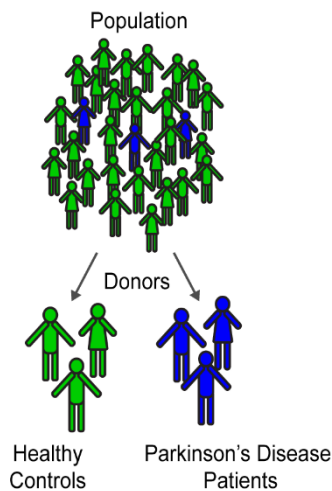


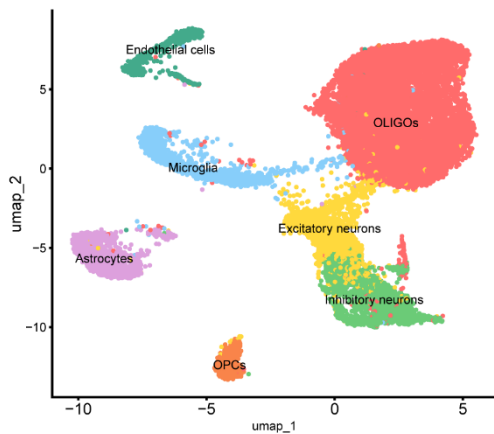
A



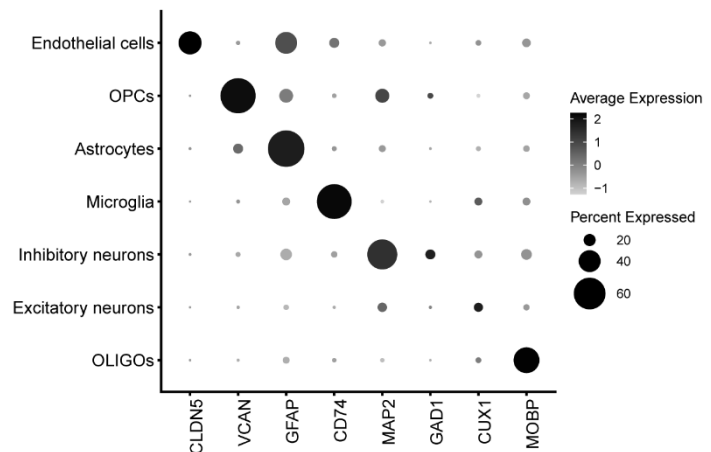
Healthy Individuals	Lab Identifier	Age of sampling	Age of onset	Sex	References
H1	200	81	-	F	Reinhardt et al., 2013
H1/G2019S	207	81	-	F	Reinhardt et al., 2013
H2	202	55-59	-	M	Qing et al., 2017
H2/G2019S	208	55-59	-	M	Qing et al., 2017
H3	201	-	-	F	GIBCO
H3/G2019S	264	-	-	F	GIBCO

Patients (PD-G2019S)	Lab Identifier	Age of sampling	Age of onset	Sex	References
PD1	209	78	70	F	Reinhardt et al., 2013
PD1/gc	212	78	70	F	Reinhardt et al., 2013
PD2	228	51	40	F	Reinhardt et al., 2013
PD2/gc	229	51	40	F	Reinhardt et al., 2013

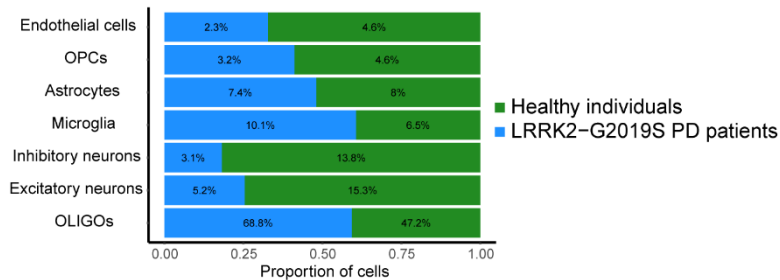
B



C

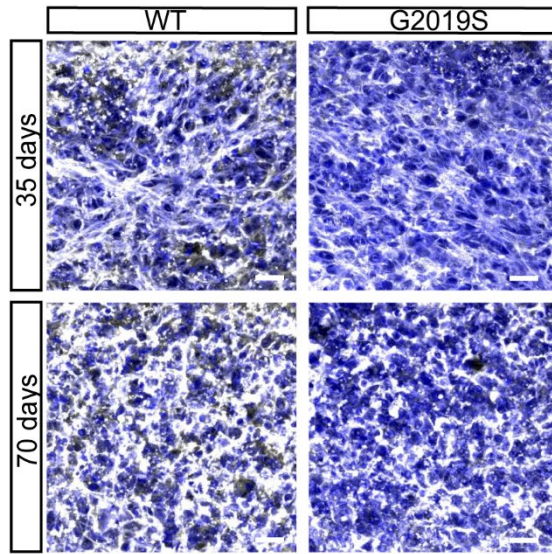
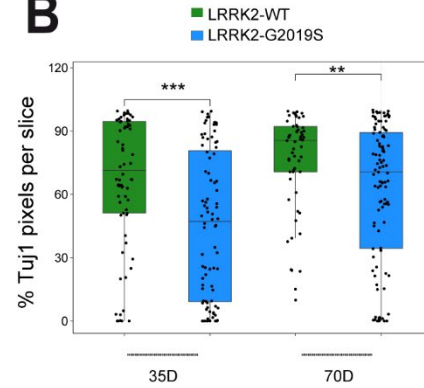
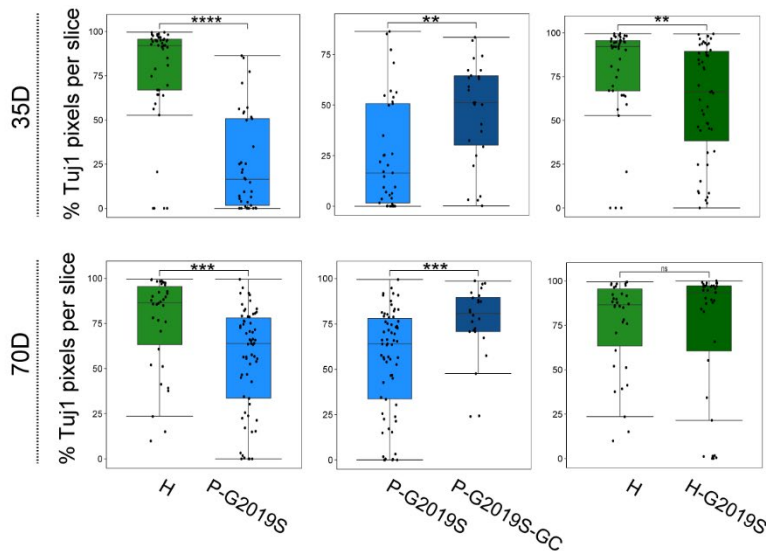


D



Supplementary Figure 1. Lines used in the experimental setup and snRNA sequencing in midbrain autaptic brain samples

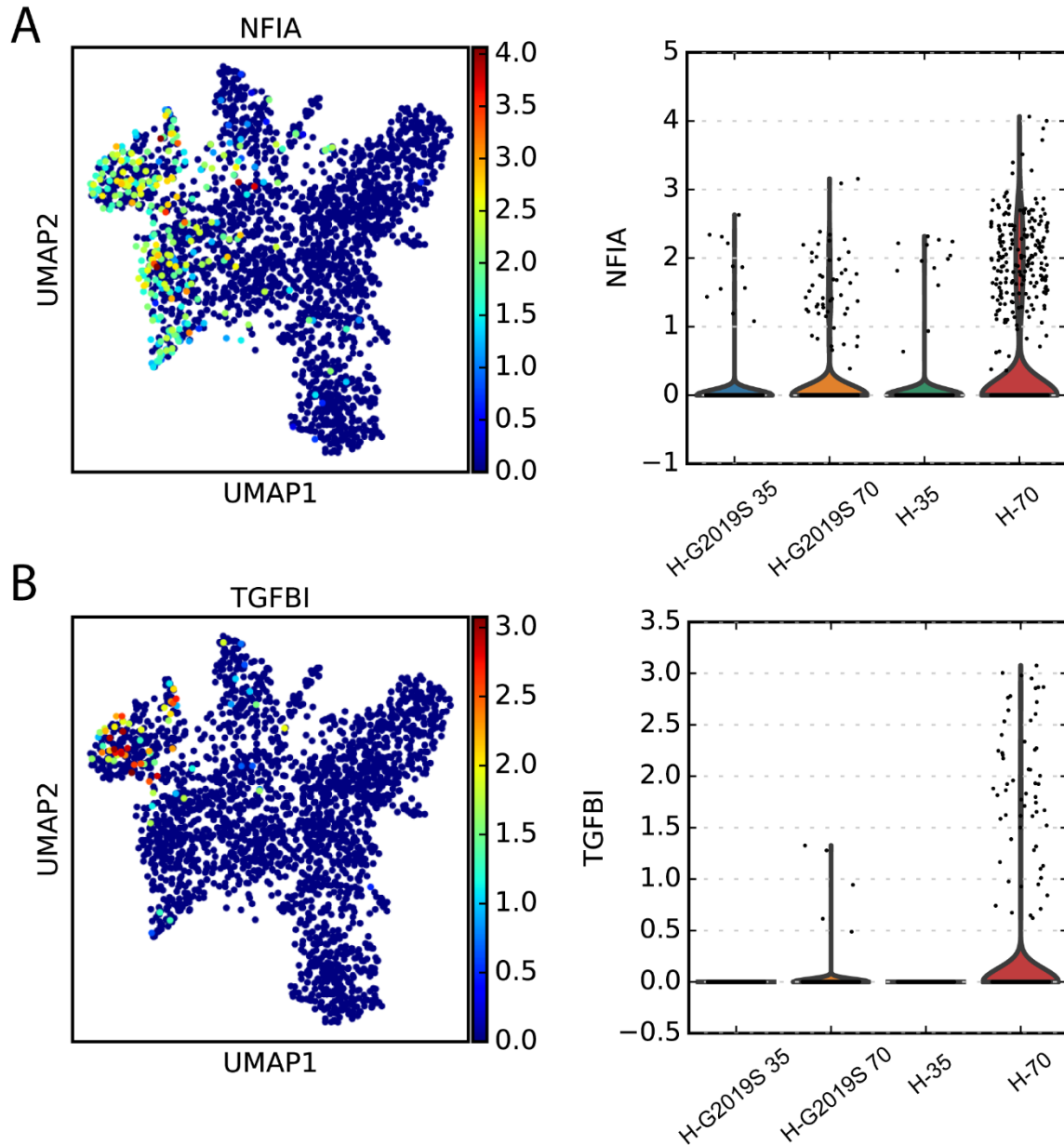
(a) Table showing the iPSC lines used in the study. **(b)** UMAP representation of the single-nuclei RNA-sequencing performed on autaptic brain samples. Each point corresponds to an individual cell positioned according to transcriptional similarity in low-dimensional space. Colours indicate different cellular populations. **(c)** Dot plot showing marker gene expression across identified cell clusters. The size of each dot reflects the proportion of cells in the cluster expressing the marker gene, while the colour scale represents the average expression level across all cells in that cluster. **(d)** Comparison of cell cluster proportions between the healthy individuals and PD patients carrying the LRRK2-G2019S mutation.

A**B****C**

Supplementary Figure 2. Patient-specific organoids carrying the LRRK2-G2019S mutation show reduced neuronal expression

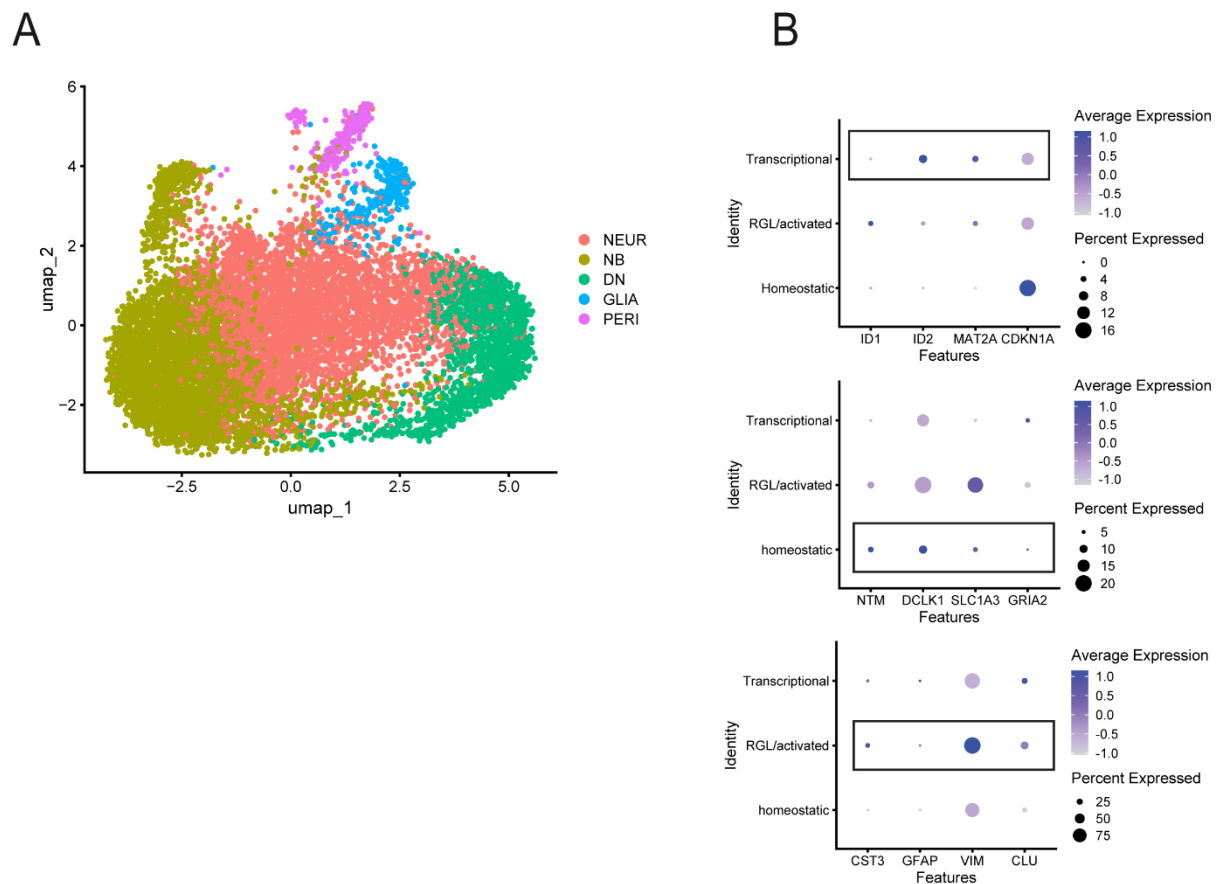
(a) Representative confocal pictures showing Tuj1 (white) staining and Hoechst (blue) after 35 and 70 days of differentiation. **(b)** Box plots showing the % of Tuj1-positive pixels per organoid slice. ** $P < 0.01$, *** $P < 0.001$ Wilcoxon test performed in RStudio. **(c)** Boxplots showing the % of Tuj1 positive pixels after 35 days in the lines grouped according to the genetic background: PD-patients carrying the LRRK2-G2019S, H, healthy individuals. The following pairs of isogenic lines were used with the indicated number of organoid slices (n): H2(n=22)/H2-G2019S(n=28), H3(n=21)/H3-G2019S(n=18), PD1-G2019S(n=19)/PD1-G2019S-GC(n=4), PD2-G2019S(n=19)/PD2-G2019S-GC(n=20). **(d)** Boxplots showing the % of Tuj1 positive pixels after 70 days in the lines grouped according to the genetic background: P, PD-patients carrying the LRRK2-G2019S, H, healthy individuals. The following pairs of isogenic lines were used: H2(n=19)/H2-G2019S(n=34), H3(n=13), PD1-G2019S(n=27)/PD1-G2019S-GC(n=11), PD2-G2019S(n=37)/PD2-G2019S-GC(n=11). ** $P < 0.01$,

*** $P < 0.001$, **** $P < 0.0001$, Wilcox test performed in RStudio. The image acquisition was performed at 20x. The total number of slices per line at each time point was collected over 3 independent replicates.



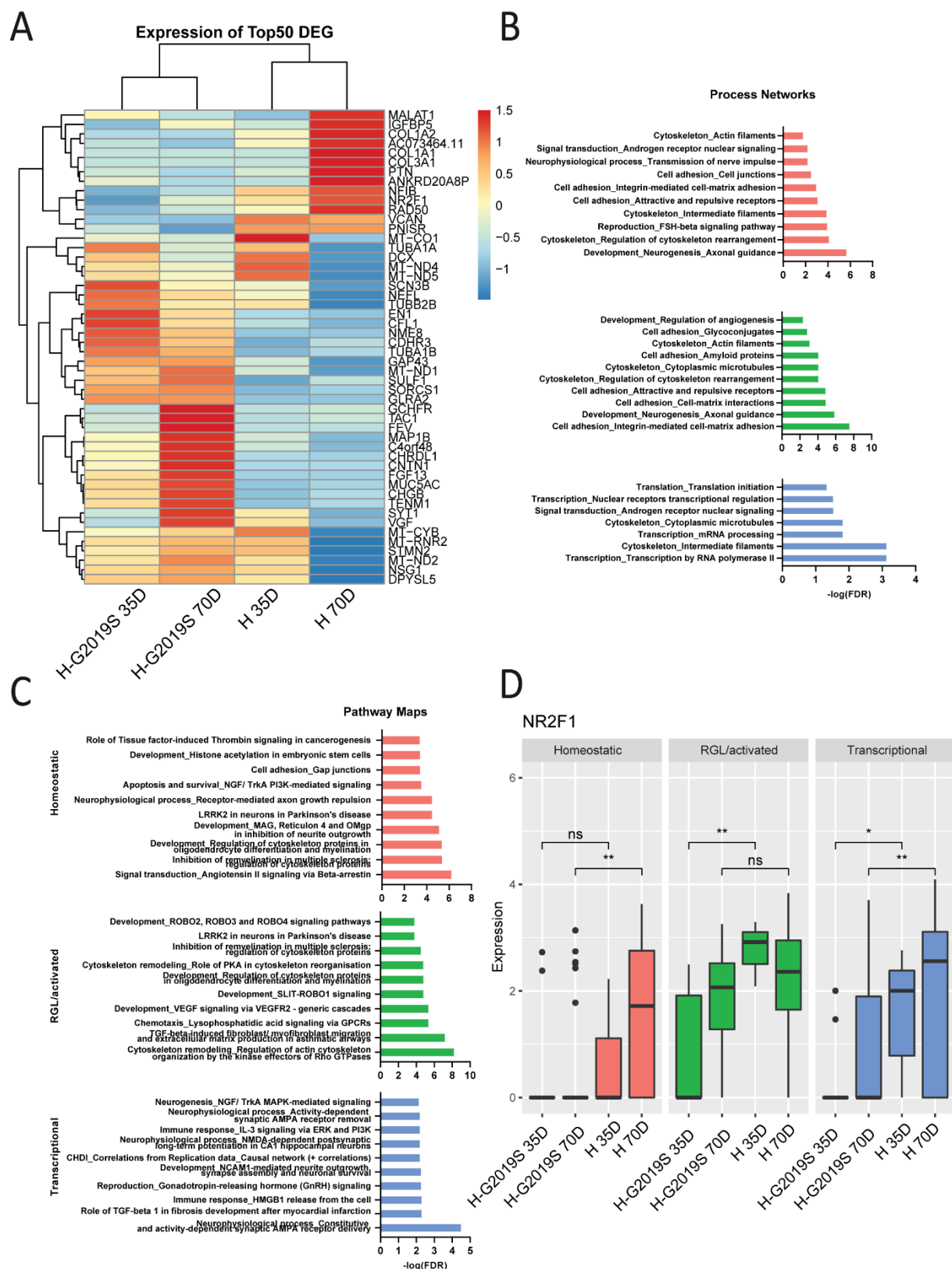
Supplementary Figure 3. Astrocyte transcriptomic profile in LRRK2-G2019S organoids.

(a, left) Dimensionality reduction of the scRNA-seq data with cells colored according to the expression of the gene NFIA. **(a, right)** Distributions of cells over the gene expression for the gene NFIA (violin plots). **(b, left)** Dimensionality reduction of the scRNA-seq with cells colored according to the expression of the gene TGF β 1. **(b, right)** Distributions of cells over the gene expression for the gene TGF β 1 (violin plot). Both NFIA and TGF β 1 genes were almost not expressed at day 30. All the data in this figure refers to the isogenic pair H2-H2/G2019S.



Supplementary Figure 4. Identification of astrocyte subpopulations.

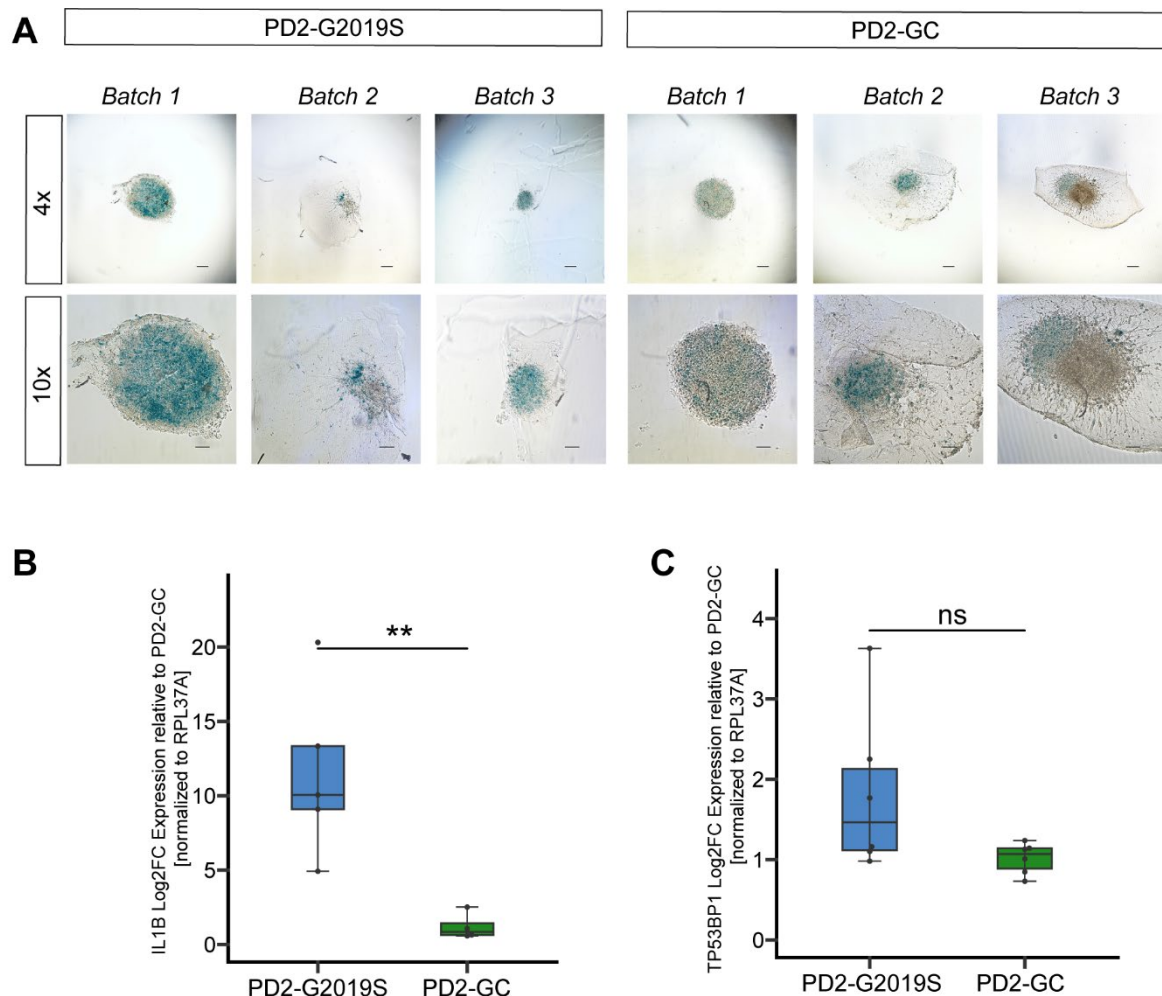
(a) UMAP plot showing the projection of all cells in the scRNA-sequencing data used for identifying astrocyte subtypes, here named Glia **(b)** Dotplot showing cluster names based on the highest average marker expression³⁵. All the data in this figure refers to the isogenic pair H2-H2/G2019S. NEUR=neurons; NB= neuroblasts; DN= dopaminergic neurons; PERI=pericytes.



Supplementary Figure 5. Pathway enrichment analysis shows differential process networks and pathway maps in LRRK2-G2019S astrocytes

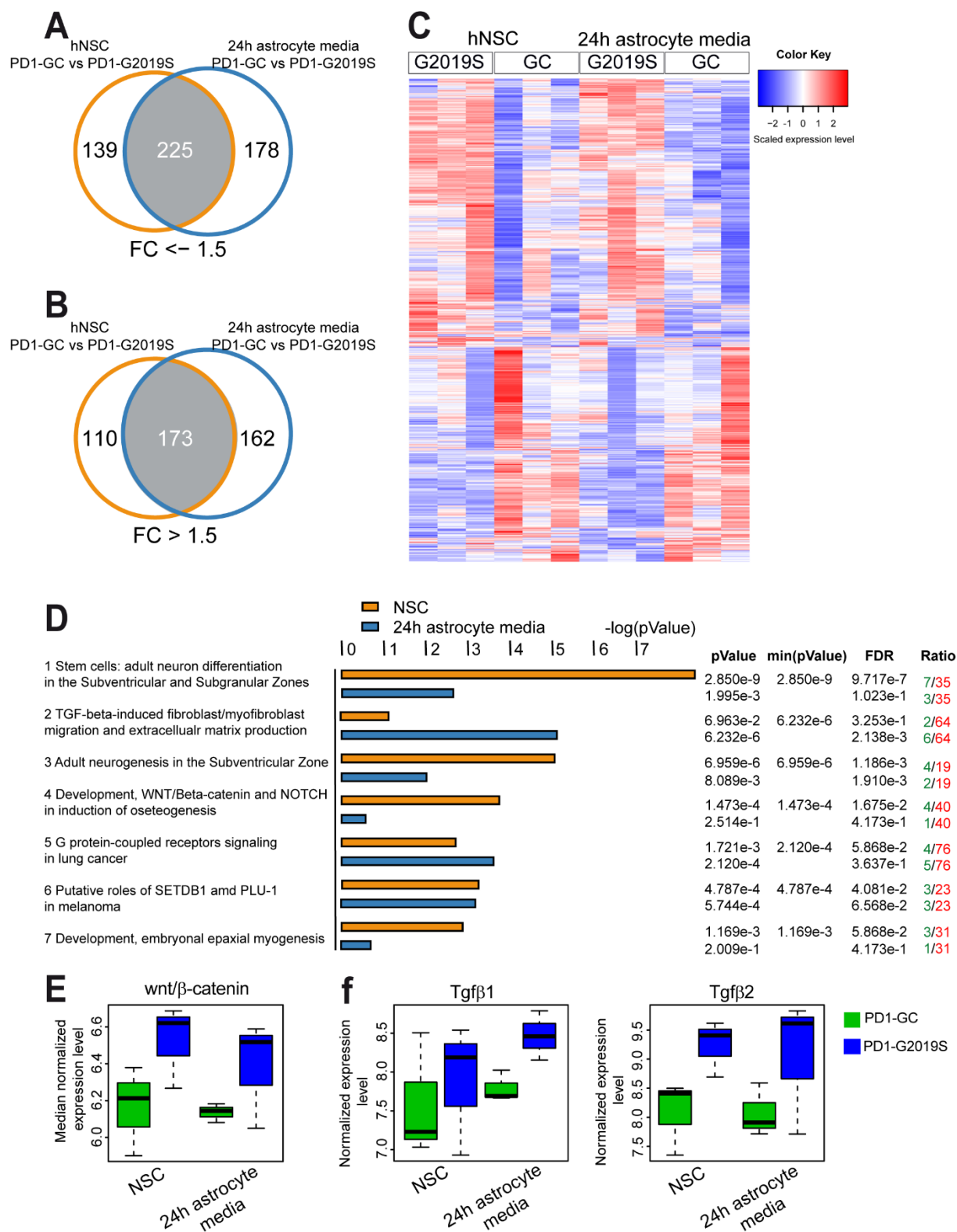
(a) Heatmap of top 50 DEG between H-G2019S and H midbrain organoid in astrocytes, showing the two cell lines at the two time points (35 and 70 days). **(b-c)** Pathway enrichment analysis of DEGs

(p.adj<0.05) of each subpopulation showing process networks **(b)** and pathway maps **(c)**. **(d)** Expression of NR2F1 in astrocyte subpopulation between cell lines and time points. Non-parametric Kruskal-Wallis test $p < 0.05$ *, $p < 0.01$ **. All the data in this figure refers to the isogenic pair H2-H2/G2019S.



Supplementary Figure 6. Senescence-like phenotype markers in midbrain organoids carrying the LRRK2-G2019S mutation.

(a) Senescence-associated β -galactosidase staining (blue) of 70-day-old midbrain organoids (scale bars, 200 μ m, 4 $\times$; 100 μ m, 10 $\times$). Images from organoids from three different batches are shown. Quantitative RT-PCR data of *IL1B* (b) and *TP53BP1* (c) in 70-day-old midbrain organoids. Both genes were normalised against *RPL37A* as a housekeeping gene. Statistical analyses were conducted in R (version 4.5.1) using the Student's t-test, $p < 0.05$ *. Organoids from at least four different batches were used. All the data in this figure refers to the isogenic pair PD2-G2019S and PD2-G2019S-GC.



Supplementary Figure 7. Pathways associated with Wnt/ β -catenin and TGF- β signaling are activated upon astrocytic differentiation in 2D cultures carrying the LRRK2-G2019S mutation

(a) Venn diagram showing the overlap of under-expressed genes between PD-G2019S and PD-G2019S-GC NSCs in maintenance and after 24h of exposure to the astrocyte media. **(b)** Venn diagram showing the overlap of over-expressed genes between PD-G2019S and PD-G2019S-GC NSCs in maintenance and after 24h of exposure to the astrocyte media. **(c)** Heat map visualisation of the genes differentially expressed between PD-G2019S and PD-G2019S-GC NSCs under maintenance or after 24h exposure to the astrocyte media. For all pairwise comparisons, only genes with an absolute expression fold change above 1.5 were chosen. The colour intensities reflect the Z-score standardised gene expression levels. The isogenic line PD1/PD1-gene corrected (GC) was used.

Supplementary Data 1. Reagents and resources

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies and dyes		
GFAP mouse	Millipore	Cat# MAB3402, RRID:AB_94844
GFAP chicken	Millipore	Cat# AB5541, RRID:AB_177521
Tuj1	OptimAB Eurogentec	Cat# PRB-435P-050
LaminB1	Abcam	Cat# ab16048, RRID:AB_443298
S100 β	Sigma-Aldrich	Cat# S2532, RRID:AB_477499
Cleaved Caspase-3 (Asp175) Antibody	Cell Signaling Technology	Cat# 9661, RRID:AB_2341188
Nestin	Millipore	Cat# MAB5326, RRID:AB_2251134
TH	Millipore	Cat# MAB318, RRID:AB_2201528
Tetramethylrhodamine methyl ester (TMRM)	Thermo Scientific	T668
Cell Mask	Invitrogen	C10046
Mitotracker Green	Invitrogen	M46750
Hoechst33342	Thermo Fisher Scientific	H21492
Chemicals, peptides, and recombinant proteins		
hBDNF	PeproTech EC Ltd.	450-02
N-2 supplement	Thermo Fisher Scientific	17502048
E8 iPSC medium	Thermo Fisher Scientific	A1517001
DMEM/F12	Thermo Fisher Scientific	12634010

Neurobasal Medium	Thermo Fisher Scientific	21103049
Y-27632	Merck Millipore	688000-100mg
B-27 Supplement	Thermo Fisher Scientific	17504044
L-Ascorbic acid	Sigma-Aldrich	A4544-100g
dbcAMP	Sigma-Aldrich	D0627
CHIR99021	Axon Medchem BV	AXON1386
SB 431542	Abcam	ab120163
SAG	STEMCELL Technologies	73412
LDN-193189	Sigma-Aldrich	SML0559
hGDNF	PeproTech EC Ltd.	450-44B
hLIF	Merck	L-5283
Purmorphamin	Enzo Life science	ALX-420-045-M005
B-27 Supplement Minus Vitamin A	Thermo Fisher Scientific	12587-010
Penicillin-Streptomycin	Thermo Fisher Scientific	15140122
Accutase	Thermo Fisher Scientific	A11105-01
GelTrex	Thermo Fisher Scientific	A1413302
TGF- β 3	PeproTech EC Ltd.	100-36E
Paraformaldehyde	Sigma-Aldrich	P6148
Tween20	Sigma-Aldrich	P7949
TritonX	Carl Roth	3051.3
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A4503

Fetal Bovine Serum (FBS)	Thermo Fisher Scientific	10270106
Fluoromount-G	Southern Biotech	SOUT0100-01
Mli-2	Tocris	5756/10
GlutaMAX	Thermo Fisher Scientific	35050061
ActivinA	Thermo Fisher Scientific	PHC9561
DAPT	R&D Systems	2634 /50
Normal Goat Serum (NGS)	Thermo Fisher Scientific	10000C
Deposited data		
Single-cell RNA sequencing data on midbrain organoids (isogenic pair H2/H2-G2019S)		GEO: GSE128040
Single-nuclei RNA sequencing on autaptic brain samples		GEO: GSE311604
Single-nuclei RNA sequencing on 70-day-old midbrain organoids		GEO: GSE313031
Figures and Source codes	This paper	https://doi.org/10.17881/p1vt-kg48
Experimental models: Cell lines		
iPSC line H1	Reinhardt et al., 2013	N/A
iPSC line H2	Dr. Bill Skarnes (The Wellcome Trust Sanger Institute, Cambridge, UK)	N/A
iPSC line H3	A13777	GIBCO
iPSC line PD1	Reinhardt et al., 2013	N/A
iPSC line PD2	Reinhardt et al., 2013	N/A

Experimental models: Human Tissue		
PD patient, LRRK2-G2019S carrier, male, 84-year-old	UK Parkinson's Disease Society Tissue Bank at Imperial College, London, UK	PD235
Healthy individual, male, 83-year-old	UK Parkinson's Disease Society Tissue Bank at Imperial College, London, UK	PDC051
Healthy individual, male, 83-year-old	UK Parkinson's Disease Society Tissue Bank at Imperial College, London, UK	PDC084
PD patient, LRRK2-G2019S carrier, male, 65-year-old	Queen Square Brain Bank for Neurological Disorders at University College of London, London, UK	P71/99
Healthy individual, female, 84-year-old	Queen Square Brain Bank for Neurological Disorders at University College of London, London, UK	P32/09
Healthy individual, male, 63-year-old	Queen Square Brain Bank for Neurological Disorders at University College of London, London, UK	P58/08

PD patient, LRRK2-G2019S carrier, female, 80-year-old	Queen Square Brain Bank for Neurological Disorders at University College of London, London, UK	P72/01
Healthy individual, male, 64-year-old	Queen Square Brain Bank for Neurological Disorders at University College of London, London, UK	P10/20
Software and algorithms		
SEURAT R package	http://satijalab.org/seurat/	-
Monocle R package	http://cole-trapnell-lab.github.io/monocle-release/	2.4.0
GraphPad Prism	GraphPad Software, Inc.	7.03
MatLab	The MathWorks, Inc.	R2017a (9.2.0.556244)
Illustrator	Adobe Systems, Inc.	19.2.1
Photoshop	Adobe Systems, Inc.	2015.1.2 20160113.r.355
t-SNE	(Maaten and Hinton, 2008)	https://cran.r-project.org/web/packages/Rtsne/index.html

Supplementary Data 2. Gene lists to identify cell clusters in scRNA-sequencing on organoids.

Supplementary Data 3. Lists of differentially expressed genes in scRNA-sequencing on organoids.

Supplementary Data 4. Senescence gene set from the Kyoto Encyclopedia of Genes and Genomes (KEGG).