



**Figure S5** - The TECPR domain of TECPR2 associates with autophagosomes and lysosomes. **(A,B)** HeLa cells were transfected for expression of mCherry-fused *TECPR2* variants using JetPrime reagent for 48 h. **(A)** Extracted proteins were immunoblotted for RFP to evaluate the levels of expressed fusion protein. **(B)** Cells fixed with methanol were immunostained for RFP to visualize their distribution by confocal microscopy. Scale bar: 20  $\mu$ m. **(C)** HeLa cells were transfected with siRNA targeting *VAMP8* and/or *STX17* siRNAs, or a non-targeting (siNT) control, using DharmaFECT1 transfection reagent for 72 h, and optionally treated for the last 4 h with 0.1  $\mu$ M bafilomycin A1. Extracted proteins were immunoblotted for *VAMP8* and *STX17*. **(D)** HeLa cells were transfected with siRNA targeting *VAMP8* and/or *STX17* siRNAs, or a non-targeting (siNT) control, using DharmaFECT1 transfection reagent. After 24 h cells were transfected for mCherry-*TECPR* or mCherry-*TECPR*[ $\Delta$ LIR] expression using JetPrime reagent for additional 24 h. Cells were fixed with methanol, immunostained for LC3B and LAMP1 and examined by confocal microscopy. Scale bar: 20  $\mu$ m.