Electronic Supplementary Information

Ultrahigh yields of giant vesicles obtained through mesophase evolution and breakup

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Calculation of Debye screening length

We follow reference[1] to calculate the Debye screening length. The range of the electrostatic repulsion depends on the Debye length, $\frac{1}{\kappa_D}$, where κ_D is:

$$\kappa_D = \sqrt{\frac{N_a e^2}{\varepsilon_0 \varepsilon k_B T} \sum_i [C]_i z_i^2} \quad (1)$$

In this equation, N_A is Avogadro's number, e is the elementary charge, ε_0 is the permittivity of free space, ε is the dielectric constant, k_B is the Boltzmann constant, T is the absolute temperature, $[C]$ is the concentration of ionic species i, and z is the charge of ionic species i. In unbuffered ultrapure water with dissolved nonionic solutes in equilibrium with atmosphere, the Debye length is ~ 170 nm while in 1 mM NaCl it is 9.6 nm. In 1× PBS, the Debye length is 0.75 nm.

Supplementary reference

1. Cooper, A., Girish, V. & Subramaniam, A.B. Osmotic Pressure Enables High-Yield Assembly of Giant Vesicles in Solutions of Physiological Ionic Strengths. *Langmuir* **39**, 5579–5590 (2023).

Supplementary Figures

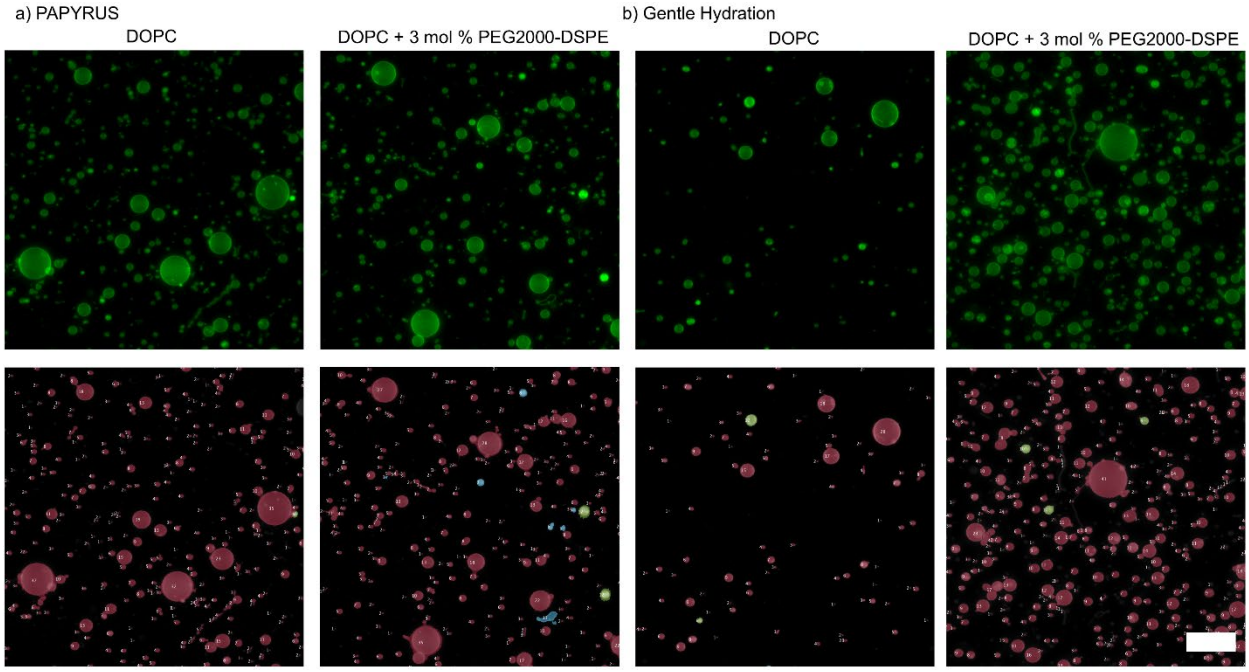

Fig. S1 Representative images of harvested objects from samples shown in Fig. 1 in the main text. The bottom row show montages of harvested objected that are selected as GUVs (red) and rejected as non-GUV structures (green and blue). The scale bar is 50 µm.

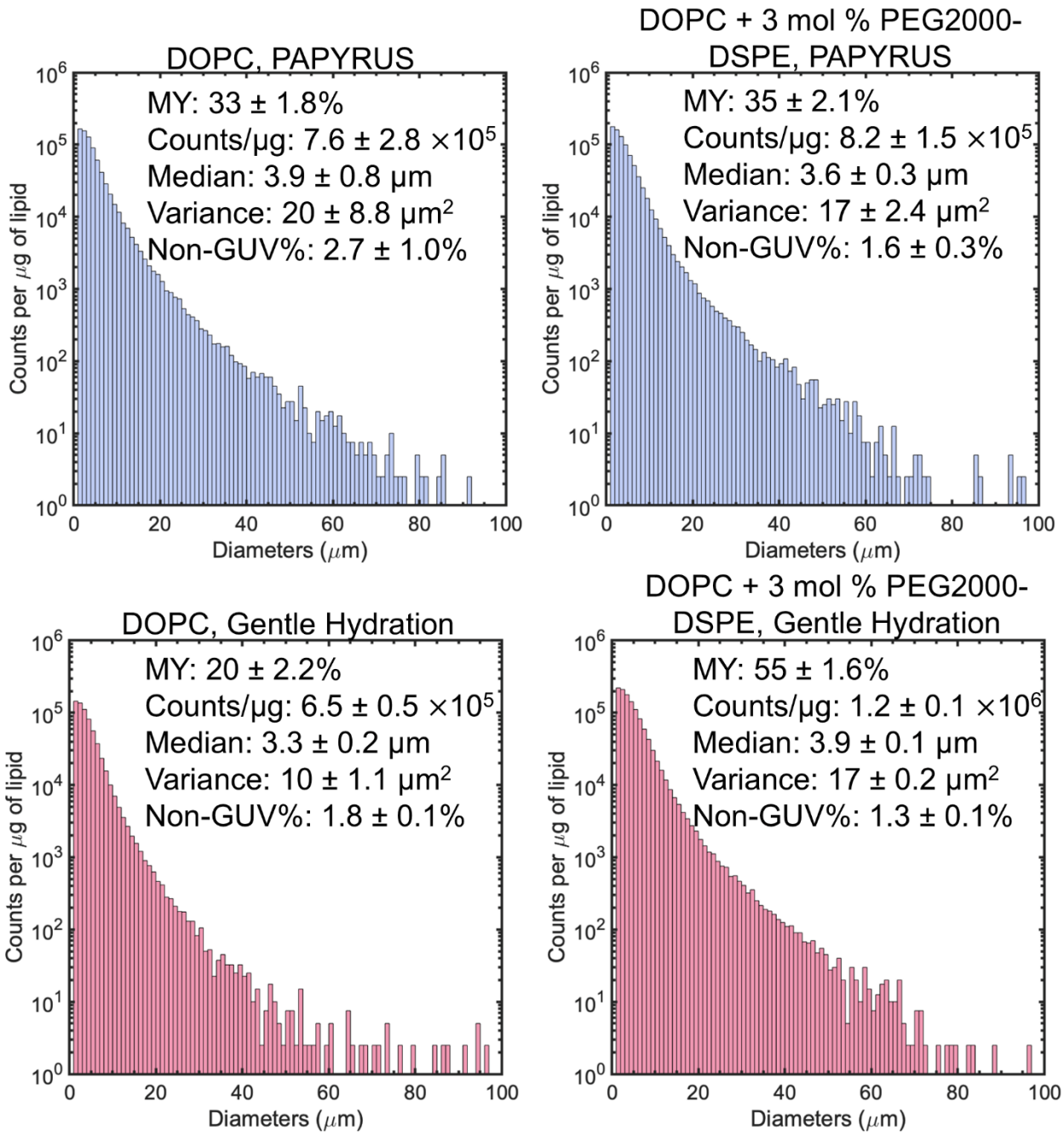

Fig. S2 Histograms of GUV diameters of the samples shown in Fig. 1 in the main text. Each histogram is the average of N=3 independent repeats. Note the logarithmic scale on the *y*-axis. Bin widths are 1 μm. The molar yield (MY), total counts per μg of lipid (counts/ μg), median diameter, non-GUV % and variance are reported in the text inserts.

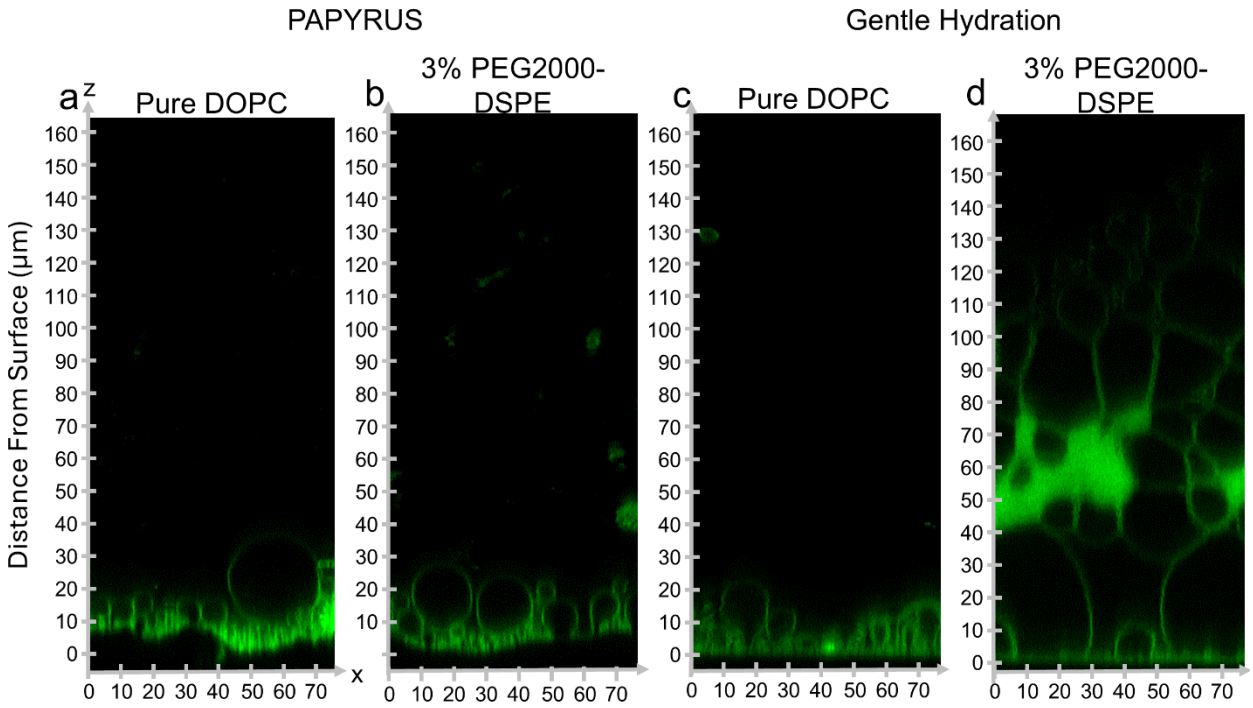

Fig. S3 Orthogonal *x*-*z* images shown in Fig. 2 in the main text without histogram equalization.

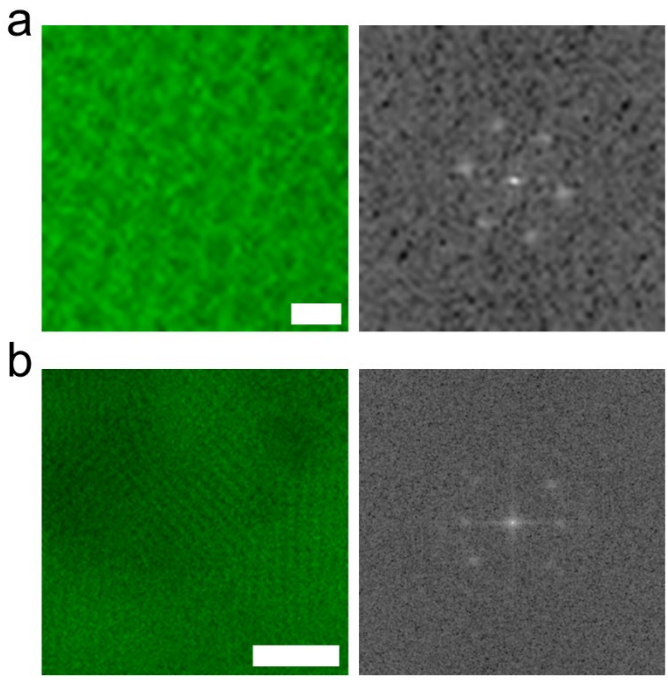

Fig. S4 Zoomed images of the lipid dense region (left) and its corresponding fast Fourier transform, FFT (right). Scale bars are a) 1 μm and b) 5 μm.

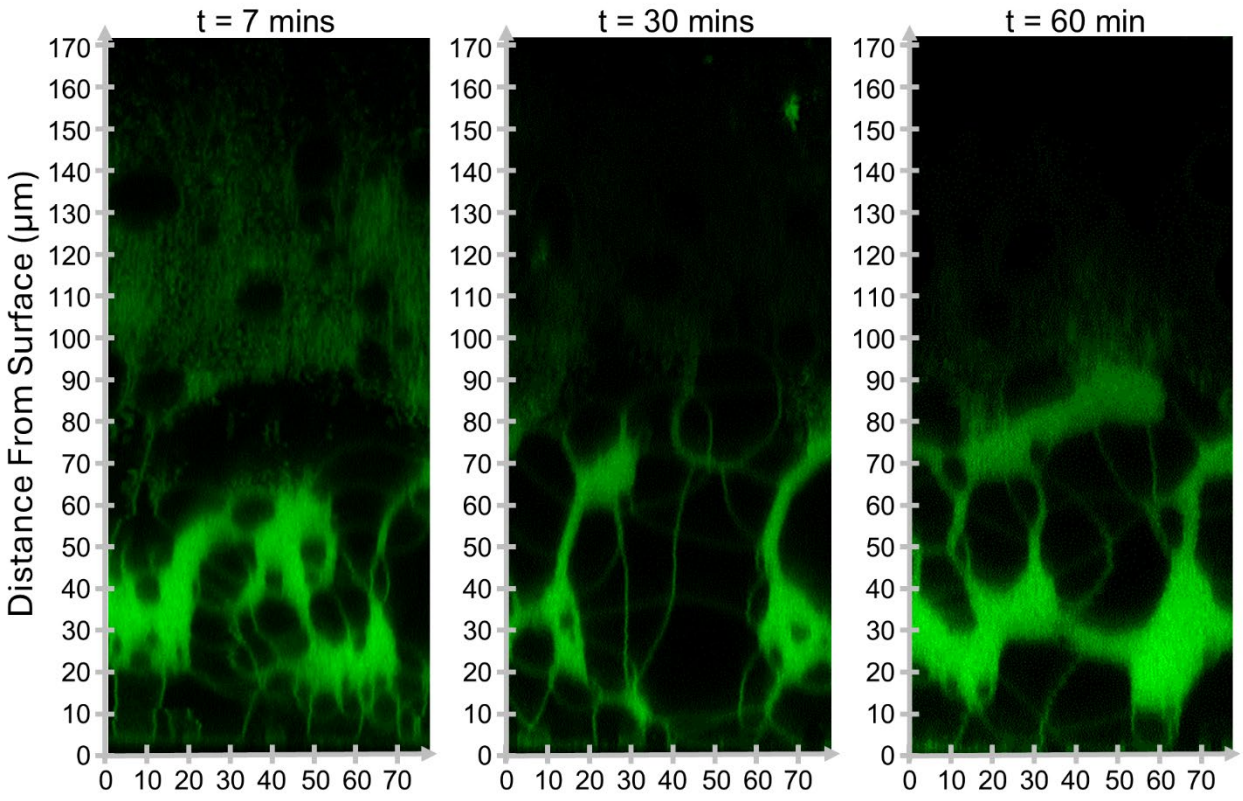

Fig. S5 Orthogonal *x*-*z* images without histogram equalization shown in Fig. 3 in the main text.

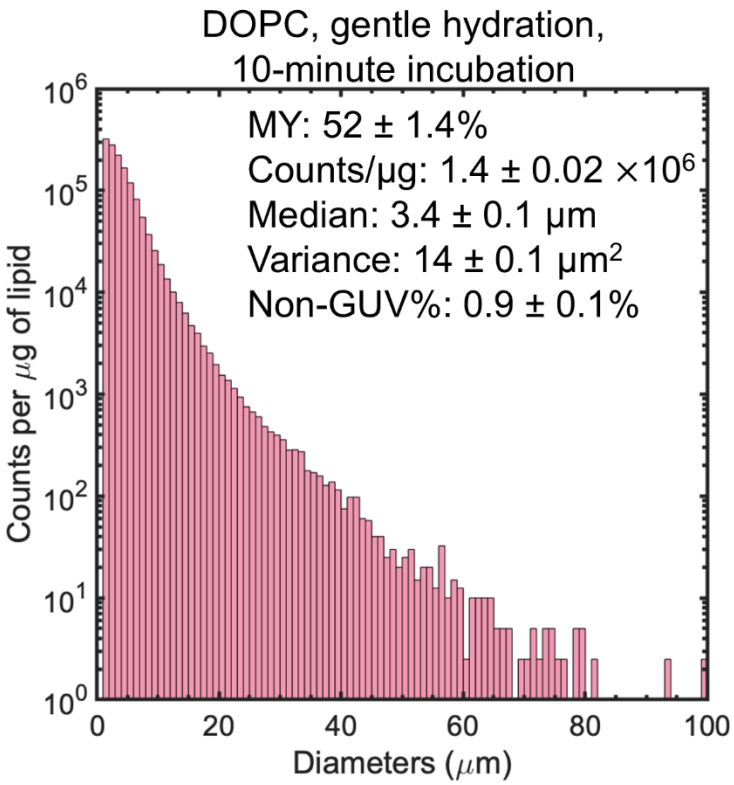

Fig. S6 Histogram of GUV diameters of the data shown in Figure 3. The histogram is the average of N=3 independent repeats. Note the logarithmic scale on the *y*-axis. Bin widths are 1 μm. The molar yield (MY), total counts per μg of lipid (counts/ μg), median diameter, non-GUV% and variance are shown in the text insert on the plots.

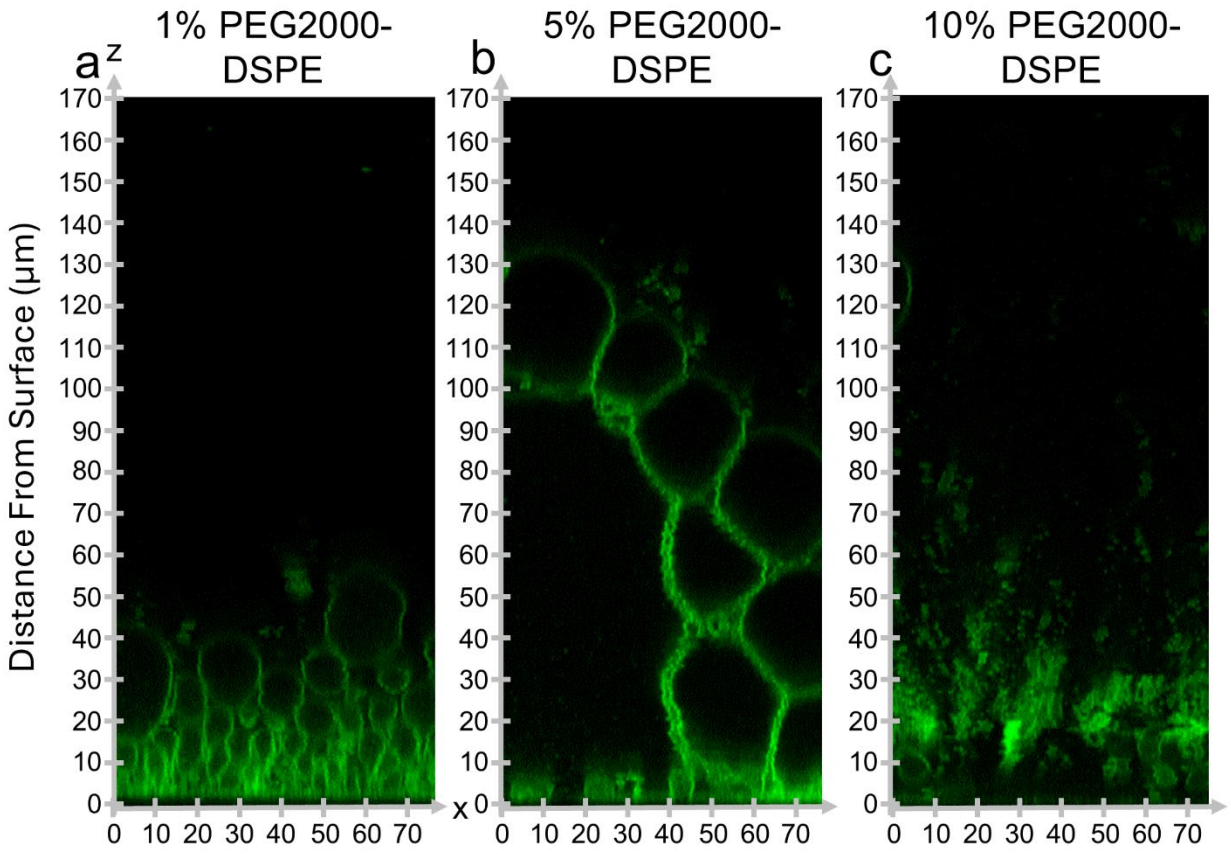

Fig. S7 Orthogonal *x*-*z* images without histogram equalization shown in Fig. 4 in the main text.

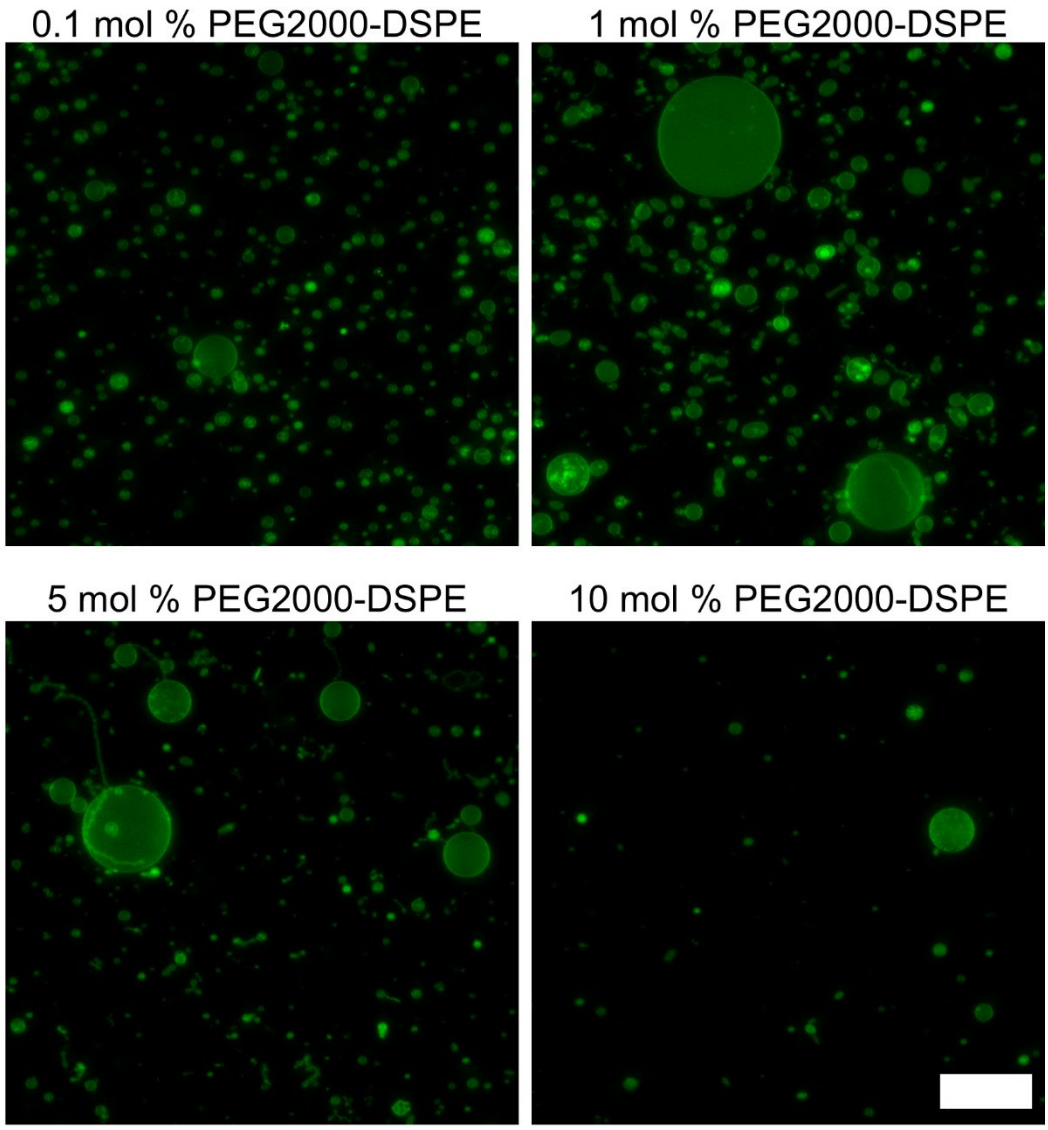

Fig. S8 Representative images of harvested GUVs from the samples shown in Fig. 4f in the main text. The scale bar is 50 μm.

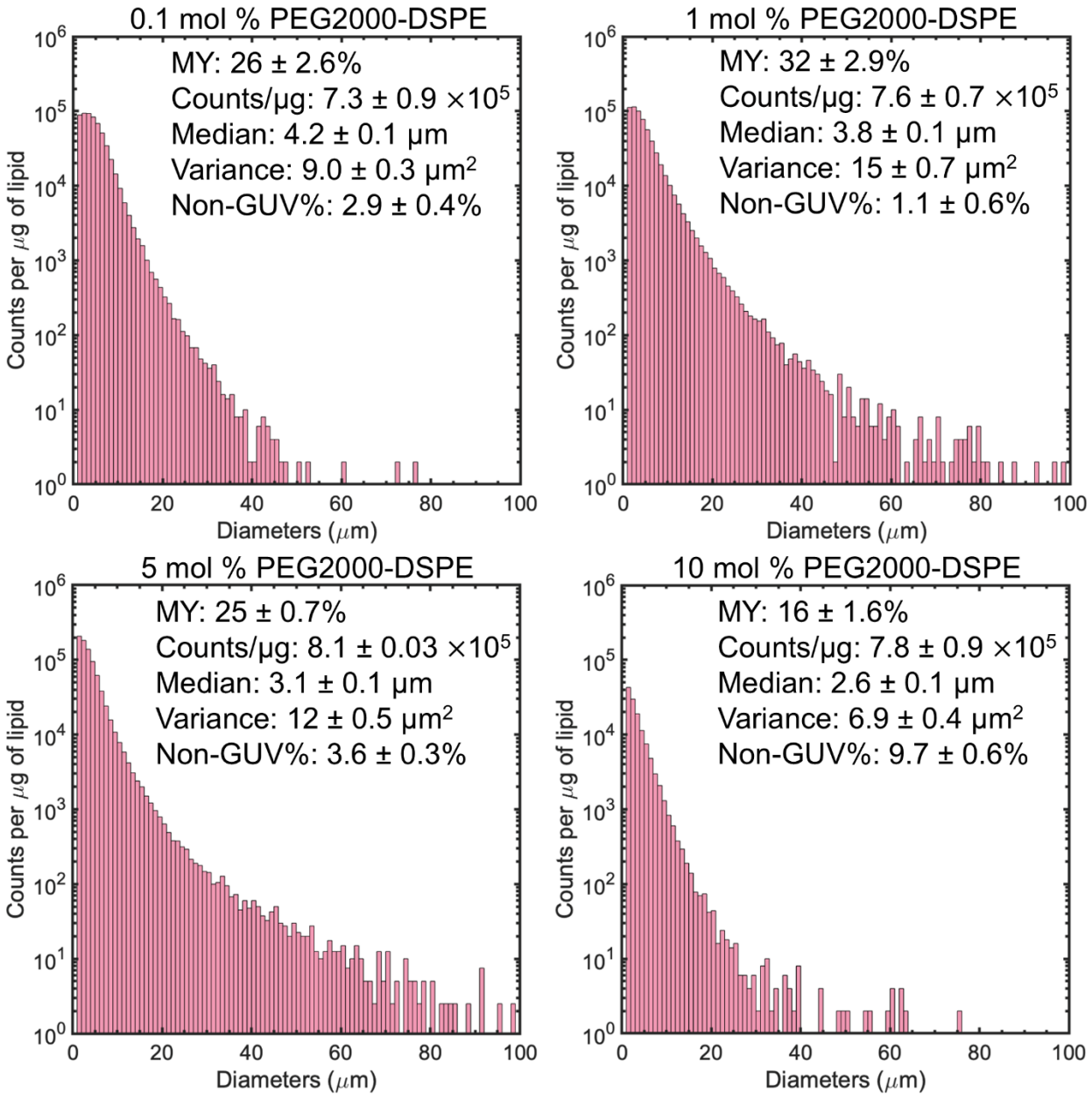

Fig. S9 Histograms of GUV diameters of samples shown in Fig. 4f in the main text. Each histogram is the average of N=3 independent repeats per sample. Note the logarithmic scale on the y-axis. Bin widths are 1 μm. The molar yield (MY), total counts per μg of lipid (counts/ μg), median diameter, non-GUV% and variance are shown in the text insert on the plots.

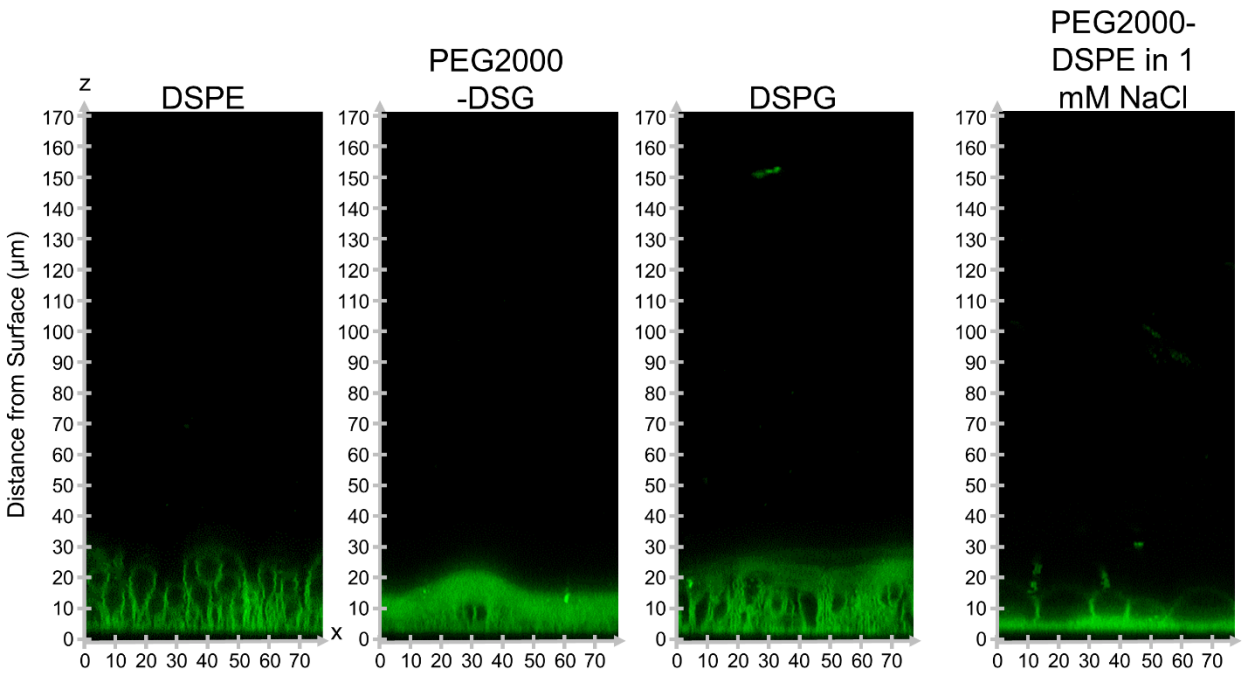

Fig. S10 Orthogonal *x*-*z* images without histogram equalization shown in Fig. 5 in the main text.

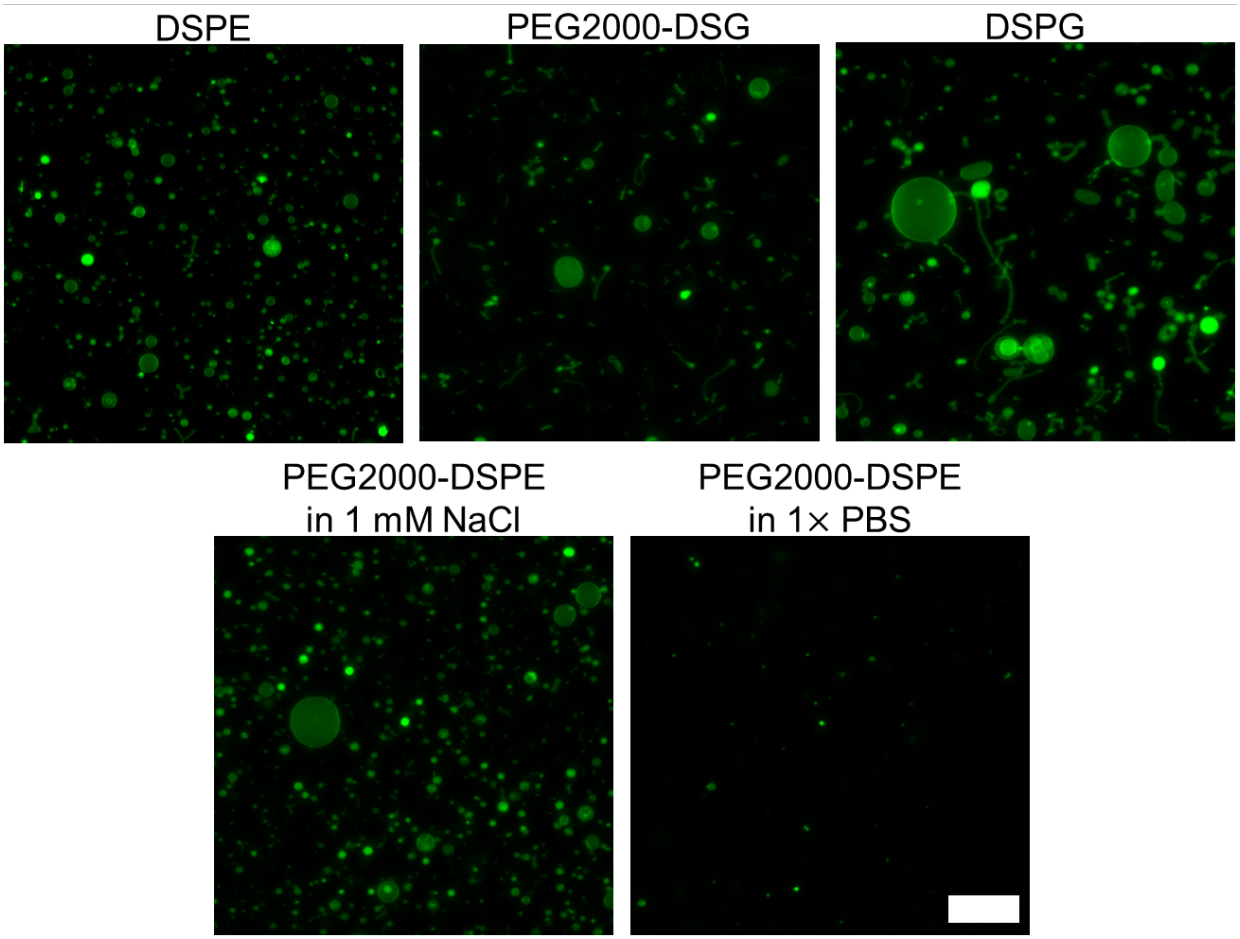

Fig. S11 Representative harvested images from the samples imaged for Fig. 6 in the main text. Scale bar is 50 μm.

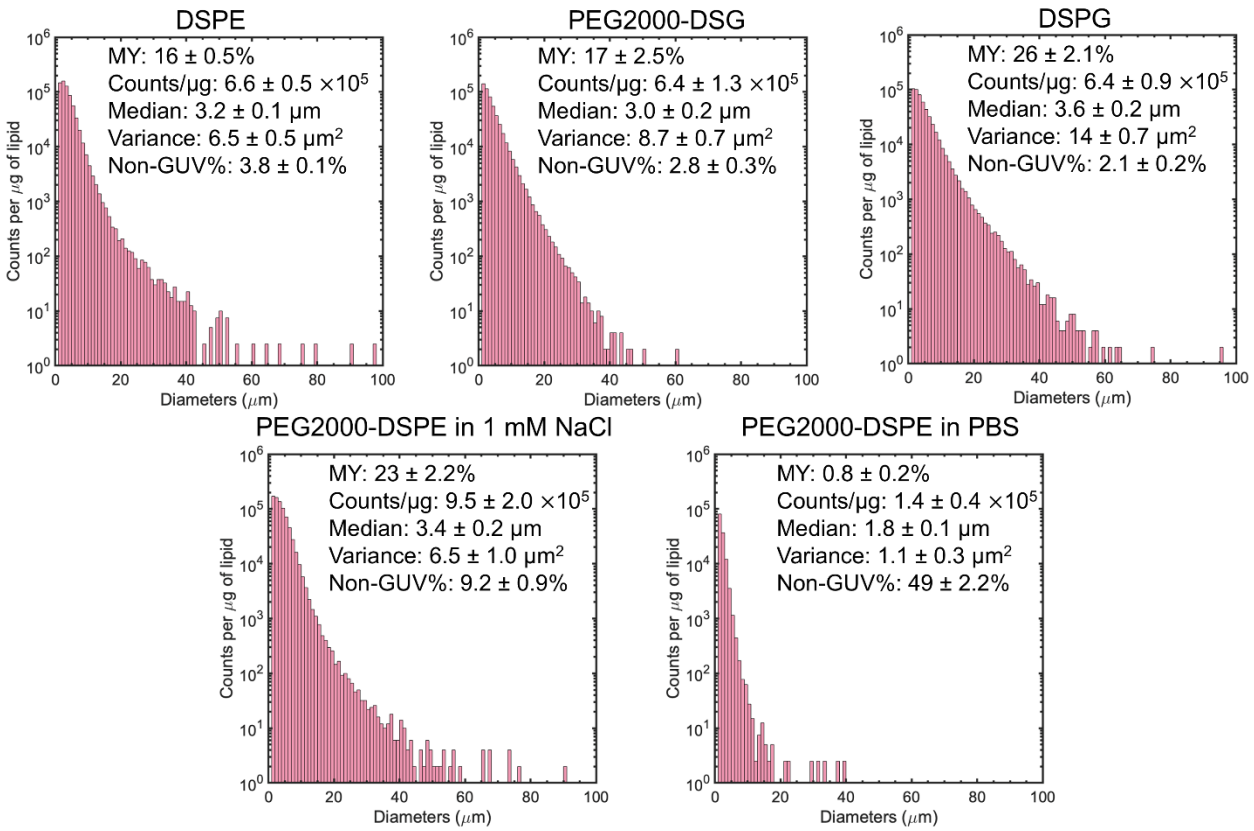

Fig. S12 Histograms of GUV diameters of samples shown in Fig. 6 in the main text. Each histogram is the average of N=3 independent repeats per sample. Note the logarithmic scale on the *y*-axis. Bin widths are 1 μm. The molar yield (MY), total counts per μg of lipid (counts/ μg), median diameter, non-GUV% and variance are shown in the text insert on the plots.

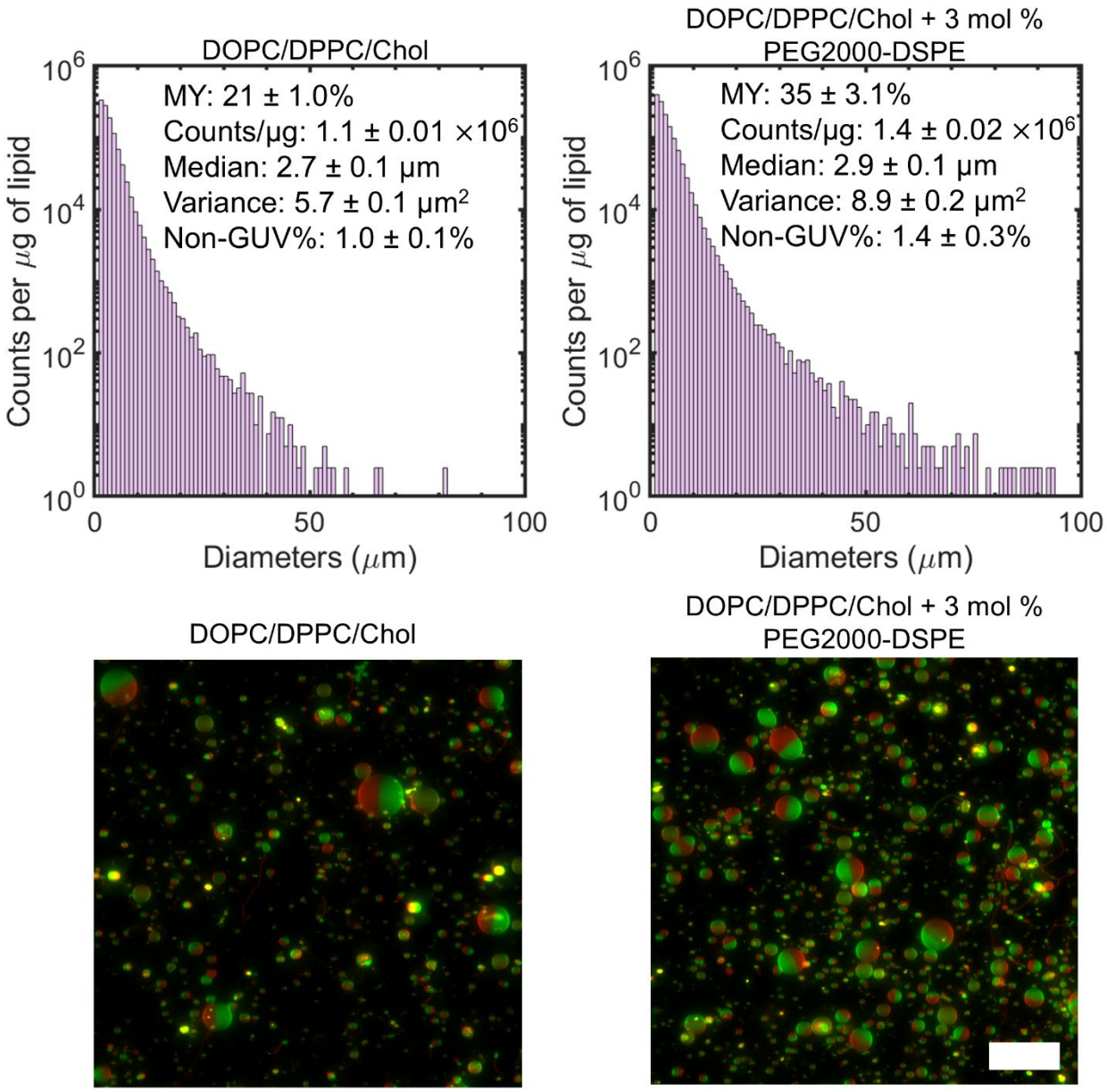

Fig. S13 Histograms of GUV diameters and representative images from DOPC/DPPC/Chol and DOPC/DPPC/Chol + 3 mol % PEG2000-DSPE samples in Figure 7. The histograms in the top row show the average of N=3 independent repeats per sample. Note the logarithmic scale on the y-axis. Bin widths are 1 μm. The molar yield (MY), total counts per μg of lipid (counts/μg), median diameter, non-GUV% and variance are shown in the text insert on the plots. Scale bar is 50 μm.

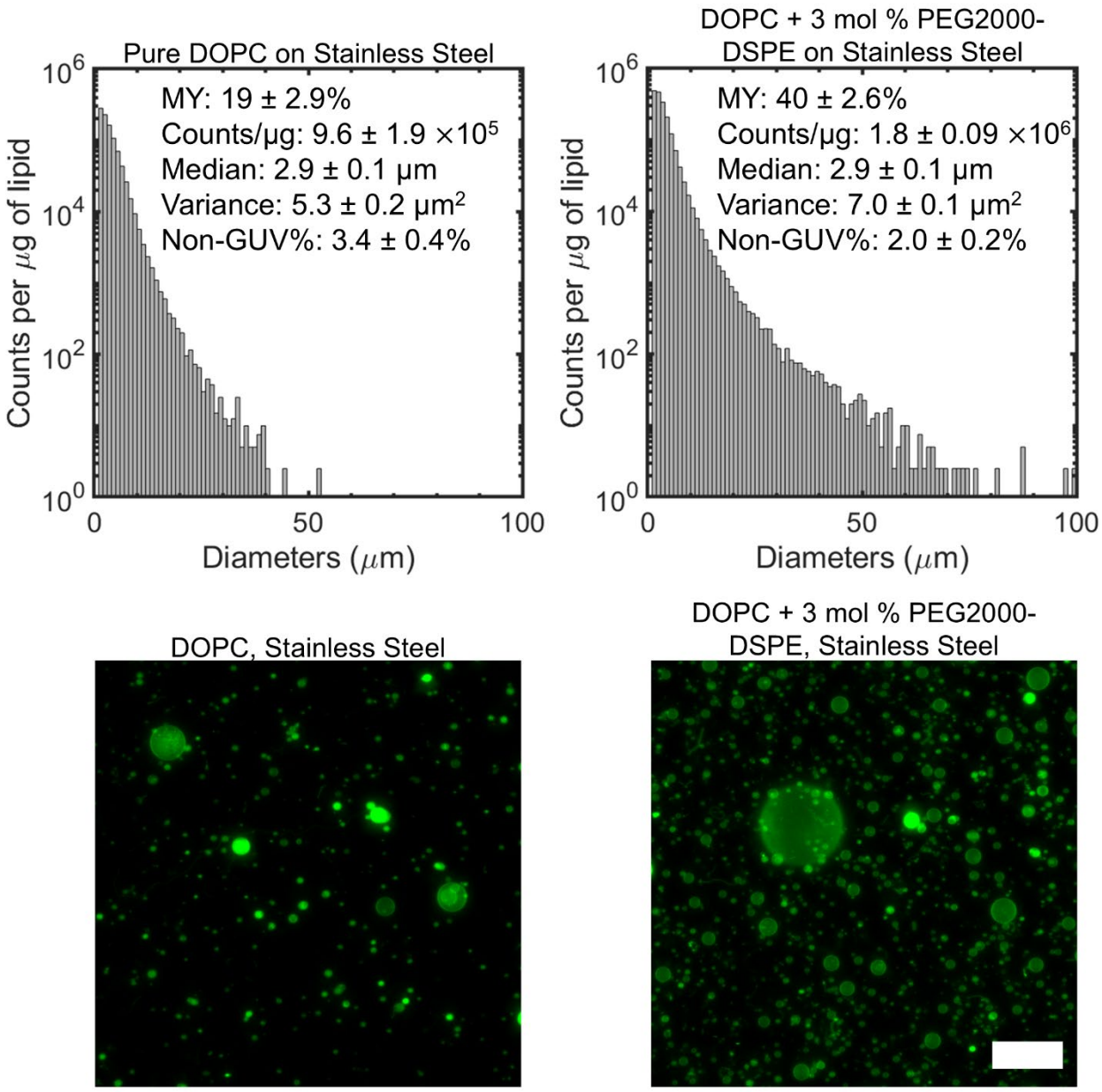

Fig. S14 Histograms of GUV diameters and representative images from DOPC/DPPC/Chol and DOPC/DPPC/Chol + 3 mol % PEG2000-DSPE samples in Fig. 7 in the main text. The histograms in the top row show the average of N=3 independent repeats per sample. Note the logarithmic scale on the y-axis. Bin widths are 1 µm. The molar yield (MY), total counts per µg of lipid (counts/µg), median diameter, non-GUV% and variance are shown in the text insert on the plots. Scale bar is 50 µm.

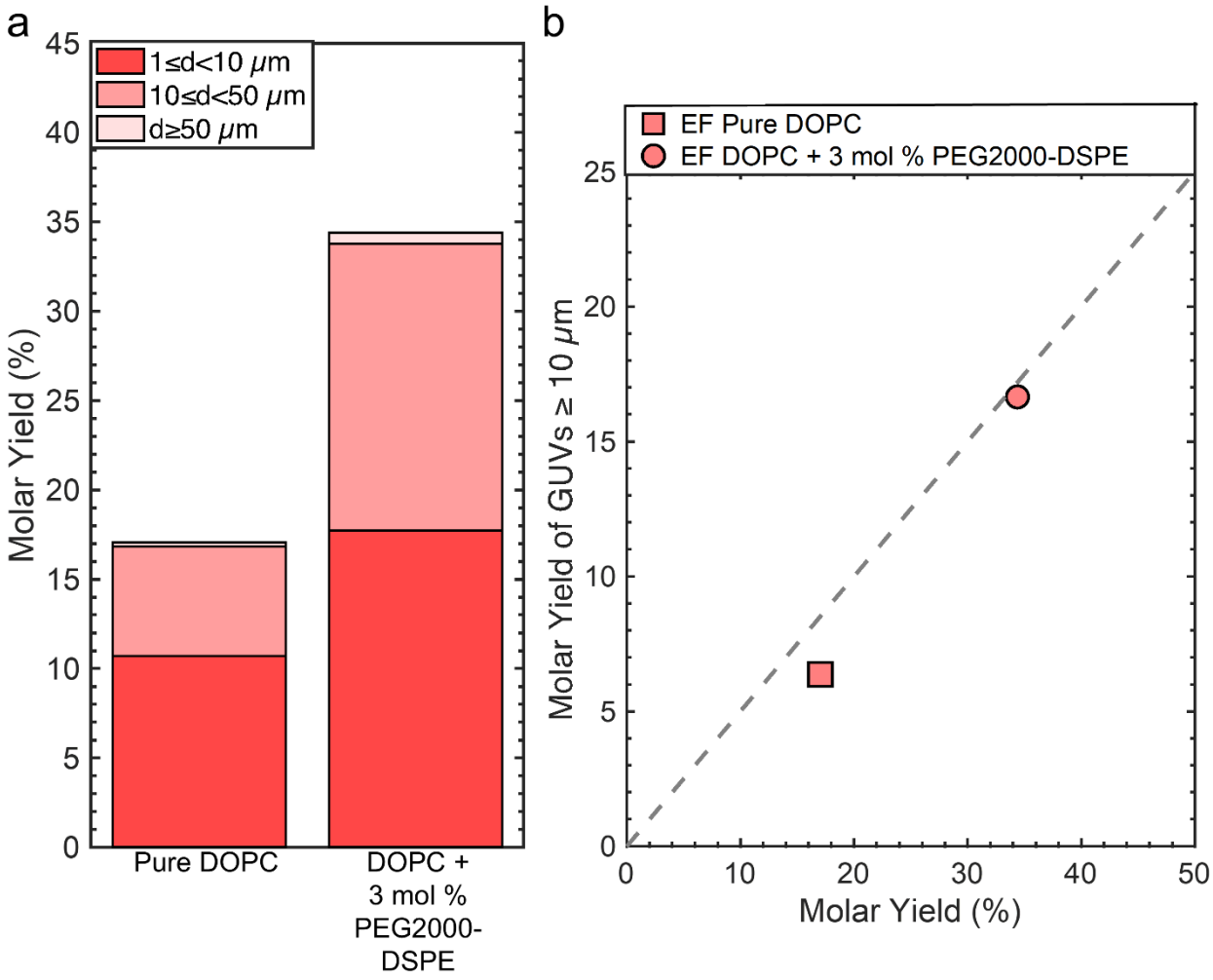

Fig. S15 Molar yields of PEGylated and PEG-free GUVs obtained from electroformation on ITO glass. a) Stacked bar plots showing molar yields of GUVs obtained from electroformation on ITO glass. The stacks show the percentage of the molar yield that is comprised of the size classifications as listed in the legend. b) Scatter plot showing the molar yield of GUVs with diameters ≥ 10 μm versus the total molar yield. The grey dashed line indicates where half the lipid molecules are in GUVs with diameters ≥ 10 μm.

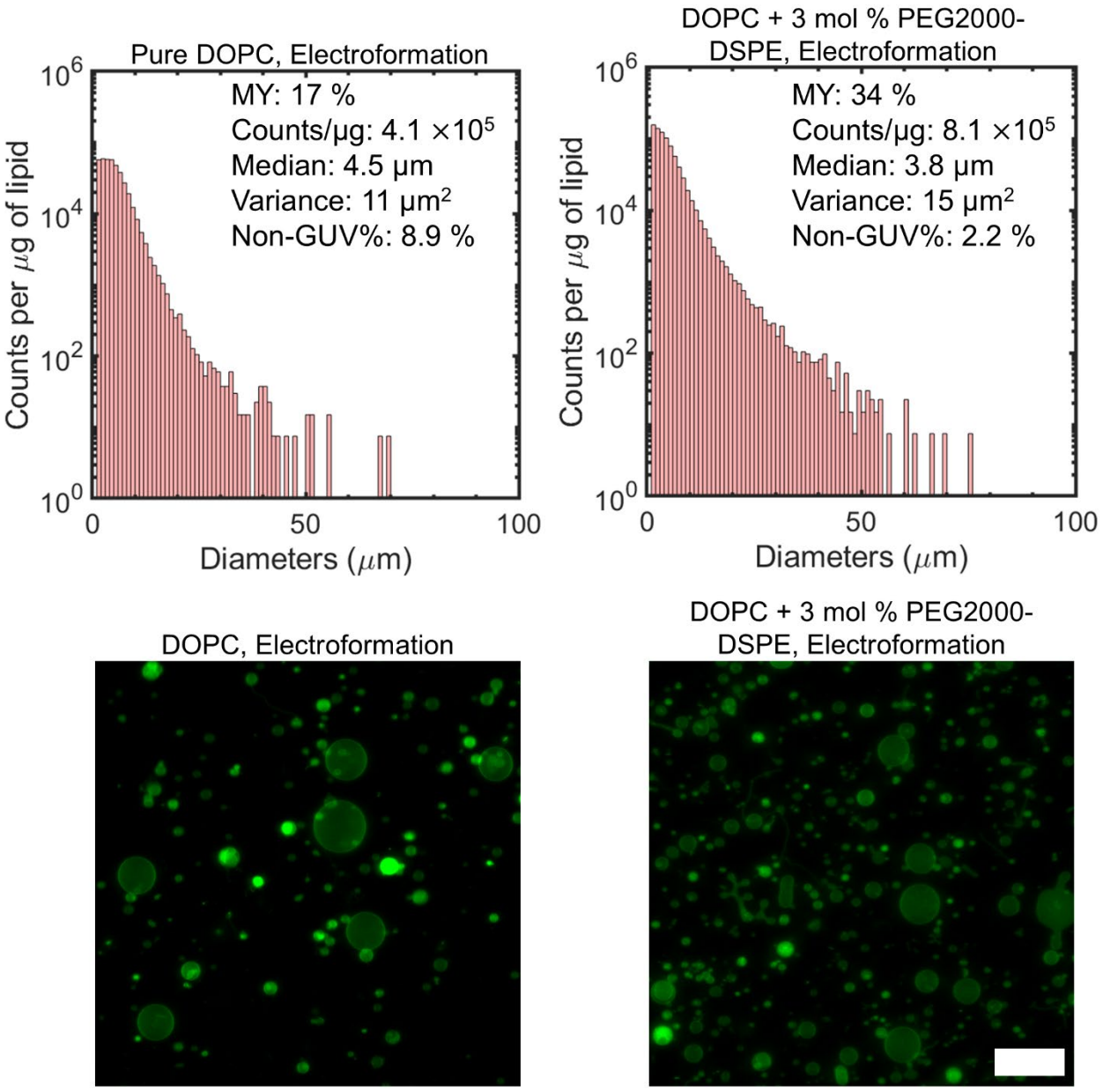

Fig. S16 Histograms of GUV diameters and representative images from PEGylated and PEG-free GUVs obtained from electroformation on ITO glass. The histograms are from N=1 independent repeats per sample. Note the logarithmic scale on the y-axis. Bin widths are 1 µm. The molar yield (MY), total counts per µg of lipid (counts/µg), median diameter, non-GUV% and variance are shown in the text insert on the plots. Scale bar is 50 µm.

Group 1	Group 2	*p*-value	Significance	Interpretation
PAPYRUS, Pure DOPC	PAPYRUS, DOPC + 3 mol % PEG2000-DSPE	0.451	NS	3 mol % PEG2000-DSPE did not result in a significant change in molar yield using PAPYRUS
Gentle Hydration on Glass, Pure DOPC	Gentle Hydration on Glass, DOPC + 3 mol % PEG2000-DSPE	5.22×10^{-5}	***	3 mol % PEG2000-DSPE results in a significant increase in molar yield using gentle hydration on glass

Table S1. Table of p-values from Student's t-tests of the molar yields of GUVs obtained from PAPRYUS and gentle hydration on glass using DOPC with and without 3 mol % PEG2000-DSPE. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, NS: not significant.

Group 1	Group 2	*p*-value	Significance	Interpretation
Gentle Hydration on Glass, 60 minute incubation	Gentle Hydration on Glass, 10 minute incubation	0.118	NS	Incubation for 10 minutes is not significantly different than incubation for 60 minutes

Table S2. The *p*-value from a Student's t-test of the molar yields of GUVs obtained from gentle hydration on glass using DOPC with 3 mol % PEG2000-DSPE harvested at 60 minutes and 10 minutes. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, NS: not significant.

Source	SS	'df'	'MS'	'F'	'Prob>F'
Columns	2860.373	5	572.074	89.746	4.43×10^{-9}
Error	76.492	12	6.3743		
Total	2936.866	17			

Group 1	Group 2	*p*-value	Significance	Interpretation
0 mol %	0.1 mol %	0.119	NS	The addition of 0.1 mol % of PEG2000-DSPE did not result in a significant change in molar yield.
0 mol %	1 mol %	7.76×10^{-4}	***	The addition of 1 mol % of PEG2000-DSPE resulted in a significant change in molar yield.
0 mol %	3 mol %	1.11×10^{-8}	***	The addition of 3 mol % of PEG2000-DSPE resulted in a significant change in molar yield.
0 mol %	5 mol %	0.170	NS	The addition of 5 mol % of PEG2000-DSPE did not result in a significant change in molar yield.
0 mol %	10 mol %	0.439	NS	The addition of 10 mol % of PEG2000-DSPE did not result in a significant change in molar yield.
0.1 mol %	1 mol %	0.079	NS	There is no significant difference between the addition of 0.1 and 1 mol % PEG2000-DSPE.
0.1 mol %	3 mol %	9.32×10^{-8}	***	There is a significant difference between 0.1 and 3 mol % PEG2000-DSPE.

0.1 mol %	5 mol %	0.999	NS	There is no significant difference between the addition of 0.1 and 5 mol % PEG2000-DSPE.
0.1 mol %	10 mol %	0.005	**	There is a significant difference between the addition of 0.1 and 10 mol % PEG2000-DSPE.
1 mol %	3 mol %	1.47×10^{-6}	***	There is a significant difference between the addition of 1 and 3 mol % PEG2000-DSPE.
1 mol %	5 mol %	0.054	NS	There is no significant difference between the addition of 3 and 5 mol % PEG2000-DSPE.
1 mol %	10 mol %	5.38×10^{-5}	***	There is a significant difference between the addition of 1 and 10 mol % PEG2000-DSPE.
3 mol %	5 mol %	7.75×10^{-8}	***	There is a significant difference between the addition of 1 and 10 mol % PEG2000-DSPE.
3 mol %	10 mol %	3.01×10^{-9}	***	There is a significant difference between the addition of 3 and 10 mol % PEG2000-DSPE.
5 mol %	10 mol %	0.007	**	There is a significant difference between the addition of 5 and 10 mol % PEG2000-DSPE.

Table S3. ANOVA table and table of p-values from post hoc Tukey's HSD tests of the molar yields of GUVs obtained from gentle hydration on glass with DOPC with 0-10 mol % PEG2000-DSPE. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, NS: not significant.

Source	SS	'df'	'MS'	'F'	'Prob>F'
Columns	172.811	3	57.604	9.741	0.00478
Error	47.307	8	5.913		
Total	220.118	11			

Group 1	Group 2	*p*-value	Significance	Interpretation
DOPC	DSPE	0.184	NS	Addition of 3 mol % DSPE does not result in a significant change in yield from pure DOPC
DOPC	PEG2000-DSG	0.541	NS	Addition of 3 mol % PEG2000-DSG (steric repulsion) does not result in a significant change in yield from pure DOPC
DOPC	DSPG	0.091	NS	Addition of 3 mol % DSPG (electrostatic repulsion) does not result in a significant change in yield from pure DOPC
DSPE	PEG2000-DSG	0.811	NS	The yields of mixtures containing 3 mol % DSPE and 3 mol % PEG2000-DSG are not significantly different from each other
DSPE	DSPG	4.34×10^{-3}	**	The yields of mixtures containing 3 mol % DSPE and 3 mol % DSPG are significantly different from each other
PEG2000-DSG	DSPG	1.34×10^{-2}	*	The yields of mixtures containing 3 mol % PEG2000-DSG and 3 mol % DSPG are significantly different from each other

Table S4. ANOVA table and table of p-values from post hoc Tukey's HSD tests of the molar yields of GUVs obtained from gentle hydration on glass with pure DOPC and DOPC + 3 mol % PEG2000-DSG, DSPG, or DSPE. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, NS: not significant.

Group	*Group 2*	*p*-value	Significance	Interpretation
DOPC/DPPC/Chol	DOPC/DPPC/Chol + 3 mol % PEG2000-DSPE	4.06×10^{-3}	**	Addition of 3 mol % PEG2000-DSPE results in a significant increase in yield

Table S5. The *p*-value from a Student's t-test of the molar yields of GUVs obtained from gentle hydration on glass using DOPC/DPPC/Cholesterol with and without 3 mol % PEG2000-DSPE. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, NS: not significant.

Group 1	Group 2	*p*-value	Significance	Interpretation
Pure DOPC on stainless steel	Pure DOPC + 3 mol % PEG2000-DSPE on stainless steel	1.40×10^{-3}	**	Addition of 3 mol % PEG2000-DSPE results in a significant increase in yield

Table S6. The *p*-value from a Student's t-test of the molar yields of GUVs obtained from gentle hydration on glass using DOPC with and without 3 mol % PEG2000-DSPE on stainless steel. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, NS: not significant.