

were surveyed over a range of ionic strengths (0-100 mM NaCl, 25 mM Tris pH8.0, 5 mM β -mercaptoethanol), molar protein ratios (0.5-2), and final protein concentrations (50-120 μ M). Reactions containing isolated CHMP2A or CHMP3, mixtures of full length CHMP2A+CHMP3, and mixtures of CHMP2A+CHMP3_{V59D, V62D, I168D, L169D} did not assemble into regular structures under any conditions tested, mixtures of CHMP2A+CHMP3₁₋₁₅₀ and of CHMP2A+CHMP3_{I168D, L169D} assembled under all conditions tested, and mixtures of CHMP2A+CHMP3_{V48D, A64D} assembled under the higher protein (≥ 100 μ M) and salt (≥ 50 mM NaCl) conditions. Assembly conditions for the images shown in Fig. 7 were: a) CHMP2A (120 μ M)+CHMP3 (120 μ M) in 0 M NaCl, b) CHMP2A (50 μ M)+CHMP3₁₋₁₅₀ (50 μ M) in 0 M NaCl, c) CHMP2A (120 μ M)+CHMP3_{V48D, A64D} (120 μ M) in 50 mM NaCl, d) CHMP2A (120 μ M)+CHMP3_{I168D, L169D} (120 μ M) in 0 M NaCl, e) CHMP2A+CHMP3_{V59D, V62D, I168D, L169D} (120 μ M) in 100 M NaCl.

GST pulldown assays. We induced 5ml *E. coli* cultures to express GST-CHMP3 proteins using the autoinduction method, pelleted and lysed them by treatment (4°C, 20 minutes) with 1 mg ml⁻¹ lysozyme in 4 ml binding buffer (10 mM Tris-HCl (pH 8.0), 100 mM NaCl, 5 mM β -mercaptoethanol, 5% (v/v) glycerol, 0.2% (v/v) NP40) followed by addition of 5% w/v sodium deoxycholate (4 °C, 20 minutes). Clarified lysates (1 ml) were incubated (1 h, 4°C) with 50 μ l glutathione sepharose 4B matrix (pre-washed in binding buffer) followed by three binding buffer washes. Pure CHMP2A protein (35 μ M in 1 ml binding buffer, ~5-fold molar excess over GST-CHMP3 proteins) was incubated with the GST-CHMP3/matrix for 1 hour at 4 °C and