

SUPPLEMENTARY METHODS

Protein expression and purification. We expressed CHMP3, CHMP3₈₋₂₂₂, CHMP3₁₋₁₅₀, CHMP3_{V48D, A64D}, CHMP3_{V59D, V62D, I168D, L169D}, CHMP3_{I168D, L169D} and CHMP1B proteins with N-terminal GST affinity tags in BL21 (RIPL) *E. coli* grown in auto-induction media ZYP-5052⁹ (3 L cultures for CHMP3 proteins, 6 L cultures for CHMP1B). Cells were grown at 37 °C for 6 hours with vigorous shaking in baffled flasks before moving to 23 °C and growing to saturation for 16-18 hours. Subsequent purification steps were performed at 4 °C and were the same for all proteins except where specified. Cells were lysed with sonication, lysozyme and sodium deoxycholate treatment (50 mM Tris pH 7.0, 300 mM NaCl, 5 mM β -mercaptoethanol (BME)), and the supernatant was clarified by low speed centrifugation. 0.1 equivalent (v:v) of 10% polyethyleneamine (PEI) in 0.2 M NH₄SO₄ was added and nucleic acid precipitates were removed by centrifugation. The clarified supernatant was dialysed for 12 h against glutathione column loading buffer (10 mM potassium phosphate, pH 7.4, 150 mM NaCl, 5 mM BME), filtered, and applied to a Glutathione Sepharose 4 Fast Flow column (GE Healthcare) at 0.5 ml min⁻¹. The bound protein was washed extensively with loading buffer, and eluted with 50 mM Tris, pH 8.0, 150 mM NaCl, 20 mM reduced glutathione, 5 mM BME. The eluted protein (~70 ml) was dialyzed against 50 mM Tris, pH 8.0, 50 mM NaCl, 5 mM BME while incubating with TEV protease (0.6 mg per 70 ml) to remove the GST tag (12 h for CHMP3 proteins, 24 h for CHMP1B, 23 °C). The processed protein was dialyzed against SP column loading buffer (25 mM Tris, pH 6.5, 150 mM NaCl, 5 mM BME), applied to a SP Sepharose Fast Flow column (GE Healthcare),