

# SUPPLEMENTARY INFORMATION

FOR

## **Imaging flow cytometry for high-throughput phenotyping of synthetic cells**

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In the sequences below, the following regulatory elements have been highlighted as indicated:

- **T7 promoter**
- T7 terminator
- vsv terminator
- phi29 origins of replication

### Sequence of *yfp* (5'→3', complete linear construct)

AAAGTAAGCCCCACCCTCACATGATACCATTCTCCTAATATCGACATAATCCGTCGATCCTCGGCATACCATGATCAGGGAGGGAAA  
CTACTACTTAATATATCAATCTATAGACCTACTAGATAGGTTTGTCAATGAACAACATAAAAACGACACAGAATCCCACGTTTTAGCGC  
TTCGTCTGTGTCGCATGTGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAAATAATTTTGTTTAACTTTAA  
GAAGGAGATATACATATGCGGGGTTTCATCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGCAAATGGGTCCGGAT  
CTGTACGACGATGACGATAAGGATCCGATGGTTAGCAAAGGCGAAGAACTGTTTACGGGCGTGGTGCCGATTCTGGTGAACTGGAC  
GGCGACGTGAACGGTCACAAATTCAGCGTTTCGGGCGAAGGTGAAGGCGATGCGACCTATGGTAACTGACGCTGAAATTTATTTGC  
ACCACCGGTAAACTGCCGGTGCCGTGGCCGACCCTGGTTACCACGTTTGGTTATGGCCTGCAGTGTTTCGCGCGCTACCCGGATCATA  
TGAAACAACACGACTTTTTCAAATCTGCCATGCCGAAGGTTATGTGACGGAACGTACGATTTTCTTTAAAGATGACGGCAACTACAA  
AACCCGCGCAGAAGTCAAATTTGAAGGTGATACGCTGGTGAACCGTATTGAACTGAAAGGCATCGATTTCAAAGAAGACGGTAATAT  
CCTGGGCCATAAACTGGAATACAACACTACAACCTCCACAACGTTTACATCATGGCAGATAAACAGAAAAACGGTATCAAAGTCAACTT  
CAAAATCCGCCATAACATCGAAGATGGCTCAGTGAACCTGGCTGACCCTACCAGCAAAAACACCCCGATCGGTGATGGCCCGGTTCT  
GCTGCCGACAATCATTATCTGAGCTACCAGTCTGCACTGAGTAAAGATCCGAACGAAAAACGTGACCACATGGTCTCTGCTGGAATTT  
GTGACGGCGGCTGGTATTACGCTGGGCATGGATGAACTGTATAAATGAAAGCTTCCCGGGAAAGTATATATGAGTAAAGATATCGAC  
GCAACTGAATGAAATGGTGAAGGACGGGTCCAGGTGTGGCTGCTTCGGCAGTGCAGCTTGTGAGTAGAGTGTGAGCTCCGTAAC  
GTGCGCTCGATATCCCGGGCTAGCATAACCCCTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTTGCTCTATGATTGGTTGTCTT  
ATTACCTTACTTCTATTATAGTATAACATGTTAAACGATAGTTTGTCTACCCTTTTCGACAAATTGATGATAATAAATAGTATAGGTAT  
ATAGTCGTGATTAGTTGTTAGATTCTTGTGCAAGATAGTCGGTCAATGGGGAAATGGTGTATGTTGTCGCTGTACCCTACTTT

### Sequence of *p2-p3* (5'→3', complete linear construct)

AAAGTAAGCCCCACCCTCACATGATACCATTCTCCTAATATCGACATAATCCGTCGATCCTCGGCATACCATGATCAGGGAGGGAAA  
CTACTACTTAATATATCAATCTATAGACCTACTAGATAGGTTTGTCAATGAACAACATAAAAACGACACAGAATCCCACGTTTTAGCGC  
TTCGTCTGTGTCGCATGTGAAATTAATACGACTCACTATAGGGAGACCACAACGGTTTCCCTCTAGAAAATAATTTTGTTTAACTTTAA  
GAAGGAGATATACATATGCCCGTAAAATGTACAGCTGCGATTTTGAACGACGACGAAAGTTGAAGATTGCCGTGTCTGGGCCTAT  
GGTTATATGAACATCGAAGACCATTGAGAATATAAAATGGCAACTCGCTGGATGAAATTTATGGCGTGGGTGCTGAAAGTTCAGGCC  
GACCTGTACTTCCACAATCTGAAATTTGATGGTGCCTTATTATCAACTGGCTGGAACGTAATGGCTTTAAATGGAGCGCCGATGGTC  
TGCCGAACACCTATAATACGATTATCTCTCGTATGGGCCAATGGTATATGATTGATATCTGCCTGGGCTACAAAGGTAAACGCAAAAT  
TCATACCGTGATCTATGACAGCTGAAAAAATGCGGTTTCCGGTGAAGAAAATTGCGAAAGATTTCAAACGACCGTCTGAAAGG  
CGATATTGACTATCACAAGAACGTCGGGTTGGTTACAAAATCACGCCGGAAGAATATGCGTACATTAACGATATCCAGATTAT  
CGCAGAAGCTCTGCTGATTAGTTAAACAAGGCCTGGATCGCATGACCGCCGACGACTCCCTGAAAGGTTTCAAAGATATCATC  
ACCACGAAAAAATTAAGAAAGTGTCCCGACCCTGAGCCTGGGTCTGGATAAAGAAAGTTTCGTTATGCATACCGCGGCGGTTTTACGT  
GGCTGAACGACCGTTTCAAAGAAAAAGAAATTTGGCGAGGGTATGGTCTTTGATGTGAATAGTCTGTATCCGGCTCAGATGACTCCCG  
CCTGCTGCCGTATGGCGAACCGATCGTTTTTCGAGGGTAAATATGTCTGGGATGAAGACTACCCGCTGCATATTCAGCACATCCGTTGT  
GAATTTGAACTGAAAGAAGGCTATATTCCGACCATTCAAATCAAACGTAGCCGCTTCTATAAGGGTAACGAATACCTGAAAAGCTCT  
GGCGGTGAAATCGCAGACCTGTGGCTGAGTAACGTCGATCTGAACTGATGAAAGAACATTACGATCTGTACAACGTTGAATACATC  
TCCGGCCTGAAATTTAAAGCCACCACGGGTCTGTTAAAGATTTTATTGACAAATGGACCTACATCAAAACCACGCTGTAAGGTGCAA  
TCAAACAGCTGGCTAAACTGATGCTGAACAGCCTGTATGGCAAATTTGCATCTAATCCGGATGTTACCGGTAAGTCCCCTACCTGAA  
AGAAAATGGCGCTCTGGGTTTTCGCCTGGGCGAAGAAGAAACCAAGATCCGGTGTATACGCCGATGGGTGTTTTTATTACCGCTG  
GGCAGGTTACACCACCATCACCGCCGACAAGCGTGCTATGACCGCATTATCTACTGTGATACCGACTCAATTCATCTGACCGGCACG  
GAAATCCCGATGTGATTAAGATATCGTTGACCCGAAAAAATGGGTTATTGGGCACACGAATCGACCTTTAAACGTGCTAAATAC  
CTGCGCCAGAAAACGTACATCCAAGACATCTACATGAAAGAAGTCGATGGCAAACCTGGTGAAGGTTACCGGATGACTATACCGAC  
ATTAATTTTCGGTGAATGCGCCGGCATGACCGATAAAATTAAGAAAGAAGTGCAGTTTCGAAAATTTCAAAGTGGGTTTTAGTCCG  
AAAATGAAACCGAAACCGGTCCAAGTTCCGGGCGGCGTTGTGCTGGTTCGATGACACCTTCACGATCAAAATAAGAATTGTACTAGAGT

ATCTGTTAGTTTTTTCACTAGAGTACTAGAGTATCTGTTAGTTTTTTCATCGGATCCCGGGCCCGTCGACTGCTAATACGACTC  
ACTATAGGGCCCTCTGGAGACACCAGAGGGTTACATGTTTATTTGTTAACTTTAAGAAGGAGATATACTAATGGCACGCAGCCCGC  
GCATCCGCATCAAAGATAACGACAAAGCCGAATACGCCCGCTGGTGA AAAATACGAAAGCTAAAATCGCACGTACCAAGAAAAA  
TATGGCGTGGATCTGACGGCTGAAATTGACATCCCGGATCTGGACTCATTGAAACCCGCGCGCAGTTCAATAAATGAAAAGAACA  
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TAACATGTTAAACGATAGTTTGTCTACCCTTTTCGACAAATTGATGATAATAAATAGTATAGGTATATAGTCGTGATTTAGTTGTTAGA  
TTCTTGTGCAAGATAGTCGGTCAATGGGGAAATGGTGTATGTTGTCGCTGTACCCTACTT

### Sequence of *btubA* (5'→3', complete linear construct)

CAGTCACGACGTTGTA AAAACGACGGCCAGTCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTC  
TAGAAATAATTTTGTAACTTTAAGAAGGAGATATACATATGAAAGTTAATAATACAATTGTAGTTAGTATTGGTCAGGCGGGCAAC  
CAAATCGCGGCGAGCTTCTGAAAACCGTGTGCCTGGAGCACGGTATTGACCCGCTGACCGGTCAGACCGCGCCGGGCGTTGCGCCG  
CGTGGTAACTGGAGCAGCTTCTTTAGCAAGCTGGGCGAGAGCAGCAGCGGTAGCTACGTGCCGCGTGCATCATGGTTGATCTGGAA  
CCGAGCGTGATTGACAACGTTAAAGCGACCGCGCAGCCTGTTCAACCCGGCGAACCTGATTAGCCGTACCGAGGGCGCGGGTGGC  
AACTTTGCGGTTGGTTACCTGGGTGCGGGTCTGAGGTGCTGCCGGAAGTTATGAGCCGCTGGATTATGAAATCGACAAGTGGGATA  
ACGTGGGTGGCATTGTTCTGCATGCGATCGGTGGTGGCACCGGCAGCGGTTTTGGCGCGCTGCTGATCGAGAGCCTGAAGGAAA  
AATACGGCGAGATTCCGGTGTGAGCTGCGCGGTTCTGCCGAGCCCGCAGGTGAGCAGCGTGGTTACCGAGCCGTATAACACCGTTTT  
TGCGCTGAACACCTGCGTCTGAGCGGGATGCGTGCCTGATCTTCGATAACGAAGCGCTGTTGACCTGGCGCACCGTAAATGGAAC  
ATTGAGAGCCCGACCGTGGACGATCTGAACCTGCTGATCACCGAAGCGCTGGCGGGCATTACCGCGAGCATGCGTTTCAGCGGTTTTC  
TGACCGTGGAAATCACCTGCGTGAAGTCTGACCAACCTGGTTCGCAACCGAGCCTGCACTTCTGATGTGCGCGTTTTGCGCCGCT  
GACCCCGCCGATCGTAGCAAGTTCGAGGAACTGGGTATCGAGGAAATGATTA AAAAGCCTGTTTCGACAACCGCAGCGTGTTCGCGC  
GTGACGCCGATGGAAGGTGCTTTTCTGAGCACCGGTTCTGTATCGTGGCATCATGGAGGATAAACCCGCTGGCGGATGCGGCGCTG  
CGGCGATGCGTGA AAAAGCTGCCGCTGACCTACTGGATTCCGACCGCGTTCAAAAATTGGCTATGTTGAGCAGCCGGTATTAGCCACC  
GTAAAAGCATGGTGTCTGCGGAACAACACCGAAATCGCGCGTGTCTGATCGTATTTGCCACAACCTTCGACAAGCTGTGGCAAC  
GTAAAGCGTTTTCGAACTGGTATCTGAACGAGGGTATGAGCGAGGAACAGATCAACGTGCTGCGTGCAGCGCGCAAGA ACTGGTGC  
AGAGCTATCAAGTTGCGGAGGAAAGCGGCGGAAGGCGAAAGTTCAAGACAGCGGGTGATACCGGTATGCGTGCAGCGCGGGCG  
GGTGTGAGCGACGATGCGCGTGGTAGCATGAGCCTGCGTACCTGGTTGATCGTGCCTTAAGCGATCACTAGCATAACCCCTTGGG  
GCCTCTAAACGGGTCTTGAGGGTTTTTTGGGCGTAATCATGGTCATAGCTGTTTCTGTGTG

### Sequence of *btubB* (5'→3', complete linear construct)

CAGTCACGACGTTGTA AAAACGACGGCCAGTCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTC  
TAGAAATAATTTTGTAACTTTAAGAAGGAGATTTGAAAATGAGAGAAATATTAAGTATACATGTAGGTCAATGCGGCAACCAGAT  
CGCGGATAGCTTTTGGCGTCTGGCGCTGCGTGAACACGGCCTGACCGAGGCGGGCACCCGTAAGGAAGGTAGCAACGCGGCGGGGAA  
CAGCAACATGGAAGTGTCTTCCACAAGTTCTGTGACGGTAAATACGTGCCGCGTGGTGTGTTGATCTGGAGCCGGGCGTTATC  
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GGCTTCTGATGACCCATAGCATCGGTGGCGGTAGCGGCAGCGGTCTGGGCAGCCTGATTCTGGAACGTCTGCGTCAGGCGTACCCGA  
AGAAACGTATCTTACCTTTAGCGTGGTCCGAGCCCGCTGATTAGCGACAGCGCGGTGGAGCCGTATAACCGCATCCTGACCTGCA  
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CTGAGCGAGTTCGTGACCAACCTGGTTCGGTTCGCCGGCAACCACTTCTGACCGCGAGCTTCGCGCCGATGCGTGGTGGGTCAGG  
AAGGTCAAGTGCATACCAACTTTCCGACCTGGCGCGTGA AACCTTTGCGCAGGACAACCTCACCGCGGCGATCGATTGGCAGCAAG

GTGTTTATCTGGCGGCGAGCGCGCTGTTCCGTGGCGATGTGAAGGCGAAAGACGTTGATGAAAACATGGCGACCATTTCGTAAGAGCC  
TGAACACGCGAGCTATATGCCGGCGAGCGGGCTGAAACTGGGCTATGCGGAAACCGCGCCGGAAGTTTTGCGAGCAGCGGCC  
TGGCGCTGGTGAACCACACCGGTATCGCGCGGTTTTTCGAGCGTCTGATCGCGCAATTCGACATTATGTTTGATAACCACGCGTACAC  
CCACTGGTATGAAAACGCGGGTGTAGCCGTGACATGATGGCGAAAAGCGCGTAACCAGATTGCGACCCTGGCGCAGAGCTATCGTGA  
TGCGAGCTAAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTGGGCGTAATCATGGTCATAGCTGT  
TTCCTGTGTG

### Sequence of *minD* (5'→3', complete linear construct)

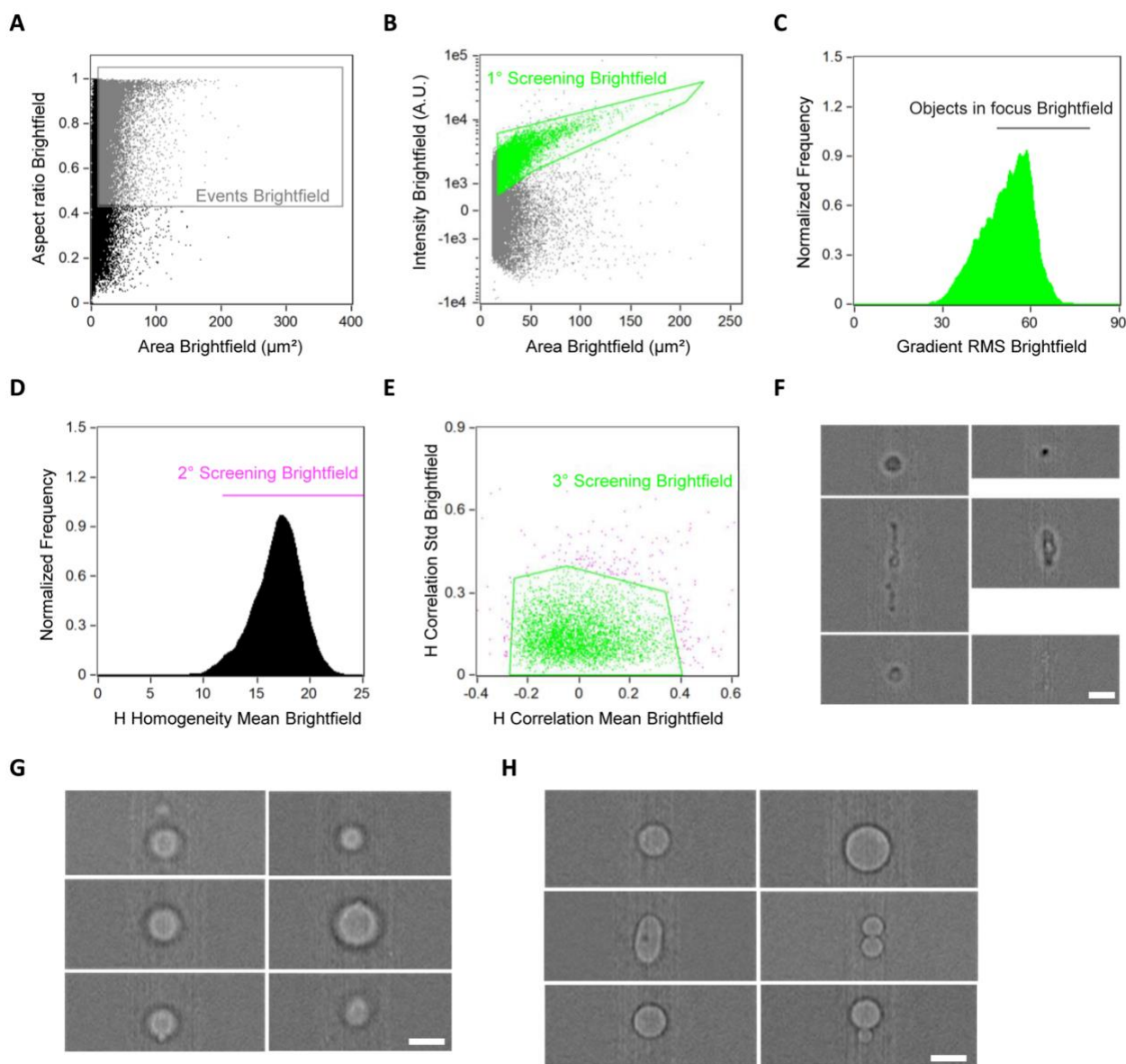
TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTGTTTAACTTTAAGAAGGAGATATACA  
TATGGCACGCATTATTGTTGTTACTTCGGGCAAAGGGGGTGTGGTAAGACAACCTCCAGCGCGCCATCGCCACTGGTTTTGCCAG  
AAGGGAAAAGAAAAGTGTCTGTATAGATTTTGATATCGGCCTGCGTAATCTCGACCTGATTATGGGTTGTGAACGCCGGTCTTTACG  
ATTTTCGCAACGTCATTCAGGGCGATGCAACGCTAAATCAGGCGTTAATTAAGATAAGCGTACTGAAAATCTCTATATTCTGCCGGC  
ATCGAAAACACGCGATAAAGATGCCCTCACCCGTGAAGGGTTCGCCAAAGTTCCTGATGATCTGAAAGCGATGGATTTTGAATTTATC  
GTTTGTGACTCCCGGCGAGGGATTGAAAACGGTGCCTAATGGCACTCTATTTTGCAGACGAAAGCCATTATTACCACCAACCCGGAAGT  
CTCCTCAGTACGCGACTCTGACCGTATTTTAGGCATTCTGGCGTCAAATCACGCCGCGCAGAAAATGGCGAAGAGCCTATTAAGAG  
CACCTGCTGTTAACGCGCTATAACCCAGGCCGCGTAAGCAGAGGTGACATGCTGAGCATGGAAGATGTGCTGGAGATCTGCGCATC  
AAACTCGTCGGCGTGATCCAGAGGATCAATCAGTATTGCGCGCCTTAACCAGGGTGAACCGGTCATTCTCGACATTAACGCCGATG  
CGGGTAAAGCCTACGAGATAACCGTAGAACGCTCTGTTGGGAGAAGAAGCTCCTTTCCGCTTCATTGAAGAAGAGAAGAAAGGCTTCC  
TCAAACGCTTGTTCGGAGGATAAGGATCCGGCTGCTAACAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAA  
CTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG

### Sequence of *pssA* (5'→3', complete linear construct)

AAAGTAAGCCCCACCCTCACATGATACCATTCTCCTAATATCGACATAATCCGTCGATCCTCGGCATACCATGATCAGGGAGGGAAA  
CTACTACTTAATATATCAATCTATAGACCTACTAGATAGGTTTTGTCAATGAACAACATAAAAACGACACAGAATCCCACGTTTTAGCGC  
TTCGTCTGTGTCGATGTGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATCCCCTCTAGAAATAATTTGTTT  
AACTTTAAGAAGGAGATATACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGGCTGCATGTTGTCAAAATTTA  
AGCGTAATAAACATCAACAACACCTTGCCCAACTACCCAAGATTTCTCAATCAGTTGATGATGTCGATTTCTTTACGCTCCCGCGAC  
TTCCGGGAGACGCTGCTGGAAAAAATAGCCAGCGGAAGCAGCGCATTTGCATTGTCGCCCTGTATCTCGAACAGGATGACGGTGGC  
AAAGGCATTCTGAACGCGTTGTATGAGGCTAAAAGGCAGCGTCCGGAAGTGGATGTGCGGGTGTGGTTCGACTGGCATCGTGCACAA  
CGTGGACGCATTGGCGCTGCGGCATCTAACACTAACGCTGACTGGTACTGCCGATGGCGCAGGAAAATCCGGGCGTAGATGTTCCG  
GTTTATGGCGTTCAATCAATACTCGTGAAGCCCTTGGTGTCTGCACCTTAAAGGCTTTATCATCGACGATAGCGTACTTTATAGCGG  
TGCCAGCCTGAACGATGTTTACCTGCATCAGCACGATAAATATCGCTACGACCGTTATCATCTGATCCGTAACCGTAAGATGTCAGAC  
ATTATGTTTGAATGGGTTACACAGAATATTATGAATGGCCGCGCGTTAATCGTCTGGATGATGTTAATCGGCCAAAAAGCCCGGAAA  
TCAAGAACGATATTCGTCTGTTCGCCAGGAGCTGCGTGATGCCGCTTATCATTTCAGGGCGATGCCGACAACGATCAGCTTTCTGT  
AACGCCGCTAGTGGGGCTGGGGAAATCGAGTCTGTTGAACAAGACCATTTCCATCTTATGCCTTGTGCCGAGCAGAAACTAACCATC  
TGTACGCCATACTTCAACCTGCCAGCAATCCTTGTGCGCAATATTATCCAGTTGCTGCGCGAAGGAAAAAGTGCAAAATTATTGTTG  
GTGATAAAAACCGCAATGACTTCTACATTCCGGAAGATGAACCTTTCAAGATAATTGGCGCATTGCCTTATCTCTATGAGATCAATCT  
GCGTCGTTTCTGAGCCGTTTGCAGTATTACGTCAATACTGACCAGCTAGTGGTTCCGGTTATGGAAAAGATGACGACAACACCTATCAC  
CTGAAAGGGATGTGGGTTGATGATAAGTGGATGTTGATCACCGGTAATAACCTGAACCCGCGCGCTGGCGTCTGGATCTGGAAAAC  
GCCATTTTATCCACGATCCGCAACTTGAGCTGGCGCCACAGCGAGAGAAAGAACTGGAGCTGATCCGCGAGCATAACCACATCGTT  
AAGCACTATCGCGATCTGCAAAGTATTGCCGATTATCCGGTGAAGGTTTCGTAAACTCATCCGCCGTTTTCGCGGATCCGCATCGACC  
GATTAATTAGCCGATCCTGTAACAAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAATAGCATAACCCCTG  
GGGCTCTAAACGGGTCTTGAGGGGTTTTTTGCTCCTATGATTGGTTGCTTATTACCTACTTCTATTATAGTATAACATGTTAAACG  
ATAGTTTGTCTACCCTTTTCGACAAATTGATGATAATAAATAGTATAGGTATATAGTCGTGATTTAGTTGTTAGATTCTTGTGCAAGAT  
AGTCGGTCAATGGGAAATGGTGTATGTTGTCGCTGTACCCTACTT

**Table S1:** List of primers used in this study

<b>Name</b>	<b>Sequence (5'→ 3')</b>
ChD491	AAAGTAAGCCCCACCCTCACATG
ChD492	AAAGTAGGGTACAGCGACAACATACAC
ChD709	CAAAAAACCCTCAAGACCCGTTAGAGG
ChD757	TAATACGACTCACTATAGGG



**Figure S1: Liposome identification using the brightfield channel.** The brightfield channel was used to identify liposome that did not have a membrane signal. The pipeline is similar to the one reported in Figure 2. A mask fitting the area to be used during the analysis in the brightfield channel was created. Identifying liposomes via the brightfield channel was more challenging than using a membrane dye signal. Structural characterization was less precise and stricter cut-off values were needed for the selection of actual liposomes. This stringency can, however, discard some “good” liposomes. We always recommend visual inspection of all gated populations for validation. **A)** Scatter diagram of the *area* of the mask and *aspect ratio* features. Objects having a surface area > 30  $\mu\text{m}^2$  and an aspect ratio > 0.4 were gated as relevant events (grey gate). **B)** Scatter plot of the *intensity* of the brightfield signal and the *area* of the mask (green gate). **C)** In-focus events were selected based on the *gradient RMS* feature. The events having a gradient RMS > 45 were selected for further analysis (black gate). **D)** Selection of liposomes with high *H-homogeneity* values (magenta gate). **E)** Scatter diagram of the *H-Correlation mean* and *standard deviation*. The selected objects (green gate) correspond to the final liposome population, which is cleared from undesired events. **F)** Images of debris, aggregates with different sizes and shapes that were eliminated. **G)** Images of out-of-focus liposomes that were excluded from the analysis. **H)** Gallery of liposome images that passed the gating steps. Analysis in the brightfield channel was performed on a small set of 8,000 liposomes from one sample. Scale bars are 7  $\mu\text{m}$ .