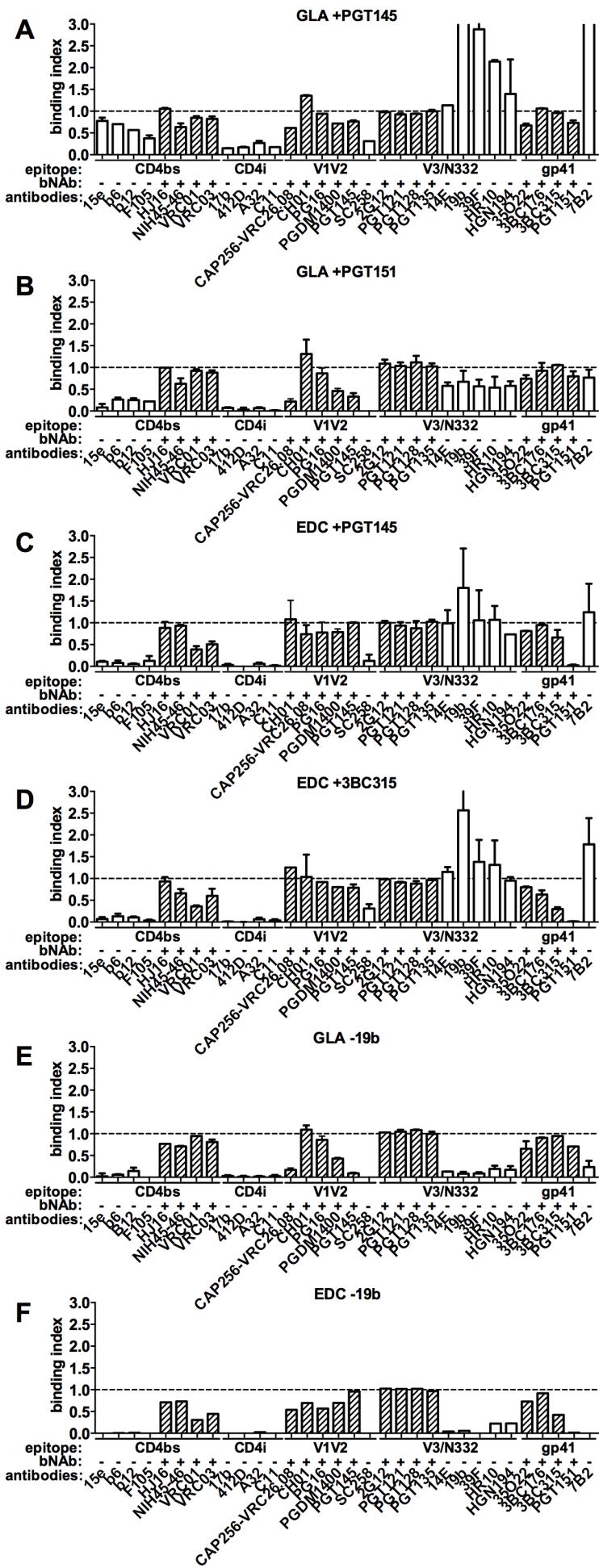


| mAb | GLA | GLA +145 | GLA +151 | GLA -19b | EDC | EDC +145 | EDC +315 | EDC -19b |
|-----------------------------|------------|---------------------|---------------------|---------------------|------------|---------------------|---------------------|---------------------|
| 15e | 0.21 | 0.78 | 0.08 | 0.04 | 0.06 | 0.11 | 0.10 | 0.00 |
| b6 | 0.46 | 0.70 | 0.26 | 0.07 | 0.01 | 0.08 | 0.17 | 0.00 |
| B12 | 0.28 | 0.57 | 0.25 | 0.09 | 0.01 | 0.05 | 0.09 | 0.01 |
| F105 | 0.31 | 0.38 | 0.22 | 0.00 | 0.13 | 0.13 | 0.05 | 0.00 |
| HJ16 | 0.84 | 1.05 | 0.99 | 0.77 | 0.87 | 0.88 | 0.86 | 0.71 |
| NIH45-46 | 0.61 | 0.64 | 0.62 | 0.71 | 0.72 | 0.93 | 0.73 | 0.73 |
| VRC01 | 0.86 | 0.85 | 0.92 | 0.95 | 0.39 | 0.39 | 0.38 | 0.31 |
| VRC03 | 0.94 | 0.82 | 0.88 | 0.77 | 0.20 | 0.51 | 0.72 | 0.45 |
| 17b | 0.12 | 0.15 | 0.07 | 0.03 | 0.07 | 0.03 | 0.01 | 0.00 |
| 412D | 0.20 | 0.17 | 0.03 | 0.00 | 0.27 | 0.00 | 0.00 | 0.00 |
| A32 | 0.15 | 0.27 | 0.06 | 0.01 | 0.02 | 0.05 | 0.09 | 0.02 |
| C11 | 0.19 | 0.17 | 0.01 | 0.02 | 0.18 | 0.01 | 0.02 | 0.00 |
| CAP256- VRC26.08 | 0.13 | 0.61 | 0.22 | 0.17 | 0.60 | 0.74 | 1.25 | 0.54 |
| CH01 | 1.06 | 1.36 | 1.31 | 1.02 | 0.31 | 1.08 | 1.40 | 0.69 |
| PG16 | 0.74 | 0.94 | 0.86 | 0.86 | 0.49 | 0.78 | 0.92 | 0.57 |
| PGDM1400 | 0.36 | 0.72 | 0.46 | 0.43 | 0.67 | 0.79 | 0.80 | 0.70 |
| PGT145 | 0.27 | 0.76 | 0.33 | 0.09 | 0.86 | 1.00 | 0.84 | 0.96 |
| SC258 | 0.00 | 0.31 | 0.00 | 0.00 | 0.07 | 0.13 | 0.24 | 0.00 |
| 2G12 | 0.99 | 0.98 | 1.09 | 1.03 | 0.99 | 1.00 | 0.99 | 1.02 |
| PGT121 | 0.90 | 0.93 | 1.03 | 1.05 | 0.87 | 0.94 | 0.92 | 1.02 |
| PGT128 | 0.97 | 0.94 | 1.12 | 1.08 | 0.94 | 0.87 | 0.84 | 1.02 |
| PGT135 | 1.01 | 1.00 | 1.02 | 0.96 | 0.86 | 1.02 | 0.98 | 0.97 |
| 14E | 0.67 | 1.13 | 0.58 | 0.13 | 0.51 | 0.99 | 1.23 | 0.04 |
| 19b | 0.56 | 3.49 | 0.67 | 0.08 | 0.79 | 1.80 | 3.06 | 0.05 |
| 39F | 0.35 | 2.88 | 0.57 | 0.09 | 0.52 | 1.06 | 1.74 | 0.00 |
| HR10 | 0.63 | 2.14 | 0.54 | 0.19 | 0.38 | 1.07 | 0.92 | 0.22 |

| | | | | | | | | |
|---------------|------|------|------|------|------|------|------|------|
| HGN194 | 0.63 | 1.40 | 0.58 | 0.19 | 0.48 | 0.73 | 0.89 | 0.23 |
| 35022 | 0.80 | 0.67 | 0.74 | 0.60 | 0.70 | 0.81 | 0.81 | 0.73 |
| 3BC176 | 1.00 | 1.06 | 0.93 | 0.90 | 0.84 | 0.95 | 0.70 | 0.92 |
| 3BC315 | 1.04 | 0.96 | 1.05 | 0.95 | 0.60 | 0.66 | 0.33 | 0.42 |
| PGT151 | 0.62 | 0.73 | 0.80 | 0.70 | 0.09 | 0.02 | 0.01 | 0.01 |
| 7B2 | 0.56 | 3.70 | 0.77 | 0.33 | ND | 1.24 | 2.21 | 0.00 |

Supplementary table 1: Complete dataset of binding indices. GLA (GLA) or EDC (EDC) cross-linked material was positively selected with PGT145 (+145), PGT151 (+151) or 3BC315 (+315) or negatively selected with 19b (-19b). Binding indices were calculated compared to unmodified trimers. Data shown are means of all (n=1 for EDC -19b, n=2-6 for all other antigens) independent experiments. Negative values are shown as 0.00. ND: not determined.



Supplementary Fig. 1. Effects on antigenicity of positive and negative selection. BG505 was cross-linked with either GLA or EDC/NHS (EDC) and positively (+) or negatively (-) selected with the indicated antibody. Binding indices were calculated similarly to Fig. 2B and 4C. **(A)** GLA cross-linked trimers were positively selected on a PGT145 column. **(B)** GLA cross-linked trimers were positively selected on a PGT151 column. **(C)** EDC/NHS cross-linked trimers were positively selected on a PGT145 column. **(D)** EDC/NHS cross-linked trimers were positively selected on a 3BC315 column. **(E)** GLA cross-linked trimers were negatively selected on a 19b column. **(F)** EDC/NHS cross-linked trimers were negatively selected on a 19b column.