

SUPPLEMENTARY INFORMATION

Clonal amplification-enhanced gene expression for cell-free directed evolution

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Supplementary Table 1. DNA primer sequences and purpose.

| Name | Purpose | Sequence | Modifications |
|----------|---------------------------|---|--------------------|
| 1106 ChD | YFP gene subcloning | CCGTTTAGAGCCCAAGGG | none |
| 1107 ChD | YFP gene subcloning | CTTCGTCTGTGTCGCATGTGAaATTAATACGACTCACTATAGGGAGACCACAACG | none |
| 1104 ChD | amplification of vector | TAGCATAACCCCTTGGGGC | none |
| 1105 ChD | amplification of vector | CCTATAGTGAGTCGTATTAATtTCACATGCGAC | none |
| 1115 ChD | pssA gene subcloning | CTTCGTCTGTGTCGCATGTGAaATTAATACGACTCACTATAGGGGAATTGTGAGC | none |
| 1116 ChD | pssA gene subcloning | AACCCCTCAAGACCCGTTTAGAG | none |
| 961 ChD | TP gene cloning | acgtgtaccAAAGTAAGCCCCACCCTCACATG | none |
| 962 ChD | TP gene cloning | agctaagcttAAAGTAGGGTACAGCGACAACATACAC | none |
| 491 ChD | preparative PCR for IVTTR | 5-phos/AAAGTAAGCCCCACCCTCACATG | 5'-phosphorylation |
| 492 ChD | preparative PCR for IVTTR | 5-phos/AAAGTAGGGTACAGCGACAACATACAC | 5'-phosphorylation |
| 1121 ChD | YFP detection | TGCAACTGGCTGACCACTAC | none |
| 1122 ChD | YFP detection | AATGATTGTCCGGCAGCAGA | none |
| 980 ChD | p3 detection | ACGGCTGAAATTGACATCCCG | none |
| 981 ChD | p3 detection | CCAGGCGTTGAACCTCTTTGG | none |
| 1125 ChD | pssA-qPCR-F | AACAGGATGACGGTGGCAAA | none |
| 1126 ChD | pssA-qPCR-R | GGAACATCTACGCCGGATT | none |
| 1208 ChD | MinD detection | CGCGACTCTGACCGTATTT | none |
| 1209 ChD | MinD detection | AGCATGTCACCTCTGCTTAC | none |

Supplementary Table 2. Plasmid DNA description.

| plasmid name | plasmid description |
|--------------|--|
| G365 | Contains the DNA unit for the expression of YFP fluorescence protein. Transcription is regulated by a T7 promoter and T7 terminator sequences. The entire CDS unit is placed in between right and left origins of replication from the Φ 29 DNA replication machinery. |
| G368 | Contains the DNA unit for the expression of the phospholipid biosynthesis protein PssA. Transcription is regulated by a T7 promoter and T7 terminator sequences. The entire CDS unit is placed in between right and left origins of replication from the Φ 29 DNA replication machinery. |
| G437 | Contains the DNA unit for the expression of MinD protein. Transcription is regulated by a T7 promoter and T7 terminator sequences. The entire CDS unit is placed in between right and left origins of replication from the Φ 29 DNA replication machinery. |
| G338 | Contains the DNA unit for the expression of the Φ 29 terminal protein TP. Transcription is regulated by a T7 promoter and T7 terminator sequences. The entire CDS unit is placed in between right and left origins of replication from the Φ 29 DNA replication machinery. |
| G85 | Contains the DNA unit for the expression of Φ 29 DNA polymerase. Transcription is regulated by a T7 promoter and vsv terminator sequences. |
| G95 | Contains the DNA sequence for the expression of DNAP and TP. Each protein expression is independently regulated by a T7 promoter and a terminator sequence. DNAP unit uses a vsv terminator. TP unit utilizes a T7 terminator sequence. The entire CDS encoding for DNAP and TP is placed in between right and left origins of replication from the Φ 29 DNA replication machinery. |

Supplementary Table 3. Transitions of the MS/MS measurements for the proteolytic peptides of the indicated proteins.

| Protein | Compound name | Precursor ion (m/z) | Product ion (m/z) | Collision energy (eV) | Accelerator voltage (eV) | Ion name |
|--|--------------------|---------------------|-------------------|-----------------------|--------------------------|----------|
| PSSA | DLQSIADYPVK.light | 624.8272 | 805.4454 | 20.4 | 4 | y7 |
| PSSA | DLQSIADYPVK.light | 624.8272 | 692.3614 | 20.4 | 4 | y6 |
| PSSA | DLQSIADYPVK.light | 624.8272 | 506.2973 | 20.4 | 4 | y4 |
| PSSA | DLQSIADYPVK.light | 624.8272 | 343.2340 | 20.4 | 4 | y3 |
| PSSA | DLQSIADYPVK.light | 624.8272 | 357.1769 | 20.4 | 4 | b3 |
| PSSA. QconCAT | DLQSIADYPVK.heavy | 631.3079 | 813.4217 | 20.4 | 4 | y7 |
| PSSA. QconCAT | DLQSIADYPVK.heavy | 631.3079 | 699.3406 | 20.4 | 4 | y6 |
| PSSA. QconCAT | DLQSIADYPVK.heavy | 631.3079 | 511.2825 | 20.4 | 4 | y4 |
| PSSA. QconCAT | DLQSIADYPVK.heavy | 631.3079 | 347.2221 | 20.4 | 4 | y3 |
| PSSA. QconCAT | DLQSIADYPVK.heavy | 631.3079 | 361.1650 | 20.4 | 4 | b3 |
| YFP | FEGDTLVNR.light | 525.7644 | 903.4530 | 17.3 | 4 | y8 |
| YFP | FEGDTLVNR.light | 525.7644 | 774.4104 | 17.3 | 4 | y7 |
| YFP | FEGDTLVNR.light | 525.7644 | 717.3890 | 17.3 | 4 | y6 |
| YFP | FEGDTLVNR.light | 525.7644 | 602.3620 | 17.3 | 4 | y5 |
| YFP | FEGDTLVNR.light | 525.7644 | 501.3144 | 17.3 | 4 | y4 |
| YFP | FEGDTLVNR.light | 525.7644 | 449.1667 | 17.3 | 4 | b4 |
| YFP. QconCAT | FEGDTLVNR.heavy | 532.2451 | 915.4175 | 17.3 | 4 | y8 |
| YFP. QconCAT | FEGDTLVNR.heavy | 532.2451 | 785.3778 | 17.3 | 4 | y7 |
| YFP. QconCAT | FEGDTLVNR.heavy | 532.2451 | 727.3593 | 17.3 | 4 | y6 |
| YFP. QconCAT | FEGDTLVNR.heavy | 532.2451 | 611.3354 | 17.3 | 4 | y5 |
| YFP. QconCAT | FEGDTLVNR.heavy | 532.2451 | 509.2906 | 17.3 | 4 | y4 |
| YFP. QconCAT | FEGDTLVNR.heavy | 532.2451 | 453.1548 | 17.3 | 4 | b4 |
| Ribosomal protein S4 (YFP quantification) | LSDYGVQLR.light | 525.7826 | 850.4417 | 17.3 | 4 | y7 |
| Ribosomal protein S4 (YFP quantification) | LSDYGVQLR.light | 525.7826 | 735.4148 | 17.3 | 4 | y6 |
| Ribosomal protein S4 (YFP quantification) | LSDYGVQLR.light | 525.7826 | 572.3515 | 17.3 | 4 | y5 |
| Ribosomal protein S4 (YFP quantification) | LSDYGVQLR.light | 525.7826 | 635.3035 | 17.3 | 4 | b6 |
| Ribosomal protein S4. QconCAT (YFP quantification) | LSDYGVQLR.heavy | 525.7826 | 861.4091 | 17.3 | 4 | y7 |
| Ribosomal protein S4. QconCAT (YFP quantification) | LSDYGVQLR.heavy | 525.7826 | 745.3851 | 17.3 | 4 | y6 |
| Ribosomal protein S4. QconCAT (YFP quantification) | LSDYGVQLR.heavy | 525.7826 | 581.3248 | 17.3 | 4 | y5 |
| Ribosomal protein S4. QconCAT (YFP quantification) | LSDYGVQLR.heavy | 525.7826 | 641.2857 | 17.3 | 4 | b6 |
| Ribosomal protein L6 (YFP quantification) | APVVVPAGVDVK.light | 575.8452 | 883.5247 | 18.9 | 4 | y9 |
| Ribosomal protein L6 (YFP quantification) | APVVVPAGVDVK.light | 575.8452 | 784.4563 | 18.9 | 4 | y8 |
| Ribosomal protein L6 (YFP quantification) | APVVVPAGVDVK.light | 575.8452 | 685.3879 | 18.9 | 4 | y7 |

| | | | | | | |
|---|--------------------|----------|-----------|------|---|-----|
| Ribosomal protein L6 (YFP quantification) | APVVVPAGVDVK.light | 575.8452 | 268.1656 | 18.9 | 4 | b3 |
| Ribosomal protein L6 (YFP quantification) | APVVVPAGVDVK.light | 575.8452 | 367.2340 | 18.9 | 4 | b4 |
| Ribosomal protein L6 (YFP quantification) | APVVVPAGVDVK.light | 575.8452 | 466.3024 | 18.9 | 4 | b5 |
| Ribosomal protein L6. QconCAT (YFP quantification) | APVVVPAGVDVK.heavy | 582.3259 | 893.4951 | 18.9 | 4 | y9 |
| Ribosomal protein L6. QconCAT (YFP quantification) | APVVVPAGVDVK.heavy | 582.3259 | 793.4296 | 18.9 | 4 | y8 |
| Ribosomal protein L6. QconCAT (YFP quantification) | APVVVPAGVDVK.heavy | 582.3259 | 693.3642 | 18.9 | 4 | y7 |
| Ribosomal protein L6. QconCAT (YFP quantification) | APVVVPAGVDVK.heavy | 582.3259 | 271.1567 | 18.9 | 4 | b3 |
| Ribosomal protein L6. QconCAT (YFP quantification) | APVVVPAGVDVK.heavy | 582.3259 | 371.2221 | 18.9 | 4 | b4 |
| Ribosomal protein L6. QconCAT (YFP quantification) | APVVVPAGVDVK.heavy | 582.3259 | 471.2876 | 18.9 | 4 | b5 |
| Ribosomal protein S1 (PSSA quantification) | GVVVAIDK.light | 400.7475 | 644.3978 | 13.4 | 4 | y6 |
| Ribosomal protein S1 (PSSA quantification) | GVVVAIDK.light | 400.7475 | 545.3293 | 13.4 | 4 | y5 |
| Ribosomal protein S1 (PSSA quantification) | GVVVAIDK.light | 400.7475 | 446.2609 | 13.4 | 4 | y4 |
| Ribosomal protein S1 (PSSA quantification) | GVVVAIDK.light | 400.7475 | 426.2711 | 13.4 | 4 | b5 |
| Ribosomal protein S1. QconCAT (PSSA quantification) | GVVVAIDK.heavy | 405.234 | 651.3770 | 13.4 | 4 | y6 |
| Ribosomal protein S1. QconCAT (PSSA quantification) | GVVVAIDK.heavy | 405.234 | 551.3115 | 13.4 | 4 | y5 |
| Ribosomal protein S1. QconCAT (PSSA quantification) | GVVVAIDK.heavy | 405.234 | 451.2461 | 13.4 | 4 | y4 |
| Ribosomal protein S1. QconCAT (PSSA quantification) | GVVVAIDK.heavy | 405.234 | 431.2563 | 13.4 | 4 | b5 |
| Ribosomal protein L1 (PSSA quantification) | VVGQLGQVLGPR.light | 611.8670 | 1024.5898 | 20 | 4 | y10 |
| Ribosomal protein L1 (PSSA quantification) | VVGQLGQVLGPR.light | 611.8670 | 839.5098 | 20 | 4 | y8 |
| Ribosomal protein L1 (PSSA quantification) | VVGQLGQVLGPR.light | 611.8670 | 726.4257 | 20 | 4 | y7 |
| Ribosomal protein L1 (PSSA quantification) | VVGQLGQVLGPR.light | 611.8670 | 442.2772 | 20 | 4 | y4 |
| Ribosomal protein L1 (PSSA quantification) | VVGQLGQVLGPR.light | 611.8670 | 329.1932 | 20 | 4 | y3 |
| Ribosomal protein L1 (PSSA quantification) | VVGQLGQVLGPR.heavy | 620.3418 | 1039.5453 | 20 | 4 | y10 |
| Ribosomal protein L1 (PSSA quantification) | VVGQLGQVLGPR.heavy | 620.3418 | 851.4742 | 20 | 4 | y8 |
| Ribosomal protein L1 (PSSA quantification) | VVGQLGQVLGPR.heavy | 620.3418 | 737.3931 | 20 | 4 | y7 |
| Ribosomal protein L1 (PSSA quantification) | VVGQLGQVLGPR.heavy | 620.3418 | 449.2565 | 20 | 4 | y4 |
| Ribosomal protein L1 (PSSA quantification) | VVGQLGQVLGPR.heavy | 620.3418 | 335.1754 | 20 | 4 | y3 |
| Ribosomal protein S4 (PSSA quantification) | AAELEAEQR.light | 500.7747 | 745.3839 | 16.5 | 4 | y6 |
| Ribosomal protein S4 (PSSA quantification) | AAELEAEQR.light | 500.7747 | 616.3413 | 16.5 | 4 | y5 |

| | | | | | | |
|--|-----------------|----------|----------|------|---|----|
| Ribosomal protein S4 (PSSA quantification) | AALELAEQR.light | 500.7747 | 503.2572 | 16.5 | 4 | y4 |
| Ribosomal protein S4 (PSSA quantification) | AALELAEQR.light | 500.7747 | 432.2201 | 16.5 | 4 | y3 |
| Ribosomal protein S4 (PSSA quantification) | AALELAEQR.light | 500.7747 | 385.2082 | 16.5 | 4 | b4 |
| Ribosomal protein S4 (PSSA quantification) | AALELAEQR.heavy | 507.2555 | 755.3542 | 16.5 | 4 | y6 |
| Ribosomal protein S4 (PSSA quantification) | AALELAEQR.heavy | 507.2555 | 625.3146 | 16.5 | 4 | y5 |
| Ribosomal protein S4 (PSSA quantification) | AALELAEQR.heavy | 507.2555 | 511.2335 | 16.5 | 4 | y4 |
| Ribosomal protein S4 (PSSA quantification) | AALELAEQR.heavy | 507.2555 | 439.1994 | 16.5 | 4 | y3 |
| Ribosomal protein S4 (PSSA quantification) | AALELAEQR.heavy | 507.2555 | 389.1963 | 16.5 | 4 | b4 |

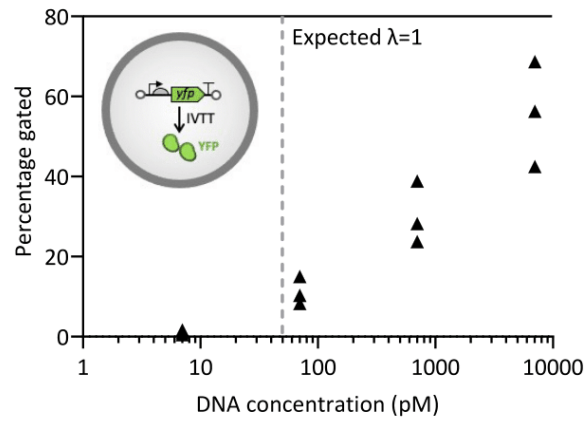


Fig S1. Effect of DNA concentration on YFP protein expression without gene amplification. Fluorescence from individual liposomes was analyzed by flow cytometry.

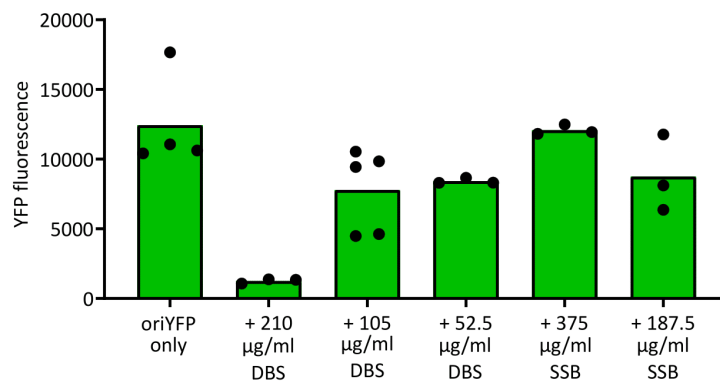


Fig S2. End-point YFP fluorescence measurements from *ori-yfp* bulk IVTT reactions. Protein expression can be inhibited under high DBS concentrations (210 $\mu\text{g/ml}$).

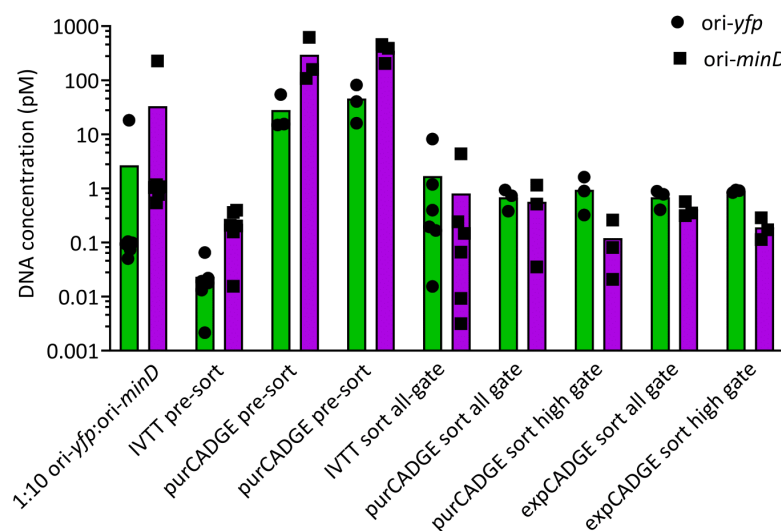


Fig S3. Individual qPCR data from Figure 4h. Enrichment of *ori-yfp* over *ori-minD*. Each symbol represents a biological repeat. 'IVTT' indicates a reaction without DNA replication.

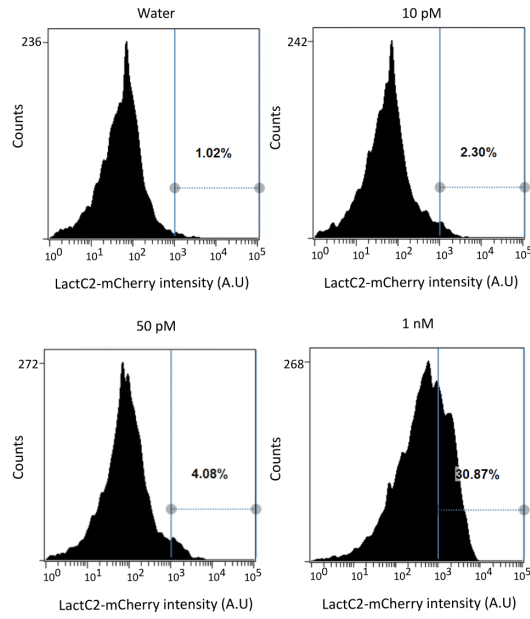


Fig S4. Raw FACS data of liposome samples with appended gating line as used in Figure 5c,d.

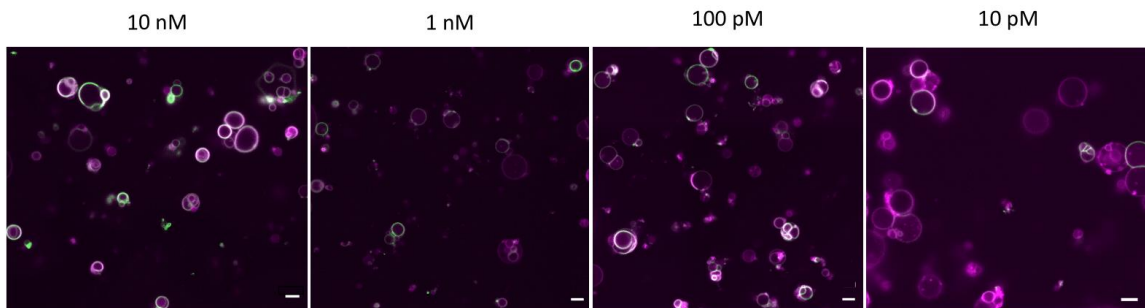


Fig S5. In-liposome expression of *ori-pssA* under different DNA concentrations (10 nM to 10 pM) without DNA replication. Liposome membrane dye (Texas-red) is colored in magenta and PS binding protein LactC2-eGFP is colored in green. Overlay of the two colors is displayed in white. Lowering the concentration of *pssA* gene reduces the number of liposomes with membrane-recruited LactC2-eGFP. Scale bars are 5 μ m.

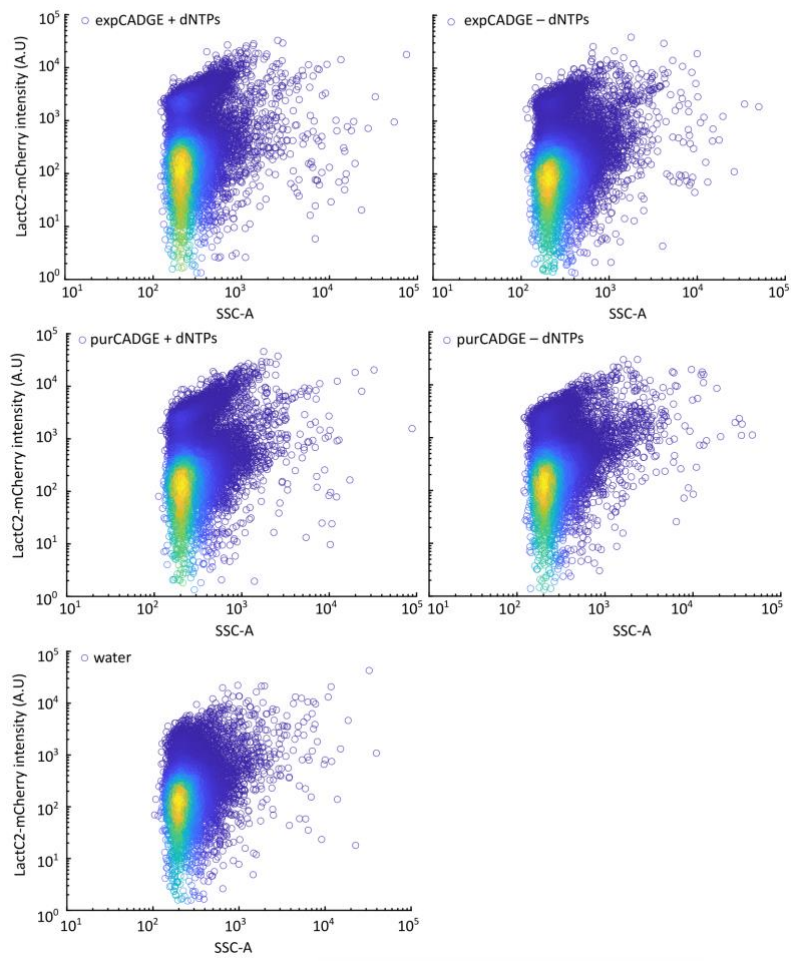


Fig S6. FACS data of liposome samples analyzed in Figure 5g.

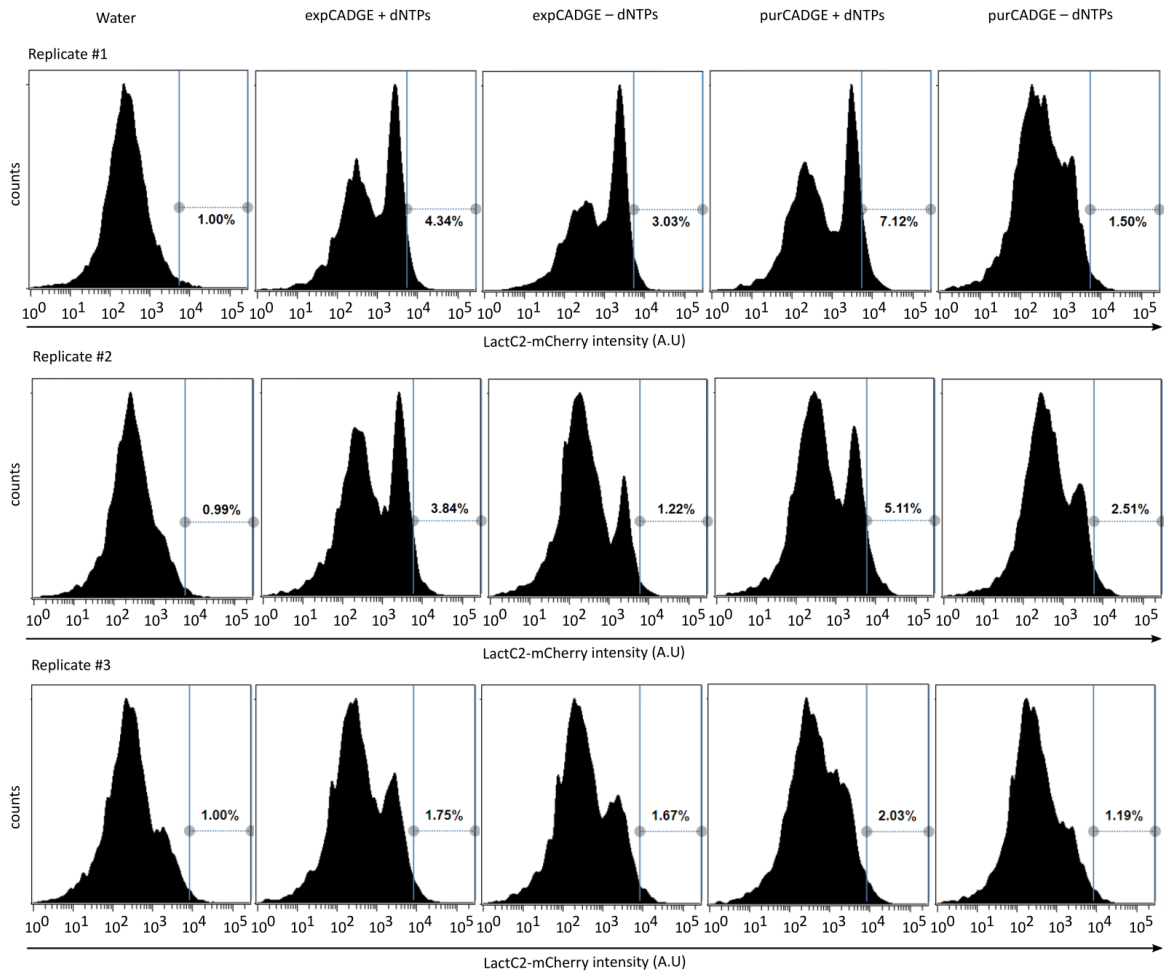


Fig S7. Liposomes FACS data and gating strategy for Figure 5h.

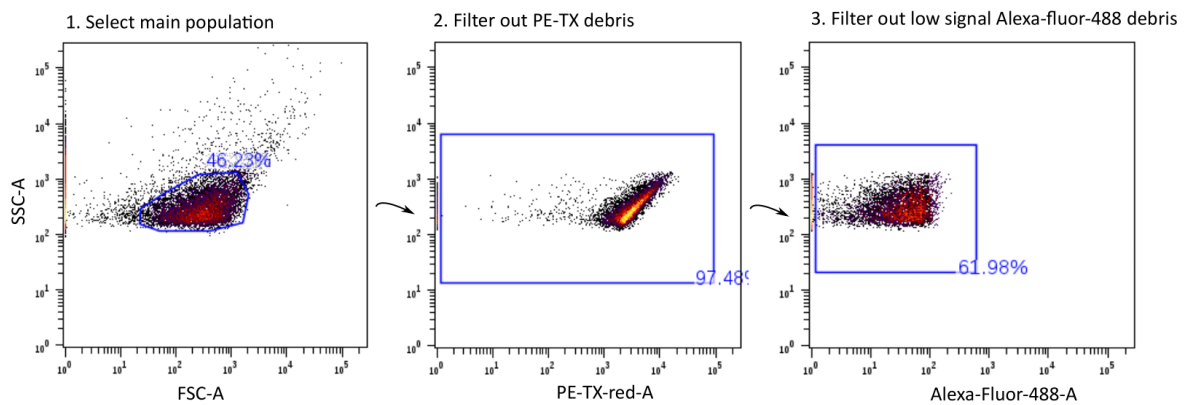


Fig S8. Data processing for FACS data. Application of the filtering gate to remove liposomal debris in PssA expressing-liposomes.