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Hippocampal snRNA-seq In Collaborative Cross Reveals Molecular Signatures of Cognitive Resilience Independent of Chronological Aging

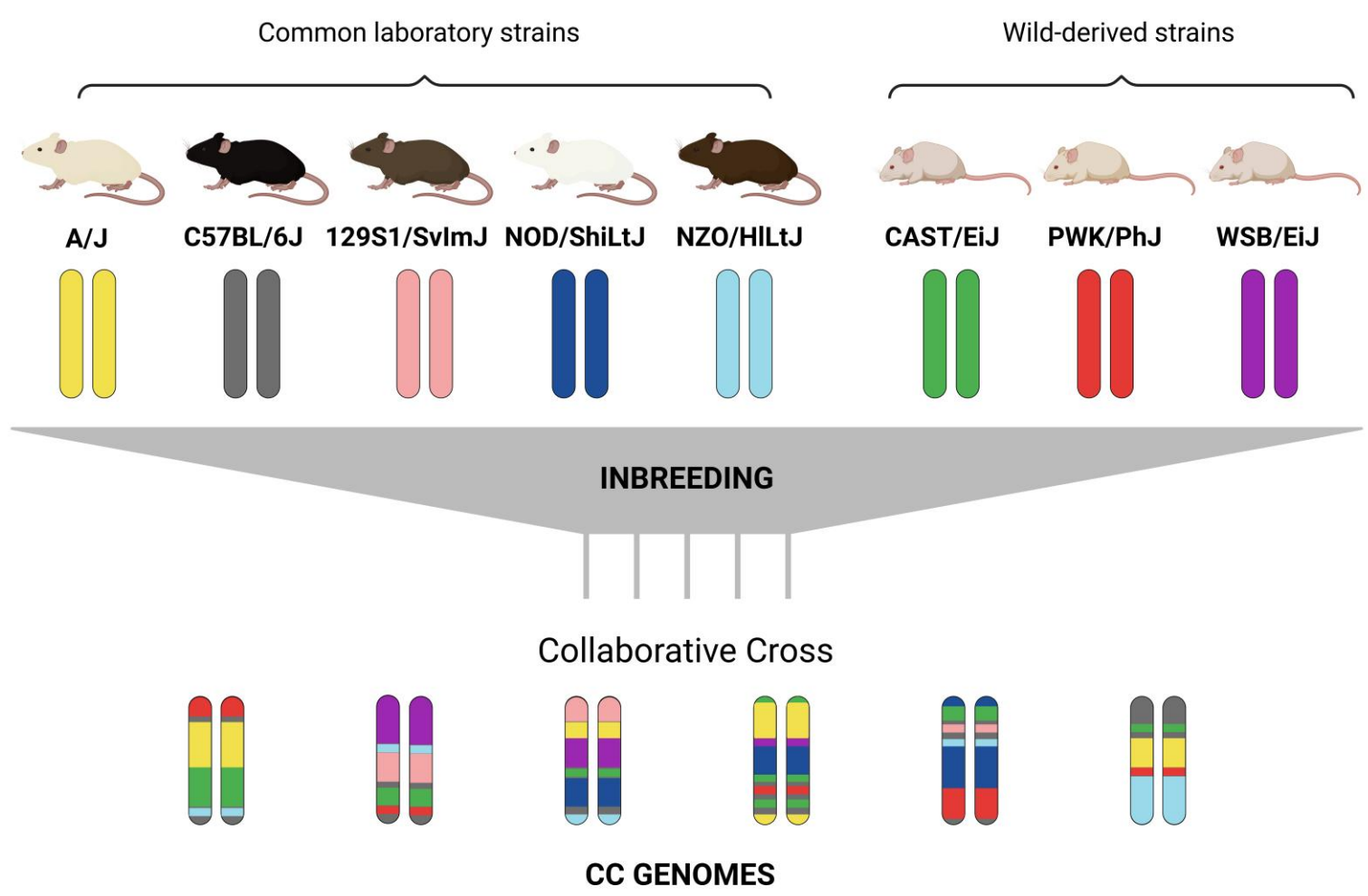
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Collaborative Cross mice: A genetically diverse model for studying cognitive resilience and age-related cognitive decline

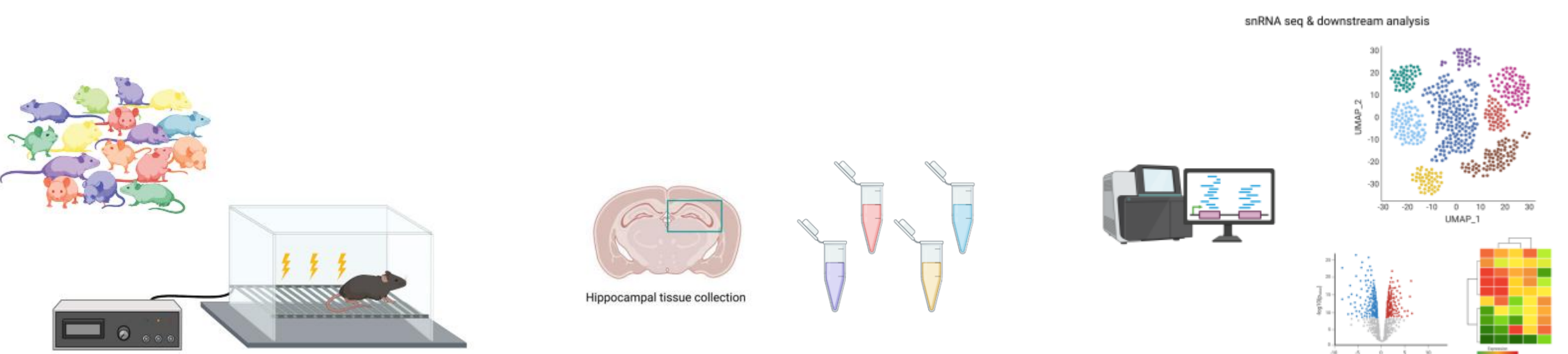
Aging is the strongest risk factor for cognitive decline, yet substantial variability exists in cognitive aging trajectories. While some individuals experience pronounced age-related cognitive impairment, others maintain cognitive function well into late life, suggesting the presence of biological mechanisms that promote cognitive resilience. Genetic factors are thought to contribute significantly to this variability, but the molecular pathways underlying resilience remain poorly understood. Here, we leveraged the genetically diverse Collaborative Cross mouse population and hippocampal single-nucleus RNA sequencing to determine whether cognitive decline reflects the same molecular processes as chronological aging or represents a distinct biological program.



The Collaborative Cross (CC) consists of recombinant inbred strains derived from eight founder mice. The resulting population captures extensive genetic diversity and serves as a powerful resource for studying complex traits.

Study Design and Single-Nucleus RNA Sequencing Workflow

Hippocampal tissue was collected from 107 Collaborative Cross mice spanning eight strains, three age groups (6, 12, and 18 months), and both sexes following contextual fear memory testing. Single-nucleus RNA sequencing (snRNA-seq) was performed to characterize cell-type-specific transcriptional changes associated with chronological aging and cognitive decline. Differential expression and pathway enrichment analyses were conducted across major hippocampal cell populations and excitatory neuron subtypes.



Overview of the study workflow. Mice from eight Collaborative Cross strains underwent contextual fear memory testing prior to hippocampal tissue collection. Isolated nuclei were processed for single-nucleus RNA sequencing, followed by cell type annotation, differential expression analysis, and pathway enrichment analyses.

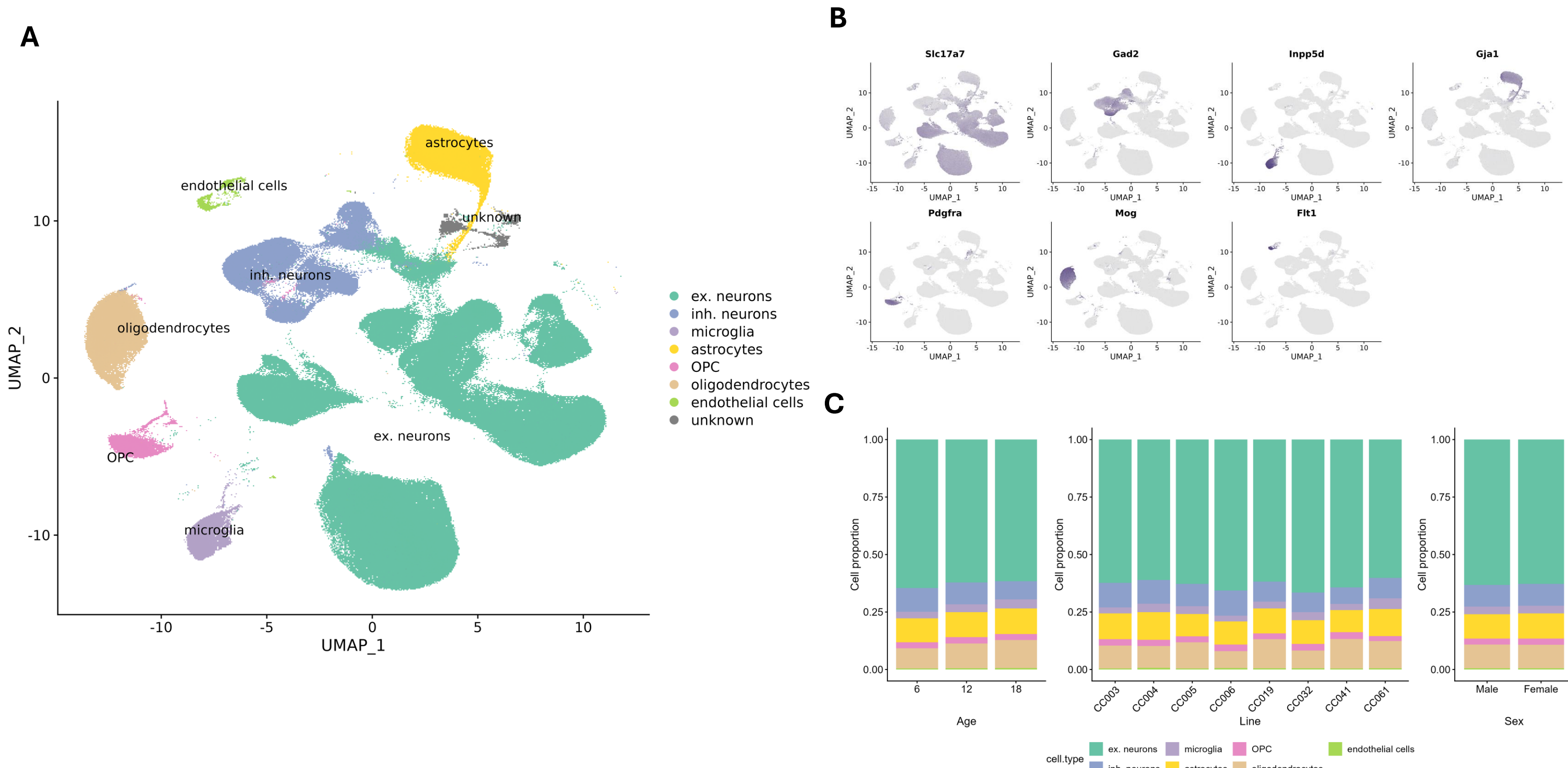
Strain	Female	Male	6 months	12 months	18 months
CC019	6	4	4	4	2
CC003	6	6	4	4	4
CC041	4	4	4	4	0
CC051	6	5	4	4	3
CC052	6	6	4	4	4
CC006	4	4	4	4	0
CC005	6	6	4	4	4
CC004	6	6	4	4	4

Distribution of sequenced animals across Collaborative Cross strains, sex, and age groups. A total of 107 mice spanning eight CC strains and three age points (6, 12, and 18 months) were included in the snRNA-seq dataset.

Acknowledgement

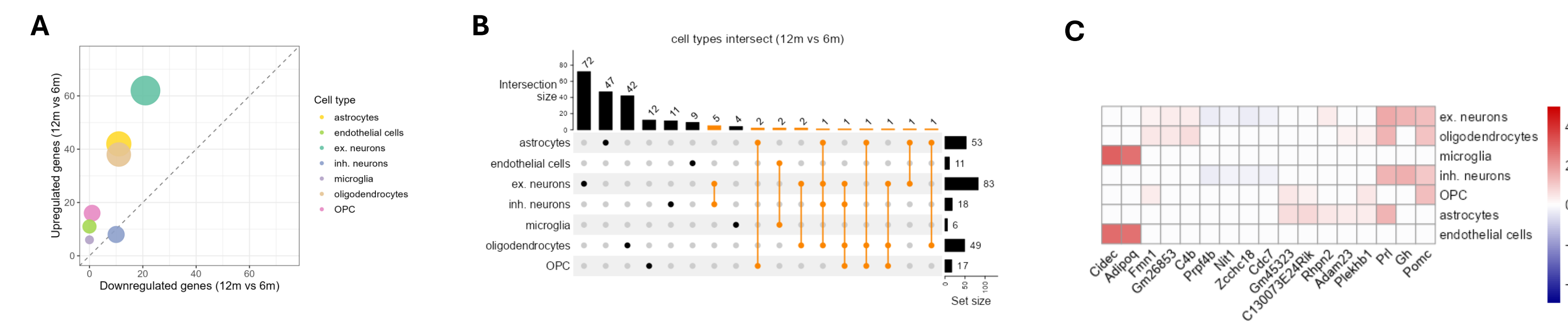
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Hippocampal snRNA-seq identifies major cell populations across genetically diverse aging mice



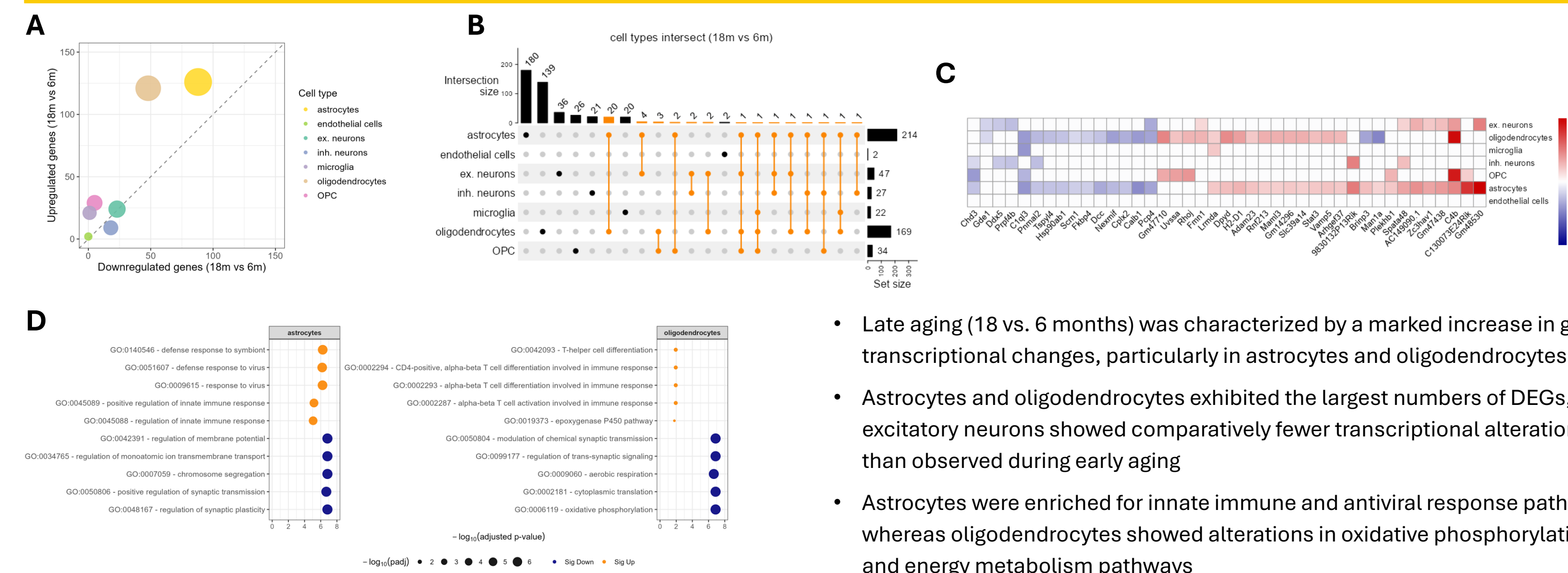
- snRNA-seq identified seven major hippocampal cell populations, including excitatory neurons, inhibitory neurons, astrocytes, microglia, oligodendrocytes, OPCs, and endothelial cells
- Excitatory neurons represented the largest cell population across all Collaborative Cross strains and age groups
- Overall cellular composition remained largely stable across aging, strain and sex, with no major shifts in neuronal populations
- Genetic background and age primarily influenced glial cell proportions, particularly oligodendrocytes and microglia

Early aging induces cell-type-specific transcriptional responses in the hippocampus, with excitatory neurons showing the greatest dysregulation



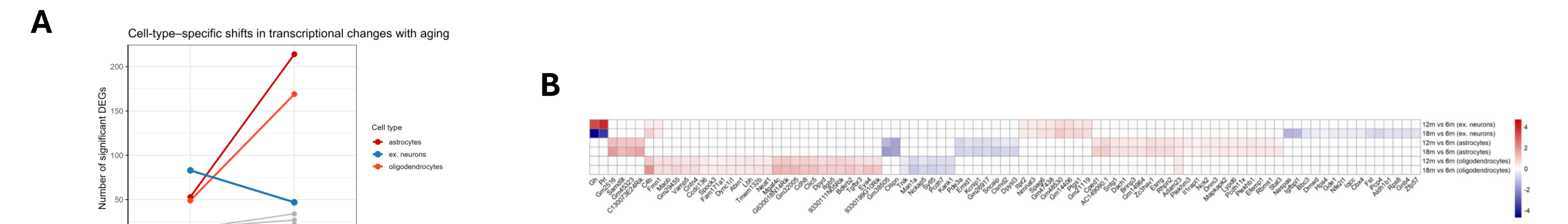
- Excitatory neurons displayed the greatest number of DEGs during early aging (12 vs. 6 months)
- Most aging-associated DEGs were cell-type specific, with limited overlap across hippocampal populations.
- Shared aging-responsive genes included Gh, Prl, Cidec, and Adipoq, suggesting common molecular responses across multiple cell types.
- Pathway analysis identified enrichment of growth factor signaling and developmental pathways in excitatory neurons, JAK-STAT signaling in astrocytes, and protein folding/chaperone pathways in oligodendrocytes

Late-stage aging shifts transcriptional burden to glia with coordinated astrocyte and oligodendrocyte dysregulation



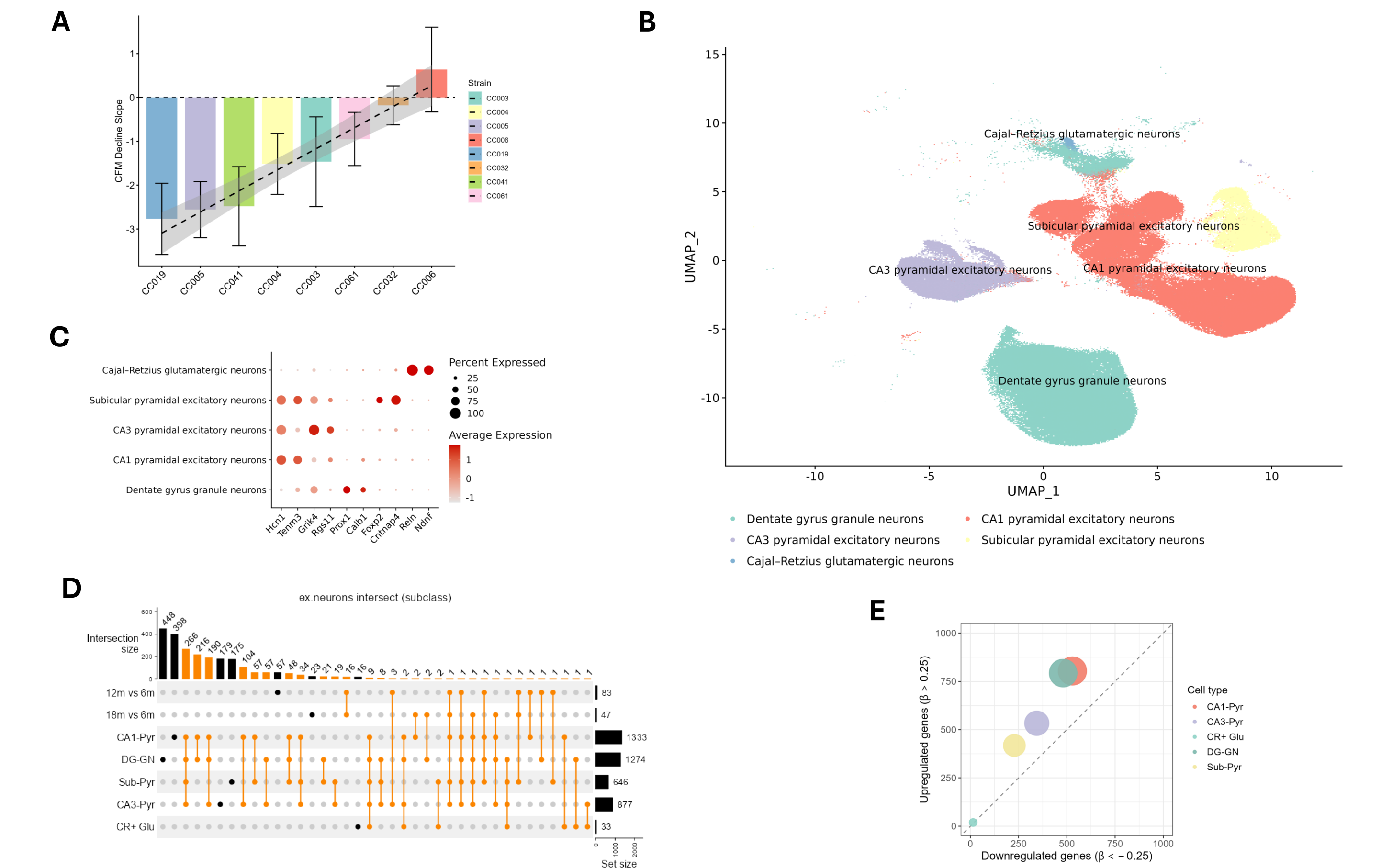
- Late aging (18 vs. 6 months) was characterized by a marked increase in glial transcriptional changes, particularly in astrocytes and oligodendrocytes
- Astrocytes and oligodendrocytes exhibited the largest numbers of DEGs, while excitatory neurons showed comparatively fewer transcriptional alterations than observed during early aging
- Astrocytes were enriched for innate immune and antiviral response pathways, whereas oligodendrocytes showed alterations in oxidative phosphorylation and energy metabolism pathways

Aging drives a progressive shift from neuronal responses to glial remodeling



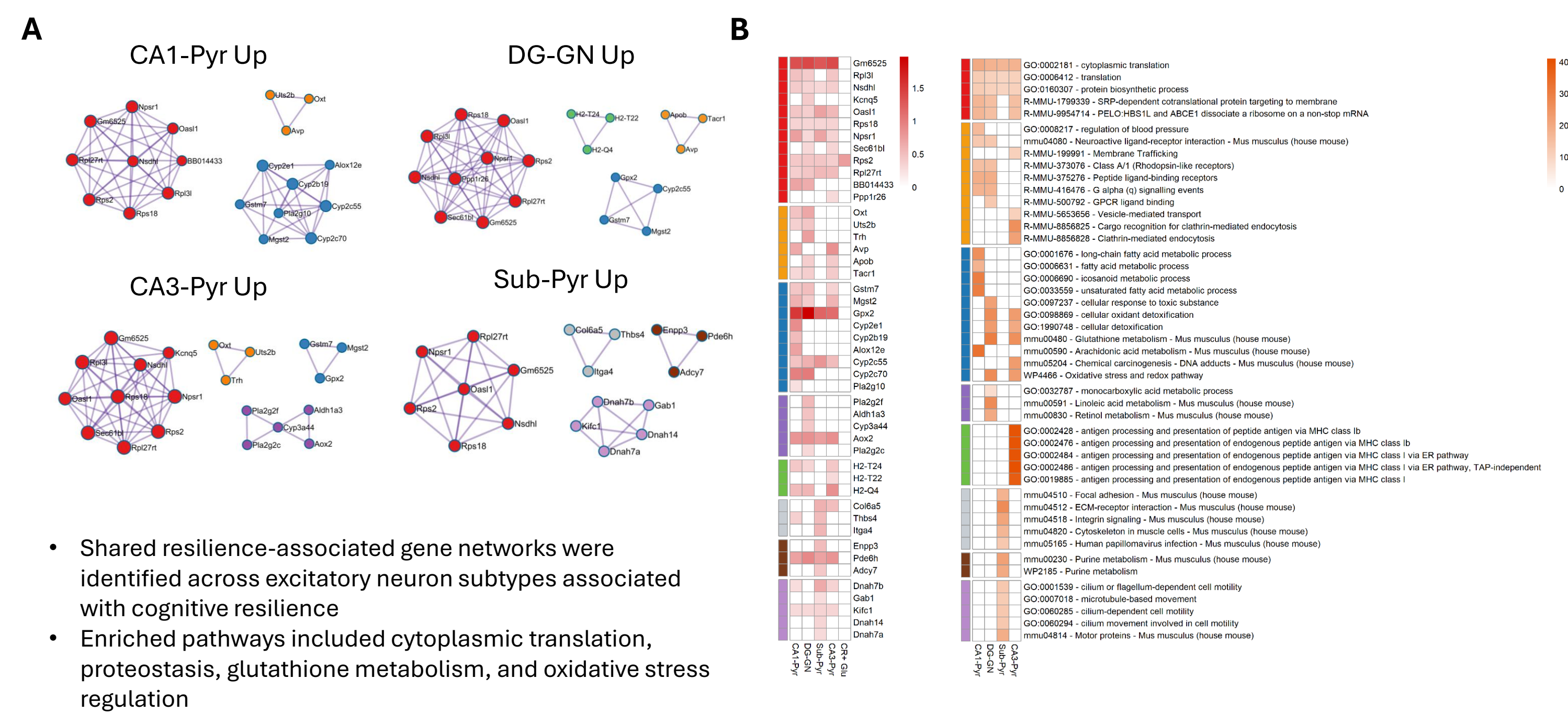
- Excitatory neurons exhibited an early transcriptional response that diminished at later ages
- Astrocytes and oligodendrocytes accumulated progressively more DEGs with advancing age, indicating sustained glial remodeling

Cognitive decline-associated transcriptional changes are driven by excitatory neurons and with little overlap with chronological aging



- Initial differential expression analysis revealed that excitatory neurons contributed the largest number of cognitive decline-associated DEGs across all major hippocampal cell types
- Therefore, excitatory neurons were further subdivided into five hippocampal populations: CA1 pyramidal, CA3 pyramidal, dentate gyrus granule, subicular pyramidal, and Cajal-Retzius neurons
- CA1 pyramidal and dentate gyrus granule neurons exhibited the strongest transcriptional responses, contributing the greatest numbers of cognitive decline-associated DEGs
- The majority of cognitive decline-associated DEGs showed little overlap with chronological aging-associated DEGs, indicating that cognitive decline and aging are driven by largely distinct transcriptional programs

Proteostasis and glutathione pathways emerge as conserved signatures of cognitive resilience



- Shared resilience-associated gene networks were identified across excitatory neuron subtypes associated with cognitive resilience
- Enriched pathways included cytoplasmic translation, proteostasis, glutathione metabolism, and oxidative stress regulation