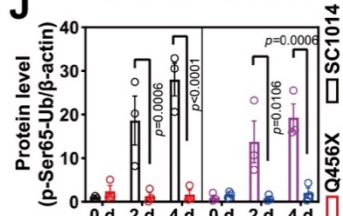
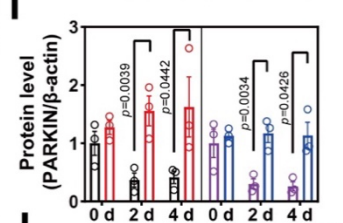
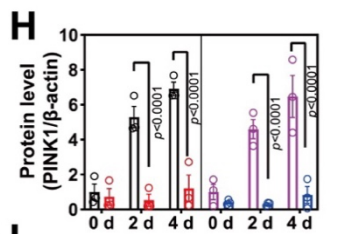
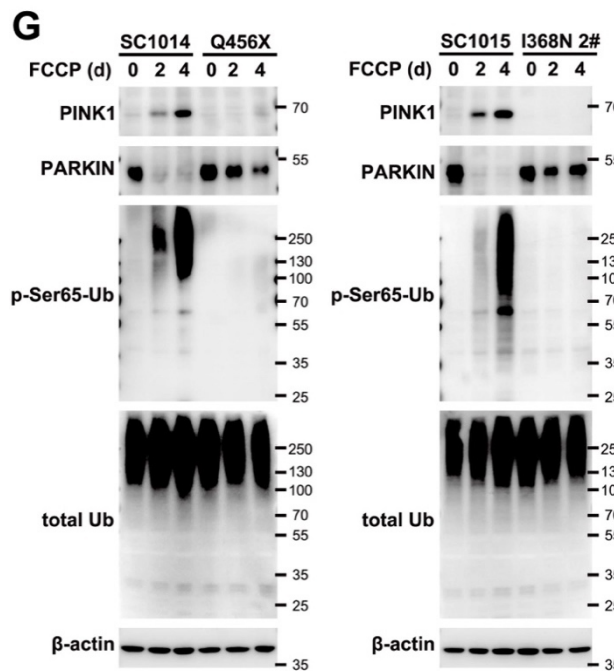
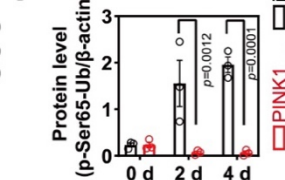
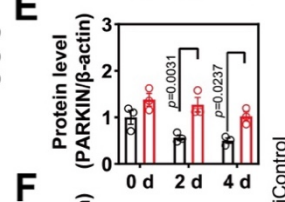
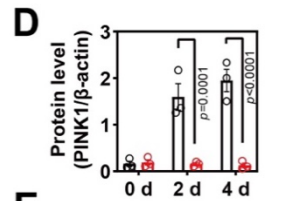
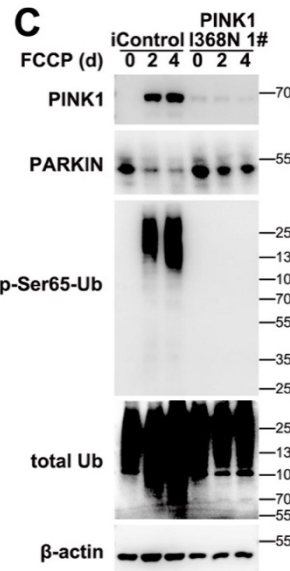
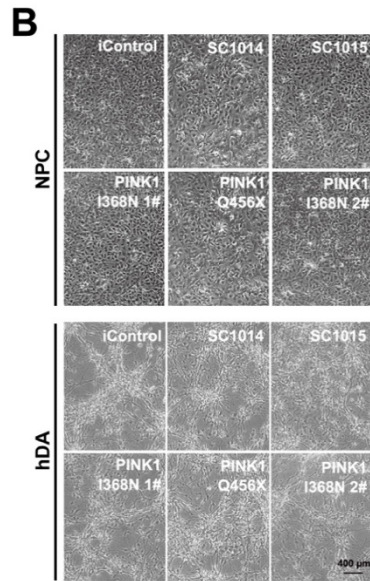
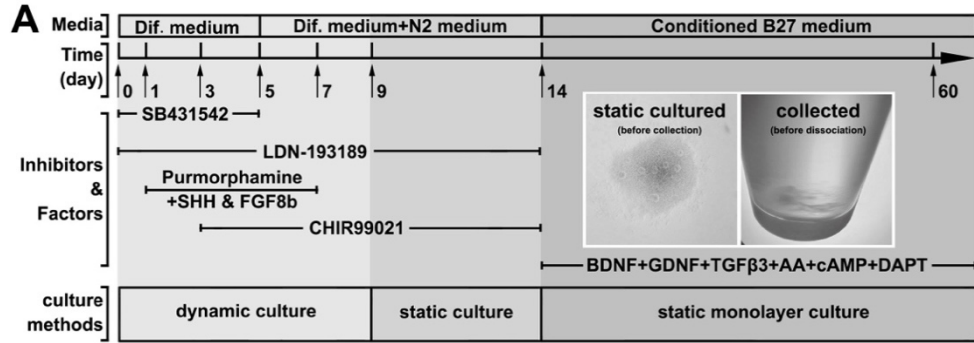


## Supplementary Information

### **Defects in Mitochondrial Biogenesis Drive Mitochondrial Alterations in PINK1-deficient Human Dopamine Neurons.**

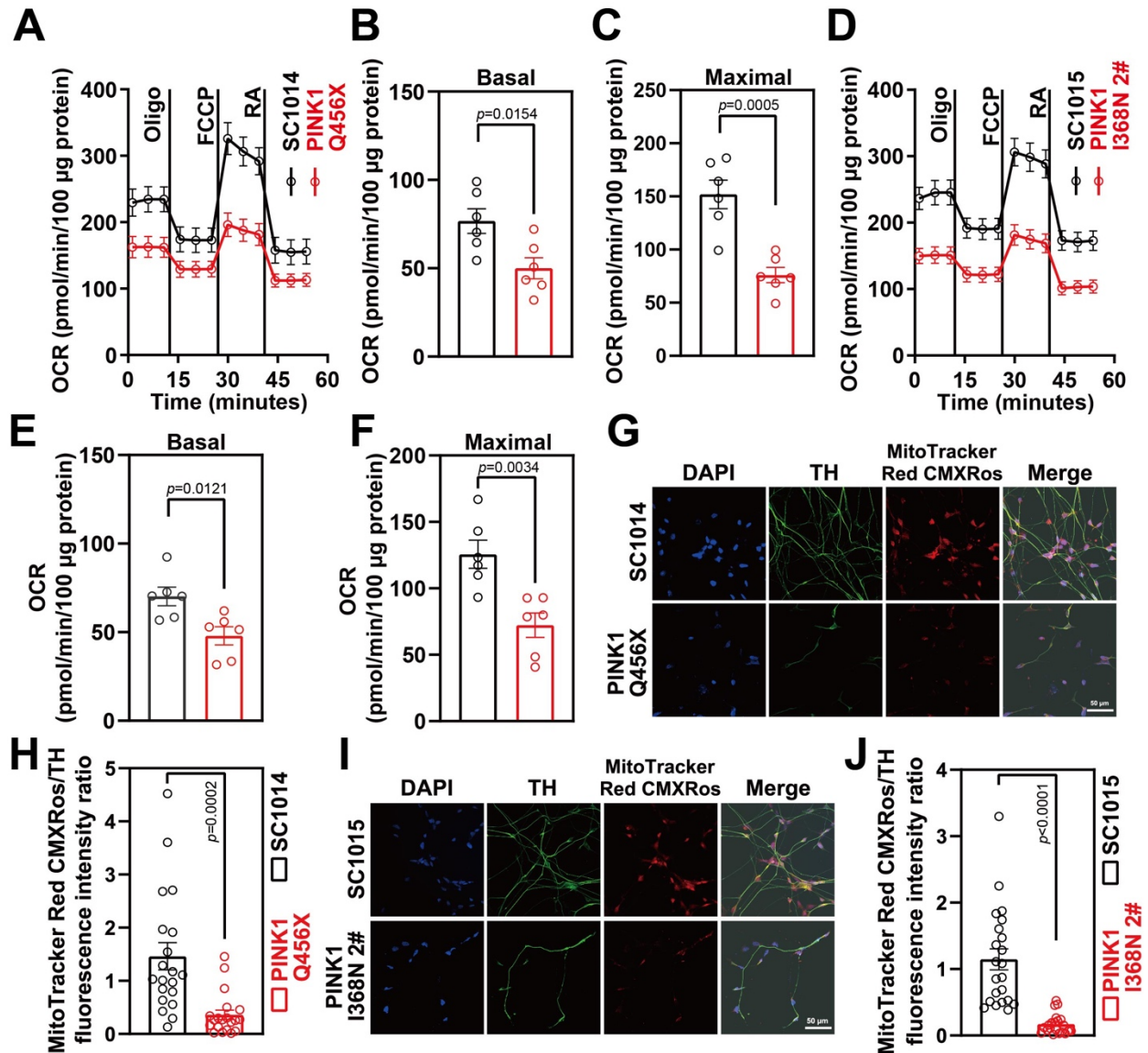
Correspondence to: Ted M. Dawson, M.D Ph.D. ([tdawson@jhmi.edu](mailto:tdawson@jhmi.edu)) or Valina L. Dawson, Ph.D. ([vdawson1@jhmi.edu](mailto:vdawson1@jhmi.edu))

# Supplementary Figure and Figure Legends



## Figure S1 Differentiation protocol optimization and DA neuron characterization

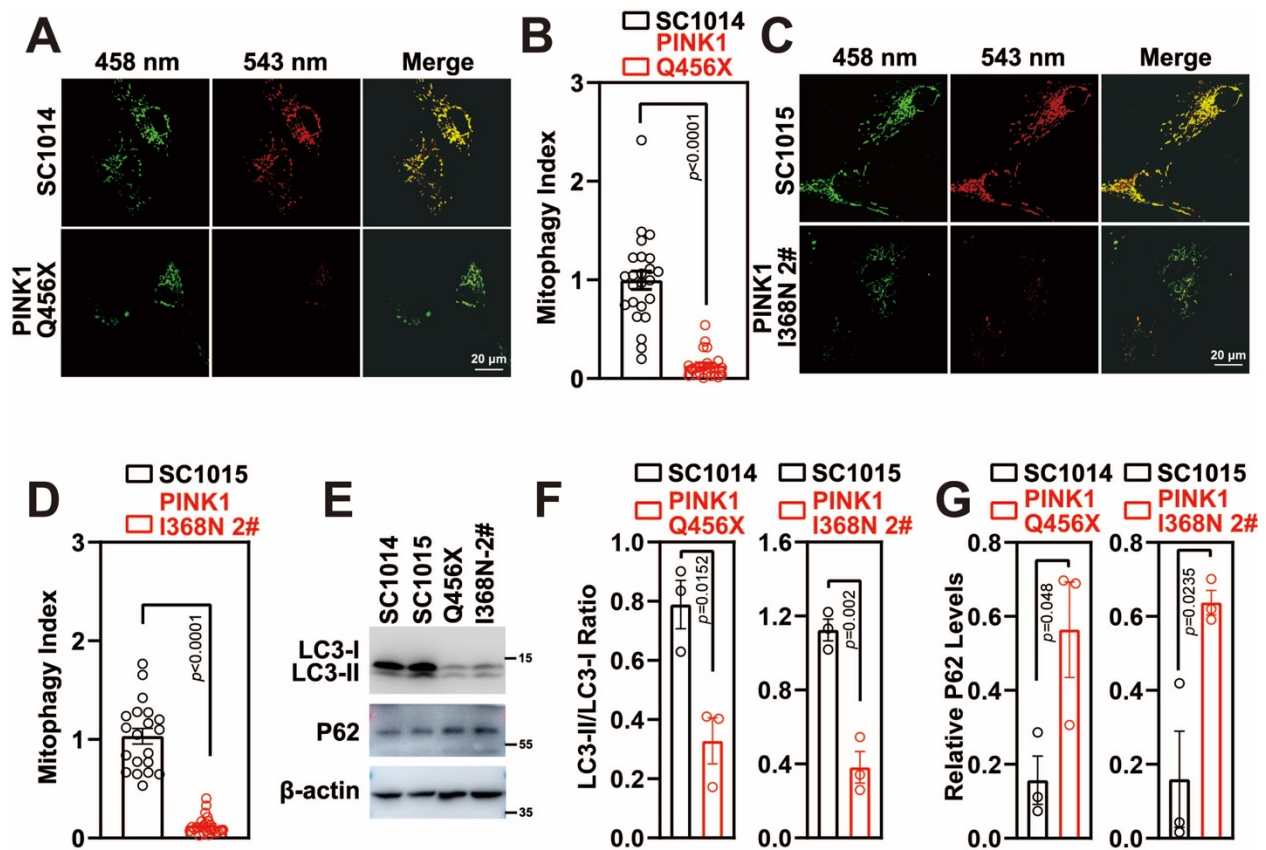
- A. Schematic illustration of hDA neuron differentiation protocol used, differentiation time, medium, factors or inhibitors and culture method were included, progenitor clumps were collected and dissociated before replating.
- B. Representative images of progenitors and mature hDA neurons differentiated from isogenic control and PINK1 p.I368N 1# lines, wildtype SC1014 and SC1015, PINK1 p.Q456X and PINK1 p.I368N 2# mutant lines.
- C. Immunoblot analysis of PINK1, PARKIN, pSer65-Ub, an Ub protein level in isogenic control and PINK1 p.I368N 1# neurons.
- D. Quantification of PINK1 protein levels normalized by  $\beta$ -actin. N=3 independent experiments.
- E. Quantification of PARIN protein levels by  $\beta$ -actin. N=3 independent experiments.
- F. Quantification of pSer65-Ub protein levels normalized by  $\beta$ -actin. N=3 independent experiments.
- G. Immunoblot analysis of PINK1, PARKIN, pSer65-Ub, an Ub protein level in wildtype SC1014 and SC1015, PINK1 p.Q456X and PINK1 p.I368N 2# neurons.
- H. Quantification of PINK1 protein levels normalized by  $\beta$ -actin. N=3 independent experiments.
- I. Quantification of PARIN protein levels by  $\beta$ -actin. N=3 independent experiments.
- J. Quantification of pSer65-Ub protein levels by  $\beta$ -actin. N=3 independent experiments.



**Figure S2 Mitochondrial dysfunction in PINK1 p.Q456X mutant DA neurons**

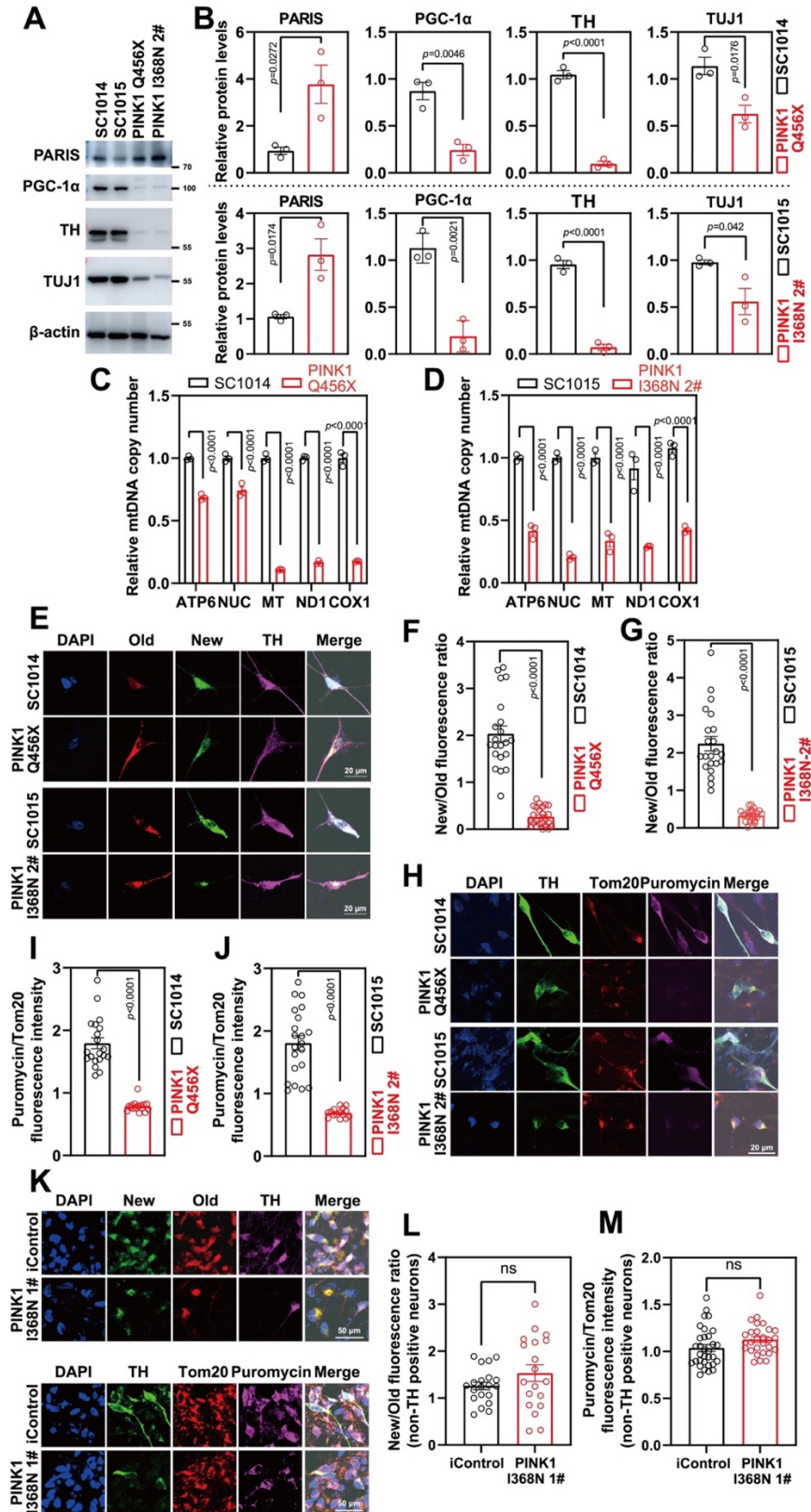
- Mitochondrial oxygen consumption rate curve generated using Seahorse platform showing the mitochondrial dysfunction in wildtype SC1014 and PINK1 p.Q456X mutant neurons in the presence of oligomycin, FCCP, and rotenone respectively.
- Quantification of basal respiration in wildtype SC1014 and PINK1 p.Q456X mutant neurons. N=3 independent experiments.
- Quantification of maximal respiration in wildtype SC1014 and PINK1 p.Q456X mutant neurons. N=3 independent experiments.
- Mitochondrial oxygen consumption rate curve generated using Seahorse platform showing the mitochondrial dysfunction in wildtype SC1015 and PINK1 p.I368N 2# mutant neurons in the presence of oligomycin, FCCP, and rotenone respectively.
- Quantification of basal respiration in wildtype SC1015 and PINK1 p.I368N-2# mutant neurons. N=3 independent experiments.
- Quantification of maximal respiration in wildtype SC1015 and PINK1 p.I368N 2# mutant neurons. N=3 independent experiments.

- G. Representative immunostaining images of TH-positive neurons and MitoTracker Red CMXRos from wildtype SC1014 and PINK1 p.Q456X mutant neurons.
- H. Quantification of TH and MitoTracker Red CMXRos intensity. N=20 TH positive neurons in each group. N=15-30 TH positive neurons in each group.
- I. Representative immunostaining images of TH-positive neurons and MitoTracker Red CMXRos from wildtype SC1015 and PINK1 p.I368N 2# mutant neurons.
- J. Quantification of TH and MitoTracker Red CMXRos intensity. N=20 TH positive neurons in each group. N=15-30 TH positive neurons in each group.



**Figure S3 Mitophagy defects in PINK1 p.Q456X mutant neurons**

- Representative confocal live images of DA neurons infected with lentivirus encoding for MitoKeima.
- Quantified mitophagy index in wildtype SC1014 and PINK1 p.Q456X mutant neurons. N=20 neurons in each group.
- Representative confocal live images of DA neurons infected with lentivirus encoding for MitoKeima.
- Quantified mitophagy index in wildtype SC1015 and PINK1 p.I368N 2# mutant neurons. N=20 neurons in each group.
- Analysis of LC3-I/II and autophagy marker P62 protein level by immunoblot.
- Quantification of immunoblot of LC3-I/II ratio are shown. N=3 independent experiments.
- Quantification of immunoblot of P62 normalized to  $\beta$ -actin. N=3 independent experiments.



**Figure S4 Defects of mitochondrial biogenesis in PINK p.Q456X mutant DA neurons**

- A. Analysis of PARIS, PGC-1 $\alpha$ , TUJ1 and TH protein level in wildtype SC1014 and SC1015, PINK1 p.Q456X and PINK1 p.I368N 2# mutant neurons by immunoblot.
- B. Quantification of immunoblot of PARIS, PGC-1 $\alpha$ , TUJ1 and TH normalized to  $\beta$ -actin. N=3 independent experiments.
- C. Representative confocal images of SNAP-TAG Cox8a labeled mitochondria with TH staining in wildtype SC1014 and SC1015, PINK1 p.Q456X and PINK1 p.I368N 2# mutant neurons.
- D. Fluorescence intensity quantification of old and new mitochondria labeled by Cox8a in TH positive neurons. N=20 TH positive neurons in each group.
- E. Fluorescence intensity quantification of old and new mitochondria labeled by Cox8a in TH positive neurons. N=20 TH positive neurons in each group.
- F. Representative confocal images of puromycin labelling SUnSET assay in wildtype SC1014 and SC1015, PINK1 p.Q456X and PINK1 p.I368N 2# mutant neurons.
- G. Immunofluorescence intensity quantification of Tom20 and puromycin within TH positive neurons. N=20 TH positive neurons in each group.
- H. Immunofluorescence intensity quantification of Tom20 and puromycin within TH positive neurons. N=20 TH positive neurons in each group.
- I. Representative confocal images of SNAP-TAG and SUnSET assay in isogenic control and PINK1 p.I368N 1# mutant neurons.
- J. Fluorescence intensity quantification of old and new mitochondria labeled by Cox8a in non-TH positive neurons. N=20 TH positive neurons in each group.
- K. Immunofluorescence intensity quantification of Tom20 and puromycin within non-TH positive neurons. N=20 TH positive neurons in each group.
- L. Relative mitochondrial DNA copy number quantification of ATP6, NUC, ND1, MT, and COX1 in wildtype SC1014 and PINK1 p.Q456X mutant neurons. N=3 independent experiments.
- M. Relative mitochondrial DNA copy number quantification of ATP6, NUC, ND1, MT, and COX1 in wildtype SC1015 and PINK1 p.I368N 2# mutant neurons. N=3 independent experiments.



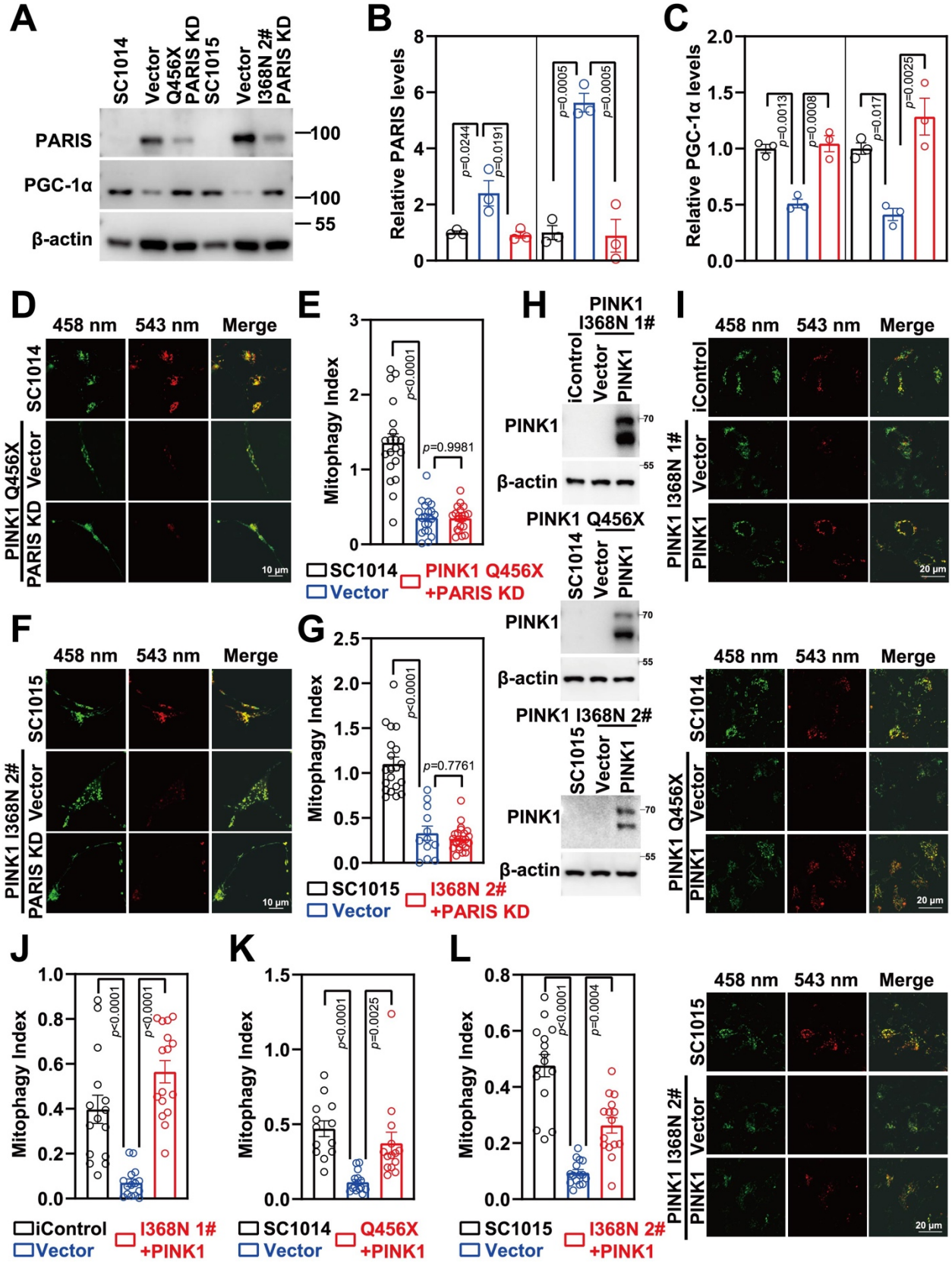


Figure S5 Characterization of PARIS knockdown in PINK1 p.Q456X mutant neurons.

- A. Immunoblot analysis of PARIS, and PGC-1 $\alpha$  protein level in wildtype SC1014 and SC1015, PINK1 p.Q456X and PINK1 p.I368N 2# mutant neurons with or without PARIS knockdown.
- B. Quantified intensities of PARIS are shown, relative protein level was normalized to  $\beta$ -actin. N=3 independent experiments.
- C. Quantified intensities of PGC-1 $\alpha$  are shown, relative protein level was normalized to  $\beta$ -actin. N=3 independent experiments.
- D. Representative confocal images of wildtype SC1014 and PINK1 p.Q456X mutant neurons infected with lentivirus encoding Mito-Keima mitophagy reporter systems.
- E. Quantified mitophagy index in wildtype SC1014 and PINK1 p.Q456X mutant neurons with or without PARIS knockdown. N=20 TH positive neurons in each group.
- F. Representative confocal images of wildtype SC1015 and PINK1 p.I368N 2# mutant neurons infected with lentivirus encoding Mito-Keima mitophagy reporter systems.
- G. Quantified mitophagy index in wildtype SC1015 and PINK1 p.I368N-2# mutant neurons with or without PARIS knockdown. N=20 TH positive neurons in each group.
- H. Immunoblot analysis of PINK1 protein level in isogenic control and PINK1 p.I368N 1# mutant, wildtype SC1014 and SC1015, PINK1 p.Q456X and PINK1 p.I368N-2# mutant neurons with or without PINK overexpression.
- I. Representative confocal images of isogenic control and PINK1 p.I368N 1# mutant, wildtype SC1014 and SC1015, PINK1 p.Q456X and PINK1 p.I368N-2# mutant neurons infected with lentivirus encoding Mito-Keima mitophagy reporter systems.
- J. Quantified mitophagy index in isogenic control and PINK1 p.I368N 1# mutant neurons with or without PINK overexpression. N=20 TH positive neurons in each group.
- K. Quantified mitophagy index in wildtype SC1014 and PINK1 p.Q456X mutant neurons with or without PINK overexpression. N=20 TH positive neurons in each group.
- L. Quantified mitophagy index in wildtype SC1015 and PINK1 p.I368N 2# mutant neurons with or without PINK overexpression. N=20 TH positive neurons in each group.

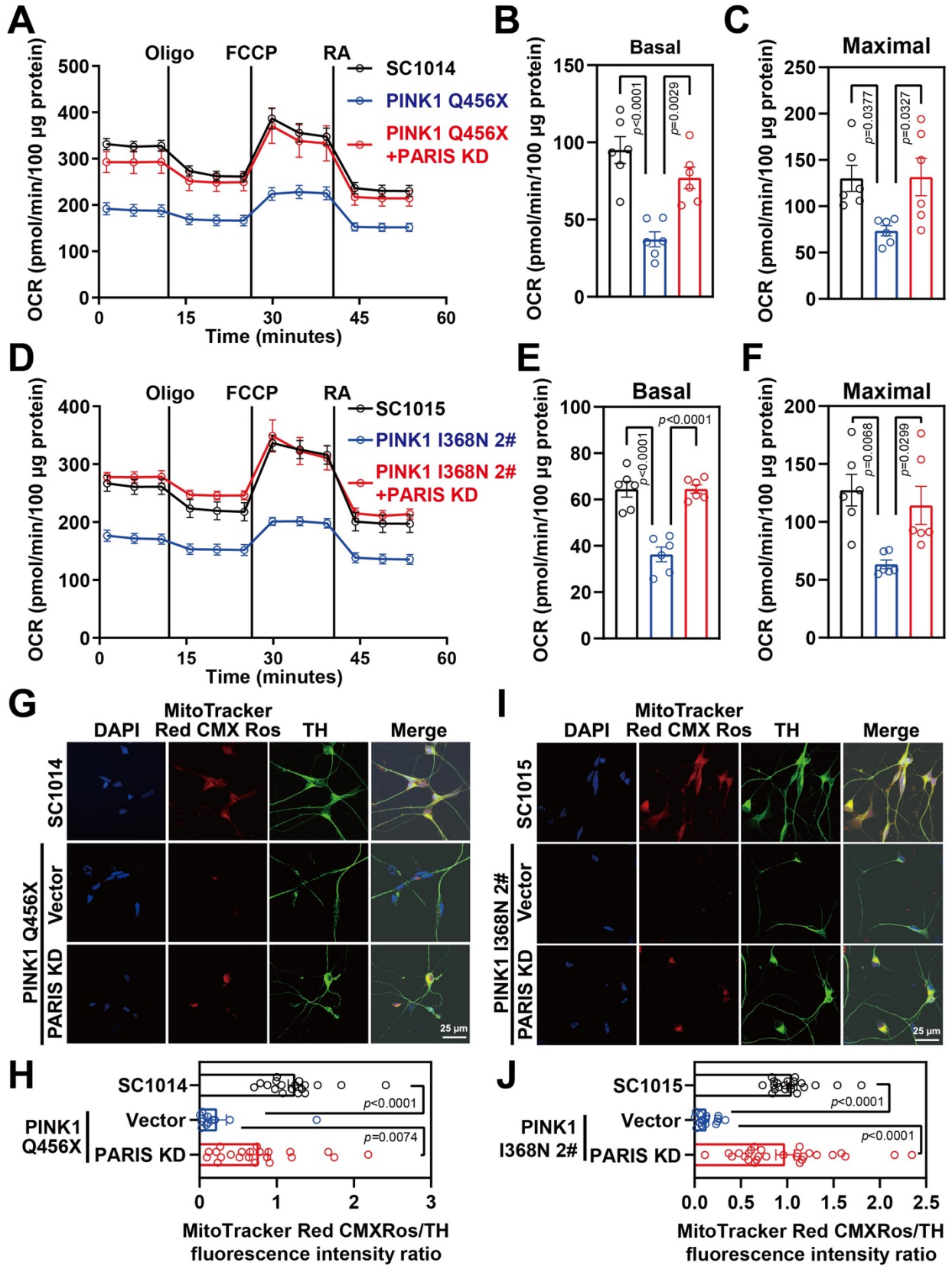
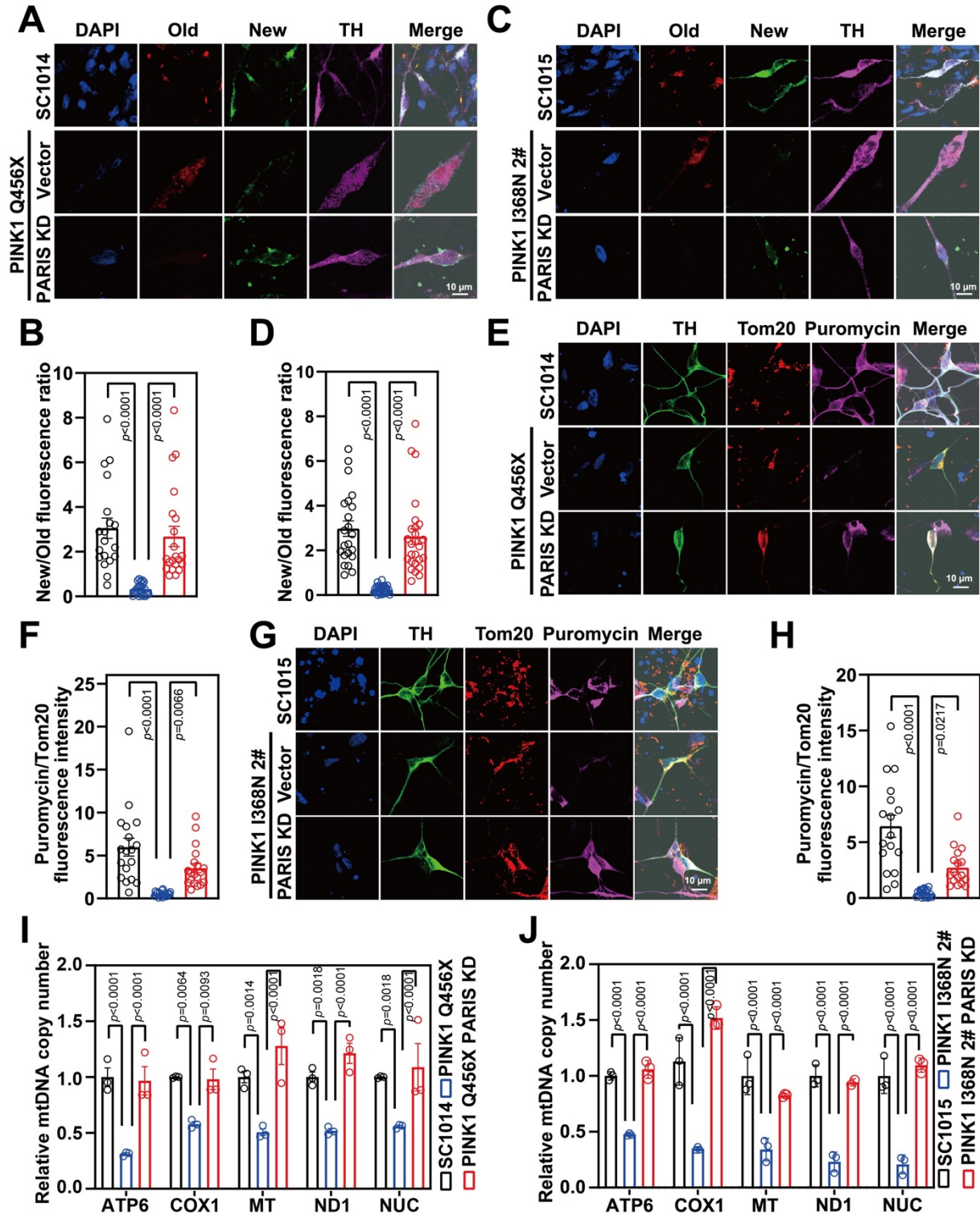


Figure S6 Mitochondrial function rescued in PINK1 p.Q456X mutant neurons by PARIS

## knockdown

- A. Mitochondrial oxygen consumption rate curve generated using Seahorse platform showing the mitochondrial function of wildtype SC1014 and PINK1 p.Q456X mutant with or without PARIS knockdown.
- B. Quantification of basal respiration in wildtype SC1014 and PINK1 p.Q456X mutant neurons with or without PARIS knockdown. N=3 independent experiments.
- C. Quantification of maximal respiration in wildtype SC1014 and PINK1 p.Q456X mutant neurons with or without PARIS knockdown. N=3 independent experiments.
- D. Mitochondrial oxygen consumption rate curve generated using Seahorse platform showing the mitochondrial function of wildtype SC1015 and PINK1 p.I368N 2# mutant neurons with or without PARIS knockdown.
- E. Quantification of basal respiration in wildtype SC1015 and PINK1 p.I368N 2# mutant neurons with or without PARIS knockdown. N=3 independent experiments.
- F. Quantification of maximal respiration in wildtype SC1015 and PINK1 p.I368N 2# mutant neurons with or without PARIS knockdown. N=3 independent experiments.
- G. Representative immunostaining images of TH-positive neurons and MitoTracker Red CMXRos from wildtype SC1014 and PINK1 p.Q456X mutant neurons with or without PARIS knockdown.
- H. Quantification of TH and MitoTracker Red CMXRos intensity. N=20 TH positive neurons in each group. N=15-30 TH positive neurons in each group.
- I. Representative immunostaining images of TH-positive neurons and MitoTracker Red CMXRos from wildtype SC1015 and PINK1 p.I368N 2# mutant neurons with or without PARIS knockdown.
- J. Quantification of TH and MitoTracker Red CMXRos intensity. N=20 TH positive neurons in each group. N=15-30 TH positive neurons in each group.



**Figure S7 Mitochondrial biogenesis rescued in PINK1 p.Q456X mutant neurons by PARIS knockdown**

A. Representative confocal images of SNAP-TAG Cox8a labeled mitochondria with TH staining in wildtype SC1014 and PINK1 p.Q456X mutant neurons.

- B. Fluorescence intensity quantification of old and new mitochondria labeled by Cox8a in TH positive neurons. N=20 TH positive neurons in each group.
- C. Representative confocal images of SNAP-TAG Cox8a labeled mitochondria with TH staining in wildtype SC1015 and PINK1 p.I368N 2# mutant neurons.
- D. Fluorescence intensity quantification of old and new mitochondria labeled by Cox8a in TH positive neurons. N=20 TH positive neurons in each group.
- E. Representative confocal images of puromycin labelling SUnSET assay in wildtype SC1014 and PINK1 p.Q456X mutant neurons with or without PARIS knockdown.
- F. Immunofluorescence intensity quantification of Tom20 and puromycin within TH positive neurons with or without PARIS knockdown. N=20 TH positive neurons in each group.
- G. Representative confocal images of puromycin labelling SUnSET assay in wildtype SC1015 and PINK1 p.I368N 2# mutant neurons with or without PARIS knockdown.
- H. Immunofluorescence intensity quantification of Tom20 and puromycin within TH positive neurons with or without PARIS knockdown. N=20 TH positive neurons in each group.
- I. Relative mitochondrial DNA copy number quantification of ATP6, NUC, ND1, MT, and COX1 from wildtype SC1014 and PINK1 p.Q456X mutant neurons with or without PARIS knockdown. N=3 independent experiments.
- J. Relative mitochondrial DNA copy number quantification of ATP6, NUC, ND1, MT, and COX1 from wildtype SC1015 and PINK1 p.I368N 2# mutant neurons with or without PARIS knockdown. N=3 independent experiments.

## Supplementary Table

**Table S1. Primers used for RT-PCR**

Gene	Sequence
ATP6	F: CGCCACCCTAGCAATATCA
	R: TTAAGGCGACAGCGATTTTC
MT	F: CCCCACAAACCCCATTAATAAATCCCA
	R: TTTCATCATGCGGAGATGTTGGATGG
COX1	F: CGATGCATACACCACATGA
	R: AGCGAAGGCTTCTCAAATC
NUC	F: ATACCCCGATTCCGCTACGA
	R: GTTTGAGGGGGAATGCTGGAG
ND1	F: ATACCCCGATTCCGCTACGA
	R: GTTTGAGGGGGAATGCTGGAG
B2-micoglobulin	F: TGCTGTCTCCATGTTTGATGTATCT
	R: TCTCTGCTCCCCACCTCTAAGT