Supplemental Table 1: List of IMC by HIV-1 Subtypes and Neutralization Tier

	Subtype A	Subtype AE	Subtype B	Subtype C
Tier 1	Q32.17	92TH023	BaL SF162	MW96.5
Tier 2		CM235 CM244 C1080.C03 427299 816763	CH040 CH058 SUMA WITO YU2	CAP45 CH505 DU151 DU422 TV1 1086.C 246-F3.C10

Supplemental Table 2. Equilibrium Dissociation Constants (K_D) for Binding of A32xCD3 and 7B2xCD3 to Recombinant Env and CD3 Protein

Antigen	DART	k_a (±SD), $M^{-1}s^{-1}$	k_d (±SD), s^{-1}	K_D (±SD), nM
Human CD3ε/δ	A32xCD3	6.8 (± 0.1) $x10^{5}$	2.4 (± 0.0) $x10^{-3}$	3.6 (± 0.1)
	7B2xCD3	4.1 (± 0.0) $x10^{4}$	2.5 (± 0.0) $x10^{-4}$	6.1 (± 0.0)
M.ConS gp140	A32xCD3	1.7 (± 0.0) $x10^{4}$	8.1 (± 1.8) $x10^{-4}$	47.7 (± 10.8)
JR-FL gp140	7B2xCD3	2.0 (± 0.1) $x10^{4}$	3.1 (± 0.9) $x10^{-4}$	15.1 (± 3.5)

Supplemental Table 3: Clinical Characteristics

Patient	Virus used for infection in VCA*	Pre-ART Viral Load (copies/ml)	Nadir CD4	Current CD4 count	Duration of suppression (years)
493	JR-CSF	78,115	257	637	2.5
532	JR-CSF	unknown	130	623	14
673	JR-CSF	Unknown	unknown	707	7
527	JR-CSF, AR	184,781	600	718	4.5
728	JR-CSF, AR	45,000	354	456	4
749	JR-CSF, AR	unknown	404	402	1.5
725	JR-CSF, AR	234.048	475	789	3
720	JR-CSF, AR	586,930	166	446	3
425	Autologous	750,001	604	783	8
407	Autologous	1,042,734 (AHI)	499	706	2
408	Autologous	6095 (AHI)	830	1168	2
795	Autologous	175,718	526	850	2.5
674	Autologous	185,042	338	1045	4.5

* Virus Clearance Assay

AR = autologous reservoir virus

Autologous indicates patient cells used in latency clearance assay, no superinfection used.

AHI = Acute HIV Infection

Supplemental Table 4. DARTs redirect patient T cells against JR-CSF infected autologous target cells, absolute p24 concentration

Patient	E:T Ratio	HIV-1 gag p24 concentration (ng/ml) (SEM)					
		No DART	CD3x4420	7B2x4420	7B2xCD3	A32xCD3	Combo
493	No CD8s	10.6(2)	12.6(4)	NT	1.44(1.0)	10.8 (2.2)	NT
	1:10	4.7(1.0)	7.35 (2.1)	NT	1.05 (0.6)	3.5 (1.3)	NT
	1:1	.92 (0.2)	1.29 (.03)	NT	.04 (.02)	.343 (.01)	NT
532	No CD8s	12.9 (2.7)	12.6 (3.9)	NT	1.44(1.0)	10.8(4.1)	NT
	1:10	8.9(1.7)	7.35(2.1)	NT	1.05(.629)	3.5(1.3)	NT
	1:1	0.92(0.2)	1.5(0.16)	NT	0.04(0.02)	0.16 (0.03)	NT
673	No CD8s	345(6.4)	194(25)	253(17)	213(28)	139(4.6)	NT
	1:10	329(1.2)	137(5.2)	184(12)	73(4.6)	57(5.2)	NT
	1:1	94(6.9)	8.8(0.2)	13.5(0.5)	4.4(0.5)	11.4(1.0)	NT
527	No CD8s	154(5.2)	185(22)	106(9.4)	27.3(12.3)	54.2(29.3)	62.9(3.4)
	1:10	164(16)	210(11)	78(6.6)	12.1(2.1)	21(2.8)	68(5.9)
	1:1	116(53)	139(10.8)	80(21)	13(6)	8.4(0.9)	34.9(5.6)
728	No CD8s	103(25)	111(10)	92(24)	19(10)	122(51)	30(11)
	1:10	114(18)	117(13)	90(2.2)	3.7(2.6)	54(11)	15.9(5.4)
	1:1	178(14)	121(32)	60(23)	1.9(1.1)	44(9.5)	0.9(0.4)
749	No CD8s	27.9(11)	7.3(6.8)	13.8(2.9)	0.9(0.5)	19.3(7.7)	16.5(12.2)
	1:1	11.4(0.5)	6.8(3.3)	5.5(3)	**ND**	**ND**	**ND**
725	No CD8s	191(3.7)	177(37)	189(24)	29(21)	134(16)	45(13)
	1:10	234(53)	187(14)	163(22)	6.5(5.5)	122(17)	**ND**
	1:1	60(22)	71(5)	53(17)	**ND**	4.5(3.3)	**ND**
720	No CD8s	15(4.8)	26.3(4.8)	22(2.3)	0.7(0.2)	5.9(2.6)	0.6(0.2)
	1:10	5.7(2.5)	17(2.3)	16(4.6)	0.4(0.1)	2.5(0.5)	1.0(0.6)
	1:1	1.1(0.1)	0.4(0.3)	1.1(0.3)	**ND**	1.3(0.4)	0.2(0.02)

SEM=standard error of the mean of 3 replicates
ND = not detected
NT = not tested due to cell availability

Supplemental Table 5: Absolute # of Positive Wells in Latency Clearance Assay with DARTs

LRA	Patient	#wells plated	#positive wells after addition of DART MOLECULE					
			No Effectors	No DART	4420 xCD3	7B2 xCD3	A32 xCD3	Combo
PHA	**425**	12	8	8	9	6	7	7
	728	12	7	9	6	NT	NT	0
	725	12	11	7	7	6	3	5
	749	12	7	5	3	3	1	2
	674	12	6	4	4	NT	NT	2
	795*	12	10	4	6	NT	NT	0
VOR	**674**	12	6	6	7	NT	NT	3
	408	12	2	1	1	0	0	NT
	407	24	6	5	6	NT	NT	2
	795	36	28	17	21	NT	NT	22
	795*	12	3	1	2	NT	NT	0

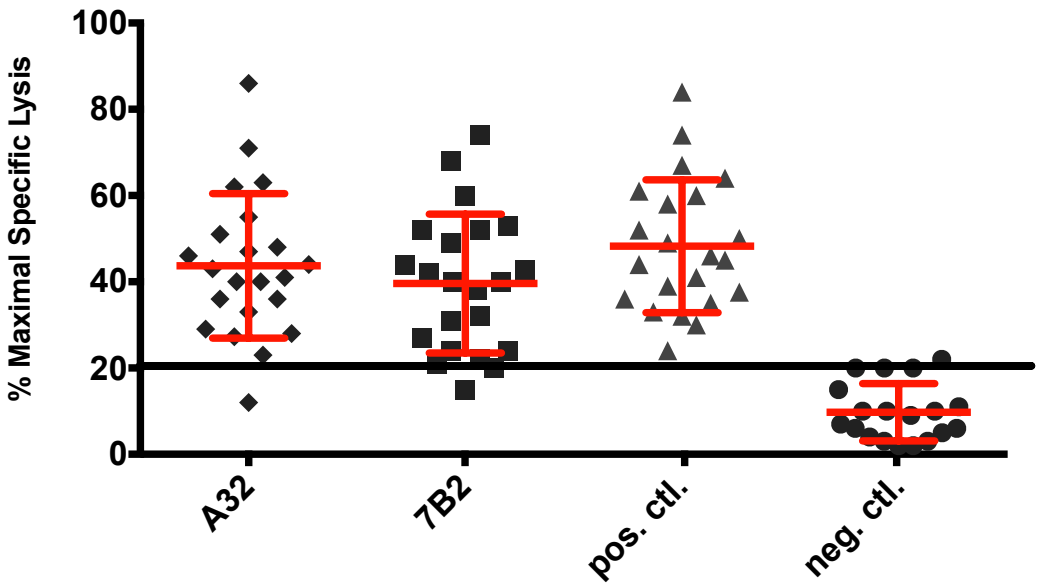
LRA=latency reversing agent

NT=not tested due to cell availability

combo= Addition of 50ng/ml of 7B2xCD3 DART and 50ng/ml of A32xCD3 DART

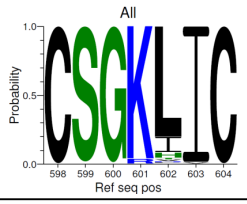
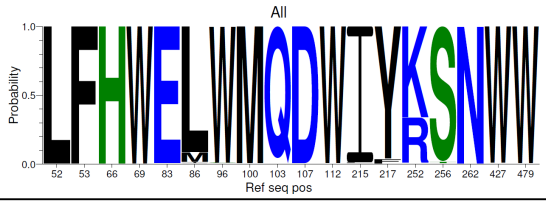
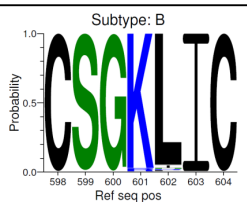
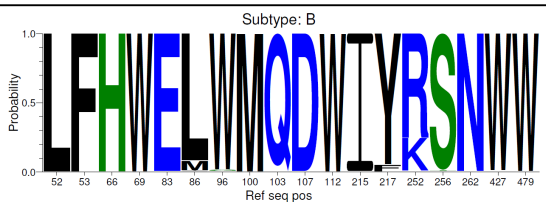
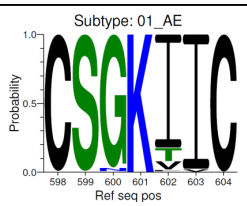
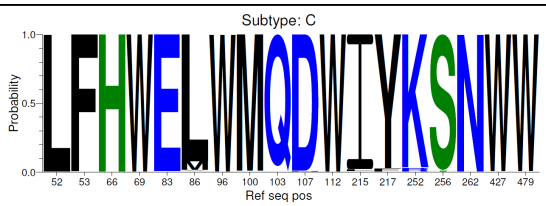
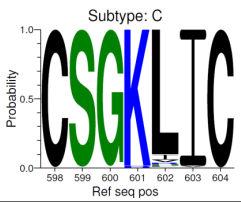
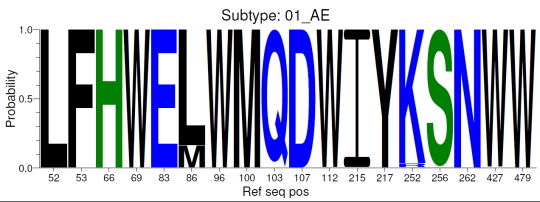
* evaluated using 96 hours co-culture

VOR=vorinostat



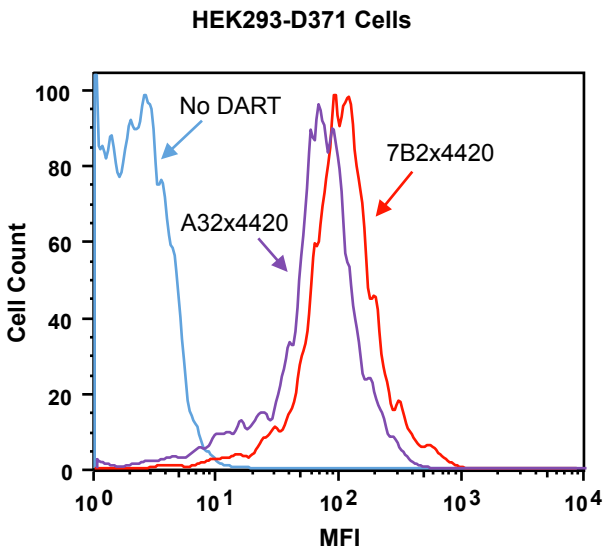
IMC Recognized	**21**	**20**	**22**	**1**
Percentage	**95**	**91**	**100**	**5**
Mean Max %SL	**43.69**	**39.58**	**48.25**	**9.73**
Range %SL	**12-86**	**15-74**	**24-84**	**2-22**

Supplemental Figure 1. Potency and breadth of ADCC-mediating mAbs. The ADCC activities of the A32 (anti-gp120 C1/C2) mAb (◆) and 7B2 (anti-gp41 cluster I) mAb (■) are reported as maximum percentage of specific lysis (%SL) against each of the 22 HIV-1 IMC . Each dot represents one HIV-1 IMC. The results obtained with plasma from one HIV-1 seropositive (positive control; pos ctrl) and one seronegative (negative control; neg ctrl) donor are also reported. The lines represent the mean ± standard deviation. The black line represents the cut-off for positive response.

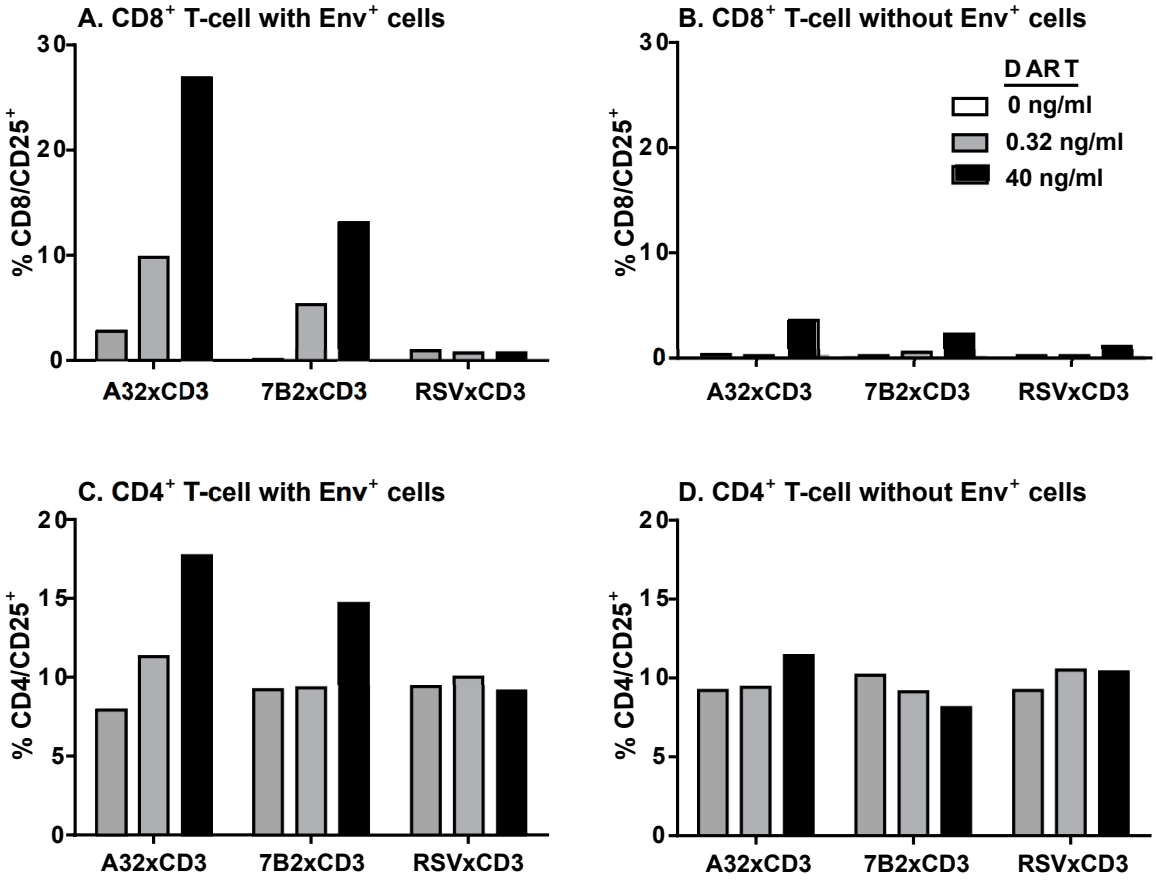
HIV-1 *Env* (# isolates)	Residues Influencing 7B2 Binding	Residues Influencing A32 Binding
All subtypes (4556)		
Subtype B (1529)		
Subtype AE (426)		
Subtype C (1191)		

Supplemental Figure 2. Conservation of HIV-1 *Env* residues known to influence the binding of 7B2 and A32 mAbs. A linear 7-residue sequence in gp41 (gp160 positions 598-604; immunodominant cluster I) is reported to contain the binding site for 7B2 mAb *(28, 29)*. Discontinuous residues in gp120 C1-C4 known to influence A32 mAb binding (based on point mutagenesis studies) occur at positions 52, 53, 66, 69, 83, 86, 96, 100, 103, 107, 112, 215, 217, 252, 256, 262, 427 and 479 *(37, 39, 68)*. The conservation of these residues in the Los Alamos National Laboratory (LANL) HIV1 Env Amino acid Filtered web alignment, a database consisting of 4556 HIV-1 Env sequences with representation of all subtypes, was assessed by QuickAlign analysis (http://www.hiv.lanl.gov/content/sequence/QUICK_ALIGNv2/QuickAlign.html). The height of the residue at each position of Env is proportional to its frequency of distribution among the HIV-1 isolates. Residues are colored according to hydrophobicity: black, hydrophilic; green,

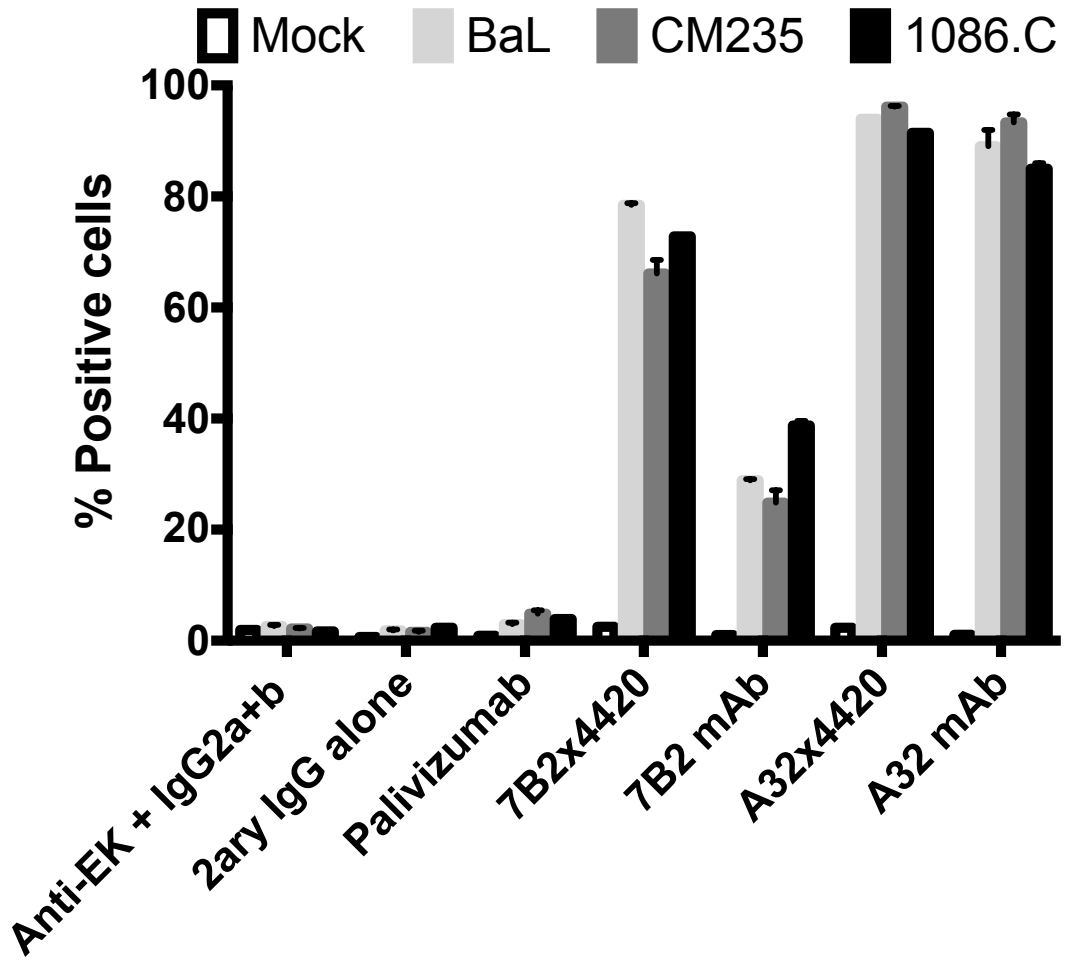
neutral; blue, hydrophobic. Based on a crystal structure of a CD4-stabilized gp120 core complexed with a Fab fragment of N5-i5 (an A32-like mAb), residues at 52, 53, 69, 103, 107 and 217 (located in C1-C2) may be direct epitope contacts *(27)*.



Supplemental Figure 3. Cell surface Env binding of A32x4420 and 7B2x4420 control DARTs. DART binding to HEK293-D371 cells expressing HIV-1 Env, CM244, subtype AE was measured and data are reported as mean fluorescence intensity (MFI). A32 and 7B2 are targeting arms that recognize HIV-1 gp120 and gp41, respectively; 4420 is an irrelevant, negative control arm.

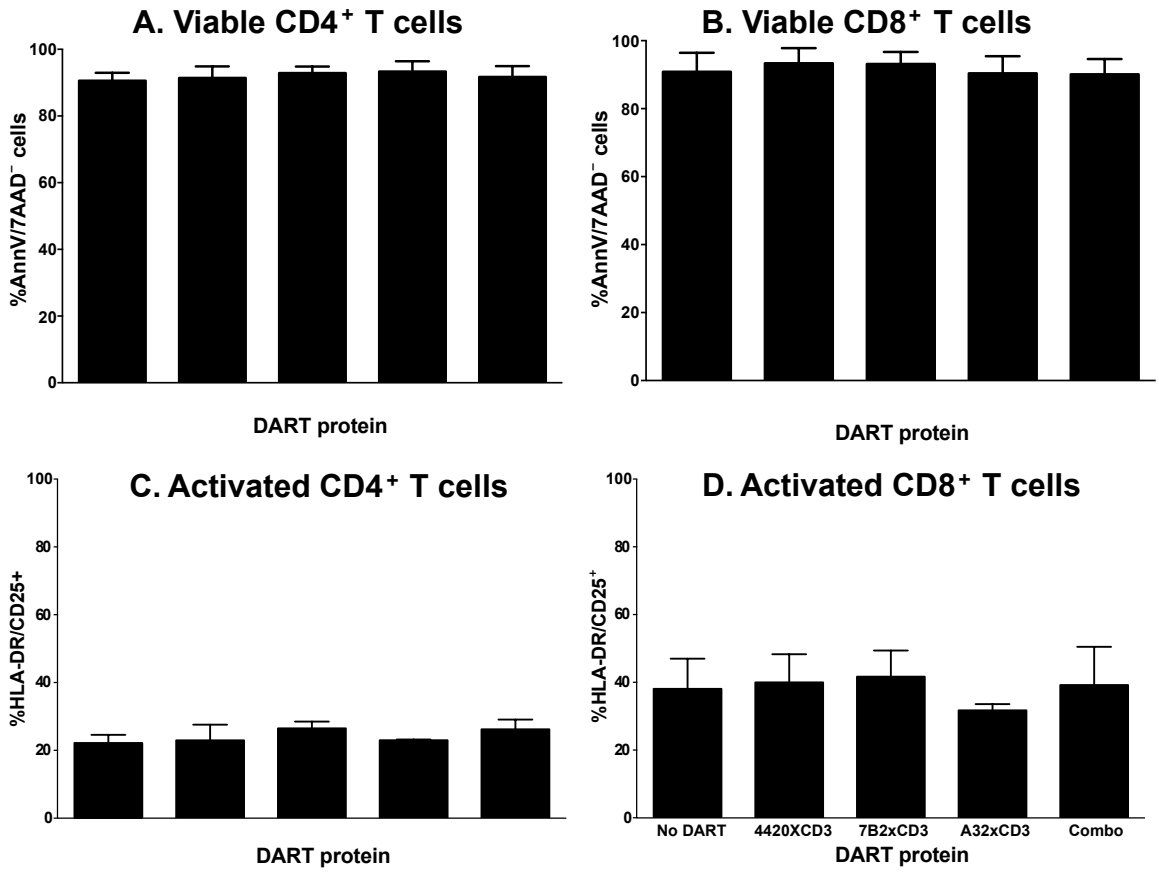


Supplemental Figure 4. HIVxCD3 DART-mediated T-cell activation depends on co-engagement with target cells. Unstimulated $CD4^+$ or $CD8^+$ T-cells from healthy seronegative donors were incubated with (**A, C**) and without (**B, D**) Env expressing Jurkat-522 F/Y cell line in the absence or presence of control (RSVxCD3) or HIVxCD3 (A32xCD3, 7B2xCD3) DARTs at 40, 0.32, and 0 ng/mL for 48 hours. $CD8^+$ (**A-B**) and $CD4^+$ (**C-D**) T cell activation was assessed by staining with CD25 Ab cells. The data are reported as frequency (%) of activated ($CD25^+$) T cells. Each bar represent the average of results obtained from 2 different donors.



Supplemental Figure 5. HIV DARTs bind specifically to HIV-1 IMC infected CD4$^+$ T cells. Activated CD4$^+$ T cells obtained from healthy HIV-1 seronegative donors were infected for 48 hours with HIV-1 IMCs representing the HIV-1 subtype B BaL, AE CM235, and C 1086.C as reported in the methods section. Non-infected CD4$^+$ T cells (mock) were utilized as negative control. The cells were stained using the 7B2x4420 and A32x4420 DART where the CD3 arm was substituted with the irrelevant 4420 protein to avoid binding to the CD3 receptor. After incubation with the DART, the cells were stained with the secondary anti-EK-IgG2a-biotinylated complex to reveal binding of the DARTs. The staining with 7B2 and A32 mAbs, utilizing an indirect staining technique with the secondary mouse anti-human-IgG mAb, was performed as control. The secondary fluoresceinated anti-human IgG Abs and the Palivizumab mAb were

utilized as negative controls. The frequency of infected cells was determined by intracellular staining using the anti-p24 mAb as reported in the method section. Each bar represents $CD4^+$ T cells infected with the IMCs and controls as indicated above the graph. The results are reported as frequency (%) of viable infected ($p24^+$) $CD4^+$ T cells that were stained by each of the DARTs, mAbs, and controls as listed on the x-axis.



Supplemental Figure 6. Lack of HIVxCD3 DART effects on T cell viability or activation status in the absence of added target cells using PBMC from HIV-1 infected donors. Unstimulated $CD4^+$ or $CD8^+$ T-cells from HIV-infected, ART suppressed were incubated in the absence or presence of control (4420xCD3, 7B2x4420, A32x4420) or active (A32xCD3, 7B2xCD3) DARTs at 100ng/mL for 7 days. **A-B**) T cell viability was assessed by staining cells for Annexin V/7-AAD. Viable cells were identified as those that were Annein V and 7-AAD negative. **C-D**) T cell activation was assessed by staining cells for HLA-DR and CD25 expression. Data points for both analyses are from n=3 patients performed on 3 independent occasions. Error bars represent standard error mean.