Supplementary Information for

Quantifying the contribution of Fc-mediated effector functions to the antiviral activity of anti-HIV-1 IgG1 antibodies in vivo

Pengfei Wang¹, Mili R. Gajjar¹, Jian Yu¹, Neal N. Padte¹, Agegnehu Gettie¹, James L. Blanchard², Kasi Russell-Lodrigue², Laura E. Liao³, Alan S. Perelson³, Yaoxing Huang¹, & David D. Ho¹*

¹Aaron Diamond AIDS Research Center, Columbia University Vagelos College of Physicians and Surgeons, 701 W. 168th Street, New York, NY 10032, USA.

²Tulane National Primate Research Center, Covington, LA 70433, USA.

³Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, NM 87545, USA.

*To whom correspondence should be addressed: dh2994@cumc.columbia.edu.

This PDF file includes:

Figures S1 to S5
Tables S1 to S2

www.pnas.org/cgi/doi/10.1073/pnas.2008190117
\[
\frac{dT^*}{dt} = kVT - \delta T^* \quad (1)
\]

\[
\frac{dV}{dt} = N\delta T^* - cV \quad (2)
\]

Fig. S1. Mathematical equations describing the dynamics of productively HIV-1-infected CD4 T cells (T*) and plasma virus particles (V). T, uninfected but susceptible CD4 T cells; k, infectivity constant of virions for susceptible CD4 T cells; \( \delta \), the death rate of productively infected CD4 T cells; c, HIV particle clearance rate; and N, the burst size or the number of virions produced by an infected CD4 T cell over its lifespan.
Fig. S2. *In vitro* characterization and *in vivo* PK profiles of 117/1400 variants and 10E8.2/iMab used for humanized mouse experiments. (A) A schematic depiction of 117/1400. (B) Antiviral coverage of 117/1400 and its parental antibodies against a panel of 118 multi-clade HIV-1 strains. (C) Size exclusion chromatography (SEC) analysis of 117/1400 variants. (D) *In vitro* neutralization of HIV-1JR-CSF by 117/1400 variants and 10E8.2/iMab. (E) Biacore sensograms of 117/1400 variants binding to human FcγRs. (F and G) PK profiles of 117/1400 variants and 10E8.2/iMab in the first (F) and second (G) experiment in HIV-1-infected humanized mice.
Fig. S3. Plasma viral load before antibody administration in the first humanized mouse experiment.
Fig. S4. *In vitro* characterization and *in vivo* PK profiles of 117/1400 variants and 10E8.4/iMab used for rhesus macaque experiments. (A) Experimental schema for the viral dynamics experiment performed in rhesus macaques. Open purple circles indicate time points when insufficient PBMCs were collected from some of the monkeys to measure cell-associated SHIV RNA. (B) SEC analysis of WT and Null variants of 117/1400 that were separately manufactured at Wuxi Biologics, Inc. (C) *In vitro* neutralization of SHIV.A.BG505 by 117/1400 variants, and by 10E8.4/iMab. (D and E)
Biacore sensograms of 117/1400 WT (D) and Null variants (E) binding to rhesus FcγRs. (F) PK profiles of 117/1400 variants and 10E8.4/iMab in the two groups of macaques. (G and H) Rhesus anti-human IgG response against 117/1400 (G) or 10E8.4/iMab (H) detected in serum by ELISA.
Fig. S5. *In vitro* characterization and *in vivo* PK profiles of N6-LS-WT and N6-LS-Null in the final humanized mouse experiment. (A) SEC analysis of N6-LS variants. (B) *In vitro* neutralization against HIV-1pNL(AD8) by N6-LS variants. (C) Biacore sensograms of N6-LS variants binding to human FcγRIIIa. (D) PK profiles of N6-LS-WT and N6-LS-Null in the final humanized mouse experiment.
### Table S1: Baseline characteristics of HIV-1-infected humanized mice

| 1st Experiment |
|----------------|
| **WT Group** |
| Mouse # | Gender | % huCD45 | % CD4 | VL (copies /mL) |
| 50 | Female | 72.1 | 38.8 | $2.17 \times 10^5$ |
| 51 | Female | 81.6 | 65.7 | $5.65 \times 10^4$ |
| 57 | Female | 77.2 | 54.4 | $1.66 \times 10^5$ |
| 61 | Male | 54.8 | 63.5 | $1.53 \times 10^5$ |
| 67 | Female | 80.5 | 59.3 | $1.37 \times 10^5$ |
| 70 | Male | 62.0 | 68.8 | $1.03 \times 10^5$ |
| **Mean** | 71.4 | 58.4 | $1.39 \times 10^5$ |
| **Median** | | | $1.45 \times 10^5$ |

| **Null Group** |
| Mouse # | Gender | % huCD45 | % CD4 | VL (copies /mL) |
| 54 | Male | 16.3 | 69.6 | $2.40 \times 10^5$ |
| 64 | Female | 77.9 | 49.9 | $1.20 \times 10^5$ |
| 68 | Female | 78.8 | 49.2 | $2.82 \times 10^5$ |
| 72 | Female | 71.2 | 74.4 | $3.94 \times 10^4$ |
| 73 | Female | 57.9 | 55.3 | $3.21 \times 10^4$ |
| 79 | Female | 80.7 | 60.5 | $1.18 \times 10^5$ |
| **Mean** | 60.4 | 59.7 | $1.39 \times 10^5$ |
| **Median** | | | $1.19 \times 10^5$ |

| **GASDALIE Group** |
| Mouse # | Gender | % huCD45 | % CD4 | VL (copies /mL) |
| 11 | Male | 73.8 | 57.0 | $9.14 \times 10^4$ |
| 14 | Female | 91.6 | 53.4 | $1.11 \times 10^5$ |
| 29 | Female | 28.8 | 78.5 | $1.36 \times 10^5$ |
| 91 | Female | 76.3 | 52.5 | $1.11 \times 10^4$ |
| **Mean** | 67.6 | 60.4 | $3.94 \times 10^5$ |
| **Median** | | | $1.01 \times 10^5$ |

| 2nd Experiment |
|----------------|
| **WT Group** |
| Mouse # | Gender | % huCD45 | % CD4 | VL (copies /mL) |
| 3 | Female | 94.1 | 63.8 | $2.10 \times 10^5$ |
| 4 | Female | 89.8 | 44.0 | $6.24 \times 10^4$ |
| 32 | Female | 77.5 | 40.9 | $1.20 \times 10^4$ |
| 59 | Male | 50.1 | 55.5 | $7.32 \times 10^5$ |
| 82 | Male | 75.4 | 52.0 | $1.71 \times 10^5$ |
| **Mean** | 77.4 | 51.2 | $2.38 \times 10^5$ |
| **Median** | | | $1.71 \times 10^5$ |

| **Null Group** |
| Mouse # | Gender | % huCD45 | % CD4 | VL (copies /mL) |
| 23 | Female | 75.9 | 61.6 | $1.37 \times 10^5$ |
| 60 | Male | 68.3 | 15.4 | $1.55 \times 10^5$ |
| 88 | Female | 80.6 | 50.6 | $2.97 \times 10^5$ |
| 89 | Male | 56.3 | 48.1 | $4.32 \times 10^4$ |
| **Mean** | 70.3 | 43.9 | $1.58 \times 10^5$ |
| **Median** | | | $1.46 \times 10^5$ |

| **GASDALIE Group** |
| Mouse # | Gender | % huCD45 | % CD4 | VL (copies /mL) |
| 11 | Male | 73.8 | 57.0 | $9.14 \times 10^4$ |
| 14 | Female | 91.6 | 53.4 | $1.11 \times 10^5$ |
| 29 | Female | 28.8 | 78.5 | $1.36 \times 10^5$ |
| 91 | Female | 76.3 | 52.5 | $1.11 \times 10^4$ |
| **Mean** | 67.6 | 60.4 | $3.94 \times 10^5$ |
| **Median** | | | $1.01 \times 10^5$ |

| 3rd Experiment |
|----------------|
| **WT Group** |
| Mouse # | Gender | % huCD45 | % CD4 | VL (copies /mL) |
| 545 | Male | 61.4 | 49.9 | $5.99 \times 10^6$ |
| 556 | Male | 57.6 | 43.1 | $1.90 \times 10^7$ |
| 557 | Male | 61.1 | 43.6 | $1.76 \times 10^6$ |
| 559 | Female | 47.2 | 34.5 | $3.52 \times 10^6$ |
| 561 | Female | 70.0 | 41.8 | $3.94 \times 10^6$ |
| 562 | Female | 21.4 | 42.2 | $3.79 \times 10^6$ |
| **Mean** | 53.1 | 42.5 | $6.34 \times 10^6$ |
| **Median** | | | $3.86 \times 10^6$ |

| **Null Group** |
| Mouse # | Gender | % huCD45 | % CD4 | VL (copies /mL) |
| 548 | Female | 70.8 | 54.1 | $4.26 \times 10^6$ |
| 549 | Female | 67.8 | 45.0 | $2.55 \times 10^6$ |
| 551 | Female | 59.9 | 47.2 | $4.04 \times 10^6$ |
| 554 | Male | 61.0 | 39.0 | $7.08 \times 10^6$ |
| 555 | Male | 46.6 | 43.7 | $1.38 \times 10^7$ |
| 558 | Male | 48.0 | 57.8 | $5.86 \times 10^6$ |
| **Mean** | 59.0 | 47.8 | $6.36 \times 10^6$ |
| **Median** | | | $4.26 \times 10^6$ |
Table S2: Baseline characteristics of SHIV-infected rhesus macaques

| Animal # | Weight, Kg | Age  | CD4 count (cells / µL) | VL (copies /mL) | TRIM5α allele |
|----------|------------|------|------------------------|-----------------|---------------|
| JK67     | 14.3       | 7.33 | 661                    | $2.14 \times 10^6$ | TFP/Q         |
| JL22     | 15.0       | 7.32 | 534                    | $3.35 \times 10^4$ | TFP/TFP       |
| JT22     | 14.2       | 7.09 | 810                    | $1.25 \times 10^4$ | TFP/Q         |
| JT35     | 16.5       | 7.06 | 647                    | $1.25 \times 10^3$ | TFP/Q         |
| JT52     | 16.0       | 7.07 | 786                    | $1.45 \times 10^4$ | TFP/Q         |
| JV73     | 11.0       | 6.28 | 664                    | $4.07 \times 10^3$ | TFP/TFP       |
| Mean     | 14.5       | 7.03 | 684                    | $3.68 \times 10^5$ |               |
| Median   |            |      |                        | $1.35 \times 10^4$ |               |

| Animal # | Weight, Kg | Age  | CD4 count (cells / µL) | VL (copies /mL) | TRIM5α allele |
|----------|------------|------|------------------------|-----------------|---------------|
| JL72     | 15.5       | 7.30 | 462                    | $3.66 \times 10^3$ | TFP/Q         |
| JM03     | 14.0       | 7.29 | 1042                   | $9.90 \times 10^2$ | TFP/Q         |
| JM71     | 11.7       | 7.28 | 586                    | $3.11 \times 10^4$ | TFP/Q         |
| JV62     | 16.0       | 6.42 | 1193                   | $7.04 \times 10^5$ | TFP/Q         |
| KC46     | 12.1       | 6.28 | 646                    | $2.13 \times 10^4$ | TFP/TFP       |
| KF30     | 14.1       | 6.24 | 560                    | $6.45 \times 10^3$ | TFP/Q         |
| Mean     | 13.9       | 6.80 | 748                    | $1.28 \times 10^5$ |               |
| Median   |            |      |                        | $1.39 \times 10^4$ |               |