Supporting Information

Lackner et al. 10.1073/pnas.1215232110

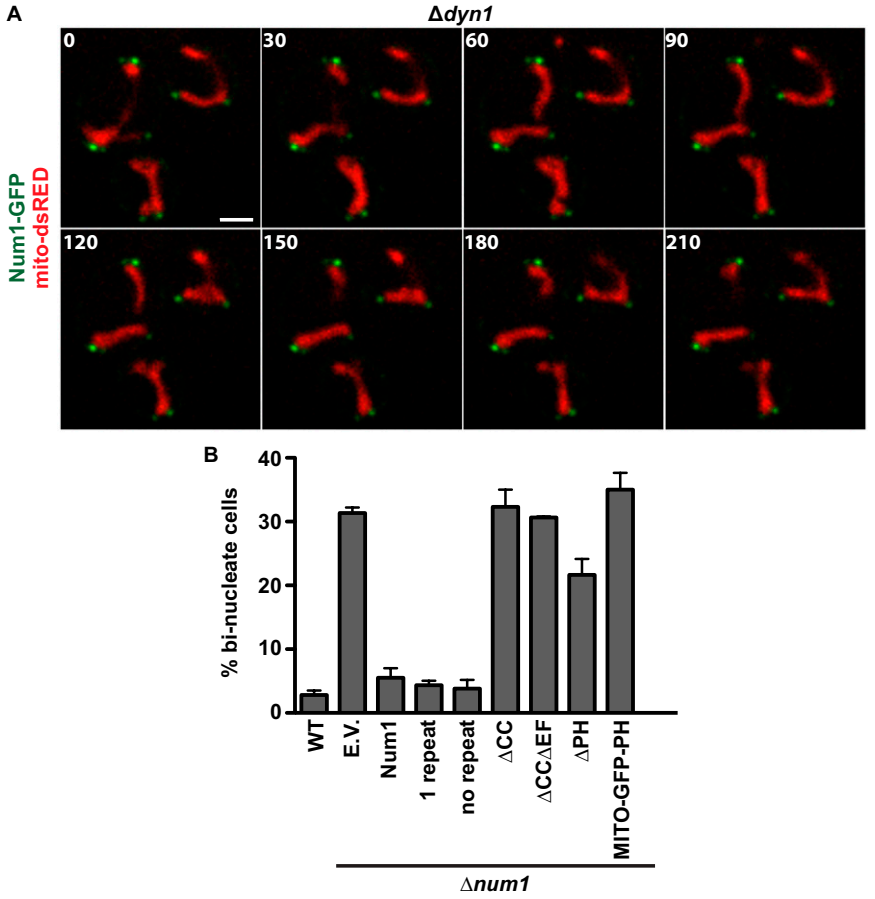


Fig. S1. The role of Num1 in Dyn1-mediated nuclear migration is distinct from its role in mitochondrial tethering and distribution. (*A*) Time-lapse images of Δ*dyn1* cells expressing Num1-GFP and mito-dsRED. A single focal plane is shown. Time is shown in seconds. (Scale bar, 2 μm.) (*B*) Quantification of the percent of binucleate cells observed, as assessed by DAPI staining, after growth of the indicated strains at 12 °C for 16 h. Data are shown as the mean ± SE of three independent experiments, in each of which 200 cells were counted. E.V., empty vector.

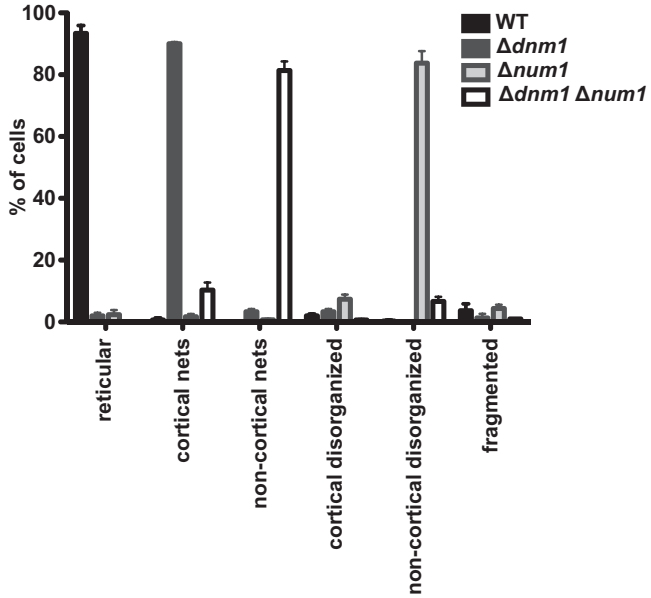


Fig. S2. Characterization and quantification of the mitochondrial-morphology defects observed in wild-type, Δ*dnm1*, Δ*num1*, and Δ*dnm1* Δ*num1* cells. The morphology of mitochondria in the indicated strains expressing mitochondrial matrix-targeted dsRED (mito-dsRED) was analyzed by fluorescence microscopy. Data are shown as the mean ± SE of three independent experiments, in each of which 100 cells were counted.

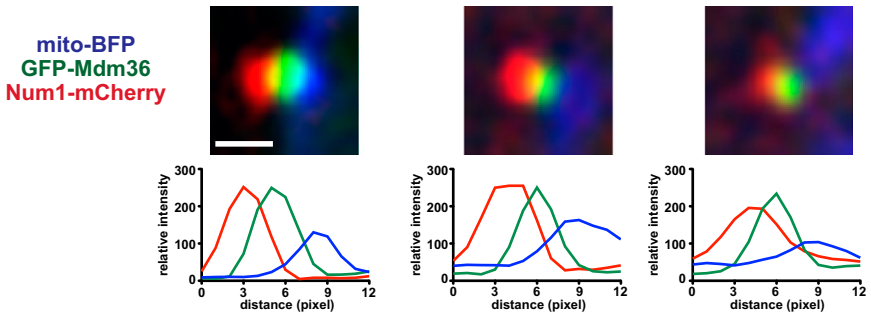


Fig. S3. Additional examples of the localization of Mdm36 foci relative to Num1 and mitochondria. Wild-type cells expressing mitochondrial-targeted blue fluorescent protein (mito-BFP), GFP-Mdm36, and Num1-mCherry were analyzed by fluorescence microscopy. A single focal plane is shown. The graphs show the relative fluorescence intensities of Num1 (red), Mdm36 (green), and mitochondria (blue) along a line drawn through the center of the Num1 and Mdm36 foci. (Scale bar, 500 nm.)

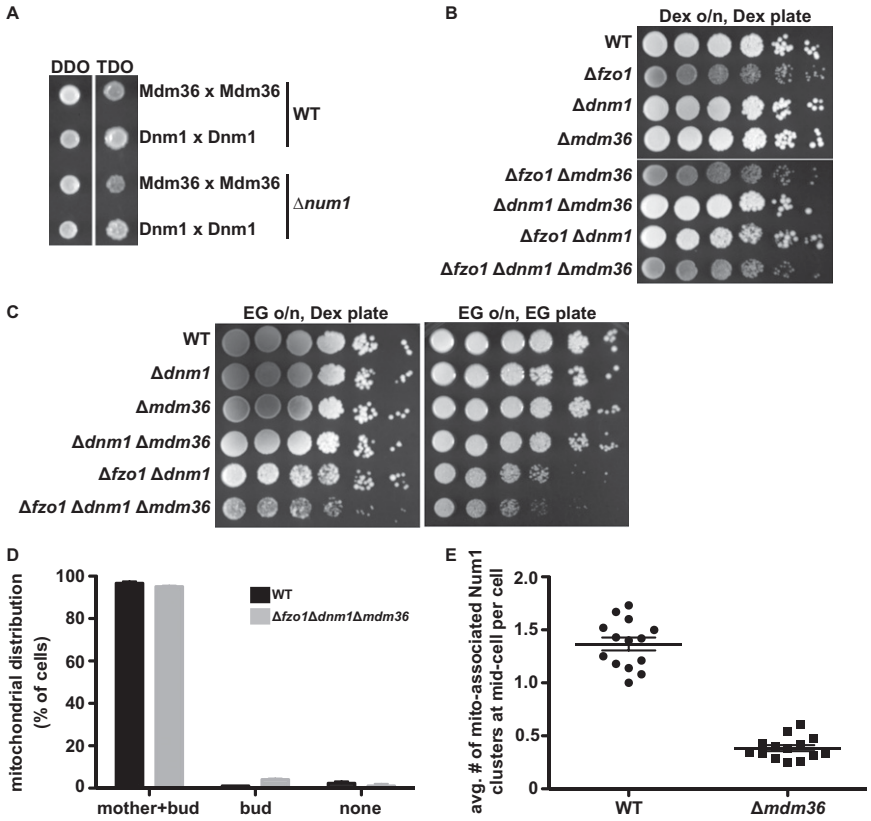


Fig. S4. Mdm36 is important but not essential for Num1-mediated mitochondrial tethering. (*A*) Yeast two-hybrid analysis to assess the interaction of a Gal4AD-Mdm36 fusion with a Gal4BD-Mdm36 fusion in the presence (WT) or absence (Δ*num1*) of Num1. Dnm1 self-interaction was used a positive control. Protein–protein interactions were assessed as in Fig. 5*D*. DDO, double dropout; TDO, triple dropout. (*B*) Δ*fzo1* Δ*dnm1* Δ*mdm36* cells exhibit a growth defect. After growth in yeast extract/peptone/dextrose (YPD) medium, serial dilutions of the indicated strains were plated on YPD medium and grown at 30 °C. (*C*) After growth in yeast extract/peptone/ethanol/glycerin (YPEG) medium to ensure that all cells possessed mtDNA at the start of the growth assay, serial dilutions of the indicated strains were plated on YPD or YPEG medium, as indicated, and were grown at 30 °C. (*D*) The distribution of mitochondria in Δ*fzo1* Δ*dnm1* Δ*mdm36* cells expressing mito-dsRED was analyzed by fluorescence microscopy after growth at 30 °C. Cells were stained with calcofluor to image septa and bud scars to distinguish mother and daughter cells. Data are shown as the mean ± SE of three independent experiments, in each of which >70 cells were counted. (*E*) The number of mitochondrial-associated Num1 clusters at midcell was quantified in wild-type and Δ*mdm36* cells expressing Num1-GFP and mito-dsRED. Each point represents the average number of mitochondrial-associated Num1 clusters found at midcell per cell in a field of ≥10 cells. A total of 14 fields and >200 cells were analyzed.

		Δ*mdm36* Num1-yEGFP
	Num1	340 ± 17 (67 ± 2)
PM/mito	Pil1	52 ± 4 (71 ± 3)
	Lsp1	27 ± 3 (52 ± 5)
PM	Seg1	17 ± 2 (20 ± 2)
	Eis1	10 ± 1 (15 ± 2)
	Msc3	27 ± 5 (35 ± 4)
	YGR130C	3 ± 0.3 (5 ± 4)
mito/ER	Rtn1	15 ± 0.3 (40 ± 3)
ER	Scs2	6 ± 1 (26 ± 1)
nucleus/ nucleolous	Nop 7	4 ± 2 (7 ± 4)
	Rpf4	2 ± 0.3 (7 ± 2)
	Nop16	2 ± 1 (8 ± 4)
unknown	Dcr2	1 ± 0 (1 ± 0)

Fig. S5. Proteomic analysis of proteins that copurify with Num1-GFP in the absence of Mdm36. Proteomic analysis of Num1-GFP immunoprecipitated from Δ*mdm36* cells as described in *Methods*. The number of peptides (and percent coverage) is shown for each identified protein. Data are shown as the mean ± SE of four independent experiments.

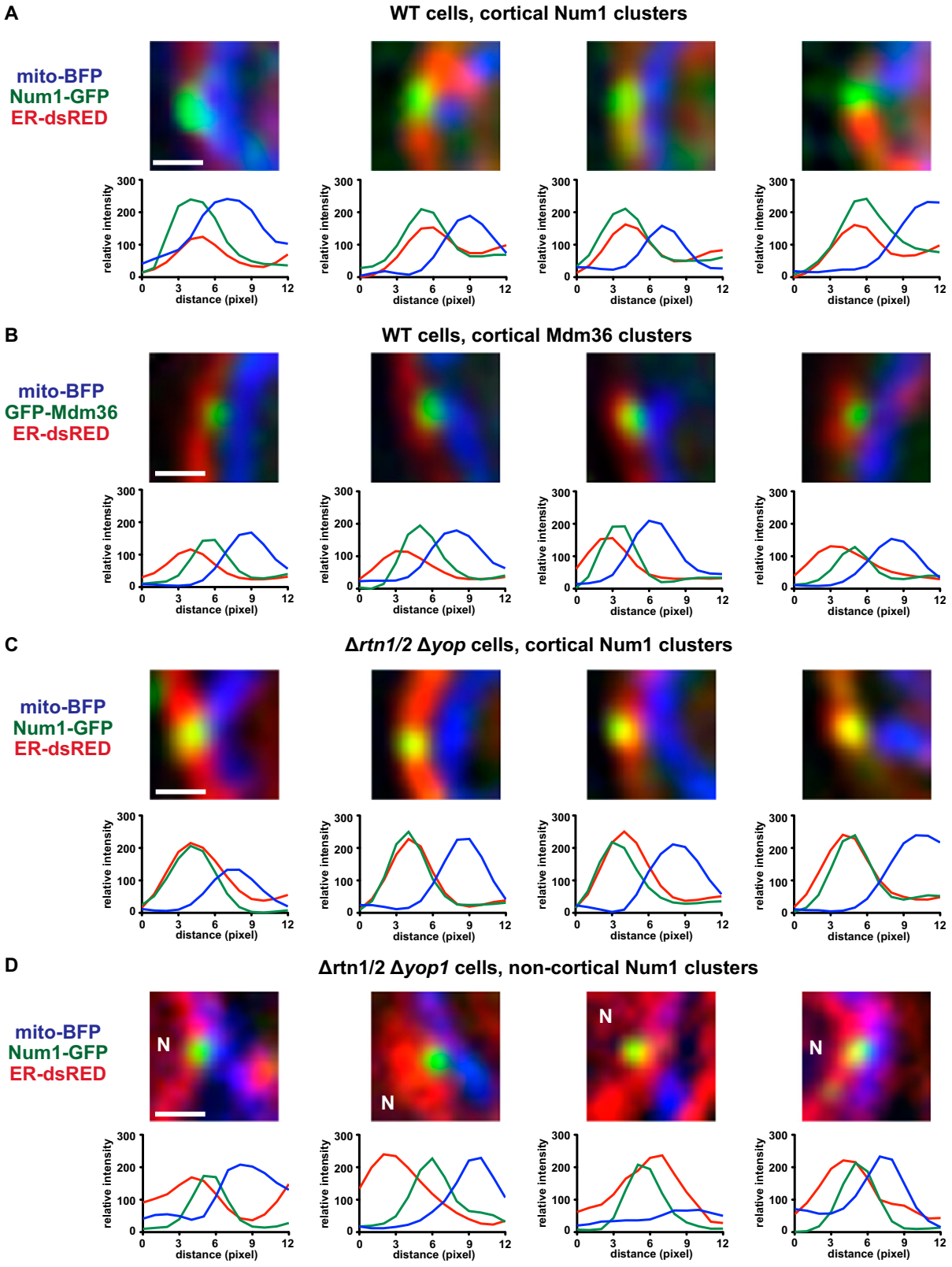
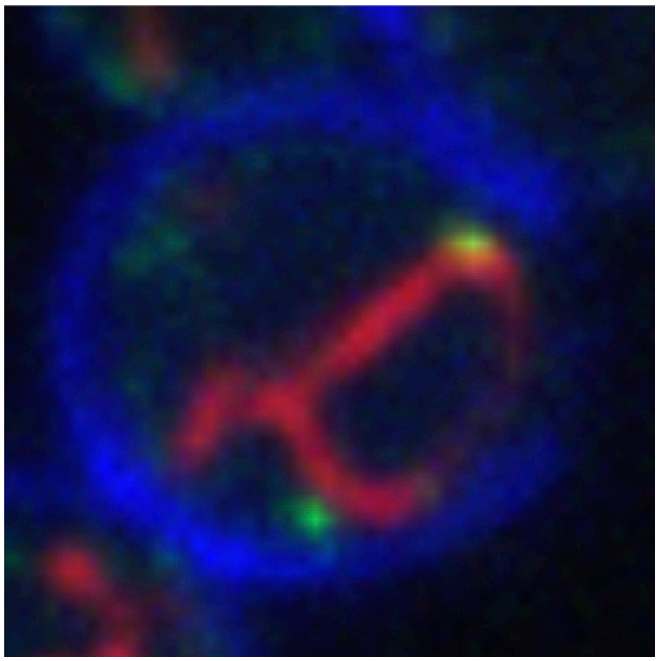


Fig. S6. Additional examples of Num1 and Mdm36 localization relative to the endoplasmic reticulum (ER) and mitochondria. (*A*) Wild-type cells expressing Num1-GFP, mito-BFP, and ER-dsRED were analyzed by fluorescence microscopy. The graphs show the relative fluorescence intensities of the ER (red), Num1 (green), and mitochondria (blue) along a line drawn through the center of the Num1 cluster. (*B*) Wild-type cells expressing GFP-Mdm36, mito-BFP, and ER-dsRED were analyzed by fluorescence microscopy. The graphs show the relative fluorescence intensities of the ER (red), Mdm36 (green), and mitochondria (blue) along a line drawn through the center of the Mdm36 foci. (*C* and *D*) Δ*rtn1/2* Δ*yop1* cells expressing Num1-GFP, mito-BFP, and ER-dsRED were analyzed as described in *A*. In *D*, the nucleus is marked with an "N" for spatial reference. In all panels, a single focal plane is shown. (Scale bars, 500 nm.)



Movie S1. Num1-marked mitochondria–cortex anchor points. Movie of time-lapse images shown in Fig. 2*A*. (Scale bar, 2 μm.)

Movie S1