# Disruption of lysosomal proteolysis in astrocytes facilitates midbrain organoid proteostasis failure in an early-onset PD model
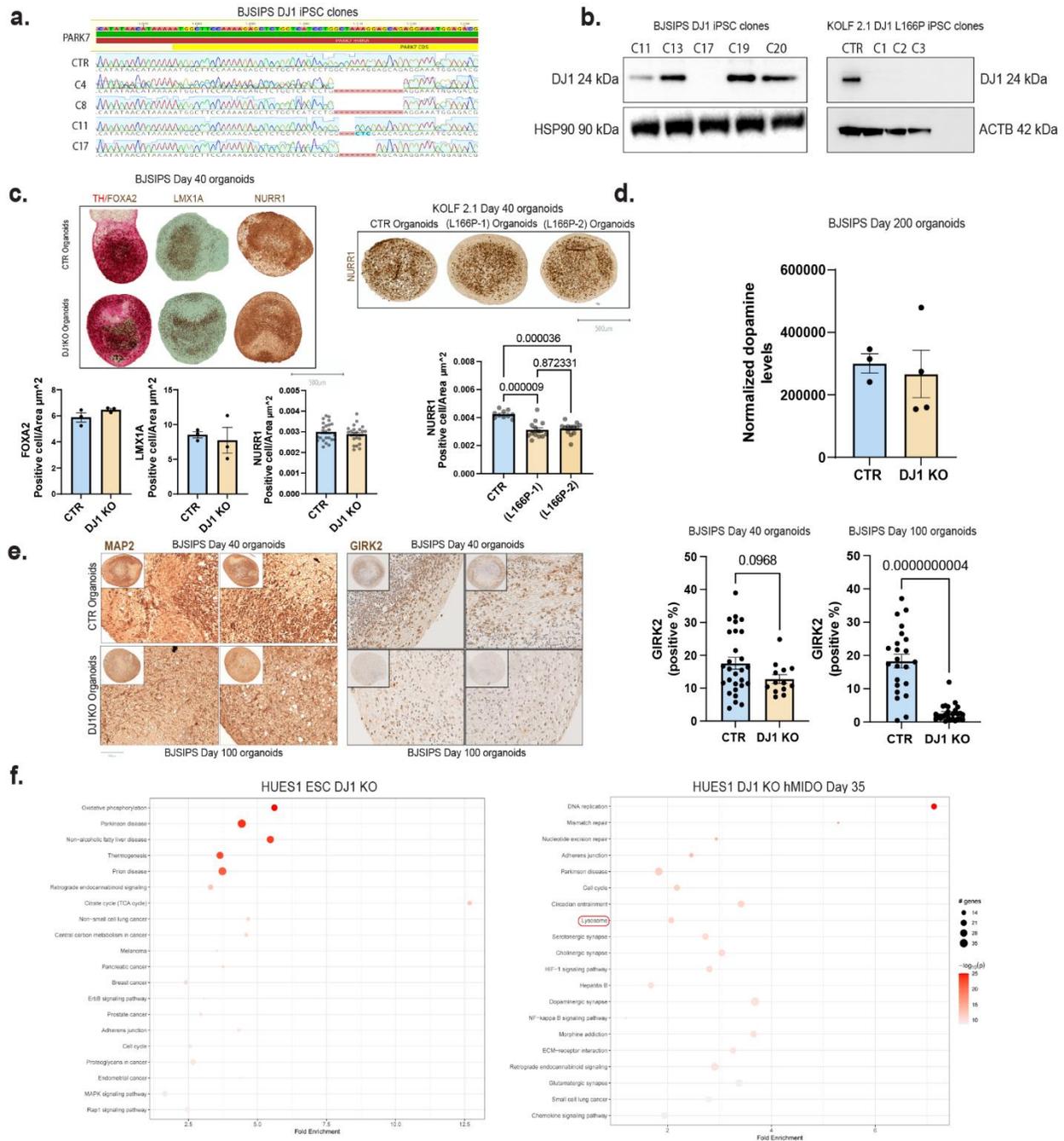


**Figure S1. iPSC and midbrain organoid quality controls and validation**. a. Representation of the Sanger sequencing chromatogram for DJ1 CRISPR clones in BJ-SIPS iPSC line. b. KO confirmation immunoblot for DJ1 CRISPR clones in BJ-SIPS and KOLF 2.1J iPSC lines. c.

Midbrain organoids staining and quantification of TH/FOXA2 ($n = 3$, Two-tailed t test was used for mean comparisons) and LMX1A ($n = 3$, Two-tailed t test was used for mean comparisons) markers at day 20 and NURR1 at day 40 positive cells for CTR and KO BJ-SIPS ($n = 24$, Two-tailed t test was used for mean comparisons) or KOLF 2.1J CTR ($n = 10$), DJ1 L166P KO1 ($n = 13$), and KO2 ($n = 13$, One-way ANOVA followed by Tukey's for the multiple comparisons test). d. Mass spectrometry dopamine quantification of midbrain organoids at day 200 (CTR, $n = 3$ and DJ1 KO, $n = 4$). e. Representative 40x images of hMIDO staining for MAP2 and GIRK2 at different time points and quantification of GIRK2 positive cells for day 40 ($n = 3$) and day 100 organoids ($n = 3$, Two-tailed t-test was used for mean comparisons). f. Pathway enrichment analysis in DJ1 KO generated from HUES1 iPSC and day 35 midbrain organoids. All data are represented in mean ± S.E.M, data points are individual organoids, and the p-value was reported on the graph highlighted comparison. All measurements were taken from distinct samples.
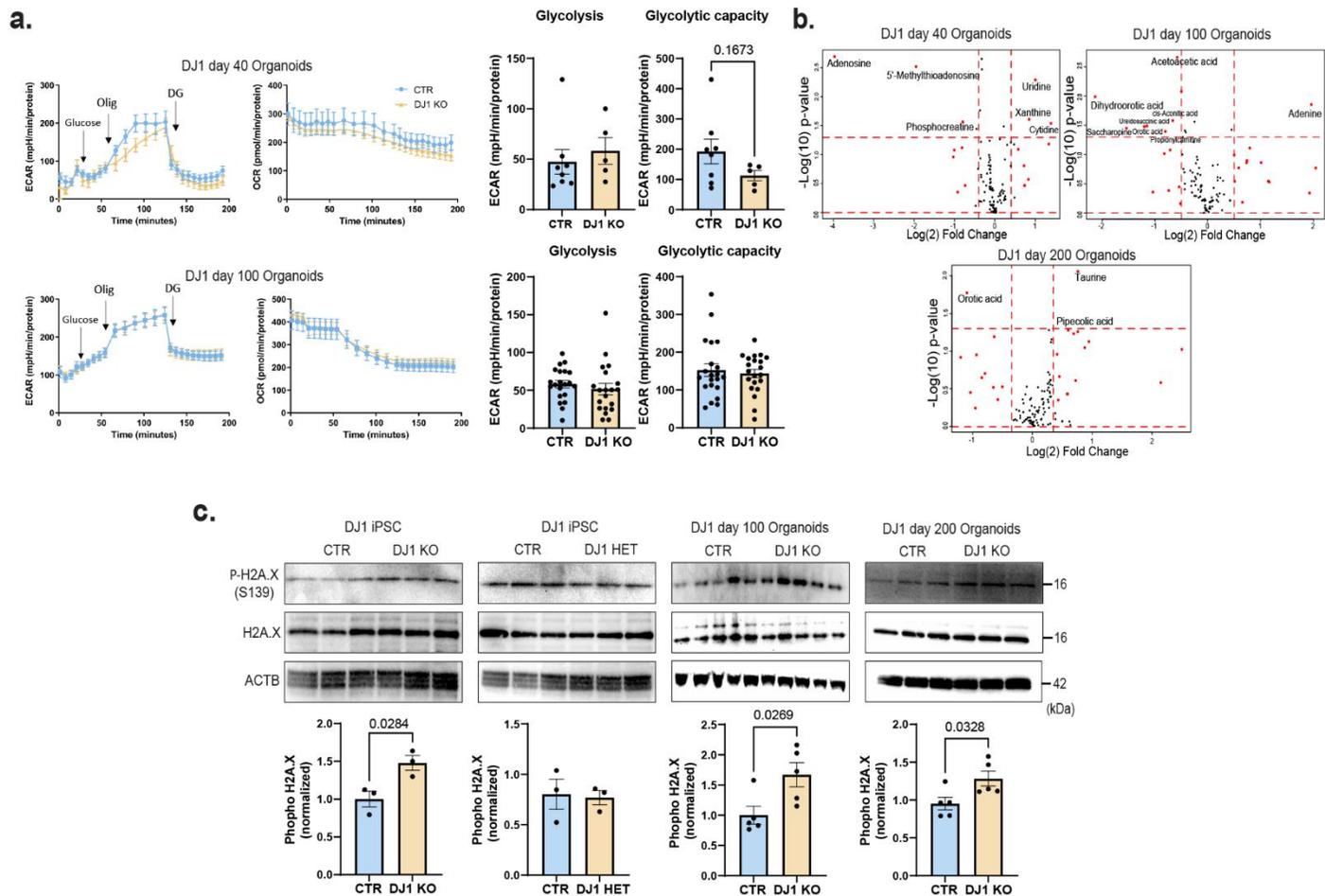
**Figure S2**. **Metabolic and DNA damage quantifications in midbrain organoids.** a. Glycolysis stress seahorse assay in day 40 (CTR, $n = 8$ and DJ1 KO, $n = 5$, Two-tailed t-test was used for mean comparisons) and day 100 (CTR, $n = 21$ and DJ1 KO, $n = 20$, Two-tailed t test was used for mean comparisons) midbrain organoids showing ECAR and OCR levels pre- glucose and after glucose, oligomycin, and DG treatments. b. Volcano plot representation of metabolomics panel for energy-related metabolites in day 40 ($n = 6$), 100 ($n = $ CTR, $n = 3$ and DJ1 KO, $n = 5$), and 200 (CTR, $n = 5$ and DJ1 KO, $n = 8$) midbrain organoids. c. Immunoblots for native H2AX, phospho-H2AX (S139), and actin (ACTB) loading control for CTR ($n = 3$), DJ1 HET ($n = 3$), DJ1 KO iPSC ($n = 3$) and day 100 ($n = 5$) and 200 ($n = 5$) and respective CTR ($n = 5$) midbrain organoids (Two-tailed t test was used for mean comparisons). All data are represented in mean ± S.E.M, data points from seahorse assay are individual organoids, and data points for Wb are individual well differentiations. p-value was reported on the graph highlighted comparison. All measurements were taken from distinct samples.
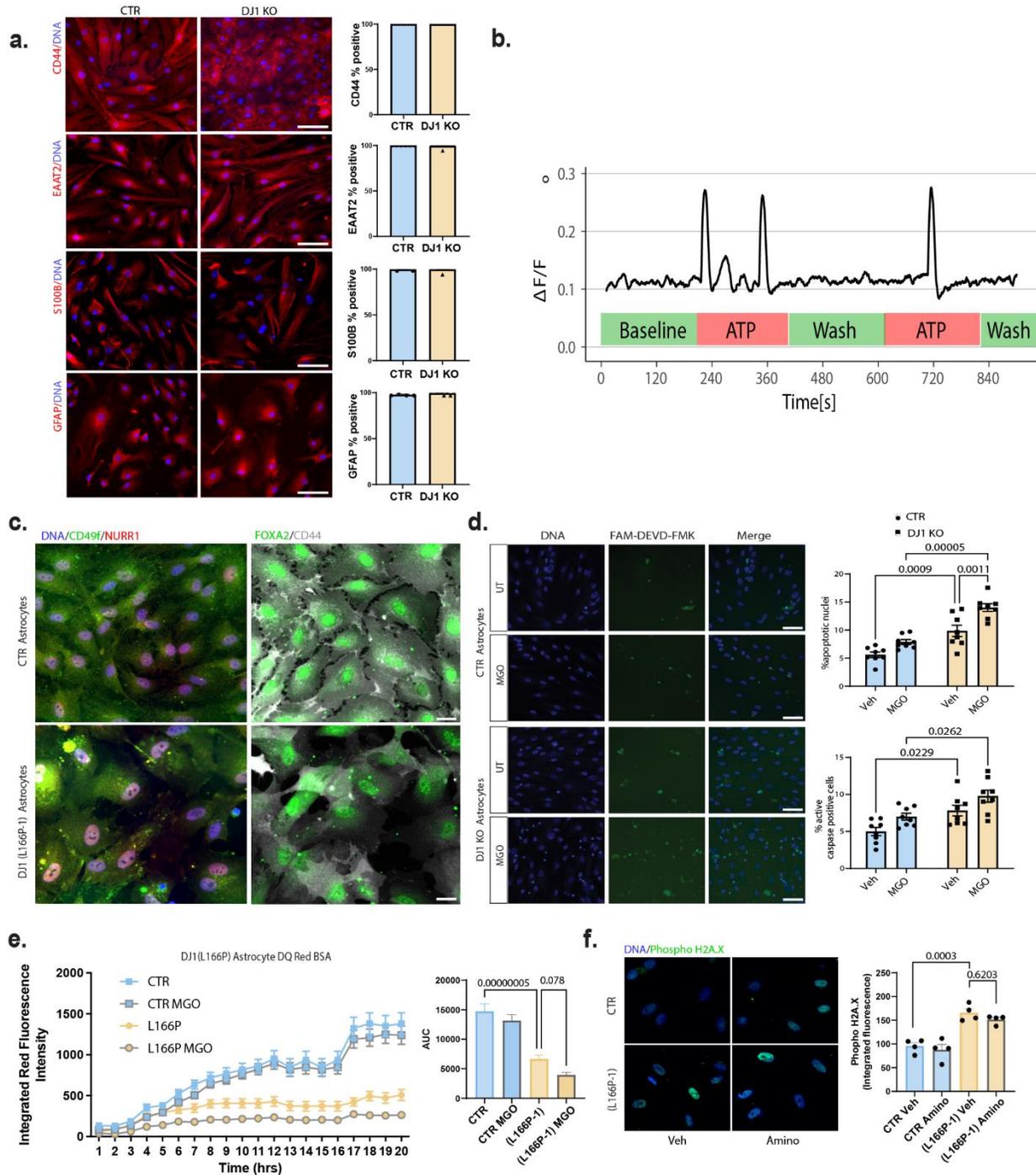
**Figure S3**. **Astrocyte markers and functional validation.** a. Immunostaining panel of CD44 (CTR, $n$ = 8, DJ1 HET $n$ = 12 and, DJ1 KO, $n$ = 12), EAAT2 (CTR, $n$ = 6, DJ1 HET $n$ = 10 and, DJ1 KO, $n$ = 10), S100B (CTR, $n$ = 9, DJ1 HET $n$ = 13 and, DJ1 KO, $n$ = 13), and GFAP (CTR, $n$ = 4, DJ1 HET $n$ = 8 and, DJ1 KO, $n$ = 8) counterstained with DAPI in midbrain astrocytes and % of positive cells (Two-tailed t test was used for mean comparisons). b. Astrocyte GCAMP8s calcium imaging δF/F trace before and after ATP stimulation. c. Immunostaining panel of midbrain

markers NUUR1 and FOXA2 co-stained with CD44. D. Poli-caspase reporter peptide FAM-DEVD-FMK staining in MGO treated or untreated CTR ($n$ = 8) or DJ1 KO ($n$ = 8) astrocytes counterstained with DAPI (Two-way ANOVA followed by Tukey's for the multiple comparisons test). d. Quantification of the standing showing percentage apoptotic nuclei and caspase-positive cells. e. DQBSA proteolysis live imaging assay of vehicle or MGO-treated astrocytes of CTR ($n$ = 24) or DJ1 L166P ($n$ = 24, Two-way ANOVA followed by Tukey's for the multiple comparisons test) genotypes. f. Immunostaining for phospho H2A.X in Amino-treated astrocytes ($n$ = 4, Two-way ANOVA followed by Tukey's for the multiple comparisons test). Scale bars, 100 μm for A.; 20 μm for C; 50 μm for D. All data are represented in mean ± S.E.M, data points are individual organoids, and the p-value was reported on the graph highlighted comparison. All measurements were taken from distinct samples.

**a.**

Principal Component 2 vs Principal Component 1

DJ1 (L166P-2)
DJ1 (L166P-1)
DJ1 CTR

**b.**

DJ1 L166P 1 vs. 2

-Log1Up vs Log2FC

**c.**

Aggregation risk

Log2 FC vs ZaggSC

C6orf132
RASGRF2
SERP1    NAV3
F11R    MOCOS
RRAD    CCDC9B    GRB14
COX14    MISP
CCND2

Aggregation risk

Log2 FC vs TANGO

ZC2HC1B
GLDC
ATP6B1
KIT
OCLN
TUBB4A
ALDH1A3
GYG2
ALDH9A1
MICA
GALNT3
RFX5
SSR3
SEC61G
TUSC1    SPECC1
SLC35E1    SHROOM3
TULP4
JADE1    T3C1D22A
RAB6B    AKT1S1
SCG2
SNAHC5
PPDPF
APOA1

**CDK1, CDK2, CDK6 and MAPK8**

GO Enrichment Analysis

protein depolymerization
negative regulation of microtubule polymerization or depolymerization
regulation of protein depolymerization
regulation of small GTPase mediated signal transduction
microtubule depolymerization
protein-containing complex disassembly
negative regulation of organelle organization
regulation of Ras protein signal transduction
regulation of supramolecular fiber organization
regulation of protein-containing complex assembly
negative regulation of protein depolymerization
negative regulation of microtubule depolymerization
regulation of microtubule depolymerization
establishment of cell polarity
negative regulation of protein-containing complex disassembly

GeneRatio

p.adjust
0.006
0.009
0.012
0.015
0.018

**d.**

Top phosphosite(s) for each predicted kinase

family
group

PLEKHA4;S535;
PDGFRA;S767;
IRAK4;S144;
GAB1;S454;
TLN1;S2170;
NFIX;T267;
AKAP1;S444;
GATAD2B;S134;
TNS1;S962;
NFIX;S268;
STIM1;S667;
PSMF1;T251;
SYNC;T131;
FUBP1;T629;
SHROOM2;S922;
PJA1;S78;
PHF6;T182;
CLMP;S288;
MRTFB;T556;
IK;T48;
SEC16B;S234;
NOL8;S662;
TCF7L1;S77;
PHACTR2;S434;
RIN2;S533;
CRTC3;S410;
TTN;S4761;
ITGB4;T1394;
TBL1X;T43;
VPS51;S652;

ABL2 HIPK2 CDK6 CDK2 CDK1 MAPK3 MTOR MAPK8 CDK5 MAPK13 AURKA PAK1 STK38 LATS1 LATS2 RPS6KB1 SGK1 PRKCA PRKCB CSNK2A1 CAMK2A PRKACA PRKAA1 RPS6KA3 MELK ROCK1

family
Abl
AUR
CAMK2
CAMKL
CDK
CK2
DMPK
DYRK
MAPK
NDR
PIKK
PKA
PKC
RSK
SGK
STE20

group
AGC
Atypical
CAMK
CMGC
Other
STE
TK

Upregulated
Downregulated

**Figure S4. Astrocyte proteomics quality control analysis and phosphoproteomics analysis**
a. PCA analysis of the TMT-labelled proteomics in KOLF 2.1J CTR ($n$ = 3), L166P-1 ($n$ = 3), and L166P-2 ($n$ = 3) midbrain astrocytes (related to KN073-96 dataset). b. Volcano plot comparing the two DJ1 L166P clones highlighting selected proteins (related to KN073-96 dataset). c. Log2FC DJ1 L166P proteomics integration with aggregation risk scores for the human proteome ZaggSC and TANGO score showing selected proteins (related to KN073-96 dataset). d. Clustering plot of phospho-proteomics kinase activity prediction showing the top phosphosites, IPA Kinase/phosphosite network plots, and GO Enrichment analysis for CDK1, CDK2, CDK6, and MAPK8. All measurements were taken from distinct samples (related to KN043-066 and KN067-072 datasets).

a.
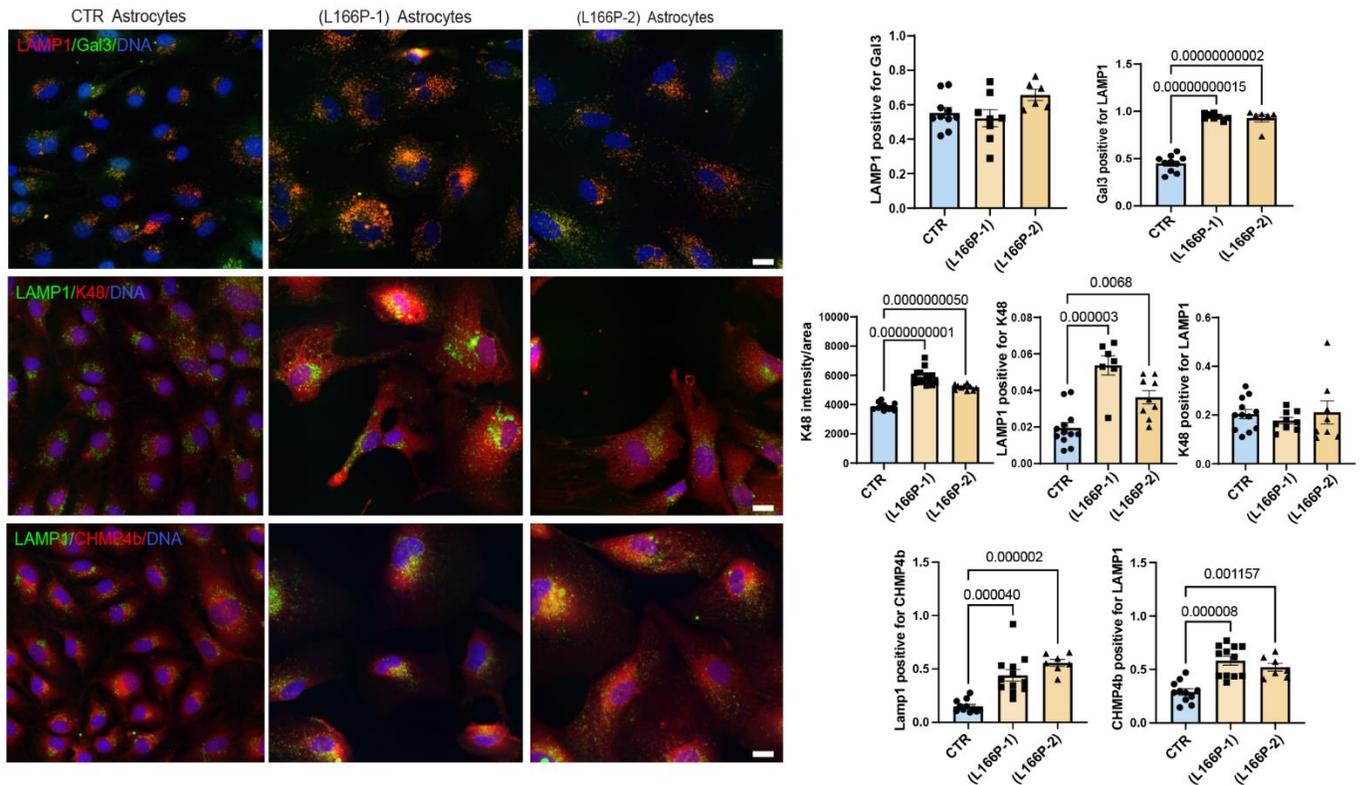


**Figure S5. Astrocyte DJ1 loss of function lysosome damage and repair analysis.** a. Immunostaining panel showing Gal3 co-stained with LAMP1 in KOLF 2.1J CTR, L166P-1, and L166P-2 midbrain astrocytes. Co-localization analysis of Gal3 co-stained with LAMP1(CTR, $n$ = 10, L166P-1 $n$ = 8 and, L166P-2, $n$ = 6, One-way ANOVA followed by Tukey's for the multiple comparisons test). Immunostaining panel showing K48 Ub chain co-stained with LAMP1 in KOLF 2.1J CTR, L166P-1, and L166P-2 midbrain astrocytes. Co-localization analysis of K48 Ub chain

co-stained with LAMP1 (CTR, $n$ = 12, L166P-1 $n$ = 9 and, L166P-2, $n$ = 8, One-way ANOVA followed by Tukey's for the multiple comparisons test). Immunostaining panel showing CHMP4b co-stained with LAMP1 in KOLF 2.1J CTR, L166P-1, and L166P-2 midbrain astrocytes. Co-localization analysis of CHMP4b co-stained with LAMP1 (CTR, $n$ = 11, L166P-1 $n$ = 12 and, L166P-2, $n$ = 7, One-way ANOVA followed by Tukey's for the multiple comparisons test). Scale bars, 15 μm. All data are represented in mean ± S.E.M, data points are individual wells, and the p-value was reported on the graph highlighted comparison. All measurements were taken from distinct samples.

| Case | Age | Sex | Clinical Diagnosis | Neuropathological Diagnosis |
|------|-----|-----|--------------------|-----------------------------|
| 1 | 84 | M | Parkinson's disease | Parkinson's disease |
| 2 | 69 | M | Parkinson's disease | Parkinson's disease |
| 3 | 82 | M | Parkinson's disease | Parkinson's disease |
| 4 | 88 | M | Parkinson's disease | Parkinson's disease |
| 5 | 64 | M | Parkinson's disease | Parkinson's disease |
| 6 | 77 | M | Parkinson's disease | Parkinson's disease |
| 7 | 89 | M | Pancreatic cancer | Control |
| 8 | 65 | F | ST-segment elevation myocardial infarction | Control |
| 9 | 82 | M | Cholecystitis | Control |
| 10 | 77 | F | Diabetes | Control |
| 11 | 65 | M | Acute myeloid leukemia | Control |
| Case | Age | Sex | Clinical Diagnosis | Neuropathological Diagnosis |

**Supplementary Table1.** Clinical information on the PD patients cohort.

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Antibodies/ Concentration | | |
| Donkey anti-Mouse IgG, Alexa Fluor 488 (1:300) | Invitrogen | Cat#A32766 |
| Donkey anti-Mouse IgG, Alexa Fluor 647 (1:300) | Invitrogen | Cat#A-31571 |
| Donkey anti-Mouse IgG, Alexa Fluor 555 (1:300) | Invitrogen | Cat#A-31570 |
| Donkey anti-Rabbit IgG, Alexa Fluor 488 (1:300) | Invitrogen | Cat#A-21206 |
| Donkey anti-Rabbit IgG, Alexa Fluor 647 (1:300) | Invitrogen | Cat#A-31573 |
| Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L) (1:5000) | Jackson Laboratory | Cat#115-035-166; RRID: AB_2338511 |
| Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L) (1:5000) | Jackson Laboratory | Cat#111-035-144; RRID: AB_2307391 |
| FOXA2 (1:100) | Abcam | Cat#Ab108422; RRID: AB_11157157 |
| GAPDH (1:3000) | Abcam | Cat#Ab9485; RRID: AB_307275 |
| GFAP (1:100 IF, 1:1000 WB) | Millipore | Cat#MAB360; RRID: AB_11212597 |
| GFAP (1:10 IHC) | Roche | Cat#MAB360; RRID: N/A |
| TH (1:100 IF, 1:2000 WB) | Millipore | Cat#MAB318; RRID: AB_2201528 |
| TH (1:100 IF, 1:2000 WB) | ABCAM | Cat#ab112;RRID: N/A |

| | | |
|---|---|---|
| FOXA2 (1:500) | ABCAM | Cat#ab40874;RRID: N/A |
| PARK7/DJ1 (1:2000) | ABCAM | Cat#ab169520;RRID: N/A |
| LMX1A (1:500) | SIGMA | Cat#HPA030088;RRID: N/A |
| FOXA2 (1:500) | ABCAM | Cat#ab60721;RRID: N/A |
| alpha Synuclein (1:1000) | ABCAM | Cat#ab138501;RRID:AB_2537217 |
| alpha Synuclein (Phosphorylated S129) (1:1000) | ABCAM | Cat#ab168381;RRID:AB_2728613 |
| alpha Synuclein (Filament) (1:1000) | ABCAM | Cat#ab209538;RRID:AB_2714215 |
| EAAT2 (1:100) | ABCAM | Cat#ab41621;RRID: N/A |
| LC3A/B (1:1000) | Cell Signaling | Cat#4108S;RRID: N/A |
| RAGE (1:1000) | ABCAM | Cat#ab37647;RRID: AB_777613 |
| Histone H2A.X (1:1000) | Millipore | Cat#07-627; RRID:AB_2233033 |
| CD49f (1:50) | Biolegend | Cat#313602;RRID:AB_345296 |
| Methylglyoxal (MGO) (1:1000) | Cell Biolabs | Cat#STA-011,RRID: N/A |
| S100 – Beta (1:1000) | ABCAM | Cat#ab52642;RRID: AB_882426 |
| LAMP1 (1:1000 WB, 1:50 IF) | ABCAM | Cat#ab25630;RRID: AB_470708 |
| NR4A2 (NURR1) (1:100 IF, 1:3000 IHC) | SIGMA | Cat#N6413;RRID:AB_1841046 |

| | | |
|---|---|---|
| CHMP4B (1:100) | ptglab | Cat#13683-1-AP;RRID: N/A |
| Galectin-3 (Mac-2) (1:100) | Biolegend | Cat#125401;RRID:AB_1134237 |
| CD44 (1:100) | ABCAM | Cat#ab157107;RRID:AB_2847859 |
| Ubiquitin (Lys48-Specific), clone Apu2 (1:100 IF, 1:1000 Wb) | Millipore | Cat#05-1307;RRID:AB_1587578 |
| Phospho-Histone H2A.X (Ser139), clone JBW301 (1:1000) | Millipore | Cat#05-636;RRID:AB_309864 |
| oxDJ-1 (Cys106), clone M149 (1:400) | Millipore | Cat#MABN1773;RRID: N/A |
| β-Actin (C4) HRP (1:1000) | Santa Cruz | Cat#sc-47778;RRID:AB_2714189 |
| β-Actin (AC-15) 1:5000) | Invitrogen | Cat#AM4302; RRID:AB_2536382 |
| P62 (1:1000) | Progen | Cat#GP62-C;RRID:AB_2687531 |
| GBA (1:1000) | Abnova | Cat#H00002629-M01;RRID:AB_464151 |
| EAAT2 (1:100) | ABCAM | Cat#ab41621;RRID:AB_941782 |
| Chemicals, peptides, and recombinant proteins | | |
| Accutase® Cell Detachment Solution | Innovative Cell Technologies | Cat#AT104; RRID: AB_2869384 |
| B-27™ Supplement (50×), Minus Vitamin A | Gibco | Cat#12587010; RRID: N/A |
| Bovine Albumin Fraction V (7.5% Solution) | Gibco | Cat#15260037; RRID: N/A |

| | | |
|---|---|---|
| CHIR99021 | Tocris | Cat#4423; RRID: N/A |
| DAPT | Cayman Chemical | Cat#13197; RRID: N/A |
| DB-cAMP/Dibutyryl-cAMP | Biolog | Cat#D 009; RRID: N/A |
| DirectPCR Lysis Reagent (Cell) | Viagen Biotech | Cat#302-C; RRID: N/A |
| DMEM/F-12, GlutaMAX™ Supplement | Gibco | Cat#10565018; RRID: N/A |
| Astrocyte Medium | Sciencell | Cat#1801; RRID: N/A |
| DMEM, High Glucose | Gibco | Cat#11965092; RRID: N/A |
| Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix | Gibco | Cat#A1413302; RRID: N/A |
| Hoechst 33342, Trihydrochloride, Trihydrate - 10 mg/mL Solution in Water | Invitrogen | Cat#H3570; RRID: N/A |
| Laminin Mouse Protein, Natural | Gibco | Cat#23017015; RRID: N/A |
| L-Ascorbic Acid (White Crystalline Powder) | Fisher Scientific | Cat#BP351-500; RRID: N/A |
| LDN193189 | Stemgent | Cat#04-0074; RRID: N/A |
| Leibovitz's L-15 Medium | Gibco | Cat#11415064; RRID: N/A |
| N-2 Supplement (100×) | Gibco | Cat#17502048; RRID: N/A |
| Opti-MEM™ I Reduced Serum Medium | Gibco | Cat#31985062; RRID: N/A |

| | | |
|---|---|---|
| PBS, pH 7.4 (-CaCl2, -MgCl2) | Gibco | Cat#10010023; RRID: N/A |
| Penicillin-Streptomycin (10,000 U/mL) | Gibco | Cat#15140122; RRID: N/A |
| Poly-L-Ornithine Hydrobromide | Sigma-Aldrich | Cat#P3655; RRID: N/A |
| Proteinase K Solution (20 mg/mL) | Viagen Biotech | Cat#501-PK; RRID: N/A |
| Purmorphamine | STEMCELL Technologies | Cat#72204; RRID: N/A |
| Puromycin Dihydrochloride | Gibco | Cat#A1113803; RRID: N/A |
| Recombinant Human BDNF Protein | R&D Systems | Cat#248-BD; RRID: N/A |
| Recombinant Human CNTF Protein | Peprotech | Cat#450-13;RRID: N/A |
| Recombinant Human GDNF Protein | R&D Systems | Cat#212-GD; RRID: N/A |
| SAG | Cayman Chemical | Cat#11914; RRID: N/A |
| SB431542 | Stemgent | Cat#04-0010-10; RRID: N/A |
| StemFlex™ Medium | Gibco | Cat#A3349401; RRID: N/A |
| Thiazovivin | Selleck Chemicals | Cat#S1459; RRID: N/A |
| Trypan Blue Solution, 0.4% | Gibco | Cat#15250061; RRID: N/A |
| Aminoguanidine hydrochloride | Sigma | Cat#1937-19-5; RRID: N/A |
| Bafilomycin A1 from Streptomyces griseus | Sigma | Cat#B1793; RRID: N/A |

| | | |
|---|---|---|
| (R)-MG-132 | Selleck Chemicals | Cat#S2619; RRID: N/A |
| Non-essential Amino Acids (NEAA) | Gibco | Cat#11140050;RRID: N/A |
| GlutaMax Supplement | Gibco | Cat#35050061;RRID: N/A |
| Triton X-100 | Sigma | Cat# T9284;RRID: N/A |
| RIPA buffer | Sigma | Cat# R0278;RRID: N/A |
| DQ™ Red BSA | ThermoFisher | Cat#D12051;RRID: N/A |
| Y-27632 Dihydrochloride | Tocris | Cat#1254; RRID: N/A |
| Critical commercial assays | | |
| Seahorse XF Glycolysis Stress Test Kit | Agilent | Cat#103020-100;RRID: N/A |
| Image-iT™ LIVE Red and Green Caspase Apoptosis Detection Kits for microscopy | ThermoFisher | Cat#I35101;RRID: N/A |
| Lipofectamine™ Stem Transfection Reagent | Invitrogen | Cat#STEM00003; RRID: N/A |
| PROTEOSTAT | Enzo | Cat#ENZ-51023;RRID: N/A |
| P3 Primary Cell 4D-Nucleofector™ X Kit S | Lonza | Cat#V4XP-3032; RRID: N/A |
| Experimental models: Cell lines | | |
| Human: BJ SiPS-D induced pluripotent stem cells | Harvard University | Cat#BJ SiPS-D: RRID: CVCL_X741 |
| KOLF2.1J | Jackson Laboratory | RRID: N/A |

| | | |
|---|---|---|
| KOLF2.1J DJ1 L166P 1 | Jackson Laboratory | RRID: N/A |
| KOLF2.1J DJ1 L166P 2 | Jackson Laboratory | RRID: N/A |
| Human: BJ SiPS-D induced pluripotent stem cells Dj1 HET #11 | This paper | RRID: N/A |
| Human: BJ SiPS-D induced pluripotent stem cells Dj1 KO #17 | This paper | RRID: N/A |
| Oligonucleotides | | |
| PARK7 _L166P F    ATGCATACCCGCCTC CATTACGTTG | This paper | RRID: N/A |
| PARK7 _L166P R    ATAAGCAGAGAAAAT CACAAGCCTC | This paper | RRID: N/A |
| PARK7 _L166P seq    AATGGATTCCTAA CGGCCTG | This paper | RRID: N/A |
| PARK7 R ATGGCTAAAAATCGATGTG G | This paper | RRID: N/A |
| PARK7 F TGGGGTATCTCAGGGTTG CA | This paper | RRID: N/A |
| Recombinant DNA | | |
| Plasmid: pTAHR TH-p2a-TD:Tomato (floxed selection Puro) | Ahfeldt et al., 2020; Addgene | Cat#135814; RRID: N/A |
| CRISPR 1: pTACR TH1-p2aGFP | Ahfeldt et al., 2020; Addgene | Cat#135815; RRID: N/A |

| | | |
|---|---|---|
| CRISPR 2: pTACR TH2-p2aGFP | Ahfeldt et al., 2020; Addgene | Cat#135816; RRID: N/A |
| Plasmid: pCAG-Cre:GFP | Matsuda and Cepko, 2007; Addgene | Cat#13776; RRID: Addgene_13776 |
| Software and algorithms | | |
| CellProfiler | https://cellprofiler.org/ | RRID:SCR_007358 |
| Rstudio | https://www.rstudio.com/ | RRID:SCR_000432 |
| Ingenuity Pathway Analysis (IPA) | Qiagen | RRID:SCR_008653 |
| QuPath | https://QuPath.github.io/ | RRID:SCR_018257 |
| Pathfinder | https://github.com/egeulgen/pathfindR | Ulgen et al., 2019 |
| PhosR  R packages | https://github.com/PYangLab/PhosR | Kim et al., 2021 |
| MetaboAnalyst 4.0 | https://www.metaboanalyst.ca | RRID:SCR_015539 |
| Fiji | https://fiji.sc/ | Chong et al., 2019; RRID:SCR_002285 |
| Other | | |
| 4D-Nucleofector™ Core Unit | Lonza | Cat#AAF-1002B; RRID: N/A |
| Cell Culture Microplate, 96-well, PS, F-bottom (Chimney Well), µClear®, Black, CELLCOAT®, Poly-L-Lysine, Lid with Condensation Rings, 5 pcs./bag | Greiner | Cat#655936; RRID: N/A |
| Corning® 125 mL Disposable Spinner Flask | Corning | Cat#3152; RRID: N/A |

| with 70 mm Top Cap and 2 Angled Sidearms, Sterile | | |
|---|---|---|
| Corning® Costar® Ultra-Low Attachment Multiple Well Plate | MilliporeSigma | Cat#CLS3471; RRID: N/A |
| Countess™ Cell Counting Chamber Slides | Invitrogen | Cat#C10228; RRID: SCR_019815 |
| Countess™ II FL Automated Cell Counter | Invitrogen | Cat#AMQAF1000; RRID: N/A |
| Falcon® Round-Bottom Tubes with Cell Strainer Cap, 5 mL | STEMCELL Technologies | Cat#100-0087; RRID: N/A |
| Fisherbrand™ Disposable Borosilicate Glass Pasteur Pipets | Fisher Scientific | Cat#13-678-20D; RRID: N/A |
| Fisherbrand™ Sterile Cell Strainers (70 µm) | Fisher Scientific | Cat#22-363-548; RRID: N/A |
| pluriStrainer® 300 µm, 25 pcs. – Sterile (Cell Strainer) | pluriSelect | Cat#43-50300-03; RRID: N/A |
| pluriStrainer® 500 µm, 25 pcs. – Sterile (Cell Strainer) | pluriSelect | Cat#43-50500-03; RRID: N/A |
| Stirrers, Magnetic, Nine-position, Dura-Mag | Chemglass Life Sciences | Cat#CLS-4100-09; RRID: N/A |
| Variable Speed 2D Rocker | USA Scientific | Cat#2527-2000; RRID: N/A |

**Supplementary Table2.** List of reagents.

Exp1_AO

4plex TMTpro
proteomics
dataset:

| Sample | Channel | Clone | Cell type | Lysate preparation |
|--------|---------|-------|-----------|--------------------|
| WT_1 | 132c | KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |
| WT_2 | 133n | KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |
| L166P_1 | 133c | L166P-1/KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |
| L166P_2 | 134n | L166P-1/KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |

| Source files | Experiment |
|--------------|------------|
| KN043-066 | Total proteome (FAIMS-MS3) |
| KN067-072 | Phospho proteome (FAIMS-MS2) |

Exp2_AO

9plex TMT (FAIMS-hrMS2) proteomics
dataset:

| Sample | Channel | Clone | Cell type | Lysate preparation |
|--------|---------|-------|-----------|--------------------|
| WT_1 | 126 | KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |
| WT_2 | 127n | KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |
| WT_3 | 127c | KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |
| L166P_A_1 | 128n | L166P-1/KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |
| L166P_A_2 | 128c | L166P-1/KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |
| L166P_A_3 | 129n | L166P-1/KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |
| L166P_B_1 | 129c | L166P-2/KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |
| L166P_B_2 | 130n | L166P-2/KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |
| L166P_B_3 | 130c | L166P-2/KOLF 2.1J background | Midbrain patterned astrocytes of 100 days | RIPA |

| Source files | Experiment |
|--------------|------------|
| KN073-96 | Total proteome(FAIMS-MS2) |

**Supplementary Table3.** Metadata for the proteomics and phosphoproteomics experiments.