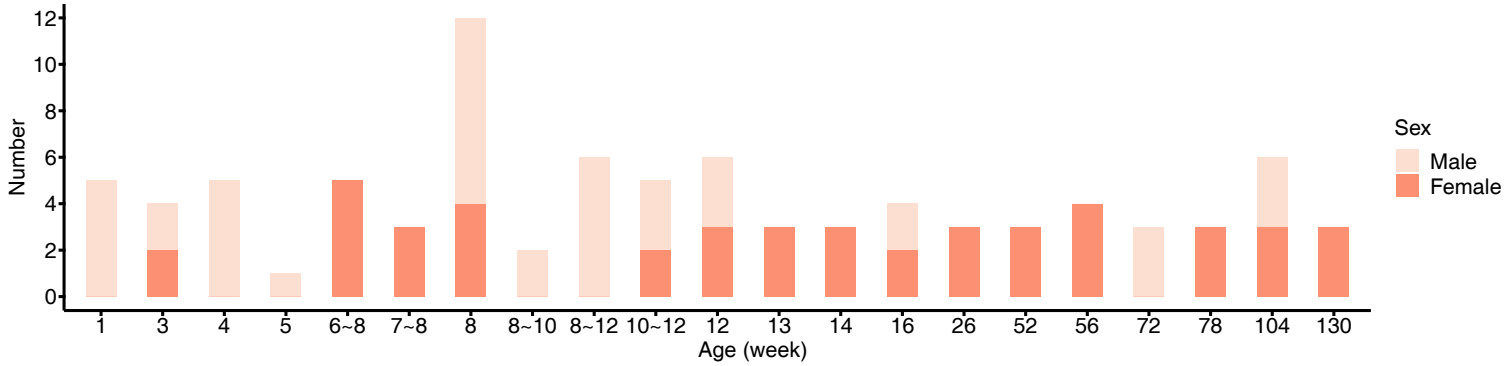
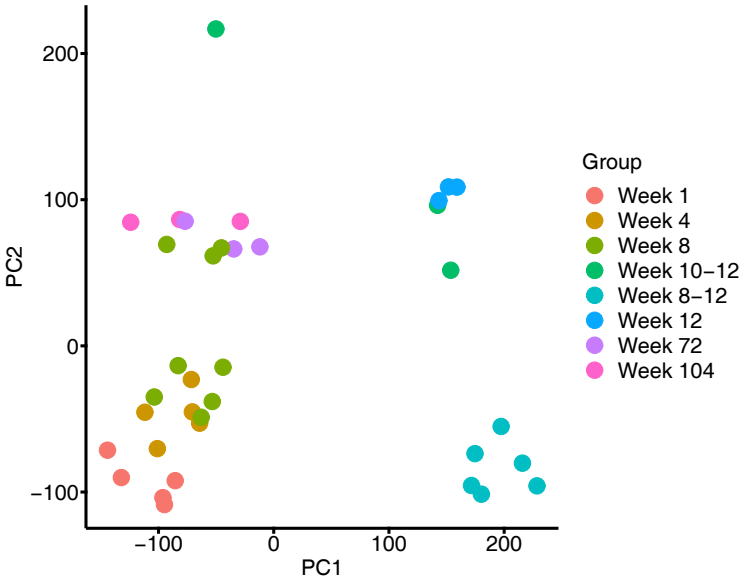
Supplementary Figure S1

The age distribution of 89 individual mouse data sets retrieved from 15 studies.
See supplementary Table S1 for further details. Because of the gender disproportionate distribution of genomic data sets, we excluded data from female mice.



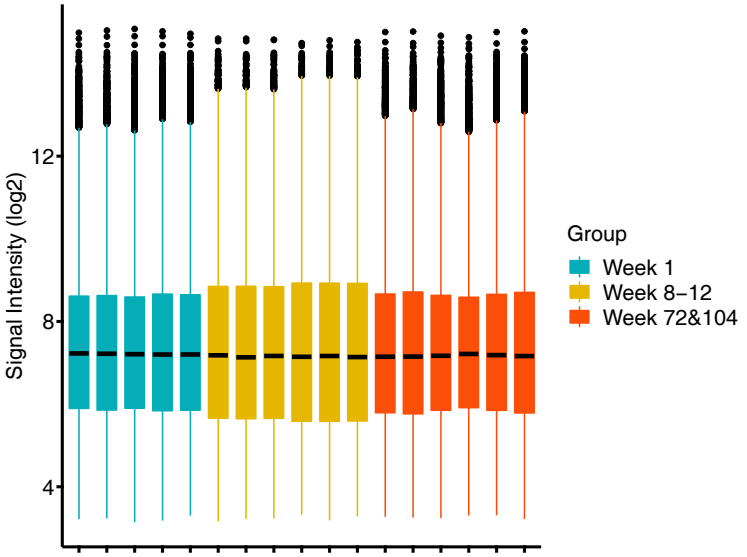
Supplementary Figure S2

PCA of 36 male mouse genomic data, which are not well separated by age.
See also supplementary Figure S12 for further information on the final selection of individual samples.



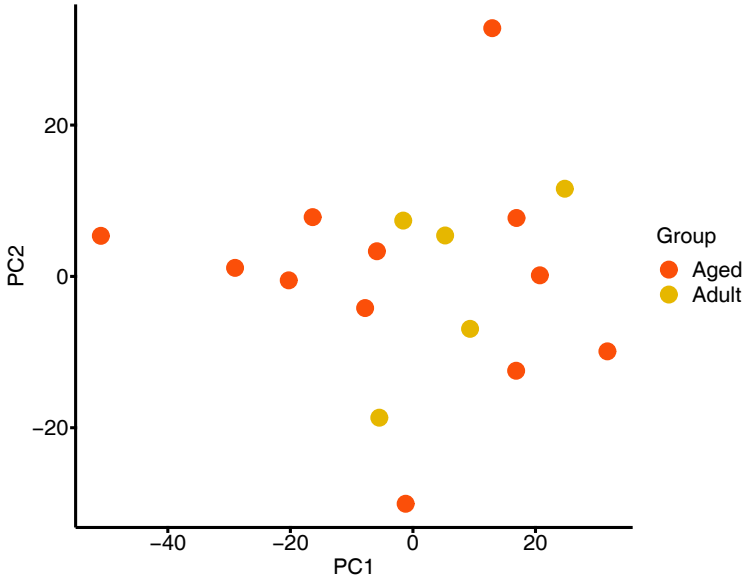
Supplementary Figure S3

Box plots of whole genome signal intensities for 17 mouse lung samples, which we included for final analysis.
The signal intensities are evenly distributed.



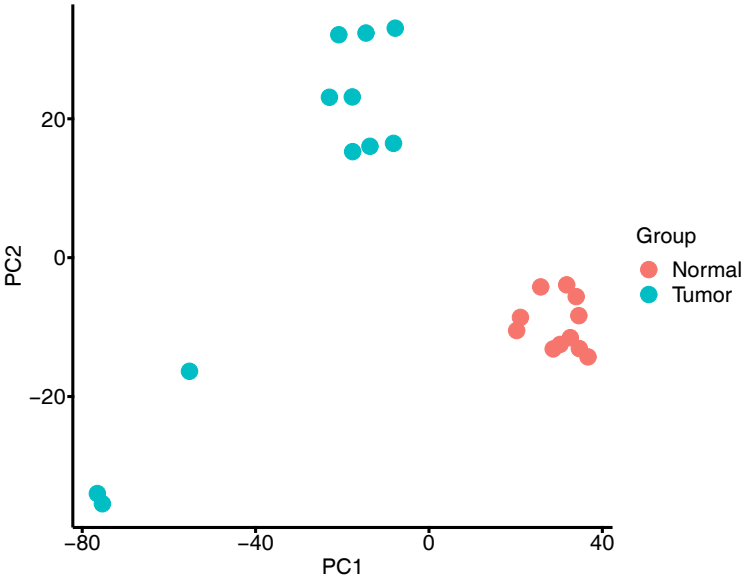
Supplementary Figure S4

PCA of 17 human test set genomic data.
The individual data sets are not well separated by age.



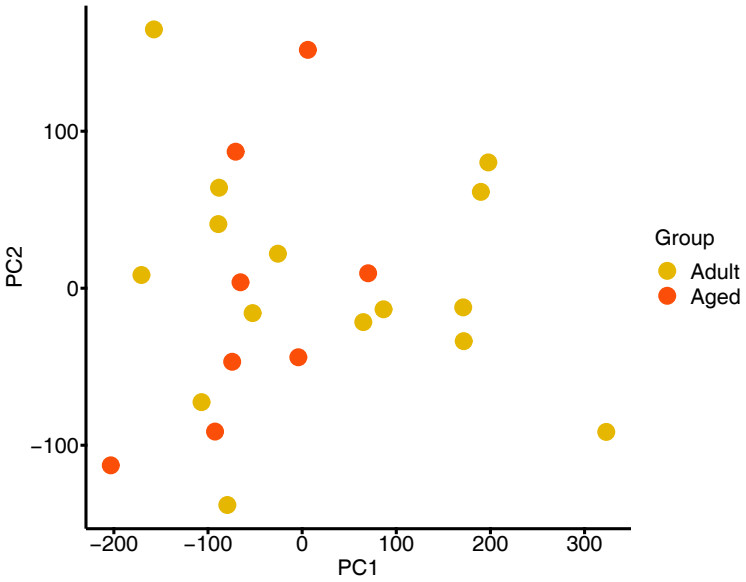
Supplementary Figure S5

PCA of 11 samples, which we included for final analysis as human test set. Shown is the distribution of histological normal tissue samples and the matched tumor samples.



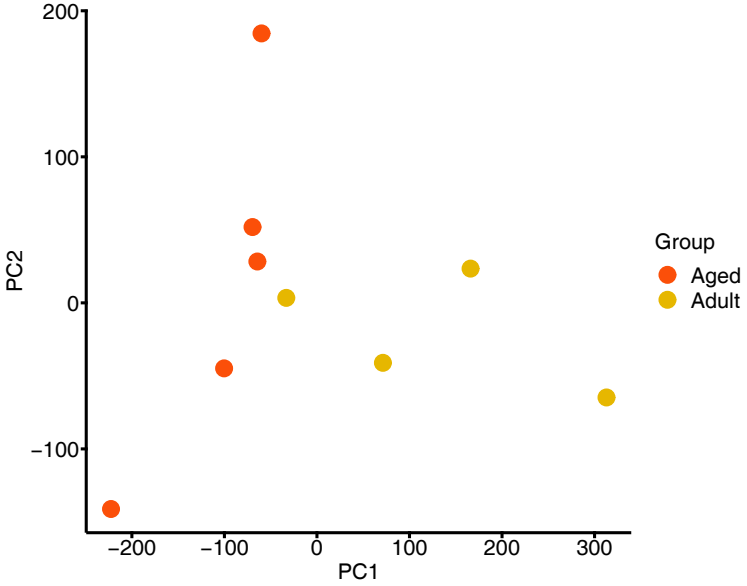
Supplementary Figure S6

PCA of 23 (cohort I, GSE1643) human validation set.
The individual genomic data are not well separated by age.



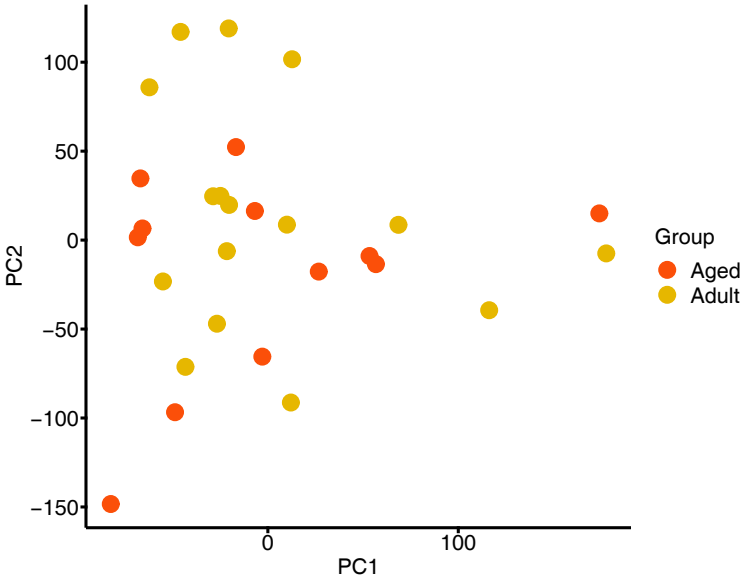
Supplementary Figure S7

PCA of 9 samples, which we included for final analysis as human validation set.



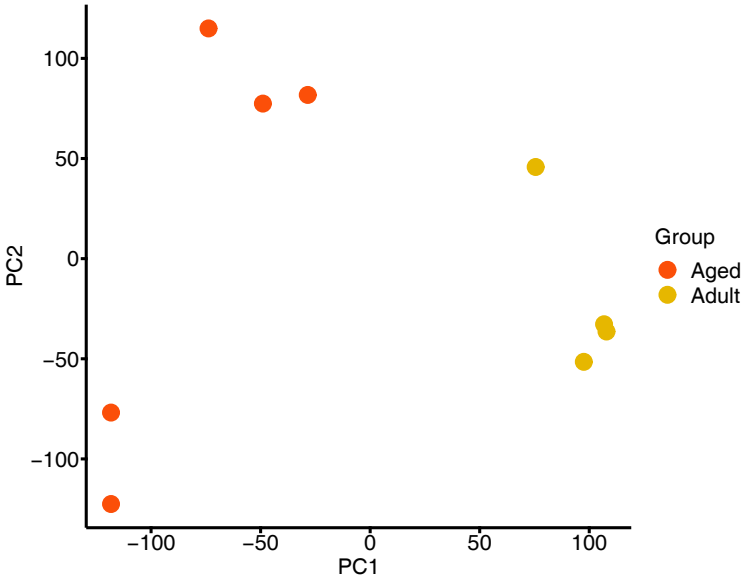
Supplementary Figure S8

PCA of 28 (cohort II, GSE71181) human validation set.
The individual genomic data are not well separated by age.



Supplementary Figure S9

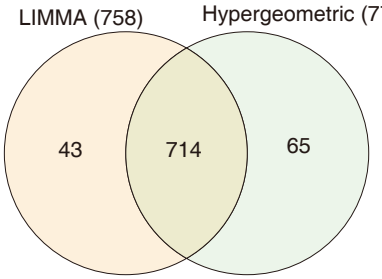
PCA of 9 samples, which we included for final analysis as human va lidation set. Together, the human validation set consisted of 18 individual data sets derived from GSE1643 and GSE71181.



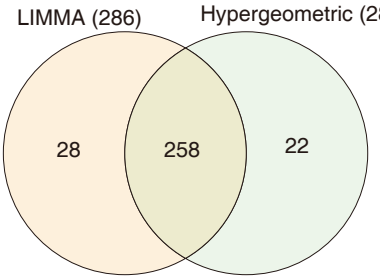
Supplementary Figure S10

Computation of DEGs for the mouse lung by two methods, i.e. Linear Models for Microarray data (LIMMA) and the hypergeometric test. We compared DEGs from the two statistical tests using the following criteria: BH-adjusted p-value <0.05 and FC ≥ 3-fold.

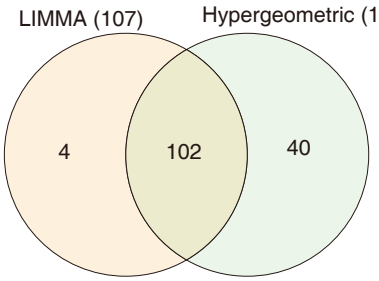
A Venn diagram of the comparison Week 1 vs Week 8-12, for upregulated DEGs



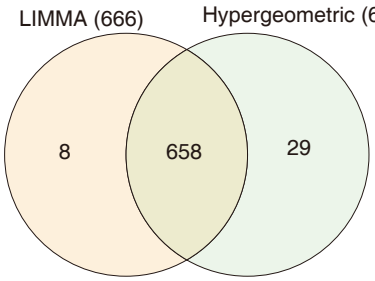
B Venn diagram of the comparison Week 1 vs Week 8-12, for downregulated DEGs



C Venn diagram of the comparison Week 8-12 vs Week 72&104 for upregulated DEGs

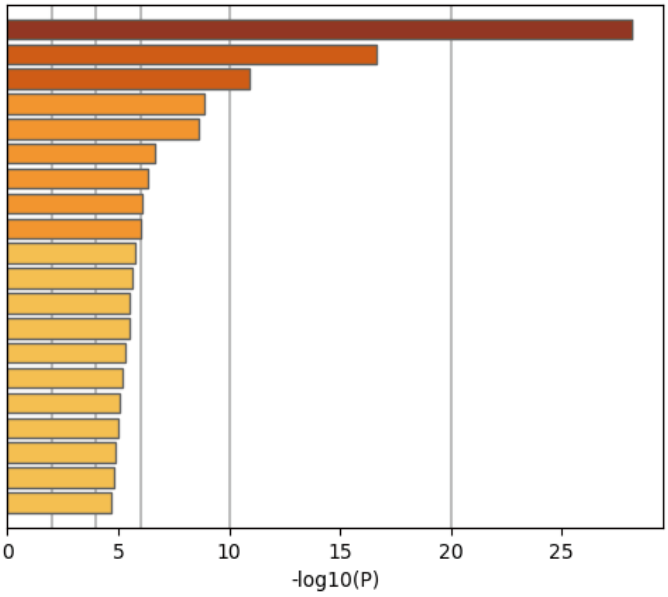


D Venn diagram of the comparison Week 8-12 vs Week 72&104 for downregulated DEGs



Supplementary Figure S11

We considered 1200 housekeeping genes of which 342 changed in expression with age.
Shown is the gene enrichment analysis for regulated housekeeping genes, and the enriched terms do not implicate pulmonary biology for these genes. Finally, we used the 798 unregulated housekeeping genes as a normalizer for further data analysis.

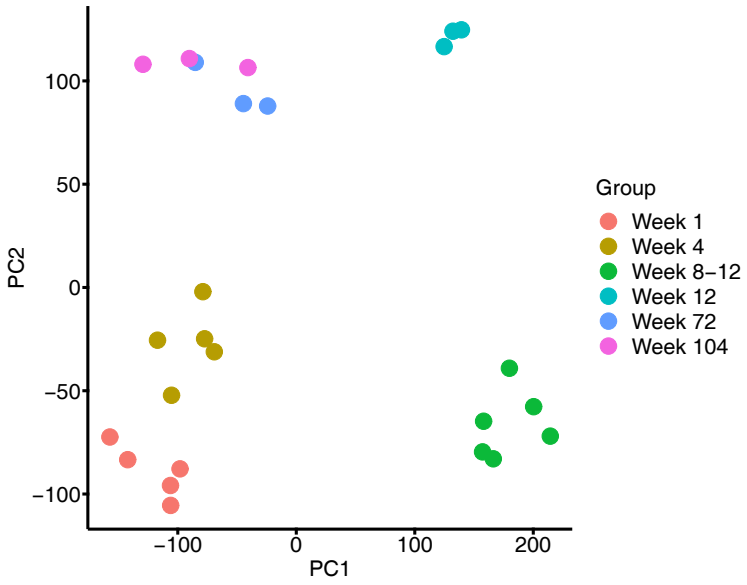


R-MMU-8953854: Metabolism of RNA
GO:1903311: regulation of mRNA metabolic process
R-MMU-9711123: Cellular response to chemical stress
WP246: TNF-alpha NF-kB Signaling Pathway
GO:0006412: translation
GO:0050657: nucleic acid transport
ko05132: Salmonella infection
R-MMU-6798695: Neutrophil degranulation
R-MMU-3700989: Transcriptional Regulation by TP53
GO:0097190: apoptotic signaling pathway
mmu05152: Tuberculosis
GO:0022613: ribonucleoprotein complex biogenesis
mmu04520: Adherens junction
GO:0016032: viral process
R-MMU-199991: Membrane Trafficking
ko04140: Autophagy - animal
GO:0050686: negative regulation of mRNA processing
GO:0010638: positive regulation of organelle organization
GO:1901355: response to rapamycin
WP1763: PluriNetWork: mechanisms associated with pluripotency

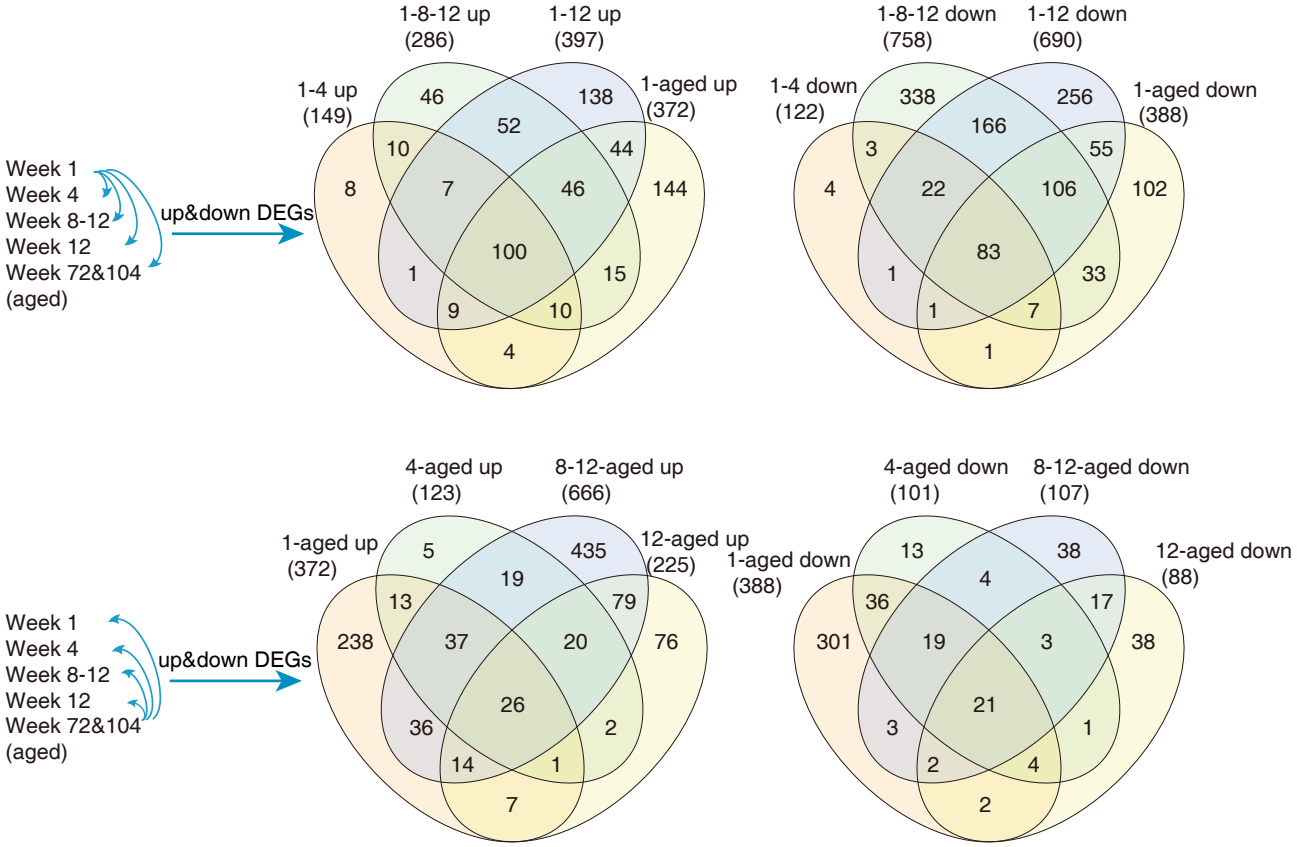
Supplementary Figure S12A

Selection of mouse genomic data sets for final analysis.
Based on the PCA shown in supplementary Figure S2, we removed 11 samples, which were intermingled among the various age groups. The subsequently computed PCA revealed DEGs of mice aged 4 and 12 weeks to be very different from their age counterparts and therefore were excluded.

A PCA of 25 individual genomic data sets

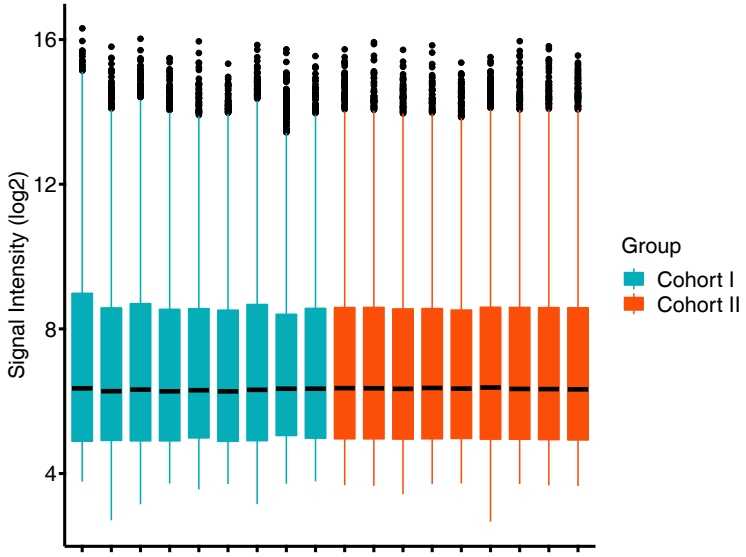


B Venn diagrams to identify commonly regulated DEGs among the different age groups.



Supplementary Figure S13

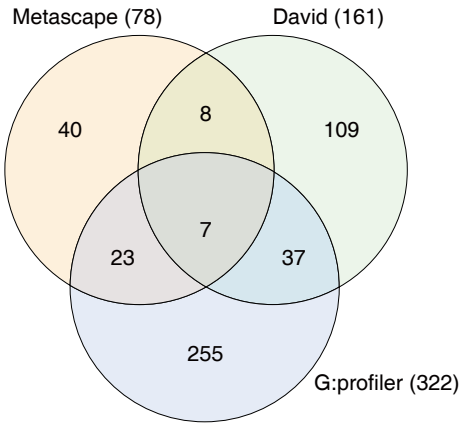
Box plots of whole genome signal intensities for 18 human lung samples, which we included for final analysis.
The signal intensities are evenly distributed.



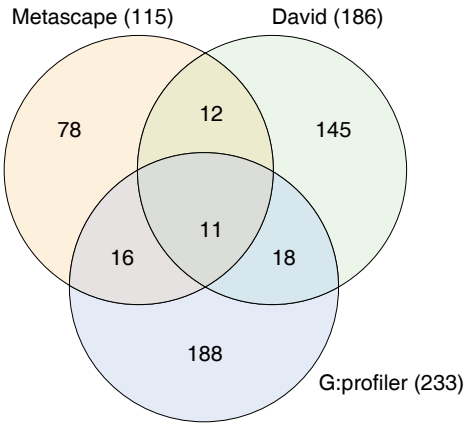
Supplementary Figure S14

Consensus among different gene ontology tools

A Shown are Venn diagrams for enriched terms for upregulated genes in mice aged 1 week compared to 8-12 week ones.

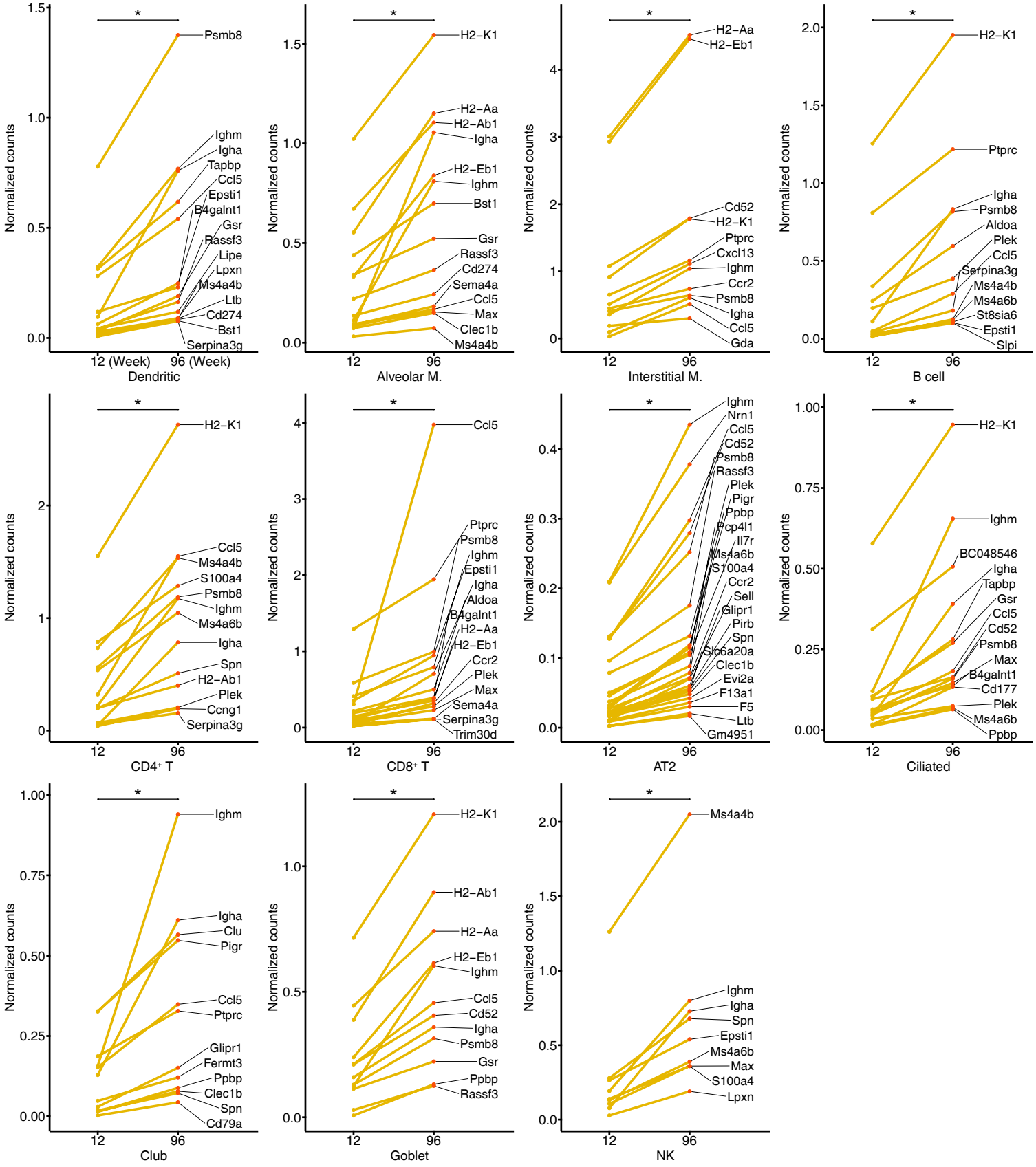


B Shown are Venn diagrams enriched terms for downregulated genes in mice aged 1 week compared to 8-12 week ones.



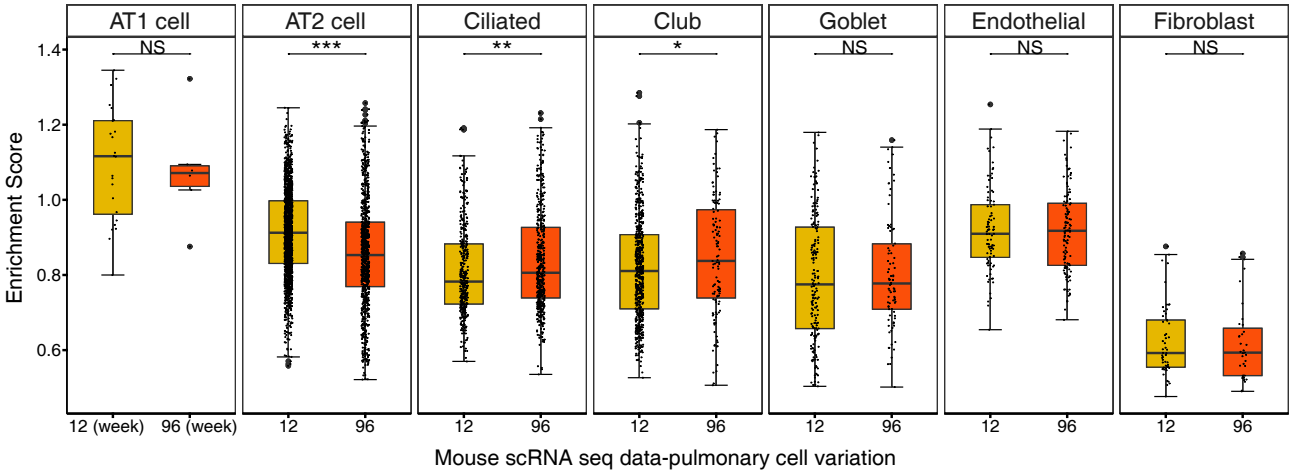
Supplementary Figure S15

Validation of continuous upregulated genes by single cell RNA sequencing.
We probed for the regulation of 87 continuous upregulated genes among immune and other resident cells of the lung.
Here we compared animals aged week 12 to animals aged 96 weeks and assessed their expression in dendritic, alveolar and interstitial macrophages, CD4+ and CD8+ T-cells, B-cells as well as AT2, ciliated, club, goblet and NK cells.
In total we confirm the age-related regulation of 45 genes among individual pulmonary cells.
We computed statistical significance with the “Wilcoxon rank-sum” test. *p<0.05.



Supplementary Figure S16

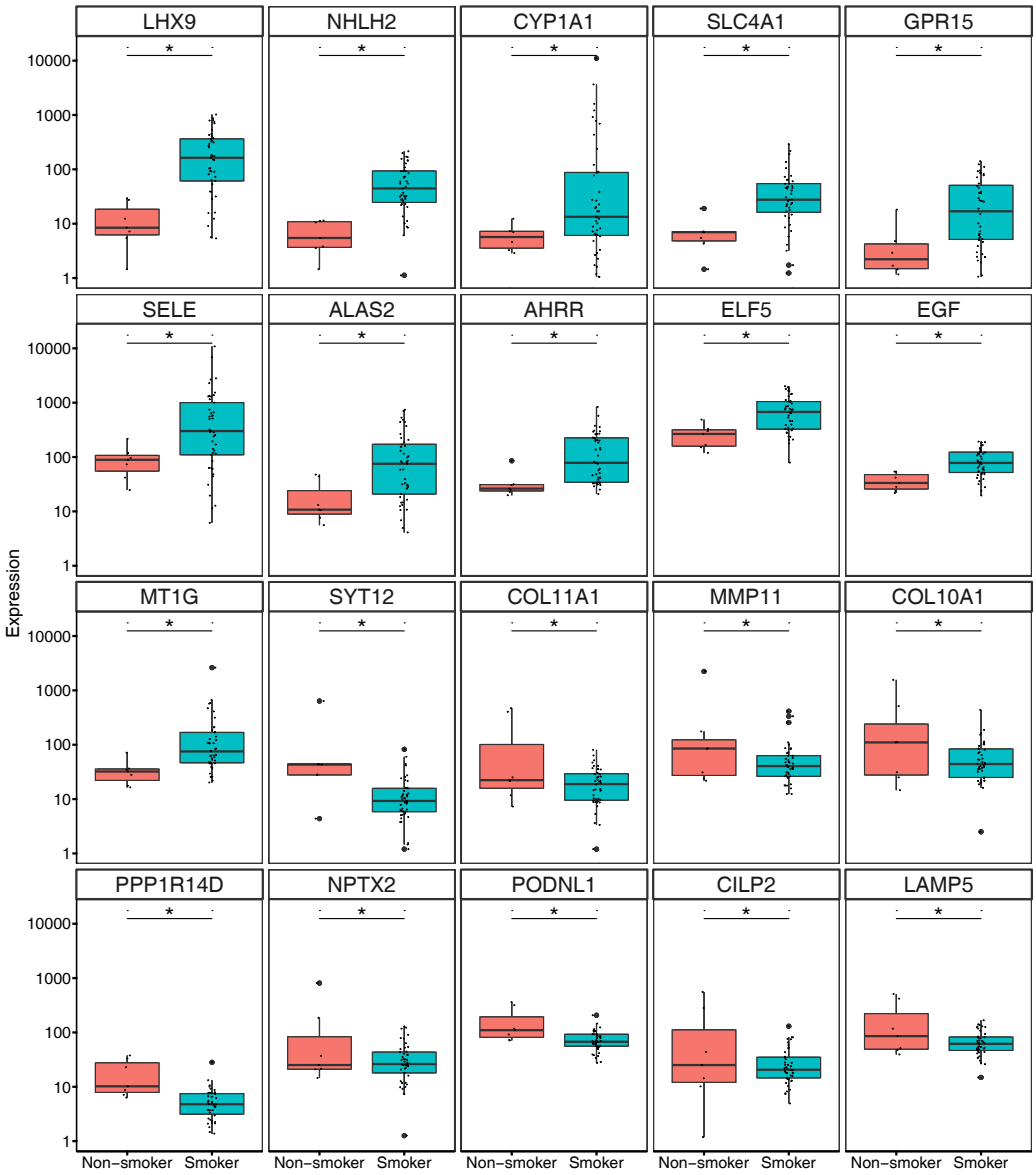
Validation of marker genes by single cell RNA sequencing of pulmonary cells.
We retrieved marker genes from various databases and considered their age-dependent regulation in pulmonary cells by single cell RNA sequencing. We computed enrichment scores based on a large set of genes (range 44-434 genes for the mouse, supplementary Table S16). By interrogating single cell RNAseq data of 7 different pulmonary cells, we confirmed the results in Figure 10B&C by an independent method. We computed statistical significance with the "Wilcoxon rank-sum" test. ns: not significant, *p<0.05, **p<0.01, ***p<0.001.



Supplementary Figure S17

Comparative human pulmonary genomics of smokers and non-smokers.
In order to examine the influence of tobacco product use on age-related gene expression changes,
we compared the genomic data of morphologically unaltered lung tissue of 45 smokers to 7 non-smokers.
We show that tobacco product use per se did not influence the expression of age-regulated genes reported
in the present study; however, tobacco smoke exposure caused marked induction of xenobiotic defense genes.
ns: not significant, Wilcoxon rank sum test, *p<0.05.

A Box plots of 20 genes highly regulated following tobacco smoke exposure.



B None of the age-related gene expression changes in the human lung were influenced by tobacco use.