Supplemental Material

The pleural tuberculosis-associated microenvironment promotes HIV persistence by impairing CD8+ T-cell mediated viral control

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Supplementary tables

Table S1. Participant Characteristics for HIV-negative donors with Pleural Effusion.

	TB-PE	**HF-PE**
Age (years, range)	35 (19-52)	50 (35-66)
Gender, male %	M, 78%	M, 80%
Nationality, %	Argentina, 56% Bolivia, 33% Peru, 11%	Argentina, 100%
TB disease %	Pleural, 22% Pleural and Pulmonary, 78%*	-
Etiology	*M. tuberculosis*	ND

*Confirmed by chest X-Ray

Table S2. Clinical data for PLWH included in this study

Participant ID	Age (years)	Sex	Time on therapy[A] (years)	Viral load[A] (copies/ml)	Antiretroviral regimen[A]
PS02017	29	Male	3	<50	NVP, TRU
PS02018	46	Male	3	<50	3TC, DDI, EFV, TRU
PS03022	20	Male	4	<50	ABC, 3TC
PS01008	29	Male	2	<50	SQV
PS01007	42	Male	4	<50	ABC, 3TC

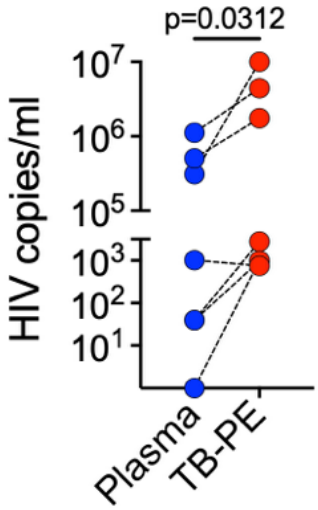
3TC, lamivudine; ABC, abacavir; DDI, didanosine; EFV, efavirenz; NVP, nevirapine; TRU, truvada, SQV, saquinavir.

[A]At time of sampling.

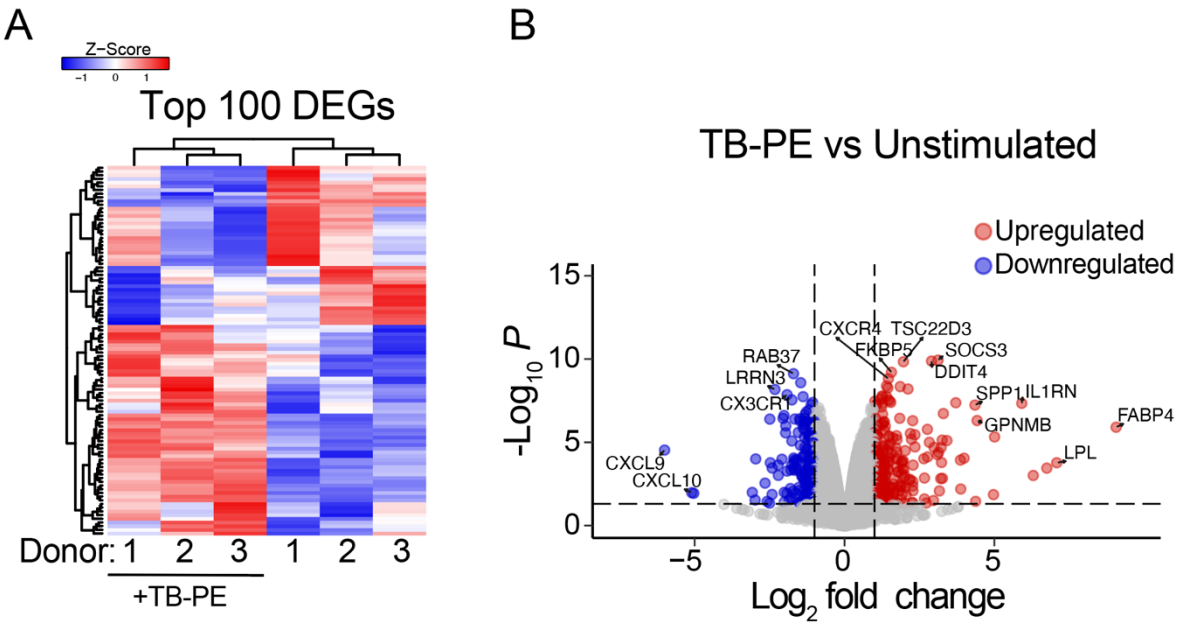
Table S3. Antibodies Used for High-Parameter Flow Cytometry

Antibody Name	Clone	Source	Catalogue Number
CD28-BUV395	CD28.2	BD Biosciences	740308
CD8-BUV496	SK1	BD Biosciences	741199
HLADQ-BUV563	TU169	BD Biosciences	748563
CD161-BUV615	HP-3G10	BD Biosciences	751374
CD154-BUV737	TRAP1	BD Biosciences	748983
CD4-BUV805	OKT4	BD Biosciences	750976
CCR7-BV421	2-LI-A	BD Biosciences	562555
CD39-BV480	TU66	BD Biosciences	746454
HLADR-BV605	G46-6	BD Biosciences	562845
PD1-BV711	EH12.1	BD Biosciences	564017
CD27-BV786	LI27	BD Biosciences	563327
CCR5-VioB515	REA245	Miltenyi Biotec	130120022
CD3-NovaFluorB610	UCHT1	Thermo Fisher Scientific	H002T03B06-A
CD69-BB700	FN50	BD Biosciences	569399
TIGIT-PE	A15153G	BioLegend	372704
CD103-PEVio615	REA803	Miltenyi Biotec	130111837
CD127-PECY7	R34.34	Thermo Fisher Scientific	25-1271-82
CD38-APCFire810	HIT2	BioLegend	303550

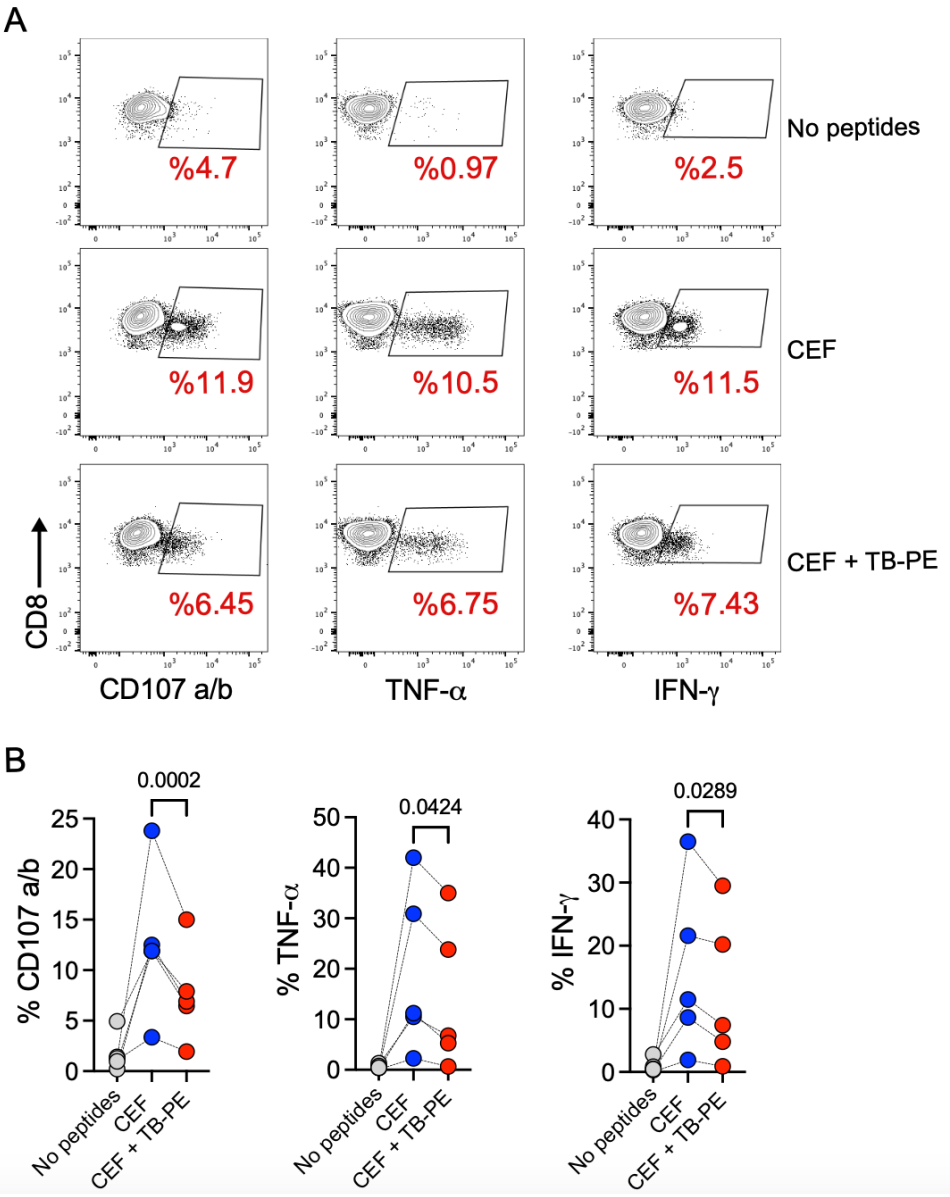
Supplementary Figures



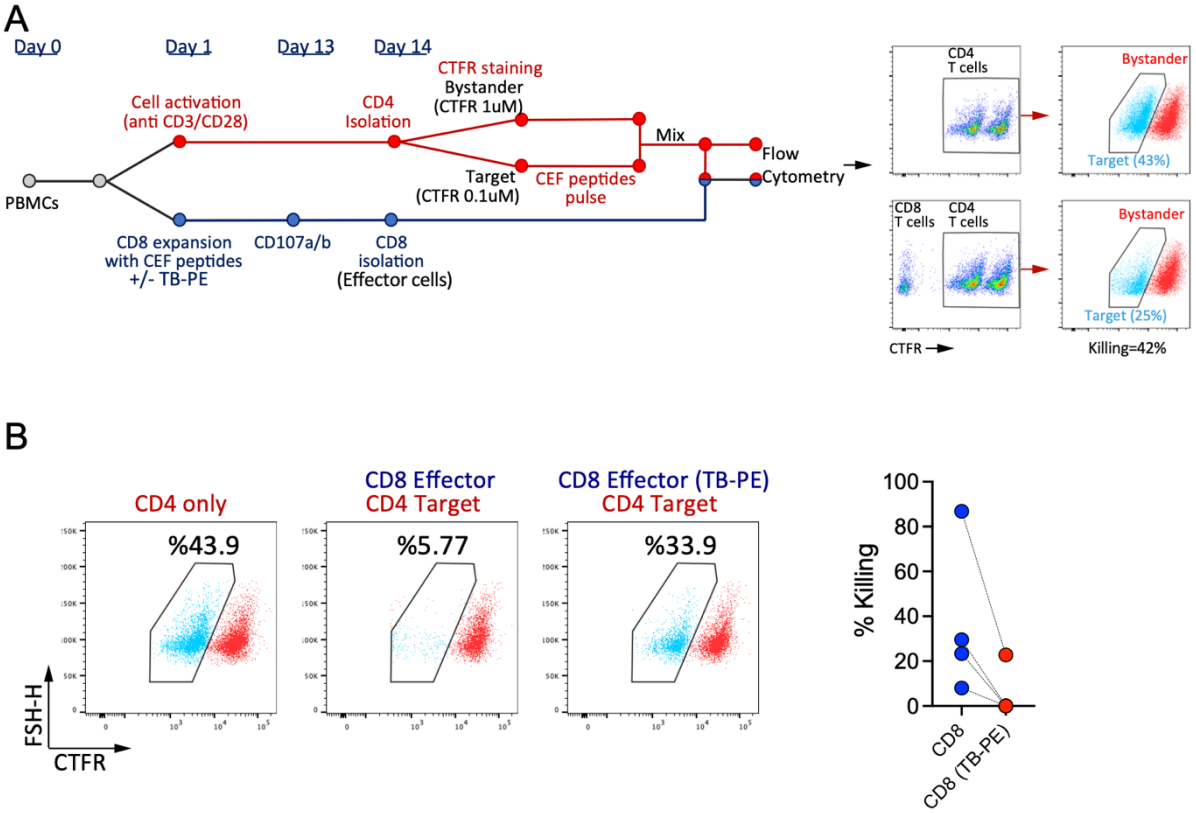
Supplementary Figure 1. HIV-1 viral load is higher in TB-PE. *HIV-1 RNA copies per ml in plasma (blue) and tuberculous pleural effusion (red) from seven people living with HIV-1 and pleural TB.*



Supplementary Figure 2. TB-PE treatment alters the transcriptome of CD8+ T cells. *(A) Heatmap showing hierarchical clustering of the top 100 differentially expressed genes (DEGs) between TB-PE treated and control CD8+ T cells. (B) Volcano plot of differentially expressed genes with significantly upregulated genes in red and significantly downregulated genes in blue (p<0.05).*



Supplementary Figure 3. The effector antiviral responses of CD8+ T cells are inhibited by TB-PE. *(A) Representative flow cytometry of healthy donor-derived CD8+ T cells expressing CD107a/b, TNF-α, and IFN-γ as markers of effector response upon restimulation with pooled peptides from cytomegalovirus, influenza, and Epstein-Barr virus (CEF peptide pool) in the presence (red) or absence (blue) of TB-PE. Cells exposed to no peptides (grey) were used as an experimental control. (B) Quantification of the expression of CD107a/b, TNF-α, and IFN-γ in healthy donor-derived CD8+ T cells exposed to TB-PE (n=5). Each data point represents one donor. Statistical significance was assessed by paired t-test with $p<0.05$ considered significant.*



Supplementary Figure 4. TB-PE inhibits virus-specific CD8+ T cell cytotoxicity. *(A) Schematic of the experimental model used to study the effect of TB-PE on virus-specific CD8+ T cell cytotoxicity. CD8+ T cells were expanded with a pool of peptides derived from Cytomegalovirus, Epstein Barr Virus and Influenza (CEF peptide pool) in the presence/absence of TB-PE. (B) Representative flow cytometry plots showing the proportion of target cells after coculture with CEF-specific CD8+ T cells. As a control, CD4+ T cells were cultured without CD8+ T cells (left). Quantification of CD8+ T cell-mediated killing of cells pulsed with CEF peptides (right). Each dot represents a different donor.*