Supplementary Information (SI) for Faraday Discussions.
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Melting Point Matters: Designing Lipid Nanocarriers for Improved T Cell Activation

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Supplementary

Table S1**:** DNA sequence of αCD3 scFv SNAP-tag protein.

αCD3 scFV SNAP-tag	TAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAATAATT TTGTTTAACTTTAAGAAGGAGATATACATATGGACTATAAAGACGATGACG ATAAACATATGGGCAGCATGGATAAAGATTGCGAAATGAAACGTACCACA CTGGATAGTCCGCTGGGTAAACTGGAACTGAGCGGTTGTGAACAGGGTCTG CATGAAATTAAACTGTTAGGTAAAGGCACCAGCGCAGCAGATGCAGTTGA AGTTCCGGCACCGGCAGCAGTTTTAGGTGGTCCGGAACCGCTGATGCAGGC AACCGCATGGCTGAATGCATATTTTCATCAGCCGGAAGCCATTGAAGAATT TCCGGTTCCTGCACTGCATCATCCGGTTTTTCAGCAAGAAAGCTTTACCCGT CAGGTTCTGTGGAAACTGCTGAAAGTTGTTAAATTTGGCGAGGTGATTAGC TATCAGCAGCTGGCAGCACTGGCAGGTAATCCGGCAGCAACCGCAGCAGT TAAAACCGCACTGAGCGGCAATCCGGTTCCGATTCTGATTCCGTGTCATCG TGTTGTTAGCAGCAGCGGTGCAGTTGGTGGTTATGAAGGTGGTCTGGCAGT TAAAGAATGGCTGCTGGCACATGAAGGCCATCGTCTGGGCAAACCTGGTTT AGGCGGTAGCAGTGGTGCAAGCCCTGCAGCACCGGCTCCGGCAAGTCCAG CAGCACCAGCACCTAGCGCACCAGCCGGTGGTGATATCAAACTGCAGCAG AGCGGTGCAGAACTGGCACGTCCGGGTGCAAGCGTTAAAATGAGCTGTAA AACCAGCGGTTATACCTTTACACGTTATACCATGCATTGGGTTAAACAGCG TCCTGGTCAAGGTCTGGAATGGATTGGTTATATCAATCCGAGCCGTGGTTA TACCAATTACAACCAGAAATTCAAAGACAAAGCAACCCTGACCACCGATA AAAGCAGCAGCACCGCCTATATGCAGCTGAGCAGCCTGACCAGCGAAGAT AGCGCAGTTTATTACTGTGCACGCTATTATGATGATCACTATTGCCTGGATT ATTGGGGTCAGGGCACCACACTGACCGTTAGCAGCGTTGAAGGTGGTAGC GGTGGTTCAGGTGGTAGTGGCGGTAGTGGTGGTGTTGATGATATTCAGCTG ACCCAGAGTCCGGCAATTATGAGCGCAAGTCCGGGTGAAAAAGTTACCAT GACCTGTCGTGCCAGCAGCAGCGTTAGCTATATGAATTGGTATCAGCAGAA AAGCGGTACAAGCCCGAAACGTTGGATTTATGATACCAGCAAAGTTGCAA GCGGTGTTCCGTATCGTTTTAGCGGTAGCGGTTCAGGCACCAGCTATAGTC TGACCATTAGCTCAATGGAAGCAGAAGATGCAGCAACCTATTATTGTCAGC AGTGGTCAAGCAATCCGCTGACCTTTGGTGCAGGTACAAAACTGGAACTGA AATAAGTCGACCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTG CTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGG TCTTGAGGGGTTTTTTG

Table S2: Results of dynamic light scattering for LUVs used for activation without functionalization.

Lipid type	**z-Average (SD) in nm**	**Polydispersity Index (SD)**
18:1 PC	116 (8)	0.07 (0.01)
18:1 PS	131 (9)	0.07 (0.01)
12:0 PS	116 (4)	0.15 (0.01)
14:0 PG	118 (22)	0.11 (0.02)
14:0 PC	126 (7)	0.05 (0.03)
18:0-14:0 PC	129 (2)	0.05 (0.01)
14:0-16:0 PC	138 (16)	0.06 (0.08)
14:0 PS	124 (5)	0.03 (0.01)
18:1 (Delta9-Trans) PE	168 (5)	0.25 (0.05)
14:0-18:0 PC	136 (12)	0.05 (0.05)
16:0 PC	129 (1)	0.06 (0.02)
16:0 PG	125 (6)	0.05 (0.02)
17:0 PC	142 (2)	0.08 (0.03)
16:0 PS	145 (5)	0.09 (0.01)
18:0 PG	151 (3)	0.14 (0.01)

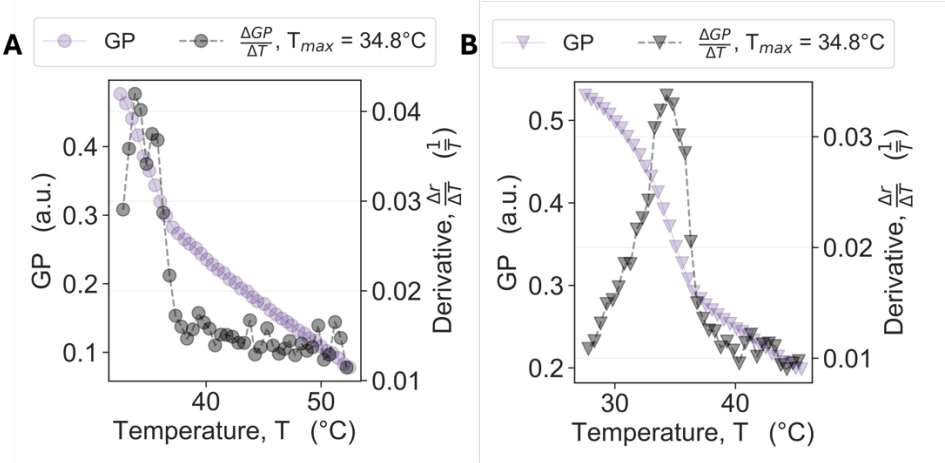

Figure S1: Laurdan GP as function of temperature for 18:1 (Δ9-Trans) PE liposomes in their composition similar to the activation study (including 29mol% cholesterol). Melting transition measured in PBS (A) and RPMI (B).

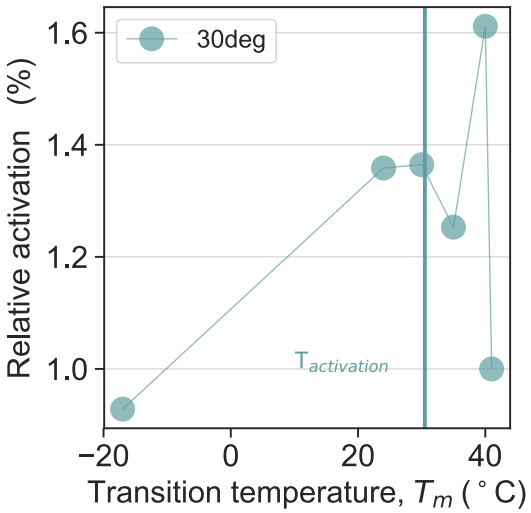

Figure S2: Results for activation at 30°C normalized to 14:0-18:0 PC. Data set includes PC lipids and the DPPG replicates (relative activation: 1.61). Comparing only PC lipids the maximal activation is shifted to 14:0-16:0PC (T_m = 30°C), but overall DPPG is the best activating sample contrary to the trend.

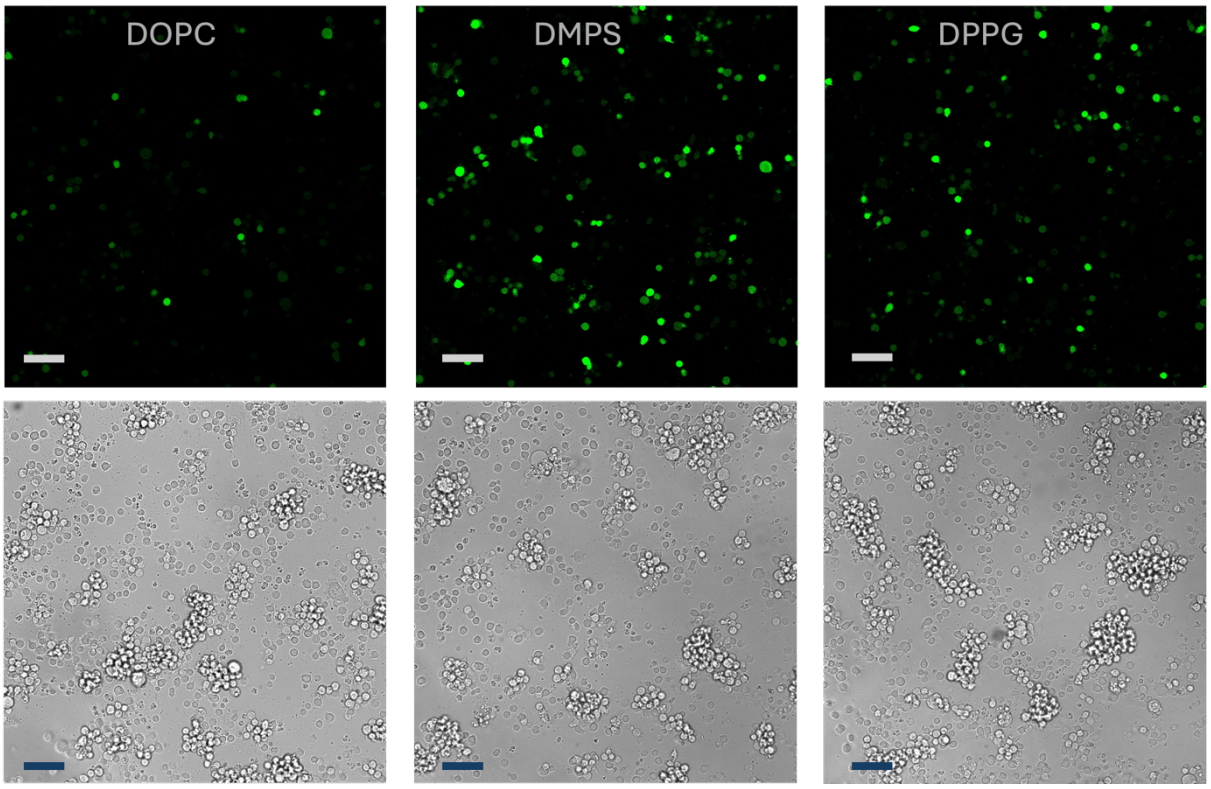

Figure S3: Activation of Jurkats with functionalized GUVs via αCD3-conjugation after overnight incubation. GFP expression upon activation of Jurkat reporter cell line is visible in microscopy images in all three tested conditions.