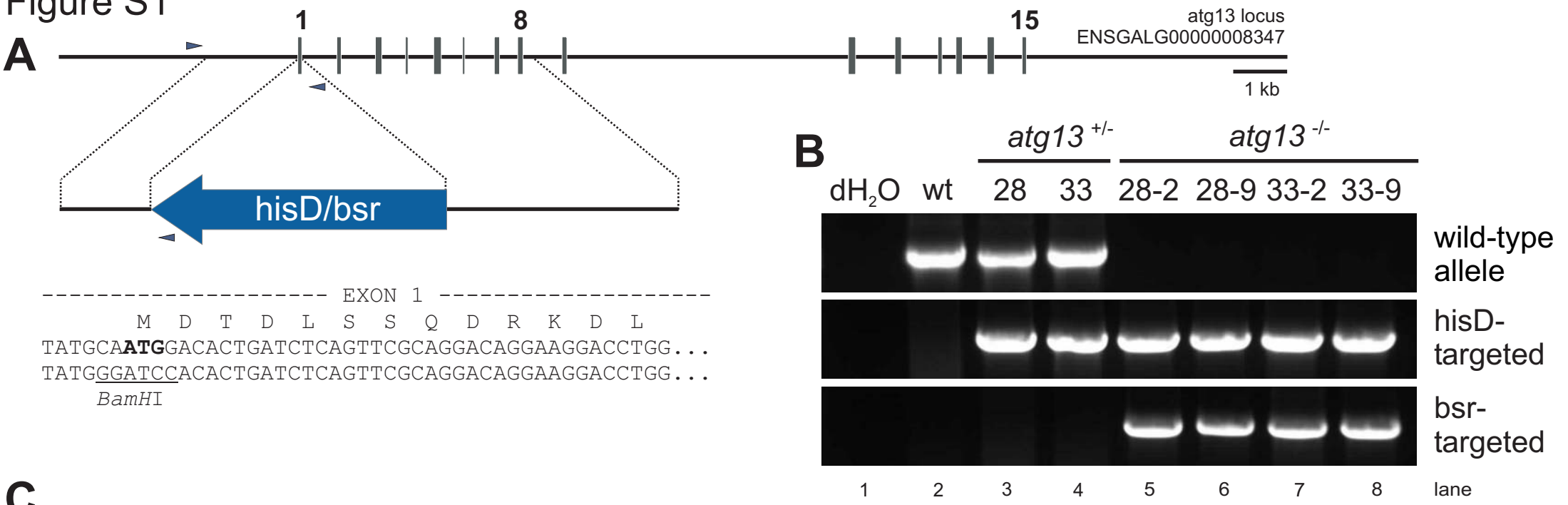
Figure S1



C

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Homo sapiens  METDLNSQDRKDLDKFIKFFALKTVQVIVQARLGEKICTRSSSSPTGSDWFNLAIKDIPEVTHEAKKALAGQLPAVGRSMCVEISLKTSEGDSMELEIWCLEMNEKCDKEIKVSYTVYNR  120
Gallus gallus MDTDLSSQDRKDLDKFIKFFALKTVQVIVQARLGEKICTRSSSSPTGSDWFNLAIKDIPEVTHEAKKALAGQLPAVGRSMCVEISLKTSEGDSMELEIWCLEMNEKCDKEIKVSYTVYNR  120
              *:***.**********************************************************************************************************

Homo sapiens  LSLLLKSLLAITRVTPAYRLSRKQGHEYVILYRIYFGEVQLSGLGEGFQTVRVGTVGTPVGTITLSCAYRINLAFMSTRQFERTPPIMGIIIDHFVDRPYPSSSPMHPCNYRTAGEDTGV  240
Gallus gallus LSLLLKSLLAITRVTPAYRLSRKQGHEYVILYRIYFGEVQLSGLGEGFQTVRVGTVGTPVGTITLSCAYRINLAFMSTRQFERTPPIMGIIIDHFVDRPYPSSSPMHPCNYRAG-EDNGA  239
              **********************************************************************************************************:. **.*.

Homo sapiens  IYPSVEDSQEVCTTSFSTSPPSQLMVPGKEGGVPLAPNQPVHGTQADQERLATCTPSDRTHCAATPSSSEDTETVSNSSEG-RASPHDVLETIFVRKVGAFVNKPINQVTLTSLDIPFAM  359
Gallus gallus VYPSVEDSQEVCTTSFSTSPPSQLIGPGKEGGVPPVPSQPAHGTQADQERMCTPLDG-VHYSAATPSSSEDTETVSNSSEGKCGSPHDLLETIFIRKVGAFVNKPINQVTMANLDIPFAM  358
              :************************: ******** .*.**.*********:.*   .   :.******************  .****:*****:***************::.*******

Homo sapiens  FAPKNLELEDTDPMVNPPDSPETESPLQGSLHSDGSSGGSSGNTHDDFVMIDFKPAFSKDDILPMDLGTFYREFQNPPQLSSLSIDIGAQSMAEDLDSLPEKLAVHEKNVREFDAFVETLQ 480
Gallus gallus FAPKNVELEDNDPMVNPPDSPETESPLQGSLHSEGSSGSSAGNTHDDFVMIDFKPAFSKDDILPMDLGTFYREFQNPPQLSSLSIDIGAQSMAEDLDSLPEKLAVHEKNVKEFDAFVETLQ 479
              *****:****.**********************:****.*:**************************************************************:**********
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Figure S1 (A) Schematic representation of *atg13* locus (ENSGALG00000008347) and pAtg13-hisD and pAtg13-bsr targeting construct. The start codon (ATG) in exon 1 was mutated and hisD and bsr resistance cassettes were ligated into this newly generated *Bam*HI site. (B) Successful targeting was confirmed by genomic PCR using primers specific for wild-type, hisD- or bsr-targeted alleles. Primer annealing sites are indicated by grey arrow heads in (A). Two heterozygous clones (28 and 33, lanes 3-4) were subjected to a second round of transfection and selection, resulting in four independent homozygous clones (28-2, 28-9, 33-2 and 33-9, lanes 5-8). Both wild-type alleles have been replaced by hisD and bsr resistance cassette, respectively. (C) ClustalW amino acid sequence alignment of *Homo sapiens* Atg13 isoform 2 (NP_055556.2) and *Gallus gallus* Atg13 isoform A using BLOSUM matrix.

Figure S2

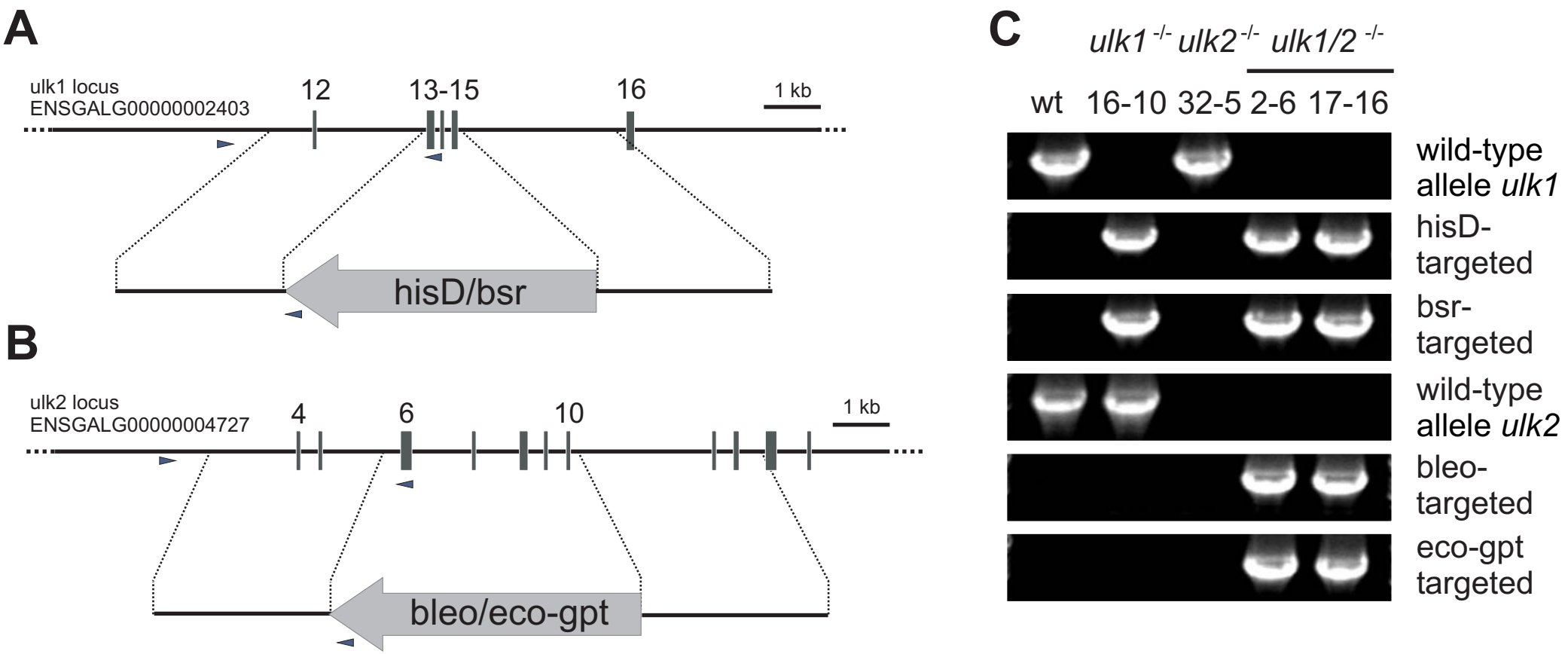
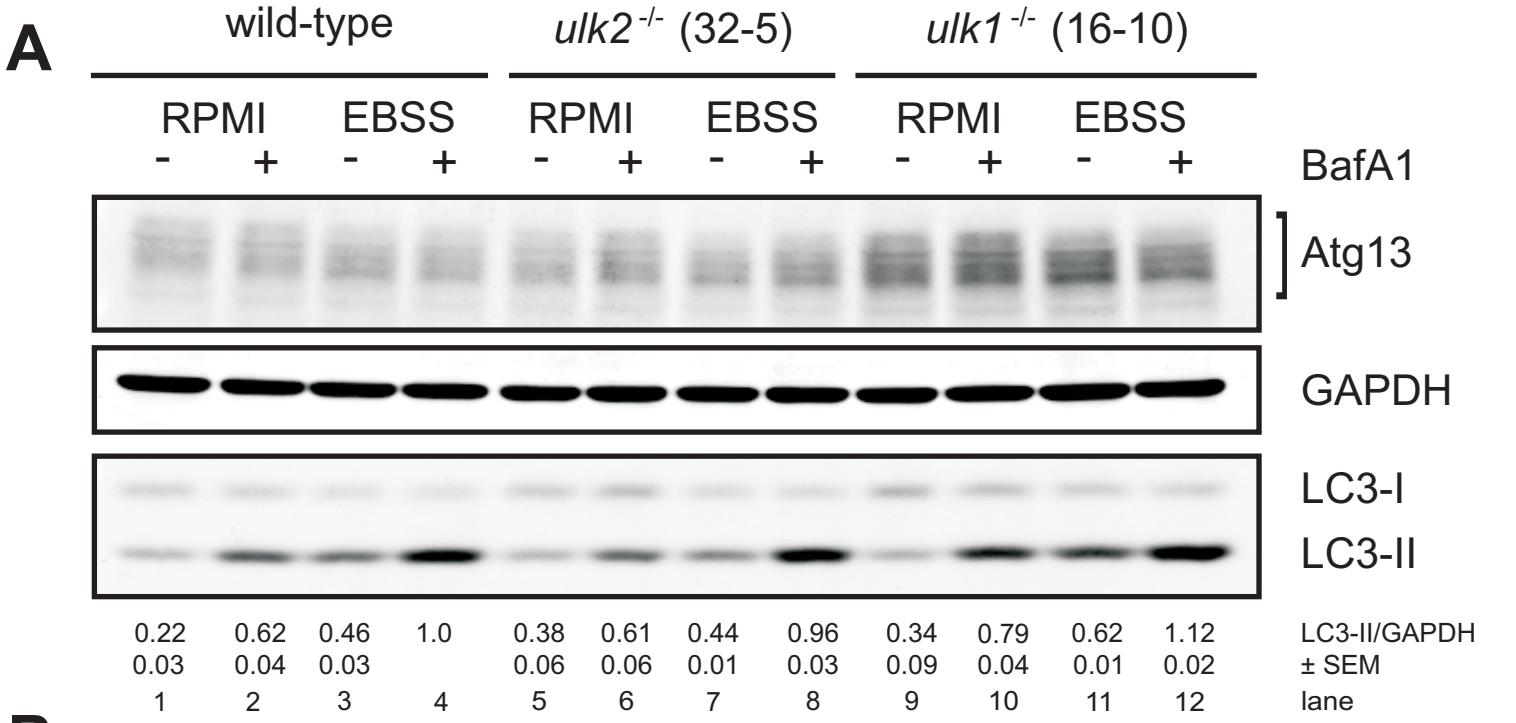
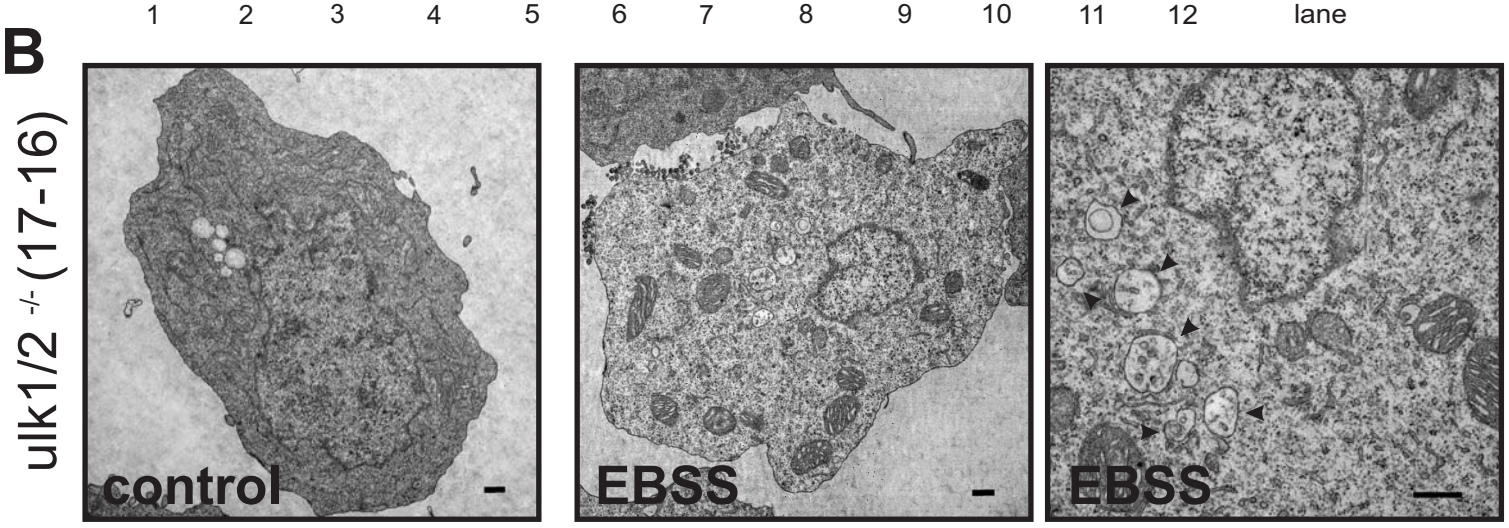


Figure S2 (A) Schematic representation of *ulk1* (ENSGALG00000002403) locus and pUlk1-hisD and pUlk1-bsr targeting constructs. (B) Schematic representation of *ulk2* (ENSGALG00000004727) locus and pUlk2-bleo and pUlk2-eco-gpt targeting constructs. (C) DT40 cells deficient for Ulk1 (*ulk1*$^{-/-}$), Ulk2 (*ulk2*$^{-/-}$) or Ulk1 and Ulk2 (*ulk1/2*$^{-/-}$) were generated by gene targeting and loss of wild-type alleles was confirmed by genomic PCR using primers specific for wild-type, hisD-, bsr-, bleo- or eco-gpt-targeted alleles. Primer annealing sites are indicated by grey arrow heads in (A) and (B). *ulk1/2*$^{-/-}$ cells were generated from *ulk1*$^{-/-}$ cells (16-10) using the targeting construct depicted in (B). *ulk2*$^{-/-}$ cells were generated using the modified targeting constructs pUlk2-hisD and pUlk2-bsr. For further details see Supplementary Material and Methods.

Figure S3





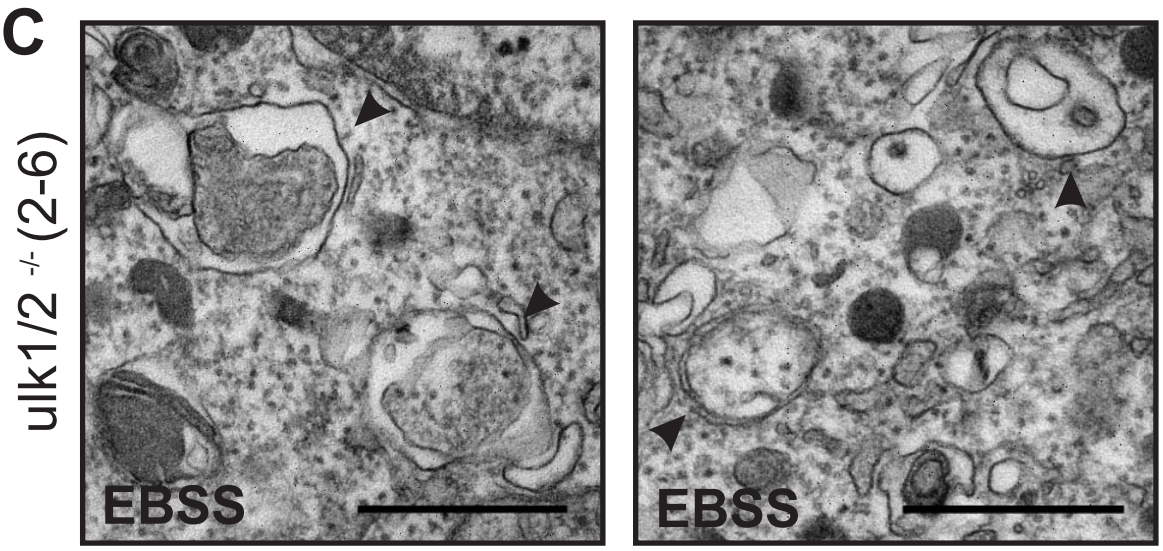
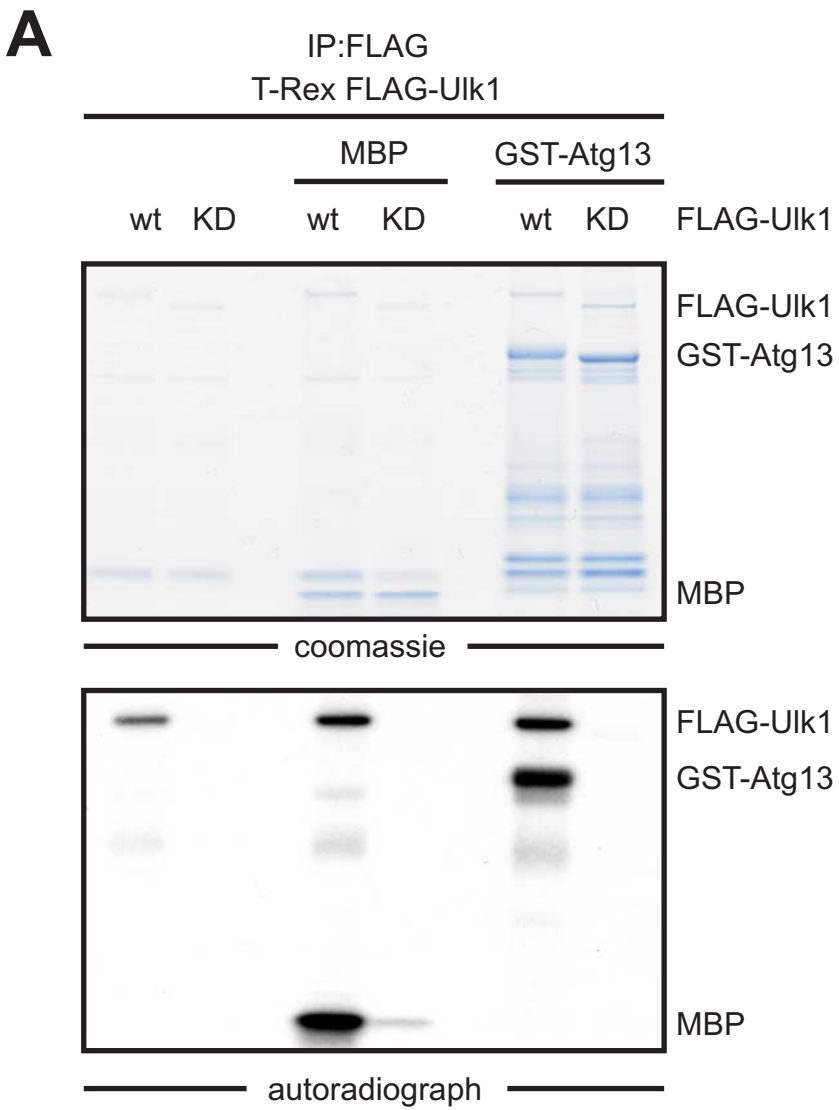


Figure S3 (A) Wild-type cells, *ulk1*$^{-/-}$ (clone 16-10) and *ulk2*$^{-/-}$ cells (clone 32-5) were incubated in full medium (RPMI) or EBSS in the presence or absence of 10 nM bafilomycin A_1 (BafA1) for 1 h. Equal amounts of protein from cleared cellular lysates were analyzed for Atg13, GAPDH and LC3 by immunoblotting. (B) *ulk1/2*$^{-/-}$ cells (clone 17-16) were treated as described in Figure 3C and analyzed by transmission electron microscopy. For starvation condition, a representative cell is shown in two different magnifications. Autophagosomes are indicated by black arrow heads in the image with higher magnification (bars: 500 nm). (C) Higher magnification from *ulk1/2*$^{-/-}$ cells (clone 2-6) described in Figure 3C (bars: 500 nm). Black arrow heads indicate autophagosomes.

Figure S4



B

Observed (m/z)	Theoretical Mass (Da)	Score	Peptide Sequence	Phosphorylated Residue
639.905218	1277.521698	45	R.EFDAFVE**pT**LQ.	**T 478**
782.592573	1562.749405	70	R.ASPHDVLE**pT**IFVR.K	**T 331**
818.523256	1634.705154	48	K.DDILPMDLG**pT**FYR.E	**T 428**
889.109473	1775.776733	71	R.SSSSPTG**pS**DWFNLAIK.D	**S 48**
1090.709015	2179.035095	34	R.IYFGEVQLSGLGEGFQ**pT**VR.V	**T 170**

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Homo sapiens    METDLNSQDRKDLDKFIKFFALKTVQVIVQARLGEKICTRSSSSPTGSDWFNLAIKDIPE 60
Gallus gallus   MDTDLSSQDRKDLDKFIKFFALKTVQVIVQARLGEKICTRSSSSPTGSDWFNLAIKDIPE 60
                *:***.**************************************************

Homo sapiens    VTHEAKKALAGQLPAVGRSMCVEISLKTSEGDSMELEIWCLEMNEKCDKEIKVSYTVYNR 120
Gallus gallus   VTHEAKKALAGQLPAVGRSMCVEISLKTSEGDSMELEIWCLEMNEKCDKEIKVSYTVYNR 120
                ************************************************************

Homo sapiens    LSLLLKSLLAITRVTPAYRLSRKQGHEYVILYRIYFGEVQLSGLGEGFQTVRVGTVGTPV 180
Gallus gallus   LSLLLKSLLAITRVTPAYRLSRKQGHEYVILYRIYFGEVQLSGLGEGFQTVRVGTVGTPV 180
                ************************************************************

Homo sapiens    GTITLSCAYRINLAFMSTRQFERTPPIMGIIIDHFVDRPYPSSSPMHPCNYRTAGEDTGV 240
Gallus gallus   GTITLSCAYRINLAFMSTRQFERTPPIMGIIIDHFVDRPYPSSSPMHPCNYR-AGEDNGA 239
                ************************************************** ****.*.

Homo sapiens    IYPSVEDSQEVCTTSFSTSPPSQLMVPGKEGGVPLAPNQPVHGTQADQERLATCTPSDRT 300
Gallus gallus   VYPSVEDSQEVCTTSFSTSPPSQLIGPGKEGGVPPVPSQPAHGTQADQER--MCTPLDGV 297
                :**********************: ******** .*.**.*********   *** * .

Homo sapiens    HC-AATPSSSEDTETVSNSSEGRA-SPHDVLETIFVRKVGAFVNKPINQVTLTSLDIPFA 358
Gallus gallus   HYSAATPSSSEDTETVSNSSEGKCGSPHDLLETIFIRKVGAFVNKPINQVTMANLDIPFA 357
                *  ******************:. ****:*****:***************::.******

Homo sapiens    MFAPKNLELEDTDPMVNPPDSPETESPLQGSLHSDGSSGGSSGNTHDDFVMIDFKPAFSK 418
Gallus gallus   MFAPKNVELEDNDPMVNPPDSPETESPLQGSLHSEGSSGSSAGNTHDDFVMIDFKPAFSK 417
                ******:****.**********************:****.*:******************

Homo sapiens    DDILPMDLGTFYREFQNPPQLSSLSIDIGAQSMAEDLDSLPEKLAVHEKNVREFDAFVETLQ 480
Gallus gallus   DDILPMDLGTFYREFQNPPQLSSLSIDIGAQSMAEDLDSLPEKLAVHEKNVKEFDAFVETLQ 479
                *********************************************:**********
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Figure S4 (A) For *in vitro* kinase assay, FLAG-Ulk1 wild-type (wt) and FLAG-Ulk1 kinase dead (kd) were immunopurified from lysates of Flp-In™ T-REx™ 293 cells and incubated with GST-Atg13 or myelin basic protein (MBP) as substrates for the kinase reaction under radioactive conditions using [^{32}P]ATP. The reaction was subjected to SDS-PAGE, stained with Coomassie (upper panel), and analyzed by autoradiography (lower panel). (B) For mapping of *in vitro* Atg13 phosphorylation sites, GST-Atg13 was used as substrate in a non-radioactive kinase assay. Following SDS-PAGE and staining with Coomassie, GST-Atg13 was excised, enzymatically digested and analyzed by mass spectrometry. Atg13 phospho-peptides identified by MS analysis are listed. Individual ions scores > 29 indicate identity or extensive homology ($p<0.05$). The putative Ulk1-dependent phosphorylation sites in human Atg13 and the corresponding sites in chicken Atg13 are highlighted by red rectangles.

Figure S5

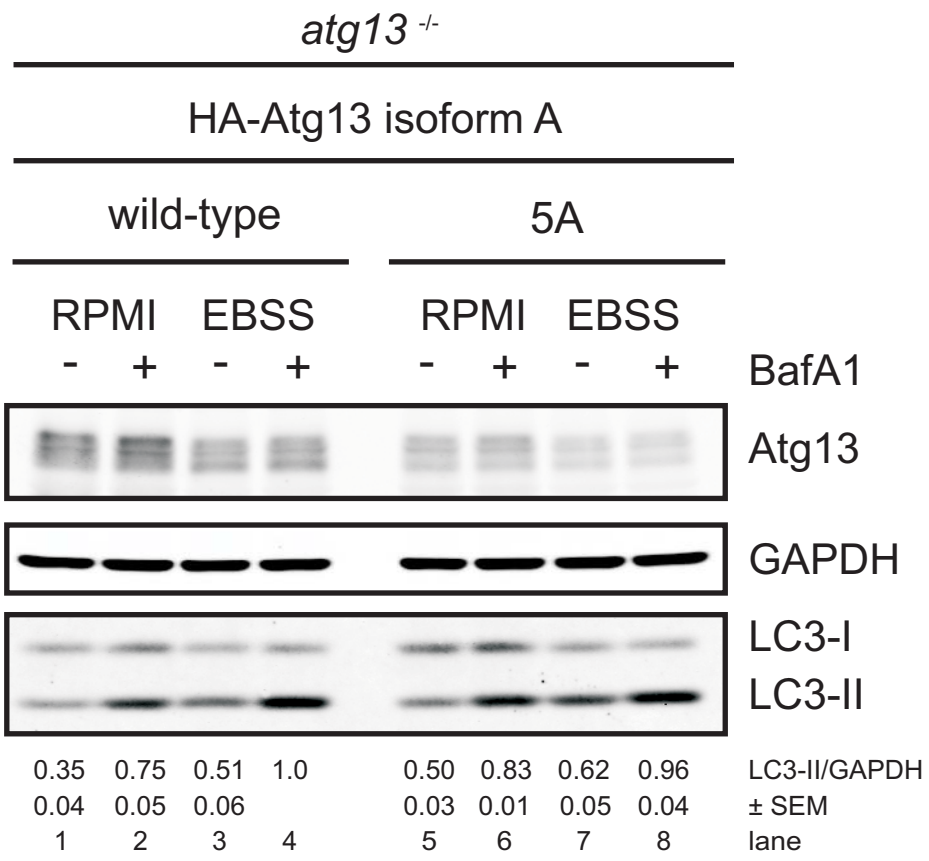


Figure S5 *atg13*$^{-/-}$ cells were reconstituted with cDNA coding for a HA-tagged quintuple mutant of full-length chicken Atg13 (isoform A), in which the corresponding residues of all five putative phosphorylation sites mapped for human Atg13 were mutated to alanine (see Figure S4). These cells were incubated in full medium (RPMI) or starvation medium (EBSS) either in the presence or absence of 10 nM bafilomycin A_1 (BafA1) for 1 h and equal amounts of protein were subjected to immunoblotting for Atg13, GAPDH and LC3 (lanes 5-8). As control *atg13*$^{-/-}$ cells were reconstituted with wild-type HA-Atg13 isoform A (lanes 1-4) and analyzed in parallel. LC3-II/GAPDH ratios from three independent experiments are represented as mean values ± SEM.

Figure S6

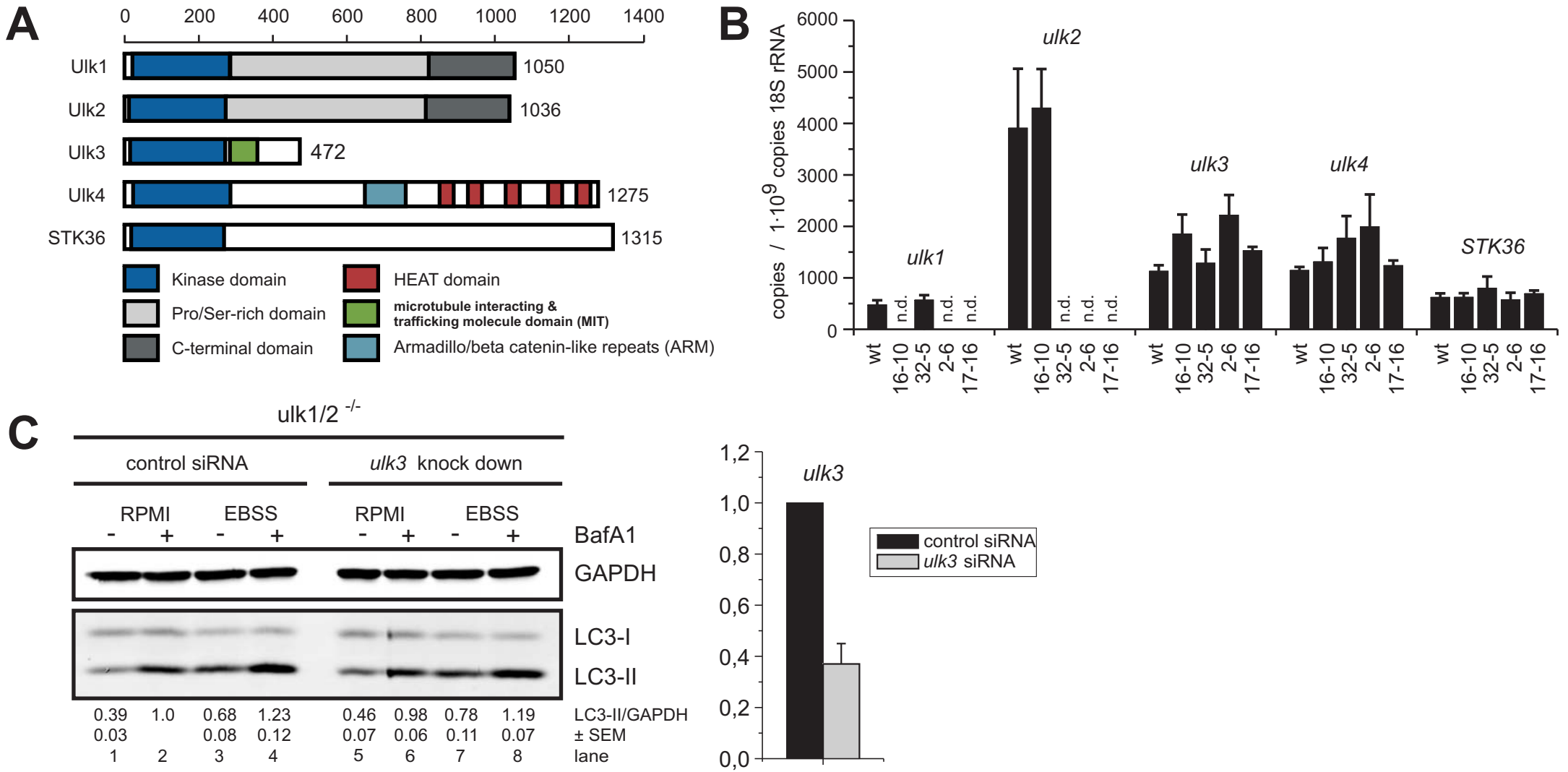


Figure S6 (A) Schematic representation of human Ulk1 [NP_003556.1], Ulk2 [NP_055498.3], Ulk3 [NP_001092906.1], Ulk4 [NP_060356.2] and STK36 (Fused) [NP_056505.2] domain architecture. (B) The absolute abundance of all known Ulk homologs in DT40 cells was analyzed by qRT-PCR and was normalized to the absolute copy numbers of 18S rRNA. Results are displayed as copies per 1 x 10^9 copies 18S rRNA. Mean value from duplicates ± SD over 3 dilutions (80/8/0.8 ng cDNA per reaction) are given (for primer sequences see Supplementary Material and Methods). (C) *ulk1*$^{-/-}$*ulk2*$^{-/-}$ cells were transfected with 1µM ON-TARGETplus SMARTpool® siRNA for *ulk3* using Amaxa Nuclefector® Kit T. Cells were incubated for 30 hrs and resuspended in normal growth medium or EBSS, either containing bafilomycin A_1 (BafA1, 10 nM) or DMSO, and incubated for 1 h at 37°C. Equal total protein amounts were analyzed by immunoblotting for GAPDH and LC3 and quantified using ImageJ. The LC3-II/GAPDH ratios are represented as mean values from three independent experiments ± SEM. In parallel, knock down efficiency was assessed by qRT-PCR and normalized to 18S rRNA. The mean value from duplicates of three independent experiments ± SEM is shown.

Figure S7

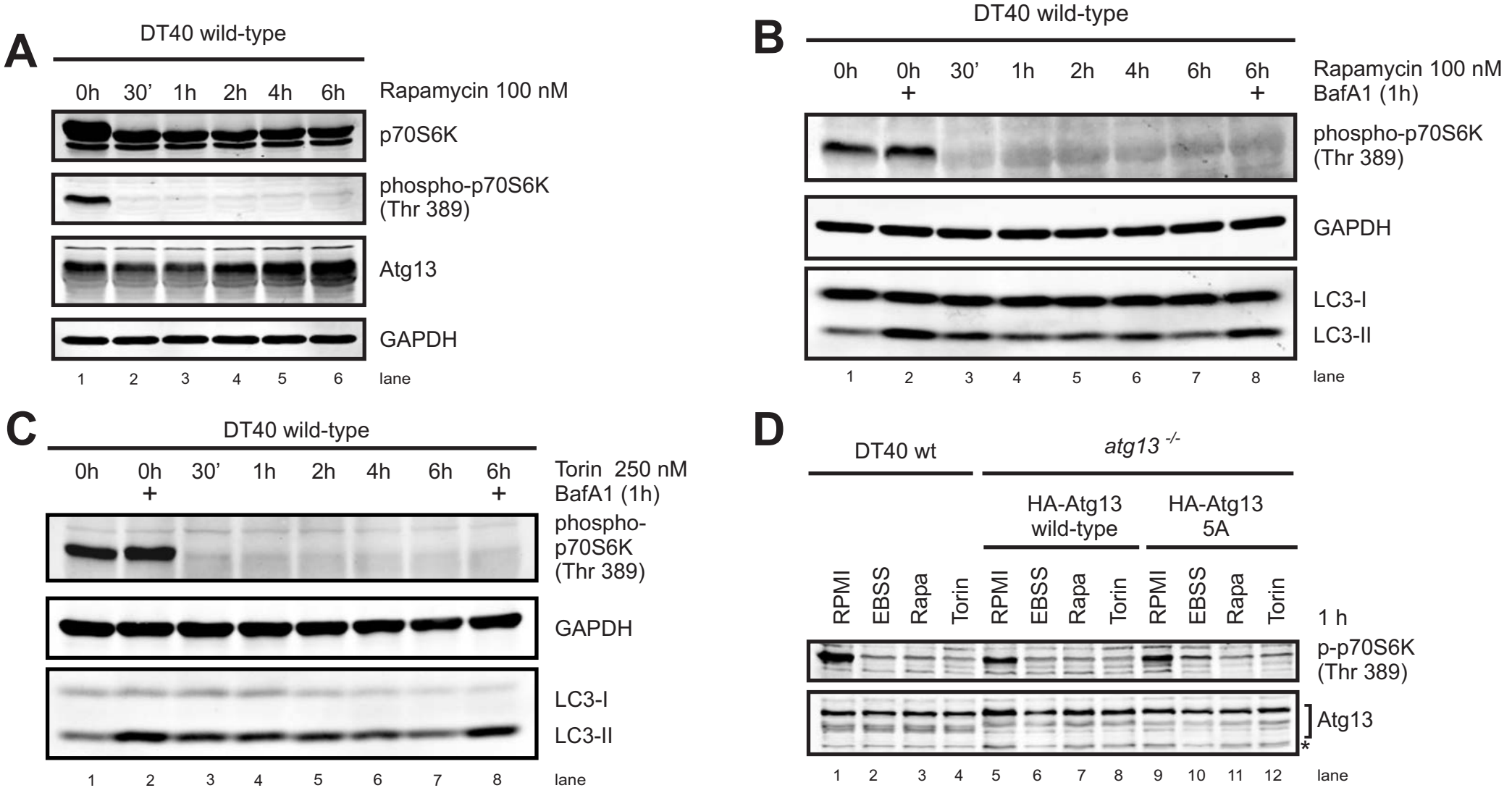
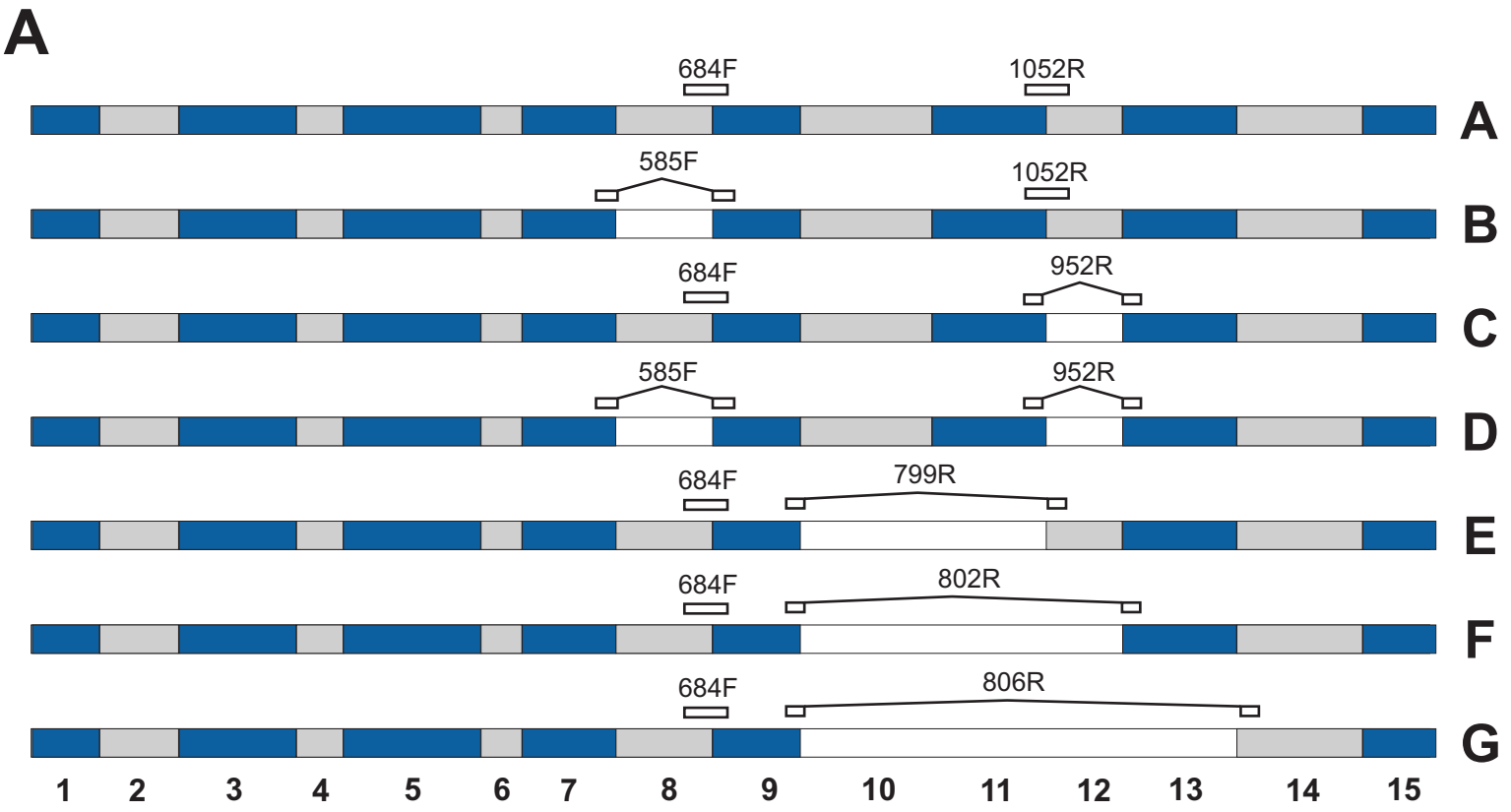


Figure S7 (A) DT40 wt cells were treated with 100 nM rapamycin for the indicated time. Cleared cellular lysates were subjected to anti-p70S6K (total), anti-phospho-p70S6K (Thr389), anti-Atg13 and anti-GAPDH immunoblotting. (B) DT40 wt cells were incubated in medium containing 100 nM rapamycin (lanes 3-8) for the indicated time. As control, cells were treated with DMSO for 6 hrs (lanes 1-2). In order to analyze the autophagic flux, 10 nM bafilomycin A_1 (BafA1) was added for the last 1 h of treatment (lanes 2 and 8). Cleared cellular lysates were analyzed for phospho-p70S6K (Thr389), GAPDH and LC3 by immunoblotting. (C) The experiment was performed according to (B) except that the cells were incubated in medium containing 250 nM Torin1. (D) DT40 wt cells and *atg13*$^{-/-}$ cells either reconstituted with HA-Atg13 wild-type (isoform A) or the quintuple mutant HA-Atg13 5A were incubated in normal growth medium containing DMSO as control (lanes 1, 5 and 9), 100 nM rapamycin (lanes 3, 7 and 11), 250 nM Torin1 (lanes 4, 8 and 12), or were incubated in EBSS (lanes 2, 6 and 10) for 1h. Equal total protein amounts from cleared cellular lysates were analyzed by anti-phospho-p70S6K (Thr389) and anti-Atg13 immunoblotting. Asterisk indicates an unspecific background band.

Figure S8



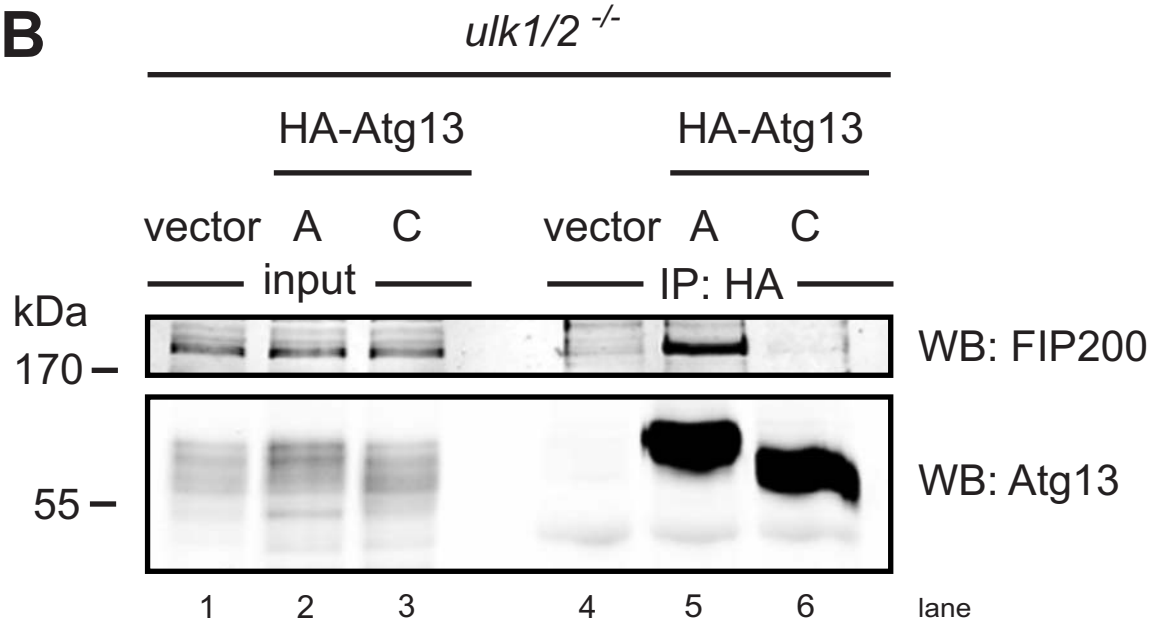


Figure S8

(A) Primer annealing sites in *atg13* cDNA for quantitative real-time PCR (for primer sequences see Supplementary Material and Methods). Primers were used in the depicted combinations to ensure splice variant-specific amplification. (B) *ulk1*$^{-/-}$*ulk2*$^{-/-}$ cells were retrovirally transfected with HA-tagged versions of splice variants A and C or the empty vector. Equal total protein amounts from cleared cellular lysates were subjected to anti-HA immunopurification and analyzed for Atg13 and FIP200 by immunoblotting.

Supplementary Material and Methods

Generation of *atg13* targeting constructs

The targeting vectors pAtg13-hisD and pAtg13-bsr were designed to disrupt the start codon in exon 1 of both *atg13* alleles by histidinol (hisD) and blasticidin (bsr) resistance cassettes, respectively. For schematic representation see Supplementary Figure S1A.

The 6 kb fragment for *atg13* targeting construct was amplified from DT40 genomic DNA using primers 5'-TTGTCGACCGCTTCCTTATAGCTTTCTCCAGG-3' and 5'-TTGCGGCCGCCAGTATGATCGACCCTAATCCTCC-3' and subcloned into pBluescript II SK(-) vector via *Sal*I and *Not*I. The start codon (ATG) in exon 1 was mutated by site directed mutagenesis using primers 5'-CAGACTGCCATATGGGATCCACACTGATCTCAGTTC-3' and 5'- GAACTGAGATCAGTGTGGATCCCATATGGCAGTCTG-3' to generate a *Bam*HI restriction site. HisD and bsr resistance cassettes were inserted into this newly generated *Bam*HI site, respectively. After electroporation, cells were selected in medium containing 1 mg/ml histidinol and 50 µg/ml blasticidin. Atg13-deficient clones were screened by genomic PCR using primer combinations Atg13_screen_for/ Atg13_screen_rev (wt allele), Atg13_screen_for/hisD_rev (hisD targeted allele) and Atg13_screen_for/bsr_rev (bsr targeted allele).

Generation of *ulk1* targeting constructs

The targeting vectors pUlk1-hisD and pUlk1-bsr were designed to replace exons 13-15 of both *ulk1* alleles by hisD and bsr resistance cassettes, respectively. For schematic representation see Supplementary Figure S2A.

The genomic 3034 bp fragment for the left targeting arm was amplified using primers 5'-TTGAATTCGTTGTAATGCACTGGGGAAGGCAC-3' and 5'-TTGGATCCGCCAGAGGCAGATGTTCAGCTTAC-3'; genomic 3132 bp fragment for the right targeting arm was amplified using primers 5'-TTGGATCCCTGCTGAGGAAGCAGGACATCTTTC-3' and 5'-TTACTAGTGTGGATTTATCCTGGGAGGCAGTTC-'3. Both fragments were subcloned into pBluescript II SK(-) vector via *Eco*RI / *Bam*HI and *Bam*HI / *Spe*I, respectively. HisD and bsr resistance cassettes were ligated into *Bam*HI site. After electroporation, cells were selected in medium containing 1 mg/ml histidinol and 50 µg/ml blasticidin. Positive clones were screened by genomic PCR using primer combinations Ulk1_screen_for/ Ulk1_screen_rev (wt allele), Ulk1_screen_for/hisD_rev (hisD targeted allele) and Ulk1_screen_for/bsr_rev (bsr-targeted allele).

Generation of *ulk2* targeting constructs

The targeting vectors pUlk2-hisD, pUlk2-bsr (for generation of ulk2$^{-/-}$ cells), pUlk2-bleo and pUlk2-eco-gpt (for generation of *ulk1*$^{-/-}$*ulk2*$^{-/-}$ cells) were designed to replace exons 6-10 of both *ulk2* alleles by the respective resistance cassettes. For schematic representation see Supplementary Figure S2B. The genomic 3146 bp fragment for the left targeting arm was amplified using primers
5'-TTGTCGACATGTTGGAGATGGGTATGCATGCC-3' and
5'-TTGGATCCCCACAGGAGCCAGGAACATTAAAG-3'; genomic 3269 bp fragment for the right targeting arm was amplified using primers 5'-TTGGATCCTCCTCACCTTTTGTCTACAGGCTG-3' and
5'-TTGCGGCCGCAGCAGGGAGGGAGAAGTAAGAAAC-'3. Both fragments were subcloned into pBluescript II SK(-) vector via *Sal*I / *Bam*HI and *Bam*HI / *Not*I, respectively. A premature stop codon (TAA) was introduced into exon 4 by site directed mutagenesis using primers
5'-TTTCTTCTCTTTTAGTAAATGCCCAGTTCTG-3' and
5'-CAGAACTGGGCATTTACTAAAAGAGAAGAAA-3'. HisD, bsr, bleomycin (bleo) and eco-gpt resistance cassettes were introduced via *Bam*HI, respectively. After electroporation, double-deficient cells were selected in medium containing 80 µg/ml Bleocin® and 10 µg/ml mycophenolic acid, single-deficient cells in medium containing 1 mg/ml histidinol and 50 µg/ml blasticidin. Positive clones were screened by genomic PCR using primer combinations Ulk2_screen_for/ Ulk2_screen_rev (wt allele), Ulk2_screen_for/bleo_rev (bleo targeted allele) and Ulk2_screen_for/gpt_rev (eco-gpt targeted allele) for *ulk1*$^{-/-}$*ulk2*$^{-/-}$ cells; Ulk2_screen_for/hisD_rev (hisD targeted allele) and Ulk2_screen_for/bsr_rev (bsr targeted allele) for ulk2$^{-/-}$ cells.

***In vitro* kinase assay**

FLAG-Ulk1 (human) was immunopurified from Flp-In™ T-REx™ 293 cells, either stably expressing FLAG-Ulk1 wt or FLAG-Ulk1 kinase dead, washed three times with lysis buffer [50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, 1 mM Na_3VO_4, 10 mM sodium β-glycerophosphate, 50 mM NaF, 5 mM NaPP, 0.27 M sucrose, 0.1% DTT, 1 mM benzamidine and 0.1 mM PMSF] and once with buffer A [50 mM Tris-HCl pH 7.5, 0.1 mM EGTA and 0.1% DTT]. *Atg13* cDNA (NM_014741) was amplified by RT-PCR from human RNA and cloned into pGEX6. GST-Atg13 was purified from BL21 bacteria and subsequently used as substrate for the *in vitro* kinase assay. 1-2 µg of substrate (GST-Atg13 or MBP) was incubated with precipitated FLAG-Ulk1 in kinase reaction buffer [50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1% DTT, 5 mM magnesium acetate and 0.1 mM [^{32}P]ATP] for 40 min at 30 °C. The reaction was stopped by the addition of SDS sample buffer and subjected to SDS-PAGE, Coomassie staining, and autoradiography.

Mass spectrometric analysis

For mapping of *in vitro* phosphorylation sites, a non-radioactive kinase reaction was separated by SDS-PAGE and stained with Colloidal Coomassie blue (LC6025, Invitrogen). The respective bands were excised, washed and digested with trypsin. Peptides were extracted with 2.5% (v/v) formic acid / 50% (v/v) MeOH, dried under vacuum, reconstituted and analyzed by LC-MS on a Dionex Ultimate 3000 HPLC system interfaced to an Applied Biosystems 4000 QTrap mass spectrometer. Peptides were typically injected onto a PepMap 100 reverse phase C_{18} 3 µm column with a flowrate of 300 nl/min and eluted with a 40 min linear gradient of 95% solvent A (2% acetonitrile, 0.1% formic acid in H_2O) to 50% solvent B (90% acetonitrile, 0.08% formic acid in H_2O). A Harvard syringe pump was used to deliver isopropanol at a flowrate of 100 nl/min with mixing occurring at a T-junction after the LC and prior to the MS. Phosphopeptides were identified and analyzed by the use of a precursor ion scan of m/z −79 in negative ion mode followed by an ion trap high resolution scan (an enhanced resolution scan) and a high sensitivity MS/MS scan (enhanced product ion scan) in positive mode (Williamson et al., 2006). All MS/MS spectra were searched against local databases using the Mascot search engine (www.MatrixScience.com) run on a local server, and sites of phosphorylation were manually assigned from individual MS/MS spectra viewed using a combination of the Mascot results and Analyst software (AppliedBiosystems/MDS-Sciex).

siRNA mediated knock down

For siRNA mediated knock down of Ulk3 expression, 5 x 10^6 *ulk1*$^{-/-}$*ulk2*$^{-/-}$ cells were resuspended in 100 µl Nucleofector® Solution T (Lonza) and transfected with 1 µM ON-TARGETplus SMARTpool® siRNA (Dharmacon) using Amaxa Nuclefector® II program B-023 (for siRNA sequences see below). Cells were resuspended in prewarmed medium and incubated for 30 hrs prior to experiments. Knock down efficiency was assessed by qRT-PCR using the primer combination chUlk3-1F/chUlk3-2R (for primer sequences see below) and normalized to 18S rRNA.

Primer sequences for genomic screening PCR

Atg13_screen_for	5'-CCTAACAGACCAGTGAGCCTAAC-3'
Atg13_screen_rev	5'-CACTGTAGCAGATTCATAGGCCC-3'
Ulk1_screen_for	5'-GATCCAGAGCCGTGTGTTTTAGCAG-3'
Ulk1_screen_rev	5'-GGGACCATAACGAAGTCATCAGTG-3'.
Ulk2_screen_for	5'- CCTTTAAGTGAAGTGGTCCTCAGC-3'

Ulk2_screen_rev	5’-CGTATGCCACTGACACTTGACTTC-3’
hisD_rev	5’-GCCGTGACCCTGCGCGTAAACGCCCTCAAGG-3’
bsr_rev	5’-CGATTGAAGAACTCATTCCACTCAAATATACCC-3’
bleo_rev	5’-CAACTGCGTGCACTTCGTGGCC-3’
ecogpt_rev	5’-CTGGATTGAACAGCCGTGGGATATG-3’

Primer sequences for RT-PCR

chUlk1_RT_for	5’-GGCCTTCTGCAGCGCAATCATAAG -3’
chUlk1_RT_rev	5’-CCAGAGAACTCCCACTGTACATCAG -3’
chUlk2_RT_for	5’-CTTACCTGGCTGACCTGCTGTTG-3’
chUlk2_RT_rev	5’-TGCCAGAGACTGAGCCTGCATATG-3’
chGAPDH_RT_for	5’-AATGCATCGTGCACCACCAAC-3’
chGAPDH_RT_rev	5’-TCACTCCTTGGATGCCATGTG-3’

Primer sequences for quantitative real-time PCR

chAtg13-684F	5’-CTGCAATTACAGAGCTGGTGAAGA-3’
chAtg13-585F	5’-CATGTCAACCAGAGCTGGTGAA-3’
chAtg13-1052R	5’-TTGGCCATGGTCACCTGG-3’
chAtg13-952R	5’-CAGGAGGATTGACCTGGTTAATAGG-3’
chAtg13-799R	5’-GTTGGCCATGGTCACCTGAG-3’
chAtg13-802R	5’-AATCAGGAGGATTGACCTGAGATG-3’
chAtg13-806R	5’-TCTTTTGAAAATGCTGGTTTCTGAG-3’
chUlk1-13F	5’-TTTCTGCATGGATCGAAGGAC-3’
chUlk1-14R	5’-GCCTCTGCAGTCATTTCACTTG-3’
chUlk2-538F	5’-TACATGGCCCCTGAAGTGATTA-3’
chUlk2-651R	5’-TTGGCCTGAAAAGGTGGTTT-3’
chUlk3-1F	5’-GCGTACGGCAAGTGGGAC-3’
chUlk3-2R	5’-GGATGAAGCGGGAGAGGTC-3’
chUlk4-8F	5’-TGTACCTGTGTCTGAGGCATTTATACT-3’
chUlk4-10R	5’-TACCTCCTCGCAGGCTATGAG-3’
chUlk5-18F	5’-GCTTCCTGGTGCAACAATGG-3’

chUlk5-20R	5’-TCCTGAGCAACCTGCTGCTT-3’
chUlk3-S-F	5’-ACCTACGCCACGGTGTACAAA-3’
chUlk3-S-R	5’-ATAAAGGATGACGCCCACTGAC-3’
chUlk4-S-7F	5’-TGTTAATACAGCAGCTGCGTGC-3’
chUlk4-S-11R	5’-AAATGGTTCACTATCCGGTCTTCC-3’
chUlk5-S-17F	5’-AGCCTGCGAGAGGAGAATGAG-3’
chUlk5-S-22R	5’-GGACCTTGCCCTTTGAGAGC-3’
18SrRNA_for	5’-CGGCTACCACATCCAAGGAA-3’
18SrRNA_rev	5’-GCTGGAATTACCGCGGCT-3’

siRNA sequences for knock-down of chicken *ulk3*

ulk3-01	CTGAGAAGGTGGCACGTAT
ulk3-02	AGGCGCAATCCATGGGCAA
ulk3-03	CCACGGTGTACAAAGCGTA
ulk3-04	CGGTGGTGGTTTGTGGCAA

Supplementary Reference

Williamson BL, Marchese J, Morrice NA. Automated identification and quantification of protein phosphorylation sites by LC/MS on a hybrid triple quadrupole linear ion trap mass spectrometer. Mol Cell Proteomics 2006; 5:337-46.