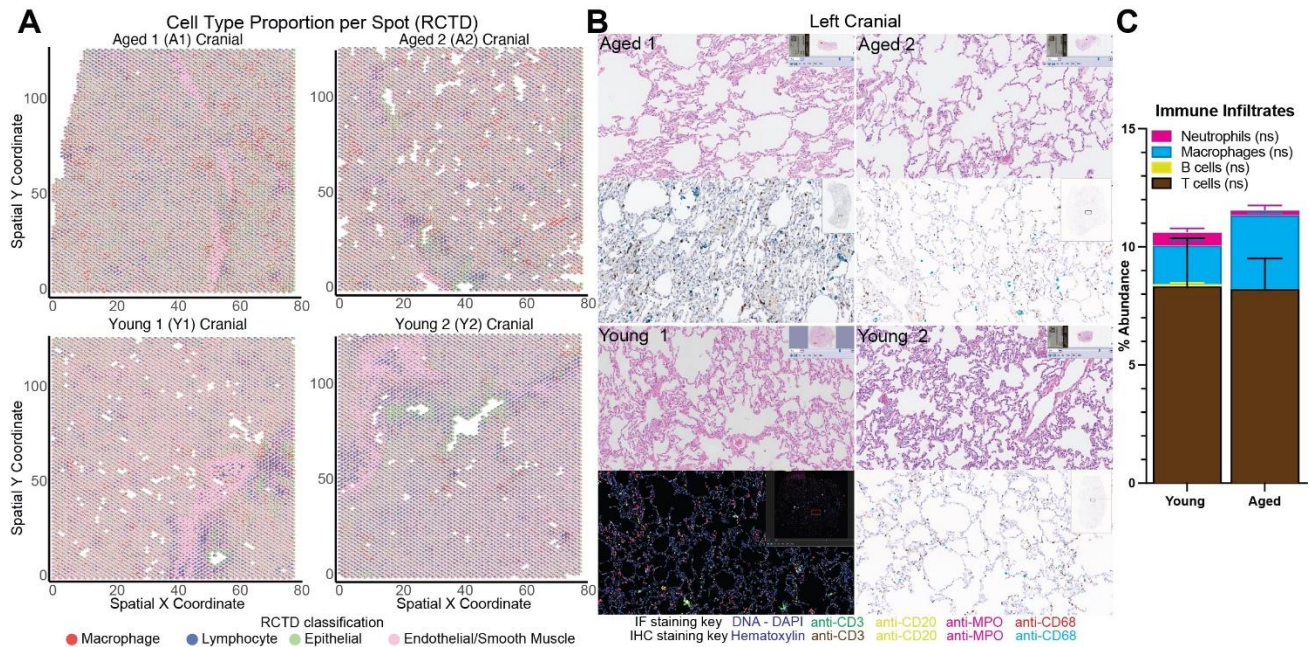


SUPPLEMENTARY DATA

Spatial Transcriptomic Analysis Reveals Dysregulated Pro-Inflammatory Signaling in the Aged Lung

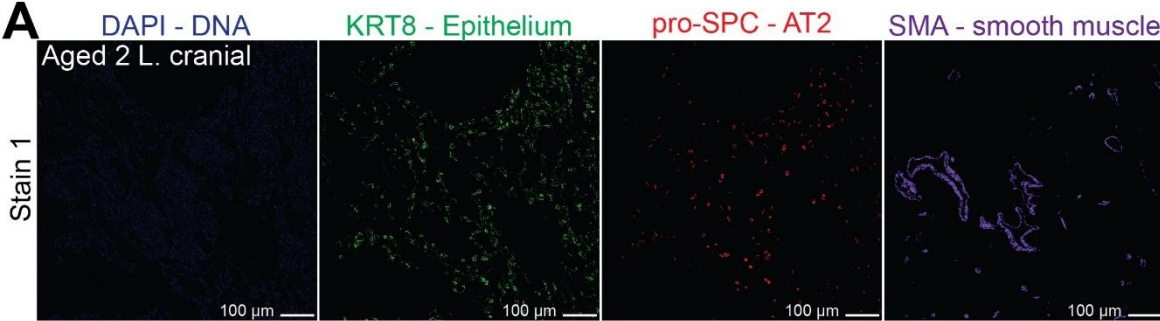
**Brianna M. Doratt, Ethan G. Napier, Mahdi Eskandarian Boroujeni, Michael H. Davies,
Christine F. Brainson, Eliot R. Spindel, Ilhem Messaoudi**

SUPPLEMENTARY DATA

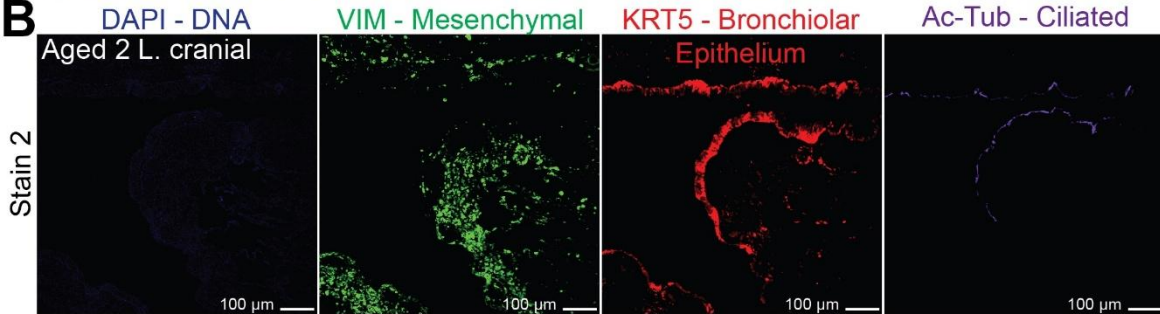
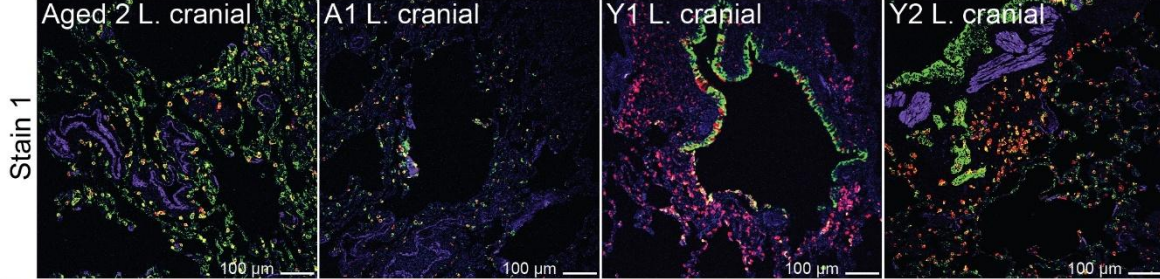


Supplementary Figure 1. Histological analysis of lung samples shows comparable abundance of immune cells in young and aged animals. (A) Pie scatter plot of deconvolution analysis where each pie represents the frequency of the indicated cell type in the spot on the Visium assay. (B) Representative H&E and immunostaining of left cranial lobes at 5x magnification of Aged 1, Aged 2, Young 1, and Young 2. Immunofluorescence was done for a general DAPI stain (Blue), anti-CD3 for T-cell identification (Green), anti-CD20 for B-cell identification (Yellow), anti-MPO for neutrophil identification (Pink), and anti-CD68 for identification of macrophages and monocytes (Red). Immunohistochemistry staining incorporated hematoxylin for a general DNA stain (Blue), anti-CD3 for T-cell identification (Brown), anti-CD20 for B-cell identification (Yellow), anti-MPO for neutrophil identification (Pink), and anti-CD68 for identification of macrophages and monocytes (Cyan). (C) The average percent abundance, as determined by HALO of T-cells, macrophages, B cells, and neutrophils detected in the left lungs grouped by young and aged. 1 lung section from each animal was used (n=2 aged, n=2 young). Multiple unpaired, nonparametric, Mann-Whitney t tests with FDR correction were performed between the young and aged data. Error bars represent SEM.

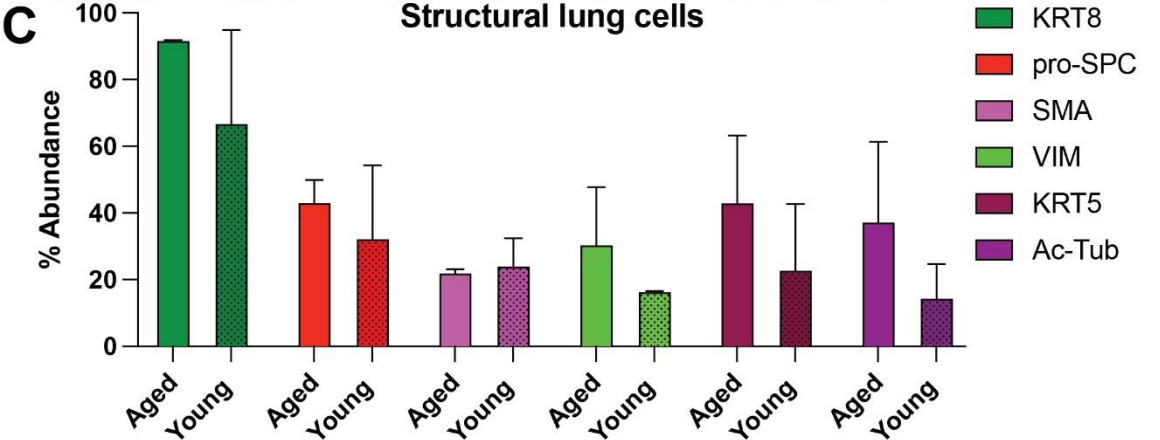
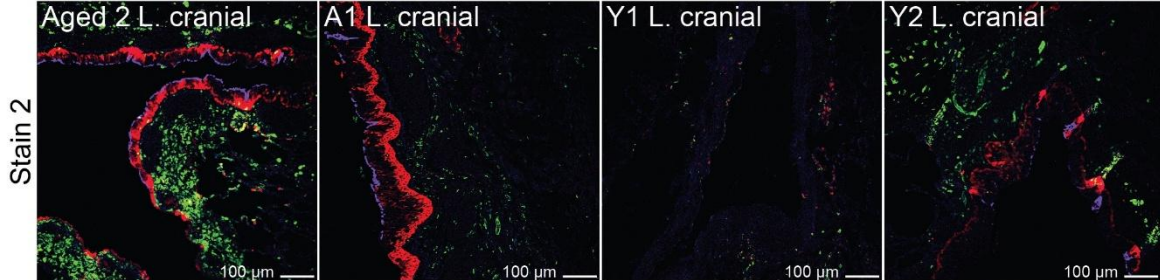
SUPPLEMENTARY DATA



Merge

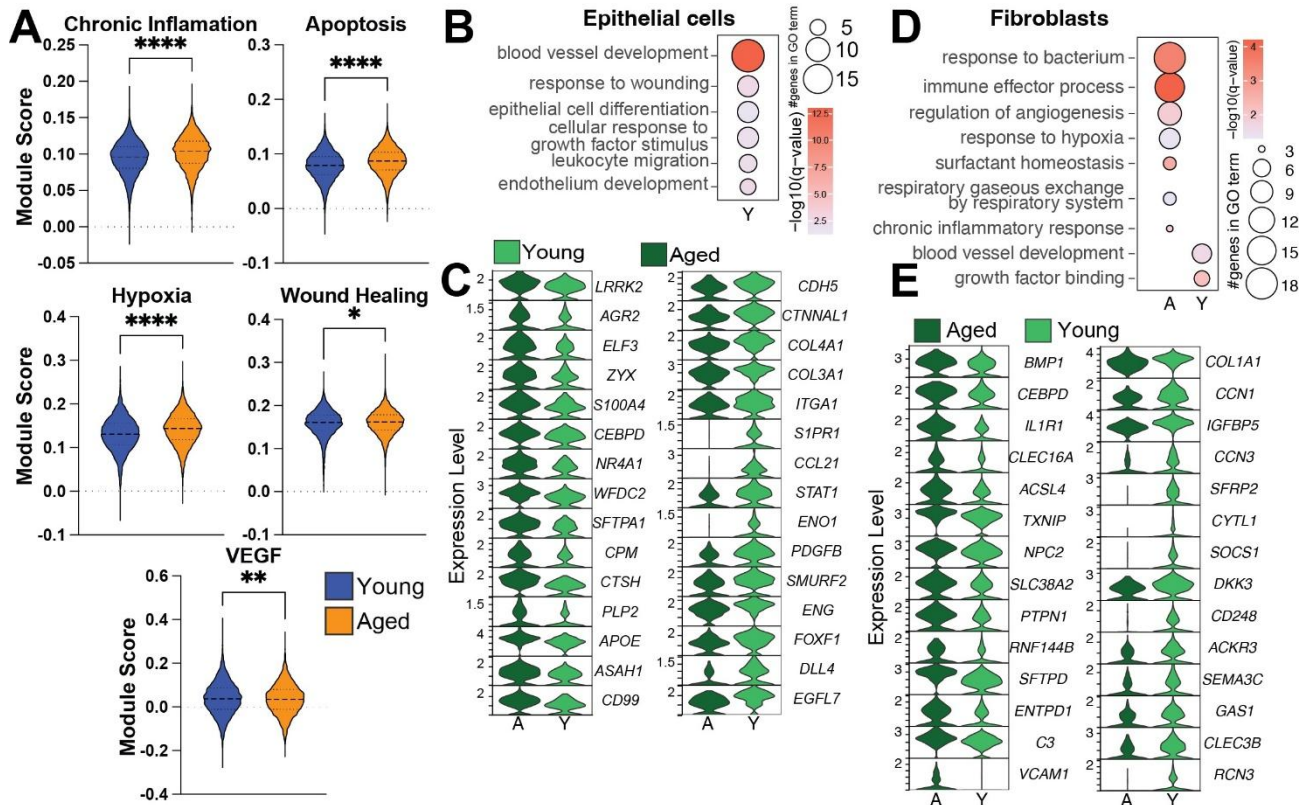


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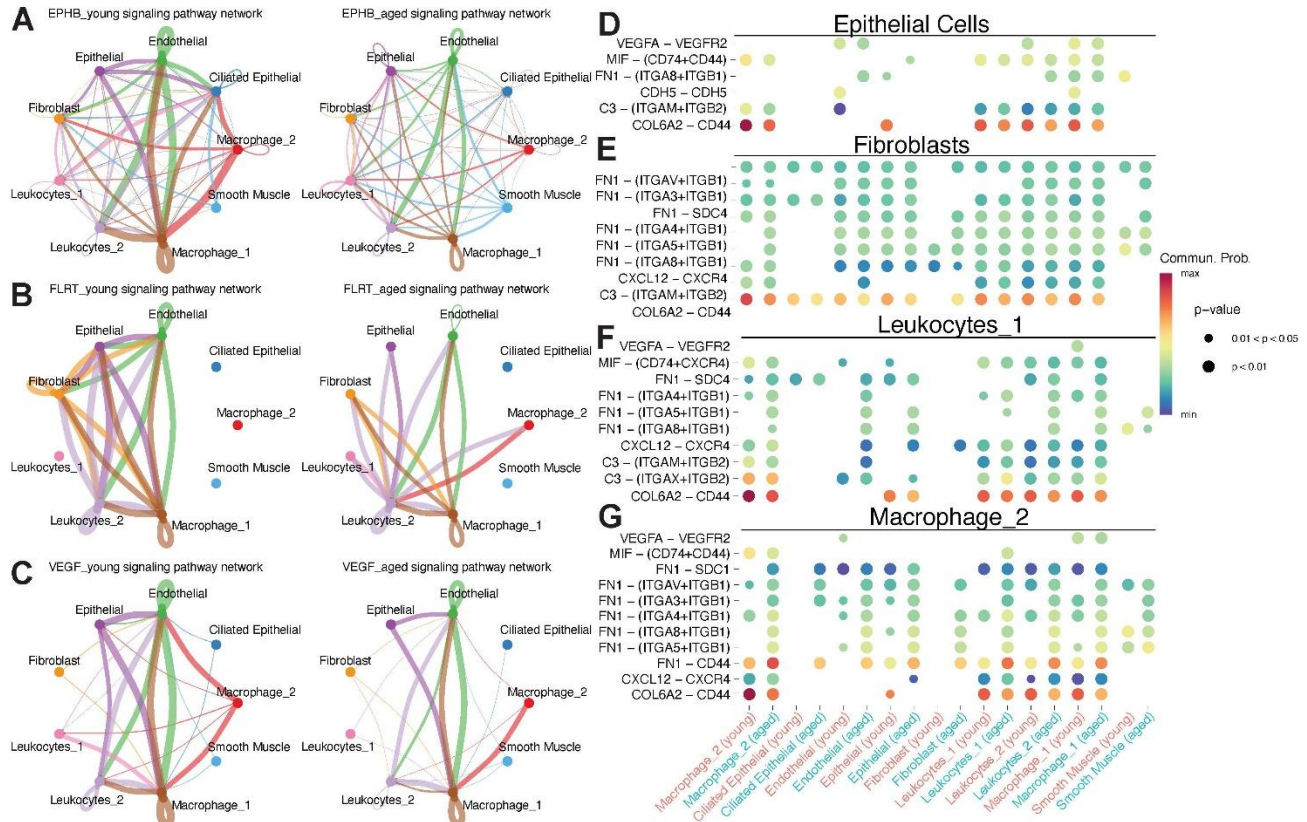
SUPPLEMENTARY DATA

Supplementary Figure 2. Histological analysis shows comparable distribution of canonical structural cells. (A) Immunofluorescence of the indicated lung tissue at 10x magnification for a general DAPI stain (Blue), anti-KRT8 for club cell identification (Green), anti-pro-SPC for alveolar type 2 cells (Red), and anti-SMA for smooth muscle cell identification (Purple). (B) Immunofluorescence for a general DAPI stain (Blue), anti-VIM for mesenchymal cell identification (Green), anti-KRT5 for epithelial cells (Red), and anti-Ac-Tub for ciliated epithelial cell identification (Purple). (C) The average percent abundance, as determined by HALO of KRT8, pro-SPC, SMA, VIM, KRT5, or Ac-Tub expressing cells detected in the left lungs grouped by young and aged. 1 lung section from each animal was used (n=2 aged, n=2 young). Multiple unpaired, nonparametric, Mann-Whitney t tests with FDR correction were performed between the young and aged data. Error bars represent SEM.



Supplementary Figure 3. Epithelial cells and fibroblasts are hyperinflammatory in aged lungs. (A) Violin plots of chronic inflammation, apoptosis, hypoxia, wound healing, and VEGF module scores across all cell clusters. 575 cells were captured for the aged group (n=2) and 752 cells were captured for the young group (n=2). Unpaired, nonparametric, two-tailed, Mann-Whitney t tests were performed between the young and aged data. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Bubble plots representing Gene Ontology (GO) terms and violin plots depicting differentially expressed genes (DEG) between the left lungs from young and aged animals in (B, C) epithelial cells, or (D, E) fibroblasts. For bubble plots, the size of the bubble indicates the number of genes that enriched to that GO term and color indicates the significance compared to aged samples.

SUPPLEMENTARY DATA



Supplementary Figure 4. Disrupted network signaling and ligand-receptor pair interactions with age. Network signaling diagrams for the (A) EPHB, (B) FLRT, and (C) VEGF signaling pathways among cell subsets in the young group (on left) and the aged group (on right). Nodes represent cell subsets and edge width is proportional to the CellChat communication probability. Bubble plot of differential ligand-receptor pair signaling in young or aged lungs from (D) the epithelial, (E) fibroblast, (F) leukocytes_1, and (G) macrophage_2 cells to the other indicated cell types. Color indicates the communication probability and size indicates the p-value.

SUPPLEMENTAL TABLES

Supplementary Table 1. Metadata of lung tissue.					
ID	Side	Lobe	RIN	DV200	Clinical Characteristics
A1	Left	Cranial	2.5	54%	Unremarkable
A2	Left	Cranial	2.4	42%	Unremarkable
Y1	Left	Cranial	2.3	33%	Unremarkable
Y2	Left	Cranial	2.2	54%	Unremarkable

SUPPLEMENTARY DATA

Supplementary Table 4: Predicted counts and weights of each cell type from young and aged lung cell chat data.

Aged Count									
	Macrophage_2	Ciliated Epithelial	Endothelial	Epithelial	Fibroblast	Leukocytes_1	Leukocytes_2	Macrophage_1	Smooth Muscle
Macrophage_2	204	170	88	159	155	225	282	186	154
Ciliated Epithelial	243	247	163	229	205	272	336	256	197
Endothelial	125	124	75	173	85	144	319	266	127
Epithelial	219	200	158	246	159	228	358	298	185
Fibroblast	216	200	146	217	179	243	314	240	171
Leukocytes_1	306	279	210	284	236	343	411	331	227
Leukocytes_2	355	323	335	401	284	428	571	505	263
Macrophage_1	251	228	240	330	193	322	512	426	215
Smooth Muscle	194	169	154	205	160	220	291	239	158
Aged Weight									
	Macrophage_2	Ciliated Epithelial	Endothelial	Epithelial	Fibroblast	Leukocytes_1	Leukocytes_2	Macrophage_1	Smooth Muscle
Macrophage_2	5.842647011	2.243961156	2.279876767	4.082584216	2.462606244	4.793071049	5.531535988	5.071641431	3.856036507
Ciliated Epithelial	4.562977081	2.171185367	2.23980854	3.545275334	1.927577743	3.885097934	4.555629245	4.045033209	3.008812994
Endothelial	4.139414202	1.174610718	1.605782984	3.204746212	1.173565198	3.719110971	6.086607593	6.090162876	3.323245965
Epithelial	6.023980726	2.664524019	4.132695618	6.360538793	2.272344373	5.307932421	8.267205756	8.007268653	4.035858259
Fibroblast	7.461645393	4.348497735	5.206448143	6.818441533	4.722417735	6.918146508	8.001214212	7.19330411	5.417461573
Leukocytes_1	6.115765244	2.849467892	3.597184218	5.498710654	2.275447779	5.100606777	6.509548468	5.946780993	3.934447078
Leukocytes_2	7.541004366	3.912455325	6.484703523	8.529102546	3.548142879	7.306159243	10.43662721	10.425674	5.031858509
Macrophage_1	5.761067188	2.121022216	4.0073092	5.584777381	2.280393432	4.805344187	7.915334266	7.956017748	4.641246893
Smooth Muscle	6.928160124	3.945163996	5.099716656	6.783122101	4.14280472	6.720636388	7.986036951	7.566981348	5.018454928
Young Count									
	Macrophage_2	Ciliated Epithelial	Endothelial	Epithelial	Fibroblast	Leukocytes_1	Leukocytes_2	Macrophage_1	Smooth Muscle
Macrophage_2	165	169	86	123	195	148	161	152	128
Ciliated Epithelial	293	305	246	272	297	273	302	310	218
Endothelial	195	227	314	263	186	161	344	379	145
Epithelial	203	190	182	182	189	166	252	267	144
Fibroblast	347	359	332	339	354	315	382	379	240
Leukocytes_1	247	251	141	185	257	218	230	222	166
Leukocytes_2	278	256	304	295	244	227	377	384	164
Macrophage_1	240	240	300	271	208	194	349	373	156
Smooth Muscle	151	154	136	147	148	132	176	177	111
Young Weight									
	Macrophage_2	Ciliated Epithelial	Endothelial	Epithelial	Fibroblast	Leukocytes_1	Leukocytes_2	Macrophage_1	Smooth Muscle
Macrophage_2	5.422045804	2.20658527	1.74680462	2.695815747	1.621629005	2.667865687	2.842301106	3.339639196	3.098669824
Ciliated Epithelial	5.564920772	2.904337168	3.671643695	4.670079849	2.168857742	4.122648192	5.05252699	5.209266099	3.740988988
Endothelial	6.905616148	3.148592983	7.965839001	7.457915591	2.017919818	4.589224908	8.858998067	10.28578396	5.069862986
Epithelial	7.351976995	2.67092353	5.685509242	6.330472748	1.751667693	4.845808491	7.842005725	9.201713736	5.152417031
Fibroblast	8.213823098	4.776350622	7.272796539	7.977467952	4.071708231	6.038891542	8.530510027	8.613598813	5.306095452
Leukocytes_1	6.119829657	2.381142535	1.337423537	3.529259073	1.626243739	3.781666705	4.53071126	5.005532893	3.963455571
Leukocytes_2	8.158772955	4.056276333	9.092000494	9.442918764	2.538704343	6.096093057	10.52565679	11.48397856	5.690801578
Macrophage_1	7.078060358	3.012674191	7.316904856	6.755319479	2.028465813	4.701123248	8.828560045	10.13632187	4.969322369
Smooth Muscle	6.354591822	3.753924916	5.654071038	6.30206302	2.658493922	4.507539155	6.806952127	6.775506247	4.4174365

Supplementary Table 6. Number of differentially expressed genes in each cell type from young and aged tissues

Cell type	Up in Young	Up in Aged
Ciliated Epithelial	13	122
Endothelial	101	93
Epithelial	67	47
Fibroblast	48	175
Leukocytes 1	1	5
Leukocytes 2	74	87
Macrophage 1	124	127
Macrophage 2	7	6
Smooth muscle	201	515