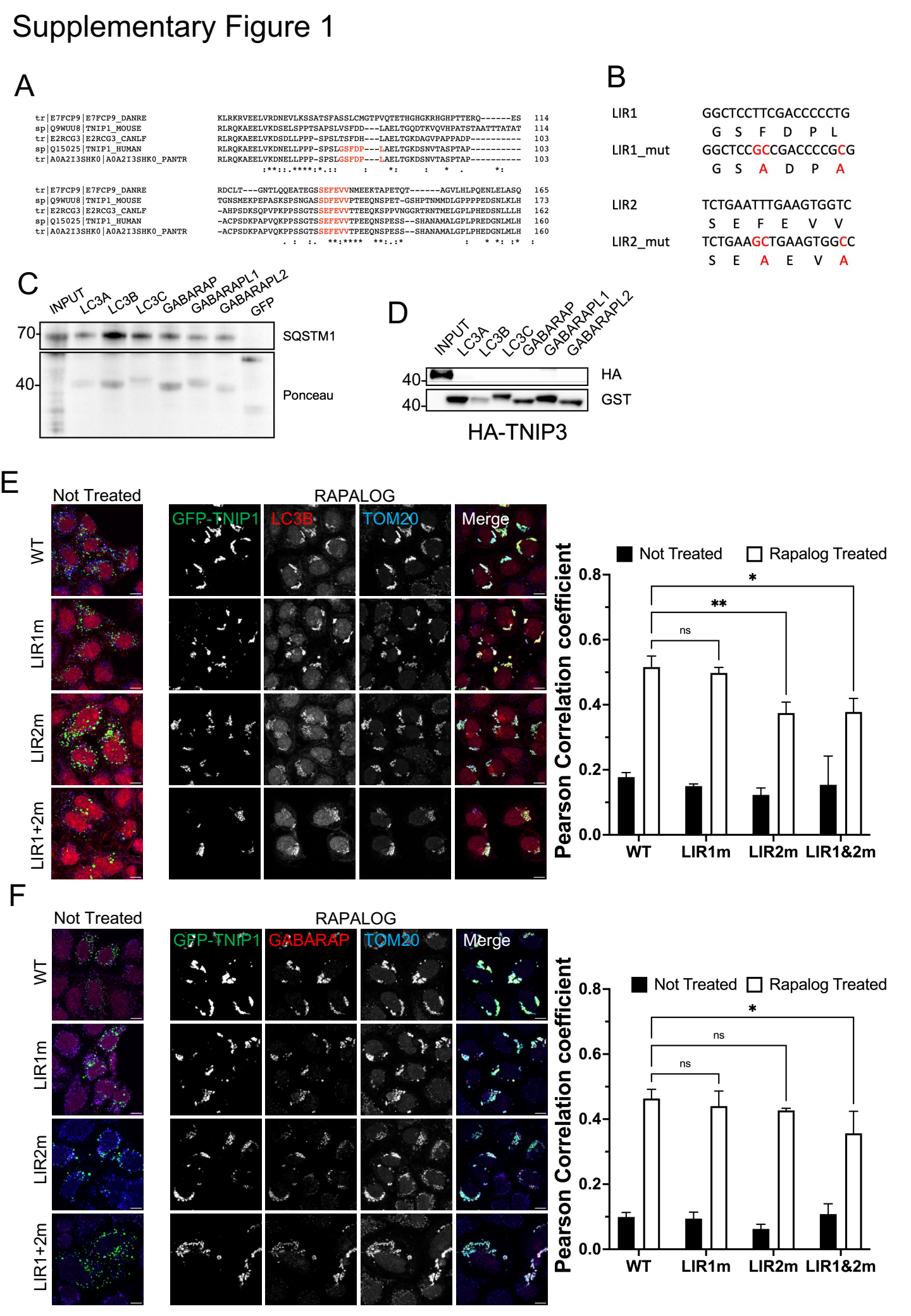
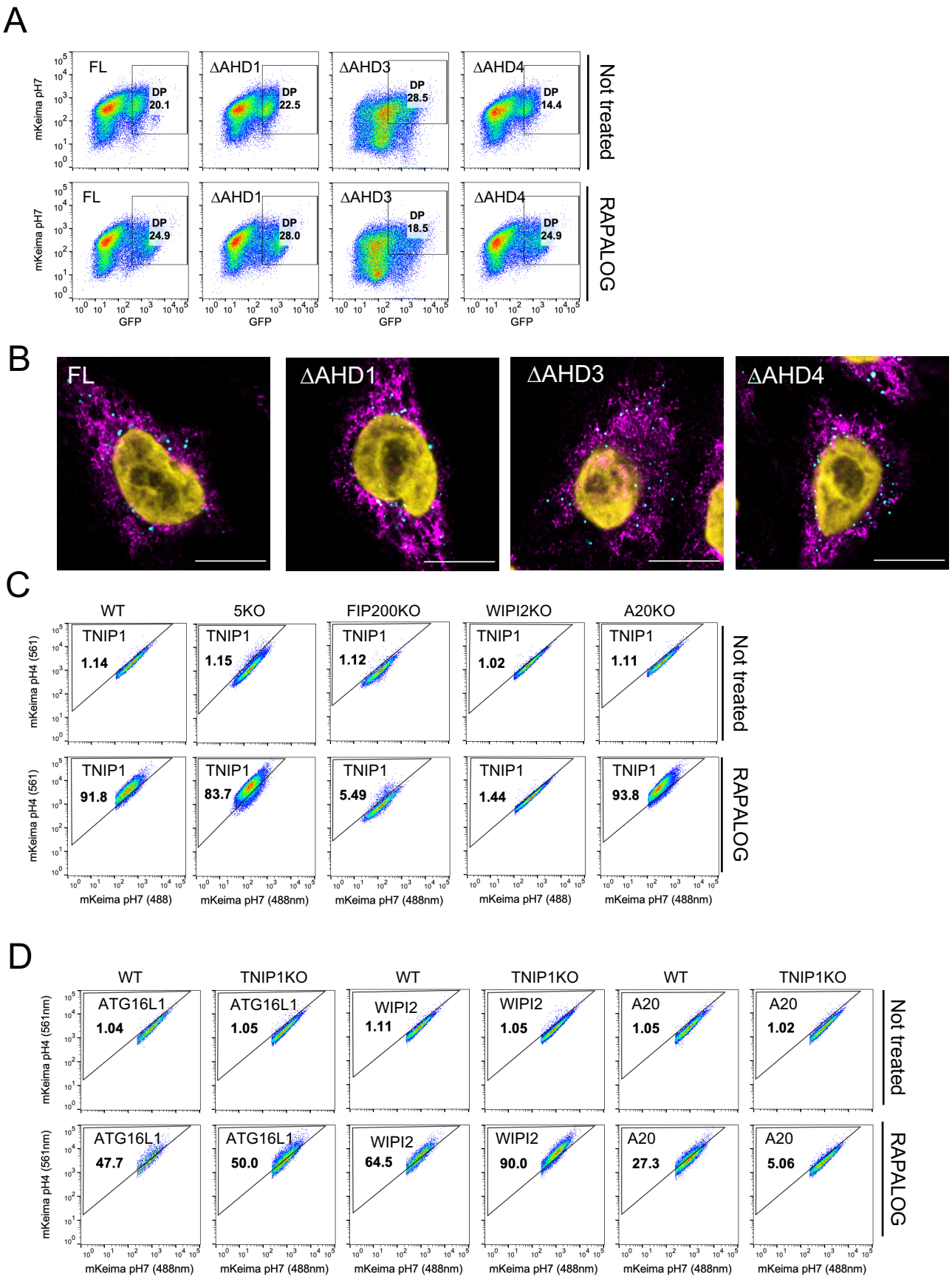
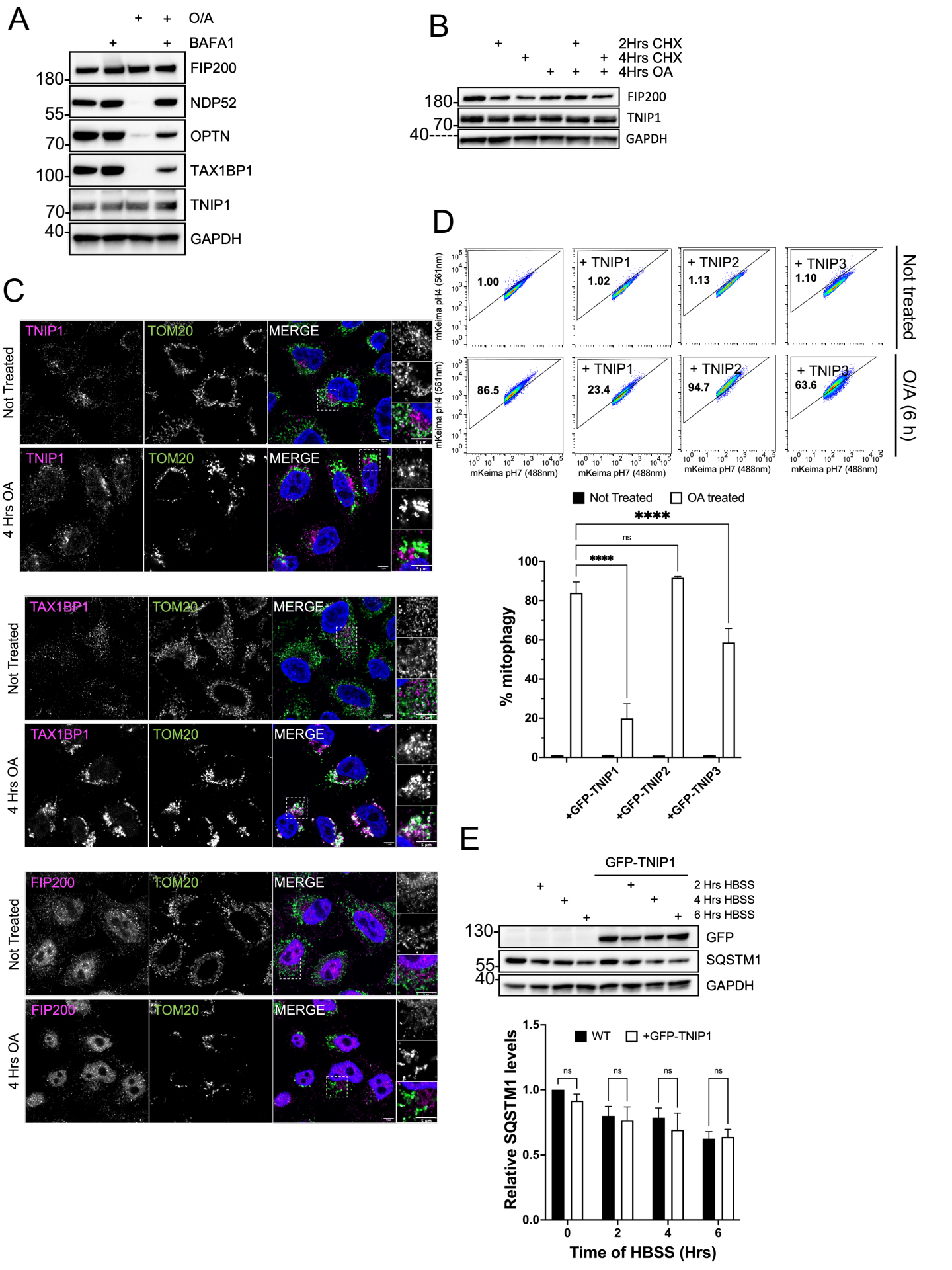
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



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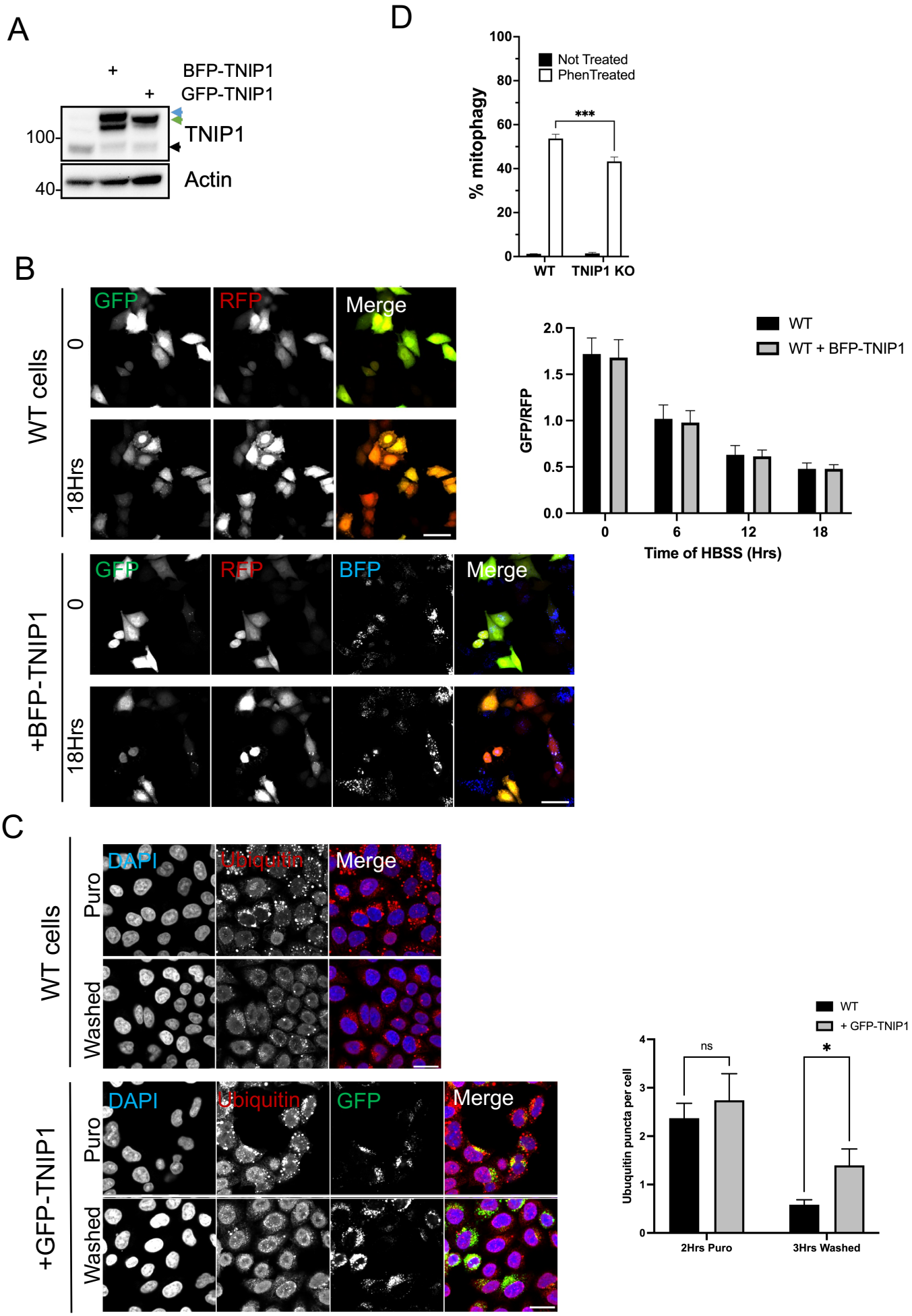
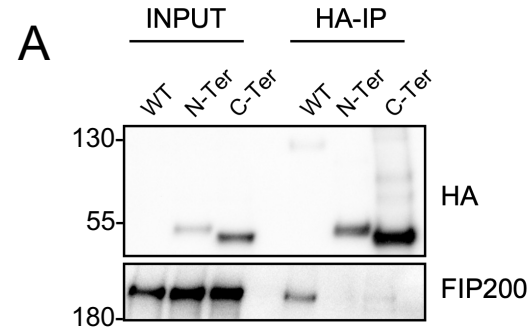
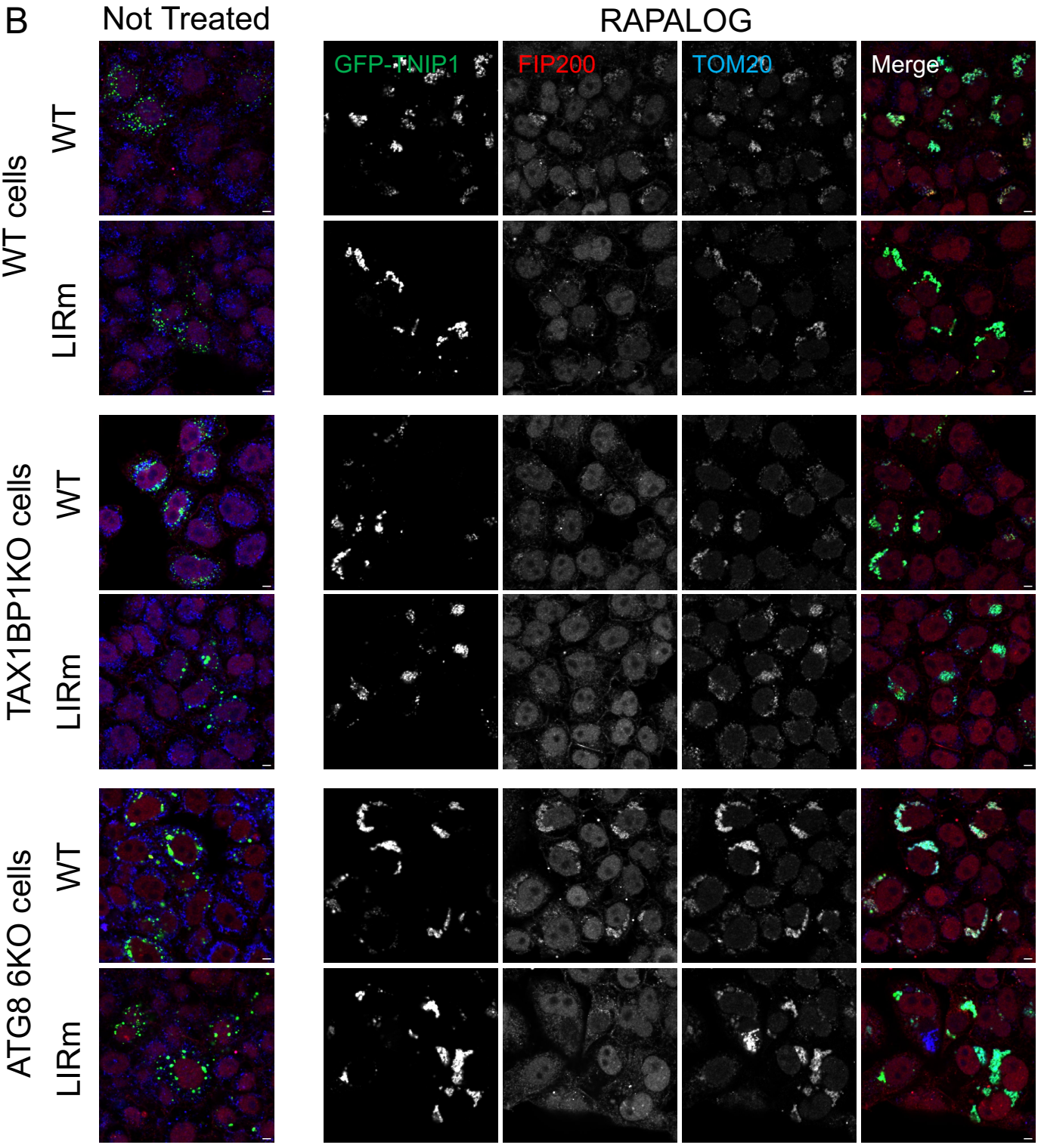
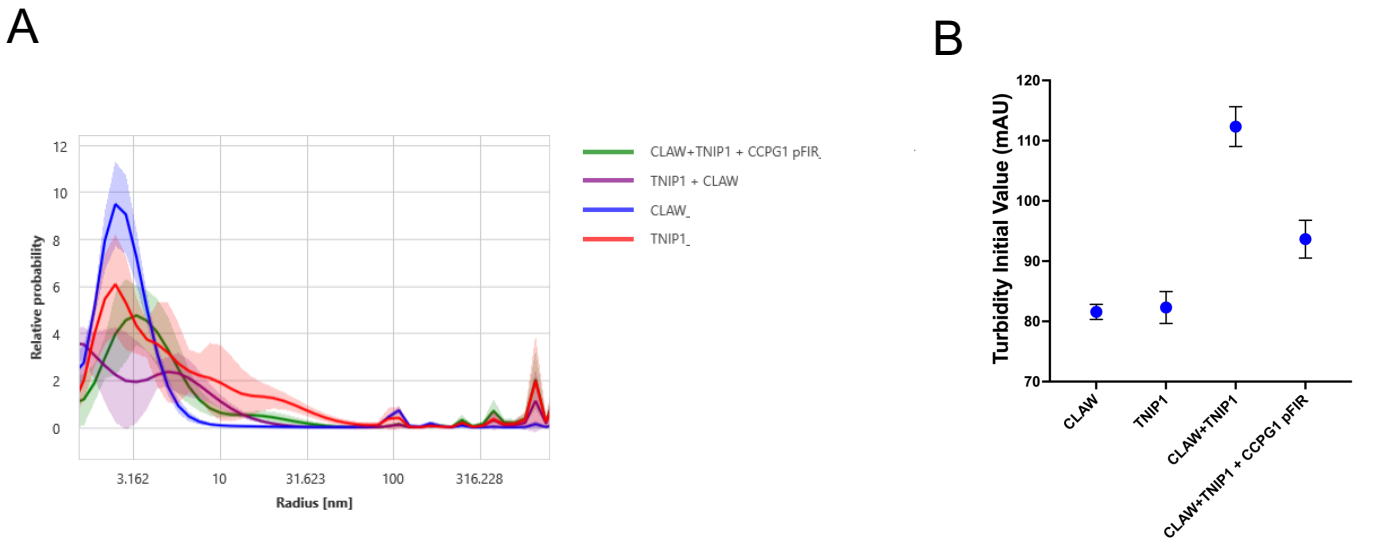


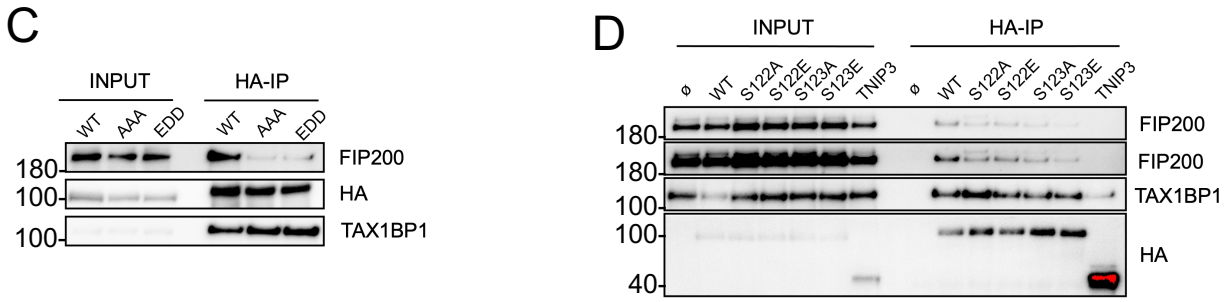
Supplementary Figure 5





Supplementary Figure 6





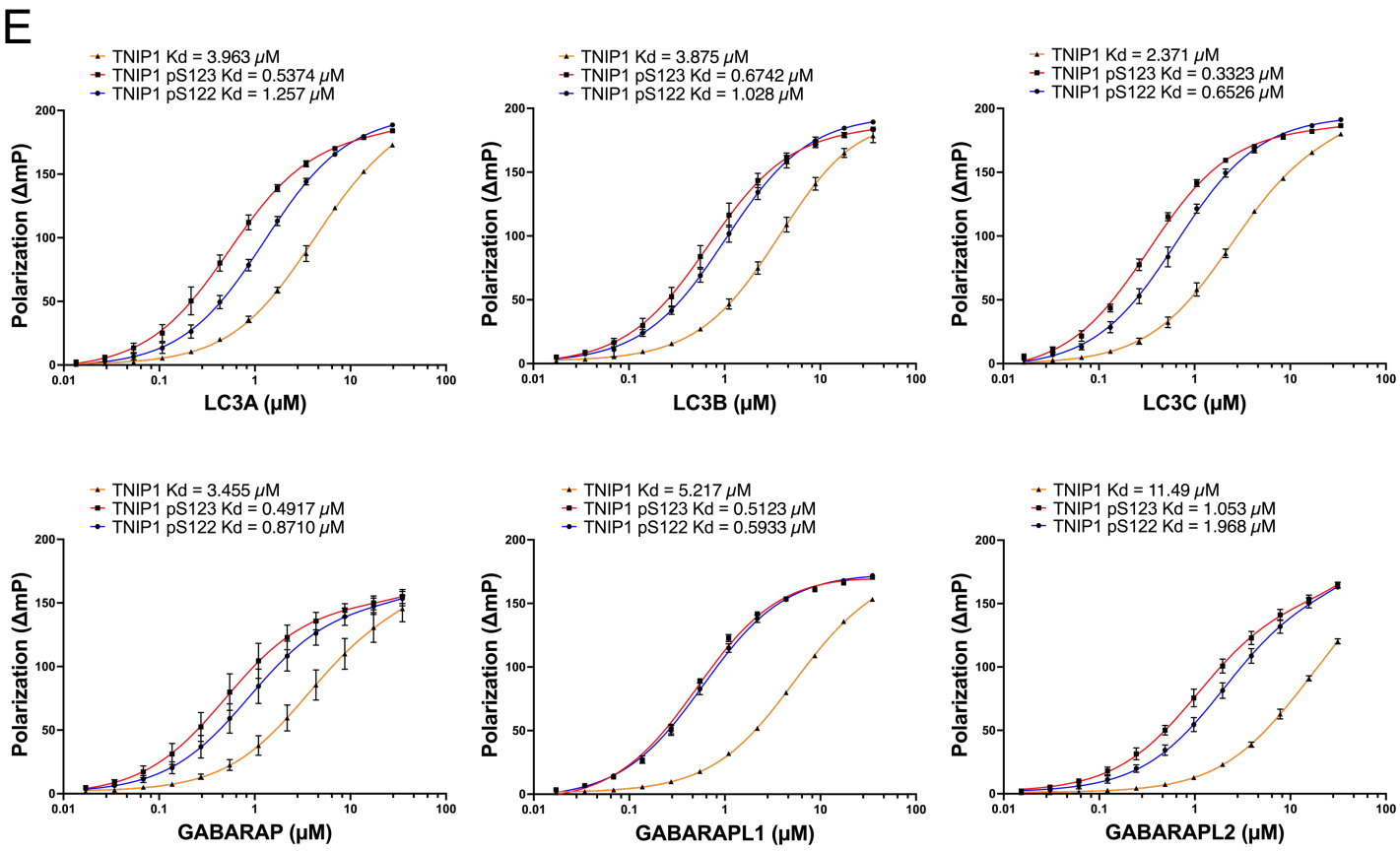


Figure legend Supplementary Fig. 1

Characterization of the LIR motif of TNIP1. Related to Figure 1

(A) Alignment of TNIP1 protein sequences from Human, Zebrafish (DANRE), Mouse, Dog (CANLF) and Chimpanzee (PANTR). Highlighted in red are the identified putative LIR motifs.

(B) LIR1 mutant and LIR2 mutant showing the altered nucleotide and amino acid sequences in red.

(C) Co-IP and IB of HeLa cells stably expressing HA-SQSTM1 with purified recombinant GST tagged mATG8 proteins or GFP.

(D) Co-IP and IB of HeLa cells stably expressing HA-TNIP3 with purified recombinant GST tagged mATG8 proteins.

(E) HeLa cells stably expressing FRB-FIS1 and FKBP-GFP-TNIP1 WT, FKBP-GFP-TNIP1 LIR1 mutant, FKBP-GFP-TNIP1 LIR2 mutant or FKBP-GFP-TNIP1 LIR1&LIR2 double mutant were left untreated or treated for 24 h with Rapalog and stained for the mitochondrial protein TOM20 and endogenous LC3B before immunofluorescence acquisition. Representative images. Scale bar: 10µm. Right, quantifications as mean ± SEM of Pearson correlation coefficient representing colocalization between LC3B and GFP.

(F) HeLa cells stably expressing FRB-FIS1 and FKBP-GFP-TNIP1 WT FKBP-GFP-TNIP1 LIR1 mutant, FKBP-GFP-TNIP1 LIR2 mutant or FKBP-GFP-TNIP1 LIR1&LIR2 double mutant were left untreated or treated for 24 h with Rapalog and stained for the mitochondrial protein TOM20 and endogenous GABARAP before immunofluorescence acquisition on a confocal microscope. Representative images. Scale bar: 10µm. Right, quantifications as mean ± SEM of Pearson correlation coefficient representing colocalization between GABARAP and GFP.

Figure legend Supplementary Fig. 2

Characterization of TNIP1's domains. Related to Figure 2

(A) Related to 2B. Representative gating FACS plots of HeLa cells stably expressing mito-mKeima, FRB-FIS1 and FKBP-GFP-TNIP1 Full Length or FKBP-GFP-TNIP1 mutants ΔAHD1, ΔAHD3 and ΔAHD4 treated with Rapalog for 24 h and subjected to FACS analysis. DP: double positive.

(B) HeLa cells stably expressing FRB-FIS1 and FKBP-GFP-TNIP1 FL, FKBP-GFP-TNIP1 mutants ΔAHD1, ΔAHD3 and ΔAHD4 (Cyan) left untreated and stained for the mitochondrial protein TOM20 (magenta) and DAPI (Yellow) before immunofluorescence acquisition on a confocal microscope. Airyscan representative images. Scale bar: 10µm.

(C) Related to 2E. Representative FACS plot.

(D) Related to 2F. Representative FACS plot.

Figure legend Supplementary Fig. 3

TNIP1 is not an autophagy receptor of mitophagy. Related to Figure 3

(A) IB of HeLa cells stably expressing HA-Parkin treated for 4 h with Oligomycin and Antimycin (O/A) and/or Bafilomycin A1 (BAFA1).

(B) IB of HeLa cells stably expressing HA-Parkin treated for 4 h with O/A and/or 2 h and 4 h of cycloheximide (CHX).

(C) HeLa cells stably expressing HA-Parkin were treated for 4 h with O/A and stained for the mitochondrial protein TOM20 (Green) and endogenous TNIP1 (Magenta), FIP200 (Magenta) or TAX1BP1 (Magenta) and DAPI (Blue) before immunofluorescence acquisition on a confocal microscope. Airyscan representative images. Scale bar: 5µm.

(D) HeLa cells stably expressing mito-mKeima, HA-Parkin and overexpressing FKBP-GFP-TNIP1, FKBP-GFP-TNIP2 or FKBP-GFP-TNIP3 constructs were treated with O/A for 6 h and subjected to FACS acquisition. Top, representative FACS plot. Bottom, bar graph representing data as mean ± SEM obtained from 3 independent replicates.

(E) IB of HeLa cells stably expressing BFP-Parkin and GFP-TNIP1 WT starved for 2 h, 4 h or 6 h by incubation with HBSS. Right, bar graph representing data as mean ± SEM obtained from 3 independent replicates.

Figure legend Supplementary Fig. 4

TNIP1 is specifically involved in selective autophagy. Related to Figure 3

(A) HeLa cells overexpressing BFP-TNIP1 or GFP-TNIP1 subjected to immunoblot analysis. Blue arrow, BFP-TNIP1 construct. Green arrow, GFP-TNIP1 construct. Black arrow, endogenous TNIP1.

(B) HeLa cells stably expressing YFP-LC3B_RFP-LC3BΔG and BFP-TNIP1 were starved for 24 h by incubation with HBSS and monitored every hour under a confocal microscope. Left, representative images at the 0 h and 18 h time points. Scale bar: 50µm. Right, bar graph of the GFP/RFP ratio for time points 0, 6, 12 and 18 h are displayed representing data as mean ± SEM obtained from 3 independent replicates.

(C) HeLa cells stably expressing or not GFP-TNIP1 were treated for 2 h with Puromycin and subsequently washed for 3 h or treated with Puromycin without washing and stained for Ubiquitin before immunofluorescence acquisition on a confocal microscope. Left, representative images of unwashed and washed cells. Scale bar: 50µm. Right, bar graph representing data as mean ± SEM obtained from 3 independent replicates.

(D) HeLa WT cells and TNIP1 KO cells stably expressing mito-mKeima, were treated with Phenanthroline for 16 h and subjected to FACS analysis. Bar graph representing data as mean ± SEM obtained from 3 independent replicates.

Figure legend Supplementary Fig. 5

TNIP1 recruitment of FIP200 is independent of TAX1BP1 and mATG8 proteins. Related to Figure 5

(A) Co-IP and IB of HEK293T cells stably expressing HA-TNIP1 full length or the N-terminal and C-terminal constructs using magnetic HA beads.

(B) Related to Fig 5B. HeLa wild type, TAX1BP1 KO and LC3/GABARAP 6KO cells stably expressing FRB-FIS1 and FKBP-GFP-TNIP1 WT or LIR2m were treated for 24 h with Rapalog and stained for the mitochondrial protein TOM20 and endogenous FIP200 before immunofluorescence acquisition on a confocal microscope. Representative images. Scale bar: 10µm.

Figure legend Supplementary Fig. 6

TNIP1 forms a complex with FIP200. Related to Figure 6

(A) Dynamic Light Scattering assay using purified recombinant full length TNIP1 in complex with CLAW domain of FIP200 and phosphorylated CCPG1 FIR peptides. The average relative probability distribution fit is shown as a solid line with the shaded region above and below representing the standard deviation in fits across four independent experiments.

(B) Turbidity assay using purified recombinant full length TNIP1 in complex with CLAW domain of FIP200 and phosphorylated CCPG1 FIR peptides. Error bars represent the standard deviation from the mean for three independent experiments.

(C) Co-IP and IB of HEK293T cells stably expressing HA-TNIP1 WT, AAA or EDD mutants using magnetic HA beads.

(D) Co-IP and IB of HEK293T cells stably expressing HA-TNIP1 WT, S122A, S122E, S123A, S123E mutants or HA-TNIP3 using magnetic HA beads. Ø: no overexpressed construct.

(E) Fluorescence polarization of unmodified and phosphorylated FIR peptides of TNIP1 and binding to the purified LC3A (top left), LC3B (top middle), LC3C (top right), GABARAP (bottom left), GABARAPL1 (bottom middle) or GABARAPL2 (bottom right) with increasing concentrations of recombinant proteins. Error bars represent the standard deviation measured across 3 independent experiments for each condition.