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## Supplemental information

# Reconstruction of a polyclonal ADCC antibody repertoire from an HIV-1 non-transmitting mother

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**Supplemental Information**

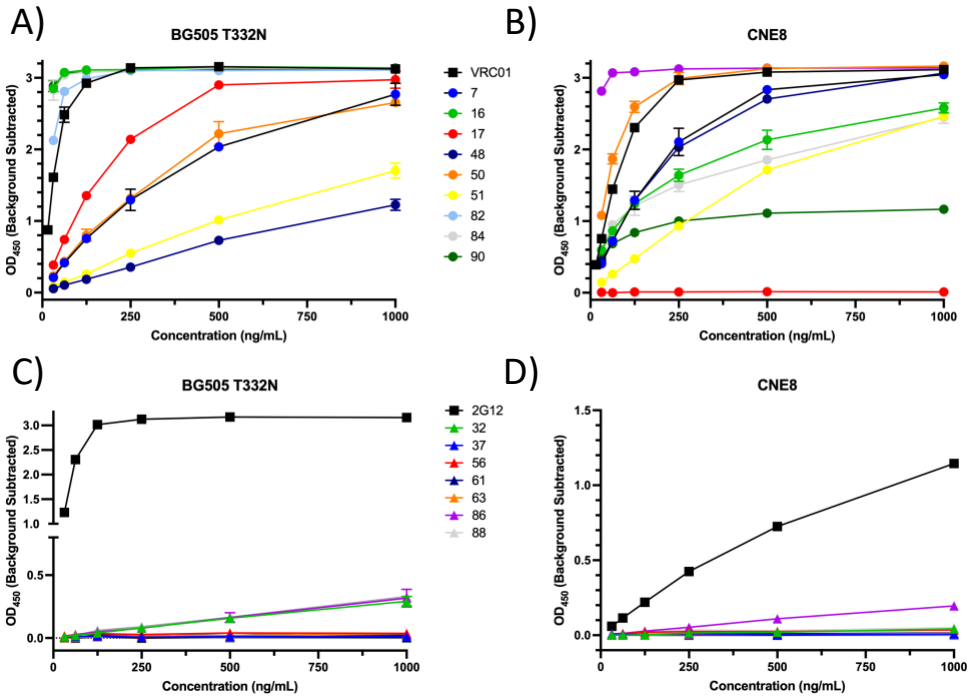


**Figure S1. Representative SOSIP ELISA data, related to Table 1**

Raw background-subtracted ELISA activity (OD450) of the mAbs plotted against antibody concentration (ng/mL). Gp120- (A, B) and gp41-specific (C-D) mAbs were tested for ELISA activity against BG505 T332N (A,C) or CNE8 (B,D) SOSIP trimer-coated wells. Data are representative of two independent experiments. Gp120-specific positive control ADCC mAbs were C11 and A32 and gp41-specific positive controls were QA255.067 and 167-D. 2G12 was also included as an Env trimer-specific positive control. 2G12 activity was much higher than the other mAbs in (C) so the axis was split to more clearly display activity for all mAbs.

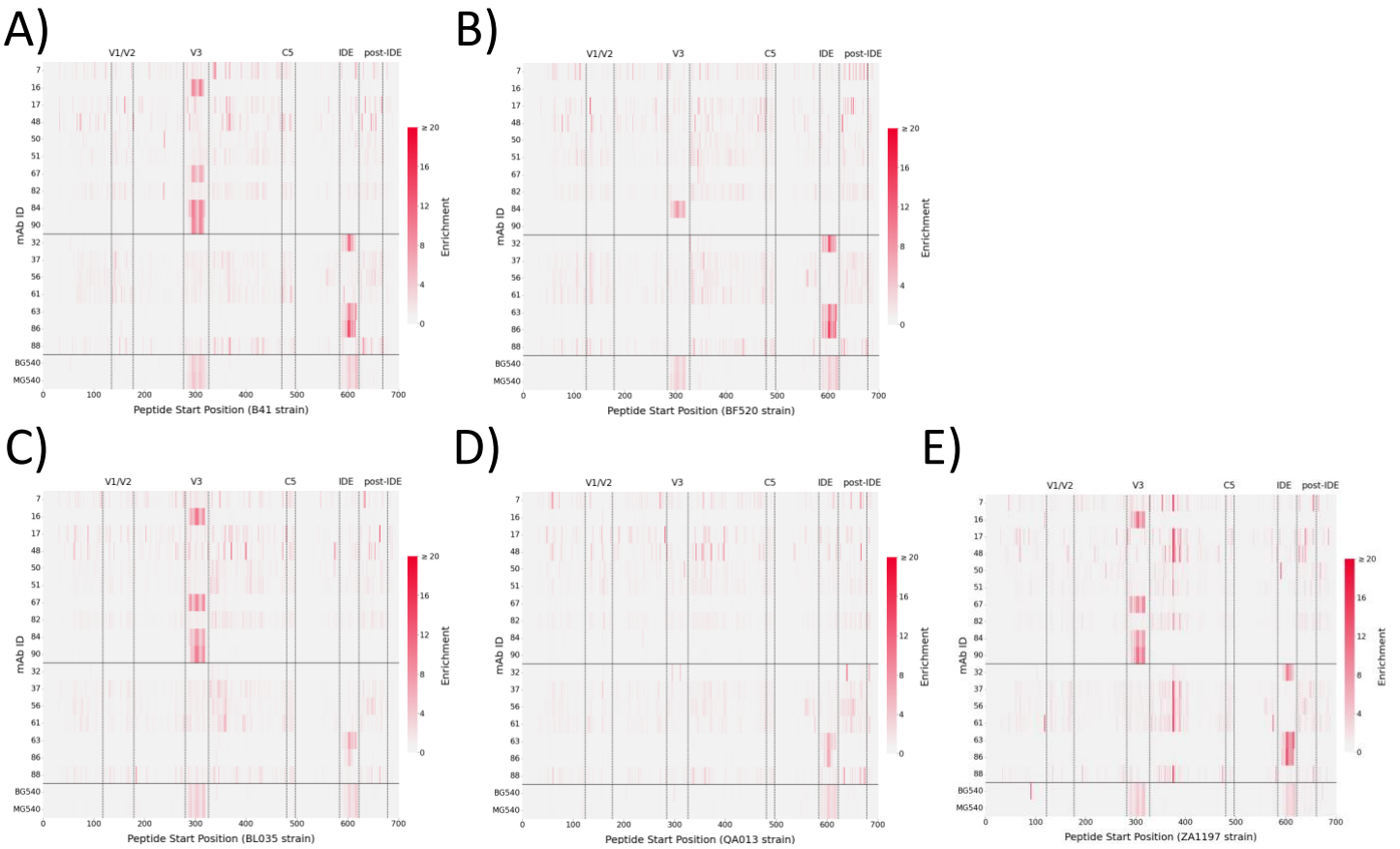


**Figure S2. PhIP-Seq data for additional library strains, related to Figure 4**

Peptide enrichment (red intensity) data for Env strains B41 (A), BF520 (B), BL035 (C), QA013 (D), and ZA1197 (E) are shown for representative MG540 mAbs. Full HIV strain information provided in Methods. Peptides ordered based on start position of the N-terminal amino acid.

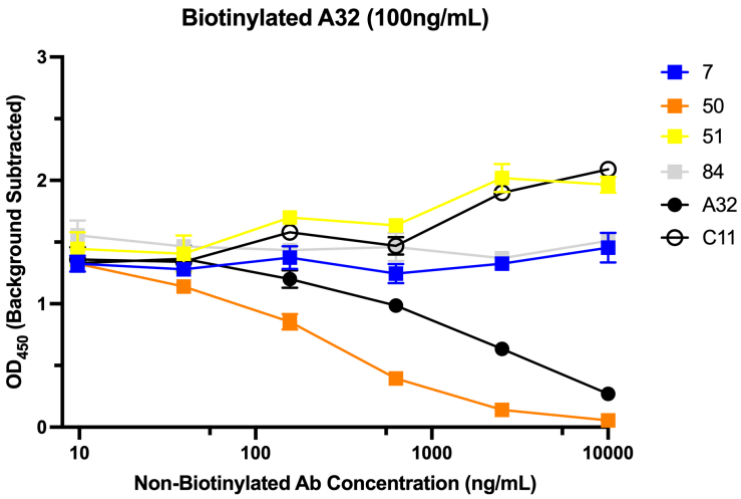


**Figure S3. Enhancement of A32 binding in competition ELISA, related to Figure 4**

Background-subtracted ELISA activity of 100 ng/mL biotinylated A32 mAb is shown for BG505 gp120-coated wells pre-incubated with the indicated MG540 and control mAbs at the indicated concentrations (x-axis). Data is from a single experiment, with two technical replicates per condition.

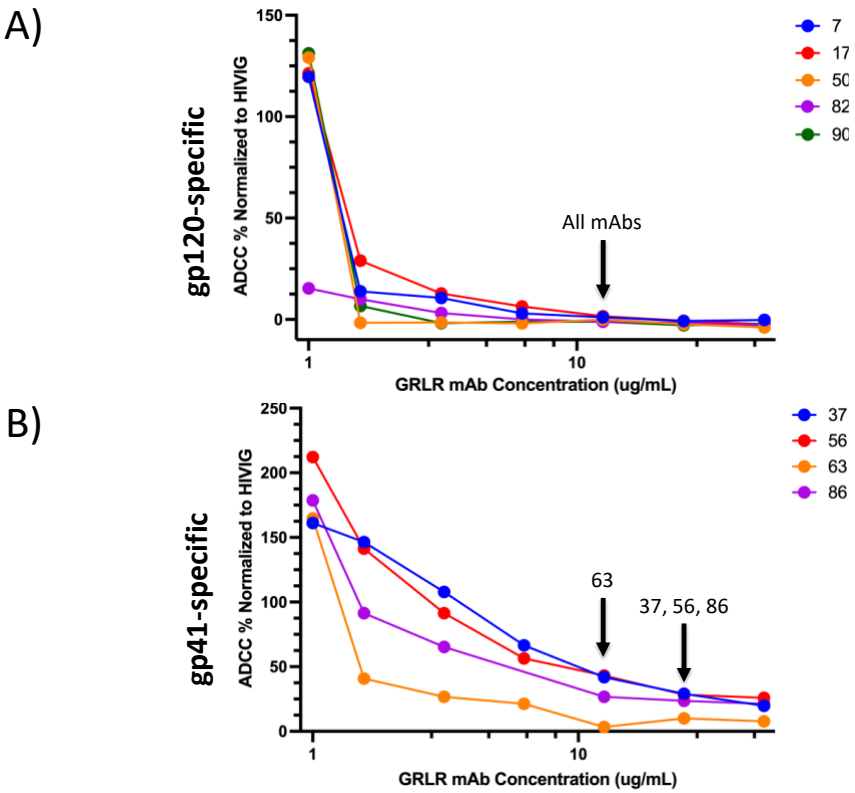


**Figure S4. Validation of GRLR variant competition of wildtype mAb ADCC, related to Figure 5**

Relative ADCC of the indicated gp120- (A) or gp41-specific (B) wildtype mAbs in the presence or absence of the corresponding GRLR mAb variant at the indicated concentration (µg/mL). WT mAbs were added to target cells at their $EC_{95}$ concentrations. A placeholder value of 1 was used for the no competition condition to allow for plotting on a logarithmic scale. The GRLR mAb concentrations selected for subsequent competition experiments are indicated by labeled arrows. Data are representative of two replicate experiments.

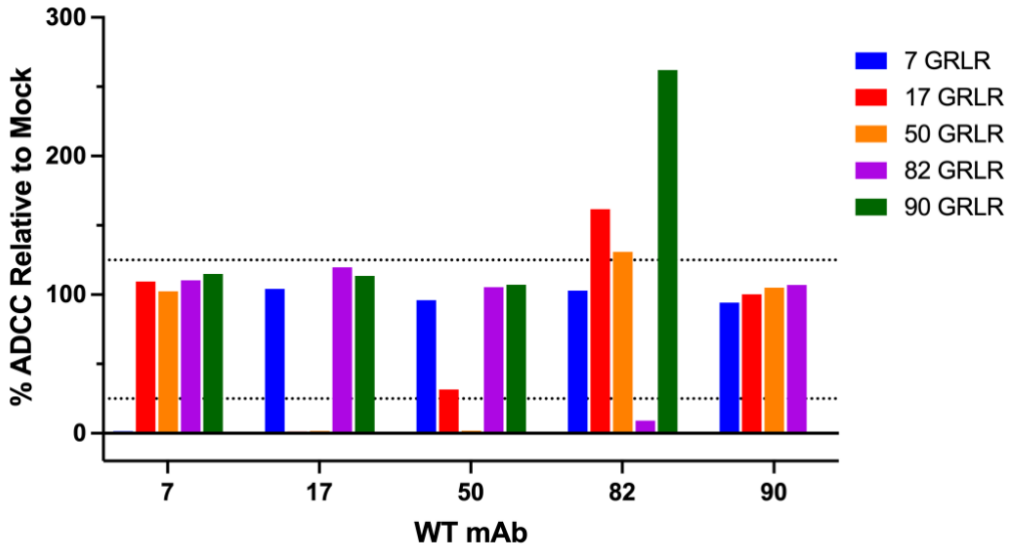


**Figure S5. Cross-clonotype competition or enhancement by GRLR mAbs at $EC_{50}$ wildtype mAb concentrations, related to Figure 5**

Additional competition experiments using wildtype antibodies at the corresponding $EC_{50}$ concentration. Data points indicate the relative ADCC activity of the indicated wildtype antibody in the presence of GRLR variants normalized to the activity of the mock (no GRLR antibody) condition. Dashed lines indicate 25% and 125% of mock competition ADCC. Data are from two replicate experiments.

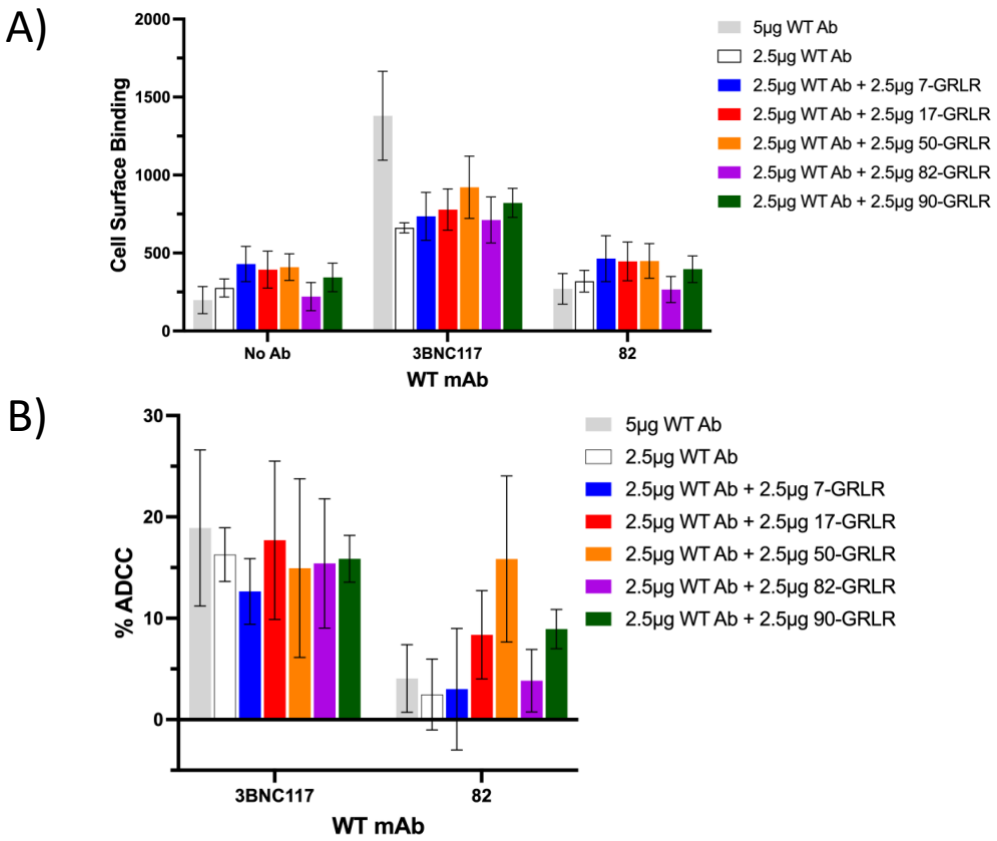


**Figure S6. GRLR mAb ADCC enhancement experiments using BG505-infected CD4⁺ T cells, related to Figure 5**

Surface antibody binding (A) and ADCC (B) of indicated mAbs against BG505 T332N-infected primary CD4⁺ T cells in the presence or absence of the indicated GRLR mAbs. The antibody listed at the bottom indicates the wildtype antibody tested. The right legend indicates the combination of mAbs tested and their concentrations. 3BNC117 was included as a positive ADCC control. Data are the average and standard deviation of five replicate experiments, except for MG540.7-GRLR and .17-GRLR, which were only tested three times.

| MG540 P34 Ab characteristics | | Neutralization | | | | | |
|---|---|---|---|---|---|---|---|
| | | N/A | Tier 1 | | Tier 2 | | |
| | | | Clade B | Clade A | Clade A | Clade C | Clade D |
| **Family** | | **SIV** | **SF162** | **Q461.D1** | **BG505.C2 T332N** | **QC406.F3** | **QD435.A4** |
| plasma | **MG540 P34 plasma** | <1:100 | >3200 | 528.0 | <1:100 | <1:100 | <1:100 |
| 1 | MG540.7 | >20 | >20 | >20 | >20 | NT | NT |
| 2 | MG540.16 | >20 | <0.625 | 3.1 | >20 | >20 | >20 |
| | MG540.67 | >20 | >20 | 5.4 | >20 | >20 | >20 |
| | MG540.90 | >20 | <0.625 | 2.7 | >20 | >20 | >20 |
| 3 | MG540.17 | >20 | >20 | >20 | >20 | NT | NT |
| 4 | MG540.48 | >20 | >20 | >20 | >20 | NT | NT |
| 5 | MG540.50 | >20 | >20 | >20 | >20 | NT | NT |
| 6 | MG540.51 | >20 | >20 | >20 | >20 | NT | NT |
| 7 | MG540.82 | >20 | >20 | >20 | >20 | NT | NT |
| 8 | MG540.84 | >20 | <0.625 | 0.8 | >20 | >20 | >20 |
| 9 | MG540.32 | >20 | >20 | >20 | >20 | NT | NT |
| | MG540.86 | >20 | >20 | >20 | >20 | NT | NT |
| 10 | MG540.37 | >20 | >20 | >20 | >20 | NT | NT |
| 11 | MG540.56 | >20 | >20 | >20 | >20 | NT | NT |
| 12 | MG540.61 | >20 | >20 | >20 | >20 | NT | NT |
| 13 | MG540.63 | >20 | >20 | >20 | >20 | NT | NT |
| 14 | MG540.88 | >20 | >20 | >20 | >20 | NT | NT |

**Table S1. Heterologous virus neutralization by MG540 mAbs and plasma, related to Table 1**

Neutralization of heterologous HIV viruses by MG540 P34 plasma (top row) and MG540 mAbs. Reported $IC_{50}$ values are the reciprocal dilution of plasma or mAb concentration in µg/mL. Antibodies that did not neutralize the Tier 1 viruses SF162 and Q461.D1 were not tested against additional viruses. Data are the average of two replicate experiments. NT: not tested.