Appendix

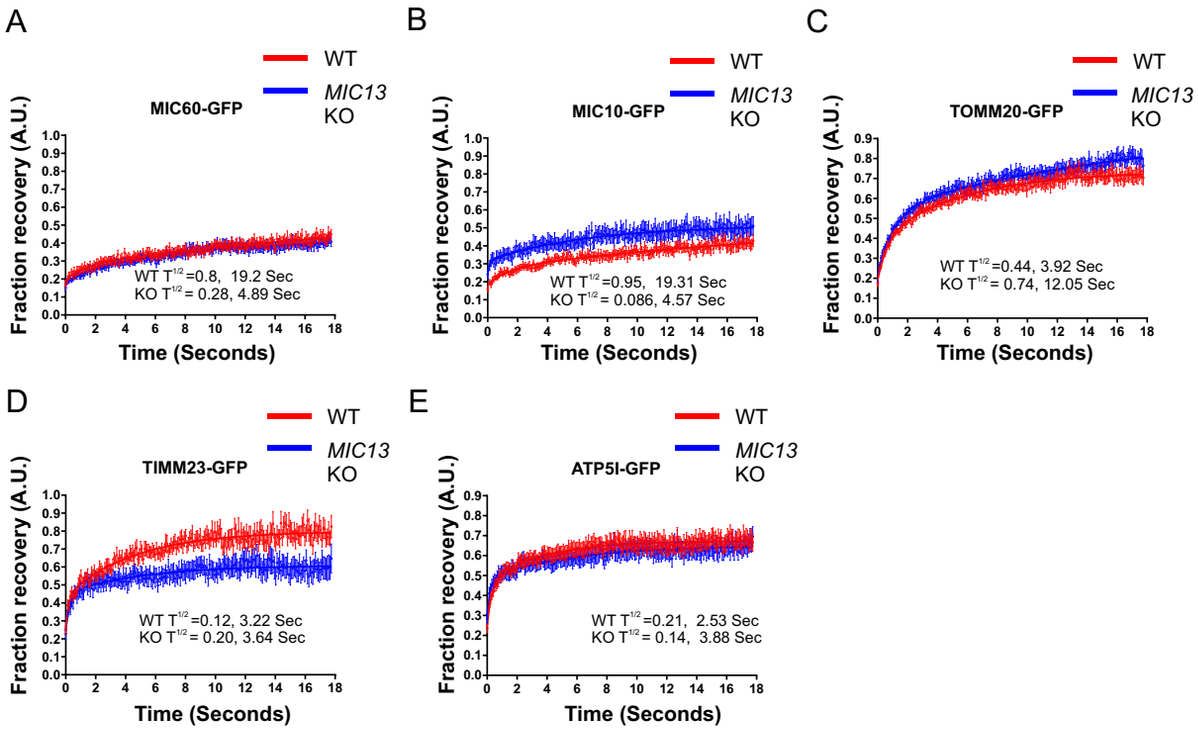
Cristae undergo continuous cycles of membrane remodelling in a MICOS-dependent manner

Arun Kumar Kondadi, Ruchika Anand, Sebastian Hänsch, Jennifer Urbach, Thomas Zobel, Dane M. Wolf, Mayuko Segawa, Marc Liesa, Orian S. Shirihai, Stefanie Weidtkamp-Peters, Andreas S. Reichert

Table of Content - Appendix

Appendix Figures

Appendix S1



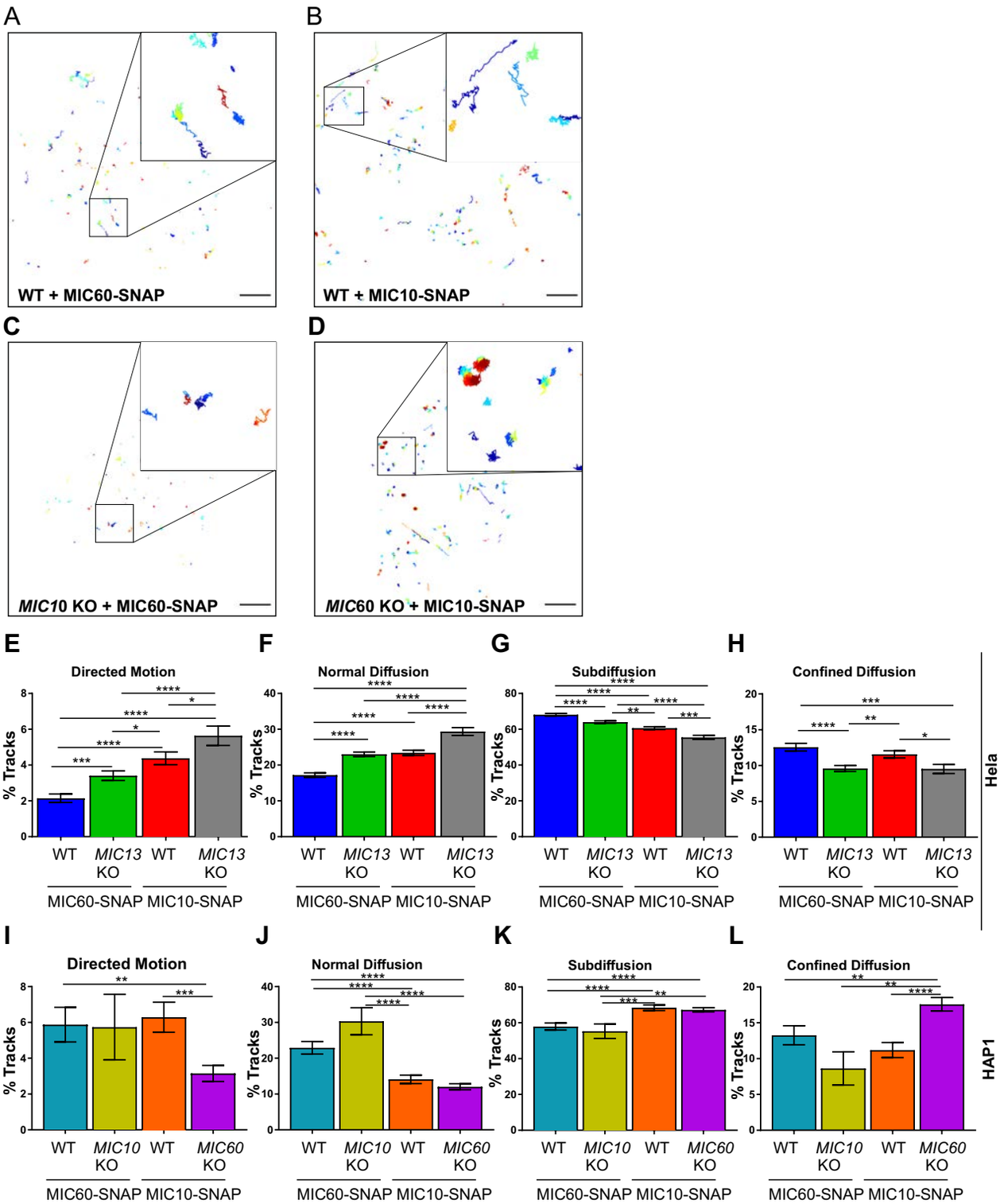
F **$T^{1/2}$ recovery time (seconds) of different molecules**

	Mitochondrial Compartment	Molecule	WT $T^{1/2}$ First Phase (seconds)	*Mic13* KO $T^{1/2}$ First Phase (seconds)	WT $T^{1/2}$ Second Phase (seconds)	*Mic13* KO $T^{1/2}$ Second Phase (seconds)
1	**CJs**	MIC60-GFP	0.8	0.28	19.26	4.89
2	**CJs**	MIC10-GFP	0.95	0.086	19.31	4.57
3	**OM**	TOMM20-GFP	0.44	0.74	3.92	12.05
4	**IBM**	TIMM23- GFP	0.12	0.20	3.22	3.64
5	**Cristae**	ATP5I-GFP	0.21	0.14	2.53	3.88

Appendix Figure S1. Detailed analysis of FRAP measurements of fusion proteins localizing to different mitochondrial subcompartments in WT and *MIC13* KO Hela cells.

A-E FRAP curves (curve-fitted) of MIC60-GFP **(A)** MIC10-GFP **(B)** TOMM20-GFP **(C)** TIMM23-GFP **(D)** ATP5I-GFP **(E)** in WT and *MIC13* KO Hela cells. Average FRAP curves (intensities in arbitrary units) for each protein were obtained (3 independent experiments, 6-10 mitochondria for each experiment). Error bars for each time point representing SEM are shown. **F** $T^{1/2}$ recovery time of the above indicated proteins are shown in the table.

Appendix S2


A
B
WT + MIC60-SNAP
WT + MIC10-SNAP
C
D
MIC10 KO + MIC60-SNAP
MIC60 KO + MIC10-SNAP
E
F
G
H
Directed Motion
Normal Diffusion
Subdiffusion
Confined Diffusion
% Tracks
WT
MIC13 KO
MIC60-SNAP
MIC10-SNAP
Hela
I
J
K
L
MIC10 KO
MIC60 KO
HAP1

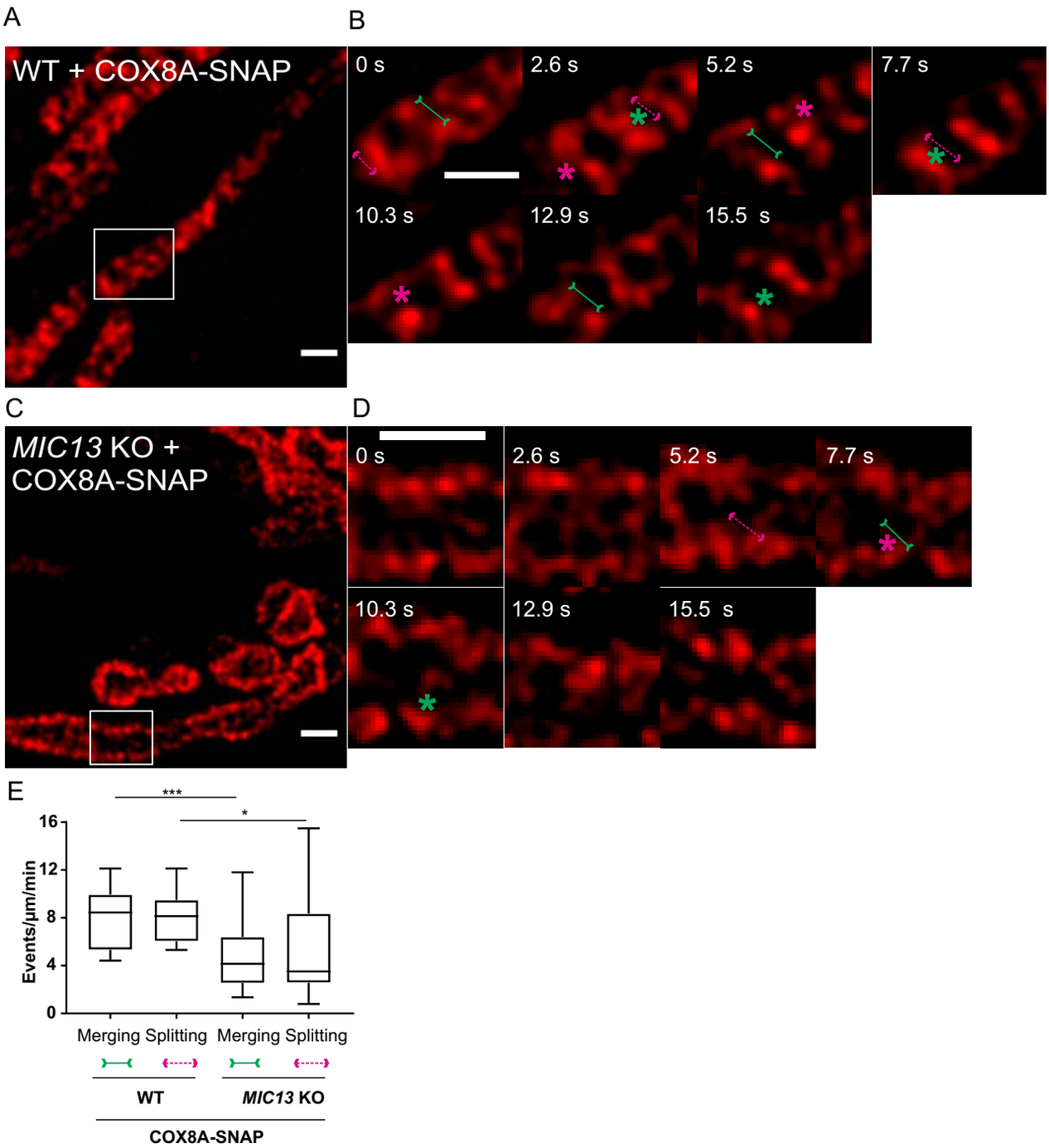
Appendix Figure S2. Single particle tracking reveals requirement of MIC60 for high directionality of MIC10.

A-D Representative single particle tracks of cells expressing MIC60- **(A)** and MIC10-SNAP **(B)** in WT HAP1 cells and MIC60- **(C)** and MIC10-SNAP **(D)** in *MIC10* and *MIC60* KO HAP1 cells respectively, stained with silicone-rhodamine dye, and imaged at a rate of 33 ms/frame. Single tracks were color-coded according to temporal appearance. Scale Bar 5 µm. Insets in each image **(A-D)** show zoomed-in images of few representative tracks.

E-L Percentage of subtracks having directed motion **(E & I)**, normal diffusion **(F & J)**, subdiffusion **(G & K)** and confined diffusion **(H & L).**

(E-H) WT or *MIC13* KO Hela cells either expressing MIC60- and MIC10-SNAP (Number of tracks analysed obtained from 2 independent experiments: n = 2541, 2540, 3560 and 1441 in WT Hela cells expressing MIC60-SNAP and MIC10-SNAP, *MIC13* KO Hela cells expressing MIC60-SNAP and MIC10-SNAP, respectively). **(I –L)** WT or *MIC10* KO HAP1 cells expressing MIC60-SNAP and WT or *MIC60* KO HAP1 cells expressing MIC10-SNAP (Number of tracks analysed obtained from 2 independent experiments: n = 505, 134, 561 and 1110 in WT and *MIC10* KO HAP1 cells expressing MIC60-SNAP and WT and *MIC60* KO HAP1 cells expressing MIC10-SNAP respectively). Data represents mean ± SEM. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ****$P \leq 0.0001$, unpaired Student's *t*-test.

Appendix S3


A
B
WT + COX8A-SNAP
0 s
2.6 s
5.2 s
7.7 s
10.3 s
12.9 s
15.5 s
C
D
MIC13 KO +
COX8A-SNAP
0 s
2.6 s
5.2 s
7.7 s
10.3 s
12.9 s
15.5 s
E
***
*
16
12
8
4
0
Events/µm/min
Merging Splitting Merging Splitting
WT
MIC13 KO
COX8A-SNAP

Appendix Figure S3. Cristae undergo balanced merging and splitting events in a MICOS-dependent manner.

A, C Representative live-cell STED super-resolution images (t=0 s) showing WT **(A)** and *MIC13* KO **(B)** Hela cells expressing COX8A-SNAP respectively stained with silicone-rhodamine. Box in **A** and **C** mark selection shown as a zoom in panel **B** and **D** respectively. Scale bar 500 nm.

B, D Time-lapse image series of a mitochondrion expressing COX8A-SNAP in WT **(B)** and *MIC13* KO **(D)** Hela cells respectively (2.5 s/frame). Green and magenta asterisks show merging and splitting events of cristae marked by COX8A-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 cristae merging and splitting events in WT and *MIC13* KO Hela cells expressing COX8A-SNAP represented as boxplots. Boxplot shows median and interquartile range from 25 to 75 percentile and whiskers represent minimum and maximum value. Data from 3 independent experiments, 5-10 mitochondria for each experiment; ***P = 0.0007 (Merging events of WT vs. *MIC13* KO) and *P = 0.016 (Splitting events of WT vs. *MIC13* KO), unpaired Student's *t*-test.