

Supporting Information

Programmable pH-Responsive DNA Inter-Strand Matching (PRISM) for Precision Molecular Band-Pass Actuation

Xiaole Han^{1,2#}, Hongyan Yu^{1,#}, Xiaomei Lin^{1,#}, Li Zhang^{1,#}, Weitao Wang¹, Yaoyi

Zhang¹, Jianbo Jiang¹, Xingyu Liu¹, Ke Lv^{*,1} and Guoming Xie^{*,1,2}

¹Department of Neurosurgery, Laboratory of Neurological Diseases and Interdisciplinary Medicine, The First Affiliated Hospital of Chongqing Medical University, No.1 Youyi Road, Chongqing, 400016, China.

²Key Laboratory of Clinical Laboratory Diagnostics (Chinese Ministry of Education), College of Laboratory Medicine, Chongqing Medical University, Chongqing, 400016, PR China.

* To whom correspondence should be addressed. Tel: +86 23 68485240; Fax: +86 23 68485239; Email: guomingxie@cqmu.edu.cn. Correspondence may also be addressed to Ke Lv Email: luke@hospital.cqmu.edu.cn.

[#]The authors wish it to be known that, in their opinion, the authors should be regarded as Joint First Authors.

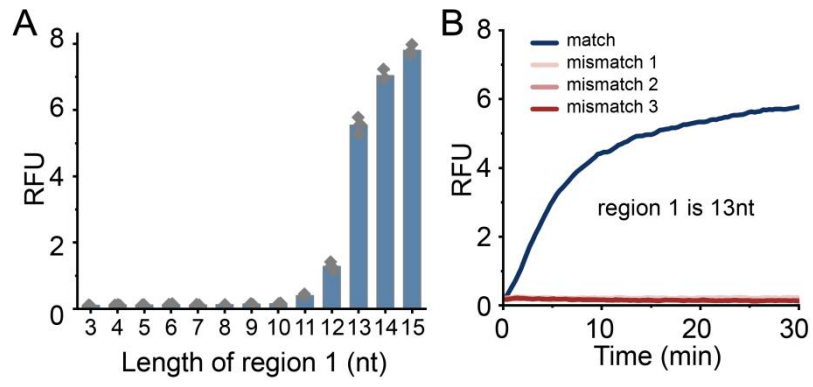


Figure S1. Fluorescence of region 1 of different lengths (A) and the effect of introducing mismatch in region 1 (B).

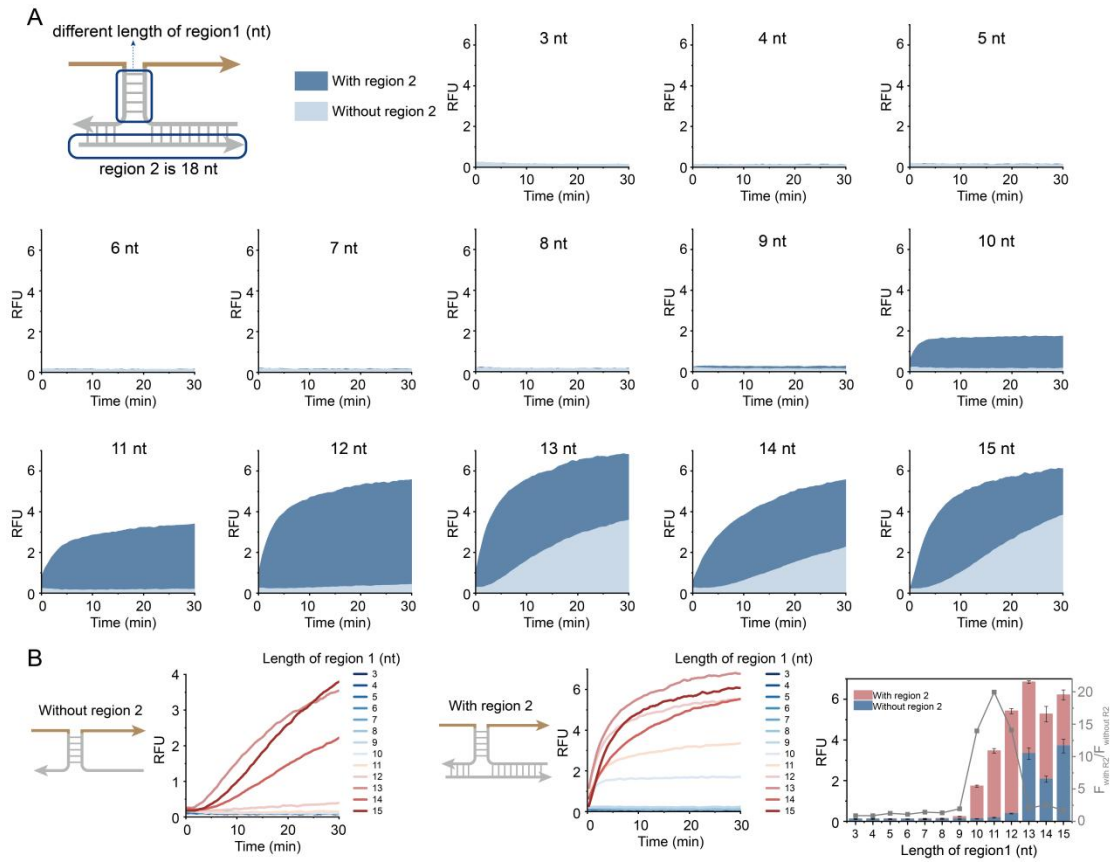


Figure S2. When region 2 is 18nt, the fluorescence curves of region 1 of different lengths (A), and the signal comparison of whether region 2 exists (B).

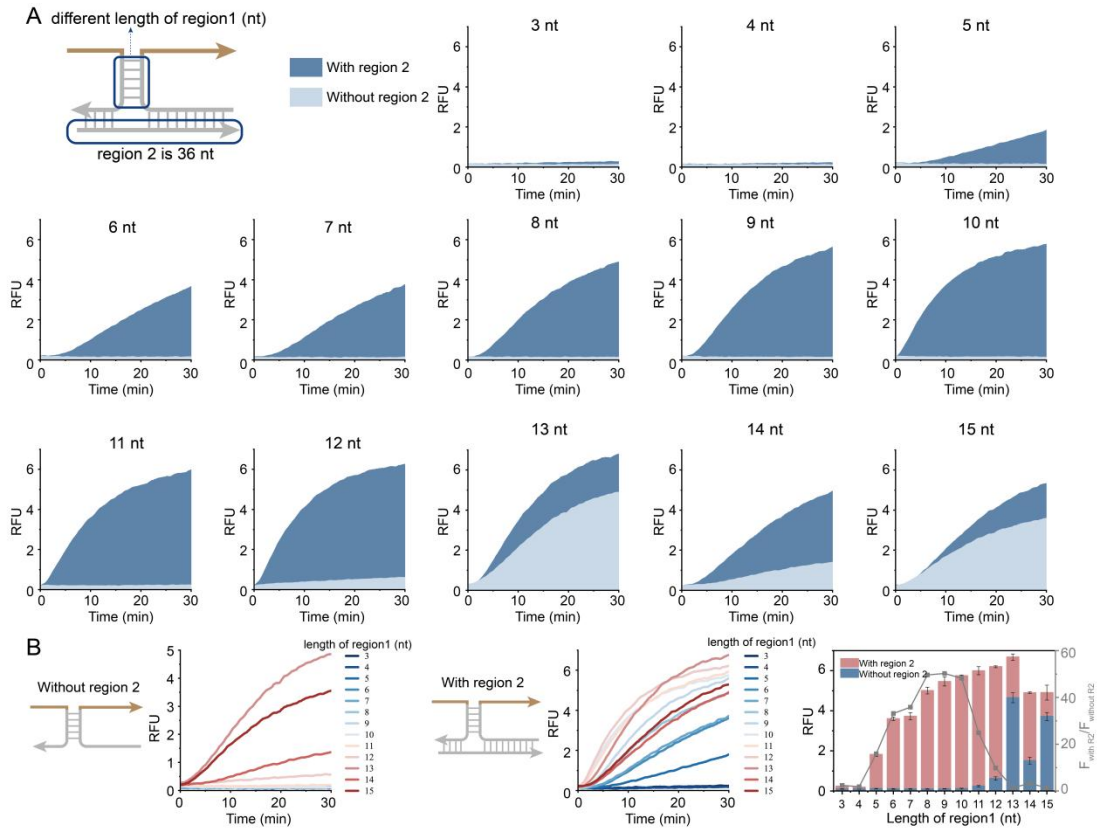


Figure S4. When region 2 is 36nt, the fluorescence curves of region 1 of different lengths (A), and the signal comparison of whether region 2 exists (B).

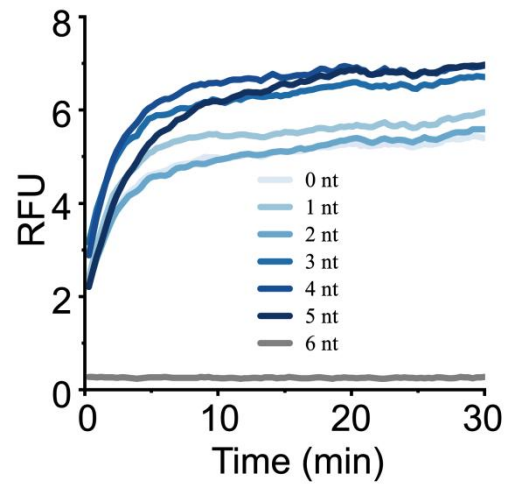


Figure S5. Fluorescence curves of different spacer lengths.

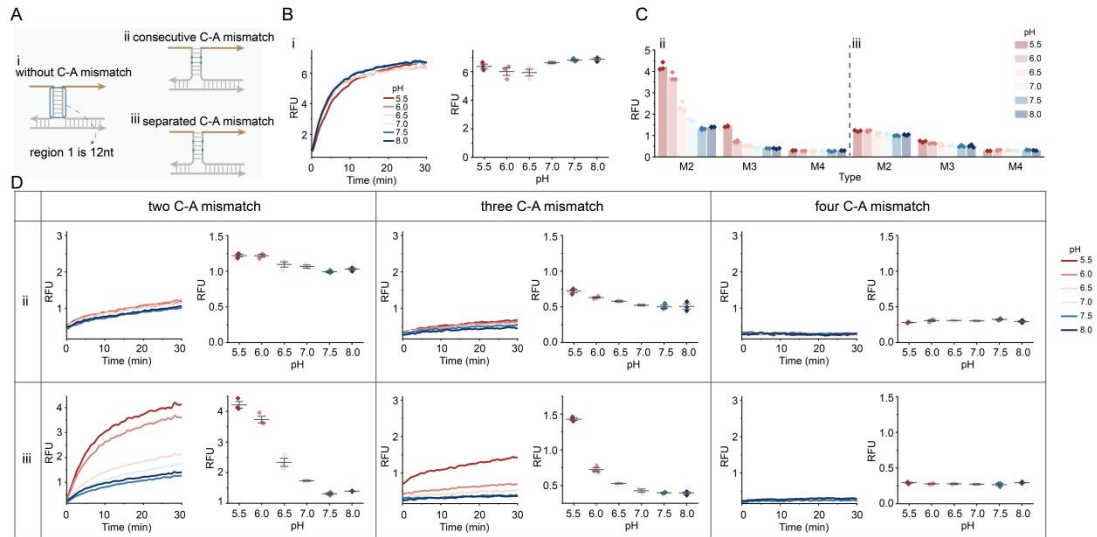


Figure S6. (A) Schematic diagram of different types of C-A mismatches with 12nt region 1. (B) Fluorescence curve of fully matched region 1 without C-A mismatches. (C) Effect of C-A mismatch arrangement and quantity on the pH-dependent signal profile. (D) Fluorescence curves of different types of C-A mismatches.

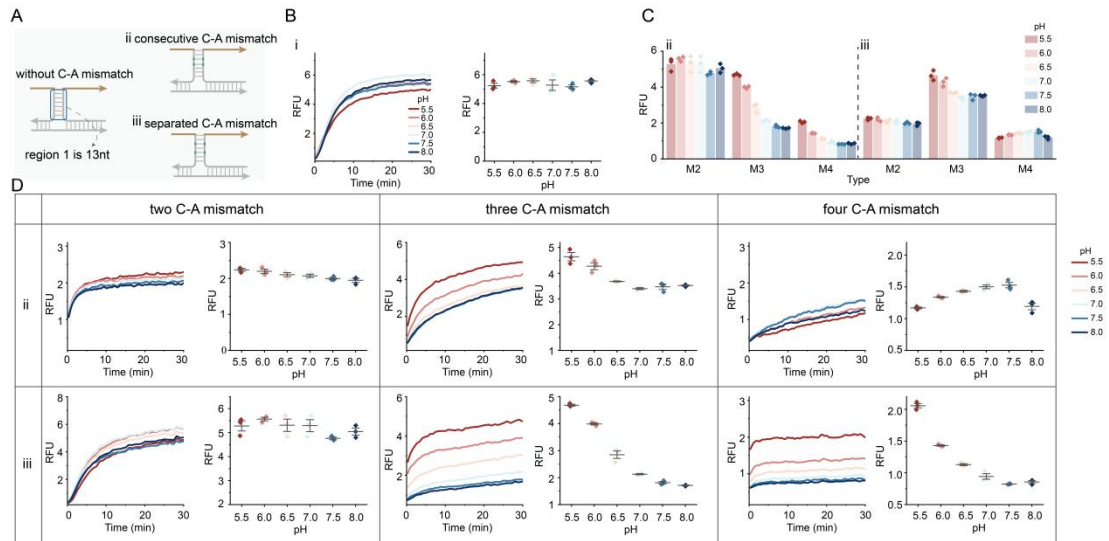


Figure S7. (A) Schematic diagram of different types of C-A mismatches with 13nt region 1. (B) Fluorescence curve of fully matched region 1 without C-A mismatches. (C) Effect of C-A mismatch arrangement and quantity on the pH-dependent signal profile. (D) Fluorescence curves of different types of C-A mismatches.

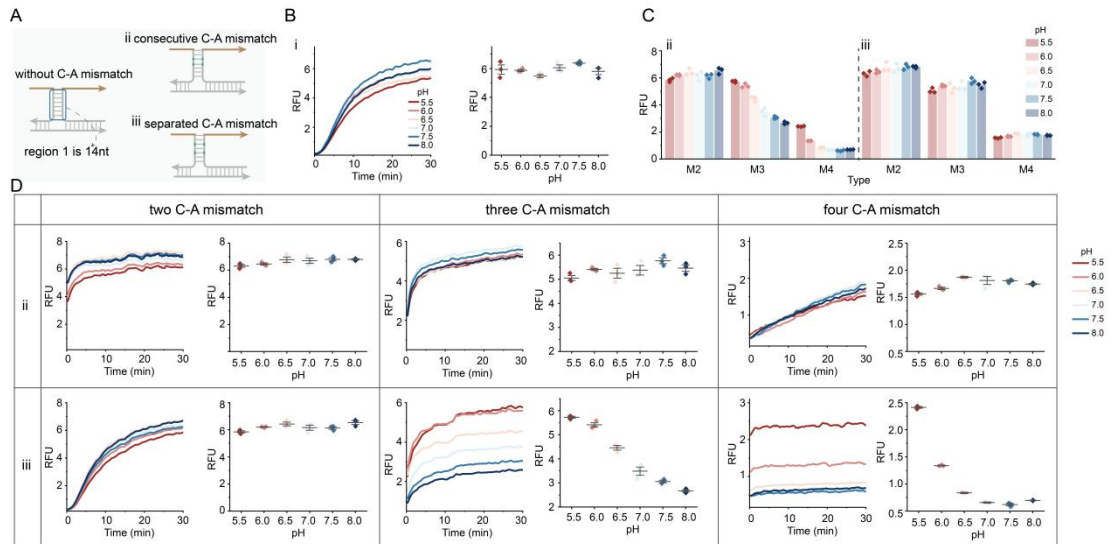


Figure S8. (A) Schematic diagram of different types of C-A mismatches with 14nt region 1. (B) Fluorescence curve of fully matched region 1 without C-A mismatches. (C) Effect of C-A mismatch arrangement and quantity on the pH-dependent signal profile. (D) Fluorescence curves of different types of C-A mismatches.

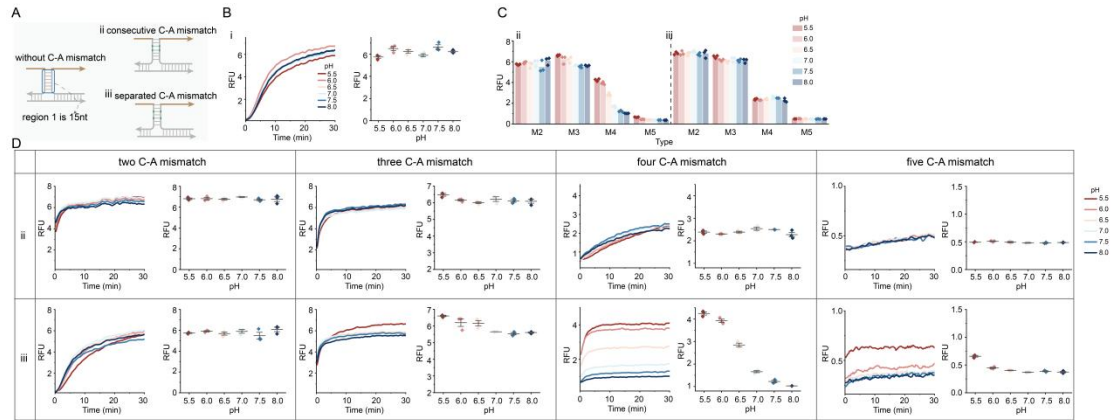


Figure S9. (A) Schematic diagram of different types of C-A mismatches with 15nt region 1. (B) Fluorescence curve of fully matched region 1 without C-A mismatches. (C) Effect of C-A mismatch arrangement and quantity on the pH-dependent signal profile. (D) Fluorescence curves of different types of C-A mismatches.

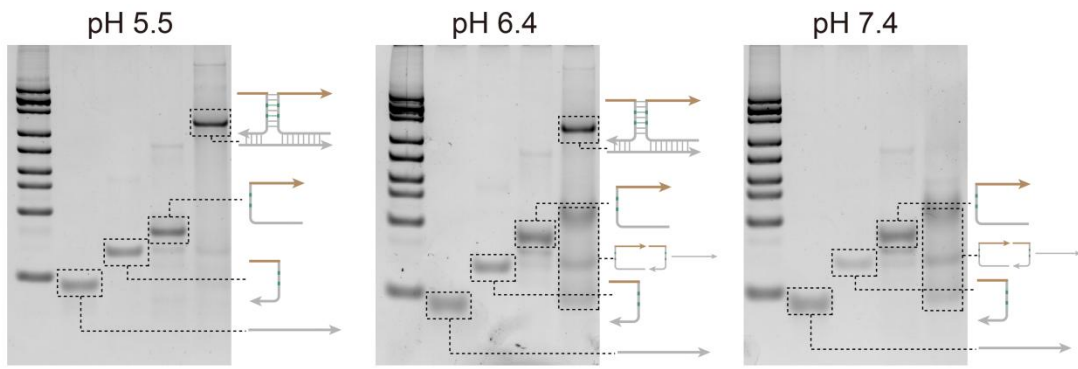


Figure S10. PAGE validation of C-A mismatch-mediated high-pH OFF module.

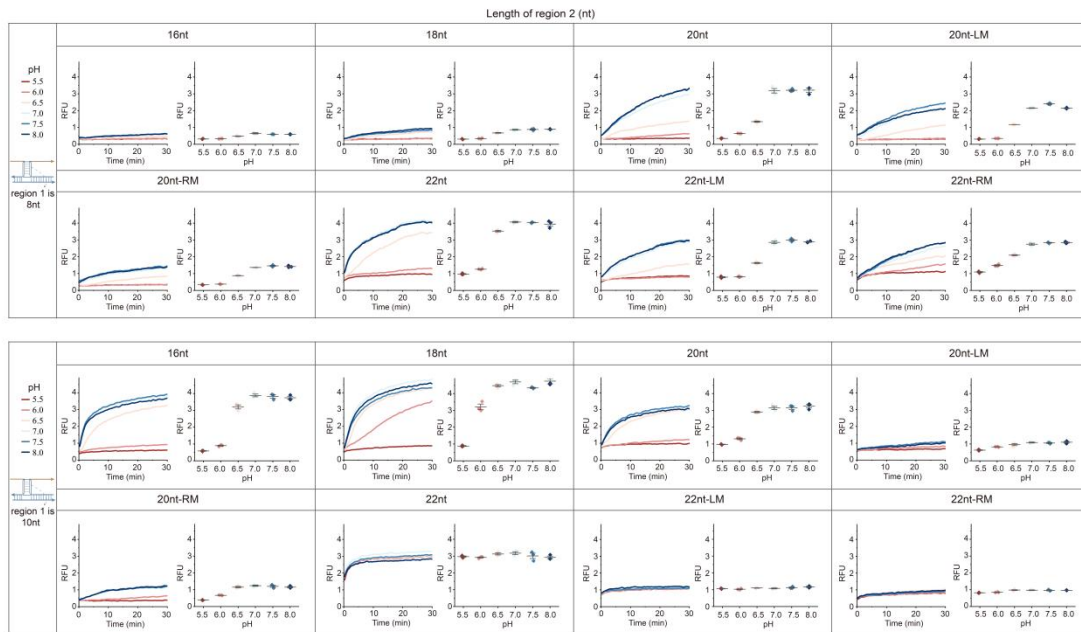


Figure S11. Fluorescence curve of C6 i-motif and framework topology (region 1: 8 vs 10 nt; region 2: 16-22 nt). LM1 refers to a mismatch on the left side of spacer in region 2, and RM1 indicates a mismatch on the right side of the spacer.

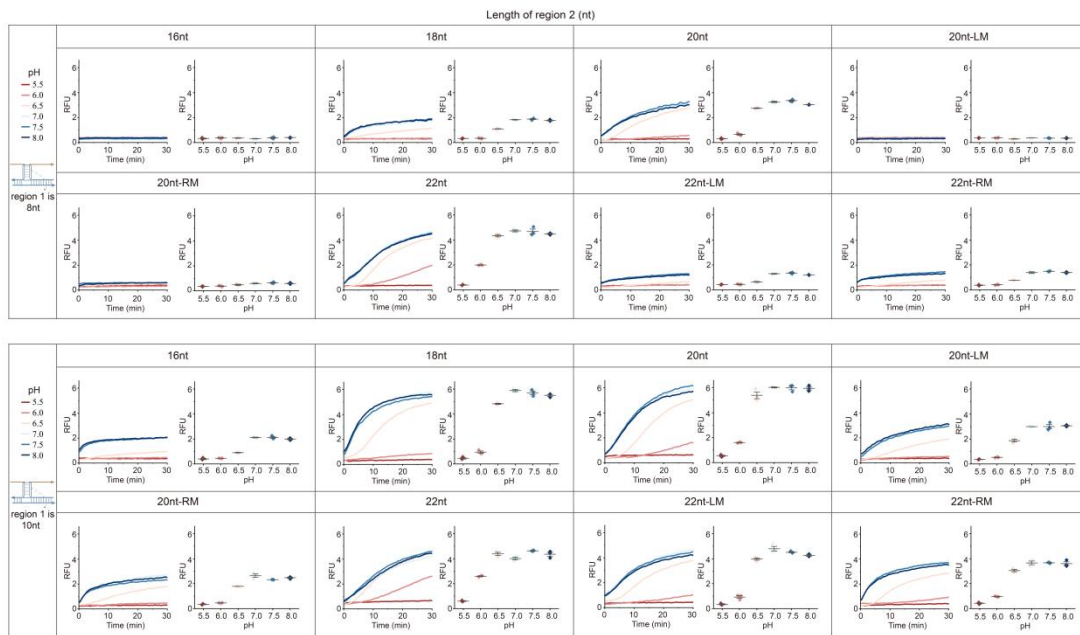


Figure S12. Fluorescence curve of C5 i-motif and framework topology (region 1: 8 vs 10 nt; region 2: 16-22 nt). LM1 refers to a mismatch on the left side of spacer in region 2, and RM1 indicates a mismatch on the right side of the spacer.

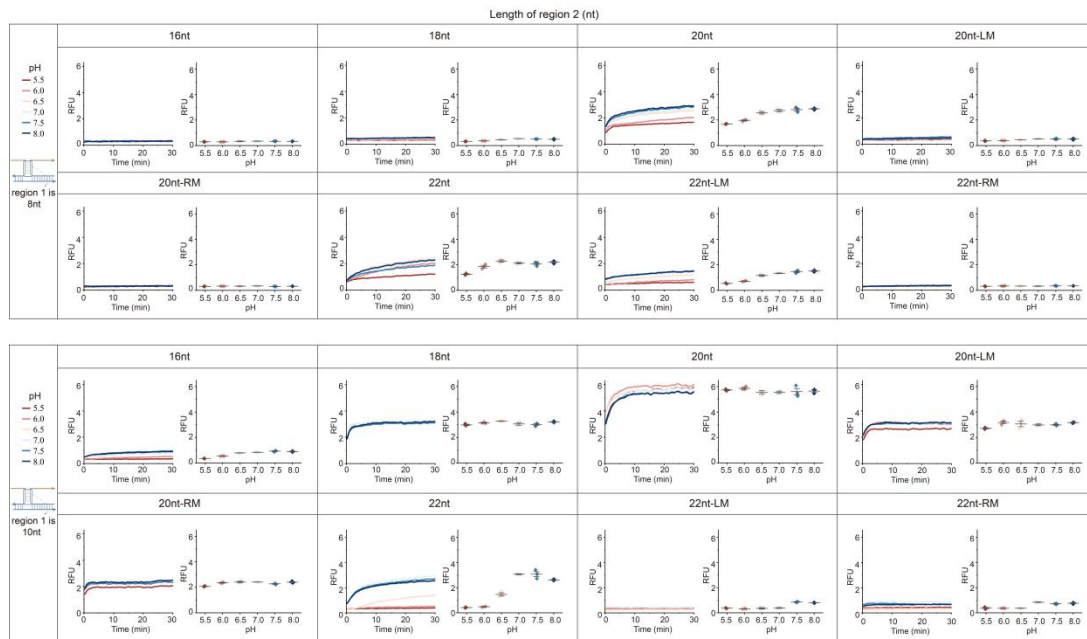


Figure S13. Fluorescence curve of C4 i-motif and framework topology (region 1: 8 vs 10 nt; region 2: 16-22 nt). LM1 refers to a mismatch on the left side of spacer in region 2, and RM1 indicates a mismatch on the right side of the spacer.

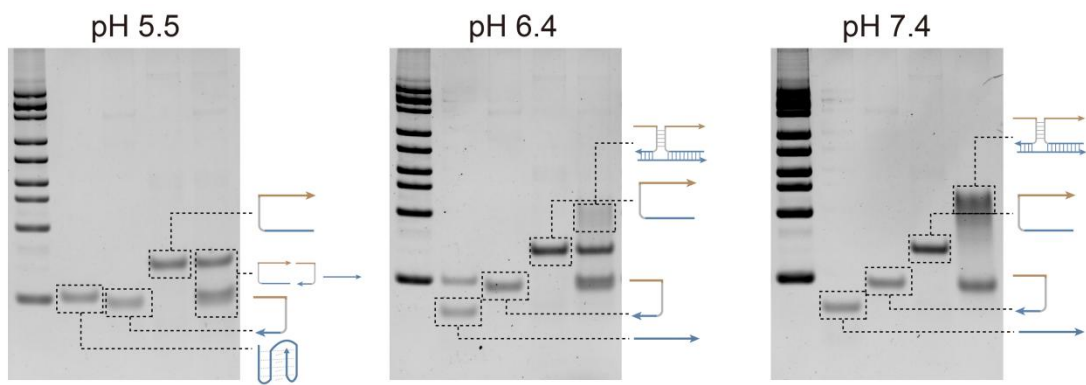


Figure S14. PAGE validation of i-motif-mediated low-pH OFF module.

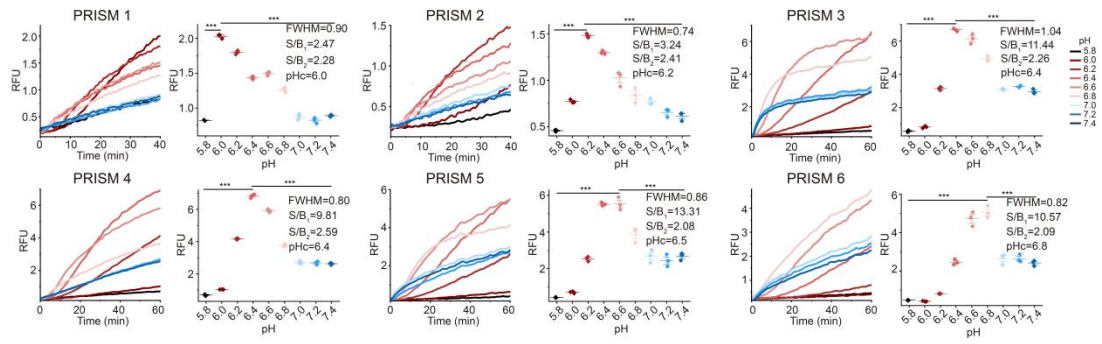


Figure S15. Different types of PRISM probes. Statistical significance between peak signal and outer boundaries (pH 5.8 and pH 7.4) was analyzed using a two-tailed Student's t-test (***) denotes $p < 0.001$).

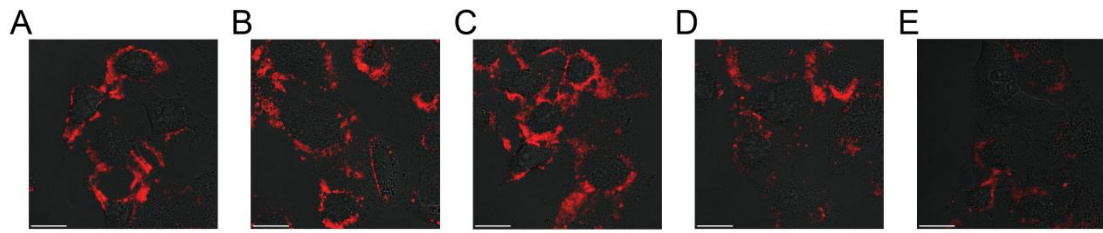


Figure S16. Confocal laser scanning microscopy (CLSM) characterization for the optimization of probe incubation time. HeLa cells were incubated with 300 nM of the c-MET-anchored PRISM probe in Phy buffer (pH 6.4) at room temperature for (A) 10 min, (B) 20 min, (C) 30 min, (D) 60 min, and (E) 120 min. Scale bars: 20 μ m.

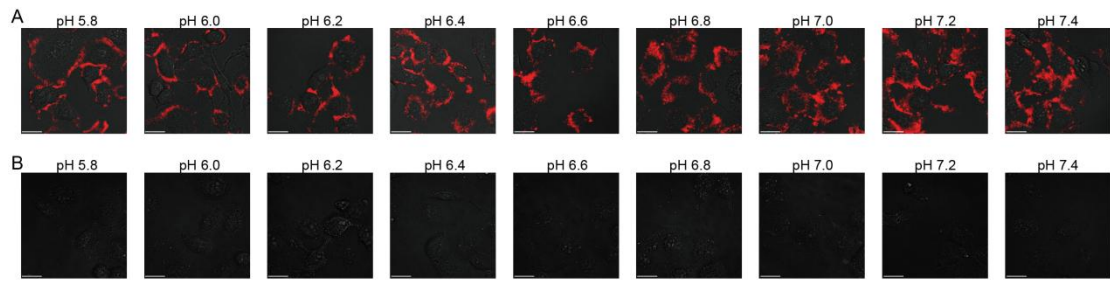


Figure S17. At different pH values, imaging results of aptamer (A) and PRISM probe with random aptamer sequences (B). Scale bars: 20 μm .

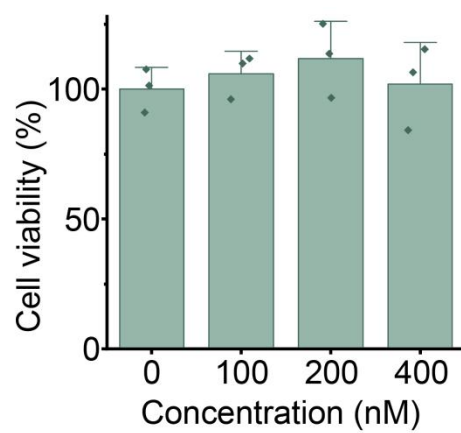


Figure S18. Cytotoxicity evaluation of the PRISM probe on HeLa cells. Cell viability was measured after 4 days (96 h) of incubation with the PRISM probe at different concentrations. The cell viability of the untreated group (0 nM) was defined as 100%.

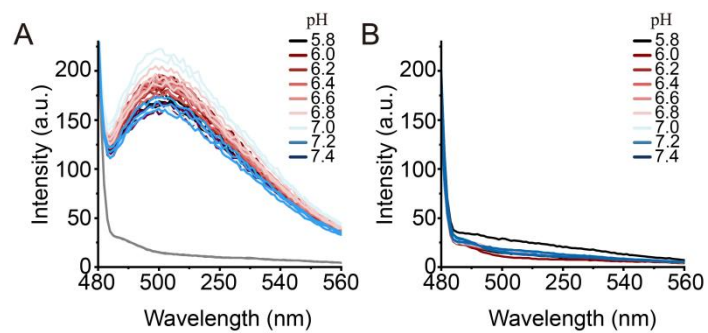


Figure S19. Fluorescence measurements of the intact DNA Lettuce (A) and the free DFHBI-1T dye (B) across a pH range of 5.5 to 8.0.

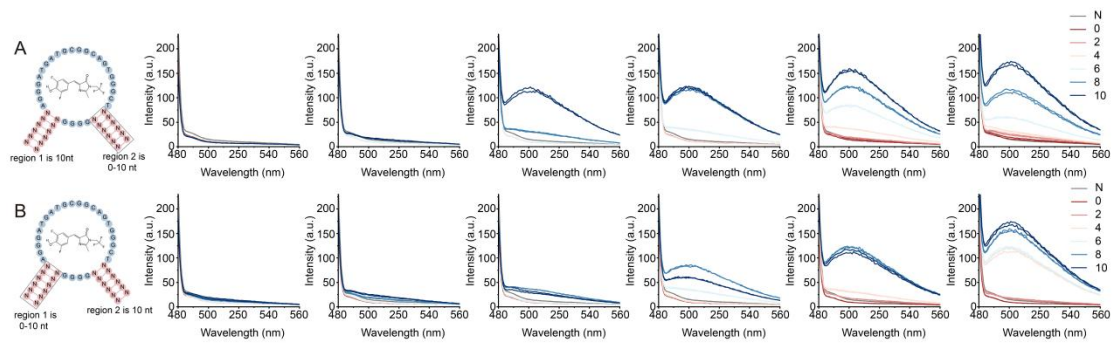


Figure S20. An orthogonal fluorescence measurements analysis of the lengths of region 2 (A) and region 1 (B).

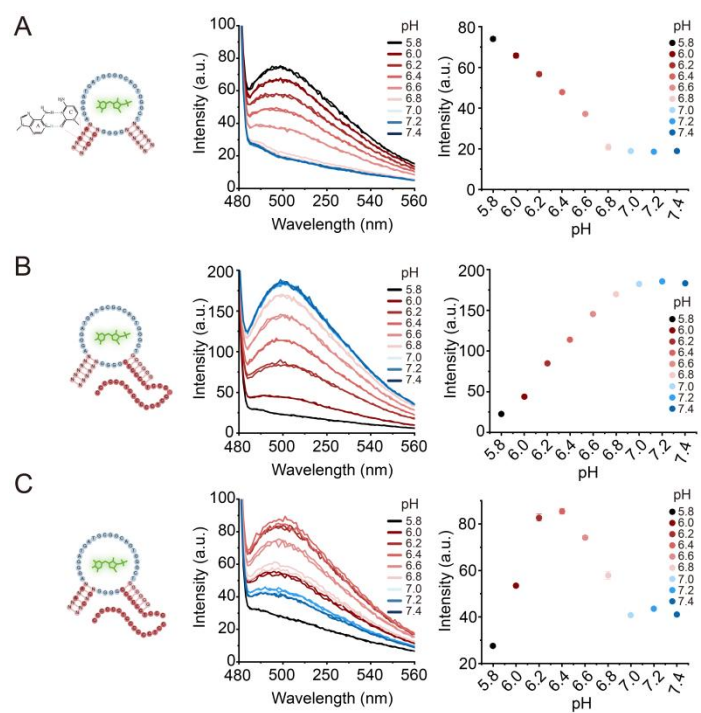


Figure S21. An fluorescence measurements of DNA Lettuce with acidic response (A), DNA Lettuce with alkaline response (B) and DNA Lettuce with narrow window pH response (C).

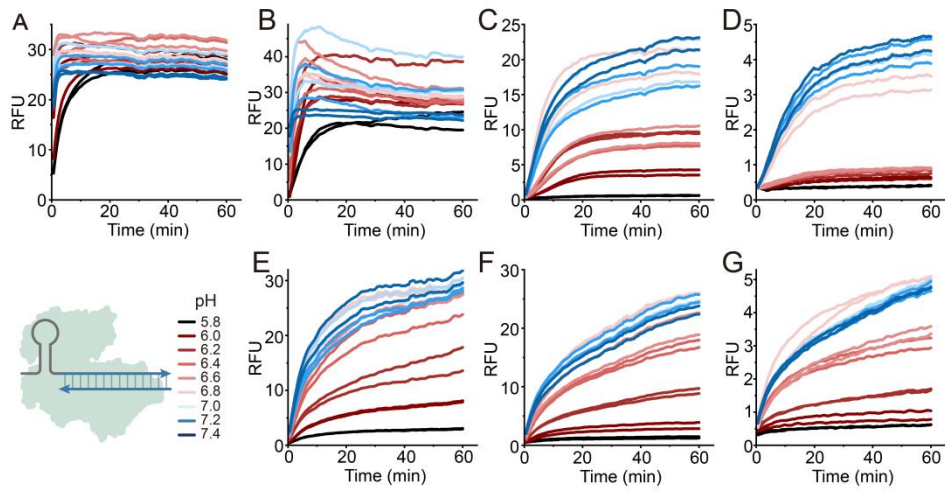


Figure S22. The curve of pH-dependent fluorescence across various concentrations of Cas enzyme and target RNA. (A) 10 nM Cas+10 nM targetRNA, (B) 2.5 nM Cas+10 nM targetRNA, (C) 1.25 nM Cas+10 nM targetRNA, (D) 0.625 nM Cas+10 nM targetRNA, (E) 10 nM Cas+2 nM targetRNA, (F) 10 nM Cas+0.4 nM targetRNA, (G) 10 nM Cas+0.08 nM targetRNA.

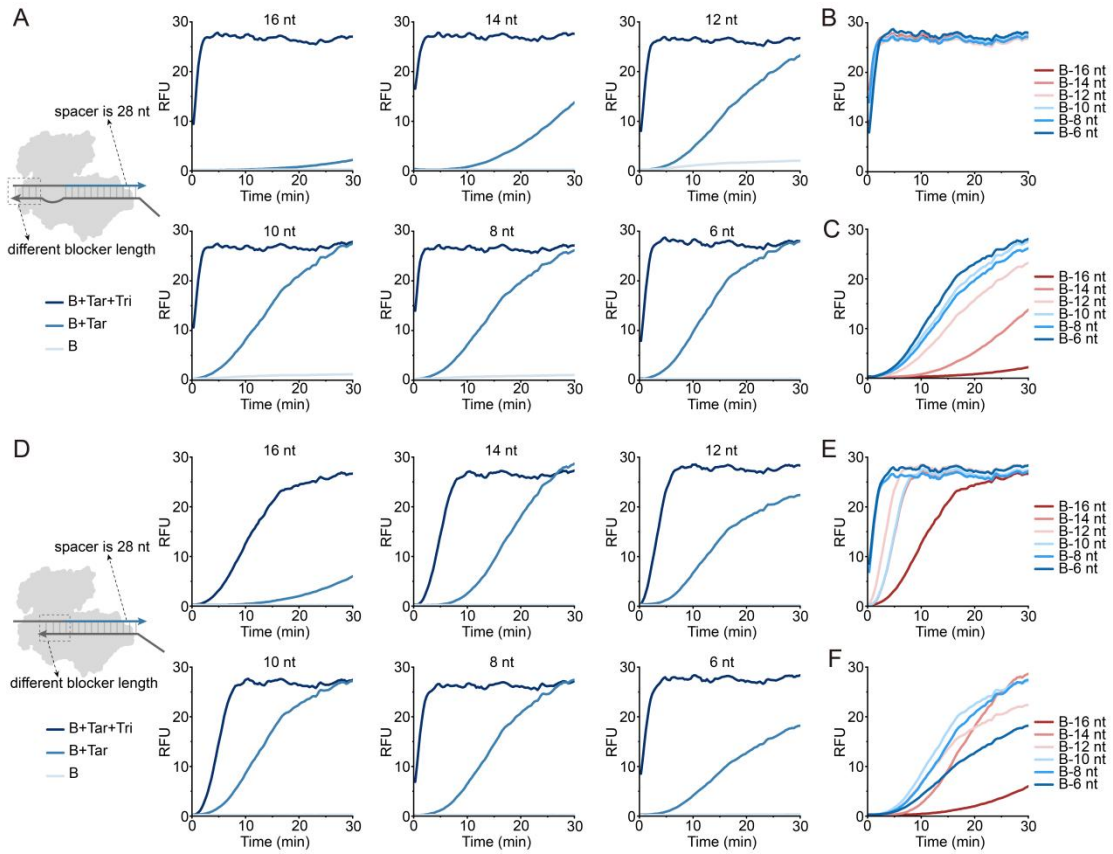


Figure S23. Fluorescence curves of blocker in the 5'-end (A-C) or 3'-end (D-F) of DR region when the spacer region of crRNA is 28nt. Comparison of trigger-induced deblocking signal (B, E, B+Tar+Tri) and the background signal (C, F, B+Tar).

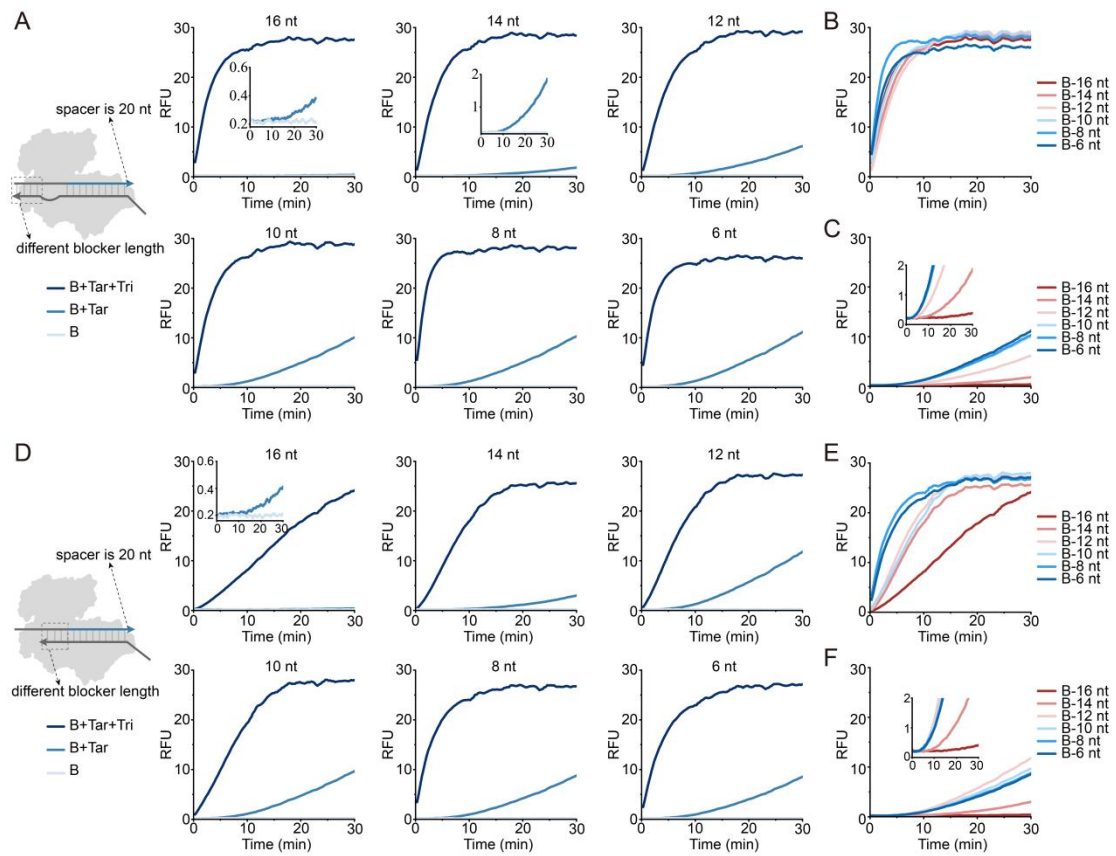


Figure S24. Fluorescence curves of blocker in the 5'-end (A-C) or 3'-end (D-F) of DR region when the spacer region of crRNA is 20nt. Comparison of trigger-induced deblocking signal (B, E, B+Tar+Tri) and the background signal (C, F, B+Tar).

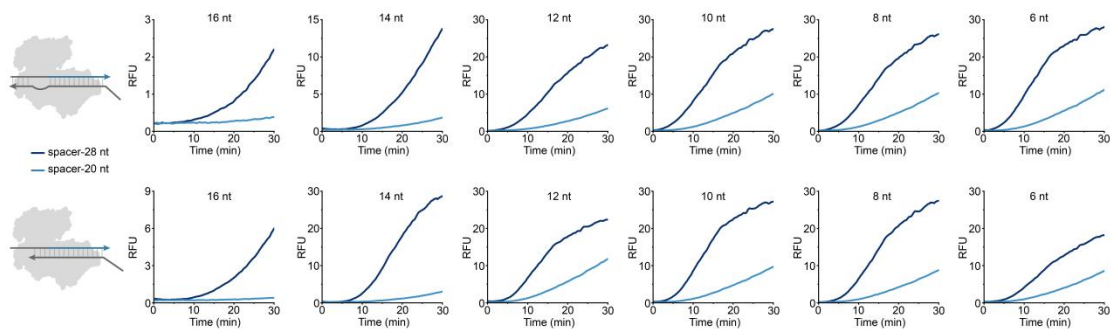


Figure S25. Comparison of spacers of crRNA with different blocker positions and lengths.

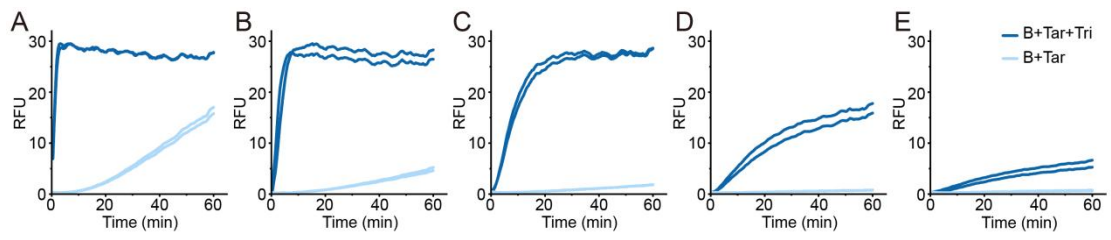


Figure S26. Fluorescence curves of target RNA concentrations at 50 nM (A), 10 nM (B), 2 nM (C), 0.4 nM (D), and 0.08 nM (E).

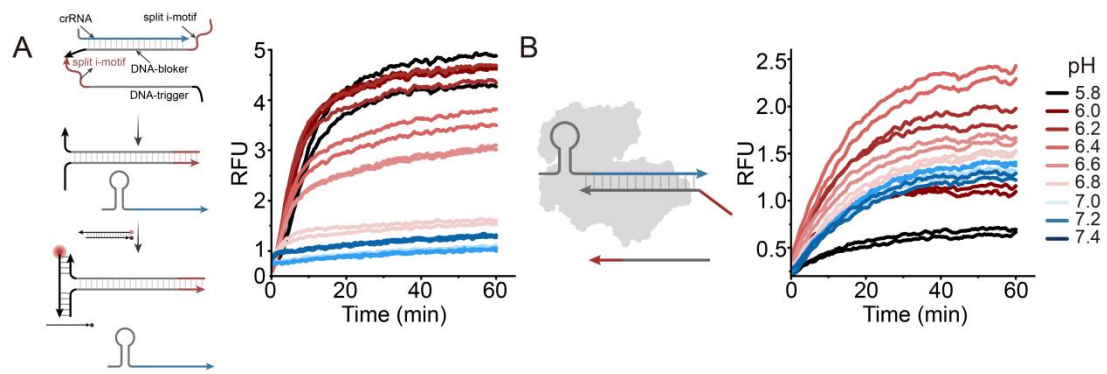


Figure S27. (A) Engineering a split i-motif into the toehold region of the TMSD reaction. (B) Synergizing the split i-motif-driven "Acidic-ON" logic with the intrinsic "Base-ON" activity of Cas enzyme.

Table S1. The sequences of DNA used in experiment. (Region 1 is bolded, and region 2 is underlined. The mismatch base is red. The C-A mismatches are green, the i-motif is blue, and the aptamer is italic.)

Name	Sequence (5'-3')
Tri-probe	TTCACATCATCGCAGGGTAGGTAGGTAGGGTCATAG
Tri-probe-1	TTCACATCAT
Tri-probe-2	CGCAGGGTAGGTAGGTAGGGTCATAG
Probe-ROX	/ROX/CTATGACCCTACCTACCTACCCTGCGATGATGTGAA
Probe-BHQ2	CGCAGGGTAGGTAGGTAGGGTCATAG/BHQ2/
R ₁ 3-1	TTCACATCATT TGTT
R ₁ 3-2	AACCGCAGGGTAGGTAGGTAGGGTCATAG
R ₁ 4-1	TTCACATCATT TGTT
R ₁ 4-2	AACCCGCAGGGTAGGTAGGTAGGGTCATAG
R ₁ 5-1	TTCACATCATT TAGTT
R ₁ 5-2	AACCTCGCAGGGTAGGTAGGTAGGGTCATAG
R ₁ 6-1	TTCACATCATT TAGTTC
R ₁ 6-2	GAACCTCGCAGGGTAGGTAGGTAGGGTCATAG
R ₁ 7-1	TTCACATCATT TAAGTTC
R ₁ 7-2	GAACCTTCGCAGGGTAGGTAGGTAGGGTCATAG
R ₁ 8-1	TTCACATCATT TCTAAGTT
R ₁ 8-2	AACCTTAGCGCAGGGTAGGTAGGTAGGGTCATAG
R ₁ 9-1	TTCACATCATT TCTAAGTTC
R ₁ 9-2	GAACCTTAGCGCAGGGTAGGTAGGTAGGGTCATAG
R ₁ 10-1	TTCACATCATT TCTAAGTTCA
R ₁ 10-2	TGAACCTTAGCGCAGGGTAGGTAGGTAGGGTCATAG
R ₁ 11-1	TTCACATCATT TCTAAGTTCAC
R ₁ 11-2	GTGAACCTTAGCGCAGGGTAGGTAGGTAGGGTCATAG
R ₁ 12-1	TTCACATCATT TCTAAGTTCACA
R ₁ 12-2	TGTGAACCTTAGCGCAGGGTAGGTAGGTAGGGTCATAG
R ₁ 13-1	TTCACATCATT TCCTAAGTTCACA
R ₁ 13-2	TGTGAACCTTAGGCGCAGGGTAGGTAGGTAGGGTCATA G
R ₁ 14-1	TTCACATCATT CTACTAAGTTCACA
R ₁ 14-2	TGTGAACCTTAGTGCAGGGTAGGTAGGTAGGGTCAT AG
R ₁ 15-1	TTCACATCATT CTACTAAGTTCACA

R ₁ 15-2	TGTGAACCTTAGTTGCGCAGGGTAGGTAGGTAGGGTCA TAG
R ₂ 18	<u>AGCGGTTGATTTTGTGGGC</u>
R ₁ 11-1-mismatch1	TTCACATCATTT CCTAAGCTTCACA
R ₁ 11-1-mismatch2	TTCACATCATTT CCTCAGCTTCACA
R ₁ 11-1-mismatch3	TTCACATCATTT CCTCAGCTACACA
R ₁ 3R ₂ 18-1	TTCACATCATTT <u>GTTTCAACCGCT</u>
R ₁ 3R ₂ 18-2	<u>GCCCAACAAAACCGCAGGGTAGGTAGGTAGGGTCATAG</u>
R ₁ 4R ₂ 18-1	TTCACATCATTT <u>GGTTCAACCGCT</u>
R ₁ 4R ₂ 18-2	<u>GCCCAACAAAACCGCAGGGTAGGTAGGTAGGGTCATA G</u>
R ₁ 5R ₂ 18-1	TTCACATCATTT <u>AGGTTCAACCGCT</u>
R ₁ 5R ₂ 18-2	<u>GCCCAACAAAACCTCGCAGGGTAGGTAGGTAGGGTCAT AG</u>
R ₁ 6R ₂ 18-1	TTCACATCATTT <u>AGGTTCTCAACCGCT</u>
R ₁ 6R ₂ 18-2	<u>GCCCAACAAGAACCTCGCAGGGTAGGTAGGTAGGGTCA TAG</u>
R ₁ 7R ₂ 18-1	TTCACATCATTT <u>AAGGTTCTCAACCGCT</u>
R ₁ 7R ₂ 18-2	<u>GCCCAACAAGAACCTTCGCAGGGTAGGTAGGTAGGGTC ATAG</u>
R ₁ 8R ₂ 18-1	TTCACATCATTT <u>CCTAAGGTTCAACCGCT</u>
R ₁ 8R ₂ 18-2	<u>GCCCAACAAAACCTTAGCGCAGGGTAGGTAGGTAGGGT CATAG</u>
R ₁ 9R ₂ 18-1	TTCACATCATTT <u>CCTAAGGTTCTCAACCGCT</u>
R ₁ 9R ₂ 18-2	<u>GCCCAACAAGAACCTTAGCGCAGGGTAGGTAGGTAGGG TCATAG</u>
R ₁ 10R ₂ 18-1	TTCACATCATTT <u>CCTAAGGTTCAATCAACCGCT</u>
R ₁ 10R ₂ 18-2	<u>GCCCAACAATGAACCTTAGCGCAGGGTAGGTAGGTAGG GTCATAG</u>
R ₁ 11R ₂ 18-1	TTCACATCATTT <u>CCTAAGGTTCACTCAACCGCT</u>
R ₁ 11R ₂ 18-2	<u>GCCCAACAAGTGAACCTTAGCGCAGGGTAGGTAGGTAG GGTCATAG</u>
R ₁ 12R ₂ 18-1	TTCACATCATTT <u>CCTAAGGTTACATCAACCGCT</u>
R ₁ 12R ₂ 18-2	<u>GCCCAACAATGTGAACCTTAGCGCAGGGTAGGTAGGTA GGGTCATAG</u>
R ₁ 13R ₂ 18-1	TTCACATCATTT CCTAAGGTTACATCAACCGCT
R ₁ 13R ₂ 18-2	<u>GCCCAACAATGTGAACCTTAGGCGCAGGGTAGGTAGGT AGGGTCATAG</u>
R ₁ 14R ₂ 18-1	TTCACATCATTT CACTAAGGTTACATCAACCGCT

R₁14R₂18-2 GCCCAACAATGTGAACCTTAGTGCGCAGGGTAGGTAG
GTAGGGTCATAG

R₁15R₂18-1 TTCACATCATTTCAACTAAGGTTCAATCAACCGCT

R₁15R₂18-2 GCCCAACAATGTGAACCTTAGTTGCGCAGGGTAGGTAG
GTAGGGTCATAG

R₂26 AAAGAGCGGTTGATATTTGTTGGGCGGCT

R₁3R₂26-1 TTCACATCATTTGTTTCAACCGCTCTTT

R₁3R₂26-2 AGCCGCCCAACAAAACCGCAGGGTAGGTAGGTAGGGTC
ATAG

R₁4R₂26-1 TTCACATCATTTGGTTTCAACCGCTCTTT

R₁4R₂26-2 AGCCGCCCAACAAAACCGCAGGGTAGGTAGGTAGGGT
CATAG

R₁5R₂26-1 TTCACATCATTTAGGTTTCAACCGCTCTTT

R₁5R₂26-2 AGCCGCCCAACAAAACCTCGCAGGGTAGGTAGGTAGGG
TCATAG

R₁6R₂26-1 TTCACATCATTTAGGTTCTCAACCGCTCTTT

R₁6R₂26-2 AGCCGCCCAACAAGAACCTCGCAGGGTAGGTAGGTAGG
GTCATAG

R₁7R₂26-1 TTCACATCATTTAAGGTTCTCAACCGCTCTTT

R₁7R₂26-2 AGCCGCCCAACAAGAACCTTCGCAGGGTAGGTAGGTAG
GGTCATAG

R₂6R₂26-1 TTCACATCATTTCTAAGGTTTCAACCGCTCTTT

R₂6R₂26-2 AGCCGCCCAACAAAACCTTAGCGCAGGGTAGGTAGGTA
GGGTCATAG

R₁9R₂26-1 TTCACATCATTTCTAAGGTTCTCAACCGCTCTTT

R₁9R₂26-2 AGCCGCCCAACAAGAACCTTAGCGCAGGGTAGGTAGGT
AGGGTCATAG

R₁10R₂26-1 TTCACATCATTTCTAAGGTTCAATCAACCGCTCTTT

R₁10R₂26-2 AGCCGCCCAACAATGAACCTTAGCGCAGGGTAGGTAGG
TAGGGTCATAG

R₁11R₂26-1 TTCACATCATTTCTAAGGTTCACTCAACCGCTCTTT

R₁11R₂26-2 AGCCGCCCAACAAGTGAACCTTAGCGCAGGGTAGGTAG
GTAGGGTCATAG

R₁12R₂26-1 TTCACATCATTTCTAAGGTTCAATCAACCGCTCTTT

R₁12R₂26-2 AGCCGCCCAACAATGTGAACCTTAGCGCAGGGTAGGTA
GGTAGGGTCATAG

R₁13R₂26-1 TTCACATCATTTCTAAGGTTCAATCAACCGCTCTTT

R₁13R₂26-2 AGCCGCCCAACAATGTGAACCTTAGGCGCAGGGTAGGT

AGGTAGGGTCATAG

R₁14R₂26-1 TTCACATCATTTCACTAAGGTTACATCAACCGCTCTTT

R₁14R₂26-2 AGCCGCCCAACAATGTGAACCTTAGTGCGCAGGGTAG
GTAGGTAGGGTCATAG

R₁15R₂26-1 TTCACATCATTTCAACTAAGGTTACATCAACCGCTCTT
T

R₁15R₂26-2 AGCCGCCCAACAATGTGAACCTTAGTTGCGCAGGGTA
GGTAGGTAGGGTCATAG

R₂36 GCTCCAAAGAGCGGTTGATATTTGTTGGGCGGCTCAGCT

R₁3R₂36-1 TTCACATCATTTGTTTCAACCGCTCTTTGGAGC

R₁3R₂36-2 AGCTGAGCCGCCCAACAAAACCGCAGGGTAGGTAGGTA
GGGTCATAG

R₁4R₂36-1 TTCACATCATTTGGTTTCAACCGCTCTTTGGAGC

R₁4R₂36-2 AGCTGAGCCGCCCAACAAAACCGCAGGGTAGGTAGGT
AGGGTCATAG

R₁5R₂36-1 TTCACATCATTTAGGTTTCAACCGCTCTTTGGAGC

R₁5R₂36-2 AGCTGAGCCGCCCAACAAAACCTCGCAGGGTAGGTAGG
TAGGGTCATAG

R₁6R₂36-1 TTCACATCATTTAGGTTCTCAACCGCTCTTTGGAGC

R₁6R₂36-2 AGCTGAGCCGCCCAACAAGAACCTCGCAGGGTAGGTAG
GTAGGGTCATAG

R₁7R₂36-1 TTCACATCATTTAAGGTTCTCAACCGCTCTTTGGAGC

R₁7R₂36-2 AGCTGAGCCGCCCAACAAGAACCTTCGCAGGGTAGGTA
GGTAGGGTCATAG

R₁8R₂36-1 TTCACATCATTTCTAAGGTTTCAACCGCTCTTTGGAGC

R₁8R₂36-2 AGCTGAGCCGCCCAACAAAACCTTAGCGCAGGGTAGGT
AGGTAGGGTCATAG

R₁9R₂36-1 TTCACATCATTTCTAAGGTTCTCAACCGCTCTTTGGAGC

R₁9R₂36-2 AGCTGAGCCGCCCAACAAGAACCTTAGCGCAGGGTAGG
TAGGTAGGGTCATAG

R₁10R₂36-1 TTCACATCATTTCTAAGGTTCAACCGCTCTTTGGAG
C

R₁10R₂36-2 AGCTGAGCCGCCCAACAATGAACCTTAGCGCAGGGTAG
GTAGGTAGGGTCATAG

R₁11R₂36-1 TTCACATCATTTCTAAGGTTCACTCAACCGCTCTTTGGA
GC

R₁11R₂36-2 AGCTGAGCCGCCCAACAAGTGAACCTTAGCGCAGGGTA
GGTAGGTAGGGTCATAG

R₁12R₂36-1 TTCACATCATTTCTAAGGTTACATCAACCGCTCTTTGG
AGC

R₁12R₂36-2 AGCTGAGCCGCCCAACAATGTGAACCTTAGCGCAGGGT

AGGTAGGTAGGGTCATAG

R₁13R₂36-1 TTCACATCATTTCTAAGGTTCACATCAACCGCTCTTTG
GAGC

R₁13R₂36-2 AGCTGAGCCGCCCAACAATGTGAACCTTAGGCGCAGG
 GTAGGTAGGTAGGGTCATAG

R₁14R₂36-1 TTCACATCATTTCACTAAGGTTCACATCAACCGCTCTTT
GGAGC

R₁14R₂36-2 AGCTGAGCCGCCCAACAATGTGAACCTTAGTGCGCAG
 GTAGGTAGGTAGGGTCATAG

R₁15R₂36-1 TTCACATCATTTCAACTAAGGTTCACATCAACCGCTCTT
TGGAGC

R₁15R₂36-2 AGCTGAGCCGCCCAACAATGTGAACCTTAGTTGCGCA
 GGGTAGGTAGGTAGGGTCATAG

R₂26-spacer0 AAAGAGCGGTTGATTGTTGGGCGGCT

R₂26-spacer1 AAAGAGCGGTTGATTTGTTGGGCGGCT

R₂26-spacer2 AAAGAGCGGTTGATTTTGTGGGCGGCT

R₂26-spacer4 AAAGAGCGGTTGATATATTGTTGGGCGGCT

R₂26-spacer5 AAAGAGCGGTTGATATATTGTTGGGCGGCT

R₁13R₂26-1-mis2 TTCACATCATTTCTACGGTACACATCAACCGCTCTTT

R₁13R₂26-1-mis3 TTCACATCATTTCCAAACGTACACATCAACCGCTCTTT

R₁13R₂26-1-mis4 TTCACATCATTTCCAAACGTACCCATCAACCGCTCTTT

R₂26-mis1 AAAGAGCCGTTGATATTTGTTGGGCGGCT

R₂26-mis2 AAAGAGCCGTTGATATTTGTTGCGCGGCT

R₂26-mis3 AAAGAGCGGTTGATATTTGTTGCGCGGCT

R₂26-mis4 AAACAGCGCTTGATATTTGTTGCGCGGCT

R₂26-mis5 AAAGAGCCGTTGATATTTGTTGCGCGGCT

R₁12R₂26-2-2C_A_{sp} AGCCGCCCAACAATGCGAACCTAGCGCAGGGTAGGT
 AGGTAGGGTCATAG

R₁13R₂26-2-2C_A_{sp} AGCCGCCCAACAATGTAAACCTCAGGCGCAGGGTAGGT
 AGGTAGGGTCATAG

R₁14R₂26-2-2C_A_{sp} AGCCGCCCAACAATGTAAACCTCAGTGCGCAGGGTAG
 GTAGGTAGGGTCATAG

R₁15R₂26-2-2C_A_{sp} AGCCGCCCAACAATGTAAACCTTAATTGCGCAGGGTAG
 GTAGGTAGGGTCATAG

R₁12R₂26-1-2C_A_{con} TTCACATCATTTCTAAAATTCACATCAACCGCTCTTT

R₁13R₂26-1-2C_A_{con} TTCACATCATTTCTAAAATTCACATCAACCGCTCTTT

R₁14R₂26-1-2C_A_{con} TTCACATCATTTCACTAAAATTCACATCAACCGCTCTTT

R₁15R₂26-1-2C_A_{con} TTCACATCATTTCAACTAAAATTCACATCAACCGCTCTT

R₁12R₂26-1-3CA_{sp} TTCACATCATTTCCAAAGTCCACATCAACCGCTCTTT
R₁13R₂26-1-3CA_{sp} TTCACATCATTTCCCAAGTCCACATCAACCGCTCTTT
R₁14R₂26-1-3CA_{sp} TTCACATCATTTCACCAAAGTCCACATCAACCGCTCTTT
R₁15R₂26-1-3CA_{sp} TTCACATCATTTCAACCAAAGTCCACATCAACCGCTCTT
I
R₁12R₂26-1-3CA_{con} TTCACATCATTTCTAAGACCCACATCAACCGCTCTTT
R₁13R₂26-1-3CA_{con} TTCACATCATTTCCTAAGACCCACATCAACCGCTCTTT
R₁14R₂26-1-3CA_{con} TTCACATCATTTCACTAAAACCTCACATCAACCGCTCTTT
R₁15R₂26-1-3CA_{con} TTCACATCATTTCAACTAAAACCTCACATCAACCGCTCTT
I
R₁12R₂26-2-4CA_{sp} AGCCGCCCAACAATATAAACCTAACGCAGGGTAGGTA
GGTAGGGTCATAG
R₁13R₂26-2-4CA_{sp} AGCCGCCCAACAATATAAACCTCAAGCGCAGGGTAGGT
AGGTAGGGTCATAG
R₁14R₂26-2-4CA_{sp} AGCCGCCCAACAATATAAACCTAATGCGCAGGGTAGG
TAGGTAGGGTCATAG
R₁15R₂26-2-4CA_{sp} AGCCGCCCAACAATATAAACCTAGTCGCGCAGGGTAG
GTAGGTAGGGTCATAG
R₁12R₂26-1-4CA_{con} TTCACATCATTTCTAAAACCCACATCAACCGCTCTTT
R₁13R₂26-1-4CA_{con} TTCACATCATTTCCTAAAACCCACATCAACCGCTCTTT
R₁14R₂26-1-4CA_{con} TTCACATCATTTCACTAAAACCCACATCAACCGCTCTTT
R₁15R₂26-1-4CA_{con} TTCACATCATTTCAACTAAAACCCACATCAACCGCTCTT
I
R₁15R₂26-2-5CA_{sp} AGCCGCCCAACAATATAAACCTAATCGCGCAGGGTAG
GTAGGTAGGGTCATAG
R₁15R₂26-1-5CA_{con} TTCACATCATTTCAACTAAAACCCACATCAACCGCTCTT
I
R₁15R₂26-2-5CA_{con} AGCCGCCCAACAATGTGAACCCTAGTTGCGCAGGGTA
GGTAGGTAGGGTCATAG
R₁12R₂26-1-reset /ROX/CTAAGGTTACATCAACCGCTCTTT
R₁12R₂26-2-reset AGCCGCCCAACAATGCGAACCCCTAG/BHQ2/
R₁14R₂26-1-G TTCACATCATTTACGAAGGTGCACATCAACCGCTCTTT
R₁14R₂26-1-T TTCACATCATTTCACTAATGTTACATCAACCGCTCTTT
R₁14R₂26-1-A TTCACATCATTTCACAAAAGTACACATCAACCGCTCTTT
R₁14R₂26-1-C TTCACATCATTTACCAACGTCCACATCAACCGCTCTTT
R₁14R₂26-2-G AGCCGCCCAACAATGTGGACGTTGGTGCGCAGGGTAG
GTAGGTAGGGTCATAG
R₁14R₂26-2-T AGCCGCCCAACAATGTGTACTTTTGTGCGCAGGGTAGG
TAGGTAGGGTCATAG
R₁14R₂26-2-A AGCCGCCCAACAATGTGAACATTAGTGCGCAGGGTAG

GTAGGTAGGGTCATAG

R ₁ 14R ₂ 26-2-C	<u>AGCCGCCCAACAATGTGCACCTTCGTGCGCAGGGTAG</u> GTAGGTAGGGTCATAG
R ₂ -imotif-C6	CCCCCTCCCCCTCCCCCTCCCCC
R ₁ 8R ₂ 16-1-C6	TTCACATCATTTCTAAGGTTGGGGGGAG
R ₁ 8R ₂ 16-2-C6	<u>GAGGGGGGAACCTTAGCGCAGGGTAGGTAGGTAGGGTC</u> ATAG
R ₁ 8R ₂ 18-1-C6	TTCACATCATTTCTAAGGTTGGGGGGAGG
R ₁ 8R ₂ 18-2-C6	<u>GGAGGGGGGAACCTTAGCGCAGGGTAGGTAGGTAGGGT</u> CATAG
R ₁ 8R ₂ 20-1-C6	TTCACATCATTTCTAAGGTTGGGGGGAGGG
R ₁ 8R ₂ 20-2-C6	<u>GGGAGGGGGGAACCTTAGCGCAGGGTAGGTAGGTAGG</u> GTCATAG
R ₁ 8R ₂ 20-1-C6-mis	TTCACATCATTTCTAAGGTTGGGTGGAGGG
R ₁ 8R ₂ 20-2-C6-mis	<u>GGGAGGTGGGAACCTTAGCGCAGGGTAGGTAGGTAGGG</u> TCATAG
R ₁ 8R ₂ 22-1-C6	TTCACATCATTTCTAAGGTTGGGGGGAGGGG
R ₁ 8R ₂ 22-2-C6	<u>GGGGAGGGGGGAACCTTAGCGCAGGGTAGGTAGGTAG</u> GGTCATAG
R ₁ 8R ₂ 22-1-C6-mis	TTCACATCATTTCTAAGGTTGGGGTGAGGGG
R ₁ 8R ₂ 22-2-C6-mis	<u>GGGGAGTGGGGAAACCTTAGCGCAGGGTAGGTAGGTAGG</u> GTCATAG
R ₁ 10R ₂ 16-1-C6	TTCACATCATTTCTAAGGTTTCAGGGGGGAG
R ₁ 10R ₂ 16-2-C6	<u>GAGGGGGGTGAACCTTAGCGCAGGGTAGGTAGGTAGG</u> GTCATAG
R ₁ 10R ₂ 18-1-C6	TTCACATCATTTCTAAGGTTTCAGGGGGGAGG
R ₁ 10R ₂ 18-2-C6	<u>GGAGGGGGGTGAACCTTAGCGCAGGGTAGGTAGGTAG</u> GGTCATAG
R ₁ 10R ₂ 20-1-C6	TTCACATCATTTCTAAGGTTTCAGGGGGGAGGG
R ₁ 10R ₂ 20-2-C6	<u>GGGAGGGGGGTGAACCTTAGCGCAGGGTAGGTAGGTA</u> GGGTCATAG
R ₁ 10R ₂ 20-1-C6-mis	TTCACATCATTTCTAAGGTTTCAGGGTGGAGGG
R ₁ 10R ₂ 20-2-C6-mis	<u>GGGAGGTGGGTGAACCTTAGCGCAGGGTAGGTAGGTAG</u> GGTCATAG
R ₁ 10R ₂ 22-1-C6	TTCACATCATTTCTAAGGTTTCAGGGGGGAGGGG
R ₁ 10R ₂ 22-2-C6	<u>GGGGAGGGGGGTGAACCTTAGCGCAGGGTAGGTAGGT</u> AGGGTCATAG
R ₁ 10R ₂ 22-1-C6-mis	TTCACATCATTTCTAAGGTTTCAGGGGTGAGGGG
R ₁ 10R ₂ 22-2-C6-mis	<u>GGGGAGTGGGGTGAACCTTAGCGCAGGGTAGGTAGGTA</u> GGGTCATAG

R ₂ -imotif-C5	CCCCCTTCCCCCTTCCCCCTTCCCC
R ₁ 8R ₂ 16-1-C5	TTCACATCATTTCTAAGGTTGGGGGAAA
R ₁ 8R ₂ 16-2-C5	<u>AAAGGGGGAACCTTAGCGCAGGGTAGGTAGGGT</u> ATAG
R ₁ 8R ₂ 18-1-C5	TTCACATCATTTCTAAGGTTGGGGGAAAG
R ₁ 8R ₂ 18-2-C5	<u>GAAAGGGGGAACCTTAGCGCAGGGTAGGTAGGGT</u> CATAG
R ₁ 8R ₂ 20-1-C5	TTCACATCATTTCTAAGGTTGGGGGAAAGG
R ₁ 8R ₂ 20-2-C5	<u>GGAAAGGGGGAACCTTAGCGCAGGGTAGGTAGG</u> GTCATAG
R ₁ 8R ₂ 20-1-C5-mis	TTCACATCATTTCTAAGGTTGGGTGAAAGG
R ₁ 8R ₂ 20-2-C5-mis	<u>GGAAAGTGGGAACCTTAGCGCAGGGTAGGTAGGG</u> TCATAG
R ₁ 8R ₂ 22-1-C5	TTCACATCATTTCTAAGGTTGGGGGAAAGGG
R ₁ 8R ₂ 22-2-C5	<u>GGGAAAGGGGGAACCTTAGCGCAGGGTAGGTAG</u> GGTCATAG
R ₁ 8R ₂ 22-1-C5-mis	TTCACATCATTTCTAAGGTTGGGTGAAAGGG
R ₁ 8R ₂ 22-2-C5-mis	<u>GGGAAAGTGGGAACCTTAGCGCAGGGTAGGTAGG</u> GTCATAG
R ₁ 10R ₂ 16-1-C5	TTCACATCATTTCTAAGGTTTCAGGGGGAAA
R ₁ 10R ₂ 16-2-C5	<u>AAAGGGGGTGAACCTTAGCGCAGGGTAGGTAGG</u> GTCATAG
R ₁ 10R ₂ 18-1-C5	TTCACATCATTTCTAAGGTTTCAGGGGGAAAG
R ₁ 10R ₂ 18-2-C5	<u>GAAAGGGGGTGAACCTTAGCGCAGGGTAGGTAG</u> GGTCATAG
R ₁ 10R ₂ 20-1-C5	TTCACATCATTTCTAAGGTTTCAGGGGGAAAGG
R ₁ 10R ₂ 20-2-C5	<u>GGAAAGGGGGTGAACCTTAGCGCAGGGTAGGTAG</u> GGTCATAG
R ₁ 10R ₂ 20-1-C5-mis	TTCACATCATTTCTAAGGTTTCAGGGGTGAAAGG
R ₁ 10R ₂ 20-2-C5-mis	<u>GGAAAGTGGGTGAACCTTAGCGCAGGGTAGGTAG</u> GGTCATAG
R ₁ 10R ₂ 22-1-C5	TTCACATCATTTCTAAGGTTTCAGGGGGAAAGGG
R ₁ 10R ₂ 22-2-C5	<u>GGGAAAGGGGGTGAACCTTAGCGCAGGGTAGGTAG</u> AGGGTCATAG
R ₁ 10R ₂ 22-1-C5-mis	TTCACATCATTTCTAAGGTTTCAGGGGTGAAAGGG
R ₁ 10R ₂ 22-2-C5-mis	<u>GGGAAAGTGGGTGAACCTTAGCGCAGGGTAGGTAG</u> GGTCATAG
R ₂ -imotif-C4	CCCCTAACCCTAACCCTAACCCTAACCCT
R ₁ 8R ₂ 16-1-C4	TTCACATCATTTCTAAGGTTGGGGTTAG
R ₁ 8R ₂ 16-2-C4	<u>GTTAGGGGAACCTTAGCGCAGGGTAGGTAGGGT</u> C

ATAG

R ₁ 8R ₂ 18-1-C4	<u>TTCACATCATTTCTAAGGTTGGGGTTAGG</u>
R ₁ 8R ₂ 18-2-C4	<u>GGTTAGGGGAACCTTAGCGCAGGGTAGGTAGGGT</u> CATAG
R ₁ 8R ₂ 20-1-C4	<u>TTCACATCATTTCTAAGGTTGGGGTTAGGG</u>
R ₁ 8R ₂ 20-2-C4	<u>GGGTTAGGGGAACCTTAGCGCAGGGTAGGTAGGG</u> TCATAG
R ₁ 8R ₂ 20-1-C4-mis	<u>TTCACATCATTTCTAAGGTTGGGTTTAGGG</u>
R ₁ 8R ₂ 20-2-C4-mis	<u>GGGTTATGGGAACCTTAGCGCAGGGTAGGTAGGG</u> TCATAG
R ₁ 8R ₂ 22-1-C4	<u>TTCACATCATTTCTAAGGTTGGGGTTAGGGG</u>
R ₁ 8R ₂ 22-2-C4	<u>GGGGTTAGGGGAACCTTAGCGCAGGGTAGGTAGGTAGG</u> GTCATAG
R ₁ 8R ₂ 22-1-C4-mis	<u>TTCACATCATTTCTAAGGTTGGGTTTAGGGG</u>
R ₁ 8R ₂ 22-2-C4-mis	<u>GGGGTTATGGGAACCTTAGCGCAGGGTAGGTAGGTAGG</u> GTCATAG
R ₁ 10R ₂ 16-1-C4	<u>TTCACATCATTTCTAAGGTTTCAGGGGTTAG</u>
R ₁ 10R ₂ 16-2-C4	<u>GTTAGGGGTGAACCTTAGCGCAGGGTAGGTAGGTAGGG</u> TCATAG
R ₁ 10R ₂ 18-1-C4	<u>TTCACATCATTTCTAAGGTTTCAGGGGTTAGG</u>
R ₁ 10R ₂ 18-2-C4	<u>GGTTAGGGGTGAACCTTAGCGCAGGGTAGGTAGGTAGG</u> GTCATAG
R ₁ 10R ₂ 20-1-C4	<u>TTCACATCATTTCTAAGGTTTCAGGGGTTAGGG</u>
R ₁ 10R ₂ 20-2-C4	<u>GGGTTAGGGGTGAACCTTAGCGCAGGGTAGGTAGGTAG</u> GGTCATAG
R ₁ 10R ₂ 20-1-C4-mis	<u>TTCACATCATTTCTAAGGTTTCAGGGTTTAGGG</u>
R ₁ 10R ₂ 20-2-C4-mis	<u>GGGTTATGGGTGAACCTTAGCGCAGGGTAGGTAGGTAG</u> GGTCATAG
R ₁ 10R ₂ 22-1-C4	<u>TTCACATCATTTCTAAGGTTTCAGGGGTTAGGGG</u>
R ₁ 10R ₂ 22-2-C4	<u>GGGGTTAGGGGTGAACCTTAGCGCAGGGTAGGTAGGTA</u> GGGTCATAG
R ₁ 10R ₂ 22-1-C4-mis	<u>TTCACATCATTTCTAAGGTTTCAGGGTTTAGGGG</u>
R ₁ 10R ₂ 22-2-C4-mis	<u>GGGGTTATGGGTGAACCTTAGCGCAGGGTAGGTAGGTA</u> GGGTCATAG
R ₁ 8R ₂ 20-1-reset	<u>/ROX/CTAAGGTTGGGGGGAGGG</u>
R ₁ 8R ₂ 20-2-reset	<u>GGGAGGGGGGAACCTTAG/BHQ2/</u>
PRISM-1-3CA	<u>TTCACATCATTTACCAAAGTCCACAGGGGTTAGGGGA</u> <u>G</u>
PRISM-2	<u>GAGGGTTTAGGGGTGTGAACCTTAGTGCGCAGGGTAG</u> GTAGGTAGGGTCATAG

PRISM-3 CTCCCCTAACCCCTAACCCCTAACCCCTC

PRISM-4-3CA TTCACATCATTTCACCAAAGTCCACAGGGGGGAGGGGG
GAA

PRISM-5 AAGGGGGGAGTGGGGTGTGAACCTTAGTGCGCAGGGT
AGGTAGGTAGGGTCATAG

PRISM-6 TCCCCCTCCCCCTCCCCCTCCCCCT

PRISM-7 TTCACATCATTCTAAGGTTACAGGGGGGAGGGGGGA
A

PRISM-8-3CA AAGGGGGGAGTGGGGTGCGAACCCTAGCGCAGGGTAG
GTAGGTAGGGTCATAG

PRISM-9 TTCACATCATTCTAAGGTTACAGGGGGGAAAGGGGG

PRISM-10-2CA GGGGGAAAGGGGGGTGCGAACCTAGCGCAGGGTAGG
TAGGTAGGGTCATAG

PRISM-11 CCCCCTCCCCCTCCCCCTCCCCC

PRISM-12 TTCACATCATTCTAAGTTCACAGGGGGGAGGGGGGAA

PRISM-13-2CA AAGGGGGGAGTGGGGTGCGAACCCTAGCGCAGGGTAGG
TAGGTAGGGTCATAG

PRISM-14-3CA GGGGGAAAGTGGGGTGCGAACCCTAGCGCAGGGTAGG
TAGGTAGGGTCATAG

PRISM₄-1-reset /ROX/CTAAGGTTACAGGGGGGAAAGGGGG

PRISM₄-2-reset GGGGGAAAGGGGGGTGCGAACCTAG/BHQ2/

Lettuce CTTAGTAGGGATGATGCGGCAGTGGGCTTCGCAGT

Le-R₁10R₂10-1 TTCTCTTAGTAGGGATGATGCGGCAGTGGGCTCGCAGG
CTCT

Le-R₁10R₂10-2 AGAGCCTGCGGGGGACTAAGAGAA

Le-R₁10R₂8-1 TTCTCTTAGTAGGGATGATGCGGCAGTGGGCTCGCAGG
CT

Le-R₁8R₂10-2 AGAGCCTGCGGGGGACTAAGAG

Le-R₁10R₂6-1 TTCTCTTAGTAGGGATGATGCGGCAGTGGGCTCGCAGG

Le-R₁6R₂10-2 AGAGCCTGCGGGGGACTAAG

Le-R₁10R₂4-1 TTCTCTTAGTAGGGATGATGCGGCAGTGGGCTCGCA

Le-R₁4R₂10-2 AGAGCCTGCGGGGGACTA

Le-R₁10R₂2-1 TTCTCTTAGTAGGGATGATGCGGCAGTGGGCTCG

Le-R₁2R₂10-2 AGAGCCTGCGGGGGAC

Le-R₁10R₂0-1 TTCTCTTAGTAGGGATGATGCGGCAGTGGGCT

Le-R₁0R₂10-2 AGAGCCTGCGGGGG

Le-R₁10R₂10-2-2CA AGAGCCTGCGGGGGACCAAAGAA

Le-R₁10R₂10_{motif}-1 TTCTCTTAGTAGGGATGATGCGGCAGTGGGCTAGGGGG
GAGG

Le-R ₁ 10R ₂ 10 _{imotif-2}	CCCCCCTCCCCCCTCCCCCCTCCCCCCTGGGGACTAAGA GAA
Le-R ₁ 10R ₂ 10 _{imotif-2-2C} A	CCCCCCTCCCCCCTCCCCCCTCCCCCCTGGGGACCAAAA GAA
R ₁ 12R ₂ 26-1-apt	ATCAGGCTGGATGGTAGCTCGGTCGGGGTGGGTGGGTTGG CAAGTCTGATTCACATCATTTCTAAGGTTACATCAACC GCTCTTT
R ₁ 8R ₂ 20-1-C6-apt	ATCAGGCTGGATGGTAGCTCGGTCGGGGTGGGTGGGTTGG CAAGTCTGATTCACATCATTTCTAAGGTTGGGGGGAGG G
PRISM-9-apt	ATCAGGCTGGATGGTAGCTCGGTCGGGGTGGGTGGGTTGG CAAGTCTGATTCACATCATTTCTAAGGTTACAGGGGG GAAAGGGGG
PRISM-9-apt-random	AACACCCTGGTTGGTAGCTCCCTCGGGGTGGGAGCCTTGG CAAGTCACATTCACATCATTTCTAAGGTTACAGGGGG GAAAGGGGG
Probe-Cy5	/Cy5/CTATGACCCTACCTACCTACCCTGCGATGATGTGAA
Probe-BHQ3	CGCAGGGTAGGTAGGTAGGGTCATAG/BHQ3/
Cr-28	GACCACCCCAAAAAUGAAGGGGACUAAAACCGCCACA AGCUCCAACUACCACAAGUUU
Cr-20	GACCACCCCAAAAAUGAAGGGGACUAAAAC CGCCACAAGCUCCAACUACC
RNA-tar	AAACUUGUGGUAGUUGGAGCUUGUGGCG
B-5-16	AAACTTGTGGTAGTTGGAGCTTGTGGCGCATTTTTGGGG TGCTGGAAGGAGG
B-5-14	AAACTTGTGGTAGTTGGAGCTTGTGGCGTTTTTGGGGTG GTCTGGAAGGAGG
B-5-12	AAACTTGTGGTAGTTGGAGCTTGTGGCGTTTTGGGGTGGT CTGGAAGGAGG
B-5-10	AAACTTGTGGTAGTTGGAGCTTGTGGCGTGGGGTGGTCT GGAAGGAGG
B-5-8	AAACTTGTGGTAGTTGGAGCTTGTGGCGGGTGGTCTGG AAGGAGG
B-5-6	AAACTTGTGGTAGTTGGAGCTTGTGGCGGTGGTCTGGAA GGAGG
B-3-16	AAACTTGTGGTAGTTGGAGCTTGTGGCGTTTTAGTCCC CTTCATGGAAGGAGG
B-3-14	AAACTTGTGGTAGTTGGAGCTTGTGGCGTTTTAGTCCC CTTTGGAAGGAGG
B-3-12	AAACTTGTGGTAGTTGGAGCTTGTGGCGTTTTAGTCCC CTGGAAGGAGG
B-3-10	AAACTTGTGGTAGTTGGAGCTTGTGGCGTTTTAGTCCT GGAAGGAGG

B-3-8	AAACTTGTGGTAGTTGGAGCTTGTGGCGGTTTTAGTTGG AAGGAGG
B-3-6	AAACTTGTGGTAGTTGGAGCTTGTGGCGGTTTTATGGAA GGAGG
Tri-5-16	CCTCCTTCCAGACCACCCCAAAAATGCGCCACAAGCTCC AACTACCACAAGTTT
Tri-5-14	CCTCCTTCCAGACCACCCCAAAAACGCCACAAGCTCCA ACTACCACAAGTTT
Tri-5-12	CCTCCTTCCAGACCACCCCAAACGCCACAAGCTCCA ACTACCACAAGTTT
Tri-5-10	CCTCCTTCCAGACCACCCCAACGCCACAAGCTCCA ACTACCACAAGTTT
Tri-5-8	CCTCCTTCCAGACCACCCCGCCACAAGCTCCA ACTACCACAAGTTT
Tri-5-6	CCTCCTTCCAGACCACCGCCACAAGCTCCA ACTACCACAAGTTT
Tri-3-16	CCTCCTTCCATGAAGGGGACTAAAACCGCCACAAGCTCC AACTACCACAAGTTT
Tri-3-14	CCTCCTTCCAAAGGGGACTAAAACCGCCACAAGCTCCA ACTACCACAAGTTT
Tri-3-12	CCTCCTTCCAGGGGACTAAAACCGCCACAAGCTCCA ACTACCACAAGTTT
Tri-3-10	CCTCCTTCCAGGACTAAAACCGCCACAAGCTCCA ACTACCACAAGTTT
Tri-3-8	CCTCCTTCCAATAAAAACCGCCACAAGCTCCA ACTACCACAAGTTT
Tri-3-6	CCTCCTTCCATAAAAACCGCCACAAGCTCCA ACTACCACAAGTTT
B-5-14-imotif-probe	CCTCCTTCCAAATACTTGGTAGTTGGAGCTTGTGGCGTT TTTGGGGTGGTCCCCCTCCCC
Tri-5-16-imotif-probe	CCCCCTCCCCCGACCACCCCAAAAACGCCACAAGCT CCAATACTACCATACTCCCCAGGTG
B-5-14-imotif	GGTAGTTGGAGCTTGTGGCGTTTTTGGGGTGGTCCCC CTCCCC
Tri-5-16-imotif	CCCCCTCCCCCGACCACCCCAAAAACGCCACAAGCT CCAATACTACC
Cas-reporter	/ROX/UUUUU/BHQ2/