Expanded View Figures

Figure EV1. CHK2 is involved in oxidative stress-induced autophagy.

A Intracellular ROS levels detected in H1299 cells treated with HBSS starvation.

B Western blot detection of p62 in H1299 cells transfected with the indicated shRNA in normal medium or after HBSS starvation.

C Western blot detection of p62 in H1299 cells cotransfected with the indicated shRNA and plasmid and cultured in normal or HBSS starvation medium. CHK2 NTm, shRNA nontargetable mutant CHK2 rescue plasmid.

D, E Quantification of p62 and LC3-II/I protein levels in H1299 cells transfected with the indicated shRNA in normal medium or after H_2O_2 (500 μM) treatment. Results are presented as mean ± s.e.m. from three independent experiments, $^*P < 0.05$, $^{**}P < 0.01$ (Student's *t*-test).

F, G Quantification of p62 and LC3-II/I protein levels in H1299 cells cotransfected with the indicated shRNA and plasmid and cultured in normal medium or after H_2O_2 (500 μM) treatment. Results are presented as mean ± s.e.m. from three independent experiments, $^*P < 0.05$ (Student's *t*-test).

H Quantification of p62 protein levels in H1299 cells transfected with the indicated shRNA in normal medium or after HBSS starvation. Results are presented as mean ± s.e.m. from three independent experiments, $^*P < 0.05$ (Student's *t*-test).

I Quantification of p62 protein levels in H1299 cells cotransfected with the indicated shRNA and plasmid and cultured in normal medium or after HBSS starvation. Results are presented as mean ± s.e.m. from three independent experiments, $^*P < 0.05$ (Student's *t*-test).

J Quantification of p62 protein levels in MEFs of indicated genotype during growth in normal medium or after HBSS starvation. Results are presented as mean ± s.e.m. from three independent experiments, $^{**}P < 0.01$ (Student's *t*-test).

K Quantification of p62 protein levels in MEFs of the indicated genotype during growth in normal medium or after H_2O_2 (500 μM) treatment. Results are presented as mean ± s.e.m. from three independent experiments, $^{**}P < 0.01$ (Student's *t*-test).

L Western blot detection of p62 in MEFs of the indicated genotype during growth in normal medium or after HBSS starvation.

M Western blot detection of p62 in MEFs of the indicated genotype during growth in normal medium or after H_2O_2 (500 μM) treatment.

N Representative images of GFP-LC3 puncta in HeLa/GFP-LC3 cells transfected with the indicated shRNA, pretreated with 60 nM CQ or vehicle (DMSO), and cultured for 1 h in normal, HBSS starvation medium or treated with H_2O_2 (500 μM). Scale bar, 50 μm.

O Quantification of GFP-LC3 puncta in HeLa/GFP-LC3 cells transfected with the indicated shRNA, pretreated with 60 nM CQ or vehicle (DMSO), and cultured for 1 h in normal, HBSS starvation medium or treated with H_2O_2 (500 μM; $n = 30$). Results are presented as mean ± s.e.m. from three independent experiments, $^{***}P < 0.001$ (Student's *t*-test).

P Western blot detection of LC3 in H1299 cells transfected with the indicated shRNA, pretreated with 60 nM CQ or vehicle (DMSO), and cultured for 3 h in normal or treated with H_2O_2 (500 μM).

Source data are available online for this figure.

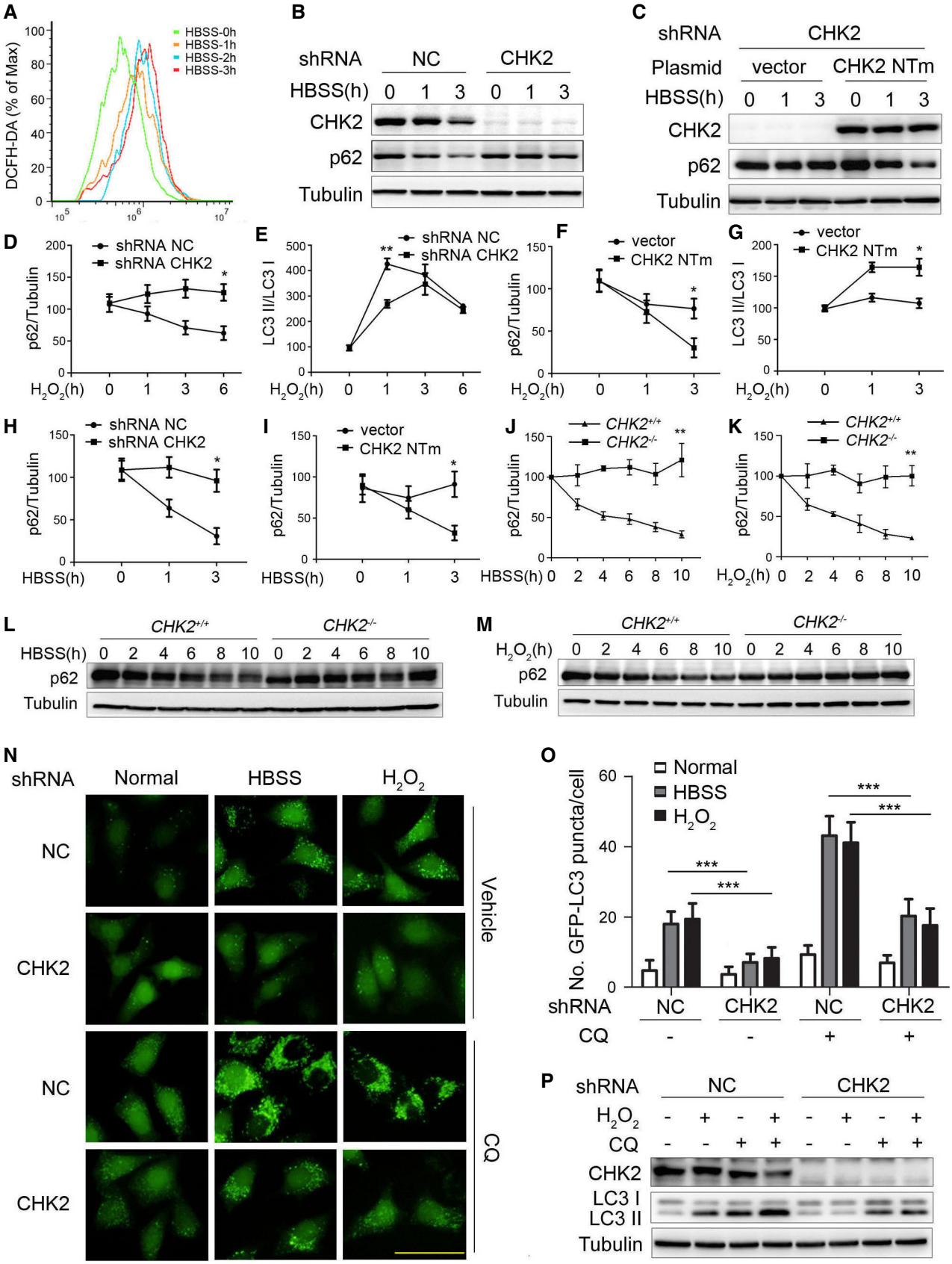
A
DCFH-DA (% of Max)
HBSS-0h
HBSS-1h
HBSS-2h
HBSS-3h
B
shRNA NC CHK2
HBSS(h) 0 1 3 0 1 3
CHK2
p62
Tubulin
C
shRNA CHK2
Plasmid vector CHK2 NTm
HBSS(h) 0 1 3 0 1 3
CHK2
p62
Tubulin
D
shRNA NC
shRNA CHK2
p62/Tubulin
H2O2(h) 0 1 3 6
E
shRNA NC
shRNA CHK2
LC3 II/LC3 I
H2O2(h) 0 1 3 6
F
vector
CHK2 NTm
p62/Tubulin
H2O2(h) 0 1 3
G
vector
CHK2 NTm
LC3 II/LC3 I
H2O2(h) 0 1 3
H
shRNA NC
shRNA CHK2
p62/Tubulin
HBSS(h) 0 1 3
I
vector
CHK2 NTm
p62/Tubulin
HBSS(h) 0 1 3
J
CHK2+/+
CHK2-/-
p62/Tubulin
HBSS(h) 0 2 4 6 8 10
K
CHK2+/+
CHK2-/-
p62/Tubulin
H2O2(h) 0 2 4 6 8 10
L
CHK2+/+ CHK2-/-
HBSS(h) 0 2 4 6 8 10 0 2 4 6 8 10
p62
Tubulin
M
CHK2+/+ CHK2-/-
H2O2(h) 0 2 4 6 8 10 0 2 4 6 8 10
p62
Tubulin
N
shRNA Normal HBSS H2O2
NC
CHK2
Vehicle
NC
CHK2
CQ
O
Normal
HBSS
H2O2
No. GFP-LC3 puncta/cell
shRNA NC CHK2 NC CHK2
CQ - - + +
P
shRNA NC CHK2
H2O2 - + - + - + - +
CQ - - + + - - + +
CHK2
LC3 I
LC3 II
Tubulin

Figure EV1.

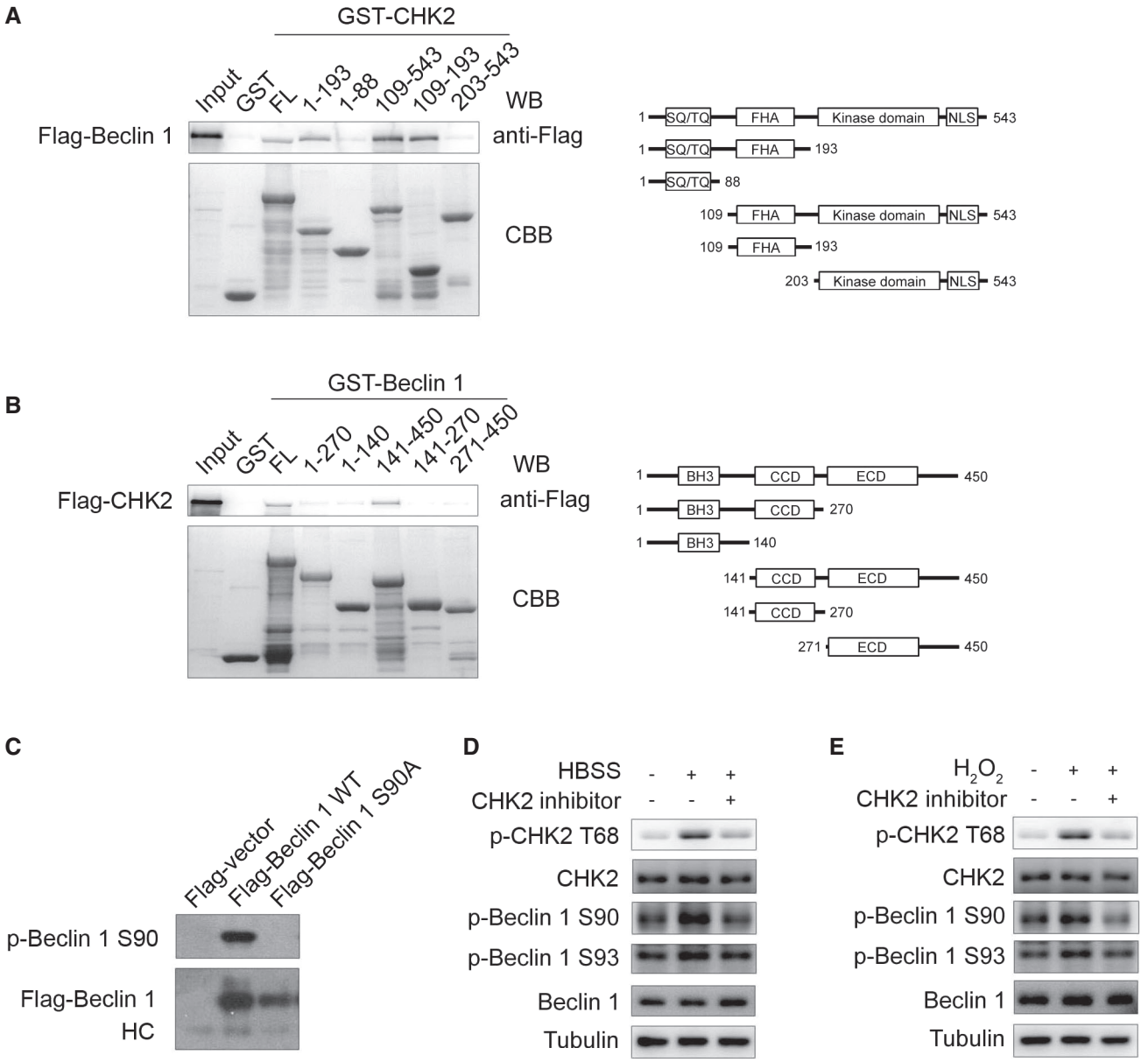

Figure EV2. The interaction domain between CHK2 and Beclin 1 and characterization of Beclin 1 Ser90 phosphorylation antibody.

A GST-CHK2 full-length (FL) and fragments fusion proteins were expressed in bacteria and purified; these proteins were then incubated with *in vitro*-translated Flag-Beclin 1 protein. Western blotting was performed to detect the interaction of Beclin 1 with CHK2.

B GST-Beclin 1 FL and fragments fusion proteins were expressed in bacteria and purified; these proteins were then incubated with *in vitro*-translated Flag-CHK2 protein. Western blotting was performed to detect the interaction of CHK2 with Beclin 1.

C Cell lysates from 293 cells transfected with WT or mutant forms of Flag-Beclin 1 were immunoprecipitated with Flag-M2 beads followed by Western blot using antibody against Beclin 1 Ser90 or Flag.

D, E H1299 cells were pretreated with CHK2 inhibitor II in complete medium for 4 h and then cultured for 1 h in HBSS starvation (D) or H_2O_2 (500 μM) stimulation (E). Western blot detection of p-CHK2 Thr68, CHK2, p-Beclin 1 Ser90, p-Beclin 1 Ser93, and Beclin 1.

Source data are available online for this figure.

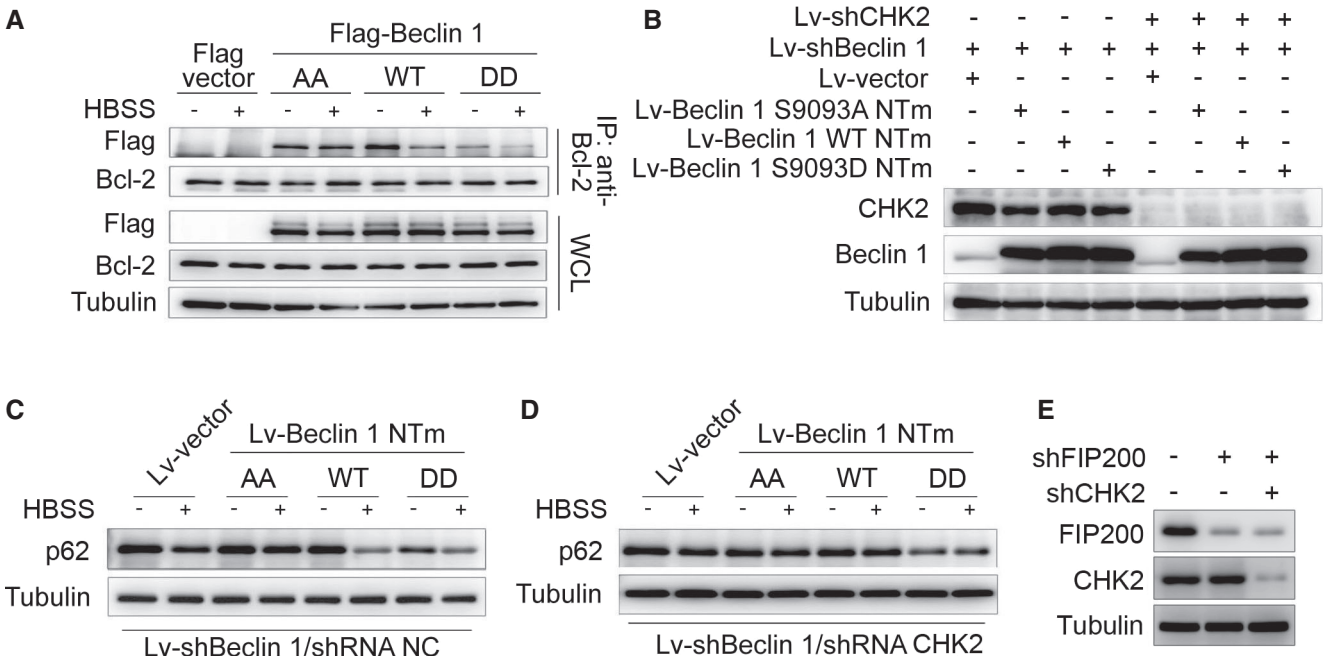

Figure EV3. CHK2-mediated Beclin 1 phosphorylation promotes autophagy.

A Immunoprecipitation of Bcl-2 with Flag-Beclin 1 WT, AA mutant, and DD mutant in HCT116 cells in normal medium or after HBSS starvation.

B Beclin 1-depleted H1299 cells with reconstituted expression of the indicated proteins.

C, D Autophagy level was determined in Beclin 1-depleted H1299 cells with reconstituted expression of Beclin 1 WT, AA mutant, or DD mutant in normal medium or after HBSS starvation in the presence (C) or absence (D) of CHK2.

E FIP200- and CHK2-depleted H1299 cells with reconstituted expression of the indicated proteins.

Source data are available online for this figure.

Figure EV4. ROS acts as a signaling molecule to activate the ATM/CHK2/Beclin 1 axis under glucose starvation and hypoxic stress.

A Autophagic flux is shown by representative confocal microscopic images for 293 cells stably expressing GFP-mCherry-LC3 transfected with the indicated shRNA. Scale bar, 10 μm. Quantitation of autophagosomal (green) and autolysosomal (red) LC3 puncta following 12-h glucose starvation and 36-h hypoxia (n = 30). Data are presented as mean ± s.e.m. from three independent experiments; $^{**}P < 0.01$ (Student's t-test).

B Western blot detection of LC3 in H1299 cells transfected with the indicated shRNA, pretreated with 30 nM CQ or vehicle (DMSO), and cultured for 12 h in normal or glucose starvation.

C Western blot detection of LC3 in H1299 cells transfected with the indicated shRNA, pretreated with 30 nM CQ or vehicle (DMSO), and cultured for 36 h in normal or hypoxia.

D, E Intracellular ROS (D) or ATP (E) levels detected in H1299 cells treated with glucose starvation. Data are presented as mean ± s.e.m. from three independent experiments.

F, G Intracellular ROS (F) or ATP (G) levels detected in H1299 cells treated with hypoxia. Data are presented as mean ± s.e.m. from three independent experiments.

H H1299 cells detected with γH2AX staining followed by HBSS starvation (1 h), H_2O_2 treatment (200 μM, 3 h), glucose starvation (10 h), hypoxia (36 h), and DOX (0.5 μM, 3 h). Scale bar, 10 μm.

I Intracellular ATP levels detected in H1299 cells treated with 2DG (5 mM) and oligomycin (2.5 μM) treatment. Data are presented as mean ± s.e.m. from three independent experiments.

Source data are available online for this figure.

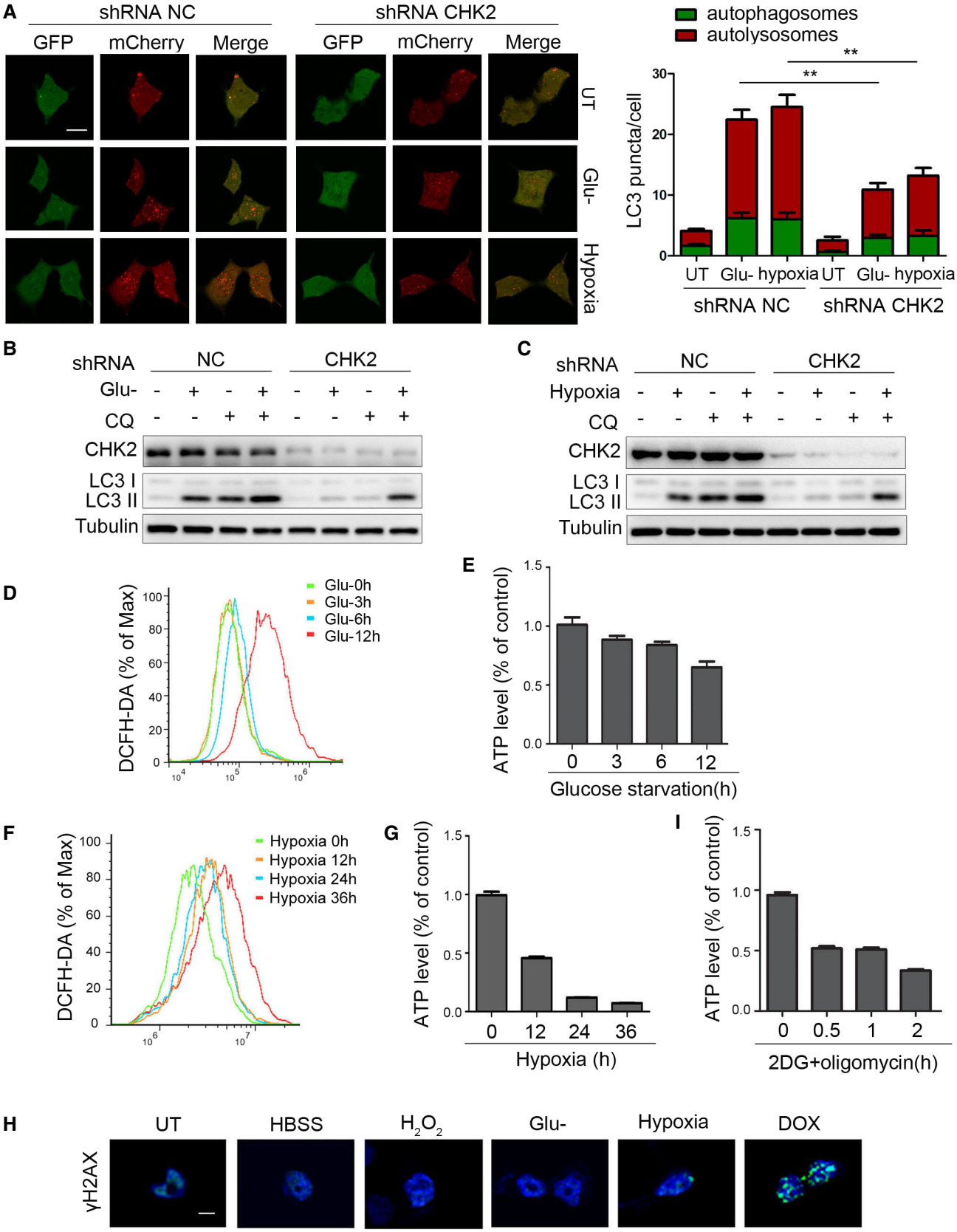

Figure EV4.

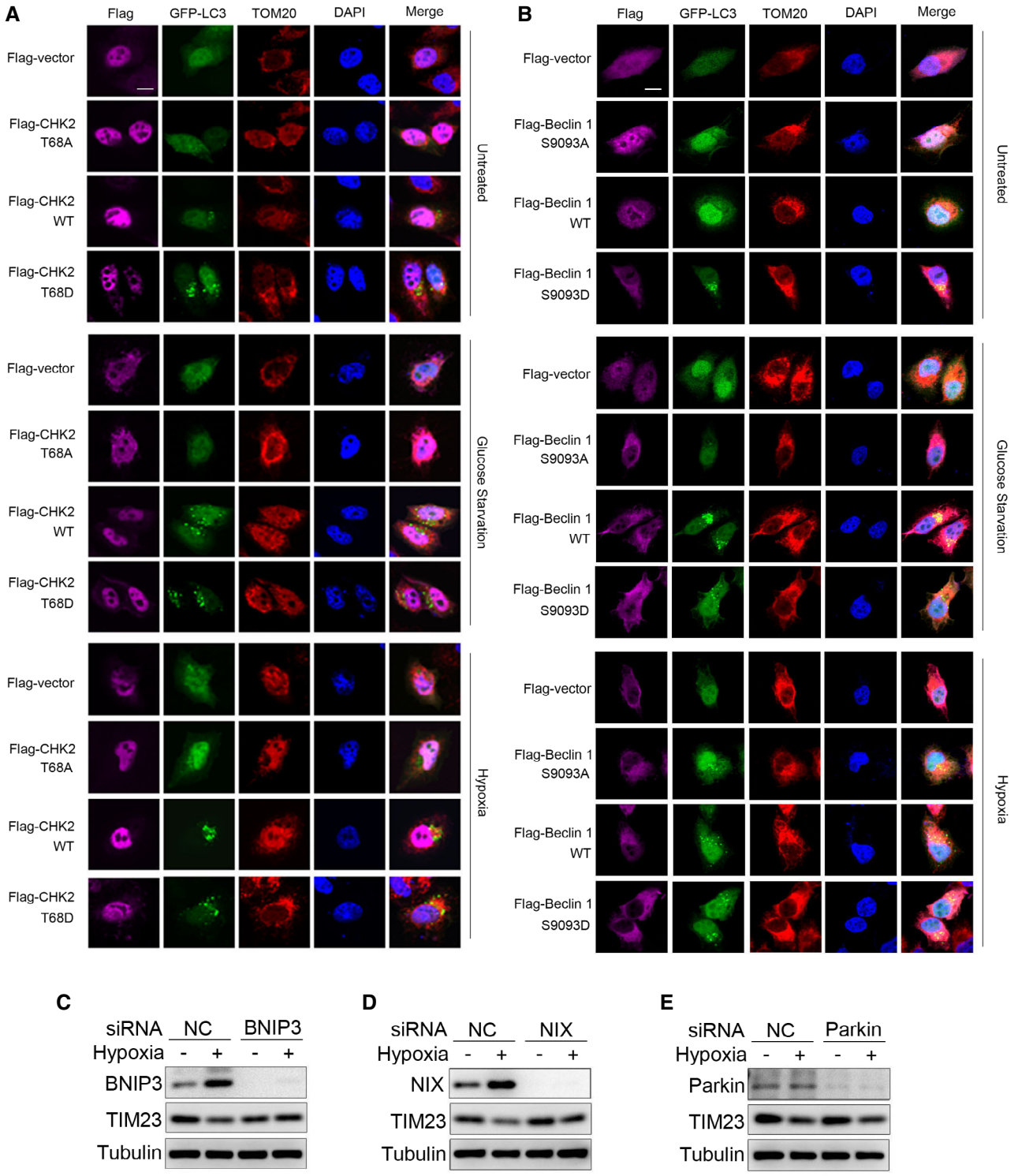

Figure EV5. CHK2-mediated autophagy limits ROS levels by clearing damaged mitochondria.

A, B Fluorescence microscopy of the colocalization of GFP-LC3 with mitochondria (identified with the mitochondrial stain TOM20) in H1299 cells transfected with the indicated plasmid and cultured in untreated (UT), glucose starvation for 12 h, or subjected to hypoxia for 36 h. DAPI, DNA-binding dye. Scale bar, 10 μm.

C Western blot detection of BNIP3 and TIM23 in H1299 cells transfected with the indicated siRNA and untreated or subjected to hypoxia for 36 h.

D Western blot detection of NIX and TIM23 in H1299 cells transfected with the indicated siRNA and untreated or subjected to hypoxia for 36 h.

E Western blot detection of parkin and TIM23 in H1299 cells transfected with the indicated siRNA and untreated or subjected to hypoxia for 36 h.

Source data are available online for this figure.