Supporting Information for

PAM-independent ultra-specific activation of CRISPR-Cas12a via sticky-end dsDNA

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SI 1 Supplementary tables

1.1 Table S1. DNA sequences used in Figure 1

Name	Sequences (5'→3')
Probe-1	FAM -TTATTTATTTAA-BHQ
gRNA-1	UAAUUUCUACUAAGUGUAGAU UAUACAUAUUUAUGGGUUUG
G1-B-1	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCATAAATATG TATAGAAAGATCCT
G1-B-2	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCATAAATATG TATTGAAAGATCCT
G1-B-3	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCATAAATATG TGTAGAAAGATCCT
G1-B-4	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCATAAATACG TATAGAAAGATCCT
G1-B-5	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCATAACTATG TATAGAAAGATCCT
G1-B-6	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCCTAAATATG TATAGAAAGATCCT
G1-B-7	TGCAGACTGTACCTCACGACTACCACTCTACAAACTCATAAATATG TATAGAAAGATCCT
G1-PAM$^+$-T-1	AGGATCTTTCTATACATATTTATGGGTTTGTAGAGTGGTAGTCGTG AGGTACAGTCTCGA
G1-PAM$^+$-T-2	AGGATCTTTCAATACATATTTATGGGTTTGTAGAGTGGTAGTCGTG AGGTACAGTCTCGA
G1-PAM$^+$-T-3	AGGATCTTTCTACACATATTTATGGGTTTGTAGAGTGGTAGTCGTG AGGTACAGTCTCGA
G1-PAM$^+$-T-4	AGGATCTTTCTATACGTATTTATGGGTTTGTAGAGTGGTAGTCGTG AGGTACAGTCTCGA
G1-PAM$^+$-T-5	AGGATCTTTCTATACATAGTTATGGGTTTGTAGAGTGGTAGTCGTG AGGTACAGTCTCGA
G1-PAM$^+$-T-6	AGGATCTTTCTATACATATTTAGGGGTTTGTAGAGTGGTAGTCGTG AGGTACAGTCTCGA
G1-PAM$^+$-T-7	AGGATCTTTCTATACATATTTATGAGTTTGTAGAGTGGTAGTCGTGA GGTACAGTCTCGA
G1-Blocker-1	ATATTTATGGGTTTGTAGAG
G1-Blocker-2	ATACATAGTTATGGGTTTGTAGAG
G1-Blocker-3	TACATAGTTATGGGTTTGTAGAG
G1-Blocker-4	ACATAGTTATGGGTTTGTAGAG
G1-Blocker-5	CATAGTTATGGGTTTGTAGAG
G1-Blocker-6	ATAGTTATGGGTTTGTAGAG
G1-Blocker-7	TAGTTATGGGTTTGTAGAG
G1-Blocker-8	AGTTATGGGTTTGTAGAG
G1-Blocker-9	GTTATGGGTTTGTAGAG
G1-Blocker-10	TATTTATGGGTTTGTAGAG

G1-Blocker-12	TATTTCTGGGTTTGTAGAG
G1-Blocker-13	TATTTATGAGTTTGTAGAG
G1- SE^{+}-T-1	TATACATATTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-2	ATACATATTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-3	TACATATTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-4	ACATATTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-5	CATATTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-6	ATATTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-7	TTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-8	ATACATAGTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-9	TACATAGTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-10	ACATAGTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-11	CATAGTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-12	ATAGTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-13	TAGTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-14	AGTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-15	GTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-16	GTATTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-17	ATATTTAGGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE^{+}-T-18	ATATTTATGAGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
gRNA-2	UAAUUUCUACUAAGUGUAGAU UGAAGUAGAUAUGGCAGCAC
G2-B-1	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCATATCTACTTCAGAAAGATCCT
G2-B-2	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCATATCTACTTCTGAAAGATCCT
G2-B-3	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCATATCTACTGCAGAAAGATCCT
G2-B-4	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCATATCTTCTTCAGAAAGATCCT
G2-B-5	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCATACCTACTTCAGAAAGATCCT
G2-B-6	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCCTATCTACTTCAGAAAGATCCT
G2-B-7	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTTCCATATCTACTTCAGAAAGATCCT
G2-PAM^{+}-T-1	AGGATCTTTCTGAAGTAGATATGGCAGCACTAGAGTGGTAGTCGT

	GAGGTACAGTCTGCA
G2-PAM$^+$-T-2	AGGATCTTTCAGAAGTAGATATGGCAGCACTAGAGTGGTAGTCGT GAGGTACAGTCTGCA
G2-PAM$^+$-T-3	AGGATCTTTCTGCAGTAGATATGGCAGCACTAGAGTGGTAGTCGT GAGGTACAGTCTGCA
G2-PAM$^+$-T-4	AGGATCTTTCTGAAGAAGATATGGCAGCACTAGAGTGGTAGTCGT GAGGTACAGTCTGCA
G2-PAM$^+$-T-5	AGGATCTTTCTGAAGTAGGTATGGCAGCACTAGAGTGGTAGTCGT GAGGTACAGTCTGCA
G2-PAM$^+$-T-6	AGGATCTTTCTGAAGTAGATAGGGCAGCACTAGAGTGGTAGTCGT GAGGTACAGTCTGCA
G2-PAM$^+$-T-7	AGGATCTTTCTGAAGTAGATATGGAAGCACTAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G2-Blocker-1	AGATATGGCAGCACTAGAG
G2-Blocker-2	AGGTATGGCAGCACTAGAG
G2-Blocker-3	AGATAGGGCAGCACTAGAG
G2-Blocker-4	AGATATGGAAGCACTAGAG
G2- SE$^+$-T-1	TAGATATGGCAGCACTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G2- SE$^+$-T-2	AAGATATGGCAGCACTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G2- SE$^+$-T-3	TAGGTATGGCAGCACTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G2- SE$^+$-T-4	TAGATAGGGCAGCACTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G2- SE$^+$-T-5	TAGATATGGAAGCACTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
gRNA-3	UAAUUUCUACUAAGUGUAGAU ACAAUAUGUGCUUCUACACA
G3-B-1	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGAAGCACATA TTGTGAAAGATCCT
G3-B-2	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGAAGCACATA TTGAGAAAGATCCT
G3-B-3	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGAAGCACATA TCGTGAAAGATCCT
G3-B-4	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGAAGCACAGA TTGTGAAAGATCCT
G3-B-5	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGAAGCCCATA TTGTGAAAGATCCT
G3-B-6	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGACGCACATA TTGTGAAAGATCCT
G3-B-7	TGCAGACTGTACCTCACGACTACCACTCTATGTGTTGAAGCACATA TTGTGAAAGATCCT
G3-PAM$^+$-T-1	AGGATCTTTCACAATATGTGCTTCTACACATAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G3-PAM$^+$-T-2	AGGATCTTTCTCAATATGTGCTTCTACACATAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G3-PAM$^+$-T-3	AGGATCTTTCACGATATGTGCTTCTACACATAGAGTGGTAGTCGTG AGGTACAGTCTGCA

G3-PAM$^+$-T-4	AGGATCTTTCACAATCTGTGCTTCTACACATAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G3-PAM$^+$-T-5	AGGATCTTTCACAATATGGGCTTCTACACATAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G3-PAM$^+$-T-6	AGGATCTTTCACAATATGTGCGTCTACACATAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G3-PAM$^+$-T-7	AGGATCTTTCACAATATGTGCTTCAACACATAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G3-Blocker-1	TGTGCTTCTACACATAGAG
G3-Blocker-2	TGGGCTTCTACACATAGAG
G3-Blocker-3	TGTGCGTCTACACATAGAG
G3-Blocker-4	TGTGCTTCAACACATAGAG
G3- SE$^+$-T-1	ATGTGCTTCTACACATAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G3- SE$^+$-T-2	CTGTGCTTCTACACATAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G3- SE$^+$-T-3	ATGGGCTTCTACACATAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G3- SE$^+$-T-4	ATGTGCGTCTACACATAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G3- SE$^+$-T-5	ATGTGCTTCAACACATAGAGTGGTAGTCGTGAGGTACAGTCTGCA
gRNA-4	UAAUUUCUACUAAGUGUAGAU GCAGUUUGGCCCGGCCAAAA
G4-B	TGCAGACTGTACCTCACGACTACCACTCTATTTTGGCCGGGCCAAA CTGCGAAAGATCCT
G4-PAM$^+$-T	AGGATCTTTCGCAGTTTGGCCCGGCCAAAATAGAGTGGTAGTCGT GAGGTA CAGTCTGCA
G4- SE$^+$-T	TTGGCCCGGCCAAAATAGAGTGGTAGTCGTGAGGTACAGTCTGCA
gRNA-5	UAAUUUCUACUAAGUGUAGAU AAACAUUACAACCCCAUAAA
G5-B	TGCAGACTGTACCTCACGACTACCACTCTATTTATGGGGTTGTAAT GTTTGAAAGATCCT
G5-PAM$^+$-T	AGGATCTTTCAAACATTACAACCCCATAAATAGAGTGGTAGTCGTG AGGTA CAGTCTGCA
G5- SE$^+$-T	TTACAACCCCATAAATAGAGTGGTAGTCGTGAGGTACAGTCTGCA
gRNA-6	UAAUUUCUACUAAGUGUAGAU UGGGUUUGUAAUGUUUUAAU
G6-B	TGCAGACTGTACCTCACGACTACCACTCTAATTAAAACATTACAAAC CCA GAAAGATCCT
G6-PAM$^+$-T	AGGATCTTTCTGGGTTTGTAATGTTTTAATTAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G6- SE$^+$-T	TTGTAATGTTTTAATTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
gRNA-7	UAAUUUCUACUAAGUGUAGAU AAAAAAAAAAAAAAAAAAAA
G7-B	TGCAGACTGTACCTCACGACTACCACTCTATTTTTTTTTTTTTTTTTT TTGAAAGATCCT
G7-PAM$^+$-T	AGGATCTTTCAAAAAAAAAAAAAAAAAAAATAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G7- SE$^+$-T	AAAAAAAAAAAAAAATAGAGTGGTAGTCGTGAGGTACAGTCTGCA

The mutation bases are marked in red or green. The binding region between B-stands and gRNAs are marked in purple and brown. The PAM region of T-strands marked in red. The initiation region of B-strands marked in blue. The tables below are also marked with the same. The gRNA is shown in bold.

1.2 Table S2. DNA sequences used in Figure 2

Name	Sequences (5'→3')
gRNA-8	UAAUUUCUACUAAGUGUAGAU UAUACACAUUUAUGGGUUUG
gRNA-9	UAAUUUCUACUAAGUGUAGAU UAUACAUAUCUAUGGGUUUG
gRNA-10	UAAUUUCUACUAAGUGUAGAU UAUACAUAUUUAUCGGUUUG
gRNA-11	UAAUUUCUACUAAGUGUAGAU UAUACAUAUUUAUUGGUUUG
G1-B-1	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCATAAATATG TATAGAAAGATCCT
G1-B-4	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCATAAATACG TATAGAAAGATCCT
G1-B-5	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCATAACTATG TATAGAAAGATCCT
G1-B-6	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCCTAAATATG TATAGAAAGATCCT
G1-B-7	TGCAGACTGTACCTCACGACTACCACTCTACAAACTCATAAATATG TATAGAAAGATCCT
G1-PAM$^+$-T-1	AGGATCTTTCTATACATATTTATGGGTTTGTAGAGTGGTAGTCGTG AGGTACAGTCTCGA
G1-PAM$^+$-T-4	AGGATCTTTCTATACGTATTTATGGGTTTGTAGAGTGGTAGTCGTG AGGTACAGTCTCGA
G1-PAM$^+$-T-5	AGGATCTTTCTATACATAGTTATGGGTTTGTAGAGTGGTAGTCGTG AGGTACAGTCTCGA
G1-PAM$^+$-T-6	AGGATCTTTCTATACATATTTAGGGGTTTGTAGAGTGGTAGTCGTG AGGTACAGTCTCGA
G1-PAM$^+$-T-7	AGGATCTTTCTATACATATTTATGAGTTTGTAGAGTGGTAGTCGTGA GGTACAGTCTCGA
G1- SE$^+$-T-6	ATATTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE$^+$-T-12	ATAGTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE$^+$-T-16	GTATTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE$^+$-T-17	ATATTTAGGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
G1- SE$^+$-T-18	ATATTTATGAGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA
gRNA-12	UAAUUUCUACUAAGUGUAGAU UGAAAUAGAUAUGGCAGCAC
gRNA-13	UAAUUUCUACUAAGUGUAGAU UGAAGUAGACAUGGCAGCAC
gRNA-14	UAAUUUCUACUAAGUGUAGAU UGAAGUAGAUCUGGCAGCAC
gRNA-15	UAAUUUCUACUAAGUGUAGAU UGAAGUAGAUAUGTCAGCAC
G2-B-1	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCATATCTAC TTCAGAAAGATCCT
G2-B-4	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCATATCTTC TTCAGAAAGATCCT
G2-B-5	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCATACCTAC TTCAGAAAGATCCT
G2-B-6	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCCTATCTAC TTCAGAAAGATCCT

G2-B-7	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTTCCATATCTAC TTCAGAAAGATCCT
G2-PAM$^+$-T-1	AGGATCTTTCTGAAGTAGATATGGCAGCACTAGAGTGGTAGTCGT GAGGTACAGTCTGCA
G2-PAM$^+$-T-4	AGGATCTTTCTGAAGAAGATATGGCAGCACTAGAGTGGTAGTCGT GAGGTACAGTCTGCA
G2-PAM$^+$-T-5	AGGATCTTTCTGAAGTAGGTATGGCAGCACTAGAGTGGTAGTCGT GAGGTACAGTCTGCA
G2-PAM$^+$-T-6	AGGATCTTTCTGAAGTAGATAGGGCAGCACTAGAGTGGTAGTCGT GAGGTACAGTCTGCA
G2-PAM$^+$-T-7	AGGATCTTTCTGAAGTAGATATGGAAGCACTAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G2- SE$^+$-T-1	TAGATATGGCAGCACTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G2- SE$^+$-T-2	AAGATATGGCAGCACTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G2- SE$^+$-T-3	TAGGTATGGCAGCACTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G2- SE$^+$-T-4	TAGATAGGGCAGCACTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G2- SE$^+$-T-5	TAGATATGGAAGCACTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
gRNA-16	UAAUUUCUACUAAGUGUAGAU ACAACAUGUGCUUCUACACA
gRNA-17	UAAUUUCUACUAAGUGUAGAU ACAAUAUGUACUUCUACACA
gRNA-18	UAAUUUCUACUAAGUGUAGAU ACAAUAUGUGCCUCUACACA
gRNA-19	UAAUUUCUACUAAGUGUAGAU ACAAUAUGUGCUUGUACACA
G3-B-1	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGAAGCACATA TTGTGAAAGATCCT
G3-B-4	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGAAGCACAGA TTGTGAAAGATCCT
G3-B-5	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGAAGCCCATA TTGTGAAAGATCCT
G3-B-6	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGACGCACATA TTGTGAAAGATCCT
G3-B-7	TGCAGACTGTACCTCACGACTACCACTCTATGTGTTGAAGCACATA TTGTGAAAGATCCT
G3-PAM$^+$-T-1	AGGATCTTTCACAATATGTGCTTCTACACATAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G3-PAM$^+$-T-4	AGGATCTTTCACAATCTGTGCTTCTACACATAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G3-PAM$^+$-T-5	AGGATCTTTCACAATATGGGCTTCTACACATAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G3-PAM$^+$-T-6	AGGATCTTTCACAATATGTGCGTCTACACATAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G3-PAM$^+$-T-7	AGGATCTTTCACAATATGTGCTTCAACACATAGAGTGGTAGTCGTG AGGTACAGTCTGCA
G3- SE$^+$-T-1	ATGTGCTTCTACACATAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G3- SE$^+$-T-2	CTGTGCTTCTACACATAGAGTGGTAGTCGTGAGGTACAGTCTGCA

G3- SE^{+}-T-3	ATGGGCTTCTACACATAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G3- SE^{+}-T-4	ATGTGCGTCTACACATAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G3- SE^{+}-T-5	ATGTGCTTCAACACATAGAGTGGTAGTCGTGAGGTACAGTCTGCA

1.3 Table S3. DNA sequences used in Figure 3

Name	Sequences (5'→3')
gRNA-1	UAAUUUCUACUAAGUGUAGAU UAUACAUAUUUAUGGGUUUG
G1-B-8	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCATAAATATG TATA
G1-B-9	TGCAGACTGTACCTCACGACTACCACTCT(FAM)ACAAACCCATAAA TATGTATA
G1- SE⁺-T-19	BHQ-ATATTTATGGGTTTGT(FAM)AGAGTGGTAGTCGTGAGGTACAGTCT CGA
G1- SE⁺-T-20	ATA(BHQ)TTTATGGGTTTGT(FAM)AGAGTGGTAGTCGTGAGGTACA GTCTCGA
G1- SE⁺-T-21	ATATT(BHQ)TATGGGTTT(FAM)GTAGAGTGGTAGTCGTGAGGTACA GTCTCGA
gRNA-2	UAAUUUCUACUAAGUGUAGAU UGAAGUAGAUAUGGCAGCAC
G2-B-8	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTTCCATATCTAC TTCA
G2- SE⁺-T-6	BHQ-TAGATATGGCAGCACT(FAM)AGAGTGGTAGTCGTGAGGTACAGTC TGCA

1.4 Table S4. DNA sequences of gel electrophoresis used in this work

Name	Sequences (5'→3')
gRNA-1	UAAUUUCUACUAAGUGUAGAUUAUACAUAUUUAUGGGUUUG
G1-B-10	ACCTCACGACTACCACTCTACAAACCCATAAATATGTATA
G1-B-11	ACCTCACGACTACCACTCTA
G1- SE^{+}-T-19	ATCATCATCATCATCATCATCATCATCTATCATCATCATCGTCTCAG
	ATATTTATGGGTTTGTAGAGTGGTAGTCGTGAGGT
G1- SE^{+}-T-20	TTTGTAGAGTGGTAGTCGTGAGGTACAGTCTCGA

1.5 Table S5. DNA sequences used in Figure 4

Name	Sequences (5'→3')
gRNA-8	UAAUUUCUACUAAGUGUAGAUUAUACACAUUUAUGGGUUUG
gRNA-10	UAAUUUCUACUAAGUGUAGAUUAUACAUAUUUACGGGUUUG
G1-B-1	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCATAAATATG TATAGAAAGATCCT
G1-B-4	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCATAAATACG TATAGAAAGATCCT
G1-B-6	TGCAGACTGTACCTCACGACTACCACTCTACAAACCCCTAAATATG TATAGAAAGATCCT
G1-Helper-1	GTATTTATGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G1-Helper-2	ATATTTAGGGGTTTGTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
gRNA-12	UAAUUUCUACUAAGUGUAGAU UGAAAUAGAUAUGGCAGCAC
gRNA-14	UAAUUUCUACUAAGUGUAGAU UGAAGUAGAUAUGTCAGCAC
G2-B-1	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCATATCTAC TTCAGAAAGATCCT
G2-B-4	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCATATCTTC TTCAGAAAGATCCT
G2-B-6	TGCAGACTGTACCTCACGACTACCACTCTAGTGCTGCCCTATCTAC TTCAGAAAGATCCT
G2-Helper-1	AAGATATGGCAGCACTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G2-Helper-2	TAGATAGGGCAGCACTAGAGTGGTAGTCGTGAGGTACAGTCTGCA
gRNA-16	UAAUUUCUACUAAGUGUAGAUACAACAUGUGCUUCUACACA
gRNA-18	UAAUUUCUACUAAGUGUAGAUACAAUAUGUGCCUCUACACA
G3-B-1	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGAAGCACATA TTGTGAAAGATCCT
G3-B-4	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGAAGCACAGA TTGTGAAAGATCCT
G3-B-6	TGCAGACTGTACCTCACGACTACCACTCTATGTGTAGACGCACATA TTGTGAAAGATCCT
G3-Helper-1	CTGTGCTTCTACACATAGAGTGGTAGTCGTGAGGTACAGTCTGCA
G3-Helper-2	ATGTGCGTCTACACATAGAGTGGTAGTCGTGAGGTACAGTCTGCA
EGFR T790M-gRNA	UAAUUUCUACUAAGUGUAGAUAAGGGCAUGAGCUAGGUGAU
EGFR-T790M-MT	CTGCCTCACCTCCACCGTGCAGCTCATCACGTAGCTCATGCCCTT
EGFR-T790M-WT	CTGCCTCACCTCCACCGTGCAGCTCATCACGCAGCTCATGCCCTT
EGFR-T790M-Helper	CATGAGCTGCGTGATGAGCTGCACGGTGGAGGTGAGGCAG
EGFR S768I-gRNA	UAAUUUCUACUAAGUGUAGAUCGUGAUGGCCCUCGUGGAC

EGFR S768I - MT	GCAGGCGGCACACGTGGGGGTTGTCCACGATGGCCATCACG
EGFR S768I - WT	GCAGGCGGCACACGTGGGGGTTGTCCACGCTGGCCATCACG
EGFR S768I - Helper	TGGCCAGCGTGGACAACCCCCACGTGTGCCGCCTGC
TP53 R248W-gRNA	UAAUUUCUACUAAGUGUAGAUGATGGGCCTCCATTTCATG
TP53 R248W-MT	TGTGTAACAGTTCCTGCATGGGCGGCATGAACTGGAGGCCCATC
TP53 R248W-WT	TGTGTAACAGTTCCTGCATGGGCGGCATGAACCGGAGGCCCATC
TP53 R248W-Helper	GCCTCCGGTTCTGCCGCCCATGCAGGAACTGTTACACA
PARP1/T>G-gRNA	UAAUUUCUACUAAGUGUAGAUAAGAAGGGAUUCCUCCAUCGA
PARP1/T>G-MT	CACAATGCGTATGACTTGGAAGTCATCGATGGAAGAATCCCTTCTT
PARP1/T>G-WT	CACAATGCGTATGACTTGGAAGTCATCGATGTAAGAATCCCTTCTT
PARP1/T>G-Helper	GGGATTCTTACATCGATGACTTCCAAGTCATACGCATTGTG
BARF V600E-gRNA	UAAUUUCUACUAAGUGUAGAUAUCGAGAUUUCUAUGUAGC
BARF V600E-MT	AGTAAAAATAGGTGATTTTGGTCTAGCTACAGAGAAATCTCGAT
BARF V600E-WT	AGTAAAAATAGGTGATTTTGGTCTAGCTACAGTGAAATCTCGAT
BARF V600E-Helper	GATTTCACTGTAGCTAGACCAAAATCACCTATTTTTACT
PIK3CA E545D-gRNA	UAAUUUCUACUAAGUGUAGAUUGAAAUCACUGCCCAGGAG
PIK3CA E545D - MT	ACCTGTGACTCCATAGAAAATCTTTCTCCTGGTCAGTGATTTCA
PIK3CA E545D - WT	ACCTGTGACTCCATAGAAAATCTTTCTCCTGCTCAGTGATTTCA
PIK3CA E545D - Helper	TCACTGAGCAGGAGAAAGATTTTCTATGGAGTCACAGGT

1.6 Table S6. DNA sequences used in Figure 5

Name	Sequences (5'→3')
EGFR-T790M-MT-L	CACGTGTGCCGCCTGCTGGGCATCTGCCTCACCTCCACCGTGCAGCTCATCACGCAGCTCATGCCCTTCGGCTGCCTCCTGGACTATGTCCGGG
EGFR-T790M-WT-L	CACGTGTGCCGCCTGCTGGGCATCTGCCTCACCTCCACCGTGCAGCTCATCACGCAGCTCATGCCCTTCGGCTGCCTCCTGGACTATGTCCGGG
RPA-FP-1	CACGTGTGCCGCCTGCTGGGCATCTGCCTC
RPA-RP-1	CCCGGACATAGTCCAGGAGGCAGCCGAAGG
EGFR-S768I-MT-L	AGCTGCACGGTGGAGGTGAGGCAGATGCCCAGCAGGCGGCACACGTGGGGGTT GTCCACGATGGCCATCACGTAGGCTTCCTGGAGGGAGGGAGAGAGGCACGTCAGTGTGGCTTCG
EGFR-S768I-WT-L	AGCTGCACGGTGGAGGTGAGGCAGATGCCCAGCAGGCGGCACACGTGGGGGTT GTCCACGCTGGCCATCACGTAGGCTTCCTGGAGGGAGGGAGAGAGGCACGTCAGTGTGGCTTCG
FP-2	AGCTGCACGGTGGAGGTGA
RP-2	CGAAGCCACACTGACGTGCCT
TP53 R248W-MT-L	GGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGCGGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAGGTCAG
TP53 R248W-WT-L	GGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGCGGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAGGTCAG
FP-3	GGCTCTGACTGTACCACCAT
RP-3	CTGACCTGGAGTCTTCCAGT
PARP1/T>G-MT-L	AGAACACTCATGCAACCACACACAATGCGTATGACTTGGAAGTCATCGATGGAAGAATCCCTTCTTCCCCCACTTCCTTCTGTGTCCTGCCAGCTCTTCCCTG
PARP1/T>G-WT-L	AGAACACTCATGCAACCACACACAATGCGTATGACTTGGAAGTCATCGATGTAAGAATCCCTTCTTCCCCCACTTCCTTCTGTGTCCTGCCAGCTCTTCCCTG
FP-4	AGAACACTCATGCAACCACACA
RP-4	CAGGGAAGAGCTGGCAGGA
BARF V600E-MT-L	TCTTCATGAAGACCTCACAGTAAAAATAGGTGATTTTGGTCTAGCTACAGAGAAATCTCGAT GGAG TGG GTC CCA TCA GTT TGA ACA GTT GTC TGG ATC CA
BARF V600E-WT-L	TCTTCATGAAGACCTCACAGTAAAAATAGGTGATTTTGGTCTAGCTACAGTGAAATCTCGAT GGAG TGG GTC CCA TCA GTT TGA ACA GTT GTC TGG ATC CA
FP-4	TCTTCATGAAGACCTCACAGT
RP-4	TGGATCCAGACAACTGTTCA

PIK3CA E545D -MT-L	CAGAGAATCTCCATTTTAGCACTTACCTGTGACTCCATAGAAAATCTTTCTCCTGGTCAGTGATTTCAGAGAGAGGATCTCGTGTAGAAATTGCTTTGAGCTGTTC
PIK3CA E545D -WT-L	CAGAGAATCTCCATTTTAGCACTTACCTGTGACTCCATAGAAAATCTTTCTCCTGCTCAGTGATTTCAGAGAGAGGATCTCGTGTAGAAATTGCTTTGAGCTGTTC
FP-4	CAGAGAATCTCCATTTTAGCAC
RP-4	GAACAGCTCAAAGCAATTTCTA

1.7 Table S7. DNA sequences used in Figure 6

Name	Sequences (5'→3')
Probe-2	HS-AAAAAAAAAAAAAAATTGATTAGTTGATTTGATTAT-FAM

SI 2 Supplementary figures

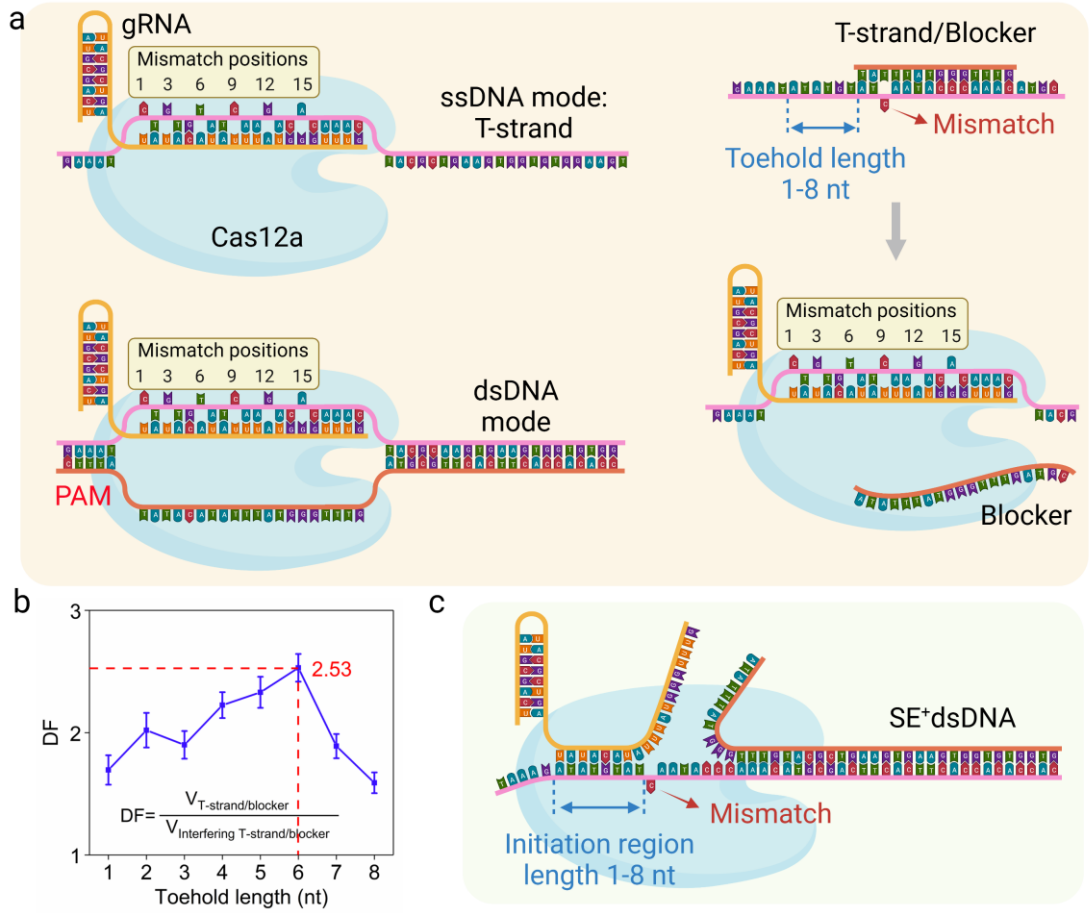

Figure S1

(a) Schematic illustration of single-base-mismatch at different positions 1，3，6，9，12 and 15 between gRNA and dsDNA, ssDNA and ssDNA/blocker. (b) The influence of the length of the initiation region on the cleavage efficiency of CRISPR-cas12a activated by ssDNA/blocker complex. Reaction setup: 100 nM B-strand, 200 nM blocker strands, 20 nM LbaCas12a, 10 nM gRNA, 200 nM ssDNA FQ probe (labeled with FAM and BHQ-1). (c) Schematic diagram of the length adjustment of the initiation region.

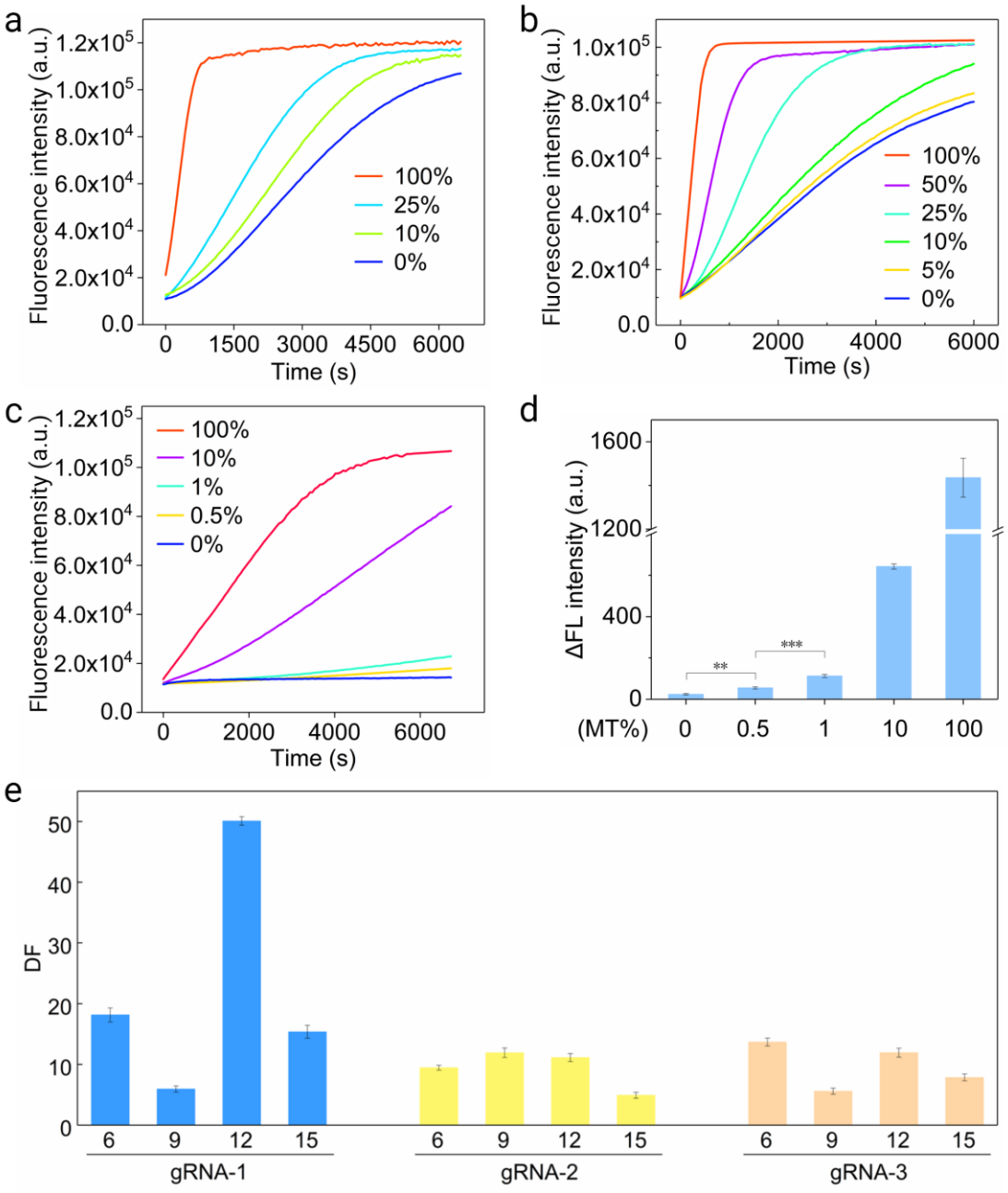

Figure S2

(a) and (b) Detection of low-abundance targeting PAM^{-}SE^{+5} dsDNA when the single-base-mismatch located at position 6 (a) and 12 (b). gRNA-1 were used. (c) and (d) The detection limit for low-abundance targeting PAM^{-}SE^{+5} dsDNA with an artificial mismatch when the non-artificial mismatch located at position 6. gRNA-1 were used. All the above experiments were conducted in triplex. Results were demonstrated in mean ±SD. Student's t-test, ns, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. (e) The DFs of CRISPR-Cas12a system toward targeting PAM^{-}SE^{+5} dsDNA and single-base-difference interfering PAM^{-}SE^{+5} dsDNAs when the non-

artificial mismatch located at position 6, 9, 12 and 15. Three randomly designed gRNAs were tested. Reaction setup: 200 nM B-strand, 400 nM T-strand, 20 nM LbaCas12a, 10 nM gRNA, 200 nM ssDNA FQ probe (labeled with FAM and BHQ-1).

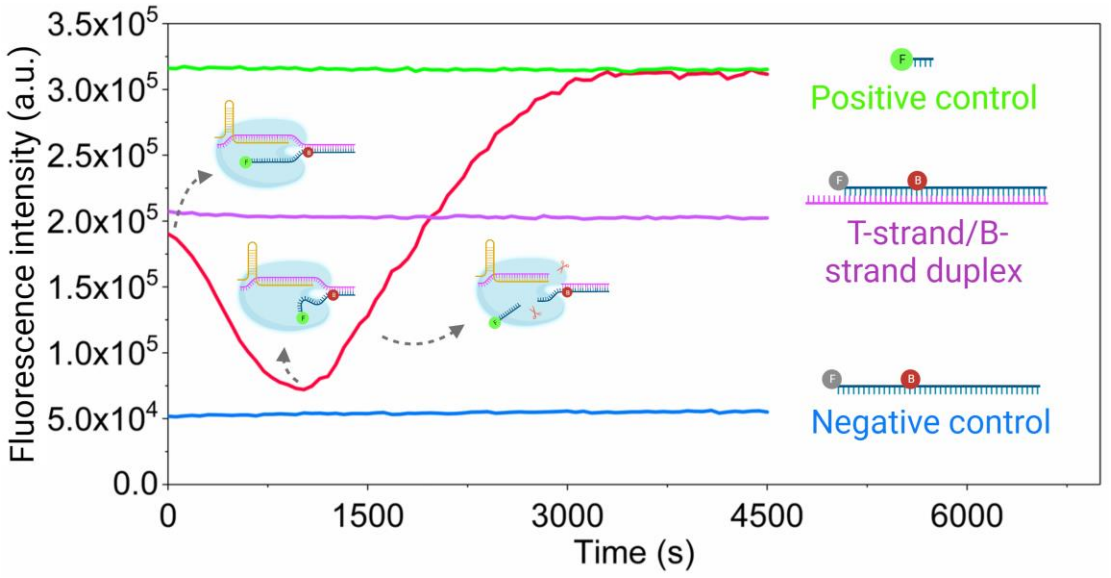

Figure S3

The CRISPR-cas12a system activated by the PAM^{-}SE^{+5} dsDNA which T-strand was labelled with a FAM at its 5' end and a BHQ-1 at the 16th nucleotide to the 5' end. Reaction system: reaction system, 200 nM B-strand, 200 nM T-strand (labeled with FAM and BHQ-1), 400 nM LbaCas12a, 200 nM gRNA, positive control, 200 nM FAM-labeled ssDNA, negative control, 200 nM T-strand (labeled with FAM and BHQ-1).

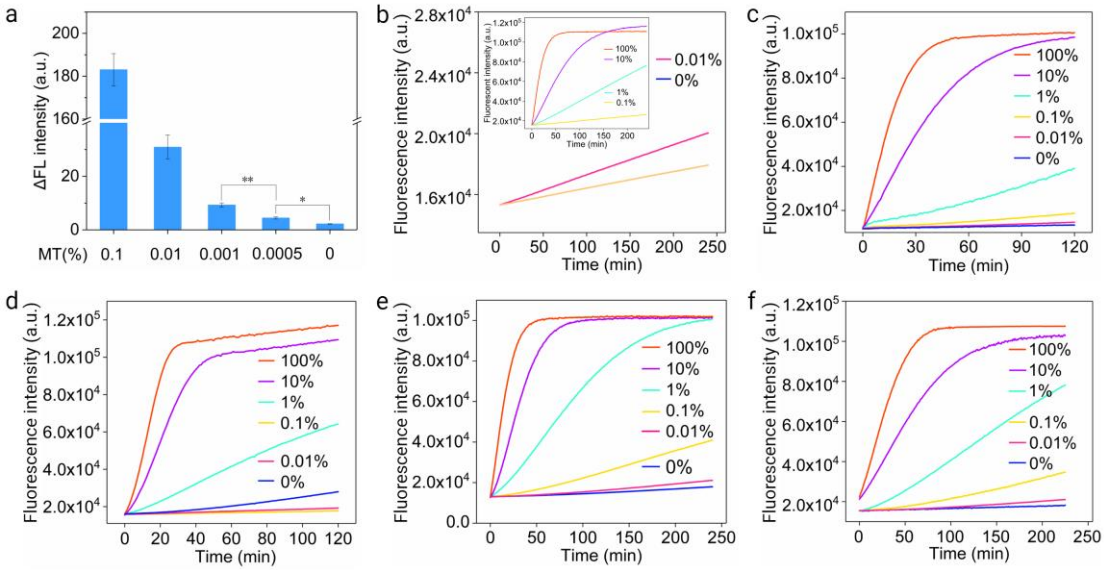

Figure S4

(a) Statistical analysis of the detection limit for low-abundance targeting PAM^{-}SE^{+5} dsDNA when the non-artificial mismatch located at position 12. gRNA-1 were used. All the experiments above repeat three times. Results were demonstrated in mean±SD. Student's t-test, ns, P>0.05; *, P<0.05; **, P<0.01; ***, P<0.001. (b)-(f) The detection limit for low-abundance synthesized MT strand of EGFR T790M (b), EGFR S768I (c), TP53 R248W (d), BRAF V600E (e) and PIK3CA E545D (f). Reaction setup: 200 nM substrate DNA, 1000 nM Helper strand, 20 nM LbaCas12a, 10 nM gRNA, 200 nM ssDNA FQ probe (labeled with FAM and BHQ-1).

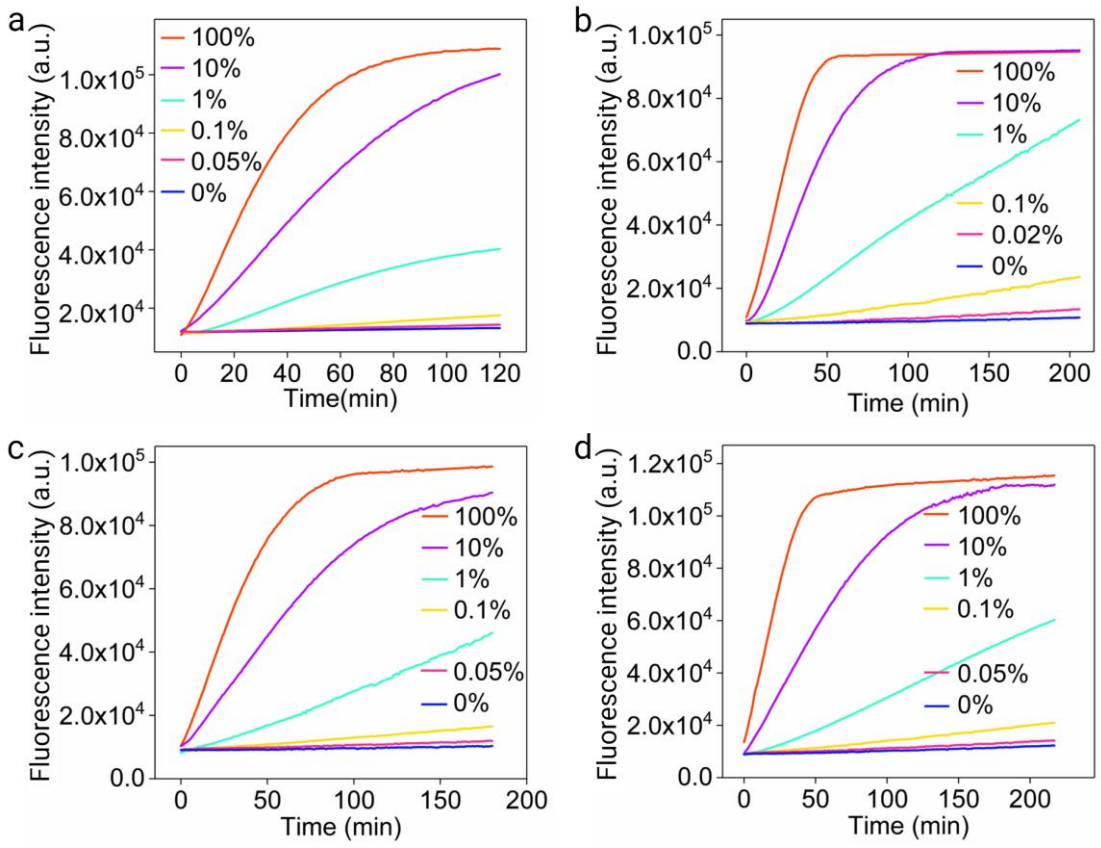

Figure S5

(a)-(d) The detection limit for low-abundance targeting MT plasmids of EGFR S768I (a), TP53 R248W (b), BARF V600E (c) and PIK3CA E545D (d) mutations by workflow 1. Reaction setup: 10 ng plasmid DNA, 4000 nM Helper strand, 40 nM LbaCas12a, 20 nM gRNA, 200 nM ssDNA FQ probe (labeled with FAM and BHQ-1).

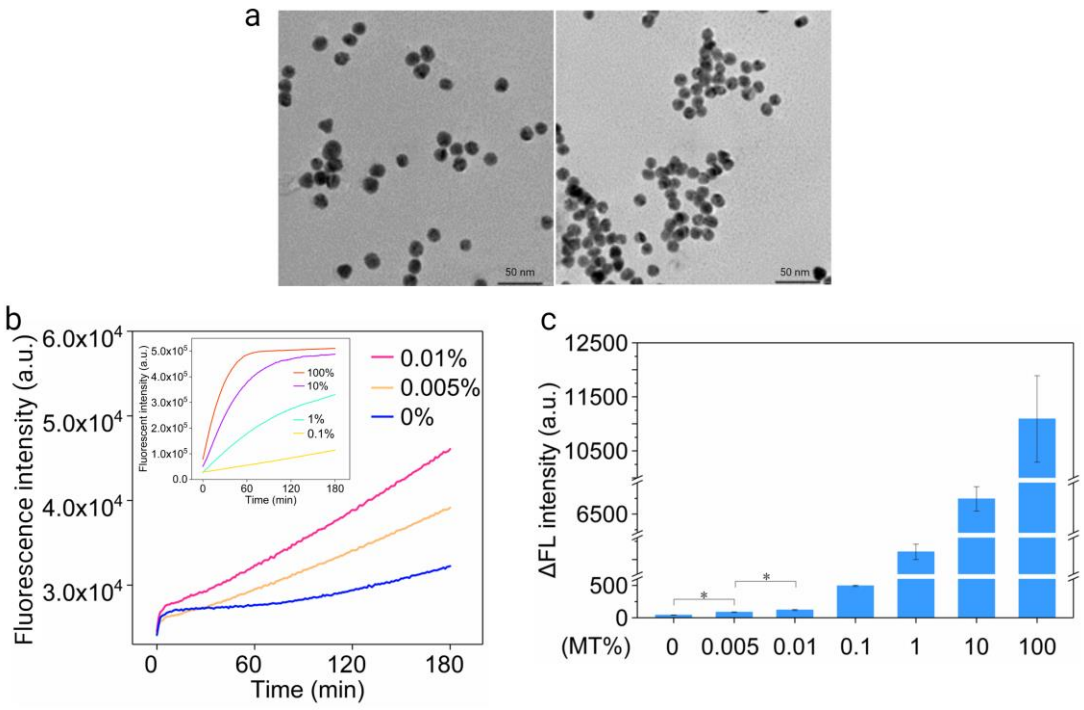

Figure S6

(a) Transmission electron microscopy (TEM) of AuNPs-DNA probe. (b)-(c) The detection limits for synthesized MT strand of EGFR T790M by AuNPs-DNA probe. Reaction setup: 400 nM substrate DNA, 4000 nM Helper strand, 40 nM LbaCas12a, 20 nM gRNA, 0.2 nM AuNPs-DNA probe. All the experiments above repeat three times. Results were demonstrated in mean±SD. Student's t-test, ns, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

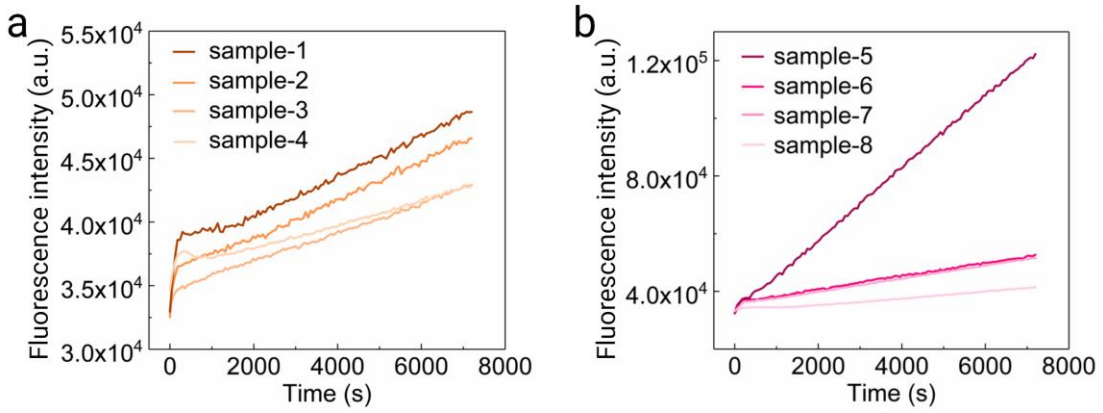

Figure S7

(a)- (b) The detection results of 8 clinical samples for EGFR T790M.

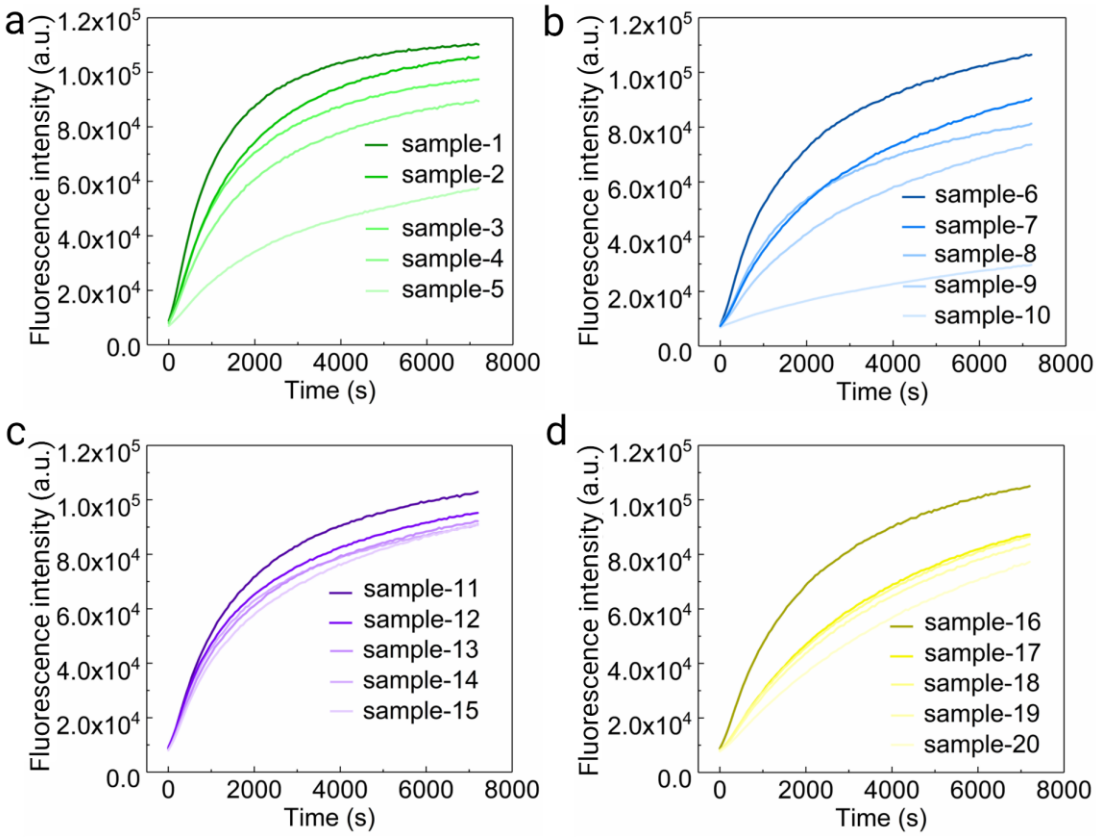

Figure S8

(a)- (d) The detection results of 20 clinical samples for TP53 R248W.