Expanded View Figures

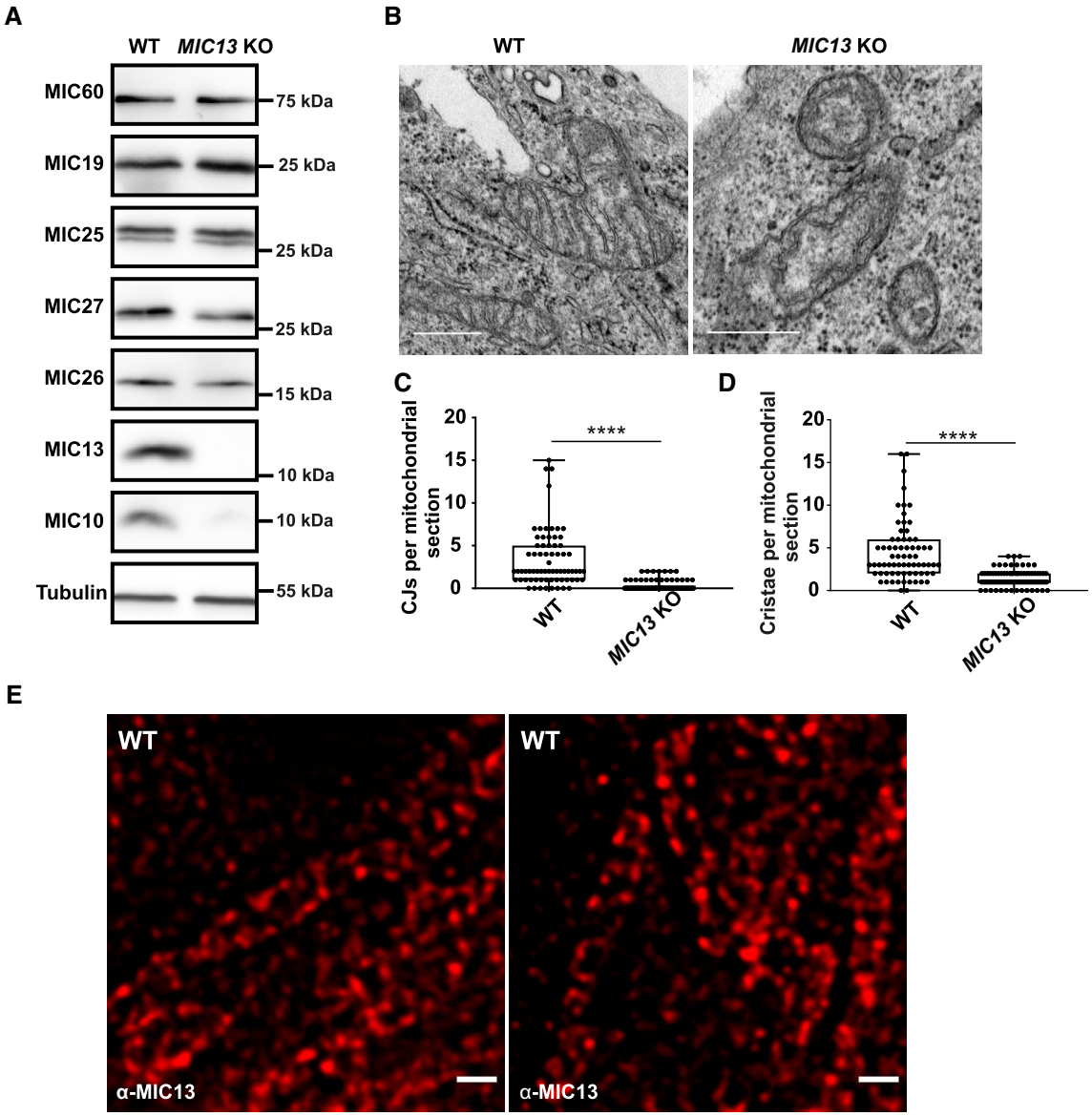

Figure EV1. Deletion of *MIC13* in HeLa cells leads to a loss of crista junctions.

A Western blot analysis of WT and *MIC13* KO HeLa cells showing a reduction in MIC10, MIC26 and MIC27 protein levels in *MIC13* KOs.

B Representative electron micrographs of WT and *MIC13* KO HeLa cells show loss of CJs in *MIC13* KOs. Scale bar 500 nm.

C Boxplot showing quantification of CJs per mitochondrial section from different mitochondria in WT and *MIC13* KO HeLa cells represented as boxplots. Boxplots show median and interquartile range from 25 to 75 percentile, and whiskers represent minimum and maximum value. Data from n = 70–90 mitochondria (from two independent experiments) are shown as data points in the boxplots. ****$P < 0.0001$ (WT versus *MIC13* KO), unpaired Student's t-test.

D Boxplot showing quantification of cristae per mitochondrial section in WT and *MIC13* KO HeLa cells represented as boxplots. Boxplots show median and interquartile range from 25 to 75 percentile, and whiskers represent minimum and maximum value. Data from n = 70–90 mitochondria (from two independent experiments) are shown as data points in the boxplots. ****$P < 0.0001$, unpaired Student's t-test.

E Representative STED super-resolution images of WT HeLa cells stained with anti-MIC13 antibody shows punctate distribution of MIC13. Scale bar 500 nm.

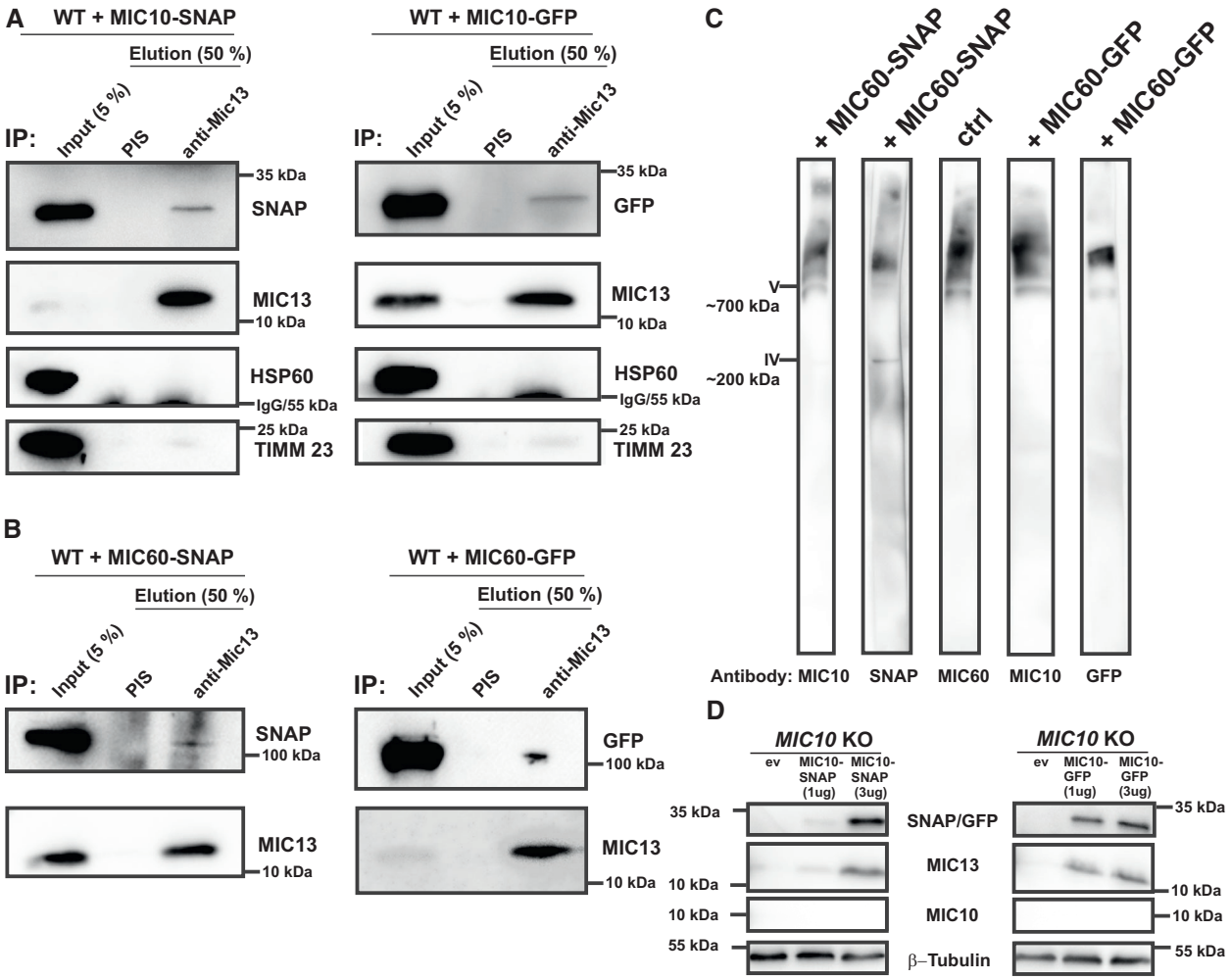

Figure EV2. Functionality and localization of MIC10 and MIC60 are not impaired by C-terminal protein tags.

A, B HEK293 cells expressing either MIC10-SNAP/GFP (A) or MIC60-SNAP/GFP (B) were used for coimmunoprecipitation using anti-MIC13 antibody. Endogenous MIC13 could pull down MIC10-SNAP or MIC10-GFP (A) as well as MIC60-SNAP or MIC60-GFP (B).

C Isolated mitochondria expressing MIC60-SNAP or MIC60-GFP were probed for proper incorporation of fusion proteins into the native MICOS complex. The complex containing MIC60-GFP or MIC60-SNAP runs at similar molecular weight as MICOS complex in native state marked by MIC60 or MIC10 antibody, confirming their incorporation into native complex.

D *MIC10* KO HAP1 cells expressing an empty vector (ev), 1 and 3 μg of MIC10-SNAP/GFP and probed with antibodies against SNAP/GFP, MIC10 and MIC13. MIC13 protein levels in *MIC10* KO HAP1 cells are rescued by the expression of MIC10-SNAP (3 μg) and MIC10-GFP (1 and 3 μg) showing that the GFP and SNAP tags do not interfere with the MIC10 function.

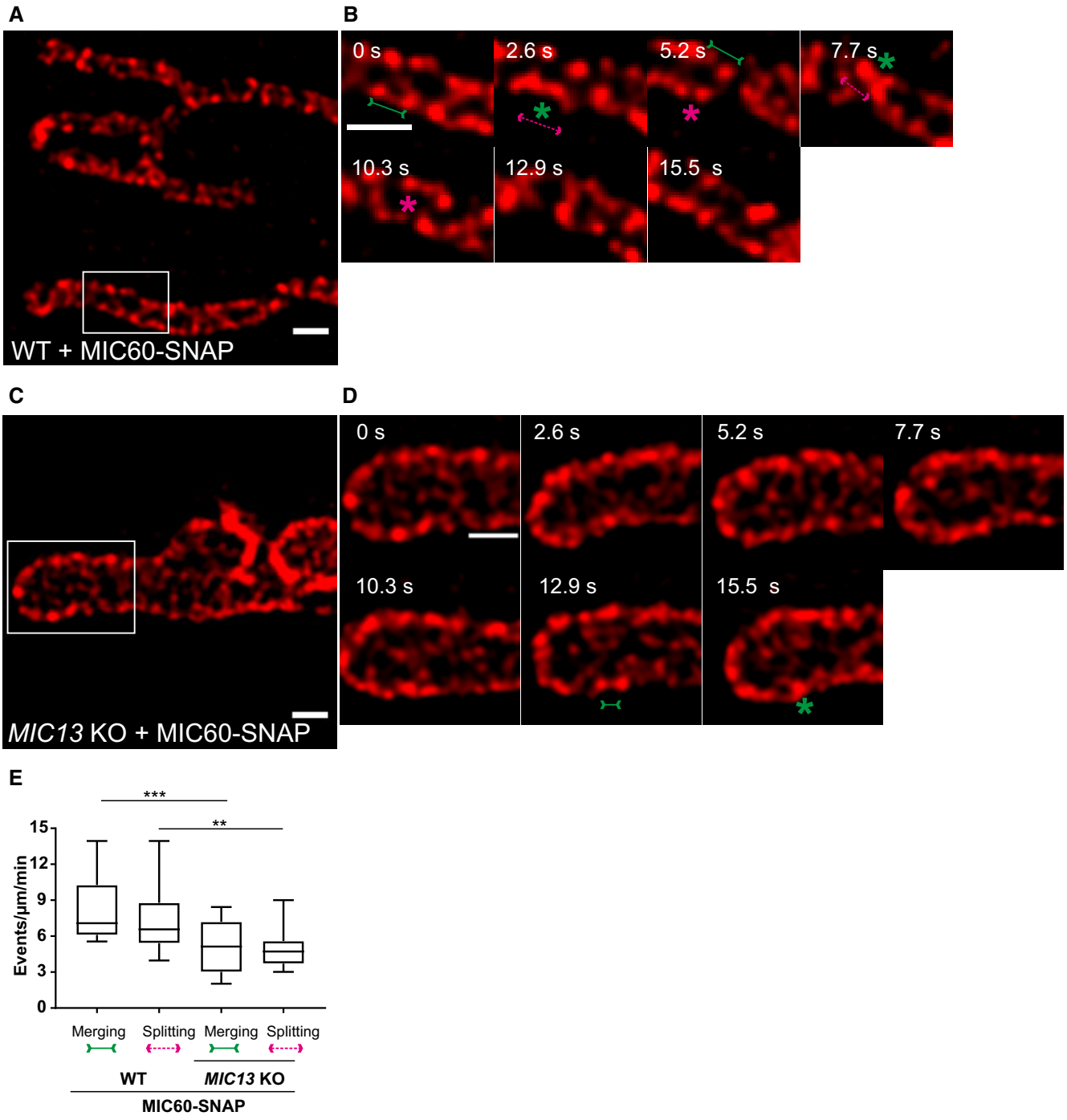
A
B
0 s
2.6 s
5.2 s
7.7 s
10.3 s
12.9 s
15.5 s
WT + MIC60-SNAP
C
D
0 s
2.6 s
5.2 s
7.7 s
10.3 s
12.9 s
15.5 s
MIC13 KO + MIC60-SNAP
E
***
**
15
12
9
6
3
0
Events/µm/min
Merging
Splitting
Merging
Splitting
WT
MIC13 KO
MIC60-SNAP

Figure EV3.

Figure EV3. Crista junctions marked by MIC60-SNAP dynamically merge and split within a mitochondrion in a MIC13-dependent manner.

A Representative live-cell STED super-resolution images (t = 0 s) showing WT HeLa cells expressing MIC60-SNAP stained with silicon rhodamine. Box in (A) marks selection shown as a zoom in panel (B). Scale bar 500 nm.

B Time-lapse image series of a mitochondrion expressing MIC60-SNAP in WT HeLa cells (2.6 s/frame). Green and magenta asterisks show merging and splitting events of MIC60-SNAP, respectively. Green arrows pointing inward connected by solid line and magenta arrows pointing outward connected by dotted line show sites of imminent merging and splitting events, respectively. Scale bar 500 nm.

C Representative live-cell STED super-resolution images (t = 0 s) showing *MIC13* KO HeLa cells expressing MIC60-SNAP stained with silicon rhodamine. Box in (C) marks selection shown as a zoom in panel (D). Scale bar 500 nm.

D Time-lapse image series of a mitochondrion expressing MIC60-SNAP in *MIC13* KO HeLa cells (2.6 s/frame). Green and magenta asterisks show merging and splitting events of MIC60-SNAP, respectively. Green arrows pointing inward connected by solid line and magenta arrows pointing outward connected by dotted line show sites of imminent merging and splitting events, respectively. Scale bar 500 nm.

E Blind quantification of merging and splitting events of CJs in WT and *MIC13* KO HeLa cells expressing MIC60-SNAP represented as boxplots. Boxplots show median and interquartile range from 25 to 75 percentile, and whiskers represent minimum and maximum value. Data obtained from three independent experiments, 5–7 mitochondria for each experiment. ***P = 0.0008 (merging events WT versus *MIC13* KO) and **P = 0.0019 (splitting events WT versus *MIC13* KO), unpaired Student's t-test.

Figure EV4. Raw data of CJ and cristae dynamics.

A Raw data of live-cell STED super-resolution images (t = 0 s) showing WT HeLa cells expressing MIC13-SNAP stained with silicon rhodamine. Box in (A) marks selection shown as a zoom in panel (B). Raw data used for Fig 6A. Scale bar 500 nm.

B Raw data of time-lapse image series of a mitochondrion expressing MIC13-SNAP in WT HeLa cells (2.5 s/frame). Green and magenta asterisks show merging and splitting events of cristae, respectively. Green arrows pointing inward connected by solid line and magenta arrows pointing outward connected by dotted line show sites of imminent merging and splitting events, respectively. Green "X" and "Y" represent X- and Y-type imminent mergence events. Raw data used for Fig 6B. Scale bar 500 nm.

C Raw data of live-cell STED super-resolution images (t = 0 s) showing WT HeLa cells expressing MIC13-SNAP stained with silicon rhodamine. Box in (C) marks selection shown as a zoom in panel (D). Raw data used for Fig 6C. Scale bar 500 nm.

D Raw data of time-lapse image series of a mitochondrion expressing MIC13-SNAP in WT HeLa cells (2.5 s/frame). Green and magenta asterisks show merging and splitting events of cristae, respectively. Green arrows pointing inward connected by solid line and magenta arrows pointing outward connected by dotted line show sites of imminent merging and splitting events, respectively. Raw data used for Fig 6D. Scale bar 500 nm.

E Raw data of live-cell STED super-resolution images (t = 0 s) showing WT HeLa cells expressing ATP5I-SNAP stained with silicon rhodamine. Box in (E) marks selection shown as a zoom in panel (F). Raw data used for Fig 7A. Scale bar 500 nm.

F Raw data of time-lapse image series of a mitochondrion expressing ATP5I-SNAP in WT HeLa cells (2.5 s/frame). Green and magenta asterisks show merging and splitting events of cristae respectively. Green arrows pointing inward connected by solid line and magenta arrows pointing outward connected by dotted line show sites of imminent merging and splitting events, respectively. Green "X" and "Y" represent X- and Y-type imminent merging events. Raw data used for Fig 7B. Scale bar 500 nm.

G Raw data of live-cell STED super-resolution images (t = 0 s) showing *MIC13* KO HeLa cells expressing ATP5I-SNAP stained with silicon rhodamine. Box in (G) marks selection shown as a zoom in panel (H). Raw data used for Fig 7C. Scale bar 500 nm.

H Raw data of time-lapse image series of a mitochondrion expressing ATP5I-SNAP in *MIC13* KO HeLa cells (2.5 s/frame). Green and magenta asterisks show merging and splitting events of cristae marked by ATP5I-SNAP, respectively. Green arrows pointing inward connected by solid line and magenta arrows pointing outward connected by dotted line show sites of imminent merging and splitting events, respectively. Raw data used for Fig 7D. Scale bar 500 nm.

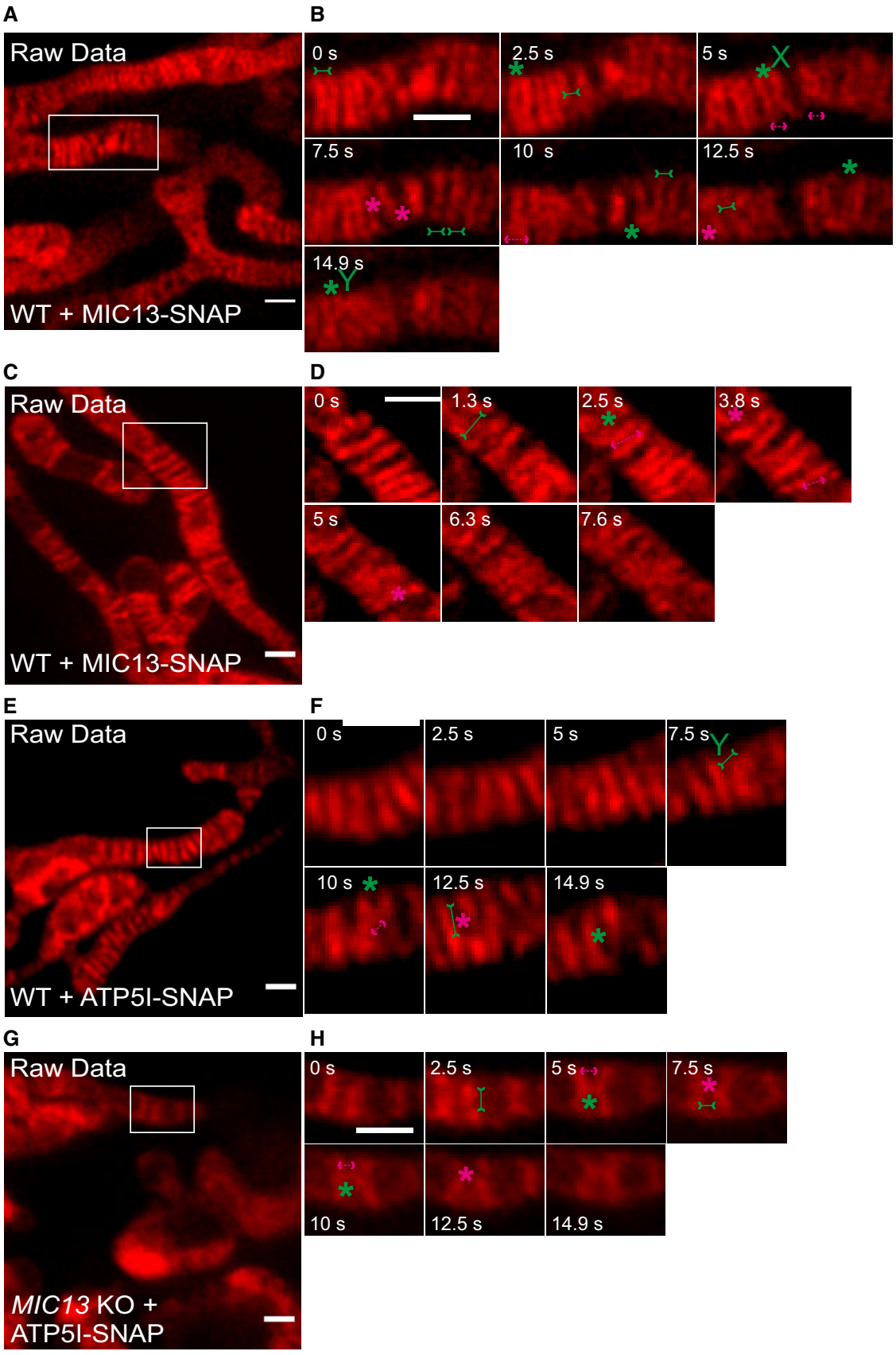

Figure EV4.

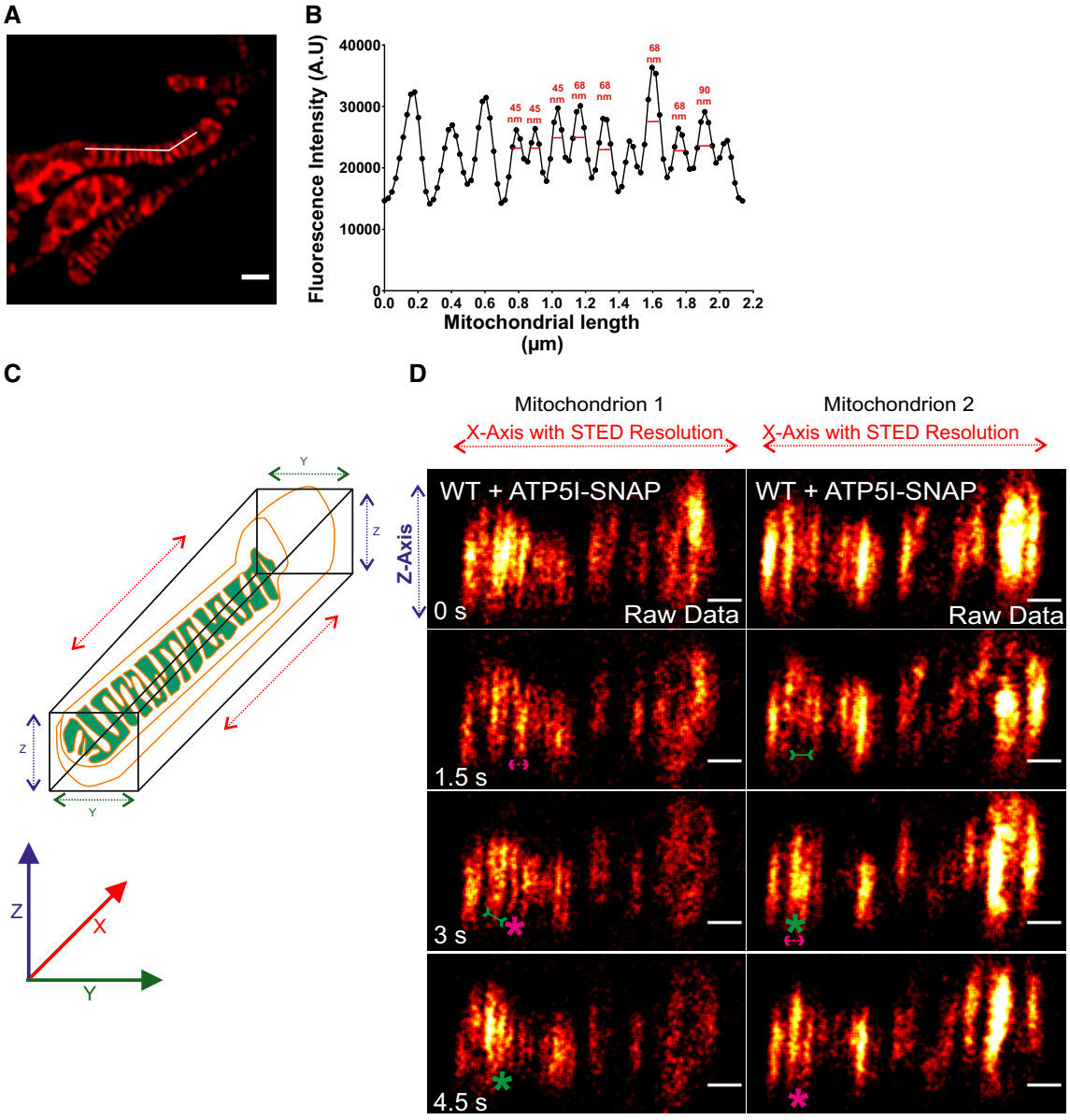

Figure EV5. Image depicting the resolution of live-cell STED nanoscopy and axial dimension (XZ) of mitochondria showing cristae dynamics in lateral dimension (X-axis) at STED resolution.

A, B Line scan (white line) along the length of mitochondria (A) from a representative live-cell STED super-resolution image from WT HeLa cells expressing ATP5I-SNAP stained with silicon rhodamine marking cristae intensities (B). Image in panel (A) is already represented in Fig 7A. FWHM (full width at half maximum) of fluorescence intensities of cristae show a resolution of 50–60 nm using live-cell STED conditions. Scale bar in (A) 500 nm.

C Cartoon depicting the XYZ axes of mitochondria. Mitochondria were imaged by STED super-resolution imaging in lateral axis (XY plane), while images were acquired in axial axis (XZ plane, diffraction-limited).

D Time-lapse image series of axial plane of two mitochondria expressing ATP5I-SNAP, stained with silicon rhodamine (red hot LUT) in WT HeLa cells (raw data, 1.5 s/frame), as visualized by STED super-resolution imaging in lateral plane. Green and magenta asterisks show merging and splitting events of cristae, respectively. Green arrows pointing inward connected by solid line and magenta arrows pointing outward connected by dotted line show sites of imminent merging and splitting events, respectively. Scale bar 500 nm.