Molecular and network disruptions in neurodevelopment uncovered by single cell transcriptomics analysis of CHD8 heterozygous cerebral organoids

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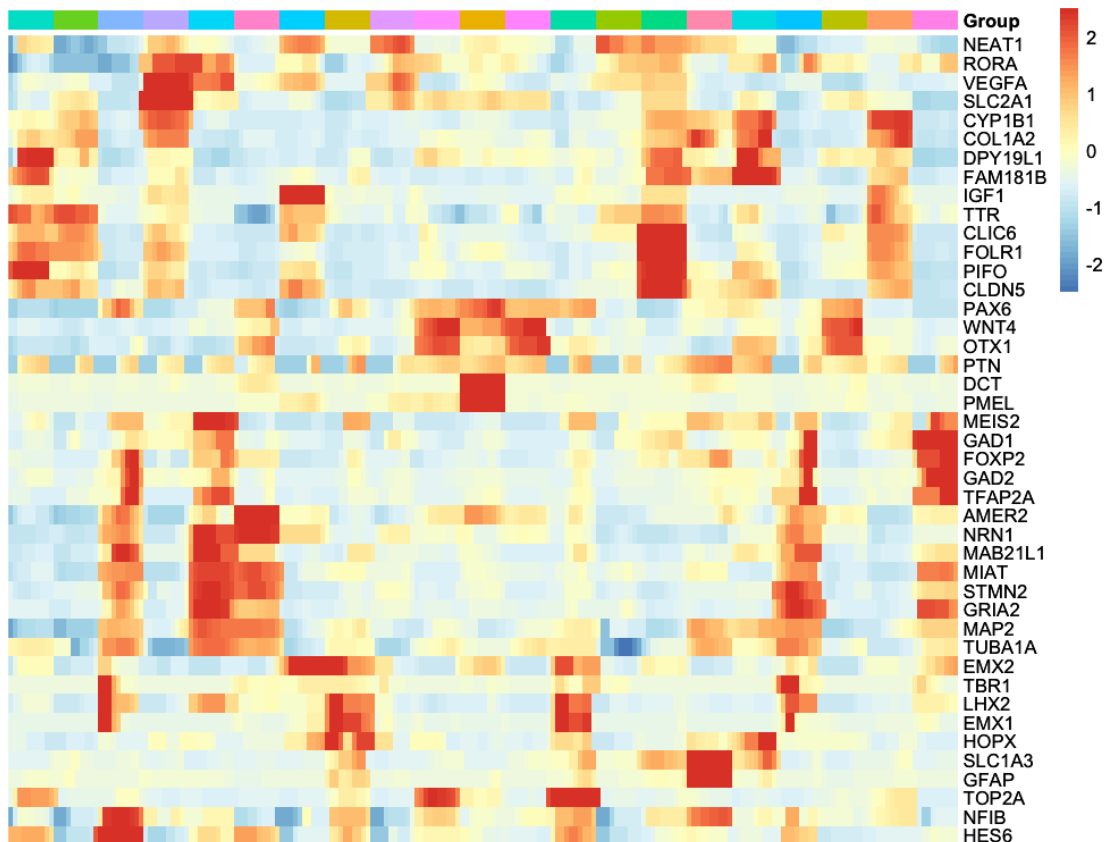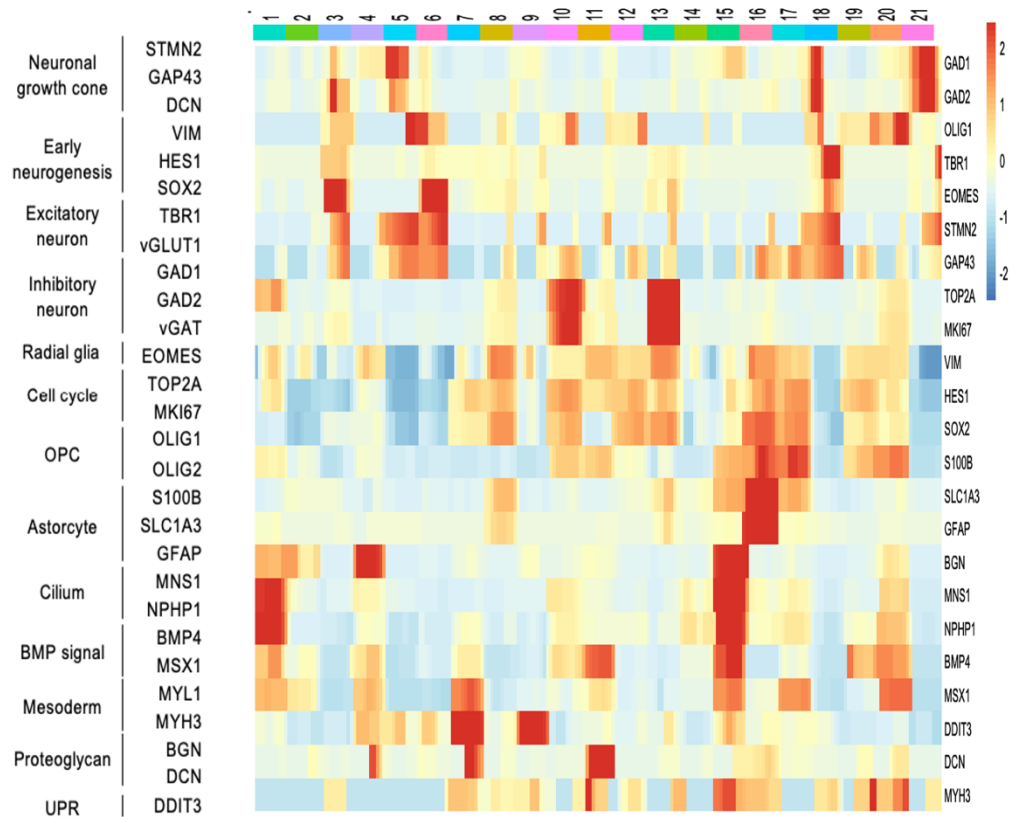
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Supplemental Figures

Figure S1. Heatmaps for the expression pattern of selected marker genes used in previous publication publications, and a bubble plot for key genes used in our cluster annotation.
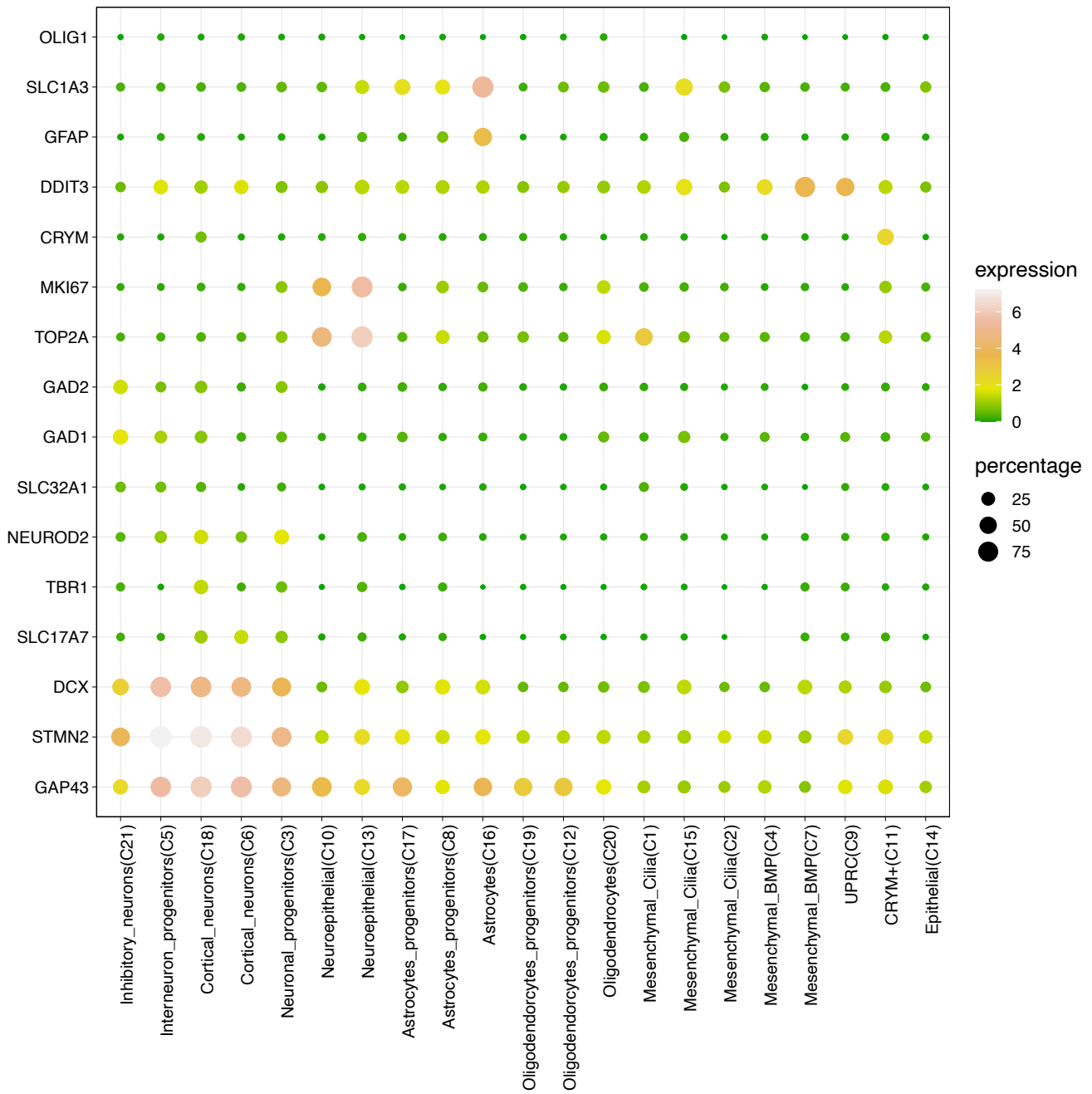
Figure S2. Dendrogram for cluster similarity based on mean gene expression averaged for all cells in each cluster.
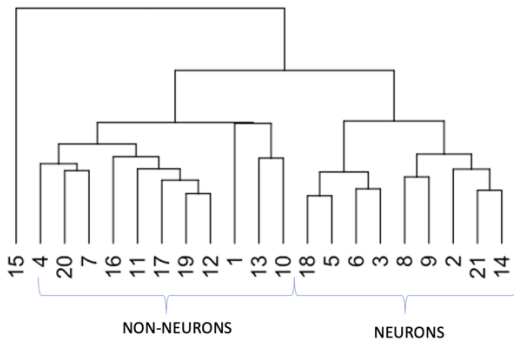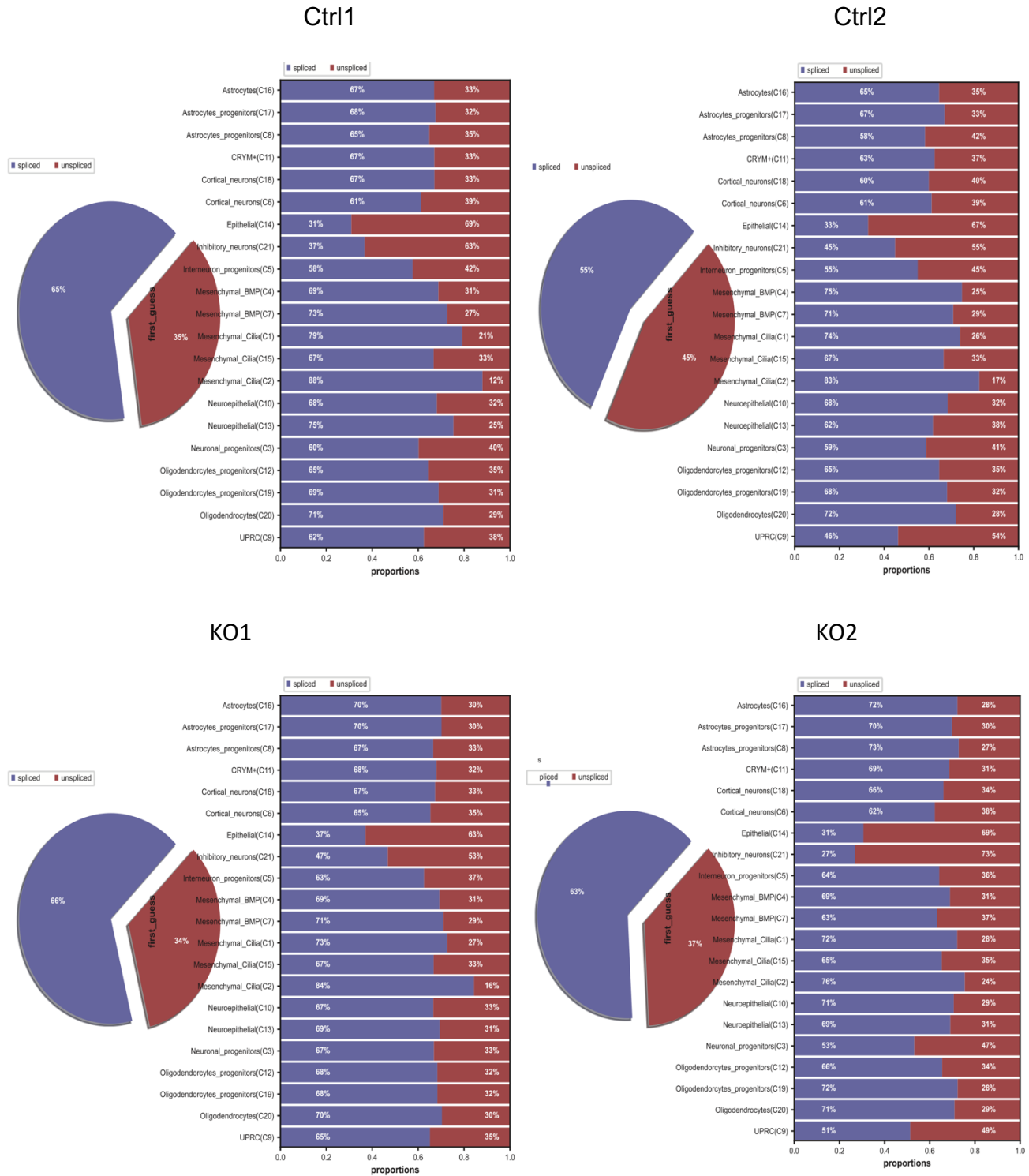
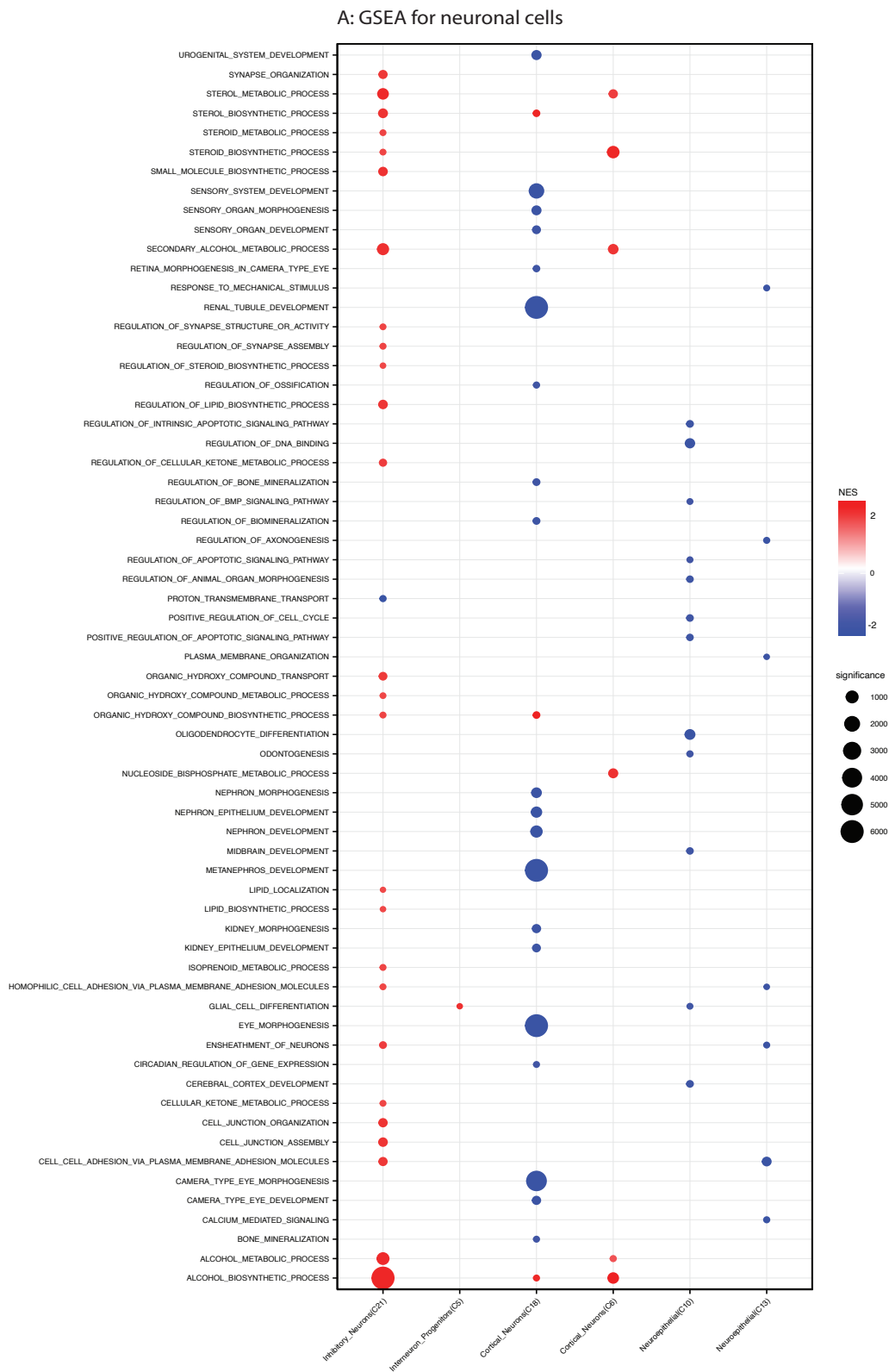# Figure S3. Proportion of spliced/unspliced reads for each cell cluster.

Ctrl1

Cell cluster	spliced	unspliced
Astrocytes(C16)	67%	33%
Astrocytes_progenitors(C17)	68%	32%
Astrocytes_progenitors(C8)	65%	35%
CRYM+(C11)	67%	33%
Cortical_neurons(C18)	67%	33%
Cortical_neurons(C6)	61%	39%
Epithelial(C14)	31%	69%
Inhibitory_neurons(C21)	37%	63%
Interneuron_progenitors(C5)	58%	42%
Mesenchymal_BMP(C4)	69%	31%
Mesenchymal_BMP(C7)	73%	27%
Mesenchymal_Cilia(C1)	79%	21%
Mesenchymal_Cilia(C15)	67%	33%
Mesenchymal_Cilia(C2)	88%	12%
Neuroepithelial(C10)	68%	32%
Neuroepithelial(C13)	75%	25%
Neuronal_progenitors(C3)	60%	40%
Oligodendorcytes_progenitors(C12)	65%	35%
Oligodendorcytes_progenitors(C19)	69%	31%
Oligodendrocytes(C20)	71%	29%
UPRC(C9)	62%	38%

Pie chart: spliced 65%, unspliced 35%

Ctrl2

Cell cluster	spliced	unspliced
Astrocytes(C16)	65%	35%
Astrocytes_progenitors(C17)	67%	33%
Astrocytes_progenitors(C8)	58%	42%
CRYM+(C11)	63%	37%
Cortical_neurons(C18)	60%	40%
Cortical_neurons(C6)	61%	39%
Epithelial(C14)	33%	67%
Inhibitory_neurons(C21)	45%	55%
Interneuron_progenitors(C5)	55%	45%
Mesenchymal_BMP(C4)	75%	25%
Mesenchymal_BMP(C7)	71%	29%
Mesenchymal_Cilia(C1)	74%	26%
Mesenchymal_Cilia(C15)	67%	33%
Mesenchymal_Cilia(C2)	83%	17%
Neuroepithelial(C10)	68%	32%
Neuroepithelial(C13)	62%	38%
Neuronal_progenitors(C3)	59%	41%
Oligodendorcytes_progenitors(C12)	65%	35%
Oligodendorcytes_progenitors(C19)	68%	32%
Oligodendrocytes(C20)	72%	28%
UPRC(C9)	46%	54%

Pie chart: spliced 55%, unspliced 45%

KO1

Cell cluster	spliced	unspliced
Astrocytes(C16)	70%	30%
Astrocytes_progenitors(C17)	70%	30%
Astrocytes_progenitors(C8)	67%	33%
CRYM+(C11)	68%	32%
Cortical_neurons(C18)	67%	33%
Cortical_neurons(C6)	65%	35%
Epithelial(C14)	37%	63%
Inhibitory_neurons(C21)	47%	53%
Interneuron_progenitors(C5)	63%	37%
Mesenchymal_BMP(C4)	69%	31%
Mesenchymal_BMP(C7)	71%	29%
Mesenchymal_Cilia(C1)	73%	27%
Mesenchymal_Cilia(C15)	67%	33%
Mesenchymal_Cilia(C2)	84%	16%
Neuroepithelial(C10)	67%	33%
Neuroepithelial(C13)	69%	31%
Neuronal_progenitors(C3)	67%	33%
Oligodendorcytes_progenitors(C12)	68%	32%
Oligodendorcytes_progenitors(C19)	68%	32%
Oligodendrocytes(C20)	70%	30%
UPRC(C9)	65%	35%

Pie chart: spliced 66%, unspliced 34%

KO2

Cell cluster	spliced	unspliced
Astrocytes(C16)	72%	28%
Astrocytes_progenitors(C17)	70%	30%
Astrocytes_progenitors(C8)	73%	27%
CRYM+(C11)	69%	31%
Cortical_neurons(C18)	66%	34%
Cortical_neurons(C6)	62%	38%
Epithelial(C14)	31%	69%
Inhibitory_neurons(C21)	27%	73%
Interneuron_progenitors(C5)	64%	36%
Mesenchymal_BMP(C4)	69%	31%
Mesenchymal_BMP(C7)	63%	37%
Mesenchymal_Cilia(C1)	72%	28%
Mesenchymal_Cilia(C15)	65%	35%
Mesenchymal_Cilia(C2)	76%	24%
Neuroepithelial(C10)	71%	29%
Neuroepithelial(C13)	69%	31%
Neuronal_progenitors(C3)	53%	47%
Oligodendorcytes_progenitors(C12)	66%	34%
Oligodendorcytes_progenitors(C19)	72%	28%
Oligodendrocytes(C20)	71%	29%
UPRC(C9)	51%	49%

Pie chart: spliced 63%, unspliced 37%

Supplementary Figure S4: Dot plot showing enriched pathways from GSEA for neuronal (A) and non-neuronal (B) cell clusters. Red and blue indicate higher and lower activities (GSEA NES) in the *CHD8* KO samples compared to controls, respectively. Dot sizes indicate statistical significance defined as 1/FDR.

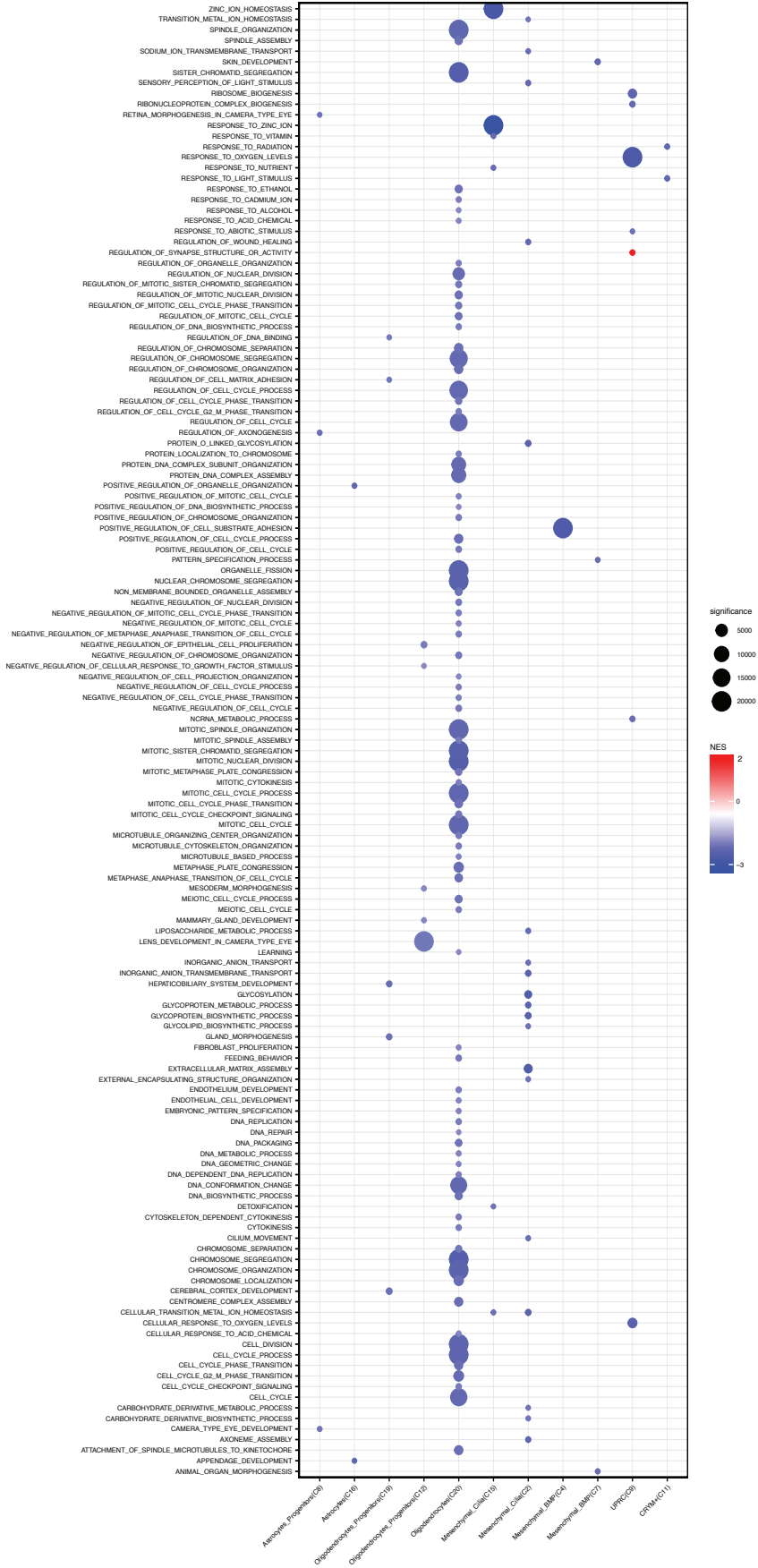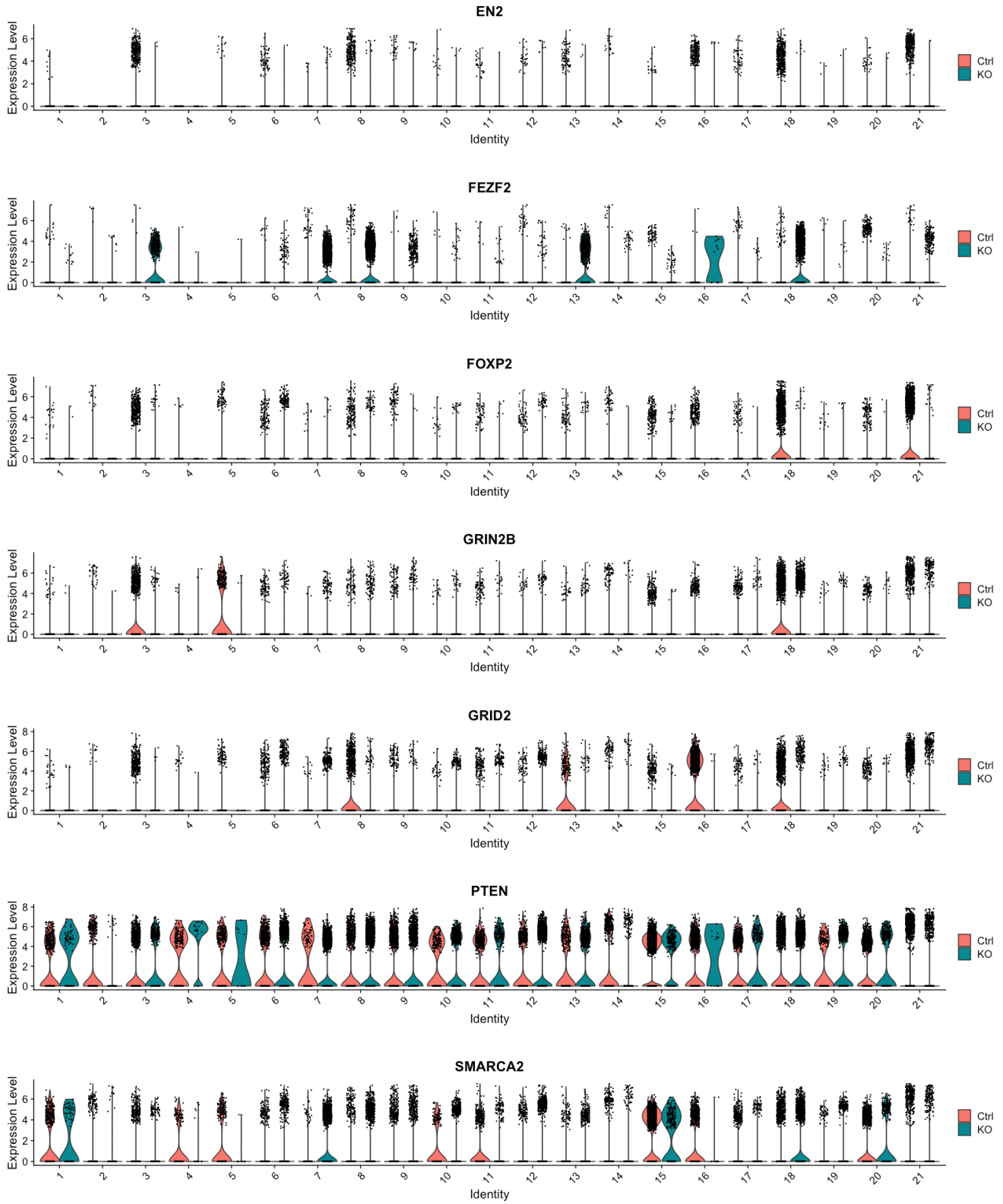A: GSEA for neuronal cells

B: GSEA for non-neuronal cells

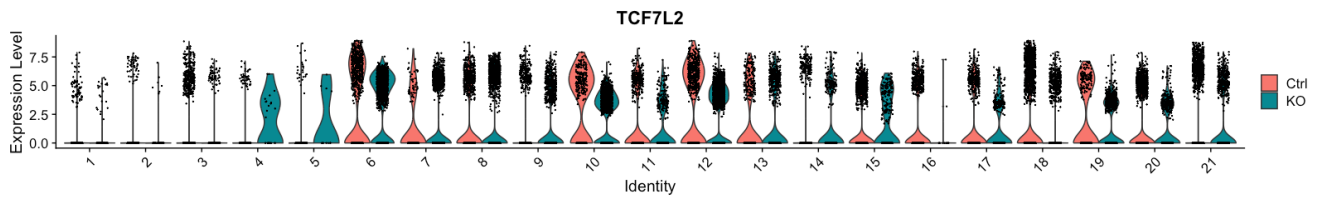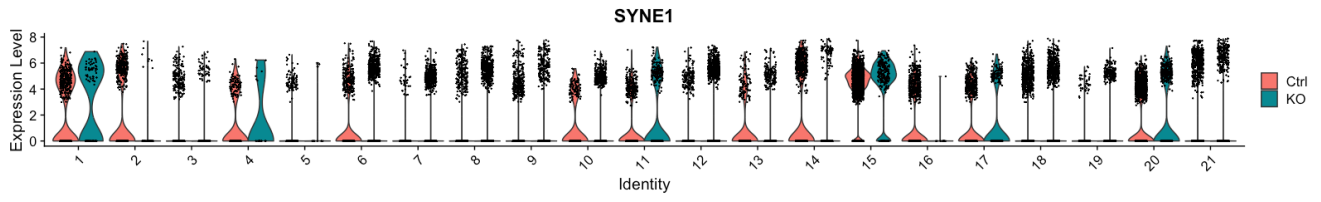# Figure S5: Violin plots showing differential expression of genes implicated in ASD

RELN

SYNE1

TBR1

TCF4

TCF7L2

Figure S6. Heatmap showing cell-cell communication count differences between control and CHD8 KO samples. The rows and columns indicate signaling sending and receiving cells, respectively.
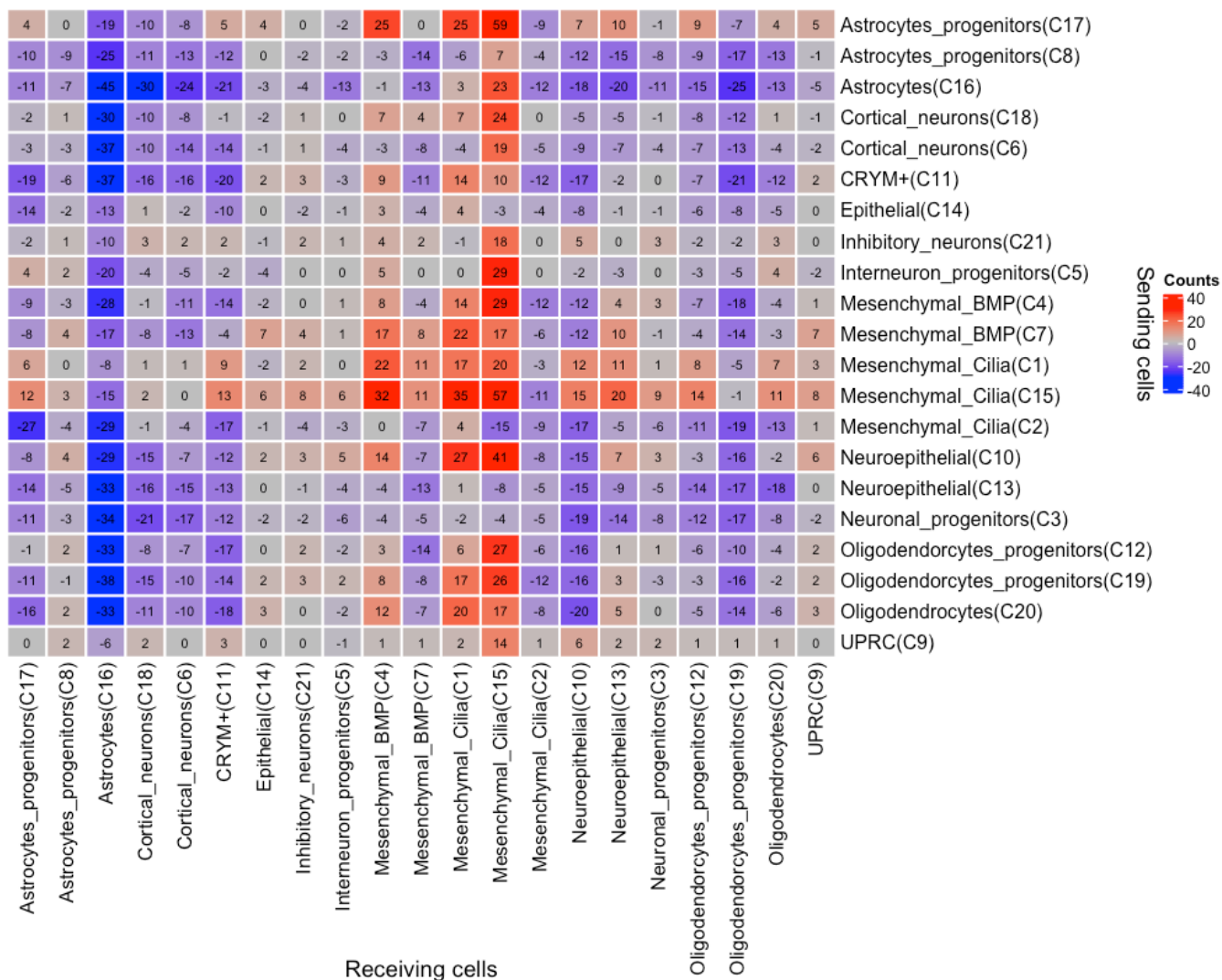
Figure S7. Heatmap showing cell-cell communication strength differences between control and CHD8 KO samples. The rows and columns indicate signaling sending and receiving cells, respectively.
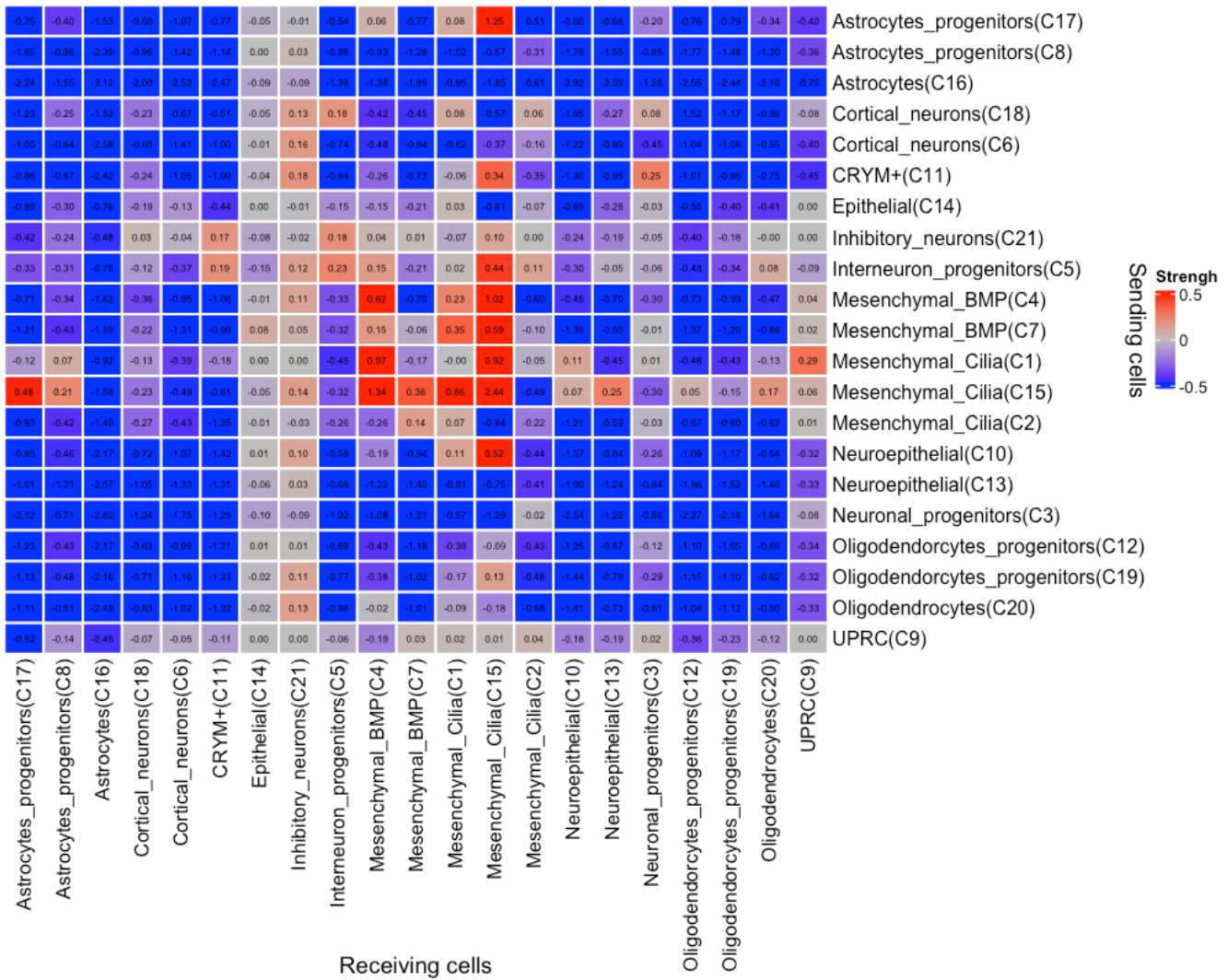
Figure S8. Violin plots showing differential expression of genes encoding ligands or receptors