



Supplementary Figure S1. Beclin 1 is co-localized with RB in nuclei. Knockdown of *BECN1* has no significant effect on the steady-state *RB1* mRNA levels.

a. Low *RB1* expression is associated with poor overall survival of human colorectal cancer patients. Kaplan-Meier plots of overall survival (OS) of human colorectal cancer patients were stratified by the *RB1* mRNA expression levels obtained from the TCGA COADREAD dataset (access on 3 May 2022).

b-d. There was no significant correlation between *BECN1* and *RB1* mRNA levels in colon cancer patient samples. Pearson correlation analyses were conducted between *BECN1* and *RB1* mRNA levels in colon cancer patient samples. **(b)** The mRNA expression datasets include TCGA Firehose Legace (access on 10 September 2022), TCGA PanCancer Atlas and CPTAC-2 Prospective from cBioPortal (access on 10 September 2022). The gene expression data were transformed to z-scores relative to all samples based on log2(RSEM) values in each dataset. **(c)** Data from GDC TCGA COAD. The gene expression data were expressed as log2(fpkm-ug+1) (access on 10 September 2022). **(d)** Data from GEPIA. (access on 10 September 2022) The gene expression data were expressed as log2(TPM). Transcripts Per Million, TPM; Fragments Per Kilobase of exon Per million read, FPKM; RNA-Seq by Expectation-Maximization, RSEM.

e-f. Knockdown of *BECN1* upregulates RB protein expression in nuclei.

- (e) HCT116 p53^{-/-} cells stably expressing shC or shBeclin 1 were subjected to immunofluorescent (IF) staining for endogenous Beclin 1 (red) or RB (green) and counter-staining nuclei with DAPI (blue). Scale bar=50 µm. (f) Total cell lysates derived from HCT116 p53^{-/-} cells stably expressing shC or shBeclin 1 were subjected to a nuclear-cytoplasmic fractionation assay.
- g. Caco-2 and A549 cells stably expressing shC or shBeclin 1 were subjected to western blot.
- h. HCT116 or HCT116 p53^{-/-} cells stably expressing shC or shBeclin 1 (#1 or #2) were subjected to qPCR analyses for *RB1* or *MDMX* mRNA levels, *GAPDH* was used as an internal control. Three independent experiments were performed in triplicates. ns: not significant.

Supplement Table S1.

qPCR primers

| Gene name | Primer sequences |
|-----------------------|--------------------------------------------------------------|
| Human <i>BECN1</i> | F-AGGTTGAGAAAGGCGAGACA R-GCTTTTGTCCACTGCTCCTC |
| Human <i>GAPDH</i> | F-CCCGTGGAATGGAATGAGATT R-CTCAGGAGAGGAGCCATTT |
| Human <i>MDMX</i> | F-GCAAGAAATTTAACTCTCCAAGCAA R-CTTTGAACAATCTGAATACCAATCCTT |
| Human <i>MDM2</i> | F-TTGCGGTGCCAAGCTTCTCT R-TACCTGAGTCCGATGATTCC |
| Human <i>RB1</i> | F-GACCCAGAAGCCATTGAAATCT R-GGTGTGCTGGAAAAGGGTCC |

Short-hairpin RNA oligo sequences.

| Name | Oligo sequences |
|---------------------|------------------------|
| pLKO.1-shGFP | TCCTAAGGTTAAGTCGCCCTCG |
| pLKO.1-shBeclin 1#1 | CCCGTGGAATGGAATGAGATT |
| pLKO.1-shBeclin 1#2 | CTCAGGAGAGGAGCCATTT |
| pLKO.1-shRB#1 | TTTGGACTAGAAATAATGTGG |
| pLKO.1-shRB#2 | ATCGCTGTGATCCAATTTTCG |

Small interfering RNA oligo sequences.

| Name | Sense chain (5'-3') |
|---------------------------|-----------------------|
| hMDM2 | GCCTGGCTCTGTGTGTAAT |
| hMDMX | CACCTAGAAGTAATGGCTCAA |
| shScramble (target hMDMX) | ATAACTAGGAGGCAACACTCT |
| shScramble (target hMDM2) | ATGCTCGGTCCATGTGTTG |