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Supplemental Information

A Strain-Specific Inhibitor

of Receptor-Bound HIV-1

Targets a Pocket near the Fusion Peptide

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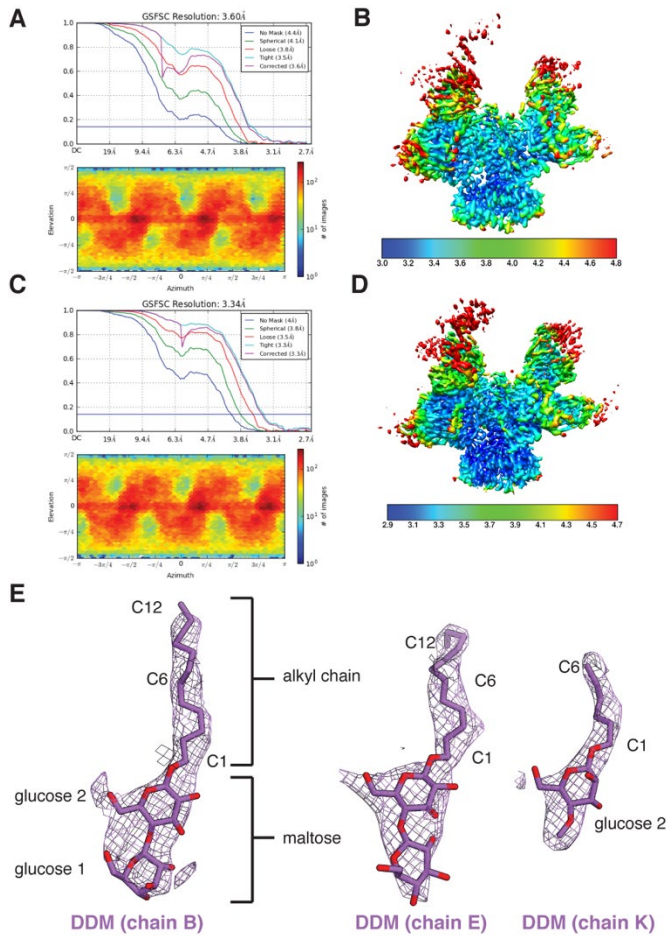


Fig. S1. Cryo-EM maps and models of detergent-bound HIV Env in complex with receptor. Related to figure 1. (A) Fourier shell correlation (*top*) and distribution plot of particle orientations (*bottom*) and (B) local resolution estimates (colored by Å) of C1 reconstruction of B41-CD4-17b. (C) Fourier shell correlation (*top*) and distribution plot of particle orientations (*bottom*) and (D) local resolution estimates (colored by Å) of C3 reconstruction of B41-CD4-17b. (E) Density and modeled atoms for DDM in three protomers of the asymmetric reconstruction of B41-CD4-17b.

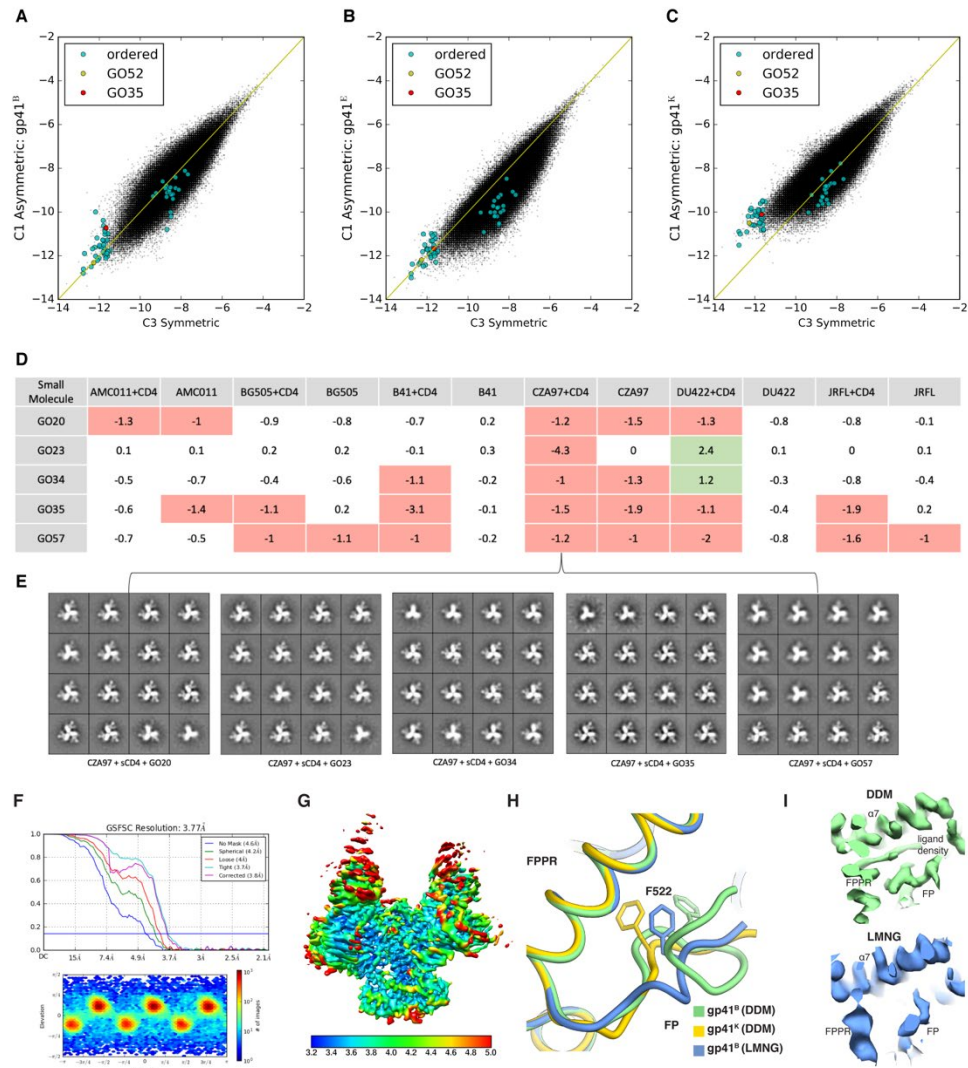


Fig. S2. Selection of small molecule candidates by *in silico* screening and differential scanning fluorimetry. Related to figure 2. Plot of scores obtained in the C1 asymmetric structure (y-axis) against scores obtained in the C3 symmetric structure (x-axis). Each of the three panels corresponds to a different FP conformation as represented in **Fig. 1A**: **(A)** gp41^B, **(B)** gp41^E and **(C)** gp41^K. **(D)** Top 5 candidates based on a selection criteria of a ΔT_m value equal or greater than $\pm 1.0^\circ\text{C}$ against at least 2 different Env genotypes, in the presence or absence of sCD4. **(E)** Negative-stain 2D class averages of CZA97 SOSIP+sCD4 complexed with the top 5 candidates in **(D)** reveals that changes in stability are not due to trimer dissociation/denaturation. **(F)** Fourier shell correlation (*top*) and distribution plot of particle orientations (*bottom*) and **(G)** local resolution estimates (colored by Å) of C3 reconstruction of B41-CD4-17b frozen with LMNG. **(H)** Comparison of fusion peptides from B41-CD4-17b frozen with either DDM (asymmetric model) or LMNG. **(I)** Comparison of EM map (contoured at 1.5σ) near the ligand binding pocket of B41-CD4-17b frozen with either DDM (green) or LMNG (blue).

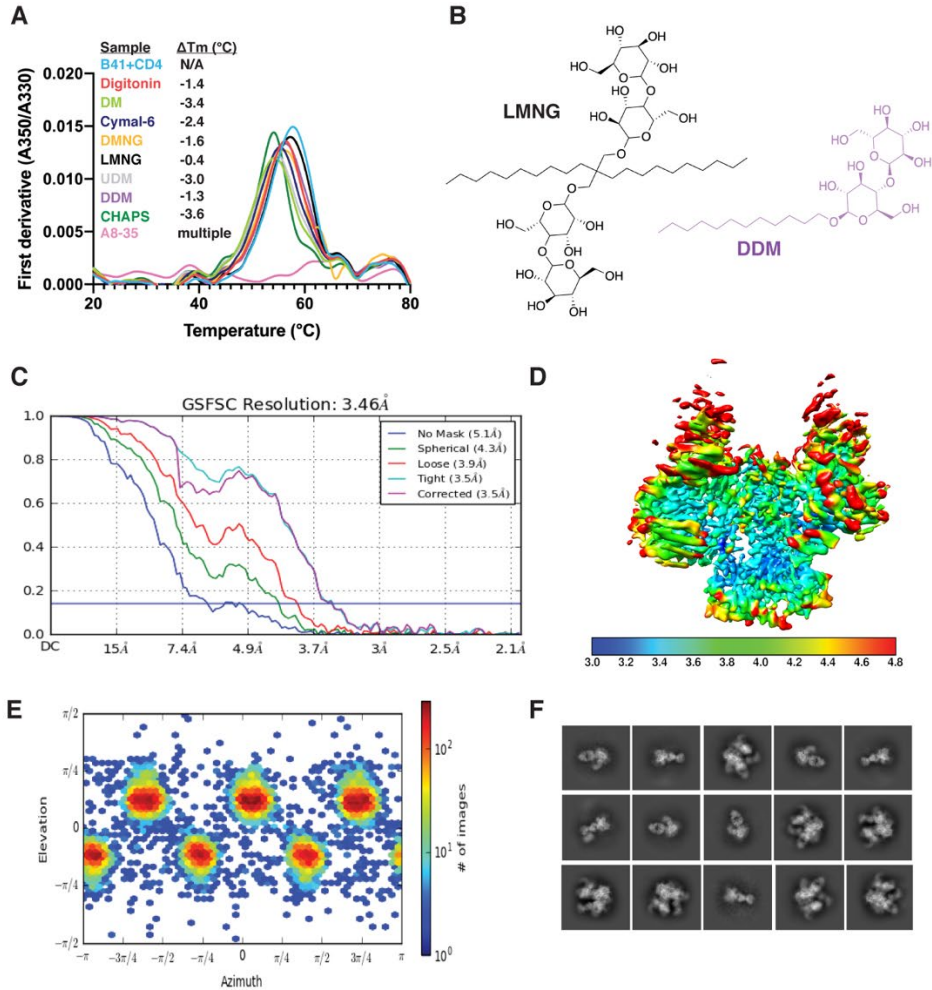


Fig. S3. Targeting the DDM-pocket of gp41 with small molecules. Related to figure 3. (A) Change in T_m of B41 SOSIP in the presence of various detergents. **(B)** Comparison of DDM and LMNG structures. **(C)** Global Fourier shell correlation (FSC), **(D)** side view of the final 3D reconstruction colored by local resolution estimates (in Å), and **(E)** distribution plot of particle orientations of B41+CD4+17b+GO35. **(F)** Select 2D class averages of B41+CD4+17b+GO35.

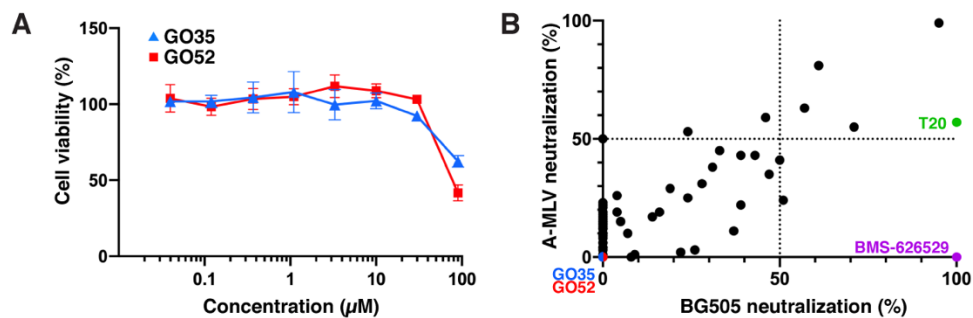


Fig. S4. Cytotoxicity and neutralization assay profiles of small molecules. Related to figure 4. (A) Cytotoxicity measurements of GO35 and GO52. Assays performed as duplicates. Mean values plotted with standard deviation represented by vertical bars. (B) Neutralization activity of 80 small molecules against BG505 N332 HIV-1 and A-MLV viruses. All molecules tested at 30 μ M final concentration. Assay performed as duplicates ($N=2$) and mean value is plotted. BMS-626529 and T20 (enfuvirtide) are known HIV-1 fusion inhibitors included as controls.

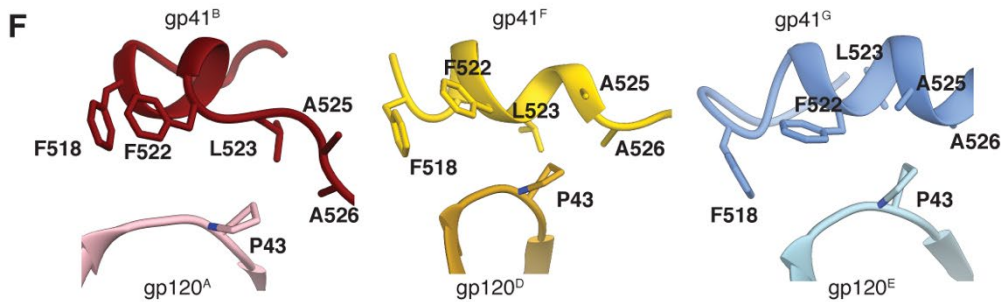
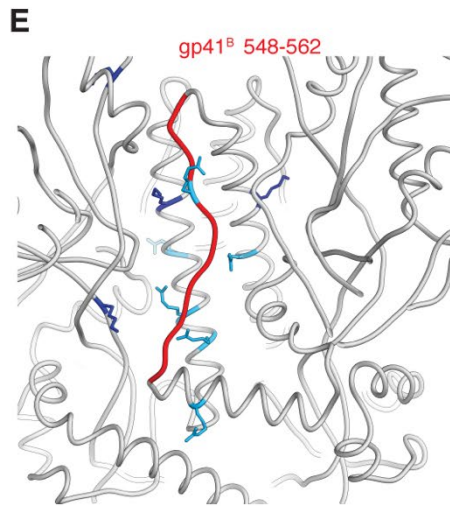
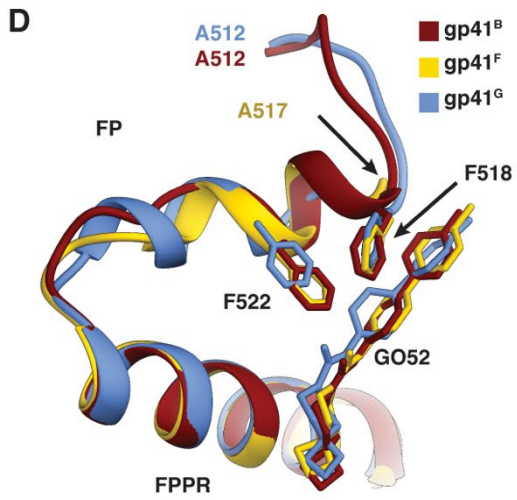
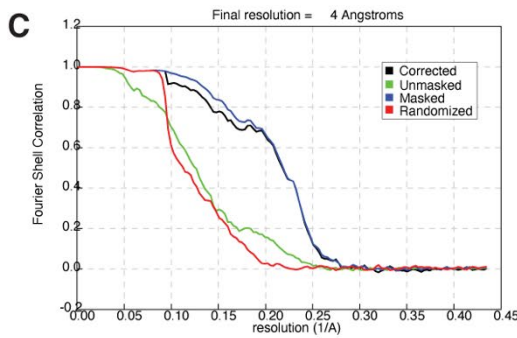
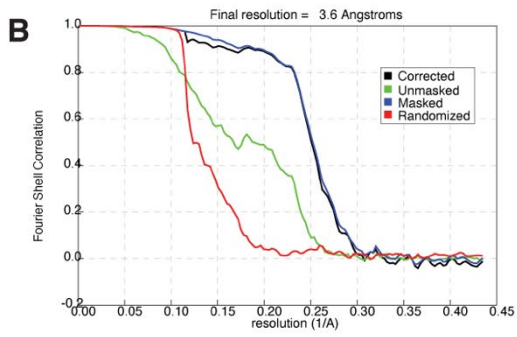
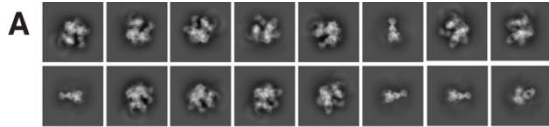


Fig. S5. Cryo-EM reconstructions of B41+CD4+17b+GO52. Related to figure 5. (A) Select 2D class averages of B41+CD4+17b+GO52. Global Fourier shell correlations (FSC; *left*), local resolution estimates (colored by resolution in Å; *middle*) and distribution plot of particle orientations (*right*), of the (B) C3 symmetric and (C) asymmetric reconstructions of B41+CD4+17b+GO52. (D) Superimposition of the 3 asymmetric gp41 chains of (C) with a focus on the FP and FPPR regions. (E) Lysine (dark blue) and arginine (light blue) residues within 10 Å of gp41 HR1 region 548-562. (F) Comparison of P43 (gp120) interactions with the FP in the three asymmetric protomers of (C).

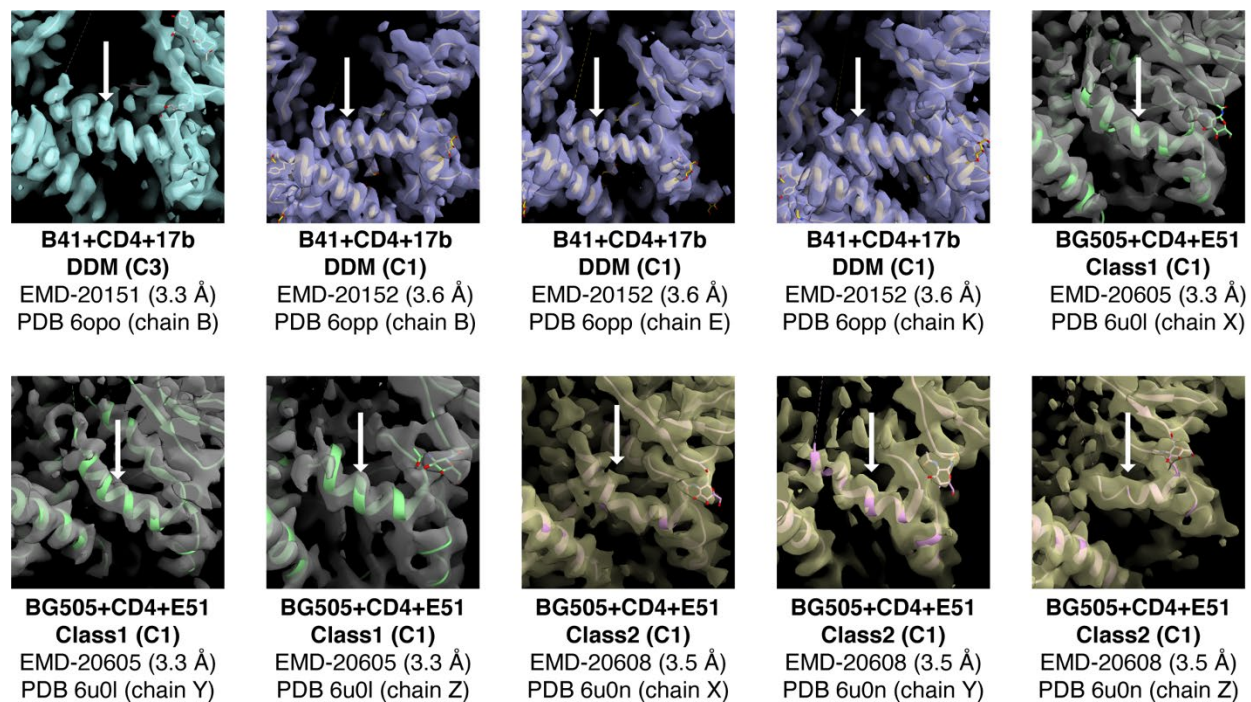


Fig. S6. The B41 binding FPPR is more ordered than in BG505. Related to figure 6. Comparison of CD4- and DDM-bound C3-symmetric and asymmetric B41 cryo-EM maps with asymmetric reconstructions of CD4-bound BG505, with special focus on the FPPR (denoted by a white arrow).

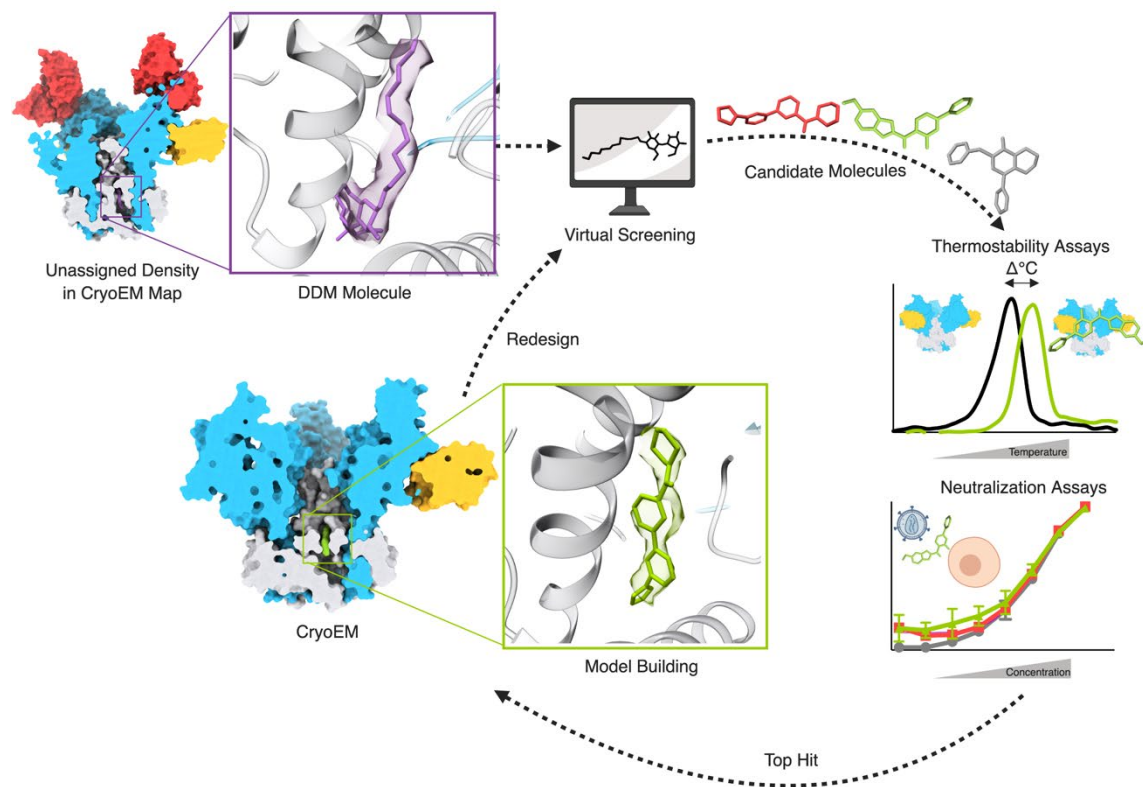


Fig. S7. A schematic of iterative drug design using a combination of cryo-EM, virtual screening, and assays. Related to figures 1, 2, 3, 4, and 5. Created with BioRender.com.

