Supporting Information

Jans et al. 10.1073/pnas.1301820110

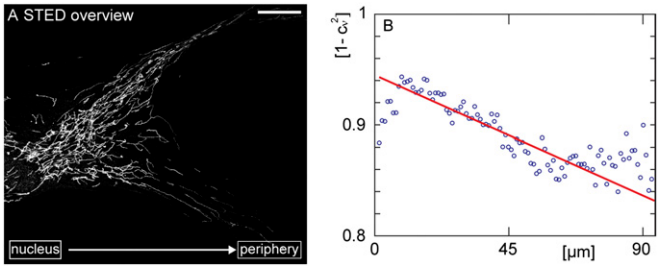

Fig. S1. Mitofilin cluster distribution is denser in the perinuclear mitochondria. (*A*) Representative primary human fibroblasts used for the analysis shown in *B*. The image is identical to that shown in Fig. 2*A*. The cell was labeled with antiserum against mitofilin and imaged with stimulated emission depletion (STED) microscopy. (*B*) Plot of [1-c_v^2] radiating from the center of the cell to its border. The normalized variance value, c_v^2, reflects several physical parameters, including the distribution and size of the protein clusters, cluster-to-mitochondrial background ratio, and others. c_v^2 was determined as described previously (1). In brief, first the local variance of the fluorescence intensity of raw STED images was determined in round regions of interest (ROIs) with a diameter of 7 pixels (~140 nm). The resulting variance values were assigned to the central pixel of the analyzed ROI. To evaluate the results of the analysis independent from the absolute brightness of the structures, the individual variance values were normalized to the squared average fluorescence intensity of the respective ROI, giving the c_v^2 values. The variance calculation was repeated using each pixel successively as an ROI center, resulting in an image in which each pixel represents the local normalized variance. The mitochondria-containing fraction of the image was selected by image segmentation using masks. Finally, the [1-c_v^2] values were plotted against the distance from the nucleus. Blue circles indicate 100 bins that pool ~9×10^5 individual [1-c_v^2] values. The red line is the linear fit based on the individual [1-c_v^2] values. The negative slope of the curve indicates that the density of the mitofilin cluster distribution is greater in mitochondria around the nucleus.

1. Wurm CA, et al. (2011) Nanoscale distribution of mitochondrial import receptor Tom20 is adjusted to cellular conditions and exhibits an inner-cellular gradient. *Proc Natl Acad Sci USA* 108(33):13546–13551.

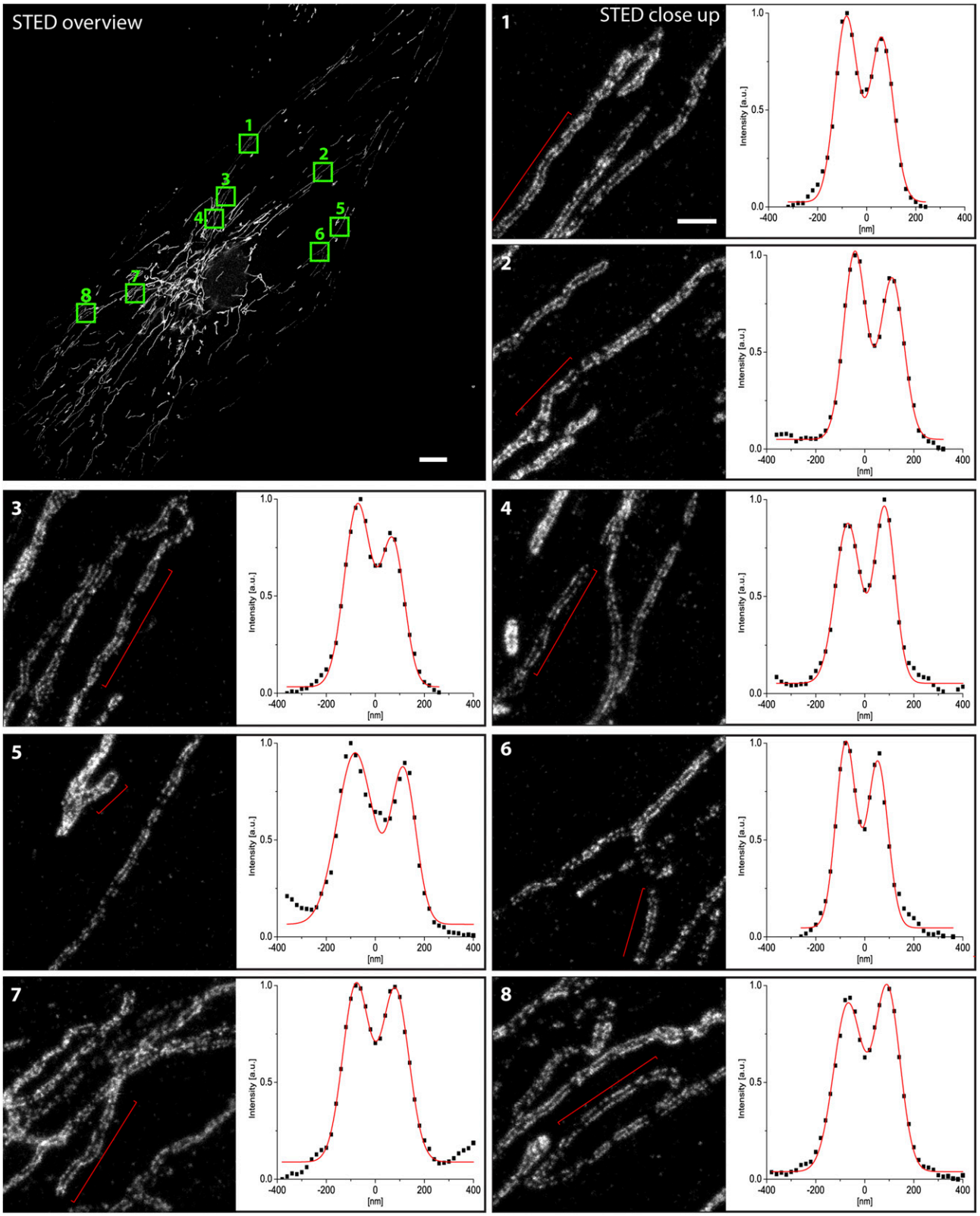

Fig. S2. Mitofilin is localized at the rim of the mitochondria. The large image shows an overview of a primary adult human fibroblast labeled with an antiserum against mitofilin and imaged by STED microscopy. The numbered small images are magnifications of the boxed areas, as indicated. The graphs present averaged intensity profiles across the lengths of the indicated mitochondrial tubule sections. (Scale bars: 10 μm in the large image; 1 μm in the small images.)

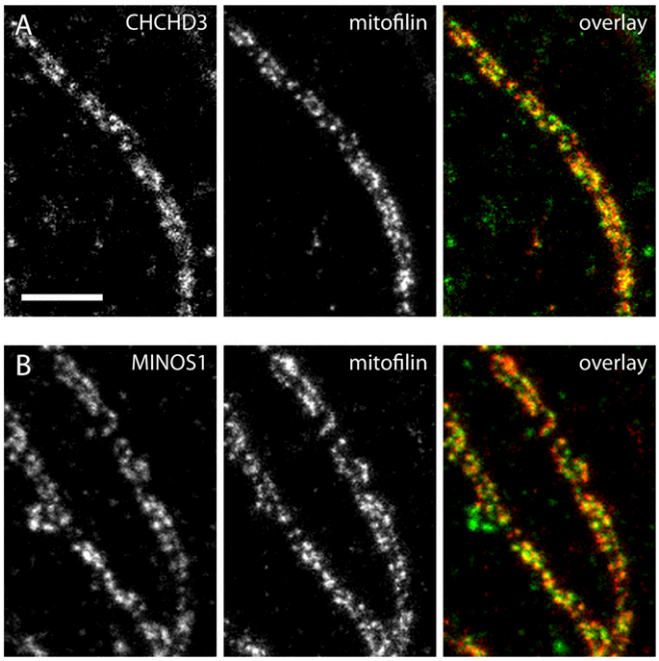

Fig. S3. Two-color STED microscopy of primary adult human fibroblast mitochondria labeled with antisera against mitofilin and CHCHD3 (*A*) and against mitofilin and mitochondrial inner membrane organizing system 1 (MINOS1) (*B*). The primary antibodies were detected with fluorescently labeled Fab fragments. Because the primary antibodies were all derived from rabbits, a sequential labeling strategy was implemented. To this end, cells grown on coverslips were fixed with 4% (wt/vol) prewarmed formaldehyde in PBS for 10 min at 37 °C, extracted with 0.5% Triton X-100 in PBS, blocked with 5% (wt/vol) BSA, and incubated with the first primary rabbit antibodies. After incubation with the first fluorophore-coupled Fab fragments and several washing steps, potential free binding sites of the primary antibodies were blocked with unlabeled anti-rabbit Fab fragments. After a brief additional fixation with formaldehyde, the second labeling round was performed analogously. Finally, the samples were washed in PBS for 30 min and mounted in Mowiol with 0.1% 1,4-Diazabicyclo[2.2.2]octan (DABCO). In the overlay images, mitofilin is shown in red, and CHCHD3 and MINOS1 are in green. (Scale bar: 1 μm.)