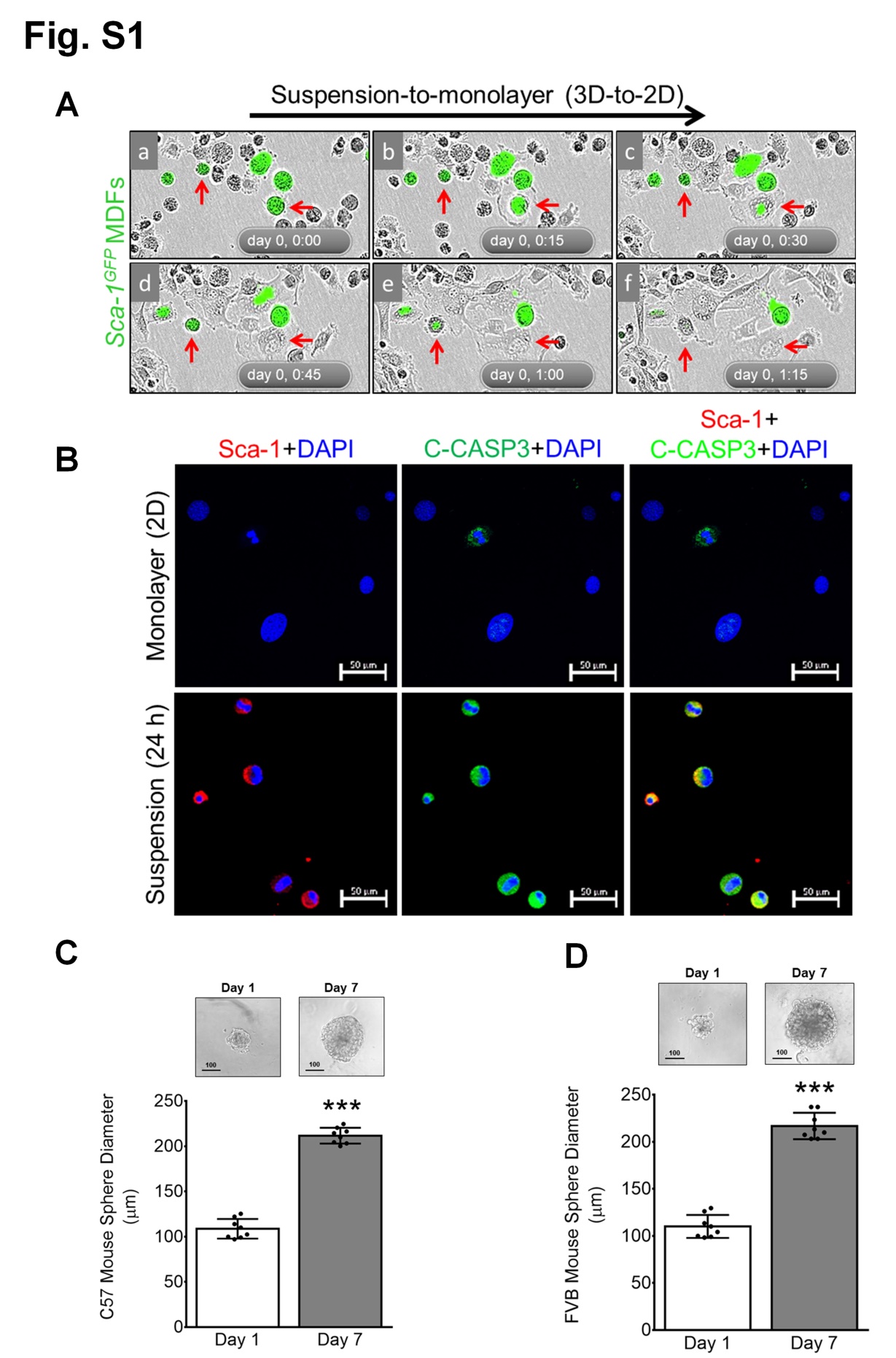
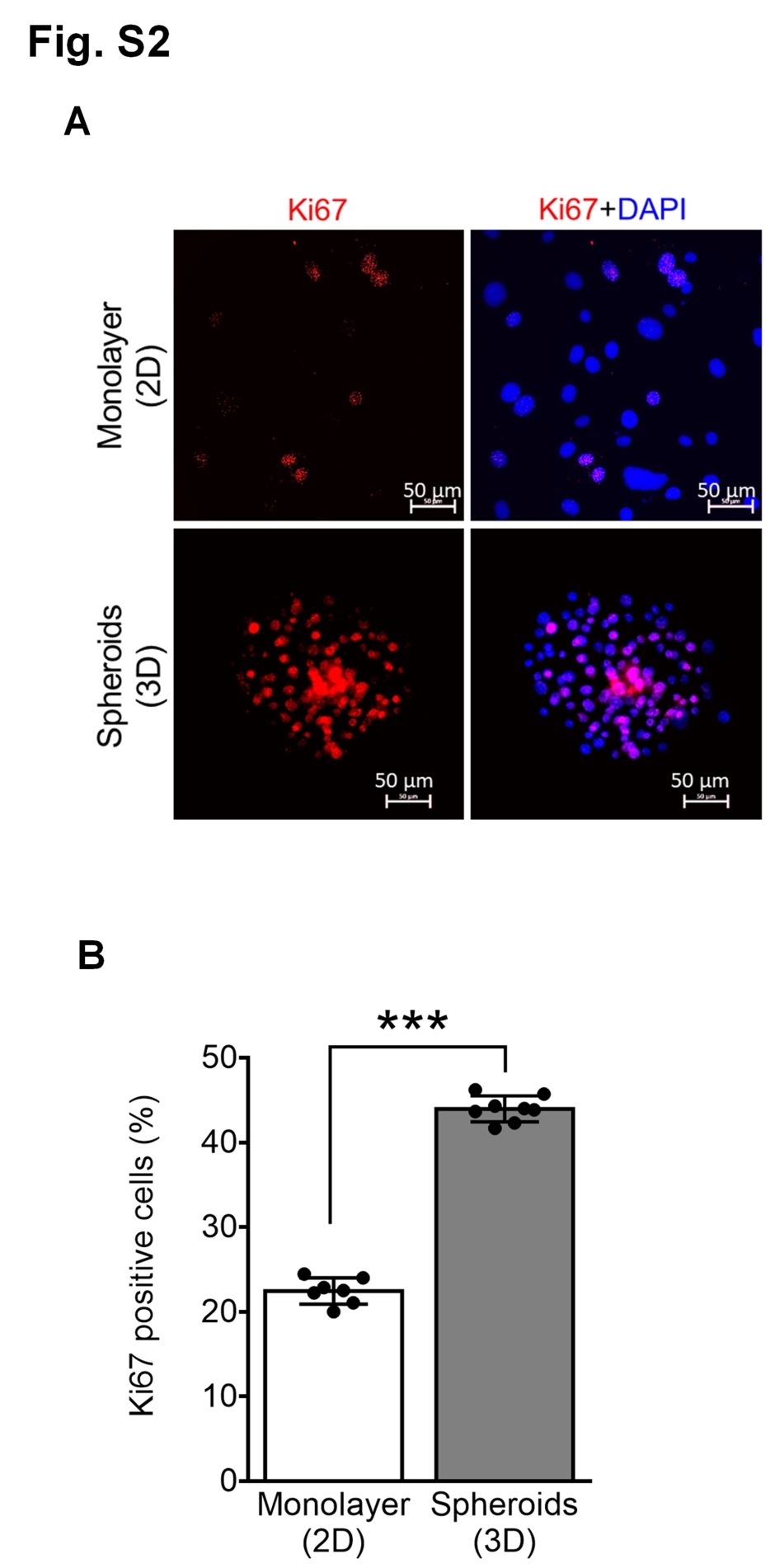
Suspension-Induced Stem Cell Transition: A Non-Transgenic Method to Generate Adult Stem Cells from Mouse and Human Somatic Cells

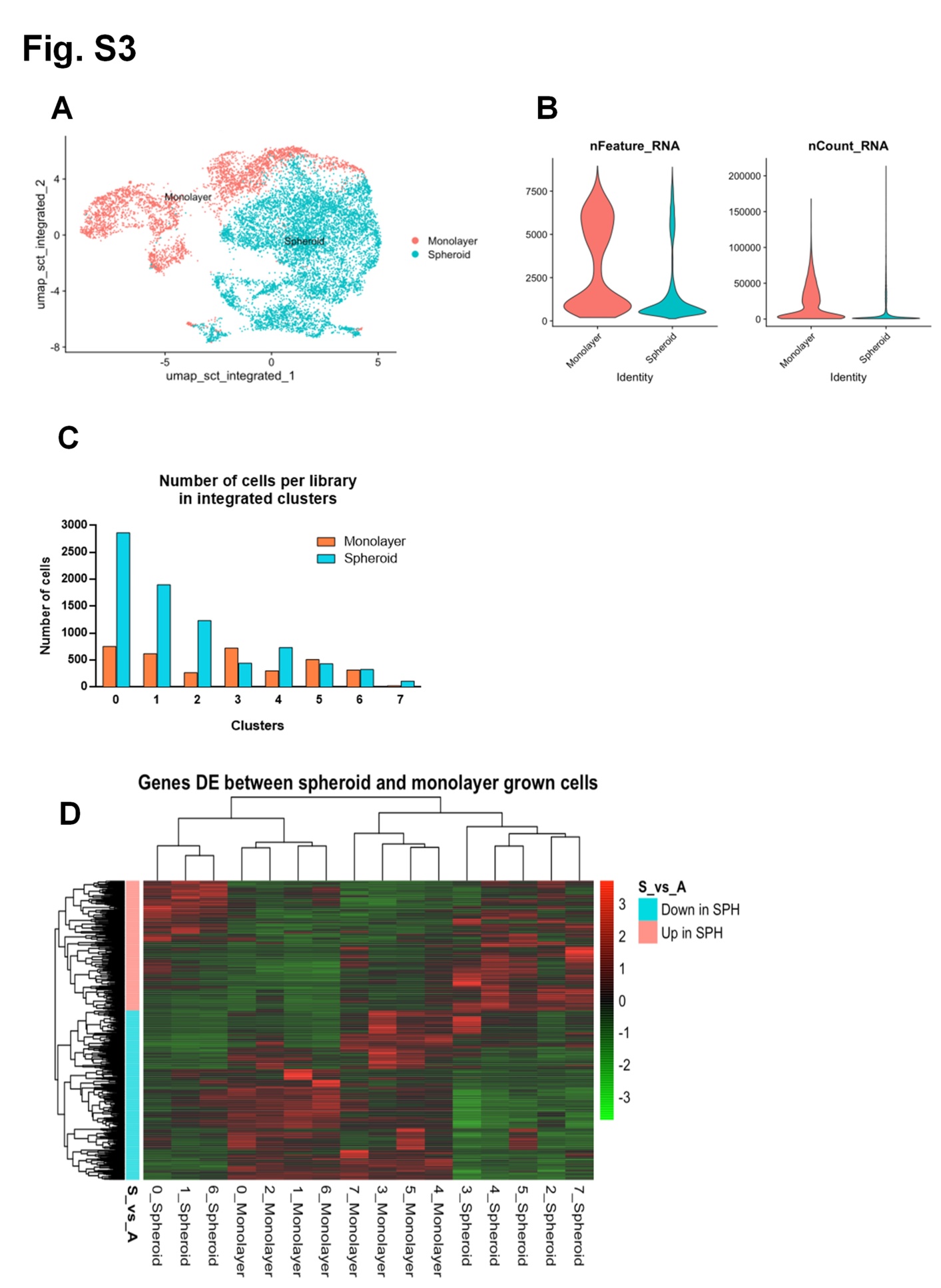
**Authors:** Behzad Yeganeh1,2\*, Azadeh Yeganeh3, Kyle Malone1,4, Shawn T Beug1,4,5, Robert P. Jankov1,2,6



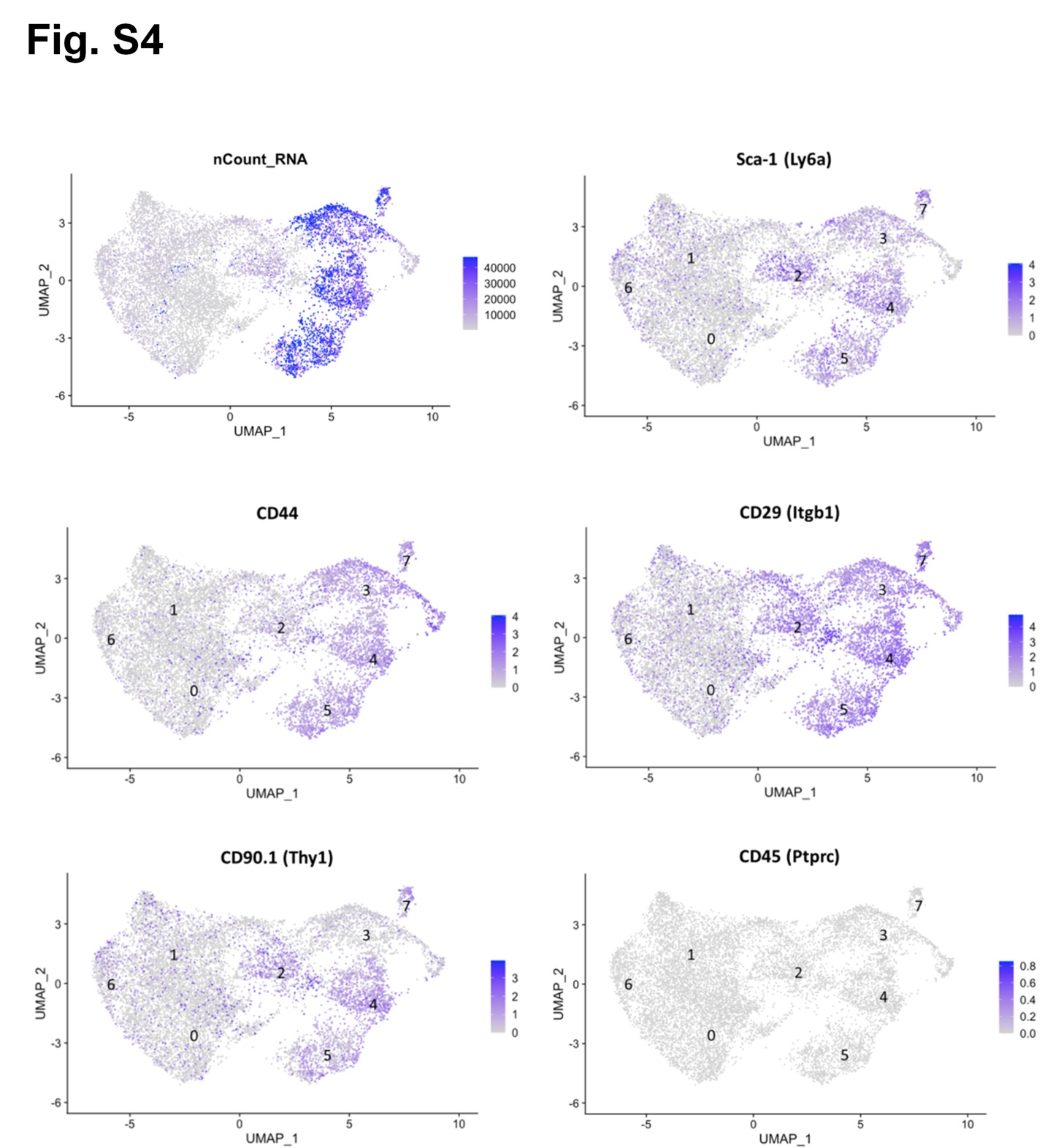
**Figure S1. Mouse TEFs grown in suspension culture and expressing stem cell markers undergo cell death. (A)** Co-immunofluorescence staining of Sca-1 (red) with apoptosis marker cleaved Caspase 3 (C.CASP3) (green) in TEFs grown for 24 h in adherent (top) and suspension culture (bottom). Representative images of spheres of TEFs isolated from **(B)** C57 (top) and **(C)** FVB (bottom) wild-type mice and quantification of their spheroids size at day-7 compared to day one. (mean ± SEM, n = 8 spheroids). Scale bars are indicated in the images. \*\*\*p<0.001. Scale bars: 50 µm.

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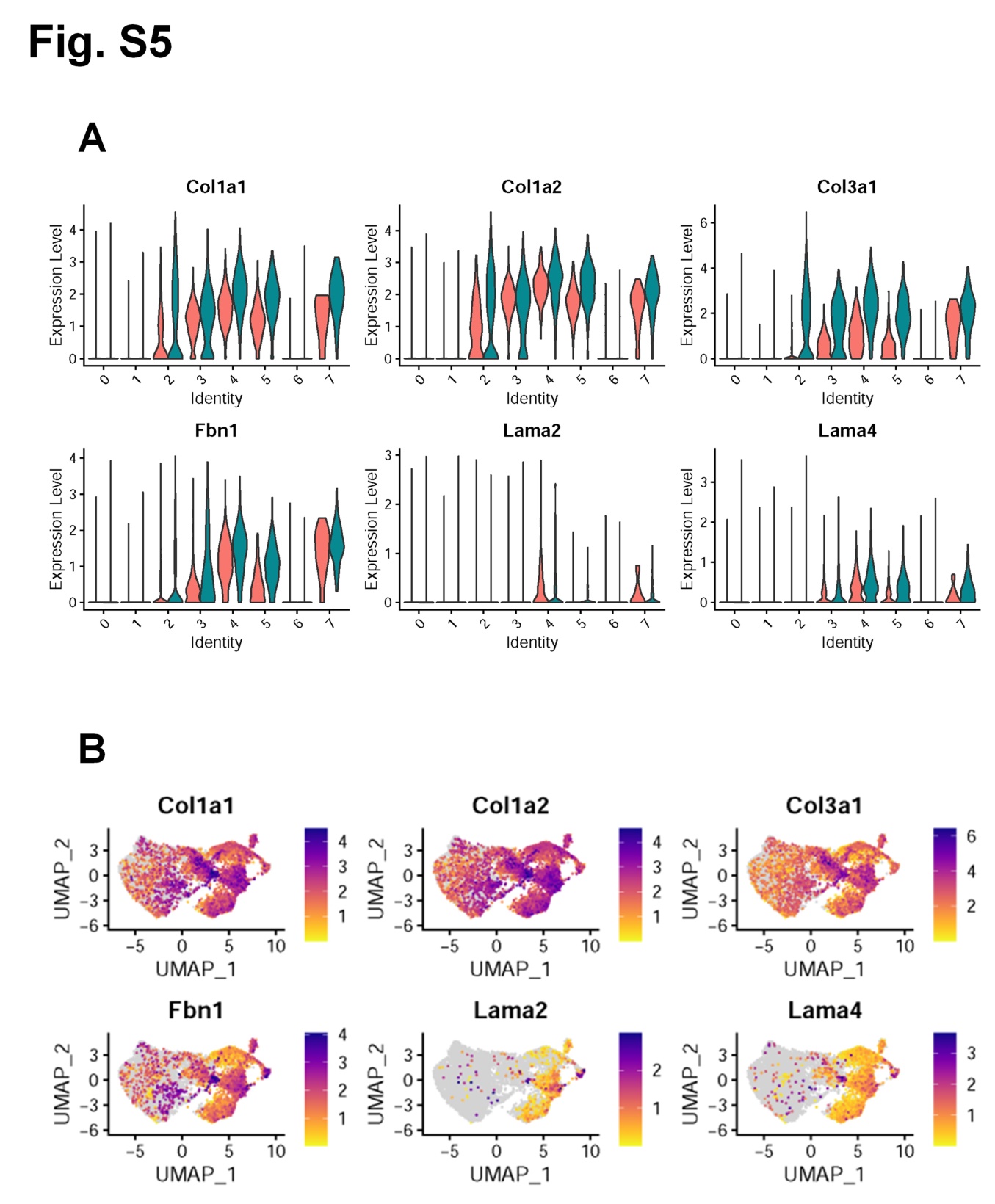
**Figure S2. Mouse TEF-derived spheroids are highly proliferative. (A)** Representative IF staining for Ki67 expression in mouse fibroblasts grown in monolayer (2D) and spheroids grown in suspension culture (3D). Spheroids cultured for 7 days were stained for the presence of the proliferation marker Ki-67 (anti-Ki-67) and nuclei were stained with DAPI. **(B)** Quantitative analysis of Ki67-positive as a % of total cells. (mean ± SEM, n = 7-8 spheroids). Scale bars are indicated in the images. \*\*\*p<0.001.

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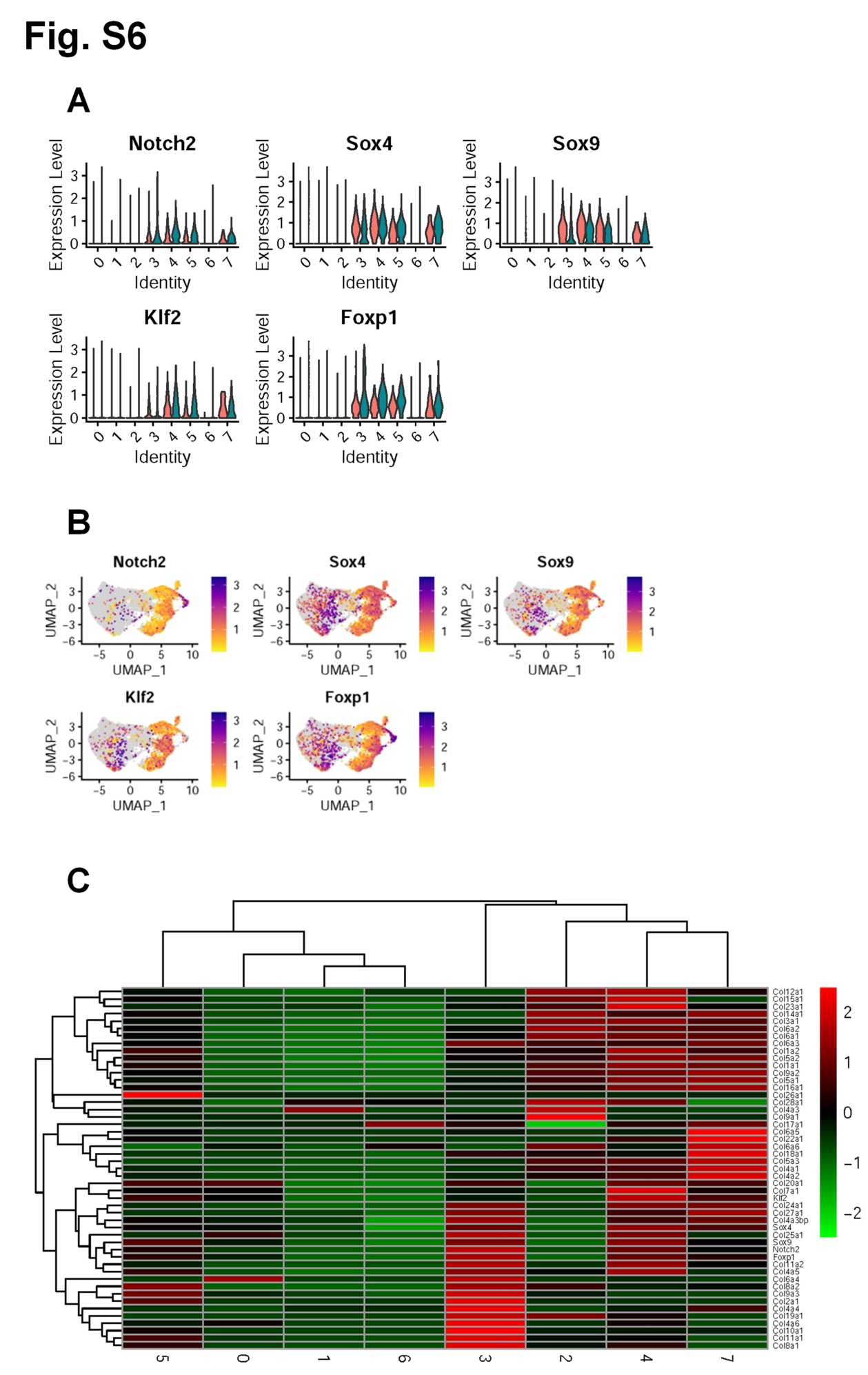
**Figure S3. Transcriptome analysis using RNA-seq in TEFs cultured as a monolayer and spheroids. (A)** (A) UMAP projection of cells from the attached (monolayer) and suspension (spheroid) cells. UMAP embeddings calculated from a PCA reduction of the SCTransformed UMI counts, showing that the cells from the two libraries have generally non-overlapping UMAP embeddings. **(B)** Violin plots showing distributions of the number of detected genes (nFeature\_RNA) and number of UMIs (nCount\_RNA) per cell in the two libraries. Cells fall into two populations, with ‘high’ (> ~2,500) or ‘low’ (< ~2,500) numbers of detected genes. The absolute number of ‘high’ gene cells is similar in the two libraries, but the Spheroid library has a large excess of ‘low’ gene cells. (**B**) (**C**) Bar plot showing the number of cells from each library in each of the resolution 0.3 clusters identified on the integrated assay. (**D**) Heatmap illustrating differentially expressed genes (DEGs) of mouse TEFs cultured as a monolayer and spheroids in across 8 clusters, illustrating that each cluster exhibited unique gene expression.

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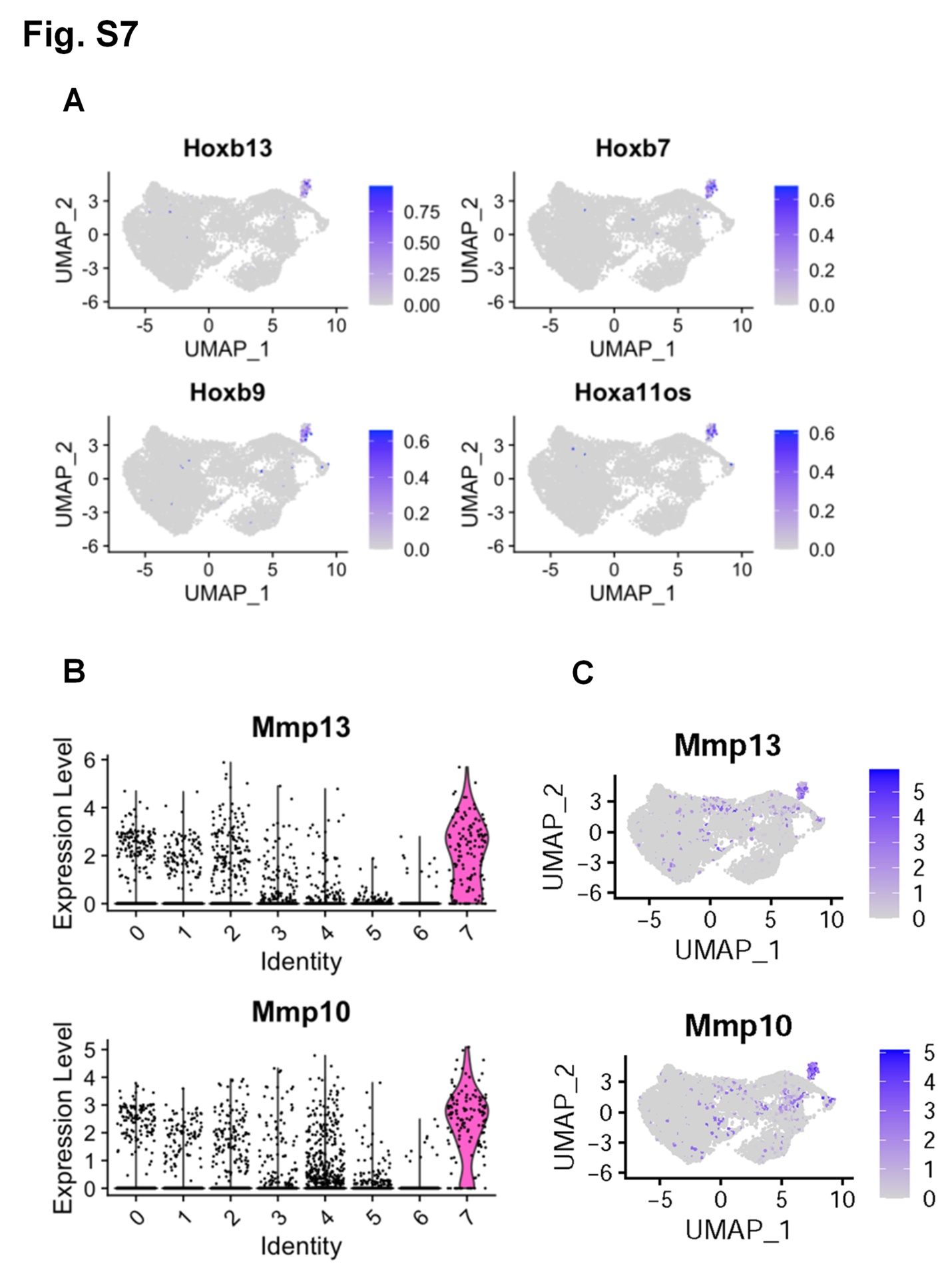
**Figure S4. Expression distribution for selected mouse MSCs-specific genes. (A)** UMAP projections (integrated assay) showing the distribution of UMI counts, and the five MSC surface markers genes observed in IF staining in Figure 2D across the dataset (negative for *CD45 (Ptprc)* gene).

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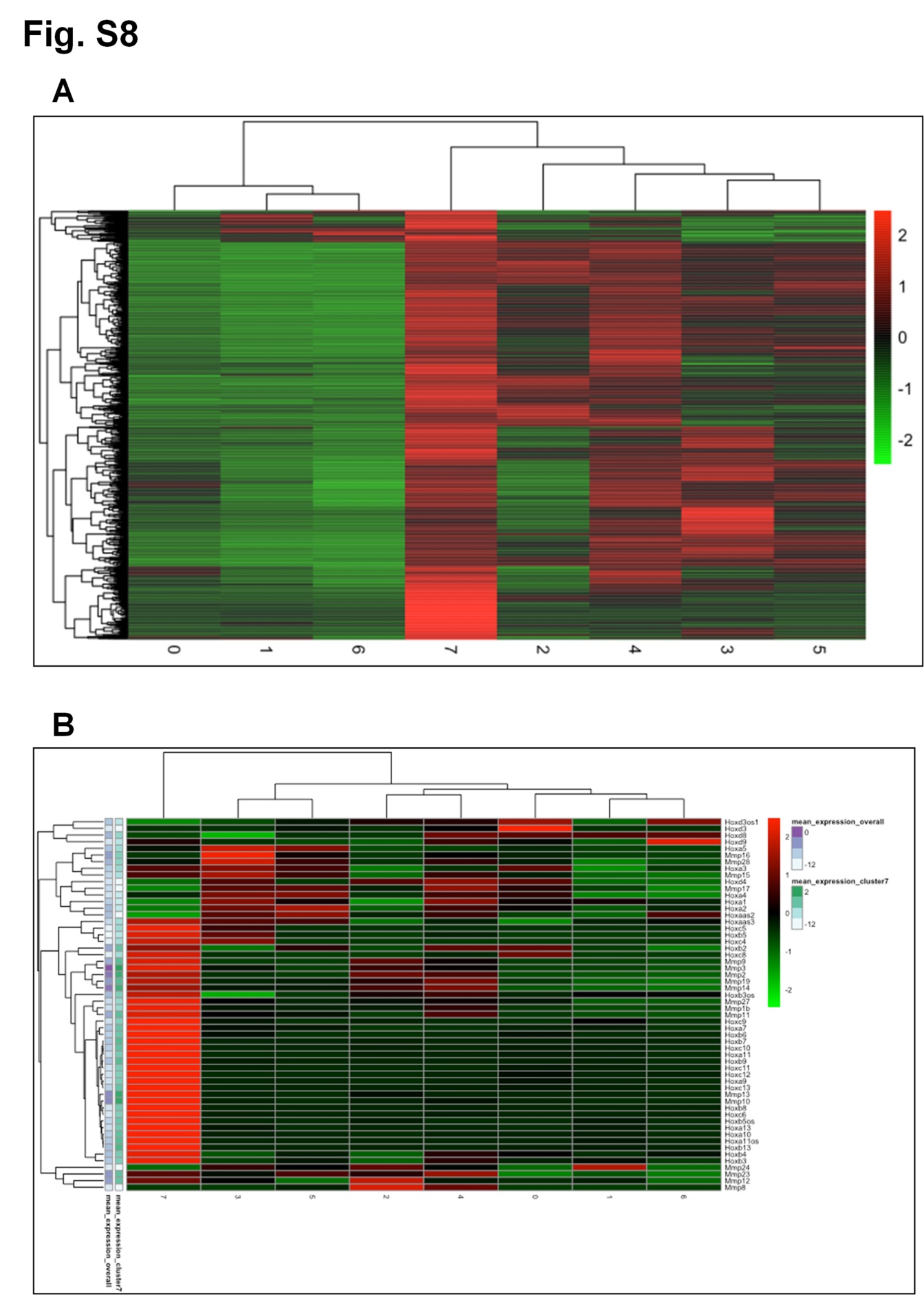
**Figure S5. Expression distribution of selected ECM genes in monolayer and spheroid cells. (A)** Violin plot illustrating expression of the six selected ECM genes in monolayer and spheroid cells across eight clusters. **(B)** Feature plots of expression distribution for six selected ECM genes with the lowest p-value in in the dataset. Expression levels for each cell are color-coded and overlaid onto the UMAP plot.

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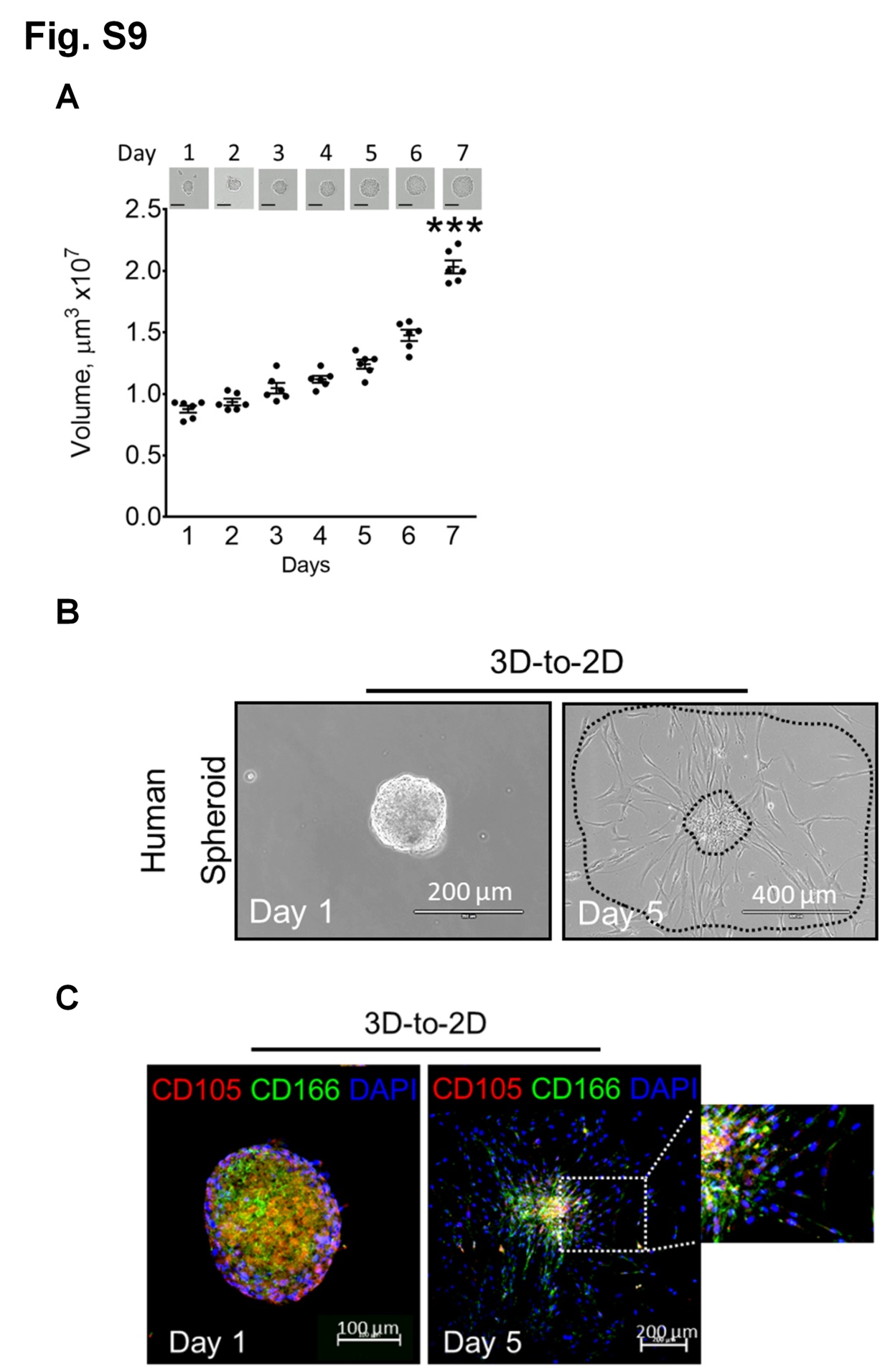
**Figure S6. Expression distribution for self-renewal genes in monolayer and spheroid cells. (A)** Violin plot showing expression of self-renewal genes *Notch2, Sox4, Sox9, Klf2*, and *Foxp1* across eight clusters. **(B)** Feature plots of expression distribution of self-renewal genes *Notch2, Sox4, Sox9, Klf2*, and *Foxp1*. Expression levels for each cell are color-coded and overlaid onto the UMAP plot. **(C)** Heatmap shows the average expression level of all collagen genes present in the dataset and selected self-renewal genes across clusters.

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**Figure S7. Feature plots of expression distribution for *Hox* and *Mmp* genes. (A)** UMAP visualization of expression distribution of selected *Hox* genes illustrating their higher expression in cluster 7. **(B)** Violin plot and (**C**) UMAP showing expression of *Mmp*13 and *Mmp*10 genes expressed more highly in cluster 7.

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**Figure S8. *Hox* and *Mmp* genes are highly expressed in cluster 7. (A)** Heatmaps showing average normalized expression per cluster of genes expressed at a significantly higher level (adjusted p-value < 0.05) in cluster 7 than in all other cells in the dataset, in which clusters 4 and 7 look much more similar to each other; and **(B)** All *Hox* and *Mmp* genes with measured expression in the dataset. Normalized counts per cell were averaged for each cluster, and values were scaled and centered row-wise to plot Z-scores for each gene; rows and columns were hierarchically clustered using complete linkage clustering with a Euclidian distance metric. Sidebars in **(B)** show the log2 average normalized expression of each gene across the whole dataset and specifically in the cells in cluster 7.



**Figure S9. Human dermal fibroblast-derived spheroids are proliferative. (A)** Quantification of spheroid volume using real-time cell imaging by IncuCyte® live-cell analysis system over a 7-day time course (n = 6). (B) A single sphere of human fibroblasts after seeding on a coverslip after 1 (left) and 5 days (right) in culture. (C) Co-immunofluorescence staining of CD105 (red) with another marker of human MSCs CD166 (green) in a single sphere after 1 (left panel) and 5 days in culture (right panel).