Cell Reports, Volume *41*

Supplemental information

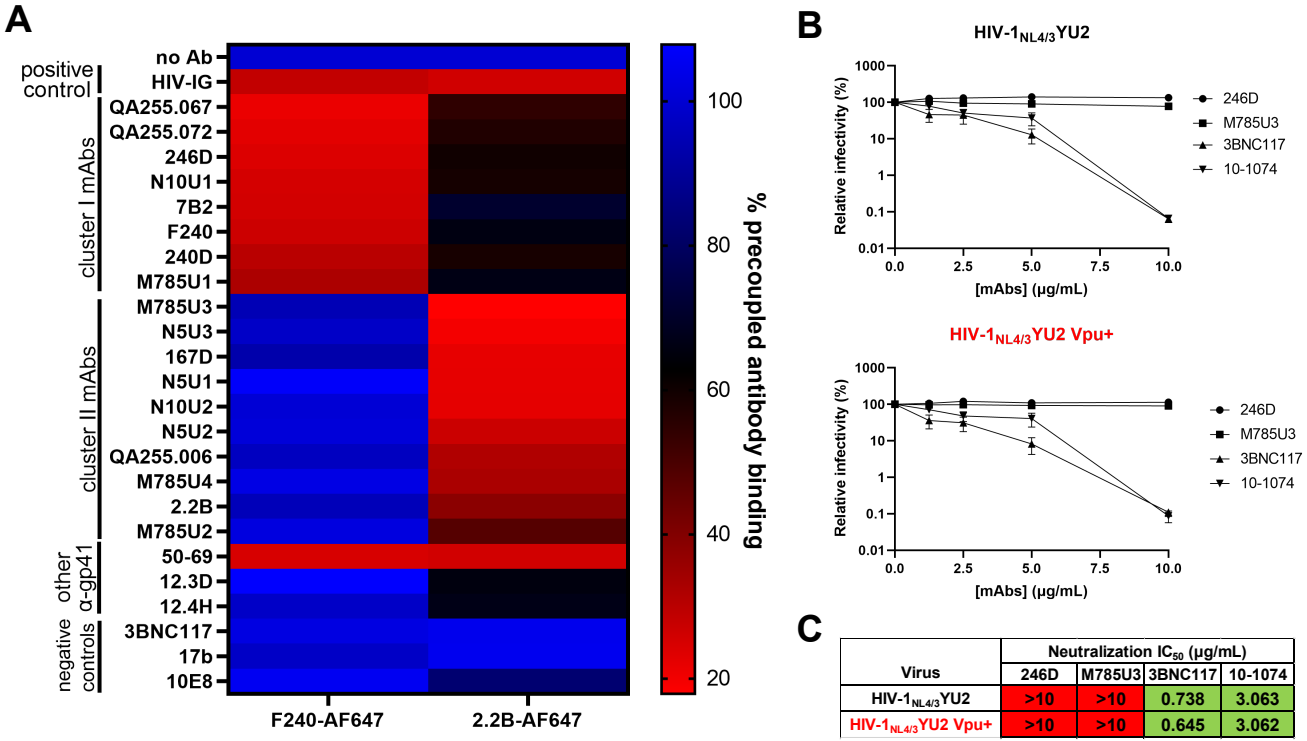
HIV-1 Vpu restricts Fc-mediated effector functions *in vivo*

Jérémie Prévost, Sai Priya Anand, Jyothi Krishnaswamy Rajashekar, Li Zhu, Jonathan Richard, Guillaume Goyette, Halima Medjahed, Gabrielle Gendron-Lepage, Hung-Ching Chen, Yaozong Chen, Joshua A. Horwitz, Michael W. Grunst, Susan Zolla-Pazner, Barton F. Haynes, Dennis R. Burton, Richard A. Flavell, Frank Kirchhoff, Beatrice H. Hahn, Amos B. Smith III, Marzena Pazgier, Michel C. Nussenzweig, Priti Kumar, and Andrés Finzi

Table S1. Cohort of HIV-1-infected individuals. Related to Figure 1.

Group	Gender		Age (years)	Days since infection	Days since ART	Days between infection and ART	Viral load (copies/mL)	CD4 count (cells/mm^3)
	Male	Female						
Group 1 0-90 days untreated	10	0	40 (18-55)	68 (42-97)	N/A	N/A	60848 (132-391113)	470 (430-829)
Group 2 91-180 days untreated	10	0	38 (20-58)	135 (109-177)	N/A	N/A	21663 (6641-195302)	650 (420-1235)
Group 3 181-365 days untreated	10	0	31 (24-45)	240 (203-314)	N/A	N/A	27785 (1866-260852)	490 (230-770)
Group 4 2-5 years untreated	11	1	36 (23-54)	1158 (856-1810)	N/A	N/A	29234 (14255-809600)	421 (200-1311)
Group 5 2-5 years ART-treated	7	1	37 (23-60)	983 (794-1570)	690 (19-879)	428 (192-986)	50 (40-3337)	615 (170-1149)

ART :antiretroviral therapy
N/A : not applicable



Virus	Neutralization IC_{50} (µg/mL)			
	246D	M785U3	3BNC117	10-1074
HIV-$1_{NL4/3}$YU2	>10	>10	0.738	3.063
HIV-$1_{NL4/3}$YU2 Vpu+	>10	>10	0.645	3.062

Figure S1. Classification of anti-gp41 non-neutralizing antibodies in two main clusters. Related to Figures 3 and 5.
(**A**) The binding of Alexa Fluor 647 (AF647)-precoupled anti-gp41 cluster I F240 mAb or anti-gp41 cluster II 2.2B mAb was evaluated on HIV-$1_{NL4/3}$YU2-infected cells in presence of a panel of unlabeled anti-gp41 nnAbs to determine epitope cross-competition. A pool of purified immunoglobulins from HIV-1-infected individuals (HIV-IG) was used as a positive control. Monoclonal antibodies with known non-competing epitopes (3BNC117, 17b, 10E8) were used as negative controls. (**B**) Lentiviral particles were produced from HIV-$1_{NL4/3}$YU2 IMC expressing Vpu or not. Viruses were incubated with serial dilutions of anti-Env mAbs (246D, M785U3, 3BNC117, 10-1074) at 37°C for 1 h prior to infection of TZM-bl target cells. The infectivity at each Ab concentration tested is shown as the percentage of infection without Ab for each virus. Quadruplicate samples were analyzed in each experiment. The data shown are the means of results obtained in three independent experiments. Error bars indicate means ± the SEM. Neutralization half maximal inhibitory concentration (IC_{50}) values are summarized in (**C**).

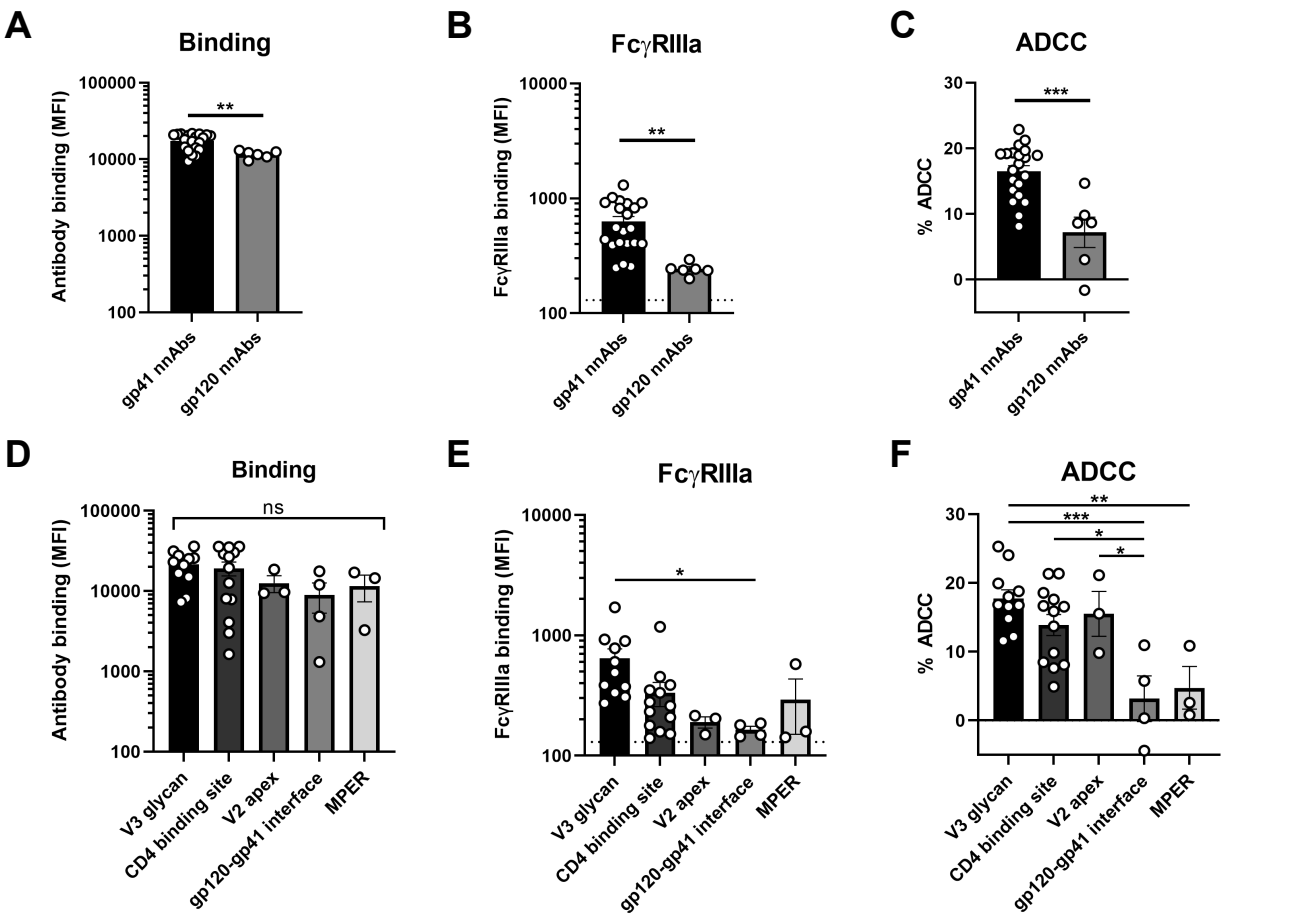


Figure S2. Epitope specificity dictates anti-Env ADCC responses mediated by nnAbs and bNAbs. Related to Figures 3 and 4. (**A-F**) Levels of antibody binding, FcγRIIIa binding and ADCC responses mediated by (**A-C**) nnAbs and (**D-F**) bNAbs as classified by epitope specificity (gp41 nnAbs, gp120 nnAbs; V3 glycan, CD4-binding site, V2 apex, gp120-gp41 interface, MPER). Statistical significance was tested using (**A-C**) an unpaired t test or a Mann-Whitney U test and (**D-F**) a one-way ANOVA with a Holm-Sidak post-test or a Kruskal-Wallis test with a Dunn's post-test based on statistical normality (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, nonsignificant).

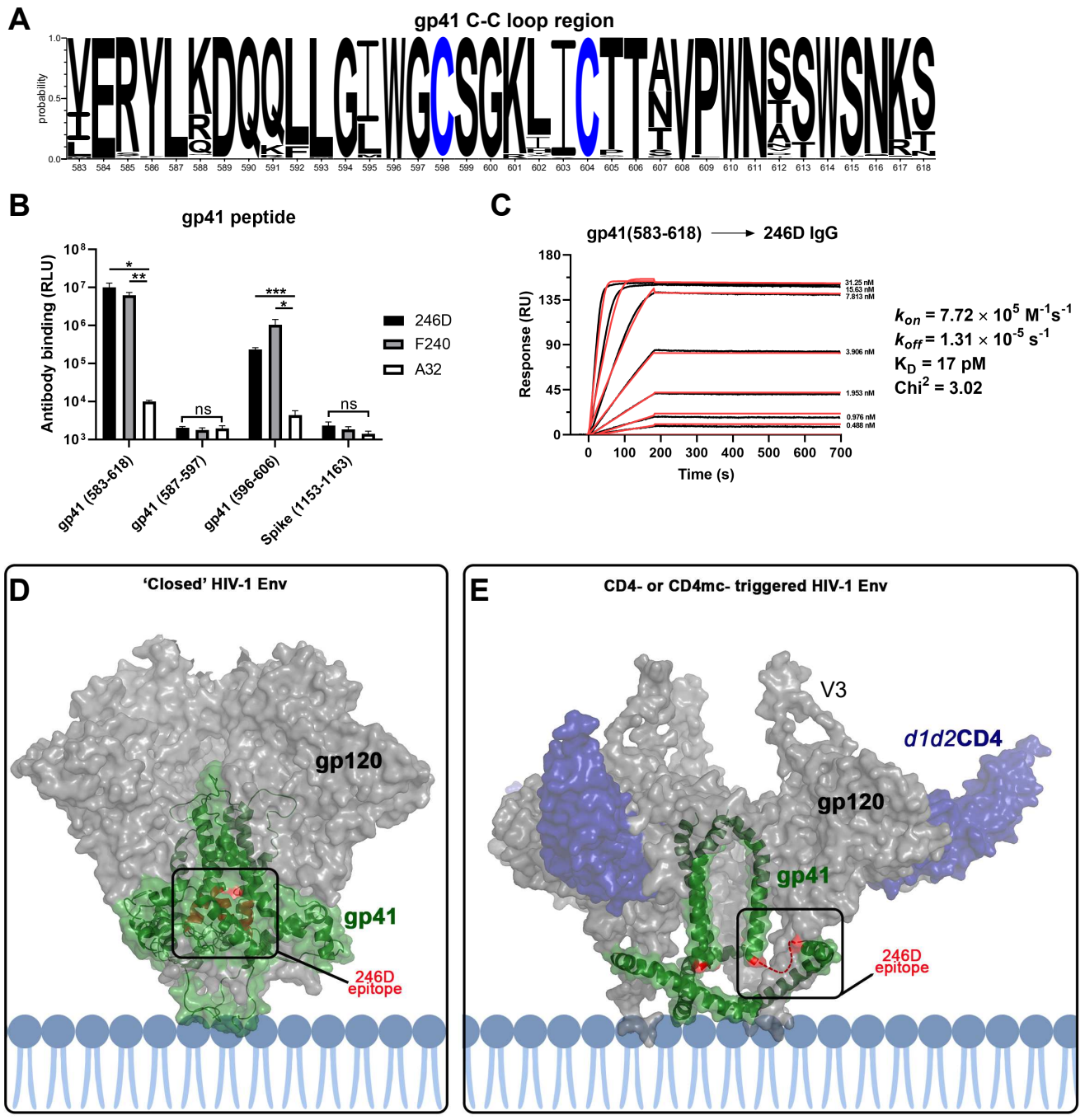


Figure S3. Monoclonal antibody 246D recognizes a gp41 linear peptide occluded in the closed Env trimer. Related to Figure 7. (**A**) Logo depiction of the frequency of each amino acid from the HIV-1 Env gp41 C-C loop region (residues 583-618) in all HIV-1 isolates. The height of the letter indicates its frequency among all strains. The 2019 Los Alamos database-curated filtered web Env alignment was used as the basis for this figure, which contains 6,223 individual Env amino acid sequences. Residue numbering is based on the HXB2 reference strain of HIV-1. (**B**) Indirect ELISA was performed using HIV-1 Env gp41 peptides corresponding to the C-C loop region, or a SARS-CoV-2 Spike S2 peptide as a negative control. Peptide-coated wells were incubated with anti-gp41 246D and F240 mAbs, as well as anti-gp120 cluster A A32 mAb as a negative control. Antibody binding was detected using HRP-conjugated anti-human IgG and was quantified by relative light units (RLU). The data shown are the means of results obtained in three independent experiments. Error bars indicate means ± the SEM. (**C**) 246D binding affinity and kinetics to gp41 C-C loop using surface plasmon resonance (SPR). The 246D IgG was immobilized as the ligand on a Protein A chip and HIV-1 gp41 (583-618) peptide used as analyte from 0.488 to 31.25 nM (2-fold serial dilution). Kinetic constants were determined using a 1:1 Langmuir model in bimolecular interaction analysis (BIA) evaluation software (experimental readings depicted in black and fitted curves in red). (**D-E**) Mapping of the ^{596}WGCSGKLICTT^{606} epitope within available structures of HIV-1 Env. (**D**) The closed conformation of HIV-1 Env (PDB: 6ULC) (Pan et al., 2020) from a cryo-EM structure of full-length HIV-1 Env bound to the Fab of the antibody PG16 (not shown), with the 246D epitope highlighted in red. The 246D epitope is fully occluded in the closed the trimer and not accessible for antibody binding. (**E**) The CD4-triggered HIV-1 Env trimer (PDB: 3J70) (Rasheed et al., 2015) from a computational model of full-length HIV-1 Env bound to the d1d2 domain of CD4 and the Fab of antibody 17b (not shown). In the CD4 triggered trimer the 246D epitope is largely disordered (highlighted with a broken red line for one of three gp41 protomers), but it is exposed at the surface of trimer and available for antibody recognition.

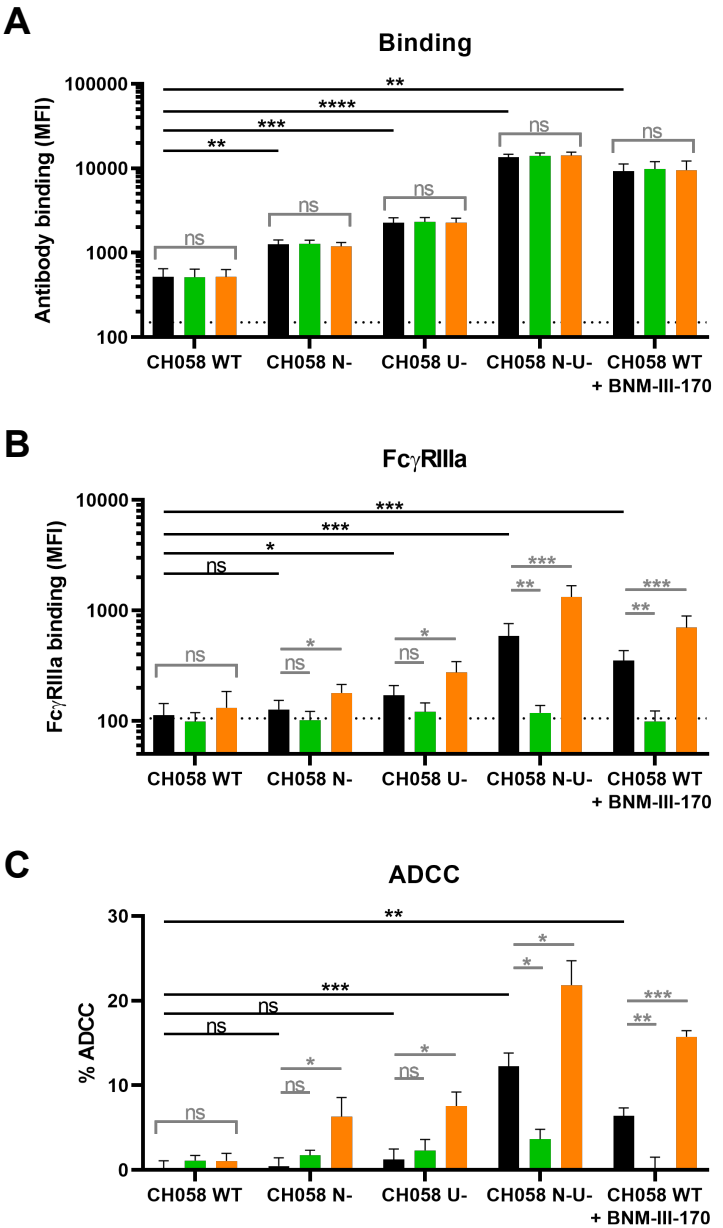


Figure S4. CD4 mimetics and Fc modifications boost the capacity of anti-gp41 nnAbs to mediate ADCC responses. Related to Figure 7.

(**A-C**) Primary CD4+ T cells were either infected with transmitted/founder virus CH058 wild-type (WT) or its Nef and/or Vpu-deleted counterparts (N-, U-, N-U-). Forty-eight hours post-infection, cell surface staining and ADCC responses were measured in presence of the anti-gp41 nnAb 246D WT or Fc-mutated to impair (G236R/L328R; GRLR) or enhance (G236A/S239D/A330L/I332E; GASDALIE) Fc-effector functions. Alternatively, cells infected with CH058 WT were treated with CD4mc BNM-III-170 during staining and ADCC experiments. Antibody binding was detected using (**A**) Alexa Fluor 647-conjugated anti-human secondary Abs or (**B**) by using biotinylated recombinant soluble dimeric FcγRIIIa followed by the addition of Alexa Fluor 647-conjugated streptavidin. (**A-B**) The graphs represent the mean fluorescence intensities (MFI) obtained from the infected p24+ cell population using cells from five different healthy donors. The horizontal dotted lines represent the signal obtained in absence of mAb. (**C**) Infected primary CD4+ T cells were used as target cells and autologous PBMCs were used as effector cells in a FACS-based ADCC assay. The graph represents the percentages of ADCC obtained in the presence of the respective antibodies using cells from five different healthy donors. Error bars indicate means ± standard errors of the means (SEM). (**A-C**) Statistical significance was tested using a one-way ANOVA with a Holm-Sidak post-test or a Kruskal-Wallis test with a Dunn's post-test when comparing between the different 246D Fc variants and an unpaired t test or a Mann-Whitney U test when comparing between viruses or treatment. Appropriate statistical test (parametric or nonparametric) was applied based on dataset distribution normality (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns, nonsignificant).