Supplementary Data

A New heavy lanthanide-dependent DNAzyme displaying strong metal cooperativity and unrescuable phosphorothioate effect

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Table S1. DNA oligonucleotides used for in vitro selection and assays.

DNA Name	**Sequence and modifications (from 5′ to 3′)**
Lib-FAM-N_{35}	pGGCGAAACATCTT**N$_{35}$**TAGTGGGTAAGCTTGGCAC-**FAM**
Lib-rA	AATACGAGTCACTAT**rA**GGAAGAT
splint	AAGATGTTTCGCCATCTTCCTATAGTCCACCACCA
P1 primer	GTGCCAAGCTTACCG
P2 primer	CTGCAGAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACA
P3 primer	**FAM**-AAATGATCCACTAATACGACTCACTAT**rA**GG
P4 primer	AACAACAACAAC-**iSp18**-GTGCCAAGCTTACCG
Tm4	TTTCGCCATCTTCAAAATTCAGTCTGACTCGTGAC
Tm7	TTTCGCCATCTTCGATACTCTCTTTGACTCGTGAC
Tm8	TTTCGCCATCTTCATACACGTTTTTTA ACTCGTGAC
Er32	TTTCGCCATCTTTTGTACATTGATCTGACTCGTGAC
Er36	TTTCGCCATCTTCTAAACGTACATCATCTCTAACTCGTGAC
Ho11	TTTCGCCATCTTCTAAAATTCCCTCTAACTCGTGAC
Ho15	TTTCGCCATCTTCTAATAATTCTTTTAACTCGTGAC
RNA-sub	GTCACGAGTCAC**rUrArUrArGrGrA**AGATGGCGAAA-**FAM**
Sub-FAM	GTCACGAGTCACTAT**rA**GGAAGATGGCGAAA-**FAM**
Tm7-Q	**Iowa Black ® FQ**-CGCCATCTTCGATACTCTCTTTGACTCGTGAC

Table S2. Sequence alignment for the three selection (aligned together).

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Ho7             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TAG--A- 51
Ho13            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TAG--A- 51
Ho14            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TAG--A- 51
Er5             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TTGG-C- 52
Er1             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TCGAGA- 53
Er16            CTGCAG-AATTCTAATACSAGTCACYMTAGGAAGATGGCGAAACATCT-----T--AGA- 51
Er18            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TGA--T- 51
Er39            CTGCAG-AATTCTAATAC------CTATAGGAAGATGGCGAAACATCT-----TGA--T- 45
Er11            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TGA--T- 51
Tm4             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TCA--A- 51
Tm7             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TCG--A- 51
Tm26            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TAA--A- 51
Ho12            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TATATG- 53
Tm27            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TCTATG- 53
Ho9             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TCAGTA- 53
Er34            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TCGAAAG 54
Tm14            CTGCAG-AATTCTAATA-GAGTCACTAT-GGAAGATGGCGAAACATCT-----TCAAAA- 51
Ho2             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TAGCTC- 53
Er22            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTC---TAGAAT- 55
Ho6             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TAATAA- 53
Er32            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TTTGTA- 53
Er24            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TTAAACA 54
Er30            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TTAAA-- 52
Ho4             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TCAAT-- 52
Ho11            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TCTAA-- 52
Er36            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TTAAA-- 52
Er4             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TAGTA-- 52
Tm16            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TAAGA-- 52
Tm32            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGARGGCGAAACATCT-----TAAGA-- 52
Er23            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCC-----TCAAACT 54
Ho15            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TCTAATA 54
Tm8             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TCATACA 54
Ho1             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TAGAGGT 54
Er6             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TAGA--- 51
Er13            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT-----TCGAAAA 54
Er31            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT---ATAGGTAA 56
Er25            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCT------TGGAGA 53
Er35            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTAGGGGTAGTGC 59
Tm17            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT--GAGAAGCAC 57
Er12            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----G--TATA 53
Er26            CTGCAG-AATT-TAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----CGACATA 54
Er27            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TTATAGA 55
Er33            CGCCCTTAATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TTATAGA 56
Tm1             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTTG---TACAAGA 56
Er9             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGC 55
Er20            CTGCAG-AATTCTAATACGA-TCACTATAGGAAGATGGCGAAACATCTT----TACAAGC 54
Er10            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGC 55
Tm3             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGC 55
Tm22            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGC 55
Er37            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGC 55
Ho3             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGC 55
Ho10            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGC 55
Er2             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGG 55
Er17            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGG 55
Er28            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGG 55
Ho8             CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGG 55
Er19            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGG 55
Er38            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACAAGG 55
Ho5             CTGCAG-AATTCCAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TACGAGA 55
Er15            CTGCAG-AATTCTAATACGAGTCACTATAGGAAGATGGCGAAACATCTT----TGTATAC 55
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Ho7              -AATTTTC---------TATAACGAGTTTGAT-CATGTACCGTTAGT--CGGTAAGCTTG 98
Ho13             -AATTTTC---------TATAACGAGTTTGAT-CATGTACCGTTAGT--CGGTAAGCTTG 98
Ho14             -AATTTTC---------TATAACGAGTTTGAT-CATGTACCGTTAGT--CGGTAAGCTTG 98
Er5              -AAATCTC---------TTTAACGAATTTAGT-AGTGTACCG-TAGT--CGGTAAGCTTG 98
Er1              -TGATTTA---------TATAACGAGTAAAGG-ACCGA--TTGTAGT--CGGTAAGCTTG 98
Er16             -CAAATTA---------TATAACGAGTATAGG-AGCGAGCACATAGT--CGGTAAGCTTG 98
Er18             -TCT-CTC---------TTTAACGAGTATCAGTGGCTRCAACGTAGT--CGGTAAGCTTG 98
Er39             -TCT-CTC---------TTTAACGAGTATCAGTGGCTCCAACGTAGT--CGGTAAGCTTG 92
Er11             -TCT-CTC---------TCTAACGAGTATCAGTGGCTCCACCGTAGT--CGGTAAGCTTG 98
Tm4              -AATTCAG---------TCTGACGCG-GTAGGTGGGTCCACCATAGT--CGGTAAGCTTG 98
Tm7              -TACTCTC---------TTTGACGTTCGTATAAACGAGACA-ATAGT--CGGTAAGCTTG 98
Tm26             -TATAATC---------TTTAACG--AGTAACCCAGACACATATAGT--CGGTAAGCTTG 97
Ho12             -ATATCT----------TTTAACGAGTATTAA--ACCATAAGATAGT--CGGTAAGCTTG 98
Tm27             -TTACTA----------TCTAACGAGAAGAGA--ACCATAA-CTAG---CGGTAAGCTTG 96
Ho9              -CCATCAA---------TCTAACGAGTGTTAG--AACGCAA-ATAGT--CGGTAAGCTTG 98
Er34             CAAATTTA---------TTTGACGAGAAT-----GCATACAAACAGT--CGGTAAGCTTG 98
Tm14             ---TTTAC---------TATAACGAGTGTTTA--ACGTGGAAATTAG--CGGTAAGCTTG 95
Ho2              ---AATCA--------GCACGCATGCGTGATT-ATAGTGAAG-TAGT--CGGTAAGCTTG 98
Er22             ---CATTT--------ATTTGACCACG-GATT-GCAGATTA--TAG---CGGTAAGCTTG 97
Ho6              ---CTTT---------ATCTAACGAAGTATTA-CTCAAAGAGGTAGT--CGGTAAGCTTG 98
Er32             ---CATTG--------ATCTGACTCACTCATG-CT-GTGGACCTAGT--CGGTAAGCTTG 98
Er24             GGAATTTC---------TTTAACTCGCTGTAG-GTG-GACT---AGT--CGGTAAGCTTG 98
Er30             --ACTTTA---------TTTGACTCGGAGGAA-GAG-AATTGGTAGT--CGGTAAGCTTG 97
Ho4              --TTATTC---------TTTGATGAGAAAAAG-GTGGAACTATTAGT--CGGTAAGCTTG 98
Ho11             -AATTCC---------CTCTAACTCGTACAAT-GAC-CCTTGTTAGT--CGGTAAGCTTG 98
Er36             -CGTACAT-----CATCTCTAACTCGTTGATA-GAA-C----TTAGT--CGGTAAGCTTG 98
Er4              -CCCAAAGGGAGGATGTTATAACTCGTATCT------C-----TAGT--CGGTAAGCTTG 98
Tm16             GCCGTCAG--------GTATGCCCTGTCAAAA-ACT-CGT--ATAGT--CGGTAAGCTTG 98
Tm32             GCCGTCAG--------GTATGCCCTGTCAAAA-ACT-CGT--ATAGT--CGGTAAGCTTG 98
Er23             GATATCAG--------AAGATCAACTCCTATA-ACT-CG----TAGT--CGGTAAGCTTG 98
Ho15             A-TTCTTT-----------TAACTCGATTATG-TAG-CGTATATAG---CGGTAAGCTTG 97
Tm8              CGTTTTTT-----------TAACTCGCT-ATG-TAT-GGAAGGTAG---CGGTAAGCTTG 97
Ho1              ACGTATAG---------AGACGAG-GTGACGCAATT----GAGTAGT--CGGTAAGCTTG 98
Er6              ---TATGG---------AAACGATTGTGAGGCTATATGACGAGGTAG--CGGTAAGCTTG 97
Er13             AGGTATCT---------GAGTTTATTTGATTCGTTG----AT-TAGT--CGGTAAGCTTG 98
Er31             TGGAAACT---------AA---TATTTGACTCGCTG----ATATAGT--CGGTAAGCTTG 98
Er25             GAGAAACT---------CAATTTATTTGACTCAATG----GGTTAGT--CGGTAAGCTTG 98
Er35             GTAGGACG---------AGATTTATTTGGCTCGTAG-------------CGGTAAGCTTG 97
Tm17             GAAGTGC----------AGGTTTATTTGACAAATAAT-------AGT--CGGTAAGCTTG 98
Er12             ACTTAAGT---------AGGTTATTTTAACGAGTATT----ATTAGT--CGGTAAGCTTG 98
Er26             ACGAATGT---------TACCCCCTTTAACGAGTA------ATTAGC--CGGTAAGCTTG 97
Er27             TCGATAGA---------GGATTTATTTGACAGGTAT------TTAGT--CGGTAAGCTTG 98
Er33             TCGATAGA---------GGATTTATTTGACAGGTAT------T-AGT--CGGTAAGCTTG 98
Tm1              ACGGGAGA---------GGGTATATTTAAATCGTAG------T--AG--CGGTAAGCTTG 97
Er9              ATCAGTAG-------------ATTGGAAATGCGGTT------ATAGTGACGGTAAGCTTG 96
Er20             ATCAGTAG-------------ATTGGAAATGCGGTT------ATAGTGACGGTAAGCTTG 95
Er10             ATCAGTAG-------------ATTGGAAATGCGGTT------ATAGTGACGGTAAGCTTG 96
Tm3              ATCAGTAG-------------ATTGGAAATGCGGTT------ATAGTGACGGTAAGCTTG 96
Tm22             ATCAGTAG-------------ATTGGAAATGCGGTT------ATAGTGACGGTAAGCTTG 96
Er37             ATCAGTAG-------------ATTGGAGATGCGGTT------ATAGTGACGGTAAGCTTG 96
Ho3              ACAAATAG-------------ATTGGAAATGCGGTT------ATAGTGACGGTAAGCTTG 96
Ho10             ACCGGACA---------AACTATGGGAGGAGCGGTT------ATAGT--CGGTAAGCTTG 98
Er2              AAGGGTCC---------A---CTATGCACAACGGTT------ATAGTGACGGTAAGCTTG 97
Er17             AAGGGTCC---------A---CTATGCACAACGGTT------ATAGTGACGGTAAGCTTG 97
Er28             AAGGGTCC---------A---CTATGCACAACGGTT------ACAGTGACGGTAAGCTTG 97
Ho8              AAGGGTCC---------A---CTATGCACAACGGTT------GTAGTGACGGTAAGCTTG 97
Er19             AAGGGTCC---------A---CTATGCACAACGGTT------ATAGTGGCGGTAAGCTTG 97
Er38             AAGTGTCC---------A---CTATGCACAACGGTT------ATAGTGACGGTAAGCTTG 97
Ho5              TAGAATGT---------TG--TACAGTCTAGCGGTT------ATAGT--CGGTAAGCTTG 96
Er15             CGGAAAGG---------TAAAATTAATTTAACGAGA------GTAGT--CGGTAAGCTTG 98
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Note: the bold A in red is the cleavage site. The bases in blue are conserved for Lu12. The green T denotes for the starting of the sequence alignment in the randomized region.

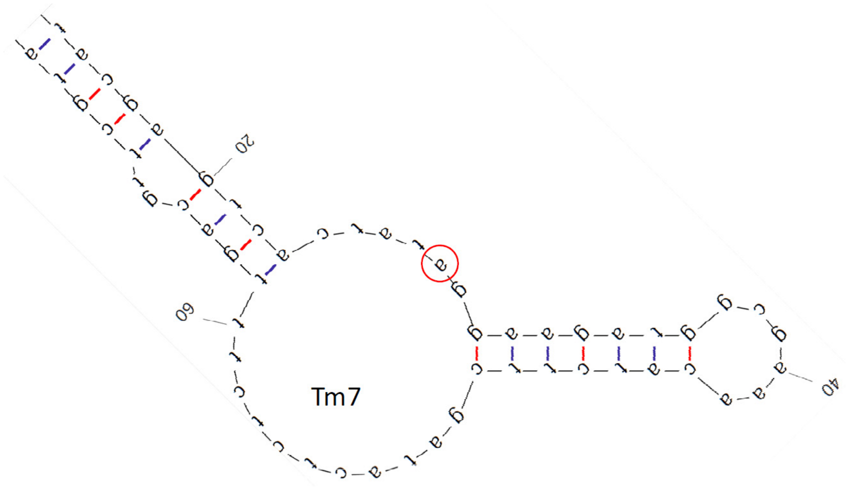

Figure S1. M-fold predicted Tm7 secondary structure in the cis-cleaving form. The numbering starts from the 5′-end of the sequence. The cleavage site rA is marked in the red circle. To engineer this into a trans-cleaving DNAzyme, the loop around nucleotide number 40 is removed and the base pairs are fixed as shown in Figure 1E of the main paper.

We tested a total of seven sequences in the family and six of them showed activity as presented in the paper. The last one, Tm8, is put here in SI due to limited space. The trans-cleaving Tm8 is presented in Figure S2A, which also has the pyrimidine stretch followed by a mixed purines and pyrimidines. However, it is also active active with the heavy lanthanides but not the lighter ones (Figure S2B).

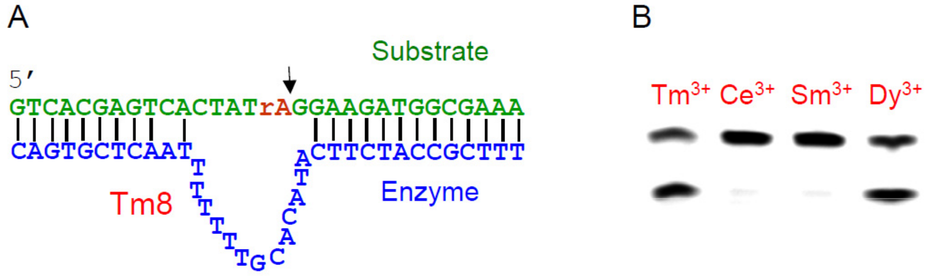

Figure S2. (A) The secondary structure of the trans-cleaving Tm8 DNAzyme. (B) Its activity in the presence of four lanthanides. Again, only the heavy lanthanides Tm^{3+} and Dy^{3+} produced activity.

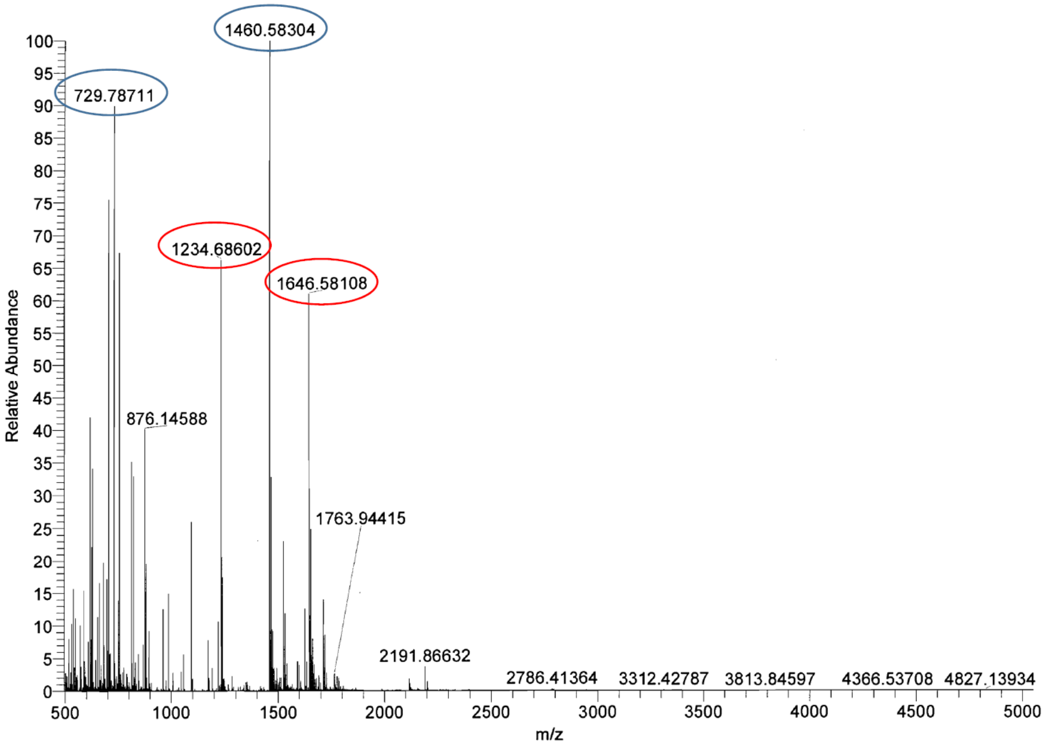

Figure S3. Mass spectrometry characterization of the Tm7 cleavage product. The peaks in red are the cyclic phosphate product from the 5′-fragment of the substrate (molecular weight = 4942.7, 3 and 4 charges for the two marked peaks), and the peaks in blue are from the 3′-fragment (molecular weight = 4384.7, 3 and 6 charges for the two marked peaks). These samples were prepared by reacting a non-labeled DNAzyme substrate with Tm7 or 17E DNAzymes at 1 µM substrate and 1.5 µM enzyme concentration in 50 mM MES buffer (pH 6) with 25 mM NaCl. Then the samples were desalted using a Sep-Pak column and dried. After rehydration in water to ~20 µM substrate concentration, the samples were analyzed using an ESI mass spectrometer.

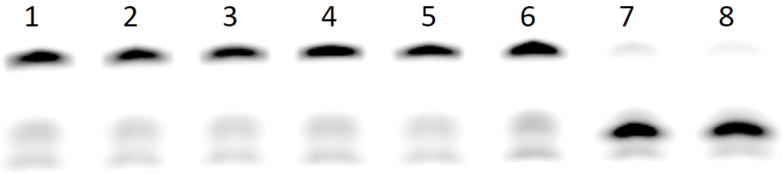

Figure S4. Tm7 and 17E cleavage of a RNA substrate (RNA-sub in Table S1 for sequence). Lane 1 is the substrate alone, and some degradation was observed. Lane 2 is the substrate with 10 µM Er^{3+}. Lane 3 is the Tm7 DNAzyme complex without metal. Lane 4 is the complex with 10 µM Er^{3+} for 30 min incubation. Lane 6 is the 17E DNAzyme complex without metal. Lane 7 and 8 are the 17E DNAzyme complex with 10 µM Pb^{2+} after 30 and 60 min incubation. The 17E DNAzyme is known to cleave full RNA, and it serves as a positive control. Cleavage was observed only with the 17E DNAzyme, but not Tm7.

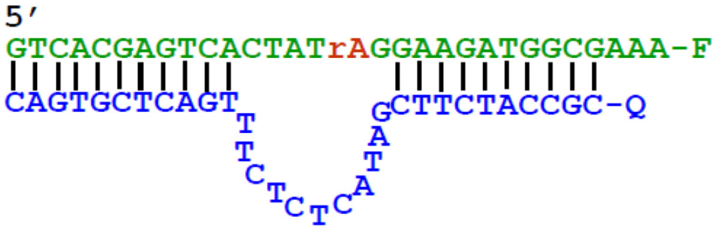

Figure S5. Catalytic beacon design using the Tm7 DNAzyme. F = FAM. Q = Iowa Black ® FQ dark quencher.