Yamashita et al., https://doi.org/10.1083/jcb.201605093

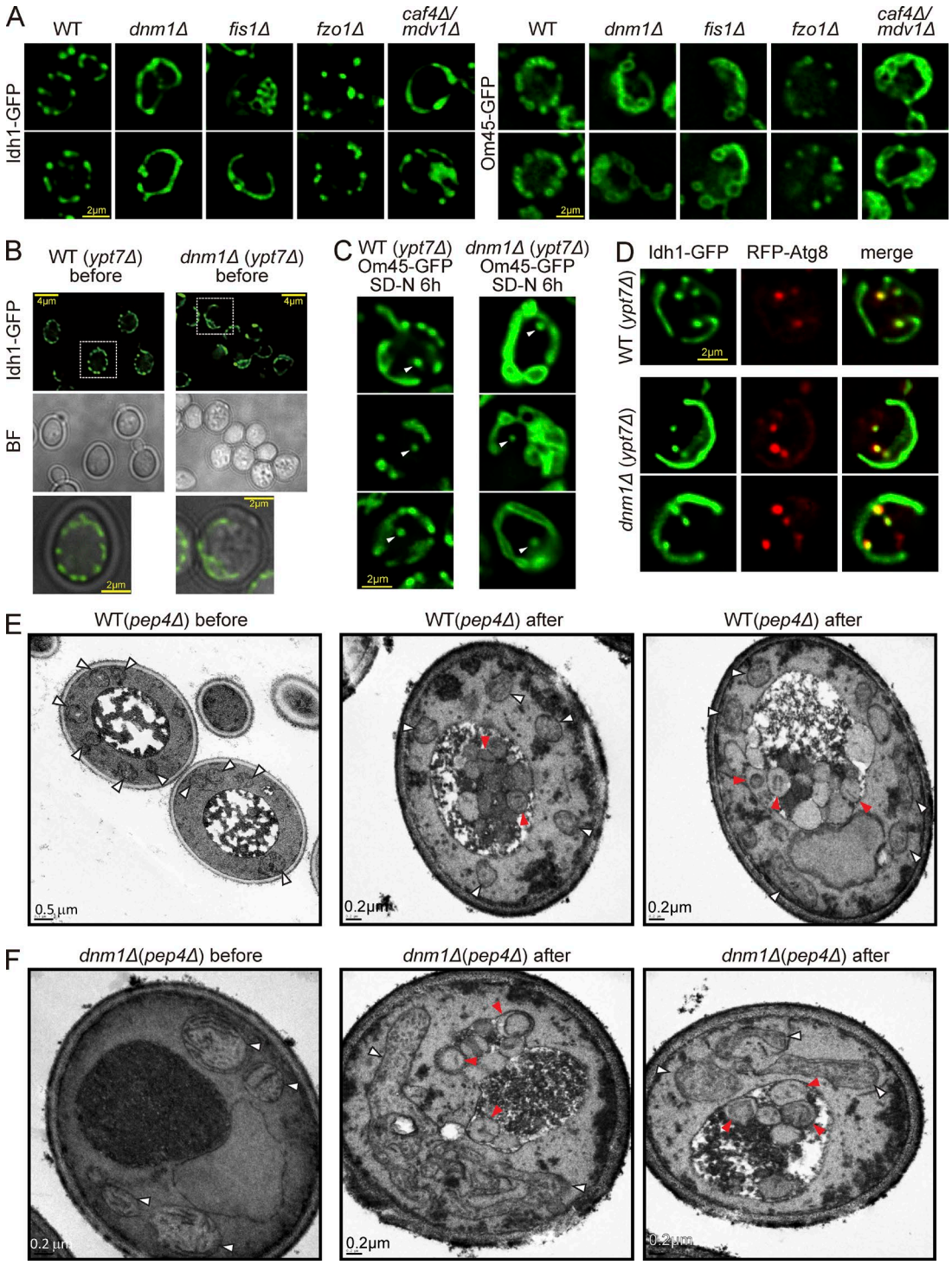

Figure S1. **Dnm1-independent mitochondrial division occurs during mitophagy in yeast.** (A and B) The indicated mutant cells expressing Idh1-GFP or Om45-GFP were cultured in YPL medium until the mid-log growth phase. The morphology of mitochondria was observed by fluorescence microscopy. (C) The indicated cells expressing Om45-GFP were cultured in YPL medium until the mid-log growth phase and then shifted to SD-N medium for 6 h. GFP signals were observed by fluorescence microscopy. Arrowheads indicate mitochondria presumed to be within the mitophagosome. (D) The indicated cells expressing Idh1-GFP and RFP-Atg8 were cultured in SML-Ura medium until the mid-log growth phase and then shifted to SD-N medium for 6 h. GFP and RFP signals were observed by fluorescence microscopy. (E and F) The indicated cells were cultured in YPL medium until the mid-log growth phase and then shifted to SD-N medium for 6 h. Cells were collected and treated for EM and observed with a transmission electron microscope. White arrowheads indicate cytosolic mitochondria, and red arrowheads indicate mitophagic bodies.

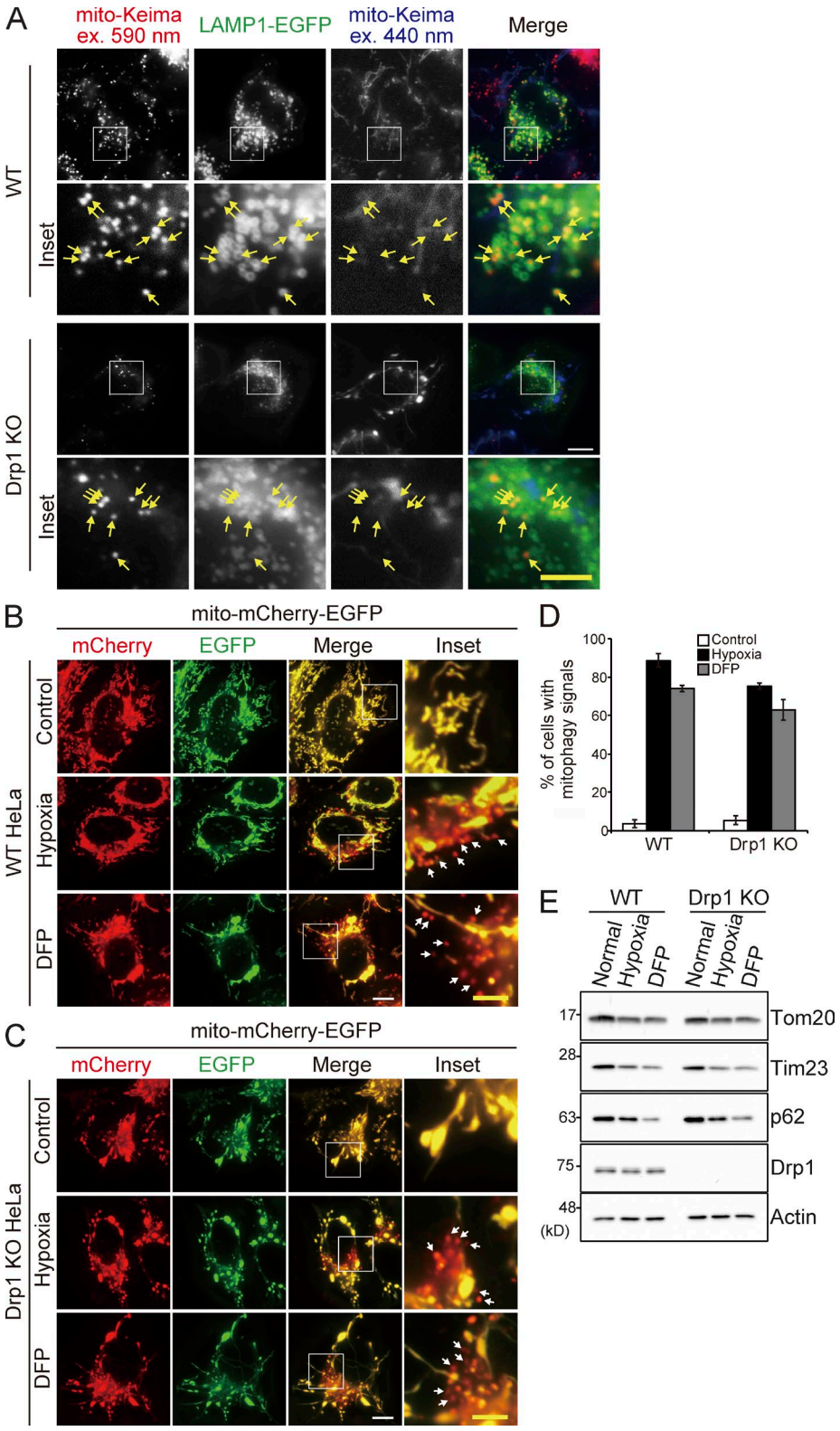

Figure S2. **Hypoxia-induced and DFP-induced mitophagy can be observed by tandem fluorescent protein reporter and immunoblot analysis in Drp1 KO HeLa cells.** (A) WT and Drp1 KO cells stably expressing mito-Keima were transfected with LAMP1-EGFP expression vector and cultured under hypoxic condition for 24 h. Colocalization of mito-Keima dots excited by 590-nm light and LAMP1-EGFP are indicated by yellow arrows. (B–D) WT (B) and Drp1 KO cells (C) expressing mito-mCherry–EGFP were cultured under the condition of mitophagy as in Fig. 2 A and analyzed by fluorescence microscopy. Mitophagy signals shown as punctate mCherry signals without EGFP are indicated by white arrows. Bars: (merged view) 10 µm; (inset) 5 µm. (D) The cells undergoing mitophagy that have >10 mitophagy signals were calculated from at least 50 cells under control, hypoxic, and DFP treatment conditions as shown in B and C. Data are shown as the mean ± SD of three independent experiments. (E) WT and Drp1 KO HeLa cells were cultured under hypoxic conditions or normal conditions in the presence of 1 mM DFP for 24 h. The cells were then lysed and analyzed by immunoblotting with anti-Tom20, anti-Tim23, anti-p62, anti-Drp1, and anti-actin (loading control) antibodies.

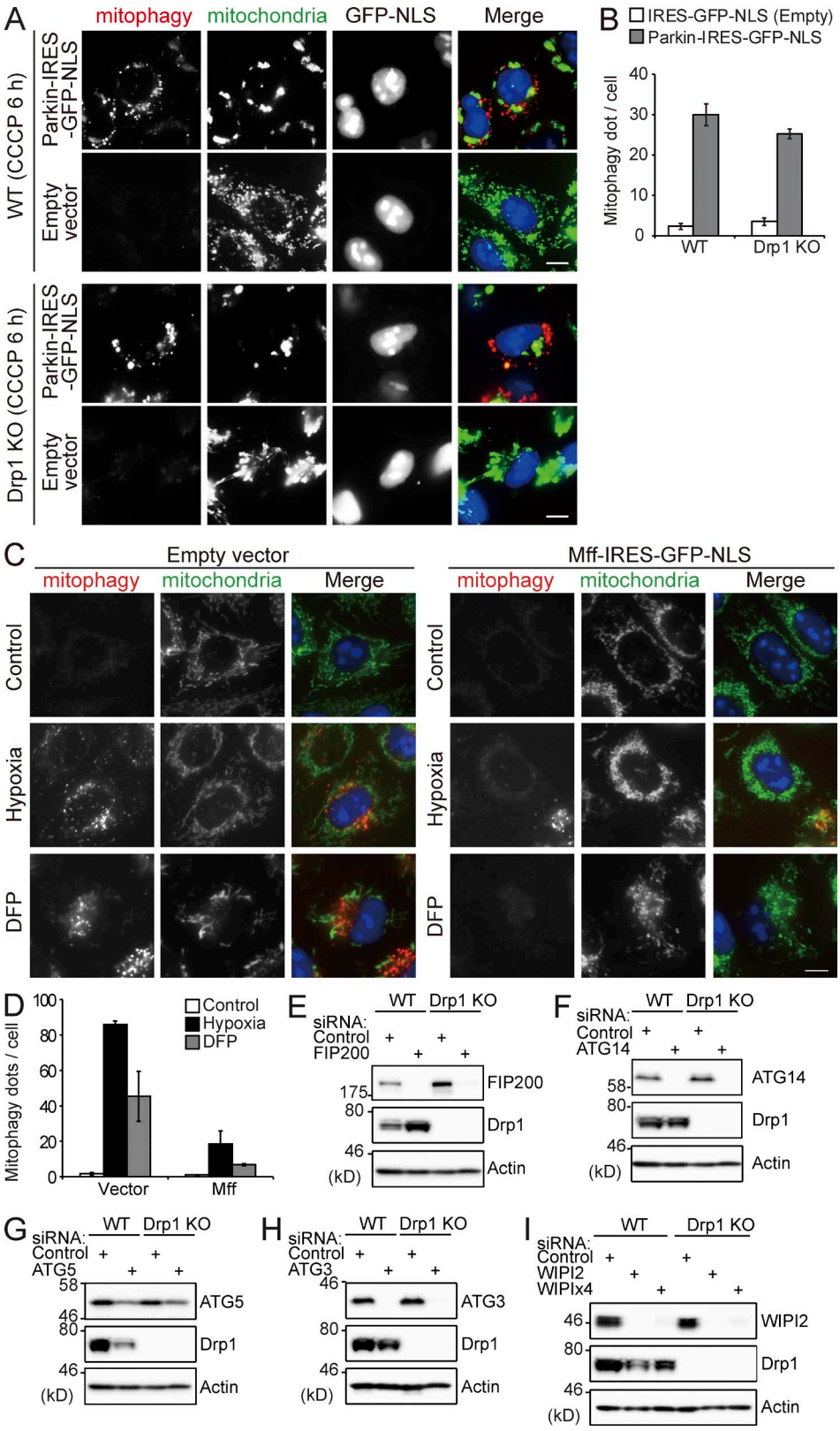

Figure S3. **Mitochondrial fragmentation does not enhance mitophagy flux, and Drp1 is dispensable for Parkin-mediated mitophagy.** (A and C) WT and Drp1 KO HeLa cells stably expressing mito-Keima were transfected with Parkin-IRES-GFP-NLS or IRES-GFP-NLS (empty vector; A) or with Mff-IRES-GFP-NLS or empty vector (C) and cultured under the condition of mitophagy. Parkin-expressing cells were cultured with 10 µM CCCP for 6 h. Mff-expressing cells were cultured as in Fig. 2 A. Cells were analyzed by fluorescence microscopy. Bars, 10 µm. (B and D) Mitophagy dots shown in A and C, respectively, were calculated as in Fig. 2 C. Data are shown as the mean ± SD of three independent experiments. (E–I) WT and Drp1 KO HeLa cells were transfected with siRNAs against FIP200, ATG14, ATG5, ATG3, WIPI2, and WIPI family genes (*WIPI1*, *WIPI2*, *WDR45L*, and *WDR45*) and cultured as described in Materials and methods. The cells were lysed and analyzed by immunoblotting with antibodies against FIP200, Drp1, ATG14, ATG5, ATG3, WIPI2, and actin.

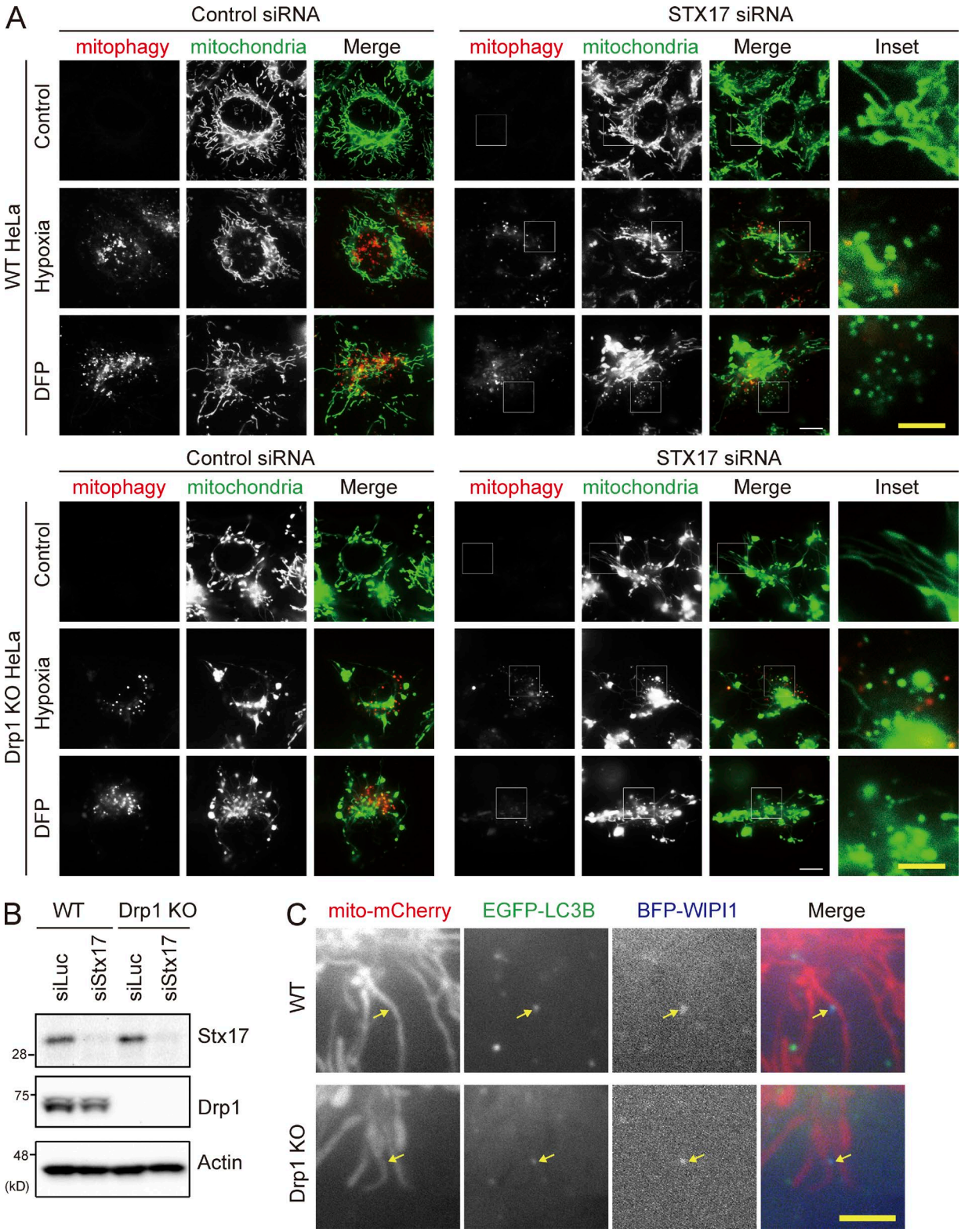

Figure S4. **Drp1-independent mitochondrial fragmentation appears under the condition of mitophagy in HeLa cells.** (A) WT and Drp1 KO cells expressing mito-Keima were transfected with control and STX17 siRNAs and cultured under the condition of mitophagy as in Fig. 2 A. Bars: (merged views) 10 µm; (insets) 5 µm. (B) The cells transfected with control and STX17 siRNAs were lysed and analyzed by immunoblotting with anti-STX17, anti-Drp1, and anti-actin antibodies. (C) WT and Drp1 KO cells expressing mito-mCherry, EGFP-LC3B, and BFP-WIPI1 were cultured in the presence of 1 mM DFP for 12 h and analyzed by fluorescence microscopy. Colocalization of EGFP-LC3B with BFP-WIPI1 on the mitochondria is indicated by yellow arrows. Bar, 5 µm.

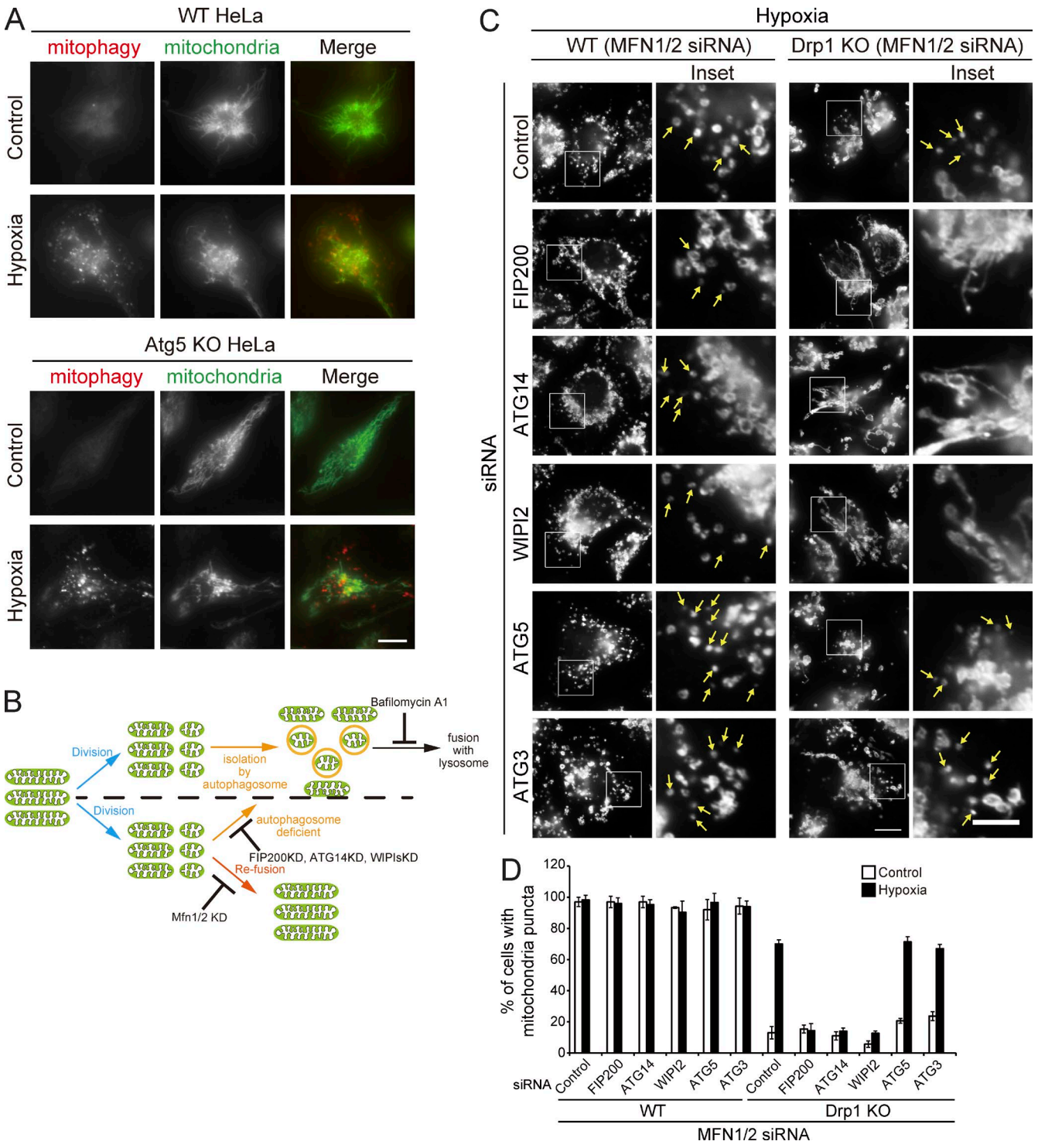

Figure S5. **Drp1-independent mitochondrial division in mitophagy requires isolation membrane extension, even in MFN knockdown cells.** (A) ATG5 KO HeLa cells expressing mito-Keima were cultured under hypoxic conditions for 24 h. Cells were then analyzed by fluorescence microscopy as in Fig. 2 A. (B) Schematic representation of the strategy for analysis of whether mitochondrial division in mitophagy only requires isolation membrane extension in Drp1 KO cells. (C) WT and Drp1 KO HeLa cells were transfected with *MFN1/2* siRNAs and siRNAs against the indicated genes, and mitochondrial morphology was monitored upon hypoxia-induced mitophagy as in Fig. 9 A. Small mitochondrial puncta are indicated by yellow arrows in insets. Bars: (main images) 10 µm; (insets) 5 µm. (D) Percentages of cells with small mitochondrial puncta were calculated as in Fig. 9 B. Data are shown as the mean ± SD of three independent experiments.

Table S1. **List of *S. cerevisiae* strains used in this study**

Strain	Genotype	Source
BY4742	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Open Biosystems
TKYM94	BY4742 *Idh1-GFP::HIS3MX6*	This study
KSY66	BY4742 *atg11Δ::KanMX6 Idh1-GFP::HIS3MX6*	This study
KSY153	BY4742 *fis1Δ::KanMX6 Idh1-GFP::HIS3MX6*	This study
KSY154	BY4742 *fzo1Δ::KanMX6 Idh1-GFP::HIS3MX6*	This study
KSY155	BY4742 *pep4Δ::KanMX6*	This study
KSY158	BY4742 *pep4Δ::KanMX6 dnm1Δ::LEU2*	This study
KSY161	BY4742 *dnm1Δ::KanMX6 Idh1-GFP::HIS3MX6*	This study
KSY165	BY4742 *mdv1Δ::KanMX6 caf4Δ::LEU2 Idh1-GFP::HIS3MX6*	This study
KSY181	SEY6210 *pep4Δ:: LEU2*	This study
KSY182	SEY6210 *pep4Δ:: LEU2 dnm1Δ::KanMX6*	This study
SEY6210	MATα *his3-Δ200 leu2-3,112 lys2-801 trp1-Δ901 ura3-52 suc2-Δ9 GAL*	Robinson et al., 1988
YKF100	BY4742 *ypt7Δ::LEU2 Idh1-GFP::HIS3MX6*	This study
YKF101	BY4742 *atg11Δ::KanMX6 ypt7Δ::LEU2 Idh1-GFP::HIS3MX6*	This study
YKF102	BY4742 *dnm1Δ::KanMX6 ypt7Δ::LEU2 Idh1-GFP::HIS3MX6*	This study
TKYM280	BY4742 *Om45-GFP::HIS3MX6*	Aoki et al., 2011
YKF117	BY4742 *dnm1Δ::KanMX6 Om45-GFP::HIS3MX6*	This study
YKF118	BY4742 *fis1Δ::KanMX6 Om45-GFP::HIS3MX6*	This study
YKF119	BY4742 *fzo1Δ::KanMX6 Om45-GFP::HIS3MX6*	This study
YKF122	BY4742 *caf4Δ::LEU2 mdv1Δ::KanMX6 Om45-GFP::HIS3MX6*	This study
YKF124	BY4742 *ypt7Δ::LEU2 Om45-GFP::HIS3MX6*	This study
YKF126	BY4742 *dnm1Δ::KanMX6 ypt7Δ::LEU2 Om45-GFP::HIS3MX6*	This study

Table S2. **List of *P. pastris* strains used in this study**

Strain	Genotype	Source
PPY12	*arg4 his4*	Sakai et al., 1998
MAYP6	PPY12 *Idh1-GFP::ARG4*	Aihara et al., 2014
MAYP14	PPY12 *atg11Δ::Zeocin^r Idh1-GFP::ARG4*	Aihara et al., 2014
P21	PPY12 *dnm1Δ::Zeocin^r Idh1-GFP::ARG4*	This study
P22	PPY12 *fzo1Δ::Zeocin^r Idh1-GFP::ARG4*	This study

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