

FIG S1. Structural details of NCI05-peptide V2c complex and comparison with other antibody-bound V2. (A) Electron density map of the V2c peptide and glycan on CDR H3 of NCI05. Map is shown in gray mesh around the peptide residues in sticks representation and contoured at 1σ . Glycans are shown in sticks representation. (B) Superposition of V2 fragments in prefusion closed SIV-1 Env, HIV-1 Env, and NCI05- and NCI09-bound conformation. Residues 168 and 173 are shown in sticks representation to mark the relative locations. (C) Comparison of NCI05 and NCI09 binding modes with superpositioned V2 peptides. (D) NCI05 binding mode is incompatible with prefusion closed state of SIV-1 Env. Prefusion-specific antibody PGT145 is shown in brown. NCI05-binding surface on V2 faces the inside of the Env volume. (E) Comparison of antibody-bound V2 peptides. Residues at positions 168 and 173 are shown in sticks representation to mark their locations. (F) Env in CD4-induced partially open state may be more compatible with NCI05 binding. Binding of CD4 is thought to induce a partially open state of the Env, which potentially exposes epitopes on V1/V2 that are not accessible in the prefusion state, such as that targeted by NCI05. (G) mAB is shown as electrostatic surface, V2 as a ribbon backbone, and stick side-chains with atom size proportional to the contact area of that atom with the antibody. Alignment of the four indicated V2 antibody-bound epitopes, with key contact residues indicated and α -helical segments shaded. (H) Ramachandran plots of the backbone phi-psi angles of the four antibody-bound V2 epitopes. Generic Ramachandran plot conformation zone legend: Superposition of the α -helical turn in NCI05 and CH59.

FIG S2. Inhibition of gp120 costimulation of CD4⁺ T-cells by V2 mAbs. Flow cytometric plots displaying the percentage of activated and proliferating CD25⁺Ki67⁺CD4⁺ T-cells by SIVM766 gp120 stimulation in the presence of ITS12, ITS03, NCI05, and NCI09.

FIG S3. Binding of NCI05 and NCI09 to SIV-infected cells. (A-B) Binding of NCI05 to V2 of various SIV-infected CEM.NKR-CCR5-sLTR-Luc cells in the (A) absence or (B) presence of soluble CD4. (C) ADCC mediated by KHYG-1 NK cells recognizing NCI05 bound to V2 of various SIVs in the absence of soluble CD4. (D-E) Binding of NCI09 to V2 of various SIV-infected CEM.NKR-CCR5-sLTR-Luc cells in the (D) absence or (E) presence of soluble CD4. (F) ADCC mediated by NCI09 bound to V2 of various SIV-infected CEM.NKR-CCR5-sLTR-Luc cells in the absence of soluble CD4. (G) ADCC mediated by non-specific binding of control dengue virus antibody to various SIV-infected CEM.NKR-CCR5-sLTR-Luc cells in the absence of soluble CD4.

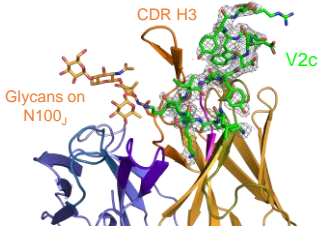
FIG S4. Miscellaneous data. (A) Staining of SIV-infected cells by Dengue3 control antibody. (B) Spearman rank correlation between the geometric mean of NCI05 measured in plasma prior to infection by ELISA with peptide 150 and the time of SIV acquisition. (C) Normalized percentage of ADCC mediated by human PBMC effectors obtained prior to passive immunization incubated with NCI09 plasma obtained one week after infection.

Table S1. Crystallographic data and refinement statistics.

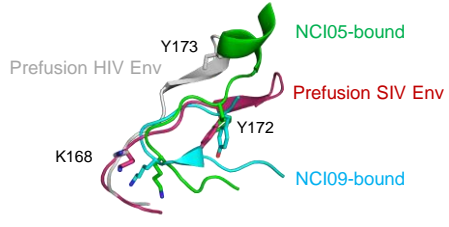
Table S2. Interactions between antibody NCI05 and V2c peptide.

A

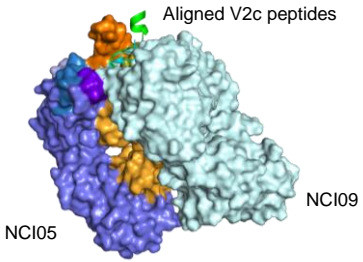
Electron density of V2c peptide and CDR H3 glycan

**B**

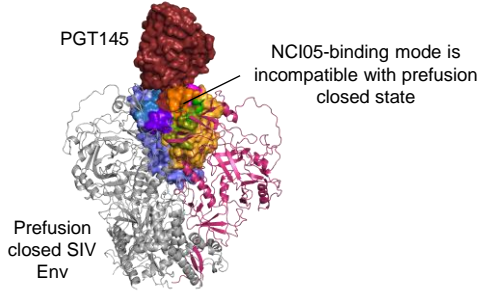
V2c conformations in NCI05, NCI09 and prefusion closed Env

**C**

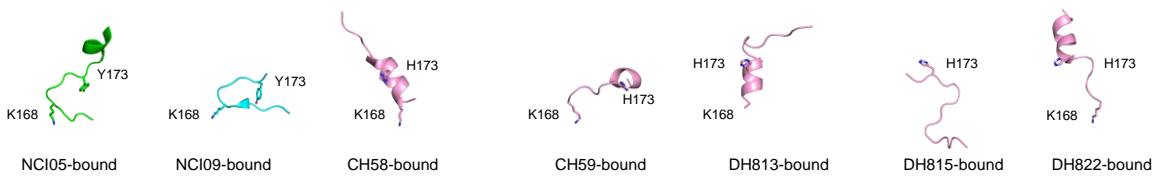
V2c-bound NCI05 and NCI09

**D**

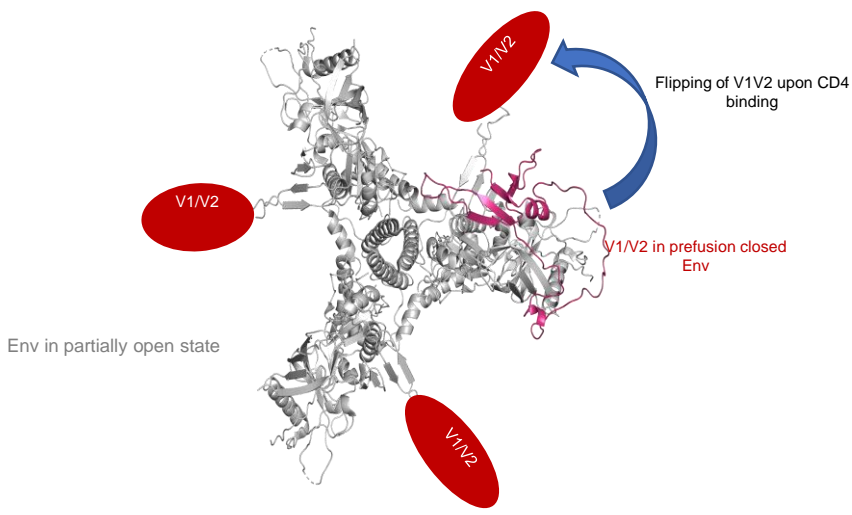
NCI05-binding is incompatible with prefusion closed state

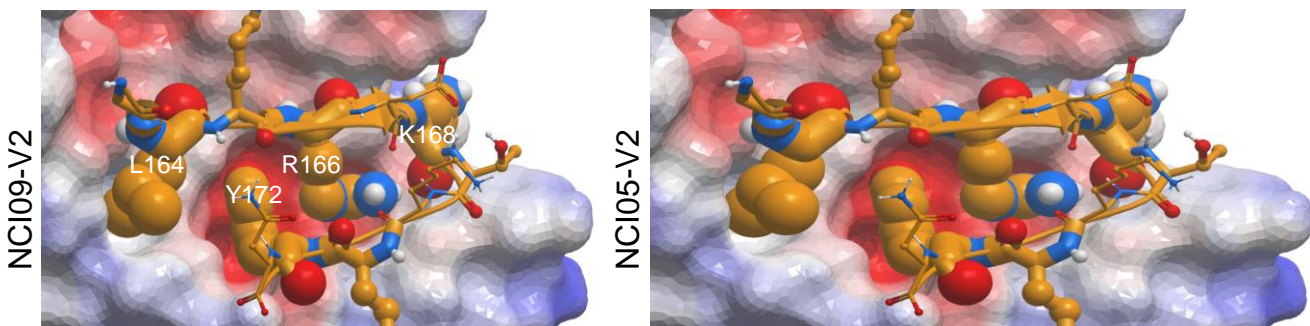
**E**

Conformation of antibody-bound V2 peptides

**F**

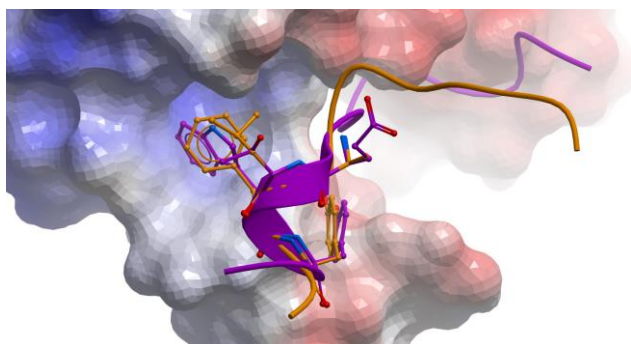
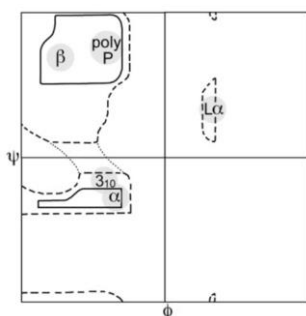
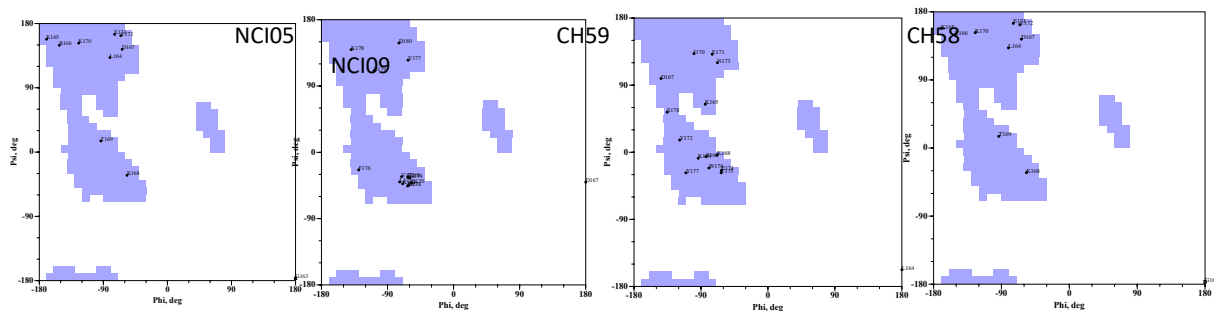
CD4-induced partially open state maybe compatible with NCI05 binding

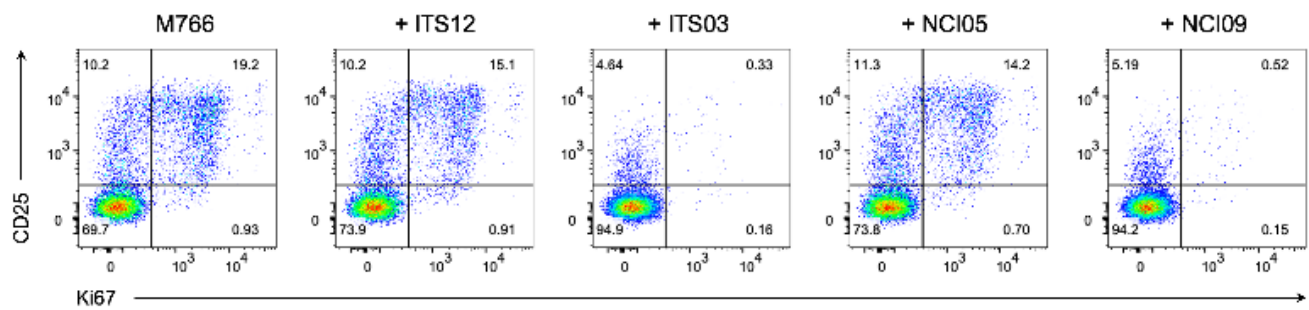
**FIG S1**

G

Residue with strong contact with antibody α -helix

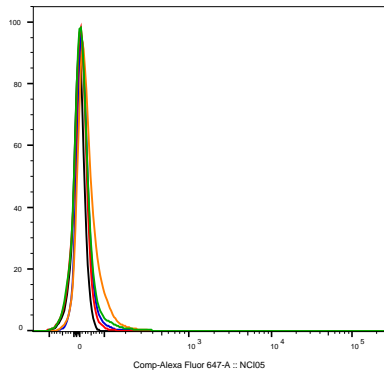
5%		K...#.....
NCI05V2	164	-	LKSD K KIEYNET W YSR--
NCI09V2	163	G	L K R D K TKEY N -----
CH59	168	-	----- K K QKVHAL F Y K---
CH58	167	-	----- D K K QKVHAL F Y KLDI

H**FIG S1**

A**FIG S2**

A

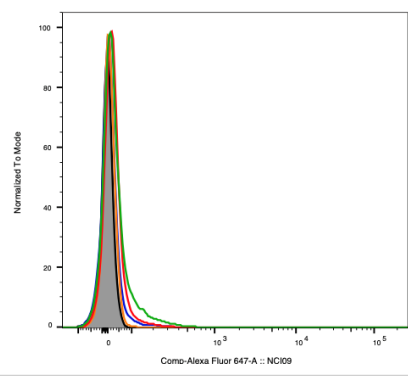
NCI05



Sample Name	Geometric Mean : Comp-Alexa Fluor 647-A
Specimen_001_4-SIVmac251-swarm_004.fcs	24.4
Specimen_001_3-SIVmac251-M766r_003.fcs	15.7
Specimen_001_2-SIVsmE660-FL14_002.fcs	41.9
Specimen_001_1-SIVmac239_001.fcs	27.8
Specimen_001_26-SIVmac239_026.fcs	8.92

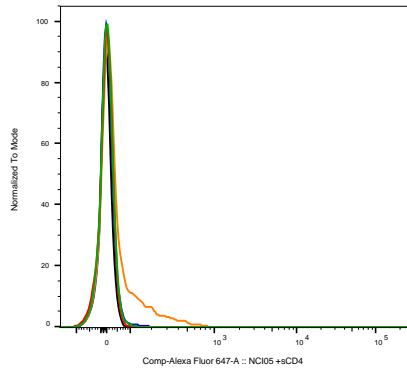
D

NCI09



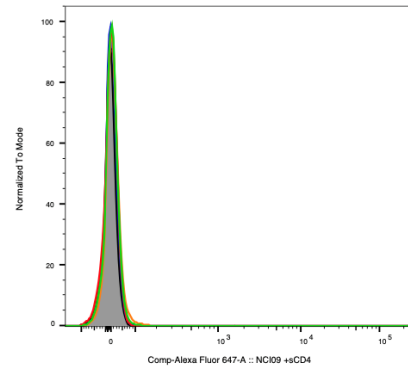
Sample Name	Geometric Mean : Comp-Alexa Fluor 647-A
Specimen_001_9-SIVmac251-swarm_008.fcs	39.9
Specimen_001_8-SIVmac251-M766r_008.fcs	31.5
Specimen_001_7-SIVsmE660-FL14_007.fcs	17.6
Specimen_001_6-SIVmac239_006.fcs	21.6
Specimen_001_26-SIVmac239_026.fcs	8.92

NCI05+sCD4

B

Sample Name	Geometric Mean : Comp-Alexa Fluor 647-A
Specimen_001_14-SIVmac251-swarm_014.fcs	15.5
Specimen_001_13-SIVmac251-M766r_013.fcs	12.1
Specimen_001_12-SIVsmE660-FL14_012.fcs	49.9
Specimen_001_11-SIVmac239_011.fcs	16.1
Specimen_001_26-SIVmac239_026.fcs	8.92

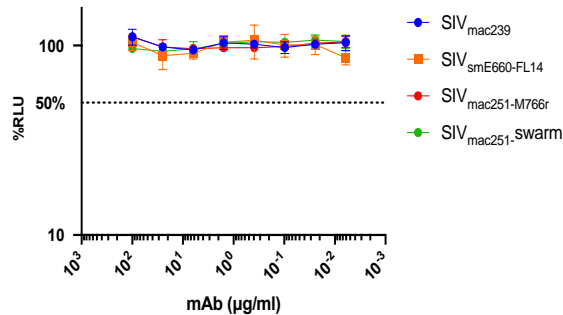
NCI09+sCD4

E

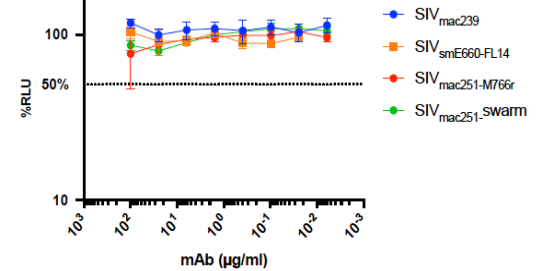
Sample Name	Geometric Mean : Comp-Alexa Fluor 647-A
Specimen_001_19-SIVmac251-swarm_019.fcs	15.9
Specimen_001_18-SIVmac251-M766r_018.fcs	11.8
Specimen_001_17-SIVsmE660-FL14_017.fcs	19.4
Specimen_001_16-SIVmac239_016.fcs	13.7
Specimen_001_26-SIVmac239_026.fcs	8.92

C

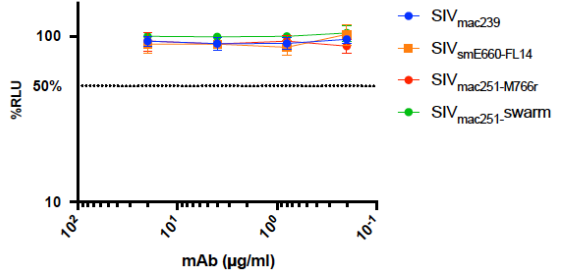
NCI05

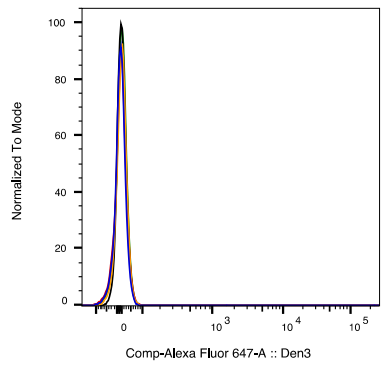
**F**

NCI09

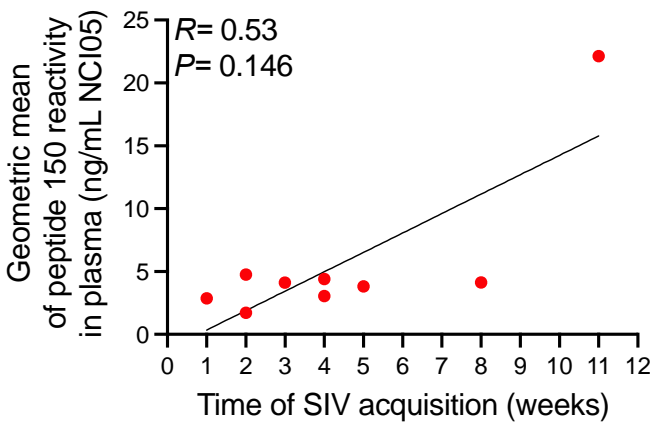
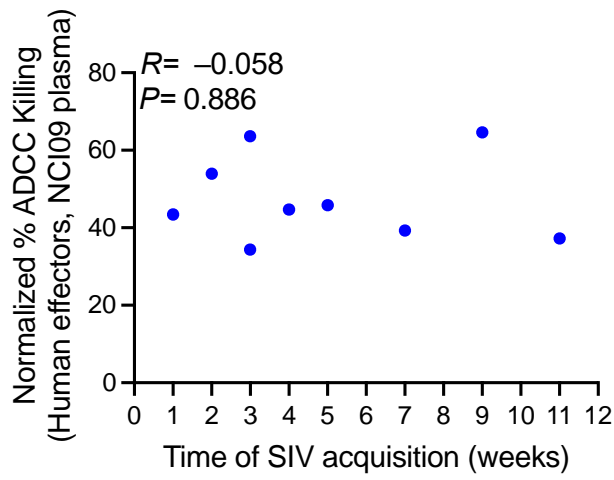
**G**

Control plate-Dengue3



A

Sample Name	Geometric Mean : Comp-Alexa Fluor 647-A
Specimen_001_26-SIVmac239_026.fcs	8.92
Specimen_001_27-SIVsmE660-FL14_027.fcs	14.2
Specimen_001_28-SIVmac251-M766r_028.fcs	10.5
Specimen_001_30-NKR28-uninfected_030.fcs	15.1
Specimen_001_29-SIVmac251-swarm_029.fcs	13.4

B**C****FIG S4**