Expanded View Figures

Figure EV1. The ubiquitination of FIP200 and ATG13 is not promoted by TRIM27, and ULK1 mRNA levels are not affected by TRIM27.

A, B Ubiquitination analysis of HA-FIP200 (A) or HA-ATG13 (B) co-expressed with Flag-TRIM27 and His-Ub in HEK293T cells by Western blot.

C Ubiquitination analysis of HA-ULK1 co-expressed with Flag-TRIM27 or Flag-ΔR-TRIM27 and His-Ub in HEK293T cells.

D Quantification of data presented in Fig 2D showing the mean ± SEM of three independent experiments. *P*-values were determined by a one-way ANOVA with Dunnett's multiple comparisons test, $*P < 0.05$.

E Relative ULK1 mRNA levels in HeLa cells with or without knockdown of TRIM27 using qPCR. Data are mean ± SEM of three independent experiments. *P*-values were determined by a one-way ANOVA with Dunnett's multiple comparisons test. ns, not significant.

F Relative ULK1 mRNA levels in HeLa cells with or without over-expression of Flag-TRIM27. Data are mean ± SEM of three independent experiments. *P*-values were determined by a two-tailed unpaired *t*-test. ns, not significant.

G Quantification of data presented in Fig 2E shows mean ± SEM of three independent experiments. *P*-values were determined by an unpaired *t*-test with Holm-Sidak's method for multiple *t*-tests, $*P < 0.05$. ns, not significant.

H Ubiquitination analysis of HA-ULK1 truncation constructs co-expressed with Flag-TRIM27 and His-Ub in HEK293T cells.

I Alignment of human and rodent ULK1 aa sequences shows conservation of TRIM27-mediated ubiquitination sites.

J Quantification of data presented in Fig 2L shows mean ± SEM of three independent experiments. *P*-values were determined by an unpaired *t*-test with Holm-Sidak's method for multiple *t*-tests, $**P < 0.01$. ns, not significant.

K Quantification of data presented in Fig 2O shows mean ± SEM of three independent experiments. *P*-values were determined by an unpaired *t*-test with Holm-Sidak's method for multiple *t*-tests, $**P < 0.01$. ns, not significant.

Source data are available online for this figure.

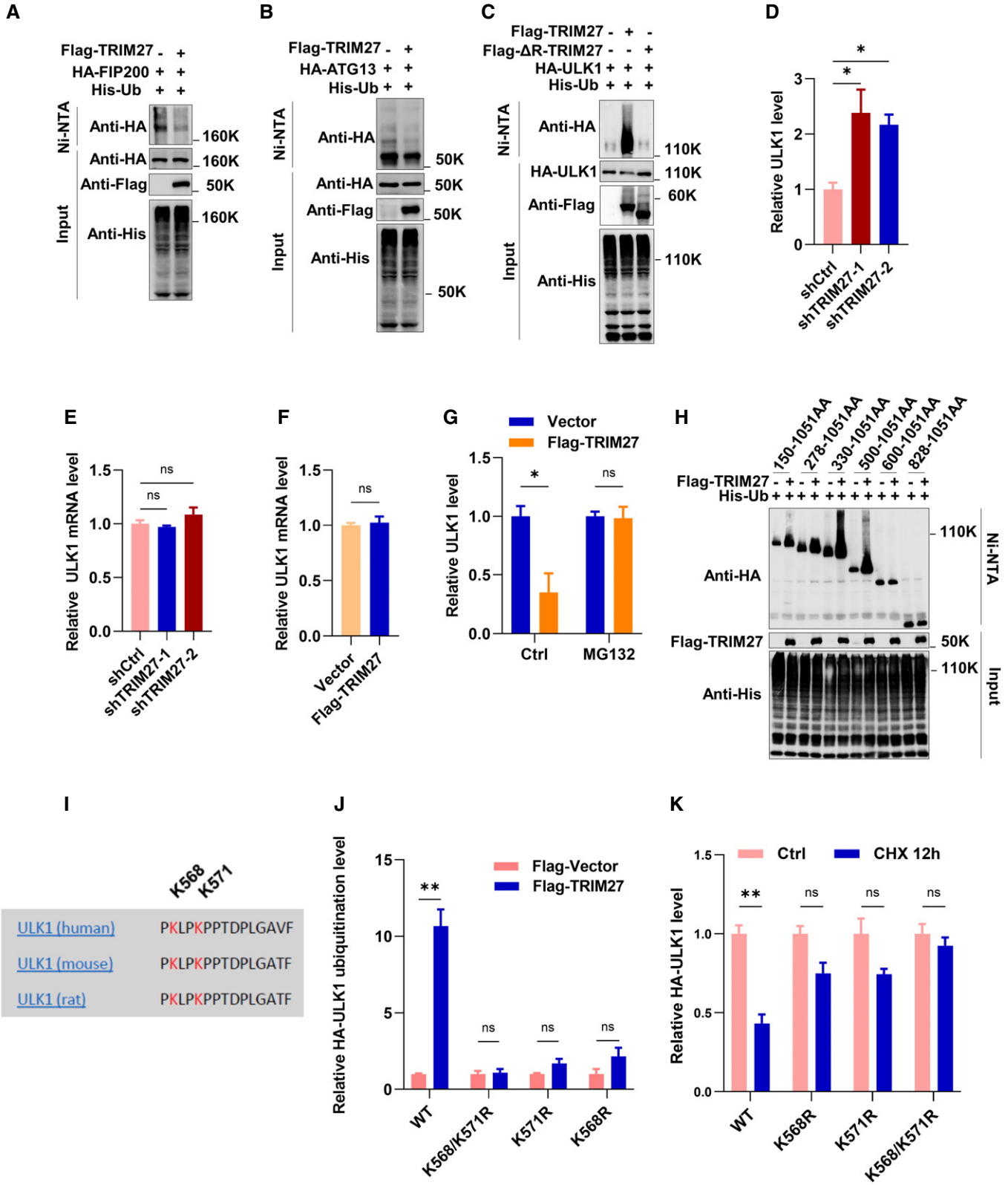
A
Flag-TRIM27 - +
HA-FIP200 + +
His-Ub + +
Ni-NTA
Anti-HA
160K
Anti-HA
160K
Anti-Flag
50K
Input
Anti-His
160K
B
Flag-TRIM27 - +
HA-ATG13 + +
His-Ub + +
Ni-NTA
Anti-HA
50K
Anti-HA
50K
Anti-Flag
50K
Input
Anti-His
50K
C
Flag-TRIM27 - + -
Flag-ΔR-TRIM27 - - +
HA-ULK1 + + +
His-Ub + + +
Ni-NTA
Anti-HA
110K
HA-ULK1
110K
Anti-Flag
60K
Input
Anti-His
110K
D
Relative ULK1 level
shCtrl
shTRIM27-1
shTRIM27-2
E
Relative ULK1 mRNA level
ns
shCtrl
shTRIM27-1
shTRIM27-2
F
Relative ULK1 mRNA level
ns
Vector
Flag-TRIM27
G
Vector
Flag-TRIM27
Relative ULK1 level
Ctrl
MG132
H
150-1051AA
278-1051AA
330-1051AA
500-1051AA
600-1051AA
828-1051AA
Flag-TRIM27 - + - + - + - + - + - +
His-Ub + + + + + + + + + + + +
Anti-HA
110K
Ni-NTA
Flag-TRIM27
50K
Anti-His
110K
Input
I
K568
K571
ULK1 (human) PKLPKPPTDPLGAVF
ULK1 (mouse) PKLPKPPTDPLGATF
ULK1 (rat) PKLPKPPTDPLGATF
J
Relative HA-ULK1 ubiquitination level
Flag-Vector
Flag-TRIM27
WT
K568/K571R
K571R
K568R
K
Ctrl
CHX 12h
Relative HA-ULK1 level
WT
K568R
K571R
K568/K571R

Figure EV1.

Figure EV2. Knockout of TRIM27 leads to an increase of autophagy in multiple tissues.

A Verification of ULK1 knockout in HeLa cells by Western blot.

B p62 or LC3 levels in HeLa ULK1 KO cells co-expressing vector control, HA-ULK1 WT, or HA-ULK1 K568/K571R.

C Quantification of LC3-II/LC3-I ratio, and LC3-II/actin ratio with Bafilomycin A1 treatment related to Fig 3C. Data are mean ± SEM for three independent experiments. *P*-values were determined by an unpaired *t*-test with Holm-Sidak's method for multiple *t*-tests, ***$P < 0.001$.

D Representative images of GFP fluorescence localization in HeLa ULK1 KO cells co-expressing GFP-LC3 together with vector control, HA-ULK1 wildtype, or HA-ULK1 K568/K571R, respectively.

E Quantitation of data presented in (D) shows mean number of GFP-LC3 puncta per cell ± SD ($n = 30$ cells per condition). *P*-values were determined by a one-way ANOVA with Tukey's multiple comparisons test, ***$P < 0.001$.

F, G Western blot analysis of ULK1 accumulation and LC3 conversion in the liver of 3-month-old $Trim27^{+/+}$ and $Trim27^{-/-}$ mice. Each lane represents a different mouse (F). Quantification of ULK1, LC3-II/LC3-I, and LC3-II/actin ratios in F (G).

H, I Western blot analysis of ULK1 accumulation and LC3 conversion in the spleen of 3-month-old $Trim27^{+/+}$ and $Trim27^{-/-}$ mice. Each lane represents a different mouse (H). Quantification of ULK1, LC3-II/LC3-I, and LC3-II/actin ratios in H (I).

J, K Western blot analysis of ULK1 accumulation and LC3 conversion in the brain of 3-month-old $Trim27^{+/+}$ and $Trim27^{-/-}$ mice. Each lane represents a different mouse (J). Quantification of ULK1, LC3-II/LC3-I, and LC3-II/actin ratios in J (K).

L, M Western blot analysis of ULK1 accumulation and LC3 conversion in the thymus of 3-month-old $Trim27^{+/+}$ and $Trim27^{-/-}$ mice. Each lane represents a different mouse (L). Quantification of ULK1, LC3-II/LC3-I, and LC3-II/actin ratios in L (M).

Data information: (G, I, K and M) Data are mean ± SEM for three mice per genotype. *P*-values were determined by an unpaired *t*-test with Holm-Sidak's method for multiple *t*-tests, *$P < 0.05$, **$P < 0.01$; ***$P < 0.001$.
Source data are available online for this figure.

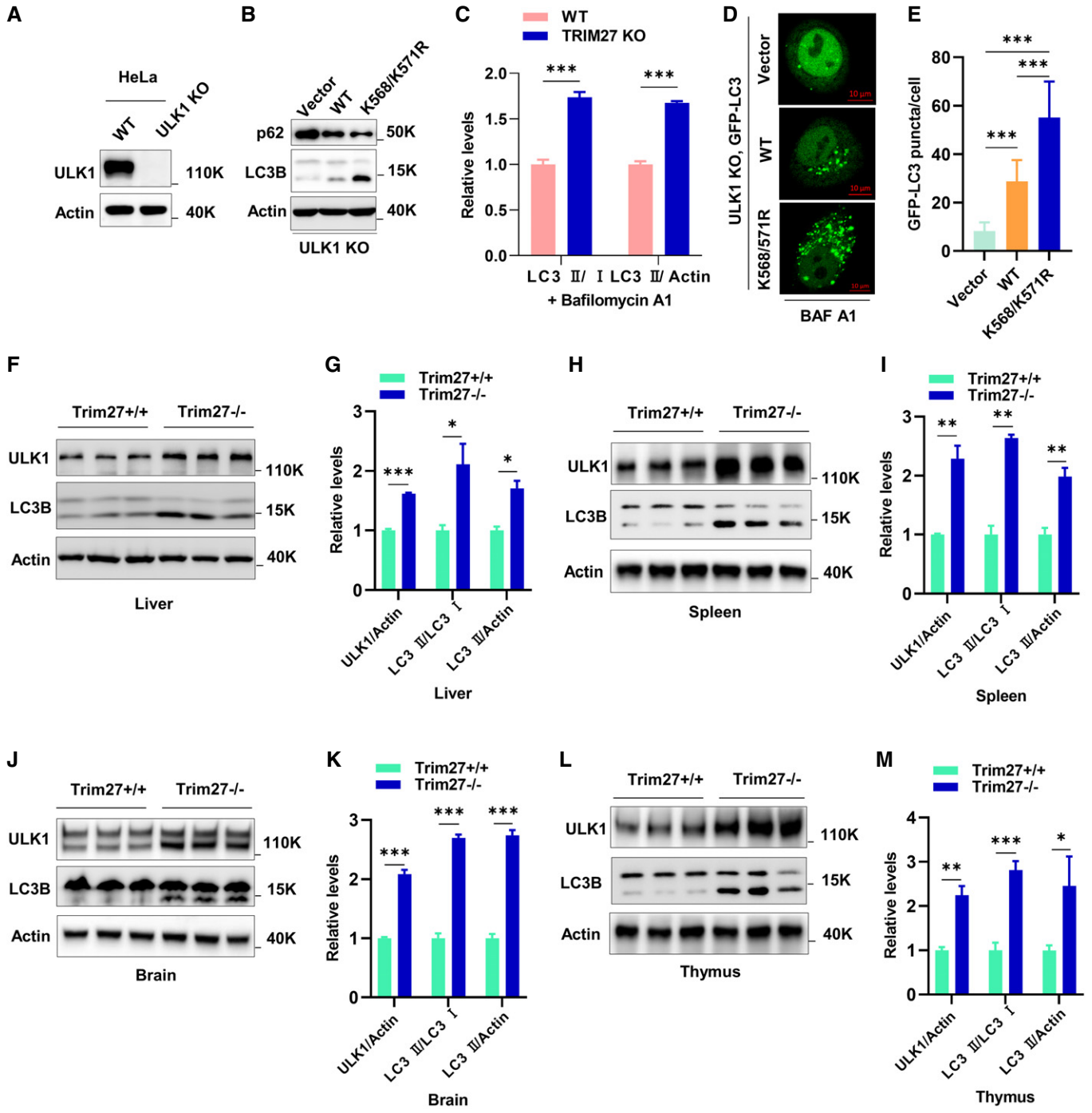
A
HeLa
WT
ULK1 KO
ULK1
110K
Actin
40K
B
Vector
WT
K568/K571R
p62
50K
LC3B
15K
Actin
40K
ULK1 KO
C
WT
TRIM27 KO
Relative levels
LC3 Ⅱ/ Ⅰ
LC3 Ⅱ/ Actin
+ Bafilomycin A1
D
ULK1 KO, GFP-LC3
Vector
WT
K568/571R
BAF A1
E
GFP-LC3 puncta/cell
Vector
WT
K568/K571R
F
Trim27+/+
Trim27-/-
ULK1
LC3B
Actin
Liver
G
Relative levels
ULK1/Actin
LC3 Ⅱ/LC3 Ⅰ
LC3 Ⅱ/Actin
Liver
H
Spleen
I
Spleen
J
Brain
K
Brain
L
Thymus
M
Thymus

Figure EV2.

Figure EV3. The phosphorylation modification of ULK1 S494 (mouse) mediated by STK38L promotes the binding of TRIM27 to ULK1.

A Quantification of data presented in Fig 5A shows mean ± SEM of three independent experiments. *P*-values were determined by a one-way ANOVA with Dunnett's multiple comparisons test, $*P < 0.05$, ns, not significant.

B Phosphorylation of Myc-TRIM27 in HeLa STK38L knockout cells with or without expression of Flag-STK38L. Myc-TRIM27 was immunoprecipitated and then analyzed by Western blot using anti-phospho-serine or –threonine antibodies. The presence of Myc-TRIM27 and Flag-STK38L were revealed using anti-Myc and anti-Flag antibodies as indicated.

C *In vitro* kinase assay using GST-STK38L, or GST-STK38L-K119A (a kinase-inactive mutant) and HA-ULK1 as substrate. GST-STK38L or GST-STK38L-K119A was pulled down using glutathione Sepharose beads. Recombinant HA-ULK1 was immunopurified from HEK293T using anti-HA and treated with lambda phosphatase. Reaction products were analyzed by Western blot using anti-phospho-serine along with anti-HA and anti-GST to confirm inputs.

D Co-immunoprecipitation between Flag-TRIM27 and the indicated HA-ULK1 mutants from co-transfected HEK293T cells.

E Co-immunoprecipitation between Flag-TRIM27 and HA-ULK1 or HA-ULK1 S494A in HEK293T cells.

F Western blot measuring STK38L levels in HEK293T with or without over-expression of Flag-TRIM27.

G Western blot measuring STK38L levels in HeLa with or without knockout of TRIM27.

H Quantification of data presented in Fig 5M shows mean ± SEM of three independent experiments. *P*-values were determined by a one-way ANOVA with Dunnett's multiple comparisons test, $*P < 0.05$, $***P < 0.001$.

I Quantification of data presented in Fig 5N shows mean ± SEM of three independent experiments. *P*-values were determined by an unpaired *t*-test with Holm-Sidak's multiple comparisons test, $**P < 0.01$, ns, not significant.

Source data are available online for this figure.

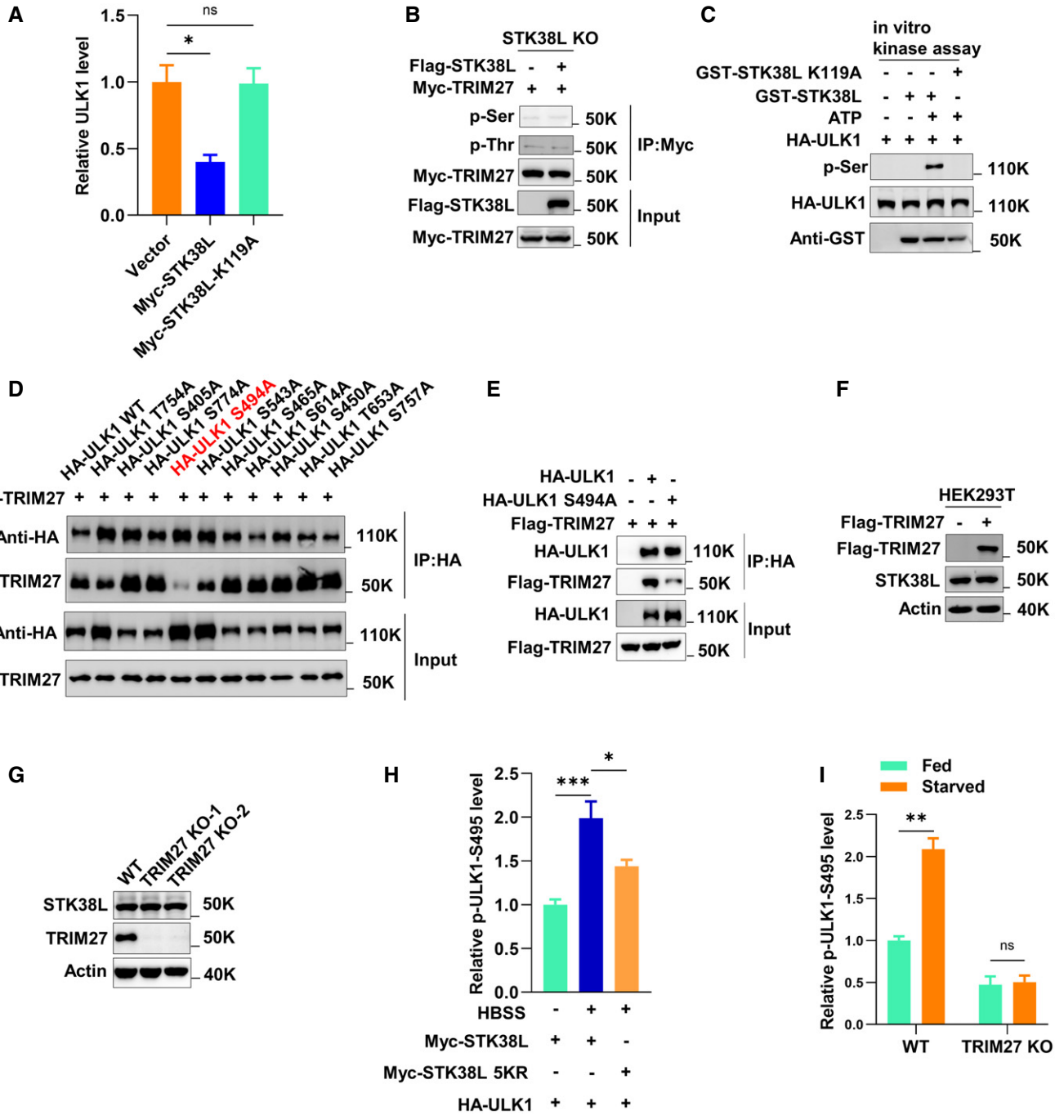
A
Relative ULK1 level
1.5
1.0
0.5
0.0
ns
*
Vector
Myc-STK38L
Myc-STK38L-K119A
B
STK38L KO
Flag-STK38L - +
Myc-TRIM27 + +
p-Ser 50K
p-Thr 50K
Myc-TRIM27 50K
IP:Myc
Flag-STK38L 50K
Myc-TRIM27 50K
Input
C
in vitro kinase assay
GST-STK38L K119A - - - +
GST-STK38L - + + -
ATP - - + +
HA-ULK1 + + + +
p-Ser 110K
HA-ULK1 110K
Anti-GST 50K
D
HA-ULK1 WT
HA-ULK1 T754A
HA-ULK1 S405A
HA-ULK1 S774A
HA-ULK1 S494A
HA-ULK1 S543A
HA-ULK1 S465A
HA-ULK1 S614A
HA-ULK1 S450A
HA-ULK1 T653A
HA-ULK1 S757A
Flag-TRIM27 + + + + + + + + + + +
Anti-HA 110K
Flag-TRIM27 50K
IP:HA
Anti-HA 110K
Flag-TRIM27 50K
Input
E
HA-ULK1 - + -
HA-ULK1 S494A - - +
Flag-TRIM27 + + +
HA-ULK1 110K
Flag-TRIM27 50K
IP:HA
HA-ULK1 110K
Flag-TRIM27 50K
Input
F
HEK293T
Flag-TRIM27 - +
Flag-TRIM27 50K
STK38L 50K
Actin 40K
G
WT
TRIM27 KO-1
TRIM27 KO-2
STK38L 50K
TRIM27 50K
Actin 40K
H
Relative p-ULK1-S495 level
2.5
2.0
1.5
1.0
0.5
0.0
***
*
HBSS - + +
Myc-STK38L + + -
Myc-STK38L 5KR - - +
HA-ULK1 + + +
I
Fed
Starved
Relative p-ULK1-S495 level
2.5
2.0
1.5
1.0
0.5
0.0
**
ns
WT
TRIM27 KO

Figure EV3.

Figure EV4. Identification of TRIM27-mediated ubiquitination sites in STK38L.

A Ubiquitination analysis of Flag-STK38L in HEK293T cells co-expressing Myc-TRIM27 together with either His-Ub WT, or the His-Ub mutants as indicated, respectively. Total cell lysates were subject to ubiquitination analysis involving recovery of His-labelled proteins by Ni-NTA followed by Western blot against Flag (top panel). Transfection and loading efficiency were determined by Western blot of input samples (bottom panels).

B The amino acid sequence of STK38L contains a total of 38 lysine residues (yellow) ordered from the N- to C-terminus. Five numbered lysine residues in red (K181, K215, K224, K432 and K434) relate to subsequent findings in panels (C) and (D).

C Identification of STK38L ubiquitination sites. Ubiquitination analysis of Myc-STK38L or the indicated K to R substitution mutants in HEK293T cells co-expressing HA-TRIM27 and His-Ub before analysis of STK38L ubiquitination. Myc-STK38L K1-38R represents the Myc-STK38L mutant with all lysine (K) residues mutated to arginine (R). K1-K13R represents the mutant that the 1st to 13th lysine residues of STK38L all mutated to arginine. The other mutant designations were similarly named. As anticipated, mutation of all lysines (K1-38R) ablated the total ubiquitination signals in the presence of TRIM27 while mutation of lysines 1–19 also showed a large decrease in STK38L ubiquitination. Lesser effects observed with other mutant constructs indicated the ubiquitination sites were primarily located between lysine residues 16–19 and in the C-terminal region. Some ubiquitination signals for K1-19R indicate other potential sites exist.

D Refinement of the principal C-terminal ubiquitination sites in STK38L. Myc-STK38L mutants co-expressing HA-TRIM27 and His-Ub were co-transfected with individual Myc-STK38L mutants into HEK293T cells before analysis of STK38L ubiquitination. Myc-STK38L-K432/K434 represents the Myc-STK38L mutant only containing K432 and K434 lysine residues, while all other lysine (K) residues mutated to arginine (R). The other mutants were similarly named. Interpretation of the data indicates the major ubiquitination sites as K181, K215, K224, K432 and K434.

Source data are available online for this figure.

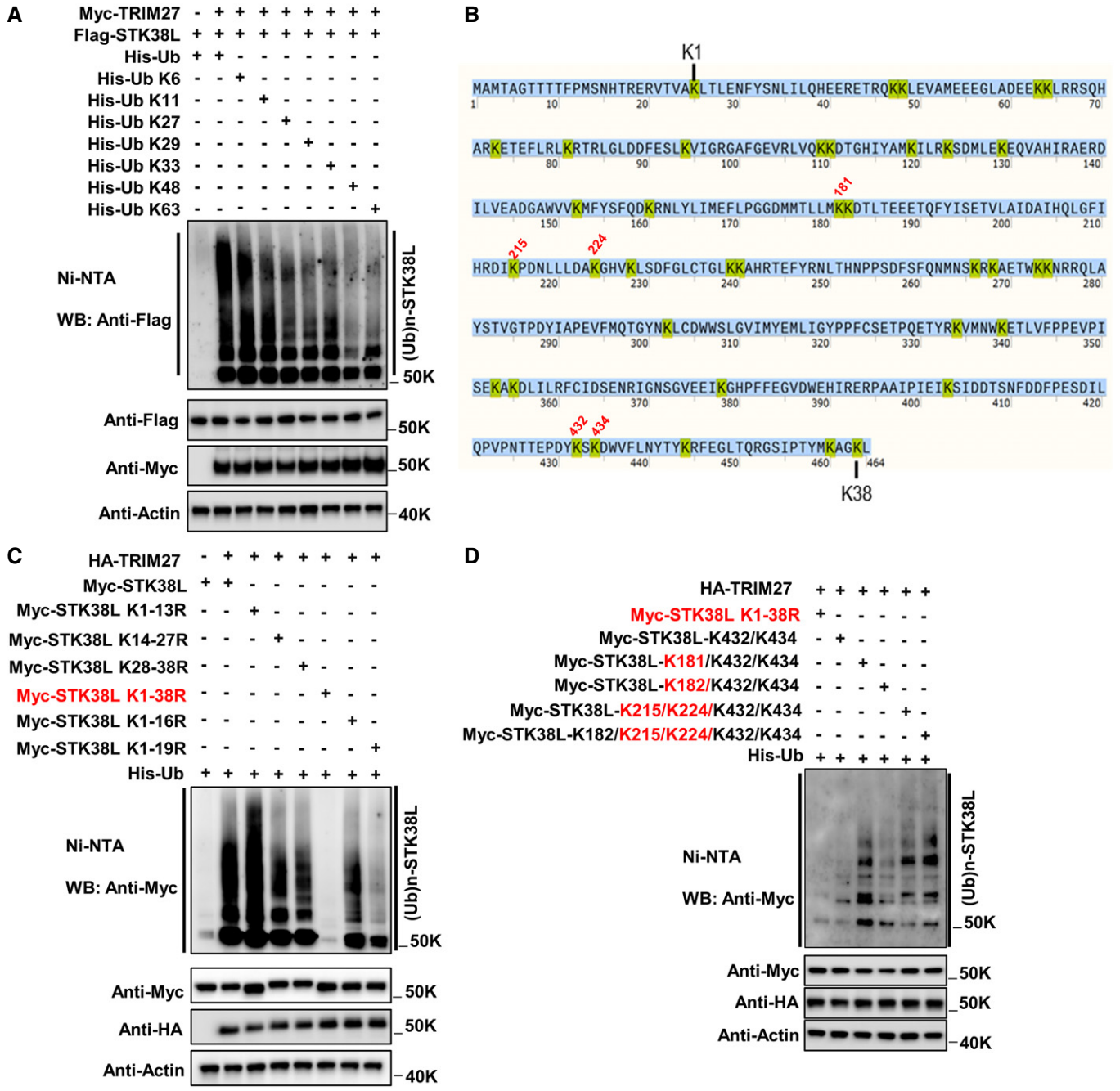

Figure EV4.

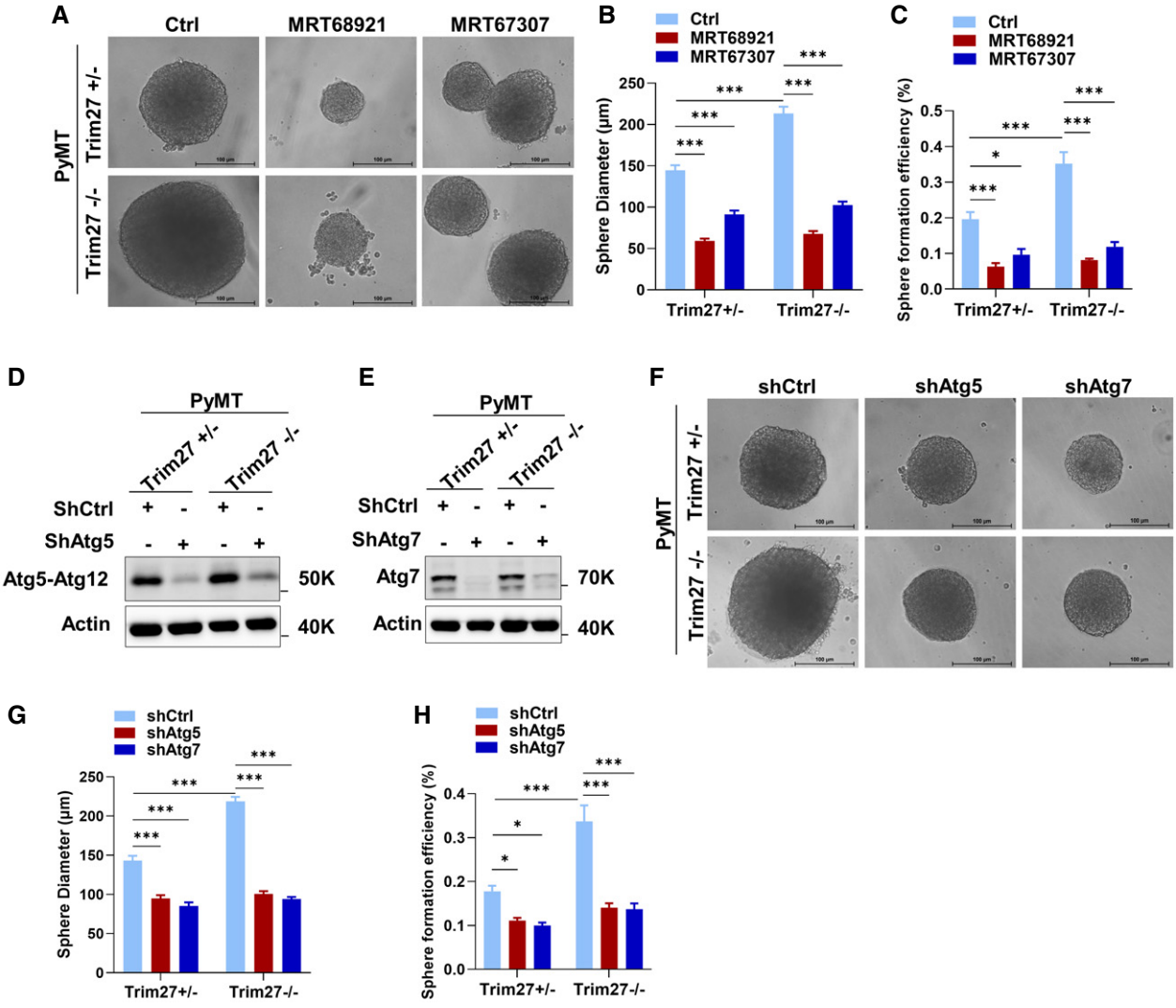

Figure EV5. Organoid growth promotion in TRIM27 knockout tumors and metastasis *in vivo* is dependent on autophagy.

A Representative morphology of Trim27$^{+/-}$, Trim27$^{-/-}$ PyMT organoids in 3D sphere formation assays treated with vehicle (DMSO), 100 nM MRT68921 or MRT67307. Scale bar, 100 μm.

B, C Quantification of sphere diameter (B) (n = 24) and sphere formation efficiency (diameter ≥ 50 μm) (C) in the indicated groups from three independent pairs of tumors. Data are mean ± SEM; P-values were determined by a two-way ANOVA with Tukey's multiple comparisons test, *P < 0.05, ***P < 0.001.

D, E Verification of Atg5 (D) and Atg7 (E) knockdown in PyMT Trim27$^{+/-}$ and Trim27$^{-/-}$ tumor cells by Western blot.

F Representative morphology of Trim27$^{+/-}$, Trim27$^{-/-}$ PyMT organoids in 3D sphere formation assays transfected with control or Atg5 or Atg7 knockdown vectors. Scale bar, 100 μm.

G, H Quantification of sphere diameter (G) (n = 24) and sphere formation efficiency (diameter ≥ 50 μm) (H) in the indicated groups from three independent pairs of tumors. Data are mean ± SEM; P-values were determined by a two-way ANOVA with Tukey's multiple comparisons test, *P < 0.05, ***P < 0.001.

Source data are available online for this figure.