Methods for the Figures in Boxes 1 and 2

Single-cell RNA-seq count data resources: Single-cell count tables were downloaded from the following studies:

1. Tabula Muris Senis (Almanzar et al., 2020) (only droplet-based count data was used for the analysis)
2. Aging mouse brain (Ximerakis et al., 2019)
3. Aging mouse lung (Angelidis et al., 2019)
4. Aging mouse T cells (Elyahu et al., 2019)
5. Aging human pancreas (Enge et al., 2017)

Single-cell RNA-seq data processing: The count matrices and meta-data were imported into a Seurat object (Stuart et al., 2019) (without applying any filtering), and the Seurat workflow was executed, including normalisation, finding most variable features, scaling, dimension reduction (PCA and UMAP), finding neighboring cells, and clustering. In order to observe if the data needed further processing to rule out the possibility of batch effects, the UMAP plots were visually inspected to observe whether the cells cluster by tissue, age, or cell type. The primary factor that explains the variation between cells was confirmed to be the "cell type" rather than the source tissue or the age of the tissue donor.

The meta-data tables from different studies were further processed to enable cross-study comparisons. When the cells were not annotated as "young" or "old" by the study authors, the cells were categorised as "young" if the source sample is from mice younger than 3 months; "old" if the source sample is from mice older than 15 months. The human samples younger than 30 were categorised as "young", while those older than 30 were categorised as "old". Cell type and tissue annotations were used as readily annotated by the study authors.

Selected marker genes: We selected 10 senescence associated marker genes that were discovered in the Tabula Muris Senis study (Almanzar et al., 2020) to be most strongly enriched in the older mice compared to the younger mice, namely: Cdkn2a (p16), Parp14, Il1b, Itgam, Itgax, Tnf, Bst1, Lmnb1, E2f2, Il10.

Fraction of cells expressing marker genes: A marker gene was considered to be expressed in a given cell, if the marker gene had an expression value greater than zero. The fraction of cells that express the markers were compared between young and old mice at various levels, considering the full dataset, tissues/organs, and cell types.

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