Expanded View Figures

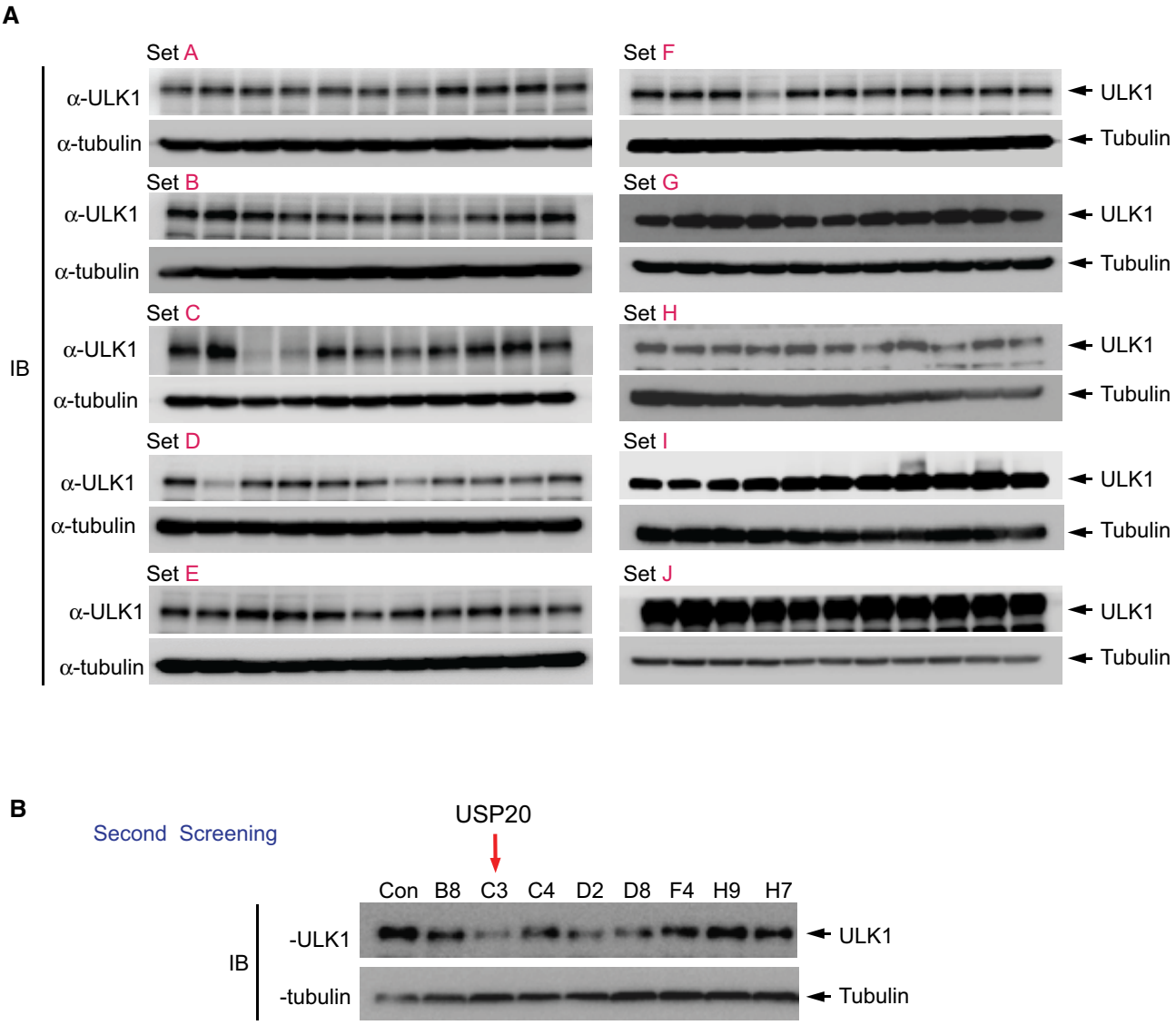

Figure EV1. Screening of the deubiquitinases (DUBs) involved in the regulation of ULK1 expression by siRNA libraries targeting 99 human DUBs in HeLa cells.

A, B HeLa cells were reverse-transfected by siRNAs, and the expression of ULK1 was monitored by immunoblot analysis through the first screening (A) and the second screening (B). Expression of α-tubulin was used as a loading control.

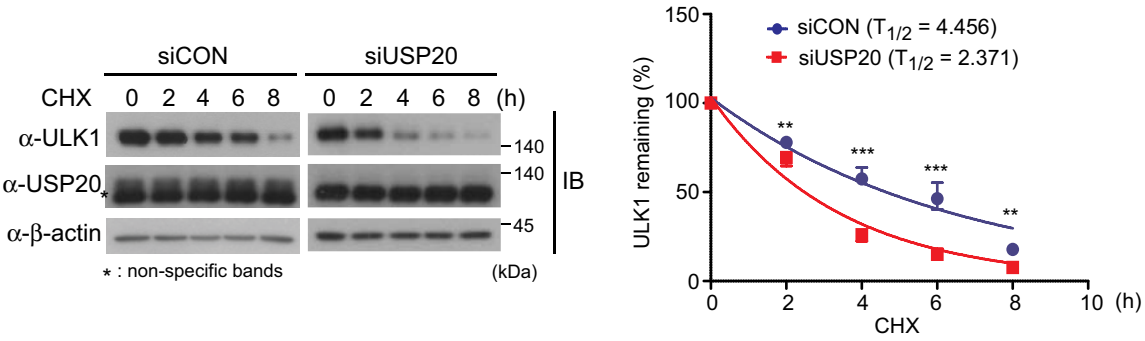

Figure EV2. USP20 depletion reduces the stability of ULK1 protein.
HeLa cells were reverse-transfected with 20 nM control siRNA (siCON) or USP20-specific siRNA (siUSP20-1). After 24 h, cells were subsequently treated with 10 μM cycloheximide (CHX) for the indicated time points. To address the decreased stability of ULK1 in USP20-depleted cells, we adjusted the amount of starting materials and endogenous levels of ULK1 and USP20 protein were measured by immunoblotting with the indicated antibodies (left). ULK1 levels were quantified using ImageJ software (right). For normalization, expression of non-specific bands below the USP20 was used as a control. The data were statistically analyzed by two-way ANOVA followed by Bonferroni's multiple comparison test (**$P < 0.01$, ***$P < 0.001$ compared to siUSP20, $n = 3$). The bars represent the mean ± SD. The result in this figure is representative of three independent experiments.

Source data are available online for this figure.

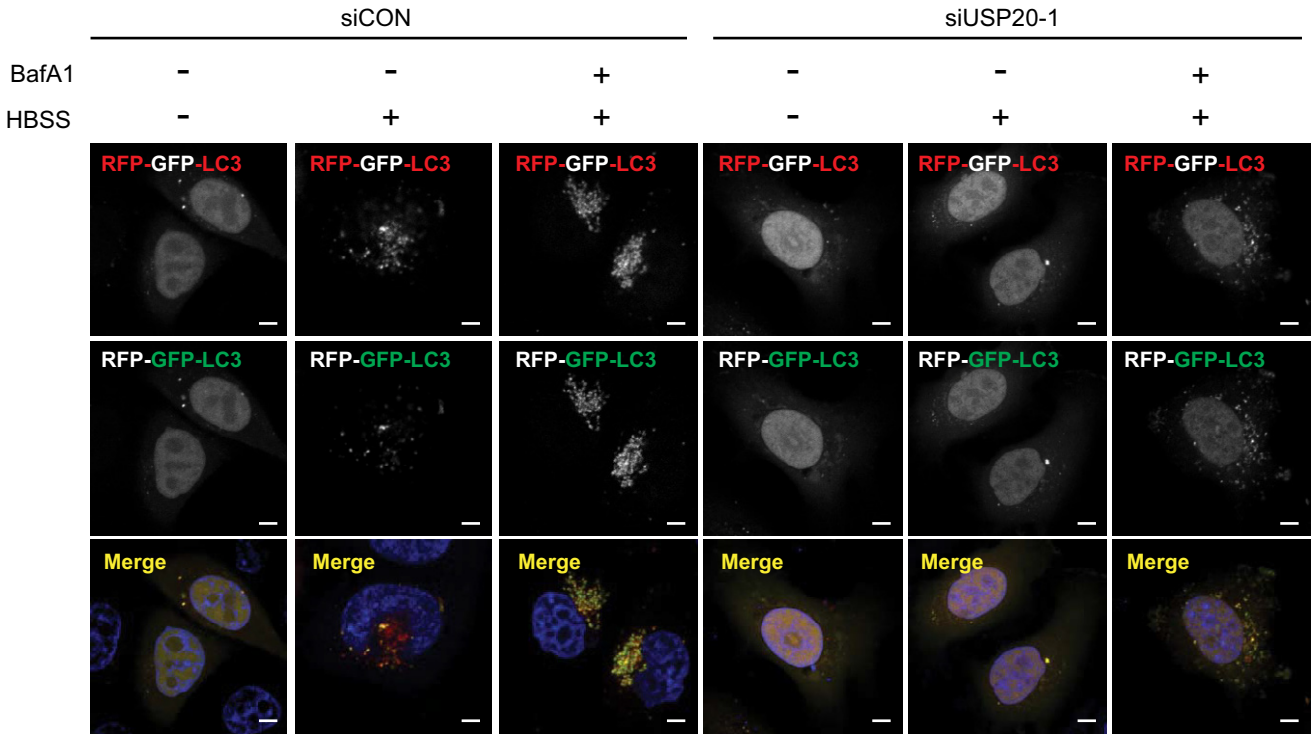

Figure EV3. USP20 is required for autophagy induction.
Either control HeLa cells or USP20-depleted cells expressing a GFP-RFP-LC3 plasmid were incubated with HBSS alone for 8 h or in combination with bafilomycin A1 (BafA1). Individual channels of LC3 puncta containing green and red fluorescence shown in Fig 3A were converted to images in grayscale. Scale bar, 5 μm.

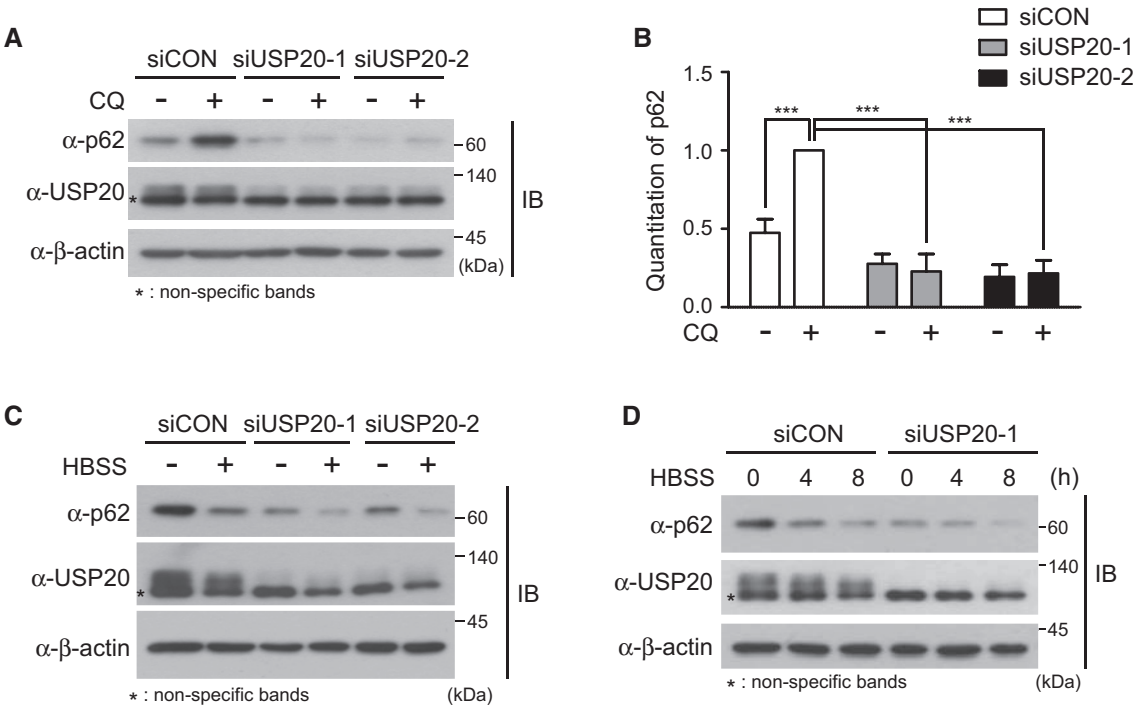

Figure EV4. Decreased expression of p62 protein in USP20-knockdown cells under nutrient starvation.

A HeLa cells were reverse-transfected with two independent USP20-specific siRNAs or control siRNA for 24 h. After cells were treated with 50 μM chloroquine (CQ) for 1 h, expressions of p62 and USP20 proteins were observed by immunoblotting with the indicated antibodies. β-actin expression was used as a loading control.

B p62 levels were quantified using ImageJ software. For normalization, β-actin expression was used as a control. The data were statistically analyzed by two-way ANOVA followed by Sidak's multiple comparison test (***$P < 0.001$ compared to the indicated points, $n = 3$). The bars represent the mean ± SD.

C HeLa cells were reverse-transfected with two independent USP20-specific siRNAs or control siRNA for 24 h. Cells were starved with HBSS medium for 8 h. p62 and USP20 expressions were observed by immunoblotting with the indicated antibodies. β-actin expression was used as a loading control.

D HeLa cells were reverse-transfected with USP20-specific siRNA or control siRNA for 24 h. After transfection, cells were starved with HBSS medium for the indicated times. Cell lysates were immunoblotted with the indicated antibodies. β-actin expression was used as a loading control. All results in this figure are representative of three independent experiments.

Source data are available online for this figure.

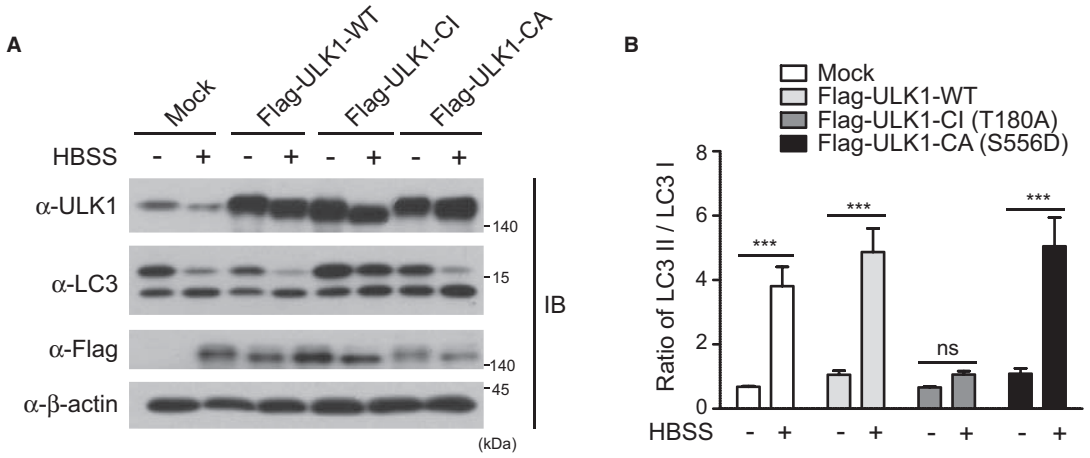

Figure EV5. Autophagy flux in wild-type ULK1-, catalytically inactive (CI) ULK1-, or catalytically active (CA) ULK1-expressing HeLa cells under nutrient starvation.

A HeLa cells stably expressing Flag-ULK1-WT, Flag-ULK1-CI, or Flag-ULK1-CA were starved with HBSS medium for 1 h, respectively. Expressions of ULK1 and LC3 proteins were observed by immunoblotting with the indicated antibodies. β-actin expression was used as a loading control.

B The ratio of LC3II to LC3I was quantified using ImageJ software. For normalization, β-actin expression was used as a control. The data were statistically analyzed by two-way ANOVA followed by Sidak's multiple comparison test (***$P < 0.001$ compared to the indicated points; ns, not significant; $n = 3$). The bars represent the mean ± SD.

Data information: All results in this figure are representative of three independent experiments.
Source data are available online for this figure.