

Appendix

TOLLIP acts as a cargo adaptor to promote lysosomal degradation of aberrant ER membrane proteins

Table of Contents

Appendix Table S1.....	page 2–11
Appendix Figure S1 and S2.....	page 12–14

Appendix Table S1. Reagents and Tools Table.

Reagent/Resource	Reference or Source	Identifier or Catalog Number
Experimental Models		
Human: HEK293 T-REx/A53T-HA-HP cells	This study	N/A
Human: HEK293A cells	Invitrogen	Cat#R70507
Human: HEK293A T-REx/mCherry-A53T-HA-HP cells	This study	N/A
Human: HEK293A T-REx/mCherry-HA-VAPB cells	This study	N/A
Human: HEK293A T-REx/mCherry-HA-P56S VAPB cells	This study	N/A
Human: HEK293A <i>CCPG1</i> KO cells	This study	N/A
Human: HEK293A <i>FAM134B</i> KO cells	This study	N/A
Human: HEK293A <i>RTN3</i> KO cells	This study	N/A
Human: HEK293A <i>SEC62</i> KO cells	This study	N/A
Human: HEK293A <i>TEX264</i> KO cells	This study	N/A
Human: HEK293A <i>FIP200</i> KO cells	This study	N/A
Human: HEK293A <i>ATG7</i> KO cells	This study	N/A
Human: HEK293A <i>TOLLIP</i> KO cells clone1	This study	N/A
Human: HEK293A <i>TOLLIP</i> KO cells clone2	This study	N/A
Human: HEK293A <i>TOLLIP</i> KO clone1/empty vector cells	This study	N/A
Human: HEK293A <i>TOLLIP</i> KO clone1/Venus-TOLLIP cells	This study	N/A
Human: HeLa cells	ATCC	Cat#CCL-2
Human: SH-SY5Y cells	ATCC	N/A
Mouse: NSC34 cells	Cedarlane	Cat#CLU140
Recombinant DNA		
pcDNA6/TR	Invitrogen	N/A

pcDNA4/TO	Invitrogen	N/A
pcDNA4/TO/A53T-HA-HP	This study	N/A
pcDNA4/TO/mCherry-A53T-HA-HP	This study	N/A
pcDNA4/TO/mCherry-A53T-FLAG-HP	This study	N/A
pcDNA4/TO/mCherry-EGFP-A53T-HA-HP	This study	N/A
pcDNA4/TO/mCherry-A53T-HA	This study	N/A
pcDNA4/TO/mCherry-E-Syt1	This study	N/A
pcDNA4/TO/VN-A53T-HA-HP	This study	N/A
pcDNA4/TO/mCherry-HA-VAPB	This study	N/A
pcDNA4/TO/mCherry-FLAG-VAPB	This study	N/A
pcDNA4/TO/mCherry-HA-P56S VAPB	This study	N/A
pcDNA4/TO/mCherry-FLAG-P56S VAPB	This study	N/A
pcDNA4/TO/Seipin-HA-mCherry	This study	N/A
pcDNA4/TO/Seipin-FLAG-mCherry	This study	N/A
pcDNA4/TO/N88S Seipin-HA-mCherry	This study	N/A
pcDNA4/TO/N88S Seipin-FLAG-mCherry	This study	N/A
pcDNA4/TO/S90L Seipin-HA-mCherry	This study	N/A
pcDNA4/TO/S90L Seipin-FLAG-mCherry	This study	N/A
pcDNA3/GW	Invitrogen	N/A
pcDNA3/GW/Venus-p62	This study	N/A
pcDNA3/GW/Venus-OPTN	This study	N/A
pcDNA3/GW/Venus-NBR1	This study	N/A
pcDNA3/GW/Venus-TOLLIP	This study	N/A
pcDNA3/GW/Venus-TOLLIP Δ TBD (Δ 1–44)	This study	N/A
pcDNA3/GW/Venus-TOLLIP TBD ^{mut} (F21A)	This study	N/A
pcDNA3/GW/Venus-TOLLIP Δ C2 (Δ 53–178)	This study	N/A

pcDNA3/GW/Venus-TOLLIP C2 ^{mut #1} (R123A)	This study	N/A
pcDNA3/GW/Venus-TOLLIP C2 ^{mut #2} (R78A)	This study	N/A
pcDNA3/GW/Venus-TOLLIP ΔIDR (Δ181–229)	This study	N/A
pcDNA3/GW/Venus-TOLLIP IDR ^{mut} (181– 229>(GGGGS) ₉ GGGG)	This study	N/A
pcDNA3/GW/Venus-TOLLIP ΔCUE (Δ231–274)	This study	N/A
pcDNA3/GW/Venus-TOLLIP CUE ^{mut} (L267A, L268A)	This study	N/A
pcDNA3/GW/Venus-TOLLIP IDR (181–229)	This study	N/A
pcDNA3/GW/FLAG-TOLLIP	This study	N/A
pcDNA3/GW/FLAG-TOLLIP ΔTBD (Δ1–44)	This study	N/A
pcDNA3/GW/FLAG-TOLLIP C2 ^{mut #1} (R123A)	This study	N/A
pcDNA3/GW/FLAG-TOLLIP C2 ^{mut #2} (R78A)	This study	N/A
pcDNA3/GW/FLAG-TOLLIP LIR ^{mut} (W133A, T134A, H135A, I136A, W151A, Y152A, S153A, L154A)	This study	N/A
pcDNA3/GW/FLAG-TOLLIP ΔIDR (Δ181–229)	This study	N/A
pcDNA3/GW/FLAG-TOLLIP IDR ^{mut} (181– 229>(GGGGS) ₉ GGGG)	This study	N/A
pcDNA3/GW/FLAG-TOLLIP ΔCUE (Δ231–274)	This study	N/A
pcDNA3/GW/FLAG-TOLLIP CUE ^{mut} (L267A, L268A)	This study	N/A
pcDNA3/GW/FLAG-NEDD4	This study	N/A
pcDNA3/GW/LAMP1-ECFP	This study	N/A
pcDNA3/GW/FLAG-C867S NEDD4	This study	N/A
pcDNA3/GW/Venus-2xFYVE	This study	N/A
pcDNA3/GW/mCherry- 2xFYVE	This study	N/A
pcDNA3/ECFP-2xFYVE	This study	N/A
pcDNA3/GW/FLAG-CFP	This study	N/A

pcDNA3/GW/VC-FLAG-TOLLIP	This study	N/A
pcDNA3/GW/H79G SAR1A-Venus	This study	N/A
pcDNA3/GW/CD147-6xMyc	This study	N/A
pcDNA3/GW/ss-mCherry-EGFP-KDEL	This study	N/A
pcDNA3/GW/FLAG-CCPG1	This study	N/A
pcDNA3/GW/FLAG-mouse Tollip	This study	N/A
pcDNA3/GW/NHK-HA	This study	N/A
pcDNA3/GW/D18G TTR-FLAG	This study	N/A
pGEX-6P-1	Cytiva	N/A
pGEX-6P-1/GST-TOLLIP IDR (181–230)	This study	N/A
pGEX-6P-1/GST-TOLLIP CUE (229–274)	This study	N/A
pGEX-6P-1/GST-HSP70	This study	N/A
pX459	(Ran <i>et al.</i> , 2013)	Addgene Plasmid #62988
pTRE-Tight-BI-DsRed-Express	Clontech	N/A
pTRE-Tight-BI-DsRed-Express/mCherry-HA-VAPB	This study	N/A
pTRE-Tight-BI-DsRed-Express/mCherry-HA-P56S VAPB	This study	N/A
pTRE-Tight-BI-DsRed-Express/Seipin-HA-mCherry	This study	N/A
pTRE-Tight-BI-DsRed-Express/N88S Seipin-HA-mCherry	This study	N/A
pTet-Off	Clontech	N/A
Antibodies		
Rat monoclonal anti-BAP31	Thermo Fisher Scientific	Cat#MA3-003; RRID: AB_325096
Goat polyclonal anti-Histone H3	Santa Cruz Biotechnology	Cat#sc-8654; RRID: AB_2118303
Mouse monoclonal anti-TOM20	Santa Cruz Biotechnology	Cat#sc-17764; RRID: AB_628381
Rabbit polyclonal anti-E-Syt1	Sigma-Aldrich	Cat#HPA016858; RRID: AB_1848404
Rat monoclonal anti- α -tubulin	Bio-Rad	Cat#MCA77G; RRID: AB_325003
Mouse monoclonal anti- β -catenin	Santa Cruz Biotechnology	Cat#sc-7963; RRID: AB_626807

Mouse monoclonal anti-SQSTM1/p62	Santa Cruz Biotechnology	Cat#sc-28359; RRID: AB_628279
Mouse monoclonal anti-KDEL (BiP)	Enzo Life Sciences	Cat#ADI-SPA-827; RRID: AB_10618036
Mouse monoclonal anti-GM130	BD Biosciences	Cat#610822; RRID: AB_398141
Mouse monoclonal anti-Actin	Sigma-Aldrich	Cat#A3853; RRID: AB_262137
Rabbit polyclonal anti-TOLLIP	Thermo Fisher Scientific	Cat#PA5-30747; RRID: AB_2548221
Rabbit polyclonal anti-OPTN	Proteintech	Cat#10837-1-AP; RRID: AB_2156665
Rabbit monoclonal anti-RAB7	Cell Signaling Technology	Cat#9367; RRID: AB_1904103
Rat monoclonal anti-HSC70	Enzo Life Sciences	Cat#ADI-SPA-815; RRID: AB_10617277
Rabbit polyclonal anti-SOD1	Enzo Life Sciences	Cat#ADI-SOD-100; RRID: AB_10616253
Mouse monoclonal anti-Ubiquitin	Santa Cruz Biotechnology	Cat#sc-8017; RRID: AB_628423
Goat polyclonal anti-Luciferase	Promega	Cat#G745A; RRID: AB_2335880
Rabbit monoclonal anti-NEDD4	Cell Signaling Technology	Cat#3607; RRID: AB_2149311
Mouse monoclonal anti-VPS34	Santa Cruz Biotechnology	Cat#sc-365404; RRID: AB_10846189
Rabbit polyclonal anti-CCPG1	Proteintech	Cat#13861-1-AP; RRID: AB_2074010
Rabbit polyclonal anti-FAM134B	Proteintech	Cat#21537-1-AP; RRID: AB_2878879
Mouse monoclonal anti-RTN3	Santa Cruz Biotechnology	Cat#sc-374599; RRID: AB_10986405
Rabbit polyclonal anti-SEC62	Bethyl	Cat#A303-981A; RRID: AB_2620330
Mouse monoclonal anti-TEX264	Santa Cruz Biotechnology	Cat#sc-100944; RRID: AB_2201887
Mouse monoclonal anti-LC3	Cosmo Bio	Cat#CAC-CTB-LC3-1-50; RRID: AB_1962479
Mouse monoclonal anti-ATG7	Santa Cruz Biotechnology	Cat#sc-376212; RRID: AB_10988418
Rabbit polyclonal anti-HERP	(Fujisawa <i>et al</i> , 2012)	N/A
Mouse monoclonal anti-IRE1 α	Santa Cruz Biotechnology	Cat#sc-390960
Rat monoclonal anti-HA	Roche	Cat#11867431001; RRID: AB_390919
Rabbit polyclonal anti-mCherry	Abcam	Cat#ab183628; RRID: AB_2650480
Mouse monoclonal anti-GFP	MBL International	Cat#M048-3; RRID: AB_591823
Mouse monoclonal anti-DYKDDDDK/FLAG (IB)	FUJIFILM Wako Shibayagi	Cat#012-22384; RRID: AB_10659717

Rabbit polyclonal anti-DYKDDDDK/FLAG (IF)	Thermo Fisher Scientific	Cat#PA1-984B; RRID: AB_347227
Mouse monoclonal anti-Myc	(Nishitoh <i>et al</i> , 2008)	N/A
Rat IgG1 isotype control	Bio X Cell	Cat#BE0290; RRID: AB_2687813
FITC-conjugated anti-mouse IgG	Jackson ImmunoResearch Labs	Cat#115-095-146; RRID: AB_2338599
Alexa Fluor 488-conjugated anti-mouse IgG	Molecular Probes	Cat#A-11029; RRID: AB_2534088
Alexa Fluor 488-conjugated anti-rat IgG	Molecular Probes	Cat#A-11006; RRID: AB_141373
Alexa Fluor 633-conjugated anti-rabbit IgG	Thermo Fisher Scientific	Cat#A-21070; RRID: AB_2535731
Alexa Fluor 633-conjugated anti-rat IgG	Thermo Fisher Scientific	Cat#A-21094; RRID: AB_2535749
HRP-conjugated anti-rabbit IgG	Cell Signaling Technology	Cat#7074; RRID: AB_2099233
HRP-conjugated anti-mouse IgG	Cell Signaling Technology	Cat#7076; RRID: AB_330924
HRP-conjugated anti-rat IgG	Cell Signaling Technology	Cat#7077; RRID: AB_10694715
HRP-conjugated anti-goat IgG	Santa Cruz Biotechnology	Cat#sc-2033; RRID: AB_631729
Oligonucleotides and other sequence-based reagents		
siRNA targeting sequence: Non-targeting: UGGUUACAUGUCGACUA A	Horizon	Cat#D-001810-01-05
siRNA targeting sequence: TOLLIP (#1): UCACGGUGGUACAGGCAA A	Horizon	Cat#J-016930-05
siRNA targeting sequence: TOLLIP #2: GGAAUAAGGUCAUCCACU G	Horizon	Cat#J-016930-07
siRNA targeting sequence: TOLLIP #3: GCAGGGCGUUGGCUAUGU G	Horizon	Cat#J-016930-08
p62 siRNA	Thermo Fisher Scientific	Cat#HSS113118
siRNA targeting sequence: OPTN:	Horizon	Cat#D-016269-01-0010

GCACGGCAUUGUCUAAAU
A

siRNA targeting sequence: NEDD4 #1: GAUCACAAUCCAGAACG A	Horizon	Cat#J-007178-07
siRNA targeting sequence: NEDD4 #2: GAACUAGAGCUUCUUAUG U	Horizon	Cat#J-007178-08
siRNA targeting sequence: VPS34: GAACAACGGUUUCGCUCU U	Horizon	Cat#J-007178-08
qPCR Primer: mCherry-HA- A53T-HP	Eurofins Genomics	Forward: AGTTCATGCGCTTCAAGGTG, Reverse: TTGGTCACCTTCAGCTTGGC
qPCR Primer: mCherry-HA- VAPB	Eurofins Genomics	Forward: CCCACAACGAGGACTACACC, Reverse: TGAATTCGAGGCTTGCATAA
qPCR Primer: Seipin-HA- mCherry	Eurofins Genomics	Forward: TTCCCTCGAGTATCCGTATGA, Reverse: CGCCCTCGATCTCGAACT
qPCR Primer: <i>BiP</i>	Eurofins Genomics	Forward: TGTTACAATCAAGGTCTATGAAG GTG, Reverse: CAAAGGTGACTTCAATCTGTGG
qPCR Primer: <i>HERP</i>	Eurofins Genomics	Forward: ATGGAGTCCGAGACCGAAC, Reverse: TTGGTGATCCAACAACAGCTT
qPCR Primer: <i>sXBP1</i>	Eurofins Genomics	Forward: TGCTGAGTCCGCAGCAGGTG, Reverse: GCTGGCAGGCTCTGGGGAAG
qPCR and semi-quantitative PCR Primer: human <i>RPS18</i>	Eurofins Genomics	Forward: TTTGCGAGTACTCAACACCAA, Reverse: GCATATCTTCGGCCCACA
qPCR Primer: mouse <i>Rps18</i>	Eurofins Genomics	Forward: CTTCCACAGGAGGCCTACAC, Reverse: TGGTGTTGAGTACTCGCAAAAT
semi-quantitative PCR Primer: <i>XBP1</i>	Eurofins Genomics	Forward: TTACGAGAGAAACTCA TGGC, Reverse: GGGTCCAAGTTGTCCAG AATGC

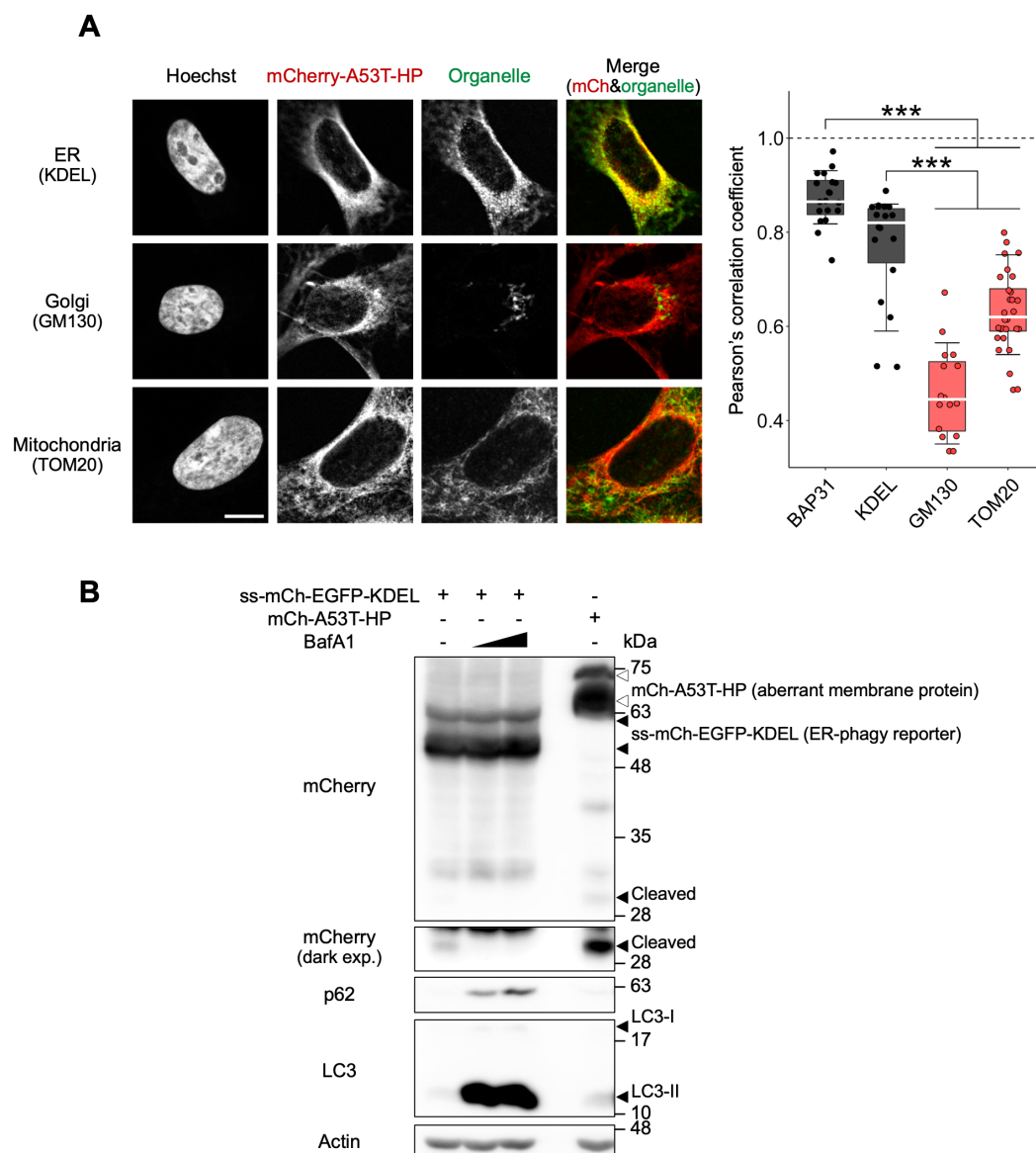
Chemicals, Enzymes and other reagents		
Polyethylenimine “MAX”	Polysciences	Cat#24765
Lipofectamine RNAiMAX	Thermo Fisher Scientific	Cat#133778500
Lipofectamine 3000	Thermo Fisher Scientific	Cat#L3000008
Hoechst 33342	Dojindo	Cat#346-07951
LysoTracker Red DND-99	Invitrogen	Cat#L7528
Tetracycline	Sigma-Aldrich	Cat#T7660
MG132	Enzo Life Sciences	Cat#BML-PI102
Bafilomycin A1	Merck Millipore	Cat#196000
Bafilomycin A1	Cayman	Cat#11038
SAR405	Med Chem Express	Cat#HY-12481
Rapamycin	Selleck	Cat#S1039
Tunicamycin	FUJIFILM Wako	Cat#202-08241
Protein G Sepharose 4 Fast Flow	Cytiva	Cat#17061802
Glutathione Sepharose 4B	Cytiva	Cat#17075601
Anti-FLAG M2-Agarose Gel	Sigma-Aldrich	Cat#A2220
Anti DYKDDDDK tag Antibody Beads	FUJIFILM Wako	Cat#016-22784
3xFLAG peptide	Sigma-Aldrich	Cat#F4799
3xFLAG peptide	M&S TechnoSystems	Cat#GEN-3-FLAG-5
QuantiLum Recombinant Luciferase	Promega	Cat#E1701
Ponceau S solution	APRO Science	Cat#SP-4030
ECL Select Western Blotting Detection Reagent	Cytiva	Cat#RPN2235
ISOGEN	Nippongene	Cat#319-90211
dNTP mix	Promega	Cat#U1515
5x Green Go Taq Reaction Buffer	Promega	Cat#M7911
Taq polymerase	This study	N/A
Software		
Fiji	(Schindelin <i>et al</i> , 2012)	https://fiji.sc
RStudio	RStudio	https://www.rstudio.com

R	The R Foundation	https://www.r-project.org
Adobe Illustrator	Adobe	https://www.adobe.com/products/illustrator.html
CHOPCHOP	(Labun <i>et al</i> , 2019)	https://chopchop.cbu.uib.no
DISOPRED3	(Jones & Cozzetto, 2015)	http://bioinf.cs.ucl.ac.uk/psipred
PONDR	(Romero <i>et al</i> , 2001)	http://www.pondr.com
SPOT-Disorder2	(Hanson <i>et al</i> , 2019)	https://sparks-lab.org/server/spot-disorder2
Other		
ReverTra Ace qPCR RT Master Mix	TOYOBO	Cat#FSQ-301
FastStart Essential DNA Green Master	Roche	Cat#06924204001
KAPA SYBR Fast qPCR Kit	Kapa Biosystems	Cat#KK4602
DC Protein Assay Reagents Package Kit	Bio-Rad	Cat#5000116

References

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Appendix Figure S1

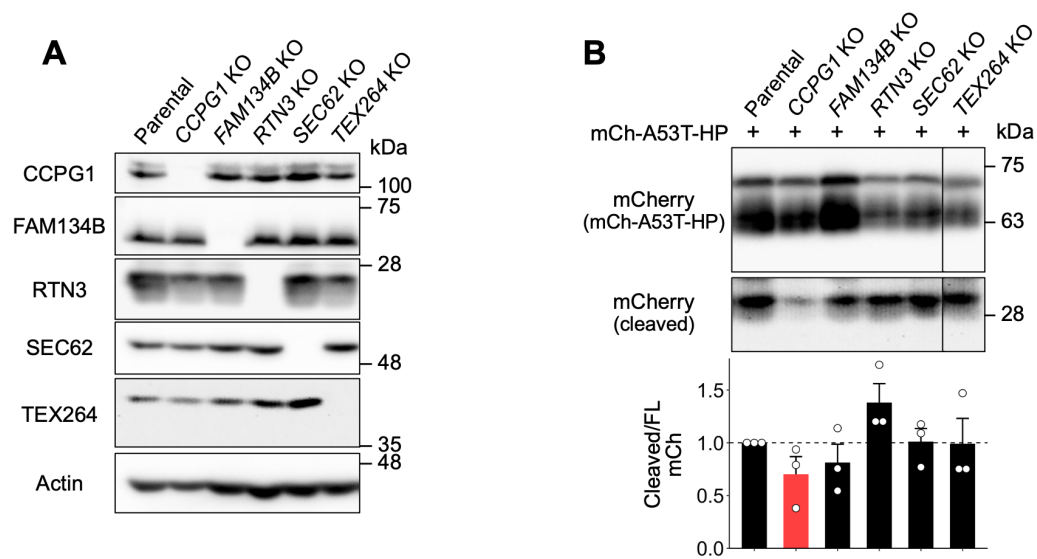


Appendix Figure S1. ER localization of a model aberrant membrane protein.

- A** Colocalization analysis of A53T-HP and organelle markers. HEK293A cells expressing tetracycline-inducible mCherry-A53T-HP were immunostained with anti-KDEL, GM130, or TOM20 antibodies. The graph represents Pearson's correlation coefficient measured between mCherry-A53T-HP and organelle markers. A total of 16–29 cells in each condition pooled from $n = 2$ independent experiments were analyzed. Box centers indicate medians, box edges represent first and third quartiles, and whiskers show the 10th to 90th percentile. Scale bar, 10 μ m. *** $P < 0.001$ by one-way ANOVA with Tukey's multiple comparison test. A representative micrograph of BAP31 staining is shown in Fig 1B.

- B Lysosomal delivery of the ER-phagy reporter ss-mCh-EGFP-KDEL and the aberrant membrane protein A53T-HP monitored by mCherry processing. HEK293A cells transfected with indicated constructs were treated with increasing concentrations of BafA1 (200 or 400 nM) for 24 h. The rightmost lane was duplicated from the leftmost lane of Fig 1E to confirm that mCherry fragments of the same size were produced from both mCherry-tagged constructs.

Appendix Figure S2



Appendix Figure S2. Involvement of ER-phagy receptors in lysosomal degradation of an aberrant ER membrane protein.

- A Validation of ER-phagy receptor KO in HEK293A cells as determined by immunoblotting.
- B Lysosomal degradation of A53T-HP in HEK293A cells with indicated genotypes transfected with mCherry-A53T-HP. The graph represents mean $\pm$ SEM of mCherry processing ($n = 3$ independent experiments).