Supplemental Material

Mouse husbandry

All animals were treated and housed in accordance to the Guide for Care and Use of Laboratory Animals. All experimental procedures were approved by Stanford's Administrative Panel on Laboratory Animal Care (APLAC) and were in accordance with institutional and national guidelines. Male C57BL/6N mice at different ages (3, 12, and 29 month old animals) were obtained from the National Institute on Aging (NIA) colony at Charles Rivers, which is a specific-pathogen-free (SPF) facility, and were acclimated at the SPF animal facility at Stanford University for 1 to 2 weeks before processing. All animals were euthanized between 10am-12pm for tissue harvesting or NSCs isolation. No animals were censored.

Primary NSC cell culture

NSCs were isolated from 3 month, 12 month, and 29 month old male C57BL/6N mice from the NIA aging colony at Charles River as previously described (Renault et al. 2009; Webb et al. 2013). Briefly, the subventricular zone (SVZ) was finely microdissected and chopped into ice-cold PBS. Microdissected SVZs from 5 age-matched mice were pooled from each age group to make a single culture. Tissue chunks were digested by 10 min incubation 14 U/mL Papain (Worthington) at 37^{o}C. Following mechanical trituration, cells were purified by a 22% Percoll (GE Healthcare) gradient. NSCs were plated at a density of $<10^5$ cells/cm^2 as non-adherent spheres in NSC growth media (*i.e.* Neurobasal A medium [Life technologies] medium supplemented with 1% penicillin/streptomycin/glutamine [Life technologies], 2% B27 supplement [Life technologies] and 20 ng/mL each of FGF2 [Peprotec] and EGF [Peprotec]). Cultures from all ages were plated at the same density to minimize differences in autocrine/paracrine signaling. Cells were passaged using Accutase enzyme (Stem Cell Technologies, 07920). All datasets were generated on cells grown as non-adherent spheres that had been disassociated the day prior, replated as a suspension culture and collected at the end of passages 2-3 (14 days of culture). NSCs cultures derived from animals at these different ages were assessed at the end of passages 1 and 3/4 (**Supplemental Fig. S1C**). This analysis revealed that there were significantly fewer NSCs

in cultures from 29 month old mice compared to 3 month old mice at passage 1, although this difference seemed to have been erased by passage 3/4 (**Supplemental Fig. S1C**).

Tissue dissection and histopathology

For our aging 'omics' studies, we selected the heart, liver, cerebellum, olfactory bulb, as well as primary NSCs cultures derived from the Subventricular Zone (SVZ) because (i) these tissues and cells are known to display age-related functional decline (Enwere et al. 2004; Sussman and Anversa 2004; Zhang et al. 2010; Shioi and Inuzuka 2012; Mobley et al. 2014; Delire et al. 2016) and (ii) the tissues are all clearly defined anatomically, which guarantees reproducible isolation across animals of different ages and minimizes the risk that observed differences comes from dissection differences. For the heart, we dissected the ventricle tissue for chromatin and RNA extraction. For the liver, the top-most lobe was harvested for chromatin and RNA extraction. The complete anatomical structure of the cerebellum and olfactory bulb (both sides) was used for chromatin and RNA extraction. Detailed information for each sample is reported in **Supplemental Table S1A**.

Heart and liver tissue were harvested for histopathology evaluation in parallel to RNA-seq profiling. Tissues samples were processed as described previously (Harel et al. 2015). Briefly, tissues were collected into ice cold PBS, washed, then fixed for 24h in Bouin's solution at room temperature. The following day, tissues were rinsed in PBS and paraffin-embedded using standard procedures. Sections of 5 µm were stained with hematoxylin and eosin (HE) and Mason's trichrome for histopathologic analysis. All tissues were reviewed by a board-certified veterinary anatomic pathologist who was blinded to animal identification (K.M.C.). Microscopically, cross-sectional areas of the liver and heart were qualitatively evaluated for age-related histopathologic lesions from 3 month old (n=3), 12 month old (n=3) and 29 month old (n=3) C57BL/6N male mice. Liver tissues were specifically evaluated for hepatic capsule contour, perivascular lymphoid aggregates, hepatocellular atrophy, extramedullary hematopoiesis (foci of myeloid and erythroid precursor cells), anisocytosis (variation in hepatocellular size), anisokaryosis (variation in hepatic nuclear size), bile duct proliferation, fibrosis, sinusoidal cellularity, and the presence of Ito cells (*i.e.* major resident cell type involved in liver fibrosis, also known as hepatic stellate cells). Because of dissection of parts of the tissue for RNA-seq

analysis, representative histologic sections of all heart chambers (*i.e.* left and right atria, left and right ventricles), valves, and vessels could not be consistently obtained for all mice. Thus, comparisons could not be drawn between specific heart chambers. Based on tissue availability, heart samples were evaluated for age-related pathology including myocardial fibrosis, myocardial degeneration (*i.e.* loss of cross striations, vacuolization, necrosis), myocardial inflammation, lymphoid aggregates, mineralization, and atrial thrombosis.

Livers of 3 month old mice were characterized by smooth hepatic capsules and rare scattered foci of extramedullary hematopoiesis (**Supplemental Fig. S1B**). Normal lobar architecture and sinusoidal organization of the liver were evident, with evenly spaced centrilobular and portal regions. At 12 months of age, in addition to rare foci of extramedullary hematopoiesis, small perivascular lymphoid aggregates were identified in the liver. Slight anisocytosis and anisokaryosis were also observed across hepatocytes at 12 months of age. By 29 months of age, foci of extramedullary hematopoiesis and perivascular lymphoid aggregates were increased in number and size in the liver. Undulation of the hepatic capsule was evident and was considered a direct result of hepatic cord atrophy. Anisocytosis and anisokaryosis were more pronounced in 29 month old mice than in 12 month-old mice. Additional histologic findings that were present only in the livers from 29 month old mice included occasional bile duct hyperplasia, increased sinusoidal cellularity (notably for Kupffer cells), increased numbers of lipid-rich Ito cells, and rare sinusoidal fibrosis (**Supplemental Fig. S1B**). Overall, no observable histologic differences were identified between available heart samples from 3 month, 12 month, and 29 month old mice. Occasionally, small foci of myocardial degeneration (characterized by loss of myofiber cross-striation and vacuolization) were noted across all age groups. Surprisingly, hemangiosarcoma (an endothelial cell neoplasm) was noted within the left ventricle of one mouse within the 29 month old cohort. The tumor comprised ill-defined and infiltrative vascular channels lined by variably plump neoplastic endothelial cells. Lymphatic dilation and perivascular edema were noted adjacent to the neoplasm in the heart and were thus considered sequelae to tumor formation, rather than an age-related manifestation. The corresponding RNA-seq sample did not encompass the tumor, which is compatible with the absence of gross outlier behavior in the RNA-seq analysis. Since the

whole cerebellum and olfactory bulb samples were used to extract RNA, the histological status of these tissues could not be assessed in our cohorts.

Chromatin preparation from cells and tissues

We performed ChIP experiments on different tissues from independent animals (**Supplemental Table S1A**). Olfactory bulbs were microdissected from 3, 12, and 29 months old males C57BL/6N mice (NIA colony at Charles Rivers) and pooled from 4-8 mice of the same age per biological replicate (**Supplemental Table S1A**). Cerebella were dissected, weighed, and pooled from 2 mice of the same age per biological replicate. The entire upper left lobe of the liver was dissected and weighed from an individual mouse and used for a single biological replicate. Following removal of blood and aorta, the heart ventricles from an individual mouse were dissected and weighed, and they served as a single biological replicate. All tissue samples were finely minced with a fresh razor blade, then resuspended in ice-cold PBS. Following mincing, tissues were crosslinked via addition of 1% formaldehyde for 15min at room temperature and quenched by the addition of 0.125M glycine for 5min at room temperature. ChIP experiments on mouse primary NSC cultures were performed as previously described (Benayoun et al. 2014). Briefly, NSCs neurospheres (passages 2-3) (**Supplemental Table S1A**) were dissociated 16-18 hours prior to collection. Cells were crosslinked at a density of 100,000 cells/mL in ice cold PBS with 1% formaldehyde for 9min at room temperature, and the crosslinking reaction was quenched with 0.125M glycine for 5min. For tissues and cells, after the quenching step, all crosslinked samples were washed three times with 1X PBS with protease inhibitors cocktail (Roche). Samples were then snap-frozen in liquid nitrogen and preserved at -80^{o}C as dry cell or tissue pellets until the day of the IP. On the day of the IP, samples were resuspended in 1mL of SDS lysis buffer (50 mM Tris-HCl pH7.5, 10 mM EDTA, 1% SDS in PBS at pH7.4) with protease inhibitors (Roche). Chromatin was sheared with a Vibra-Cell Sonicator VC130 (Sonics) 8 [tissues] or 7 times [cells] for 30 seconds at 60% amplitude with probe CV188, and then diluted 1:5 fold in RIPA buffer (1% NP-40, 0.5% Sodium deoxycholate in PBS, pH 7.4) with protease inhibitors (Roche).

Chromatin quantification and immunoprecipitation

For liver, heart, and cerebellum, chromatin content was measured and equalized for all ages to enable fair comparison across samples of a tissue. To measure the chromatin content of sonicated samples, samples were incubated with 0.2 μg/mL RnaseA (Life Technologies) for 1h at 37ºC and 0.2 mg/mL Proteinase K (Life Technologies) for 1h at 55ºC, and crosslinks were reversed by incubation at 80ºC for 2h. DNA was precipitated by the addition of 0.1 volume 3M sodium acetate, 2μg glycoblue (Life Technologies), and 2.5 volumes of 100% ethanol. The resulting DNA concentration was quantified by Nanodrop technology.

We used 20μg of chromatin for the H3 ChIPs, 50μg for the H3K4me3 ChIPs, and respectively 70μg (heart) or 100μg (liver and cerebellum) for the H3K27ac ChIPs. For the olfactory bulb, chromatin from approximately 30mg of tissue was used for immunoprecipitation with anti-H3 antibody, and 60mg was used for immunoprecipitation with anti H3K4me3 and H3K27ac antibodies. For adult NSCs, we used chromatin from ~250,000 cells for the H3 ChIP, ~750,000 cells for the H3K4me3 ChIP, and ~1,000,000 for the H3K27ac ChIP. ChIP was performed as previously described (Webb et al. 2013; Benayoun et al. 2014). The corresponding amount of chromatin diluted in RIPA buffer containing protease inhibitors cocktail (Roche) [see above] was incubated overnight at 4ºC with the following antibodies: 5μg H3K4me3 antibody (Active Motif #39159, lot #1609004), 5μg Histone H3 (Abcam #1791 lot #GR178101-1), and 7μg H3K27ac (Active motif #39133, lot #1613007).

S2 cell culture for ChIP normalization control

We explored the use of spike-in for ChIP normalization in some of our samples. Indeed, ChIP-seq from spiked-in chromatin from a different species (e.g. *Drosophila*) (termed ChIP-Rx for ChIP with reference exogenous genome (ChIP-Rx) has been shown to allow genome-wide quantitative comparisons of histone modification status across cell populations (Orlando et al. 2014). For example, the fraction of histone modification ChIP-seq reads (*e.g.* H3K4me3) corresponding to the species of interest (*e.g.* mouse) compared to the exogenous control (*e.g. Drosophila*) can be used to detect global differences in the amount of that histone modification between conditions of interest (*e.g.* aging). As several studies suggested that global levels of total histone proteins or specific histone

modifications may change with aging (Feser et al. 2010; O'Sullivan et al. 2010; Liu et al. 2013), we thought that ChIP-Rx could be helpful to account for this potential change.

To this end, *Drosophila* S2 cells were grown at 25°C in Schneider's media (Invitrogen) with 10% heat inactivated fetal bovine serum and 1% Penicillin/Streptomycin/Glutamine (Invitrogen). Cells were crosslinked at a density of 1-2x10^6 cells/ml in 1X PBS with 1% formaldehyde for 8min at room temperature, and the reaction was quenched with 0.125M glycine for 5min at room temperature. Next, 10^7 S2 cells were sonicated 7 times for 30s at 60% amplitude in 1mL of SDS lysis buffer (50 mM Tris-HCl pH7.5, 10 mM EDTA, 1% SDS in PBS pH7.4) containing protease inhibitors cocktail (Roche) using a Vibra-Cell Sonicator VC130. The S2 chromatin was added to heart, liver, and cerebellum tissues at a ratio of 1µg:120µg chromatin from S2:mouse tissue. For the second set of NSCs ChIPs, S2 chromatin was added at a 1:4 S2:NSC cell number ratio. ChIP was performed using the combination of probed tissue chromatin and S2 chromatin (Orlando et al. 2014). Note that the first set of NSC ChIPs and the Olfactory bulb ChIPs were performed without S2 chromatin, because this set was done before we became aware of the possibility to perform ChIP-Rx. However, we found that too few reads per spiked sample mapped to the *Drosophila* genome build dm3 in our libraries (<50,000 on average), which may explain why there was more variation in observed percentages of *Drosophila* reads between samples of the same age than between ages. For this reason, we did not end up using the S2 chromatin mappings to normalize our data in the processing pipeline. However, these reads are present in the ChIP-seq data and could be used for re-analysis.

Next-generation sequencing ChIP library generation

For olfactory bulb libraries and the first set of NSCs libraries, libraries were generated with the Illumina TruSeq kit (IP-202-1012) according to the manufacturer instructions. Briefly, repaired and adapter-ligated DNA was size-selected in range of 250-400bp and PCR-amplified for 16 (H3), 17 (H3K4me3) and 18-19 (H3K27ac) cycles. After the TruSeq kit became backordered, we generated libraries for the liver, heart, cerebellum, and the second set of H3 and H3K4me3 NSCs libraries using the NEBNext DNA library prep kit (E6040L). Repaired and adapter-ligated DNA was size-selected in range of 250-400bp

using agarose gel electrophoresis and PCR-amplified for 14 (H3), 16-17 (H3K4me3) or 17-18 (H3K27ac) cycles. Library quality was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies). Single-end 101bp reads were generated on Illumina HiSeq 2000 machines at the Stanford Genome Center.

ChIP-seq data processing

For ChIP-seq data processing, 101 bp reads were trimmed using Trim Galore! 0.3.1 to retain high-quality bases with phred score of greater than 15. The trimming command was: Trim_galore -q 15 --stringency 3 --length 36 --phred33 data_file.fastq. Reads were mapped to the mm9 mouse genome assembly using bowtie 0.12.7 (Langmead et al. 2009). We used GRCm37 (mm9) assembly to map all sequencing reads from mouse origin throughout our study, because when we started our study, many functional annotation programs did not yet support the mm10 build. Because our study compares samples across different ages and does not perform absolute analyses in each tissue, realigning the reads to GRCm38 (mm10) should not significantly affect our conclusions. PCR duplicates were removed using FIXSeq (fixseq-e2cc14f19764) (Hashimoto et al. 2014), which accounts for overdispersed per-base read count distributions using a nonparametric method which was shown to substantially improve the performance and precision of ChIP-seq analysis compared to existing alternatives (Hashimoto et al. 2014). Regions of significant enrichment were determined using MACS2 2.0.8 (Zhang et al. 2008) using the --broad option to enable wider regions of enrichment to be detected. The '--keep-dup=all' option was used to supersede MACS2 basic duplicate removal method since the FIXSeq method had already been applied. Total H3 ChIP-seq samples were used to determine the local background of H3 modification ChIP-seq datasets. Significant ChIP peaks of interest were annotated to the gene with the closest transcription start site in the mm9 assembly using the HOMER suite (Heinz et al. 2010). Files with the mm9 coordinates of ChIP-seq peaks that significantly changed with aging can be found on our GitHub repository (https://github.com/BenayounLaboratory/Mouse_Aging_Epigenomics_2018).

Datasets quality control

Quality metrics for each sample (*e.g.* sequencing depths, unique reads, replicate correlations, *etc.*) are reported in **Supplemental Table S1B-E**. Using either Pearson's and Spearman's correlation, we find that biological replicates of chromatin marks are generally well correlated and similar (>0.96 (Pearson) and >0.97 (Spearman) for H3K4me3 normalized to total H3; >0.9 (Pearson) and > 0.9 (Spearman) for H3K27ac normalized to total H3, with the exception of H3K27ac olfactory bulb samples, which exhibit correlations of 0.694-0.837 (Pearson) and 0.606-0.802 (Spearman) between samples) (**Supplemental Table S1D,E**). The biological replicates of RNA-seq are also well correlated overall (>0.82 (Pearson) and > 0.97 (Spearman)). Though some tissues presenting better replicate Pearson's correlations (>0.91 in cerebellum and olfactory bulb samples) or lesser Pearson's replicate correlations (0.822-0.864 in heart, liver and NSCs), these differences were not observed with the Spearman's correlation values (**Supplemental Table S1D,E**). As each sample is derived from independent animals, each with its own life trajectory, and as RNA measurement is quite sensitive to small differences, this may contribute to some of the observed differences between biological replicates. In addition, accumulating evidence has shown that aging is accompanied by increased variability in transcription across cells and individuals (Southworth et al. 2009; White et al. 2015; Enge et al. 2017; Martinez-Jimenez et al. 2017), which is likely to contribute to variability between biological replicates in these tissues/samples.

Dimensionality reduction techniques for data exploration

To perform Multidimensional Scaling (MDS) analysis, we used a distance metric based on the Spearman's rank correlation value (1-Rho) between samples, which was then provided to the core R command 'cmdscale' (R Core Team 2018). Principal component analysis (PCA) was performed using the core R command 'prcomp' (R Core Team 2018). Dimensionality reduction techniques were applied to log2-transformed DESeq2 1.6.3 normalized counts.

H3K4me3 breadth remodeling analysis

To compare changes in the breadth of H3K4me3 domains, we improved upon our previously developed pipeline to computationally adjust samples such that the signal-to-

noise ratio across all peaks is equalized between samples (Benayoun et al. 2014). We created a reference peakset for all comparative analyses using pooled QC reads from all ages and replicates (hereafter referred to as 'metapeaks'). To match the signal-to-noise ratios across all aging samples, we down-sampled reads separately in each H3K4me3 ChIP-seq biological sample to match the coverage histogram across all samples over the metapeaks intervals, similar to (Benayoun et al. 2014). This procedure matches the "height" of the peaks from the peak caller's point of view. The appropriate down-sampling rate that allows the coverage histogram of higher sensitivity H3K4me3 ChIP-seq samples to be equal or lower than that of the lowest sensitivity H3K4me3 ChIP-seq sample was determined by minimizing the p-value of Kolmogorov-Smirnov test (comparison to the sample with lowest H3K4me3 ChIP-seq sensitivity). In addition, to limit the effect of variations in input sample depth, we also matched the effective depth of H3 input samples to that of the lowest available sample. Final H3K4me3 domain breadth calls per samples were performed by using MACS 2.08 with the same parameters as above. IntersectBed (BEDTools 2.16.1) (Quinlan and Hall 2010) was used to estimate the length coverage of the sample peaks over the reference metapeaks. The goal of this pipeline is to increase the likelihood that called gains/losses of breadth result from a change in breadth of the enriched region, and not simply from an underlying difference in H3K4me3 intensity. Differential breadth was then estimated using the DESeq2 R package (DESeq2 1.6.3) (Love et al. 2014). Files with the mm9 coordinates of loci that exhibit significant remodeling of H3K4me3 breadth with age (all H3K4me3 domains and top 5% broadest H3K4me3 domains) can be found on our GitHub repository (https://github.com/BenayounLaboratory/Mouse_Aging_Epigenomics_2018).

Super-enhancer calling

Super-enhancers were called as outlined in (Hnisz et al. 2013). Briefly, MACS2 H3K27ac peaks were stitched together if within 12.5kb of one another (Hnisz et al. 2013), using mergeBed from BEDTools 2.16.0. Reads mapping within these peaks were counted using intersecBed from BEDTools 2.16.0, and the ROSE algorithm (Hnisz et al. 2013) was used to determine the H3K27ac intensity inflexion point determining typical versus super-enhancers. Files with the mm9 coordinates of super-enhancers that exhibit significant

changes in H3K27ac ChIP-seq intensity with age can be found und on our GitHub repository (https://github.com/BenayounLaboratory/Mouse_Aging_Epigenomics_2018).

H3K4me3 and H3K27ac intensity remodeling analysis

Similar to above, we created a reference peak sets (*i.e.* 'metapeaks') for all comparative analyses using pooled QC reads from all ages and replicates. Intensity signals for histone H3 modifications normalized to the local H3 occupancy were obtained using the 'DiffBind' R package (DiffBind 1.12.3) (Ross-Innes et al. 2012). Normalized intensities were then used to estimate differential intensities as a function of age using the DESeq2 R package (DESeq2 1.6.3) (Love et al. 2014). Files with the mm9 coordinates of loci marked by H3K4me3 and H3K27ac peaks that exhibit intensity remodeling with age can be found on our GitHub repository (https://github.com/BenayounLaboratory/Mouse_Aging_Epigenomics_2018).

Cell and tissue isolation for RNA purification

For RNA isolation, we used a new cohort of aging male C57BL/6N mice (same ages than the ChIP-seq cohort), and RNA-seq datasets were generated at a later time than the ChIP-seq datasets (**Supplemental Table S1A**). For RNA extraction on tissues: olfactory bulbs were microdissected from 3 month, 12 month, and 29 month old C57BL/6N male mice and weighed, and tissues from 2 independent mice of the same age were pooled per biological replicate. Cerebellum samples were similarly dissected, weighed, and samples from 2 mice of the same age were pooled per biological replicate. For the liver, the leftmost part of upper left lobe of the liver was dissected and weighed from an individual mouse and was used for a single biological replicate. For the heart, following removal of blood, the bottom-most part of heart ventricles from an individual mouse was dissected, weighed and used as a single biological replicate. All tissue samples were flash-frozen in liquid nitrogen until further processing. Tissues were resuspended in 600μL of RLT buffer (RNeasy plus mini kit, Qiagen) supplemented with 2-mercaptoethanol, then homogenized on Lysing Matrix D 2mL tubes (MP Biomedicals) on a FastPrep-24 machine (MP Biomedicals) with a speed setting of 6. Heart tissue was homogenized for 4 times 30 seconds, and all other tissues were homogenized for 40 seconds. Subsequent RNA extraction was performed using the

RNeasy plus mini kit (Qiagen) following the manufacturer's instructions. Primary NSC neurospheres (passages 2-3) were dissociated 16-18 hours prior to collection and seeded in 12-well plates. Cells were spun down and collected in RLT buffer supplemented with 2-mercaptoethanol and processed as above.

RNA-seq library preparation

For RNA-seq library preparation, 1μg of total RNA was combined to 2μL of a 1:100 dilution of ERCC RNA Spike-in mix (Thermo Fisher Scientific) in nuclease-free water, as recommended by the manufacturer. The resulting mix was then subjected to rRNA depletion using the NEBNext rRNA Depletion Kit (NEB), according to the manufacturer's protocol. Strand-specific RNA-seq libraries were then constructed using the SMARTer Stranded RNA-Seq Kit (Clontech), according to the manufacturer's protocol. Paired-end 75bp reads were generated on the Illumina NextSeq 500 platform.

RNA-seq analysis pipeline

Paired-end 75bp reads were trimmed using Trim Galore! 0.3.1 (github.com/FelixKrueger/TrimGalore) to retain high-quality bases with phred score > 15 and a remaining length > 35bp. Read pairs were then mapped to the UCSC mm9 genome build using STAR 2.4.0j (Dobin et al. 2013). Read counts were assigned to genes using subread 1.4.5-p1 (Liao et al. 2014). Read counts were imported into R to estimate differential gene expression as a function of age using the DESeq2 R package (DESeq2 1.6.3) (Love et al. 2014). Because no overt variation of ERCC spike-in levels were observed from samples to samples with a tissue/cell type, and because their use can increase technical noise, ERCC reads were not used after the initial quality-checking step.

To map repetitive element expression, we used the TEtranscripts 1.5.1 software (Jin et al. 2015), with mm9 RepeatMasker data (Smit 1996-2005)downloaded from the UCSC Table Browser. Read counts were imported into R to estimate differential gene expression as a function of age using the DESeq2 R package (DESeq2 1.6.3) (Love et al. 2014). We also used the 'analyzeRepeats.pl' functionality of the HOMER software (Heinz et al. 2010). In that case, read counts were imported into R to estimate differential gene expression as a function of age using the DESeq2 R package (DESeq2 1.16.1) (Love et al.

2014). We used a more recent version of DESeq2 for this because these analyses were run at a later time than the rest of the study. Because there were no major changes between these versions, the overall results should not be significantly affected.

Differential nucleosome calling using total H3 ChIP-seq

To compare changes in the local H3 deposition landscape, we used DANPOS 2.2 (Chen et al. 2013) and DiNUP 1.3 (Fu et al. 2012) nucleosome-calling softwares. For higher confidence results, we considered nucleosomes to be significantly remodeled if the position were called differential by DANPOS ($p < 1x10^{-15}$) and DiNUP (FDR < 0.05) following the same direction (*i.e.* increased *vs.* decreased signal). Consistently with a previously published MNase study on mouse aging liver tissue (Bochkis et al. 2014), we found that significant nucleosome remodeling with chronological aging seems to be restricted to a limited number of loci. Based on our observations and previously published reports, it is possible that decreased nucleosome occupancy may only be a cell-type or context specific effect of aging. Differential nucleosome calls were used as features in our machine learning models [see machine-learning section].

Machine-Learning analysis

Machine-learning models were built for each tissue, but not in NSCs since no gene was found to be significantly misregulated by RNA-seq in these cells. We built classification models in each tissue independently using 4 different classification algorithms as implemented through R package 'caret' (caret 6.0-80). Classification algorithms for neural nets (NNET; 'pcaNNet') are directly implemented in the 'caret' 6.0-80 package. Auxiliary R packages were used with 'caret' to implement random forests (RF; 'randomForest' 4.6-14), gradient boosting (GBM; 'gbm' 2.1.3) and radial support vector machines (SVM; kernlab 0.9-27). These package versions for machine-learning are used throughout our machine-learning analyses.

'Caret' was allowed to optimize final model parameters on the training data using 10-fold cross validation. Accuracies, sensitivities and specificities for all classifiers in their cognate tissue were estimated using a test set of randomly held out 1/3 of the data (thus not used in the training phase) obtained using the 'createDataPartition' function in 'caret' 6.0-

80 package (**Supplemental Table S3**). Feature importance estimation was only done using random forests and gradient-boosted trees, since other algorithms do not natively allow for it. The Gini score for feature importance was computed by 'caret' for each feature in the GBM and RF models, and the maximum in each model was scaled to '100' for ease of visualization.

Feature extraction and machine-learning analysis

For each expressed gene in each tissue, we extracted two types of 'features': (i) **dynamic** features, which encode changes to the chromatin landscape with age, and (ii) **static** features, which reflect the status of the chromatin and transcriptional landscape in young healthy animals. For dynamic features, we included: number of H3 nucleosomes with increased occupancy between 3 and 29 months, number of H3 nucleosomes with decreased occupancy between 3 and 29 months, maximum $\log_2$ fold change in H3 occupancy between 3 and 29 months, slope of H3K4me3 intensity change at annotated promoter with aging, slope of H3K27ac intensity change at annotated promoter with aging, slope of H3K4me3 breadth quantile change at annotated promoter with aging, and slope of H3K27ac intensity at stitched enhancers with aging (*i.e.* enhancer score).

For static features using the data we generated, we included the enhancer presence and type (*i.e.* None, Typical, or Super), the distance of the closest super-enhancer to the transcriptional start site of the gene, the broad H3K4me3 domain status (*i.e.* None, Typical, or Broad), the H3K4me3 breadth quantile in the young sample, the breadth of the broadest H3K4me3 domain associated to gene in the young sample, the average promoter intensity for H3K4me3 and H3K27ac in the young samples (promoters defined as -300bp;+300bp with regards to the transcriptional start sites defined in mm9 build, downloaded from the UCSC Genome Browser on 2016/1/21), the enhancer score in the young sample (as defined in (Hnisz et al. 2013)). In addition, we took advantage of the mouse ENCODE datasets (Shen et al. 2012; Yue et al. 2014) in the same tissues (heart, liver, cerebellum and olfactory bulb) in 2 months old mice to select additional potentially informative features (see complete list of datasets in **Supplemental Table S2A**). Using the H3K4me1 and H3K27me3 ENCODE ChIP-seq datasets, we collected the average promoter intensity for these marks (same promoter definition as above). In addition, we intersected our young

H3K4me3 ChIP-seq datasets to the H3K27me3 peaks to identify whether a gene had a potentially bivalent domain (*i.e.* with overlapping H3K27me3 and H3K4me3 peaks) (Bernstein et al. 2006) in the young animals or not. Using RNA polymerase II subunit POLR2A ChIP-seq datasets, we included the number of POLR2A peaks associated with each gene, the absolute distance of the closest POLR2A peak to the transcriptional start site of the gene, the maximal MACS2 score for POLR2A associated to the gene, the average promoter intensity for POLR2A. We also extracted the Traveling Ratio (TR) of POLR2A, which gives a measure of RNA polymerase II pausing, as described before (Benayoun et al. 2014). Using the CCCTC-binding factor (CTCF) ChIP-seq datasets, we included the number of CTCF peaks associated with each gene, the absolute distance of the closest CTCF peak to the transcriptional start site of the gene, the maximal MACS2 2.08 score for CTCF associated to the gene, and the average promoter intensity for CTCF. We also included the average promoter intensity for DNase I signals, which quantifies how accessible the gene promoter is to the transcriptional machinery. Finally, we included several DNA sequence features from the mm9 build associated to each gene: the presence of a CpG island, the percentage of CG nucleotide and the percentage of CpG dinucleotide in promoters computed using HOMER, as well as the number of exons in each gene.

Because absolute gene expression levels can influence the ability of differential gene expression pipelines to identify differentially expressed genes (Tarazona et al. 2011), we also included the average expression level of the genes in the young samples, expressed in FPKM (fragments per read per million). This should allow us to account for potential non-biological effects (*i.e.* effects due to differential expression pipelines) in our models. Any additional feature that is significantly predictive of differential gene expression with age should then be associated independently of the expression level effect, since the expression level is taken into account by the model at the same time when evaluating the importance of features.

We then trained machine-learning classification models in two different ways: (i) either only comparing genes called by DESeq2 1.6.3 as up *vs.* down with age at FDR < 10% (2-class models) or (ii) comparing genes called by DESeq2 1.6.3 as up, down, or unchanged with age at FDR < 10% (3-class models) (**Fig. 3, Supplemental Fig. S3**). These 2 types models allow us to ask different questions: the former interrogates whether there

are chromatin differences between upregulated and downregulated genes, without taking the rest of the genes into account, whereas the latter also attempts to identify differences between the genes that change and the ones that do not change during aging. To note, 2-class models systematically outperformed 3-class models in terms of model accuracy (**Fig. 3B,C and Supplemental Fig. S3B,C; Supplemental Table S3**), which is consistent with the increased complexity of a classification problem with the number of classes to discriminate.

The relative number of genes that change transcriptionally with age (< 500) is low compared to the number of expressed genes (>3000) (class sizes in each tissue can be found in **Supplemental Table S3**). Thus, to avoid biases in the 3-class classification output due to the imbalanced number of genes called as transcriptionally changed and unchanged with aging, we generated a list of unchanged genes for each sampling that are equal in number to the changed genes in the training set of the cognate tissue (*e.g.* sample 500 unchanged genes to be compared to 500 changed genes in each iteration). We repeated the sampling procedure 50-250 times to eliminate random sampling biases (NNET and SVM: 100 samplings; RF: 250 samplings; GBM: 50 samplings). Notably, to improve the signal-to-noise ratio and decrease the inclusion of false negatives (*i.e.* genes called as "unchanged" because of lack of statistical power) in our dataset in the learning and testing of the machine-learning, the constant class was restricted to genes with a $\log_2$ fold change (as called by DESeq2 1.6.3) within half a standard deviation of the $\log_2$ fold change distribution both above and below 0 (no change).

Importantly, for all machine-learning models, performance was evaluated using the 1/3 randomly held out genes (not used in the training phase) for testing, without artificially correcting the natural class imbalances created by the large number of unchanged genes with aging compared to changed genes.

Ingenuity Pathway Analysis

Upstream regulator analysis was performed using QIAGEN's Ingenuity Pathway Analysis (IPA QIAGEN Redwood City) software, using the genes that passed the filter in our datasets as reference genome, the cutoff of 0.05 for FDR-corrected p-values and species parameter was restricted to mouse. Results in **Supplemental Table S5** are shown for gene

sets that passed significance in IPA in at least 4 of the analyzed sets (each tissues and tissue-independent analysis).

Analysis of chromatin remodeling at repetitive elements

To assess chromatin remodeling at repetitive elements with aging, we used an approach based on extracting uniquely mapping reads to the mm9 genome in the vicinity of annotated repeats from the UCSC mm9 RepeatMasker track. We focused on repeats which overlapped H3K4me3 or H3K27ac metapeaks in each tissue, and combined reads mapping to all instances of a repeat family in this catalog. This strategy is similar to what the HOMER software performs for RNA-level repetitive element analysis, and akin to the method implemented to analysis chromatin at repetitive elements in a recent study (Liu et al. 2018). We used a modified version of our ChIP-seq differential pipeline, using DiffBind 2.4.8 and DESeq2 1.16.1, to calculate differential ChIP intensity at each repeat family and identified significant chromatin remodeling at several TE families with FDR < 5%. However, we note that because our ChIP data is single-ended, our ability to robustly and uniquely map reads around repetitive loci is limited because of the inherent repetitiveness of these loci and the existence of multiple annotated insertion sites in the genome (Liu et al. 2018). To note, we used a more recent version of DiffBind and DESeq2 here because these analyses were run at a later time than the rest of this study. Because the packages did not undergo major algorithmic changes between these versions, the overall results should not be significantly affected by the versions that were used.

Comparison of RNA-seq datasets with previously published studies

We compared our RNA-seq datasets with publicly available aging datasets (Bochkis et al. 2014; White et al. 2015; Boisvert et al. 2018) (see **Supplemental Table S2C**). We modified our pipeline to accommodate shorter sequencing reads and unpaired designs used in these studies (Bochkis et al. 2014; White et al. 2015; Boisvert et al. 2018). First, sequencing reads were trimmed using Trim Galore! 0.3.1 with a remaining length > 25bp. Read pairs were then mapped to the UCSC mm9 genome build using STAR v2.4.0j, and Read counts were assigned to genes using subread 1.4.5-p1. Read counts were imported into R to estimate differential gene expression as a function of age using the DESeq2 R

package (DESeq2 1.16.1) (Love et al. 2014). Gene-level changes were compared using DESeq2 $\log_2$ fold change values, using gene names as comparison points. Gene expression genes were considered significant when FDR < 5%. Although there are notable differences in between these RNA-seq datasets, these significant overlaps suggest that transcriptome remodeling with aging is overall robust, despite differences at both the technical (*e.g.* RNA selection methods, library construction kits, sequencing platforms) and biological (*e.g.* ages of the mice, genetic background, time of dissection) levels.

Tissue RNA-seq deconvolution using CIBERSORT

Raw data from RNA-seq datasets from purified mouse cell types was downloaded from the SRA repository. All samples were processed using a standardized pipeline, which consisted of mapping with STAR 2.4.0j (Dobin et al. 2013) to the mm9 assembly, and counting of reads over mm9 genes using subread 1.4.5-p1 (Liao et al. 2014). Read counts from all samples were imported into R for further processing and normalization. Single-cell RNA-seq datasets were aggregated into pseudo-bulks by adding reads coming from 4-150 cells of the same cell type from the same study (depending on sequencing depth and number of available cells) (**Supplemental Table S2**; **Supplemental Code**). Next, any bulk or pseudo bulk sample with more than 18,000 genes without any read detected were eliminated from further processing as too low coverage. Then, all retained quality-checked samples were normalized using the DESeq2 1.6.3 variance stabilizing transformation (VST), and subjected to $\log_2$ transformation before upload to the CIBERSORT portal (Newman et al. 2015). The CIBERSORT website was used with default parameters to build the signature matrix and to analyze RNA-seq samples from cell mixtures.

To perform the deconvolution process, CIBERSORT requires an input matrix of reference gene expression signatures (or 'signature matrix'), collectively used to estimate the relative proportions of each cell type of interest. To provide this reference framework for CIBERSORT, we curated >1500 RNA-seq datasets of purified mouse cell types (**Supplemental Fig. S5A; Supplemental Table S2B**). After undergoing standardized processing, these datasets were used to build a signature matrix for CIBERSORT (**Supplemental Table S7A**). To test the accuracy of the trained signature matrix, we randomly withheld one RNA-seq sample per cell type with at least 3 biological samples

for validation (which was thus not used in the training phase). We verified that CIBERSORT was generally sensitive enough to detect various cell types on their own, and within *in silico* mixes of cell types expected to co-occur in tissues (**Supplemental Fig. S5B,C; Supplemental Table S7B**). Using CIBERSORT and the trained signature matrix, we could detect a macrophage/microglia signature in the brain tissue of Alzheimer's mouse models with known increased inflammatory cell content (Gjoneska et al. 2015; Iaccarino et al. 2016) (**Supplemental Fig. S5D; Supplemental Table S7C**). However, when applied to our RNA-seq datasets, CIBERSORT did not identify significant change in the presence of inflammatory cell signatures, and no other significant changes could be observed (**Supplemental Fig. S5E; Supplemental Table S7D**). Moreover, CIBERSORT did not detect changes in relative proportions of other cell types (*e.g.* fibroblasts, astrocytes, hepatocytes, etc.) either. However, single-cell RNA-seq from dissociated tissues will be needed to fully elucidate this question.

Functional enrichment analysis using the minimum hypergeometric test

To perform functional enrichment analysis, we leveraged the minimum HyperGeometric (mHG) distribution, which has been spearheaded through the GOrilla enrichment tool (Eden et al. 2007; Eden et al. 2009). The mHG tests is performed using a ranked list of genes, and allows computing of an exact p-value for the observed enrichment, taking threshold multiple testing into account without the need for simulations, which enables rigorous and rapid statistical analysis of thousands of genes and thousands of functional enrichment terms (Eden et al. 2007; Eden et al. 2009). We used the R implementation of the mHG distribution ('mHG' 1.1 package) to run a minimum-hypergeometric (mHG) test as described in (Eden et al. 2007) on gene sets from MSigDB hallmarks (Liberzon et al. 2015), KEGG 2017 (Kanehisa et al. 2017), obtained using KEGG API, after excluding disease pathways to focus on core biological processes (lists in **Supplemental Table S5B,C**), and transcription factor loss-of-function targets compiled by EnrichR (list downloaded on 2015/11/19) (Kuleshov et al. 2016). For analysis of function enrichments associations on chromatin datasets, we ranked domains to run the mHG tests based on the intensity or breadth of the mark of interest and annotated each domain to the gene with the closest transcriptional start site. Genes were often associated to more than one chromatin

domain, and only the most extreme domain (*i.e.* more intense, or broadest) were retained when running the mHG enrichment test.

Rat and turquoise killifish RNA-seq processing

Tissue aging RNA-seq datasets from female and male rats were obtained from the rat Bodymap project (GEO accession number GSE53960) (Yu et al. 2014). Reads were mapped using Kallisto (0.43.0) (Bray et al. 2016). DESeq2 $\log_2$-normalized fold changes were then used to estimate differential gene expression as a function of age using the DESeq2 R package (DESeq2 1.6.3). Orthology tables between rat and mouse genes for aging trends comparison were obtained from Ensembl BioMart (2017/05/15).

The African turquoise killifish (*Nothobranchius furzeri,* wild-derived MZM-0410 strain) aging RNA-seq dataset was obtained from GEO (GSE69122) (Baumgart et al. 2014; Baumgart et al. 2016). Reads were mapped to African turquoise killifish reference transcriptome (GRZ) from NCBI Annotation Release 100 using Kallisto. Pseudo-counts for each transcript were merged and read counts per gene were obtained using Bioconductor R package 'tximportData' 1.6.0 (Love M, 2017). Linear modeling of the differential expression analysis from 5-weeks to 39-weeks old tissues was performed using DESeq2 (v1.6.3). To note, biological replicate clustering by hierarchical clustering and PCA was not clear, suggesting potential unknown covariates in the dataset. High confidence orthologs between African turquoise killifish and mouse genes were obtained using bidirectional best BLASTp analysis using longest protein sequences for each of the two species (E-value < 10^{-3}).

Human RNA-seq analysis with aging (GTEx)

Read counts of human tissue RNA-seq with aging per genes were obtained from from the GTEx Consortium ('v6p' version) (The GTEx Consortium 2015). Genotyping principal components, sex, age, as well as other provided sample meta-data (i.e. "RIN" [RNA integrity score], "Ischemic time", "Fixation time", "RNA batch"), were used as covariates for differential expression analysis. To allow for integration of the discretized age variable (by 5 year increments) into quantitative models, age ranges were converted to a single age at the middle point of the coded range (*e.g.* "50-59" coded as 55 years). DESeq2 normalized

fold-changes were then used to estimate differential gene expression as a function of age (DESeq2 1.6.3). The GTEx v6p count data on genes is reported according to GENCODE 19 models, which correspond to Ensembl 74/75 builds (NCBI assembly GRCh37.p13). Using the Biomart mirror for the Ensembl 75 build, we obtained the orthology table between human and mouse genes for aging trajectory comparisons.

Human microarray analysis with aging

We obtained an independent human liver aging transcriptome dataset (accession GSE61260; "GSE61260_datLiverNormalizedExpr.csv") (Horvath et al. 2014), along with accompanying sample meta-data (sex, age, BMI, disease status). R package 'limma' 3.32.10 normalized fold-changes were used to estimate differential gene expression as a function of age.

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