Supplementary Materials and Methods

Differential preparation of the cytoplasmic and nuclear proteins

The cytoplasmic and nuclear proteins of the 293T cells were prepared using a Cytoplasmic and Nuclear Protein Extraction Kit purchased from Beyotime Biotechnology (#P0027). The preparation of the different fractions was performed according to the manufacturer's protocol.

Oncomine bioinformatic analysis

To assess the gene expression changes in cancers and in normal tissues, an oncomine bioinformatic analysis was employed using the Oncomine online services (www.oncomine.org).

Supplementary Figure Legends

Figure S1. The repeated experiments of Figure 1I.

293T cells were transfected with a control or a USP44 plasmid for 48 h. The cells were then lysed and subjected to western blot analysis using the indicated antibodies. The p62 protein levels were semi-quantified and the data were shown in the right (n=5).

Figure S2. The H2B monoubiquitination levels are decreased during starvation-induced autophagy activation.

(**A**) HeLa cells were starved by treating with HBSS for the indicated time periods. Cell extracts were then prepared and subjected to western blot analysis using the indicated antibodies.

(**B**) Control or HBSS-treated (starvation) HeLa cells were lysed and subjected to western blot and RT-PCR analyses using specific antibodies or primers, as indicated.

(**C**) Control or HBSS-treated (starvation) HEK293T cells were lysed and subjected to western blot analyses using specific antibodies (left) or realtime RT-PCR analyses by using specific primers (right) (two-tailed unpaired *t* test, n=3), as indicated. Three biological replicates were analyzed.

(**D**) Cell extracts from HEK293T cells treated with or without starvation for the indicated time periods were prepared and subjected to Co-IP analysis using an anti-RNF20 antibody, followed by western blot assays using the indicated antibodies.

(**E**) Cytoplasmic and nuclear extracts from HEK293T cells treated with or without starvation for different time periods were separately prepared. The lysates were then subjected to western blot assays using the indicated antibodies.

(**F**) HEK293T and HeLa cells were transfected with a control or an ATG5- or ATG7-specific siRNA, and the total proteins were prepared. A western blot analysis was performed using specific antibodies, as indicated.

(**G**) Control or ATG5 RNAi or ATG7 RNAi HEK293T and HeLa cells were treated with or without starvation for 10 h. The cell extracts were then lysed and subjected to western blot assays using the indicated antibodies.

(**H**) HeLa cells treated with starvation or 2 μM rapamycin for 10 h. The cells were then lysed and subjected to western blot assays using the indicated antibodies.

Figure S3. The effect of Rapamycin treatment on the levels of H2Bub1.

H1299, SMMC, HeLa and HEK293T cells were treated with rapamycin for a time course or dose course analysis as indicated. The cells were harvested and subjected to western blot analyses with specific antibodies as indicated.

Figure S4. The expression correlation between DNMT3a and DNMT3b and USP44 in cancer and normal tissues.

The bioinformatic analyses for the gene expression changes between the normal and cancer tissues were performed using the Oncomine online services (www.oncomine.org).

Figure S5. The RNAi efficiency assay of USP44 RNAi in HEK293T cells.

(**A**) HEK293T cells were transfected with a control or USP44-specific siRNAs for 48 h. The total RNA was then prepared and subjected to an RT-PCR assay using specific primers for USP44 to determine the interference efficiency of the USP44 siRNAs.

(**B**) HEK293T cells were transfected with a control or USP44-specific siRNAs for 48 h. The cells were then treated with or without HBSS (starvation) for the indicated time periods. Cell extracts were prepared and subjected to western blot analysis using the indicated antibodies.

Supplementary Tables

Table S1. Primer Sequences

Real-Time RT-PCR primers

Gene Name	Primers
p62/SQSTM1 (human)	F: CTCACCGTGAAGGCCTACCT R: CGTCCTCATCGCGGTAGTG
USP44 (human)	F: GGGCACTACACTGCCTACTG R: CAGGAGCTCTGGAGGCAAAA
DNMT3a (human)	F: CCTGTGGGAGCCTCAATGTT R: CCACACACTCCACGCAAAAG
DNMT3b (human)	F: GCCATGTACCATGCTCTGGA R: CACGACGCACCTTCGACTTA
ZBTB42 (human)	F: ACTCGGGTGAGAAGCCCTAT R: CAGTGAAACTTGCGGACGTG
NTN4 (human)	F: CTGCTGCTGCTCTGGGG R: CCGACAAGTCAGATCCGTGT
PTGER2 (human)	F: GCTGGGGAACCTCATAGCAC R: CGTACGAAGCCAGTACCACT
TRIM32 (human)	F: TCCTGGCCAAGATCAAGCAG R: TTCCACGTTAACTGTCCGGG
TACC2 (human)	F: TGATGGCGTTGTCTGTGTTTC R: AGTTGTAAGTCCCACTGCTGG
MBP (human)	F: CCGGCAAGAACTGCTCACTA R: CCCCCAGCTAAATCTGCTCA
TRAF3IP2 (human)	F: ATCTCAGCTTTCAGCGGCTT R: TCCTGGGGCTGGGAATCATA
GPR161 (human)	F: ATGCTGGTGTGCTATGGCTT R: CATTCCTCCTGCTGCCTGAA
WFDC1 (human)	F: ATGAGTGCCACATCCTGAGC R: GCTTCTGTTGTCCCCTTCCA
CXCL12 (human)	F: TGAGCTACAGATGCCCATGC R: TAGCTTCGGGTCAATGCACA
EPHA6 (human)	F: GACCCTCGTGCCCTGTTC R: AGAAATTCCCGGACTTCGCA
TPR (human)	F: TGATGCTTCGGAACATGCCT R: GGTGGAGGGGAGATCTGACT
TNFAIP3 (human)	F: CTTGTGGCGCTGAAAACGAA R: GCAAAGCCCCGTTTCAACAA
PLAGL1 (human)	F: AAGTAGCTTGCCAGCTGAGG R: GGCTTGCAAGTGGGGAGTAT
SLC25A13 (human)	F: GCAGAAGGCCTCAGGTGATT R: AGAGCCAGTTGATCGTTGGT

CENPK (human)	F: CGCTGAACTCAGTCAATGGC R: ATCCAACCACCGTTGTTCCC
USP36 (human)	F: CATCGACGCCATGCAGAAAG R: AGTAGGGGTCGTAGGTGTCC
GABBR2 (human)	F: CCATCGAGCAGATCCGCAA R: AGACGCCTCCAAACACCATC
ZBTB10 (human)	F: AAGGAGTTGATGCAGGACGG R: GGGAAGTGCTGGACAGTCTC
NFKBIZ (human)	F: CCGCTCAACCTGAGCTACTT R: ATATGGGGCTCAACTGGCTG
C5orf41 (human)	F: TTCAAGGTCATGCCACTCCC R: TGGCTGTTCACCCAAGTTGT
USP44 (mouse)	F: CTTACTCGACGGCACCCAAT R: TCTTCGCCTCTCGTTTTGCT
USP7 (human)	F: CATGGAGATGGAAGCGGGAG R: TCACTCAGTCTGCTGAAGCG
USP12 (human)	F: CAGCAAACAGGAAGCACACA R: ATTGGGACCACTTCCACAGT
USP22 (human)	F: TGGACAACTGGAAGCAGAACC R: ACAGCCGAAGAAGACACAGTAG
USP46 (human)	F: CTGTCCGAAACATCGCCTCC R: GTACAATGCCTGAAGCACGG
USP49 (human)	F: TGGGGTCCATGTCGTCTTTG R: TGCAGTGGACCCAAAAACCT

Semi-quantitive RT-PCR primers (Figure S1B and S3A)

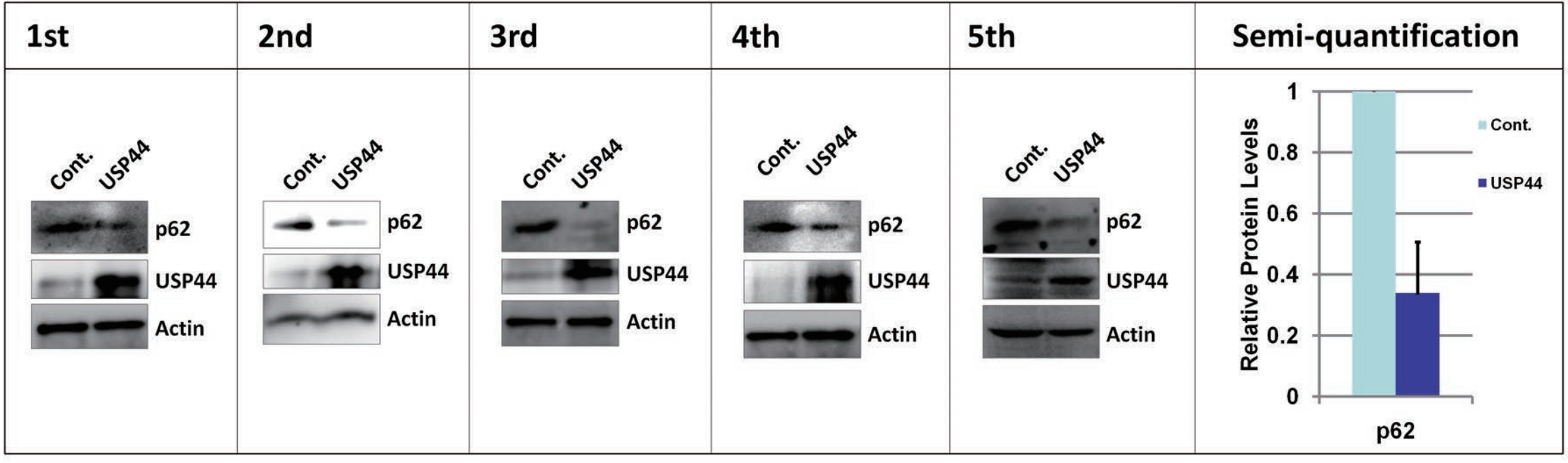
Gene Name	**Primers**
USP44 (human)	F: CTTGCCTTAGCTGCTCCCAT R: GACTGAGCGAGCCCTTGTAA

ChIP-PCR primers

Gene Name	**Primers**
USP44 (human)	F: GTTTGAGGAGAAAGTGAATTTT R: TAAAAATCAACTTCCTAAAACCA
USP44 (mouse)	F: TAAAGAATTGGCCCGGTCGT R: AAAACTTCCTCTCAGGGCGG
ZBTB42 (human)	F: GGTTTACCCTCGGGTGACTG R: TCTCTCGCGATACTTGGCG
NTN4 (human)	F: AAATCCATCTGAGTTAGCTTCCA R: ATTTCTTAACTCTTTCAGTGATGCT
PTGER2 (human)	F: AGTTGCTTTGCTTTGCTCGTT R: CGTGTTGCCGGTGTACTGA
TRIM32 (human)	F: GCACCAAGGAGACGGAAAACT R: CAGAGCTCCCTGTCCGTCAC

TACC2 (human)	F: GAGGAGAGGGCTGGAAAATGA R: GGTGCCTAGCACAGTAGATGA
MBP (human)	F: CCAAGACTCCTGGGTTCACAT R: AGAGGGTCGATGGCTACTGAA
TRAF3IP2 (human)	F: CTGAGCACTGAGCTGAGAAA R: GAGGGGTGGGTTTGTGGAAT
GPR161 (human)	F: ATTATTGGGCCACTGAGGCA R: GGAGCGTCAGCTTCCAGTTT
WFDC1 (human)	F: TGGAGAGTGGAGAGTGACAGG R: GGTCCCTCGGATCATGTGT
CXCL12 (human)	F: CCTTTGACCTTCTCAGGCTCC R: AGTTCCCGCCATCGAAAGG
EPHA6 (human)	F: TGCGAAGTCCGGGAATTTCTT R: CCAGTACCCTGGTTCTCCAAG
TPR (human)	F: TCTTCACAGGCAAGTCACAA R: TGTTGAATGAATAGATGCAGC
TNFAIP3 (human)	F: CTGGATGCTTGGTGAGCAGA R: TCCATTGTTCCTGCCAGCTC
PLAGL1 (human)	F: GCAGCCGTCATTTTGGATGT R: ACGAGACTGGGACTATGGCT
SLC25A13 (human)	F: TTCATGCGCCTCTGACCATT R: AATGCATGTTGCCCTGTTGC
CENPK (human)	F: GCAAACTCCTTAGTCACACCC R: CCTCAATCCCTGTTCTTTGGCT
USP36 (human)	F: CAGTCGCTGAACAAATCTCCG R: TGTGGCTCCCAATCTCTACC
GABBR2 (human)	F: CTTTCAGAGGAGCGCAGGAA R: TAGGCTGGGCCGGTAAATCA
ZBTB10 (human)	F: AAGTTCACTTCCTTGGGCACC R: AGGACAAACAGCTGGCGAGT
NFKBIZ (human)	F: ATGCTGTCACGTACTTGGGT R: TGCCCCAAAGCAAGTATGGC
C5orf41 (human)	F: AGTCACGCGATTTCCGGG R: GTTCCGAGCAGCGGGTG

Figure S1


1st
2nd
3rd
4th
5th
Semi-quantification
Cont.
USP44
p62
USP44
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Relative Protein Levels
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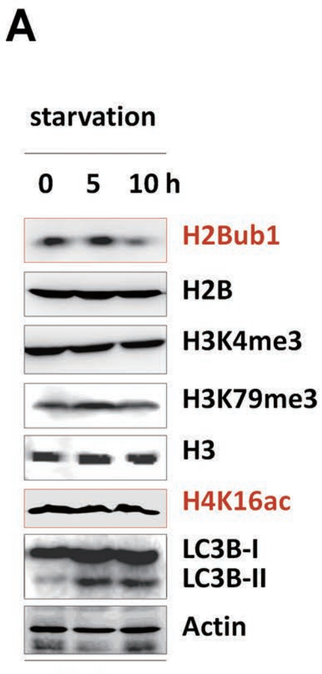
0.6
0.4
0.2
0
Cont.
USP44
p62

Figure S2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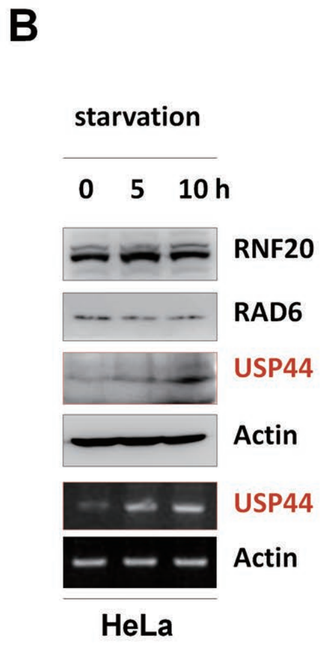
LC3B-I
LC3B-II
Actin
HeLa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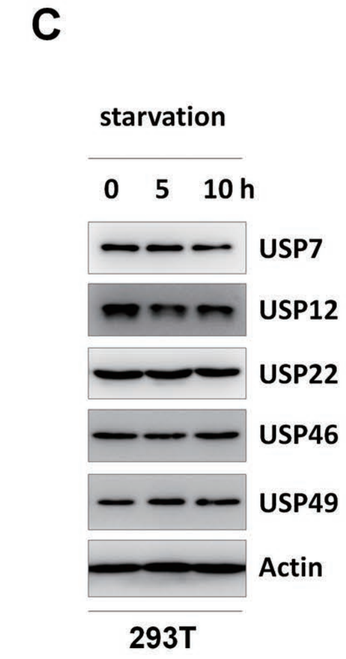
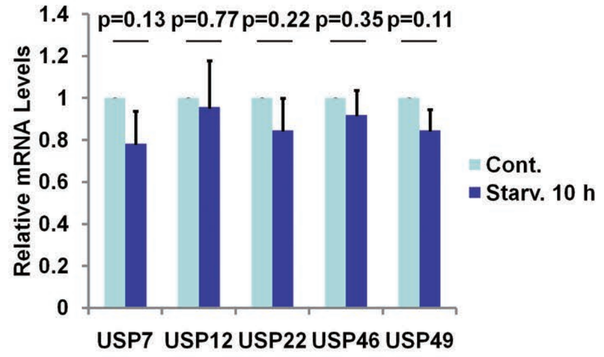
0 5 10 h
RNF20
RAD6
USP44
Actin
USP44
Actin
HeLa


C
starvation
0 5 10 h
USP7
USP12
USP22
USP46
USP49
Actin
293T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1.2
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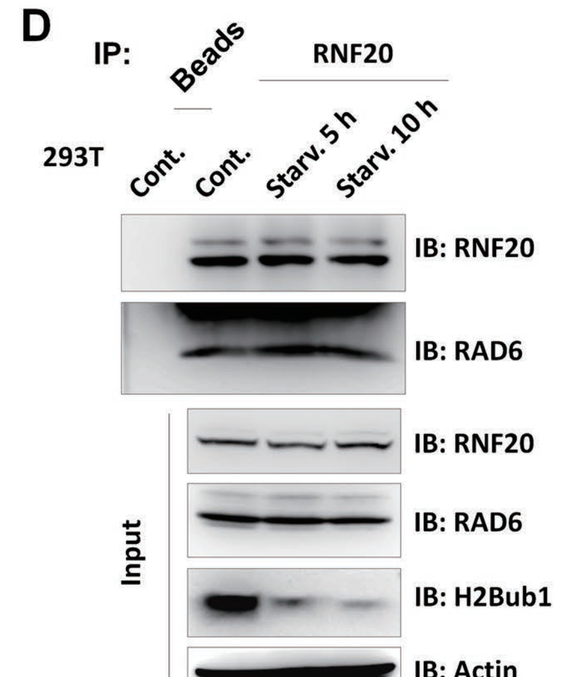
0.8
0.6
0.4
0.2
0
USP7 USP12 USP22 USP46 USP49
Cont.
Starv. 10 h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IP:
Beads
RNF20
293T
Cont.
Cont.
Starv. 5 h
Starv. 10 h
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IB: RAD6
Input
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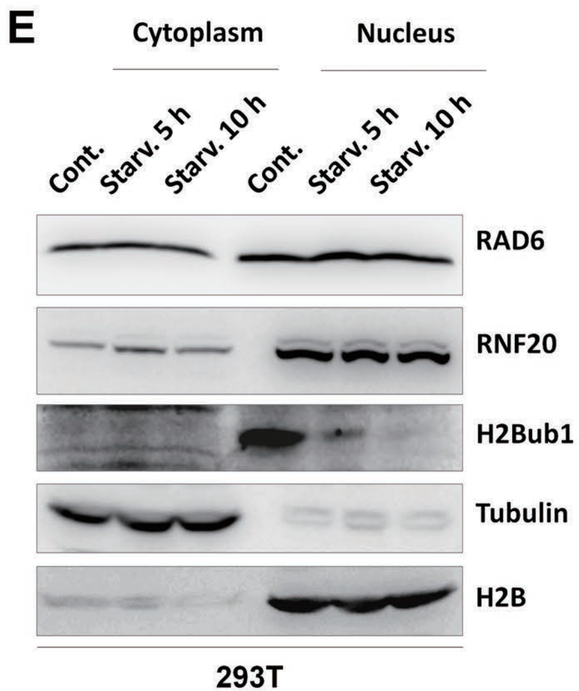
IB: RAD6
IB: H2Bub1
IB: Actin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Cytoplasm
Nucleus
Cont.
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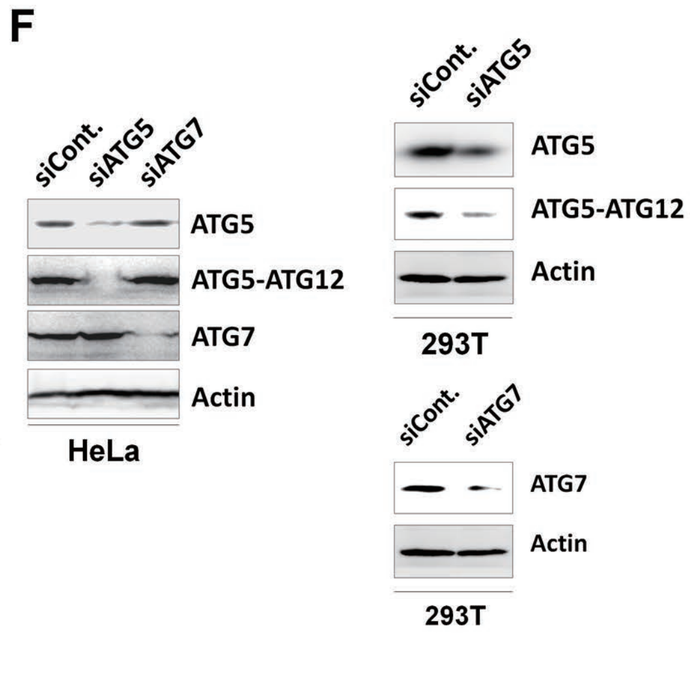
Starv. 10 h
Cont.
Starv. 5 h
Starv. 10 h
RAD6
RNF20
H2Bub1
Tubulin
H2B
293T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ATG5-ATG12
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HeLa
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Actin
293T
siCont.
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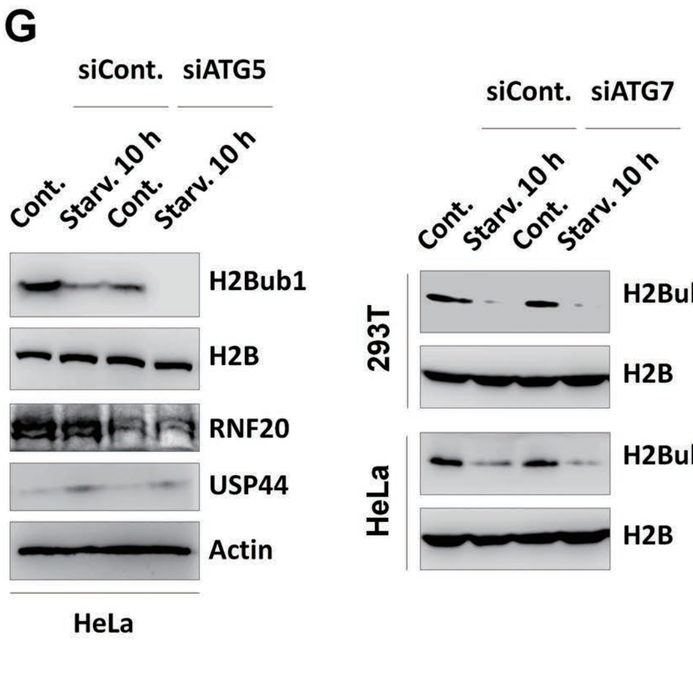
ATG7
Actin
293T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Cont.
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Cont.
Starv. 10 h
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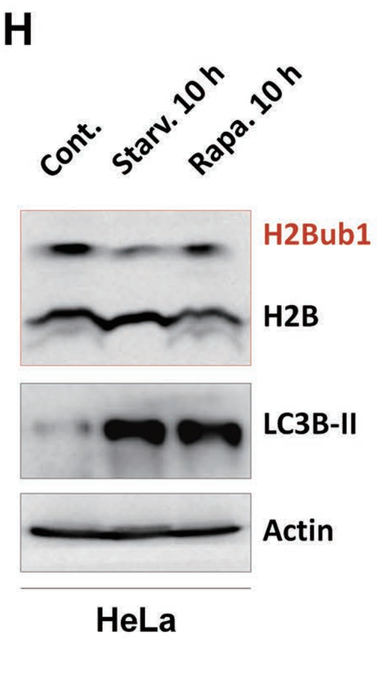
H2B
RNF20
USP44
Actin
HeLa
siCont.
siATG7
Cont.
Starv. 10 h
Cont.
Starv. 10 h
293T
H2Bub1
H2B
HeLa
H2Bub1
H2B


H
Cont.
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Rapa. 10 h
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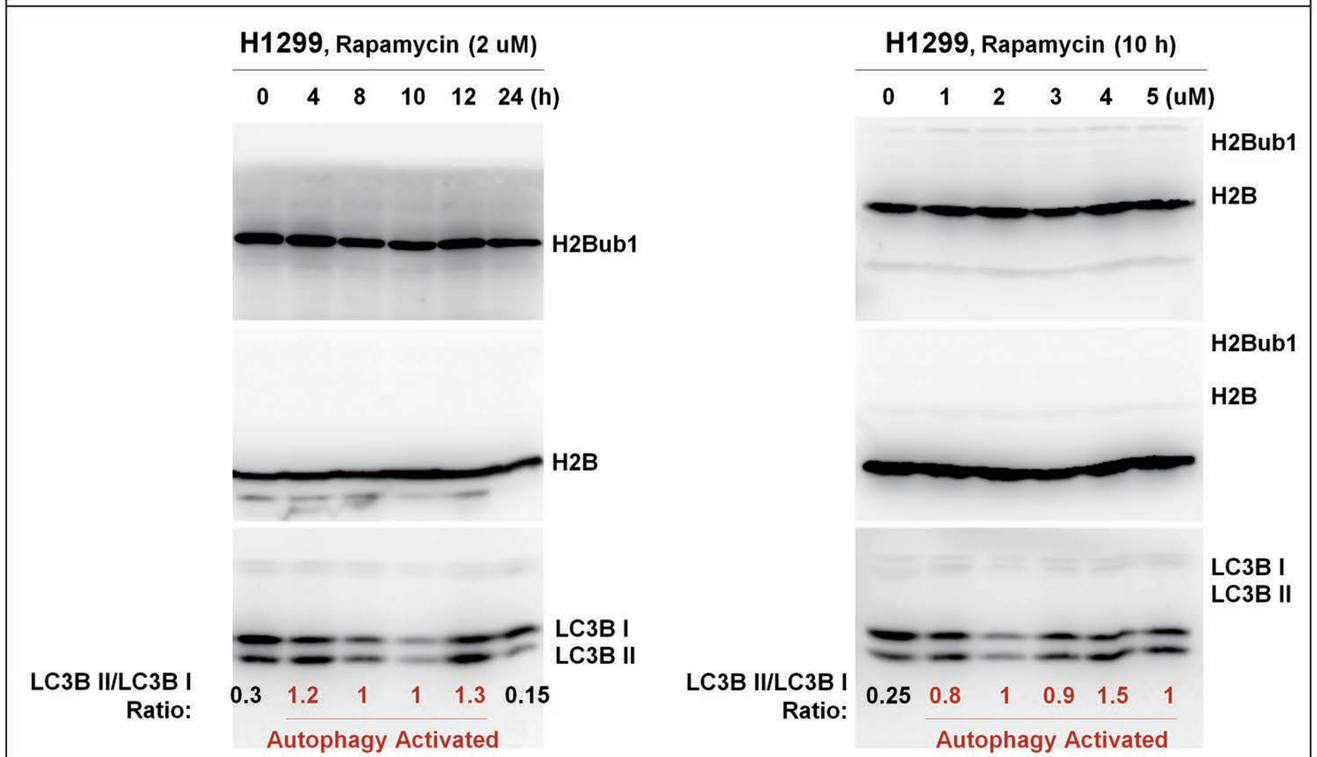
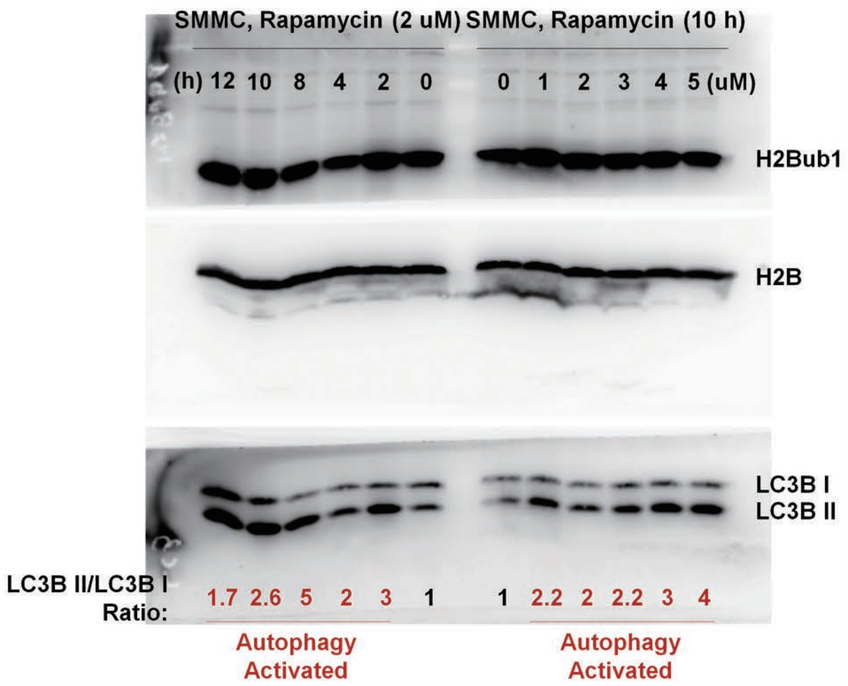
H2B
LC3B-II
Actin
HeLa

Figure S3

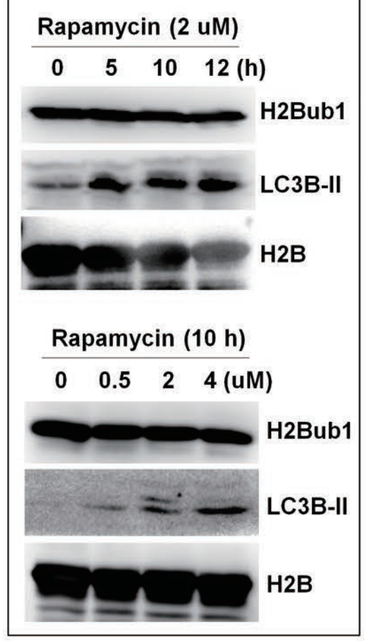
H1299 Cells



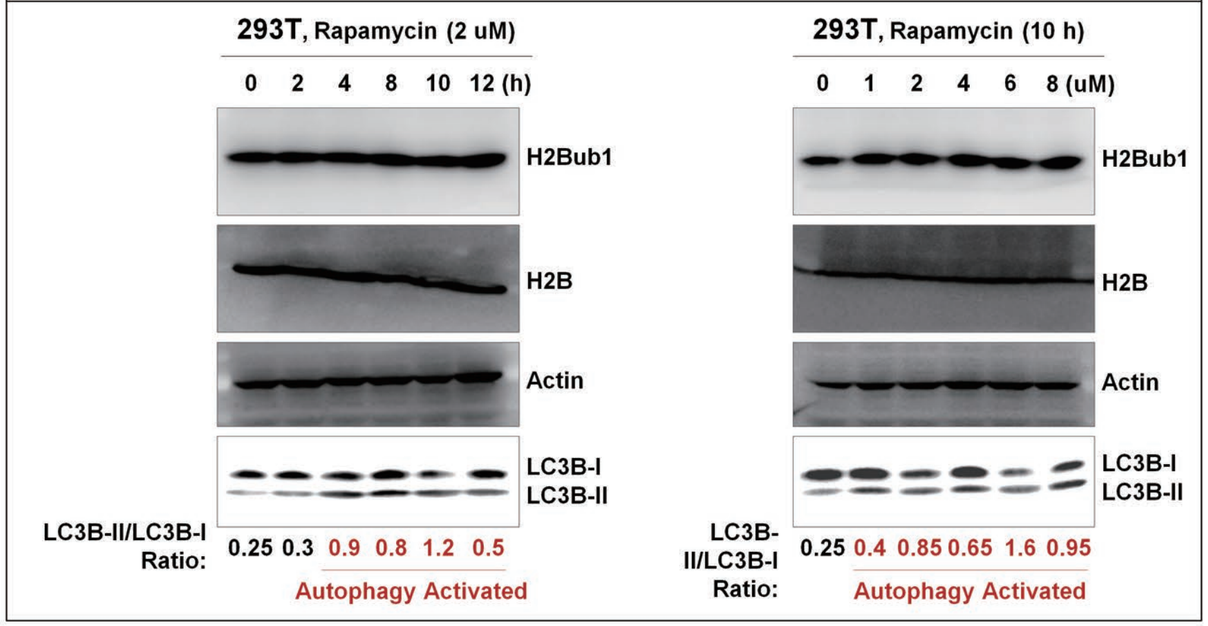
SMMC Cells



HeLa Cells



293T Cells 1st



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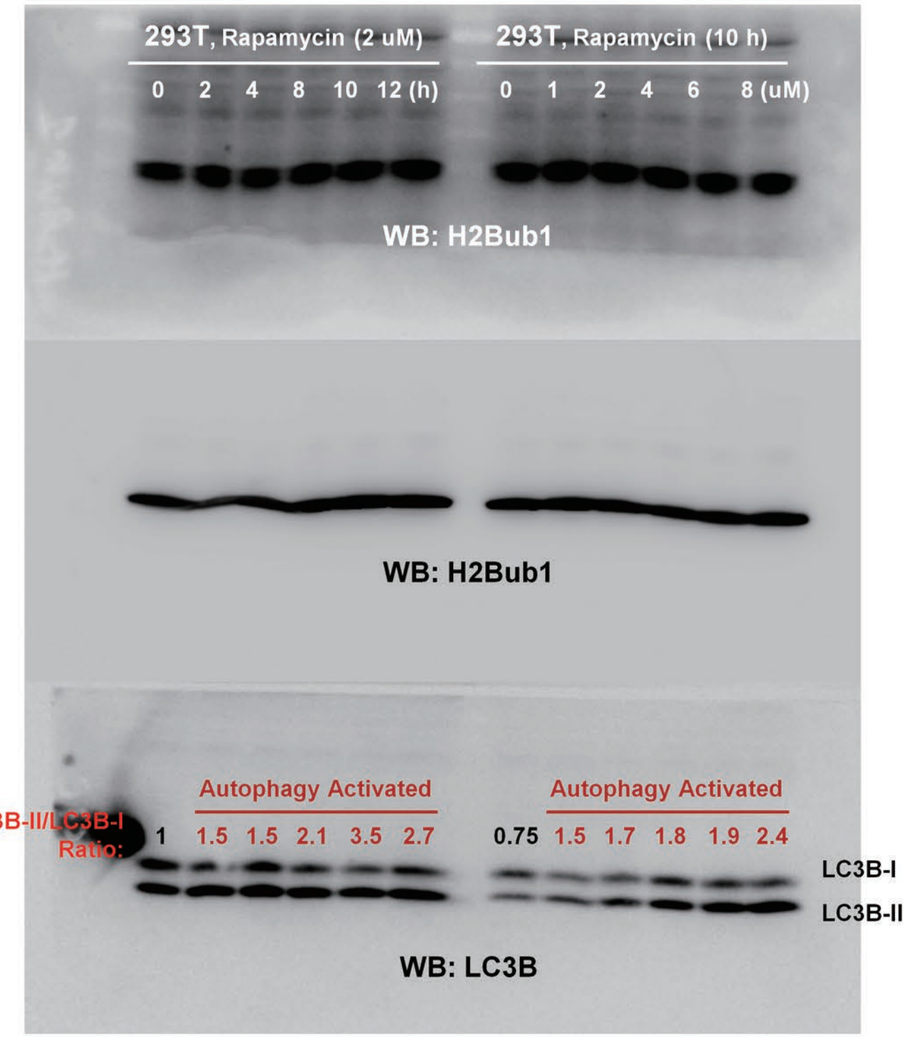
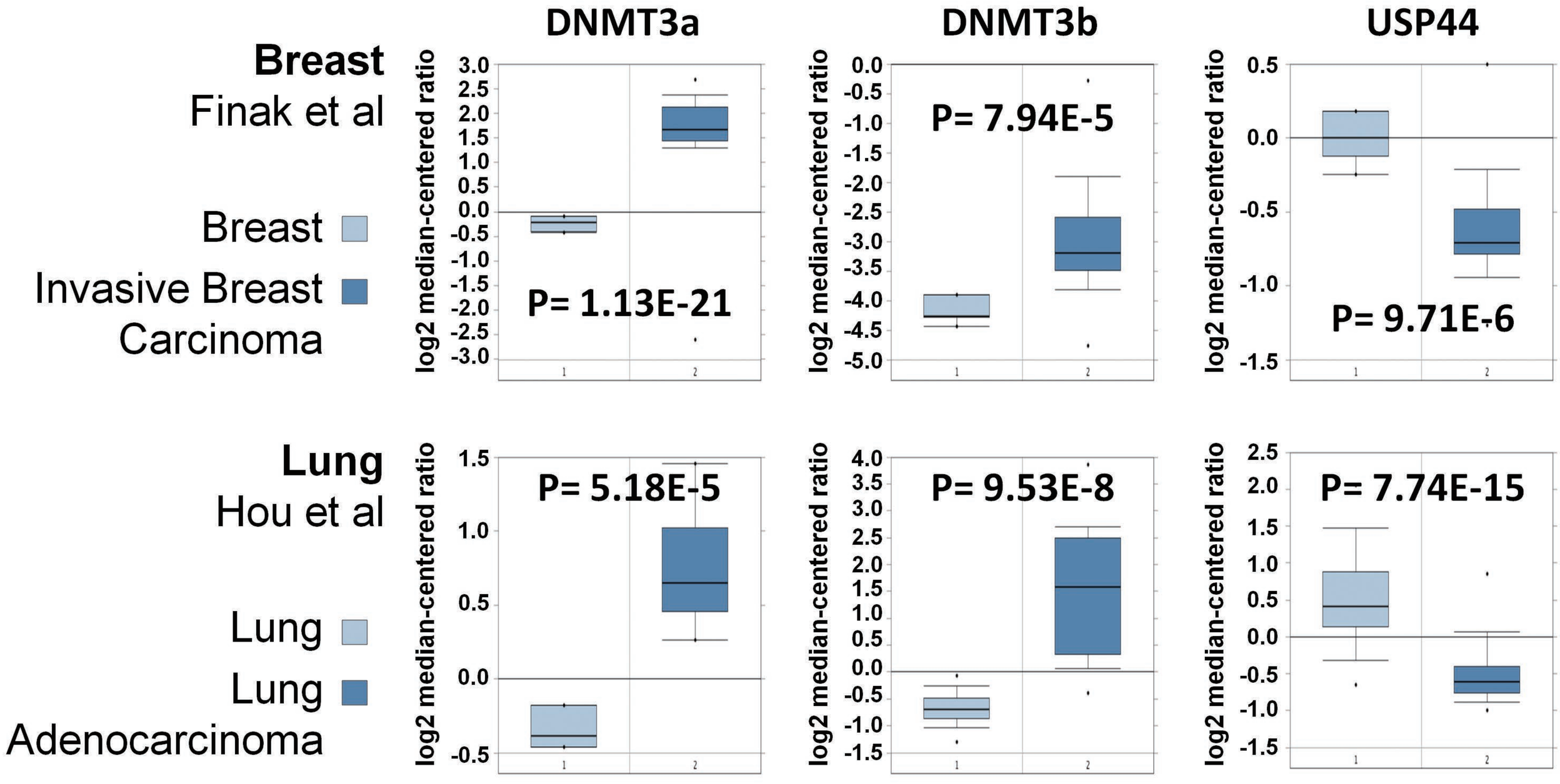


Figure S4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DNMT3b
USP44
Breast
Finak et al
Breast
Invasive Breast Carcinoma
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P= 7.94E-5
P= 9.71E-6
Lung
Hou et al
Lung
Lung Adenocarcinoma
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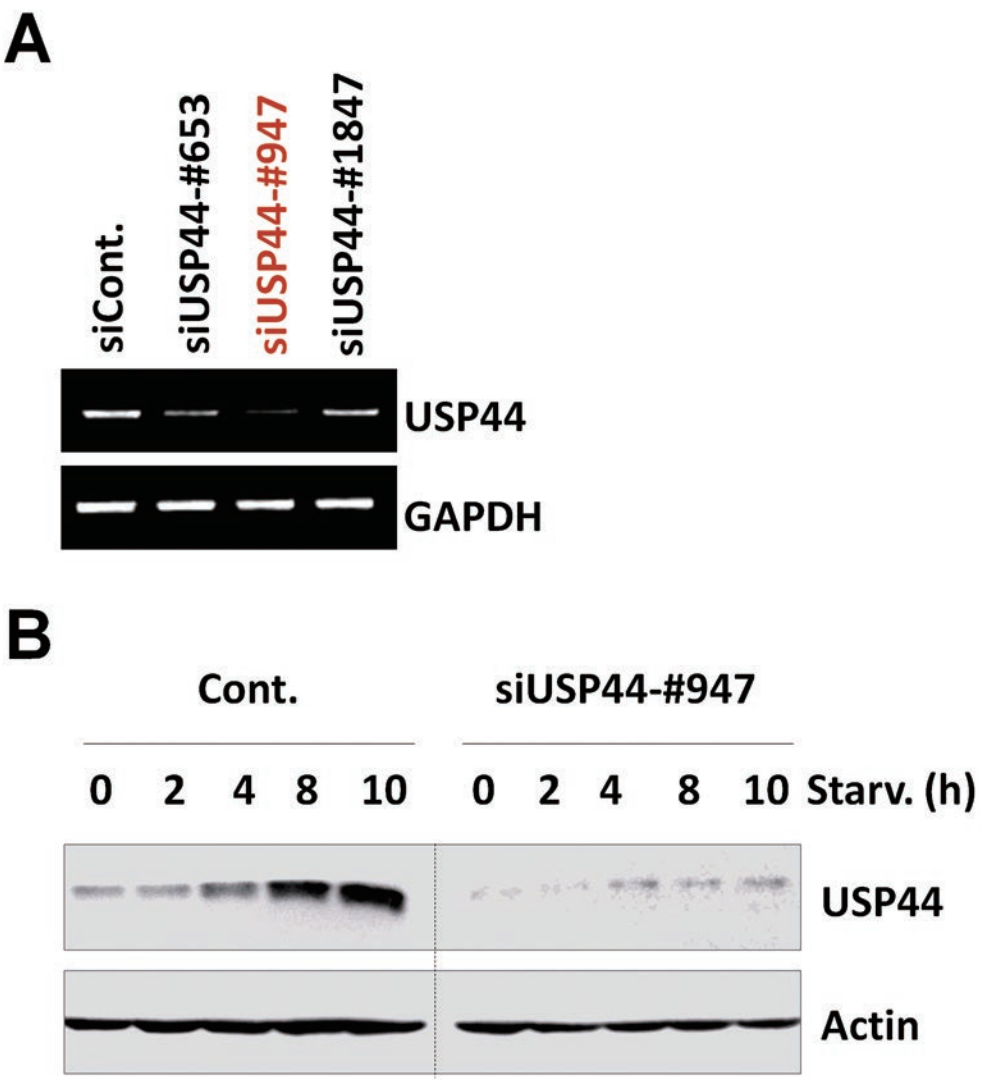
Figure S5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USP44
Actin