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Supplemental Material to:

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The accumulation of misfolded proteins in the mitochondrial matrix is sensed by PINK1 to induce PARK2/Parkin-mediated mitophagy of polarized mitochondria

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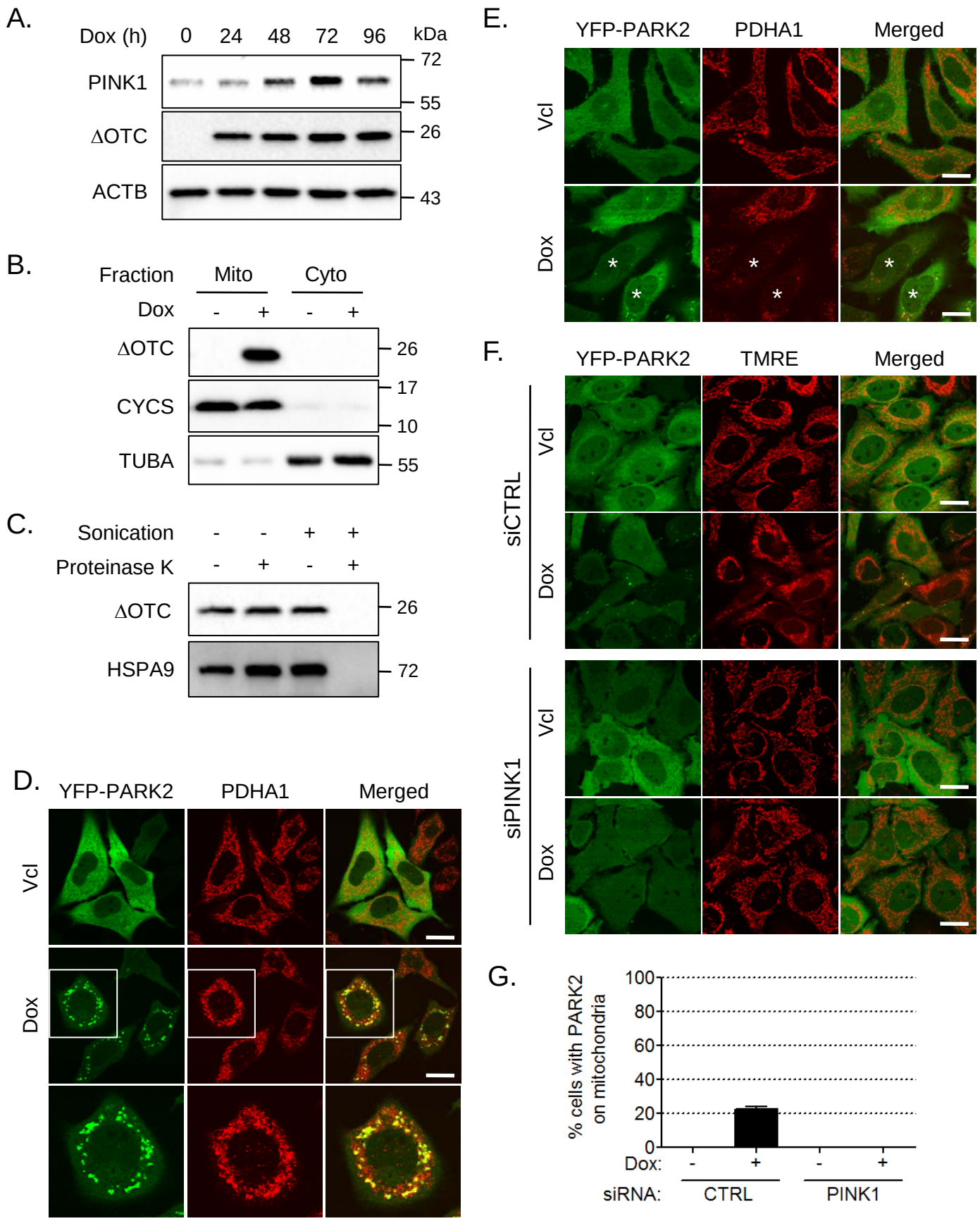
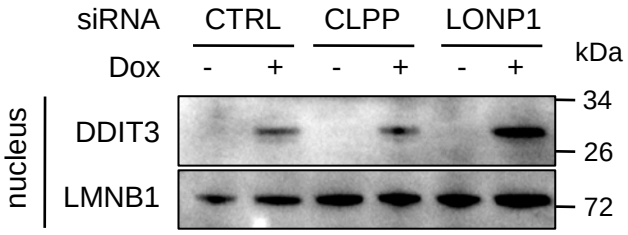
Supplementary Fig.1



Supplementary Fig.2



Supplementary Fig.3

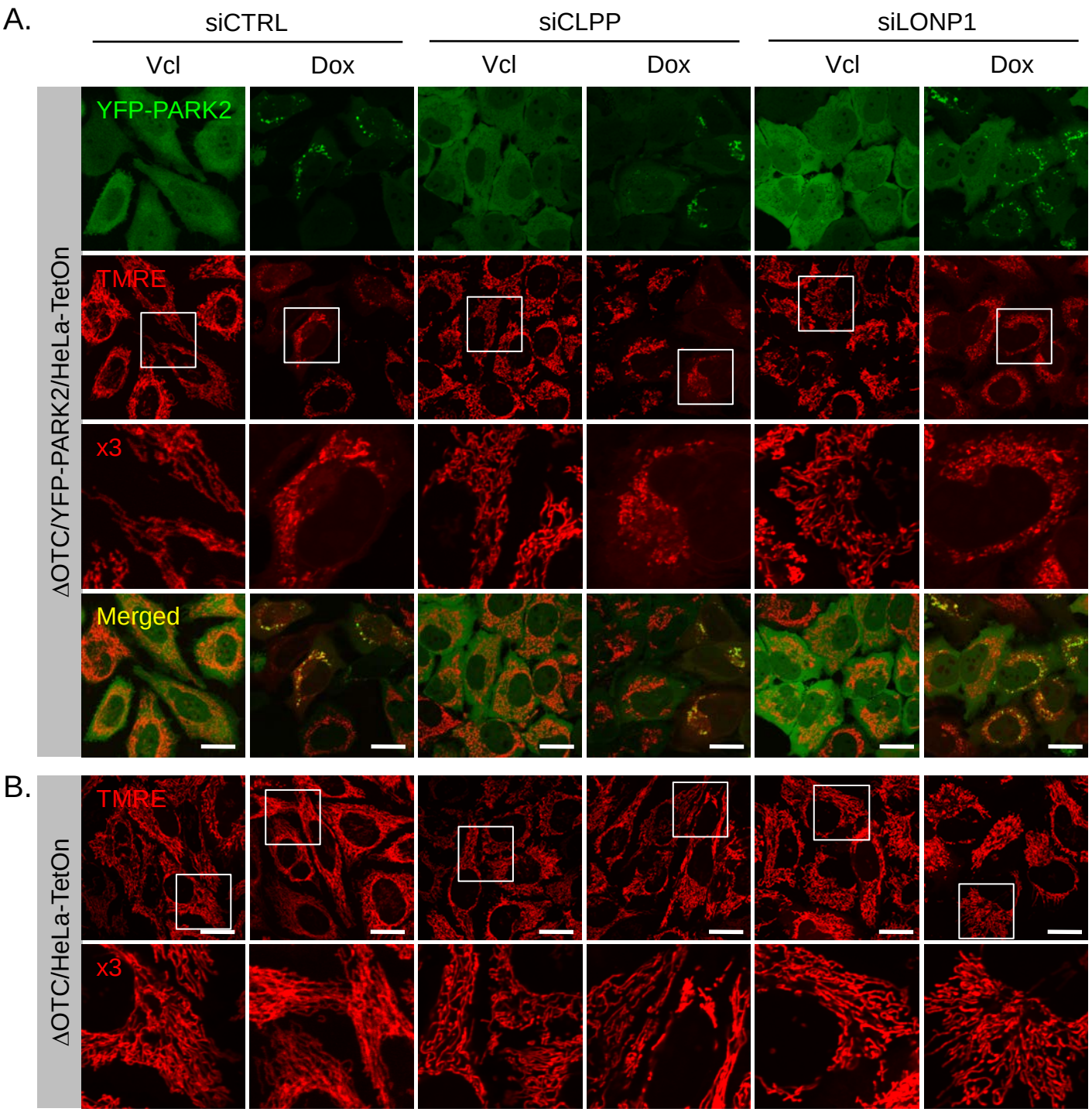


Figure S1. ΔOTC expression in ΔOTC/HeLa-TetOn stable cell line induces PINK1 accumulation, PINK1-dependent PARK2 translocation and mitophagy. (**A**) Time course of PINK1 and ΔOTC expression in the ΔOTC/HeLa-TetOn cell line following addition of Dox (1 μg/ml). ACTB served as a loading control. (**B**) ΔOTC/HeLa-TetOn cells were treated with Dox (1 μg/ml) for 72 h and fractionated as described in Materials and Methods. Twenty μg of each fraction was analyzed by western blotting with the indicated antibodies. (**C**) Aliquots of mitochondrial fraction from (**B**). (**D and E**) PARK2 translocation (**D**) and mitophagy (**E**) in the ΔOTC/YFP-PARK2/HeLa-TetOn stable cell line. Cells were treated with Dox (1 μg/ml) for 72 h for PARK2 translocation (**D**) and 96 h for mitophagy (**E**) and immunostained with anti-PDHA1 antibody as described in Materials and Methods. Scale bars, 20 μm. In (**B**), White boxes in the middle panel were magnified in the bottom panel. (**F and G**) ΔOTC/YFP-PARK2/HeLa-TetOn stable cells were transfected with nontargeting control siRNA (siCTRL) or *PINK1* siRNA (siPINK1) for 24 h, treated with Dox (1 μg/ml) for 72 h, and analyzed for PARK2 translocation. Scale bars, 20 μm (**F**).

Figure S2. The knockdown of *CLPP* or *LONP1* without ΔOTC expression does not induce DDIT3 expression. ΔOTC/HeLa-TetOn stable cells were transfected with nontargeting control (CTRL), *CLPP*, or *LONP1* siRNAs. After 24 h, cells were treated with Dox (1 μg/ml) for 72 h and fractionated. The level of DDIT3 in the nuclear fraction was analyzed by western blotting. LMNB1 was used as a loading control.

Figure S3. Mitochondrial fragmentation upon ΔOTC expression requires PARK2. (**A and B**) ΔOTC/YFP-PARK2/HeLa-TetOn (**A**), ΔOTC/HeLa-TetOn (**B**) or stable cell lines were transfected with nontargeting control (CTRL), *CLPP*, or *LONP1* siRNAs. After 24 h, cells were treated with Dox (1 μg/ml) for 72 h, stained with TMRE for 30 min, and imaged by confocal microscopy. Scale bars, 20 μm. White boxes were 3-fold magnified in the next panel.