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Supplemental information

Sox6 expression distinguishes dorsally and ventrally biased dopamine neurons in the substantia nigra with distinctive properties and embryonic origins

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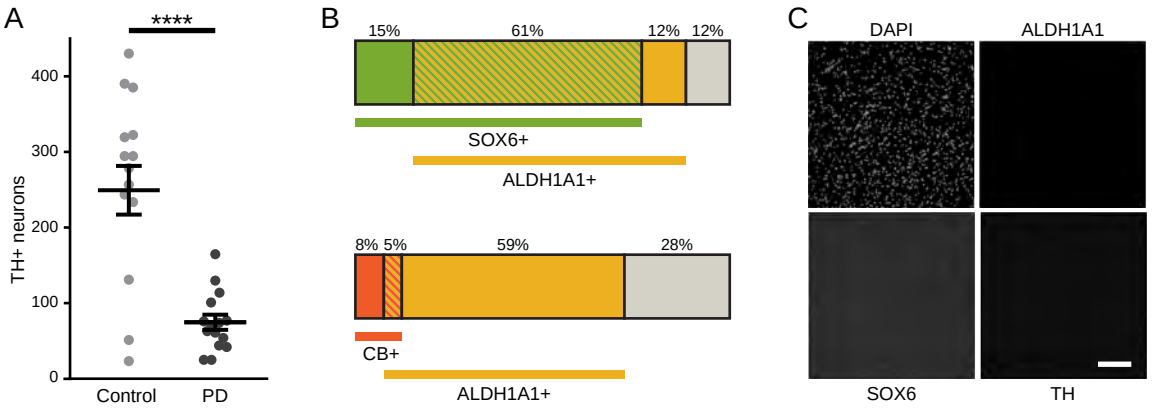

Fig. S1 (Related to Figure 1): Vulnerability and markers of DA neurons in hSNc.

A) Quantification of TH+ neurons in control and PD brains ($p = 1.89 \times 10^{-5}$, n=14 controls, n=15 PD). **B)** Percentage of SOX6+ and ALDH1A1+, and CALBINDIN-D28k+ (CB+) and ALDH1A1+ DA neurons in control brains. **C)** Primary antibody negative controls. Error bars are SEM. Scale bar: 200 µm.

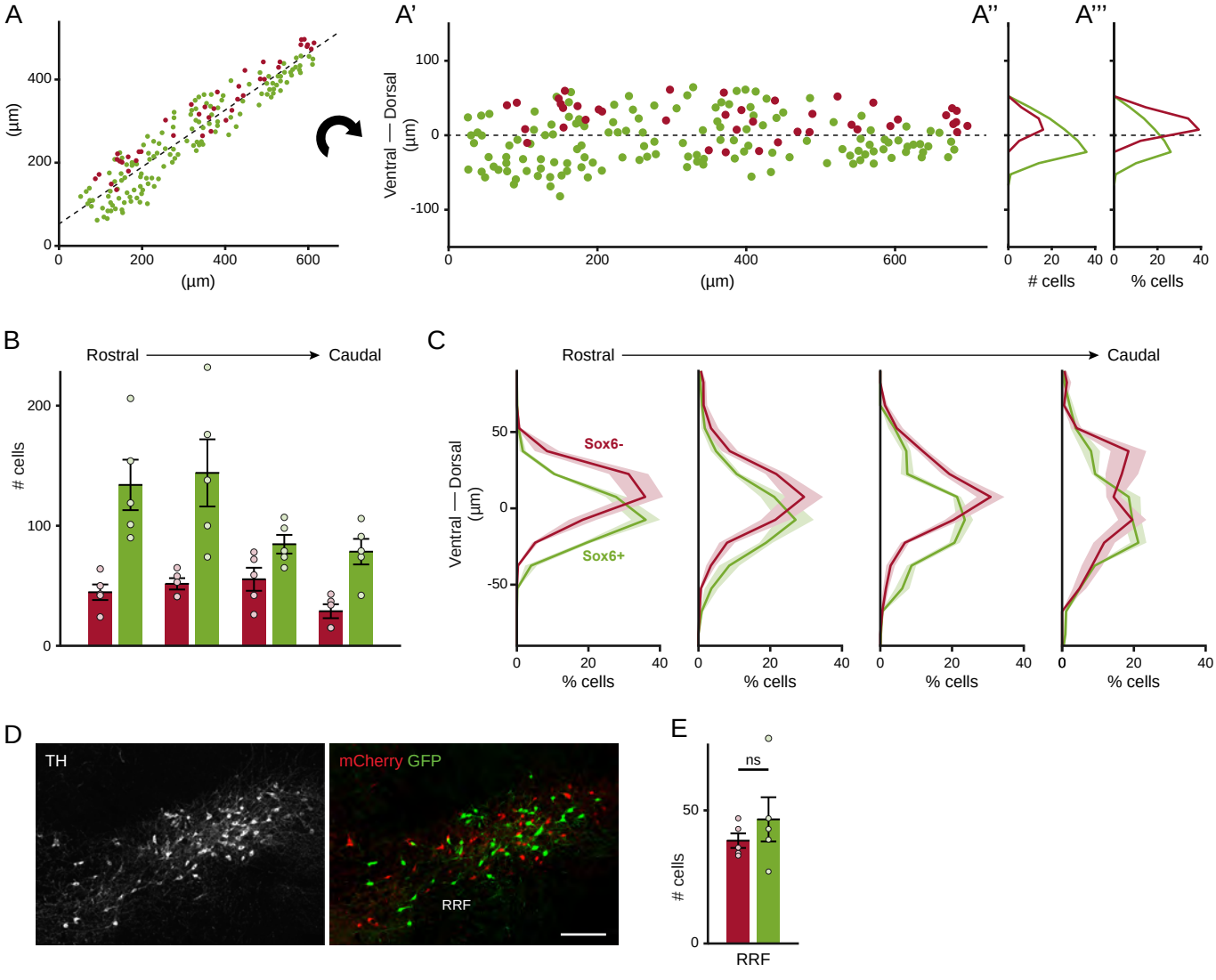

Figure S2 (Related to Figure 2): *Sox6*+ and *Sox6*- neurons distribution across the rostrocaudal axis.

A) Example of methodology used for obtaining dorso-ventral histograms in Fig. 2D,E and Fig. S2C. Coordinates are obtained for SNc Th+GFP+ and Th+mCherry+ cells. A line of best fit is obtained for all cells, which is used to set the center of the SNc. Cell coordinates are then rotated along this line (A'), and a histogram of cells is obtained, perpendicular to the center line (A''). For Fig 2E, S2C, cell counts are then normalized (A'''). **B)** Average number of GFP+ (green) and mCherry+ (red) neurons per SNc section level; GFP+ cells are more abundant than mCherry+ cells at all SNc section levels (n=5). **C)** Spatial distribution of GFP+ and mCherry+ neurons per SNc section level. mCherry+ neurons are biased dorsally at all rostro-caudal levels (n=5). **D)** In the RRF of Sox6-FSF-Cre, Th-2A-Flpo, RC-Frepe brains, GFP+ and mCherry+ cells are evenly distributed. **E)** Average numbers of GFP+ (green bars) and mCherry+ (red bars) cells in the RRF (n=5). Error bars (B, E) and shaded area (C) are SEM. Scale bar = 200 µm. RRF: retrorubral field.

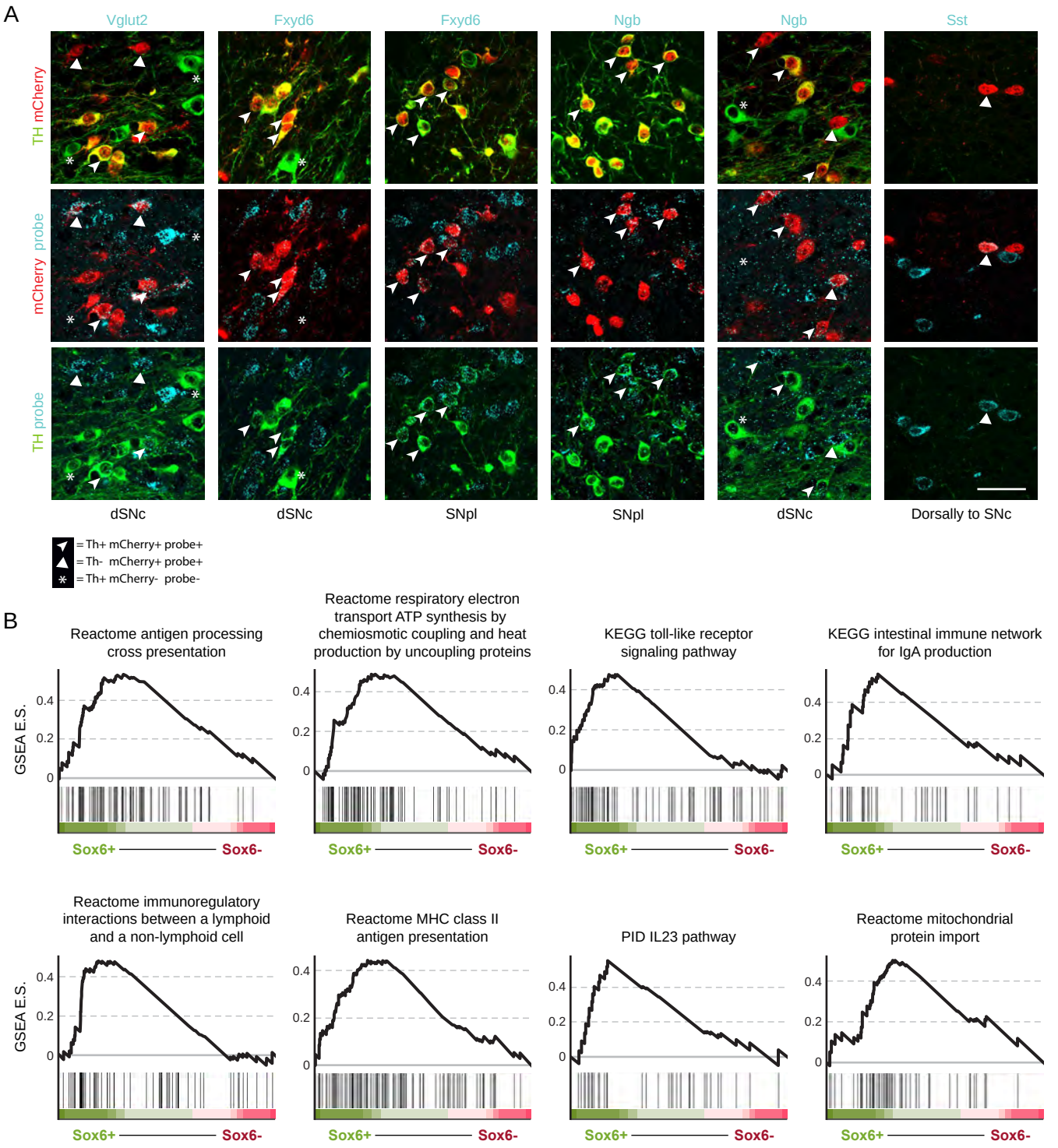

Fig. S3 (Related to Figure 3): Genes enriched in the *Sox6*- and *Sox6*+ population by in situ hybridization and GSEA, respectively.

A) RNAscope in situ hybridization of genes differentially expressed in mCherry+ neurons combined with immunofluorescence of TH and mCherry. *Vglut2* is expressed in mCherry+,TH+ (arrowheads) and mCherry+,TH- neurons (triangles), although we observed a basal nuclear expression in most cells (asterisk, fewer than 10 dots). In the SNpl and dorsal SNc (dSNc), *Fxyd6* and *Ngb* are expressed in mCherry+,TH+ neurons (arrowheads) and in mCherry+,TH- (triangles), but not in mCherry-TH+ neurons (asterisk). *Sst* was observed only in mCherry+,TH- neurons above the SNc. Scale bar is 50 µm.
B) GSEA Plots of significantly enriched gene sets in the *Sox6*+ population (q-value < 0.05). Plots are shown in increasing order of q. E.S.: Enrichment Score.

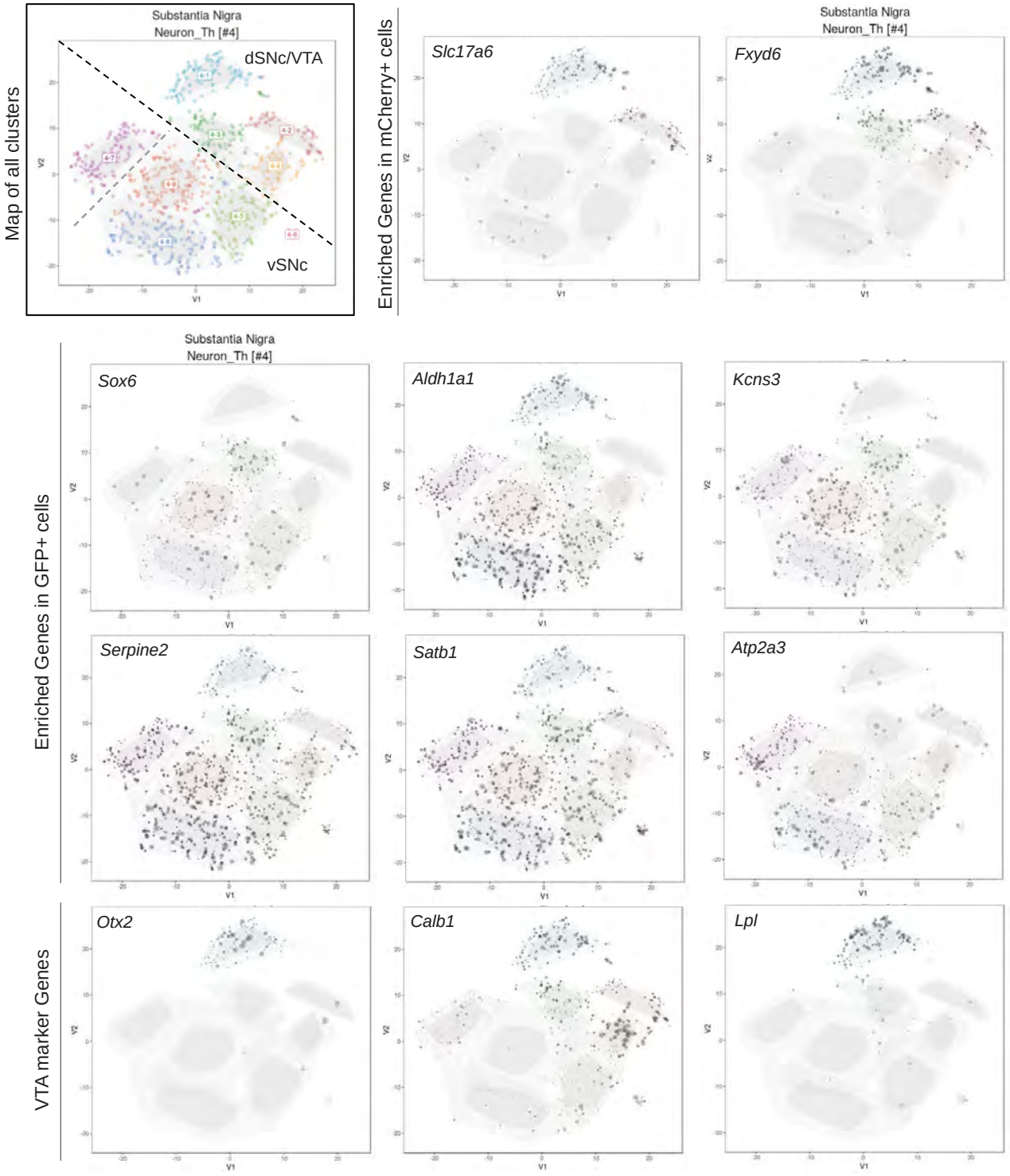

Fig. S4 (Related to Figure 3): DropViz plots of differentially expressed genes in Sox6+ and Sox6- SNc neurons, and VTA.

DropViz visualization of genes of interest within the preset "Substantia Nigra Neuron_Th #4" population. The reference panel on the top left indicates our proposed ventral SNc (vSNc) and dSNc/VTA clusters. vSNc clusters (4-5, 4-6, 4-8, 4-9) show expression of *Sox6, Aldh1a1, Kcns3, Satb1, Serpine2* and *Atp2a3*. dSNc/VTA clusters (4-1, 4-2, 4-3, 4-4) express *Slc17a6* (Vlgut2), *Fxyd6* and *Calb1* but only 4-1 shows *Otx2* and *Lpl*, VTA markers. Thus, 4-1 appears to represent the ventromedial VTA. Cluster 4-3 is *Calb1*+ and *Sox6*+, thus, it could represent parabrachial VTA since the latter includes *Sox6*+ neurons. 4-2 is *Calb1*+, *Vglut2*+, *Fxyd6*+, *Sox6*-, and thus likely represents the SNpl neurons. We were unable to disambiguate cluster 4-7.

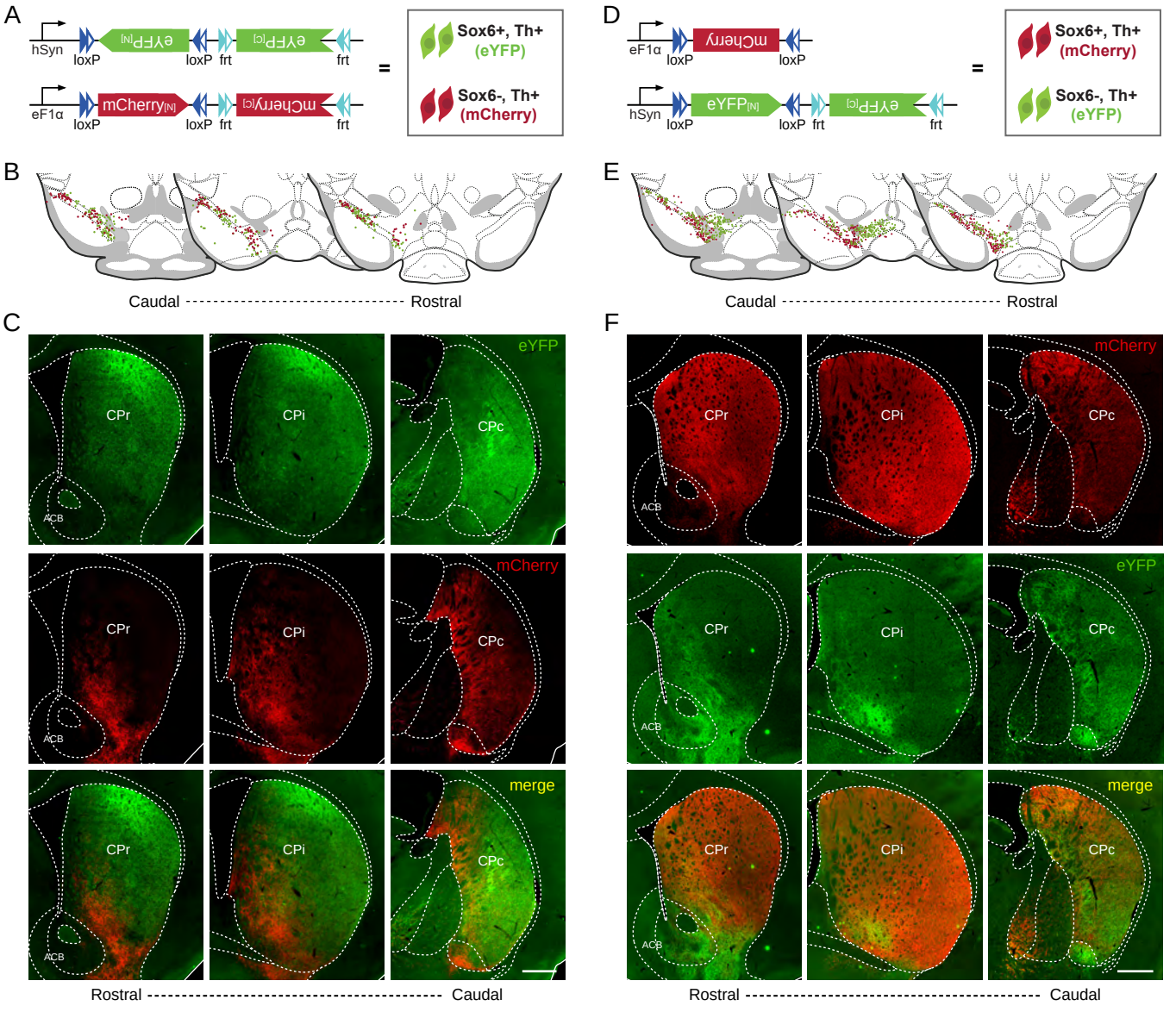

Fig. S5 (Related to Figure 4): Sox6+ and Sox6- projection patterns are similar when only SNc neurons are labelled, and when an alternative viral strategy is used.

A) Genetic strategy used to simultaneously label Sox6+ and Sox6- neurons and their projections in adult brains, as in Fig. 4. **B)** Representation of labeled cells in the midbrain corresponding to panel A. Each dot represents 1 labeled cell. Few cells in VTA and less medial SNc cells are labeled, compared to Fig. 4. **C)** Projections across the rostro-caudal axis of the striatum from Th+Sox6+ dSNc neurons (eYFP, top), from Th+Sox6- vSNc neurons (mCherry, middle), and merged images (bottom), from cells shown in Fig. 5SB. Scale bar 500 µm. **D)** Genetic strategy used to simultaneously label Sox6+ and Sox6- neurons and their projections in adult brains. The viral strategy is opposite to Fig. 4 and Fig. S5A-C, with Sox6+Th+ cells labeled by mCherry and Sox6-Th+ cells labeled by eYFP. **E)** Representation of labeled cells in the midbrain corresponding to panel D. Each dot represents 1 labeled cell. F) Projections across the rostro-caudal axis of the striatum from Th+Sox6+ dSNc neurons (mCherry, top), from Th+Sox6- vSNc neurons (eYFP, middle), and merged images (bottom), from cells shown in Fig. 5SE. Scale bar 500 µm.

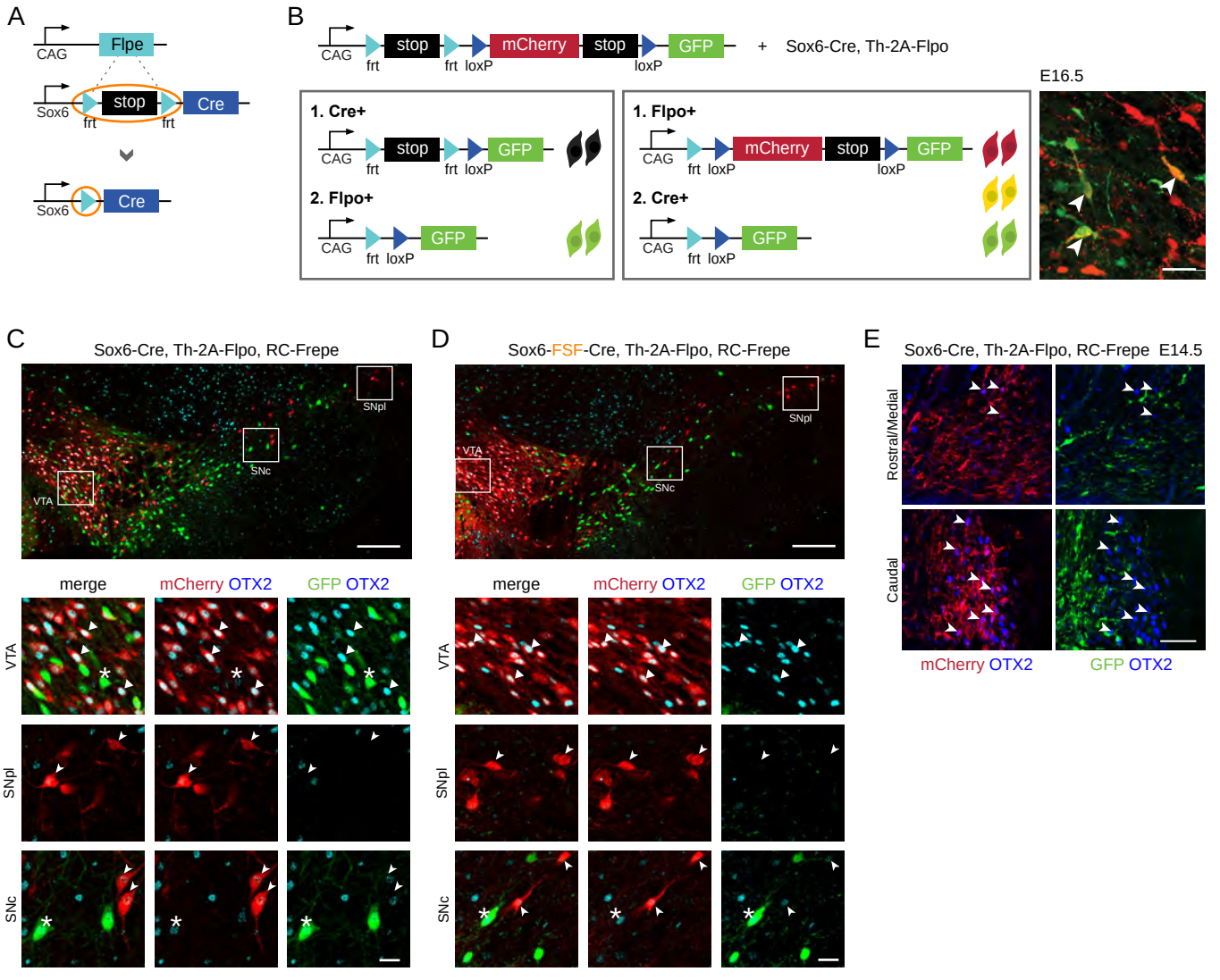

Figure S6 (Related to Figure 5): *Sox6* cumulative fate map characterization

A) When Sox6-FSF-Cre is crossed to CAG-Flpe, the frt-flanked stop cassette is deleted to obtain a Sox6-Cre line. **B)** Possible sequence of recombination events in Sox6-Cre, Th-2A-Flpo, RC-Frepe brains (all heterozygous). Left: Cell-labeling outcome if Cre expression precedes Flpo. 1. Cre expression alone does not induce fluorescence. 2. Upon subsequent Flpo expression, cells will express GFP. Middle: Cell-labeling outcome if Flpo expression precedes Cre. 1. Flpo expression induces mCherry. 2. Subsequent Cre expression in mCherry+ cells induces expression of GFP. In this scenario, cells express mCherry+ and GFP+ until mCherry is degraded and then cell expresses only GFP. Right: example of mCherry, GFP double-positive cells in E16.5 Sox6-Cre, Th-2A-Flpo, RC-Frepe brains (arrowheads, n=3). **C-D)** Comparison of OTX2 expression in Sox6-Cre vs Sox6-FSF-Cre with Th-2A-Flpo, RC-Frepe. Few GFP+OTX2+ are observed in the VTA of Sox6-FSF-Cre brains (D), some GFP+ cells have detectable OTX2 signal in Sox6-Cre brains (C, asterisks). In both fate maps, mCherry+ cells in the VTA express roubst OTX2 (triangles); mCherry+ in the dorsal SNc and SNpl have weak/residual OTX2 signal (arrowheads). In the SNc, GFP+ cells have weak/residual OTX2 signal (asterisk) (n=3, each). **E)** Sox6-Cre, Th-2A-Flpo, RC-Frepe at E14.5. Rostral-ly, there are few faint OTX2+ cells colocalizing mainly with mCherry (arrowheads). In caudal sections, there are visibly more and higher signal OTX2+ cells that co-express mCherry (arrowheads) (n=2). Scale bars: C, D: 200 µm (low magnification) and 25 µm (crops), B, E: 50 µm. VTA: ventral tegmental area, SNpl: substantia nigra pars lateralis, SNc: substantia nigra pars compacta.

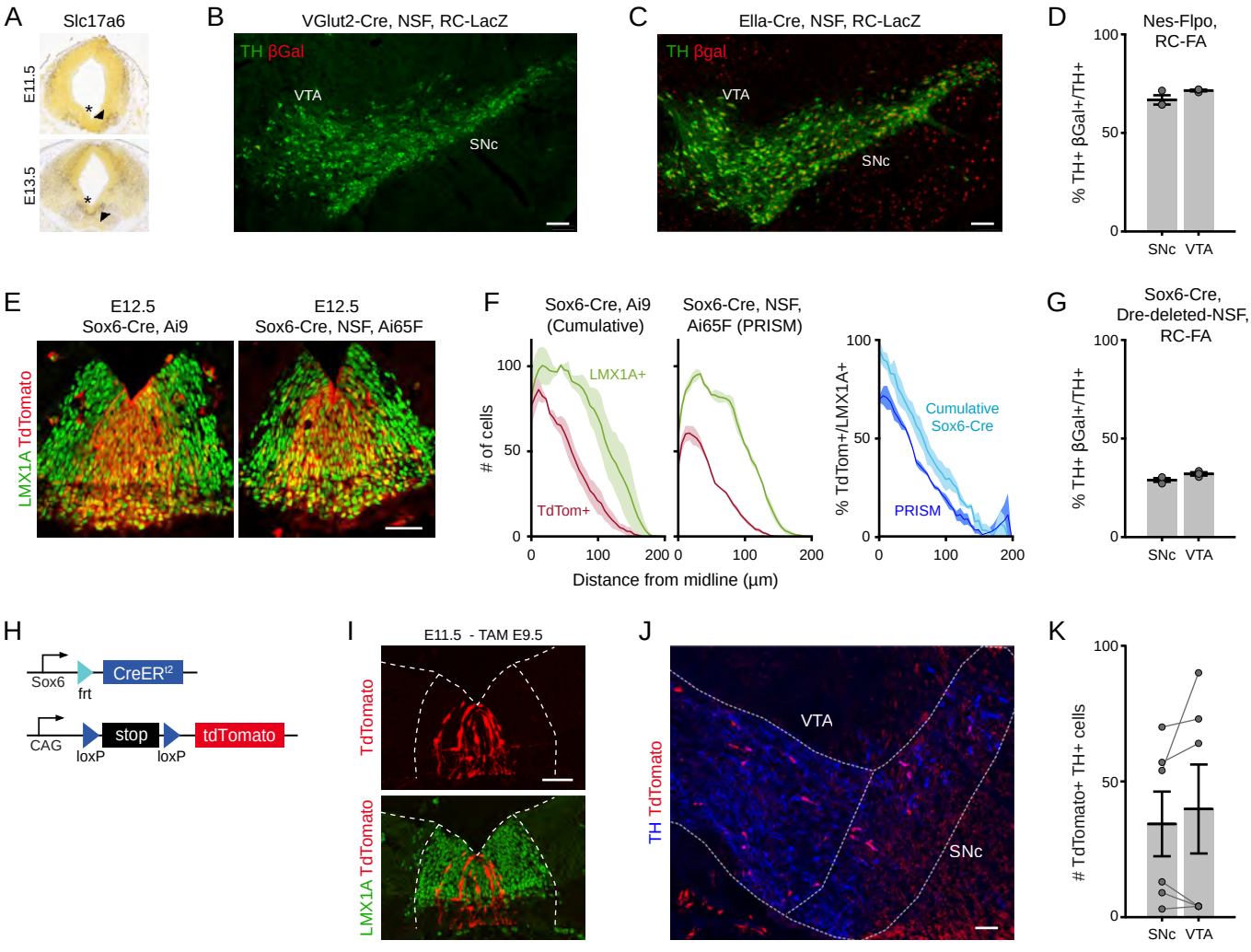

Fig. S7 (Related to Figure 6): PRISM efficiency and validation of results with a genetically induced fate map

A) Allen Atlas coronal sections of embryonic brains showing Slc17a6 (*Vglut2*) in nascent post-mitotic neurons (arrowheads) and lack thereof in the ventricular zone (asterisk). **B)** No βGal+ cells are observed in Vglut2-Cre, Nestin-LSL-Flpo (NSF), RC-LacZ adult brains, proving that PRISM does not label post-mitotic neurons (n=3). **C)** Immunolabeling for TH (green) and βGal (red) on EIIa-Cre, Nes-LSL-Flpo, RC-LacZ brains show βGal+ cells widely expressed in the SNc and VTA (n=3). **D)** Maximum recombination potential of PRISM determined in Nestin-Flpo (obtained by germline deletion of loxP-flanked STOP cassette), RC-LacZ brains (n=3). **E)** Sox6-Cre, Ai9 and Sox6-Cre, Nestin-LSL-Flpo, Ai65F embryonic floor plate at E12.5. *Sox6*+ progenitors (tdTomato+) are mainly medially located. **F)** Spatial distribution of *Sox6*+ cells in the embryonic floor plate (left) and percentage of LMX1A+ cells that express tdTomato+ in Sox6 vs PRISM fate maps (right). All tdTomato+ counted were also LMX1A+ (n=3). **G)** Quantification of TH+,βGal+/TH+ when the d4GFP cassette is germline deleted in Nestin-LSL-Flpo with a Dre-deleter (n=3). **H)** Genetically induced fate map breeding strategy. **I)** In TAM injected E9.5 → E11.5 harvested brains, tdTomato+ progenitors (*Sox6*+) are located medially in the *Lmx1a*+ floor plate (n=3). **J)** TAM injected E9.5→P0 harvested brains stained for TH and tdTomato (n=6). K) Average number of tdTomato+,TH+ cells in the SNc and VTA of Sox6-CreERT2, Ai9 P0 brains (TAM at E9.5). tdTomato+ cells (derived from Sox6+ progenitors) are observed in the SNc and VTA. Scale bars: B, C: 100 μm, all other scale bars 50 μm. Error bars (D, G, K) and shaded areas (F) are SEM. NSF: Nestin-LSL-Flpo, SNc: substantia nigra pars compacta, VTA: ventral tegmental area.

Control Cases				
Case	Age (yrs)	Sex	Cause of death	Post-mortem delay (hs)
1	60	F	Ovarian cancer	13
2	61	F	Ovarian cancer	15
3	67	F	Metastatic ovarian cancer	32
4	70	F	Sepsis, Stage IV peritoneal cancer, ischemic heart disease	45
5	73	F	Chronic obstructive pulmonary disease	30
6	74	F	Unknown	29
7	78	F	Myeloid leukeamia	33
8	83	F	Hyperglycemia	49
9	84	M	Bladder cancer, pneumonia	23
10	84	M	Cardiac asthenia amyloid	34
11	85	M	Unknown	12
12	89	F	Unknown	22
13	90	M	Respiratory failure secondary to bronchial cancer	12
14	91	F	Unknown	19

PD Cases						
Case	Age (yrs)	Sex	Cause of death	Post-mortem delay (hs)	Braak stage	Disease duration (yrs)
15	67	M	PD, MSA	53	VI	8
16	70	F	Ischemic heart disease, PD	34	VI	15
17	72	F	Renal cancer	17	VI	10
18	72	M	Unknown	46	VI	26
19	74	M	Unknown	52	VI	14
20	75	M	Bronchial pneumonia	26	VI	11
21	76	F	Unknown	19	VI	24
22	79	F	Congestive cardiac failure, PD	41	VI	10
23	79	M	Aspiration pneumonia secondary to advanced PD	8	V	13
24	82	F	PD, senile dementia	40	VI	14
25	82	M	Ischemic and degenerative heart disease, carcinoma liver	30	VI	11
26	83	F	Unknown	17	VI	19
27	86	F	End stage PD	48	VI	8
28	88	F	Ischemic heart disease	24	VI	21
29	92	F	Unknown	63	VI	19

Supplementary Table I (related to Figure 1): Human control and Parkinson's disease cases in this study.

Age in years, sex, cause of death/additional diagnosis, post-mortem time elapsed before tissue samples were collected (PMD), Braak stage of PD, and disease duration in years of 14 control and 15 PD patients.