Cell Reports, Volume *42*

Supplemental information

Small CD4 mimetics sensitize HIV-1-infected macrophages to antibody-dependent cellular cytotoxicity

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Table S1:Relative CD4 and BST2 levels on surface of CD4+T cells and Macrophages (Related to Figure 1)

	AD8				YU2				JRFL				CH77			
	CD4		BST2		CD4		BST2		CD4		BST2		CD4		BST2	
	T cells	Mφ	T cells	Mφ	T cells	Mφ	T cells	Mφ	T cells	Mφ	T cells	Mφ	T cells	Mφ	T cells	Mφ
Mock	100.0	100.0	100.0	100.0	100	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
WT	0.7	18.1	9.3	42.5	3.4	16.7	4.8	62.5	2.4	18.4	28.2	42.8	0.8	14.7	9.9	53.9
N-	43.7	21.3	11.1	50.4	29.1	19.6	6.9	80.9	41.8	21.2	20.5	41.7	15.1	17.6	15.9	69.5
U-	2.0	19.8	62.3	85.5	17.1	17.5	78.9	111.4	5.0	28.0	117.1	98.3	4.2	23.2	106.4	88.9
N-U-	37.6	30.6	104.3	106.4	58.5	36.9	110.5	133.7	69.2	35.6	130.5	112.6	31.3	32.2	137.1	85.4
D368R	1.1	18.8	10.7	42.2	5.2	19.3	4.4	71.2	1.7	31.4	14.2	36.0	1.2	14.0	8.6	61.9
N-U-D368R	72.3	103.2	112.1	107.2	97.2	197.1	107.7	141.0	112.5	130.0	125.9	106.1	118.7	70.2	135.7	114.3

WT : Wild type
N-: Nef Defective
U-: Vpu defective
D368R: CD4 binding site mutant
M**φ** : Macrophage

Table S2:Summary of Anti-HIV antibodies/ligands utilized for assessment

Antibody	Env subunit	Epitope/Region	Ab/Drug class	Conformational State
2G12	gp120	Outer domain/oligomannose patch	bNAb	independent
PG9	gp120	V1V2-apex	bNAb	1
PGT121	gp120	V3-glycan	bNAb	1
10.1074	gp120	V3-glycan	bNAb	1
VRC03	gp120	CD4bs	bNAb	1
3BNC.117	gp120	CD4bs	bNAb	1
PGT151	gp41	fusion peptide	bNAb	1
10E8	gp41	MPER	bNAb	2/3
F240	gp41	cluster I region of gp41	nnAb	2/3
19b	gp120	V3-tip	nnAb	2/3
17b	gp120	CoRbs	nnAb	2
A32	gp120	cluster A region of gp120 inner domain	nnAb	2A
BNM III 170	gp120	CD4bs	CD4 mimetic	2

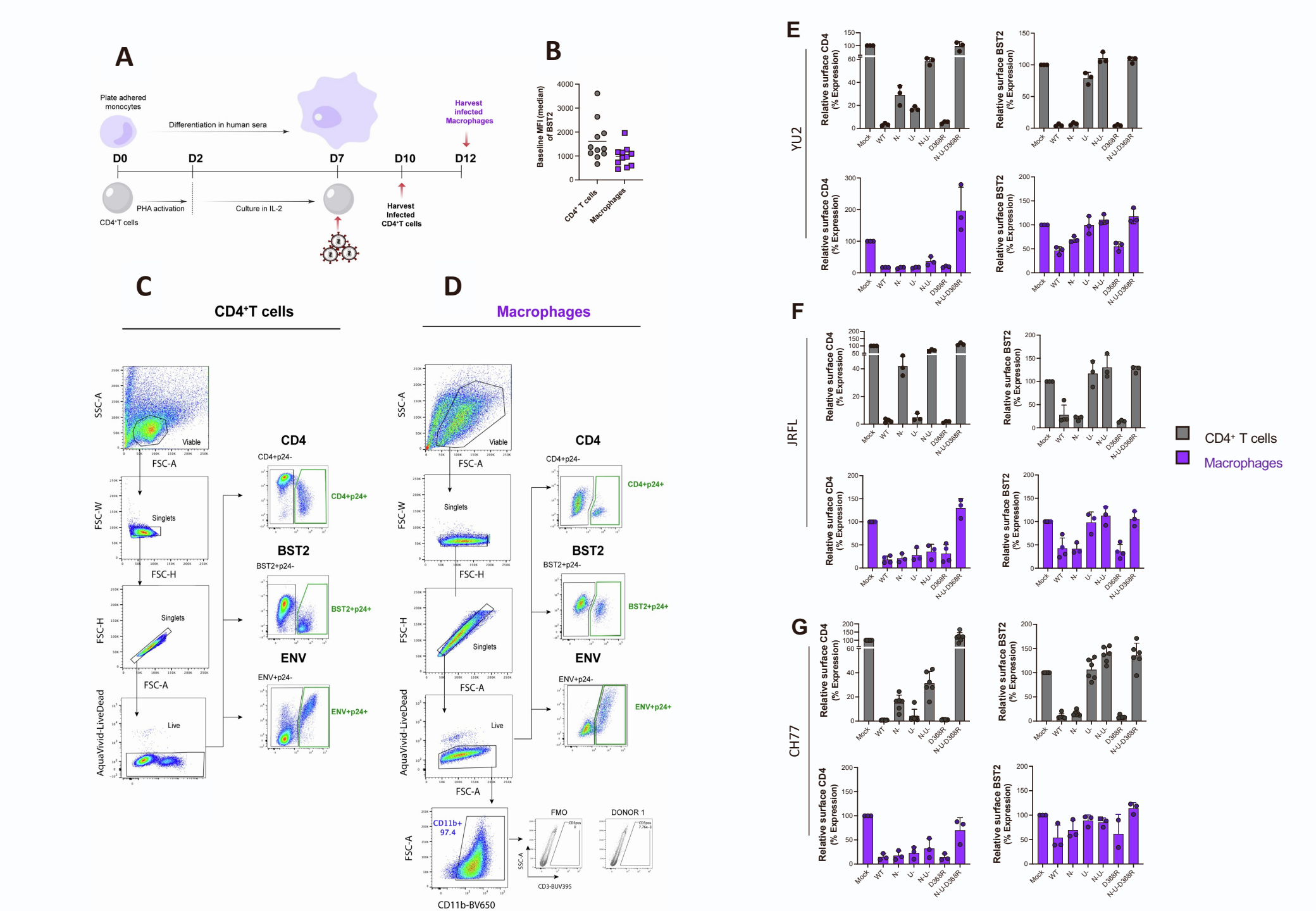

A
Plate adhered monocytes
Differentiation in human sera
Harvest infected Macrophages
D0
D2
D7
D10
D12
PHA activation
Culture in IL-2
CD4+T cells
Harvest Infected CD4+T cells
B
Baseline MFI (median) of BST2
CD4+ T cells
Macrophages
C
CD4+T cells
D
Macrophages
SSC-A
Viable
FSC-A
FSC-W
Singlets
FSC-H
AquaVivid-LiveDead
Live
CD4
CD4+p24-
CD4+p24+
BST2
BST2+p24-
BST2+p24+
ENV
ENV+p24-
ENV+p24+
CD11b+ 97.4
FMO
DONOR 1
CD3-BUV395
CD11b-BV650
E
YU2
F
JRFL
G
CH77
Relative surface CD4 (% Expression)
Relative surface BST2 (% Expression)
Mock
WT
N-
U-
N-U-
D368R
N-U-D368R
CD4+ T cells
Macrophages

Figure S1. Effect of Nef, Vpu and Env on the surface expression of CD4 and BST2 in autologous $CD4^+$ T cells and macrophages infected with different HIV-1 strains (Related to Figure 1).

(*A*) Schematic of experimental timeline. Details are described in STAR METHODS. (*B*) Baseline Mean Fluorescence Intensity (MFI) of BST2 on uninfected $CD4^+$ T cells and macrophages from 12 different experiments. Gating strategy for measurement of expression of Env, BST and CD4 on (*C*) $CD4^+$ T cells and (*D*) macrophages; (*E-G*) Relative surface levels of CD4 and BST2 on autologous $CD4^+$T cells and macrophages infected with panel of viruses [wt, nef-defective (N-), Vpu-defective (U-), or both Nef and Vpu (N-U-), CD4BS mutant (D368R) and N-U-D368R mutant] from (*E*) HIV-1_{YU2} (*F*) HIV-1_{JR-FL} and (*G*)HIV-1_{CH77}. Bar graphs represent percent fold change in CD4 expression relative to Mock (p24+/uninfected cells) or percent fold change in BST2 expression of $p24^+$ relative to $p24^-$ cells (p24+/p24-). Shown are data from at least 3 donors.

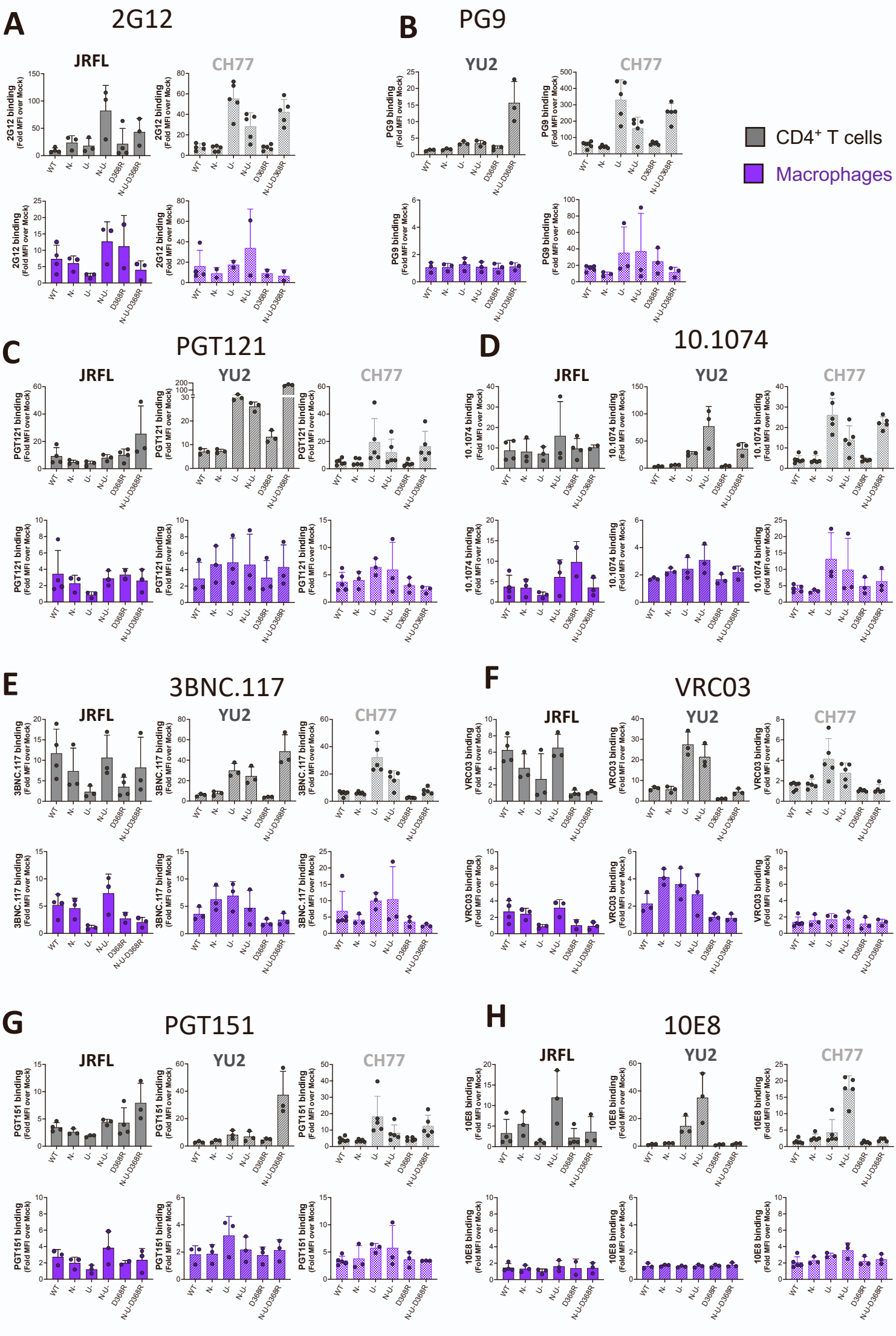

A
2G12
JRFL
CH77
2G12 binding (Fold MFI over Mock)
B
PG9
YU2
CH77
PG9 binding (Fold MFI over Mock)
CD4+ T cells
Macrophages
C
PGT121
JRFL
YU2
CH77
PGT121 binding (Fold MFI over Mock)
D
10.1074
10.1074 binding (Fold MFI over Mock)
E
3BNC.117
3BNC.117 binding (Fold MFI over Mock)
F
VRC03
VRC03 binding (Fold MFI over Mock)
G
PGT151
PGT151 binding (Fold MFI over Mock)
H
10E8
10E8 binding (Fold MFI over Mock)
WT
N-
U-
N-U-
D368R
N-U-D368R

Figure S2. HIV-1 Env detection by a panel of mAbs at the surface of autologous CD4$^+$ T cells and macrophages infected with different HIV-1 strains. (Related to Figure 2).
Autologous CD4$^+$T cells and macrophages were infected with panel of viruses [wt, nef-defective (N-), Vpu-defective (U-), or both Nef and Vpu (N-U-) and CD4BS mutant (D368R) and N-U-D368R mutant] from HIV-1_{JR-FL}, HIV-1_{CH77} and HIV-1_{YU2}. 48h later (CD4$^+$ T cells) or 5 days post-infection (Macrophages). Cells were then stained with (*A*) 2G12 a conformation independent antibody; (*B*) the V1V2-apex antibody PG9; V3glycan antibodies (*C*) PGT121 and (*D*) 10.1074; CD4BS antibodies (*E*) 3BNC117 and (*F*) VRC03; *(G)*PGT151 which targets the gp120-gp41 interface; and the *(H)* anti-MPER antibody 10E8. Bar graphs show Fold antibody binding over Mock cells. Shown are data from a minimum of 3 donors.

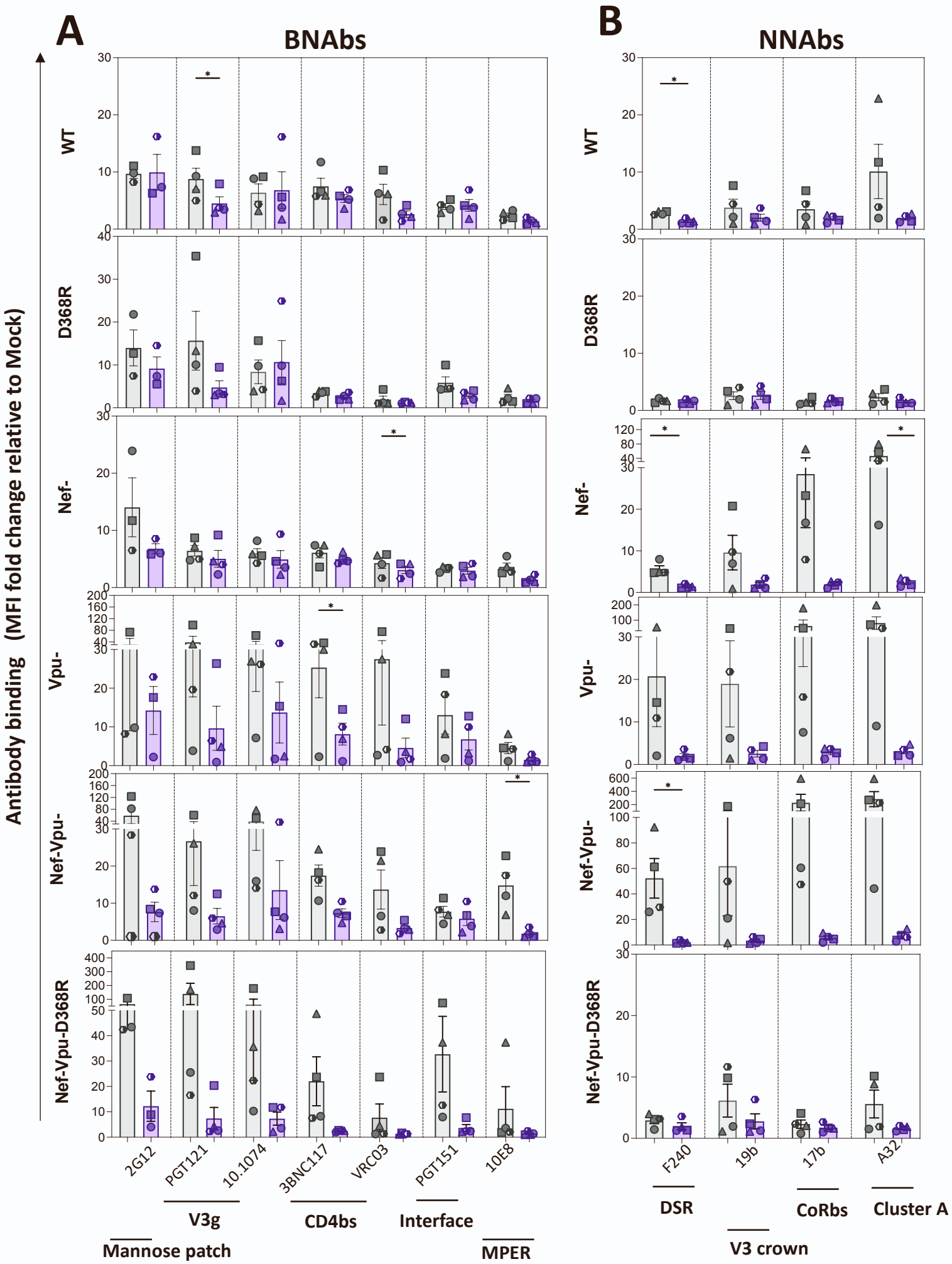

A
BNAbs
B
NNAbs
Antibody binding (MFI fold change relative to Mock)
WT
D368R
Nef-
Vpu-
Nef-Vpu-
Nef-Vpu-D368R
2G12
PGT121
10.1074
3BNC117
VRC03
PGT151
10E8
F240
19b
17b
A32
V3g
CD4bs
Interface
Mannose patch
MPER
DSR
V3 crown
CoRbs
Cluster A

Figure S3. HIV Env conformational landscape on the surface of autologous CD4$^+$ T cells and macrophages infected with different HIV strains (Related to Figures 2 and 3)

Summary of Env recognition on Autologous (CD4$^+$T cells and macrophages infected for 48h (CD4$^+$ T cells) or 5 days (Macrophages) with panel of viruses [wt, nef-defective (N-), Vpu-defective (U-), or both Nef and Vpu (N-U-) and CD4BS mutant (D368R) and N-U-D368R mutant] of different IMCs (AD8, YU2, JRFL, CH77). *(A)* Recognition by bNAbs. *(B)* Recognition by nnAbs. Statistical significance was tested using paired *t* test (*p<0.05; **p<0.001; ***p<0.0001); only comparison less than or equal to 0.05 are shown. Shown are data from at least 3 donors.

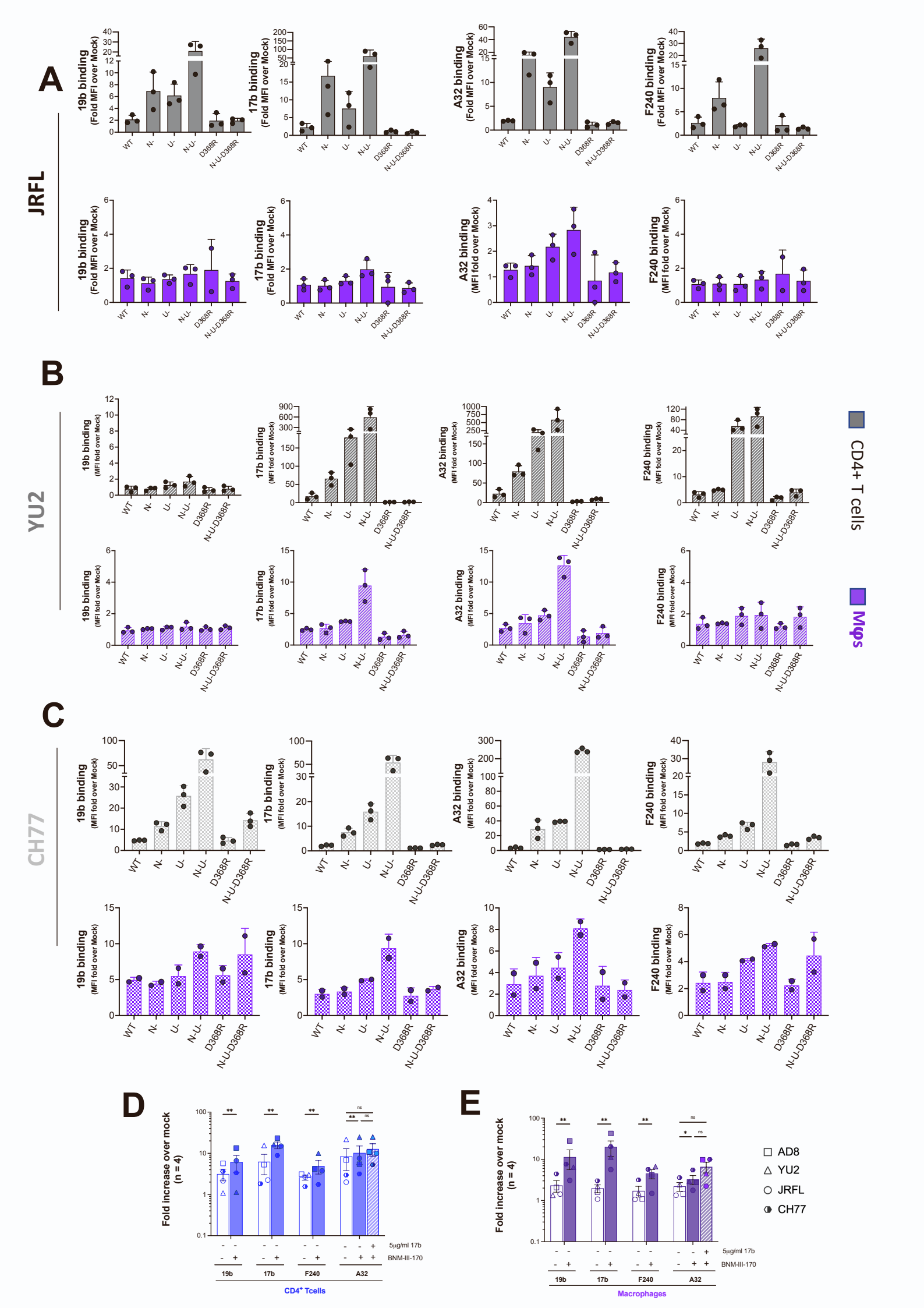

A
JRFL
B
YU2
C
CH77
CD4+ T cells
Mφs
19b binding (Fold MFI over Mock)
17b binding (Fold MFI over Mock)
A32 binding (Fold MFI over Mock)
F240 binding (Fold MFI over Mock)
19b binding (MFI fold over Mock)
17b binding (MFI fold over Mock)
A32 binding (MFI fold over Mock)
F240 binding (MFI fold over Mock)
WT
N-
U-
N-U-
D368R
N-U-D368R
D
Fold increase over mock (n = 4)
5μg/ml 17b
BNM-III-170
19b
17b
F240
A32
CD4+ Tcells
E
AD8
YU2
JRFL
CH77
Macrophages

Figure S4. **Impact of HIV-1 accessory proteins Nef, Vpu and small molecule CD4mc on Env conformation (Related to Figure 3).**
Autologous $CD4^+$T cells and macrophages were infected with panel of viruses [wt, nef-defective (N-), Vpu-defective (U-), or both Nef and Vpu (N-U-) and CD4BS mutant (D368R) and N-U-D368R mutant] from (*A*) HIV-1_{JR-FL}, (*B*) HIV-1_{YU2} and (*C*) HIV-1_{CH77}. 48h later ($CD4^+$ T cells) or 5 days post-infection (Macrophages), cells were stained with non-neutralizing antibodies 19b (V3 crown), 17b (Co-receptor binding site), A32 (Anti-cluster A) and F240 (gp41-Disulfide loop region). Fold increase in nnAb binding in the presence of the CD4mc for (*D*) $CD4^+$T cells and *(E)* macrophages. Bar graphs show Fold antibody binding over Mock cells. Statistical significance for panels D and E was tested using Mixed Effect analysis (*$p<0.05$; **$p<0.001$; ***$p<0.0001$; ns, non-significant). Data shown are from minimum of 2 donors.

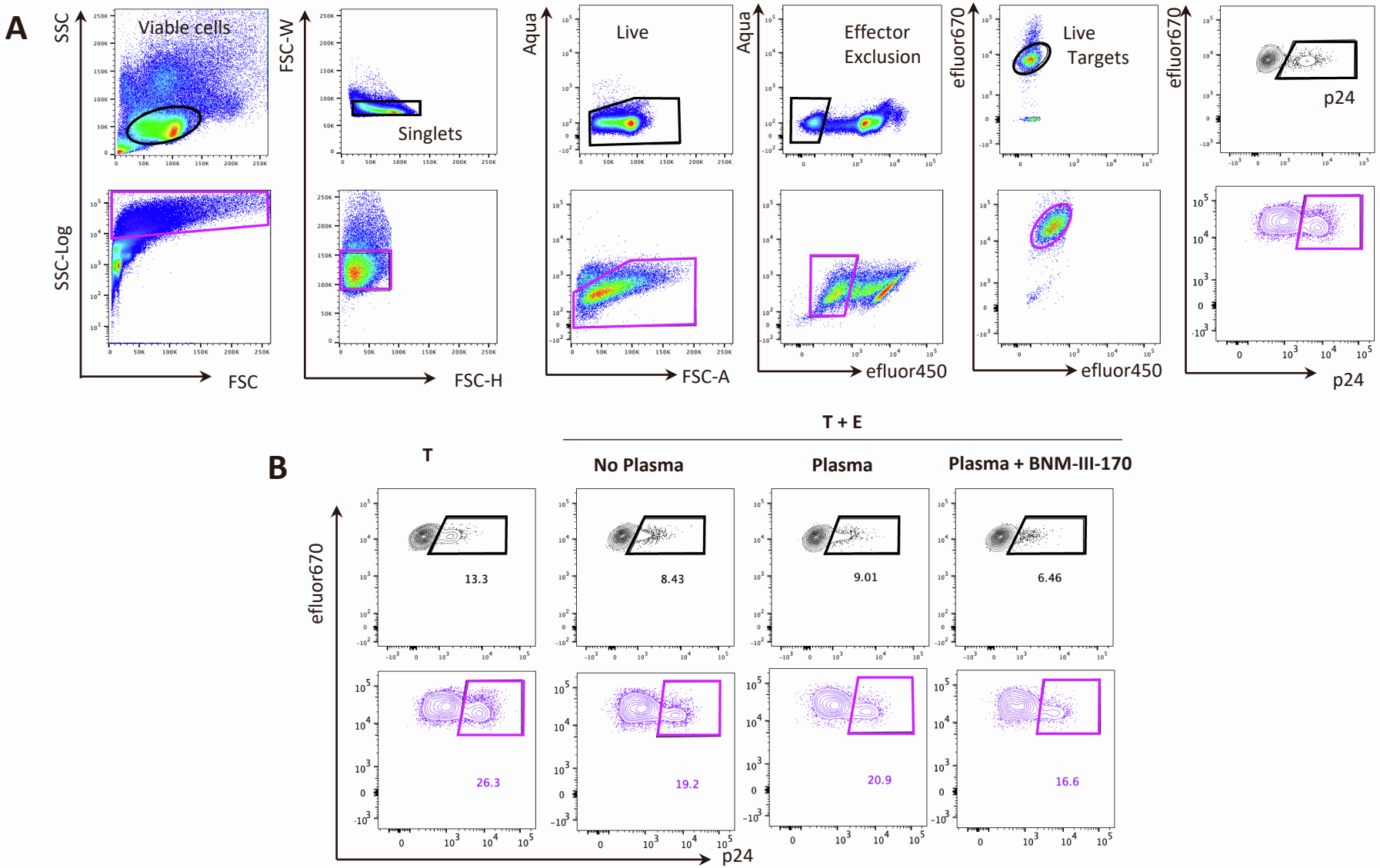
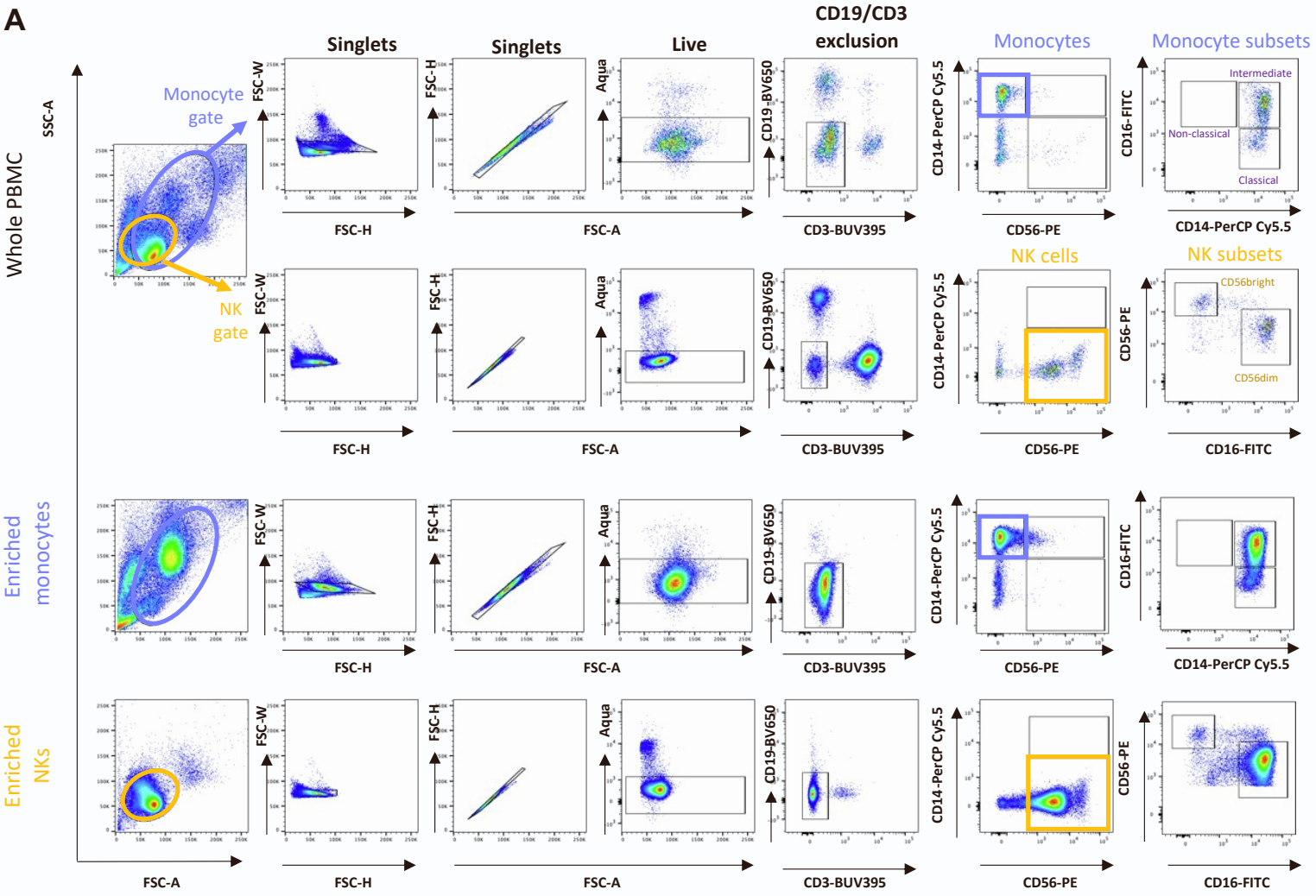
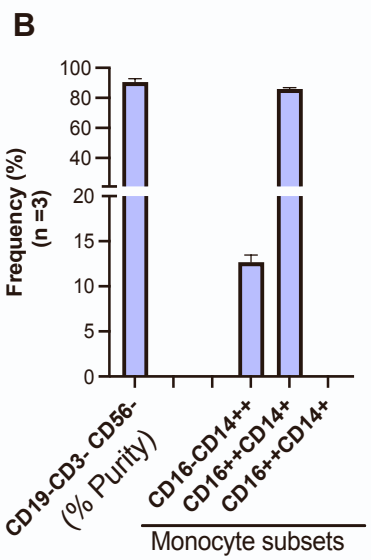


Figure S5. Gating strategy for CD4⁺T cells and Macrophages ADCC Assays (Related to Figure 4).
Gating strategy for ADCC assays performed for CD4⁺T cells (*Top panel*) and Macrophages (*Bottom panel*) infected with HIV-1_{AD8} wildtype (WT) or virus defective to Nef and Vpu (N-U-) and incubated with autologous PBMCs for 5h. Macrophages and CD4⁺T cells were gated for viable cells. Side Scatter Axis for macrophages were transformed to log axis to visualize both the large macrophage targets and the small PBMC effectors. Subsequent doublet exclusion was performed, followed by live dead exclusion and effector exclusion. Targets were then selected and subsequent gating was done on the p24+ population. *(B)* Representative plots for the p24+ gate for Targets alone (T), and Targets with Effectors (T+E) in presence or absence of HIV+ plasma and/or CD4mc BNM-III-170


A
Whole PBMC
Enriched monocytes
Enriched NKs
SSC-A
FSC-A
Monocyte gate
NK gate
Singlets
Singlets
Live
CD19/CD3 exclusion
Monocytes
Monocyte subsets
NK cells
NK subsets
FSC-W
FSC-H
Aqua
CD19-BV650
CD3-BUV395
CD14-PerCP Cy5.5
CD56-PE
CD16-FITC
Intermediate
Non-classical
Classical
CD56bright
CD56dim


B
Frequency (%) (n =3)
100
80
60
40
20
15
10
5
0
CD19-CD3- CD56- (% Purity)
CD16-CD14++
CD16++CD14+
CD16++CD14+
Monocyte subsets

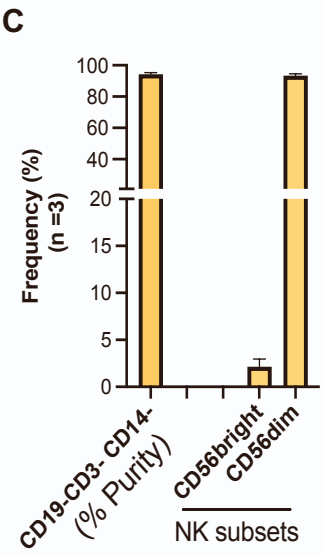

C
Frequency (%) (n =3)
100
80
60
40
20
15
10
5
0
CD19-CD3- CD14- (% Purity)
CD56bright
CD56dim
NK subsets

Figure S6. **Effector cell gating strategy (Related to Figure 4).**
(A) Whole PBMC effectors utilized for ADCC assays (*top panel*) were stained for the NK cell marker CD56 and monocyte marker CD14 to determine presence of these populations. Cells were first gated on morphology followed by doublet exclusion, dead cell exclusion and exclusion of CD19+ and CD3+ populations. Gating on CD14 and CD56 distinguished the monocyte and NK population respectively. Presence of CD16 on both cell populations enabled subset determination. Similarly enriched monocytes (*middle panel*) were stained with monocyte markers CD14 and enriched NK cells with CD56 (*bottom panel*) to confirm purity of cells in the enriched cell fractions utilized for ADCC assays. Similar gating strategy as whole PBMCs was applied for purity confirmation and subset determination. **(B)** Purity of monocytes and proportion of classical (CD16-CD14++), intermediate (CD14+CD16++) and non-classical (CD16+CD14+) subsets present during ADCC **(C)** Purity of NK cells and proportion of CD56bright and CD56dim present during ADCC.

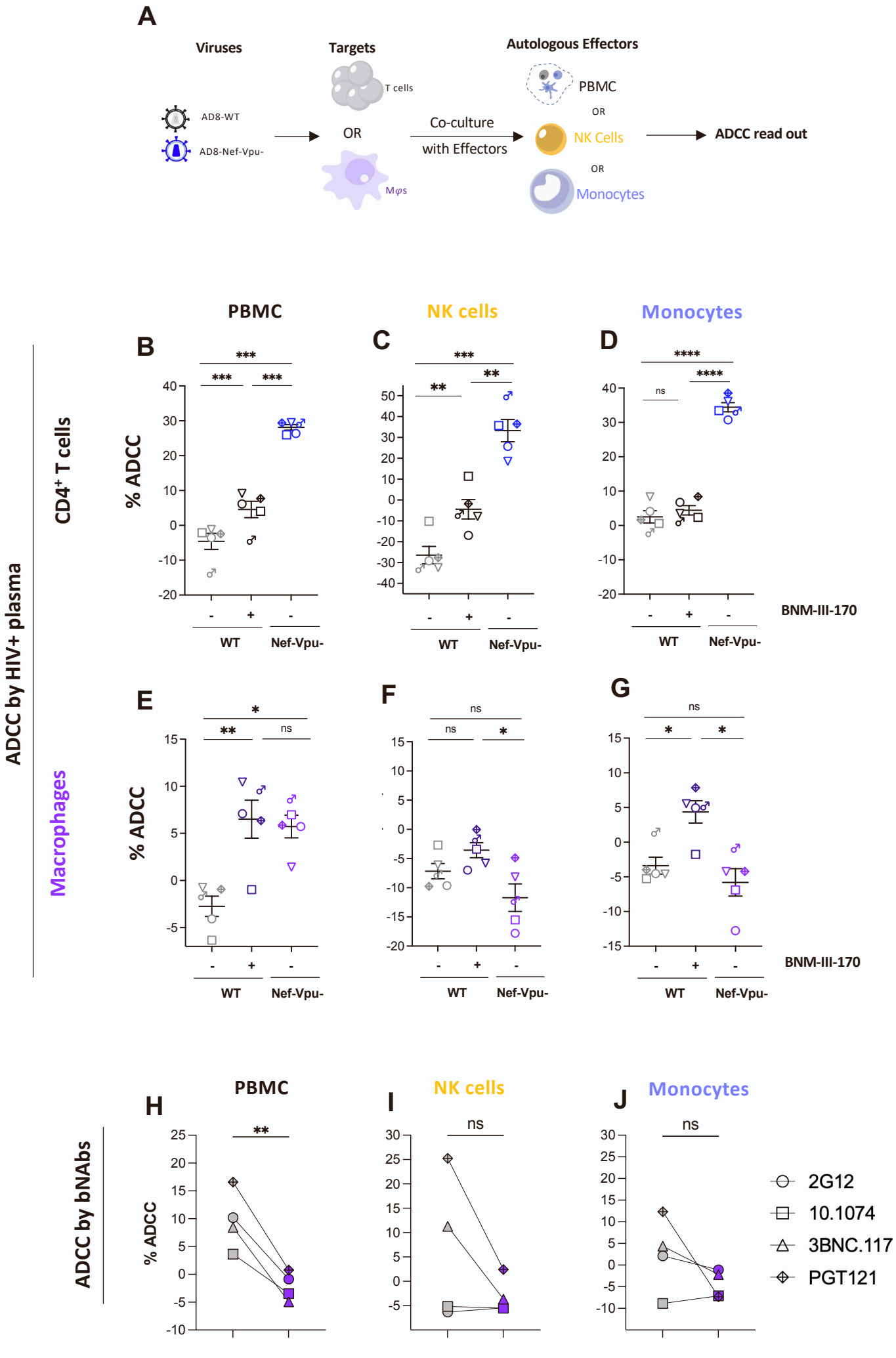

A
Viruses
Targets
Autologous Effectors
AD8-WT
AD8-Nef-Vpu-
OR
T cells
Mφs
Co-culture
with Effectors
PBMC
OR
NK Cells
OR
Monocytes
ADCC read out
PBMC
NK cells
Monocytes
ADCC by HIV+ plasma
CD4+ T cells
Macrophages
B
C
D
E
F
G
% ADCC
WT
Nef-Vpu-
BNM-III-170
ns
ADCC by bNAbs
H
I
J
2G12
10.1074
3BNC.117
PGT121
CD4+T cells
Macrophages

Figure S7. **ADCC of HIV-1 infected CD4$^+$T cells and Macrophages by autologous Effectors: PBMCs, Monocytes or NK cell effectors (Related to Figure 4).**

Autologous CD4$^+$T cells and macrophages were infected with HIV-1_{AD8} and subsequently incubated with autologous Effectors, PBMC (**B,E,H**), NK cells (**C,F,I**) or monocytes (**D,G,J**) for 5h in the presence of 5 different HIV+ plasma (represented by the different symbols) with or without the CD4mc BNMIII170 (**A-G**); or select bNAbs (2G12, PGT121, 10.1074, 3BNC.117) (**H,I,J**). Percent (%) ADCC of CD4$^+$ T cells (**B, C, D**) and macrophages (E,F,G) were determined as outlined in STAR METHODS. Statistical significance was tested using paired *t*-test (*p<0.05; **p<0.001; ***p<0.0001; ns, non-significant). Data show ADCC for 4 donors.