

Supplementary Materials for

Restoration of striatal neuroprotective pathways by kinase inhibitor treatment of Parkinson's disease-linked *LRRK2*-mutant mice

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The PDF file includes:

Figs. S1 to S5
Table S1

Other Supplementary Material for this manuscript includes the following:

MDAR Reproducibility Checklist

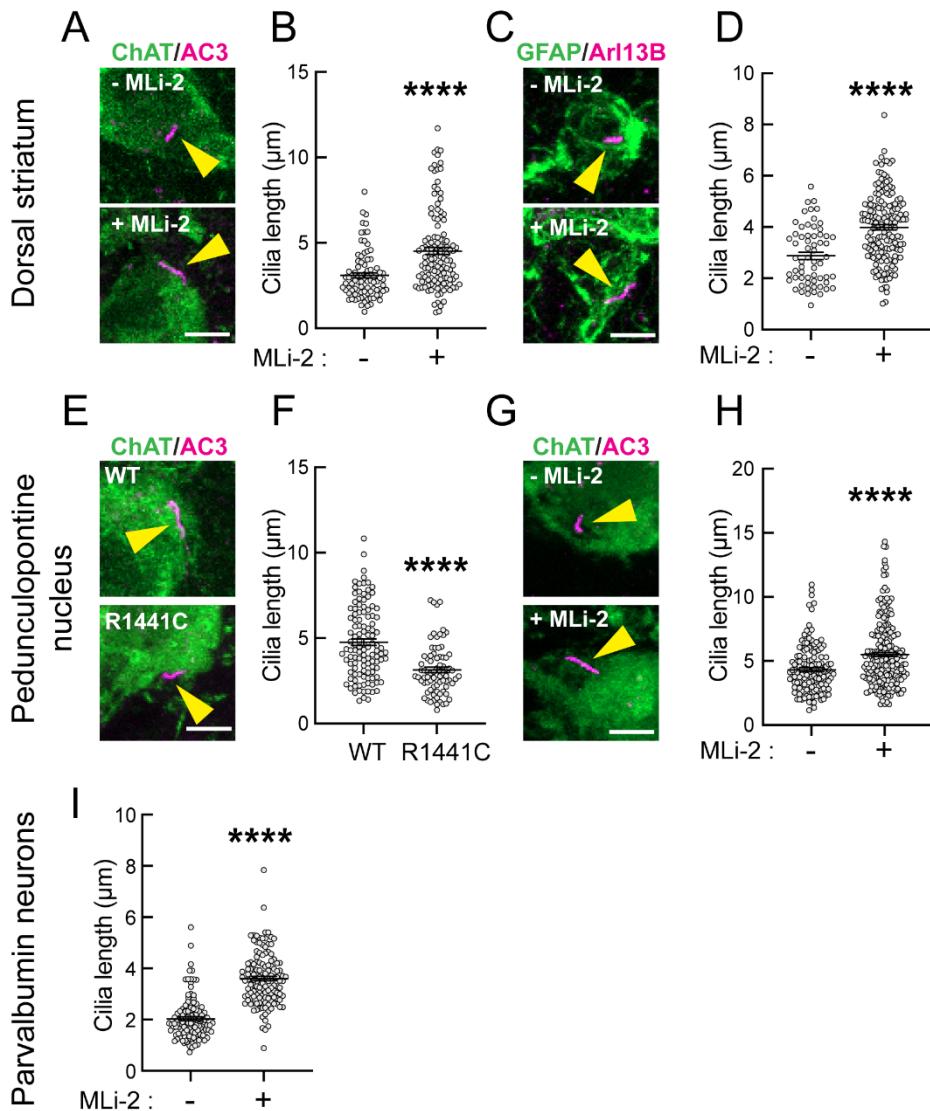


Figure S1: Examples and data for cilia length determinations. **(A)** Confocal images of dorsal striatum sections from R1441C *LRRK2* mice fed \pm MLi-2-containing chow for 3 months, representative of 6 mice in each group. Cholinergic interneurons were stained for choline acetyltransferase; cilia were stained for adenylate cyclase 3 -. Yellow arrowheads point to cilia. **(B)** Summary of cilia length from ≥ 85 ChAT⁺ interneurons from 6, R1441C *LRRK2* mice fed \pm MLi-2 chow. Each dot represents the length of one cilium. **(C)** Confocal images of striatal astrocytes from R1441C *LRRK2* mice fed \pm MLi-2 chow for 3 months. Astrocytes were immunostained for GFAP; their cilia were labeled with Arl13B. **(D)** Summary of cilia length from >60 astrocytes from 6 R1441C *LRRK2* mice fed \pm MLi-2 chow. **(E)** Confocal images of sections of pedunculopontine nucleus (PPN) from 4-month-old WT and R1441C *LRRK2* mice. Cholinergic neurons and cilia were labeled as in (A). **(F)** Summary of cilia length from >75 cholinergic neurons from 4 WT and 4 R1441C *LRRK2* animals. **(G)** Confocal images of sections of pedunculopontine nucleus (PPN) from 6, R1441C *LRRK2* mice fed \pm MLi-2 for 3 months labeled as in (A). Images in A, C, and G are representative of 6 mice in each group; images in E are representative of 4 mice. **(H)** Summary of cilia length from >140 cholinergic neurons from 6 R1441C *LRRK2* mice fed \pm MLi-2 diet. **(I)** Summary of cilia length from >120 parvalbumin interneurons from 6 R1441C *LRRK2* mice fed \pm MLi-2. Statistical significance was determined using unpaired t-test. **** $p < 0.0001$. Scale bars, 5 μm .

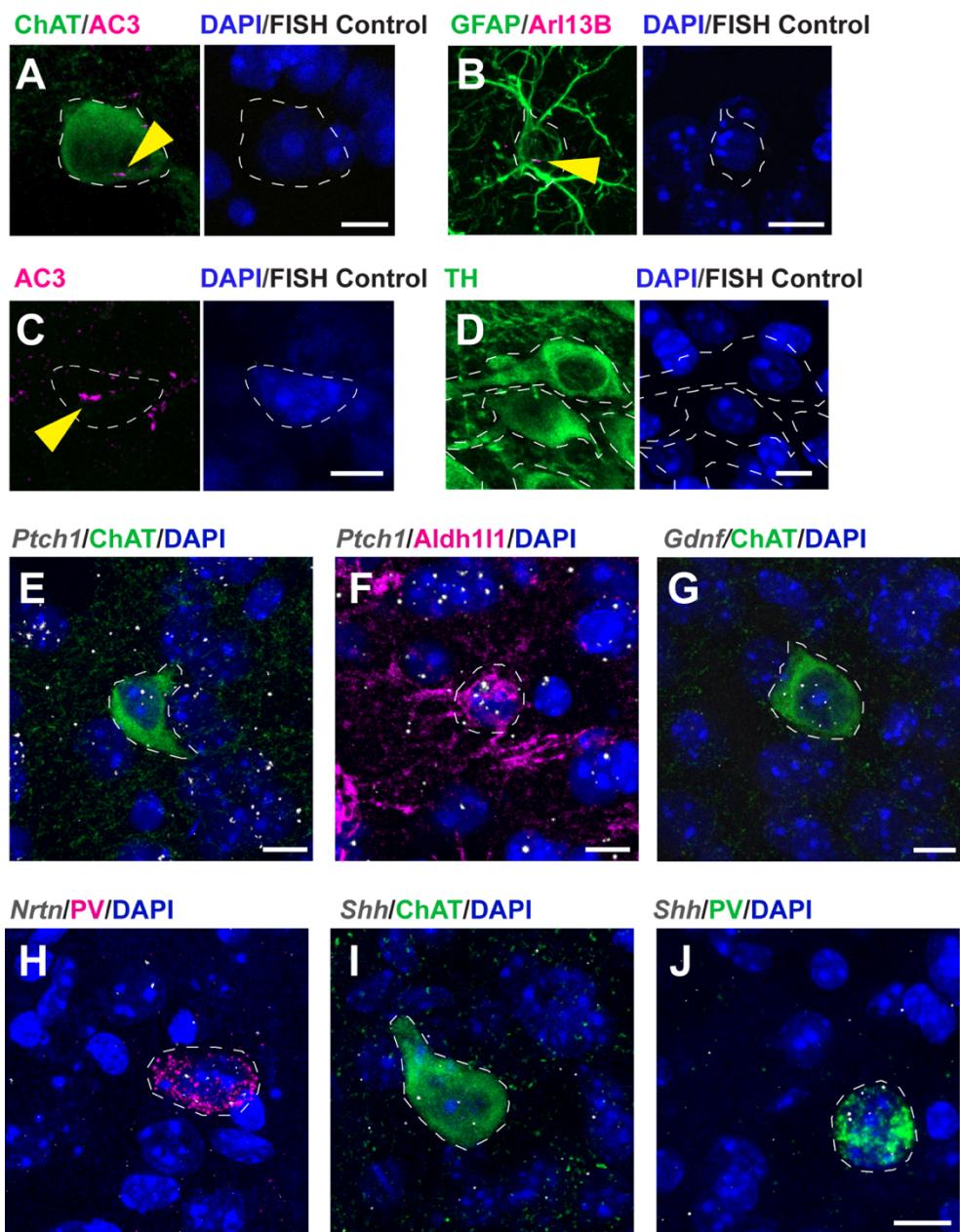


Figure S2: Control RNAscope reactions. (A to D) Representative images of the RNAscope for the indicated markers alongside images of the controls in (A) cholinergic interneurons, (B) astrocytes, (C) parvalbumin interneurons, and (D) nigral dopaminergic neurons in wild-type mice. Cells and their cilia were labeled as in Figures 1, 6 and 8. (E and F) RNAscope analysis of *Ptch1* RNA (white dots) in striatal (E) cholinergic interneurons or (F) astrocytes labeled using an antibody specific for *Aldh1l1*. (G) RNAscope analysis of *Gdnf* RNA (white dots) in striatal cholinergic interneurons. (H) RNAscope analysis of *Nrtn* RNA (white dots) in striatal parvalbumin interneurons. (I and J) RNAscope analysis of *Shh* RNA (white dots) in (I) striatal cholinergic and (J) parvalbumin interneurons. Images in (A to J) are representative of 20 cells from at least 5 wild-type mice per group. Scale bars, 10 μ m. Dashed lines indicate cell soma.

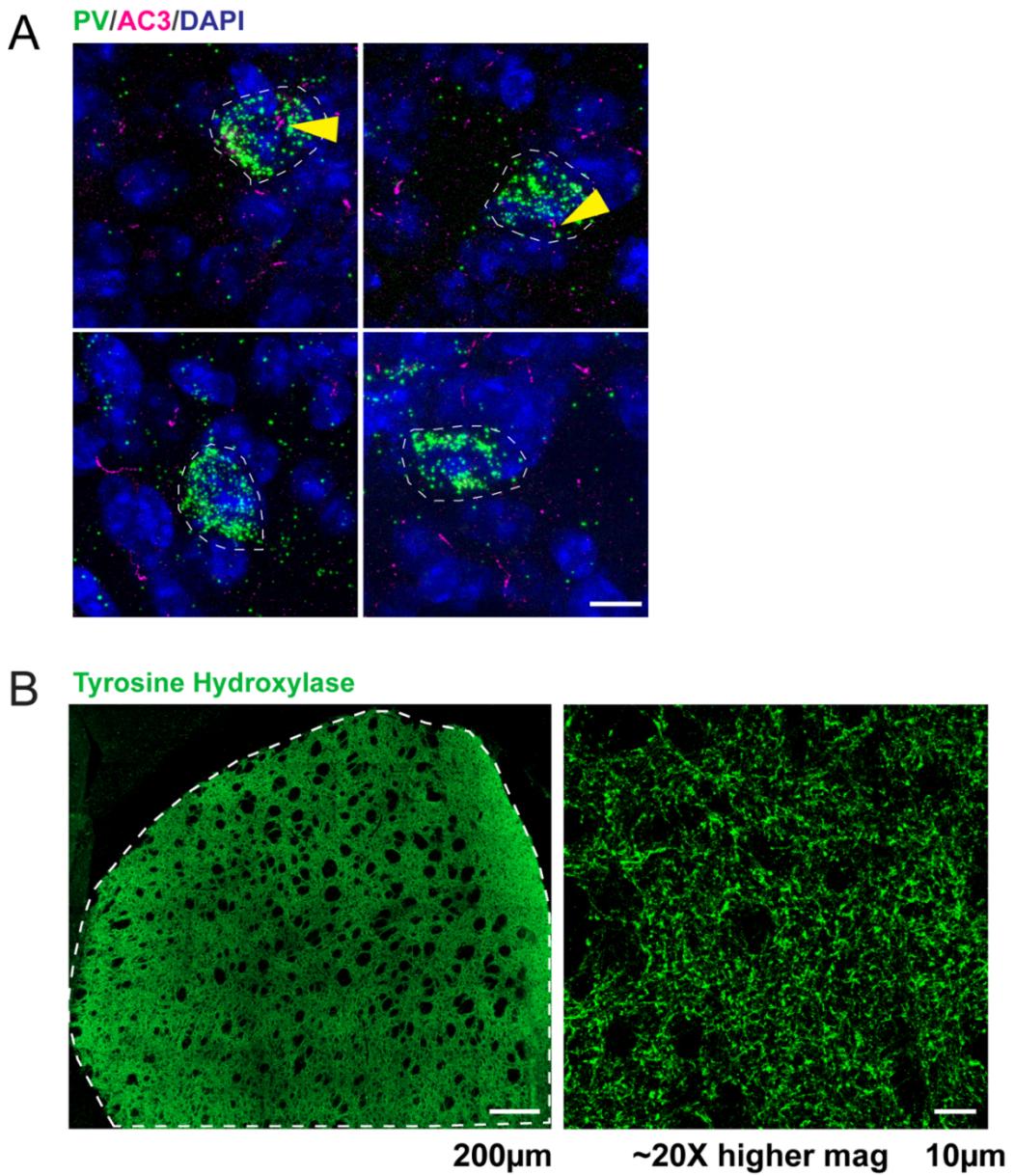


Figure S3: Specificity of a chicken antibody raised against AC3 for cilia identification, and examples of fine dopamine processes in the striatum. (A) Example images of ciliated (top) or unciliated (bottom) parvalbumin interneurons (green) and cilia (magenta) labeled using chicken anti-AC3 as in Fig 6 wild-type mice. Special care was taken to scan through a series of Z stacks to ensure that the linear cilium (and not pink dots) was associated with a specific cell body and emanated from near the nucleus of every cell scored. Scale bar, 10 μ m. Small pink dots are background staining. The rabbit anti-AC3 antibody was used in all other figures. (B) Comparison of striatal tyrosine hydroxylase staining at low and high magnification to assess fine processes, quantified in Figure 7. Scale/magnification bar values are indicated beneath the images.

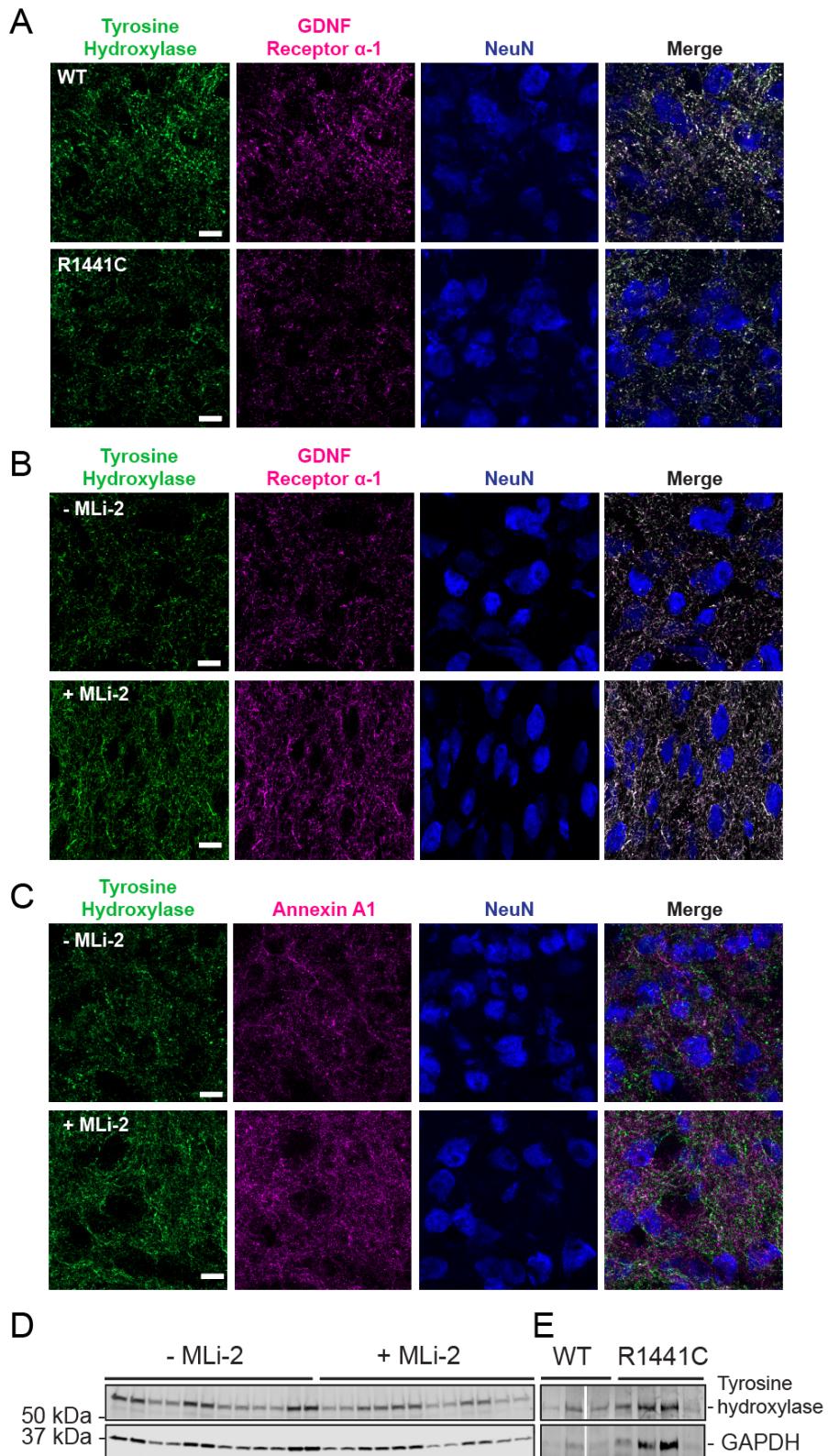


Figure S4: Example images showing tyrosine hydroxylase, GDNF receptor alpha-1, and NeuN staining in dorsal striatal sections. (A) Sections from 5-month-old WT and R1441C *LRRK2* mice, labeled as indicated. Note that the nuclei here are not from TH⁺ neurons as those nuclei are in the Substantia

nigra. **(B)** Examples from R1441C *LRRK2* mice (10 weeks old) fed \pm MLi-2 for 3 months labeled as in (A). **(C)** Example confocal images showing tyrosine hydroxylase, Annexin A1, and NeuN staining as indicated in dorsolateral striatal sections of R1441C *LRRK2* mice (10 weeks old) fed \pm MLi-2 for 3 months. **(D)** Immunoblot of SDS-solubilized 25 μ m sections (2 per lane) of dorsolateral striatum surgically dissected from fixed mouse brain cryosections of R1441C *LRRK2* mice (10 weeks old \pm MLi-2 for 3 months as indicated. Each pair of lanes represents a single animal, with two solubilized sections per lane analyzed in duplicate. Analysis is presented in Figure 7D. **(E)** Immunoblot analysis of 3 WT and 4 R1441C *LRRK2* mice (3-month-old). Each lane represents a different animal. Analysis is presented in Figure 7J. Scale bars (A to C), 10 μ m.

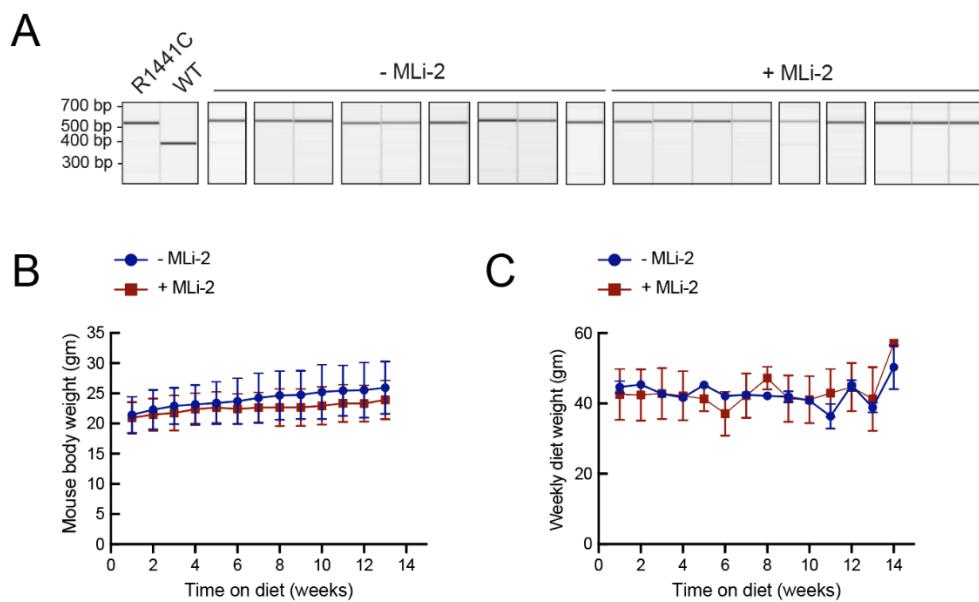


Figure S5: Mouse genotyping, average body weights, and average diet consumption of R1441C *LRRK2* mice fed an MLi-2 containing diet or control chow. (A) PCR genotyping of wild type and R1441C *LRRK2* homozygous mice. Primers were: CTGCAGGCTACTAGATGGTCAAGGT (forward primer) and CTAGATAGGACCGAGTGTCGCAGAG (reverse primer) as per Jackson Labs. A 520 bp band is expected for homozygous knock-in; a 386 bp band is expected for wild type. R1441C *LRRK2* mice were fed an MLi-2 containing diet or control chow as indicated. Each lane represents the PCR product from a different animal. **(B and C)** Average body weights (B) and average diet consumption (C) of R1441C *LRRK2* mice fed \pm MLi-2. Values represent mean \pm SEM from 6 *LRRK2* mice fed \pm MLi-2 as indicated.

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Genetic reagent (Mus musculus)	B6.Cg-Lrrk2tm1.1Shn/J	Jackson Laboratory	#009346, RRID:IMSR_JAX:009346	C57BL/6J; R1441C KI
Genetic reagent (Mus musculus)	Constitutive KI Lrrk2tm4.1Arte	Taconic	#13940, RRID:IMSR_TAC:13940	C57BL/6J; G2019S
Chemical Compound, drug	MLi-2	MRC PPU Reagents and Services, U. Dundee	CAS No. : 1627091-47-7	
Rodent diet	Control diet	Research Diets, Inc. New Brunswick, NJ	Research Diets D01060501	
MLi-2 modified rodent diet	Diet containing MLi-2 at 360 mg/Kg.	Research Diets, Inc. New Brunswick, NJ	Research Diets D01060501	
Antibody	anti-Choline Acetyltransferase (goat polyclonal)	Millipore	AB144P-1ML (RRID:AB_2079751)	(1:200)
Antibody	anti-Adenylate cyclase III (rabbit polyclonal)	EnCOR	RPCA-ACIII (RRID:AB_2572219)	(1:10000)
Antibody	anti-GFAP (chicken polyclonal)	EnCOR	CPCA-GFAP (RRID:AB_2109953)	(1:2000)
Antibody	anti-Arl13B (mouse monoclonal)	Neuromab	N295B/66 (RRID:AB_2877361)	(1:500)
Antibody	anti-Adenylate cyclase III (chicken polyclonal)	EnCOR	CPCA-ACIII (RRID:AB_2744500)	(1:5000)
Antibody	anti-GFR alpha-1/GDNF R alpha-1 (goat polyclonal)	R&D Systems	AF560 (RRID:AB_2110307)	(1:500)
Antibody	anti-tyrosine hydroxylase (sheep polyclonal)	Novus Biologicals	NB300-110 (RRID:AB_10002491)	(1:500)
Antibody	anti-NeuN(chicken polyclonal)	Millipore	ABN91 (RRID:AB_11205760)	(1:1000)
Antibody	H+L Donkey anti-goat Alexa 488	Life Technologies	A11055 (RRID:AB_2534102)	(1:2000)
Antibody	H+L Donkey anti-Rabbit Alexa 568	Life Technologies	A10042 (RRID:AB_2534017)	(1:2000)
Antibody	H+L Donkey anti-chicken Alexa 488	Jackson ImmunoResearch Laboratories	#703-545-155 (RRID:AB_2340375)	(1:2000)
Antibody	H+L Donkey anti-mouse 568	Life Technologies	A10037 (RRID:AB_11180865)	(1:2000)
Antibody	H+L Donkey anti-sheep Alexa 488	Life Technologies	A-11015 (RRID:AB_2534082)	(1:2000)
Antibody	H+L Donkey anti-goat Alexa 568	Life Technologies	A-11057 (RRID:AB_2534104)	(1:2000)
Antibody	H+L Donkey anti-chicken Alexa 647	Jackson ImmunoResearch Laboratories	#703-605-155 (RRID:AB_2340379)	(1:2000)
Antibody	LRRK2 (C-terminus)	Antibodies Incorporated/NeuroM ab	75-253 (RRID:AB_10675136)	(1:1000)
Antibody	LRRK2 pSer935	MRC PPU Reagents and Services, U. Dundee	UDD2 10(12) (RRID:AB_2921228)	(1 µg/ml)
Antibody	Rab10 pThr ⁷³	Abcam Inc.	ab230261 (RRID:AB_2811274)	(1:1000)

Antibody	Rab10	Nanotools	0680–100/Rab10-605B11 (RRID:AB_2921226)	(1:500)
Antibody	Rab12 pSer ¹⁰⁶	Abcam Inc.	ab256487 (RRID:AB_2884880)	(1:1000)
Antibody	Anti-Annexin A1	Abcam	ab214486 (RRID:AB_2890907)	(1:1000)
Antibody	Anti-tyrosine hydroxylase (sheep polyclonal)	Millipore Sigma	AB1542 (RRID:AB_90755)	(1:1000)
Antibody	Anti-GAPDH (mouse monoclonal)	Santa Cruz Biotechnology	sc-32233 (RRID:AB_627679)	(1:2000)
Antibody	DyLight™ 800 rabbit anti- sheep IgG (H+L)	Invitrogen	SA5-10060 (RRID:AB_2556640)	(1:10,000)
Antibody	IRDye 680RD Donkey anti- Mouse IgG	LI-COR	926-68072 (RRID:AB_10953628)	(1:10,000)
Antibody	Rab12	ABclonal	A26172 (RRID AB_3166186)	(1:20,000)
Antibody	IRDye 800CW Donkey anti- Rabbit IgG	LI-COR	926-32213 (RRID:AB_621848)	(1:20,000)
Antibody	IRDye 680LT Donkey anti- Mouse IgG	LI-COR	926-68 022 (RRID:AB_10715072)	(1:20,000)
Commercial assay or kit	RNAscopeMultiplexFluoresce ntReagent Kit v2	Advanced Cell Diagnostics	#323100	
Commercial assay or kit	RNAscope Probe- Mm-Ptch1- C2	Advanced Cell Diagnostics	#402811-C2	(1:5)
Commercial assay or kit	RNAscope Probe- Mm-Gdnf	Advanced Cell Diagnostics	#421951	(1:20)
Commercial assay or kit	RNAscope Probe- Mm-Nrtn- C2	Advanced Cell Diagnostics	#441501-C2	(1:3)
Commercial assay or kit	RNAscope Probe- Mm-Pvalb- C3	Advanced Cell Diagnostics	#421921-C3	(1:10)
Commercial assay or kit	RNAscope Probe- Mm-Shh- C2	Advanced Cell Diagnostics	#314361-C2	
Commercial assay or kit	OPAL 690 REAGENT PACK	Akoya Biosciences	FP1497001KT	
Commercial assay or kit	OPAL 570 REAGENT PACK	Akoya Biosciences	FP1488001KT	
Software, Algorithm	FIJI	PMID:29187165	RRID:SCR_002285	
Software, Algorithm	CellProfiler version 4.2.6	PMID:29969450	RRID:SCR_007358	
Software, Algorithm	Graphpad Prism	Prism 10 version 10.2.3	RRID:SCR_002798	
Software, Algorithm	ZEN	Zeiss ZEN Microscopy Software	RRID:SCR_013672 www.zeiss.com/microscopy/ en/products/software/zeiss-zen.html	
Software, Algorithm	Image Studio Lite (version 5.2.5)		RRID:SCR_013715)	

Table S1: Key resources. A list of the key resources, their sources and catalog numbers, as well as additional information where applicable (mouse strain, antibody dilutions) are provided in the table.