Supplementary Figure 1. Annotation of skin T and NK cell clusters from single cell RNA sequencing dataset GSE195452
**Supplementary Figure 2. Single cell RNA sequencing analysis of dataset GSE138669**

A Unsupervised Uniform Manifold Approximation and Projection (UMAP) clustering of 5,061 cells detects 8 transcriptionally distinct cell clusters: tissue-resident memory T cells (Trm), cytotoxic T cells (CTLs), regulatory T cells (Tregs), hypofunctional tissue resident T cells (Thprm), naïve/central memory (Tncm), proliferating T cells (Tprolif) and NK cells (NK) and a cluster containing a mixture of MAIT, INKT CD8+ T and γδ T cells (T mix). B UMAP showing the cells belonging to healthy individuals (control) or patients (SSc). C Heatmap illustrating the top 5 differentially expressed genes in each distinguished cell cluster: Trm (CD69, ZFP36L2, CXCR4, IL7R), CTLs (GZMK, IFNG, CCL5, CCL4, CD8A), Tregs (CD4, FOXP3, CTLA4, IL2RA), Thprm (NR4A1, CD69, CXCR4, DUSP1), Tncm (TCF7, SELL, IL7R), Tprolif (MKI67), NK (NKG7, FCGR3A, FGFBP2, KLRD1, GZMB, PRF1) and Tmix (CD8A, CCL5, TRGC2, NKG7, GZMB, PRF1, FCGR3A, FGFBP2, KLRE1). D Heatmap demonstrating the top 10 upregulated genes in each of the 5 different clusters of isolated CD8+ T cells: naïve (Tn); IL7R, Granzyme K+ (GZK+); GZMK, IFNG, CCL4, Granzyme B+ (GZB+); PRF1, GNLY, NKG7, GZMB, GZM2, GZMH, exhausted (Tex); NR4A2, NR4A3, and proliferating (Tprolif): LASP1, TMPO, ANP32B. E UMAP representing positive (red) and negative (grey) gene expression of PDCD1 (PD-1) among CD8+ T cell clusters.
IL7R, TCF7, SELL, ANXA1, CTLs (CD8A, GZMK, GZMA), Tregs (CD4, CD27, CTLA4, IL2RA), Thprm (NR4A1, DUSP1), Tncm (TCF7, IL7R), Tprolif (MKI67), NK (NKG7, FCGR3A, KLRD1, PRF1). C (left) Gene set enrichment analysis with Wiki pathways as reference dataset. Examples of top pathways (p < 1e-10) represented by NK and CTL clusters are shown. Statistics were performed with Kolmogorov-Smirnov (KS) test. (right) Comparison of the enrichment scores of the overview of proinflammatory and profibrotic (q=0.68) and lung fibrosis (q=6e-15) pathways in HD versus SSC skin T and NK cell clusters (here for GSE138669, Fig 1 for GSE195452).

Supplementary Figure 3. Pairwise correlations of CD7 with pro-fibrotic genes

The Wiki gene pathways lung fibrosis and pro-inflammatory and pro-fibrotic manifestations were merged and potential correlation of CD7 gene expression with the included genes was evaluated separately for the cluster of A cytotoxic T cells (CTLs) and B NK cells. Statistical significance for every
Supplementary figure 4. CD3 and CD7 immunohistochemistry in SSc affected versus non-affected skin of patients with systemic sclerosis. CD7 gene expression in skin immune and stromal cells is also depicted.

A Representative images of CD3 immunohistochemistry (IHC) staining of the affected and non-affected skin biopsies from one SSc patient. Scale is 100 µm. B Quantification of CD3+ T cells in the
affected vs non lesion SSc skin (n=20). Bars are mean ±SD. Non-parametric Wilcoxon test, p=0.19. C In SSc affected skin large infiltration of CD7+ cells is found in perivascular areas while in matched non-affected skin, a smaller number of CD7+ cells is present around blood vessels. Scale is 100 µm. Here, representative images of one SSc patient with early diffuse disease are depicted. D 2-D dot plot comparing CD7 gene expression in skin immune and stromal cells. Cell cluster annotations were retrieved from metadata information as have been described in the single-cell RNA sequencing dataset GSE195452. Circle size shows the percentage of cells expressing CD7 and color intensity depicts average expression. Numbers indicate average of normalized counts.

Supplementary Figure 5. SECTM1-CD7 axis in activation of cytotoxic T and NK cells in SSc affected skin.

A Schematic model for the proposed involvement of SECTM1-CD7 axis in cytotoxic T cell and NK cell activation (Created with BioRender.com). In SSc affected skin, CD7 and IFNG is predominantly expressed in cytotoxic T and NK cells while its receptor IFNGR and SECTM1 in antigen presenting cells (APCs) and stromal cells (mainly fibroblasts). This suggests a cytokine-mediated positive feedback loop in the communication between CD7+ cytotoxic immune cells and SECTM1 producing APCs and fibroblasts, with IFN-γ being a key cytokine. B 2-D dot plots comparing expression levels of selected genes in skin immune (myeloid and lymphoid) and stromal cell populations. Cell cluster annotations were retrieved from metadata information as have been described in the single-cell RNA sequencing dataset GSE195452. Circle size shows the percentage of cells expressing each gene and color intensity depicts average expression. Numbers indicate average of normalized counts. C Pair-wise correlation plots in SSc affected skin (GSE195452) show a positive correlation between CD7/IFNG and SECTM1/IFNGR1. Abbreviations; TCR: T cell receptor, NKR: NK cell receptor, MHC: major histocompatibility complex, APC: antigen-presenting cell.
Supplementary Figure 6. CD3/CD7-IT specifically eliminates only the activated cytotoxic T and NK cells in vitro.
Concentration of IL-2 (pg/ml) was measured in cell supernatant of cells with or without treatment with α-CD3/CD7-IT. **Pie charts illustrating the proportion of effector (CD8^+CD45RA^+CD27^−), memory (CD8^+CD45RA^+CD27^−) and naïve (CD8^+CD45RA^−CD27^+) cells among the CD8^+ T cell population in the depicted stimulation and treatment culture conditions (percentages in the pie charts are mean values of n=6 SSc patients).**

**Response to TCR mediated (PHA) restimulation of cells treated with α-CD3/CD7-IT was evaluated by intracellular flow cytometry. Values are represented as fold change of the re-stimulated compared to the values before stimulation.**

**Comparison of absolute counts (cells/µl) of CD19^+ B cells after in-vitro treatment with CD3/CD7-IT compared to non-treated peripheral blood mononuclear cells (n=6).** (top) Representative flow cytometry plots of one experiment.

**Normalized cell viability, of M2 macrophages and CD19^+ B cells that were isolated from SSc patients’ blood for the depicted different culture/treatment conditions. Cycloheximide was used a positive control.**

**Flow cytometric histograms of one representative experiment exhibiting elevated expression of CD3 and CD7 in CD8^+GZMB^+ cells and CD7 in CD56^+GZMB^+ NK cells upon stimulation with phytohemagglutinin (PHA) that is further quantified in G. CD3 and CD7 expression is presented as mean fluorescence intensity (MFI). Statistics were performed with Student’s t-test, **p<0.01, ****p<0.0001.**

**The percentage of necrotic cytotoxic T (CD8^+7-AAD^-Annexin V^+) and NK (CD56^+7-AAD^-Annexin V^+) cells in the depicted conditions was measured with flow cytometry of the enzymatically digested collagen plugs (n=5).**
Supplementary Figure 7. Fibroblasts co-cultured with CD7+ T cells and NK cells exhibit increased contractility that is accompanied by an elevated myofibroblast-like phenotype.

A. Collagen contraction assay

B. Flow cytometry analysis

C. Graph showing % of no control

D. Western blot for collagen type 1

E. Graph showing % of α-SMA+ fibroblasts

F. Flow cytometry for α-SMA

G. Relative gene expression of COL1A1 and ACTA2

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A Schematic representation of the experimental design in the developed 3D in-vitro collagen contraction fibroblast : immune cell co-culture model. B Flow cytometry gating strategy that was used to sort CD7+ versus CD7- T cell and NK cell populations from healthy peripheral blood (n=3). First, we gated on the lymphoid cell population based on cell size (FSC) and granularity (SSC). Following, we excluded dead cells based on 7-AAD+ staining and we then sorted CD19+CD14- lymphoid cells that were either positive or negative for CD7 expression. C The level of fibroblast contraction was quantified compared to no-cells control and plotted graphically (n=3). Bars are mean ±SD. An image of a representative experiment is depicted on the right part of this panel. D (left) Representative images of Collagen type 1 immunohistochemistry of the collagen plugs that contained only fibroblasts or fibroblasts co-cultured with CD7+ T cells and NK cells. (right) Quantification of Collagen type 1 positive fibroblasts in control versus fibroblasts that were co-cultured with either CD7- or CD7+ cells (n=3). Bars are mean ±SD. E The percentage of CD45-a-SMA-IL-6+ fibroblasts in the depicted conditions was measured with flow cytometry of the enzymatically digested collagen plugs (n=3). F Expression levels (mean fluorescence intensity-MFI) of a-SMA in CD45-a-SMA+ fibroblasts in the depicted conditions was measured with flow cytometry of the enzymatically digested collagen plugs (n=3). (right) Flow cytometry histograms of one representative experiment exhibiting increased expression of a-SMA in fibroblasts co-cultured with CD7+ compared to CD7- T cells and NK cells is shown. G CD7+ versus CD7- T and NK cells (n=3) were co-cultured with primary dermal fibroblasts in the developed 3D hydrogel collagen co-culture model and fibroblasts were analyzed for expression of genes reflective of a myofibroblast phenotype such as COL1A1 and ACTA2. Values represent relative gene expression (\(\Delta\Delta^Ct\)) as measured with qPCR. GAPDH and RPS27A were used as reference genes. Data represents mean ± SEM.