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Dynamic subunit turnover in ESCRT-III assemblies is regulated by Vps4 to mediate membrane remodelling during cytokinesis

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29 **ABSTRACT**

30 The Endosomal Sorting Complex Required for Transport (ESCRT)-III mediates membrane
31 fission in fundamental cellular processes, including cytokinesis. ESCRT-III is thought to form
32 persistent filaments that over time increase their curvature to constrict membranes. Unexpectedly,
33 we found that ESCRT-III at the midbody of human cells rapidly turns over subunits with
34 cytoplasmic pools while gradually forming larger assemblies. ESCRT-III turnover depended on
35 the ATPase VPS4, which accumulated at the midbody simultaneously with ESCRT-III subunits,
36 and was required for assembly of functional ESCRT-III structures. *In vitro*, the Vps2/Vps24
37 subunits of ESCRT-III formed side-by-side filaments with Snf7 and inhibited further
38 polymerization, but the growth inhibition was alleviated by the addition of Vps4 and ATP. High-
39 speed atomic force microscopy further revealed highly dynamic arrays of growing and shrinking
40 ESCRT-III spirals in presence of Vps4. Continuous ESCRT-III remodeling by subunit turnover
41 might facilitate shape adaptations to variable membrane geometries, with broad implications for
42 diverse cellular processes.

43

44

45 **INTRODUCTION**

46 The Endosomal Sorting Complex Required for Transport-III (ESCRT-III) promotes membrane
47 fission from the inner side of membrane necks in various cellular processes¹, including the
48 biogenesis of multivesicular bodies², cytokinetic abscission³⁻⁷, nuclear envelope sealing⁸⁻¹¹,
49 plasma membrane repair¹², HIV budding^{13,14}, and exosome or microvesicle shedding¹⁵⁻¹⁷. ESCRT-
50 III forms polymers that are thought to constrict membrane necks until they split¹⁸⁻²⁵, but the
51 mechanism underlying constriction is unknown.

52 ESCRT-III is evolutionary conserved from humans to archaea, and is composed of four
53 structurally related core subunits with distinct functions¹⁸⁻²⁷. Budding yeast Vps20 (human
54 homolog is CHMP6) functions as a nucleation factor, Snf7 (human homolog CHMP4 has three
55 isoforms, A-C) serves as a main polymer subunit, Vps24 (CHMP3 in humans) and Vps2
56 (CHMP2A and B isoforms in humans) inhibit Snf7 polymerization²⁸⁻³² and recruit the ATPase
57 Vps4, which is thought to predominantly disassemble ESCRT-III polymers^{30,31,33-38}. How
58 different ESCRT-III components coordinately assemble and remodel polymer structures has
59 remained unclear.

60 Purified ESCRT-III subunits polymerize into filaments that form spirals on flat membranes or
61 helices on membrane tubes^{36,39-45}. ESCRT-III also forms filament spirals and helices in intact
62 cells^{44,46,47}, and it is required for the assembly of large filament helices that constrict the
63 intercellular bridge during cytokinetic abscission⁴.

64 Prevailing models propose that ESCRT-III mediates membrane fission by sequential assembly of
65 distinct subunits^{18-23,25}, whereby late-binding Vps2/Vps24 (CHMP2/CHMP3) subunits might
66 form a rigid dome-shaped scaffold to guide attached membranes towards the fission site³⁶ or
67 induce changes in the curvature of pre-assembled Snf7 filament spirals to promote membrane
68 neck constriction⁴¹. These models rely on the sequential addition of distinct subunits and the
69 persistence of ESCRT-III polymers, yet this has not been directly observed under physiological
70 conditions. We hence set out to systematically quantify the assembly kinetics, dynamics, and
71 structure of ESCRT polymers in live human cells and in an *in vitro* reconstitution system.

72

73

74 **RESULTS**

75

76 **ESCRT-III assemblies continuously turn over their subunits with the cytoplasm**

77 To investigate the dynamics of ESCRT-III polymers at the abscission site, we generated stable
78 HeLa cell lines expressing fluorescently tagged subunits. We found that CHMP4B tagged with
79 GFP via a long flexible linker (Localization and Affinity Purification tag, LAP) and expressed
80 close to endogenous levels⁴ did not perturb abscission (Fig. 1a). To probe the functionality of
81 LAP-tagged CHMP4B, we depleted endogenous CHMP4B in wild-type HeLa cells or in HeLa
82 cells stably expressing siRNA-resistant mouse CHMP4B-LAP (Supplementary Fig. 1a).
83 Cytokinetic abscission was substantially perturbed upon depletion of endogenous CHMP4B in
84 wild-type HeLa cells, but was not affected in mouse-CHMP4B-LAP-expressing HeLa cells (Fig.
85 1a), validating the functionality of CHMP4B-LAP.

86 We next investigated the dynamics of midbody-localized ESCRT-III by fluorescence recovery
87 after photobleaching (FRAP) experiments. Unexpectedly, we found that CHMP4B-LAP rapidly
88 re-accumulated at the midbody following photobleaching (Fig. 1b, c and Supplementary Video
89 1). A single exponential function constrained to initial fluorescence values did not fit the FRAP
90 kinetics (Fig. 1c), indicating the presence of two populations of CHMP4B-LAP with distinct
91 residence times at the midbody. We determined the residence times for the two midbody-
92 localized fractions by a double-exponential fit (Fig. 1b, h, i). A highly mobile fraction of $64 \pm 6\%$
93 (mean \pm SEM, as in the rest of the paper, if not otherwise noted) of CHMP4B-LAP had a
94 residence time of 19.5 ± 2.7 s, whereas a stably-bound fraction of $36 \pm 2\%$ had a residence time
95 of 716.0 ± 91.3 s. Modeling the stably-bound fraction as completely immobile also yielded a
96 good approximation (Supplementary Fig. 1b, c). Importantly, both methods of model fitting
97 yielded consistent values for the fraction and residence time of the highly mobile pool

98 (Supplementary Fig. 1d). Thus, the majority of CHMP4B-LAP molecules at the midbody
99 continuously turns over with a cytoplasmic pool – at a rate up to two orders of magnitude faster
100 than the macroscopic accumulation of ESCRT-III at the midbody^{4,5}.

101 ESCRT-III initially localizes within two cortical regions adjacent to the midbody, which later
102 constrict to split the plasma membrane^{4,5}. Photobleaching of CHMP4B-LAP prior to or during
103 constriction stages revealed similar recovery kinetics (Fig. 1b, d, e, and Supplementary Video 1),
104 indicating that midbody-localized ESCRT-III dynamically turns over subunits with cytoplasmic
105 pools during its macroscopic accumulation and constriction.

106 We next investigated the localization and dynamics of other ESCRT-III subunits. We tagged
107 human CHMP2B, CHMP3 and CHMP4B with the same design used for tagging mouse
108 CHMP4B. All three LAP-tagged ESCRT-III subunits localized to the midbody (Fig. 2f, g, and
109 Supplementary Video 2) and did not perturb abscission (Supplementary Fig. 2a-d). CHMP2B,
110 CHMP3, and CHMP4B subunits accumulated at the midbody with indistinguishable kinetics
111 (Fig. 2f, g, and Supplementary Video 2). Hence, ESCRT-III assembles at the midbody with a
112 fairly constant proportion of different core subunits during the progression of abscission.

113 We next probed the dynamics of human CHMP2B, CHMP3, and CHMP4B at the midbody.
114 FRAP experiments showed that all three subunits had highly mobile fractions with residence
115 times similar to mouse CHMP4B (Fig. 1h, i, and Supplementary Fig. 2g-i). We noticed somewhat
116 variable kinetics at late stages of FRAP recovery, which resulted in inaccurate fitting of single
117 exponential functions (Supplementary Fig. 2e-g). Given the technical difficulty to accurately
118 measure long residence times, the relevance of the observed variations remains unclear.
119 Importantly, however, all FRAP experiments consistently show that highly mobile fractions of
120 CHMP2B, CHMP3, and CHMP4B dynamically turn over with similar residence times. Overall,
121 our experiments show that ESCRT-III forms highly dynamic assemblies at the midbody.

122

123 **Dynamic subunit turnover in ESCRT-III assemblies depends on VPS4**

124 We wondered if VPS4 could be responsible for ESCRT-III turnover, as it is the only known
125 nucleotide hydrolase in the ESCRT-III pathway^{30,33}. VPS4 was previously detected at the
126 midbody only during late stages of abscission⁵, which would be inconsistent with its contribution
127 to the high ESCRT-III turnover observed during early stages. However, previous measurements
128 of VPS4 accumulation were based on overexpression from a viral promoter⁵, which could limit
129 its detection at the midbody owing to high cytoplasmic background. We thus re-examined VPS4
130 accumulation in cells stably expressing LAP-tagged murine VPS4B from its endogenous
131 promoter (Supplementary Fig. 3a). In these cells, VPS4B-LAP indeed accumulated at the
132 midbody simultaneously with CHMP4B-LAP (Fig. 2a, b, and Supplementary Video 3). Thus,

133 VPS4 is present at early stages and could contribute to ESCRT-III dynamics throughout the entire
134 abscission process.

135 To investigate the role of VPS4 in ESCRT-III dynamics, we depleted both isoforms VPS4A and
136 VPS4B in CHMP4B-LAP-expressing cells using RNAi. Depletion of endogenous VPS4A/B to
137 undetectable levels at 48 h after siRNA transfection (Supplementary Fig. 3b) substantially
138 reduced the amount of CHMP4B-LAP at the midbody (Fig. 2c, and Supplementary Fig. 3c).
139 FRAP experiments revealed that under this condition, CHMP4B-LAP turnover at the midbody
140 was almost completely suppressed (Fig. 2e, f). We considered that this phenotype may arise from
141 the strong reduction of the cytoplasmic CHMP4B concentration, owing to the accumulation of
142 ESCRT-III at endosomes upon complete VPS4 depletion^{47,48} (Fig. 3c, d). However, partial
143 depletion of VPS4A/B at 20 h after siRNA transfection also reduced the fluorescence recovery
144 after photobleaching (Fig. 2e, f) without altering the cytoplasmic CHMP4B-LAP concentration
145 (Fig. 2c, d). The cytoplasmic levels of CHMP2B-LAP or CHMP3-LAP were also not affected
146 upon partial VPS4A/B depletion (Supplementary Fig. 3d, e). Furthermore, microinjection of
147 recombinant human CHMP4B protein into telophase cells resulted in rapid accumulation at the
148 midbody in control cells, but a much slower rate in VPS4A/B-depleted cells (Supplementary Fig.
149 4). Together, these experiments show that VPS4 is required for dynamic turnover of ESCRT-III
150 at the midbody.

151

152 **VPS4 is required for constriction of the intercellular bridge**

153 We next studied how VPS4 contributes to abscission. RNAi depletion of VPS4A/B delayed
154 abscission and frequently caused cleavage furrow regression (Fig. 3a, b). These abscission
155 failures did not occur in cells stably expressing murine VPS4B-LAP, which is resistant to siRNA
156 targeting human VPS4A/B (Fig. 3b, and Supplementary Fig. 3a), validating that this phenotype is
157 caused by on-target depletion. To gain further insight into the underlying defect, we investigated
158 the ultrastructure of intercellular bridges in cryo-immobilized telophase cells. Most of the control
159 cells contained constriction zones adjacent to the midbody with regularly spaced 17 nm diameter
160 filaments and compressed bundles of microtubules (Fig. 3c; 4 out of 7 cells), as previously
161 observed⁴. After partial VPS4A/B depletion at 26 h after siRNA transfection, only a small
162 fraction of cells had 17 nm filaments (Fig. 3d; 4 out of 27 cells), and narrow constriction zones
163 were never observed. Together, these data indicate that VPS4 is required for the formation and
164 constriction of a functional ESCRT-III apparatus at intercellular bridges.

165

166 **Vps2 and Vps24 inhibit Snf7 polymerization *in vitro***

167 The high ESCRT-III dynamics in cells prompted us to dissect the specific contribution of each
168 subunit *in vitro*. As purified human ESCRT-III proteins are difficult to spontaneously polymerize
169 on flat membranes under physiological concentrations⁴⁹, we considered to use the evolutionarily
170 conserved budding yeast proteins. We first tested whether budding yeast Snf7 (homolog of
171 human CHMP4) can in principle recapitulate the cellular dynamics observed for human proteins
172 and therefore expressed a LAP-tagged version in HeLa cells. Budding yeast Snf7-LAP
173 specifically localized to the midbody during abscission and rapidly recovered after
174 photobleaching similar to human and mouse CHMP4B-LAP (Supplementary Fig. 5a-d),
175 validating the use of yeast proteins for *in vitro* analysis of ESCRT-III dynamics.

176 Previous work showed that Snf7 spontaneously polymerizes on membrane covered areas of
177 coverslips to form large patches composed of densely packed filament spirals⁴³. We studied patch
178 growth kinetics only in central areas of membrane-covered regions, as patches stop growth at the
179 edge of membrane-covered regions (Supplementary Fig. 5e-g). We first investigated how Vps24
180 (homolog of human CHMP3) and Vps2 (CHMP2) affected the kinetics of Snf7 polymerization.
181 We therefore incubated supported lipid bilayers with fluorescently labeled Snf7 until patches
182 formed and then simultaneously added Vps2 and Vps24 (Fig. 4a, b, and Supplementary Video 4;
183 22 min). Following rapid binding, Vps2 and Vps24 suppressed patch growth and strongly
184 reduced further accumulation of Snf7 in patches (Fig. 4c, d, and Supplementary Fig. 6a-c).
185 Sequential injection of Vps2 and Vps24 into the fluid chamber further showed that these subunits
186 depend on each other in their Snf7 growth-inhibitory function (Fig. 4e, Supplementary Fig. 6d, e,
187 and Supplementary Videos 5 and 6). Thus, prolonged phases of ESCRT-III assembly, as observed
188 during cytokinetic abscission^{4,5}, are not recapitulated by mixed solutions of Snf7, Vps2, and
189 Vps24.

190 The inhibition of Snf7 patch growth by Vps2/Vps24 could be caused by lower rates of Snf7
191 subunit accumulation or by an increase of the Snf7 dissociation rate. To investigate this, we
192 incubated supported lipid bilayers with fluorescently labeled Snf7 until patches formed and then
193 washed out soluble Snf7. We subsequently added fluorescently labeled Vps2 and Vps24, which
194 enriched at the edge of the patch, where newly growing Snf7 filament spirals localize⁴³ (Fig. 4f,
195 g, and Supplementary Video 7). Snf7 remained stably bound to patches throughout the entire
196 imaging period, indicating that Snf7 polymers have extremely low intrinsic subunit dissociation
197 rates irrespective of their association with Vps2 and Vps24. The inhibition of Snf7 patch growth
198 imposed by Vps2 and Vps24 is thus independent of Vps4 and caused by a reduced rate of Snf7
199 subunit incorporation.

200

201 **Vps2 and Vps24 form filament bundles with Snf7**

202 We next investigated if growth inhibition by Vps2 and Vps24 could arise from an ultrastructural
203 change in ESCRT-III polymers. Transmission electron microscopy showed that Snf7 alone
204 polymerized on liposomes to form one-start spirals containing a single 4.5 nm wide filament,
205 which occasionally paired between neighboring turns (Fig. 5a, b), as previously observed⁴³. When
206 Vps2 and Vps24 were added after Snf7 polymerization, filaments appeared double-stranded and
207 neighboring spiral turns occasionally bundled to form quadruple strands with an approximate
208 width of 15 nm (Fig. 5c, d) – close to the width of ESCRT-III-dependent filaments observed at
209 the abscission site in vertebrate cells⁴ (Fig. 5c-f; compare Fig. 3c). Given the one-start single-
210 stranded geometry of Snf7 spirals prior to addition of Vps2/Vps24, the paired filaments likely
211 represent lateral copolymers of Vps2/Vps24 along Snf7.

212 To further characterize the morphological changes of Snf7 filaments upon addition of Vps2/24,
213 we visualized ESCRT-III assemblies by high-speed atomic force microscopy (HS-AFM) (Fig.
214 5g). Snf7 alone formed spirals with pronounced filaments, but subsequent addition of
215 Vps2/Vps24 induced a compact disc-like morphology (Fig. 5g-i). This is consistent with a
216 filament thickening and bundling limiting access of the AFM tip in between neighboring spiral
217 turns. Together, these data suggest that Vps2/Vps24 might reduce the rate of Snf7 polymerization
218 through the formation of bundled filaments.

219

220 **Vps4 induces subunit turnover and net growth of ESCRT-III assemblies in vitro**

221 In cells, Vps2 and Vps24 are both present in the cytoplasm, raising the question of how ESCRT-
222 III polymerization is sustained over prolonged periods. Our *in vivo* observations imply the
223 possibility that Vps4 might leverage Vps2/Vps24-mediated growth inhibition by ESCRT-III
224 turnover. To explore whether Vps4 promotes ESCRT-III turnover *in vitro*, we separately
225 measured ESCRT-III subunit association and dissociation kinetics. We first determined the rate
226 by which Vps4 disassembles Snf7 patches in the absence of Vps2 and Vps24. We polymerized
227 Snf7 on supported lipid membranes, washed out soluble Snf7, and then added Vps4 and ATP.
228 This did not cause detectable disassembly of Snf7 patches even at very high Vps4 concentrations
229 (Fig. 6a, b), indicating that Vps4-mediated Snf7 depolymerization strictly depends on
230 Vps2/Vps24, as shown before³⁷. We thus quantified the rate of Vps4-mediated ESCRT-III patch
231 disassembly in presence of Vps2 and Vps24. We first polymerized Snf7 patches on supported
232 lipid membranes, then removed the soluble pool of Snf7, and subsequently added a mix of
233 fluorescently labeled Vps2 and unlabeled Vps24, Vps4, and ATP. Vps2 first bound to Snf7
234 patches and subsequently partially disassembled together with Snf7 (Fig. 6c, d, Supplementary
235 Fig. 7a, b, and Supplementary Video 8). Thus, Vps4-mediated Snf7 depolymerization is fast
236 enough to account for dynamic turnover of its homolog CHMP4B at the midbody in human cells.

237 To characterize the Snf7-disassembly process at the molecular level, we visualized morphological
238 changes of individual ESCRT-III spirals by HS-AFM. We polymerized Snf7 patches on
239 supported lipid bilayers, then added Vps2 and Vps24, and subsequently washed out the soluble
240 components. We then added Vps4 and ATP and acquired HS-AFM movies, which showed that
241 ESCRT-III spirals reduced their diameter (Fig. 6e, f, and Supplementary Video 9). When
242 omitting ATP from the reaction, Vps4 did not disassemble ESCRT-III spirals (Fig. 6g, and
243 Supplementary Video 10), confirming that ESCRT-III disassembly is an energy-consuming
244 process. Given that the ESCRT-III spirals did not depolymerize below a certain diameter, these
245 data suggest that Vps4 mediates Snf7 filament disassembly predominantly from the outer regions
246 of spirals, whereby inner spiral segments are refractory to disassembly.

247 We next tested whether Vps4 can mediate Snf7 turnover *in vitro*. For this purpose, we used Snf7
248 subunits labeled with two distinct fluorophores. We first incubated supported lipid bilayers with
249 AlexaFluor-488-labeled Snf7 until patches formed and then added Vps24 and Vps2 to inhibit
250 further patch growth (Fig. 7a-c, see 0 - 45 min, and Supplementary Video 11). We then
251 exchanged the soluble pool of Snf7-AlexaFluor-488 with Snf7-Atto-647N while maintaining
252 Vps2 and Vps24 in the reaction. At this point, we also added Vps4. Snf7 polymer patches
253 maintained constant size and did not incorporate Atto-647N-Snf7, presumably because ATP was
254 not yet present (Fig. 7a-c, see 45 - 54 min). Indeed, subsequent ATP addition caused dissociation
255 of AlexaFluor-488-Snf7 from patches, but also concomitant binding of Atto-647N-Snf7 (Fig. 7a-
256 c, see 54 - 88 min). Thus, an ATP-dependent activity of Vps4 promotes not only disassembly of
257 ESCRT-III but also the formation of new ESCRT-III polymers on membranes.

258 Interestingly, Vps4 and ATP also restored the macroscopic growth of Snf7 patches despite the
259 continued presence of Vps2 and Vps24 (Fig. 7a, b, see 58 - 88 min, and Supplementary Video
260 11). We thus tested if a solution containing Vps4, ATP, and all three ESCRT-III subunits
261 supports nucleation and growth of ESCRT-III polymers as observed *in vivo*. Strikingly, this
262 combination resulted in efficient nucleation and growth of ESCRT-III patches, in contrast to a
263 mix that lacked Vps4 (Fig. 7d, e). Furthermore, the net ESCRT-III assembly rate increased with
264 higher concentrations of Vps4 (Supplementary Fig. 7c). Thus, Vps4 promotes net growth of
265 ESCRT-III assemblies in presence of growth-inhibiting Vps2 and Vps24 subunits.

266

267 **Vps4 induces dynamic growth and shrinkage of ESCRT-III filament spirals**

268 To study Vps4-mediated polymer remodeling at the molecular level, we visualized ESCRT-III
269 spirals by HS-AFM. We assembled Snf7 spirals on supported lipid bilayers and then added Vps2,
270 Vps24, Vps4, and ATP. This induced a drastic reorganization of ESCRT-III polymers:
271 preexisting spirals reduced their size, while many new spirals nucleated and grew in between the

272 original spirals (Fig. 8a, b, and Supplementary Video 12). The innermost parts of ESCRT-III
273 spirals appeared refractory to disassembly and thus might represent the stably-bound fraction of
274 ESCRT-III observed by fluorescence microscopy. In contrast, pre-existing spirals remained
275 unchanged in the absence of ATP (Fig. 8c, and Fig. 6g). Thus, Vps4 induces a dynamic steady-
276 state with both growing and shrinking ESCRT-III spirals when Snf7, Vps2, Vps24 are present in
277 solution.

278 Furthermore, while ESCRT-III spirals formed an immobile array in the absence of ATP, the
279 addition of ATP induced substantial lateral mobility of individual ESCRT-III spirals relative to
280 each other (Fig. 8d-f, and Fig. 6g). Thus, in the presence of Vps4 and ATP, ESCRT-III forms
281 highly dynamic polymer structures on membranes.

282

283

284

285 **DISCUSSION**

286 Our study shows that membrane-bound ESCRT-III polymers rapidly turn over subunits with
287 cytoplasmic pools while they assemble into larger structures. The dynamic subunit turnover is
288 driven by Vps4 and is necessary to sustain efficient net growth of ESCRT-III assemblies in
289 presence of inhibitory Vps2 and Vps24 subunits.

290 Most previous models for ESCRT-III-mediated membrane fission imply sequential recruitment of
291 different ESCRT-III subunits and Vps4 to establish distinct phases of polymer growth,
292 reorganization/maturation, and disassembly^{5,14,18-21,23-25,28,30,31}. However, our study shows that
293 different ESCRT-III subunits and VPS4 accumulate at the midbody with indistinguishable
294 kinetics. This does not rule out sequential subunit binding at the single-filament level, but
295 indicates that the distinct stages of ESCRT-III accumulation, constriction, and disassembly during
296 abscission cannot be explained by a sequence of subunit binding.

297 Our findings call for re-interpretation of the terminal phenotype resulting from Vps4 depletion –
298 the accumulation of ESCRT-III polymers in endosomes (termed class E compartment in yeast)
299 that has led to the model of Vps4 serving predominantly as a disassembly factor^{26,28,33,50}. We
300 show that Vps4 is important for efficient ESCRT-III assembly, yet we also note that residual slow
301 ESCRT-III polymerization in the absence of Vps4 is sufficient to ultimately capture all
302 cytoplasmic subunits in class E compartments – owing to the extremely low intrinsic subunit
303 dissociation rates.

304 At the molecular level, Vps4 might promote the net growth of ESCRT-III assemblies by constant
305 turnover of Vps2 and Vps24 in side-by-side co-polymers along Snf7 filaments – to create growth-

306 competent free Snf7 filament tips (Fig. 8g). Furthermore, Vps4 might induce subunit turnover at
307 the core of ESCRT-III filament bundles, in analogy to interaction of the Vps4 homolog Spastin
308 with the lattice of microtubules⁵¹ (Fig. 8g). In contrast to previous models^{5,52}, however, Vps4 does
309 not cut an ESCRT-III helix during a single definite time point to induce tension release, but rather
310 continuously remodels filaments. Within bundled filaments, such turnover does not necessarily
311 lead to a complete breakage of larger structures.

312 The innermost parts of ESCRT-III spirals appear refractory to disassembly even at high
313 concentrations of Vps4, suggesting that ESCRT-III interaction with Vps4 might depend on
314 mechanical stress owing to low filament curvature or on filament polarity. Potential stochastic
315 fluctuations in subunit turnover rates could then lead to dynamic growth and shrinkage of
316 ESCRT-III spirals.

317 Vps4-induced subunit turnover in ESCRT-III assemblies might directly contribute to membrane
318 constriction. Indeed, macroscopic shape changes of many other cellular polymer structures
319 critically depend on dynamic subunit turnover within the constituent filaments, as for example
320 mitotic spindles⁵³ or actomyosin rings⁵⁴. In ESCRT-III assemblies, Vps4-induced subunit
321 turnover might facilitate sliding of adjacent helix turns, thereby promoting constriction of
322 mechanically pre-stressed, low-curved filaments into more relaxed high-curvature states⁴³. The
323 underlying bending forces could be generated by binding of Vps2 and Vps24 to Snf7⁴¹, or by
324 shortening-induced increase of filament rigidity. By revealing dynamic subunit turnover in
325 ESCRT-III assemblies, our study provides a framework for understanding how this highly
326 conserved membrane fission machinery adapts to diverse membrane geometries.

327

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349

AUTHOR CONTRIBUTIONS

350 B.E.M. designed, conducted, and analyzed all cell biological experiments, and analyzed part of
351 the HS-AFM data. N.C. designed, conducted, and analyzed in vitro reconstitution experiments
352 based on fluorescence microscopy. L.R-M. designed, conducted, and analyzed HS-AFM
353 experiments. J.M-v-F. and N.C. designed, conducted, and analyzed electron microscopy of in
354 vitro-assembled ESCRT-III polymers. J.K. and T.M-R designed, conducted, and analyzed
355 electron microscopy experiments of intercellular bridges. J.L. established the CHMP4B
356 purification and produced labelled CHMP4B. I.P. generated HeLa cells stably expressing
357 mmVPS4B-LAP. B.E.M., N.C., D.W.G., A.R. and S.S. conceived the project, analyzed data, and
358 wrote the manuscript.

359

COMPETING FINANCIAL INTERESTS

361 The authors declare no competing financial interests. Correspondence and requests for materials
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363 (aurelien.roux@unige.ch).

364

365 **REFERENCES**

- 366 1 Hurley, J. H. ESCRTs are everywhere. *EMBO J* **34**, 2398-2407 (2015).
- 367 2 Hanson, P. I. & Cashikar, A. Multivesicular body morphogenesis. *Annu Rev Cell Dev Biol*
368 **28**, 337-362 (2012).
- 369 3 Carlton, J. G. & Martin-Serrano, J. Parallels between cytokinesis and retroviral budding: a
370 role for the ESCRT machinery. *Science* **316**, 1908-1912 (2007).
- 371 4 Guizetti, J. *et al.* Cortical constriction during abscission involves helices of ESCRT-III-
372 dependent filaments. *Science* **331**, 1616-1620 (2011).
- 373 5 Elia, N., Sougrat, R., Spurlin, T. A., Hurley, J. H. & Lippincott-Schwartz, J. Dynamics of
374 endosomal sorting complex required for transport (ESCRT) machinery during cytokinesis
375 and its role in abscission. *Proc Natl Acad Sci U S A* **108**, 4846-4851 (2011).
- 376 6 Lafaurie-Janvore, J. *et al.* ESCRT-III assembly and cytokinetic abscission are induced by
377 tension release in the intercellular bridge. *Science* **339**, 1625-1629 (2013).
- 378 7 Mierzwa, B. & Gerlich, D. W. Cytokinetic abscission: molecular mechanisms and temporal
379 control. *Dev Cell* **31**, 525-538 (2014).
- 380 8 Vietri, M. *et al.* Spastin and ESCRT-III coordinate mitotic spindle disassembly and nuclear
381 envelope sealing. *Nature* **522**, 231-235 (2015).
- 382 9 Olmos, Y., Hodgson, L., Mantell, J., Verkade, P. & Carlton, J. G. ESCRT-III controls
383 nuclear envelope reformation. *Nature* **522**, 236-239 (2015).
- 384 10 Raab, M. *et al.* ESCRT III repairs nuclear envelope ruptures during cell migration to limit
385 DNA damage and cell death. *Science* **352**, 359-362 (2016).
- 386 11 Denais, C. M. *et al.* Nuclear envelope rupture and repair during cancer cell migration.
387 *Science* **352**, 353-358 (2016).
- 388 12 Jimenez, A. J. *et al.* ESCRT machinery is required for plasma membrane repair. *Science* **343**,
389 1247136 (2014).
- 390 13 von Schwedler, U. K. *et al.* The protein network of HIV budding. *Cell* **114**, 701-713 (2003).
- 391 14 Bleck, M. *et al.* Temporal and spatial organization of ESCRT protein recruitment during
392 HIV-1 budding. *Proc Natl Acad Sci U S A* **111**, 12211-12216 (2014).
- 393 15 Baietti, M. F. *et al.* Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. *Nat Cell*
394 *Biol* **14**, 677-685 (2012).
- 395 16 Choudhuri, K. *et al.* Polarized release of T-cell-receptor-enriched microvesicles at the
396 immunological synapse. *Nature* **507**, 118-123 (2014).
- 397 17 Matusek, T. *et al.* The ESCRT machinery regulates the secretion and long-range activity of
398 Hedgehog. *Nature* **516**, 99-103 (2014).
- 399 18 Henne, W. M., Stenmark, H. & Emr, S. D. Molecular mechanisms of the membrane
400 sculpting ESCRT pathway. *Cold Spring Harb Perspect Biol* **5** (2013).
- 401 19 McCullough, J., Colf, L. A. & Sundquist, W. I. Membrane fission reactions of the
402 mammalian ESCRT pathway. *Annu Rev Biochem* **82**, 663-692 (2013).
- 403 20 Peel, S., Macheboeuf, P., Martinelli, N. & Weissenhorn, W. Divergent pathways lead to
404 ESCRT-III-catalyzed membrane fission. *Trends Biochem Sci* **36**, 199-210 (2011).
- 405 21 Hurley, J. H. & Hanson, P. I. Membrane budding and scission by the ESCRT machinery: it's
406 all in the neck. *Nat Rev Mol Cell Biol* **11**, 556-566 (2010).

- 407 22 Guizetti, J. & Gerlich, D. W. ESCRT-III polymers in membrane neck constriction. *Trends*
408 *Cell Biol* **22**, 133-140 (2012).
- 409 23 Schmidt, O. & Teis, D. The ESCRT machinery. *Curr Biol* **22**, R116-120 (2012).
- 410 24 Schoneberg, J., Lee, I. H., Iwasa, J. H. & Hurley, J. H. Reverse-topology membrane scission
411 by the ESCRT proteins. *Nat Rev Mol Cell Biol* **18**, 5-17 (2017).
- 412 25 Christ, L., Raiborg, C., Wenzel, E. M., Campsteijn, C. & Stenmark, H. Cellular Functions
413 and Molecular Mechanisms of the ESCRT Membrane-Scission Machinery. *Trends Biochem*
414 *Sci* **42**, 42-56 (2017).
- 415 26 Babst, M., Katzmann, D. J., Estepa-Sabal, E. J., Meerloo, T. & Emr, S. D. Escrt-III: an
416 endosome-associated heterooligomeric protein complex required for mvb sorting. *Dev Cell*
417 **3**, 271-282 (2002).
- 418 27 Samson, R. Y., Obita, T., Freund, S. M., Williams, R. L. & Bell, S. D. A role for the ESCRT
419 system in cell division in archaea. *Science* **322**, 1710-1713 (2008).
- 420 28 Teis, D., Saksena, S. & Emr, S. D. Ordered assembly of the ESCRT-III complex on
421 endosomes is required to sequester cargo during MVB formation. *Dev Cell* **15**, 578-589
422 (2008).
- 423 29 Teis, D., Saksena, S., Judson, B. L. & Emr, S. D. ESCRT-II coordinates the assembly of
424 ESCRT-III filaments for cargo sorting and multivesicular body vesicle formation. *EMBO J*
425 **29**, 871-883 (2010).
- 426 30 Saksena, S., Wahlman, J., Teis, D., Johnson, A. E. & Emr, S. D. Functional reconstitution of
427 ESCRT-III assembly and disassembly. *Cell* **136**, 97-109 (2009).
- 428 31 Wollert, T., Wunder, C., Lippincott-Schwartz, J. & Hurley, J. H. Membrane scission by the
429 ESCRT-III complex. *Nature* **458**, 172-177 (2009).
- 430 32 Carlson, L. A. & Hurley, J. H. In vitro reconstitution of the ordered assembly of the
431 endosomal sorting complex required for transport at membrane-bound HIV-1 Gag clusters.
432 *Proc Natl Acad Sci U S A* **109**, 16928-16933 (2012).
- 433 33 Babst, M., Wendland, B., Estepa, E. J. & Emr, S. D. The Vps4p AAA ATPase regulates
434 membrane association of a Vps protein complex required for normal endosome function.
435 *EMBO J* **17**, 2982-2993 (1998).
- 436 34 Obita, T. *et al.* Structural basis for selective recognition of ESCRT-III by the AAA ATPase
437 Vps4. *Nature* **449**, 735-739 (2007).
- 438 35 Stuchell-Brereton, M. D. *et al.* ESCRT-III recognition by VPS4 ATPases. *Nature* **449**, 740-
439 744 (2007).
- 440 36 Lata, S. *et al.* Helical structures of ESCRT-III are disassembled by VPS4. *Science* **321**,
441 1354-1357 (2008).
- 442 37 Adell, M. A. *et al.* Coordinated binding of Vps4 to ESCRT-III drives membrane neck
443 constriction during MVB vesicle formation. *J Cell Biol* **205**, 33-49 (2014).
- 444 38 Yang, B., Stjepanovic, G., Shen, Q., Martin, A. & Hurley, J. H. Vps4 disassembles an
445 ESCRT-III filament by global unfolding and processive translocation. *Nat Struct Mol Biol*
446 **22**, 492-498 (2015).
- 447 39 Ghazi-Tabatabai, S. *et al.* Structure and disassembly of filaments formed by the ESCRT-III
448 subunit Vps24. *Structure* **16**, 1345-1356 (2008).
- 449 40 Pires, R. *et al.* A crescent-shaped ALIX dimer targets ESCRT-III CHMP4 filaments.
450 *Structure* **17**, 843-856 (2009).

- 451 41 Henne, W. M., Buchkovich, N. J., Zhao, Y. & Emr, S. D. The Endosomal Sorting Complex
452 ESCRT-II Mediates the Assembly and Architecture of ESCRT-III Helices. *Cell* **151**, 356-
453 371 (2012).
- 454 42 Shen, Q. T. *et al.* Structural analysis and modeling reveals new mechanisms governing
455 ESCRT-III spiral filament assembly. *J Cell Biol* **206**, 763-777 (2014).
- 456 43 Chiaruttini, N. *et al.* Relaxation of Loaded ESCRT-III Spiral Springs Drives Membrane
457 Deformation. *Cell* **163**, 866-879 (2015).
- 458 44 McCullough, J. *et al.* Structure and membrane remodeling activity of ESCRT-III helical
459 polymers. *Science* **350**, 1548-1551 (2015).
- 460 45 McMillan, B. J. *et al.* Electrostatic Interactions between Elongated Monomers Drive
461 Filamentation of Drosophila Shrub, a Metazoan ESCRT-III Protein. *Cell Rep* **16**, 1211-1217
462 (2016).
- 463 46 Hanson, P. I., Roth, R., Lin, Y. & Heuser, J. E. Plasma membrane deformation by circular
464 arrays of ESCRT-III protein filaments. *J Cell Biol* **180**, 389-402 (2008).
- 465 47 Cashikar, A. G. *et al.* Structure of cellular ESCRT-III spirals and their relationship to HIV
466 budding. *Elife* **3** (2014).
- 467 48 Babst, M., Sato, T. K., Banta, L. M. & Emr, S. D. Endosomal transport function in yeast
468 requires a novel AAA-type ATPase, Vps4p. *EMBO J* **16**, 1820-1831 (1997).
- 469 49 Lee, I. H., Kai, H., Carlson, L. A., Groves, J. T. & Hurley, J. H. Negative membrane
470 curvature catalyzes nucleation of endosomal sorting complex required for transport
471 (ESCRT)-III assembly. *Proc Natl Acad Sci U S A* **112**, 15892-15897 (2015).
- 472 50 Cashikar, A. G. *et al.* Structure of cellular ESCRT-III spirals and their relationship to HIV
473 budding. *Elife*, e02184 (2014).
- 474 51 Roll-Mecak, A. & Vale, R. D. Structural basis of microtubule severing by the hereditary
475 spastic paraplegia protein spastin. *Nature* **451**, 363-367 (2008).
- 476 52 Elia, N., Fabrikant, G., Kozlov, M. M. & Lippincott-Schwartz, J. Computational model of
477 cytokinetic abscission driven by ESCRT-III polymerization and remodeling. *Biophys J* **102**,
478 2309-2320 (2012).
- 479 53 Kline-Smith, S. L. & Walczak, C. E. Mitotic spindle assembly and chromosome segregation:
480 refocusing on microtubule dynamics. *Mol Cell* **15**, 317-327 (2004).
- 481 54 Pollard, T. D., Blanchoin, L. & Mullins, R. D. Molecular mechanisms controlling actin
482 filament dynamics in nonmuscle cells. *Annu Rev Biophys Biomol Struct* **29**, 545-576 (2000).
- 483 55 Zemp, I. *et al.* Distinct cytoplasmic maturation steps of 40S ribosomal subunit precursors
484 require hRio2. *J Cell Biol* **185**, 1167-1180 (2009).
- 485 56 Poser, I. *et al.* BAC TransgeneOmics: a high-throughput method for exploration of protein
486 function in mammals. *Nat Methods* **5**, 409-415 (2008).
- 487 57 Hein, M. Y. *et al.* A human interactome in three quantitative dimensions organized by
488 stoichiometries and abundances. *Cell* **163**, 712-723 (2015).
- 489 58 Schmitz, M. H. & Gerlich, D. W. Automated live microscopy to study mitotic gene function
490 in fluorescent reporter cell lines. *Methods Mol Biol* **545**, 113-134 (2009).
- 491 59 Lukinavicius, G. *et al.* Fluorogenic probes for live-cell imaging of the cytoskeleton. *Nat*
492 *Methods* **11**, 731-733 (2014).
- 493 60 Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nat Methods*
494 **9**, 676-682 (2012).

- 495 61 Morita, E. *et al.* Human ESCRT-III and VPS4 proteins are required for centrosome and
496 spindle maintenance. *Proc Natl Acad Sci U S A* **107**, 12889-12894 (2010).
- 497 62 Wollert, T. & Hurley, J. H. Molecular mechanism of multivesicular body biogenesis by
498 ESCRT complexes. *Nature* **464**, 864-869 (2010).
- 499 63 Aguet, F., Van De Ville, D. & Unser, M. Model-based 2.5-d deconvolution for extended
500 depth of field in brightfield microscopy. *IEEE Trans Image Process* **17**, 1144-1153 (2008).

501
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FIGURE LEGENDS

505

506 **Figure 1 | ESCRT-III assemblies at the midbody dynamically turn over subunits in early**
507 **and late abscission stages.** (a) Validation of mmCHMP4B-LAP functionality by RNAi
508 phenotype rescue. Cumulative histograms indicate the duration from complete cleavage furrow
509 ingresson until abscission for wildtype HeLa cells and for HeLa cells expressing mmCHMP4B-
510 LAP at 55-80 h after transfection of siRNAs (3 replicates with combined sample numbers of n =
511 48 for wildtype+siControl, n = 38 for wildtype+siCHMP4B, n = 60 for mmCHMP4B-
512 LAP+siControl, and n = 46 for mmCHMP4B-LAP+siCHMP4B). siCHMP4B (hs) targets only
513 endogenous human CHMP4B but not mmCHMP4B-LAP. (b) FRAP of mmCHMP4B-LAP at a
514 HeLa cell midbody at early and late abscission stages, stained with SiR-tubulin. Dashed circles
515 indicate photobleaching region; time 0 indicates first image after photobleaching. (c-d)
516 Fluorescence recovery curves for (c) early abscission (n = 18 from 4 replicates) or (d) late
517 abscission stages (n = 17 from 4 replicates). Single exponential function $f(t)=1-e^{-k*t}$, or
518 double exponential function $f(t)=A1*(1-e^{-k1*t})+(1-A1)*(1-e^{-k2*t})$ were fitted to the data.
519 Points and shaded areas indicate mean \pm SEM of fluorescence; dashed lines indicate fits of single
520 or double exponential functions. (e) Quantification of highly mobile fractions by fitting double
521 exponential functions to data from c, d. Dots represent individual cells. (f) 3D live-cell confocal
522 microscopy of the intercellular bridge during telophase, in HeLa cells expressing hsCHMP2B-
523 LAP or hsCHMP3-LAP, respectively, stained with SiR-tubulin. Arrowheads indicate abscission.
524 (g) Quantification of hsCHMP2B-LAP (n = 17 from 4 replicates), hsCHMP3-LAP (n = 13 from 3
525 replicates), and hsCHMP4B-LAP (n = 17 from 3 replicates) accumulation at the midbody. Points
526 and shaded areas indicate mean \pm SEM; normalized to intercellular bridge fluorescence after
527 cleavage furrow ingresson, and temporally aligned to abscission (time point 0). (h) Highly
528 mobile fractions of LAP-tagged ESCRT-III subunits derived from double exponential fits to
529 FRAP curves as in c, d. Each dot represents a single FRAP experiment acquired in 3 replicates;
530 bars indicate medians. (i) Residence times of highly mobile fractions for cells shown in h. Scale
531 bars, 1 μ m in b, f.

532

533 **Figure 2 | VPS4 is required for ESCRT-III accumulation and turnover.** (a) Confocal
534 microscopy of the intercellular bridge in HeLa cells expressing mmCHMP4B-LAP or
535 mmVPS4B-LAP, respectively, stained with SiR-tubulin. Arrowheads indicate abscission. (b)
536 Midbody accumulation of mmCHMP4B-LAP (n = 15 from 3 replicates) or mmVPS4B-LAP (n =
537 16 from 3 replicates) relative to abscission (time point 0). Points and shaded areas indicate mean
538 and SEM. (c) Live-cell images of telophase cells expressing mmCHMP4B-LAP after transfection
539 of a non-targeting control siRNA, or siRNAs targeting hsVPSP4A/B after 20 h or 48 h. Insets

540 show enlarged midbody regions. The same contrast settings were used for all panels. **(d)**
541 Quantification of cytoplasmic mmCHMP4B-LAP levels from data in e, f. Dots represent
542 individual cells from 3 replicates; bars indicate medians. **(e)** FRAP curves and double exponential
543 fits for mmCHMP4B-LAP at pre-constriction stages transfected with control siRNAs (n = 18
544 from 3 replicates) or siRNAs targeting VPS4A/B (n = 18 for siVPS4A/B 20h, and n = 17 for
545 siVPS4A/B 48h from 3 replicates). Points and shaded areas indicate mean \pm SEM. **(f)** Highly
546 mobile fractions of mmCHMP4B-LAP determined by double exponential fits to FRAP curves
547 shown in e (3 replicates with combined sample numbers of n = 9 for siControl 48h, n = 18 for
548 siVPS4A/B 20h, and n = 13 for siVPS4A/B 48h). Statistical test using the two-sided
549 Kolmogorov–Smirnov test yielded $P = 6.562e^{-3}$ for siControl 48h relative to siVPS4A/B 20h, and
550 $P = 4.021e^{-6}$ for siControl 48h relative to siVPS4A/B 48h. Dots represent individual cells; bars
551 indicate medians. Scale bars, 1 μ m in a; 5 μ m or 1 μ m (inset) in c.
552

553 **Figure 3 | VPS4 is required for constriction of the intercellular bridge.** **(a-b)** Transfection of
554 siRNAs targeting hsVPS4A/B causes abscission failure in wild-type HeLa cells, but not in HeLa
555 cells stably expressing mmVPS4B-LAP. **(a)** Progression from cleavage furrow ingression (time
556 point 0) until abscission in wildtype HeLa cells at 30-50 h after transfection of indicated siRNAs
557 (n = 84 for wildtype+siControl, and n = 80 for wildtype+siVPS4A/B for 3 fields of view from 2
558 replicates). **(b)** Rescue of abscission failure in HeLa cells stably expressing mmVPS4B-LAP
559 (data from a, n = 54 for mmVPS4B-LAP+siControl, and n = 45 for mmVPS4B-
560 LAP+siVPS4A/B). Bars and error bars indicate mean \pm SEM. **(c)** Representative electron
561 micrograph of an intercellular bridge of a control cell (n = 10, out of which 3 cells had filaments
562 without constriction, and 4 showed filaments with constriction). Arrowheads indicate 17 nm
563 diameter filaments. **(d)** Intercellular bridge of a cell 26 h after transfection of VPS4A/B siRNA (n
564 = 26, out of which 4 cells showed filaments without constriction). Arrowheads indicate 17 nm
565 diameter filaments. Scale bars, 200 nm in c, d.

566

567 **Figure 4 | Vps2 and Vps24 cooperatively bind Snf7 patches and inhibit ESCRT-III**
568 **polymerization.** **(a)** Time-lapse microscopy of ESCRT-III polymerization on supported lipid
569 membranes in a microfluidic flow chamber. Recombinant Snf7-AlexaFluor-488 was injected at t
570 = 0 min; Vps2-Atto-565 and Vps24 were added at t = 22 min. **(b)** Kymograph of a single
571 ESCRT-III patch from a. **(c-d)** Quantification of **(c)** mean fluorescence and **(d)** patch diameters
572 from 24 patches (quantified from 4 fields of view within a representative experiment, and
573 consistent results in 3 additional replicates using differently labeled proteins, e.g. Supplementary
574 Fig. 6a-c). Curves and shaded areas represent mean \pm SEM. **(e)** Kymograph of an experiment
575 where Snf7-AlexaFluor-647N was added at t = 0 min, followed by sequential addition of Vps24-
576 AlexaFluor-488 and Vps2-Atto-565 (representative image from 24 patches within the shown
577 experiment, and one additional replicate). **(f)** Kymograph of an ESCRT-III patch, where Snf7-
578 AlexaFluor-488 was added at t = 0 min, then washed out during 28-32 min (shaded area),
579 followed by addition of Vps2-Atto-565 and Vps24 at t = 47 min. The transient increase of Vps2
580 signal during washout resulted from background ambient light. **(g)** Fluorescence quantification of
581 37 patches (analyzed from 4 fields of view within the shown experiment, and 3 additional
582 replicates). Curves and shaded areas represent mean \pm SEM. Scale bars, 5 μ m in a; 5 μ m
583 (vertical) and 5 min (horizontal) in b, e, f.
584

585 **Figure 5 | Vps2 and Vps24 polymerize side-by-side with Snf7 to form filament bundles.** (a)
586 Transmission electron microscopy of Snf7 spirals polymerized on liposomes. Colored overlays
587 indicate the number of parallel filament strands. (b) Distribution of filament bundle lengths
588 quantified in 11 spirals (from 3 replicates). (c) Snf7 was polymerized on liposomes, followed by
589 Vps2 and Vps24 addition. Colored overlays indicate the number of parallel filament strands. (d)
590 Quantification of 17 spirals (from 2 replicates). (e) Examples of filament morphologies with
591 different strand numbers, corresponding to colored overlays used in a-d. (f) Averaged line
592 profiles across ESCRT-III filament bundles from a-d (n = 3 for 1 strand, n = 8 for 2 strands, n = 3
593 for 3 strands, n = 8 for 4 strands; and n = 3 for 6 strands). Curves and shaded areas indicate mean
594 \pm SEM. (g-i) HS-AFM imaging of ESCRT-III polymers on supported lipid membranes. Snf7 was
595 polymerized on lipid membranes, followed by addition of Vps2 and Vps24 at t = 0. (g) Spiral
596 morphology before and after addition of Vps2 and Vps24. Green and magenta lines indicate line
597 profiles used to (h) measure height variability. (i) Height variability was measured as coefficient
598 of variation along radial line profiles within spirals before and after addition of Vps2 and Vps24,
599 respectively, as shown in g, h (n = 28 for Snf7, and n = 26 for Snf7+Vps2+Vps24 from 2
600 replicates). Statistical test using the two-sided Kolmogorov–Smirnov test yielded $P = 2.875e-14$
601 for Snf7 relative to Snf7+Vps2+Vps24. Dots represent individual line profile measurements; bars
602 indicate medians. Scale bars, 50 nm in a, c, e; 200 nm in g.

603

604 **Figure 6 | Kinetics of ESCRT-III patch disassembly by Vps4.** (a) Kymograph of a
605 representative patch from time-lapse microscopy of Snf7 patches on supported lipid membranes
606 in a flow chamber. A solution of Snf7-AlexaFluor-488 was injected into the flow chamber and
607 incubated until patches polymerized on the membrane. Snf7 was washed out at t = 22 min,
608 followed by addition of ATP and 8 μ M Vps4 at t = 33 min. (b) Quantification of mean
609 fluorescence of 13 patches as in a (4 different fields of view within the shown experiment, and
610 one additional replicate). Curves and shaded areas indicate mean \pm SEM. (c) A solution of Snf7-
611 AlexaFluor-488 was injected into the flow chamber and incubated until patches polymerized. At t
612 = 34 min, Snf7 was removed from the solution and Vps2-Atto-565, Vps24, Vps4, and ATP were
613 injected. (d) Quantification of mean fluorescence of 33 patches from c (4 fields of view within
614 the shown experiment, and one additional replicate). Curves and shaded areas indicate mean \pm
615 SEM. (e-g) Vps4-induced dynamic turnover and lateral mobility of ESCRT-III filament spirals
616 depends on ATP. (e) HS-AFM imaging of ESCRT-III spirals. Assemblies were generated by
617 polymerization of Snf7 on supported lipid membranes, followed by addition of Vps2 and Vps24.
618 After washout of all soluble components, Vps4 was injected. Then, ATP and Mg^{2+} were added,
619 and imaging was started 22 s later (t = 0). Images represent averages of 2 consecutive time frames
620 to improve signal-to-noise ratio. (f) Quantification of spiral diameters from f (119 spirals from a
621 representative experiment, out of 6 independent replicates). Dots represent single spirals; bars
622 indicate medians. (g) Snf7 was polymerized on supported lipid membranes, followed by addition
623 of Vps2 and Vps24, and subsequent addition of Vps4, as in Fig. 8a but without ATP. Imaging
624 was started 30 s after addition of Vps4 (t = 0). Images represent averages of 3 consecutive time
625 frames. Corresponding spiral diameter quantification is shown in Fig. 8c. Scale bars, 5 μ m
626 (vertical) and 5 min (horizontal) in a, c; 100 nm in e; 200 nm in g.

627

628 **Figure 7 | Vps4 induces subunit turnover and net growth of ESCRT-III assemblies.** (a)
629 Time-lapse microscopy of ESCRT-III polymerization on supported lipid membranes. Snf7-
630 AlexaFluor-488 was injected at t = 0 min. Vps2-Atto-565 and Vps24 were added at t = 36 min

631 while maintaining Snf7 in the solution. At $t = 45$ min, Snf7-AlexaFluor-488 was removed and a
632 mix containing Snf7-Atto-647N, Vps2-Atto-565, Vps24 and Vps4 was added, followed by
633 addition of ATP at $t = 54$ min. **(b)** Kymograph of a single patch from a. **(c)** Mean fluorescence
634 quantification of 35 patches (4 fields of view within the shown experiment, and consistent results
635 in 2 additional replicates using differently labeled proteins). Curves and shaded areas represent
636 mean \pm SEM. **(d-e)** Time-lapse microscopy of in vitro polymerization as in a, but for a mixed
637 solution containing Snf7-AlexaFluor-488, Vps2-Atto-565, Vps24, and ATP **(d)** in the presence of
638 Vps4, or **(e)** without Vps4. Representative images of 2 replicates per condition are shown. Scale
639 bars, 10 μm in a, d, e; 5 μm (vertical) and 5 min (horizontal) in b.

640

641 **Figure 8 | Vps4 induces dynamic reorganization of ESCRT-III assemblies.** HS-AFM imaging
642 of ESCRT-III polymers on supported lipid membranes. **(a)** Snf7 was polymerized on supported
643 lipid membranes, followed by addition of Vps2, Vps24, and Vps4. Then, Mg^{2+} and ATP were
644 added and imaging was started 5.5 min later ($t = 0$). Overlays highlight pre-formed spirals (blue)
645 or newly formed spirals (orange). Bottom panels show a close-up of the nucleation of a new
646 spiral. Images represent averages of 3 consecutive time frames to improve the signal-to-noise
647 ratio. Scale bars, 200 nm (top panel) or 5 nm (bottom panel). **(b)** Quantification of spiral
648 diameters from a (274 spirals from a representative experiment, out of 3 independent replicates).
649 Dots represent single spirals; bars indicate medians. **(c)** Quantification of spiral diameters from
650 Fig. 6g (175 spirals from a representative experiment, from a total of 6 independent replicates).
651 Dots represent single spirals; bars indicate medians. **(d)** Tracking of spiral centers from a. **(e)**
652 Tracking as in d, but for an experiment without ATP and Mg^{2+} as shown in Fig. 6g. **(f)**
653 Quantification of mean velocity of spiral centers from d, e ($n = 34$ for with ATP, and $n = 31$ for
654 without ATP). Statistical test using the two-sided Kolmogorov–Smirnov test yielded $P = 4.441\text{e-}$
655 16 for spiral velocities in the presence of ATP relative to velocities in the absence of ATP. Dots
656 represent single tracks; bars indicate medians. **(g)** Model of dynamic ESCRT-III assembly and
657 constriction. Vps4 mediates continuous subunit turnover in ESCRT-III assemblies during growth
658 and constriction. (1) At the tip, dynamic turnover of growth-inhibitory Vps2 and Vps24 subunits
659 could sustain extension of inward-curving filaments. (2) At the core of filament bundles, Vps4-
660 mediated subunit turnover could facilitate sliding of neighboring helical turns to promote
661 constriction.

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664

665 SUPPLEMENTARY INFORMATION

666 Supplementary Information includes:

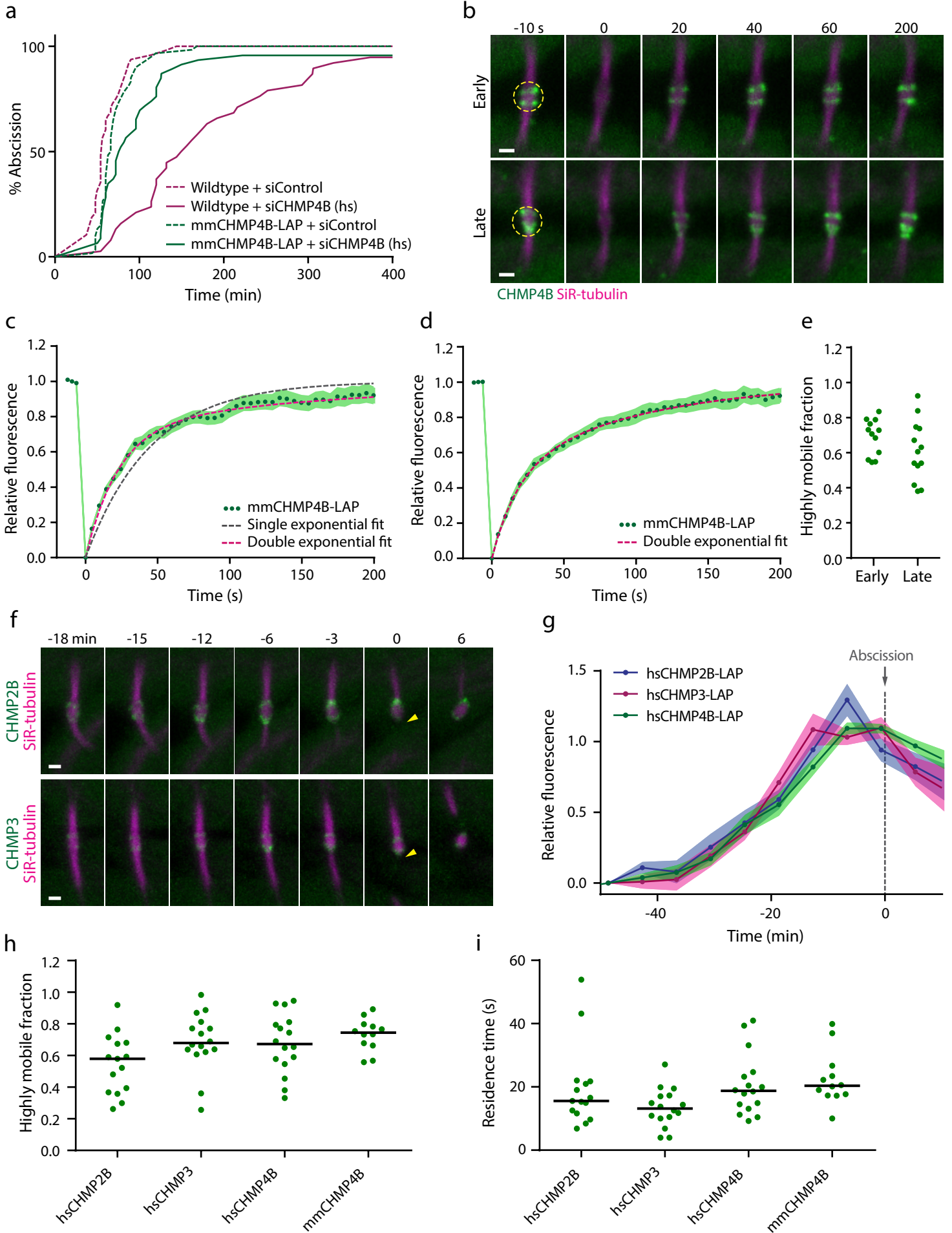
667 Supplementary Figures 1-8

668 Supplementary Videos 1-12

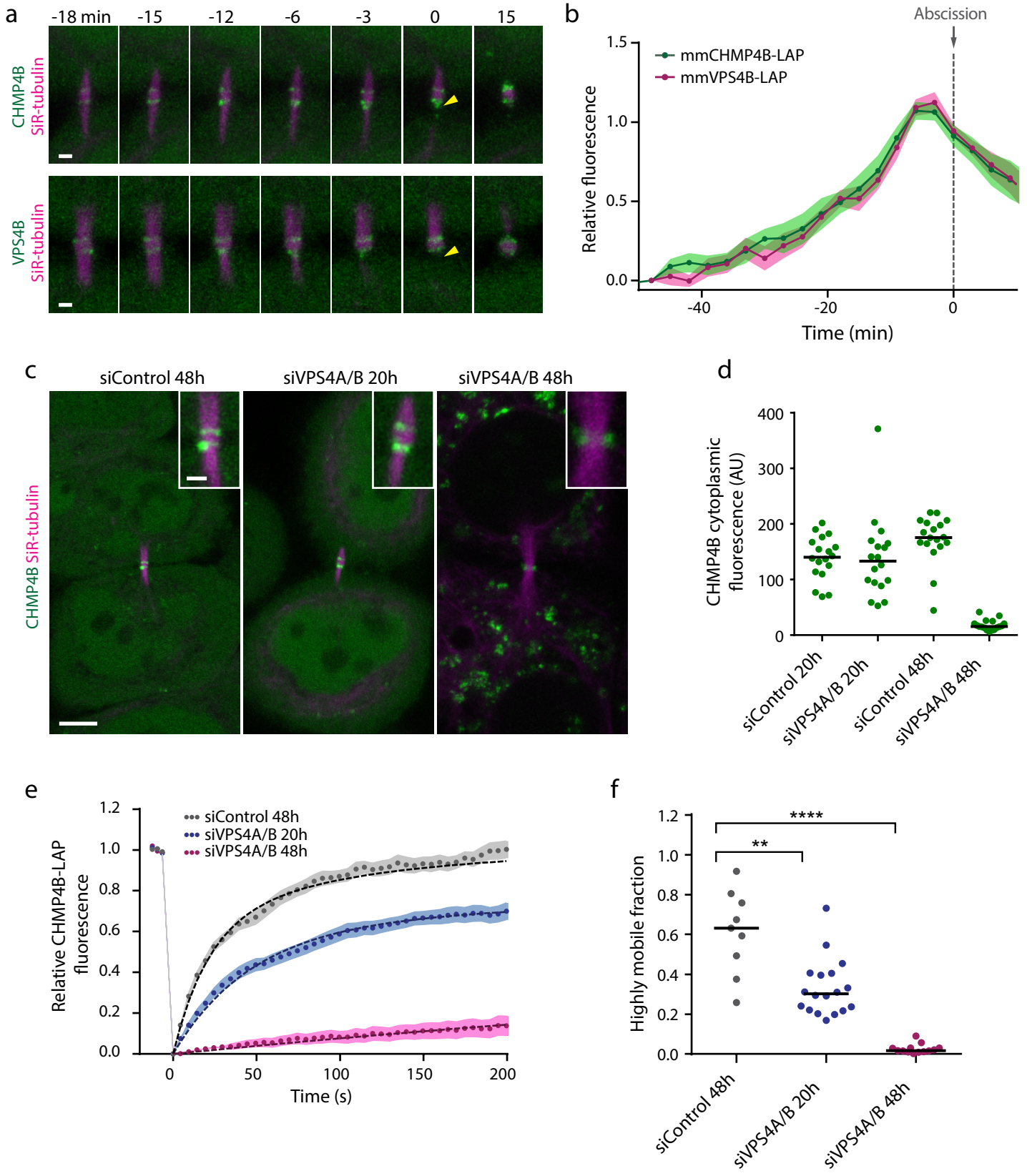
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670

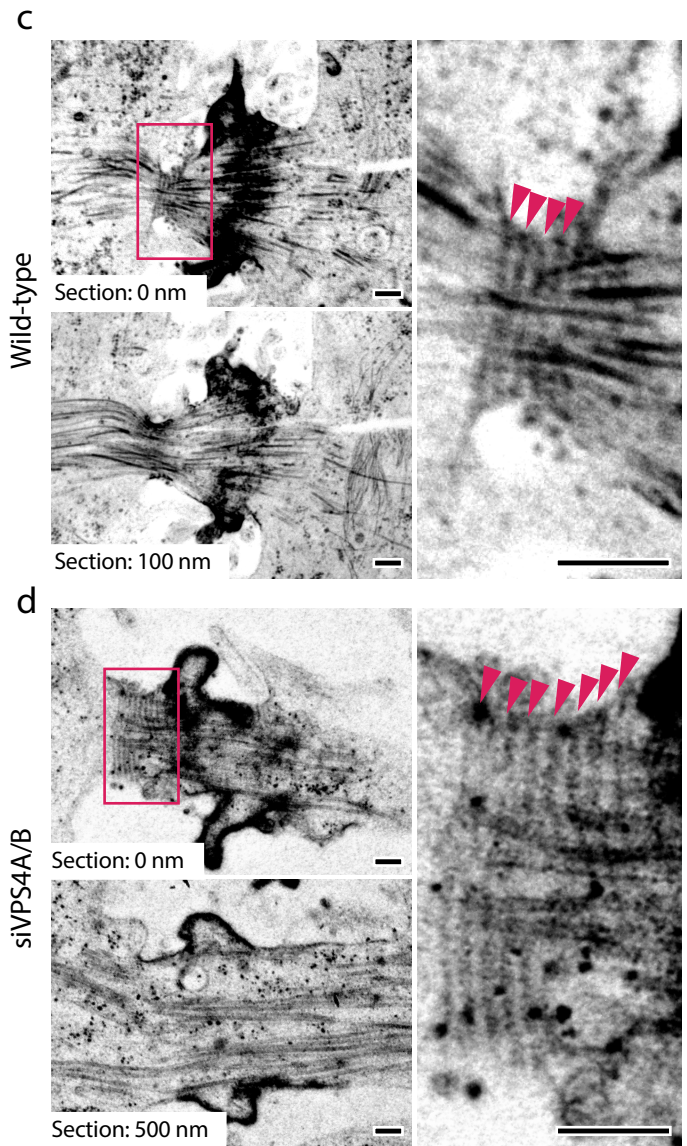
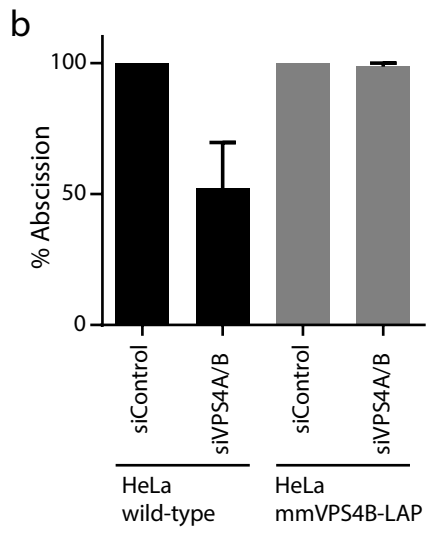
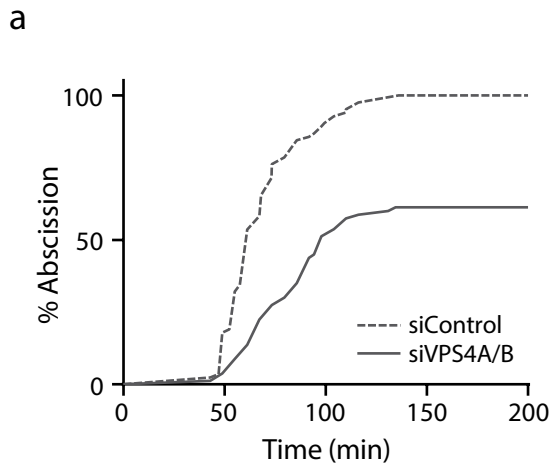
Mierzwa et al., Fig. 1



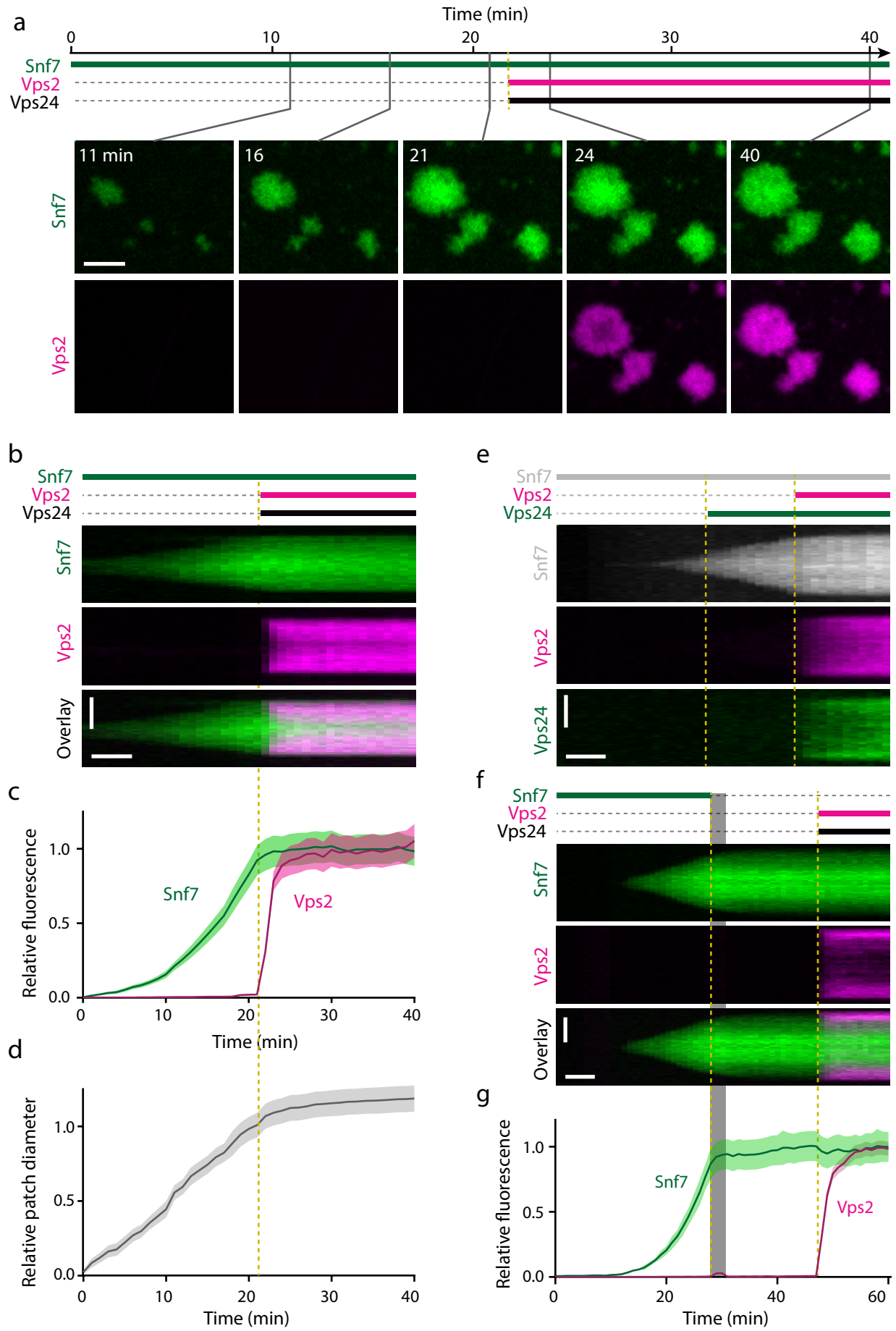
Mierzwa et al., Fig. 2



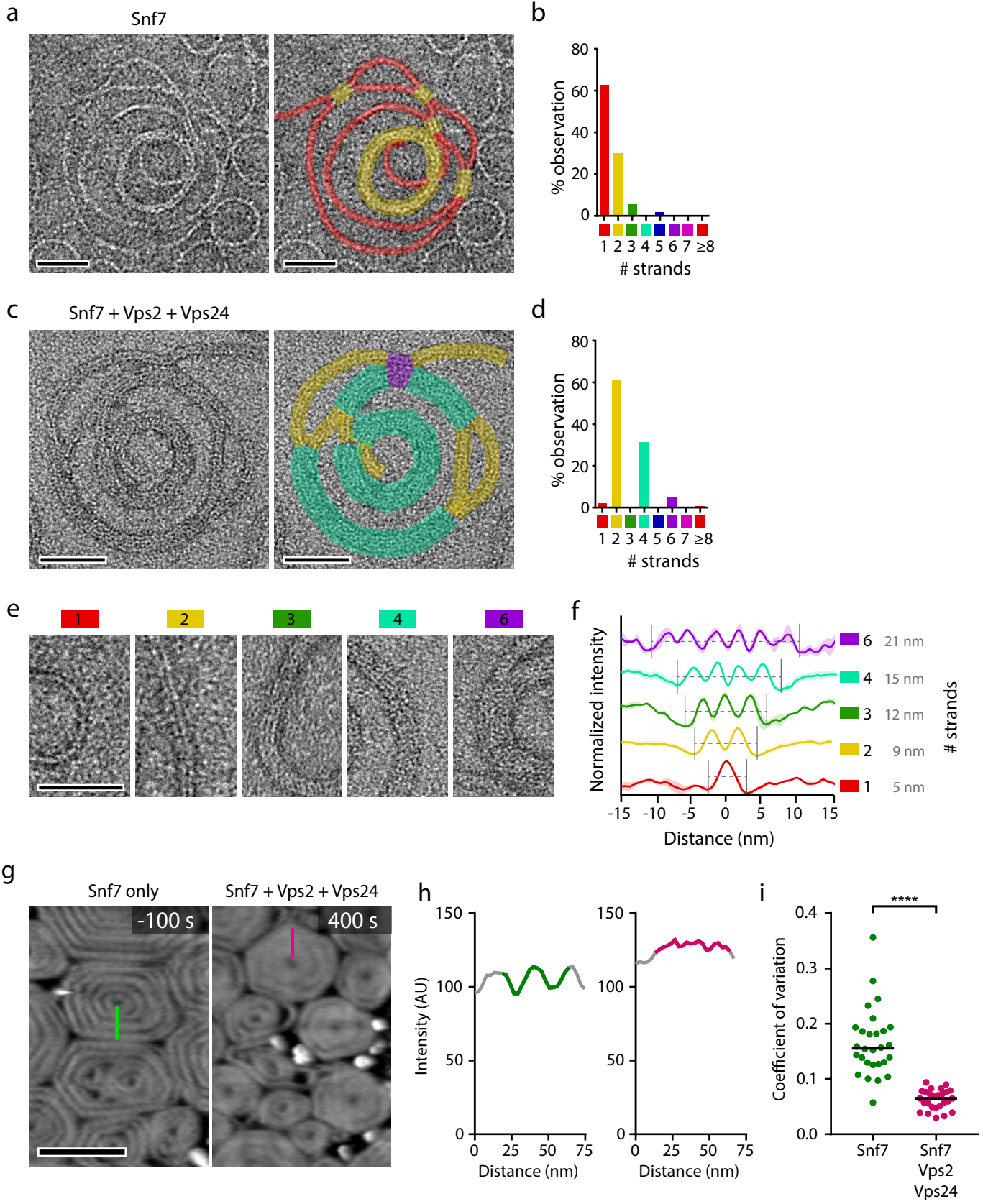
Mierzwa et al., Fig. 3



Mierzwa et al., Fig. 4



Mierzwa et al., Fig. 5



Mierzwa et al., Fig. 6

