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## **Structural basis of CHMP2A–CHMP3 ESCRT-III polymer assembly and membrane cleavage**

Kimi Azad, Delphine Guilligay, Cecile Boscheron, Sourav Maity, Nicola de Franceschi, Guidenn Sulbaran, Gregory Effantin, Haiyan Wang, Jean-Philippe Kleman, Patricia Bassereau, et al.

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1 **Structural basis of CHMP2A-CHMP3 ESCRT-III polymer assembly and membrane**  
2 **cleavage**

3  
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## 28 **Abstract**

29 The endosomal sorting complex required for transport (ESCRT) is a highly conserved multi-protein  
30 complex machinery that drives a diverse set of physiological and pathological membrane  
31 remodeling processes. Membrane remodeling is essentially catalyzed by ESCRT-III filaments  
32 adopting different geometries that stabilize either negatively or positively curved membranes <sup>1</sup>.  
33 The filaments are remodeled and ultimately recycled by the AAA-type ATPase VPS4, which sets  
34 the stage for membrane fission <sup>2-4</sup>. However, the structural basis of ESCRT-III polymers stabilizing,  
35 constricting and cleaving negatively curved membranes is unknown. Here we reconstituted the  
36 ESCRT-III CHMP2A and CHMP3 polymer within narrow membrane tubes. Cryo electron  
37 microscopy structures of the membrane-coated CHMP2A-CHMP3 filaments of two different  
38 diameters at 3.3 and 3.6 Å resolution, respectively, show helical filaments assembled by  
39 CHMP2A-CHMP3 heterodimers in the open ESCRT-III conformation <sup>5</sup>. Polymerization via helical  
40 hairpin stacking generates a partially positive charged membrane interaction surface, positions  
41 short N-terminal motifs for membrane interaction and the C-terminal VPS4 target sequence  
42 towards the tube interior. Inter-filament interactions are electrostatic, which facilitate filament  
43 sliding upon VPS4-mediated polymer remodeling. Fluorescence microscopy as well as high speed  
44 atomic force microscopy imaging corroborate that VPS4 and CHMP2A-CHMP3 polymers can  
45 constrict and cleave narrow membrane tubes, thus acting as a minimal membrane fission  
46 machinery resembling the ancestral ESCRT-III-VPS4 function.

47

## 48 **Main**

49 The endosomal sorting complex required for transport (ESCRT) machinery catalyzes  
50 many divergent membrane remodeling processes including the formation of multivesicular  
51 endosomes, cytokinesis, nuclear envelope reformation, membrane repair, autophagy, exosome  
52 biogenesis, neuronal pruning, dendritic spine maintenance, enveloped virus budding, release of  
53 peroxisomes and of recycling endosomes <sup>6-14</sup>. Common to all ESCRT-catalyzed processes in  
54 eukaryotes, archaea and bacteria is the recruitment of ESCRT-III proteins that polymerize to  
55 generate and/or to stabilize membranes with either flat, negatively or positively curved geometries  
56 <sup>1-3,15-19</sup>. The principal function of the polymers is to induce membrane constriction via outside-in  
57 fission of tubular structures or inside-out fission from within membrane neck structures <sup>1-4,20,21</sup>.

58 Humans express eight ESCRT-III proteins that are in a closed conformation in the cytosol  
59 <sup>22-24</sup>. Membrane recruitment is thought to activate ESCRT-III entailing opening up of the small  
60 helical assembly <sup>25-27</sup>, which leads to an extended polymer-competent open ESCRT-III  
61 conformation <sup>5,28,29</sup> that is conserved across all kingdoms of life <sup>17-19</sup>. While most of these structures

62 demonstrate filament formation to stabilize positively curved membranes<sup>5,17-19</sup> only low resolution  
63 models for filaments stabilizing negatively curved membranes have been imaged revealing single  
64 and multi-stranded polymers *in vitro*<sup>15,16,23,30-36</sup> and *in vivo*<sup>35,37-42</sup>. Notably CHMP3 copolymerizes  
65 with CHMP2A into helical tubular structures with constant diameters of +/- 50 nm *in vitro*<sup>30,43</sup>,  
66 which are remodeled and cleaved in the absence of membrane by VPS4<sup>44</sup>.

67 Many ESCRT processes act on negatively curved membranes to catalyze inside-out  
68 membrane fission. Here, we determined the structural basis of a late acting ESCRT-III polymer  
69 stabilizing negatively curved membranes by cryo electron microscopy, which shows how the  
70 filaments can slide upon VPS4 remodeling to induce constriction and membrane fission.

71

### 72 **Structure of the CHMP2A-CHMP3 polymer assembled within membrane tubes**

73 CHMP3 full length (residues 1-222) and C-terminally truncated CHMP2A (residues 1-164) were  
74 assembled into helical tubular structures as described<sup>30</sup>. After removal of the N-terminal tags, the  
75 tubular structures were coated with lipid bilayer, which tightly associated with the protein layer as  
76 shown by cryo electron microscopy (cryo-EM) (**Extended Data Fig.1A**). 2D classification of the  
77 manually picked tube-like structures generated a dataset composed of 5 different diameters  
78 ranging from 380 Å to 490 Å, with the 410 Å (51.4%) and 430 Å (31.2%) diameters representing  
79 the most populated classes (**Extended Data Fig.1B**). The power spectra of the segments of the  
80 class averages of the two diameters (**Extended Data Fig. 1C and D**) were then employed to  
81 explore possible helical symmetries and combined with helical real-space reconstruction<sup>45</sup> to  
82 validate the symmetry parameters. This revealed that the 410 Å and 430 Å diameter tubes are  
83 formed by elementary helices composed of respectively 6.3 and 6.6 units per turn with a small  
84 pitch of 9 Å for the 410 Å diameter and 18 Å for the 430 Å diameter. The latter displays an  
85 additional C2 symmetry around the helical axis, explaining the doubling of the pitch (**Extended**  
86 **Data Fig.1C and D**). Although, the asymmetric units along the elementary helix are not  
87 biochemically connected, they translate into filaments with extended interaction surfaces between  
88 subsequent asymmetric units. The symmetry parameters of the filaments are relatively similar for  
89 both diameters (Rise/Twist of 8.227 Å /16.877° and 8.641 Å /17.701° for the 410 Å and 430 Å  
90 diameters, respectively), and therefore likely represent the preferred polymerization mode of the  
91 repeating unit, the CHMP2A-CHMP3 heterodimers (**Extended Data Fig.1C and D**).

92 Six filaments (2\*3 in the case of the C2 symmetric helix) form left-handed six-start helices  
93 with helical pitches of ~175 Å for both diameters (**Figures 1A and B**). The 430 Å filament is  
94 composed of 21.33 units per turn (**Figures 1A and B**) and the 410 Å filament contains 20.33 units  
95 per turn (**Figures 1C and D**), indicating a helical repeat after three turns and similar inter-filament

96 interactions along the helical axis. Comparison of the 430 and 410 Å demonstrates that removal  
97 of one heterodimer reduces the tube diameter by 20 Å.

98 The three-dimensional (3D) helical reconstruction shows an overall resolution of 3.6 Å for  
99 the 410 Å and 3.3 Å for the 430 Å diameter (**Figure 1 and Extended Data Fig. 2**) with local  
100 resolutions ranging from 3.3 Å to 4.6 Å for the 430 Å diameter and 3.6 to 5.2 for the 410 Å diameter  
101 (**Extended Data Fig. S3A, B and C**). The map of the 430 Å diameter was employed (**Figure 1E**)  
102 to build the atomic model of the repeating unit of the filament, formed by the CHMP2A-CHMP3  
103 heterodimer revealing both protomers in the open ESCRT-III conformation (**Figure**  
104 **1F, Extended Data Fig. 3D, Extended Data Table 1**).

105 Comparison of the closed<sup>22,23</sup> and open CHMP3 conformations showed the conformational  
106 transitions upon CHMP3 activation, which involves extension of the helical hairpin (residues P12-  
107 A101) that is identical in both conformations (r.m.s.d. of 1.082 Å) by a linker and helix 3 (L117).  
108 The following short connection forms an elbow and translates helix 4 by ~10 Å positioning helix 4  
109 in a 140° angle with respect to the hairpin axis. Helix 4 is composed of the closed conformation  
110 helix 4 and most of the disordered linker connecting to helix 5 via a 90° kink at positions M151 to  
111 D152 (**Figure 2A**). The remaining CHMP3 residues 170 to 220 are flexible and disordered in the  
112 structure. Both CHMP3 and CHMP2A open conformations are similar as superposition of their C $\alpha$   
113 atoms revealed an r.m.s.d. of 0.934 Å (**Extended Data Fig.4A**), suggesting that CHMP2A can  
114 fold into the same closed conformation structure as CHMP3.

115 The repeating unit of the filament is the CHMP2A-CHMP3 heterodimer formed by parallel  
116 interaction of their hairpins with the CHMP2A hairpin tip shifted by six helical turns with respect to  
117 CHMP3 (**Figure 2B**). The heterodimer interaction covers 2026 Å<sup>2</sup> of CHMP2A and 1997 Å<sup>2</sup> of  
118 CHMP3 surfaces involving 55 and 51 interface residues, respectively. The structure of the  
119 heterodimer was further confirmed by mutagenesis. Introducing pairs of cysteine demonstrated  
120 that CHMP2A\_D57C together with CHMP3\_S75C and CHMP2A\_N18C together with  
121 CHMP3\_V110C (**Extended Data Fig.5A**) assembled into disulfide-linked heterodimers upon  
122 polymerization into tube-like structures as shown by SDS-PAGE analyses and negative staining  
123 EM (**Extended Data Fig.5B and C**). Furthermore, mutagenesis of CHMP2A-CHMP3 interface  
124 residues (**Extended Data Fig.5D**) prevented polymerization as expected (**Extended Data**  
125 **Fig.5E**). The principle of the heterodimer hairpin stacking is employed to assemble the filament,  
126 which is further stabilized by lateral interactions of CHMP3<sub>i</sub> elbow helix 4 with CHMP2A<sub>i</sub>,  
127 CHMP3<sub>i+1</sub> and CHMP2A<sub>i+1</sub>. In addition, CHMP3<sub>i</sub> helix 5 interacts with the tip of the CHMP3<sub>i+2</sub>  
128 hairpin (**Figure 2B**), as observed in the closed conformation (**Figure 2A**)<sup>22,23</sup>. Similar to CHMP3,  
129 CHMP2A helix 4 exerts the same domain exchange interactions (**Figure 2B**). C $\alpha$  superposition of

130 the open CHMP3 conformation revealed the closest match with the *S. cerevisiae* Snf7 protomer  
131 (**Extended Data Fig.4B**) and considerable differences with CHMP1B, Vipp1, PspA and Vps24<sup>5,17-</sup>  
132 <sup>19,46</sup> (**Extended Data Fig.4C-F**). Notably, different hairpin interactions and orientations of the  
133 helical arms upon polymerization determine the filament geometry that leads to positively curved  
134 membrane interaction by CHMP1B, Vipp1 and PspA<sup>5,17-19</sup> underlining the extensive structural  
135 plasticity of ESCRT-III proteins.

### 136 137 **CHMP2A-CHMP3 polymer interaction with membrane**

138 The CHMP2A-CHMP3 polymer is tightly associated with the lipid bilayer (**Extended Data Fig.1E**)  
139 and both CHMP2A and CHMP3 expose the same regions to the membrane. The polymerization  
140 mode positions the N-terminal regions of both CHMP2A and CHMP3 at the membrane interface.  
141 Although CHMP3 residues 1-10 and CHMP2A residues 1- 7 are disordered, they are both oriented  
142 by conserved prolines towards the lipid bilayer (**Figure 3A**) consistent with previous suggestions  
143 that short amphipathic N-terminal helices insert into the bilayer<sup>38,47</sup>. The main membrane  
144 interaction surfaces locate to the elbow formed by helices 3 and 4 (residues K104 to R131)  
145 exposing six basic residues of CHMP2A (K104, K108, R115, K118, K124, R131) and five basic  
146 residues of CHMP3 (K106, K112, K119, K132, K136) prone to interact with negative charges of  
147 the membrane (**Figure 3B**). The electrostatic potential map shows in addition to the stretch of  
148 basic surfaces some negative and non-charged regions of the outer polymer surface (**Figure 3C**).  
149 Most of the basic residues are conserved in *S. cerevisiae* Vps2 and Vps24 (**Extended Data**  
150 **Fig.7A**). Notably, alanine mutagenesis of some CHMP3 basic residues within the membrane  
151 interaction surface did not interfere with CHMP2A-CHMP3 polymerization *in vitro* (**Extended Data**  
152 **Fig.6A**) nor did they affect the dominant negative effect of C-terminally truncated CHMP3 on VLP  
153 release (**Extended Data Fig.6B**), indicating that membrane binding is complex and not only  
154 electrostatic, consistent with plasma membrane localization of the CHMP3 mutant (**Extended**  
155 **Data Fig.6C**).

### 156 157 **Inter-filament interactions**

158 Conserved basic helix 1 residues of CHMP2A and CHMP3 (**Extended Data Fig.7A**) are at the  
159 filament interface opposed by a stretch of conserved acidic residues within helices 4 and 5  
160 (**Extended Data Fig.7A**) of neighboring filaments (**Figure 3D**), which indicate electrostatic inter-  
161 filament interactions (**Figure 3C**). Mutation of the helix 1 basic cluster within either CHMP2A or  
162 CHMP3 prevented polymer formation *in vitro* (**Extended Data Fig.6D**), indicating that the basic  
163 charge of helix 1 is important for filament polymerization, which is in line with mutagenesis of a

164 similar cluster of basic residues within helix 1 of CHMP3 abolishing its dominant negative effect  
165 on HIV-1 budding <sup>22</sup>. To further test the electrostatic inter-filament interactions, we exposed the  
166 helical tubular CHMP2A-CHMP3 polymers to high ionic strength. This led to the partial unwinding  
167 of the filaments producing single and multi-stranded filaments (**Extended Data Fig.8A-E**) in  
168 agreement with the presence of single and multi-start helices upon CHMP2A-CHMP3  
169 polymerization *in vitro* <sup>43</sup>. We suggest that these electrostatic interactions between filaments  
170 enable filament sliding upon VPS4-catalyzed remodeling. The acidic cluster in helices 4 and 5 is  
171 conserved in CHMP4A, B, C, CHMP5 and CHMP6 (**Extended Data Fig.7B**) indicating potential  
172 similar involvement in inter-filament interaction for the formation of mixed filaments. Notably, the  
173 acidic cluster is not conserved in CHMP1A and B, which stabilizes positively curved membranes  
174 via basic charges present on the inside of the protein tube-like polymer <sup>5</sup>. We therefore suggest  
175 that the acidic cluster is a hallmark of ESCRT-III stabilizing negatively curved membrane  
176 structures.

177

#### 178 **VPS4 remodels and cleaves CHMP2A-CHMP3 membrane tubes**

179 We next tested whether VPS4B can remodel the CHMP2A-CHMP3 membrane coated tubes as  
180 we have shown before for CHMP2A-CHMP3 tubes without membrane <sup>44</sup>. When we incubated  
181 CHMP2-CHMP3 and VPS4B containing membrane tubes (**Extended Data Fig.9A**) with ATP and  
182 Mg<sup>2+</sup>, complete disassembly of the tubes was observed (**Extended Data Fig.9B**). In order to  
183 image tube remodeling by fluorescence microscopy, VPS4B and caged ATP were incorporated  
184 into the tubes wrapped with fluorescently labelled membrane. Imaging of tubes containing either  
185 only caged ATP (**Figures 4A and B; movie 1; Extended Data Fig.10A and B; movie 2**) or only  
186 VPS4B (**Figures 4C and D; movie 3**) demonstrated that photolysis used to uncage ATP did not  
187 affect the tube structure. However, tubes containing both caged ATP and VPS4B revealed  
188 constriction and tube cleavage upon ATP activation at different sites starting at 30s leading to  
189 complete disassembly within 270s (**Figure 4E and F; movie 4**) or starting at 69s (**Extended Data**  
190 **Fig.10C and D; movie 5**) or 50 s (**movie 6**).

191 Tube cleavage was further confirmed by high-speed AFM (HS-AFM) imaging. First,  
192 CHMP2A-CHMP3 tubes with and without membrane were imaged (**Extended Data Fig.11A-C**).  
193 A comparative height histogram showed an increase in tube height of ~8 nm for the membrane  
194 coated tubes (**Extended Data Fig.11D**), as expected for an unilamellar membrane coating. Next,  
195 membrane-coated tubes loaded with 10 μM caged ATP with and without UV exposure (**Extended**  
196 **Data Fig.11E; movie 7**) were recorded by HS-AFM. In the absence of VPS4B, photolysis of the  
197 caged ATP did not induce changes in tube morphology, consistent with the observations using

198 fluorescence microscopy. Further, no changes in tube morphology were observed without UV  
199 exposure for an extended period of time of CHMP2A-CHMP3 tubes coated with membrane and  
200 loaded with VPS4B and caged ATP (**Figure 5A, movie 8**). However, upon UV exposure,  
201 constriction and cleavage of the membrane coated tubes was observed (**Figure 5B; movie 9**).  
202 Kymographs along the tube cross section (**Figure 5C**) and the evolution of the height at the  
203 constriction sites over time (**Figure 5D**) reveal that complete cleavage of the membrane tube  
204 occurs within a time period of ~ 200 s of UV exposure. We conclude that VPS4B can constrict the  
205 CHMP2A-CHMP3 filaments bound to membranes that leads to membrane cleavage, reminiscent  
206 of membrane fission.

207

## 208 **Discussion**

209 The structure of the CHMP2A-CHMP3 heteropolymer demonstrates how ESCRT-III  
210 filaments polymerize into rigid structures that can stabilize and/or shape negatively curved  
211 membrane necks with diameters of approximately 50 nm. Such membrane structures are present  
212 at many ESCRT-catalyzed processes including vesicle and virus budding or at later stages during  
213 cytokinetic midbody constriction<sup>2,48</sup>. Although the structural principles of the open ESCRT-III  
214 conformation are highly conserved between CHMP2A/CHMP3 and CHMP1B<sup>5</sup>, Snf7/Shrub  
215 (CHMP4)<sup>28,29</sup> and bacterial PspA and Vipp1<sup>17-19</sup>, differences in helical hairpin stacking and  
216 orientations of the helical arms dictates the geometry of the filaments that stabilize positively  
217 curved membrane or negatively curved membrane as in case of CHMP2A-CHMP3 polymerization.  
218 Furthermore, assembly modes of ESCRT-III monomers can vary as shown for CHMP2A, CHMP3  
219 and CHMP4B, which can yet adapt another filament geometry that stabilizes positively curved  
220 membranes<sup>15,16</sup>. Thus the plastic nature of ESCRT-III protein conformations can lead to variable  
221 ESCRT-III filament geometries that can adapt a wide range of curvatures to accommodate ESCRT  
222 function in different membrane remodeling processes<sup>1</sup>.

223 A striking feature of the CHMP2A-CHMP3 polymer is the narrow range of tube diameters,  
224 which is consistent with their late recruitment to catalyze last steps in membrane constriction.  
225 During MVB biogenesis, *S. cerevisiae* ESCRT-III assembles first Vps20/Snf7 (CHMP6/CHMP4)  
226 which in turn recruits Vps24/Vps2 (CHMP3/CHMP2)<sup>49</sup>, which were suggested to block Snf7  
227 (CHMP4) polymerization by capping ESCRT-III assembly prior to recycling<sup>50,51</sup>. Likewise, CHMP4  
228 isoforms recruit CHMP3 and CHMP2A to HIV-1 budding sites<sup>52</sup> consistent with live cell imaging  
229 of Gag and ESCRT-III recruitment<sup>53,54</sup>. The narrow range of diameters of the CHMP2A-CHMP3  
230 polymer structure suggests that recruitment of CHMP3 and CHMP2A molds membrane necks into  
231 approximately 40 to 50 nm diameters, which sets the stage for further constriction. Comparison of



232 the 410 and 430 Å wide structures shows that removal of only one CHMP2A-CHMP3 heterodimer  
233 per turn reduces the tube diameter by 20 Å, indicating how a stepwise removal of heterodimers  
234 can successively induce membrane constriction.

235 Another feature of the tubular polymer is that it can assemble from single or multi-stranded  
236 filaments<sup>43</sup> the latter being the preferred assembly *in vitro*. Interaction between filaments is driven  
237 by complementary positive and negative charges. However, surprisingly, high ionic strength did  
238 not disassemble the tube into individual filaments but preserved the multi-strand architecture. The  
239 17.5 nm width of the six-stranded helix present in the structure fits the 17 nm wide helices imaged  
240 at the midbody<sup>40</sup>, suggesting that such spirals contain six ESCRT-III filaments. Since inter-  
241 filament interactions are electrostatic, different ESCRT-III filaments may contribute to the  
242 formation of mixed multi-stranded filaments<sup>35,55</sup>. In line, acidic residues within helix 4 and basic  
243 residues within helix 1 have been implicated in *S. cerevisiae* Snf7-Vps24 interaction<sup>56</sup>. Because  
244 basic and acidic charges within these regions are conserved in CHMP6, CHMP4A, B, C, CHMP2B  
245 and CHMP5, filaments thereof may also form side by side via homo- or hetero-filament assembly.  
246 The loose electrostatic inter-filament interactions likely facilitate sliding of filaments upon ESCRT-  
247 III filament remodeling by VPS4, which catalyzes filament constriction prior to complete  
248 disassembly<sup>44</sup>. Notably, dynamic VPS4-mediated turnover of ESCRT-III has been proposed in  
249 different membrane remodeling processes<sup>35,57,58</sup>.

250 CHMP2A-CHMP3 polymers have been suggested to interact with negatively charged  
251 membranes<sup>59-61</sup>, which is confirmed by the cluster of basic residues within the membrane  
252 interaction surface. Furthermore, the structure indicates that short N-terminal hydrophobic motifs,  
253 implicated in ESCRT-III function<sup>38 47</sup> are positioned to insert into the membrane. Although this N-  
254 terminal motif is helical in a filament structure assembled by an intermediate Vps24 (CHMP3)  
255 conformation<sup>46</sup>, the corresponding helices of CHMP2A and CHMP3 are not visible in the  
256 membrane-bound structure suggesting that the putative amphipathic helices can adopt different  
257 membrane insertion angles. Polymer interaction with membrane is tight, which excludes other  
258 membrane proteins thereby serving as a diffusion barrier<sup>61</sup>.

259 CHMP3 is dispensable for HIV-1 budding<sup>52</sup> and *S. cerevisiae* Vps24 can be substituted  
260 by Vps2 overexpression restoring partial endosomal cargo sorting<sup>62</sup>. Both processes depend on  
261 CHMP2A and Vps2 interaction with CHMP4 or Snf7. The structure suggests that CHMP3 can be  
262 structurally replaced by CHMP2A in the polymer, indicating that CHMP2A filaments on their own  
263 may form similar helices. Although CHMP2A can polymerize into circular filaments with  
264 approximate diameters of 40 nm that often coil up, no regular CHMP2A tube-like structures have  
265 been yet imaged *in vitro*<sup>43</sup>.

266 VPS4B remodels CHMP2A-CHMP3 helical tubular structures *in vitro*<sup>30,63</sup> inducing filament  
267 constriction and cleavage that generates dome-like end caps prior to complete disassembly, which  
268 was proposed to drive membrane fission<sup>44,64</sup>. Here, we show that VPS4B constricts and cleaves  
269 CHMP2A-CHMP3 membrane-coated tubes via membrane fission likely via the formation of dome-  
270 like end-caps<sup>44</sup>. Cleavage of membrane tubes pulled from GUVs has been reported previously  
271 by employing a minimal system composed of *S. cerevisiae* Snf7, Vps24, Vps2 and Vps4<sup>65</sup>, while  
272 another model proposed sequential recruitment of *S. cerevisiae* Snf7, followed by Vps2-Vps24,  
273 Vps2-Did2 and Did2-Ist1 for final constriction<sup>55</sup>. Our data suggests that CHMP2A-CHMP3  
274 filaments constitute together with VPS4 a minimal ESCRT-III membrane fission machinery that  
275 can constrict membrane necks with 40 to 50 nm large diameters to the point of fission. *In vivo*,  
276 CHMP2A and CHMP3 are recruited by CHMP4 polymers, which nucleate CHMP2A-CHMP3  
277 polymerization<sup>50, 52</sup>. It is yet unclear how many helical turns are required for constriction *in vivo*,  
278 although more than one, as estimated from imaging<sup>58</sup>, would allow filament sliding powered by  
279 ATP-driven forces that can drive filament-induced membrane constriction and cleavage. Finally,  
280 catalyzing membrane fission with a minimal machinery is well in line with ancestral ESCRT-III  
281 function<sup>66,67</sup>.

282

283

## 284 **Methods**

285

### 286 **Expression and purification**

287 CHMP2A $\Delta$ C containing residues 1 to 161 was subcloned in a pMAL-c5X vector with an additional  
288 TEV site at the amino terminal end expressed as a MBP fusion protein in the C41 (DE3) *E. coli*  
289 bacterial strain (Lucigen). Expression was induced for 1h at 37°C. Bacteria were lysed by  
290 sonication in a buffer containing 50 mM HEPES pH 7.5, 300 mM NaCl, 1 mM DTT, 5 mM EDTA  
291 and protease inhibitors. Cleared lysate was applied onto an amylose resin (New England Biolabs),  
292 washed with buffer A (25mM HEPES 7.5, 150 mM NaCl, 1mM DTT), then with Buffer B (25 mM  
293 HEPES pH 7.5, 1 M NaCl, 1 M KCl, 1mM DTT) followed by a last wash with Buffer A. Finally,  
294 protein was eluted with buffer C (25 mM HEPES 7.5, 150 mM NaCl, 10 mM maltose). The most  
295 concentrated fraction was directly applied to size exclusion chromatography (SEC) Superdex 200  
296 column (GE Healthcare) in buffer D (25 mM HEPES pH 7.5, 150 mM NaCl).

297 Full-length CHMP3 was subcloned in a pProEX-HTb vector (Life Technologies, Thermo Fisher)  
298 and expressed in BL21Gold (DE3) *E. coli* bacterial strain (Agilent). Expression was induced for 3h  
299 at 37°C and purified as described<sup>30</sup> with minor modifications. Bacteria were lysed by sonication

300 in buffer E (25 mM HEPES pH 7.5, 150 mM NaCl, 10mM imidazole) containing protease inhibitors  
301 and the cleared lysate was applied onto a Ni<sup>2+</sup>-chelating sepharose (Cytivia), washed extensively  
302 with lysis buffer E, and subsequently with buffer F (25 mM HEPES pH 7.5, 300 mM NaCl, 300 mM  
303 KCl, 20 mM imidazole) and buffer G (25 mM HEPES pH 7.5, 300 mM NaCl, 300 mM KCl, 50 mM  
304 imidazole). Finally, CHMP3 was eluted with buffer H (25 mM HEPES 7.5, 150 mM NaCl, 350 mM  
305 imidazole) and cleaved overnight at 4°C with Tobacco Etch Virus (TEV) protease at 1:100 (w/w)  
306 ratio in the presence of 10 mM β-mercaptoethanol. Cleaved protein was then applied on a second  
307 Ni<sup>2+</sup>-chelating sepharose in order to remove TEV, the His-tag and uncleaved protein. The final  
308 step included size exclusion chromatography (SEC) on a Superdex 75 column (GE Healthcare)  
309 in buffer D. CHMP3 concentrated at 300 μM was frozen for further use.

310 CHMP2AΔC-mutants containing residues 9 to 161 and CHMP3-mutants containing residues 9 to  
311 183 were synthesized (ThermoFisher), subcloned in a pETM40 vector (PEPcore facility-EMBL  
312 Heidelberg) and the pProEX-HTb (ThermoFisher) vector, respectively. Mutants were expressed  
313 and purified as described above for wild type sequences.

314

315 **CHMP2A-CHMP3 membrane tube generation**

316 For helical tube formation as described previously<sup>30</sup>, 10 μM CHMP2AΔC was mixed with 20 μM  
317 full-length CHMP3 and incubated for 48-72h at 4°C. After incubation, tubes were harvested by  
318 centrifugation at 20,000g for 30min and the pellet containing CHMP2AΔC-CHMP3 tubes was  
319 resuspended in buffer D. In order to wrap the CHMP2AΔC-CHMP3 tubes with a lipid bilayer, the  
320 following lipid film was produced containing 70% Egg phosphatidyl choline (Egg PC), 10% dioleoyl  
321 glycerophosphoserine (DOPS), 10% dioleoyl glycerol phosphoethanolamine (DOPE), 10% brain  
322 phosphatidylinositol-4,5-bisphosphate (PI(4,5)P<sub>2</sub>) and 2μL of dioleoyl-sn-glycero-3-  
323 phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (LISS-Rhodamin PE) (all Avanti Polar  
324 lipids). The lipid film was resuspended in water at a final concentration of 5 mg/mL. The  
325 CHMP2AΔC-CHMP3 tubes (25 μL) were mixed with 25 μL of 2% CHAPS, 25 μL of lipids and 0.1  
326 mg/mL of TEV protease (to remove the MBP from CHMP2AΔC) and incubated at room  
327 temperature for 2h. To remove free lipids/micelles and CHAPS, the tubes were dialyzed twice for  
328 48-72h against buffer I (25mM Tris pH 7.4, 25 mM NaCl, 1 mM β-mercaptoethanol and 0.5 g of  
329 Bio-Beads (Biorad). After dialysis, CHMP2AΔC-CHMP3 tubes wrapped with bilayer were  
330 incubated with Bio-Beads overnight at 4°C and removed by centrifugation. The quality of the  
331 bilayer wrapped CHMP2AΔC-CHMP3 tubes was assessed by negative staining EM prior to cryo  
332 EM data collection, fluorescence microscopy imaging and HS-AFM analysis.

333 To test remodeling by VPS4, CHMP2A-CHMP3 tubes were incubated with 10  $\mu$ M VPS4B, 5  $\mu$ M  
334  $MgCl_2$  and 5  $\mu$ M caged ATP (#A1048 Invitrogen) prior to membrane wrapping, following the  
335 protocol described above. Because deposition of the membrane onto the CHMP2A-CHMP3  
336 protein coat requires extensive dialysis, the final VPS4 concentration present within the tubes can  
337 be only estimated from SDS-PAGE; however, the final concentration of caged ATP inserted into  
338 the tubes cannot be determined.

339

#### 340 **CHMP2A-CHMP3-VPS4 membrane tube imaging**

341 Epifluorescence video microscopy of CHMP2A-CHMP3 membrane tubes containing VPS4B and  
342 caged ATP was performed using an Olympus IX83 optical microscope equipped with a UPFLN  
343 100X O-2PH/1.3 objective and an ORCA-Flash4.0 Digital sCMOS camera (Hamamatsu). A 5  $\mu$ L  
344 aliquot of ESCRT-III tube suspension was spread on a slide, covered with a glass coverslip (#1)  
345 and sealed with twinstil speed 22 (Picodent, ref 13001002) for imaging. Caged-ATP was uncaged  
346 using a 10s 10% 365-nm LED illumination (Figures 4 A-F and movies S1-4) or using at each time  
347 point a 100ms 30% 365-nm LED illumination (Extended Data Fig.10 and movies S4 and S5).  
348 ESCRT tubes were fluorescently imaged using a 550nm LED (10% with an exposure time of  
349 100ms) at 1 frame/s. Images were acquired using the Volocity software package. Images were  
350 analyzed, adjusted, and cropped using ImageJ software.

351

#### 352 **HS-AFM analysis**

353 The AFM images were acquired in amplitude modulation tapping mode in liquid, using high-speed  
354 atomic force microscopes (RIBM, Japan)<sup>44,68</sup>. The HS-AFM imaging was performed using USC-  
355 F1.2-k0.15 cantilevers (NanoWorld, Switzerland), an ultrashort cantilever with a nominal spring  
356 constant of 0.15 N/m and a resonance frequency  $\approx$  0.5 MHz. All HS-AFM recordings were done  
357 at room temperature and in buffer D. Uncaging of the caged ATP was performed by directly  
358 irradiating 365 nm UV light at the AFM sample stage using an optical fiber. The membrane coated  
359 tubes were immobilized at the surface using streptavidin on top of a lipid bilayer (DOPC) on mica  
360 containing 0.01% biotinylated lipid<sup>69</sup>. HS-AFM images were analyzed using Igor Pro, and ImageJ  
361 with additional home written plugins<sup>70</sup>. Height measurements were performed on raw images after  
362 tilt correction.

363

#### 364 **Dominant negative effect CHMP150 wild type and mutants**

365 CHMP3 residues 1–150 wt or mutants were synthesized (ThermoFisher) and cloned into the  
366 pEGFP-N1 vector using restriction sites *Xho*1- *Hind*III. To determine the effect of GFP-CHMP3

367 (1–150) on virus like particle (VLP) production upon HIV-1 Gag expression, 293T cells were seeded  
368 into 10mm dishes and transfected 24 hr later using a Jetprime (Polyplus) technique. The cultures  
369 were cotransfected with 0.5 µg of Rev-independent HIV-1 Gag construct and with 2 µg of either  
370 pcDNA or wild type and mutant versions of GFP-CHMP3(1–150). Twenty-four hours post  
371 transfection, VLPs released into the culture medium were pelleted through sucrose. HIV-1 Gag  
372 proteins in VLPs and cell lysates were detected by Western blotting with a mouse anti-p24  
373 antibody (183-H12-5C). For live cell imaging cells were seeded in glass bottomed µ-dishes and  
374 co-transfected with 0.8 µg of Rev-independent HIV-1 Gag construct, 0.2 µg of Gag-mCherry <sup>71</sup>  
375 and with 1 µg of either wild type or mutant GFP-CHMP3(1–150). Live cell fluorescent protein  
376 localization was analyzed by spinning disc confocal microscopy 24 hr post transfection in HeLa  
377 cells.

### 378 379 **Cryo-EM sample preparation and data collection**

380 *Cryo-electron microscopy.* 3.5 µL of sample were applied to glow discharged (45s 30 mA) 1.2/1.3  
381 Ultrafoil holey grids (Quantifoil Micro Tools GmbH, Germany) and they were plunged frozen in  
382 liquid ethane with a Vitrobot Mark IV (Thermo Fisher Scientific) (100% humidity, temperature 20°C,  
383 6 s blot time, blot force 0). The grids were pre-screened on the 200kV Glacios electron microscope  
384 (Thermo Fischer Scientific) at the IBS (Grenoble) and data were collected at the beamline CM01  
385 of the ESRF (Grenoble, France) <sup>72</sup> on a Titan Krios G3 (Thermo Fischer Scientific) at 300 kV  
386 equipped with an energy filter (Bioquantum LS/967, Gatan Inc, USA) (slit width of 20 eV). 5028  
387 movies were recorded automatically on a K2 summit direct detector (Gatan Inc., USA) with EPU  
388 (Thermo Fisher Scientific) for a total exposure time of 5 s and 200 ms per frame resulting in 25  
389 frame movies with a total dose of ~24 e<sup>-</sup>/Å<sup>2</sup>. The magnification was 130,000x (1.052 Å/pixel at the  
390 camera level). The defocus of the images was adjusted between -0.5 and -1.5 µm in 0.2 µm  
391 steps. For the high ionic strength unwinding of the CHMP2A-CHMPA filament the same grid and  
392 freezing conditions have been used as described above and images have been recorded on the  
393 Glacios electron microscope using a K2 direct electron detector.

### 394 395 **EM image analysis and 3D reconstructions**

396 The workflow of the image analysis is shown in **Extended Data Fig.2**. Electron beam-induced  
397 sample motion on the recorded movie frames was corrected using MotionCor2 <sup>73</sup> and the contrast  
398 transfer function (CTF) was estimated with CTFFIND4 <sup>74</sup>. 9,207 filaments were manually picked  
399 from 5,027 micrographs using the EMAN2 program e2helixboxer.py <sup>75</sup>. All subsequent data  
400 processing steps were carried out in RELION3 <sup>76,77</sup> unless mentioned otherwise. Initially, 89,122

401 overlapping segments were extracted with ~90% overlap between boxes of 768 x 768 pixels and  
402 downsampled to a pixel size of 2.104 for initial classification steps. Several rounds of 2D  
403 classification resulted in class averages that could be classified into 5 main different groups based  
404 on the filament diameter, without membrane (380 Å diameter (7.4%), 410 Å diameter (51.4%),  
405 430 Å diameter (31.2%), 470 Å diameter (1.9%) and 490 Å diameter (0.3%)). In order to  
406 compensate for potential mis-assignment of diameters to the segments due to inaccuracies in 2D  
407 classification, we assigned to each entire tube a diameter based on the class assignment of the  
408 corresponding segments. If more than 80% of segments of a particular tube were belonging to  
409 classes assigned to a particular diameter, this entire tube would be assigned this diameter for the  
410 subsequent steps. After re-extraction of segments with ~95% overlap, another round of 2D  
411 classification was performed with the for each diameter group. Most populated 2D classes with  
412 filament diameter of 430 Å and 410 Å were chosen for further processing and analysis. For  
413 determination of helical symmetry, the sum of power spectra from a smaller subset (1,904 and  
414 3,993 segments from one 2D class each for 430 Å and 410 Å respectively) was calculated for both  
415 filament groups. The resulting average power spectrum (**Extended Data Fig.1C and D**) was  
416 analyzed for estimation of helical symmetry parameters using the web tool helixplorer  
417 (<http://rico.ibs.fr/helixplorer/>). Based on a prior visual inspection of the PS, we made following  
418 hypotheses: the layer line with a maximum seemingly on the meridian could be the helical rise or  
419 the pitch (given the large diameter of the tube and possibilities that selected 2D class averages  
420 contained a number of slightly out-of-plane tilted segments). Given those two hypotheses, and  
421 allowing any cyclic symmetries, we explored possible helical symmetries matching the  
422 experimental PS, giving a list of 20 and 15 symmetries to test for the 430 Å and 410 Å diameter  
423 classes, respectively. Those symmetries were applied on the real-space 2D class-averages using  
424 SPRING program segclassreconstruct.py<sup>78</sup> in order to generate initial models and narrowing down  
425 possible symmetry solutions to 14 and 10, by discarding those giving aberrant density distribution.  
426 Using those initial models, each of the remaining symmetry solutions was tested for 3D refinement  
427 in RELION3, and the resulting maps inspected for high-resolution features such as clear  
428 secondary structures, allowing to determine the helical parameters to be 18 Å pitch, 2.72 Å rise,  
429 54.39° twist, 6.6 units/turn and C2 point symmetry for 430 Å, and 9 Å pitch, 1.43 Å rise, 57° twist,  
430 6.3 units/turn and C1 point symmetry for 410 Å diameter filaments.

431 In order to select a more homogeneous subset of segments, we applied 3D classification and the  
432 classes (containing 25,353 and 11,396 segments for diameters 430 and 410 Å) were chosen for  
433 a final round of 3D auto-refine reconstruction that converged to a 2.74 Å rise and 54.37° twist for  
434 430 Å, and 1.44 Å rise and 57.04° twist for 410 Å diameter filaments. Using soft protein-only

435 masks, the final resolutions were estimated at 3.3 Å and 3.6 Å for the 430 Å and 410 Å diameter  
436 filaments, at the FSC (Fourier shell correlation) 0.143 cutoff criterion<sup>79</sup>. The maps were sharpened  
437 with b-factors of -96.57 Å<sup>2</sup> (430 Å) and -101.52 Å<sup>2</sup> (410 Å). Local resolution was estimated in  
438 RELION3<sup>76</sup> and the density maps were rendered in UCSF Chimera<sup>80</sup>. The statistics of the EM  
439 map are summarized in **Extended Data Table 1**.

440

#### 441 **Atomic modelling and validation**

442 The SWISS-MODEL<sup>81</sup> server was used to create homology models of human CHMP3 and  
443 CHMP2A, using the open conformation of CHMP1B (PDB ID 6TZ4) as a reference model. Helices  
444 with residues 15-52, 57-117 and 120-151 for CHMP3 and 14-51, 56-116 and 119-150 for CHMP2A  
445 were initially fit into the EM density as separate rigid bodies using Chimera and then adjusted in  
446 Coot<sup>82</sup>. Further, the rest of the N-terminal and C-terminal residues, as well as the connecting loops  
447 were manually built and adjusted in Coot. The CHMP2A-CHMP3 heterodimer model was then  
448 expanded by helical symmetry in each direction in order to get 10 such dimers surrounding the  
449 central dimer. Thus, a total of 11 helical symmetry-related dimers were again checked in Coot,  
450 before applying the first round of real-space refinement in PHENIX<sup>83</sup> with non-crystallographic  
451 symmetry (NCS) restraints. NCS, along with SS (secondary structure) restraints were then used  
452 for a second round of real-space refinement. At last, the symmetry-related dimers were removed  
453 and the central CHMP2A-CHMP3 dimer was saved as the final model. The statistics of the final  
454 models were tabulated using MolProbity<sup>84</sup> and are summarized in **Extended Data Table 11** and  
455 map versus atomic model FSC plots were computed in PHENIX<sup>85</sup>. All structure figures were  
456 generated with UCSF Chimera, ChimeraX<sup>86</sup> and PyMOL (W. Delano; The PyMOL Molecular  
457 Graphics System, Version 1.8 Schrödinger, LLC, <http://www.pymol.org>). Sequence alignments  
458 were performed with Clustal Omega<sup>87</sup> and ESPript<sup>88</sup>.

459

#### 460 **Data Availability**

461 CryoEM maps and models were deposited to the PDB and EMDB with the following codes:  
462 membrane-bound CHMP2A-CHMP3, 430 Å diameter (PDB ID 7ZCG, EMD-14630) and  
463 membrane-bound CHMP2A-CHMP3, 410 Å diameter (PDB ID 7ZCH, EMD-14631).

464

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477  
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479 experiments, supervised and received funding for the study. K.A. performed all cryo-EM data  
480 analyses. N.D.F. established the membrane coating protocol. D.G. prepared wild type and mutant  
481 CHMP2A-CHMP3 polymers for all analyses. G.S. performed negative staining EM analyses. C.B.  
482 and J.P.K. performed fluorescence microscopy imaging and H.W. mutant analyses. S.M.  
483 performed AFM analyses and W.H.R. supervised AFM analyses. G.E. and G.S. collected cryoEM  
484 data and A.D. supervised all aspects of cryoEM data analyses, structure solution and  
485 interpretation. W.W. wrote the paper with input from all authors.

486  
487 **Competing interests:** The authors declare no competing interests.

488  
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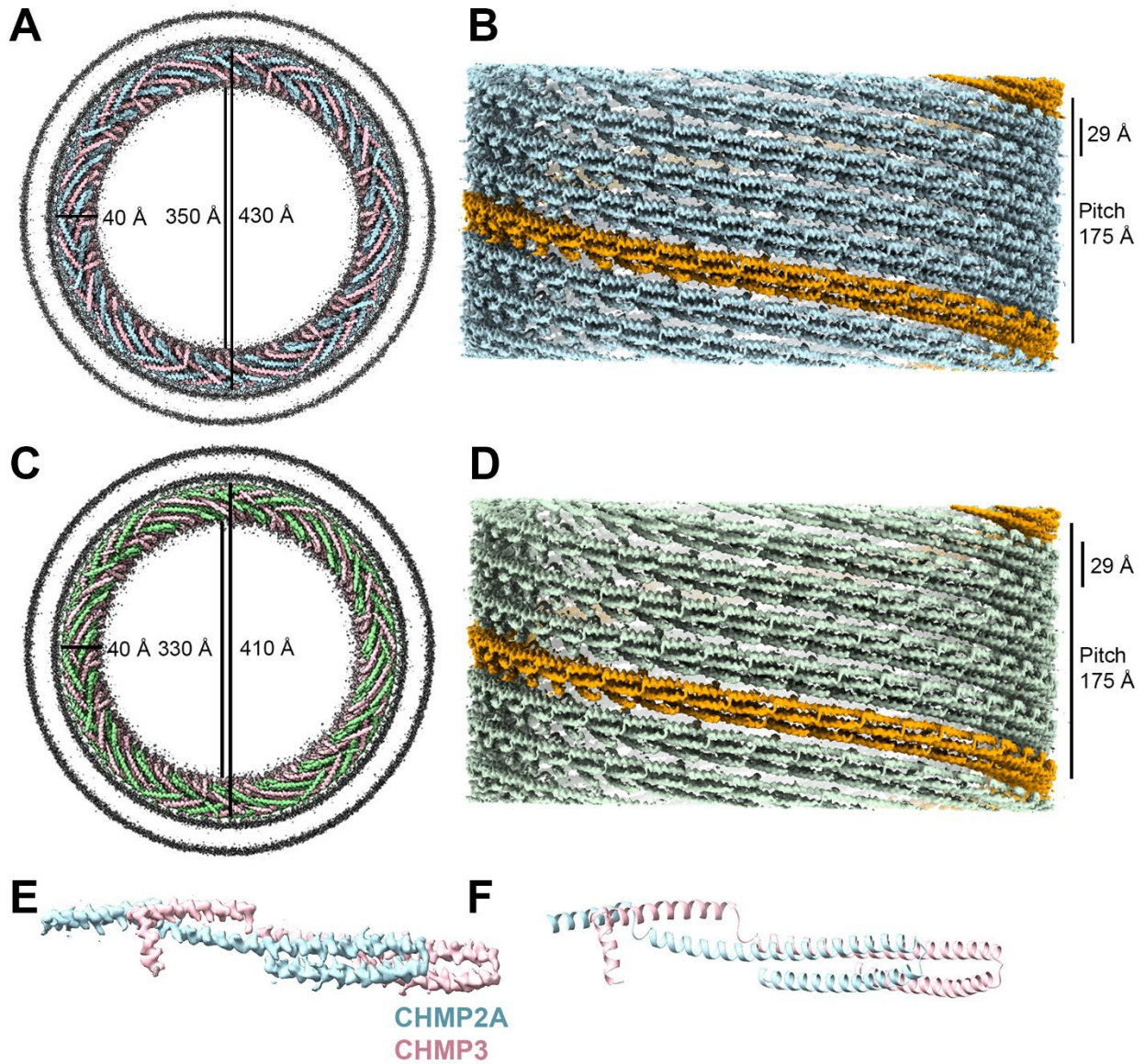
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718 **Figures**

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721 **Figure 1: Cryo-EM structure of CHMP2A-CHMP3 membrane-coated helical polymers**

722 **(A)** Density map of the reconstructed 430 Å diameter CHMP2A-CHMP3 membrane tube with the  
723 top view looking down the helical axis. The helical arrangement of CHMP2A (light blue) and  
724 CHMP3 (pink) inside the bilayer membrane (dark grey) is shown. The thickness, and the inner  
725 and outer diameter of the helical protein tube are also marked in Å.

726 **(B)** Side view of the helical polymer without the lipid membrane. One left-handed filament is  
727 indicated in orange, and the thickness of one filament and the pitch of the helical assembly are  
728 also marked.

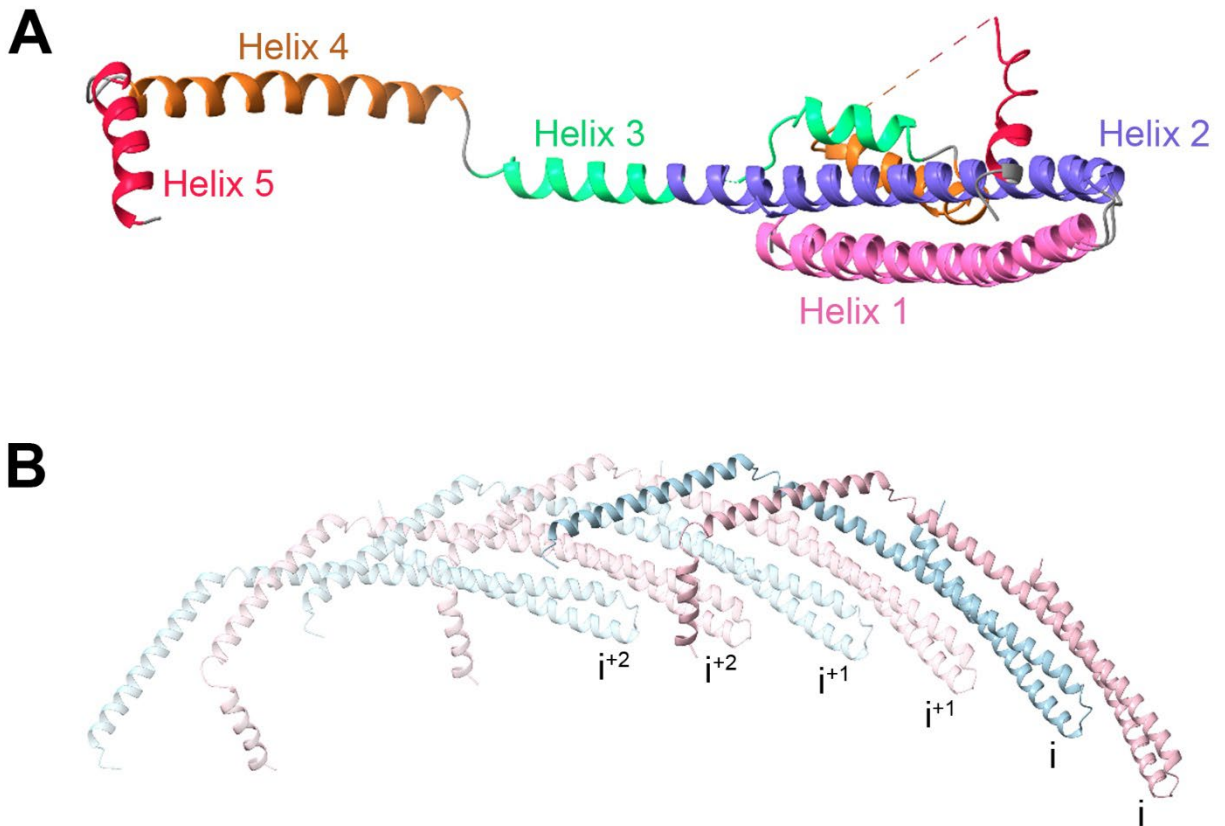
729 **(C)** Density map of the reconstructed 410 Å diameter CHMP2A-CHMP3 membrane tube with the  
730 top view looking down the helical axis. The helical arrangement of CHMP2A (green) and CHMP3  
731 (pink) inside the bilayer membrane (dark grey) is shown. The thickness, and the inner and outer  
732 diameter of the helical protein tube are indicated in Å.

733 **(D)** Side view of the helical polymer without the lipid membrane. One left-handed filament is  
734 indicated in orange, and the thickness of one filament and the pitch of the helical assembly are  
735 indicated.

736 **(E)** Cryo-EM density of the single repeating unit of the 430 Å diameter polymer formed by the  
737 heterodimer of CHMP2A (light blue) and CHMP3 (pink) is indicated.

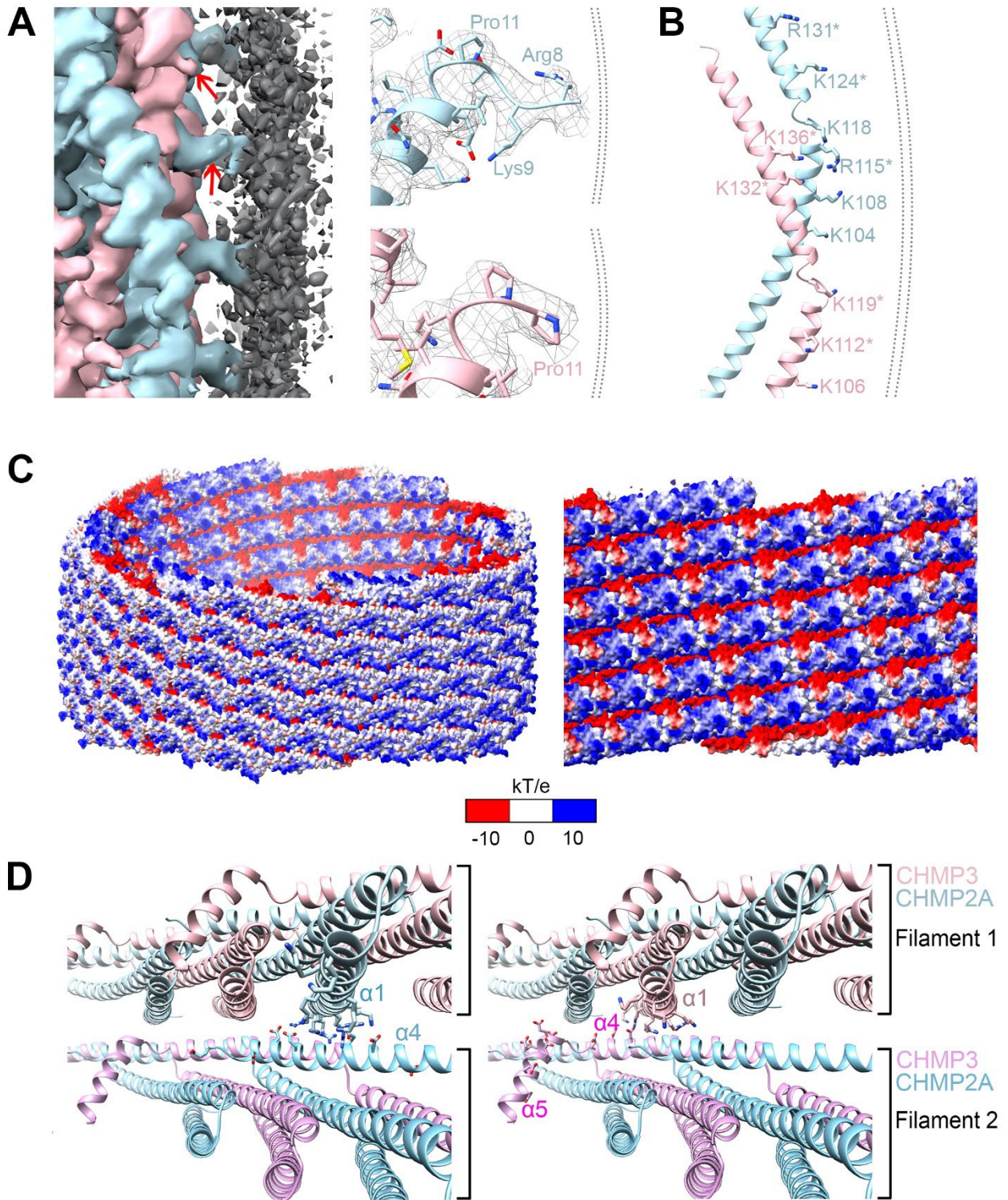
738 **(F)** Ribbon representation of the atomic model of CHMP2A (light blue) - CHMP3 (pink)  
739 heterodimer.

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 742 **Figure 2: Atomic model and architecture of the CHMP2A-CHMP3 helical polymer.**  
 743 **(A)** Ribbon diagram of Ca superposition of the closed and open CHMP3 conformations (Helix 1:  
 744 pink, Helix 2: purple, Helix 3: light green, Helix 4: brown, Helix 5: red).  
 745 **(B)** Three interlocked copies of CHMP2A-CHMP3 heterodimer are shown as ribbons. Helix 4 and  
 746 5 of CHMP3 (pink) interact with four subsequent protomers. Helix 4 of CHMP2A (light blue) also  
 747 makes similar interactions.  
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752 **Figure 3: Membrane interaction of the CHMP2A-CHMP3 filament.**

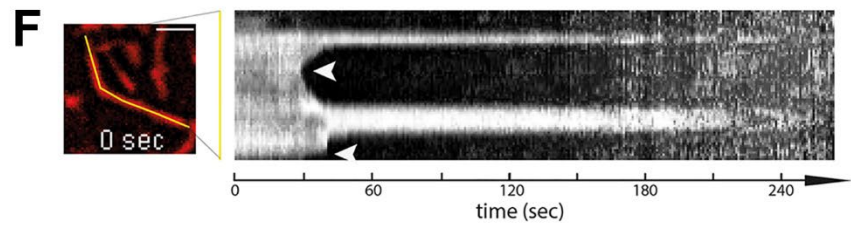
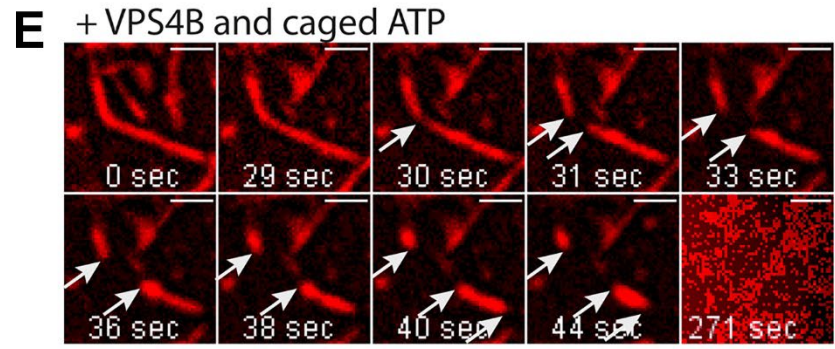
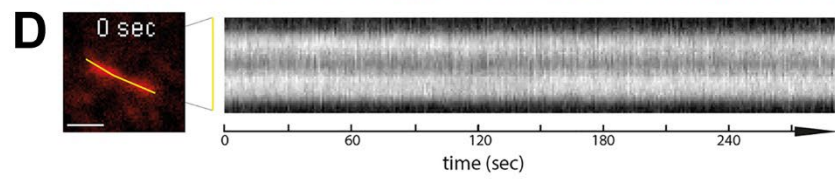
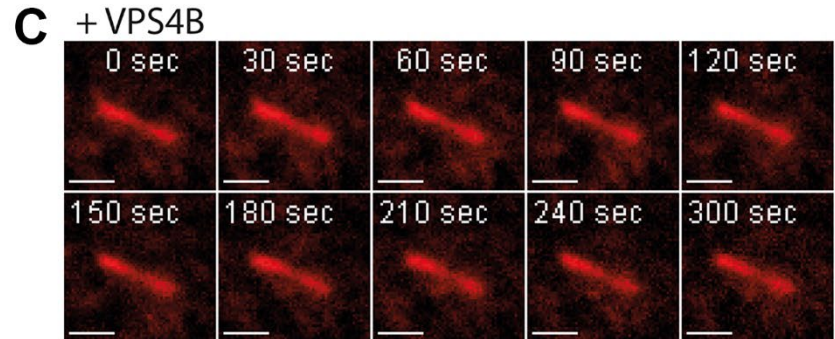
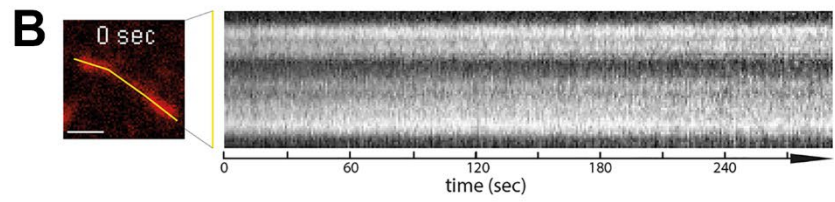
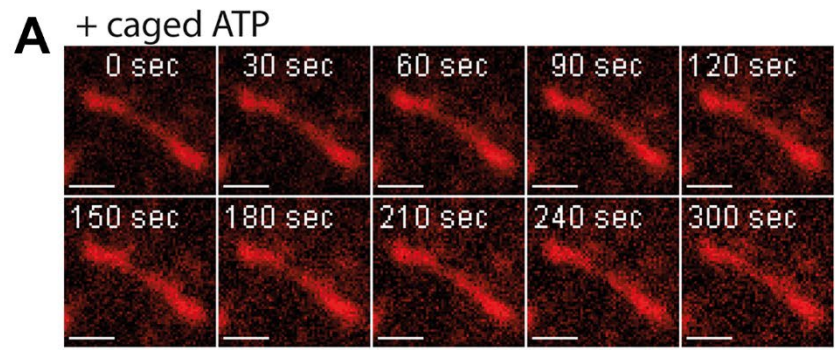
753 **(A)** Left panel, zoomed-in view of the membrane-bound CHMP2A-CHMP3 filament, highlighting  
754 the interface between lipid membrane (dark grey) and CHMP2A (light blue) and CHMP3 (pink).  
755 Red arrows are pointing to the N-termini of both CHMP2A and CHMP3 that are oriented towards  
756 the lipid bilayer. Right panel, the orientation of the N-termini is determined by Pro11 of CHMP2A  
757 (light blue) and CHMP3 (pink) shown with the density map.

758 **(B)** Ribbon diagram of the CHMP2A-CHMP3 heterodimer indicating the basic residues (sticks)  
759 oriented towards the membrane. Basic residues conserved in yeast Vps2 and Vps24 are marked  
760 by asterisks.

761 **(C)** Electrostatic potential map of the CHMP2A-CHMP3 filament (left panel, tilted, side view),  
762 revealing the exposure of the cluster of basic charges, a small negatively charged surface and a  
763 neutral surface to the membrane. Right panel, zoomed-in view of the electrostatic surface of the  
764 inside of the polymer showing clusters of negative charges in one filament juxtaposed to positive  
765 charges of the neighboring filament.

766 **(D)** Close-up of the inter-filament interactions. Ribbon diagram of two neighboring filaments  
767 showing the basic and acidic residues of CHMP2A (left panel) and CHMP3 (right panel) implicated  
768 in electrostatic inter-filament interactions.

769



771 **Figure 4: Imaging of VPS4B and ATP induced cleavage of CHMP2A-CHMP3 membrane**  
772 **coated tubes.**

773 **(A)** A CHMP2A-CHMP3-caged ATP containing membrane coated tube was activated at 365 nm  
774 (10%, 10s) to uncage ATP and imaged over 300s (movie S1).

775 **(B)** The kymograph of the tube shows that the tube stays intact over the imaging time.

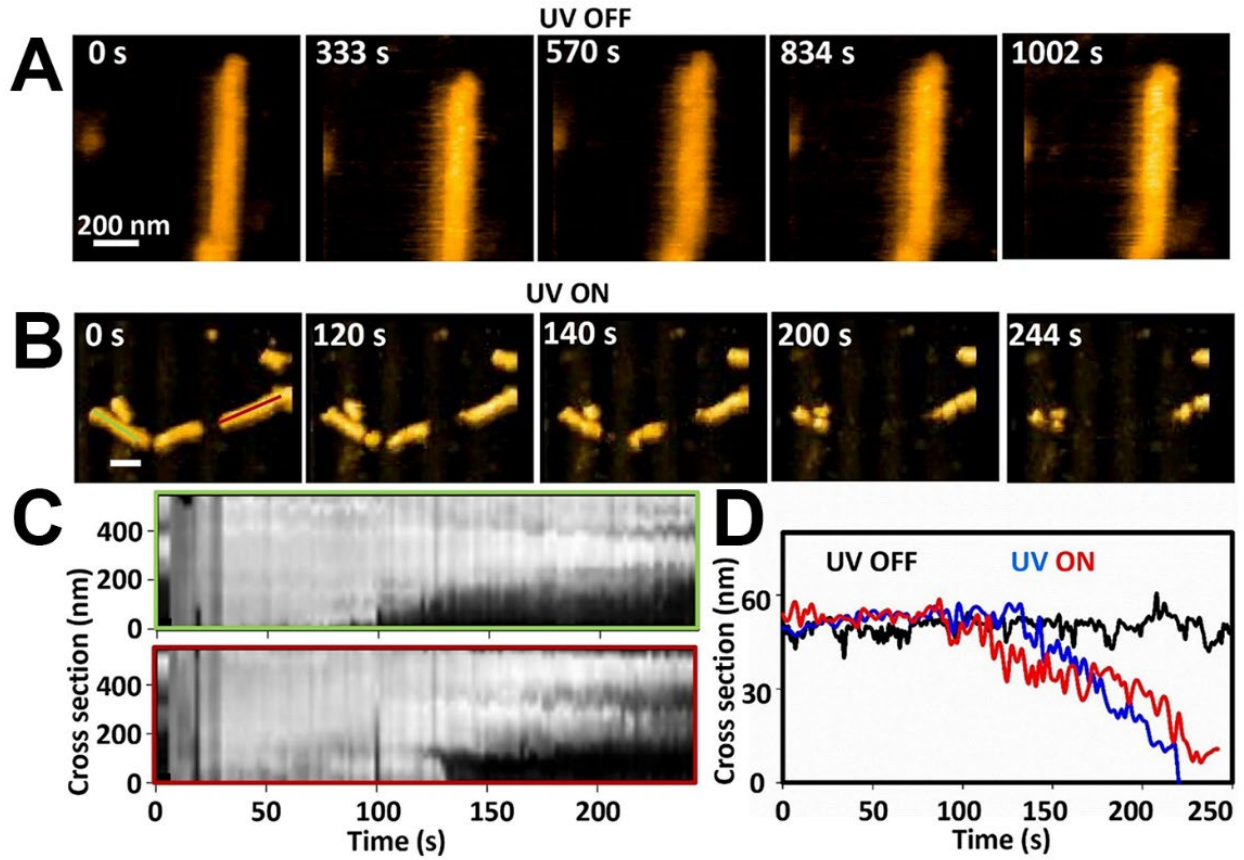
776 **(C)** A CHMP2A-CHMP3-VPS4B containing membrane coated tube was activated at 365 nm (10%,  
777 10s) to uncage ATP and imaged over 300s.

778 **(D)** The kymograph of the tube shows that the tube stays intact over the imaging time. **(A)** to **(D)**  
779 demonstrate that imaging at 550 nm to visualize the membrane tube and ATP uncaging at 365  
780 nm did not change the tube structures (movie S2).

781 **(E)** Imaging of a CHMP2A-CHMP3-VPS4B-caged ATP containing membrane-coated tubes  
782 following ATP uncaging (265 nm, 10%, 10s) reveals constriction and cleavage of the tube at 30  
783 s followed by a shrinking event from both sides. Another shrinking event is observed at 40s.  
784 Eventually all tubes were fully disassembled 271 s (movie S3 and S4). Bar, 1 $\mu$ m.

785 **(F)** The kymograph of the tube indicate the kinetics of cleavage and shrinking.

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 788 **Figure 5. Constriction, cleavage, and disassembly of membrane coated CHMP2A-CHMP3**  
 789 **tube.**

790 **(A)** Snapshots of HS-AFM images of CHMP2A-CHMP3 tubes coated with membrane and loaded  
 791 with 5  $\mu\text{M}$  VPS4B, 10 mM caged ATP, in absence of UV irradiation. Scale bar 200 nm.

792 **(B)** As in A, but upon 365 nm UV irradiation. Scale bar 200 nm.

793 **(C)** Kymograph representation of the height vs time along the two lines in panel B (leftmost image)  
 794 throughout the movie S10.

795 **(D)** Example of height vs time profile of the membrane coated tube in absence of UV irradiation  
 796 (in black), and in presence of UV irradiation (in blue and red).

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**Supplementary data**

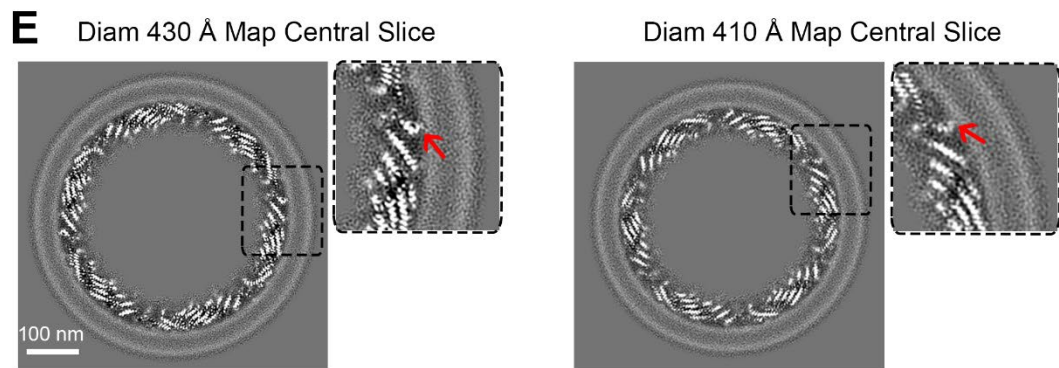
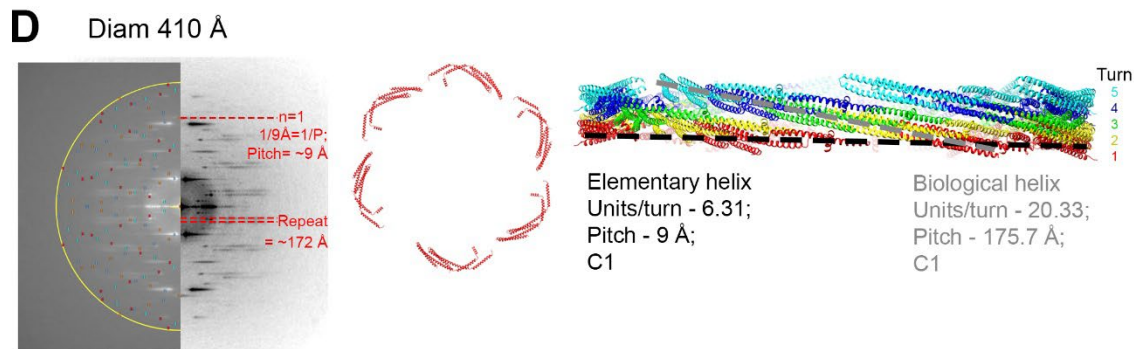
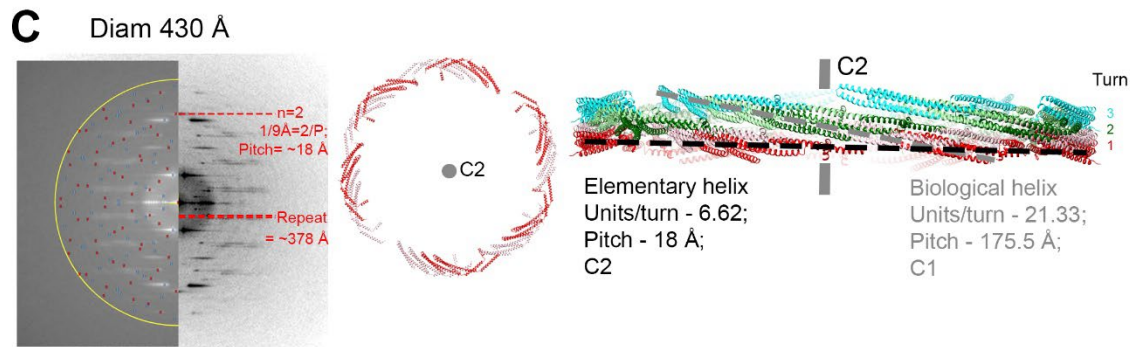
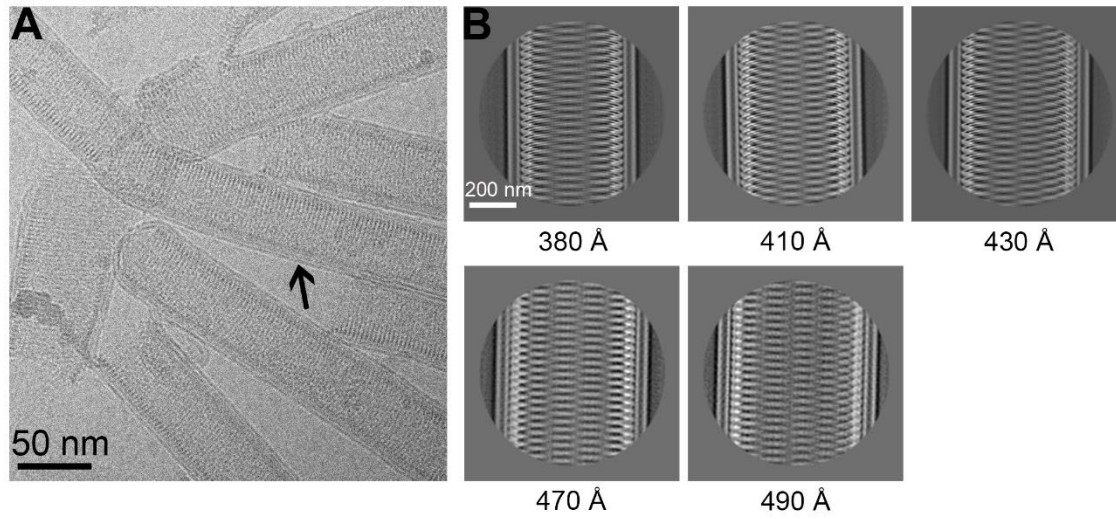
**Structural basis of CHMP2A-CHMP3 ESCRT-III polymer assembly and membrane cleavage**

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Guidenn Sulbaran<sup>1</sup>, Gregory Effantin<sup>1</sup>, Haiyan Wang<sup>1</sup>, Jean-Philippe Kleman<sup>1</sup>, Patricia  
Bassereau<sup>3</sup>, Guy Schoehn<sup>1</sup>, Wouter H Roos<sup>2</sup>, Ambroise Desfosses<sup>1\*</sup>, Winfried Weissenhorn<sup>1\*</sup>

<sup>1</sup> Univ. Grenoble Alpes, CEA, CNRS, Institut de Biologie Structurale (IBS), Grenoble, France.

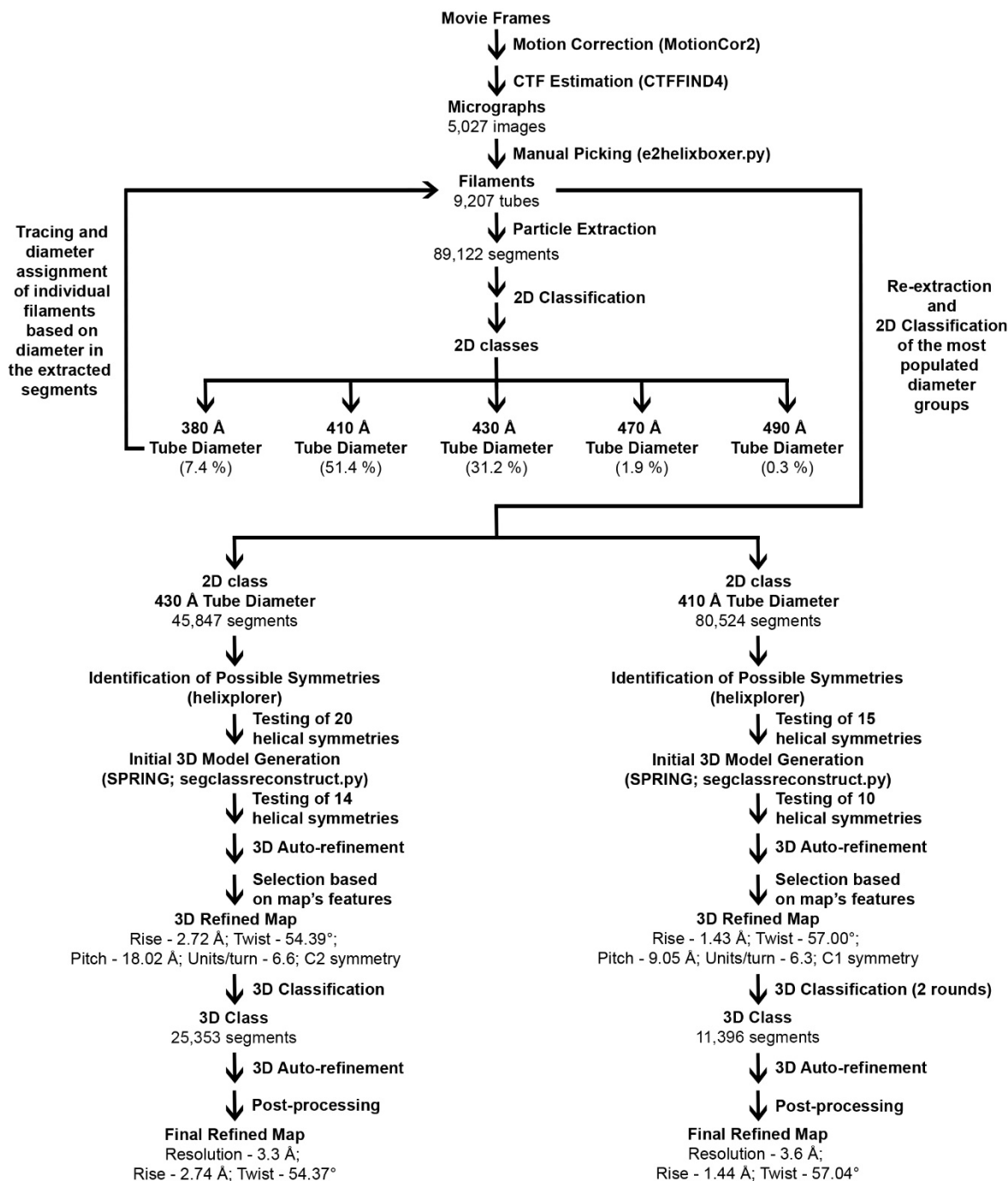
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Paris, France.



814 **Extended Data Fig.1: Cryo-EM data processing of CHMP2A-CHMP3 membrane-coated tubes and**  
815 **helical symmetry analyses**  
816 **(A)** Representative cryo-electron micrograph of CHMP2A-CHMP3 membrane tubes, with an arrow pointing  
817 to the lipid bilayer. Scale bar, 50 nm.  
818 **(B)** Selected 2D class averages of manually picked datasets, arranged according to the tube diameter  
819 ranging from 380 to 490 Å as indicated.  
820 **(C), (D)** Helical symmetry determination and representation of the elementary and biological helices for the  
821 430 Å and 410 Å diameter tubes. Left panel, the sum of the 2D power spectra of segments corresponding  
822 to one class-average show in both cases a maximum on or near the meridian corresponding to the pitch  
823 (Bessel order  $n=1$  for C1 helix;  $n=2$  for C2 helix) instead of the axial rise, due to the large helical diameter  
824 and possible inclusion of slightly out-of-plane tilted segments in the class-average. The left half of the sum  
825 of the power spectra show the calculated position from helixplorer of the two first maxima of each Bessel  
826 function corresponding to the determined symmetry. Middle panel, six asymmetric units (CHMP2A-CHMP3  
827 dimers; in red) of the elementary helix are represented. The symmetry related protomers of the 430 Å  
828 diameter are colored in light red.  
829 **(C), (D)** Right panel, side view of the elementary helix with turns (red, yellow, green, blue and aqua) indicated  
830 with a black dashed line. The grey dashed line follows one turn of the biological helix. The central grey line  
831 (in C) highlights the C2 symmetry axis. Symmetry parameters of both the elementary and biological helices  
832 are indicated.  
833 **(E)** Central slices looking down the helical axis of the cryo-EM 3D reconstructions of the 430 and 410 Å  
834 diameter tubes. The red arrows in the right zoom-in images indicate the density of the N-terminal region  
835 prone to insert into the lipid bilayer.  
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838 **Extended Data Fig.2: Cryo-EM image processing workflow of 430 and 410 Å diameter tubes**

839 **structure determination.**

840 Basic image processing strategy used for helical 3D reconstruction and refinement of 430 and 410 Å

841 diameter tubes is shown. Helical filaments were segmented and classified based on the tube diameter.

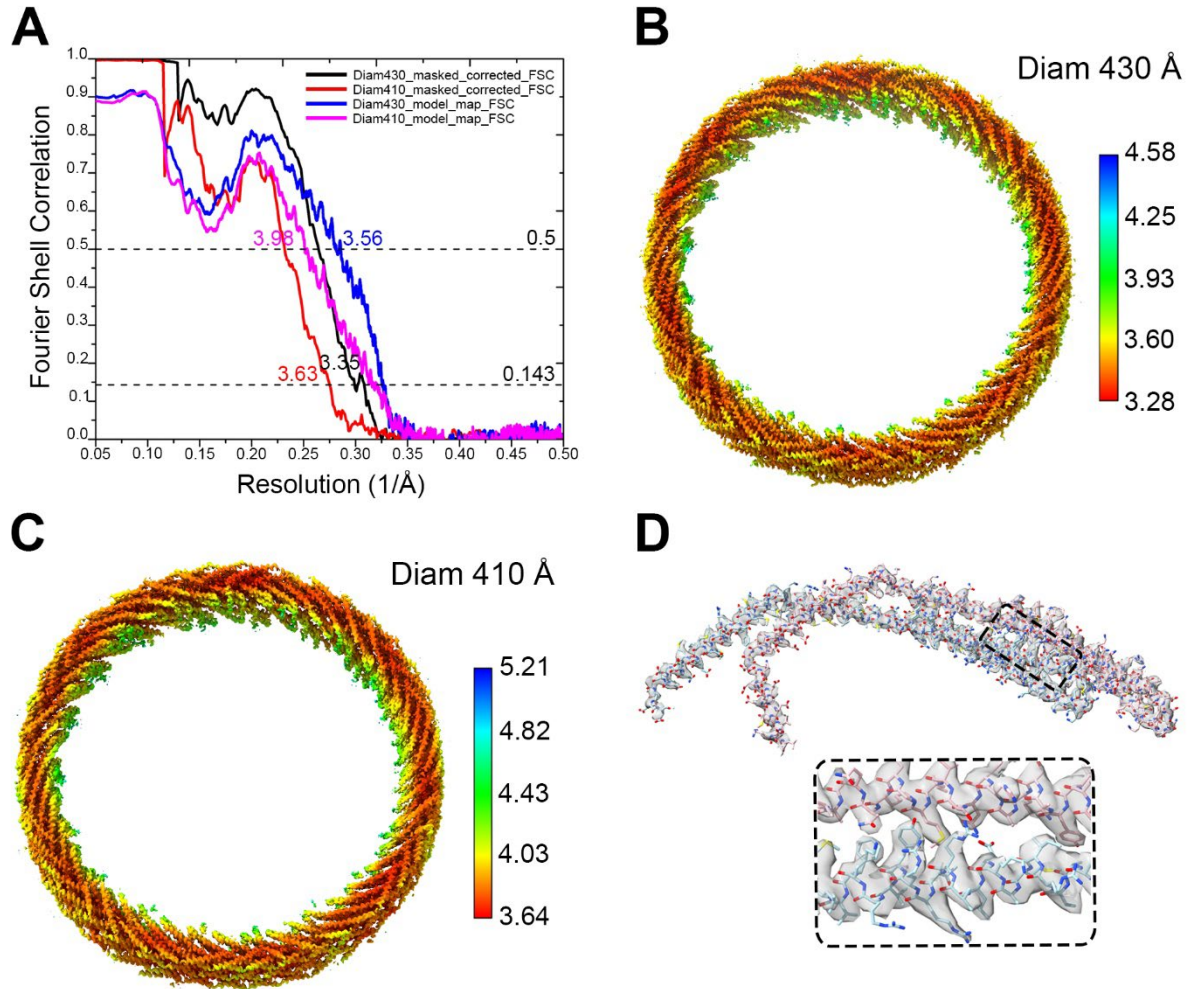
842 Segment subsets were subjected to symmetry determination (<http://rico.ibs.fr/helixplorer/>) and initial 3D

843 model generation in SPRING, followed by symmetry refinement and final 3D structure refinement in

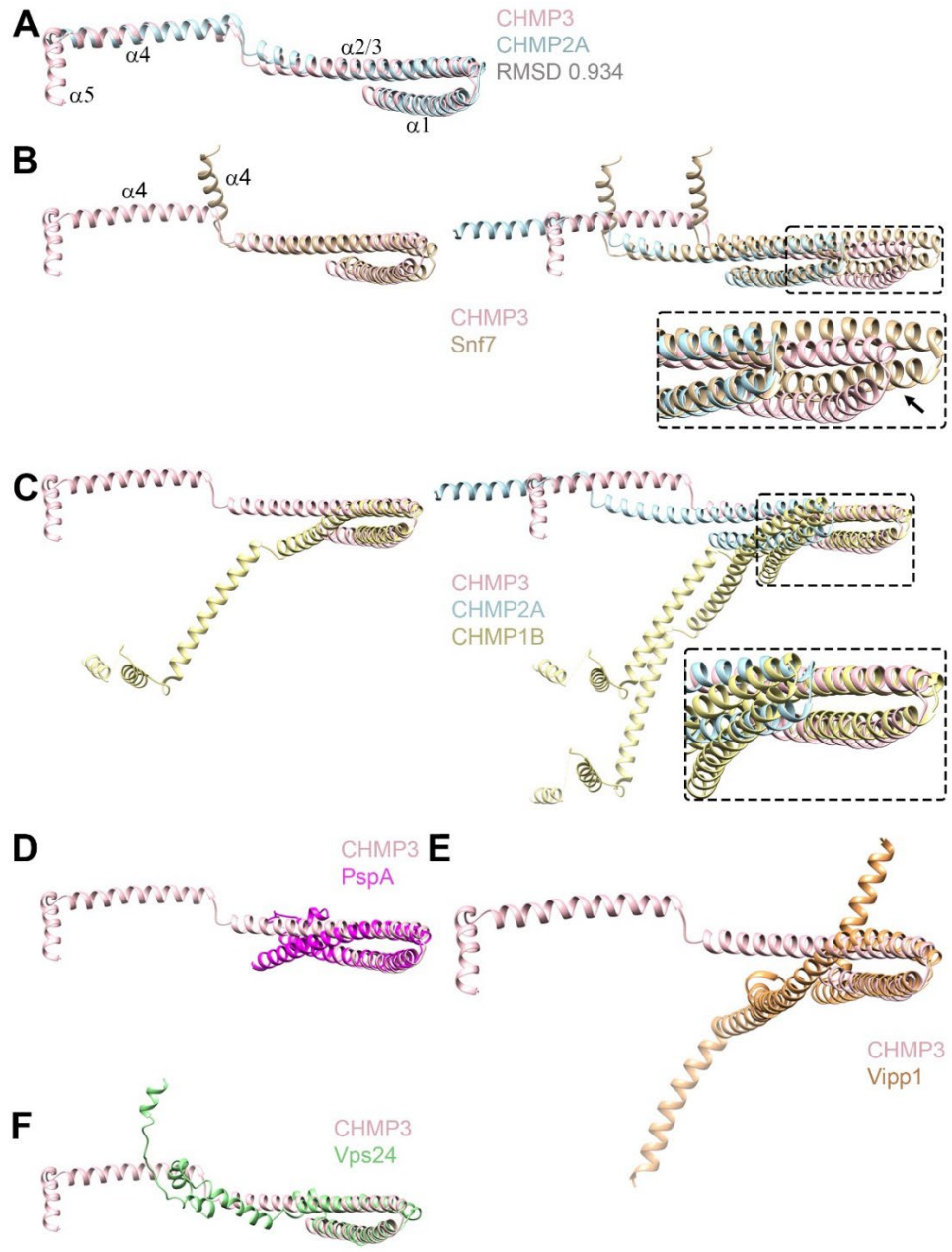
844 RELION. A complete description of the processing workflow is provided in 'Materials and Methods' section.

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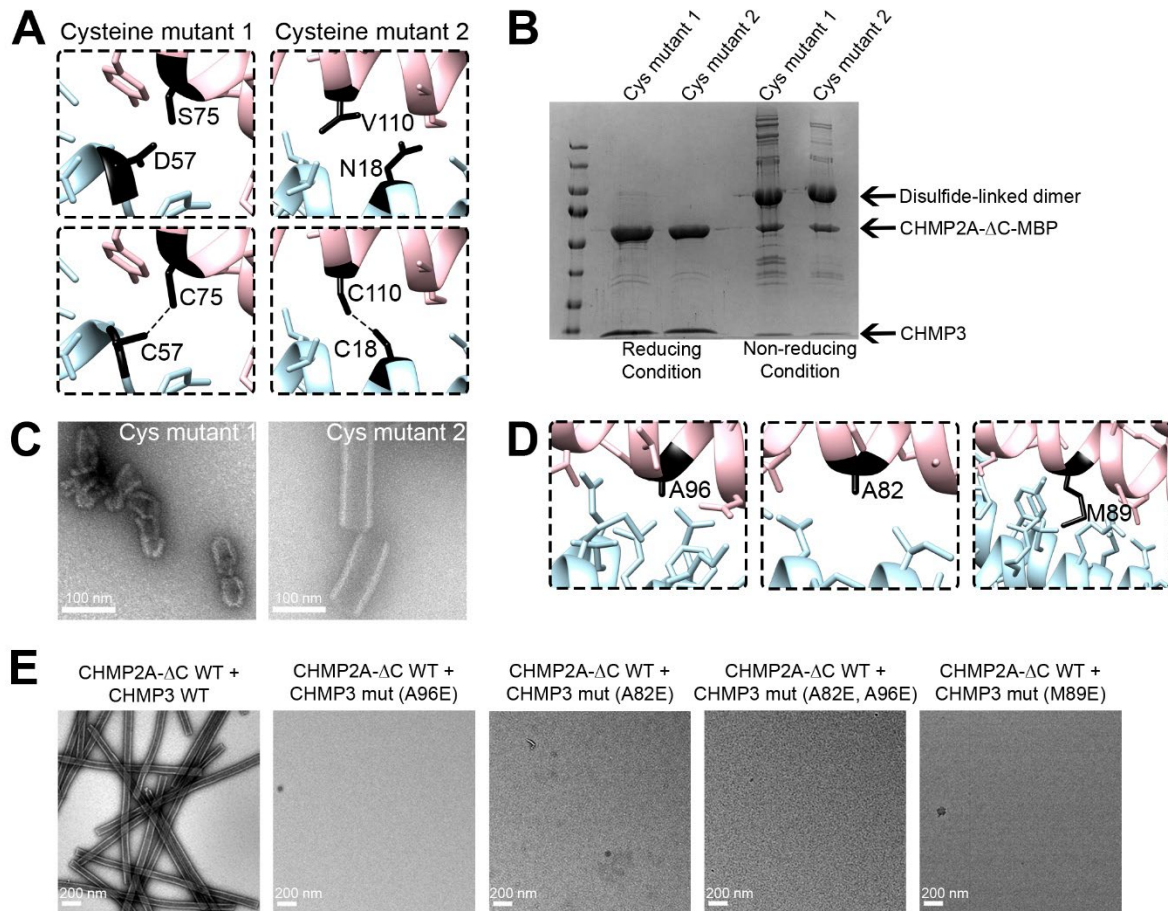
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 848 **Extended Data Fig.3: 430 and 410 Å diameter tubes FSC curves, local resolution maps and atomic**  
 849 **model fitting.**  
 850 **(A)** FSC curves for the 430 Å (black) and 410 Å (red) diameter tube maps, with the resolutions at the FSC  
 851 cut-off of 0.143 are indicated. Model versus map FSC curves, with the resolutions at the FSC cut-off of 0.5  
 852 are indicated for the 430 Å (blue) and 410 Å (pink) diameter tube maps.  
 853 Local resolution estimates are mapped onto the 430 Å **(B)**, and 410 Å **(C)** diameter tube cryo-EM density  
 854 maps and the color keys (right) highlight the local resolution values in Å.  
 855 **(D)** The refined atomic model of CHMP2A-CHMP3 dimer was fit into the corresponding cryo-EM density  
 856 map of the 430 Å diameter tube. The inset (below) represents the zoomed-in view of the fitted model,  
 857 indicating CHMP2A and CHMP3 helices and the corresponding map.  
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 861 **Extended Data Fig.4: Comparison of ESCRT-III open conformations highlights their versatile**  
 862 **polymerization modes**  
 863 **(A)** Ribbon diagram of C $\alpha$  superposition of CHMP2A and CHMP3 reveals an RMSD of 0.934 Å.  
 864 **(B)** Ribbon diagram of C $\alpha$  superposition of CHMP3 with an Snf7 monomer (left panel) and with the  
 865 homodimer (crystallographic dimer) (right panel), which indicates different hairpin interaction (arrow) for the  
 866 second protomer and different orientations of Snf7 helix 4.  
 867 **(C)** Ribbon diagram of C $\alpha$  superposition of CHMP3 and CHMP1B (left panel) and the superposition of the  
 868 CHMP2A-CHMP3 heterodimer onto the CHMP1B homodimer (right panel) indicate the differences in helical  
 869 hairpin stacking (zoom, right panel) and orientations of the C-terminal helical arms (helices 3 to 5).  
 870 **(D)** Ribbon diagram of C $\alpha$  superposition of CHMP3 (pink) with PspA and **(E)** with Vipp1.  
 871 **(F)** Ribbon diagram of C $\alpha$  superposition of CHMP3 with an intermediate Vps24 conformation that forms  
 872 filaments on its own <sup>46</sup>.



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874 **Extended Data Fig.5: Structure-based mutagenesis of CHMP2A-CHMP3 heterodimer formation and**  
 875 **polymerization *in vitro*.**

876 (A) Close-up views of the pairs of residues (black) mutated to cysteine to induce the formation of disulfide-  
 877 linked CHMP2A (light blue) - CHMP3 (pink) heterodimers upon polymerization.

878 (B) Cysteine cross-linking of the CHMP2A-CHMP3 heterodimer. Mutant CHMP2A\_D57C was incubated  
 879 with CHMP3\_S75C and CHMP2A\_N18C with CHMP3\_V110C to induce polymerization as reported for wild-  
 880 type CHMP2A and CHMP3<sup>30</sup>. SDS-PAGE analysis showing that both CHMP2A\_D57C-CHMP3\_S75C and  
 881 CHMP2A\_N18C-CHMP3\_V110C formed disulfide-linked dimers under non-reducing SDS PAGE  
 882 conditions.

883 (C) Negative staining electron micrographs showing regular tube formation for CHMP2A\_N18C-  
 884 CHMP3\_V110C (right), while CHMP2A\_D57C-CHMP3\_S75C (left) produced only shorter tubes. Scale bar,  
 885 100 nm.

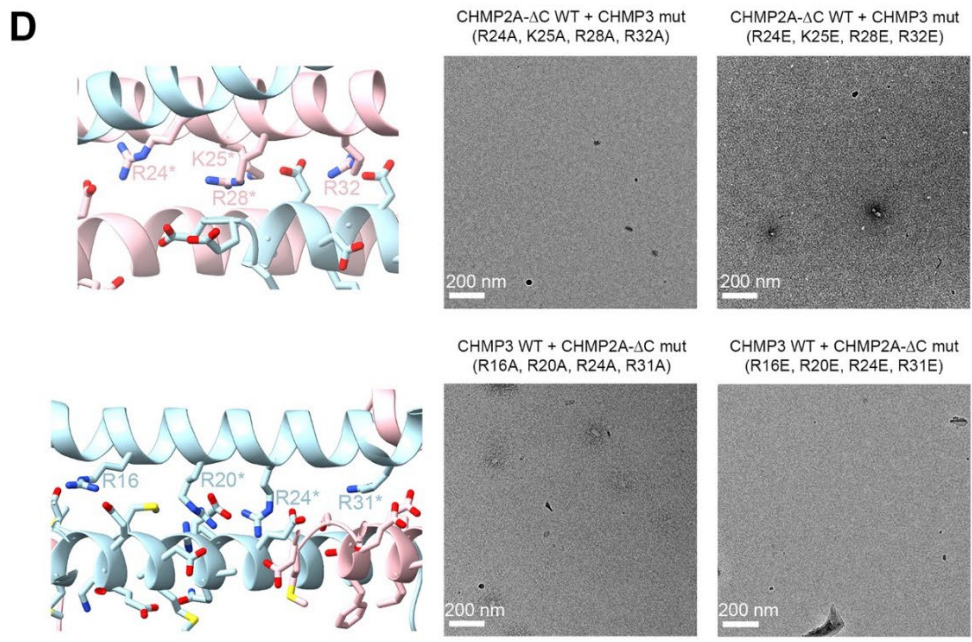
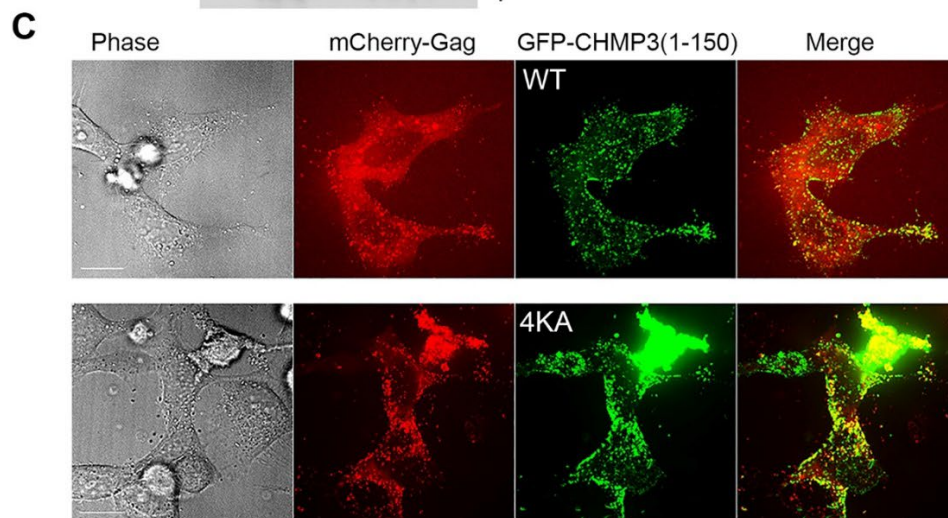
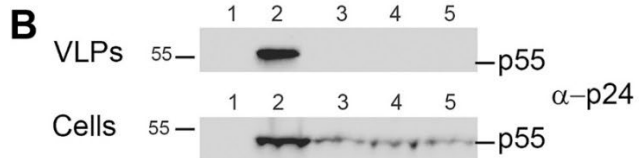
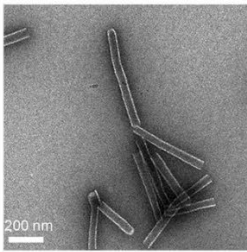
886 (D) Close-up views of the CHMP3 interface residues A96, A82 and M89E tested for heterodimer formation  
 887 and polymerization.

888 (E) Negative staining electron micrographs of CHMP2A-CHMP3 wild-type and mutants (CHMP3\_A96E,  
 889 CHMP3\_A82E, CHMP3\_A82E\_A96E and CHMP3\_M89E) assemblies as indicated. Scale bar, 200 nm.

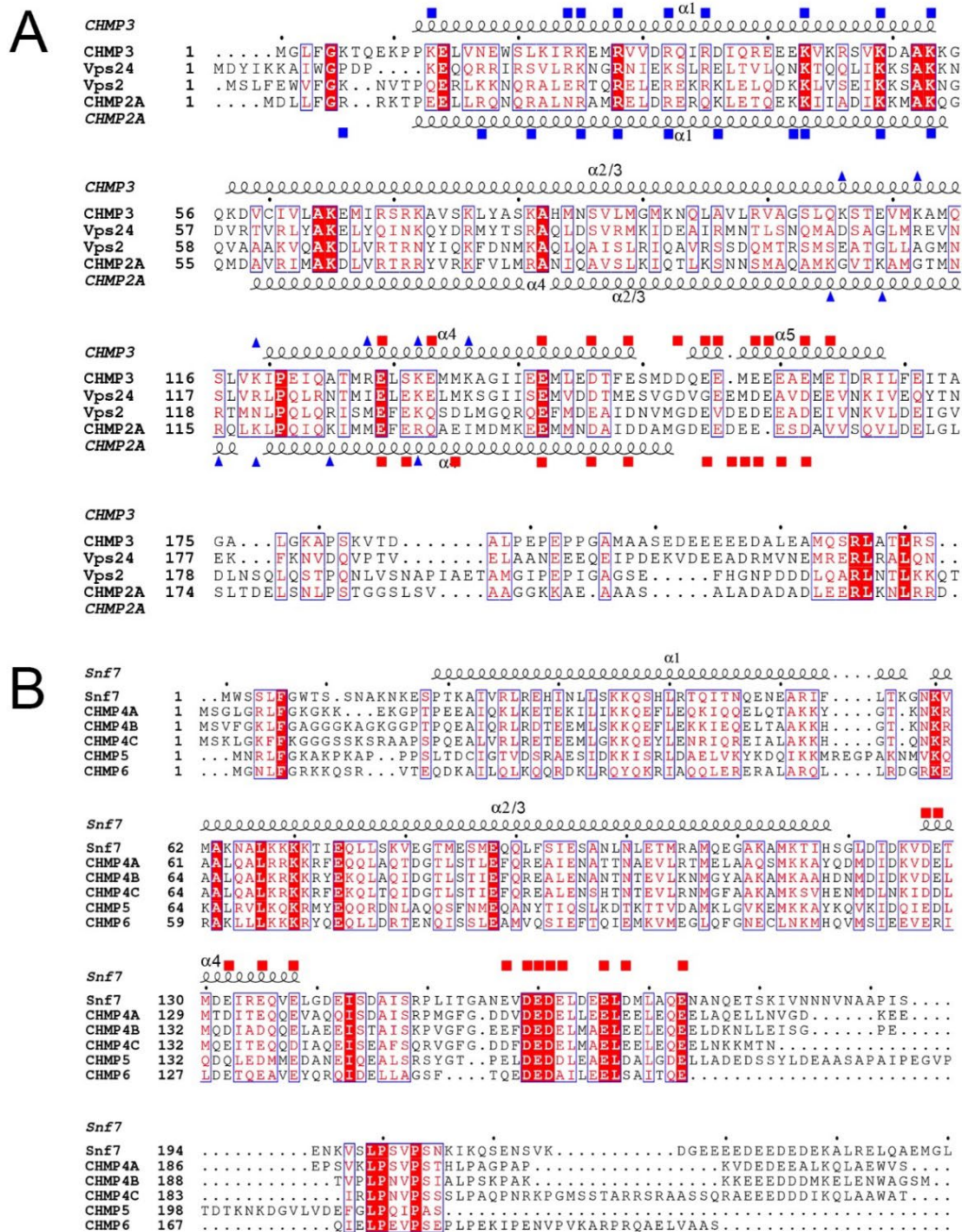
890

891

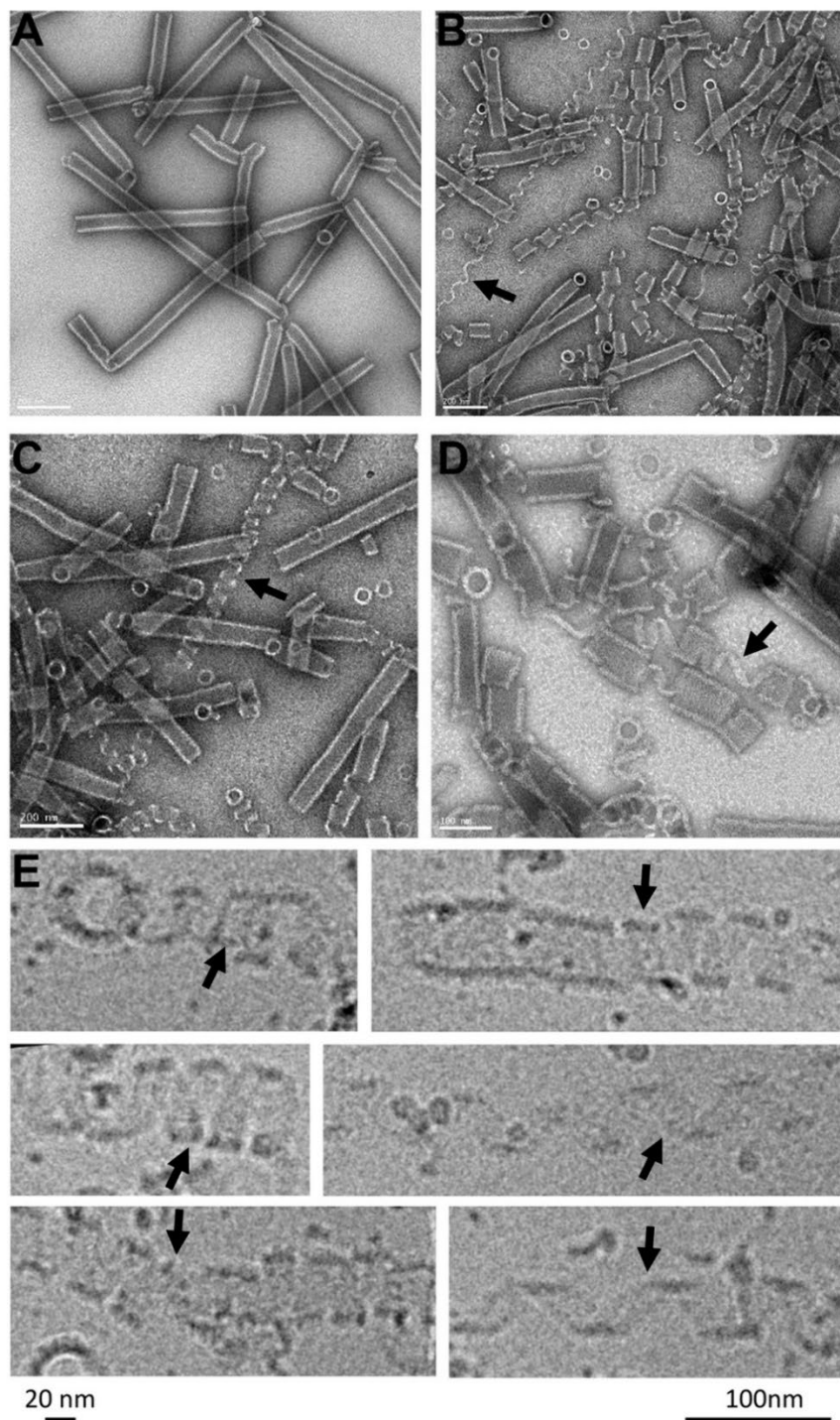
**A** CHMP2A-ΔC WT + CHMP3 mut  
(K112A, K119A, K132A, K136A)



893 **Extended Data Fig.6: Structure-based mutagenesis of CHMP2A-CHMP3 polymer formation**  
894 **(A)** Negative staining electron micrograph showing regular tube formation by CHMP2A-CHMP3\_K112A,  
895 K119A, K132A, K136A polymerization. Scale bar, 200 nm.  
896 **(B)** The substitution of four basic residues in GFP-CHMP3(1–150) 4KA (K112A, K119A, K132A, K136A)  
897 does not diminish its dominant-negative effect on HIV-1 budding. Western blot analyses of Gag released  
898 from Gag expressing cells as Gag-VLPs (upper panel) and detection of Gag in total cell extracts (lower  
899 panel): lane 1, Gag expression; lane 2, Gag and GFP-VPS4A E228Q expression; lane 3, Gag and GFP-  
900 CHMP3(1-150) expression; lane 4, Gag and GFP-CHMP3(1-150) 4KA expression.  
901 **(C)** Representative fluorescence images of HeLa cells transfected with Gag/mCherry-Gag and CHMP1(1-  
902 150) or CHMP3(1-150)4KA. Cellular distribution of wild-type and mutant KA GFP-CHMP3(1–150) indicates  
903 predominantly plasma membrane and intracellular localization as well as co-localization with mCherry-Gag.  
904 Scale bar, 10  $\mu$ m.  
905 **(D)** Negative staining electron micrographs showing no polymer formation of CHMP3 mutants (upper left  
906 panel, close-up of a ribbon diagram illustrating the interface residues) R24A, K25A, R28A, R32A (upper  
907 middle panel) and R24E, K25E, R28E, R32E (upper right panel) with CHMP2A. (Lower panel) CHMP2A  
908 mutants (lower left panel, close-up of a ribbon diagram illustrating the interface residues) R16A, R20A,  
909 R24A, R31A (lower middle panel) and R16E, R20E, R24E, R31E (lower right panel) did not polymerize with  
910 CHMP3 *in vitro*. Scale bar, 200 nm.  
911  
912



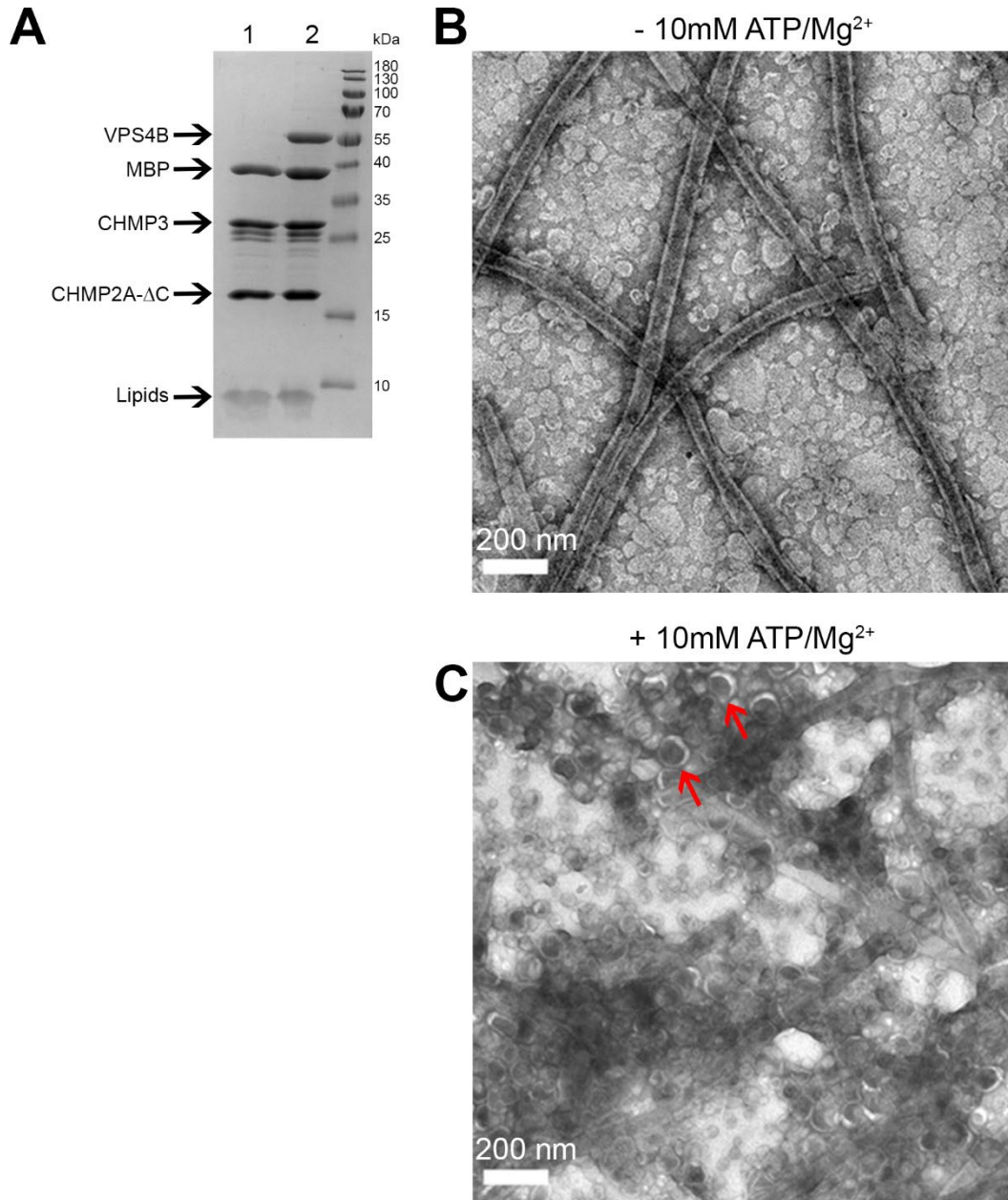
913  
 914 **Extended Data Fig.7: ESCRT-III sequence alignment.**  
 915 **(A)** Sequence alignment of CHMP3 (AF219226), *S. cerevisiae* Vps24 (QHB09957), *S. cerevisiae*  
 916 Vps2 (P36108.2) and CHMP2A (NM\_198426.3). Secondary structure elements are shown for  
 917 CHMP3 above the sequence and for CHMP2A below the sequence alignment. Blue triangles  
 918 indicate basic residues of CHMP3 (above) and CHMP2A (below) exposed at the membrane  
 919 binding interface. Blue rectangles show basic residues and red squares conserved acidic residues  
 920 exposed at the interface between filaments.  
 921 **(B)** Sequence alignment of *S. cerevisiae* Snf7 (Z73197.1) and its secondary structure (pdb 5FD9),  
 922 CHMP4A (NM\_014169.5), CHMP4B (NM\_176812.5) CHMP4C (NM\_152284), CHMP5  
 923 (NM\_016410.6) and CHMP6 (NM\_024591.5). Conserved acidic residues implicated in inter-  
 924 filament interactions in the CHMP2A-CHMP3 polymer are indicated as red squares.



926

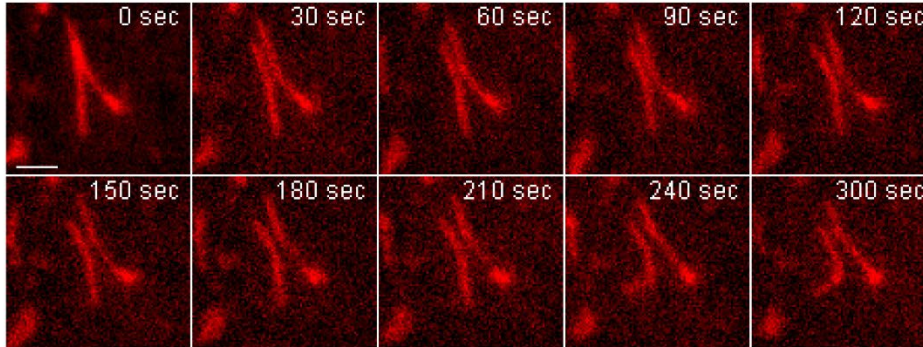
927 **Extended Data Fig.8: High ionic strength unwinds the CHMP2A-CHMP3 filaments.**928 **(A)** Negative staining electron micrographs showing CHMP2A-CHMP3 wild-type polymers after treatment  
929 with 1M NaCl **(B, C)** and 1M KCl **(D)**.930 **(E)** Close-up of cryo-EM images shows unwinding of ~20 nm wide filaments corresponding to the six-start  
931 helix observed in the structure. Single and multi-stranded unwound filaments are indicated by arrows.  
932



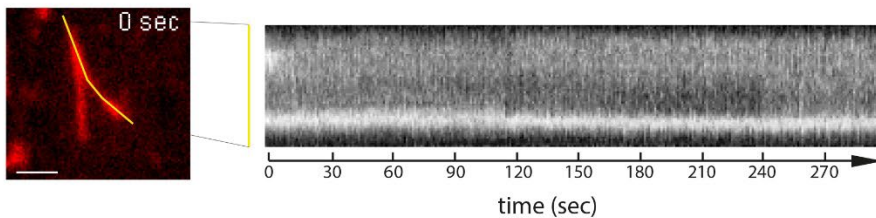


933  
 934 **Extended Data Fig.9: Incorporation of VPS4B and ATP into CHMP2A-CHMP3 membrane tubes**  
 935 **induces their disassembly.**  
 936 **(A)** SDS-PAGE analyses of purified CHMP2A-CHMP3 polymers; lane 1, CHMP2A-CHMP3 polymers  
 937 cleaved with TEV and coated with a lipid bilayer; lane 2 CHMP2A-CHMP3 polymers, TEV cleaved and  
 938 incorporation of VPS4B prior to lipid bilayer coating.  
 939 Negative staining electron micrographs of CHMP2A-CHMP3-VPS4B membrane-coated polymers before  
 940 **(B)** and after **(C)** incubation with ATP and Mg<sup>2+</sup>. Red arrows point to membrane vesicles resulting from tube  
 941 cleavage. Scale bar, 200 nm.  
 942

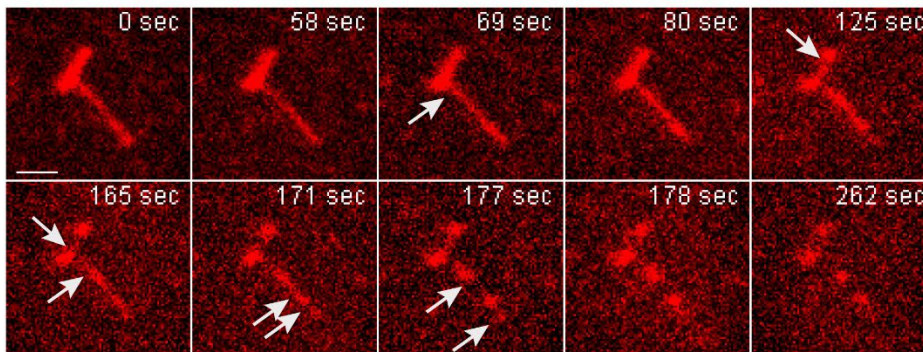
**A** + caged ATP



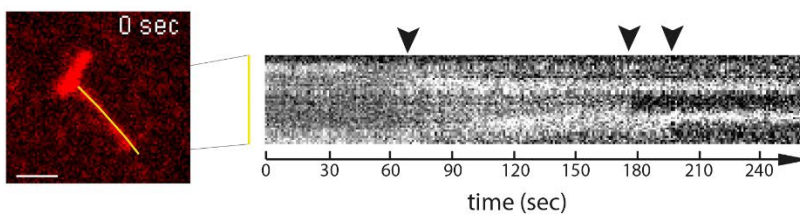
**B**



**C** + VPS4B and caged ATP

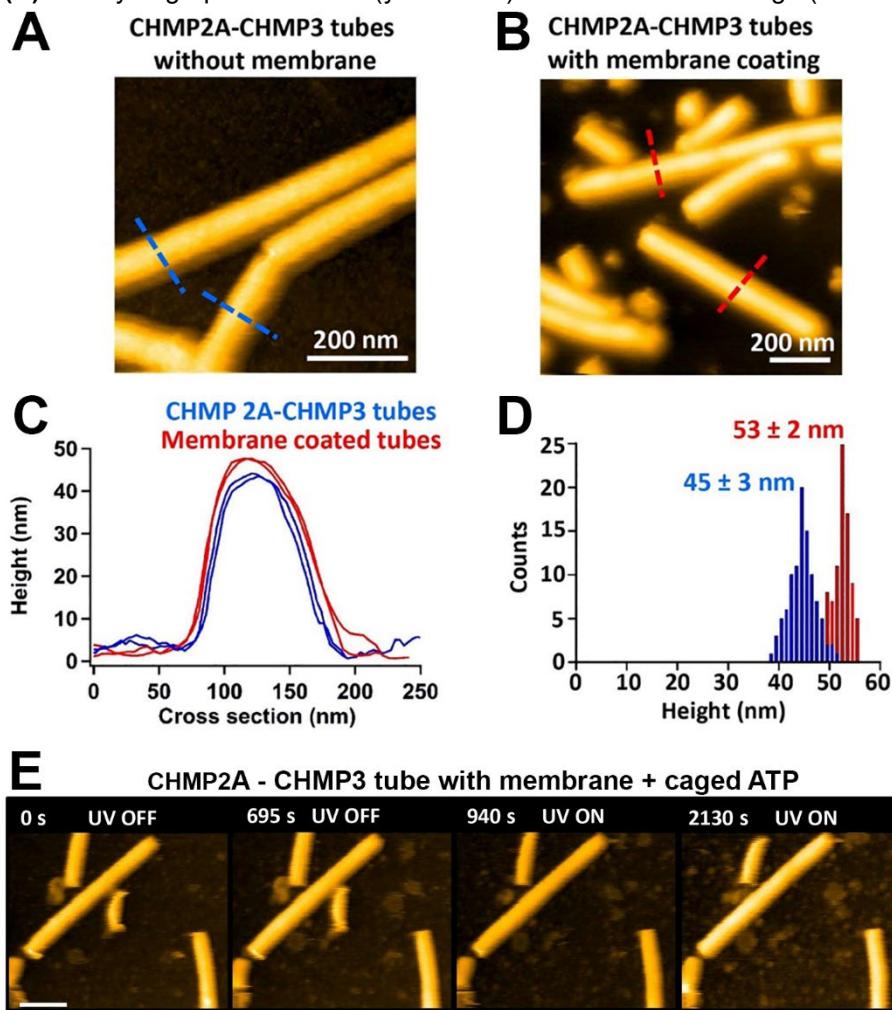


**D**



943  
944 **Extended Data Fig.10: Imaging of VPS4B and ATP induced cleavage of CHMP2A-CHMP3 membrane**  
945 **coated tubes.**  
946 **(A)** A CHMP2A-CHMP3-caged ATP membrane coated tube was activated at 365 nm (30%, 100 ms) to  
947 uncage ATP and imaged over 282s, which indicated that uncaging did not change the tube structure (movie  
948 S3).  
949 **(B)** The kymograph of the tube (yellow line) shows that the tube stays intact over the imaging time.  
950 **(C)** Imaging of a CHMP2A-CHMP3-VPS4B-caged ATP membrane-coated tube following ATP uncaging  
951 (365 nm, 30%, 100 ms) reveals cleavage of the tube at several sites over the imaging time (movie S5).  
952 Scale Bar, 1 $\mu$ m.

953 (D) The kymograph of the tube (yellow line) indicates tube cleavage (arrows) over the imaging time.



954 **Extended Data Fig.11. Height distribution of CHMP2A-CHMP3 tubes with and without membrane-**  
 955 **coating.**

957 (A) AFM image of CHMP2A-CHMP3 tubes without membrane.

958 (B) AFM image of CHMP2A-CHMP3 tubes coated with membrane.

959 (C) Cross-section of AFM images of CHMP2A-CHMP3 in panel A (blue dotted line) and panel B (red dotted  
 960 line).

961 (D) Height histogram of CHMP2A-CHMP3 tubes with (red) and without (blue) membrane coating with  
 962 respect to the surface.

963 (E) Snapshots HS-AFM images of membrane-coated tubes loaded with 10 mM caged ATP taken with and  
 964 without UV irradiation. The UV was turned on from 700 s onwards. Scale bar, 200 nm.

965  
 966

967 **Movies**

968  
969 **Movie 1: Fluorescence microscopy imaging of CHMP2A-CHMP3 membrane coated tubes.** A  
970 CHMP2A-CHMP3-caged ATP membrane-coated tube was activated at 365 nm to uncage ATP and imaged  
971 over 291s.

972 **Movie 2: Fluorescence microscopy imaging of CHMP2A-CHMP3 membrane coated tubes.** Another  
973 dataset of CHMP2A-CHMP3-caged ATP membrane-coated tube activated at 365 nm 100 ms at each time  
974 point to uncage ATP and imaged over 300s.

975 **Movie 3: Fluorescence microscopy imaging of CHMP2A-CHMP3 membrane coated tubes.** A  
976 CHMP2A-CHMP3-VPS4B membrane-coated tube was activated at 365 nm to uncage ATP and imaged  
977 over 251s.

978 **Movie 4: Fluorescence microscopy imaging of CHMP2A-CHMP3 membrane coated tubes.** Imaging of  
979 a CHMP2A-CHMP3-VPS4B-caged ATP membrane-coated tube following ATP uncaging reveals a tube  
980 fission followed by a shrinking event from both sides.

981 **Movie 5: Fluorescence microscopy imaging of CHMP2A-CHMP3 membrane coated tubes.** Another  
982 dataset of CHMP2A-CHMP3-VPS4B-caged ATP membrane-coated tube following ATP uncaging (100 ms  
983 at each time point) reveals cleavage of the tube at several sites over the imaging time.

984 **Movie 6: Fluorescence microscopy imaging of CHMP2A-CHMP3 membrane coated tubes.** Imaging of  
985 a CHMP2A-CHMP3-VPS4B-caged ATP membrane-coated tube following ATP uncaging showing at 36s a  
986 shrinking event from the end of a tube and at 51s a cleavage of the tube.

987 **Movie 7: HS-AFM imaging of CHMP2A-CHMP3 membrane-coated tubes.** HS-AFM movie of membrane-  
988 coated CHMP2A-CHMP3 tubes loaded with 10 mM caged ATP, taken before and after UV (365 nm)  
989 irradiation. Imaging time 5 seconds/frame.

990 **Movie 8: HS-AFM imaging of CHMP2A-CHMP3 membrane-coated tubes.** HS-AFM movie of membrane-  
991 coated CHMP2A-CHMP3 tubes loaded with 5  $\mu$ M VPS4B and 10 mM caged ATP, taken without UV  
992 irradiation. Imaging time 3 seconds/frame.

993 **Movie 9: HS-AFM imaging of CHMP2A-CHMP3 membrane-coated tubes.** HS-AFM movie of membrane-  
994 coated CHMP2A-CHMP3 tubes loaded with 5  $\mu$ M VPS4B and 10 mM caged ATP, taken with 365 nm UV  
995 irradiation. Imaging time 2 seconds/frame.

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997  
998

**Extended Data Table 1 – Cryo-EM data collection, refinement and validation statistics**

	Membrane-bound CHMP2A + CHMP3 (Tube Diameter 430 Å) (EMD-14630, PDB 7ZCG)	Membrane-bound CHMP2A + CHMP3 (Tube Diameter 410 Å) (EMD-14631, PDB 7ZCH)
<b>Data collection and processing</b>		
Magnification	130,000x	130,000x
Voltage (kV)	300	300
Electron exposure per frame (e <sup>-</sup> /Å <sup>2</sup> )	0.96	0.96
Number of frames	25	25
Defocus range (μm)	0.5-1.5	0.5-1.5
Pixel size (Å)	1.052	1.052
Refined helical symmetry	54.37°, 2.74 Å	57.04°, 1.44 Å
Point group symmetry	C2	C1
Initial particle images (no.)	45,847	80,524
Final particle images (no.)	25,353	11,396
Map resolution (Å)	3.3	3.6
FSC threshold	0.143	0.143
<b>Refinement</b>		
Initial model used (PDB code)	<i>de novo</i>	<i>de novo</i>
Model resolution (Å)	3.6	4.0
FSC threshold	0.5	0.5
Map sharpening <i>B</i> factor (Å <sup>2</sup> )	-96.57	-101.52
Model-to-map fit		
Correlation coefficient, CC (mask)	0.80	0.77
Model Composition		
Chains	22	22
Nonhydrogen atoms	27,148	27,148
Protein residues	3,366	3,366
<i>B</i> factor (Å <sup>2</sup> )		
Protein	74.18	90.17
R.m.s. deviations		
Bond lengths (Å)	0.004	0.004
Bond angles (°)	0.758	0.762
Validation		
MolProbity score	1.86	1.89
Clashscore	15.23	21.23
Rotamer outliers (%)	0.00	0.00
Cbeta deviations (%)	0.00	0.00
Ramachandran plot		
Favored (%)	97.02	97.68
Allowed (%)	2.98	2.32
Disallowed (%)	0.00	0.00