



**Supplementary Figure 5** Secondary structural models and alignments of human ESCRT-III proteins.

**(a)** Secondary structural models of human ESCRT-III proteins. The figure shows known (CHMP3 and IST1<sub>NTD</sub>) or predicted secondary structures of representative members from the eight different families of human ESCRT-III proteins. Helices are shown as blocks, and colors match the scheme of Fig. 2a-c. ESCRT-III core domain helices ( $\alpha$ 1- $\alpha$ 4) are colored purple-green, and autoinhibitory helices are shown in orange. Approximate residue boundaries for each helix are provided above each secondary structure. Downstream elements show predicted helices (boxes), the two known classes of VPS4 MIT domain interacting motifs (MIM1, magenta and MIM2, pink), the binding site for the Spastin MIT domain on CHMP1B, and the binding site for the ALIX Bro1 domain on CHMP4B. Note that the C-terminal core domain of CHMP7 was used in the illustration and that, in several cases,  $\alpha$ 2- $\alpha$ 4 segments were predicted as continuous helices and were manually separated into  $\alpha$ 2,  $\alpha$ 3, and  $\alpha$ 4 helices.

**(b)** Structure-based sequence alignment between the N-terminal domains of IST1<sub>NTD</sub> and CHMP3. Uppercase residues were included in the alignment (126 residues aligned, RMSD=3.0 $\text{\AA}$ ). Red residues are conserved between the two proteins (8% overall sequence identity). Secondary structures are depicted above and below their corresponding sequences, with color coding that matches Fig. 2 and Supplementary Fig. 5a. Red stars denote residues within the  $\alpha$ 5- $\alpha$ 1/ $\alpha$ 2 interface and cyan stars denote tip-to-tip mutants that inhibit polymerization *in vitro*.