**Supplementary information**

**The ubiquitin-specific protease USP8 directly deubiquitinates SQSTM1/p62**

**to suppress its autophagic activity**

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**Figure S1.** Schematic representation of Flag-tagged SQSTM1 truncated mutants (left panel). HEK293T cells were co-transfected with USP8-GFP and Flag-tagged SQSTM1 truncated mutants, immunoprecipitated with anti-GFP beads and immunoblotted with antibodies against Flag and GFP (right panel).



**Figure S2.** USP8 overexpression did not reduce K48- and K63-linked ubiquitination of the SQSTM1K420R mutant. HEK293T cells transfected with HA-K48- or K63-Ub and His-tagged SQSTM1 or SQSTM1K420R mutant with or without USP8-Flag for 48 h. The cells were subjected to pull down using the Ni2+-NTA beads, followed by immunoblotting.



**Figure S3.** USP8 knockdown promoted autophagic degradation of SQSTM1. (**A**) Immunoblotting of SQSTM1 and USP8 protein in scramble (scr) or sh*USP8*-infected MCF7 and AtT20 cells. (**B**) Immunoblotting of SQSTM1 protein in scramble (scr) or sh*Usp8*-infected MEFs in the presence of cycloheximide (CHX, 50 µg/ml) for indicated times.



**Figure S4.** USP8 overexpression delayed GFP-Q46 degradation induced by rapamycin through deubiquitinating SQSTM1 at K420. Wild-type (**A**) *SQSTM1*-knockdown (**B**) or SQSTM1K420R mutant-expressing HEK293T cells (**C**) were co-transfected with GFP-Q46 and USP8-HA. 48 h later, cells were treated with 500nM rapamycin for 12 h, followed by western blotting using indicated antibodies. *SQSTM1*-knockdown HEK293T cells were generated by infection of specific shRNA against SQSTM1 (sh*SQSTM1*). The knockdown efficiency of SQSTM1 in HEK293T cells was confirmed by immunoblotting (**B,** left). The lentivirus encoding the SQSTM1K420R-His mutant was used to infect *SQSTM1-*knockdown HEK293T cells to generate SQSTM1K420R -His mutant-expressing HEK293T cells.

**Table S1.** The sequence of primers used in this study.

**Primers for subcloning and point mutation**

SQSTM1-FL- Forward: 5-CCGGAATTCatggcgtcgctcaccgtgaa-3

SQSTM1-FL- Reverse: 5-CCGCTCGAGcaacggcgggggatgctttg-3

SQSTM1-T1- Forward: 5-CCGGAATTC atggccatgtcctacgtgaa-3

SQSTM1-T1- Reverse: 5-CCGCTCGAGcaacggcgggggatgctttg-3

SQSTM1-T2- Forward1: 5-CCGGAATTCatggcgtcgctcaccgtgaa-3

SQSTM1-T2- Reverse1: 5-tgtcaattcctcgtcactggaaaaggcaacccccaacgttcttcaggaaattcac-3

SQSTM1-T2- Forward2: 5-cttttccagtgacgaggaattgacagtgaatttcctgaagaacgttggggagagt-3

SQSTM1-T2- Reverse2: 5-CCGCTCGAGcaacggcgggggatgctttg-3

SQSTM1-T3- Forward1: 5-CCGGAATTCatggcgtcgctcaccgtgaa-3

SQSTM1-T3- Reverse1: 5-cgccctcaacattcccacccggcttactcggatcctccgatggaccagaa-3

SQSTM1-T3- Forward2: 5-ttctggtccatcggaggatccgagtaagccgggtgggaatgttgagggcg-3

SQSTM1-T3- Reverse2: 5-CCGCTCGAGcaacggcgggggatgctttg-3

SQSTM1-T4- Forward1: 5-CCGGAATTC atggcgtcgc tcaccgtgaa-3

SQSTM1-T4- Reverse1: 5-gccgcgggtcagcctctggcgggaggctggggtcagagcagcagctgctt-3

SQSTM1-T4- Forward2: 5-agcagctgctgctctgaccccagc ctcccgccagaggctgacccgcggc-3

SQSTM1-T4- Reverse2: 5-CCGCTCGAGcaacggcgggggatgctttg-3

SQSTM1-T5- Forward: 5-CCGGAATTCatggcgtcgctcaccgtgaa-3

SQSTM1-T5- Reverse: 5-CCGCTCGAGgcgggagatgtgggtacaaggcagcttc-3

SQSTM1delZZ- Forward1: 5-CCGGAATTCatggcgtcgctcaccgtgaa-3

SQSTM1delZZ- Reverse1: 5-gcccgaaggggctggggaacatgttgcggggcgcctcctgag-3

SQSTM1delZZ- Forward2: 5-gccccgcaacatgttccccagccccttcgggcac-3

SQSTM1delZZ- Reverse2: 5-CCGCTCGAGcaacggcgggggatgctttg-3

SQSTM1K420R-forward: 5-TCACCAGGCTCCTGCAGACCaggAACTATGAC-3

SQSTM1K420r-reverase: 5-gccgctccgatgtcatagttcctggtctgcag-3