Supplementary Materials for

Mitofusosome exocytosis, a mitophagy-independent mitochondrial quality control in flunarizine-induced parkinsonism-like symptoms

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Other Supplementary Material for this manuscript includes the following:

Data S1 to S3
Fig. S1. FNZ decreased motor function and memory *in vivo*.

(A) FNZ+ treated group (n = 22 mice) received FNZ for 14 days at the dose of 30 mg/kg per day; FNZ- group (n = 25 mice) received 50% PEG400. Mice were weighed daily. Error bars = SEM. Differences were established using two-way ANOVA (*P < 0.05).

(B to D) Open field test for mice treated with FNZ or PEG400 for 1 week. Error bars = SEM. Differences were established using t-test (n ≥ 10 mice, *P < 0.05).

(E to H) Latency to locating the platform during the training phase in the Morris water maze in the mice with or without FNZ treatment for 1 week (E) and 2 weeks (G). Latency to first entry to the platform zone in the probe trial in mice post FNZ treatment for 1 week (F) and 2 weeks (H) are shown. Error bars = SEM (n ≥ 11 mice).
Fig. S2. FNZ did not affect the amount of TH positive cells.

After 2 weeks of FNZ treatment, midbrain slices of mice were stained for TH by immunohistochemistry and TH positive neurons in SN were counted. Representative photomicrographs of TH stained sections from the SN are shown in (A). Quantification of the number of TH positive neurons by stereological analysis is shown in (B). Representative photomicrographs from the striatum are shown in (C). Scale bar: 1 mm. Quantification of the striatal TH positive fiber density is shown in (D). (n ≥ 4 mice). Error bars = SD.
Supplementary Figure 3

A

1 week brain

Time (s)

Activity (%ID/g)

FNZ-  FNZ+

0  0.04  0.08

0  1000  2000  3000  4000

0  0.04  0.08

1 week vena cava

Time (s)

Activity (%ID/g)

FNZ-  FNZ+

0  0  0.5  1

0  1000  2000  3000  4000

0  0.5  1

B

2 week brain

Time (s)

Activity (%ID/g)

FNZ-  FNZ+

0  0.04  0.08

0  2000  4000

0  0.04  0.08

2 week vena cava

Time (s)

Activity (%ID/g)

FNZ-  FNZ+

0  0  0.5  1

0  2000  4000

0  0.5  1

C

Value of K2

Baseline  1W  2W

FNZ-  FNZ+

0.0  0.3  0.6

P = 0.3443  P = 0.2003

D

Value of K3

Baseline  1W  2W

FNZ-  FNZ+

0.0  0.02  0.04

P = 0.0816  P = 0.3535

E

Value of K1

Baseline  1W  2W

FNZ-  FNZ+

0.0  0.01  0.02

P = 0.4830  P = 0.2758
Fig. S3. PET/CT analysis of mice treated with FNZ or PEG400.

(A and B) Time activity curves from the mean activities of brain and vena cava are shown.
(C to E) Quantification of K2 (C), K3 (D), and Ki (E) in FNZ or PEG400 treated mice, waterfall plots of the percentage change after treatment are shown in the right panels, each bar representing an individual mouse. (n = 12 mice). Error bars = SEM.
Fig. S4. Mitochondrial mass was not affected in muscle, liver, kidney, heart or spleen of mice post FNZ treatment.
Western blot detection of mitochondrial OMM proteins (TOM20, VDAC), IMM protein (PHB1) and matrix protein (HSP60) in mouse muscle (A) liver (B), kidney (C), heart (D) and spleen (E) homogenates. Quantification of protein levels is shown in lower histograms. Error bars = SD. Differences were established using t-test (n ≥ 4 mice, *P < 0.05).
Fig. S5. Mitochondrial content decreases in mice TH negative neurons and in cultured human neurons and astrocytes.

(A to C) Mice were treated with FNZ for 2 weeks. Immunostaining was used to detect mitochondria (TOM20) in SN TH negative TUJ positive neurons. Representative confocal images are shown in (A). Scale bar: 10 μm. Quantification of relative mitochondrial area per cell is shown in (B and C). Error bars = SD. Differences were established using t-test (79 cells from 4 mice, ***P < 0.001).

(D to F) Cultured neurons were treated with FNZ for 3 days, then immunostaining was used to detect mitochondria (TOM20) in TUJ positive cultured neurons. Representative confocal microscopy images with magnified boxed region are shown in (D). Scale bar: 10 μm. Quantification of relative mitochondrial area in axons and cell bodies are shown in (E) (≥ 30 cell bodies from three independent experiments) and (F) (≥ 30 neurites from three independent experiments), respectively. Error bars = SD. Differences were established using t-test (***P < 0.001).

(G and H) Human primary astrocytes were treated with FNZ for 3 days, then immunostaining was used to detect mitochondria (TOM20) in GFAP positive astrocytes. Representative confocal microscopy images are shown in (G). Scale bar: 10 μm. Quantification of relative mitochondrial area per cell is shown in (H). Error bars = SD. Differences were established using t-test (n = 30 cells from three independent experiments, ***P < 0.001).
Fig. S6. The effect of FNZ on glycolysis in NPCs.

(A) NPCs were treated with or without FNZ for 3 days before Seahorse analysis, followed by sequential addition of glucose, oligomycin (which inhibits ATP synthesis), and 2-DG (which inhibits glycolysis). ECAR, i.e. extracellular acidification rate, was measured in real time. (B) Glycolysis (calculated by subtraction of the basal ECAR from the glucose ECAR), (C) Glycolytic capacity (calculated by subtraction of the basal ECAR from the oligomycin ECAR), and (D) Glycolytic reserve (calculated by subtraction of the glucose ECAR from the oligomycin ECAR) are shown. Error bars = SD. Differences were established using t-test (n = 2 independent experiments; *P < 0.05).
Fig. S7. FNZ eliminated mitochondria in human fibroblasts, MEFs and Hela cells.
mito-GFP expressing normal human fibroblasts (GZF2), MEFs and Hela cells were treated with FNZ for 3 days. Mitochondrial elimination occurred in all the three cell types. Representative microscopy images (A) and quantification of the fraction of cells without mitochondria (B) are shown. Scale bar: 10 μm. Western blot analysis of mitochondrial proteins (TOM20 and HSP60) in cells with or without FNZ treatment for 3 days is shown in (C) and quantification of protein
levels shown in the lower panes. Error bars = SD. Differences were established using t-test (n ≥ 3 independent experiments; *P < 0.05, **P < 0.01, ***P < 0.001).
Fig. S8. ER, lysosome and Golgi apparatus remained intact in FNZ-induced mitochondrial depleted NPCs.

NPCs were treated with FNZ for 3 days, used mito-GFP for labeling mitochondria, and ER, lysosome, Golgi apparatus with ER-DsRed, LysoTracker for lysosome, DsRed-Golgi for Golgi apparatus, respectively. Representative microscopy images are shown. Scale bar: 10 μm.
Supplementary Figure 9

![Image of Supplementary Figure 9 with various concentration levels and corresponding graphs showing relative mitochondrial area per cell.

- Mito-GFP
- FNZ
- CNZ
- CPZ
- DOM
- HP
- MPTP

The graphs depict concentration (μM) on the x-axis and relative mitochondrial area per cell on the y-axis. The data points are accompanied by statistical symbols indicating statistical significance.]
NPCs expressing mito-GFP were treated for 3 days with parkinsonism-inducing drugs including typical antipsychotics (chlorpromazine (CPZ) and haloperidol (HP)), gastrointestinal prokinetic drug domperidone (DOM), neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and calcium channel blocker Cinnarizine (CNZ) and FNZ. Representative microscopy images are shown. Scale bar: 10 μm. Quantification of relative mitochondrial area per cell is shown in the upper panes. Error bars = SD. Differences were established using one-way ANOVA (n = 60 cells from three independent experiments; *P < 0.05, **P < 0.01, ***P < 0.001).
Fig. S10. Knockdown of mitophagy-related genes in NPCs.

qPCR detection of ATG5 (A), PINK1 (B), FUNDC1 (C), RAB9 (D) and ULK1 (E) in NPCs transduced with control TRC, ATG5, PINK1, FUNDA1, RAB9 or ULK1 shRNA vectors. Error bars = SD. Differences were established using t-test (n = 3 independent experiments, **P < 0.005, ***P < 0.001).
**Fig. S11. Mitochondria entry into lysosomes post FNZ treatment.**

(A) Representative 3D images of NPCs expressing mito-DsRed and LAMP1-GFP treated with FNZ for 24 h. The 3D images were reconstructed by Imaris software. The arrow in the middle panel indicates a mitochondrion engulfed by a lysosome. Scale bar: 1 μm.

(B and C) NPC lysosomes were labeled with LAMP1-GFP, and mitochondrial matrix and outer membrane with COX subunit VIII (mito-DsRed) and OMP25-mCherry, respectively. Cells were treated with FNZ for 24 h. Representative confocal microscopy images are shown in (B). Scale bar: 10 μm. Quantification of the fraction of mitochondrial matrix and outer membrane puncta in lysosomes (C). Error bars = SD, Differences were established using t-test (n ≥ 18 cells from two independent experiments, **P < 0.005, ***P < 0.001).

(D and E) Dual-excitation ratiometric imaging of mito-mKeima in FNZ-treated NPCs, with representative confocal microscopy images shown in (D). Scale bar: 10 μm. (E) The histogram shows the ratio of 543:458, normalized to the FNZ- group’s ratio. Error bars = SD. Differences were established using t-test (n = 30 cells from three independent experiments, ***P < 0.001).

(F and G) Effects of 3MA and CLQ on mitochondrial entry into lysosomes. Mito-DsRed and LAMP1-GFP-expressing NPCs were treated with FNZ for 24 h in the presence of 3MA or CLQ. Representative confocal microscopy images are shown in (F). White lines demarcated the edges of cells. Scale bar: 10 μm. Quantification of the ratio of mitolysosome to total mitochondria (G). Error bars = SD, Differences were established using t-test (n ≥ 40 cells from three independent experiments, ***P < 0.001).
Supplementary Figure 12

A
FM1-43 mito-DsRed

FNZ-  FNZ+  FNZ+

B
| FNZ+ (h) | 0  | 8  | 16 | 24 | M | P | H2O |
|----------|----|----|----|----|---|---|------|
| MT-ND1   |    |    |    |    |   |   |      |
| MT-ND4   |    |    |    |    |   |   |      |
| GAPDH    |    |    |    |    |   |   |      |

C
FM1-43 mito-DsRed phalloidin

D

E
Extracellular mito. (%)

FNZ-  FNZ+

*
Fig. S12. Mitolysosome exocytosis occurred in FNZ-treated NPCs.

(A) NPCs expressing mito-DsRed (Red) were treated with FNZ for 24 h, followed by FM1–43FX (green) staining. Representative confocal microscopy images are shown. Scale bars, 10 μm.

(B) Two mitochondrial DNA-encoded genes (MT-ND1 and MT-ND4) and one nuclear DNA-encoded gene (GAPDH) were amplified by PCR from culture supernatant during FNZ treatment. DNAs isolated from NPCs were used as the positive control (P). Medium (M) and H2O were used as negative controls.

(C) NPCs expressing mito-DsRed were treated with FNZ for 24 h, followed by FM1–43FX and phalloidin staining (to label Actin). Representative confocal microscopy image with magnified boxed region is shown. Scale bars, 10 μm.

(D and E) FACS showed that the number of extracellular mitolysosome increased following FNZ treatment in NPCs. Representative FACS plots are shown in (D). Quantification of extracellular mitochondria is shown in (E). Error bars = SD. Differences were established using t-test (n = 3 independent experiments; *P < 0.05).
Fig. S13. FNZ treatment did not increase expression of TOM20 on the cell surface.

FACS analysis of cell surface staining of TOM20 in non-fixed NPCs. Representative plots are shown in (A). Quantification of the fraction of TOM20 positive cells is shown in (B). Error bars = SD (n = 3 independent experiments).
Fig. S14. Addition of calcium blocked FNZ-induced mitochondrial elimination.

NPCs were treated with FNZ, ionomycin and varying concentrations of calcium for 3 days. The fraction of cells without mitochondria is shown. Error bars = SD. Differences were established using one-way ANOVA (n = 3 independent experiments, *P < 0.05, **P < 0.001).
Fig. S15. Transcriptome analysis revealed upregulation of genes involved in vesicle-mediated transport.

(A and B) Gene enrichment analysis using Gene Ontology (GO) annotations of up-regulated (A) and down-regulated (B) genes from NPCs treated with FNZ for 1 day.

(C and D) Fuzzy c-means clustering (FCM) of up-regulated (C) and down-regulated (D) genes over the course of FNZ treatment. The profile was centered to mean and scaled to variance. The solid line represents the mean value of the cluster and the dashed lines representing 95% confidence intervals.

(E and F) Gene enrichment analysis of the up-regulated (E) and down-regulated (F) genes from FCM, using GO annotation (upper panels).
Supplementary Figure 16

A. LAMP1-GFP, mito-DsRed, VAMP2

B. VAMP2, mito-DsRed, DAPI

C. Relative expression level of VAMP2

D. Relative expression level of STX4

E. LAMP1-GFP, mito-DsRed

F. Ratio of mitochondria to total mitochondria

Legend:
- shTRC
- shVAMP2-a
- shSTX4-a
Fig. S16. 

**VAMP2/STX4 knockdown had no effect on mitochondrial entry into lysosome.**

(A) NPCs expressing mito-DsRed and LAMP1-GFP were treated with FNZ for 24 h and then immuno-labeled for VAMP2. Representative confocal microscopy images are shown. The boxed region is magnified in the lower panels. Arrows indicate lysosomal engulfing of mitochondria. Scale bars, 10 μm.

(B) NPCs expressing mito-DsRed were treated with FNZ for 24 h, and then immune-labeled for VAMP2. Representative confocal microscopy images are shown. The boxed region is magnified. Scale bars, 10 μm.

(C and D) qPCR detection for VAMP2 and STX4 in NPCs transduced with control TRC, VAMP2 or STX4 shRNA vectors. Error bars = SD. Differences were established using one-way ANOVA (n ≥ 2 independent experiments; ***P < 0.001).

(E and F) Effect of VAMP2/STX4 knockdown on mitochondrial entry into lysosome. NPCs expressing mito-DsRed and LAMP1-GFP were transduced with control TRC, VAMP2 or STX4 shRNA vectors, followed by treatment with FNZ for 24 h. Representative confocal microscopy images are shown in (E). Scale bars, 10 μm. Quantification of the ratio of mitolysosome is shown in (F). Error bars = SD (n ≥ 30 cells from three independent experiments).
Fig. S17. Knockout of selected genes did not affect mitochondrial mass and mitolysosome secretion in NPCs without FNZ treatment.

(A) FNZ-treated NPC-GeCKO library cells were sorted, based on the intensity of mito-GFP fluorescence, for further deep sequencing.

(B) Boxplot of sgRNA distribution in the NPC-GeCKO library before and after screen. Biological replicates are shown as rep1 and rep2, values are shown in log2 scale, sgRNAs are median-normalized to account for differences in total Illumina read counts. Each point represents an individual sgRNA. sgRNAs are distributed by quartile, where the boxes represent the middle quartiles (25%–75% distribution), and the lines and dots represent sgRNAs in the upper and the lower 25% of the distribution.

(C) Gene ontology analysis of hits from the GeCKO screen.

(D) Scatterplot showing enrichment of specific sgRNAs after FNZ treatment and FACS sorting.

(E and F) Knockout of selected genes did not affect mitochondrial mass and mitolysosome exocytosis without FNZ treatment. Cell lysates and culture supernatants from selected NPC KOs were subjected to western blot for TOM20 and Actin. Quantification of the protein level is shown in (F) (n = 2 independent experiments).
Fig. S18. Effect of FNZ on mitochondrial elimination in serum medium.
NPCs expressing mito-GFP were treated with FNZ for 3 days in medium containing 15% serum. Representative microscopy images are shown. Scale bar: 10 μm. Quantification of fraction of cells without mitochondria is shown in the upper panes. Error bars = SD. Differences were established using one-way ANOVA (n = 3 independent experiments; **P < 0.01, ***P < 0.001).
Supplementary Figure 19

A

Mito-GFP  Untreated  FNZ  EGTA  BAPTA

B

Fraction of cells without mitochondria

C

Mito-GFP

TMRM - 20 min  0 min  30 min

D

Relative Fl

Time (h)
Fig. S19. FNZ damaged mitochondrial function in NPCs.

(A and B) Mito-GFP labeled NPCs were treated with FNZ, EGTA or BAPTA-AM for 3 days. Representative confocal images are shown in (A), Scale bar: 10 μm. Quantification of the fraction of cells without mitochondria is shown (B). Error bars = SD. Differences were established using t-test (n = 3 independent experiments; ***P < 0.001).

(C and D) FNZ dissipated mitochondrial membrane potential. Mitochondria of mt-GFP-expressing NPCs were stained with TMRM, followed by FNZ addition. Representative microscopy images are shown in (C). (D) Time course of relative fluorescence intensity of TMRM and mt-GFP is shown in the lower panel. Error bars = SD (n = 4 cells from two independent experiments).
Fig. S20. Effect of FNZ-induced mitochondrial depletion on cell proliferation and differentiation of NPCs.

(A) Proliferation curves for NPCs with or without FNZ treatment. Error bars = SD. Differences were established using two-way ANOVA (n = 5 independent experiments; **P < 0.005, ***P < 0.001).

(B) NPCs expressing mito-DsRed were treated with FNZ, followed by immunolabeling with stem cell marker NESTIN. Representative confocal microscopy images are shown. Scale bars, 10 μm.

(C to F) FNZ-induced mitochondrial elimination affected neuronal differentiation of NPCs. NPCs were treated with FNZ for mitochondrial elimination, followed by differentiation for 2 weeks. Then cells were immuno-labeled with stem cell marker NESTIN, neuron marker TUJ, or glia marker GFAP. Representative confocal microscopy images are shown in (C). Scale bars, 10 μm. Quantification of the fraction of NESTIN-positive (D), TUJ-positive (E) and GFAP-positive (F) cells is shown. Error bars = SD. Differences were established using t-test (n = 3 independent experiments; *P < 0.05, ***P < 0.001).
Supplementary Table

Table S1. Sequence analysis of sgRNA target sites in KO cells. Briefly, a nearly 250bp region flanking the sgRNA target site was amplified by PCR and cloned into a T vector for each KO cell line. ~8-12 T clones for each KO cell line were analyzed by Sanger sequencing for disruptions at the sgRNA target site.

| Gene   | Mutation                                      |
|--------|-----------------------------------------------|
| SYT5   | 4 nt del, 8 nt del, 24 nt del, 1 nt in        |
| FBXO30 | 4 nt del, 1 nt in                             |
| AGMAT  | 8 nt del, 13 nt del, 5 nt del, 14 nt del, 8 nt in |
| BAX    | 1 nt del, 2 nt del, 3 nt del, 10 nt del, 179 nt del, 1 nt in, 2 nt in |
| P2RX7  | 1 nt del, 3 nt del, 8 nt del, 10 nt in, 1 nt in, 2 nt in |
| PTPRN  | 4 nt del, 1 nt in, 2 nt in, 3 nt in, 17 nt in, wt |
| FEM1C  | 1 nt del, 2 nt del, 4 nt del, 10 nt del, 162 nt del, 1 nt in, 2 nt in |
| LGI3   | 3 nt del, 6 nt del, 8 nt del, 34 nt del       |
| NDUFS4 | 3 nt del, 4 nt del, 7 nt del, 1 nt in, 14 nt in, wt |
| ATP5A1 | 10 nt del, 36 nt del, 1 nt in, 24 nt in, wt   |
| HKDC1  | 8 nt del, 26 nt del, 1 nt in, 2 nt in, 6 nt in, 7 nt in, wt |
| BACE1  | 3 nt del, 4 nt del, 7 nt del, 10 nt del, 13 nt del, 18 nt del, 1 nt in |
| GBAS   | 1 nt del, 3 nt del, 20 nt del, 22 nt del, 64 nt del, 109 nt del, wt |
| FNBP1  | 1 nt del, 6 nt del, 7 nt del, 20 nt del, 1 nt in, 2 nt in, 14 nt in, 23 nt in |
| UBR7   | 2 nt del, 4 nt del, 14 nt del, 1 nt in, 7 nt in, 8 nt in, 20 nt in, 22 nt in |
Table S2. Target sequence for shRNA.

| Gene | ID     | Target sequence         |
|------|--------|-------------------------|
| ULK1 | shULK1-a | GCCCTTTGCGTTATATTGTAT  |
|      | shULK1-b | CGCATGGACTTCGATGAGTTT  |
| ATG5 | shATG5-a | CCTTTCATTCCAGAAAGCTGTTC |
|      | shATG5-b | CCTGAACAGAATCATCCTTAA  |
| PINK | shPINK1-a | CGGCTGGAGGAGTATCTGATA  |
|      | shPINK1-b | GAAGCCACCATTGCCTACATTG |
| RAB9 | shRAB9-a | CCGAGGATAGGTCAGATCATT  |
|      | shRAB9-b | GACAACGGCGACTATCCTTAT  |
| FUNDC1 | sh-FUNDC1-a | GATTAAGAAACGAGCGAACAA |
|      | shFUNDC1-b | GCAGCACCTGAAATCAACA   |
| VAMP2 | shVAMP2-a | CATCATCCTCATCACTCATC   |
|      | shVAMP2-b | CATGAGGTTAACGTGGACAA   |
| STX4 | shSTX4-a | CCGTCAACAACAAGAATGAGAA |
|      | shSTX4-b | GCTGCACGACATATTCACTTT  |
| Antibodies                                      | Company       | Cat#         |
|------------------------------------------------|---------------|--------------|
| Rabbit polyclonal anti TOM20                   | Proteintech   | Cat#11802-1-AP |
| Rabbit polyclonal anti TOM20                   | Abcam         | Cat# ab78547 |
| Mouse monoclonal anti TOM20                    | Abcam         | Cat#ab56783  |
| Mouse monoclonal anti TUJ                       | Abcam         | Cat#ab7751   |
| Rabbit polyclonal anti TUJ                       | Abcam         | Cat#ab18207  |
| Rat monoclonal anti GFAP                        | Invitrogen    | Cat#13-0300  |
| Mouse Monoclonal anti GFAP                      | Millipore     | Cat#MAB360   |
| Chicken polyclonal anti TH                      | Millipore     | Cat# AB9702  |
| Rabbit polyclonal anti PHB1                     | Cell signaling| Cat#2426s    |
| Rabbit polyclonal anti HSP60                    | Abcam         | Cat#ab46798  |
| Rabbit polyclonal anti VDAC                     | Cell signaling| Cat#4661     |
| mouse monoclonal anti MTCO1                    | Abcam         | Cat#ab14705  |
| Rabbit polyclonal anti ATP8                     | Santa Cruz    | Cat#sc-84231 |
| Rabbit polyclonal anti ATP5a                    | Proteintech   | Cat#14676-1-AP |
| Rabbit polyclonal anti H3                       | Abcam         | Cat#ab1791   |
| Rabbit monoclonal anti VAMP2                    | Abcam         | Cat#ab181869 |
| Rabbit polyclonal anti beta TUBLIN              | Abcam         | Cat#ab134185 |
| mouse monoclonal anti ACTIN                     | Sigma-Aldrich. | Cat#A2228   |
**Table S4. Primers for PCR.**

| Gene     | Species | Forward primer sequence | Reverse primer sequence |
|----------|---------|-------------------------|-------------------------|
| **FEM1C** | Human   | ATAGGGCTAAAGGCCTG        | TGGTTAICTCTGCCCCCAAAC  |
|          |         | CTG                     | A                       |
| **BAX**  | Human   | CCCCGGGGAATGTAG          | TAGAAAAAGGCGACACCCC    |
|          |         | GAT                     | G                       |
| **UBR7** | Human   | GCAACGCAACTGTGGTTTT      | AAAGGCTCTAGAAACAACCT   |
|          |         | GGT                     | CTCTCC                  |
| **FBXO30**| Human   | GGCTCTGAATTTGTGCAG       | TTTTTGATTGCATGGGTCGC   |
|          |         | TTTTG                  | A                       |
| **BACE1**| Human   | TCTGTTGGTCGTGGTGAAC      | ATGTGGGGTTTTCGTCTCCC   |
|          |         | AC                      |                          |
| **ATP5A1**| Human  | ACAGGTCAAGTCGTGAAGAT     | ACACGTAGTACAGGCCGTGA   |
|          |         | AGAT                   | A                       |
| **NDUFS4**| Human  | TCTGCCGAGCAACAGCTCT     | CAGTGTTACTTAACAGCGTC   |
|          |         | TGT                    | A                       |
| **LGI3** | Human   | GGGGCAACTCACTCAACGT      | GTTGAAACGATGGAGCCCCACT |
|          |         | TGT                    |                          |
| **P2RX7**| Human   | AGTCTGCCTAGTCTCTGC      | GAGAGTTGGTGTGATCGGGGT  |
|          |         | CT                     |                          |
| **PTPRN**| Human   | TGATCTTCACAACAGCCCC     | GTGACCATTCCTGCACCTCT   |
|          |         | CC                     |                          |
| **SYT5** | Human   | CAGGTGGCAGACAAGCCAGTGA  | AGGGTGAAGGCAAGCAGTTG   |
|          |         | TGA                    | G                       |
| **HKDC1**| Human   | TTAGTCCGCAACACCTCG      | GCTTCCGCATCCAATACCTG   |
|          |         | CT                     |                          |
| **GBAS** | Human   | AAGGTTAACTCCACATGGCGT   | AATGCCCCATGTTTGGCCTATG |
|          |         |                         |                          |
| **FNBP1**| Human   | CTCCGGGCTTTCCGCC        | CCTCCAGAGCGAAGACGG     |
|          |         |                         |                          |
| **sgRNA-GECKO**| Human | AATGGACTATCATATGCTTACCGT | ACTATTTTCCCTGGCACTGT   |
| Gene   | Species | Forward primer sequence   | Reverse primer sequence         |
|--------|---------|---------------------------|---------------------------------|
| MT-ND1 | Human   | ACGCCATAAAAACTCTCCACC     | GGGTTGATAGTAGAAGAGCG            |
|        |         | AAAG                       | ATGG                            |
| MT-ND4 | Human   | ACCTTGGCTATCATCACC         | AGTGCGATGAGTAGGGGAAGG           |
|        |         | AT                         | G                               |
| MT-CO1 | Human   | GATTTTTTCGGTGACC          | CTCAGACCATACTATGATC             |
|        |         | CCCTGAAA                    | G                               |
| GAPDH  | Human   | GGAGCGAGATCCCCCTCAA        | GGCTGTGTCATACTTCATG             |
|        |         | AT                         | GG                             |
| ACTIN  | Human   | CATGTACGTTGCTATCCAG        | CTCCTTAATGCACGCAG               |
|        |         | C                          | G                               |
| ULK1   | Human   | GGCAAGCTTCGAGTTCTCCCCG     | CGACCTCCAATCAGCAG               |
|        |         |                            | CACCCGGTGACCAACGTCA             |
| ATG5   | Human   | AAAGATGTGTCTCGGAGATGT      | CACTTTGTCACTAATCCAAACGT         |
|        |         | GT                         | CA                             |
| PINK1  | Human   | CCCAAGCAACTAGCCCCCTC       | GGCAGACACATCCAGGTAAGTC          |
| RAB9   | Human   | AGGGACAACGGGCGACTATC       | TCTGACCTATCTGCCGTAGCA           |
| FUNDC1 | Human   | CCTCCCAGCAGCTATGAAA        | AAAACACTCGATTCCACCAGCT          |
|        |         | GTGA                       |                                 |
| VAMP2  | Human   | CTCAAGCGCAAATACTGTTG       | TGATGGCGCAAATCAGCTCC            |
|        |         | GG                         |                                 |
| STX4   | Human   | CGGACAATTTCGGCAGACTATT     | TTCTGGGGCTCTATGGCCTT           |
|        |         |                            |                                 |
| Gene  | sgRNA Target sequence          |
|-------|-------------------------------|
| NT1   | CACCGCTGAAAAAGGAAGGAGTTGA     |
| BAX   | AGCGAGTGTCACACACATCAAACCTGC  |
| UBR7  | CAAGCTAAACAAATTTCCTGC         |
| FBXO30| TCTCGTGTCACACACATCAAACCTGC  |
| FEM1C | TGTAAAGAGCAGCGATATGC          |
| BACE1 | TCCTGCATCGCTACTACCAG          |
| ATP5AI| GACAGACCAACGTTAATTGC          |
| NDUFS4| GACTGCATTTATTGCAGAATGC       |
| LGI3  | GAGTTTGATGCATGCCATCACCAC     |
| P2RX7 | ATCTCTTACCAGACCAGAAGT        |
| PTPRN | CACCCAGTATGTGATCTCTCTAG      |
| SYT5  | TCGTACCGGCCTCCGTGGTGC        |
| HKDC1 | AATGTTTGCGGTCCACTTGGAGAGT    |
| GBAS  | GGGACTTGGAAACAGTGGAATG       |
| FNBP1 | TGAGCTGGGGCAACGGAGCTC        |
Other Supplementary Materials for this manuscript

Data S1. (separate file)
RNA-seq analysis for cells collected at 1 day treatment.

Data S2. (separate file)
Gene ontology analysis of genes showing increasing or decreasing expression at multiple time-points from RNA-seq.

Data S3. (separate file)
GeCKO result with gene ontology analysis.