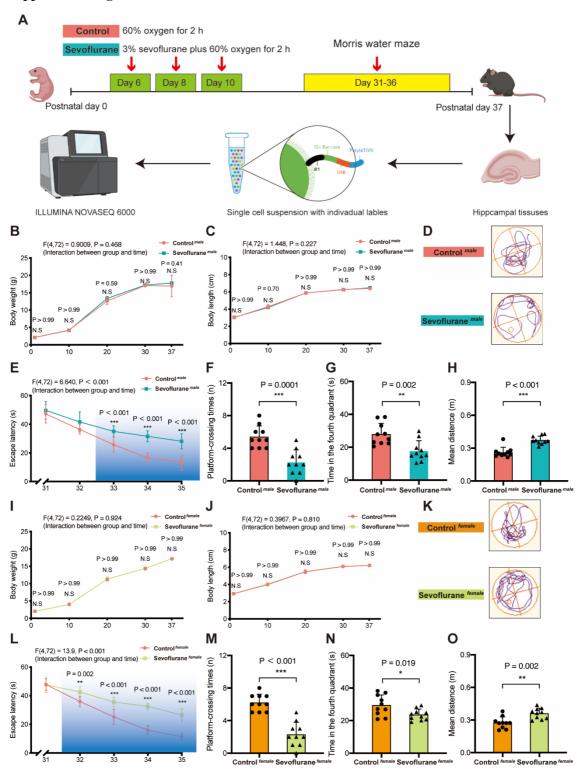
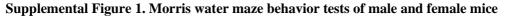
### Supplemental digital content

# Single-nucleus atlas of sevoflurane-induced hippocampal cell-type- and sex-specific effects during development in mice

Shao-yong Song, Ke Peng, Xiao-wen Meng, Xi-sheng Shan, Qing-cai Chen, Wei-ming Zhao, Biyu Shen, Hong Qiu, Hong Liu, Hua-yue Liu, Fu-hai Ji

#### **Supplemental Figure 1.**

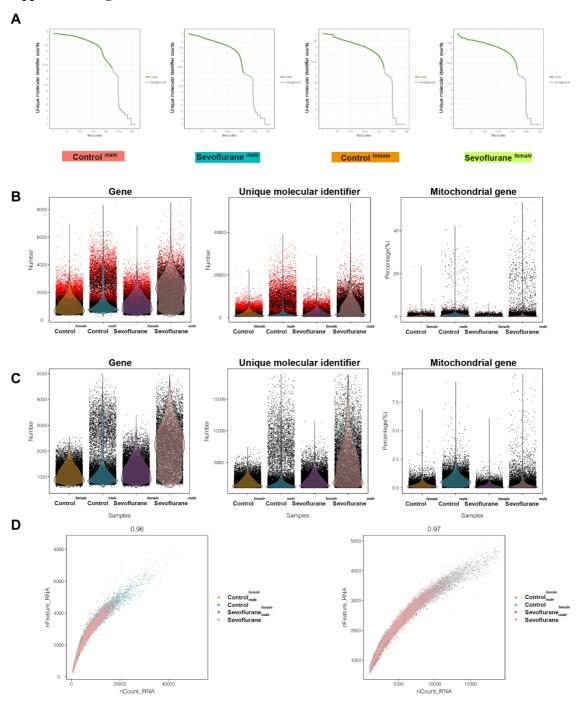


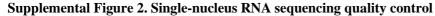


(A) Schematic diagram: 3% sevoflurane or control treatment for 2 h on postnatal day 6, 8, and 10, Morris water maze tests on postnatal day 31–36, and single-nucleus RNA sequencing of hippocampus on postnatal day 37. (B) Body weight of male mice on postnatal day 1, 10, 20, 30, and 37. (C) Body length of male mice on postnatal day 1, 10, 20, 30, and 37. (D) Trace plots of male mice on postnatal day 36. (E) Escape latency of male mice on postnatal day 31–35. (F) Platform-crossing times of male

mice on postnatal day 36. (G) Time spent in the fourth quadrant of male mice on postnatal day 36. (H) Mean distance from the platform of male mice on postnatal day 36. (I) Body weight of female mice on postnatal day 1, 10, 20, 30, and 37. (J) Body length of female mice on postnatal day 1, 10, 20, 30, and 37. (K) Trace plots of female mice on postnatal day 36. (L) Escape latency of female mice on postnatal day 31-35. (M) Platform-crossing times of female mice on postnatal day 36. (N) Time spent in the fourth quadrant of female mice on postnatal day 36. (O) Mean distance from the platform of female mice on postnatal day 36. Data are means  $\pm$  SD. n = 10 mice/group. Student's t-test or two-way repeated measures ANOVA with Bonferroni correction. \*\*P < 0.01, \*\*\*P < 0.001.

#### **Supplemental Figure 2.**





(A) Estimated cells (green) and background (grey) in the control <sup>male</sup>, sevoflurane <sup>male</sup>, control <sup>female</sup>, and sevoflurane <sup>female</sup> groups using the Cell Ranger software. (B) Distribution of genes, unique molecular identifier, and mitochondrial genes before filtering in the 4 groups. (C) Distribution of genes, unique molecular identifier, and mitochondrial genes after filtering in the 4 groups. (D) Correlation between the number of unique molecular identifier and the number of genes before filtering (left) and after filtering (right).

#### 5

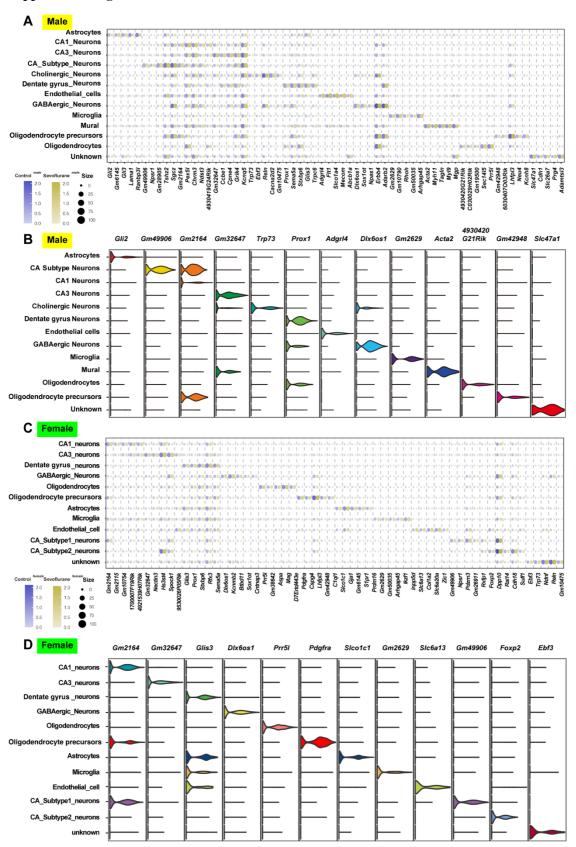
#### **Supplemental Figure 3.**

A <mark>Male</mark>													
Astrocytes (294)	89	13	6	23	26	6	6	5	5	10	10	13	Apoe,Rgs20,Tspan7,Lsamp,Nnat
Endothelial_cell (150)		29	12	8	9	_	2	3	0	3	8	5	ltgb1,Utrn,Timp3,Tsc22d1,Atp1a2
Microglia (256)	7	22	87	12	21	1 3	3	3	1	4	6	8	Man2b1,Csf1r,Lyn,Hsp90b1,Srsf5
Oligodendrocyte precursors (308)	27	14	10	65	27	7 4	1	2	7	6	16	4	Bcan,Dner,Kank1,Ppfibp1,Cspg5
Oligodendrocytes (440)	10	12	17	29	154	4 6	3	2	5	13	12	11	Cfl2,Pcdh9,Cmtm5,Lgi3,Fbxl5
CA3_neurons (392)	24	13	6	3	5	2	2	14	16	17	4	4	Neurod6,Chgb,Crym,Nrxn1,Cacna2d3
CA_Subtype1_neurons (357)	16	4	2	6	5	9	)	3	15	11	14	6	Ly6h,Stmn2,Crtac1
CA_Subtype2_neurons (462)	23	11	5	7	14	1 9	)	12	33	14	14	4	Apba2,Arap2,St3gal5,Osbpl1a,Phactr1
CA1_neurons (391)	24	9	3	2	6	1	5	10	13	50	5	6	Arhgap12,Atp2b1,Cck,Tmem200a,Pkp2
GABAergic_Neurons (483)	26	10	13	8	16	5 1	0	5	12	6	62	5	Ndfip1,Rnpc3,Scn9a,Dner,Slc6a1
Dentate gyrus_neurons (385)		9	5	9	9	4	1	2	2	16	6	82	Stxbp6,Abr,Kbtbd11,Camk2b,Grina
	<b>A</b> SITO	Ender Sol	Mich Othelial (S	0113 09118 (340	Oli	ioodenat	A3 (64)	CA SUD	CA SUIT	CAT CO.	,39, (38,84 (39)	Deni, etgic	<b>b</b> <b>b</b> <b>1</b> 0.1 1e-07
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B Female Astrocytes (555)	69	39	13	24	31	4	7	<b>*39</b>	6	14	13	13	10-00
		39 66	13 13						6 8	14 5	13 7	13 8	0
Astrocytes (555)	15			24	31	4	7	6	<u> </u>				Apoe,Atp1a2,Msi2,Gm3764,Tcf4
Astrocytes (555) Endothelial_cells (128)	15 13 32	66 25 25	13 109 8	24 4 15 83	31 7 29 41	4 9 5 3	7 2 3 4	6 1 1 11	8 8 5	5 12 21	7 9 11	8 2 7	Apoe,Atp1a2,Msi2,Gm3764,Tcf4 Fcho2,Cobll1,Sico1c1,Sic39a10,Esyt2
Astrocytes (555) Endothelial_cells (128) Microglia (488)	15 13 32 12	66 25 25 29	13 109 8 14	24 4 15 83 34	31 7 29 41 199	4 9 5 3 4	7 2 3 4 2	6 1 1 11 4	8 8 5 10	5 12 21 16	7 9 11 13	8 2 7 4	Apoe,Atp1a2,Msi2,Gm3764,Tcf4 Fcho2,Cobll1,Sico1c1,Sic39a10,Esyt2 Rrbp1,Elmo1,Srgap2,Ptgs1,Rap1b Abhd17b,Ncam1,Sema3d,Top2b,Pdgfra Ncam1,Map7,Fam168a,Fgfr2,Ptgds
Astrocytes (555) Endothelial_cells (128) Microglia (488) Oligodendrocyte precursors (593) Oligodendrocytes (780) CA3_Neurons (341)	15 13 32 12 21	66 25 25 29 8	13 109 8 14 3	24 4 15 83 34 3	31 7 29 41 199 4	4 9 5 3 4 17	7 2 3 4 2 10	6 1 1 11 4 6	8 8 5 10 19	5 12 21 16 2	7 9 11 13 15	8 2 7 4 2	Apoe,Atp1a2,Msi2,Gm3764,Tcf4 Fcho2,Cobll1,Sico1c1,Sic39a10,Esyt2 Rrbp1,Elmo1,Srgap2,Ptgs1,Rap1b Abhd17b,Ncam1,Sema3d,Top2b,Pdgfra Ncam1,Map7,Fam168a,Fgfr2,Ptgds Epha4,Sic24a2,Nell2,Asph,Hs3st4
Astrocytes (555) Endothelial_cells (128) Microglia (488) Oligodendrocyte precursors (593) Oligodendrocytes (780) CA3_Neurons (341) CA_Subtype_Neurons (631)	15 13 32 12 21 17	66 25 25 29 8 8 7	13 109 8 14 3 2	24 4 15 83 34 3 7	31 7 29 41 199 4 10	4 9 5 3 4 17 8	7 2 3 4 2 10 3	6 1 11 4 6 21	8 8 5 10 19 9	5 12 21 16 2 16	7 9 11 13 15 17	8 2 7 4 2 3	Apoe,Atp1a2,Msi2,Gm3764,Tcf4 Fcho2,Cobll1,Sico1c1,Sic39a10,Esyt2 Rrbp1,Elmo1,Srgap2,Ptgs1,Rap1b Abhd17b,Ncam1,Sema3d,Top2b,Pdgfra Ncam1,Map7,Fam168a,Fgfr2,Ptgds Epha4,Sic24a2,Nell2,Asph,Hs3st4 Fut9,Lrrtm3,Phactr1,Vsnl1,Etl4
Astrocytes (555) Endothelial_cells (128) Microglia (488) Oligodendrocyte precursors (593) Oligodendrocytes (780) CA3_Neurons (341) CA_Subtype_Neurons (631) CA1_Neurons (239)	<ol> <li>15</li> <li>13</li> <li>32</li> <li>12</li> <li>21</li> <li>17</li> <li>28</li> </ol>	66 25 25 29 8 7 4	13 109 8 14 3 2 2 2	24 4 15 83 34 3 4 3 7 4	31 7 29 41 199 4 10 8	4 9 5 3 4 17 8 18	7 2 3 4 2 10 3 9	6 1 11 4 6 21 10	8 8 5 10 19 9 32	5 12 21 16 2 16 8	7 9 11 13 15 17 10	8 2 7 4 2 3 1	Apoe,Atp1a2,Msi2,Gm3764,Tcf4 Fcho2,Cobll1,Sico1c1,Sic39a10,Esyt2 Rrbp1,Elmo1,Srgap2,Ptgs1,Rap1b Abhd17b,Ncam1,Sema3d,Top2b,Pdgfra Ncam1,Map7,Fam168a,Fgfr2,Ptgds Epha4,Sic24a2,Nell2,Asph,Hs3st4 Fut9,Lrrtm3,Phactr1,Vsnl1,Etl4 Pde1a,Grin2b,Fth1,Calm2,Nrgn
Astrocytes (555) Endothelial_cells (128) Microglia (488) Oligodendrocyte precursors (593) Oligodendrocytes (780) CA3_Neurons (341) CA_Subtype_Neurons (631) CA1_Neurons (239) GABAergic_Neurons (667)	<ol> <li>15</li> <li>13</li> <li>32</li> <li>12</li> <li>21</li> <li>17</li> <li>28</li> <li>19</li> </ol>	66 25 25 29 8 7 4 12	13 109 8 14 3 2 2 2 4	24 4 15 83 34 3 4 7 4 10	31 7 29 41 199 4 10 8 8 14	4 9 5 3 4 17 8 18 3	7 2 3 4 2 10 3 9 9	6 1 11 4 6 21 10 11	8 8 5 10 19 9 32 7	5 12 21 16 2 16 8 59	7 9 11 13 15 17 10 10	8 2 7 4 2 3 1 1	Apoe,Atp1a2,Msi2,Gm3764,Tcf4 Fcho2,Cobll1,Sico1c1,Sic39a10,Esyt2 Rrbp1,Eimo1,Srgap2,Ptgs1,Rap1b Abhd17b,Ncam1,Sema3d,Top2b,Pdgfra Ncam1,Map7,Fam168a,Fgfr2,Ptgds Epha4,Sic24a2,Nell2,Asph,Hs3st4 Fut9,Lrrtm3,Phactr1,Vsnl1,Etl4 Pde1a,Grin2b,Fth1,Calm2,Nrgn Col25a1,Sic6a1,Sic32a1,Akap9,Lamp5
Astrocytes (555) Endothelial_cells (128) Microglia (488) Oligodendrocyte precursors (593) Oligodendrocytes (780) CA3_Neurons (341) CA_Subtype_Neurons (631) CA1_Neurons (239) GABAergic_Neurons (667) Dentate gyrus_Neurons (671)	<ol> <li>15</li> <li>13</li> <li>32</li> <li>12</li> <li>21</li> <li>17</li> <li>28</li> <li>19</li> <li>21</li> </ol>	66 25 25 29 8 7 4 12 10	13 109 8 14 3 2 2 2 4 3	24 4 15 83 34 3 4 3 7 4 10 8	31 7 29 41 199 4 10 8 10 8 14 18	4 9 5 3 4 17 8 18 3 3 3	7 2 3 4 2 10 3 9 9 4 4 2	6 1 11 4 6 21 10 11 2	8 8 5 10 19 9 32 7	5 12 21 16 2 16 8 59	7 9 11 13 15 17 10 10	8 2 7 4 2 3 1 1 6	Apoe,Atp1a2,Msi2,Gm3764,Tcf4 Fcho2,Cobll1,Sico1c1,Sic39a10,Esyt2 Rrbp1,Elmo1,Srgap2,Ptgs1,Rap1b Abhd17b,Ncam1,Sema3d,Top2b,Pdgfra Ncam1,Map7,Fam168a,Fgfr2,Ptgds Epha4,Sic24a2,Nell2,Asph,Hs3st4 Fut9,Lrrtm3,Phactr1,Vsnl1,Etl4 Pde1a,Grin2b,Fth1,Calm2,Nrgn Col25a1,Sic6a1,Sic32a1,Akap9,Lamp5 Abr,Elmod1,Prox1,Cdh8,Mapk1
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# Supplemental Figure 3. Overlap of cluster-specific gene signatures with the previously defined hippocampal cell types markers

(A) Male mice. (B) Female mice. Cluster-specific gene numbers are indicated in the parenthesis. Enrichment was assessed using Fisher's exact test with Bonferroni adjusted p values. Color intensity indicates statistical significance of overlapping genes, and the numbers of overlapping cluster-specific genes between our cluster-specific gene and previously known markers are shown in the cells. Top cell marker genes determined by our single-nucleus RNA sequencing data are listed on the right of the plot.

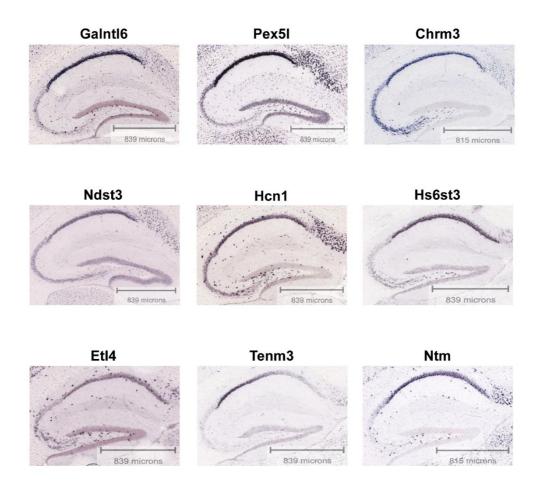
#### **Supplemental Figure 4.**



Supplemental Figure 4. Identification and cross-validation of novel cell type-specific marker genes in male and female mice

(A) Dot plot showing the expression of top 5 genes for each specific hippocampal cell type of male mice. Dot size is proportional to the fraction of cells expressing specific genes, and color intensity corresponds to the level of gene expression. (B) Violin plot showing the normalized expression of top cell type-specific marker genes (columns) in specific cell types (rows) of male mice. (C) Dot plot showing the expression of top 5 genes for each specific hippocampal cell type of female mice. Dot size is proportional to the fraction of cells expressing specific genes, and color intensity corresponds to the level of gene expression. (D) Violin plot showing the normalized expression of top cell type-specific marker genes (columns) in specific cell types (rows) of female mice. Criteria for specific marker genes: mincell\_pct  $\geq 0.25$ , P value  $\leq 0.01$ , and log<sub>2</sub> (fold change)  $\geq 0.361$  (Seurat R package).

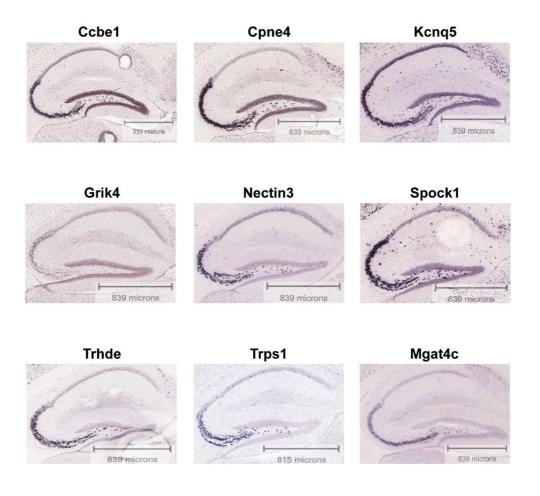
### **Supplemental Figure 5.**



### Supplemental Figure 5. Validation of CA1 neuron-specific marker genes

The top marker genes for CA1 neurons identified by our single cell sequencing approach were validated using the in-situ hybridization images in the Allen Brain Atlas (https://mouse.brain-map.org).

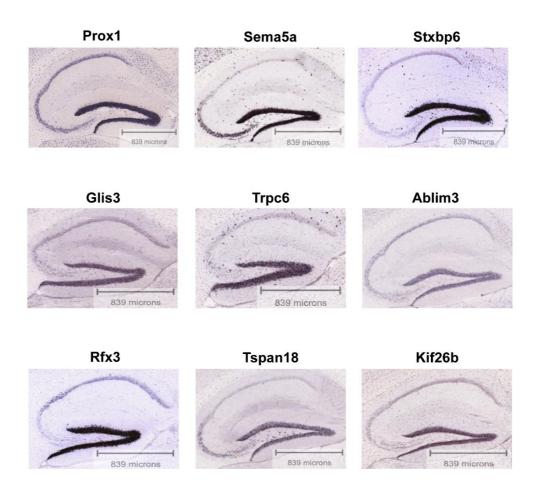
### Supplemental Figure 6.



### Supplemental Figure 6. Validation of CA3 neuron-specific marker genes

The top marker genes for CA3 neurons identified by our single cell sequencing approach were validated using the in-situ hybridization images in the Allen Brain Atlas (https://mouse.brain-map.org).

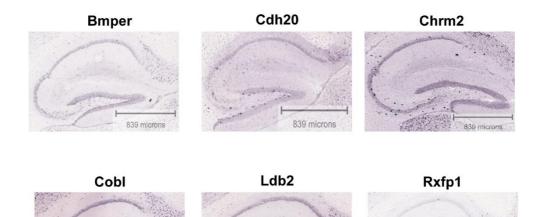
### Supplemental Figure 7.



### Supplemental Figure 7. Validation of dentate gyrus granule cell-specific marker genes

The top marker genes for dentate gyrus granule cells identified by our single cell sequencing approach were validated using the in-situ hybridization images in the Allen Brain Atlas (https://mouse.brainmap.org).

### **Supplemental Figure 8.**





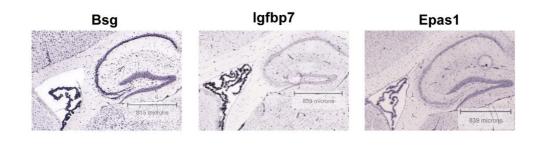
839 mic

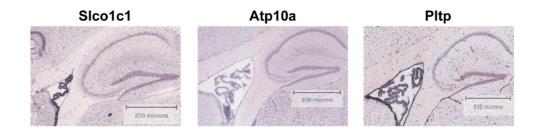
### Supplemental Figure 8. Validation of CA subtype neuron-specific marker genes

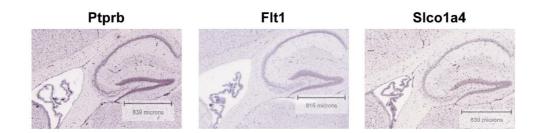
839 micron

The top marker genes for CA subtype neurons identified by our single cell sequencing approach were validated using the in-situ hybridization images in the Allen Brain Atlas (https://mouse.brain-map.org).

839 microns

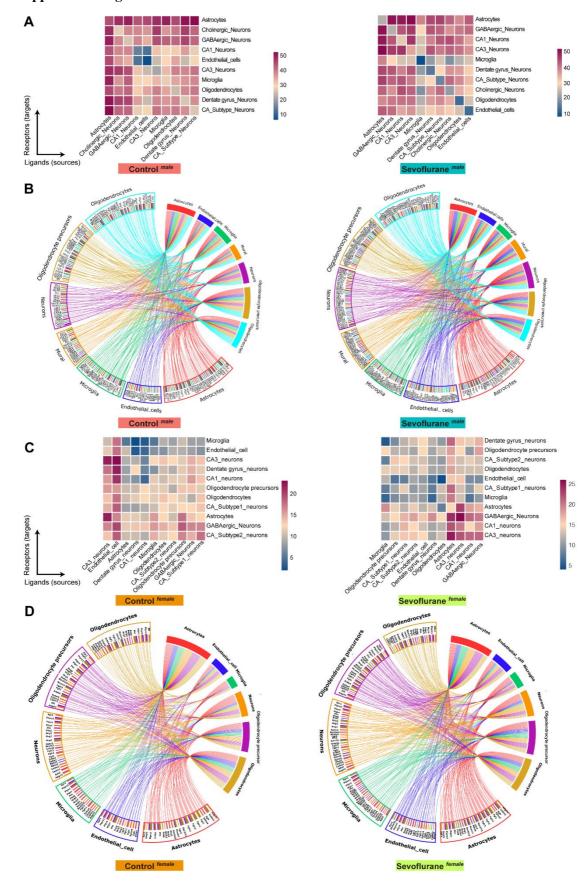






# Supplemental Figure 9. Validation of endothelial cell-specific marker genes

The top marker genes for endothelial cells identified by our single cell sequencing approach were validated using the in-situ hybridization images in the Allen Brain Atlas (https://mouse.brain-map.org).

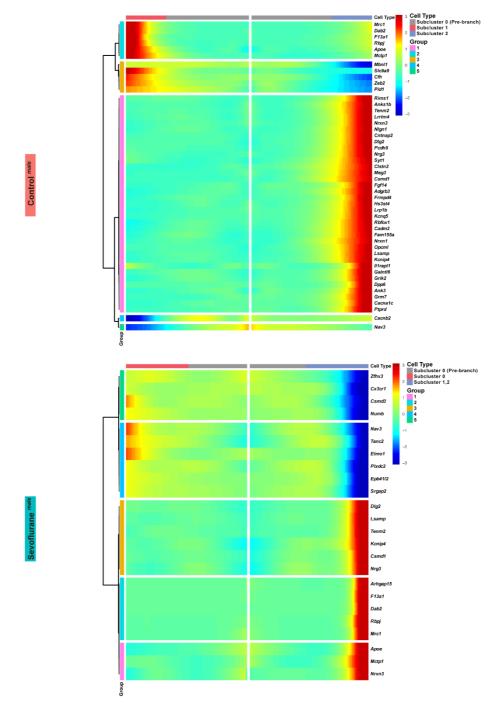


#### Supplemental Figure 10.

# Supplemental Figure 10. Alterations in hippocampal cellular cross-talk induced by sevoflurane exposures in male and female mice

(A) Heatmaps showing the abundance of ligand-receptor interactions among source cells (columns) and target cells (rows) of hippocampus in the control <sup>male</sup> group (left) and sevoflurane <sup>male</sup> group (right). Darker purple indicates stronger interactions. (B) Circos plots showing significant cell-cell gene co-expression among source hippocampal cell types with secreted peptides (bottom half of the circus) and target cell types (top half of the circus) in the control <sup>male</sup> group (left) and sevoflurane <sup>male</sup> group (right). (C) Heatmaps showing the abundance of ligand-receptor interactions among source cells (columns) and target cells (rows) of hippocampus in the control <sup>female</sup> group (left) and sevoflurane <sup>female</sup> group (right). Darker purple indicates stronger interactions. (D) Circos plots showing significant cell-cell gene co-expression among source hippocampal cell types with secreted peptides (bottom half of the circus) and target cell types (top half of the circus) in the control <sup>female</sup> group (left) and sevoflurane <sup>female</sup> group (right). Darker purple indicates stronger interactions. (D) Circos plots showing significant cell-cell gene co-expression among source hippocampal cell types with secreted peptides (bottom half of the circus) and target cell types (top half of the circus) in the control <sup>female</sup> group (left) and sevoflurane <sup>female</sup> group (right). Criteria for significant enriched ligand-receptor pair: mincell\_pct  $\geq$  30% and P value < 0.05 (CellPhoneDB software).

#### **Supplemental Figure 11.**

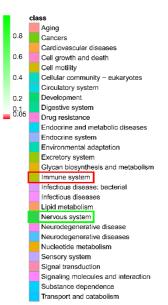


Supplemental Figure 11. Key genes that determined the differentiation branches of microglia in male mice

Heatmaps showing that the differential expression of key genes among subclusters 0, 1, and 2 determined microglia differentiation branches in the control <sup>male</sup> group (upper) and sevoflurane <sup>male</sup> group (lower). Criteria for key genes: false discovery rate < 1e-7 (Monocle 2 package).

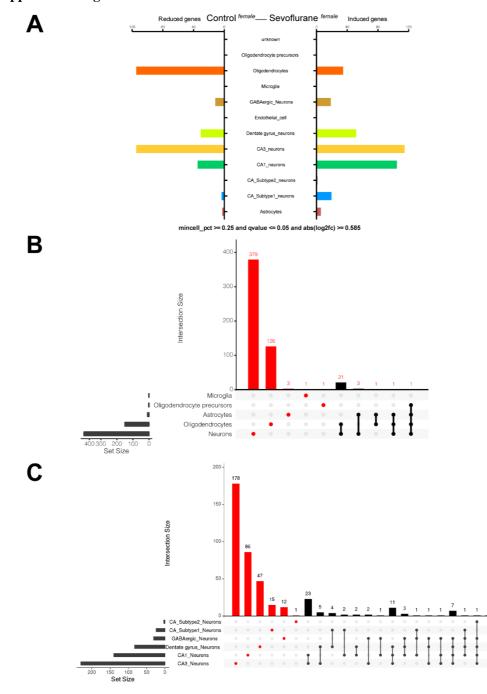
### Supplemental Figure 12.

	0.040	NA	ko04213 Longevity regulating pathway - multiple species
	0.478	0.023	ko05200 Pathways in cancer
			ko05202 Transcriptional misregulation in cancers
	0.158	1.662	ko05205 Proteoglycans in cancer ko05214 Glioma
			ko05220 Chronic myeloid leukemia
	0.000	).417	ko05231 Choline metabolism in cancer
	0.000	).145	ko05410 Hypertrophic cardiomyopathy (HCM)
	0.000	1824	ko04114 Dilated cardiomyopathy (DCM)
	0.001	).034	kc05414 Dilated cardiomyopathy (ICM) kc04114 Oocyte meiosis kc04410 Regulation of actin cytoskeleton
	0.039	J.158	kou4510 Focal adhesion
	0.001	0.020	ko04520 Adherens junction ko04530 Tight junction
	0.013	1.698	ko04530 Fight junction ko04540 Gap junction
			ko04260 Cardiac muscle contraction
			ko04270 Vascular smooth muscle contraction
	0.000	). 120	ko04360 Axon guidance
	0.329	1.313	ko04380 Osteoclast differentiation ko04970 Salivary secretion
	0.000	0.667	ko04971 Gastric acid secretion
	0.000	).796	ko04972 Pancreatic secretion ko04973 Carbohydrate digestion and absorption
	0.003	).490	ko04973 Carbohydrate digestion and absorption
	0.032 (	). 109	ko04978 Mineral absorption ko01521 EGFR tyrosine kinase inhibitor resistance
	0.000	0.505	ko04930 Type II diabetes mellitus
			ko04934 Cushing syndrome
			ko04911 Insulin secretion ko04912 GnRH signaling pathway
			ko04912 Girkh signaling pathway ko04914 Progesterone-mediated oocyte maturation
	0.001	0.307	ko04915 Estrogen signaling pathway
	0.000	).652	ko04918 Thyroid hormone synthesis
	0.003	1.654	ko04919 Thyroid hormone signaling pathway ko04921 Oxytocin signaling pathway
	0.004	).447	ko04922 Glucagon signaling pathway
	0.028	). 195	ko04923 Regulation of lipolysis in adipocyte
	0.002	0.667	ko04924 Renin secretion ko04925 Aldosterone synthesis and secretion
	0.000	1.773	ko04925 Aldosterone synthesis and secretion ko04926 Relaxin signaling pathway
H			ko04928 Parathyroid hormone synthesis, secretion and action
	0.000	NA	ko04929 GnRH secretion
			ko04935 Growth hormone synthesis, secretion and action
			ko04713 Circadian entrainment ko04960 Aldosterone-regulated sodium reabsorption
	0.000	).585	ko04961 Endocrine and other factor-regulated calcium reabsorption
	0.004	NA	ko04964 Proximal tubule bicarbonate reclamation
	0.010	NA	kc00512 Mucin type O-glycan biosynthesis
	0.041		kc00514 Other types of O-glycan biosynthesis kc00534 Glycosaminoglycan biosynthesis - heparan sulfate / hepara
	0.121	0.019	ko04062 Chemokine signaling pathway
			ko04610 Complement and coagulation cascades
	0.004 0		ko04611 Platelet activation ko04640 Hematopoietic cell lineage
	0.379		ko04659 Th17 cell differentiation
	0.208		kc04662 B cell receptor signaling pathway
	0.051		ko04664 Fc epsilon RI signaling pathway ko04666 Fc gamma R-mediated phagocytosis
۲	0.012 (		ko05135 Yersinia infection
	0.036		ko05142 Chagas disease (American trypanosomiasis)
	NA 0.095		ko05150 Staphylococcus aureus infection ko05152 Tuberculosis
	NA	0.005	ko00565 Ether lipid metabolism
	NA	0.009	ko00590 Arachidonic acid metabolism
			ko04720 Long-term potentiation
		NA	ko04721 Synaptic vesicle cycle ko04722 Neurotrophin signaling pathway
			ko04723 Retrograde endocannabinoid signaling
	0.000	).810	ko04724 Glutamatergic synapse
	0.000	0.807	ko04725 Cholinergic synapse
	0.000	).729 ).855	ko04727 GABAergic synapse ko04728 Dopaminergic synapse
	0.000	0.056	ko04730 Long-term depression
	0.000	NΔ	ko05017 Spinocerebellar ataxia
	0.024	1.863	ko05010 Alzheimer disease ko05010 Alzheimer disease ko05020 Prion diseases
	0.078	1.012	ko05020 Prion diseases
	0.003	).319	ko00230 Purine metabolism
			ko04750 Inflammatory mediator regulation of TRP channels
			ko04010 MAPK signaling pathway ko04012 ErbB signaling pathway
			ko04014 Ras signaling pathway
	0.000	.202	ko04015 Rap1 signaling pathway
_	0.000	0.530	ko04020 Calcium signaling pathway
-	0.120	1.236	ko04022 cGMP - PKG signaling pathway ko04066 HIE-1 signaling pathway
	0.000	).417	ko04066 HIF-1 signaling pathway ko04070 Phosphatidylinositol signaling system
	0.004	0.252	ko04071 Sphingolipid signaling pathway
_	0.000	1.065	ko04072 Phospholipase D signaling pathway
	0.814	0.043	ko04151 PI3K-Akt signaling pathway ko04330 Notch signaling pathway
	0.031	).573	ko04370 VEGF signaling pathway
			kc04371 Apelin signaling pathway
			ko04080 Neuroactive ligand-receptor interaction ko04514 Cell adhesion molecules (CAMs)
	0.000	0.631	ko05031 Amphetamine addiction
	0.000	).387	ko05032 Morphine addiction
	0.000	NA	ko05033 Nicotine addiction ko04140 Autophagy – animal
	0.913	000.0	ko04140 Autophagy – animal ko04142 Lysosome ko04145 Phagosome
	0.897	0.037	ko04145 Phagosome
355	0	<u>-</u>	
ö	Cluster	Cluster	
	CIL	ü	



# Supplemental Figure 12. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of microglia subclusters of female mice

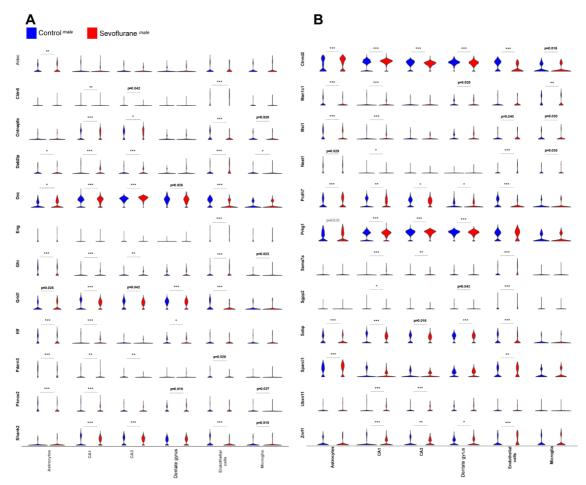
Significantly enriched Kyoto Encyclopedia of Genes and Genomes pathways for subcluster 0 and 1 in relation to the nervous system and immune system, respectively. False discovery rate  $\leq 0.05$  as a threshold to determine a significant enrichment.



Supplemental Figure 13.

Supplemental Figure 13. Differentially expressed genes induced by sevoflurane exposures in hippocampal cell types in female mice

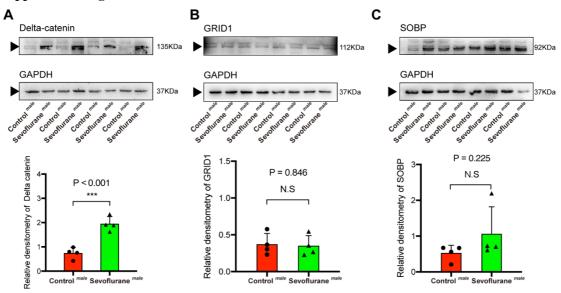
(A) Histogram showing the number of differentially expressed genes in each hippocampal cell type. (B) Histogram showing the number of differentially expressed genes in hippocampal cell types, with plots (red) indicating differentially expressed genes unique to a cell type and plots and lines (black) indicating common differentially expressed genes between  $\geq 2$  cell types. (C) Histogram with plots and lines showing the unique and common differentially expressed genes in hippocampal neuron subtypes. Criteria for differentially expressed genes: mincell\_pct  $\geq 0.25$ , q value  $\leq 0.05$ , and  $|log_2|$  (fold change)  $|\geq 0.585$  (Model-based Analysis of Single-cell Transcriptomics test). **Supplemental Figure 14.** 



# Supplemental Figure 14. Normalized expression of common differentially expressed genes in specific hippocampal cell types in male mice

Violin plots showing the normalized expression of 24 common differentially expressed genes in specific hippocampal cell types in the control <sup>male</sup> group (blue) and sevoflurane <sup>male</sup> group (red). Data are means  $\pm$  SD. n = 15–3154 cells/group. Student's t-test. \*P < 0.02, \*\*P < 0.01, \*\*\*P < 0.001.

## Supplemental Figure 15.



Supplemental Figure 15. Protein expression of delta-catenin, GRID1, and SOBP in male mice using Western blot

(A) Protein bands and expression analysis showing that the sevoflurane <sup>male</sup> group had upregulated expression of delta-catenin (mean difference = 1.20, 95%CI: 0.74 to 1.67, P < 0.001). (**B**, **C**) Protein bands and expression analysis showing that the expression of GRID1 and SOBP was comparable between groups. Data are means  $\pm$  SD. n = 4 mice/group. Student's t-test.