A

Control siRNA

parkin-mCherry

mito-GFP

merge

Afg3L2 siRNA

parkin-mCherry

mito-GFP

merge

Control siRNA + antimycin A

parkin-mCherry

mito-GFP

merge

B

Control siRNA

Afg3L2 siRNA

Control siRNA + CCCP

TMRM

mito-GFP

C

Experiment 1

Experiment 2

TMRM staining (arbitrary fluorescence units)

Control siRNA

Afg3L2 siRNA

Control siRNA + CCCP

n = 26 25 11 21 20 11
Figure S2. Depletion of AFG3L2 does not recruit parkin to mitochondria or affect the mitochondrial membrane potential

(A) Representative images of axons from neurons transfected with parkin-mCherry, mito-GFP and either control or Afg3l2 siRNAs. As a positive control, neurons were treated with antimycin A. Arrows point to colocalisation of parkin-mCherry and mito-GFP. Bar: 10 µm. (B) Representative images of soma from neurons transfected with mito-GFP and either control or Afg3l2 siRNAs, and stained with TMRM. As a positive control, neurons were treated with CCCP. The top panel shows the TMRM signal as pseudocolour-coded intensities, while the bottom panel displays the corresponding mitochondrial staining by mito-GFP. Bar: 10 µm. (C) Boxplots of TMRM intensities for two independent experiments.