USP19 modulates autophagy and antiviral immune responses by deubiquitinating Beclin-1

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Appendix Materials and Methods

Antibodies

Anti-USP19 (ab68527) and anti-VPS34 (ab68527) were purchased from Abcam; anti-ULK1 (sc-33182), anti-IRF3 (sc-9082), anti-MAVS (sc-166583) and anti-ubiquitin (sc-8017) were purchased from Santa Cruz Biotechnology; anti-LC3 (PM036) and anti-ATG14L (PD026) were purchased from MBL; anti-LC3B (3868), anti-Beclin-1 (3738), anti-p62 (8025), antibody to IRF3 phosphorylated at Ser396 (4947), antibody to TBK1 phosphorylated at S172 (5483) and anti-TBK1 (3013) and were purchased from Cell Signaling Technology; horseradish peroxidase (HRP)-anti-Flag (M2) (A8592) and anti-β-actin (A1978) were purchased from Sigma; HRP-anti-hemagglutinin (clone 3F10), anti-c-Myc-HRP (11814150001) and unlabeled anti-c-Myc (11667203001) were purchased from Roche Applied Science; anti-USP19 (YT4832) was purchased from ImmunoWay.

Generation of stable expression and knockout cell lines

The retroviral vectors (15 g) were co-transfected with 5 μg of an expression plasmid for the vesicular stomatitis virus G protein into the 293T cell line. At 48 h later, the supernatant containing the retroviral particles was recovered and supplemented with Polybrene (8 μg ml^{-1}). For USP19 ectopic expression, lentiviral particles were produced by transfecting 293T cells with FG-EH-DEST-USP19, VSGV and Δ8.9. HeLa cells were infected by incubation with retrovirus-containing supernatant for 48 h. For USP19 KO and Beclin-1 KO cells, target sequences were cloned into *Bsm*BI-digested pLentiCRISPRv2 plasmid. The target sequences used were as follows:

USP19 target #1: 5′-CGTACCCGGCTGTTCTTTCC-3′;

USP19 target #2: 5′-GGTGATTGTCAAGCTTCGTG-3′;

USP19 target #3: 5′-GTGATTGTCAAGCTTCGTGT-3′;

BECN 1 target #1: 5′-GGGTCTCTCCTGGTTTCGCC-3′;

BECN 1 target #2: 5′-GGTCTCTCCTGGTTTCGCCT-3′;

BECN 1 target #3: 5′-ATTTATTGAAACTCCTCGCC-3′.

Reporter assays

Luciferase reporter assays were performed as described previously (Cui et al, 2014).

Quantitative RT-PCR

Total RNA was extracted from cells using the Trizol reagent (Invitrogen) according to the manufacturer's instructions. For RT-PCR analysis, cDNA was generated with HiScript® II Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme, R223-01) and was analyzed by quantitative real-time RT-PCR using the 2×RealStar SYBR Mixture (Genestar). All data were normalized to *Gapdh* expression. Primer sequences were:

Gapdh: 5′-CGGAGTCAACGGATTTGGTC-3′,
5′-GACAAGCTTCCCGTTCTCAG-3′;

BECN1: 5′-TCAATAACTTCAGGCTGGGTCG-3′,
5′-TTCCGTAAGGAACAAGTCGGTA-3′;

USP19: 5′-CACGCTCATCTTCCAGACCAGG-3′,
5′-ATGCGAGAAGCCGTGAAACAGA-3′;

IFNB: 5′-GATGAACTTTGACATCCCTGAG-3′,
5′-TCAACAATAGTCTCATTCCAGC-3′;

ISG15: 5′-CGCAGATCACCCAGAAGATCG-3′,
5′-TTCGTCGCATTTGTCCACCA-3′;

ISG54: 5′-TATTGGTGGCAGAAGAGGAAGA-3′,
5′-CAGGTGAAATGGCATTTTAGTT-3′;

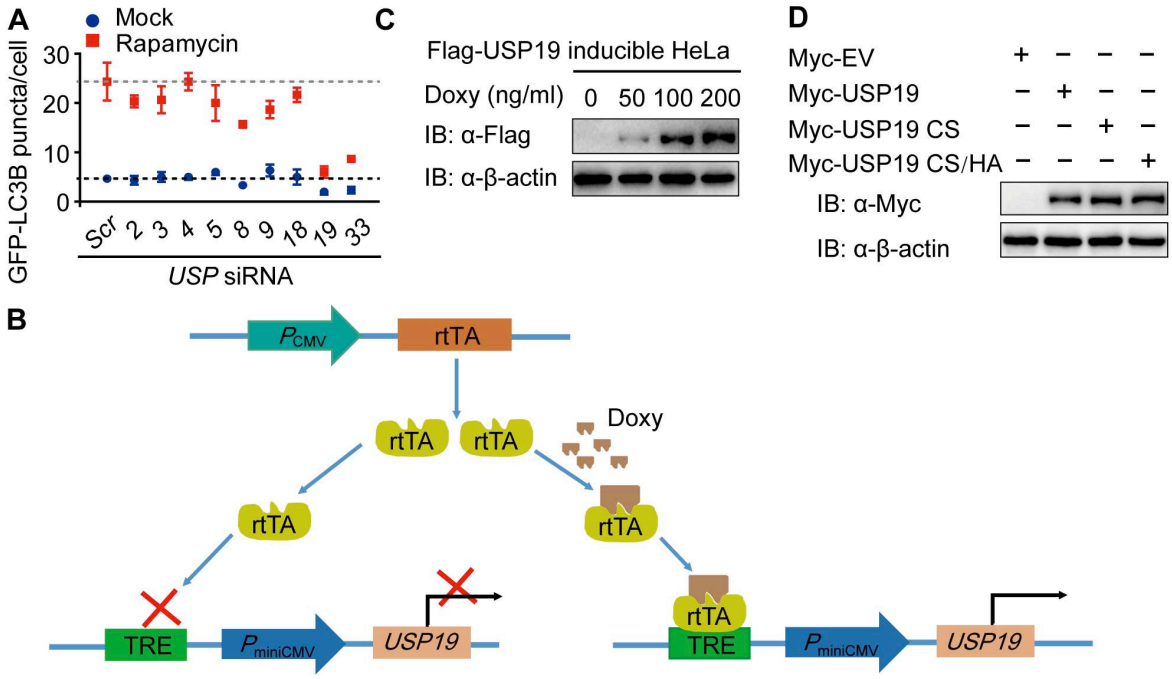
ISG56: 5′-TCAGGTCAAGGATAGTCTGGAG-3′,
5′-AGGTTGTGTATTCCCACACTGTA-3′;

SeV P: 5′-TGTTATCGGATTCCTCGACGCAGTC-3′,
5′- TACTCTCCTCACCTGATCGATTATC-3′;

VSV G: 5′-CAAGTCAAAATGCCCAAGAGTCACA-3′,
5′-TTTCCTTGCATTGTTCTACAGATGG-3′;

Nucleoprotein (*NP*): 5′-TGTGTATGGACCTGCCGTAGC-3′,
5′-CCATCCACACCAGTTGACTCTTG-3′.

Appendix Fig S1



Appendix Fig S1. Identification of USP19 as a positive regulator in autophagy.

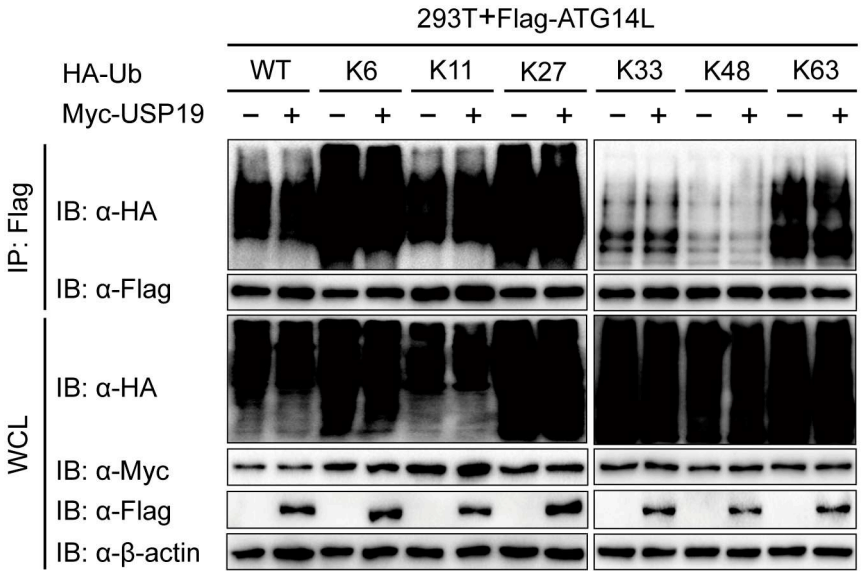
(A) HeLa-GFP-LC3B cells were subjected to DUB siRNA and treated with rapamycin (250 nM) for 12 h. Pictures were taken using Leica DMI3000 B with a×100 oil-immersion objective and average GFP-LC3B puncta per cell were calculated.

(B) Schematic of gene regulation in the BD^{TM} Tet-On Systems. The transcription is turned on by the reverse tetracycline-responsive transcriptional activator (rtTA), which binds to the tetracycline-responsive element (TRE) promoter and activates transcription in the presence of doxycycline (Doxy).

(C) Lysates of Flag-USP19 inducible HeLa cells incubated with Doxy as indicated concentration for 12 h were analyzed by immunoblot.

(D) 293T cells were transfected with pcDNA3.1 empty vector and plasmid expressing the wide-type (WT) and the mutant form of USP19 (CS and CS/HA), the expression levels of Myc-tagged USP19 were detected by immunoblot. Data are representative of three independent experiments, n=3. Error bars indicate the SEM.

Appendix Fig S2



Appendix Fig S2. USP19 does not affect the ubiquitination of ATG14L.

Lysates of 293T cells transfected with plasmid for Flag-ATG14L and HA-tagged ubiquitin and their indicated mutants, with or without Myc-USP19 were immunoprecipitated with anti-Flag and immunoblotted with anti-HA.