Ashrafi et al., http://www.jcb.org/cgi/content/full/jcb.201401070/DC1

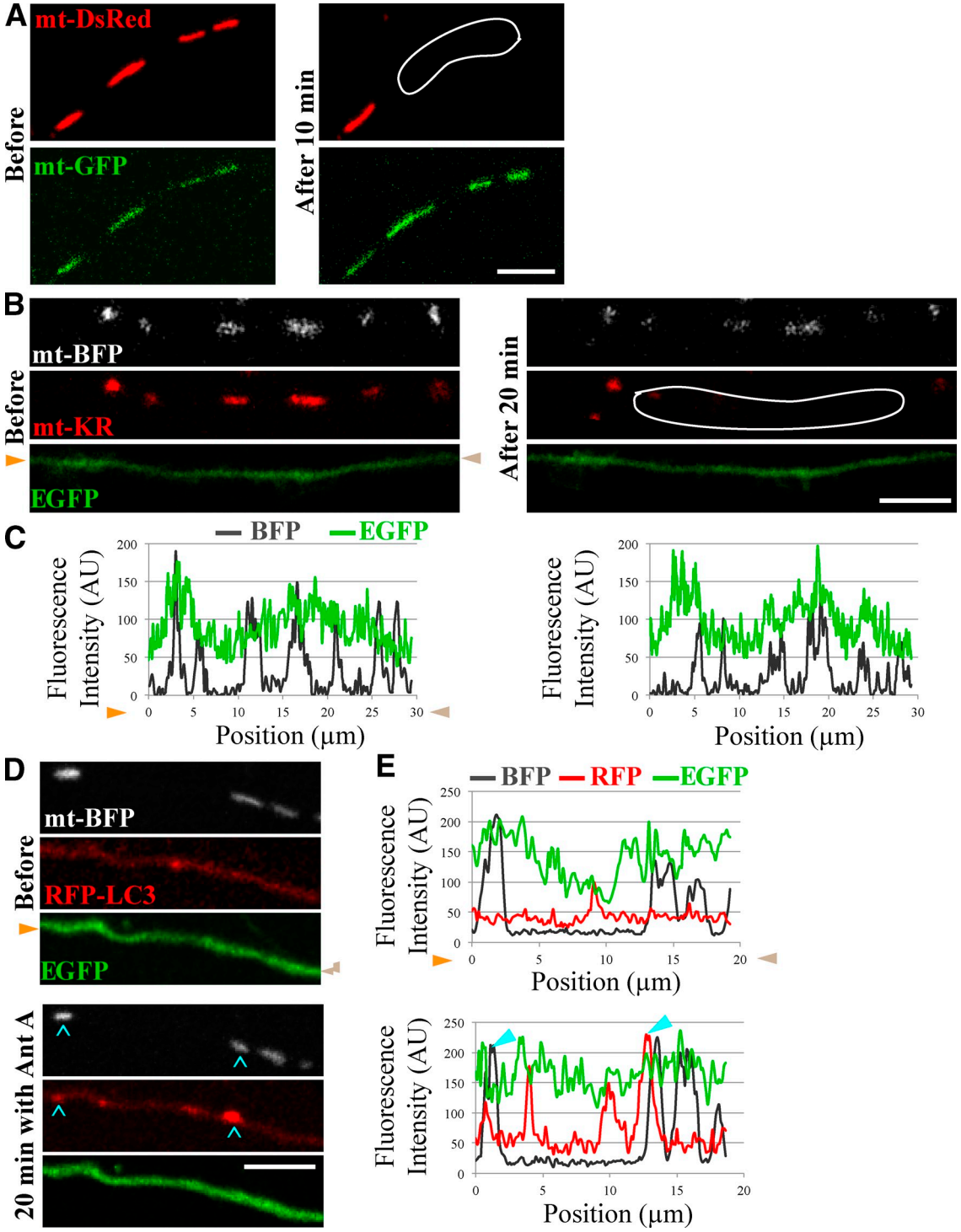

Figure S1. **Laser irradiation in the absence of mt-KR does not cause mitochondrial damage, and mitochondrial damage does not cause axonal swelling.** Irradiated areas are outlined. (A) Irradiation of mt-DsRed with the same dose given to mt-KR–expressing cells (Fig. 1) does not affect the morphology of axonal mitochondria coexpressing mt-GFP. (B and C) Activation of mt-KR in axons does not cause an accumulation of cytoplasmic EGFP in the vicinity of the mitochondria (n = 43 irradiated mitochondria). (D and E) Formation of RFP-LC3 puncta colocalizing with Antimycin A (Ant A)–treated mitochondria does not correlate with changes in cytoplasmic EGFP denoting axon morphology. The accumulations under similar conditions of GFP-LC3 in Fig. 3 and YFP-Parkin in Figs. 5–7, therefore, are caused by mitochondrial recruitment rather than axonal swelling. Cyan arrowheads point to mitochondria analyzed with line scans. (C–F) Orange and brown arrowheads denote corresponding points in images and line scans. AU, arbitrary unit. Bars, 5 µm.

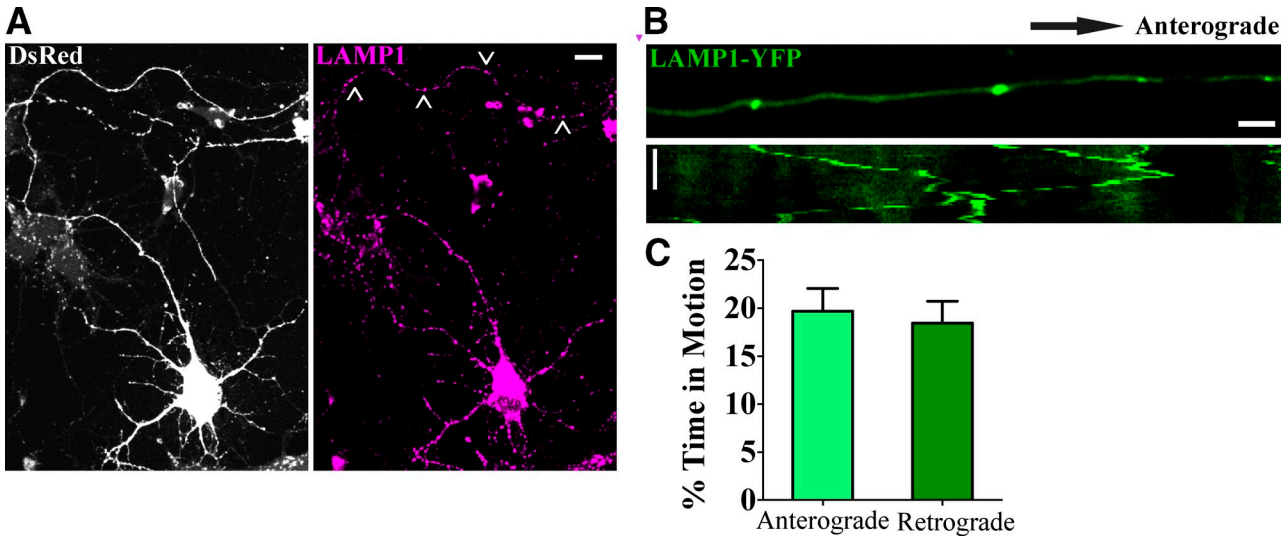

Figure S2. **Axons contain moving lysosomes.** (A) Endogenous LAMP1 staining of lysosomes in a hippocampal neuron expressing cytoplasmic DsRed. Arrowheads denote lysosomal vesicles in a distal axon. (B) Representative image and kymograph from experiments in which lysosomal movement in axons ~500 µm from the cell body was visualized with overexpression of LAMP1-YFP. Kymographs were constructed from sequential line scans of the axon depicted above it, such that the positions of mitochondria along the axon are displayed along the x axis and time proceeds from top to bottom along the y axis. Stationary lysosomes appear as vertical lines; moving lysosomes appear as diagonals. (C) The percentage of time a lysosome was in motion was calculated from kymographs and averaged. The mean anterograde and retrograde velocities were 0.34 and 0.27 µm/s, respectively. *n* = 130 lysosomes from 15 axons. Error bars represent means ± SEM. Bars: (horizontal) 10 µm; (vertical) 50 s.

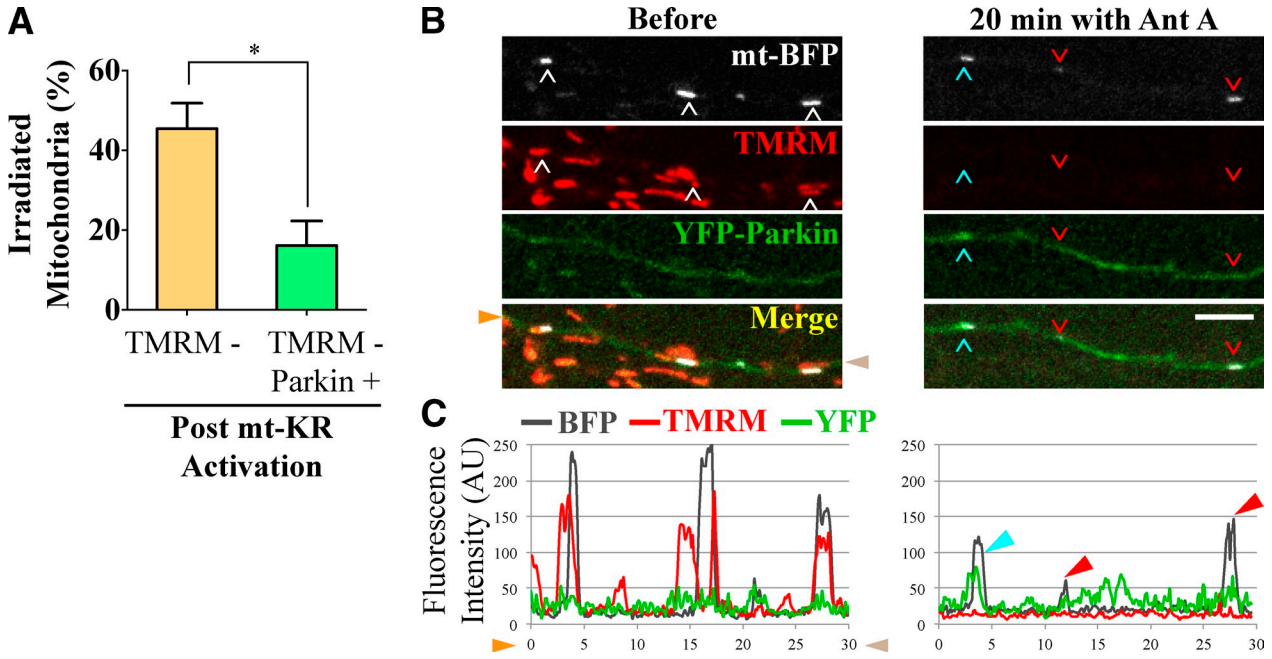

Figure S3. **Recruitment of Parkin occurs on only a subset of depolarized mitochondria.** (A) Activation of mt-KR results in loss of mitochondrial TMRM staining and recruitment of YFP-Parkin to a subset of TMRM-negative mitochondria. *n* = 63 mitochondria from eight transfections. (B and C) Application of Antimycin A (Ant A) to hippocampal axons in a microfluidic devices causes loss of TMRM staining of mitochondria and recruitment of Parkin to some (cyan arrowheads) but not all of the depolarized mitochondria (red arrowheads). White arrowheads indicate TMRM staining of mt-BFP–expressing mitochondria in the transfected axon. Orange and brown arrowheads denote corresponding points in images and line scans. *, P < 0.05. Error bars represent mean ± SEM. AU, arbitrary unit. Bar, 5 µm.

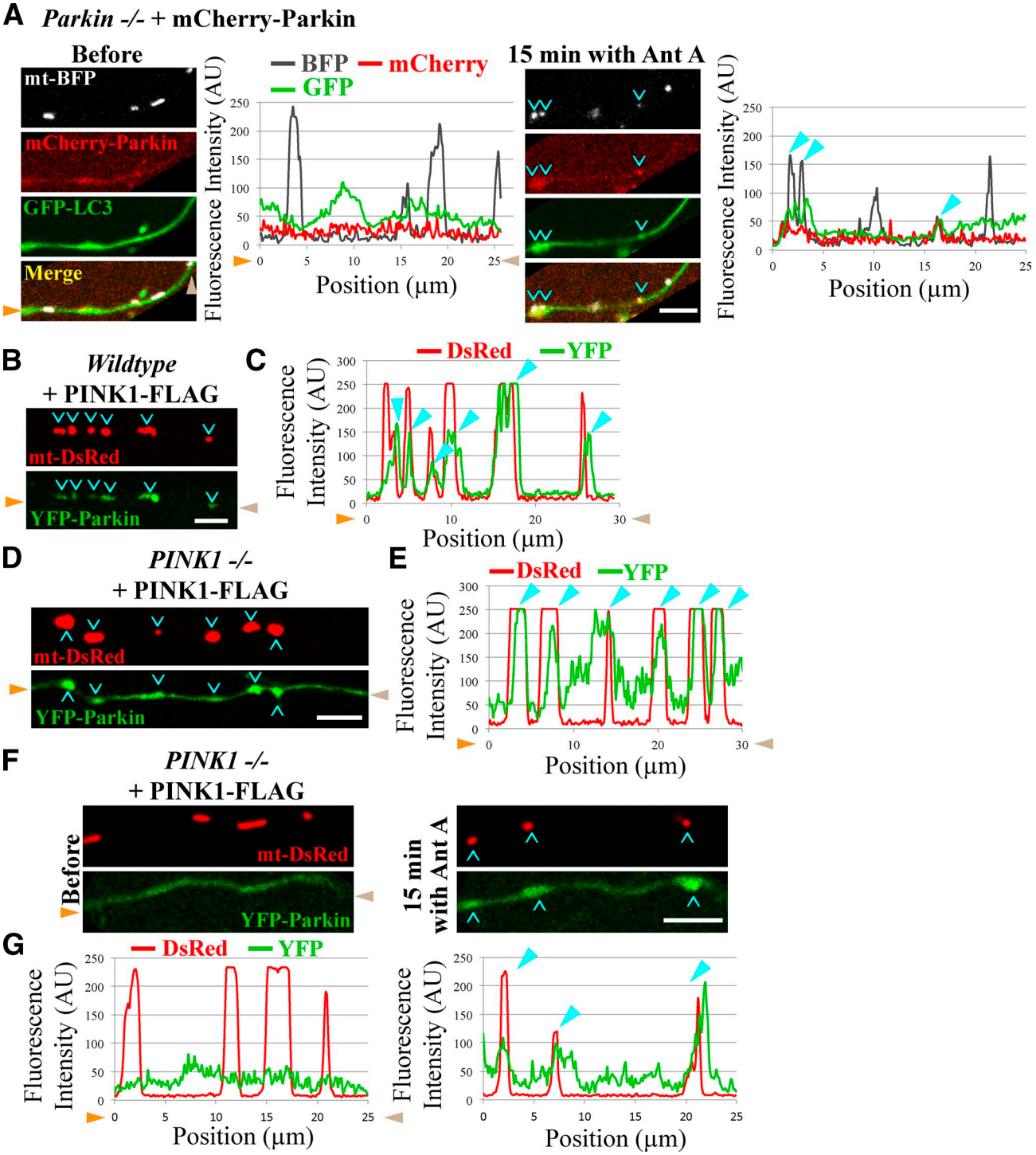

Figure S4. **Expression of Parkin and PINK1 rescues mitophagy and Parkin recruitment in *Parkin*$^{-/-}$ and *PINK1*$^{-/-}$ axons, respectively.** (A) Representative example of a *Parkin*$^{-/-}$ mouse hippocampal neurons expressing mCherry-Parkin in which axonal mitochondria were depolarized with 20 μM Antimycin A (Ant A). Within 30 min, mitochondria colocalized with mCherry-Parkin– and GFP-LC3–positive autophagosomes (cyan arrowheads). (B and C) Overexpression of PINK1-FLAG in *wild-type* hippocampal axons caused mitochondrial accumulation of YFP-Parkin in the absence of mitochondrial depolarization. (D and E) Overexpression of PINK1 in *PINK1*$^{-/-}$ axons at high levels also triggered recruitment of YFP-Parkin to mitochondria in the absence of mitochondrial depolarization. (F and G) In *PINK1*$^{-/-}$ axons expressing low levels of PINK1-FLAG, YFP-Parkin was cytoplasmic in the absence of damage but relocated to axonal mitochondrial after treatment with 40 μM Antimycin A. (A–G) Cyan arrowheads point to Parkin/LC3-positive mitochondria. Orange and brown arrowheads denote corresponding points in images and line scans. AU, arbitrary unit. Bars, 5 μm.

Supplemental material also includes a ZIP file that provides the source code for the Kymolyzer ImageJ macros used for generation of kymographs and determination of particle motility parameters.