Supplemental Data

LOSS OF PARKIN OR PINK1 FUNCTION INCREASES DRP1-DEPENDENT MITOCHONDRIAL FRAGMENTATION

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Running head: Parkin, PINK1 and mitochondria

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Figure Legends

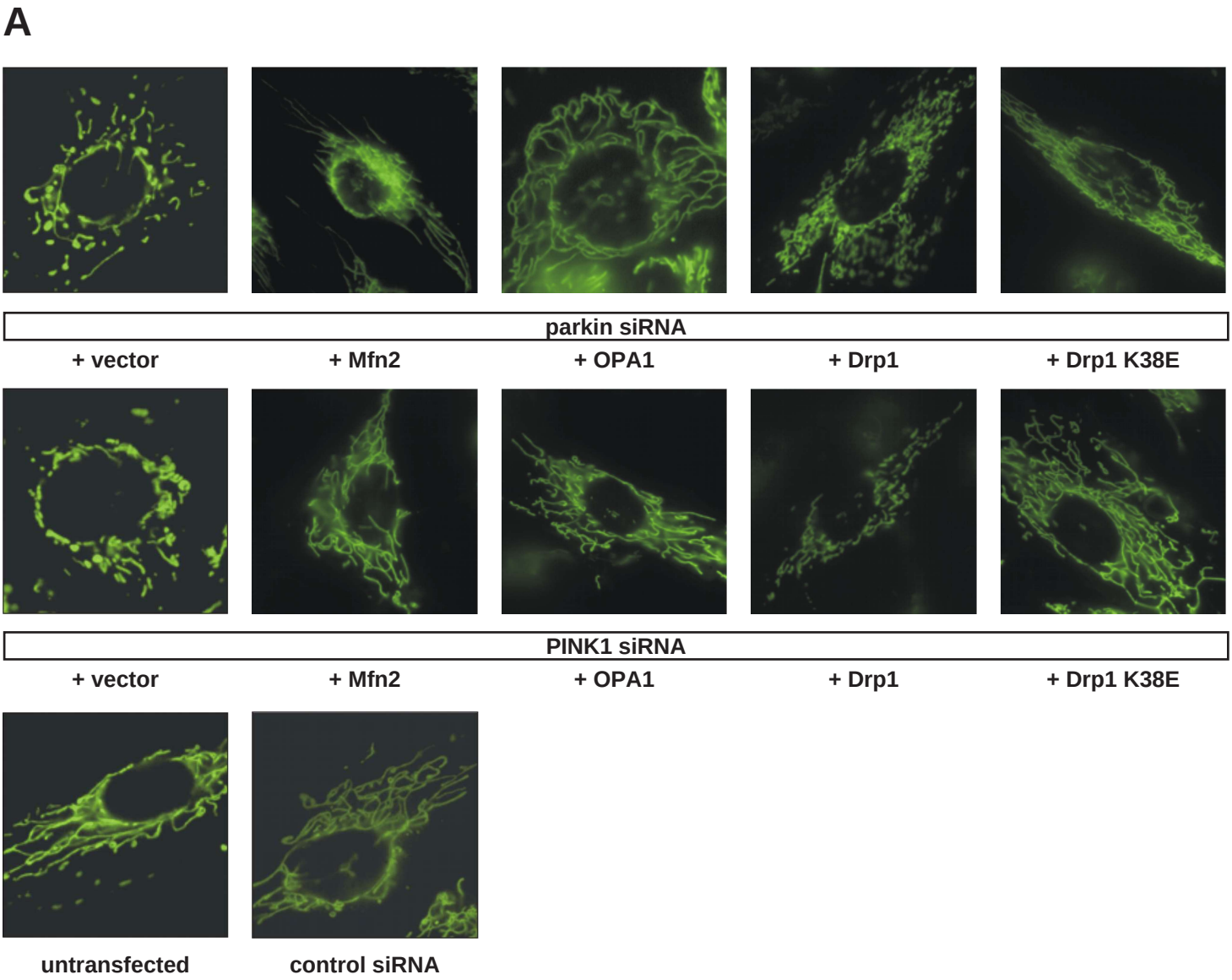
Supplemental Figure 1. A, Abnormal mitochondrial morphology caused by parkin or PINK1 loss of function can be rescued by increasing mitochondrial fusion or decreasing fission. SH-SY5Y cells transfected with control siRNA or siRNA targeting parkin were stained with the fluorescent dye $DiOC_6(3)$ to visualize mitochondria and analyzed by fluorescence microscopy. Shown are examples of mitochondrial morphologies of the experiments described in Fig. 3A, B, C, D. **B, C, D,** Expression controls to the experiments shown in Fig. 3E (B), Fig. 3F (C), Fig. 3G (D). β-Actin was used as a loading control. **B,** Parkin expression was detected by using the anti-

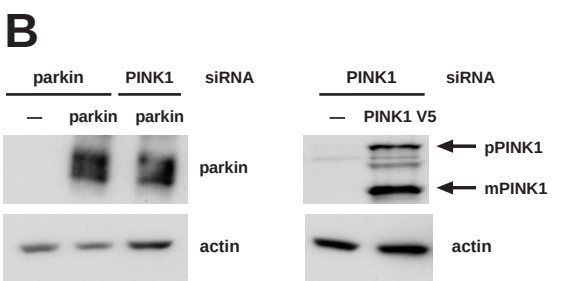
parkin polyclonal antibody 2132, expression of PINK1-V5 was detected using an anti-V5 monoclonal antibody. **C, D,** Expression of Drp1-FLAG and Mfn2-FLAG was detected using the anti-FLAG M2 monoclonal antibody, Drp1 K38E was visualized by an anti-Drp1 monoclonal antibody, and OPA1-HIS was detected by penta-HIS HRP conjugate mouse IgG.

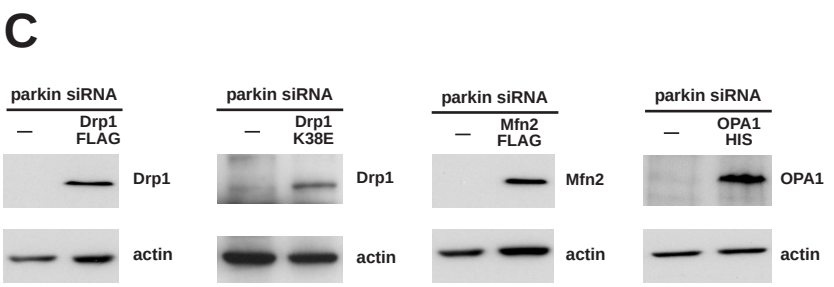
Supplemental Figure 2. Parkin and PINK1 (at higher expression levels) can reduce mitochondrial fission induced by Drp1. SH-SY5Y cells were transfected with the constructs indicated. 24 hours after transfection, mitochondrial morphology of transfected cells (identified by the coexpression of mCherry) was assessed as described in Fig. 1. Shown are examples of mitochondrial morphologies of the experiments described in Fig. 4B.

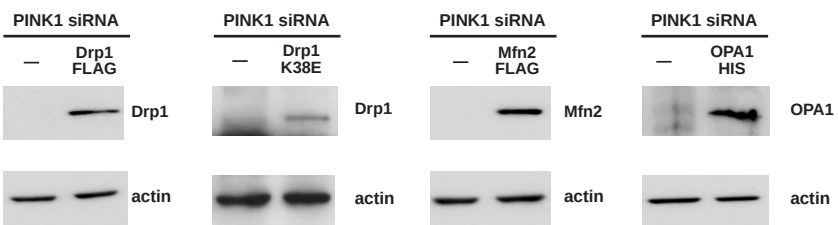
Supplemental Figure 3. Downregulation of Mfn2 causes alterations in mitochondrial morphology which are not influenced by parkin. SH-SY5Y cells were transfected with control siRNA or one of three siRNAs targeting Mfn2 (SET 1). In SET 2 Mfn2 and parkin were silenced simultaneously. In SET 3, parkin was overexpressed in Mfn2-deficient cells. Three days after transfection the cells were stained with the fluorescent dye $DiOC_6(3)$ to visualize mitochondria and analyzed by fluorescence microscopy. Cells displaying an intact network of tubular mitochondria were classified as tubular. When this network was disrupted and mitochondria appeared predominantly spherical or rod-like the cells were classified as fragmented. For quantification, the mitochondrial morphology of at least 300 cells per plate was determined in a blinded manner. Quantifications were based on triplicates of at least three independent experiments. Shown is the percentage of cells with fragmented or

truncated mitochondria. **B.** The efficiency of Mfn2 and parkin silencing/expression is shown by Western blotting using an anti-Mfn2 polyclonal antibody or the monoclonal anti-parkin antibody PRK8. β-Actin was used as a loading control. **C.** Examples of mitochondrial morphologies of the experiments described in Supplemental Fig. 3A.


A
parkin siRNA
+ vector
+ Mfn2
+ OPA1
+ Drp1
+ Drp1 K38E
PINK1 siRNA
+ vector
+ Mfn2
+ OPA1
+ Drp1
+ Drp1 K38E
untransfected
control siRNA


B
parkin
PINK1
siRNA
—
parkin
parkin
parkin
actin
PINK1
siRNA
—
PINK1 V5
pPINK1
mPINK1
actin


C
parkin siRNA
—
Drp1 FLAG
Drp1
actin
parkin siRNA
—
Drp1 K38E
Drp1
actin
parkin siRNA
—
Mfn2 FLAG
Mfn2
actin
parkin siRNA
—
OPA1 HIS
OPA1
actin


D
PINK1 siRNA
—
Drp1 FLAG
Drp1
actin
PINK1 siRNA
—
Drp1 K38E
Drp1
actin
PINK1 siRNA
—
Mfn2 FLAG
Mfn2
actin
PINK1 siRNA
—
OPA1 HIS
OPA1
actin

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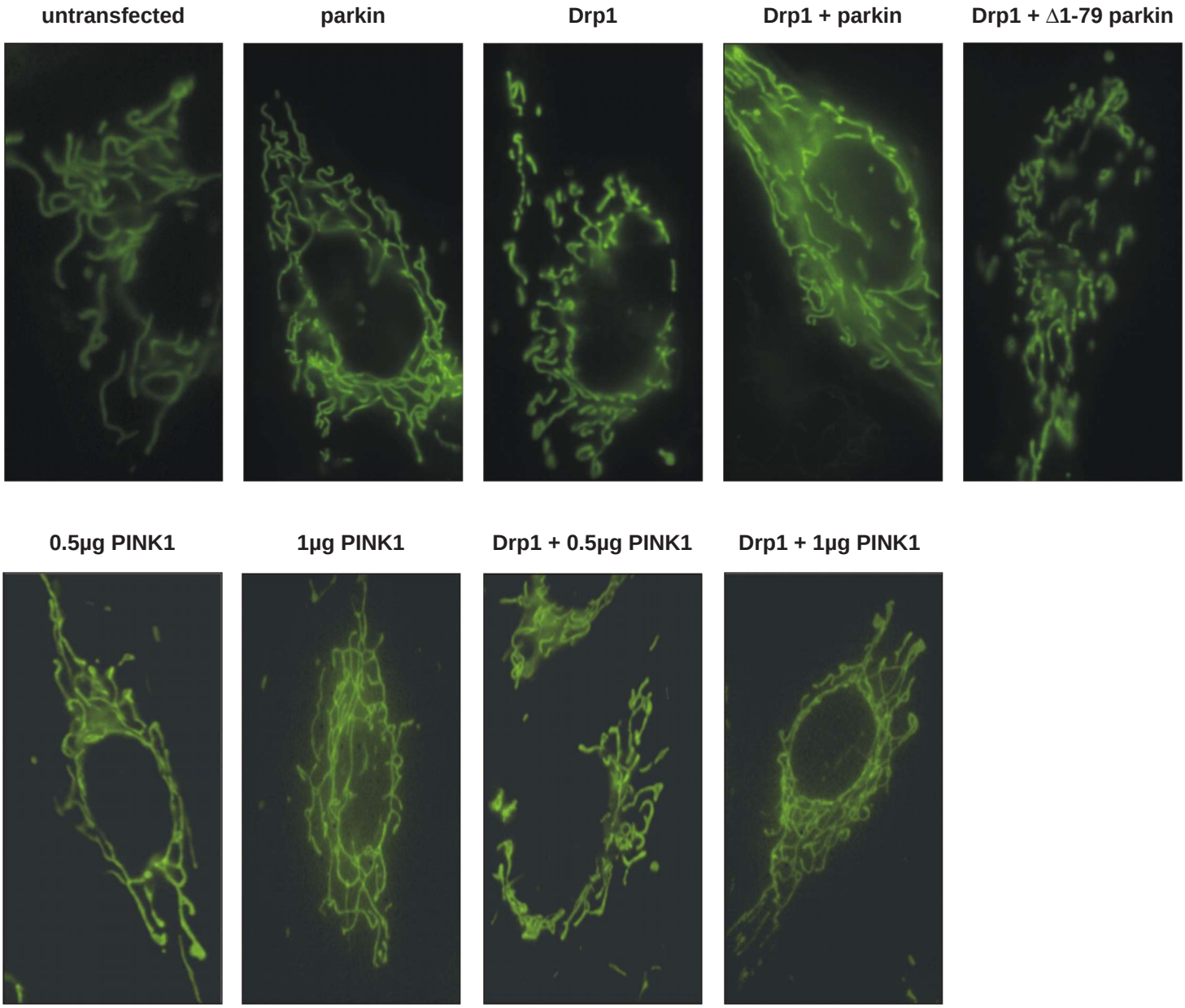
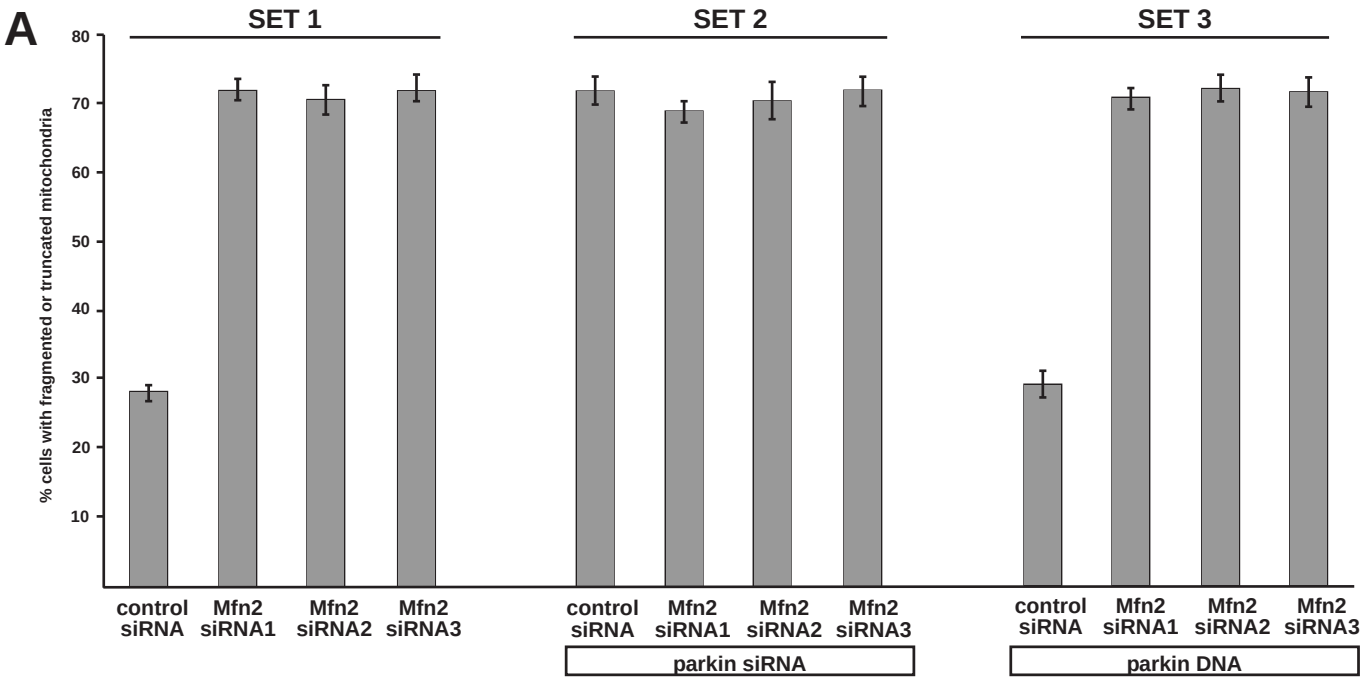
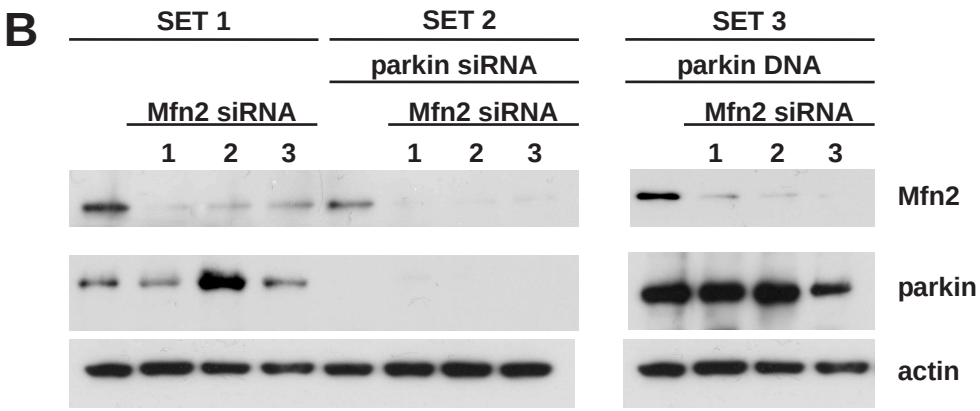
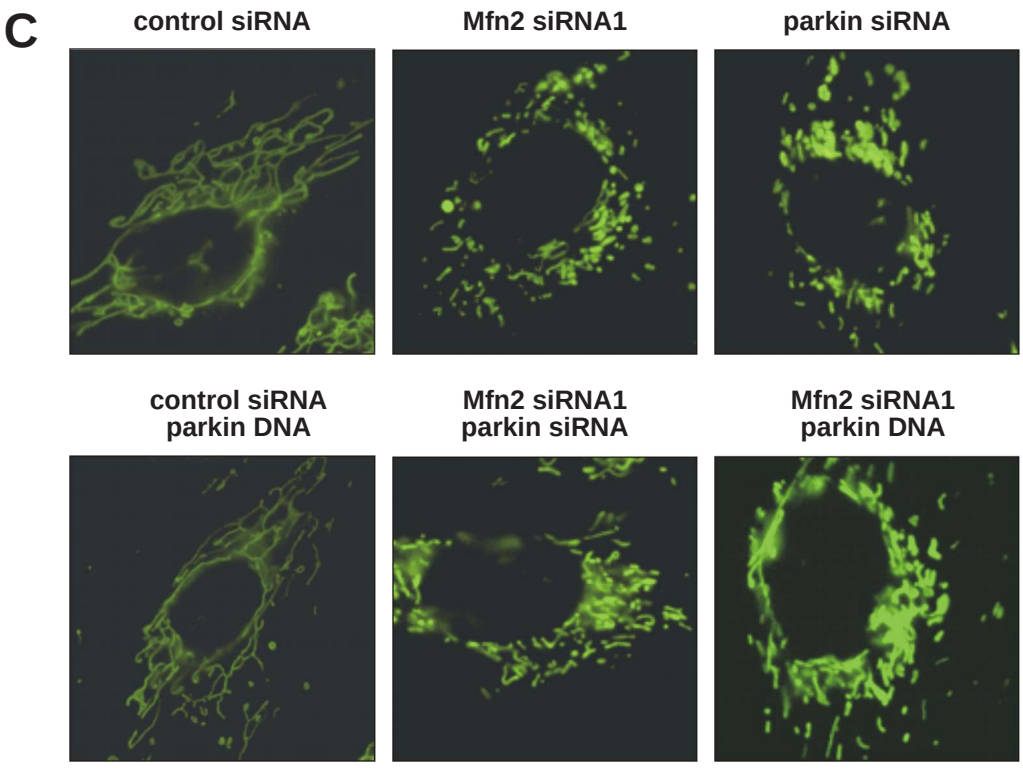



A
SET 1
SET 2
SET 3
% cells with fragmented or truncated mitochondria
80
70
60
50
40
30
20
10
control siRNA
Mfn2 siRNA1
Mfn2 siRNA2
Mfn2 siRNA3
parkin siRNA
parkin DNA


B
SET 1
SET 2
parkin siRNA
SET 3
parkin DNA
Mfn2 siRNA
1 2 3
Mfn2
parkin
actin


C
control siRNA
Mfn2 siRNA1
parkin siRNA
control siRNA
parkin DNA
Mfn2 siRNA1
parkin siRNA
Mfn2 siRNA1
parkin DNA