

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.

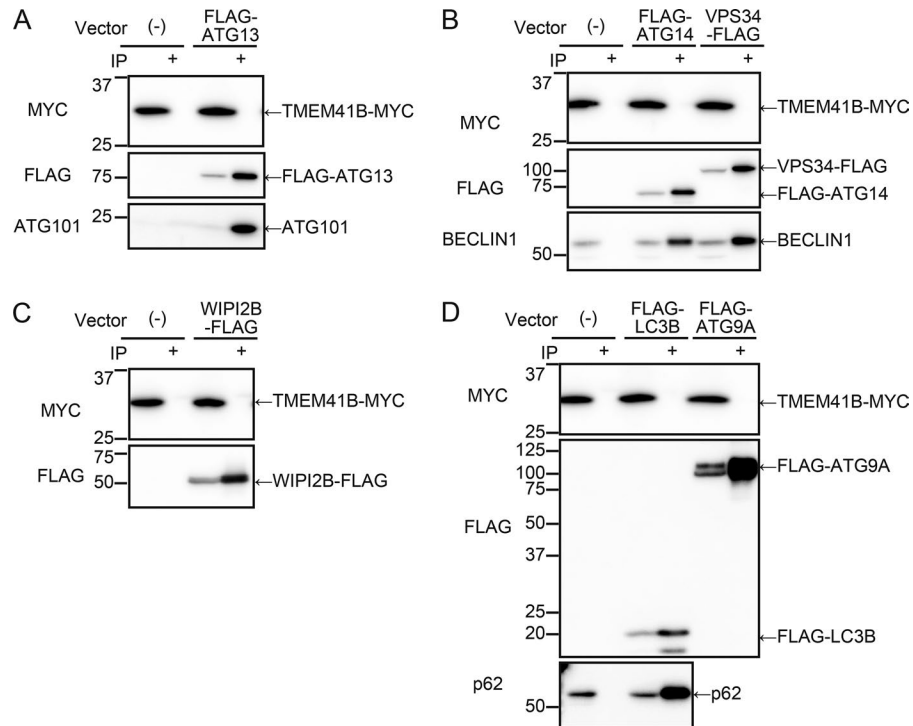


Figure S3. **TMEM41B does not interact with core ATG proteins. (A–D)** HEK293T cells stably expressing MYC-tagged TMEM41B were transfected with FLAG-tagged ATG13 (A), ATG14, VPS34 (B), WIPI2B (C), LC3B, or ATG9A (D), and their lysates were subjected to IP using anti-FLAG antibody-conjugated Sepharose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

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