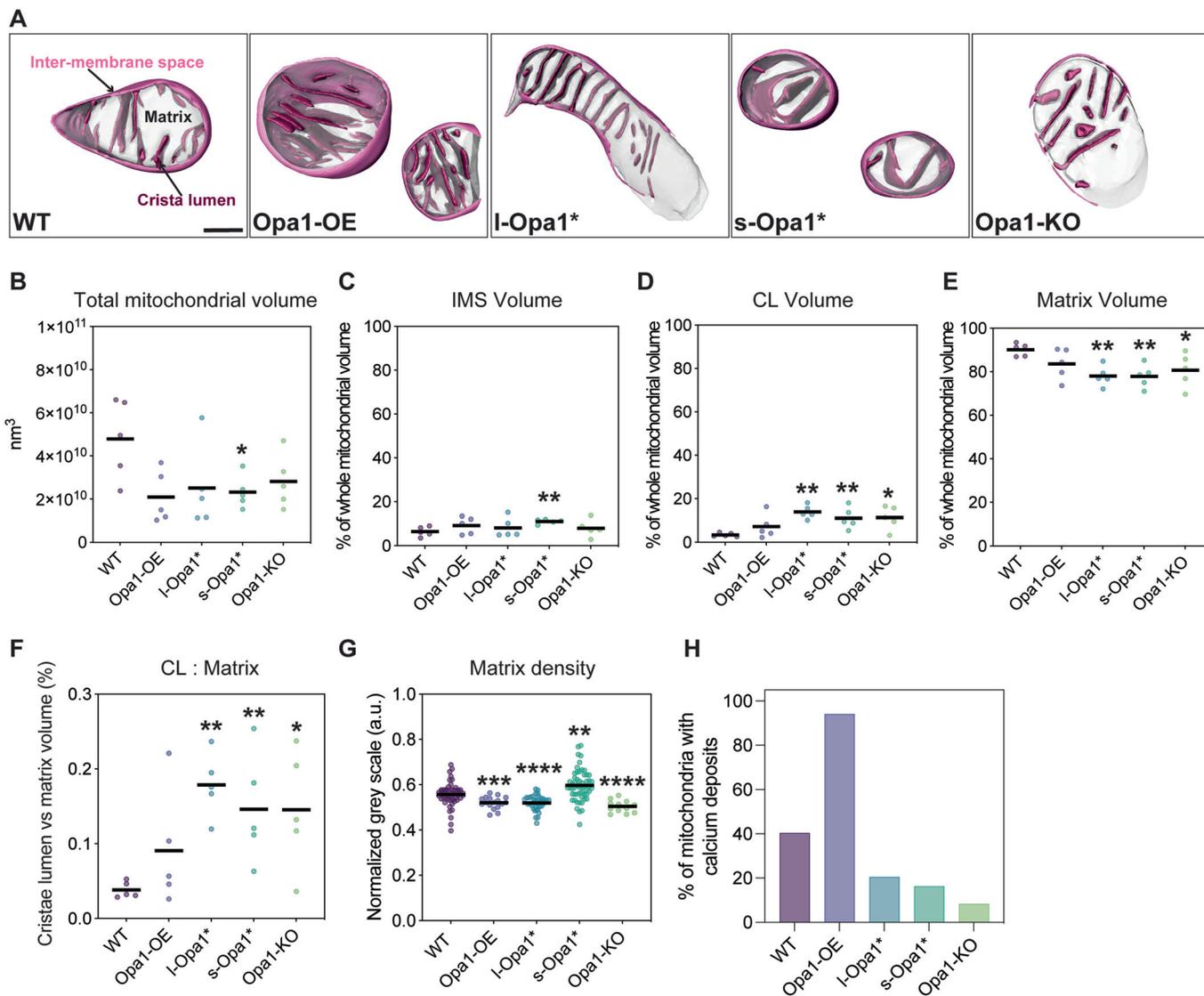
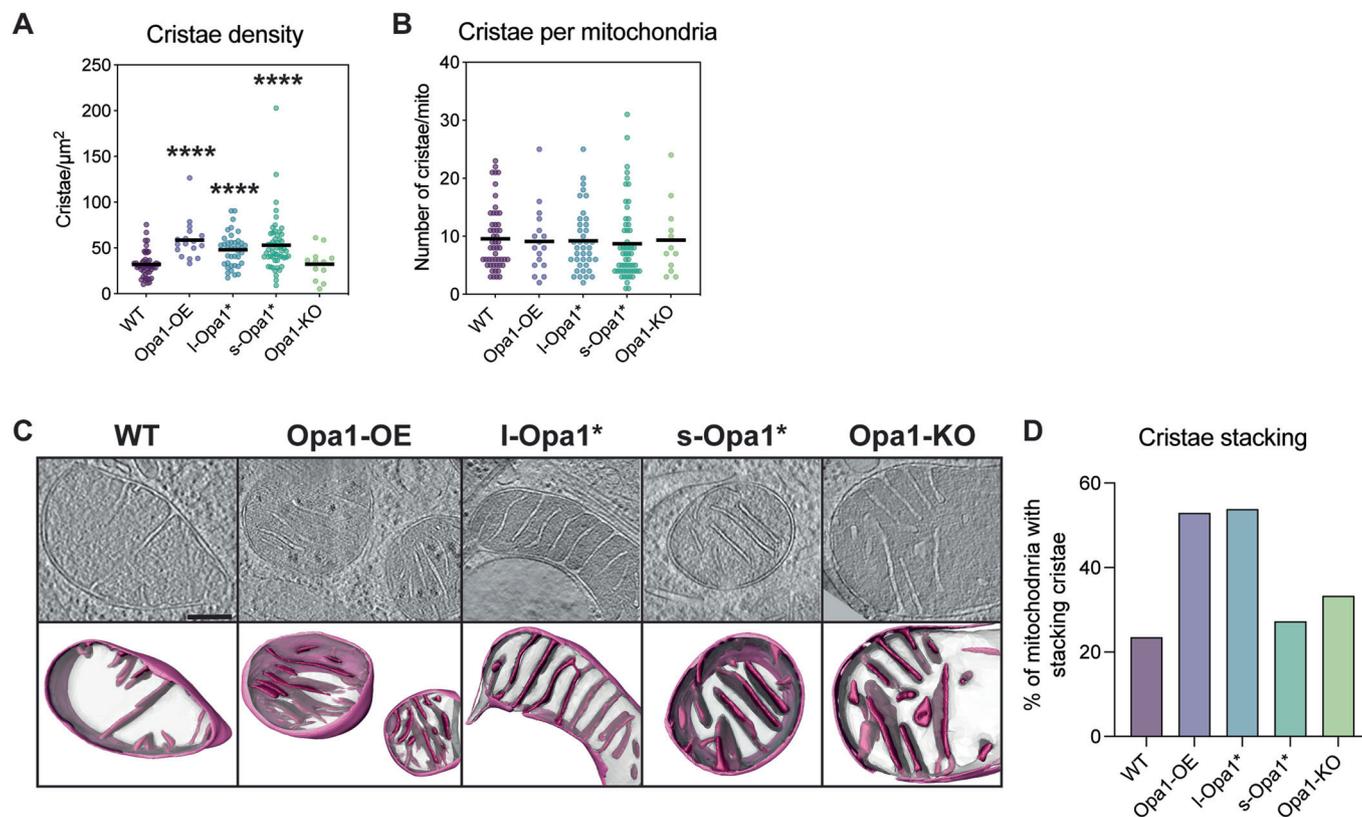


## Expanded View Figures



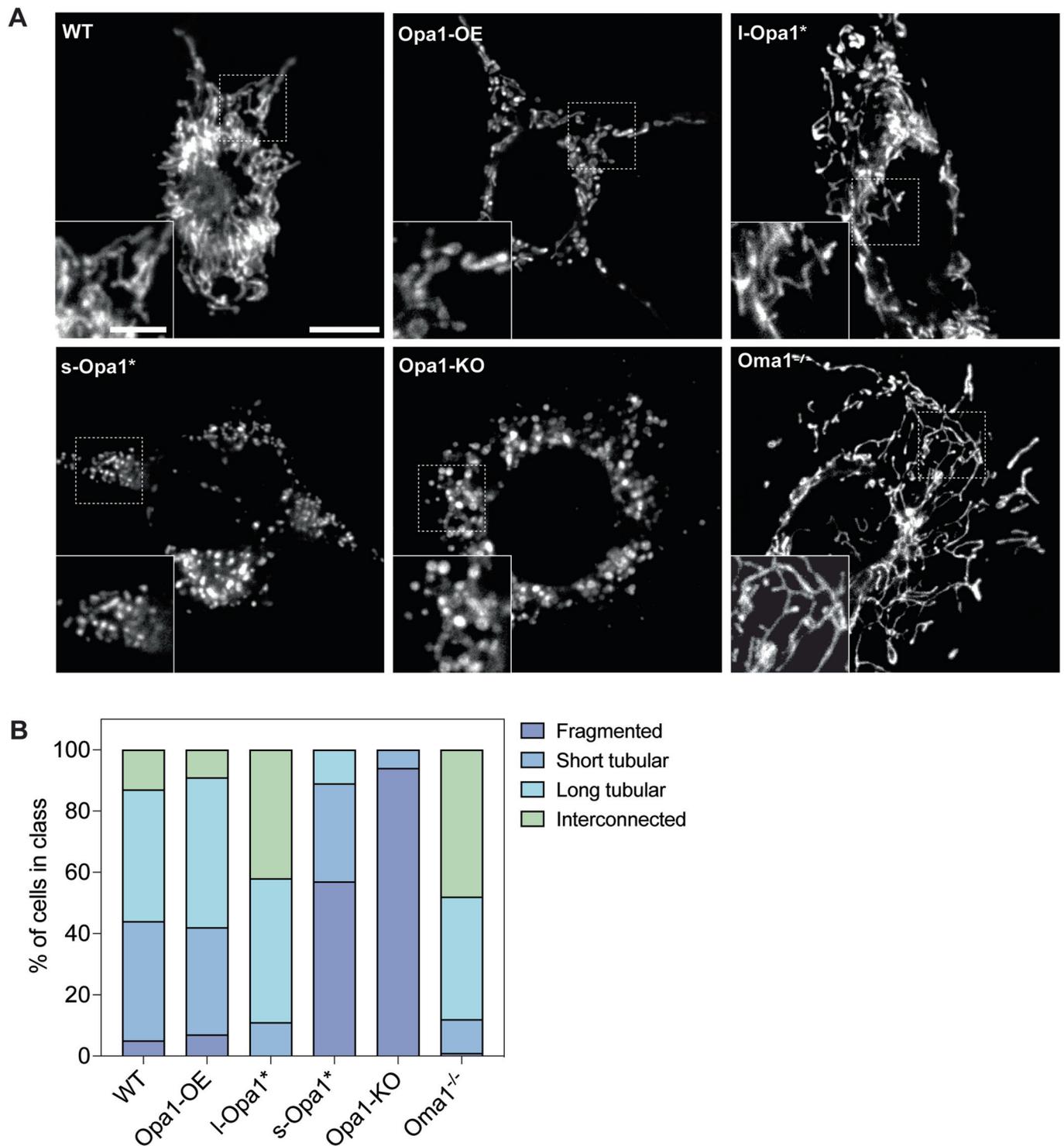
**Figure EV1. Mitochondrial subcompartment volumes.**

(A) Three-dimensional renderings of segmented inter-membrane space (IMS, pink surface), cristae lumen (CL, magenta surface), and matrix (translucent gray surface) volumes. Scale bar = 200 nm. (B) Total mitochondrial volume across indicated cell lines.  $N = 5$  cells for all cell lines. (C) Quantification of IMS volume relative to total volume of each mitochondrion indicated in (B).  $N = 5$  cells for all cell lines. (D) Quantification of CL volume relative to total volume of each mitochondrion indicated in (B).  $N = 5$  cells for all cell lines. (E) Quantification of matrix volume relative to total volume of each mitochondrion indicated in (B).  $N = 5$  cells for all cell lines. (F) CL to matrix ratio across cell lines.  $N = 5$  cells for all cell lines. (G) Normalized grey scale mitochondrial matrix value across cell lines.  $N = 5$  cells for all cell lines. (H) Graph bar representing percentage of cells with detected calcium deposits in cryo-electron tomograms.  $N$  refers to the number of mitochondria: WT = 57, Opa1-OE = 17, I-Opa1\* = 39, s-Opa1\* = 55, Opa1-KO = 12. Data information: Scatter plots show data distribution, the mean is shown by a bold black line. Significance of difference is tested relative to wild type using Mann-Whitney test in (B, D, E, G); \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ ; and unpaired  $t$  test in (C): \*\* $p < 0.01$ .



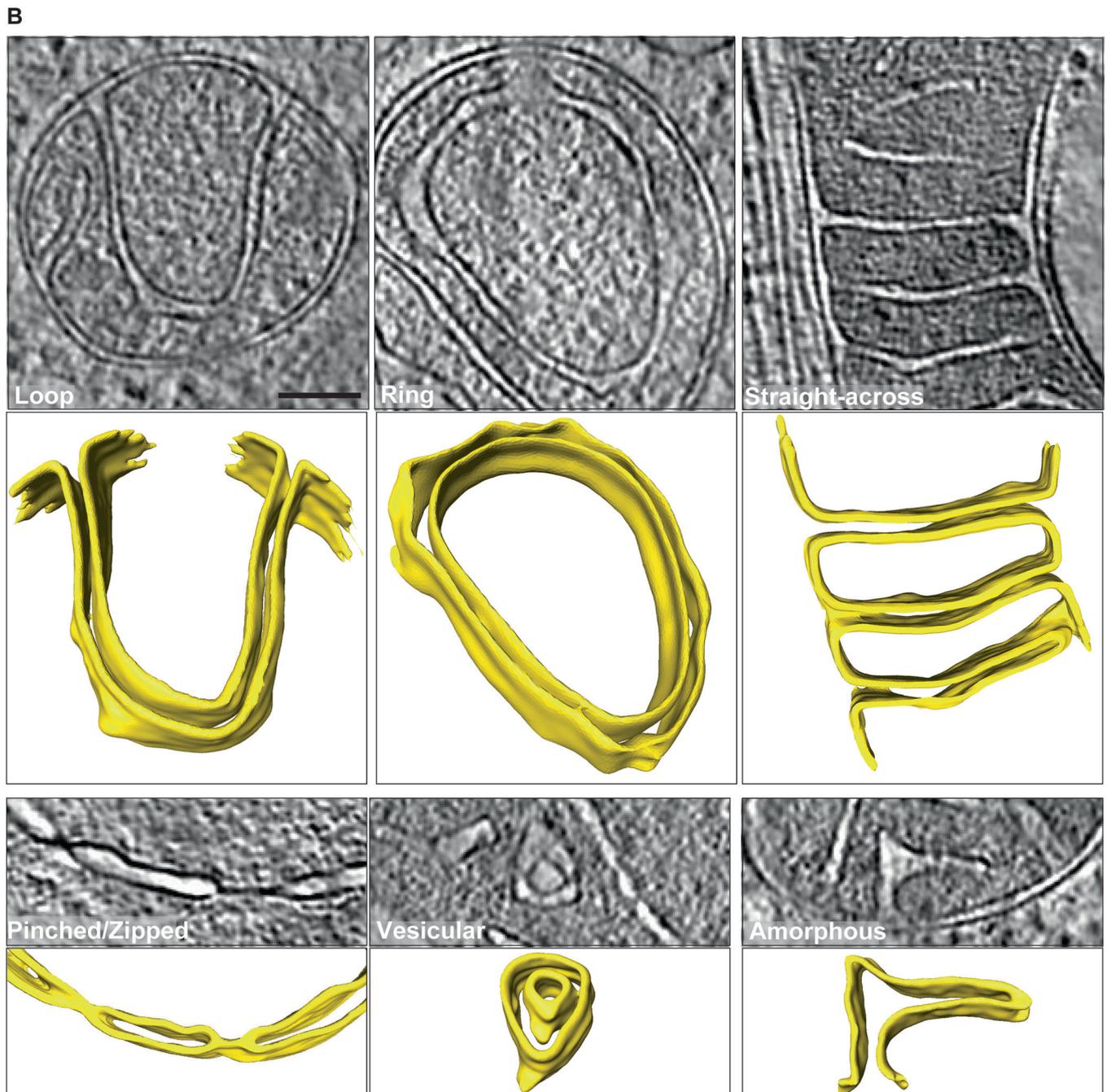
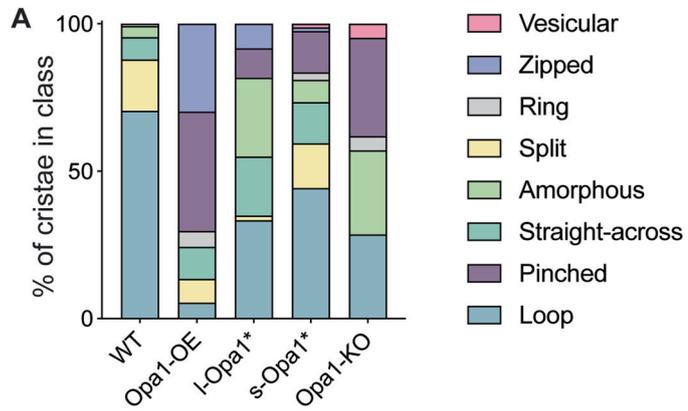
**Figure EV2. Cristae analysis.**

(A) Cristae density (cristae per  $\mu\text{m}^2$ ) represented as a scatter plot. *N* refers to number of cells; WT = 33, Opa1-OE = 7, I-Opa1\* = 21, s-Opa1\* = 28, Opa1-KO = 11. (B) Number of cristae per mitochondria represented as a scatter plot. *N* refers to number of cells; WT = 51, Opa1-OE = 17, I-Opa1\* = 39, s-Opa1\* = 55, Opa1-KO = 12. (C) (Top) Summed, projected central slices of cryo-electron tomograms visualizing mitochondria with stacking crista characteristics, supported by 3D representations consisting of their sub compartments (bottom) in indicated MEF lines. Scale bar = 200 nm. *N* refers to number of cells; WT = 57, Opa1-OE = 17, I-Opa1\* = 39, s-Opa1\* = 55, Opa1-KO = 12. The representative tomograms for WT, Opa1-OE, I-Opa1\*, and Opa1-KO are the same as in Fig. 1A. The representative s-Opa1\* tomogram is the same as the second from the left in Appendix Fig. S2A. (D) Graph bar representing percentage of mitochondria with stacking crista formation in each MEF line. *N* refers to number of cells; WT = 57, Opa1-OE = 17, I-Opa1\* = 39, s-Opa1\* = 55, Opa1-KO = 12. Data information: Significance of difference is tested relative to WT using Mann-Whitney test; \*\*\*\**p* < 0.0001.



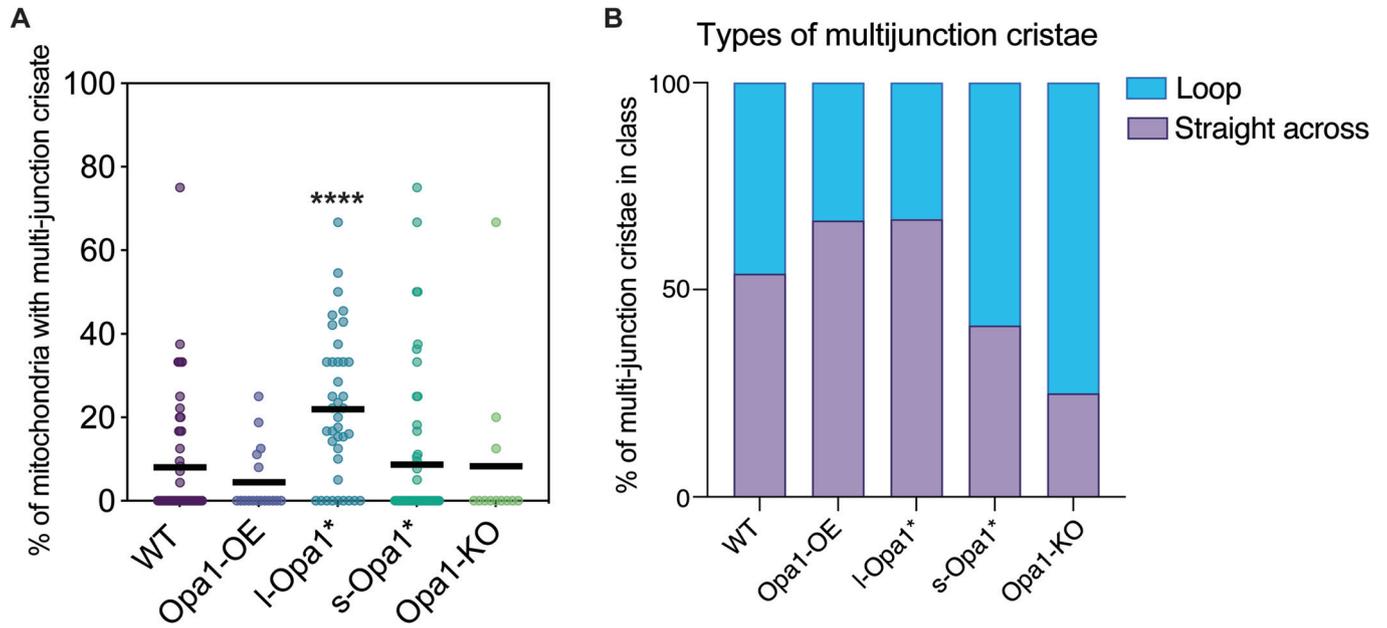
**Figure EV3. Mitochondrial network morphology in MEF lines by fluorescence microscopy.**

(A) Representative images of mitochondrial morphology in indicated MEF lines labeled with MitoTracker™ Deep Red FM. Insets show magnified view of regions indicated with dashed boxes. Scale bar = 10 μm. Inset scale bar = 5 μm. (B) Graph bar representing mitochondrial network morphology scored in indicated MEF lines. N = 100 cells analyzed per cell line.



**◀ Figure EV4. Unusual cristae morphology.**

(A) Graph bar representing the relative proportion of unusual cristae morphology observed in indicated MEF lines. Unusual cristae were categorized into vesicular, zipped, ring, split, amorphous, straight-across, pinched and loop. *N* refers to number of cristae analyzed, *N*: wild-type = 222, Opa1-OE = 430, I-Opa1\* = 323, s-Opa1\* = 653, Opa1-KO = 243. (B) Summed, projected central slices of cryo-electron tomograms showing examples of unusual cristae in mitochondria across cell lines in 2D (top) and 3D (bottom). Loop (from Fig. 1A, s-Opa1\*), ring, straight-across (from Fig. 1A, I-Opa1\*), pinched (from Appendix Fig. S2A, Opa1-KO second from the left), vesicular (from Fig. 1A, Opa1-KO), and amorphous cristae are shown. Scale bar = 200 nm.



**Figure EV5. Multijunction cristae.**

(A) Scatter plot showing the percentage of multijunction cristae per mitochondrion in indicated MEF lines. *N* refers to number of cristae; WT = 18, Opa1-OE = 5, I-Opa1\* = 30, s-Opa1\* = 16, Opa1-KO = 3. (B) Graph bar representing percentage of multijunction cristae categorized into straight-across and loop morphology in each MEF line. *N* refers to number of cristae; WT = 26, Opa1-OE = 9, I-Opa1\* = 79, s-Opa1\* = 29, Opa1-KO = 4. Data information: Scatter plot shows data distribution, the mean is marked by a bold black line. Significance of difference is tested relative to wild type using Mann-Whitney; \*\*\*\**p* < 0.0001.