

Appendix tables for Kim et al., The ubiquitinating enzyme USP20 is required for autophagy initiation through the stabilization of ULK1.

Appendix tables

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Appendix table S1. Primer sequences used to construct plasmids in this study

Construct	Direction	Sequence(5'-3')
Flag-ULK1	Forward	GATGAATTCATGGAGCCGGGCCGCGCGGC
	Reverse	GATCTCGAGTAGTCAGGCATAGACACCACTCAG
Flag-ULK1 KD	Forward	GATGAATTCATGGAGCCGGGCCGCGCGGC
	Reverse	GATCTCGAGTAGCAAGAAAGGGTGGTGGAAAAA
Flag-ULK1 S/T	Forward	GATGAATTCGATGCCAGCACCCCATCAAG
	Reverse	GATCTCGAGTAGAGCCTCGAAGGTCACAGCCCC
Flag-ULK1 CTD	Forward	GATGAATTCCCTGACCTCCAGAGGAGACC
	Reverse	GATCTCGAGTAGTCAGGCATAGACACCACTCAG
Myc-ULK1	Forward	ATGGAGCCCGGCCGCGGC
	Reverse	TCAGGCACAGATGCCAGTC
HA-USP20	Forward	ATGGGGGACTCCAGGGAC
	Reverse	TCACACGGCCCCGCGTCTC
GST-USP20	Forward	ATGGGGGACTCCAGGGAC
	Reverse	TCACACGGCCCCGCGTCTC
Flag-USP20	Forward	ATGGGGGACTCCAGGGAC
	Reverse	TCACACGGCCCCGCGTCTC
Flag-USP20 ZF-UBP	Forward	ATGGGGGACTCCAGGGAC
	Reverse	TCAGAGGCCTCGAGGTTTCAG
Flag-USP20 UCH	Forward	ACGGGCATGAAGAACCTC
	Reverse	TCAGTAGAAGAGTACGTAGCC
Flag-USP20 DUSP	Forward	AGGAAGAGCAGCGAGGAG
	Reverse	TCACACGGCCCCGCGTCTCGGC
Flag-rUSP20-CI (C154S,H643Q)	Forward	C154S - CGGCGTTCATGTAGGAGGAGTTCCTCCGAGG H643Q - GCAGGCAGTGGGCAGTACATCGCCTACTG
	Reverse	C154S - CCTCGGGAACCTCCTACATGAACGCCG H643Q - GCAGGCAGTGGGCAGTACATCGCCTACTG
Flag-rUSP20-WT	Forward	GAGAGGAGCTGCGTAGATGTGCGTCATTGTCCGGCTCTG
	Reverse	CAGAGCCGGACAATGACGCACATCTACGCAGCTCCTCTC
3XFlag-mcherry-ULK1	Forward	GATGAATTCGATGGAGCCGGGCCGCGCGGC
	Reverse	GATAGATCTTAGTCAGGCATAGACACCACTCAG
Flag-ULK1 K865R	Forward	GAGATTGCAGCCCTGAGGGGAAGTGCCAGTGAG
	Reverse	CTCACTGGCACTTCCCCTCAGGGCTGCAATCTC
Flag-ULK1 K906R	Forward	CTGGTTCTGTA CTTGAGGGTGGCTGAGCTGCTG
	Reverse	CAGCAGCTCAGCCACCCTCAAGTACAGAACCAG
Flag-ULK1 K926R	Forward	CAGATTTCGAGCTGGCAGACTCTGCCTTTCATCT
	Reverse	AGATGAAAGGCAGAGTCTGCCAGCTCGAATCTG
Flag-ULK1 K934R	Forward	CTTTCATCTACTGTGAGGCAGGTGGTACGCAGA
	Reverse	TCTGCGTACCACCTGCCTCACAGTAGATGAAAG
Flag-ULK1 K945R	Forward	CTAAATGAGCTGTACAGGGCCAGCGTGGTATCC
	Reverse	GGATACCACGCTGGCCCTGTACAGCTCATTAG
Flag-ULK1 K965R	Forward	CGCTTCTTCTGGACAGACAACGGCTGCTGGAC
	Reverse	GTCCAGCAGCCGTTGTCTGTCCAGAAAGAAGCG
Flag-ULK1 K1010R	Forward	GTACCGAGATATCACAGAGCCCTGCTATTGCTG
	Reverse	CAGCAATAGCAGGGCTCTGTGATATCTCGGTAC
Flag-ULK1 K1033R, K1035R	Forward	ATTGAGAACATTGCCAGATGCAGGCTGTGCATTGAGAGG
	Reverse	CCTCTCAATGCACAGCCTGCATCTGGCAATGTTCTCAAT

Appendix table S2. Primary antibodies used in this study

Antibody name	Company name	Cat No.	Species	Assay	Dilution / Amount	Validation	Citation
ULK1	Cell signaling	#8054	Rabbit	IB	1:2000	Datasheet by company	PLoS One. 2017 Sep 14;12(9):e0184909. doi: 10.1371/journal.pone.0184909. eCollection 2017.
ULK1	Santa Cruz	sc-33182	Rabbit	IP	1 µg	Datasheet by company	EMBO J. 2015 May 5;34(9):1214-30. doi: 10.15252/embj.201489920. Epub 2015 Mar 14.
phospho-ULK1 Ser758	Cell signaling	#6888	Rabbit	IB	1:2000	Datasheet by company	Nat Commun. 2017 Sep 18;8(1):571. doi: 10.1038/s41467-017-00628-y.
cleaved-Caspase3	Cell signaling	#9661	Rabbit	IB	1:5000	Datasheet by company	Nat Commun. 2017 Sep 19;8(1):598. doi: 10.1038/s41467-017-00590-9.
S6K	Cell signaling	#9202	Rabbit	IB	1:2000	Datasheet by company	Mol Oncol. 2017 Sep;11(9):1143-1155. doi: 10.1002/1878-0261.12079. Epub 2017 Jun 28
phospho-S6K Thr389	Cell signaling	#9234	Rabbit	IB	1:5000	Datasheet by company	Nat Commun. 2017 Sep 19;8(1):584. doi: 10.1038/s41467-017-00551-2.
LC3B	Cell signaling	#9234	Rabbit	IB	1:2000	Datasheet by company	EMBO Rep. 2017 Oct;18(10):1727-1739. doi: 10.15252/embr.201744069. Epub 2017 Aug 23.
PARP	Cell signaling	#9542	Rabbit	IB	1:5000	Datasheet by company	Exp Ther Med. 2017 Oct;14(4):3265-3272. doi: 10.3892/etm.2017.4873. Epub 2017 Aug 2.
SQSTM1/p62	Cell signaling	#D5E2	Rabbit	IB	1:2000	Datasheet by company	Cell Death Dis. 2017 Sep 14;8(9):e3048. doi: 10.1038/cddis.2017.438.
USP20/VDU2	Bethyl laboratory	A301-189A	Rabbit	IB	1:2000	Datasheet by company	J Biol Chem. 2016 Apr 1;291(14):7450-64. doi: 10.1074/jbc.M115.687129. Epub 2016 Feb 2.
ATG13	Sigma	SAB4200100	Rabbit	IB	1:5000	Datasheet by company	Autophagy. 2014 Jun;10(6):957-71. doi: 10.4161/auto.28363.
phospho-ATG13 S318	Rockland	600-401-C49	Rabbit	IB	1:5000	Datasheet by company	Elife. 2017 Feb 14;6. pii: e23063. doi: 10.7554/eLife.23063
ATG5	Cell signaling	#12994	Rabbit	IB	1:5000	Datasheet by company	PLoS One. 2017 Sep 14;12(9):e0184909. doi: 10.1371/journal.pone.0184909. eCollection 2017.
β-actin	Sigma	A5441	Mouse	IB	1:10000	Datasheet by company	Br J Cancer. 2014 Oct 28;111(9):1788-801. doi: 10.1038/bjc.2014.475. Epub 2014 Aug 28.
Flag	Sigma	F7425	Rabbit	IB	1:5000	Datasheet by company	Autophagy. 2014 Sep;10(9):1549-64. doi: 10.4161/auto.29329. Epub 2014 Jun 30.
Flag M2	Sigma	F1804	Mouse	IP	1 µg	Datasheet by company	Cell Cycle. 2017 May 19;16(10):947-956. doi: 10.1080/15384101.2017.1310350. Epub 2017 Apr 5.
FIP200	Protein Tech	17250-1-AP	Rabbit	IB	1:2000	Datasheet by company	Autophagy. 2017 May 4;13(5):955-966. doi: 10.1080/15548627.2017.1287652. Epub 2017 Feb 15.
HA-peroxidase	Roche	12013819001	Mouse	IB	1:5000	Datasheet by company	Nat Commun. 2014 Sep 11;5:4857
Myc-peroxidase	Roche	118141500001	Mouse	IB	1:10000	Datasheet by company	Mol Cell Biol. 2015 May;35(10):1741-53
Tubulin	Merck Millipore	05-829	Mouse	IB	1:10000	Datasheet by company	J Cell Mol Med. 2015 Jan;19(1):210-26. doi: 10.1111/jcmm.12418. Epub 2014 Sep 30.
FK2	Enzo	BML-PW0150	Mouse	IB	1:2000	Datasheet by company	Biochim Biophys Acta. 2016 Nov;1863(11):2658-2667. doi: 10.1016/j.bbamcr.2016.08.006. Epub 2016 Aug 16.
Annexin V	BD Pharmingen	556547	NA	F	3 µl/100 µl	Datasheet by company	J Biol Chem. 1990 Mar 25;265(9):4923-8.
7-AAD	BD Pharmingen	559763	NA	F	3 µl/100 µl	Datasheet by company	Proc Natl Acad Sci U S A. 1996 Feb 20;93(4):1624-9.
NA: Not Applicable, IB: Immunoblot, IP: Immunoprecipitation, F: Flow cytometry							

Appendix table S3. Target Sequences of siRNAs used in this study

siRNA	Species	Target Sequences
siUSP20-1	Human	GGACAATGATGCTCACCTA
siUSP20-2	Human	TCGAGTGACACGGATGAGAAA
siULK1	Human	CGGAGAGCCTGCAGGAGAA