Supplemental Materials and Methods

ULK1 phosphorylates Ser30 of BECN1 in association with ATG14 to stimulate autophagy induction

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Supplemental Figures
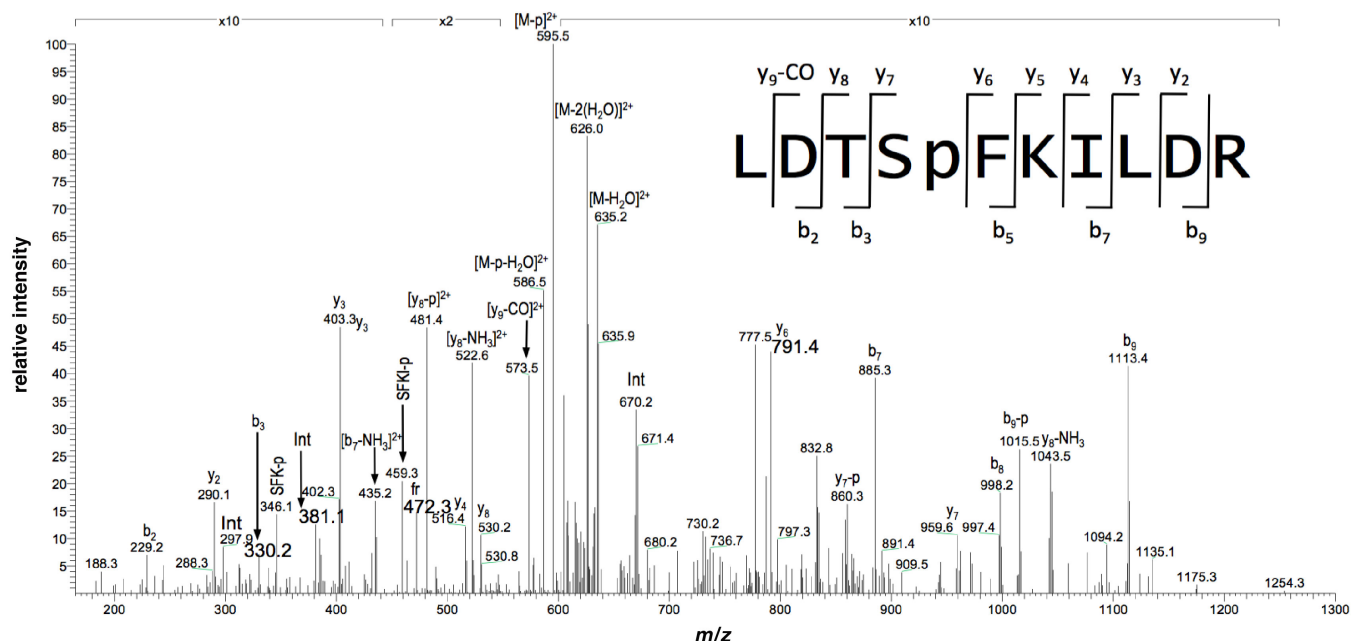
Supplemental Figures

Figure S1. MS/MS spectrum for the phosphopeptide containing BECN1 Ser30. The PEAKS software (Bioinformatics Solutions Inc.) was used for database search and the spectrum analysis. We observed the CID (collision induced dissociation and ion trap detection) tandem MS spectrum for the doubly charged, monoisotopic precursor ion 644.3193 *m/z* (2.4 ppm mass error) for peptide LDTS(phos)FKILDR at retention time 35.4 minutes, as reported by PEAKS Studio 8.0 software. The PEAKS amino acid site localization score (Ascore[1]) for phosphoserine is 1000, which is the maximum Ascore achievable. Manual inspection of the tandem MS provided additional support for the peptide match and serine phosphorylation site. Peptide fragment ions observed in the experimental data were labeled according to Biemann nomenclature[2]. We used MS Convert from Proteowizard (v 3.0.9248-x86_64) to create an mzXML file and imported the spectrum into mMass version 5.5.0 for theoretical fragment ion generation and peak assignments. The tandem MS spectrum was copied from Xcalibur 3.0.63 and the fragment ion labels were manually added to the spectrum upon comparison to the theoretical and experimental *m/z* values. More detailed information is available in another supplemental file containing PEAKS analysis data and at this html file:
file:///Users/dhkim/Library/Containers/com.apple.mail/Data/Library/Mail%20Downloads/AEB79E54-B637-4EE9-8826-25D9E61286FE/reviewer_responses_dhkim_paper/ProteinProspector_Report_beclin_Phosphopeptide_20171005.html

A

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TTACTTTCTCTTTTCTATGTCAGTATTGCAATGGTGGAGACCTCGCA-GATTA---TTTG
|||||||||||||||||||||||||||||||||||||||||||||| ||||| ||||
TTACTTTCTCTTTTCTATGTCAGTATTGCAATGGTGGAGACCTCGCAAGATTATTTTTTG

CAAGGTAACTGTGTATGTGTGTCAGTGTTCAGTAAAGACAGTGCTGCAGCCTGTTTAATC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CAAGGTAACTGTGTATGTGTGTCAGTGTTCAGTAAAGACAGTGCTGCAGCCTGTTTAATC

CTAAGAGGTACTTGTGAAATACACTACCCATTTTCTGCTCTTG
|||||||||||||||||||||||||||||||||||||||||||
CTAAGAGGTACTTGTGAAATACACTACCCATTTTCTGCTCTTG
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B


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ACTTTCTCTTTTCTATGTCAGTATTGCAATGGTGGAGACCTCGCAAGATTATTTTTTGCAAGGTAACTGTGTATGTGTGT
                  Y   C   N   G   G   D   L   A                          stop
                  87  88  89  90  91  92  93  94
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translation of 6 amino acids off the frame
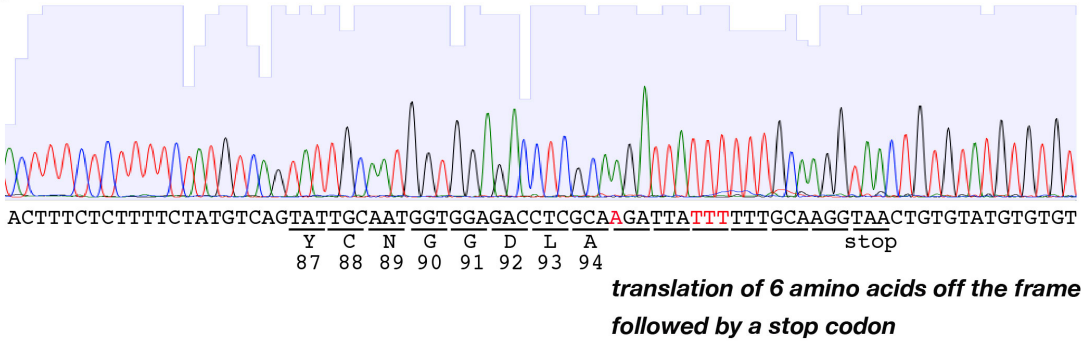*followed by a stop codon*

Figure S2. Confirmation of ULK2 allelic nucleotide insertions that cause a frame shift of ULK2 translation. (**A**) Sequence alignment for the wild type sequence (top) and a CRISPR-cas9 targeted KO clone sequence (bottom) of the ULK2 allele in HCT116 cells. (**B**) Sequencing confirms nucleotide insertions (types in the red color) in the ULK2 allele, which lead to translation of 6 amino acids off the frame followed by a stop codon.
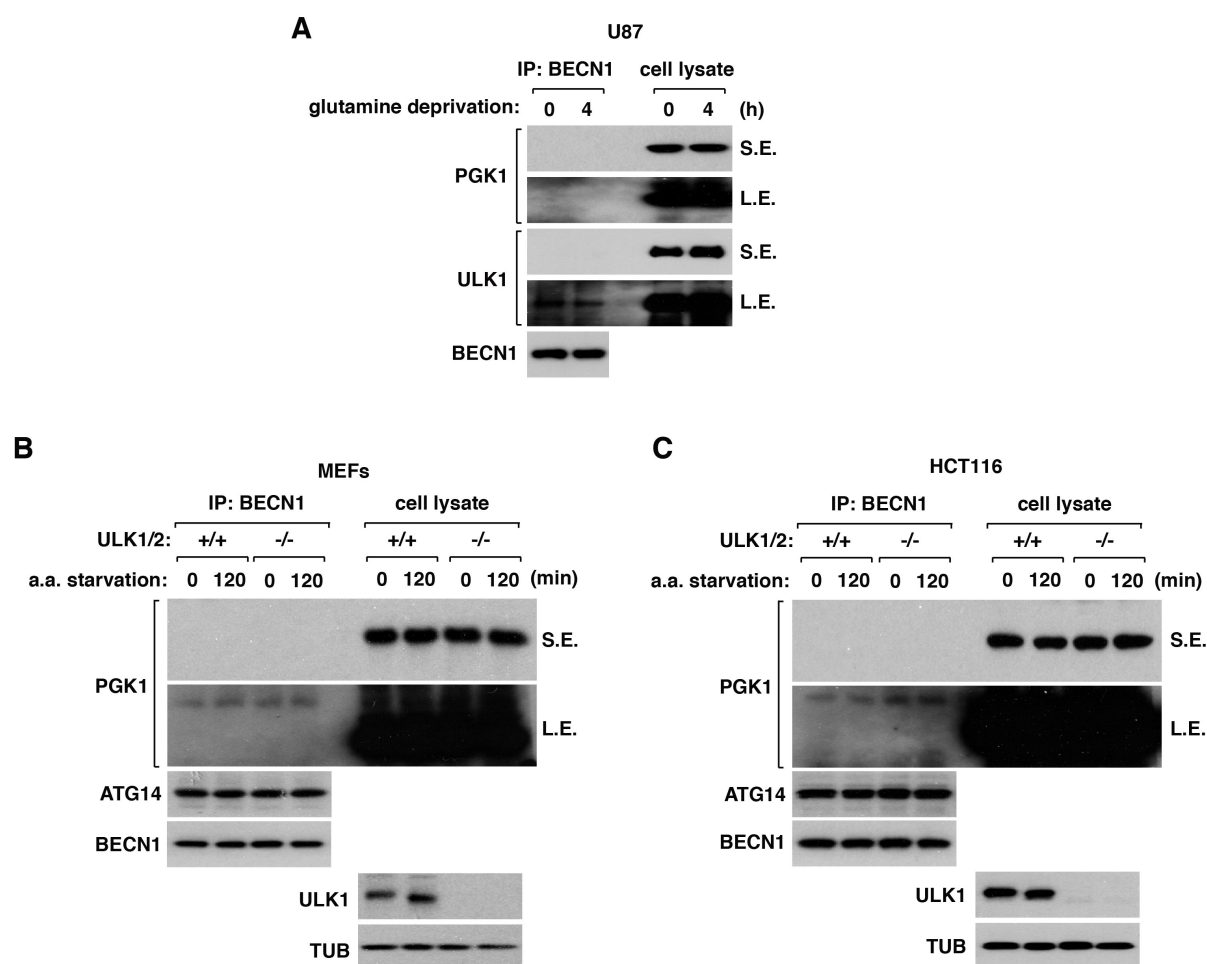
Figure S3. PGK1 is not present in BECN1 immunoprecipitates. BECN1 immunoprecipitates were obtained from U87 cells (A), MEFs (B), and HCT116 cells (C) that were treated with glutamine deprivation (A) or amino acid starvation (B and C). Western blotting was performed to detect the indicated proteins in the immunoprecipitates and cell lysates. S.E., short exposure; L.E., long exposure.

GGGCTGAGGTGGGACCTTAGAAGGGAGTCTGGGAACCCTCACGGCTCTTATTGGAGTCCCTTCCCTGACC
CTGGGCTCTAAACTGCCTTTGCTCAGGCTGTCCCGGAAGCAGGTCCTCCCCGTATCATACCATTCCTAGA
GGGAACGGCGCAGGTTGGGACTTCCCTCCCTTTACCATCGTCACCAAGGCATGTGGTAACCCCGGGCCG
GAGGTCAAGGGCGTCGCTTCTCCCTAATGTTGCCTCTTTTCCACGGCCTCAGGGATGGAAGGGTCTAAGA
CGTCCAACAACAGCACCATGCAGGTGAGCTTCGTGTGCCAGCGCTGCAGCCAGCCCCTGAAACTGGACA
CG**gc**TTTCAAGATC**CTc**GACCGTGTCACCATCCAGGAACTCACAGGTCAGCGAGACCCTTGGAAAGAGGG
TGGTCAAGAATGTGGAAGGTGGGCAGCTTTGGTGATATAATTCTTATGGTGGCTGTGTAGTCTGCCACAA
ACTCATCGTGTGAACCTAGGAAAGTCACTTCATCTGGCTCCCTGTTTTCTCCTTGGAAACATAAAGCATTC
TGACATTGTGGACTTCTGAGAGCTTTCCGACCGGGCGCGGTGGCTCACGCCTGTAATCCCAGCATTTTGG
GAGACCGAGGCGGGCAGATCACCTGAGGTCAGGAGTTCGAGACCAGCCTGGCCAACATGGCGAAACCC
CTCTCTACTAAAAATACA

Figure S4. Sequence of the replacement DNA to introduce a point mutation of BECN1 S30A into the genome of HCT116 cells. The DNA was cloned into pUC57 vector and introduced into HCT116 cells as described in Materials and Methods. The underlined codons reflect the S30A mutation (gcT) and a silence mutation (CTc) at the PAM sequence of the gRNA target site GGACACGAGTTTCAAGATCC, respectively.
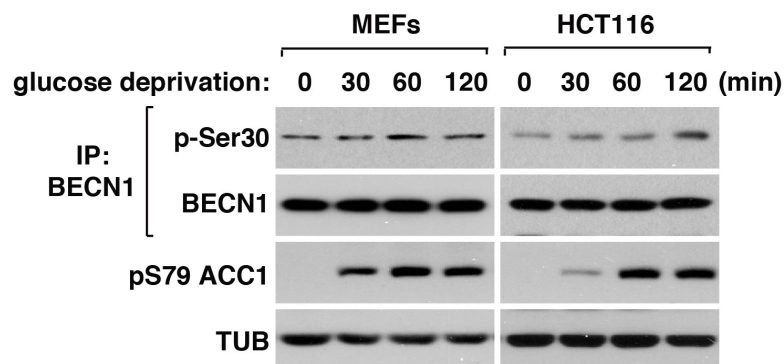
Figure S5. Glucose deprivation causes a moderate increase of BECN1 Ser30 phosphorylation. MEFs and HCT116 cells were cultured in glucose-deprived DMEM (11966-025, ThermoFisher Scientific Inc.) supplemented with 10% dialyzed fetal bovine serum for the periods of time as indicated. The phosphorylation state of Ser30 in BECN1 isolated by immunoprecipitation was analyzed by western blotting. The activation of AMPK by glucose deprivation was confirmed by monitoring the phosphorylation of acetyl-CoA carboxylase (ACC1) Ser79.

Supplemental Materials

All the sources of antibodies and chemicals used for the supplemental experiments are listed in Materials and Methods.

Supplemental Methods

MS/MS spectrometry spectrum for BECN1 Ser30 phosphorylation was obtained using the PEAKS software (Bioinformatics Solution Inc.). A detailed procedure for mass spectrometry is described in Materials and Methods and the supplemental figure legend.

See Materials and Methods for the following experimental procedures: Cell culture and treatment; immunoprecipitation and western blotting; genome editing to introduce the BECN1 S30A mutation and knockout ULK1 and ULK2.

Supplemental References

1.   Beausoleil SA, Villen J, Gerber SA, Rush J, Gygi SP. A probability-based approach for high-throughput protein phosphorylation analysis and site localization. Nature biotechnology 2006; 24:1285-92.
2.   Biemann K. Contributions of mass spectrometry to peptide and protein structure. Biomed Environ Mass Spectrom 1988; 16:99-111.