## Supplemental material

Bui et al., http://www.jcb.org/cgi/content/full/jcb.201207079/DC1



Figure S1. **Expression, interaction, and assembly properties of Dnm1 and Mdv1 variants.** (A) Steady-state abundance of N-terminal GFP-tagged WT and Dnm1<sup>InsBmut</sup> proteins expressed from a plasmid in *dnm1*Δ cells. Whole cell lysates from 0.5 OD<sub>600</sub> cell equivalents were separated by SDS-PAGE and immunoblotted with anti-GFP antibody. (B) In vitro GST pull-down assay. Bacterial expressed GST-Mdv1 β-propeller immobilized on glutathione resin was incubated for 2 h at 4°C with purified His-Dnm1 expressed in insect cells from a previously described plasmid (Ingerman et al., 2005). After washing, bound proteins were eluted, separated by SDS-PAGE, and immunoblotted with anti-Mdv1 and Dnm1 antibodies. (C) Steady-state abundance of Mdv1 suppressor proteins. Whole cell lysates from 0.5 OD<sub>600</sub> cell equivalents were separated by SDS-PAGE and immunoblotted with anti-Mdv1 and Dnm1 antibodies. (C) Steady-state abundance of Mdv1 suppressor proteins. Whole cell lysates from 0.5 OD<sub>600</sub> cell equivalents were separated by SDS-PAGE and immunoblotted with anti-Mdv1 and Dnm1 antibodies. (C) Steady-state abundance of Mdv1 suppressor proteins. Whole cell lysates from 0.5 OD<sub>600</sub> cell equivalents were separated by SDS-PAGE and immunoblotted with anti-Mdv1 antibody. (D) Representative images of GFP-Mdv1 and GFP-Mdv1<sup>DS76G</sup> localization in cells expressing Dnm1<sup>F610A</sup> from the genome. Differential interference contrast microscopy (DIC), mitochondrial matrix-targeted dsRed (mt-ffRFP), GFP, and merged images are shown. Bar, 5 µm.

## Table S1. Screen for mdv1 suppressors of dnm1<sup>F610A</sup>

Strain	25°C		37°C	
	Mitochondrial morphology	Glycerol growth	Mitochondrial morphology	Glycerol growth
fzo1-1 (ts) DNM1 MDV1	WT/Not fragmented	Yes	Fragmented	No
fzo1-1 mdv1 $\Delta$ dnm1::dnm1 <sup>F610A</sup>	Nets	Yes	Not fragmented	Yes
fzo1-1 mdv14 dnm1::dnm1 <sup>F610A</sup> expressing Mdv1 <sup>WT</sup>	Nets	Yes	Not fragmented	Yes
fzo1-1 mdv1Δ dnm1::dnm1 <sup>F610A</sup> expressing <b>Mdv1<sup>suppressor</sup></b>	WT/Not fragmented	Yes	Fragmented	No

See Materials and methods for additional description of genetic screen. Bold terms emphasize the genotype and phenotype sought in the described genetic screen.

## Table S2. Plasmids used in this study

ID number	Plasmid	Source
B363	pRS415-DNM1	Otsuga et al., 1998
B2901, B2902, B2940-2945	pRS415-DNM1 <sup>[F606A, L607A, N608A, Y609A, F610A, F611A, G612A or K613A]</sup>	This study
B2144	pRS415-MET25-GFP-DNM1	Karren et al., 2005
B2949, B3004-3006	pRS415-MET25-GFP- DNM1, pRS426-DNM1-3xHA	This study, Karren et al., 2005
B2947, B2973-2975	pRS426-DNM (F606A, L607A, F610A or F611A)-3xHA	This study
B955	pRS425-DNM1-MYC	Karren et al., 2005
B2950, B3007-3009	pRS425-DNM (F606A, L607A, F610A or F611A)-MYC	This study
B1642, B1643	pRS414-GPD-mt-ffRFP, pRS416-GPD-mt-ffRFP	Karren et al., 2005
B2053	pRS416-MET25-MDV1	Karren et al., 2005
B3312-B3323	pRS416-MET25-MDV1 <sup>suppressor</sup>	This study
B3324	pGAD-C1-DNM1	This study
B3325	pGAD-C1-DNM1 <sup>F610A</sup>	This study
B3352	pGBD-C1-DNM1	This study
B3353-3356	pGBD-C1-DNM1 <sup>F606A, L607A, F610A, F611A</sup>	This study
B3326	pGBD-C1-MDV1	This study
B3327-3338	pGBD-C1-MDV1 <sup>suppressor</sup>	This study
B2455, B2456	pGAD-C, pGBD-C1	Guthrie and Fink, 2002

## References

Guthrie, C., and G. Fink. 2002. Guide to yeast genetics and molecular biology. Methods in enzymology series. Vol. 350. San Diego: Academic Press, Inc.

Ingerman, E., E.M. Perkins, M. Marino, J.A. Mears, J.M. McCaffery, J.E. Hinshaw, and J. Nunnari. 2005. Dnm1 forms spirals that are structurally tailored to fit mitochondria. J. Cell Biol. 170:1021–1027. http://dx.doi.org/10.1083/jcb.200506078

Karren, M.A., E.M. Coonrod, T.K. Anderson, and J.M. Shaw. 2005. The role of Fis1p-Mdv1p interactions in mitochondrial fission complex assembly. J. Cell Biol. 171:291–301. http://dx.doi.org/10.1083/jcb.200506158

Otsuga, D., B.R. Keegan, E. Brisch, J.W. Thatcher, G.J. Hermann, W. Bleazard, and J.M. Shaw. 1998. The dynamin-related GTPase, Dnm1p, controls mitochondrial morphology in yeast. J. Cell Biol. 143:333–349. http://dx.doi.org/10.1083/jcb.143.2.333