Supplemental Information

EXTENDED EXPERIMENTAL PROCEDURES

Analysis of Antibody Framework Amino Acid Replacements versus CDR H3 Length

Antibodies with limited (Table S1A) and broad (Table S1B) neutralizing activity were analyzed for correlations between CDRH3 length and the number of amino acid replacements in the four framework regions (FWR1-4) in heavy chain only, light chain only, and both heavy and light chains. For the broad neutralizers, MPER antibody 10E8 (Huang et al., 2012) was added, and antibodies NIH45-46, 12A12, and 3BNC117 were excluded to avoid skewing of the data because these antibodies were closely related to VRC01, 12A21, and 3BNC60, respectively. The Spearman rank correlation coefficient was used for the analysis.

Analysis of Antibody Framework Amino Acid Replacements versus Interactive Surface Area on Antibody

The following antibodies with broad neutralization activity from Figures 2 and 3 were included in the structural analysis: VRC01 (PDB id: 3ngb [Zhou et al., 2010], PGT128 (3tyg [Pejchal et al., 2011]), 4E10 (2fx7 [Cardoso et al., 2007]), 2G12 (1op5 [Calarese et al., 2003]), 2F5 (1tji [Ofek et al., 2004]), as well as 3BNC117, 12A21 (4jpv and 4jpw, unpublished data), PG16 (4dqo, unpublished data). In addition, structures of antibody-antigen complexes for the following antibodies were included: 10E8 (4g6f [Huang et al., 2012]), VRC-PG04 (3se9 [Wu et al., 2011]), F105 (3h1i [Chen et al., 2009]), 17b (2nxy [Zhou et al., 2007]), 48D (3jwd [Pancera et al., 2010]), 55 (2b4c [Huang et al., 2005]), 447-52D (1q1j [Stanfield et al., 2004]), 2219 (2bi1 [Stanfield et al., 2006]), 537-10D (3ghe [Burke et al., 2009]), D5 (2cmr [Luftig et al., 2006]), 412d (2qad [Huang et al., 2007]), 21c (3lqa [Diskin et al., 2010]), F425-B4e8 (2qsc [Bell et al., 2008]), as well as VRC-PG20 and VRC-CH31 (unpublished data). Antibodies NIH45-46 (3u7y [Diskin et al., 2011]), VRC03 (3se8 [Wu et al., 2011]), and PG9 (3u2s [McLellan et al., 2011]) were excluded to avoid skewing of the data because these antibodies were closely related to VRC01 and PG16. Interactive surface areas were computed using NACCESS (Hubbard and Thornton, 1993) and in-house scripts. The Spearman rank correlation coefficient was used for the analysis. The black line shown in Figure S4C was derived by linear regression of the values labeled in black.

SUPPLEMENTAL REFERENCES

### Figure S1. FWR Mutations in HIV-1-Reactive Antibodies with Limited Neutralizing Activity, Related to Figure 1

Position of FWR mutations in heavy and light chain of 9 investigated antibodies with limited neutralizing activity. Indicated are silent (black) mutations and replacement (red) mutations. Number of replacement mutations within CDR1/2 and FWR1-4 are listed in the two columns at the very right. For 17b only amino acid changes (red) are shown.
Figure S2. Binding and Neutralization Activity of Mature, CDR1/2-GL, FWR-GL and FWR-GLIMGT Antibodies, Related to Figure 2

(A–C) Binding to gp140ELISA (ELISA; left) and neutralization activity (right) of mature antibodies (green) and (A) antibodies with CDR1 and CDR2 reverted to germline (CDR1/2-GL; orange); (B) antibodies with limited neutralization activity and FWRs reverted to germline (FWR-GL, blue) and (C) antibodies with FWRs reverted to germline according to IMGT alignment (FWR-GLIMGT, purple). Neutralization panels on the right compare IC50 values for neutralization of Tier 1 (MW965.26, SF162.LS, Bal.26, SS1196.1, DJ263.8, 6535.3) Tier 2 (RHPA4259.7, SC422661.8, TRO.11, YU2.DG), and Tier 3 (PVO.4) viruses. Neutralization activity is color coded (dark red, IC50 below 0.01 μg/ml; red, 0.01–<0.1 μg/ml; orange, 0.1–<1 μg/ml; light orange, 1–10 μg/ml; yellow: >10 μg/ml; white, IC50 was not achieved up to the tested concentration).
Figure S3. Illustration of Mutations in 2G12 Responsible for Domain Swapping, Related to Figure 2

(A) Ribbon representation of the domain swapped (Fab)2 of 2G12 (PDB code 1OP3). Somatically mutated FWR residues are highlighted in red and yellow, with yellow representing residues that were shown to be important for domain swapping (Huber et al., 2010). Black arrows point to canonical antigen combining sites located at the VH-VL interface of each Fab. Red arrow points to an additional antigen-binding site formed at the VH-VH’ interface (Calarese et al., 2003).

(B) Amino acid sequence of 2G12 and 2G12 FWR-GL. Residues implicated in domain swapping are highlighted in yellow. FWRs and FWR mutations are highlighted in gray and red, respectively.

2G12
QGLIVSGGGLVQ NQGL1SCQVSNFVTQLMLMPHSQDNYASTLFTSSSRFQDGFFVTELDDYDFKQGKRGIDPTVLYIPGQLEWTVQFAYRWCYTIHSSSTYVYQDDLGNLCSADDVSGDYNSLSDSDYQAGK

2G12 FWR-GL
QGLIVSGGGLVQ NQGL1SCQVSNFVTQLMLMPHSQDNYASTLFTSSSRFQDGFFVTELDDYDFKQGKRGIDPTVLYIPGQLEWTVQFAYRWCYTIHSSSTYVYQDDLGNLCSADDVSGDYNSLSDSDYQAGK
Figure S4. Relationships between Framework Amino Acid Replacements and Both Antibody CDRH3 Length and Interactive Surface Area

(A and B) Each symbol represents one HIV-1-directed antibody with limited (triangles) or broad (dots) neutralization activity. No significant correlation (Spearman) was observed for either limited (A) or broad (B) neutralizers when comparing CDRH3 lengths against FWR1-4 amino acid replacements in heavy chain only, light chain only, or both heavy and light chains.

(C) Relationship between antibody interactive surface area and framework amino acid replacements. A significant correlation (Spearman) was observed between the total interactive surface area and the number of amino acid replacements in FWR1-4 for a set of 23 HIV-1-directed antibodies with known antibody-antigen structures. In order to avoid skewing NIH45-46, VRC03, and PG9 (gray dots) were excluded from analysis because these mAbs are clonal members of the included mAbs VRC01 and PG16.
Figure S5. Structure of the V_{H} Domain of 3BNC117 in Its gp120-Bound Conformation, Related to Figure 4

(A and B) gp120-bound 3BNC117 IGVH with CDRs highlighted in green and FWRs in gray as assigned by Kabat (A) and IMGT (B). The side chains of Pro61 (orange) and the 3BNC60 WDFD insertion (red) are highlighted. Pro61 and nearby residues (59–65) differ in the structure of free 3BNC60 due to a crystal contact involving this region (Scheid et al., 2011).
Figure S6. Overview of the Packing in Crystals of 3BNC60 and 3BNC60P61A, Related to Figure 6

3BNC60 (green) and 3BNC60P61A (blue) crystallized in the same space group utilizing nearly identical crystal packing interactions. Residues 59–65 of 3BNC60 (red), which deviate from the canonical variable domain fold, contribute to packing interactions in the 3BNC60 crystals (pink ovals). Although the 3BNC60P61A crystal packing is almost identical, the extra hydrogen bond that Ala61 forms with the C’ β strand (Figure 6A) prevents residues 59–65 of 3BNC60P61A from adopting a similar conformation and interacting with a crystallographic neighbor.