

Supplemental material

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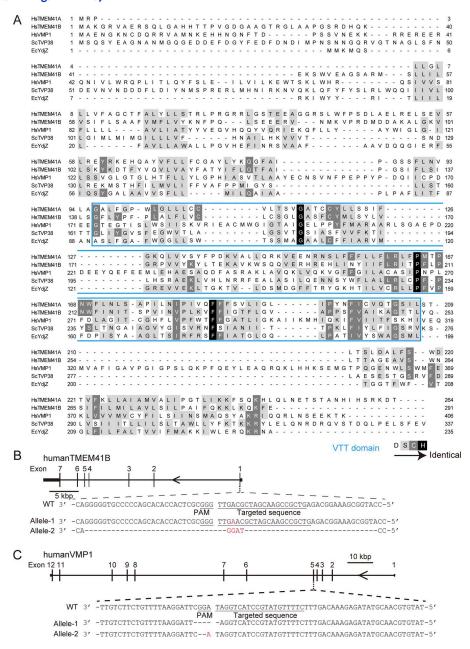


Figure S1. Similarity of TMEM41B and VMP1 and generation of TMEM41B-KO and VMP1-KO cells. (A) Multiple sequence alignment of sequences of full-length HsTMEM41A, HsTMEM41B, HsVMP1, ScTvp38, and EcYdjZ. Positions are colored according to increasing sequence identity: white, not conserved; gray, similar; black, identical. (B and C) Positions of sgRNA-targeted sequences in human TMEM41B (B) and VMP1 (C) genes and sequences of the resultant mutant alleles in HEK293T cells. PAM, protospacer-adjacent motif.



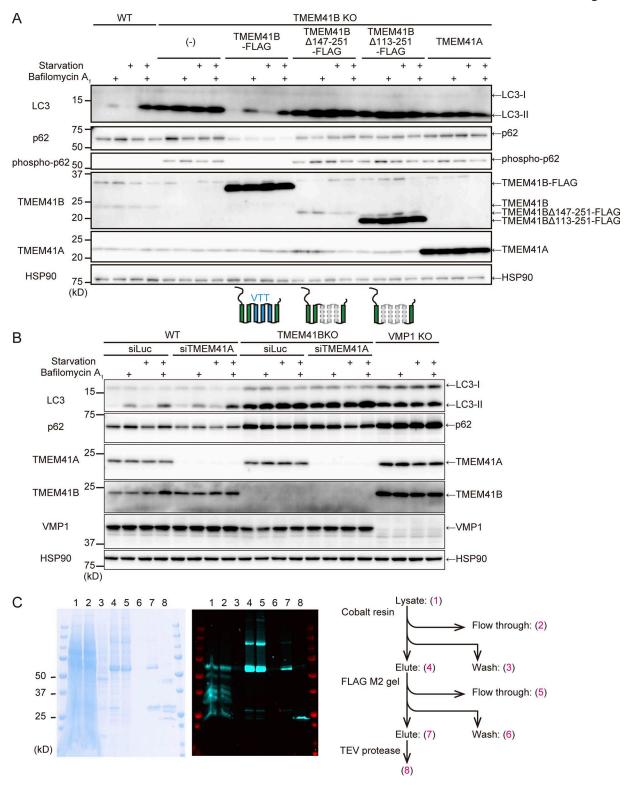


Figure S2. Requirement of the VTT domain and TMEM41B for autophagy and purification of TMEM41B and VMP1. (A) TMEM41B-KO cells stably expressing TMEM41B-FLAG, TMEM41B-FLAG lacking the VTT domain (TMEM41B Δ 147–251 and TMEM41B Δ 113–251), and exogenous TMEM41A were cultured with or without bafilomycin A₁ under nutrient-rich or starvation conditions for 2 h. (B) WT and TMEM41B-KO cells were transfected twice with siLuciferase (siLuc) or siTMEM41A. After 2 d, cells were cultured with or without bafilomycin A₁ under nutrient-rich or starvation conditions for 2 h. (C) VMP1-TEV-GFP-His and TMEM41B-FLAG were coexpressed in Sf9 cells. The VMP1-TMEM41B complex was purified by cobalt-affinity chromatography followed by FLAG M2 affinity chromatography. The left panel shows CBB-stained SDS-PAGE, and the right panel shows in-gel fluorescence signals of GFP (460 nm) and markers (630 nm) colored with cyan and magenta, respectively.



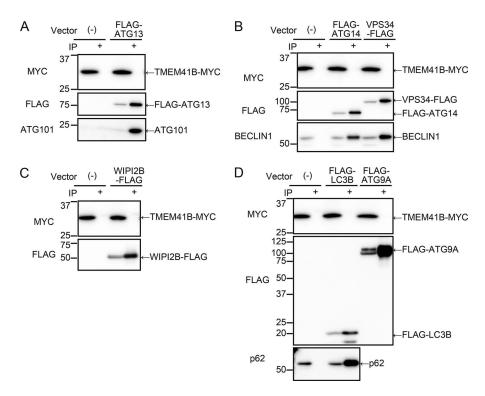


Figure S3. **TMEM41B does not interact with core ATG proteins. (A–D)** HEK293T cells stably expressing MYC-tagged TMEM41B were transfected with FLAG-tagged ATG13 (A), ATG14, VPS34 (B), WIPI2B (C), LC3B, or ATG9A (D), and their lysates were subjected to IP using anti-FLAG antibody–conjugated Sepharose beads. Molecular masses are given in kilodaltons.

Table S1 is a separate Excel file showing results of the genome-wide pooled CRISPR screen.