

Supplementary Table 1 | Data collection and refinement statistics

	MTR1-m ¹ A	MTR1-bn ¹ A	MTR1-ab ¹ A	MTR1-m ¹ A; no Mg ²⁺	MTR1-m ¹ A; 2'-SeMeU	MTR1-m ¹ A; TI ⁺
	(Crystal I)	(Crystal II)	(Crystal III)	(Crystal IV)	(Crystal V)	(Crystal VI)
PDB code	7Q7X	7Q7Y	7Q7Z	7Q80	7Q81	7Q82
Data collection						
Space group	P 4 ₁ 2 ₁ 2	P 4 ₁ 2 ₁ 2	P 4 ₁ 2 2	P 4 ₁ 2 2	P 4 ₁ 2 ₁ 2	P 4 ₁ 2 ₁ 2
Cell dimensions						
a, b, c (Å)	71.0 71.0 82.8	71.3 71.3 83.1	117.9 117.9 83.3	120.9 120.9 82.2	71.1 71.1 83.6	72.0 72.0 84.5
α, β, γ (°)	90 90 90	90 90 90	90 90 90	90 90 90	90 90 90	90 90 90
Resolution (Å)	42.94 - 2.80 (2.90 - 2.80) ¹	43.09-2.90 (3.08-2.90)	44.54 - 3.26 (3.38 - 3.26)	45.17 - 3.15 (3.26 - 3.15)	43.07 - 2.85 (2.95 - 2.85)	43.59 - 2.95 (3.06 - 2.95)
R _{merge}	0.11 (>1.0)	0.14 (>1.0)	0.14 (>1.0)	0.12 (>1.0)	0.07 (>1.0)	0.10 (>1.0)
I/σI	18.74 (1.13)	11.4 (1.20)	11.92 (0.60)	13.52 (0.56)	27.07 (1.38)	18.01 (0.81)
Completeness (%)	99.9 (99.8)	99.9 (100)	99.71 (98.83)	99.1 (91.8)	99.9 (99.6)	99.8 (100)
Redundancy	24.8 (25.5)	11.0 (11.4)	15.4 (15.7)	25.3 (23.1)	24.5 (25.7)	24.9 (26.7)
CC1/2 (%)	99.6 (66.4)	99.5 (56.1)	99.9 (23.6)	99.4 (25.8)	99.9 (69.3)	99.9 (36.6)
Unique reflections	5592 (533)	5134 (799)	9584 (937)	11036 (1054)	5365 (515)	5037 (487)
Wilson B-factor (Å ²)	90.4	111.1	162.3	138.3	122.5	145.5
Wavelength (Å)	0.9198	0.9716	1.0332	1.0332	0.9786	0.9751
No. mol/AU	1	1	2	2	1	1
Refinement						
Resolution						
No. reflections	5587 (531)	5091 (492)	9568 (928)	10973 (993)	5363 (515)	5037 (487)
R _{work} / R _{free} (%)	21.3/25.5	19.8/24.4	21.0/26.4	17.6/20.3	18.8/21.4	20.9/22.5
No. of non-H atoms	1340	1346	2671	2647	1347	1334
Macromolecules	1312	1289	2578	2624	1313	1312
Ligands/ions	28	57	93	23	34	22
Average B-factors (Å ²)	104.4	98.4	166.2	151.9	127.1	123.9
Macromolecules (Å ²)	121.3	106.9	186.4	189.5	125.1	114.7
Ligands/ions (Å ²)	87.5	89.8	146.0	114.4	129.0	133.2
r.m.s deviations						
Bond lengths (Å)	0.001	0.002	0.001	0.001	0.001	0.001
Bond angles (°)	0.20	0.41	0.23	0.27	0.29	0.31
Coord. Precis. ² (Å)	0.33	0.34	0.46	0.37	0.16	0.37

¹ Statistics for the highest-resolution shell are shown in parentheses. ² Calculated by Maximum-Likelihood Estimation.

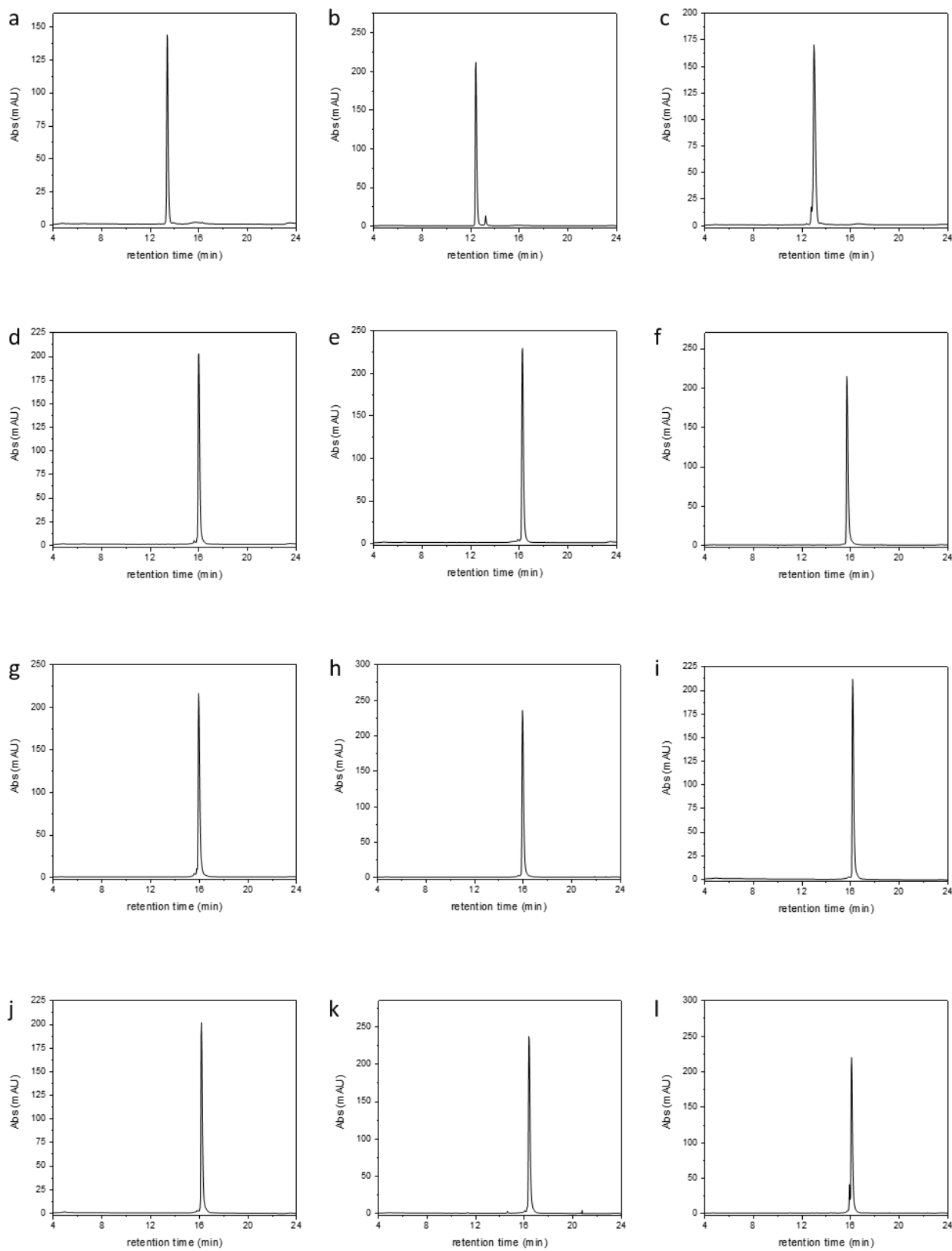
Supplementary Table 2 | RNAs used in this study

RNAs prepared by solid-phase synthesis

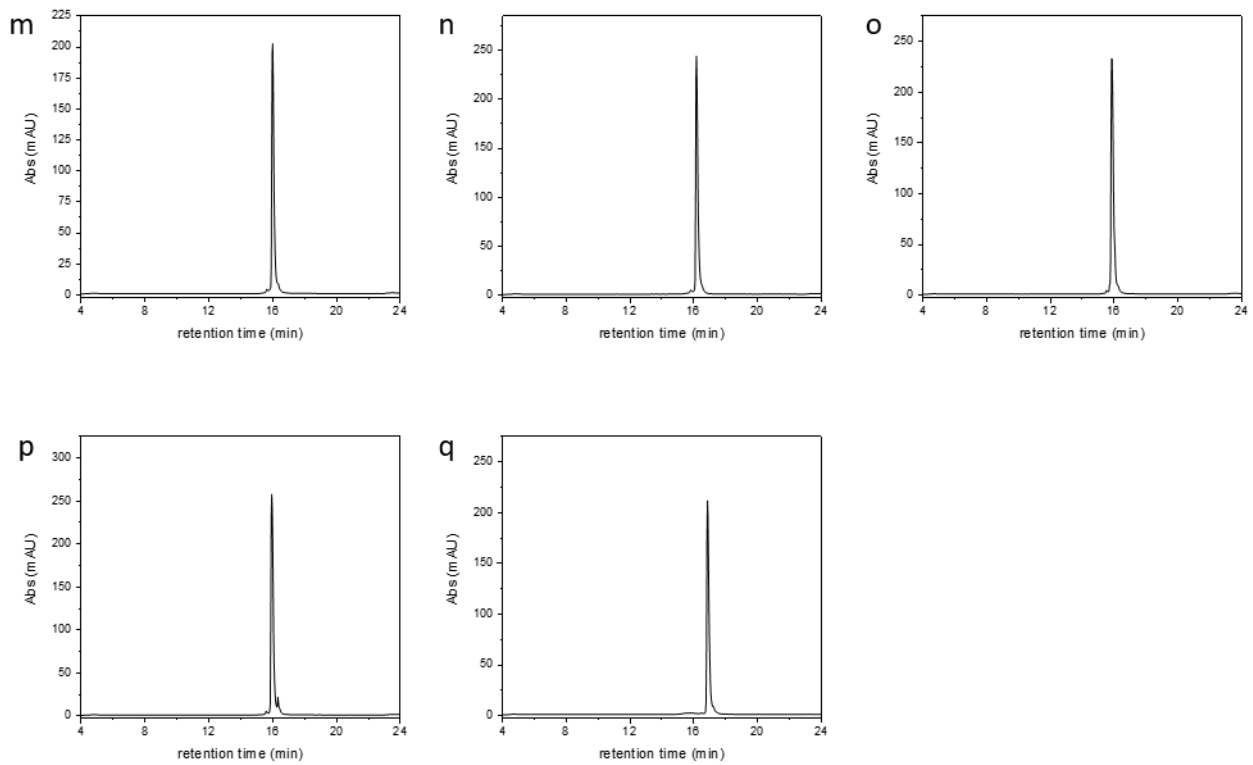
description	5'-sequence-3'	Mass calculated	Mass found
R1 (unmodified substrate)	CCACUGAGAGCUUC	4403.62	4403.63
m1A R1 (methylated product)	CCACUG ^m AGAGCUUC	4418.65	4418.65
R1 with SeMeU12	CCACUGAGAGC ^U Se ^m UC	4481.56	4481.57
MTR1 5' fragment (R2)	GGAAGCUCUGACCGACCCCCAGCC	7621.13	7621.14
MTR1 3' fragment (R3)	GCUGGGACAACUAGACAUACAGUG	7735.10	7735.13
MTR1 5' fragment C12U	GGAAGCUCUGA ^U CGACCCCCAGCC	7622.08	7622.11
MTR1 3' fragment U42C	GCUGGGACAACUAGACA ^C ACAGUG	7734.12	7734.17
MTR1 5' fragment m5C12	GGAAGCUCUGA ^m 5 ^C CGACCCCCAGCC	7635.11	7635.14
MTR1 5' fragment m4C12	GGAAGCUCUGA ^m 4 ^C CGACCCCCAGCC	7635.11	7635.14
MTR1 5' fragment dC12	GGAAGCUCUGA ^d C ^C CGACCCCCAGCC	7605.10	7605.13
MTR1 3' fragment dU42	GCUGGGACAACUAGACA ^d UACAGUG	7719.11	7719.13
MTR1 5' fragment Cf12	GGAAGCUCUGA ^C f ^C CGACCCCCAGCC	7623.09	7623.13
MTR1 3' fragment Uf42	GCUGGGACAACUAGACA ^U f ^f ACAGUG	7737.10	7737.11
MTR1 5' fragment Cm12	GGAAGCUCUGA ^C m ^C CGACCCCCAGCC	7635.11	7635.17
MTR1 3' fragment Um42	GCUGGGACAACUAGACA ^U m ^m ACAGUG	7749.12	7749.13
MTR1 3' fragment USem42	GCUGGGACAACUAGACA ^U Se ^m ACAGUG	7813.04	7816.09
MTR1 3' fragment Am41	GCUGGGACAACUAGACA ^A m ^m UACAGUG	7749.12	7749.13

Ribozymes prepared by in vitro transcription

description	5'-sequence-3' (binding arms underlined)
wt MTR1 ribozyme	<u>GGAAGCUCUGACCGACCCCCGAGUUCGCUCGGGGACAACUAGACAUACAGUGG</u>
M1 C17U	<u>GGAAGCUCUGACCGACU</u> CCCCGAGUUCGCUCGGGGACAACUAGACAUACAGUGG
M2 A15G	<u>GGAAGCUCUGACCGG</u> CCCCCGAGUUCGCUCGGGGACAACUAGACAUACAGUGG
M3 ΔC17A31	<u>GGAAGCUCUGACCGAC</u> -CCCCGAGUUCGCUCGGGG-CAACUAGACAUACAGUGG
M4 A15U+C16G+C17U	<u>GGAAGCUCUGACCGUGU</u> CCCCGAGUUCGCUCGGGGACAACUAGACAUACAGUGG
M5 A15G+ C16U+C32U+A33G	<u>GGAAGCUCUGACCGGU</u> CCCCCGAGUUCGCUCGGGGGAUGACUAGACAUACAGUGG
M6 G14A+C35U	<u>GGAAGCUCUGACC</u> AACCCCCGAGUUCGCUCGGGGACA <u>AU</u> UAGACAUACAGUGG
M7 C13U+G38A	<u>GGAAGCUCUGACU</u> GACCCCCCGAGUUCGCUCGGGGACAACUA <u>AA</u> CAUACAGUGG
M8 U9C	<u>GGAAGCUC</u> CGACCGACUCCCCGAGUUCGCUCGGGGACAACUAGACAUACAGUGG
M9 U42C	<u>GGAAGCUCUGACCGACCCCCGAGUUCGCUCGGGGACAACUAGACA</u> <u>C</u> ACAGUGG



(Supplementary Figure 1. Continued on next page)



Supplementary Figure 1. Anion exchange HPLC chromatograms to confirm purity of RNA oligonucleotides prepared by solid-phase synthesis. (a) R1, (b) m¹A R1, (c) R1_USem12 (USem = 2'-SeMe-U), (d) R2, (e) R3, (f) R2_C12U, (g) R3_U42C, (h) R3_dU42, (i) R2_dC12, (j) R2_Cm12, (k) R3_Um42, (l) R3_USem42, (m) R2_Cf12, (n) R3_Uf42, (o) R2_m⁵C12, (p) R2_m⁴C12, (q) R3_Am43. Dionex DNAPAc PA200, 2x250 mm, 60°C, monitored at 260 nm.