Supplementary Information for

Co-allosteric hairpin probe for detecting microRNA with high specificity

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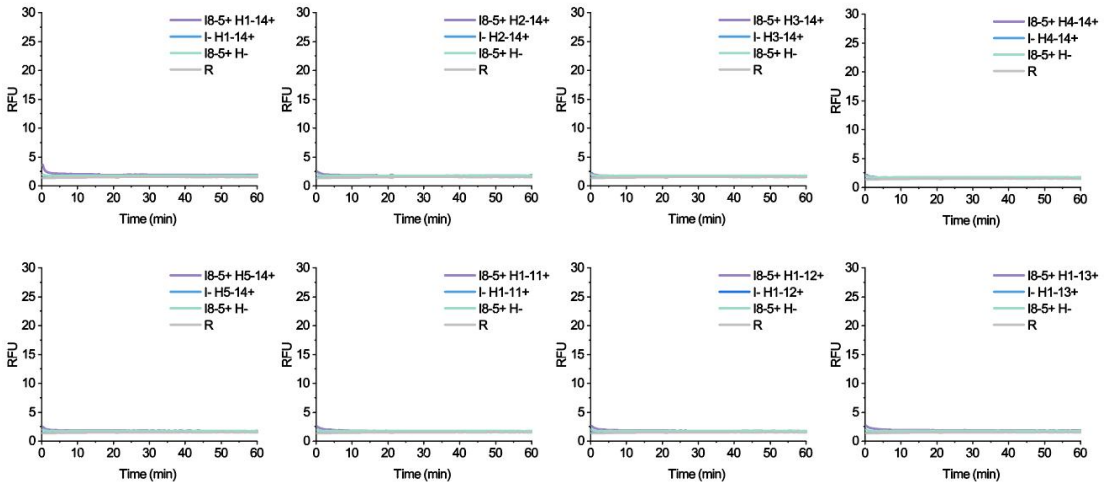

Figure S1. CA-HP of I8-5.

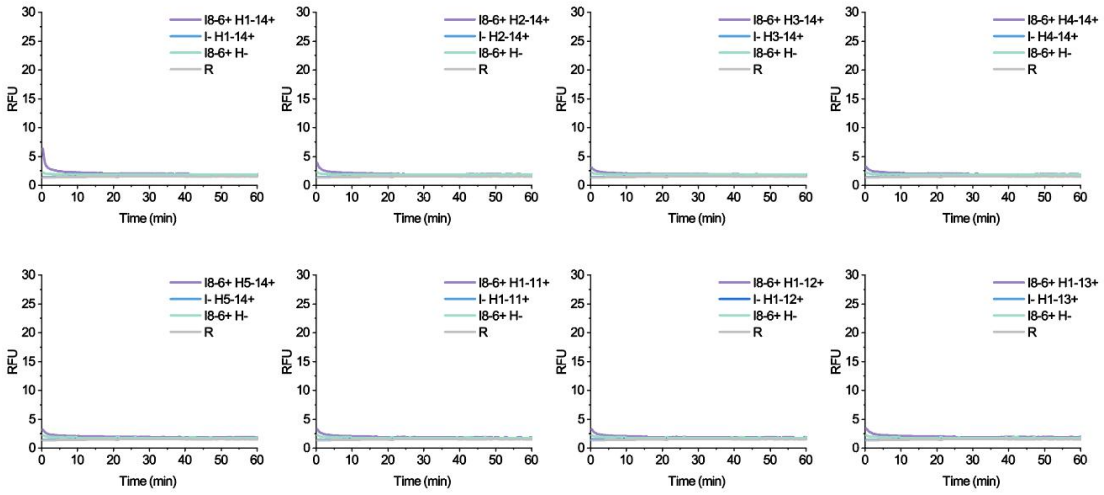

Figure S2. CA-HP of I8-6.

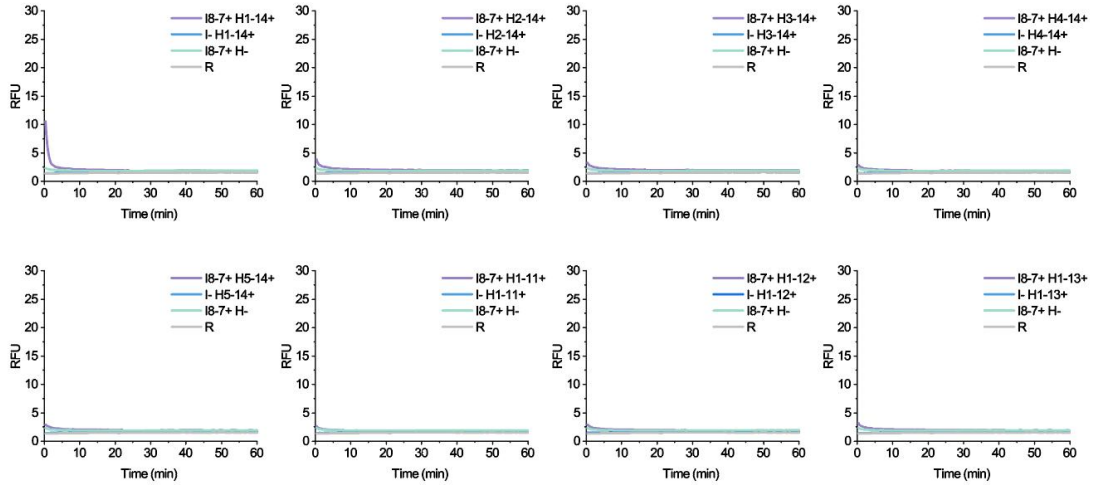

Figure S3. CA-HP of I8-7.

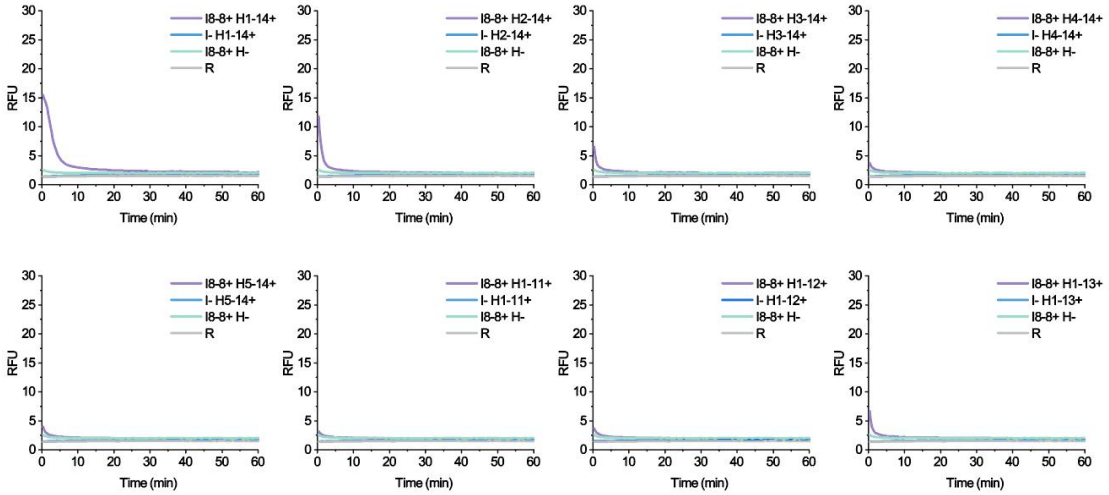

Figure S4. CA-HP of I8-8.

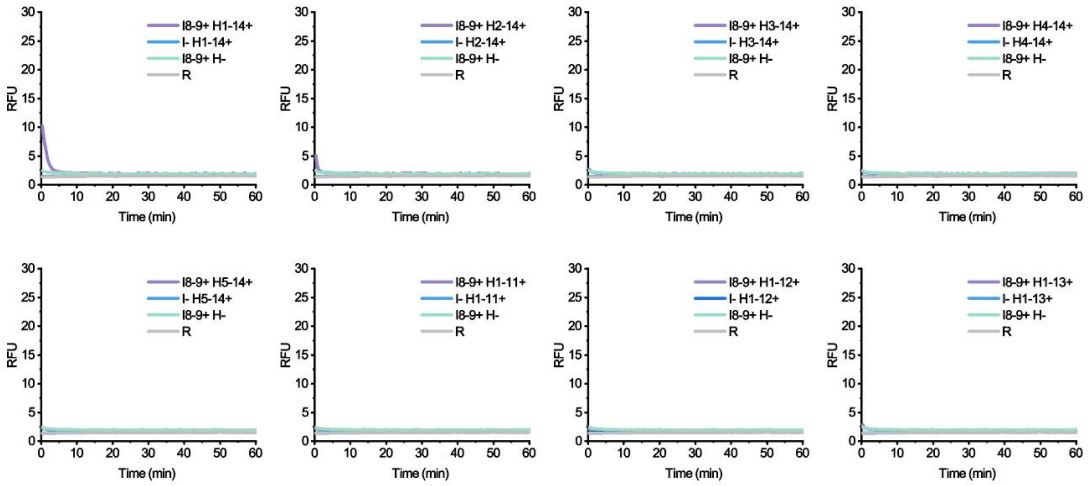

Figure S5. CA-HP of I8-9.

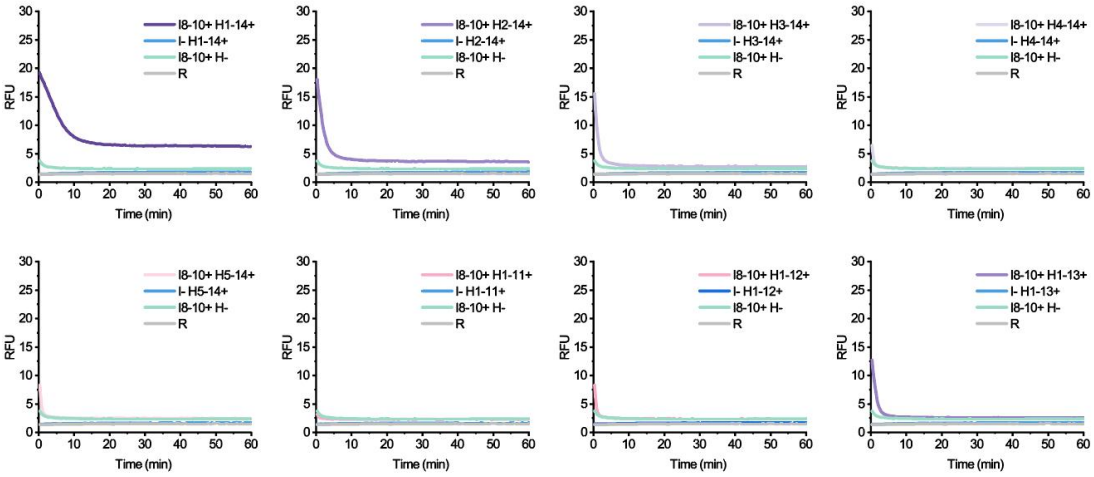

Figure S6. CA-HP of I8-10.

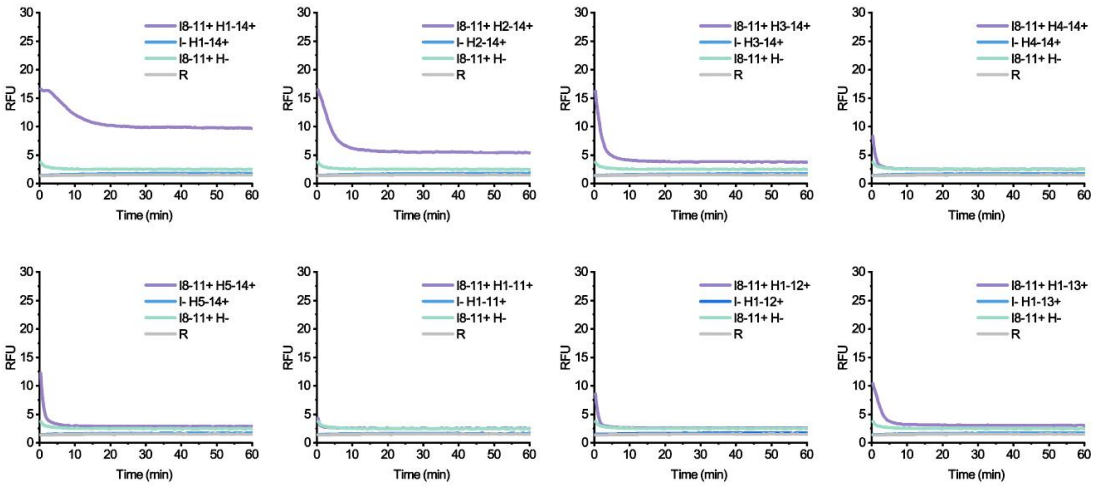

Figure S7. CA-HP of I8-11.

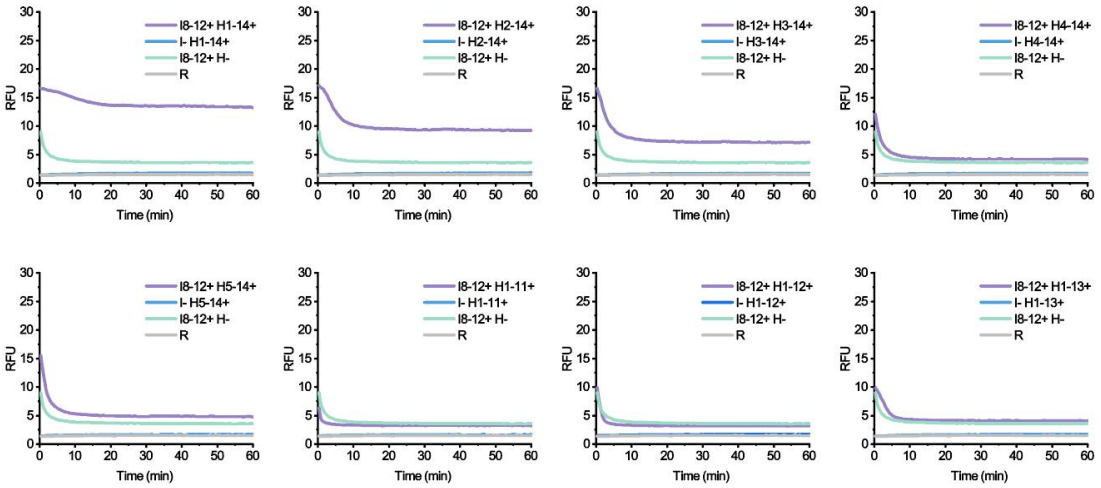

Figure S8. CA-HP of I8-12.

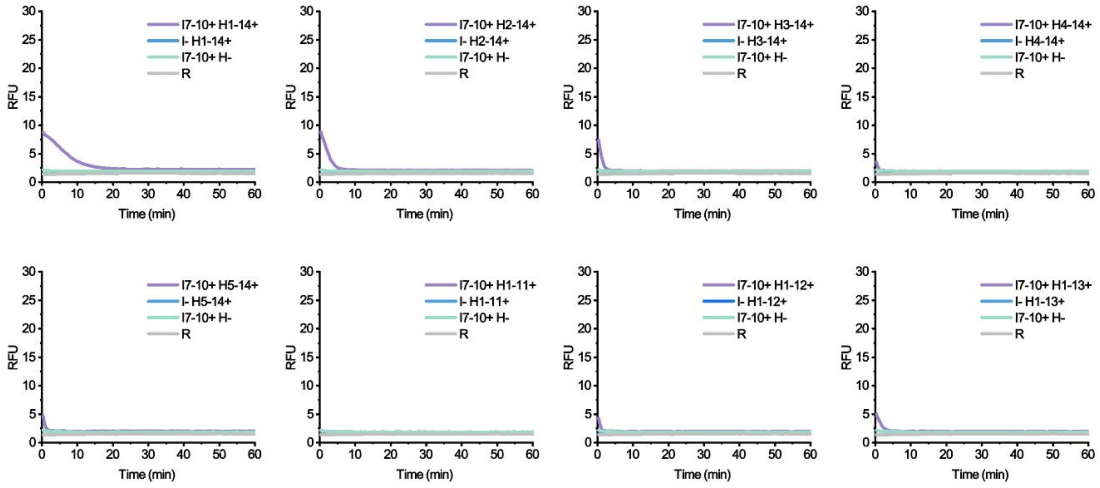

Figure S9. CA-HP of I7-10.

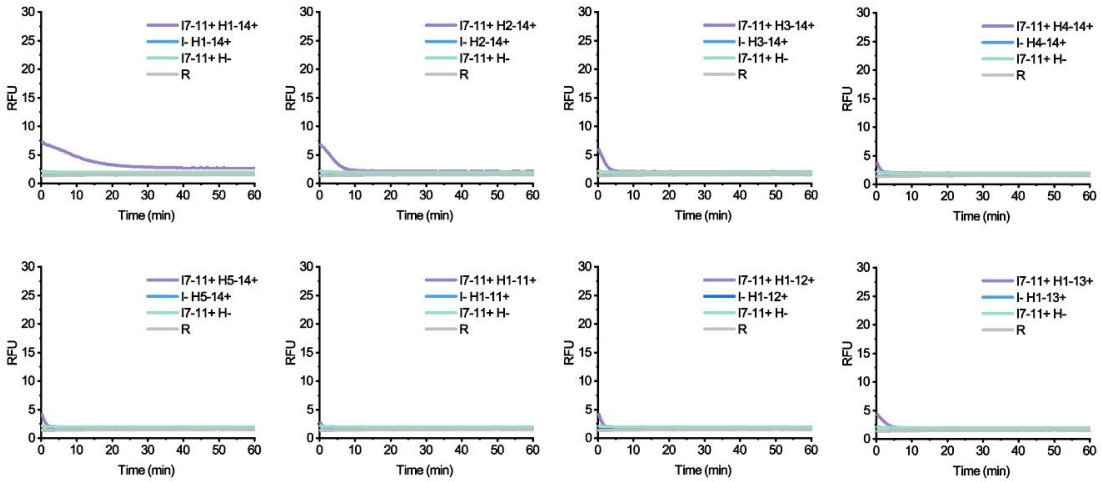

Figure S10. CA-HP of I7-11.

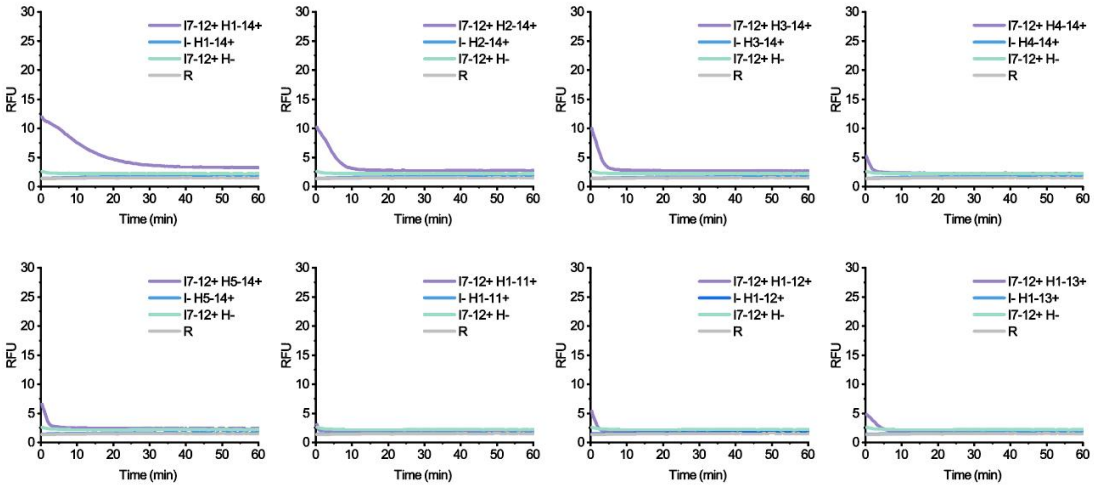

Figure S11. CA-HP of I7-12.

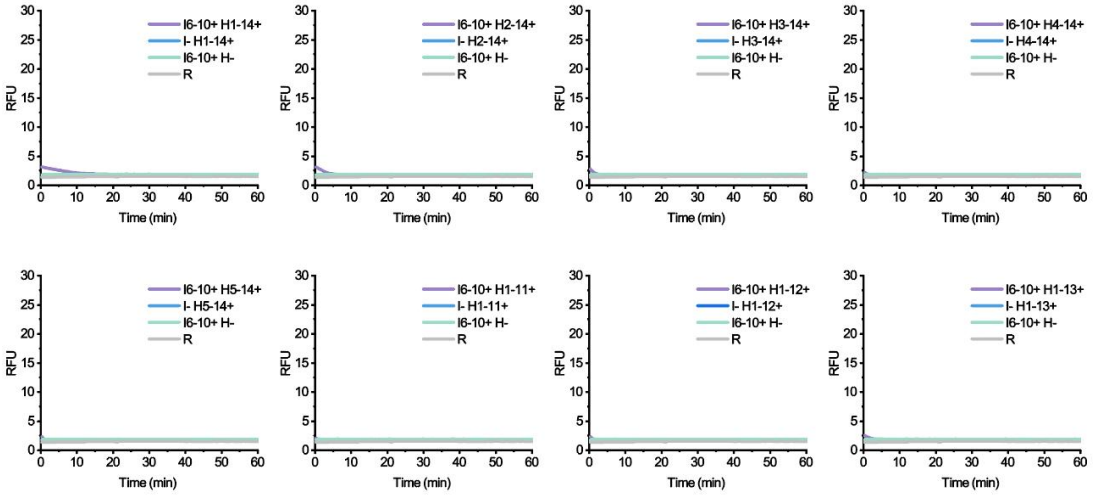

Figure S12. CA-HP of I6-10.

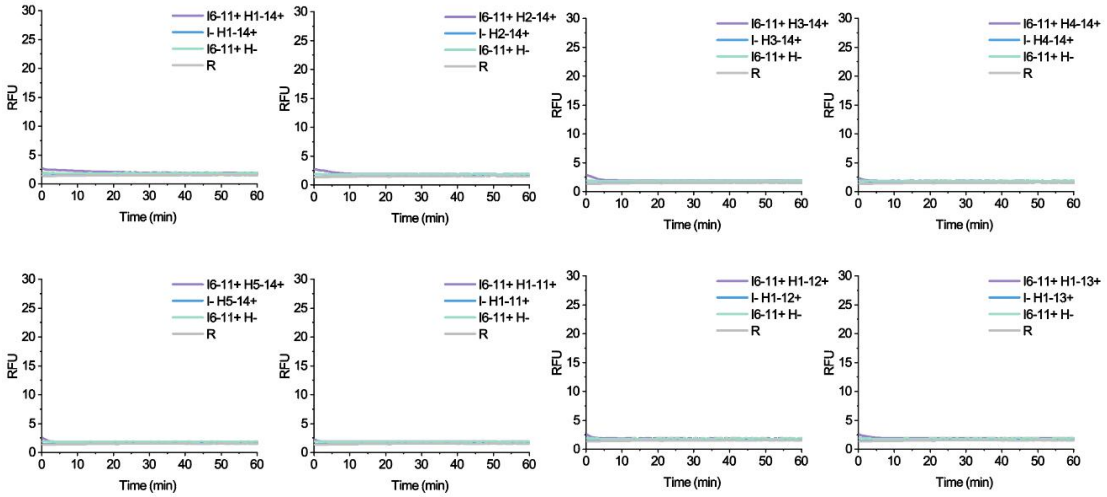

Figure S13. CA-HP of I6-11.

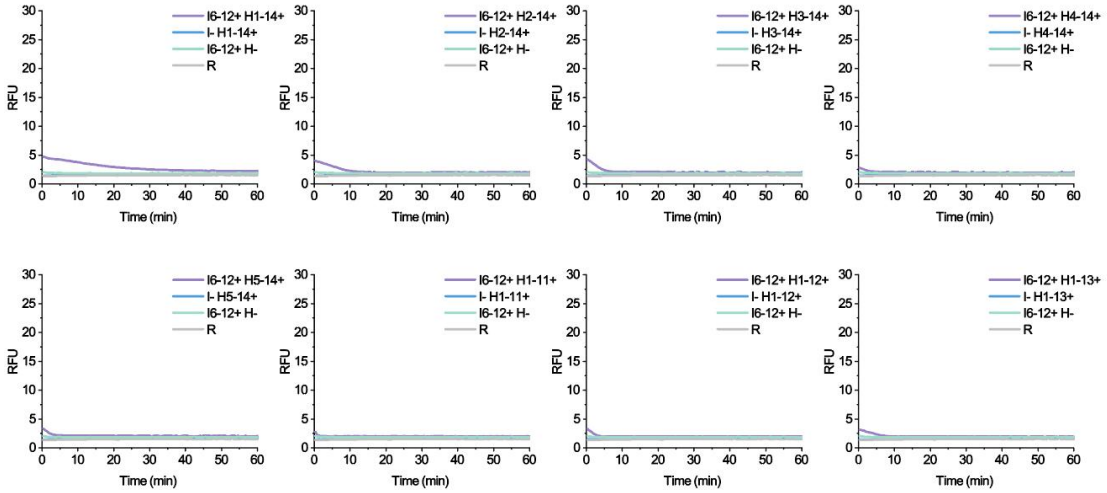

Figure S14. CA-HP of I6-12.

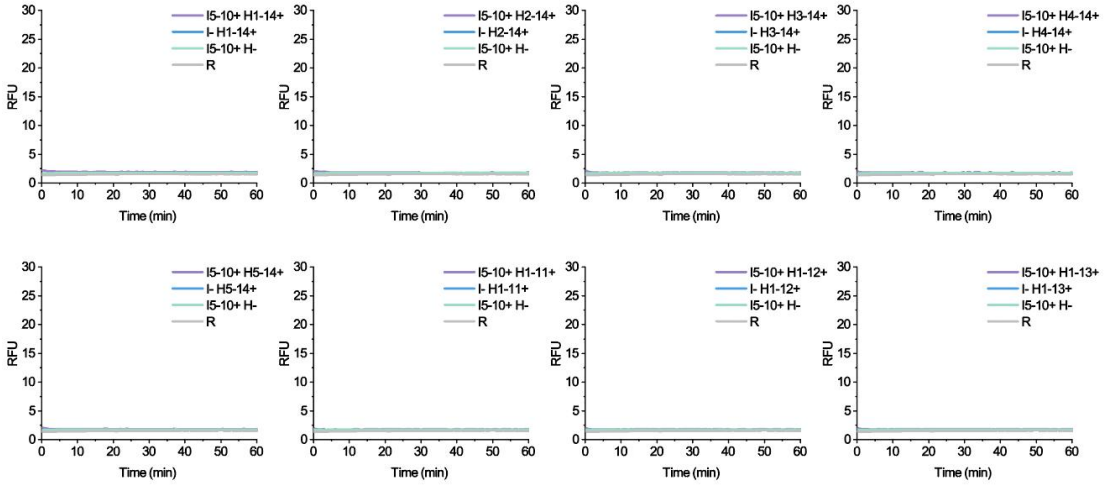

Figure S15. CA-HP of I5-10.

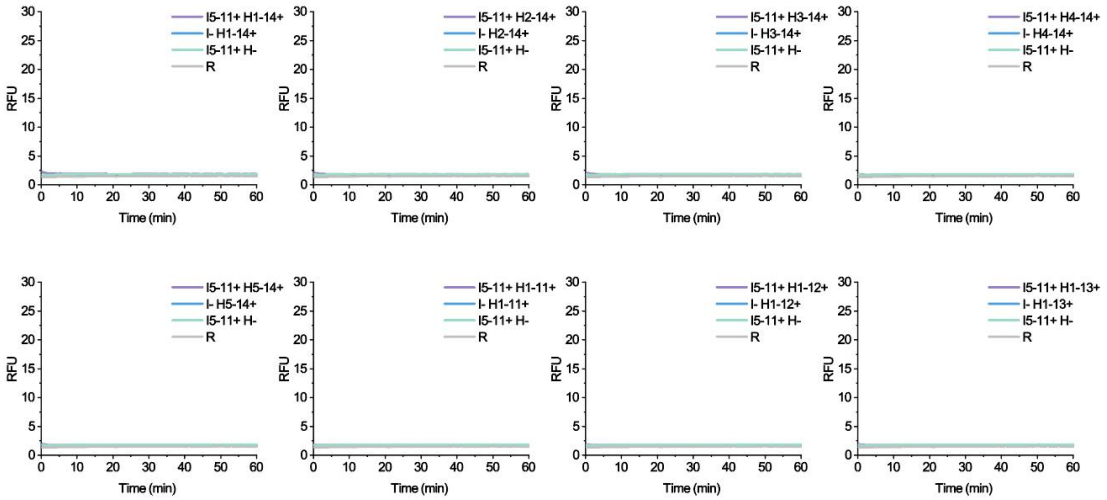

Figure S16. CA-HP of I5-11.

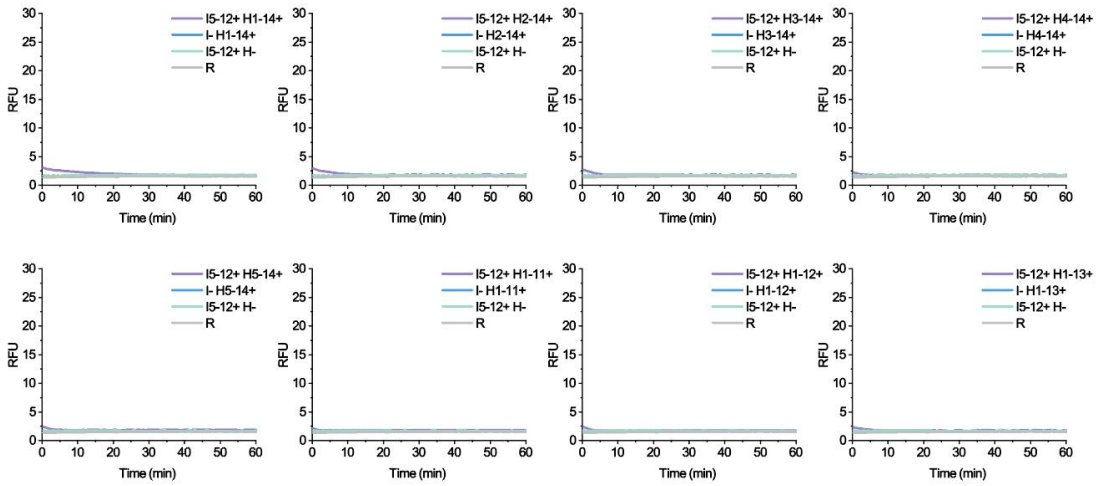

Figure S17. CA-HP of I5-12.

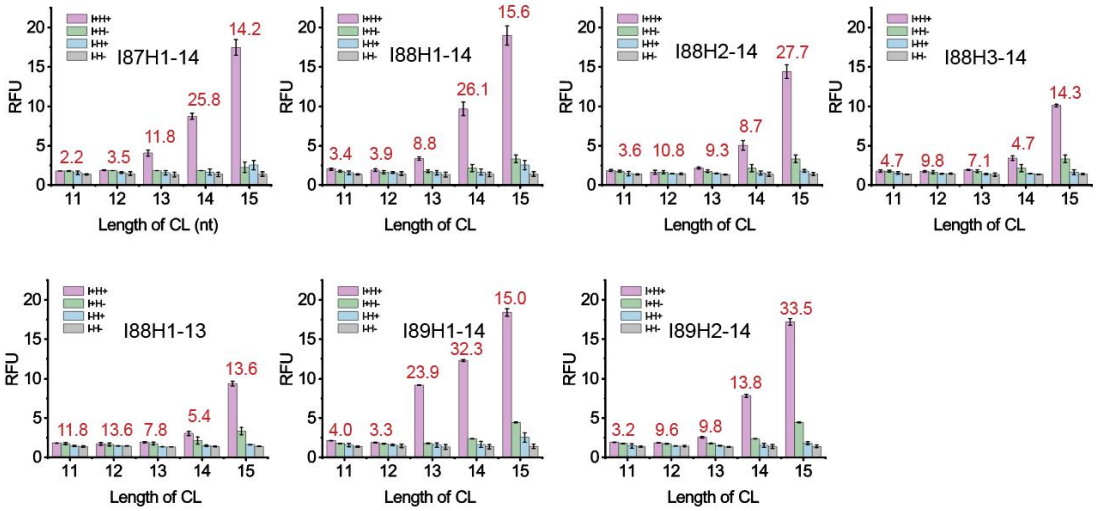

Figure S18. Different CL length of I8-7、I8-8 and I8-9. The numbers in red are the DF values. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

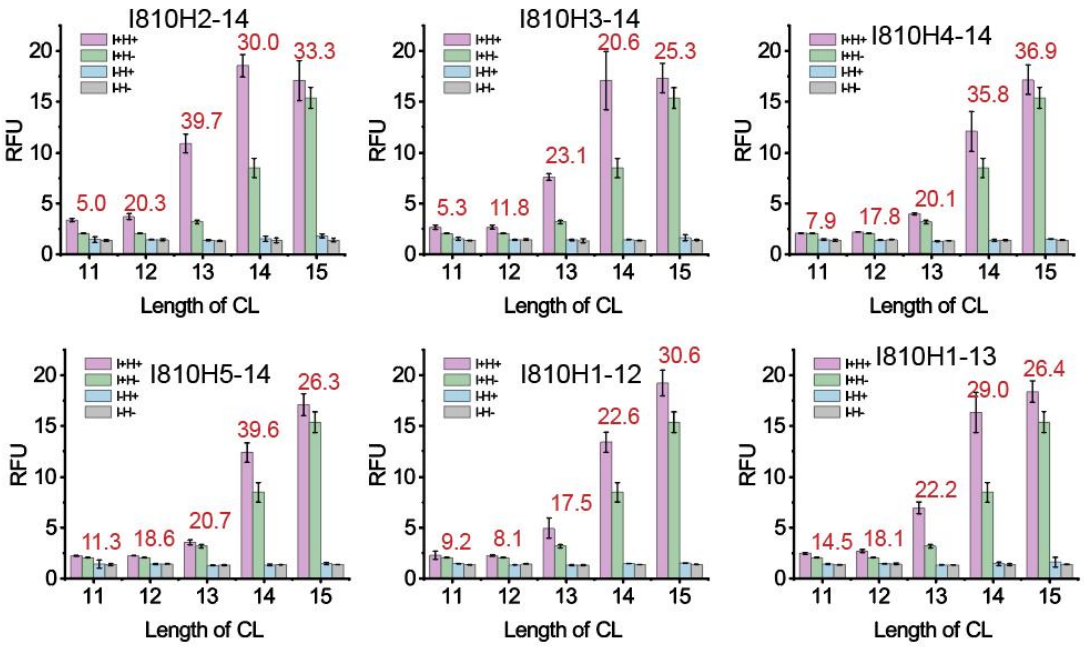

Figure S19. Different CL length of I8-10. The numbers in red are the DF values. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

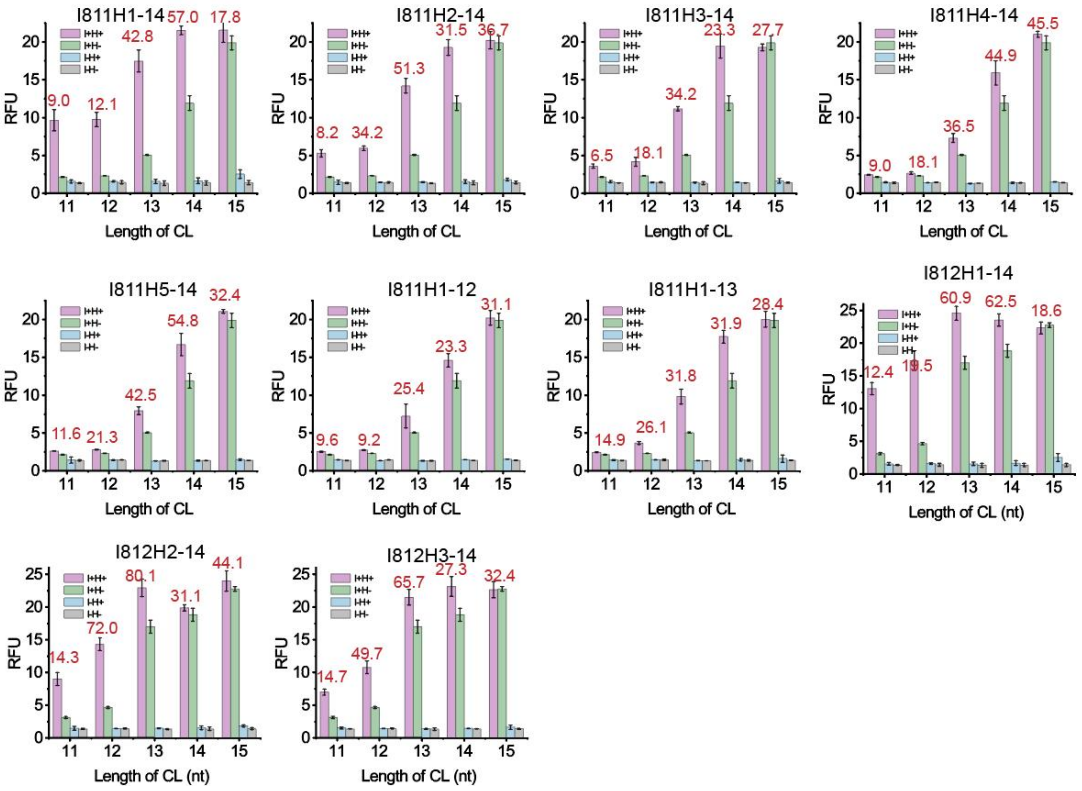

Figure S20. Different CL length of I8-11、I8-12. The numbers in red are the DF values. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

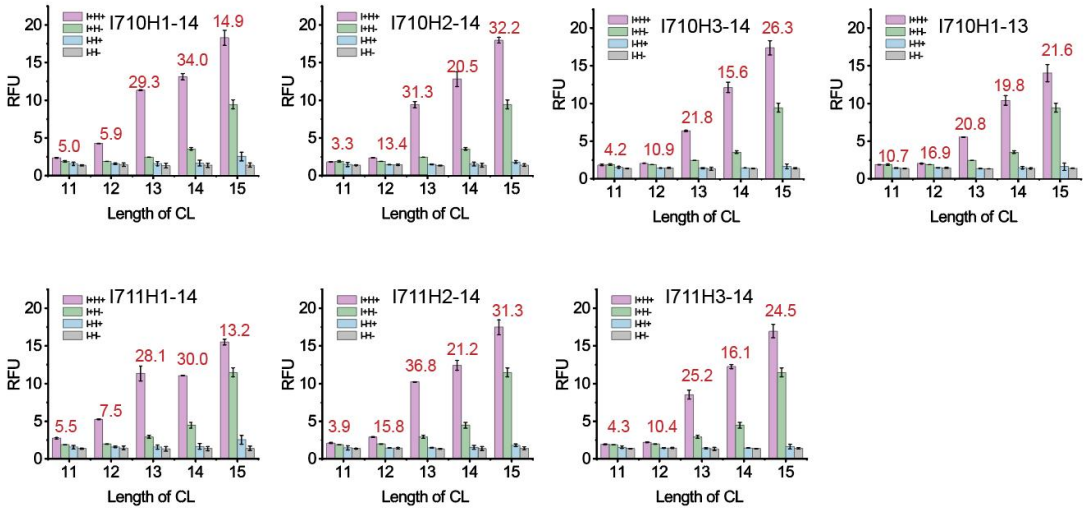

Figure S21. Different CL length of I7-10、I7-11. The numbers in red are the DF values. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

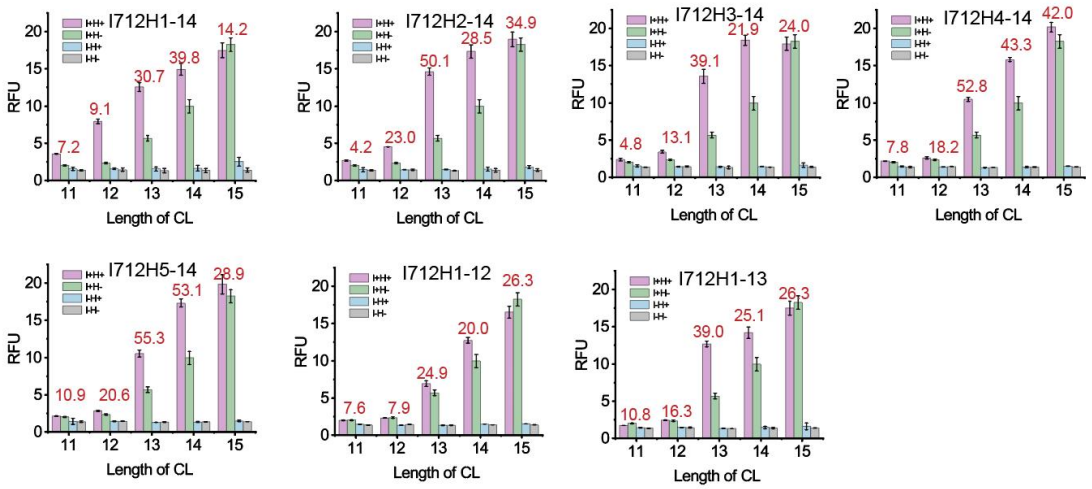

Figure S22. Different CL length of I7-12. The numbers in red are the DF values. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

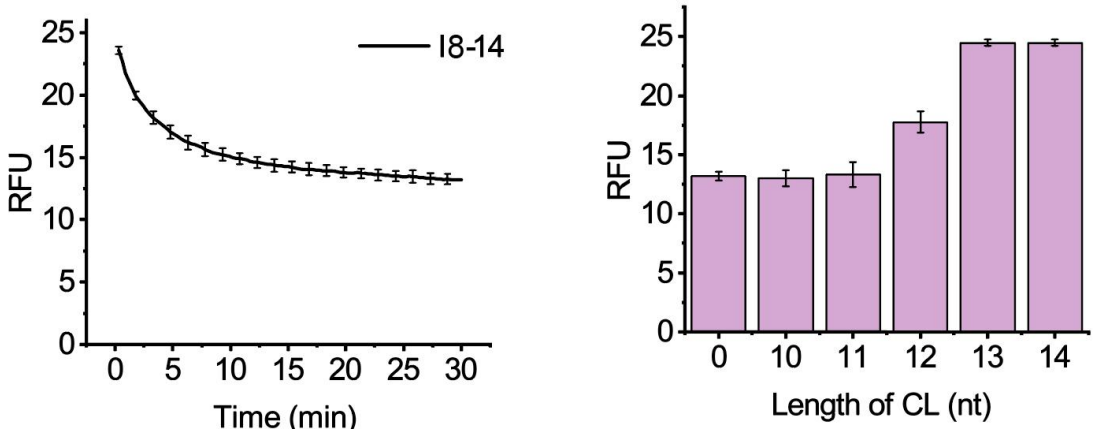

Figure S23. Normal hairpin probe of I8-14 with different CL length. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

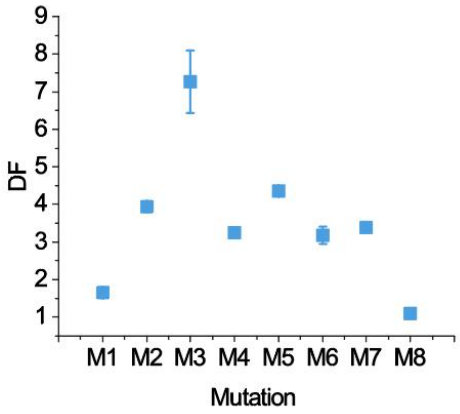

Figure S24. DF of normal hairpin probe. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

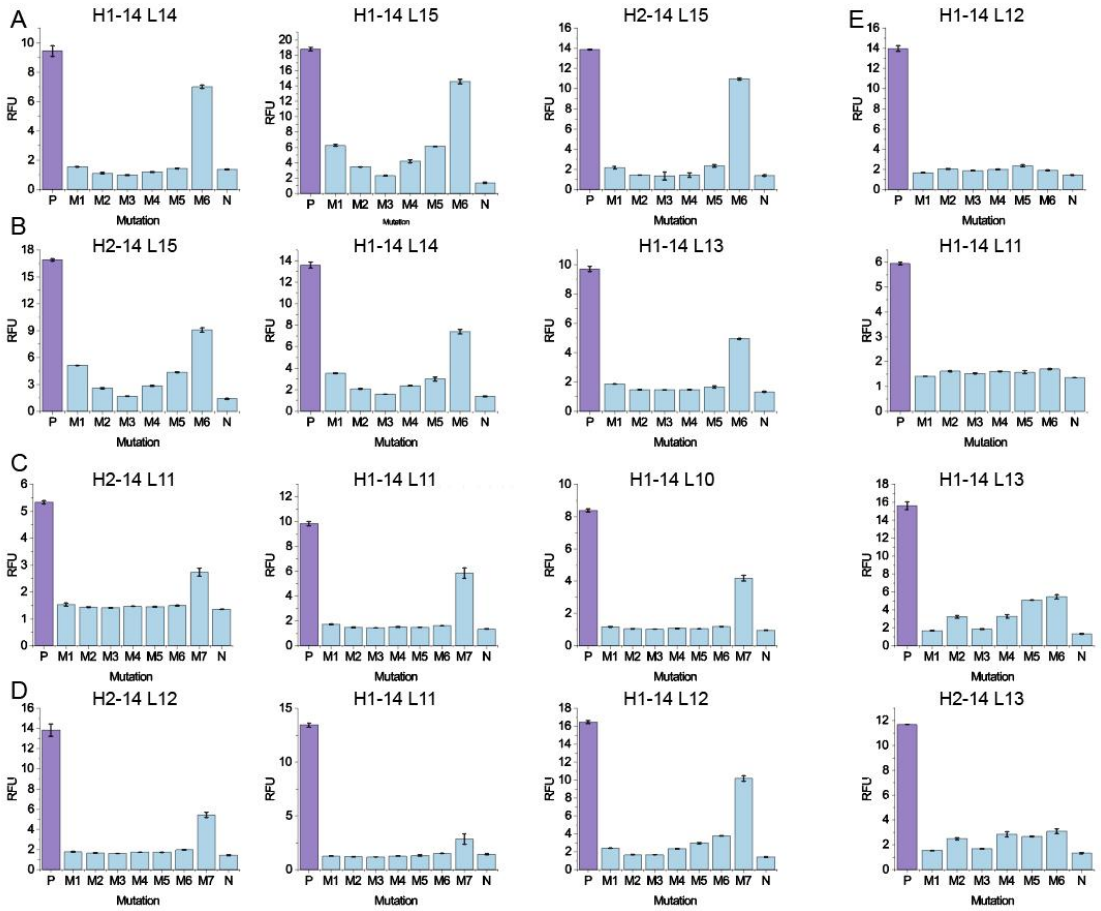

Figure S25. The fluorescence of I8-8(A)、I8-9(B)、I8-10(E)、I8-11(C) and I8-12(D) with different mutations. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

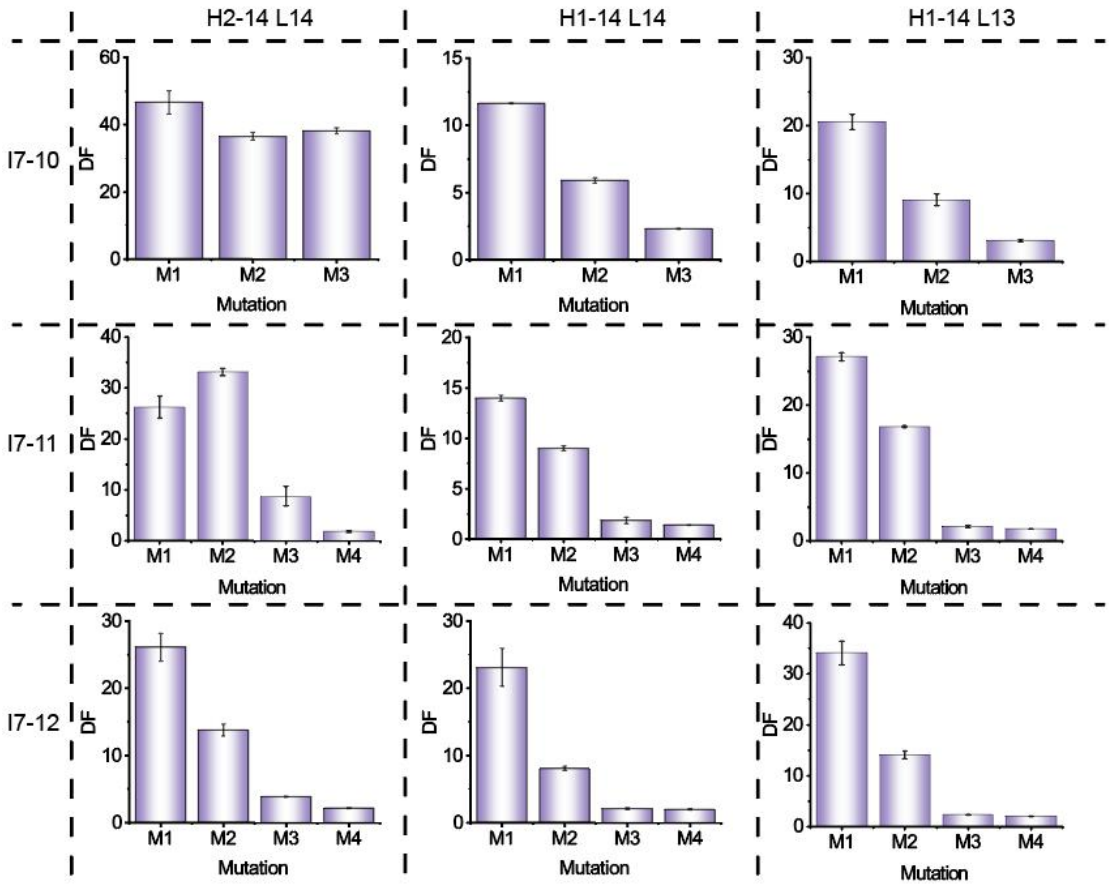

Figure S26. The DF of I with 7nt toehold. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

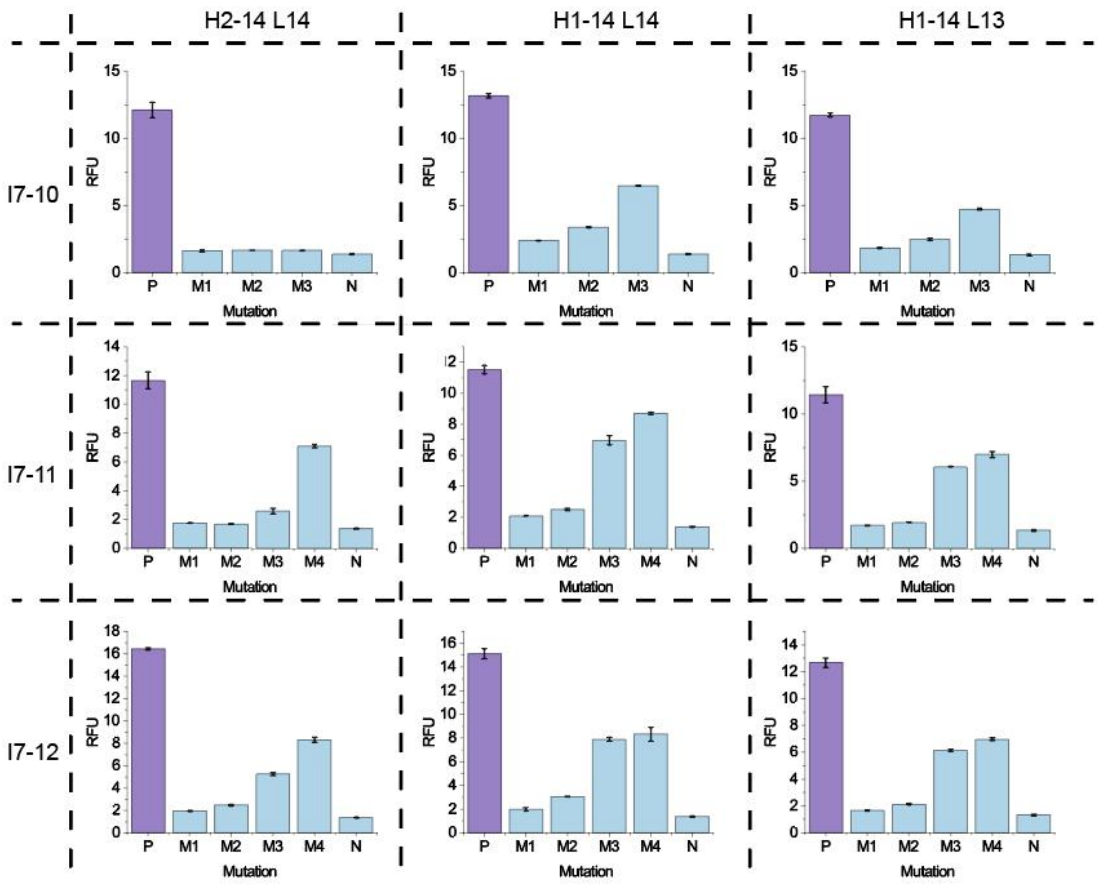

Figure S27. The fluorescence of I with 7nt toehold. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

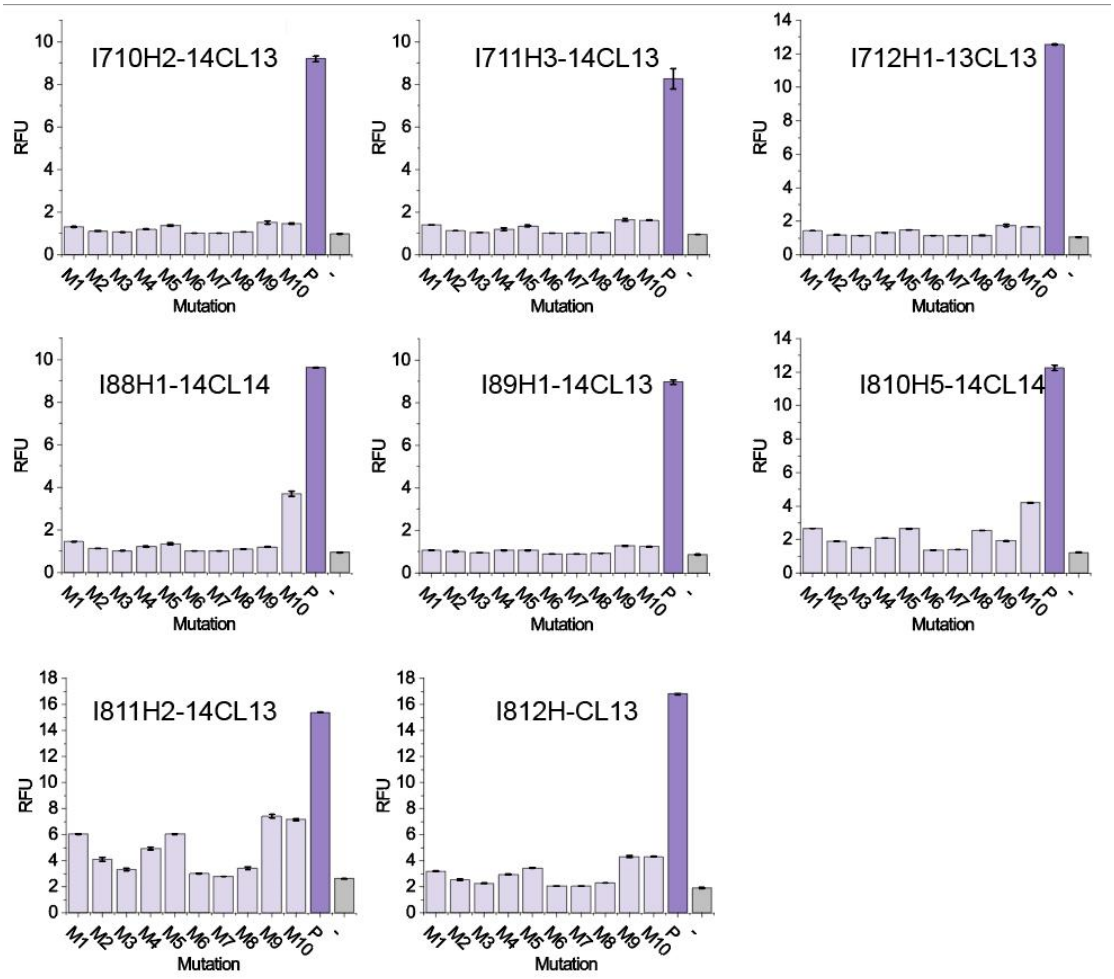

Figure S28. The fluorescence of I with different CL. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

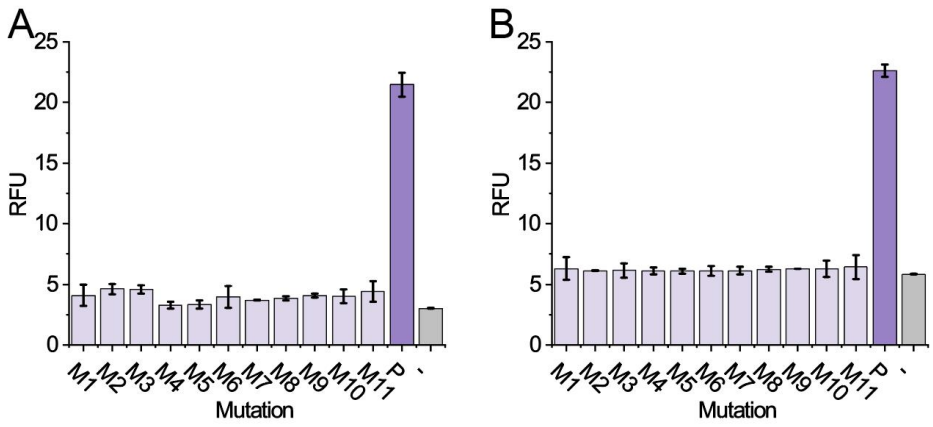

Figure S29. Fluorescence of I8-16 with H3-24 (A) or I8-18 with H3-24 (B). Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

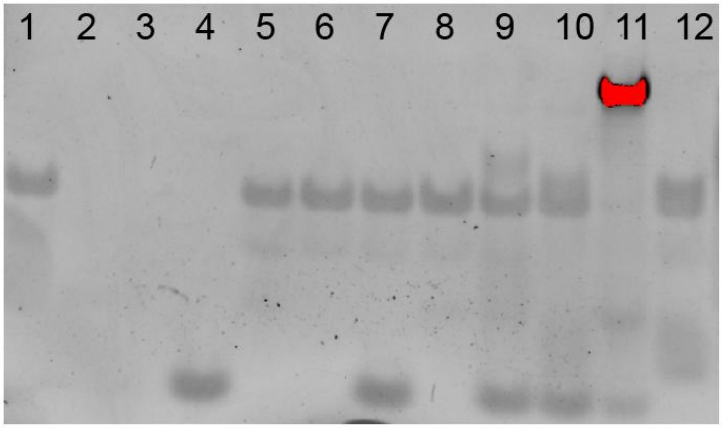

Figure S30. PAGE analysis. Lane1: R; Lane2: I8-18; Lane3: H3-24; Lane4: micro-122; Lane5: R\I8-18; Lane6: R\H3-24; Lane7: R\micro-122; Lane8:R\I8-18\H3-24; Lane9:R\I8-18\micro-122; Lane10: R\H3-24\micro-122; Lane11:R\I8-18\H3-24\micro-122; Lane12:R\I8-18\H3-24\micro-122M6.

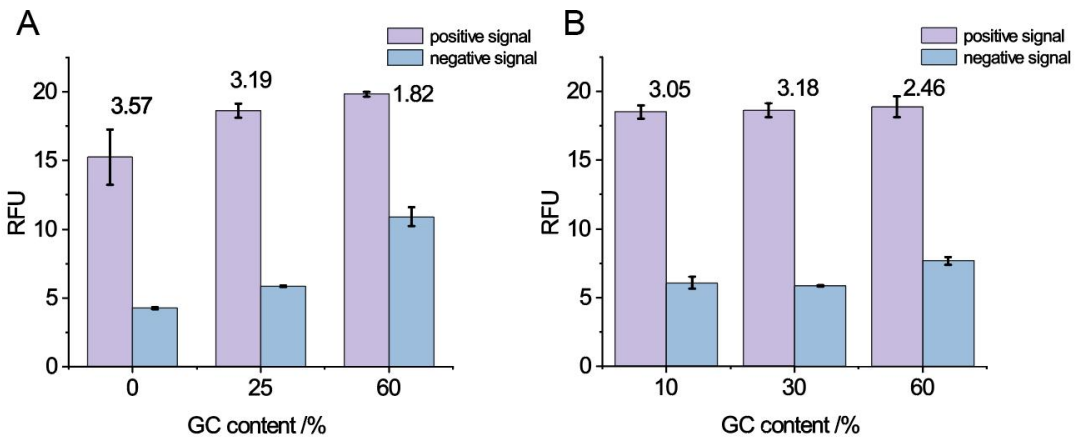

Figure S31. The effect of GC content in toehold (A) and stem domain(B) on the reaction.The number is the ratio of the positive signal to the negative signal. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

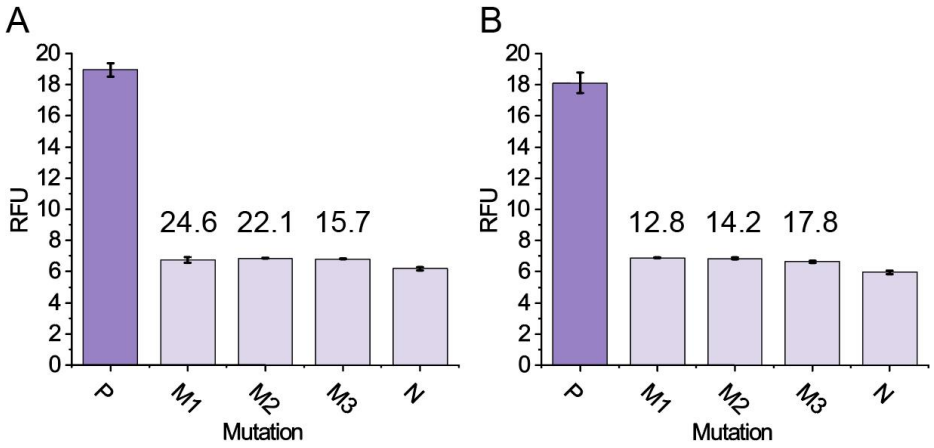

Figure S32. The Specificity of micro-122 and let-7a. Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

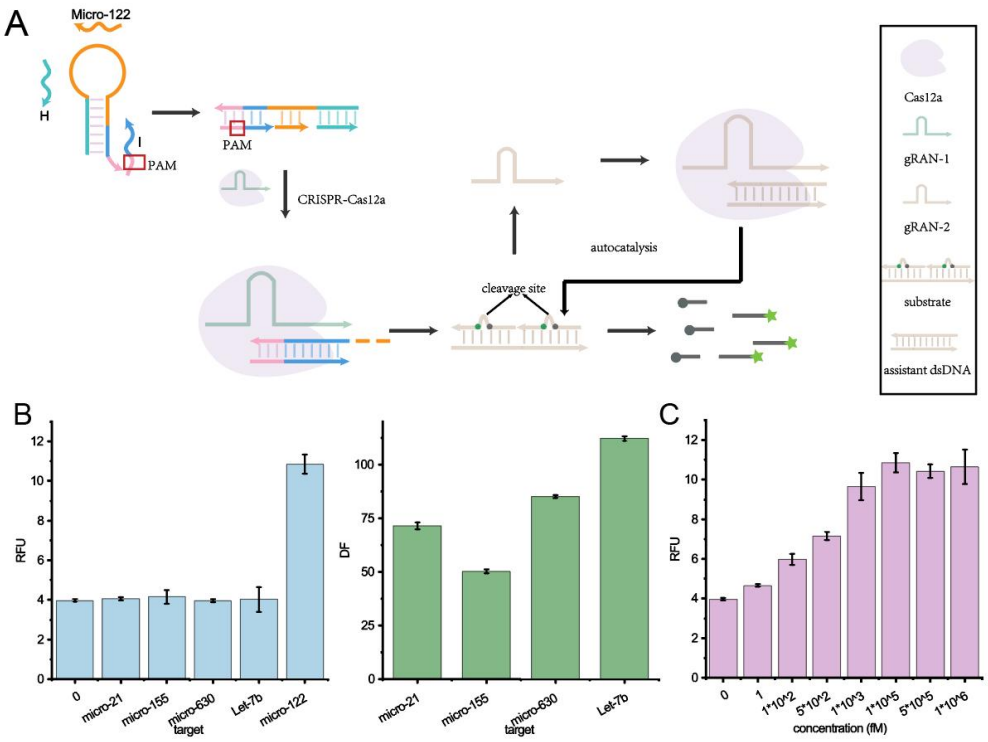

Figure S33. (A) The principle of CA-HP combined with autocatalytic CRISPR-Cas12a. Specificity (B) and fluorescence values of micro-122 with different concentrations(C). Error bars represent ± standard deviation (SD) from three independent experiments (n = 3).

679 **Table S1.** Sequence in experiment. The toehold dimain is red, mismatch base is blue,

680 and the complementary dimain is underlined.

Name	Sequence（5'→3'）
R	BHQ1- AAGATGATTTACTTTGACTGAGATCATCTTTAGGTGCCACTCAGTC AAAGTAA-FAM
I8-5	AGTAAATCATCTT
I8-6	AAGTAAATCATCTT
I8-7	AAAGTAAATCATCTT
I8-8	CAAAGTAAATCATCTT
I8-9	TCAAAGTAAATCATCTT
I8-10	GTCAAAGTAAATCATCTT
I8-11	AGTCAAAGTAAATCATCTT
I8-12	CAGTCAAAGTAAATCATCTT
I8-14	CTCAGTCAAAGTAAATCATCTT
I7-10	GTCAAAGTAAATCATCT
I7-11	AGTCAAAGTAAATCATCT
I7-12	CAGTCAAAGTAAATCATCT
I6-10	GTCAAAGTAAATCATC
I6-11	AGTCAAAGTAAATCATC
I6-12	CAGTCAAAGTAAATCATC
I5-10	GTCAAAGTAAATCAT
I5-11	AGTCAAAGTAAATCAT
I5-12	CAGTCAAAGTAAATCAT
H1-14	TTACTTTGACTGAG
H2-14	TACTTTGACTGAG
H3-14	ACTTTGACTGAG
H4-14	CTTTGACTGAG
H5-14	TTTGACTGAG
H1-11	TTACTTTGACT
H1-12	TTACTTTGACTG
H1-13	TTACTTTGACTGA
CL11	CACCTAAAGAT
CL12	CACCTAAAGATG
CL13	GCACCTAAAGATG
CL14	GCACCTAAAGATGA

CL15	GGCACCTAAAGATGA
I8-14-M1	CTCAGTCAAAGTAAATCATATT
I8-14-M2	CTCAGTCAAAGTAAATAATCTT
I8-14-M3	CTCAGTCAAAGTAACTCATCTT
I8-14-M4	CTCAGTCAAAGTCAATCATCTT
I8-14-M5	CTCAGTCAACGTAAATCATCTT
I8-14-M6	CTCAGTAAAAGTAAATCATCTT
I8-14-M7	CTCCGTCAAAGTAAATCATCTT
I8-14-M8	ATCAGTCAAAGTAAATCATCTT
I8-8-M1	CAAAGTAAATCATATT
I8-8-M2	CAAAGTAAATAATCTT
I8-8-M3	CAAAGTAACTCATCTT
I8-8-M4	CAAAGTCAATCATCTT
I8-8-M5	CAACGTAAATCATCTT
I8-8-M6	AAAAGTAAATCATCTT
I8-9-M1	TCAAAGTAAATCATATT
I8-9-M2	TCAAAGTAAATAATCTT
I8-9-M3	TCAAAGTAACTCATCTT
I8-9-M4	TCAAAGTCAATCATCTT
I8-9-M5	TCAACGTAAATCATCTT
I8-9-M6	TAAAAGTAAATCATCTT
I8-10-M1	GTCAAAGTAAATCATATT
I8-10-M2	GTCAAAGTAAATAATCTT
I8-10-M3	GTCAAAGTAACTCATCTT
I8-10-M4	GTCAAAGTCAATCATCTT
I8-10-M5	GTCAACGTAAATCATCTT
I8-10-M6	GTAAAAGTAAATCATCTT
I8-11-M1	AGTCAAAGTAAATCATATT
I8-11-M2	AGTCAAAGTAAATAATCTT
I8-11-M3	AGTCAAAGTAACTCATCTT
I8-11-M4	AGTCAAAGTCAATCATCTT
I8-11-M5	AGTCAACGTAAATCATCTT
I8-11-M6	AGTAAAAGTAAATCATCTT
I8-11-M7	CGTCAAAGTAAATCATCTT
I8-12-M1	CAGTCAAAGTAAATCATATT
I8-12-M2	CAGTCAAAGTAAATAATCTT
I8-12-M3	CAGTCAAAGTAACTCATCTT

I8-12-M4	CAGTCAAAGTCAATCATCTT
I8-12-M5	CAGTCAACGTAAATCATCTT
I8-12-M6	CAGTAAAAGTAAATCATCTT
I8-12-M7	CCGTCAAAGTAAATCATCTT
I7-10-M1	GTCAAAGTAAATCATAT
I7-10-M2	GTCAACGTAAATCATCT
I7-10-M3	GTAAAAGTAAATCATCT
I7-11-M1	AGTCAAAGTAAATCATAT
I7-11-M2	AGTCAACGTAAATCATCT
I7-11-M3	AGTAAAAGTAAATCATCT
I7-11-M4	CGTCAAAGTAAATCATCT
I7-12-M1	CAGTCAAAGTAAATCATAT
I7-12-M2	CAGTCAACGTAAATCATCT
I7-12-M3	CAGTAAAAGTAAATCATCT
I7-12-M4	CCGTCAAAGTAAATCATCT
CL13-M1	TCACCTAAAGATG
CL13-M2	GAACCTAAAGATG
CL13-M3	GCCCCTAAAGATG
CL13-M4	GCAACTAAAGATG
CL13-M5	GCACCGAAAGATG
CL13-M6	GCACCTACAGATG
CL13-M7	GCACCTAAATATG
CL13-M8	GCACCTAAAGCTG
CL13-M9	GCACCTAAAGAGG
CL13-M10	GCACCTAAAGATT
CL14-M1	TCACCTAAAGATGA
CL14-M2	GAACCTAAAGATGA
CL14-M3	GCCCCTAAAGATGA
CL14-M4	GCAACTAAAGATGA
CL14-M5	GCACCGAAAGATGA
CL14-M6	GCACCTACAGATGA
CL14-M7	GCACCTAAAGCTGA
CL14-M8	GCACCTAAAGAGGA
CL14-M9	GCACCTAAAGATTA
CL14-M10	GCACCTAAAGATGC
micro-122	UGGAGUGUGACAAUGGUGUUUG
R-micro-122	BHQ1-

	AAGATGATCCTTACTTACTTACTTTGACTGAGAACAAACACCATTGT CACACTCCAAA CTCAGTCAAAGTAAGTAAGTAAGG-FAM
micro-122-M1	UUGAGUGUGACAAUGGUGUUUG
micro-122-M2	UGUAGUGUGACAAUGGUGUUUG
micro-122-M3	UGGCGUGUGACAAUGGUGUUUG
micro-122-M4	UGGAGGGUGACAAUGGUGUUUG
micro-122-M5	UGGAGUGGGACAAUGGUGUUUG
micro-122-M6	UGGAGUGUGCCAAUGGUGUUUG
micro-122-M7	UGGAGUGUGACCAUGGUGUUUG
micro-122-M8	UGGAGUGUGACAAGGGUGUUUG
micro-122-M9	UGGAGUGUGACAAUGGUUUUUG
micro-122-M10	UGGAGUGUGACAAUGGUGUGUG
micro-122-M11	UGGAGUGUGACAAUGGUGUUGG
micro-122-I8-16	AAGTAAGTAAGTAAGGATCATCTT
micro-122-I8-18	CAAAGTAAGTAAGTAAGGATCATCTT
micro-122-H1-22	CCTTACTTACTTACTTTGACTG
micro-122-H3-24	TTACTTACTTACTTTGACTGAG
R-micro-122-loop-GC10%	BHQ1-AAGATGATTATTAATTACTTACTTTAACTGAGAACAAACACCATTGT CACACTCCAAACTCAGTTAAAGTAAGTAATTAATA-FAM
micro-122-I8-18-loop-GC10%	TAAAGTAAGTAATTAATAATCATCTT
micro-122-H3-24-loop-GC10%	TTAATTACTTACTTTAACTGAG
R-micro-122-loop-GC60%	BHQ1-AAGATGATCCCTACCTACCGACTCTGACTGAGAACAAACACCATT GTCACACTCCAAACTCAGTCAGAGTCGGTAGGTAGGG-FAM
micro-122-I8-18-loop-GC60%	CAGAGTCGGTAGGTAGGGATCATCTT
micro-122-H3-24-loop-GC60%	CTACCTACCGACTCTGACTGAG
R-micro-122-toe-GC0%	BHQ1-AATATTATCCTTACTTACTTACTTTGACTGAGAACAAACACCATTGT CACACTCCAAACTCAGTCAAAGTAAGTAAGTAAGG-FAM
micro-122-I8-	CAAAGTAAGTAAGTAAGGATAATATT

18-loop-GC60%	
R-micro-122-toe-GC60%	AGGAGGACCCTTACTTACTTACTTTGACTGAGAACAAACACCATTGTCACACTCCAAACTCAGTCAAAGTAAGTAAGTAAGG-FAM
micro-122-l8-18-loop-GC60%	CAAAGTAAGTAAGTAAGGGTCCTCCT
R-micro-21	BHQ1-AAGATGATCCTTACTTACTTACTTTGACTGAGAATCAACATCAGTCTGATAAGCTAAA CTCAGTCAAAGTAAGTAAGTAAGG-FAM
micro-21	UAGCUUAUCAGACUGAUGUUGA
micro-21-M1	UAGAUUAUCAGACUGAUGUUGA
micro-21-M2	UAGCUUAUCAGCCUGAUGUUGA
micro-21-M3	UAGCUUAUCAGACUGAUGGUGA
R-let-7a	BHQ1-AAGATGATCCTTACTTACTTACTTTGACTGAGAAAACTATACAACCTACTACCTCAAA CTCAGTCAAAGTAAGTAAGTAAGG-FAM
let-7a	UGAGGUAGUAGGUUGUAUAGUU
let-7a-M1	UGCGGUAGUAGGUUGUAUAGUU
let-7a-M2	UGAGGUAGUAGGGUGUAUAGUU
let-7a-M3	UGAGGUAGUAGGUUGUAUCGUU
R-Cas	CCTTACTTACTTACTTTGACTGAGAACAAACACCATTGTCACACTCCAAACTCAGTCAAAGTAAGTAAGTAAGGCAAATGAT
gRNA-1	UAAUUUCUACUAAGUGUAGAUCCUUACUUACUUACUUUG
gRNA-2	UAAUUUCUACUAAGUGUAGAUGAUCGUUACGCUAACUAUGA
Cas-l	ATCATTTGCCTTACTTACTTACTTTG
Cas-H	CTCAGTCAAAGTAAGTAAGTAA
gRNA-2-cDNA1	TCATAGTTAG/i6FAMdT/TTATT/iBHQ1dT/CGTAACGATC
gRNA-2-cDNA2	ATCTACACTT/i6FAMdT/TTATT/iBHQ1dT/AGTAGAAATTA
assistant DNA-1	GATCGTTACGCTAACTATGACAAA
assistant DNA-2	TTTGTCATAGTTAGCGTAACGATC
micro-155	UUAAUGCUAAUCGUGAUAGGGGU
micro-630	AGUAUUCUGUACCAGGGAAGGU
Let-7b	UGAGGUAGUAGGUUGUGUGGUU

685 **Table S2.** The recovery rates of micro-122 in human serum.

Sample	Added (nM)	Detected(nM)	Recovery (%)	RSD (%)
1	0.1	0.105	105.0	2.3
2	0.2	0.212	106.1	3.0
3	0.5	0.528	105.6	5.3
4	1.0	1.077	107.7	5.2
5	2.0	2.016	100.8	9.2

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688 **Table S3.** The comparison with other miRNA detection methods.

methods	**Analytes**	**sensitivity**	**specificity**	**Reference**
Fluorescent assay	miR-141	100 fM	miR-200 family	1
Fluorescent assay	miR-21	10 fM	single-nucleotide polymorphisms	2
Fluorescent assay	miR-21	10 fM	miR-155、miR-122、miR-16、miR-let7a	3
Fluorescent assay	miR-21	130 pM	miRNA-122 or miRNA-194	4
TIRFM	Let7a	500 fM	let-7c, let-7f, let-7g, and let-7i	5
Electrochemical assay	miR-21	260 fM	2.6 times	6
Electrochemiluminescence assay	miR-21	6.6 fM	None	7
Fluorescent assay	miR-122	1 fM	This work	

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