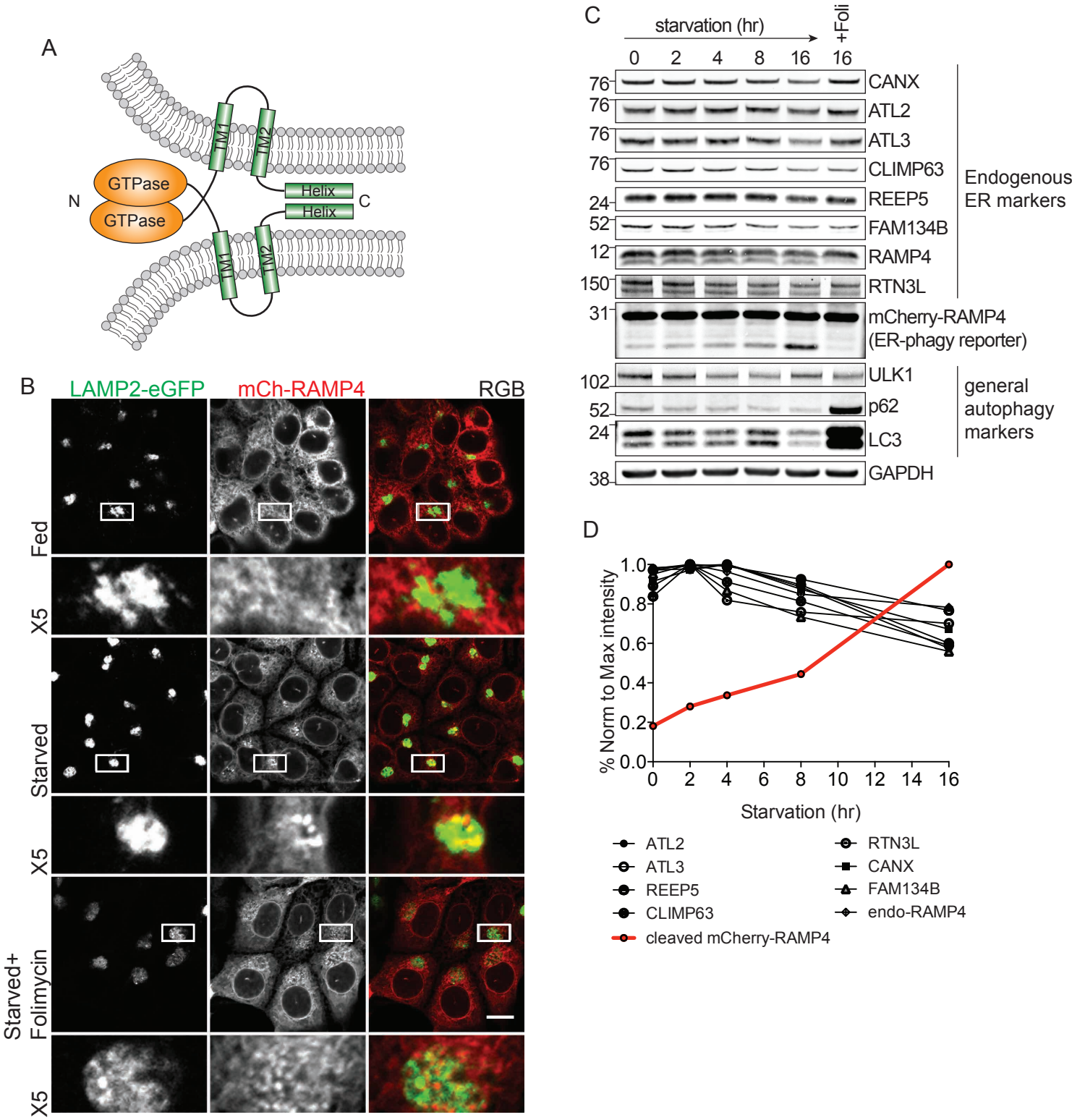
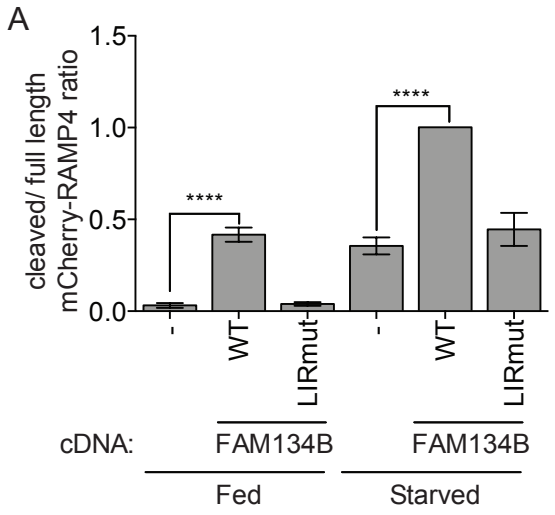
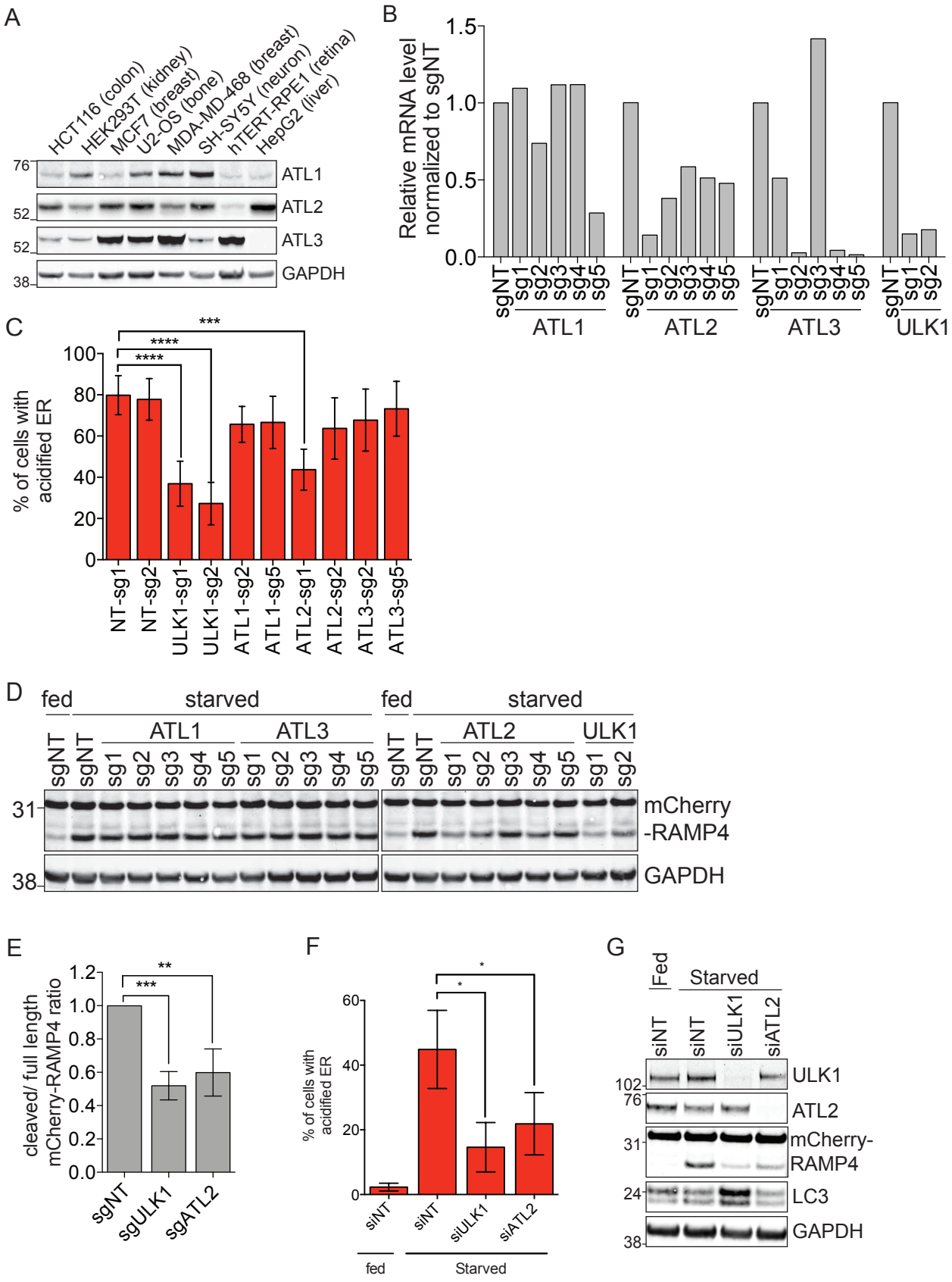
Supplementary Figure 1



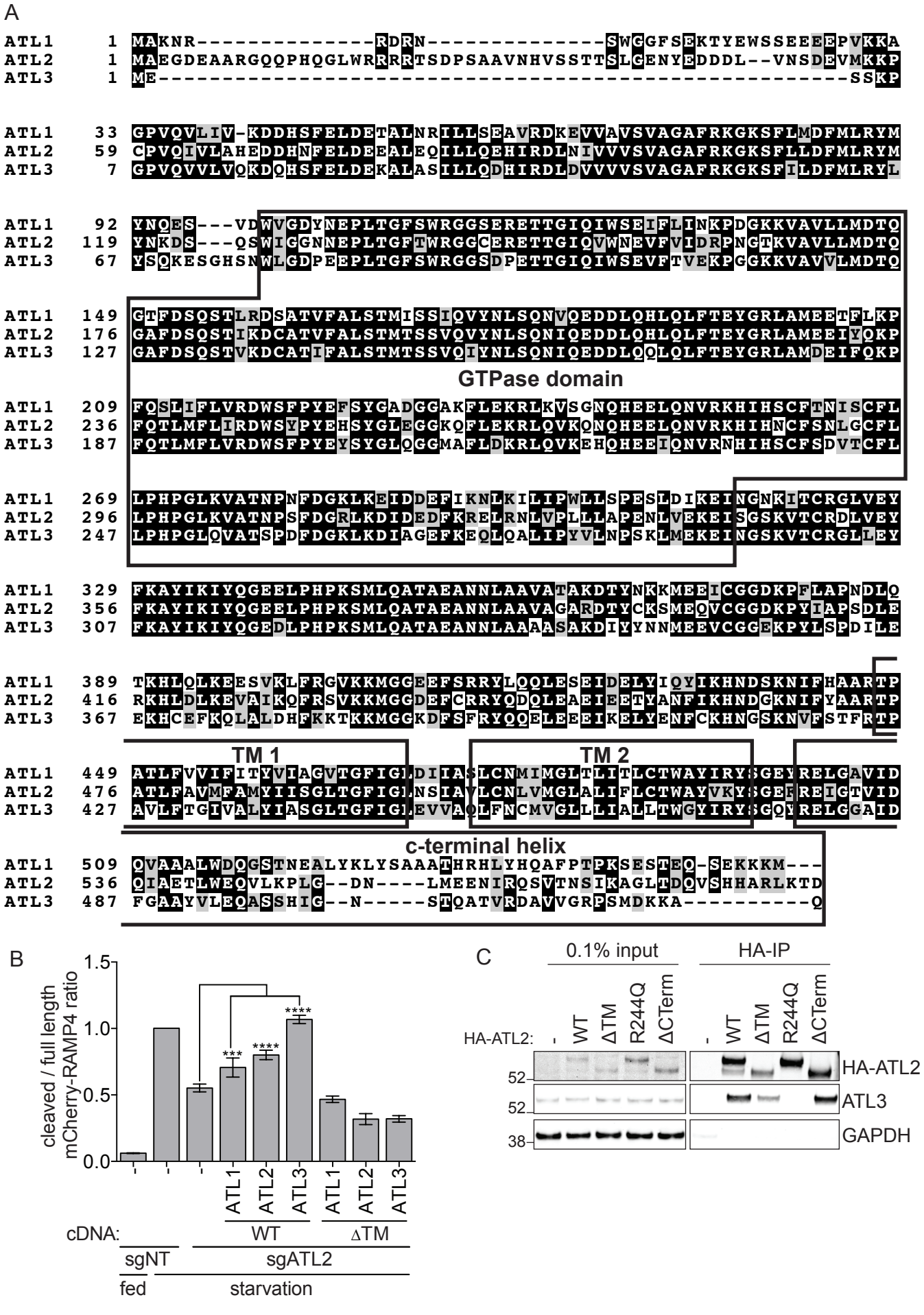
Supplementary Figure 2



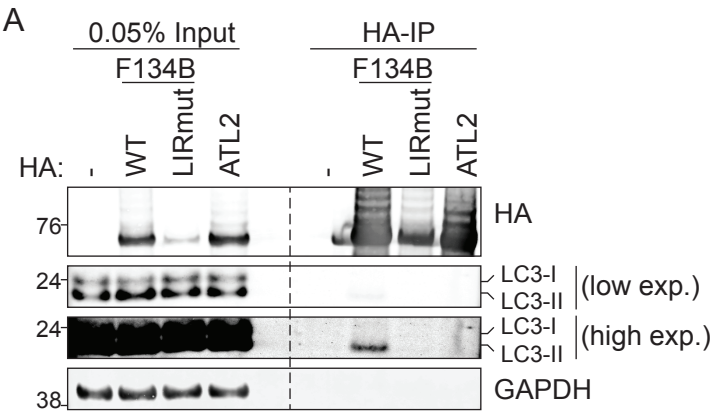
Supplementary Figure 3

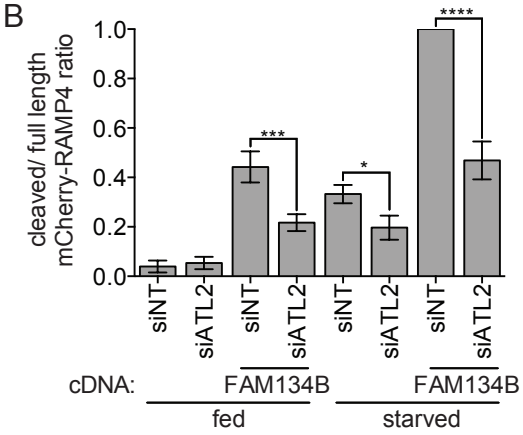


Supplementary Figure 4

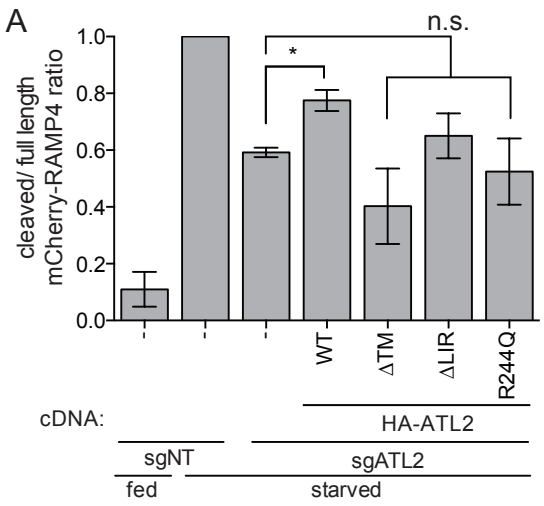


Supplementary Figure 5





Supplementary Figure 6



Supp Table 1: sgRNAs Targeting Transcription Start Site for CRIPSRi

sgRNAs targeting TSS	Protospacer	Forward Primer	Reverse Primer	Vector backbone
pLG1-puro Non-targeting sgRNA 1	GCGCCAAACGTGCCCTGACGG	TTGGGCGCCAAACGTGCCCTGACGGTTTAAGAGC	TTAGCTCTTAAACCCGTCAGGGCACGTTTGGCGCCAACAAG	pLG1-Puro
pLG1-puro Non-targeting sgRNA 3	GGCTCGGTCCCGCGTCGTCG	TTGGGGCTCGGTCCCGCGTCGTCGTTTAAGAGC	TTAGCTCTTAAACCGACGACGCGGGACCGAGCCCAACAAG	pLG1-Puro
pLG1-puro-sgULK1-1	GGCGGCGGCACAGAGACCGT	TTGGGCGGCGGCACAGAGACCGTGTTTAAGAGC	TTAGCTCTTAAACACGGTCTCTGTGCCGCCGCCCAACAAG	pLG1-Puro
pLG1-puro-sgULK1-2	GCCGACTCCGGCTCCAACTA	TTGGCCGACTCCGGCTCCAACTAGTTTAAGAGC	TTAGCTCTTAAACTAGTTGGAGCCGGAGTCGGCCAACAAG	pLG1-Puro
pLG1-puro-sgATL1-1	GCAACCTGCGGCCCCGGAGA	TTGGCAACCTGCGGCCCCGGAGAGTTTAAGAGC	TTAGCTCTTAAACTCTCCGGGGCCGCAGGTTGCCAACAAG	pLG1-Puro
pLG1-puro-sgATL1-2	GGCGCTCGCTGCCTTCTCCG	TTGGGCGCTCGCTGCCTTCTCCGGTTTAAGAGC	TTAGCTCTTAAACCGGAGAAGGCAGCGAGCGCCCAACAAG	pLG1-Puro
pLG1-puro-sgATL1-3	GGGAACCCACAAGTCCCCGG	TTGGGGAACCCACAAGTCCCCGGGTTTAAGAGC	TTAGCTCTTAAACCCGGGGACTTGTGGGTTCCCCAACAAG	pLG1-Puro
pLG1-puro-sgATL1-4	GCGGGGCCGCAGGTTGCTGG	TTGGCGGGGCCGCAGGTTGCTGGGTTTAAGAGC	TTAGCTCTTAAACCCAGCAACCTGCGGCCCCGCCAACAAG	pLG1-Puro
pLG1-puro-sgATL1-5	GTCCTCCCAACCGATCGCTA	TTGGTCCTCCCAACCGATCGCTAGTTTAAGAGC	TTAGCTCTTAAACTAGCGATCGGTTGGGAGGACCAACAAG	pLG1-Puro
pLG1-puro-sgATL2-1	GAGGGCAGCAACCGCACCAG	TTGGAGGGCAGCAACCGCACCAGGTTTAAGAGC	TTAGCTCTTAAACCTGGTGCGGTTGCTGCCCTCCAACAAG	pLG1-Puro
pLG1-puro-sgATL2-2	GTAGCTGCTGGGAGAACCAG	TTGGTAGCTGCTGGGAGAACCAGGTTTAAGAGC	TTAGCTCTTAAACCTGGTTCTCCCAGCAGCTACCAACAAG	pLG1-Puro
pLG1-puro-sgATL2-3	GTCGGTACAAGATGGCGGAG	TTGGTCGGTACAAGATGGCGGAGGTTTAAGAGC	TTAGCTCTTAAACCTCCGCCATCTTGTACCGACCAACAAG	pLG1-Puro
pLG1-puro-sgATL2-4	GAAGGTCGGGGCGGACACGG	TTGGAAGGTCGGGGCGGACACGGGTTTAAGAGC	TTAGCTCTTAAACCCGTGTCCGCCCCGACCTTCCAACAAG	pLG1-Puro
pLG1-puro-sgATL2-5	GAGGCCGCCCCCTAAGGTCG	TTGGAGGCCGCCCCCTAAGGTCGGTTTAAGAGC	TTAGCTCTTAAACCGACCTTAGGGGGCGGCCTCCAACAAG	pLG1-Puro
pLG1-puro-sgATL3-1	GTGGCCCAACGGACAGCCcg	TTGGTGGCCCAACGGACAGCCcgGTTTAAGAGC	TTAGCTCTTAAACcgGGCTGTCCGTTGGGCCACCAACAAG	pLG1-Puro
pLG1-puro-sgATL3-2	GAGCAGGGGTGCAGAGGAGA	TTGGAGCAGGGGTGCAGAGGAGAGTTTAAGAGC	TTAGCTCTTAAACTCTCCTCTGCACCCCTGCTCCAACAAG	pLG1-Puro
pLG1-puro-sgATL3-3	GCGGCCCTTGGGCCGCCCAG	TTGGCGGCCCTTGGGCCGCCCAGGTTTAAGAGC	TTAGCTCTTAAACCTGGGCGGCCCAAGGGCCGCCAACAAG	pLG1-Puro
pLG1-puro-sgATL3-4	GCAGAGGAGAGGGACGGGTG	TTGGCAGAGGAGAGGGACGGGTGGTTTAAGAGC	TTAGCTCTTAAACCACCCGTCCCTCTCCTCTGCCAACAAG	pLG1-Puro
pLG1-puro-sgATL3-5	GGTGCGGGCGGGAACGAACC	TTGGGTGCGGGCGGGAACGAACCGTTTAAGAGC	TTAGCTCTTAAACGGTTCGTTCCCGCCCGCACCCAACAAG	pLG1-Puro

*** Unless specified otherwise, sgRNA highlighted in bold were used as the default sgRNAs.**

Protospacer sequences were obtained from Gilbert et al. 2014.

shRNA constructs	Target sequence	Forward Primer	Reverse Primer	Reference
pLKO.1-puro-shNT	CCTAAGGTTAAGTCGCCCTCG	CCGGCCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGGTTTTTG	AATTCAAAAACCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG	sequence obtained from Addgene #26701, subcloned into pLKO.1 puro vector
pLKO.1-puro-shFAM134B*	GAGGTATCCTGGACTGATAAT	CCGGGAGGTATCCTGGACTGATAATCTCGAGATTATCAGTCCAGGATACCTCTTTTTG	AATTCAAAAAGAGGTATCCTGGACTGATAATCTCGAGATTATCAGTCCAGGATACCTC	sequence obtained from Khaminets et al. (2015) Nature

Supp Table 2: Overexpression plasmids

cDNA expression constructs	Reference
pEF1a-dCas9-HA-BFP-KRAB-NLS	Addgene #102244
TetOn-mCherry-eGFP-RAMP4	cDNA of HCT116 cells; NM_014445.3
pLenti-X1-Hygro-mCherry-RAMP4	cDNA of HCT116 cells; NM_014445.3
pLenti-X1-Blast-mCherry-RAMP4	cDNA of HCT116 cells; NM_014445.3
pLenti-X1-Blast-mLAMP1-eGFP	mLAMP1 subcloned from Addgene #55073
pLenti-X1-Neo-GFP-ATL1	Derived from Harvard plasmID (HsCD00326984); NM_015915.4
pLenti-X1-Neo-GFP-ATL1-ΔTM	Derived from pLenti-X1-Neo-GFP-ATL1; aa 1-449 only
pLenti-X1-Neo-GFP-ATL2	cDNA of HCT116 cells, NM_001135673.3
pLenti-X1-Neo-GFP-ATL2-ΔTM	Derived from pLenti-X1-Neo-GFP-ATL2; aa 1-476 only
pLenti-X1-Neo-GFP-ATL2-Δcterm	Derived from pLenti-X1-Neo-GFP-ATL2; aa 1-523 only
pLenti-X1-Neo-GFP-ATL2-R244Q	Derived from pLenti-X1-Neo-GFP-ATL2; with Q to Q mutation at aa 244
pLenti-X1-Neo-GFP-ATL3	cDNA of HCT116 cells, NM_001290048.1
pLenti-X1-Neo-GFP-ATL3-ΔTM	Derived from pLenti-X1-Neo-GFP-ATL3; aa 1- 427 only
pLenti-X1-Neo-HA-ATL1	Derived from Harvard plasmID (HsCD00326984); NM_015915.4
pLenti-X1-Neo-HA-ATL1-ΔTM	Derived from pLenti-X1-Neo-HA-ATL1; AA 1-449 only
pLenti-X1-Neo-HA-ATL2	cDNA of HCT116 cells, NM_001135673.3
pLenti-X1-Neo-HA-ATL2-ΔTM	Derived from pLenti-X1-Neo-HA-ATL2; aa 1-476 only
pLenti-X1-Neo-HA-ATL2-Δcterm	Derived from pLenti-X1-Neo-HA-ATL2; aa 1-523 only
pLenti-X1-Neo-HA-ATL2-R244Q	Derived from pLenti-X1-Neo-HA-ATL2; with R to Q mutation at aa 244
pLenti-X1-Neo-HA-ATL2-K107A	Derived from pLenti-X1-Neo-HA-ATL3; with K to A mutation at aa 107
pLenti-X1-Neo-HA-ATL3	cDNA of HCT116 cells, NM_001290048.1
pLenti-X1-Neo-HA-ATL3-ΔTM	Derived from pLenti-X1-Neo-HA-ATL3; aa 1-427 only
pLenti-X1-Neo-BFP-ATL2	cDNA of HCT116 cells, NM_001135673.3
pLenti-X1-Neo-BFP-ATL2-ΔTM	Derived from pLenti-X1-Neo-BFP-ATL2; aa 1-476 only
pLenti-X1-Neo-BFP-ATL2-Δcterm	Derived from pLenti-X1-Neo-BFP-ATL2; aa 1-523 only
pLenti-X1-Neo-BFP-ATL2-R244Q	Derived from pLenti-X1-Neo-BFP-ATL2; with R to Q mutation at aa 244
pLenti-X1-Neo-GFP-FAM134B	cDNA of HCT116 cells, NM_001034850.2
pLenti-X1-Neo-HA-FAM134B	cDNA of HCT116 cells, NM_001034850.2
pLenti-X1-Neo-GFP-FAM134B-LIRmut	Derived from pLenti-X1-Neo-GFP-FAM134B; DDFELL to DDAEAL aa 453-458
pLenti-X1-Neo-HA-FAM134B-LIRmut	Derived from pLenti-X1-Neo-HA-FAM134B; DDFELL to DDAEAL aa 453-458

Supp Table 3: siRNA sequences

siRNA	target sequence	Reference
siNT	Dharmacon ON-TARGETplus non-targeting #1	Cat No.: D-001810-01-05
siULK1	Dharmacon siGenome Smartpool	Cat No.: M-005049-00-0005
siATL2	GGAGCUAUCCUUAUGAACAUUCAUA	Rismanchi et al. (2008) Hum Mol Genet.
siATL3	GGUUAGAGAUUGGAGUUUCCCUUAU	Rismanchi et al. (2008) Hum Mol Genet.
siFAM134B	AGGUAUCCUGGACUGAUAAUG	Rivera-Monroy et al. (2016) Sci Rep.

Supp Table 4: qRT-PCR primers

Target	Primer	Sequence
ULK1	Forward	GTCGCCGTCAAGTGCATTAACA
	Reverse	CGTACAGGGCCACGATGTTTTC
ATL1	Forward	CAGCACCTCCAGCTTTTCACTG
	Reverse	CACCACCATCGGCTCCATATGA
ATL2	Forward	TCCTTTTGCCACATCCTGGTCT
	Reverse	GCAAGCAGCAATGGAACCAGAT
ATL3	Forward	AAGATCTGCCTCACCCCAAGTC
	Reverse	CTCCCCCACAAACCTCTTCCAT
β-actin	Forward	GGGTCAGAAGGATTCCTATG
	Reverse	GGTCTCAAACATGATCTGGG

Supp Table 5: Primary and secondary antibodies for western blotting and immunofluorescence

Target	Company	Cat. No.	Species	WB Dilution	IF Dilution
ATL1	Cell Signaling	#12728	rabbit	1 in 1000	
ATL2	Novus Biologicals	NBP1-78733	rabbit	1 in 1000	
ATL2	Santa Cruz	sc-109213	goat	1 in 1000	
ATL3	Bethyl Laboratories	A303-313A	rabbit	1 in 1000	
CANX	Cell Signaling	#2679	rabbit	1 in 2000	1 in 250
CANX	Santa Cruz	sc-46669	mouse	1 in 1000	1 in 250
CLIMP63/CKAP4	Bethyl Laboratories	A302-257A	rabbit	1 in 2000	
FAM134B	Abcam	ab151755	rabbit	1 in 1000	
GAPDH	Cell Signaling	#97166	mouse	1 in 2000	
GFP	Abcam	ab6556	rabbit	1 in 2000	
GFP	Santa Cruz	sc-9996	mouse	1 in 2000	
HA epitope	Cell Signaling	#3724	rabbit	1 in 2000	
LAMP2	Santa Cruz	sc-18822	mouse	1 in 1000	1 in 250
LC3	Nanotools	0231-100	mouse	1 in 200	1 in 200
LC3B	Novus Biologicals	NB100-2220	rabbit	1 in 1000	1 in 250
mCherry	Abcam	ab183628	rabbit	1 in 2000	
p62	Santa Cruz	sc-28359	mouse	1 in 1000	
PDI	Santa Cruz	sc-20132	rabbit	1 in 2000	
RAMP4	Abcam	ab184571	rabbit	1 in 1000	
REEP5	ProteinTech	14643-1-AP	rabbit	1 in 1000	
RTN3	Novus Biologicals	A302-860A	rabbit	1 in 500	
ULK1	Cell Signaling	#8054	rabbit	1 in 1000	

Secondary antibodies for Western	Catalogue No.	Company	Dilution	Application
Donkey anti-mouse IRDye 680CW	926-32222	Li-Cor	1 in 10,000	near-infrared fluorescence
Donkey anti-mouse IRDye 800CW	926-32212	Li-Cor	1 in 10,000	near-infrared fluorescence
Donkey anti-rabbit IRDye 680CW	926-32223	Li-Cor	1 in 10,000	near-infrared fluorescence
Donkey anti-rabbit IRDye 800CW	926-32213	Li-Cor	1 in 10,000	near-infrared fluorescence
Donkey anti-goat IgG HRP	sc-2020	Santa Cruz	1 in 5000	HRP chemiluminescence

Secondary Antibody for Immunofl	Catalogue No.	Company	Dilution
Donkey anti-mouse AF350	A10035	Invitrogen	1 in 200
Donkey anti-mouse AF488	A21202	Invitrogen	1 in 200
Donkey anti-mouse AF594	A21203	Invitrogen	1 in 500
Donkey anti-rabbit AF350	A10039	Invitrogen	1 in 200
Donkey anti-rabbit AF488	A21206	Invitrogen	1 in 500
Donkey anti-rabbit AF594	A21207	Invitrogen	1 in 500