

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33

---

## Endocytosis caused by liquid-liquid phase separation of proteins

Louis-Philippe Bergeron-Sandoval<sup>1</sup>, Hossein Khadivi Heris<sup>2</sup>, Adam G. Hendricks<sup>2</sup>, Allen J. Ehrlicher<sup>2</sup>, Paul François<sup>3</sup>, Rohit V. Pappu<sup>4</sup> and Stephen W. Michnick<sup>1,5\*</sup>

### Affiliations

<sup>1</sup>Département de Biochimie, Université de Montréal, C.P. 6128, Succursale centre-ville, Montréal, Québec, H3C 3J7, Canada.

<sup>2</sup>Department of Bioengineering, McGill University, Montreal, Quebec, H3A0C3, Canada.

<sup>3</sup>Ernest Rutherford Physics Building, 3600 rue University, McGill University, Montreal, Québec, H3A2T8

<sup>4</sup>Department of Biomedical Engineering and Center for Biological Systems Engineering, Washington University in St. Louis, One Brookings Drive, Campus Box 1097, St. Louis, Missouri 63130, USA.

<sup>5</sup>Centre Robert-Cedergren, Bio-Informatique et Génomique, Université de Montréal, C.P. 6128, Succursale centre-ville, Montréal, Québec, H3C 3J7, Canada.

\*Correspondence to: S.W.M. ([stephen.michnick@umontreal.ca](mailto:stephen.michnick@umontreal.ca)).

### Summary

Clathrin-mediated endocytosis (CME) underlies intra- and extracellular material trafficking in eukaryotes, and is essential to protein metabolism, intercellular signaling, membrane remodeling and other cell regulatory processes. Although CME is usually driven by F-actin polymerization, membrane invagination can also occur through unknown actin independent mechanisms. Here, we present evidence that CME is driven by the accumulation of proteins at sites of endocytosis initiation that undergo liquid-liquid phase separation to form viscoelastic droplets. The surfaces of these droplets, bind to the membrane and surrounding cytosol and generate the work required to drive membrane invagination. The proposed mechanism expands the repertoire of functions of membraneless organelles that form via liquid-liquid phase separation to include their ability to do work due to soft interfaces that shape and organize cellular matter.

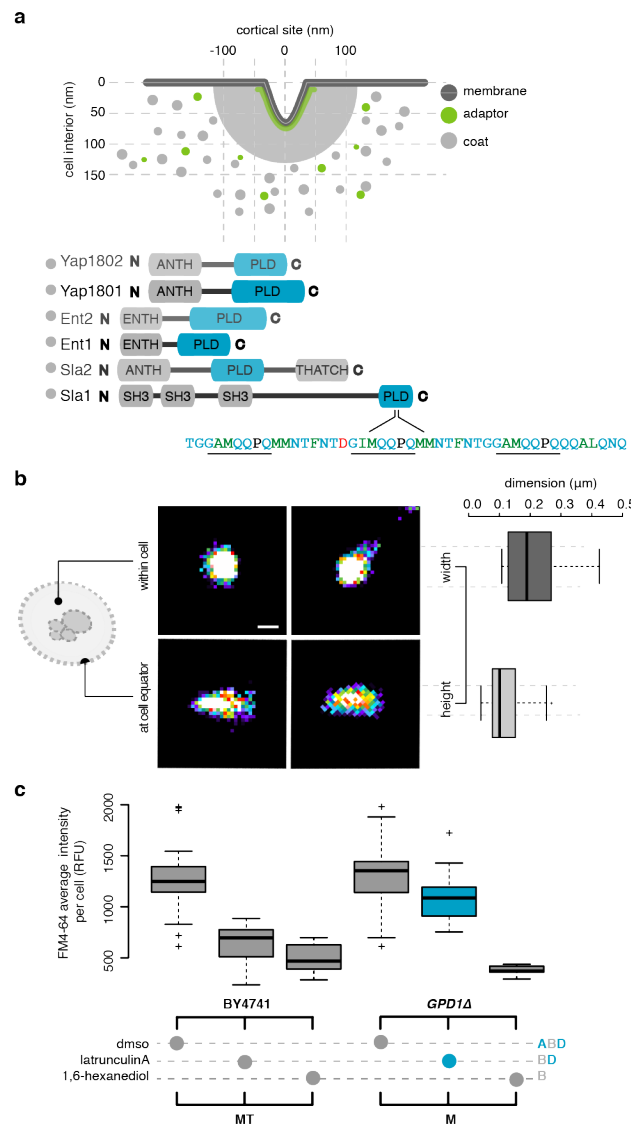
## 34 **Introduction**

35 Evolution has resulted in numerous innovations by which morphogenesis of organisms  
36 occurs within limits imposed by physical and chemical constraints on the underlying biochemical  
37 processes (Darwin 1859, Thompson 1917). At the cellular level these biochemical processes  
38 include active polymerization of cytoskeletal proteins (Pollard and Cooper 2009), motor protein  
39 regulation of polymerization (Haviv, Gillo et al. 2008) and cytoplasmic transport (Hendricks,  
40 Holzbaur et al. 2012, Guo, Ehrlicher et al. 2014), the generation and maintenance of osmotic  
41 gradients (Meshcheryakov, Steudle et al. 1992) and peptidoglycan cell walls (Harold 1990).  
42 However, the mechanical forces that drive some subcellular mechanics remain a mystery. This  
43 includes the membrane invagination and vesicle formation process called clathrin-mediated  
44 endocytosis (CME) (Aghamohammadzadeh and Ayscough 2009, Boulant, Kural et al. 2011, Li,  
45 Shao et al. 2015). In the baker's yeast *Saccharomyces cerevisiae* CME is preceded by the  
46 spatially focused accumulation of a number of proteins on the plasma membrane, among which  
47 are those that encode low amino acid sequence complexity and structurally disordered "prion-  
48 like domains" (PLD) (Fig. 1a) (Kaksonen, Toret et al. 2005, Hom, Vora et al. 2007, Idrissi,  
49 Blasco et al. 2012, Malinovska, Kroschwald et al. 2013, Brach, Godlee et al. 2014, Avinoam,  
50 Schorb et al. 2015). Here, we demonstrate that membrane invagination can arise from liquid-  
51 liquid phase separation (demixing) of PLD-containing proteins from the cytosol. Demixing of  
52 these proteins results in formation of a droplet, which, by virtue of its viscoelastic properties,  
53 binds to and deforms plasma membrane and cytosol to which it binds. Demonstration that phase  
54 separated droplets can perform mechanical work expands the repertoire of known protein droplet  
55 functions to include the ability to do work at the droplet surfaces and their interfaces. Similar  
56 mechanisms may govern or contribute to other membrane shaping, invagination and budding  
57 processes that are involved in the cellular material uptake, secretion, and cell shape remodeling.

58  
59 In *S. cerevisiae*, the dominant force for vesicle generation in CME is branched actin assembly,  
60 which is required to compete against intracellular turgor pressure and membrane tension to drive  
61 the invagination of the plasma membrane (Drubin, Kaksonen et al. 2005, Carlsson and Bayly  
62 2014, Dmitrieff and Nedelec 2015, Li, Shao et al. 2015). If, however, turgor pressure is  
63 eliminated, CME in *S. cerevisiae* cells can also occur independent of actin polymerization  
64 (Aghamohammadzadeh and Ayscough 2009).

65  
66 Alternative mechanisms to explain actin-independent membrane invagination in CME include  
67 intrinsic twisting of the membrane by the clathrin matrix, binding of curved BAR  
68 (Bin/Amphiphysin/Rvs) domain-containing proteins, protein domain insertion in the membrane  
69 bilayer, or steric repulsion of coat and adaptor proteins due to their crowding at cortical CME  
70 nucleation sites. Although these models are physically plausible, as demonstrated in model  
71 systems, their importance *in vivo* remain controversial (detailed in Material and Methods)  
72 (Carlsson and Bayly 2014, Kukulski, Picco et al. 2016, Scher-Zagier and Carlsson 2016). For  
73 instance, deletion of the only F-BAR domain-containing protein that accumulates on cortical

74 sites prior to excision, Syp1, does not affect membrane invagination (Fig. S1) (Boettner,  
 75 D'Agostino et al. 2009).  
 76



77  
 78  
 79 **Figure 1 | Assembly of proteins into a cortical droplet drive clathrin-mediated endocytosis** (a)  
 80 (Upper panel) Illustration of the geometry of a plasma membrane (dark grey) invagination into the cell  
 81 during clathrin-mediated endocytosis (CME). 70 nm invagination is required for vesicle scission to occur.  
 82 Electron microscopic data suggest that clathrin-coated plasma membrane patches are surrounded by a  
 83 cortical body of ~200 nm diameter (light grey) before appearance of actin structures. Clathrin heavy and  
 84 light chains (Chc1 and Clc1) interact with adaptor proteins (Ede1 and Syp1) to form a lattice on the  
 85 membrane (in green). Subsequently, early coat proteins (light grey), such as Sla1/2, Ent1/2, and  
 86 Yap1801/2, directly bind to the adaptor-clathrin lattice and form the cortical body (in grey). (lower panel)  
 87 Coat proteins contain “Prion-like domains” (PLD, in blue) that include tandem repeats of asparagine and  
 88 glutamine. (b) Geometry and size distribution of coat protein Sla1-GFP at cortical sites measured using  
 89 super-resolution microscopy (dSTORM). Lateral x, y resolution was ~10 nm. Pseudo-color reconstructed  
 90 images show circular structures (left panels) when viewed from the top, or within cells (left, upper), but

91 form narrow ellipses when imaged at the equator of cells (left, lower). Automatic segmentation and  
92 morphological analysis (right) were performed on these reconstructed images to determine the width (209  
93  $\pm 10$  nm) and height ( $118 \pm 6$  nm) of cortical bodies (mean  $\pm$  sd;  $n = 250$ ), consistent with other electron  
94 and light micrographic evidence. (c) Lipophilic cargo membrane-labelling dye FM4-64 is taken up into  
95 vesicles by CME in wild type (left) and *GPD1* $\Delta$  cells (eliminates turgor pressure; right) treated with either  
96 DMSO, latrunculin A (prevents F-actin polymerization) or 1,6-hexanediol (disrupts liquid-liquid phase  
97 separated protein droplets). Each boxplot shows the relative fluorescence units of  $n = 50$  cells. Note that  
98 *GPD1* $\Delta$  cells can undergo CME in the absence of F-actin polymerization (blue) because there is no turgor  
99 pressure in these cells (Fig. S2-3).

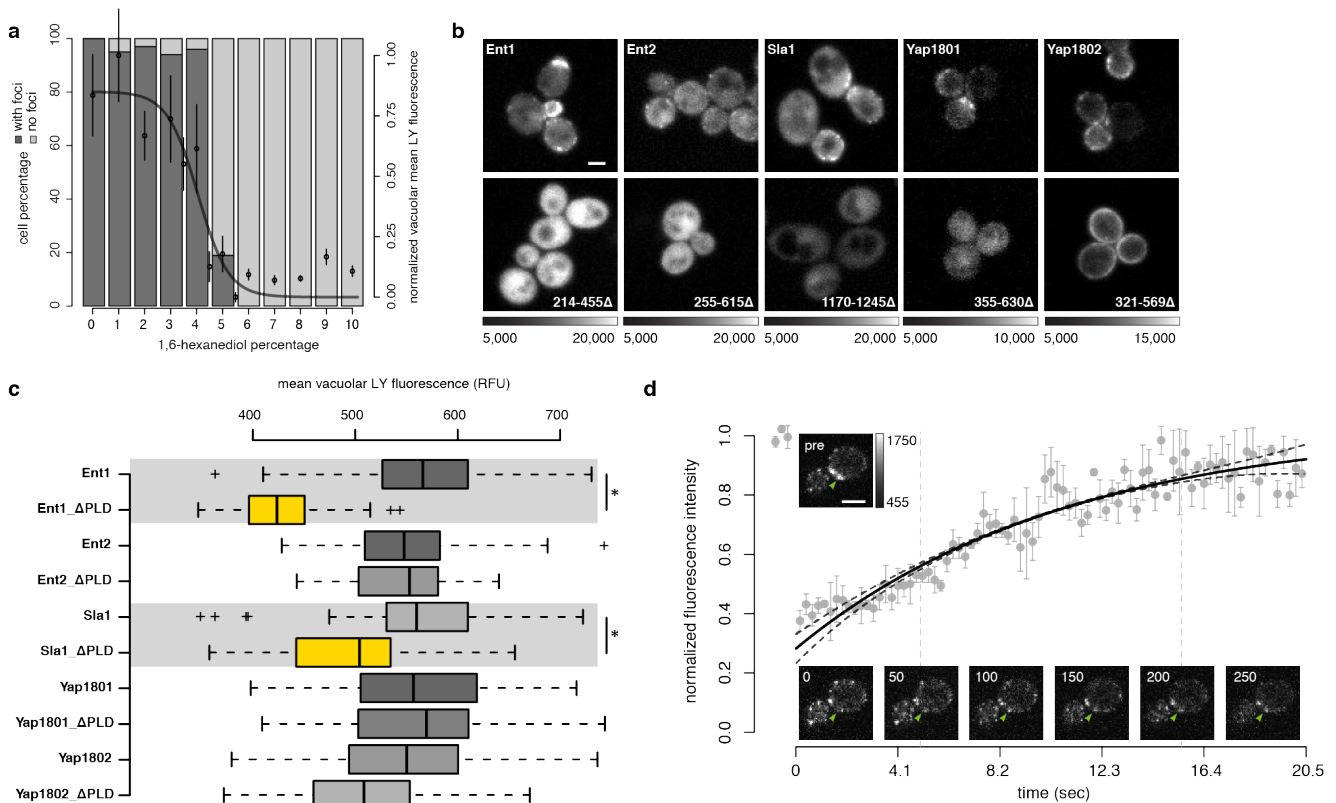
100  
101 A potential mechanism that could drive actin-independent CME in yeast was suggested to us by  
102 the observation that there is a common amino acid sequence pattern found among coat and  
103 adapter proteins. Several of these proteins have PLDs (Fig. 1a) (Alberti, Halfmann et al. 2009,  
104 Malinowska, Kroschwald et al. 2013). Such proteins are known to phase separate *in vitro* and in  
105 cells to form spherical condensates or droplets that are hundreds of nanometers to micrometers in  
106 size and having a range of viscoelastic properties (Guilak, Tedrow et al. 2000, Pappu, Wang et  
107 al. 2008, Brangwynne, Eckmann et al. 2009, Hyman, Weber et al. 2014, Banjade, Wu et al.  
108 2015, Jiang, Wang et al. 2015, Kroschwald, Maharana et al. 2015, Molliex, Temirov et al. 2015,  
109 Nott, Petsalaki et al. 2015, Zhang, Elbaum-Garfinkle et al. 2015). We postulate that such droplets  
110 exist at CME initiation sites and that, owing to their viscoelastic properties and interfacial  
111 tension bind to the plasma membrane adaptors and generate a force that drives invagination of  
112 the membrane (Hertz 1882, Johnson 1971, Ewers, Romer et al. 2010, Style, Hyland et al. 2013).

### 113 114 **PLD-containing CME proteins accumulate and phase separate at cortical sites**

115  
116 Evidence that a protein droplet (henceforth called the cortical droplet) could form at CME sites  
117 include first, electron and light microscopic studies that reveal a region surrounding CME  
118 membrane invaginations and mature vesicles of  $\sim 200$  nm diameter that is devoid of ribosomes  
119 (Kukulski, Schorb et al. 2012, Picco, Mund et al. 2015). This “exclusion zone” thus appears to  
120 present a physical barrier to large molecular complexes at least as large as ribosomes ( $> 10$  nm)  
121 (Kukulski, Schorb et al. 2012). Furthermore, we and as others have observed an object at cortical  
122 sites of  $\sim 200$  nm diameter by super-resolution imaging of Sla1 in cells treated with Latrunculin  
123 A (LatA), an inhibitor of actin polymerization. The exclusion zone cannot, thus, be attributed  
124 only to F-actin bundles (Fig. 1b, Fig. S4) (Picco, Mund et al. 2015). Our results agree with  
125 quantitative immuno-EM data which show that many endocytic coat proteins (including Sla1/2  
126 and Ent1/2) assemble into a hemisphere of similar dimensions, consistent with a protein droplet  
127 that associates with membrane on cortical sites (Idrissi, Grotsch et al. 2008, Idrissi, Blasco et al.  
128 2012).

129  
130 The simple alcohol 1,6-hexanediol (HD) has been demonstrated to prevent liquid-liquid phase  
131 separation of proteins to form droplets *in vivo* and *in vitro* (Updike, Hachey et al. 2011,  
132 Kroschwald, Maharana et al. 2015, Molliex, Temirov et al. 2015, Wheeler, Matheny et al. 2016,

133 Rog, Kohler et al. 2017). CME, as measured by cell uptake of a lipophilic membrane-bound  
 134 fluorescent dye (FM4-64), was inhibited by HD, whether or not turgor pressure and actin  
 135 polymerization were present (Fig. 1c, left *versus* right panels, respectively). Furthermore, an HD  
 136 dose-response of uptake of the fluorescent dye (Lucifer Yellow) into vacuoles and formation of  
 137 puncta monitored as Sla1-GFP fluorescence at cortical sites were prevented, but not in cells  
 138 treated with the related alcohol 1,2,3-Hexanetriol that does not disrupt droplets (Fig. 2a, Fig. S5).  
 139 The other PLD-containing proteins, including Sla2, Ent1, Ent2, Yap1801 and Yap1802, all failed  
 140 to form puncta in cells treated with HD (Fig. S5). Pulse-chase experiments showed that HD-  
 141 dependent dissolution of Sla1 puncta was reversible (Fig. S6 and movie 1). Finally, PLD-  
 142 containing proteins can also form amyloid aggregates, which can be diagnosed by binding and  
 143 co-localization of Thioflavin T (ThT) to the aggregates (Khurana, Coleman et al. 2005). We  
 144 observed no colocalization of ThT with Sla1-mCherry-labelled puncta (Fig. S7).  
 145



146  
 147

148 **Figure 2 | CME adaptor and coat proteins phase separate to form droplets** (a) 1,6-hexanediol (HD),  
 149 disrupts cortical droplets in an all-or-none manner. Barplot shows percentage of cells that contain Sla1-  
 150 GFP foci (dark grey), or not (light grey), as a function of HD concentration monitored by counting  
 151 fluorescent puncta containing Sla1-GFP at cortical sites 5 minutes after HD treatment (n = 150 cells). Plot  
 152 overlay (in black) shows quantification of lucifer yellow fluorescent dye uptake in CME vesicles (mean ±  
 153 sd; n = 25; logistic fit) (b) Prion-like domains (PLDs) are essential for localization of proteins to the  
 154 cortical sites. Fluorescence images of cortical localization of Ent1, Ent2, Sla1, Yap1801 and Yap1802  
 155 fused to Venus YFP. Localization of full-length (upper panels) *versus* C-terminal PLD truncation mutants  
 156 of the proteins (lower panels). Amino acid positions of the deleted PLDs are indicated for respective

157 images. Grayscale dynamic range for image pairs are indicated below. Scale bar, 2  $\mu\text{m}$ . (c) Quantification  
158 by fluorescence microscopy of lucifer yellow dye uptake for strains that express either full-length or  
159 PLD-truncated Ent1, Ent2, Yap1801, Yap1802 and Sla1 (as detailed in panel b). We observed a  
160 significant decrease in CME for PLD truncation mutants of Sla1 and Ent1 (two-sided t-test; star indicates  
161  $P < 0.001$ ;  $n = 100$  cells). (d) Coat proteins exchange with cortical droplets at rates typical of those  
162 observed for proteins that compose other protein droplets. Fluorescence recovery after photo bleaching  
163 (FRAP) of Sla2-GFP, GFP signal recovery was measured within a segmented Sla1-mCherry region of  
164 interest to ensure that FRAP was acquired within the cortical droplet (mean  $\pm$  sd;  $n = 5$ ); photo bleached  
165 Sla1-mCherry region identified arrows in image inserts. Data was fitted to a single term recovery equation  
166 (full line) and corrected for the known rate of molecule accumulation and depletion that occurs during the  
167 droplet lifecycle (dashed lines) (Material and Methods). Incomplete fluorescence recovery suggests that  
168 cortical droplets are viscoelastic. Representative cell images before bleaching, upon bleaching, and after  
169 recovery are shown in inserts; frame numbers are indicated in the upper left of each image. Grayscale  
170 values, 455 to 1750. Scale bar, 2  $\mu\text{m}$ .

171  
172 The PLDs of cortical CME proteins were essential to their localization to cortical sites (Fig. 2b).  
173 Furthermore, CME was significantly reduced in cells where the PLDs of Sla1 and Ent1 were  
174 deleted and with substitutions of proline for other residues in the Sla1 PLD, which weakens the  
175 driving force for phase separation (Fig. 2c, Fig. S8) (Toombs, McCarty et al. 2009, Crick, Ruff et  
176 al. 2013). Our results support evidence that there is a functional redundancy among most of the  
177 PLD-containing proteins with the two that are more essential, perhaps required for specific  
178 functions mediated by other domains within their sequences (Watson, Cope et al. 2001, Godlee  
179 and Kaksonen 2013).

180  
181 The interactions among proteins in liquid-liquid phase separated droplets are expected to be  
182 weak and this is assessed by their rapid exchange within and between droplets and their  
183 surroundings (Brangwynne, Eckmann et al. 2009, Elbaum-Garfinkle, Kim et al. 2015, Lin,  
184 Protter et al. 2015, Feric, Vaidya et al. 2016). In fluorescence recovery after photobleaching  
185 (FRAP) experiments we measured both mobile ( $0.7 \pm 0.2$ ) and immobile ( $0.3 \pm 0.2$ ) fractions for  
186 the coat protein Sla2 and rapid recovery time (7.5 seconds), similar to other protein and nucleic  
187 acid droplets including the dense internal fibrillar component of *X. laevis* nucleoli (Fig. 2d)  
188 (Feric, Vaidya et al. 2016). Taken together, these results support the hypothesis that the cortical  
189 bodies are phase separated viscoelastic droplets. We next set out to determine the material  
190 properties of the cortical droplets and to test our postulate that their binding to the plasma  
191 membrane generates the force that drives invagination of the membrane.

### 192 193 **Cortical droplets can mechanically deform both cytosol and membrane**

194  
195 We hypothesized that free energy released by cortical droplet phase separation is converted into  
196 mechanical work to deform the membrane and the cytosol. Simply stated, we posit that binding  
197 of the droplet to cytosol draws the droplet inward as it grows, while the membrane follows,  
198 mediated by its own binding to the droplet and the requirement that the volume of the droplet be  
199 conserved. This idea is captured by the well-known Johnson-Kendall-Roberts (JKR) theory,

200 which describes how non-flat material surfaces stick to and conform to one another in order to  
201 minimize their interfacial energy (Young 1805, Dupré and Dupré 1869, Kendall 1994). When  
202 they stick to one another, soft and compliant materials such as the membrane and cytoplasm are  
203 deformed to a degree limited by their elasticity. Style, *et al.* adapted the JKR theory of contact  
204 mechanics to describe the contact surface geometry between a microscopic rigid particle and a  
205 soft substrate (Style, Hyland et al. 2013). We followed a similar approach in a simple  
206 phenomenological model expressed as the sum of mechanical strain energy ( $\phi$  term) and work ( $\psi$   
207 term), respectively;

$$208 \quad U \sim \phi \cdot \delta^{1+\varepsilon} - \psi \cdot \delta ; \quad (1)$$

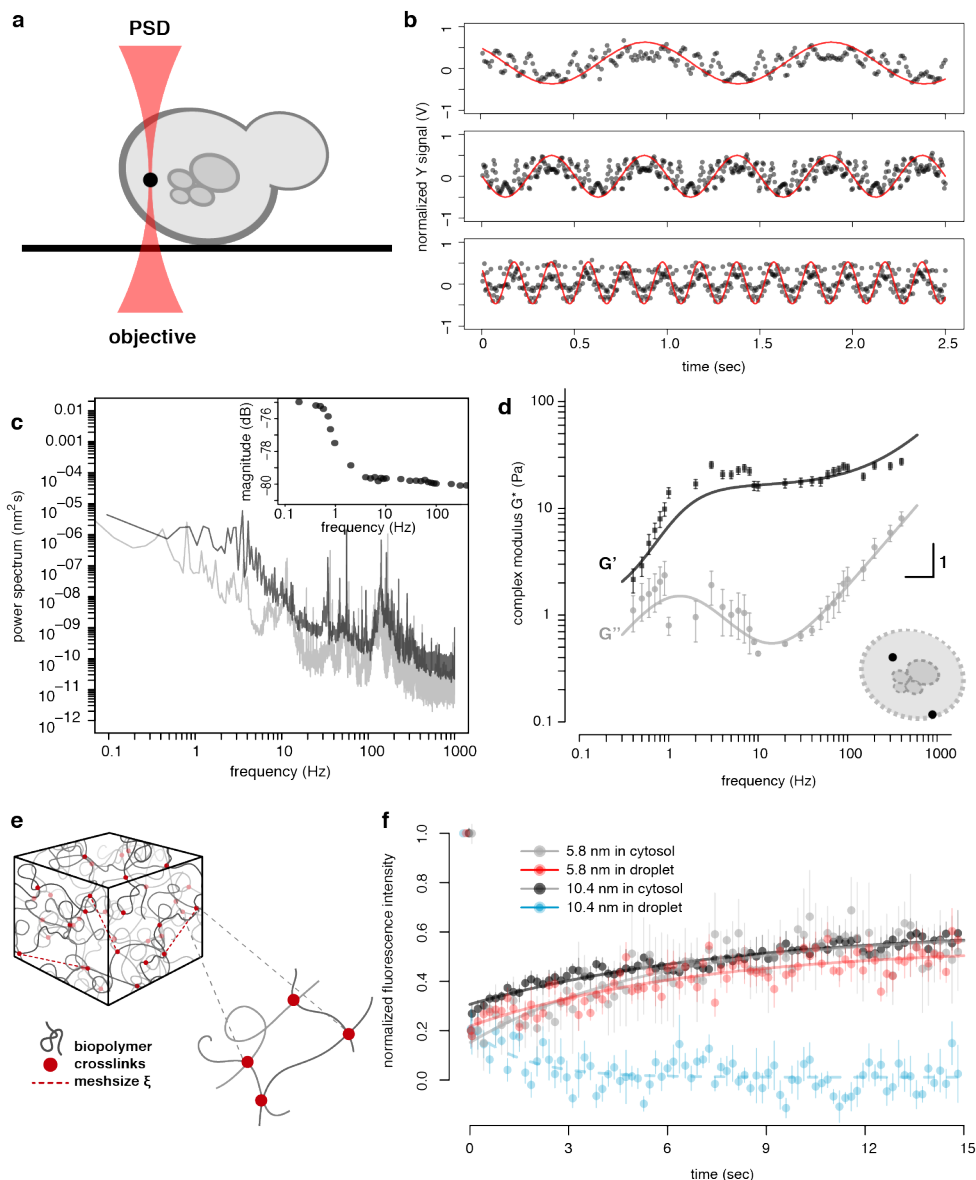
209  
210 Here,  $U$  is the mean-field energy,  $\delta$  is the invagination depth of both the membrane and cytosol  
211 (which we assume to be coupled to each other by virtue of conservation of volume of the  
212 droplet) and the exponent  $\varepsilon > 0$  reflects the deformation geometry (Material and Methods) (Hertz  
213 1882, Johnson 1971). Close to equilibrium (as  $\partial U/\partial \delta$  approaches 0) we expect invagination to  
214 balance the two contributions so that  $\delta^*$  minimizes energy in (1) resulting in,  
215

$$216 \quad \delta^* = \left( \frac{\psi}{\phi (1 + \varepsilon)} \right)^{\frac{1}{\varepsilon}} ; \quad (2)$$

217  
218 Equation (2) shows that the invagination depth  $\delta$  is determined by the ratio  $\psi/\phi$  and the  
219 deformation geometry  $\varepsilon$ . Values of  $\phi$  and  $\psi$  can be determined as functions of individual  
220 geometries, elasticities, and viscosities of cytosol, droplet and membrane and interfacial tensions  
221 among them (Material and Methods). These in turn can be determined by super-resolution  
222 imaging (geometries) and elastic and viscous moduli, taken from the literature or determined by  
223 active micro-rheology experiments as described next.  
224

225  
226 We used active rheology to determine the material properties of the cytosol in which cortical  
227 droplets are embedded and then, because the droplets are too small to probe directly, we  
228 calculated their properties through well-understood relationships between the properties of  
229 materials in contact and their resulting geometries, as described below. Specifically, we used  
230 optical tweezers to examine the frequency-dependent amplitude and phase responses of  
231 polystyrene beads that are embedded in cells (Fig. 3a, Material and Methods). 200 nm diameter  
232 polystyrene beads were integrated into cells by osmoporation (Fig. S9) (da Silva Pedrini, Dupont  
233 et al. 2014). Measurements of passive diffusion of the beads showed mean square displacements  
234 (MSD) close to that of random mechanical noise caused by vibration of the microscope (Fig.  
235 S9). Furthermore, we established that the osmoporation procedure did not affect rheological  
236 properties of cells by measuring the MSD of expressed viral capsid microNS particles labeled

237 with GFP in untreated or osmoporated cells and showing that their diffusion behaviors were  
 238 identical (Fig. S10) (Munder, Midtvedt et al. 2016).  
 239



240  
 241  
 242 **Figure 3 | Cytosol and cortical droplets are composed of a viscoelastic amorphous network of**  
 243 **interacting proteins** (a) We used optical tweezers (red beam between the microscope objective and a  
 244 position sensitive detector (PSD) coupled to an acousto-optic device (AOD) to oscillate polystyrene beads  
 245 in cells. Two pulses of osmotic shock were used to osmoporate 200 nm polystyrene beads (black) into  
 246 latA-treated haploid yeast *GPD1Δ* cells. (b) PSD output signal in volts (V) as a function of time for  
 247 acquisitions made at 1Hz (top), 2 Hz (middle) and 5 Hz (bottom). A bead, located in the cell periphery,  
 248 was oscillated with the AOD in the Y-axis of the specimen plane with fixed tweezer movement amplitude  
 249 (normalized red curve) at different frequencies. The recorded PSD raw traces (black points) were also  
 250 normalized to a corresponding magnitude range (coherence cutoff of 0.9). (c) Power spectrum of the  
 251 oscillated bead (black) with magnitude of response as a function of frequency (insert). (d) Decomposition



252 of  $G^*$  as a function of frequency into  $G'$  (storage modulus; darker squares) and  $G''$  (loss modulus; light  
253 shade circles) for beads distributed at both the cell periphery and interior (see schematic insert; mean  $\pm$   
254 sd;  $n = 17$ ) with an average trap stiffness  $k_{\text{trap}}$  (mean  $\pm$  se;  $8.0 \times 10^{-5} \pm 2.7 \times 10^{-5} \text{ N m}^{-1}$ ) and photodiode  
255 sensitivity factor  $\beta$  (mean  $\pm$  se;  $10.7 \times 10^3 \pm 2.3 \times 10^3 \text{ nm V}^{-1}$ ). Data was fitted to a model of an entangled  
256 and crosslinked network of flexible polymers (Material and Methods; Eq. 2.9-2.10). (e) 3D illustration  
257 and zoom-in of the latticework composed of amorphous protein chains (grey filaments) with the binding  
258 sites (red dots) through which they are non-covalently associate and the mesh size (dashed red line). (f)  
259 Fluorescence recovery after photo bleaching (FRAP) of fluorescent dye (FITC)-conjugated dextran of  
260 different size (see legend) within a Sla1-mCherry focus (red and blue) or neighbouring cytosolic regions  
261 without Sla1 signal (grey and black) in latA-treated *GPD1Δ* cells. Data points represent normalized  
262 fluorescence recovery (mean  $\pm$  sd;  $n=3$ ). Values for distinct dextran-FITC sizes and cell region were fitted  
263 to a single term recovery equation (Material and Methods).

264  
265 For active rheology experiments, we used an acousto-optic device to oscillate the position of the  
266 optical trap in the specimen plane at frequencies over four orders of magnitude and measured the  
267 displacement of trapped beads from the trap center using back focal plane interferometry (Fig.  
268 3b). We could thus measure the viscoelastic properties of the cytosol surrounding the beads by  
269 measuring their phase and amplitude response to the oscillations of the optical tweezers. Then by  
270 calculating the power spectrum of unforced fluctuations of the bead we obtained storage ( $G'$ ) and  
271 loss ( $G''$ ) moduli as a function of frequency (Fig. 3c-d, Fig. S11, Material and Methods) (Fischer,  
272 Richardson et al. 2010, Hendricks, Holzbaaur et al. 2012, Hendricks and Goldman 2017).

273  
274 In addition to obtaining quantities essential to calculate material properties of the cytoplasm and  
275 droplet, active rheology combined with spatiotemporal dynamics of interacting materials can  
276 inform of their structures. The mechanical properties of living cells can be compared to that of  
277 the popular children's toy "Silly Putty" (Cross 2012). Like this material, cells and underlying  
278 structures show different mechanical properties depending on the rates at which forces are  
279 applied to them (Hendricks, Holzbaaur et al. 2012, Guo, Ehrlicher et al. 2013, Guo, Ehrlicher et  
280 al. 2014). Thus if a force is applied at a low velocity, the cell behaves like a viscous fluid; taking  
281 on whatever shape it is forced into. When a force is applied at higher velocity, however, the  
282 material behaves like an elastic object, bouncing back to its original shape. As we discuss below,  
283 these behaviors reflect the manner and strengths with which the molecules that make up a  
284 material interact with each other and their environment.

285  
286 In specific terms, the material properties of the yeast cytoplasm and its interactions with the  
287 cortical droplet could be interpreted from the complex modulus versus frequency plot as follows  
288 (Fig. 3d): The inflection of the  $G'$  modulus at 2 Hz results in similar  $G'$  and  $G''$  values at low  
289 frequencies, which indicates that the cytosol is more viscous near rest. When deformed by the  
290 droplet growth (at a velocity of growth  $=2360 \pm 120 \text{ nm s}^{-1}$ ; corresponding to a stress at  $\sim 30 \pm 2$   
291 Hz) the cytosol is more elastic, whereas membrane invagination occurs at a rate at which the  
292 cytoplasm is more viscous (a velocity of  $7.4 \pm 2.5 \text{ nm s}^{-1}$ ; corresponding to  $0.1 \pm 0.04 \text{ Hz}$ ) (Fig.  
293 1b, 3d, Fig. S12). The  $G'$  and  $G''$  we measured are similar to the cytoplasm of adherent  
294 mammalian cells and indicate that the beads are confined within a dense network of interacting

295 molecules (Hendricks, Holzbaur et al. 2012, Guo, Ehrlicher et al. 2013, Guo, Ehrlicher et al.  
296 2014). We observed that the cytosol has a more malleable behavior at high frequencies than  
297 expected for an exclusively crosslinked network of flexible polymers; this may be attributed to  
298 an entangled, rather than crosslinked, network or due to compliant polymers that act more like  
299 entropic springs (Fig. 3d-e) (Koenderink, Atakhorrami et al. 2006, Lieleg, Schmoller et al. 2009,  
300 Broedersz, Depken et al. 2010, Sunthar 2010, Guo, Bourret et al. 2012, Dooling, Buck et al.  
301 2016).

302  
303 We could now determine the mechanical properties of the cortical droplet as follows. First, our  
304 micro-rheological data is consistent with both cortical droplets and cytosol behaving as elastic  
305 materials at rates above 1 Hz (Fig. 3d). Classic Hertz theory relates contact geometries of elastic  
306 materials to their mechanical properties. We could thus, use the geometry of the cortical droplets  
307 determined in our super-resolution imaging experiments and elasticity of the cytosol in which  
308 they are embedded to estimate the cortical droplet elastic modulus as 59 Pa (Fig. 1b, 3d, Material  
309 and Methods; Eq. 3.7-3.10) (Hertz 1882). These results suggest that the cortical droplets have  
310 similar material properties as the surrounding cytosol, which as an elastic (or Young's) modulus  
311 of 45 Pa at 1 Hz (Material and Methods). We estimated the mesh size of the cortical droplets by  
312 probing them with fluorophore-conjugated dextran molecules of 2.4, 5.8, and 10.4 nm in size and  
313 measured FRAP and colocalization of dextrans with Sla1-mCherry puncta (Fig. 3g-h, Fig. S13,  
314 14). Both 2.4 nm and 5.8 nm dextran-FITC recovered equally in the Sla1 droplet and cytosolic  
315 zones, with a mobile fraction of  $0.37 \pm 0.1$  and recovery rate constant of  $12.7 \pm 1.1$  s. In contrast,  
316 the 10.4 nm dextran-FITC molecules cannot be replaced once the PLD-rich protein network in  
317 the droplet is formed whereas they recover in the neighbour cytosol. These results are consistent  
318 with an exclusion zone for ribosomes as discussed above and with exclusion of dextrans of 5.8  
319 nm and more by known protein-RNA phase separated droplets called P bodies (Updike, Hachey  
320 et al. 2011, Kukulski, Schorb et al. 2012).

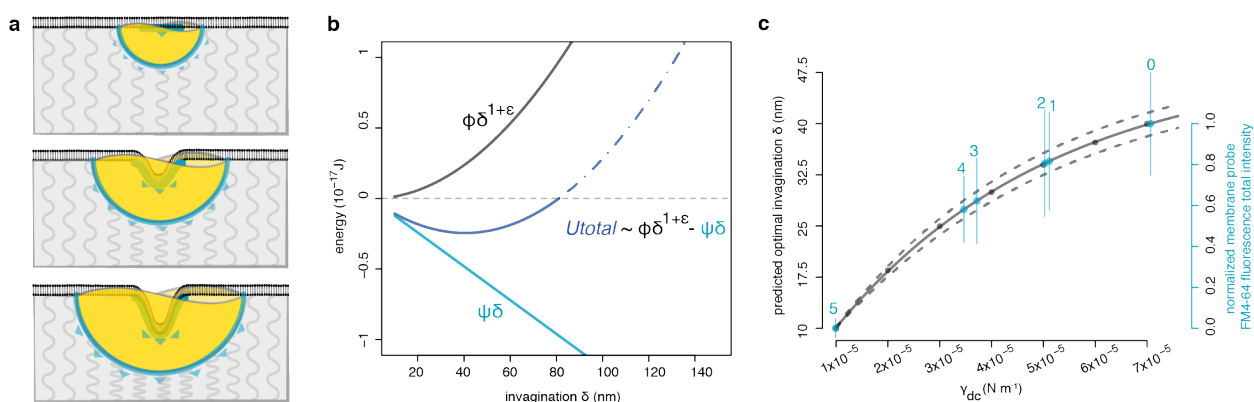
### 321 322 **Cortical droplet binding to cytosol provides energy to drive membrane invagination**

323  
324 The deformation of the membrane in response to contact of a soft viscoelastic object depends on  
325 the geometries and mechanical properties of the object and the vessel it is in (in our case the  
326 cytosol of a cell) and the membrane (Fig. 4a). From all electron and super-resolution  
327 fluorescence microscopic evidence, we know that the favored geometry of the membrane is flat  
328 with invagination centered in the middle of the droplet (Fig. 4a, lower). Such geometries could  
329 be explained by a local radial stress-gradient generated by the droplet adhesion to both the  
330 membrane and cytosol. Simply stated, as the droplet grows the binding to the cytosol draws it  
331 inward and the membrane follows, mediated by its own binding to the droplet and the  
332 requirement that the volume of the droplet be conserved.

333

334 We could now quantify the work performed by the droplet to invaginate the membrane using the  
 335 storage and loss moduli obtained from the micro-rheology experiments, geometric data obtained  
 336 from super-resolution imaging and other data available from the literature, to solve the explicit  
 337 form of the  $\phi$  and  $\psi$  terms (mechanical strain and work, respectively) in Equation (1) as  
 338 functions of membrane and cytosol invagination  $\delta$  (Material and Methods; Eq. 4.25-4.26). We  
 339 first estimated with the Young-Laplace equation an interfacial tension for the droplet-cytosol  
 340 interface,  $\gamma_{dc}$ , of  $7 \times 10^{-5} \text{ N}\cdot\text{m}^{-1}$ , from the pressure difference across the cytosolic interface and  
 341 the droplet mean curvature (Material and Methods; Eq. 4.6). This value falls within the range of  
 342  $10^{-5} \text{ N}\cdot\text{m}^{-1}$  to  $10^{-4} \text{ N}\cdot\text{m}^{-1}$  that has been reported for other protein droplets, including nucleoli and  
 343 P granules (Materials and Methods; Eq. 4.9) (Brangwynne, Mitchison et al. 2011, Elbaum-  
 344 Garfinkle, Kim et al. 2015).

345



346

347

348 **Figure 4 | Cortical droplets do mechanical work to deform the membrane and cytosol** (a) Illustration  
 349 of a cortical droplet (gold) that binds to (wets) a bilayer membrane (black) and drives membrane  
 350 invagination (top to bottom). Resultant membrane deformations reflect how forces balance under a  
 351 Young-Dupré adhesion gradient (blue lines and arrows). Resistance to deformation is represented by grey  
 352 curved lines. (b) Equation (1) (insert) was used to calculate the energy penalties ( $\phi$ ) and contributions ( $\psi$ )  
 353 at the cytosol and membrane interfaces with the cortical droplet. Total energy of the system (dark blue),  
 354 energy penalties (black) and energy contributions (light blue) are presented as a function of membrane  
 355 invagination ( $\delta$ ). The energy of membrane invagination is favourable for  $\delta$  between about 15-80 nm (solid  
 356 blue line) and unfavourable above 80 nm (dashed blue line). Quantities used to calculate energies are  
 357 detailed in Figure S15 and Tables S3-S4. (c) Our model predicts that vesicle size is proportional to the  
 358 strength of droplet intermolecular protein-protein interactions that are proportional to  $\gamma_{dc}$ , the droplet-to-  
 359 cytosol interfacial tension. Predicted membrane invagination  $\delta$  as a function of  $\gamma_{dc}$  (left axis and black  
 360 points). Data points were fitted to an exponential decay function (full line) with 95% confidence interval  
 361 (dashed lines). Titration of 1,6-hexanediol was used to reduce intermolecular cohesion and therefore  $\gamma_{dc}$   
 362 resulting in reduced vesicle size as measured by uptake of the lipophilic membrane probe FM4-64 into  
 363 cells (Right axis, red) *versus* % HD (blue numbers,  $n = 25$ , mean  $\pm$  sd) expressed as a function of the  
 364 droplet-cytosol interfacial tension  $\gamma_{dc}$  (Material and Methods).

365

366 With estimates of  $\gamma_{dc}$  we also determined the work of adhesion that is released when the droplet  
 367 surfaces are created, as described by the Young-Dupré equation (Materials and Methods; Eq.

368 4.11). Again, we assumed a radial adhesion gradient around the invagination peak in the middle  
369 of the cortical droplet (Fig. 4a). We calculated an adhesion energy ( $\psi$ ) of  $4.9 \times 10^{-18}$  J from  
370 interactions between the cortical droplet and both the membrane and cytosol (Fig. 4b, Fig. S15,  
371 Materials and Methods; Eq. 4.26). Our results suggest that the most significant contribution of  
372 the mechanical energy comes from the droplet-cytosol interface, where the adhesion energy of  
373  $2.9 \times 10^{-18}$  J is enough to overcome energy penalty of  $2.4 \times 10^{-18}$  J to deform both membrane and  
374 cytosol. This energy cost includes the elastic, viscous, and interfacial stress penalties (Fig. 4b,  
375 Fig. S15, Table S4). We also calculated an average adhesion energy of  $1.3 \text{ kJ}\cdot\text{mol}^{-1}$  at the  
376 droplet-cytosol interface (Material and Methods), which is consistent with the free energies  
377 expected of non-covalent interactions (Mahadevi and Sastry 2016).

378  
379 Our model provides a physical framework to explain how cortical droplets do the mechanical  
380 work needed to induce invagination of membranes in CME. The interface between droplets,  
381 formed, by phase separation of disordered proteins into cortical bodies, and the cytosol-  
382 membrane interface deforms the surrounding materials through adhesive interactions.  
383 Invagination occurs when  $\psi$  dominates  $\phi$  and this is favored within the observed  $\delta$  interval of 40  
384 nm to 80 nm (Fig. S15). Notably, this predicted  $\delta$  interval is within the range of plasma  
385 membrane invagination of  $\sim 70$  nm at which point a membrane scission mechanism is activated  
386 and vesicle generation is completed (Idrissi, Blasco et al. 2012).

387  
388 We propose that cortical droplets store and dissipate mechanical energy in the form of surface  
389 tension, whereby the composition of the droplets determines their interfacial interactions and  
390 provides the energy for adhesion and invagination of membranes. Accordingly, the underlying  
391 energy stored within the droplets and the balance of interactions amongst droplet components  
392 and solvent governs the nature of the interface. The potential energy  $\psi$  of droplets, which by the  
393 work-energy theorem should be equivalent to the total work of adhesion, should be dictated by  
394 the density and strengths of physical interactions amongst proteins within the droplet (the droplet  
395 cohesion and interfacial tensions). We tested this assumption by weakening the favorable free  
396 energies of the protein-protein interactions that hold droplet components together using 1,6-  
397 hexanediol (HD). These are the interactions that drive the phase separation of cortical droplets,  
398 and so would correspond to a decrease of the droplet surface tension ( $\gamma_{dc}$  or  $\psi$ ). Our model  
399 predicts that invagination depth should continuously vary with  $\psi$  from Equation (2). We titrated  
400 HD below the effective concentration that prevents protein phase separation and quantified  
401 individual membrane excision events by quantifying uptake of the lipophilic membrane probe  
402 FM4-64 into cells by fluorescence microscopy (Fig. 2a, Material and Methods). In LatA treated  
403 *GPD1A* cells, this measures the amount of labeled membrane taken up into cells under the action  
404 of cortical droplets alone. By increasing subcritical HD concentration (corresponding to a  
405 decrease in  $\psi$ ), the average fluorescence-labeled membrane per vesicle (a proxy for invagination  
406  $\delta$ ) was continuously reduced over one order of magnitude in the value of  $\gamma_{dc}$  (Fig. 4c, Material  
407 and Methods; Eq. 2.8). This observation fits with the reduced membrane invagination that we

408 predicted at the outset (*i.e.*, that  $\delta$  scales with the  $\psi/\phi$  ratio) when the droplet cohesion ( $\gamma_{dc}$  or  $\psi$ )  
409 is also reduced (Fig. 4c, Material and Methods; Eq. 4.2).

410

## 411 **Discussion**

412

413 Our results provide a framework for answering many questions regarding CME and other  
414 membrane budding processes. Given our observations, how is CME coupled to multiple  
415 signaling pathways that integrate to regulate vesicle formation? For instance, the PLD-containing  
416 CME proteins we investigated are enriched for multiple phosphorylation sites, which undergo  
417 changes in response to activation of a CME-regulating signaling pathway (Kanshin, Bergeron-  
418 Sandoval et al. 2015). Since the amount and distribution of charge in disordered regions of  
419 proteins regulate their interactions and conformations (Das and Pappu 2013), such post-  
420 translational modifications may be important to regulating CME. Our fluorescence microscopy  
421 and electron micrographic evidence from the literature suggests that the cortical droplet remains  
422 associated temporarily with mature vesicles (Kukulski, Schorb et al. 2012). Does the droplet play  
423 any role in trafficking and fusing with, for instance, plasma membrane (protein recycling) or  
424 lysosome (protein degradation)? CME underlies several fundamental mechanisms of vesicle  
425 trafficking and attendant membrane and vesicle protein cargo transport, including late secretory  
426 pathways, endocytosis and neuronal synaptic vesicle recycling. Yeast and human proteins  
427 implicated in clathrin-mediated vesicle trafficking are enriched for long disordered protein  
428 domains (47/23% of proteins with long consecutive disordered regions of 30 residues and more  
429 for humans and yeast, respectively) whereas those involved in two other vesicle trafficking  
430 systems are not (COPI: 8/5%; COPII: 8/5%) (Pietrosemoli, Panca et al. 2013, Busch, Houser et  
431 al. 2015). These observations argue for investigating the generality and conservation of protein  
432 droplet adhesion-driven membrane invagination as the basis of clathrin-mediated vesicle  
433 trafficking in the absence of actin polymerization.

434

435 It is possible that other liquid-liquid phase separated protein and protein nucleic acid droplets  
436 may influence cellular sub-structural dynamics and shape thus contributing to cell, tissue, and  
437 organism morphology (Bergeron-Sandoval, Safaee et al. 2016). More broadly, interfacial contact  
438 potentials between different biological materials could represent a vastly underestimated source  
439 of complex pattern formation in biology, such as has been observed in embryonic tissue layers  
440 (Foty, Pflieger et al. 1996) or recently in a model of growing brain convolutions (Tallinen, Chung  
441 et al. 2016) or in protein stabilization (Gupta, Donlan et al. 2017).

442

443

## 444 **Acknowledgments**

445 The authors acknowledge support from CIHR grants MOP-GMX-152556 (SWM) and the US  
446 National Institutes of Health grant R01NS056114 (RVP). We thank Simon Alberti for insightful  
447 discussions and microNS plasmid, Jackie Vogel for strains, Jacqueline Kowarzyk for technical  
448 assistance, Susan Liebman for the Sup35 plasmids, Daniel Zenklussen and Pascal Raymond for  
449 help with FRAP experiments.

450

## 451 **Author contributions**

452 L.P.S.B. and S.W.M. designed all of the research and R.V.P. helped in designing part of the  
453 research; L.P.S.B. performed biological research; L.P.S.B. and H.K.H. performed micro  
454 rheology experiments; L.P.B.S., H.K.H., A.J.E. and A.G.H analyzed micro rheology data;  
455 L.P.S.B., A.J.E. and S.W.M. analyzed biological data; L.P.B.S., H.K.H. and P.F. developed  
456 physical droplet model; L.P.S.B., R.V.P., and S.W.M. combined physical models with data  
457 analysis; all authors wrote the paper.

458

## 459 **Supplementary Materials**

460 Materials and Methods

461 Figures S1-S15

462 Tables S1-S4

463 Movies S1

464 References

465

## 466 **References**

467

- 468 1. Aghamohammadzadeh, S. and K. R. Ayscough (2009). "Differential requirements for actin  
469 during yeast and mammalian endocytosis." *Nat Cell Biol* 11 (8): 1039-1042.
- 470 2. Alberti, S., R. Halfmann, O. King, A. Kapila and S. Lindquist (2009). "A systematic survey  
471 identifies prions and illuminates sequence features of prionogenic proteins." *Cell* 137 (1): 146-  
472 158.
- 473 3. Avinoam, O., M. Schorb, C. J. Beese, J. A. Briggs and M. Kaksonen (2015). "ENDOCYTOSIS.  
474 Endocytic sites mature by continuous bending and remodeling of the clathrin coat." *Science* 348  
475 (6241): 1369-1372.
- 476 4. Banjade, S., Q. Wu, A. Mittal, W. B. Peeples, R. V. Pappu and M. K. Rosen (2015). "Conserved  
477 interdomain linker promotes phase separation of the multivalent adaptor protein Nck." *Proc Natl*  
478 *Acad Sci U S A* 112 (47): E6426-6435.
- 479 5. Bergeron-Sandoval, L. P., N. Safaee and S. W. Michnick (2016). "Mechanisms and  
480 Consequences of Macromolecular Phase Separation." *Cell* 165 (5): 1067-1079.
- 481 6. Boettner, D. R., J. L. D'Agostino, O. T. Torres, K. Daugherty-Clarke, A. Uygur, A. Reider, B.  
482 Wendland, S. K. Lemmon and B. L. Goode (2009). "The F-BAR protein Syp1 negatively

- 483 regulates WASp-Arp2/3 complex activity during endocytic patch formation." *Curr Biol* 19 (23):  
484 1979-1987.
- 485 7. Boulant, S., C. Kural, J. C. Zeeh, F. Ubelmann and T. Kirchhausen (2011). "Actin dynamics  
486 counteract membrane tension during clathrin-mediated endocytosis." *Nat Cell Biol* 13 (9): 1124-  
487 1131.
- 488 8. Brach, T., C. Godlee, I. Moeller-Hansen, D. Boeke and M. Kaksonen (2014). "The initiation of  
489 clathrin-mediated endocytosis is mechanistically highly flexible." *Curr Biol* 24 (5): 548-554.
- 490 9. Brangwynne, C. P., C. R. Eckmann, D. S. Courson, A. Rybarska, C. Hoege, J. Gharakhani, F.  
491 Julicher and A. A. Hyman (2009). "Germline P granules are liquid droplets that localize by  
492 controlled dissolution/condensation." *Science* 324 (5935): 1729-1732.
- 493 10. Brangwynne, C. P., T. J. Mitchison and A. A. Hyman (2011). "Active liquid-like behavior of  
494 nucleoli determines their size and shape in *Xenopus laevis* oocytes." *Proc Natl Acad Sci U S A*  
495 108 (11): 4334-4339.
- 496 11. Broedersz, C. P., M. Depken, N. Y. Yao, M. R. Pollak, D. A. Weitz and F. C. MacKintosh  
497 (2010). "Cross-link-governed dynamics of biopolymer networks." *Phys Rev Lett* 105 (23):  
498 238101.
- 499 12. Busch, D. J., J. R. Houser, C. C. Hayden, M. B. Sherman, E. M. Lafer and J. C. Stachowiak  
500 (2015). "Intrinsically disordered proteins drive membrane curvature." *Nat Commun* 6: 7875.
- 501 13. Carlsson, A. E. and P. V. Bayly (2014). "Force generation by endocytic actin patches in budding  
502 yeast." *Biophys J* 106 (8): 1596-1606.
- 503 14. Crick, S. L., K. M. Ruff, K. Garai, C. Frieden and R. V. Pappu (2013). "Unmasking the roles of  
504 N- and C-terminal flanking sequences from exon 1 of huntingtin as modulators of polyglutamine  
505 aggregation." *Proceedings of the National Academy of Sciences* 110 (50): 20075-20080.
- 506 15. Cross, R. (2012). "Elastic and viscous properties of Silly Putty." *American Journal of Physics* 80:  
507 870-875.
- 508 16. da Silva Pedrini, M. R., S. Dupont, A. de Anchieta Camara, Jr., L. Beney and P. Gervais (2014).  
509 "Osmoporation: a simple way to internalize hydrophilic molecules into yeast." *Appl Microbiol*  
510 *Biotechnol* 98 (3): 1271-1280.
- 511 17. Darwin, C. (1859). *On the origin of species by means of natural selection, or, The preservation of*  
512 *favoured races in the struggle for life*. London, J. Murray.
- 513 18. Das, R. K. and R. V. Pappu (2013). "Conformations of intrinsically disordered proteins are  
514 influenced by linear sequence distributions of oppositely charged residues." *Proc Natl Acad Sci U*  
515 *S A* 110 (33): 13392-13397.
- 516 19. Dmitrieff, S. and F. Nedelec (2015). "Membrane Mechanics of Endocytosis in Cells with  
517 Turgor." *PLoS Comput Biol* 11 (10): e1004538.
- 518 20. Dooling, L. J., M. E. Buck, W. B. Zhang and D. A. Tirrell (2016). "Programming Molecular  
519 Association and Viscoelastic Behavior in Protein Networks." *Adv Mater* 28 (23): 4651-4657.
- 520 21. Drubin, D. G., M. Kaksonen, C. Toret and Y. Sun (2005). "Cytoskeletal networks and pathways  
521 involved in endocytosis." *Novartis Found Symp* 269: 35-42; discussion 43-36, 223-230.
- 522 22. Dupré, A. and P. Dupré (1869). *Théorie mécanique de la chaleur* Gauthier-Villars.
- 523 23. Elbaum-Garfinkle, S., Y. Kim, K. Szczepaniak, C. C. Chen, C. R. Eckmann, S. Myong and C. P.  
524 Brangwynne (2015). "The disordered P granule protein LAF-1 drives phase separation into  
525 droplets with tunable viscosity and dynamics." *Proc Natl Acad Sci U S A* 112 (23): 7189-7194.
- 526 24. Ewers, H., W. Romer, A. E. Smith, K. Bacia, S. Dmitrieff, W. Chai, R. Mancini, J. Kartenbeck,  
527 V. Chambon, L. Berland, A. Oppenheim, G. Schwarzmann, T. Feizi, P. Schwille, P. Sens, A.  
528 Helenius and L. Johannes (2010). "GM1 structure determines SV40-induced membrane  
529 invagination and infection." *Nat Cell Biol* 12 (1): 11-18; sup pp 11-12.
- 530 25. Feric, M., N. Vaidya, T. S. Harmon, D. M. Mitrea, L. Zhu, T. M. Richardson, R. W. Kriwacki, R.  
531 V. Pappu and C. P. Brangwynne (2016). "Coexisting Liquid Phases Underlie Nucleolar  
532 Subcompartments." *Cell* 165 (7): 1686-1697.
- 533 26. Fischer, M., A. C. Richardson, S. N. Reihani, L. B. Oddershede and K. Berg-Sorensen (2010).

- 534 "Active-passive calibration of optical tweezers in viscoelastic media." *Rev Sci Instrum* 81 (1):  
535 015103.
- 536 27. Foty, R. A., C. M. Pflieger, G. Forgacs and M. S. Steinberg (1996). "Surface tensions of  
537 embryonic tissues predict their mutual envelopment behavior." *Development* 122 (5): 1611-1620.
- 538 28. Godlee, C. and M. Kaksonen (2013). "Review series: From uncertain beginnings: initiation  
539 mechanisms of clathrin-mediated endocytosis." *J Cell Biol* 203 (5): 717-725.
- 540 29. Guilak, F., J. R. Tedrow and R. Burgkart (2000). "Viscoelastic properties of the cell nucleus."  
541 *Biochem Biophys Res Commun* 269 (3): 781-786.
- 542 30. Guo, H., G. Bourret, R. B. Lennox, M. Sutton, J. L. Harden and R. L. Leheny (2012).  
543 "Entanglement-controlled subdiffusion of nanoparticles within concentrated polymer solutions."  
544 *Phys Rev Lett* 109 (5): 055901.
- 545 31. Guo, M., A. J. Ehrlicher, M. H. Jensen, M. Renz, J. R. Moore, R. D. Goldman, J. Lippincott-  
546 Schwartz, F. C. Mackintosh and D. A. Weitz (2014). "Probing the stochastic, motor-driven  
547 properties of the cytoplasm using force spectrum microscopy." *Cell* 158 (4): 822-832.
- 548 32. Guo, M., A. J. Ehrlicher, S. Mahammad, H. Fabich, M. H. Jensen, J. R. Moore, J. J. Fredberg, R.  
549 D. Goldman and D. A. Weitz (2013). "The role of vimentin intermediate filaments in cortical and  
550 cytoplasmic mechanics." *Biophys J* 105 (7): 1562-1568.
- 551 33. Gupta, K., J. A. Donlan, J. T. Hopper, P. Uzdavinys, M. Landreh, W. B. Struwe, D. Drew, A. J.  
552 Baldwin, P. J. Stansfeld and C. V. Robinson (2017). "The role of interfacial lipids in stabilizing  
553 membrane protein oligomers." *Nature* 541 (7637): 421-424.
- 554 34. Harold, F. M. (1990). "To shape a cell: an inquiry into the causes of morphogenesis of  
555 microorganisms." *Microbiol Rev* 54 (4): 381-431.
- 556 35. Haviv, L., D. Gillo, F. Backouche and A. Bernheim-Groswasser (2008). "A cytoskeletal  
557 demolition worker: myosin II acts as an actin depolymerization agent." *J Mol Biol* 375 (2): 325-  
558 330.
- 559 36. Hendricks, A. G. and Y. E. Goldman (2017). "Measuring Molecular Forces Using Calibrated  
560 Optical Tweezers in Living Cells." *Methods Mol Biol* 1486: 537-552.
- 561 37. Hendricks, A. G., E. L. Holzbaur and Y. E. Goldman (2012). "Force measurements on cargoes in  
562 living cells reveal collective dynamics of microtubule motors." *Proc Natl Acad Sci U S A* 109  
563 (45): 18447-18452.
- 564 38. Hertz, H. R. (1882). *Ueber die Beruehrung elastischer Koerper (On Contact Between Elastic*  
565 *Bodies)*. Leipzig, Germany, 1895.
- 566 39. Hom, R. A., M. Vora, M. Regner, O. M. Subach, W. Cho, V. V. Verkhusha, R. V. Stahelin and T.  
567 G. Kutateladze (2007). "pH-dependent binding of the Epsin ENTH domain and the AP180 ANTH  
568 domain to PI (4,5)P2-containing bilayers." *J Mol Biol* 373 (2): 412-423.
- 569 40. Hyman, A. A., C. A. Weber and F. Julicher (2014). "Liquid-liquid phase separation in biology."  
570 *Annu Rev Cell Dev Biol* 30: 39-58.
- 571 41. Idrissi, F. Z., A. Blasco, A. Espinal and M. I. Geli (2012). "Ultrastructural dynamics of proteins  
572 involved in endocytic budding." *Proc Natl Acad Sci U S A* 109 (39): E2587-2594.
- 573 42. Idrissi, F. Z., H. Grottsch, I. M. Fernandez-Golbano, C. Presciatto-Baschong, H. Riezman and M.  
574 I. Geli (2008). "Distinct acto/myosin-I structures associate with endocytic profiles at the plasma  
575 membrane." *J Cell Biol* 180 (6): 1219-1232.
- 576 43. Jiang, H., S. Wang, Y. Huang, X. He, H. Cui, X. Zhu and Y. Zheng (2015). "Phase transition of  
577 spindle-associated protein regulate spindle apparatus assembly." *Cell* 163 (1): 108-122.
- 578 44. Johnson, K. L. K., K.;Roberts, A. D. (1971). "Surface energy and the contact of elastic solid."  
579 *Proc. R. Soc. Lond. A.* 324: 301-313.
- 580 45. Kaksonen, M., C. P. Toret and D. G. Drubin (2005). "A modular design for the clathrin- and  
581 actin-mediated endocytosis machinery." *Cell* 123 (2): 305-320.
- 582 46. Kanshin, E., L. P. Bergeron-Sandoval, S. S. Isik, P. Thibault and S. W. Michnick (2015). "A cell-  
583 signaling network temporally resolves specific versus promiscuous phosphorylation." *Cell Rep* 10  
584 (7): 1202-1214.



- 585 47. Kendall, K. (1994). "Adhesion: molecules and mechanics." *Science* 263 (5154): 1720-1725.
- 586 48. Khurana, R., C. Coleman, C. Ionescu-Zanetti, S. A. Carter, V. Krishna, R. K. Grover, R. Roy and
- 587 S. Singh (2005). "Mechanism of thioflavin T binding to amyloid fibrils." *J Struct Biol* 151 (3):
- 588 229-238.
- 589 49. Koenderink, G. H., M. Atakhorrami, F. C. MacKintosh and C. F. Schmidt (2006). "High-
- 590 frequency stress relaxation in semiflexible polymer solutions and networks." *Phys Rev Lett* 96
- 591 (13): 138307.
- 592 50. Kroschwald, S., S. Maharana, D. Mateju, L. Malinowska, E. Nuske, I. Poser, D. Richter and S.
- 593 Alberti (2015). "Promiscuous interactions and protein disaggregases determine the material state
- 594 of stress-inducible RNP granules." *Elife* 4: e06807.
- 595 51. Kukulski, W., A. Picco, T. Specht, J. A. Briggs and M. Kaksonen (2016). "Clathrin modulates
- 596 vesicle scission, but not invagination shape, in yeast endocytosis." *Elife* 5.
- 597 52. Kukulski, W., M. Schorb, M. Kaksonen and J. A. Briggs (2012). "Plasma membrane reshaping
- 598 during endocytosis is revealed by time-resolved electron tomography." *Cell* 150 (3): 508-520.
- 599 53. Li, D., L. Shao, B. C. Chen, X. Zhang, M. Zhang and B. Moses (2015). "Extended-resolution
- 600 structured illumination imaging of endocytic and cytoskeletal dynamics." *Science* (New York,
- 601 NY).
- 602 54. Lieleg, O., K. M. Schmoller, M. M. Claessens and A. R. Bausch (2009). "Cytoskeletal polymer
- 603 networks: viscoelastic properties are determined by the microscopic interaction potential of cross-
- 604 links." *Biophys J* 96 (11): 4725-4732.
- 605 55. Lin, Y., D. S. W. Protter, M. K. Rosen and R. Parker (2015). "Formation and Maturation of
- 606 Phase-Separated Liquid Droplets by RNA-Binding Proteins." *Molecular Cell* 60 (2): 208-219.
- 607 56. Mahadevi, A. S. and G. N. Sastry (2016). "Cooperativity in Noncovalent Interactions." *Chem Rev*
- 608 116 (5): 2775-2825.
- 609 57. Malinowska, L., S. Kroschwald and S. Alberti (2013). "Protein disorder, prion propensities, and
- 610 self-organizing macromolecular collectives." *Biochim Biophys Acta* 1834 (5): 918-931.
- 611 58. Meshcheryakov, A., E. Steudle and E. Komor (1992). "Gradients of turgor, osmotic pressure, and
- 612 water potential in the cortex of the hypocotyl of growing ricinus seedlings : effects of the supply
- 613 of water from the xylem and of solutes from the Phloem." *Plant Physiol* 98 (3): 840-852.
- 614 59. Molliex, A., J. Temirov, J. Lee, M. Coughlin, A. P. Kanagaraj, H. J. Kim, T. Mittag and J. P.
- 615 Taylor (2015). "Phase separation by low complexity domains promotes stress granule assembly
- 616 and drives pathological fibrillization." *Cell* 163 (1): 123-133.
- 617 60. Munder, M. C., D. Midtvedt, T. Franzmann, E. Nuske, O. Otto, M. Herbig, E. Ulbricht, P.
- 618 Muller, A. Taubenberger, S. Maharana, L. Malinowska, D. Richter, J. Guck, V. Ziburdaev and S.
- 619 Alberti (2016). "A pH-driven transition of the cytoplasm from a fluid- to a solid-like state
- 620 promotes entry into dormancy." *Elife* 5.
- 621 61. Nott, T. J., E. Petsalaki, P. Farber, D. Jervis, E. Fussner, A. Plochowietz, T. D. Craggs, D. P.
- 622 Bazett-Jones, T. Pawson, J. D. Forman-Kay and A. J. Baldwin (2015). "Phase transition of a
- 623 disordered nuage protein generates environmentally responsive membraneless organelles." *Mol*
- 624 *Cell* 57 (5): 936-947.
- 625 62. Pappu, R. V., X. Wang, A. Vitalis and S. L. Crick (2008). "A polymer physics perspective on
- 626 driving forces and mechanisms for protein aggregation." *Arch Biochem Biophys* 469 (1): 132-
- 627 141.
- 628 63. Picco, A., M. Mund, J. Ries, F. Nedelec and M. Kaksonen (2015). "Visualizing the functional
- 629 architecture of the endocytic machinery." *Elife* 4.
- 630 64. Pietrosevoli, N., R. Pancsa and P. Tompa (2013). "Structural disorder provides increased
- 631 adaptability for vesicle trafficking pathways." *PLoS Comput Biol* 9 (7): e1003144.
- 632 65. Pollard, T. D. and J. A. Cooper (2009). "Actin, a central player in cell shape and movement."
- 633 *Science* 326 (5957): 1208-1212.
- 634 66. Rog, O., S. Kohler and A. F. Dernburg (2017). "The synaptonemal complex has liquid crystalline
- 635 properties and spatially regulates meiotic recombination factors." *Elife* 6.

- 636 67. Scher-Zagier, J. K. and A. E. Carlsson (2016). "Local Turgor Pressure Reduction via Channel  
637 Clustering." *Biophys J* 111 (12): 2747-2756.
- 638 68. Style, R. W., C. Hyland, R. Boltyanskiy, J. S. Wettlaufer and E. R. Dufresne (2013). "Surface  
639 tension and contact with soft elastic solids." *Nat Commun* 4: 2728.
- 640 69. Sunthar, P. (2010). *Polymer Rheology*, Springer New York.
- 641 70. Tallinen, T., J. Chung, F. Rousseau, N. Girard, J. Lefèvre and L. Mahadevan (2016). "On the  
642 growth and form of cortical convolutions." *Nature Physics* 12: 588–593.
- 643 71. Thompson, D. A. W. (1917). *On growth and form*. Cambridge Eng., University press.
- 644 72. Toombs, J. A., B. R. McCarty and E. D. Ross (2009). "Compositional Determinants of Prion  
645 Formation in Yeast." *Molecular and Cellular Biology* 30 (1): 319-332.
- 646 73. Updike, D. L., S. J. Hachey, J. Kreher and S. Strome (2011). "P granules extend the nuclear pore  
647 complex environment in the *C. elegans* germ line." *J Cell Biol* 192 (6): 939-948.
- 648 74. Watson, H. A., M. J. Cope, A. C. Groen, D. G. Drubin and B. Wendland (2001). "In vivo role for  
649 actin-regulating kinases in endocytosis and yeast epsin phosphorylation." *Mol Biol Cell* 12 (11):  
650 3668-3679.
- 651 75. Wheeler, J. R., T. Matheny, S. Jain, R. Abrisch and R. Parker (2016). "Distinct stages in stress  
652 granule assembly and disassembly." *Elife* 5.
- 653 76. Young, T. (1805). "An essay on the cohesion of fluids." *Philosophical Transactions of the Royal  
654 Society of London* 95: 65-87.
- 655 77. Zhang, H., S. Elbaum-Garfinkle, E. M. Langdon, N. Taylor, P. Occhipinti, A. A. Bridges, C. P.  
656 Brangwynne and A. S. Gladfelter (2015). "RNA Controls PolyQ Protein Phase Transitions."  
657 *Molecular Cell* 60 (2): 220-230.  
658