

## Supplementary Figures

**Figure S1:** (A) Schematic representation of CHMP2B and CHMP2B mutants used throughout this study. MIM: MIT domain interacting motif. (B) Sequence alignment of the N-terminal amino-acids of human CHMP proteins. Conserved LF residues are shown in bold.

**Figure S2:** (A) Western-blot analysis of cells used in the experiment shown in figure 1 demonstrates the effect of siRNAs on VPS4A and VPS4B proteins. Lysates of HeLa cells were prepared 72 h after transfection with siRNAs and western-blot revealed using the indicated antibodies.  $\alpha$ -Actin was used as loading control. (B) VPS4 and B downregulation induces accumulation of endogeneous CHMP2B at the plasma membrane. HeLa cells were depleted of VPS4A and B and immunostained with antibodies against CHMP2B as described in Figure 1. The left photograph shows maximum intensity projections. Confocal sections of the boxed areas shown on the right demonstrate the concentration of CHMP2B at the plasma membrane. Bars: 20  $\mu$ m.

**Figure S3:** (A) CHMP2B overexpression in post-mitotic neurons induces formation of cell protrusions in which it accumulates. Rat hippocampal neurons (E19) were prepared and transfected with CHMP2B after 14 DIV as described in (39). Three days later, CHMP2B was revealed by immunostaining with a polyclonal antibody against CHMP2B. (B) Western-blot analysis of cells used in the experiment shown in figure 2 reveals comparable levels of expression of all CHMP2B proteins. Lysates of HeLa cells were prepared 36 h after transfection and western blots revealed using the indicated antibodies.

**Figure S4:** (A) Cryo electron microscopy image of a CHMP2B tube. (B) Part of a 2D average made of 35 boxes (900 x 900 pixels) chosen along the tube shown in A. Bars 50 nm. (C) Power spectrum of the 2D average shown in B. The arrow indicates the layer line corresponding to the 1/32Å spacing.

**Movie S1:** 3D reconstruction of a cell expressing CHMP2B immunostained with anti-CHMP2B antibody.

**Movie S2:** 3D reconstruction of a cell expressing CHMP2B-Flag immunostained with anti-Flag antibody.

Figure S1

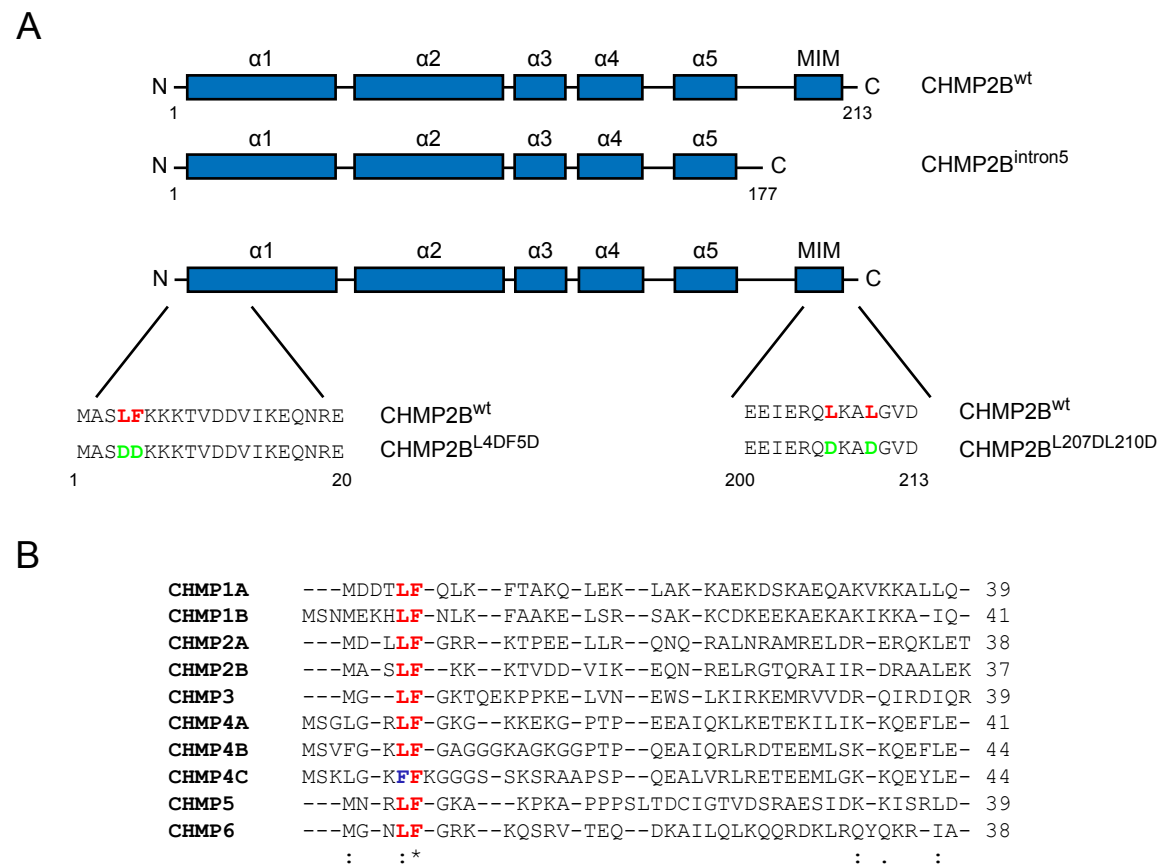


Figure S2

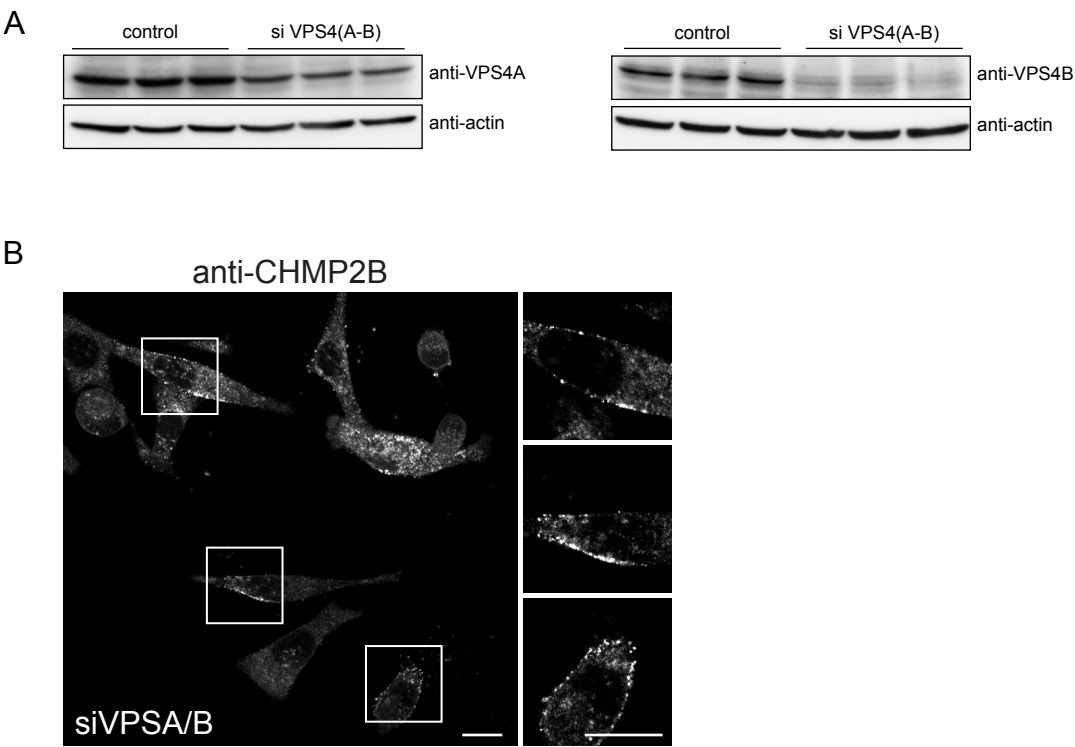
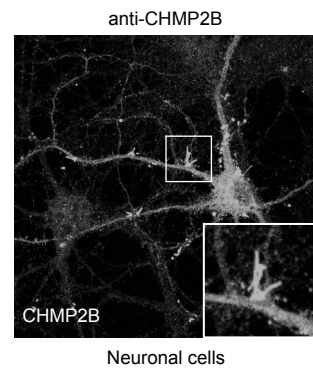


Figure S3

A



B

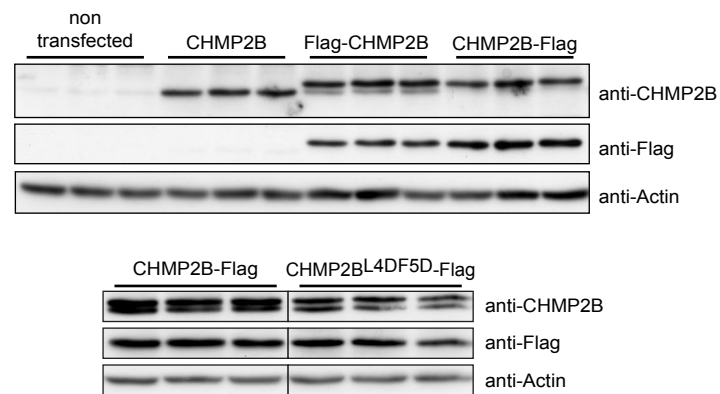


Figure S4

