Supporting Information

Stronger together for in cell translation: natural and unnatural base modified mRNA

*Lisa Bornewasser, Christof Domnick and Stephanie Kath-Schorr**

Department of Chemistry, University of Cologne, Greinstrasse 4, 50939 Cologne, Germany.

E-mail: skathsch@uni-koeln.de

Supplementary Information

General methods

DNA template preparation

The DNA templates for ensuing *in vitro* transcriptions were prepared by PCR amplification from the *pmCherry-N1* plasmid employing either a six letter expanded genetic alphabet or standard PCR approach. Applying a forward primer holding the T7 promotor sequence as 5′-overhang (**mCh_FW**) and either single or double **dNaM** modified or single or double T↔C mutated or unmodified reverse primer, nucleotides 618 to 1430 from the *pmCherry-N1* plasmid were amplified for **mCh_DNA** resulting in either none (**mCh_RV**), or one or two dA (plasmid) : d**NaM** (primer) or one or two dA (plasmid) : dC (primer) mismatches at positions 1418 (**mCh_RV$^{1\ dNaM}$** or **mCh_RV$^{1\ PM}$**) or 1411 and 1425 (**mCh_RV$^{2\ dNaM}$** or **mCh_RV$^{2\ PM}$**), respectively. For **mCh_DNA$_{UTR\ 2}$** the same forward primer was used, but together with the **mCh_RV$_{UTR\ 2}$** reverse primer including a 5′ overhang sequence introducing an alternative sequence at the DNA coding strand's 3′-end. Applying the **mCh_FW** and **mCh_RV$_{UTR\ 2}$** primer pair, nucleotides 618 to 1412 from the *pmCherry-N1* plasmid were amplified for **mCh_DNA$_{UTR\ 2}$.** Accordingly, either one or two dA (plasmid) : d**NaM** (primer) or one or two dA (plasmid) : dC (primer) mismatches were used to introduce modifications at position 1420 for **mCh_DNA$^{1\ UBP}_{UTR\ 2}$** and **mCh_DNA$^{1\ PM}_{UTR\ 2}$** or positions 1413 and 1427 for or **mCh_DNA$^{2\ UBP}_{UTR\ 2}$** and **mCh_DNA$^{2\ PM}_{UTR\ 2}$**, respectively**.** PCR reactions were performed in 100 µL scale containing a final concentration of 20 mM Tris-HCl pH 8.9, 22 mM NH_4Cl, 22 mM KCl, 1.8 mM $MgCl_2$, 0.06 % IGEPAL® CA-630, 0.05 % Tween® 20 (*OneTaq*® Standard Reaction Buffer, *New England Biolabs*), 375 µM each canonical dNTP (*Jena Bioscience*), optionally 200 µM d**NaM** TP and d**TPT3** TP, 1 µM forward and reverse primer (**mCh_FW**/**mCh_RV** for **mCh_DNA** or **mCh_FW**/**mCh_RV$^{1\ dNaM}$** for **mCh_DNA$^{1\ UBP}$** or **mCh_FW**/**mCh_RV$^{2\ dNaM}$** for **mCh_DNA$^{2\ UBP}$** or **mCh_FW**/**mCh_RV$^{1\ PM}$** for **mCh_DNA$^{1\ PM}$** or **mCh_FW**/**mCh_RV$^{2\ PM}$** for **mCh_DNA$^{2\ PM}$** or **mCh_FW**/**mCh_RV$_{UTR\ 2}$** for **mCh_DNA$_{UTR\ 2}$** or **mCh_FW**/**mCh_RV$^{1\ dNaM}_{UTR\ 2}$** for **mCh_DNA$^{1\ UBP}_{UTR\ 2}$** or **mCh_FW**/**mCh_RV$^{2\ dNaM}_{UTR\ 2}$**for **mCh_DNA$^{2\ UBP}_{UTR\ 2}$**or **mCh_FW**/**mCh_RV$^{1\ PM}_{UTR\ 2}$**for **mCh_DNA$^{1\ PM}_{UTR\ 2}$**or **mCh_FW**/**mCh_RV$^{2\ PM}_{UTR\ 2}$** for **mCh_DNA$^{2\ PM}_{UTR\ 2}$**), 0.5 ng µL^{-1} *pmCherry-N1* as template and 0.025 U µL^{-1} *OneTaq*® DNA Polymerase (*New England Biolabs*). PCR was performed with an initial denaturing step at 94 °C for 2 min, followed by 30 cycles of denaturing at 94 °C for 30 s, annealing at indiviual temperature for each sequence for 40 s, elongation at 68 °C for 1 min and a final elongation step at 68 °C for 3 min. Annealing temperatures for **mCh_DNA** were set to 56 °C for **mCh_DNA^{WT}** or 51 °C for **mCh_DNA$^{1\ UBP}$** or 55 °C for **mCh_DNA$^{1\ PM}$**, 46 °C for **mCh_DNA$^{2\ UBP}$** or 57 °C for **mCh_DNA$^{2\ PM}$**. For **mCh_DNA$_{UTR\ 2}$** annealing temperatures were set to 61 °C for **mCh_DNA$^{WT}_{UTR\ 2}$** or 63 °C for **mCh_DNA$^{1\ PM}_{UTR\ 2}$**, and **mCh_DNA$^{2\ PM}_{UTR\ 2}$**. For **mCh_DNA$^{1\ UBP}_{UTR\ 2}$** and **mCh_DNA$^{2\ UBP}_{UTR\ 2}$** annealing temperatures for the first five cycles were set to 51 °C and 46 °C, respectively, then changed to 55 °C and 51 °C, respectively, for subsequent 25 cycles. PCR products were analyzed by agarose gel electrophoresis and purified using the *NucleoSpin*® Gel and PCR Clean-Up Kit (*Macherey-Nagel*) according to the manufacturer's protocol.

mRNA *in vitro* transcription

For **mCh_mRNA** and **mCh_mRNA$_{UTR\ 2}$** *in vitro* transcription, the *HiScribe™ T7 ARCA mRNA Kit (with tailing)* (*New England Biolabs*) was used. According to the manufacturer's protocol, co-transcriptionally ARCA-capped RNA was transcribed in a first step. Therefore 0.05 µg µL^{-1} of purified template DNA per reaction was used. For *in vitro* transcriptions of **mCh_mRNA$^{1\ CP}$** and **mCh_mRNA$^{2\ CP}$** from the UBP modified DNA templates, **mCh_DNA$^{1\ UBP}$** and **mCh_DNA$^{2\ UBP}$**, or **mCh_mRNA$^{1\ CP}_{UTR\ 2}$** and **mCh_mRNA$^{2\ CP}_{UTR\ 2}$** from the UBP modified DNA templates, **mCh_DNA$^{1\ UBP}_{UTR\ 2}$** and **mCh_DNA$^{2\ UBP}_{UTR\ 2}$**,, respectively, a concentration of 0.25 mM r**TPT3^{CP}** TP was additionally added to the reaction. For additional Ψ and 5mC modifications, 1.25 mM Ψ TP and 1.25 mM 5mC TP were added to the IVT reaction mix. Following DNase template digestion by addition of 4 U DNase I to the reaction mix and incubation for 15 min, the transcribed RNA was polyadenylated with approximately 150 nt poly(A). Therefore a 50 µL tailing reaction was prepared including 10x Poly(A) Polymerase Buffer, Poly(A) Polymerase (unlisted concentrations of kit components) according to the manufacturer's protocol and incubated at 37 °C for 30 min. Subsequently the obtained mRNA was purified via LiCl precipitation according to the manufacturer's protocol.

***In vitro* click reaction and fluorescence analysis**

8 pmol each of **mCh_mRNA^{WT}**, **mCh_mRNA$^{1\ CP}$**, **mCh_mRNA$^{2\ CP}$**, **mCh_mRNA$^{1\ PM}$**, **mCh_mRNA$^{2\ PM}$** as well as **mCh_mRNA$^{WT}_{UTR\ 2}$**, **mCh_mRNA$^{1\ CP}_{UTR\ 2}$**, **mCh_mRNA$^{2\ CP}_{UTR\ 2}$**, **mCh_mRNA$^{1\ PM}_{UTR\ 2}$**, **mCh_mRNA$^{2\ PM}_{UTR\ 2}$** and H_2O as control were incubated with 71 µM AF Dye 488 tetrazine (*Click Chemistry Tools*) in a total reaction volume of 7 µL at 25 °C in the dark for 1 h. Control samples of all **mCh_mRNA** sequences were treated equally but without addition of AF Dye 488 tetrazine, instead a compensational volume of H_2O was added. Then, agarose gel electrophoresis without addition of EtBr was performed and the resulting bands were analyzed first by fluorescence scanning, λ_{ex} = 473 nm λ_{em} = 520 nm, using a *Phosphorimager FLA-3000* (*Fujifilm*), before EtBr post-staining and visualization by UV illumination.

Cell culture and mRNA transfection

Adherent HeLa cells were cultured in *Dulbecco's Modified Eagle Medium* (*1x DMEM) GlutaMAX™* (*gibco™, Thermo Fisher Scientific*) supplemented with 10 % foetal calf serum (FCS) (*Sigma, Batch No.: 054M3399*), 1 % *Minimum Essential Medium* (*MEM) non-essential amino acids solution* (100x, *gibco™, Thermo Fisher Scientific*) and 1 % sodium pyruvate (100 mM, *gibco™, Thermo Fisher Scientific*) in *TC Flask T75, Standard* (*Sarstedt*) cell culture flasks at 37 °C and 5 % CO_2. Cell confluence was kept between 80-90 %, therefore cells were split at regular intervals of two to three days. Therefore, cells were washed with 8 mL *Dulbecco's Phosphate Buffered Saline (1x DPBS)* (*gibco™, Thermo Fisher Scientific*), trypsinized with 2 mL Trypsin-EDTA (0.05%) (*gibco™, Thermo Fisher Scientific*) and incubated at 37 °C and 5 % CO_2 for 5 to 10 min, before partly transfered into a new cell culture flask with fresh *DMEM* medium.

For **mCh_mRNA** transfection experiments cells were seeded in a *TC Plate 24 well, Standard* (*Sarstedt*) to a density of 70 000 cells per well in 500 µL *DMEM* medium the day before transfection. For fixed cell imaging with confocal fluorescence microscopy, additionally glas coverslips (ø: 15 mm, thickness 1, *Marienfeld*) were placed in each well prior to cell seeding. HeLa cells were transfected using *Lipofectamine® MessengerMAX™* (*Invitrogen*) transfection reagent. According to the manufacturer's protocol, for each well of cells to be transfected 1.5 µL *MessengerMAX™* was diluted in 25 µL *Opti-MEM® Reduced-Serum Medium* (*gibco™, Thermo Fisher Scientific*), vortexed thouroughly and incubated at room temperature (RT) for 10 min. Likewise 0.5 µg µL^{-1} **mCh_mRNA** (consistend for all different **mCh_mRNA** sequences) or 1 µL H_2O as control was diluted in 25 µL *Opti-MEM®.* Diluted mRNA and diluted *MessengerMAX™* were mixed in an 1:1 ratio, vortexed thouroughly and incubated at RT for 5 min. The mRNA-lipid complex was then added dropwisely to the cell medium and cells were incubated for 6, 24 or 48 h at 37 °C and 5 % CO_2. 4 h after transfection start the cell medium was exchanged for fresh *DMEM* medium.

Live cell click reaction and confocal fluorescence microscopy

After implementation of **mCh_mRNA** transfection and incubation for 6, 24 or 48 h for **mCh_mRNA** translation and mCherry reporter protein expression, 1 µL AFDye 488 tetrazine (500 µM) (*Click Chemistry Tools*) was added to the cell medium of each well. Cells were incubated at 37 °C and 5 % CO_2 for 1 h for implementation of the iEDDA click reaction. Then, cells were washed once with 500 µL *1x DPBS* per well before addition of 500 µL of 3.7 % formaldehyde solution (*Formaldehyde, 37 wt% solution in water, stabilized with 5-15 % methanol, Acros Organics*, diluted 1:10 in *1x DPBS*) per well and incubation at RT for 10 min for cell fixation. Subsequently cells were washed trice with 500 µL *1x DPBS* per well and washing step. Cell nuclei counterstaining was performed adding 1 mL of *4',6-Diamidino-2-phenylindole* (DAPI) (*Merck*, 1 µg µL^{-1} solution in H_2O) per well and incubation at RT for 2 min. Afterwards cells were again washed trice with 500 µL *1x DPBS* per well and washing step. Cell cover slips were mounted on microscope slides (90° ground edges, frosted, *Carl Roth*) using *Fluoro-Gel (with Tris buffer)* mounting medium (*Electron Microscopy Sciences*), let dry at RT overnight and sealed with nail polish.

Confocal fluorescence fixed cell imaging of **mCh_mRNA** transfected and live cell clicked HeLa cells was performed on a *LSM 710* (*Zeiss*) Laser Scanning Microscope equipped with a 40x/1.4 DIC oil objective. Red fluorescence emission of translated and expressed mCherry reporter protein was imaged with Ex/Em = 543/637 nm. Green fluorescence emission of AFDye 488 clicked **mCh_mRNA$^{1\ CP}$** and **mCh_mRNA$^{2\ CP}$** or **mCh_mRNA$^{1\ CP}_{UTR\ 2}$** and**mCh_mRNA$^{2\ CP}_{UTR\ 2}$**, respectively, was imaged with Ex/Em = 488/516 nm. DAPI counterstained cell nuclei were imaged with Ex/Em = 405/460 nm. For each image and channel a number of four scans was averaged. Z-stack images for signal localization analysis were taken with intervals of 3.426 µm (corresponds 1 AU).

mCherry protein quantification

mCherry protein quantification was performed using a *mCherry Quantification Kit* (*BioVision*) in combination with *RIPA Lysis and Extraction Buffer* (*Thermo Fisher Scientific*). After implementation of **mCh_mRNA** transfection and incubation for 6, 24 or 48 h for **mCh_mRNA** translation and mCherry reporter protein expression, the cell medium

was removed and 130 µL assay buffer (*RIPA Lysis and Extraction Buffer* supplemented with 1 mM *Phenylmethylsulfonyl fluoride* (PMSF), *Thermo Fisher Scientific*) added. After incubation on ice for 20 min, the samples were centrifuged at 10 *x g*, 4 °C for 5 min. The supernatants were directly taken for accomplishment of the quantification assay or stored at -20 °C until all time course samples were collected. Using a 10 ng/µL mCherry working solution a mCherry standard series of 0, 20, 40, 60, 80, 100 ng/well was prepared as duplicate in a 96-well plate and all volumes adjusted to 100 µL with assay buffer. Frozen samples were gently thawed on ice. Each 100 µL of supernatant was transferred into the plate. mCherry fluorescence was measured at Ex/Em = 587/610 nm at 25 °C using an *EnSpire Multimode Plate Reader* (*Perkin Elmer*). All measured fluorescence values were offset corrected and the mCherry standard curve was plotted. The fluorescence readings of samples were applied to the mCherry standard curve to calculate the amount of mCherry protein in the sample wells. Mean values of of mCherry protein amounts were calculated for technical duplicates of each transfection condition (**mCh_mRNA^{WT}** or **mCh_mRNA$^{1\ CP}$** or **mCh_mRNA$^{2\ CP}$** or **mCh_mRNA$^{1\ PM}$** or **mCh_mRNA$^{2\ PM}$** or **mCh_mRNA$^{WT,\ \Psi+5mC}$** or **mCh_mRNA$^{1\ CP,\ \Psi+5mC}$** or **mCh_mRNA$^{2\ CP,\ \Psi+5mC}$** or **mCh_mRNA$^{WT}_{UTR\ 2}$** or **mCh_mRNA$^{1\ CP}_{UTR\ 2}$** or **mCh_mRNA$^{2\ CP}_{UTR\ 2}$** or **mCh_mRNA$^{1\ PM}_{UTR\ 2}$** or **mCh_mRNA$^{2\ PM}_{UTR\ 2}$**). Also, mCherry protein amounts for corresponding transfection conditions from biologically independend experiments were averaged (n=20 for **mCh_mRNA^{WT}**, **mCh_mRNA$^{1\ CP}$** and **mCh_mRNA$^{2\ CP}$**, n=10 for **mCh_mRNA$^{1\ PM}$** or **mCh_mRNA$^{2\ PM}$** or **mCh_mRNA$^{WT,\ \Psi+5mC}$**, **mCh_mRNA$^{1\ CP,\ \Psi+5mC}$** or **mCh_mRNA$^{2\ CP,\ \Psi+5mC}$** or **mCh_mRNA$^{WT}_{UTR\ 2}$** or **mCh_mRNA$^{1\ CP}_{UTR\ 2}$** or **mCh_mRNA$^{2\ CP}_{UTR\ 2}$** or **mCh_mRNA$^{1\ PM}_{UTR\ 2}$** or **mCh_mRNA$^{2\ PM}_{UTR\ 2}$**).

RT-qPCR analysis of mRNA levels

After implementation of **mCh_mRNA** transfection and incubation for 6, 24 or 48 h, the cells were lysed and total cellular RNA was isolated. Total RNA isolation was accomplished using the NucleoSpin® RNA Kit (Macherey-Nagel) according to the manufacturer's protocol.

RT-qPCR reactions were performed applying the GoTaq® Probe 2 step RT-qPCR System (Promega). Initial experiments were performed to ensure compatibility of multiplexing qPCR and to determine amplification efficiencies (see Supplementary Information). Reverse transcription was accomplished separately for each transfection condition and controls. First, denaturing of RNA templates and primers was performed in a 7 µL scale with 100 ng of isolated cellular total RNA, 0.5 µg Oligo(dT)$_{15}$ primer and 0.5 µg random primers. RNA/primer mixes were pipetted on ice, then incubated at 70 °C for 5 min and subsequently chilled in an ice-water bath for at least 5 min. According to the manufacturers' protocol, master mixes for reverse transcription (RT) and no-reverse transcriptase (NRT) controls were prepared on ice with a total volume of 13 µL per each reaction. 13 µL each of RT or NRT master mix were added to 7 µL each of RNA/primer mixes for a final reaction volume of 20 µL containing GoScript™ Reaction Buffer (unlisted concentrations of GoTaq® Probe 2 step RT-qPCR System component), 2 mM $MgCl_2$, 500 µM PCR nucleotide mix, 50 U recombinant RNasin ribonuclease inhibitor and GoScript™ reverse transcriptase (unlisted concentration of GoTaq® Probe 2 step RT-qPCR System component) for RT or compensational amount of ddH_2O for NRT controls, respectively. RT and NRT reaction mixes were incubated at 25 °C for 5 min to hybridize RNA and primers, followed by 42 °C for 45 min for implementation of reverse

transcription and subsequent enzyme inactivation at 70 °C for 15 min. For usage in qPCR, the reverse transcribed cDNA samples and NRT control reactions were diluted 1:2 with ddH_2O. 0.625 µL of diluted cDNA or NRT control were then taken per each qPCR reaction. According to the manufacturer's protocol, qPCR master mixes were prepared on ice and added to diluted cDNA or NRT control, respectively. Each qPCR reaction was complemented in a total volume of 20 µL containing GoTaq® Probe qPCR master mix (unlisted concentration of GoTaq® Probe 2 step RT-qPCR System component), 200 nM **mCh_qPCR^{internal}_FW** forward primer, 200 nM **mCh_qPCR^{internal}_RV** reverse primer, 300 nM **mCh_qPCR^{internal}_probe**, 200 nM **mCh_qPCR$^{3'\text{-}end}$_FW** forward primer, 200 nM **mCh_qPCR$^{3'\text{-}end}$_RV** reverse primer, 300 nM **mCh_qPCR$^{3'\text{-}end}$_probe**, 200 nM **GAPDH_qPCR_FW** forward primer, 200 nM **GAPDH_qPCR_RV** reverse primer, 300 nM **GAPDH_qPCR_probe**, 200 nM **β-Actin_qPCR_FW** forward primer, 200 nM **β-Actin _qPCR_RV** reverse primer and 300 nM **β-Actin _qPCR_probe**. qPCR master mix and cDNA or NRT dilutions, respectively, as well as a no-template control (NRT) for qPCR were pipetted into 96-well PCR plates (Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white, Bio-Rad) on ice. The qPCR plate was sealed with adhesive foil (Microseal 'B' PCR Plate Sealing Film, adhesive, optical, Bio-Rad), briefly centrifuged and placed into the qPCR thermal cycler (CFX96 Touch Real-Time PCR Detection System, Bio-Rad). qPCR cycling was performed with an initial denaturing step at 95 °C for 2 min, followed by 40 cycles of denaturing at 95 °C for 15 s and a combined step for annealing and elongation at 60 °C for 1 min. All qPCR reactions were performed as technical triplets. C_t values for each target gene were calculated by the CFX Maestro software (Bio-Rad). C_t mean values and standard deviation (s.d.) of technical triplicate samples were calculated first, then ΔC_t values of either **mCh_qPCR^{internal}** C_t mean values or **mCh_qPCR$^{3'\text{-}end}$** C_t mean values were calculated by substraction with the corresponding **GAPDH_qPCR** reference gene C_t mean values. Next, calculated ΔC_t values of either **mCh_qPCR^{internal}** or **mCh_qPCR$^{3'\text{-}end}$** of two biologically independend experiments were averaged. $\Delta\Delta C_t$ values of either **mCh_qPCR^{internal}** or **mCh_qPCR$^{3'\text{-}end}$** were calculated by substracting the averaged ΔC_t value of the corresponding sample transfected with **mCh_mRNA^{WT}** and lysed 6 h post transfection from the respective averaged ΔC_t value of a sample transfected with modified **mCh_mRNA** (**mCh_mRNA$^{1\ CP}$** or **mCh_mRNA$^{2\ CP}$** or **mCh_mRNA$^{1\ PM}$** or **mCh_mRNA$^{2\ PM}$** or **mCh_mRNA$^{WT,\ \Psi+5mC}$** or **mCh_mRNA$^{1\ CP,\ \Psi+5mC}$** or **mCh_mRNA$^{2\ CP,\ \Psi+5mC}$** or **mCh_mRNA$^{WT}_{UTR\ 2}$** or **mCh_mRNA$^{1\ CP}_{UTR\ 2}$** or **mCh_mRNA$^{2\ CP}_{UTR\ 2}$** or **mCh_mRNA$^{1\ PM}_{UTR\ 2}$** or **mCh_mRNA$^{2\ PM}_{UTR\ 2}$**) and lysed at 6, 24 or 48 h post transfection. Fold changes of $2^{-\Delta\Delta Ct}$ were then calculated from the corresponding $\Delta\Delta C_t$ values.

Cell viability assays

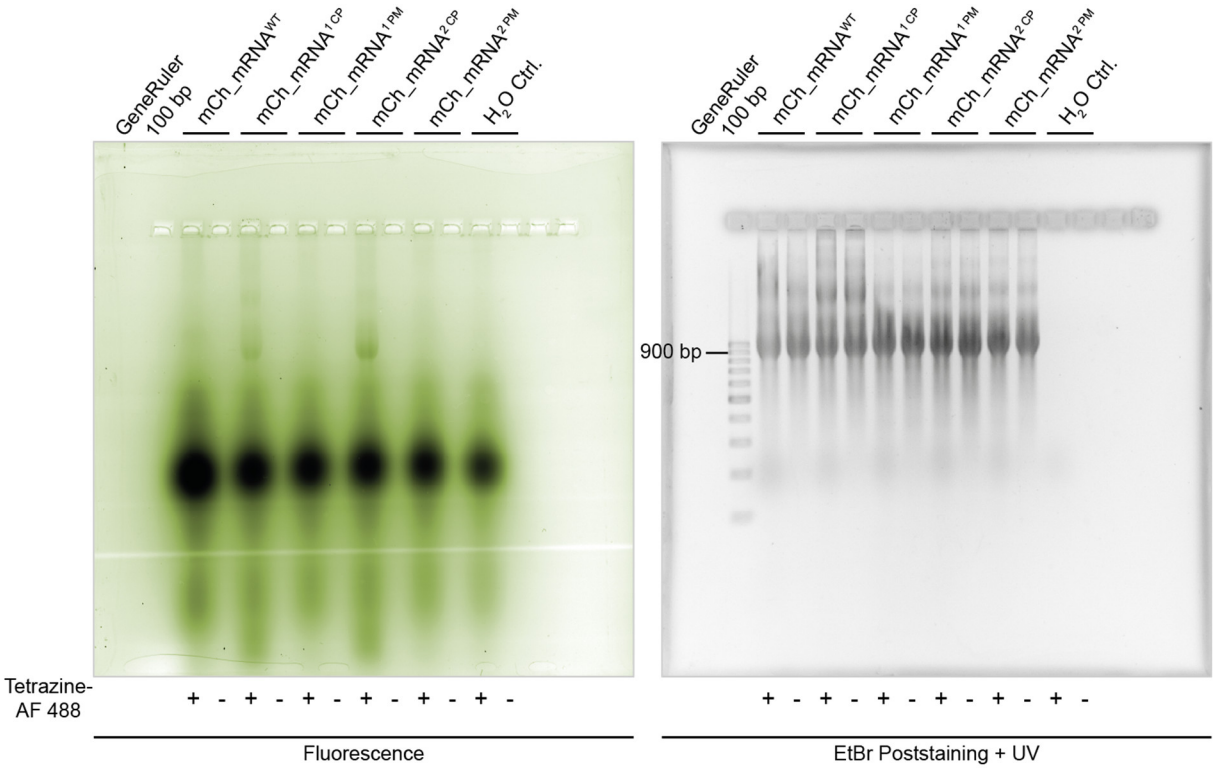
For cell viability assays the RealTime-Glo™ MT Cell Viability Assay (Promega) was used. Other than for protein quantification and RT-qPCR analysis, cells were seeded in a *96 well microplate, PS, µClear® bottom, chimney well* (*Greiner Bio-One)* to a density of 2 500 cells per well in 100 µL *DMEM* medium the day before transfection. HeLa cells were transfected using *Lipofectamine® MessengerMAX™* (*Invitrogen*) transfection reagent. According to the manufacturer's protocol, for each well of cells to be transfected 0.3 µL *MessengerMAX™* was diluted in 4.7 µL *Opti-MEM® Reduced-Serum Medium* (*gibco™, Thermo Fisher Scientific*), vortexed thouroughly and incubated at room temperature (RT) for 10 min. Likewise 100 ng μL^{-1} **mCh_mRNA** (consistend for all different **mCh_mRNA**

sequences) or an equivalent volume of H_2O as control was diluted in 5 µL *Opti-MEM®.* Diluted mRNA and diluted *MessengerMAX™* were mixed in an 1:1 ratio, vortexed thouroughly and incubated at RT for 5 min. The mRNA-lipid complex was then added dropwisely to the cell medium and cells were incubated for 24 h at 37 °C and 5 % CO_2. 4 h after transfection start the cell medium was exchanged for 100 µL fresh *DMEM* medium including 1x MT Cell Viability Substrate and 1x NanoLuc® Enzyme (RealTime-Glo™ MT Cell Viability Assay Kit, Promega). Luminescence was measured 6, 8, 22, 24 h post transfection with an integration time of 0.1 s, at 37 °C using an *EnSpire Multimode Plate Reader* (*Perkin Elmer*). Then, 0.2 µL AFDye 488 tetrazine (500 µM) (*Click Chemistry Tools*) was added to the cell medium of each well. Likewise, 2 µL Triton X-100 (10% in H_2O) were added to control wells without mRNA treatment for induced cell lysis. Luminescence was measured 1 and 2 h post addition of AFDye 488 tetrazine, corresponding to 25 and 26 post transfection. Mean values ± S.D. of measured luminescence were calculated for technical triplicates of each transfection condition (**mCh_mRNA^{WT}** or **mCh_mRNA$^{1\ CP}$** or **mCh_mRNA$^{2\ CP}$** or **mCh_mRNA$^{1\ PM}$** or **mCh_mRNA$^{2\ PM}$** or **mCh_mRNA$^{WT,\ \Psi+5mC}$** or **mCh_mRNA$^{1\ CP,\ \Psi+5mC}$** or **mCh_mRNA$^{2\ CP,\ \Psi+5mC}$** or H_2O Ctrl.), likewise for the untreated cells Ctrl. and lysed cells Ctrl..

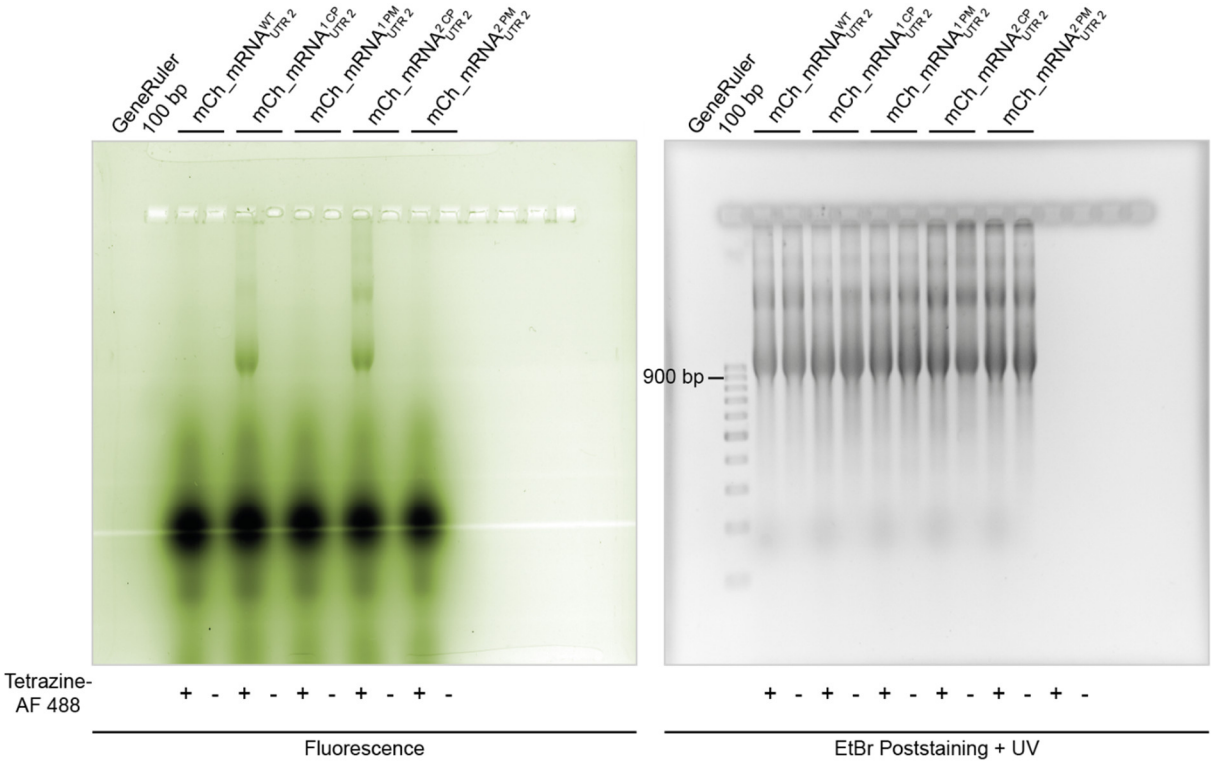
Agarose gel electrophoresis

Analytical agarose gel electrophoresis

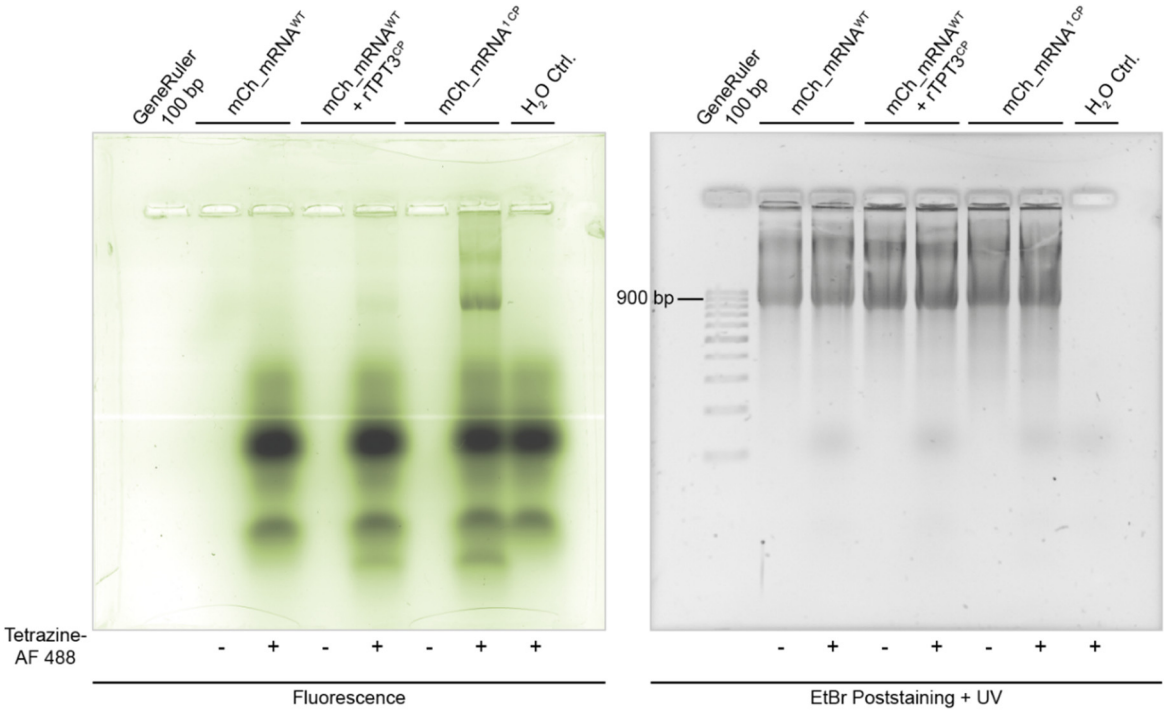
For analytical agarose gel electrophoresis, either a 2 % (w/v) (for mCh_DNA and mCh_RNA sequences) or 4 % (w/v) (for qPCR sequences) solution of agarose high resolution (*Sigma*) in 0.5 % Tris-borate-EDTA buffer (0.5 x TBE, Tris, *Carl Roth*, Boric acid, *Labochem International*, EDTA, *AppliChem*) supplemented with 1 mg mL^{-1} ethidium bromide (EtBr, *Carl Roth*) was used. Samples were prepared using a homemade 2 x loading buffer (50 mM Tris-HCl pH 8.3, 15 % (w/v) Ficoll® 400, 6 mM EDTA, 0.25 % (w/v) bromophenol blue). 0.5 x TBE was employed as running buffer. Gel electrophoresis was performed at 150 V const. for 20 to 30 min. Gene Ruler 100 bp DNA Ladder (*Thermo Fisher Scientific*) was used as dsDNA standard for mCh_DNA and mCh_mRNA sequences. Gene Ruler Ultra Low Range DNA Ladder (*Thermo Fisher Scientific*) was used as dsDNA standard for mCh_qPCR sequences. Analytical agarose gels were visualized by UV illumination using a Gel Doc 2000 gel documentation system (*Bio-Rad*).



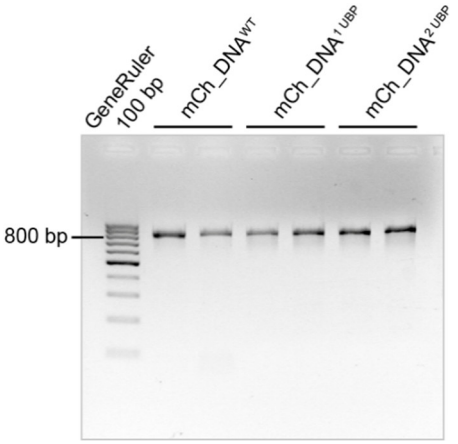
Supplementary Figure 1. mCh_mRNA *in vitro* click reaction with AF Dye 488. Fluorescence scan on the left, EtBr post-staining and UV-scan on the right. Segments shown in Figure 2.



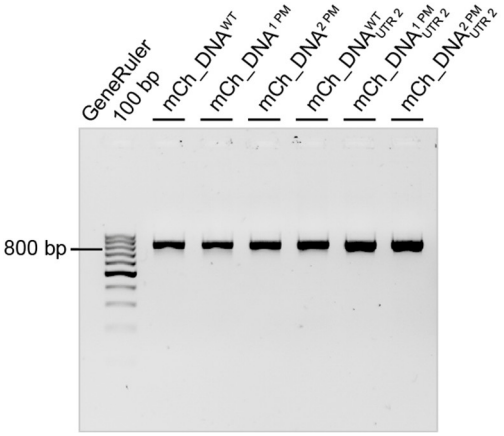
Supplementary Figure 2. mCh_mRNA$_{UTR\,2}$ *in vitro* click reaction with AF Dye 488. Fluorescence scan on the left, EtBr post-staining and UV-scan on the right.



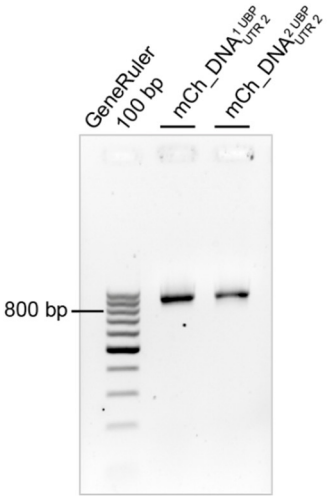
Supplementary Figure 3. mCh_mRNA and mCh_mRNA$^{2\,CP}$ *in vitro* click reaction with AF Dye 488. Fluorescence scan on the left, EtBr post-staining and UV-scan on the right. To check for unspecific rTPT3^{CP} incorporation into UB-unmodified mCh_mRNA, two samples of mCh_mRNA were prepared by *in vitro* transcription from the unmodified mCh_DNA, one with and the other without extra addition of the UB rTPT3^{CP}.



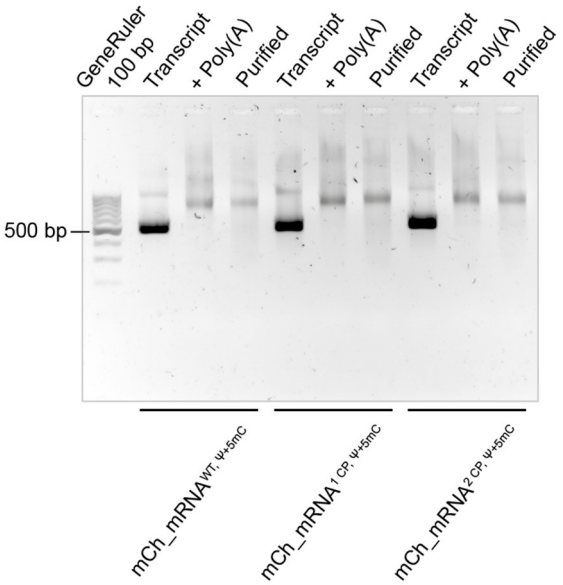
Supplementary Figure 4. PCR preparation of unmodified and UBP-modified **mCh_DNA** templates. Segments shown in Figure 2.



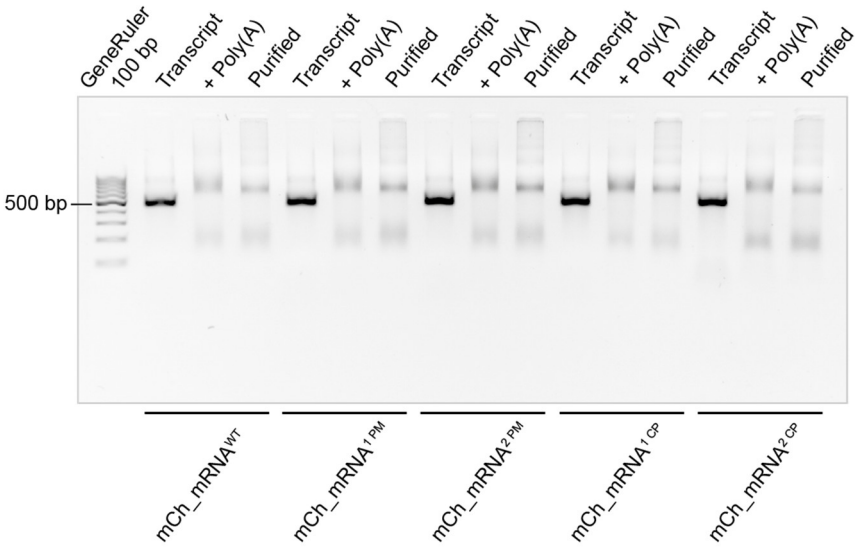
Supplementary Figure 5. PCR preparation of **mCh_DNA** and **mCh_DNA$_{UTR\ 2}$** templates.



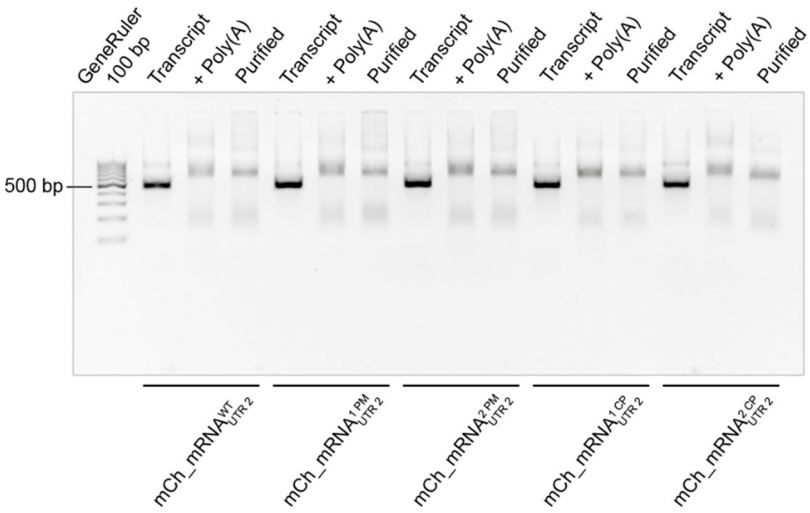
Supplementary Figure 6. PCR preparation of UBP-modified **mCh_DNA$_{UTR\ 2}$** templates.



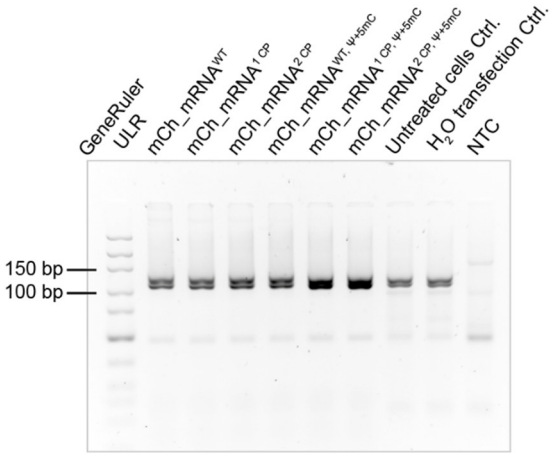
Supplementary Figure 7. *In vitro* transcribed mCh_mRNA$^{\Psi+5mC}$ with optional r**TPT3^{CP}** modification. Samples were taken after DNase digestion post transcription (Transcript), after Poly(A) tailing reaction reaction (+ Poly(A)) and after LiCl precipitation and mRNA purification (Purified). Segments shown in Figure 2.



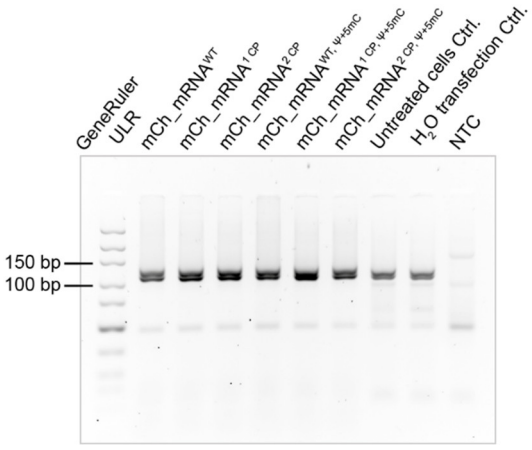
Supplementary Figure 8. *In vitro* transcribed mCh_mRNA with optional r**TPT3^{CP}** modification. Samples were taken after DNase digestion post transcription (Transcript), after Poly(A) tailing reaction reaction (+ Poly(A)) and after LiCl precipitation and mRNA purification (Purified). Segments shown in Figure 2.



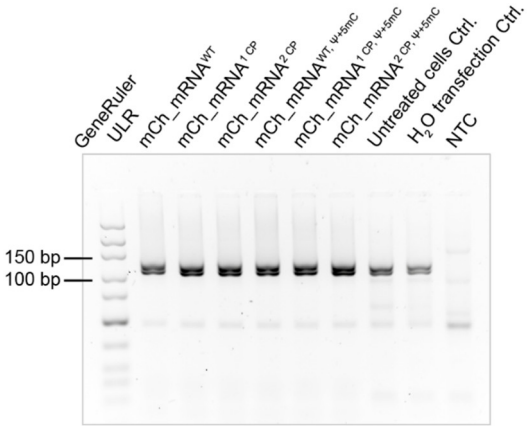
Supplementary Figure 9. *In vitro* transcribed mCh_mRNA$_{UTR\ 2}$ with optional r**TPT3^{CP}** modification. Samples were taken after DNase digestion post transcription (Transcript), after Poly(A) tailing reaction reaction (+ Poly(A)) and after LiCl precipitation and mRNA purification (Purified).



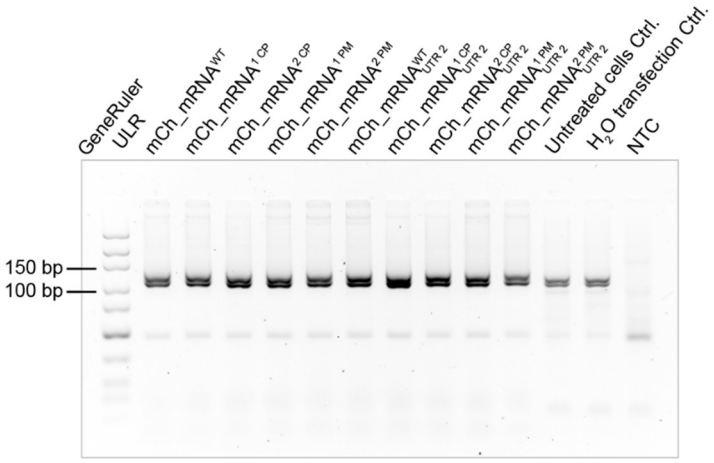
Supplementary Figure 10. RT-qPCR products from cells transfected with mCh_mRNA and mCh_mRNA$^{\Psi+5mC}$. Cell lysis and total cellular RNA isolation was performed 6 h post transfection.



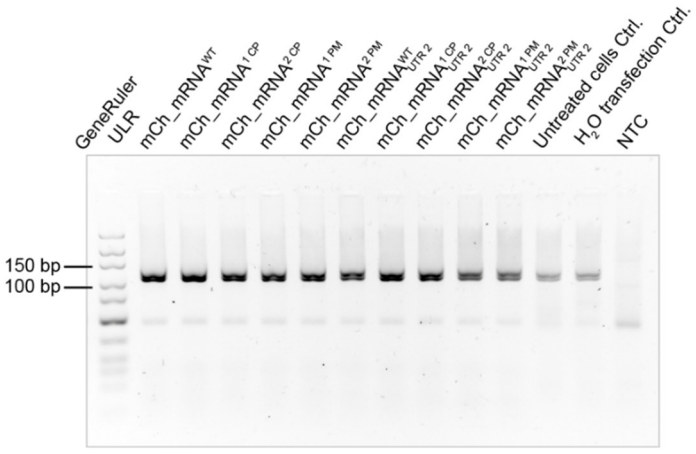
Supplementary Figure 11. RT-qPCR products from cells transfected with mCh_mRNA and mCh_mRNA$^{\Psi+5mC}$. Cell lysis and total cellular RNA isolation was performed 24 h post transfection.



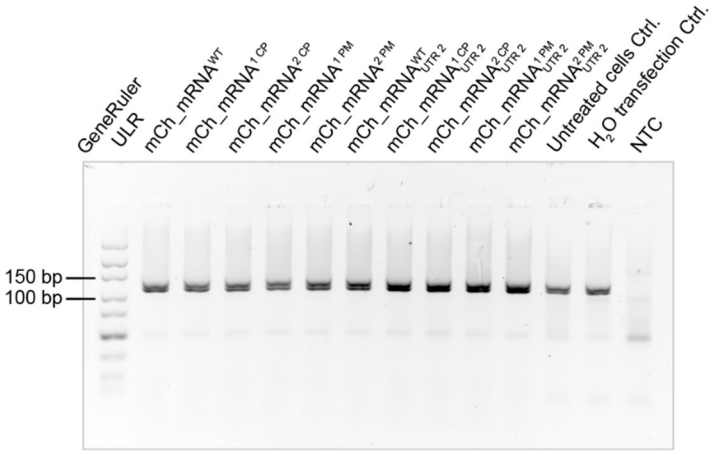
Supplementary Figure 12. RT-qPCR products from cells transfected with mCh_mRNA and mCh_mRNA$^{\Psi+5mC}$. Cell lysis and total cellular RNA isolation was performed 48 h post transfection.



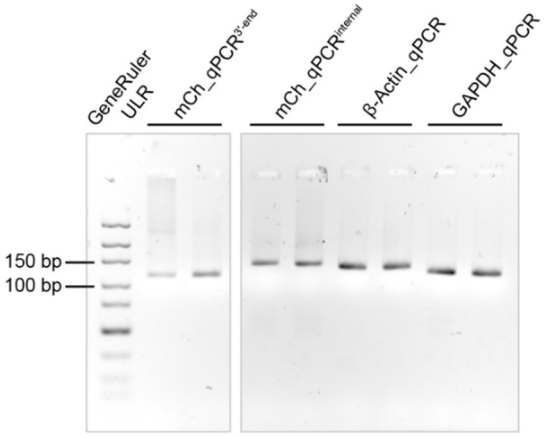
Supplementary Figure 13. RT-qPCR products from cells transfected with mCh_mRNA and mCh_mRNA$_{UTR\ 2}$. Cell lysis and total cellular RNA isolation was performed 6 h post transfection.



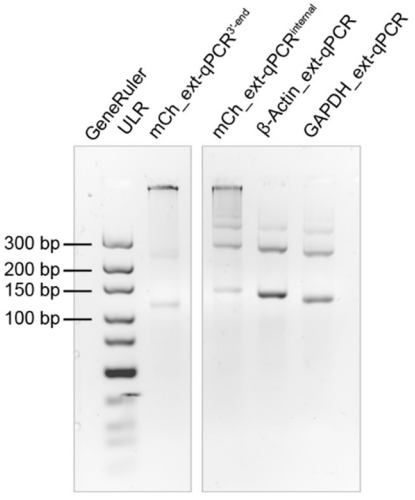
Supplementary Figure 14. RT-qPCR products from cells transfected with mCh_mRNA and mCh_mRNA$_{UTR\ 2}$. Cell lysis and total cellular RNA isolation was performed 24 h post transfection.



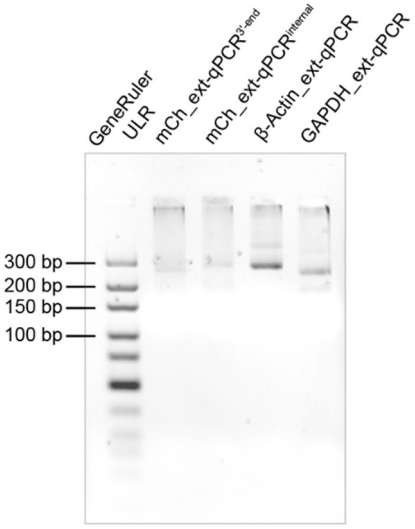
Supplementary Figure 15. RT-qPCR products from cells transfected with mCh_mRNA and mCh_mRNA$_{UTR\ 2}$. Cell lysis and total cellular RNA isolation was performed 48 h post transfection.



Supplementary Figure 16. PCR amplification of qPCR products.



Supplementary Figure 17. Extended qPCR ligation products.



Supplementary Figure 18. PCR amplification of extended qPCR products.

Nucleic acid concentration determination

Concentrations of DNA and RNA samples were determined by absorption at 260 nm (A_{260}) using a *Nanodrop UV-spectrometer 2000c* (*Thermo Fisher Scientific*). Concentrations were obtained from the A_{260} value and software-assisted calculation (native sequences containing canonical bases were plotted for modified RNA or DNA oligonucleotides, *http://biotools.nubic.northwestern.edu/OligoCalc.html*)

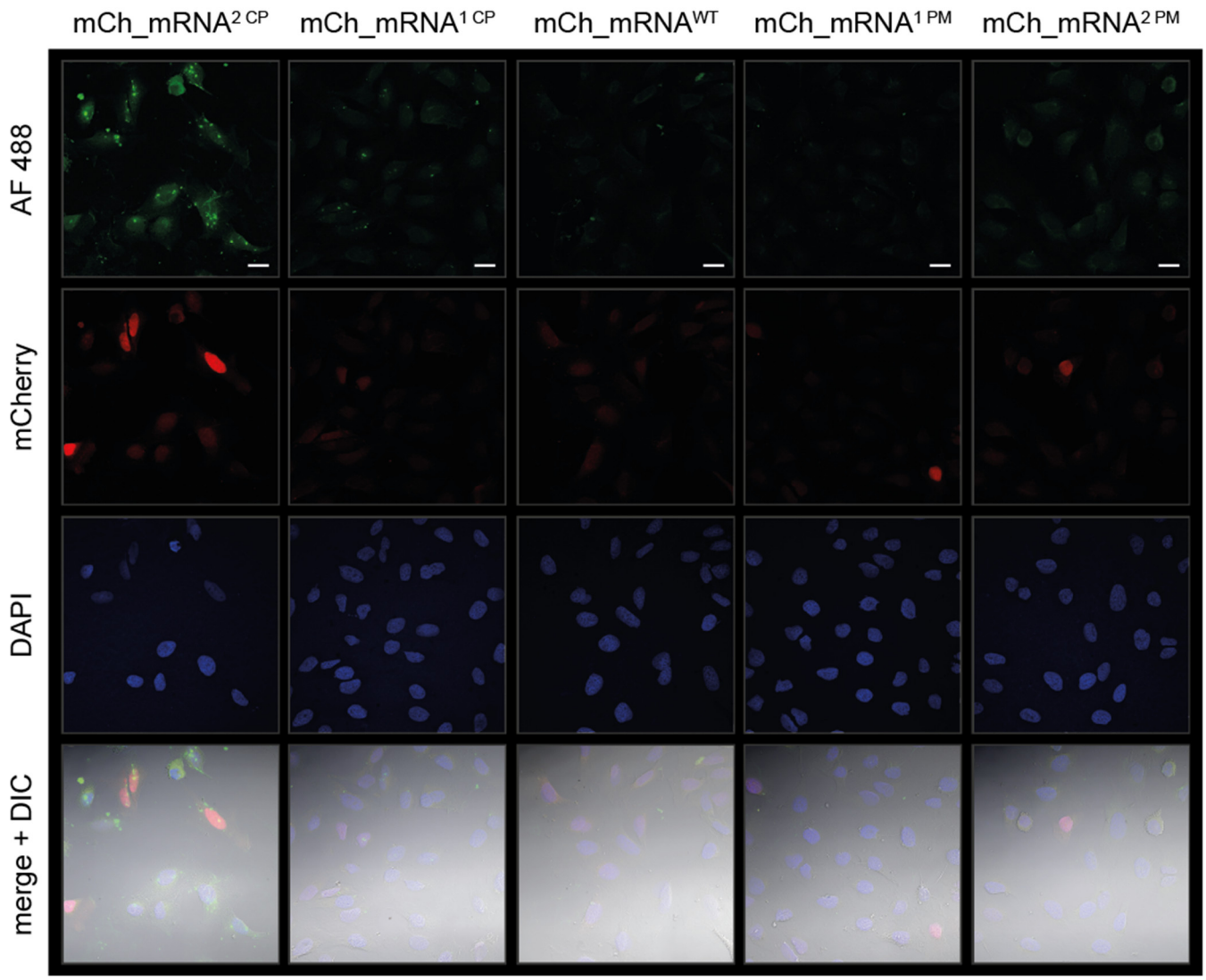
Microscopy data analysis

Raw microscopy images were exported to tagged image file format (TIF) with Zeiss Zen 3.3 (blue edition) and processed with Adobe Photoshop CS5. Different fluorescent and transmitted light signals were divided into single layers for each signal. Tonal correction was performed for each layer. Red (mCherry protein) and green (clicked CP labeled mRNA) signal layers were treated identically during tonal correction for all different mRNA transfection samples and treatment controls and for all different time points. Also, red and green signal layers were treated identically during subsequent brightness adjustment for all different mRNA transfection samples and treatment controls and for all different time points. Blue (DAPI cell nuclei counterstain) and transmitted light signal layers were treated individually during tonal correction for each image. To enhance visibility of the blue signal, the blue signal layer was copied, converted to greyscale and placed behind the blue signal layer. The blue greyscale layer's opacity was reduced to 10 percent and the blend mode was changed to "luminosity". For the transmitted light signal, brightness and contrast adjustments were performed, discriminative for each image. To create a merged image, the blend modes of all fluorescent signal layers were changed to "screen". Scale bars graphics were calculated and added to the raw microscopy images by the Zen 3.3 software, then measured in pixel size with Photoshop CS5 and added as fixed size boxes in a new layer of the processed image.

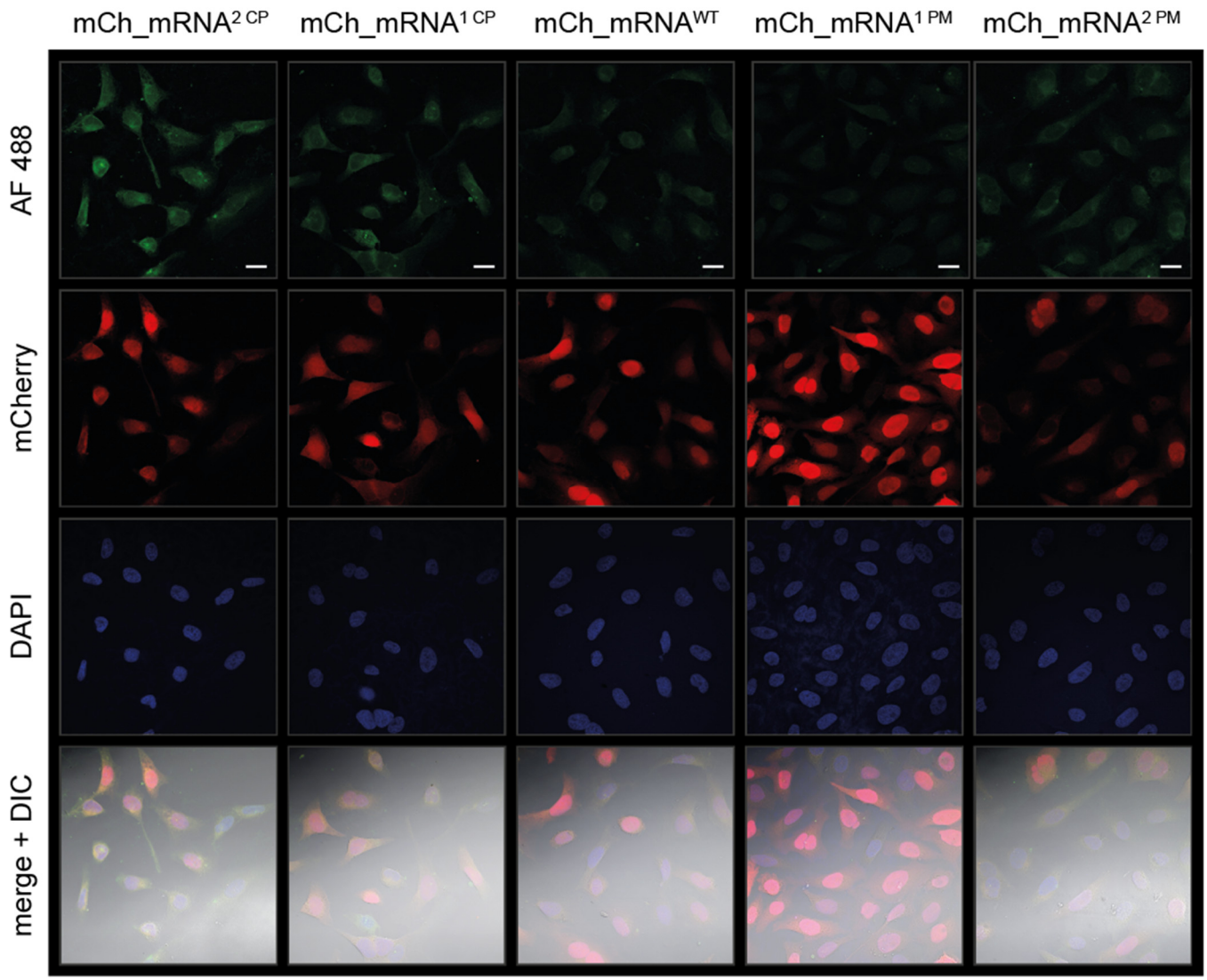
Overview images: Scale bars corresponds to 20 µm.

Z-stack: Scale bar corresponds to 10 µm.

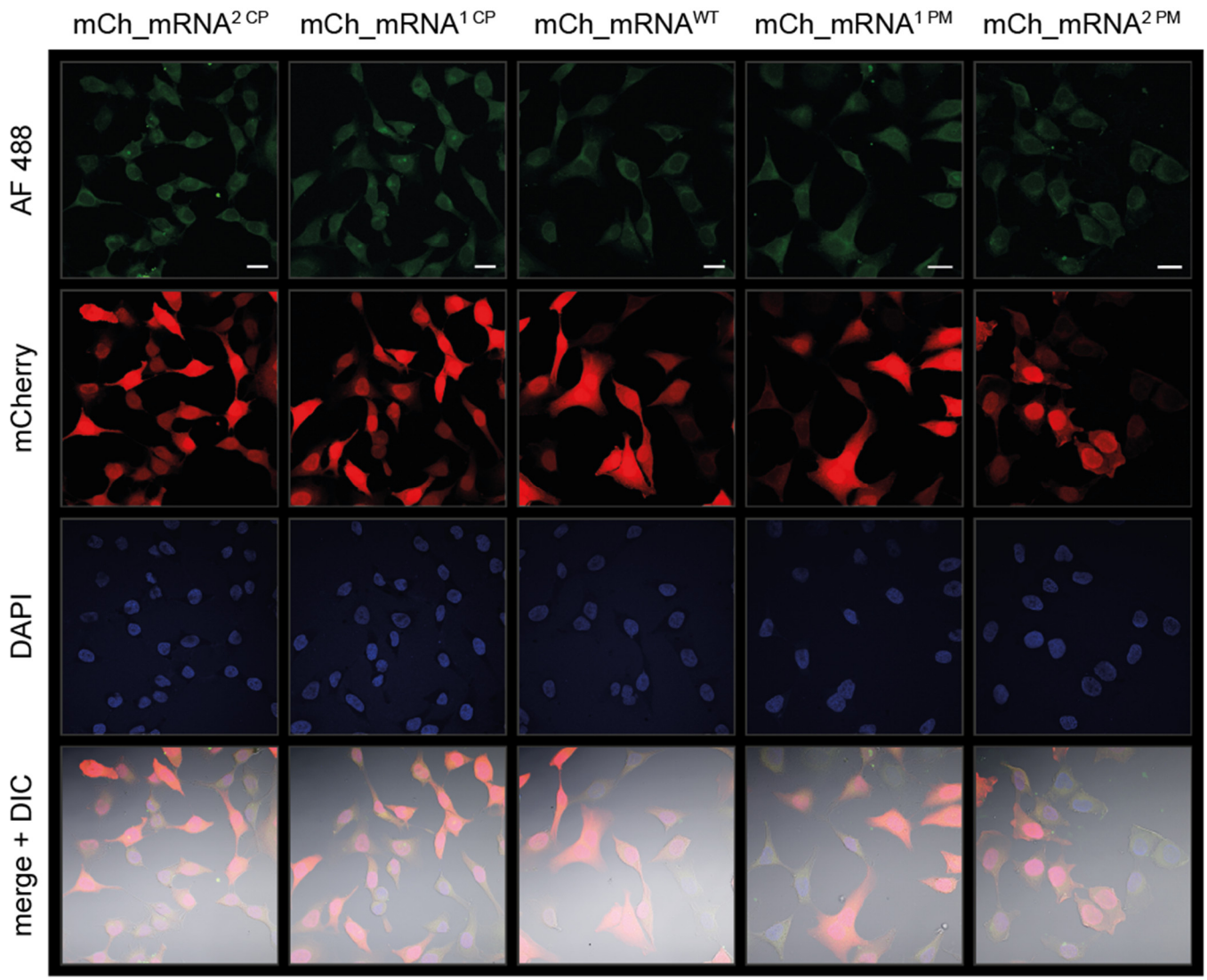
Close-up images: Scale bars corresponds to 10 µm.



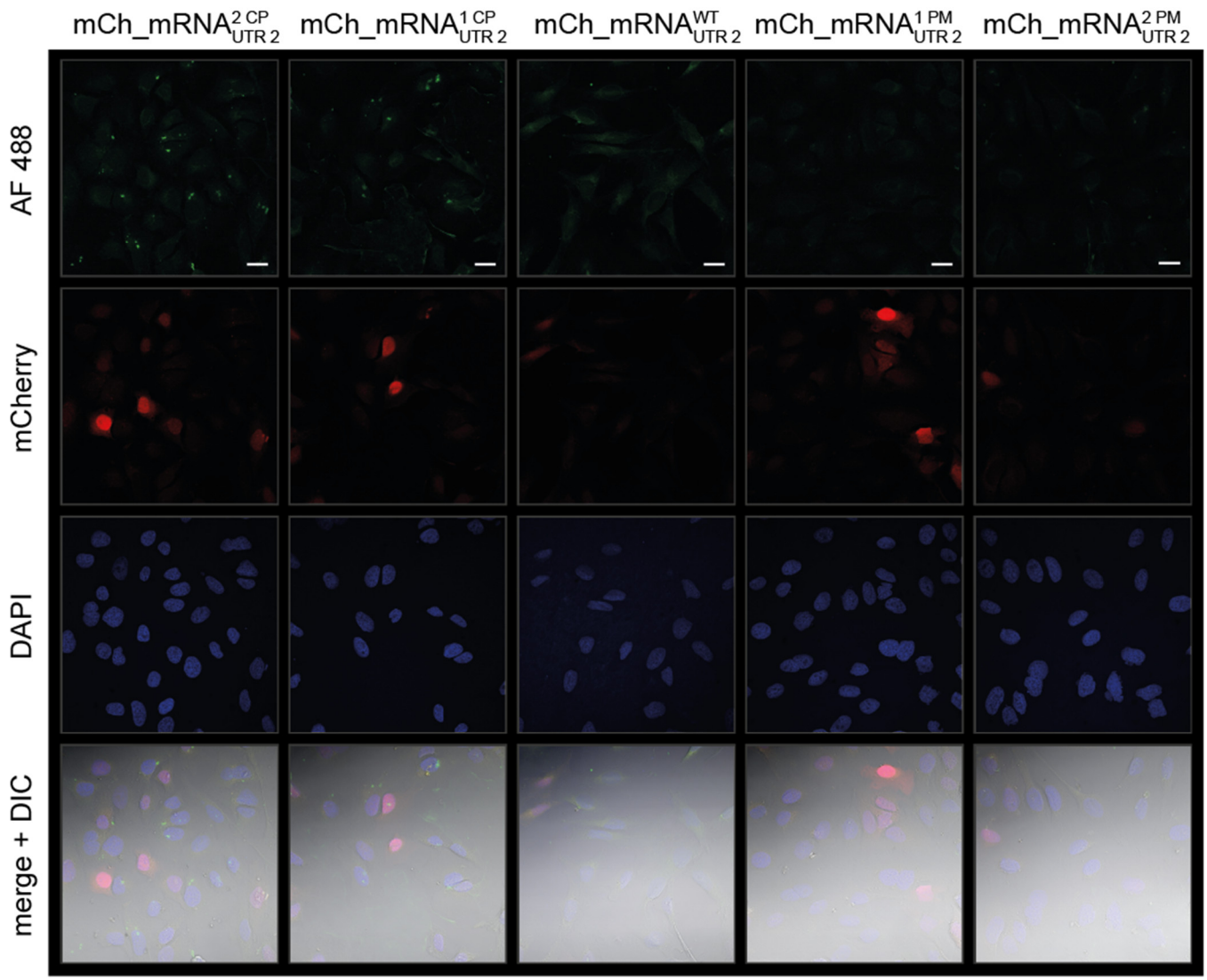
Supplementary Figure 19. Confocal fluorescence images of cells transfected with **mCh_mRNA**, 6h post transfection. Excerpts are shown in Figure 3, main text.



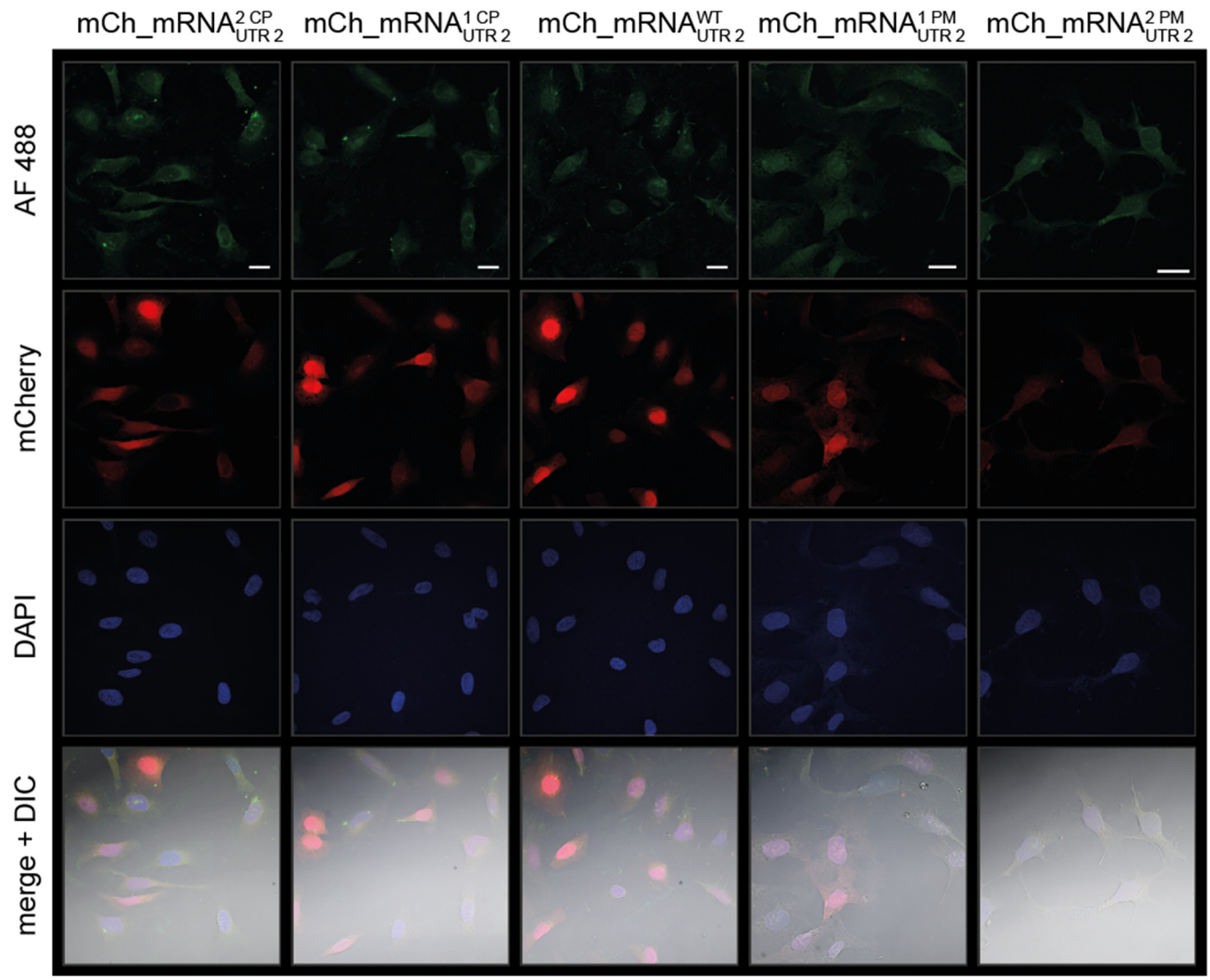
Supplementary Figure 20. Confocal fluorescence images of cells transfected with **mCh_mRNA**, 24 h post transfection. Excerpts are shown in Figure 3, main text.



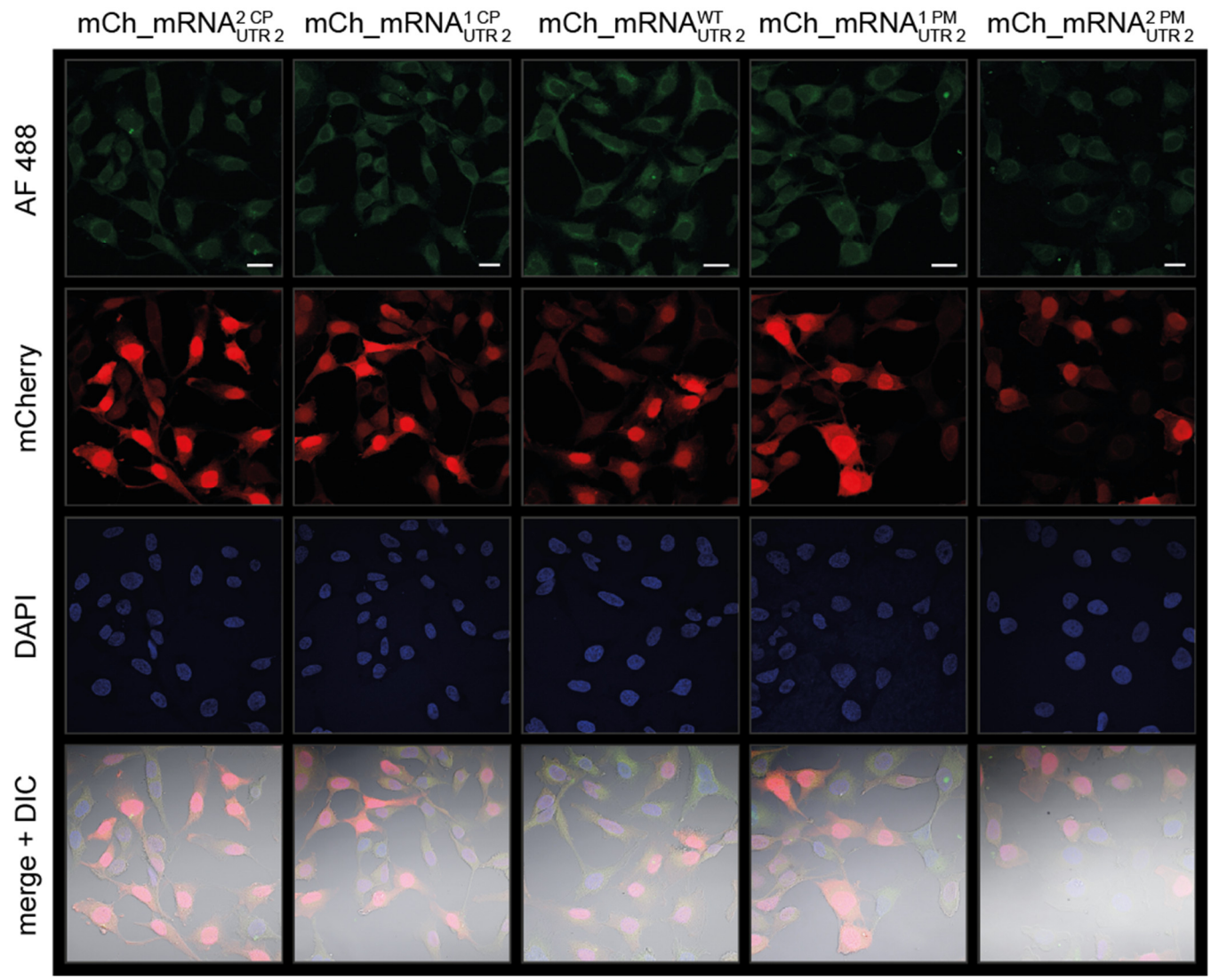
Supplementary Figure 21. Confocal fluorescence images of cells transfected with **mCh_mRNA**, 48 h post transfection. Excerpts are shown in Figure 3, main text.



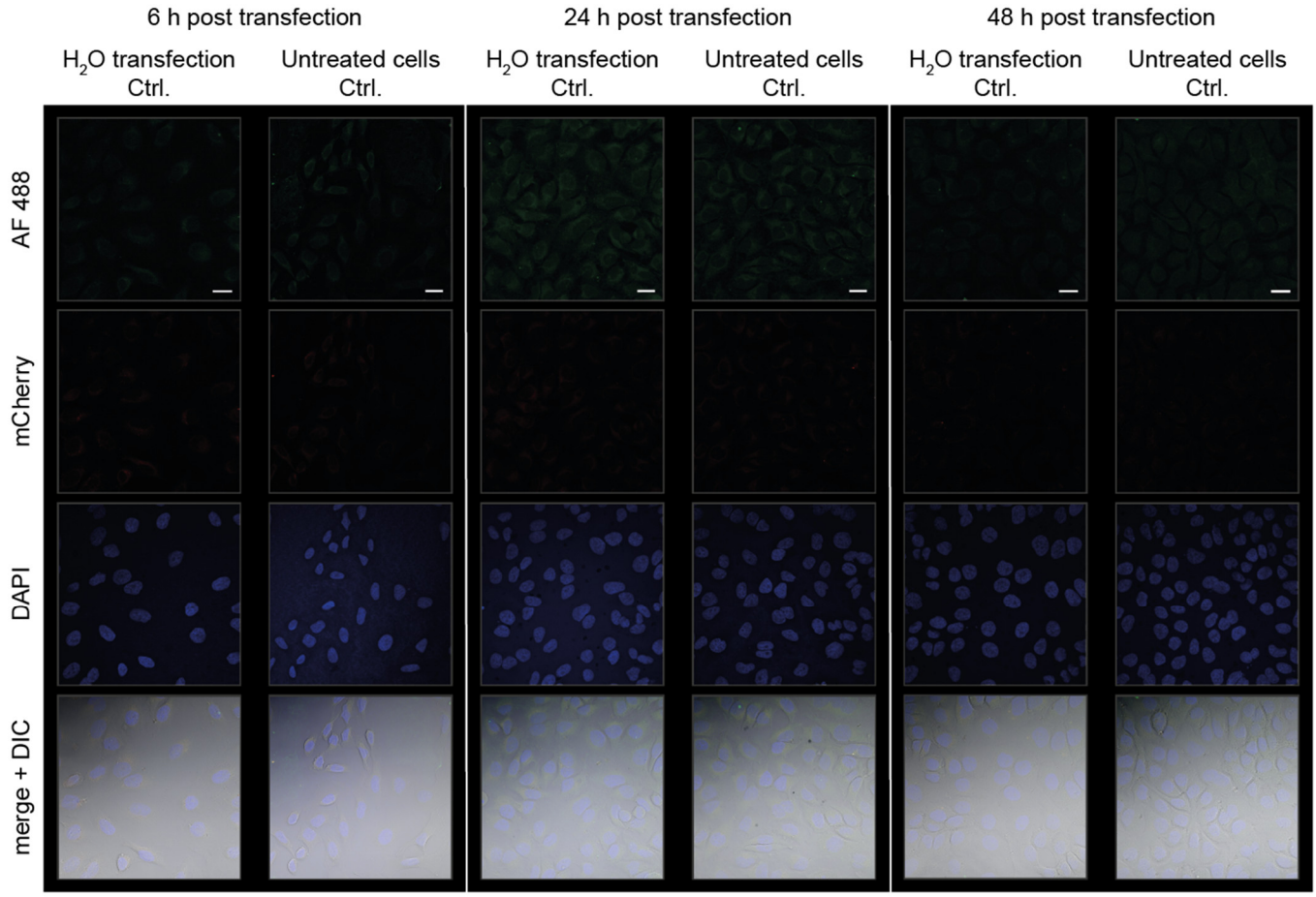
Supplementary Figure 22. Confocal fluorescence images of cells transfected with **mCh_mRNA$_{UTR\ 2}$**, 6 h post transfection.



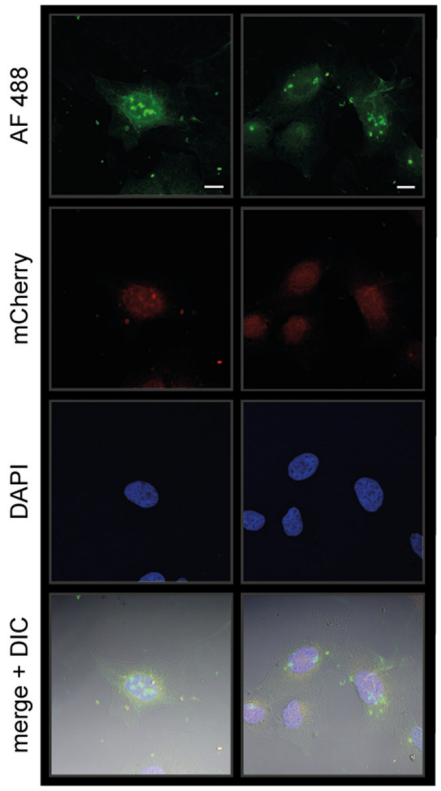
Supplementary Figure 23. Confocal fluorescence images of cells transfected with **mCh_mRNA$_{UTR\ 2}$**, 24 h post transfection.



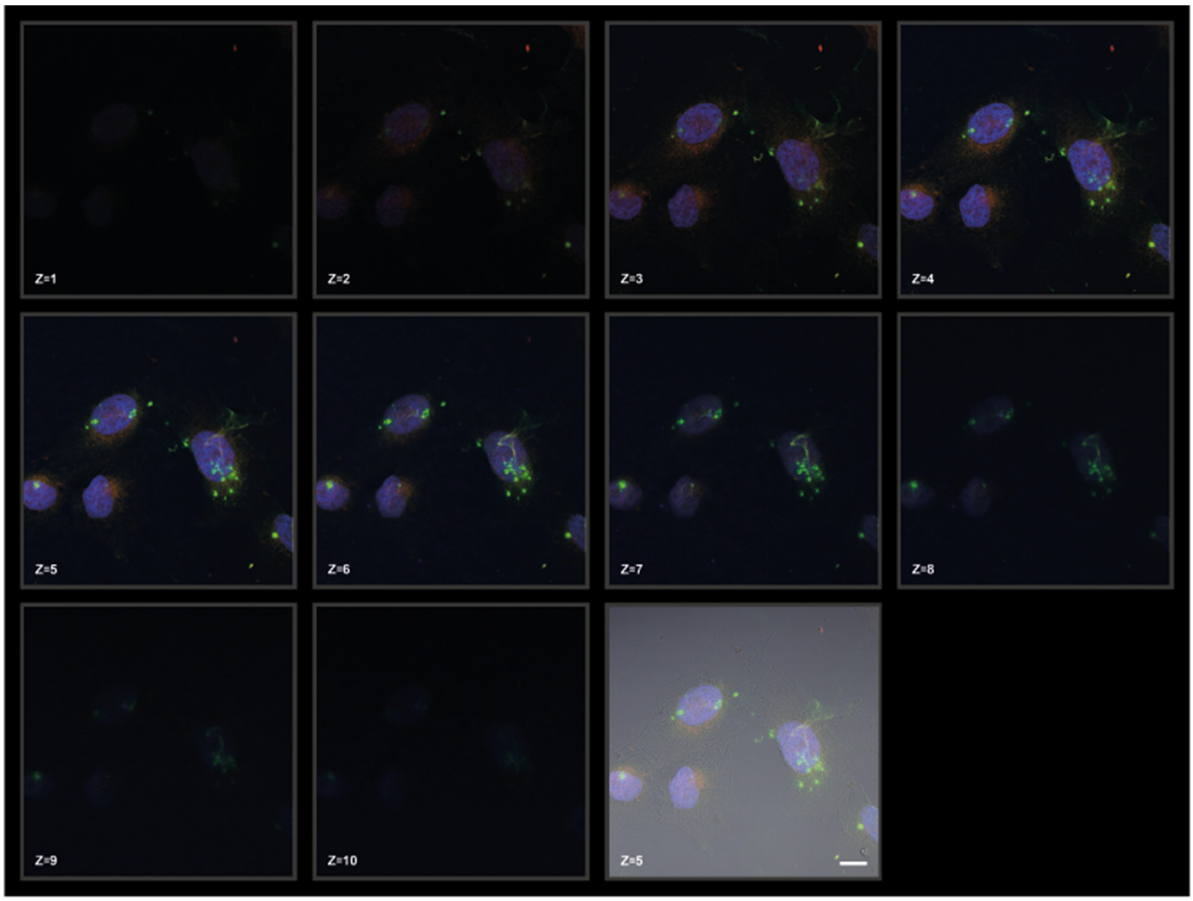
Supplementary Figure 24. Confocal fluorescence images of cells transfected with **mCh_mRNA$_{UTR\,2}$**, 48 h post transfection.



Supplementary Figure 25. Confocal fluorescence images of cells transfected with H_2O instead of mRNA or left untreated, 6, 24 and 48 h post transfection.



Supplementary Figure 26. Close-up images of mCh_mRNA$^{2\ CP}$, 6 h post transfection.

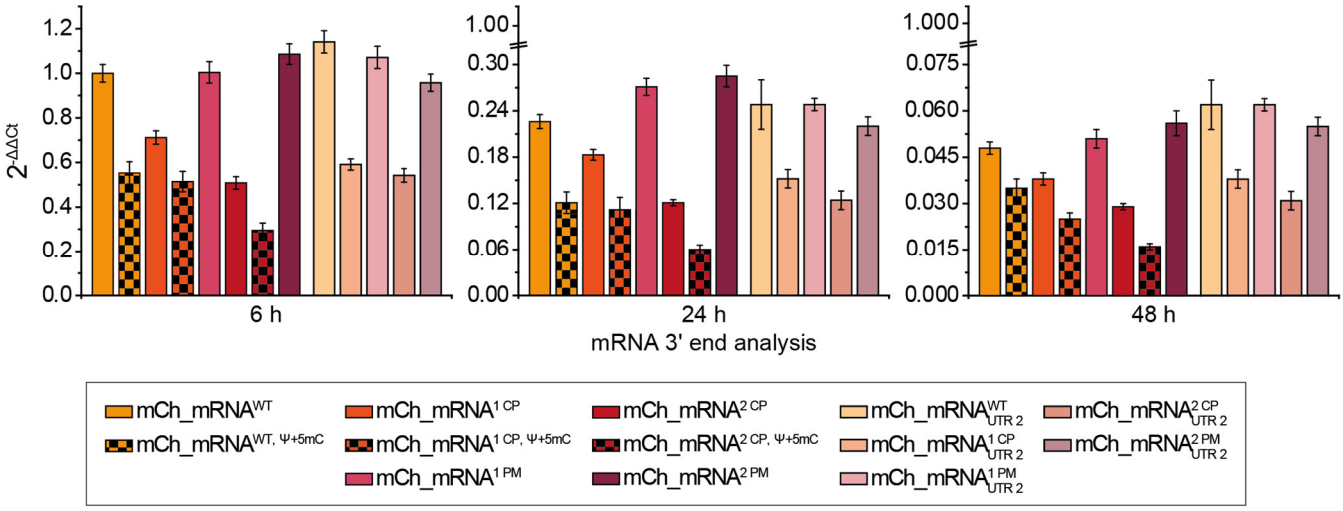


Supplementary Figure 27. Z-stack images of mCh_mRNA$^{2\ CP}$, 6 h post transfection.

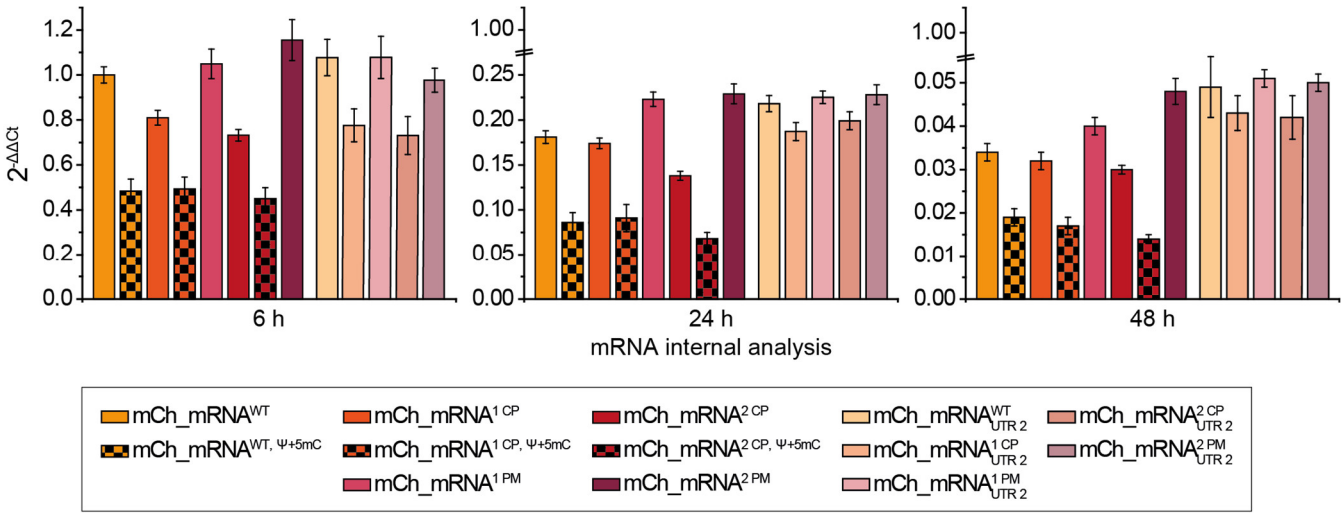
RT-qPCR analysis

qPCR was performed in a multiplex assay allowing for simultaneous analysis of mCh_mRNA at the mRNA 3' end and internal position together with GAPDH and β-Actin mRNA sequences which we choose as reference genes. ΔC_t values were calculated using GAPDH as the only reference gene. $\Delta\Delta C_t$ values were calculated by substracting the mCh_mRNA^{WT} ΔC_t value at 6 h post transfection from all other ΔC_t values of variant mCh_mRNAs and various time points. β-Actin C_t values were discarded for further data analysis due to lower accuracy in the overall course of experiments. Samples qPCR reaction products for each reaction were analysed by agarose gel electrophoresis. Also, sequences of obtained qPCR products were veryfied by Sanger sequencing and therefore needed to be extended. First, samples of qPCR products were taken and amplified by standard PCR reactions. PCR amplification was performed separately for all four qPCR amplicon sequences (mCh_qPCR^{internal}, mCh_qPCR$^{3'\text{-end}}$, GAPDH_qPCR and β-Actin_qPCR) with 2 µL of qPCR product in 100 µL scales containing a final concentration of 20 mM Tris-HCl pH 8.9, 22 mM NH_4Cl, 22 mM KCl, 1.8 mM $MgCl_2$, 0.06 % IGEPAL® CA-630, 0.05 % Tween® 20 (*OneTaq*® Standard Reaction Buffer, *New England Biolabs*), 375 µM each canonical dNTP (*Jena Bioscience*), 1 µM forward primer (mCh_qPCR^{internal}_FW or mCh_qPCR$^{3'\text{-end}}$_FW or GAPDH_qPCR_FW or β-Actin_qPCR_FW), 1 µM reverse primer (mCh_qPCR^{internal}_RV or mCh_qPCR$^{3'\text{-end}}$_RV or GAPDH_qPCR_RV or β-Actin_qPCR_RV) and 0.025 U µL^{-1} *OneTaq*® DNA Polymerase (*New England Biolabs*). PCR was performed with an initial denaturing step at 94 °C for 2 min, followed by 30 cycles of denaturing at 94 °C for 30 s, annealing at 60 °C for 40 s, elongation at 68 °C for 30 s and a final elongation step at 68 °C for 5 min. PCR products were analysed by agarose gel electrophoresis and purified using the *NucleoSpin*® Gel and PCR Clean-Up Kit (*Macherey-Nagel*) according to the manufacturer's protocol. Then, amplified and purified qPCR products were first phosphorylated in a 50 µL reaction mix with 2 µM qPCR product and containing a final concentration of 70 mM Tris-HCl pH 7.6, 10 mM $MgCl_2$, 5 mM DTT (T4 PNK Reaction Buffer, Thermo Fisher Scientific), 1 mM ATP and 0.2 U T4 Polynucleotide Kinase (Thermo Fisher Scientific). Phosphorylation reactions were incubated at 37 °C for 30 min, then heated to 65 °C for 20 min for enzyme inactivation and subsequently purified using a G-25 gel filtration column (GE healthcare) according to the manufacturers' protocol. Phosphorylated qPCR products were lyophilized and resuspended in 10 µL ddH_2O. Next, phosphorylated qPCR products were extended by ligation with a universal adapter and sequence specific splint DNA oligonucleotides (sequences in SI) for each qPCR product (mCh_qPCR^{internal} or mCh_qPCR$^{3'\text{-end}}$ or GAPDH_qPCR or β-Actin_qPCR). Ligation reactions were performed in a 20 µL reaction volume containing 50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 1 mM ATP, 10 mM DTT (T4 DNA Ligase Buffer, Thermo Fisher Scientific), 2.5 µM qPCR_adapter DNA oligonucleotide, 2.5 µM splint DNA oligonucleotide (mCh_qPCR^{internal}_splint or mCh_qPCR$^{3'\text{-end}}$_ splint or GAPDH_qPCR_ splint or β-Actin_qPCR_ splint), 2.5 µM phosphorylated and purified qPCR product (mCh_qPCR^{internal} or mCh_qPCR$^{3'\text{-end}}$ or GAPDH_qPCR or β-Actin_qPCR) and 40 U T4 DNA Ligase (Thermo Fisher Scientific). Before adding the enzyme, the remaining ligation mix was prepared separately and heated to 95 °C for 2 min, then chilled to 50 °C and incubated for 5 min followed by slow cooling to 16 °C with a cooling rate of 1 °C min^{-1}. Then, the T4 DNA Ligase was added, the ligation reaction mix was incubated at 16 °C overnight and finally heated to 65 °C for 10 min for enzyme inactivation. Ligation products were analysed and purified by analytic and preparative agarose gel electrophoresis, before ligated qPCR products were again amplified

via PCR. Amplification of ligated qPCR products was performed individually for each sequence (mCh_ext-qPCR^{internal} or mCh_ext-qPCR$^{3'\text{-end}}$ or GAPDH_ext-qPCR or β-Actin_ext-qPCR) with 70 ng ligated qPCR product in a 100 µL PCR reaction mix containing a final concentration of 20 mM Tris-HCl pH 8.9, 22 mM NH_4Cl, 22 mM KCl, 1.8 mM $MgCl_2$, 0.06 % IGEPAL® CA-630, 0.05 % Tween® 20 (*OneTaq*® Standard Reaction Buffer, *New England Biolabs*), 375 µM each canonical dNTP (*Jena Bioscience*), 1 µM ext-qPCR_FW forward primer, 1 µM reverse primer (mCh_ext-qPCR^{internal}_RV or mCh_ext-qPCR$^{3'\text{-end}}$_RV or GAPDH_ext-qPCR_RV or β-Actin_ext-qPCR_RV) and 0.025 U µL^{-1} *OneTaq*® DNA Polymerase (*New England Biolabs*). PCR was performed with an initial denaturing step at 94 °C for 2 min, followed by 30 cycles of denaturing at 94 °C for 30 s, annealing at 57 °C for 40 s, elongation at 68 °C for 30 s and a final elongation step at 68 °C for 5 min. PCR products were analysed by agarose gel electrophoresis and purified using the *NucleoSpin*® Gel and PCR Clean-Up Kit (*Macherey-Nagel*) according to the manufacturer's protocol.



Supplementary Figure 28. RT-qPCR analysis at 3'-end section of mCh_mRNA and mCh_mRNA$_{UTR\ 2}$ sequences. Subsets shown in Figure 4 of the main text.



Supplementary Figure 29. RT-qPCR analysis at internal section of mCh_mRNA and mCh_mRNA$_{UTR\ 2}$ sequences. Subsets shown in Figure 4 of the main text.

mCh_mRNA + mCh_mRNA$_{UTR2}$ data set 1, cell lysis 6 h post transfection

C_t values

	Duplicate Samples	Technical Triplicats	Ct values			
			mCh 3' end: FAM	mCh internal: HEX	β-Actin: ROX	GAPDH: Cy5
mCh_mRNA^{WT}	#1	#1.1	16.45	16.43	19.04	22.21
		#1.2	16.32	16.32	19.02	22.15
		#1.3	16.29	16.35	19	22.15
	#1 NRT		36.19	34.9	0	0
	#2	#2.1	16.32	16.33	17.69	22.02
		#2.2	16.14	16.17	18.78	22.04
		#2.3	16.1	16.2	18.73	21.92
	#2 NRT		0	0	0	0
	mean		16.27	16.3	18.71	22.08
	SD		0.08	0.06	0.26	0.04
mCh_mRNA$^{1\ CP}$	#1	#1.1	16.96	16.88	17.76	21.89
		#1.2	16.96	16.85	20.34	21.93
		#1.3	16.92	16.89	18.02	21.87
	#1 NRT		0	0	0	38.55
	#2	#2.1	16.69	16.6	20.26	21.95
		#2.2	16.64	16.43	20.24	21.79
		#2.3	16.55	16.36	17.23	21.83
	#2 NRT		0	0	0	40.22
	mean		16.79	16.67	18.98	21.88
	SD		0.04	0.06	1.29	0.05
mCh_mRNA$^{2\ CP}$	#1	#1.1	17.32	16.72	16.63	21.77
		#1.2	17.2	16.96	15.54	21.66
		#1.3	17.18	16.72	16.52	21.67
	#1 NRT		0	0	0	0
	#2	#2.1	17.12	16.57	16.7	21.78
		#2.2	17.09	16.57	16.57	21.8
		#2.3	17.25	16.87	15.88	21.82
	#2 NRT		0	0	0	37.14
	mean		17.19	16.74	16.31	21.75
	SD		0.07	0.13	0.42	0.03
mCh_mRNA$^{1\ PM}$	#1	#1.1	16.43	16.37	16.94	21.91
		#1.2	16.25	16.33	15.23	21.72
		#1.3	16.25	16.32	20.18	21.75
	#1 NRT		0	0	0	0
	#2	#2.1	16.3	16.29	20.19	21.77
		#2.2	16.31	16.9	15.77	21.8

		#2.3	16.35	16.57	15.95	21.82
	#2 NRT		0	0	0	0
	mean		16.32	16.46	17.38	21.80
	SD		0.05	0.14	2.05	0.05
mCh_mRNA$^{2\ PM}$	**#1**	**#1.1**	16.33	16.6	16.61	22.01
		#1.2	16.19	16.18	16.29	21.79
		#1.3	16.23	16.22	17.23	21.89
	#1 NRT		0	0	0	41.51
	#2	**#2.1**	16.23	16.11	16.34	21.85
		#2.2	16.23	16.54	17.16	21.89
		#2.3	16.24	16.54	16.88	21.83
	#2 NRT		0	0	0	39.46
	mean		16.24	16.37	16.75	21.88
	SD		0.03	0.20	0.37	0.06
mCh_mRNA$_{UTR2}^{WT}$	**#1**	**#1.1**	16.06	16.28	16.77	21.86
		#1.2	16.1	16.38	20.61	21.72
		#1.3	16.18	16.49	17	21.86
	#1 NRT		0	0	0	0
	#2	**#2.1**	16.28	16.83	17.09	21.97
		#2.2	16.17	17.06	17.11	21.92
		#2.3	16.14	16.36	20.75	21.86
	#2 NRT		0	0	0	0
	mean		16.16	16.57	18.22	21.87
	SD		0.06	0.19	1.74	0.06
mCh_mRNA$_{UTR2}^{1\ CP}$	**#1**	**#1.1**	17.06	17.12	17.27	21.83
		#1.2	17.14	17.35	16.69	21.71
		#1.3	17.15	17.33	15.51	21.78
	#1 NRT		0	0	0	38.62
	#2	**#2.1**	16.92	17.31	15.54	21.71
		#2.2	16.77	17.11	16.08	21.65
		#2.3	16.81	16.39	16.87	21.53
	#2 NRT		0	0	0	0
	mean		16.98	17.10	16.33	21.70
	SD		0.05	0.25	0.64	0.06
mCh_mRNA$_{UTR2}^{2\ CP}$	**#1**	**#1.1**	17.22	16.79	15.09	21.72
		#1.2	17.06	16.68	14.99	21.71
		#1.3	16.98	16.66	15.62	21.65
	#1 NRT		0	0	0	0
	#2	**#2.1**	17.49	17.88	17.18	22.09
		#2.2	17.44	18.06	15.68	22.04
		#2.3	17.21	16.77	16.56	21.82

	#2 NRT		0	0	0	0
	mean		17.23	17.14	15.85	21.84
	SD		0.11	0.31	0.45	0.07
mCh_mRNA$_{UTR2}$$^{1\ PM}$	**#1**	**#1.1**	16.19	16.5	20.16	21.82
		#1.2	16.2	16.32	15.48	21.67
		#1.3	16.19	16.15	16.72	21.69
	#1 NRT		0	0	0	38.66
	#2	**#2.1**	16.24	16.34	16.27	21.8
		#2.2	16.23	16.86	14.61	21.79
		#2.3	16.18	16.3	16.35	21.78
	#2 NRT		0	0	0	0
	mean		16.21	16.41	16.60	21.76
	SD		0.02	0.20	1.39	0.04
mCh_mRNA$_{UTR2}$$^{2\ PM}$	**#1**	**#1.1**	16.54	16.86	16.11	21.82
		#1.2	16.47	16.61	16.23	21.77
		#1.3	16.44	17.02	16.96	21.71
	#1 NRT		0	0	0	35.67
	#2	**#2.1**	16.48	16.56	16.19	21.69
		#2.2	16.4	16.51	15.35	21.7
		#2.3	16.43	16.62	15.51	21.73
	#2 NRT		0	0	0	0
	mean		16.46	16.70	16.06	21.74
	SD		0.04	0.11	0.37	0.03
H_2O Ctrl.	**#1**	**#1.1**	0	0	18.77	21.57
		#1.2	0	0	16.8	21.54
	#1 NRT		0	0	0	41.76
	#2	**#2.1**	0	0	15.84	21.41
		#2.2	0	0	16.66	21.38
	#2 NRT		0	0	0	37.91
	mean		0	0	17.02	21.48
	SD		0	0	0.70	0.02
Untreated Cells	**#1**	**#1.1**	0	0	17.89	21.64
		#1.2	0	0	15.7	21.47
		#1.3	0	0	15.66	21.47
	#1 NRT		0	0	0	39.81
	#2	**#2.1**	0	0	16.46	21.45
		#2.2	0	0	15.65	21.43
		#2.3	0	0	15.46	21.41
	#2 NRT		0	0	0	0
	mean		0	0	16.14	21.48
	SD		0	0	0.74	0.05
NTC	**#1**		0	0	0	0
	#2		0	0	0	0

$\Delta C_t = C_t(mCh) - C_t(GAPDH)$

	ΔCt (mCh 3'end)	error	ΔCt (mCh internal)	error
mCh_mRNA^{WT}	-5.81	0.09	-5.78	0.07
mCh_mRNA$^{1\ CP}$	-5.09	0.06	-5.21	0.08
mCh_mRNA$^{2\ CP}$	-4.56	0.07	-5.02	0.13
mCh_mRNA$^{1\ PM}$	-5.48	0.07	-5.33	0.15
mCh_mRNA$^{2\ PM}$	-5.64	0.07	-5.51	0.20
mCh_mRNA$_{UTR2}^{WT}$	-5.71	0.08	-5.30	0.20
mCh_mRNA$_{UTR2}^{1\ CP}$	-4.73	0.08	-4.60	0.26
mCh_mRNA$_{UTR2}^{2\ CP}$	-4.61	0.13	-4.70	0.32
mCh_mRNA$_{UTR2}^{1\ PM}$	-5.55	0.04	-5.35	0.20
mCh_mRNA$_{UTR2}^{2\ PM}$	-5.28	0.05	-5.04	0.11
H_2O Ctrl.	-21.48	0.02	-21.48	0.02
Untreated cells	-21.48	0.05	-21.48	0.05

mCh_mRNA + mCh_mRNA$_{UTR2}$ data set 1, cell lysis 24 h post transfection

C_t values

	Duplicate Samples	Technical Triplicats	Ct values			
			mCh 3' end: FAM	mCh internal: HEX	β-Actin: ROX	GAPDH: Cy5
mCh_mRNA^{WT}	#1	#1.1	18.7	19.1	19.15	21.71
		#1.2	18.54	19.1	19.44	21.75
		#1.3	18.54	19.09	19.17	21.73
	#1 NRT		0	0	0	0
	#2	#2.1	19.02	19.59	19.92	22.37
		#2.2	18.9	19.62	19.17	22.33
		#2.3	18.95	19.56	19.01	22.39
	#2 NRT		0	0	0	0
	mean		18.78	19.34	19.31	22.05
	SD		0.06	0.01	0.26	0.02
mCh_mRNA$^{1\ CP}$	#1	#1.1	19.2	19.49	19.43	21.79
		#1.2	19.16	19.48	18.99	21.75
		#1.3	19.02	19.44	19.19	21.72
	#1 NRT		0	0	0	0
	#2	#2.1	19.21	19.52	18.66	21.84
		#2.2	19.1	19.6	19.24	21.92
		#2.3	19.17	19.59	19.18	21.9
	#2 NRT		0	0	0	36.91
	mean		19.14	19.52	19.12	21.82

	SD		0.06	0.03	0.22	0.03
mCh_mRNA$^{2\ CP}$	**#1**	**#1.1**	19.8	19.82	19.25	21.88
		#1.2	19.72	20.07	19.44	21.81
		#1.3	19.75	19.95	18.26	21.83
	#1 NRT		0	0	0	39.9
	#2	**#2.1**	19.7	19.83	18.61	21.82
		#2.2	19.59	19.9	18.86	21.74
		#2.3	19.59	19.92	18.81	21.72
	#2 NRT		0	0	0	0
	mean		19.69	19.92	18.87	21.80
	SD		0.04	0.07	0.31	0.04
mCh_mRNA$^{1\ PM}$	**#1**	**#1.1**	18.82	19.27	19.57	21.98
		#1.2	18.69	19.29	18.76	21.91
		#1.3	18.72	19.29	18.43	21.89
	#1 NRT		0	0	0	0
	#2	**#2.1**	18.76	19.29	19.22	22.05
		#2.2	18.68	19.28	18.71	22
		#2.3	18.7	19.24	18.43	22.04
	#2 NRT		0	0	0	41.58
	mean		18.73	19.28	18.85	21.98
	SD		0.04	0.02	0.40	0.03
mCh_mRNA$^{2\ PM}$	**#1**	**#1.1**	18.7	19.2	19.05	21.86
		#1.2	18.52	19.07	18.12	21.71
		#1.3	18.58	19.11	18.67	21.75
	#1 NRT		0	0	0	38.95
	#2	**#2.1**	18.78	19.32	19.18	22.04
		#2.2	18.64	19.21	19.02	22
		#2.3	18.62	19.26	18.94	21.96
	#2 NRT		0	39.72	0	37.18
	mean		18.64	19.20	18.83	21.89
	SD		0.07	0.05	0.24	0.05
mCh_mRNA$_{UTR2}^{WT}$	**#1**	**#1.1**	18.65	19.17	17.69	21.78
		#1.2	18.56	19.15	18.56	21.73
		#1.3	18.6	19.13	18.71	21.76
	#1 NRT		0	0	0	0
	#2	**#2.1**	18.42	18.91	18.27	21.69
		#2.2	18.42	19.09	18.11	21.73
		#2.3	18.33	18.83	18.65	21.66
	#2 NRT		0	0	0	0
	mean		18.50	19.05	18.33	21.73
	SD		0.04	0.06	0.34	0.02

mCh_mRNA$_{UTR2}$$^{1\ CP}$	**#1**	**#1.1**	19.51	19.47	19.54	22.01
		#1.2	19.27	19.21	18.55	21.74
		#1.3	19.28	19.19	17.51	21.74
	#1 NRT		0	0	0	0
	#2	**#2.1**	19.35	19.24	18.53	21.73
		#2.2	19.3	19.21	18.02	21.71
		#2.3	19.3	19.24	18.56	21.74
	#2 NRT		0	0	0	0
	mean		19.34	19.26	18.45	21.78
	SD		0.07	0.07	0.54	0.07
mCh_mRNA$_{UTR2}$$^{2\ CP}$	**#1**	**#1.1**	19.59	19.45	19.02	21.92
		#1.2	19.5	19.36	18.16	21.84
		#1.3	19.44	19.22	18.55	21.76
	#1 NRT		0	0	0	0
	#2	**#2.1**	19.67	19.44	18.84	21.89
		#2.2	19.62	19.46	18.8	21.86
		#2.3	19.62	19.6	17.74	21.89
	#2 NRT		0	0	0	40.95
	mean		19.57	19.42	18.52	21.86
	SD		0.04	0.08	0.43	0.04
mCh_mRNA$_{UTR2}$$^{1\ PM}$	**#1**	**#1.1**	18.64	19.14	19.31	21.92
		#1.2	18.53	19.04	18.64	21.85
		#1.3	18.52	19.09	19.12	21.81
	#1 NRT		0	0	0	37.4
	#2	**#2.1**	18.73	19.22	19.51	22
		#2.2	18.62	19.19	18.31	21.94
		#2.3	18.61	19.19	19.16	21.95
	#2 NRT		0	0	0	38.98
	mean		18.61	19.15	19.01	21.91
	SD		0.05	0.03	0.39	0.04
mCh_mRNA$_{UTR2}$$^{2\ PM}$	**#1**	**#1.1**	18.74	19.12	18.92	21.94
		#1.2	18.75	19.19	18.67	21.94
		#1.3	18.65	19.04	18.17	21.83
	#1 NRT		0	0	0	0
	#2	**#2.1**	18.66	19.05	17.87	21.71
		#2.2	18.8	19.23	18.3	22
		#2.3	18.67	19.13	19.03	21.9
	#2 NRT		0	0	0	40.25
	mean		18.71	19.13	18.49	21.89
	SD		0.05	0.07	0.40	0.09
H_2O Ctrl.	**#1**	**#1.1**	0	0	20.43	22.02
		#1.2	0	0	18.88	21.93
	#1 NRT		0	0	0	0

	#2	**#2.1**	0	0	18.11	21.84
		#2.2	0	0	18.62	21.67
	#2 NRT		0	0	0	0
	mean		0.00	0.00	19.01	21.87
	SD		0.00	0.00	0.52	0.06
Untreated Cells	**#1**	**#1.1**	0	0	19.73	21.87
		#1.2	0	0	19.03	21.9
		#1.3	0	0	19.01	21.82
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	18.33	21.93
		#2.2	0	0	19.38	21.86
		#2.3	0	0	18.8	21.88
	#2 NRT		0	0	0	0
	mean		0.00	0.00	19.05	21.88
	SD		0.00	0.00	0.38	0.03
NTC	**#1**		0	0	0	0
	#2		0	0	0	0

$\Delta C_t = C_t(mCh) - C_t(GAPDH)$

	ΔCt (mCh 3'end)	**error**	**ΔCt (mCh internal)**	**error**
mCh_mRNA^{WT}	-3.27	0.07	-2.70	0.03
mCh_mRNA$^{1\ CP}$	-2.68	0.07	-2.30	0.04
mCh_mRNA$^{2\ CP}$	-2.11	0.06	-1.89	0.08
mCh_mRNA$^{1\ PM}$	-3.25	0.05	-2.70	0.03
mCh_mRNA$^{2\ PM}$	-3.25	0.09	-2.69	0.07
mCh_mRNA$_{UTR2}^{WT}$	-3.23	0.05	-2.68	0.07
mCh_mRNA$_{UTR2}^{1\ CP}$	-2.44	0.10	-2.52	0.10
mCh_mRNA$_{UTR2}^{2\ CP}$	-2.29	0.06	-2.44	0.09
mCh_mRNA$_{UTR2}^{1\ PM}$	-3.30	0.07	-2.77	0.05
mCh_mRNA$_{UTR2}^{2\ PM}$	-3.18	0.10	-2.76	0.11
H_2O Ctrl.	-21.87	0.06	-21.87	0.06
Untreated cells	-21.88	0.03	-21.88	0.03

mCh_mRNA + mCh_mRNA$_{UTR2}$ data set 1, cell lysis 48 h post transfection

C_t values

	Duplicate Samples	**Technical Triplicats**	**Ct values**			
			mCh 3' end: FAM	**mCh internal: HEX**	**β-Actin: ROX**	**GAPDH: Cy5**
mCh_mRNA^{WT}	**#1**	**#1.1**	20.92	21.5	19.65	21.68
		#1.2	21.25	21.74	19.65	22.1

		#1.3	21.17	21.66	19.7	22.01
	#1 NRT		0	0	0	0
	#2	**#2.1**	21.47	22.07	20.17	22.14
		#2.2	21.27	21.85	19	21.94
		#2.3	21.2	21.82	18.56	21.91
	#2 NRT		38.33	40.72	30.2	31.91
	mean		21.21	21.77	19.46	21.96
	SD		0.13	0.11	0.35	0.14
mCh_mRNA$^{1\ CP}$	**#1**	**#1.1**	22.05	22.37	20.06	22.28
		#1.2	21.73	22.15	19.24	22.13
		#1.3	21.55	22.01	19.56	21.96
	#1 NRT		0	0	0	41.42
	#2	**#2.1**	21.67	22.06	19.14	22.18
		#2.2	21.46	21.82	19.09	22.02
		#2.3	21.33	21.71	19.01	21.9
	#2 NRT		0	0	0	0
	mean		21.63	22.02	19.35	22.08
	SD		0.17	0.15	0.20	0.12
mCh_mRNA$^{2\ CP}$	**#1**	**#1.1**	22.15	22.04	20.01	22.15
		#1.2	22.14	22.08	19.09	22.17
		#1.3	22.14	22.12	19.99	22.16
	#1 NRT		0	0	0	0
	#2	**#2.1**	22.2	22.15	19.55	22.29
		#2.2	22.05	22.03	18.92	22.17
		#2.3	21.76	21.83	19.15	22.02
	#2 NRT		0	0	0	0
	mean		22.07	22.04	19.45	22.16
	SD		0.09	0.08	0.34	0.06
mCh_mRNA$^{1\ PM}$	**#1**	**#1.1**	21.34	21.77	19.85	22.19
		#1.2	21.06	21.43	18.91	21.77
		#1.3	21.04	21.57	18.82	22.01
	#1 NRT		0	0	0	0
	#2	**#2.1**	21.28	21.99	19.91	22.28
		#2.2	21.23	21.93	19.87	22.24
		#2.3	21.2	21.99	19.74	22.25
	#2 NRT		0	0	0	0
	mean		21.19	21.78	19.52	22.12
	SD		0.08	0.08	0.27	0.09
mCh_mRNA$^{2\ PM}$	**#1**	**#1.1**	21.13	21.46	19.53	22.09
		#1.2	20.77	21.32	19.44	21.74
		#1.3	21.25	21.69	20.28	22.2

	#1 NRT		0	0	0	0
	#2	**#2.1**	21.09	21.56	19.6	22.11
		#2.2	20.96	21.34	19.47	22.13
		#2.3	20.83	21.27	19.8	22.01
	#2 NRT		0	0	0	0
	mean		21.01	21.44	19.69	22.05
	SD		0.16	0.14	0.26	0.12
mCh_mRNA$_{UTR2}^{WT}$	**#1**	**#1.1**	20.64	21.15	19.06	21.76
		#1.2	20.54	21.07	18.68	21.7
		#1.3	20.86	21.34	19.31	22.1
	#1 NRT		0	0	0	0
	#2	**#2.1**	20.41	20.92	18.56	21.45
		#2.2	21.3	21.87	20.01	22.37
		#2.3	20.71	21.23	19.32	21.78
	#2 NRT		0	0	0	0
	mean		20.74	21.26	19.16	21.86
	SD		0.25	0.25	0.43	0.28
mCh_mRNA$_{UTR2}^{1\ CP}$	**#1**	**#1.1**	22.03	22.04	19.34	22.28
		#1.2	21.58	21.63	19.04	22
		#1.3	21.6	21.63	18.78	21.97
	#1 NRT		0	0	0	0
	#2	**#2.1**	21.69	21.8	18.83	22.12
		#2.2	21.47	21.55	19.1	21.92
		#2.3	21.29	21.38	18.86	21.72
	#2 NRT		0	0	0	0
	mean		21.61	21.67	18.99	22.00
	SD		0.19	0.18	0.17	0.15
mCh_mRNA$_{UTR2}^{2\ CP}$	**#1**	**#1.1**	22.3	22.11	19.96	22.29
		#1.2	22.18	21.99	19.27	22.15
		#1.3	21.64	21.52	19.25	21.59
	#1 NRT		0	0	0	0
	#2	**#2.1**	21.88	21.56	18.99	21.89
		#2.2	22.03	21.76	18.86	22.04
		#2.3	22.14	21.99	19.6	22.21
	#2 NRT		0	0	0	0
	mean		22.03	21.82	19.32	22.03
	SD		0.20	0.22	0.33	0.22
mCh_mRNA$_{UTR2}^{1\ PM}$	**#1**	**#1.1**	21	21.34	19.6	22.11
		#1.2	20.95	21.36	18.57	21.98
		#1.3	20.99	21.41	19.21	22.02
	#1 NRT		0	0	0	39.63
	#2	**#2.1**	21.09	21.53	19.3	22.18

		#2.2	21	21.43	19.17	22.1
		#2.3	21.06	21.57	19.34	22.18
	#2 NRT		0	0	0	0
	mean		21.02	21.44	19.20	22.10
	SD		0.03	0.04	0.25	0.05
mCh_mRNA$_{UTR2}^{2\ PM}$	**#1**	**#1.1**	21.01	21.25	19.33	22.05
		#1.2	20.77	21.05	19.16	21.85
		#1.3	21.03	21.31	19.1	21.95
	#1 NRT		0	0	0	0
	#2	**#2.1**	21.08	21.39	19.14	22.04
		#2.2	21.15	21.52	19.29	22.12
		#2.3	21.13	21.5	19.3	22.11
	#2 NRT		0	0	0	0
	mean		21.03	21.34	19.22	22.02
	SD		0.07	0.08	0.09	0.06
H_2O Ctrl.	**#1**	**#1.1**	0	0	21.35	22.69
		#1.2	0	0	19.81	22.31
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	19.68	22.47
		#2.2	0	0	19.51	22.52
	#2 NRT		0	0	0	0
	mean		0.00	0.00	20.09	22.50
	SD		0.00	0.00	0.43	0.11
Untreated Cells	**#1**	**#1.1**	0	0	20.11	22.89
		#1.2	0	0	19.33	22.34
		#1.3	0	0	19.33	22.35
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	19.5	22.43
		#2.2	0	0	19.57	22.51
		#2.3	0	0	19.09	22.41
	#2 NRT		0	0	0	0
	mean		0.00	0.00	19.49	22.49
	SD		0.00	0.00	0.29	0.15
NTC	**#1**		0	0	0	0
	#2		0	0	0	0

$\Delta C_t = C_t(mCh) - C_t(GAPDH)$

	ΔCt (mCh 3'end)	**error**	**ΔCt (mCh internal)**	**error**
mCh_mRNA^{WT}	-0.75	0.19	-0.19	0.18
mCh_mRNA$^{1\ CP}$	-0.45	0.21	-0.06	0.19
mCh_mRNA$^{2\ CP}$	-0.09	0.11	-0.12	0.10
mCh_mRNA$^{1\ PM}$	-0.93	0.13	-0.34	0.13

mCh_mRNA$^{2\ PM}$	-1.04	0.20	-0.61	0.19
mCh_mRNA$_{UTR2}^{WT}$	-1.12	0.38	-0.60	0.38
mCh_mRNA$_{UTR2}^{1\ CP}$	-0.39	0.24	-0.33	0.24
mCh_mRNA$_{UTR2}^{2\ CP}$	0.00	0.29	-0.21	0.31
mCh_mRNA$_{UTR2}^{1\ PM}$	-1.08	0.05	-0.65	0.06
mCh_mRNA$_{UTR2}^{2\ PM}$	-0.99	0.09	-0.68	0.10
H_2O Ctrl.	-22.50	0.11	-22.50	0.11
Untreated cells	-22.49	0.15	-22.49	0.15

mCh_mRNA + mCh_mRNA$_{UTR2}$ data set 2, cell lysis 6 h post transfection

C_t values

	Duplicate Samples	Technical Triplicats	Ct values			
			mCh 3' end: FAM	mCh internal: HEX	β-Actin: ROX	GAPDH: Cy5
mCh_mRNA^{WT}	**#1**	**#1.1**	16.38	16.55	18.7	21.31
		#1.2	16.19	16.37	20.86	21.2
		#1.3	16.22	16.43	18.24	21.21
	#1 NRT		0	0	0	0
	#2	**#2.1**	16.19	16.44	18.62	21.12
		#2.2	16.2	16.46	18.12	21.2
		#2.3	16.25	16.5	18.15	21.24
	#2 NRT		0	0	0	0
	mean		16.24	16.46	18.78	21.21
	SD		0.05	0.05	0.69	0.05
mCh_mRNA$^{1\ CP}$	**#1**	**#1.1**	16.97	17.01	18.35	21.25
		#1.2	16.96	16.83	18.48	21.27
		#1.3	16.71	16.82	18.7	21.25
	#1 NRT		0	0	0	0
	#2	**#2.1**	16.72	16.87	18.17	21.23
		#2.2	16.78	16.98	18.23	21.3
		#2.3	16.68	16.87	17.64	21.22
	#2 NRT		0	0	0	0
	mean		16.80	16.90	18.26	21.25
	SD		0.08	0.07	0.20	0.02
mCh_mRNA$^{2\ CP}$	**#1**	**#1.1**	17.29	16.84	18.33	21.23
		#1.2	17.28	16.83	18.1	21.24
		#1.3	17.29	16.88	18.52	21.26
	#1 NRT		0	0	0	0
	#2	**#2.1**	17.28	17.09	18.38	21.14
		#2.2	17.25	17.07	18.36	21.15

		#2.3	17.27	17.1	18.09	21.18
	#2 NRT		0	36.48	0	0
	mean		17.28	16.97	18.30	21.20
	SD		0.01	0.02	0.15	0.01
mCh_mRNA$^{1\ PM}$	**#1**	**#1.1**	16.45	16.62	18.76	21.46
		#1.2	16.42	16.64	21.39	21.46
		#1.3	16.41	16.61	18.14	21.28
	#1 NRT		0	0	0	0
	#2	**#2.1**	16.45	16.7	18.2	21.2
		#2.2	16.53	16.75	18.18	21.34
		#2.3	16.57	16.82	18.48	21.35
	#2 NRT		0	0	0	0
	mean		16.47	16.69	18.86	21.35
	SD		0.03	0.03	0.77	0.08
mCh_mRNA$^{2\ PM}$	**#1**	**#1.1**	16.39	16.57	18.48	21.29
		#1.2	16.34	16.54	18.58	21.27
		#1.3	16.35	16.56	20.92	21.43
	#1 NRT		0	0	0	0
	#2	**#2.1**	16.31	16.46	18.5	21.32
		#2.2	16.32	16.52	21.36	21.19
		#2.3	16.32	16.54	20.84	21.22
	#2 NRT		0	39.9	0	0
	mean		16.34	16.53	19.78	21.29
	SD		0.01	0.02	1.19	0.06
mCh_mRNA$_{UTR2}^{WT}$	**#1**	**#1.1**	16.23	16.51	20.82	21.25
		#1.2	16.13	16.38	20.75	21.16
		#1.3	16.15	16.39	20.8	21.21
	#1 NRT		0	0	0	0
	#2	**#2.1**	16.14	16.39	18.31	21.08
		#2.2	16.17	16.37	18.42	21.21
		#2.3	16.11	16.38	17.83	21.12
	#2 NRT		0	0	0	0
	mean		16.16	16.40	19.49	21.17
	SD		0.03	0.03	0.14	0.05
mCh_mRNA$_{UTR2}^{1\ CP}$	**#1**	**#1.1**	17.21	16.82	18.45	21.3
		#1.2	17.12	16.71	18.01	21.22
		#1.3	17.09	16.65	17.52	21.19
	#1 NRT		0	37.21	33.7	36.41
	#2	**#2.1**	17.02	16.59	17.92	21.12
		#2.2	17.02	16.59	17.73	21.14

		#2.3	17.03	16.63	18.22	21.13
	#2 NRT		0	41.37	0	0
	mean		17.08	16.67	17.98	21.18
	SD		0.03	0.04	0.29	0.03
mCh_mRNA$_{UTR2}^{2\ CP}$	**#1**	**#1.1**	17.37	16.94	18.03	21.45
		#1.2	17.28	17.03	18	21.38
		#1.3	17.29	17.03	18.01	21.37
	#1 NRT		0	0	0	0
	#2	**#2.1**	17.38	17.12	17.7	21.22
		#2.2	17.33	17.11	18.3	21.2
		#2.3	17.36	17.12	18.04	21.22
	#2 NRT		0	0	0	0
	mean		17.34	17.06	18.01	21.31
	SD		0.03	0.02	0.13	0.02
mCh_mRNA$_{UTR2}^{1\ PM}$	**#1**	**#1.1**	16.42	16.64	18.51	21.28
		#1.2	16.37	16.6	18.47	21.26
		#1.3	16.38	16.59	20.76	21.39
	#1 NRT		0	0	0	0
	#2	**#2.1**	16.4	16.62	18.27	21.49
		#2.2	16.26	16.49	17.9	21.24
		#2.3	16.39	16.9	18.37	21.51
	#2 NRT		0	0	0	0
	mean		16.37	16.64	18.71	21.36
	SD		0.04	0.10	0.64	0.09
mCh_mRNA$_{UTR2}^{2\ PM}$	**#1**	**#1.1**	16.52	16.68	17.98	21.5
		#1.2	16.44	16.63	18.19	21.45
		#1.3	16.45	16.6	18.24	21.43
	#1 NRT		0	41.06	0	40.2
	#2	**#2.1**	16.51	16.61	18.3	21.3
		#2.2	16.4	16.56	18.3	21.25
		#2.3	16.34	16.79	21.31	21.4
	#2 NRT		0	40.45	0	0
	mean		16.44	16.65	18.72	21.39
	SD		0.05	0.07	0.77	0.05
H_2O Ctrl.	**#1**	**#1.1**	0	39.85	18.85	21.51
		#1.2	0	40.34	17.97	21.43
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	18.2	21.31
		#2.2	0	0	18.94	21.31
	#2 NRT		0	0	0	0
	mean		0.00	20.05	18.49	21.39
	SD		0.00	0.12	0.41	0.02
Untreated Cells	**#1**	**#1.1**	34.49	37.09	18.33	21.41
		#1.2	33.39	36.73	17.94	21.34

		#1.3	34.61	37.27	17.95	21.37
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	18.13	21.3
		#2.2	0	0	18.38	21.29
		#2.3	0	0	18.13	21.33
	#2 NRT		0	0	0	0
	mean		17.08	18.52	18.14	21.34
	SD		0.27	0.11	0.15	0.02
NTC	**#1**		0	0	0	0
	#2		0	0	0	0

$\Delta C_t = C_t(mCh) - C_t(GAPDH)$

	ΔCt (mCh 3'end)	**error**	**ΔCt (mCh internal)**	**error**
mCh_mRNA^{WT}	-4.98	0.07	-4.76	0.07
mCh_mRNA$^{1\ CP}$	-4.45	0.08	-4.36	0.07
mCh_mRNA$^{2\ CP}$	-3.92	0.02	-4.23	0.02
mCh_mRNA$^{1\ PM}$	-4.88	0.08	-4.66	0.08
mCh_mRNA$^{2\ PM}$	-4.95	0.06	-4.76	0.07
mCh_mRNA$_{UTR2}^{WT}$	-5.02	0.06	-4.77	0.06
mCh_mRNA$_{UTR2}^{1\ CP}$	-4.10	0.04	-4.52	0.05
mCh_mRNA$_{UTR2}^{2\ CP}$	-3.97	0.04	-4.25	0.03
mCh_mRNA$_{UTR2}^{1\ PM}$	-4.99	0.10	-4.72	0.13
mCh_mRNA$_{UTR2}^{2\ PM}$	-4.95	0.07	-4.74	0.08
H_2O Ctrl.	-21.39	0.02	-1.34	0.12
Untreated cells	-4.26	0.28	-2.83	0.11

mCh_mRNA + mCh_mRNA$_{UTR2}$ data set 2, cell lysis 24 h post transfection

C_t values

	Duplicate Samples	**Technical Triplicats**	**Ct values**			
			mCh 3' end: FAM	**mCh internal: HEX**	**β-Actin: ROX**	**GAPDH: Cy5**
mCh_mRNA^{WT}	**#1**	**#1.1**	19.08	19.59	19.12	22.1
		#1.2	18.94	19.44	18.73	22.03
		#1.3	19.11	19.68	19.12	22.05
	#1 NRT		0	0	0	0
	#2	**#2.1**	18.78	19.41	18.29	21.93
		#2.2	18.94	19.43	17.71	21.96
		#2.3	18.73	19.38	18.34	21.94
	#2 NRT		0	0	0	0
	mean		18.93	19.49	18.55	22.00

	SD		0.08	0.06	0.23	0.02
mCh_mRNA$^{1\ CP}$	**#1**	**#1.1**	18.95	19.14	18.97	21.69
		#1.2	19.16	19.37	18.32	21.97
		#1.3	19.28	19.53	17.73	22.11
	#1 NRT		0	0	0	0
	#2	**#2.1**	19.47	19.49	18.39	22.07
		#2.2	19.44	19.46	17.66	22.08
		#2.3	19.4	19.5	17.84	22.04
	#2 NRT		0	0	0	0
	mean		19.28	19.42	18.15	21.99
	SD		0.08	0.09	0.41	0.10
mCh_mRNA$^{2\ CP}$	**#1**	**#1.1**	19.4	19.25	17.59	21.87
		#1.2	19.46	19.39	17.04	22.01
		#1.3	19.48	19.42	17.48	22.03
	#1 NRT		0	0	0	0
	#2	**#2.1**	19.46	19.43	17.8	21.92
		#2.2	19.49	19.4	17.66	22.01
		#2.3	19.52	19.48	18.56	22.04
	#2 NRT		0	0	0	0
	mean		19.47	19.40	17.69	21.98
	SD		0.03	0.05	0.32	0.06
mCh_mRNA$^{1\ PM}$	**#1**	**#1.1**	18.75	19.26	18.23	22.06
		#1.2	18.62	19.13	17.62	21.97
		#1.3	18.65	19.13	16.72	22
	#1 NRT		0	0	0	0
	#2	**#2.1**	18.72	19.21	18.07	22.02
		#2.2	18.74	19.26	18.18	22.05
		#2.3	18.79	19.33	18.15	22.12
	#2 NRT		0	0	0	0
	mean		18.71	19.22	17.83	22.04
	SD		0.04	0.06	0.33	0.04
mCh_mRNA$^{2\ PM}$	**#1**	**#1.1**	18.53	19.18	18.07	21.91
		#1.2	18.5	19.09	18.45	21.9
		#1.3	18.63	19.24	18.78	22.02
	#1 NRT		0	0	0	0
	#2	**#2.1**	18.48	18.96	18.93	22.02
		#2.2	18.56	19.06	18.39	22.12
		#2.3	18.6	19.15	18.55	22.17
	#2 NRT		0	0	0	0
	mean		18.55	19.11	18.53	22.02
	SD		0.05	0.07	0.26	0.06

mCh_mRNA$_{UTR2}^{WT}$	**#1**	**#1.1**	18.77	19.31	17.9	22.1
		#1.2	18.62	19.22	17.37	22
		#1.3	18.66	19.27	18.01	22.04
	#1 NRT		0	0	0	0
	#2	**#2.1**	18.64	19.23	18.04	21.98
		#2.2	18.52	19.13	17.44	21.92
		#2.3	18.58	19.2	17.95	22.01
	#2 NRT		0	0	0	0
	mean		18.63	19.23	17.79	22.01
	SD		0.06	0.04	0.27	0.04
mCh_mRNA$_{UTR2}^{1\ CP}$	**#1**	**#1.1**	19.62	19.66	18.31	22.11
		#1.2	19.45	19.66	18.23	22
		#1.3	19.54	19.73	18.44	22.1
	#1 NRT		0	0	0	0
	#2	**#2.1**	19.15	19.31	18.48	21.94
		#2.2	19.19	19.37	18.01	22.01
		#2.3	19.19	19.56	17.96	22.07
	#2 NRT		0	0	0	0
	mean		19.36	19.55	18.24	22.04
	SD		0.04	0.07	0.16	0.05
mCh_mRNA$_{UTR2}^{2\ CP}$	**#1**	**#1.1**	19.51	19.3	17.56	22.09
		#1.2	19.47	19.23	17.41	22.04
		#1.3	19.46	19.25	16.82	22.06
	#1 NRT		0	0	0	0
	#2	**#2.1**	19.43	19.22	18.38	21.92
		#2.2	19.56	19.35	17.49	22.06
		#2.3	19.61	19.38	18.56	22.12
	#2 NRT		0	0	0	0
	mean		19.51	19.29	17.70	22.05
	SD		0.05	0.05	0.39	0.05
mCh_mRNA$_{UTR2}^{1\ PM}$	**#1**	**#1.1**	18.72	19.26	17.77	22.1
		#1.2	18.71	19.24	17.42	22.1
		#1.3	18.74	19.33	17.35	22.14
	#1 NRT		0	0	0	0
	#2	**#2.1**	18.87	19.45	18.52	22.13
		#2.2	18.86	19.43	18.3	22.19
		#2.3	19	19.45	17.23	22.18
	#2 NRT		0	0	0	0
	mean		18.82	19.36	17.77	22.14
	SD		0.04	0.02	0.37	0.02
mCh_mRNA$_{UTR2}^{2\ PM}$	**#1**	**#1.1**	18.86	19.3	17.59	22.12
		#1.2	19.01	19.3	18.53	22.1

		#1.3	18.94	19.22	17.98	22.07
	#1 NRT		0	0	0	0
	#2	**#2.1**	18.94	19.19	17.46	22.03
		#2.2	18.95	19.22	17.42	22.07
		#2.3	18.91	19.23	17.34	22.04
	#2 NRT		0	0	0	0
	mean		18.94	19.24	17.72	22.07
	SD		0.04	0.03	0.22	0.02
H_2O Ctrl.	**#1**	**#1.1**	0	0	20.2	22.16
		#1.2	0	0	17.74	22.08
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	17.59	22.05
		#2.2	0	0	18.94	21.98
	#2 NRT		0	0	0	0
	mean		0.00	0.00	18.62	22.07
	SD		0.00	0.00	0.95	0.04
Untreated Cells	**#1**	**#1.1**	0	0	18.09	22.03
		#1.2	0	0	17.78	21.9
		#1.3	0	0	17.24	21.9
	#1 NRT		0	0	0	0
	#2	**#2.1**	33.54	35.47	17.34	21.92
		#2.2	32.37	35.14	17.49	21.94
		#2.3	32.82	35.54	17.87	21.89
	#2 NRT		32.31	34.14	0	33.18
	mean		16.46	17.69	17.64	21.93
	SD		0.24	0.09	0.29	0.04
NTC	**#1**		0	0	0	0
	#2		0	0	0	0

$\Delta C_t = C_t(mCh) - C_t(GAPDH)$

	ΔCt (mCh 3'end)	**error**	**ΔCt (mCh internal)**	**error**
mCh_mRNA^{WT}	-3.07	0.08	-2.51	0.06
mCh_mRNA$^{1\ CP}$	-2.71	0.13	-2.58	0.13
mCh_mRNA$^{2\ CP}$	-2.51	0.07	-2.58	0.08
mCh_mRNA$^{1\ PM}$	-3.33	0.06	-2.82	0.07
mCh_mRNA$^{2\ PM}$	-3.47	0.08	-2.91	0.09
mCh_mRNA$_{UTR2}^{WT}$	-3.38	0.07	-2.78	0.06
mCh_mRNA$_{UTR2}^{1\ CP}$	-2.68	0.07	-2.49	0.09
mCh_mRNA$_{UTR2}^{2\ CP}$	-2.54	0.07	-2.76	0.07
mCh_mRNA$_{UTR2}^{1\ PM}$	-3.32	0.04	-2.78	0.03
mCh_mRNA$_{UTR2}^{2\ PM}$	-3.14	0.04	-2.83	0.03
H_2O Ctrl.	-22.07	0.04	-22.07	0.04

Untreated cells	-5.48	0.24	-4.24	0.10

mCh_mRNA + mCh_mRNA$_{UTR2}$ data set 2, cell lysis 48 h post transfection

C_t values

	Duplicate Samples	Technical Triplicats	Ct values			
			mCh 3' end: FAM	mCh internal: HEX	β-Actin: ROX	GAPDH: Cy5
mCh_mRNA^{WT}	#1	#1.1	21.39	22.06	19.44	22.19
		#1.2	21.24	21.94	19.59	22.11
		#1.3	21.16	21.93	17.91	22.07
	#1 NRT		0	0	0	0
	#2	#2.1	21.24	22.01	17.33	22.1
		#2.2	21.31	22.08	17.71	22.15
		#2.3	21.23	22.03	16.84	22.09
	#2 NRT		0	0	0	0
	mean		21.26	22.01	18.14	22.12
	SD		0.07	0.04	0.56	0.04
mCh_mRNA$^{1\ CP}$	#1	#1.1	21.73	22.04	17.15	22.11
		#1.2	21.63	22.13	17.98	22.04
		#1.3	21.65	22.19	17.53	22.15
	#1 NRT		0	0	0	0
	#2	#2.1	21.66	22.2	17.43	22.26
		#2.2	21.58	22.11	16.57	22.19
		#2.3	21.57	22.08	15.28	22.09
	#2 NRT		0	0	0	0
	mean		21.64	22.13	16.99	22.14
	SD		0.04	0.06	0.61	0.06
mCh_mRNA$^{2\ CP}$	#1	#1.1	21.87	21.9	18.12	22.14
		#1.2	21.81	21.92	17.23	22.12
		#1.3	21.8	21.94	17.83	22.02
	#1 NRT		0	0	0	0
	#2	#2.1	21.78	21.84	17.86	22.2
		#2.2	21.75	21.81	16.89	22.17
		#2.3	21.78	22	16.49	22.18
	#2 NRT		0	0	0	0
	mean		21.80	21.90	17.40	22.14
	SD		0.02	0.05	0.47	0.03
mCh_mRNA$^{1\ PM}$	#1	#1.1	21.45	22.08	17.01	22.27
		#1.2	21.43	21.98	17.46	22.26
		#1.3	21.41	21.96	16.64	22.22

	#1 NRT		0	0	0	0
	#2	**#2.1**	21.35	21.9	16.03	22.09
		#2.2	21.25	21.77	16.94	22.03
		#2.3	21.22	21.91	17.46	22.07
	#2 NRT		0	0	0	0
	mean		21.35	21.93	16.92	22.16
	SD		0.04	0.06	0.46	0.02
mCh_mRNA$^{2\ PM}$	**#1**	**#1.1**	21.07	21.56	18.31	22.06
		#1.2	20.98	21.5	16.59	21.98
		#1.3	21	21.51	16.67	21.96
	#1 NRT		0	0	0	0
	#2	**#2.1**	21.05	21.63	16.69	22.06
		#2.2	21.03	21.59	17.85	22.04
		#2.3	20.95	21.55	16.73	22.01
	#2 NRT		0	0	0	0
	mean		21.01	21.56	17.14	22.02
	SD		0.04	0.03	0.67	0.03
mCh_mRNA$_{UTR2}^{WT}$	**#1**	**#1.1**	20.94	21.45	18.67	22.09
		#1.2	20.84	21.51	17.25	22.01
		#1.3	20.82	21.46	17.41	22.1
	#1 NRT		0	0	0	0
	#2	**#2.1**	20.76	21.36	16.05	21.94
		#2.2	20.7	21.33	17.17	21.9
		#2.3	20.7	21.4	13.95	21.9
	#2 NRT		0	38.36	31.23	0
	mean		20.79	21.42	16.75	21.99
	SD		0.04	0.03	0.98	0.03
mCh_mRNA$_{UTR2}^{1\ CP}$	**#1**	**#1.1**	21.7	21.74	16.79	22.21
		#1.2	21.52	21.57	17.54	22.02
		#1.3	21.52	21.62	15.75	22.08
	#1 NRT		0	0	0	0
	#2	**#2.1**	21.58	21.66	17.87	22.12
		#2.2	21.63	21.69	17.6	22.06
		#2.3	21.59	21.67	16.69	22.05
	#2 NRT		0	0	0	0
	mean		21.59	21.66	17.04	22.09
	SD		0.05	0.04	0.62	0.06
mCh_mRNA$_{UTR2}^{2\ CP}$	**#1**	**#1.1**	21.73	21.58	17.69	22.1
		#1.2	21.68	21.55	17.87	22.13
		#1.3	21.66	21.52	17.86	22.05
	#1 NRT		0	0	0	0

	#2	**#2.1**	21.79	21.66	17.62	22.08
		#2.2	21.77	21.67	16.55	22.13
		#2.3	21.82	21.72	18.04	22.09
	#2 NRT		0	0	0	0
	mean		21.74	21.62	17.61	22.10
	SD		0.02	0.03	0.35	0.03
mCh_mRNA$_{UTR2}$$^{1\ PM}$	**#1**	**#1.1**	21.16	21.86	17.15	22.3
		#1.2	21.11	21.8	16.68	22.25
		#1.3	21.04	21.71	15.9	22.18
	#1 NRT		0	0	0	0
	#2	**#2.1**	20.73	21.33	16.62	22.04
		#2.2	20.67	21.31	16.69	22.08
		#2.3	20.71	21.37	17.21	22.04
	#2 NRT		0	0	0	0
	mean		20.90	21.56	16.71	22.15
	SD		0.04	0.04	0.39	0.03
mCh_mRNA$_{UTR2}$$^{2\ PM}$	**#1**	**#1.1**	21.1	21.61	18.02	22.19
		#1.2	21.06	21.57	17.5	22.08
		#1.3	21.16	21.56	16.51	22.15
	#1 NRT		0	40.5	0	0
	#2	**#2.1**	21.3	21.71	16.64	22.2
		#2.2	21.12	21.65	17.36	22.15
		#2.3	21.17	21.72	16.57	22.19
	#2 NRT		0	0	0	0
	mean		21.15	21.64	17.10	22.16
	SD		0.06	0.03	0.49	0.03
H_2O Ctrl.	**#1**	**#1.1**	0	0	19.72	22.23
		#1.2	0	0	15.57	22.25
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	16.72	22.3
		#2.2	0	0	17.6	22.32
	#2 NRT		0	0	0	0
	mean		0.00	0.00	17.40	22.28
	SD		0.00	0.00	1.26	0.01
Untreated Cells	**#1**	**#1.1**	0	0	17.57	22.37
		#1.2	0	0	18.33	22.29
		#1.3	0	0	17.57	22.32
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	15.8	22.35
		#2.2	0	0	16.72	22.32
		#2.3	0	0	16.55	22.35
	#2 NRT		0	0	0	0
	mean		0.00	0.00	17.09	22.33
	SD		0.00	0.00	0.38	0.02

NTC	**#1**		0	0	0	0
	#2		0	0	0	0

$\Delta C_t = C_t(mCh) - C_t(GAPDH)$

	ΔCt (mCh 3'end)	error	ΔCt (mCh internal)	error
mCh_mRNA^{WT}	-0.86	0.08	-0.11	0.06
mCh_mRNA$^{1\ CP}$	-0.50	0.07	-0.02	0.08
mCh_mRNA$^{2\ CP}$	-0.34	0.04	-0.24	0.06
mCh_mRNA$^{1\ PM}$	-0.81	0.04	-0.22	0.06
mCh_mRNA$^{2\ PM}$	-1.01	0.05	-0.46	0.04
mCh_mRNA$_{UTR2}^{WT}$	-1.20	0.05	-0.57	0.04
mCh_mRNA$_{UTR2}^{1\ CP}$	-0.50	0.08	-0.43	0.07
mCh_mRNA$_{UTR2}^{2\ CP}$	-0.35	0.04	-0.48	0.04
mCh_mRNA$_{UTR2}^{1\ PM}$	-1.24	0.05	-0.58	0.06
mCh_mRNA$_{UTR2}^{2\ PM}$	-1.01	0.07	-0.52	0.04
H_2O Ctrl.	-22.28	0.01	-22.28	0.01
Untreated cells	-22.33	0.02	-22.33	0.02

mCh_mRNA + mCh_mRNA$^{\Psi+5mC}$ data set 1, cell lysis 6 h post transfection

C_t values

	Duplicate Samples	Technical Triplicats	Ct values			
			mCh 3' end: FAM	mCh internal: HEX	β-Actin: ROX	GAPDH: Cy5
mCh_mRNA^{WT}	**#1**	**#1.1**	17.09	17.52	20.72	21.88
		#1.2	17.01	17.49	19.52	21.85
		#1.3	17.02	17.51	20.62	21.88
	#1 NRT		0	0	25.71	0
	#2	**#2.1**	17.27	17.75	21.08	22.27
		#2.2	17.1	17.81	20.3	22.11
		#2.3	17	17.55	20.81	22.02
	#2 NRT		0	0	36.67	0
	mean		17.08	17.61	20.51	22.00
	SD		0.07	0.06	0.43	0.06
mCh_mRNA$^{1\ CP}$	**#1**	**#1.1**	17.3	17.44	20.41	21.8
		#1.2	17.29	17.45	20.4	21.78
		#1.3	17.28	17.5	20.52	21.81
	#1 NRT		0	0	0	0
	#2	**#2.1**	16.91	17.27	20.25	21.69
		#2.2	17.33	17.66	20.9	22.13

		#2.3	17.07	17.41	20.39	21.88
	#2 NRT		0	0	0	0
	mean		17.20	17.46	20.48	21.85
	SD		0.09	0.09	0.17	0.10
mCh_mRNA$^{2\ CP}$	**#1**	**#1.1**	17.75	17.54	20.56	21.8
		#1.2	16.69	17.53	20.38	21.75
		#1.3	17.69	17.54	20.38	21.76
	#1 NRT		0	0	0	0
	#2	**#2.1**	17.77	17.65	19.72	21.83
		#2.2	17.73	17.73	19.4	21.8
		#2.3	17.83	17.68	19.34	21.86
	#2 NRT		0	0	0	0
	mean		17.58	17.61	19.96	21.80
	SD		0.26	0.02	0.13	0.02
mCh_mRNA$^{WT,\ \Psi+5mC}$	**#1**	**#1.1**	17.38	18.05	20.36	21.69
		#1.2	17.31	17.97	19.25	21.62
		#1.3	17.25	17.73	20.29	21.57
	#1 NRT		0	0	0	0
	#2	**#2.1**	17.47	18.13	19.39	21.67
		#2.2	17.43	18.11	20.4	21.65
		#2.3	17.5	18.19	20.46	21.73
	#2 NRT		0	0	0	0
	mean		17.39	18.03	20.03	21.66
	SD		0.04	0.08	0.50	0.04
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	**#1**	**#1.1**	17.56	18.06	20.51	21.75
		#1.2	17.41	17.96	19.64	21.6
		#1.3	17.42	17.96	20.31	21.65
	#1 NRT		0	0	0	0
	#2	**#2.1**	17.42	17.76	20.4	21.67
		#2.2	17.43	17.96	20.37	21.68
		#2.3	17.41	17.96	20.3	21.67
	#2 NRT		0	0	0	0
	mean		17.44	17.94	20.26	21.67
	SD		0.04	0.07	0.21	0.03
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	**#1**	**#1.1**	18.62	18.19	20.11	21.88
		#1.2	18.44	18.06	19.05	21.73
		#1.3	18.4	17.89	19.31	21.74
	#1 NRT		0	0	0	0
	#2	**#2.1**	17.97	17.91	19.55	21.71
		#2.2	18.09	17.92	19.3	21.7
		#2.3	17.93	17.86	20.37	21.7

	#2 NRT		0	0	0	0
	mean		18.24	17.97	19.62	21.74
	SD		0.08	0.07	0.45	0.04
Untreated Cells	**#1**	**#1.1**	0	0	21.03	21.79
		#1.2	0	0	21.08	21.86
		#1.3	0	0	20.94	21.83
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	20.82	21.61
		#2.2	0	0	20.9	21.75
		#2.3	0	0	20.76	21.53
	#2 NRT		0	0	0	0
	mean		0.00	0.00	20.92	21.73
	SD		0.00	0.00	0.06	0.06
H_2O Ctrl.	**#1**	**#1.1**	0	0	20.79	21.73
		#1.2	0	0	20.62	21.45
		#1.3	0	0	20.61	21.45
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	39.35	20.61	21.53
		#2.2	0	38.93	20.66	21.49
		#2.3	0	38.69	18.91	21.68
	#2 NRT		0	0	0	0
	mean		0.00	19.50	20.37	21.56
	SD		0.00	0.14	0.45	0.11
NTC	**#1**		0	0	0	0
	#2		0	0	0	0

$\Delta C_t = C_t(mCh) - C_t(GAPDH)$

	ΔCt (mCh 3'end)	**error**	**ΔCt (mCh internal)**	**error**
mCh_mRNA^{WT}	-4.92	0.09	-4.40	0.09
mCh_mRNA$^{1\ CP}$	-4.65	0.13	-4.39	0.13
mCh_mRNA$^{2\ CP}$	-4.22	0.26	-4.19	0.03
mCh_mRNA$^{WT,\ \Psi+5mC}$	-4.27	0.06	-3.63	0.09
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	-4.23	0.05	-3.73	0.08
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	-3.50	0.09	-3.77	0.08
H_2O Ctrl.	-21.73	0.06	-21.73	0.06
Untreated cells	-21.56	0.11	-2.06	0.17

mCh_mRNA + mCh_mRNA$^{\Psi+5mC}$ data set 1, cell lysis 24 h post transfection

C_t values

	Duplicate Samples	Technical Triplicats	Ct values			
			mCh 3' end: FAM	mCh internal: HEX	β-Actin: ROX	GAPDH: Cy5
mCh_mRNA^{WT}	#1	#1.1	19.41	19.71	21.63	22.29
		#1.2	19.3	19.57	21.54	22.22
		#1.3	19.27	19.7	21.54	22.22
	#1 NRT		0	0	0	0
	#2	#2.1	19.12	19.53	21.38	22.02
		#2.2	19.09	19.52	21.35	22.01
		#2.3	19.37	19.92	21.61	22.17
	#2 NRT		0	0	0	0
	mean		19.26	19.66	21.51	22.16
	SD		0.09	0.13	0.08	0.05
mCh_mRNA$^{1\ CP}$	#1	#1.1	19.56	19.61	21.41	22.04
		#1.2	19.53	19.63	21.42	22.02
		#1.3	19.53	19.7	21.41	22.06
	#1 NRT		0	0	0	0
	#2	#2.1	19.3	19.53	21.43	22.1
		#2.2	19.28	19.49	21.45	22.1
		#2.3	19.22	19.49	21.38	22.06
	#2 NRT		0	0	0	0
	mean		19.40	19.58	21.42	22.06
	SD		0.02	0.03	0.02	0.02
mCh_mRNA$^{2\ CP}$	#1	#1.1	20.19	20.02	21.38	22.03
		#1.2	20.12	20	21.29	21.96
		#1.3	20.1	20.01	21.35	21.95
	#1 NRT		0	0	0	0
	#2	#2.1	20.11	20.11	21.25	21.86
		#2.2	20.22	20.11	21.31	21.9
		#2.3	20.2	20.11	21.35	22
	#2 NRT		0	0	0	0
	mean		20.16	20.06	21.32	21.95
	SD		0.04	0.00	0.04	0.05
mCh_mRNA$^{WT,\ \Psi+5mC}$	#1	#1.1	19.89	20.4	21.34	22.05
		#1.2	19.68	20.15	21.21	21.88
		#1.3	19.67	20.25	21.17	21.86
	#1 NRT		0	0	0	0
	#2	#2.1	19.83	20.4	21.24	21.86
		#2.2	19.92	20.49	21.3	21.97

		#2.3	19.95	20.57	21.38	22.01
	#2 NRT		0	0	0	0
	mean		19.82	20.38	21.27	21.94
	SD		0.08	0.09	0.06	0.07
mCh_mRNA[1] CP, Ψ+5mC	**#1**	**#1.1**	20.17	20.52	21.49	22.08
		#1.2	20.03	20.38	21.26	21.93
		#1.3	20.31	21.08	21.61	22.34
	#1 NRT		0	0	0	0
	#2	**#2.1**	19.86	20.26	21.18	21.78
		#2.2	20.01	20.36	21.31	21.94
		#2.3	19.99	20.4	21.35	21.94
	#2 NRT		0	0	39.47	0
	mean		20.06	20.50	21.37	22.00
	SD		0.09	0.18	0.11	0.12
mCh_mRNA[2] CP, Ψ+5mC	**#1**	**#1.1**	21.24	21.13	21.6	22.22
		#1.2	21.13	21.01	21.47	22.13
		#1.3	21.07	20.95	21.46	22.09
	#1 NRT		0	0	0	0
	#2	**#2.1**	20.8	20.72	21.25	21.89
		#2.2	20.68	20.6	21.22	21.82
		#2.3	20.68	20.63	21.26	21.83
	#2 NRT		0	0	0	0
	mean		20.93	20.84	21.38	22.00
	SD		0.06	0.06	0.04	0.04
Untreated Cells	**#1**	**#1.1**	0	0	21.58	22.13
		#1.2	0	0	21.41	21.97
		#1.3	0	0	21.42	21.94
	#1 NRT		0	0	39.14	0
	#2	**#2.1**	0	0	21.43	21.98
		#2.2	0	0	21.43	21.95
		#2.3	0	0	21.34	21.89
	#2 NRT		0	0	0	0
	mean		0.00	0.00	21.44	21.98
	SD		0.00	0.00	0.06	0.06
H_2O Ctrl.	**#1**	**#1.1**	0	0	21.75	22.11
		#1.2	0	0	21.54	22
		#1.3	0	0	21.54	21.96
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	21.65	22.07
		#2.2	0	0	21.52	22.08
		#2.3	0	0	21.66	22.12
	#2 NRT		0	0	0	0

	mean		0.00	0.00	21.61	22.06
	SD		0.00	0.00	0.08	0.04
NTC	**#1**		0	0	0	0
	#2		0	0	0	0

$\Delta C_t = C_t(mCh) - C_t(GAPDH)$

	ΔCt (mCh 3'end)	**error**	**ΔCt (mCh internal)**	**error**
mCh_mRNA^{WT}	-2.90	0.11	-2.50	0.14
mCh_mRNA$^{1\ CP}$	-2.66	0.03	-2.49	0.03
mCh_mRNA$^{2\ CP}$	-1.79	0.06	-1.89	0.05
mCh_mRNA$^{WT,\ \Psi+5mC}$	-2.12	0.11	-1.56	0.11
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	-1.94	0.15	-1.50	0.22
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	-1.06	0.08	-1.16	0.08
H_2O Ctrl.	-21.98	0.06	-21.98	0.06
Untreated cells	-22.06	0.04	-22.06	0.04

mCh_mRNA + mCh_mRNA$^{\Psi+5mC}$ data set 1, cell lysis 48 h post transfection

C_t values

	Duplicate Samples	**Technical Triplicats**	**Ct values**			
			mCh 3' end: FAM	**mCh internal: HEX**	**β-Actin: ROX**	**GAPDH: Cy5**
mCh_mRNA^{WT}	**#1**	**#1.1**	21.71	22.47	16.55	22.32
		#1.2	21.6	22.39	17.36	22.26
		#1.3	21.6	22.54	19.02	22.31
	#1 NRT		0	0	20.92	0
	#2	**#2.1**	21.54	22.26	18.69	22.31
		#2.2	21.62	22.54	15.98	22.45
		#2.3	21.65	22.58	15.79	22.48
	#2 NRT		0	0	0	0
	mean		21.62	22.46	17.23	22.36
	SD		0.05	0.10	1.18	0.05
mCh_mRNA$^{1\ CP}$	**#1**	**#1.1**	22.04	22.49	17.5	22.46
		#1.2	21.97	22.52	17.12	22.4
		#1.3	22.02	22.58	17.31	22.35
	#1 NRT		0	0	0	0
	#2	**#2.1**	22.03	22.55	13.08	22.42
		#2.2	22.16	22.8	17.78	22.56
		#2.3	22.07	22.81	17.27	22.55
	#2 NRT		0	0	0	0

	mean		22.05	22.63	16.68	22.46
	SD		0.04	0.08	1.13	0.05
mCh_mRNA$^{2\ CP}$	**#1**	**#1.1**	22.39	22.54	17.33	22.33
		#1.2	22.36	22.57	15.09	22.32
		#1.3	22.25	22.57	16.61	22.28
	#1 NRT		0	0	0	0
	#2	**#2.1**	22.63	23.04	14.24	22.53
		#2.2	22.65	23.05	17.29	22.57
		#2.3	22.57	23.02	16.21	22.51
	#2 NRT		0	0	24.86	0
	mean		22.48	22.80	16.13	22.42
	SD		0.05	0.01	1.10	0.02
mCh_mRNA$^{WT,\ \Psi+5mC}$	**#1**	**#1.1**	22	23.01	15.1	22.42
		#1.2	21.96	22.9	16.41	22.4
		#1.3	21.94	23.02	15.54	22.35
	#1 NRT		0	0	0	0
	#2	**#2.1**	21.87	22.98	17.3	22.18
		#2.2	21.87	22.99	16.16	22.26
		#2.3	21.98	22.92	14.92	22.24
	#2 NRT		0	0	0	0
	mean		21.94	22.97	15.91	22.31
	SD		0.04	0.04	0.76	0.03
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	**#1**	**#1.1**	22.49	23.24	17.24	22.45
		#1.2	22.39	23.19	16.87	22.36
		#1.3	22.39	23.15	16.3	22.35
	#1 NRT		0	0	18.07	0
	#2	**#2.1**	22.11	22.73	14.65	22.2
		#2.2	22.04	22.85	15.48	22.13
		#2.3	22.14	22.75	16.45	22.25
	#2 NRT		0	0	0	0
	mean		22.26	22.99	16.17	22.29
	SD		0.04	0.04	0.56	0.05
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	**#1**	**#1.1**	23.02	23.35	15.06	22.43
		#1.2	22.94	23.31	16.36	22.37
		#1.3	22.96	23.3	17.29	22.42
	#1 NRT		0	0	0	0
	#2	**#2.1**	22.84	23.23	16.38	22.22
		#2.2	22.88	23.22	15.6	22.33
		#2.3	22.84	23.22	14.83	22.25
	#2 NRT		0	0	0	0
	mean		22.91	23.27	15.92	22.34

	SD		0.03	0.01	0.77	0.04
Untreated Cells	**#1**	**#1.1**	0	0	17.22	22.65
		#1.2	0	0	18.01	22.64
		#1.3	0	0	16.16	22.52
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	14.48	22.48
		#2.2	0	0	16.46	22.48
		#2.3	0	0	16.26	22.5
	#2 NRT		0	0	0	0
	mean		0.00	0.00	16.43	22.55
	SD		0.00	0.00	0.82	0.03
H_2O Ctrl.	**#1**	**#1.1**	0	0	17.29	23
		#1.2	0	0	17.99	22.87
		#1.3	0	0	15.55	22.93
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	16.98	22.39
		#2.2	0	0	15.58	22.41
		#2.3	0	0	15.42	22.43
	#2 NRT		0	0	0	0
	mean		0.00	0.00	16.47	22.67
	SD		0.00	0.00	0.86	0.03
NTC	**#1**		0	0	14.31	0
	#2		0	0	0	0

$\Delta C_t = C_t(mCh) - C_t(GAPDH)$

	ΔCt (mCh 3'end)	**error**	**ΔCt (mCh internal)**	**error**
mCh_mRNA^{WT}	-0.73	0.07	0.11	0.11
mCh_mRNA$^{1\ CP}$	-0.41	0.07	0.17	0.10
mCh_mRNA$^{2\ CP}$	0.05	0.05	0.38	0.03
mCh_mRNA$^{WT,\ \Psi+5mC}$	-0.37	0.05	0.66	0.05
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	-0.03	0.06	0.70	0.06
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	0.58	0.04	0.93	0.04
H_2O Ctrl.	-22.55	0.03	-22.55	0.03
Untreated cells	-22.67	0.03	-22.67	0.03

mCh_mRNA + mCh_mRNA$^{\Psi+5mC}$ data set 2, cell lysis 6 h post transfection

C_t values

	Duplicate Samples	**Technical Triplicats**	**Ct values**			
			mCh 3' end: FAM	**mCh internal: HEX**	**β-Actin: ROX**	**GAPDH: Cy5**
mCh_mRNA^{WT}	**#1**	**#1.1**	17.04	17.14	18.34	21.99

		#1.2	16.94	17.18	18.07	21.93
		#1.3	16.86	17.04	18.39	21.89
	#1 NRT		0	0	0	0
	#2	**#2.1**	16.95	17.25	18.35	21.91
		#2.2	16.94	17.25	18.07	21.92
		#2.3	16.96	17.12	18.12	21.96
	#2 NRT		0	0	0	0
	mean		16.95	17.16	18.22	21.93
	SD		0.04	0.06	0.13	0.03
mCh_mRNA$^{1\ CP}$	**#1**	**#1.1**	17.43	17.38	18.12	21.9
		#1.2	17.34	17.36	18.6	21.78
		#1.3	17.32	17.36	17.81	21.82
	#1 NRT		0	39.02	21.22	0
	#2	**#2.1**	17.36	17.41	17.24	22.05
		#2.2	17.35	17.36	18.87	21.94
		#2.3	17.28	17.31	18.15	21.85
	#2 NRT		0	0	0	0
	mean		17.35	17.36	18.13	21.89
	SD		0.04	0.03	0.50	0.07
mCh_mRNA$^{2\ CP}$	**#1**	**#1.1**	17.78	17.39	17.94	21.89
		#1.2	17.68	17.32	18.72	21.79
		#1.3	17.71	17.23	17.32	21.84
	#1 NRT		0	0	0	0
	#2	**#2.1**	17.81	17.44	18.53	21.85
		#2.2	17.76	17.4	17.15	21.82
		#2.3	17.79	17.44	18.11	21.84
	#2 NRT		0	0	0	0
	mean		17.76	17.37	17.96	21.84
	SD		0.03	0.04	0.58	0.03
mCh_mRNA$^{WT,\ \Psi+5mC}$	**#1**	**#1.1**	17.39	17.59	18.86	21.76
		#1.2	17.27	17.4	17.87	21.64
		#1.3	17.27	17.48	18.84	21.66
	#1 NRT		0	0	0	0
	#2	**#2.1**	17.33	17.64	18.01	21.69
		#2.2	17.31	17.63	17	21.67
		#2.3	17.34	17.65	17.61	21.73
	#2 NRT		0	0	0	0
	mean		17.32	17.57	18.03	21.69
	SD		0.03	0.04	0.44	0.04
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	**#1**	**#1.1**	17.61	17.73	17.69	21.75
		#1.2	17.44	17.55	18.52	21.6

		#1.3	17.4	17.52	18.28	21.54
	#1 NRT		0	0	0	0
	#2	**#2.1**	17.5	17.61	16.55	21.68
		#2.2	17.51	17.6	18.47	21.69
		#2.3	17.51	17.63	16.97	21.88
	#2 NRT		0	40.05	0	0
	mean		17.50	17.61	17.75	21.69
	SD		0.05	0.05	0.59	0.09
mCh_mRNA[2 CP, Ψ+5mC]	**#1**	**#1.1**	18.49	18.05	18.52	21.79
		#1.2	18.32	17.88	17.58	21.62
		#1.3	18.31	17.83	17.39	21.64
	#1 NRT		0	0	0	0
	#2	**#2.1**	18.26	17.91	17.6	21.67
		#2.2	18.28	17.78	17.82	21.6
		#2.3	18.3	17.8	18	21.59
	#2 NRT		0	0	0	0
	mean		18.33	17.88	17.82	21.65
	SD		0.05	0.08	0.33	0.06
Untreated Cells	**#1**	**#1.1**	0	0	19.11	21.95
		#1.2	0	0	18.23	21.84
		#1.3	0	0	18.59	21.85
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	18.37	21.76
		#2.2	0	0	18.22	21.71
		#2.3	0	0	20.64	22.61
	#2 NRT		0	0	0	0
	mean		0.00	0.00	18.86	21.95
	SD		0.00	0.00	0.73	0.23
H_2O Ctrl.	**#1**	**#1.1**	0	40.51	17.78	21.94
		#1.2	0	39.82	18.35	21.82
		#1.3	0	40.53	17	21.76
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	17.23	21.8
		#2.2	0	0	17.67	21.69
		#2.3	0	0	17.23	21.8
	#2 NRT		0	0	0	0
	mean		0.00	20.14	17.54	21.80
	SD		0.00	0.17	0.38	0.06
NTC	**#1**		0	0	0	0
	#2		0	0	0	0

$\Delta C_t = C_t(mCh) - C_t(GAPDH)$

	ΔCt (mCh 3'end)	error	ΔCt (mCh internal)	error
mCh_mRNA^{WT}	-4.99	0.05	-4.77	0.07
mCh_mRNA$^{1\ CP}$	-4.54	0.08	-4.53	0.07
mCh_mRNA$^{2\ CP}$	-4.08	0.04	-4.47	0.05
mCh_mRNA$^{WT,\ \Psi+5mC}$	-4.37	0.05	-4.13	0.06
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	-4.20	0.10	-4.08	0.10
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	-3.33	0.07	-3.78	0.09
H_2O Ctrl.	-21.95	0.23	-21.95	0.23
Untreated cells	-21.80	0.06	-1.66	0.18

mCh_mRNA + mCh_mRNA$^{\Psi+5mC}$ data set 2, cell lysis 24 h post transfection

C_t values

	Duplicate Samples	Technical Triplicats	Ct values			
			mCh 3' end: FAM	mCh internal: HEX	β-Actin: ROX	GAPDH: Cy5
mCh_mRNA^{WT}	**#1**	**#1.1**	19.14	19.83	19.56	21.93
		#1.2	18.98	19.63	19.08	21.8
		#1.3	18.98	19.68	19.21	21.82
	#1 NRT		0	0	41.14	0
	#2	**#2.1**	19.09	19.79	19.24	22.02
		#2.2	19.07	19.96	17.54	22.02
		#2.3	19.09	19.97	19.47	22.04
	#2 NRT		0	0	0	0
	mean		19.06	19.81	19.02	21.94
	SD		0.04	0.08	0.53	0.03
mCh_mRNA$^{1\ CP}$	**#1**	**#1.1**	19.5	20.02	19.5	22.2
		#1.2	19.42	19.97	18.62	22.16
		#1.3	19.3	19.82	19.47	22.14
	#1 NRT		0	0	41.52	0
	#2	**#2.1**	19.24	19.88	19.15	22.12
		#2.2	19.23	19.9	18.24	22.14
		#2.3	19.19	19.86	19.33	22.11
	#2 NRT		0	0	0	0
	mean		19.31	19.91	19.05	22.15
	SD		0.05	0.05	0.44	0.02
mCh_mRNA$^{2\ CP}$	**#1**	**#1.1**	20.08	20.26	19.22	22.14
		#1.2	19.97	20.16	17.87	22.08
		#1.3	20.01	20.2	19.16	22.09
	#1 NRT		0	0	0	0

	#2	**#2.1**	20.03	20.2	18.37	22.13
		#2.2	19.97	20.15	18.56	22.09
		#2.3	19.99	20.21	19.04	22.13
	#2 NRT		0	0	0	0
	mean		20.01	20.20	18.70	22.11
	SD		0.04	0.03	0.45	0.02
mCh_mRNA$^{WT, \Psi+5mC}$	**#1**	**#1.1**	19.69	20.57	18.47	21.72
		#1.2	19.58	20.48	18.73	21.69
		#1.3	19.55	20.45	18.35	21.64
	#1 NRT		0	0	0	0
	#2	**#2.1**	19.7	20.59	19.15	21.78
		#2.2	19.69	20.75	18.45	21.93
		#2.3	19.74	20.71	18.98	21.99
	#2 NRT		0	0	0	0
	mean		19.66	20.59	18.69	21.79
	SD		0.04	0.06	0.23	0.06
mCh_mRNA$^{1\ CP, \Psi+5mC}$	**#1**	**#1.1**	19.83	20.41	19.01	21.97
		#1.2	19.66	20.27	18.28	21.75
		#1.3	19.71	20.29	19.13	21.77
	#1 NRT		0	0	0	0
	#2	**#2.1**	20.01	20.78	19.24	22.06
		#2.2	20.03	20.66	18.48	22.08
		#2.3	20	20.69	18.07	22.08
	#2 NRT		0	0	0	0
	mean		19.87	20.52	18.70	21.95
	SD		0.04	0.06	0.43	0.05
mCh_mRNA$^{2\ CP, \Psi+5mC}$	**#1**	**#1.1**	20.94	21.15	18.49	22.03
		#1.2	20.79	21.07	18.73	21.82
		#1.3	20.73	21.01	18.2	21.74
	#1 NRT		0	0	0	0
	#2	**#2.1**	20.57	20.83	18.25	21.88
		#2.2	20.53	20.81	18.55	21.87
		#2.3	20.51	20.7	16.81	21.85
	#2 NRT		0	0	0	0
	mean		20.68	20.93	18.17	21.87
	SD		0.06	0.06	0.49	0.07
Untreated Cells	**#1**	**#1.1**	0	0	18.32	22.06
		#1.2	0	0	18.34	21.96
		#1.3	0	0	18.36	21.89
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	18.27	22.11

		#2.2	0	0	18.89	22.12
		#2.3	0	0	17.56	22.14
	#2 NRT		0	0	0	0
	mean		0.00	0.00	18.29	22.05
	SD		0.00	0.00	0.28	0.04
H_2O Ctrl.	**#1**	**#1.1**	0	0	18.49	22.11
		#1.2	0	0	18.26	22.01
		#1.3	0	0	17.67	21.97
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	18.21	21.99
		#2.2	0	0	18.12	21.92
		#2.3	0	0	18.15	21.93
	#2 NRT		0	0	0	0
	mean		0.00	0.00	18.15	21.99
	SD		0.00	0.00	0.19	0.04
NTC	**#1**		0	0	0	0
	#2		0	0	0	0

$\Delta C_t = C_t(mCh) - C_t(GAPDH)$

	ΔCt (mCh 3'end)	**error**	**ΔCt (mCh internal)**	**error**
mCh_mRNA^{WT}	-2.88	0.05	-2.13	0.09
mCh_mRNA$^{1\ CP}$	-2.83	0.06	-2.24	0.05
mCh_mRNA$^{2\ CP}$	-2.10	0.04	-1.91	0.04
mCh_mRNA$^{WT,\ \Psi+5mC}$	-2.13	0.07	-1.20	0.08
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	-2.08	0.07	-1.44	0.08
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	-1.19	0.09	-0.94	0.09
H_2O Ctrl.	-22.05	0.04	-22.05	0.04
Untreated cells	-21.99	0.04	-21.99	0.04

mCh_mRNA + mCh_mRNA$^{\Psi+5mC}$ data set 2, cell lysis 48 h post transfection

C_t values

	Duplicate Samples	**Technical Triplicats**	**Ct values**			
			mCh 3' end: FAM	**mCh internal: HEX**	**β-Actin: ROX**	**GAPDH: Cy5**
mCh_mRNA^{WT}	**#1**	**#1.1**	21.38	22.18	19.77	22.39
		#1.2	21.42	22.15	19.65	22.37
		#1.3	21.37	22.15	20.34	22.35
	#1 NRT		0	0	0	0
	#2	**#2.1**	21.84	22.74	20.09	22.45
		#2.2	21.8	22.74	20.39	22.43

		#2.3	21.83	22.78	21.11	22.47
	#2 NRT		0	0	0	0
	mean		21.61	22.46	20.23	22.41
	SD		0.02	0.02	0.36	0.02
mCh_mRNA[1 CP]	**#1**	**#1.1**	22.19	22.7	20.19	22.54
		#1.2	22.16	22.72	20.31	22.5
		#1.3	22.04	22.68	20.44	22.49
	#1 NRT		0	0	0	0
	#2	**#2.1**	21.87	22.4	19.7	22.37
		#2.2	21.88	22.52	20.29	22.39
		#2.3	21.78	22.38	19.63	22.41
	#2 NRT		0	0	29.18	0
	mean		21.99	22.57	20.09	22.45
	SD		0.05	0.04	0.20	0.02
mCh_mRNA[2 CP]	**#1**	**#1.1**	22.86	23.11	20.47	22.73
		#1.2	22.77	23.11	19.52	22.64
		#1.3	22.77	23.1	19.57	22.65
	#1 NRT		0	0	0	0
	#2	**#2.1**	22.65	23.01	19.43	22.5
		#2.2	22.58	22.96	19.77	22.47
		#2.3	22.59	22.97	18.97	22.46
	#2 NRT		0	0	0	0
	mean		22.70	23.04	19.62	22.58
	SD		0.04	0.01	0.38	0.03
mCh_mRNA[WT, Ψ+5mC]	**#1**	**#1.1**	22.22	23.35	20.39	22.54
		#1.2	21.97	23.25	19.87	22.45
		#1.3	21.96	23.2	19.73	22.42
	#1 NRT		0	0	0	0
	#2	**#2.1**	22.5	23.8	20.36	22.64
		#2.2	22.52	23.71	20.37	22.68
		#2.3	22.57	23.94	19.83	22.75
	#2 NRT		0	0	0	0
	mean		22.29	23.54	20.09	22.58
	SD		0.07	0.08	0.27	0.05
mCh_mRNA[1 CP, Ψ+5mC]	**#1**	**#1.1**	23.17	24.05	20.13	22.73
		#1.2	22.88	23.85	20.24	22.59
		#1.3	22.88	23.73	18.67	22.59
	#1 NRT		0	0	0	0
	#2	**#2.1**	23	23.99	20.19	22.73
		#2.2	23.01	24.03	19.52	22.73
		#2.3	22.98	24.02	20.46	22.74

	#2 NRT		0	0	0	0
	mean		22.99	23.95	19.87	22.69
	SD		0.07	0.07	0.56	0.04
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	**#1**	**#1.1**	23.86	24.34	19.43	22.8
		#1.2	23.68	24.23	20.31	22.63
		#1.3	23.64	24.21	19.77	22.62
	#1 NRT		0	0	0	0
	#2	**#2.1**	23.66	24.19	19.69	22.61
		#2.2	23.56	24.11	19.33	22.57
		#2.3	23.58	24.14	19.4	22.62
	#2 NRT		0	0	0	0
	mean		23.66	24.20	19.66	22.64
	SD		0.07	0.05	0.26	0.05
Untreated Cells	**#1**	**#1.1**	0	0	20.75	23
		#1.2	0	0	19.86	22.84
		#1.3	0	0	20.28	22.81
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	20.75	22.86
		#2.2	0	0	20.86	22.9
		#2.3	0	0	20.5	22.86
	#2 NRT		0	0	0	0
	mean		0.00	0.00	20.50	22.88
	SD		0.00	0.00	0.26	0.05
H_2O Ctrl.	**#1**	**#1.1**	0	0	20.42	22.57
		#1.2	0	0	19.55	22.46
		#1.3	0	0	19.23	22.47
	#1 NRT		0	0	0	0
	#2	**#2.1**	0	0	20.15	22.53
		#2.2	0	0	19.41	22.47
		#2.3	0	0	20.15	22.47
	#2 NRT		0	0	0	0
	mean		0.00	0.00	19.82	22.50
	SD		0.00	0.00	0.43	0.04
NTC	**#1**		0	0	0	0
	#2		0	0	0	0

ΔC_t= C_t(mCh) – C_t(GAPDH)

	ΔCt (mCh 3'end)	**error**	**ΔCt (mCh internal)**	**error**
mCh_mRNA^{WT}	-0.80	0.03	0.05	0.02
mCh_mRNA$^{1\ CP}$	-0.46	0.06	0.12	0.04
mCh_mRNA$^{2\ CP}$	0.13	0.05	0.47	0.03

mCh_mRNA$^{WT, \Psi+5mC}$	-0.29	0.09	0.96	0.09
mCh_mRNA$^{1\ CP, \Psi+5mC}$	0.30	0.08	1.26	0.08
mCh_mRNA$^{2\ CP, \Psi+5mC}$	1.02	0.09	1.56	0.07
H_2O Ctrl.	-22.88	0.05	-22.88	0.05
Untreated cells	-22.50	0.04	-22.50	0.04

Combined ΔCt values of all data sets

mCh_mRNA 3' end primer + probe set

	6 h		24 h		48 h	
	ΔCt (mCh 3'end)	**error**	**ΔCt (mCh 3'end)**	**error**	**ΔCt (mCh 3'end)**	**error**
mCh_mRNA^{WT}	-5.17	0.04	-3.03	0.04	-0.79	0.05
mCh_mRNA$^{1\ CP}$	-4.68	0.05	-2.72	0.04	-0.46	0.06
mCh_mRNA$^{2\ CP}$	-4.20	0.07	-2.13	0.03	-0.06	0.03
mCh_mRNA$^{WT, \Psi+5mC}$	-4.32	0.12	-2.12	0.17	-0.33	0.12
mCh_mRNA$^{1\ CP, \Psi+5mC}$	-4.21	0.12	-2.01	0.20	0.14	0.13
mCh_mRNA$^{2\ CP, \Psi+5mC}$	-3.41	0.15	-1.13	0.15	0.80	0.11
mCh_mRNA$^{1\ PM}$	-5.18	0.06	-3.29	0.04	-0.87	0.07
mCh_mRNA$^{2\ PM}$	-5.29	0.05	-3.36	0.06	-1.02	0.10
mCh_mRNA$_{UTR2}^{WT}$	-5.36	0.05	-3.30	0.04	-1.16	0.19
mCh_mRNA$_{UTR2}^{1\ CP}$	-4.41	0.04	-2.56	0.06	-0.45	0.13
mCh_mRNA$_{UTR2}^{2\ CP}$	-4.29	0.07	-2.41	0.05	-0.18	0.15
mCh_mRNA$_{UTR2}^{1\ PM}$	-5.27	0.05	-3.31	0.04	-1.16	0.04
mCh_mRNA$_{UTR2}^{2\ PM}$	-5.11	0.04	-3.16	0.06	-1.00	0.06

mCh_mRNA internal primer + probe set

	6 h		24 h		48 h	
	ΔCt (mCh internal)	**error**	**ΔCt** (mCh internal)	**error**	**ΔCt** (mCh internal)	**error**
mCh_mRNA^{WT}	-4.93	0.04	-2.46	0.04	-0.04	0.05
mCh_mRNA$^{1\ CP}$	-4.62	0.05	-2.40	0.04	0.05	0.06
mCh_mRNA$^{2\ CP}$	-4.48	0.04	-2.07	0.03	0.12	0.03
mCh_mRNA$^{WT, \Psi+5mC}$	-3.88	0.16	-1.38	0.17	0.81	0.12
mCh_mRNA$^{1\ CP, \Psi+5mC}$	-3.91	0.15	-1.47	0.24	0.98	0.13
mCh_mRNA$^{2\ CP, \Psi+5mC}$	-3.77	0.15	-1.05	0.14	1.25	0.10
mCh_mRNA$^{1\ PM}$	-5.00	0.08	-2.76	0.04	-0.28	0.07
mCh_mRNA$^{2\ PM}$	-5.13	0.11	-2.80	0.06	-0.53	0.10
mCh_mRNA$_{UTR2}^{WT}$	-5.03	0.10	-2.73	0.04	-0.58	0.19
mCh_mRNA$_{UTR2}^{1\ CP}$	-4.56	0.13	-2.50	0.07	-0.38	0.12

mCh_mRNA$_{UTR2}^{2\ CP}$	-4.47	0.16	-2.60	0.06	-0.34	0.15
mCh_mRNA$_{UTR2}^{1\ PM}$	-5.03	0.12	-2.77	0.03	-0.62	0.04
mCh_mRNA$_{UTR2}^{2\ PM}$	-4.89	0.07	-2.79	0.06	-0.60	0.06

$\Delta\Delta C_t$= ΔC_t(mCh_mRNA modified, any time) – ΔC_t(mCh_mRNA^{WT}, 6h)

mCh_mRNA 3' end primer + probe set

	6 h		**24 h**		**48 h**	
	ΔΔCt (mCh 3'end)	**error**	**ΔΔCt (mCh 3'end)**	**error**	**ΔΔCt (mCh 3'end)**	**error**
mCh_mRNA^{WT}	0.00	0.06	2.14	0.06	4.39	0.07
mCh_mRNA$^{1\ CP}$	0.49	0.06	2.45	0.06	4.72	0.07
mCh_mRNA$^{2\ CP}$	0.98	0.08	3.04	0.05	5.11	0.05
mCh_mRNA$^{WT,\ \Psi+5mC}$	0.85	0.13	3.05	0.17	4.84	0.13
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	0.96	0.13	3.16	0.20	5.31	0.14
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	1.76	0.16	4.05	0.15	5.97	0.12
mCh_mRNA$^{1\ PM}$	-0.01	0.07	1.89	0.06	4.30	0.08
mCh_mRNA$^{2\ PM}$	-0.12	0.06	1.81	0.07	4.15	0.11
mCh_mRNA$_{UTR2}^{WT}$	-0.19	0.06	1.87	0.06	4.02	0.19
mCh_mRNA$_{UTR2}^{1\ CP}$	0.76	0.06	2.61	0.07	4.73	0.13
mCh_mRNA$_{UTR2}^{2\ CP}$	0.88	0.08	2.76	0.06	5.00	0.15
mCh_mRNA$_{UTR2}^{1\ PM}$	-0.10	0.07	1.86	0.06	4.01	0.05
mCh_mRNA$_{UTR2}^{2\ PM}$	0.06	0.06	2.02	0.07	4.17	0.07

mCh_mRNA internal primer + probe set

	6 h		**24 h**		**48 h**	
	ΔΔCt (mCh internal)	**error**	**ΔΔCt** (mCh internal)	**error**	**ΔΔCt** (mCh internal)	**error**
mCh_mRNA^{WT}	0.00	0.05	2.47	0.06	4.89	0.07
mCh_mRNA$^{1\ CP}$	0.30	0.06	2.53	0.05	4.98	0.07
mCh_mRNA$^{2\ CP}$	0.45	0.05	2.86	0.05	5.05	0.05
mCh_mRNA$^{WT,\ \Psi+5mC}$	1.05	0.16	3.55	0.18	5.74	0.13
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	1.02	0.15	3.46	0.24	5.90	0.14
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	1.15	0.16	3.88	0.15	6.17	0.11
mCh_mRNA$^{1\ PM}$	-0.07	0.09	2.17	0.05	4.64	0.08
mCh_mRNA$^{2\ PM}$	-0.21	0.11	2.13	0.07	4.39	0.10
mCh_mRNA$_{UTR2}^{WT}$	-0.11	0.11	2.20	0.06	4.34	0.19
mCh_mRNA$_{UTR2}^{1\ CP}$	0.37	0.14	2.42	0.08	4.55	0.13
mCh_mRNA$_{UTR2}^{2\ CP}$	0.45	0.17	2.33	0.07	4.58	0.16
mCh_mRNA$_{UTR2}^{1\ PM}$	-0.11	0.13	2.15	0.05	4.31	0.06
mCh_mRNA$_{UTR2}^{2\ PM}$	0.03	0.08	2.13	0.07	4.32	0.07

Fold change $2^{-\Delta\Delta Ct}$

mCh_mRNA 3' end primer + probe set

	6 h		24 h		48 h	
	$2^{-\Delta\Delta Ct}$ (mCh 3'end)	error	$2^{-\Delta\Delta Ct}$ (mCh 3'end)	error	$2^{-\Delta\Delta Ct}$ (mCh 3'end)	error
mCh_mRNA^{WT}	1.00	0.04	0.23	0.01	0.048	0.002
mCh_mRNA$^{1\ CP}$	0.71	0.03	0.18	0.01	0.038	0.002
mCh_mRNA$^{2\ CP}$	0.51	0.03	0.12	0.00	0.029	0.001
mCh_mRNA$^{WT,\ \Psi+5mC}$	0.55	0.05	0.12	0.01	0.035	0.003
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	0.51	0.05	0.11	0.02	0.025	0.002
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	0.30	0.03	0.06	0.01	0.016	0.001
mCh_mRNA$^{1\ PM}$	1.00	0.05	0.27	0.01	0.051	0.003
mCh_mRNA$^{2\ PM}$	1.09	0.05	0.28	0.01	0.056	0.004
mCh_mRNA$_{UTR2}^{WT}$	1.14	0.05	0.27	0.01	0.062	0.008
mCh_mRNA$_{UTR2}^{1\ CP}$	0.59	0.02	0.16	0.01	0.038	0.003
mCh_mRNA$_{UTR2}^{2\ CP}$	0.54	0.03	0.15	0.01	0.031	0.003
mCh_mRNA$_{UTR2}^{1\ PM}$	1.07	0.05	0.28	0.01	0.062	0.002
mCh_mRNA$_{UTR2}^{2\ PM}$	0.96	0.04	0.25	0.01	0.055	0.003

mCh_mRNA internal primer + probe set

	6 h		24 h		48 h	
	$2^{-\Delta\Delta Ct}$ (mCh internal)	error	$2^{-\Delta\Delta Ct}$ (mCh internal)	error	$2^{-\Delta\Delta Ct}$ (mCh internal)	error
mCh_mRNA^{WT}	1.00	0.04	0.18	0.01	0.034	0.002
mCh_mRNA$^{1\ CP}$	0.81	0.03	0.17	0.01	0.032	0.002
mCh_mRNA$^{2\ CP}$	0.73	0.03	0.14	0.00	0.030	0.001
mCh_mRNA$^{WT,\ \Psi+5mC}$	0.48	0.05	0.09	0.01	0.019	0.002
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	0.49	0.05	0.09	0.02	0.017	0.002
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	0.45	0.05	0.07	0.01	0.014	0.001
mCh_mRNA$^{1\ PM}$	1.05	0.07	0.22	0.01	0.040	0.002
mCh_mRNA$^{2\ PM}$	1.15	0.09	0.23	0.01	0.048	0.003
mCh_mRNA$_{UTR2}^{WT}$	1.08	0.08	0.22	0.01	0.049	0.007
mCh_mRNA$_{UTR2}^{1\ CP}$	0.78	0.07	0.19	0.01	0.043	0.004
mCh_mRNA$_{UTR2}^{2\ CP}$	0.73	0.08	0.20	0.01	0.042	0.005
mCh_mRNA$_{UTR2}^{1\ PM}$	1.08	0.09	0.22	0.01	0.051	0.002
mCh_mRNA$_{UTR2}^{2\ PM}$	0.98	0.05	0.23	0.01	0.050	0.002

Ratios

	3′ end			
Time point [h]	**mCh_mRNA$^{WT, \Psi+5mC}$ / mCh_mRNA^{WT}**	**error**	**mCh_mRNA$^{2\ CP, \Psi+5mC}$ / mCh_mRNA^{WT}**	**error**
6	0.55	0.05	0.30	0.03
24	0.54	0.07	0.27	0.03
48	0.73	0.07	0.33	0.03

	internal			
Time point [h]	**mCh_mRNA$^{WT, \Psi+5mC}$ / mCh_mRNA^{WT}**	**error**	**mCh_mRNA$^{2\ CP, \Psi+5mC}$ / mCh_mRNA^{WT}**	**error**
6	0.48	0.06	0.45	0.05
24	0.48	0.06	0.38	0.04
48	0.56	0.07	0.41	0.04

Initial experiments for qPCR efficiencies in singleplex vs. multiplex assays

HeLa cells were transfected with **mCh_mRNA^{WT}** and incubated for 24 h. Then, cells were lysed and total cellular RNA was isolated. Total RNA isolation was accomplished using the NucleoSpin® RNA Kit (Macherey-Nagel) according to the manufacturer's protocol.

RT-qPCR reactions were performed applying the GoTaq® Probe 2 step RT-qPCR System (Promega). First, denaturing of RNA templates and primers was performed in a 7 µL scale with 100 ng of isolated cellular total RNA, 0.5 µg Oligo(dT)$_{15}$ primer and 0.5 µg random primers. RNA/primer mixes were pipetted on ice, then incubated at 70 °C for 5 min and subsequently chilled in an ice-water bath for at least 5 min. According to the manufacturers' protocol, master mixes for reverse transcription (RT) and no-reverse transcriptase (NRT) controls were prepared on ice with a total volume of 13 µL per each reaction. 13 µL each of RT or NRT master mix were added to 7 µL each of RNA/primer mixes for a final reaction volume of 20 µL containing GoScript™ Reaction Buffer (unlisted concentrations of GoTaq® Probe 2 step RT-qPCR System component), 2 mM $MgCl_2$, 500 µM PCR nucleotide mix, 50 U recombinant RNasin ribonuclease inhibitor and GoScript™ reverse transcriptase (unlisted concentration of GoTaq® Probe 2 step RT-qPCR System component) for RT or compensational amount of ddH_2O for NRT controls, respectively. RT and NRT reaction mixes were incubated at 25 °C for 5 min to hybridize RNA and primers, followed by 42 °C for 45 min for implementation of reverse transcription and subsequent enzyme inactivation at 70 °C for 15 min. For usage in qPCR, the reverse transcribed cDNA samples and NRT control reactions were diluted 1:2 with ddH_2O. Five or six different concentrations of two-fold serial dilutions of cDNA or NRT control were then taken for qPCR reactions. According to the manufacturer's protocol, qPCR master mixes were prepared on ice and added to diluted cDNA or NRT control, respectively. Each qPCR reaction was complemented in a total volume of 20 µL containing GoTaq® Probe qPCR master mix (unlisted concentration of GoTaq® Probe 2 step RT-qPCR System component), 200 nM forward primers, 200 nM reverse primers and 300 nM hydrolysis probe. For mCh_qPCR^{internal} singleplex qPCR reactions only mCh_qPCR^{internal}_FW forward primer, 200 nM mCh_qPCR^{internal}_RV reverse primer

and 300 nM mCh_qPCR^{internal}_probe as for mCh_qPCR$^{3'\text{-}end}$ singleplex qPCR reactions only 200 nM mCh_qPCR$^{3'\text{-}end}$_FW forward primer, 200 nM mCh_qPCR$^{3'\text{-}end}$_RV reverse primer and 300 nM mCh_qPCR$^{3'\text{-}end}$_probe as for GAPDH_qPCR singleplex qPCR reactions only 200 nM GAPDH_qPCR_FW forward primer, 200 nM GAPDH_qPCR_RV reverse primer and 300 nM GAPDH_qPCR_probe as for β-Actin singleplex qPCR reactions only 200 nM β-Actin_qPCR_FW forward primer, 200 nM β-Actin _qPCR_RV reverse primer and 300 nM β-Actin _qPCR_probe were used, respectively. For multiplex reactions a combination thereof was used. qPCR master mix and cDNA or NRT dilutions, respectively, were pipetted into 96-well PCR plates (Hard-Shell® 96-Well PCR Plates, low profile, thin wall, skirted, white/white, Bio-Rad) on ice. The qPCR plate was sealed with adhesive foil (Microseal 'B' PCR Plate Sealing Film, adhesive, optical, Bio-Rad), briefly centrifuged and placed into the qPCR thermal cycler (CFX96 Touch Real-Time PCR Detection System, Bio-Rad). qPCR cycling was performed with an initial denaturing step at 95 °C for 2 min, followed by 40 cycles of denaturing at 95 °C for 15 s and a combined step for annealing and elongation at 60 °C for 1 min. All qPCR reactions were performed as technical triplets. C_t values for each target gene were calculated by the CFX Maestro software (Bio-Rad). C_t mean values and standard deviation (s.d.) of technical duplicate samples were calculated. C_t mean values were plotted against the logarithm of diluted cDNA concentrations. Lines of best fit were computed with Excel (Microsoft Office) and qPCR efficiencies were calculated as $(10^{-1/slope}-1)*100$.

singleplex mCh_qPCR$^{3'\text{-}end}$					
V_{cDNA} [µL]	**log(V_{cDNA})**	**Ct Duplicate #1**	**Ct Duplicate #2**	**Ct mean value**	**S.D.**
0.156	-0.807	24.40	24.32	24.36	0.04
0.313	-0.504	23.17	23.19	23.18	0.01
0.625	-0.204	22.10	22.01	22.06	0.045
1.25	0.097	20.93	20.98	20.96	0.025
2.5	0.398	19.88	19.86	19.87	0.01
5	0.699	18.73	19.10	18.92	0.185

Ctrls	**Ct**
NRT	0.0
NTC	0.0

multiplex mCh_qPCR$^{3'\text{-}end}$ together w/ β-Actin_qPCR + GAPDH_qPCR					
V_{cDNA} [µL]	**log(V_{cDNA})**	**Ct Duplicate #1**	**Ct Duplicate #2**	**Ct mean value**	**S.D.**
0.156	-0.807	24.16	24.07	24.12	0.045
0.313	-0.504	23.10	22.94	23.02	0.08
0.625	-0.204	22.03	21.92	21.98	0.055
1.25	0.097	21.01	20.97	20.99	0.02
2.5	0.398	20.08	19.95	20.02	0.065
5	0.699	18.99	18.92	18.96	0.035

Ctrls	Ct
NRT	0.0
NTC	0.0

Multiplex mCh_qPCR$^{3'\text{-end}}$ together w/ mCh_qPCR^{internal}+ β-Actin_qPCR + GAPDH_qPCR					
V_{cDNA} [µL]	log(V_{cDNA})	Ct Duplicate #1	Ct Duplicate #2	Ct mean value	S.D.
0.156	-0.807	24.10	24.04	24.07	0.03
0.313	-0.504	23.05	23.08	23.07	0.015
0.625	-0.204	22.00	21.98	21.99	0.01
1.25	0.097	20.96	20.95	20.96	0.005
2.5	0.398	20.01	20.02	20.02	0.005
5	0.699	24.10	24.04	24.07	0.03

Ctrls	Ct
NRT	0.0
NTC	0.0

singleplex mCh_qPCR^{internal}					
V_{cDNA} [µL]	log(V_{cDNA})	Ct Duplicate #1	Ct Duplicate #2	Ct mean value	S.D.
0.156	-0.807	24.62	24.73	24.68	0.055
0.313	-0.504	23.44	23.51	23.48	0.035
0.625	-0.204	21.71	22.26	21.99	0.275
1.25	0.097	21.21	21.19	21.20	0.01
2.5	0.398	20.12	20.15	20.14	0.015
5	0.699	19.05	19.09	19.07	0.02

Ctrls	Ct
NRT	0.0
NTC	0.0

multiplex mCh_qPCR^{internal} together w/ β-Actin_qPCR + GAPDH_qPCR					
V_{cDNA} [µL]	log(V_{cDNA})	Ct Duplicate #1	Ct Duplicate #2	Ct mean value	S.D.
0.156	-0.807	25.43	25.48	25.46	0.025
0.313	-0.504	24.18	24.09	24.14	0.045
0.625	-0.204	23.05	23.06	23.06	0.005
1.25	0.097	21.98	21.93	21.96	0.025
2.5	0.398	21.05	20.89	20.97	0.08
5	0.699	20.20	19.85	20.03	0.175

Ctrls	Ct

NRT	0.0
NTC	0.0

multiplex mCh_qPCR^{internal} together w/ mCh_qPCR^{internal}+ β-Actin_qPCR + GAPDH_qPCR					
V_{cDNA} [µL]	**log(V_{cDNA})**	**Ct Duplicate #1**	**Ct Duplicate #2**	**Ct mean value**	**S.D.**
0.156	-0.807	24.97	25.00	24.99	0.015
0.313	-0.504	23.76	23.79	23.78	0.015
0.625	-0.204	22.64	22.64	22.64	0
1.25	0.097	21.59	21.58	21.59	0.005
2.5	0.398	20.54	20.53	20.54	0.005
5	0.699	24.97	25.00	24.99	0.015

Ctrls	**Ct**
NRT	0.0
NTC	0.0

singleplex β-Actin_qPCR					
V_{cDNA} [µL]	**log(V_{cDNA})**	**Ct Duplicate #1**	**Ct Duplicate #2**	**Ct mean value**	**S.D.**
0.156	-0.807	24.27	24.19	24.23	0.04
0.313	-0.504	23.17	23.07	23.12	0.05
0.625	-0.204	21.98	21.91	21.95	0.035
1.25	0.097	20.87	20.85	20.86	0.01
2.5	0.398	19.73	19.47	19.60	0.13
5	0.699	18.68	18.46	18.57	0.11

Ctrls	**Ct**
NRT	0.0
NTC	0.0

multiplex β-Actin_qPCR together w/ mCh_qPCR$^{3'\text{-}end}$ + mCh_qPCR^{internal} + GAPDH_qPCR					
V_{cDNA} [µL]	**log(V_{cDNA})**	**Ct Duplicate #1**	**Ct Duplicate #2**	**Ct mean value**	**S.D.**
0.156	-0.807	23.37	23.29	23.33	0.04
0.313	-0.504	22.28	22.54	22.41	0.13
0.625	-0.204	21.62	21.60	21.61	0.01
1.25	0.097	20.58	19.42	20.00	0.58
2.5	0.398	19.54	19.55	19.55	0.005
5	0.699	23.37	23.29	23.33	0.04

Ctrls	**Ct**
NRT	0.0

NTC	0.0

singleplex GAPDH_qPCR					
V_{cDNA} [µL]	**log(V_{cDNA})**	**Ct Duplicate #1**	**Ct Duplicate #2**	**Ct mean value**	**S.D.**
0.156	-0.807	25.14	25.20	25.17	0.03
0.313	-0.504	24.05	24.06	24.06	0.005
0.625	-0.204	22.83	23.21	23.02	0.19
1.25	0.097	21.82	21.78	21.80	0.02
2.5	0.398	20.78	20.82	20.80	0.02
5	0.699	19.72	19.87	19.80	0.075

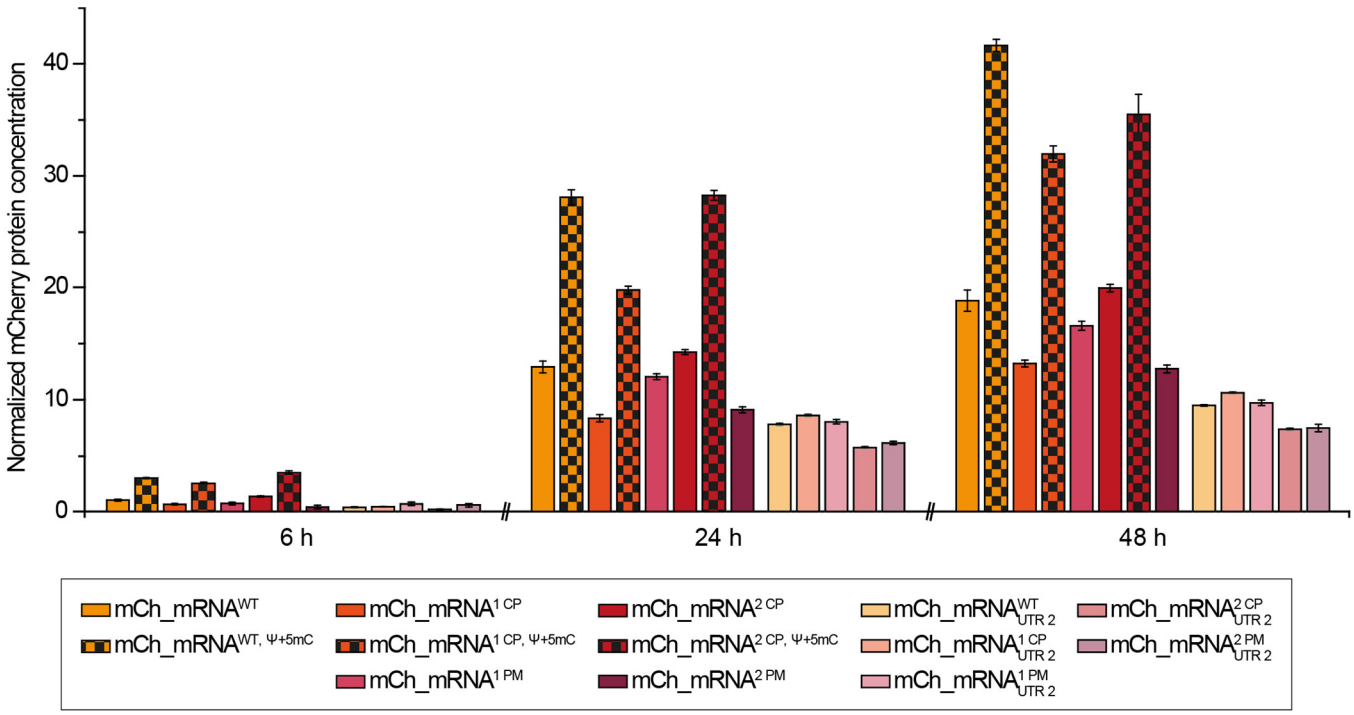
Ctrls	**Ct**
NRT	0.0
NTC	0.0

multiplex GAPDH_qPCR together w/ mCh_qPCR$^{3'\text{-end}}$ + mCh_qPCR^{internal} + β-Actin_qPCR					
V_{cDNA} [µL]	**log(V_{cDNA})**	**Ct Duplicate #1**	**Ct Duplicate #2**	**Ct mean value**	**S.D.**
0.156	-0.807	24.54	24.58	24.56	0.02
0.313	-0.504	23.51	23.56	23.54	0.025
0.625	-0.204	22.50	22.49	22.50	0.005
1.25	0.097	21.54	21.48	21.51	0.03
2.5	0.398	20.52	20.45	20.49	0.035
5	0.699	24.54	24.58	24.56	0.02

Ctrls	**Ct**
NRT	0.0
NTC	0.0

qPCR target		**qPCR efficiency [%]**
mCh_qPCR$^{3'\text{-end}}$	singleplex	88.56
	multiplex (+β-Actin_qPCR +GAPDH_qPCR)	96.94
	multiplex (+ mCh_qPCR^{internal}+ β-Actin_qPCR + GAPDH_qPCR)	97.07
mCh_qPCR^{internal}	singleplex	86.8
	multiplex (+β-Actin_qPCR +GAPDH_qPCR)	90.18
	multiplex (+ mCh_qPCR$^{3'\text{-end}}$+ β-Actin_qPCR + GAPDH_qPCR)	86.85
β-Actin_qPCR	singleplex	83.57
	multiplex (+ mCh_qPCR$^{3'\text{-end}}$+ mCh_qPCR^{internal} + GAPDH_qPCR)	100.31
GAPDH_qPCR	singleplex	89.81
	multiplex (+ mCh_qPCR$^{3'\text{-end}}$+ mCh_qPCR^{internal} + β-Actin_qPCR)	97.66

mCherry protein quantification



Supplementary Figure 30. mCherry protein quantification of **mCh_mRNA** and **mCh_mRNA$_{UTR\ 2}$** sequences. Subsets are shown in Figure 4 of the main text.

Raw data of fluorescence (RFU) and mCherry protein standard curves for quantification

mCh_mRNA + mCh_mRNA$_{UTR\ 2}$

Exp. #1

	WT #1	WT #2	1 CP #1	1 CP #2	2 CP #1	2 CP #2	1 PM #1	1 PM #2	2 PM #1	2 PM #2	H_2O Transf. Ctrl. #1	H_2O Transf. Ctrl. #2	Untr. Cells Ctrl. #1	Untr. Cells Ctrl. #2
6 h mCh_mRNA	72	57	0	120	96	90	95	0	82	2				
6 h mCh_mRNA$_{UTR2}$	80	94	74	74	77	0	74	135	47	74				
6 h H_2O Transf. Ctrl.											41	78		
6 h Untr. Cells Ctrl.													16	66
24 h mCh_mRNA	416	365	227	364	391	501	426	460	397	351				
24 h mCh_mRNA$_{UTR2}$	351	211	319	376	250	270	372	314	321	245				
24 h H_2O Transf. Ctrl.											51	0		
24 h Untr. Cells Ctrl.													2	43
48 h mCh_mRNA	561	608	196	509	576	645	583	564	402	553				
48 h mCh_mRNA$_{UTR2}$	355	331	444	511	464	378	443	467	427	347				
48 h H_2O Transf. Ctrl.											83	25		
48 h Untr. Cells Ctrl.													55	29

mCherry protein standard [ng/well]	0	20	40	60	80	100
Standard curve	60	119	208	396	587	620
Standard curve #2	80	82	331	148	499	544

Background (mean value for 0 ng/well)	70
Slope line of best fit	6.52
Coefficient of determination R^2	0.988

Exp. #2

	WT #1	WT #2	1 CP #1	1 CP #2	2 CP #1	2 CP #2	1 PM #1	1 PM #2	2 PM #1	2 PM #2	H_2O Transf. Ctrl. #1	H_2O Transf. Ctrl. #2	Untr. Cells Ctrl. #1
6 h mCh_mRNA	5	25	0	0	5	0	0	30	35	0			
6 h mCh_mRNA$_{UTR2}$	46	0	32	16	0	0	15	76	42	41			
6 h H_2O Transf. Ctrl.											0	0	
6 h Untr. Cells Ctrl.													0
24 h mCh_mRNA	300	398	137	260	336	349	336	313	251	246			
24 h mCh_mRNA$_{UTR2}$	270	187	276	259	171	192	195	256	204	258			
24 h H_2O Transf. Ctrl.											0	0	
24 h Untr. Cells Ctrl.													0
48 h mCh_mRNA	516	470	285	292	506	529	310	511	350	394			
48 h mCh_mRNA$_{UTR2}$	347	343	381	398	200	250	384	361	348	145			
48 h H_2O Transf. Ctrl.											5	0	
48 h Untr. Cells Ctrl.													26

mCherry protein standard [ng/well]	0	20	40	60	80	100
Standard curve #1	0	118	94	214	306	317
Standard curve #2	0	33	145	198	318	429

Background (mean value for 0 ng/well)	0
Slope line of best fit	3.68
Coefficient of determination R^2	0.996

Exp. #3

	WT #1	WT #2	1 CP #1	1 CP #2	2 CP #1	2 CP #2	1 PM #1	1 PM #2	2 PM #1	2 PM #2	H_2O Transf. Ctrl. #1	H_2O Transf. Ctrl. #2	Untr. Cells Ctrl. #1	Untr. Cells Ctrl. #2
6 h mCh_mRNA	132	115	63	1	124	147	137	71	23	52				
6 h mCh_mRNA$_{UTR2}$	77	88	12	38	51	55	40	91	47	36				
6 h H_2O Transf. Ctrl.											73	28		
6 h Untr. Cells Ctrl.													2	69
24 h mCh_mRNA	872	830	236	374	543	577	635	741	505	605				
24 h mCh_mRNA$_{UTR2}$	439	326	304	462	221	296	377	466	330	336				
24 h H_2O Transf. Ctrl.											4	15		

24 h Untr. Cells Ctrl.												45	39
48 h mCh_mRNA	1050	1193	331	512	608	787	957	1141	698	827			
48 h mCh_mRNA$_{UTR2}$	493	490	447	472	379	485	437	528	473	411			
48 h H_2O Transf. Ctrl.											27	32	
48 h Untr. Cells Ctrl.												1	54

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	38	136	170	256	424	432
Standard curve #2	43	95	192	336	346	430

Background (mean value for 0 ng/well)	40.5
Slope line of best fit	4.60
Coefficient of determination R^2	0.992

Exp. #4

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**1 PM #1**	**1 PM #2**	**2 PM #1**	**2 PM #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	89	65	0	69	104	157	93	82	26	60				
6 h mCh_mRNA$_{UTR2}$	31	75	52	59	17	56	101	78	53	90				
6 h H_2O Transf. Ctrl.											60	9		
6 h Untr. Cells Ctrl.													0	44
24 h mCh_mRNA	985	1075	323	509	761	869	967	996	656	718				
24 h mCh_mRNA$_{UTR2}$	607	537	438	543	363	436	515	560	406	486				
24 h H_2O Transf. Ctrl.											59	8		
24 h Untr. Cells Ctrl.													34	39
48 h mCh_mRNA	1561	1424	492	592	1204	1259	1335	1480	1047	1170				
48 h mCh_mRNA$_{UTR2}$	851	776	600	671	599	547	817	700	563	613				
48 h H_2O Transf. Ctrl.											43	17		
48 h Untr. Cells Ctrl.													76	10

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	49	122	266	282	289	548
Standard curve #2	50	102	301	304	393	413

Background (mean value for 0 ng/well)	49.5
Slope line of best fit	4.84
Coefficient of determination R^2	0.976

Exp. #5

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**1 PM #1**	**1 PM #2**	**2 PM #1**	**2 PM #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	42	125	19	13	92	103	8	36	94	98				

6 h mCh_mRNA$_{UTR2}$	13	0	21	41	0	0	26	89	43	42				
6 h H_2O Transf. Ctrl.											0	0		
6 h Untr. Cells Ctrl.													0	0
24 h mCh_mRNA	694	670	117	390	833	683	659	782	582	574				
24 h mCh_mRNA$_{UTR2}$	487	407	396	332	173	316	381	471	329	332				
24 h H_2O Transf. Ctrl.											1	0		
24 h Untr. Cells Ctrl.													0	0
48 h mCh_mRNA	1194	1031	373	606	1219	1024	1234	1205	806	991				
48 h mCh_mRNA$_{UTR2}$	610	566	597	570	390	321	635	653	526	480				
48 h H_2O Transf. Ctrl.											15	0		
48 h Untr. Cells Ctrl.													0	7

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	10	104	263	367	523	616
Standard curve #2	13	135	241	389	480	653

Background (mean value for 0 ng/well)	11.5
Slope line of best fit	6.31
Coefficient of determination R^2	1

Exp. #6

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**1 PM #1**	**1 PM #2**	**2 PM #1**	**2 PM #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	91	106	55	22	180	155	116	92	102	1				
6 h mCh_mRNA$_{UTR2}$	52	46	49	67	57	31	52	38	54	34				
6 h H_2O Transf. Ctrl.											63	0		
6 h Untr. Cells Ctrl.													37	0
24 h mCh_mRNA	717	647	226	371	750	975	696	742	463	614				
24 h mCh_mRNA$_{UTR2}$	409	383	424	388	256	322	457	462	331	299				
24 h H_2O Transf. Ctrl.											43	0		
24 h Untr. Cells Ctrl.													45	9
48 h mCh_mRNA	1072	833	279	520	1138	1243	1162	1196	799	866				
48 h mCh_mRNA$_{UTR2}$	610	543	555	551	369	318	543	615	484	502				
48 h H_2O Transf. Ctrl.											62	4		
48 h Untr. Cells Ctrl.													37	13

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	4	106	202	304	435	507
Standard curve #2	0	154	222	387	394	564

Background (mean value for 0 ng/well)	2
Slope line of best fit	5.18

Coefficient of determination R^2	0.999

Exp. #7

	WT #1	WT #2	1 CP #1	1 CP #2	2 CP #1	2 CP #2	1 PM #1	1 PM #2	2 PM #1	2 PM #2	H_2O Transf. Ctrl. #1	H_2O Transf. Ctrl. #2	Untr. Cells Ctrl. #1	Untr. Cells Ctrl. #2
6 h mCh_mRNA	72	124	51	56	90	67	140	96	107	79				
6 h mCh_mRNA$_{UTR2}$	77	47	101	95	87	46	68	89	95	75				
6 h H_2O Transf. Ctrl.											20	1		
6 h Untr. Cells Ctrl.													33	61
24 h mCh_mRNA	589	517	244	421	372	216	534	548	449	414				
24 h mCh_mRNA$_{UTR2}$	514	492	604	625	365	423	463	433	343	381				
24 h H_2O Transf. Ctrl.											15	33		
24 h Untr. Cells Ctrl.													88	120
48 h mCh_mRNA	692	691	301	443	320	333	668	726	513	603				
48 h mCh_mRNA$_{UTR2}$	511	516	635	720	399	377	515	530	356	396				
48 h H_2O Transf. Ctrl.											38	8		
48 h Untr. Cells Ctrl.													76	45

mCherry protein standard [ng/well]	0	20	40	60	80	100
Standard curve #1	17	251	281	415	564	669
Standard curve #2	53	170	361	438	632	706

Background (mean value for 0 ng/well)	35
Slope line of best fit	7.24
Coefficient of determination R^2	0.993

Exp. #8

	WT #1	WT #2	1 CP #1	1 CP #2	2 CP #1	2 CP #2	1 PM #1	1 PM #2	2 PM #1	2 PM #2	H_2O Transf. Ctrl. #1	H_2O Transf. Ctrl. #2	Untr. Cells Ctrl. #1	Untr. Cells Ctrl. #2
6 h mCh_mRNA	91	103	0	51	22	6	45	39	30	8				
6 h mCh_mRNA$_{UTR2}$	42	23	96	85	13	23	81	55	73	80				
6 h H_2O Transf. Ctrl.											34	41		
6 h Untr. Cells Ctrl.													20	45
24 h mCh_mRNA	587	616	231	378	260	389	536	538	395	480				
24 h mCh_mRNA$_{UTR2}$	443	447	545	620	372	304	421	395	309	303				
24 h H_2O Transf. Ctrl.											65	0		
24 h Untr. Cells Ctrl.													35	0
48 h mCh_mRNA	632	700	366	516	306	334	723	692	580	628				
48 h mCh_mRNA$_{UTR2}$	549	446	714	682	474	409	474	515	401	374				
48 h H_2O Transf. Ctrl.											8	0		
48 h Untr. Cells Ctrl.													37	0

mCherry protein standard [ng/well]	0	20	40	60	80	100
Standard curve #1	39	207	264	361	507	654
Standard curve #2	31	119	341	440	467	734

Background (mean value for 0 ng/well)	35
Slope line of best fit	6.72
Coefficient of determination R^2	0.994

Exp. #9

	WT #1	WT #2	1 CP #1	1 CP #2	2 CP #1	2 CP #2	1 PM #1	1 PM #2	2 PM #1	2 PM #2	H_2O Transf. Ctrl. #1	H_2O Transf. Ctrl. #2	Untr. Cells Ctrl. #1	Untr. Cells Ctrl. #2
6 h mCh_mRNA	0	2	0	59	4	0	32	39	0	41				
6 h mCh_mRNA$_{UTR2}$	0	8	0	9	14	55	0	18	57	14				
6 h H_2O Transf. Ctrl.											0	0		
6 h Untr. Cells Ctrl.													0	0
24 h mCh_mRNA	589	528	161	411	276	256	384	555	378	366				
24 h mCh_mRNA$_{UTR2}$	437	326	526	583	275	359	430	441	285	285				
24 h H_2O Transf. Ctrl.											0	0		
24 h Untr. Cells Ctrl.													0	0
48 h mCh_mRNA	545	566	205	389	246	298	546	571	358	492				
48 h mCh_mRNA$_{UTR2}$	355	299	511	548	326	319	399	264	256	228				
48 h H_2O Transf. Ctrl.											0	0		
48 h Untr. Cells Ctrl.													0	0

mCherry protein standard [ng/well]	0	20	40	60	80	100
Standard curve #1	0	67	202	307	456	511
Standard curve #2	0	102	197	273	369	557

Background (mean value for 0 ng/well)	0
Slope line of best fit	5.16
Coefficient of determination R^2	0.998

Exp. #10

	WT #1	WT #2	1 CP #1	1 CP #2	2 CP #1	2 CP #2	1 PM #1	1 PM #2	2 PM #1	2 PM #2	H_2O Transf. Ctrl. #1	H_2O Transf. Ctrl. #2	Untr. Cells Ctrl. #1	Untr. Cells Ctrl. #2
6 h mCh_mRNA	1	18	0	25	6	0	19	0	0	51				
6 h mCh_mRNA$_{UTR2}$	0	21	31	22	54	0	0	21	54	0				
6 h H_2O Transf. Ctrl.											0	0		
6 h Untr. Cells Ctrl.													0	0
24 h mCh_mRNA	655	510	228	371	299	435	623	712	409	523				
24 h mCh_mRNA$_{UTR2}$	519	391	585	529	402	383	436	506	359	318				
24 h H_2O Transf. Ctrl.											0	0		

24 h Untr. Cells Ctrl.													0	0
48 h mCh_mRNA	506	572	283	389	298	330	554	526	458	397				
48 h mCh_mRNA$_{UTR2}$	390	363	615	585	312	414	337	396	254	256				
48 h H_2O Transf. Ctrl.											0	0		
48 h Untr. Cells Ctrl.													0	0

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	0	15	122	312	436	506
Standard curve #2	0	69	245	236	282	502

Background (mean value for 0 ng/well)	0
Slope line of best fit	4.72
Coefficient of determination R^2	0.991

mCh_mRNA + mCh_mRNA$^{\Psi+5mC}$

Exp. #1

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**WT Ψ+5mC #1**	**WT Ψ+5mC #2**	**1 CP Ψ+5mC #1**	**1 CP Ψ+5mC #2**	**2 CP Ψ+5mC #1**	**2 CP Ψ+5mC #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	130	116	109	83	131	148	190	222	130	126	128	144				
6 h H_2O Transf. Ctrl.													13	71		
6 h Untr. Cells Ctrl.															44	67
24 h mCh_mRNA	869	1083	572	549	846	767	1850	1980	637	611	929	890				
24 h H_2O Transf. Ctrl.													26	2		
24 h Untr. Cells Ctrl.															61	64
48 h mCh_mRNA	1317	1468	775	834	1203	1204	2540	2509	901	921	1154	1141				
48 h H_2O Transf. Ctrl.													35	48		
48 h Untr. Cells Ctrl.															37	47

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	6	127	262	347	354	450
Standard curve #2	86	138	238	307	386	383

Background (mean value for 0 ng/well)	46
Slope line of best fit	4.68
Coefficient of determination R^2	0.978

Exp. #2

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**WT Ψ+5mC #1**	**WT Ψ+5mC #2**	**1 CP Ψ+5mC #1**	**1 CP Ψ+5mC #2**	**2 CP Ψ+5mC #1**	**2 CP Ψ+5mC #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	121	98	96	92	189	161	231	249	138	106	217	346				
6 h H_2O Transf. Ctrl.													18	0		
6 h Untr. Cells Ctrl.															2	40

24 h mCh_mRNA	1236	1447	667	746	1405	1343	1681	1845	1216	1234	1869	1793				
24 h H_2O Transf. Ctrl.													6	27		
24 h Untr. Cells Ctrl.															36	3
48 h mCh_mRNA	1936	2360	1201	1219	1880	2339	2716	2921	2364	1868	2777	2471				
48 h H_2O Transf. Ctrl.													19	25		
48 h Untr. Cells Ctrl.															13	21

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	0	143	215	304	466	595
Standard curve #2	0	150	256	385	365	514

Background (mean value for 0 ng/well)	0
Slope line of best fit	5.53
Coefficient of determination R^2	0.997

Exp. #3

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**WT Ψ+5mC #1**	**WT Ψ+5mC #2**	**1 CP Ψ+5mC #1**	**1 CP Ψ+5mC #2**	**2 CP Ψ+5mC #1**	**2 CP Ψ+5mC #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	0	92	0	27	76	37	91	65	75	64	147	178				
6 h H_2O Transf. Ctrl.													0	0		
6 h Untr. Cells Ctrl.															0	0
24 h mCh_mRNA	404	1017	432	545	1159	1141	1704	1275	1080	1078	1685	1643				
24 h H_2O Transf. Ctrl.													0	0		
24 h Untr. Cells Ctrl.															0	0
48 h mCh_mRNA	594	1465	812	794	1477	1405	1687	1832	1541	1504	330	1835				
48 h H_2O Transf. Ctrl.													0	0		
48 h Untr. Cells Ctrl.															0	0

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	0	55	187	271	411	472
Standard curve #2	0	72	199	215	402	481

Background (mean value for 0 ng/well)	0
Slope line of best fit	4.77
Coefficient of determination R^2	0.995

Exp. #4

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**WT Ψ+5mC #1**	**WT Ψ+5mC #2**	**1 CP Ψ+5mC #1**	**1 CP Ψ+5mC #2**	**2 CP Ψ+5mC #1**	**2 CP Ψ+5mC #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	38	22	21	76	83	112	177	131	111	78	167	246				
6 h H_2O Transf. Ctrl.													0	0		
6 h Untr. Cells Ctrl.															0	52
24 h mCh_mRNA	313	761	439	521	1036	999	1082	1087	1042	909	1667	1352				

24 h H_2O Transf. Ctrl.													5	0		
24 h Untr. Cells Ctrl.															0	15
48 h mCh_mRNA	504	1389	897	950	1584	1198	1446	1788	1442	1492	2055	1862				
48 h H_2O Transf. Ctrl.													0	55		
48 h Untr. Cells Ctrl.															0	0

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	0	139	282	418	480	605
Standard curve #2	0	121	283	286	475	648

Background (mean value for 0 ng/well)	0
Slope line of best fit	6.18
Coefficient of determination R^2	0.998

Exp. #5

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**WT Ψ+5mC #1**	**WT Ψ+5mC #2**	**1 CP Ψ+5mC #1**	**1 CP Ψ+5mC #2**	**2 CP Ψ+5mC #1**	**2 CP Ψ+5mC #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	101	68	43	62	104	106	110	85	140	79	171	126				
6 h H_2O Transf. Ctrl.													0	0		
6 h Untr. Cells Ctrl.															0	0
24 h mCh_mRNA	503	673	216	291	950	992	915	1114	643	667	1024	951				
24 h H_2O Transf. Ctrl.													20	0		
24 h Untr. Cells Ctrl.															0	0
48 h mCh_mRNA	287	1092	548	616	1460	1400	1520	1689	975	959	1538	1449				
48 h H_2O Transf. Ctrl.													0	0		
48 h Untr. Cells Ctrl.															0	0

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	0	101	159	315	389	535
Standard curve #2	0	103	219	324	434	523

Background (mean value for 0 ng/well)	0
Slope line of best fit	5.33
Coefficient of determination R^2	1

Exp. #6

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**WT Ψ+5mC #1**	**WT Ψ+5mC #2**	**1 CP Ψ+5mC #1**	**1 CP Ψ+5mC #2**	**2 CP Ψ+5mC #1**	**2 CP Ψ+5mC #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	89	90	46	34	118	65	65	107	50	73	84	127				
6 h H_2O Transf. Ctrl.													0	0		
6 h Untr. Cells Ctrl.															0	0
24 h mCh_mRNA	524	647	327	367	1142	1073	1071	1145	687	713	1152	1242				
24 h H_2O Transf. Ctrl.													0	0		

24 h Untr. Cells Ctrl.															0	2
48 h mCh_mRNA	1110	1468	742	789	1751	1608	1710	1992	1175	1167	1584	1451				
48 h H_2O Transf. Ctrl.													0	0		
48 h Untr. Cells Ctrl.															0	0

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	0	96	260	314	449	505
Standard curve #2	6	117	203	344	484	465

Background (mean value for 0 ng/well)	3
Slope line of best fit	5.34
Coefficient of determination R^2	0.994

Exp. #7

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**WT Ψ+5mC #1**	**WT Ψ+5mC #2**	**1 CP Ψ+5mC #1**	**1 CP Ψ+5mC #2**	**2 CP Ψ+5mC #1**	**2 CP Ψ+5mC #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	94	177	169	186	144	148	441	443	359	362	373	321				
6 h H_2O Transf. Ctrl.													65	37		
6 h Untr. Cells Ctrl.															101	92
24 h mCh_mRNA	683	857	875	872	1009	900	2732	3041	1951	2176	2071	1891				
24 h H_2O Transf. Ctrl.													71	101		
24 h Untr. Cells Ctrl.															93	87
48 h mCh_mRNA	1019	1347	1523	1476	1488	1454	4304	4424	2931	2923	3027	2894				
48 h H_2O Transf. Ctrl.													119	96		
48 h Untr. Cells Ctrl.															83	81

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	87	226	298	488	633	787
Standard curve #2	112	243	413	514	575	739

Background (mean value for 0 ng/well)	99.5
Slope line of best fit	7.96
Coefficient of determination R^2	0.991

Exp. #8

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**WT Ψ+5mC #1**	**WT Ψ+5mC #2**	**1 CP Ψ+5mC #1**	**1 CP Ψ+5mC #2**	**2 CP Ψ+5mC #1**	**2 CP Ψ+5mC #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	45	67	104	58	35	87	298	290	229	233	259	266				
6 h H_2O Transf. Ctrl.													2	0		
6 h Untr. Cells Ctrl.															0	0
24 h mCh_mRNA	592	728	734	796	794	771	2512	2818	1696	1880	1961	1759				
24 h H_2O Transf. Ctrl.													0	15		
24 h Untr. Cells Ctrl.															2	9

48 h mCh_mRNA	970	1270	1288	1390	1323	1321	4006	4200	2715	2682	2752	2671				
48 h H_2O Transf. Ctrl.													13	0		
48 h Untr. Cells Ctrl.															0	29

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	0	120	250	345	492	627
Standard curve #2	5	138	226	356	526	596

Background (mean value for 0 ng/well)	2.5
Slope line of best fit	6.14
Coefficient of determination R^2	0.999

Exp. #9

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**WT Ψ+5mC #1**	**WT Ψ+5mC #2**	**1 CP Ψ+5mC #1**	**1 CP Ψ+5mC #2**	**2 CP Ψ+5mC #1**	**2 CP Ψ+5mC #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	59	37	99	132	81	88	117	110	247	176	246	242				
6 h H_2O Transf. Ctrl.													32	76		
6 h Untr. Cells Ctrl.															0	0
24 h mCh_mRNA	284	683	884	827	1057	889	709	727	928	1154	2096	1883				
24 h H_2O Transf. Ctrl.													18	0		
24 h Untr. Cells Ctrl.															0	0
48 h mCh_mRNA	369	1137	1274	1273	1208	1091	1075	1142	2000	2521	2477	2139				
48 h H_2O Transf. Ctrl.													18	71		
48 h Untr. Cells Ctrl.															0	0

mCherry protein standard [ng/well]	**0**	**20**	**40**	**60**	**80**	**100**
Standard curve #1	32	131	206	383	545	634
Standard curve #2	0	161	284	369	576	774

Background (mean value for 0 ng/well)	16
Slope line of best fit	6.84
Coefficient of determination R^2	0.997

Exp. #10

	WT #1	**WT #2**	**1 CP #1**	**1 CP #2**	**2 CP #1**	**2 CP #2**	**WT Ψ+5mC #1**	**WT Ψ+5mC #2**	**1 CP Ψ+5mC #1**	**1 CP Ψ+5mC #2**	**2 CP Ψ+5mC #1**	**2 CP Ψ+5mC #2**	**H_2O Transf. Ctrl. #1**	**H_2O Transf. Ctrl. #2**	**Untr. Cells Ctrl. #1**	**Untr. Cells Ctrl. #2**
6 h mCh_mRNA	91	150	176	143	133	191	196	152	291	281	321	316				
6 h H_2O Transf. Ctrl.													66	153		
6 h Untr. Cells Ctrl.															95	48
24 h mCh_mRNA	355	813	898	848	1063	869	761	719	1040	1216	1984	1847				
24 h H_2O Transf. Ctrl.													93	50		
24 h Untr. Cells Ctrl.															88	85
48 h mCh_mRNA	469	1186	1307	1314	1164	1175	1137	1115	1977	2409	2341	2299				

48 h H_2O Transf. Ctrl.													77	52		
48 h Untr. Cells Ctrl.															107	18

mCherry protein standard [ng/well]	0	20	40	60	80	100
Standard curve #1	70	204	290	372	654	635
Standard curve #2	86	283	298	425	549	807

Background (mean value for 0 ng/well)	78
Slope line of best fit	7.31
Coefficient of determination R^2	0.985

Mean values [ng mCherry protein/well] and standard deviation of duplicate samples, background subtracted and divided by slope of linear standard curve (individual for each measurement)

mCh_mRNA + mCh_mRNA$_{UTR\ 2}$

Exp. #1

	WT	error	1 CP	error	2 CP	error	1 PM	error	2 PM	error	H_2O Transf. Ctrl.	error	Untr. Cells Ctrl.	error
6 h mCh_mRNA	-0.84	1.15	-1.53	9.20	3.53	0.46	-3.45	7.29	-4.30	6.14				
6 h mCh_mRNA$_{UTR2}$	2.61	1.07	0.61	0.00	-4.83	5.91	5.29	4.68	-1.46	2.07				
6 h H_2O Transf. Ctrl.											-1.61	2.84		
6 h Untr. Cells Ctrl.													-4.45	3.83
24 h mCh_mRNA	49.16	3.91	34.59	10.51	57.68	8.44	57.22	2.61	46.63	3.53				
24 h mCh_mRNA$_{UTR2}$	32.37	10.74	42.57	4.37	29.15	1.53	41.88	4.45	32.67	5.83				
24 h H_2O Transf. Ctrl.											-6.83	3.91		
24 h Untr. Cells Ctrl.													-7.29	3.14
48 h mCh_mRNA	78.92	3.60	43.33	24.01	82.91	5.29	77.23	1.46	62.51	11.58				
48 h mCh_mRNA$_{UTR2}$	41.88	1.84	62.51	5.14	53.84	6.60	59.06	1.84	48.63	6.14				
48 h H_2O Transf. Ctrl.											-2.45	4.45		
48 h Untr. Cells Ctrl.													-4.30	1.99

Exp. #2

	WT	error	1 CP	error	2 CP	error	1 PM	error	2 PM	error	H_2O Transf. Ctrl.	error	Untr. Cells Ctrl.	error
6 h mCh_mRNA	4.08	2.72	0,00	0,00	0.68	0.68	4.08	4.08	4.76	4.76				
6 h mCh_mRNA$_{UTR2}$	6.25	6.25	6,53	2,18	0.00	0.00	12.37	8.29	11.28	0.14				
6 h H_2O Transf. Ctrl.											0.00	0.00		
6 h Untr. Cells Ctrl.													0.00	0.00
24 h mCh_mRNA	94.90	13.32	53,97	16,72	93.13	1.77	88.23	3.13	67.57	0.68				
24 h mCh_mRNA$_{UTR2}$	62.13	11.28	72,74	2,31	49.35	2.86	61.32	8.29	62.81	7.34				
24 h H_2O Transf. Ctrl.											0.00	0.00		
24 h Untr. Cells Ctrl.													0.00	0.00

48 h mCh_mRNA	134.05	6.25	78,45	0,95	140.71	3.13	111.62	27.33	101.15	5.98				
48 h mCh_mRNA$_{UTR2}$	93.81	0.54	105,91	2,31	61.18	6.80	101.29	3.13	67.03	27.60				
48 h H_2O Transf. Ctrl.											0.68	0.68		
48 h Untr. Cells Ctrl.													3.53	3.53

Exp. #3

	WT	**error**	**1 CP**	**error**	**2 CP**	**error**	**1 PM**	**error**	**2 PM**	**error**	**H_2O Transf. Ctrl.**	**error**	**Untr. Cells Ctrl.**	**error**
6 h mCh_mRNA	18.04	1.85	-1.85	6.74	20.65	2.50	13.80	7.17	-0.65	3.15				
6 h mCh_mRNA$_{UTR2}$	9.13	1.20	-3.37	2.83	2.72	0.43	5.43	5.54	0.22	1.20				
6 h H_2O Transf. Ctrl.											2.17	4.89		
6 h Untr. Cells Ctrl.													-1.09	7.28
24 h mCh_mRNA	176.18	4.56	57.49	15.00	112.92	3.70	140.75	11.52	111.84	10.87				
24 h mCh_mRNA$_{UTR2}$	74.34	12.28	74.45	17.17	47.39	8.15	82.82	9.67	63.58	0.65				
24 h H_2O Transf. Ctrl.											-6.74	1.20		
24 h Untr. Cells Ctrl.													0.33	0.65
48 h mCh_mRNA	234.97	15.54	82.82	19.67	142.81	19.45	219.22	20.00	156.94	14.02				
48 h mCh_mRNA$_{UTR2}$	98.03	0.33	91.08	2.72	85.10	11.52	96.08	9.89	87.27	6.74				
48 h H_2O Transf. Ctrl.											-2.39	0.54		
48 h Untr. Cells Ctrl.													-2.83	5.76

Exp. #4

	WT	**error**	**1 CP**	**error**	**2 CP**	**error**	**1 PM**	**error**	**2 PM**	**error**	**H_2O Transf. Ctrl.**	**error**	**Untr. Cells Ctrl.**	**error**
6 h mCh_mRNA	5.68	2.48	-3.10	7.13	16.73	5.47	7.85	1.14	-1.34	3.51				
6 h mCh_mRNA$_{UTR2}$	0.72	4.54	1.24	0.72	-2.69	4.03	8.26	2.38	4.54	3.82				
6 h H_2O Transf. Ctrl.											-3.10	5.27		
6 h Untr. Cells Ctrl.													-5.68	4.54
24 h mCh_mRNA	202.56	9.30	75.72	19.21	158.14	11.16	192.54	3.00	131.70	6.40				
24 h mCh_mRNA$_{UTR2}$	107.94	7.23	91.11	10.85	72.31	7.54	100.82	4.65	81.91	8.26				
24 h H_2O Transf. Ctrl.											-3.31	5.27		
24 h Untr. Cells Ctrl.													-2.69	0.52
48 h mCh_mRNA	298.11	14.15	101.75	10.33	244.19	5.68	280.55	14.98	218.78	12.71				
48 h mCh_mRNA$_{UTR2}$	157.83	7.75	121.06	7.33	108.15	5.37	146.47	12.09	111.25	5.16				
48 h H_2O Transf. Ctrl.											-4.03	2.69		
48 h Untr. Cells Ctrl.													-1.34	6.82

Exp. #5

	WT	**error**	**1 CP**	**error**	**2 CP**	**error**	**1 PM**	**error**	**2 PM**	**error**	**H_2O Transf. Ctrl.**	**error**	**Untr. Cells Ctrl.**	**error**
6 h mCh_mRNA	11.42	6.58	0.71	0.48	13.64	0.87	1.67	2.22	13.40	0.32				
6 h mCh_mRNA$_{UTR2}$	-0.79	1.03	3.09	1.59	-1.82	0.00	7.30	5.00	4.92	0.08				

6 h H_2O Transf. Ctrl.											-1.82	0.00		
6 h Untr. Cells Ctrl.													-1.82	0.00
24 h mCh_mRNA	106.34	1.90	38.38	21.65	118.39	11.89	112.44	9.75	89.84	0.63				
24 h mCh_mRNA$_{UTR2}$	69.07	6.34	55.90	5.07	36.95	11.34	65.74	7.14	50.59	0.24				
24 h H_2O Transf. Ctrl.											-1.74	0.08		
24 h Untr. Cells Ctrl.													-1.82	0.00
48 h mCh_mRNA	174.61	12.93	75.81	18.48	176.04	15.46	191.58	2.30	140.67	14.67				
48 h mCh_mRNA$_{UTR2}$	91.43	3.49	90.71	2.14	54.56	5.47	100.31	1.43	77.95	3.65				
48 h H_2O Transf. Ctrl.											-0.63	1.19		
48 h Untr. Cells Ctrl.													-1.27	0.56

Exp. #6

	WT	**error**	**1 CP**	**error**	**2 CP**	**error**	**1 PM**	**error**	**2 PM**	**error**	**H_2O Transf. Ctrl.**	**error**	**Untr. Cells Ctrl.**	**error**
6 h mCh_mRNA	18.63	1.45	7.05	3.19	31.96	2.41	19.69	2.32	9.56	9.75				
6 h mCh_mRNA$_{UTR2}$	9.07	0.58	10.81	1.74	8.11	2.51	8.30	1.35	8.11	1.93				
6 h H_2O Transf. Ctrl.											5.70	6.08		
6 h Untr. Cells Ctrl.													3.19	3.57
24 h mCh_mRNA	131.30	6.76	57.25	14.00	166.15	21.72	138.44	4.44	103.59	14.58				
24 h mCh_mRNA$_{UTR2}$	76.07	2.51	78.01	3.48	55.42	6.37	88.34	0.48	60.44	3.09				
24 h H_2O Transf. Ctrl.											3.77	4.15		
24 h Untr. Cells Ctrl.													4.83	3.48
48 h mCh_mRNA	183.53	23.07	76.75	23.27	229.48	10.14	227.26	3.28	160.36	6.47				
48 h mCh_mRNA$_{UTR2}$	110.93	6.47	106.39	0.39	65.94	4.92	111.41	6.95	94.80	1.74				
48 h H_2O Transf. Ctrl.											5.99	5.60		
48 h Untr. Cells Ctrl.													4.44	2.32

Exp. #7

	WT	**error**	**1 CP**	**error**	**2 CP**	**error**	**1 PM**	**error**	**2 PM**	**error**	**H_2O Transf. Ctrl.**	**error**	**Untr. Cells Ctrl.**	**error**
6 h mCh_mRNA	8.70	3.59	2.56	0.35	6.01	1.59	11.47	3.04	8.01	1.93				
6 h mCh_mRNA$_{UTR2}$	3.73	2.07	8.70	0.41	4.35	2.83	6.01	1.45	6.91	1.38				
6 h H_2O Transf. Ctrl.											-3.39	1.31		
6 h Untr. Cells Ctrl.													1.66	1.93
24 h mCh_mRNA	71.57	4.97	41.10	12.23	35.78	10.78	69.91	0.97	54.78	2.42				
24 h mCh_mRNA$_{UTR2}$	64.66	1.52	80.07	1.45	49.60	4.01	57.06	2.07	45.18	2.63				
24 h H_2O Transf. Ctrl.											-1.52	1.24		
24 h Untr. Cells Ctrl.													9.53	2.21
48 h mCh_mRNA	90.71	0.07	46.56	9.81	40.28	0.90	91.47	4.01	72.26	6.22				
48 h mCh_mRNA$_{UTR2}$	66.11	0.35	88.77	5.87	48.77	1.52	67.36	1.04	47.11	2.76				
48 h H_2O Transf. Ctrl.											-1.66	2.07		
48 h Untr. Cells Ctrl.													3.52	2.14

Exp. #8

	WT	error	1 CP	error	2 CP	error	1 PM	error	2 PM	error	H_2O Transf. Ctrl.	error	Untr. Cells Ctrl.	error
6 h mCh_mRNA	9.23	0.89	-1.41	3.80	-3.13	1.19	1.04	0.45	-2.38	1.64				
6 h mCh_mRNA$_{UTR2}$	-0.37	1.41	8.26	0.82	-2.53	0.74	4.91	1.94	6.18	0.52				
6 h H_2O Transf. Ctrl.											0.37	0.52		
6 h Untr. Cells Ctrl.													-0.37	1.86
24 h mCh_mRNA	84.35	2.16	40.13	10.94	43.11	9.60	74.75	0.15	59.93	6.33				
24 h mCh_mRNA$_{UTR2}$	61.05	0.30	81.52	5.58	45.12	5.06	55.54	1.94	40.35	0.45				
24 h H_2O Transf. Ctrl.											-0.37	4.84		
24 h Untr. Cells Ctrl.													-2.61	2.61
48 h mCh_mRNA	93.96	5.06	60.45	11.17	42.44	2.08	100.14	2.31	84.72	3.57				
48 h mCh_mRNA$_{UTR2}$	68.87	7.67	98.72	2.38	60.53	4.84	68.42	3.05	52.49	2.01				
48 h H_2O Transf. Ctrl.											-4.62	0.60		
48 h Untr. Cells Ctrl.													-2.46	2.75

Exp. #9

	WT	error	1 CP	error	2 CP	error	1 PM	error	2 PM	error	H_2O Transf. Ctrl.	error	Untr. Cells Ctrl.	error
6 h mCh_mRNA	0.19	0.19	5.72	5.72	0.39	0.39	6.88	0.68	3.97	3.97				
6 h mCh_mRNA$_{UTR2}$	0.78	0.78	0.87	0.87	6.69	3.97	1.74	1.74	6.88	4.17				
6 h H_2O Transf. Ctrl.											0.00	0.00		
6 h Untr. Cells Ctrl.													0.00	0.00
24 h mCh_mRNA	108.28	5.91	55.45	24.24	51.57	1.94	91.03	16.58	72.13	1.16				
24 h mCh_mRNA$_{UTR2}$	73.97	10.76	107.51	5.53	61.46	8.14	84.44	1.07	55.26	0.00				
24 h H_2O Transf. Ctrl.											0.00	0.00		
24 h Untr. Cells Ctrl.													0.00	0.00
48 h mCh_mRNA	107.70	2.04	57.58	17.84	52.74	5.04	108.28	2.42	82.40	12.99				
48 h mCh_mRNA$_{UTR2}$	63.40	5.43	102.66	3.59	62.53	0.68	64.27	13.09	46.92	2.71				
48 h H_2O Transf. Ctrl.											0.00	0.00		
48 h Untr. Cells Ctrl.													0.00	0.00

Exp. #10

	WT	error	1 CP	error	2 CP	error	1 PM	error	2 PM	error	H_2O Transf. Ctrl.	error	Untr. Cells Ctrl.	error
6 h mCh_mRNA	2.01	1.80	2.65	2.65	0.64	0.64	2.01	2.01	5.41	5.41				
6 h mCh_mRNA$_{UTR2}$	2.23	2.23	5.62	0.95	5.73	5.73	2.23	2.23	5.73	5.73				
6 h H_2O Transf. Ctrl.											0.00	0.00		
6 h Untr. Cells Ctrl.													0.00	0.00
24 h mCh_mRNA	123.53	15.37	63.51	15.16	77.83	14.42	141.55	9.44	98.82	12.09				
24 h mCh_mRNA$_{UTR2}$	96.49	13.57	118.12	5.94	83.24	2.01	99.88	7.42	71.78	4.35				
24 h H_2O Transf. Ctrl.											0.00	0.00		
24 h Untr. Cells Ctrl.													0.00	0.00
48 h mCh_mRNA	114.30	7.00	71.25	11.24	66.59	3.39	114.52	2.97	90.66	6.47				

48 h mCh_mRNA$_{UTR2}$	79.84	2.86	127.24	3.18	76.98	10.82	77.72	6.26	54.08	0.21				
48 h H_2O Transf. Ctrl.											0.00	0.00		
48 h Untr. Cells Ctrl.													0.00	0.00

mCh_mRNA + mCh_mRNA$^{\Psi+5mC}$

Exp. #1

	WT	error	**1 CP**	error	**2 CP**	error	**WT Ψ+5mC**	error	**1 CP Ψ+5mC**	error	**2 CP Ψ+5mC**	error	**H_2O Transf. Ctrl.**	error	**Untr. Cells Ctrl.**	error
6 h mCh_mRNA	16.44	1.49	10.68	2.78	19.96	1.81	34.16	3.42	17.51	0.43	19.22	1.71				
6 h H_2O Transf. Ctrl.													-0.85	6.19		
6 h Untr. Cells Ctrl.															2.03	2.46
24 h mCh_mRNA	198.57	22.85	109.85	2.46	162.38	8.43	399.05	13.88	123.41	2.78	184.37	4.16				
24 h H_2O Transf. Ctrl.													-6.83	2.56		
24 h Untr. Cells Ctrl.															3.52	0.32
48 h mCh_mRNA	287.49	16.12	161.95	6.30	247.14	0.11	529.19	3.31	184.69	2.14	235.18	1.39				
48 h H_2O Transf. Ctrl.													-0.96	1.39		
48 h Untr. Cells Ctrl.															-0.85	1.07

Exp. #2

	WT	error	**1 CP**	error	**2 CP**	error	**WT Ψ+5mC**	error	**1 CP Ψ+5mC**	error	**2 CP Ψ+5mC**	error	**H_2O Transf. Ctrl.**	error	**Untr. Cells Ctrl.**	error
6 h mCh_mRNA	19.79	2.08	16.99	0.36	31.63	2.53	43.38	1.63	22.05	2.89	50.88	11.66				
6 h H_2O Transf. Ctrl.													1.63	1.63		
6 h Untr. Cells Ctrl.															0.18	0.18
24 h mCh_mRNA	242.49	19.07	127.70	7.14	248.36	5.60	318.67	14.82	221.43	1.63	330.97	6.87				
24 h H_2O Transf. Ctrl.													2.98	1.90		
24 h Untr. Cells Ctrl.															3.25	3.25
48 h mCh_mRNA	388.27	38.32	218.72	1.63	381.31	41.48	509.46	18.53	382.48	44.83	474.31	27.66				
48 h H_2O Transf. Ctrl.													3.98	0.54		
48 h Untr. Cells Ctrl.															1.17	1.17

Exp. #3

	WT	error	**1 CP**	error	**2 CP**	error	**WT Ψ+5mC**	error	**1 CP Ψ+5mC**	error	**2 CP Ψ+5mC**	error	**H_2O Transf. Ctrl.**	error	**Untr. Cells Ctrl.**	error
6 h mCh_mRNA	9.65	9.65	2.83	2.83	11.85	4.09	16.36	2.73	14.57	1.15	34.07	3.25				
6 h H_2O Transf. Ctrl.													0.00	0.00		
6 h Untr. Cells Ctrl.															0.00	0.00
24 h mCh_mRNA	148.98	64.27	102.43	11.85	241.14	1.89	312.32	44.98	226.25	0.21	348.91	4.40				
24 h H_2O Transf. Ctrl.													0.00	0.00		
24 h Untr. Cells Ctrl.															0.00	0.00
48 h mCh_mRNA	215.87	91.32	168.38	1.89	302.15	7.55	368.94	15.20	319.24	3.88	226.98	157.79				

48 h H_2O Transf. Ctrl.													0.00	0.00		
48 h Untr. Cells Ctrl.															0.00	0.00

Exp. #4

	WT	error	**1 CP**	error	**2 CP**	error	**WT Ψ+5mC**	error	**1 CP Ψ+5mC**	error	**2 CP Ψ+5mC**	error	**H_2O Transf. Ctrl.**	error	**Untr. Cells Ctrl.**	error
6 h mCh_mRNA	4.86	1.30	7.85	4.45	15.79	2.35	24.94	3.72	15.30	2.67	33.44	6.40				
6 h H_2O Transf. Ctrl.													0.00	0.00		
6 h Untr. Cells Ctrl.															4.21	4.21
24 h mCh_mRNA	86.95	36.27	77.72	6.64	164.75	3.00	175.60	0.40	157.95	10.77	244.42	25.50				
24 h H_2O Transf. Ctrl.													0.40	0.40		
24 h Untr. Cells Ctrl.															1.21	1.21
48 h mCh_mRNA	153.26	71.65	149.53	4.29	225.23	31.25	261.82	27.69	237.54	4.05	317.12	15.63				
48 h H_2O Transf. Ctrl.													4.45	4.45		
48 h Untr. Cells Ctrl.															0.00	0.00

Exp. #5

	WT	error	**1 CP**	error	**2 CP**	error	**WT Ψ+5mC**	error	**1 CP Ψ+5mC**	error	**2 CP Ψ+5mC**	error	**H_2O Transf. Ctrl.**	error	**Untr. Cells Ctrl.**	error
6 h mCh_mRNA	15.85	3.10	9.85	1.78	19.70	0.19	18.29	2.34	20.54	5.72	27.86	4.22				
6 h H_2O Transf. Ctrl.													0.00	0.00		
6 h Untr. Cells Ctrl.															0.00	0.00
24 h mCh_mRNA	110.30	15.94	47.55	7.03	182.15	3.94	190.31	18.66	122.87	2.25	185.24	6.85				
24 h H_2O Transf. Ctrl.													1.88	1.88		
24 h Untr. Cells Ctrl.															0.00	0.00
48 h mCh_mRNA	129.34	75.50	109.17	6.38	268.25	5.63	300.98	15.85	181.40	1.50	280.16	8.35				
48 h H_2O Transf. Ctrl.													0.00	0.00		
48 h Untr. Cells Ctrl.															0.00	0.00

Exp. #6

	WT	error	**1 CP**	error	**2 CP**	error	**WT Ψ+5mC**	error	**1 CP Ψ+5mC**	error	**2 CP Ψ+5mC**	error	**H_2O Transf. Ctrl.**	error	**Untr. Cells Ctrl.**	error
6 h mCh_mRNA	16.18	0.09	6.92	1.12	16.56	4.96	15.53	3.93	10.95	2.15	19.18	4.02				
6 h H_2O Transf. Ctrl.													-0.56	0.00		
6 h Untr. Cells Ctrl.															-0.56	0.00
24 h mCh_mRNA	108.99	11.51	64.37	3.74	206.66	6.46	206.75	6.92	130.41	2.43	223.41	8.42				
24 h H_2O Transf. Ctrl.													-0.56	0.00		
24 h Untr. Cells Ctrl.															-0.37	0.19
48 h mCh_mRNA	240.62	33.49	142.67	4.40	313.69	13.38	345.78	26.38	218.54	0.75	283.38	12.44				
48 h H_2O Transf. Ctrl.													-0.56	0.00		
48 h Untr. Cells Ctrl.															-0.56	0.00

Exp. #7

	WT	error	1 CP	error	2 CP	error	WT Ψ+5mC	error	1 CP Ψ+5mC	error	2 CP Ψ+5mC	error	H_2O Transf. Ctrl.	error	Untr. Cells Ctrl.	error
6 h mCh_mRNA	4.52	5.22	9.80	1.07	5.84	0.25	43.04	0.13	32.80	0.19	31.10	3.27				
6 h H_2O Transf. Ctrl.													-6.10	1.76		
6 h Untr. Cells Ctrl.															-0.38	0.57
24 h mCh_mRNA	84.26	10.93	97.27	0.19	107.45	6.85	350.24	19.42	246.82	14.14	236.45	11.31				
24 h H_2O Transf. Ctrl.													-1.70	1.89		
24 h Untr. Cells Ctrl.															-1.19	0.38
48 h mCh_mRNA	136.16	20.61	175.94	2.95	172.36	2.14	535.92	7.54	355.33	0.50	359.54	8.36				
48 h H_2O Transf. Ctrl.													1.01	1.45		
48 h Untr. Cells Ctrl.															-2.20	0.13

Exp. #8

	WT	error	1 CP	error	2 CP	error	WT Ψ+5mC	error	1 CP Ψ+5mC	error	2 CP Ψ+5mC	error	H_2O Transf. Ctrl.	error	Untr. Cells Ctrl.	error
6 h mCh_mRNA	8.71	1.79	12.78	3.74	9.52	4.23	47.45	0.65	37.19	0.33	42.32	0.57				
6 h H_2O Transf. Ctrl.													-0.24	0.16		
6 h Untr. Cells Ctrl.															-0.41	0.00
24 h mCh_mRNA	107.02	11.07	124.11	5.05	126.96	1.87	433.38	24.90	290.63	14.97	302.35	16.44				
24 h H_2O Transf. Ctrl.													0.81	1.22		
24 h Untr. Cells Ctrl.															0.49	0.57
48 h mCh_mRNA	181.90	24.42	217.54	8.30	214.78	0.16	667.44	15.79	438.83	2.69	440.95	6.59				
48 h H_2O Transf. Ctrl.													0.65	1.06		
48 h Untr. Cells Ctrl.															1.95	2.36

Exp. #9

	WT	error	1 CP	error	2 CP	error	WT Ψ+5mC	error	1 CP Ψ+5mC	error	2 CP Ψ+5mC	error	H_2O Transf. Ctrl.	error	Untr. Cells Ctrl.	error
6 h mCh_mRNA	4.68	1.61	14.54	2.41	10.01	0.51	14.25	0.51	28.57	5.19	33.32	0.29				
6 h H_2O Transf. Ctrl.													5.55	3.22		
6 h Untr. Cells Ctrl.															-2.34	0.00
24 h mCh_mRNA	68.33	29.16	122.70	4.17	139.88	12.28	102.60	1.32	149.81	16.52	288.45	15.57				
24 h H_2O Transf. Ctrl.													-1.02	1.32		
24 h Untr. Cells Ctrl.															-2.34	0.00
48 h mCh_mRNA	107.72	56.13	183.80	0.07	165.67	8.55	159.68	4.90	328.06	38.07	335.00	24.70				
48 h H_2O Transf. Ctrl.													4.17	3.87		
48 h Untr. Cells Ctrl.															-2.34	0.00

Exp. #10

	WT	error	1 CP	error	2 CP	error	WT Ψ+5mC	error	1 CP Ψ+5mC	error	2 CP Ψ+5mC	error	H_2O Transf. Ctrl.	error	Untr. Cells Ctrl.	error
6 h mCh_mRNA	5.82	4.04	11.15	2.26	11.50	3.97	13.14	3.01	28.46	0.68	32.91	0.34				

6 h H_2O Transf. Ctrl.													4.31	5.95		
6 h Untr. Cells Ctrl.															-0.89	3.22
24 h mCh_mRNA	69.25	31.34	108.80	3.42	121.52	13.27	90.59	2.87	143.69	12.04	251.46	9.37				
24 h H_2O Transf. Ctrl.													-0.89	2.94		
24 h Untr. Cells Ctrl.															1.16	0.21
48 h mCh_mRNA	102.57	49.06	168.67	0.48	149.37	0.75	143.42	1.51	289.44	29.56	306.82	2.87				
48 h H_2O Transf. Ctrl.													-1.85	1.71		
48 h Untr. Cells Ctrl.															-2.12	6.09

Merged data from both data sets

	6 h	**error**	**24 h**	**error**	**48 h**	**error**
mCh_mRNA^{WT}	9.18	0.78	118.66	4.86	172.70	8.64
mCh_mRNA$^{1\ CP}$	5.71	0.88	75.01	2.78	119.56	2.66
mCh_mRNA$^{2\ CP}$	12.17	0.59	130.80	2.12	182.91	3.11
mCh_mRNA$^{WT,\ \Psi+5mC}$	27.05	0.81	257.95	6.20	382.26	5.13
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	22.80	0.91	181.33	3.13	293.55	6.62
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	32.43	1.53	259.60	3.98	325.94	16.39
mCh_mRNA$^{1\ PM}$	6.50	1.21	110.69	2.53	152.19	3.78
mCh_mRNA$^{2\ PM}$	3.64	1.51	83.68	2.40	117.04	3.24
mCh_mRNA$^{WT}_{UTR\ 2}$	3.34	0.46	71.81	0.87	87.21	0.61
mCh_mRNA$^{1\ CP}_{UTR\ 2}$	3.80	0.35	78.99	0.83	97.45	0.64
mCh_mRNA$^{2\ CP}_{UTR\ 2}$	1.57	0.51	53.00	0.76	67.76	0.77
mCh_mRNA$^{1\ PM}_{UTR\ 2}$	6.19	1.29	73.78	1.79	89.24	2.30
mCh_mRNA$^{2\ PM}_{UTR\ 2}$	5.33	0.87	56.46	1.39	68.75	3.01
H_2O Transfection Ctrl.	0.10	0.69	-1.08	0.54	0.09	0.52
Untreated Cells Ctrl.	-0.34	0.60	0.30	0.34	-0.28	0.63

Normalized to mCh_mRNA^{WT}

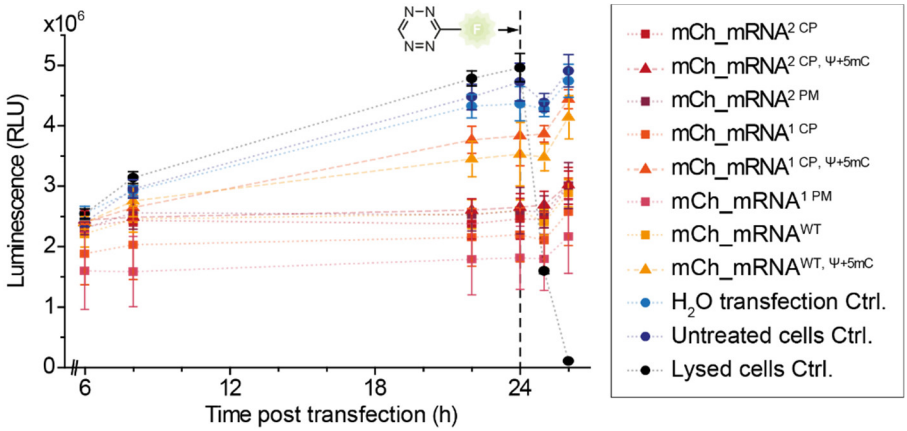
	6 h	**error**	**24 h**	**error**	**48 h**	**error**
mCh_mRNA^{WT}	1.00	0.08	12.92	0.53	18.81	0.94
mCh_mRNA$^{1\ CP}$	0.62	0.10	8.17	0.30	13.02	0.29
mCh_mRNA$^{2\ CP}$	1.33	0.06	14.24	0.23	19.92	0.34
mCh_mRNA$^{WT,\ \Psi+5mC}$	2.95	0.09	28.09	0.67	41.63	0.56
mCh_mRNA$^{1\ CP,\ \Psi+5mC}$	2.48	0.10	19.75	0.34	31.97	0.72
mCh_mRNA$^{2\ CP,\ \Psi+5mC}$	3.53	0.17	28.27	0.43	35.50	1.78
mCh_mRNA$^{1\ PM}$	0.71	0.13	12.05	0.28	16.57	0.41
mCh_mRNA$^{2\ PM}$	0.40	0.16	9.11	0.26	12.75	0.35
mCh_mRNA$^{WT}_{UTR\ 2}$	0.36	0.05	7.82	0.10	9.50	0.07
mCh_mRNA$^{1\ CP}_{UTR\ 2}$	0.41	0.04	8.60	0.09	10.61	0.07
mCh_mRNA$^{2\ CP}_{UTR\ 2}$	0.17	0.06	5.77	0.08	7.38	0.08
mCh_mRNA$^{1\ PM}_{UTR\ 2}$	0.67	0.14	8.04	0.19	9.72	0.25

mCh_mRNA$^{2\ PM}{}_{UTR\ 2}$	0.58	0.10	6.15	0.15	7.49	0.33
H_2O Transfection Ctrl.	0.01	0.07	-0.12	0.06	0.01	0.06
Untreated Cells Ctrl.	-0.04	0.07	0.03	0.04	-0.03	0.07

Ratios

Time point [h]	mCh_mRNA$^{WT,\ \Psi+5mC}$ / mCh_mRNA^{WT}	error	mCh_mRNA$^{2\ CP,\ \Psi+5mC}$ / mCh_mRNA^{WT}	error
6	2.95	0.26	3.53	0.34
24	2.17	0.10	2.19	0.10
48	2.21	0.11	1.89	0.13

MT cell viability assay



Supplementary Figure 31. Cell viability assay of mCh_mRNA sequences. Subset shown in Figure 5 of the main text.

Raw Data of Luminescence (RLU)

Time [h]	6	8	22	24	25	26
mCh mRNA$^{2\ CP}$	2432900	2554490	2685350	2725420	2643830	3255060
	2409160	2454330	2416200	2704730	2627880	3233770
	2215920	2334510	2075270	1997020	2117430	2593080
mCh mRNA$^{1\ CP}$	1174660	1244560	1518930	1614330	1498030	1851460
	2209430	2340810	2635460	2690920	2606540	3198450
	2313670	2557930	2356800	2328160	2263850	2738030
mCh mRNA^{WT}	1919750	2165110	2217530	2378180	2129170	2592010
	2374180	2623030	2778790	2795050	2621650	3105210
	2375480	2632540	2659840	2673440	2511330	3019900
mCh mRNA$^{1\ PM}$	811560	867730	982170	1093620	1086630	1333650
	1662180	1650180	2121930	2190380	2079090	2522180
	2375030	2292610	2313820	2208820	2273910	2703040
mCh mRNA$^{2\ PM}$	2465640	2948460	2843250	3069840	2907870	3503480
	2267220	2469010	2588280	2641080	2614210	3016440
	2225750	2308760	2195970	2126190	2256640	2572390
mCh mRNA$^{2\ CP,\ \Psi+5mC}$	2378570	2421430	2494960	2693910	2531250	2891690
	2673200	2673550	2888130	2934110	3023130	3366430
	2315690	2415560	2473890	2379340	2568100	2872290
mCh mRNA$^{1\ CP,\ \Psi+5mC}$	2454960	2895930	3934790	4433470	3916380	4539120
	2396240	2719690	3944030	3894430	4032920	4598720
	2394170	2368090	3467600	3222260	3701690	4242190
mRNA$^{WT,\ \Psi+5mC}$	2425740	3039910	3654140	4121950	3681070	4491290
	2531230	2851830	3692630	3662330	3625490	4330610
	2272830	2423550	3053540	2851040	3180800	3664090
H_2O Ctrl.	2665420	2951370	4445670	4552470	4314760	4921590
	2630750	3039010	4510970	4609090	4443640	4992870
	2414720	2826700	4067910	3980930	4136510	4375440
Untreated Cells Ctrl.	2494110	3062580	4509860	4963310	4550240	5130990
	2434950	3093910	4744170	4961430	4458690	5104830
	2320500	2757400	4225280	4297340	4199930	4548590
Lysed Cells Ctrl.	2456100	3169160	4836790	5141860	1665630	128210
	2659520	3270530	4929130	5146020	1627770	137680
	2552340	3029590	4627390	4643430	1558040	120080
Medium Only	13060	14430	13740	14260	13840	17180
	14230	16360	14560	15280	15720	19340
	16280	16350	16070	16170	17570	20180

	16420	16480	16710	16790	17800	21280
	17400	16640	16940	16600	19010	21020

Background Subtracted Mean Values of Luminescence (RLU) = Data points

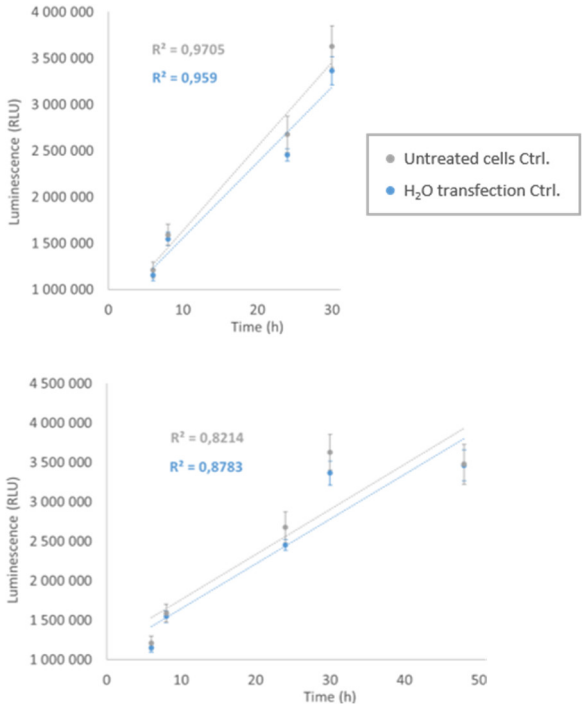
Time [h]	mCh mRNA$^{2\ CP}$	mCh mRNA$^{1\ CP}$	mCh mRNA^{WT}	mCh mRNA$^{1\ PM}$	mCh mRNA$^{2\ PM}$	mCh mRNA$^{2\ CP,\ \Psi+5mC}$	mCh mRNA$^{1\ CP,\ \Psi+5mC}$	mRNA$^{WT,\ \Psi+5mC}$	H_2O Ctrl.	Untreated Cells Ctrl.	Lysed Cells Ctrl.
6	2337182	1883775,33	2207658,67	1600778,67	2304058,67	2440342	2399645,33	2394455,33	2554818,67	2401042	2540508,67
8	2431724,67	2031714,67	2457508	1587454,67	2559358	2487461,33	2645184,67	2755711,33	2922974,67	2955244,67	3140374,67
22	2376669,33	2154792,67	2536449,33	1790369,33	2526896	2603389,33	3766536	3451166	4325912,67	4477499,33	4782166
24	2459903,33	2195316,67	2599736,67	1815120	2596550	2653300	3834233,33	3529286,67	4365010	4724873,33	4961283,33
25	2446258,67	2106018,67	2403928,67	1796422	2576118,67	2690705,33	3866875,33	3478998,67	4281515,33	4386165,33	1600358,67
26	3007503,33	2576180	2885906,67	2166490	3010970	3023670	4440210	4142196,67	4743500	4908336,67	108856,667

Standard Deviation of Luminescence (RLU) = Error bars

Time [h]	mCh mRNA$^{2\ CP}$	mCh mRNA$^{1\ CP}$	mCh mRNA^{WT}	mCh mRNA$^{1\ PM}$	mCh mRNA$^{2\ PM}$	mCh mRNA$^{2\ CP,\ \Psi+5mC}$	mCh mRNA$^{1\ CP,\ \Psi+5mC}$	mRNA$^{WT,\ \Psi+5mC}$	H_2O Ctrl.	Untreated Cells Ctrl.	Lysed Cells Ctrl.
6	97174,3025	514129,114	214527,426	639109,445	104688,676	155839,708	28181,4506	106081,818	110916,114	72064,1228	83085,8902
8	89925,9315	574828,082	218141,639	582640,286	271777,78	120257,959	219417,958	257918,483	87113,5368	151787,637	98774,5724
22	249638,092	474495,846	241476,943	587760,945	266226,352	190502,66	222445,354	292619,941	195297,173	212167,068	126237,018
24	338599,744	447237,709	175039,061	521418,319	385778,027	227161,244	495469,059	525426,201	283715,19	313499,088	235948,789
25	244474,621	463406,604	211016,934	519889,463	266289,945	223695,268	137188,711	223882,014	125924,119	148344,657	44561,1448
26	307165,258	559004,572	224534,553	607411,893	380250,934	228504,502	155932,046	358132,007	275797,926	268592,43	7192,10833

Validation of assay linearity

Assay linearity was measured in initial experiments. Cell seeding, transfection and assay implementation were performed identically as described for the final viability measurements. Assay linearity was ensured for up to 30 h but not up to 50 h (see Supplementary Figure 32).



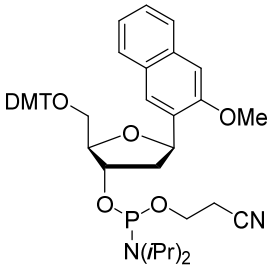
Supplementary Figure 32. Measurements for assay linearity of cell viability assay.

Preparation of DNA primers, oligonucleotides and hydrolysis probes

DNA primer oligonucleotides, splint and adapter oligonucleotides as well as qPCR primer oligonucleotides and hydrolysis probes were synthesized by *Biomers.net*, Germany.

Preparation of dNaM-modified DNA primers and DNA oligonucleotides

d**NaM** cyanoethyl phosphoramidite (Supplmentary Figure 30) was purchased from *Berry & Associates Inc.*, USA. dNaM-modified primer oligonucleotides were prepared by solid phase synthesis in 200 nmol scale by *Ella Biotech*, Germany. d**NaM** triphosphate (TP) was purchased from *MyChem LLC*, USA. d**TPT3** TP was synthesized according to literature.[1]



Supplementary Figure 33. dNaM cyanoethyl phosphoramidite.

Preparation of variant mCh_DNA and mCh_RNA sequences

Two different 3'-UTR sequences of **mCh_mRNAs** were used for experiments. For **mCh_DNA** and **mCh_RNA** the 3'-UTR sequence was adopted as it stands from the *pmCherry-N1* plasmid 3'-UTR following the mCherry protein coding region. To investigate possible effects of the UB modifications placed at different positions within the 3'-UTR sequence, we also prepared **mCh_DNA$_{\mathbf{UTR\ 2}}$** and **mCh_RNA$_{\mathbf{UTR\ 2}}$**. Therefore, a reverse primer was designed that hybridizes to an earlier section (G1393 to A1412) of the *pmCherry-N1* plasmid 3'-UTR during PCR amplification of the mCh_DNA$_{UTR\ 2}$ template. To obtain sequences of equal length for both **mCh_DNA** and **mCh_DNA$_{\mathbf{UTR\ 2}}$**, a compensatory overhang sequence was adapted from the *pmCherry-C1* plasmid (G1501 to T1520) and added to the 5' end of reverse primer mCh_RV$_{UTR\ 2}$.

In order to generate all dsDNA templates for subsequent mCh_mRNA IVT, two different PCR amplification techniques were performed. On the one hand, canonical 4-letter PCRs applying unmodified reverse primer were carried out to yield unmodified mCh_DNA. On the other hand, site-specific UBP modified mCh_DNA^{UBP} was yielded using expanded 6-letter PCRs applying d**NaM**-modified reverse primers and the unnatural desoxynucleoside triphosphates d**TPT3** TP and d**NaM** TP. Samples of UBP modified PCR products as well as canonical PCR products were send to Sanger sequencing to approve the desired sequence identities (for sequencing results see below).

Subsequently, mCh_mRNA was obtained by either standard T7 IVT for mCh_mRNA^{WT}, mCh_mRNA$^{1\ PM}$ and mCh_mRNA$^{2\ PM}$, or GENAEXT for mCh_mRNA$^{1\ CP}$ and mCh_mRNA$^{2\ CP}$, using r**TPT3**^{CP} TP as additional ribonucleoside triphosphate (Figure 2e and Supplementary Figures 8, 9). Furthermore, to check compatibility of the UB modification with additional chemical RNA alterations 1.25 mM Ψ TP and 1.25 mM 5mC TP were added to the

transcription mix. By this, RNA variants of either wild type and UB modified mCh_mRNA bearing multiple Ψ and 5mC modifications were synthesized (Figure 2f and Supplementary Figure 7). A complete replacement of Ψ for U or 5mC for C was not aimed. Instead, a random exchange of approx. 50% of canonical U and C was reached.

List of DNA and RNA sequences

Plasmid sequence:

pmCherry-N1 (4724 bp), insertion of two nucleotides **AA** at position 1388 (bold and underlined), silent mutation of stop codon, no influence on mCherry protein coding sequence (underlined with dotted line)

5'-TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTT
ACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCC
CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGC
AGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCA
TTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCA
TGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCC
ACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTC
CGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGA
ACCGTCAGATCCGCTAGCGCTACCGGACTCAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTACC
GCGGGCCCGGGATCCACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAG
GAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGG
GCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTT
CGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCC
CCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGC
GTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCAC
CAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGT
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AGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCAC
TCCACCGGCGGCATGGACGAGCTGTACAAGT**AA**AGCGGCCGCGACTCTAGATCATAATCAGCCATACCACA
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TATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAAC
CATCACCCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGGAACCCTAAAGGGAGCCCCC
GATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAGAAAGCGAAAGGAGCGGG
CGCTAGGGCGCTGGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCG
CTACAGGGCGCGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATAC
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CTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGG
CCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAGATC
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TGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCG
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ACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCAC
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CTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCAT
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CTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGAT
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GTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATA
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GCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATA
GTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACG
ACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGC
GGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGC
CTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGG
GGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCT
CACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCATGCAT-3'

Primer sequences:

mCh_FW (39 nt, T7 promotor sequence underlined, after promotor sequence two additional nucleotides GG (dotted line) were added for enhanced T7 RNA polymerase transcription start)

5'-TAATACGACTCACTATAGGGCTCAAGCTTCGAATTCTGC-3'

mCh_RV (24 nt)

5'-ACAAATGTGGTATGGCTGATTATG-3'

mCh_RV$^{1\ dNaM}$ (N=d**NaM**) or **mCh_RV$^{1\ PM}$** (N=C) (24 nt)

5'-ACAAATGTGGTANGGCTGATTATG-3'

mCh_RV$^{2\ dNaM}$ (N=d**NaM**) or **mCh_RV$^{2\ PM}$** (N=C) (24 nt)

5'-ACAAANGTGGTATGGCTGANTATG-3'

mCh_RV$_{UTR\ 2}$
(alternative 5' end overhang sequence marked with dashed line, 40 nt)

5'- ATAAACAAGTTAACAACAACTATGATCTAGAGTCGCGGCC-3'

mCh_RV$^{1\ dNaM}_{UTR\ 2}$ (N=d**NaM**) or **mCh_RV$^{1\ PM}_{UTR\ 2}$** (N=C)
(alternative 5' end overhang sequence marked with dashed line, 40 nt)

5'-ATAAACAAGTTAACAACAACTATGATCNAGAGTCGCGGCC-3'

mCh_RV$^{2\ dNaM}_{UTR\ 2}$ (N=d**NaM**) or **mCh_RV$^{2\ PM}_{UTR\ 2}$** (N=C)
(alternative 5' end overhang sequence marked with dashed line, 40 nt)

5'- ATAAACAAGTTAACAACAACTANGATCTAGAGNCGCGGCC -3'

mCh_qPCR^{internal}_FW (21 nt)

5'-CCCGACTACTTGAAGCTGTCC-3'

mCh_qPCR^{internal}_RV (22 nt)

5'-TCACCTTGTAGATGAACTCGCC-3'

mCh_qPCR$^{3'\text{-}end}$_FW (22 nt)

5'-ATCAAGTTGGACATCACCTCCC-3'

mCh_qPCR$^{3'\text{-}end}$_RV (22 nt)

5'-CGCTTTACTTGTACAGCTCGTC-3'

GAPDH_qPCR_FW (22 nt)

5'-AGCCTCAAGATCATCAGCAATG-3'

GAPDH_qPCR_RV (23 nt)

5'-ATGGACTGTGGTCATGAGTCCTT-3'

β-Actin_qPCR_FW (20 nt)

5'-GGTCATCACCATTGGCAATG-3'

β-Actin_qPCR_RV (23 nt)

5'-CGTCACACTTCATGATGGAGTTG-3'

ext-qPCR_FW (17 nt)

5'-GCGTTTACCCGCCATCC-3'

mCh_ext-qPCR^{internal}_RV (21 nt)

5'-TCACCTTGTAGATGAACTCGC-3'

mCh_ext-qPCR$^{3'\text{-}end}$_RV (22 nt)

5'-CGCTTTACTTGTACAGCTCGTC-3'

GAPDH_ext-qPCR_RV (21 nt)

5'-ATGGACTGTGGTCATGAGTCC-3'

β-Actin_ext-qPCR_RV (23 nt)

5'-CGTCACACTTCATGATGGAGTTG-3'

Hydrolysis probe sequences for qPCR:

mCh_qPCR^{internal}_probe (23 nt)

5'-HEX-TGACCGTGACCCAGGACTCCTCC-BMN-Q535-3'

mCh_qPCR$^{3'\text{-}end}$_probe (26 nt)

5'-FAM-ACACCATCGTGGAACAGTACGAACGC-BMN-Q535-3'

GAPDH_qPCR_probe (23 nt)

5'-Cyanine 5-CCAACTGCTTAGCACCCCTGGCC-BMN-Q620-3'

β-Actin_qPCR_probe (29 nt)

5'-Cyanine 3.5-ATGGAGTCCTGTGGCATCCACGAAACTAC-BMN-Q590-3'

Adapter and splint sequences for qPCR extension:

qPCR_adapter (130 nt)

5'-GCGTTTACCCGCCATCCGCACATGCCACCCTCCAGATATATTCGTCTCGACCAAATCACTTGGCAGTCTAG
GCGATCGCGATGGCCTGAGTCTAGAGCTCGAGTTCGAAGCTTAAGACGTCAGCTGCCAT-3'

mCh_qPCR^{internal}_splint (30 nt)

5'-GCTTCAAGTAGTCGGGATGGCAGCTGACGT-3'

mCh_qPCR$^{3'\text{-}end}$_ splint (32 nt)

5'-GGTGATGTCCAACTTGATATGGCAGCTGACGT-3'

GAPDH_qPCR_ splint (31 nt)

5'-CTGATGATCTTGAGGCTATGGCAGCTGACGT-3'

β-Actin_qPCR_ splint (30 nt)

5'-GCCAATGGTGATGACCATGGCAGCTGACGT-3'

Primer sequences for Sanger sequencing:

mCh_Seq (17 nt)

5'-GTGGAACAGTACGAACG-3'

T7_Seq (20 nt)

5'-TAATACGACTCACTATAGGG-3'

DNA sequences:

mCh_DNA^{WT} (834 bp, mCherry protein coding region underlined)

5'-TAATACGACTCACTATAGGGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACC
GGTCGCCACCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGG
TGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGA
GGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCC
CCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCC
TTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGG
ACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGC
CCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCC
TGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCAC
CTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCC
ACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGA
CGAGCTGTACAAGTAAAGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGT-3'

mCh_DNA$^{1\ UBP}$ (N=d**TPT3**) or **mCh_DNA$^{1\ PM}$** (N=G)
(834 bp, mCherry protein coding region underlined)

5'-TAATACGACTCACTATAGGGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACC
GGTCGCCACCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGG
TGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGA
GGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCC
CCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCC
TTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGG
ACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGC
CCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCC
TGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCAC
CTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCC
ACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGA
CGAGCTGTACAAGTAAAGCGGCCGCGACTCTAGATCATAATCAGCCNTACCACATTTGT-3'

mCh_DNA$^{2\ UBP}$ (834 bp, N=d**TPT3**) or **mCh_DNA$^{2\ PM}$** (N=G)
(834 bp, mCherry protein coding region underlined)

5'-TAATACGACTCACTATAGGGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACC
GGTCGCCACCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGG
TGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGA
GGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCC
CCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCC
TTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGG
ACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGC
CCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCC
TGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCAC
CTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCC
ACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGA
CGAGCTGTACAAGTAAAGCGGCCGCGACTCTAGATCATANTCAGCCTTACCACNTTTGT-3'

mCh_DNA$^{WT}_{UTR\ 2}$
(834 bp, mCherry protein coding region underlined)

5'-TAATACGACTCACTATAGGGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACC
GGTCGCCACCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGG
TGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGA
GGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCC
CCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCC
TTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGG
ACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGC
CCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCC
TGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCAC
CTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCC
ACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGA
CGAGCTGTACAAGTAAAGCGGCCGCGACTCTAGATCATAGTTGTTGTTAACTTGTTTAT-3'

mCh_DNA$^{1\ UBP}_{UTR\ 2}$ (N=d**TPT3**) or **mCh_DNA$^{1\ PM}_{UTR\ 2}$** (N=G)
(834 bp, mCherry protein coding region underlined)

5'-TAATACGACTCACTATAGGGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACC
GGTCGCCACCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGG
TGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGA
GGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCC
CCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCC
TTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGG
ACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGC
CCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCC
TGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCAC
CTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCC
ACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGA
CGAGCTGTACAAGTAAAGCGGCCGCGACTCTNGATCATAGTTGTTGTTAACTTGTTTAT-3'

mCh_DNA$^{2\ UBP}_{UTR\ 2}$ (N=d**TPT3**) or **mCh_DNA$^{2\ PM}_{UTR\ 2}$** (N=G)
(834 bp, mCherry protein coding region underlined)

5'-TAATACGACTCACTATAGGGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACC
GGTCGCCACCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGG
TGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGA
GGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCC
CCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCC
TTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGG
ACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGC
CCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCC
TGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCAC
CTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCC
ACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGA
CGAGCTGTACAAGTAAAGCGGCCGCGNCTCTAGATCNTAGTTGTTGTTAACTTGTTTAT-3'

mCh_qPCR^{internal} (130 bp)

5'-CCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGC
GGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGA-3'

mCh_qPCR$^{3'\text{-}end}$ (112 bp)

5'-ATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCC
GCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAAGCG-3'

GAPDH_qPCR (111 bp)

5'-AGCCTCAAGATCATCAGCAATGCCTCCTGCACCACCAACTGCTTAGCACCCCTGGCCAAGGTCATCCATG
ACAACTTTGGTATCGTGGAAGGACTCATGACCACAGTCCAT-3'

β-Actin_qPCR (122 bp)

5'-GGTCATCACCATTGGCAATGAGCGGTTCCGCTGCCCTGAGGCACTCTTCCAGCCTTCCTTCCTGGGCATG
GAGTCCTGTGGCATCCACGAAACTACCTTCAACTCCATCATGAAGTGTGACG-3'

mCh_ext-qPCR^{internal} (260 bp)

5'-GCGTTTACCCGCCATCCGCACATGCCACCCTCCAGATATATTCGTCTCGACCAAATCACTTGGCAGTCTAG
GCGATCGCGATGGCCTGAGTCTAGAGCTCGAGTTCGAAGCTTAAGACGTCAGCTGCCATCCCGACTACTTGA
AGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGT
GACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGA-3'

mCh_ ext-qPCR$^{3'\text{-}end}$ (242 bp)

5'-GCGTTTACCCGCCATCCGCACATGCCACCCTCCAGATATATTCGTCTCGACCAAATCACTTGGCAGTCTAG
GCGATCGCGATGGCCTGAGTCTAGAGCTCGAGTTCGAAGCTTAAGACGTCAGCTGCCATATCAAGTTGGACA
TCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGC
GGCATGGACGAGCTGTACAAGTAAAGCG-3'

GAPDH_ ext-qPCR (241 bp)

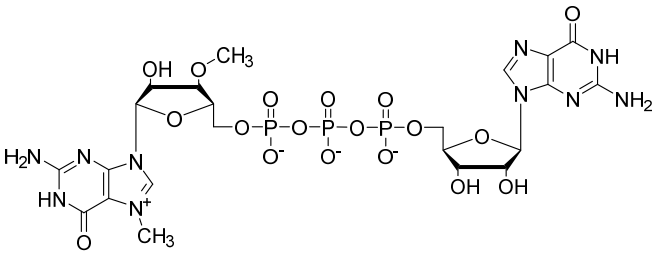
5'-GCGTTTACCCGCCATCCGCACATGCCACCCTCCAGATATATTCGTCTCGACCAAATCACTTGGCAGTCTAG
GCGATCGCGATGGCCTGAGTCTAGAGCTCGAGTTCGAAGCTTAAGACGTCAGCTGCCATAGCCTCAAGATCA
TCAGCAATGCCTCCTGCACCACCAACTGCTTAGCACCCCTGGCCAAGGTCATCCATGACAACTTTGGTATCG
TGGAAGGACTCATGACCACAGTCCAT-3'

β-Actin_ ext-qPCR (252 bp)

5'-GCGTTTACCCGCCATCCGCACATGCCACCCTCCAGATATATTCGTCTCGACCAAATCACTTGGCAGTCTAG
GCGATCGCGATGGCCTGAGTCTAGAGCTCGAGTTCGAAGCTTAAGACGTCAGCTGCCATGGTCATCACCATT

GGCAATGAGCGGTTCCGCTGCCCTGAGGCACTCTTCCAGCCTTCCTTCCTGGGCATGGAGTCCTGTGGCAT
CCACGAAACTACCTTCAACTCCATCATGAAGTGTGACG-3'

RNA sequences:



Supplementary Figure 34. Structure of anti-reverse cap analog (ARCA).

mCh_mRNA^{WT} (817 nt, mCherry protein coding region underlined)

5'-ARCA-GGGCUCAAGCUUCGAAUUCUGCAGUCGACGGUACCGCGGGCCCGGGAUCCACCGGUCGCCACC
AUGGUGAGCAAGGGCGAGGAGGAUAACAUGGCCAUCAUCAAGGAGUUCAUGCGCUUCAAGGUGCACAUG
GAGGGCUCCGUGAACGGCCACGAGUUCGAGAUCGAGGGCGAGGGCGAGGGCCGCCCCUACGAGGGCAC
CCAGACCGCCAAGCUGAAGGUGACCAAGGGUGGCCCCCUGCCCUUCGCCUGGGACAUCCUGUCCCCUCA
GUUCAUGUACGGCUCCAAGGCCUACGUGAAGCACCCCGCCGACAUCCCCGACUACUUGAAGCUGUCCUU
CCCCGAGGGCUUCAAGUGGGAGCGCGUGAUGAACUUCGAGGACGGCGGCGUGGUGACCGUGACCCAGG
ACUCCUCCCUGCAGGACGGCGAGUUCAUCUACAAGGUGAAGCUGCGCGGCACCAACUUCCCCUCCGACG
GCCCCGUAAUGCAGAAGAAGACCAUGGGCUGGGAGGCCUCCUCCGAGCGGAUGUACCCCGAGGACGGCG
CCCUGAAGGGCGAGAUCAAGCAGAGGCUGAAGCUGAAGGACGGCGGCCACUACGACGCUGAGGUCAAGA
CCACCUACAAGGCCAAGAAGCCCGUGCAGCUGCCCGGCGCCUACAACGUCAACAUCAAGUUGGACAUCAC
CUCCCACAACGAGGACUACACCAUCGUGGAACAGUACGAACGCGCCGAGGGCCGCCACUCCACCGGCGG
CAUGGACGAGCUGUACAAGUAAAGCGGCCGCGACUCUAGAUCAUAAUCAGCCAUACCACAUUUGU-poly(A)-
3'

mCh_mRNA$^{1\ CP}$ (N=r**TPT3^{CP}**) or **mCh_mRNA$^{1\ PM}$** (N=G)
(817 nt, mCherry protein coding region underlined)

5'-ARCA-GGGCUCAAGCUUCGAAUUCUGCAGUCGACGGUACCGCGGGCCCGGGAUCCACCGGUCGCCACC
AUGGUGAGCAAGGGCGAGGAGGAUAACAUGGCCAUCAUCAAGGAGUUCAUGCGCUUCAAGGUGCACAUG
GAGGGCUCCGUGAACGGCCACGAGUUCGAGAUCGAGGGCGAGGGCGAGGGCCGCCCCUACGAGGGCAC
CCAGACCGCCAAGCUGAAGGUGACCAAGGGUGGCCCCCUGCCCUUCGCCUGGGACAUCCUGUCCCCUCA
GUUCAUGUACGGCUCCAAGGCCUACGUGAAGCACCCCGCCGACAUCCCCGACUACUUGAAGCUGUCCUU
CCCCGAGGGCUUCAAGUGGGAGCGCGUGAUGAACUUCGAGGACGGCGGCGUGGUGACCGUGACCCAGG
ACUCCUCCCUGCAGGACGGCGAGUUCAUCUACAAGGUGAAGCUGCGCGGCACCAACUUCCCCUCCGACG
GCCCCGUAAUGCAGAAGAAGACCAUGGGCUGGGAGGCCUCCUCCGAGCGGAUGUACCCCGAGGACGGCG
CCCUGAAGGGCGAGAUCAAGCAGAGGCUGAAGCUGAAGGACGGCGGCCACUACGACGCUGAGGUCAAGA
CCACCUACAAGGCCAAGAAGCCCGUGCAGCUGCCCGGCGCCUACAACGUCAACAUCAAGUUGGACAUCAC
CUCCCACAACGAGGACUACACCAUCGUGGAACAGUACGAACGCGCCGAGGGCCGCCACUCCACCGGCGG
CAUGGACGAGCUGUACAAGUAAAGCGGCCGCGACUCUAGAUCAUAAUCAGCCNUACCACAUUUGU-poly(A)
-3'

mCh_mRNA$^{2\ CP}$ (817 nt, N=r**TPT3^{CP}**) or **mCh_mRNA$^{2\ PM}$** (817 nt, N=G)
(817 nt, mCherry protein coding region underlined)

5'-ARCA-GGGCUCAAGCUUCGAAUUCUGCAGUCGACGGUACCGCGGGCCCGGGAUCCACCGGUCGCCACC
AUGGUGAGCAAGGGCGAGGAGGAUAACAUGGCCAUCAUCAAGGAGUUCAUGCGCUUCAAGGUGCACAUG
GAGGGCUCCGUGAACGGCCACGAGUUCGAGAUCGAGGGCGAGGGCGAGGGCCGCCCCUACGAGGGCAC
CCAGACCGCCAAGCUGAAGGUGACCAAGGGUGGCCCCCUGCCCUUCGCCUGGGACAUCCUGUCCCCUCA
GUUCAUGUACGGCUCCAAGGCCUACGUGAAGCACCCCGCCGACAUCCCCGACUACUUGAAGCUGUCCUU
CCCCGAGGGCUUCAAGUGGGAGCGCGUGAUGAACUUCGAGGACGGCGGCGUGGUGACCGUGACCCAGG
ACUCCUCCCUGCAGGACGGCGAGUUCAUCUACAAGGUGAAGCUGCGCGGCACCAACUUCCCCUCCGACG
GCCCCGUAAUGCAGAAGAAGACCAUGGGCUGGGAGGCCUCCUCCGAGCGGAUGUACCCCGAGGACGGCG
CCCUGAAGGGCGAGAUCAAGCAGAGGCUGAAGCUGAAGGACGGCGGCCACUACGACGCUGAGGUCAAGA
CCACCUACAAGGCCAAGAAGCCCGUGCAGCUGCCCGGCGCCUACAACGUCAACAUCAAGUUGGACAUCAC
CUCCCACAACGAGGACUACACCAUCGUGGAACAGUACGAACGCGCCGAGGGCCGCCACUCCACCGGCGG
CAUGGACGAGCUGUACAAGUAAAGCGGCCGCGACUCUAGAUCAUANUCAGCCAUACCACNUUUGU-poly(A)
-3'

mCh_mRNA$^{WT}_{UTR\ 2}$ (817 nt, mCherry protein coding region underlined)

5'-ARCA-GGGCUCAAGCUUCGAAUUCUGCAGUCGACGGUACCGCGGGCCCGGGAUCCACCGGUCGCCACC
AUGGUGAGCAAGGGCGAGGAGGAUAACAUGGCCAUCAUCAAGGAGUUCAUGCGCUUCAAGGUGCACAUG
GAGGGCUCCGUGAACGGCCACGAGUUCGAGAUCGAGGGCGAGGGCGAGGGCCGCCCCUACGAGGGCAC
CCAGACCGCCAAGCUGAAGGUGACCAAGGGUGGCCCCCUGCCCUUCGCCUGGGACAUCCUGUCCCCUCA
GUUCAUGUACGGCUCCAAGGCCUACGUGAAGCACCCCGCCGACAUCCCCGACUACUUGAAGCUGUCCUU
CCCCGAGGGCUUCAAGUGGGAGCGCGUGAUGAACUUCGAGGACGGCGGCGUGGUGACCGUGACCCAGG
ACUCCUCCCUGCAGGACGGCGAGUUCAUCUACAAGGUGAAGCUGCGCGGCACCAACUUCCCCUCCGACG
GCCCCGUAAUGCAGAAGAAGACCAUGGGCUGGGAGGCCUCCUCCGAGCGGAUGUACCCCGAGGACGGCG
CCCUGAAGGGCGAGAUCAAGCAGAGGCUGAAGCUGAAGGACGGCGGCCACUACGACGCUGAGGUCAAGA
CCACCUACAAGGCCAAGAAGCCCGUGCAGCUGCCCGGCGCCUACAACGUCAACAUCAAGUUGGACAUCAC
CUCCCACAACGAGGACUACACCAUCGUGGAACAGUACGAACGCGCCGAGGGCCGCCACUCCACCGGCGG
CAUGGACGAGCUGUACAAGUAAAGCGGCCGCGACUCUAGAUCAUAGUUGUUGUUAACUUGUUUAU-poly(A)
-3'

mCh_mRNA$^{1\ CP}_{UTR\ 2}$ (N=r**TPT3^{CP}**) or **mCh_mRNA$^{1\ PM}_{UTR\ 2}$** (N=G)
(817 nt, mCherry protein coding region underlined)

5'-ARCA-GGGCUCAAGCUUCGAAUUCUGCAGUCGACGGUACCGCGGGCCCGGGAUCCACCGGUCGCCACC
AUGGUGAGCAAGGGCGAGGAGGAUAACAUGGCCAUCAUCAAGGAGUUCAUGCGCUUCAAGGUGCACAUG
GAGGGCUCCGUGAACGGCCACGAGUUCGAGAUCGAGGGCGAGGGCGAGGGCCGCCCCUACGAGGGCAC
CCAGACCGCCAAGCUGAAGGUGACCAAGGGUGGCCCCCUGCCCUUCGCCUGGGACAUCCUGUCCCCUCA
GUUCAUGUACGGCUCCAAGGCCUACGUGAAGCACCCCGCCGACAUCCCCGACUACUUGAAGCUGUCCUU
CCCCGAGGGCUUCAAGUGGGAGCGCGUGAUGAACUUCGAGGACGGCGGCGUGGUGACCGUGACCCAGG
ACUCCUCCCUGCAGGACGGCGAGUUCAUCUACAAGGUGAAGCUGCGCGGCACCAACUUCCCCUCCGACG
GCCCCGUAAUGCAGAAGAAGACCAUGGGCUGGGAGGCCUCCUCCGAGCGGAUGUACCCCGAGGACGGCG
CCCUGAAGGGCGAGAUCAAGCAGAGGCUGAAGCUGAAGGACGGCGGCCACUACGACGCUGAGGUCAAGA
CCACCUACAAGGCCAAGAAGCCCGUGCAGCUGCCCGGCGCCUACAACGUCAACAUCAAGUUGGACAUCAC
CUCCCACAACGAGGACUACACCAUCGUGGAACAGUACGAACGCGCCGAGGGCCGCCACUCCACCGGCGG
CAUGGACGAGCUGUACAAGUAAAGCGGCCGCGACUCUNGAUCAUAGUUGUUGUUAACUUGUUUAU-poly(A)
-3'

mCh_mRNA$^{2\,CP}_{UTR\,2}$ (N=r**TPT3^{CP}**) or **mCh_mRNA$^{2\,PM}_{UTR\,2}$** (N=G)
(817 nt, mCherry protein coding region underlined)

5'-ARCA-GGGCUCAAGCUUCGAAUUCUGCAGUCGACGGUACCGCGGGCCCGGGAUCCACCGGUCGCCACC
AUGGUGAGCAAGGGCGAGGAGGAUAACAUGGCCAUCAUCAAGGAGUUCAUGCGCUUCAAGGUGCACAUG
GAGGGCUCCGUGAACGGCCACGAGUUCGAGAUCGAGGGCGAGGGCGAGGGCCGCCCCUACGAGGGCAC
CCAGACCGCCAAGCUGAAGGUGACCAAGGGUGGCCCCCUGCCCUUCGCCUGGGACAUCCUGUCCCCUCA
GUUCAUGUACGGCUCCAAGGCCUACGUGAAGCACCCCGCCGACAUCCCCGACUACUUGAAGCUGUCCUU
CCCCGAGGGCUUCAAGUGGGAGCGCGUGAUGAACUUCGAGGACGGCGGCGUGGUGACCGUGACCCAGG
ACUCCUCCCUGCAGGACGGCGAGUUCAUCUACAAGGUGAAGCUGCGCGGCACCAACUUCCCCUCCGACG
GCCCCGUAAUGCAGAAGAAGACCAUGGGCUGGGAGGCCUCCUCCGAGCGGAUGUACCCCGAGGACGGCG
CCCUGAAGGGCGAGAUCAAGCAGAGGCUGAAGCUGAAGGACGGCGGCCACUACGACGCUGAGGUCAAGA
CCACCUACAAGGCCAAGAAGCCCGUGCAGCUGCCCGGCGCCUACAACGUCAACAUCAAGUUGGACAUCAC
CUCCCACAACGAGGACUACACCAUCGUGGAACAGUACGAACGCGCCGAGGGCCGCCACUCCACCGGCGG
CAUGGACGAGCUGUACAAGUAAAGCGGCCGCGNCUCUAGAUCNUAGUUGUUGUUAACUUGUUUAU-poly(A
)-3'

Sanger sequencing of UBP modified and unmodified DNA

Sanger sequencing

Purified PCR amplicons of **mCh_DNA**, **mCh_DNA$^{1\ PM}$** and **mCh_DNA$^{2\ PM}$** and also **mCh_DNA$_{UTR\ 2}$**, **mCh_DNA$^{1\ PM}_{UTR\ 2}$** and **mCh_DNA$^{2\ PM}_{UTR\ 2}$** were submitted to Sanger sequencing (*GATC services, Eurofins Genomics*) using the self-designed **mCh_Seq** sequencing primer and the **T7_Seq** primer provided by the sequencing company. Purified, extended qPCR amplicons of **mCh_ext-qPCR^{internal}**, **mCh_ext-qPCR$^{3'\text{-}end}$**, **GAPDH_ext-qPCR** and **β-Actin_ext-qPCR** were submitted to Sanger sequencing (*GATC services, Eurofins Genomics*) using the self-designed **ext-qPCR_FW** primer. Purified PCR amplicons of **mCh_DNA$^{1\ UBP}$** and **mCh_DNA$^{2\ UBP}$** were used with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (*Applied Biosystems™*) together with the **mCh_FW** primer and then submitted to Sanger sequencing at the Cologne Center for Genomics.

pmCherry-N1 **plasmid with AA-insertion**
(AA-insertion highlighted; mutated stop codon underlined; mCherry protein coding region underlined)

5'-aGCaGaGCTGGTTtaGTGAaCCGTCAGATCCGCTAGCGCTACCGGaCTCAGATCTCGAGCTCAA
GCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCGGGATCCACCGGTCGCCACCATGGTGAG
CAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAG
GGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGG
CACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCT
GTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTAC
TTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGC
GTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGC
GCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCT
CCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGC
TGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGC
AGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACAC
CATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTA
CAAGTAAAGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTTGTAGAGGTTTTACTTGCT
TTAAAAAaCCTCCCACACCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTGTTAACT
TGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATT
TTTTTCaCTGCATTCTAGTTgtGgTTtGTCCAAaCTCATCAATGTATCTTAAGGCGTAAaTTGTAAGCG
TTAAnaTTTtGTTAAAaTTcnnGTTAAaTTTTTggtAAaTCAGcTcATTTTTtAaCCAanaGgCCGAAaTCGG
cAAanTCCcTTanAAatCAAa-3'

mCh_DNA^{WT}
(stop codon underlined; mCherry protein coding region underlined)

T7_seq primer results:

5'-antctgCagTCGAACGGTACCGCGGggCCCggGATCCACCGGTCGCCACCATGGTGAGCAAGGGC
GAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCG
TGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAG
ACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCT
CAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGC
TGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGA
CCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCA
CCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCG
AGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGG

ACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGC
CCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGT
GGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTA
AAGCGGCCGCGACTCTAGATCATAATCAGCCAtaCCc-3'

mCh_seq primer results:

5'-atGgnnGAgCTGTACaAGTAAAGCGGCCGCGACTCTAGATCATAATCAGCCATACCACATTT-3'

mCh_DNA[1 PM]
(stop codon underlined; mCherry protein coding region underlined, mutated base in red)

T7_seq primer results:

GngTCGaangGtcCGCGGGGCCCGGGATcACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGG
ATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGG
CCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCA
AGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCAT
GTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTC
CCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACC
CAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCC
CCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGT
ACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGC
CACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCC
TACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTA
CGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAAGCGGCC
GCGACTCTAGATCATAATCAGc

mCh_seq primer results:

cgaagcttgtacaagtaaagcggccgcgactctagatcataatcagccgtaccacatt

mCh_DNA[2 PM]
(stop codon underlined; mCherry protein coding region underlined, mutated bases in red)

T7_seq primer results:

gTcgaacGGtnnCGCGGGgCCCGGGancCACCGGtncGCCACCATGGTGAGCAAGGGCGAGGAGGAT
AACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCC
ACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAG
CTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGT
ACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCC
CGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCA
GGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCC
TCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTAC
CCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCA
CTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTA
CAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACG
AACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGC
GACTCTAGATCATAGTCAGct

mCh_seq primer results:

acgagctgtacaagttaaagcggccgcgactctagatcatagtcagccataccacgtttgt

mCh_DNA$^{WT}_{UTR\ 2}$
(stop codon underlined; mCherry protein coding region underlined)

T7_seq primer results:

gTCGaangGtaCnGCGGGGCCCGggnatCCACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGGA
TAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGC
CACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAA
GCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATG
TACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCC
CCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCC
AGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCC
CTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTA
CCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCC
ACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCT
ACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTAC
GAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAAGCGGCCG
CGACTCTAGATCATAGTTGTTGTAcctTtnnttt

mCh_seq primer results:

tGgnnGAgCTGTaCaAGTAAAGCGGCCGCGACTCTAGATCATAGTTGTTGTTAACTTGTT

mCh_DNA$^{1\ PM}_{UTR\ 2}$
(stop codon underlined; mCherry protein coding region underlined, mutated base in red)

T7_seq primer results:

GcAgTCGaacGgGtaccGCGGGGCCCggGgatCCACCGGTCGCCACCATGGTGAGCAAGGGCGAGG
AGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAA
CGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCG
CCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTT
CATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCC
TTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTG
ACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACT
TCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGA
TGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCG
GCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCG
CCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAG
TACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAAGCGGC
CGCGACTCTGGATCATAGTTGTTGTA

mCh_seq primer results:

gcatggacgagctgtacaagttaaagcggccgcgactctggatcatagttgttgttaacttgtttat

mCh_DNA$^{2\ PM}_{UTR\ 2}$
(stop codon underlined; mCherry protein coding region underlined, mutated bases in red)

T7_seq primer results:

gngTcgaacgGtncGCGGGCCCGGGATcCaCCGgnccGCCACCATGGTGAGCAAGGGCGAGGAGGAT
AACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCC
ACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAG
CTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGT
ACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCC
CGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCA

GGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCC
TCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTAC
CCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCA
CTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTA
CAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACG
AACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAAGCGGCCGC
GGCTCTAGATCGTAGTTGTGTAannTtttttTT

mCh_seq primer results:

acgaagcttgtacaagtaaagcggccgcggctctagatcgtagttgttgttaacttgttta

mCh_DNA[1] UBP
(stop codon underlined; mCherry protein coding region underlined, unnatural base position in red)

nnnnnnnnnCGCGGnnnnGGnnCnnCCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGGATAAnnTG
GCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGT
TCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAG
GTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCT
CCAAGGCCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGG
CTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTC
CTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGAC
GGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAG
GACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGAC
GCTGAnGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCA
ACATCAAGTTGGACATCACCTCCCACAACGAGGACTACACCATCGTGnAACAGTACGAACGCGCC
GAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAAAnCGnnCGCGACTCTnnnnn
nnnAATCAGCCnnnnnnn

mCh_DNA[2] UBP
(stop codon underlined; mCherry protein coding region underlined, unnatural base position in red)

nnnnnnnnnnnnnGnnCCnCCGGTCGCCnCCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATC
ATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGA
TCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACC
AAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGG
CCTACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAA
GTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCT
GCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCC
CGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGG
CGCCCTGAAGGGCGAGATCAAGCAGAnGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGA
nGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATC
AAGTTGGACATCACCTCCCACnACGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGG
GCCGCCACTCCACCGGCGGCATGGACGAnCnGTACAnGTAAAGCGnCCGCGACTCTnGATCAnAn
nnnnnnCnnnCnnnnnn

mCh_ext-qPCR[internal]

5'-tTGgcagTCtAGGCGAtCGCgATGGCCTGAGTCTAGAGCTCGAgTTcnaAgCTtAagACGTCaGCTGC
CATCCCgACtACTTGAanCTGtCCTTCCCcgAgGgCTtcaagTGGgAaCgCGTGATGAACTTCgAGGACG
GCGGCgTGgTGACCGTGACCCAGGACTCCtCCCTGCAGGACGGCaAGTTCATCTACaAGGTGA-3'

mCh_ext-qPCR$^{3'\text{-end}}$

5'-aGGCGAtcGcGATGGCCTGAGTCTAgAGCTCGAGTTCGAAgCTtAagACGTCngCTGCCATATCAg
GTTGGACaTCaCCTCCCacAacGAGGACTACaCCaTcgtGgAacAGTACgAAcGCgCCgAGGGCCgCCa
cTCCaCCGGcGGcatGgacaAgCTGTACAAGTAAagcG-3'

GAPDH_ext-qPCR

5'-tTGgCAGTCTAGGCGATCGcgATGGCCTGAGTCTAGAGCTCgAgTTCgAagCTtAAGACGTCagCT
GCcaTAgCCtCAagATCATCAgCaaTGCctCctGcaCCaCCAactGcTTAACaCCCctgAnnnAGGTCATCCa
TGACaaCTTTGgTATCgtGgAAGGACTCatGAccaCagtcca-3'

β-Actin_ext-qPCR

5'-tcaCTTGGCAGTCTAGGCGATCGCGATGGCCTGAGTCTAGAGCTCGAGTTCGAAGCTTAAgACg
TCAgCTGCcatGGTCATCACCATTGgcAATGAgCGGTTCCgctGCCcgGaGGCACTCTTCCagCCTTCC
TTCCgGGGcATGGAaTCctGtGgCATCcacnaaACtaCcTTCAacTCcnTCatgaagTGtga -3'

cNLS Mapper results for mCherry protein sequence

For prediction of nuclear localization signals (NLS) specific to the importin αβ pathway[2] the cNLS Mapper tool was used (*http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi*). The result for the mCherry protein coding amino acid (aa) sequence is displayed in Supplementary Figure 33. The cut-off score for prediction was set to 3.0 as a protein with scores of 3, 4 and 5 (scale range from 1 to 10) is localized to both the nucleus and the cytoplasm. It was searched for bipartite NLS with a long linker (13-20 aa) throughout the entire region. Two predicted bipartite NLS were found with a score of 3 and 3.3, as well as three predicted bipartite NLS were found with scores of 4.1, 4.3 and 4.5.

cNLS Mapper Result

Predicted NLSs in query sequence	
MVSKGEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAK	50
LKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWER	100
VMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEA	150
SSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNV	200
NIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYK	236

Predicted monopartite NLS		
Pos.	Sequence	Score

Predicted bipartite NLS		
Pos.	Sequence	Score
51	LKVTKGGPLPFAWDILSPQFMYGSKAYVK	3
74	SKAYVKHPADIPDYLKLSFPEGFKWERV	4.1
142	QKKTMGWEASSERMYPEDGALKGEIKQRLK	3.3
163	KGEIKQRLKLKDGGHYDAEVKTTYKAKKP	4.3
163	KGEIKQRLKLKDGGHYDAEVKTTYKAKKPV	4.5

Supplementary Figure 35. cNLS mapper results for mCherry protein amino acid sequence.

References

1 L. Li *et al.*, Natural-like replication of an unnatural base pair for the expansion of the genetic alphabet and biotechnology applications, *J. Am. Chem. Soc.*, 2014, **136**, 826–829.

2 S. Kosugi *et al.*, Systematic identification of cell cycle-dependent yeast nucleocytoplasmic shuttling proteins by prediction of composite motifs, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 10171–10176.