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# Immunofluorescence and confocal imaging in brain sections and image analysis V.1

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

Single cell profiling studies and research on both postmortem PD tissues and preclinical mouse models demonstrated that the Sox6/Aldh1a1 markers molecularly define the vulnerable ventral dopamine neurons, while Calbindin1 (Calb1) marks the resilient dorsal neurons. These distinct dopamine neuronal subtypes not only differ in their molecular profiling but also exhibit unique projection patterns and functional responses. Therefore, studying these neurons in a subtype-specific manner can yield important insights into cell-intrinsic mechanisms of vulnerability in PD. We first addressed whether LRRK2 is expressed in dopamine neurons, as this is a prerequisite for any cell-autonomous effects. We performed immunolabeling on mouse brain sections and confirmed LRRK2 expression in SNc dopamine neurons. We also employed confocal imaging and 3D reconstructions to assess Th+ fibers in the dorsolateral striatum.

## Safety warnings

These protocols need prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee

## Immunofluorescence

1 Mice were perfused with 50 ml of PBS, followed by 50 ml of 4% paraformaldehyde in PBS. The brains were dehydrated using 30% sucrose in PBS for 48 hours and then sectioned coronally at 30 µm thickness with a cryostat (Leica Biosystems, CM305). The slices were collected in PBS containing 0.1% sodium azide and stored at 4°C for immunohistochemistry.

2 Sections were incubated in 5% goat serum with 0.2% Triton X-100 for 2 hours.

3 Antigen retrieval was performed by incubating the sections in antigen unmasking solution (Vector, H-3300) at 100°C for 30 minutes

4 cooling to room temperature for another 30 minutes

5 wash with PBS for 5 mins

6 sections were incubated overnight at 4°C in 5% goat serum with 0.2% Triton X-100

7 incubated for an additional 48 to 72 hours at 4°C with primary antibodies in 5% goat serum with 0.2% Triton X-100

7.1 ***Lrrk2 signal in SNc sections:*** anti-Th (1:1000, SYSY), anti-Aldh1a1 (1:300, Abcam), anti-LRRK2/Dardarin clone N241A/34 (1:200, NeuroMab), anti-GFP (1:1000, invitrogen).

7.2 ***Striatal Th expressing fibers :***: anti-Th (1:1000, SYSY), anti-Aldh1a1 (1:300, Abcam), and anti-Vmat2 (1:200, Immunostar).

8 washing with PBS for 5 mins

9 sections were incubated with secondary antibodies—Alexa Fluor™ 488, Alexa Fluor™ 568, and Alexa Fluor™ 647 (1:300, invitrogen) in 5% goat serum with 0.2% Triton X-100 for 3hrs RT

10 all slices were washed with PBS and mounted using ProLong™ Diamond Antifade Mountant (invitrogen)

## Confocal imaging

11 Confocal images of the fixed 30 µm thick striatal and midbrain sections were obtained using the Nikon A1R microscope

12 Fluorescence images were captured with a 20x objective for Lrrk2 expression, Th area, intensity (Supplementary Figure 6) and APEX2 expression, and 100x objective for Th fluorescence at a resolution of 1,024 × 1,024 pixels, maintaining constant laser power for each channel across genotypes.
Images for volume measurements of Th fibers were captured with Nikon CSU-W1 SoRa with a 60x objective at 0.1 mm intervals at 1,024 × 1,024 pixel resolution

## Image analysis: The protein signals for Th, Aldh1a1, and Lrrk2 were measured using Imaris 10.1 software (Bitplane, Concord, USA).

13 ***Lrrk2 signal in SNc sections***

13.1 The surface rendering function was used to segment Th/Aldh1a1 cells

13.2 Background subtraction was enabled; the diameter for the largest sphere was set to 10 µm and automatically thresholded, with a smoothing surface set to 3 µm

13.3 Mean intensity of Aldh1a1 protein within the Th surface above 1.5 times the average Th protein channel mean intensity was considered positive

13.4 The Lrrk2 intensity within Th-positive cells was measured automatically by the software

14 ***Striatal Th expressing fibers analysis:***

14.1 Volume measurements of Th fibers
Surface rendering function was used to segment the Th fiber. Background subtraction was enabled, the diameter of the largest sphere was set at 0.805 µm and threshold set at 2000.

14.2 Segments were filtered with “Number of voxels Img=1” set above 50. Th volume was measured automatically by software.

14.3 Intensity and area of Th expressing axons.
Rolling ball background subtraction was performed using Nikon Elements software.

14.4 For the high magnification analysis
The surface rendering function was used to segment TH, Aldh1a1, and Vmat2 proteins. Background subtraction was disabled and automated thresholds were applied with manual adjustments made as needed.

14.5 :
Segments were filtered with “Number of voxels Img=1” set above 50.

14.6 For the Aldh1a1 positive Th axon, filtering was applied based on the “overlapped area ratio to surface surface=TH-aldh1a1” above 0.4.

14.7 Vmat2 within Th axonal terminal was filtered with “overlapped area ratio to surface surface= Th-VMAT2” above 0.4.

14.8 Area and intensity were measured automatically by software. For the low magnification analysis

14.9 For the low magnification analysis
The surface rendering function was used to segment TH. Background subtraction was disabled and the threshold was set to 1500 with manual adjustments made if needed

14.10 Segments were filtered with “Number of voxels Img=1” set above 50. Th area and intensity were measured automatically by software.