Material and Method

Antibodies: The information of antibodies and cell dyes used in this study are shown in the **Supplementary Table 5**. The HLA-F-specific mAb, 4D11 was a generous gift from Dr. Dan Geraghty, Fred Hutchinson Cancer Research Institute, Seattle, WA.

Generation of an HLA-E knockout 221 cell line and its HLA class I transductants: Since HLA-E tetramers were unstable, a panel of 721.221 derivative cell lines were generated to discriminate between classical HLA- Ia and HLA-E restricted T-cell responses. The CRISPR/Cas9 system designed with the guide RNA (5'-cctCCTTTTACTCCTCTCGGAGGC-3') targeting the first exon of *HLA-E* was used to disrupt the *HLA-E* gene in 221 cells (Plasmid 42230, Addgene). An *HLA-E* knockout clone with loss-of-function heterozygous deletions by genotyping was isolated ($221\Delta^{E}$). Individual HLA-Ia and HLA-E*01 alleles were subsequently transduced into the HLA-E^{KO} cells by using lentiviral plasmids encoding HLA-A*02:01, HLA-E*01:01 and HLA-E*01:03, and HLA-B*57:01, HLA-B*57:03, and human CD4 co-receptor. Lentiviruses were produced by co-transfection of 293T cells with ViraPower Packaging Mix (ThermoFisher) using Lipofectamine (Invitrogen) and concentrated by centrifugation (100,000 x g for 2 hrs. at 4°C). Transduction was achieved by spinfection at 1,065 x g for 2hrs. and surface expression of the target gene by transduced cells monitored by immunostaining. Derivative lines expressing a single class Ia or class Ib allele were generated from $221\Delta^{E}$ cells ($221^{\Delta E}$.A*02:01, $221^{\Delta E}$.B*57:01, $221^{\Delta E}$.B*57:03, $221^{\Delta E}$.E*01:01 and $221^{\Delta E}$.E*01:03). Additionally, four of these were rendered HIV permissive by the transduction of the T cell co-receptor CD4 ($221^{\Delta E}$.CD4, $221^{\Delta E}$.B*57:01.CD4, $221^{\Delta E}$.E*01.01.CD4 and $221^{\Delta E}$.E*01.03.CD4).

Ex-vivo intracellular cytokine staining (ICS) based antigen sensitivity assay: Untouched CD8 T cells were isolated from PBMCs of chronically HIV infected individuals with detectable antigen-specific CD8 T-cell response as measured by ex-vivo ELISpot assay. Cell lines, 721.221-AEH (HLA-E), K562-B*57:01 (HLA-B57) as well as CD4.E01, CD4.E03 and CD4.B57 expressing cell lines were used as antigen presenting cells (APCs). APCs were pulsed with three log fold serial dilutions of peptide (10, 1 and 0.1 μg/ml) for 2 hrs. and washed three times before adding to the CD8 T cells at 1:1 ratio. Costimulatory molecules (anti-CD28 and CD49d), anti-CD107a-FITC antibodies and Monensin and Brefeldin-A (5 μg/ml, eBioscience) were added and the cells co-cultured for 12 hrs. Cytokine/effector molecules were detected using flow cytometry (IFN-γ, TNF-α, IL-2, CD107a, perforin and granzyme B) at 37ºC/5% CO_2. Following incubation, cells were stained with Dead cell dye-UV, anti-CD3-Alexa 780, anti-CD8-V500 and anti-CD19-Percp/CY5.5 at 4ºC for 30 min. Cells were then fixed and permeabilized using Cytofix/Cytoperm and intracellularly stained with anti-IFNγ-Alexa 700, anti-IL2-APC, anti-TNFα-PECy7, anti-Perforin-PE, and anti-Granzyme B-V450 at 4ºC for 30 min. Cells cultured in absence of peptide or in presence of SEB were served as negative or positive control respectively. At least 200,000 total events were acquired on an LSR II flow cytometer (BD Immunocytometry Systems), and data were analyzed using FlowJo (version 10.6.1, TreeStar Inc.).

Supplemental Table 1. IFN-γ ELISpot data showing recognition of overlapping Gag peptides (7954 and 7955) by CD8 T cells primed from two healthy donors.

Donor	OLP	Location in Gag	OLP Sequence	Net SFCs / 10^6 cells
HD1	**7912**	161-175	E K A F S P E V I P M F S A L	2,520
	7919	189-203	N T V G G H Q A A M Q M L K E	280
	7920	193-207	G H Q A A M Q M L K E T I N E	120
	7937	261-275	I Y K R W I I L G L N K I V R	1,000
	7938	265-279	W I I L G L N K I V R M Y S P	400
	7954	329-343	D C K T I L **K A L G P A A T L**	**12,840**
	7955	333-347	I L **K A L G P A A T L** E E M M	**7,720**
	7978	425-439	D C T E R Q A N F L G K I WP	400
	7979	429-443	R Q A N F L G K I WP S H K G	760
	7980	433-447	F L G K I WP S H K G R G P N	1,920
HD2	7879	29-43	Y K L K H I V WA S R E L E R	25
	7880	33-47	H I V WA S R E L E R F A V N	27
	7908	145-159	Q A I S P R T L N A WV K V V	3,760
	7909	149-163	P R T L N A WV K V V E E K A	2,760
	7910	153-167	N A WV K V V E E K A K S P E	42
	7911	157-171	K V V E E K A K S P E V I P M	148
	7916	177-191	E G A T P Q D L N T M L N T V	30
	7917	181-195	P Q D L N T M L N T V G G H Q	499
	7938	265-401	W I I L G L N K I V R M Y S P	219
	7939	269-283	G L N K I V R M Y S P T S I L	456
	7943	285-299	I R Q G P K E P F R D Y V D R	20
	7954	329-343	D C K T I L **K A L G P A A T L**	**369**
	7955	333-347	I L **K A L G P A A T L** E E M M	**175**
	7985	453-467	P E P T A P P E E S F R F G E	565

OLP= overlapping peptide; HD= healthy donor; KL9 epitope and its response is bolded and underlined; data are representative of at least two independent experiments. HD=healthy donor; HLA of HD1 is A0101/A0201; B3905/B5701 and HD2 is A0201/A68; B51/B58

Supplementary Table 2. HLA-I and clinical characteristics of chronically HIV-infected (CHI) individuals used in this study.

PID*	HLA-A and -B genotype				HLA-E genotype	CD4 (cells/μL)	VL (copies/μL)	ART
CHI-2*	A*02:01	A*02:01	B*35:01	B*57:03	E*01:03, E*01:03	854	49	Naïve
CHI-4	A*30:01	A*68:02	B*42:01	B*57:03	E*01:03, E*01:03	930	208	Naïve
CHI-5	A*30:01	A*66:01	B*42:01	B*57:03	E*01:03, E*01:03	409	280	Yes
CHI-6	A*30:01	A*30:02	B*15:16	B*57:03	E*01:01, E*01:03	992	12,407	Yes
CHI-7	A*29:02	A*30:01	B*15:01	B*57:02	E*01:01, E*01:03	167	1,680	Yes
CHI-9*	A*24:02	A*30:01	B*53:01	B*57:03	E*01:03, E*01:03	885	613	Yes
CHI-10	A*02:01	A*03:01	B*07:02	B*15:01	E*01:01, E*01:03	291	176	Yes
CHI-11	A*30:02	A*33:01	B*57:03	B*78:01	E*01:01, E*01:01	1049	167	Yes
CHI-12*	A*02:02	A*03:01	B*56:01	B*57:03	E*01:01, E*01:03	1241	398	Yes
CHI-13	A*03	A*03	B*14	B*57	E*01:01, E*01:03	943	420	Naïve
CHI-14	A*68	A*80:01	B*40	B*57:01	E*01:01, E*01:03	693	226	Naive
CHI-15	A*02:01	A*30:01	B*13:02	B*57:01	E*01:01, E*01:01	551	47	Yes
CHI-16	A*03:01	A*68:01	B*51:01	B*57:01	E*01:03, E*01:03	403	74	Yes
CHI-17	A*01:01	A*02:05	B*49:01	B*57:01	E*01:01, E*01:01	520	2,950	No
CHI-18	A*02	A*30	B*49	B*57	E*01:03, E*01:03	420	38,425	Yes
CHI-19	A*02:01	A*02:01	B*40:02	B*57:01	E*01:03, E*01:03	818	456	Naïve
CHI-20	A*02:01	A*30:01	B*08:01	B*57:02	E*01:01, E*01:03	1763	19	Naïve
CHI-21	A*01:01	A*02(01, 09)	B*44:02	B*57:03	E*01:01, E*01:03	511	1665	Naive
CHI-23*	A*02:01	A*30:10	B*07:02	B*57:01	E*01:01, E*01:01	587	1250	No
CHI-25*	A*68:02	A*68:02	B*57:03	B*57:03	E*01:01, E*01:03	572	1477	Naïve

** used in both in-vitro and ex-vivo assays*

Supplementary Table 3. Detection of HLA-E*01 restricted KL9 and KF11 specific CD8 T cells in chronically HIV-infected individuals.

PID	% Specific Degranulation (% Nonspecific Degranulation)				
	KL9 Restricted by			KF11 Restricted by	
	A*02:01	B*57	E*01	B*57	E*01
CHI-2	-	**7.2 (2.2)**	3.4 (2.2)	**34.6 (1.2)**	**20.9 (1.5)***
CHI-4	-	6.8 (4.9)	4.1 (3.4)	**37.0 (5.4)**	**16.5 (3.5)***
CHI-5	-	3.3 (2.8)	1.8 (1.9)	**9.6 (2.8)**	1.7 (1.9)
CHI-6	-	2.2 (1.4)	0.8 (1.3)	**28.0 (1.4)****	**18.8 (1.3)****
CHI-7	-	6.7 (2.9)*	**32.4 (1.2)***	1.0 (2.9)	0.2 (2.6)
CHI-9	-	2.3 (0.2)*	1.6 (0.4)*	**28.6 (5.0)**	**17.5 (5.5)**
CHI-10	**19.9 (2.3)***	-	**21.6 (2.8)***	-	-
CHI-11	-	9.3 (7.9)	11.1 (8.4)	17.8 (7.9)	10.4 (8.4)
CHI-12	-	-	-	**30.1 (0.2)**	**30.0 (0.4)***
CHI-13	-	12.0 (11)	8.6 (6.2)	**68.8 (11.0)**	8.9 (6.2)
CHI-14	-	2.1 (11.4)	7.9 (9.5)	28.1 (11.0)	9.3 (9.5)
CHI-15	-	-	-	20.6 (8.0)	14.9 (12.0)
CHI-16	-	1.3 (1.2)	1.3 (1.2)	**64.5 (1.2)**	**11.9 (1.2)**
CHI-17	-	1.5 (1.2)*	1.7 (1.4)*	3.4 (1.2)	**13.7 (1.4)**
CHI-18	-	2.4 (2.2)	3.1 (3.5)	3.0 (2.2)	**11.6 (3.5)**
CHI-19	-	5.2 (4.1)	5.3 (5.6)	**26.6 (4.1)**	11.8 (5.6)
CHI-20	-	6.4 (5.0)	5.0 (6.5)	4.8 (5.0)	5.6 (6.5)
CHI-21	-	**25.5 (1.2)**	0.5 (0.4)	**14.4 (1.2)**	0.4 (0.4)
CHI-23	-	11.9 (4.9)	6.5 (4.4)	14.0 (5.4)	6.5 (4.4)
CHI-25	-	-	-	**29.0 (7.8)**	**31.0 (5.07)**
# Positive / # Tested	**1/1**	**2/16**	**2/17**	**11/19**	**9/19**

***Representative results from two independent experiments using different PBMC aliquots*

****Representative results from three experiments. Bolded= positive based on 3 times the background*

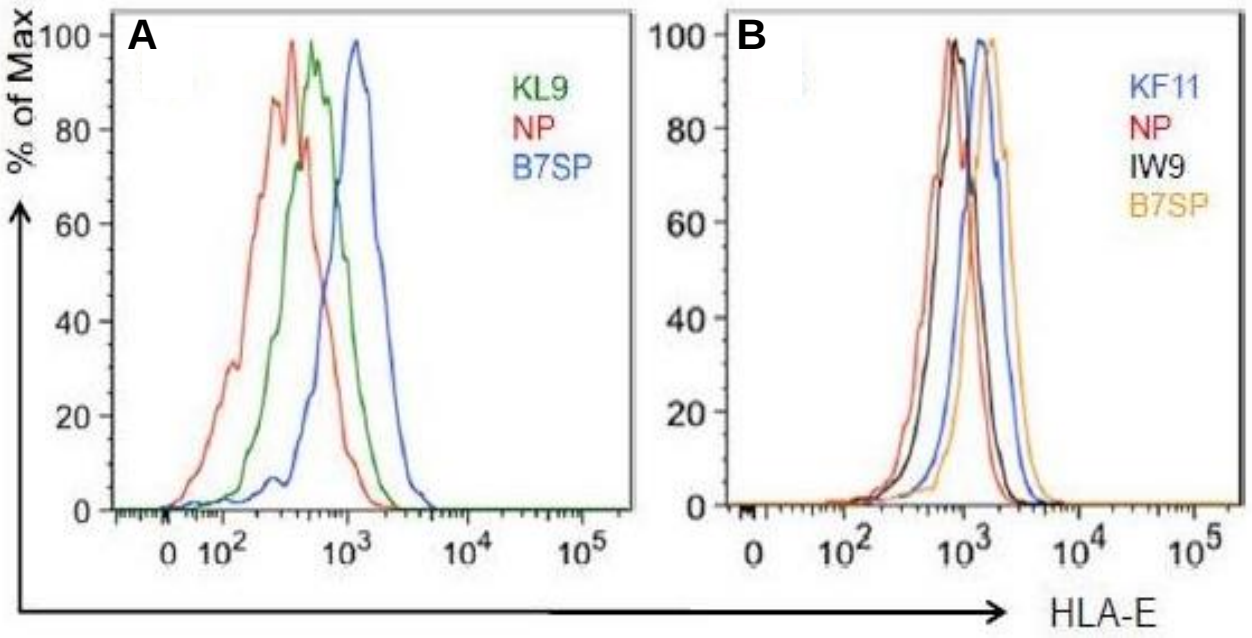
Supplementary Table 4. Functionality of CD8 T cells stimulated ex-vivo with antigen presenting cells expressing HLA-E or B57

			CD107 plus				
PTID	**APC**	**peptide**	**IFNγ**	**IL2**	**TNFα**	**Perforin**	**GranzymeB**
P1	AEH	Media	0.1	0	0.16	0.066	0.016
	AEH	KF11	1.11	0.006	0.81	0.23	0.019
	AEH	KL9	0.085	0	0.15	0.077	0.017
	B57	Media	0	0	0	0.003	0.003
	B57	KF11	0.32	0.003	0.25	0.019	0.003
	B57	KL9	0.028	0	0.038	0.041	0.01
P2	AEH	Media	0.17	0.001	0.17	0.092	0.064
	AEH	KF11	0.74	0.001	0.29	0.18	0.078
	AEH	KL9	0.21	0	0.19	0.12	0.072
	B57	Media	0.004	0	0.011	0.02	0.012
	B57	KF11	0.097	0	0.055	0.036	0.021
	B57	KL9	0.13	0.003	0.064	0.043	0.026
P3	AEH	Media	0.012	0	0.026	0.038	0.014
	AEH	KF11	0.18	0	0.088	0.09	0.028
	AEH	KL9	0.012	0	0.032	0.037	0.022
	B57	Media	0	0	0	0.002	0.002
	B57	KF11	0.008	0	0	0.006	0.003
	B57	KL9	0.017	0.003	0.015	0.015	0.015
P4	AEH	Media	0.004	0	0	0.006	0.009
	AEH	KF11	0.07	0.002	0.037	0.016	0.016
	AEH	KL9	0.006	0	0.004	0.008	0.014
	B57	Media	0	0	0	0	0.005
	B57	KF11	0.028	0	0.02	0.002	0.004
	B57	KL9	0.007	0	0.002	0.002	0.005
P5	AEH	Media	0.06	0	0.072	0.13	0.019
	AEH	KF11	0.98	0	0.64	0.59	0.06
	B57	Media	0.005	0	0.005	0.01	0.002
	B57	KF11	0.14	0	0.095	0.1	0.021
P6	AEH	Media	0.11	0	0.19	0.22	0.078
	AEH	KF11	2.13	0.002	2.04	1.4	0.28
	AEH	KL9	0.14	0	0.19	0.22	0.049
	B57	Media	0.002	0	0.005	0.039	0.025
	B57	KF11	0.039	0	0.041	0.058	0.02
	B57	KL9	0.3	0	0.32	0.16	0.11
P7	AEH	Media	0.024	0.001	0.039	0.027	0.01
	AEH	KF11	0.65	0.011	0.51	0.078	0.01
	B57	Media	0.015	0	0.023	0.017	0.002
	B57	KF11	0.17	0	0.16	0.043	0.002
	AEH	Media	0.012	0.002	0.022	0.032	0.015
	AEH	KL9	0.021	0	0.026	0.038	0.023
	B57	Media	0.014	0.005	0.017	0.045	0.024
	B57	KL9	0.24	0.003	0.14	0.069	0.013
P8	AEH	Media	0.088	0	0.089	0.22	0.12
	AEH	KF11	3.11	0	1.36	2.89	0.8
	AEH	KL9	0.091	0	0.062	0.21	0.13
	B57	Media	0.002	0.002	0.009	0.009	0.017
	B57	KF11	0.36	0	0.23	0.33	0.16
	B57	KL9	0.088	0	0.034	0.11	0.088
P9	AEH	Media	0.097	0	0.17	0.065	0.047
	AEH	KF11	0.39	0.002	0.39	0.083	0.034
	AEH	KL9	0.14	0	0.22	0.073	0.056
	B57	Media	0.005	0	0.005	0.002	0.002
	B57	KF11	0.073	0.003	0.064	0.005	0.007
	B57	KL9	0.12	0	0.15	0.022	0.017

Supplementary Table 5. List of antibodies used in this study.

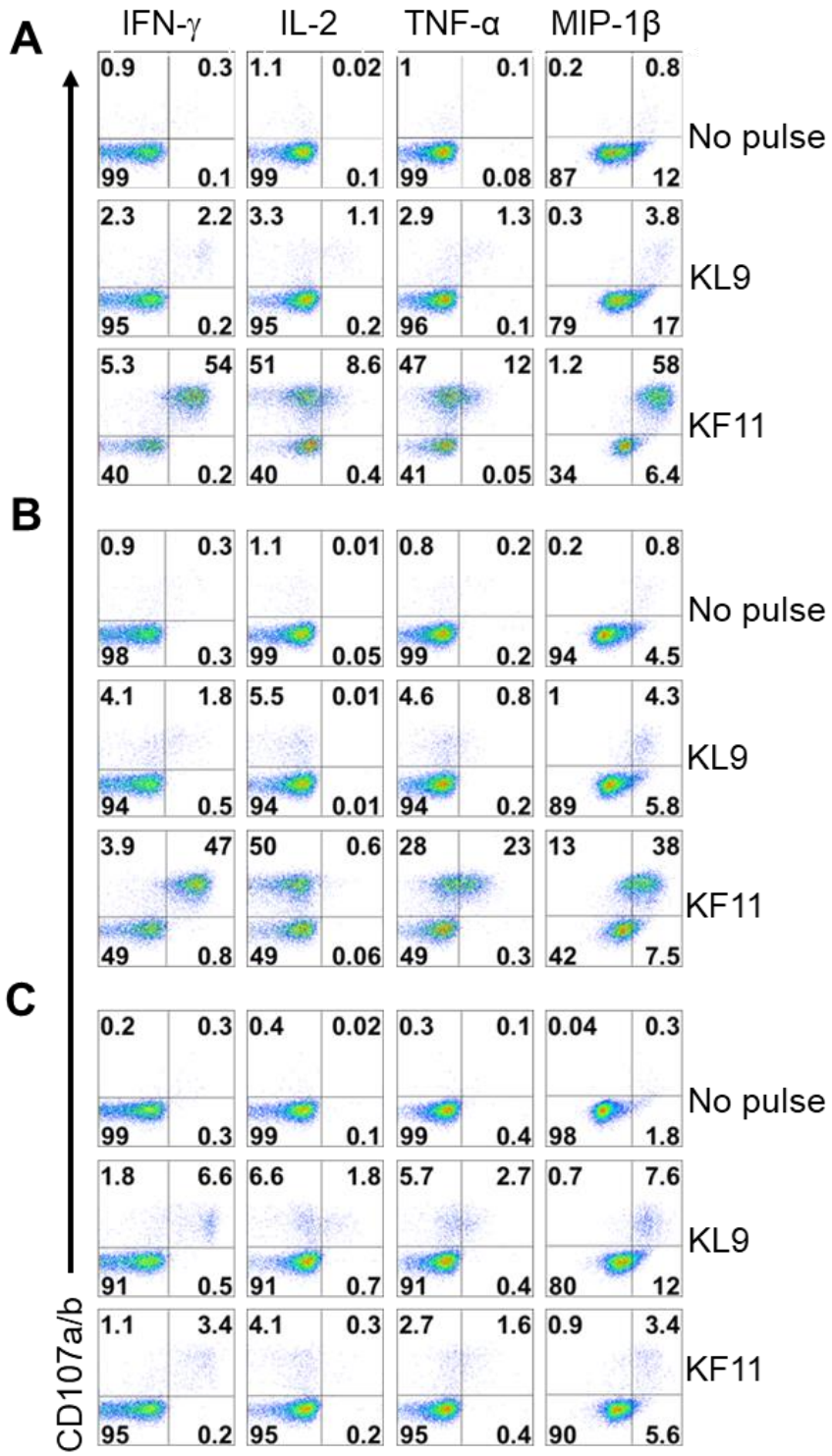
Marker	Fluorochrome	Supplier	Cat. #	Clone
CD8	PE	BD Biosciences	557086	RPA-T8
CD8	V500	BD Biosciences	560774	RPA-T8
CD8	FITC	BD Biosciences	561947	RPA-T8
CD4	FITC	BD Biosciences	561005	RPA-T4
CD19	Percp-Cy 5.5	BD Biosciences	561295	HIB19
CD69	APC	BD Biosciences	555533	FN50
CD137	PE	BD Biosciences	555956	4B4-1
IFN-γ	ALEXA 700	BD Biosciences	557995	B27
IFN-γ	V500	BD Biosciences	561980	B27
IL-2	APC	BD Biosciences	555434	M-A251
IL-2	Percp-Cy 5.5	BD Biosciences	560503	M-A251
TNF-α	PE-Cy7	BD Biosciences	557647	MAb11
CD107a	FITC	BD Biosciences	555800	H4A3
CD107b	FITC	BD Biosciences	555804	H4B4
Granzyme B	V450	BD Biosciences	561151	GB11
Granzyme B	Alexa 647	BD Biosciences	561999	GB11
MIP-1β	PE	BD Biosciences	550078	D21-1351
CD3	APC-eFluor 780	eBioscience	47-0032-82	17A2
HLA-E	APC	eBioscience	17-9953-41	3D12
HLA-A2	APC	eBioscience	17-9876-42	BB7.2
CD19	APC	eBioscience	47-0199-42	HIB19
TNF-a	Alexa700	eBioscience	56-7349-42	MAb11
CD94	APC	eBioscience	17-5094-42	HP-3D9
NKG2D	PE-Cy7	eBioscience	14-5878-82	1D11
NKG2C	PerCp-e Fluor 710	eBioscience	46-5896-82	20D5
Perforin	PE	cell sciences	CDM247	B-D48
Dead cell dye	blue dead cell stain	Invitrogen	-	L23105
Dead cell dye	aqua dead cell stain	Invitrogen	-	L34977

Supplementary Figure 1



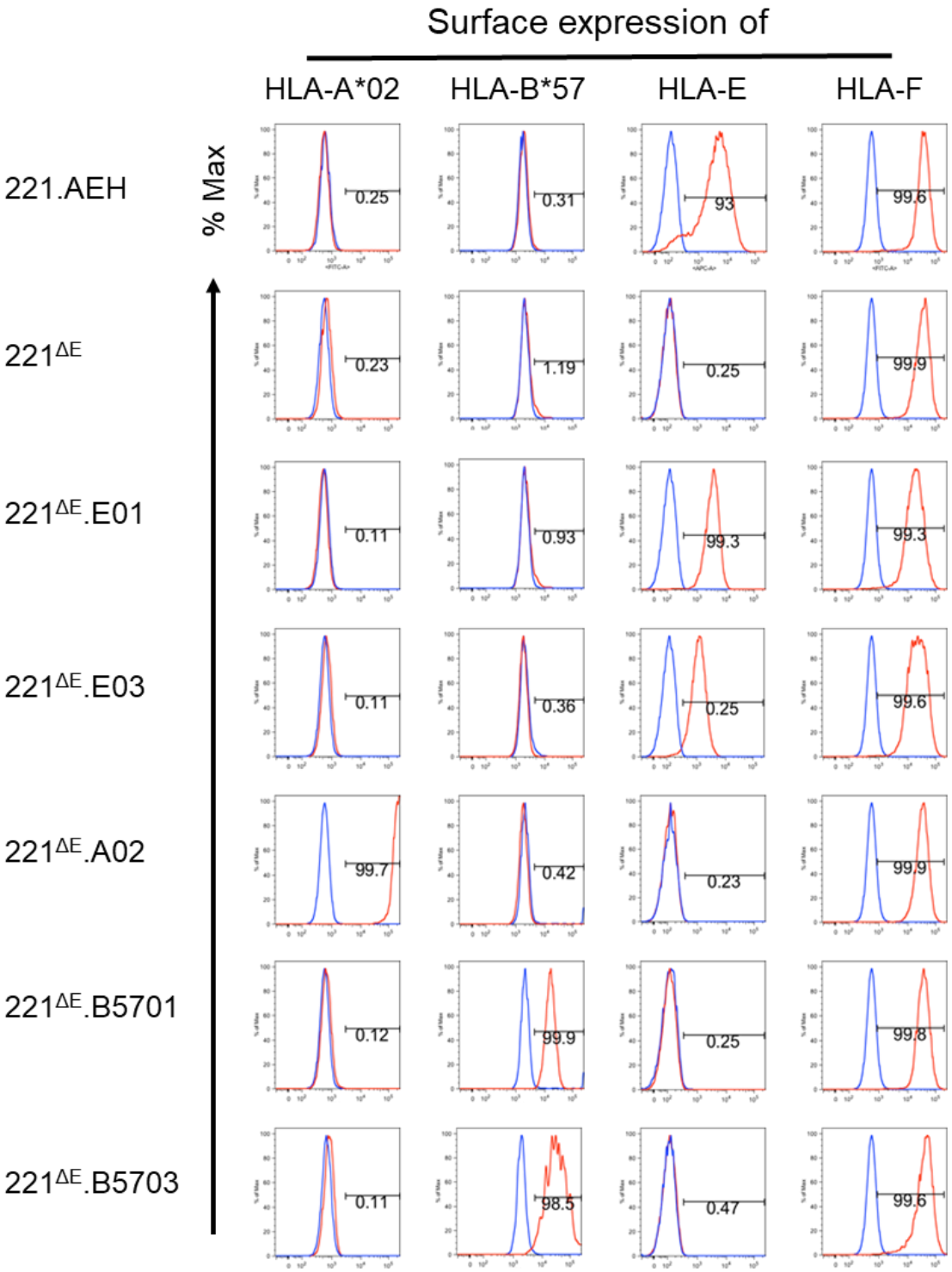
Supplementary Figure 1. Binding of KL9 and KF11 to HLA-E*01:01 expressed on 721.221 cells. The histogram of cells incubated with an isotype control IgG1κ was essentially identical to that of cells incubated without peptide (NP) or with 100 μg/ml IW9, an HLA-B*57:01-restricted HIV Gag peptide. All peptides used were >95% pure. (**A)** and **(B)** show HLA-E*01:01 binding of KL9 and KF11, respectively.

Supplementary Figure 2



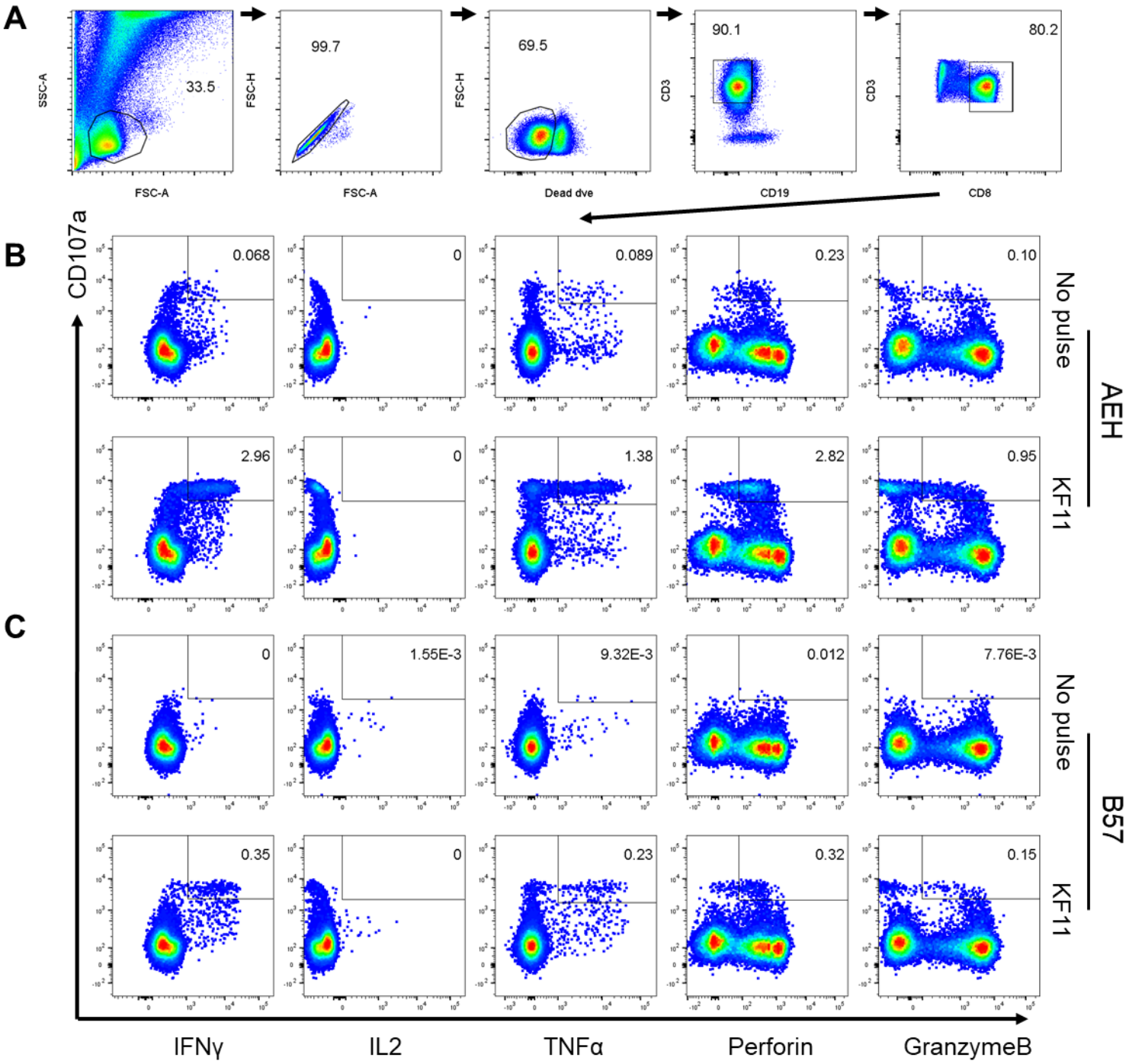
Supplementary Figure 2. Polyfunctionality of KL9 specific CD8 T-cell responses elicited in chronically HIV infected individuals. In–vitro cultured antigen specific CD8 T-cells from three HIV infected individuals were stimulated with KL9 peptide (10 μg/ml loaded AEH cells for 5 hrs. in presence of anti-CD28/49d and golgi stop/plug. No peptide and Gag-KF11 peptide were used as negative and positive controls respectively. The production of IFN-γ, IL-2, TNF-α and MIP-1β are shown.

Supplementary Figure 3



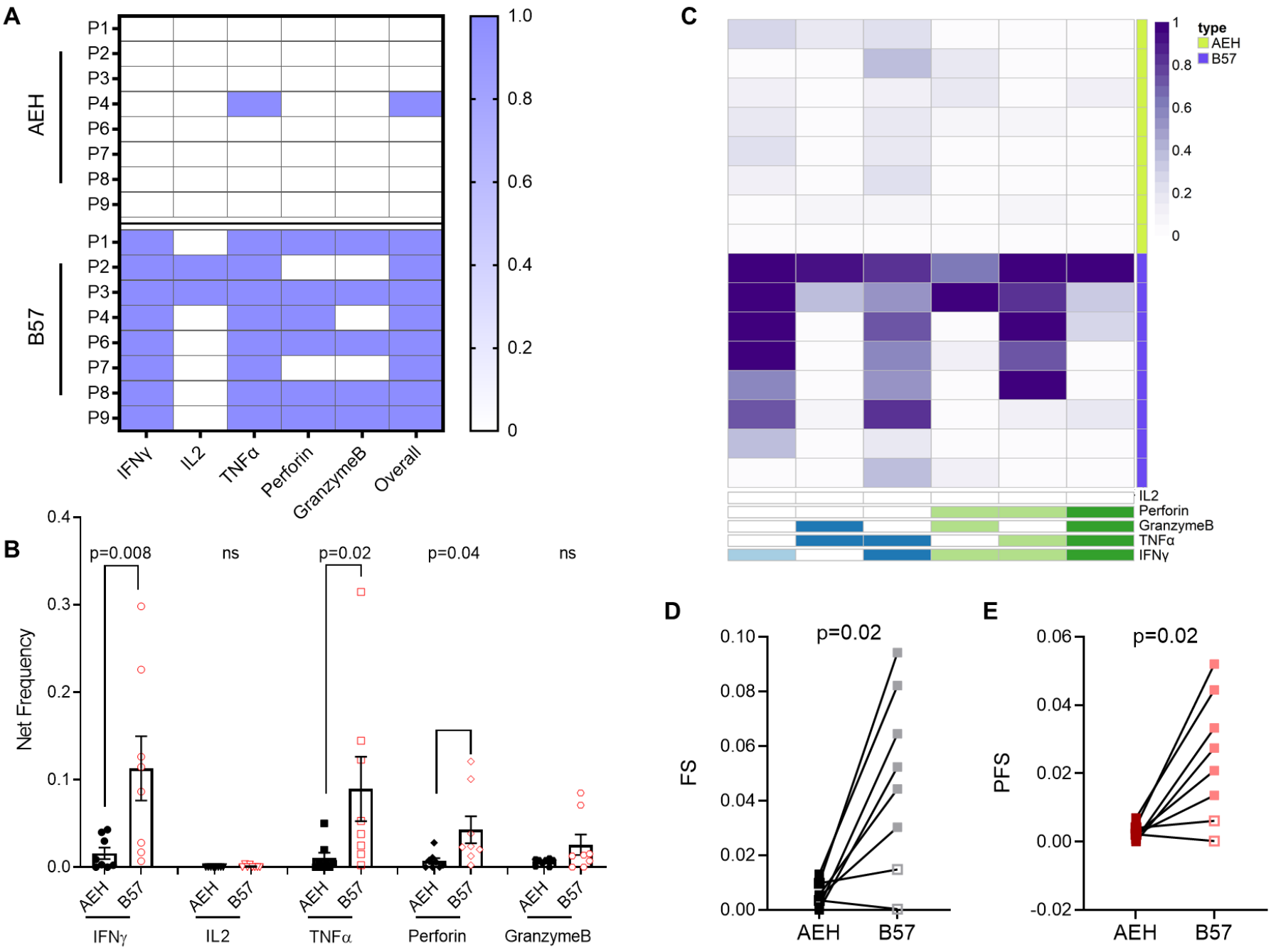
Supplementary Figure 3. Expression of HLA class I alleles by 221$^{\Delta E}$ and derivative single HLA-Ia or -Ib transductants. Histograms showing the surface expression of HLA class I antigens (HLA-A*02:01, HLA-B*57, HLA-E*01, and HLA-F) by immunostaining (red line) in each of the five single allele expressing derivatives of 221$^{\Delta E}$ cell line. Isotype-matched mAbs were used as negative controls in these assays (blue line).

Supplementary Figure 4



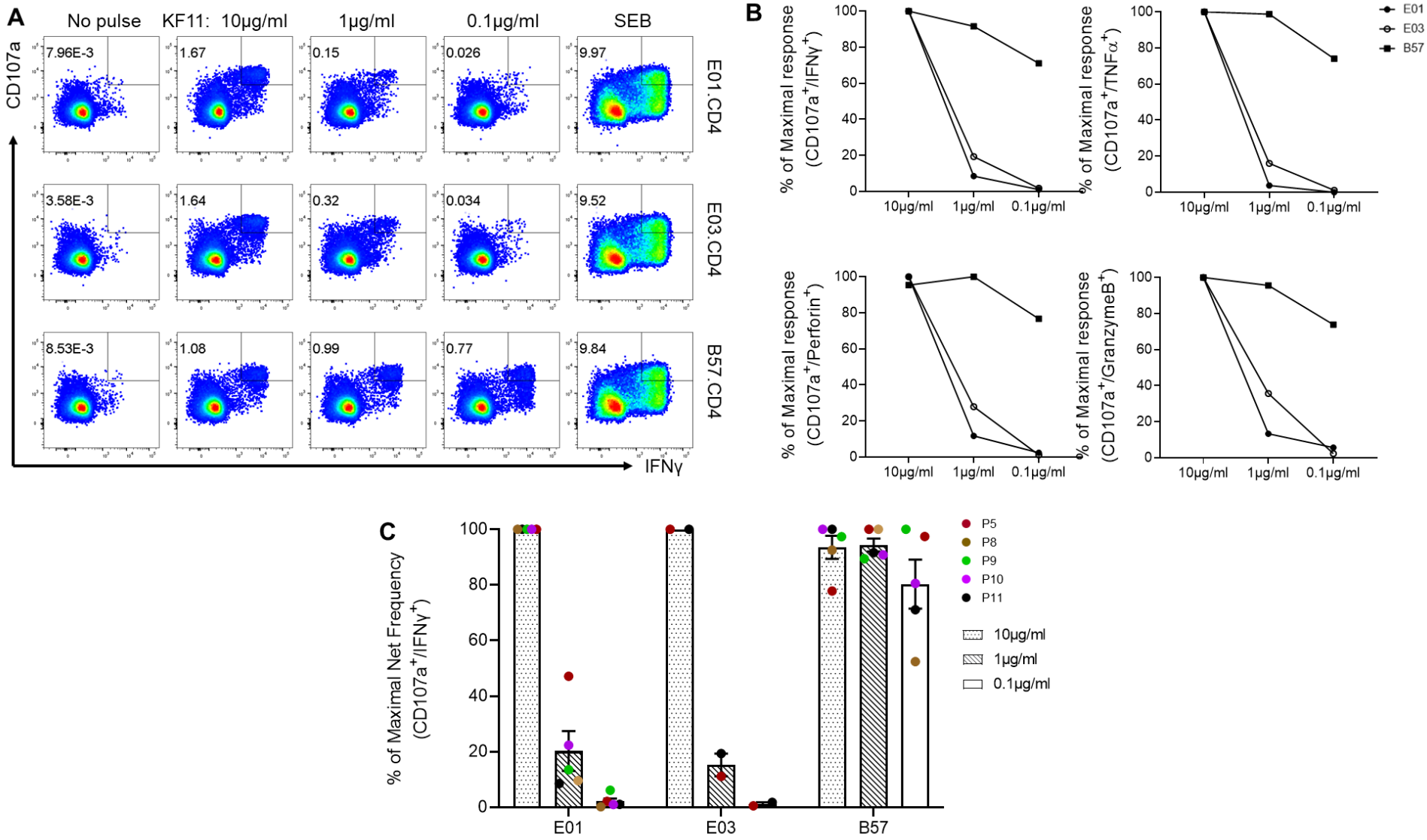
Supplementary Figure 4. Representative gating strategy for measuring functionality of KF11 specific CD8 T-cell responses restricted by HLA-E and B*57 in an ex-vivo assay. (A) Top most panel shows representative gating strategy. Dual production of CD107a and either IFN-γ, IL2, TNF-α, perforin and granzyme B is shown for CD8 T cells recognizing KF11 presented by 221.AEH **(B)** or K562-B*57 targets **(C).** In both **B** and **C**, expression of cytokines/effector molecules are shown relative to negative control with no peptide pulse.

Supplementary Figure 5



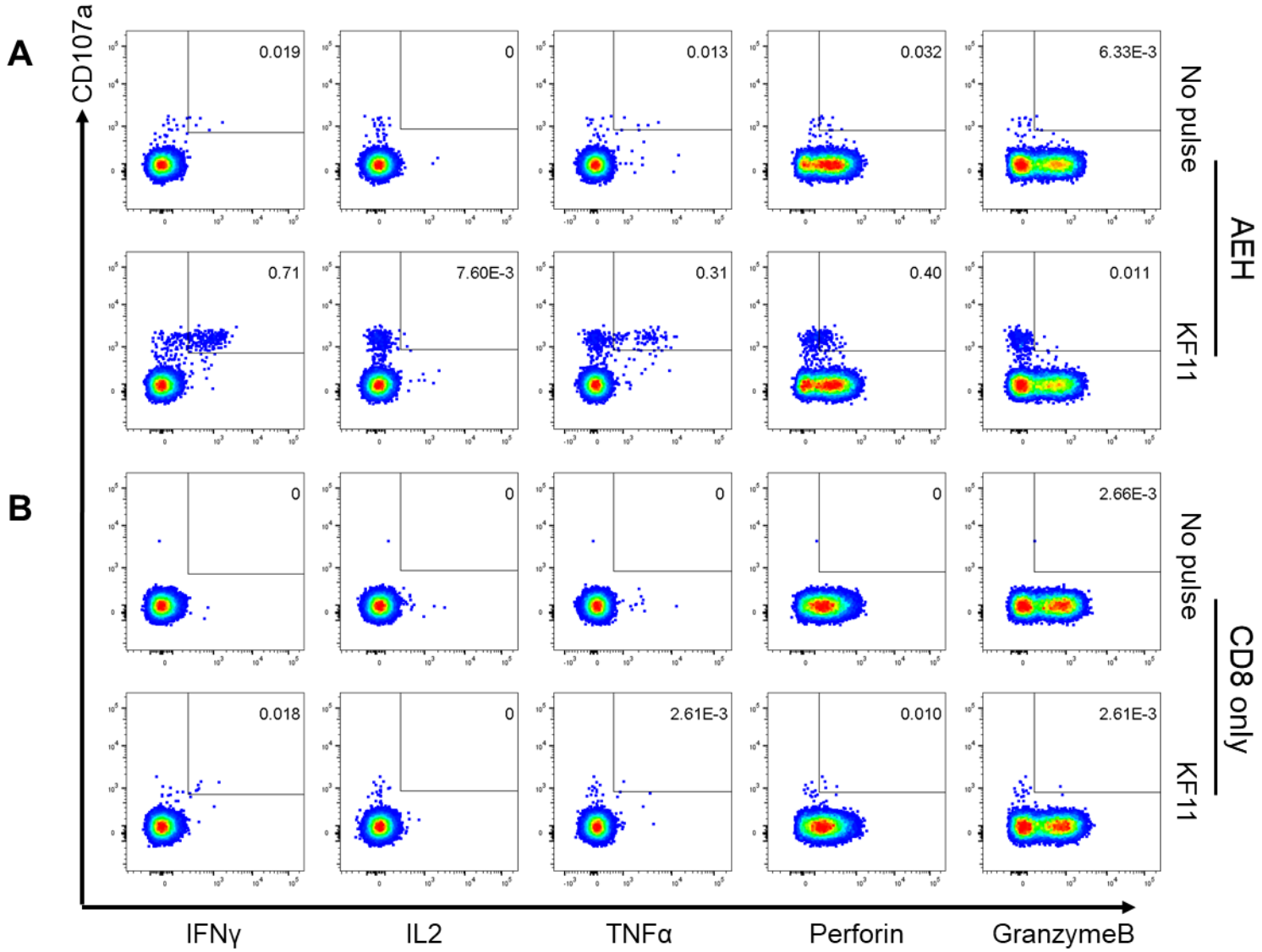
Supplementary Figure 5. Ex-vivo ICS based polyfunctional profile comparison of KL9 specific responses restricted by HLA-B*57 versus HLA-E in chronically HIV infected individuals. (A) Heatmap showing the KL9 response restricted by HLA-E or B57 in each of the nine individuals tested (positive response was assigned with value of 1, negative response was assigned with value of 0). **(B)** The net frequency of cytokine/effector molecules production by CD8 T cells responding to KL9 presented by AEH cell line and K562.B57 cell line is shown. Net frequency was calculated by subtracting the no peptide pulsed control of the identical APC. **(C)** Heatmap of COMPASS functional analysis is shown. Data for functional score (FS) and polyfunctional score (PFS) are shown in panels **(D)** and **(E)**. Error bars represent mean ± SEM. Significant changes are indicated.

Supplementary Figure 6



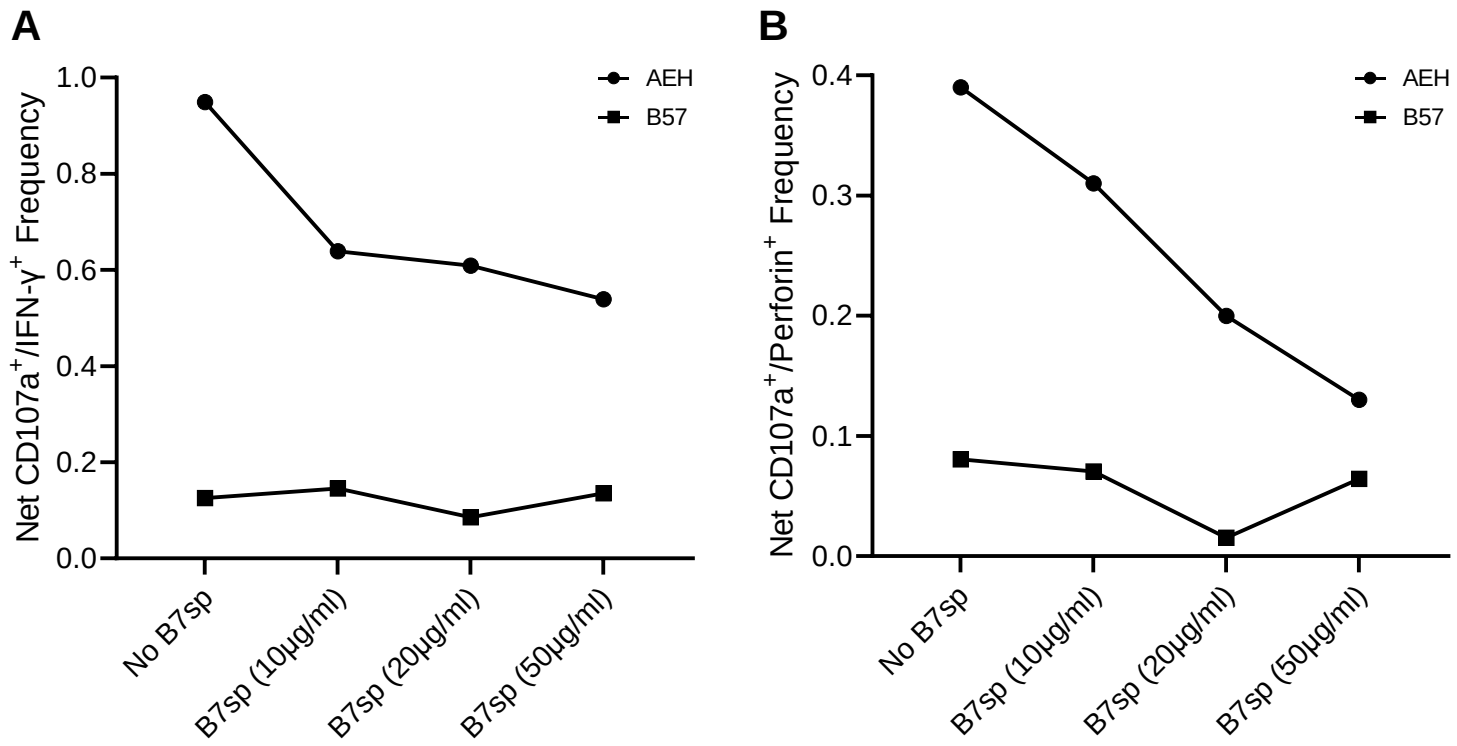
Supplementary Figure 6. Antigen sensitivity of HLA-E and B57 restricted KF11 responses as measured by ex-vivo ICS assay. (A) Representative flow plot showing dual CD107a/ IFN-γ production from CD8 T cells responding to stimulation by single HLA allele expressing APCs that were pulsed with KF11 at three log fold peptide dilutions in patient P11. Expression of cytokines/effector molecules are shown relative to negative control with no peptide pulse. CD8/APC co-cultures stimulated with SEB were used as positive controls. **(B)** Summary of antigen sensitivity data generated from P11 for multiple cytokine/effector molecules is shown. These data shown induction of dual production of CD107a and either IFN-γ, TNF-α, perforin and granzyme B when CD8 T cells respond to KF11 presented by E01, E03 or B57 targets. **(C)** Cumulative data on antigen sensitivity as measured by dual CD107a/ IFN-γ production in 5 chronically infected individuals is shown. Error bars represent mean ± SEM.

Supplementary Figure 7



Supplementary Figure 7. CD8 T cells are not activated via cross presentation. Dual production of CD107a and either of IFN-γ, IL2, TNF-α, perforin and granzyme B is shown for CD8 T cells relative to no peptide pulse under conditions of co-culturing with KF11 pulsed AEH target cells **(A)** or cultured in presence of KF11 peptide only **(B)** from a single HIV infected person.

Supplementary Figure 8



Supplementary Figure 8. Blocking of HLA-E mediated KF11 presentation to antigen specific CD8 T cells by leader sequence derived B7 signal peptide. CD8 T cells were positively isolated from PBMCs of a chronically HIV infected individual. HLA-E expressing AEH cells and HLA-B*57 expressing K562.B57 cells that were pretreated with B7 signal peptide at three different concentrations (10, 20 and 50 µg/ml) for 2 hrs. after which excess peptide was washed off. These cell lines were next pulsed with KF11 (10 µg/ml) for 2 hrs. and then excess peptide washed off and these target cells co-cultured with CD8 T cells at 1:1 ratio for 12 hours. Net frequency of cytokine /effector molecule is shown (after subtracting the no peptide pulsed control using the identical APC). **(A)** CD107a/IFN-γ and **(B)** CD107a/Perforin. Blocking of HLA-E mediated presentation is shown relative to that by an HLA-B*57 expressing line.