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Article

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Generation of functionally distinct T-cell populations by altering the viscoelasticity of their extracellular matrix

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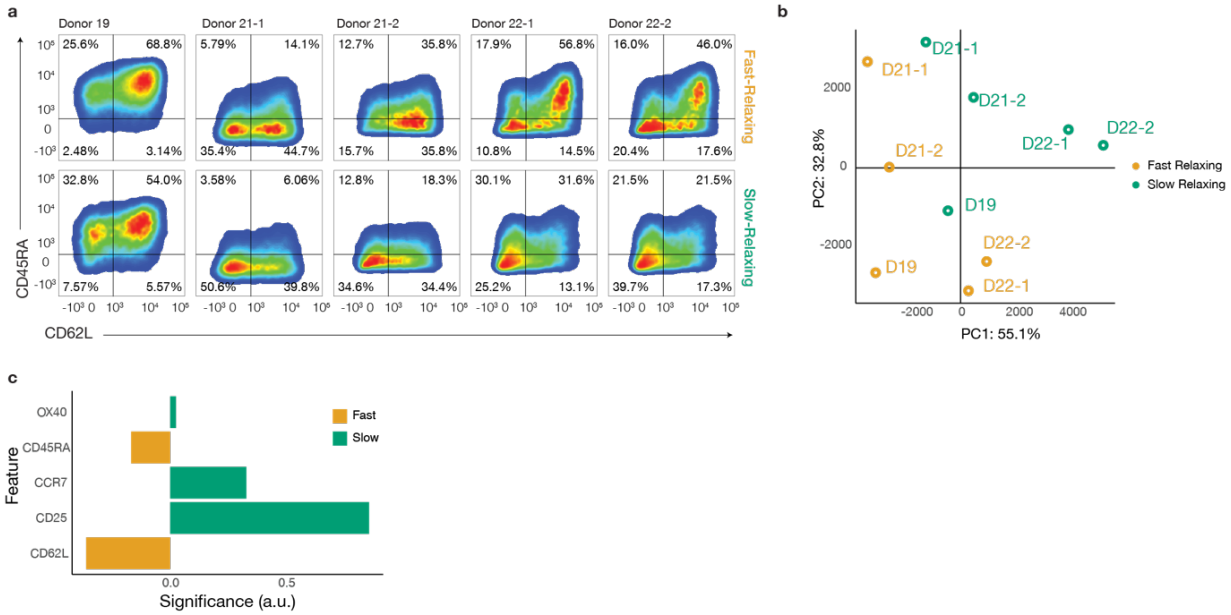
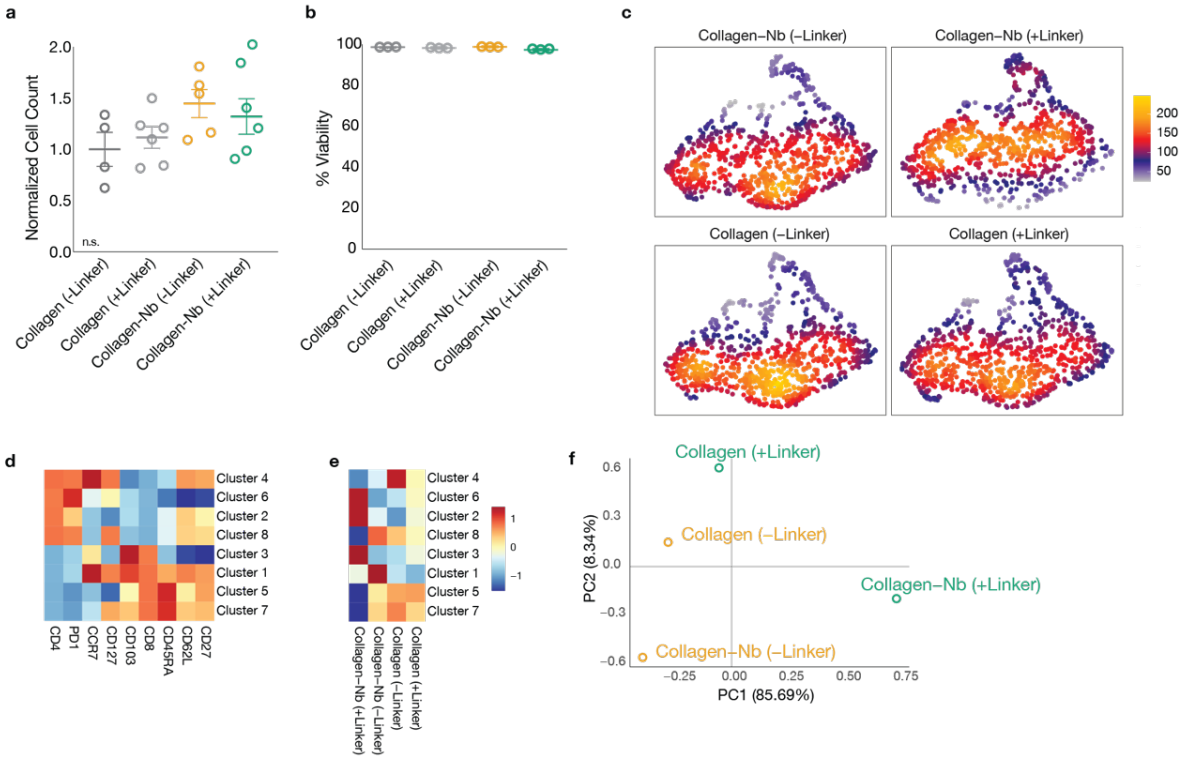
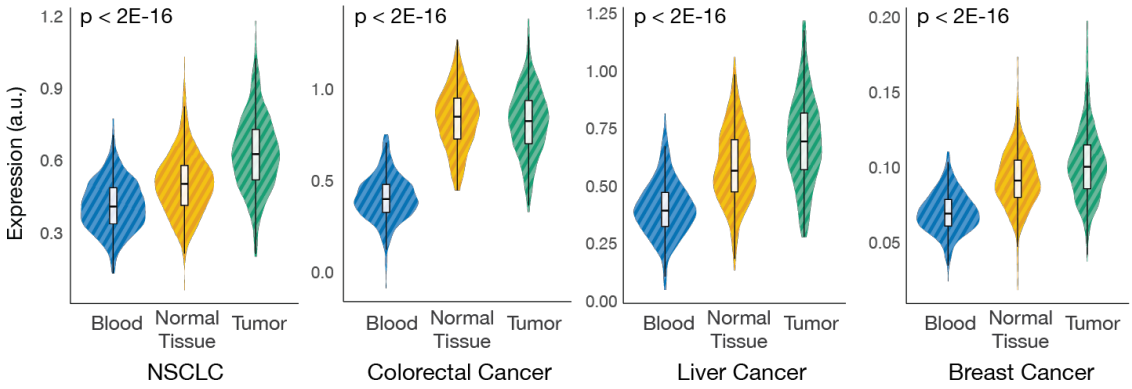


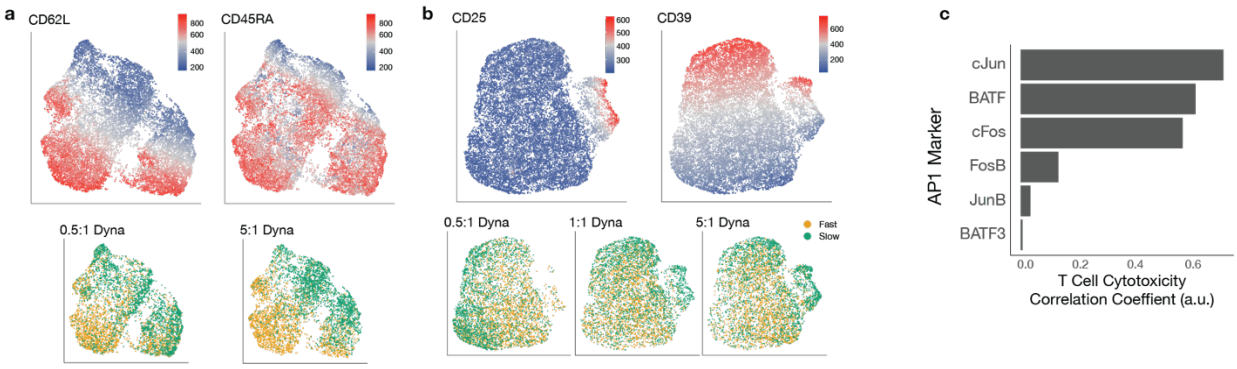
Supplementary Fig. 1 | ECM modulation of T cell phenotype using multiple donors and across independent experiments. a. Representative flow cytometry plots showing CD62L and CD45RA expression for T cells cultured in soft fast and soft slow relaxing gels after 4 days of activation for multiple donors and across multiple independent studies. For donors used for independent studies, the study number is indicated after the donor number. b. PCA plot showing that ECM viscoelasticity drives phenotypic differences along both principal components 1 and 2. c. PCA loadings for PC1 showing relative importance of input features. Data shows pooled samples n=2-3 for 3 donors across 5 independent experiments.



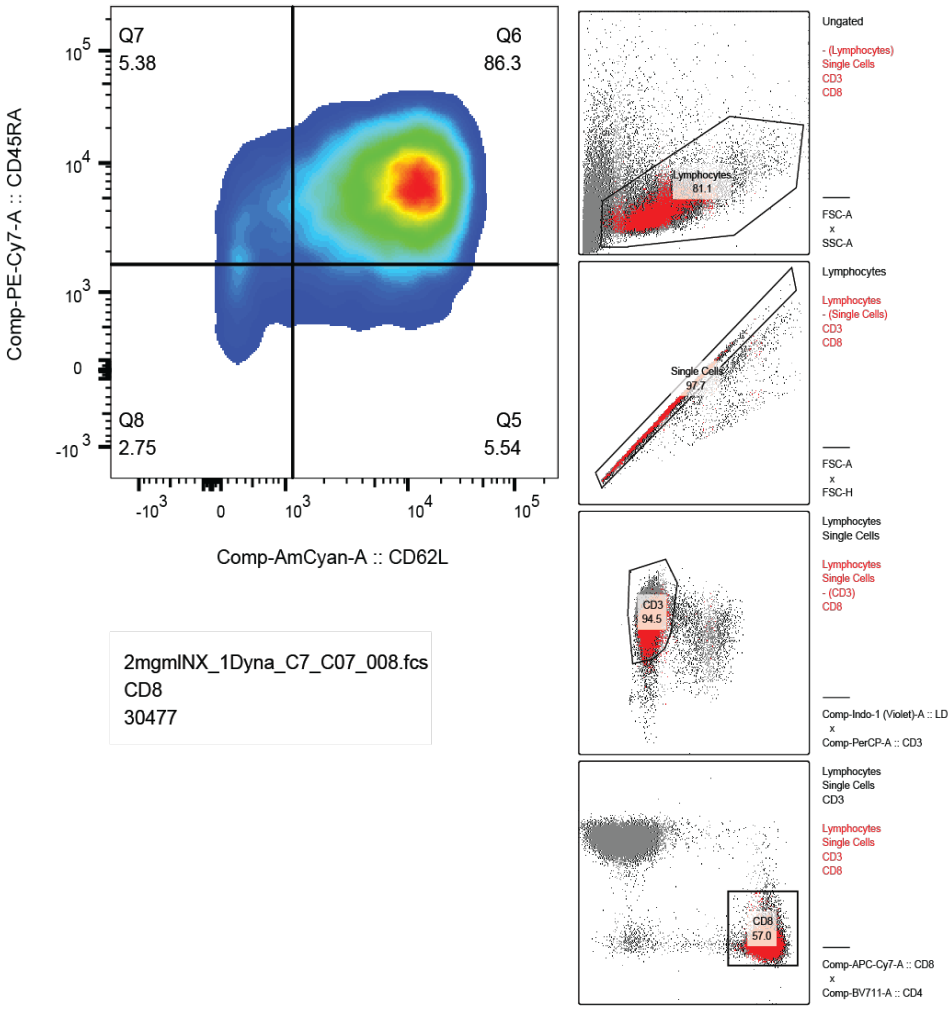
Supplementary Fig. 2 | Collagen modification and crosslinker have minimal effects on T cell adhesion and phenotype. a. Adhesion of T cells seeded on top of unmodified collagen or norbornene modified collagen (Collagen-Nb) with or without methyltetrazine linkers (n=4-6 biological replicates). Data are normalized to cell counts from Collagen (-Linker) group and represents mean ± s.e.m. b. Viability of T cells embedded in collagen matrices with or without norbornene modification and crosslinker. Data are mean ± s.e.m. (n=3 biological replicates) c. Umap plots of T cells phenotyped by flow cytometry after embedding in collagen matrices with or without norbornene modification and crosslinker. d-e. K-means clustering was performed on pooled T cells from all experimental conditions. d. Heatmap plot showing characteristic markers for each cluster. e. Heatmap plot showing the frequencies of cells per condition for each cluster. f. PCA plot showing relative similarities between the different conditions. Data for c-f show pooled samples for n=3 replicates.



Supplementary Fig. 3 l Application of in vitro generated modules to pan T cells in tumor patients. Violin plots comparing expression levels of SModule for pan CD8+ T cells tumors, adjacent normal tissues and blood for NSCLC, liver cancer and colorectal cancer. P-values were determined by using two-tailed one-way Anova.



Supplementary Fig. 4 l Characterizing T cell phenotype and AP-1 correlation with T cell cytotoxicity. a. Representative umap plots of CD8+ T cells showing expression of CD62L (left) and CD45RA (right), as well as localization of T cells (bottom) cultured in either fast relaxing or slow relaxing gels, after T cells are activated with different dynabead to T cell ratios. b. Representative umap plots showing expression of CD25 (left), CD39 (right) and T cell localization (bottom) of the same collagen conditions used in a. Data shows pooled samples n=3. c Correlation analyses showing how the expression of indicated AP-1 proteins for T cells cultured in the different mechanical conditions correlate with their observed killing of Raji cells.



Supplementary Fig. 5 l Flow-cytometry gating strategy. Representative flow cytometry gating strategy showing steps from ungated population to CD62L and CD4RA populations.