Supplemental Figure Legends:

A novel fission-independent role of DRP1 in cardiac mitochondrial respiration

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Figure S1. Image processing for mitochondrial morphology quantification in adult cardiomyocytes. The original image was first enhanced by subtracting background, adjusting contrast and then filtering with a kernel to obtain the enhanced image. The enhanced image was then calculated by the ImageJ software, which output the mask showing the selected areas (in black) and the parameters (e.g., size, circularity, and aspect ratio) for each selected area.
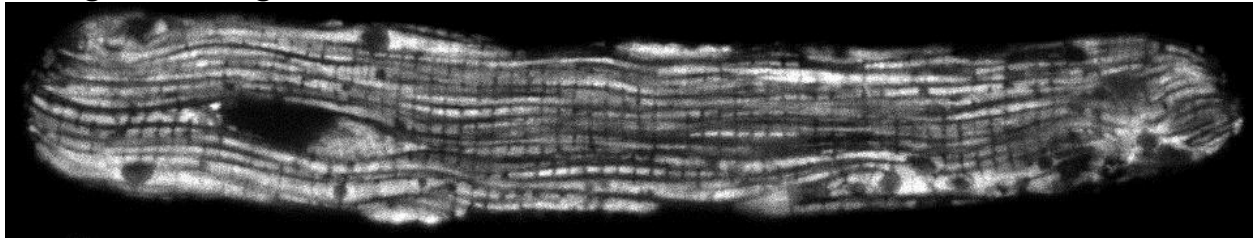
Figure S2. Western blot showing the expression of K38A mutation (A) or the wild type DRP1 in H9C2 (B). Similar results were obtained from at least 3 independent experiments.

Figure S3. DRP1 manipulation on OCR and extracellular acidification rate (ECAR) in adult cardiomyocytes. A, State 4 and State 3 OCR in isolated mitochondria from adult mouse heart. N = 3. **B**, FCCP-induced maximal OCR in permeabilized adult cardiomyocytes with or without cyclosporine A (CsA, 1 µM) or Mdivi-1 (50 µM) treatment. N = 4-5. **C**, Basal, leak (after 2.5 µM oligomycin A), and maximal (after 1 µM FCCP) OCR in intact adult cardiomyocytes by Seahorse system. N = 4-12. **D**, ECAR monitored by Seahorse system at basal, leak (oligomycin A), and maximal (FCCP) conditions. N = 4-12. The treatments in **A-D** are the same as in Figure 3. *: $P<0.05$ vs Control.

Figure S4. In gel Complex I activity. NADH (5 mM) and Nitrotereazolium blue (0.5 mM) were used as substrates in the staining buffer and the blue native gel was stained for 1 hr at room temperature then the image was taken by a gel documentation system (Bio-Rad). The mitochondria from Ndufs4H-/- mouse hearts (cardiac specific Complex I deficiency) were used as positive control showing decreased Complex I activity.

Figure S1
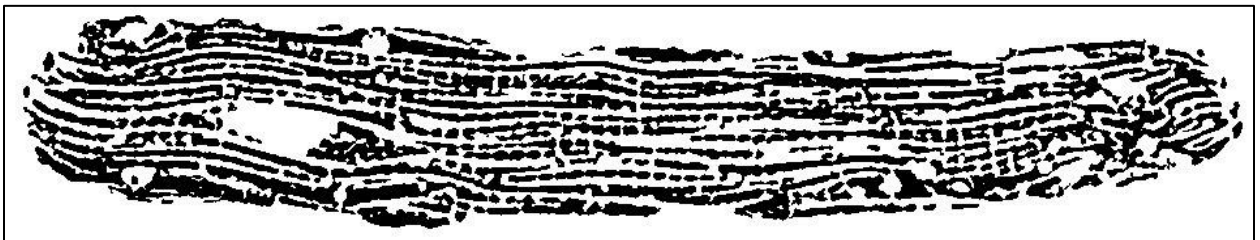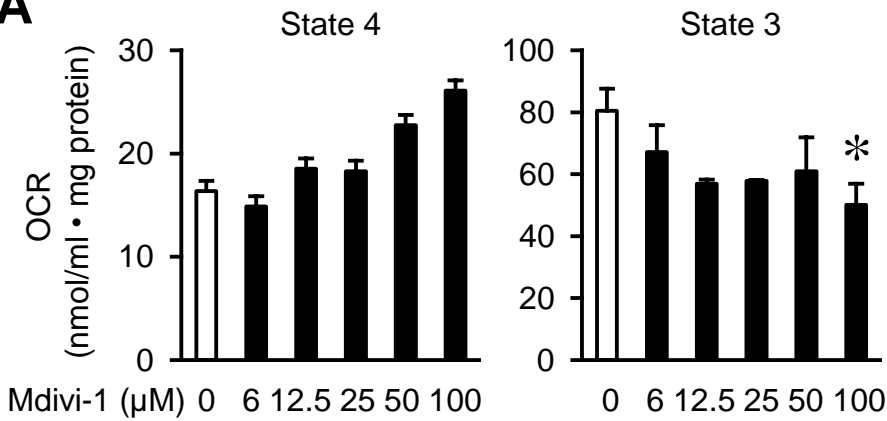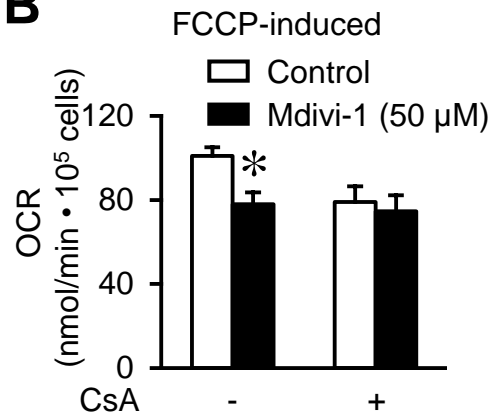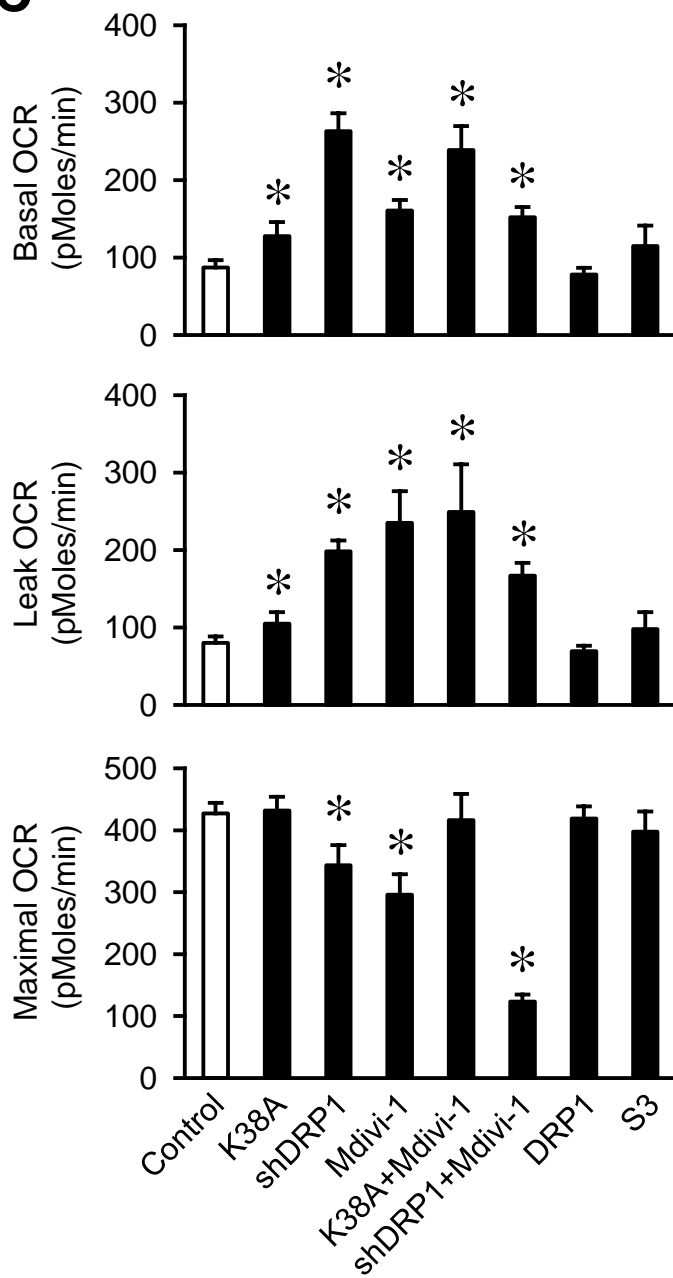
Original image

Enhanced image

Mask

Figure S2

A

B

Figure S3

Figure S4

Super complexes

Complex I Monomeric →

DMSO　　Mdivi-1　　Ndufs4H-/-

← Complex I disassembled