Ageing hallmarks exhibit organ-specific temporal signatures

Ageing is the single greatest cause of disease and death worldwide, and understanding the associated processes could vastly improve quality of life. Although major categories of ageing damage have been identified—such as altered intercellular communication, loss of proteostasis and eroded mitochondrial function—these deleterious processes interact with extraordinary complexity within and between organs, and a comprehensive, whole-organism analysis of ageing dynamics has been lacking. Here we performed bulk RNA sequencing of 17 organs and plasma proteomics at 10 ages across the lifespan of *Mus musculus*, and integrated these findings with data from the accompanying *Tabula Muris Senis* or ‘Mouse Ageing Cell Atlas’—which follows on from the original *Tabula Muris*. We reveal linear and nonlinear shifts in gene expression during ageing, with the associated genes clustered in consistent trajectory groups with coherent biological functions—including extracellular matrix regulation, unfolded protein binding, mitochondrial function, and inflammatory and immune response. Notably, these gene sets show similar expression across tissues, differing only in the amplitude and the age of onset of expression. Widespread activation of immune cells is especially pronounced, and is first detectable in white adipose depots during middle age. Single-cell RNA sequencing confirms the accumulation of T cells and B cells in adipose tissue—including plasma cells that express immunoglobulin J—which also accrue concurrently across diverse organs. Finally, we show how gene expression shifts in distinct tissues are highly correlated with corresponding protein levels in plasma, thus potentially contributing to the ageing of the systemic circulation. Together, these data demonstrate a similar yet asynchronous inter- and intra-organ progression of ageing, providing a foundation from which to track systemic sources of declining health at old age.

To uncover ageing dynamics across the whole organism, we measured plasma proteins and sequenced RNA from 17 organ types isolated from C57BL/6JN male mice (*n* = 4; aged 1, 3, 6, 9, 12, 15, 18, 21, 24 and 27 months; equivalent to humans aged 13, 20, 30, 43, 50, 56, 63, 69 and 75 years, respectively) and female mice (*n* = 2; aged 1, 3, 6, 9, 12, 15, 18 and 21 months) (Fig. 1a, b). This encompasses development at 1 month of age to maturity at 3–6 months, as well as ageing through adulthood to the median lifespan of 27 months. We isolated all 17 organs from each mouse, including bone (femurs and tibiae), brain (hemibrain), brown adipose tissue (BAT, interscapular depot), gonadal adipose tissue (GAT, inguinal depot), heart, kidney, limb muscle (tibialis anterior), liver, lung, bone marrow, mesenteric adipose tissue (MAT), pancreas, skin, small intestine (duodenum), spleen, subcutaneous adipose tissue (SCAT, posterior depot), and white blood cells (buffy coat). Raw data are available from the Gene Expression Omnibus (accession code GSE132040), and an interactive data browser is available at https://twc-stanford.shinyapps.io/maca/. Concurrently, we performed single-cell RNA sequencing on 529,823 cells from 20 organs across the lifespan of the mouse, generating a *Tabula Muris Senis* that is presented in the accompanying Article.

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Check for updates
of the data in d, f. Scatterplot displaying gene-wise enrichment scores for tissue, age and tissue/age (see Methods section ‘Specificity of gene expression for tissues and ages’). g, Tissue-wise expression changes with age (within each column, from left to right) for the top 15 genes exhibiting shifts in most tissues. h–j, Igj expression in marrow (h), spleen (I) and GAT (J). n = S1, S4 and S2 independent samples, respectively. The black lines indicate locally estimated scatterplot smoothing (LOESS) regression. Data are mean ± s.e.m.

**Pairwise differential gene expression with age**

Ageing instigates functional decline across organs, disrupting intricate crosstalk that is essential for maintaining healthy organismal processes. Although individual organ types transcriptionally segregate by age (Fig. 1c), we lack a basic comparative understanding of ageing between these organs, including differences in the onset and rate of ageing. We therefore performed pairwise differential expression to determine when differentially expressed genes (DEGs) arise and whether they persist with advancing age. Although a few DEGs were observed between organs at neighbouring ages, in most organs the number of DEGs increases markedly in older mice relative to 3-month-old adults, suggesting progressive, gradual changes in gene expression that are detectable only after sufficient time (Fig. 1d, e). Some organs—such as the pancreas and marrow—seem to be relatively refractory to gene expression changes upon ageing; this might be in part explained by the relatively small proportion of global variance due to ageing in the dataset (Extended Data Fig. 1a, b). Whereas the core profiles of ageing mice are maintained relative to those of 6-month-old mice, the number of DEGs increases greatly when compared with organs from 1-month-old mice that are still undergoing development (Extended Data Fig. 2a–c). The clear outlier is the spleen, which displays large numbers of DEGs relative to 3 months old (18-month) DEGs between the sexes suggests that these differences in fat storage, sex hormone regulation and renal haemodynamics*7 (Extended Data Figs. 1c, d, 3b). Differential gene expression analysis between the sexes at each age revealed that these four tissues consistently displayed the most DEGs across the lifespan (Extended Data Fig. 4a, b), and the large overlap between young (3-month) and old (18-month) DEGs between the sexes suggests that these differences are established early and are maintained throughout life (Extended Data Fig. 4c, d). However, the biological pathways that comprise these sex DEGs largely differ from those comprising ageing DEGs, therefore providing no evidence that sex differences influence the transcriptional ageing profiles we observe here (Extended Data Fig. 4e–h).

We next asked whether early-life DEGs persist with advancing age, or whether they give way to new DEGs at older ages. In bone, for example, the early-life expression of ossification genes decreases as bone formation is completed, and these genes are highly correlated with late-life DEGs for which expression is increased, typifying age-related bone loss (Supplementary Table 1). Overall, most organs show correlation between early and late DEGs, which is exemplified by nearly every pairwise comparison in GAT, liver, kidney and heart (Supplementary Table 1). Few genes are unique to any individual age, with organ specificity outweighing age specificity (Fig. 1f). Differential expression common between ageing organs is of particular interest, because ubiquitous ageing pathways may present new therapeutic opportunities. When we isolated genes that are most frequently differentially expressed across organs, we found strong enrichment for those relating to immune response pathways (Fig. 1g, Extended Data Fig. 2e–h, Supplementary Table 2). Notably, the expression of *Jchain* (also known as *Igj*)—which encodes the plasma B cell marker immunoglobulin J—is persistently increased throughout life in 11 out of 17 organs (Fig. 1g–k). The circadian clock genes *Bhlhe40*, *Bhlhe41*, *Arntl*, *Npas2*, *Per3*, *Ciart* and *Dbp* also feature among the top DEGs
Ageing gene expression dynamics across organs. **a**, Whole-organism gene expression trajectory clustering. The trajectory for each gene was averaged across all 17 organs, and those average trajectories were grouped into eight clusters. The number of genes and the top functionally enriched pathway for each cluster are reported. Within each cluster, the average trajectory for each individual organ is overlaid. Cluster trajectories ± s.d. (n = 17 tissue trajectories) are indicated in black and grey. Enrichment was tested using Fisher’s exact test (Gene Ontology, GO) and the hypergeometric test (Reactome and Kyoto Encyclopedia of Genes and Genomes, KEGG). q values were estimated with the Benjamini–Hochberg correction for each database separately, and for GO classes (molecular function, cellular component, biological process) independently. **b**, Identification of stable and variable clusters between organs. For each cluster in **a**, an amplitude index and a variability index were calculated. **c**, The amplitude and variability indices for the four clusters in **b** that showed the greatest trajectory change with age are shown, and adipose tissues are indicated. **d**, Unsupervised hierarchical clustering was used to group genes with similar trajectories in GAT (n = 9,832). **e**, Clustering dendrogram and cut-off used to define five independent clusters in GAT. **f**, Gene trajectories of the five clusters in **e** are shown by grey lines. Purple lines outlined with white represent the average trajectory for each cluster ± s.d. **g**, The top five pathways for each cluster in **e**. The number of genes is as in **e**, with the 15,000 most highly expressed genes as background. Enrichment and q values are as in **a**.

Gene expression dynamics with age

Pairwise comparisons are inherently limited, and our data enable the examination of gene expression dynamics with high temporal resolution across the lifespan. To reveal organism-wide processes, we first searched for gene expression trajectories across the lifespan with common behaviour between organs. We calculated the average trajectory for each gene across all 17 organs and clustered these averaged trajectories, revealing functional enrichment for hallmarks of ageing such as increased inflammation, mitochondrial dysfunction, and loss of proteostasis (Fig. 2a, Supplementary Tables 3, 4). Notably, these hallmarks have distinct dynamic patterns. For example, cluster 3 declines linearly across the lifespan and is strongly enriched for mitochondrial genes, whereas cluster 7—encoding heat shock proteins that are important for protein folding—demonstrates a sharp decline that begins only at 12 months of age. This is in contrast to cluster 8, which contains extracellular matrix genes that decline rapidly until 6 months, after which a more gradual decline prevails. Genes encoding immune response pathways feature in clusters 4 and 6; the expression of cluster 4 genes such as beta-2 microglobulin (B2m) and Igf1 increases steadily throughout life. Conversely, the expression of immune genes of cluster 6—such as Cdh74 and complement C1qa—exhibits a nonlinear increase, with a plateau between 9 and 15 months.

Each cluster contains genes with similar global trajectories, but organ-specific differences in the phase and magnitude of these trajectories suggest that similar processes have unique dynamics. For each cluster we assigned an amplitude index (absolute change in z-score of the mean trajectory between 1 month and 30 months) and a variability index (a measure of the spread from the mean trajectory) (Fig. 2b). This revealed that clusters with the largest amplitudes also show the strongest organ-specific behaviour, with adipose tissues featuring prominently in clusters 4 and 6 (immune response), cluster 7 (protein folding) and cluster 8 (extracellular matrix) (Fig. 2c). The lifelong increase in expression of genes relating to immune response pathways is especially evident when organs are analysed independently, specifically for adipose tissues such as GAT (Fig. 2d–g, Extended Data Figs. 5, 6, Supplementary Table 5). Indeed, genes of cytokine-mediated inflammatory pathways (such as GO:0019221) show a pronounced increase in expression in GAT that begins at 18 months (Extended Data Fig. 7a, Supplementary Table 9). This includes Ccl8, which shows very high correlation in the majority of tissue types (Extended Data Fig. 7b). Notably, however, even though many downstream processes are shared across organs (Fig. 2a), there is little overlap of transcription factor regulatory networks (Supplementary Tables 6, 9). Additionally, although tissue-specific trajectory clusters (Extended Data Fig. 5) show that several transcription factor genes are common between tissues—for example Irf1 in the kidney, lung and heart—the clusters often differ in behaviour between tissues, or are enriched for genes of disparate biological pathways (Supplementary Table 7). It therefore seems that, even though common biological pathways emerge between tissues, these are not—for the most part—driven by an underlying change to common transcription factor regulatory networks. It is of course possible that more distant
regulatory elements—including enhancers or super enhancers—may provide a missing link, and it is anticipated that such regulatory elements could be analysed in future studies.

**RNA sequencing confirms plasma B cell infiltration**

A fundamental question emerging from transcriptomics of whole organs is whether the observed shifts in gene expression are driven by cell-intrinsic changes with age or by changes in cell composition. Using the Tabula Muris Senis database, a database of the ageing mouse obtained by single-cell RNA sequencing (scRNA-seq), we first asked whether the top genes correlated with age from bulk RNA-seq (Supplementary Table 8) were specific to an individual cell type or were expressed across multiple cell types, demonstrating an organ-wide decline of mitochondrial function (Extended Data Fig. 8a–f). Other genes, such as Ms4a7, are positively correlated with age but are expressed only in kidney macrophages (Fig. 3a–c). This is characteristic of many other upregulated inflammatory genes that are specific to these cells (Extended Data Fig. 8b). In addition to the dispersion score, we used deconvolution software with cell-type-specific gene expression profiles from the two datasets of the Tabula Muris Senis separately—fluorescence activated cell sorting (FACS) coupled with Smart-seq2 data (denoted ‘FACS’) and microfluidic-droplet-based scRNA-seq data (denoted ‘droplet’) — in order to estimate changes in abundance of cell types in each tissue with age (Extended Data Fig. 8h). Although the cell types and profiles captured with these two methods do not always overlap, in the nine cases for which both methods found the same overlap, in the nine cases for which both methods found the same overlap, the correlations are highly concordant. In GAT and in the liver, a large increase in the number of B cells with age was observed, providing further evidence that accumulating immune cells are a driver of the whole-organ inflammatory signal. Furthermore, cell fractions as profiled by the four methods (FACS scRNA-seq, droplet scRNA-seq, FACS bulk deconvolution and droplet bulk deconvolution) show strong agreement, indicating that the results are highly stable (Extended Data Fig. 8g). Finally, as demonstrated by the Tabula Muris Senis, a combination of changes in the abundance of different

**Fig. 3 | Integration of bulk and single-cell transcriptomic data identifies cross-tissue infiltration of Igj⁺⁺ plasma B cells.** a. Expression of Aco1 and Ms4a7 mRNA in the kidney. The black line indicates LOESS regression, and the Spearman’s rank correlation coefficient is indicated. Data are mean ± s.e.m. b. t-SNE plots of genes with ‘disperse’ (Aco1) and ‘specific’ (Ms4a7) single-cell expression patterns in the kidney, n = 1,108 cells. c. Single-cell dispersion scores (scRNA-seq) plotted against Spearman’s rank correlation coefficient (≥0.6; bulk RNA-seq) for a given tissue. The colours represent different organ scores (scRNA-seq) plotted against Spearman’s rank correlation coefficient. d. t-SNE visualization of scRNA-seq data (FACS) from GAT, coloured by age. A cluster of B cells that is present only in aged GAT is circled. e. GAT B and T cells as a percentage of all analysed cells, n = 4 independent mice. f. Distribution of Igj⁺⁺ plasma cells in kidney and marrow, n = 4 independent mice. Significance was assessed using Student’s t-test; data are mean ± s.e.m.f. Expression of B cell marker Cd79α and plasma B cell marker Igj. g. t-SNE visualization of scRNA-seq data (droplet) of all Cd79α-expressing cells present in the Tabula Muris Senis dataset (17 tissues), coloured by the plasma B cell markers Igj and Xbp1.h. GO terms enriched among the top 300 marker genes of Igj⁺⁺ (n = 1,198 cells) compared with B cells (n = 22,598 cells), with 1,886 genes passing filtering as background. q-values are estimated using the Benjamini–Hochberg correction for each database separately, and for GO classes (molecular function, cellular component, biological process) independently. i. Distribution of Igj⁺⁺ cells, shown as percentages of Cd79α-expressing cells for each tissue type. j. Representative FACs scatterplots from 2 independent experiments, showing increased plasma cell abundance in aged marrow. Cd138, plasma cell marker; B220, B cell marker. k. FACs quantification of plasma cells in kidney and marrow, n = 4 independent mice. Significance was assessed using Student’s t-test; data are mean ± s.e.m. I. Representative images from 2 independent experiments showing the visualization of Igj in the kidneys of 3-month-old and 24-month-old mice using RNAscope. Almost no Igj signal was present in young kidneys. Scale bars, 100 μm.
cell types and changes in cell-intrinsic gene expression is present in most tissues; however, overall, cell-type composition seems to be more important.

Changes of cell-type composition with age, and in particular the accumulation of immune cells in tissues such as visceral fat, is well established. However, temporal and cell-specific resolution is lacking. Given that expansion of visceral fat predicts morbidity and mortality\(^{15}\), we aimed to discover the origin of the age-related adipose inflammatory signature by using single-cell transcriptomic data from the Tabula Muris Senis. We identified increasing numbers of T cells and B cells in the gonadal adipose tissue with age, including a unique cluster of Cd79\(a/b\)-positive plasma cells that were present only in old mice (Fig. 3d, e); this is consistent with increased expression of adaptive immune response genes in whole organs (Fig. 2a, g). Unbiased screening of genes enriched in this population revealed high \(Igj\) expression (Fig. 3f, Supplementary Tables 11, 13). Considering that we observed differential expression of \(Igj\) in all of 17 whole organs (Fig. 1g), we analysed thousands of Cd79\(a/b\)-positive plasma cells across organs, revealing a unique cluster of \(Igj^{\text{ph}}\) cells in both the FACs scRNA-seq and droplet datasets, concordant with high expression of plasma B cell markers \(xbp1\) and \(Derl3\) (Fig. 3g, Extended Data Fig. 9a, b, f, g). Relative to \(Igj^{\text{ph}}\) cells, these cells show increased expression of genes relating to the unfolded protein response and endoplasmic reticulum stress pathways, characteristic of highly secretory plasma B cells\(^{14}\) (Fig. 3h, Extended Data Fig. 9c, Supplementary Tables 12, 14). Notably, these plasma B cells originate almost entirely from aged mice, and accumulate across diverse organs (Fig. 3i–l, Extended Data Fig. 9d, e, h, Supplementary Fig. 1). Taking advantage of the high temporal resolution available with the whole-organ dataset, we also traced these cells across the lifespan through the expression of \(Igj\).

We observed an initial increase of \(Igj\) in marrow, bone and spleen—organs responsible for producing adaptive immune cells (Extended Data Fig. 9j). Notably, the expression of \(Igj\) and \(Derl3\) subsequently increases in GAT and in the kidney at around 12 months of age, preceding the increase of \(Igj\) in BAT, heart and lung. \(Igj\) expression also increases in human visceral and subcutaneous fat (Genotype-Tissue Expression (GTEx) data; Extended Data Fig. 9j). It is possible that the changes in expression of genes encoding chemokine signalling and cell-surface receptors and ligands that we observe here could explain this differential accumulation. We then reconstructed the B cell receptor locus using scRNA-seq data, and found that \(Igj^{\text{ph}}\) plasma cells are predominantly of the IgM class (Extended Data Fig. 9k). Notably, we detected several clones present across diverse tissues, suggesting that these cells are trafficked to tissues from a common origin (Extended Data Fig. 9i). The role of these cells—or the specificity of the antibodies they produce—is currently unknown, but it is tempting to speculate that they may contribute to the global increase in autoantibodies that has been reported to occur upon ageing\(^{24}\).

**Correlation between plasma proteins and organ mRNA**

Investigating organs individually can reveal detailed ageing processes, and even common phenotypes that are potentially susceptible to intervention. However, ageing occurs systematically, with the decline of one organ possibly inciting or accelerating dysfunction throughout the body. In part, this may be due to alterations in blood-borne factors that mediate intercellular and organ–organ communication. Encouraged by heterochronic parabiosis experiments demonstrating rejuvenation\(^{15,16}\), we and others have identified plasma proteins with detrimental
or rejuvenating functions in ageing brain, muscle, pancreas, bone and other organs, as well as hundreds more proteins that are correlated with human ageing and are associated with traits such as cognition and grip strength. However, the origins of these factors remain largely unknown.

Here we attempted to determine which organs contribute to age-related changes in the plasma proteome by correlating plasma protein age trajectories with their corresponding gene expression trajectories in each organ (Fig. 4a, b). This analysis revealed 25 plasma proteins that are correlated (Spearman's correlation coefficient $\rho > 0.6$) with gene expression in at least one organ, totalling 35 unique plasma protein/organ pairs. We discovered high correlation for several, such as vascular cell adhesion molecule 1 (VCAM1) in the kidney and fibroblast growth factor 10 (FGF10) in the spleen, and other notable pairs such as glial fibrillary acidic protein (GFAP) in the brain. Of particular interest are VCAM1 and periostin (POSTN), which both show exceptional correlation across several organs (Fig. 4c–j). VCAM1 was recently identified as a critical mediator of brain ageing by old plasma, and the loss of POSTN in adipose tissues contributes to impaired lipid metabolism. Furthermore, both are implicated in extracellular matrix regulation and fibrotic diseases, perhaps indicating age-related fibrosis. Thrombospondin-4 (TBS4), levels of which decreased with age and were highly correlated with gene expression in muscle in this study, has recently been shown to promote synapse formation and be enriched in young blood. Notably, white adipose tissues emerge from this analysis as well, with five plasma proteins highly correlated with gene expression in visceral MAT and VAT, and three in SCAT (Fig. 4a). In limb muscle, which has a modest number of DEGs, seven plasma proteins are correlated with gene expression across the lifespan, including POSTN, bone morphogenic protein-1 (BMP1), matrix metalloprotease-2 (MMP2) and other proteins associated with the extracellular matrix (Extended Data Fig. 10a). Such extracellular-matrix-associated proteins constitute a majority of the 25 plasma proteins we identified (Extended Data Fig. 10b). Although these findings are intriguing, the abundance of plasma proteins may change independently of differences in gene expression. For example, a tissue may preferentially release greater amounts of certain proteins with age. Furthermore, some tissues lack age correlation but show high expression of genes encoding plasma proteins (Supplementary Table 10). Future research will determine whether these changes in gene expression contribute to functionally relevant age-related differences in the plasma proteome, or whether they mediate other processes such as immune cell adhesion and infiltration.

**Discussion**

Our dataset provides temporal resolution of the transcriptome and the plasma proteome for all major organs across the entire lifespan of the mouse, and can serve as a fundamental resource for biologists across many disciplines. We discovered gene expression trajectories consistent with previously identified deleterious processes such as mitochondria dysfunction, impaired protein folding and inflamming. Furthermore, DEGs emerging in middle age are highly correlated with those in late life, suggesting that these harmful processes emerge early across diverse organs. With rejuvenation strategies such as senescent cell ablation (senolytics), nutrient sensing manipulation (by treatment with rapamycin and metformin) and plasma proteome alteration progressing rapidly, we need an improved understanding of where and when to apply these therapies. Indeed, the common ageing patterns observed here may help to explain the profound health-span benefits of such interventions. In summary, this organism-wide characterization of ageing dynamics may accelerate the development of therapeutics, and the insights into circadian rhythm disruption, plasma cell accumulation and adipose decline suggest avenues for renewed focus.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of code availability are available at https://doi.org/10.1038/s41586-020-2499-y.
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Methods

Mice and organ collection
Male and virgin female C57Bl/6JN mice were shipped from the National Institute on Ageing colony at Charles River (housed at 19–23 °C) to the Veterinary Medical Unit (VMU; housed at 20–24 °C) at the VA Palo Alto (VA). At both locations, mice were housed on a 12-h light/dark cycle, and provided food and water ad libitum. The diet at Charles River was NIH-31, and Teklad 2918 at the VA VMU. Littermates were not recorded or tracked, and mice were housed at the VA VMU for no longer than 2 weeks before euthanasia, with the exception of mice older than 18 months, which were housed at the VA VMU beginning at 18 months of age. After anaesthesia with 2.5% v/v Avertin, mice were weighed, shaved, and blood was drawn via cardiac puncture before transcardial perfusion with 20 ml PBS. Whole organs were then dissected in the following order: pancreas, spleen, brain, heart, lung, kidney, mesenteric adipose tissue, intestine (duodenum), gonadal adipose tissue, muscle (tibialis anterior), skin (dorsal), subcutaneous adipose tissue (inguinal pad), brown adipose tissue (interscapular pad), bone and bone marrow (femurs and tibiae). Mice were randomized and organs collected from 8:30–16:00 over several days. Organs were immediately snap-frozen on dry ice. All animal care and procedures were carried out in accordance with institutional guidelines approved by the VA Palo Alto Committee on Animal Research.

Sample size, randomization and blinding
No sample size choice was performed before the study. Randomization was performed in the case of mouse dissection order and during the preparation of 96-well plates for cDNA creation. Blinding was not performed: the authors were aware of all data and metadata-related variables during the entire course of the study.

RNA isolation and preparation
Snap-frozen bone and skin was crushed on liquid nitrogen with a mortar and pestle. Snap-frozen whole organs, or crushed bone or skin, were placed in TRizol and immediately homogenized with a TissueRuptor in 50 ml conical flasks (see Supplementary Table 15 for organ-specific details). Debris from the homogenate was pelleted in 1.5-ml tubes at 12,000g for 5 min at 4 °C. The supernatant was then transferred to a new 1.5-ml tube to which chloroform was added. After vortexing on maximum speed for 10 s, samples were transferred to 1.5-ml or 2-ml Phase Lock Gel tubes, to which water was added before spinning at 12,000g for 5 min at 4 °C. The aqueous phase was then transferred to a new tube, and after adding isopropanol, mixtures were vortexed at max speed for 10 s. Solutions were then run through RNeasy columns according to the manufacturer’s instructions, and eluted with the indicated volume of water. RNA was then quantified with a nanodrop, and stored to exclude low-coverage samples. Data visualization and analysis were performed using custom Rstudio scripts and the following Bioconductor packages: Rsne, Deseq2, topGO, destiny and org. Mn.eg.db. To assess the quality of our dataset, the raw count matrix was normalized using DESeq2 before conducting the built-in variance stabilizing transformation. Principal component analysis revealed that samples clustered mostly by tissue, with the exception of the white fat tissues. We also plotted our data after running t-SNE, using 500 iterations and retaining either 50 or 6 principal components—that is, with most principal components or only the ones explaining the most variance, respectively. We further complemented these analyses with hierarchical clustering using Ward’s clustering algorithm. In order to detect whether samples within a given tissue would show profound clustering by age, we finally calculated diffusion maps using the R package destiny with default parameters.

To identify significant differential expression changes with age, we used the raw count matrix as recommended for the DEseq2 standard analysis pipeline. Factors and dispersion estimates were calculated for each tissue separately. We conducted differential expression analysis comparing samples from 3-month-old mice to each consecutive time point, using age and sex as covariates. P values were adjusted for multiple testing, and genes with an adjusted P value of less than 0.05 were determined to be statistically significant. In addition, we ran similar analyses using 1-month-old or 6-month-old mice as reference.

To rank genes on the basis of their regulation across tissues, we summarized in how many tissues a given gene would be called as significantly regulated in at least one comparison between samples from 3-month-old mice and any following sampling time point.

Gene expression trajectory analysis
To estimate gene trajectories during ageing, normalized counts from DEseq2 were z-scored and LOESS regression was fitted for each gene using the median expression per age group in each tissue. Whole-organism trajectory per gene was estimated using the average trajectory across the 17 tissues. Organism-wide analysis focused on 11,403 genes expressed in all tissues (that is, genes among the 15,000 most expressed genes in each tissue).

The distance matrix between whole-organism gene trajectories was computed using the Euclidian distance and hierarchical clustering was performed using the complete method. We identified 10 clusters of genes changing with age, ranging from 1 to 4,571 genes. Clusters 9 and 10 were excluded from further analysis as they included fewer than 10 genes.

To identify clusters that changed the most between tissues, we computed an amplitude and variability index. The amplitude index corresponds to the change in the z-score (absolute value) of the average trajectory between 1 month and 27 months. The variability index, which measures the spread of organ trajectories, corresponds to the average Euclidian distance between each organ-specific trajectory and the organism-wide trajectory.

Reactome, KEGG and GO databases were queried to understand the biological functions of each cluster. We used the R TopGO package for GO analysis and the R clusterprofiler package for KEGG and Reactome analyses. The 11,403 genes expressed in all tissues served as the background set of genes against which to test for over-representation. Because clusterprofiler requires EntrezID as input, we mapped gene symbols to EntrezID using the org.Mm.eg.db package. When Gene Symbols were mapped to multiple EntrezID, only the first EntrezID was used. q values were estimated using the Benjamini–Hochberg approach for the different databases taken separately. In addition, for GO analysis, q values were calculated for the three GO classes (molecular function, cellular component, biological process) independently.

Organ-specific clustering was performed using the 15,000 most expressed genes per tissue. For each tissue, five clusters were considered for further analysis and the pathways analysis used the corresponding background set of genes against which to test for over-representation.

Single-cell RNA-sequencing analysis
Pre-processed and annotated scRNA-seq data (FACS followed by Smart-seq2 protocol) from gonadal adipose tissue (3 and 24 months old) were obtained from the Tabula Muris Senis consortium. Given
the lack of data from aged female mice, we focused our analyses on samples derived from male mice. Additionally, cells with fewer than 200 or more than 6,500 genes were excluded. This yielded 1,962 high-quality cell transcriptomes derived from four young and four old biological replicates. Data visualization and analysis were performed using custom RStudio scripts and the following Bioconductor packages: Seurat (v.3)30 and topGO. Data normalization and scaling was performed using Seurat’s built-in SCTransform function with default parameters. A shared-nearest-neighbours graph was constructed using the first 30 principal components before clustering cells using Seurat’s built-in FindClusters function with a resolution of 0.8 and default parameters. Annotations for B cells and T cells were adopted from the Tabula Muris Senis31. Cell numbers were normalized to the total number of detected cells and compared using standard t-tests. \( \text{Ig}_j \) B cells formed a separate cluster and were identified using Seurat’s FindMarkers function (parameters: only.pos = T min.pct = 0.15 thresh. use = 0.25, test = ‘MAST’).

To profile \( \text{Ig}^\mu \) B cells organism-wide, we obtained the complete and pre-processed scRNA-seq dataset (FACS followed by Smart-seq2 protocol) from the Tabula Muris Senis—encompassing cells isolated from all major tissues. Focusing on data from samples from male mice only, we filtered for cells showing detectable expression of the \( \text{Cd79a} \) gene (alpha chain of the B cell receptor). Cell transcriptomes were thus derived from four young and four old biological replicates. The resulting 10,867 cells were analysed using Seurat, as described above. A shared-nearest-neighbours graph was constructed using the first 10 PC dimension before clustering cells using Seurat’s built-in FindClusters function with a resolution of 0.4 and default parameters. \( \text{Ig}^\mu \) B cells formed a separate cluster (cluster 11; 129 cells). To characterize \( \text{Ig}^\mu \)-specific expression profile, Seurat’s FindMarkers function (parameters: only.pos = F min.pct = 0.15 thresh.use = 0.25, test = ‘MAST’) was run comparing the cluster of \( \text{Ig}^\mu \) B cells against all other \( \text{Cd79a} \) cells in the dataset. Functional enrichment analysis of the top 300 differentially expressed genes (sorted by adjusted \( p \)-value) were compared to all 1,051 genes passing the filtering parameters for the test. Top-ranked GO terms were selected and visualized using the CellPlot package (https://github.com/dieterich-lab/CellPlot). The full-length GO terms were shortened to fit into the figure format; the complete table of significantly enriched GO terms and associated genes can be found in Supplementary Table 12.

Single-cell RNA-sequencing analysis (microfluidic droplet)
The Tabula Muris Senis encompasses scRNA-seq data generated with microfluidic droplets, enabling the profiling of more cells without prior selection of surface markers32. To profile \( \text{Ig}^\mu \) B cells organism-wide, we obtained the complete and pre-processed droplet dataset. Focusing on data from samples from male mice only, we filtered for cells showing detectable expression of the \( \text{Cd79a} \) gene. Cell transcriptomes were derived from the following sampling time points: 2× 1 month, 2× 3 months, 2× 18 months, 4× 24 months and 3× 30 months. The resulting 23,796 cells were analysed using Seurat, as described above. A shared-nearest-neighbours graph was constructed using the first 10 PC dimension before clustering cells using Seurat’s built-in FindClusters function with a resolution of 0.4 and default parameters. \( \text{Ig}^\mu \) B cells formed a separate cluster (cluster 5; 1,198 cells). To characterize \( \text{Ig}^\mu \)-specific expression profile, Seurat’s FindMarkers function (parameters: only.pos = F min.pct = 0.15 thresh.use = 0.25, test = ‘MAST’) was run comparing the cluster of \( \text{Ig}^\mu \) B cells against all other \( \text{Cd79a} \) cells in the dataset. Functional enrichment analysis of the top 300 differentially expressed genes (sorted by adjusted \( p \)-value) were compared to all 1,886 genes passing the filtering parameters for the test. Top-ranked GO terms were selected and visualized using the CellPlot package. The full-length terms were shortened to fit into the figure format; the complete table of significantly enriched GO terms and associated genes can be found in Supplementary Table 14.

Plasma proteomic analysis
All animal care and procedures were carried out in accordance with institutional guidelines approved by the VA Palo Alto Committee on Animal Research. Sixty-five male (n = 5–6 per age group: 1, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 months old) and 16 virgin female (n = 4 per age group: 3, 12, 18 and 21 months old) C57BL/6J mice were shipped from the National Institute on Ageing colony at Charles River (housed at 19–23°C) to the Veterinary Medical Unit (VMU; housed at 20–24°C) at the VA Palo Alto (VA). Mice were provided food (NIH-31 at Charles River, and Teklad 2918 at the VA VMU) and water ad libitum. Mice were housed on a 12 h/12 h light/dark cycle at both places. Mice older than 18 months were housed at the VA VMU until they reached the experimental age. Mice younger than 18 months were housed for less than 2 weeks at the VA VMU. After anaesthetization with 2.5% v/v Avertin, blood was drawn via cardiac puncture. EDTA-plasma was isolated by centrifugation at 1,000g for 10 min at 4°C. Samples were aliquoted, stored at −80°C and sent on dry ice to SomaLogic.

The SomaLogic platform is primarily designed to detect and measure human proteins. To reduce the influence of cross-species effects on our analysis, we first determined proteins in our dataset with high evolutionary conservation between mice and humans. To this end, we downloaded the plain text file containing all homologies between mouse and human along with sequence identifiers for each species (HOM_MouseHumanSequence.rpt) from MGI (http://www.informatics.jax.org/). Next, reference protein sequences for human and mouse were downloaded from UniProt (https://www.uniprot.org/). Using the R “Biostrings” library a global pairwise sequence alignment was carried out between the human and mouse sequences. Only sequences with identity of 80% across the whole alignment were included in the downstream analyses.

To determine the effect of age on the plasma proteome, relative fluorescent units (RFUs) provided by SomaLogic were log_{10}-transformed and linear models adjusted for age and sex were used. Type II sum of squares (SS) were calculated using the R package car33 and \( q \)-values were estimated using the Benjamini–Hochberg approach34.

Raw protein abundance data, as measured by the Somatologic platform, were scaled (z-transformed) before calculating the median across all replicates for each sampled age time point to obtain an average trajectory. Normalized RNA-seq counts after pre-processing with DESeq2 were transformed for each tissue alike. To compare plasma protein changes with shifts in mRNA expression in any tissue, we calculated pairwise Spearman’s rank correlation coefficients between a given protein trajectory with the expression trajectory for its corresponding gene in each tissue separately. Thus, a given protein could be correlated with expression changes in multiple tissues. To limit our analysis to mRNA/protein pairs reflecting robust changes, we filtered the resulting mRNA/protein correlations as follows: (1) The protein had to exhibit a sequence homology between human and mouse of at least 75% (770 proteins). (2) The protein had to change significantly with age according to the linear modelling analysis (\( q < 0.05; 115 proteins \)). (3) The corresponding gene had to be differentially expressed in the given tissue in at least one pairwise comparison between 3 months old mice and any consecutive time point (\( q < 0.05; 95 proteins \)). (4) The mRNA/protein profiles had to exhibit a Spearman’s rank correlation of at least 0.6 (25 proteins; 35 protein/gene pairs). Benjamini–Hochberg correction per tissue was applied to assess significance of proteins/mRNA profiles correlations. Given that genes can be expressed at differing levels across tissues, we additionally calculated average mRNA expression ranks for each gene. To this end, we ranked for a given gene each tissue on its average mRNA expression, based on baseMean in DESeq2.

To investigate connectivity networks between top proteins correlated with organ gene expressed, we used String v.11.0, available at https://string-db.org/35.
Correlation analysis of gene expression and ageing using self-organizing maps

For every gene a tissue-wise Spearman's rank correlation coefficient was computed on the basis of expression and age. Similarly, we computed Spearman's rank correlation coefficients for expression and sex. The resulting correlation matrix was then filtered, such that only genes that were significantly correlated with either age or sex in any tissue \( (P < 0.01) \) after false discovery rate (FDR) adjustment with the Benjamini–Hochberg procedure \(^6\) were considered. This matrix was then used to create a self-organizing map (SOM) \(^5\) with the Kohonen R package \((v.3.0.8)\) \(^8\). In addition to Spearman's rank correlation coefficients for comparing gene expression and sex we also tested other measures, such as log transformed \(P\) values from one-sided Wilcoxon rank sum test (testing both—that is, 'greater' and 'less' alternatives and flipping the sign if the 'greater' alternative yielded a lower \(P\) value) and Somers' \(D\). Because the different approaches gave similar results we continued to use Spearman's correlation for gender and age for better comparability.

Specificity of gene expression for tissues and ages

To identify how specific a gene is expressed at a certain time point or in a certain tissue, we used an approach known from gene set analysis, so-called gene set enrichment analysis (GSEA). Instead of computing how significantly a biological category is enriched in a sorted list of genes, we computed how enriched a certain tissue, age, or pair of tissue/age is in each gene. For each gene we computed 10 enrichment scores for the 10 ages, 17 enrichment scores for the 17 tissues and 170 enrichment scores for their combinations. The specificity is defined as the difference between the maximal enrichment and second maximal enrichment for all tissues, time points and combinations. The higher the difference the more specific the gene either for a tissue, time point or the combination thereof. Notably, the maximal enrichment score can be translated into \(P\) values—for example, by random sampling. For better comparability, however, we present the running sum directly and do not translate them to \(P\) values. Here, the approach is referred to as sample set enrichment analysis (SSEA).

Estimating the variance of the data depending on metadata

To estimate the variance in the data depending on age, tissue or gender we made use of principal variance component analysis (PVCA) as implemented in the Bioconductor Package peca. PVCA combines the strength of principal component analysis and variance components analysis (VCA). Originally it was applied to quantify batch effects in strength of principal component analysis and variance components analysis \((pvca)\). We then selected those cells expressing it \((\text{log}(1 + \text{cpm}) > 0)\), and calculated their weighted centre in the single-cell latent space, where we considered normalized gene expression values as weights. We then defined the 'single-cell dispersion' as the weighted mean distance of the cells from their centre, normalizing within each tissue to enable cross-tissue comparisons. Finally, we defined methodology to systematically analyse gene expression: we computed for each gene per tissue the Spearman correlation of its bulk DESeq2 normalized gene expression with ageing. We then plotted the single-cell dispersion against the Spearman's rank correlation coefficient for each bulk ageing DEG.

Cell intrinsic gene expression versus cell abundance

We first selected FACS cells from the Tabula Muris Senis with more than 500 distinct genes and 50k reads. Owing to the lower number of replicates of female mice, we considered only data from male mice. We then used the log \((1 + \text{cpm})\) transformed data, and considering each tissue separately, we binned these cells into young \((\leq 3\text{ months}; Y)\) and old \((> 3\text{ months}; O)\). For each gene, we then calculated the log-fold-change of cell counts and read counts between \(Y\) and \(O\), where cell count is defined as the fraction of cells within the tissue expressing the gene \((\log(1 + \text{cpm}) > 0)\), and read count is defined as the mean read count of the gene in the cells that express it. Next, we analysed each gene in the bulk data, first computing the Spearman's correlation of DESeq2 normalized gene expression with age. We then binned genes into those increasing with age (Spearman > 0.7; I) and decreasing with age (Spearman < 0.7; D). Finally, we compare the single-cell log \(Y\) versus \(O\) cell and read counts with the bulk correlations I and D by running the Wilcoxon–Mann–Whitney test, to determine whether single-cell data based cell or read count changes separate the bulk groups I and D. We then plot the resultant read and cell count \(U\) statistics against the corresponding \(P\) value for each tissue for the droplet and FACS single-cell data separately.

Deconvolution with CIBERSORTx

We first used the signature matrix creation feature of CIBERSORTx \(^{11}\), which detects cell-type-specific signature genes using annotated single-cell data. As input, we used male cells from Tabula Muris Senis of every age to create tissue-specific signature matrices from the FACS and droplet data, separately. Consistent with cell selection criteria used through the manuscript, we selected droplet cells with more than 500 distinct genes and 5k reads, and more than 500 distinct genes and 50k reads from FACS cells. We input single-cell normalized cpm data without log transformation, as well as cpm bulk tissue data on which to perform deconvolution. Deconvolution was performed in 5-mode owing to the possibility of high technical variance, with all other parameters set to default, and the resultant inferred cell type fractions were correlated with age using Spearman's rank correlation.

Igj and plasma cell validation experiments

RNAseq Multicolor Fluorescent Reagent Kit v2 was used on fresh frozen kidney sections 20-μm thick from 3-month-old and 24-month-old C57BL/6J male mice, with Igj-C1 probes and Opal 690 Reagent Pack (Akoya Biosciences FP1497001KT), with no modifications to the kit instructions. Images were acquired at 20X on a Keyence BX-710 fluorescence microscope.

Cell suspensions for FACS of plasma cells were generated from GAT and kidney. After cardiac perfusion with PBS, tissues were immediately dissected and minced to a paste with scissors. Samples were resuspended in 40 ml buffer \((\text{GAT}, 10\% \text{ horse serum in F12}; \text{kidney}, 2\% \text{ FBS in RPMI})\) and passed through a 100-μm filter into a 50 ml conical tube, grinding with a syringe plunger to further dissociate clumps. Filters were washed with 5 ml buffer, and all 45 ml was then passed through a 35-μm filter, washing with another 5 ml buffer. Filtered cells were then pelleted \((500g, 5\text{ min}, 4^\circ C)\) and treated with 10 ml ACK lysis buffer for 5 min at room temperature. After pelleting, washing with 5 ml buffer and pelleting again, cells were resuspended in 1 ml FACS buffer \((2\% \text{ FBS in PBS})\), and aliquoted through 35-μm strainer caps into FACS tubes. Cells were pelleted and resuspended in the antibody cocktail: Cd138-PE \((1:400); \text{BioLegend}142504), \text{Cd19-BV421} \((1:200); \text{BioLegend}115538), \)
B220-APC (1:100; BioLegend 103212) for 30 min on ice. After pelleting and resuspending, cells were stained with 1 μl 1:1,000 SYTOX Green (Thermo Fisher S7020) immediately before sorting on a BD FACS Aria III. Gates were set to capture live singlets and reduce debris according to standard procedures, and Cd138high cells were quantified.

**Data availability**

Raw data are available from the Gene Expression Omnibus under accession code GSE132040. Source data are provided with this paper.
Extended Data Fig. 1 | Gene expression variance analysis. a, Visualization of the principal variance component analysis, displaying the gene expression variance explained by residuals (that is, biological and technical noise) or experimental factors such as tissue, age, sex and respective combinations. n = 904 total samples. b, c, t-SNE visualization of all samples, based on the first six principal components coloured by age (b) and sex (c). d, Hierarchical clustering of all samples using Ward’s algorithm. Samples are annotated by tissue, sex and age. Highlighted are samples clustering by sex in selected tissues. Non-specific clustering of samples derived from white adipose tissues is further highlighted.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Validation of differential gene expression analysis.

a, Heat map displaying the number of DEGs per tissue for pairwise analysis on adjacent time points. b, Heat map displaying the number of DEGs per tissue for pairwise comparisons, referenced to tissues from 1-month-old mice. c, Heat map displaying the number of DEGs per tissue for pairwise comparisons, referenced to 6-month tissues. d, Box plot displaying the number of DEGs per tissue (n = 17 tissues) for pairwise comparisons with a 3-month reference. In box plots, the centre line denotes the mean, box boundaries denote the first and third quartiles. Outliers show tissues undergoing exceptionally strong expression shifts at a given age. e, Enrichment for functional categories in the top 100 genes that are differentially expressed in the most tissues (ranked using pairwise comparisons with a 3-month reference). Pathway enrichment with GO, Reactome and KEGG databases. Enrichment was tested using Fisher’s exact test (GO) and the hypergeometric test (Reactome and KEGG). To estimate the contribution of each tissue, we used the number of genes per pathway in the top 100 DEGs and estimated the percentage of significant genes per tissue. q-values were estimated using the Benjamini–Hochberg procedure for each database separately, and for GO classes (molecular function, cellular component, biological process) independently. Values of n are as in d.

f, Cumulative sum of DEGs per tissue in the ranked top 100 genes. g, Number of DEGs per tissue in the top 100 genes. n = 54 (MAT), 52 (kidney), 52 (GAT), 54 (spleen), 50 (liver), 54 (lung), 50 (intestine), 55 (SCAT), 51 (skin), 53 (BAT), 52 (heart), 52 (muscle), 53 (brain), 52 (WBC), 54 (bone), 51 (marrow), 46 (pancreas). q-values are as in e.

h, STRING analysis of the top 30 genes in Fig. 1g.
**Extended Data Fig. 3 | SOMs of gene correlation with age and sex.**

(a, b) SOMs were generated from transcriptome-wide gene expression correlation (Spearman’s rank correlation coefficient) of each gene ($n = 12,462$ genes) with age (a) and sex (b). Genes with similar correlation are mapped to the same cell, and cells are grouped by similarity. The SOM cell layout is common across organs, with the average across all organs at the bottom.
**Extended Data Fig. 4** | Sex-specific expression changes across organs.

(a) Smoothed line plot displaying the number of DEGs between female and male mice at each age. Positive (negative) values represent upregulated (downregulated) genes. Grey lines represent all other tissues.

(b) Heat map representation of data from (a).

(c) Expression of Apoe mRNA in GAT and Axin2 mRNA in spleen. The black line indicates LOESS regression. \( n = 45 \) (GAT) and \( n = 47 \) (spleen) independent samples.

(d) Venn diagrams depicting the overlap of DEGs in female and male mice detected at 3 months and 18 months of age in GAT, SCAT, liver and kidney. One-sided Fisher’s exact test; *** \( P < 0.0001 \).

(e–h) Top 10 GO terms enriched among the DEGs between female and male mice at 18 months of age in GAT (e), SCAT (f), liver (g) and kidney (h). Data are mean ± s.e.m. \( n = 2 \) (female) and \( n = 4 \) (male) independent mice for each organ. \( q \) values were estimated using the Benjamini–Hochberg procedure for each database separately, and for GO classes (molecular function, cellular component, biological process) independently.
Extended Data Fig. 5 | Organ-specific gene expression dynamics. For each of the 17 organs (rows), the average trajectory of the 15,000 most highly expressed genes is represented in column 1. Five clusters were used (columns 2–6) for further analysis. Average trajectories for each cluster ± s.d. are represented.
Extended Data Fig. 6 | Pathway enrichment analysis of organ-specific clusters. Clusters from Extended Data Fig. 5 show enrichment for genes in functional categories. Pathway enrichment was tested using GO, Reactome and KEGG databases. Enrichment was tested using Fisher’s exact test (GO) and the hypergeometric test (Reactome and KEGG). The top five pathways for each cluster are shown. $q$ values were estimated with the Benjamini–Hochberg procedure for each database separately, and for GO classes (molecular function, cellular component, biological process) independently. Sample size per cluster or tissue is indicated in Extended Data Fig. 5.
Extended Data Fig. 7 | Analysis of cytokines and transcription factors.

a, Age-related changes in inflammatory cytokine and chemokines (cytokine-mediated signalling pathways GO:0019221; n = 501 genes), and transcription factors (TRANSFAC database; n = 334 genes). Thicker lines outlined with white represent the average trajectory for each cluster ± s.d.

b, c, Spearman correlation coefficient for ageing genes in a.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Integration of bulk and single-cell transcriptomic data. a, b, Representative GO terms enriched among the genes with highly disperse (a) and cell-specific (b) expression patterns. n = 1,108 cells. q values were estimated using the Benjamini–Hochberg procedure for each database separately, and for GO classes (molecular function, cellular component, biological process) independently. c, Expression of Aco2 mRNA in the kidney. Black line shows LOESS regression; ρ, Spearman’s rank correlation coefficient. n = 52 independent samples. Data are mean ± s.e.m. d, e, t-SNE visualization of scRNA-seq data (FACS) from the kidney, coloured by expression of Aco2 (d) and Cs (e). n = 1,108 cells. f, Violin plot representing expression of Aco1 and Aco2 across all profiled cell types in the kidney. Points indicate cell-wise expression levels and the violin indicates average distribution split by age. Significance was assessed using a Student’s t-test. n = 325 cells (3 months) and 783 cells (24 months). g, Spearman’s rank correlation for cell type fractions significantly (P < 0.05) changing with age, based on deconvolution with FACS or droplet scRNA-seq expression signatures. n = 38 (FACS, BAT), n = 37 (droplet, GAT), n = 37 (FACS, GAT), n = 34 (droplet, kidney), n = 35 (FACS, kidney), n = 35 (droplet, liver), n = 35 (FACS, liver), n = 37 (droplet, lung), n = 37 (FACS, lung), n = 38 (droplet, marrow), n = 36 (FACS, marrow), n = 38 (droplet, MAT), n = 39 (FACS, MAT), n = 34 (droplet, pancreas), n = 32 (FACS, pancreas), n = 37 (droplet, SCAT), n = 38 (FACS, SCAT), n = 35 (droplet, skin), n = 33 (FACS, skin), n = 36 (droplet, spleen), n = 37 (FACS, spleen) independent samples. h, Pairwise comparisons cell fractions between scRNA-seq (FACS), scRNA-seq (droplet), FACS-based bulk RNA-seq deconvolution and droplet-based bulk RNA-seq deconvolution. Each point represents an individual cell type in an individual tissue type.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Identifying IgJ\textsuperscript{high} B cells with FACS and droplet scRNA-seq. a, t-SNE visualization of all Cd79a-expressing cells present in the Tabula Muris Senis FACS dataset (17 tissues). Coloured clusters as identified with the Seurat software toolkit. IgJ\textsuperscript{high} B cell cluster 11 is highlighted. \(n=10,867\) cells. b, t-SNE in a coloured by the IgJ\textsuperscript{high} B cell markers Igj, Xbp1 and Derl3. c, GO terms enriched among the top 300 marker genes of IgJ\textsuperscript{high} (n = 129 cells) versus IgJ\textsuperscript{low} (n = 10,738 cells) (FACS). \(q\) values were estimated using the Benjamini–Hochberg procedure for each database separately, and for GO classes (molecular function, cellular component, biological process) independently. d, Distribution of IgJ\textsuperscript{high} as percentages of Cd79a-expressing cells per tissue. e, Percentage of IgJ\textsuperscript{high} B cells of all Cd79a-expressing cells across all tissues. \(n=5\) (3 months) and \(n=4\) (24 months) independent mice. Significance was assessed using a Student’s \(t\) test. Data are mean ± s.e.m. f, t-SNE visualization of all Cd79a-expressing cells present in the Tabula Muris Senis droplet dataset (17 tissues). Coloured clusters as identified with the Seurat software toolkit. IgJ\textsuperscript{high} B cell cluster 5 is highlighted. \(n=23,796\) cells. g, t-SNE in f coloured by the B cell marker Cd79a and IgJ\textsuperscript{high} B cell marker Derl3. h, Percentage of IgJ\textsuperscript{high} B cells of all Cd79a-expressing cells across all tissues. I, Heat map of the z-transformed IgJ expression trajectories across bone (n = 54), marrow (n = 51), spleen (n = 54), liver (n = 50), GAT (n = 52), kidney (n = 52), heart (n = 52) and muscle (n = 52). j, Change in IgJ mRNA expression in human visceral fat (aged 20–29, n = 25; aged 50–59, n = 124; aged 70–79, n = 12) and subcutaneous fat (aged 20–29, n = 32; aged 50–59, n = 149; aged 70–79, n = 13) (data from GTEx consortium). In the box plot, the median is represented by the centre line and the box boundaries represent the first and third quartiles. k, Number of IgJ\textsuperscript{high} B cells with a successfully assembled B cell receptor locus, split by mouse and immunoglobulin class. l, Clonally amplified IgJ\textsuperscript{high} B cells as detected in mouse 1 and mouse 3, grouped by tissue of origin (colour) and immunoglobulin class (shape).
Extended Data Fig. 10 | STRING analysis of top correlating plasma proteins. **a.** The top seven plasma proteins correlated with gene expression in muscle, coloured by pathway. **b.** The top 25 plasma proteins correlated with gene expression in any organ.
Reporting Summary

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When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

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☐ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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☐ Only common tests should be described solely by name; describe more complex techniques in the Methods section.
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☐ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
☐ Clearly defined error bars
☐ State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

| Data collection | FACS data was collected with BD FACS Diva version 8. Sequencing data was collected with the NovaSeq Control Software version 1.6.0. |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------|
| Data analysis   | FACS data was analyzed with FlowJo version 10.6.1. Sequences from the NovaSeq were de-multiplexed using bcl2fastq version 2.20. Reads were aligned to the GRCh38.p6 (mus musculus) genome with Gencode v.M19 annotations using using STAR version 2.5.2b with parameters TK. Gene counts were produced using HTSEQ version 0.6.1p1 with default parameters, except “stranded” was set to “false”, and “mode” was set to “intersection-nonempty”. Seurat version 3 String version 11.0 Kohonen R package (version 3.0.8) Rtsne version 0.15 topGO version 2.36 destiny version 2.14 org.Mm.eg.db version 3.8.2 clusterProfiler version 3.12 car version 3.0.4 DESeq2 version 1.26.0 CellPlot version 1.0 |
Data

Policy information about availability of data
All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Raw data are available on GEO (GSE132040).

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Life sciences

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
No statistical methods were used to pre-determine sample size. The number of mice was restricted due to costs and practical constraints of collecting and processing organs. However, the study is designed such that there are many closely related experimental groups (up to 10 different ages in close proximity across the lifespan), allowing for pooling of closely related ages, and for analyses across the lifespan that include >50 samples. Additionally, as this is the largest study of its kind, so far, we believe the sample size is sufficient.

Data exclusions
Exclusion criteria were pre-establish. Bulk: samples with fewer than 4 million uniquely mapped reads were excluded.
Preprocessed data from Tabula Muris Senis was filtered as follows: We removed genes not expressed in at least 3 cells and then cells that did not have at least 250 detected genes. For FACS we removed cells with less than 5000 counts and for droplet cells with less than 2500 UMIs.
FACS single-cell adipose data was filtered as follows: cells with less than 200 or more than 6,500 genes were excluded.

Replication
We have not replicated the RNA-sequencing findings in this study, except in the case of IgJ-high plasma cells, which were verified with RNAscope and FACS. All attempts (that were technically successful) at RNAscope and FACS for plasma cells successfully replicated the age-related increase.

Randomization
Randomization was performed in the case of mouse dissection order and during the preparation of 96-well plates for cDNA creation.

Blinding
Blinding was not applicable for the transcriptomic data analysis. Blinding was performed for the RNAscope and FACS analysis.

Reporting for specific materials, systems and methods

Materials & experimental systems
n/a Involved in the study
□ Unique biological materials
□ Antibodies
□ Eukaryotic cell lines
□ Palaeontology
□ Animals and other organisms
□ Human research participants

Methods
n/a Involved in the study
□ ChiP-seq
□ Flow cytometry
□ MRI-based neuroimaging
Antibodies

| Antibodies used | Cd138-PE (1:400; Biolegend142504, Clone 281-2, Lot B246403), Cd19-BV421 (1:200; Biolegend 115538, Clone 6D5, Lot B263601), B220-APC (1:100; Biolegend 103212, Clone RA3-682, Lot B271008) |

Validation

Antibodies were validated for murine flow cytometry:

CD138-PE and CD19-BV421
Pracht, K. et al. A new staining protocol for detection of murine antibody-secreting plasma cell subsets by flow cytometry. Eur. J. Immunol. 47, 1389–1392 (2017).

B220-APC
Wilmore, J. R., Jones, D. D. & Allman, D. Protocol for improved resolution of plasma cell subpopulations by flow cytometry. Eur. J. Immunol. 47, 1386–1388 (2017).

Additional sources include:

Biolegend142504:
Jalkanen M, et al. 1985. J. Cell. Biol. 101:976. (FC)
Miettinen H, et al. 1994. J. Cell. Sci. 107:1571. (IF)
Li Q, et al. 2002. Cell 111:635. (IF, IHC)
McCarthy BA, et al. 2012. BMC Cancer. 12:203. (IHC)

Biolegend 115538
Shoham T, et al. 2003. J. Immunol. 171:4062. (FC)
Goodyear CS, et al. 2004. J. Immunol. 172:2870. (FC)
Kamimura D, et al. 2006. J. Immunol. 177:306. (FC)
Andoniou CE, et al. 2005. Nat. Immunol. 6:1011. (FC)
Lawson BR, et al. 2007. J. Immunol. 178:5366. (FC)
Phan TG, et al. 2007. Nat. Immunol. 8:992. (FC)
Hayashida K, et al. 2008. J. Biol. Chem. 283:19895. (IF) PubMed
Charles N, et al. 2010. Nat. Med. 16:701. (FC) PubMed
Bankoti J, et al. 2010. Toxical. Sci. 115:422. (FC) PubMed
Stadnisky MD, et al. 2011. Blood. 117:5133. (FC) PubMed
Pellet T, et al. 2012. J. Immunol. 188:1201. (FC) PubMed
Olive V, et al. 2013. Elife. 2:822. PubMed

Biolegend 103212
Coffman RL. 1982. Immunol. Rev. 69:5. (IP)
George A, et al. 1994. J. Immunol. 152:1014. (Activ)
Asensi V, et al. 1989. Immunology 68:204. (Activ)
Domati-Saad R, et al. 1993. J. Immunol. 151:5936. (Activ)
Hata H, et al. 2004. J. Clin. Invest. 114:582. (IHC)
Monteith CE, et al. 1996. Can. J. Vet. Res. 60:193. (IHC)
Shih FF, et al. 2006. J. Immunol. 176:3438. (FC)
Chang C-L-T, et al. 2007. J. Immunol. 178:6984.
Fazilleau N, et al. 2007. Nature Immunol. 8:753.
Lang GL, et al. 2008. Blood 111:2158. PubMed
Charles N, et al. 2010. Nat. Med. 16:701. (FC) PubMed
del Rio ML, et al. 2011. Transpl. Int. 24:501. (FC) PubMed

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals
CS7BL/6N males (n=4, aged 1, 3, 6, 9, 12, 15, 18, 21, 24, 27 months; and females (n=2, ages 1, 3, 6, 9, 12, 15, 18, 21 months)

Wild animals
This study did not involve wild animals

Field-collected samples
This study did not involve field-collected samples
Flow Cytometry

Plots

Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Cell suspensions for fluorescence activated cell sorting (FACS) of plasma cells were generated from gonadal adipose tissue (GAT) and kidney. Following cardiac perfusion with PBS, tissues were immediately dissected and minced to a paste with scissors. Samples were resuspended in 40ml buffer (GAT: 10% horse serum in F12; kidney 2% FBS in RPMI) and passed through a 100um filter into a 50ml conical tube, grinding with a syringe plunger to further dissociate clumps. Filters were washed with 5ml buffer, and all 45ml was then passed through a 35um filter, washing with another 5ml buffer. Filtered cells were then pelleted (500 x g, 5 minutes, 4°C) and treated with 10ml ACK lysis buffer for 5 minutes at room temperature. After pelleting, washing with 5ml buffer, and pelleting again, cells were resuspended in 1ml FACS buffer (2% FBS in PBS), and aliquoted through 35um strainer caps into FACS tubes. Cells were pelleted and resuspended in the antibody cocktail: Cd138-PE (1:400; Biolegend142504), Cd19-BV421 (1:200; Biolegend 115538), B220-APC (1:100; Biolegend 103212) for 30min on ice. After pelleting and resuspending, cells were stained with 1ul 1:1000 Sytox Green (Thermo S7020) immediately before sorting on a BD FACS Aria III. Gates were set to capture live singlets and reduce debris according to standard procedures, and Cd138high cells were quantified.

Instrument

BD FACS Aria III

Software

FACS data was collected with BD FACS Diva version 8
FACS data was analyzed with Flowjo version 10.6.1

Cell population abundance

Plasma cell abundance in young marrow: 0.007-0.023%; old marrow: 0.1-0.35%.
Plasma cell abundance in young kidney: 0-0.012%; old kidney: 0.041-0.19%

Gating strategy

FSC-A vs. SSC-A - gated on cells
FSC-A vs. FSC-H - gated for singlets
SytoxGreen-FITC vs. SSC-A - gated for live cells on SytoxGreen-FITC
CD138-PE vs. B220-APC - gated positive on CD138-PE > 10^4

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.