Supporting Information

for

Chemical tagging for sensitive determination of uridine modifications in RNA

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The Supporting Information includes following items:

Quantification of modifications in RNA

As for the quantification of modifications in RNA, 1/10 of the enzymatically digested nucleosides was analyzed by HPLC with UV detection to quantify the amount of RNA. The calibration curves of the four canonical nucleosides (A, C, G and U) were constructed by plotting peak areas of canonical nucleosides against the molar amounts of canonical nucleosides with triplicate measurements.

The rest of 9/10 of the digested nucleosides were subjected to CMCT labelling and CeO_2 extraction followed by LC-ESI-MS/MS analysis. The amount of each modification was normalized with the amount of uridine quantified by HPLC analysis.

CeO_2 extraction of CMCT labelled nucleosides

The procedure of CeO_2 extraction was performed according to the previous study [1]. Briefly, 600 mg of CeO_2 was added into a 5 mL centrifuge tube followed by sequential washing with 1 mL of 1% formic acid in water, 1 mL of water and 1 mL of 1% ammonia solution for 3 min each time. The sample mixture (110 μL) was diluted with 1% ammonia solution (2 mL) to 2.1 mL and then added into the tube and vortexed vigorously for 5 min. After centrifugation at 12,000 rpm for 4 min, the resulting CeO_2 with extracted compounds were sequentially washed with 2 mL water and 1 mL methanol for 3 min. Then 1 mL of 1% formic acid in water was used to elute the adsorbed compounds by vortexing for 3 min. The eluent was collected, lyophilized to dryness, and then dissolved in 50 μL water for subsequent LC-ESI-MS/MS analysis.

In-vitro transcription of RNA

We synthesized a RNA strand by *in-vitro* transcription as the control sample. A 558-bp duplex DNA containing a T7 promoter was used as template for the *in-vitro* transcription according to previous study.[2] *In-vitro* transcription was performed in a 20-μL reaction mixture containing 100 ng of DNA template, 2 μL of 10 × reaction buffer, 1 μL of NTP (10 mM for each), 2 μL of DTT (50 mM) and 2 μL of T7 RNA polymerase (Sangon Biotech, Shanghai, China). The reaction was incubated at 37°C for 2 h and then 1 μL DNase (2 U) was added. The mixture was incubated at 37°C for 15 min to digest the template DNA. The *in-vitro* transcribed RNA was purified by chloroform extraction and isopropanol precipitation. The integrity of purified RNA was confirmed using 5% denaturing PAGE. The sequence of the *in-vitro* transcribed RNA is listed in Table S7 in Supporting Information.

Evaluation of the purity of isolated mRNA by real-time quantitative PCR

The polyA-extracted mRNA was further purified by 0.8% agarose gel of low melting point. The agarose gel electrophoresis was carried out with 0.5 × TBE at 130 V for 10 min. Then the agarose gel was stained with GelRed (Invitrogen) and visualized by a gel documentation system (Tanon, Shanghai, China). mRNA was excised from the agarose gel and recovered using Zymoclean Gel RNA Recovery Kit (Zymo Research, Catalog No: R1011) according to the manufacture's recommended procedure.

The purity of the isolated mRNA was evaluated by real-time quantitative PCR. The primer sequences are: 18S rRNA forward primer: 5'-GTAACCCGTTGAACCCCATT-3', 18S rRNA reverse primer: 5'-CCATCCAATCGGTAGTAGCG-3'; 28S rRNA forward primer: 5'-

TCATCAGACCCCAGAAAAGG-3', 28S rRNA reverse primer: 5'-GATTCGGCAGGTGAGTTGTT-3'; $tRNA^{HisGUG}$ forward primer: 5'-GCCCGTGATGGTATAGTGGTTAGTA-3', $tRNA^{HisGUG}$ reverse primer: 5'-CGTGACTCTGATTCGTACCGAGGTT -3'.

To evaluate the 18S rRNA and 28S rRNA contents in mRNA, the equal amounts of mRNA and total RNA were used as the template for real-time quantitative PCR. It has been know that 18S rRNA and 28S rRNA account for approximately 80% of total RNA, and the length of 18S rRNA and 28S rRNA are ~ 1900 nt and ~ 5000 nt, respectively [3]. Therefore, the contents of 18S rRNA and 28S rRNA in mRNA are calculated as follows:

$\Delta Ct = Ct^{18S} - Ct^{mRNA}$

Percentage of 18S rRNA in mRNA = $2^{\Delta Ct} \times 80\% \times 1900/(5000 + 1900)$

Percentage of 28S rRNA in mRNA = $2^{\Delta Ct} \times 80\% \times 5000/(5000 + 1900)$

To evaluate the small RNA (< 200 nt) content in mRNA, the equal amounts of mRNA and small RNA (< 200 nt) were used as the template for real-time quantitative PCR. The content of small RNA (< 200 nt) in mRNA was calculated as follows:

$\Delta Ct = Ct^{small\ RNA} - Ct^{mRNA}$

Percentage of small RNA in mRNA = $2^{\Delta Ct} \times 100\%$

Using the above method, the measured percentages of 18S rRNA, 28S rRNA and small RNA (< 200 nt) in mRNA was 0.948%, 1.140% and 0.023%, respectively (Figure S8 in Supporting Information). The results showed that the potential contamination of rRNA is ~ 2.088% (0.948% + 1.140%). Since the level of m^5U in mRNA and rRNA is comparable, 2.088% rRNA in the isolated mRNA should not compromise the detection of m^5U in mRNA.

For example, if mRNA contains no m^5U, the measured content of m^5U in mRNA (with 2.088% rRNA) of HEK293T cells should be 48 times lower, i.e., 0.000094% (m^5U/U). But the measured level of m^5U in mRNA of HEK293T cells is 0.0034% (m^5U/U), suggesting that mRNA indeed contains m^5U modification.

The real-time quantitative PCR analysis demonstrated that the potential contamination of small RNA was less than 0.023% in mRNA (Figure S8 in Supporting Information), i.e. less than 0.12 ng of small RNA in 500 ng of mRNA. We then analyzed m^5U from 500 ng of mRNA. The peak of m^5U from 500 ng of mRNA was clear and distinct (Figure S9 in Supporting Information). However, no signal of m^5U was observed from 0.12 ng of small RNA (Figure S9 in Supporting Information), indicating that the m^5U signal from 500 ng of mRNA indeed comes from mRNA, but not the trace level of small RNA.

Western blotting analysis of TRMT2A

The overexpression and siRNA knockdown of *TRMT2A* were examined by western blot. Briefly, human HEK293T cell lysates were prepared using RIPA lysis buffer (Beyotime Biotech Inc., Shanghai, China). Protein concentration was measured by the BCA (bicinchoninic acid) assay according to the manufacture's recommended procedure (Beyotime Biotech Inc., Shanghai, China). Antibodies that specifically recognized TRMT2A (Abcam, ab205616, Cambridge, MA) and GAPDH (Abcam, ab181602, Cambridge, MA) were used at 1:2,000 and 1:8,000 dilutions, respectively. Horseradish peroxidase-conjugated secondary goat anti-rabbit antibody (Abcam, ab6721, Cambridge, MA) was used at a 1:10,000 dilution.

Analysis of mRNA by high-throughput sequencing

The isolated mRNA was processed using KAPA Stranded RNA-Seq Library Prep Kit (Illumina) for sequencing library construction following the manufacturer's protocol. RNA-seq was carried out on Illumina HiSeq 4000 (Illumina) platform by Aksomics Inc (Shanghai, China). Solexa pipeline version 1.8 (O□-Line Base Caller software, version 1.8) software was used for image processing and base recognition. The sequencing quality of the raw data was assessed by FastQC (v 0.11.7). High-quality reads were aligned to the (hg19) human reference genome though Hisat2 (v 2.1.0) software [4]. Gene expression by RNA-seq was available in the Gene Expression Omnibus (GEO) database at NCBI with the accession number of GSE135049.

The results showed that 12299 genes were detected in the isolated mRNA (Table S5 in and Table S6 Supporting Information), indicating the good quality of the isolated mRNA. And the potential contamination of rRNA is approximately 0.46% (Table S5 in Supporting Information).

Table S1. The MRM transition ions and optimized parameters for the analysis of nucleosides and CMCT/D_3-CMCT labelled nucleosides by LC-ESI-MS/MS.

Analytes	MRM transition ions	DP/V	EP / V	CEP / V	CE / V	CXP / V
A	268.2 → 136.2	32	5	16.6	24	3
U	245.2 → 113.2	34	5	15.8	17	4
C	244.2 → 112.1	25	4	16.3	20	3
G	284.2 → 152.2	32	5	15.8	24	3
m^5U	259.1 → 127.1	20	7	16.3	20	3
m^6U	259.1 → 127.1	20	7	16.3	20	3
$m^1\Psi$	259.1 → 139.0	44	4	16.3	24	3
hm^5U	275.2 → 125.1	20	9	16.8	22	3
mcm^5U	317.1 → 153.4	36	4	18.2	22	3
Ψ	245.1 → 125.0	35	5	15.9	24	3
mo^5U	275.1 → 143.0	21	6	16.8	18	4
i^6A	336.2 → 204.1	35	6	21.4	30	5
t^6A	413.1 → 281.2	20	10	17.1	30	5
m^1G	298.1 → 166.1	30	5	17.1	25	4
m^2G	298.1 → 166.1	30	5	17.1	25	4
m^5U-CMCT	510.3 → 252.2	45	7	24.6	35	3
m^6U-CMCT	510.3 → 252.2	45	7	24.6	35	3
$m^1\Psi$-CMCT	510.3 → 252.2	45	7	24.6	35	3
hm^5U-CMCT	526.3 → 252.2	45	9	25.4	42	3
mcm^5U-CMCT	568.3 → 252.2	45	6	26.5	35	3
Ψ-CMCT	496.3 → 252.2	40	8	24.1	42	3
U-CMCT	496.3 → 252.2	40	8	24.1	42	3
mo^5U-CMCT	526.3 → 252.2	45	9	25.1	42	3
G-CMCT	535.2 → 252.2	45	8	24.3	35	3
m^5U-D_3-CMCT	513.3 → 255.2	45	8	24.6	35	3
m^6U-D_3-CMCT	513.3 → 255.2	45	8	24.6	35	3
$m^1\Psi$-D_3-CMCT	513.3 → 255.2	45	8	24.6	35	3
hm^5U-D_3-CMCT	529.3 → 255.2	45	9	25.4	42	3
mcm^5U-D_3-CMCT	571.3 → 255.2	45	6	26.5	35	3
Ψ-D_3-CMCT	499.3 → 255.2	40	8	24.1	42	3
U-D_3-CMCT	499.3 → 255.2	40	8	24.1	42	3

mo^5U-D_3-CMCT	529.3 → 255.2	45	9	25.4	42	3

Table S2. CMCT labelling efficiencies for uridine and uridine modifications.

Analytes	Labelling efficiency (%)
m^5U	91.1
U	95.5
m^6U	98.0
$m^1\Psi$	91.8
Ψ	100
mcm^5U	98.0
hm^5U	100.0
mo^5U	97.0

Table S3. Calibration curves for the analysis of m^5U, m^6U, $m^1\Psi$, Ψ, mcm^5U, hm^5U, and mo^5U by CMCT labelling coupled with LC-ESI-MS/MS analysis.

Analytes	Linear range (pmol)	Linear regression	R^2
m^5U	0.016 - 16	y = 2.5774 x - 0.0738	0.9998
m^6U	0.016 - 16	y = 1.7650 x - 0.0724	0.9997
$m^1\Psi$	0.016 - 16	y = 1.7322 x + 0.0080	0.9994
Ψ	0.016 - 16	y = 0.1609 x - 0.0114	0.9994
mcm^5U	0.016 - 16	y = 0.9859 x - 0.0218	0.9998
hm^5U	0.016 - 16	y = 1.1040 x - 0.0224	0.9999
mo^5U	0.016 - 16	y = 0.3042 x - 0.0050	0.9999

Table S4. Accuracy and precision for the detection of uridine modifications by CMCT labeling coupled with LC-ESI-MS/MS analysis.

Analytes	Theoretical value (pmol)	Measured value (pmol)	Relative error (%)	Intra-day (RSD%, n = 5)	Inter-day (RSD%, n = 3)
	0.08	0.083	3.5	10.6	10.0
m^5U	0.80	0.81	1.1	2.4	1.9
	8.00	7.37	-7.8	1.5	3.1
	0.08	0.082	2.5	5.1	5.3
m^6U	0.80	0.78	-2.5	3.2	2.9
	8.00	8.4	5.0	5.5	6.6
	0.08	0.077	-3.8	6.0	5.2
$m^1\Psi$	0.80	0.77	-3.8	2.8	0.6
	8.00	7.41	-7.3	3.3	2.5
	0.08	0.082	2.5	11.3	15.3
Ψ	0.80	0.79	-1.3	5.1	6.0
	8.00	8.26	3.3	2.8	3.9
	0.08	0.082	2.5	13.9	12.1
mcm^5U	0.80	0.81	1.3	4.8	4.1
	8.00	7.95	-1.0	6.1	8.6
	0.08	0.085	2.5	11.1	10.3
hm^5U	0.80	0.81	-1.3	5.5	6.5
	8.00	8.4	3.3	1.1	2.6
	0.08	0.0826	2.5	5.5	6.1
mo^5U	0.80	0.79	-1.3	2.7	3.0
	8.00	7.90	-1.3	6.8	5.7

Table S5. Mapped read pairs and detected number of genes in isolated mRNA of HEK293T cells by RNA-seq.

Raw Pairs	Trimmed	rRNA	Mapped	Unmapped	Detected number of genes
25407861	25405130	0.46%	94.62%	5.38%	12299

Table S7. The sequence of the in-vitro transcribed RNA.

5’GGGAGACAGACUAAACUGGCUGACGGAAUUUAUGCCUCUUCCGACCAUCAA

GCAUUUUAUCCGUACUCCUGAUGAUGCAUGGUUACUCACCACUGCGAUCCCCG

GGAAAACAGCAUUCCAGGUAUUAGAAGAAUAUCCUGAUUCAGGUGAAAAUAU

UGUUGAUGCGCUGGCAGUGUUCCUGCGCCGGUUGCAUUCGAUUCCUGUUUGU

AAUUGUCCUUUUAACAGCGAUCGCGUAUUUCGUCUCGCUCAGGCGCAAUCACG

AAUGAAUAACGGUUUGGUUGAUGCGAGUGAUUUUGAUGACGAGCGUAAUGGC

UGGCCUGUUGAACAAGUCUGGAAAGAAAUGCAUAAACUUUUGCCAUUCUCAC

CGGAUUCAGUCGUCACUCAUGGUGAUUUCUCACUUGAUAACCUUAUUUUUGA

CGAGGGGAAAUUAAUAGGUUGUAUUGAUGUUGGACGAGUCGGAAUCGCAGAC

CGAUACCAGGAUCUUGCCAUCCUAUGGAACUGCCUCGGUGAGUUUUCUCCUUC

AUUACAGAAACGGCU-3’

Figure S1. Chemical reaction of the synthesis of D_3-CMCT.

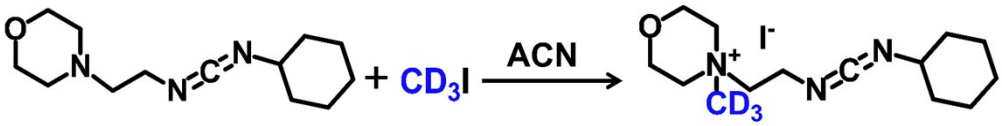


Figure S2. Examination the CMCT standard and synthesized D_3-CMCT by the high-resolution mass spectrometry. (A) The extracted-ion chromatogram (left panel), MS spectrum (middle panel) and MS/MS spectrum (right panel) of CMCT standard. (B) The extracted-ion chromatogram (left panel), MS spectrum (middle panel) and MS/MS spectrum (right panel) of synthesized D_3-CMCT. Highlighted in red are the theoretical *m/z*.

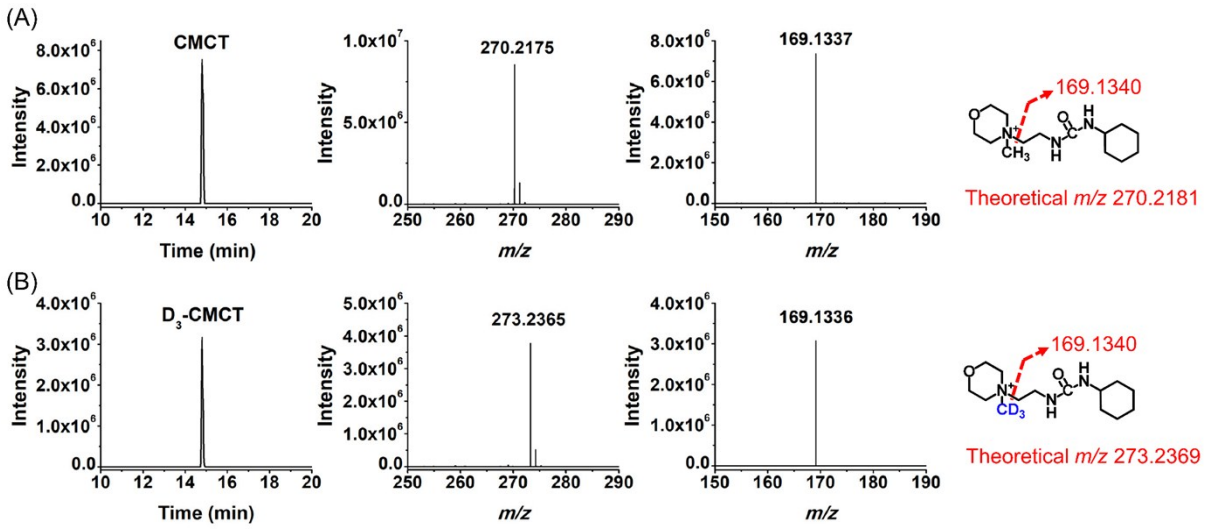
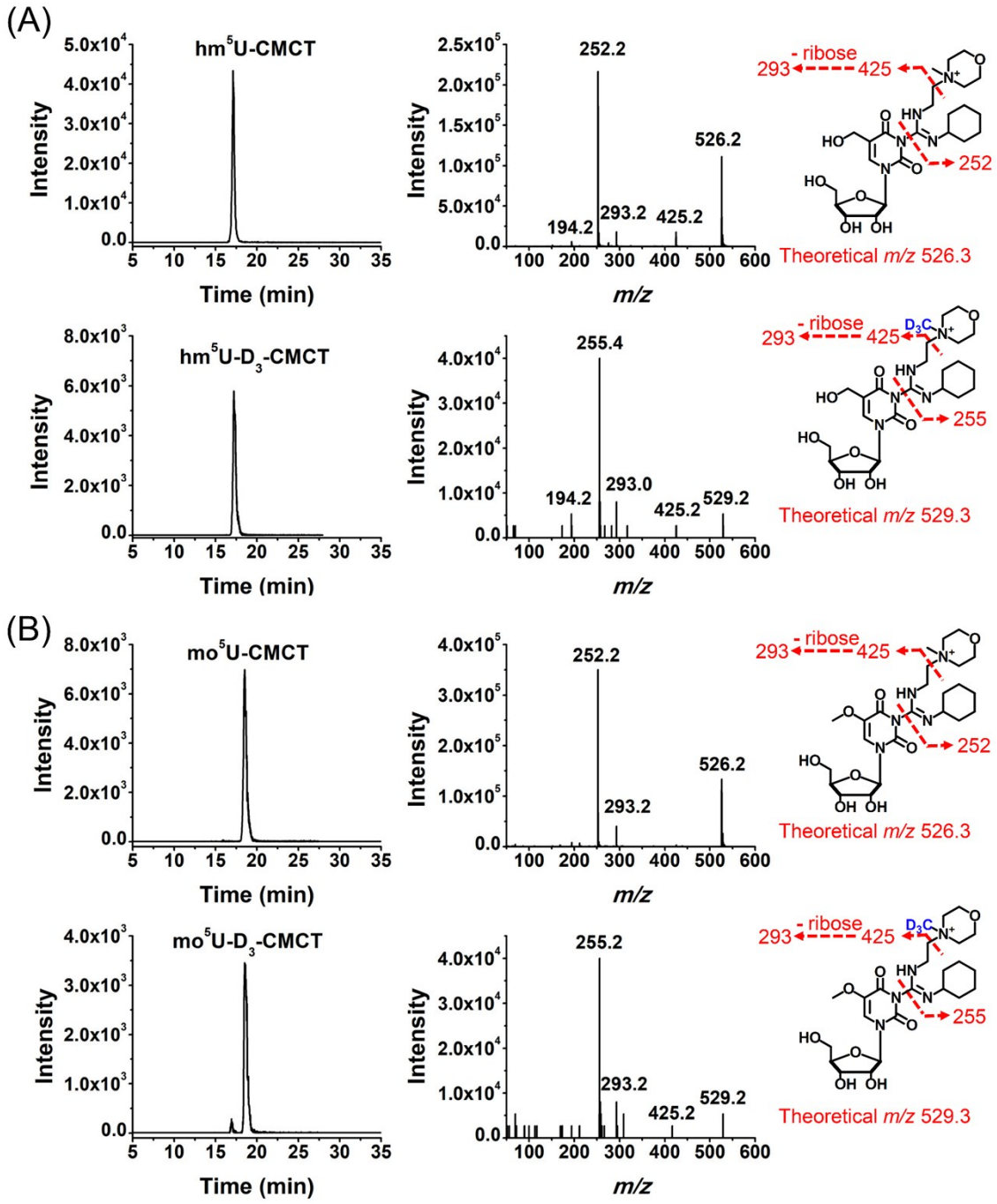
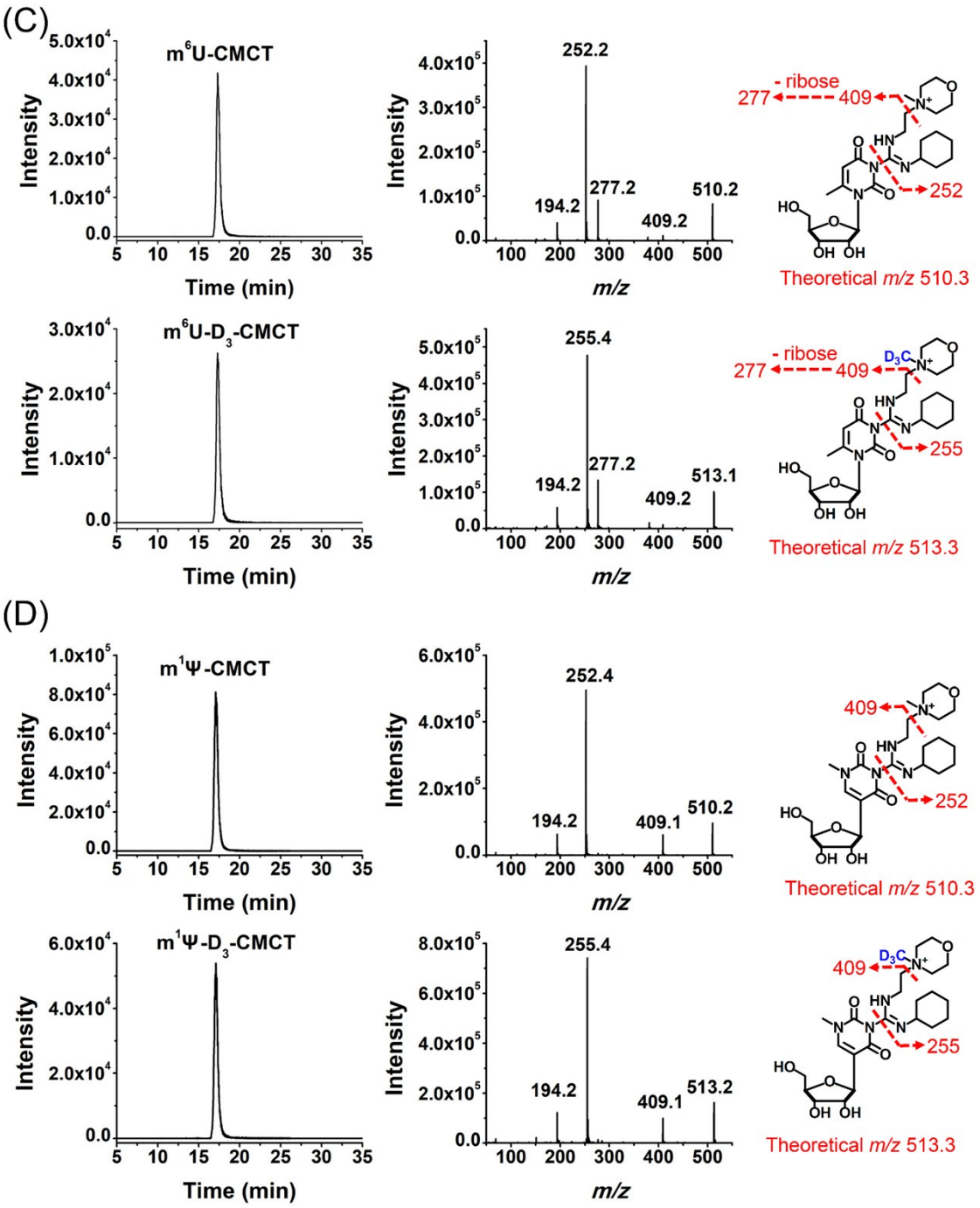


Figure S3. Examination of the CMCT/D_3-CMCT labelled standards of uridine modifications. The extracted-ion chromatograms (left panel) and the product-ion spectra (right panel) of the CMCT/D_3-CMCT labelled hm^5U (A), mo^5U (B), m^6U (C), $m^1\Psi$ (D), mcm^5U (E), and Ψ (F). Highlighted in red are the theoretical *m/z*.




(C)
m6U-CMCT
Intensity
Time (min)
252.2
277.2
194.2
409.2
510.2
m/z
- ribose
277
409
252
Theoretical m/z 510.3
m6U-D3-CMCT
255.4
277.2
194.2
409.2
513.1
255
Theoretical m/z 513.3
(D)
m1Ψ-CMCT
252.4
194.2
409.1
510.2
Theoretical m/z 510.3
m1Ψ-D3-CMCT
255.4
194.2
409.1
513.2
Theoretical m/z 513.3

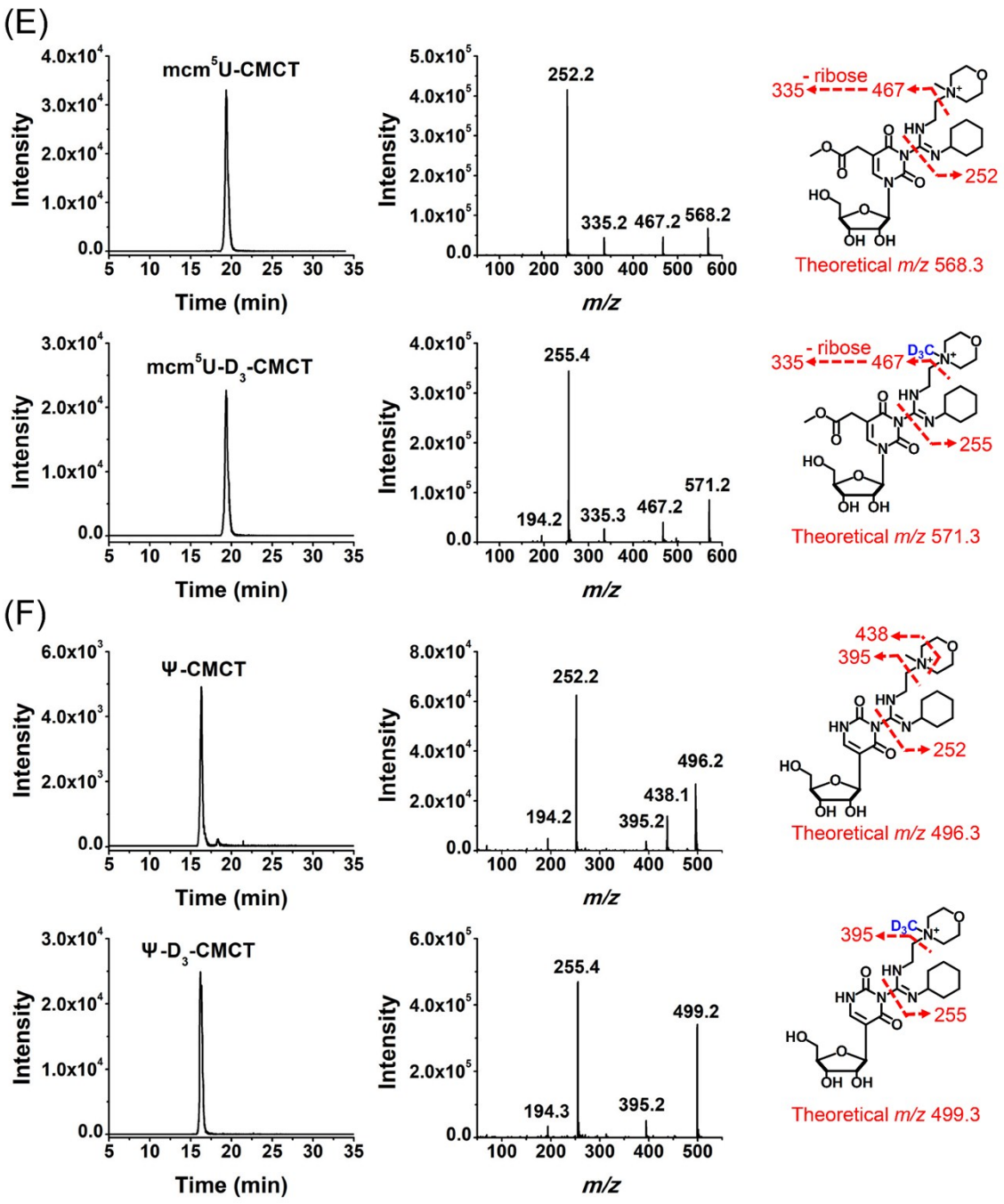
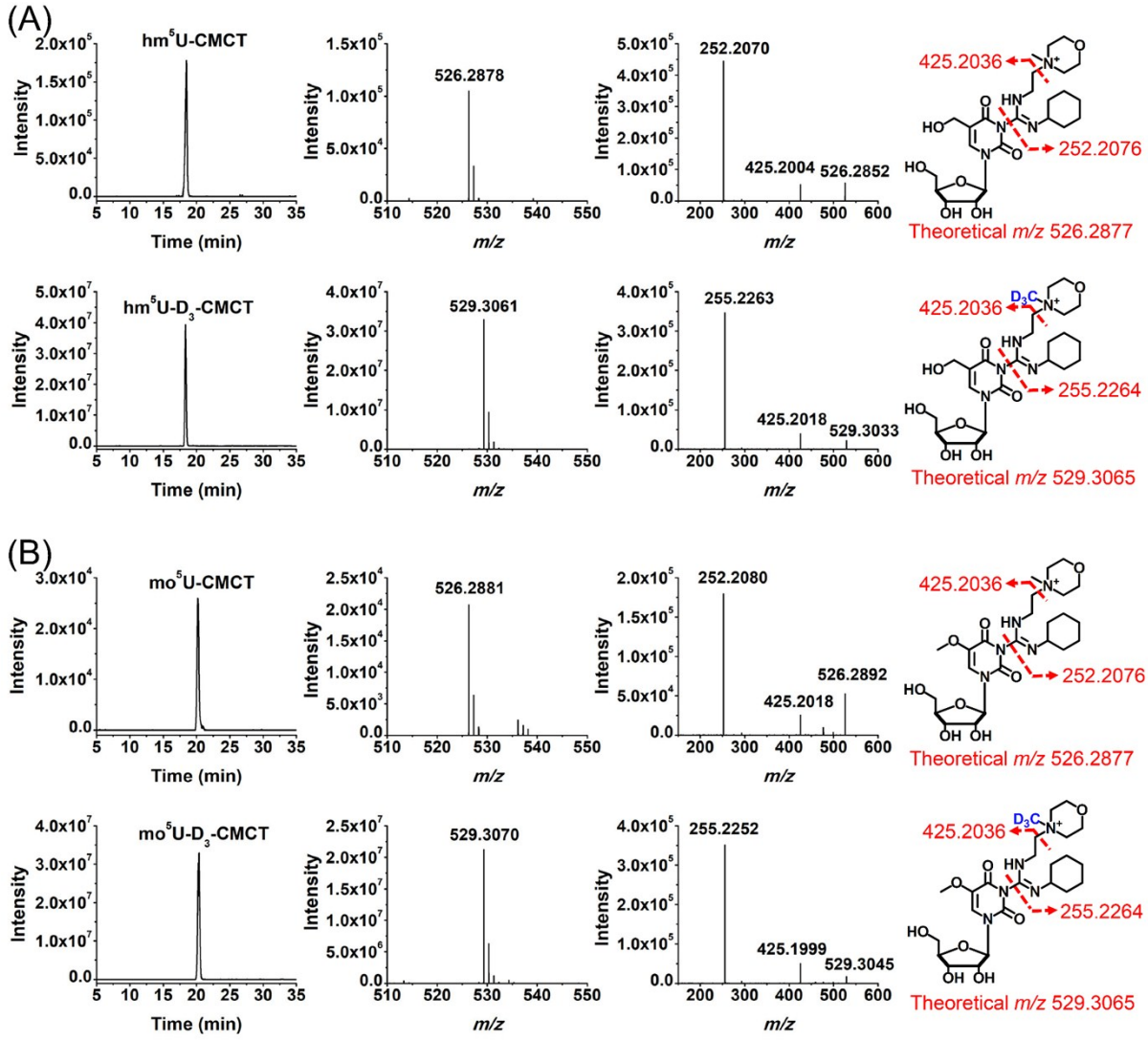
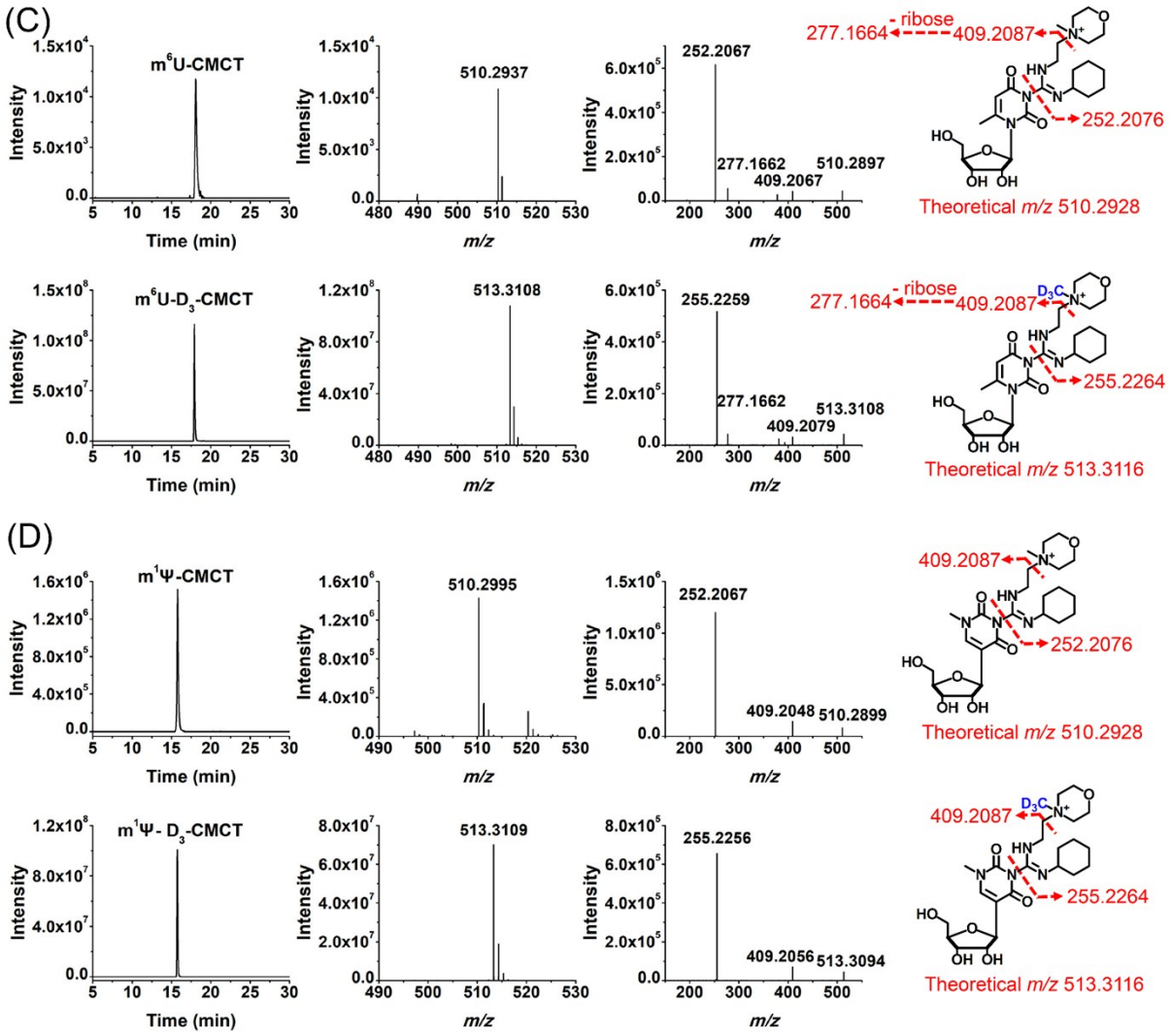

(E)
mcm5U-CMCT
Intensity
Time (min)
252.2
335.2 467.2 568.2
m/z
- ribose
335
467
252
Theoretical m/z 568.3
mcm5U-D3-CMCT
255.4
194.2 335.3 467.2 571.2
255
Theoretical m/z 571.3
(F)
Ψ-CMCT
252.2
194.2 395.2 438.1 496.2
438
395
252
Theoretical m/z 496.3
Ψ-D3-CMCT
255.4
194.3 395.2 499.2
Theoretical m/z 499.3

Figure S4. Examination of CMCT/D_3-CMCT labelled standards of uridine modifications by high-resolution mass spectrometry analysis. The extracted-ion chromatograms (left panel), MS spectra (middle panel) and MS/MS spectra (right panel) of CMCT/D_3-CMCT labelled hm^5U (A), mo^5U (B), m^6U (C), $m^1\Psi$ (D), U (E), Ψ (F), mcm^5U (G), and m^5U (H). Highlighted in red are the theoretical *m/z*.




(C)
m6U-CMCT
Intensity
Time (min)
510.2937
m/z
252.2067
277.1662
510.2897
409.2067
277.1664
- ribose
409.2087
252.2076
Theoretical m/z 510.2928
m6U-D3-CMCT
513.3108
255.2259
277.1662
513.3108
409.2079
D3C
255.2264
Theoretical m/z 513.3116
(D)
m1Ψ-CMCT
510.2995
252.2067
409.2048
510.2899
409.2087
252.2076
Theoretical m/z 510.2928
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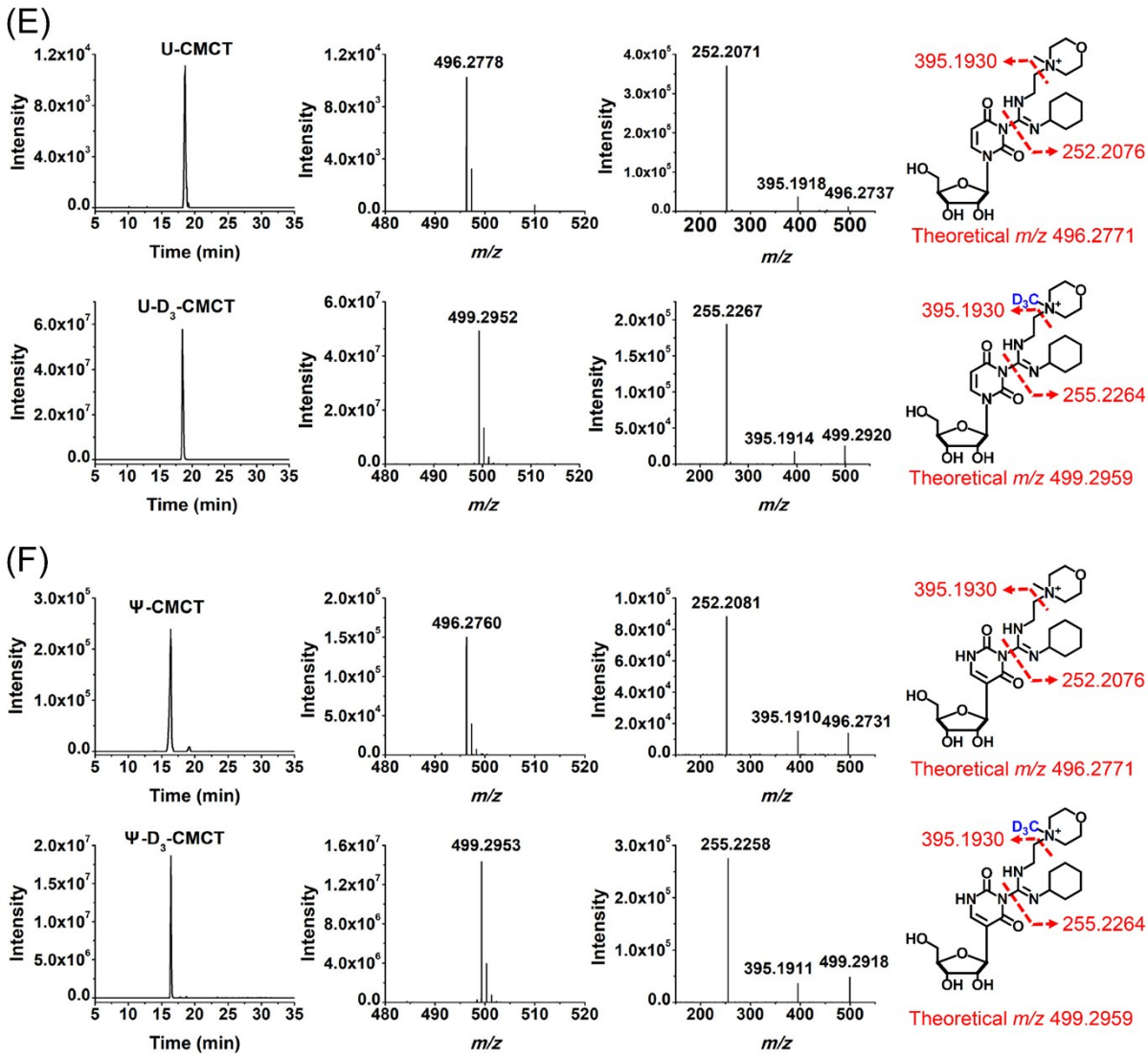
513.3094
255.2264
Theoretical m/z 513.3116


(E)
U-CMCT
Intensity
Time (min)
496.2778
m/z
252.2071
395.1918
496.2737
395.1930
252.2076
Theoretical m/z 496.2771
U-D3-CMCT
499.2952
255.2267
395.1914
499.2920
D3C
255.2264
Theoretical m/z 499.2959
(F)
Ψ-CMCT
496.2760
252.2081
395.1910
496.2731
Theoretical m/z 496.2771
Ψ-D3-CMCT
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395.1911
499.2918
Theoretical m/z 499.2959

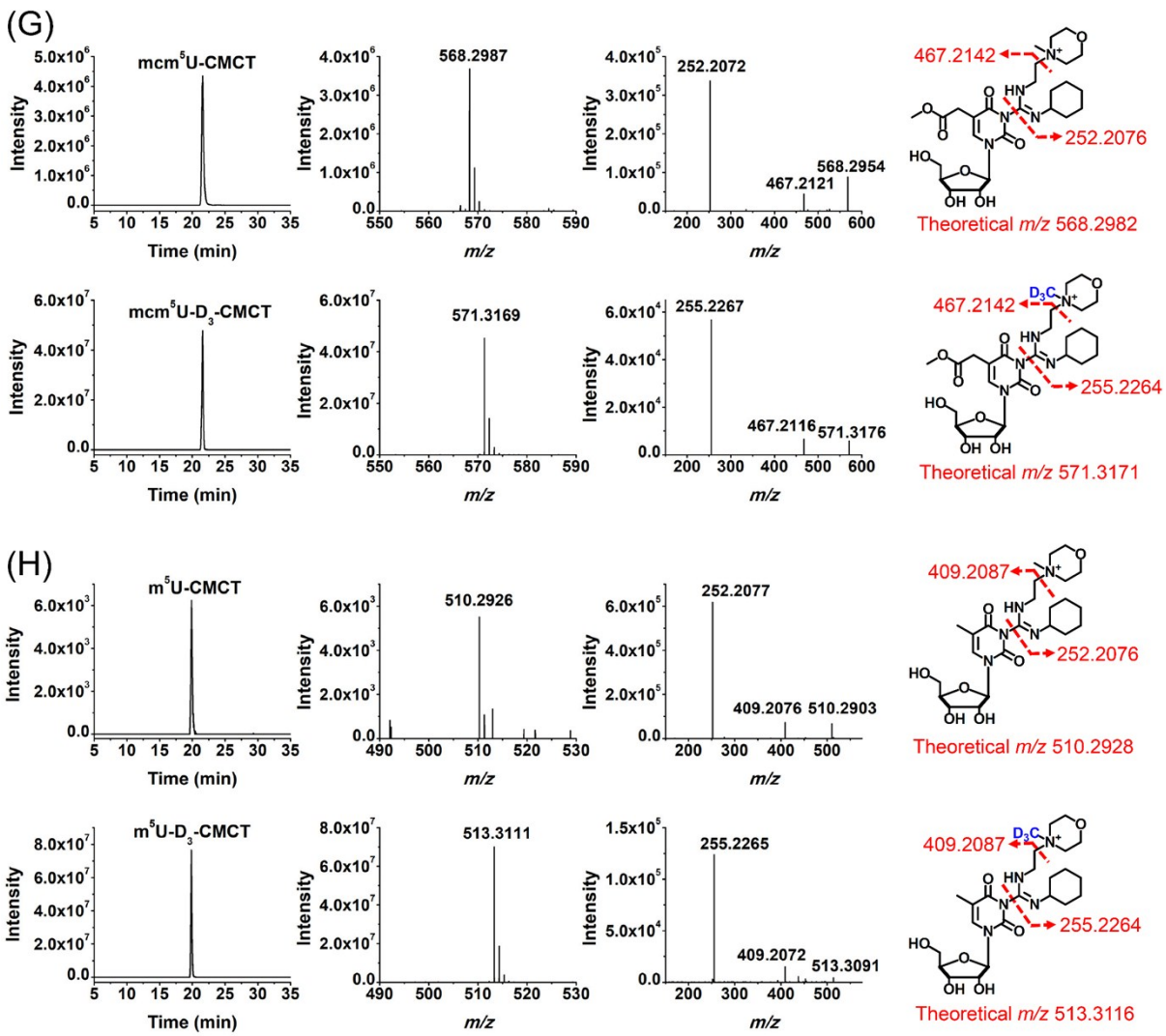

(G)
mcm5U-CMCT
568.2987
252.2072
568.2954
467.2121
467.2142
252.2076
Theoretical m/z 568.2982
mcm5U-D3-CMCT
571.3169
255.2267
467.2116
571.3176
467.2142
255.2264
Theoretical m/z 571.3171
(H)
m5U-CMCT
510.2926
252.2077
409.2076
510.2903
409.2087
252.2076
Theoretical m/z 510.2928
m5U-D3-CMCT
513.3111
255.2265
409.2072
513.3091
409.2087
255.2264
Theoretical m/z 513.3116
Intensity
Time (min)
m/z

Figure S5. The fragmentation pathway of CMCT labelled m^5U. The CMCT labelled m^5U is constantly and positively charged with carrying a quaternary ammonium group and CMCT labelled m^5U undergoes charge-remote fragmentation to generate the fragment ion of *m/z* 252.2. The CMCT labelled m^5U can also lose a neutral fragment of 4-methylmorpholine and generate the fragment ion of *m/z* 409.2, which can further lose a ribose to form the fragment ion of *m/z* 277.2.

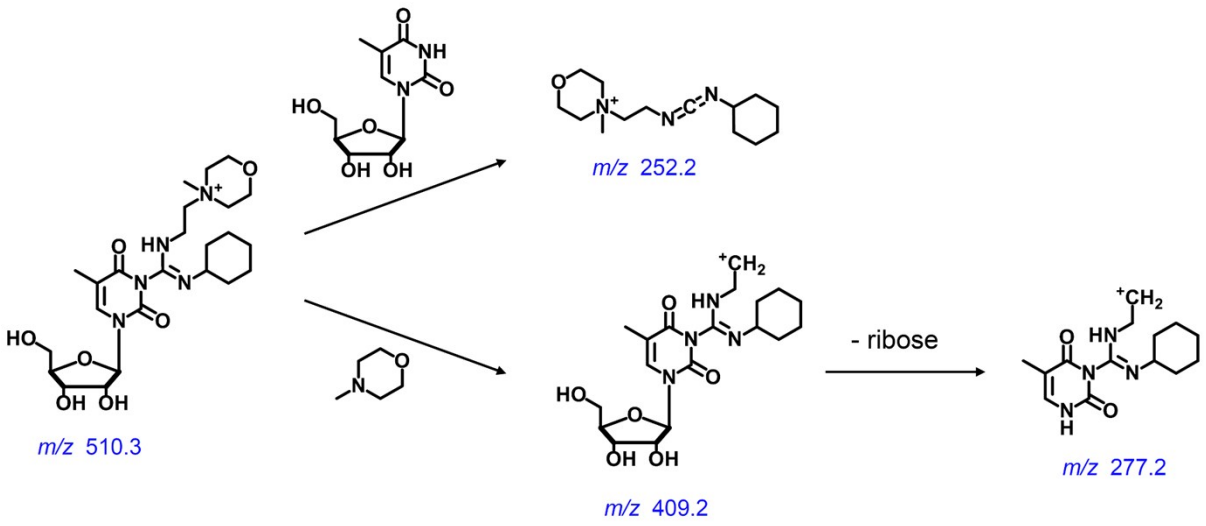


Figure S6. The fragmentation of CMCT labelled m^5U with different collision energy ranging from 1 eV to 30 eV.

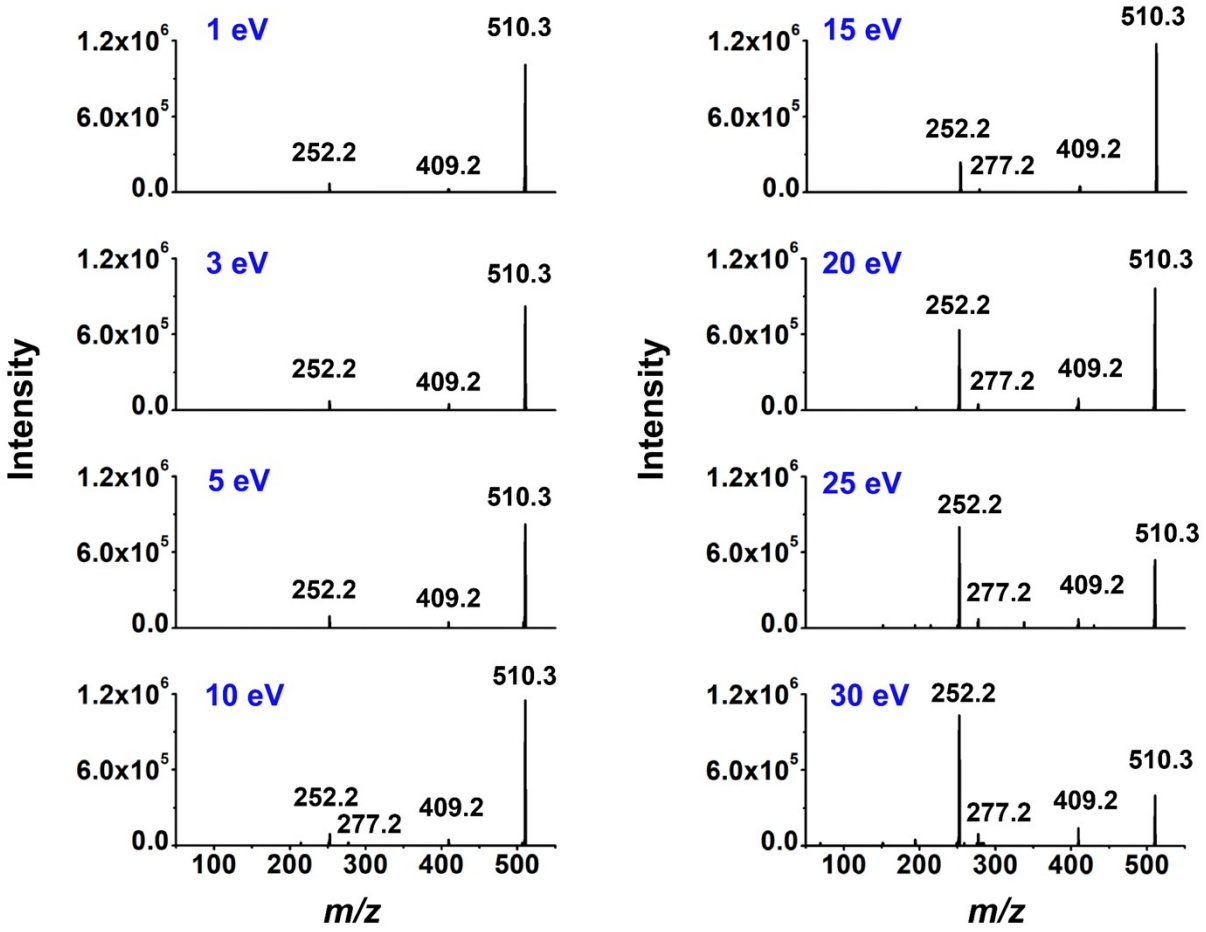


Figure S7. Optimization of CMCT labelling conditions. (A) Optimization of reaction temperature. (B) Optimization of reaction time. (C) Optimization of CMCT concentration. (D) Evaluation of the stability of the CMCT labelled derivatives.

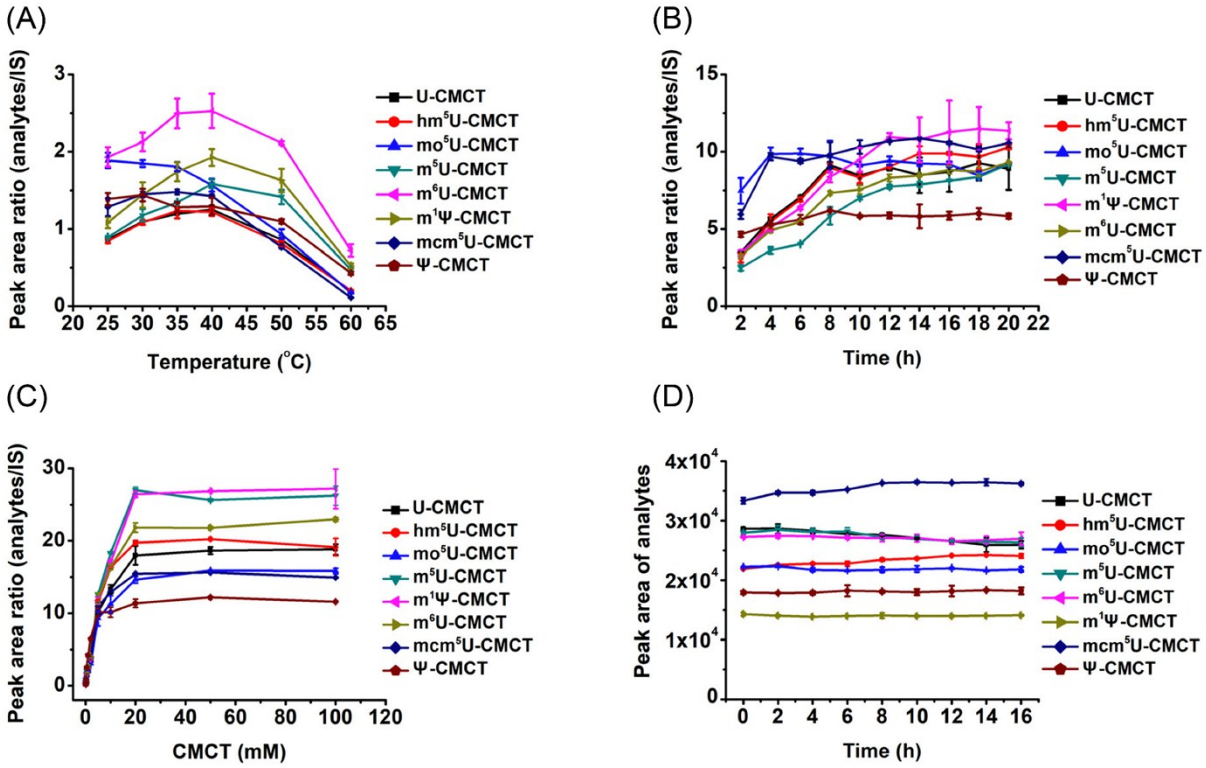
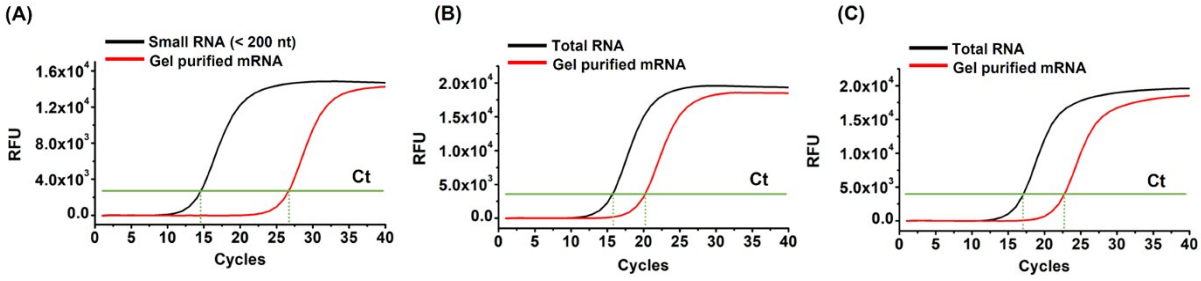


Figure S8. Evaluation of the purity of isolated mRNA by examining the potential trace levels of (A) small RNA (< 200 nt), (B) 18S rRNA, and (C) 28S rRNA by real-time quantitative PCR. The percentages of small RNA (< 200 nt), 18S rRNA, and 28S rRNA in isolated mRNA were calculated by the equations listed in the text in Supporting Information. Small RNA (< 200 nt) was evaluated by examining $tRNA^{HisGUG}$; 18S rRNA was evaluated using the specific primers targeting 18S rRNA; 28S rRNA was evaluated using the specific primers targeting 28S rRNA.



(A)

	Small RNA (< 200 nt) (20 ng)	Isolated mRNA (20 ng)	ΔCt	Percentage of small RNA (< 200 nt) in isolated mRNA
Ct ($tRNA^{HisGUG}$)	14.68 ± 0.04	26.76 ± 0.05	12.08	0.023%

(B)

	Total RNA (0.04 ng)	Isolated mRNA (0.04 ng)	ΔCt	Percentage of 18S rRNA in isolated mRNA
Ct (18S rRNA)	15.75 ± 0.05	20.26 ± 0.08	4.51	0.948%

(C)

	Total RNA (0.04 ng)	Isolated mRNA (0.04 ng)	ΔCt	Percentage of 28S rRNA in isolated mRNA
Ct (28S rRNA)	17.04 ± 0.13	22.70 ± 0.22	5.66	1.140%

Figure S9. Confirmation of the detected m^5U in mRNA. The real-time quantitative PCR analysis demonstrated that the potential contamination of small RNA was less than 0.023% in mRNA, i.e. less than 0.048 ng or 0.12 ng of small RNA in 200 ng or 500 ng of mRNA, respectively. We then analyzed m^5U from 200 ng and 500 ng of mRNA. The peak of m^5U from 200 ng and 500 ng of isolated mRNA was clear and distinct. However, no signal of m^5U was observed from 0.048 ng or 0.12 ng of small RNA, indicating that the m^5U signal from 200 ng and 500 ng of mRNA indeed come from mRNA, but not the potential trace level of small RNA.

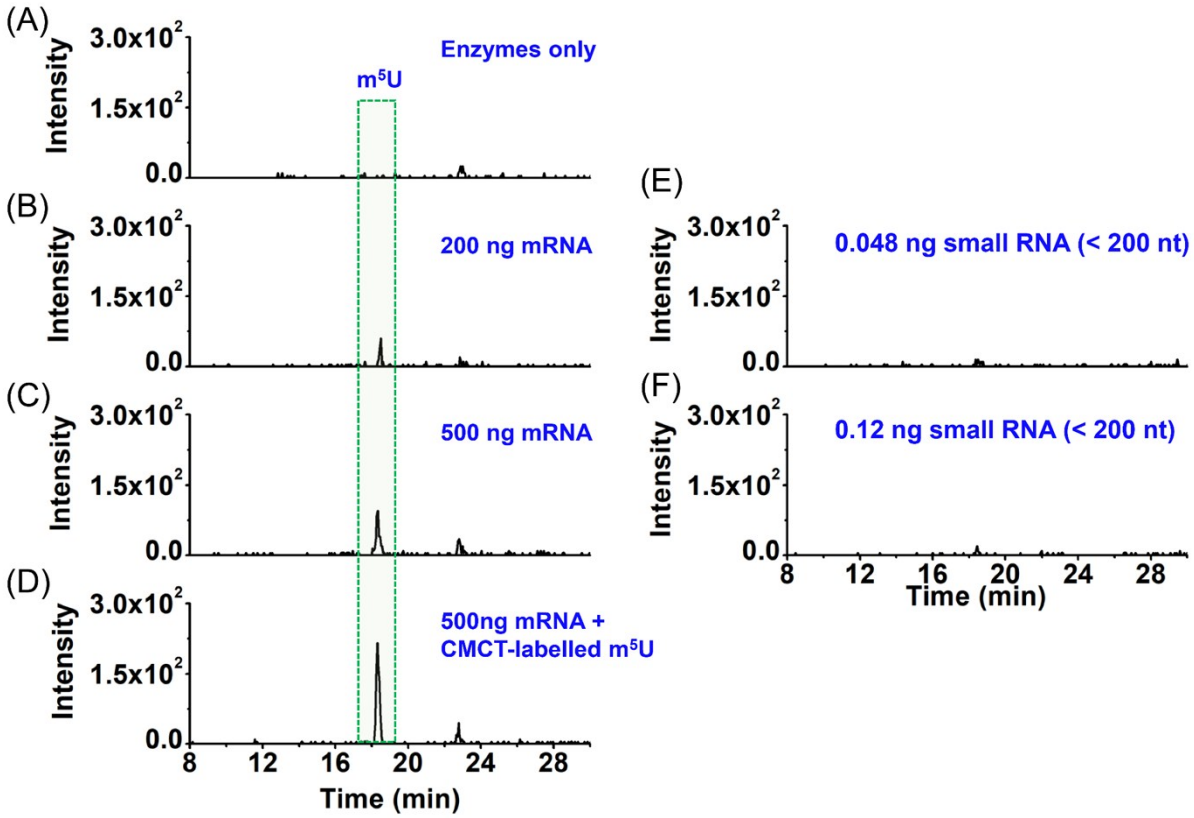


Figure S10. Extracted-ion chromatograms of (A) CMCT labelled m^5U, m^6U, and $m^1\Psi$ standards and (B) detected m^5U from mRNA of HEK293T cells. The separation of CMCT labelled uridine modifications was performed on an Inersil ODS-3 reversed-phase column (5 μm, 2.1 × 250 mm; GL Sciences Lnc., Tokyo, Japan). NH_4HCO_3 (2 mM, pH 4.5, solvent A) and ACN (solvent B) were used as the mobile phases. A gradient of 0 - 3 min, 5 % B; 3 - 26 min, 5 - 40% B; 26 - 30 min, 40 % B; 30 - 32 min, 40 - 5 % B; and 32 - 45 min, 5 % B was used. The flow rate was set at 200 μL/min.

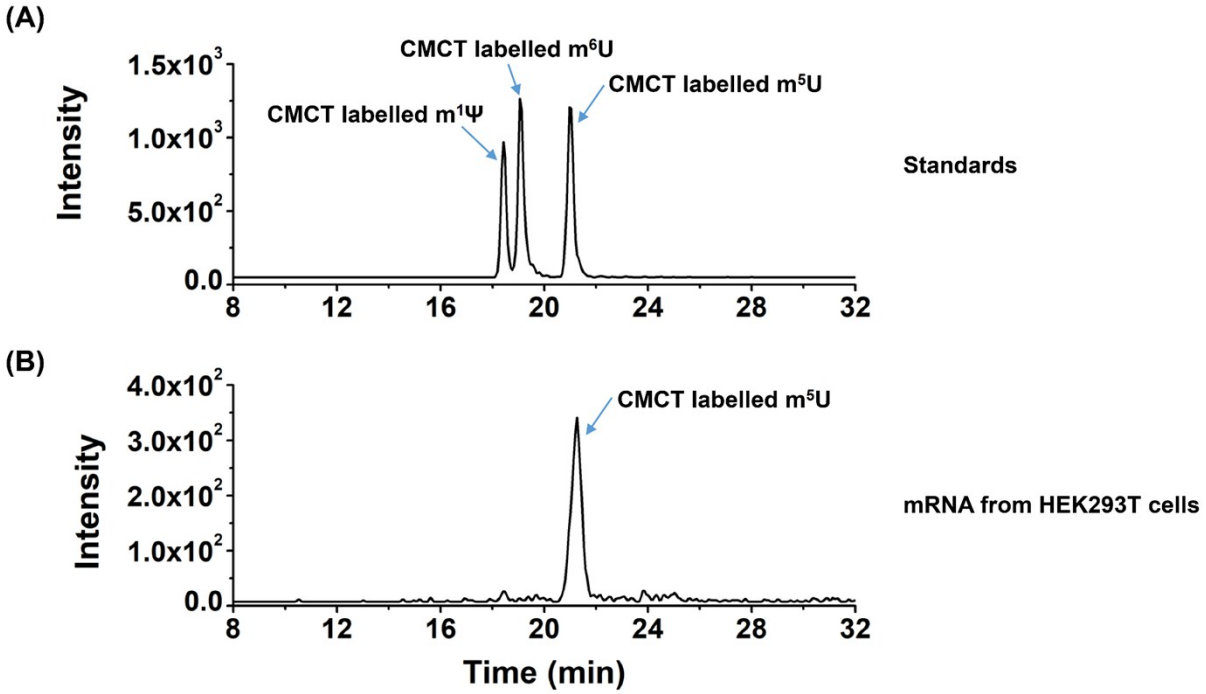


Figure S11. Identification of Ψ in mRNA of HEK293T cells by high-resolution mass spectrometry analysis. (A) The extracted-ion chromatogram (left panel), MS spectrum (middle panel) and MS/MS spectrum (right panel) of CMCT labelled Ψ standard. (B) The extracted-ion chromatogram (left panel), MS spectrum (middle panel) and MS/MS spectrum (right panel) of CMCT labelled Ψ from mRNA of HEK293T cells. (C) The extracted-ion chromatogram (left panel), MS spectrum (middle panel) and MS/MS spectrum (right panel) of D_3-CMCT labelled Ψ standard. (D) The extracted-ion chromatogram (left panel), MS spectrum (middle panel) and MS/MS spectrum (right panel) of D_3-CMCT labelled Ψ from mRNA of HEK293T cells.

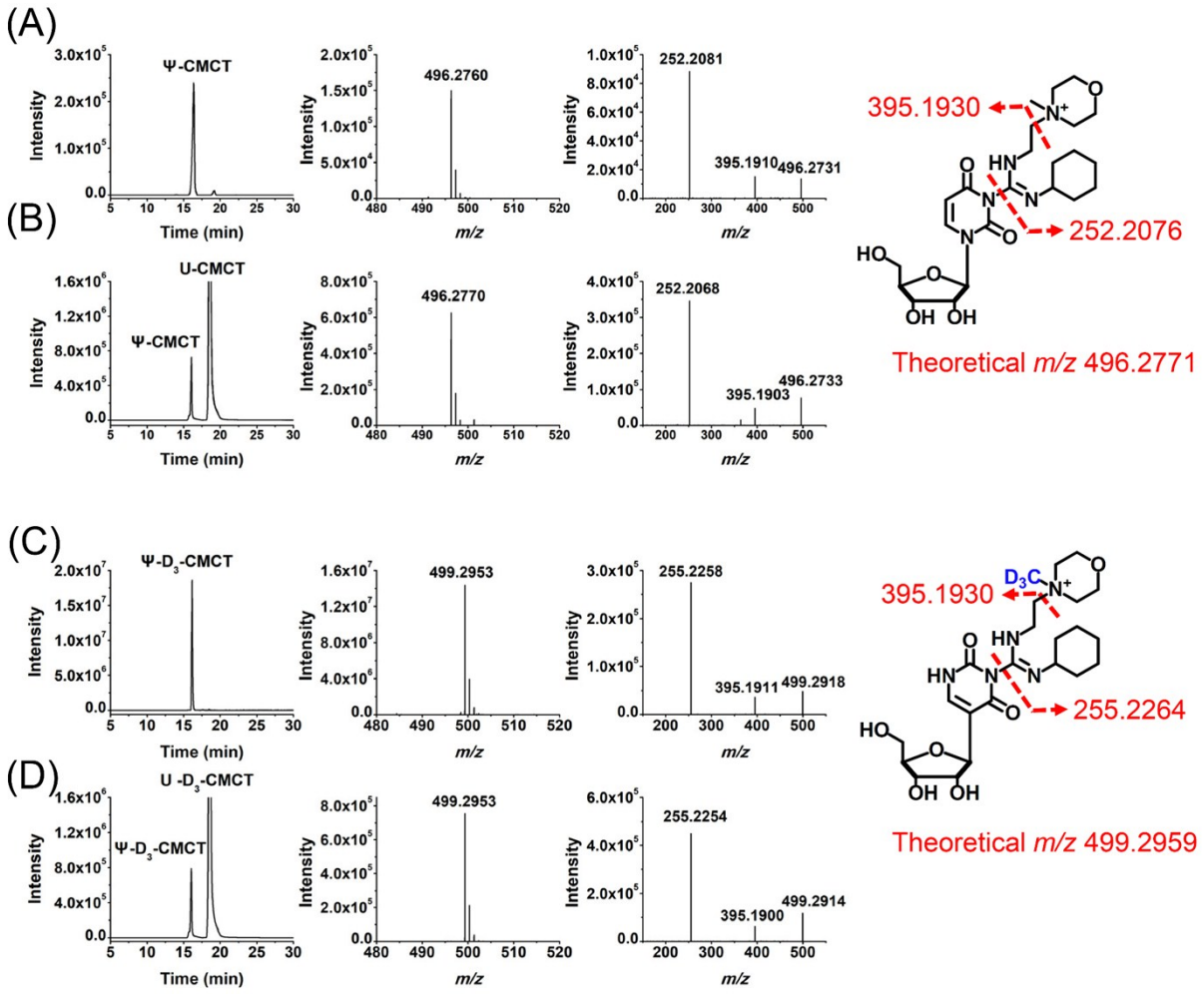


Figure S12. Detection of m^5U in mRNA of HEK293T cells by direct LC-ESI-MS/MS analysis without CMCT labelling. (A) m^5U standard. (B) m^5U in mRNA of HEK293T cells. The mass transition of 259.1→127.1 was used for the detection under MRM detection mode. 5 μg mRNA was used for the analysis.

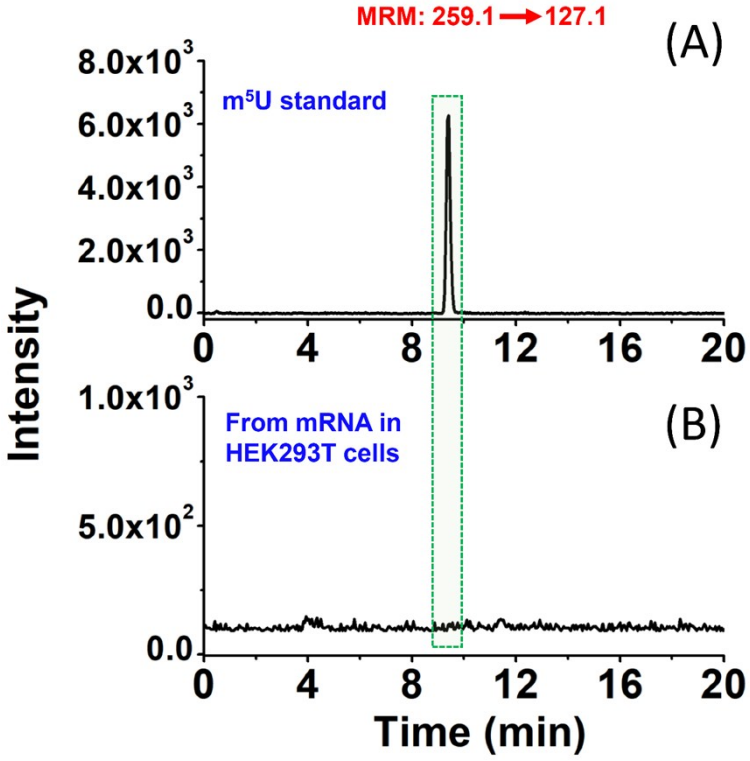


Figure S13. Examination of the isolated 18S rRNA, 28S rRNA and small RNA (< 200 nt). (A) Separation of 28S rRNA and 18S rRNA by size-exclusion chromatography (SEC). The fraction containing 28S rRNA was collected between 3.1 min and 4.1 min; the fraction containing 18S rRNA was collected between 4.6 min and 5.4 min. (B) Examination of the purified 18S rRNA and 28S rRNA by agarose gel electrophoresis. (C) Examination of the isolated small RNA (< 200 nt) by polyacrylamide gel electrophoresis.

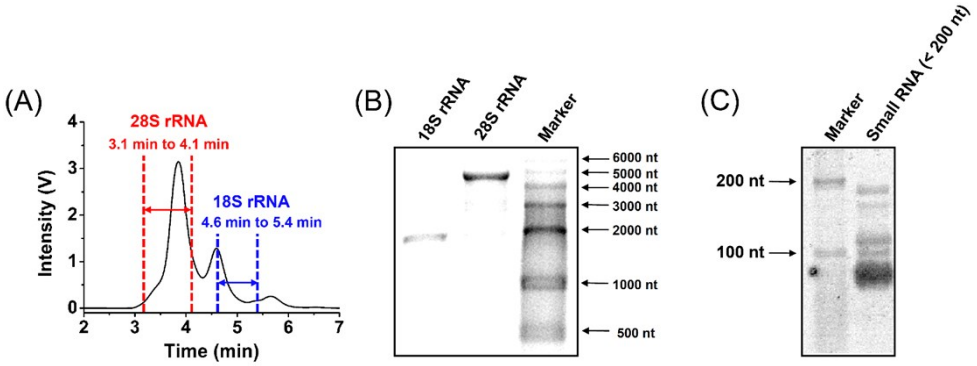
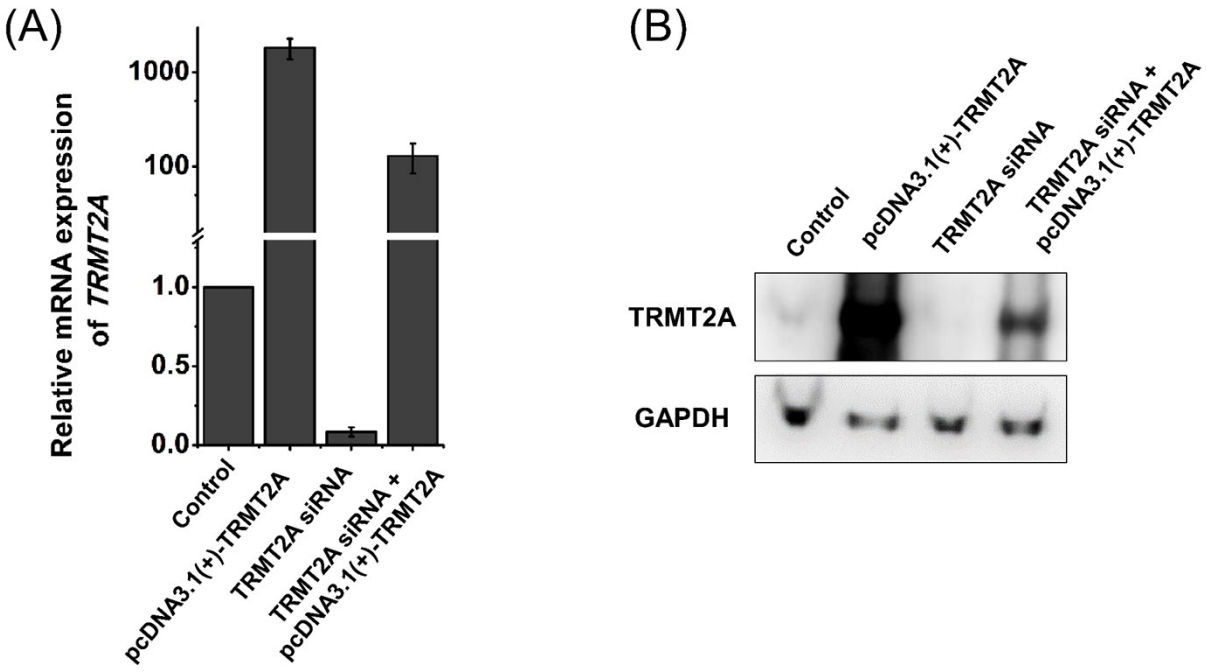


Figure S14. Evaluation of the overexpression and siRNA knockdown of *TRMT2A* gene. (A) Quantitative real-time PCR analysis of the *TRMT2A* gene level upon overexpression, siRNA knockdown, or rescue. The levels of *TRMT2A* gene expression were normalized to glyceraldehyde 6-phosphate dehydrogenase gene (*GAPDH*). (B) Western blotting analysis of TRMT2A protein level upon overexpression, siRNA knockdown, or rescue.



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