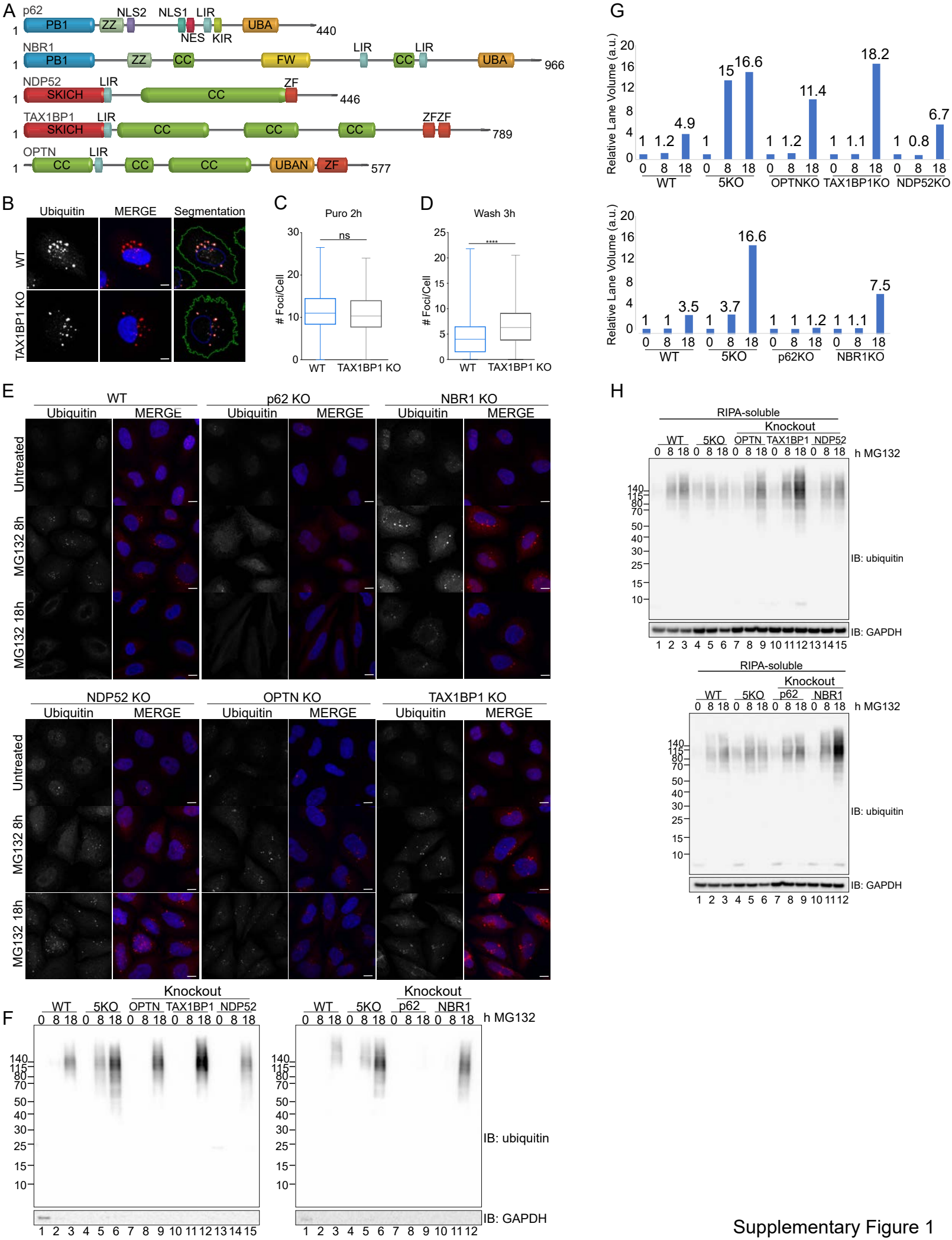
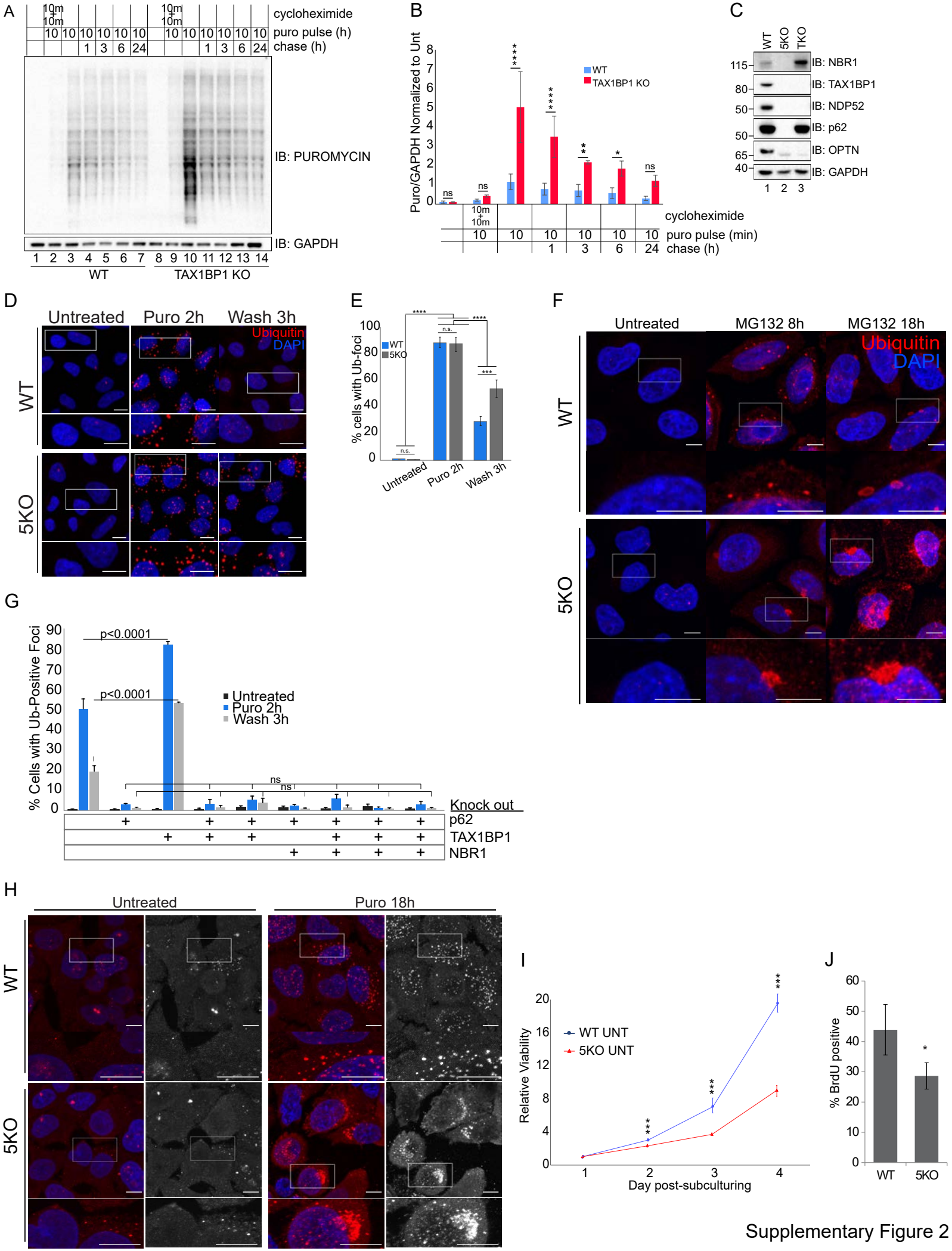


Supplementary Figure 1

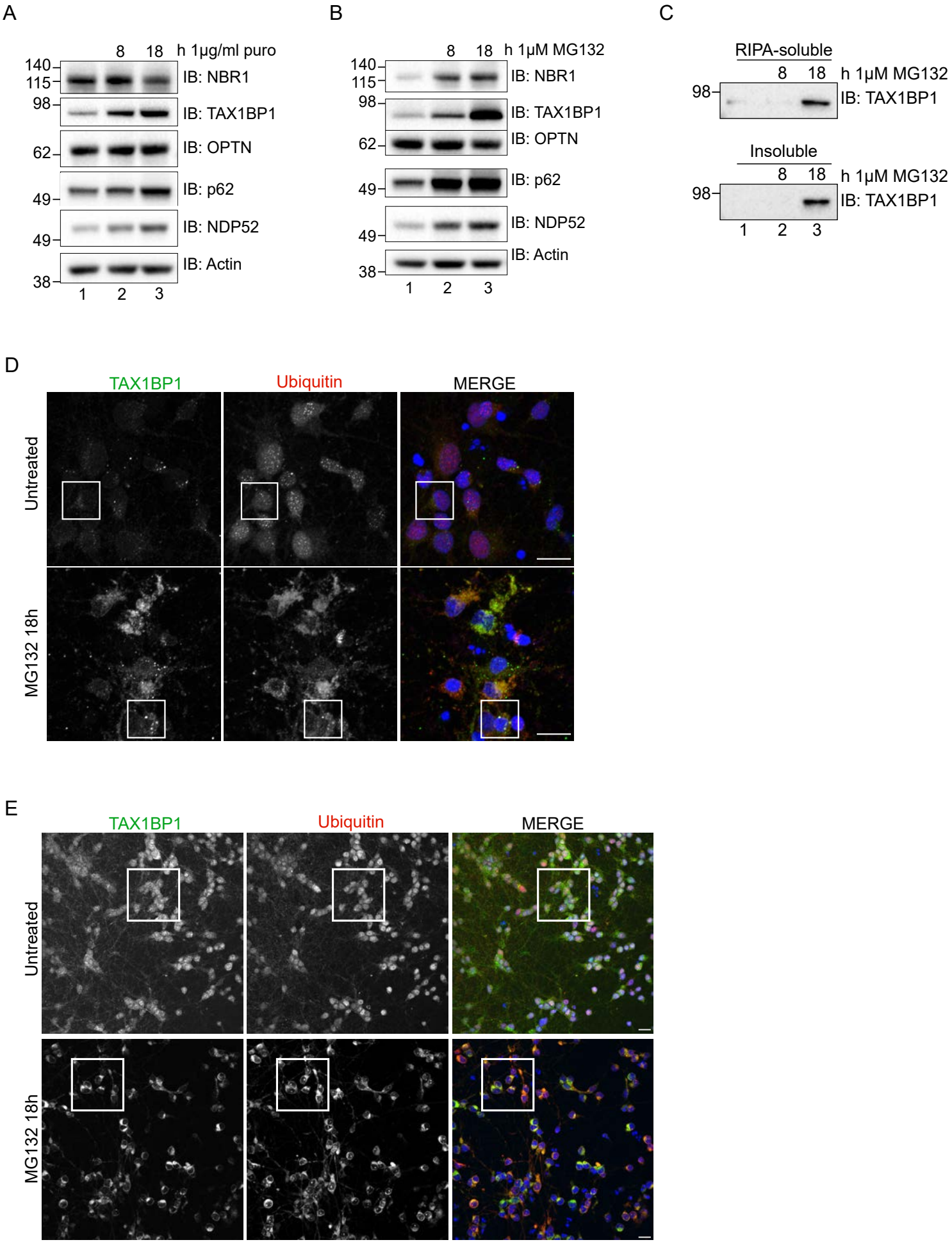
Supplementary Figure 1. Depletion of TAX1BP1 impairs clearance of protein aggregates. Related to Figure 1. **(A)** Schematic illustration of protein domain architectures of mammalian autophagy receptors OPTN, NDP52, TAX1BP1, p62, and NBR1. PB1, Phox and Bem1 domain; ZZ, ZZ-type zinc finger domain; NLS1 and NLS2, nuclear localization signals 1 and 2; NES, nuclear export signal; LIR, LC3-interacting region; KIR, Keap-interacting region; UBA, ubiquitin-associated domain; CC, coiled-coil domain; FW, four tryptophan domain; SKICH, SKIP carboxyl homology domain; ZF, Zinc-finger domain; UBAN ubiquitin binding in ABIN and NEMO domain. The size of the receptors (in number of amino acids) is indicated. **(B)** Representative image of segmentation analysis performed using CellProfiler. **(C), (D)** Quantification of (B) using CellProfiler: number of foci per cell in WT or TAX1BP1 KO cells in 3 independent experiments at (C) 2 h puromycin or (D) 2 h puromycin followed by 3 h washout (For box plots, center line = median, box limits = first to third quartile, whiskers = minimum and maximum). **(E)** WT and individual knockouts for p62, NBR1, NDP52, OPTN, and TAX1BP1 cell lines were exposed to 1 μM MG132 for 8 or 18 h, after which cells were either fixed for imaging or washed and followed for a further 3 h in full media; scale bar 10 μm. **(F)** WT, pentaKO, or individual KO lines for each autophagy receptor were treated with 1 μM MG132 for 8 or 18 h, fractionated into RIPA-soluble or -insoluble fractions and immunoblotted for total ubiquitin. **(G)** Quantification of (F) determined by densitometry and normalized first to soluble GAPDH and subsequently to WT levels within each fraction. **(H)** Soluble fractions corresponding to insoluble fractions in (F).



Supplementary Figure 2

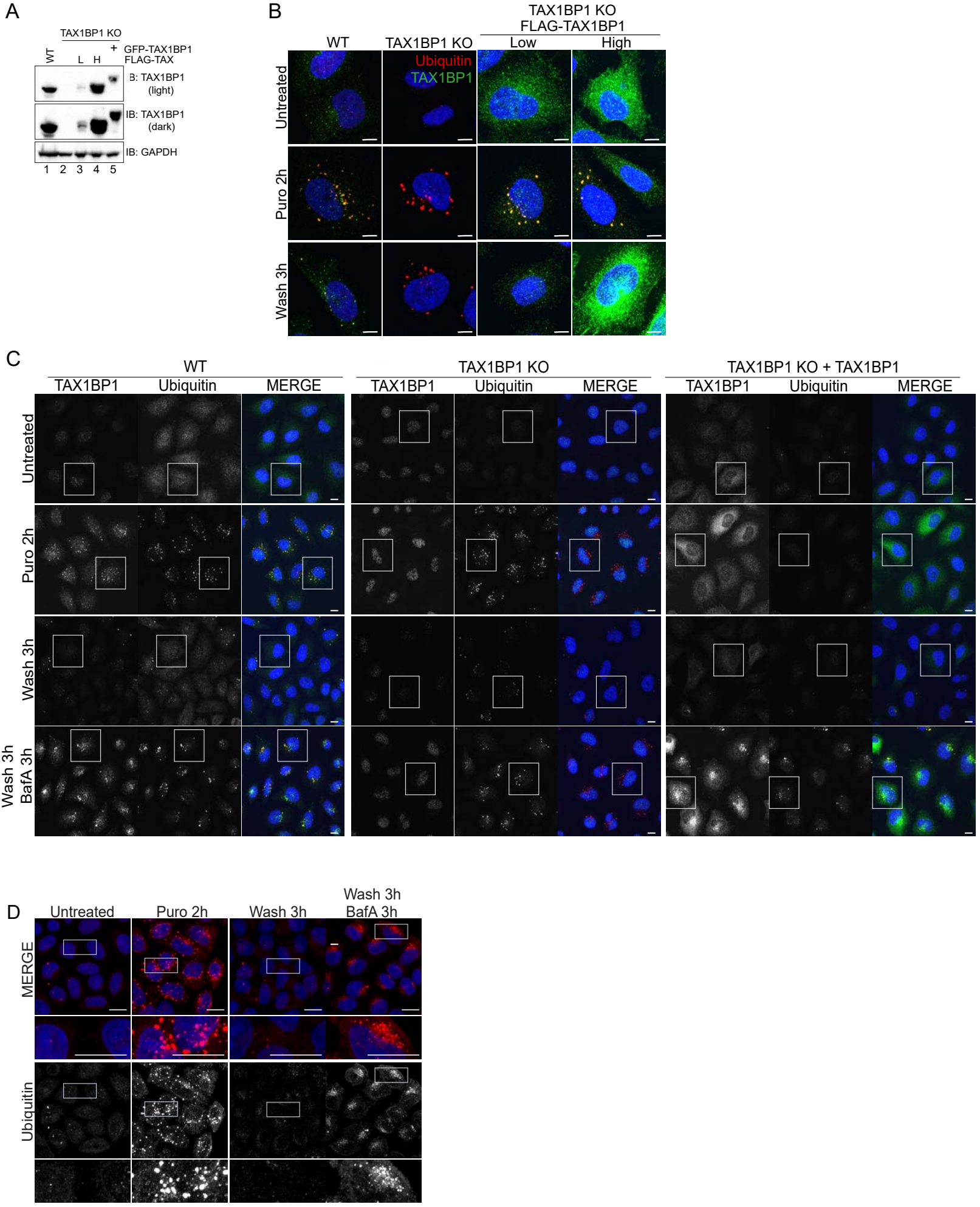
Supplementary Figure 2. Depletion of TAX1BP1 impairs clearance of protein aggregates. Related to Figure 1. **(A)** WT or TAX1BP1 KO cell lines were pulsed with 5 μg/ml puromycin and 15 μM cycloheximide as indicated, then chased/harvested at the indicated time points and immunoblotted for puromycin or GAPDH (loading control). **(B)** Quantification of (A) determined by densitometry and normalized first to GAPDH and subsequently to the untreated condition for each cell line. **(C)** Immunoblot validation of pentaKO and TKO (NDP52, OPTN, and TAX1BP1 triple knockout) cell lines. **(D)** WT or pentaKO cell lines were exposed to 5 μg/ml puromycin for 2 hours, after which cells were either fixed for imaging or washed and followed for a further 3 hours in full media. **(E)** Quantification of (D): percent of cells containing Ub-positive foci was assessed in > 200 cells per condition in 3 independent experiments. Quantification is displayed as mean ± s.d. from 3 independent experiments using one-way ANOVA test (***P<0.001, ****P<0.0001) and Tukey's post hoc test. **(F)** WT or pentaKO HeLa cell lines were exposed to 1 μM MG132 for 8 or 18 hours, fixed for imaging and stained for ubiquitin. Scale bars 10 μm. **(G)** WT HeLa cells or the indicated single and combinatorial knock out lines were exposed to 5 μg/ml puromycin for 2 h, after which cells were either fixed for imaging or washed and followed for a further 3 h in full media or media then fixed for imaging. Quantification represents percent of cells containing Ub-positive foci which was assessed in ~ 200 cells per condition in 3 independent experiments. Quantification is displayed as mean ± s.d. from 3 independent experiments using two-way ANOVA test and Tukey's post hoc test. **(H)** WT or pentaKO HeLa cell lines were exposed to 5 μg/ml puro for 18 hours, fixed for imaging and stained for ubiquitin. **(I)** WT or pentaKO cells were followed for 4 days during which viability was measured by quantification of ATP production. Relative viability represents normalized luminescence displayed as mean ± s.d. from 3 independent experiments; significance was

assessed using two-way ANOVA test (****P<0.0001, ***P<0.001, **P<0.01) with Tukey's post hoc test. **(J)** Percent of WT or pentaKO cells positive for BrdU. Error bars indicate standard deviation; n=3 independent experiments. All blots and microscopy images are representative of at least 3 independent experiments; scale bar 10 μm.



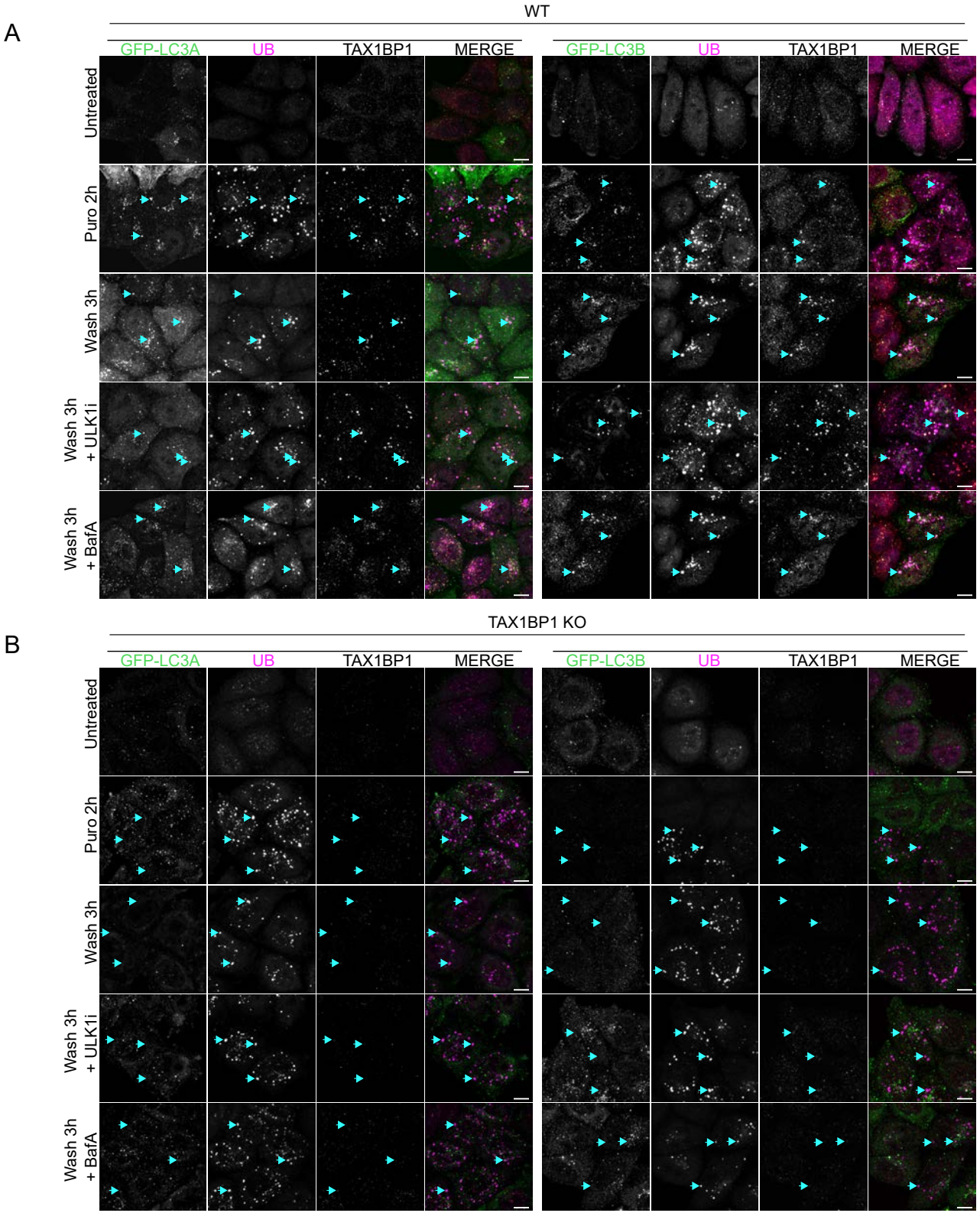
Supplementary Figure 3

Supplementary Figure 3. TAX1BP1 responds to proteotoxic stress and associates with insoluble protein. Related to Figure 2. **(A), (B)** WT HeLa cells were treated with either (A) puromycin or (B) MG132 as indicated, lysed in 2% SDS and immunoblotted for TAX1BP1, OPTN, NBR1, p62, or NDP52. **(C)** WT HeLa cells treated with 1 μM MG132 for 8 or 18 h were fractionated into RIPA-soluble or -insoluble fractions and immunoblotted for TAX1BP1. **(D)** Full field of view images of primary rat cortical neurons treated with 1 μM MG132 for 18 h, after which cells were fixed for imaging and stained with antibodies for TAX1BP1 and Ub; scale bar 10 μm. **(E)** Full field of view images of neurons derived from human induced pluripotent stem cells (iPSCs) treated with 1 μM MG132 for 18 h, fixed and stained with antibodies targeting TAX1BP1 and Ub; scale bar 20 μm. All images are representative of at least 3 independent experiments.



Supplementary Figure 4

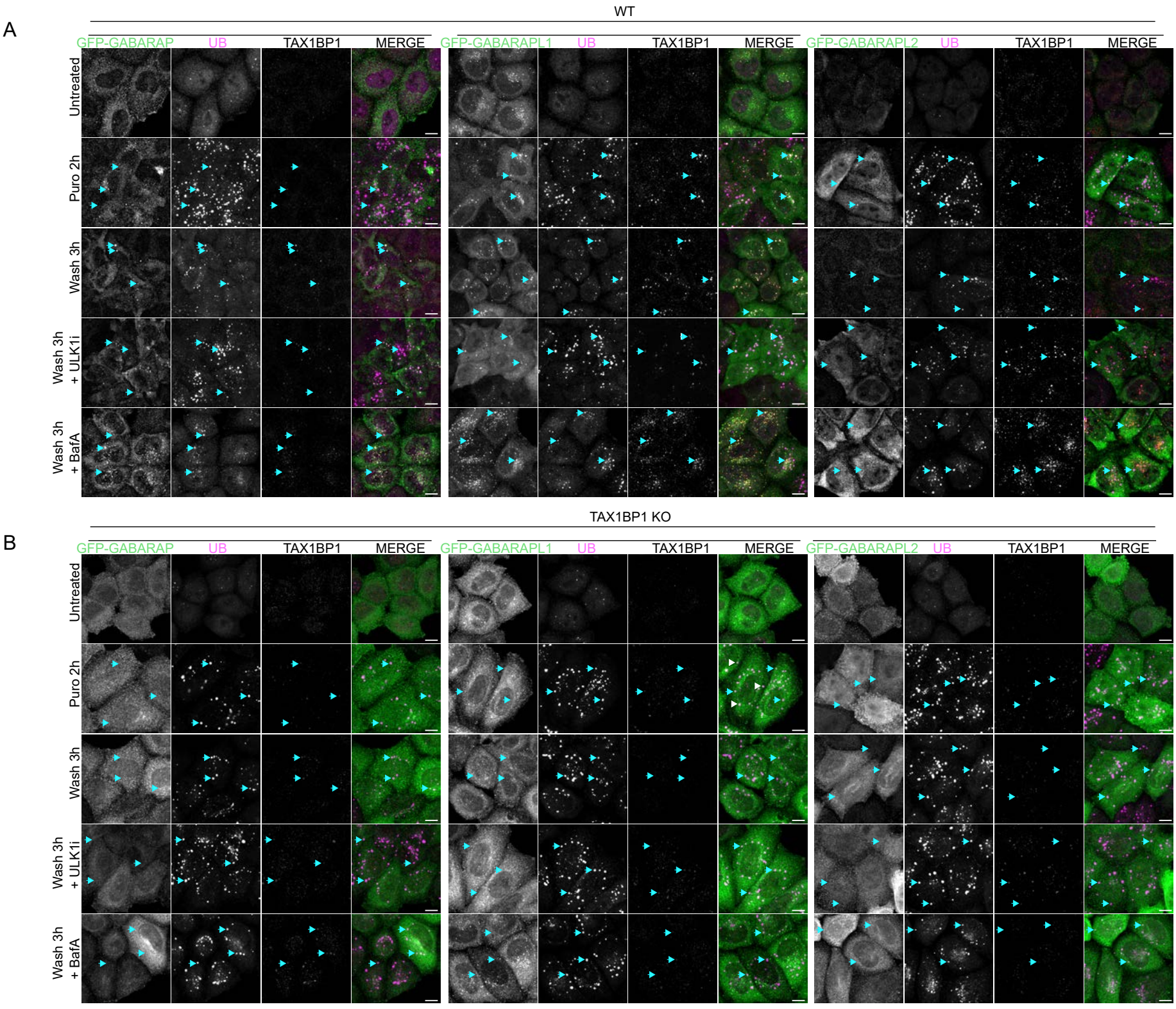
Supplementary Figure 4. TAX1BP1 mediates aggregate clearance. Related to figure 3. **(A)** GFP- or FLAG-tagged TAX1BP1 was stably reintroduced into TAX1BP1 KO cells via viral infection. TAX1BP1 expression levels were titered for use in rescue experiments: L = low expression, H = high expression. **(B)** Cell lines in (A) were exposed to 5 μg/ml puromycin for 2 h, after which cells were either fixed for imaging or washed and followed for a further 3 h in full media; scale bar 10 μm. **(C)** Full field of view images associated with Figure 3E, F showing WT, TAX1BP1 KO, or TAX1BP1 KO + FLAG-TAX1BP1 (H) cell lines exposed to 5 μg/ml puromycin in the presence or absence of 100 nM Bafilomycin A, after which cells were either fixed for imaging or washed and followed for a further 3 h in full media or in media containing Bafilomycin A. **(D)** WT HeLa cells exposed to 5 μg/ml puromycin for 2 h in the presence or absence of Bafilomycin A, after which cells were either fixed for imaging or washed and followed for a further 3 h in full media with or without Bafilomycin A; scale bar 10 μm. All images are representative of at least 3 independent experiments.



Supplementary Figure 5

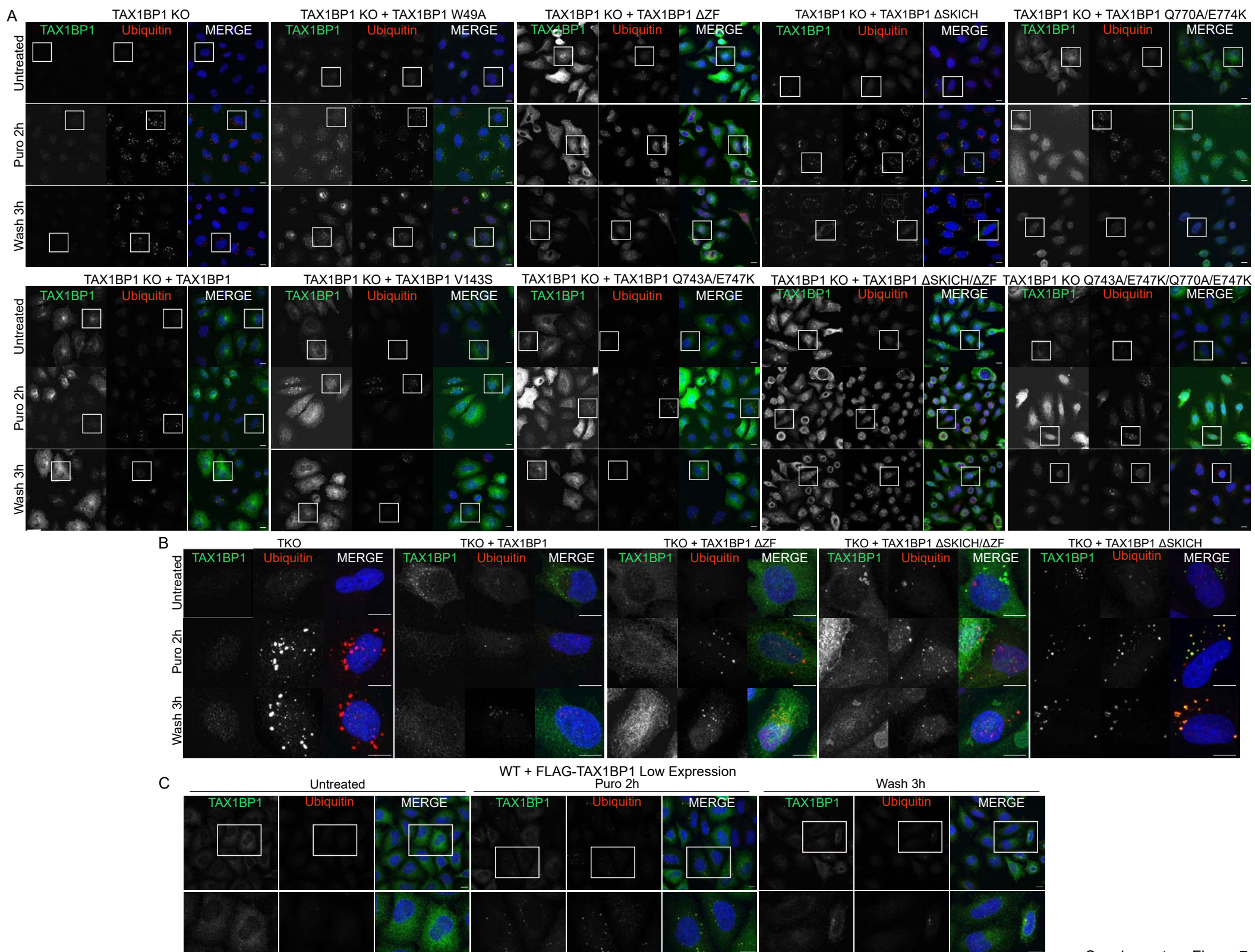
Supplementary Figure 5. TAX1BP1 and LC3 proteins localize to protein aggregates.

Related to figure 3. **(A)** WT and **(B)** TAX1BP1 KO cell lines stably expressing EGFP-LC3A or EGFP-LC3B were exposed to 5 μg/ml puromycin for 2 h in the presence or absence of 1 mM ULK1 inhibitor or 100 nM Bafilomycin A after which cells were either fixed for imaging or washed and followed for a further 3 h in full media in the presence or absence of ULK1 inhibitor or Bafilomycin A; scale bar 10 μm.



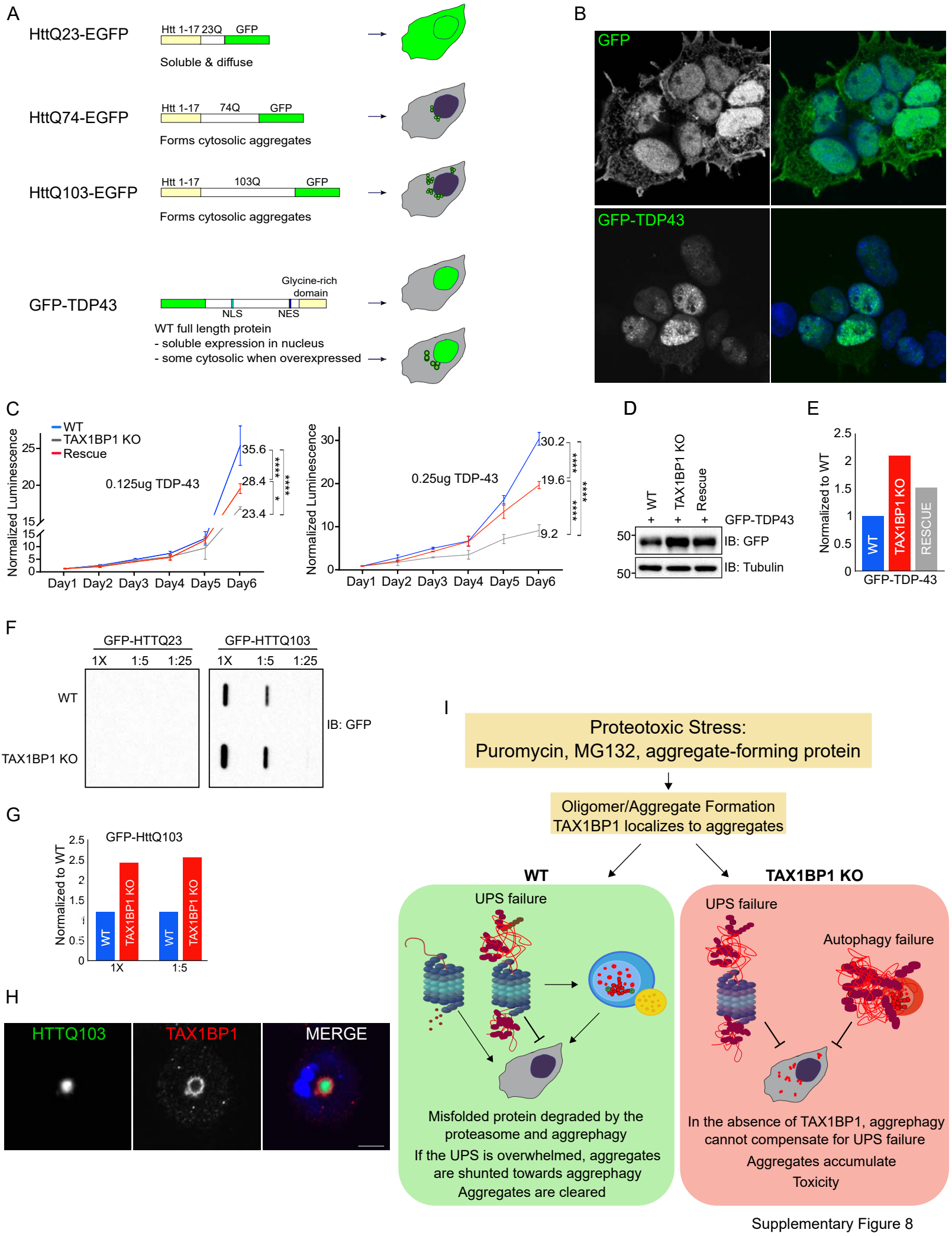
Supplementary Figure 6

Supplementary Figure 6. GABARAP proteins minimally localize to protein aggregates. Related to figure 3. **(A)** WT and **(B)** TAX1BP1 KO cell lines stably expressing EGFP-GABARAP, EGFP-GABARAPL1, or EGFP-GABARAPL2 were exposed to 5 μg/ml puromycin for 2 h in the presence or absence of 1 mM ULK1 inhibitor or 100 nM Bafilomycin A after which cells were either fixed for imaging or washed and followed for a further 3 h in full media in the presence or absence of ULK1 inhibitor or Bafilomycin A; scale bar 10 μm.



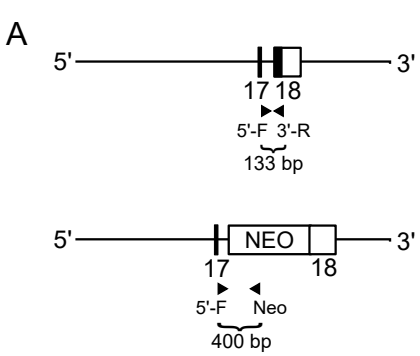
Supplementary Figure 7

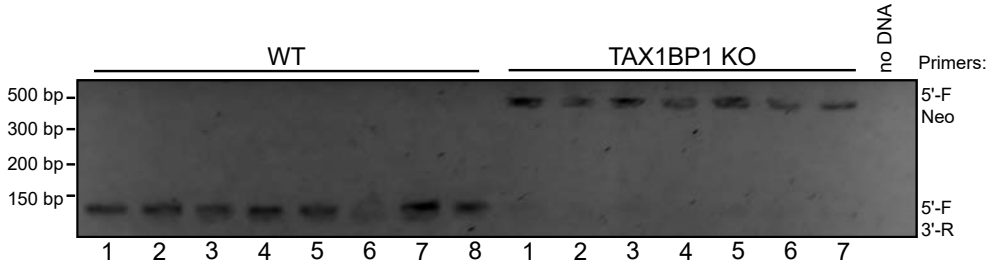
Supplementary Figure 7. Requirements for TAX1BP1 domains in aggrephagy. Related to figure 4. **(A)** Full field of view images of all TAX1BP1 stable mutant expression cell lines exposed to 5 μg/ml puromycin for 2 h, after which cells were either fixed for imaging or washed and followed for a further 3 h in full media. Associated with Figure 4B, C, D. **(B)** TKO (triple knockout: TAX1BP1, OPTN, NDP52) cell line with stable expression of TAX1BP1 mutants exposed to 5 μg/ml puromycin after which cells were either fixed for imaging or washed and followed for a further 3 h in full media; scale bar 10 μm. **(C)** WT cells stably expressing low levels of FLAG-TAX1BP1 were exposed to 5 μg/ml puromycin for 2 h, after which cells were either fixed for imaging or washed and followed for a further 3 h in full media; scale bar 10 μm. All images are representative of at least 3 independent experiments.

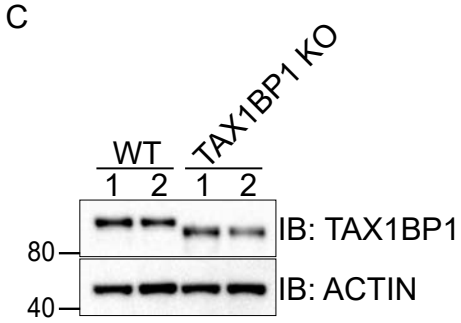


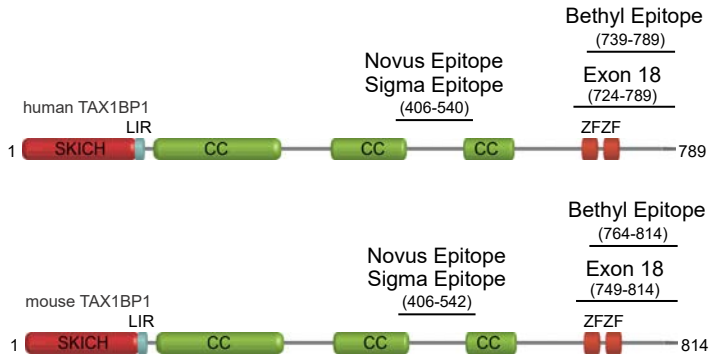
Supplementary Figure 8

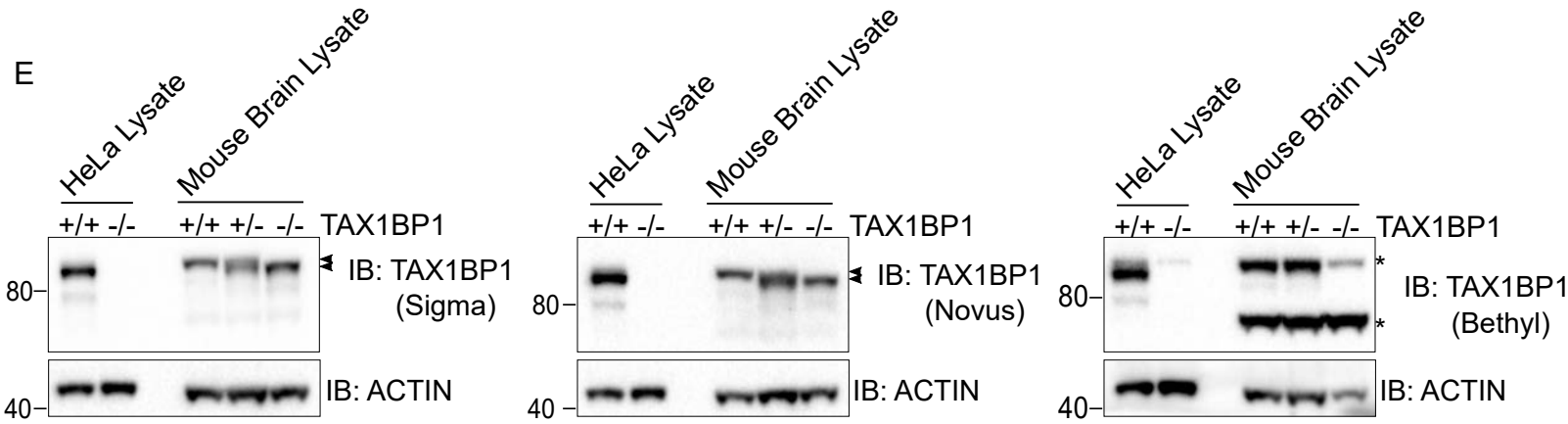
Supplementary Figure 8. TAX1BP1 mediates aggrephagy of cytotoxic aggregation-prone proteins. Related to figure 5. **(A)** Constructs used in this study. **(B)** Expression of GFP control or GFP-TDP-43. **(C)** WT, TAX1BP1 knockout, or TAX1BP1-rescue cells transfected with DNA expressing EGFP-TDP-43 at the indicated concentrations on Day 1, then followed for 6 days during which viability was measured by quantification of ATP production. Relative viability represents normalized luminescence displayed as mean ± s.d. from 3 independent experiments; significance was assessed using two-way ANOVA test (****P<0.0001, ***P<0.001, **P<0.01) with Tukey's post hoc test. P values and normalized viability measurements shown on graphs are for day 6 comparisons. **(D)** WT, TAX1BP1 knockout, or rescue cells expressing EGFP-TDP43, were assessed for TDP-43 clearance 4 days post-transfection. **(E)** Quantification of EGFP-TDP43 observed in (D). **(F)** Filter-trap assay assessing the degree of insoluble material retained in WT or TAX1BP1 KO cells infected with virus expressing HttQ23-EGFP or HttQ103-EGFP 4 days post-infection. **(G)** Quantification of HttQ103-EGFP retained on the filter in (F). **(H)** A single 1 μm slice is shown from images taken of immunofluorescence labeling of endogenous TAX1BP1 in cells infected with HttQ103-EGFP; scale bar 10 μm. **(I)** Proteotoxic stress, induced by translational stress, proteasome inhibition, or expression of aggregate-promoting proteins causes misfolded or damaged proteins to assemble into toxic oligomers or aggregates. In WT cells (green panel), the proteasome and aggrephagy both function to remove potentially toxic protein products. If the proteasome is overwhelmed, aggregated protein is shunted to the autophagy pathway. In the absence of TAX1BP1 (red panel), aggrephagy is deficient - once the proteasome has become overwhelmed by misfolded or aggregated protein, there is decreased backup clearance via aggrephagy, and insoluble protein accumulates, leading to toxicity and cell death.











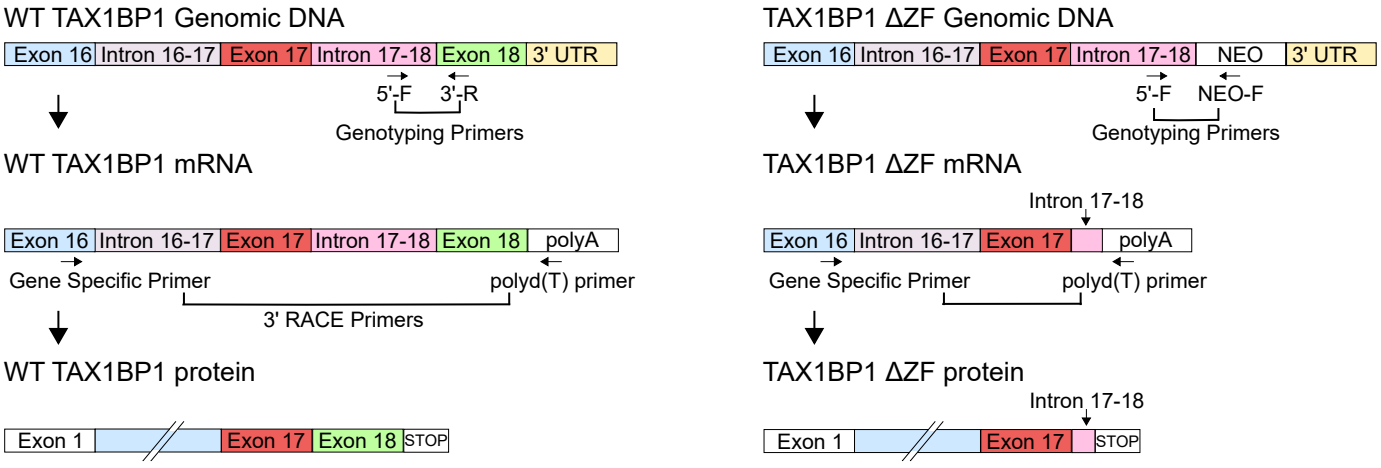
F

Ions detected	Peptide	TAX1BP1 knockout HeLa cell line	WT mouse	TAX1BP1 knockout mouse
m/z 480.79 z = 2	ALLLTTSNK aa 338-346			+
m/z 734.87 z = 2	LSDQAASTNSVFTK aa 466-479			+
m/z 661.83 z = 2	LELAEVEDNYK aa 580-590			+
m/z 789.91 z = 2	EINGLASYLENLSR aa 599-612		+	+
m/z 614.82 z = 2	LEGQSPQQVSR aa 628-638		+	

Supplemental Figure 9

Supplementary Figure 9. TAX1BP1 knockout mice express a truncated TAX1BP1 construct. Related to figure 7. **(A)** Detail of knockout strategy used by Iha et al. 2008 to create TAX1BP1 knockout (ΔZF) mouse. **(B)** Genotyping of animals used in this study. **(C)** Immunoblot against TAX1BP1 in cortical lysate from WT or TAX1BP1 knockout (ΔZF) mice showing truncated protein in TAX1BP1 knockout (ΔZF) animals. **(D)** Schematic demonstrating regions targeted by TAX1BP1 antibodies used in (E) and the region targeted in (A). **(E)** Immunoblot against TAX1BP1 in whole brain lysate using 3 different antibodies on lysate from WT or TAX1BP1 knockout (ΔZF) mice showing truncated protein in TAX1BP1 knockout (ΔZF) animals. *, nonspecific band present in both the HeLa TAX1BP1 knockout and mouse TAX1BP1 KO (ΔZF). **(F)** Mass spectrometry identifying TAX1BP1 peptides present in TAX1BP1 knockout (ΔZF) mouse whole brain lysate, performed in replicate.

A



B

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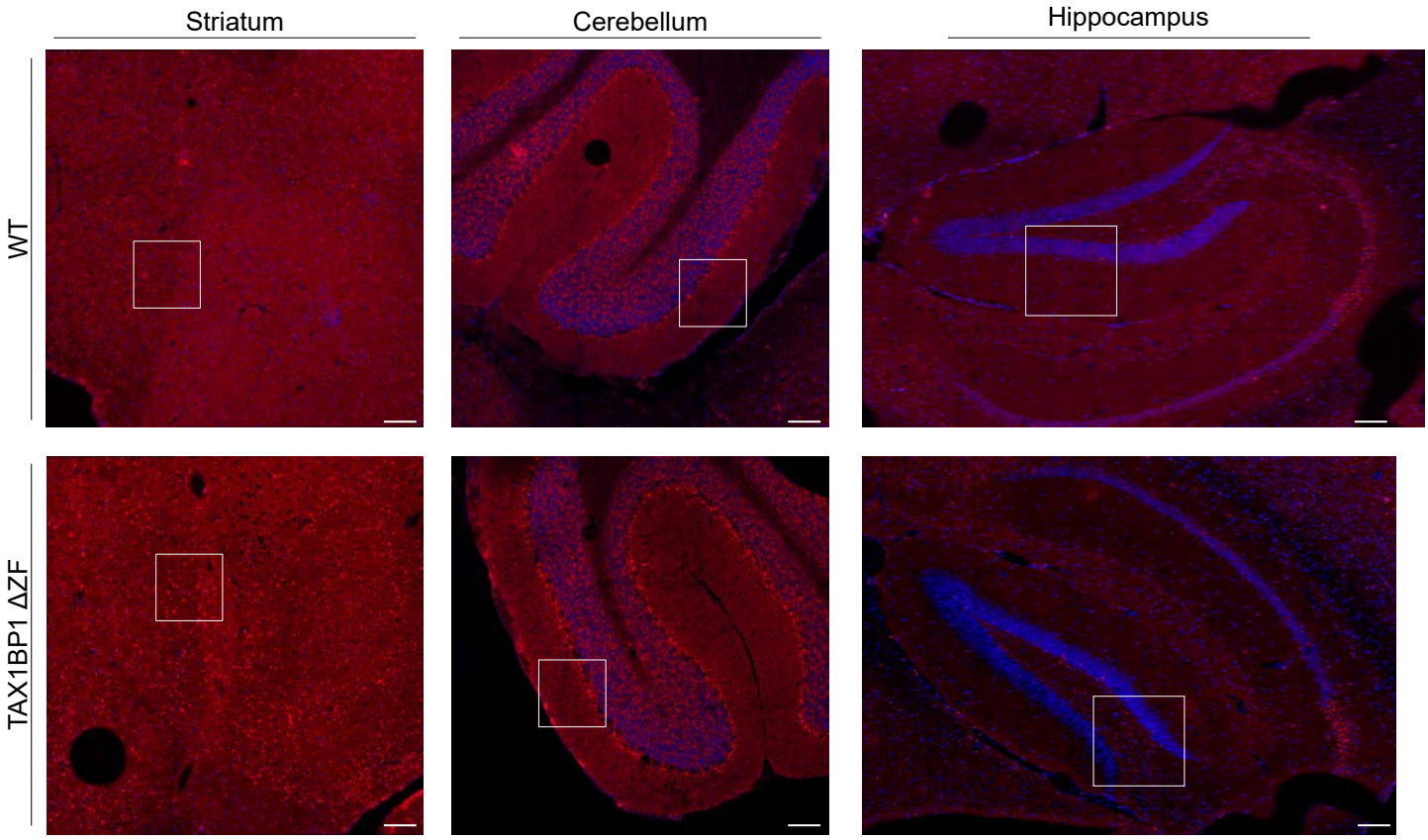
WT                693  ...SWEDNVVCSQPARNLSRPDGLEDPEDSREDENVPIPPDPANQHLRSHGAG
TAX1BP1 ΔZF            ...SWEDNVVCSQPARNLSRPDGLEDPEDSREDENVPIPPDPANQHLRSHGAG

WT                743  FCFDSSFDVHKKCPLCELMFPPNYDQTKFEEHVESHWKVCPMCSEQFPPDYDQ
TAX1BP1 ΔZF            FCFDSFCFDSRQVFPL*----------------------------------

WT                796  QGFERHVQTHFDQNVLNFD*
TAX1BP1 ΔZF            ------------------
```

Exon 16
Exon 17
Exon 18

C



Supplemental Figure 10

Supplementary Figure 10. TAX1BP1 knockout mice express a truncated TAX1BP1 construct and accumulate lipofuscin in multiple brain regions. Related to figure 7. **(A)** Comparison between WT and TAX1BP1 knockout (ΔZF) mouse genomic regions as determined by genotyping PCR and mRNA and predicted protein as determined by 3'RACE PCR and sequencing. **(B)** Predicted 3' terminus of TAX1BP1 protein produced in WT versus TAX1BP1 knockout (ΔZF) mice based on 3' RACE PCR. **(C)** Representative tiled images showing entire regions imaged in assessing lipofuscin deposits (red puncta) in WT and TAX1BP1 knockout (ΔZF) mouse striatum, cerebellum, and hippocampus sections. Nuclei are stained with DAPI. Scale bars, 100 μm

Supplemental Table 1. CRISPR-mediated knockout lines created in this study. Related to Figure 1 and Supplementary Figure 2.

Single Knock out Lines

Name	Gene	Clone #	Exon Targeted	CRISPR guide sequence	Editing Results	
p62 KO	p62	20	3	GGCGCCTCCTGAGCACACGG	1	1 basepair insertion
					2	1 basepair deletion
NBR1 KO	NBR1	4	5	GCCAGAGGATCCTGCAGTGC	1	1 basepair insertion
					2	19 basepair deletion
					3	13 basepair deletion

Double Knock out Lines

Name	Gene	Clone #	Exon Targeted	CRISPR or TALEN Sequence	Editing Results	
p62/NBR1 DKO	NBR1	10	5	GCCAGAGGATCCTGCAGTGC	1	5 basepair deletion
					2	5 basepair deletion
	p62 KO	10, (parent 20)				
p62/TAX1BP1 DKO	TAX1BP1	1	3	GATTGTGTACTAGCATTCCA	1	1 basepair insertion
					2	14 basepair deletion
					3	2 basepair deletion
	p62 KO	1, (parent 20)				
p62/TAX1BP1 DKO	TAX1BP1	3	3	GATTGTGTACTAGCATTCCA	1	2 basepair insertion
					2	20 basepair deletion
					3	20 basepair deletion
	p62 KO	3, (parent 20)				

Triple Knock out Lines

Name	Gene	Clone #	Exon Targeted	CRISPR or TALEN Sequence	Editing Results	
p62/NBR1/TAX1BP1	TAX1BP1	1	3	GATTGTGTACTAGCATTCCA	1	1 basepair insertion
					2	7 basepair deletion
					3	9 basepair deletion
					4	7 basepair deletion
	p62/NBR1	1, (parent 10)				
p62/NBR1/TAX1BP1	TAX1BP1	7	3	GATTGTGTACTAGCATTCCA	1	1 basepair deletion
					2	1 basepair insertion
					3	7 basepair deletion
	p62/NBR1	7, (parent 10)				
p62/NBR1/TAX1BP1	TAX1BP1	11	3	GATTGTGTACTAGCATTCCA	1	1 basepair deletion
					2	2 basepair insertion
					3	3 basepair deletion
					4	13 basepair deletion
	p62/NBR1	11, (parent 10)				