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Supplementary Materials for

Disruption of the autism gene and chromatin regulator KDM5A alters hippocampal cell identity

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The PDF file includes:

Figs. S1 to S9
Legends for tables S1 to S3

Other Supplementary Material for this manuscript includes the following:

Tables S1 to S3

Fig. S1.
Quality control metrics. (**A**) Number of UMIs per nucleus in each sample. All nuclei sequenced had >= 500 UMIs. (**B**) Number of genes per nucleus in each sample. All nuclei sequenced had > 300 genes. (**C**) Number of UMIs per nucleus in each cluster. (**D**) Number of genes per nucleus in each cluster. (**E**) Scatter plot showing the number of genes (y axis) versus the number of UMIs (x axis). KO, knockout (*Kdm5a*$^{-/-}$).

Fig. S2.
Cell identities of the 24 clusters identified by single-nucleus RNA-seq from WT and *Kdm5a*$^{-/-}$ hippocampi. (**A**) UMAP plot of WT and *Kdm5a*$^{-/-}$ nuclei with each cluster annotated with its respective cell identity. (**B**) Scaled expression of *Kdm5a* across clusters. (**C**) Dot plot showing the relative expression of marker genes for specific hippocampal cell subtypes. The dot size represents the percentage of nuclei expressing each marker gene and the dot color intensity represents the average expression of the gene (light color, low expression; dark color, high expression).

Fig. S3.
Cell type proportions from each WT and *Kdm5a*$^{-/-}$ biological replicate. (**A**) UMAP of the 24 hippocampal clusters identified by snRNA-seq to visualize changes in cell proportion within specific clusters between WT (purple) and *Kdm5a*$^{-/-}$ (yellow) cells. WT cells are superimposed on *Kdm5a*$^{-/-}$ cells (left) and vice versa (right). (**B**) Bar graph representing the proportion of cell types from the major groups in each biological replicate. (**C**) Bar graph representing the proportion of cell types from each cluster belonging to a biological replicate (CA1.1, $^{**}P$=0.0023; CA1.4, $^{***}P$<0.0001; CA2, $^{\#}P$=0.3216; CA3.4, $^{***}P$<0.0001; Inh1, $^{*}P$=0.0223; Inh2, $^{**}P$=0.004). Data were analyzed using X^2 test.

Fig. S4.
Gene ontology for unique differentially expressed genes in the cell clusters sensitive to loss of KDM5A. Gene ontology analysis on the exclusive DEGs in excitatory clusters CA1.1, CA1.4, CA3.4, and CA2 (**A**), and in inhibitory clusters PVALB+ and SST+ (**B**). Data from DEGs with an FDR-corrected $P \leq 0.05$ and $\log_2$ fold change $\geq |0.3|$.

Fig. S5.
KDM5A regulates hundreds of genes in the hippocampus. (**A**) Overlap between genes dysregulated in all *Kdm5a*$^{-/-}$ cells compared to all WT cells identified by snRNA-seq and DEGs identified by hippocampal bulk RNA-seq from El Hayek et al., 2020 (*30*), irrespective of their direction of dysregulation (P<2.2 x 10^{-16}, OR=2.96) (top). Overlap between genes that are commonly upregulated (P=0.2078, OR=1.41) and commonly downregulated (P=1.33 x 10^{-5}, OR=1.82) in both the snRNA-seq and the bulk RNA-seq datasets (bottom). DEGs changing in opposite directions between the two datasets are not captured in these Venn diagrams (bottom) (**B**) Overlap between mouse embryonic stem cell anti-KDM5A ChIP-seq peaks from Beshiri et al., 2012 (*35*) and DEGs identified by hippocampal bulk RNA-seq from El Hayek et al., 2020 (*30*) (top). Overlap between the ChIP-seq peaks and upregulated and downregulated DEGs (bottom). Possible direct targets of KDM5A are defined as DEGs that have an anti-KDM5A ChIP-seq peak, and possible indirect targets are defined as the remaining DEGs. (**C**) High-priority possible direct targets of KDM5A are defined as the genes that are commonly upregulated or commonly

downregulated in the bulk RNA-seq and the snRNA-seq datasets and that have KDM5A ChIP-seq peaks.

Fig. S6.
KDM5A regulates the development of subtypes of CA2 and CA3 neurons. *In situ* hybridization on brain tissue for *Rps6*, *Nme2*, *Shisa6*, and *Hs6st3* shows differential expression between WT and *Kdm5a*$^{-/-}$. Hippocampal sections from WT (top) and *Kdm5a*$^{-/-}$ (bottom) mice show decreased expression of *Rps6* (**A**) and increased expression of *Nme2* (**C**) in the CA2 region of the hippocampus, decreased expression of *Shisa6* (**E**) in the CA3 region of the hippocampus, and increased expression of *Hs6st3* (**G**) in the CA1 region of the hippocampus in *Kdm5a*$^{-/-}$ compared to WT. The data is quantified in **B**, **D**, **F**, and **H**, respectively (WT n=3, *Kdm5a*$^{-/-}$ n=3, (**B**) **P*=0.0363, (**D**) **P*=0.0233, (**F**) ***P*=0.003, (**H**) ***P*=0.0068). Data were analyzed using unpaired t test. All values are mean ± SEM.

Fig. S7.
KDM5A regulates the development of *Pvalb* and *Sst* subtypes of inhibitory neurons. smFISH on brain tissue shows decreased number of cells expressing *Pvalb* and *Sst* in *Kdm5a*$^{-/-}$ hippocampi compared to WT. (**A**) Representative images from WT (left) and *Kdm5a*$^{-/-}$ (right) show decreased number of *Gad1*$^{+}$*Pvalb*$^{+}$ and *Gad1*$^{+}$*Sst*$^{+}$ cells in *Kdm5a*$^{-/-}$ compared to WT hippocampi. Scale bars, 50 μm. The data is quantified in **B** and **C** (WT n=3, *Kdm5a*$^{-/-}$ n=3, (**B**) **P*=0.0391, (**C**) **P*=0.0327). Data were analyzed using unpaired t test. All values are mean ± SEM.

Fig. S8.
Developmental trajectory tracing using pseudotime analysis shows that the CA1.1 cluster has a more mature identity while the CA1.4 cluster has a younger identity. (**A**) Pseudotime UMAP analysis of hippocampal cells colored by pseudotime (early, blue; late yellow). Data shown for one of two Monocle clusters that contained the majority of CA1 cells. (**B**) Mapping the pseudotime analysis onto the original UMAP of the 24 hippocampal clusters. (**C**) Enlarged view of the CA1 subclusters from panel (**B**).

Fig. S9.
SATB2 expression decreases in the hippocampus following loss of KDM5A. (**A**) Western blot analysis of hippocampal tissue shows a decrease in SATB2 in the *Kdm5a*$^{-/-}$ compared to WT. (**B**) Western blot quantification. SATB2 was normalized to actin (loading control) and the normalized values were plotted relative to WT (WT n=3, *Kdm5a*$^{-/-}$ n=3, **P*=0.0478). Data were analyzed using unpaired t test. All values are mean ± SEM.

Table S1.
Differentially expressed genes between WT and *Kdm5a*$^{-/-}$ in all cells and in each of the 24 hippocampal cell clusters. Genes with an FDR-corrected $P \leq 0.05$ and $\log_2$ fold change $\geq |0.3|$ were selected as differentially expressed genes (DEGs). The pink tab (snRNA-seq DEGs) represents DEGs in all *Kdm5a*$^{-/-}$ cells compared to all WT cells. The "Overlap" tab shows the percentage of overlap between the DEGs in the six clusters sensitive to KDM5A loss. Purple tabs (CA1.1, CA1.4, CA2, CA3.4, Inh1, Inh2) correspond to cell clusters sensitive to loss of KDM5A. CA, *cornu ammonis* excitatory neurons; Inh, inhibitory neurons; OPC, oligodendrocyte progenitor cells; Oligo, oligodendrocytes; Astro, astrocytes.

Table S2.
KDM5A signature genes. DEGs with an FDR-corrected $P \leq 0.05$ and $\log_2$ fold change $\geq |0.3|$ in the combined affected excitatory clusters (CA1.1, CA1.4, CA2, and CA3.4) and combined affected inhibitory clusters (Inh1 and Inh2) compared to unaffected excitatory and inhibitory clusters, respectively, were defined as KDM5A signature genes.

Table S3.
DEGs and candidate KDM5A targets. DEGs identified through bulk RNA-seq on WT and *Kdm5a*$^{-/-}$ hippocampi from El Hayek et al., 2020 (*30*). Common DEGs represent the overlap between dysregulated genes in all *Kdm5a*$^{-/-}$ cells compared to all WT cells from the hippocampal snRNA-seq and dysregulated genes from the hippocampal bulk RNA-seq. Genes with anti-KDM5A ChIP-seq peaks from Beshiri et al., 2012 (*35*) overlap with DEGs from the bulk RNA-seq dataset. Common DEGs from the snRNA-seq dataset are marked in yellow.

Figure S1

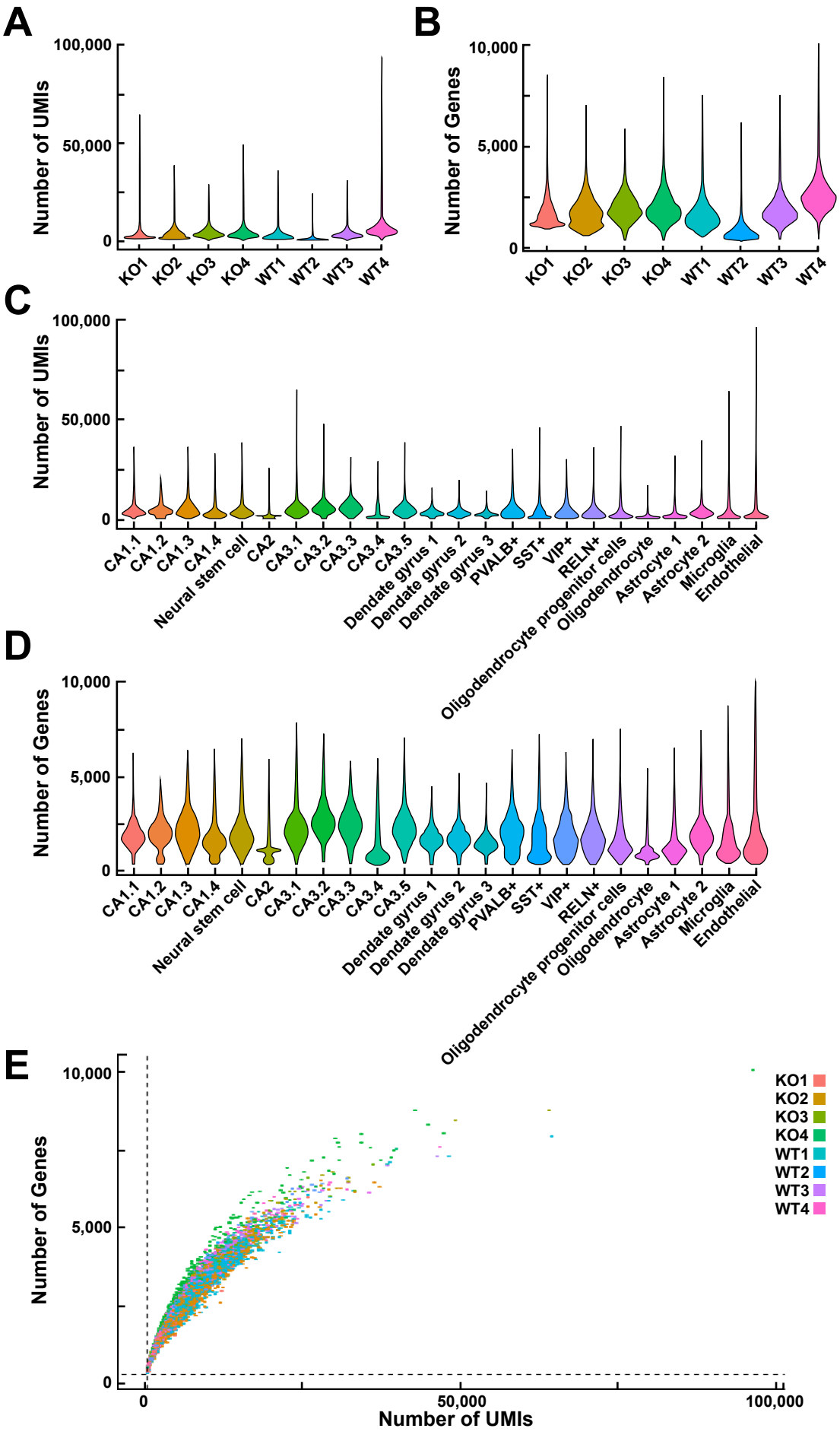

A
Number of UMIs
100,000
50,000
0
KO1
KO2
KO3
KO4
WT1
WT2
WT3
WT4
B
Number of Genes
10,000
5,000
0
C
CA1.1
CA1.2
CA1.3
CA1.4
Neural stem cell
CA2
CA3.1
CA3.2
CA3.3
CA3.4
CA3.5
Dendate gyrus 1
Dendate gyrus 2
Dendate gyrus 3
PVALB+
SST+
VIP+
RELN+
Oligodendrocyte progenitor cells
Oligodendrocyte
Astrocyte 1
Astrocyte 2
Microglia
Endothelial
D
E
Number of UMIs
KO1
KO2
KO3
KO4
WT1
WT2
WT3
WT4

Figure S2

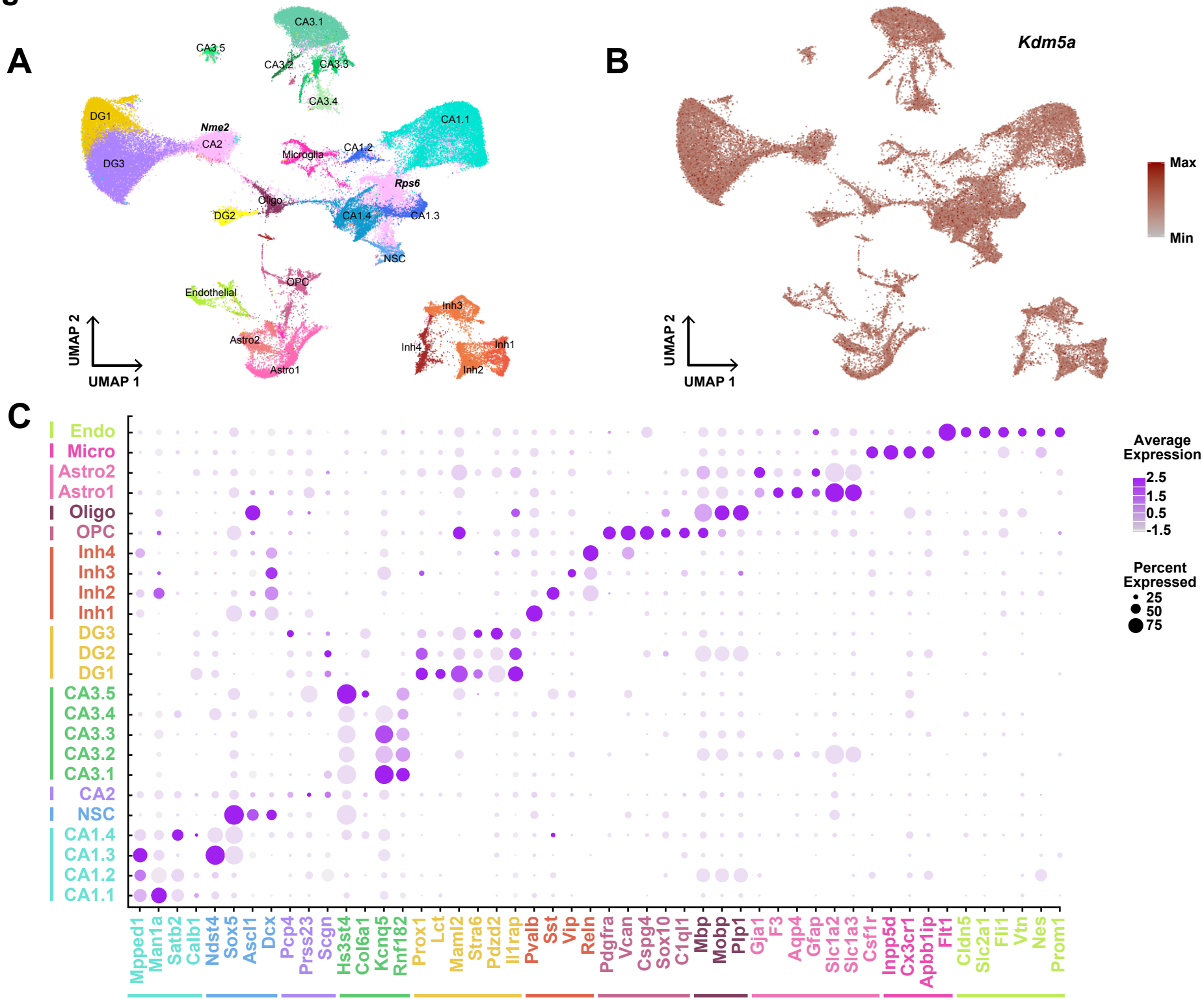

A
CA3.1
CA3.5
CA3.2
CA3.3
CA3.4
DG1
Nme2
CA2
CA1.1
DG3
Microglia
CA1.2
Rps6
Oligo
DG2
CA1.4
CA1.3
NSC
OPC
Endothelial
Inh3
Astro2
Inh4
Inh1
Inh2
Astro1
UMAP 2
UMAP 1
B
Kdm5a
Max
Min
UMAP 2
UMAP 1
C
Endo
Micro
Astro2
Astro1
Oligo
OPC
Inh4
Inh3
Inh2
Inh1
DG3
DG2
DG1
CA3.5
CA3.4
CA3.3
CA3.2
CA3.1
CA2
NSC
CA1.4
CA1.3
CA1.2
CA1.1
Mpped1
Man1a
Satb2
Calb1
Ndst4
Sox5
Ascl1
Dcx
Pcp4
Prss23
Scgn
Hs3st4
Col6a1
Kcnq5
Rnf182
Prox1
Lct
Maml2
Stra6
Pdzd2
Il1rap
Pvalb
Sst
Vip
Reln
Pdgfra
Vcan
Cspg4
Sox10
C1ql1
Mbp
Mobp
Plp1
Gja1
F3
Aqp4
Gfap
Slc1a2
Slc1a3
Csf1r
Inpp5d
Cx3cr1
Apbb1ip
Flt1
Cldn5
Slc2a1
Fli1
Vtn
Nes
Prom1
Average Expression
2.5
1.5
0.5
-1.5
Percent Expressed
25
50
75

Figure S3

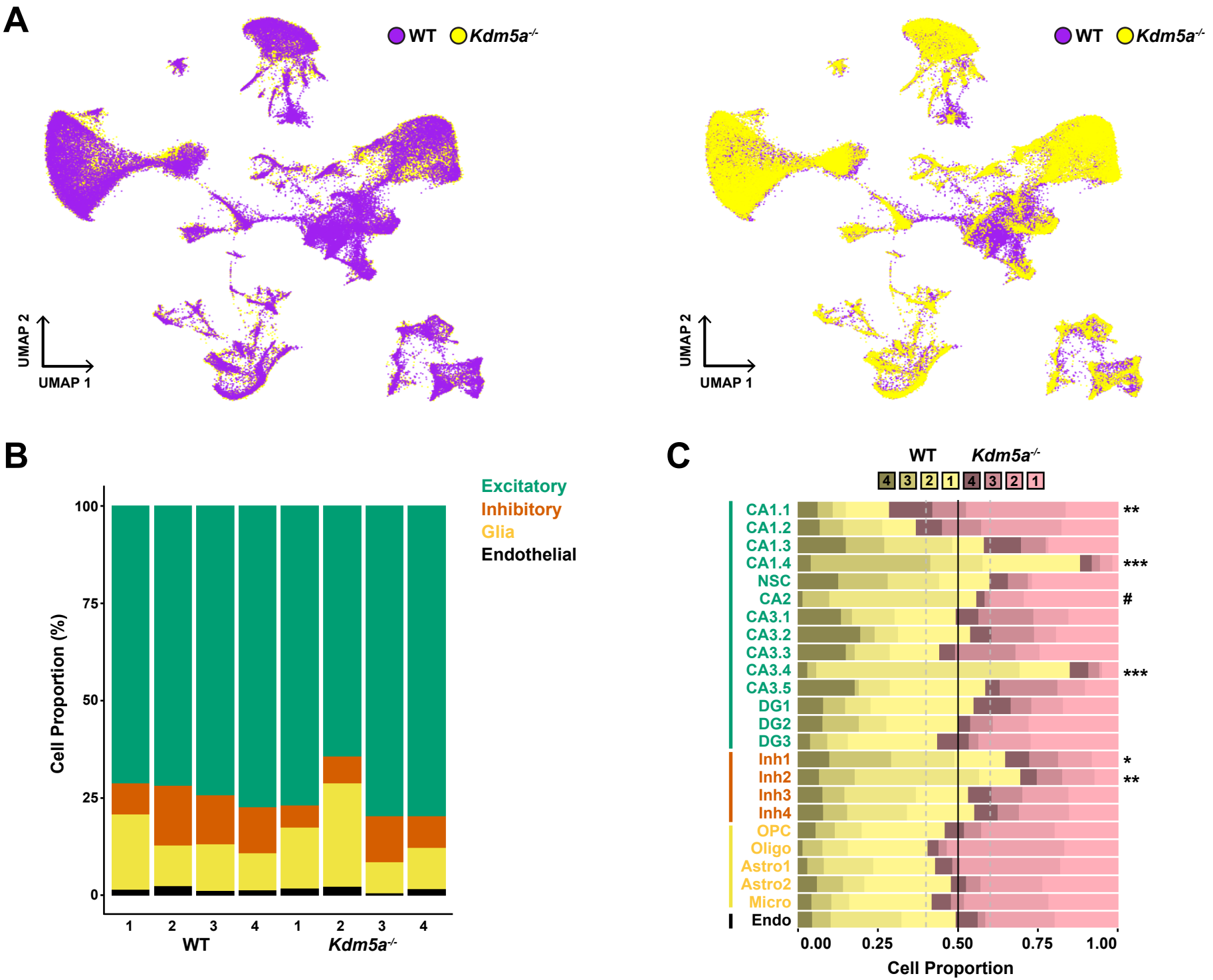

A
WT
Kdm5a-/-
UMAP 2
UMAP 1
WT
Kdm5a-/-
UMAP 2
UMAP 1
B
Excitatory
Inhibitory
Glia
Endothelial
Cell Proportion (%)
100
75
50
25
0
1 2 3 4 1 2 3 4
WT
Kdm5a-/-
C
WT
Kdm5a-/-
4 3 2 1 4 3 2 1
CA1.1
CA1.2
CA1.3
CA1.4
NSC
CA2
CA3.1
CA3.2
CA3.3
CA3.4
CA3.5
DG1
DG2
DG3
Inh1
Inh2
Inh3
Inh4
OPC
Oligo
Astro1
Astro2
Micro
Endo
**
***
#
***
*
**
0.00
0.25
0.50
0.75
1.00
Cell Proportion

Figure S4

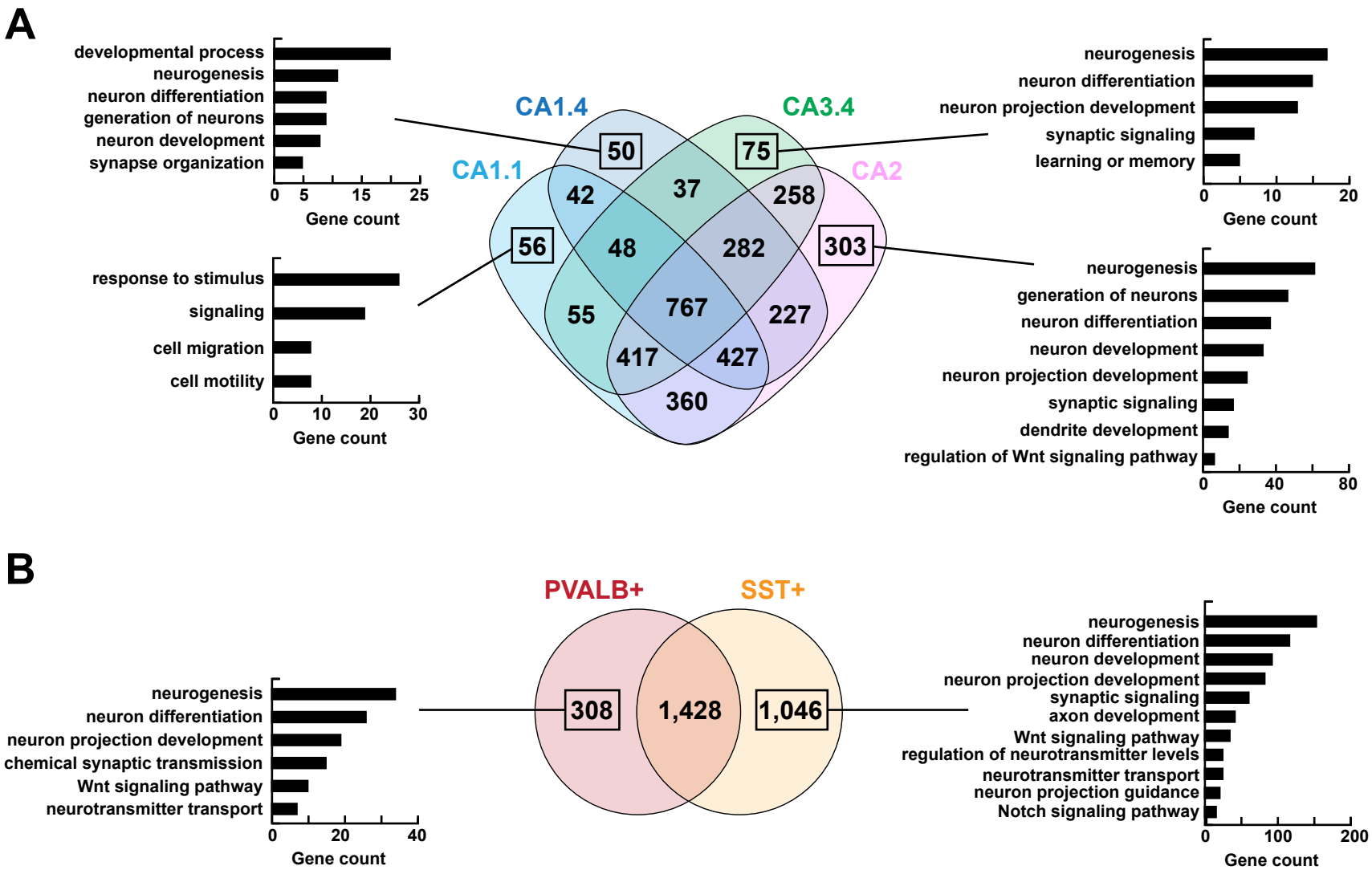

A
developmental process
neurogenesis
neuron differentiation
generation of neurons
neuron development
synapse organization
0 5 15 25
Gene count
response to stimulus
signaling
cell migration
cell motility
0 10 20 30
Gene count
CA1.4
CA3.4
CA1.1
CA2
50
75
56
303
42
37
258
48
282
55
767
227
417
427
360
neurogenesis
neuron differentiation
neuron projection development
synaptic signaling
learning or memory
0 10 20
Gene count
neurogenesis
generation of neurons
neuron differentiation
neuron development
neuron projection development
synaptic signaling
dendrite development
regulation of Wnt signaling pathway
0 40 80
Gene count
B
PVALB+
SST+
308
1,428
1,046
neurogenesis
neuron differentiation
neuron projection development
chemical synaptic transmission
Wnt signaling pathway
neurotransmitter transport
0 20 40
Gene count
neurogenesis
neuron differentiation
neuron development
neuron projection development
synaptic signaling
axon development
Wnt signaling pathway
regulation of neurotransmitter levels
neurotransmitter transport
neuron projection guidance
Notch signaling pathway
0 100 200
Gene count

Figure S5

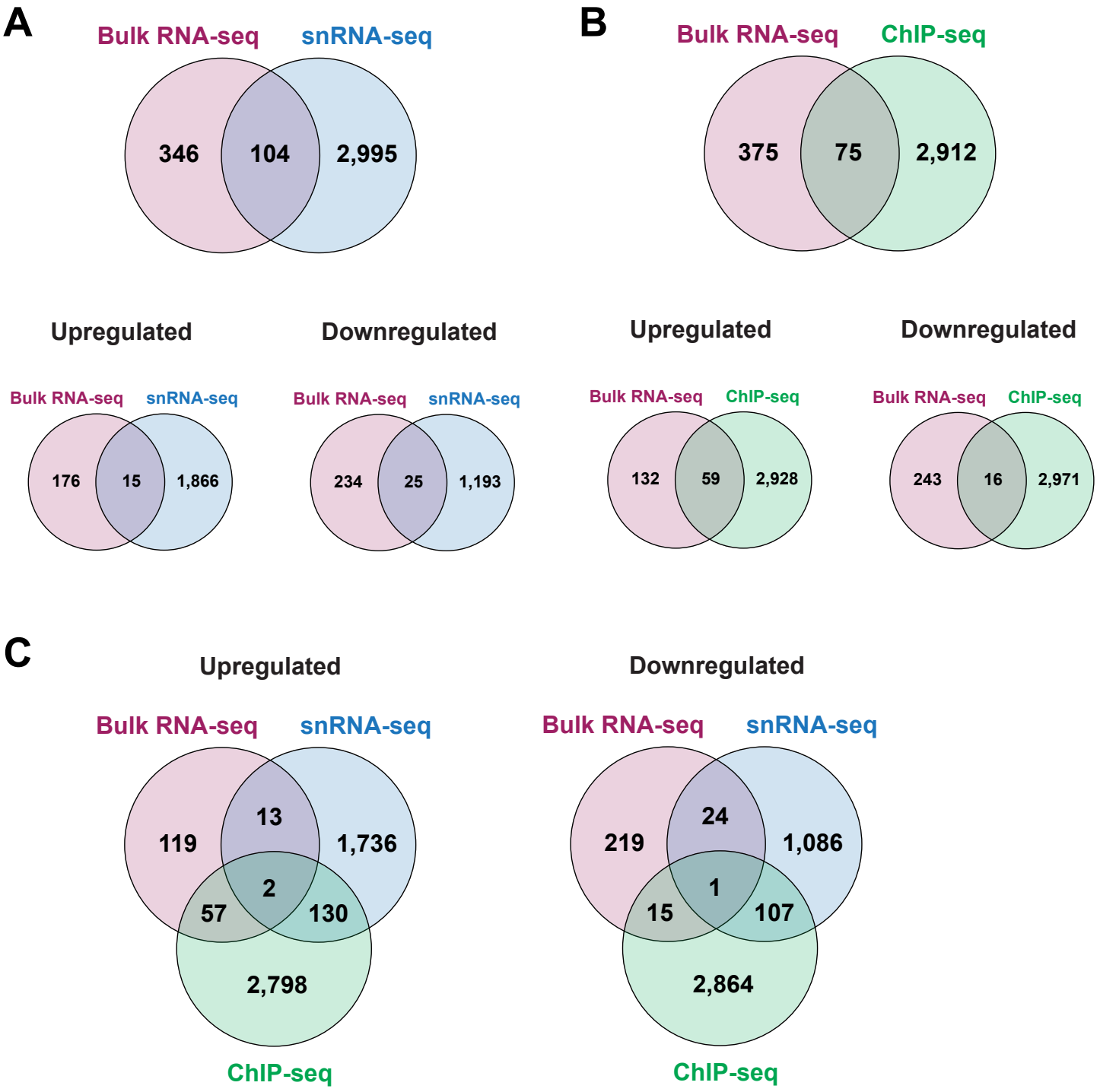

A
Bulk RNA-seq
snRNA-seq
346
104
2,995
Upregulated
Bulk RNA-seq
snRNA-seq
176
15
1,866
Downregulated
Bulk RNA-seq
snRNA-seq
234
25
1,193
B
Bulk RNA-seq
ChIP-seq
375
75
2,912
Upregulated
Bulk RNA-seq
ChIP-seq
132
59
2,928
Downregulated
Bulk RNA-seq
ChIP-seq
243
16
2,971
C
Upregulated
Bulk RNA-seq
snRNA-seq
119
13
1,736
2
57
130
2,798
ChIP-seq
Downregulated
Bulk RNA-seq
snRNA-seq
219
24
1,086
1
15
107
2,864
ChIP-seq

Figure S6

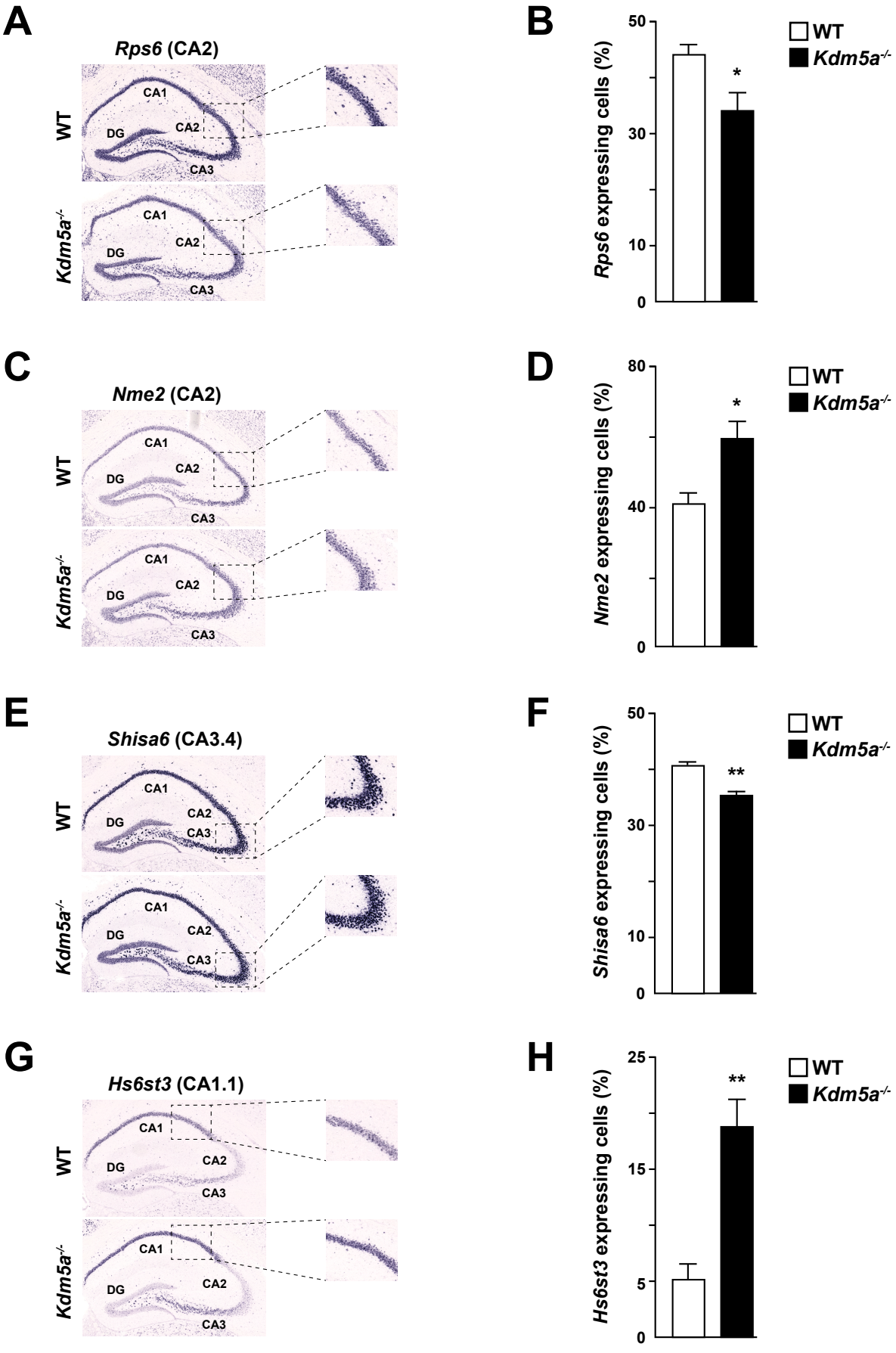

A
Rps6 (CA2)
WT
Kdm5a-/-
CA1
CA2
CA3
DG
B
Rps6 expressing cells (%)
50
30
10
0
*
WT
Kdm5a-/-
C
Nme2 (CA2)
D
Nme2 expressing cells (%)
80
40
0
E
Shisa6 (CA3.4)
F
Shisa6 expressing cells (%)
**
G
Hs6st3 (CA1.1)
H
Hs6st3 expressing cells (%)
25
15
5

Figure S7

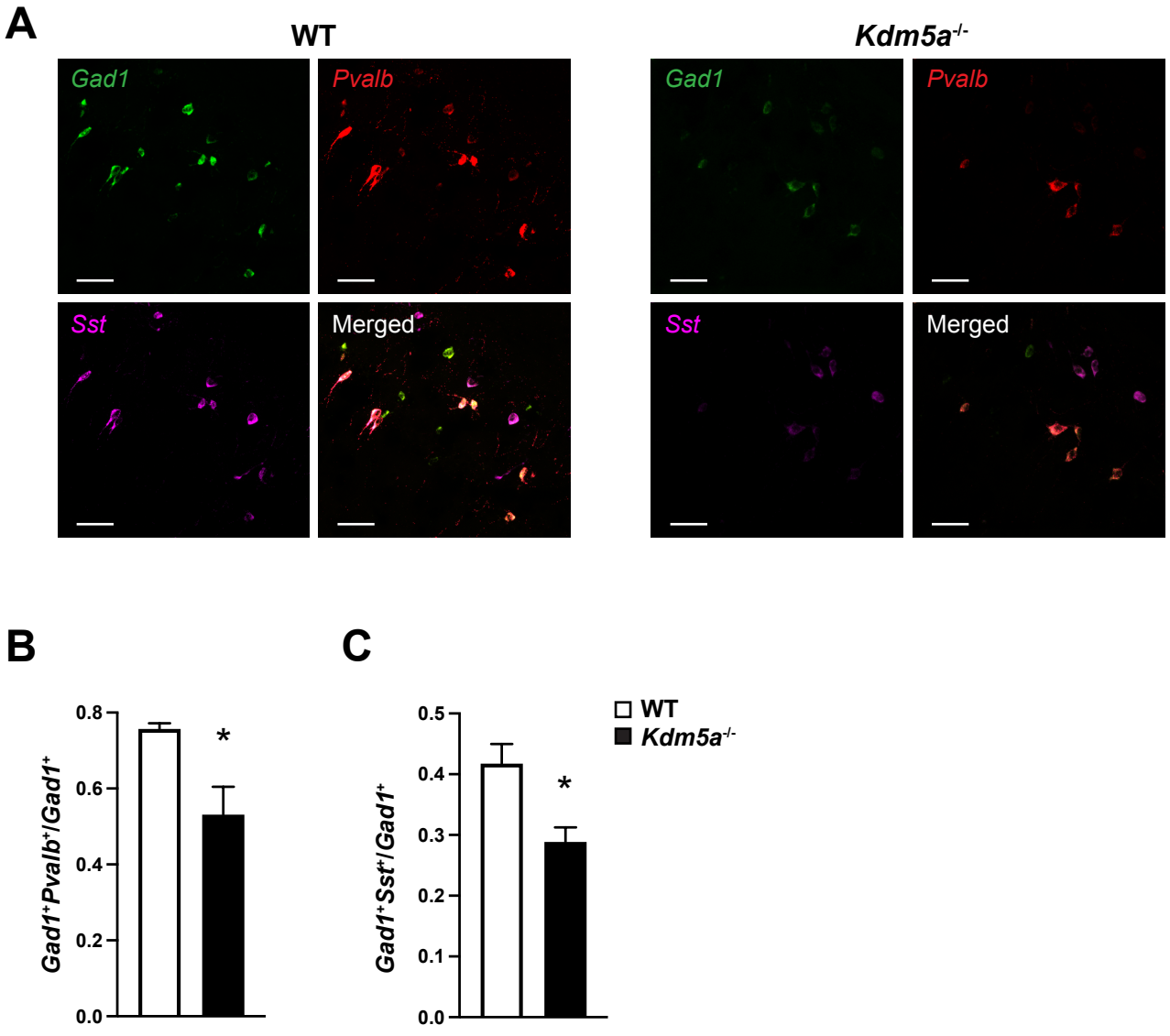

A
WT
Kdm5a-/-
Gad1
Pvalb
Sst
Merged
B
Gad1+Pvalb+/Gad1+
0.8
0.6
0.4
0.2
0.0
*
C
Gad1+Sst+/Gad1+
0.5
0.4
0.3
0.2
0.1
0.0
*
WT
Kdm5a-/-

Figure S8

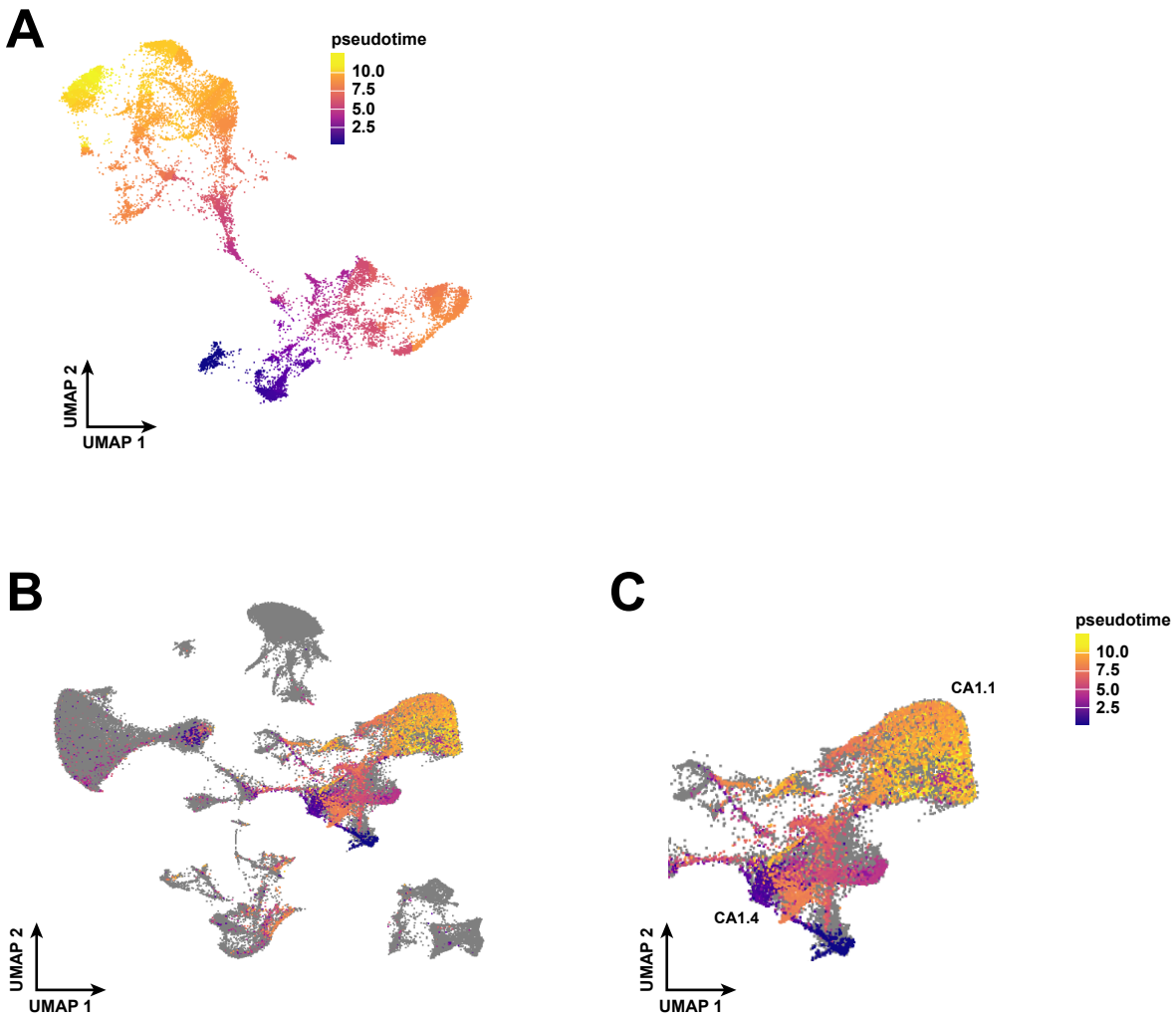
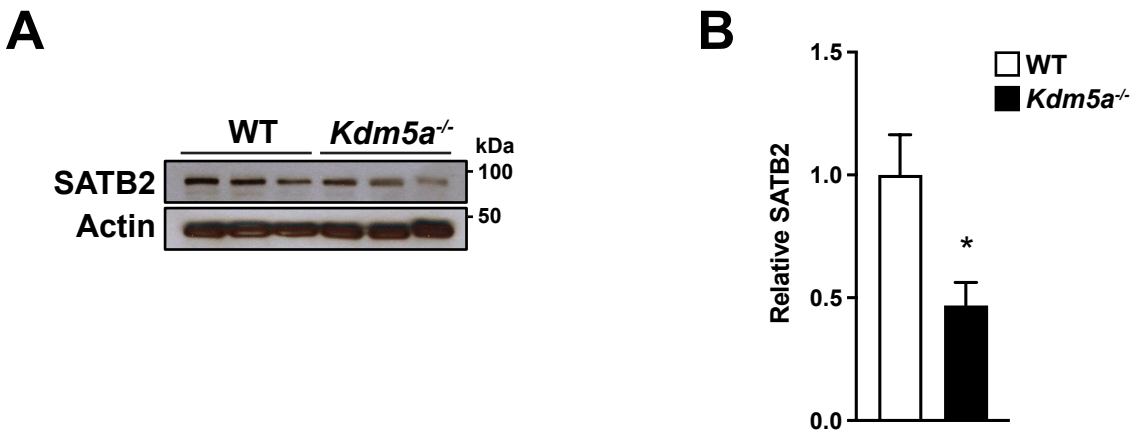

A
pseudotime
10.0
7.5
5.0
2.5
UMAP 2
UMAP 1
B
UMAP 2
UMAP 1
C
pseudotime
10.0
7.5
5.0
2.5
CA1.1
CA1.4
UMAP 2
UMAP 1

Figure S9


A
WT
Kdm5a-/-
kDa
SATB2
100
Actin
50
B
WT
Kdm5a-/-
Relative SATB2
1.5
1.0
0.5
0.0
*