Supporting Information

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SI Materials and Methods

Microscopy. For the generation of overview images and confocal images for the quantification of mtDNA clusters and mitochondrial transcription factor A (TFAM) clusters in whole cells a confocal microscope (TCS SP5; Leica Microsystems) was applied. The images were recorded sequentially. Each image was averaged at least twice. Except for contrast stretching and smoothing, no image processing was applied.

For stimulated emission depletion (STED) micrographs and the corresponding confocal images, a custom build setup (1) was applied. In brief, the fluorophores were excited with a pulsed diode laser emitting 70-ps pulses at 635 nm (PicoQuant). Stimulated emission depletion was performed using a mode-locked Titanium Sapphire laser (MIRA900; Coherent) operating at 760 nm at 76 MHz. The delay between the excitation and STED pulse was realized electronically by using the output signal of the photo diode of the STED laser to trigger the pulsed excitation diode. The initial duration of the STED pulses of less than 100 fs was stretched to about 300 ps by guiding the light through 100 m of single mode, polarization-maintaining fiber. The STED beam was converted into a doughnut shape by passing the light through a polymeric phase plate (vortex pattern; RPC Photonics) and subsequently overlaid with the excitation beam using a dichroic mirror (AHF Analysentechnik). The excitation and the STED beams were coupled onto a 100× oil immersion lens (NA 1.4 PL APO, 100×; Leica). The fluorescence signal was collected by the same lens and detected from 650 to 690 nm confocally by using a multimode fiber (diameter ~0.7 Airy discs). Scanning was realized by a two-axis beam scanner (Yanus IV; Till Photonics). The fluorescence was finally directed onto four counting avalanche photodiodes (SPCM-AQRH13; Perkin-Elmer). Using this microscope, a resolution of 230–260 nm in confocal images and between 40 and 50 nm in STED images was achieved in biological samples.

Image Analysis. For the automated analysis of confocal and STED images, several algorithms were devised and applied. All algorithms were programmed using MATLAB (Mathworks).

Determination of Average Nucleoid Diameters. First, the positions of the nucleoids in confocal and STED images were determined. To this end, the nucleoids were singled out by reduction of background and of out-of-focus signals, as well as of large scale inhomogeneities by applying sequential cycles of image filtering and segmentation (i.e., median filtering, Gaussian filtering, edge detection, binarization). After deconvolution and binarization of the resulting images, the positions and coarse outlines of the nucleoids were generated based on local maxima in the images.

To determine the diameter (full width at half maximum) of the nucleoids, the coarse outlines of the individual nucleoids were enlarged by at least 100 nm in the case of the STED images and by at least 150 nm in the case of the confocal images. These enlarged outlines of the nucleoids were used to create a binary mask to select a fraction of the raw image containing the nucleoids. Finally, the average diameter of each nucleoid was determined by binarisation applying a threshold of 50% of the fluorescence intensity within each individual nucleoid outline. The diameter of the area above this threshold was determined for every nucleoid and is regarded as the size of the respective nucleoid.

To determine the number of nucleoids assigned by STED microscopy in a nucleoid assigned by confocal microscopy, the STED and the confocal images were automatically compared. We determined for every nucleoid assigned on the confocal image the number of nucleoids in the corresponding STED image. Finally, the average values for the nucleoid diameters and the average number of nucleoids found in the STED images per nucleoid assigned in the confocal images were calculated for each image.

Determination of the Number of Nucleoids per Cell and of the Local Densities of Nucleoids. The nucleoid numbers and densities were analyzed on projections of confocal image stacks. To analyze cells individually, the cells were separated using manually created binary masks encompassing only a single cell. As in the previous analysis, the nucleoids were singled out by elimination of background as well as of large-scale inhomogeneities by applying sequential cycles of image filtering and segmentation (i.e., deconvolution, median filtering, Gaussian filtering, edge detection, binarization). After binarization of the resulting images and shinking the identified structures to a single pixel, the positions of the nucleoids were registered. Then, the distance of each nucleoid to its nearest neighbor was determined. Finally, the position of the center of the nucleus was determined on the basis of manually created binary masks delineating the nucleus. The nearest neighbor distance of each nucleoid was plotted in dependence of its respective distance to the nucleus.

1. Harke B, et al. (2008) Resolution scaling in STED microscopy. *Opt Express* 16:4154–4162.

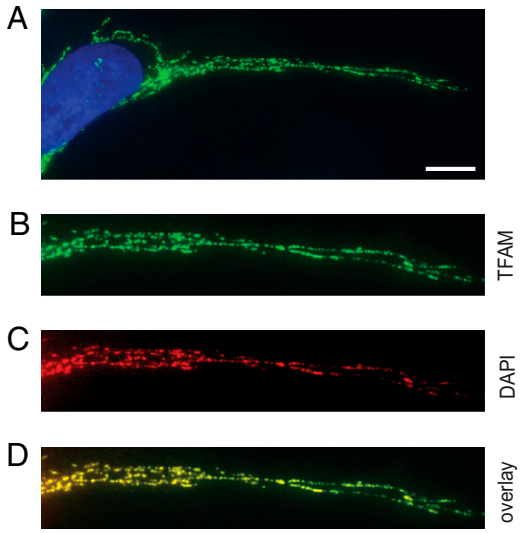

Fig. S1. (*A*) Human fibroblasts stained by antibodies against TFAM (green) and counterstaining by DAPI (blue). (*B*) Magnification of *A*: antibodies against TFAM (green). (*C*) Magnification of *A*. DAPI-staining in pseudocolor (red). (*D*) Magnification of *A*. Overlay of TFAM and DAPI. Colocalization results in yellow. (Scale bar, 10 μm.)

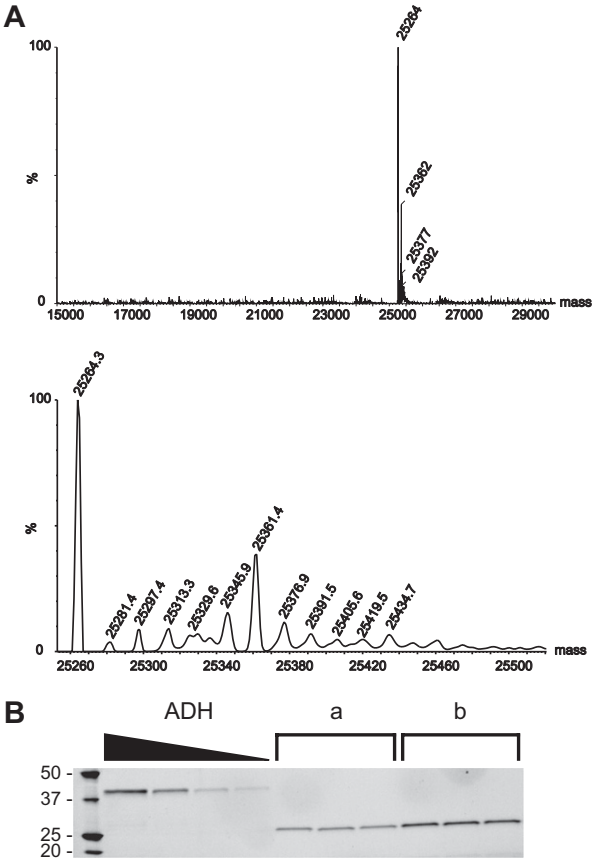

Fig. S2. Quality control of the recombinant human TFAM. (*A*) The figure shows a spectrum calculated with a maximum entropy calculation (MaxEnt 1, plug in of MassLynx 4.1; Waters) from the original multiple charge envelope from *m/z* 957 to 1,435. As can be seen in the overview spectrum there were no other proteins besides TFAM. The zoom in shows the main peak with 25,264 Da, which corresponds to the His-tagged mature human TFAM without the initial methionine and with acetylated serine. The oxidations (+16 Da) of the methionines of TFAM are depicted in the smaller peaks. For further calculations the mean value of the main peaks was used: 25.31 kD. (*B*) Representative stain-free gel for the quantification of recombinant human TFAM. "a" and "b" indicate His-tagged, purified recombinant TFAM in triplicates. Alcohol dehygrogenase (ADH) was used as standard in known concentrations. The protein marker corresponds to 20, 25, 37, and 50 kD.