Park7 deletion leads to sex-specific transcriptome changes involving NRF2-CYP1B1 axis in mouse midbrain astrocytes

Supplementary Information

Supplementary Figures

Supplementary Table Legends

Supplementary Information

The Supplementary Information contains seven Supplementary Figures and five Supplementary Tables.

Supplementary Figure 1. Distribution of the per-gene dispersion in *Park7*$^{-/-}$ mouse midbrain. Per-gene dispersions to estimate the median dispersion each sample set show similar results in all sample sets, except for the second cohort of 8-month-old male samples. Lack of changes in 3 and 8-month-old females are demonstrated to not be due to technical or biological (oestrus cycle) variation between the female samples. Increased dispersion in the second cohort of 8-month-old male samples partially explains the lower number of DEGs compared to the first cohort.

Supplementary Figure 2. Loss of *Park7* in female mice does not lead to gene expression changes associated with NRF2 signaling, epithelial-to-mesenchymal transition, focal adhesion, and extracellular matrix composition. (a-d) Pathway enrichment analysis for the 101 DEGs altered in 8-month-old female mice. The top 5 results from (a) KEGG pathways, (b) WikiPathway pathways, (c) MSigDB Hallmark pathways, and (d) GO terms for cellular components based on the significance of enrichment are shown.The x-axis represents the -log10 p-value of pathway enrichment. Complete results of enrichment analysis are available in the Supplementary Table S4. (e) Top 5 TFs associated with DEGs from Park7-/- female mice based on the significance of enrichment for primary TF targets from the ENCODE project and ChEA database. The x-axis represents the -log10 p-value. (f) Top5 Upstream Regulators associated with DEGs from Park7-/- female mice predicted by Ingenuity Pathway Analysis (IPA). The x-axis represents the -log10 p-value of pathway enrichment.

Supplementary Figure 3. RNAscope and RT-qPCR validation of gene expression levels. (**a-b)** RNAscope validation of *Cdh1* expression in the (a) midbrain of *Park7*$^{-/-}$ female mice and (b) cortex of *Park7*$^{-/-}$ male mice at 8-months of age. *Cdh1* mRNA is stained in purple, DAPI staining of DNA is in blue. Representative images from analysis of 3 independent mice per genotype are shown. No differences were observed. **(c-d)** RT-qPCR analysis of (c) *Irs2* and (d) *Crim1* expression in the midbrain of *Park7*$^{-/-}$ male and female mice and in male cortex at 8-months of age. Values represent mean ± SEM (n=4-8 mice per group). Statistical significance was tested by unpaired t-test. * = p-value < 0.05. **(e-f)** RNAscope validation of *Gstm2* expression in the (a) midbrain of *Park7*$^{-/-}$ female mice and (b) cortex of *Park7*$^{-/-}$ male mice at 8-months of age. *Gstm2* mRNA is stained in purple, DAPI staining of DNA is in blue. Representative images from analysis of 3 independent mice per genotype are shown. No differences were observed.

Supplementary Figure 4. Analysis of Cyp1b1 expression. (**a-b)** RNAscope validation of *Cyp1b1* expression in the (a) midbrain of *Park7*$^{-/-}$ female mice and (b) cortex of *Park7*$^{-/-}$ male mice at 8-months of age. *Cyp1b1* mRNA is stained in red, DAPI staining of DNA is in blue. Representative images from analysis of 3 independent mice per genotype are shown. No differences were observed. **(c)** Immunohistochemistry of CYP1B1 in mouse kidney sections was used to test the specificity of the CYP1B1 antibody. CYP1B1 is stained in red, DRAQ7 staining of DNA is in purple.

Supplementary Figure 5. Genotyping of newborn mice prior to glial cell isolation. *Ube* primers were used to test whether DNA from newborn mouse tails carried XY (two separate bands) or XX (one stronger band) genotype. L = Ladder.

Supplementary Figure 6. Expression analysis of cell type-purity and astrocyte reactivity marker genes in primary glial cultures. (a-b) Expression levels of the indicated **(a)** cell type-specific marker genes in cultures of isolated primary astrocytes, microglia, and oligodendrocytes, and **(b)** markers of reactive astrocytes in primary astrocytes and microglia, as determined by RNA-seq analysis. Red dots represent female and blue dots male samples, asterisk indicates the mean of three independent samples, and x-axis represents transcripts per million (TPM).

Supplementary Figure 7. Enrichment analysis of upregulated genes upon *Park7* knock-down. (a-c) Heatmaps showing the differences in pathway enrichments of upregulated DEGs from **panels a-c** using the (**a)** ENCODE project and ChEA databases, **(b)** BioPlanet, and **(c)** MSigDB Hallmark databases, focusing on the top 5 most enriched pathways or TFs upon *Park7* depletion. The -$\log_{10}$ p-values of the enrichments are depicted as color scale.

Supplementary Table 1. List of DEGs from *Park7*$^{-/-}$ mice compared to wild type littermate controls. The separate worksheets include DEGs from 3-month-old female mice, 3-month-old male mice, 8-month-old female mice, 8-month-old male mice (first cohort), 8-month-old male mice (second cohort), 8-month-old male mice (first and second cohort combined using batch correction), with FDR < 0.05. The table includes ENSEMBL IDs, base mean, $\log_2$-fold change, p-value, FDR (padj), and gene symbol for each DEG.

Supplementary Table 2. Mapped reads for the 4 groups of mice and the 3 groups of astrocytes.

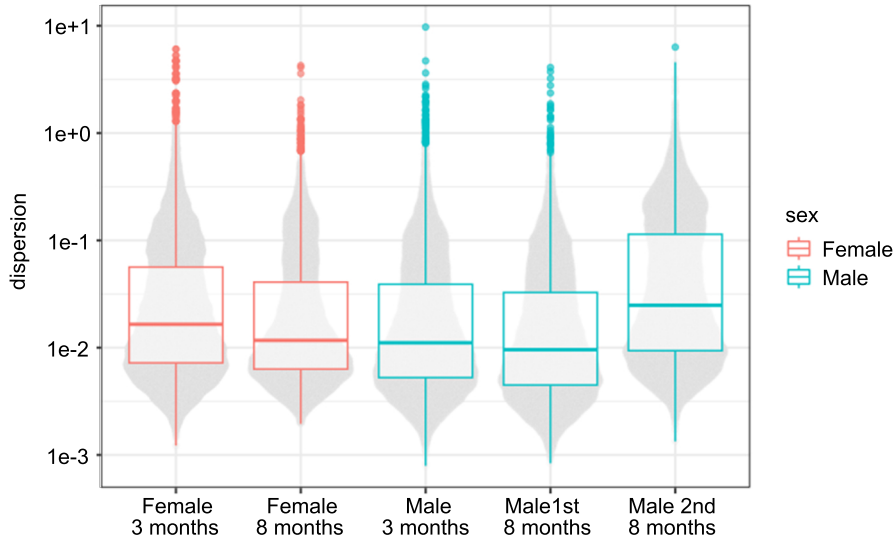
Supplementary Table 3. Lists of enriched pathways in males. Separate worksheets include enrichments for analysis using DEGs obtained from the comparison of 8-month-old male *Park7*$^{-/-}$ mice with the wild type littermate controls (first and second cohort together) from KEGG, WikiPathway, MSigDB, and BioPlanet databases, and the GO terms for Cellular components.

Supplementary Table 4. Lists of enriched pathways in females. Separate worksheets include enrichments for analysis using DEGs obtained from the comparison of 8-month-old female *Park7*$^{-/-}$ mice with the wild type littermate controls from KEGG, WikiPathway, MSigDB, and BioPlanet databases, and the GO terms for Cellular components.

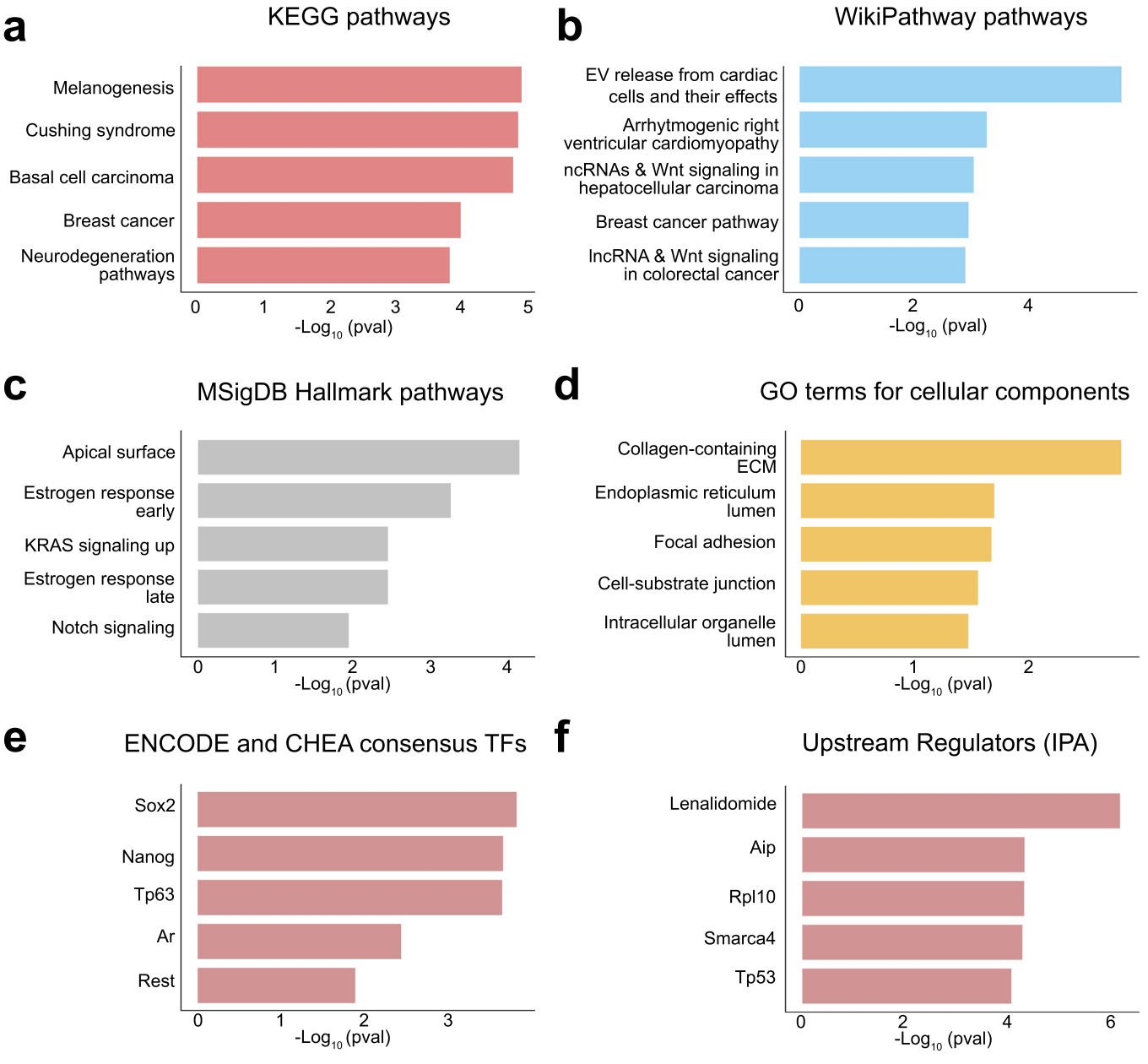
Supplementary Table 5. Lists DEGs in primary mouse astrocytes upon the knock-down of *Park7*, *Nfe2l2*, or *Cyp1b1*. DEGs were defined by applying FDR < 0.05. The table includes ENSEMBL IDs, base mean, $\log_2$-fold change, p-value, FDR (padj), and gene symbol for each DEG.

Supplementary Figure 1

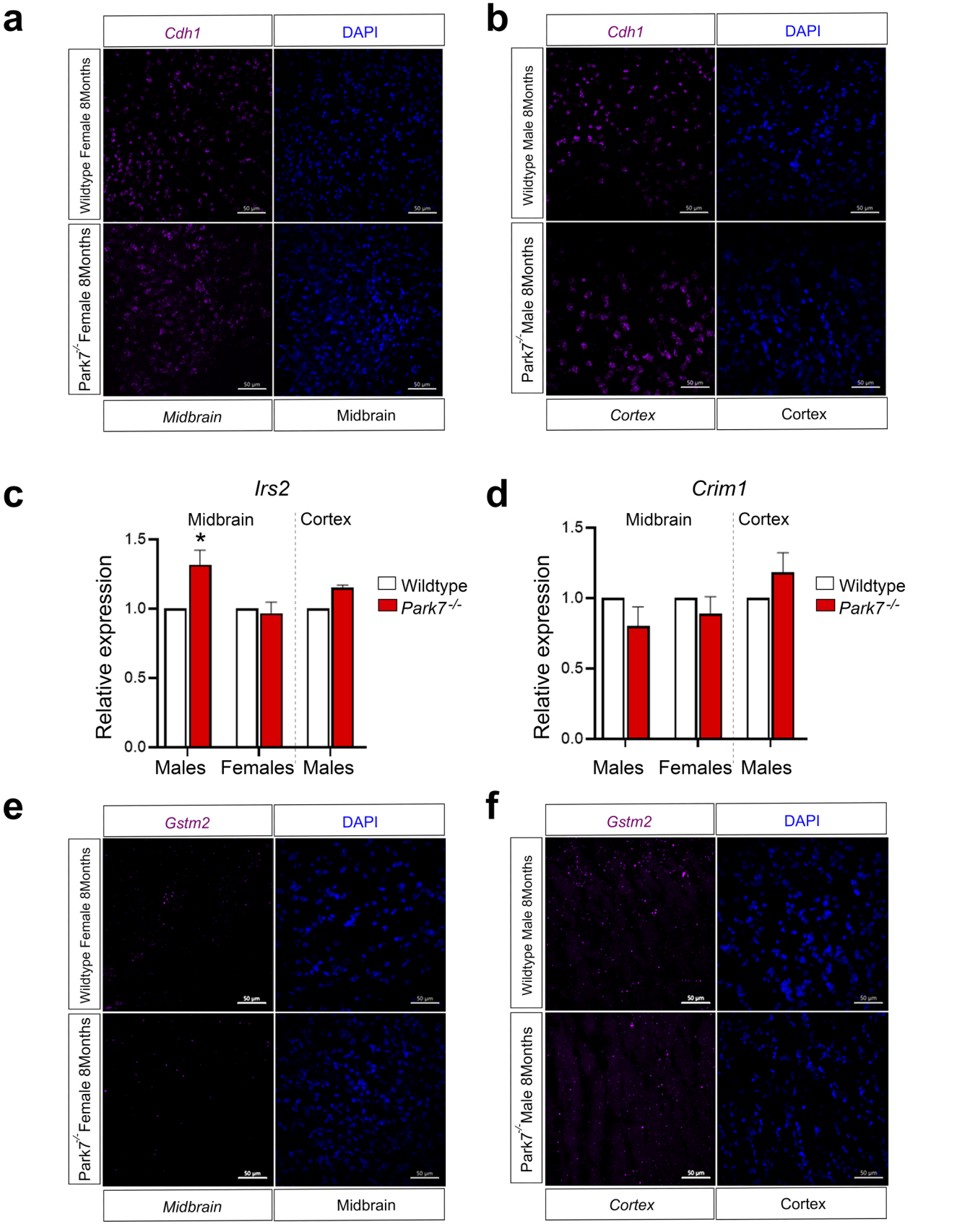
a



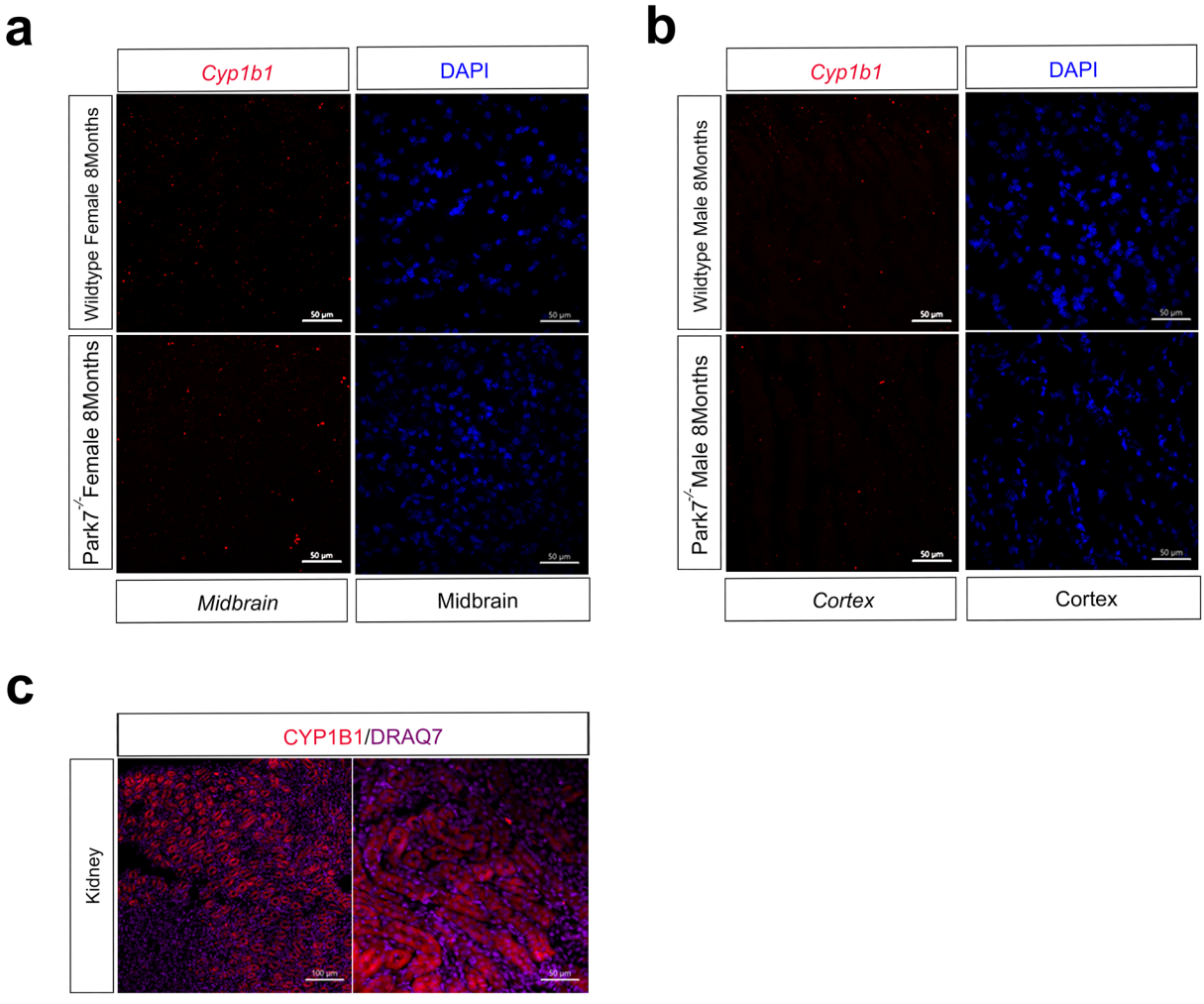
Supplementary Figure 2



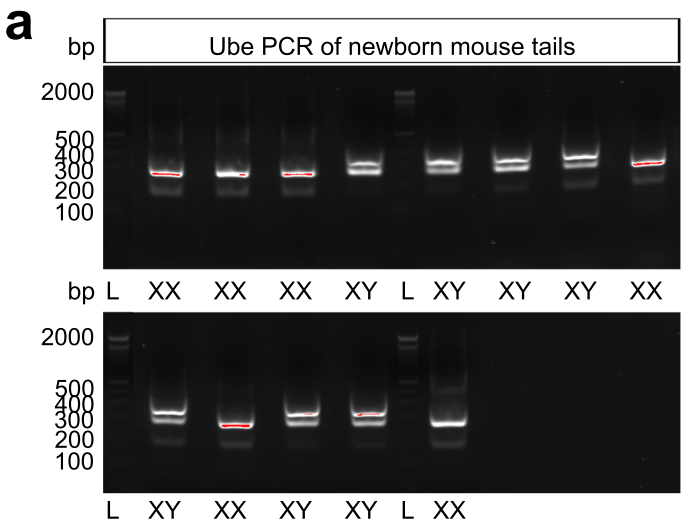
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



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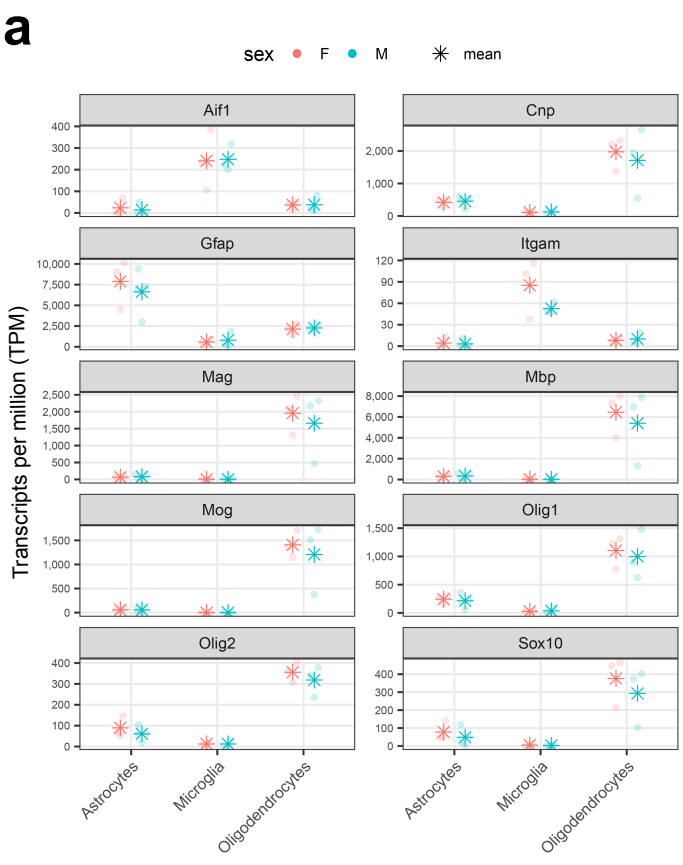

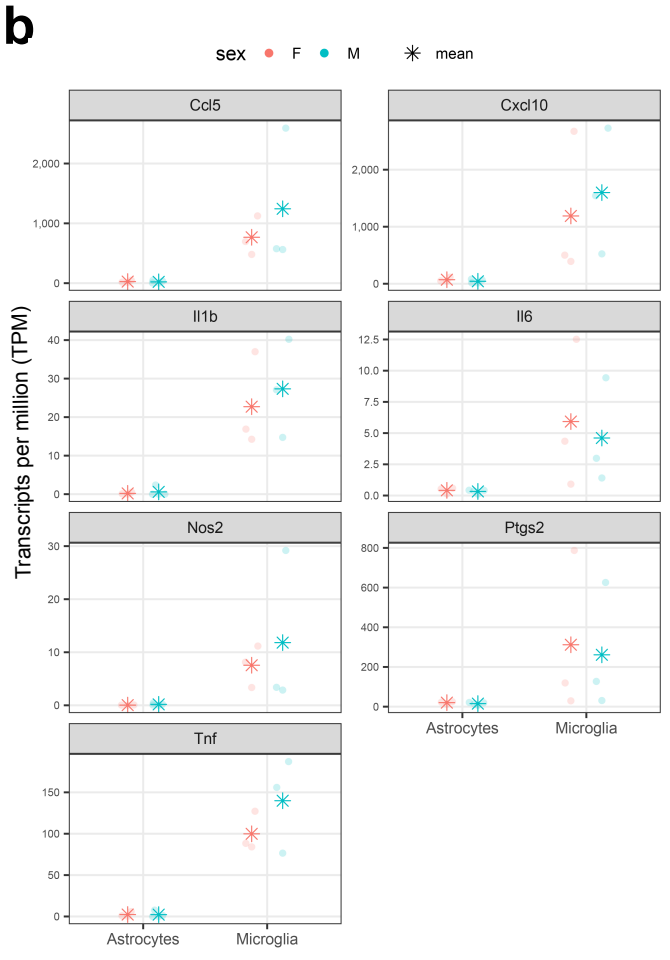

Supplementary Figure 7