Supplementary Information for

Carotenoids Improve Bacterial Tolerance Towards Biobutanol Through Membrane Stabilization

Geraldine W.N. Chia[a,b], Thomas Seviour[b,c], Staffan Kjelleberg[b,d], Jamie Hinks[b, §]

[a] Interdisciplinary Graduate School (IGS), Nanyang Technological University, Singapore
[b] Singapore Centre for Environmental Sciences Engineering (SCELSE), Nanyang Technological University, Singapore
[c] Centre for Water Technology (WATEC), Department of Engineering, Aarhus University, Nørrebrogade 44, DK-8000, Aarhus C, Denmark
[d] School of Biological, Earth and Environmental Sciences, University of New South Wales, Australia
[§] Corresponding authors: jhinks@ntu.edu.sg

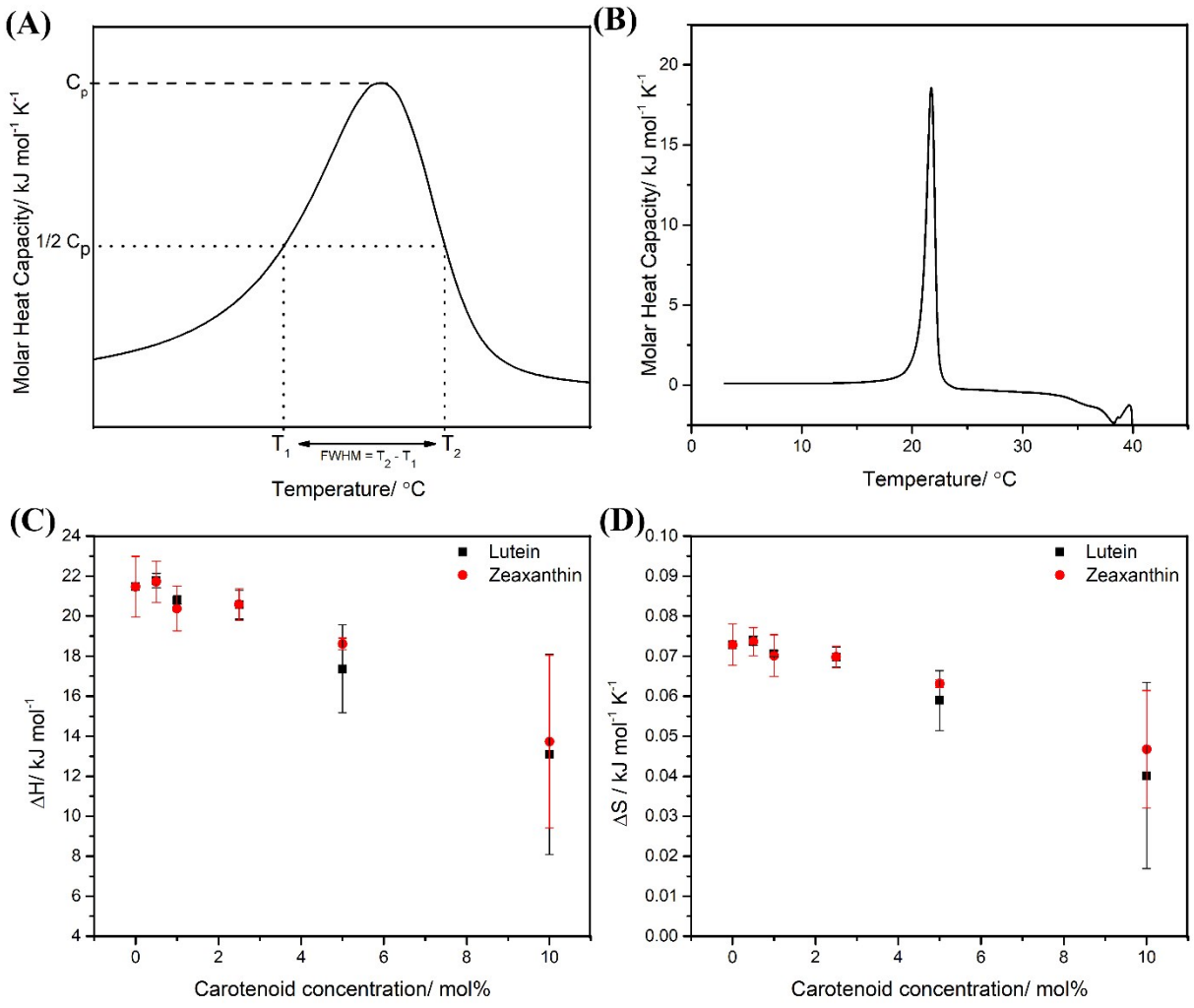

Figure S1. (A) Molar heat capacity (C_p, kJ mol^{-1} K^{-1}) and full width at half maximum (FWHM, °C) determination. (B) DSC cooling thermogram of control PEPG MLV (C) Change in enthalpy, ΔH (kJ mol^{-1}) and (D) change in entropy, ΔS (kJ mol^{-1} K^{-1}) as a function of carotenoid concentration.

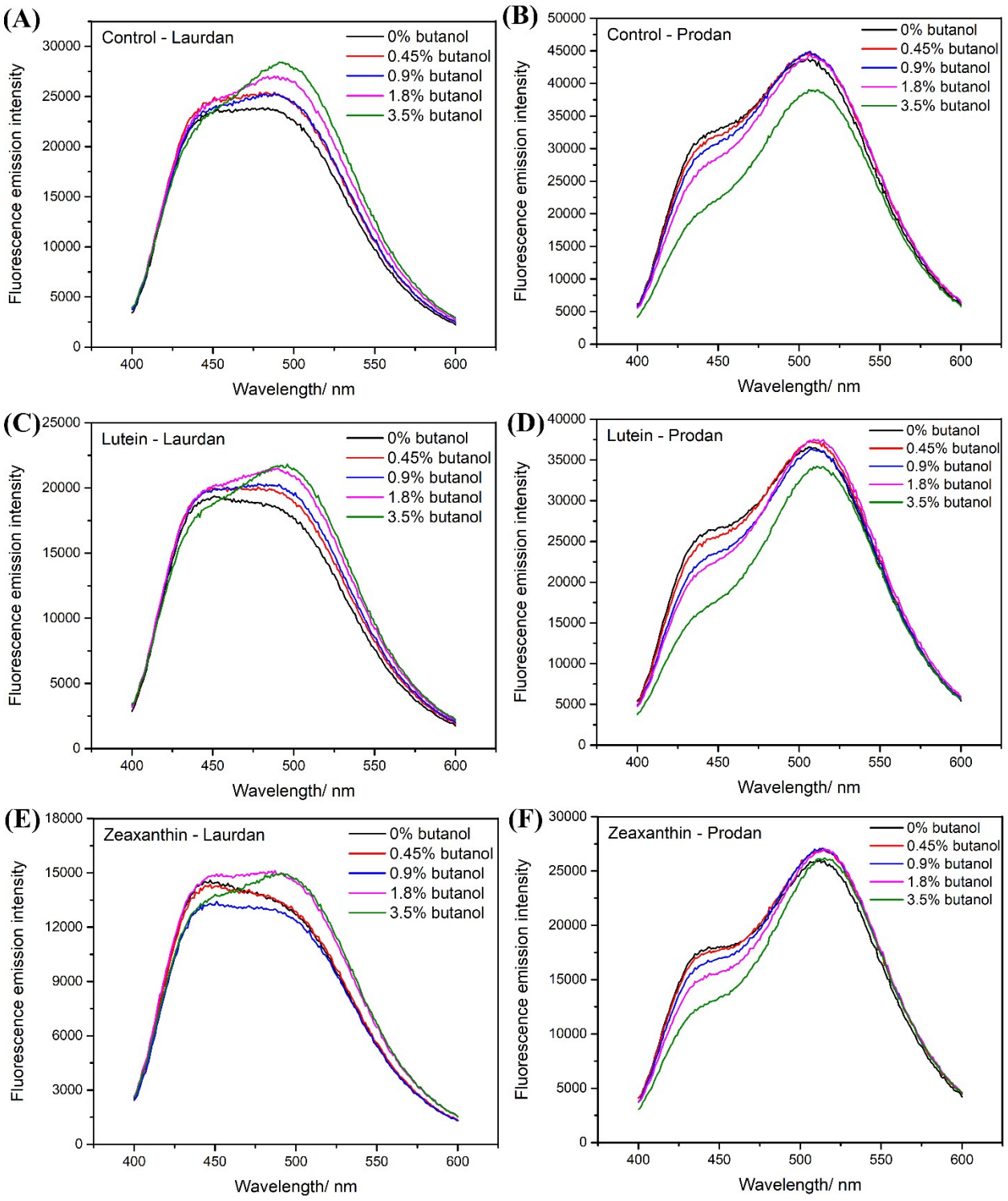

Figure S2. Representative fluorescence emission spectrum from Laurdan (A, C, E) and Prodan (B, D, F) in control MLVs (A, B), 0.5 mol% LUT-MLVs (C, D) and 0.5 mol% ZEA-MLVs (E, F).

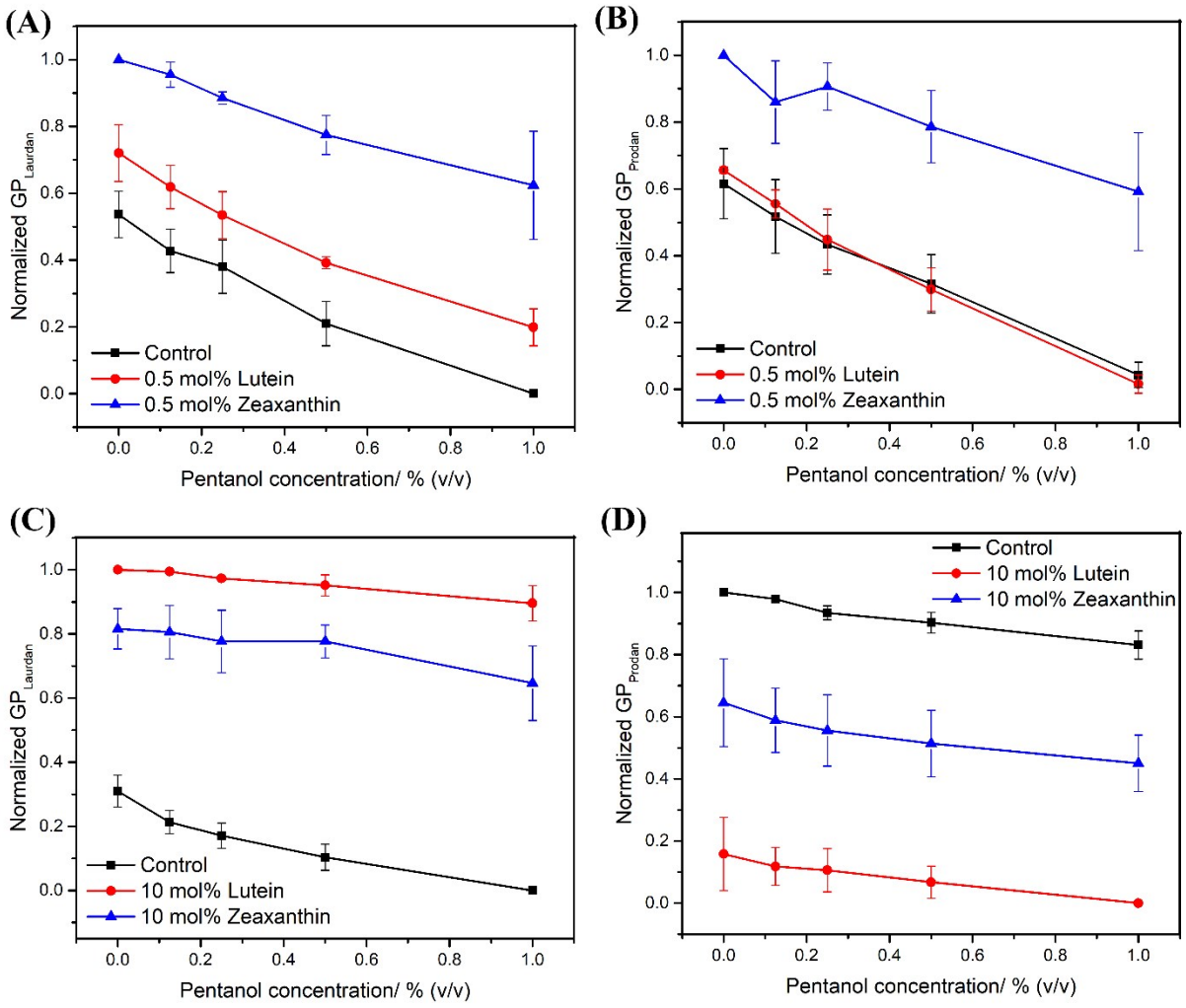

Figure S3. Generalized polarization (GP) measured using Laurdan (A, C) and Prodan (B, D) in control MLVs and MLVs with 0.5 mol% (A, B) or 10 mol% (C, D) carotenoids that were challenged with pentanol up to 1% (v/v).

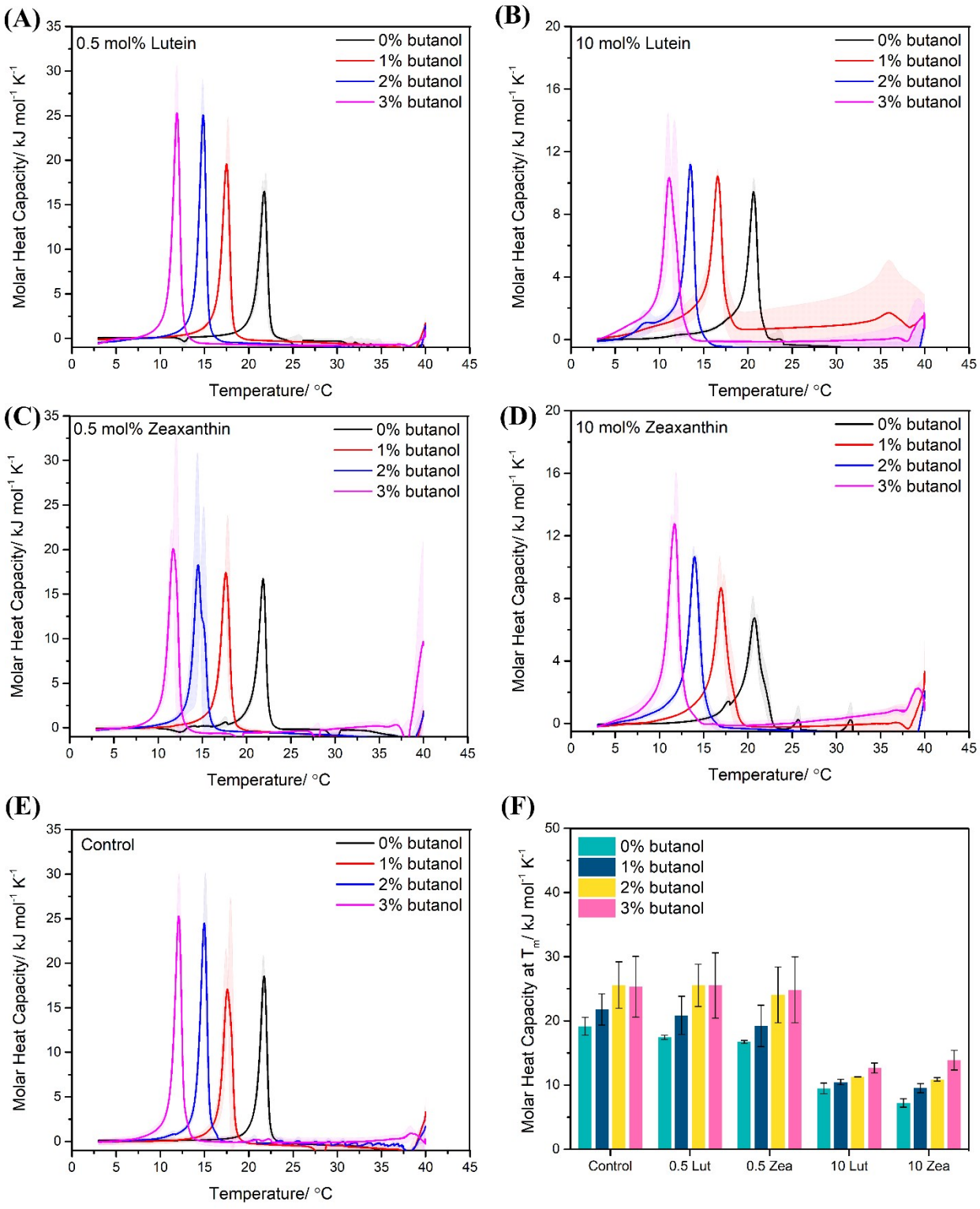

Figure S4. Butanol disorders acyl tails in MLVs and high carotenoid concentrations mitigate the disordering effects. (A) DSC cooling thermograms of 0.5 mol% LUT-MLVs, (B) 10 mol% LUT-MLVs (C), 0.5 mol% ZEA-MLVs, (D) 10 mol% ZEA-MLVs and (E) control MLVs that were challenged with butanol from 0-3% (v/v).

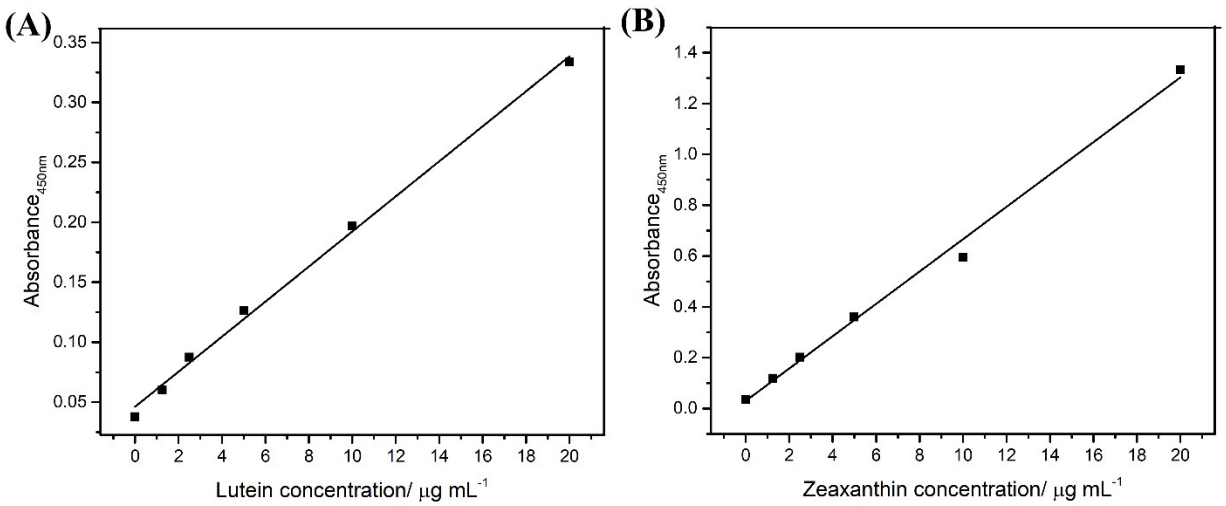

Figure S5. Calibration curves of lutein and zeaxanthin in methanol.

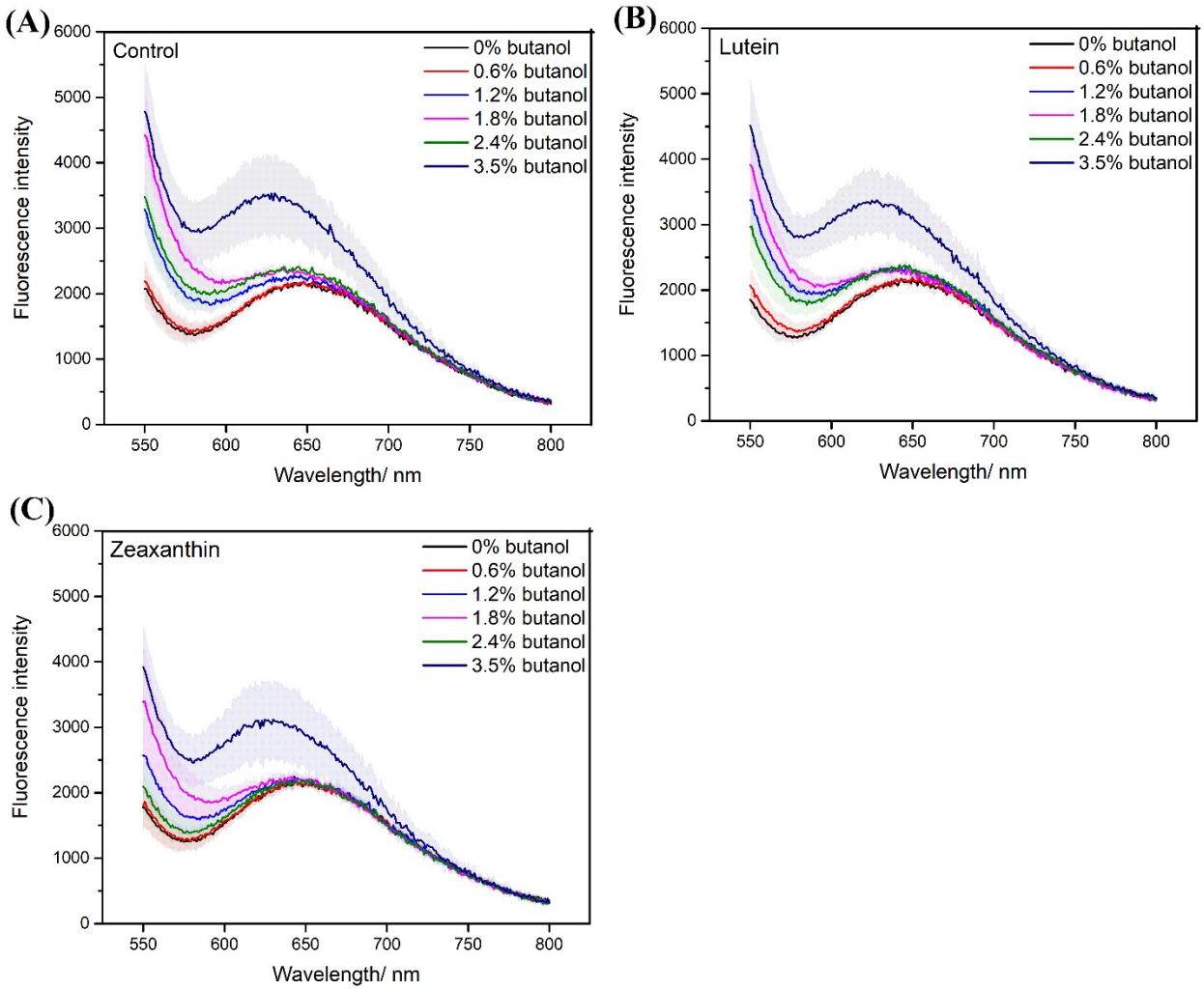

Figure S6. Fluorescence spectrum of propidium iodide (PI) signal at 630 nm (λ_{ex} = 465 nm) as a function of increasing butanol concentrations used to treat control cells (A), cells treated with 10 μg/mL lutein (B) and zeaxanthin (C).

Table S1. Average molecular weight of MLVs used in this study. [a]Full width at half maximum (FWHM) values of the main phase transition peaks in the cooling DSC thermograms in Figure 2A and 2B. [b]Lutein and zeaxanthin are positional isomers and have the same molecular weight (569), thus the average molecular weights of the MLVs are the same.

Carotenoid concentration/ mol%	Lutein[a]/ °C	Zeaxanthin[a]/°C	Molecular weight[b]
0	0.89 ±0.05	0.89 ±0.05	726
0.5	0.99 ±0.08	0.98 ±0.09	725
1	1.01 ±0.01	1.07 ±0.09	724
2.5	1.05 ±0.12	1.10 ±0.10	722
5	2.22 ±1.25	1.30 ±0.38	718
10	1.2 ±0.06	1.67 ±0.46	707

Table S2. ΔT_m calculated from cooling DSC thermograms of control MLVs and MLVS with carotenoids.

Butanol/ % (v/v)	ΔT_m/°C				
	Control	**0.5 mol% Lutein**	**0.5 mol% Zeaxanthin**	**10 mol% Lutein**	**10 mol% Zeaxanthin**
1	4.30 (± 0.13)	4.25 (± 0.35)	4.21 (± 0.20)	4.02 (± 0.05)	3.75 (± 0.0)
2	7.22 (± 0.35)	6.89 (± 0.09)	7.09 (± 0.52)	7.11 (± 0.04)	6.80 (± 0.13)
3	9.48 (± 0.40)	9.85 (± 0.11)	10.07 (± 0.28)	9.31 (± 0.56)	9.14 (± 0.57)

Table S3. Parameters to derive K_p from the gradient (m) of the linear fit, $\Delta H_{m,0}$, $T_{m,0}$ for control MLVs and MLVs with carotenoids.

MLV	[Carotenoid]/% mol	Gradient, m	$\Delta H_{m,0}$/ kJ mol^{-1}	$T_{m,0}$/°C
Control	**-**	30.8 (± 0.4)	21.5 (± 1.5)	21.7 (± 0.1)
Lutein	**0.5**	31.1 (± 0.6)	21.8 (± 0.35)	21.8 (± 0.2)
Lutein	**10**	30.2 (± 1.2)	13.1 (± 5.0)	20.6 (± 0.1)
Zeaxanthin	**0.5**	31.8 (± 0.01)	21.7 (± 1.0)	21.8 (± 0.1)
Zeaxanthin	**10**	29.3 (± 1.3)	13.7 (± 4.3)	20.8 (± 0.3)