ORIGINAL RESEARCH

Characterizing CD8+ TEMRA Cells in CP/CPPS Patients: Insights from Targeted Single-cell Transcriptomic and Functional Investigations

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Table S1| Conserved markers for the eight cell types.

Cell cluster	Gene ID
CD8+Naïve T	CXCR1, CXCL8, NAMPT, ITGAX, VNN2, DUSP1, TLR2, LAT2, IL32, C10orf54, CD52, CD3E, TRBC2, TRAC, CD3D, CD48, GAPDH, SELL, HLA.C, LCK, CCL5, CD6, CD69, CD2, ICAM1, CTSW, NKG7, RUNX3, HLA.DPB1, FYN, PIK3IP1, IL7R, ARL4C, LAT, CD5, TARP-refseq, LGALS1, GIMAP2, IL2RB, ITK, CD8A, DUSP2, TNFRSF1B, GZMH, FOSB, CD44, GZMA, ITGAL, LEF1, JUN, CD7, CD247, APOBEC3G, CST7, SELPLG, CBLB, SPOCK2, KLRK1, PRF1, ITGA4, HLA.DPA1, TNFSF10, CD27, CCR7, GNLY, KLRB1, ZAP70, TSPAN32, GZMB, IER5, CD300A, TRIB2, BAX, IFNGR1, PECAM1, S1PR1, TBX21, BCL6, TCF7, RORA, GIMAP5, NCR3, LAIR2, CXCR3, TNFRSF25, LGALS3, HLA.DRB3, TRAT1, KLRG1, CD4, CD8B, ICOS, IL23R, BCL11B, IL18R1, CD244, IER3, ZNF683, STAT4, STAT1, HLA.DQA1, CCND2, TIAF1, IFNG, CD3G, LTA, CLEC2D, FOXO1, TRDC, ITGAE, GZMK, STAT6, TXK, SLAMF1, DPP4, ANXA5, CNOT2, STAT3, GZMM, CX3CR1, IL6R, PASK, KLRC4, CCL4, CASP3, LAG3, TNF, IL4R, EGR1, CTLA4
CD8+TCM	LEF1, PIK3IP1, CCL5, CST7, NKG7, CD8B, GZMA, CCR7, GZMH, LGALS1, TARP-refseq, GNLY, SELL, CCR9, CD9, GZMB, PRF1, IFNG, TBX21, IL23R, HLA.DPA1, HLA.DPB1, TGFB3, KLRB1, CCL4, KLRK1, LAG3, FYB, BTLA, CD3E, BTG1, APOBEC3G, CD48, CD300A, CD27, KLRG1, EGR1, IL18RAP, ZNF683, KLRF1, TXK, ANXA5, TNF, SPOCK2, TNFRSF1B, GAPDH, PASK, TIGIT, IL7R, B3GAT1, IER5, KLRC3, LCK, FOXP1, ITK, TRBC2, CD7, IL2RB, ITGAM, TCF7, NCR3, NT5E, IL6R, ARL4C, CXCR4, ITGB2, CD8A, GZMK, RUNX3, IL12A, JUN, FYN, HLA.DRA, CCR2, DUSP4, HLA.DRB3, LAT, CD6, CCL20, CD4, PECAM1, BCL11B, CXCL8, CD244, IL15, HLA.DMA, TSPAN32, FOXP3, IER3, STAT6, CD160, CASP3, RORC, GIMAP2, FOXO1, CCND2, STAT4, CD40LG, NAMPT, TRDC, LAIR2, CTLA4, CD5, CX3CR1, LTA, KLRC4, FAS, BCL2, CXCR3, PRDM1, ITGAX, IFNGR1, NINJ2, SLAMF1, ZAP70, GIMAP5
CD8+TEM	HLA.DQB1, FOSB, RORA, GZMK, CCL5, CD52, CD4, LEF1, LGALS1, PASK, TRAC, IL12RB2, CCR7, DUSP2, TRBC2, TARP-refseq, LAIR2, SELL, FYB, ZNF683, KLRB1, PIK3IP1,

	IL18RAP, GZMB, TRDC, ICOS, ITK, CST7, ZBTB16, TRIB2, LTB, CD5, GNLY, TCF7, HMGB2, IL4R, GZMA, GZMH, IL23R, CD27, CTLA4, CD40LG, ITGAM, CLEC2D, LTA, TNFSF10, IL2RB, VNN2, BCL11B, KLRC3, BCL2, LAG3, ANXA5, HLA.DRB3, B3GAT1, CX3CR1, KLRG1, CD300A, JUN, IL18R1, NCR3, CD160, CCR5, TXK, FOXO1, HLA.DQA1, CBLB, HLA.DRA, CCL4, KLRC1, CCL3
CD8+TEMRA	GZMB, GZMH, GNLY, PRF1, TBX21, NKG7, TARP-refseq, CST7, CD8A, CTSW, CCL5, RUNX3, BTG1, DUSP1, CD48, LAT, JUNB, CD69, DUSP2, FYB, TRIB2, KLRB1, PIK3IP1, IL23R, IL7R, TNFRSF25, TRAT1, CD4, TCF7, GZMK, LTB, SELL, LEF1, CD27, CCR7, CD3E, PASK, TRBC2, HLA.DPB1, GZMA, CXCR4, LGALS1, ZNF683, GIMAP2, MYC, KLRK1, FOSB, CD7, GIMAP5, ITGAL, TNFSF10, TRAC, ITGAM, ICOS, STAT6, CX3CR1, HLA.DPA1, BCL11B, IL2RB, APOBEC3G, LCK, STAT5A, IL4R, B3GAT1, JUN, ITGAE, CD5, CD300A, ITK, SPOCK2, CD44, TXK, CD6, BAX, CNOT2, CD40LG, CCL4, C10orf54, DPP4, FOXP3, STAT1, KLRC3, BCL2, CCND2, KLRG1, VNN2, HMGB2, IFNG, CTLA4, LAP3, IFNGR1, KLRF1, LTA, LGALS3, SLAMF1, CD244, NAMPT, FOXO1, CHI3L2, KLRC4, LAIR2, IKZF2, HLA.DRB3, HLA.DQA1, TNF, LAG3, EGR1
CD4+TCM	CD4, LEF1, IL23R, SELL, FYB, IL7R, PIK3IP1, TRBC2, BTG1, TRAC, CD48, HLA.DPB1, HLA.DPA1, KLRG1, TBX21, CCL5, CD8B, GZMA, CTSW, KLRK1, PRF1, TARP-refseq, CST7, CD8A, ZNF683, CCL4, NKG7, GNLY, GZMB, GZMH, CX3CR1, CD300A, CD27, ITGAM, CCR7, CD3E, LGALS1, LAT, APOBEC3G, EGR1, TRIB2, IFNG, CD69, KLRC3, CD6, RUNX3, HLA.DRB3, JUNB, IL2RB, LCK, ITK, TCF7, TRAT1, HLA.DRA, B3GAT1, LTB, CD244, KLRC4, LAG3, TRDC, NCR3, GIMAP2, CD5, PASK, LAIR2, CD44, STAT6, GIMAP5, TNF, HLA.DQA1, TNFRSF25, IL18RAP, BCL11B, CXCR5, ICOS, GZMK, IL4R, SPOCK2, HLA.DQB1, KLRC1, MYC, HLA.DMA, TXK, KLRF1, GZMM, CD7, CD40LG, STAT5A, ARL4C, C10orf54, IL6R, RORA, TNFSF10, CCND2, TIAF1, FOXO1, LTA, LGALS3, CTLA4, FOXP3, CNOT2, STAT1, BCL2, S1PR1, SLAMF1, DPP4
Double negative TEMRA	FOSB, GIMAP2, CD7, IL2RB, HLA.DPA1, CD52, APOBEC3G, CD48, C10orf54, CD3D, GZMA, SELPLG, CD8B, CTSW, IL32,

	HLA.DPB1, LAT, CD27, TRAT1, GAPDH, RUNX3, CD3E, GZMB, CCL4, CD8A, LGALS1, GZMH, NKG7, CST7, HLA.DRB3, LAIR2, LAG3, TRBC2, TARP-refseq, SPOCK2, CD300A, ZNF683, ICOS, CD244, KLRB1, TRAC, HMGB2, STAT6, CD3G, ZAP70, BTG1, KLRC4, LTA, EGR1, CNOT2, LGALS3, TIAF1, BCL11B, CCR7, STAT3, HLA.DQB1, STAT4, TNFRSF25, VNN2, FAS, CCL5, PASK, CD40LG, GNLY, KLRF1, ITK, TNF, TRDC, CBLB, FOXO1, ITGA4, IFNGR1, IL18RAP, B3GAT1, LEF1, TXK, TNFRSF1B, IL18R1, FYB, CASP3, RORA
Doublet TEMRA	DUSP4, FOXP3, CTLA4, IL2RA, IL32, HLA.DRA, ICOS, IKZF2, HLA.DQB1, F5, CCL5, GAPDH, TIGIT, HLA.DRB3, TRIB2, CCR4, NKG7, CTSW, ANXA5, CD4, ARL4C, LAIR2, NINJ2, TNFRSF1B, HLA.DMA, HLA.DQA1, LGALS3, TNFSF10, CD27, CD44, SELL, CD8A, KLRK1, TARP-refseq, SELPLG, FAS, GZMH, CST7, PRDM1, RUNX3, LGALS1, GNLY, FOSB, IRF4, PRF1, PTTG2, GZMA, GZMB, CD300A, IL6R, CXCR3, NCR3, SLAMF1, CCL4, CD244, TBX21, KLRG1, CD8B, IL7R, DPP4, TRDC

Note: TCM, T central memory cell; TEM, T effector memory cell; TEMRA, T effector memory RA cell

Table S2| Baseline information for enrolled healthy volunteers and CP-LS patients.

Variable	Overall (n=32)	Healthy control (n=16)	CP-LS (n=16)	Stat	*P* value
Age, median [IQR]	26.00 [24.00,28.00]	26.00 [24.00,27.00]	27.00 [25.00,30.00]	-1.04	0.304
BMI, median [IQR]	23.81 [21.97,24.73	23.85 [22.60,24.54]	23.77 [21.46,25.06]	-0.40	0.706
Pain score, median [IQR]	5.00 [0.00,6.00]	/	6.00 [5.00,7.00]	-4.82	<0.001
NIH-CPSI Score, mean (±SD)	7.41±7.49	/	14.81±1.55	-37.01	<0.001

Note: CP-LS, chronic prostatitis-like symptoms; IQR, interquartile range; BMI, body mass index; NIH-CPSI, National Institutes of Health Chronic Prostatitis Symptom Index; SD, standard deviation.

Table S3| Antibodies used in the current study.

Antibody	Brand name	Item No.
FITC Mouse Anti-Human CD45RA（HI100）	BD Pharmingen	555488
PerCP-Cy™5.5 Mouse Anti-Human CD3（UCHT1）	BD Pharmingen	560835
APC anti-human CD197 (CCR7) Clone G043H7	BioLegend	353214
BV605 Mouse Anti-Human CD4(RPA-T4)	BD Horizon	562658
APC-Cy™7 Mouse Anti-Human CD8	BD Pharmingen	557834
BV421 Mouse Anti-Human Perforin（δG9）	BD Horizon	563393
BV510 Mouse Anti-Human Granzyme B(GB11)	BD Horizon	563388
BV786 Mouse Anti-Human IFN-γ(4S.B3)	BD Horizon	563731
PE anti-human TNF-α（MAb11）	BioLegend	502909
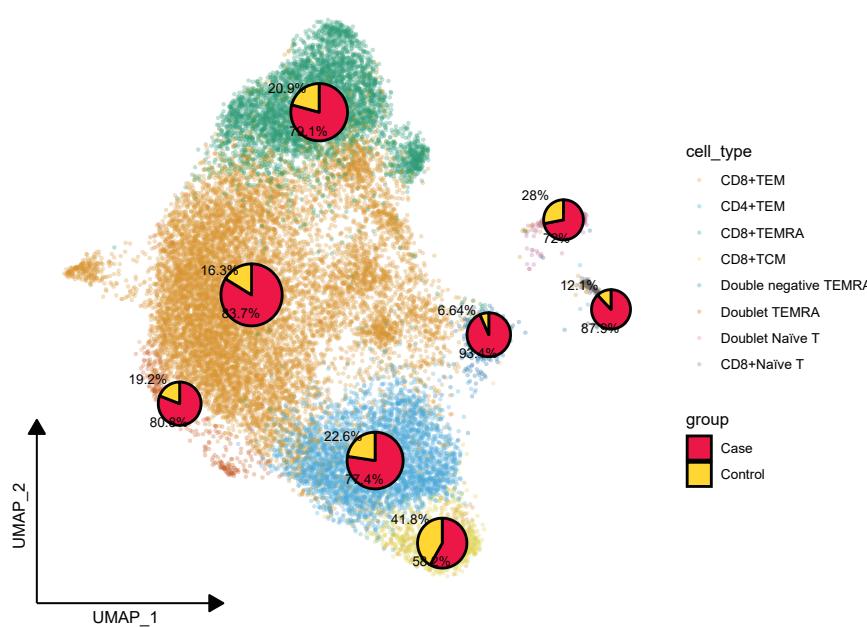
Fig. S1| The representative genes among different cell clusters, and the proportion of case and control in each cell clusters. A. Heatmap showed the representative genes of each cluster. **B.** The proportion of case and control in each cell cluster was displayed in Uniform Manifold Approximation and Projection (UMAP).
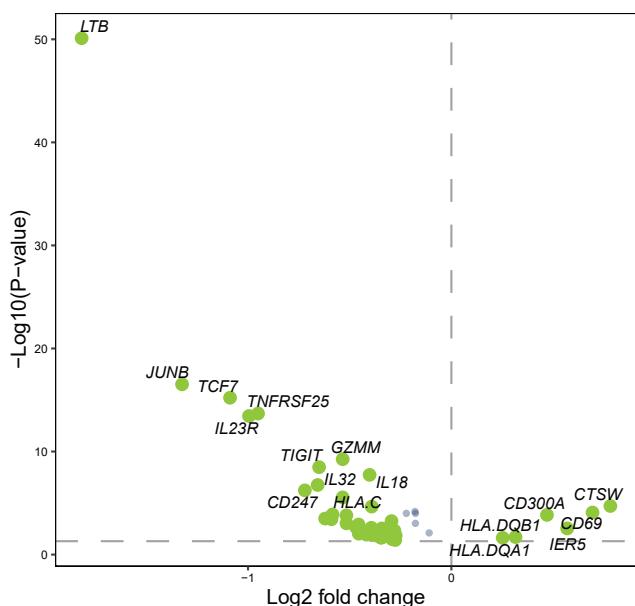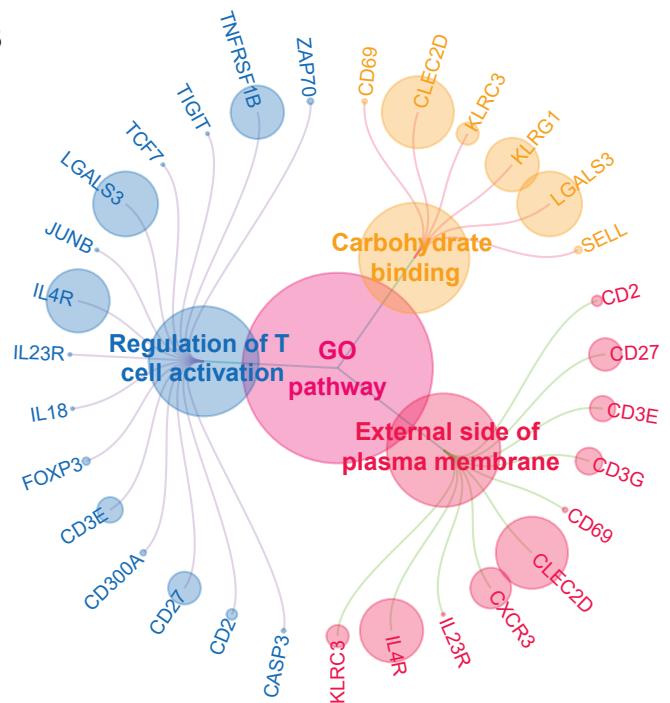
Fig. S2| DEGs filtration and pathway enrichment analysis. A. Significant DEGs between case and control groups. B. GO enrichment analysis. The top three GO pathway items were visualized.
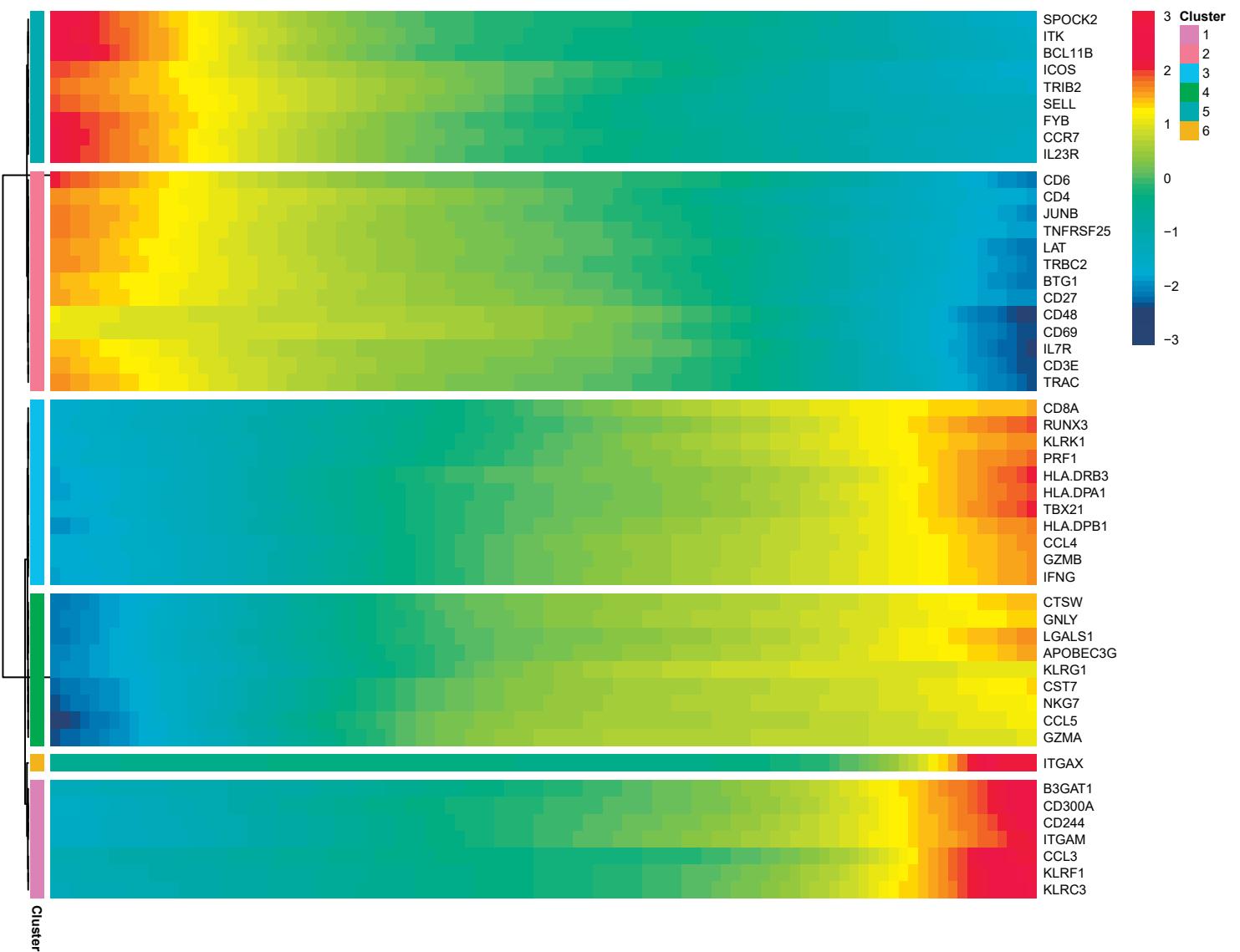
**Fig. S3| Branch expression analysis modeling (BEAM analysis).**
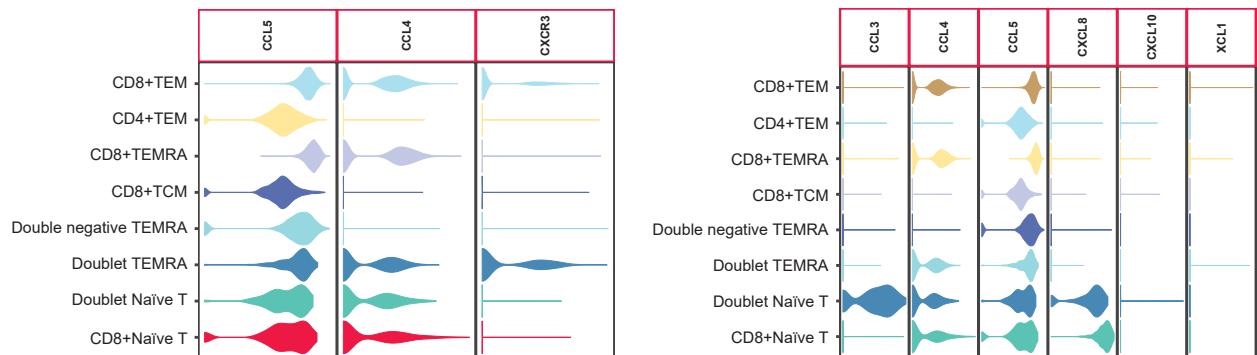
Fig. S4| Violin plot displayed the expression of selected tropic chemokines, classical cytotoxicity activation genes, and downregulated in Exhausted T cells Effector molecules in subtype clusters.
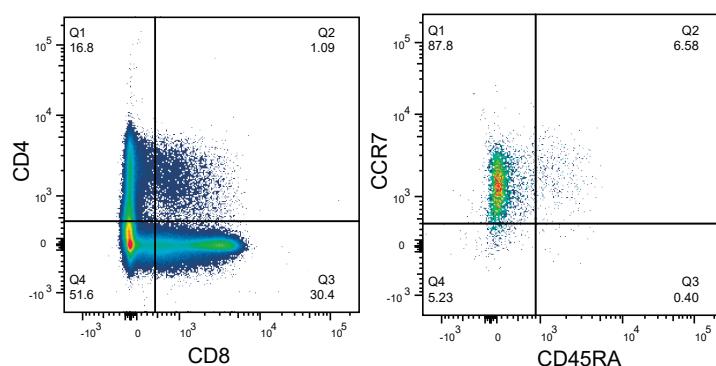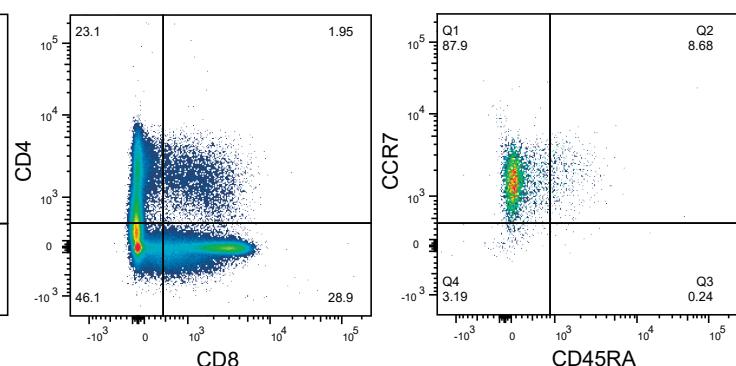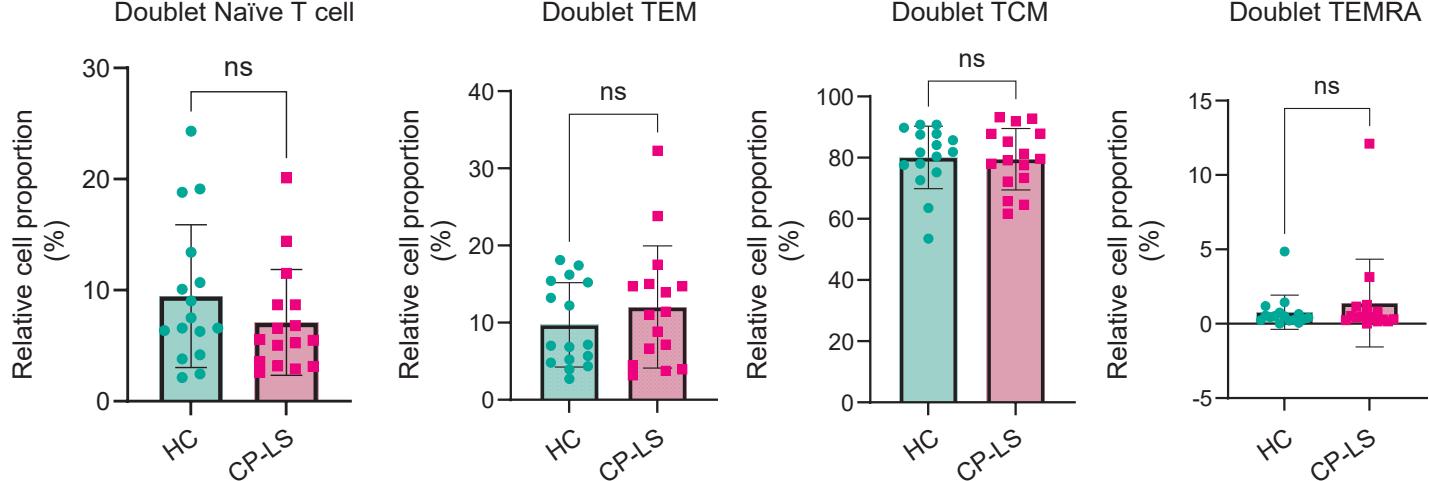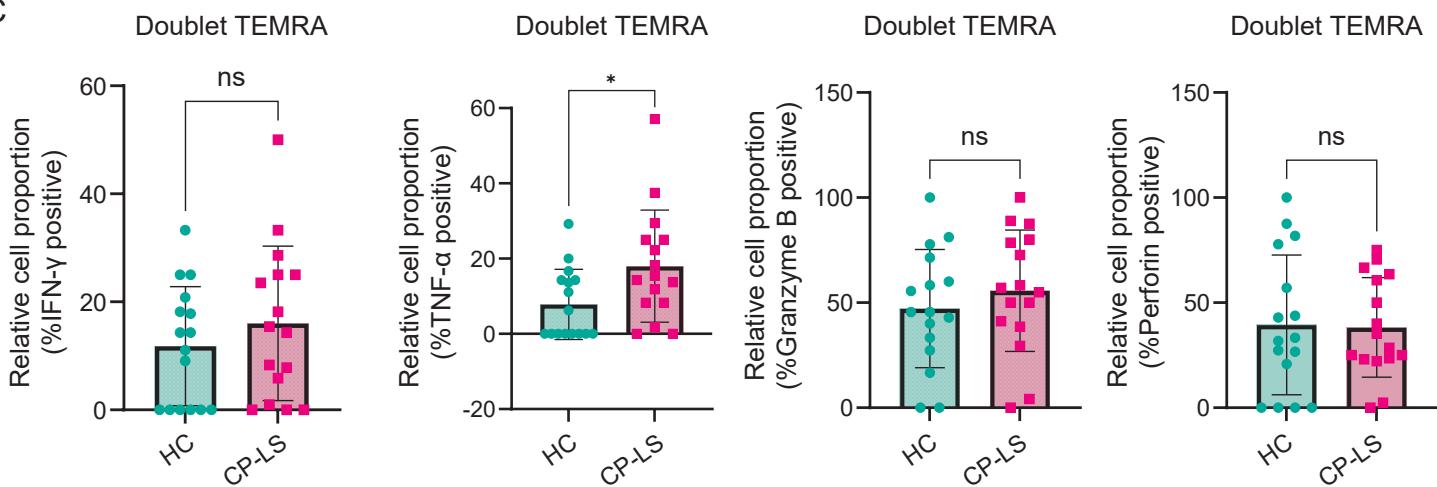
Fig. S5| Characterization of doublet TEMRA in CP-LS. PBMCs from patients with CP-LS and HCs were stimulated for 16 hours and analyzed with flow cytometry for intracellular IFN-γ, TNF-α, Granzyme B, and Perforin cytokine expression. (A) Representative flow cytometry dot plots of CD45RA and CCR7 expression to identify four CD4+CD8+ T-cell subsets in the PBMCs of a patient with CP-LS (right plot) and a HC (left plot); (B) Percentages of CD45RA+CCR7+ (Tnaive), CD45RA-CCR7+ (TCM), CD45RA-CCR7- (TEM) and CD45RA+CCR7− (TEMRA) subsets within the CD4+CD8+T-cell population in PBMCs of patients with CP-LS (n=16) and HCs (n=16); (C) Percentages of IFN-γ+, TNF-α+, Granzyme B+, Perforin+ doublet TEMRA cells from patients with CP-LS (n=16) and HCs (n=16). **Note:** HC, healthy control; CP-LS, chronic prostatitis-like symptoms; TEM, T Effector Memory; TCM, T Central Memory; TEMRA, T Effector Memory RA. Values of *p<0.05 was considered significant; ns, not significant.
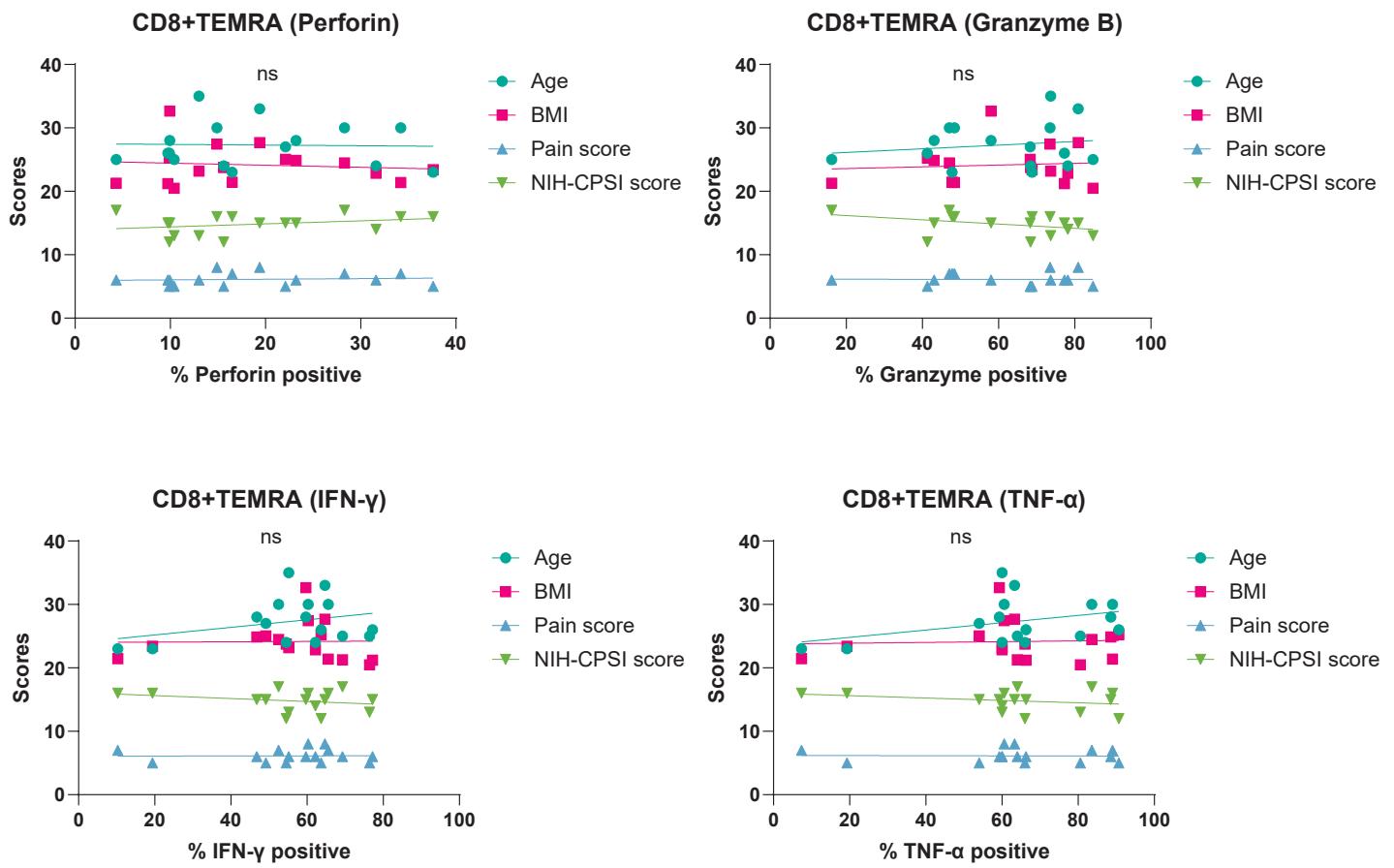
Fig. S6| Correlation between percent of Perforin/Granzyme B/IFN-γ/TNF-α positive cells and Age/ BMI/Pain score/NIH-CPSI score. Note: ns, not significant.