and washed with loading buffer before elution with a gradient from 150 mM NaCl to 1 M NaCl in 25 mM Tris, pH 8.0, 5 mM BME. For CHMP3 proteins, residual GST was removed by an additional pass through a Glutathione Sepharose 4 Fast Flow Resin column. Finally, monomeric CHMP3 and CHMP1B proteins were separated from aggregates by gel filtration chromatography in 10 mM Tris, pH 8.0, 100 mM NaCl, 5 mM BME on a HiLoad 16/60 Superdex S75 column (GE Healthcare). Yields were ~30 mg for the wild type CHMP3 protein (3 L cultures) and ~1 mg CHMP1B (6 L culture), and the proteins could be concentrated to up to 30 mg ml^{-1} (CHMP3) or ~1 mg ml^{-1} (CHMP1B) in gel filtration buffer.

We expressed CHMP2A as a GST fusion, lysed (50 mM potassium phosphate, pH 7.2, 200 mM NaCl, 1 mM DTT) and purified as described for the CHMP3 proteins except for the following modifications: 1) The protein was precipitated from clarified lysate following the 10% PEI treatment by addition of 0.4 equivalents (v:v) saturated ammonium sulfate solution. The pellet was then dissolved in lysis buffer and applied to a Glutathione Sepharose 4 Fast Flow column. 2) After TEV treatment, the processed protein was dialyzed against Q column loading buffer (50 mM Tris, pH 8.0), applied to a Q Sepharose Fast Flow column (GE Healthcare) and washed with loading buffer before elution with a gradient from 0 to 250 mM NaCl in 50 mM Tris, pH 8.0. 3) Monomeric CHMP2A protein was separated from aggregates by gel filtration chromatography in 10 mM Tris pH 8.0, 100 mM NaCl, 1 mM DTT on a HiLoad 16/60 Superdex S75 column (GE Healthcare). This procedure typically yielded 45 mg CHMP2A from 6 L cultures and CHMP2A could be concentrated to 20 mg ml^{-1} in gel filtration buffer.

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