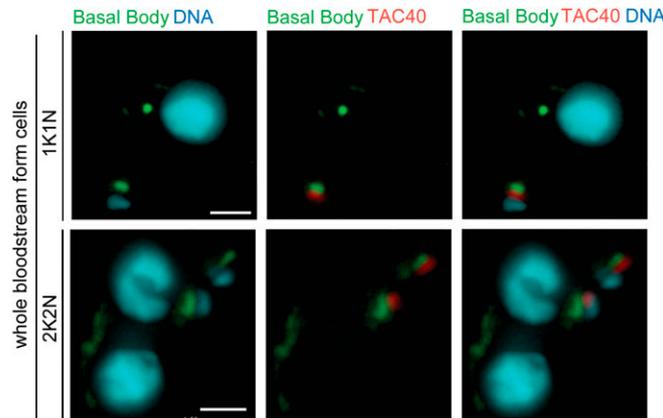
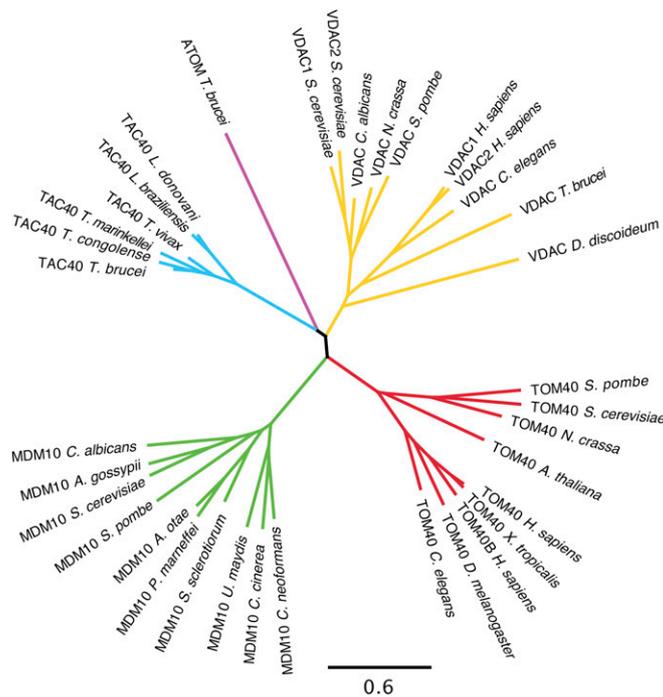


# Supporting Information

Schnarwiler et al. 10.1073/pnas.1404854111

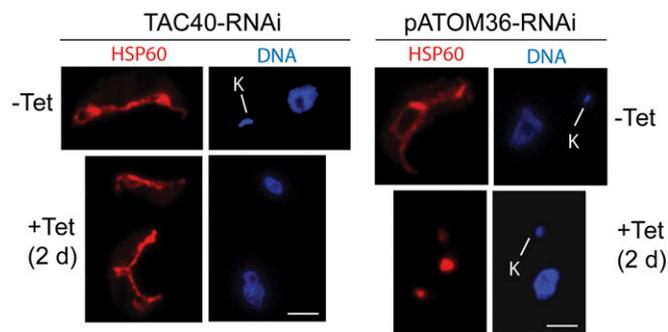


**Fig. S1.** Tripartite attachment complex 40 (TAC40) of bloodstream form cells localizes to the TAC. Maximum-intensity projections of immunofluorescence (IF) confocal microscopy images from whole bloodstream form *Trypanosoma brucei* cells expressing C-terminally hemagglutinin (HA)-tagged TAC40 are shown. Green, basal body region stained with the YL1/2 antibody; blue, 4',6-diamidino-2-phenylindole (DAPI)-stained nuclear DNA and kinetoplast DNA (kDNA); red, HA-tagged TAC40. Two cell cycle stages containing one kDNA and one nucleus (1K1N, *Upper*) and two kDNA and two nuclei (2K2N, *Lower*) are shown. The bright green spot (*Upper*) represents background staining. (Scale bars, 3  $\mu\text{m}$ .)



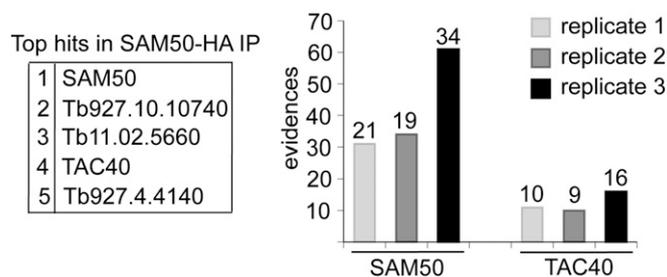
**Fig. S2.** TAC40 in the mitochondrial porin superfamily. Evolutionary tree is calculated from an alignment of 9 translocase of the mitochondrial outer membrane 40 (Tom40), 10 MDM10, 10 voltage-dependent anion channel (VDAC), 1 ATOM, and 6 TAC40 sequences. The evolutionary origin of ATOM is controversial; see refs. 1–4 for discussion. (Scale bar, Jukes–Cantor genetic distances.) The five resulting family trees have been colored for visual clarity.

1. Pusnik M, et al. (2011) Mitochondrial preprotein translocase of trypanosomatids has a bacterial origin. *Curr Biol* 21(20):1738–1743.
2. Zarsky V, Tachezy J, Dolezal P (2012) Tom40 is likely common to all mitochondria. *Curr Biol* 22(12):R479–R481, author reply R481–R482.
3. Pusnik M, et al. (2012) Response to Zarsky et al. *Curr Biol* 22:R481–R482.
4. Harsman A, et al. (2012) Bacterial origin of a mitochondrial outer membrane protein translocase: New perspectives from comparative single channel electrophysiology. *J Biol Chem* 287(37):31437–31445.

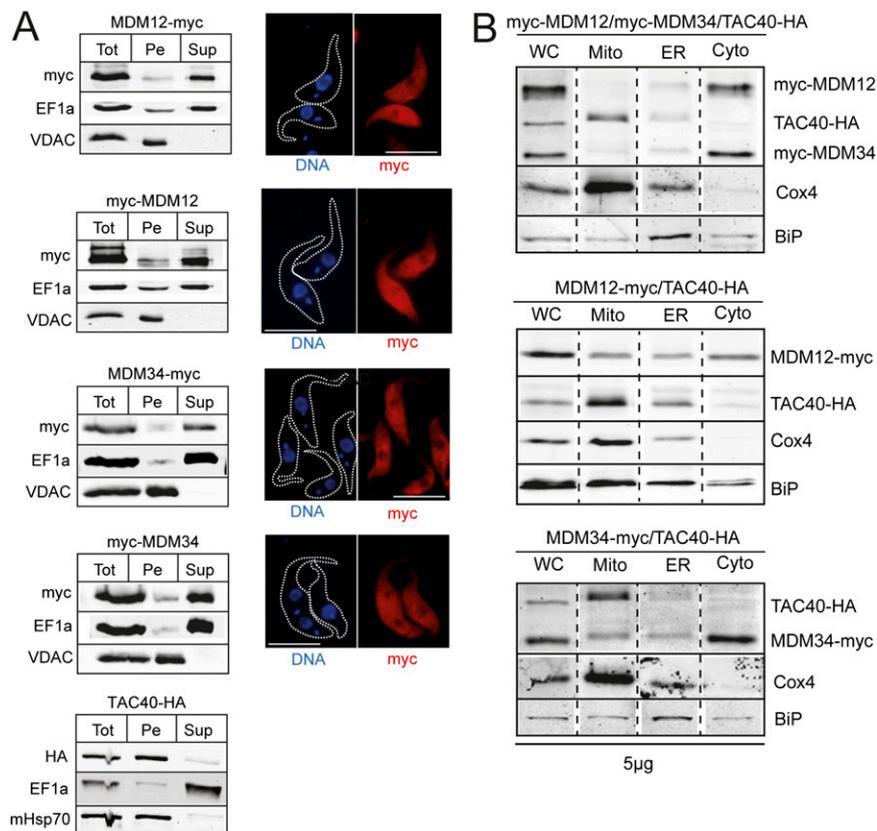


**Fig. S3.** Ablation of TAC40 does not affect mitochondrial morphology in bloodstream forms. Bloodstream form TAC40-RNAi and pATOM36 (1) RNAi cell lines were tested for alterations of mitochondrial morphology. Uninduced (–Tet) and induced (+Tet) cells were stained with DAPI (DNA). The mitochondrion was visualized using a heat-shock protein 60 (Hsp60) antiserum (red). The position of the kDNA (K) is indicated. (Scale bars, 3  $\mu$ m.)

1. Pusnik M, et al. (2012) An essential novel component of the noncanonical mitochondrial outer membrane protein import system of trypanosomatids. *Mol Biol Cell* 23(17):3420–3428.

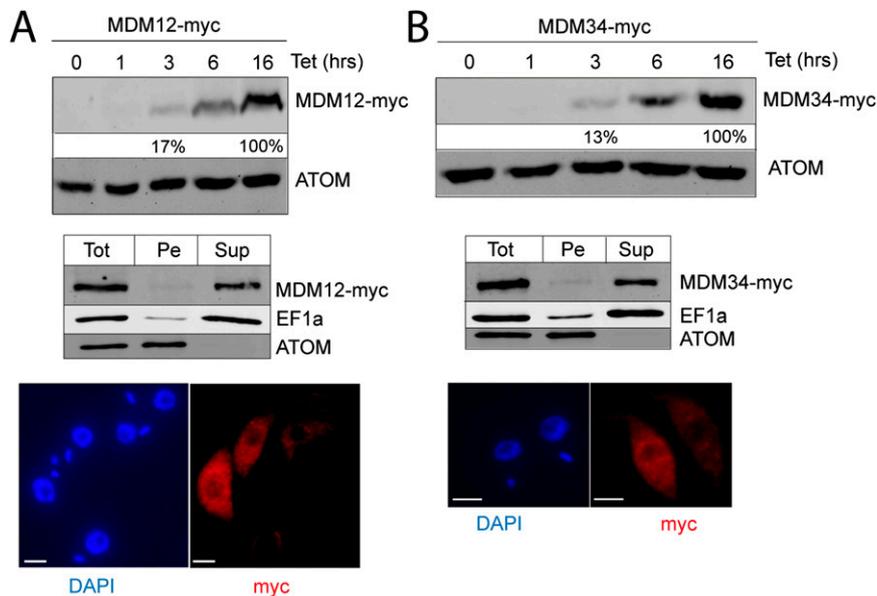


**Fig. S4.** A fraction of TAC40 associates with SAM50. A 0.15% digitonin lysate of isolated HA-tagged SAM50 containing mitochondria was immunoprecipitated using anti-HA antibodies. The corresponding eluates were analyzed using mass spectrometry. (Left) List shows the top five proteins that were detected in the eluates. (Right) Graph depicts the numbers of evidence for SAM50 and TAC40 that were detected in the eluates of each of three biological replicates. The number of unique peptides identified for each protein is depicted on the top of each column. Immunoprecipitations using wild-type mitochondria lacking the tagged protein served as controls. Neither SAM50 nor TAC40 was recovered in the eluates of nontagged wild-type mitochondria.



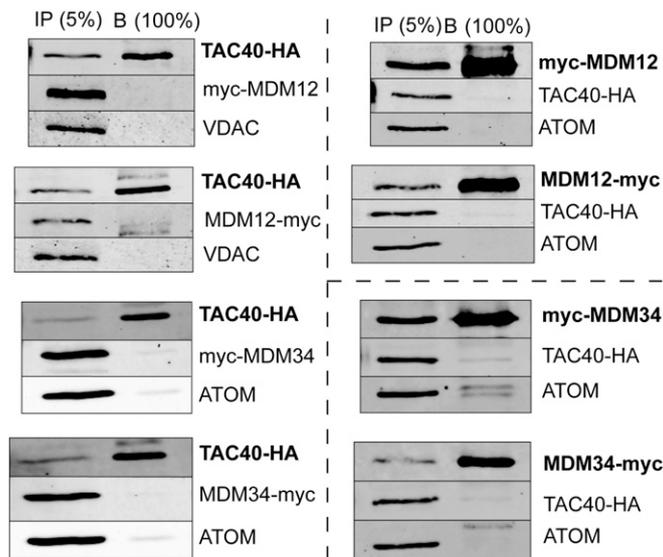
**Fig. 55.** Localization of the putative MDM12 and MDM34 orthologs of *T. brucei*. (*A, Left*) Immunoblots of total (Tot), digitonin-extracted crude mitochondrial (Pe), and cytosolic (Sup) fractions (1) of cell lines expressing the N- or C-terminally myc-tagged MDM12/MDM34 and the C-terminally HA-tagged TAC40 of *T. brucei*, respectively, were probed with anti-myc (myc) and anti-HA antibodies, respectively. Expression of all tagged MDM12/MDM34 versions was induced by adding tetracycline for 16 h. VDAC/mHsp70 and EF1a serve as mitochondrial and cytosolic markers, respectively. (*Right*) The same cell lines were analyzed by IF, using the corresponding anti-tag antibodies and DAPI for DNA. The outlines of the cells have been traced in the phase contrast channel and are projected onto the DAPI-stained pictures. (Scale bar, 10 µm.) The IF results for TAC40-HA are shown in Fig. 1. (*B*) Cell lines expressing N- and C-terminally myc-tagged MDM12/MDM34 in combination with C-terminally HA-tagged TAC40 were mixed as indicated at the top of each panel and subjected to subcellular fractionation as outlined below. Immunoblots containing 5 µg of each fraction were analyzed for the tagged proteins and for a mitochondrial (Cox4) and an ER (BiP) marker, respectively. For subcellular fractionation whole cells (WC) ( $4 \times 10^{10}$  cells each) were lysed at isotonic conditions by  $N_2$  cavitation. The resulting suspension was sedimented for 10 min at  $15,000 \times g$ , resulting in a pellet corresponding to a crude mitochondrial fraction (Mito) (2). Next, the supernatant was fivefold diluted in 10 mM Mops, pH 7.2, and 2.5 mM EDTA and centrifuged for 30 min at  $30,000 \times g$  to eliminate residual mitochondrial vesicles. Finally, ER vesicles were collected from the supernatant by ultracentrifugation (2 h/ $100,000 \times g$ ). The resulting pellet corresponds to a crude ER fraction (ER), whereas the supernatant represents the cytosol (Cyto). All steps were done at 4 °C.

1. Pusnik M, et al. (2011) Mitochondrial preprotein translocase of trypanosomatids has a bacterial origin. *Curr Biol* 21(20):1738–1743.
2. Niemann M, et al. (2013) Mitochondrial outer membrane proteome of *Trypanosoma brucei* reveals novel factors required to maintain mitochondrial morphology. *Mol Cell Proteomics* 12(2):515–528.



**Fig. 56.** Reduced expression of the putative MDM12 and MDM34 does not change their cytosolic localization. (A, Top) Immunoblot of total cellular extracts from a cell line allowing tetracycline-inducible expression of C-terminally myc-tagged MDM12. Samples were collected at the indicated time points after addition of 0.5 μg of tetracycline. The relative levels of the tagged MDM12 at 3 h compared with 16 h of induction are indicated. ATOM serves as a loading control. For the subsequent analyses expression of the C-terminally myc-tagged MDM12 was induced for 3 h only. (Middle) Immunoblot of total (Tot), digitonin-extracted crude mitochondrial (Pe), and cytosolic (Sup) fractions (1) was probed with anti-myc antibodies. ATOM and EF1a serve as mitochondrial and cytosolic markers, respectively. (Bottom) The same cell line was analyzed by DAPI staining (DNA) and by IF, using the anti-myc antibody (myc). At such short induction times the levels of expression differed in individual cells. (Scale bars, 3 μm.) (B) The same as in A but analysis was done for the cell line expressing C-terminally myc-tagged MDM34.

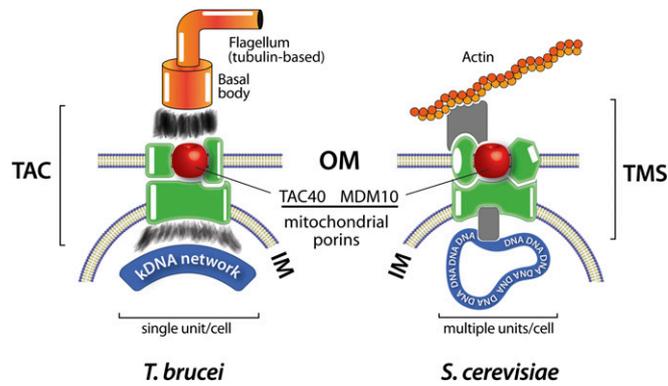
1. Pusnik M, et al. (2011) Mitochondrial preprotein translocase of trypanosomatids has a bacterial origin. *Curr Biol* 21(20):1738–1743.



**Fig. 57.** Reciprocal immunoprecipitations (IPs) do not provide evidence for interaction of TAC40 with MDM12/MDM34. Whole cell lysates from cell lines coexpressing the TAC40-HA with the indicated tagged MDM12/MDM34 were immunoprecipitated using anti-HA antibodies or anti-myc antibodies bound to agarose beads. The tagged proteins targeted in the different IP experiments are indicated in boldface type. IPs were done as described in ref. 1. In short,  $10^8$  cells each expressing the corresponding tagged proteins were solubilized in 400 μL of lysis buffer: 20 mM Tris-HCl, pH 7.4, 0.1 mM EDTA, 200 mM NaCl containing 0.5% (wt/vol) digitonin, and a protease inhibitor mixture. The lysate was cleared by centrifugation and the supernatant was incubated for 3 h at 4 °C with 25 μL or 75 μL of a 1:1 slurry of anti-myc or anti-HA agarose, respectively. Subsequently, the beads were washed in lysis buffer containing 0.1% (wt/vol) digitonin before elution with SDS sample buffer.

1. Pusnik M, et al. (2012) An essential novel component of the noncanonical mitochondrial outer membrane protein import system of trypanosomatids. *Mol Biol Cell* 23(17):3420–3428.





**Fig. S10.** Model for mitochondrial DNA segregation in *T. brucei* and *Saccharomyces cerevisiae* (not to scale). In both systems a hetero-oligomeric protein complex (green) containing the conserved mitochondrial OM  $\beta$ -barrel protein TAC40/MDM10 (red) connects the mitochondrial DNA (blue) across the two mitochondrial membranes with the cytoskeleton (orange). (Left) The situation in *T. brucei*. Its mitochondrial genome (kDNA network) is of single-unit nature and linked to the basal body of the tubulin-based flagellum via the TAC. The TAC consists of filaments that link the kDNA network to a protein complex in the mitochondrial membranes and cytosolic filaments that connect the cytosolic side of the complex to the basal body (1). The mitochondrial outer membrane (OM) mitochondrial porin TAC40 (red) is an essential component of the structure. The only previously characterized component of the TAC is p166, which is either a matrix or an inner membrane protein (2). The TAC couples the segregation of the old and the newly assembled flagella to the segregation of the replicated kDNA networks (3). (Right) The situation postulated for *S. cerevisiae*. Each cell contains multiple two-membrane spanning structures (TMS) that each link to one or two mitochondrial genomes (4). The TMS consists of a protein complex in the mitochondrial membranes that links to the mitochondrial DNA on the inside and to actin filaments on the outside (5). Actin filaments are required for the movement of mitochondria to the bud that will become the daughter cell. The mitochondrial OM protein Mdm10 (red) is an essential component of the structure. The TMS may include the whole ER-mitochondria encounter structure (ERMES) complex in which case it would bridge not only two but three membranes (not indicated).

1. Ogbadoyi EO, Robinson DR, Gull K (2003) A high-order trans-membrane structural linkage is responsible for mitochondrial genome positioning and segregation by flagellar basal bodies in trypanosomes. *Mol Biol Cell* 14(5):1769–1779.
2. Zhao Z, Lindsay ME, Roy Chowdhury A, Robinson DR, Englund PT (2008) p166, a link between the trypanosome mitochondrial DNA and flagellum, mediates genome segregation. *EMBO J* 27(1):143–154.
3. Robinson DR, Gull K (1991) Basal body movements as a mechanism for mitochondrial genome segregation in the trypanosome cell cycle. *Nature* 352(6337):731–733.
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5. Boldogh IR, et al. (2003) A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to the cytoskeleton-based segregation machinery. *Mol Biol Cell* 14(11):4618–4627.