

## Supplementary materials for

### A topographical atlas of $\alpha$ -synuclein dosage and cell type-specific expression in adult mouse brain and peripheral organs

Haley M. Geertsma<sup>1,2,3,\*</sup>, Zoe A. Fisk<sup>1,2,3,\*</sup>, Lillian Sauline<sup>3,4,\*</sup>, Alice Prigent<sup>3,4</sup>, Kevin Kurgat<sup>3,4</sup>, Steve M. Callaghan<sup>1,2,3</sup>, aSCENT-PD Consortium<sup>3</sup>, Michael X. Henderson<sup>3,4,#</sup>, Maxime W.C. Rousseaux<sup>1,2,3,#</sup>

<sup>1</sup> University of Ottawa Brain and Mind Research Institute, Ottawa, ON, K1H8M5

<sup>2</sup> Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, K1H8M5

<sup>3</sup> Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, USA

<sup>4</sup> Department of Neurodegenerative Science, Van Andel Institute, Grand Rapids, MI, USA

\* Authors contributed equally

# Co-corresponding authors

Correspondence: [max.rousseau@uottawa.ca](mailto:max.rousseau@uottawa.ca) or [michael.henderson@vai.org](mailto:michael.henderson@vai.org)

aSCENT-PD Consortium: Benjamin R. Arendt<sup>3,6</sup>, Brit Mollenhauer<sup>3,7</sup>, Michael G. Schlossmacher<sup>3,8</sup>, Christine Stadelmann<sup>3,9</sup>, Julianna J. Tomlinson<sup>3,8</sup>

<sup>6</sup> Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030

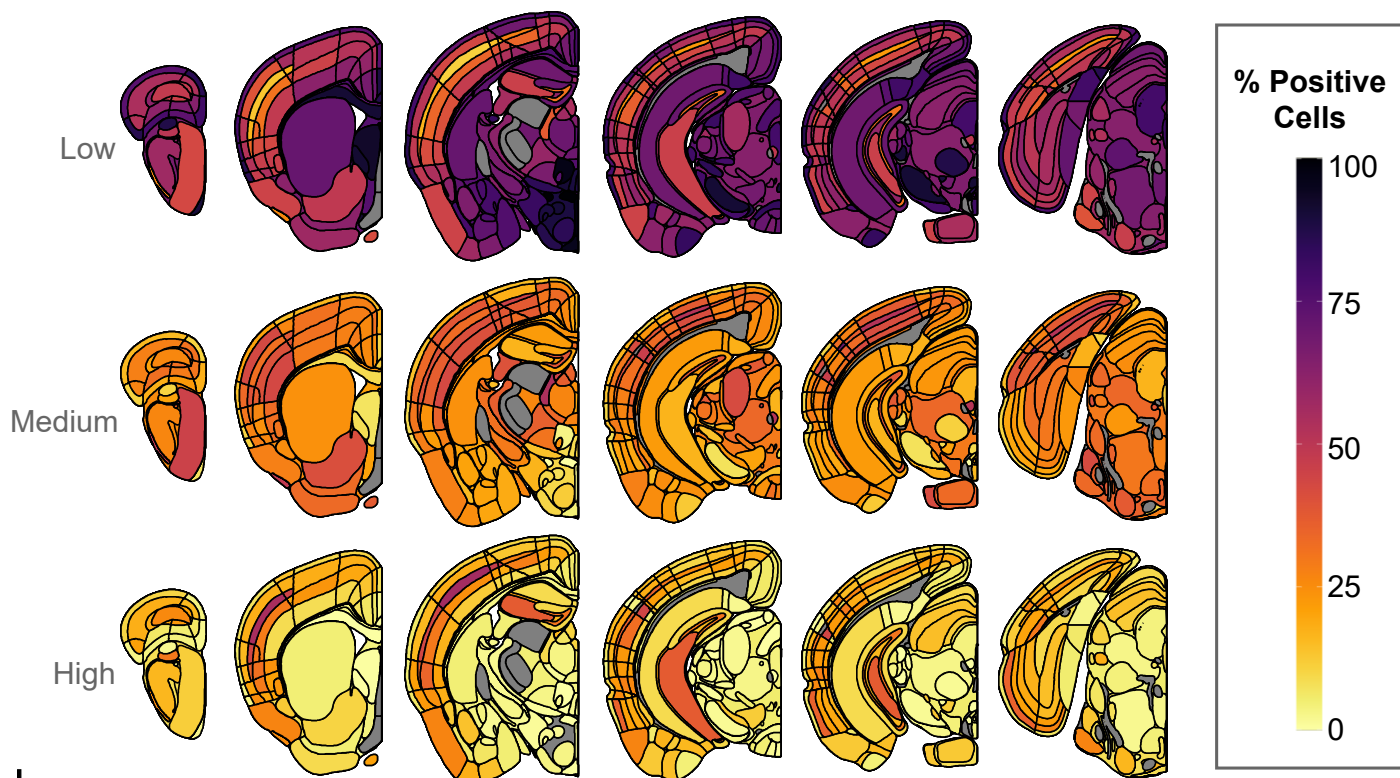
<sup>7</sup> Paracelsus-Elena-Klinik, Kassel, Germany

<sup>8</sup> Program in Neuroscience, Ottawa Hospital Research Institute, University of Ottawa Brain and Mind Research Institute, Ottawa, ON, Canada K1H8M5

<sup>9</sup> Institute of Neuropathology, University Medical Center Göttingen, Göttingen, Germany

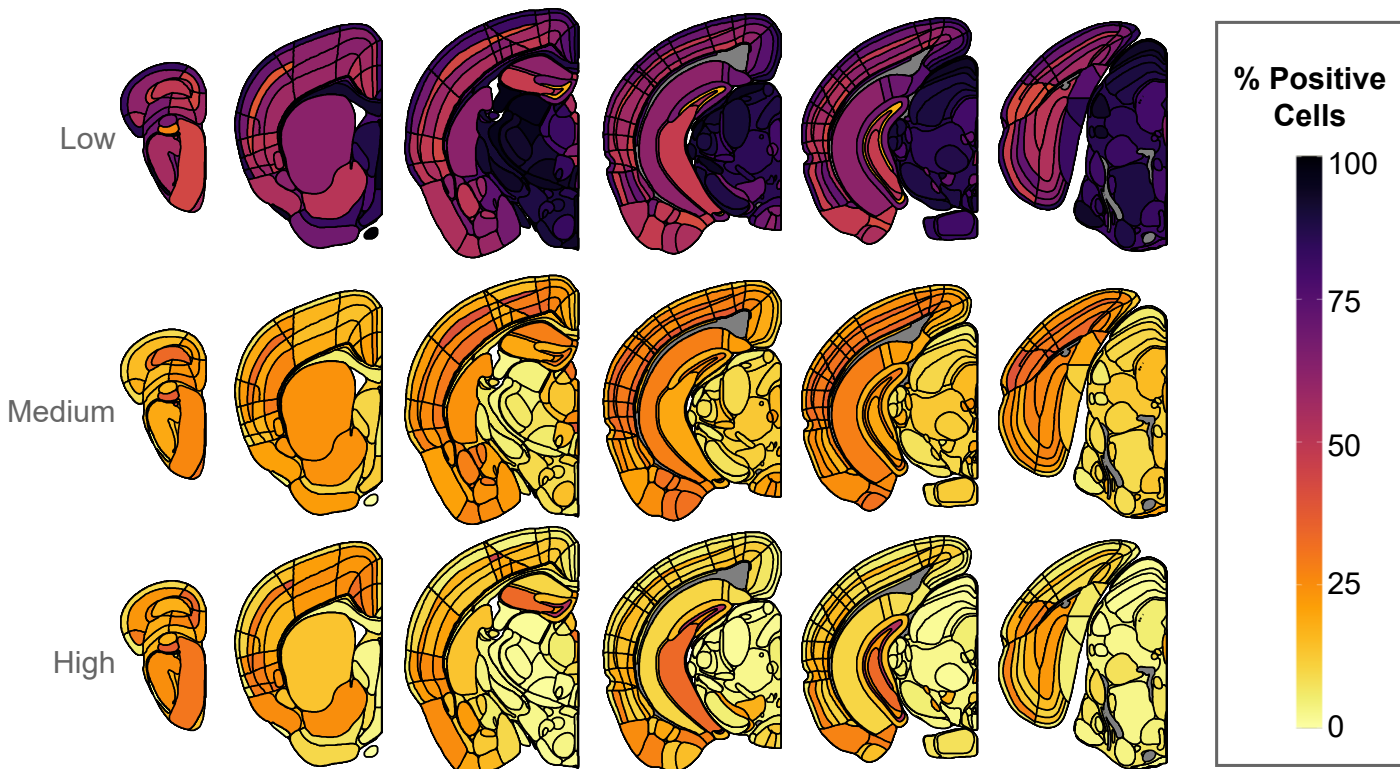
a

### *Snca* Expression Atlas

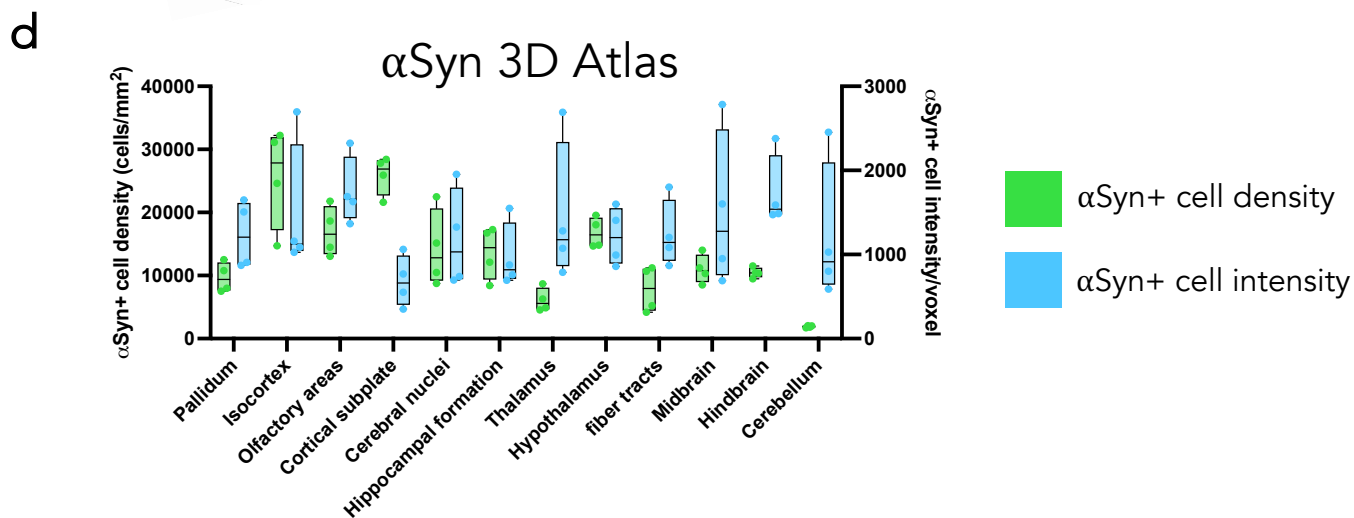
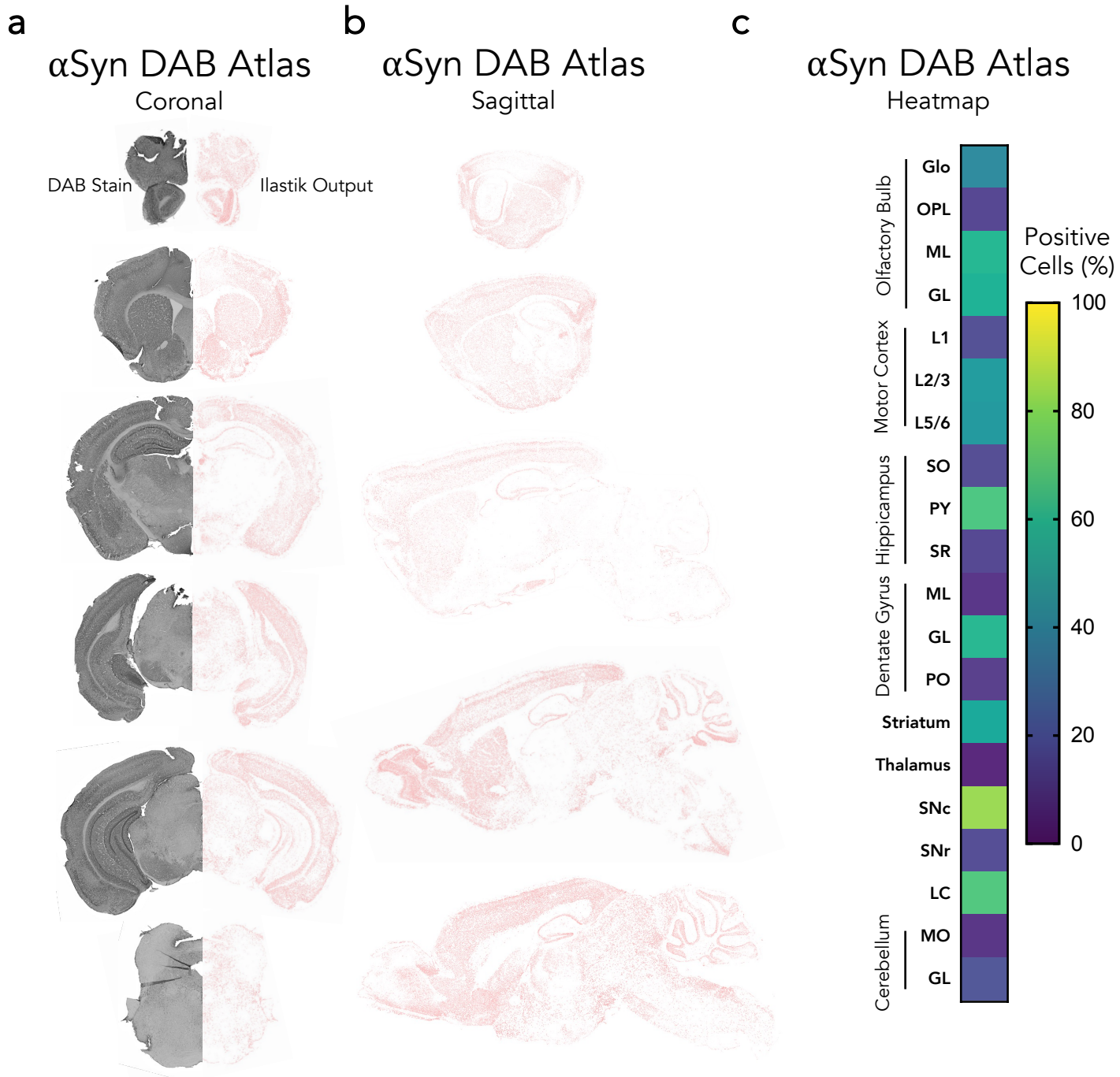


b

### $\alpha$ Syn<sup>NLS</sup> Atlas

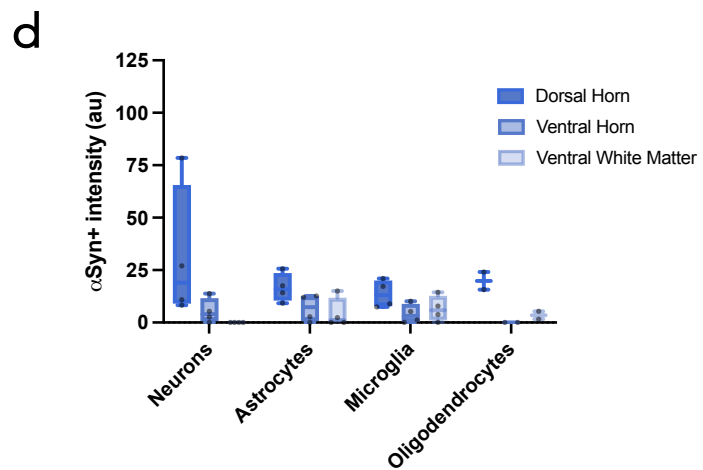
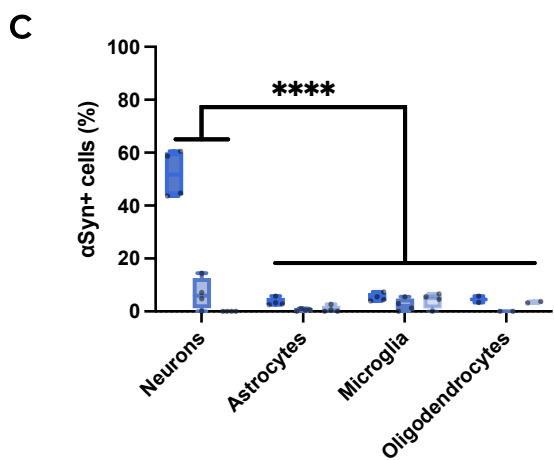
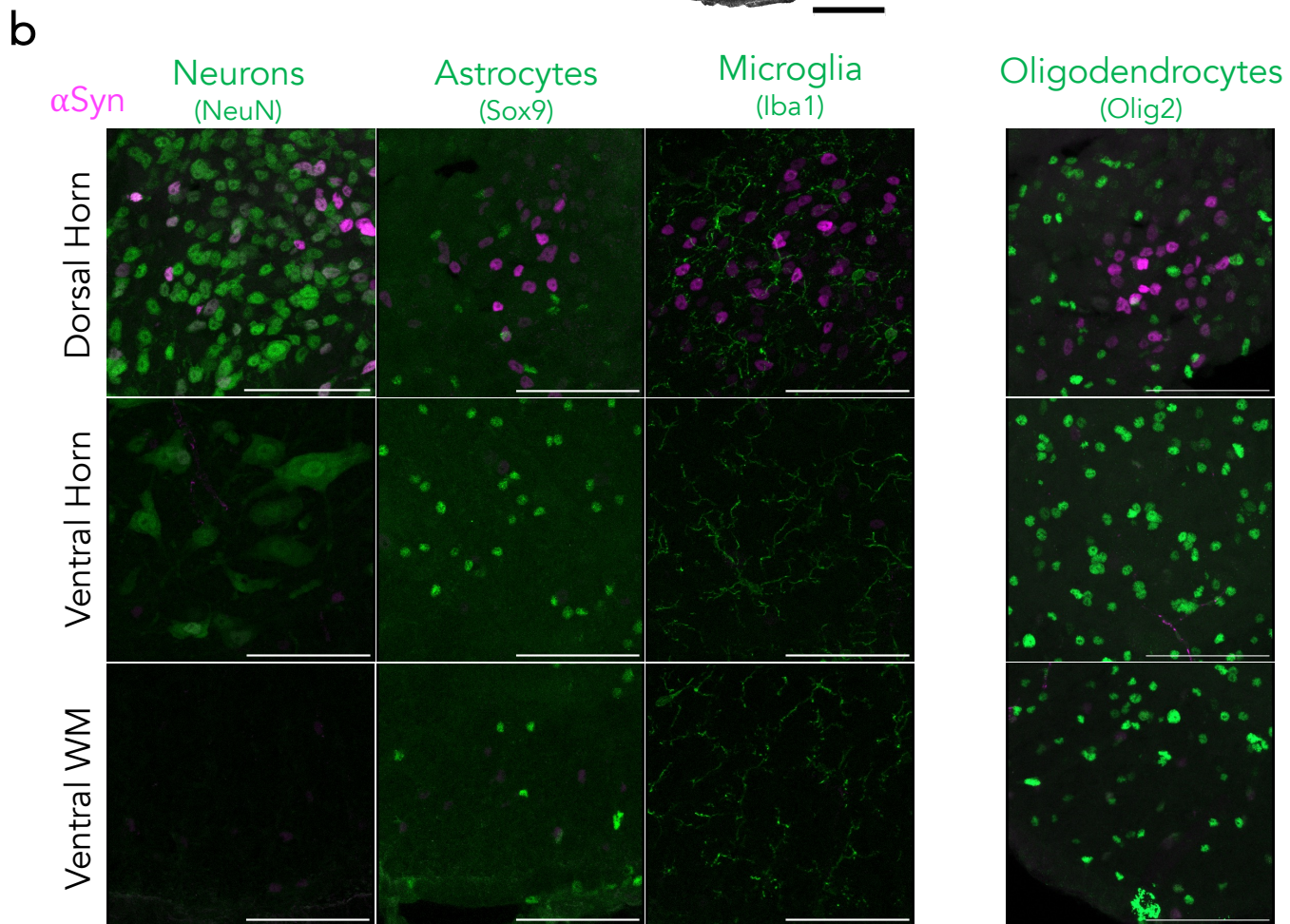
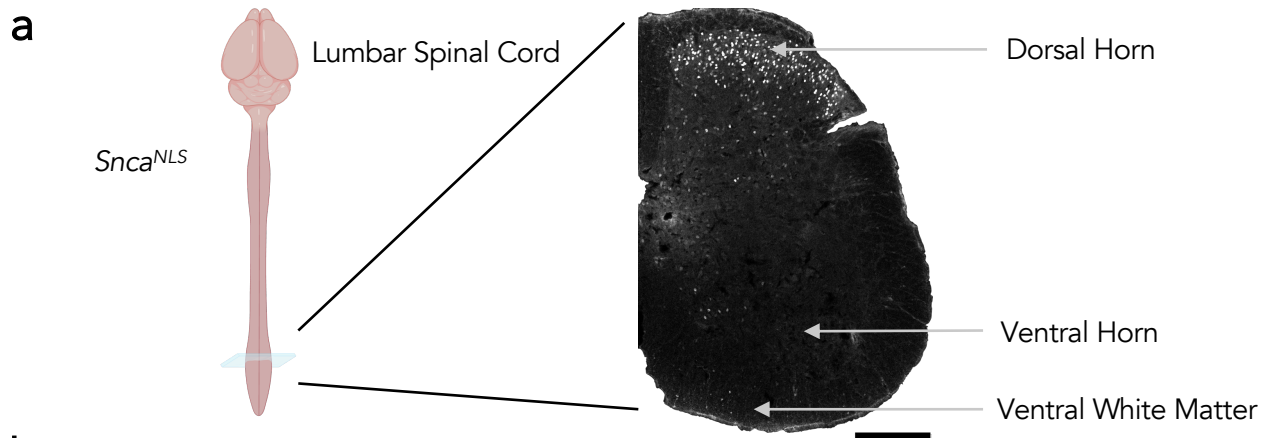


**Supplementary Figure 1: Brain-wide heatmaps of *Snca* expression and  $\alpha$ Syn protein density.** **a)** *Snca* intensity from RNAScope of a wild-type mouse binned into low (upper panel), medium (middle panel), and high intensity (lower panel) prior to generating the heatmaps. **b)**  $\alpha$ Syn staining intensity of a *Snca*<sup>NLS</sup> mouse binned into low (upper panel), medium (middle panel), and high intensity (lower panel) prior to generating the heatmaps.

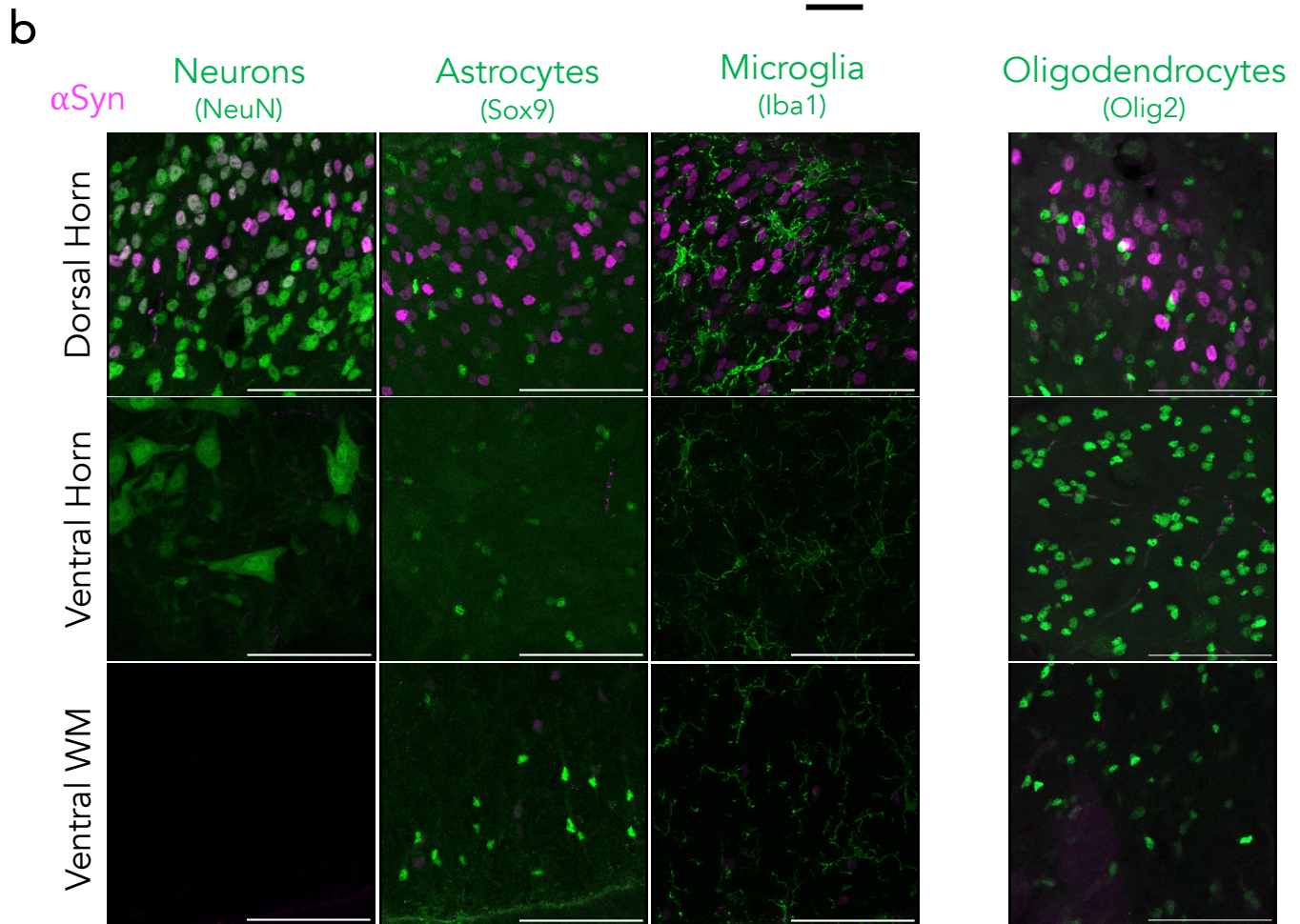
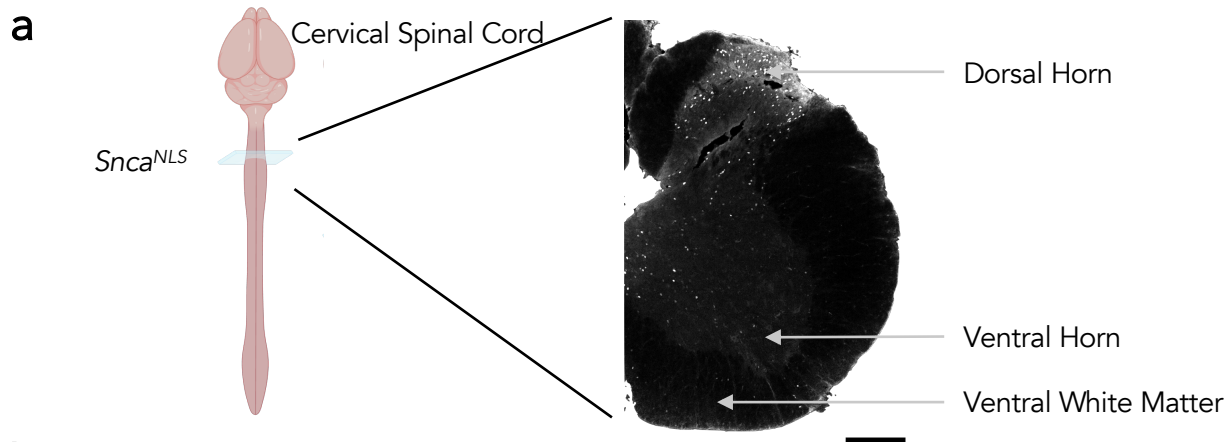




**Supplementary Figure 2: Brain-wide atlas of  $\alpha$ Syn protein density and intensity.** **a)** Coronal atlas of  $\alpha$ Syn DAB staining (left) and ilastik segmentation (right). **b)** Sagittal atlas of  $\alpha$ Syn ilastik segmentation following DAB staining. **c)** Quantification of  $\alpha$ Syn density in targeted brain regions. **d)** Quantification of  $\alpha$ Syn<sup>+</sup> density and intensity from whole brain staining and imaging. Plotted as box plots with min to max bars.

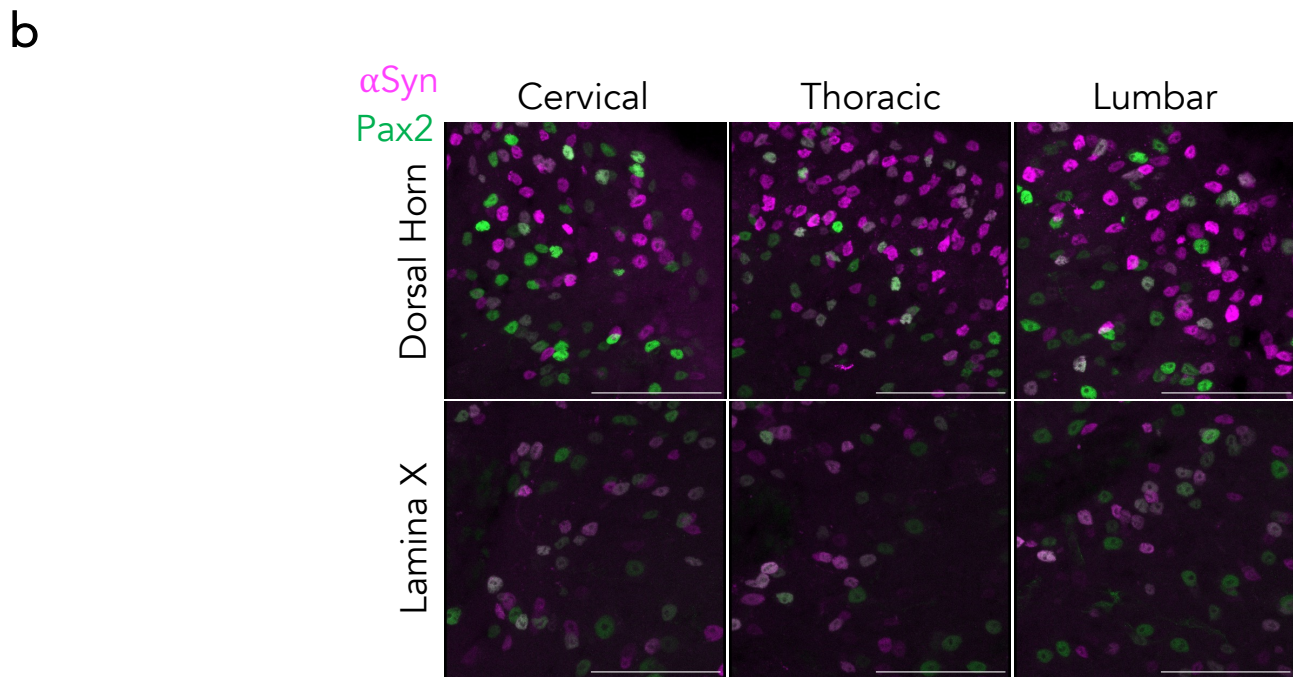
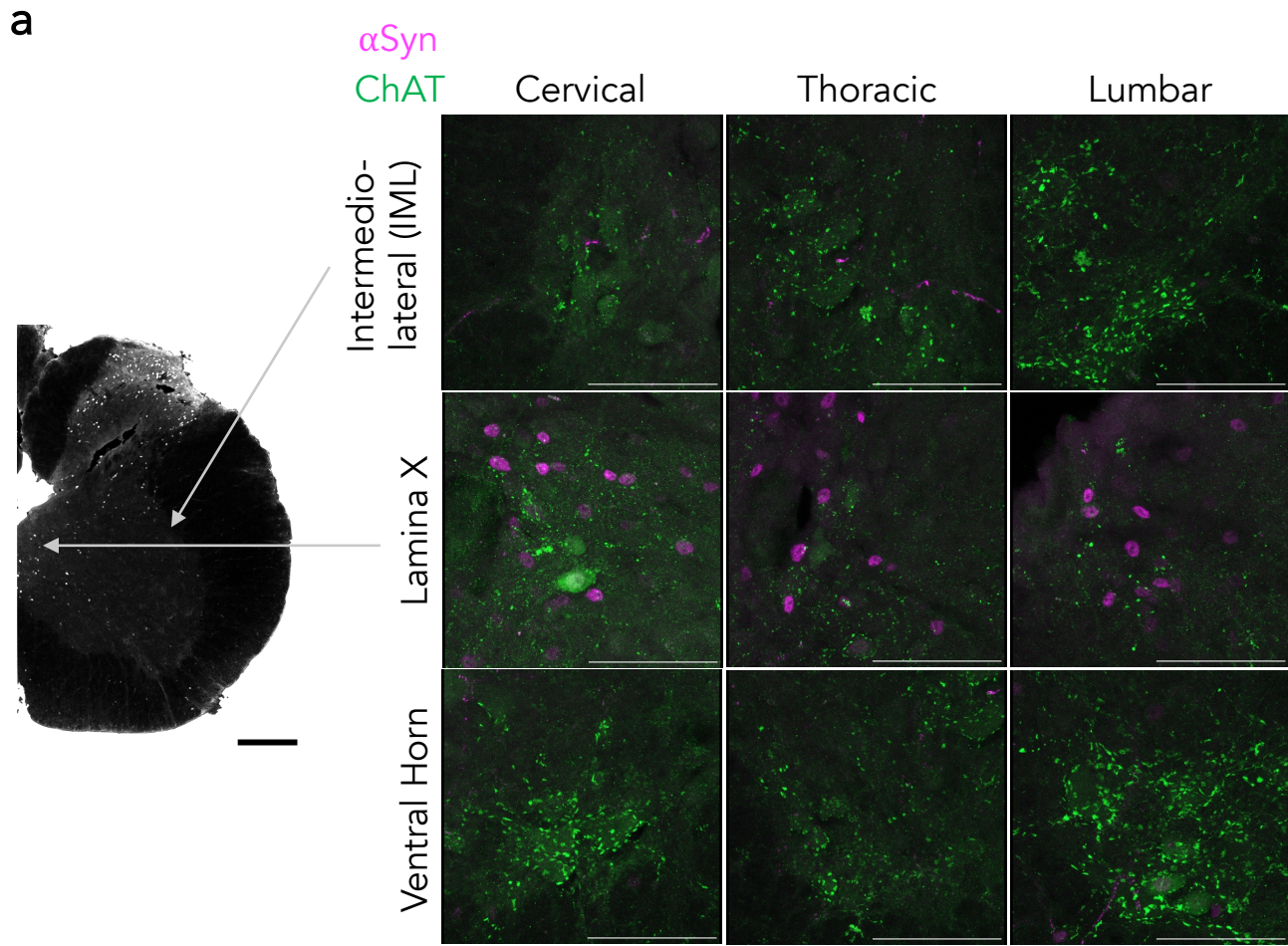


**Supplementary Figure 3:  $\alpha$ Syn density and intensity of the lumbar spinal cord.** **a)** Coronal plane chosen to assess different regions of the lumbar spinal cord in *Snc $\alpha$ <sup>NLS</sup>* mice (left) with annotations for the dorsal and ventral horns and ventral white matter (right) from a section stained for  $\alpha$ Syn. Scale bar: 1,000 $\mu$ m. **b)** Merged micrographs from the dorsal horn (upper), ventral horn (middle), and ventral white matter (bottom) staining for neurons (left), astrocytes (middle left), microglia (middle right), and oligodendrocytes (right; different staining paradigm). From these,  $\alpha$ Syn density (**c**) and intensity (**d**) were quantified. Scale bars: 75 $\mu$ m. Plotted as box plot with min to max bars. Two-way ANOVA with Tukey's post hoc, \*\*\*\* denotes  $p < 0.0001$ .



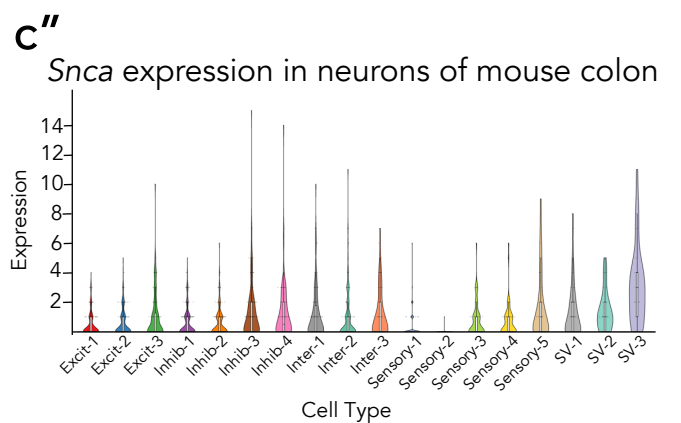
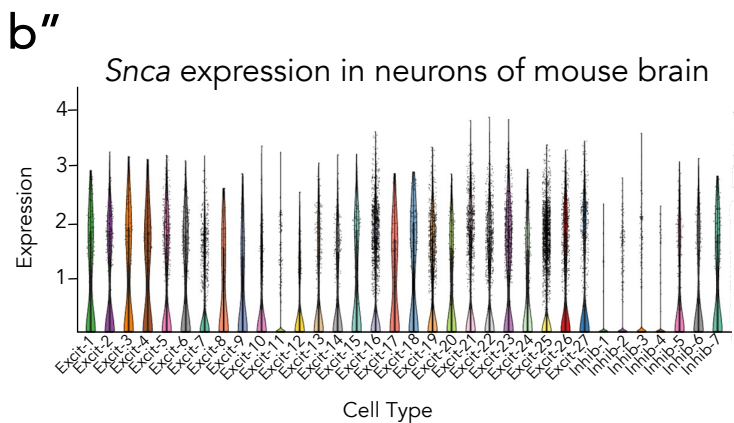
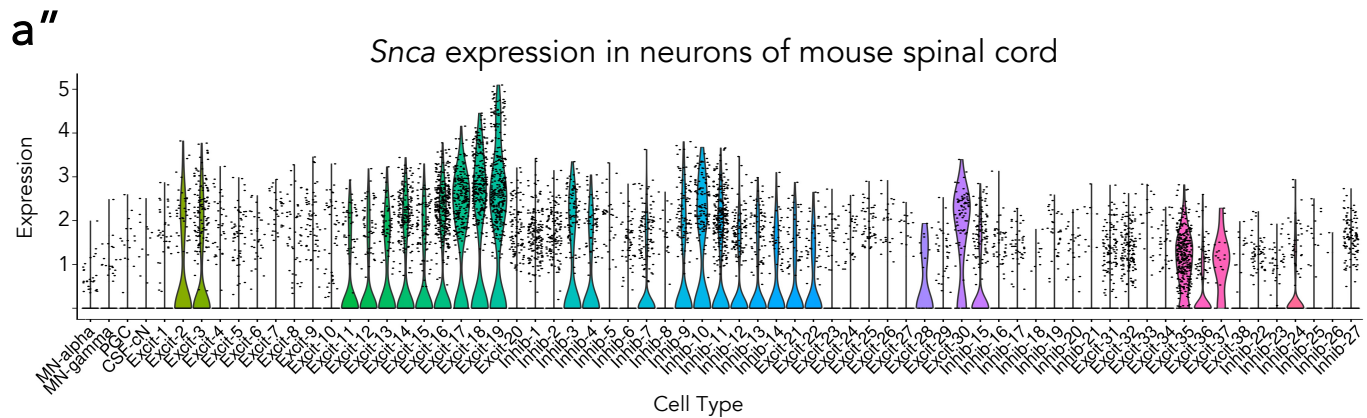
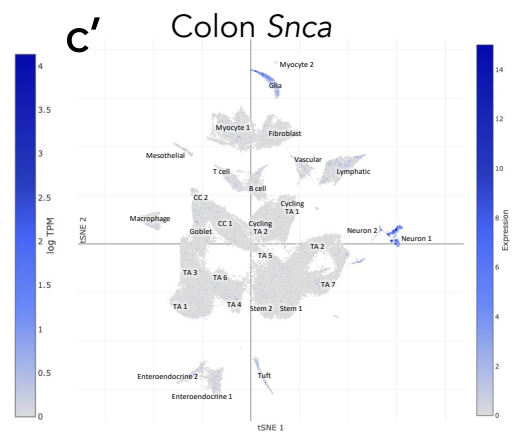
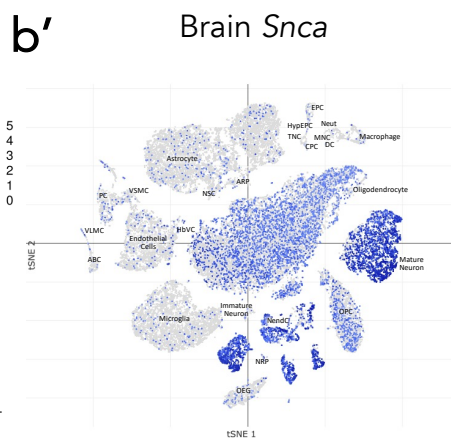
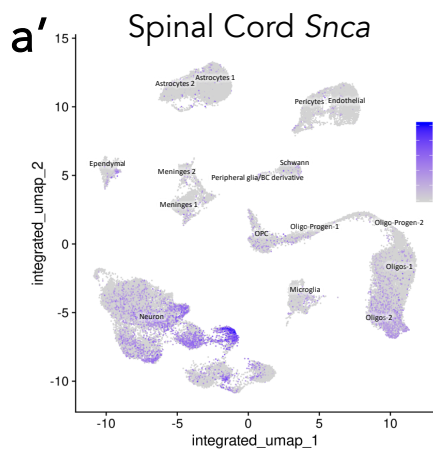
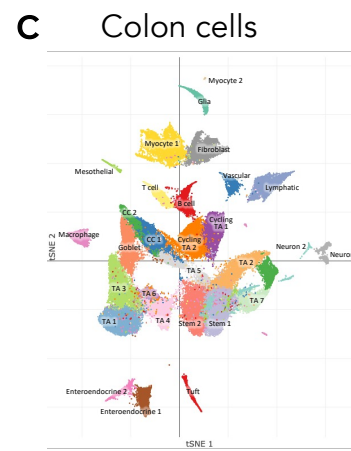
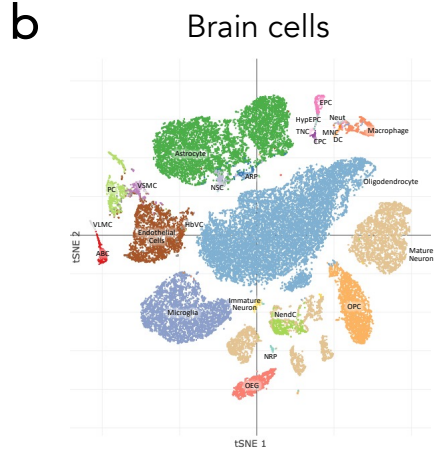
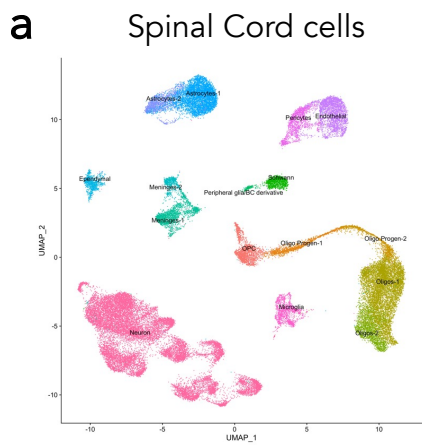
**Supplementary Figure 4:  $\alpha$ Syn staining of the cervical spinal cord.** a) Coronal plane chosen to assess different regions of the lumbar spinal cord in *Snc $\alpha$ <sup>NLS</sup>* mice (left) with annotations for the dorsal and ventral horns and ventral white matter (right) from a section stained for  $\alpha$ Syn. Scale bar: 1,000 $\mu$ m. b) Merged micrographs from the dorsal horn (upper), ventral horn (middle), and ventral white matter (bottom) staining for neurons (left), astrocytes (middle left), microglia (middle right), and oligodendrocytes (right; different staining paradigm). Scale bars: 75 $\mu$ m.







**Supplementary Figure 5:  $\alpha$ Syn staining patterns throughout the spinal cord with neuron-specific markers for ChAT and Pax2.** **a)** Coronal plane chosen to assess cervical (left) thoracic (middle) or lumbar (right) *Snc $\alpha$ <sup>NLS</sup>* spinal cord in the intermediolateral (upper), lamina X (middle), and ventral horn (lower) co-stained for  $\alpha$ Syn and ChAT. Scale bar or coronal plane: 1000 $\mu$ m. Scale bars for immunofluorescent images: 75 $\mu$ m. **b)** Coronal plane chosen to assess cervical (left) thoracic (middle) or lumbar (right) *Snc $\alpha$ <sup>NLS</sup>* spinal cord in the dorsal horn (upper) and ventral horn (lower) co-stained for  $\alpha$ Syn and Pax2. Scale bars: 75  $\mu$ m.



**Supplementary Figure 6: Mining scRNA-Seq datasets to explore  $\alpha$ Syn expression patterns in the mouse spinal cord, brain, and colon.** **a)** UMAP plot from the Harmonized Atlas of Mouse Spinal Cord with **a')** *Snca* expression pattern and **a'')** neuron-specific sub-clustering. **b)** tSNE plot from the Aging Mouse Brain dataset from the Broad Institute's Single Cell Portal with **b')** *Snca* expression pattern and **b'')** neuron-specific sub-clustering from the study Dissecting Cell-Type Composition and Activity-Dependent Transcriptional State in Mammalian Brains by Massively Parallel Single-Nucleus RNA-Seq. **c)** tSNE plot from the Human and Mouse Enteric Nervous System at Single Cell Resolution from the Broad Institute's Single Cell Portal with **c')** *Snca* expression pattern and **c'')** neuron-specific sub-clustering. ABC (arachnoid barrier cells), ARP (astrocyte-restricted precursors), CC (colonocyte), CPC (choroid plexus epithelial cells), CSF-cN (cerebrospinal fluid contacting neurons), DC (dendritic cells), EPC (ependymocytes), Excit- (excitatory neurons), HbVC (hemoglobin-expressing vascular cells), HypEPC (hypependymal cells), Inhib- (inhibitory neurons), Inter- (interneurons), MN (motor neurons), MNC (monocytes), NendC (neuroendocrine cells), Neut (neutrophils), NRP (neuronal-restricted precursors), NSC (neural stem cells), OEG (olfactory ensheathing glia), OPC (oligodendrocyte precursor cells), PC (pericytes), PGC (preganglionic cells), Sensory (sensory neurons), Stem (stem cells), SV (secretomotor/vasodilator neurons), TA (transit-amplifying cells), TNC (tanycytes), VLMC (vascular and leptomeningeal cells).

**Supplementary Table 1 Antibodies used in this study with their application**

	Antibodies (alphabetical)	Provider, Catalog #, RRID	Species	Application	Concentration
Primary	αSynuclein	BD Biosciences, 610787, RRID:AB_398108	Mouse	Immunofluorescence, αSyn <sup>NLS</sup> Atlas	1:2000
				Immunofluorescence, cell-specific staining & Immunohistochemistry, αSyn DAB Atlas	1:1000
		Cell Signaling Technology, 4179, RRID:AB_1904156	Rabbit	ENS immunofluorescence	1:250
	Calbindin D28K	Invitrogen, PA5-85669, RRID:AB_2792808	Rabbit	Immunofluorescence, cell-specific staining	1:1000
	Calretinin	Invitrogen, PA5-143562, RRID:AB_2942791	Chicken	Immunofluorescence, cell-specific staining	1:1000
	Choline acetyltransferase (ChAT)	EMD Millipore, AB144P, RRID:AB_2079751	Goat	Immunofluorescence, cell-specific staining	1:200
	DAPI	Millipore Sigma, D9542	-	Immunofluorescence, cell-specific staining	1:1000
	Flag (DDDDK)*	Abcam, ab1257, RRID:AB_299216	Goat	Immunofluorescence, αSyn 3D Atlas	4μg
	HuC/HuD	ThermoFisher Scientific, A-21271, RRID:AB_221448	Mouse	ENS immunofluorescence	1:100
	Iba1	Fujifilm Wako, 019-19741, RRID:AB_839504	Rabbit	Immunofluorescence, cell-specific staining	1:1000
	NeuN	Millipore Sigma, 3881957, RRID:AB_10807954	Rabbit	Immunofluorescence, αSyn <sup>NLS</sup> Atlas	1:500
		Cell Signaling Technologies, 12943S, RRID:AB_2630395	Rabbit	Immunofluorescence, cell-specific staining	1:1000
	Olig2	EnCor Biotechnology, MCA-1B7, RRID:AB_2572267	Mouse	Immunofluorescence, αSyn 3D Atlas	5μg
		R&D Systems, AF2418, RRID:AB_2157554	Goat	Immunofluorescence, cell-specific staining	1:200
	Pax2	R&D Systems, AF3364-SP, RRID:AB_10889828	Goat	Immunofluorescence, cell-specific staining	1:200
Sox9	Abcam, ab185966, RRID:AB_2728660	Rabbit	Immunofluorescence, cell-specific staining	1:1000	
Tuj1 (βIII-tubulin)	Sigma-Aldrich, T8660, RRID:AB_477590	Mouse	ENS immunofluorescence	1:500	
Tyrosine hydroxylase	Millipore, AB152, RRID:AB_390204	Rabbit	Immunofluorescence, cell-specific staining	1:1000	
Secondary	Donkey-anti-Goat Alexa Fluor 488	ThermoFisher Scientific, A32814, RRID:AB_2762838	Goat	Immunofluorescence	1:500
	Goat-anti-Mouse IgG (H+L) Alexa Fluor 488	Thermo Fisher Scientific, A-11001, RRID:AB_2534069	Mouse		
	Goat-anti-Mouse IgG2b Alexa Fluor 568	Thermo Fisher Scientific, A-21144, RRID:AB_2535780			
	Donkey-anti-Mouse Alexa Fluor 594	ThermoFisher Scientific, R37115, RRID:AB_2556543			
	Goat-anti-Rabbit IgG (H+L) Alexa Fluor 647	ThermoFisher Scientific, A-21244, RRID:AB_2535812	Rabbit		
	Goat-anti-Chicken IgY (H+L) Alexa Fluor 647	Thermo Fisher Scientific, A-21449, RRID:AB_2535866	Chicken		
	Goat-anti-Mouse IgG1 Alexa Fluor 488	Invitrogen, A21121, RRID:AB_2535764	Mouse		1:1000
	Goat-anti-Rabbit Alexa Fluor 546	Invitrogen, A11010, RRID:AB_2534077	Rabbit		
Staining Kits	VectaStain Elite ABC Peroxidase Kit	Vector Laboratories, PK-6102, RRID:AB_2336821	Mouse	Immunohistochemistry, αSyn DAB Atlas	-
	DAB Substrate Kit, Peroxidase HRP, with Nickel (3,3'-diaminobenzidine)	Vector Laboratories, SK-4100, RRID:AB_2336382	-	Immunohistochemistry, αSyn DAB Atlas	-

\*this antibody has since been discontinued