Supporting Information

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SI Materials and Methods

Plasmid Construction. Plasmids used in this study are listed in Table S3. Plasmids B1642, B1808, and B2053 were described previously (1). To construct B1607 and B1816, the DNA sequences encoding full-length yeast Fis1 (yFis1) and human dynamin-related protein 1 (hDrp1) isoform 3 were PCR amplified and cloned into BamHI and SalI sites of the *pRS415MET25* and *pRS416MET25* vectors (Stratagene). To create B2729, DNA sequences encoding amino acids 1–51 of yeast translocase of outer membrane (yTom20) (2) were PCR amplified and cloned into the XbaI and BamHI sites of B1808 [in-frame with the existing full-length (FL) mitochondrial division protein 1 (*MDV1*) coding region]. B2731 and B2732 were constructed by replacing BamHI-*MDV1*-SalI in B2729 with the indicated *MDV1* coding sequences. For B3090, a three-way ligation reaction was performed with the *pRS415MET25* vector (Stratagene) and PCR-amplified fragments encoding monomeric GFP^{A207K} yFis1 (amino acids 131–155) to generate the *pRS415MET25-BamHI-mOMGFP-BsiWI-yFIS1-SalI-pRS415* vector. For B3162, the StarGate cloning system (IBA) was used to introduce *PreScission Protease Cleavage Site-BamHI-hDRP1 isoform 3* DNA into the EcoRI and SalI sites of the *pYSG-IBA167* vector. For B3259, B3262, and B3294, the indicated coding sequences were exchanged for human dynamin-related protein 1 (*hDRP1*) using existing BamHI and SalI sites. For B3265, a two-step cloning protocol was used. First, the PCR-amplified copper homeostasis 1 (*CUP1*) promoter sequence was introduced into the SacII and SalI sites of the *pRS416* vector (Stratagene) to create *pRS416CUP1*. This cloning step also introduced EagI and BamHI sites upstream of the SalI site. Second, a three-way ligation reaction was performed with *pRS416CUP1* and PCR-amplified fragments encoding monomeric GFP^{A207K} and hDrp1 using EagI, BamHI, and SalI sites. B3265 contains the following order of genes and restriction sites: *pRS416 vector-SacII-CUP1-EagI-mGFP-BamHI-hDRP1-SalI-pRS416 vector*. B3357 was created by cloning PCR-amplified sequences encoding residues of human Mff (amino acids 1–198) into the EcoRI and HinDIII sites of the pMAL-c2x vector (New England BioLabs). For plasmids B2821, B2925, B2927, B2928, B3237, B3238, B3239, B3244, and B3247, a PCR fragment encoding the indicated inserts in frame with C-terminal or N-terminal 3HA were cloned into *pRS415MET25*. For B2933, a PCR fragment encoding dynamin-related protein 1 (Dnm1) was cloned into the BamHI/SalI sites of *pRS415MET25-T20*. For B2934, a PCR fragment encoding Dnm1 was cloned upstream of a fragment encoding yFis1 (amino acids 131–155) in *pRS415MET25*.

Analysis of Protein Expression. Protein expression was analyzed in whole-cell extracts prepared as described (3). For each blot in Fig. S1 *B–H*, cell equivalents were separated by SDS/PAGE and analyzed by Western blotting using anti-HA (1:1,000), anti-3 phosphoglycerate kinase (1:1,000), and anti-Dnm1 (1:1,000) primary antibodies. After incubation with the appropriate HPR-conjugated or fluorescent secondary antibodies, proteins were detected by ECL (GE Healthcare) or a fluorescent scanner (Odyssey; Li-COR Biosciences).

Analytical Equilibrium Sedimentation. The purified dynamin-related protein 1 (Drp1), mitochondrial dynamics protein 49 (MiD49), and mitochondrial fission factor (Mff) proteins were each centrifuged at a minimum of three concentrations (see the legend of Fig. S2) and two speeds 98,000 and 10,000 rpm for Drp1; 8,000, 10,000, and 12,000 rpm for MiD49; 10,000 and 12,000 rpm for Mff, using the An-50-Ti rotor, Beckman Coulter, Brea, CA) at 4 °C until equilibrium was established. Data were fit globally to an ideal single species model with a floating molecular weight using nonlinear least squares analysis as implemented in HeteroAnalysis (4). Representative data are shown for 10,000 rpm, with the MW fit and oligomeric state indicated. Buffers used for the analysis were Drp1 (20 mM Hepes 7.4, 500 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT); MiD49 (100 mM Tris·Cl 8.0, 150 mM NaCl, 1 mM EDTA, 1 mM TCEP); and Mff (50 mM sodium phosphate 7.4, 150 NaCl). Panels below each graph in Fig. S2 show the residual differences between the data and the fit. Buffer densities and protein partial specific volumes were calculated with SEDNTERP (version 1.09) (5). For Drp1, 11% of the sample was lost during centrifugation (either to self-assembly or aggregation).

1. Karren MA, Coonrod EM, Anderson TK, Shaw JM (2005) The role of Fis1p-Mdv1p interactions in mitochondrial fission complex assembly. *J Cell Biol* 171(2):291–301.
2. Ramage L, Junne T, Hahne K, Lithgow T, Schatz G (1993) Functional cooperation of mitochondrial protein import receptors in yeast. *EMBO J* 12(11):4115–4123.
3. Kushnirov VV (2000) Rapid and reliable protein extraction from yeast. *Yeast* 16(9): 857–860.
4. Cole JL (2004) Analysis of heterogeneous interactions. *Methods Enzymol* 384: 212–232.
5. Laue T, Shah B, Ridgeway T, Pelletier S (1992) Computer-aided interpretation of analytical sedimentation data for proteins. *Analytical Ultracentrifugation in Biochemistry and Polymer Science* (Royal Society of Chemistry, Cambridge, UK).

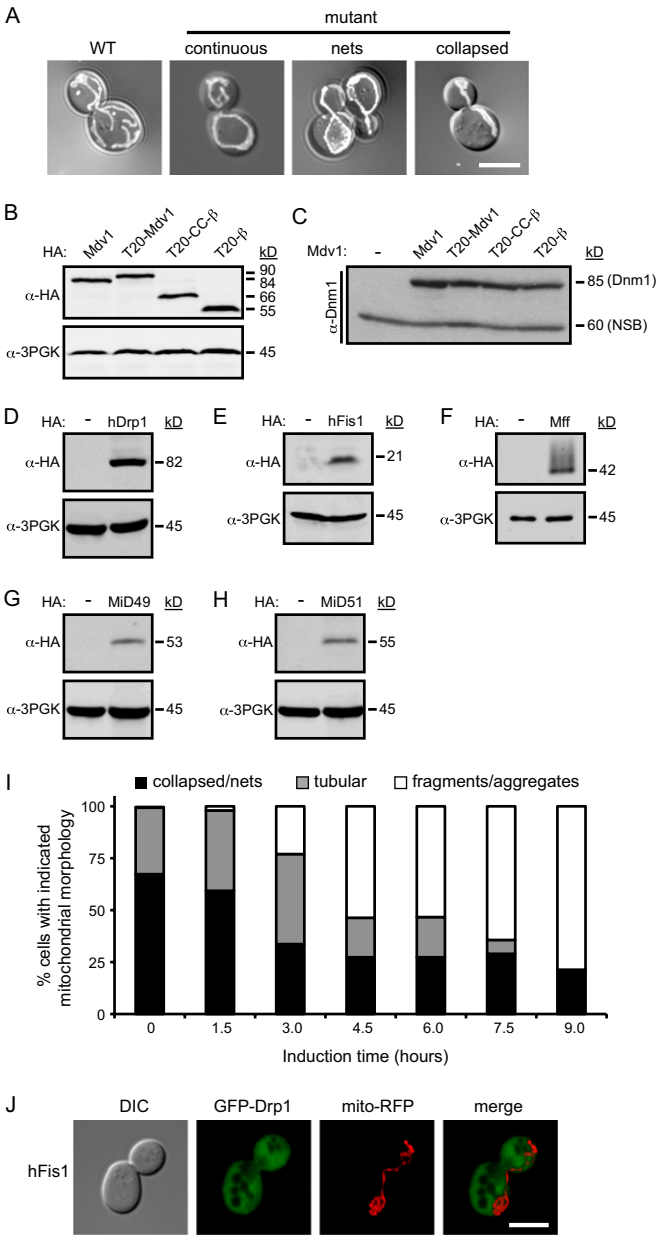

Fig. S1. Mitochondrial morphologies scored and protein expression levels for strains used in this study. (*A*) Representative images of mitochondrial morphologies scored as WT or fission mutant in this study. Superimposed differential interference contrast (DIC) and mito-RFP images are shown. (Scale bar, 5 μm.) (*B*) The steady-state abundance of C-terminal 3HA-tagged WT and tethered Mdv1 proteins was analyzed in whole-cell extracts by immunoblotting with anti-HA or anti-3PGK (loading control). (*C*) Steady-state abundance of WT Dnm1 protein expressed from the endogenous locus in strains shown in *B*. Anti-Dnm1 antibody detects Dnm1 (85 kDa) and a nonspecific band (NSB, 60-kD loading control). (*D–H*) Steady-state abundance of the indicated HA-tagged proteins expressed from the *pRS415MET25* plasmid in strain JSY9307 was analyzed in whole-cell extracts by immunoblotting with anti-HA or anti-3PGK (loading control). *(I)* Quantification of mitochondrial morphologies observed in cells during induction of Drp1 and MiD49 from the *MET25* promoter. *(J)* Representative DIC, GFP-Drp1, mito-RFP, and merged GFP/RFP images showing GFP-Drp1 localization in cells expressing hFis1 (JSY10005). (Scale bar, 5 μm.)

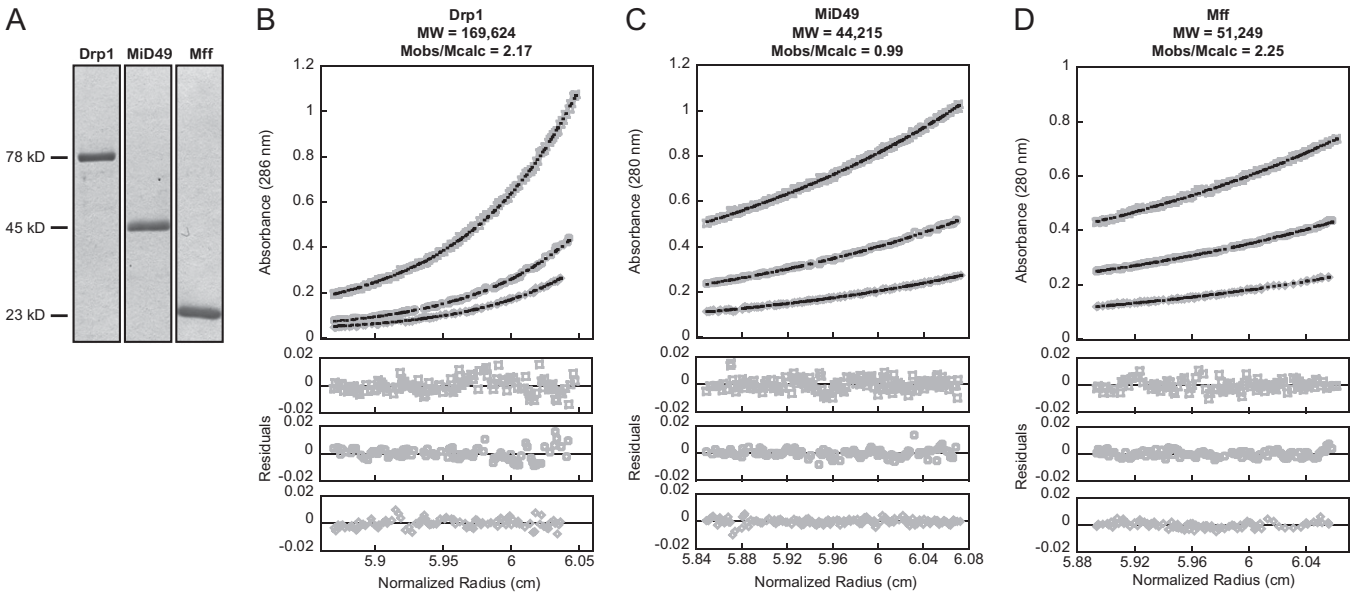

Fig. S2. Purification and analytical equilibrium sedimentation analysis of Drp1, MiD49, and Mff. (*A*) SDS/PAGE of the indicated purified proteins stained with Coomassie Brilliant Blue. (*B–D*) Analytical equilibrium sedimentation analysis of Drp1 (14.8 μM, 7.4 μM, 3.7 μM) (*B*), MiD49 (9.4 μM, 4.7 μM, 2.3 μM) (*C*), and Mff (130.0 μM, 65.1 μM, 32.6 μM) (*D*). In *B–D*, corresponding fits [observed molecular weight (MW_{obs})/calculated molecular weight (M_{calc})] are indicated above each graph, and residuals for the nonlinear least squared fits are shown below. See also *SI Materials and Methods*.

Table S1. Mitochondrial morphology in the JSY9307 tester strain expressing the indicated proteins

Protein expressed	Tubular (%)	Fission mutant (%)
None	0	100
WT Dnm1	0	100
$T20^{1\text{-}51aa}$-Dnm1	0	100
Dnm1-$Fis1^{131\text{-}155aa}$	0	100
$T20^{1\text{-}51aa}$-$Mdv1^{1\text{-}714aa\ (FL)}$	0	100
$T20^{1\text{-}51aa}$-$Mdv1^{218\text{-}714aa\ (CCWD)}$	0	100
$T20^{1\text{-}51aa}$-$Mdv1^{317\text{-}714aa\ (WD)}$	0	100
$hFis1^{1\text{-}119aa}$-$Fis1^{131\text{-}155aa}$	0	100
$hMff^{1\text{-}198}$- $Fis1^{131\text{-}155aa}$	0	100
$T20^{1\text{-}51aa}$-$hMiD49^{48\text{-}454aa}$	0	100
$T20^{1\text{-}51aa}$-$hMiD51^{47\text{-}463aa}$	0	100
WT hDrp1	0	100

Mitochondrial morphology was visualized with mito-RFP expressed from plasmid B1642. T20 is amino acids 1-51 of Tom20 used as an N-terminal mitochondrial outer membrane anchor. Fis1 is amino acids 131-155 of yeast Fis1 used as a C-terminal mitochondrial outer membrane anchor. $n = 300$.

Table S2. Yeast strains used in this study

ID	Genotype
JSY5740	*MATa, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, lys2Δ202*
JSY7459	*MATα, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, fis1::HIS3, mdv1::HIS3*
JSY8614	*MATa, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, caf4::KanMX*
JSY8616	*MATa, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, mdv1::HIS3*
JSY9234	*MATα, leu2Δ1, his3Δ200, lys2Δ202, ura3-52, TRP1, caf4::KanMX, mdv1::URA3, fis1::HIS3*
JSY9307	*MATa, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, lys2Δ202, dnm1::HIS3, fis1::HIS3, caf4::KanMX, mdv1::HIS3*
JSY9493	*MATa, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, lys2Δ202, dnm1::GFP-DNM1, fis1::HIS3, mdv1::HIS3, caf4::KanMX*
JSY9548	*MATa, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, lys2Δ202, dnm1::GFP-DNM1, caf4::KanMX*
JSY9612	*MATa, can1, ade2, trp1, ura3, his3, leu2, pep4::HIS3, prb1::LEU2, bar1::HISG, lys2::GAL1/10-GAL4*
JSY9801	*MATα, leu2Δ1, his3Δ200, ura3-52, lys2Δ202, fis1::HIS3, caf4::KanMX, mdv1::MET25-TOM20(1-51aa)-MDV1(1-714aa)*
JSY9802	*MATa, leu2Δ1, his3Δ200, ura3-52, lys2Δ202, fis1::HIS3, caf4::KanMX, mdv1::MET25-TOM20(1-51aa)-MDV1(218-714aa)*
JSY9803	*MATα, leu2Δ1, his3Δ200, ura3-52, lys2Δ202, fis1::HIS3, caf4::KanMX, mdv1::MET25-TOM20(1-51aa)-MDV1(317-714aa)*
JSY9804	*MATa, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, lys2Δ202, fis1::HIS3, caf4::KanMX, dnm1::GFP-DNM1, mdv1::MET25-TOM20(1-51aa)-MDV1(1-714aa)*
JSY9805	*MATa, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, lys2Δ202, fis1::HIS3, caf4::KanMX, dnm1::GFP-DNM1, mdv1::MET25-TOM20(1-51aa)-MDV1(218-714aa)*
JSY9806	*MATa, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, lys2Δ202, fis1::HIS3, caf4::KanMX, dnm1::GFP-DNM1, mdv1::MET25-TOM20(1-51aa)-MDV1(317-714aa)*
JSY9807	*MATα, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, lys2Δ202, caf4::KanMX, mdv1:: MET25-MDV1*
JSY10005	*MATα, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, lys2Δ202, dnm1::HIS3, fis1::HIS3, caf4::KanMX, mdv1::MET25-hFIS1(1-119aa)-yFIS1(122-155aa)*
JSY10006	*MATα, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, lys2Δ202, dnm1::HIS3, fis1::HIS3, caf4::KanMX, mdv1::MET25-hMff(1-198aa)-yFIS1(127-155aa)*
JSY10007	*MATα, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, lys2Δ202, dnm1::HIS3, fis1::HIS3, caf4::KanMX, mdv1::MET25-TOM20(1-51aa)-hMiD49(48-454aa)*
JSY10009	*MATα, leu2Δ1, his3Δ200, trp1Δ63, ura3-52, lys2Δ202, dnm1::HIS3, fis1::HIS3, caf4::KanMX, mdv1::MET25-TOM20(1-51aa)-hMiD51(47-463aa)*

Table S3. Plasmids used in this study

ID	Plasmid	Protein expressed
B363	*pRS415-DNM1*	Dnm1
B493	*pRS415-MET25*	none
B1607	*pRS415MET25-yFIS1*	yFis1
B1642	*p414GPD-mito-ffRFP*	*N. crassa* ATP9$^{1\text{-}69aa}$ + fast folding DsRed
B1816	*pRS416MET25-hDRP1*	hDrp1 (isoform 3)
B1808	*pRS415MET25-MDV1*	Mdv1
B2053	*pRS416MET25-MDV1*	Mdv1
B2729	*pRS415MET25-T20-MDV1^{FL}*	T20$^{1\text{-}51aa}$-Mdv1$^{1\text{-}714aa}$
B2731	*pRS415MET25-T20-MDV1^{CCWD}*	T20$^{1\text{-}51aa}$-mdv1$^{218\text{-}714aa}$
B2732	*pRS415MET25-T20-MDV1^{WD}*	T20$^{1\text{-}51aa}$-mdv1$^{317\text{-}714aa}$
B2821	*pRS415MET25-MDV1-3HA*	Mdv1-3HA
B2925	*pRS415MET25-T20-MDV1^{FL}-3HA*	T20$^{1\text{-}51aa}$-Mdv1$^{1\text{-}714aa}$-3HA
B2927	*pRS415MET25-T20- MDV1^{CCWD}-3HA*	T20$^{1\text{-}51aa}$-Mdv1$^{218\text{-}714aa}$-3HA
B2928	*pRS415MET25-T20- MDV1^{WD}-3HA*	T20$^{1\text{-}51aa}$-Mdv1$^{317\text{-}714aa}$-3HA
B2933	*pRS415MET25-T20-DNM1*	T20$^{1\text{-}51aa}$-Dnm1
B2934	*pRS415MET25-DNM1-yFIS1*	Dnm1-yFis1$^{131\text{-}155aa}$
B3090	*pRS415MET25-mOMGFP-yFIS1*	mOMGFP-yFis1$^{131\text{-}155aa}$
B3162	*pYSG-IBA167-hDRP1*	Flag-Strep-PP-hDrp1 (isoform 3)
B3237	*pRS415MET25- hDRP1-3HA*	hDrp1-3HA
B3238	*pRS415MET25-T20-hMiD49^{cyto}-3HA*	T20$^{1\text{-}51aa}$-hMiD49$^{48\text{-}454aa}$-3HA
B3239	*pRS415MET25-T20-hMiD51^{cyto}-3HA*	T20$^{1\text{-}51aa}$-hMiD51$^{47\text{-}463aa}$-3HA
B3244	*pRS415MET25-3HA-hMff^{cyto}-yFIS1*	3HA-hMff$^{1\text{-}198aa}$-yFis1$^{131\text{-}155aa}$
B3247	*pRS415MET25-3HA-hFIS1^{cyto}-yFIS1*	3HA-hFis1$^{1\text{-}119aa}$-yFis1$^{131\text{-}155aa}$
B3259	*pYSG-IBA167-hMiD49^{cyto}*	Flag-Strep-PP-hMiD49$^{48\text{-}454aa}$
B3262	*pYSG-IBA167-hMff^{cyto}*	Flag-Strep-PP-hMff $^{1\text{-}198aa}$
B3265	*pRS416CUP1-mGFP-hDRP1*	mGFP-hDrp1 (isoform 3)
B3294	*pYSG-IBA167-10HIS-hMiD49^{cyto}*	Flag-Strep-PP-10His-hMiD49 $^{48\text{-}454aa}$
B3357	*pMAL-c2x-hMff^{cyto}*	MBP-10xHIS-PP-hMff $^{1\text{-}198aa}$

T20 encodes yTom20, amino acids 1–51, N-terminal mitochondrial outer membrane anchor. yFIS1 encodes yFis1, amino acids 131–155, C-terminal mitochondrial outer membrane anchor. CCWD, coiled coil + WD repeat; cyto, cytoplasmic domain; h, human; FL, full length; mOMGFP, monomeric mitochondrial outer membrane GFP; PP, prescission protease cleavage site; T20, Tom20 membrane targeting domain; TM, transmembrane domain; WD, WD repeat; y, yeast.