Supplementary information

Recharacterization of Hammerhead Ribozyme as Molecular Tools for Intermolecular Cleavage

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Materials

T7 RNA polymerase, Fast AP Thermosensitive Alkaline Phosphatase, T4 polynucleotide kinase were purchased from Thermo Fisher Scientific (Massachusetts, USA). dNTP and all oligonucleotides were purchased from Sangon Biotech (Shanghai, China). [γ-^{32}P]-ATP was purchased from Furui Biological Engineering (Beijing,China).

Table S1 Sequences of HHRz

oligonucleotide	sequence
SM-S1	GGGACUGUUGUGCGUCCUGGAUUCCACUGCUCAG
SM-S2	GGGACUGUUGUGCGUGCUGGAUUCCACUGCUCAG
SM-R	GGGAGCGCUGAGCAGGUACAUCCAGCUGAUGAGUCCCAAAUAGGACGAAACGCGCAACAGU
ST-S1	GGGGCGCCGAAACACCGUGUCUCGAGC
ST-S2	GGGGCGCCGAAACACCGUGUGUCGAGC
ST-S3	GGGGCGCCGAAACACGCCCGGUGUGUCUCGAGC
ST-R	GGGGGCUCGACUGAUGAGGCGC

Obtaining of HHRz by in vitro transcription

A substrates were synthesized via transcription by T7 RNA polymerase, with wide type deoxynucleotides templates possessing the following sequence
5’- CGAAATTAATACGACTCACTATAGGGACTGTTGTGCGTCCTGGATTCCACTGCTCAG-3’, while wild type ribozymes were synthesized by the template sequence
5’-CGAAATTAATACGACTCACTATAGGGAGCGCTGAGCAGGTACATCCAGCTGATGAGTCCCAAATAGGACGAAACGCGCAACAGT-3’.
Mutations of RNA substrate and ribozymes were all subjected to alteration of nucleotides correspondingly. A transcription reaction containing 0.5μM of DNA template, 10mM of NTP, 1×T7 RNA transcription buffer(40mM Tris-HCl pH7.9, 10mM NaCl, 10mM dithiothreitol, 6mM $MgCl_2$, 2mM spermidine) and 1.25 unit per μl RNA polymerase were incubated at 37°C for 4h, followed by ethanol precipitation and purification by PAGE. The concentration of transcription was determined by ultraviolet spectrophotometer at the wavelength of 260nm.

Labeling reaction

RNA obtained by in vitro transcription was incubated with 0.05 unit per µl Fast AP in 10Mm Tris-HCl pH8.0, 5mM MgCl$_2$, 100mM KCl, at 37°C for 30min, and then extracted with phenol-chloroform and recovered by ethanol precipitation. A labeled reaction mixture containing oligonucleotides with 50mM Tris-HCl pH7.8, 40mM NaCl, 10mM MgCl$_2$, 10µCi[γ-^{32}P]-ATP and 10 unit of PNK was incubated for 4h at 37°C for RNA phosphorylation. The labeled product was purified by 20% denaturing PAGE.

Standard Conditions of HHRz cleavage reaction in vitro

A cleavage reaction mixture contains 0.5 µM 5'-labeled RNA substrate, and 50µM RNA enzyme, 50mM Tris-HCl (pH8.0), and 10mM MgCl$_2$ was incubated at 37°C. The cleavage reactions were stopped by addition of 1 volume of stopped buffer (80%[v/v] deionized formamide, 50mM EDTA pH8.0, 0.025%[w/v] bromphenolblue, 0.025%[w/v] xylene cyanole). Subsequently the products were purified on a 20% denaturing PAGE.

Determination of cleavage yield

The cleavage reactions in vitro were performed at least twice. Uncleaving substrates and cleavage products was separated by 20% denaturing PAGE. The extents of cleavage reaction were determined by quantitation of radioactivity in the substrate and product bands through Typhoon Laser scanning imaging system via phosphor imager system. The gray gradient was analyzed by ImageQuantTL, and the graphs of compare among same category were plotted by GraphPad Prism 6.01.

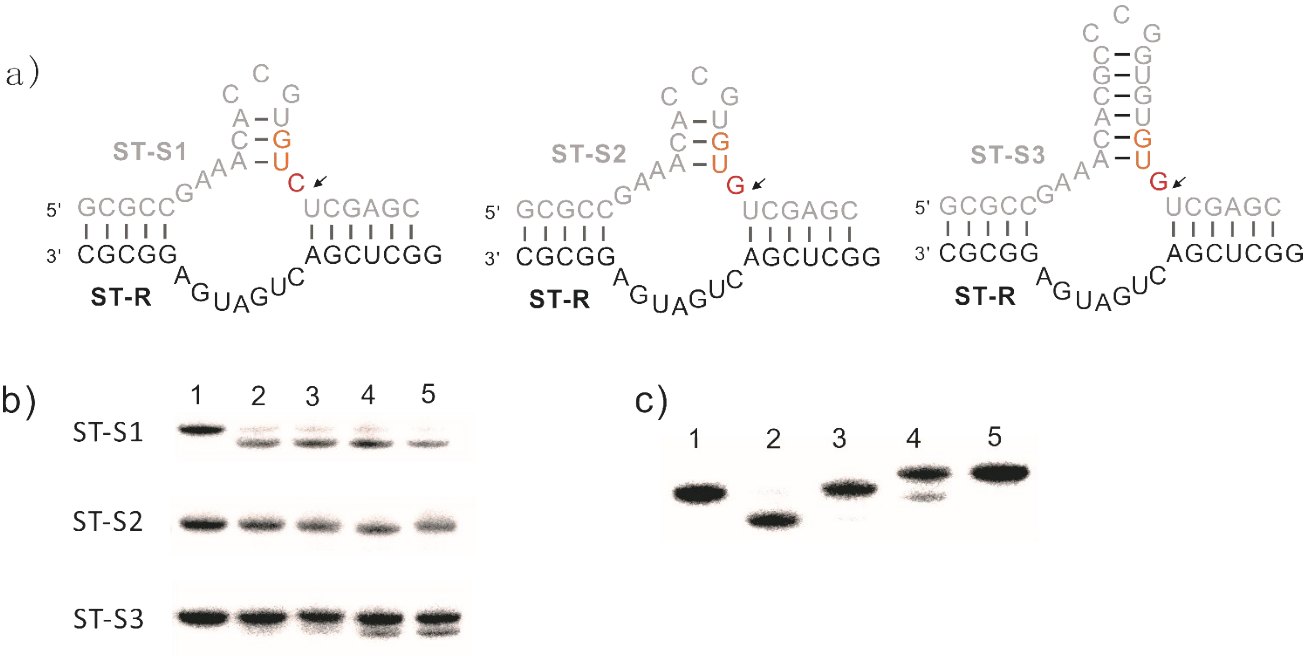

Fig. S1 The comparison of cleavage results among different ST-HHRz substrates. (a) Respective sequence and structure of **ST-S1/ST-S2/ST-S3**. Cleavage site is highlight to orange. (b) PAGE analysis of trans-cleavage reaction of ST-HHRz. Represented results are shown for a 4h time course. Lane 1, the 5'-^{32}P-radiolabeled ST-HHRz substrate; Lane2 -5, the ST-HHRz substrate cleaved by **ST-R** under standard condition for 1h/2h/3h/4h. (c) PAGE analysis of trans-cleavage reaction of ST-HHRz. Represented image are shown as a result of 4h cleavage reaction. Lane 1 and 5, the 5'-^{32}P-radiolabeled **ST-S1/S3**; Lane 2,3 and 4, the ST-HHRz substrate **ST-S1/S2/S3** cleaved by **ST-R** under standard condition.

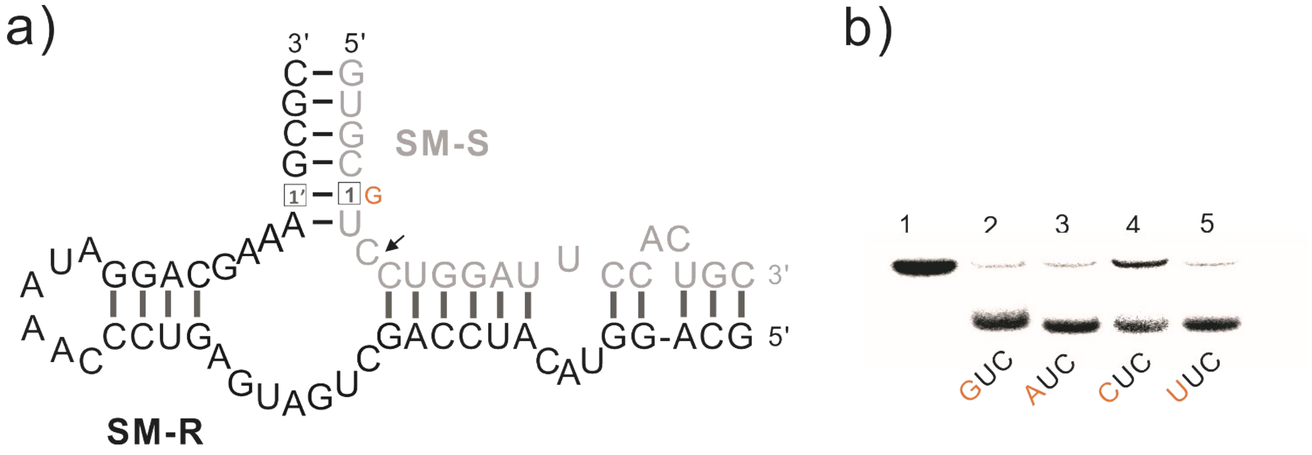

Fig. S2 The mutant pattern and cleavage assay under standard reaction condition. (a)The secondary structure of SM-HHRz with mutant position **1**. The complementary nucleotide is presented as **1’**. Original nucleotide is highlight to orange next to the position. (b) PAGE analysis of SM-HHRz mutations trans-cleaving. Lane 1, the 5’-^{32}P-radiolabeled wild type substrate **SM-S**. Lane 2-5, the cleavage results of substrates with cleavage site **GUC/AUC/CUC/UUC** cleaved by complementary enzyme part for 1min under standard single turnover condition.

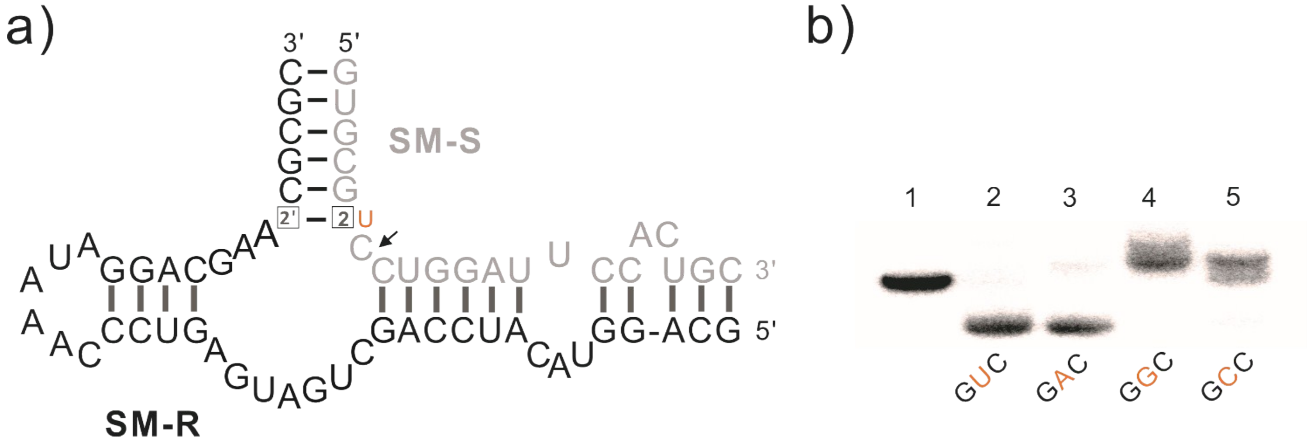

Fig. S3 The mutant pattern and cleavage assay under standard reaction condition. (a)The secondary structure of SM-HHRz with mutant position **2**. The complementary nucleotide is presented as **2’**. Original nucleotide is highlight to orange next to the position. (b) PAGE analysis of SM-HHRz mutations trans-cleaving. Lane 1, the 5’-^{32}P-radiolabeled wild type substrate SM-S. Lane 2-5, the cleavage results of substrates with cleavage site **GUC/GAC/GGC/GCC** cleaved by complementary enzyme part for 1min under standard single turnover condition.

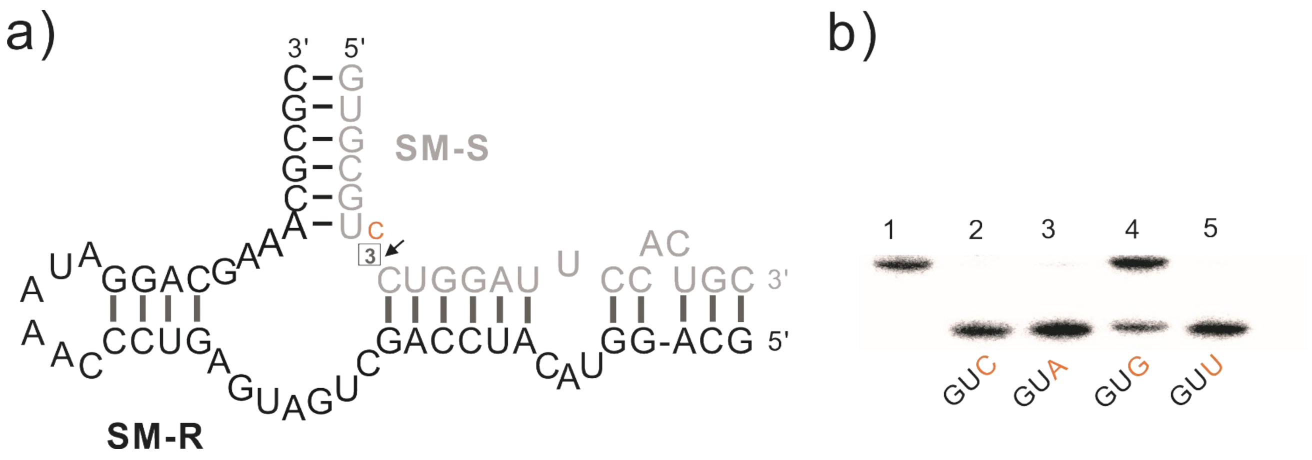

Fig. S4 The mutant pattern and cleavage assay under standard reaction condition. (A)The secondary structure of SM-HHRz with mutant position **3**. Original nucleotide is highlight to orange next to the position. (B) PAGE analysis of SM-HHRz mutations trans-cleaving. Lane 1, the 5’-^{32}P-radiolabeled wild type substrate **SM-S**. Lane 2-5, the cleavage results of substrates with cleavage site GUC/GUA/GUG/GUU cleaved by complementary enzyme part for 1min under standard single turnover condition.

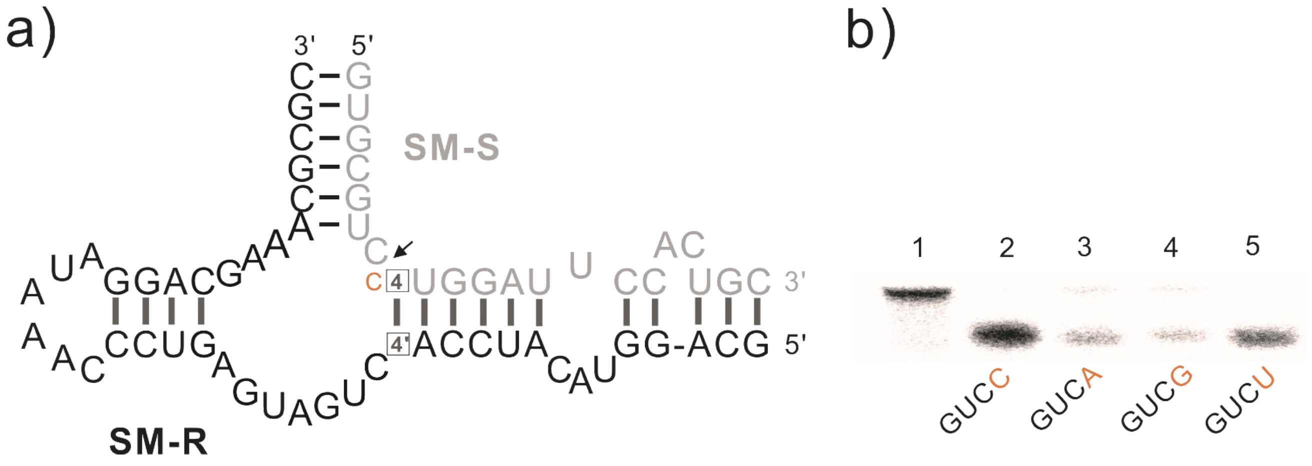

Fig. S5 The mutant pattern and cleavage assay under standard reaction condition. (a)The secondary structure of SM-HHRz with mutant position **4**. The complementary nucleotide is presented as **4’**. Original nucleotide is highlight to orange next to the position. (b) PAGE analysis of SM-HHRz mutations trans-cleaving. Lane 1, the 5’-^{32}P-radiolabeled wild type substrate **SM-S**. Lane 2-5, the cleavage results of substrates with cleavage site GUCC/GUCA/GUCG/GUCU cleaved by complementary enzyme part for 1min under standard single turnover condition.

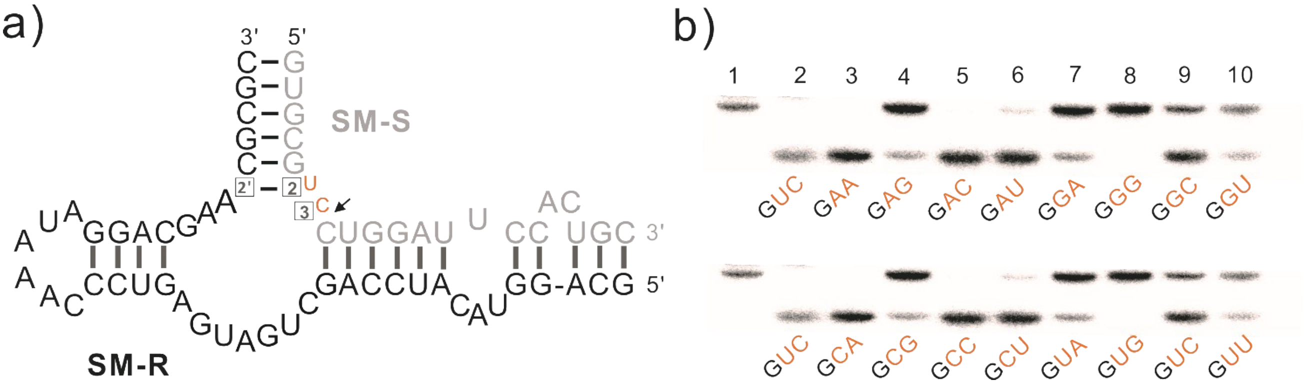

Fig. S6 The mutant pattern and cleavage assay under standard reaction condition. (a)The secondary structure of SM-HHRz with mutant position **2 3**. The complementary nucleotide is presented as **2’**. Original nucleotide is highlight to orange next to the position. (b) PAGE analysis of SM-HHRz mutations trans-cleaving. Lane 1, the 5’-^{32}P-radiolabeled wild type substrate **SM-S**. Lane 2-10, the cleavage results of substrates with their taged cleavage site cleaved by complementary enzyme part for 1min under standard single turnover condition.

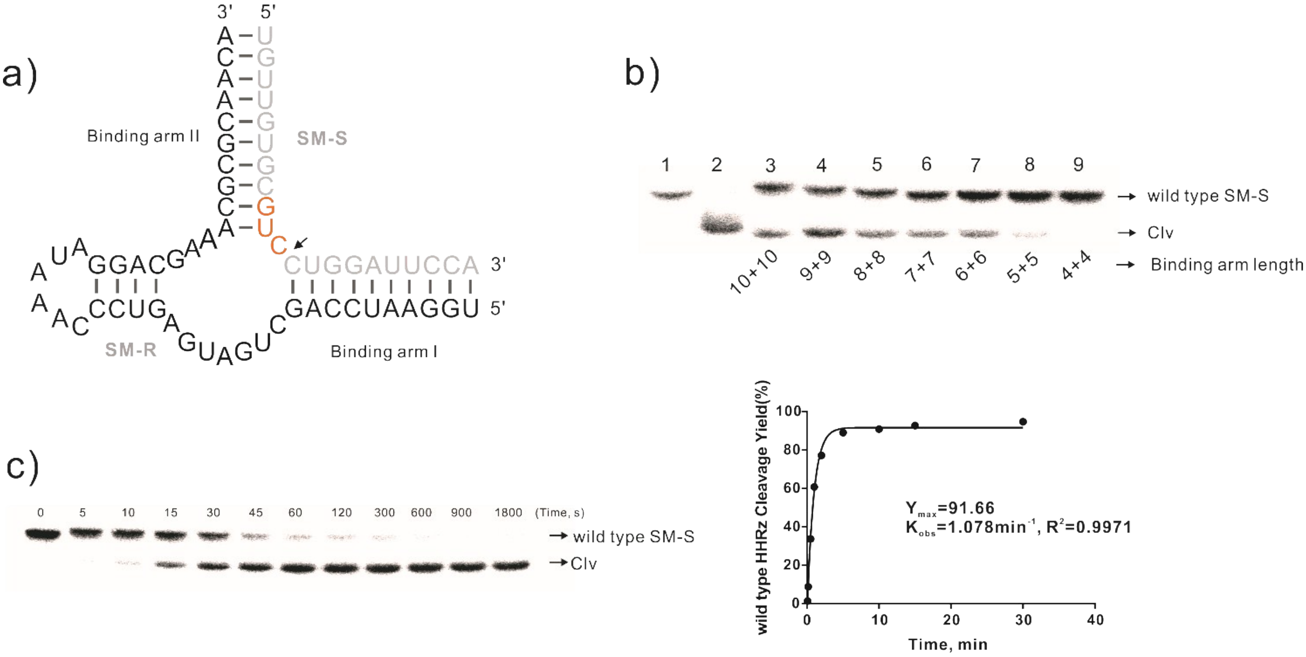

Fig. S7 (a) The intermolecular pattern of trans-cleavage of **SM-S/SM-R** with fully complementary binding arms in different lengths. (b) PAGE of trans-cleavage of **SM-S/SM-R** with fully complementary binding arms in different lengths. (c) PAGE analysis of wild type **SM-S** trans-cleaving assay under single turnover condition (about 0.5 µM 5’-labeled RNA substrate combined with 50µM RNA enzyme) at 37°C within reaction buffer containing 50mM Tris-HCl (pH 8.0) and 10mM $MgCl_2$. Represented results are shown for a 30-min time course. The observed rate constant (k_{obs}~1.078min^{-1}, R^2=0.9971) and maximum yield (Y_{max}) were derived using none-liner regression.