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The ESCRT-III isoforms CHMP2A and CHMP2B display different effects on membranes upon polymerization

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Abstract

Background: ESCRT-III proteins are involved in many membrane remodeling processes including multivesicular body biogenesis as first discovered in yeast. In humans, ESCRT-III CHMP2 exists as two isoforms, CHMP2A and CHMP2B, but their physical characteristics have not been compared yet.

Results: Here, we use a combination of techniques on biomimetic systems and purified proteins to study their affinity and effects on membranes. We establish that CHMP2B binding is enhanced in the presence of PI(4,5)P2 lipids. In contrast, CHMP2A does not display lipid specificity and requires CHMP3 for binding significantly to membranes. On the micrometer scale and at moderate bulk concentrations, CHMP2B forms a reticular structure on membranes whereas CHMP2A (+CHMP3) binds homogeneously. Thus, CHMP2A and CHMP2B unexpectedly induce different mechanical effects to membranes: CHMP2B strongly rigidifies them while CHMP2A (+CHMP3) has no significant effect.

Conclusions: We therefore conclude that CHMP2B and CHMP2A exhibit different mechanical properties and might thus contribute differently to the diverse ESCRT-III-catalyzed membrane remodeling processes.

Keywords: Endosomal sorting complexes Required for Transport (ESCRT), Reconstituted system, Bottom up approach, Lipid-protein interactions, Membrane, Mechanical properties, Giant unilamellar vesicles (GUV), Micropipette, Atomic force microscopy (AFM)

Background

The endosomal sorting complex required for transport (ESCRT-III) complex is involved in a variety of cellular processes such as biogenesis of multivesicular bodies (MVB), plasma membrane wound repair, neuron pruning, dendritic spine formation, nuclear envelope repair or nuclear envelope sealing during telophase, abscission at a late step of cytokinesis, and budding and release of some enveloped viruses from the plasma membrane of infected cells. In Saccharomyces cerevisiae, the ESCRT-III protein complex comprises four core subunits: Vps20, Vps24, Vps2, and Snf7 (vacuolar sorting proteins 20, 24, 2, and sucrose non-fermenting protein 7), whereas in Homo sapiens, up to 13 proteins form the ESCRT-III family called charged multivesicular body protein (CHMP1–8; IST1) (Additional file 1: Figure S1-A). The increased number of ESCRT-III subunits in Homo sapiens reflects the functional diversification of the complex in higher organisms.
In yeast, the sequence of recruitment of ESCRT-III proteins during MVB formation is Vps20-Snf7-Vps24-Vps2, forming a core complex [12]. Their human homologs are respectively CHMP6-CHMP4 (A, B, C)-CHMP3-CHMP2 (A, B). Both CHMP2A and CHMP2B present a high sequence homology with the yeast protein Vps2 and have therefore been considered isoforms. Indeed, CHMP2B appears to be a relatively recent acquisition in the evolution of the ESCRT-III complex resulting from a Vps2 gene duplication event [11] (Additional file 1: Figure S1-B). Together, CHMP2A and CHMP2B act in most ESCRT-catalyzed membrane remodeling processes, except in MVB formation [5], where CHMP2A but not CHMP2B is required, and neuronal pruning which requires CHMP2B but not CHMP2A. Yet so far, the dual roles of CHMP2A and CHMP2B in the interaction and remodeling of membranes remain unclear [6, 13–17] (Additional file 1: Figure S1-C).

ESCRT-III proteins cycle between an inactive cytosolic state [18–20] and an activated state [21–23] leading to filamentous polymers forming spirals or helical tubular structures in vitro and in vivo [19, 24–42].

Purified recombinant CHMP2A can coil up into flat snail-like structures [43] or form helical tubular polymers with CHMP3 in the absence of membrane [25]. On the other hand, overexpressed CHMP2B in cells [32] leads to the formation of tubular helical structures, but in vitro assembly of recombinant CHMP2B has never been visualized, neither alone nor together with CHMP3. SiRNA knockdown of individual ESCRT-III proteins demonstrated a minimal requirement of one CHMP4 and one CHMP2 member for HIV-1 release [14]. Furthermore, CHMP3 acts synergistically with CHMP2A but not CHMP2B [44], indicating a distinct role for CHMP2B independently of CHMP3. In contrast, both CHMP2A and CHMP2B are important for cytokinesis [45]. So far, CHMP2A and CHMP2B have been considered as functional homologs, but practically no study has questioned yet whether CHMP2A and CHMP2B behave similarly upon binding to membranes to validate this hypothesis.

Biophysical in vitro membrane models, albeit their limitations, have provided important new insight into membrane remodeling processes in general [46, 47] as well as into ESCRT function, such as CHMP2B acting as a diffusion fusion barrier [48], the role of membrane curvature for ESCRT-III interaction [49, 50], ESCRT-III polymerization on supported lipid bilayers [30, 51], ESCRT-III polymerization on membranes [35], and ESCRT-catalyzed membrane scission [39, 52].

Here, we have investigated in vitro the functional homology of CHMP2A and CHMP2B in the ESCRT machinery, using biomimetic membrane systems with purified CHMP proteins. We have compared their membrane binding and their mechanical effects on membrane by confocal microscopy, flow cytometry (FACS), quartz crystal microbalance with dissipation monitoring (QCM-D), and high-speed atomic force microscopy (HS-AFM). We further investigated the role of charged lipids for membrane interaction as well as the role of CHMP3 on the polymerization of CHMP2A and CHMP2B, respectively. We confirm that CHMP3 works synergistically with CHMP2A for enhancing their mutual binding towards membranes, but reduces the binding of CHMP2B. We establish that CHMP2B binding is enhanced in the presence of PI(4,5)P2 lipids forming a protein network on the membrane surface, whereas CHMP2A+CHMP3 interact homogenously with membranes via electrostatic interactions without phosphoinositide binding specificity. Moreover, we study the mechanical properties of membranes coated with these different ESCRT assemblies. We show by micropipette aspiration, osmotic shock, and HS-AFM deformation that CHMP2A and CHMP2B have different mechanical effects on the membrane. While CHMP2B highly rigidifies membranes, CHMP2A+CHMP3 have almost no effect on it. Together, our study demonstrates that CHMP2A and CHMP2B cannot be considered as functional homologs. Thus, the observed different mechanical properties are likely important for understanding the mechanics of membrane remodeling and membrane scission.

**Results**

**CHMP2B and CHMP2A display different membrane binding characteristics**

Phosphoinositides constitute a minority of the phospholipid family with a concentration lower than 1% in cell membranes. Nevertheless, PIP lipids play an essential role for signaling in cells. PI(3)P is the main phosphatidylinositol present in the endosomal compartments of the MVB pathway where the ESCRTs were first identified, and this lipid has been used in purified systems to reconstitute MVB formation using yeast proteins [53]. However, ESCRT-III-mediated processes also occur on membranes enriched in PI(4,5)P2, notably at the plasma membrane, including for instance HIV-1 egress, plasma membrane repair, and cytokinesis events, or at the nuclear envelope [54, 55]. We have thus first compared the interactions of CHMP2A and CHMP2B with membranes containing different phosphatidylinositides. To improve protein solubilization, CHMP2A was fused to MBP. In order to exclude effects of the MBP fusion, CHMP2B function was analyzed in parallel to MBP-CHMP2B.

A previous in vitro study [48] has shown that the interaction of CHMP2B with membrane is significantly enhanced in the presence of PI(4,5)P2 lipids in comparison with DOPS or PI(3,5)P2- membranes. Thus, we compared the preferential binding of CHMP2A versus
CHMP2B on GUVs containing 10% PI(4,5)P2 using confocal imaging. 10% PI(4,5)P2-GUV (see composition 1 in the “Methods” section) were incubated for 30 min with CHMP2A or CHMP2B proteins at a concentration of 500 nM in the protein binding buffer (BP buffer), which has been optimized to ensure the highest protein density on the GUV membrane (Additional file 2: Figure S2-A). CHMP2A and CHMP3, respectively. After incubation, the displacement or truncation of the C-terminal region of CHMP proteins facilitates activation of ESCRT-III proteins polymerization on membranes [19, 21, 22, 48]. Thereupon, C-terminal truncated of MBP-CHMP2A-ΔC and CHMP2B-ΔC were used in the following experiments.

While CHMP2B-ΔC shows a homogenous binding to the GUV (Fig. 1a, first panel), the interaction of MBP-CHMP2A-ΔC is rather weak under the same conditions (Fig. 1a, third panel). Although MBP cleavage increases the interaction, it also induces aggregation of CHMP2A-ΔC in solution and on the membrane, resulting in the formation of aggregates containing lipids and proteins (Additional file 2: Figure S2-B). However, to test the effect of the MBP tag, we compared the properties of MBP-CHMP2B-ΔC to that of CHMP2B-ΔC and showed they have the same membrane binding properties and mechanical effect on the membrane (Additional file 2: Figure S2-C and D), indicating that the MBP fusion does not per se affect membrane binding.

Previous experiments have shown that in solution, combinations of MBP-CHMP2A-ΔC and CHMP3-ΔC as well as MBP-CHMP2A-ΔC and CHMP3-FL co-polymerize to form tubular helical structures more efficiently than combinations of CHMP2A-FL and CHMP3-FL or CHMP2A-FL and CHMP3-ΔC [25]. We have thus tested the effect of CHMP3-FL on the polymerization of MBP-CHMP2A-ΔC. In the following experiments, MBP-CHMP2A-ΔC, CHMP2B-ΔC, and CHMP3-FL will be referred to as CHMP2A, CHMP2B, and CHMP3, respectively. After incubation of 10% PI(4,5)P2-GUVs with CHMP2A (or CHMP2B) + CHMP3 (500 nM and 2 μM respectively in BP buffer), we found that CHMP2A strongly binds to GUVs in the presence of CHMP3 (Fig. 1a, fourth panel). The quantification of the fluorescence intensity (see details in the “Methods” section) of CHMP2A on GUVs by confocal microscopy shows that the binding of CHMP2A to the membrane is increased by a factor of at least 2.5 in the presence of CHMP3 (Fig. 1b).

Unexpectedly, when CHMP3 is incubated with CHMP2B, the binding of CHMP2B is no longer homogenous and appears as patches on the GUV co-localizing with CHMP3 (Fig. 1a, second panel). The relative amount of CHMP2B on the membrane is decreased by a factor of 2 as compared to the relative CHMP2B amount measured in the absence of CHMP3 (Fig. 1b).

To quantify the amount of protein bound to the GUV membrane with higher statistics, we have used flow cytometry (FACS) [56] and 2% PI(4,5)P2-GUVs incubated with a combination of CHMP2A or CHMP2B with and without CHMP3, respectively, at 500 nM for both CHMP2A and CHMP2B proteins and 2 μM for CHMP3 for 30 min. The fluorescence intensity of the membrane and of the proteins is proportional to the amount of fluorophores in the membrane and proteins bound to it or present in the detection zone, respectively. From the plot of the protein intensity versus lipid signal for all recorded events, we could determine the signals corresponding to CHMP proteins bound to GUVs and plot the corresponding histogram of these intensities for the different conditions. The median value of this histogram is related to the average density of proteins bound to GUVs. When CHMP2A or CHMP3 are incubated alone with the 2% PI(4,5)P2-GUV suspension, an extremely weak signal is detected by FACS. However, as previously observed by confocal microscopy, binding increases significantly by almost 100×, when both proteins are incubated together, in comparison to CHMP2A alone (Fig. 1c). On the contrary, the presence of CHMP3 decreases the binding efficiency of CHMP2B—by approximately 150% (Fig. 1c). Previous experiments with surface plasma resonance (SPR) have studied the interactions of MBP-CHMP2A-ΔC with CHMP3 and CHMP2B with CHMP3 in solution [44]. CHMP2A and CHMP3 interacted with a $K_D$ of 3.2 μM and CHMP2B-CHMP3 interacted with a $K_D$ of 1.4 μM. In the present work, the protein concentrations are lower than these $K_D$ values. As a consequence, CHMP2A and CHMP2B have a stronger interaction with the membrane than their mutual interaction. We conclude that CHMP2A and CHMP3 synergize in binding to membranes, while CHMP3 might act as a negative regulator for CHMP2B membrane binding in vitro.

All the previous experiments were performed with GUVs containing PI(4,5)P2 lipids. In vivo, membranes are enriched with different PIP species depending on their localization. We thus wondered if the behavior of CHMP2A and CHMP2B in the presence or absence of CHMP3 would be affected by the incorporation of other phosphoinositides in the membrane. In the following, we tested the effect of PIP specificities and the effect of the charge. We thus performed experiments at a constant PIP fraction/concentration or at a constant charge ratio.

GUVs were produced with 2% PI(3)P, PI(3,5)P2, PI(4)P, or PI(4,5)P2 (see composition 2 in the “Methods” section), which are enriched at the early endosomes, late endosomes, endoplasmic reticulum/Golgi, and plasma membrane, respectively [54]. They were then incubated with CHMP2A+CHMP3 or CHMP2B alone or in
combination with CHMP3 for 30 min to optimize the protein coverage on the membrane. The amount of protein bound to the GUV membrane was analyzed by FACS. The median values of the histograms of binding efficiency for the different PIP species (Additional file 3: Figure S3-A) were normalized by the mean value of the

**Fig. 1** Interaction of CHMP2A versus CHMP2B with charged model membranes. a Confocal images of 10% PI (4,5)P2-GUVs incubated with 500 nM CHMP2BΔC (first line) (called CHMP2B) and MBP-CHMP2AΔC (third line) (called CHMP2A) alone or in combination with 2 μM CHMP3 (second and fourth lines, respectively). A single confocal plane is shown. Scale bar, 10 μm. Note that in the case of MBP-CHMP2AΔC (third line), the laser intensity in the protein channel has been increased (as visible by the higher background intensity) to detect protein binding on the GUV membrane. b Effect of CHMP3 on MBP-CHMP2AΔC and CHMP2BΔC binding to 10% PI (4,5)P2-GUVs (same conditions as in a). The fluorescence intensity was measured from the analysis of spinning disk microscopy images using the Cell Profiler software. The fluorescence intensity of MBP-CHMP2AΔC+CHMP3 and CHMP2BΔC+CHMP3-covered vesicles was normalized to the intensity of MBP-CHMP2AΔC and CHMP2BΔC-covered vesicles, respectively. **p-value < 0.01; ***p-value < 0.001 (Student’s t-test). N = 48. c Quantification by FACS of the fluorescence intensities of MBP-CHMP2AΔC±CHMP3, CHMP2BΔC±CHMP3 and CHMP3 co-polymers bound to 10% PI(4,5)P2-containing GUVs. The concentrations of CHMP2A/B and CHMP3 proteins are, respectively, 500 nM and 2 μM. *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001 (Student’s t-test). N = 4 (number of FACS experiment with about 10^4 counted events per experiment, per condition). d Quantification of CHMP2B-FL, CHMP2BΔC, and MBP-CHMP2AΔC (CHMP2A)+CHMP3 binding to GUVs containing DOPS and different PIPs by flow cytometry (FACS). Equimolar amount of DOPS and different PIPs (2% mol/mol of total lipids) have been used. Note that data on CHMP2BΔC binding to DOPS, PI(4,5)P2, and PI(3,4,5)P3 were already published [48]. Binding efficiencies were normalized to the fluorescence intensity of DOPS-containing vesicles (see Figure S2D). These values were then normalized to the total amount of charge for each lipid composition. *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001 (Student’s t-test). N = 6 (number of FACS experiment with about 10^4 counted events per experiment, per condition). e Resonance frequency shift ϑ5 in the QCM-D experiments when CHMP2BΔC is bound to the different types of supported lipid bilayers (light gray 30% DOPS, 70% DOPC; gray 40% DOPS, 60% DOPC; light blue 10% DOPS, 10% PI(4,5)P2, 80% DOPC; magenta 10% DOPS, 10% PI(3,4,5)P3, 80% DOPC). *p-value < 0.05; **p-value < 0.01; ***p-value < 0.001 (Student’s t-test). N = 5
distribution of proteins bound to DOPS vesicles (control GUV without PIPs) (Additional file 3: Figure S3-B). The binding of CHMP2A+CHMP3 is almost identical, within the error bars, for all the conditions tested when GUVs are doped with a nominal constant fraction of phosphoinositide.

To test the charge effect, the binding efficiencies have been normalized by the charge of each PIP species, considering that DOPS has a net charge of $-1$, $\text{P}(4,5)\text{P}_2$ (or $\text{P}(3,5)\text{P}_2$) of $-3$ at pH 7.5, and $\text{P}(4)\text{P}$ (or $\text{P}(3)\text{P}$) of $-2$ [57] (Fig. 1d). The binding of CHMP2A+CHMP3 is almost identical for all PIP species, except for $\text{P}(3,5)\text{P}_2$, which shows a decrease by a factor of 2.8 ($p$-value = 0.008) in comparison to DOPS GUVs (Fig. 1d). After charge renormalization, the binding of CHMP2B is identical for GUVs containing DOPS and all types of PIP species tested, except for $\text{P}(3,5)\text{P}_2$ (Fig. 1d). We did not measure a significantly higher binding efficiency of CHMP2B for $\text{P}(4,5)\text{P}_2$ membranes than for pure DOPS membranes (Fig. 1d, $p$-value = 0.04); nevertheless, for this specific composition, binding is much stronger for CHMP2B than CHMP2A+CHMP3 (Fig. 1d). Indeed, the binding of CHMP2B to $\text{P}(4,5)\text{P}_2$ containing membranes is 2.8 times higher than the binding of CHMP2A+CHMP3 ($p$-value = 0.03). The binding of CHMP2B is almost doubled on $\text{P}(4,5)\text{P}_2$ membranes compared to $\text{P}(3,5)\text{P}_2$ ($p$-value between $\text{P}(4,5)\text{P}_2$ and $\text{P}(3,5)\text{P}_2$ GUVs = 0.03). In contrast, we did not observe such a preference for CHMP2B-FL (Fig. 1d).

In summary, employing GUVs with a nominal constant concentration of PIP species, CHMP2A+CHMP3 do not seem to exert a specificity for PIP, whereas CHMP2B shows a strong increase in its binding capacity in the presence of $\text{P}(4,5)\text{P}_2$ before charge normalization. In addition, after charge normalization, the binding efficiency of CHMP2B and CHMP2A+CHMP3 is lower for $\text{P}(3,5)\text{P}_2$.

One of the main difficulties when working with $\text{P}(4,5)\text{P}_2$ GUV is that this lipid can exchange with the surrounding bulk [54, 55]. We thus checked with a complementary technique whether variations in PIP concentrations might have affected ESCRT-III interaction with GUV membranes. For this purpose, we used the quartz crystal microbalance with dissipation monitoring (QCM-D) to measure CHMP2B binding to supported lipid bilayers (SLBs) with a constant net charge fraction (see the “Methods” section). Indeed, the fraction of charged lipids is well preserved during SLB preparation from fusion of small unilamellar vesicles (SUVs) onto solid substrates [58]. After SLB formation with a defined DOPS or PIP composition (see lipid compositions in the “Methods” section), CHMP2B proteins were added in the chamber resulting in a shift of the resonance frequency $\Delta\theta$, of the quartz sensor, directly related to the amount of protein bound to the surface (Additional file 3: Figure S3-C). The amount of proteins adsorbed to the bilayer increased by 50% when the amount of DOPS was increased from 30 to 40% (Fig. 1e). Indeed, increasing the number of negatively charged lipids in the membrane increases the amount of protein adsorbed on it. This implies that electrostatic interactions play a key role in mediating the interaction between the proteins and the membrane in agreement with the exposure of basic surfaces in ESCRT-III polymers [35, 37]. Furthermore, in order to discriminate between the specific affinity for $\text{P}(4,5)\text{P}_2$ lipids and electrostatic interactions, we prepared SLBs with a constant total net charge with either 40% DOPS or 10% DOPS + 10% $\text{P}(4,5)\text{P}_2$, the total net charge of these SLBs being equivalent. We observe that CHMP2B density is approximately 60% higher when $\text{P}(4,5)\text{P}_2$ lipids are present in comparison with SLBs made of DOPS only (Fig. 1e). Compared to experiments on GUVs (Fig. 1d), this higher enhancement is probably due to an effective higher $\text{P}(4,5)\text{P}_2$ fraction in the SLBs as compared to the GUVs. Moreover, when $\text{P}(4,5)\text{P}_2$ lipids are replaced by the same fraction of $\text{P}(3-5)\text{P}_3$, the amount of proteins bound to the SLB decreases significantly and becomes almost equal to the amount of proteins bound to SLB with 30% DOPS only, although $\text{P}(3-5)\text{P}_3$ lipids have a higher negative net charge $(-4)$ as compared to $\text{P}(4,5)\text{P}_2$ lipids $(-3)$ [57, 59]. Altogether, these experiments show that CHMP2B preferentially interacts with $\text{P}(4,5)\text{P}_2$ lipids.

Globally, our results show that while CHMP2B is capable of binding to membrane alone, membrane binding of CHMP2A is significantly enhanced by CHMP3 (Fig. 1b, c). Additionally, CHMP3 has a modulating effect on CHMP2B and reduces its membrane association (Fig. 1b, c). Moreover, we found that the binding of CHMP2A+CHMP3 does not depend on the PIP species present in the membrane composition, in contrast to the enhanced binding of CHMP2B in the presence of $\text{P}(4,5)\text{P}_2$ lipids. This non-specificity of CHMP2A (+CHMP3) proteins to any of the PIP species including $\text{P}(4,5)\text{P}_2$ is in agreement with their presence in most cellular processes involving the ESCRT-III complex [1], contrary to CHMP2B which is only required for processes occurring at the plasma and nuclear membranes that are enriched in $\text{P}(4,5)\text{P}_2$ lipids [3, 60, 61].

CHMP2A and CHMP2B exhibit different supramolecular assemblies on membranes

Previous studies have shown that cellular overexpression of CHMP2B leads to helical scaffolds deforming the plasma membrane into long rigid tubes protruding out of the cell [32]. Similarly, CHMP2A+CHMP3 co-assemble in bulk into helical tubes in vitro [25, 44] or helical filaments on membrane tubes [41, 62]. Because the characterization of the effect of ESCRT-III on deformable model membranes is crucial to understand...
their mechanical properties, we further studied the supramolecular assemblies of CHMP2B versus CHMP2A+CHMP3 on 10%PI(4,5)P2-GUVs by spinning disk confocal microscopy.

Above 500 nM protein bulk concentration, CHMP2B proteins fully cover the surface of GUVs with no observable distinctive structure, i.e., no inward or outward tubulation (Fig. 2a, first panel). At optical resolution, CHMP2B proteins appear homogeneously distributed on the surface of the vesicles, besides some protein-lipid patches. At bulk concentration lower than 500 nM, CHMP2B proteins form a peculiar reticular-like network wrapping around the whole vesicle (Fig. 2a, second panel). Notably, the same network is observed when MBP-CHMP2B-ΔC is used (Additional file 2: Figure S2-C), indicating that the MBP fusion does not affect its function. The networks were observed after 15-min GUV incubation in the protein solution suggesting that, at high bulk concentration, a reticulum-like network forms transiently, becoming denser with time and leading to an apparent continuous coverage at optical resolution. This CHMP2B network co-localizes with PI(4,5)P2 lipids (Fig. 2b), indicating that CHMP2B recruits negatively charged PI(4,5)P2 lipids, further confirming the specific interaction between CHMP2B and PI(4,5)P2 lipids.

In contrast, the assembly of CHMP2A+CHMP3 appears to be devoid of any visible network, independent of the incubation time and protein concentration (up to 2 μM of CHMP2A) (Fig. 2c and Additional file 3: Figure S3-D). In some vesicles (approx. 10%), we observed CHMP2A (+ non-labeled CHMP3)-containing short, outward protrusions (Fig. 2c, and zoom-in). These protrusions were, however, rarely visible on most of the vesicles. We conclude that CHMP2B and CHMP2A-CHMP3 do not tubulate GUV membranes in this concentration range.

We next investigated whether these proteins perturb the mechanical properties of the membranes.

**CHMP2A and CHMP2B have different mechanical effects on model membranes**

To study the mechanical effect of CHMP2B and CHMP2A+CHMP3 on membranes, we first used the micropipette aspiration technique developed by E. Evans [63], to measure the elasticity of 10% PI(4,5)P2-GUV (lipid composition 1) coated with CHMP2A or CHMP2B in the presence or absence of CHMP3.

In the absence of CHMP proteins, micropipette aspiration of PI(4,5)P2-GUVs easily induced the formation of a characteristic tongue inside the micropipette (Fig. 3a, first panel). In contrast, PI(4,5)P2-GUVs incubated with a CHMP2B concentration leading to full coverage could not be aspirated and deformed even at high tensions (Fig. 3a, second panel) (up to 10⁻³ N m⁻¹). However, during aspiration at high tensions, in approximately 20% of the aspirated GUVs (Fig. 3b), an occasional rupture of CHMP2B protein coat could be observed, allowing the formation of a short tongue devoid of proteins inside the micropipette (Fig. 3a, third panel). This observation indicates that the CHMP2B polymer itself cannot be aspirated nor deformed and behaves as a solid shell. Surprisingly, the subsequent CHMP3 incubation with GUVs with pre-formed CHMP2B polymers on their surface resulted in the softening of the CHMP2B shell, which allowed aspiration of the GUV (Fig. 3a, fourth panel). The quantification of the percentage of aspirated vesicles at a tension of approximately 10⁻³ N m⁻¹ clearly indicates that while less than 20% of the CHMP2B-coated GUVs could be aspirated in the absence of CHMP3, generally upon shell rupture, almost 100% of the CHMP2B-coated GUVs could be aspirated when CHMP3 proteins were added (Fig. 3b). This thus suggests that CHMP2B polymers form a rigid shell around the vesicle that cannot be deformed by aspiration even at tensions as high as a few 10⁻³ N m⁻¹ unless CHMP3 is present. The presence of CHMP3 softens this rigid shell allowing its deformation by the micropipette.

In contrast, PI(4,5)P2-GUVs co-incubated with CHMP2A+CHMP3 could be easily deformed during aspiration with an increase of the tongue length inside the micropipette as a response to the aspiration increase (Fig. 3a, fifth panel). Figure 3c shows the variation of the membrane tension as a function of the fractional excess area, Δα, for two representative experiments. The stretching modulus, χ, is calculated from the slope of all the curves for both conditions (for bare lipids and membrane covered with CHMP2A+CHMP3; see the “Methods” section). The stretching modulus (Fig. 3d) is slightly decreased by the presence of CHMP2A+CHMP3 on the membrane. It is found to be equal to χ = 11 ± 6 mN m⁻¹ (N = 30 GUVs) for CHMP2A+CHMP3-covered GUVs and χ = 26 ± 19 mN m⁻¹ (N = 20 GUVs) for protein-free GUVs. This slight decrease means that CHMP2A+CHMP3 renders the GUV membrane slightly more stretchable. Note that the value of the stretching modulus for the bare lipid membrane is lower than values reported for dioleoyl, DO, chains, in the presence of cholesterol [64], probably because of the absence of a pre-stretching step in our experiments, as usually performed to suppress any pre-existing uncontrolled excess area [64]. Here, pre-incubation of the GUVs with proteins prevented any pre-stretching of the GUVs in order to limit the contact between pipette and protein-coated GUV and thus adhesion. Nevertheless, our aim was not to measure the absolute stretching modulus of the membranes coated by ESCRTs but to perform measurements relatively to bare lipid membranes in the same...
Fig. 2 Supramolecular assemblies of CHMP2A+CHMP3 versus CHMP2B on GUVs. **a** Spinning disk images of supramolecular assemblies of CHMP2B-ΔC (called CHMP2B) in BP buffer on 10% PI(4,5)P2-GUVs. After 15-min incubation of the GUVs with the protein solution, CHMP2B-ΔC at high bulk protein concentration (1 μM) (first panel) fully covers the vesicle surface, whereas at lower protein concentration (500 nM), CHMP2B-ΔC assembles into a reticular-like network on the GUV (second panel). A z-projection of the whole GUV is shown. Scale bar, 10 μm. **b** Co-localization of Fluo-PI(4,5)P2 and CHMP2B-ΔC on GUVs. A z-projection of the upper part of the GUV is shown. Scale bar, 10 μm. **c** Spinning disk images of supramolecular assemblies of MBP-CHMP2A-ΔC (500 nM) + CHMP3 (2 μM) in BP buffer on 10% PI(4,5)P2-GUVs. MBP-CHMP2A-ΔC (called CHMP2A) fluorescent signal is displayed. After 60-min incubation, the co-polymer covers the vesicle surface in a homogeneous manner with the presence of some protrusions at the surface of the GUV (zoom-in). A z-projection is shown including a zoom-in in the right panel, showing short protrusions at the surface of the GUV. Scale bar, 10 μm.
Fig. 3 (See legend on next page.)
experimental conditions. Moreover, the stretching modulus of membrane covered with CHMP2B+CHMP3 could not be measured as the tongue covered with these proteins systematically adhered to the pipette, thereby impeding any mechanical measurement. We conclude that CHMP2B strongly rigidifies membranes, whereas CHMP2A+CHMP3 membrane interaction does not alter the elastic properties of membranes.

We next applied different mechanical constraints to CHMP2B-covered GUVs to test their resistance to mechanical deformation. Spherical GUVs change shape when they are deflated upon a hyperosmotic shock since the surface/volume ratio increases and even becomes unstable when the osmotic shock is too strong [65]. We thus studied the effect of an hyperosmotic shock on 10% PI(4,5)P2-GUVs fully covered with CHMP2B by increasing the osmolarity in the external medium by salt or sugar addition. We carefully checked that the buffer change did not induce dissociation of the ESCRT-III proteins from the membrane. An osmotic shock equal to 150% (osmolarity of the external medium by salt or sugar addition. We carefully checked that the buffer change did not induce dissociation of the ESCRT-III proteins from the membrane. An osmotic shock, GUVs were completely destabilized in the presence of CHMP3 renders CHMP2B-bound membranes more deformable. This indicates that CHMP3 may counteract the effect of CHMP2B on the mechanical properties of model membranes. In contrast, the

First, a difference in surface roughness is observed between the SUV covered or not with the proteins. Whereas bare SUVs show a smooth surface, CHMP-coated vesicles possess a rougher surface, indicating the presence of the protein on the outside of the vesicles (Fig. 4c, top panels).

Next, we increased the imaging force and observed that the vesicles are progressively more deformed. The deformation of the SUVs is measured by recording the height change (Fig. 4c and Additional file 4: Figure S4). To assure that the vesicles had undergone elastic deformation, even at the maximum applied imaging force, the imaging force was reduced again to the minimum value at the end of the experiments. Only vesicles that bounced back to more than 90% of the initial height were considered for the analysis, and typically, the vesicles did recover their shape and size (Additional file 5: Movie S1). In Additional file 6: Figure S5, we show the raw data points for bare SUVs (Additional file 6: Figure S5-A), the transformation from absolute height to relative height (Additional file 6: Figure S5-B and C), and the raw data points for the relative height versus force increment (Additional file 6: Figure S5-D and E). The slope of these curves is directly related to the flexibility of the SUV membrane coated with the protein (Fig. 4d). Indeed, for a given force, a stiff membrane is less deformed than a soft one and the slope is lower. Figure 4d shows a clear difference between the different vesicles: The presence of CHMP2B stiffens the membrane by a factor 2.9±0.3. However, the addition of CHMP3 reduces the stiffening effect of CHMP2B and the corresponding membranes with CHMP2B and CHMP3 are only 1.7±0.2 times stiffer than the bare ones. In contrast, the presence of CHMP2A+CHMP3 does not modify the membrane elasticity as there is no significant difference compared to the slope of bare vesicles.

We conclude that CHMP2B stiffens membranes while the presence of CHMP3 renders CHMP2B-bound membranes more deformable. This indicates that CHMP3 may counteract the effect of CHMP2B on the mechanical properties of model membranes. In contrast, the
presence of CHMP2A+CHMP3 does not affect the mechanical properties of model membranes.

Discussion
The objective of our study was to compare the membrane binding properties of ESCRT-III proteins CHMP2A and CHMP2B in vitro in order to determine their capacity to substitute each other during membrane remodeling processes.

First, we show that CHMP2A membrane binding is strongly enhanced in the presence of CHMP3, in agreement with previous in vivo and in vitro studies [23, 26, 27].
were prepared from, as PI(4,5)P2 is only partially
GUV membrane is lower than in the lipid mixture they
in vitro work, the presence of PI(4,5)P2 lipids directly af-
dendritic spine formation [8, 55]. In vivo
repair, cytokinesis, nuclear envelope reformation, and
membranes such as HIV-1 budding, plasma membrane
context, all ESCRT-driven remodeling processes that in-
the intercellular bridge just before abscission [74]. In this
PI(4,5)P2 lipids play a crucial role in the stabilization of
sential for every aspect of cell division, and especially
5)P2 [54] upon VPS4 knockdown [32]. PIs lipids are es-
CHMP2B to the plasma membrane enriched in PI(4,
agreement with the spontaneous localization of
physiological function of ESCRT-III polymers containing
membrane binding either. In addition, many live-cell-
membrane binding in the absence of CHMP3. This is in agreement
with the synergy exerted by CHMP3 in the presence of
CHMP2A and with the absence of synergy exerted by
CHMP3 in the presence of CHMP2B on HIV-1 budding
[25, 26, 44, 66]. Our data further shows that the pres-
ence of CHMP3 confines CHMP2B to patches on the
GUV membrane. Reduced membrane binding may be
explained by a negative regulatory function of CHMP3
on CHMP2B, whereas CHMP3 interaction with
CHMP2B [44] may have a similar function than
CC2D1A/B or lgd in the negative regulation of
CHMP4B/Snf7 polymerization [67–69]. Furthermore,
the incubation of CHMP2B with CHMP3 in solution
does not induce CHMP2B polymerization in solution,
eliminating the possibility that premature CHMP2B
polymerization is responsible for reduced CHMP2B
membrane binding in the presence of CHMP3 (Add-
tional file 7: Figure S6).
CHMP2A was N-terminally fused to MBP, which
keeps CHMP2A monodisperse and monomeric in solu-
tion, while removal of MBP triggers spontaneous
polymerization or aggregation. Since we observed the
same membrane binding and mechanical properties of
CMP2B-AC and MBP-CHMP2B-ΔC, our results suggest
that the presence of MBP tag does not affect CHMP2A
membrane binding either. In addition, many live-cell-
imaging experiments performed with N-terminal fusions
of ESCRT-III proteins show no major effect on the
physiological function of ESCRT-III polymers containing
fusion proteins [8, 70–73]. Second, we confirm that
CHMP2B displays a stronger binding for PI(4,5)P2 con-
taining membranes as compared to other phosphoinosi-
tides and DOPS lipids [48]. In contrast, CHMP2A and
CHMP3 require only negatively charged membranes for
binding with no preference for specific lipid head groups.
The binding affinity for PI(4,5)P2 lipids is in agree-
ment with the spontaneous localization of
CHMP2B to the plasma membrane enriched in PI(4,
5)P2 [54] upon VPS4 knockdown [32]. PIs lipids are es-
ternal for every aspect of cell division, and especially
PI(4,5)P2 lipids play a crucial role in the stabilization of
the intercellular bridge just before abscission [74]. In this
context, all ESCRT-driven remodeling processes that in-
volve CHMP2B take place at PI(4,5)P2-containing mem-
branes such as HIV-1 budding, plasma membrane
repair, cytokinesis, nuclear envelope reformation, and
dendritic spine formation [8, 55]. In vivo, the concentra-
tion of PI(4,5)P2 depends on the membrane type. In our
in vitro work, the presence of PI(4,5)P2 lipids directly af-
A无论是CHMP2A还是
CHMP2B与CHMP3的膜
相互作用和机械性
质都不同，它们可能
诱导的膜变形是
不同的ESCRT-III亚
基在体外观察到的
ESCRT-III螺旋均
是通过不同的亚基
在体外聚合形成的。
CHMP4C is implicated in abscission control [15]. The increased rigidity imposed by the CHMP2B network might be important for dendritic spine maintenance [82] where it might limit protein diffusion, in agreement with experiments showing that CHMP2B forms a diffusion barrier at membrane necks reconstituted in vitro [48]. It might also significantly contribute to the mechanical property of the ESCRT-III spirals at the cytokinetic bridge that become very loose when CHMP2B is depleted [65]. Interestingly, CHMP2B function might be modulated by CHMP3, which limits CHMP2B-membrane interaction and softens the CHMP2B assembly. This indicates that in vivo CHMP3 either limits CHMP2B polymerization or and co-polymerizes with CHMP2B into a structure with different mechanical properties, in agreement with observations of copolymerization of ESCRT-III proteins in solution [51].

We thus propose that CHMP3 could play a key regulatory role in the sequence of recruitment of CHMP2B and CHMP2A and in their respective stoichiometry on the membranes during ESCRT-III function. In late steps of cytokinesis, pulling forces exerted by daughter cells on the intercellular bridge appear to regulate abscission, allowing daughter cells to remain connected until they have settled in their final locations. Moreover, counter-intuitively, a release of tension conducts membrane scission [83]. Thus, in the case of unmodified membrane softness, ESCRT-III would be able to carry out the membrane scission event. On the contrary, a rigid structure would impede this process. However, a certain degree of membrane rigidity might help the constriction process prior to scission, but at this stage, it is difficult to conclude on this aspect.

Conclusions
In summary, our data provides evidence that CHMP2A and CHMP2B polymerize differently on membranes and thereby impose different mechanical properties on the membrane structure. Our data thus strongly argue against a sole redundancy of the CHMP2A and CHMP2B proteins and indicate that different isoforms exert complementary functions within the ESCRT-III system.

Methods
Reagents
DOPC (1,2-dioleoyl-sn-glycero-3-phosphatidylcholine), DOPS (1,2-dioleoyl-sn-glycero-3-phospho-L-serine), DOPE (1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine), cholesterol (cholest-5-en-3ß-ol), P(3)P (1,2-dioleoyl-sn-glycero-3-phospho-(1′-myo-inositol-3′-phosphate), P(3,5)P2 (1,2-dioleoyl-sn-glycero-3-phospho-(1′-myo-inositol-3′,5′-bisphosphate)), P(4)P (L-α-phosphatidylinositol-4-phosphate), P(4,5)P2 (L-α-phosphatidylinositol-4,5-bisphosphate), BODIPY TMR-PtdIns(4,5)P2, C16 (red P(4,5)P2), 1-oleoyl-2-6-[4-(dipyrometheneboron difluoride) butanoyl] amino hexanoyl-sn-glycer-3-phosphoinositol-4,5-bisphosphate (TopFluor P(4,5)P2), and Egg Rhod PE (L-α-phosphatidylethanolamine-N-lissamine rhodamine B sulfonyl) were purchased from Avanti Polar Lipids, Inc. (Avanti Polar Lipids, USA). Stock solutions of lipids were solubilized in chloroform at a concentration of 10 mg mL\(^{-1}\), except for cholesterol and Egg Rh PE dissolved respectively at a concentration of 20 mg mL\(^{-1}\) and 0.5 mg mL\(^{-1}\) and PIPs, which were solubilized in a mixture of chloroform/methanol (70:30) (v/v) at a concentration of 1 mg mL\(^{-1}\). All stock solutions were kept under argon and stored at \(-20°C\) in amber vials (Sigma-Aldrich, France).

Expression, purification, and labeling of proteins
Expression and purification of MBP-CHMP2A-ΔC (residues 9–161) and CHMP3-FL (residues 1–122) was performed as described in [18]. A final gel filtration chromatography step on a superdex200 column was performed in a buffer containing 20 mM Hepes pH 7.6, NaCl 150 mM.

CHMP2B-FL (residues 1–222) and CHMP2B-ΔC (residues 1–154) were expressed and purified as previously described [32]. Both constructs contain a C-terminal SGSC linker for cysteine-specific labeling. Cells were lysed by sonication in 50 mM Tris pH 7.4, 1 M NaCl, 10 mM DTT, complete EDTA free, and the soluble fraction was discarded after centrifugation. The pellet was washed three times a buffer containing 50 mM Tris pH 7.4, 2 M UREA, 2% Triton X-100, 2 mM β-mercaptoethanol, and a final wash in 50 mM Tris pH 7.4, 2 mM β-mercaptoethanol. CHMP2B (-FL and -ΔC) was extracted from the pellet using a buffer composed of 50 mM Tris pH 7.4, 8 M guanidine, 2 mM β-mercaptoethanol over night at 4°C. Further purification of solubilized CHMP2B included Ni\(^{2+}\) chromatography in 50 mM Tris pH 7.4, 8 M urea, refolding by rapid dilution into a buffer containing 50 mM Tris pH 7.4, 200 mM NaCl, 2 mM DTT, 50 mM L-glutamate, 50 mM L-arginine at a final concentration of 2 μM. Refolded CHMP2B was concentrated by Ni\(^{2+}\) chromatography in a buffer containing 50 mM Tris pH 7.4, 200 mM NaCl. A final gel filtration chromatography step was performed on a superdex75 column in the buffer containing 50 mM Tris pH 7.4, 100 mM NaCl.

For MBP-CHMP2B-ΔC production, *Escherichia coli* BL21 cells were transformed with plasmids and grown at 37°C in Luria broth medium to an OD600 of 0.6. Protein expression was induced by the addition of 1 mM arabinose for 3 h at 37°C. Cells were harvested by centrifugation and the bacterial pellet was re-suspended in 50 mL of binding buffer (50 mM Hepes pH 7.6, 300 mM NaCl, 300 mM KCl). The bacteria were lysed by
sonication for 5 min and cell was pelleted by centrifugation at 20,000 rpm for 30 min. The MBP-CHMP2B-ΔC protein was purified on an amylose affinity column in binding buffer.

Following expression, CHMP proteins were concentrated, labeled overnight at 4 °C with a ratio of Alexa labeling dye per protein of 2 to 1. MBP-CHMP2A-ΔC, CHMP3-FL, and CHMP2B ( -ΔC and -FL) were labeled with Alexa 488 succimidyl ester, Alexa 633 succimidyl ester, and Alexa 488 C5 maleimide (Thermo Fisher Scientific), respectively. The excess of free dyes was removed by salt exchange chromatography except for MBP-CHMP2B-ΔC where a final gel filtration chromatography (superdex 200) step was performed in a buffer containing 50 mM Hepes pH 7.6, 150 mM NaCl. Immediately after labeling, all aliquots were frozen in liquid nitrogen with 0.1% of methyl cellulose (Sigma-Aldrich) as cryoprotectant. All aliquots were kept at −80 °C prior to experiments.

GUV preparation for confocal, spinning disk, and FACS experiments

GUVs were prepared by spontaneous swelling on polyvinyl alcohol (PVA)-based gels [84]. A thin lipid solution is deposited on a PVA gel (5% PVA, 50 mM sucrose, 25 mM NaCl and 25 mM Tris, at pH 7.5), dried under vacuum for 20 min at room temperature and rehydrated with the growth buffer at room temperature. Vesicles form within 45 min and are extracted by pipetting directly from the slides on top of the PVA gel.

Composition 1

For confocal and spinning disk microscopy experiments, lipid stock solutions were mixed to obtain DOPC/DOPS/DOPE/Cholesterol/PI(4,5)P2/PE-Rhodamine (54.2, 10:10:15:10:0.8) (molar ratio) at a concentration of 3 mg mL⁻¹ in chloroform. In the following, this GUV composition will be referred to as 10% PIP2-GUV. In order to detect the PI(4,5)P2 lipid signal, PE-Rhodamine was deposited on a PVA gel (5% PVA, 50 mM sucrose, 25 mM NaCl and 25 mM Tris, at pH 7.5), dried under vacuum for 20 min at room temperature and rehydrated with the growth buffer at room temperature. Vesicles form within 45 min and are extracted by pipetting directly from the slides on top of the PVA gel.

Composition 2

For FACS microscopy experiments, lipid stock solutions were mixed to obtain DOPC/DOPS/DOPE/Cholesterol/PI(4,5)P2/PE-Rhodamine (72.2:10:15:2:0.8) (molar ratio) at a concentration of 3 mg mL⁻¹ in chloroform. In the following, this GUV composition will be referred to as 2% PI(3)P-GUV, 2% PI(4)P-GUV, and 2% PI(3,5)P2-GUV.

SUV preparation for QCM and AFM experiments

After preparation of lipid composition 1, at 3 mg mL⁻¹ in chloroform, the solvent was evaporated by rotating the vial under a gentle stream of nitrogen, at room temperature and then was placed under vacuum for 20 min at room temperature. The dried lipid film was rehydrated in the appropriate growth buffer solution to obtain a final concentration of 1 mg mL⁻¹. The solution was vortexed for 2 min and then extruded 11 times through a polycarbonate track-etched membrane with pore sizes of 100 nm [85] or sonicated for 5 min until obtaining a clear colorless solution for small unilamellar vesicle (SUV) formation. Produced SUVs were either used freshly for QCM-D experiments and for HS-AFM indentation experiments or stored at −20 °C in amber vials (Sigma-Aldrich, France) for further use. In the following, this SUV composition will be referred to as 10% PIP2-SUV.

To compare CHMP2B protein binding in the absence of PI(4,5)P2 and to increase the net negative charge of the membrane for QCM-D experiments, SUVs were produced containing DOPC/DOPS/DOPE/Cholesterol/PE-Rhodamine (44.2:30:10:15:0.8) (molar ratio) or (34.2:40:10:15:0.8), at a concentration of 3 mg mL⁻¹ in chloroform referred to as 30% DOPS-SUV and 40% DOPS-SUV, respectively. Moreover, to compare CHMP2B protein binding to a membrane incorporating a higher amount of negative charges as well as PIP lipids, we replaced the 10% molar ratio of PI(4,5)P2 in the PIP2-SUV by 10% molar ratio of PI(3-5)P3 lipids. In all QCM-D experiments, quartz crystal resonance frequency shifts were measured at the overtone 5 of the oscillating crystal and therefore defined as Δθₜ.

CHMP supramolecular assembly on GUVs observed by fluorescence microscopy

Freshly produced 10% PIP2-GUVs were incubated with CHMP proteins at concentrations ranging from 50 nM to 2 μM in BP buffer (Tris 25 Mm, NaCl 50 mM pH 7.5) in isotonic conditions for 15 to 30 min. Then, CHMP-coated GUVs were diluted 20 times and transferred to the observation chamber, previously passivated with the β-casein solution and rinsed twice with BP buffer.

Supramolecular assembly of CHMP proteins on GUVs was visualized on an inverted Spinning Disk Confocal Roper/Nikon. The spinning disk is equipped with the camera, EMCCD 512 x 512 Andor Technology (pixel size 16 μm), an objective (×100 CFI Plan Apo VCoil NA 1.4, WD 0.13), and 3 lasers (491, 561, 633 nm 100 mW). The exposure time for all images was 50 ms.
To further characterize and compare the interaction of CHMP proteins on GUVs, we measured the total intensity of the protein on the vesicle and normalized this value by the GUV area.

Image acquisition for protein quantification was performed using a confocal microscope composed of an inverted microscope (Eclipse TE2000 from Nikon), two objectives (× 60 water immersion and × 100 oil immersion), a C1 confocal head from Nikon, three lasers (λ = 488 nm, λ = 561 nm, and λ = 633 nm). One confocal plane image was taken for each set tension.

FACS experiment for protein-lipid binding assay

2% PI-GUV and CHMP fluorescence intensity was measured with a BD LSRFORTESSA flow cytometry instrument. Data analysis was performed with BD FACS Diva software.

The collected GUVs were transferred to BP buffer and incubated 30 min with CHMP proteins at 500 nM. The vesicle concentration was adjusted in order to count about 10,000 events per condition every 60 s at high speed.

2% PI-GUVs were labeled with Egg Rhod PE (0.8% w/w), CHMP2B labeled with Alexa 488, CHMP2A labeled with Alexa 488, and CHMP3 labeled with Alexa 633. Alexa 488 was excited with a 488-nm laser, and the emission was detected through a 530/30 standard bandpass filter. Alexa 633 was excited with a 633-nm laser, and the emission was detected through a 670/30 bandpass filter. Egg Rhod PE was excited with a 532-nm laser, and the emission was detected through a 610/20 bandpass filter. Two signals were closely analyzed: the protein fluorescent signal and the lipid fluorescent signal. Thus, the fluorescence intensity of the membrane and the fluorescence intensity of the proteins are respectively proportional to the amount of fluorophores in the vesicle and proteins bound to it or present in the detection zone and unbound. The intensity plot displaying the protein fluorescence signal as a function of the lipid fluorescent signal presents 3 regions: (i) unbound proteins (single-positive for proteins only in the top left quadrant), (ii) CHMP proteins bound to GUVs (double-positive for proteins and lipids in the top right quadrant), and (iii) GUVs free of proteins (single-positive for lipids only in the lower right quadrant).

QCM-D experiments

Supported lipid bilayers (SLBs) were generated with or without PI lipids. In the absence of PI(4,5)P2, SLB made of 30% and 40% DOPS-SUV composition were produced with a buffer containing Ca2+ (150 mM NaCl, 10 mM Tris (at pH 7.5) + 2 mM Ca2+) [41]. After SLB formation, the bilayer was rinsed with the same buffer but supplemented with EDTA (150 mM NaCl, 10 mM Tris pH 7.5, 10 mM EDTA) to remove Ca2+ excess. SLBs were also produced in the presence of PIP lipids (PI(4,5)P2 or PI(3-5)P3), with PIP2-SUV or PIP3-SUV lipid compositions, respectively. SLB formation was achieved in a buffer containing 150 mM KCl, 20 mM citrate pH = 4.8 [42]. Following SLB formation, CHMP proteins were injected at a concentration of 200 nM in BP buffer. The interaction between the proteins and the lipid bilayer was directly measured from the fifth overtone of the frequency shift (∆f5).

QCM-D measurements were performed using a Q-Sense E4 system (Q sense; Gothenburg, Sweden). The mass sensor is a silicon dioxide-coated quartz crystal microbalance SiO2 (QSX-303 Lot Quantum Design France) with a fundamental frequency of 4.95 MHz. The liquid flow was controlled using a high-precision multichannel dispenser (IPC; ISMATEC—Germany). All experiments were performed at room temperature with a flow rate of 50 µL min⁻¹.

Micropipette experiments

The experimental chamber and the micropipette made of a borosilicate capillary (1-mm outer diameter and 0.58-mm inner diameter (Harvard Apparatus, UK)) introduced into the chamber are passivated with a β-casein solution at 5 mg mL⁻¹ in sucrose 25 mM, NaCl 50 mM, and Tris 25 mM (pH 7.5) for 15 min. The chamber is rinsed twice with BP buffer. Then, PIP2-GUVs pre-incubated with CHMP proteins are added to the chamber. Once the chamber is sealed with mineral oil, the zero pressure is measured and the aspiration assay can begin by decreasing the water height gradually, thus increasing the applied tension on the vesicle.

The explored tensions for the aspiration experiments with the different CHMP proteins range up to 1.6 mN m⁻¹ (corresponding to the membrane enthalpic regime). The software EZ-C1 was used for the acquisition of the confocal images.

At high tension, in the enthalpic regime, an apparent elastic stretching modulus of the membrane χ can be deduced from the linear variation of the fractional excess area ∆α (Δα = πDp(1 − Dp/Dv)ΔLP/A0 where ΔLP is the variation of the tongue length and A0 the initial area of the GUV) as a function of the applied tension σ using

\[ \Delta \alpha = \Delta \alpha_0 + \frac{1}{5} \sigma \]  

with Δα0 being the initial excess area for the reference tension ω0. According to the Young-Laplace equation, the membrane tension is equal to

\[ \sigma = \Delta P \times R_p / (2 \times (1 - \frac{R_v}{R_p})) \]

where ΔP is the difference of pressure between the interior of the micropipette and the chamber and Rp and Rv are respectively the pipette and vesicle radius [63].
Osmotic shock on GUVs

10% PI-P2-GUVs were either co-incubated with 500 nM CHMP2B-ΔC in 50 mM NaCl and 25 mM Tris, at pH 7.4 buffer (CHMP protein binding buffer referred as BP buffer) or transferred to the same buffer free of protein (osmolarity equal to 125 mOsm L⁻¹). CHMP2B-coated GUVs and CHMP2B-free GUVs were then transferred to a hyperosmotic buffer with increasing sodium chloride concentrations up to 250 mM NaCl. The effect of the osmotic shock was visualized using confocal microscopy.

HS-AFM imaging-based deformation experiment

PI-P2-SUVs were immobilized on a freshly cleaved mica surface and placed into the AFM chamber with BP buffer. For studying the vesicles with proteins, prior to immobilization to the surface, the PI-P2-SUVs were pre-incubated with either 1 μM of CHMP2B or 1 μM of CHMP2B + 2 μM of CHMP3 or 1 μM of CHMP2A + 2 μM of CHMP3 for 30 min to allow full protein coverage on the SUV surface. A high-speed amplitude modulation tapping mode AFM (RIBM, Japan) was used for imaging [87-89] and deformation experiments, with ultra-short cantilevers (spring constant 0.15 N/m, Nanoworld). Initial imaging (at minimum force) was performed at a free cantilever oscillation amplitude of 5.4 nm and a set-point amplitude at 4.3 nm. The imaging rate was 0.5 frame/s. We regulated the set-point amplitude in a stepwise manner, while keeping the free amplitude constant, in order to increase the imaging force. The imaging force can be estimated in the first approximation as \( F = k \Delta z \), where \( k \) is the spring constant of the cantilever and \( \Delta z \) is the difference between free and set-point amplitude of the cantilever oscillation. It follows that the images were acquired with an estimated minimal force of ~150 pN. For the measurement of membrane mechanics with and without proteins, image acquisition was first performed at minimal force (~150 pN). Next, step by step, the imaging force was increased with 9% increments, by decreasing the set-point amplitude. After reaching the maximal force, after ~8 steps and an estimated final imaging force of ~270 pN, the tapping force was reduced again to its lowest value (~150 pN), and the height recovery was recorded. Only those vesicles that exhibited a height recovery of at least 90% of their initial height were considered to be elastically deformed and were included in the analysis. Errors in the relative stiffness are given as standard error of the mean (SEM). Images were analyzed using IgorPro scripts of the AFM manufacturer (RIBM) and ImageJ scripts.

**Additional file 1:** Figure S1. Evolution of the ESCRT-III complex. (A) Table illustrating the ESCRT-III complex function, origin and homologs in *S. cerevisiae* and *H. sapiens*. (B) Distribution of Vps2 and Vps24 genes across Eukaryotes showing the presence of two Vps2 genes in high organisms. (C) Table illustrating the implication of ESCRT-III subunits in different subcellular locations in *S. cerevisiae* and *H. sapiens*. The names are the human homologs in cases of *S. cerevisiae*.

**Additional file 2:** Figure S2. Study of CHMP protein-membrane interaction. (A) Optimization of the buffer conditions to optimize the binding of CHMP2B-ΔC (noted here CHMP2B) at 500 nM. Pre-formed vesicles were incubated with CHMP2B-ΔC in buffers with different salt concentrations ranging from 0 mM to 100 mM NaCl (+Tris 25 mM at pH 7.5) and imaged with confocal microscope after 30-min incubation. Lipid signal is shown in magenta and protein signal in green. Scale bar: 5 μm. (B) Confocal image of MBP-CHMP2A-ΔC (noted here CHMP2A) without TEV (Top line) and in the presence of TEV to cleave the MBP tag (Bottom line). Saturated protein fluorescence signal is represented in yellow. Cleavage of MBP tag slightly increases the interaction but induces aggregation. Scale bar: 30 μm. (C) Spinning disk images of GUV incubated with MBP-CHMP2A-ΔC (noted here CHMP2A) at a concentration of 50 nM (left image, scale bar = 10 μm) and at 200 nM (right image; scale bar = 5 μm). (D) Confocal images of GUV incubated with MBP-CHMP2A-ΔC at a concentration of 1 μM (noted here MBP-CHMP2A), submitted to an osmotic pressure difference equal to 150% (Osmolarity inside and outside the GUV are respectively 120 mOsm.L⁻¹ and 315 mOsm.L⁻¹). Scale bar: 10 μm.

**Additional file 3:** Figure S3. (A) Histograms of CHMP2B-ΔC protein fluorescence intensity for PI3P, PI4P, PI(3,5)P2 and PI(4,5)P2 GUVs (lipid composition 2). (B) Comparison of the binding density of MBP-CHMP2A-ΔC + CHMP3 and of CHMP2A-ΔC to GUVs with different charged lipids, measured by FACS, corresponding to Fig. 1d. The values are normalized to their respective binding density to DOPS. ** = p-value<0.01 (Student’s t-test). N = 4 (number of FACS experiment with about 10⁴ counted events per experiment, per condition). (C) QCM-D experiment displaying the typical frequency shift of ~ 25 Hz after supported bilayer formation and a frequency shift Δf, representative of the amount of protein bound to the bilayer. (D) Spinning disk images of interaction of MBP-CHMP2A-ΔC + CHMP3 in BP buffer on 10% P(4,5)P2-containing GUVs. CHMP2A-ΔC fluorescent signal is displayed. A projection is represented. The different panels corresponding to 3 representative GUVs show the homogeneous coverage of the co-polymer as a function of protein concentration and incubation time. First panel: CHMP2A and CHMP3 are incubated at 500 nM and 2 μM, respectively, for 15 min. Second panel: CHMP2A and CHMP3 are incubated at 1 μM and 4 μM, respectively, for 15 min. Third panel: CHMP2A and CHMP3 are incubated at 500 nM and 2 μM, respectively, for 60 min. Scale bar: 10 μm.

**Additional file 4:** Figure S4. Deformation of bare vesicles and vesicles covered with CHMP2A + CHMP3 or CHMP2B + CHMP3. HS-AFM images of a vesicle covered with CHMP2A and CHMP3 left column, and a vesicle covered with CHMP2B and CHMP3 proteins (right column). The deformability of the SUVs coated with corresponding proteins upon increased applied force are shown at intermediate force increments of 27% (second panels) and at higher force increment, 81% (third panels).

**Additional file 5:** Movie S1. Typical example of vesicle response upon increasing and decreasing imaging force. It can be observed that the vesicle restores its height after the final decrease in imaging force.

**Additional file 6:** Figure S5. Deformation of bare vesicles and vesicles covered with CHMP proteins. (A) Reduction of vesicle height under increasing force for bare vesicles. Zero force increment represents the minimum imaging force (~150 pN). (B) Example of deformation for a ~60 nm vesicle over increasing force up to 80% of the initial imaging force. (C) Represents the transformation of vesicle height to relative height for each point for the curve in D. (D) Represents the relative height vs force increment for all the curves from panel A for bare SUVs. (E) Represents the relative height vs force increment for SUV covered with CHMP2B (left), CHMP2B + CHMP3 (middle) and CHMP2A + CHMP3 (right).
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Authors’ contributions
N.J.M. performed the expression, purification, and labeling of the proteins. M.A. and N.D.F. performed the GUV preparation, the confocal imaging, and the flow cytometry respectively. M.A. was a member of the France BioImaging national research infrastructure (ANR-10-INBS-04) as well as the flow Cytometry Platform of the Institute Curie for technical support in microscopy and flow cytometry respectively. M.A. was funded by the Université Pierre et Marie Curie/sorbonne Université, Doctoral school “Physique en Ile de France” (ED-564) and the Fondation pour la Recherche Médicale. N.D.F. was funded by post-doc fellowships from the Institut Curie, the Fondation pour la Recherche Médicale a EMBO non-stipendary long-term fellowship (ALTF 818-2016) and the European Union’s Horizon 2020 research and innovation program (MSCA No. 751715). P.B. performed the AFM experiments. S.Mai. performed the AFM experiments. Experiments have been designed and discussed by W.W., W.R., P.B., and S.Man. M.A., W.W., P.B., and S.Man. wrote the manuscript. The results and their interpretation were discussed by all of the authors. All authors read and approved the final manuscript.

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Availability of data and materials
The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Declarations
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Competing interests
The authors declare that they have no competing interests.

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