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# Supplemental Material to:

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## Choline dehydrogenase interacts with SQSTM1/p62 to recruit LC3 and stimulate mitophagy

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**Supplementary Figures**

**Fig. S1**

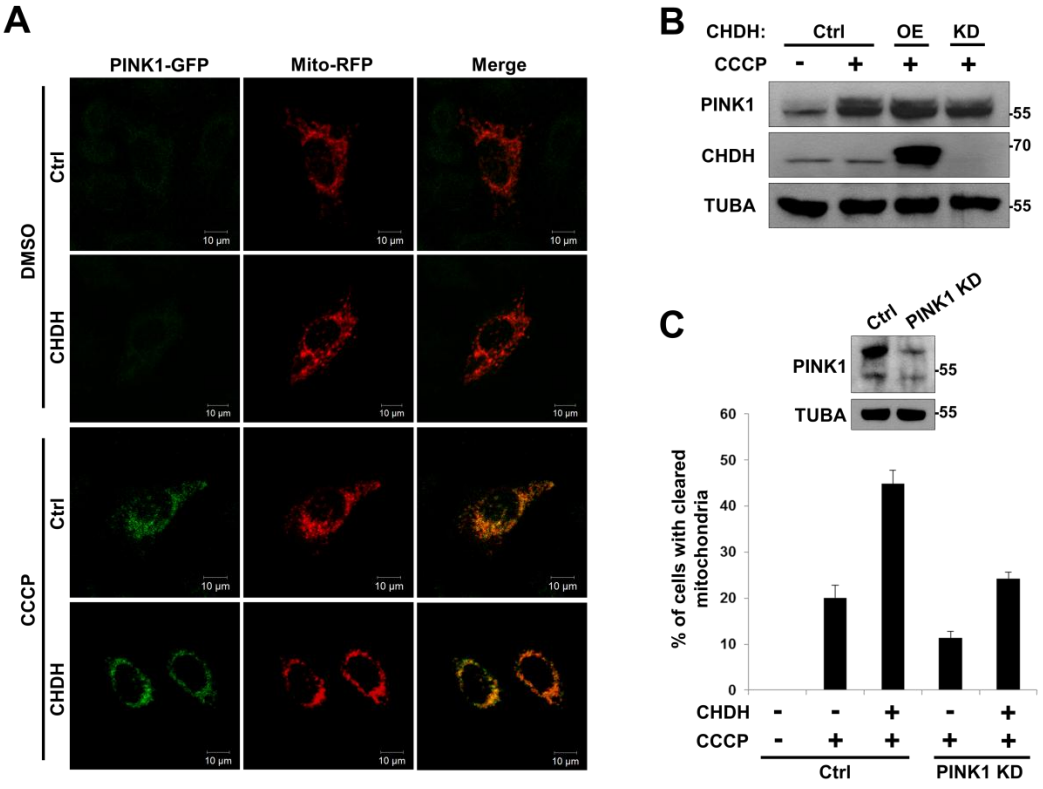


**Figure S1.** Effects of CHDH expression on PINK1-mediated mitophagy. (**A**) HeLa-Ctrl and HeLa-CHDH cells were cotransfected with PINK1-GFP and Mito-RFP and then exposed to 10 μM CCCP for 2 h, after which cells were examined under a confocal microscope. (**B**) HeLa-control (Ctrl), CHDH overexpression (OE) and sh*CHDH* knockdown (KD) stable cells were exposed to 10 μM CCCP for 2 h. Cell extracts were analyzed with western blotting. (**C**) SN4741 control (Ctrl) and PINK1 knockdown stable cells were transfected with CHDH, Mito-RFP and control GFP for 24 h and then left untreated or treated with 20 μM CCCP for 24 h. Mito-RFP-negative cells among total GFP-positive cells were counted under a fluorescence microscope. Bars represent the mean ± SD ($n > 3$).

**Fig. S2**

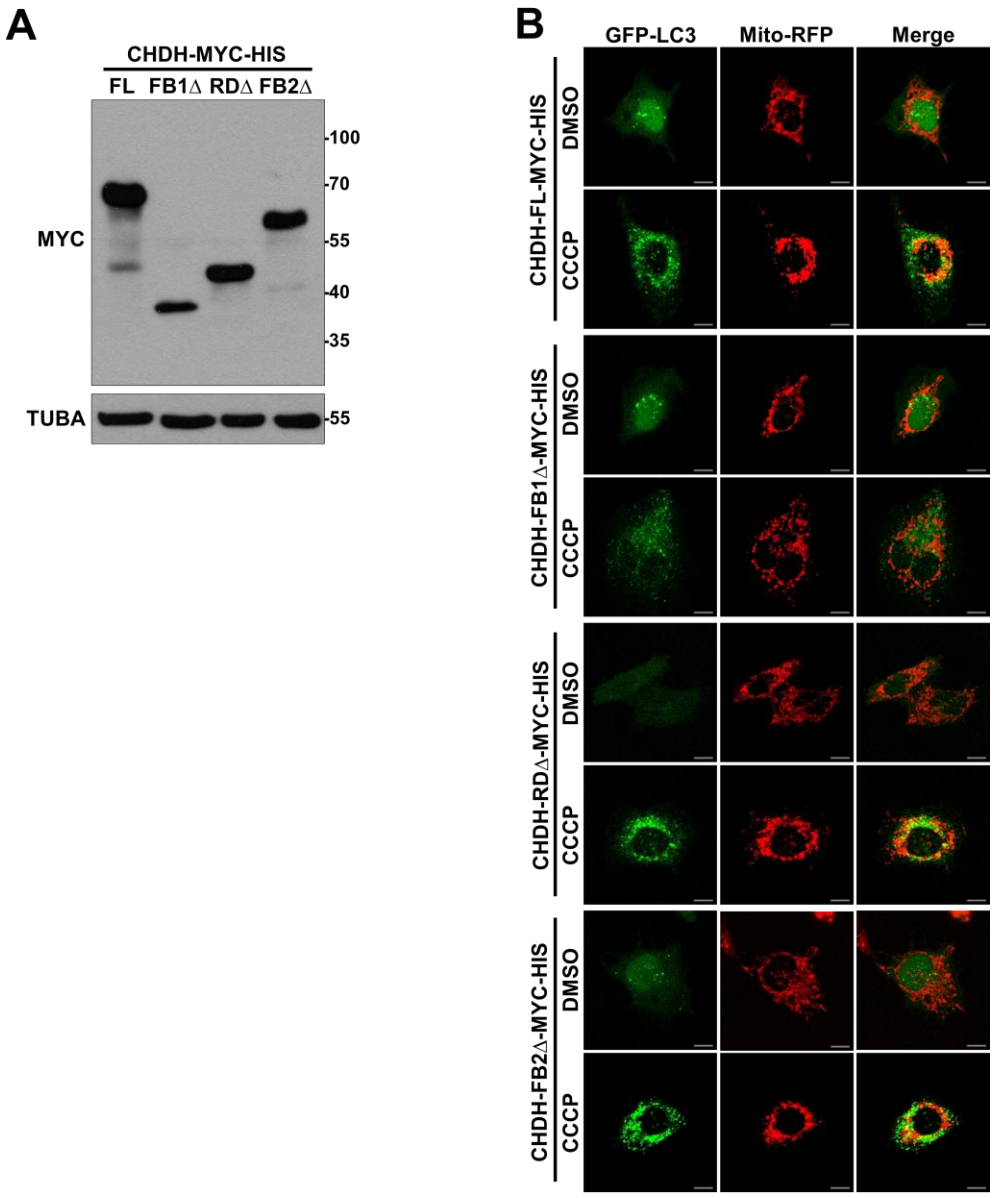


**Figure S2.** Effects of CHDH deletion mutants on mitophagy. (**A**) HeLa cells were transfected with CHDH deletion mutants and then subjected to western blot analysis using MYC antibody. (**B**) HeLa cells were transfected with GFP-LC3, Mito-RFP and either CHDH-FL or deletion mutants, and then analyzed under a confocal microscope at 4 h after treatment with 10 μM CCCP. Scale bar: 10 μm. TUBA, tubulin, α.

**Fig. S3**

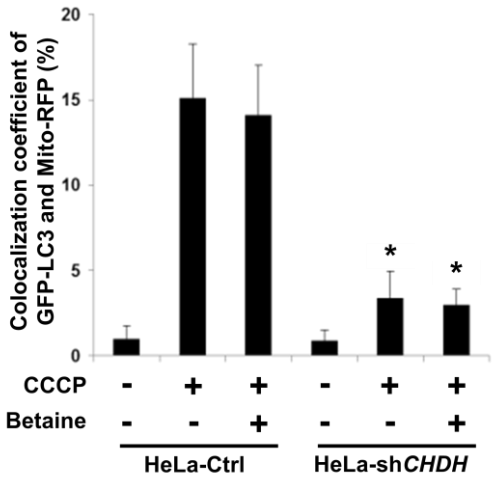


**Figure S3.** Betaine shows little effect on CHDH-mediated mitophagy. HeLa-Ctrl and HeLa-sh*CHDH* cells were cotransfected with PARK2, Mito-RFP and GFP-LC3 and then left untreated or exposed to 1 mM betaine for 2 h. After treatment with 20 μM CCCP for 2 h, cells were observed under a confocal microscope. The colocalization coefficient (%) between Mito-RFP and GFP-LC3 is represented as bars with the mean ± SD, **P* < 0.001.

**Fig. S4**

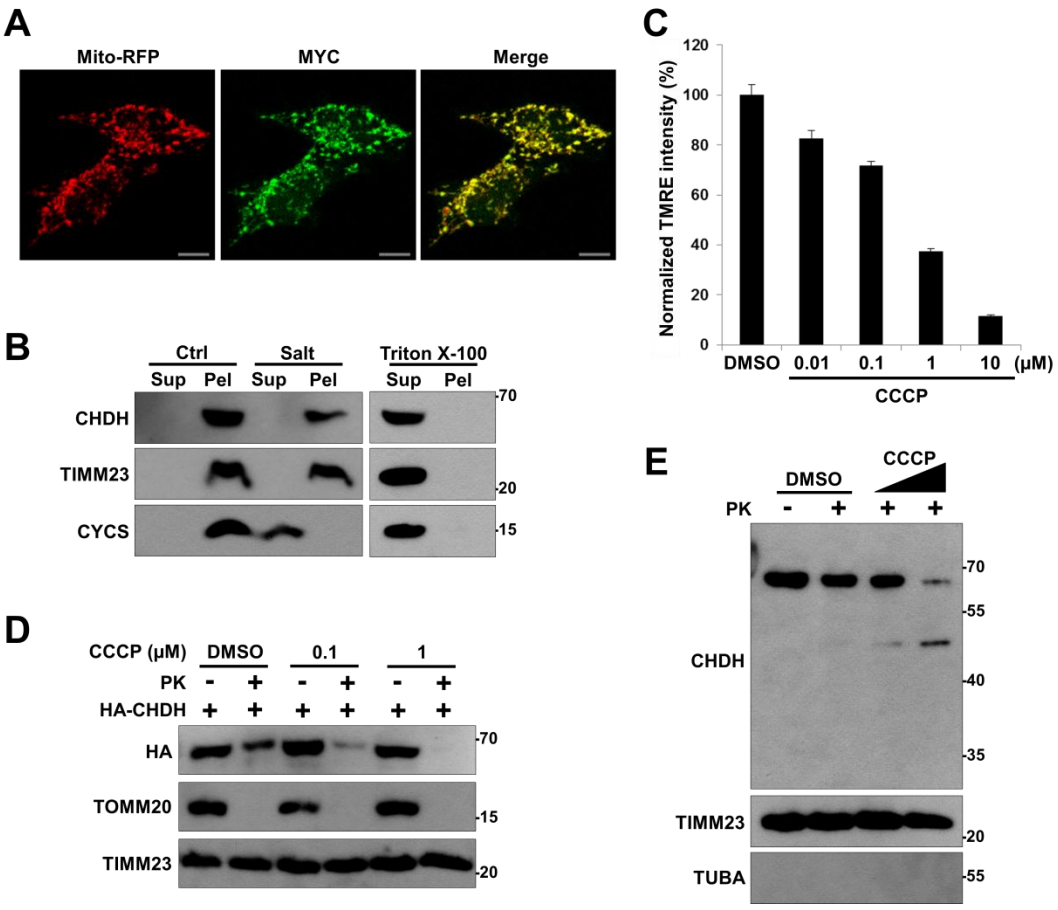


**Figure S4.** Mitochondrial localization and topology of CHDH. (**A**) HeLa cells were transfected with CHDH-MYC-HIS and Mito-RFP, followed by immunofluorescence analysis using MYC antibody (green) under a confocal microscope. (**B**) Pure mitochondria isolated from HEK293T cells were burst by sonication, incubated with Tris buffer (pH 8.0) containing 500 mM NaCl (left) or 1% Triton X-100 (right) for 30 min and then separated into the membrane-associated pellet (Pel) and soluble supernatant (Sup) fractions by ultracentrifugation. The fractions were analyzed by western blotting. Cytochrome c (CYCS)

was used as a control of soluble proteins. (**C**) HeLa cells were incubated with 100 nM TMRE for 20 min. After washing out the remaining dye with PBS, cells were treated for 30 min with the indicated concentrations of CCCP. Signal intensity of TMRE under a confocal microscope was measured using the ImageJ program and represented as bars with the mean ± SD ($n > 3$). (**D**) HeLa cells were transfected with HA-CHDH and treated with the annotated concentrations of CCCP for 30 min. Then, pure mitochondria were prepared and subjected to a proteinase K assay. (**E**) After treatment of SH-SY5Y cells with the increasing concentrations of CCCP (0.1 to 1 μM), mitochondria were purified, subjected to a proteinase K assay and analyzed by western blotting using CHDH antibody recognizing the C terminus. TUBA, tubulin, α.

**Fig. S5**

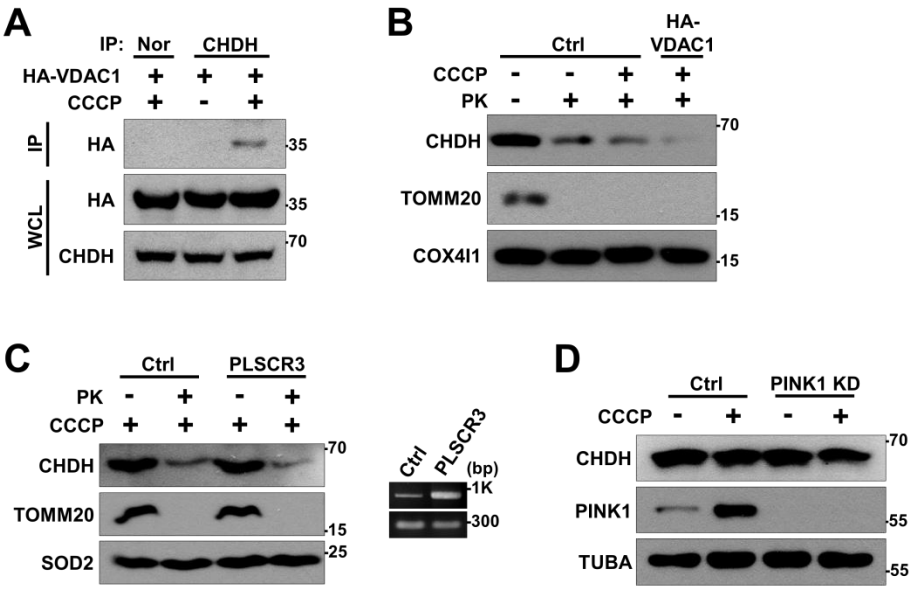


**Figure S5.** Accumulation of CHDH on the mitochondrial outer membrane by HA-VADC1. (**A**) HEK293T cells were transfected with HA-VDAC1 and treated with 1 μM CCCP for 30 min. Following immunoprecipitation (IP) with HA-antibody, the immunoprecipitates and whole cell lysates (WCL) were analyzed by western blotting. (**B**) After treatment of HEK293T cells with 0.1 μM CCCP for 30 min, cell extracts were subjected to a proteinase K assay and then analyzed by western blotting. (**C**) After transfection with PLSCR3, HEK293T cells were left untreated or treated with 0.1 μM CCCP for 30 min. Cell extracts were subjected to proteinase K assay and then analyzed by western blotting (left). The expression of PLSCR3 was confirmed by RT-PCR (right). (**D**) SN4741-Ctrl and PINK1 knockdown cells were treated with 10 μM CCCP for 2 h and cell extracts were then analyzed by western blotting. TUBA, tubulin, α.

**Fig. S6**

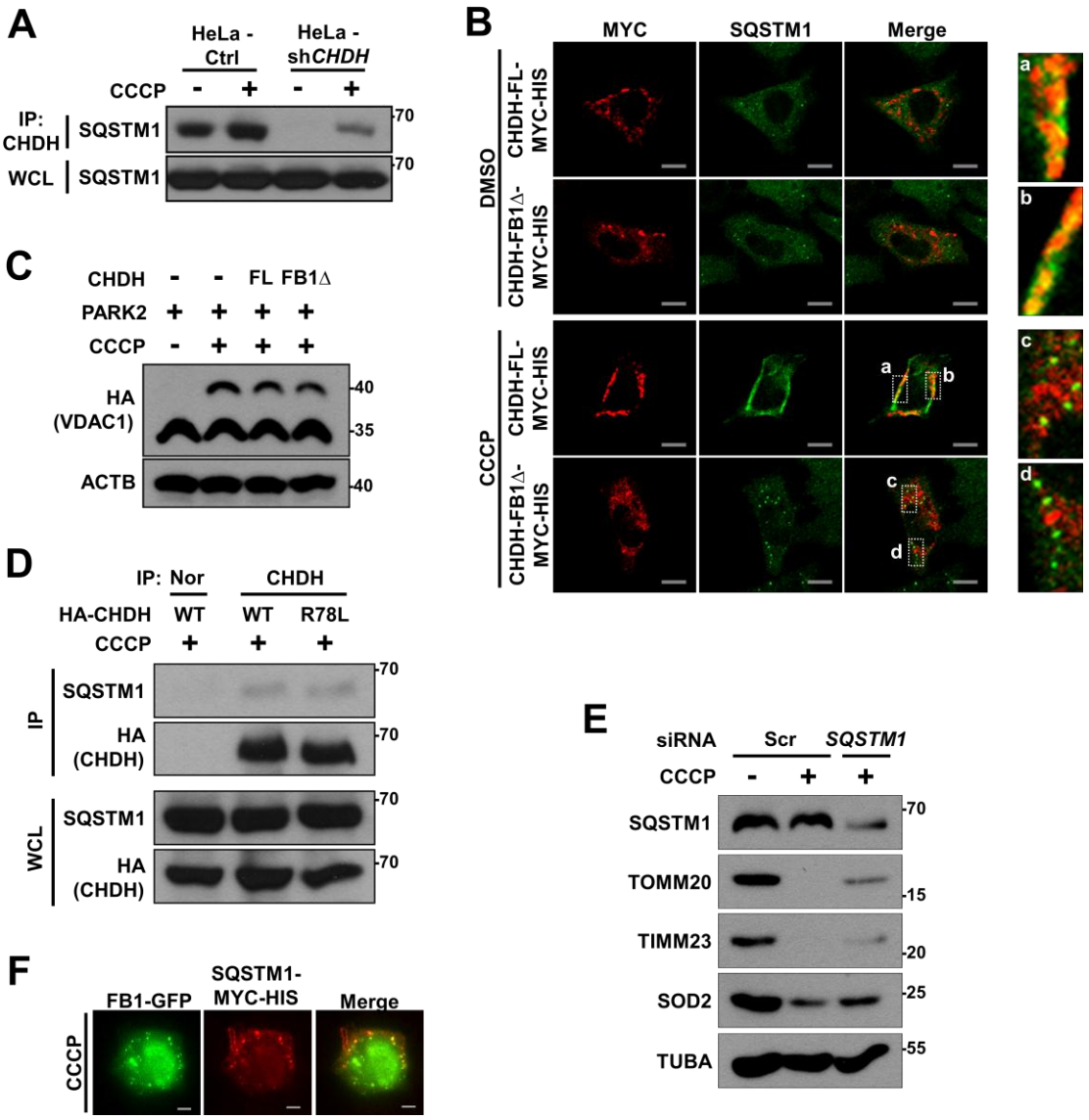


**Figure S6.** Interaction between CHDH and SQSTM1. (**A**) HeLa-Ctrl and HeLa-sh*CHDH* cells were left untreated or treated with 10 μM CCCP for 5 h and cell extracts were subjected to immunoprecipitation (IP) analysis using CHDH antibody. (**B**) HeLa cells were transfected with CHDH-FL-MYC-HIS or CHDH-FB1Δ-MYC-HIS and then exposed to 20 μM CCCP

for 3 h. Cells were examined with immunofluorescence analysis using MYC and SQSTM1 antibodies. Scale bar: 10 μm. Enlarged sections are shown on right side with labelling (a to d). (**C**) HeLa cells were transfected with HA-VDAC1, PARK2 and either CHDH-FL or FB1Δ for 24 h and incubated with 20 μM CCCP for 2 h. Cell extracts were analyzed by western blotting. (**D**) HEK293T cells were transfected with CHDH wild-type (WT) or rs12676 SNP containing CHDH-R78L and then incubated with 20 μM CCCP for 30 min. Samples were immunoprecipitated (IP) with preimmune normal (Nor) or CHDH antibody followed by western blot analysis. (**E**) HEK293T cells were transfected with scrambled siRNA (Scr) or *SQSTM1* siRNA for 48 h. After treatment with 10 μM CCCP for 24 h, cell extracts were prepared and subjected to western blot analysis. (**F**) HeLa cells were transfected with FB1-GFP and SQSTM1-MYC-HIS. After treatment with 10 μM CCCP for 24 h, cells were immunostained with MYC antibody and then observed under a confocal microscope. Scale bar: 10 μm. TUBA, tubulin, α.