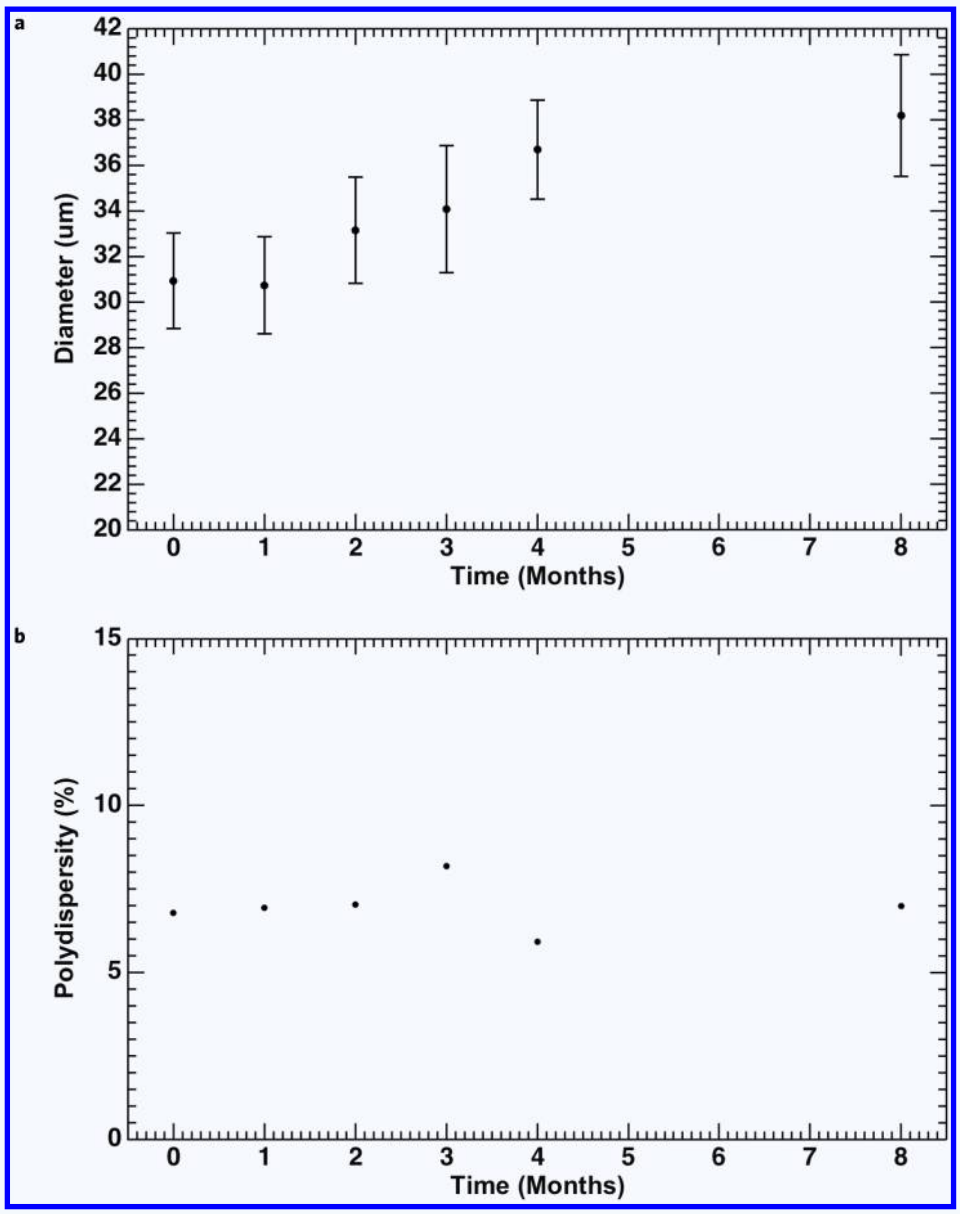
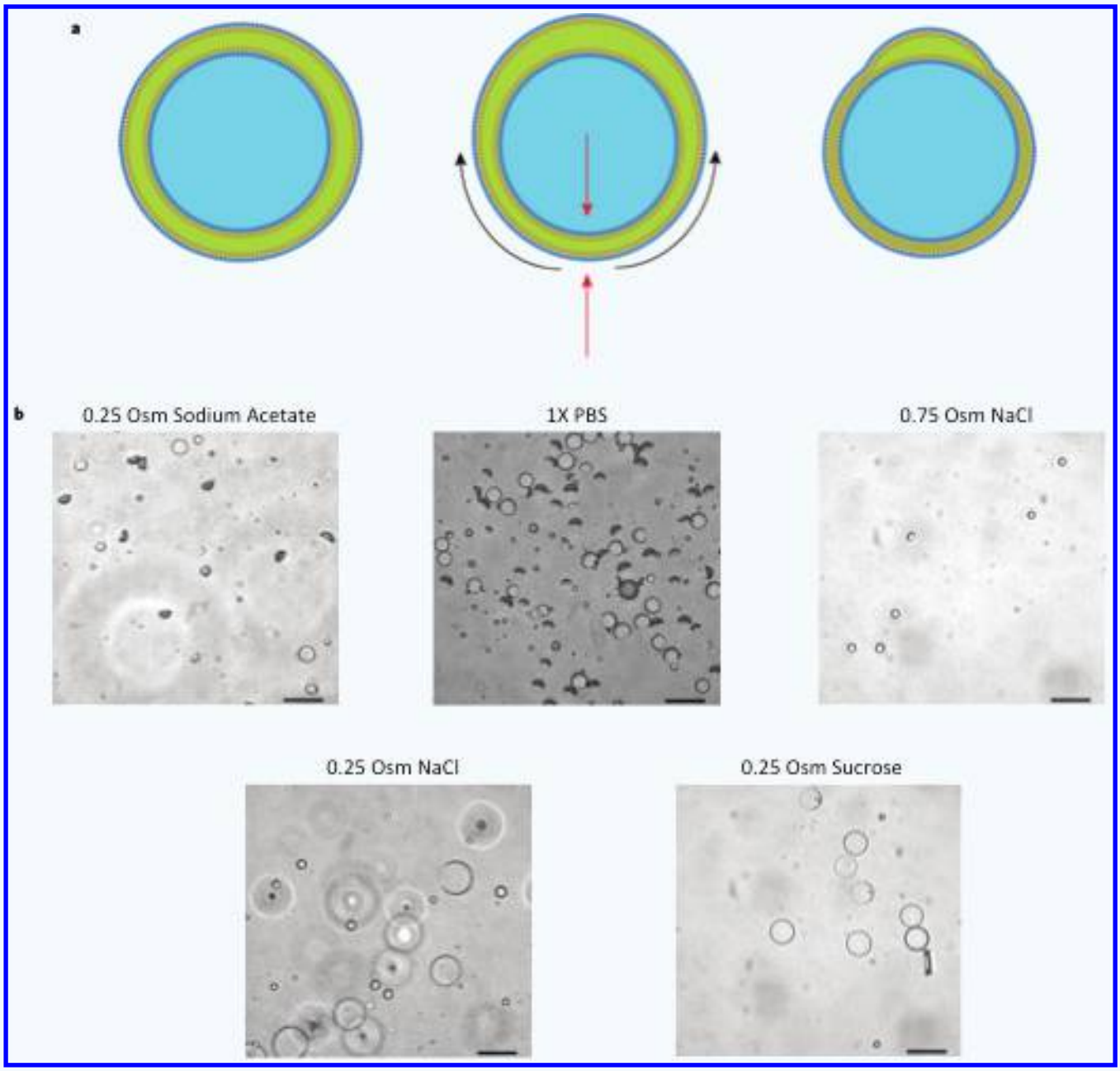
SUPPLEMENTARY INFORMATION

DOUBLE EMULSION STORAGE

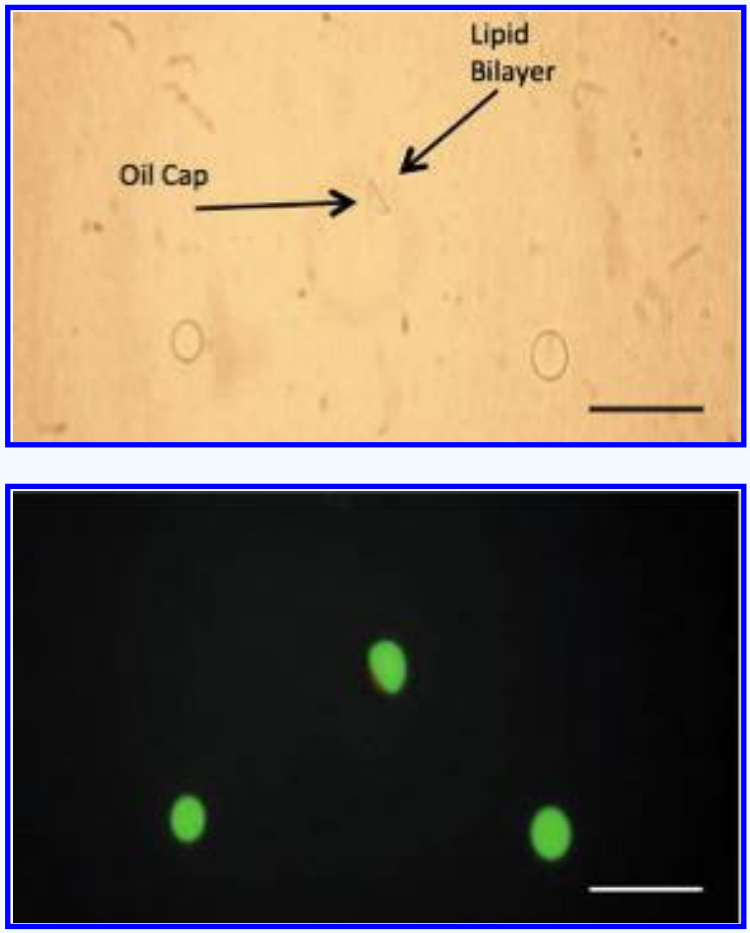


Supplementary Figure 1 Double emulsion survival. Measure of diameter (**a**) and polydispersity (**b**) over the course of eight months.

The double emulsions were stored in 0.25%–1% pluronic F68 with a 500 mM NaCl or sucrose internal phase, for osmotic pressure balance. The presence of at least 0.25% of pluronic F68 in the storage solution is necessary to prevent droplet fusion, and to maintain low interfacial energy at the inner and outer water-oil interfaces. There was a slight increase in the diameter of the double emulsions over the course of eight months, most likely due to interfacial changes that may have developed during storage, but monodispersity remained unchanged (**Supplementary Fig. 1b**).



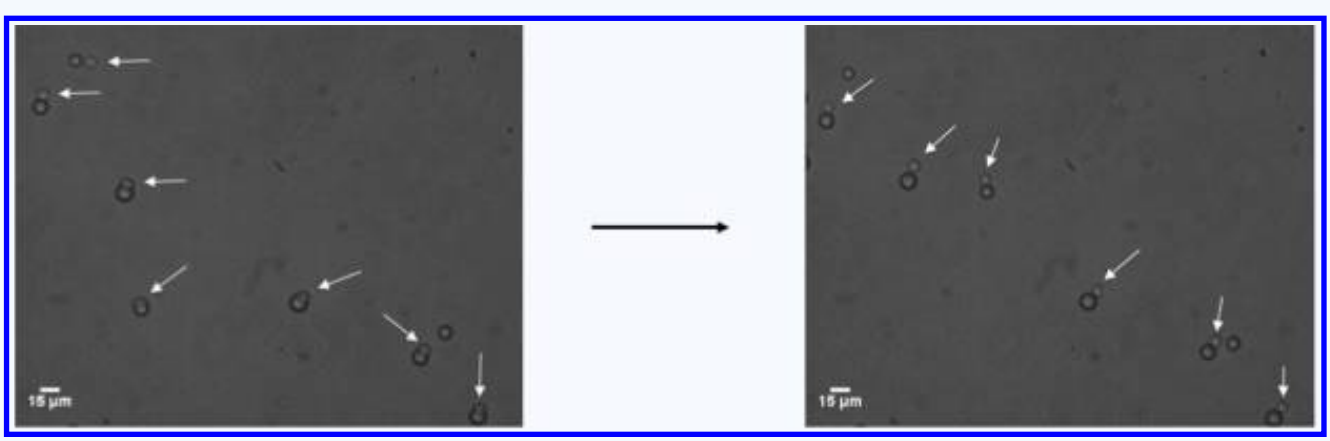
Supplementary Figure 2 Multisome formation. (**a**) Illustration of the dewetting process to form multisomes. Process is from left-to-right. Upon exposure to increased interfacial tension from electrolytes, the oil in the double emulsion begins to accumulate to one side, causing a local thinning of the oil shell and an increase in the depletion force in that area. The lipid tails can then force out the excess oil to one side and form a bilayer. (**b**) Morphological equilibrium of double emulsions when exposed to different solutions. Composition of internal and middle phase given in **Table 2**. Scale bar is 50 μm.



Supplementary Figure 3 Multisome with encapsulated dye. (**a**) Bright field and (**b**) Fluorescent image of SCM with FITC-Dextran in the internal phase. Scale bar is 50 μm.

GUV FORMATION TRIALS

After DPPC incorporation, the collected double emulsions were placed in a variety of solutions ranging from 0.5–1.2 Osm, including NaCl, PBS, sodium acetate, sucrose, sodium iodide, and KCl. Osmotic pressure differences were introduced in some scenarios to cause the double emulsions to shrink and increase the internal PBS concentration so that the condition of in Equation (5) could be realized; but most either became SCMs or remained double emulsions. Surprisingly though, it was noticed that after approximately 15 minutes of placement in either 2× PBS or > 0.6 Osm KCl, the double emulsions shrunk considerably, and began to transition into SCMs, and then GUVs (**Supplementary Fig. 4**). Since the solution used was only a few μL in volume, and was exposed to atmosphere on a glass



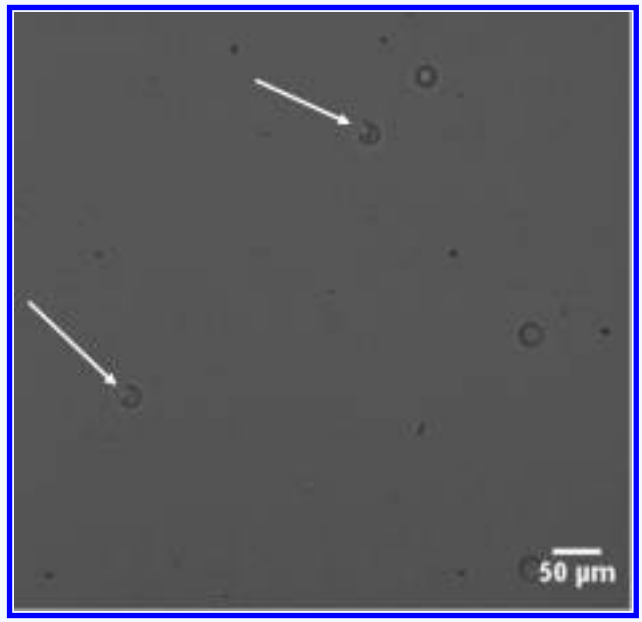
Supplementary Figure 4 Osmotic shrinkage to form GUVs. Formation of GUVs in concentrated solution of KCl. Arrows point to vesicles separating from the oil caps. Composition of internal and middle phase given in **Table 4**.

Supplementary Table 1 Spreading coefficient values promoting GUV formation. Spreading coefficients for double emulsions in external solution where GUVs were formed. Spreading coefficients are presented for both the 2× PBS and 0.6 Osm KCl conditions before (initial) and after (equilibrium) double emulsion/SCM shrinkage.

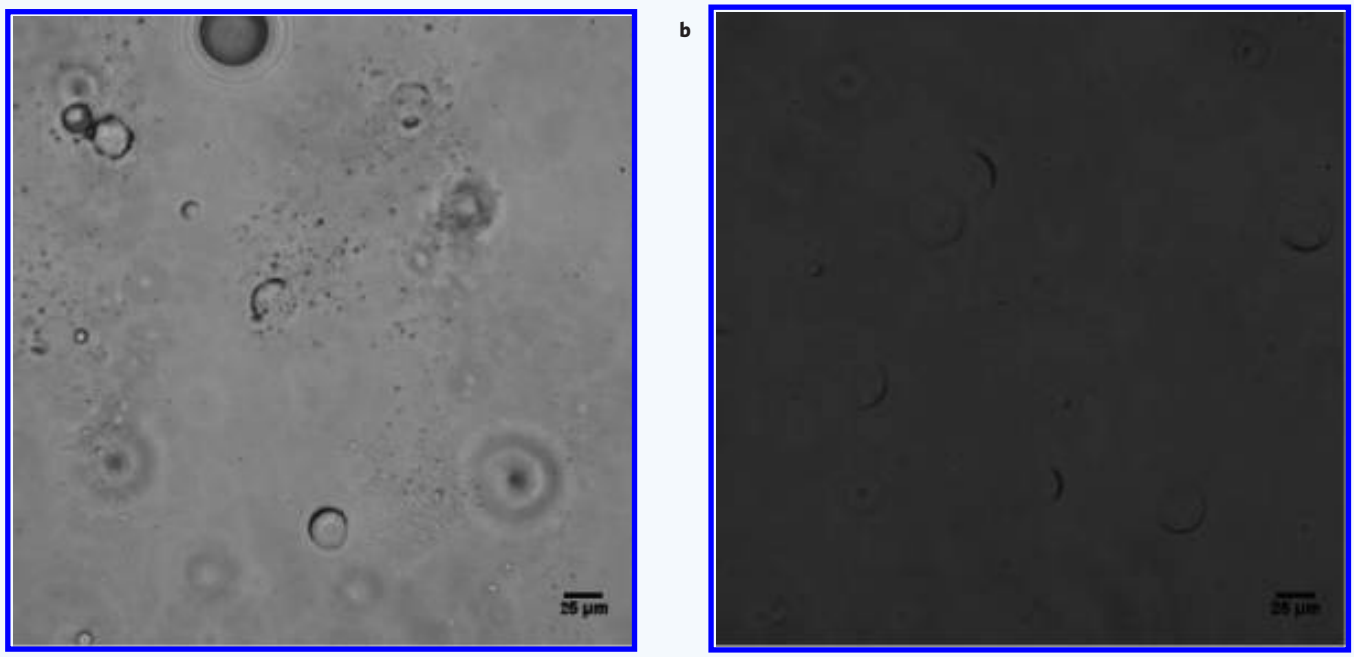
Internal Phase: 1× PBS + 1% F68 Middle Phase: 7.5 mg mL^{-1} DOPC + 2.5 mg mL^{-1} DPPC + 5 mg mL^{-1} Cholesterol in Oleic Acid	S_O	S_M	S_I
2× PBS - Initial	−7.21	−5.50	0.21
2× PBS - Equilibrium	−3.50	−9.21	−3.50
0.6 Osm KCl - Initial	−6.41	−4.70	−0.59
0.6 Osm KCl - Equilibrium	−2.70	−8.41	−4.30
0.25 Osm Sucrose + 5% F68	−0.89	0.82	−6.11

slide, it is likely that evaporation of the bulk solution lead to increased electrolyte concentration that promoted GUV formation.

The calculated spreading coefficients for this scenario are listed in **Supplementary Table 1**. As the double emulsions shrink, S_O becomes more positive, while both S_M and S_I become more negative. Assuming the trend would continue, the conditions for the non-engulfing morphology in Equation (2) would eventually be met. This is a probable scenario, as the external solution was likely evaporating and cap removal occurred towards the edge of the solution where solute concentration should be the highest. It is also likely the enhancement to the depletion effect could play a role as repeating the experiment without DPPC showed no cap removal in 0.6 Osm KCl. Even after 15 minutes, SCMs were only initially starting to form (**Supplementary Fig. 5**).



Supplementary Figure 5 Failed GUV formation without DPPC. Beginning of SCM formation for double emulsions lacking DPPC placed in 0.6 Osm KCl solution. Composition of internal and middle phase is given in **Table 3**.



Supplementary Figure 6 SCMs submerged in solution with excess surfactant. (**a**) Breakup of oil phase from double emulsions introduced into a solution of 0.5 Osm sucrose + 5% F68. Oil phase contained 5 mg mL^{-1} DOPC + 5 mg mL^{-1} DPPC + 5 mg mL^{-1} cholesterol in oleic acid. (**b**) Washing with excess solution to introduce shear removes excess oil to create structures that resemble GUVs.