

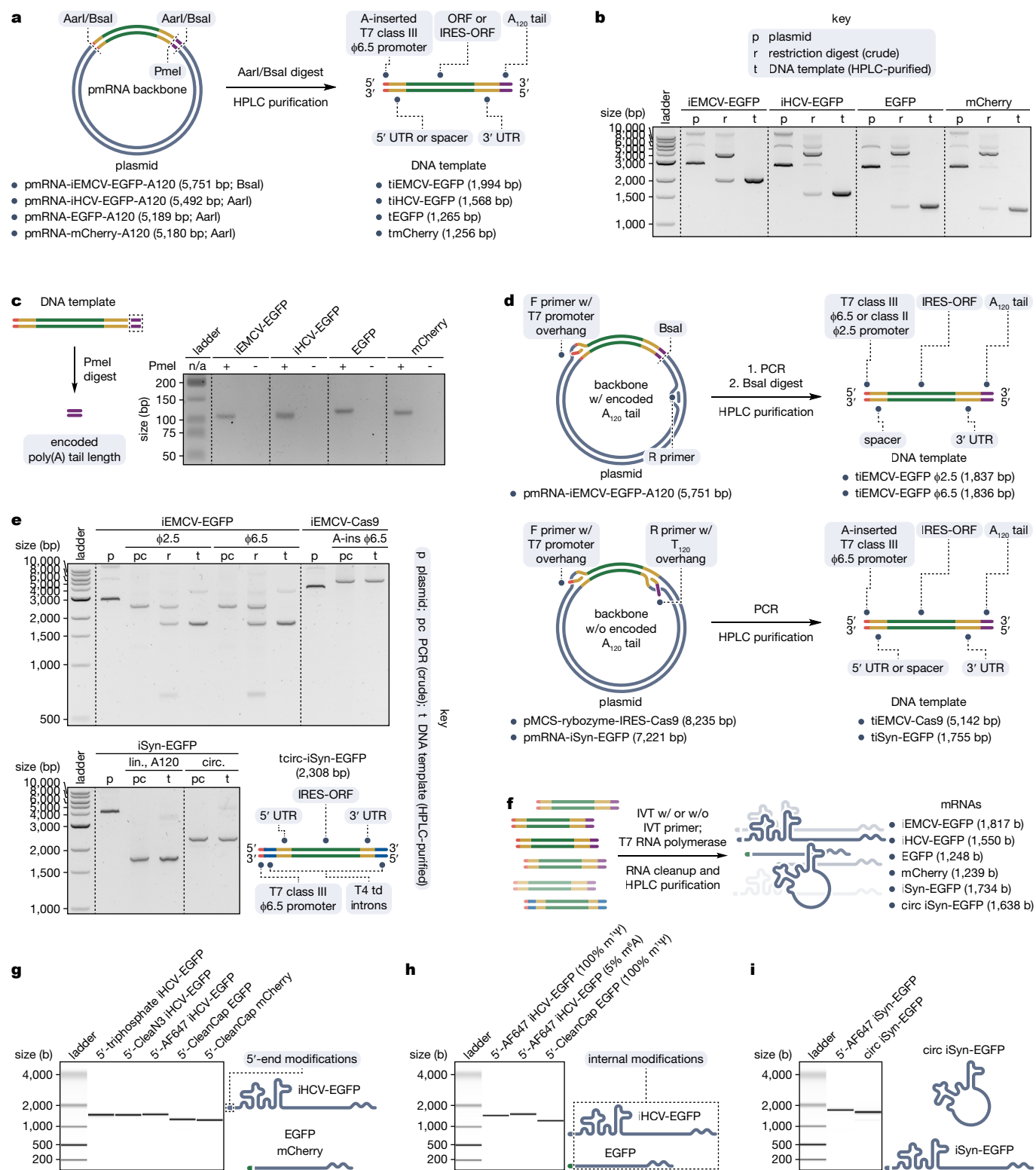
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Manufacturer's protocols

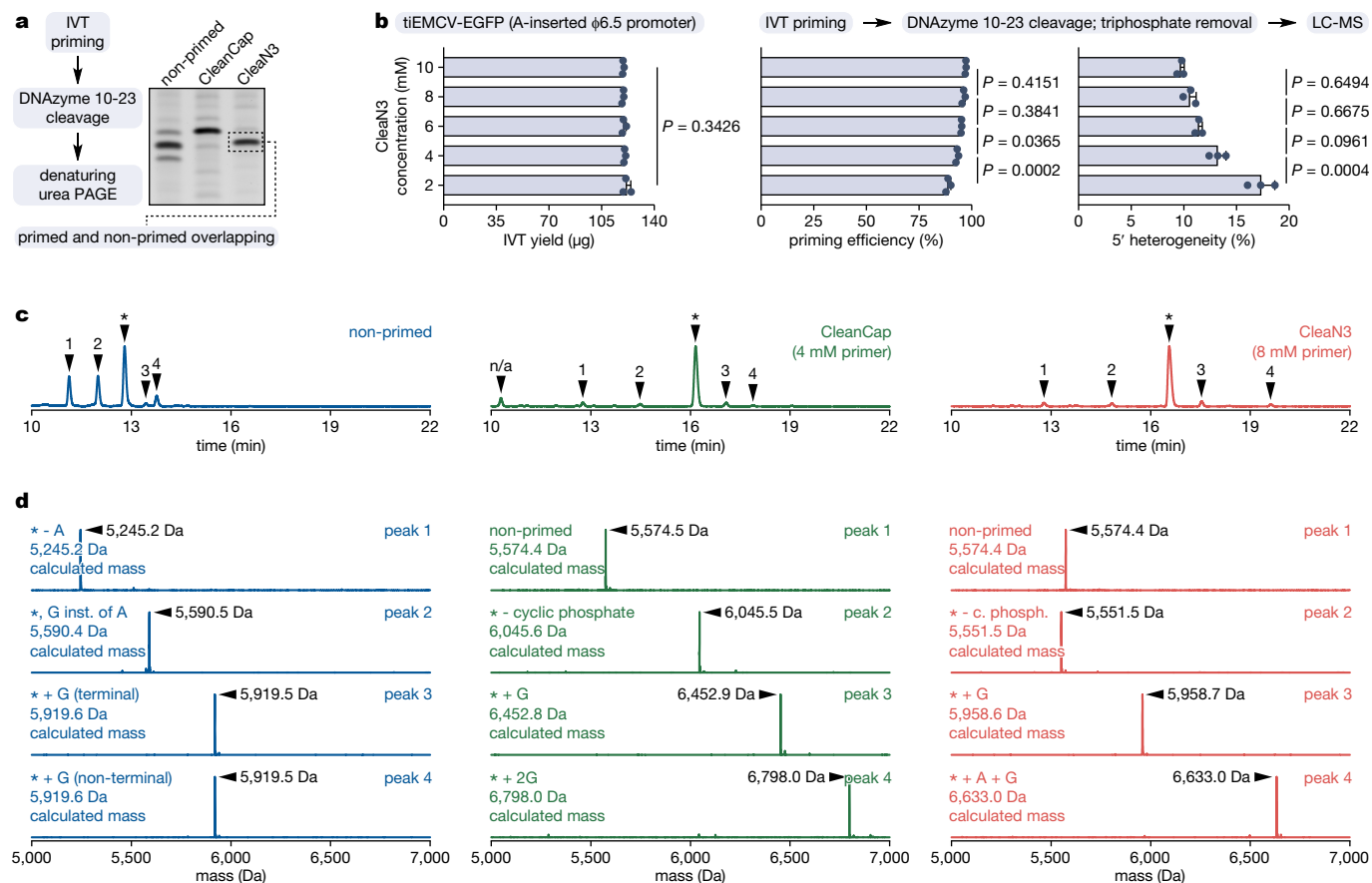
| Kit/device/reagent name | Supplier | Catalogue number | Manufacturer's protocol |
|--|--------------------------|------------------|-------------------------|
| Agilent RNA 6000 Nano Kit | Agilent Technologies | 5067-1511 | link |
| Amicon® Ultra-4 30 kDa MWCO | Merck | UFC803096 | link |
| Amicon® Ultra-4 3 kDa MWCO | | UFC800396 | |
| Monarch® DNA Gel Extraction Kit | New England Biolabs | T1020L | link |
| NEB® 5-alpha Competent E. coli | New England Biolabs | C2987H | link |
| NEB® Stable Competent E. coli (High Efficiency) | New England Biolabs | C3040H | link |
| QIAprep® Spin Miniprep Kit | Qiagen | 27104 | link |
| Plasmid Giga Kit (5) | Qiagen | 12191 | link |
| E.Z.N.A.® Plasmid DNA Mini Kit II | Omega Bio-tek | D6945-01 | link |
| Monarch® RNA Cleanup Kit | New England Biolabs | T2050L | link |
| Neon™ Transfection System 100 µL Kit | Thermo Fisher Scientific | MPK10096 | link |
| Neon™ Transfection System 10 µL Kit | | MPK1096 | |
| Monarch® Total RNA Miniprep Kit | New England Biolabs | T2010S | link |
| CellTiter-Glo® Luminescent Cell Viability Kit | Promega | G7572 | link |
| LEGENDplex™ Hu Anti-Virus Response Panel 1 (13-plex) w/VbP V02 | BioLegend | 741270 | link |

Supplementary figures

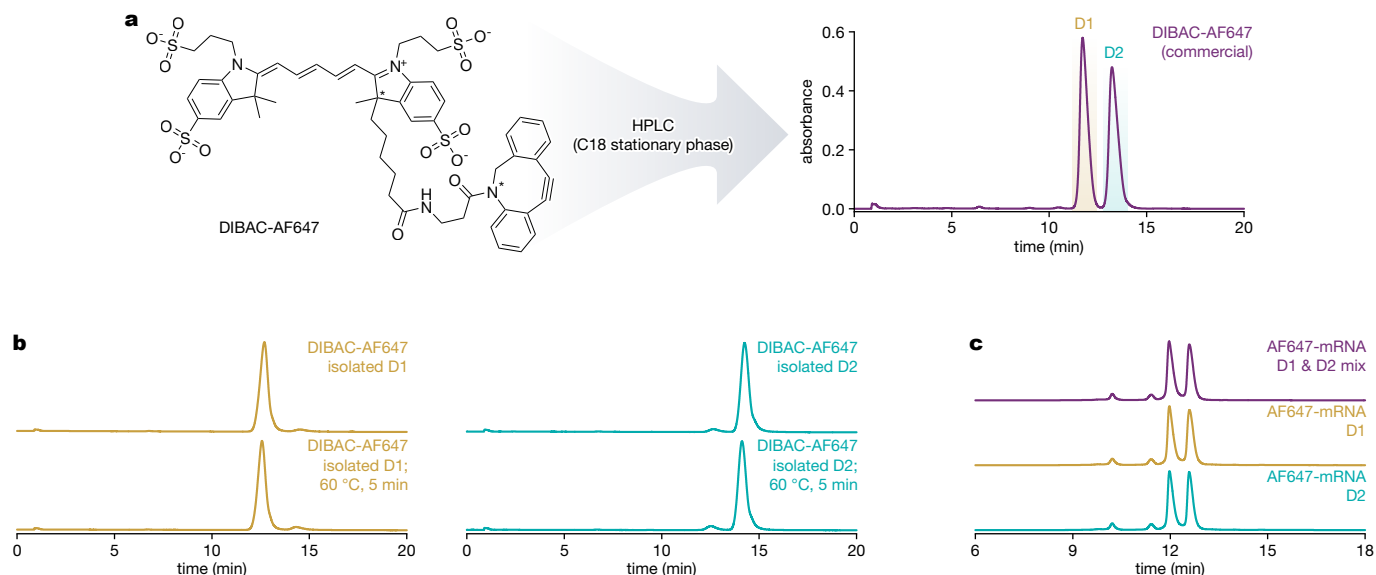


Supplementary Fig. 1 | Preparation of DNA templates and mRNAs. **a**, Preparation of tiEMCV-EGFP, tiHCV-EGFP, tEGFP, and tmCherry (containing A-inserted T7 class III $\phi 6.5$ promoter) DNA templates via restriction digest. **b**, Agarose gel electrophoresis of the plasmids, crude restriction digests, and HPLC-purified DNA templates prepared according to workflow shown in panel **a**. **c**, Long homopolymeric sequences in plasmids can undergo shortening in *E. Coli*. To verify the lengths of the plasmid-encoded poly(A) tails in DNA templates prepared via restriction digests, we performed PmeI digests and analysed the fragments using agarose gel electrophoresis. The results indicated no shortening of the encoded poly(A) tails in the analysed DNA templates. **d**, Preparation of tiEMCV-EGFP (containing T7 class II $\phi 2.5$ or T7 class III $\phi 6.5$ promoter) as well as tiEMCV-Cas9 and tiSyn-EGFP (both containing A-inserted T7 class III $\phi 6.5$ promoter) DNA templates via PCR and restriction digest or tail overhang PCR. **e**, Agarose gel electrophoresis of the plasmids, crude PCR

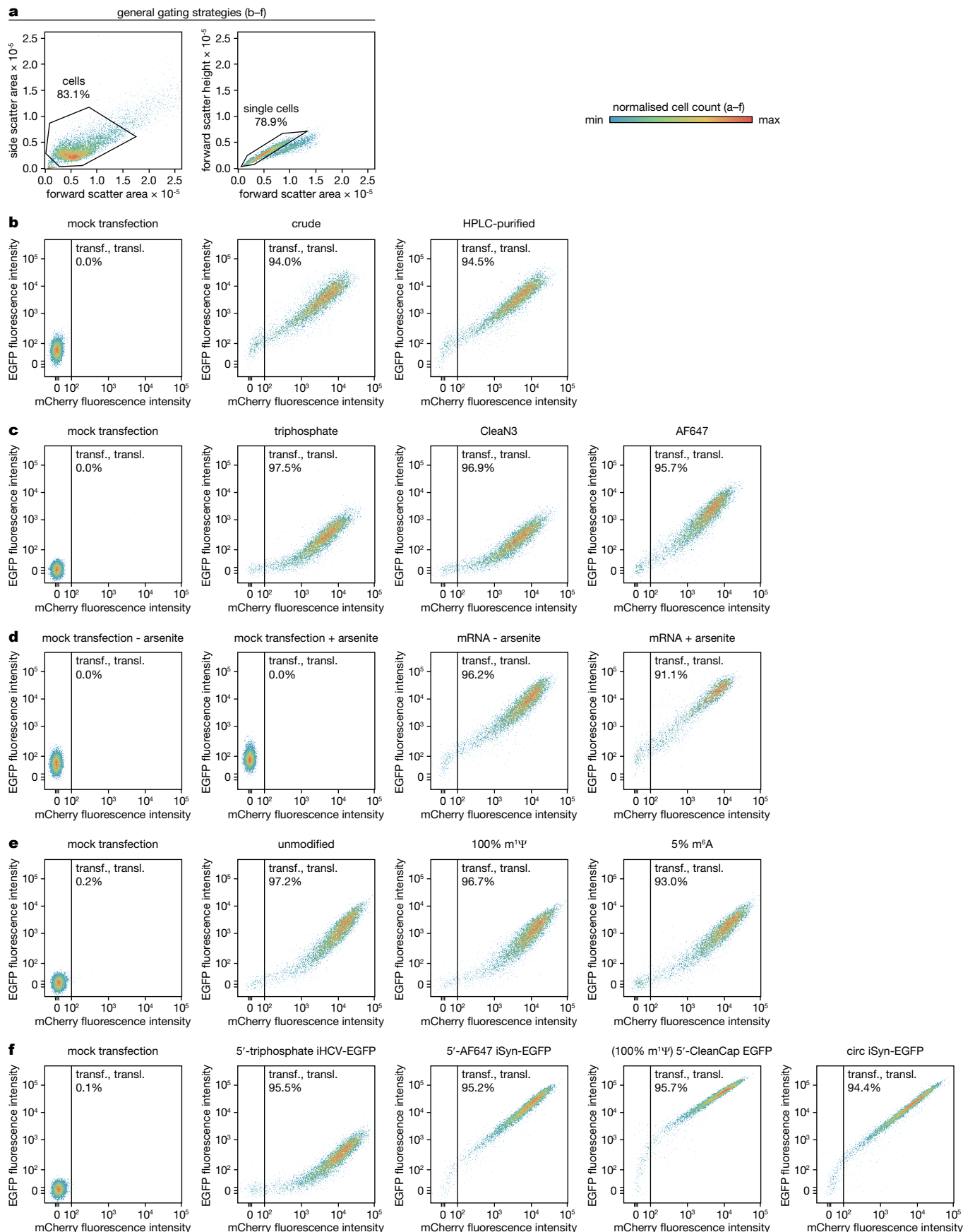
products, crude restriction digests, and HPLC-purified DNA templates prepared according to workflow shown in panel d. **f**, General workflow for preparation of mRNAs. **g**, Pseudo-gel image of 5'-triphosphate, 5'-CleanN3, and 5'-AF647 iHCV-EGFP, 5'-CleanCap EGFP, and 5'-CleanCap mCherry mRNAs. **h**, Pseudo-gel image of 5'-AF647 iHCV-EGFP and 5'-CleanCap EGFP mRNAs containing internal modifications (100% m¹Ψ and 5% m⁶A). **i**, Pseudo-gel image of 5'-AF647 iSyn-EGFP and circ iSyn-EGFP mRNAs. **b,c,e,g,h,i**, Data of one replicate. Source data are provided as a Source Data file.



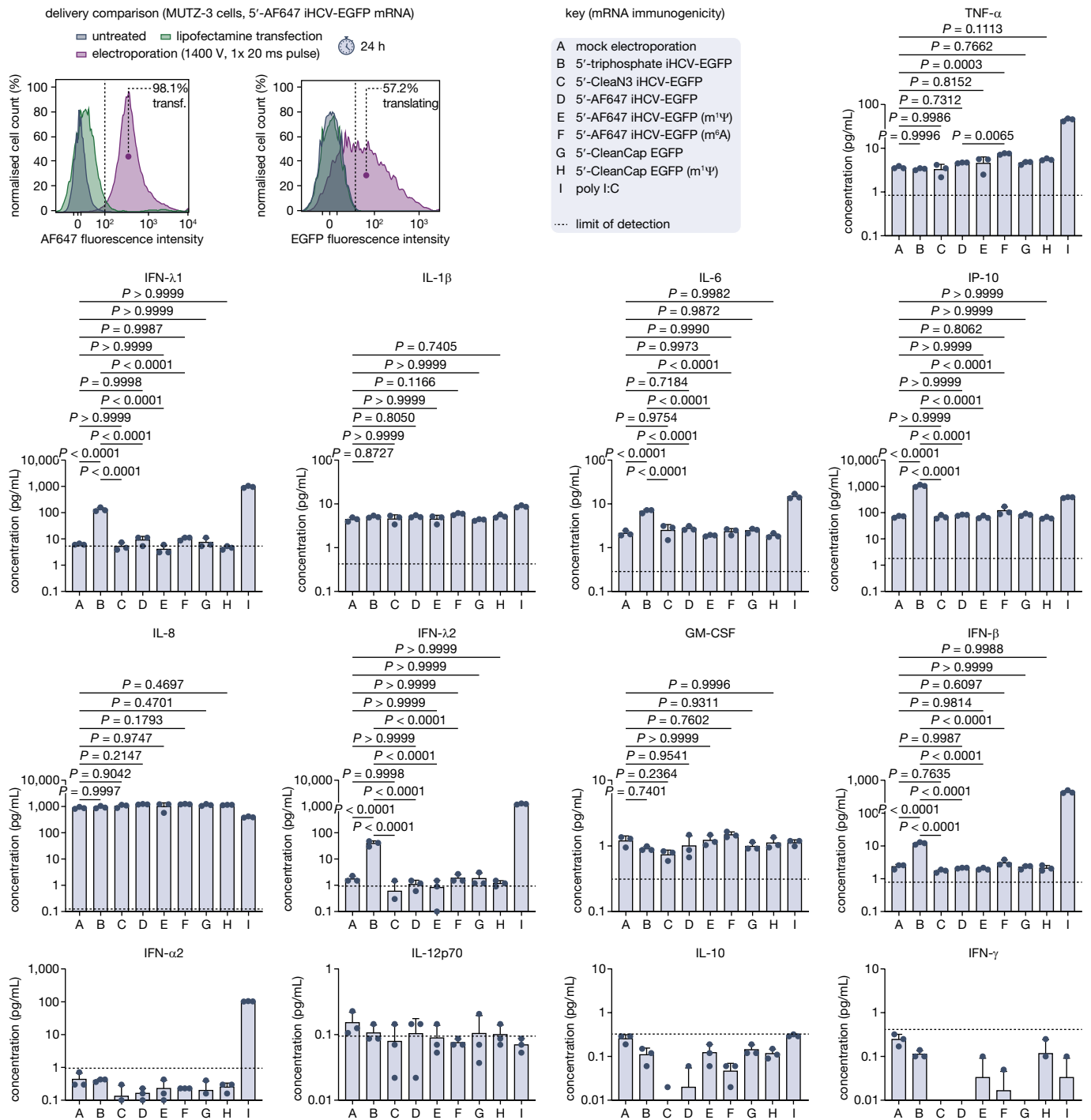
Supplementary Fig. 2 | IVT priming optimisation and transcript fragment analysis. **a**, To assess the priming efficiency and 5'-heterogeneity of transcripts primed with CleanCap and CleanN3, we trimmed the RNAs using DNAzyme 10-23 and analysed the obtained fragments using denaturing (7 M urea) 20% PAGE. For the CleanCap-primed IVT, the primed fragment separated well from its non-primed counterpart; however, for the CleanN3-primed reaction, both the primed and non-primed fragments migrated at the same height. This indicated that the analysis of the CleanN3-primed transcript fragments using denaturing PAGE can be unsuitable for the assessment of priming efficiency and transcript 5'-heterogeneity. **b**, To optimise the CleanN3-mediated IVT priming, we ran IVTs with varying concentrations of CleanN3 (2–10 mM) and analysed the reaction mixtures according to the workflow shown in Fig. 3a. The bar graphs show IVT yields (in amount of produced mRNA), priming efficiencies, and transcript 5'-heterogeneities as individual data points and the means \pm s.d. of three experimental replicates. Statistical significance was calculated using one-way ANOVA followed by Tukey's multiple comparisons test. **c**, Representative HPLC chromatograms (absorbance at 260 nm, traces normalised to maximum) of the fragments of tiEMCV-EGFP (with A-inserted ϕ 6.5 promoter)-templated transcripts primed with CleanN3 or CleanCap and non-primed. The numbers and asterisks mark peaks taken into account to calculate priming efficiencies and transcript 5'-heterogeneities. Mass spectra of the peaks marked with the numbers are shown in panel d. Mass spectra of the fragments of the main products of the *in vitro* transcriptions, marked with asterisks, are shown in Fig. 3b. **d**, Representative mass spectra (deconvolved, normalised to maximum) of fragments of the by-products of *in vitro* transcriptions together with masses calculated for anticipated by-product fragments. The fragments captioned "* - cyclic phosphate" arise from hydrolysis of the 2',3'-cyclic phosphates at the ends of the fragments of the main products of the IVTs after DNAzyme 10-23 cleavage. Thus, in the calculations of the heterogeneities and priming efficiencies in Fig. 3c, we treated these peaks as fragments of the main products of the IVTs. **a,c,d** Representative data from three experimental replicates. Source data are provided as a Source Data file.



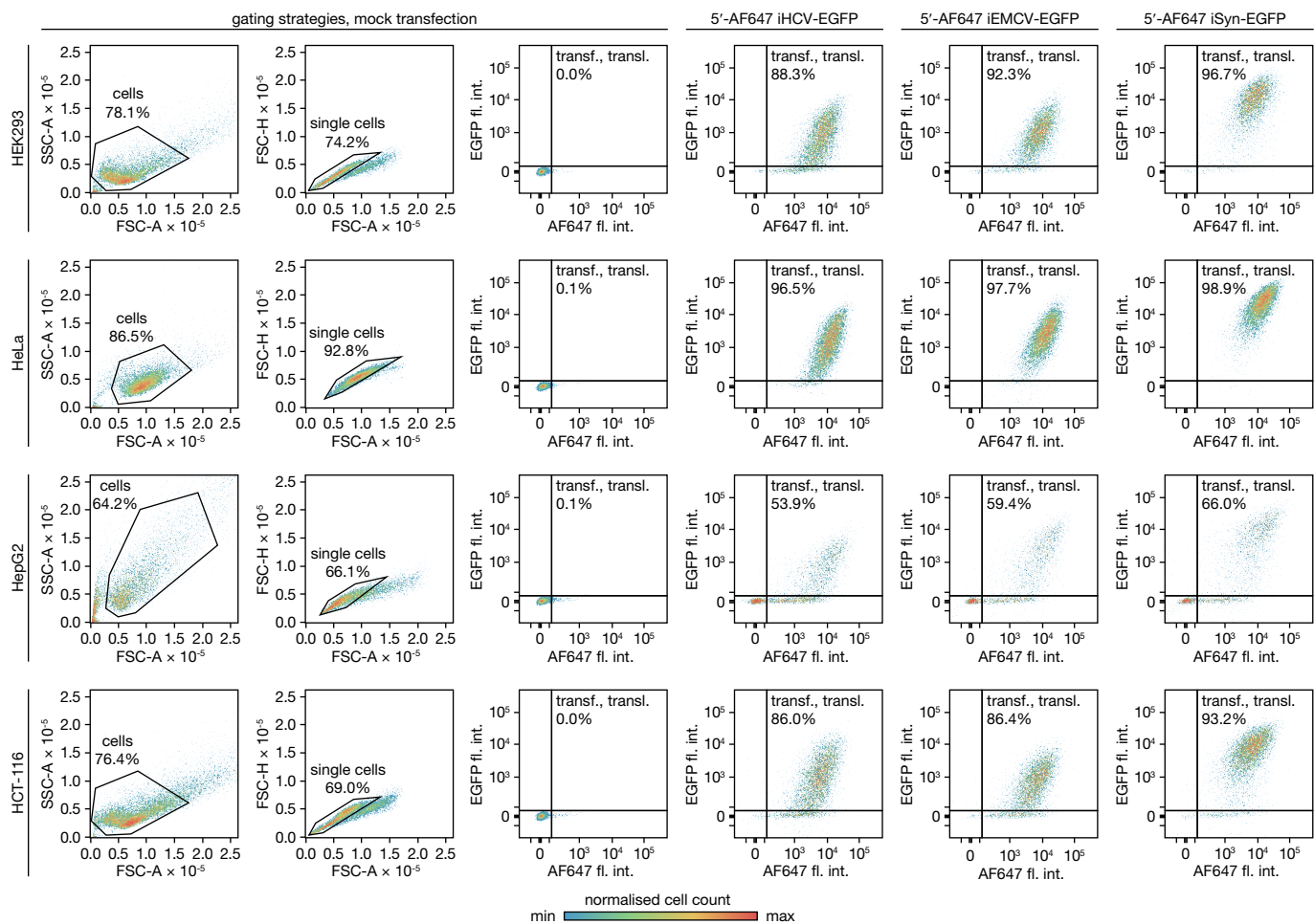
Supplementary Fig. 3 | Influence of DIBAC-AF647 isomerism on the HPLC separation of labelled mRNA. **a**, Commercial DIBAC-AF647 is a mixture of diastereomers that separate on a C18 HPLC stationary phase upon slow isocratic elution. **b**, We separated the two diastereomers of DIBAC-AF647 and confirmed their stability by heating them for 5 min at 60 °C. **c**, We conjugated 5'-Clean3 iEMCV-EGFP mRNA with DIBAC-AF647 in the form of mixture of the two diastereomers (D1 & D2 mix), isolated D1, and isolated D2. Next, we analysed the reaction mixtures using HPLC on a PS-DVB stationary phase. The results showed no influence of the DIBAC-AF647 isomerism on the HPLC separation of the labelled mRNA. **a,b,c**, HPLC chromatograms (absorbance at 260 nm; for b and c, traces normalised to maximum). Experimental details are shown in the HPLC section of the Supplementary Methods. Data of one replicate. Source data are provided as a Source Data file.



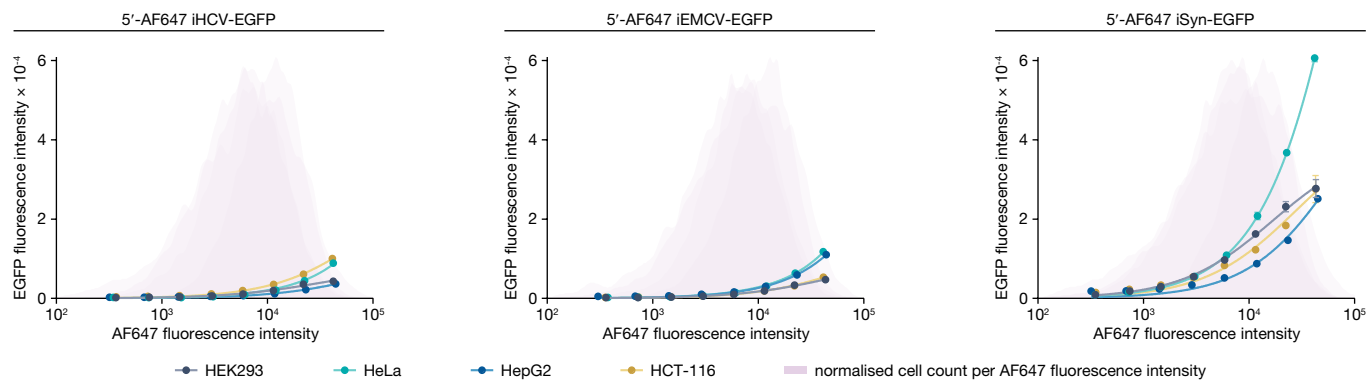
Supplementary Fig. 4 | Representative dot plots and gating strategies used for assessing translational properties of mRNAs. a, General gating strategies applied for analyses involving HEK293 cells. **b**, dot plots and gating strategy for data shown in Fig. 4b. **c**, dot plots and gating strategy for data shown in Fig. 4c. **d**, dot plots and gating strategy for data shown in Fig. 4g. **e**, dot plots and gating strategy for data shown in Fig. 4h. **f**, dot plots and gating strategy for data shown in Fig. 5f. Source data are provided as a Source Data file.



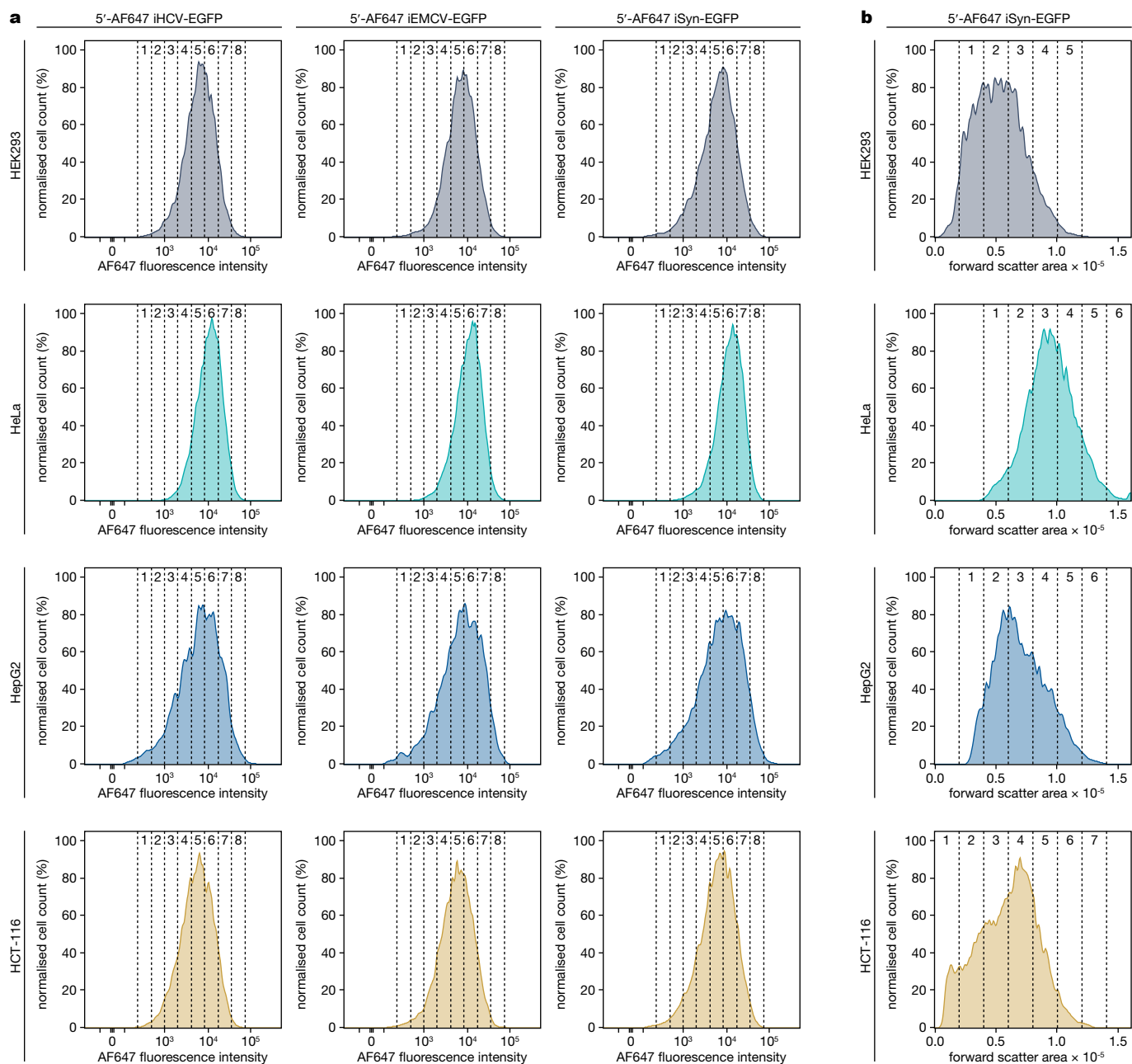
Supplementary Fig. 5 | Immunogenicity of mRNAs in MUTZ-3-derived mature dendritic cells. Top left, influence of mRNA delivery method (lipofectamine transfection and electroporation) on 5'-AF647 iHCV-EGFP mRNA uptake and EGFP expression in MUTZ-3 cells. Flow cytometry data acquired 24 h post-treatment. The histograms are normalised to mode and smoothed. Data of one replicate. Top right and below, concentrations of cytokines secreted to medium by mRNA- or control (1 mM sodium citrate buffer, pH 6.4 – mock or poly I:C)-treated MUTZ-3-derived mature dendritic cells, assessed 24 h post-electroporation. The graphs show individual data points and the means \pm s.d. of three experimental replicates. Statistical significance was calculated using one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



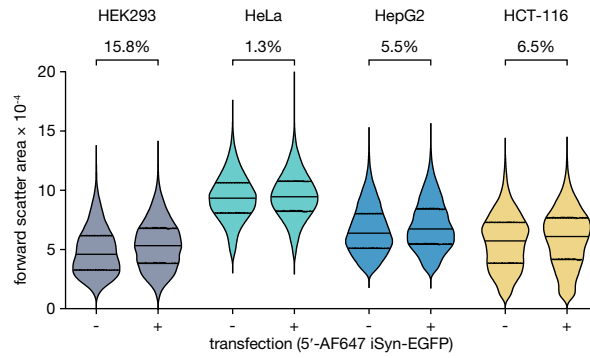
Supplementary Fig. 6 | Representative dot plots and gating strategies used for mRNA productivity studies. Dot plots and gating strategies for data shown in Fig. 5b,c. Source data are provided as a Source Data file.



Supplementary Fig. 7 | mRNA dose – EGFP expression relationships. Dose-response profiles representing the relationships between the mRNA (5'-AF647 iHCV-EGFP, 5'-AF647 iEMCV-EGFP, and 5'-AF647 iSyn-EGFP) dose (AF647 fluorescence intensity) and the EGFP expression (EGFP fluorescence intensity) 24 h post-transfection in HEK293, HeLa, HepG2, and HCT-116 cells binned according to increasing AF647 fluorescence intensity as bin means \pm s.d. of three experimental replicates. Dose-response curves were obtained by fitting the three-parameter dose-response model to the data using the least squares method. AF647 fluorescence intensity binning strategies are shown in Supplementary Fig. 8a. Representative histograms in purple (normalised to mode and smoothed) show the distribution of the collected cellular AF647 fluorescence intensity data. Source data are provided as a Source Data file.

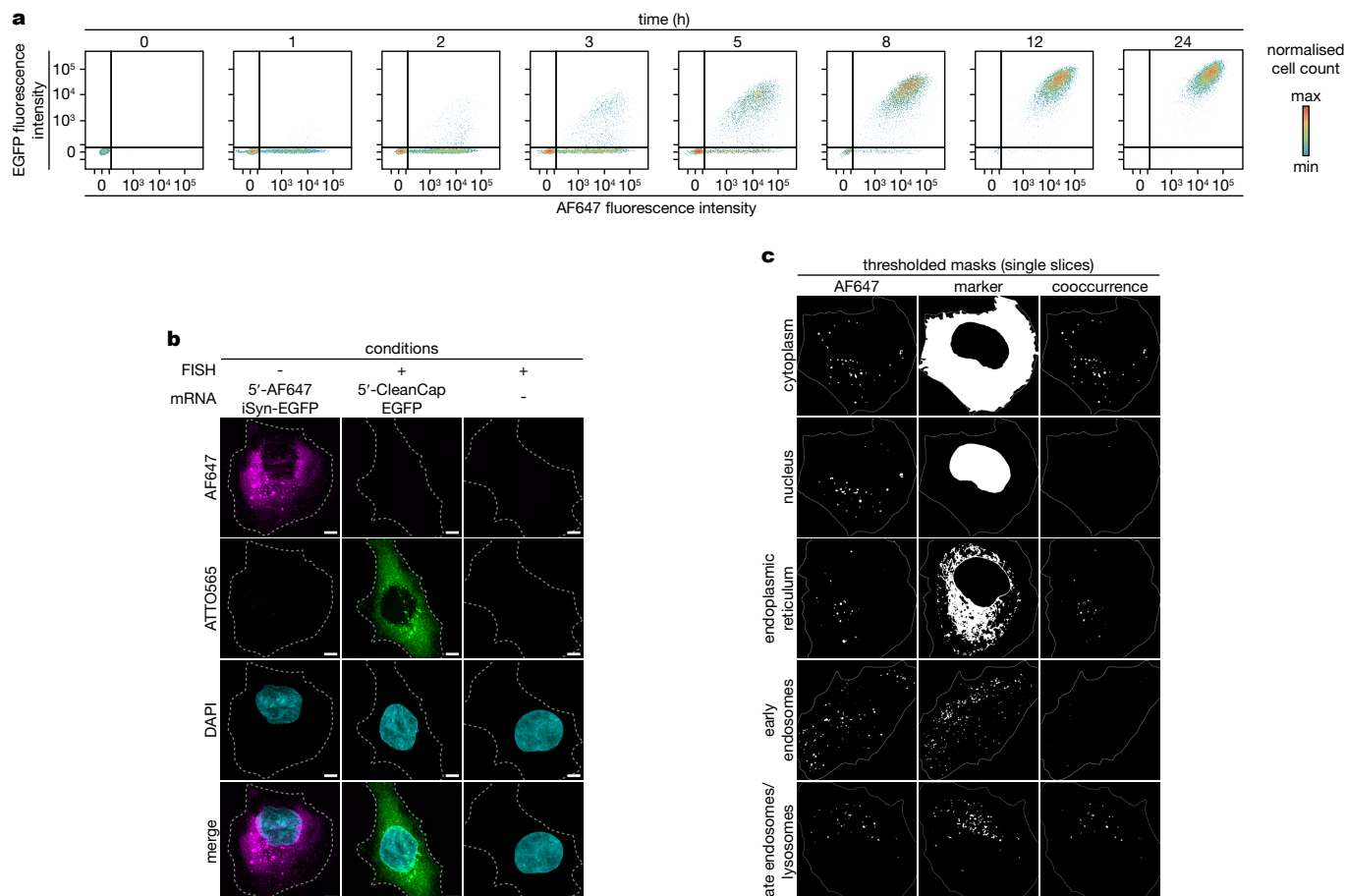


Supplementary Fig. 8 | Binning strategies. **a**, representative histograms and binning strategies for data shown in Supplementary Fig. 7. **b**, representative histograms and binning strategies for data shown in Fig. 5c. **a,b**, The histograms are normalised to mode and smoothed. Source data are provided as a Source Data file.

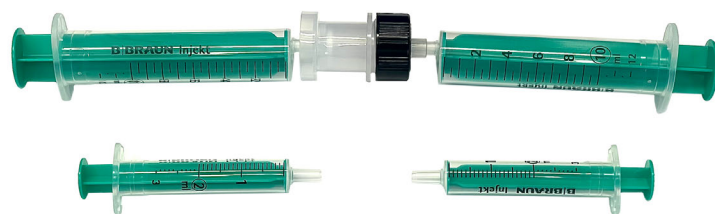


Supplementary Fig. 9 | Cell size comparison for 5'-AF647 iSyn-EGFP mRNA-transfected and non-transfected cells.

To answer whether the mRNA transfection influences the cell size, we compared HEK293, HeLa, HepG2, and HCT-116 forward scatter areas (FSC-A) before and after lipofectamine transfection with 5'-AF647 iSyn-EGFP mRNA. The violin plot of FSC-A of mRNA-transfected and non-transfected cells shows data distribution, median (solid line), and first and third quartile (dashed lines). $n = 29,476$ (HEK293, non-transfected), $n = 18,383$ (HEK293, transfected), $n = 33,601$ (HeLa, non-transfected), $n = 33,873$ (HeLa, transfected), $n = 18,930$ (HepG2, non-transfected), $n = 14,935$ (HepG2, transfected), $n = 32,728$ (HCT-116, non-transfected), and $n = 26,277$ (HCT-116, transfected) pooled single cells from three experimental replicates. Gating strategies are shown in Supplementary Fig. 6. Source data are provided as a Source Data file.



Supplementary Fig. 10 | Assessment of mRNA uptake, cellular localisation and expression. a, Representative dot plots for data shown in Fig. 6b. General gating strategy is shown in Supplementary Fig. 6. **b**, Assessment of the specificity of the FISH probes used in experiment shown in Fig. 6h. Cell segmentation based on the edge of the EGFP fluorescence or cell contours in the DIC channel. Maximum intensity projections from slices spanning the whole cell volumes. Scale bars, 5 μ m. **c**, Representative thresholded masks used for calculating Mander's overlap coefficients shown in Fig. 6i. Source data are provided as a Source Data file.



Supplementary Fig. 11 | Experimental setup for the manual synthesis on a solid support.

Nucleic acid sequences

All sequences are listed in the 5'→3' direction. 'p' denotes a phosphate. 'm⁷G' denotes *N*⁷-methylguanosine. m^{2'}0A denotes *O*^{2'}-methyladenosine. All nucleoside monomers, if not stated otherwise, are linked through phosphodiester bonds.

PCR primer sequences

IRES_GA_F

TGTACGACGATGACGATAAGCTGGAGGCCAAGGTGTGAT

IRES_GA_R

ACCATGGTGGCGAATTCTTTGCCAAGGTTGTGGCCATATTATCATCGTG

EGFP_GA_F

ACACGATGATAATATGGCCACAACCTTGGCAAAGAATTCGCCACC

EGFP_GA_R

CCGGATCAAGCTTCGAATTTTACTTGTACAGCTCGTCCATGC

mCherry_F

GCGGGCCCGGGATCCACCGGTCGCCACCATGGTGAGCAAGGG

mCherry_R

GAAATTGGACAGCAAGAAAGCGAGCTGCGGCCGCTTTACTTGTACAG

pmRNA-EGFP-A120-v1_F

CGATGATAATATGACTTCGAAAGTTTATGATCCAGAACAAATGGTGAGCAAGGGCGAGGAG

pmRNA-EGFP-A120-v1_R

ATTCGATATCAAGCTTATCGAGGCTAGCTCTAGAACGCGTGATATCT

iEMCV_F

GAGCTAGCCTCGATAAGCTTGATATCGAATTCGCCCCC

iEMCV_R

ATCATAACTTTCGAAGTCATATTATCATCGTCATATTATCATCGTGTTTTTCAAAGGAAAACCACGTCC
CCGT

pmRNA-EGFP-A120-v2_F

ATGGTGAGCAAGGGCGAG

pmRNA-EGFP-A120-v2_R

GCTAGCTCTAGAACGCGTGATATCT

iHCV_F

TATAAGGAGAGATATCACGCGTTCTAGAGCTAGCTAGTGACGTAGCCAGCCC

iHCV_R

GGTGAACAGCTCCTCGCCCTTGCTCACCATGGTTTTTCTTTGAGGTTTAGGATTCGTG

GCATATATATAACATATACTGTGATCATGGTGAGCAAGGGCGAGGAG

CACCGAGGCTCCAGCCAATTGTTATTACTTGTACAGCTCGTCCATGCC

ACTTAATACGACTCACTATAGGAGAGATATCACGCGTTCTAG

ACTTAATACGACTCACTATTAGGAGAGATATCACGCGTTCTAG

GTGTTGTTCCAGTTTGGAAACAAGAGTCC

GACTTAATACGACTCACTATAAGGAGAGATATCACGCGTCACAAATACCACTGAGATCCG

[illegible]

GACTTAATACGACTCACTATAAGGCGTCATATTCGGCG

[illegible]

AAAAAAAAAAAAAAAAAAAAAAAAAGGCCAGTGAATTGTAATACGACTCACTATAGGG

TTTTTTTTTTTTTTTTTTTTTTTTTTTCAAAAACCCCTCAAGACCCGTTTAGAGGC

AGATCCGCCACAACATCGAG

AACTCCAGCAGGACCATGTG

TCAAGGCTGAGAACGGGAAG

CGCCCCACTTGATTTTGGAG

Dz 10-23 1

GCTAGCTCTAGAAGGCTAGCTACAACGAGCGTGATATCTCTCC

Dz_10-23_2

CAGTGGTATTTGTGAGGCTAGCTACAACGAGCGTGATATCTCTCC

Dz_10-23_3

GATCCGAGCTCGGTAGGCTAGCTACAACGACAAGCTTGGGTCTCC

FISH probe sequences

X = ATTO 565

FISH_EGFP_1

XTGAACTTGTGGCCGTTTACG

FISH_EGFP_2

XTGGCGGACTTGAAGAAGTCG

FISH_EGFP_3

XTTGAAGTCGATGCCCTTCAG

FISH_EGFP_4

XTGCTCAGGTAGTGGTTGTCTG

Other oligonucleotide sequences

A_ins_φ6.5-1

pCACCTGCAACATGACTTAATACGACTCACTATAAGGAGAGATATCA

A_ins_φ6.5-2

pCGCGTGATATCTCTCCTTATAGTGAGTCGTATTAAGTCATGTTGCAGGTG

A120-AarI-1

pAAACAA
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GGTACGTTTCGTGATAAACAAACGGCC

A120-AarI-2

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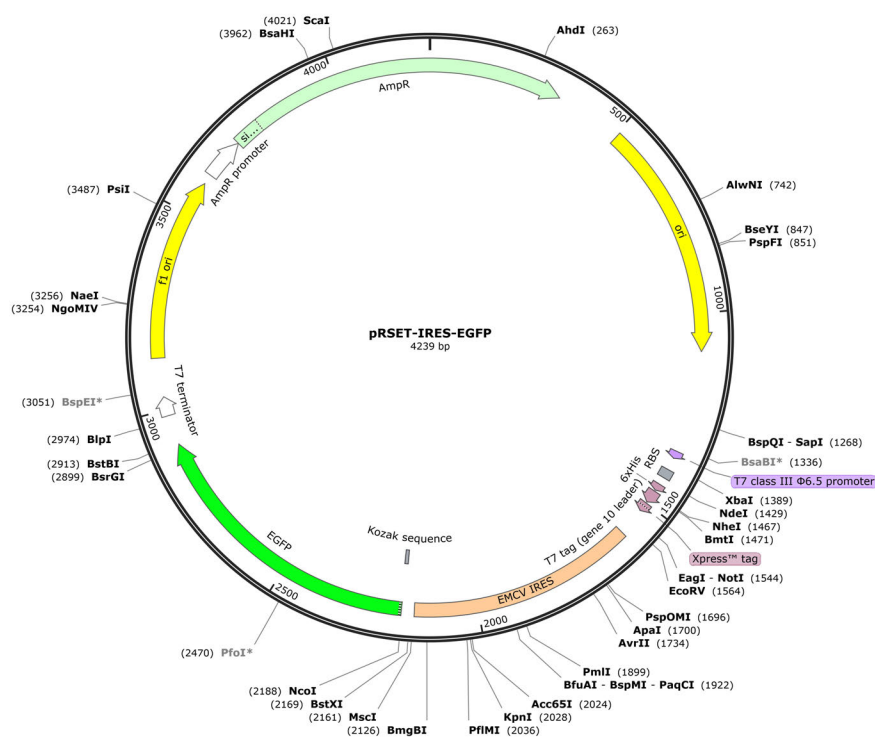
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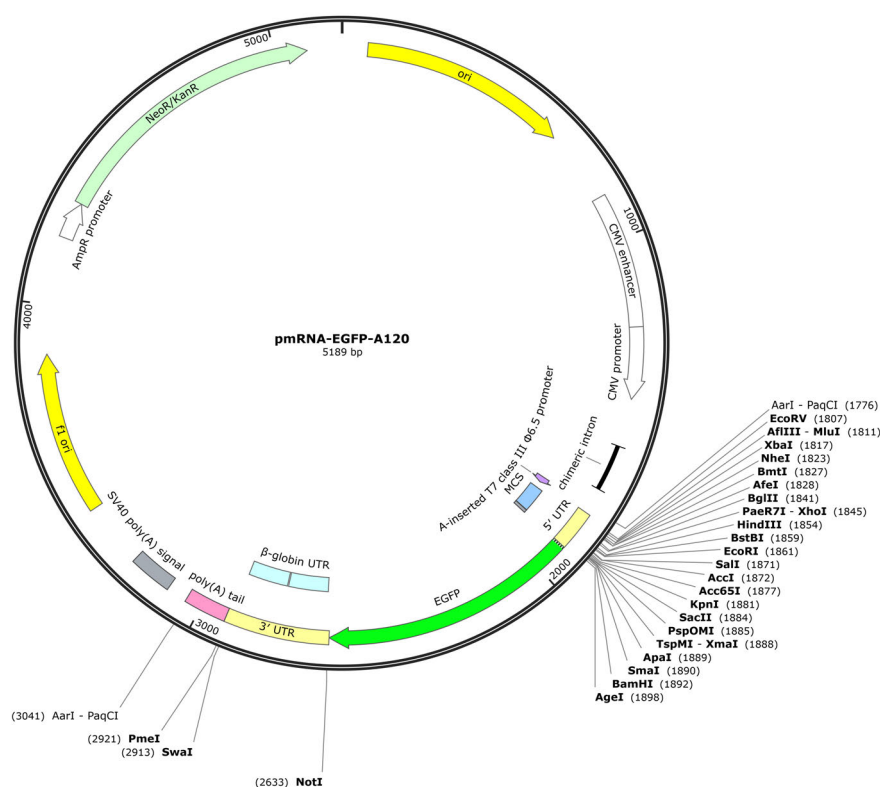
Plasmid sequences

pRSET-IRES-EGFP



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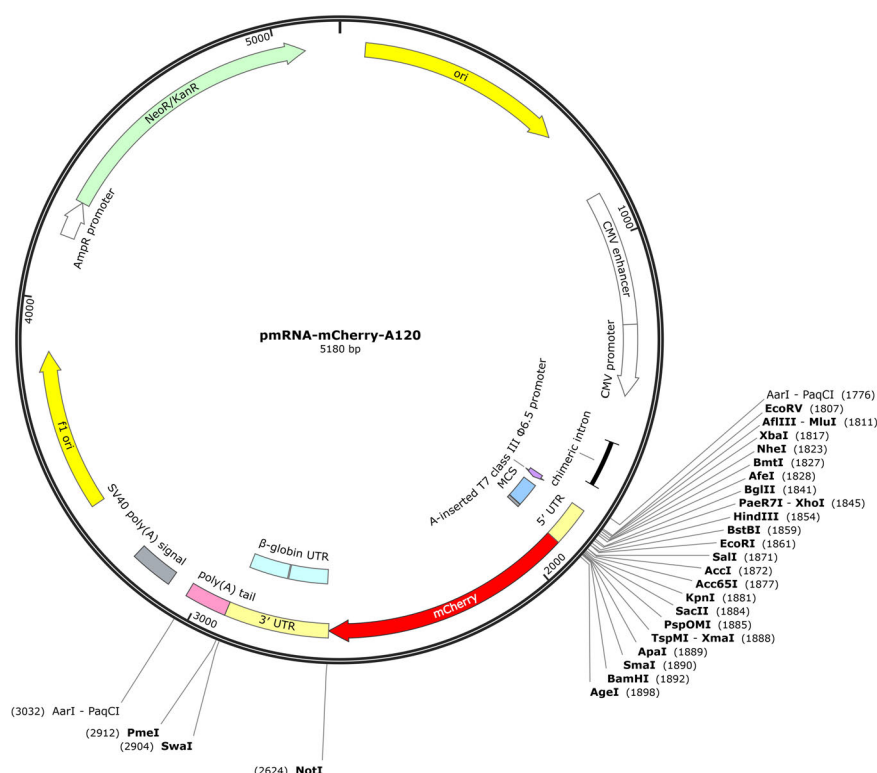


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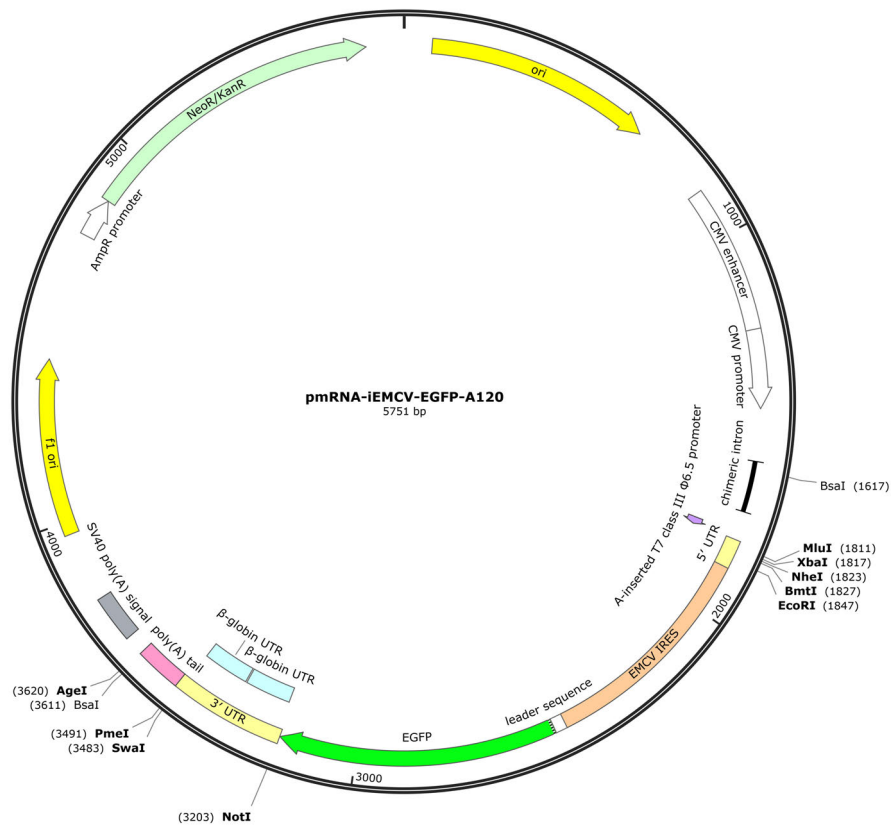


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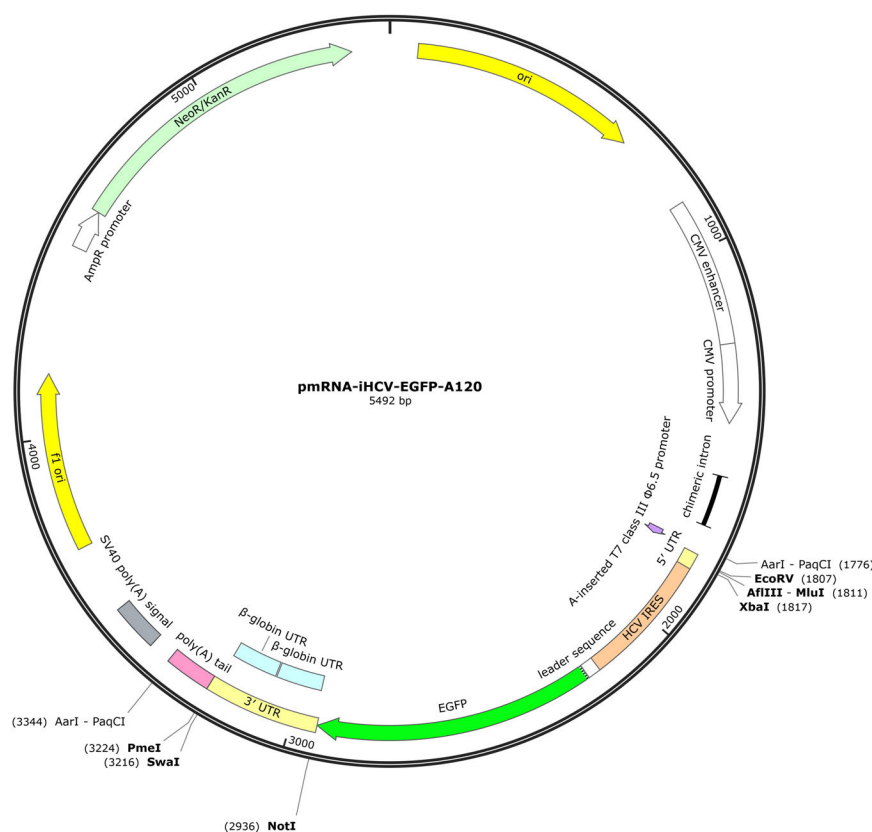


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AA

tiEMCV-EGFP (ϕ 6.5), tiEMCV-EGFP (ϕ 2.5)

ϕ 6.5: X = -; ϕ 2.5: X = T

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tiEMCV-Cas9

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tEGFP

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tmCherry

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tiSyn-EGFP

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mRNA sequences

Cluc-control

5'-triphosphate: X = pppAG; 5'-CleanN3: X = N₃ (m^{2'}°A) G; 5'-CleanCap: X = m⁷Gppp (m^{2'}°A) G

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GGGUGGAGACGAGCGAGCCUCACACGUGCUGCUUGACUACAGGGAGACGUGCGCUGCUCGCCGAAACUAGA
GGAACCUGCGUUUUGUCUGGACAUAUCUUCUACGAUACAUAUUGACAAAGCAAGAUACCAAUUCCAGGGUC
CCUGCAAGGAGAUUCUUAUGGGCCGCCGACUGUUUCUGGAACACUUGGGAUGUGAAGGUUUCACACAGGAA
UGUUGACUCUUAACACUGAAGUAGAGAAAGUACGAAUCAGGAAACAAUCGACUGUAGUAGAACUCAUUGUU
GAUGGAAAACAGAUUCUGGUUGGAGGAGAAGCCGUGUCCGUCCCGUACAGCUCUCAGAACACUCCAUCU
ACUGGCAAGAUGGUGACAUAUCUGACUACAGCCAUCCUACCUGAAGCUCUGGUGGUCAAGUUCAACUUCAA
GCAACUGCUCGUCGUACAUAUUAAGAGAUCCAUUCGAUGGUAAGACUUGCGGUAUUAUGCGGUAACUACAAC
CAGGAUUUCAGUGAUGAUUCUUUUGAUGCUGAAGGAGCCUGUGAUCUGACCCCCAACCCACCGGGAUGCA
CCGAAGAACAGAAACCUGAAGCUGAACGACUCUGCAAUAGUCUCUUCGCCGGUCAAGUGAUCUUGAUCA
GAAAUGUAACGUGUGCCACAAGCCUGACCGUGUCGAACGAUGCAUGUACGAGUAUUGCCUGAGGGGACAA
CAGGGUUUCUGUGACCACGCAUGGGAGUUCAAGAAAGAAUGCUACAUAAGCAUGGAGACACCCUAGAAG
UACCAGAUGAAUGCAAAUAGGCGGCCGCAAUAAAAUAUCUUUAUUUUAUUAACAUCUGUGUGUUGGUUUU
UUGUGUGUCUAG

iEMCV-EGFP

5'-triphosphate: X = pppAG; 5'-CleanN3: X = N₃ (m^{2'}°A) G; 5'-AF647: X = AF647 (m^{2'}°A) G;
5'-CleanCap: X = m⁷Gppp (m^{2'}°A) G

XGAGAGAUUAUCACGCGUUCUAGAGCUAGCCUCGAUAAGCUUGAUUAUCGAAUUCGCCCCCCCCCCCCUCUC
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AUUUUCCACCAUAUUGCCGUCUUUUGGCAAUGUGAGGGCCCGGAAACCUGGCCCGUCUUCUUGACGAGC
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CUCUGGAAGCUUCUUGAAGACAAACAACGUCUGUAGCGACCCUUUGCAGGCAGCGGAACCCCCCACCUGG
CGACAGGUGCCUCUGCGGCCAAAAGCCACGUGUAUAAGAUACACCUGCAAAGGCGGCACAACCCCAGUGC
CACGUUGUGAGUUGGAUAGUUGUGGAAAGAGUCAAAUGGCUCUCCUCAAGCGUAUUAACAAGGGGCUGA
AGGAUGCCCAGAAGGUACCCCAUUGUAUGGGAUCUGAUCUGGGGCCUCGGUGCACAUGCUUUACAUGUGU
UUAGUCGAGGUUAAAAAACGUCUAGGCCCCCCGAACCACGGGGACGUGGUUUUCCUUUGAAAAACACGA
UGAUAAUAUGACGAUGAUAAUAUGACUUCGAAAGUUUAUGAUCCAGAACAAAUGGUGAGCAAGGGCGAGG
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GUCCGGCGAGGGCGAGGGCGAUGCCACCUACGGCAAGCUGACCCUGAAGUUAUCUGCACCACCGGCAAG
CUGCCCCGUGCCCUGGCCACCCUCGUGACCACCCUGACCUACGGCGUGCAGUGCUUCAGCCGCUACCCCG

ACCACAUGAAGCAGCACGACUUCUUAAGUCCGCCAUGCCCCGAAGGCUACGUCCAGGAGCGCACCAUCUU
 CUUCAAGGACGACGGCAACUACAAGACCCGCGCCGAGGUGAAGUUCGAGGGCGACACCCUGGUGAACCGC
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 CUAACUGGGGGGAUAUUAUGAAGGGCCUUGAGCAUUGGAUUCUGCCUAAUAAAAACA UUUUUUUCAU
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 AACUGGGGGGAUAUUAUGAAGGGCCUUGAGCAUUGGAUUCUGCCUAAUAAAAACA UUUUUUUCAUUGC
 AUUUAAAUGUUUAAACAAA
 AA

iEMCV-Cas9

5'-triphosphate: X = pppAG; 5'-CleaN3: X = N₃ (m²'^oA) G; 5'-CleanCap: X = m⁷Gppp (m²'^oA) G

XGAGAGAUUACACGCGUCACAAUACCACUGAGAUCCGCCCCUCUCCUCCCCCCCCCUAACGUUACUG
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 CAACGUCUGUAGCGACCCUUGCAGGCAGCGGAACCCCCACCUGGCGACAGGUGCCUCUGCGGCCAAAA
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 CGCAGAUUAUACCCGCGAGAAAGAAUCGGAUCUGCUACCUGCAGGAGAUCUUUAGUAAUGAGAUGGCUAAGG
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 AAGAAGCUUGUAGACAGUACUGAUAAAGGCUGACUUGCGGUUGAUCUAUCUCGCGCUGGCGCAUAUGAUA
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 UCCUCACA UUCGGAUACCCUACUAUGUAGGCCCCCUCGCCCCGGGGAAA UCCAGAUUCGCGUGGAUGAC
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UACUGGUUGUGGCCAAAGUGGAGAAAGGGAAGUCUAAAAACUCAAAAGCGUCAAGGAACUGCUGGGCAU
CACAAUCAUGGAGCGAUCAAGCUUCGAAAAAAACCCCAUCGACUUCUUCGAGGCGAAAGGAUAUAAAGAG
GUCAAAAAAGACCUCAUCAUUAAGCUUCCCAAGUACUCUCUCUUGAGCUUGAAAACGGCCGGAAACGAA
UGCUCGCUAGUGCGGGCGAGCUGCAGAAAGGUAACGAGCUGGCACUGCCUCUAAAUACGUUAAUUUCU
GUAUCUGGCCAGCCACUAUGAAAAGCUCAAAGGGUCUCCCGAAGAUAAUGAGCAGAAGCAGCUGUUCGUG
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UUUUGUGUCUCUCACUCGGAAGGACAUAUGGGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA
AAAAAAAAAAAA

iHCV-EGFP

5'-triphosphate: X = pppAG; 5'-CleaN3: X = N₃ (m^{2'}°A) G; 5'-AF647: X = AF647 (m^{2'}°A) G

XGAGAGAUUAUCACGCGUUCUAGAGCUAGCUAGUGUACGUAGCCAGCCCCGAUUGGGGGCGACACUCCAC
CAUAGAUAUCUCCCCUGUGAGGAACUACUGUCUUCACGCAGAAAGCGUCUAGCCAUGGCGUUAGUAUGAG
AGUCGUGCAGCCUCCAGGACCCCCCUCCGGGAGAGCCAUAGUGGUCUGCGGAACCGGUGAGUACACCG
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GCAAGACUGCUAGCCGAGUAGUGUUGGGUCGCGAAAGGCCUUGUGGUACUGCCUGAUAGGGUGCUUGCGA
GUGCCCCGGGAGGUCUCGUAGACCGUGCACC AUGAGCACGAAUCCUAAACCUCAAAGAAAAACCAUGGUG
AGCAAGGGCGAGGAGCUGUUCACCGGGGUGGUGCCCAUCCUGGUCGAGCUGGACGGCGACGUAACCGGCC
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CACCACCGGCAAGCUGCCCCGUGCCCUGGCCACCCUCGUGACCACCCUGACCUACGGCGUGCAGUGCUUC
AGCCGCUACCCCGACCACAUGAAGCAGCACGACUUCUUAAGUCCGCCAUGCCC GAAGGCUACGUCCAGG
AGCGCACCAUCUUCUUAAGGACGACGGCAACUACAAGACCCGCGCCGAGGUGAAGUUCGAGGGCGACAC
CCUGGUGAACC GCAUCGAGCUGAAGGGCAUCGACUUAAGGAGGACGGCAACAUCUGGGGGCACAAGCUG
GAGUACAACUACAACAGCCACAACGUCUAUAUCAUGGCCGACAAGCAGAAGAACGGCAUCAAGGUGAACU
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CGGCGACGGCCCCGUGCUGCUGCCC GACAACCACUACCUGAGCACCCAGUCCGCCCGUGAGCAAAGACCCC
AACGAGAAGCGCGAUCACAUGGUCCUGCUGGAGUUCGUGACCGCCGCCGGGAUCACUCUCGGCAUGGACG
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CAUUUAUUUUAUUGCGUUUAGCUCGCUUUCUUGCUGUCCAAUUCUAUUAAGGUUCCUUUGUUCUUA
AGUCCAACUACUAAACUGGGGGAUUAUGAAGGGCCUUGAGCAUUUGGAUUCUGCCUAAUAAAAAAACAU
UUUUUUUAUUGCAUUUAAAGUUUAAACAAA
AA
AAAAAAAAAA

EGFP

m⁷Gppp (m^{2'}°A) GGAGAGAUUAUCACGCGUUCUAGAGCUAGCGCUACCGGACUCAGAUUCGAGCUC AAGCU
UCGAAUUCUGCAGUCGACGGUACCGCGGGGCCGGGAUCCACCGGUCGCCACCAUGGUGAGCAAGGGCGAG
GAGCUGUUCACCGGGGUGGUGCCCAUCCUGGUCGAGCUGGACGGCGACGUAACCGGCCACAAGUUCAGCG
UGUCCGGCGAGGGCGAGGGCGAUGCCACCUACGGCAAGCUGACCCUGAAGUUCAUCUGCACCACCGGCAA
GCUGCCCGUGCCCUGGCCACCCUCGUGACCACCCUGACCUACGGCGUGCAGUGCUUCAGCCGCUACCCC
GACCACAUGAAGCAGCACGACUUCUUAAGUCCGCCAUGCCC GAAGGCUACGUCCAGGAGCGCACCAUCU
UCUUAAGGACGACGGCAACUACAAGACCCGCGCCGAGGUGAAGUUCGAGGGCGACACCCUGGUGAACCG
CAUCGAGCUGAAGGGCAUCGACUUAAGGAGGACGGCAACAUCUGGGGGCACAAGCUGGAGUACAACUAC
AACAGCCACAACGUCUAUAUCAUGGCCGACAAGCAGAAGAACGGCAUCAAGGUGAACUUAAGAUC CGCC
ACAACAUCGAGGACGGCAGCGUGCAGCUCGCCGACCACUACCAGCAGAACACCCCCAUCGGCGACGGCCC
CGUGCUGCUGCCCGACAACCACUACCUGAGCACCCAGUCCGCCCGUGAGCAAAGACCCCAACGAGAAGCGC
GAUCACAUGGUCCUGCUGGAGUUCGUGACCGCCGCCGGGAUCACUCUCGGCAUGGACGAGCUGUACAAGU
AAAGCGGCCGAGCUCGCUUUCUUGCUGUCCAAUUCUAUUAAGGUUCCUUUGUUCUUAAGUCCAACU
ACUAAACUGGGGGAUUAUGAAGGGCCUUGAGCAUUUGGAUUCUGCCUAAUAAAAAAACAUUUUUUUUA
UUGCGUUUAGCUCGCUUUCUUGCUGUCCAAUUCUAUUAAGGUUCCUUUGUUCUUAAGUCCAACUACU
AAACUGGGGGAUUAUGAAGGGCCUUGAGCAUUUGGAUUCUGCCUAAUAAAAAAACAUUUUUUUUAUUG
CAUUUAAAGUUUUAAACAAA
AA

mCherry

m⁷Gppp (m²'^oA) GGAGAGAUAUACACGCGUUCUAGAGCUAGCGCUACCGGACUCAGAUCUCGAGCUCAAGCU
UCGAAUUCUGCAGUCGACGGUACCGCGGGCCCGGGAUCCACCGGUCGCCACCAUGGUGAGCAAGGGCGAG
GAGGAUAACAUGGCCAUCAUCAAGGAGUUAUGCGCUUCAAGGUGCACAUGGAGGGCUCGUGAACGGCC
ACGAGUUCGAGAUCGAGGGCGAGGGCGAGGGCCGCCCCUACGAGGGCACCCAGACCGCCAAGCUGAAGGU
GACCAAGGGUGGCCCCCUGCCCUUCGCCUGGGACAUCUGUCCCCUCAGUUAUGUACGGCUCCAAGGCC
UACGUGAAGCACCCCGCCGACAUCCCCGACUACUUGAAGCUGUCCUUCUCCCCGAGGGCUUCAAGUGGGAGC
GCGUGAUGAACUUCGAGGACGGCGGGCUGGUGACCGUGACCCAGGACUCCUCCCUGCAGGACGGCGAGUU
CAUCUACAAGGUGAAGCUGCGCGGCACCAACUCCCCUCCGACGGCCCCGUAUUGCAGAAGAAGACCAUG
GGCUGGGAGGCCUCCUCCGAGCGGAUGUACCCCGAGGACGGCGCCCUGAAGGGCGAGAUAAGCAGAGGC
UGAAGCUGAAGGACGGCGGCCACUACGACGCUGAGGUCAAGACCACCUACAAGGCCAAGAAGCCCGUGCA
GCUGCCCGGCGCCUACAACGUCAACAUCAAGUUGGACAUCACCUCCACAACGAGGACUACACCAUCGUG
GAACAGUACGAACGCGCCGAGGGCCGCCACUCCACCGGCGGCAUGGACGAGCUGUACAAGUAAAGCGGCC
GCAGCUCGCUUUCUUGCUGUCCAAUUAUUAUAAAGGUUCCUUGUUCUCCUAAGUCCAACUACUAAACUG
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GCUCGCUUUCUUGCUGUCCAAUUAUUAUAAAGGUUCCUUGUUCUCCUAAGUCCAACUACUAAACUGGGG
GAUAUUAUGAAGGGCCUUGAGCAUUUGGAUUCUGCCUAAUAAAAACAUUUAUUUUCAUUGCAUUUAAAU
GUUUAAACAA
AAA

iSyn-EGFP

AF647 (m²'^oA) GGCGUCAUAUUCGGCGACCAUUUGUGUGGUAAAAAAAAAAAAACCAAAAAAAAAAAACAA
AAAAAAAAAAUAAUUGACUAAGAUAUCUUAACAGCGGAUGGGUACCCACCAUCCGACCCACUGGGUG
UAGUACUCUGGUACUUCGUACCUUUGUACGCCUGUUCUCCCAUUGUACCCUCCUGAACUUCACCCCA
AGUAAACGUUAGAAGCUCACAUUUAGUACAACAGGAAGCACCACAUCAGUGGUGUUUAGUACAAGCACU
UCUGUUUCCCCGGAGCGAGGUUAAGGCUGUACCCACUGCCAAAACCUUUAACCGUUAUCCGCCAACCAA
CUACGUAAAAGCUAGUAGUAUUAUGUUUUUAACUAGGCGUUCGAUCAGGUGGAUUUCCCCUCCACUAGUU
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CAAGUUCAGCGUGUCCGGCGAGGGCGAGGGCGAUGCCACCUACGGCAAGCUGACCCUGAAGUUCAUCUGC
ACCACCGGCAAGCUGCCCCGUGCCCUGGCCACCCUCGUGACCACCCUGACCUACGGCGUGCAGUGCUUCA
GCCGCUACCCCGACCACAUGAAGCAGCACGACUUCUUAAGUCCGCCAUGCCCGAAGGCUACGUCCAGGA
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AGUACAACUACAACAGCCACAACGUCUAUAUCAUGGCCGACAAGCAGAAGAACGGCAUCAAGGUGAACUU
CAAGAUCGCCACAACAUCGAGGACGGCAGCGUGCAGCUCGCCGACCACUACCAGCAGAACACCCCCAUC
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CCCCUCCUCCCCUUCUGCACCCGUACCCCGUGGUCUUUGAAUAAAGUCUGAACACACAAAUGGUCGC
CGACUCAGUAGAUGAA
AAA

circ-iSyn-EGFP

CUACCGUUUAAUAUUGCGUCAUAUUCGGCGACCAUUUGUGUGGUAAAAAAAAAAAAACCAAAAAAAAAAAAA
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GUUUGGUCGAUGAGGCUAGGAUUUCCCCACGGGUGACCGUGUCCUAGCCUGCGUGGCGGCCAACCCAGCC
CACUCACUAUUUGUUUUCGCGCCCAGUUGCAAAAAGUGUCGGGGCUGGGACGCCUUUUUAUAGACAUGGU
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UGCACCACCGGCAAGCUGCCCCGUGCCCUGGCCCACCCUCGUGACCACCCUGACCUACGGCGUGCAGUGCU
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AUCGGCGACGGCCCCGUGCUGCUGCCCGACAACCACUACCUGAGCACCCAGUCCGCCCUAGAGCAAAGACC
CCAACGAGAAGCGCGAUCACAUGGUCCUGCUGGAGUUCGUGACCGCCGCCGGGAUCACUCUCGGCAUGGA
CGAGCUGUACAAGUAAUAACAUAUUGGCUGGAGCCUCGGUGGCCAUGCUUCUUGCCCCUUGGGCCUCCCCC
CAGCCCCUCCUCCCCUCCUGCACCCGUACCCCCGUGGUCUUUGAAUAAAGUCUGAACCCACACAAAUGGU
CGCCGACUCAGUAGAUGUUUUCUUGGGU

NMR and MS spectra

