

## Supporting Information Table 1 | DNA and RNA Sequences

| No          | description  | 5'-sequence-3' (DNA prepared by solid-phase synthesis)  |
|-------------|--|---|
| D1          | T7 promotor  | CTGTAATACGACTCACTATA  |
| D2          | DNA Pool   | GGTAAGGTGGACATACTG-N40-GCCTTCAAGGATGGTAGGCTGG   |
| D3          | forward primer   | CTGTAATACGACTCACTATAGGCATATACTGAGCCTTCAACCAGCCTACCATCC  |
| D4          | Reverse primer 1 <sup>st</sup> & 2 <sup>nd</sup> PCR           | GGTAAGGTGGACATACTG  |
| D5          | Forward primer 1 <sup>st</sup> PCR                             | CTTCAACCAGCCTACCATCC  |
| D6          | cloning f. primer  | TAAATAAAATAACTGTAATACGACTCACTATAGGCATATACTGAGC  |
| D7          | P1 ( <i>R. norveg.</i> tRNA-Lys; 17-34)                        | AACTTAAAAGGTTAACGC  |
| D8          | P2 ( <i>B. subtilis</i> tRNA-Ser; 30-47)                       | CCTACACGATTTCCAATC  |
| D9          | P3 ( <i>T. Thermo.</i> tRNA-Asp; 66-72)                        | TGGCGGCCCCG   |
| D10         | Disruptor 1 ( <i>R. norveg.</i> tRNA-Lys)                      | TGGTCATTGTGGAGATTTGTTGTCTCTA  |
| D11         | Disruptor 2 ( <i>B. subtilis</i> tRNA-Ser)                     | ACTCGGACAGCTCTCC  |
| D12         | P4 ( <i>E. coli.</i> tRNA-Asp GTC)                             | TGGCGGAACGG   |
| D13         | P5 ( <i>E. coli.</i> tRNA-Glu TTC)                             | TGGCGTCCCT  |
| D14         | P6 ( <i>E. coli.</i> tRNA-Gly CCC)                             | TGGAGCGGGCG   |
| D15         | P7 ( <i>E. coli.</i> tRNA-Gly TCC)                             | TGGAGCGGGCA   |
| D16         | P8 ( <i>E. coli.</i> tRNA-Ser GCT, GGA)                        | TGGCGGTGAGG   |
| D17         | P9 ( <i>E. coli.</i> tRNA-His GTG)                             | TGGGGTGGCTA   |
| D18         | P10 (cis-MTR1; 110-128)  | CAAGGATGGTAGGCTGGT  |
| description | 5'-sequence-3' (RNA prepared by solid-phase synthesis)         |   |
| R1          | Parent 5'-Alkyne-RNA(22)                                       | Hexyne-GACAUACUGAGCCUUCAAAUA  |
| R2          | Parent RNA(17)-3'-NH <sub>2</sub>                              | ACAUACUG ( <b>A</b> ) GCCUUCAA-C6-NH <sub>2</sub>   |
| R3          | Inosine 3'-NH <sub>2</sub>                                     | ACAUACUG ( <b>I</b> ) GCCUUCAA-C6-NH <sub>2</sub>   |
| R4          | 2'dA 3'-NH <sub>2</sub>  | ACAUACUG ( <b>dA</b> ) GCCUUCAA-C6-NH <sub>2</sub>  |
| R5          | 2'OMe A-3'-NH <sub>2</sub>                                     | ACAUACUG ( <b>Am</b> ) GCCUUCAA-C6-NH <sub>2</sub>  |
| R6          | Purine 3'-NH <sub>2</sub>                                      | ACAUACUG ( <b>P</b> ) GCCUUCAA-C6-NH <sub>2</sub>   |
| R7          | 2-Aminopurine-3'-NH <sub>2</sub>                               | ACAUACUG ( <b>2AP</b> ) GCCUUCAA-C6-NH <sub>2</sub>   |
| R8          | 7-deazaA-3'-NH <sub>2</sub>                                    | ACAUACUG ( <b>c7A</b> ) GCCUUCAA-C6-NH <sub>2</sub>   |
| R9          | 2'-dA-methylphosphonate-3'-NH <sub>2</sub>                     | ACAUACUG ( <b>dA-PCH<sub>3</sub></b> ) GCCUUCAA-C6-NH <sub>2</sub>                                  |
| R10a        | Parent Short RNA(13) (S*)                                      | AUACUG ( <b>A</b> ) GCCUUC-C6-NH <sub>2</sub>   |
| R10         | Parent Short RNA(13) (S)                                       | AUACUG ( <b>A</b> ) GCCUUC  |
| R11         | 2'dA   | AUACUG ( <b>dA</b> ) GCCUUC   |
| R12         | 2'dA-methylphosphate (dApm)                                    | AUACUG ( <b>dA-POCH<sub>3</sub></b> ) GCCUUC  |
| R13         | 3-deaza-2'dA   | AUACUG ( <b>c3dA</b> ) GCCUUC   |
| R14         | m <sup>6</sup> A   | AUACUG ( <b>m6A</b> ) GCCUUC  |
| R15         | m <sup>1</sup> A   | AUACUG ( <b>m1A</b> ) GCCUUC  |
| R16         | unmodified 16nt RNA (GAC)                                      | GGAAUUAACGACUCAC  |
| R17         | 1,3-dideaza-A (c <sup>1</sup> c <sup>3</sup> A) var. of R16    | GGAAUUAUCG ( <b>c<sup>1</sup>c<sup>3</sup>A</b> ) CUCAC   |
| R18         | <i>R. norvegicus</i> fragment A9                               | UUGCGAAGCUUAG   |
| R19         | <i>T. thermophil.</i> fragment A58                             | GGUUCGAGUCCCG   |
| R20         | <i>B. subtilis</i> fragment A22                                | GGUCGAAGGAGCA   |
| description | 5'-sequence-3' (RNA prepared by <i>in vitro</i> transcription) |   |
| Tr1         | Selection Library  | GGACAUACUGAGCCUUCACACAGCCUACCAUCCUUGAAGGC-N40-CAGUAUGUCCACCUUACC                                    |
| Tr2         | Parent RNA (19)  | GGACAUACUGAGCCUUCAA   |
| Tr3         | A11G   | GGACAUACUGGGCCUUCAA   |
| Tr4         | A11C   | GGACAUACUGCGCCUUCAA   |
| Tr5         | TM   | GGGUGCGUCGAGUUCUGG  |
| Tr6         | TV1  | GGUGUAUGAGAGGAAGUU  |
| Tr7         | TV2  | GGCACGCAGGAGAAGACC  |
| Tr8         | AAG  | GGACAUACUAGCCUUCAA  |
| Tr9         | CAG  | GGACAUACUCAGCCUUCAA   |
| Tr10        | UAG  | GGACAUACUUAGCCUUCAA   |
| Tr11        | GAA  | GGACAUACUGAACCUCUCAA  |
| Tr12        | GAC  | GGACAUACUGACCCUUCAA   |
| Tr13        | GAU  | GGACAUACUGAUCCUUCAA   |
| Tr14        | <i>R. norvegicus</i> tRNA-Lys A9                               | GGCAUUGCGAAGCCUUAAGCGGUUAACCUUUUAAGUUAAAGUUAGAGACAACAAUCUCCACAAUGA<br>CCA (69nt)                    |
| Tr15        | <i>B. subtilis</i> tRNA-Ser A22                                | GGAGAGCUGUCCGAGUGGUCGAAGGAGCACGAUUGGAAAUCGUGUAGGCGGUCACUCCGUCUCA<br>GGUUCGAAUCCUUGCUCUCCGCCA (92nt) |
| Tr16        | <i>T. thermophilus</i> RNA-Asp A58                             | GGCCCCGGUGUAGUUGGUUAACACACCCGCCUGUCACGUGGGAGAUCCGGGUUCGAGUCCCGU<br>CGGGGCCGCCA (77nt)               |

Supporting Information Table 1 continued

|                              |   | Ribozymes (prepared by <i>in vitro</i> transcription, binding arms underlined)   |
|------------------------------|---|--|
| Rz1                          | CA13  | <u>GGUUGAAGGCUGACCGACCC</u> CCCCGACCCUUCUCUGGGACAACUAGACAUA <u>CAGUAUGUCC</u>  |
| Rz1s                         | CA13 short binding arms   | GGAGGCUGACCGACCCCGACCCUUCUCUGGGACAACUAGACAUA <u>CAGU</u>   |
| Rz1a                         | CA13 sh binding arms for R16/17   | <u>GGUGAGUGACCGACCCCGACCCUUCUCUGGGACAACUAGACAUA</u> <u>CGUAUUUCC</u>   |
| Rz2                          | CA21  | <u>GGUUGAAGGCUGACCGACCC</u> CGUACAAUACAACAGCGACAACUAGACAUA <u>CAGU</u>   |
| Rz2s                         | CA21 short binding arms   | <u>GGAAGGCUGACCGACCCCGUACAAUACAACAGCGACAACUAGACAUA</u> <u>CAGU</u>   |
| Rz3                          | MTR1  | <u>GGUUGAAGGCUGACCGACCC</u> CGAGUUCGUCGCGGGACAACUAGACAUA <u>CAGUAUGUCC</u>   |
| Rz3s                         | MTR1 short binding arms   | GGAGGCUGACCGACCCCGAGUUCGUCGCGGGACAACUAGACAUA <u>CAGU</u>   |
| Rz4                          | CA13 U24G   | <u>GGAGGCUGACCGACCCCGACCCUUCUC</u> GGGACAACUAGACAUA <u>CAGU</u>  |
| Rz5                          | Rz4 C10G27→G10C27   | <u>GGAGGCUGACCGACCC</u> GCCCGACCCUUCUCGGGCACAACUAGACAUA <u>CAGU</u>  |
| Rz6                          | Rz4 C11G26→G11C26   | <u>GGAGGCUGACCGACCC</u> GCCCGACCCUUCUCGGCACAACUAGACAUA <u>CAGU</u>   |
| Rz7                          | Rz4 C13G24→G13C24   | <u>GGAGGCUGACCGACCC</u> CGACCCUUCUCGGGACAACUAGACAUA <u>CAGU</u>  |
| Rz8                          | Rz3 5bp   | <u>GGAGGCUGACCGACCC</u> CGUUCGCGGGACAACUAGACAUA <u>CAGU</u>  |
| Rz9                          | Rz2 Δstem   | <u>GGUUGAAGGCUGACCGACCC</u> GACAACUAGAAAUA <u>CAGUAUGUCC</u>   |
| Rz10                         | 4bp stem tetraloop  | <u>GGUUGAAGGCUGACCGACCC</u> GUAAACCAGCGACAACUAGAAAUA <u>CAGUAUGUCC</u>   |
| Rz11                         | 4bp stem hexaloop   | <u>GGUUGAAGGCUGACCGACCC</u> CGUACAAUACAGCGACAACUAGAAAUA <u>CAGUAUGUCC</u>  |
| Rz12                         | CA13 TM   | <u>GGAAUCUCCUGACCGACCC</u> CGACCCUUCUCUGGGACAACUAGACAUA <u>CUCUAACACC</u>  |
| Rz13                         | CA13 TV1  | <u>GGGGUCCUUCUGACCGACCC</u> CGACCCUUCUCUGGGACAACUAGACAUA <u>ACCUGCGUGCC</u>  |
| Rz14                         | CA13 TV2  | <u>GGUUGAAGGCUGACCGACCC</u> CGUACAAUACAACAGCGACAACUAGAAAUA <u>CAGUAUGUCC</u>   |
| Rz15                         | CA13 AAG  | <u>GGUUGAAGGCUGACCGACCC</u> CGACCCUUCUCUGGGACAACUAGACAUA <u>UAGUAUGUCC</u>   |
| Rz16                         | CA13 CAG  | <u>GGUUGAAGGCUGACCGACCC</u> CGACCCUUCUCUGGGACAACUAGACAUA <u>GAGUAUGUCC</u>   |
| Rz17                         | CA13 UAG  | <u>GGUUGAAGGCUGACCGACCC</u> CGACCCUUCUCUGGGACAACUAGACAUA <u>AAAGUAUGUCC</u>  |
| Rz18                         | CA13 GAA  | <u>GGUUGAAGGUUGACCGACCC</u> CGACCCUUCUCUGGGACAACUAGACAUA <u>CAGUAUGUCC</u>   |
| Rz19                         | CA13 GAC  | <u>GGUUGAAGGGUGACCGACCC</u> CGACCCUUCUCUGGGACAACUAGACAUA <u>CAGUAUGUCC</u>   |
| Rz20                         | CA13 GAU  | <u>GGUUGAAGGAUGACCGACCC</u> CGACCCUUCUCUGGGACAACUAGACAUA <u>CAGUAUGUCC</u>   |
| Rz21                         | MTR1_ <i>R.norvegicus</i> tRNA <sup>Lys</sup> A9  | <u>GGCUAAGCUGACCGACCC</u> CGAGUUCGUCGCGGGACAACUAGACAUA <u>UCGCAA</u>   |
| Rz22                         | MTR1_ <i>B.subtilis</i> tRNA <sup>Ser</sup> A22   | <u>GGUGCUCCUGACCGACCC</u> CGAGUUCGUCGCGGGACAACUAGACAUA <u>UCGACC</u>   |
| Rz23                         | MTR1_ <i>T.thermophilus</i> tRNA <sup>Asp</sup> A58 = MTR1_ <i>E.coli</i> tRNA <sup>Asp</sup> A58 | <u>GGACGGGACUGACCGACCC</u> CGAGUUCGUCGCGGGACAACUAGACAUA <u>CGAAGCC</u>   |
| F30-Broccoli MTR1 constructs |   |  |
| C1                           | F30-Broccoli-MTR1cis (Broccoli green, substrate red, MTR1 blue, binding arms underlined)          | <u>GGGUUGCCAUGUGUAUGUGGGAGACGGUCGGGUCCAGAUUUUGUAUCUGUCGAGUAGAGUGUGGG</u><br><u>CUCCCAUAUACUCUGAUGAUCCUCGAGGACAUACUGAGCCUUC</u> <u>CAACCAGCCUACCAUCCUUGAAGG</u><br><u>CUGACCGACCCCGAGUUCGUCGCGGGACAACUAGACAUA</u> <u>CAGUAUGUCC</u> <u>AUGGAUCAUUAUGG</u><br><u>CAAGC</u> |
| C2                           | F30-Broccoli-MTR1trans tRNA <sup>Asp</sup> (Broccoli green, MTR1 blue, binding arms underlined)   | <u>GGGUUGCCAUGUGUAUGUGGGAGACGGUCGGGUCCAGAUUUUGUAUCUGUCGAGUAGAGUGUGGG</u><br><u>CUCCCAUAUACUCUGAUGAUCCUCGAGCCACCAACGGUACCGACGGGACUGACCGACCCCGAGU</u><br><u>UCGUCGCGGGACAACUAGACAUA</u> <u>CGAACCCUAACCAUGGAUCAUUAUGGCAAGC</u>   |

## Supporting Information Table 2 | ESI-MS of synthetic RNAs and methylation products

| No                   | description  | Length (nt) | Mass calculated | Mass found |
|----------------------|--|-------------|-----------------|------------|
| R1                   | Parent 5'-Alkyne-RNA(22) [SL = S long]                     | 22          | 6821.09 Da      | 6821.03 Da |
| R2                   | Parent RNA(17)-3'-NH <sub>2</sub>                          | 17          | 5530.83 Da      | 5530.86 Da |
| R3                   | Inosine 3'-NH <sub>2</sub>                                 | 17          | 5531.82 Da      | 5531.87 Da |
| R4                   | 2'dA 3'-NH <sub>2</sub>                                    | 17          | 5514.84 Da      | 5514.86 Da |
| R5                   | 2'OMe A-3'-NH <sub>2</sub>                                 | 17          | 5544.85 Da      | 5544.87 Da |
| R6                   | Purine 3'-NH <sub>2</sub>                                  | 17          | 5515.82 Da      | 5515.85 Da |
| R7                   | 2-Aminopurine-3'-NH <sub>2</sub>                           | 17          | 5530.83 Da      | 5530.88 Da |
| R8                   | 7-deazaA-3'-NH <sub>2</sub>                                | 17          | 5529.84 Da      | 5529.88 Da |
| R9                   | 2'-dA-methylphosphonate-3'-NH <sub>2</sub>                 | 17          | 5512.96Da       | 5512.90 Da |
| R10a                 | Parent Short RNA(13) (S*)                                  | 13          | 4238.63 Da      | 4238.69 Da |
| R10                  | Parent Short RNA(13) (S)                                   | 13          | 4059.56 Da      | 4059.62 Da |
| R11                  | 2'dA   | 13          | 4043.57 Da      | 4043.62 Da |
| R12                  | 2'dA-methylphosphate (dApm)                                | 13          | 4057.58 Da      | 4057.63 Da |
| R13                  | 3-deaza-2'dA   | 13          | 4042.57 Da      | 4042.62 Da |
| R14                  | m <sup>6</sup> A   | 13          | 4073.58 Da      | 4073.62 Da |
| R15                  | m <sup>1</sup> A   | 13          | 4073.58 Da      | 4073.63 Da |
| R16                  | unmodified 16nt RNA (GAC)                                  | 16          | 5085.83 Da      | 5085.75 Da |
| R17                  | 1,3-dideaza-A (c <sup>1</sup> c <sup>3</sup> A) var.of R16 | 16          | 5083.75 Da      | 5083.77 Da |
| R18                  | <i>R.norvegicus</i> fragment A9                            | 13          | 4139.58 da      | 4139.61 Da |
| R19                  | <i>T.thermophil.</i> fragment A58                          | 13          | 4130.57 Da      | 4130.60 Da |
| R20                  | <i>B.subtilis</i> fragment A22                             | 13          | 4240.65 Da      | 4240.68 Da |
| R1-Bn-Biotin         | isolated biotinylated RNA by Rz1 with SNAP-biotin          |             | 7279.33 Da      | 7279.26 Da |
| R9-BnNH <sub>2</sub> | isolated alkylated RNA by Rz1 with BG-NH <sub>2</sub>      |             | 5631.93 Da      | 5631.99 Da |
| R10a-Me              | isolated methylation product by MTR1 (Fig 2b)              |             | 4252.65 Da      | 4252.70 Da |
| R18-Me               | isolated <i>R.norv.</i> RNA after methylation by Rz21      |             | 4153.59 Da      | 4153.58 Da |
| R19-Me               | isolated <i>T.therm.</i> RNA after methylation by Rz23     |             | 4144.59 Da      | 4144.56 Da |
| R20-Me               | isolated <i>B.subtilis.</i> RNA after methylation by Rz22  |             | 4254.66 Da      | 4254.65 Da |

## Supporting Information Table 3 | Ribozyme sequences & cis/trans activity

| No          | # clones | 5'-binding arm – core (40) – binding arm-3'                         | cis      | trans    |
|-------------|----------|---|----------|----------|
| CA7         | 2        | CUGAAGGC GCCCGAGAAACAAUGAUGCCUUGUUAGAUAACAGUAGUG CAGUAUGUCC         | -        |          |
| <b>CA9</b>  | <b>2</b> | <b>UUGAAGGC GCCCGAGAAACAAUGAUGCCUUGUUAGAUAACAGUAGUG CAGUAUGUCC</b>  | <b>+</b> | <b>-</b> |
| CA28        | 1        | UUGAAGGC GCCCGAGAAACAAUGAUGCCUUGUUAGAUAACAGGAGUG CAGUAUGUCC         | -        |          |
| <b>CA13</b> | <b>1</b> | <b>UUGAAGGC UGACCGACCCCCGACCCUUCUCUGGGACAACUAGACAU A CAGUAUGUCC</b> | <b>+</b> | <b>+</b> |
| CA23        | 1        | UUGAAGGC UGACCGACCCCCGACCAUUCUCUGGGACAACUAGACAU A CAGUAUGUCC        | +        |          |
| CA38        | 1        | UUGAGGGC UGACCGACCCCCGACCAUUCUCUGGGACAACUAGACAU A CAGUAUGUCC        | +        |          |
| <b>CA21</b> | <b>1</b> | <b>UUGAAGGC UGACCGACCCGCUACAAUACAACAGCGACAACUAGAAAUA CAGUAUGUCC</b> | <b>+</b> | <b>+</b> |