Expanded View Figures

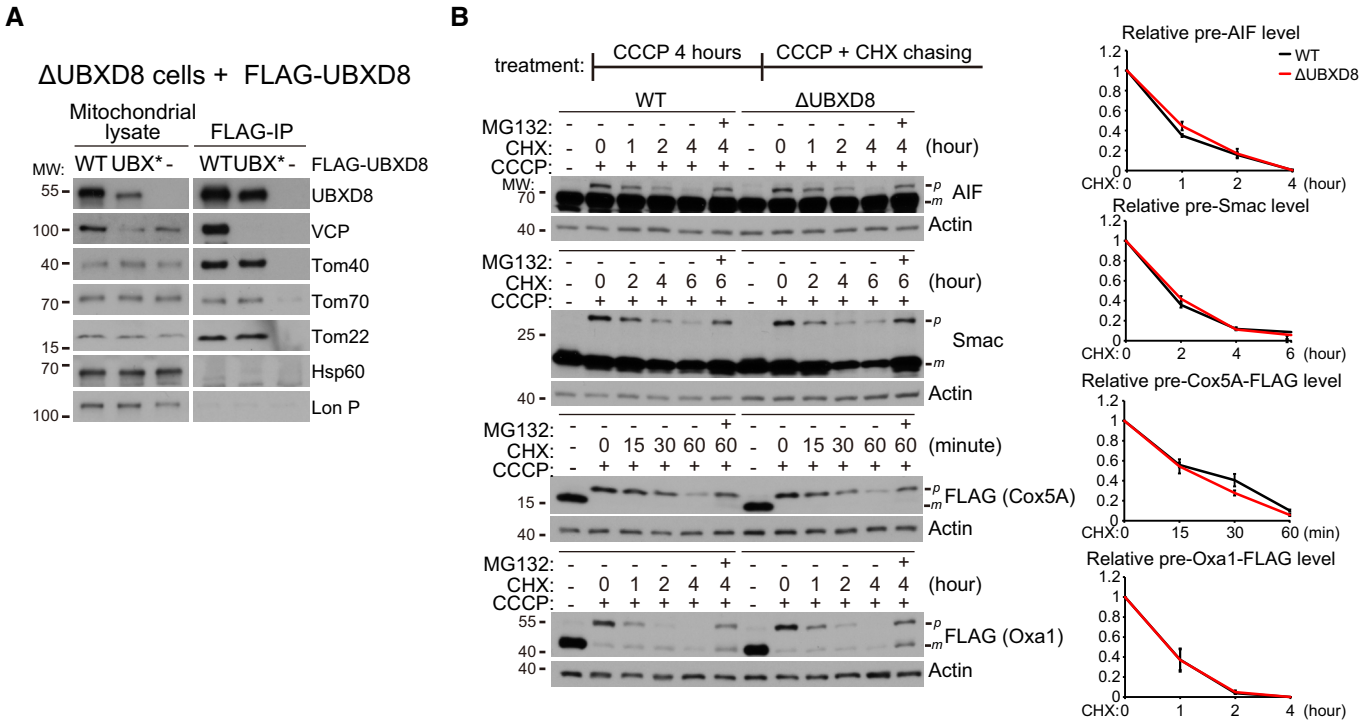

Figure EV1.

Figure EV1. UBXD8 associates with the TOM complex but is dispensable for translocation-associated degradation. Related to Fig 2.

A Immunoprecipitation analysis of the interaction between UBXD8 and the TOM complex. Mitochondrial fractions were purified from the indicated HeLa cells and lysed with lysis buffer containing 1% digitonin. Mitochondrial lysates were then subjected to anti-FLAG immunoprecipitation.

B Western blot and quantitative analysis of translocation-associated degradation with four model substrates: AIF, Smac, Cox5A-FLAG, and Oxa1-FLAG. WT and ΔUBXD8 HeLa cells were treated with 15 μM CCCP for 4 h and then subjected to CCCP + CHX (200 μg/ml) chasing for the indicated time. Cox5A-FLAG and Oxa1-FLAG expression were induced by Doxycycline (1 μg/ml) for 4 h. MG132: 20 μM. Data are shown as mean ± SE from three biological repeats.

C Western blot analysis of mitochondrial ubiquitination induced by mtDNA depletion (EB treatment) in WT and ΔUBXD8 HeLa cells. Cells were treated with EB (50 ng/ml) plus uridine (50 μg/ml), pyruvate (1 mM), and glutamine (2 mM) for the indicated time. Whole cell lysates and mitochondrial fraction lysates were analyzed.

Source data are available online for this figure.

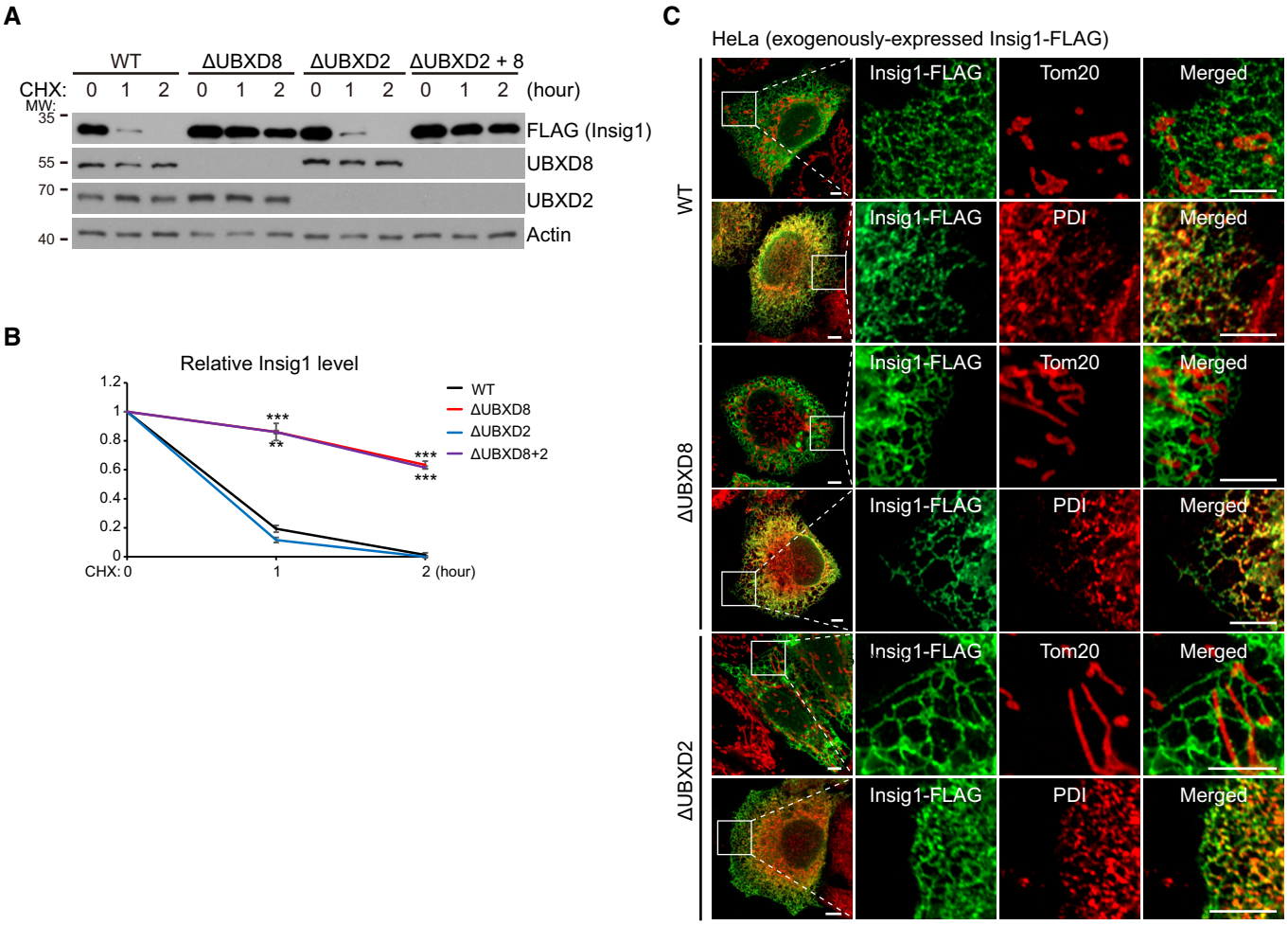

Figure EV2. The degradation and subcellular localization of Insig1-FLAG. Related to Fig 4.

A, B Western blot (A) and quantitative analysis (B) of Insig1-FLAG degradation in the indicated HeLa cells. Data are shown as mean ± SE from three biological repeats (B). Statistics: two-tailed unpaired Student's *t*-test (B); ***$P < 0.001$.

C Immunofluorescence analysis of Insig1-FLAG localization in the indicated HeLa cells. Scale bar, 5 μm.

Source data are available online for this figure.

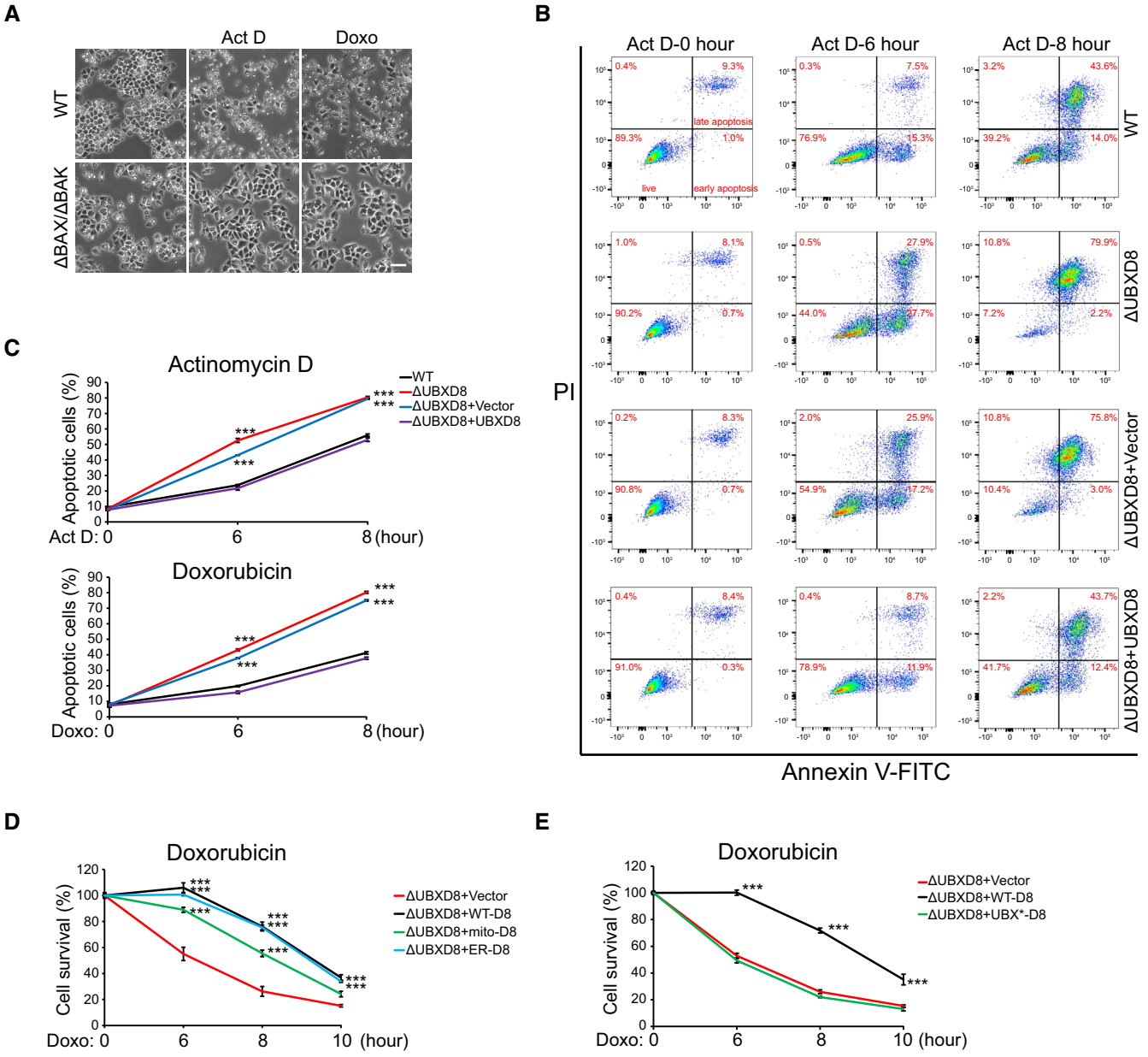

Figure EV3. UBXD8 knockout sensitizes cells to apoptotic insults. Related to Fig 5.

A Representative images of WT and ΔBAX ΔBAK HeLa cells treated with ActD and Doxo. Scale bar, 100 μm.

B, C Representative FACS (B) and quantitative analysis (C) of apoptosis in the indicated HeLa cells treated with ActD or Doxo.

D, E Quantitative analysis of apoptosis in the indicated HeLa cells treated with Doxo.

Data information: Data are shown as mean ± SE from three biological repeats (C–E). Statistics: two-tailed unpaired Student's *t*-test (C–E); ***$P < 0.001$.

Figure EV4. The mRNA level and the degradation of Noxa, Bnip3, and Bik. Related to Fig 6.

A Quantitative PCR analysis of the mRNA levels of Noxa, Bnip3, and Bik in the indicated HeLa cells. Data are shown as mean ± SE from three biological repeats.

B–G Representative western blot (B, D, F) and quantitative analysis (C, E, G) of the degradation of Mcl1, Noxa, Bnip3, and Bik in the indicated HeLa cells. In (F), VCP knockdown was induced by Doxycycline (1 μg/ml) treatment for 48 h. Data are shown as mean ± SE from three biological repeats (C, E, G). Statistics: two-tailed unpaired Student's *t*-test (C, E, G); *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Source data are available online for this figure.

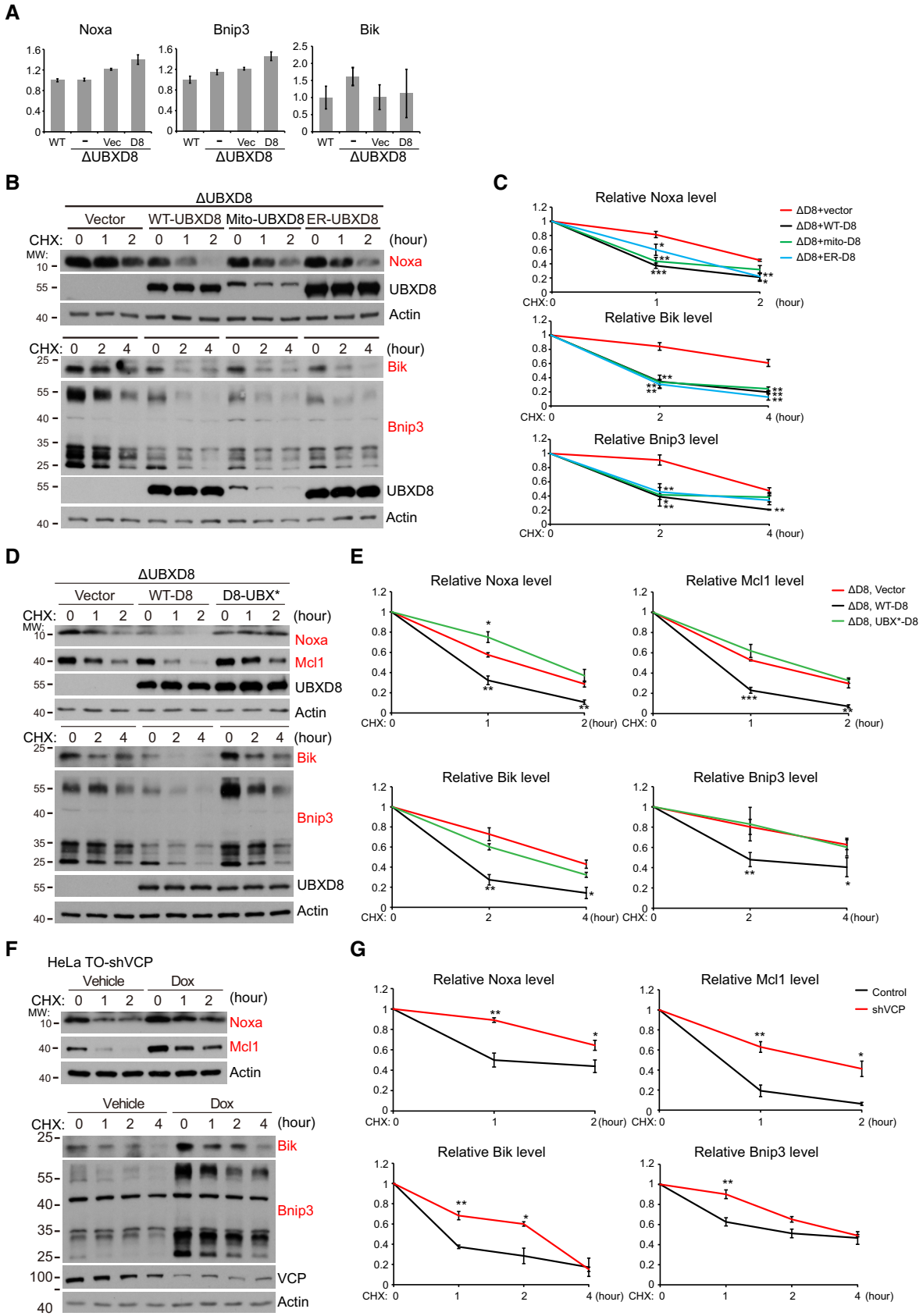
A
Noxa
Bnip3
Bik
WT
-
Vec
D8
ΔUBXD8
B
ΔUBXD8
Vector
WT-UBXD8
Mito-UBXD8
ER-UBXD8
CHX:
(hour)
MW:
Noxa
UBXD8
Actin
Bik
Bnip3
C
Relative Noxa level
Relative Bik level
Relative Bnip3 level
ΔD8+vector
ΔD8+WT-D8
ΔD8+mito-D8
ΔD8+ER-D8
D
ΔUBXD8
Vector
WT-D8
D8-UBX*
Mcl1
E
Relative Mcl1 level
ΔD8, Vector
ΔD8, WT-D8
ΔD8, UBX*-D8
F
HeLa TO-shVCP
Vehicle
Dox
VCP
G
Control
shVCP

Figure EV4.

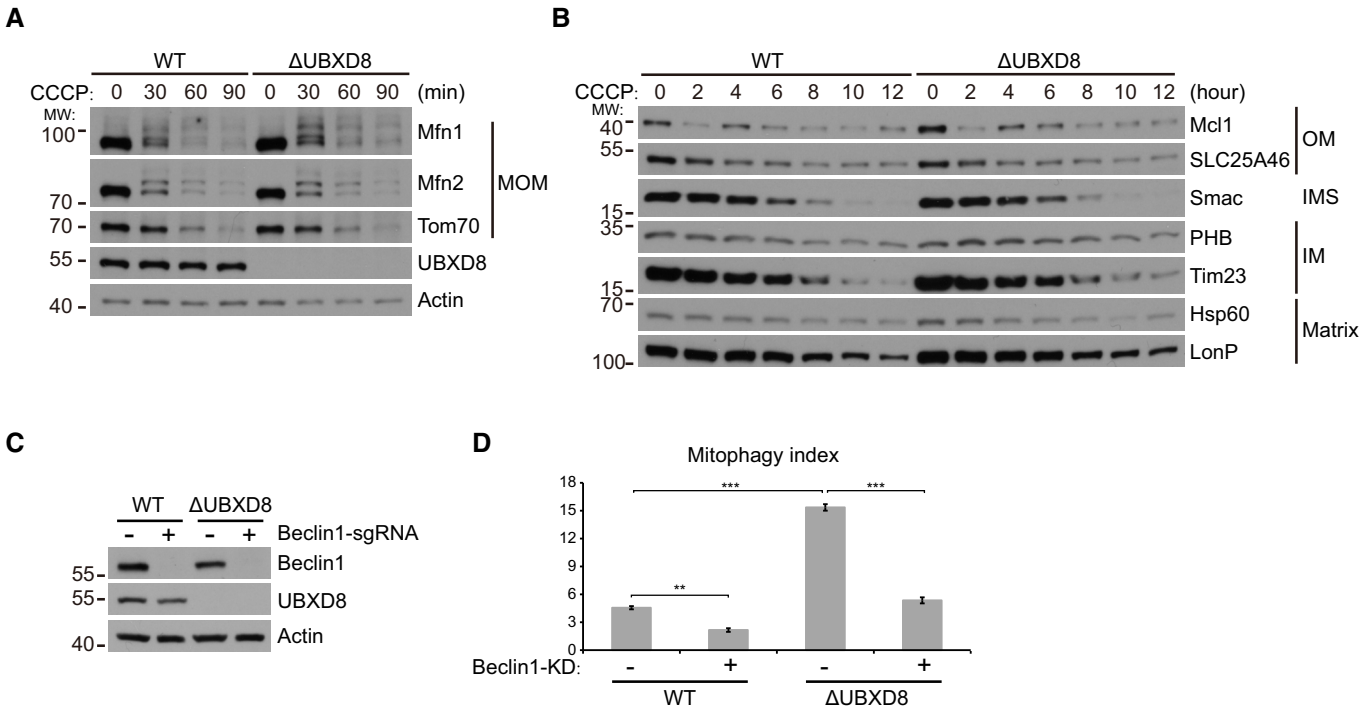

Figure EV5. UBXD8 is dispensable for Parkin-mediated mitochondrial degradation and Beclin1 knockdown blocks hyperactivated mitophagy in ΔUBXD8 cells. Related to Fig 7.

A, B HeLa cells stably expressing GFP-Parkin were treated with CCCP (15 μM) for the indicated time. The degradation of mitochondrial outer membrane (MOM), intermembrane space (IMS), inner membrane (IM), and matrix proteins were analyzed by western blot.

C Western blot analysis of Beclin1 knockdown in the indicated HeLa cells.

D Quantitative analysis of mitophagy in the indicated HeLa cells. Data are shown as mean ± SE from three biological repeats. Statistics: one-way ANOVA; $^{**}P < 0.01$, $^{***}P < 0.001$.

Source data are available online for this figure.