Expanded View Figures

Figure EV1. p13 knockdown prevents mitochondrial dysfunction and cytotoxicity.

A, B Decreased expression of *p13* mRNA (A) or p13 protein (B) in cells 72 or 96 h after infection with lentiviral vectors expressing p13 shRNA. mRNA levels were quantified by real-time RT–PCR (A). The levels of cleaved PARP were normalized to those of β-actin (B). Representative images (left) and their quantification (right) were shown.

C Decreased expression of *p13* mRNA in cells 96 h after infection with lentiviral vectors expressing p13 shRNA #2. mRNA levels were quantified by real-time RT–PCR.

D–F Prevention of the rotenone-induced decrease in $\Delta\Psi$m (D), increase in PARP cleavage (E) and decrease in complex I activity (F) in cells infected with lentiviral vectors expressing p13 shRNA #2. Complex I activity was measured on the basis of NADH-oxidizing activity. Seventy-two hours after infection, cells were exposed to vehicle or 100 nM rotenone for 24 h.

G, H Prevention of the MPP^+-induced decrease in $\Delta\Psi$m (G) and release of LDH (H) in p13 shRNA-infected cells. LDH release was measured in culture medium of SH-SY5Y cells using the LDH assay kit. Seventy-two hours after infection, cells were treated with vehicle or 5 mM MPP^+ for 24 h.

I Decreased expression of *PINK1* mRNA in cells 96 h after infection with lentiviral vectors expressing PINK1 shRNA. mRNA levels were quantified by real-time RT–PCR.

J, K Prevention of the PINK1-knockdown-induced decrease in $\Delta\Psi$m (J) and increase in PARP cleavage (K) in p13 shRNA-infected cells 96 h after infection with lentiviral vectors.

Data information: $\Delta\Psi$m was determined by measuring the TMRE fluorescence levels (D, G, J). The levels of cleaved PARP were normalized to those of β-actin (E, K). Representative images (left) and their quantification (right) were shown. All data are presented as the mean ± SEM ($n = 3$). *$P < 0.05$, **$P < 0.01$ by Student's *t*-test (A–C and I) or the Tukey–Kramer test (D–H, J and K).

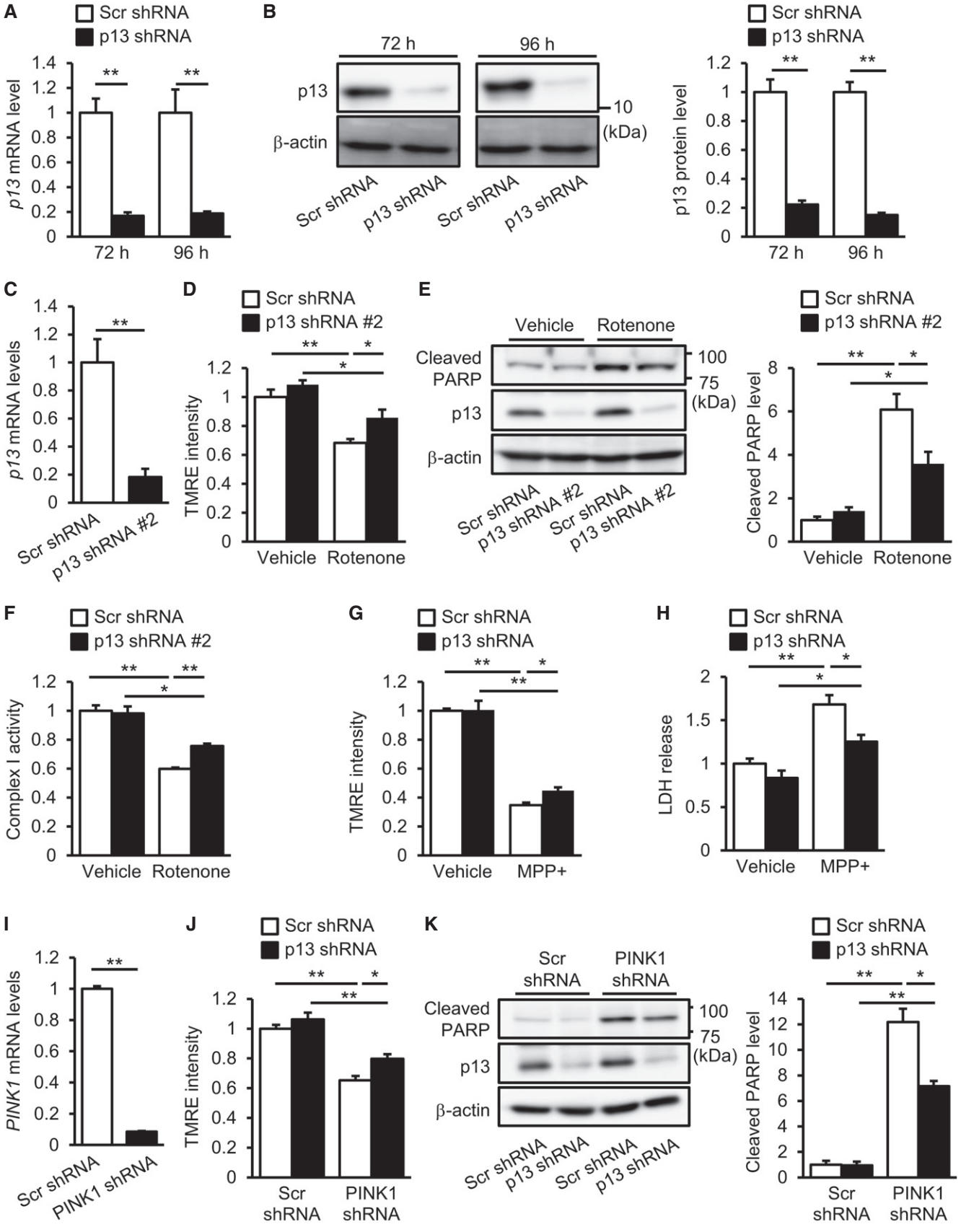

A
Scr shRNA
p13 shRNA
p13 mRNA level
72 h
96 h
B
72 h
96 h
p13
β-actin
10
(kDa)
Scr shRNA
p13 shRNA
p13 protein level
C
p13 mRNA levels
Scr shRNA
p13 shRNA #2
D
Scr shRNA
p13 shRNA #2
TMRE intensity
Vehicle
Rotenone
E
Vehicle
Rotenone
Cleaved PARP
p13
β-actin
100
75
(kDa)
Cleaved PARP level
F
Complex I activity
G
TMRE intensity
MPP+
H
LDH release
I
PINK1 mRNA levels
PINK1 shRNA
J
K
Scr shRNA
PINK1 shRNA

Figure EV1.

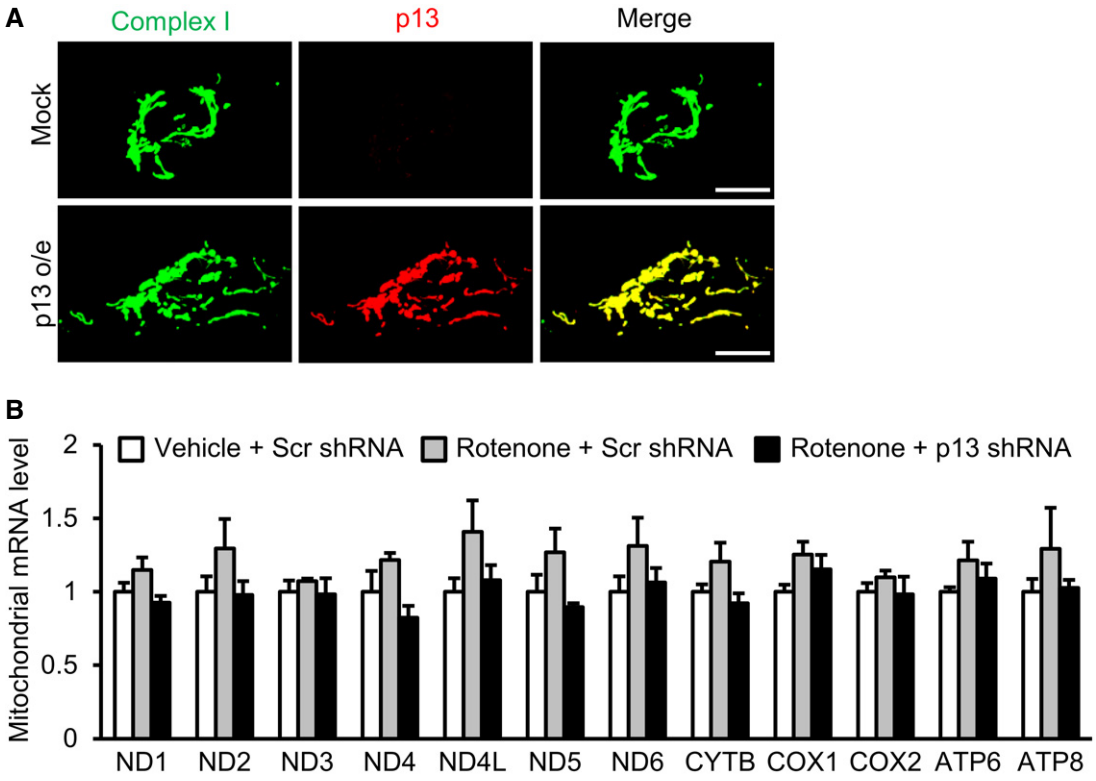


Figure EV2. Involvement of p13 in complex I and mtDNA.

A Co-localization of overexpressed p13 and complex I proteins in cells infected with lentiviral vectors expressing FLAG-tagged p13 (p13 o/e). Overexpressed p13 was detected using an antibody against p13. Scale bars, 10 μm.

B Effects of p13 knockdown on the mRNAs encoded by the mitochondrial genome in cells. Seventy-two hours after infection with lentiviral vectors expressing scrambled shRNA (Scr shRNA) or p13 shRNA, cells were treated with vehicle or rotenone for 24 h. mRNA levels of each gene were normalized to those in vehicle-treated and Scr shRNA-infected cells. Data are presented as the mean ± SEM (n = 3).

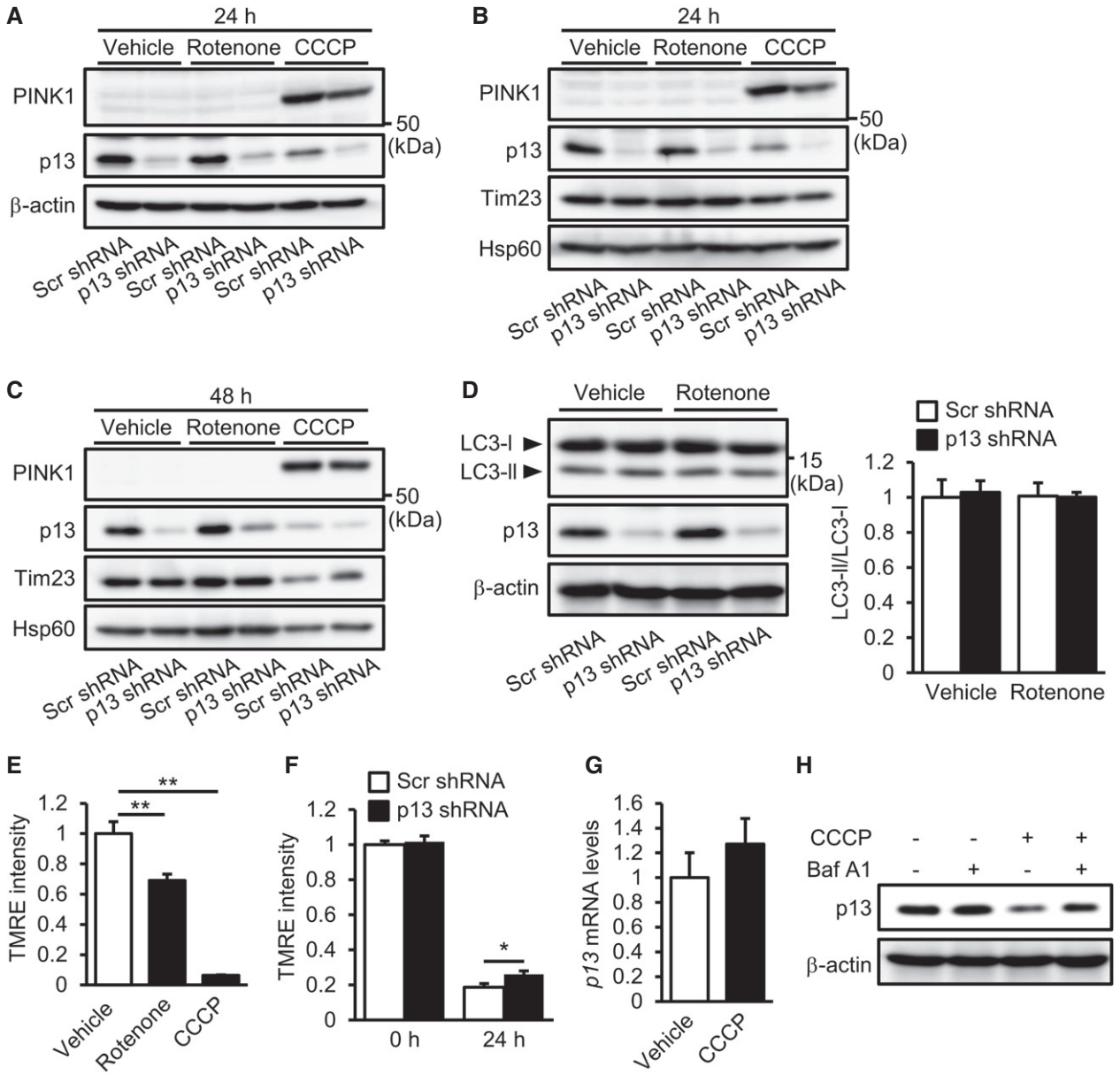


Figure EV3. p13 knockdown did not affect PINK1-associated autophagy in rotenone-treated SH-SY5Y cells.

A PINK1 accumulation in p13 shRNA-infected SH-SY5Y cells. Seventy-two hours after infection, cells were exposed to vehicle, 100 nM rotenone or 10 μM CCCP for 24 h. Total cell lysates were subjected to Western blotting with antibodies against PINK1, p13 and β-actin.

B, C PINK1 accumulation in isolated mitochondria from p13 shRNA-infected cells. Seventy-two hours after infection, cells were exposed to vehicle, 100 nM rotenone or 10 μM CCCP for 24 h (B) or 48 h (C). Mitochondrial fractions were purified and subjected to Western blotting with antibodies against PINK1, p13, Tim23 and Hsp60.

D No significant change in autophagy induction by p13 knockdown in p13 shRNA-infected cells. Seventy-two hours after infection, cells were exposed to vehicle or 100 nM rotenone for 24 h. Autophagy was measured by the conversion of cytosolic LC3-I to the autophagosome-associated LC3-II. Representative images (left) and the quantification of the ratio of LC3-II to LC3-I (right). Total cell lysates were subjected to Western blotting with antibodies against LC3, p13 and β-actin.

E Decrease in ΔΨm in SH-SY5Y cells 24 h after treatment with 100 nM rotenone or 10 μM CCCP. ΔΨm was determined by measuring the TMRE fluorescence levels.

F Attenuated CCCP-induced decrease in ΔΨm in p13 shRNA-infected SH-SY5Y cells. ΔΨm was determined by measuring the TMRE fluorescence levels. Seventy-two hours after infection, cells were exposed to vehicle or 10 μM CCCP for 24 h.

G No change in expression of *p13* mRNA 24 h after treatment of 10 μM CCCP in SH-SY5Y cells. mRNA levels were quantified by real-time RT–PCR.

H Blockage of the CCCP-induced decrease in p13 expression by bafilomycin A1 (BafA1) in SH-SY5Y cells. Cells were exposed to vehicle or 10 μM CCCP for 24 h. Cells were treated with or without 50 nM BafA1 for 2 h before harvest. Total cell lysates of these cells were subjected to Western blotting with antibodies against p13 and β-actin.

Data information: All data are presented as the mean ± SEM ($n = 3$). *$P < 0.05$, **$P < 0.01$ by Dunnett's test (E) or the Tukey–Kramer test (F).

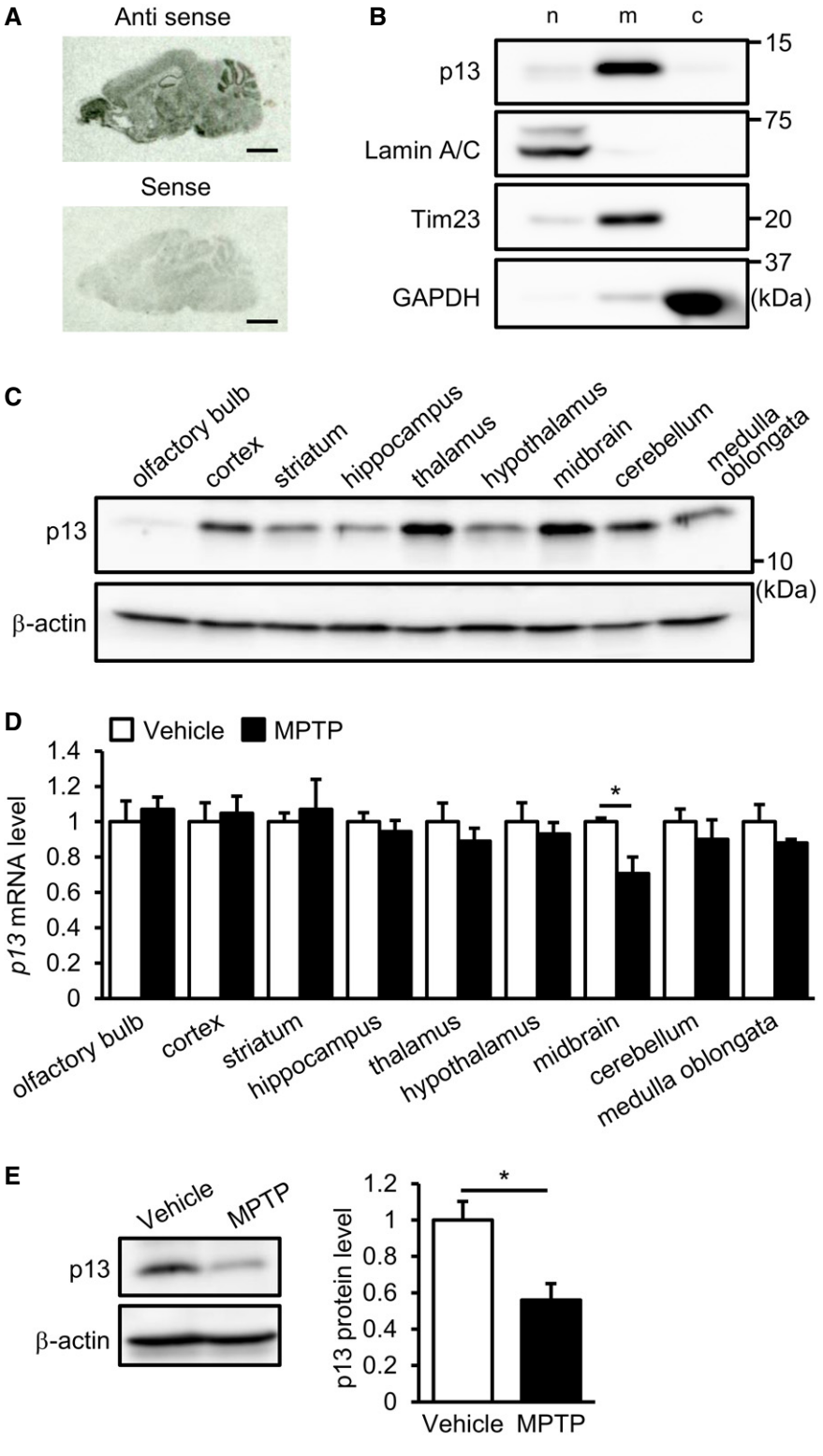


Figure EV4. Distribution of p13 expression in the brain.

A Distribution of *p13* mRNA in sagittal brain sections of mice analysed by *in situ* hybridization. Scale bars, 2 mm.

B Distribution of p13 in nuclear (n)-, mitochondria (m)- and cytosol (c)-enriched fractions of the mouse brain. The nuclear, mitochondria and cytosol fractions were purified and subjected to Western blotting with antibodies against p13, Lamin A/C (a nuclear marker), Tim23 (a mitochondrial marker) and GAPDH (a cytoplasmic marker).

C Expression of p13 protein in various brain regions of mice. Brain lysates of each region were prepared and subjected to Western blotting with antibodies against p13 and β-actin.

D Expression of *p13* mRNA in various brain regions of mice treated with 10 mg/kg MPTP. mRNA levels were quantified by real-time RT–PCR. The mRNA level in each brain region of the MPTP-treated mice was normalized to that of vehicle-treated mice.

E Expression of p13 protein in the midbrain of mice treated with 10 mg/kg MPTP. Total lysates were subjected to Western blotting with antibodies against p13 and β-actin. p13 levels were normalized to β-actin levels.

Data information: All data are presented as the mean ± SEM ($n = 3$). *$P < 0.05$ by Student's *t*-test (D and E).

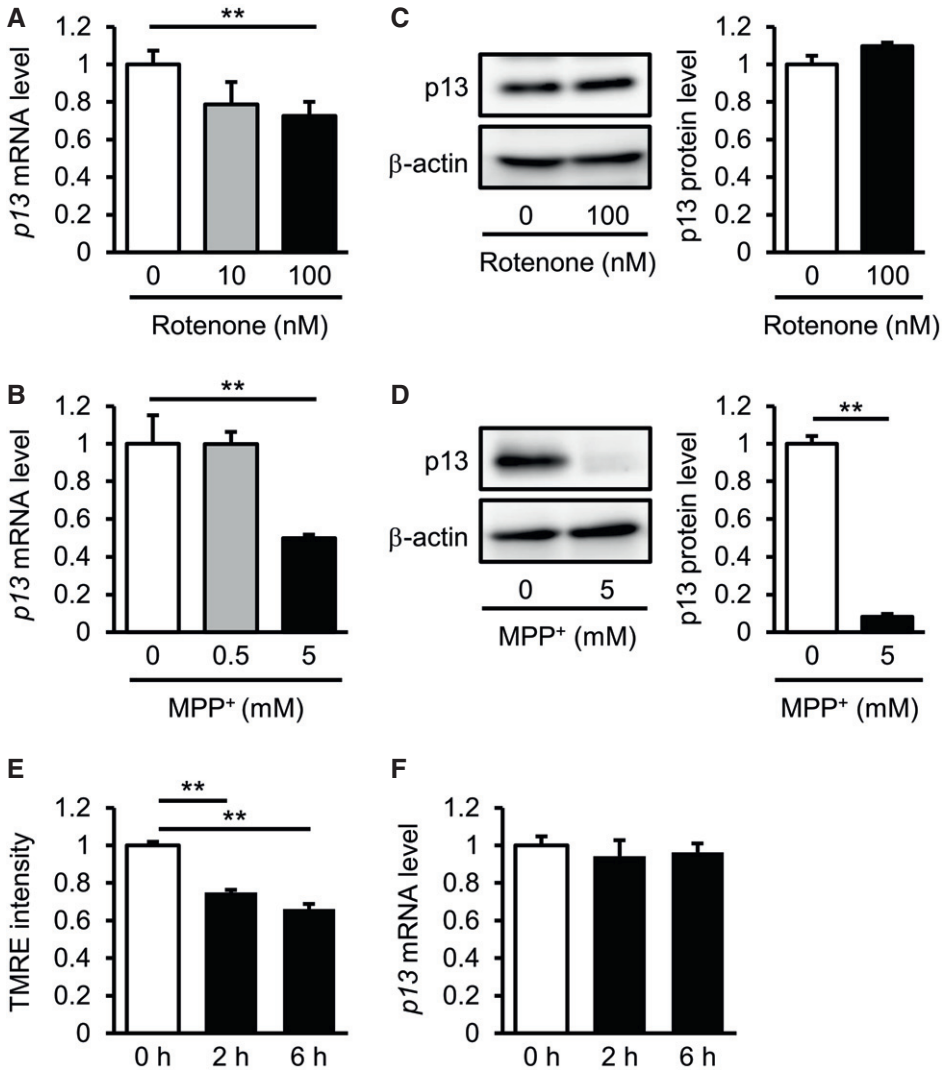


Figure EV5. p13 expression in the parkinsonian toxicant-treated SH-SY5Y cells.

A, B Decreased expression of *p13* mRNA 24 h after treatment with 100 nM rotenone (A) or 5 mM MPP^+ (B) in SH-SY5Y cells. mRNA levels were quantified by real-time RT–PCR.

C No change in expression of p13 protein 24 h after treatment with 100 nM rotenone in SH-SY5Y cells. Total cell lysates were subjected to Western blotting with antibodies against p13 and β-actin. The levels of p13 were normalized to those of β-actin.

D Decreased expression of p13 protein 24 h after treatment with 5 mM MPP^+ in SH-SY5Y cells. Total cell lysates were subjected to Western blotting with antibodies against p13 and β-actin. The levels of p13 were normalized to those of β-actin.

E Decrease in $\Delta\Psi$m in SH-SY5Y cells 2 or 6 h after treatment with 100 nM rotenone. $\Delta\Psi$m was determined by measuring the TMRE fluorescence levels.

F Expression of *p13* mRNA in SH-SY5Y cells 2 or 6 h after treatment with 100 nM rotenone. mRNA levels were quantified by real-time RT–PCR.

Data information: All data are presented as the mean ± SEM ($n = 3$). $^{**}P < 0.01$ by Dunnett's test (A, B and E) or Student's *t*-test (D).

Figure EV6. Generation of *p13* knockout mice using the CRISPR/Cas9 system.

A Schematic diagram of single-guide RNAs (sgRNAs) targeting the mouse *p13* gene. Two sgRNA sequences, sgRNA1 and sgRNA2, are shown in red. The protospacer adjacent motif (PAM) sequences are shown in green.

B Genomic sequence analysis of the obtained $p13^{+/+}$, $p13^{+/-}$ and $p13^{-/-}$ mice. $p13^{+/-}$ and $p13^{-/-}$ mice harboured a 71-bp deletion in exon 1 of the *p13* gene.

C Genotyping indicated of $p13^{+/+}$, $p13^{+/-}$ and $p13^{-/-}$ mice by genomic PCR using primers for *p13* (see Materials and Methods).

D Expression of p13 in mitochondria from the brains of $p13^{+/+}$, $p13^{+/-}$ and $p13^{-/-}$ mice. Mitochondrial fractions were purified and subjected to Western blotting with antibodies against p13 and Hsp60 (control).

E Kaplan–Meier survival curves for $p13^{+/+}$, $p13^{+/-}$ and $p13^{-/-}$ mice ($p13^{+/+}$, $n = 86$; $p13^{+/-}$, $n = 143$; and $p13^{-/-}$, $n = 65$).

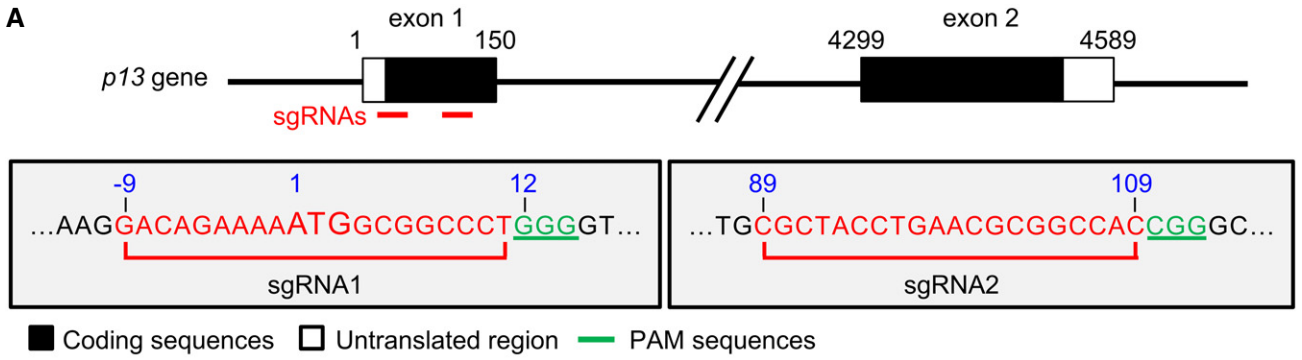
F No significant change in the MPTP-induced decrease in optical density of TH^+ fibres in the striatum of $p13^{+/+}$ and $p13^{+/-}$ mice, as analysed by immunohistochemistry ($n = 3$). Representative images (left) and the quantification of optical density of TH^+ fibres in the striatum (right). Scale bar, 1 mm.

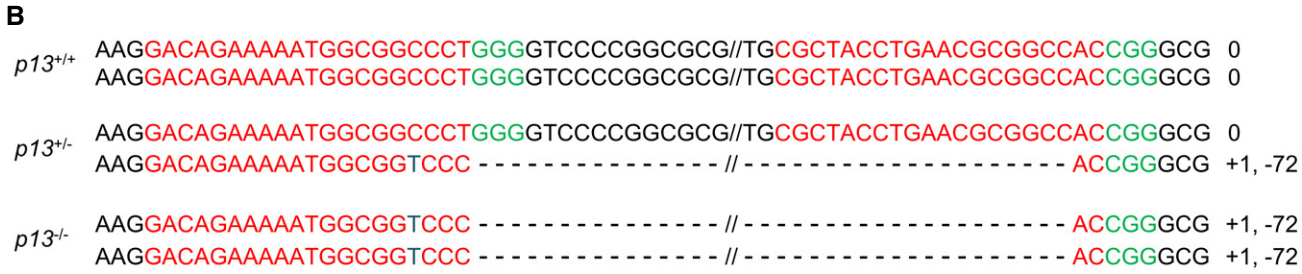
G No significant changes in autophagy induction in the substantia nigra of MPTP-treated $p13^{+/+}$ and $p13^{+/-}$ mice. Autophagy was measured by the conversion of LC3-I to LC3-II. Total lysates were subjected to Western blotting with antibodies against LC3, p13 and β-actin.

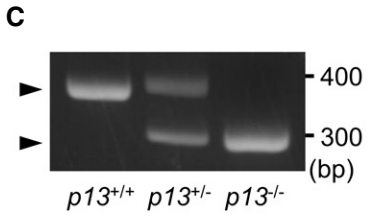
H Expression of p13, NDUFB8 and NDUFS4 in the brains of $p13^{+/+}$ and $p13^{-/-}$ mice. Total cell lysates were subjected to Western blotting with antibodies against p13, NDUFB8, NDUFS4 and β-actin.

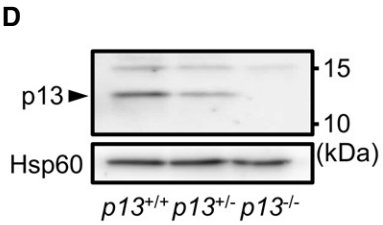
I, J Complex I activity in the brains (I) or the hearts (J) of $p13^{+/+}$, $p13^{+/-}$ and $p13^{-/-}$ mice. Brain or heart extracts were prepared, and complex I activity was measured based on NADH-oxidizing activity.

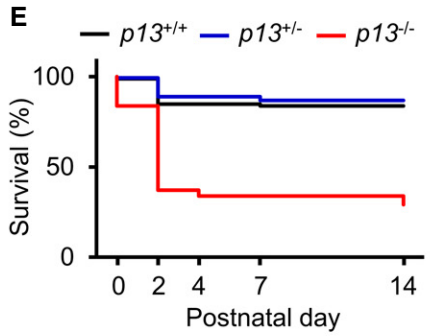
Data information: All data are presented as the mean ± SEM ($n = 3$). *$P < 0.05$ by the Tukey–Kramer test (F and J).

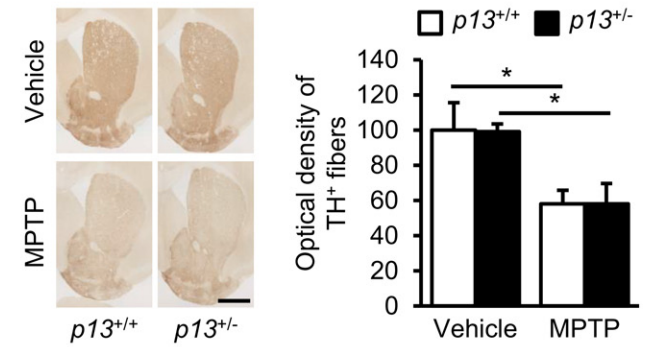


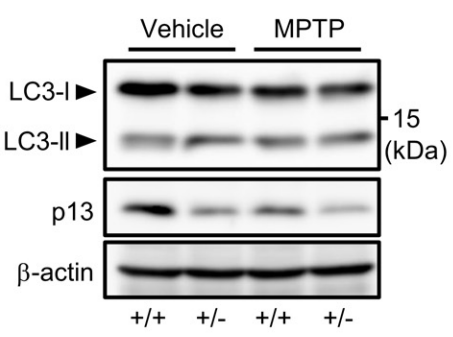


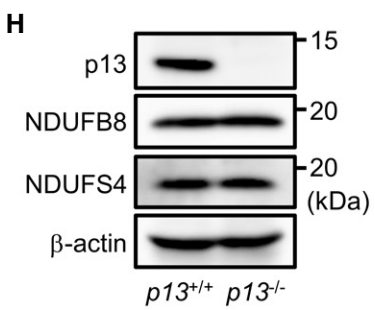


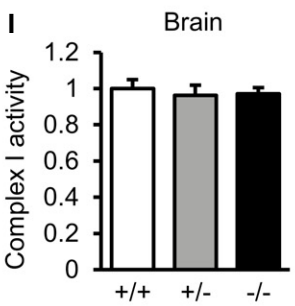












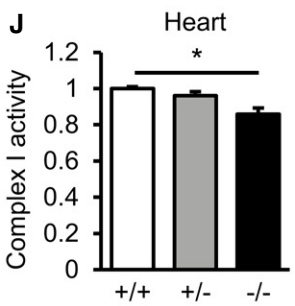


Figure EV6.