Supporting Information

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SI Materials and Methods

EMSAs. EMSA was performed as described previously (37).

Electron Microscopy.

Low-angle shadowing. DNA (10 ng/μL) was mixed with various concentrations of TFAM in binding buffer [25 mM Tris·HCl (pH 7.8), 3 mM $MgCl_2$, 150 mM NaCl] and incubated at room temperature for 30 min before being stored at 4 °C. Stored samples were diluted 1:10 with 4 mM Mg–acetate and applied to glow-discharged EM grids containing a continuous carbon support film. The grids were washed by placing them carbon-side down on 2 drops of 4 mM Mg–acetate for 5 min, stained with one drop of 0.02% (wt/vol) uranyl acetate in 30% ethanol for 1–4 min, and then washed again with 2 drops of distilled water. Excess solution was removed with filter paper and the dried grids were placed in a BAF 060 freeze-fracture system (BAL-TEC) for shadowing. Low-angle rotational shadowing was performed at room temperature, 3–4 × 10^{-7} mbar with an operating voltage and current of 1.6 V and 58–60 mA, respectively. Platinum/carbon (6 nm) was deposited at an angle of 10° using an evaporation rate of 0.05–0.09 nm/s. The thickness of deposition was monitored using a quartz crystal, and the stage revolution speed was 100 rpm. Grids were imaged at 15–25 kx using an EM208S transmission electron microscope (FEI) operating at 80 kV and equipped with a 1- × 1-k slow-scan CCD (Tietz, TVIPS) or a Gatan 2 × 2 k CCD (MSC TK 2048 1).

Cryo-ET. Samples were diluted 1:1 with fiducial markers (6-nm gold particle conjugated with protein A, Aurion) and applied to glow-discharged quantifoil EM grids (R2/2, Cu 300 mesh, Quantifoil). Excess liquid was removed with filter paper (#4, Whatman), and samples were preserved by plunge-freezing in liquid ethane (27). Single-axis tilt image series (±60°, step size 2°) were recorded using the software XPLORE (FEI) on an Polara transmission electron microscope (FEI) operating at 300 kV and equipped with a postcolumn energy filter and a 2- × 2-k CCD (GIF Tridiem 863 Gatan). Images were taken with a specimen pixel size of 0.576 nm and a defocus of 7 μm. Tilt series were aligned using the gold fiducials, and tomographic volumes were reconstructed using the IMOD software package (38). Tomograms were filtered by non-linear anisotropic diffusion to increase contrast (39) and manually segmented with the program AMIRA (FEI).

Southern Blots. Southern blots were performed as described before (40). DNA was digested with SacI or PstI endonuclease. The probes were COXI, pAM1, or 18S.

In-Solution Digestion. Samples were diluted with 100 mM ammonium bicarbonate to make a concentration of about 200 ng/μL according to the Bradford method. The tryptic digestion was performed by adding Trypsin Gold mass spectrometry grade (Promega) at a 1:50 (wt/wt) ratio and incubating at 37 °C overnight.

Protein Identification and Quantification with LC-MS/MS. Protein identification and quantification were performed with a Xevo Q-Tof (Waters) coupled with a nanoACQUITY UPLC (Waters). The digest was 10-fold diluted with 0.1% of formic acid. One microliter of standard alcohol dehydrogenase 1 tryptic digest (50 fmol/μL) (Waters) was mixed with 0.5 μL of the sample digest. A total of 1.5 μL of the mixture was loaded into a C18 trap column of 180 μm × 20 mm with 10 μL/min of 3% of solvent A (0.1% formic acid) for 2 min. The digest was then separated and eluted with an analytical column of 75 μm × 150 mm C18 BEH 1.7 μm (Waters). The gradient was 3–35% of acetonitrile in 0.1% formic acid over 10 min at a flow rate of 400 nL/min. The Xevo Q-Tof was operated in LC/MS^E mode over the *m/z* range of 50–1,800 in Nano electrospray mode. The capillary, sample cone, extraction cone, and collision energy were 3.3 kV, 25.0 V, 2.0 V, and 6.0 V, respectively. During elevated energy scan, the collision energy was ramped from 15 to 35 V. Glu–fibrinopeptide B of *m/z* 785.84 was used as lock mass for mass correction. At least three replicates of one sample were analyzed. Data were collected using MassLynx 4.1 and processed and searched using ProteinLynx Global Server 2.5.2. (Waters). The following parameters were used for the database search: enzyme "trypsin," minimal fragments ion per peptide matched "2," minimal fragments ion per protein matched "7," missed cleavages "1," variable modification "oxidation methionine," peptide tolerance "automatic," fragment tolerance "automatic,"and false positive rate "4%." The calibration protein P00330 (ADH1_YEAST) was used at a concentration of 50 fmol/μl. The mouse database was from Uniprot release knowledgebase_2012_11.

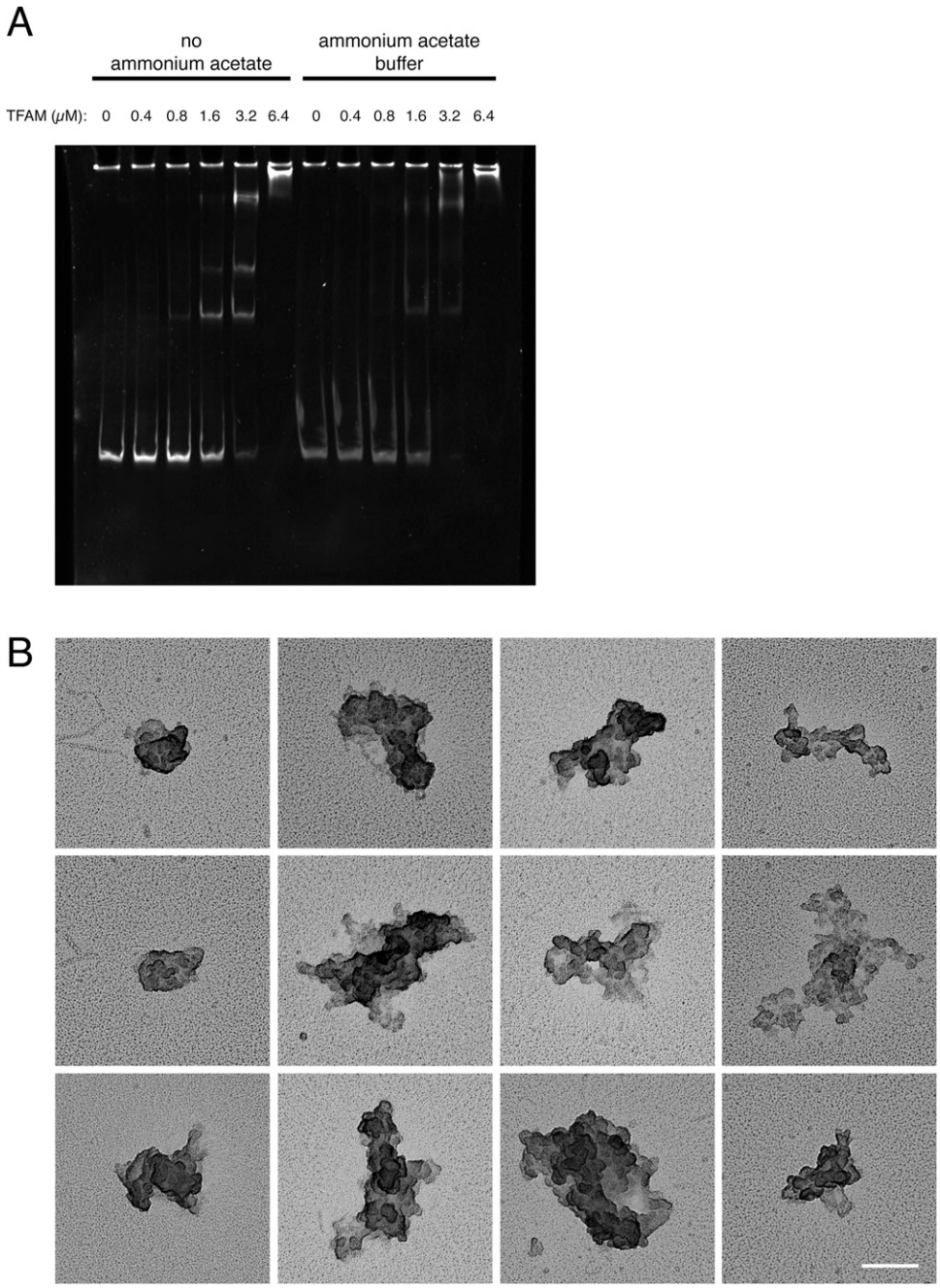


Fig. S1. (*A*) EMSA of DNA with increasing concentrations of recombinant TFAM. The stability of the formed complex was investigated with or without ammonium acetate in the buffer. Constant amounts of DNA templates were incubated with increasing amounts of recombinant TFAM protein in standard and ammonium acetate buffer. A clear shift of the DNA fragments was visible in the EMSA, indicating that the ammonium acetate required for EM does not interfere with TFAM–DNA binding. (*B*) Representative electron micrographs of in vitro-generated nucleoids with TFAM concentrations >1 TFAM/6 bp. (Scale bar: 100 nm.)

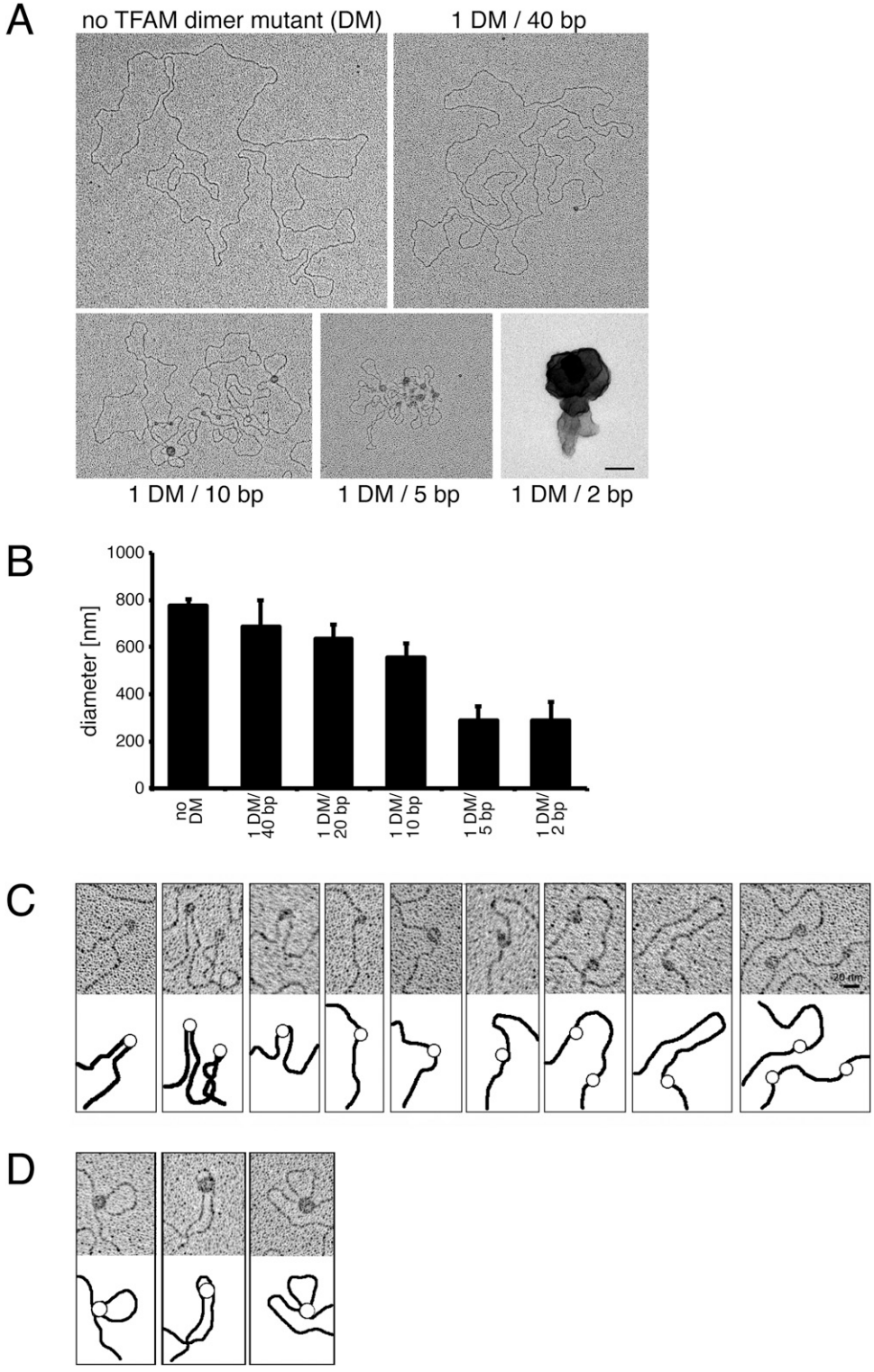


Fig. S2. (*A*) Electron micrographs of spread DNA incubated with increasing concentrations of TFAM dimer mutant (DM). (Scale bar: 100 nm.) (*B*) Quantification of the diameters of DNA incubated with increasing concentrations of TFAM DM protein by the mean of the long and short axis. Data are represented as mean ± SD; $n = 82$. (*C* and *D*) Electron micrographs showing that TFAM DM binds to DNA in two different ways. TFAM binds single DNA duplexes as beads on a string inducing bending of DNA (*C*) or bridges two DNA duplexes resulting in loops (*D*). (Scale bar: 20 nm.)

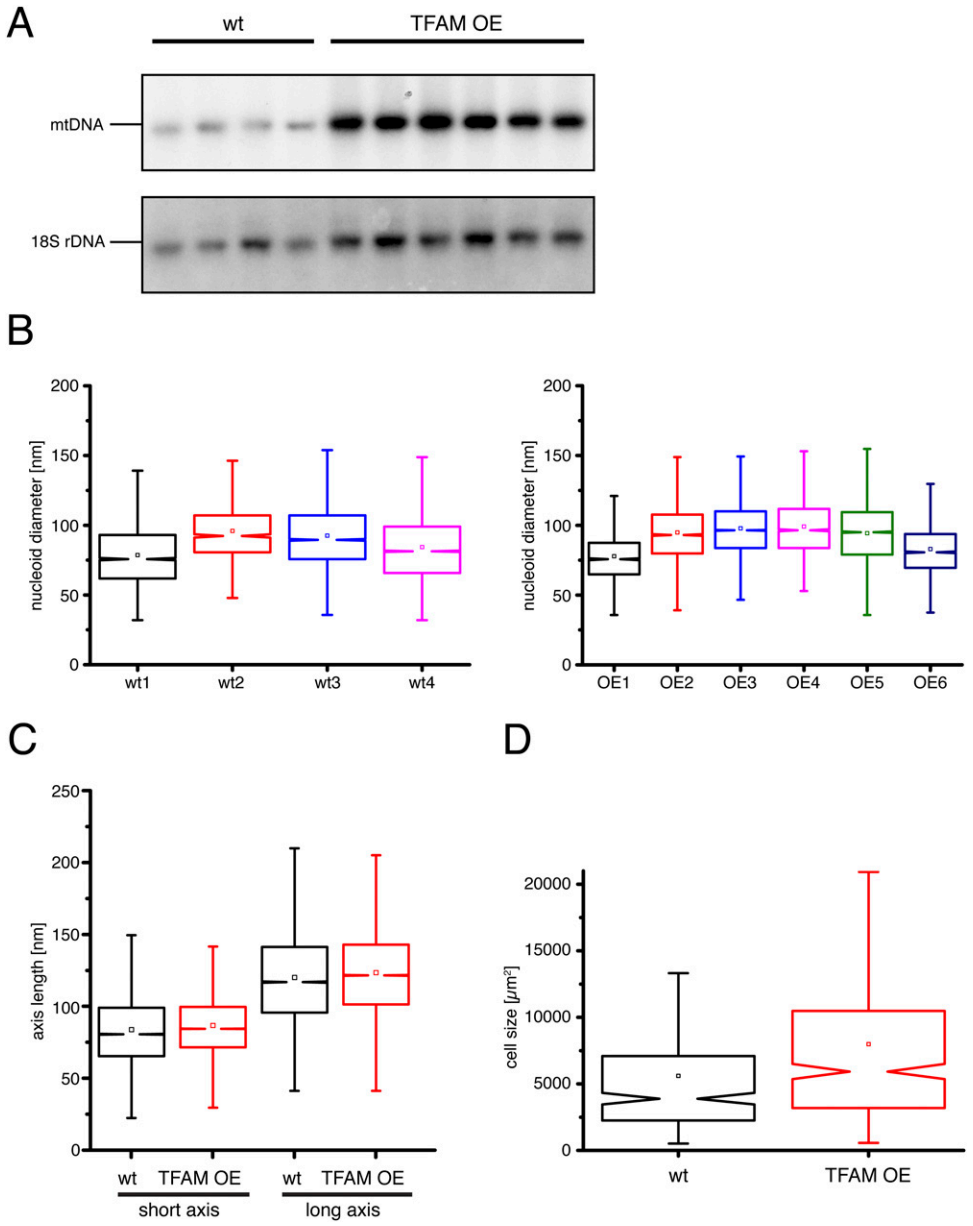


Fig. S3. (*A*) Southern blot analysis of the mtDNA content in independent MEF lines. (*B*) Diameters of mitochondrial nucleoids imaged by STED microscopy for four independent wild-type (wt) and six independent TFAM OE MEF cell lines. (*C*) Quantification of the long and short axis length of nucleoids in MEFs imaged by STED microscopy. (*D*) Cell size of MEFs. $n = 717$ cells. wt: wild-type; TFAM OE: TFAM overexpressor. Notched boxplots: the boxed intervals contain the data points from the first to third quartiles of the distribution. With a size of 1.5 times the interquartile range, the whiskers encompass ~99% of all data. The notches describe a confidence interval around the median, which equals the 95% confidence interval.

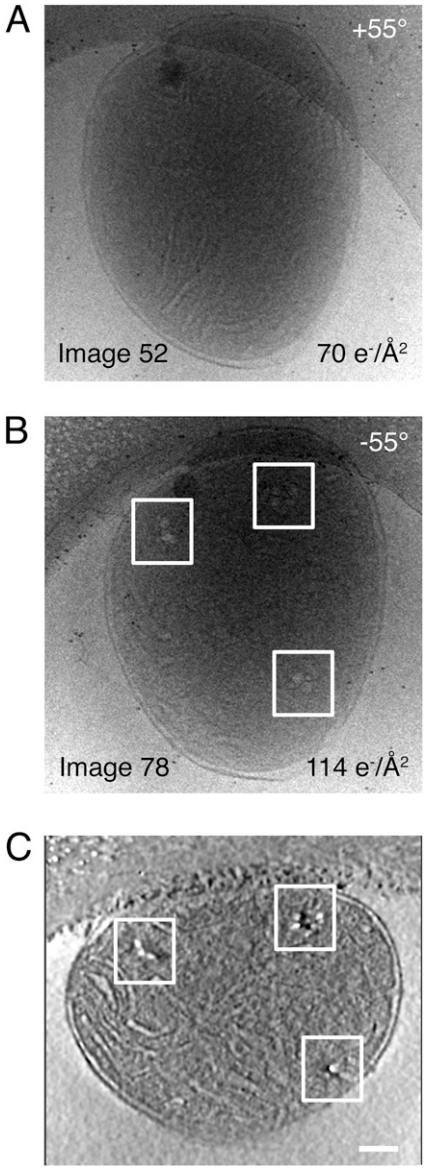


Fig. S4. (*A* and *B*) Projection images #52 and #78 from a tomographic tilt series of a bovine heart mitochondrion showing mitochondrial nucleoids (boxed areas). The total number of projection images in the tilt series is 86. Total accumulated electron dose for the series was 130 electrons per Ångström squared (e^{-}/Å^2). The total accumulated electron dose for each image is displayed at the bottom right corner of the image. (*C*) Tomographic slice through a whole bovine heart mitochondrion showing mitochondrial nucleoids (boxed areas). (Scale bar: 100 nm.)

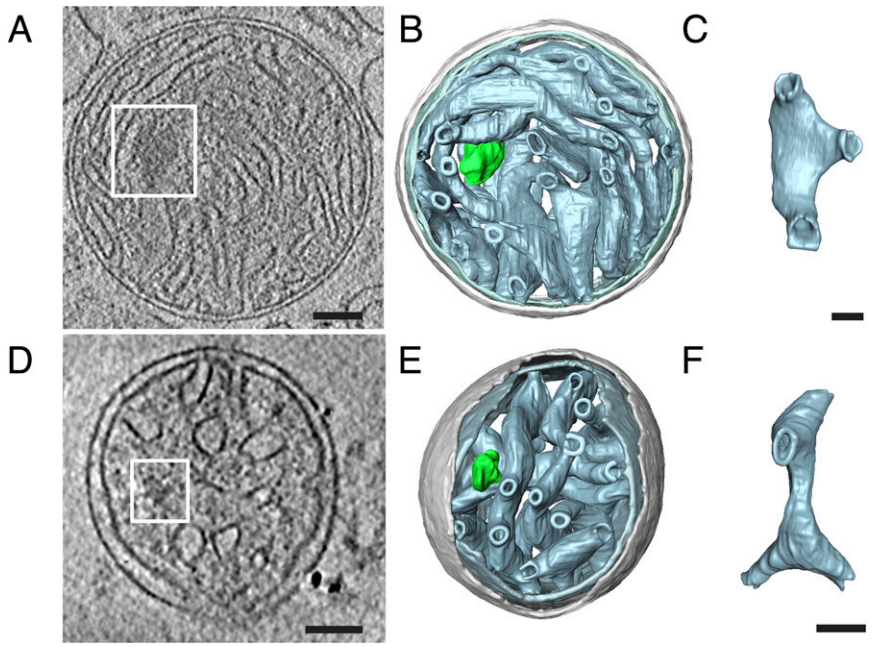
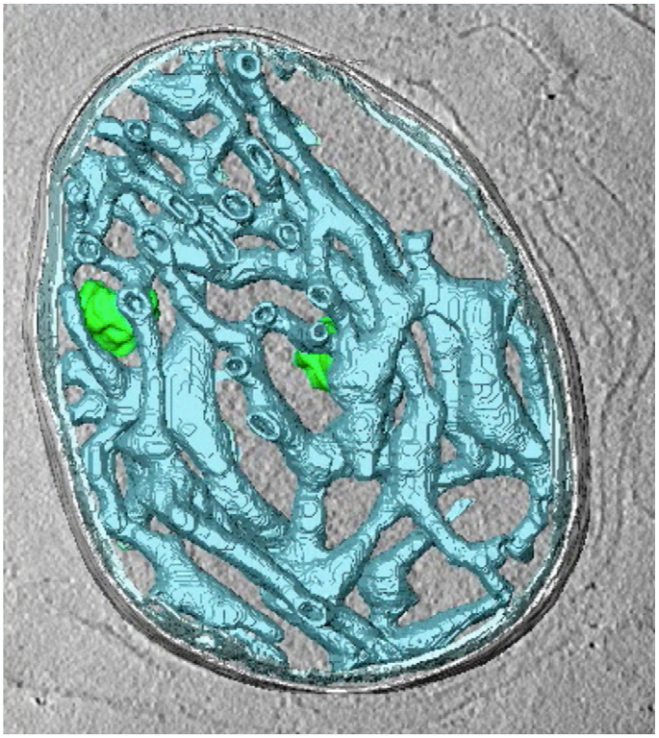


Fig. S5. (*A* and *D*) Tomographic slice through a whole bovine heart mitochondrion showing mitochondrial nucleoids (boxed areas). (Scale bar: 100 nm.) (*B* and *E*) Segmented surface representation of *A* with *D* showing position of mitochondrial nucleoids (green) in a bovine heart mitochondrion. Green: nucleoids; gray: outer membrane; gray-blue: cristae. (*C* and *F*) Crista extracted from *B* and *E* showing crista morphology. (Scale bar: 50 nm.)



Movie S1. Tomographic volume of a bovine heart mitochondrion shown in Fig. 3. Segmented surface representation showing position of mitochondrial nucleoids (green) in a bovine heart mitochondrion. Green: nucleoids; gray: outer membrane; gray-blue: cristae.

Movie S1