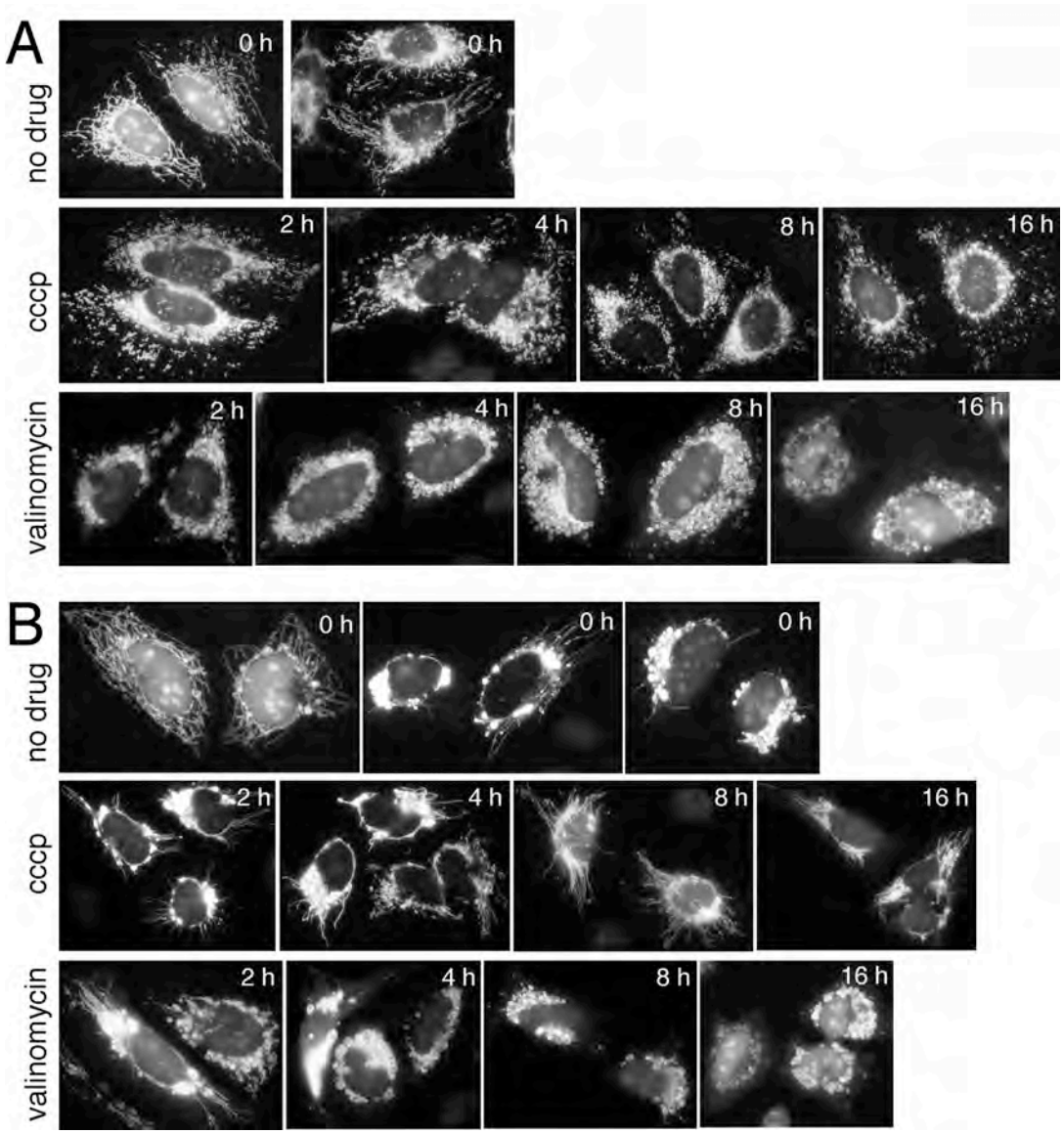
Separate fusion of outer and inner mitochondrial membranes

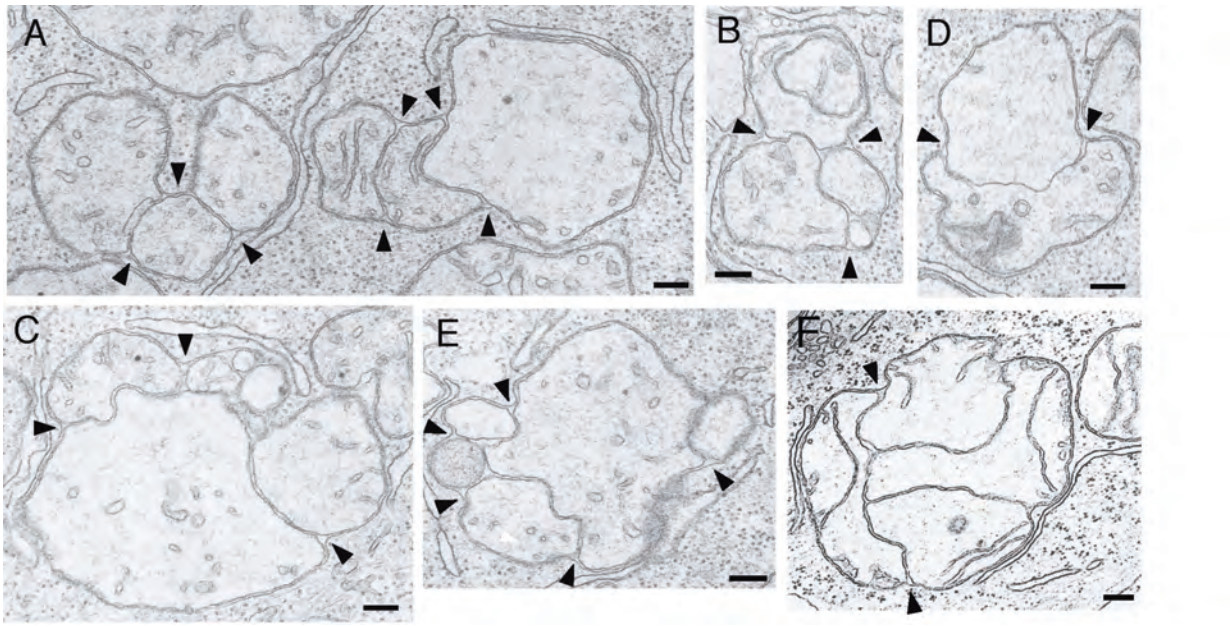
Supplementary Information

Drug	no drug	deoxyglucose	oligomycin	cccp	valinomycin	antimycin A
4 h (exp. 1)						
pmol ATP/μg protein	61,52	16,63	61,80	59,17	49,8	61,11
(% of control)	(100)	(27)	(100)	(96)	(81)	(99)
4 h (exp. 2)						
pmol ATP/μg protein	44,17	11,60	42,92	41,14	43,64	40,10
(% of control)	(100)	(26)	(97)	(93)	(99)	(91)
16 h						
pmol ATP/μg protein	51	11,14	54,60	64,1	39,23	48,99
(% of control)	(100)	(22)	(107)	(126)	(77)	(96)

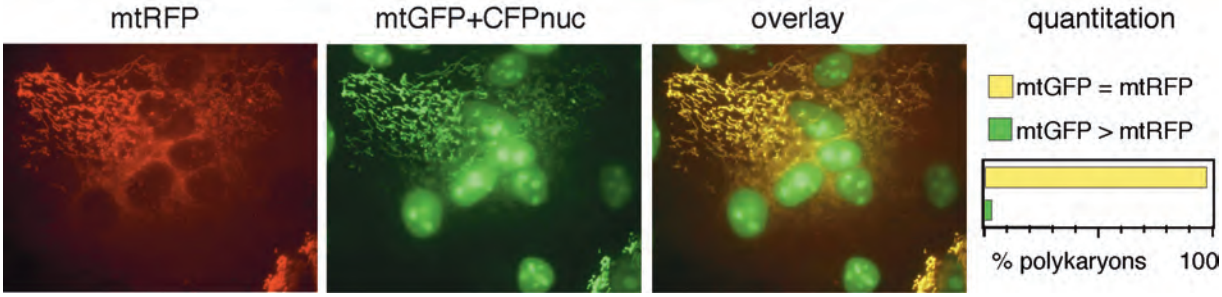
Supplementary Table 1: Modulation of cellular ATP-levels with drugs. Human 143B cells were treated with the indicated drugs for 4 or 16 hours and analyzed for ATP and protein content. Deoxyglucose treatment leads to a significant decrease of cellular ATP-levels but ATP-levels remain high upon treatment with drugs inhibiting oxidative phosphorylation. Thus, glycolysis can compensate for the inhibition of mitochondrial ATP-synthesis but mitochondrial ATP-synthesis cannot fully compensate for the inhibition of glycolysis with deoxyglucose.



Supplementary Figure S1: **Modulation of mitochondrial morphology and distribution by ionophores and dominant-negative Drp1K38A.** HeLa cells were transiently transfected with mtGFP alone (**A**) or with mtGFP and dominant negative Drp1K38A (**B**). Thirty-six hours after transfection cells were fixed without further treatment (no drug) or after treatment with cccp or valinomycin for the indicated times. **A**: Filamentous and punctate mitochondria coexist in control cells. Filamentous mitochondria disappear within 2 hours of ionophore treatment. In cccp-treated cells, small punctate mitochondria are scattered throughout cells. In valinomycin-treated cells, enlarged swollen mitochondria coalesce perinuclearly. **B**: Drp1K38A-expression modifies mitochondrial morphology and distribution in the absence of drugs: mitochondria appear as long interconnected filaments and/or form large circular structures that accumulate perinuclearly. Dominant-negative Drp1K38A inhibits cccp-mediated fragmentation but does not hamper valinomycin-induced swelling and coalescence.



Supplementary Figure S2: Mitochondrial ultrastructure in valinomycin-treated cells. Enlarged mitochondria are enveloped by continuous outer membrane (arrowheads) and contain inner membrane that entirely traverses mitochondria and delimit matrix compartments of similar or different appearance. The differences in electron density, size and/or cristae morphology of some adjacent matrices indicate separation by unfused inner membranes. Bars: 200 nm.



Supplementary Figure S3: Matrix targeted mtGFP and mtRFP display similar mobility and fluorescence intensity. RMGM cells expressing two fluorescent proteins targeted to the mitochondrial matrix (DsRed-derived mtRFP and GFP-derived mtGFP) were fused to CN cells expressing CFPnuc targeted to the nucleus. Cells were fixed after 4 hours, a time period insufficient for the distribution of matrix proteins throughout polykaryons. Both matrix fluorescent proteins show similar distribution (mtGFP = mtRFP) in the depicted polykaryon and in a majority of analyzed polykaryons (n = 90).

Supplementary Materials and Methods

Drugs, reagents and kits

Human HeLa and 143B cells were maintained as described (Legros et al., 2002). Drugs (final concentrations 2.5 μM oligomycin, 10 μM cccp, 10 μM valinomycin, 1 μM antimycin A) were added to standard culture medium containing 10% FCS (Legros et al., 2002) from 1000-fold stock solutions in ethanol (oligomycin, antimycin A) or DMSO (cccp, valinomycin) stored at 4°C (valinomycin) or –20°C (oligomycin, antimycin, cccp). Deoxyglucose (final concentration 40 mM) was added to standard culture medium devoid of glucose from 2M stock solutions in MEM stored at –20°C. Cells were scraped and homogenized by short sonication for determination of protein (Pierce Bicinchonic Acid Protein Assay) and ATP-content (Roche ATP Bioluminescence Assay Kit HS II).

Cell transfection, cell fusion and microscopy

For generation of doubly-transfected 143B cells expressing GFPOM and mtRFP, the cDNA encoding GFPOM (Nemoto and De Camilli, 1999) was inserted into pCB7, an expression plasmid carrying the hygromycin B resistance gene (Brewer, 1994). Neomycin-resistant cells expressing mtRFP (Legros et al., 2002) were transfected with pCB7-GFPOM and selected with hygromycin B. HeLa cells expressing enhanced cyan fluorescent protein targeted to the nucleus (CFPnuc) were generated by transfection of the pECFP-Nuc plasmid (BDBiosciences) and selection with neomycin. Transient transfection, polyethylene glycol-mediated cell fusion and other stably transfected cell lines were as described (Legros et al., 2002; Legros et al., 2004). Cell fixation and processing for (immuno)fluorescence and electron microscopy were as described (Rojo et al., 2002).

References

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Legros, F., Malka, F., Frachon, P., Lombès, A. and Rojo, M. (2004) Organization and dynamics of human mitochondrial DNA. *J.Cell Sci.*, 117, 2653-2662.

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Rojo, M., Legros, F., Chateau, D. and Lombes, A. (2002) Membrane topology and mitochondrial targeting of mitofusins, ubiquitous mammalian homologs of the transmembrane GTPase Fzo. *J.Cell Sci.*, 115, 1663-1674.