

Expanded View Figures

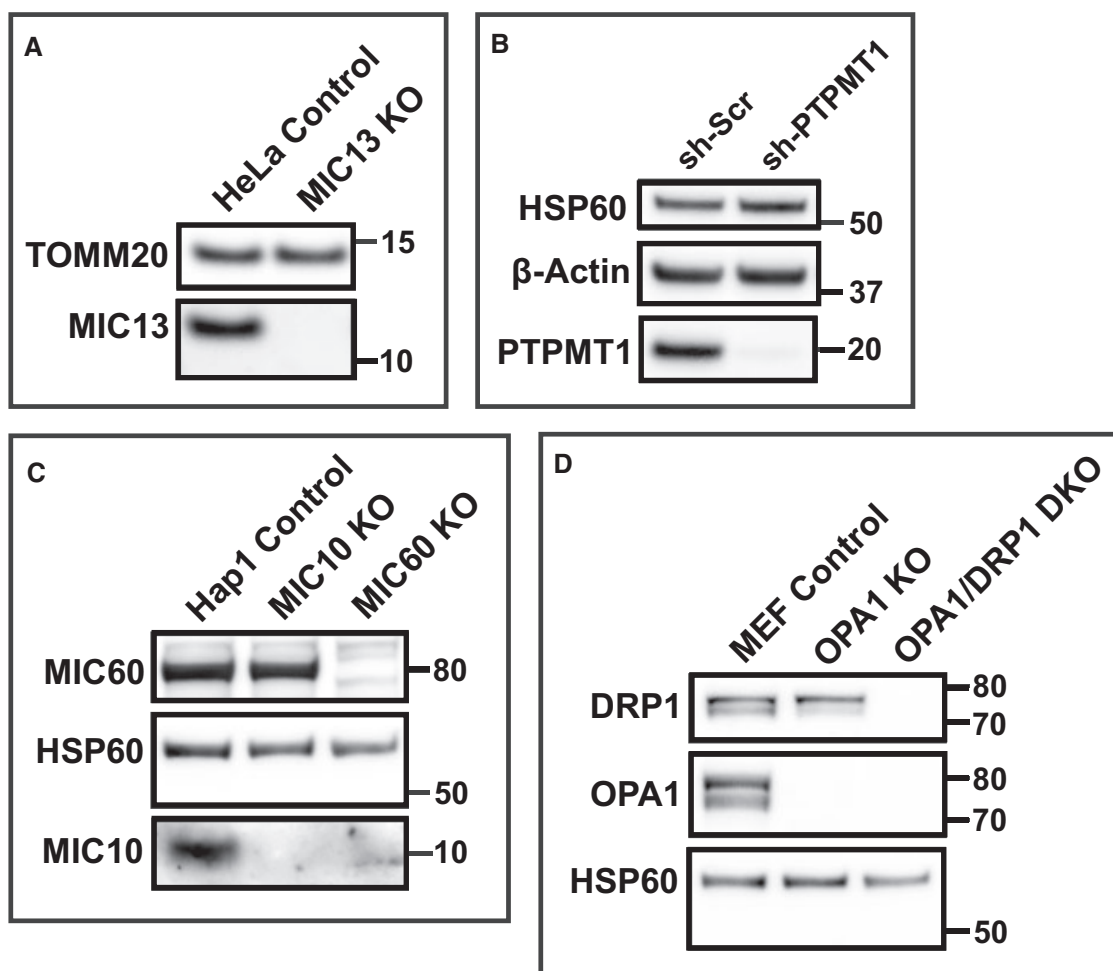


Figure EV1. Validation of KO and KD approaches of Cristae structure and Cj modulators.

- A Representative image of Western blot, showing deletion of MIC13 in HeLa cells. *N* = 3 independent experiments.
 B Representative image of Western blot, showing deletion of PTPMT1 in H1975 cells. *N* = 3 independent experiments.
 C Representative image of Western blot, showing deletion of MIC60 and MIC10 in Hap1 cells. *N* = 3 independent experiments.
 D Representative image of Western blot, showing Opa1 KO and Opa1&Drp1 DKO in MEFs. *N* = 3 independent experiments.

Source data are available online for this figure.

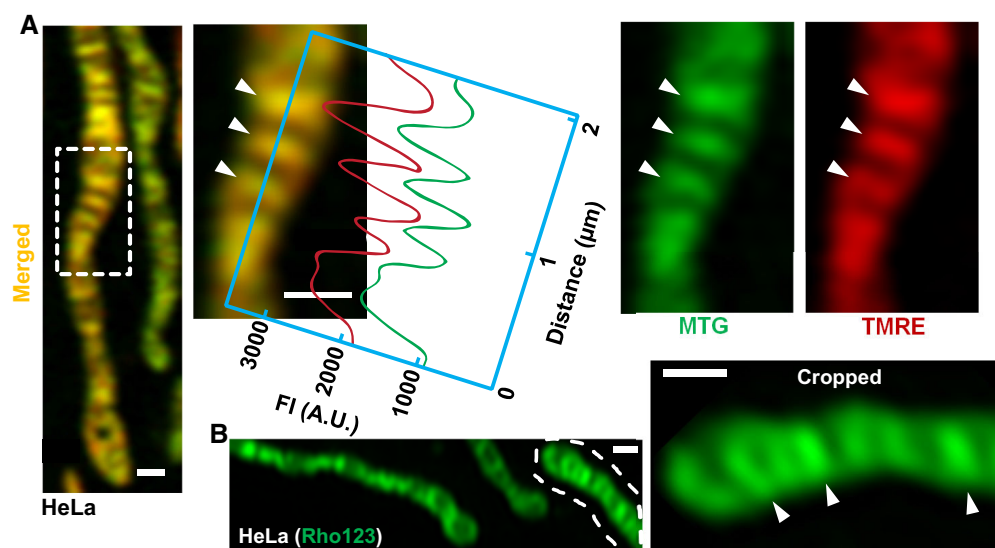


Figure EV2. Membrane potential dyes, TMRE and Rho123, are concentrated at the cristae membranes in a heterogeneous pattern.

A Mitochondria in HeLa cells co-stained with MTG and TMRE. Zoomed-in region from dashed white box highlights colocalization of dyes, showing that signal intensities vary together across the long axis (green and red lines); arrowheads indicate cristae membranes. Scale bar = 500 nm. $N = 3$ independent experiments.

B Mitochondria in HeLa cell stained with $\Delta\Psi_m$ -dependent dye, Rho123; zoomed-in mitochondrion cropped from dashed line highlights more intense signal at cristae (arrowheads). Scale bar = 500 nm. $N = 3$ independent experiments.

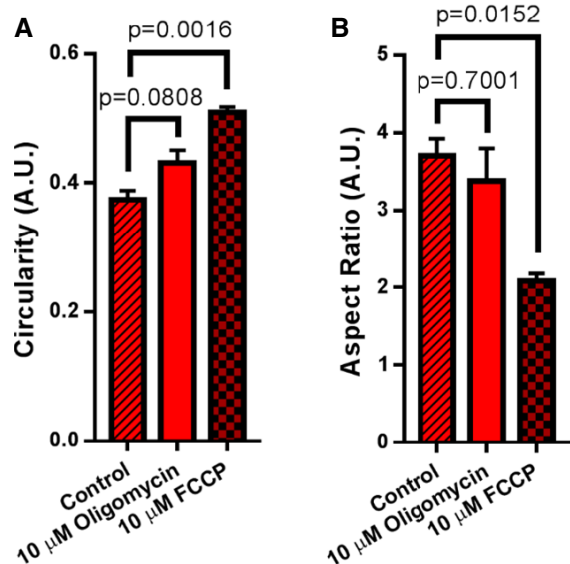


Figure EV3. Effects of FCCP and oligomycin on mitochondrial morphology.

A Quantification of mitochondrial circularity, where mitochondria with circularity closer to 1 resemble a circular object and mitochondria with circularity closer to 0 resemble a branched object. Note: FCCP results in significant increase in mitochondrial circularity, consistent with numerous studies indicating FCCP induces fragmentation. $N = 3$ independent experiments.

B Quantification of mitochondrial aspect ratio (a ratio of the long axis of the mitochondrion to the short axis), where mitochondria with an aspect ratio closer to 1 are rounder, whereas mitochondria higher and higher than 1 are increasingly elongated. Note: FCCP also leads to a significant decrease in aspect ratio, indicating loss of membrane potential results in fragmentation. $N = 3$ independent experiments.

Data information: Data were analyzed with 2-tailed Student's t -tests, and P values < 0.05 were considered statistically significant. Specific P values are indicated in the figure. Error bars indicate SEM.