Supporting Information

Deoxyribozyme-based method for absolute quantification of N^6-methyladenosine fractions at specific sites of RNA

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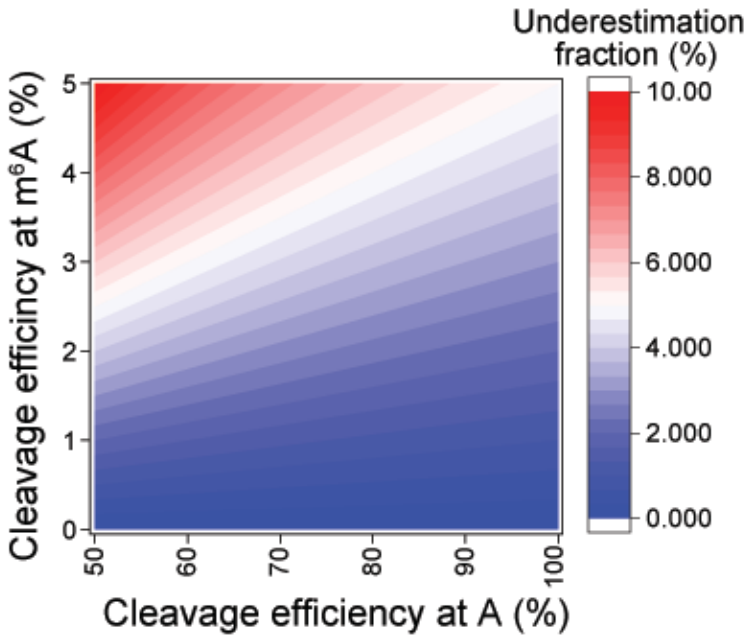

FIGURE S1. Percentage of underestimation of m^6A fraction due to cleavage of DR on m^6A sequence. The trace amount of cleavage of DR at m^6A containing sequence will cause false negative signal in the cleavage reaction and underestimation of the m^6A percentage. Considering DR cleavage efficiencies of the unmethylated A and m^6A sequence are F_{DR} and F'_{DR} respectively, and the true m^6A fraction is F'_m,

$$(1 - F'_m)F_{DR} + F_m{}'F'_{DR} = 1 - 2^{-\Delta\Delta Ct},$$

where $\Delta\Delta Ct$ is determined in Eq (2). Comparing F'_m to F_m from Eq (7) determined without the correction of F'_{DR}, the percentage of underestimation is

$$\frac{F'_m - F_m}{F'_m} = \frac{F'_{DR}}{F_{DR}}.$$

The heat map shows the percentage of underestimation as a function of F_{DR} and F'_{DR}, which increases when F_{DR} decreases, assuming F'_{DR} remains lower than 5%. When F_{DR} is 50% and F'_{DR} is 5%, the error in m6A fraction is 10%.

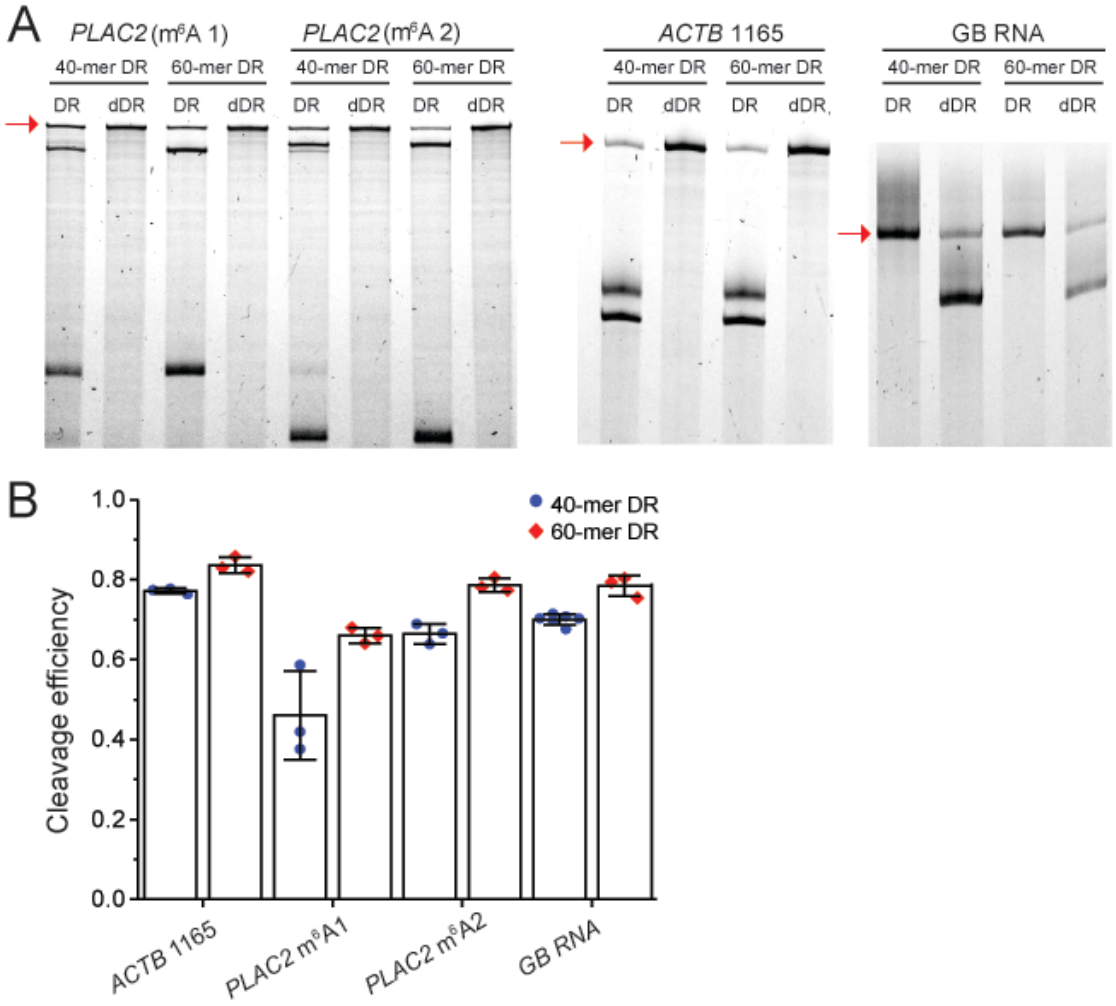

FIGURE S2. Cleavage efficiencies (F_{DR}) of unmodified *in vitro* transcribed RNA by 40-mer and 60-mer DR and dDR. (*A*) PAGE showing the DR cleavage of *PLAC2* m^{6}A1 and m^{6}A2 sites, *ACTB* 1165, and GB RNA by 40-mer and 60-mer DR. (*B*) Bar plot of the cleavage efficiencies of *PLAC2* m^{6}A1 and m^{6}A2, *ACTB* 1165, and the GB RNA by 40-mer and 60-mer DR as quantified from (*A*). Error bars indicate mean ± s.d. for 3 independent DR cleavage reactions. Red arrows point to full-length uncleaved RNA fragments. All gel splice sites are separate by white space.

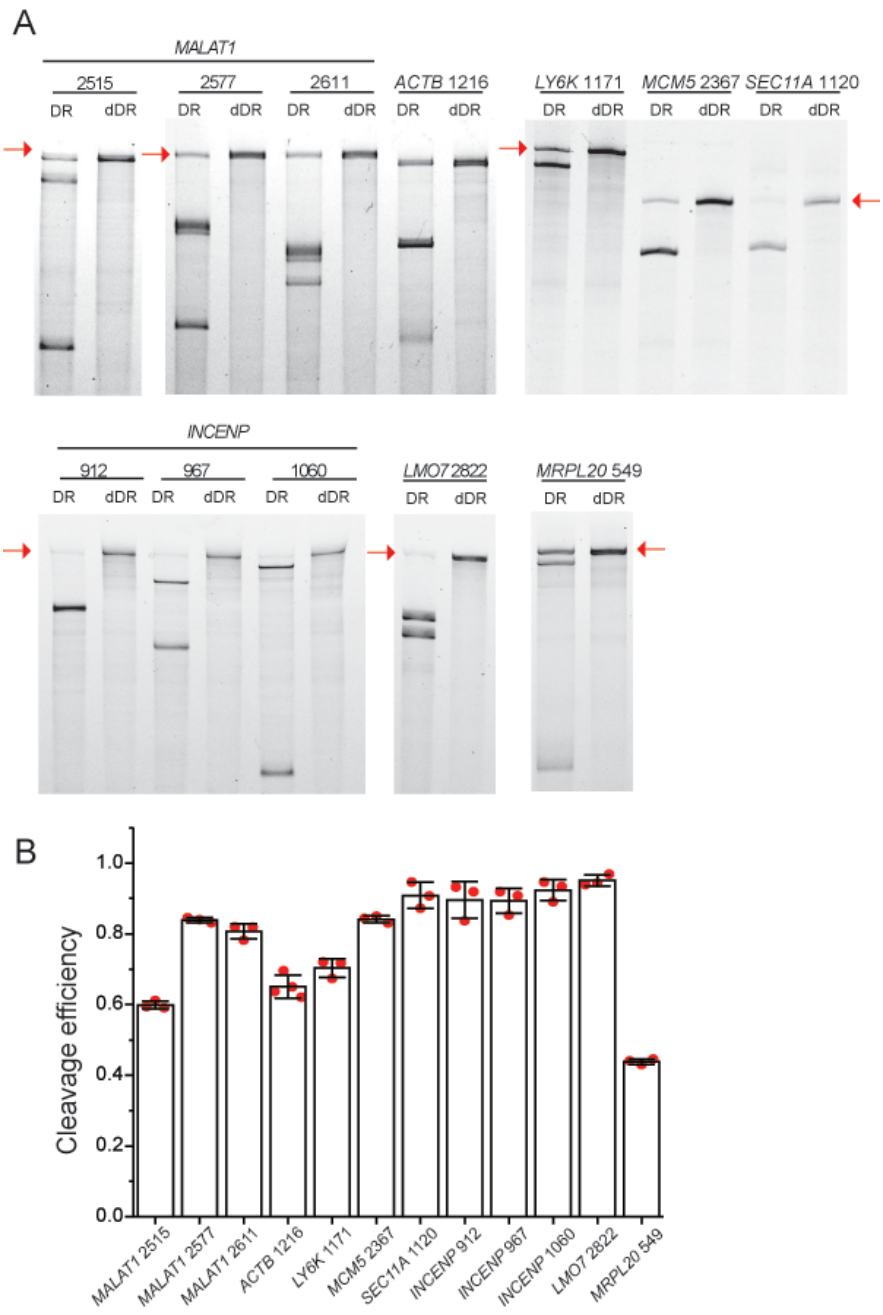

FIGURE S3. Cleavage efficiencies (F_{DR}) of unmodified *in vitro* transcribed RNA by 60-mer DR and dDR. (*A*) PAGE showing the DR cleavage of the seven endogenous targets: *MALAT1* 2515, 2577, and 2611, *ACTB* 1216, *LY6K* 1171, *MCM5* 2367, *SEC11A* 1120, *INCENP* 912, 967 and 1060, *LMO7* 2822, and *MRPL20* 549. (*B*) Bar plot of the cleavage efficiencies of endogenous targets as quantified from (*A*). Error bars indicate mean ± s.d. for 3 DR cleavage reactions. Red arrows point to full-length uncleaved RNA fragments. All gel splice sites are separate by white space.

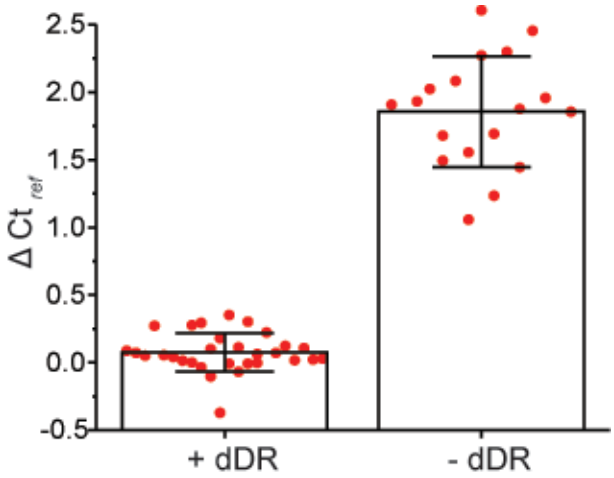

FIGURE S4. Negative control without DR and with non-functional version of DR (dDR). When the negative control does not contain DR, there is a consistent difference between $Ct_{+DR-ref}$ and $Ct_{-DR-ref}$ ($\Delta\ Ct_{ref}$), with $Ct_{-DR-ref}$ being larger. Use of dDR in the negative control eliminates $\Delta\ Ct_{ref}$. Data comes from quantification of GB RNA with 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 m^6A fraction input. Error bars indicate mean ± s.d.

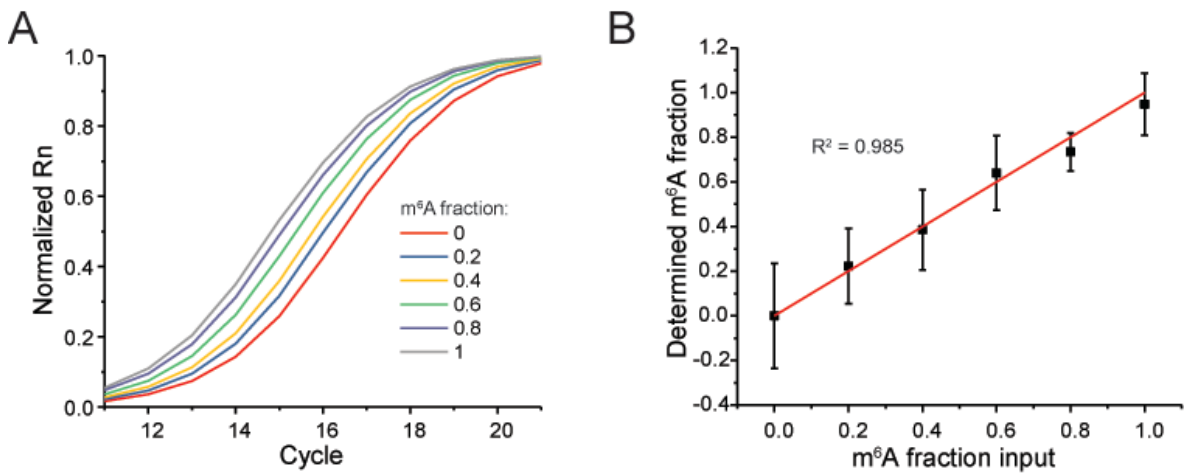

FIGURE S5. Validation of the method for absolute quantification of m^6A fraction of the GB RNA without dDR. (*A*) Normalized real-time fluorescence amplification curves for the DR cleaved synthetic RNAs with primers amplifying the m^6A site. (*B*) Estimated modification fraction as a function of input m^6A fraction for the GB RNA. Error bars indicate mean ± s.d. for 3 biological replicates.

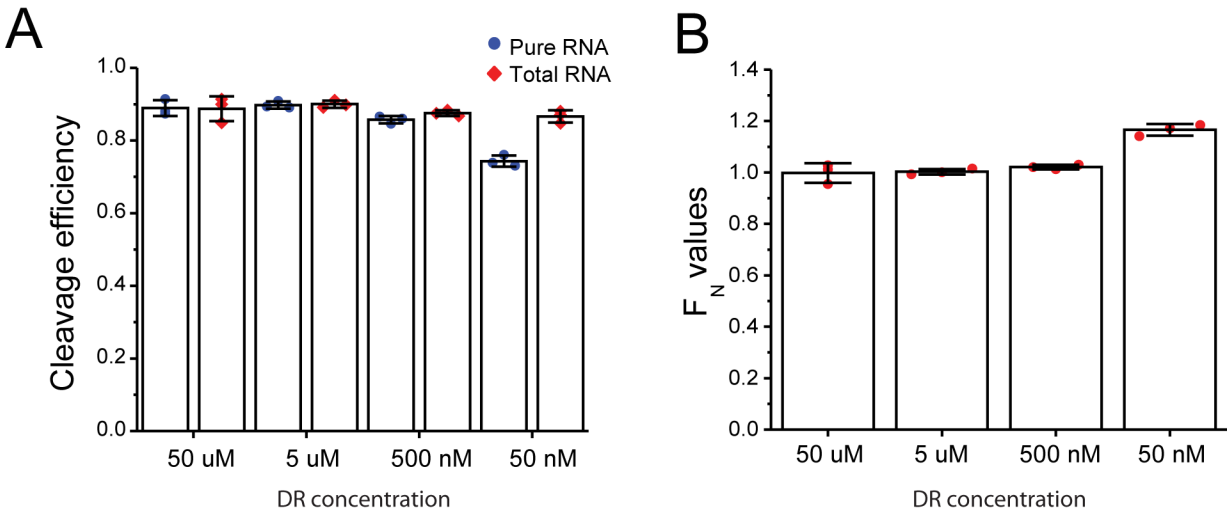

FIGURE S6. The dependence of cleavage efficiency on the concentration of DR. (A) The cleavage efficiencies of *PLAC2* m^{6}A 2 DR in presence and absence of total RNA with varying concentrations of DR. The concentration of the pure RNA was fixed at 50 nM, whereas the concentration of spike-in RNA in to the total RNA was fixed at 1 ng *PLAC2* RNA in 500 ng total RNA to better mimic the abundance of endogenous RNA. (B) F_N correction values for varying concentrations of *PLAC2* m^{6}A DR as determined from cleavage efficiencies in (A). All error bars report mean ± s.d. for 3 biological replicates. The cleavage efficiency of pure RNA is lower when using 50nM of DR, because the reaction was performed in the presence of 50 nM pure RNA, suggesting that the DR needs to be in over molar excess of the target RNA to ensure efficiency hybridization. The decrease in the cleavage efficiency for pure RNA results in a slightly larger than 1 F_N value at 50 nM DR concentration.

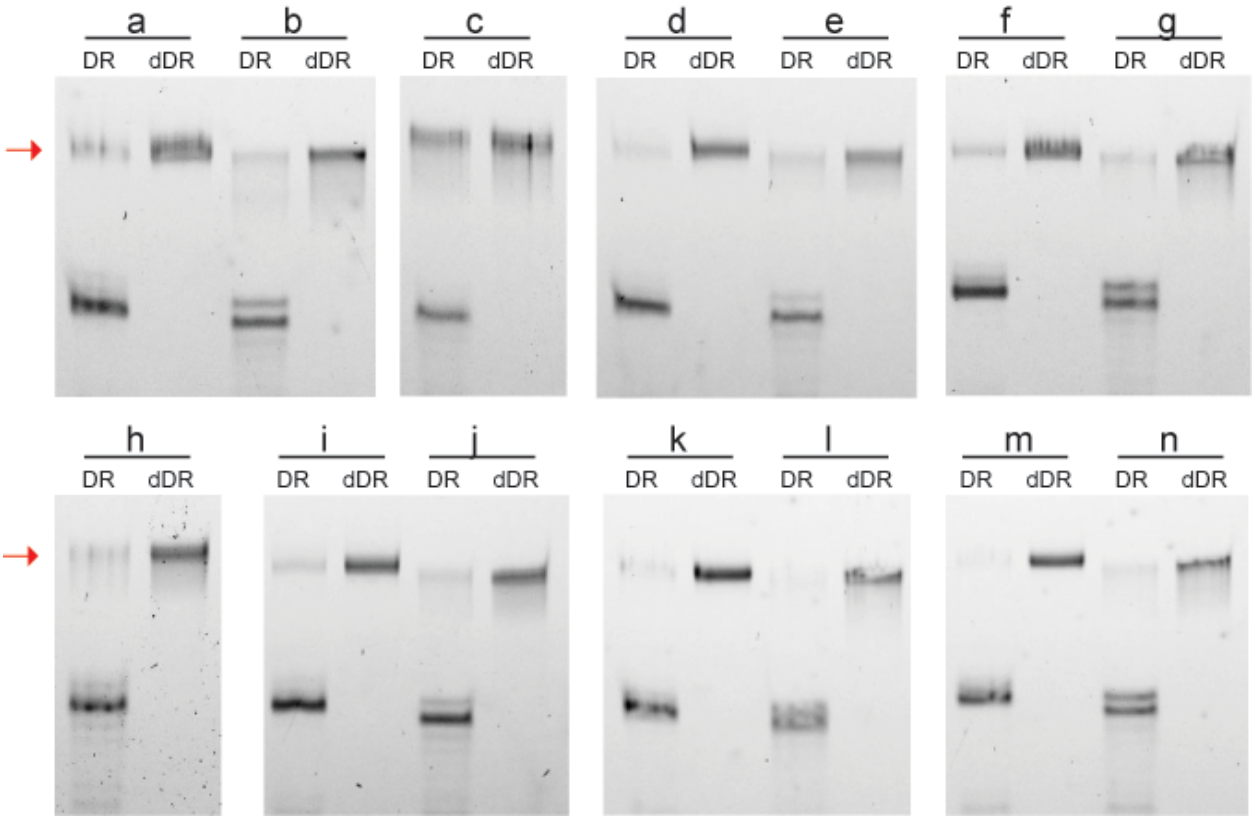

FIGURE S7. PAGE showing the DR cleavage efficiency in absence and presence of nearby m^6A, m^1A, and ψ modifications. Labels a-n correspond to 35-nt synthetic RNAs shown in Figure 6A and listed in Supplemental Table S1. Red arrows point to full-length uncleaved RNA. All gel splice sites are separate by white space.

Table S1: Synthetic DNA and RNA sequences.

Description	Sequence
GB DNA template	GGTTGCGTTGGGTGTTCCTGTTTCTTTTGGCCTTTGTCTCTGTTTCT TTCCTTTCTCCTCCTTGTCGTGTCTGTTCGTTTCGTCGCTTTCCTCC TTCCTTGTTCTCGCTCGGACTCTTCTGGGCTCTTTCTGCGTTCGCCC TTCTTGTTCTCCCTTCTCTGTGGGTCCTGTTCTTGTGCTGGTTGTGC TCCCTCCTCTTGGTGCTCCTCCTTTCTGTGGCTGCGCTGGTGTTTCT TTCTCTCGGCTGCTCTGTTTGTTGGGTCTTTGTTGTGTGTTGTTGTT CTTGTGTGCTGCGTTTTGGTGGTGTCGGTTCTGTGCTGTCTTTCGGC CTGTCGTTTTCCTTCTCGTGTTCCGTCCTGTTTTGCGTGTCTCTCCCT GTGTTCCCGCTTTCCGTGTTGGCTGTGCTTGGTGTCTTTCGCTTGTTG GTTGGTCTCCTGTCTCCTGTGCTCGTCGGTCTTGTGG
41-nt synthetic *ACTB* 1216	CGCAAAUGCUUCUAGGCGGACUAUGACUUAGUUGCGUUACU
41-nt synthetic *MALAT1* 2515	AGUUUGAAAAAUGUGAAGGACUUUCGUAACGGAAGUAAUUU
32-nt synthetic *MALAT1* 2577	AACUUAAUGUUUUUGCAUUGGACUUUGAGUUA
(a) 35-nt synthetic A 2-nt A control 1	GCCUUGUUCUCGCUCGGACUAUUCUGGGCUCUUUC
(b) 35-nt synthetic A 2-nt m^6A	GCCUUGUUCUCGCUCGGACUm^6AUUCUGGGCUCUUUC
(c) 35-nt synthetic A 2-nt m^1A	GCCUUGUUCUCGCUCGGACUm^1AUUCUGGGCUCUUUC
(d) 35-nt synthetic A 2-nt U control	GCCUUGUUCUCGCUCGGACUUUUCUGGGCUCUUUC
(e) 35-nt synthetic A 2-nt ψ	GCCUUGUUCUCGCUCGGACUψUUCUGGGCUCUUUC
(f) 35-nt synthetic A 4-nt A control 1	GCCUUGUUCUCGCUCGGACUCUACUGGGCUCUUUC
(g) 35-nt synthetic A4-nt m^6A	GCCUUGUUCUCGCUCGGACUCUm^6ACUGGGCUCUUUC
(h) 35-nt synthetic A 4-nt m^1A	GCCUUGUUCUCGCUCGGACUCUm^1ACUGGGCUCUUUC
(i) 35-nt synthetic A 4-nt U control	GCCUUGUUCUCGCUCGGACUCUUCUGGGCUCUUUC
(j) 35-nt synthetic A 4-nt ψ	GCCUUGUUCUCGCUCGGACUCUψCUGGGCUCUUUC
(k) 35-nt synthetic A 2-nt A control 2	GCCUUGUUCUCGCUAGGACUCUUCUGGGCUCUUUC
(l) 35-nt synthetic m^6A 2-nt A	GCCUUGUUCUCGCUm^6AGGACUCUUCUGGGCUCUUUC
(m) 35-nt synthetic A 4-nt A control 2	GCCUUGUUCUCGAUCGGACUCUUCUGGGCUCUUUC
(n) 35-nt synthetic m^6A 4-nt A	GCCUUGUUCUCGm^6AUCGGACUCUUCUGGGCUCUUUC

Table S2: Primers used for generating templates for *in vitro* transcription.

Description	Sequence
Forward primer GB RNA DNA template	TAATACGACTCACTATAGGGTTGCGTTGGGTGTTCCTG
Reverse primer for GB RNA DNA template	CCACAAGACCGACGAGCACA
Forward primer for *ACTB* DNA template	TAATACGACTCACTATAGGCCAACACAGTGCTGTCTGGC
Reverse primer for *ACTB* DNA template	CTGCTGTCACCTTCACCGTTCC
Forward primer for *PLAC2* DNA template	TAATACGACTCACTATAGCAAGCAAAGTGAACACGTCG
Reverse primer for *PLAC2* DNA template	GTACTGACGTCGGCATCGAT
Forward primer for *MALAT1* DNA template	TAATACGACTCACTATAGGCTACTAAAAGGACTGGTGT
Reverse primer for *MALAT1* DNA template	TTCACCACCAAATCGTTAGC
Forward primer for *LY6K* DNA template	TAATACGACTCACTATAGGCAGGCCATACCACGCAGAAG
Reverse primer for *LY6K* DNA template	CCAAGACCCTGGGAAGTCAAA
Forward primer for *MCM5* DNA template	TAATACGACTCACTATAGGGAGATGCTGAGCCGCATC
Reverse primer for *MCM5* DNA template	CAGCAGGACACTACAGCTCC
Forward primer for *SEC11A* DNA template	TAATACGACTCACTATAGGGTCTGTGATTGGTGGAATGG
Reverse primer for *SEC11A* DNA template	AAGACTTACGACCACCTCAG
Forward primer for *INCENP* DNA template	TAATACGACTCACTATAGATAACCACACCCAGTGCCAG
Reverse primer for *INCENP* DNA template	TGCGGACAACACTTTCCTGT
Forward primer for *LMO7* DNA template	TAATACGACTCACTATAGGAAATGCTGCAGGACAGGGA
Reverse primer for *LMO7* DNA template	TGAGAGCCAAAGGGTCTTGG
Forward primer for *MRPL20* DNA template	TAATACGACTCACTATAGGCCGCTACTTTCGGATCCAGG
Reverse primer for *MRPL20* DNA template	GGCCATCCCTCATGTCTGTT

Table S3: Deoxyribozyme sequences.

Description	Sequence
GB RNA DR 40-mer	CCCAGAAGAGGGGTCTCCAGCTGGACGTTCGAGCGAGAAC
GB RNA dDR 40-mer	CCCAGAAGAGGGGTCTCCTCGTGGATTTCCGAGCGAGAAC
GB RNA DR 60-mer	GCAGAAAGAGCCCAGAAGAGGGGTCTCCAGCTGGACGTT CGAGCGAGAACAAGGAAGGAG
GB RNA dDR 60-mer	GCAGAAAGAGCCCAGAAGAGGGGTCTCCTCGTGGATTTCC GAGCGAGAACAAGGAAGGAG
PLAC2 m^{6}A 1 DR 40-mer	CCTCTGAGTGGGGTCTCCAGCTGGACGTTACTCCTGCCCC
PLAC2 m^{6}A 2 DR 40-mer	TGGGAAAATGGGGTCTCCAGCTGGACGTTCTGGGCAAGAG
PLAC2 m^{6}A 1 dDR 40-mer	CCTCTGAGTGGGGTCTCCTCGTGGATTTCACTCCTGCCCC
PLAC2 m^{6}A 2 dDR 40-mer	TGGGAAAATGGGGTCTCCTCGTGGATTTCCTGGGCAAGAG
PLAC2 m^{6}A 1 DR 60-mer	AGCGGAAGTGCCTCTGAGTGGGGTCTCCAGCTGGACGTTA CTCCTGCCCCTTCTGTGCTT
PLAC2 m^{6}A 2 DR 60-mer	AAGGTGTGGCTGGGAAAATGGGGTCTCCAGCTGGACGTTC TGGGCAAGAGCGGAAGTGCC
PLAC2 m^{6}A 1 dDR 60-mer	AGCGGAAGTGCCTCTGAGTGGGGTCTCCTCGTGGATTTCA CTCCTGCCCCTTCTGTGCTT
PLAC2 m^{6}A 2 dDR 60-mer	AAGGTGTGGCTGGGAAAATGGGGTCTCCTCGTGGATTTCC TGGGCAAGAGCGGAAGTGCC
ACTB 1165 DR 40-mer	CTCGTCATACGGGTCTCCAGCTGGACGTTCTGCTTGCTGA
ACTB 1165 dDR 40-mer	CTCGTCATACGGGTCTCCTCGTGGATTTCCTGCTTGCTGA
ACTB 1165 DR 60-mer	AGGGGCCGGACTCGTCATACGGGTCTCCAGCTGGACGTTC TGCTTGCTGATCCACATCTG
ACTB 1165 dDR 60-mer	AGGGGCCGGACTCGTCATACGGGTCTCCTCGTGGATTTCCT GCTTGCTGATCCACATCTG
ACTB 1216 DR 60-mer	GTAACGCAACTAAGTCATAGGGGTCTCCAGCTGGACGTTC GCCTAGAAGCATTTGCGGTG
ACTB 1216 dDR 60-mer	GTAACGCAACTAAGTCATAGGGGTCTCCTCGTGGATTTCC GCCTAGAAGCATTTGCGGTG
MALAT1 2515 DR 60-mer	AATTACTTCCGTTACGAAAGGGGTCTCCAGCTGGACGTTCT TCACATTTTTCAAACTAAG
MALAT1 2515 dDR 60-mer	AATTACTTCCGTTACGAAAGGGGTCTCCTCGTGGATTTCCT TCACATTTTTCAAACTAAG
MALAT1 2577 DR 60-mer	AAAATAATCTTAACTCAAAGGGGTCTCCAGCTGGACGTTC AATGCAAAAACATTAAGTTG
MALAT1 2577 dDR 60-mer	AAAATAATCTTAACTCAAAGGGGTCTCCTCGTGGATTTCC AATGCAAAAACATTAAGTTG
MALAT1 2611 DR 60-mer	CAGCTGTCAATTAATGCTAGGGGTCTCCAGCTGGACGTTCT CAGGATTTAAAAAATAATC
MALAT1 2611 dDR 60-mer	CAGCTGTCAATTAATGCTAGGGGTCTCCTCGTGGATTTCCT CAGGATTTAAAAAATAATC
LY6K DR 60-mer	GAAGGCTCAGTCTGTGGCAGGGGTCTCCAGCTGGACGTTC CGTGGCTCAAGACAGGCTGA
LY6K dDR 60-mer	GAAGGCTCAGTCTGTGGCAGGGGTCTCCTCGTGGATTTCC CGTGGCTCAAGACAGGCTGA

MCM5 DR 60-mer	CAGAGGTCCCAGCAACATTGGGGTCTCCAGCTGGACGTTA ATGGCAGGCAGCGGCAGGAG
MCM5 dDR 60-mer	CAGAGGTCCCAGCAACATTGGGGTCTCCTCGTGGATTTCA ATGGCAGGCAGCGGCAGGAG
SEC11A DR 60-mer	GCTGCATTTTCATTTACAAGGGGTCTCCAGCTGGACGTTTC TGTAGGCACTTTAGAAGTG
SEC11A dDR 60-mer	GCTGCATTTTCATTTACAAGGGGTCTCCTCGTGGATTTCTC TGTAGGCACTTTAGAAGTG
INCENP 912 DR 60-mer	CTTAGACGCAGACCGCCCCGGGGTCTCCAGCTGGACGTTC CGACCCCTTGACCCTTGGGG
INCENP 912 dDR 60-mer	CTTAGACGCAGACCGCCCCGGGGTCTCCTCGTGGATTTCCC GACCCCTTGACCCTTGGGG
INCENP 967 DR 60-mer	AATCTGGAAAGGCTGGCGAGGGGTCTCCAGCTGGACGTTC CGTGGGCCAGGGGAGACCTG
INCENP 967 dDR 60-mer	AATCTGGAAAGGCTGGCGAGGGGTCTCCTCGTGGATTTCC CGTGGGCCAGGGGAGACCTG
INCENP 1060 DR 60-mer	TGTGCCGCACCGATTGAGAGGGGTCTCCAGCTGGACGTTC GTGCGAGAGCCCGTGGGCGT
INCENP 1060 dDR 60-mer	TGTGCCGCACCGATTGAGAGGGGTCTCCTCGTGGATTTCC GTGCGAGAGCCCGTGGGCGT
LMO7 DR 60-mer	GAATTTCAGTTGTTACACGGGGGTCTCCAGCTGGACGTTCT CTCTTTTTGCAAAAGTGGT
LMO7 dDR 60-mer	GAATTTCAGTTGTTACACGGGGGTCTCCTCGTGGATTTCCT CTCTTTTTGCAAAAGTGGT
MRPL20 DR 60-mer	CCTAATCAATACAGCAACAGGGGTCTCCAGCTGGACGTTC TCAGTGGTACTGCACCACTC
MRPL20 dDR 60-mer	CCTAATCAATACAGCAACAGGGGTCTCCTCGTGGATTTCCT CAGTGGTACTGCACCACTC

Table S4: Primers for reverse transcription and qPCR.

Description	Sequence
Forward primer GB RNA m^{6}A region	GGTTGCGTTGGGTGTTCCTG
Reverse primer for GB RNA m^{6}A region	GGGAGAACAAGAAGGGCGAA
Forward primer for GB RNA control region	CGTCCTGTTTTGCGTGTCTC
Reverse primer for GB RNA control region	CCACAAGACCGACGAGCACA
Forward primer for *ACTB* m^{6}A region	CCTTCCAGCAGATGTGGATC
Reverse primer for *ACTB* m^{6}A region	GCCATGCCAATCTCATCTTG
Forward primer for *ACTB* control region	CAGGATGCAGAAGGAGATCAC
Reverse primer for *ACTB* control region	CGATCCACACGGAGTACTTG
Forward primer for *PLAC2* m^{6}A region	AAGAGAAGCACAGAAGGGGC
Reverse primer for *PLAC2* m^{6}A region	ACGGCTTGGGCAAAGGTGTG
Forward primer for *PLAC2* control region	CAAGCAAAGTGAACACGTCG
Reverse primer for *PLAC2* control region	TCACTTTAACTTGCACTTTACTGC
Forward primer for *MALAT1* m^{6}A region	GGCAGAAGGCTTTTGGAAGAGT
Reverse primer for *MALAT1* m^{6}A region	CTGGGTCAGCTGTCAATTAATGC
Forward primer for *MALAT1* control region	CAGCAGCAGACAGGATTCCA
Reverse primer for *MALAT1* control region	TCCTATCTTCACCACGAACTGC
Forward primer for *LY6K* m^{6}A region	GGCCTCAGCCTGTCTTGA
Reverse primer for *LY6K* m^{6}A region	AATGCAACAGGTGACAACGG
Forward primer for *LY6K* control region	TGACTGTGCACCTTTGAGCA
Reverse primer for *LY6K* control region	ACCGAGAGAAGGCAATCACG
Forward primer for *MCM5* m^{6}A region	TCACTGGACTCATGGACTCG
Reverse primer for *MCM5* m^{6}A region	AAGTTCGAGGGCTGCAGT
Forward primer for *MCM5* control region	GAGCACAGCATCATCAAGGA
Reverse primer for *MCM5* control region	TGCATGCGATGCTGGATCT
Forward primer for *SEC11A* m^{6}A region	CAAAGCCCCCAGTGTTTGTA
Reverse primer for *SEC11A* m^{6}A region	CGTGCAGAGCTGCATTTTCAT
Forward primer for *SEC11A* control region	CACTCGAGGGGACTTTCAGT
Reverse primer for *SEC11A* control region	GGCTTTGGCTCAACCTTTTAAT
Forward primer for *INCENP* 912 and 967 m^{6}A region	TGAGCTCCCTGATGGCTACA
Reverse primer for *INCENP* 912 and 967 m^{6}A region	CTCCCGCCATGGAGAATCTG
Forward primer for *INCENP* 1060 m^{6}A region	CTCCCATCCTGCCGGATAAC
Reverse primer for *INCENP* 1060 m^{6}A region	TGGGCTAAGACTTGGGGACT
Forward primer for *INCENP* control region	CATCAGTGAGCGCCAGAATG
Reverse primer for *INCENP* control region	TGATGTCGGGATGCCCTG
Forward primer for *LMO7* m^{6}A region	GAGAGAGTAGAAGAGAAGGG
Reverse primer for *LMO7* m^{6}A region	CAAAGAGGCTGGGCTTTGTTC
Forward primer for *LMO7* control region	TCACGGAGCACACAAATGGA
Reverse primer for *LMO7* control region	TGAGAGCCAAAGGGTCTTGG
Forward primer for *MRPL20* m^{6}A region	GGGAAGGAACCTGAAGGCAT
Reverse primer for *MRPL20* m^{6}A region	TGCAAATTACTCTGTCTCTTTTCC
Forward primer for *MRPL20* control region	CCAAAGCCCGATACCTGAAGA
Reverse primer for *MRPL20* control region	GCTCCACCTGGCACTTAACTA