Supplementary information

Inhibition of mitochondrial ATPase function by IF1 changes the spatiotemporal organization of the ATP synthase

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1 SUPPLEMENTARY MOVIES

Movie S1. Fluorescence recording of single molecule ATP synthase in mitochondria in stable transfected cells expressing CV subunit γ-HaloTag^{TM} , the HaloTag was labeled with TMR^{HTL} (1 nM for 30 min). Images were taken with a frame rate of 59 Hz, 17 ms exposure time per frame. Number of images: 3000. Total recording time: 51 s. Condition: medium with 5.6 mM glucose.

Movie S2. Fluorescence recording of single molecule ATP synthase in mitochondria in stable transfected cells expressing CV subunit γ-HaloTag^{TM} , the HaloTag was labeled with TMR^{HTL}. Images were taken with a frame rate of 59 Hz. Number of images: 3000. Total recording time: 51 s. Condition: medium with 5.6 mM glucose.

Movie S3. Fluorescence recording of single molecule ATP synthase in mitochondria in stable transfected cells expressing IF1-HA and transiently transfected with CV subunit γ-HaloTag^{TM} , the HaloTag was labeled with TMR^{HTL}. Images were taken with a frame rate of 59 Hz. Number of images: 3000. Total recording time: 51 s. Condition: medium with 5.6 mM glucose.

Movie S4. Fluorescence recording of single molecule ATP synthase in mitochondria in stable transfected cells expressing IF1-H49K-HA and transiently transfected with CV subunit γ-HaloTag^{TM} , the HaloTag was labeled with TMR^{HTL}. Images were taken with a frame rate of 59 Hz. Number of images: 3000. Total recording time: 51 s. Condition: medium with 5.6 mM glucose.

Movie S5. Fluorescence recording of single molecule ATP synthase in mitochondria in stable transfected cells expressing IF1-H49K-HA and transiently transfected with CV subunit γ-HaloTag^{TM} , the HaloTag was labeled with TMR^{HTL}. Images were taken with a frame rate of 59 Hz. Number of images: 3000. Total recording time: 51 s. Condition: medium with 5.6 mM glucose.

Movie S6. Fluorescence recording of single molecule ATP synthase in mitochondria in stable transfected cells expressing IMMT-GFP (Mitofilin-GFP) and transiently transfected with CV subunit γ-HaloTag^{TM}, the HaloTag was labeled with TMR^{HTL}. Images were taken with a frame rate of 100 Hz. Number of images: 3000. Total recording time: 30 s. Condition: medium with 5.6 mM glucose.

Movie S7. Fluorescence recording of single molecule ATP synthase in mitochondria in stable transfected cells expressing CV subunit γ-HaloTag^{TM}, exposed to 5 µM CHX for 10h. The HaloTag was labeled with TMR^{HTL}. Images were taken with a frame rate of 56 Hz. Number of images: 2000. Total recording time: 34 s. Condition: medium with 5.6 mM glucose.

2 SUPPLEMENTARY FIGURES

Supplementary Figure 1 related to Figure 6 and 7

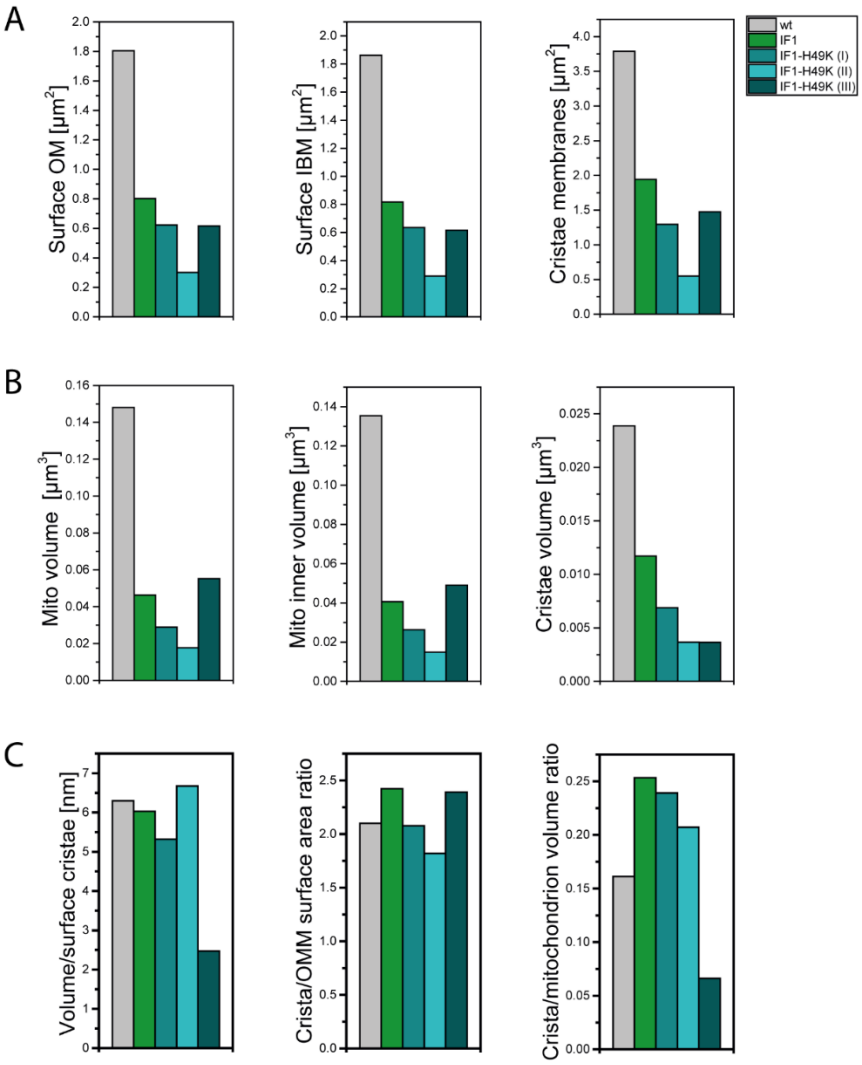

Figure S 1. Quantitative analysis of membrane surfaces and volumes of the segmented mitochondria from tomograms (Fig.6-7). (**A**) Plotted ratios of surface areas of the IMM (IBM+CM) and OMM in Hela, IF1-wt and IF1-H49K expressing cells. IF1-H49K mitochondria were classified as state I, II and III. (**B**) Relative amount of volume enclosed by cristae (intracristal space) and by the IMM (matrix space) in the segmented mitochondria shown in **Fehler! Verweisquelle konnte nicht gefunden werden.**. (**C**) Cristae volume per surface area, cristae surface area per OMM surface area, and total cristae volume per mitochondrial volume for the segmented mitochondria shown in Fig. 6 and 7.

Supplementary Figure S2 related to Figure 8

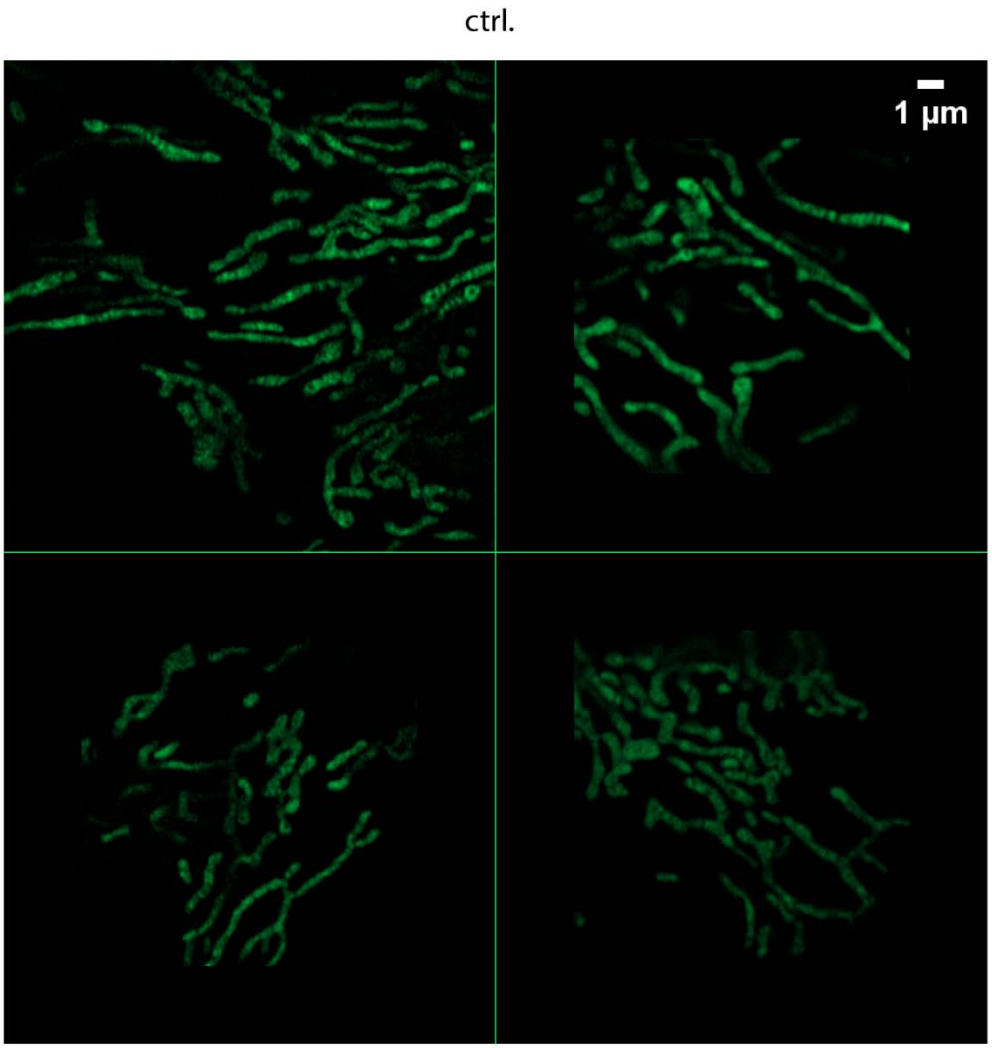

Figure S 2. Live cell imaging of control HeLa cells. Cells were stained with NAO (100 nM), present during the measurement, Scale bar: 1 µM (all panels). Images were recorded with an AiryScan (Zeiss) equipped with an alpha Plan-Apochromat 63x/1.46 Oil DIC M27 objective on the Zeiss LSM 980. Raw images were automatically processed into deconvolved Airyscan images using the Zen software.

Supplementary Figure S3 related to Figure 8

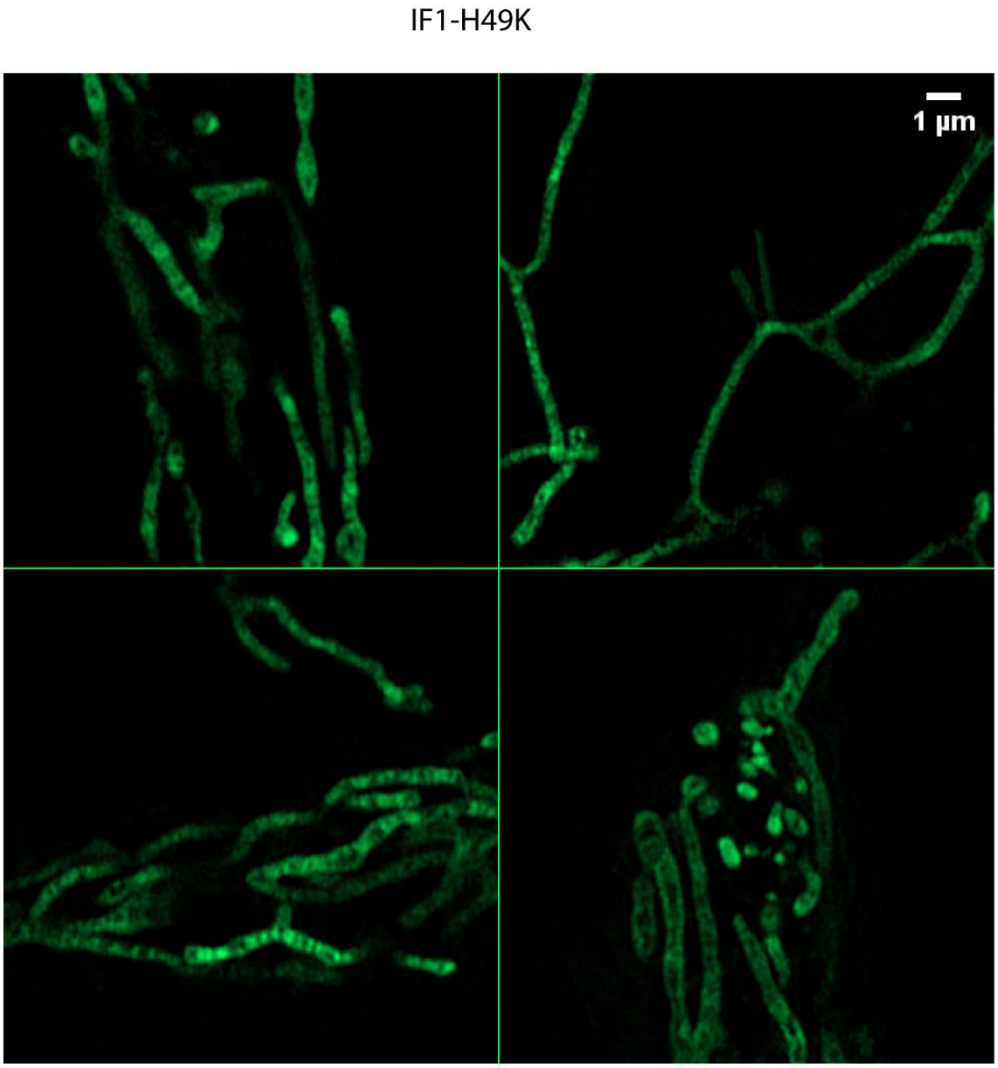

Figure S 3. Live cell imaging of cells expressing IF1-H49K. Cells were stained with NAO (100 nM), present during the measurement, Scale bar: 1 µM (all panels). Images were recorded with an AiryScan (Zeiss) equipped with an alpha Plan-Apochromat 63x/1.46 Oil DIC M27 objective on the Zeiss LSM 980. Raw images were automatically processed into deconvolved Airyscan images using the Zen software.

Supplementary Figure S4 related to Figure 10

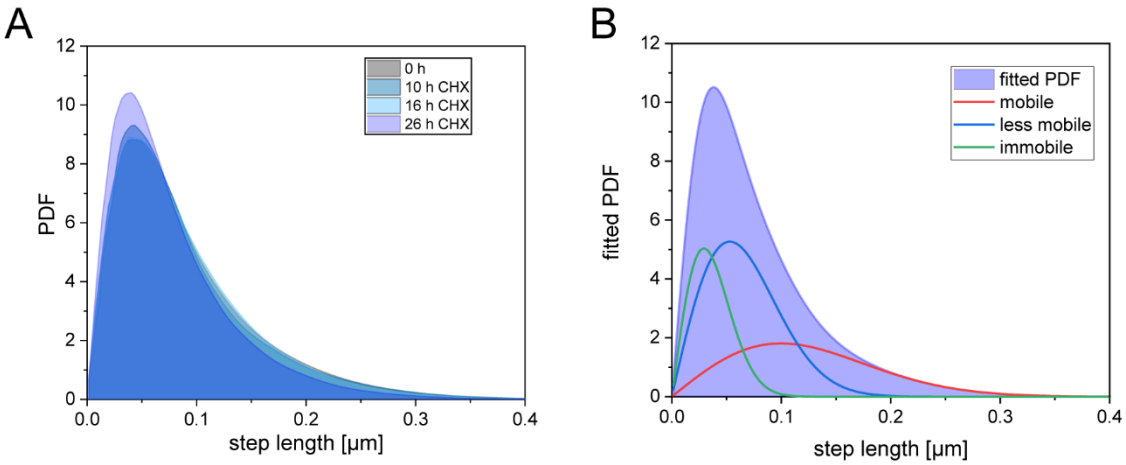

Figure S 4. (A) Step length histogram for F_1F_O ATP synthase in cells treated with and without CHX**.** CHX (5 µM) was added for the indicated times. **(B)** The step distances were fitted by a three states model [1].

Supplementary Figure S5 related to Figure 11

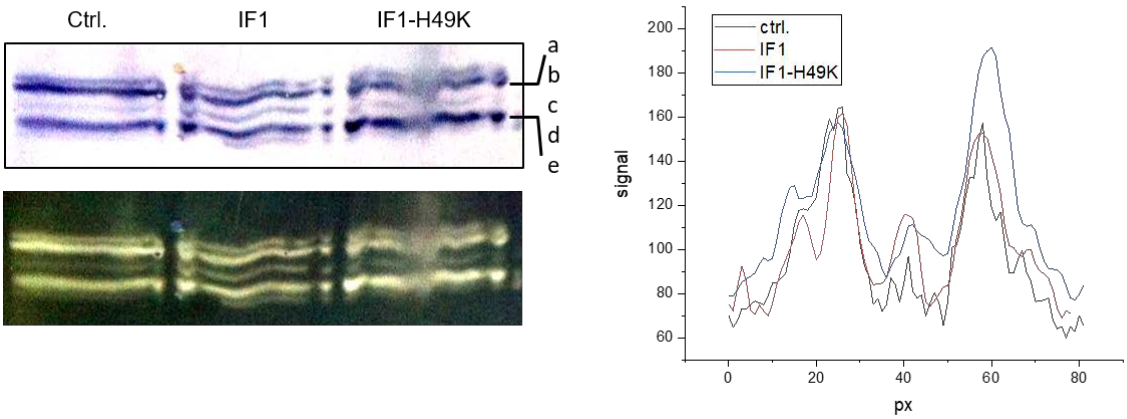

Figure S 5. (C) Immuno-staining of OPA1 in control cells and cells expressing IF1 and IF1-H49K, respectively (antibody α-OPA1: courtesy of A. Reichert lab). OPA1 appears in long and short isoforms due to proteolytic processing of L-OPA1. Line plot profile: relative levels of S- and L-OPA1 protein bands.

3 REFERENCES

[1] A.S. Hansen, M. Woringer, J.B. Grimm, L.D. Lavis, R. Tjian, X. Darzacq, Robust model-based analysis of single-particle tracking experiments with Spot-On, Elife, 7 (2018).