Figure S1. A) The number (count) of individual env-specific CD4 T cells in the scRNA Seq dataset (out of a total of 1,423 cells sequenced) as delineated by their respective cytokine protein expression determined by flow cytometry from the multiplexed cytokine capture assay.
B) Cytokine-expressing CD4 T cells (by capture assay) had significantly higher levels of the corresponding cytokine mRNA transcripts compared to cytokine protein-negative CD4 T cells. Violin plots show the *IL4, IFNG, IL2,* and D) *CD40LG* transcript levels (in logcounts) for each cell that was either positive (blue) or negative (red) for the corresponding cytokine's protein expression. Wilcoxon rank was used to determine significance. C) Transcript levels of *CD44, CD82* and *IL21* among vaccine-specific CD4 T cells with "correlate" or "non-correlate" cytokine profile.
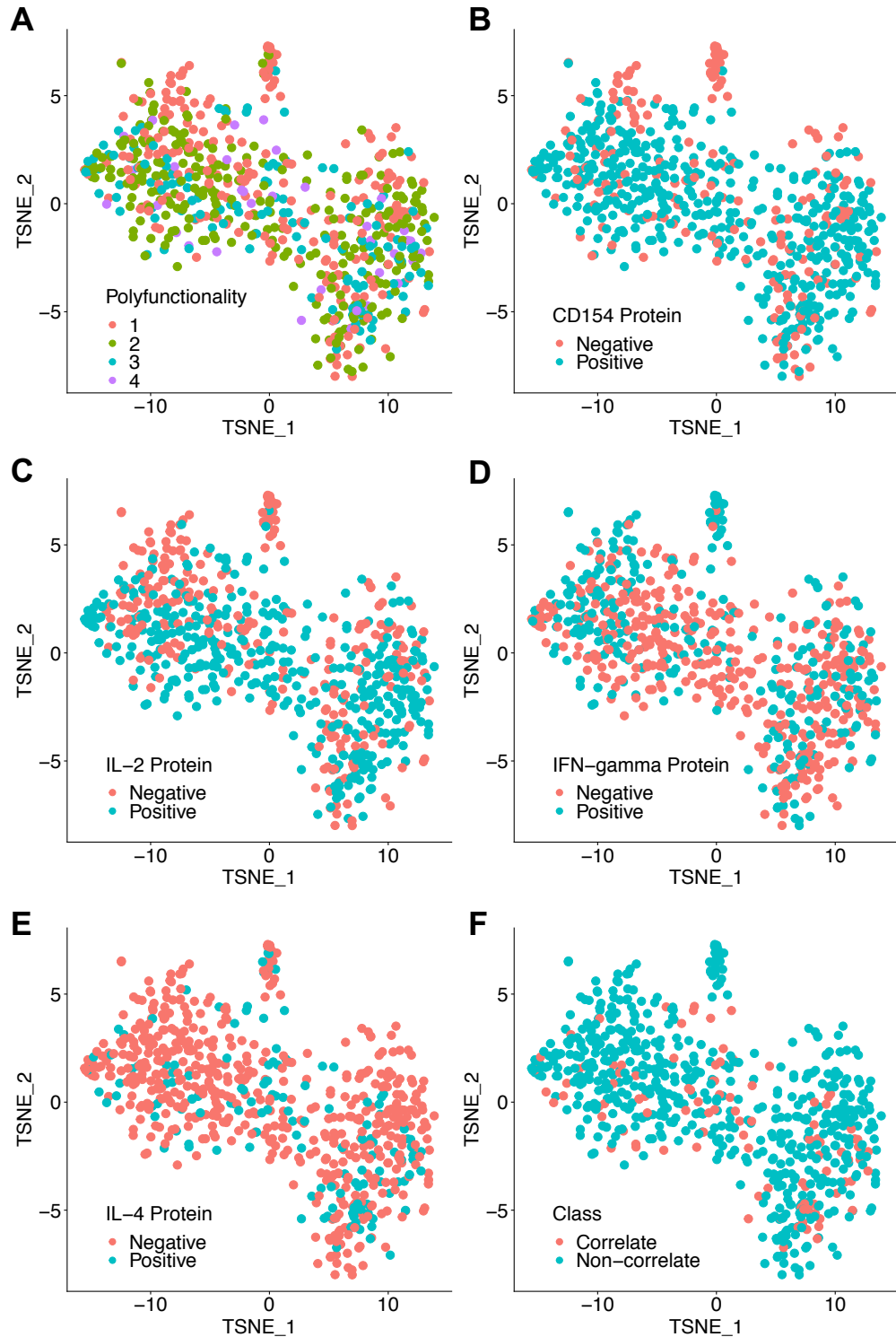
Figure S2. T-Distributed Stochastic Neighbor Embedding (t-SNE) clustering of env-specific CD4 T cells by single cell gene expression. Cells are colored by protein expression (detected by the multiplexed cytokine capture assay): A) by degree of polyfunctionality (i.e., number of contemporaneous functions/cytokines, individual functions: B) CD154, C) IL-2, D) IFN-g, E) IL-4 and F) by the polyfunctional cytokine profiles associated with reduced risk of HIV infection in the RV144 vaccine trial, i.e., "correlate" versus "non-correlate".
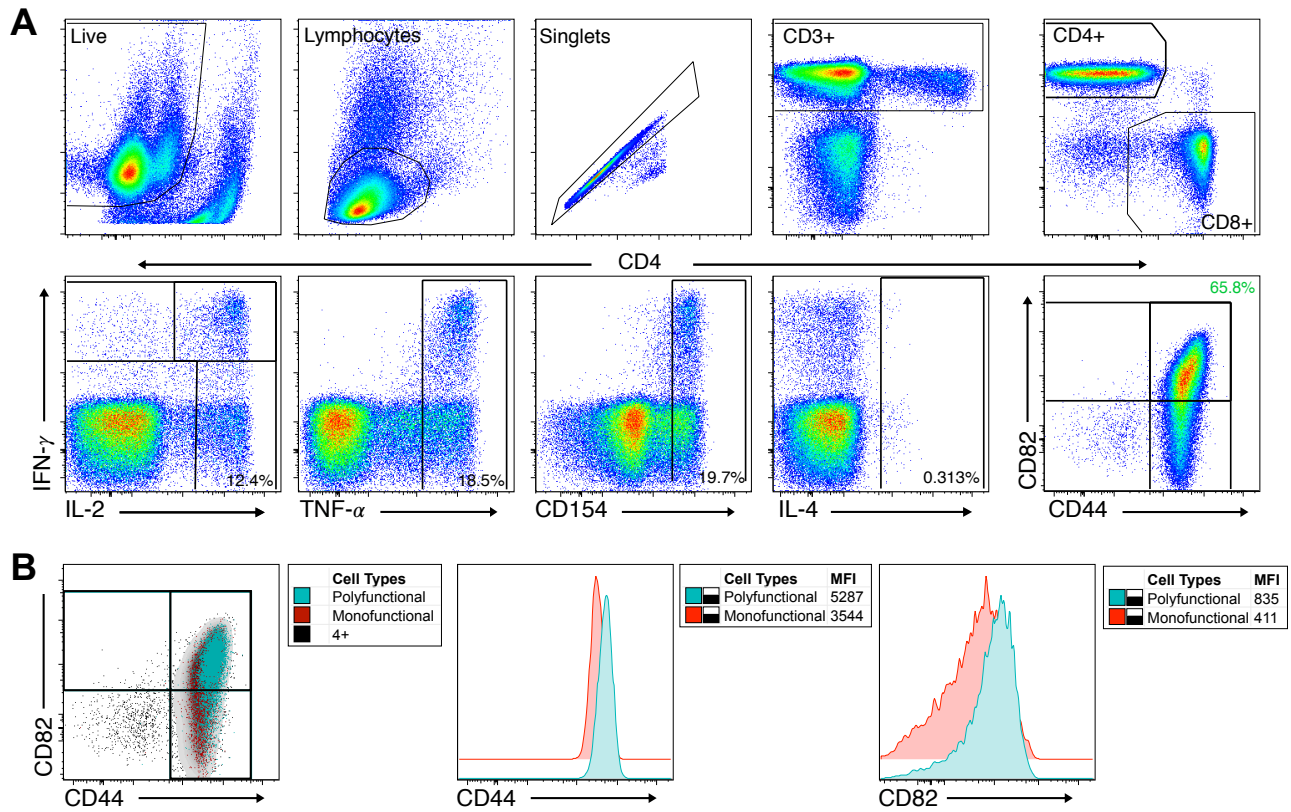
Figure S3. Surface expression of CD44 and CD82 were upregulated on polyfunctional CD4 T cells. PBMCs from a health donor were stimulated with SEB. Cytokine expression was detected by ICS assay with costaining for CD44 and CD82 surface expression. A) Top Row: the gating hierarchy shows the sequential selection of live, lymphocyte, singlet CD3+ CD4+ T cells. Bottom Row: CD4 T cell expression of cytokines and activation markers. Monofunctional CD4 T cells were defined as T cells expressing any individual cytokine (IFN-γ, IL-2, TNF, CD154 or IL-4). Polyfunctional CD4 T cells were defined as T cells co-expressing 2 or more cytokines. B) CD82 and CD44 protein expression on polyfunctional and monofunctional CD4 T cells.

Table S1. Flow cytometry and cytokine capture reagents

Assay	Antibody target and fluorochrome	Manufacturer	Clone	Catalog Number	Titer (µL)
Multiplex Capture Assay	Fixable Live/Dead Aqua	Invitrogen	N/A	L34957	0.2
	CD154 (CD40L) PE-Cy7	BioLegend	24-31	310832	0.15
	CD4 BV421	BD Bioscience	SK3	566907	0.5
	CD8 Q705	Invitrogen	3B5	Q10059	0.5
	CD14 BV510	BioLegend	M5E2	301842	1.0
	CD19 BV510	BD Bioscience	SJ25C1	562947	1.0
	CD56 APC-Cy7	BioLegend	HCD56	318332	0.25
	CD45RA BV605	BioLegend	HI100	304133	0.25
	CCR7 BV786	BioLegend	G043H7	353226	2.0
	IL-2 APC	Miltenyi Biotec	N/A	130-090-763	1.0
	IFN-γ FITC	Miltenyi Biotec	N/A	130-090-433	1.0
	IL-4 PE	Miltenyi Biotec	N/A	130-054-102	5.0
ICS Assay	Fixable Live/Dead Blue	Invitrogen	N/A	L34962	0.2
	CD3 APC-Fire750	BioLegend	UCHT1	300470	0.1
	CD4 BV480	BD Bioscience	SK3	566104	0.1
	CD8 BUV805	BD Bioscience	SK1	612889	0.05
	CD44 FITC	BioLegend	BJ18	338804	0.1
	CD82 PE-Cy7	BioLegend	ASL-24	342110	0.02
	IFN-γ V450	BD Bioscience	B27	560371	1.0
	IL-2 APC	BioLegend	MQ1-17H12	500310	0.25
	IL-4 BB700	BD Bioscience	MP4-25D2	Custom	0.05
	TNF BUV395	BD Bioscience	MAb11	563996	0.1
	CD154 (CD40L) BUV737	BD Bioscience	TRAP1	748983	0.4