SUPPLEMENTAL FIGURES AND TABLE FOR

Measurements of human CD8+ T-cell functions using high-throughput integrated single-cell analysis

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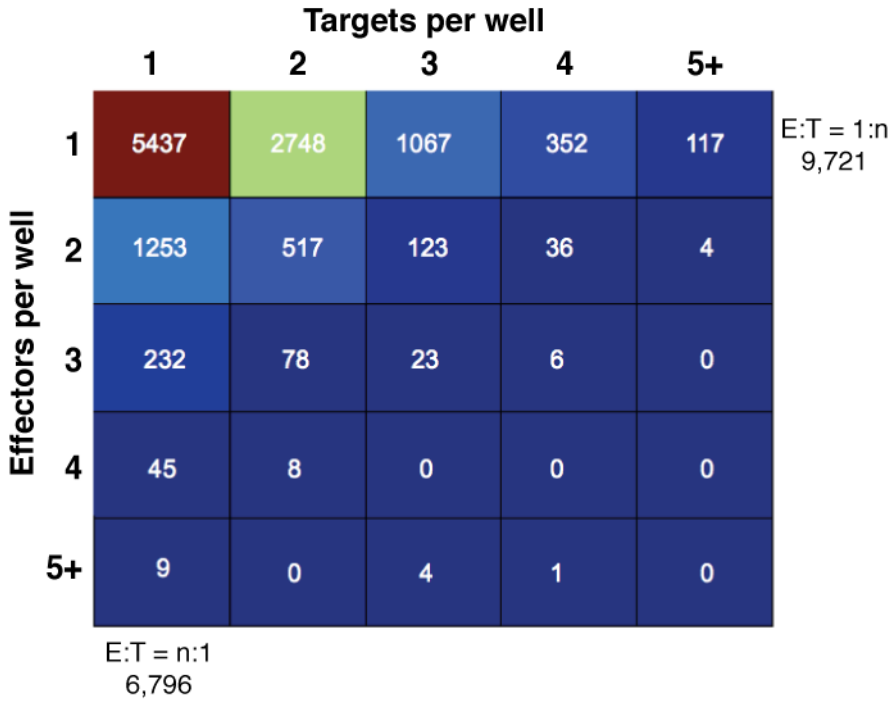
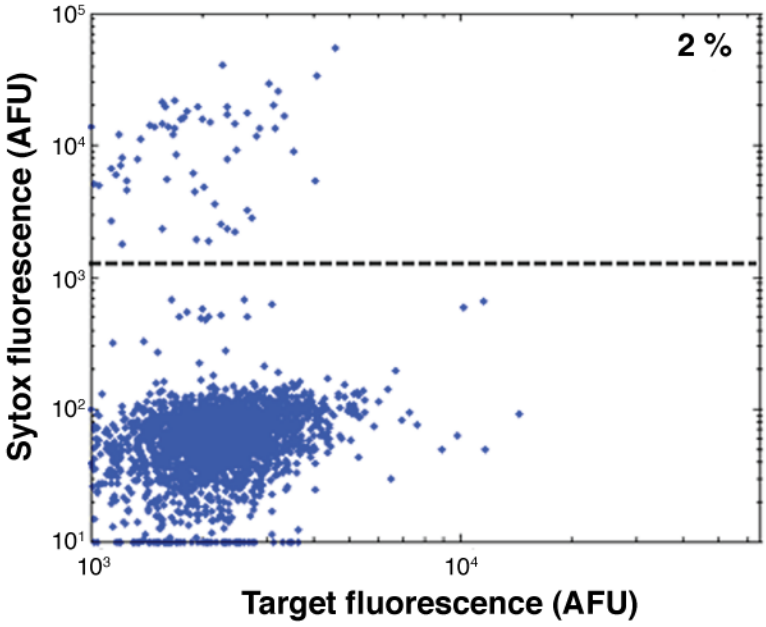
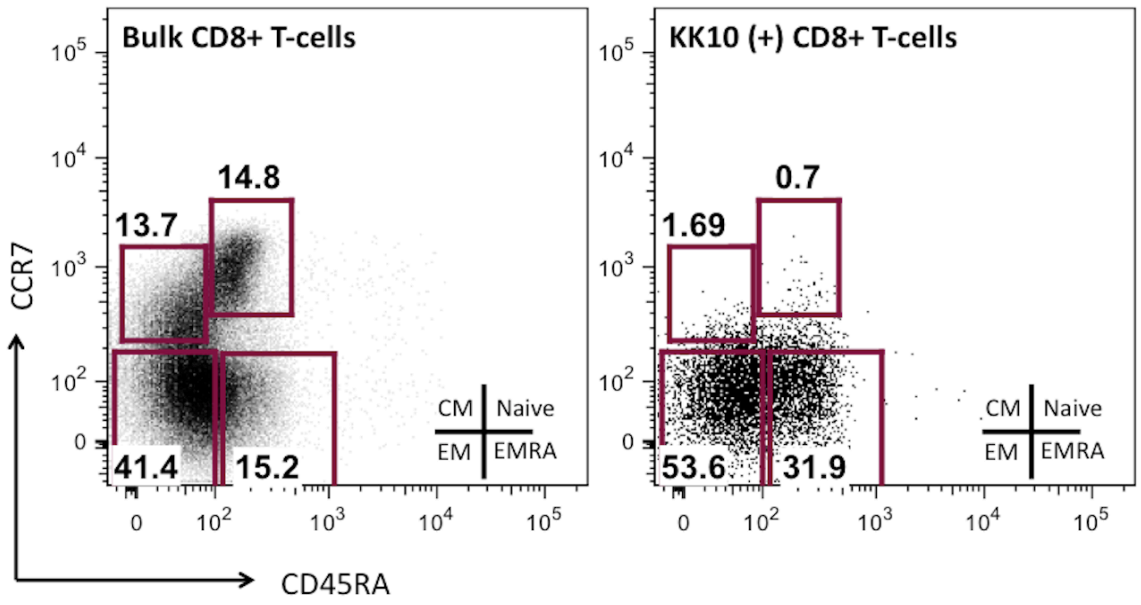


Supplemental Figure 1. Distribution of effectors and targets in individual 50 µm x 50 µm x 50 µm microwells. A density matrix indicating the number of wells containing at least a single B-cell target and a single CD8+ T-cell. The arrays comprise 84,672 microwells in total. The conditions for loading the arrays were chosen to maximize the number of wells occupied with E:T=1:1. These data are representative of typical distributions measured; data shown here are from a clinical sample (CTR40).



Supplemental Figure 2. Spontaneous loss of viability for target B-cells. Scatterplot of the percentage of dead (SYTOX) KK10-loaded, labeled B-cell targets observed in arrays of microwells in the absence of effector cells (B27-restricted T cell clones). The dashed line indicates the threshold for dead cells. These data are representative of at least three independent experiments.

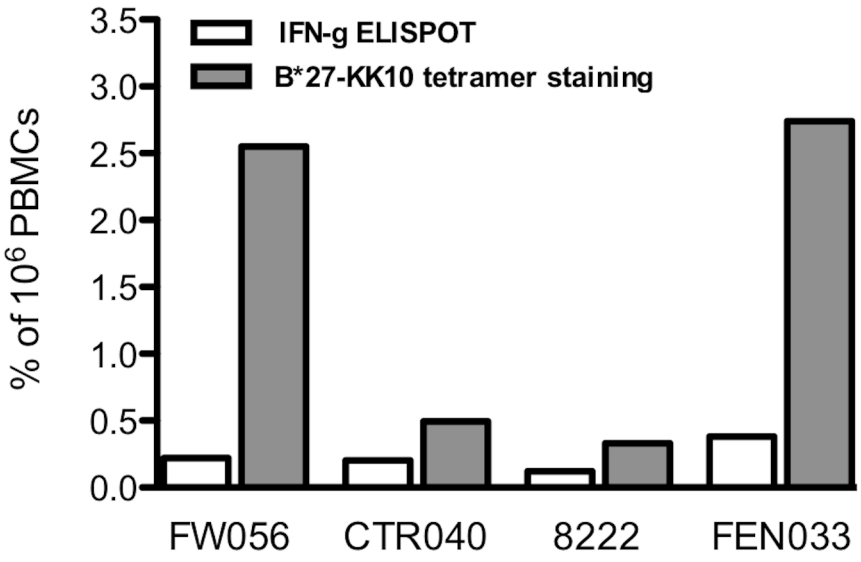


Supplemental Figure 3. **Differentiated phenotypes of bulk and KK10 tetramer (+) CD8+ T-cells from Fw056 determined by flow cytometry**. The state of differentiation was assessed by the surface expression of CCR7 and CD45RA, measured using fluorescent antibodies directed against these proteins and profiled on the flow-cytometer: EM = effector-memory ($CD45RA^-/CCR7^-$); CM = central-memory ($CD45RA^-/CCR7^+$); N = Naïve ($CD45RA^+/CCR7^+$); EMRA = effector-memory RA ($CD45RA^+/CCR7^-$). The relative percentages of each are indicated.

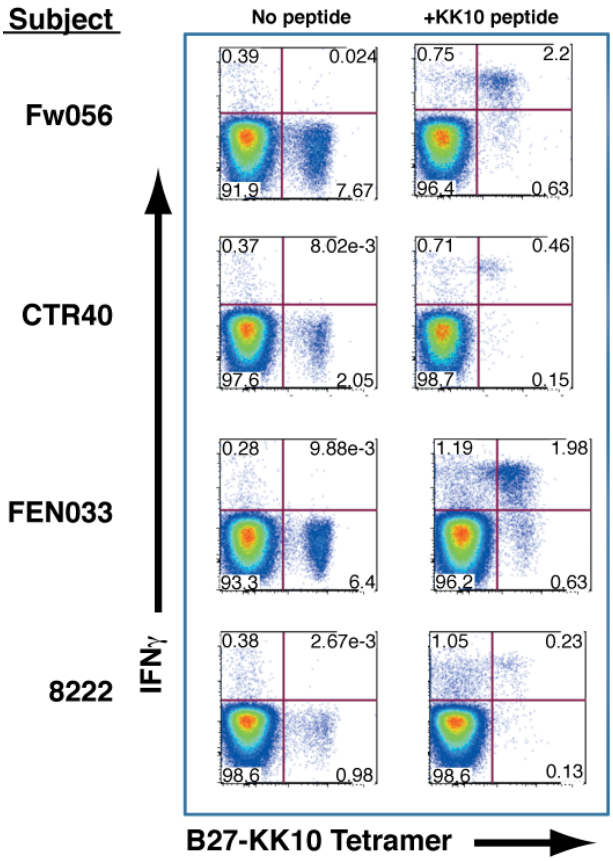
Supplemental Table 1. Clonotypic analysis of three B27-KK10-specific T-cell clones generated by screening cytolytic activity.

Clone	V region	CDR3 Sequence	J region
FW056 #2	TRBV6-5*01	CASRPGQGATEAFFGQGTRLTVV	TRBJ1-1
	TRAV30	AGRTAVLPR_SLDQGP	N/A
FW056 #3	TRBV20-1*01	CSARDRGTREVADNYGYTFGSGTRLTVV	TRBJ1-2
	TRBV15*01	CATSETGTTLEQYFGPGTRLTVT	TRBJ2-7
	TRAV14/DV4	CAMREVSSGNTGKLIFGQGTTLQVKP	TRAJ37
FW056 #4	TRBV4-3*01	CASRPGLASNEQFFGPGTRLTVL	TRBJ2-1
	TRAV14/DV4	CAMSPATARQLTFGSGTQLTVLP	TRAJ22
	TRAV8-2	CPSYRDDKIIFGKGTRLHILP	TRAJ30

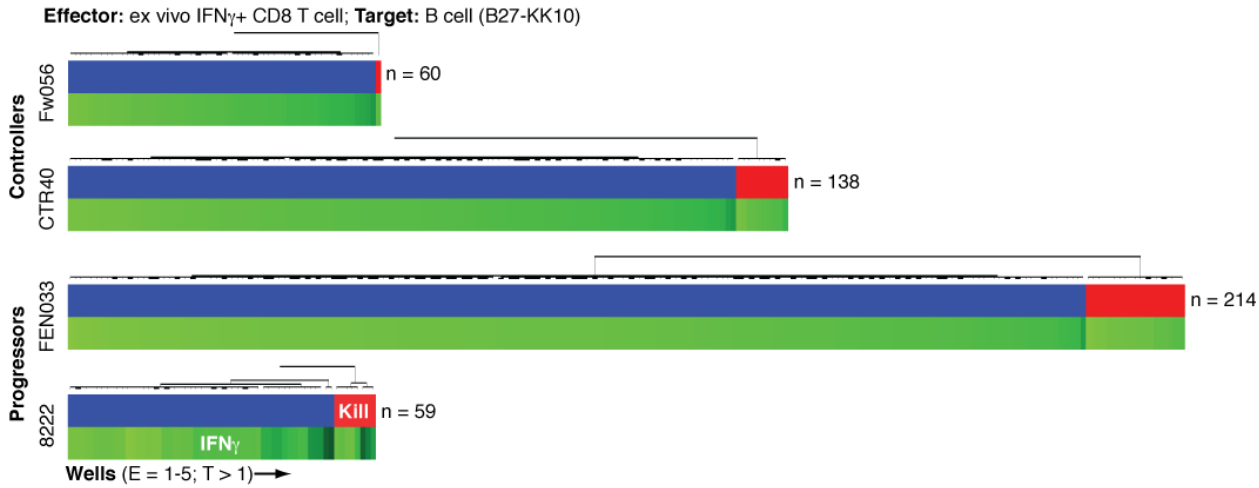
_ = not determined



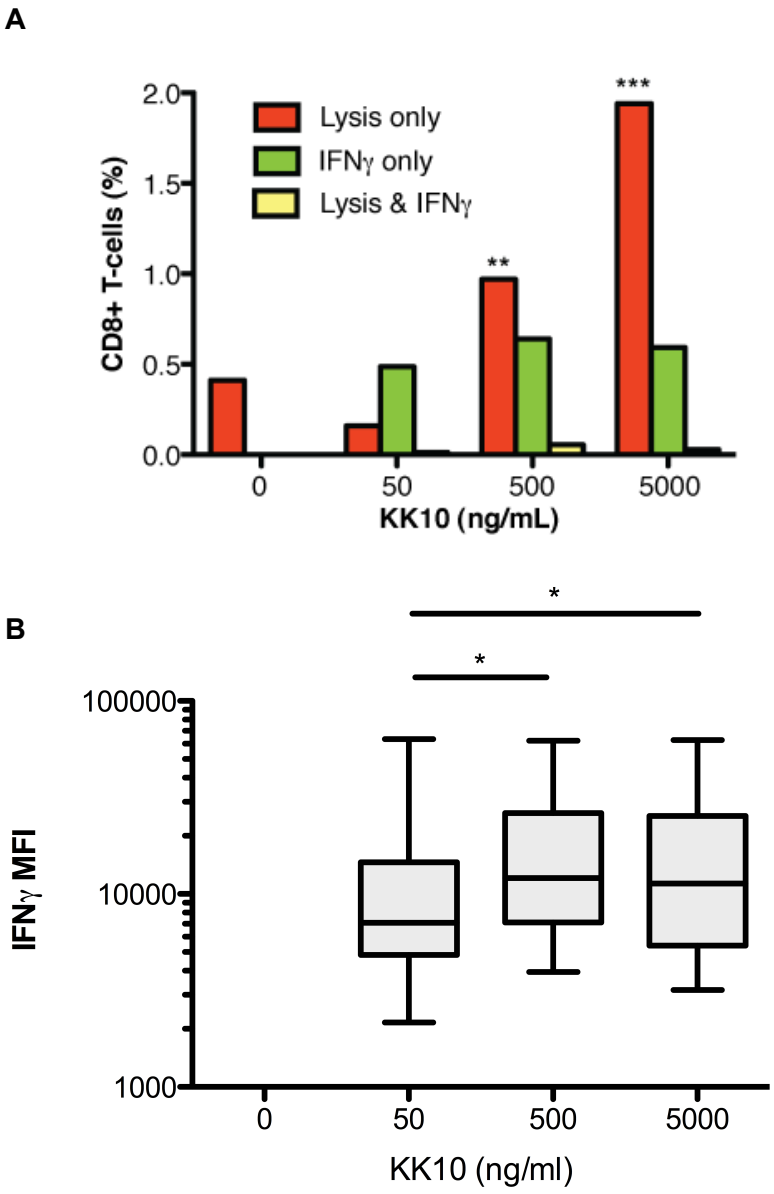
Supplemental Figure 4. Comparison of the frequencies of PBMCs specific for the HLA-B*27-restricted epitope KK10 measured by IFNγ ELISpot and $CD8^+$ T-cells binding B27-KK10 tetramers measured by flow cytometry for four HIV-infected subjects.



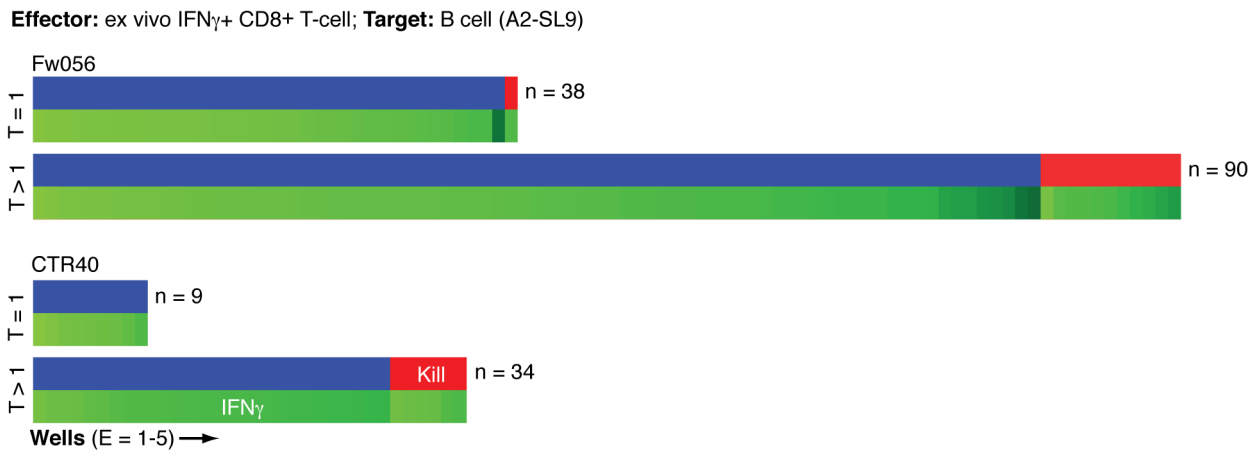
Supplemental Figure 5. Analysis of intracellular IFNγ in KK10 tetramer (+) CD8+ T-cells of 2 elite controllers and 2 viremic progressors upon stimulation with the cognate peptide. CD8+ T-cells were stained with fluorescently labeled tetramers and incubated for 5 h in the presence or absence of KK10 peptide, antibodies against the co-stimulatory molecules CD49d and CD28, and the degranulation-inhibitors Brefeldin A and Monensin.



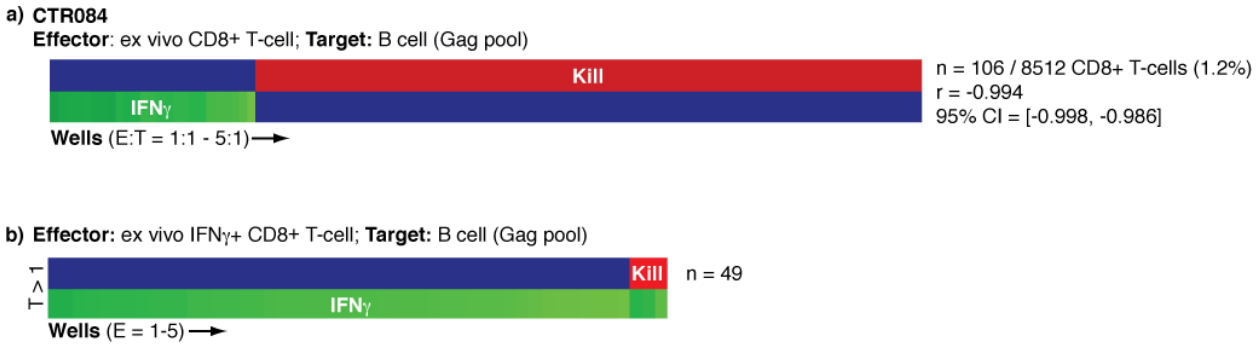
Supplemental Figure 6. Matched data for concurrent measurement of cytolysis and IFNγ secretion for ex vivo IFNγ-secreting CD8+ T-cells from four HIV+ individuals co-incubated in arrays of microwells for 6 h with multiple HLA-matched, KK10-pulsed target B-cells. The data are shown as a heatmap and organized by unsupervised hierarchical clustering (Euclidean distance, complete linkage). Cytolytic activity is shown as discrete values (blue = negative; red = death). The relative rate of secretion is shown for IFNγ (green). The dendrograms indicate the relative calculated distances and separation among clusters of wells. The number of wells scored with E = 1-5 and T > 1 with detectable IFNγ are indicated for each sample. There were no cells among any of the four samples that released either IL-2 or TNFα at a detectable rate (limit of detection: ~2 molecules/s).



Supplemental Figure 7. (A) Functional response of ex vivo CD8+ T-cells (HIV+ controller) to HLA-matched B-cell targets loaded with different doses of KK10 peptide (E = 1-5 : T = 1). The lysis rate is corrected for the background death rate of target cells on the same microwell array (E = 0 : T = 1). **p<0.01, ***p<0.0001 for lysis rate comparison to both on-chip background death rate and 0 ng/ml KK10 killing rate (Fisher's exact test, or, for large numbers of events, chi-squared with Yate's correction). The minimum number of events scored for each condition was n = 3,192. (B) Boxplot of the relative rates of secretion of IFNγ as a function of KK10 concentration (secretors only; E = 1-5 : T = 1). *p<0.05, two-tailed t-test.



Supplemental Figure 8. Matched data for concurrent measurement of cytolysis and IFNγ secretion for IFNγ-secreting CD8+ T-cells from two HIV+ controllers co-incubated in arrays of microwells for 6 h with HLA-matched (HLA-A2), SL9-pulsed target B-cells. The data are shown as a heatmap and organized by unsupervised hierarchical clustering (Euclidean distance, complete linkage). Cytolytic activity is shown as discrete values (blue = negative; red = death). The relative rate of secretion is shown for IFNγ (green). The heatmaps show all wells scored that had IFNγ secretion and (top) E:T = 1:1 – 5:1 or (bottom) E = 1-5; T > 1 for each sample (Fw056 and CTR040). There were no cells in either sample that released either IL-2 or TNFα at a detectable rate (limit of detection: ~2 molecules/s).



Supplemental Figure 9. Matched data for concurrent measurement of cytolysis and IFNγ secretion for CD8+ T-cells from an HIV+ controller (CTR084) co-incubated in microwells for 6 h with autologous target B-cells pulsed with overlapping peptides from Gag. The data are shown as a heatmap and organized by unsupervised hierarchical clustering (Euclidean distance, complete linkage). Cytolytic activity is shown as discrete values (blue = negative; red = death). The relative rate of secretion is shown for IFNγ (green). **(A)** All events observed for wells scored with E:T = 1:1 – 5:1 exhibiting any functional activity; **(B)** All events observed for wells with E = 1-5; T > 1 and IFNγ secretion.