

Fibrillin-1 Contributes To Sex- And Region-Specific Differential ENS Organization And Is Downregulated In The Aging Gut

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Background

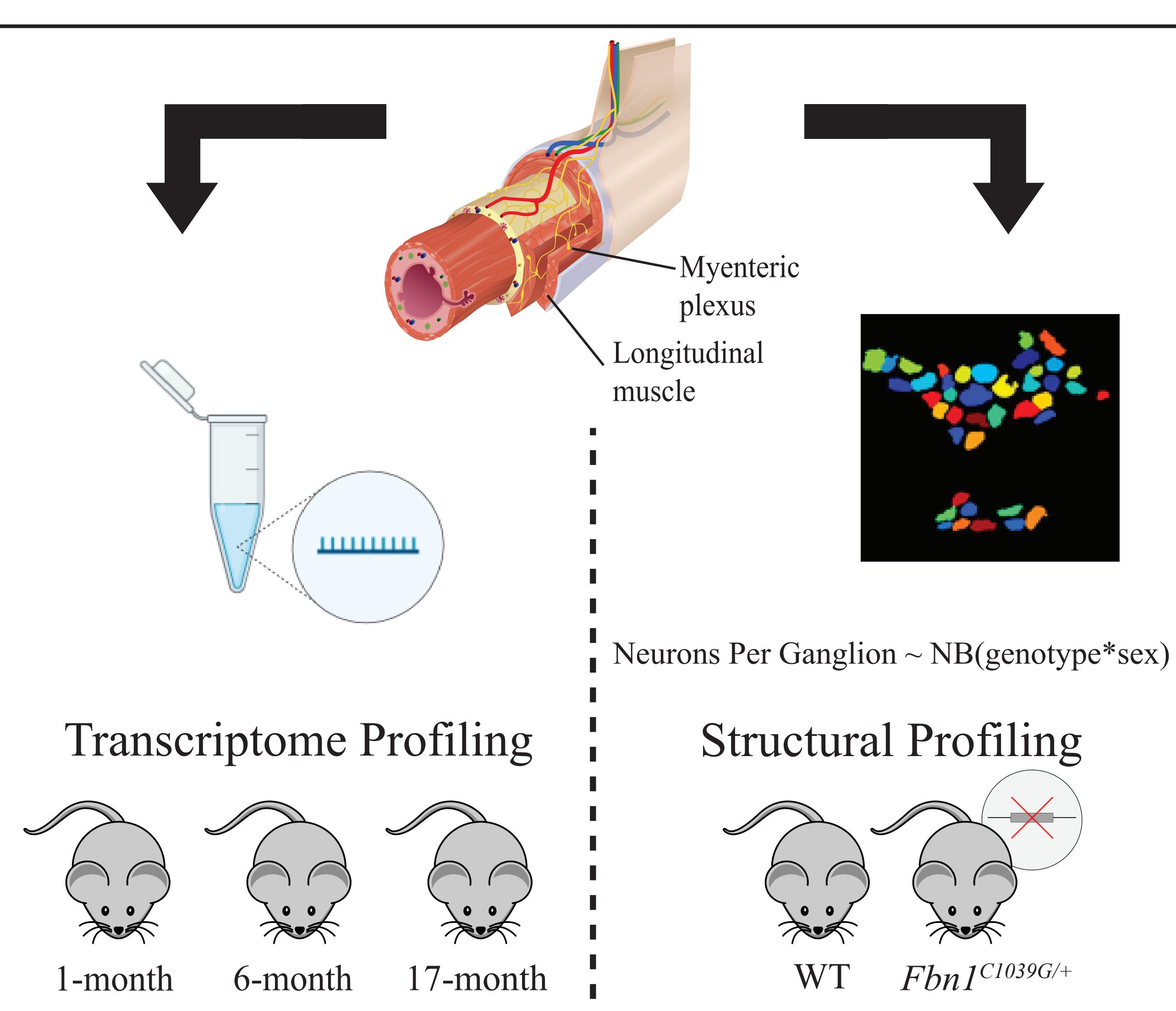
- The enteric nervous system (ENS) undergoes structural and functional changes during maturation
- The maturation phase is associated with significant changes in neuronal composition by neurochemical markers and developmental lineage
- Many of the molecular factors that drive or change during ENS maturation are unknown
- Diagnosis and treatment of functional gastrointestinal disorders, which increase in incidence with age, will be improved by a better understanding of the aging ENS

Objectives

- Investigate age-associated changes in the murine ENS and its local environment by profiling the coding transcriptome
- Understand how and to what extent the ENS is altered by the aging process

Methods

- The ileal longitudinal muscle and myenteric plexus (LM-MP) from male and female juvenile (1-month), mature adult (6-month) and elderly (17-month) C57BL6/J mice were isolated for bulk RNA-seq sequencing
- To identify molecular changes that occur with age, the coding transcriptome was profiled (1mo: N = 3 per sex, 6mo: N = 3 per sex, 17mo: N = 2 per sex)
- Genes that are differentially expressed with age were identified using the Wald test implemented in DESeq2 ($p < 0.05$)
- To further investigate the role of a dysregulated matrisome in ENS structure, ganglionic structure was studied in the *Fbn1*^{C1039G} mouse model of Marfan syndrome
- LM-MP tissue was prepared for wild-type and heterozygous *Fbn1*^{C1039G/+} mice (8-10 wks) and immunostained for HuC/D, a neuron-specific marker
- To profile ganglionic structure (size), the distribution of neurons per ganglion in the ileum and distal colon of males and females was measured on an EVOS M7000 Imaging System at 10x and 20x, respectively
- Differences in ganglia sizes between conditions were tested within each region using negative binomial regression, estimating the effects of genotype, sex, and their interaction on ganglia size



Results

- 700 genes have dynamic expression over the ages profiled, with more drastic effects observed during maturation than subsequent aging (348 genes upregulated, 309 genes downregulated, and 43 genes with inconsistent direction-of-effect, relative to 1-month)
- Transcriptional changes suggest age-associated shifts in ECM-remodeling, an increased inflammatory state, and dysregulation of neuronally-relevant gene expression
- In the *Fbn1*^{C1039G} Marfan syndrome model, ENS organization is disrupted by a heterozygous missense mutation in *Fbn1*, with opposing region- and sex-specific effects

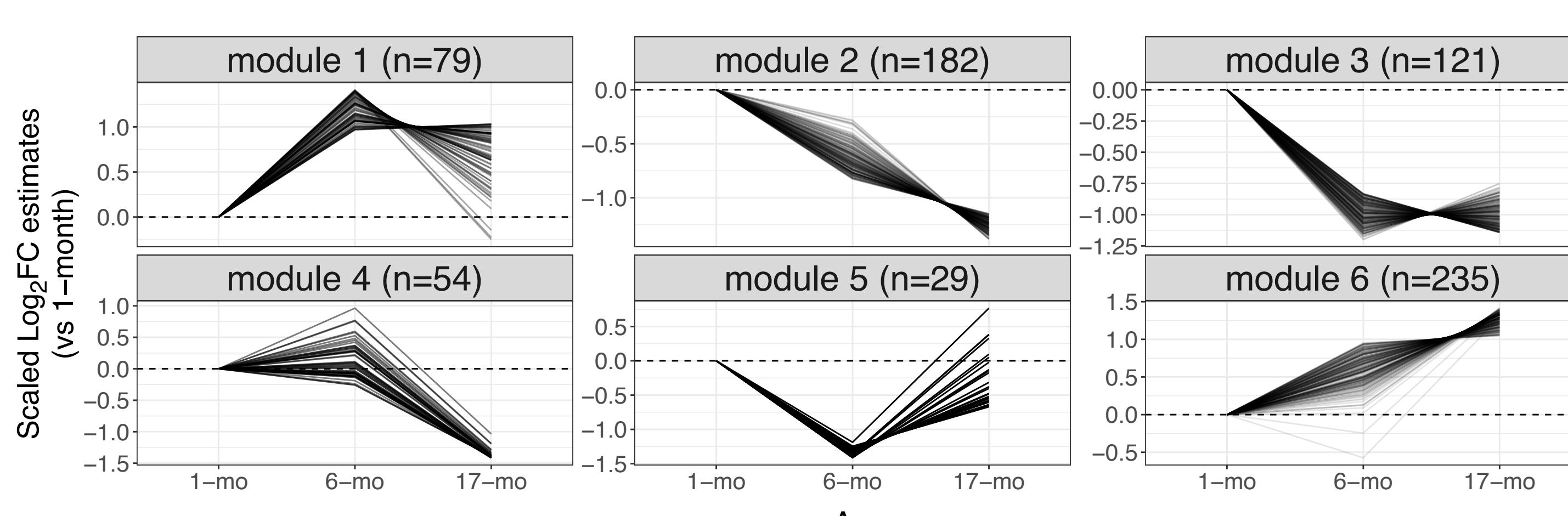
Conclusions

- Shifts in ECM gene expression with age suggest remodeling of the local environment of neurons as a potential target for therapeutics
- Fibrillin-1 has a key role in establishing or maintaining the structure of the ENS
- Partial loss of function of Fibrillin-1 results in region- and sex-specific effects to ganglionic organization, with likely implications for the neuronal circuitry

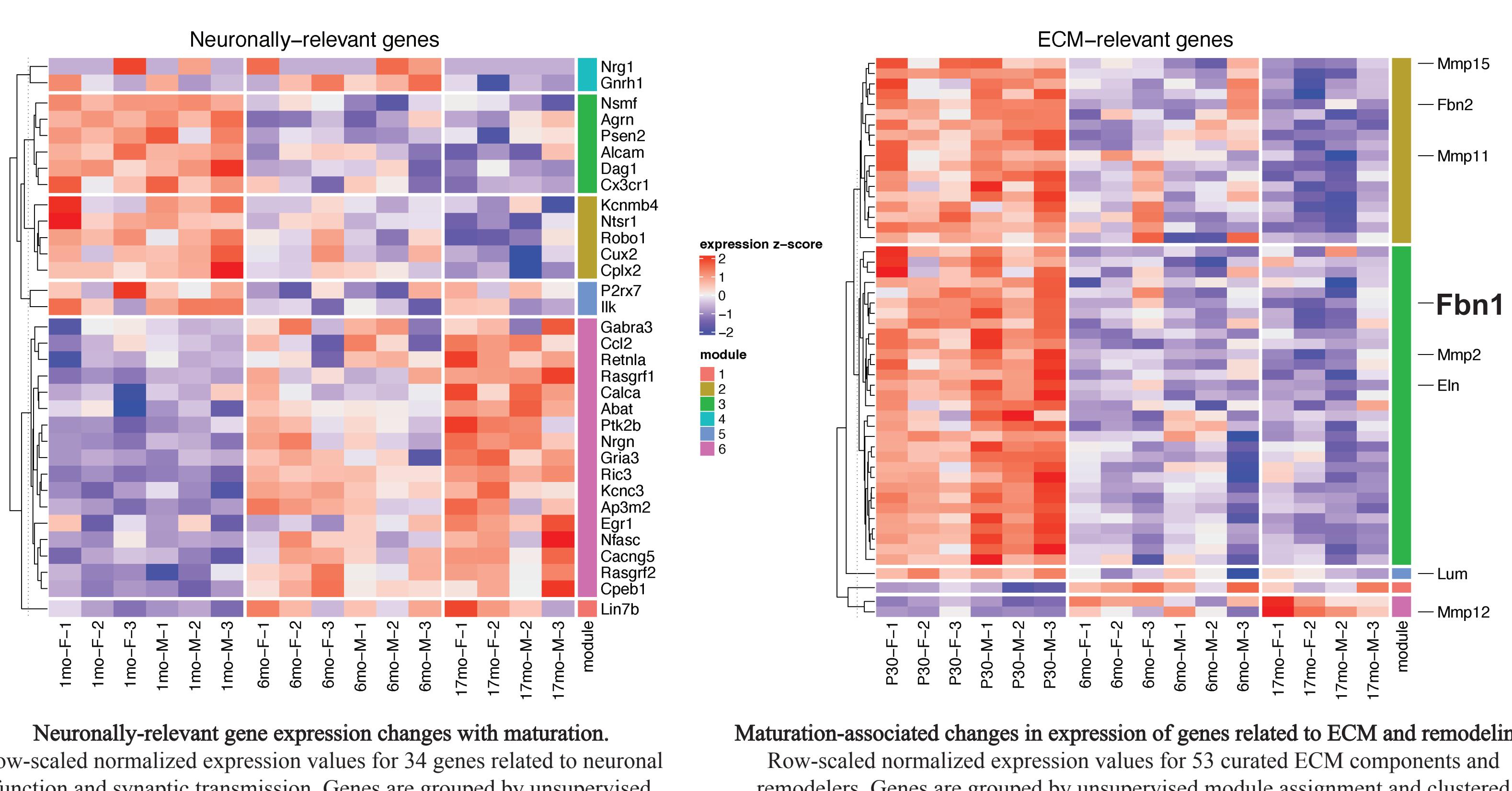
Acknowledgments

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Neuronal and extracellular matrix gene expression profiles shift with maturation and aging of the ENS



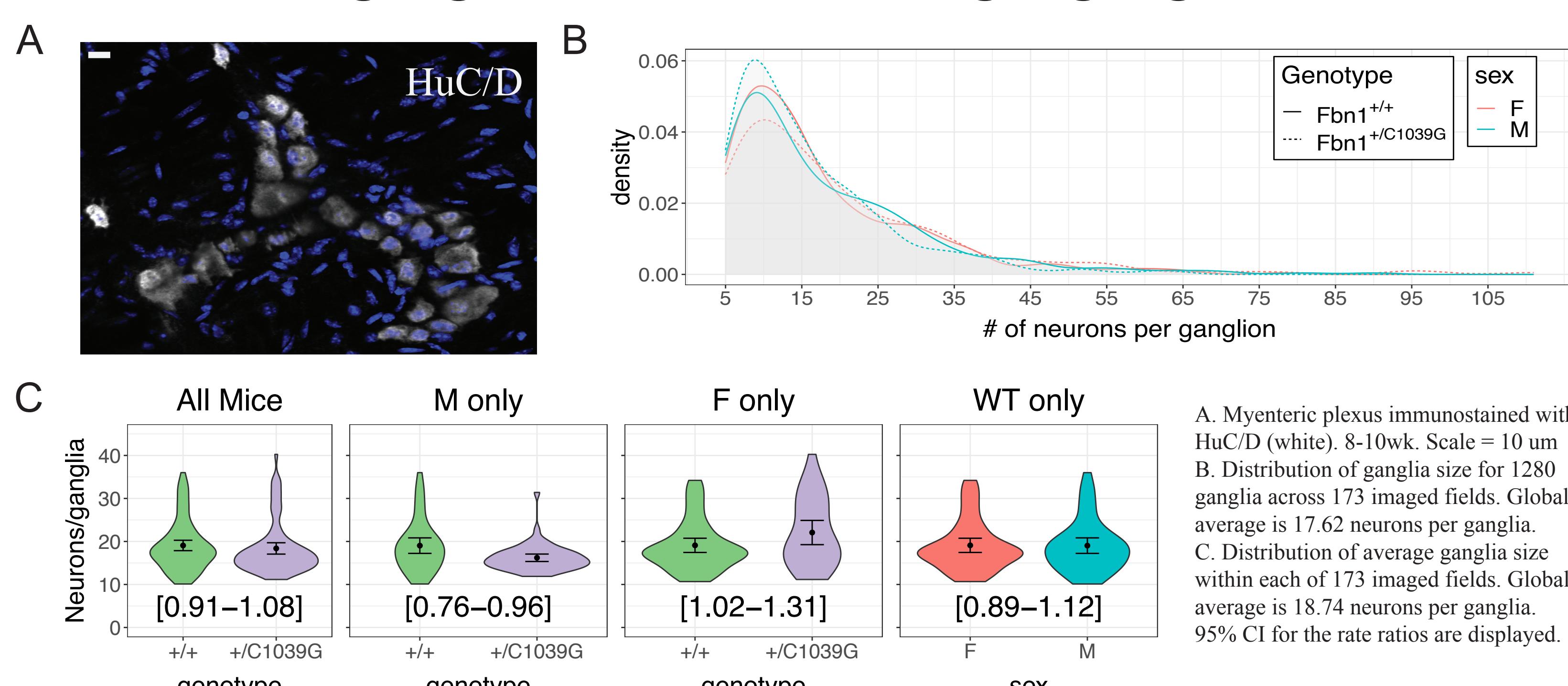
Dynamic gene modules identified from aging bulk RNA-seq analysis.
700 differentially expressed genes were clustered into modules using a cosine-distance metric of their Log₂FC relative to 1-month. 6 modules were identified that capture expression dynamics between the profiled ages. Log₂FC were scaled within each gene for visualization. Dashed line represents constant expression with age.



Neuronally-relevant gene expression changes with maturation.
Row-scaled normalized expression values for 34 genes related to neuronal function and synaptic transmission. Genes are grouped by unsupervised module assignment and clustered within each group.

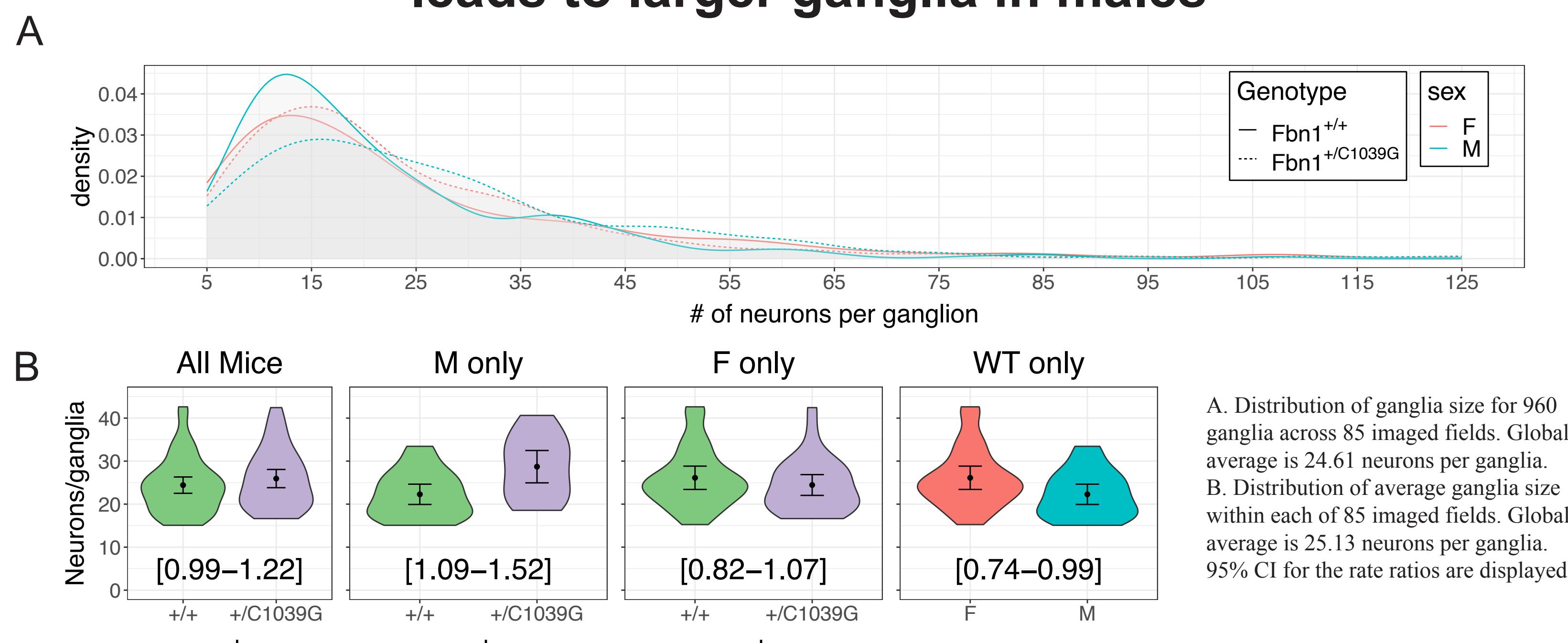
Maturation-associated changes in expression of genes related to ECM and remodeling.
Row-scaled normalized expression values for 53 curated ECM components and remodelers. Genes are grouped by unsupervised module assignment and clustered within each group. Select genes are labeled.

Ileum Myenteric Plexus: a missense mutation in *Fbn1* leads to smaller ganglia in males, and larger ganglia in females



A. Myenteric plexus immunostained with HuC/D (white). 8-10wks. Scale = 10 μ m
B. Distribution of ganglia size for 1280 ganglia across 173 imaged fields. Global average is 17.62 neurons per ganglia.
C. Distribution of average ganglia size within each of 173 imaged fields. Global average is 18.74 neurons per ganglia.
95% CI for the rate ratios are displayed.

Colon Myenteric Plexus: a missense mutation in *Fbn1* leads to larger ganglia in males



A. Distribution of ganglia size for 960 ganglia across 85 imaged fields. Global average is 24.61 neurons per ganglia.
B. Distribution of average ganglia size within each of 85 imaged fields. Global average is 25.13 neurons per ganglia.
95% CI for the rate ratios are displayed.