We expressed $IST1_{NTD}$ as a GST fusion, lysed (50 mM Tris pH 8.0, 1 M NaCl, 5% (v/v) glycerol, 5 mM BME) and purified as described for the CHMP3 proteins except for the following modifications: 1) The clarified, filtered supernatant was applied directly (without PEI treatment) to a Glutathione Sepharose 4 Fast Flow column (GE Healthcare) at 0.5 ml. min^{-1} and eluted with 50 mM Tris, pH 8.0, 1 M NaCl, 5% (v/v) glycerol, 20 mM reduced glutathione, 5 mM BME. 2) The eluted protein was dialysed against 50 mM Tris, pH 8.0, 250 mM NaCl, 1 mM DTT while incubating with TEV protease (0.4 mg per 40 ml, 24 hours, 23 °C). 3) The processed protein was dialysed for 12 hours against SP column loading buffer (50 mM Tris, pH 7.0, 150 mM NaCl, 5% (v/v) glycerol, BME), applied to a SP Sepharose Fast Flow column (GE Healthcare) and washed with loading buffer before elution with a gradient of 150 mM to 1 M NaCl in 50 mM Tris, pH 7.0, 5% (v/v) glycerol, 5 mM BME. 4) Monomeric $IST1_{NTD}$ was separated from aggregated species by gel filtration chromatography in 50 mM Tris pH 7.0, 350 mM NaCl, 5% (v/v) glycerol, 1 mM DTT on a HiLoad 16/60 Superdex S75 column (GE Healthcare). This procedure typically yielded 30 mg $IST1_{NTD}$ from 4 L cultures and the protein could be concentrated to 15 mg ml^{-1} in gel filtration buffer.

Biosensor binding assays. We performed Biosensor binding experiments as previously reported using BIACORE2000 and 3000 instruments[10]. Briefly, GST-$IST1_{NTD}$ proteins were expressed and captured directly from BL21 (DE3) codon+ (RIPL) *E. coli* extracts onto anti-GST antibody-derivatized CM5 sensor chips. Purified CHMP1B protein was diluted at the designated concentrations in binding

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