Supporting Information

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SI Materials and Methods

Primers Used for Site-Directed Mutagenesis. Primers used for site-directed mutagenesis were as follows: Drp1_S637A_f, 5′-agttcctgttgcacgaaaactagctgctcgggaac-3′; Drp1_S637A_r, 5′-gttcccgagcagctagttttcgtgcaacaggaact-3′; Drp1_S616E_f, 5′-cccattccaattatgccagccgagccacaaaaaggtcatgccgtg-3′; and Drp1_S616E_r, 5′-cacggcatgacctttttgtggctcggctggcataattggaatggg-3′.

Antibody Purchasing Information. Antibodies were purchased from the following vendors: anti-Hsp60 and anti-pS616-Drp1 from Cell Signaling; anti-Tom20, anti-Drp1, and Opa-1 from BD Biosciences; anti-Mfn1 and anti-Mfn2 from Abcam; anti-tubulin from Sigma-Aldrich; anti-chicken HRP from Aves Labs; and anti-rabbit HRP and anti-mouse HRP from Jackson ImmunoResearch.

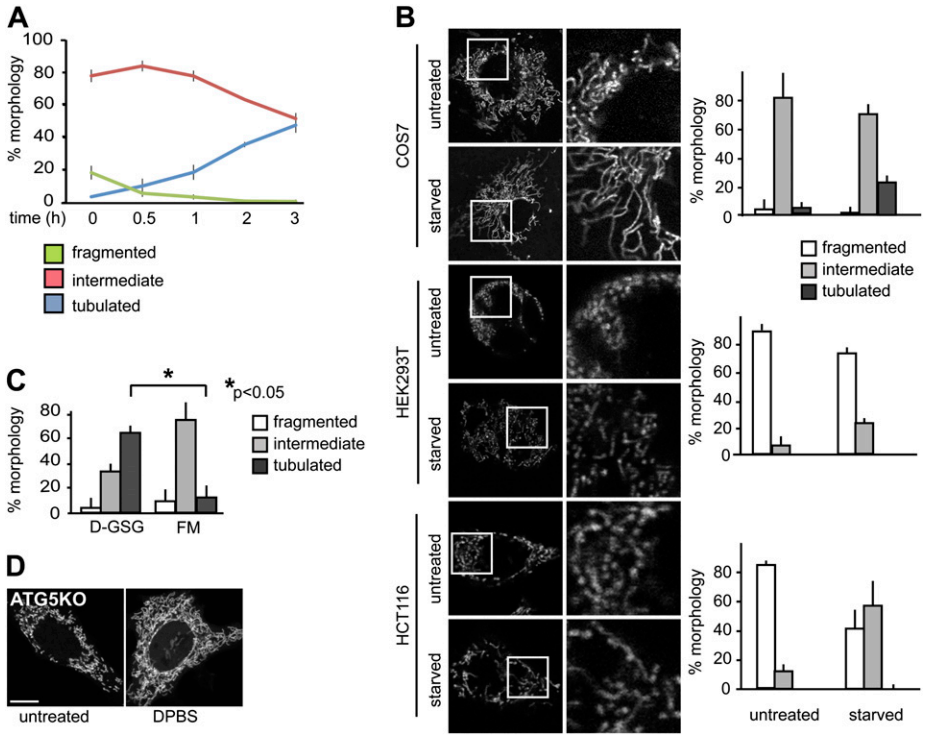

Fig. S1. Starvation induces mitochondrial tubulation rapidly, reversibly, and in several cell types. (*A*) Mouse embryonic fibroblast (MEF) cells were transfected with mitochondrially targeted YFP (mito-YFP) and, after 16 h, were starved with Dulbecco's PBS (DPBS). Mitochondrial morphology was assessed in live cells at the time points indicated. (*B*) COS7, HEK293T, and HCT116 cells were transfected with mitochondrially targeted monomeric red fluorescent protein (mito-RFP) and, after 16 h, were starved in medium containing 1 g/L glucose, no glutamine, and no serum (D-GSG) for 2 h. Images in the second column are magnified views of the boxed areas in the first column. (*C*) MEF cells were starved 16 h after mito-YFP transfection with D-GSG for 2 h then replenished back to full medium (FM) with serum, glucose, and glutamine for 2 h before quantification. (*D*) ATG5KO cells were transfected with mito-YFP and starved for 2 h in DPBS and imaged live. (*A–D*) Mitochondrial morphology was determined in live cells and scored as follows: fragmented, mainly small and round; intermediate, mixture of round and shorter tubulated; and tubulated, long and higher interconnectivity. The percentage of cells with indicated mitochondrial morphologies was determined as a percentage of the total number of transfected cells counted (≥100 cells per experiment). n = 3 independent experiments. (Scale bar: 15 μm.)

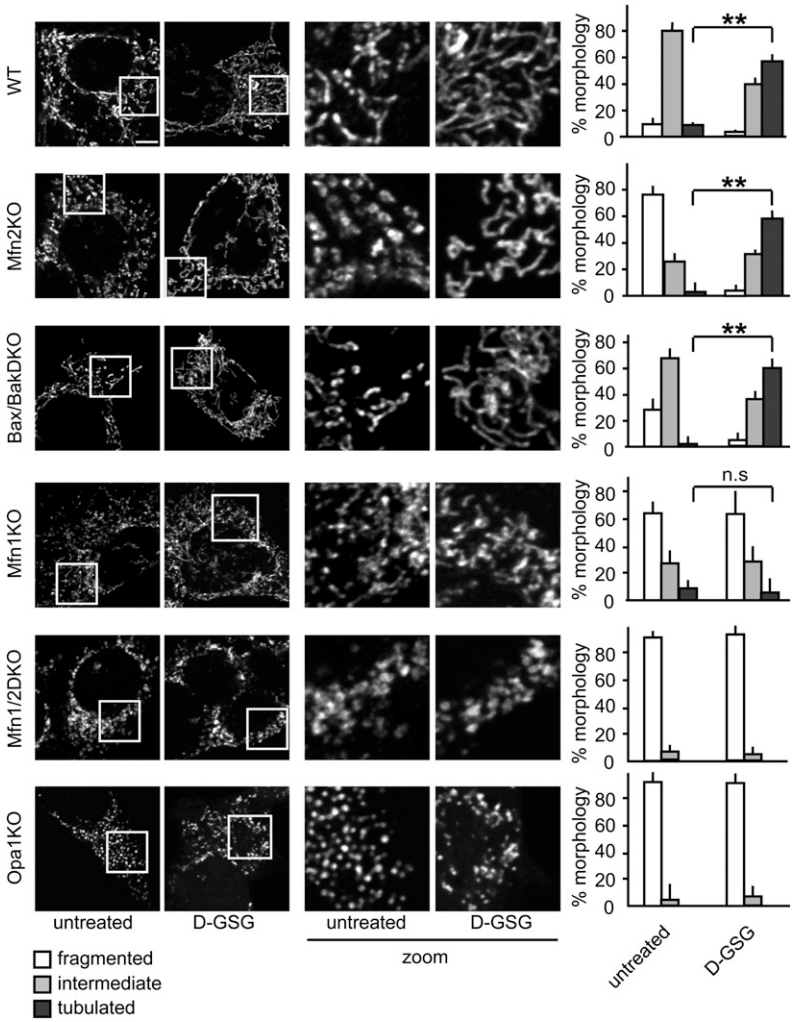

Fig. S2. The core fusion machinery is necessary for starvation-induced mitochondrial tubulation. The indicated MEF cell lines were starved in D-GSG for 2 h, fixed, and immunostained against Hsp60. Images were acquired under nonstarved (untreated) and D-GSG conditions. For better visualization of the mitochondrial morphology, higher-magnification images are provided. (Images in the third and fourth columns are magnified views of the boxed areas in the first and second columns.) Mitochondrial morphology was scored and quantified as described in Fig. S1 with $n = 5$ (**$P < 0.005$; n.s. not significant). (Scale bar: 10 μm.)

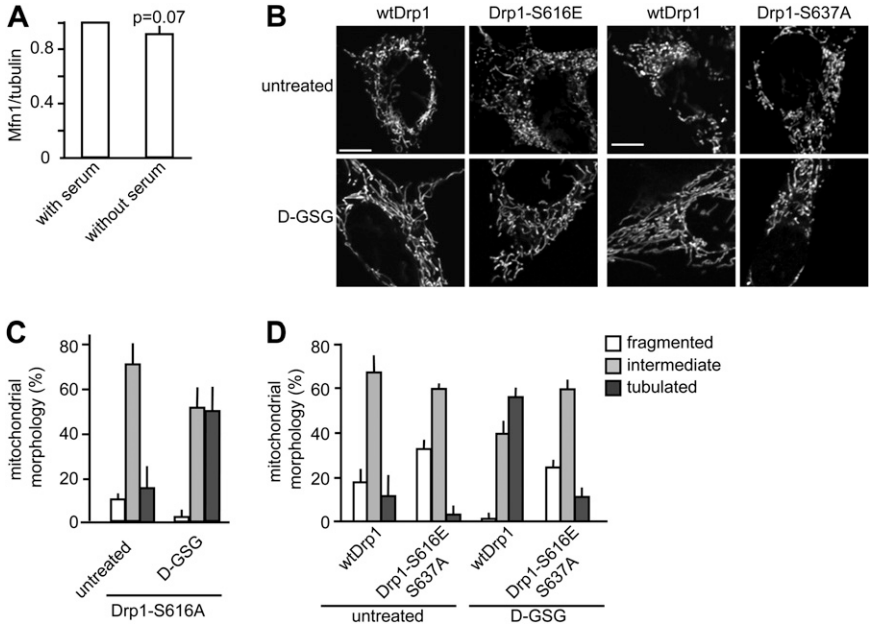

Fig. S3. Mitochondrial tubulation upon nutrient deprivation is influenced by two Drp1 posttranslational modifications. (*A*) The level of Mfn1 protein in MEF cells measured by immunoblot analysis after 6 h of serum starvation was quantified (n = 3) and determined to be not significant. (*B–D*) MEF cells were transfected with YFP-tagged wtDrp1 or Drp1-S616E or Drp1-S637A, Drp1-S616A and Drp1-S616E/S637A as indicated. At 16 h after transfection, cells were starved for 2 h with D-GSG and imaged live. Mitochondrial morphology was determined as described in Fig. S1.

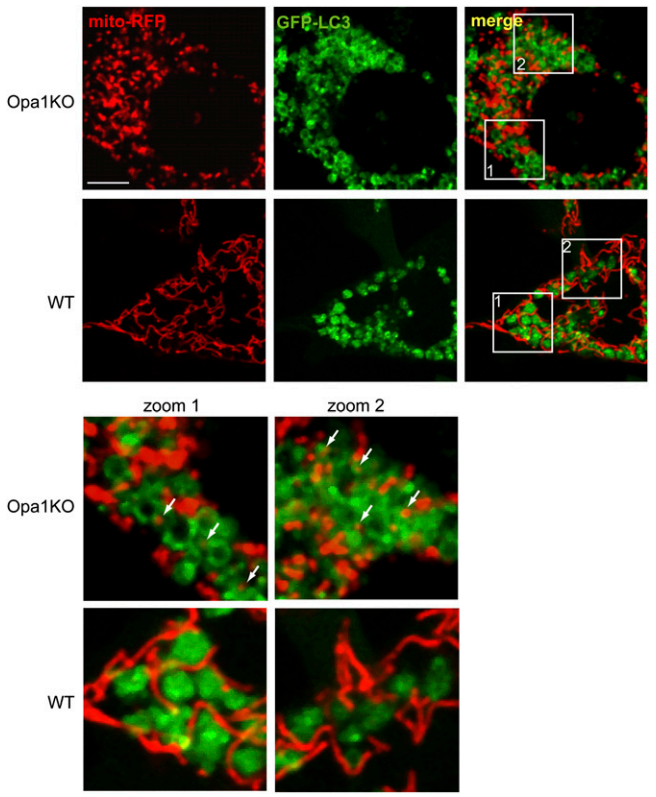

Fig. S4. Mitochondrial tubulation protects against mitophagy. WT and Opa1KO MEFs were transfected with GFP-LC3 and mito-RFP, and, after 16 h, cells were starved with HBSS in the presence of 200 nM bafilomycin A1 to inhibit the autophagosomal turnover. Live-cell images were acquired after 5 h of starvation. (Scale bar: 10 μm.)