

Improved design of hammerhead ribozyme for selective digestion of target RNA through recognition of site-specific adenosine-to-inosine RNA editing

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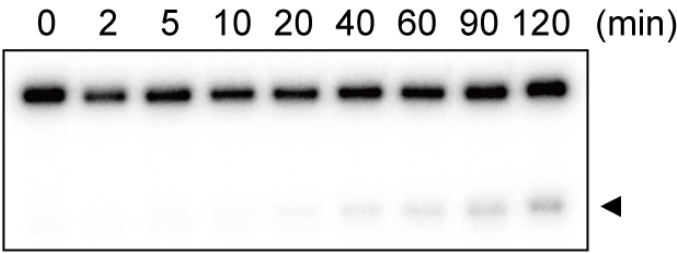
Supplementary Information

Figure S1.

Comparison of the time course of the cleavage reaction and kinetic analysis of HR-F39, HR-F39(-L), and minHR-*HTR2C*-Edit for *HTR2C*-wt under single-turnover conditions. (A) Denaturing PAGE (15%) showing time course of cleavage of *HTR2C*-wt by HR-F39(-L). Reaction times are shown for each lane, and the position of the cleavage product is indicated by the arrowhead. (B) Graphs representing the cleavage ratio as a function of reaction time. Data of HR-F39, HR-F39(-L), and minHR-*HTR2C*-Edit are represented as circles, triangles, and squares, respectively. All data were obtained from 2 independent experiments for reproducibility. Fitting curves were generated using a single-exponential equation. Kinetic parameters obtained from this experiment are summarized in Table 1.

FIGURE S1.

A



HR-F39 (-L)

B

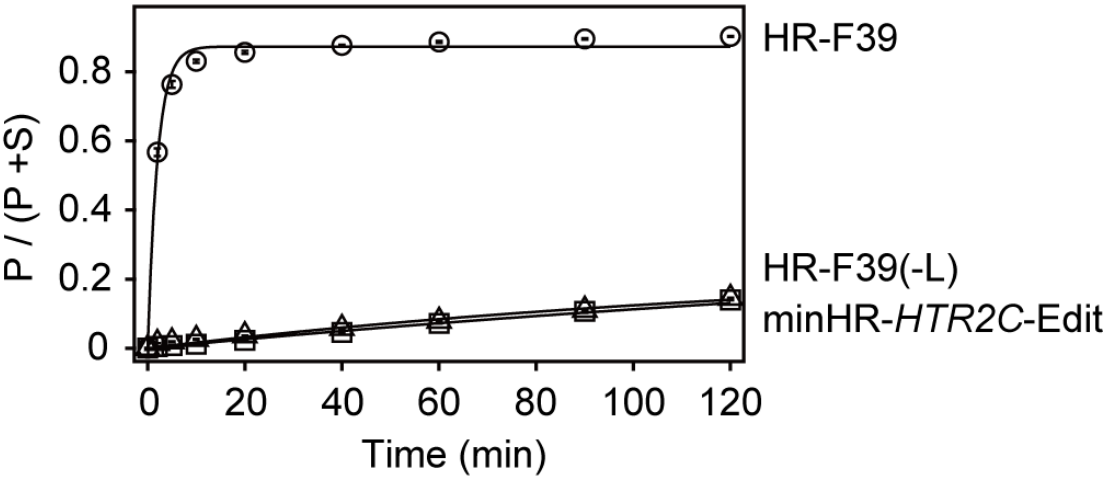


Table S1. List of sequences of synthetic oligonucleotides used in this paper.

Designation	Sequences of oligonucleotide and deoxyoligonucleotide (5' → 3')
<i>HTR2C</i> -wt (RNA)	GGAUCGGUAUGUAGCAAUACGUAAUCCUAUUGAGCAUAGCC
T7pro-F	CTAATACGACTCACTATAG
<i>HTR2C</i> -wt-R	CCGATACGAGTTATCCTAATGCATAACGATGTATGGCTAGGATATCACTCAGCATAATC
minHR- <i>HTR2C</i> -Eedit (RNA)	GCAAUAGGACUGAUGAGGCCGAAAGGCCGAAUACGUAAUUGC
minHR- <i>HTR2C</i> -Eedit-R	AGCAATACGTTTCGGCCTTTCGGCCTCATCAGATCCTATTCTATAGTGAGTCGTATTAG
HRpLib-F	CTAATACGACTCACTATAGGCTATGCTCA
HRpLib-R	GGTATGTAGCAATACGTNNNNNNNNNNNNNNNNNNNNNTCAGTCCTATNNNNNTGAGCATAGCC
HRpLib-RT	GGTATGTAGCAATACGT
HRseq-F-EcoRI	CGGAATTCTAATACGACTCACTATAG
HRseq-R-BamHI	GCGGGATCCGGTATGTAGCAATACG
HR-F39UUAU (RNA)	GGGCGGCUAGGAAUGCUCACUGACGAGGCCUCCUACGGCCGAAUAGGAUUACG
HR-F39UUAU-F	CTAATACGACTCACTATAGGGCGGCTAGGAATGCTCACTGACG
HR-F39UUAU-R	CGTAATCCTATTTCGGCCGTAGGAGGCCTCGTCAGTGAGC
HR-F39UAG (RNA)	GGGAUUACGGAAGUAUUGCUGACGAGGCCUCCUACGGCCGAAUACAUACCGA
HR-F39UAG-F	CTAATACGACTCACTATAGGGATTACGGAAGTATTG
HR-F39UAG-R	TCGGTATGTATTTCGGCCGTAGGAGGCCTCGTCAGCAATACTTCCG
HR-F39UAC (RNA)	GGGCAAUAGGGAAGAUUACCUGACGAGGCCUCCUACGGCCGAAUAAUUGCUACA
HR-F39UAC-F	CTAATACGACTCACTATAGGGCAATAGGGAAGATTACCTGACGAGGC
HR-F39UAC-R	TGTAGCAATATTTCGGCCGTAGGAGGCCTCGTCAGG
HR-F39UAA (RNA)	GGGUGCUCAGGAAAUAGGACUGACGAGGCCUCCUACGGCCGAAUACGUAAUUGC
HR-F39UAA-F	CTAATACGACTCACTATAGGGTGCTCAGGAAATAGG
HR-F39UAA-R	GCAATACGTATTTCGGCCGTAGGAGGCCTCGTCAGTCCTATTTCCTG
<i>HTR2C</i> -e4S	TGGGACTACTTGTCATGCCCTGTCTCT
<i>HTR2C</i> -ex65A2	CCAATCACAGGGATAGGAACTGAAACTCCTA
<i>HTR2C</i> -seq65A	GGAACTGAAACTCCTATTGATATTGCC
<i>GAPDH</i> -QF	CCTGCACCACCAACTGCTTAGC
<i>GAPDH</i> -QR	GATGGCATGGACTGTGGTCATGAG
<i>HTR2C</i> -qS1	CGCCATATCGCTGGACCGGTATG
<i>HTR2C</i> -qA4	CAGGGATAGGAACTGAAACTCCTA
<i>HTR2C</i> -L-T7F	CTAATACGACTCACTATAGGGTATTTGTGCCCCGTCTG
<i>HTR2C</i> -L-R	CCAGGTATAATTACCTATTGATATTGCC
HR- <i>NEIL1</i> -temp	ACAGGGGGCAATTTCGGCCGTAGGAGGCCTCGTCAGGGCTATTCCCGGGTCAGAGCCCTATAGTGA GTCGTATTAG
<i>HTR2C</i> -Cino (RNA)	GGAUCGGUAUGUAGCAAUACGUAIUCCUAUUGAGCAUAGCC
<i>HTR2C</i> -Eino (RNA)	GGAUCGGUAUGUAGCAAUACGUAIUCCUAUUGAGCAUAGCC
bio- <i>HTR2C</i> -target (RNA)	5'-biotin-CAUUACGUAAUCCUAUUGAGCAUAGCC
<i>NEIL1</i> -unedited	ACAGGGGGCAAAGGCUACGGGUCAGAG
<i>NEIL1</i> -edited	ACAGGGGGCAIAGGCUACGGGUCAGAG