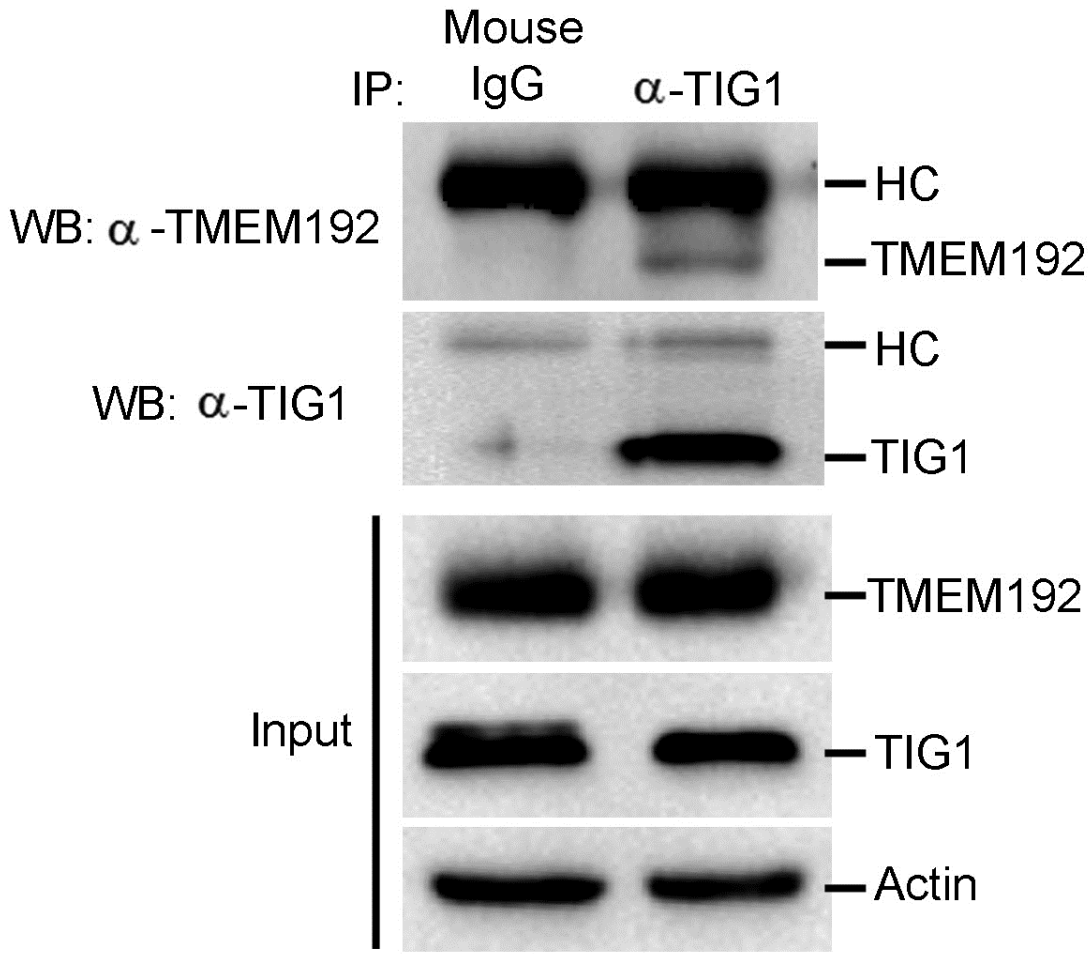
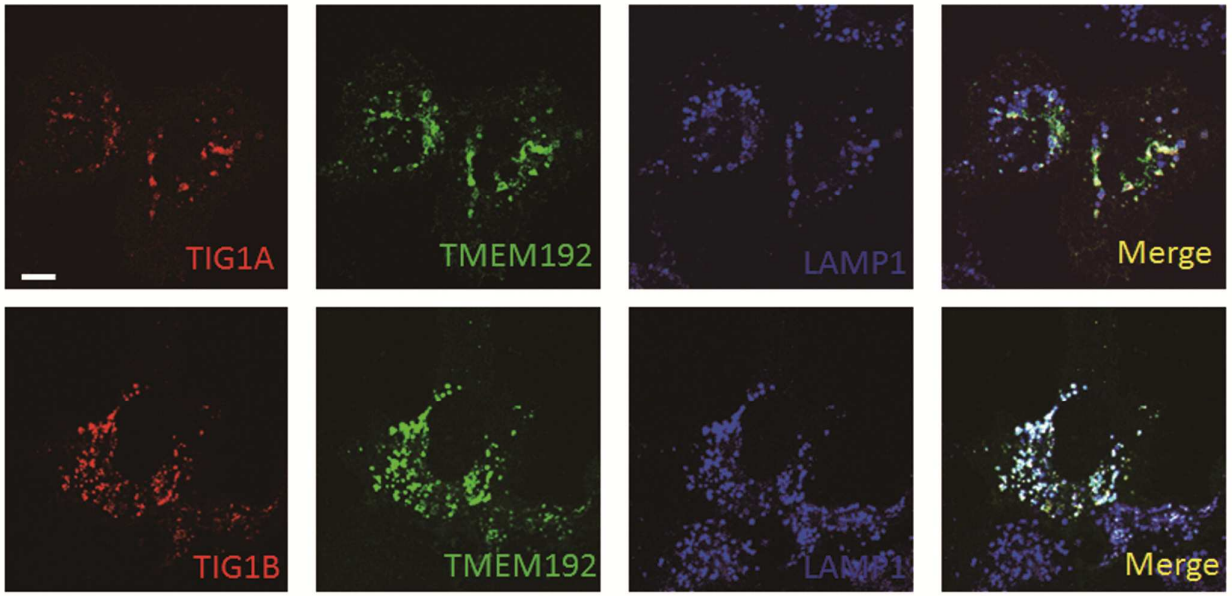
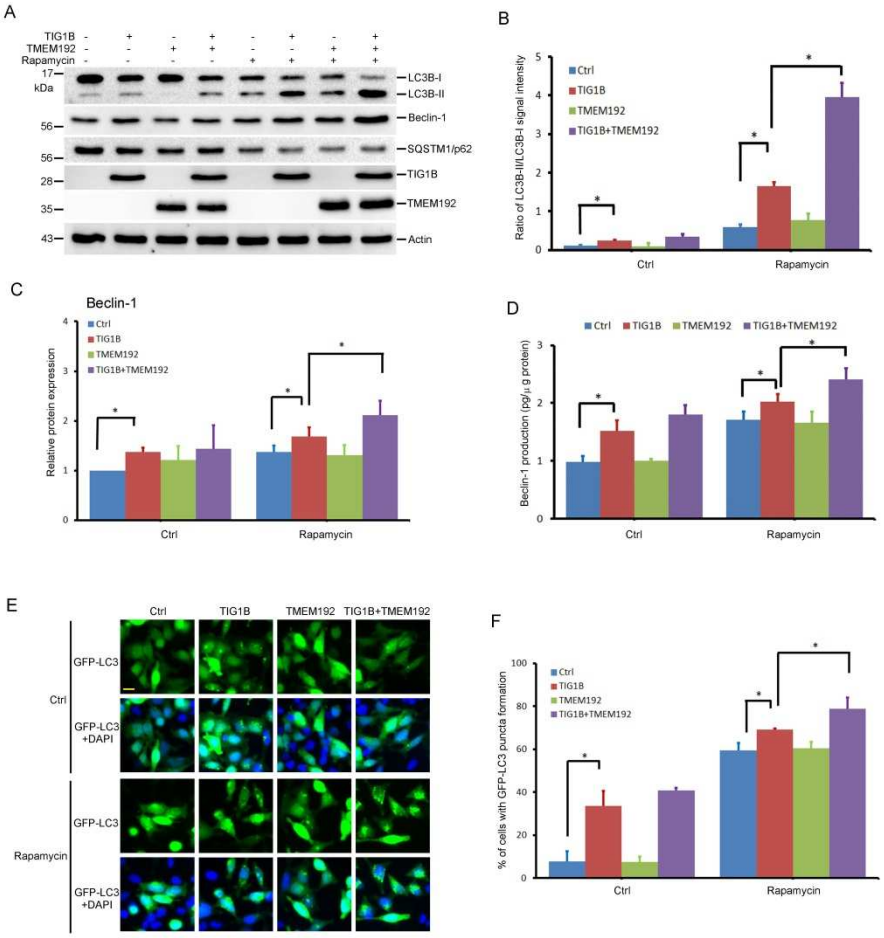
Supplementary Fig. 1. Endogenous TIG1 is associated with TMEM192. HtTA cell lysates were prepared, and the interaction between TIG1 and TMEM192 was analyzed by immunoprecipitation using anti-TIG1-specific antibody followed by Western blot analysis.



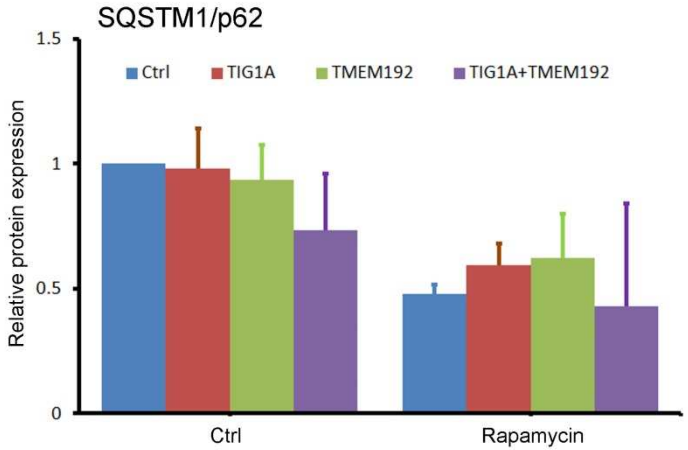
Supplementary Fig. 2. TIG1 co-localizes with TMEM192 at the lysosome. Huh7 cells were transiently transfected with the EGFP-TMEM192-Flag expression vector along with the TIG1A-myc or TIG1B-myc expression vector for 18 h. The cells were fixed and then incubated with anti-MYC and anti-LAMP1 antibodies followed by Alexa Fluor 633 goat anti-mouse IgG and Alexa Fluor 405 goat anti-rabbit IgG antibodies. The cells were then analyzed with a laser scanning confocal microscope. Scale bar: 10 μm. The localization of TIG1 (red), TMEM192 (green), and lysosomes (blue) were analyzed using a laser scanning confocal microscope. Scale bar: 10 μm.

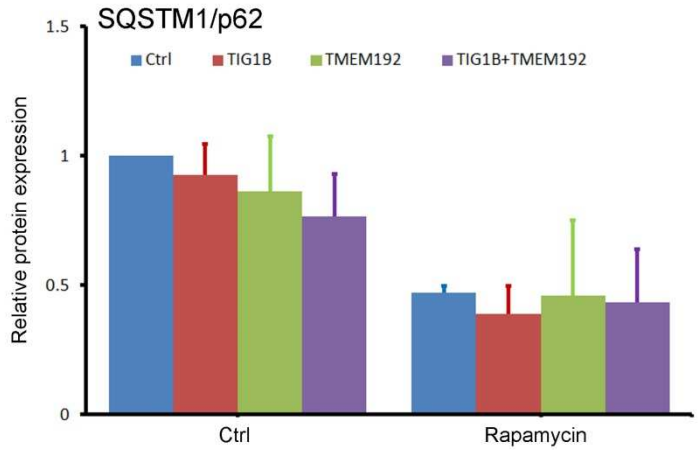


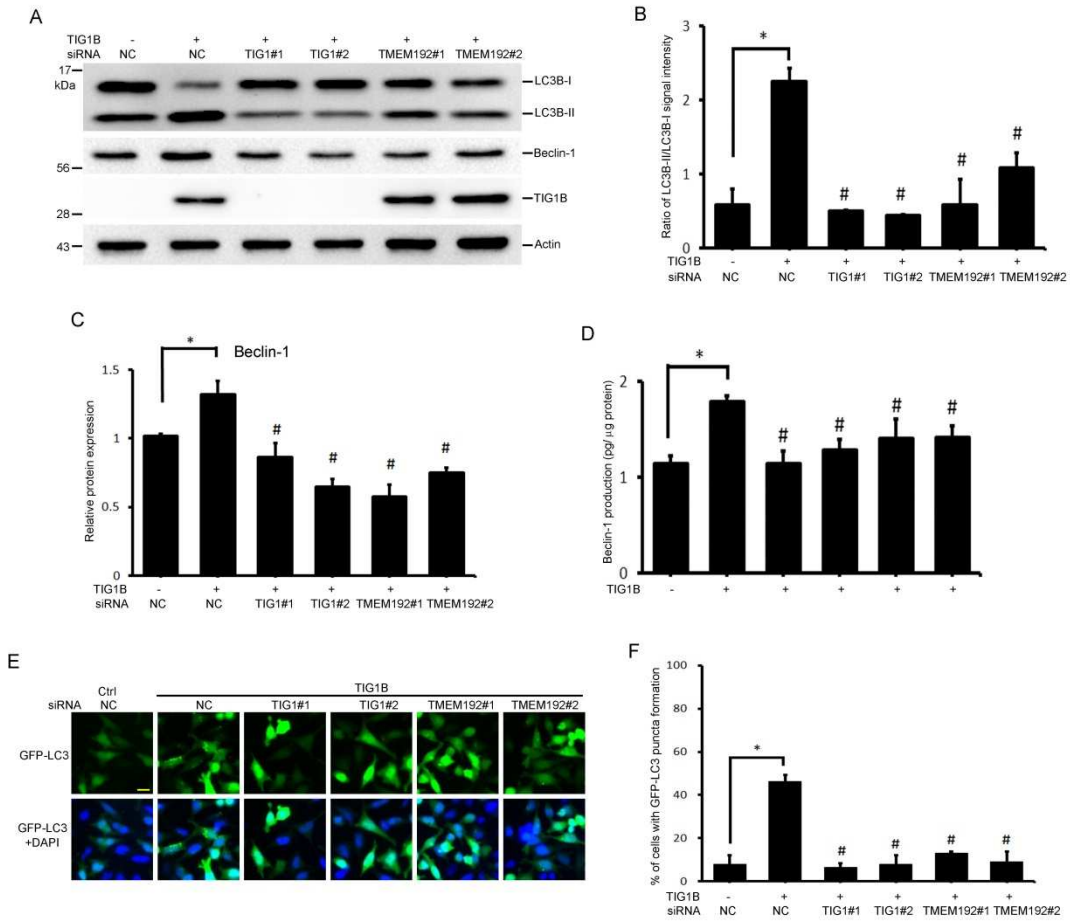


Supplementary Fig. 3. TIG1B induced expression of autophagy-related proteins. HtTA cells plated in a 6-cm dish were transfected with 1.5 μg of TMEM192-Flag expression vector along with 1.5 μg of TIG1B-myc expression vector for 24 h. Cells were refreshed in medium without serum and treated with 200 nM rapamycin for 6 h. Cell lysates were prepared, and the levels of LC-3B, Beclin-1, SQSTM1/p62, TIG1B, and actin were determined by immunoblotting (A). Experimental results are summarized as the mean percentage (±SD) of the ratio of LC3B-II to LC3B-I and the level of Beclin-1 with each sample normalized to the level of actin protein in two independent experiments (B and C). HtTA cells plated in 6-well dishes were transfected with 0.5 μg of the indicated vectors or the control vector for 24 h and were then cultured in serum-free medium with 200 nM rapamycin for 6 h. Cell lysates were prepared, and the level of Beclin-1 was detected using an enzyme immunoassay. Representative results of three independent experiments are shown (D). HtTA cells plated in triplicate in 24-well plates were transfected with 75 ng of TIG1B-myc expression vector, 75 ng of TMEM192-Flag expression vector and with 150 ng of pGFP-LC3 expression vector for 24 h. Cells were refreshed in medium without serum and treated with 400 nM rapamycin for 6 h. Representative images with GFP-LC3 puncta formation (E). Bar chart indicating the percentage of cells with GFP-LC3 puncta formation from three independent experiments (F). Scale bar: 10 μm. *Indicates p value<0.05.

Supplementary Fig. 4. Effects of TIG1 on expression of SQSTM1/p62 in HtTA cells. HtTA cells plated in a 6-cm dish were transfected with 1.5 μg of TMEM192-Flag expression vector and with 1.5 μg of TIG1A-myc or TIG1B-myc expression vector for 24 h. Cells were refreshed in medium without serum and treated with 200 nM rapamycin for 6 h. Cell lysates were prepared, and the levels of SQSTM1/p62 and actin were determined by immunoblotting. Experimental results are summarized as the mean percentage (±SD) of the level of SQSTM1/p62 with each sample normalized to the level of actin protein in two independent experiments.



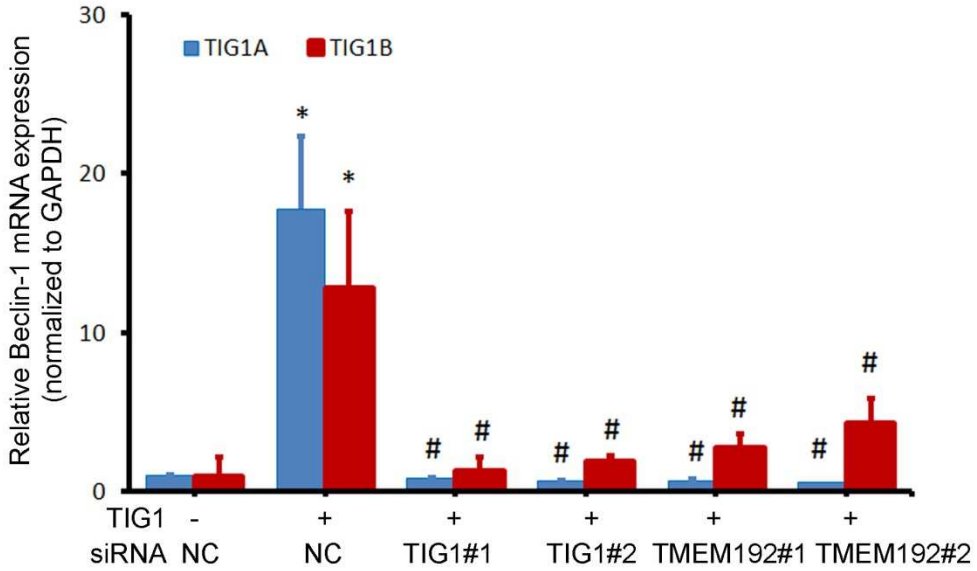




Supplementary Fig. 5. TMEM192 siRNAs alleviated TIG1B-induced expression of autophagy-related proteins. HtTA cells plated in 6-well dishes were transfected with 0.5 μg of TIG1B-myc (A-C) expression vector along with the indicated siRNA (30 nM) for 48 h and were then cultured in serum-free medium for 6 h. Cell lysates were prepared, and the expression of TIG1, TMEM192, Beclin-1, or LC3B was determined using anti-MYC, anti-FLAG, anti-Beclin-1, or anti-LC3B antibodies, respectively. Experimental results are summarized as the mean percentage (±SD) of the ratio of LC3B-II to LC3B-I and the level of Beclin-1 with each sample normalized to the level of actin protein in two independent experiments (B and C). HtTA cells plated in 6-well dishes were transfected with 0.5 μg of TIG1B-myc expression vectors along with the indicated siRNA for 48 h and were then cultured in serum-free medium for 6 h. Cell lysates were prepared, and the level of Beclin-1 was detected using an enzyme immunoassay. Representative results of three independent experiments are shown. HtTA cells plated in triplicate in 24-well plates were transfected with 75 ng of TIG1B-myc expression vector and 150 ng of pGFP-LC3 expression vector along with the indicated 30 μM siRNA for 48 h and were then cultured in serum-free medium for 6 h. Representative images with GFP-LC3 puncta formation (E). Bar chart indicating the percentage of cells with GFP-LC3 puncta formation from three independent experiments (F). Scale bar: 10 μm. *Indicates p value<0.05. #Indicates p value<0.05 when cells were co-transfected with TIG1B expression vector and indicated siRNA compared to cells that were co-transfected with TIG1B expression vector and NC siRNA.

Supplementary Fig. 6. Effects of TIG1 on the production of Beclin-1 mRNA. HtTA cells plated in 6-well dishes were transfected with 0.5 μg of either the TIG1A-myc or TIG1B-myc expression vector along with the indicated siRNA (30 nM) for 24 h and were then cultured in serum-free medium for 6 h. Total RNA was then purified, and the relative levels of Beclin-1 mRNA were quantified after normalizing for GAPDH. Representative results of three independent experiments are shown (A). Primer sequences for Beclin-1 and GAPDH used in real-time quantitative PCR (B).

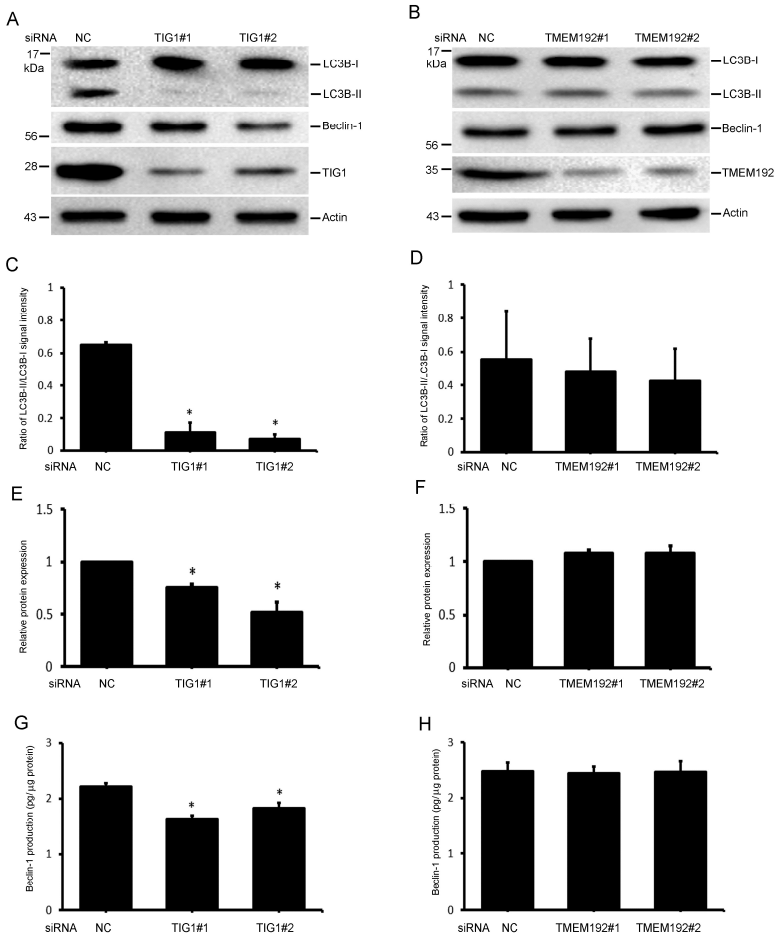
A



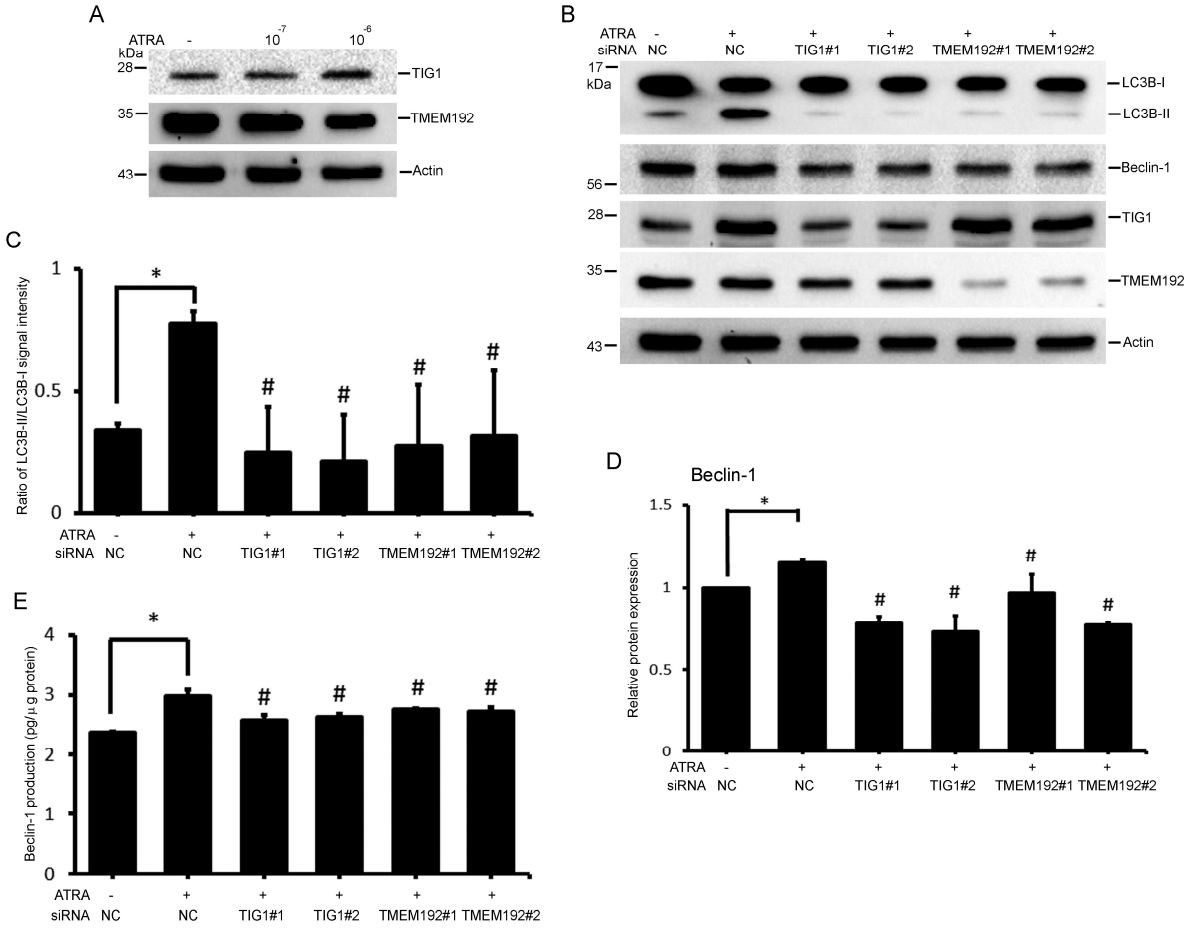
B

GAPDH	Sense	5'-gagtcaacggatttggtcgt-3'
	Antisense	5'-ttgattttggagggatctcg-3'
Beclin-1	Sense	5'-caagatcctggaccgtgtca-3'
	Antisense	5'-tggcactttctgtggacatca-3'

Supplementary Fig. 7. TIG1 siRNAs suppressed the expression of autophagy-related proteins in Huh7 cells. Huh7 cells plated in 6-well dishes were transfected with the indicated siRNA (30 nM) for 48 h. Cell lysates were prepared, and the expression of TIG1, TMEM192, Beclin-1, or LC3B was determined using anti-TIG1 (A), anti-TMEM192 (B), or anti-LC3B and Beclin-1 (A and B) antibodies, respectively. Experimental results are summarized as the mean percentage (±SD) of the ratio of LC3B-II to LC3B-I and the level of Beclin-1 with each sample normalized to the level of actin protein in two independent experiments (C-F). The level of Beclin-1 was detected using an enzyme immunoassay (G and H). Representative results of three independent experiments are shown. *Indicates p value < 0.05.



Supplementary Fig. 8. TIG1 and TMEM192 siRNAs decreases ATRA-induced upregulation of autophagy in Huh7 cells. Huh7 cells plated in 6-well dishes were treated daily with the indicated concentration of ATRA for 48 h (A). Alternatively, cells were transfected with the indicated siRNA (30 nM) and then treated daily with 10^{-6} M ATRA for 48 h. Cells were then cultured in serum-free medium for 6 h (B-D). Cell lysates were prepared, and the expression of TIG1, TMEM192, Beclin-1, or LC3B was determined using anti-TIG1, anti-TMEM192, Beclin-1, or anti-LC3B antibodies (B), respectively. Experimental results are summarized as the mean percentage (±SD) of the ratio of LC3B-II to LC3B-I and the level of Beclin-1 with each sample normalized to the level of actin protein in two independent experiments (C and D). The level of Beclin-1 was detected using an enzyme immunoassay (E). Representative results of three independent experiments are shown. *Indicates p value<0.05. #Indicates p value<0.05 when cells were transfected with the indicated siRNA and then treated with ATRA compared to cells transfected with NC siRNA and then treated with ATRA.



Supplementary Fig. 9. TIG1 and TMEM192 siRNAs decrease ATRA-induced GFP-LC3 puncta formation in HtTA cells. HtTA cells plated in triplicate in 24-well plates were transfected with 150 ng of pGFP-LC3 expression vector along with the indicated siRNA (30 nM) and then treated daily with 10^{-6} M ATRA for 48 h. Cells were then cultured in serum-free medium for 6 h. Representative images with GFP-LC3 puncta formation (A). Bar chart indicating the percentage of cells with GFP-LC3 puncta formation from three independent experiments (B). Scale bar: 10 μm. *Indicates *p* value<0.05. #Indicates *p* value<0.05 when cells were transfected with the indicated siRNA and then treated with ATRA compared to cells transfected with NC siRNA and then treated with ATRA.