted with a #. Correlations which failed to meet requirements for Spearman significance and Pearson's test assumptions but fit to a linear (or log-transformed linear) model ($R^2 >= 0.8$) are highlighted in red. For serological properties, the geometric mean was taken over each group, with 0 values converted to 1. For neutralization, a constant was added to all values, so all were >= 1 prior to geometric mean quantification. For the SH reactive linker property, Iodoacetyl was coded as 0 and Maleimide was coded as 1.

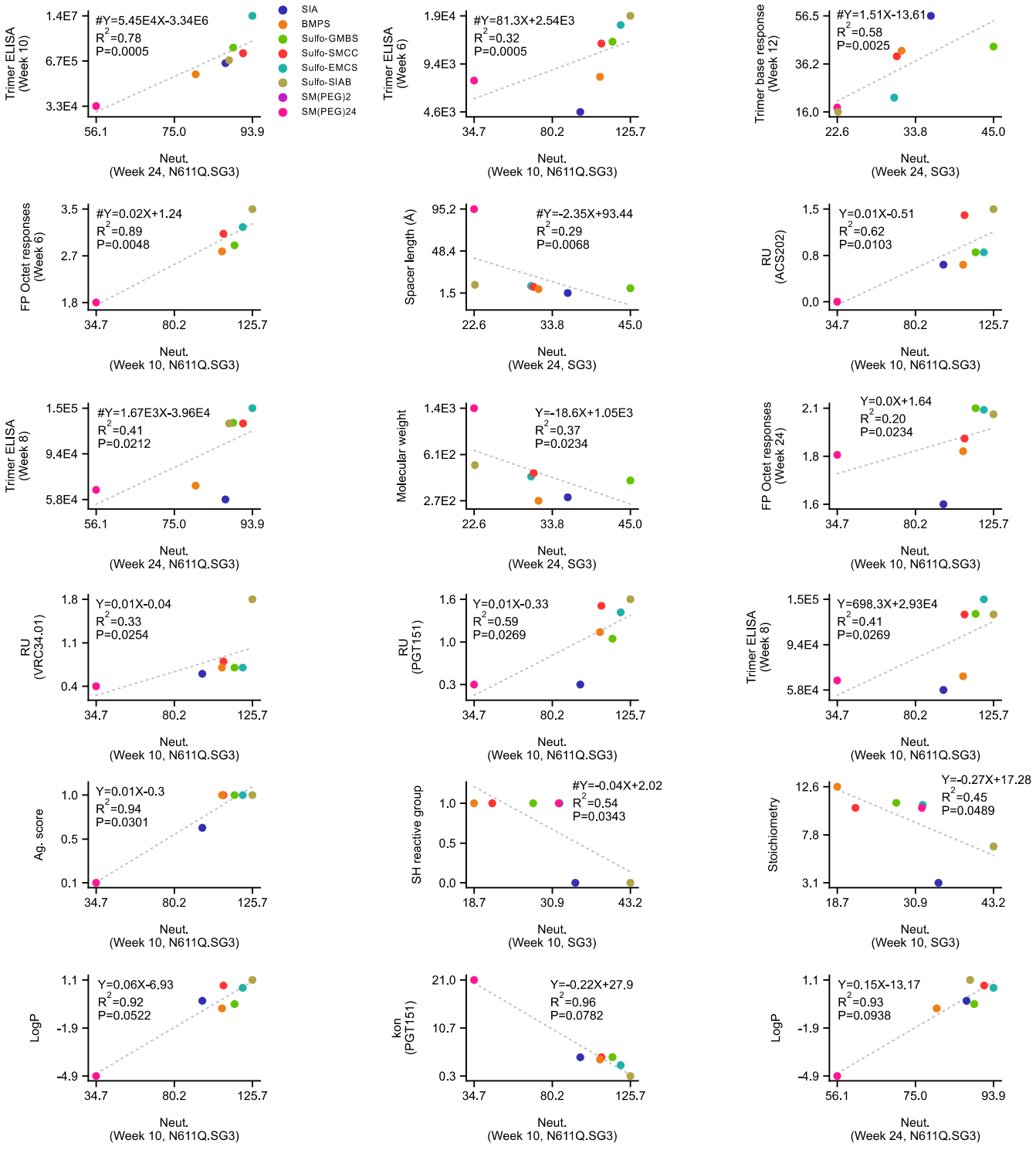



**Figure S9. Visualizing correlations between neutralization, linker, and serological properties.**
Correlation plots which 1) have high Spearman significance (p <= 0.05) or 2) fit to a linear (or log-transformed linear) model ($R^2$ >= 0.8) are shown, ordered left to right and top to bottom in terms of significance. Correlations which maintained Spearman significance when outlier SM(PEG)24 was excluded are denoted with a #. The linear (or log-transformed linear) regression model is also shown. Participant groups are colored according to linker, legend is shown.