Supplementary Materials for

Chronic inflammation partially recapitulates the gene expression signature of aging

Tomer Landsberger, Ido Amit, Uri Alon

This PDF file includes: Supplementary discussion, Supplementary figs. 1-3, Supplementary table 13-14

Discussion

The correspondence between aging and CI demonstrated in both bulk and single-cell RNA sequencing analyses is partial. This may be due to biological reasons (CI only recapitulates aging partially) and/or technical reasons of comparing data from different studies that utilize different experimental protocols, carried in different facilities and so on (i.e., batch effect), as well as intrinsic noise.

To highlight the potential combined contribution of these technical inter-study variation, we performed a 'positive control', in which we compared two different studies of the same process, aging.

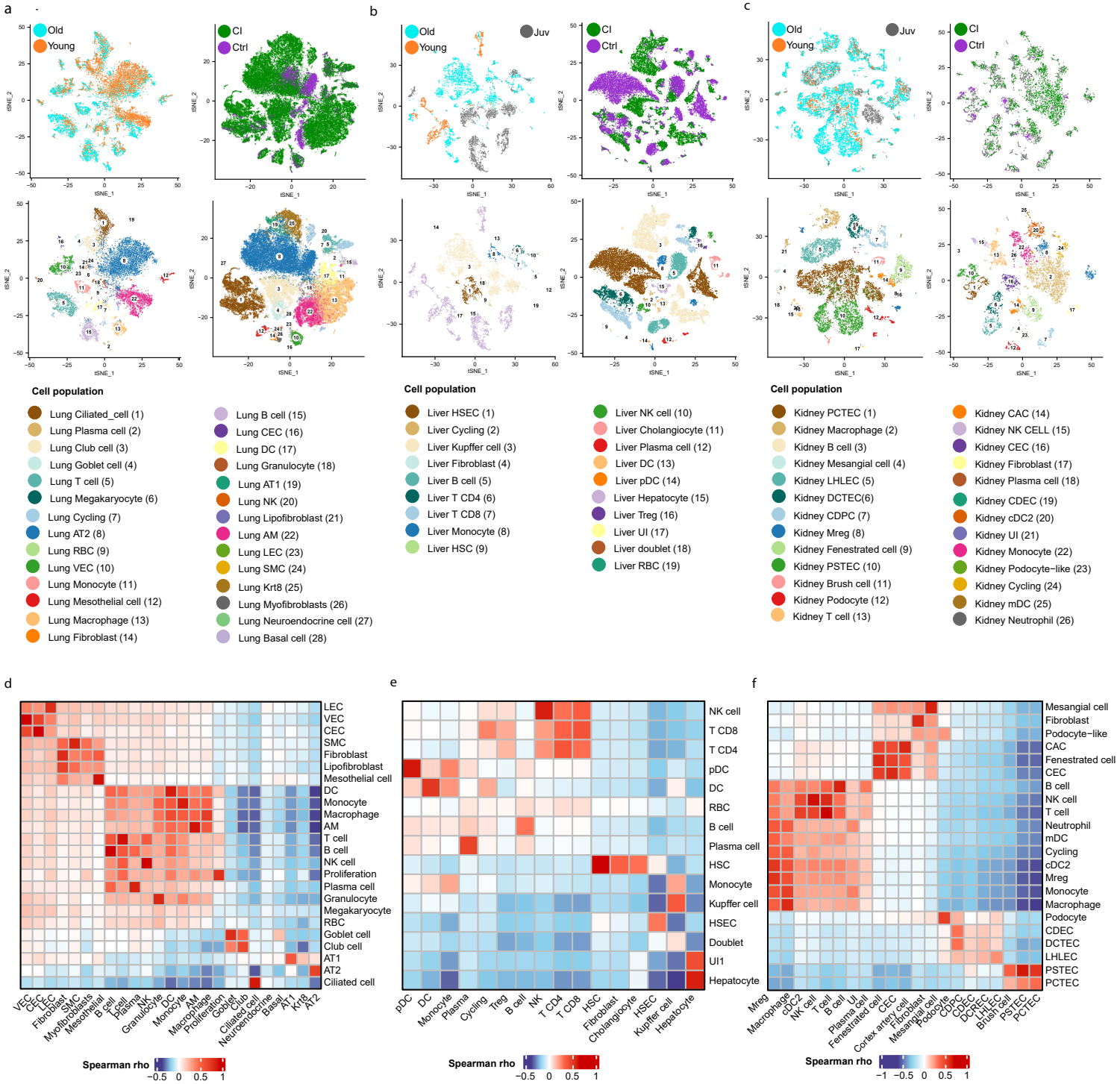
We applied the same analysis comparing the present O/Y data to O/Y from a different study with a similar design. In bulk, we used data derived from *Ovadya et al.* [1] for lung, liver and kidney (Supplementary table 13). In single-cell level, we used data derived from *The Tabula Muris (senis)* project [2] for lung (Supplementary table 14).

Ideally, one would expect almost perfect correlations, however, in reality we find comparable correlations (Supplementary Fig. 2 for bulk; Supplementary Fig. 3 for single-cell) to those found in the aging-CI comparison. This demonstrates that inter-study differences can mask the biological correspondence between conditions, limiting the strength of correlation.

References

1. Ovadya, Y. *et al.* Impaired immune surveillance accelerates accumulation of senescent cells and aging. *Nat. Commun.* **9**, 5435 (2018).
2. Almanzar, N. *et al.* A single-cell transcriptomic atlas characterizes ageing tissues in the mouse. *Nature* **583**, 590–595 (2020).

Supplementary Figure 1 - CI recapitulates age-related gene expression changes at the level of individual cell populations analyzed by scRNA-seq



Supplementary Fig. 1 ScRNA-seq analysis shows CI recapitulates age-related gene expression changes in the level of individual cell populations.

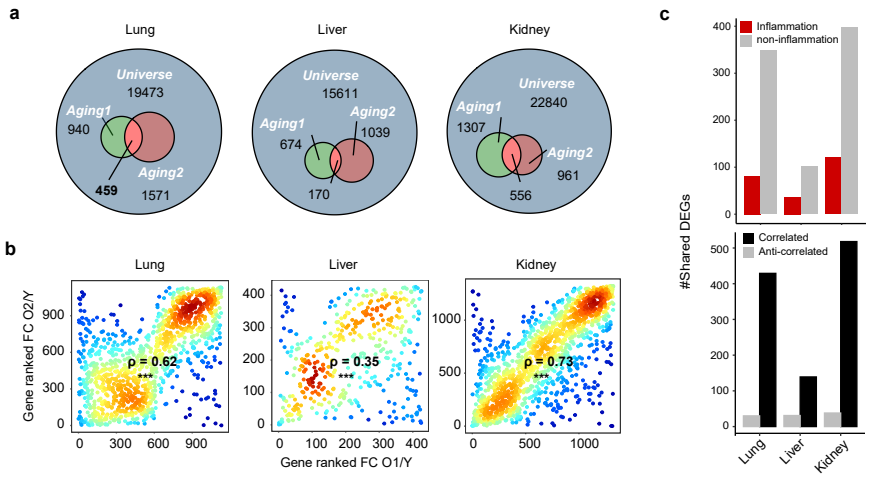
a-c, t-distributed stochastic neighbor embedding (tSNE) maps of single cells from aging (left) and CI (right) studies analyzed, color coded for experimental group (top) and for cell population (bottom), in lung (a), liver (b) and kidney (c). **d-f**, Inter-study Spearman correlation per cell population of top 3000 most variable genes in lung (d), liver (e) and kidney (f). **g**, Ranked mean fold-changes for CI/CTL (x-axis) and O/Y (y-axis) gene expression of aging DEGs shown on a density map for lung, liver and kidney cell populations. Spearman correlation coefficient presented, $p \leq 0.05$ $*$, $p \leq 0.01$ $**$, $p \leq 0.001$ $***$.

Supplementary Table 2 data sources for bulk RNA sequencing control analysis (aging vs. aging)

Source	Tissue	Context	Intervention	# mice
The Tabula Muris Consortium 2020 (senis)[11]	Lung	Aging	24/27 months vs 3 months	8 vs 7
Ovadya et al. 2018[13]	Lung	Aging	20 months vs 3 months (C57BL/6 *Perf1-/-*)	3 vs 3
The Tabula Muris Consortium 2020 (senis)[11]	Liver	Aging	24/27 months vs 3 months	7 vs 6
Ovadya et al. 2018[13]	Liver	Aging	20 months vs 3 months (C57BL/6 *Perf1-/-*)	3 vs 3
The Tabula Muris Consortium 2020 (senis)[11]	Kidney	Aging	24/27 months vs 3 months	8 vs 5
Ovadya et al. 2018[13]	Kidney	Aging	20 months vs 3 months (C57BL/6 *Perf1-/-*)	3 vs 3

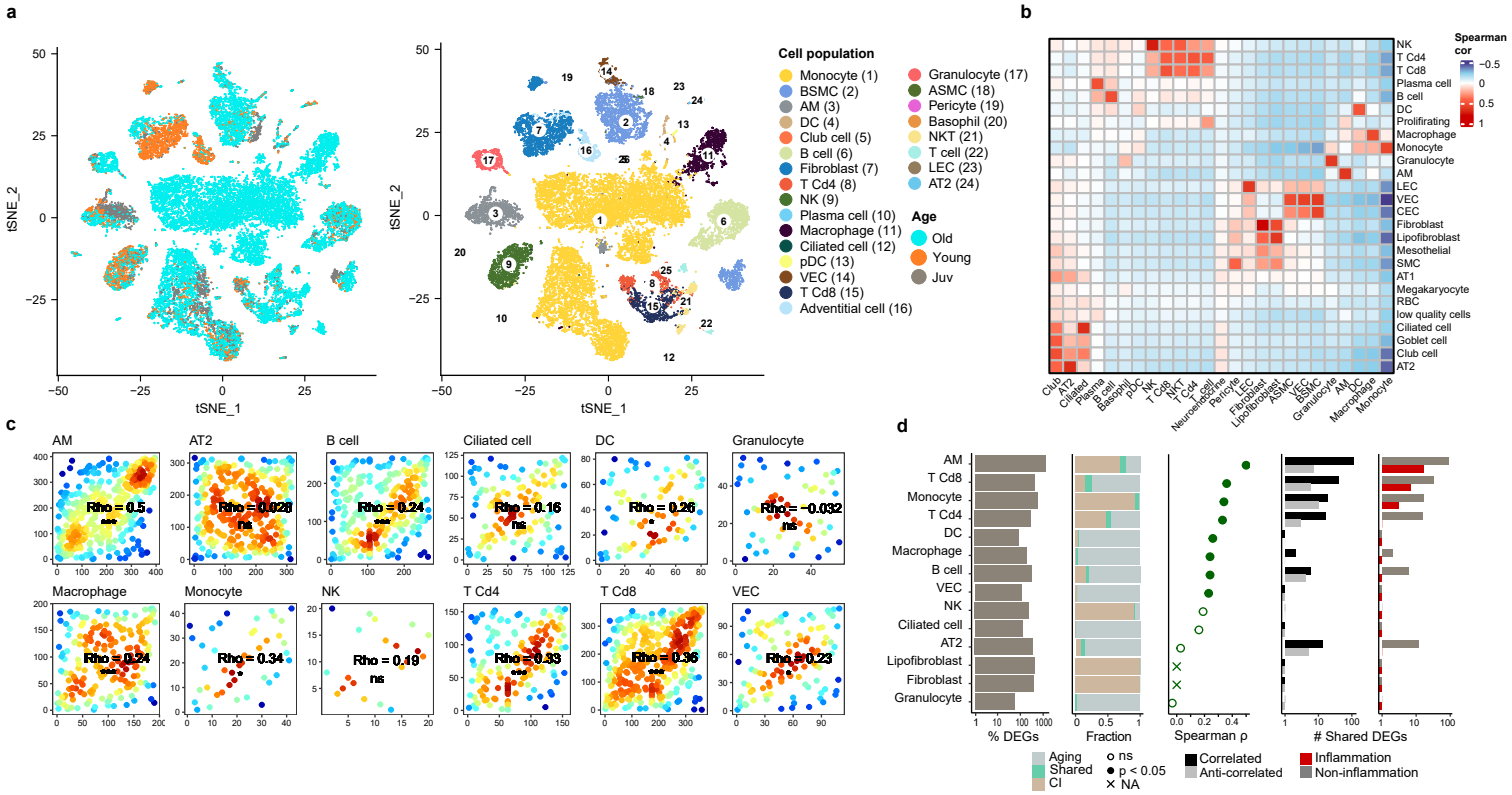
Supplementary Table 13: data sources for single-cell RNA sequencing control analysis (aging vs aging)

Source	Tissue	Context	Intervention	# mice used for downstream analysis	Clustering & annotation	Cell population for downstream analysis	# O1	# Y1	# O2	# Y2
Angelidis et al. 2019 [19]	Lung	Aging	24 months vs 3 months	8 vs 7	Published	Alveolar type 2 pneumocyte	1171	2997	11	54
The Tabula Muris Consortium 2020 (senis) [20]	Lung	Aging	30/21/18 months vs 3 months	10 vs 4	Published	Monocyte	437	68	7870	339
						Alveolar macrophage (AM)	514	1183	290	208
						Macrophage	269	179	1098	15
						Dendritic cell (DC)	266	348	85	44
						Neutrophil	26	17	22	24
						T CD8	459	148	193	70
						T CD4	449	138	106	123
						NK cell	66	123	129	534
						B cell	655	192	295	168
						Vascular EC (VEC)	444	390	73	28
						Fibroblast	35	54	26	1324



Supplementary Fig. 2: Bulk RNA-seq positive control analysis (aging vs. aging).

a, Ranked mean fold-changes for O/Y gene expression for two independent studies shown on a density map for lung, liver and kidney. Spearman correlation coefficient 0.62, 0.35 and 0.73 respectively. $p<1.0\times10^{(-16)}$ for all. **b**, Euler diagram depicting overlap of DEG sets for O vs. Y in two independent studies for lung, liver and kidney. Hypergeometric test for overlap $p<1.0\times10^{(-16)}$ for all. **c**, DEGs from the 3 overlap regions of the Euler diagrams, divided into DEGs that change in correlated (++, --) or anti-correlated (+-, -+) manners. Binomial test $p<1.0\times10^{(-16)}$ for all.



Supplementary Fig. 3: scRNA-seq positive control analysis (aging vs. aging).

a, Integrated tSNE map of single cells from TMS aging study, color coded for experimental group (left) and for cell population (right). **b**, Inter-study Spearman correlation per cell population of top 3000 most variable genes in Angelidis and TMS. **c**, Ranked mean fold-changes for Angelidis O/Y (x-axis) and TMS O/Y (y-axis) gene expression of aging DEGs shown on a density map. **d**, From left to right: total number of DGEs in aging and CI studies combined; DGE fraction shared vs study specific; Spearman correlation of Angelidis O/Y and TMS O/Y ranked mean gene expression fold-changes for Angelidis DEGs, where > 10 are present (otherwise denoted na); DEG correlation (black: ++, --, grey: +-, -+); inflammation and non-inflammation related fractions of correlated DEGs.

Supplementary Table legends

Supplementary Table 1: Shared DGEs for bulk RNA sequencing analysis in lung, liver and kidney.

Supplementary Table 2: MetaScape for shared DGEs of bulk RNA sequencing analysis in lung, liver and kidney.

Supplementary Table 3: Scaled normalized mean gene expression signature per cluster in lung from aging (Angelidis).

Supplementary Table 4: Scaled normalized mean gene expression signature per cluster in lung from CI (Strunz).

Supplementary Table 5: Scaled normalized mean gene expression signature per cluster in liver from aging (TMS).

Supplementary Table 6: Scaled normalized mean gene expression signature per cluster in liver from CI (Xiong).

Supplementary Table 7: Scaled normalized mean gene expression signature per cluster in kidney from aging (TMS).

Supplementary Table 8: Scaled normalized mean gene expression signature per cluster in kidney from CI (Conway).

Supplementary Table 9: Shared aging and CI DGEs for single-cell RNA sequencing analysis.

Supplementary Table 10: MetaScape analysis for shared DGEs of single-cell RNA sequencing analysis.

Supplementary Table 11: Shared DGEs for ABT RNA sequencing analysis in lung, liver and kidney.

Supplementary Table 12: MetaScape for shared aging, CI and ABT in lung, liver and kidney.

Supplementary Table 13: Data sources for bulk RNA sequencing control analysis (aging vs. aging).

Supplementary Table 14: Data sources for single-cell RNA sequencing control analysis (aging vs. aging).