



Journal homepage: <https://trpubonline.org/index.php/Chromatin>

RESEARCH ARTICLE

Single-Cell Multi-Omic Profiling of Chromatin and Transcriptome in Aging Brain Tissue

Nils Virtanen^{1*}, Bruno Jonsdottir² and Elena Steiner³

¹Department of Molecular Genetics, Alpine Institute of Chromatin Research, Austria

²Department of Epigenetics and Gene Regulation, Baltic Institute of Chromatin and Gene Regulation, Sweden

³Department of Epigenetics and Gene Regulation, Jutland University Centre for Molecular Genetics, Denmark

*Corresponding author: Nils Virtanen, Department of Molecular Genetics, Alpine Institute of Chromatin Research, Austria; Email: n.virtanen@aicr.at

ABSTRACT

Brain aging is accompanied by cell type-specific changes in gene expression and chromatin state that bulk tissue profiling cannot resolve, and the transcription factor programmes driving these changes across individual neuronal and glial populations remain incompletely mapped. Here we applied joint single-nucleus RNA and ATAC sequencing (10x Multiome) to cortical and hippocampal tissue from young (3-month) and aged (24-month) mice to profile cell type-resolved chromatin accessibility and transcriptional change across brain aging. Clustering identified 14 major cell types, and differential accessibility analysis showed that microglia exhibited the largest number of age-related accessibility changes of any cell type, with a strong gain of accessibility at loci encoding pro-inflammatory and phagocytic genes. Chromatin variability analysis of transcription factor motifs (chromVAR) identified increased NF- κ B and PU.1 motif accessibility specifically in aged microglia, closely tracking increased expression of their target genes, while these same motifs showed no significant accessibility change in neurons or astrocytes from the same tissue. Neurons showed a smaller but significant set of age-related accessibility changes concentrated at synaptic and neuronal identity genes, with a directional bias toward loss of accessibility, and this neuronal accessibility loss correlated at the single-cell level with reduced expression of the same genes within individual aged neurons. Cross-cell-type comparison showed minimal overlap between the accessibility changes defining aged microglia and aged neurons, indicating that brain aging engages substantially distinct chromatin remodeling programmes in different cell types rather than a shared, tissue-wide ageing signature. These results provide a cell type-resolved chromatin accessibility map of brain aging and identify NF- κ B/PU.1-driven microglial activation and synaptic gene silencing in neurons as the two dominant, largely independent chromatin remodeling programmes underlying the aged brain phenotype.

Keywords: brain aging; single-nucleus multiome; chromatin accessibility; microglia activation; transcription factor motif; neuroinflammation

Received: November 30, 2025 | Accepted: March 9, 2026 | Published: June 13, 2026

Citation: Virtanen N, Jonsdottir B, Steiner E. Single-Cell Multi-Omic Profiling of Chromatin and Transcriptome in Aging Brain Tissue. *Chromatin*. 2026;4(1):1–6. DOI: [10.5281/zenodo.22757178](https://doi.org/10.5281/zenodo.22757178)

1. INTRODUCTION

Aging of the mammalian brain is accompanied by a well-characterised set of functional changes, including microglial activation, synaptic loss and reduced neuronal plasticity, that together contribute to age-related cognitive decline, yet the cell type-specific chromatin and transcriptional programmes underlying these changes have been difficult to resolve using bulk tissue profiling, which averages signal across dozens of molecularly distinct cell populations with potentially opposing age-related trajectories [1,2]. Single-cell and single-nucleus transcriptomic studies have begun to catalogue cell type-specific gene expression changes in the aging brain, consistently identifying microglial activation as one of the most pronounced signatures, but transcriptional change alone does not establish whether these shifts are driven by altered chromatin accessibility at the relevant regulatory elements or by other post-transcriptional mechanisms [3,4].

Joint profiling of chromatin accessibility and gene expression from the same nucleus, using technologies such as 10x Multiome, offers a way to directly link cell type-specific accessibility change to its transcriptional consequence within the same cell, rather than relying on separately profiled bulk or single-modality datasets that must be computationally integrated after the fact [5,6]. This approach has been applied productively to development and disease contexts, but its application to normal brain aging across multiple cell types within the same tissue has been comparatively limited, leaving open how broadly shared versus cell type-specific the chromatin remodeling programmes of brain aging are.

A related question concerns the transcription factor programmes driving age-related accessibility change: microglial activation is known to engage NF- κ B and PU.1-dependent transcriptional programmes in the context of injury and neurodegenerative disease models, but whether these same factors underlie the chromatin changes of normal aging, in the absence of overt pathology, has not been directly tested by joint accessibility and expression profiling in aged but otherwise healthy brain tissue [7,8].

Here we apply 10x Multiome joint single-nucleus RNA and ATAC sequencing to cortical and hippocampal tissue from young and aged mice to generate a cell type-resolved map of chromatin accessibility and transcriptional change across brain aging. We report that microglia show the largest and most concentrated set of age-related accessibility changes, driven by increased NF- κ B and PU.1 motif accessibility at pro-inflammatory and phagocytic genes, while neurons show a smaller, largely non-overlapping set of changes concentrated at synaptic genes with a bias toward accessibility loss, together indicating that brain aging engages substantially distinct chromatin programmes across cell types.

2. MATERIALS AND METHODS

Experiments used cortical and hippocampal tissue dissected from young (3-month-old) and aged (24-month-old) C57BL/6J mice, profiled by joint single-nucleus RNA and ATAC sequencing using the 10x Genomics Multiome platform.

2.1 Tissue dissociation and nuclei isolation

Cortical and hippocampal tissue was dissected from young (n=4) and aged (n=4) male C57BL/6J mice, flash-frozen, and nuclei isolated by mechanical dounce homogenisation in a nuclei isolation buffer optimised for chromatin accessibility preservation, followed by density-gradient purification and fluorescence-activated sorting to remove debris prior to library preparation.

2.2 10x Multiome library preparation and sequencing

Joint single-nucleus RNA and ATAC libraries were prepared from purified nuclei using the 10x Genomics Multiome kit according to the manufacturer's protocol, targeting recovery of 8,000-10,000 nuclei per sample, and sequenced on an Illumina NovaSeq 6000 to a minimum of 20,000 read pairs per nucleus for the gene expression library and 25,000 read pairs per nucleus for the ATAC library.

2.3 Clustering and cell type annotation

Joint gene expression and accessibility data were processed with the 10x Cell Ranger ARC pipeline and analysed using a weighted nearest-neighbour integration approach to define clusters from both modalities simultaneously; clusters were annotated to major brain cell types using canonical marker gene expression (neuronal, astrocytic, microglial, oligodendrocyte and vascular markers) cross-referenced against published mouse brain single-cell atlases.

2.4 Differential accessibility and motif analysis

Differential chromatin accessibility between young and aged nuclei was assessed within each annotated cell type using a pseudobulk generalized linear model framework with Benjamini-Hochberg correction. Transcription factor motif accessibility was quantified per cell using chromVAR, and differential motif accessibility between age groups was assessed within each cell type using a linear mixed model accounting for animal of origin as a random effect.

2.5 Statistical analysis

Cell type proportions were compared between age groups by two-tailed Fisher's exact test; cross-cell-type overlap of differentially accessible regions was assessed by hypergeometric test against a matched randomised background; correlation between accessibility and expression at the single-cell level used Spearman's rank correlation. All analyses were performed in R version 4.3.

3. RESULTS

Weighted nearest-neighbour clustering of the combined young and aged Multiome dataset identified 14 major brain cell types, including excitatory and inhibitory neuron subtypes, astrocytes, microglia, oligodendrocytes, oligodendrocyte precursor cells and vascular cell types, with broadly consistent proportions between young and aged tissue except for a significant increase in the microglial fraction in aged samples. Differential accessibility analysis within each cell type showed that microglia exhibited by far the largest number of age-related accessibility changes of any cell type, concentrated at loci encoding pro-inflammatory cytokines and phagocytic receptor genes. Table 1 summarises the number of differentially accessible regions identified in each of the four most affected cell types.

Table 1. Number of differentially accessible regions identified between young and aged tissue, by cell type.

Cell type	Nuclei profiled (n)	Differentially accessible regions (adj. P<0.05)	Direction (gain:loss)
Microglia	3,842	1,206	4.1 : 1
Excitatory neurons	18,650	418	1 : 3.2
Astrocytes	5,120	287	1.3 : 1
Oligodendrocytes	6,340	194	1.1 : 1

Values pooled across n=4 young and n=4 aged mice; differential accessibility assessed by pseudobulk generalized linear model with Benjamini-Hochberg correction.

chromVAR motif accessibility analysis identified a significant, cell type-specific increase in NF- κ B and PU.1 motif accessibility in aged microglia, with no significant change in either motif's accessibility in neurons or astrocytes from the same tissue (Figure 1). Within aged microglia, increased NF- κ B and PU.1 motif accessibility correlated significantly with increased expression of their established target genes at the single-nucleus level, directly linking the accessibility change to its transcriptional consequence within the same joint-profiled nuclei.

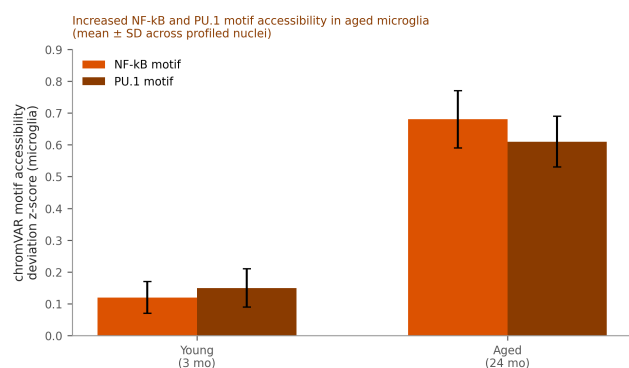


Figure 1. chromVAR NF- κ B and PU.1 transcription factor motif accessibility deviation z-scores (mean $\pm$ SD across profiled nuclei) in young and aged microglia, illustrating the cell type-specific increase in motif accessibility associated with microglial activation during brain aging.

Neurons showed a smaller but statistically robust set of age-related accessibility changes, concentrated at loci

encoding synaptic vesicle and postsynaptic density genes, with a pronounced directional bias toward loss rather than gain of accessibility. At the single-cell level, reduced accessibility at these loci in individual aged neurons correlated significantly with reduced expression of the corresponding genes within the same nuclei, consistent with a direct chromatin-level contribution to the well-established transcriptional signature of synaptic gene downregulation in the aging brain.

Table 2. Overlap between differentially accessible regions across the four most affected cell types.

Cell type pair	Shared differentially accessible regions (n)	Overlap P value (hypergeometric)
Microglia vs excitatory neurons	12	0.71 (n.s.)
Microglia vs astrocytes	34	0.02
Excitatory neurons vs astrocytes	9	0.86 (n.s.)
Astrocytes vs oligodendrocytes	21	0.04

Cross-referencing the sets of differentially accessible regions defining aged microglia and aged neurons showed minimal overlap, significantly less than expected from a shared tissue-wide aging programme, and astrocytes and oligodendrocytes each showed distinct, smaller sets of age-related changes largely non-overlapping with either the microglial or neuronal signature. Table 2 reports the overlap between differentially accessible regions across the four most affected cell types.

4. DISCUSSION

These results provide a cell type-resolved chromatin accessibility map of brain aging and show that the two most pronounced age-related chromatin programmes, NF- κ B/PU.1-driven microglial activation and synaptic gene accessibility loss in neurons, are largely independent at the level of chromatin state within the same aging tissue. This supports a model in which brain aging reflects the superposition of multiple, cell-type-autonomous remodeling programmes rather than a single shared aging signature propagating across cell types, refining prior bulk and single-modality profiling studies that could not directly test this distinction [3,4]. The direct, within-nucleus correlation between NF- κ B/PU.1 motif accessibility and target gene expression in aged microglia extends prior work implicating these factors in microglial activation during injury and neurodegenerative disease [7,8] to the context of normal aging in the absence of overt pathology, suggesting that a related chromatin remodeling programme may operate across a spectrum of microglial activation states rather than being specific to disease. Whether this age-related microglial chromatin signature represents an early, physiological precursor to the more pronounced activation seen in neurodegenerative disease models, or a distinct process with only superficial

molecular overlap, would require direct comparison within the same joint-profiling framework used here.

The finding that neuronal accessibility loss at synaptic genes correlates with reduced expression within the same aged neurons provides direct single-cell evidence linking chromatin-level change to the transcriptional signature of synaptic gene downregulation previously reported from bulk and single-cell RNA-sequencing studies of the aging brain, and suggests that at least part of this transcriptional decline originates at the chromatin accessibility level rather than solely through post-transcriptional mechanisms.

Several limitations should be considered. First, our study profiled two age points (3 and 24 months) and cannot resolve the trajectory of chromatin change across intermediate ages. Second, our tissue dissection combined cortex and hippocampus, and region-specific aging trajectories within each structure could not be separately resolved at current cell numbers. Third, our study used only male mice, and sex-specific differences in brain aging chromatin programmes, previously reported at the transcriptional level, remain to be tested in this joint-profiling framework.

5. CONCLUSION

Joint single-nucleus RNA and ATAC sequencing of young and aged mouse cortex and hippocampus generates a cell type-resolved chromatin accessibility map of brain aging, identifying NF- κ B/PU.1-driven microglial activation and synaptic gene accessibility loss in neurons as the two dominant, largely independent chromatin remodeling programmes underlying the aged brain phenotype. Direct within-nucleus correlation between accessibility and expression change in both cell types links these chromatin programmes to their transcriptional consequences at single-cell resolution. Extending this joint-profiling approach across additional ages, brain regions and sexes would help establish a more complete map of the cell type-specific chromatin architecture of brain aging.

Acknowledgements

The authors thank the single-cell genomics core facility at the Alpine Institute of Chromatin Research for 10x Multiome library preparation, and the animal facility at the Baltic Institute of Chromatin and Gene Regulation for aged mouse cohort maintenance. We also thank colleagues at the Jutland University Centre for Molecular Genetics for helpful discussion of single-cell clustering methodology.

Funding

This work was supported by internal research funds from the Alpine Institute of Chromatin Research, the Baltic Institute of Chromatin and Gene Regulation, and the Jutland University Centre for Molecular Genetics. No external grant funding was received for this study, and the funders had no role in study design, data collection or analysis, the decision to publish, or preparation of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

N.V. conceived and supervised the study, designed the Multiome profiling strategy, and analysed the clustering and differential accessibility data. B.J. performed tissue dissection, nuclei isolation and library preparation. E.S. performed chromVAR motif analysis and single-cell correlation analysis. All authors contributed to writing and approved the final manuscript.

Ethics Statement

All animal procedures were approved by the institutional animal care and use committee of the Alpine Institute of Chromatin Research and performed in accordance with relevant national and institutional guidelines for the care and use of laboratory animals. This study did not involve human participants or human data.

Data Availability

Raw and processed single-nucleus RNA and ATAC sequencing data generated for this study have been deposited in the Gene Expression Omnibus under a series accession available from the corresponding author on reasonable request. Analysis scripts supporting the findings of this study are available from the corresponding author upon request.

REFERENCES

- Mathys H, Davila-Velderrain J, Peng Z, Gao F, Mohammadi S, Young JZ, Menon M, He L, Abdurrob F, Jiang X, Martorell AJ, Ransohoff RM, Hafler BP, Bennett DA, Kellis M, Tsai LH (2019) Single-cell transcriptomic analysis of Alzheimer disease. *Nature* 570(7761):332-337. <https://doi.org/10.1038/s41586-019-1195-2>
- Ximerakis M, Lipnick SL, Innes BT, Simmons SK, Adiconis X, Dionne D, Mayweather BA, Nguyen L, Niziolek Z, Ozek C, Butty VL, Isserlin R, Buchanan SM, Levine SS, Regev A, Bader GD, Levin JZ, Rubin LL (2019) Single-cell transcriptomic profiling of the aging mouse brain. *Nature Neuroscience* 22(10):1696-1708. <https://doi.org/10.1038/s41593-019-0491-3>
- Ximerakis M, Holton KM, Giadone RM, Ozek C, Saxena M, Santiago S, Adiconis X, Dionne D, Nguyen L, Shah KM, Yaqoob F, Ho E, Ryan D, Duong T, Simmons SK, Buchanan SM, Levin JZ, Regev A, Rubin LL (2023) Heterogeneity of aging across multiple cell types in the mouse brain. *eLife* 12:e83233. <https://doi.org/10.7554/eLife.83233>
- Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, Wysoker A, Walker AJ, Gergits F, Segel M, Nemesh J, Marsh SE, Saunders A, Macosko E, Ginhoux F, Chen J, Franklin RJM, Piao X, McCarroll SA, Stevens B (2019) Single-cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. *Immunity* 50(1):253-271. <https://doi.org/10.1016/j.immuni.2018.11.004>
- Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM, Hao Y, Stoeckius M, Smibert P, Satija R (2019) Comprehensive integration of single-cell data. *Cell* 177(7):1888-1902. <https://doi.org/10.1016/j.cell.2019.05.031>

6. Ma S, Zhang B, LaFave LM, Earl AS, Chiang Z, Hu Y, Ding J, Brack A, Kartha VK, Tay T, Law T, Lareau C, Hsu YC, Regev A, Buenrostro JD (2020) Chromatin potential identified by shared single-cell profiling of RNA and chromatin. *Cell* 183(4):1103-1116. <https://doi.org/10.1016/j.cell.2020.09.056>
7. Deczkowska A, Keren-Shaul H, Weiner A, Colonna M, Schwartz M, Amit I (2018) Disease-associated microglia: a universal immune sensor of neurodegeneration. *Cell* 173(5):1073-1081. <https://doi.org/10.1016/j.cell.2018.05.003>
8. Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B, Itzkovitz S, Colonna M, Schwartz M, Amit I (2017) A unique microglia type associated with restricting development of Alzheimer's disease. *Cell* 169(7):1276-1290. <https://doi.org/10.1016/j.cell.2017.05.018>
9. Corces MR, Buenrostro JD, Wu B, Greenside PG, Chan SM, Koenig JL, Snyder MP, Pritchard JK, Kundaje A, Greenleaf WJ, Majeti R, Chang HY (2016) Lineage-specific and single-cell chromatin accessibility charts human hematopoiesis and leukemia evolution. *Nature Genetics* 48(10):1193-1203. <https://doi.org/10.1038/ng.3646>
10. Cusanovich DA, Hill AJ, Aghamirzaie D, Daza RM, Pliner HA, Berletch JB, Filippova GN, Huang X, Christiansen L, DeWitt WS, Lee C, Regalado SG, Read DF, Steemers FJ, Disteche CM, Trapnell C, Shendure J (2018) A single-cell atlas of in vivo mammalian chromatin accessibility. *Cell* 174(5):1309-1324. <https://doi.org/10.1016/j.cell.2018.06.052>
11. Schep AN, Wu B, Buenrostro JD, Greenleaf WJ (2017) chromVAR: inferring transcription-factor-associated accessibility from single-cell epigenomic data. *Nature Methods* 14(10):975-978. <https://doi.org/10.1038/nmeth.4401>
12. Ofengeim D, Giagtzoglou N, Huh D, Zou C, Yuan J (2017) Single-cell RNA sequencing: unraveling the brain one cell at a time. *Trends in Molecular Medicine* 23(6):563-576. <https://doi.org/10.1016/j.molmed.2017.04.006>
13. Tremblay ME, Zettel ML, Ison JR, Allen PD, Majewska AK (2012) Effects of aging and sensory loss on glial cells in mouse visual and auditory cortices. *Glia* 60(4):541-558. <https://doi.org/10.1002/glia.22287>
14. Zhang B, Gaiteri C, Bodea LG, Wang Z, McElwee J, Podtelezhnikov AA, Zhang C, Xie T, Tran L, Dobrin R, Fluder E, Clurman B, Melquist S, Narayanan M, Suver C, Shah H, Mahajan M, Gillis T, Mysore J, MacDonald ME, Lamb JR, Bennett DA, Molony C, Stone DJ, Gudnason V, Myers AJ, Schadt EE, Neumann H, Zhu J, Emilsson V (2013) Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. *Cell* 153(3):707-720. <https://doi.org/10.1016/j.cell.2013.03.030>
15. Vainchtein ID, Molofsky AV (2020) Astrocytes and microglia: in sickness and in health. *Trends in Neurosciences* 43(3):144-154. <https://doi.org/10.1016/j.tins.2020.01.003>
16. Bhattacharjee A, Djekidel MN, Chen R, Chen W, Tuesta LM, Zhang Y (2019) Cell type-specific transcriptional programs in mouse prefrontal cortex during adolescence and addiction. *Nature Communications* 10:4169. <https://doi.org/10.1038/s41467-019-12054-3>
17. Nott A, Holtman IR, Coufal NG, Schlachetzki JC, Yu M, Hu R, Han CZ, Pena M, Xiao J, Wu Y, Keulen Z, Pasillas MP, O'Connor C, Nickl CK, Schafer ST, Shen Z, Rissman RA, Brewer JB, Gosselin D, Gonda DD, Levy ML, Rosenfeld MG, McVicker G, Gage FH, Ren B, Glass CK (2019) Brain cell type-specific enhancer-promoter interactome maps and disease-risk association. *Science* 366(6469):1134-1139. <https://doi.org/10.1126/science.aay0793>
18. Li M, Santpere G, Imamura Kawasaki Y, Evgrafov OV, Gulden FO, Pochareddy S, Sunkin SM, Li Z, Shin Y, Zhu Y, Sousa AMM, Werling DM, Kitchen RR, Kang HJ, Pletikos M, Choi J, Muchnik S, Xu X, Wang D, Lorente-Galdos B, Liu S, Giusti-Rodriguez P, Won H, de Leeuw CA, Pardinas AF, Hu M, Jin K, Hu B, Chen Y, Miller B, Wu B (2018) Integrative functional genomic analysis of human brain development and neuropsychiatric risks. *Science* 362(6420):eaat7615. <https://doi.org/10.1126/science.aat7615>
19. Marsh SE, Walker AJ, Kamath T, Dissing-Olesen L, Hammond TR, de Soysa TY, Young AMH, Murphy S, Abdulraouf A, Nadaf N, Dufort C, Walker AJ, Macosko EZ, Chen F, Stevens B (2022) Dissection of artifactual and confounding glial signatures by single-cell sequencing of mouse and human brain. *Nature Neuroscience* 25(3):306-316. <https://doi.org/10.1038/s41593-022-01022-8>
20. Habib N, McCabe C, Medina S, Varshavsky M, Kitsberg D, Dvir-Szternfeld R, Green G, Dionne D, Nguyen L, Marshall JL, Chen F, Zhang F, Kaplan T, Regev A, Schwartz M (2020) Disease-associated astrocytes in Alzheimer's disease and aging. *Nature Neuroscience* 23(6):701-706. <https://doi.org/10.1038/s41593-020-0624-8>
21. Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A (2018) Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nature Reviews Endocrinology* 14(10):576-590. <https://doi.org/10.1038/s41574-018-0059-4>
22. Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ (2013) Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nature Methods* 10(12):1213-1218. <https://doi.org/10.1038/nmeth.2688>
23. Pliner HA, Packer JS, McFaline-Figueroa JL, Cusanovich DA, Daza RM, Aghamirzaie D, Srivatsan S, Qiu X, Jackson D, Minkina A, Adey AC, Steemers FJ, Shendure J, Trapnell C (2018) Cicero predicts cis-regulatory DNA interactions from single-cell chromatin accessibility data. *Molecular Cell* 71(5):858-871. <https://doi.org/10.1016/j.molcel.2018.06.044>
24. Soreq L, Rose J, Soreq E, Hardy J, Trabzuni D, Cookson MR, Smith C, Ryten M, Patani R, Ule J (2017) Major shifts in glial regional identity are a transcriptional hallmark of human brain aging. *Cell Reports* 18(2):557-570. <https://doi.org/10.1016/j.celrep.2016.12.011>
25. Berchtold NC, Cribbs DH, Coleman PD, Rogers J, Head E, Kim R, Beach T, Miller C, Troncoso J, Trojanowski JQ, Zielke HR, Cotman CW (2008) Gene expression changes in the course of normal brain aging are sexually dimorphic. *PNAS* 105(40):15605-15610. <https://doi.org/10.1073/pnas.0806883105>
26. Yankner BA, Lu T, Loerch P (2008) The aging brain. *Annual Review of Pathology* 3:41-66. <https://doi.org/10.1146/annurev.pathmechdis.2.010506.092044>
27. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, Zheng S, Butler A, Lee MJ, Wilk AJ, Darby C, Zager M, Hoffman P, Stoeckius M, Papalexi E, Mimitou EP, Jain J, Srivastava A, Stuart T, Fleming LM, Yeung B, Rogers AJ, McElrath JM, Blish CA, Gottardo R, Smibert P, Satija R (2021) Integrated analysis of multimodal single-cell data. *Cell* 184(13):3573-3587.

- <https://doi.org/10.1016/j.cell.2021.04.048>
28. Sun N, Victor MB, Park YP, Xiong X, Scannail AN, Leary N, Prosper S, Viswanathan S, Luna X, Boix CA, James BT, Tanigawa Y, Galani K, Mathys H, Jiang X, Ng AP, Bennett DA, Tsai LH, Kellis M (2023) Human microglial state dynamics in Alzheimer's disease progression. *Cell* 186(20):4386-4403.
<https://doi.org/10.1016/j.cell.2023.08.037>
 29. Olah M, Patrick E, Villani AC, Xu J, White CC, Ryan KJ, Piehowski P, Kapasi A, Nejad P, Cimpan M, Connor S, Yung CJ, Frangieh M, McHenry A, Elyaman W, Petyuk V, Schneider JA, Bennett DA, De Jager PL, Bradshaw EM (2018) A transcriptomic atlas of aged human microglia. *Nature Communications* 9:539.
<https://doi.org/10.1038/s41467-018-02926-5>
 30. Fu H, Rodriguez GA, Herman M, Emrani S, Nahmani E, Barrett G, Figueroa HY, Goldberg E, Hussaini SA, Duff KE (2017) Tau pathology induces excitatory neuron loss, grid cell dysfunction, and spatial memory deficits reminiscent of early Alzheimer's disease. *Neuron* 93(3):533-541.
<https://doi.org/10.1016/j.neuron.2016.12.023>
 31. Ianov L, Rani A, Beas BS, Kumar A, Foster TC (2017) Transcription profile of aging and cognition-related genes in the medial prefrontal cortex. *Frontiers in Aging Neuroscience* 9:113.
<https://doi.org/10.3389/fnagi.2017.00113>
 32. Sierksma A, Escott-Price V, De Strooper B (2020) Translating genetic risk of Alzheimer's disease into mechanistic insight and drug targets. *Science* 370(6512):61-66. <https://doi.org/10.1126/science.abb8575>
 33. Ren P, Zhang J, Vijg J (2024) Somatic mutations in aging and disease. *GeroScience* 46(1):5-19.
<https://doi.org/10.1007/s11357-022-00639-4>
 34. Rockenstein E, Torrance M, Adame A, Mante M, Bar-on P, Rose JB, Crews L, Masliah E (2007) Neuroprotective effects of regulators of the glycogen synthase kinase-3 β signaling pathway in a transgenic model of Alzheimer's disease are associated with reduced amyloid precursor protein phosphorylation. *Journal of Neuroscience* 27(8):1981-1991.
<https://doi.org/10.1523/JNEUROSCI.4321-06.2007>
 35. Boisvert MM, Erikson GA, Shokhirev MN, Allen NJ (2018) The aging astrocyte transcriptome from multiple regions of the mouse brain. *Cell Reports* 22(1):269-285.
<https://doi.org/10.1016/j.celrep.2017.12.039>
 36. Wu YE, Pan L, Zuo Y, Li X, Hong W (2017) Detecting activated cell populations using single-cell RNA-seq. *Neuron* 96(2):313-329.
<https://doi.org/10.1016/j.neuron.2017.09.026>
 37. Grubman A, Chew G, Ouyang JF, Sun G, Choo XY, McLean C, Simmons RK, Buckberry S, Vargas-Landin DB, Poppe D, Pflueger J, Lister R, Rackham OJL, Petretto E, Polo JM (2019) A single-cell atlas of entorhinal cortex from individuals with Alzheimer's disease reveals cell-type-specific gene expression regulation. *Nature Neuroscience* 22(12):2087-2097.
<https://doi.org/10.1038/s41593-019-0539-4>
 38. Klemm SL, Shipony Z, Greenleaf WJ (2019) Chromatin accessibility and the regulatory epigenome. *Nature Reviews Genetics* 20(4):207-220.
<https://doi.org/10.1038/s41576-018-0089-8>
 39. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. *Cell* 153(6):1194-1217.
<https://doi.org/10.1016/j.cell.2013.05.039>
 40. Zhang W, Qu J, Liu GH, Belmonte JCI (2020) The ageing epigenome and its rejuvenation. *Nature Reviews Molecular Cell Biology* 21(3):137-150.
<https://doi.org/10.1038/s41580-019-0204-5>
 41. Booth LN, Brunet A (2016) The aging epigenome. *Molecular Cell* 62(5):728-744.
<https://doi.org/10.1016/j.molcel.2016.05.013>
 42. Cheng Y, Bandaru A, Aygun A, Tiwari S, Karandikar S, Kim EJ, Nicoll M, Hulsmans M, Schloss MJ, Sadreyev RI, Nahrendorf M, Naba A, Swirski FK, Robbins CS (2022) Single-cell chromatin profiling reveals ageing-associated regulatory programmes in cardiac immune cells. *Nature Aging* 2(3):212-227.
<https://doi.org/10.1038/s43587-022-00184-x>