Supporting information

Multi-functional synergistic platform of Cas12a split dsDNA activators

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Materials and instruments

All oligonucleotides used in this study (Table S1) were synthesized and purified by Sangon Biotech (Shanghai, China) and subsequently diluted with 1× TE buffer (Sangon Biotech). EnGen LbaCas12a (Cpf1, LbCas12a), along with 10× NEBuffer r2.1 (100 mM Tris-HCl, 100 mM $MgCl_2$, 500 mM NaCl, 1000 μg/mL Recombinant Albumin, pH 7.9) and 10× NEBuffer 4 (200 mM Tris-acetate, 100 mM Magnesium acetate, 500 mM Potassium acetate, 10 mM DTT, pH 7.9) were procured from New England Biolabs Inc (Beijing, China). Deionized water, certified DNase/RNase free, was obtained from Tiangen Biotech (Beijing, China) and employed in all experimental procedures. Endo IV was acquired from Thermo Fisher Scientific. Additional enzymes, including APE1, T7 Exonuclease (T7), Exonuclease I (Exo I), Deoxyribonuclease I (DNase I), and Uracil glycosylase (UDG), were all sourced from New England Biolabs (NEB, MA, USA). Fluorescence measurements of all reaction solutions were conducted using a Rotor-Gene Q real-time PCR instrument (QIAGEN, Hilden, Germany), with fluorescence detection set at an excitation wavelength of 470 nm and an emission wavelength of 525 nm in the green channel.

Different combinations of split dsDNA activators for synergistic activation

In a 200 μL PCR tube, 2 μL of Cas12a (500 nM), 2 μL of crRNA (1 μM), 2 μL of 10× NEBuffer r2.1, and 2 μL of ssDNA reporter (1 μM) were combined. Subsequently, 2 μL of each splitting dsDNA activator (250 nM) was added, and the reaction mixture was adjusted to a final volume of 20 μL using deionized water. The fluorescence intensity was monitored immediately using a Rotor-Gene Q real-time PCR instrument (QIAGEN, Hilden, Germany) in the green channel, with measurements taken at 37°C in cycles of 20 seconds per cycle.

Synergistic activation platform for APE1 detection

In a 200 μl PCR tube, 2 μL of proximal split dsDNA activator (1 μM, TS-AP:NTS=1:1.5), 1 μl of 10× NEBuffer 4, and 2 μL of APE1 (at varying concentrations) were combined and thoroughly mixed in 10 uL volume. The mixture was incubated at 37°C for 30 minutes, followed by heat inactivation of APE1 at 80°C for 20 minutes. Subsequently, 2 μL of Cas12a (250 nM), 2 μL of crRNA (1 μM), 1 μL of 10× NEBuffer 4, 2 μL of ssDNA reporter (1 μM), and 2 μL of distal split dsDNA activator (1 μM) were added to the reaction. The final volume was adjusted to 20 μL using deionized water.

To assess the selectivity of this method, other potential interfering enzymes were evaluated in parallel with APE1. The enzymatic activities of APE1 and the other enzymes were analyzed using

the same protocol described above, with all enzymes standardized to a concentration of 0.00001 U/mL in the reaction solution.

For the detection of APE1 in biological samples, 2 μL of diluted cell lysate was substituted for APE1 in the aforementioned detection system. 1 uL of additional inhibitor (NCA) was used for activity inhibition experiments in cell lysate.

Synergistic activation platform for light response

In a 200 μL PCR tube, 2 μL of proximal split dsDNA activator (1 μM, TS-PC linker:NTS=1:1.5) was exposed to ultraviolet light for 1 hour to facilitate the reaction. Following illumination, 2 μL of Cas12a (250 nM), 2 μL of crRNA (1μM), 2 μL of 10× NEBuffer r2.1, and 2 μL of ssDNA reporter (1 μM) were added to the reaction. The final volume was adjusted to 20 μL using deionized water.

Synergistic activation platform for dynamic network

In a 200 μL PCR tube, 2 μL of Cas12a (500 nM), 2 μL of crRNA (1 μM), 2 μL of 10× NEBuffer r2.1, and 2 μL of ssDNA reporter (1 μM) were combined. Subsequently, 2 μL of each single-stranded probe (250 nM; A, A', B, B') was added, and the reaction mixture was adjusted to a final volume of 20 μL using deionized water.

Table S1. Detailed sequences used in experiment.

Name	Sequences from 5’ to 3’
	Synergistic activation
crRNA	UAAUUUCUACUAAGUGUAGAUCUCAGGGCGGACGAGGUUU AGGAU
reporter	FAM-TTTTTTTTTTTTTT-BHQ-1
Proximal-TS	GTCCGCCCTGAGTAAAGCGA
Proximal-TS-1Del	TCCGCCCTGAGTAAAGCGA
Proximal-TS-2Del	CCGCCCTGAGTAAAGCGA
Proximal-TS-1nt	GGTCCGCCCTGAGTAAAGCGA
Proximal-TS-2nt	AGGTCCGCCCTGAGTAAAGCGA
Proximal-TS-3nt	GAGGTCCGCCCTGAGTAAAGCGA
1nt-Proximal-TS	GTCCGCCCTGAGTAAAGCGAA
2nt-Proximal-TS	GTCCGCCCTGAGTAAAGCGAAA
3nt-Proximal-TS	GTCCGCCCTGAGTAAAGCGAAAT
Proximal-TS-M1ntEx	CGTCCGCCCTGAGTAAAGCGA
Proximal-TS-M2ntEx	TCGTCCGCCCTGAGTAAAGCGA
Proximal-TS-M3ntEx	CTCGTCCGCCCTGAGTAAAGCGA
Proximal-TS-M4ntEx	CCTCGTCCGCCCTGAGTAAAGCGA
Proximal-TS-M5ntEx	ACCTCGTCCGCCCTGAGTAAAGCGA
Proximal-NTS	TCGCTTTACTCAGGGCGGAC
Proximal-NTS-1ntDel	TCGCTTTACTCAGGGCGGA

Proximal-NTS-2ntDel	TCGCTTTACTCAGGGCGG
Proximal-NTS-1nt	TCGCTTTACTCAGGGCGGACG
Proximal-NTS-2nt	TCGCTTTACTCAGGGCGGACGA
Proximal-NTS-3nt	TCGCTTTACTCAGGGCGGACGAG
1nt-Proximal-NTS	ATCGCTTTACTCAGGGCGGA
2nt-Proximal-NTS	AATCGCTTTACTCAGGGCGGA
3nt-Proximal-NTS	TAATCGCTTTACTCAGGGCGGA
Distal-TS	ATCCTAAACCTC
1nt-Distal-TS	ATCCTAAACCTCC
2nt-Distal-TS	ATCCTAAACCTCCA
3nt-Distal-TS	ATCCTAAACCTCCAG
Distal-TS-1nt	AATCCTAAACCTC
Distal-TS-2nt	AAATCCTAAACCTC
Distal-TS-3nt	TAAATCCTAAACCTC
Distal-NTS	GAGGTTTAGGAT
1nt-Distal-NTS	CGAGGTTTAGGAT
2nt-Distal-NTS	ACGAGGTTTAGGAT
3nt-Distal-NTS	GACGAGGTTTAGGAT
Distal-NTS-1nt	CGAGGTTTAGGATA
Distal-NTS-2nt	CGAGGTTTAGGATAA
Distal-NTS-3nt	CGAGGTTTAGGATAAT
APE1 detection	
Proximal-TS-AP	GGATTTGGA*GTCCGCCCTGAGTAAAGCGA
Proximal-NTS	TCGCTTTACTCAGGGCGGACATCC
Light response	
Proximal-TS-PC linker	TGGAG/iPClink/GTCCGCCCTGAGTAAAGCGA
Proximal-NTS	TCGCTTTACTCAGGGCGGAC
Dynamic network	
A	GTCCGCCCTGAGTAAAGCGA
A’	TCGCTTTACTCAGGGCGGAC
B	TTTTTTTTTTTGGGGGGGGGGGGTCCGCCCTGAGTAAAGCGA
B’	TCGCTTTACTCAGGGCGGACGGGGGGGGGGGTTTTTTTTTTT
A-BHQ	GTCCGCCCTGAGTAAAGCGA-BHQ-1
A’-HEX	HEX-TCGCTTTACTCAGGGCGGAC

* Represents AP site modification and /IPClink/ represents PC-Linker modification

Table S2. Comparison of sensitivity with other methods

Method	Detection of limit	References
Catalytic hairpin self-assembly and G-quadruplex/hemin DNAzyme	3.37×10^{-4} U/mL	[1]
Defective PAM-driven self-catalytic biosensor	7.66×10^{-5} U/mL	[2]

Dual-locking probe self-assembly	0.05 U/mL	[3]
Synergistic platform of Cas12a split dsDNA activator	0.00001 U/mL	This work

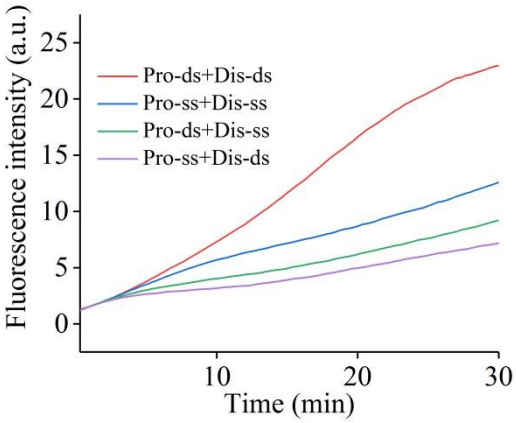

Fig. S1 Comparison of synergistic activation effects of different combinations of split activators.

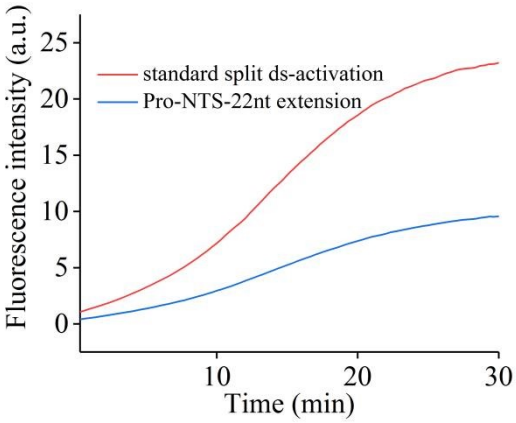

Fig. S2 The fluorescence curve of proximal NTS-22nt extension and standard activator for synergistic activation.

References:

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