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-blots revealed using the indicated antibodies. α-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 CHM2B 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 μm.

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 indicted antibodies.

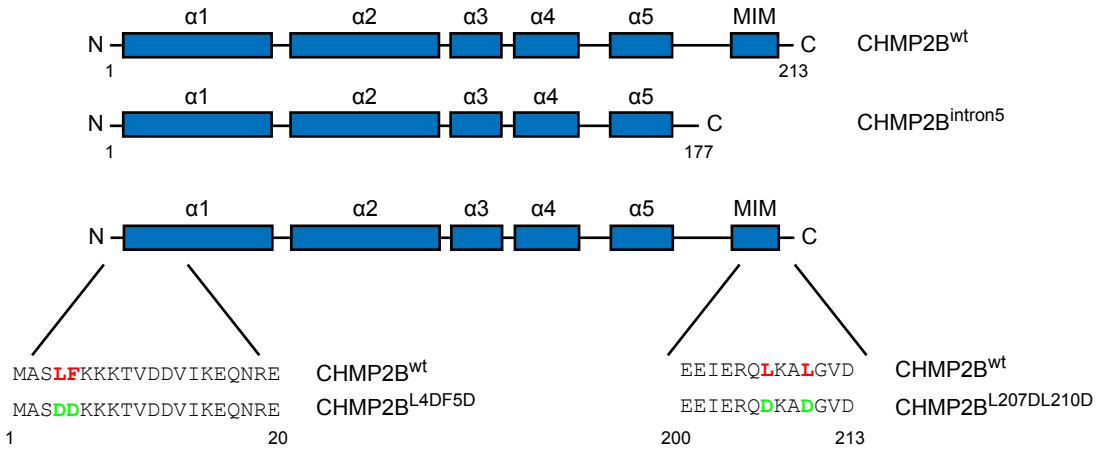
Figure S4: (A) Cryo electron microscopy image of a CHMP2B tube. (B) Part of a 2D average made of 35 boxes (900 x 900 pixels) chosen along the tube shown in A. Bars 50 nm. (C) Power spectrum of the 2D average shown in B. The arrow indicates the layer line corresponding to the 1/32Å spacing.

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

A



B

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CHMP1A    ---MDDTLF-QLK--FTAKQ-LEK--LAK-KAEKDSKAEQAKVKKALLQ- 39
CHMP1B    MSNMEKHLF-NLK--FAAKE-LSR--SAK-KCDKEEKAEKAKIKKA-IQ- 41
CHMP2A    ---MD-LLF-GRR--KTPEE-LLR--QNQ-RALNRAMRELDR-ERQKLET 38
CHMP2B    ---MA-SLF--KK--KTVDD-VIK--EQN-RELRGTQRAIIR-DRAALEK 37
CHMP3     ---MG--LF-GKTQEKPPKE-LVN--EWS-LKIRKEMRVVDR-QIRDIQR 39
CHMP4A    MSGLG-RLF-GKG--KKEKG-PTP--EEAIQKLKETEKILIK-KQEFLE- 41
CHMP4B    MSVFG-KLF-GAGGGKAGKGGPTP--QEAIQRLRDTEEMLSK-KQEFLE- 44
CHMP4C    MSKLG-KFFKGGGS-SKSRAAPSP--QEALVRLRETEEMLGK-KQEYLE- 44
CHMP5     ---MN-RLF-GKA---KPKA-PPPSLTDCIGTVDSRAESIDK-KISRLD- 39
CHMP6     ---MG-NLF-GRK--KQSRV-TEQ--DKAILQLKQQRDKLRQYQKR-IA- 38
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Figure S2

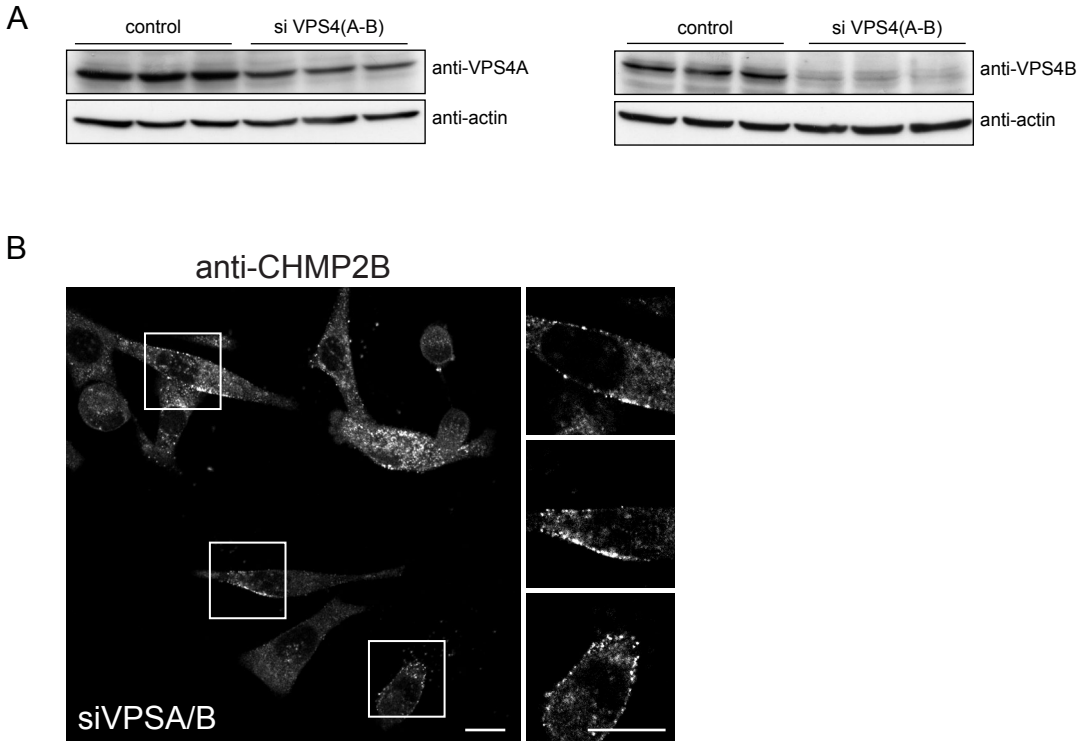
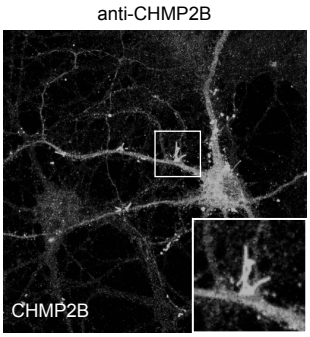

A
control
si VPS4(A-B)
anti-VPS4A
anti-actin
control
si VPS4(A-B)
anti-VPS4B
anti-actin
B
anti-CHMP2B
siVPSA/B

Figure S3

A



Neuronal cells

B

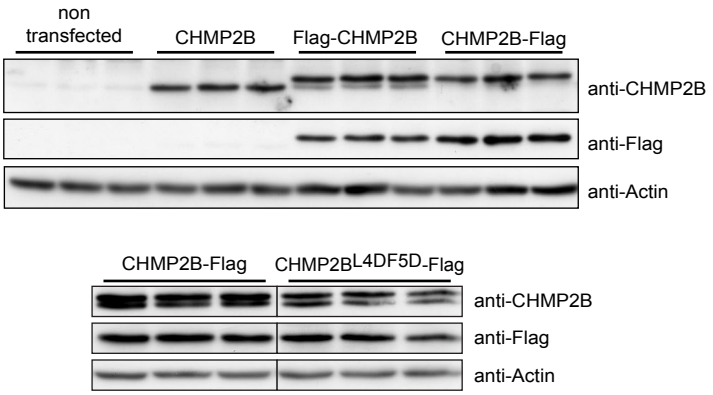


Figure S4

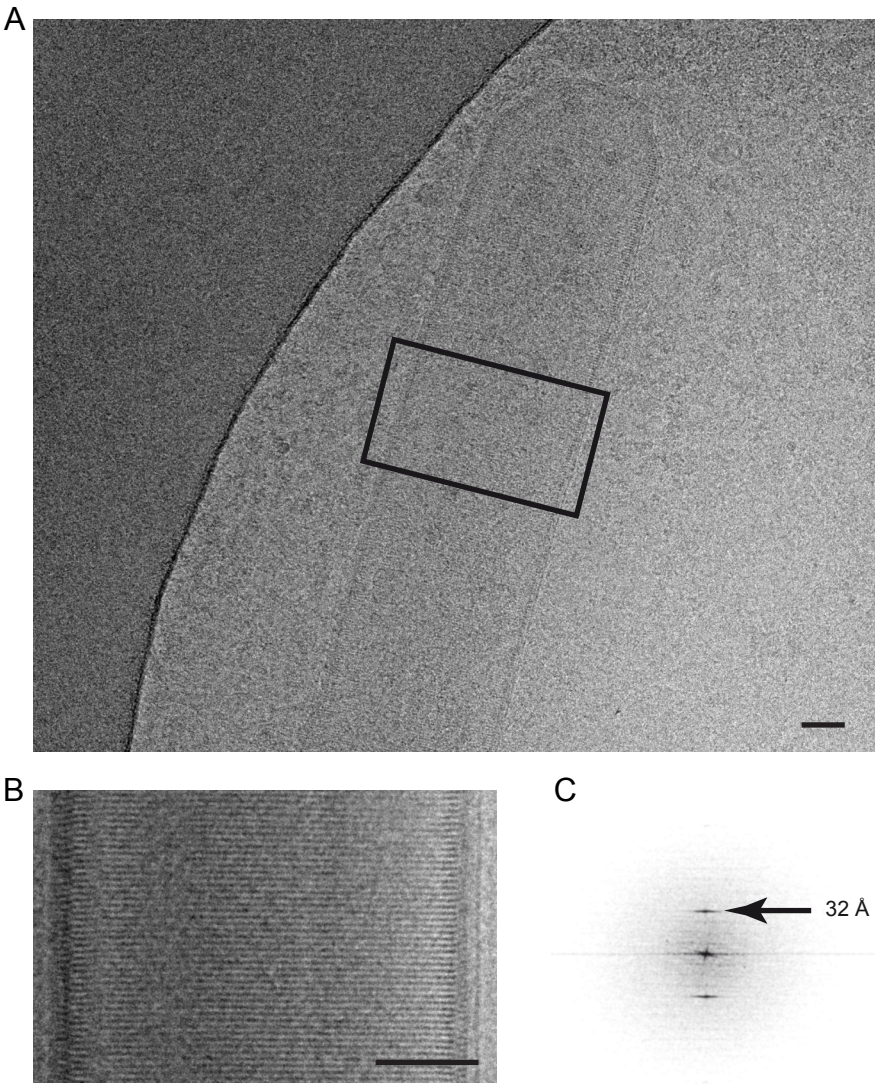


Table S1: PCR primers used in this study

Construction	Forward primer (5'-3')	Reverse primer(5'-3')
CHMP2B^{L4DF5D}	caccatggcgtccgacgacaagaag	catccacggttttcttcttgtcgtcggacgccatggtgaagggta
CHMP2B ^{L207DL210D}-Flag	caccatggcgtccctcttcaagaa	gggctacttgtcatcgtcatccttgtagtcatctactccatcagccttgtcttgccgttcaatctcttcat
CHMP2B-Flag	caccatggcgtccctcttcaagaa	gggcccctcgagctacttgtcatcgtcatccttgtagtcatctactcctaaagccttgag
CHMP2B	caccatggcgtccctcttcaagaa	gggctaatctactcctaaagccttgag
CHMP2B^{INTRON5}-Flag	caccatggcgtccctcttcaagaa	ggggggcccttacttgtcatcgtcatccttgtagtcaacctttccagaaatttcaattcc
CHMP3-Flag	caccatggggctgtttggaaag	gggcccctacttgtcatcgtcatccttgtagtcgctgcggagtggg
CHMP4A-Flag	caccatgtcgcggcggcgccc	gggtcacttgtcatcgtcatccttgtagtcggatacccactcagccaact
CHMP4B-Flag	caccatgtcggtgttcgggaagc	gggccctcacttgtcatcgtcatccttgtagtccatggatccagccagttc
CHMP4C-Flag	caccatgagcaagttgggcaagttc	ttacttgtcatcgtcatccttgtagtcggtagcccaagctgccaatt