

1 Supplementary Figures

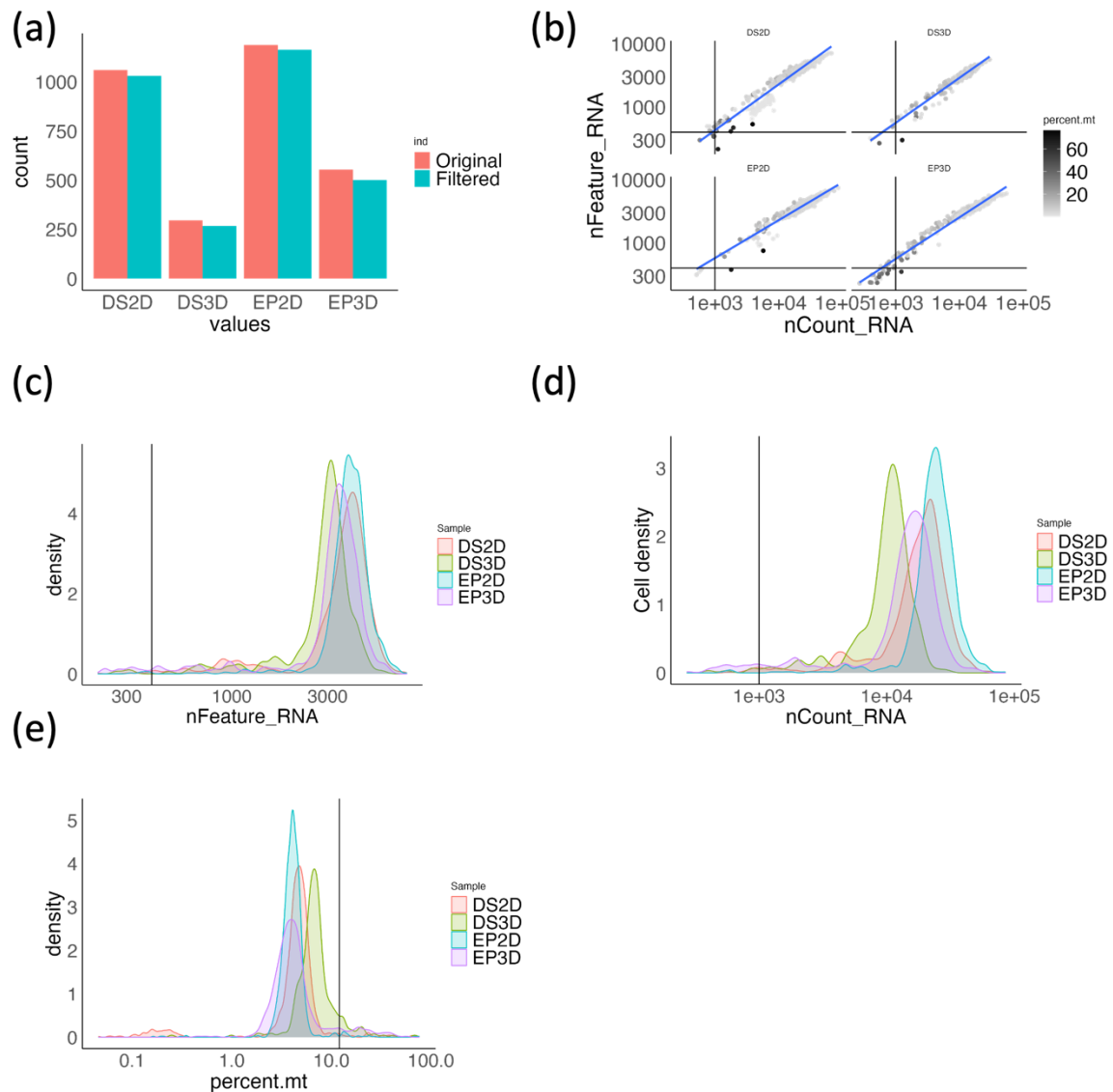


Fig S1. Quality control and filtering of scRNA-seq. (a) Number of cells sequenced before and after filtering. (b) Number of genes (nFeature_RNA) versus number of unique molecular identifiers (UMIs) (nCount_RNA), coloured by the percentage of mitochondrial RNA. (c) Density plot of nFeature_RNA for all samples. (d) Density plot of nCount_RNA for all samples. (e) Density plot of the percent.mt for all samples. The black line represents chosen filtering cut offs in all cases.

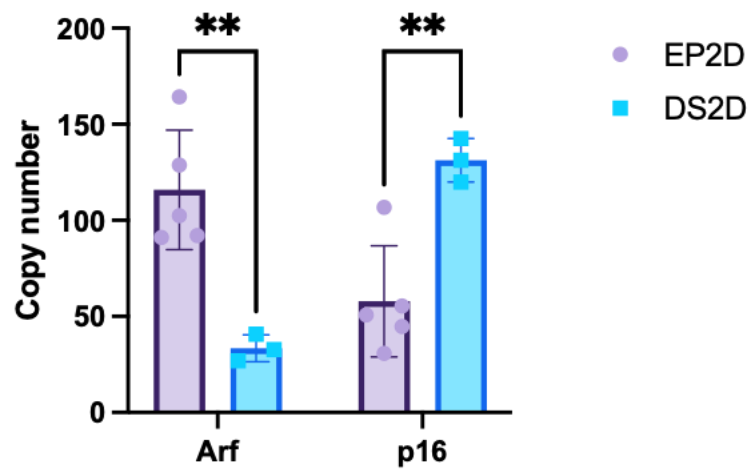


Fig S2. Digital PCR measuring ARF and p16 mRNA in Early Proliferative (EP) and Deeply Senescent (DS) Human Dermal Fibroblasts (HDFs). Copy number is normalised to 1ng/mL of cDNA loaded. Stats represents two-way ANOVA with Tukey's multiple comparison. N=5 EP, N=3 DS.

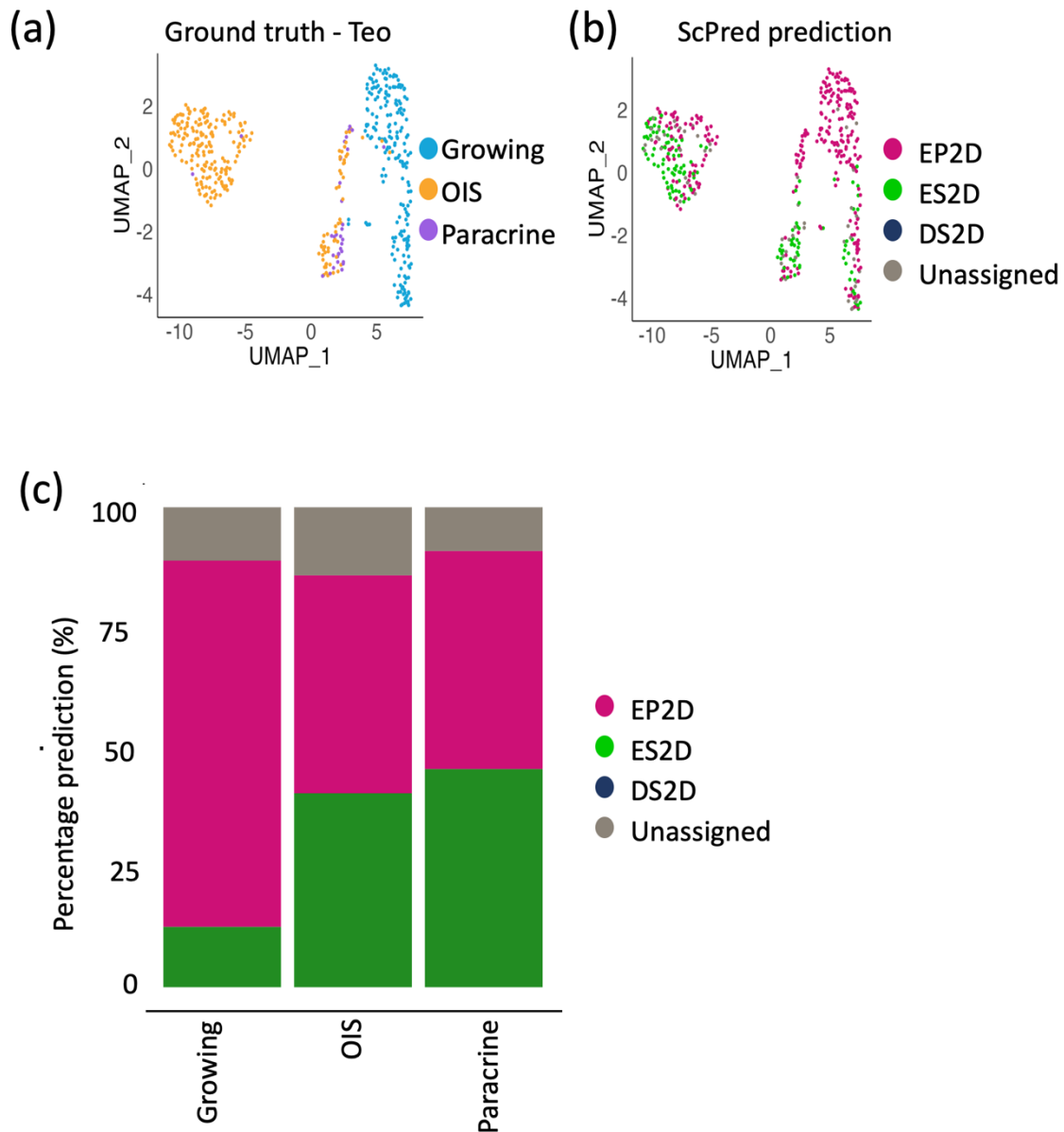
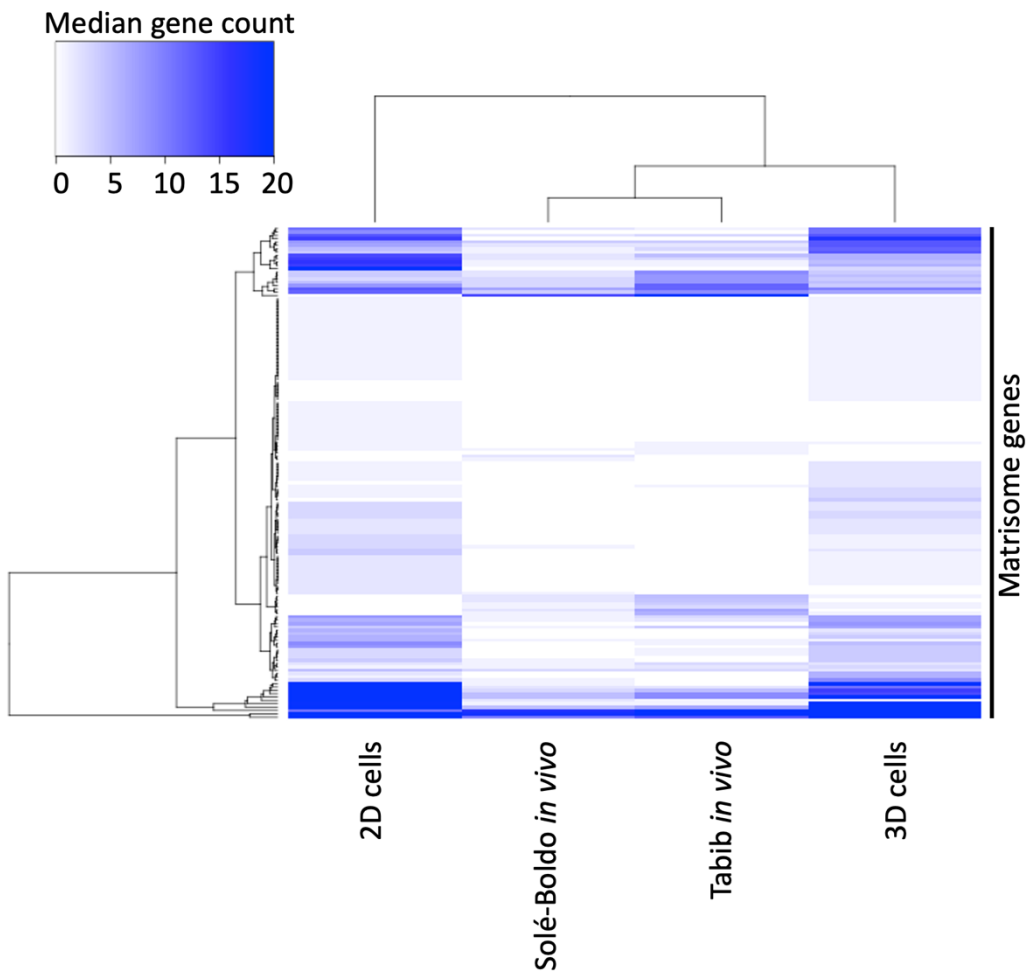
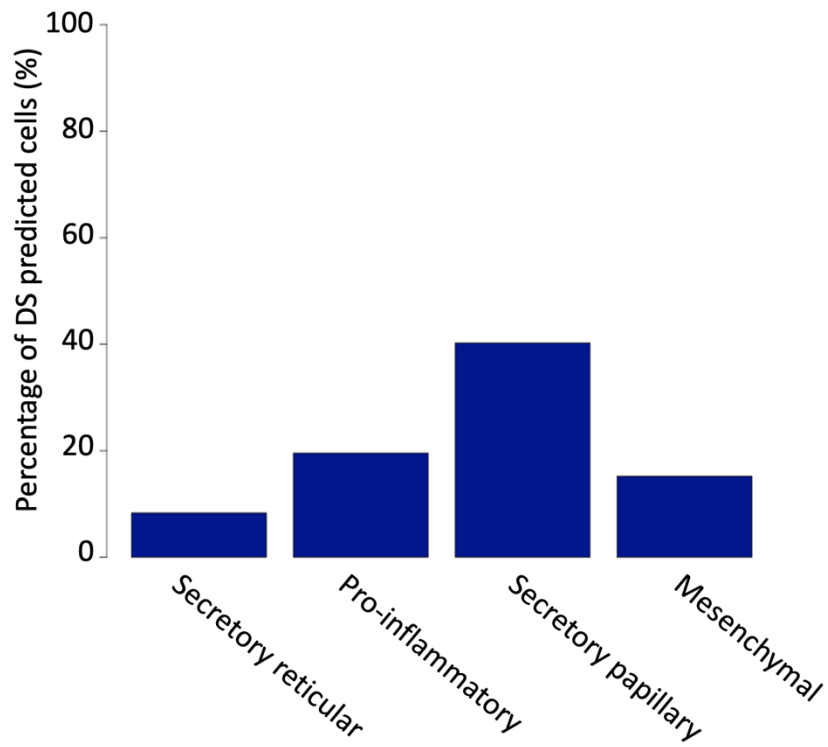


Fig S3. Applying 2D SenPred to paracrine and Oncogene Induced Senescent (OIS) cells detects early senescence. (a) UMAP of IMR90 fibroblasts from Teo et al.¹⁹, overlaid with senescence condition. (b) UMAP in (a), overlaid with SenPred prediction. (c) Percentage of predicted Early Proliferative 2D (EP2D), Early Senescent 2D (ES2D), Deeply Senescent 2D (DS2D) and Unassigned cells in each senescence condition.



17 **Fig S4. Clustering of matrisome gene expression reveals that fibroblasts grown in 3D are more**
 18 **similar to *in vivo* fibroblasts from Tabib²⁰ and Solé-Boldo²¹ datasets, than fibroblasts grown in 2D.**
 19 Heatmap displays median log-normalised gene counts for each condition, for matrisome genes listed
 20 in Supplementary List 1.



21 Fig S5. Barplot showing percentage of predicted deeply senescent cells in dataset from Solé-Boldo
22 *et al.*²¹ for each fibroblast subtype.

Supplementary Figures and Tables

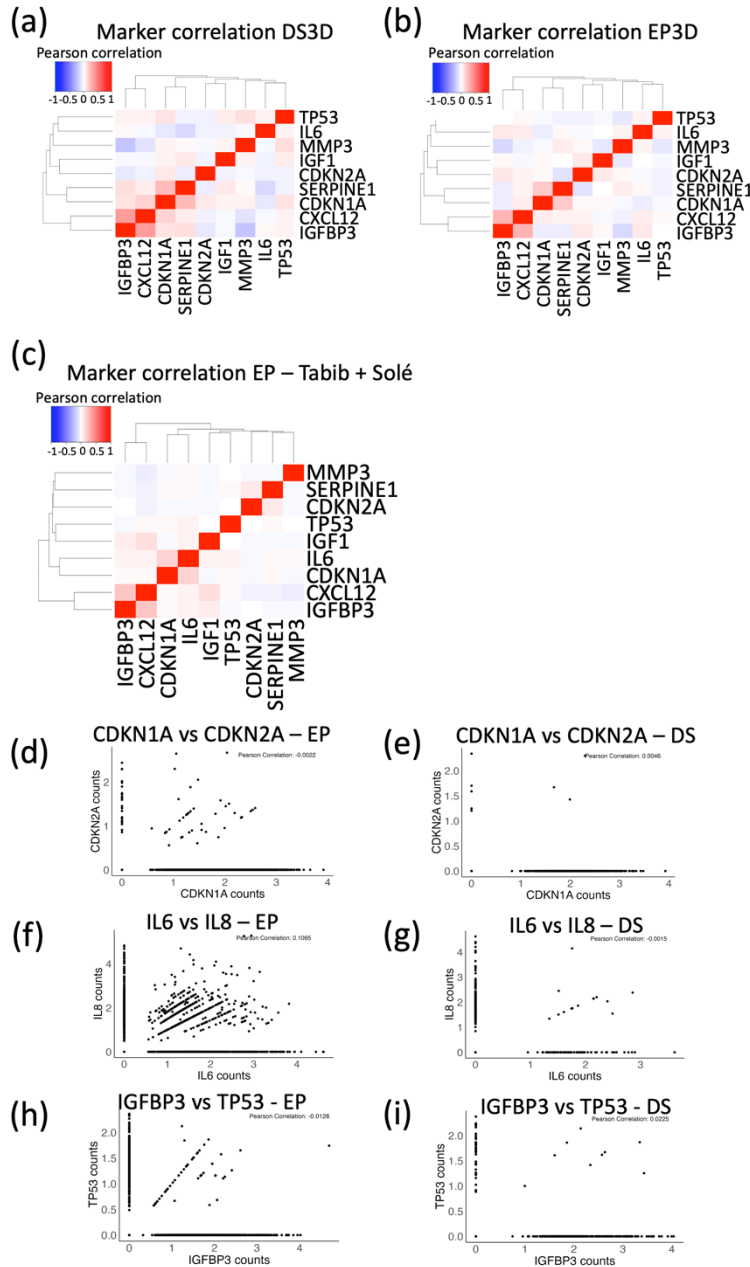
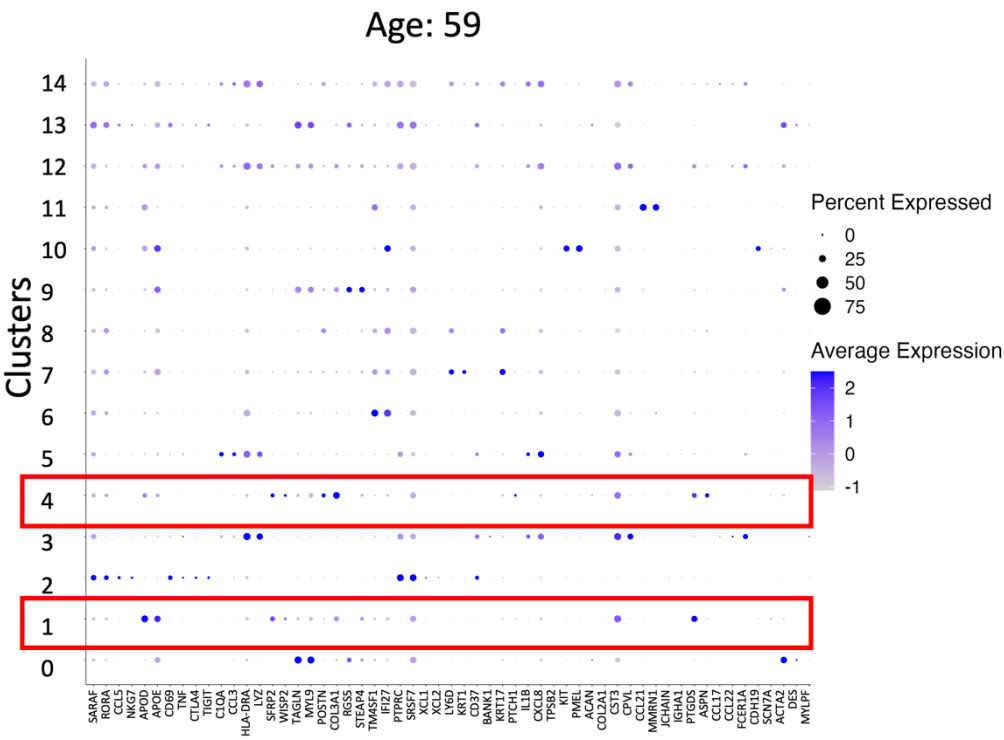
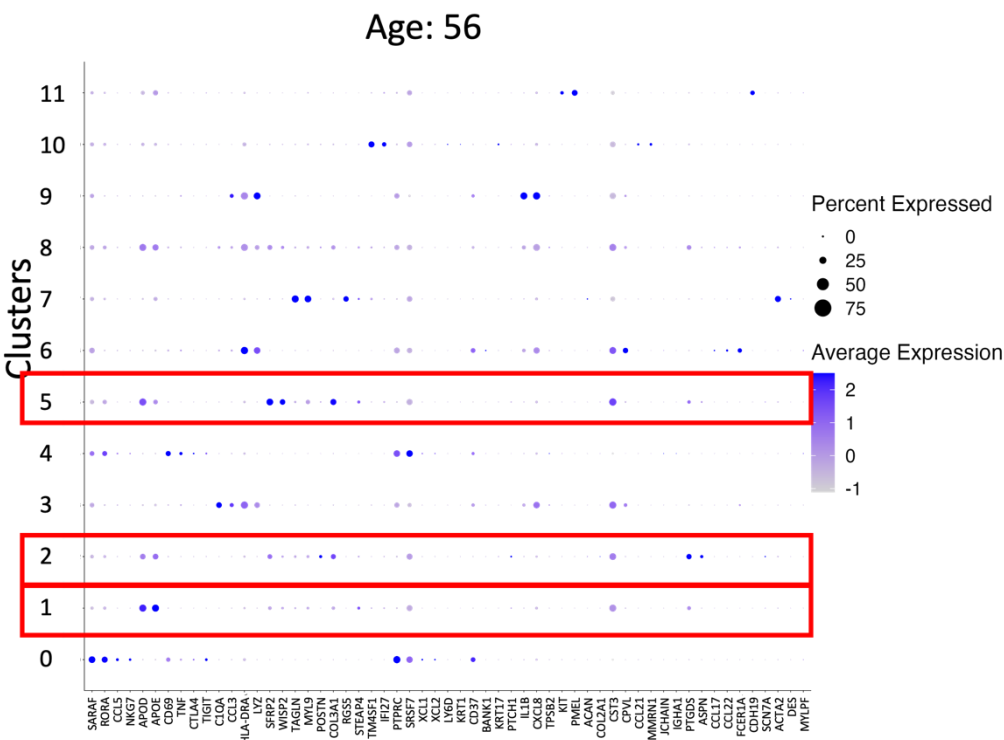
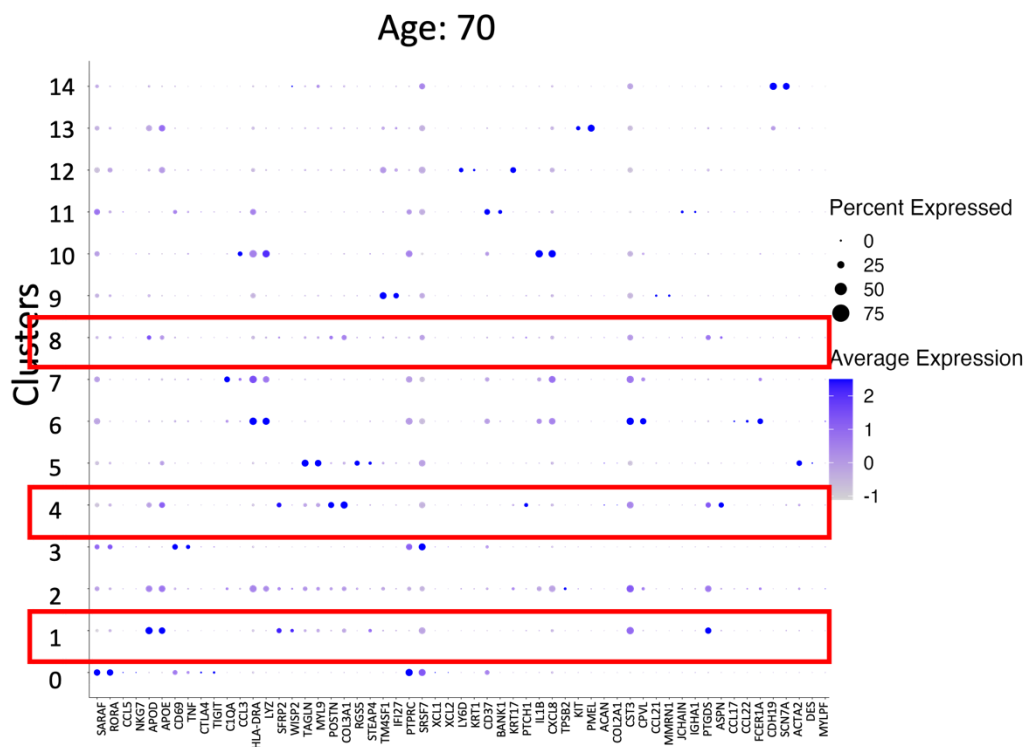
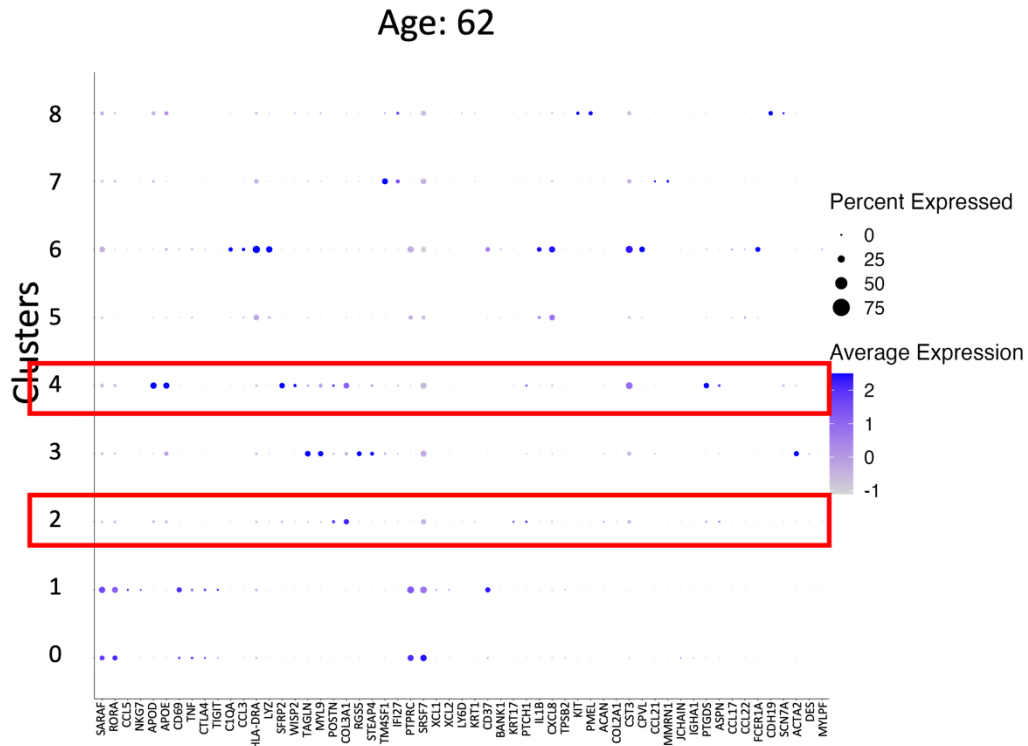


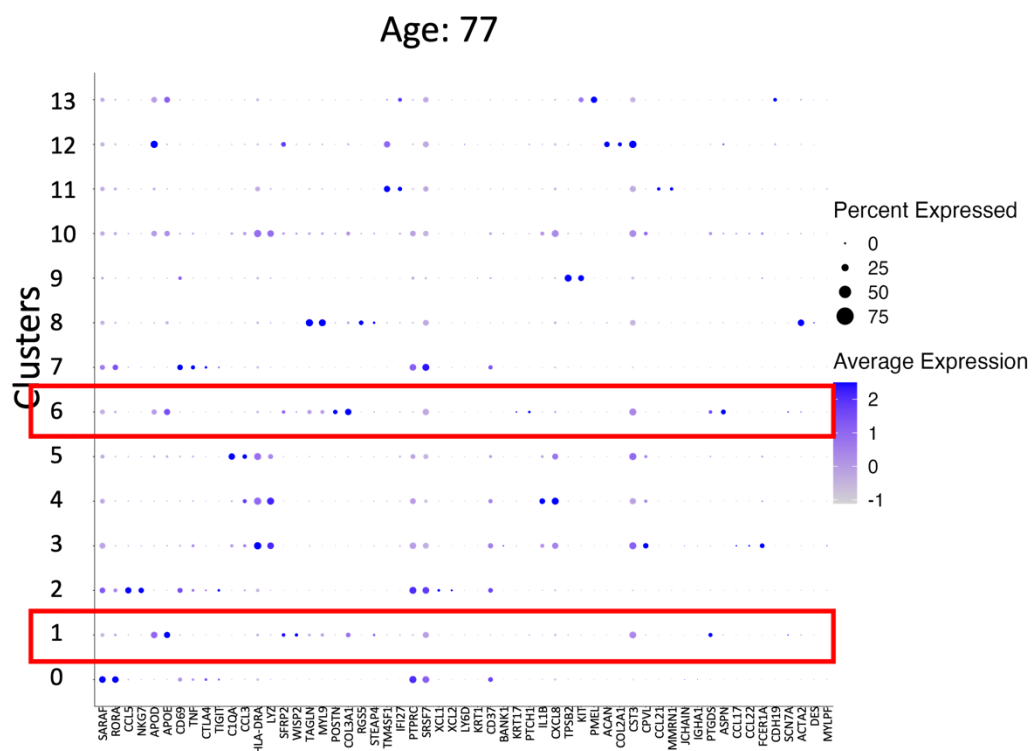
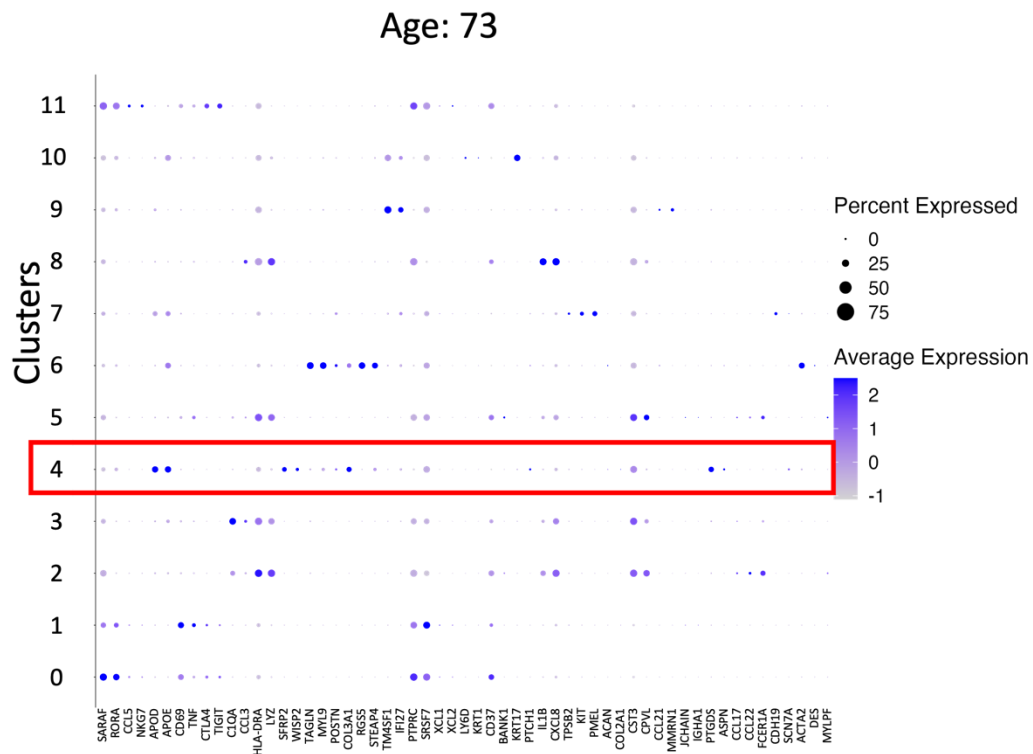
Fig S6. Pearson correlation of selected senescence markers in both Early Proliferative (EP) and Deeply Senescent (DS) cells in the 3D data, and Early Proliferative predicted cells in the Tabib²⁰ and Solé-Boldo²¹ datasets. (a) Pearson correlation matrix of normalised gene counts for a selected list of classic senescence markers, in the DS3D cells which have a state of ground truth. (b) Pearson correlation matrix of normalised gene counts for a selected list of classic senescence markers, in the EP3D cells which have a state of ground truth. (c) Pearson correlation matrix of normalised gene counts for a selected list of classic senescence markers, in the EP predicted cells from Tabib20 and Solé-Boldo21. (b) Scatterplot of CDKN1A and CDKN2A counts of EP cells from Tabib20 and Solé-Boldo21 datasets. (c) of Scatterplot CDKN1A and CDKN2A counts of DS cells from Tabib20 and Solé-Boldo21 datasets. (d) Scatterplot of IL6 and IL8 counts of EP cells from Tabib20 and Solé-Boldo21 datasets. (e) Scatterplot of IL6 and IL8 counts of DS cells from Tabib20 and Solé-Boldo21 datasets. (f)

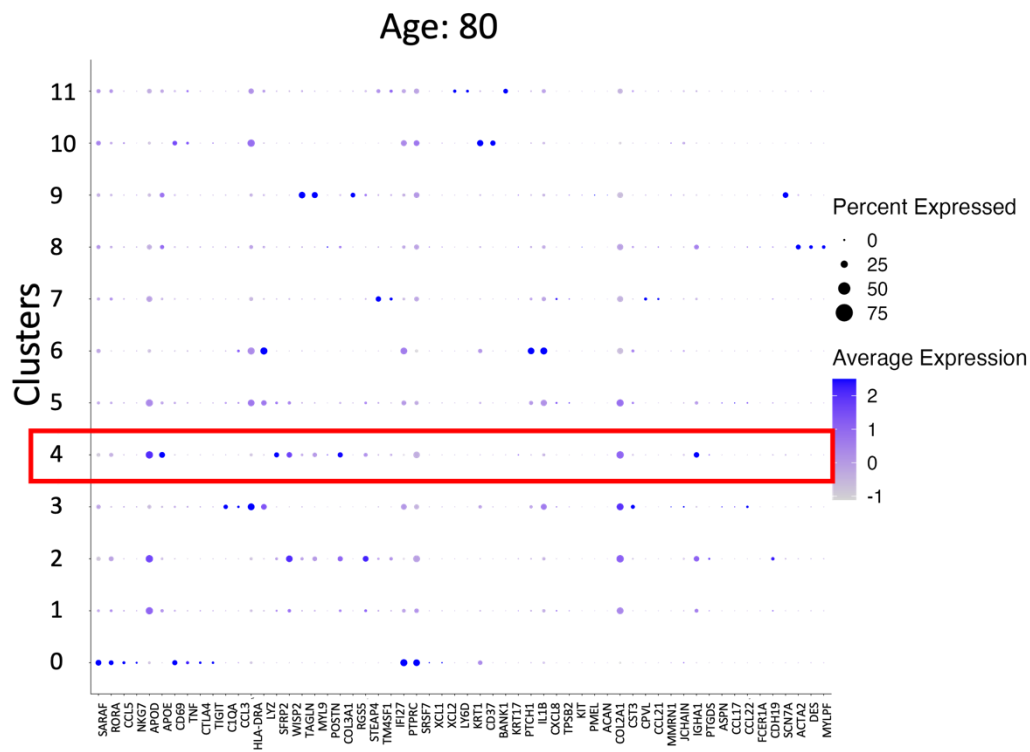
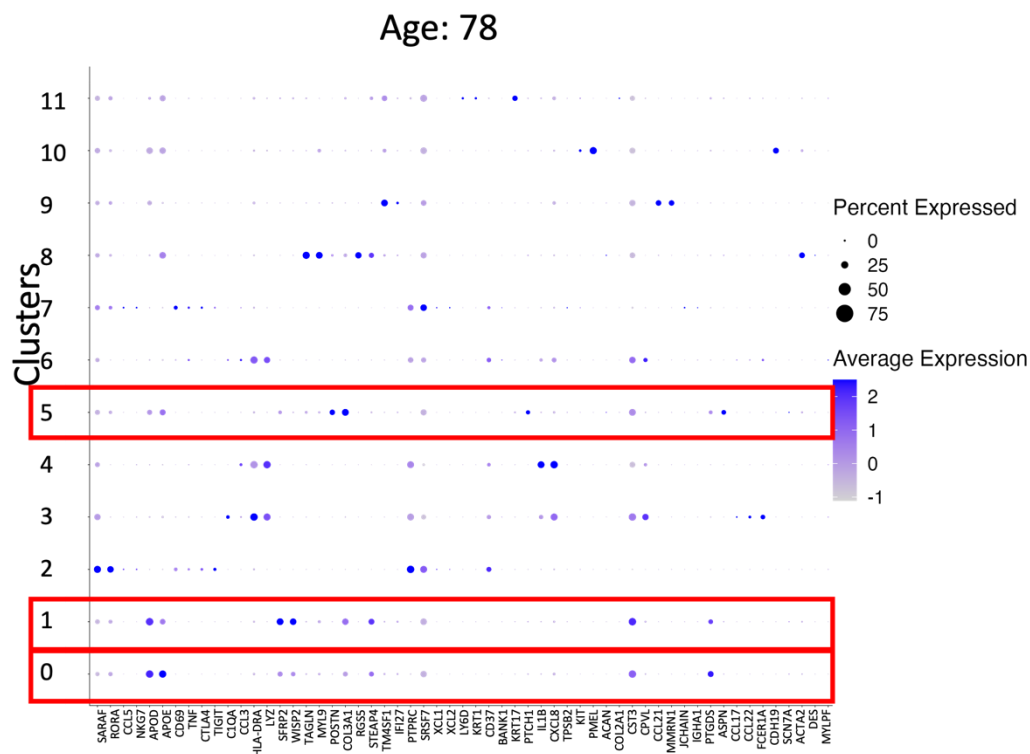
Supplementary Figures and Tables

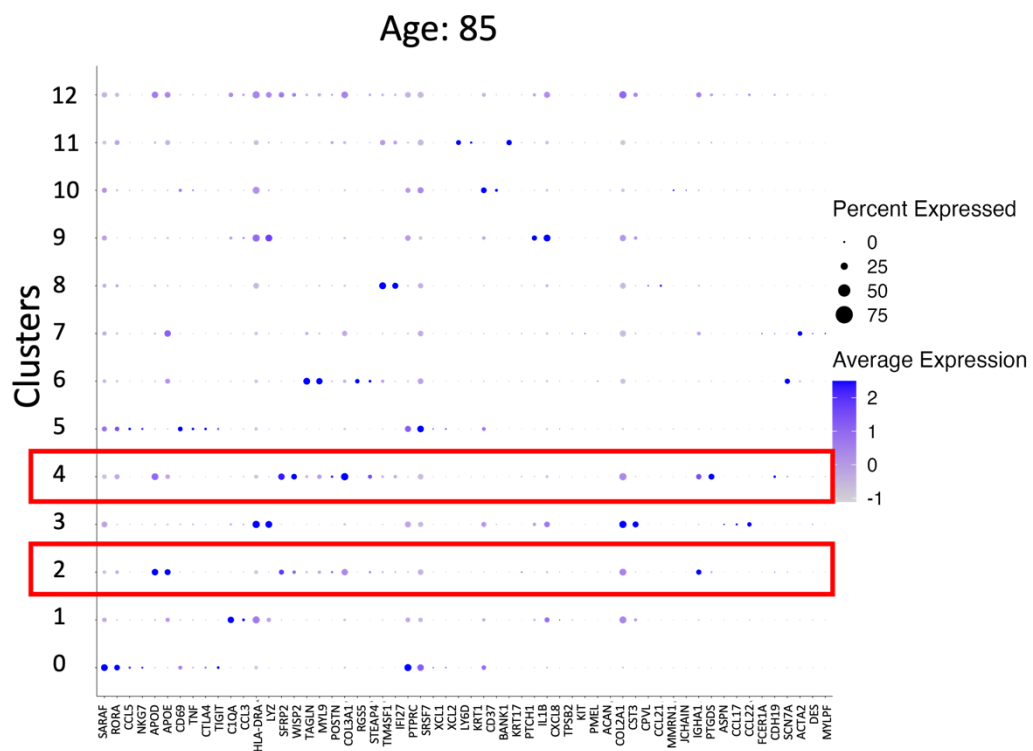
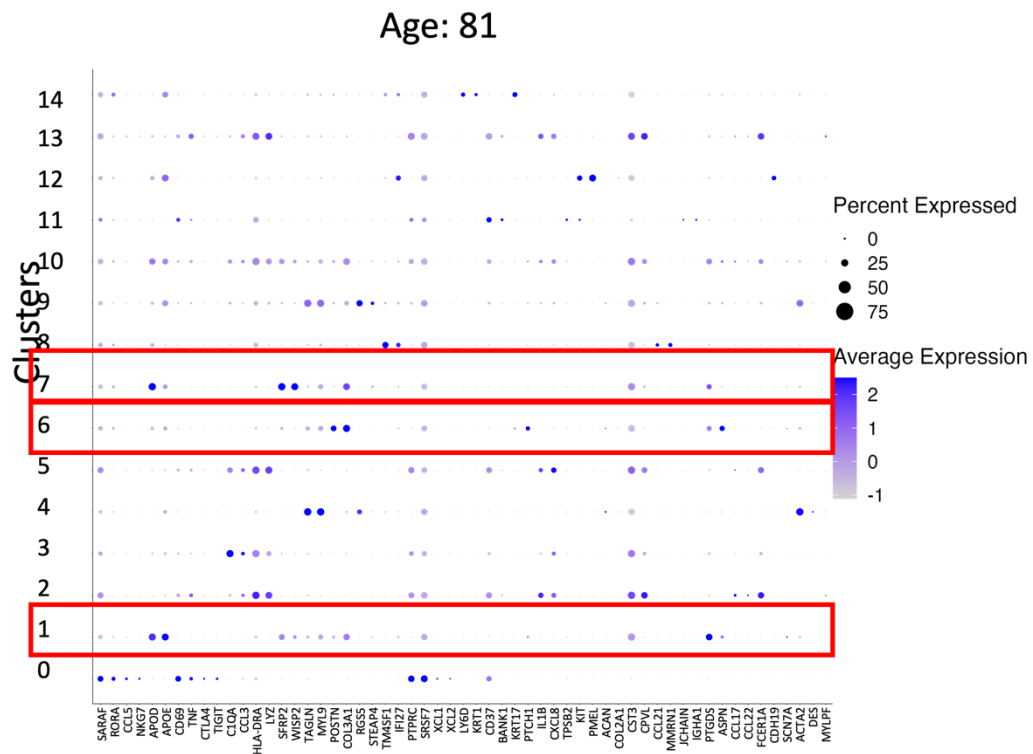
- 34 Scatterplot of IGFBP3 and TP53 counts of EP cells from Tabib20 and Solé-Boldo21 datasets. (g)
- 35 Scatterplot of IGFBP3 and TP53 counts of DS cells from Tabib20 and Solé-Boldo21 datasets.
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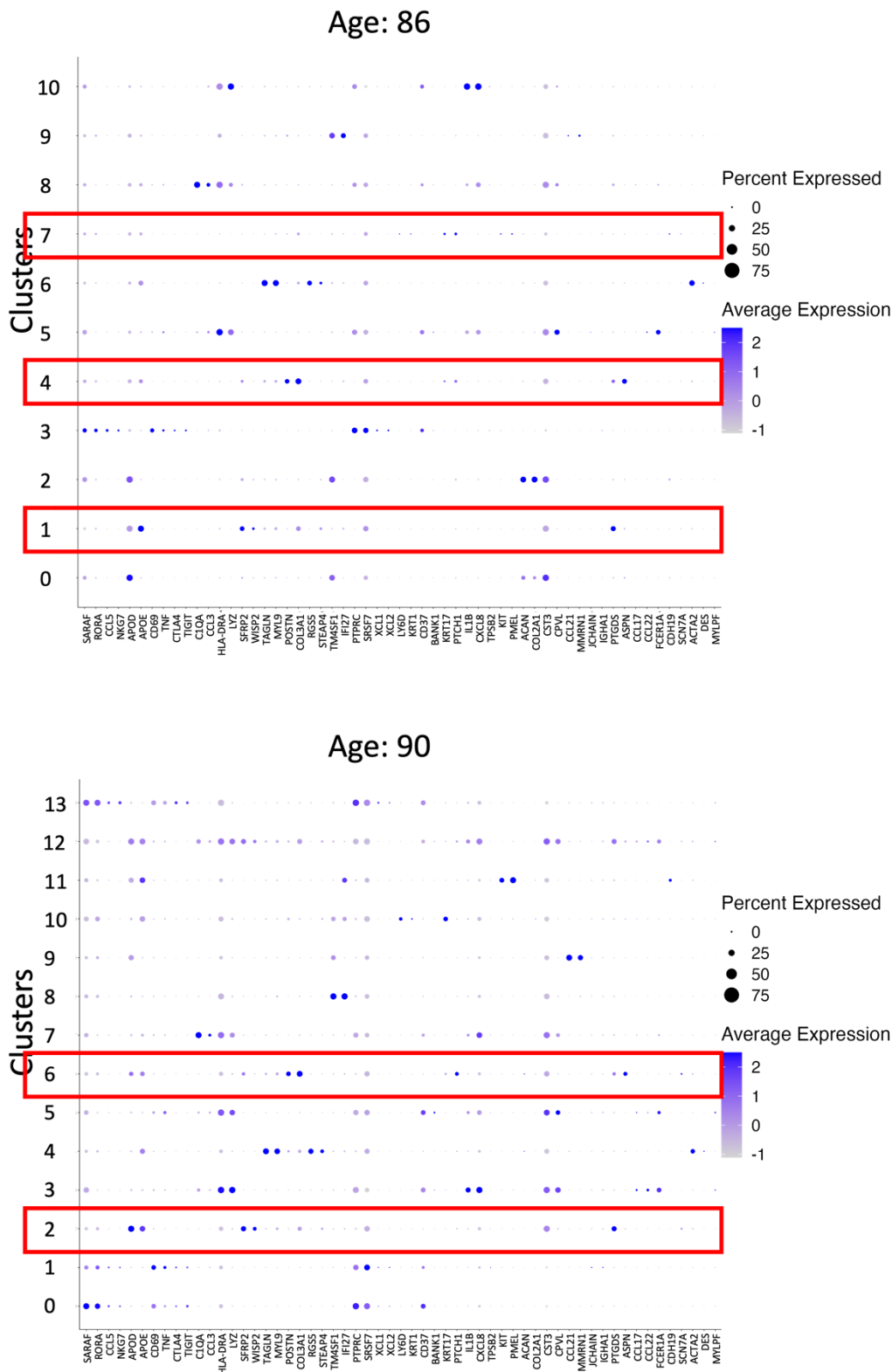


Fig S7. Dotplots of individual donor clusters from Ganier *et al.*³⁰ to identify and filter fibroblasts. Colour indicates mean normalised gene counts for each cluster, and the size of the dot indicates the percentage of cells within the cluster which express the gene.

Supplementary Figures and Tables

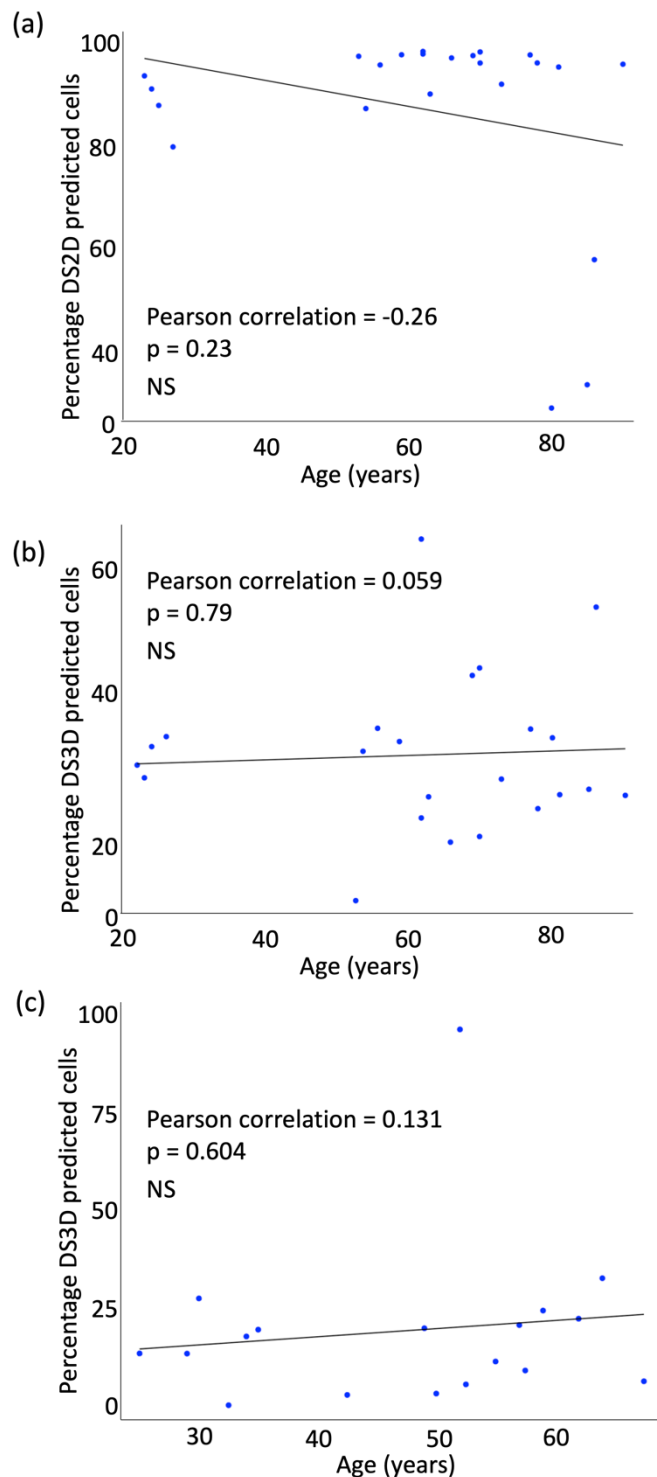


Fig S8. Scatterplots testing SenPred on dermal fibroblasts, whole skin, and lung fibroblasts. (a) Percentage of 2D SenPred senescent predicted dermal fibroblasts in Tabib²⁰, Solé-Boldo²¹, and Ganier³⁰ datasets. (b) Percentage of 3D SenPred senescent predicted skin cells in Tabib²⁰, Solé-Boldo²¹, and Ganier³⁰ datasets. (c) Percentage of 3D SenPred senescent predicted lung fibroblast cells from Sikkema *et al.*³¹

46 **Table S1. Evaluation metrics for machine learning models built using 2D Early Proliferative (EP) and**
47 **Deeply Senescent (DS) Human Dermal Fibroblasts (HDFs).**

Model	ROC	Sensitivity	Specificity
Mixture Discriminant Analysis (MDA)	1	0.999	1
<i>Support Vector Machine (SVM)</i>	<i>1</i>	<i>0.999</i>	<i>0.99</i>
K-Nearest Neighbours (KNN)	0.998	0.978	0.992
Generalised Linear Model (GLM)	0.999	0.996	0.994

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