Otera et al., http://www.jcb.org/cgi/content/full/jcb.201508099/DC1

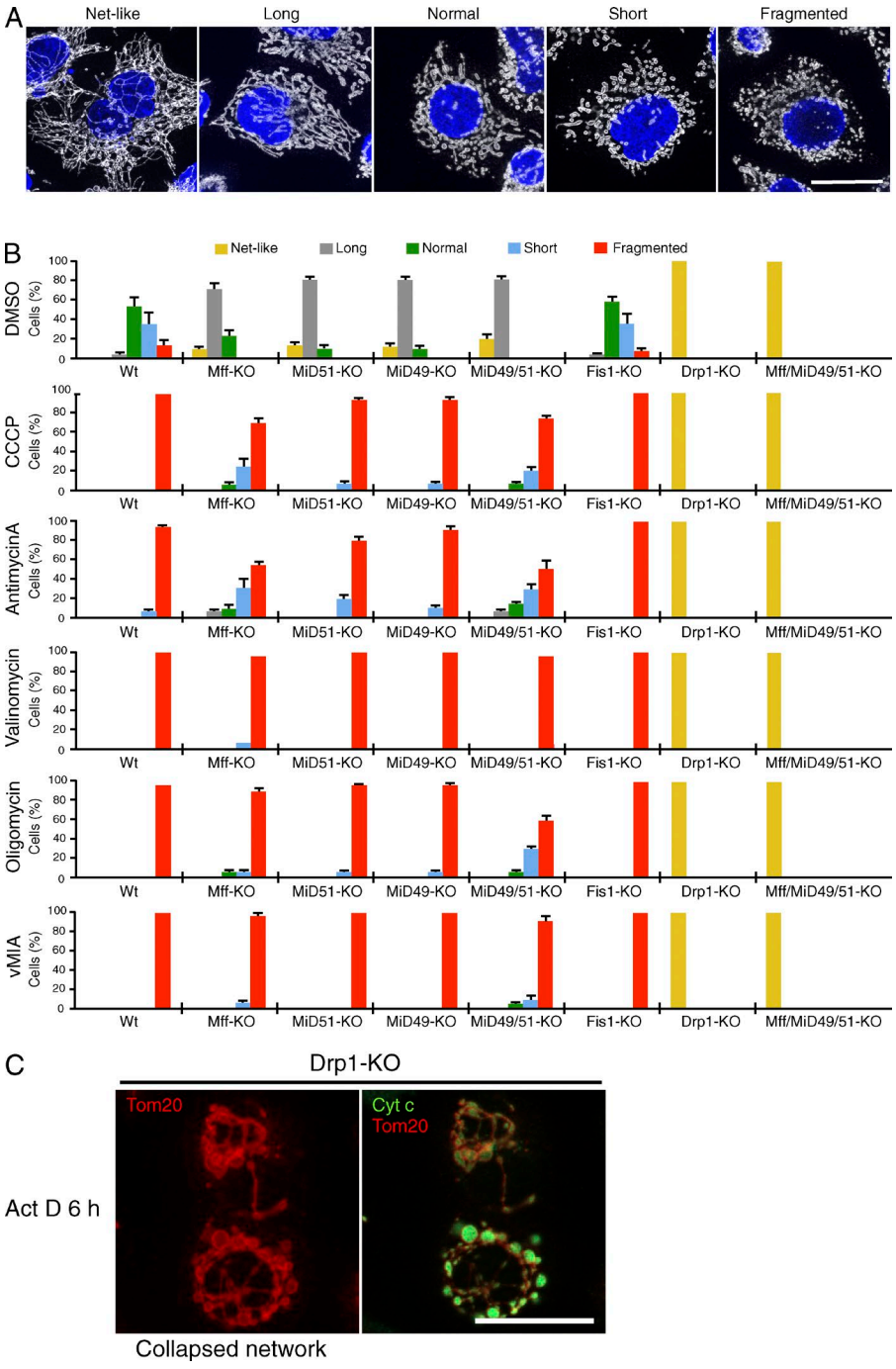

Figure S1. **Morphological response of mitochondrial fission-related protein-KO HeLa cell lines to various detrimental stressors.** (A) Representative mitochondrial morphology. Mitochondria were visualized by staining with anti-Tom20 antibody. Bar, 10 µm. (B) Wild-type (Wt) and knockout cell lines were incubated for 60 min with the indicated drugs. Mitochondria were visualized as in A. Percentages of cells with the indicated mitochondrial morphologies are shown. To assess the effect of vMIA, the indicated cells were transfected with vMIA-GFP. After 24 h, the indicated mitochondrial morphologies were counted. 200 cells each were counted in three independent experiments. $n = 3$. Error bars show SD. (C) Drp1-KO cells were treated with Act D in the presence of zVAD-FMK for 6 h. Cells were analyzed by confocal immunofluorescence microscopy for Tom20 (red) and cytochrome *c* (Cyt c; green). Bar, 20 µm.

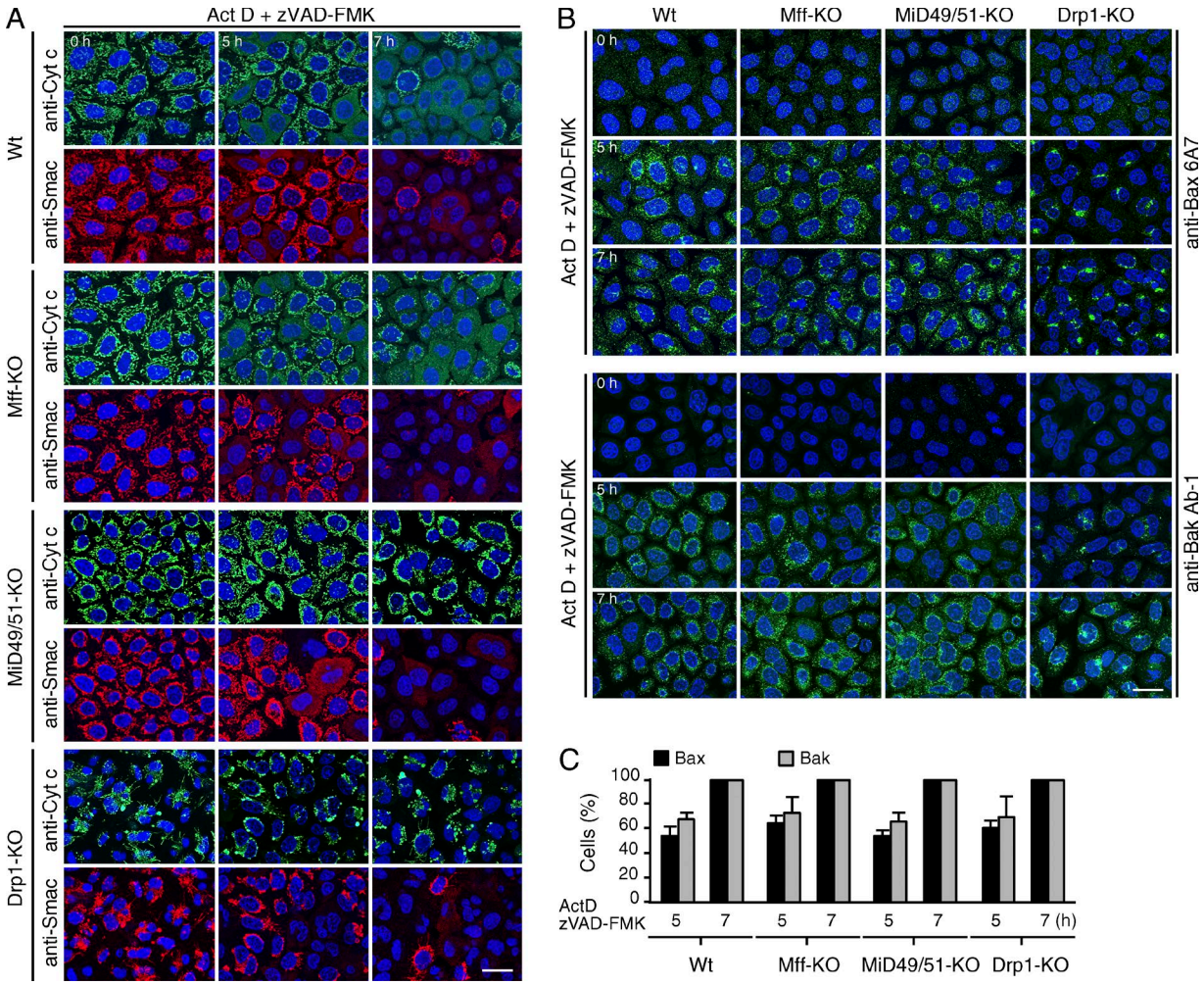

Figure S2. **Apoptotic response of mitochondrial fission-related protein-KO HeLa cell lines to actinomycin D.** HeLa cells were treated with Act D in the presence of zVAD-FMK. They were fixed and stained at fixed intervals after the addition of Act D as indicated. (A) Effects on cytochrome *c* and Smac/DIABLO release from mitochondria as detected by staining the cells with the indicated antibodies. (B) Effects on the activation of Bax or Bak as detected by staining the cells with the indicated antibodies. Bar, 20 µm. (C) Time course of Bax and Bak activation. HeLa cells (n = 300) in B positively stained for Bax or Bak were counted in three distinct fields. n = 3. Data represent mean ± SD. Wt, wild type.

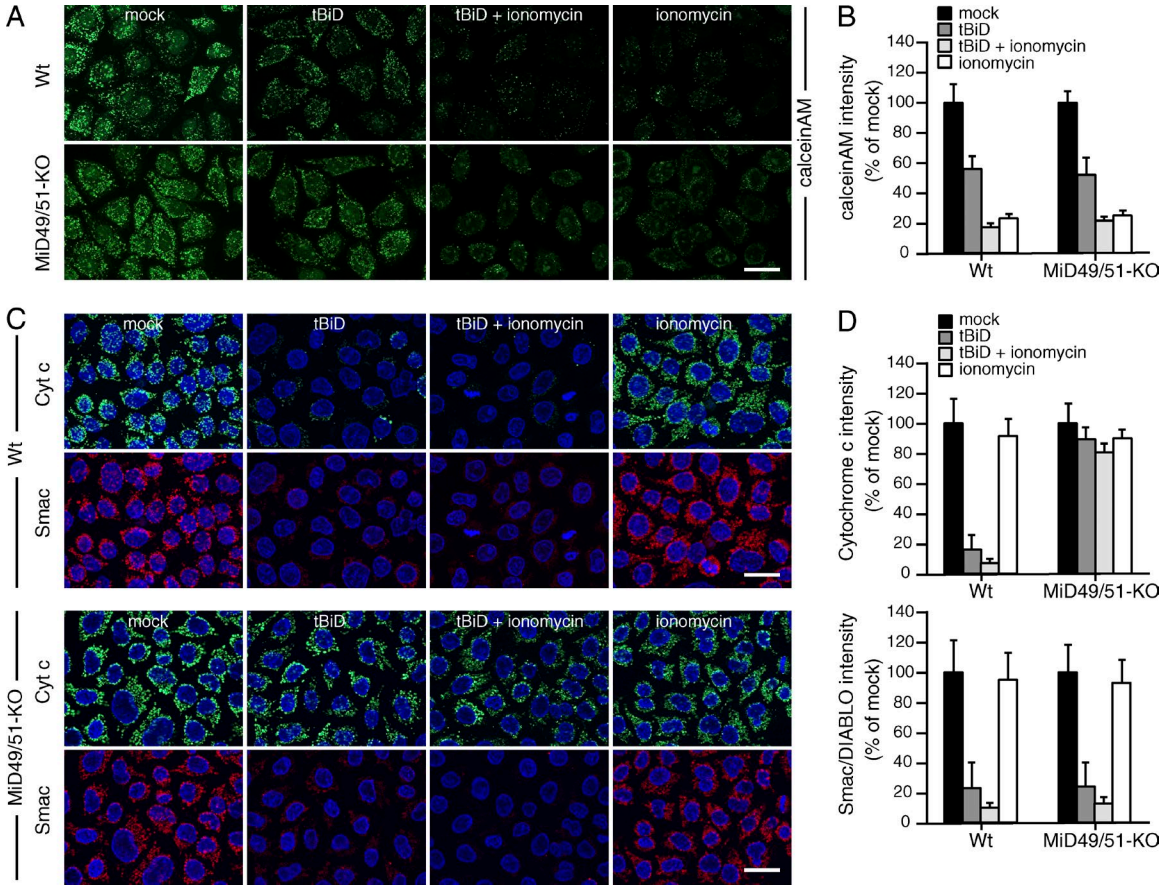

Figure S3. **MiD49/51-KO cells resist tBiD-induced cytochrome *c* release even after MPTP opening.** (A) Calcein-AM–loaded semi-intact cells were incubated with recombinant tBiD in the presence or absence of ionophore ionomycin to monitor mPTP opening. Bar, 20 μm. (B) Retained calcein-AM fluorescence was measured from randomly selected three different regions. $n = 3$. Data represent mean ± SD. (C) Digitonin-permeabilized semi-intact cells prepared from wild-type (top) and MiD49/51-KO cells (bottom) were incubated with a recombinant tBiD in the presence or absence of ionomycin, and the release of cytochrome *c* and Smac/DIABLO was analyzed by immunofluorescence microscopy with the appropriate antibodies. Bar, 20 μm. (D) Retained fluorescence of cytochrome *c* and Smac was measured from randomly selected three different regions. $n = 3$. Data represent mean ± SD. Wt, wild type.

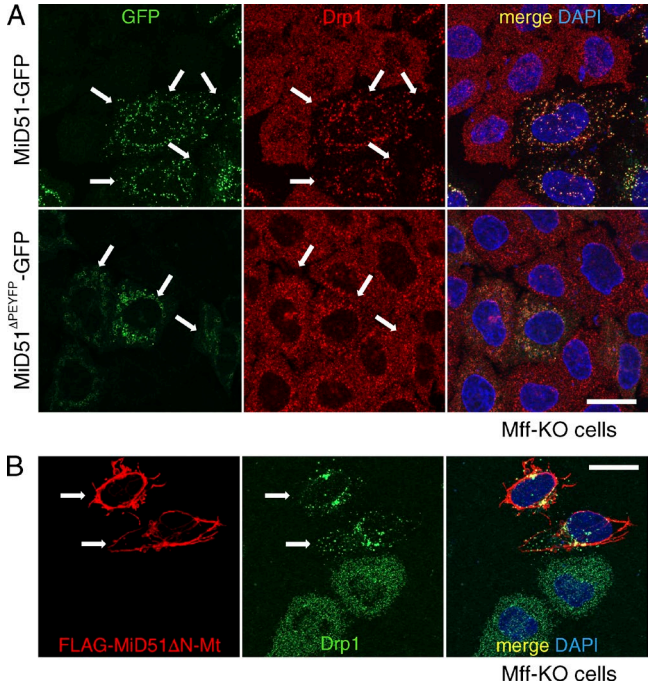

Figure S4. **Subcellular localization and Drp1 recruiting activity of MiD51 mutants.** (A) Mff-KO cells were transfected with MiD51-GFP or MiD51$^{\Delta PEYFP}$-GFP. Confocal images were obtained by immunostaining for Drp1 (red). Nuclei were stained with DAPI. Arrows indicate GFP-tagged proteins expressing cells. Bar, 20 µm. (B) Mff-KO cells were transfected with FLAG-MiD51ΔN-Mt. Confocal images were obtained by immunostaining for FLAG (red) and Drp1 (green). Arrows indicate FLAG-MiD51ΔN-Mt–expressing cells. Nuclei were stained with DAPI. Bar, 20 µm.

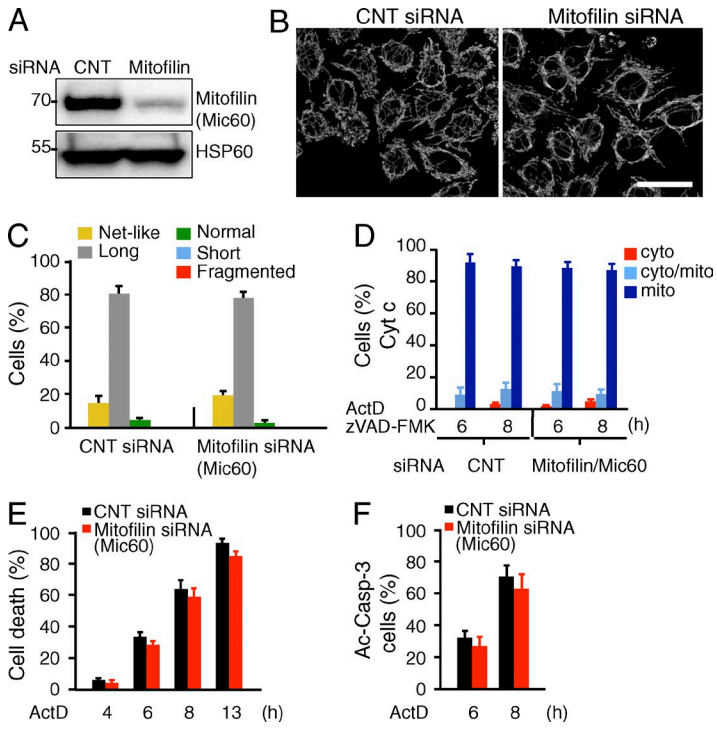

Figure S5. **Down-regulation of mitofilin/Mic60 does not affect mitochondrial morphology, cytochrome *c* release, and apoptosis in MiD49/51-KO cells.** MiD49/51-KO cells were transfected with control (CNT) or mitofilin/Mic60 siRNA. (A) Effect of mitofilin/Mic60 RNAi was confirmed by Western blotting. HSP60 serves as loading control. (B) Confocal images of MiD49/51-KO cells transfected with the indicated siRNA were obtained by immunostaining for Tom20. Bar, 20 µm. (C) Percentages of the indicated mitochondrial morphologies of MiD49/51-KO cells (n = 300) transfected with the indicated siRNA. (D) Time course of cytochrome *c* release. The indicated cells were treated with Act D in the presence of zVAD-FMK for the indicated times and immunostained with anti–cytochrome *c* antibodies. (E and F) The effect (%) of mitofilin/Mic60 knockdown on cell death and caspase-3 activation, respectively, in MiD49/51-KO cells. The percentages in C–F are means of three independent experiments with 300 cells per data point. n = 3. Data represent mean ± SD in C–F.