Miro1 Marks Parkinson's Disease Subset and Miro1 Reducer Rescues Neuron Loss in Parkinson's Models.

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Supplementary Information

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Association Tests. Related to Figure 1. The association between the ratio of Miro1 intensity (CCCP/DMSO) and PD (A), gender (B), age at sampling (C), onset age (D), years with PD (E), or clinical manifestations (F-H). The Miro1 intensities are reported in Table S1A.

Figure S2. Validation of ELISA. Related to Figure 1. (A) Relative β-actin and Miro1 protein levels in fibroblasts. No difference among all conditions for β-actin (P>0.999, One-Way ANOVA Tukey Test with adjustment). Relative protein levels are calculated from the standard plots, one of which is shown in (B) or (F). n=4. For "CCCP, 0 hr", the solvent of CCCP, DMSO, was applied for 6 hrs. (B) β-Actin signals correlate with serial dilutions of fibroblast lysates. (C) Swapping the specific coating chicken anti-β-actin with mouse anti-Miro1 eliminates β-actin protein signals in fibroblasts. Relative protein levels are calculated from the standard plots, one of which is shown in (B). n=4. (D) Miro1 signals show linear dependency on serial dilutions of lysates of HEK cells with exogenously expressed Miro1. (E) Altering the specific coating mouse anti-Miro1 by swapping it with mouse anti-β-actin or mouse anti-ATP5β, or by omitting it, eliminates Miro1 protein signals in HEK cells. Relative protein levels are calculated from the standard plots (Miro1-Myc), one of which is in (D). n=4. (F) Miro1 signals show linear dependency on concentrations of purified Miro1 protein. This plot represents the standard for (A) and (G). (G) Swapping the specific coating mouse anti-Miro1 with chicken anti-β-actin eliminates Miro1 protein signals in fibroblasts.

Figure S3. Validation of Additional Cohorts and Identification of Miro1 Reducer. Related to Figure 1-4. (A) Relative β-actin protein levels in fibroblasts used in Figure 1. No difference among all conditions (P>0.4494, One-Way ANOVA Tukey Test with adjustment). n=4. (B) Several lines used in ELISA in Figure 1 were validated by Western blotting. Fibroblast lysates were immunoblotted as indicated, and the protein band intensity of Miro1 is normalized to that of β-actin from the same blot and compared to "CCCP, 0 hr" of the same subject. n=4 independent experiments. For "CCCP, 0 hr", the solvent of CCCP, DMSO, was applied for 6 hrs. (C) The

ensemble of the poses of Miro1 Reducer in the target binding sites within the C-terminal GTPase domain of Miro1. Residues are labeled at their alpha carbons. Seven amino acids residues (K427, N428, S432, Q446, K528, D530, K454) make frequent molecular interactions, especially via hydrogen-bonds, with the poses of Miro1 Reducer. Images were generated using Molsoft ICM Browser. (D) The top 11 compounds from AI were functionally screened in flies. Wild-type flies (w^{1118}) were fed with DMSO alone (no compound) or compound in DMSO at 250 μM for 7 days, lysed, and blotted as indicated. The band intensities are normalized to those of β-actin from the same blots. n=4.

Figure S4. Miro1 Reducer in Human Cell and Fly Models. Related to Figure 2-4. (A) Fibroblasts from Healthy-1 and PD-2 were treated, lysed, and blotted as indicated. Band intensities are normalized to those of GAPDH from the same blots and compared to "Healthy-1, no treatment". n=4 independent experiments. (B) Fibroblasts from PD-2 were treated and immunostained as indicated. The intensity was quantified for each cell across 264-320 cells from 3 independent experiments, compared to "no treatment". (C) Fibroblasts were treated, immunoprecipitated (IPed) with anti-Miro1, and blotted as indicated. Similar results were observed twice. (D) Similar as in (C), fibroblasts were treated as indicated, and IPed with anti-Miro1. The GTPase activity of the IPed Miro1 protein was measured as described in Method. Bar graphs show the average of two independent experiments. (E) qPCR analysis was performed using RNA isolated from fibroblasts. *Miro1* values were normalized to those of the internal control *GAPDH*. n=4 independent experiments. (F) Fibroblasts were pretreated with Miro1 Reducer for 30 hrs, and stained with 2 μM MitoSox for 10 min at 37°C. The MitoSox intensity was quantified for each cell across 106-161 cells from 3 coverslips. P=0.1412 among all conditions. (G) Fibroblasts were pretreated with

Miro1 Reducer for 30 hrs, and stained with 25 nM TMRM for 30 min at 37°C. The TMRM intensity was measured for each cell across 48-60 cells from 3 independent experiments, and expressed as a fraction of the mean of "Healthy-1, no treatment". P=0.1085. Mitochondrial morphology is largely comparable among all conditions. (H) Fibroblasts were transfected with EGFP-peroxisome, treated with Miro1 Reducer for 30 hrs, and live imaged. The image at 0 sec is pseudo-colored in green and at 60 sec in red. The movement (the intensity of non-yellow) was quantified for each cell as described in (Tsai et al., 2014) across 22-31 cells from 3 transfections. P=0.7552 among all conditions. (I) Left: The percentage of TH-positive iPSC-derived neurons out of total cells (Dapi-positive) is calculated after immunocytochemistry under 20× as described in Figure 3. P=0.0748. n=10 fields each experiment from 3-4 independent experiments. Right: iPSC-derived neurons were treated, lysed, and immunoblotted as indicated. Band intensities of Miro1 are normalized to those of ATP5β from the same blots, compared to "no treatment" except otherwise indicated. n=4. (J) PD flies were fed with 2.5 μM Miro1 Reducer for the indicated time periods and lysed for blotting DMiro. Band intensities of DMiro are normalized to those of VDAC from the same blots. n=4 independent experiments; for each experiment 5 whole flies of mutant *LRRK2* or *PINK1*, and 15 heads of mutant *SNCA* flies were used. There is a trend of DMiro reduction in all 3 PD models fed with Miro1 Reducer, although it does not reach statistical significance. (K-N) Phenotypes of 14-day old *PINK1* null males. $PINK1^{RV}$ is the precise excision control for $PINK1^{B9}$ (imprecise excision, null). Drug administration (2.5 μM) was started from day 2. (K) ATP levels. n=1 fly each experiment, total 6 experiments. (L) Percent of total flies with thoracic indentation. (M) Percent of total flies with abnormal wing posture. (N) Percent of total flies that could not fly. For (L-N), Chi-Square Test is used because the data are categorical; comparisons with "$PINK1^{RV}$, 0 μM". n=35-61. Scale bars: (B, G, H) 50 μm; (F) 100 μm.

SUPPLEMENTARY TABLE LEGENDS

Table S2. Summary of the Miro1 Phenotype in All Subjects Used in This Study, and Miro1 Values in Cells with Different Passage Numbers. Related to Figure 1. (A) Fisher Exact Test is used to determine the P values compared to PD. The Miro1 intensities with DMSO and with CCCP are compared within the same subject in either ELISA or Western by Mann-Whitney *U* Test, and the numbers of the subjects with a P>0.05 or <0.05 are defined as "No. (Miro1 DMSO v.s. CCCP P>0.05 or <0.05)". (B) Miro1 intensities are calculated as in Table S1A. No significant difference is found among different passages within the same cell line.

Figure S1

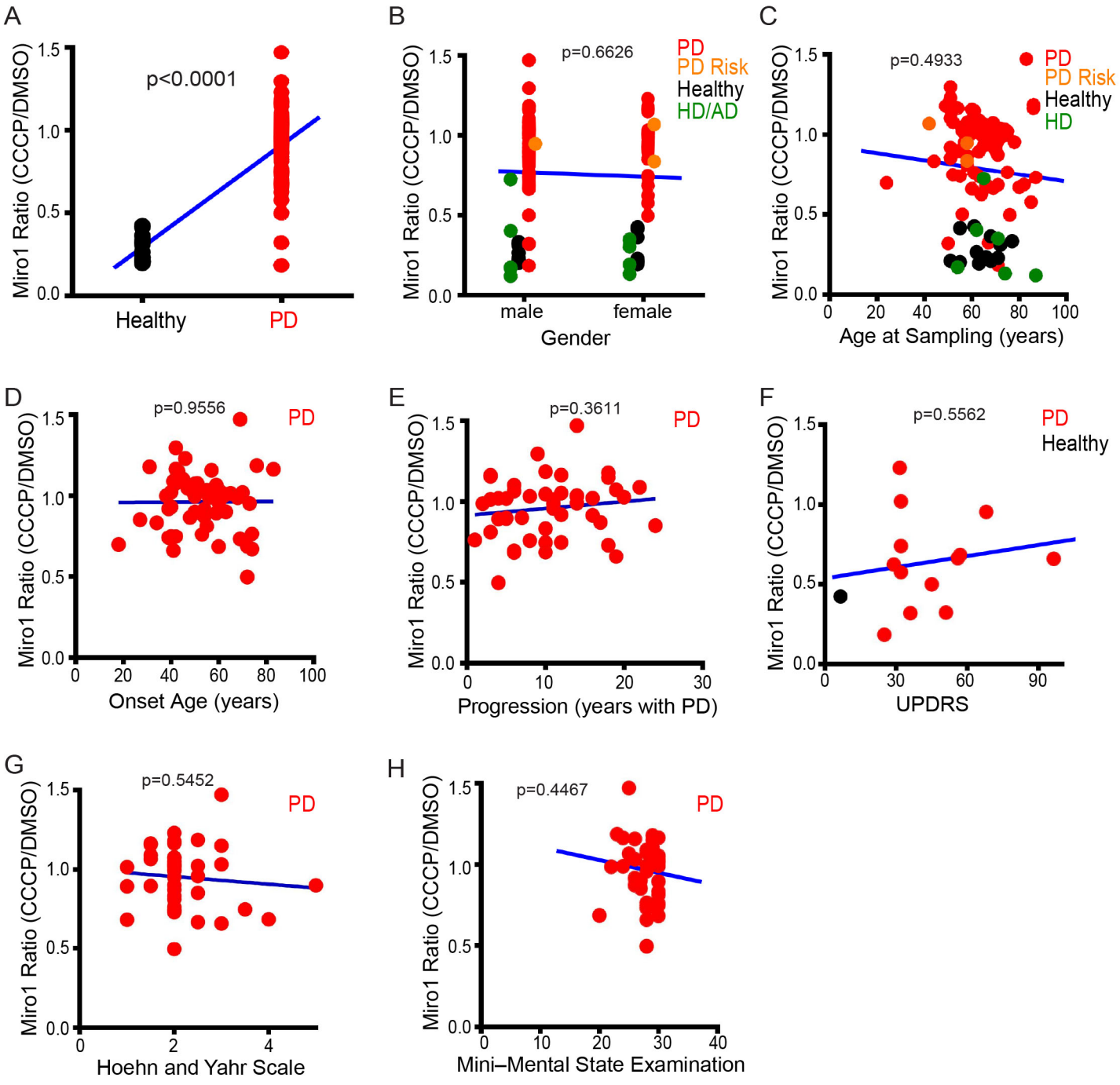
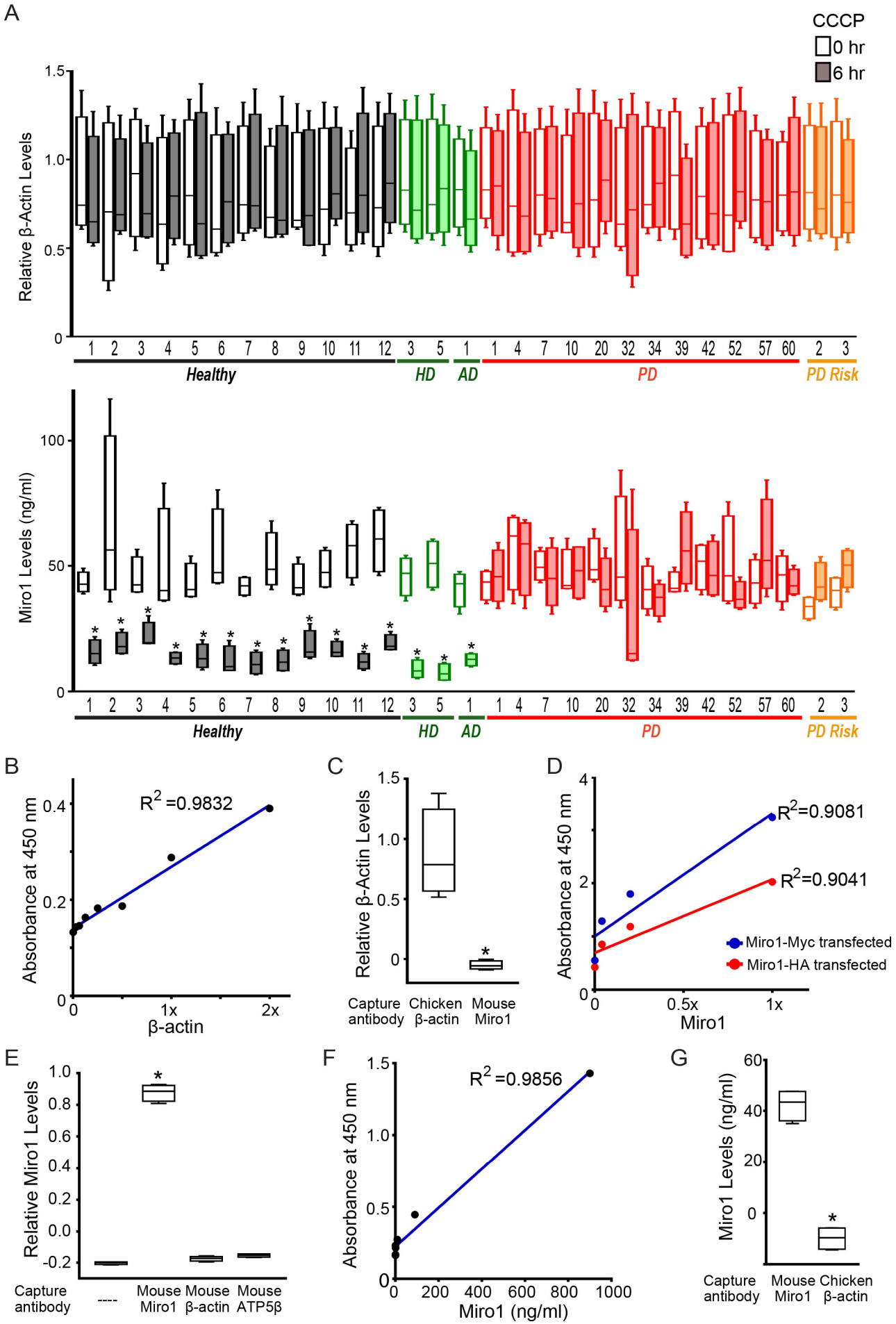
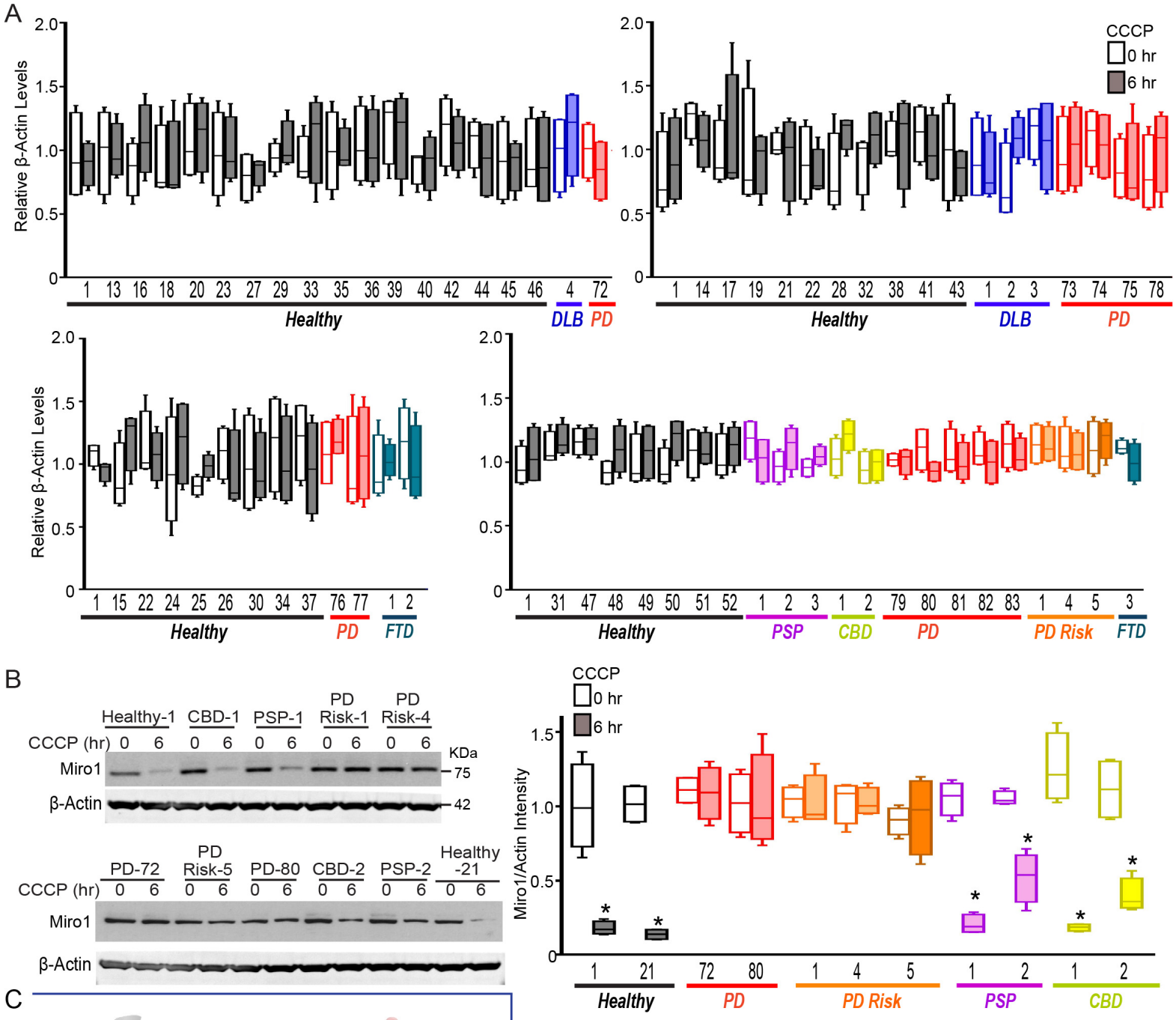

A
p<0.0001
Miro1 Ratio (CCCP/DMSO)
Healthy
PD
B
p=0.6626
PD
PD Risk
Healthy
HD/AD
male
female
Gender
C
p=0.4933
PD
PD Risk
Healthy
HD
Age at Sampling (years)
D
p=0.9556
PD
Onset Age (years)
E
p=0.3611
PD
Progression (years with PD)
F
p=0.5562
PD
Healthy
UPDRS
G
p=0.5452
PD
Hoehn and Yahr Scale
H
p=0.4467
PD
Mini–Mental State Examination

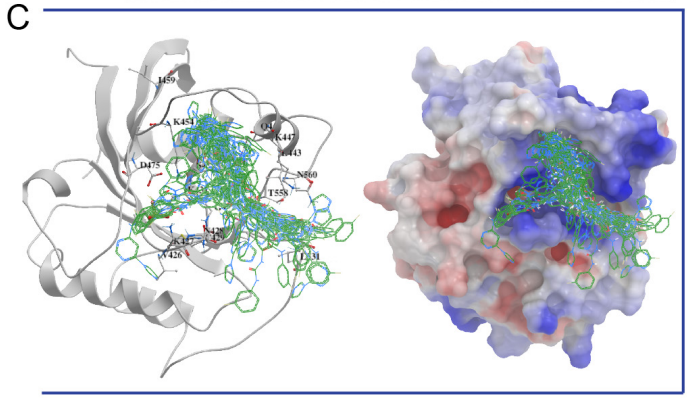
Figure S2

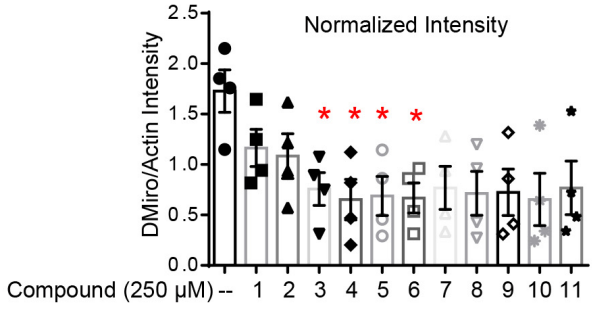

A
CCCP
0 hr
6 hr
Relative β-Actin Levels
1.5
1.0
0.5
0
1 2 3 4 5 6 7 8 9 10 11 12 3 5 1 1 4 7 10 20 32 34 39 42 52 57 60 2 3
Healthy
HD
AD
PD
PD Risk
Miro1 Levels (ng/ml)
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 3 5 1 1 4 7 10 20 32 34 39 42 52 57 60 2 3
Healthy
HD
AD
PD
PD Risk
B
Absorbance at 450 nm
0.4
0.2
0
R2 =0.9832
0
1x
2x
β-actin
C
Relative β-Actin Levels
1.5
1.0
0.5
0
*
Capture antibody
Chicken β-actin
Mouse Miro1
D
Absorbance at 450 nm
4
2
0
R2=0.9081
R2=0.9041
Miro1-Myc transfected
Miro1-HA transfected
0
0.5x
1x
Miro1
E
Relative Miro1 Levels
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
*
Capture antibody

Mouse Miro1
Mouse β-actin
Mouse ATP5β
F
Absorbance at 450 nm
1.5
1.0
0.5
0
R2 =0.9856
0
200
400
600
800
1000
Miro1 (ng/ml)
G
Miro1 Levels (ng/ml)
60
40
20
0
*
Capture antibody
Mouse Miro1
Chicken β-actin

Figure S3







D

Compound	Systemic Name
1	5-bromo-7-methyl-1H,1'H,2H,2'H-[3,3'-biindolylidene]-2,2'-dione
2	ethyl 6-(2-chloro-6-fluorophenyl)-4-methyl-2-sulfanyl-1,6-dihydropyrimidine-5-carboxylate
3	3-(3-fluorophenyl)-1-(4-{[6-(1H-imidazol-1-yl)pyrimidin-4-yl]amino}phenyl)urea
4	3-(2,3-dimethylphenyl)-1-(4-{[6-(1H-imidazol-1-yl)pyrimidin-4-yl]amino}phenyl)urea
5	3-(2-chlorophenyl)-1-(4-{[4-(dimethylamino)-6-methylpyrimidin-2-yl]amino}phenyl)urea
6	5-chloro-N4-[(3R,4S)-4-[(3,5-dimethyl-1,2-oxazol-4-yl)methoxy]oxan-3-yl]-6-methylpyrimidine-2,4-diamine
7	2-amino-6-(2,2-dimethyloxan-4-yl)-4-[2-(ethylamino)pyrimidin-5-yl]-5-propylpyridine-3-carbonitrile
8	4-(4-chloro-1-methyl-1H-indazol-3-yl)-N-methyl-6-oxo-1H,4H,5H,6H,7H-pyrazolo[3,4-b]pyridine-3-carboxamide
9	ethyl 2-{[5-(2-amino-3-cyano-5,6,7,8-tetrahydroquinolin-4-yl)pyrimidin-2-yl]amino}acetate
10	2-amino-6-(4-hydroxyphenyl)-5-methyl-4-[2-(methylsulfanyl)pyrimidin-5-yl]pyridine-3-carbonitrile
11	2-amino-6-(3-fluoro-4-methoxyphenyl)-4-(5-hydroxy-2-methoxyphenyl)pyridine-3-carbonitrile

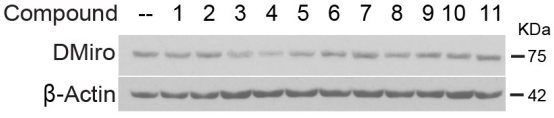
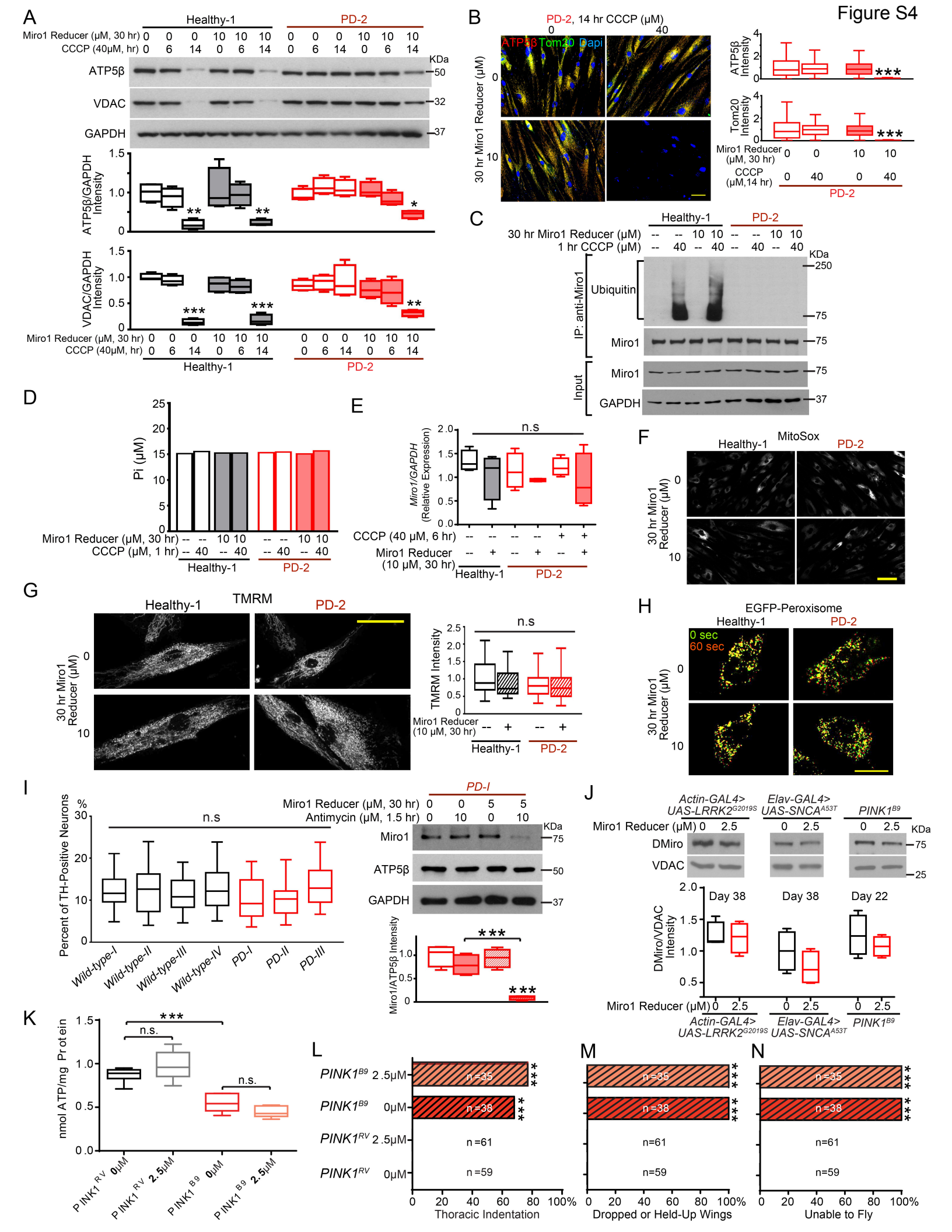


Figure S4


A
Healthy-1
PD-2
Miro1 Reducer (μM, 30 hr)
CCCP (40μM, hr)
ATP5β
VDAC
GAPDH
KDa
ATP5β/GAPDH Intensity
VDAC/GAPDH Intensity
B
PD-2, 14 hr CCCP (μM)
ATP5β Tom20 Dapi
30 hr Miro1 Reducer (μM)
ATP5β Intensity
Tom20 Intensity
CCCP (μM,14 hr)
C
30 hr Miro1 Reducer (μM)
1 hr CCCP (μM)
IP: anti-Miro1
Ubiquitin
Miro1
Input
D
Pi (μM)
E
n.s
Miro1/GAPDH (Relative Expression)
CCCP (40 μM, 6 hr)
F
MitoSox
G
TMRM
TMRM Intensity
H
EGFP-Peroxisome
0 sec
60 sec
I
PD-I
Percent of TH-Positive Neurons
Wild-type-I
Wild-type-II
Wild-type-III
Wild-type-IV
PD-I
PD-II
PD-III
Antimycin (μM, 1.5 hr)
Miro1/ATP5β Intensity
J
Actin-GAL4> UAS-LRRK2G2019S
Elav-GAL4> UAS-SNCA A53T
PINK1B9
DMiro
VDAC
DMiro/VDAC Intensity
Day 38
Day 38
Day 22
K
nmol ATP/mg Protein
n.s.
PINK1 RV 0μM
PINK1 RV 2.5μM
PINK1 B9 0μM
PINK1 B9 2.5μM
L
PINK1B9 2.5μM
PINK1B9 0μM
PINK1RV 2.5μM
PINK1RV 0μM
n=35
n=38
n=61
n=59
Thoracic Indentation
M
Dropped or Held-Up Wings
N
Unable to Fly

Disease	No. (Miro1 DMSO v.s. CCCP P<0.05)	No. (Miro1 DMSO v.s. CCCP P>0.05)	P (Fisher Exact) compared to PD	No. confirmed by ELISA	No. confirmed by Western
PD	5	78 (94%)		24	73
PD Risk	0	5 (100%)	1	5	5
Healthy	52	0 (0%)	<0.00001	52	12
HD	6	0 (0%)	<0.0001	2	6
AD	4	0 (0%)	<0.0001	1	4
DLB	4	0 (0%)	<0.0001	4	0
PSP	3	0 (0%)	0.0005	3	2
CBD	2	0 (0%)	0.0059	2	2
FTD	3	0 (0%)	0.0005	3	0

Table S2A. Summary of the Miro1 Phenotype in All Subjects Used in This Study.

Fisher Exact Test is used to determine the P values compared to PD. The Miro1 intensities with DMSO and with CCCP are compared within the same subject in either ELISA or Western by Mann-Whitney *U* Test, and the numbers of the subjects with a P>0.05 or <0.05 are defined as “No. (Miro1 DMSO v.s. CCCP P>0.05 or <0.05)”.

ID	Name	Passage	Miro1 Ratio (CCCP/DMSO)	n
1815	Healthy-1	9	0.308±0.120	6
1815	Healthy-1	10	0.400±0.049	3
1815	Healthy-1	11	0.316±0.141	3
1815	Healthy-1	12	0.241±0.173	3
1815	Healthy-1	13	0.231±0.043	6
1815	Healthy-1	14	0.196±0.052	3
1815	Healthy-1	15	0.235±0.014	3
1815	Healthy-1	16	0.153±0.018	3
1815	Healthy-1	17	0.244±0.023	3
1815	Healthy-1	18	0.298	1
1815	Healthy-1	19	0.352	1
ND27760	PD-52	8	1.079±0.129	3
ND27760	PD-52	13	0.734±0.174	3
ND27760	PD-52	14	0.925±0.076	3
ND30159	PD-3	5	0.574±0.207	3
ND30159	PD-3	7	0.379±0.080	2
ND29802	PD-41	5	0.816±0.328	3
ND29802	PD-41	9	0.699±0.034	2
ND29968	PD-56	6	0.631±0.222	3
ND29968	PD-56	9	1.038±0.274	2

Table S2B. Miro1 Values in Cells with Different Passage Numbers.

Miro1 intensities are calculated as in Table S1A. No significant difference is found among different passages within the same cell line.