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Supplemental information

Manufacture of CD22 CAR T cells following positive versus negative selection results in distinct cytokine secretion profiles and γδ T cell output

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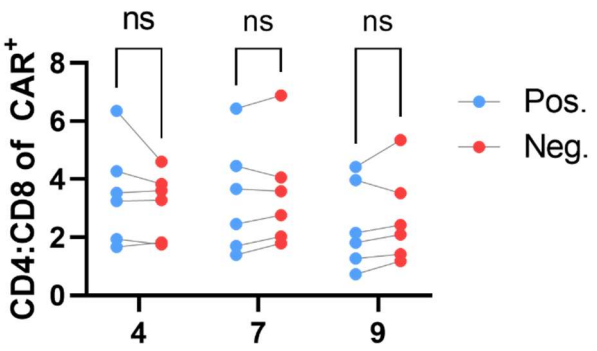

Figure S1: CD4:CD8 ratio in CAR$^+$ T cells. The ratio of CD4:CD8 cells was assessed after gating on viable CD3$^+$CAR$^+$ cells at days 4, 7, and 9 following positive and negative selection. ns, non-significant

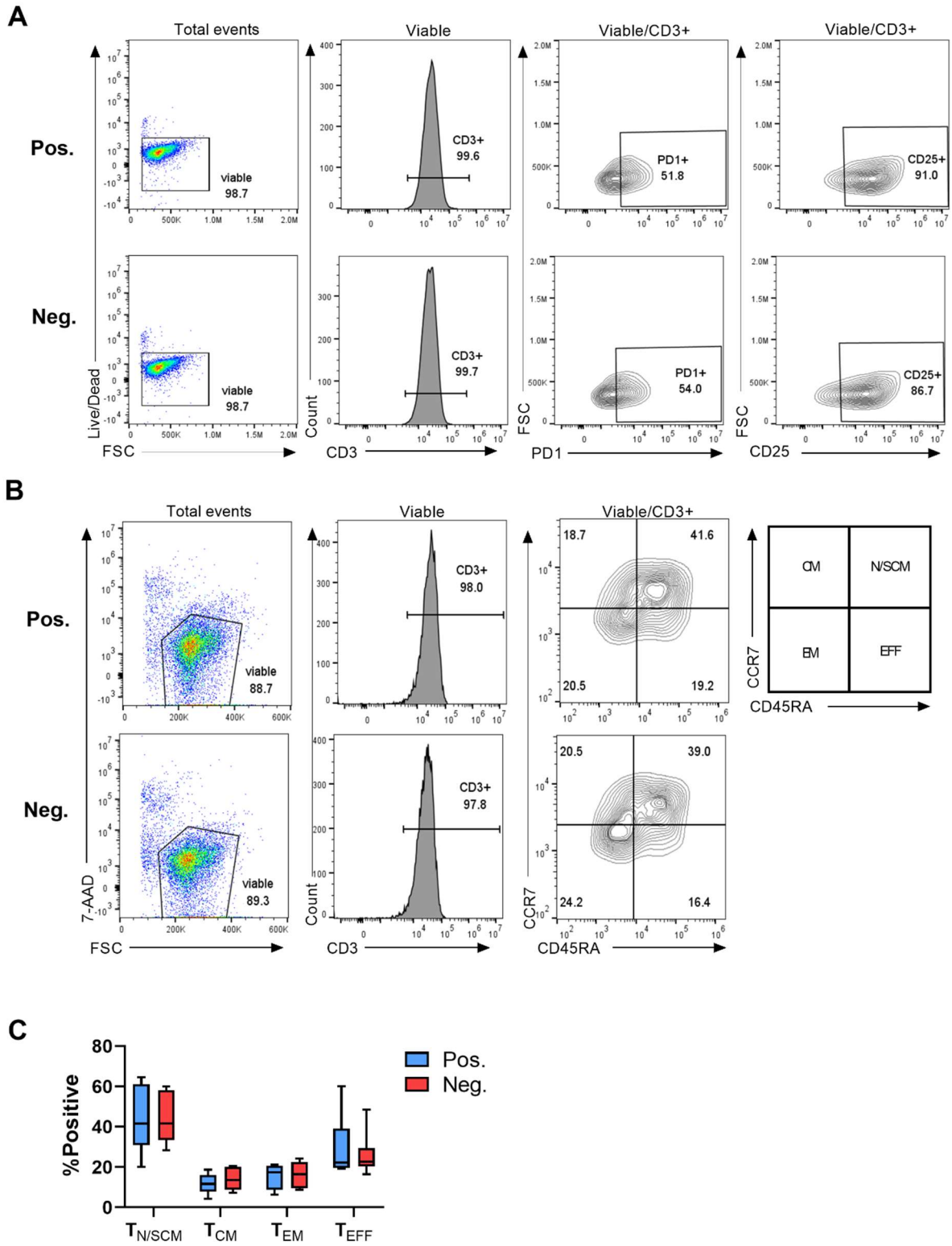

Figure S2: Representative flow cytometry plots assessing T cell phenotype and activation markers. (**A**) Representative plots showing the gating of PD-1 and CD25 activation markers on $CD3^+$ T cells at day 9. (**B**) Representative plots showing CD45RA/CCR7 profiles distinguishing naïve/stem cell memory-like (N/SCM), central memory (CM), effector (E), and terminal effector (EFF) T cells at day 9. (C) The percentages of each subset were evaluated on gated $CD3^+$ T cells at Day 9 and means ± SEM (N=6) are shown.

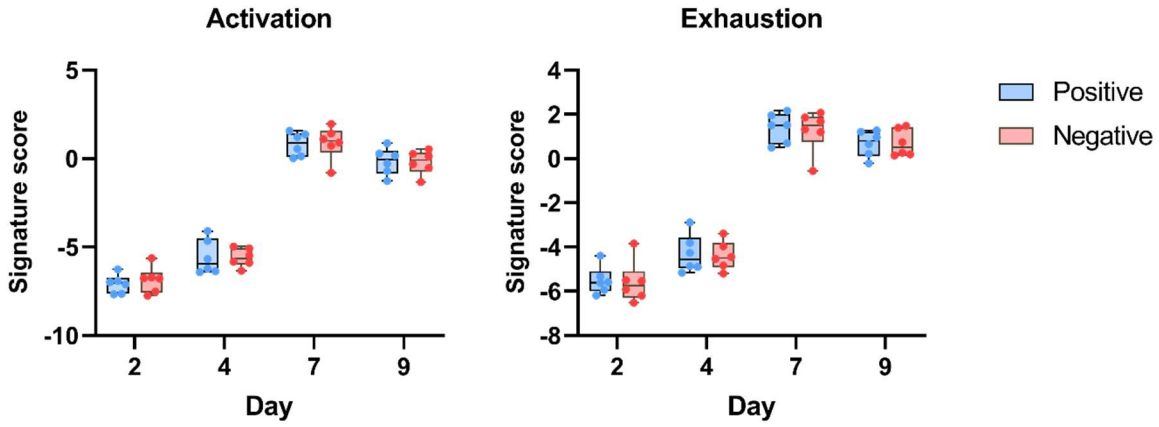

Figure S3: **Signature scores of activation and cytotoxicity pathways on negatively-selected and positively-selected T cells.** Pathway analysis of gene expression data for activation and cytotoxicity pathways were assessed at days 2, 4, 7, and 9 of culture. Means ± SEM (n=6 donors) are shown.

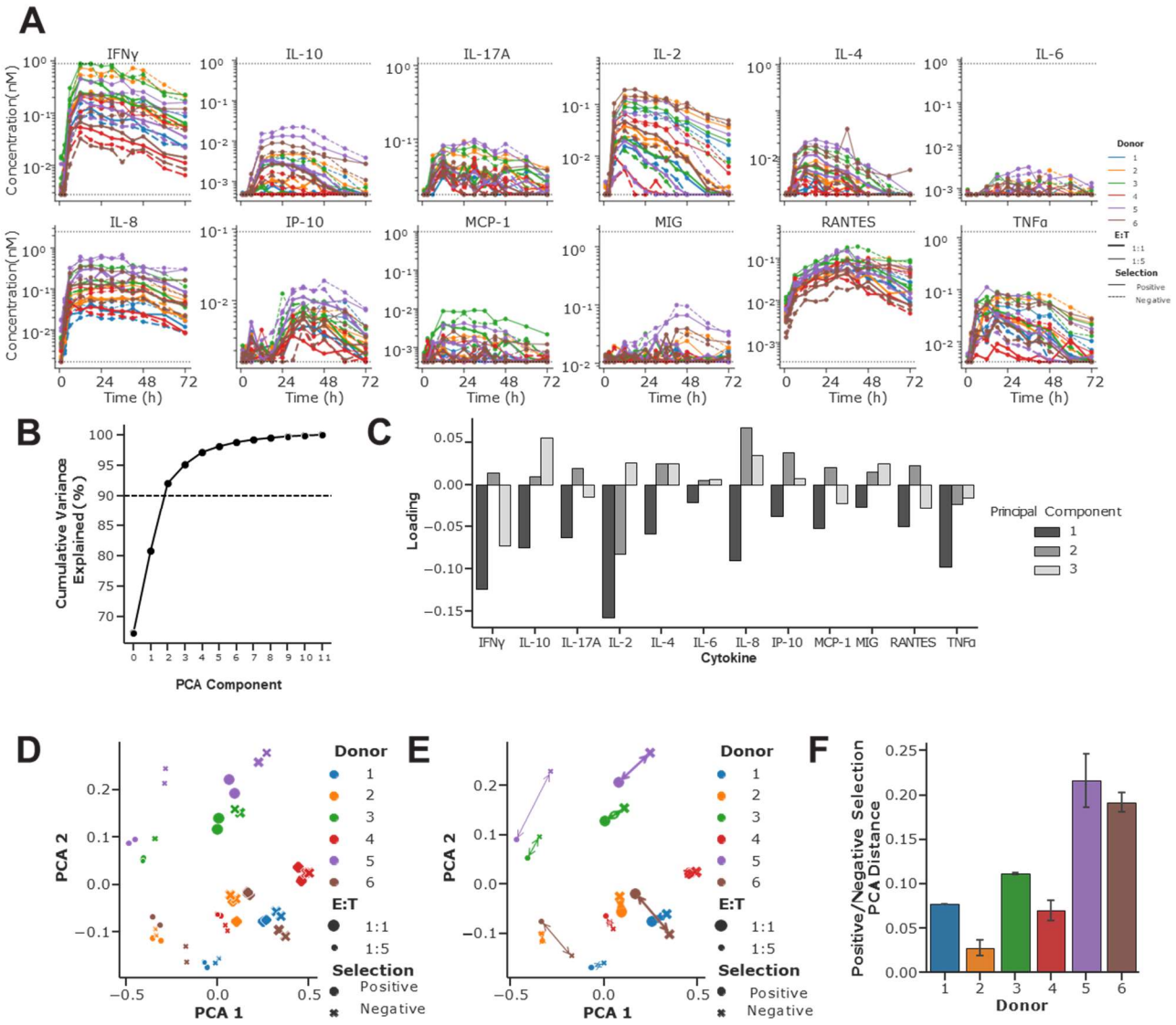

Figure S4. Dynamics of cytokine secretion by CD22 CART^{pos} and CART^{neg} in response to NALM6 leukemia. (**A**) Cytokine secretion by CD22 CART generated from 6 donors was evaluated following co-culture with NALM6 at a 1:1 effector/target (E:T) ratio. Cytokines were monitored at 1, 3, 6, 12, 18, 24, 30, 36, 42, 48, 60, and 72h timepoints on a TECAN EVO 100 robotic system. Raw Immunotron cytokine trajectories are shown; dotted lines represent the upper and lower limits of detection for each cytokine. (**B**) Cumulative variance explained for PCA on average cytokine secretion levels per sample; 3 components are sufficient to reach ~90% variance explained. (**C**) Loadings for the first 3 PCA components. (**D**) PCA1/PCA2 profiles of each sample following co-culture with NALM6 leukemia at a 1:1 and 1:5 E/T ratio. (**E**) Distances between positive and negative selections for each set of samples in PCA space. (**F**) PCA distances show considerable heterogeneity between donors, signifying that positive and negative selection had a greater impact on the cytokine secretion of cells derived from some donors over others.

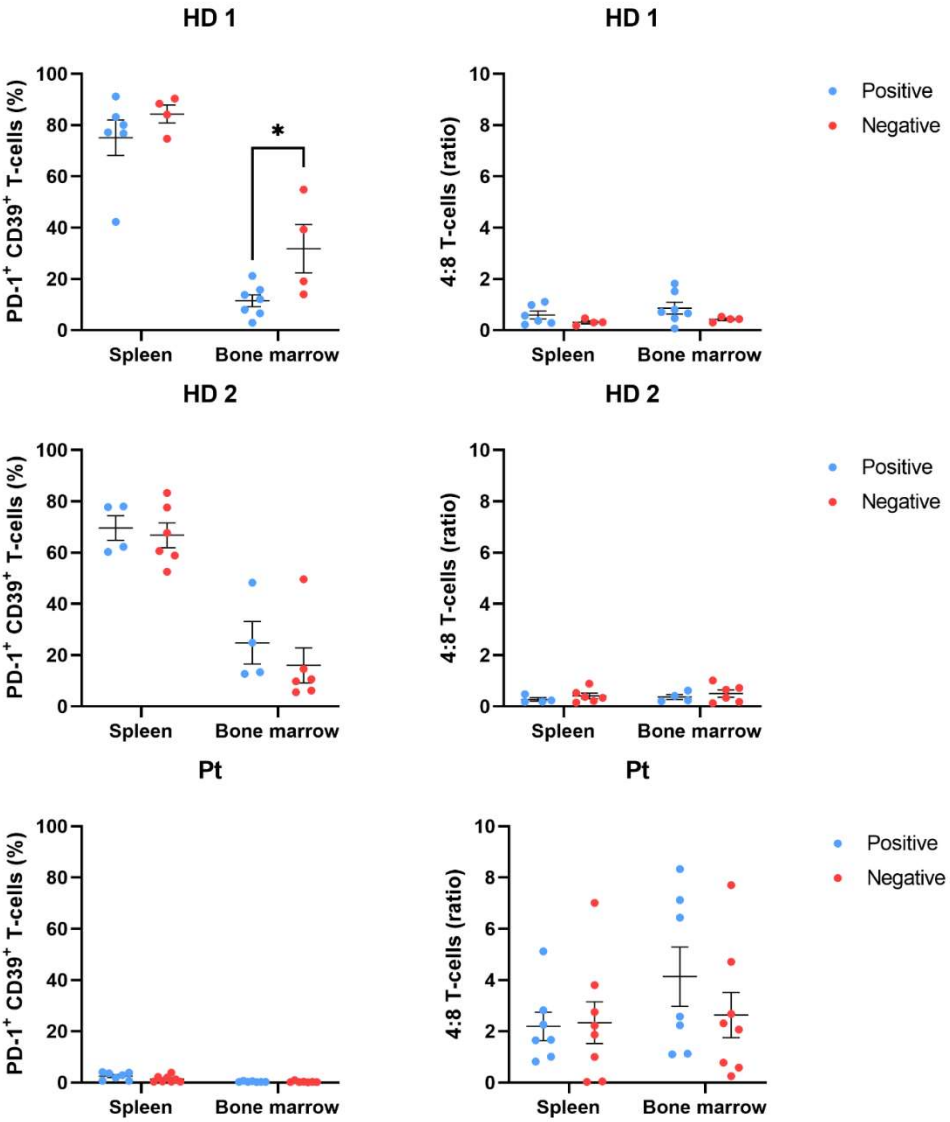

Figure S5. CD4/CD8 ratios and the PD1^{+}CD39^{+} phenotype of CD22 CART persisting in NALM6-bearing NSG mice vary as a function of donor. Following adoptive transfer of CD22 CART^{pos} and CART^{neg} from 3 donors (HD1, HD2, and Pt) into NSG mice with NALM6 leukemia, mice were sacrificed (day 10) and the phenotypes of human T cells in spleen and bone marrow were evaluated. The percentages of PD1^{+}CD39^{+} T cells and their CD4/CD8 profiles were monitored by flow cytometry and a quantification of these cells are shown for each donor (n=4-7 mice per group).

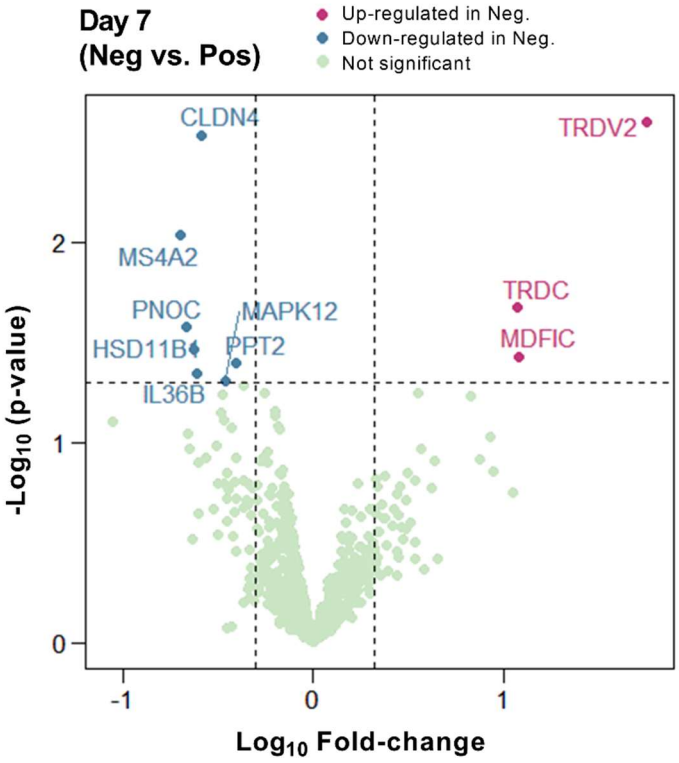

Figure S6. Differential gene expression in CD22 CART^{pos} and CART^{neg}. Volcano plot representation of differential gene expression between CD22 CART^{pos} and CART^{neg} (day 7 of expansion). Fold changes of >2 and $p<0.05$ were considered significant (dotted lines). The identities of several differentially expressed transcripts are indicated.

Table S1: Flow cytometry reagents.

Marker	Conjugate	Catalog #	Clone	Dilution
CCR7	FITC	BD 561271	150503	1:100
CD3	APC-H7	BD 641397	SK7	1:100
CD3	BV650	BD 563999	SK7	1:100
CD4	BV605	BD 562658	RPA-T4	1:100
CD4	AF-700	BD 557922	RPA-T4	1:100
CD4	PerCP	BD 344624	SK3	1:100
CD8	FITC	BD 347313	SK1	1:100
CD8	BV-510	BD 563919	SK1	1:100
CD8	BV605	BD 300936	HIT8a	1:100
CD25	PE-Cy7	BD 557741	M-A251	1:100
CD45RA	BV421	BD 562885	HI100	1:100
CD45RO	BV605	BD 562791	UCHL1	1:100
CD62L	PE-Cy7	BD 565535	DREG56	1:100
LAG3	APC-R700	BD 565774	T47-530	1:100
PD-1	BV421	BD 562516	EH12.1	1:100
Protein L	Biotin	Pierce 29997	n/a	20:100
Streptavidin	FITC	Invitrogen S32354	n/a	1:100
TCRαβ	PE	Miltenyi 130-113-537	REA652	1:100
TCRVδ1	APC	Miltenyi 130-118-968	REA173	1:100
TCRVδ2	BV421	Miltenyi 130-111-015	REA771	1:100
Viability	Fixable Aqua Dead Cell Stain	Agilent L34966	n/a	1:100
Viability	LIVE/DEAD™ Fixable Near-IR Dead Cell Stain	Biolegend L34976A	n/a	1:100
Viability	7-AAD	BD 559925	n/a	3:100