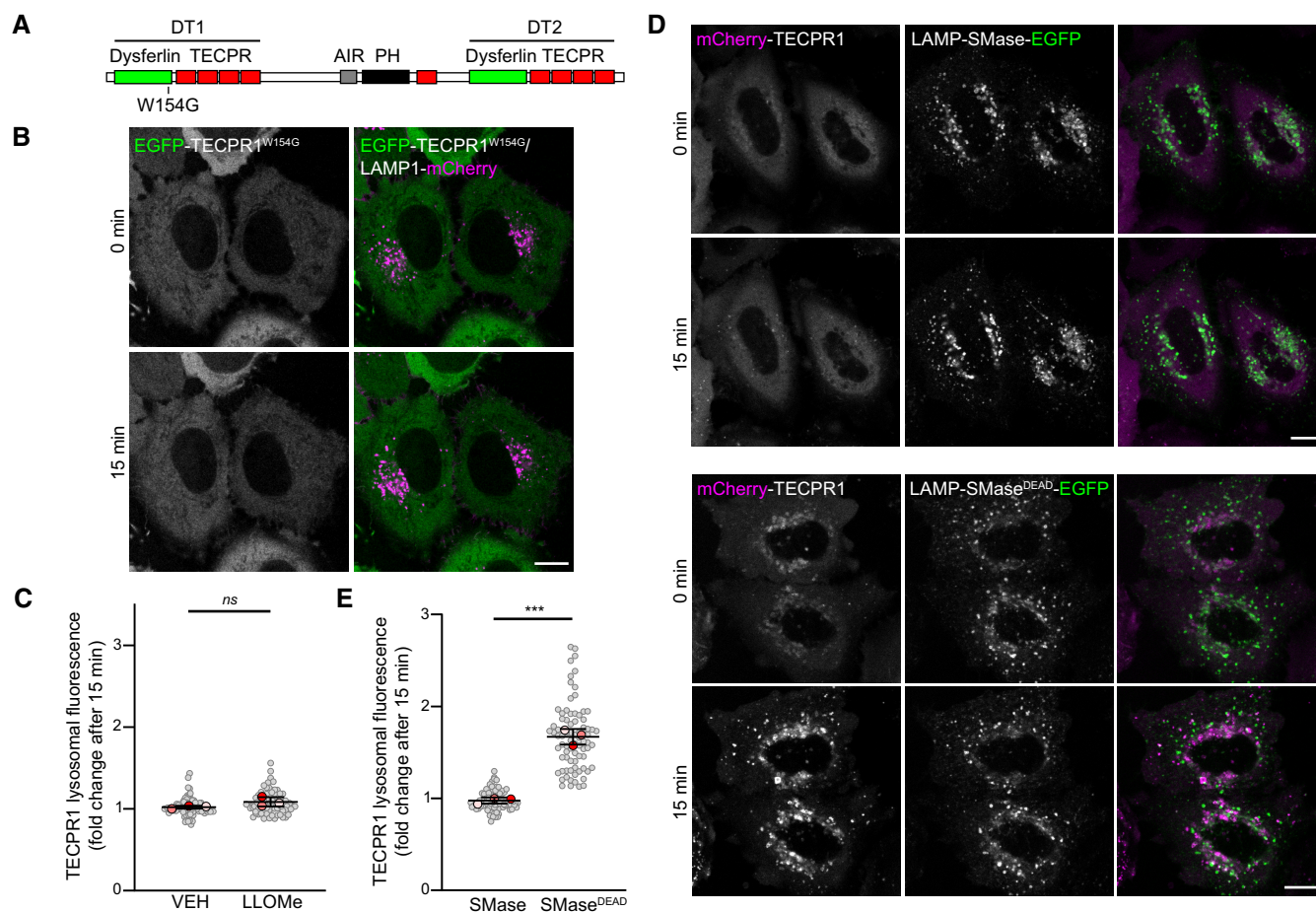
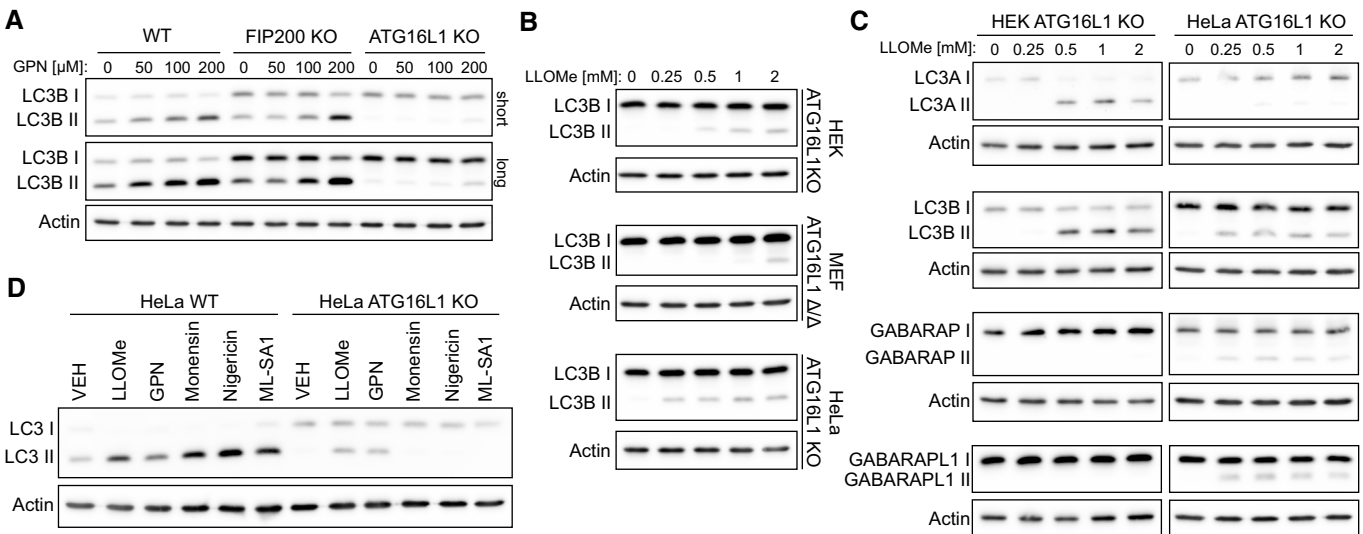


## Expanded View Figures



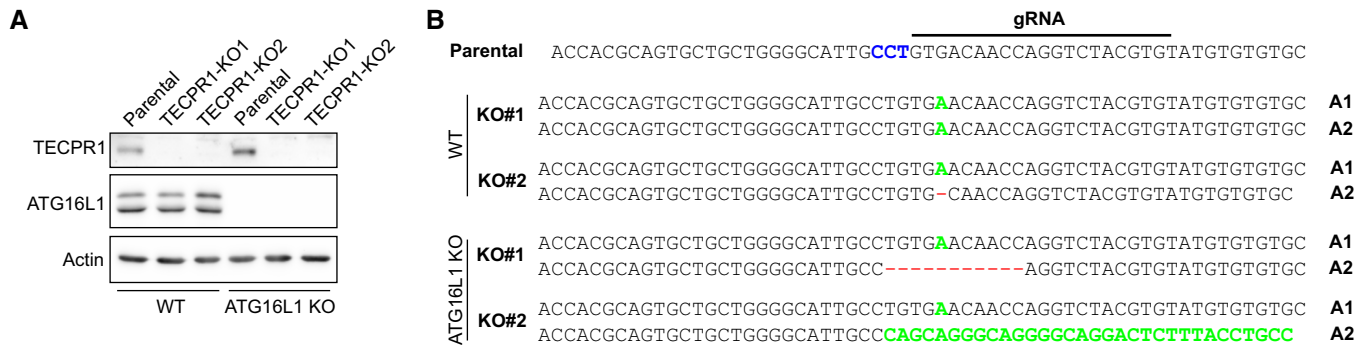
**Figure EV1. A spingomyelin binding domain in the N-terminal dysferlin domain of TECPR1 mediates lysosomal recruitment.**

- A Location of the W154G mutation in TECPR1.
- B Representative live-cell fluorescent images of HeLa cells co-transfected with EGFP-TECPR1<sup>W154G</sup> and LAMP1-mCherry before and after treatment with 1 mM LLOMe. Scale bar = 10  $\mu$ M.
- C Quantification of the fold change in EGFP-TECPR1 lysosomal fluorescence intensity after a 15-min treatment with vehicle or 1 mM LLOMe. Gray points represent individual cells from three independent experiments. Red points represent the means of individual experiments ( $n > 25$  cells per experiment). Bars represent the mean  $\pm$  SD from the three experiments. Significance was determined from biological replicates using Student's *t*-tests. ns = not significant.
- D Representative live-cell fluorescent images of HeLa cells co-transfected with mCherry-TECPR1 and LAMP1-SMase-EGFP (top) or LAMP1-SMase<sup>DEAD</sup>-EGFP (bottom) before and after treatment with 1 mM LLOMe. Scale bar = 10  $\mu$ M.
- E Quantification of the fold change in mCherry-TECPR1 lysosomal fluorescence intensity after a 15-min treatment with vehicle or 1 mM LLOMe (from D). Gray points represent individual cells from three independent experiments. Red points represent the means of individual experiments ( $n > 25$  cells per experiment). Bars represent the mean  $\pm$  SD from the three experiments. Significance was determined from biological replicates using Student's *t*-tests. \*\*\* = 0.0002.



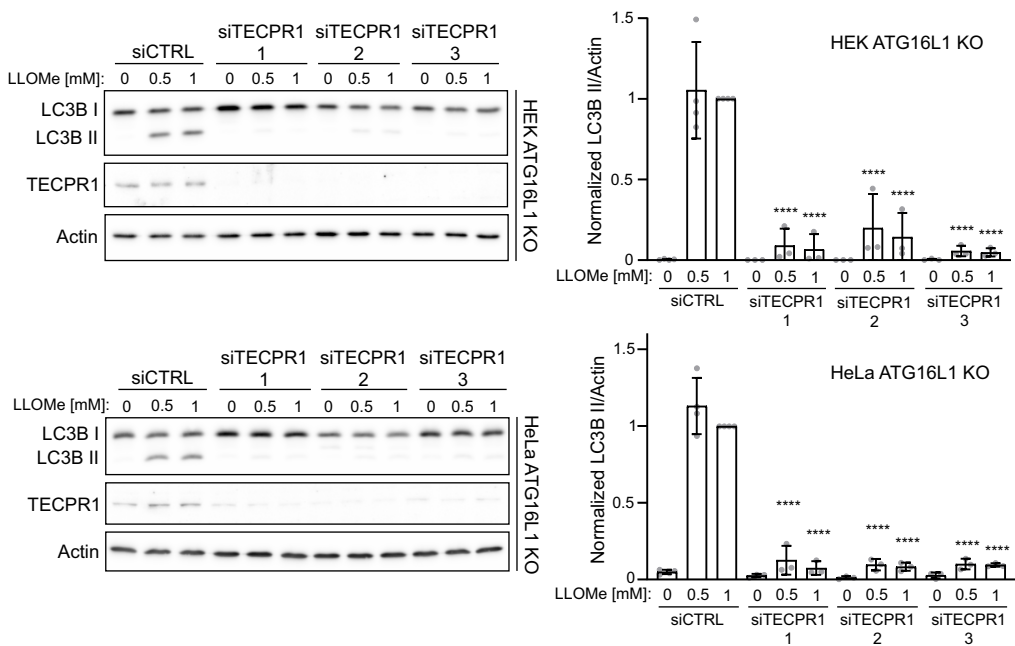
**Figure EV2. ATG8 lipidation in ATG16L1-deficient cell lines.**

A Western blot analysis of LC3 lipidation status in wild-type (WT), FIP200 KO, and ATG16L1 KO HeLa cells treated with the indicated concentrations of GPN for 30 min.  
B Western blot analysis of LC3B lipidation status in HEK, MEF, and HeLa ATG16L1-deficient cell lines treated with the indicated concentration of LLOMe for 30 min.  
C Western blot analysis of ATG8 lipidation status in HEK and HeLa ATG16L1-deficient cell lines treated with the indicated concentration of LLOMe for 30 min.  
D Western blot analysis of LC3 lipidation status in HeLa WT and ATG16L1 KO cells treated as indicated.



**Figure EV3. Validation of HEK TECPR1 KO cell lines.**

A Western blot analysis of TECPR1 and ATG16L1 protein levels in CRISPR-Cas9 clones.  
B Sequencing results from genomic PCR of TECPR1 exon 3 from CRISPR-Cas9 clones. Green represents insertions and red dashes represent deletions.



**Figure EV4. siRNA-mediated TECPR1 knockdown prevents ATG16L1-independent LC3 lipidation.** Western blot analysis of LC3 lipidation status in HEK (top) and HeLa (bottom) ATG16 KO cells transfected with TECPR1 siRNAs for 72 h and treated with the indicated concentration of LLOMe for 30 min. To the right is the corresponding quantification of LC3-II protein levels. Bars show mean  $\pm$  SD from three or four biologically independent experiments, which are represented as data points. Significance was determined from biological replicates using a one-way ANOVA with Tukey's multiple comparisons tests. \*\*\*\* $p < 0.0001$ .