Li et al., http://www.jcb.org/cgi/content/full/jcb.201404050/DC1

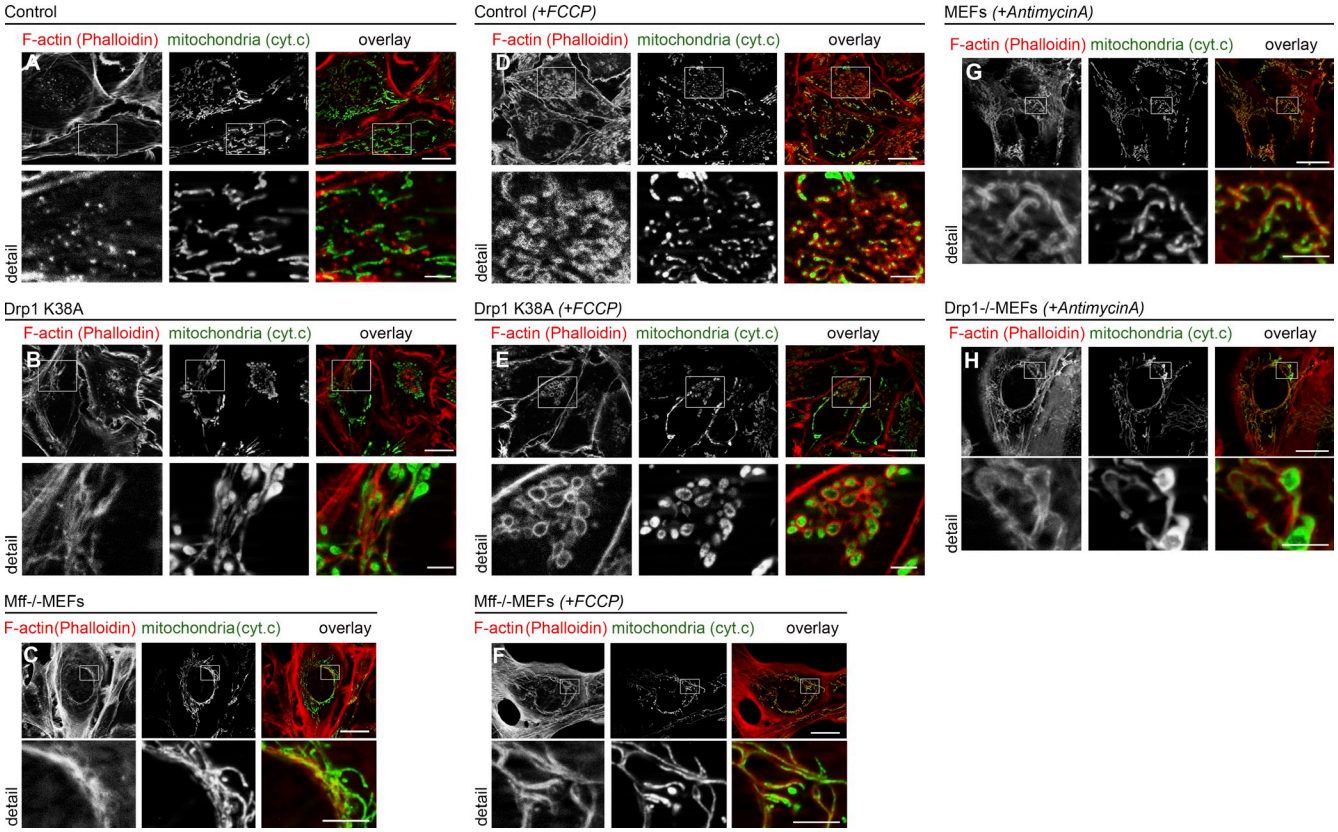

Figure S1. **Submitochondrial distribution of F-actin in FCCP- and Antimycin A–treated cells.** (A–F) Control HeLa cells (A and C), HeLa cells expressing a dominant-negative mutant of Drp1 (Drp1^{K38A}; B and E), and Mff$^{-/-}$ MEFs (C and F) were treated with either DMSO (vehicle; A–C) or FCCP (D–F) for 2 min, immunostained with anti–cytochrome *c* mAb (green on overlay images) and Alexa-phalloidin (red on overlay images), followed by structured illumination imaging. (G and H) Wild-type (G) and Drp1$^{-/-}$ (H) MEFs were treated with Antimycin A and immunostained as described above. Higher magnifications of areas marked with yellow rectangles are shown in detail images. Bars: 20 µm; (detail) 5 µm.

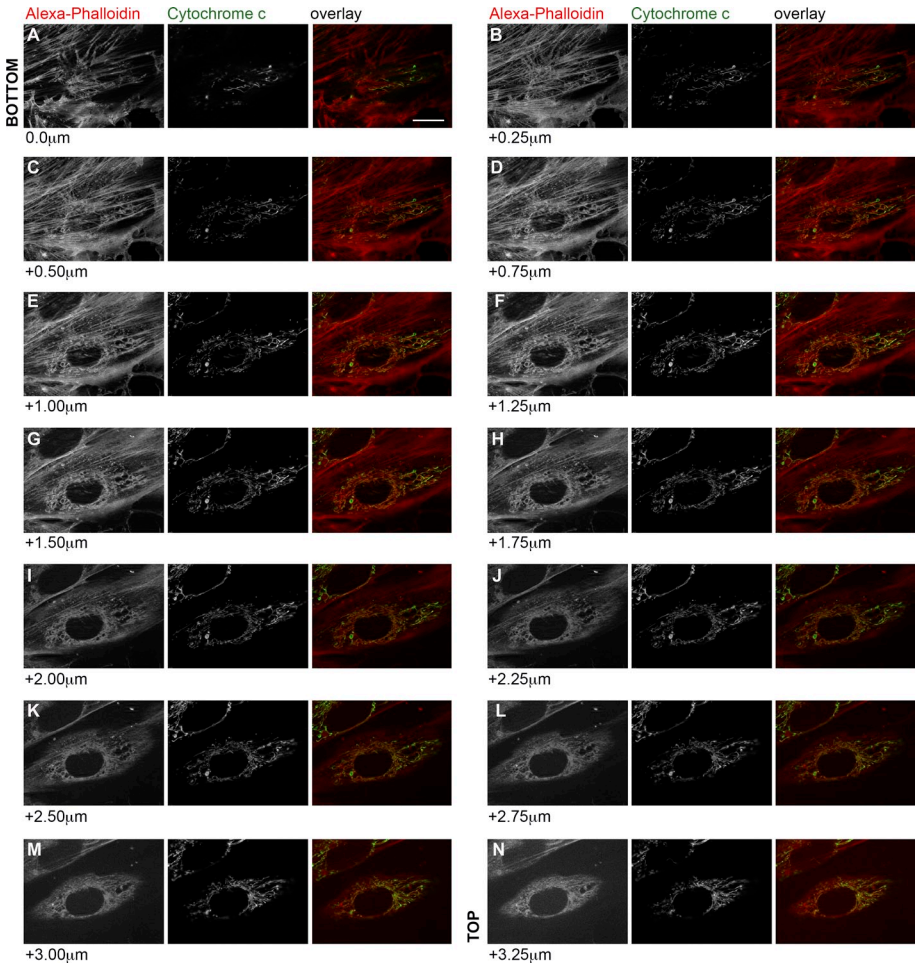

Figure S2. **Spatial relation of F-actin and mitochondria in FCCP-treated Drp1$^{-/-}$ MEFs.** Drp1$^{-/-}$MEFs treated with FCCP for 2 min were immunostained with anti–cytochrome *c* mAb (green on overlay images) and Alexa-phalloidin (red on overlay images), followed by structured illumination imaging. The sequence of single z-sections (0.25 µm intervals), from the bottom (**A**) to the top (**N**) of the cells is shown. Bar, 20 µm.

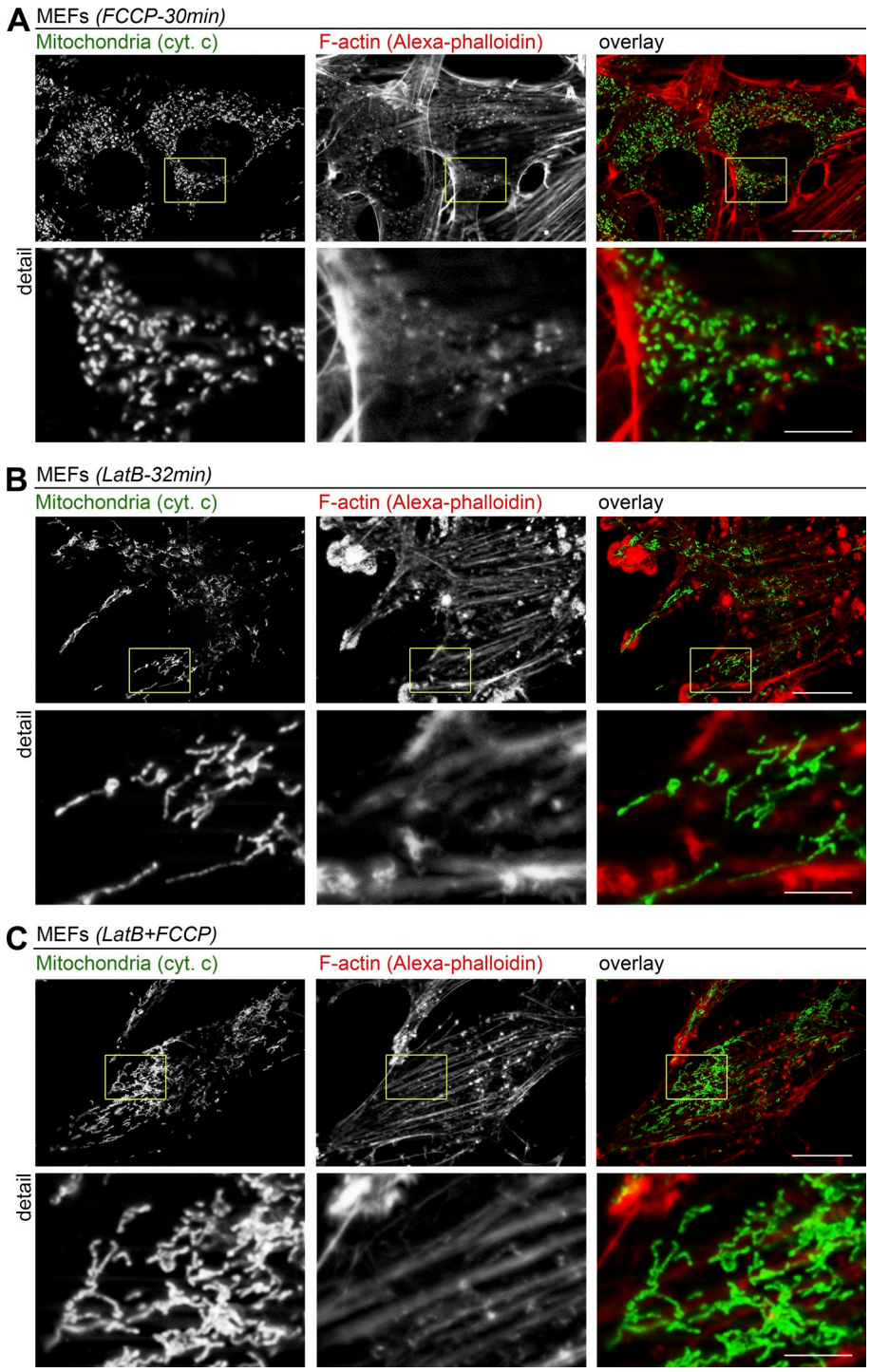

Figure S3. **Effect of actin polymerization inhibitor LatB on the mitochondrial morphology.** Wild-type MEFs (A–C) were treated with FCCP for 30 min (A), LatB for 32 min (B), or pretreated with LatB for 2 min, followed by addition of FCCP for additional 30 min (C). Cells were labeled with Alexa-phalloidin to detect F-actin (red on overlay images) and immunostained with anti–cytochrome *c* mAb (green on overlay images) to detect mitochondria. Bars: 20 μm; (detail) 5 μm.

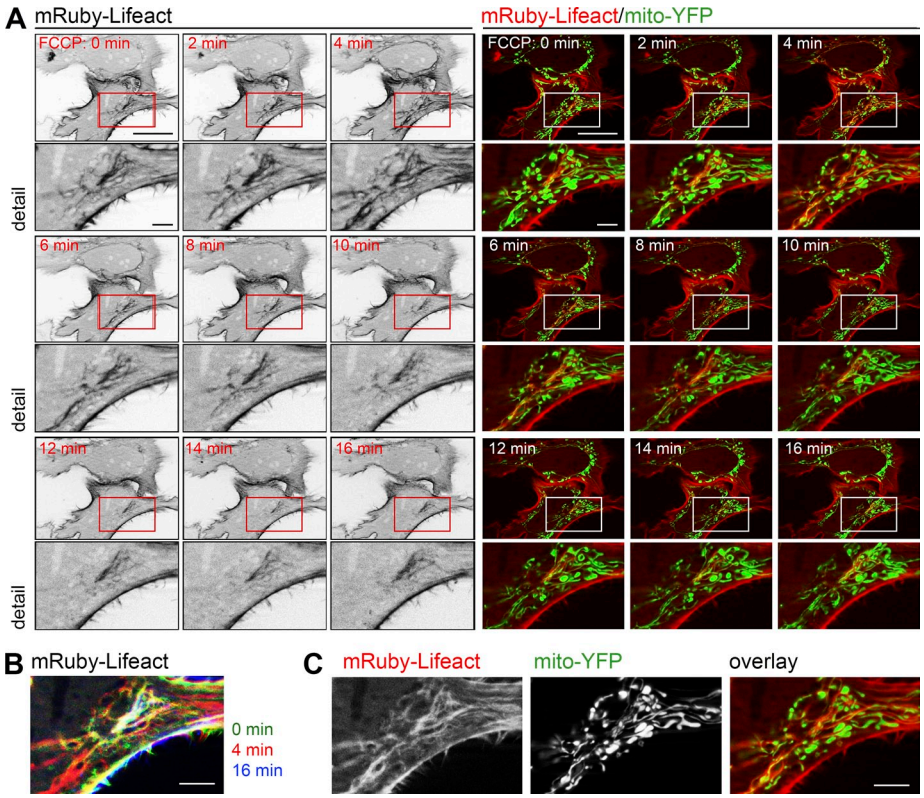

Figure S4. **Mitochondrial assembly of F-actin in Drp1^{K38A}-expressing living HeLa cells.** (A) HeLa cells expressing Drp1^{K38A} (determined by mitochondrial morphology), mRuby-Lifeact (red), and mito-YFP (green) were treated with FCCP as indicated, followed by a time-lapse structural illumination imaging. Bars, 20 µm. Left panels show fluorescence images of mRuby-Lifeact. To enable easier interpretation of the data, fluorescent images were inverted. Bars: 20 µm; (detail) 5 µm. (B) Pseudocolored images showing the mitochondrial F-actin assembly at 0 min (green), 4 min (red), and 16 min (blue) after addition of FCCP within the area shown in A. Bars, 5 µm. Note a dominant red signal in B indicating high level of mitochondrial F-actin at 4 min of FCCP treatment, as compared with 0 min and 16 min. Details from marked area in the image taken at 4 min (shown in A) of FCCP treatment.

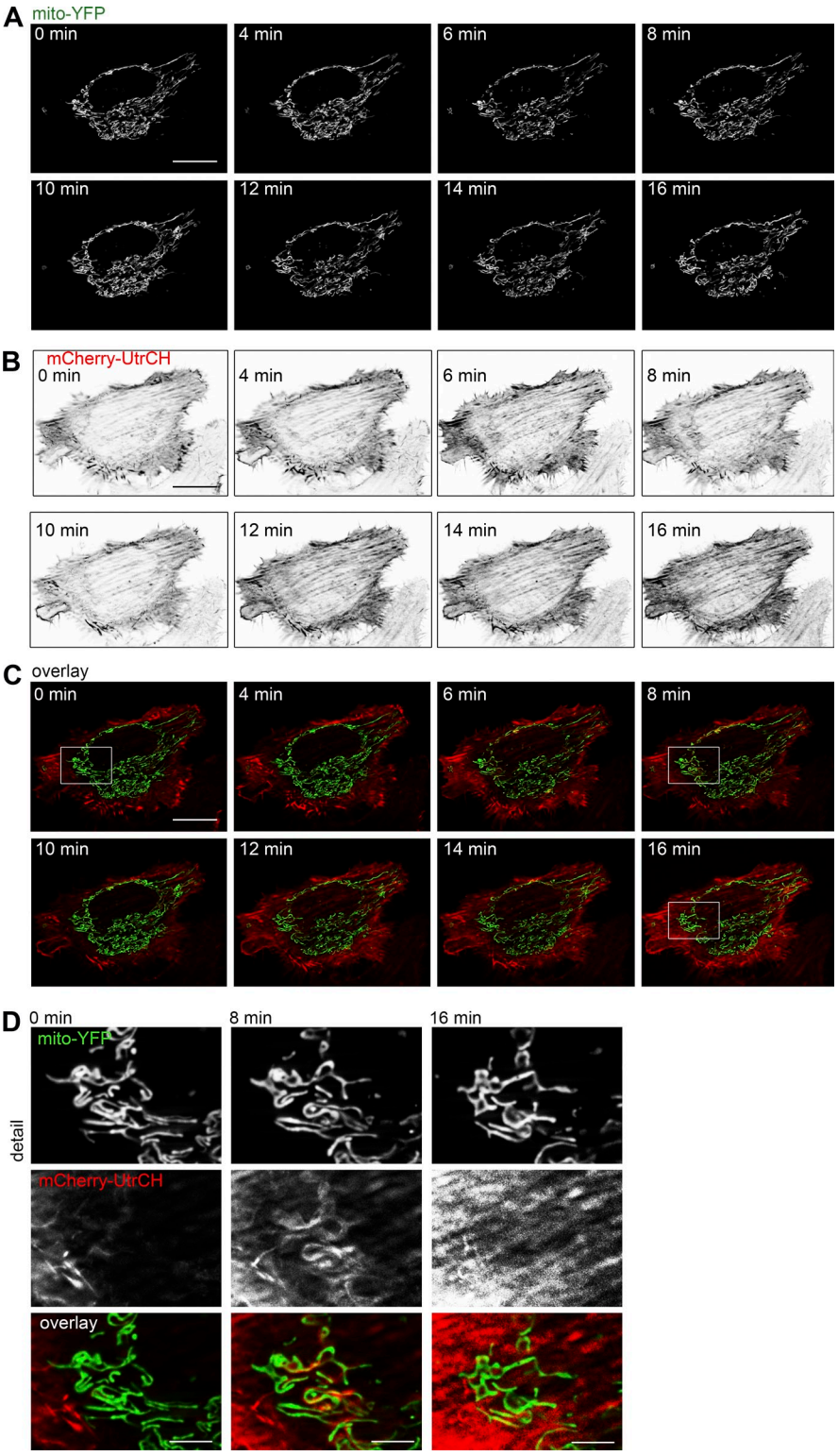

Figure S5. **Mitochondrial assembly of mCherry-UtrCH in living HeLa cells.** HeLa cells expressing mito-YFP (A, and green in C) and mCherry-UtrCH (B, and red in C) were treated with FCCP as indicated, followed by a time-lapse structural illumination imaging. Bars, 20 μm. (D) Details from marked areas in C at indicated time points. Bars, 5 μm.