Supporting Information

DNA Domino Circuits Based on a Hairpin Exonuclease Assistance Signal Transmission Architecture for Temporal Logic Operation

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Material and Method

Materials:

The DNA oligonucleotides (oligos) used in this study were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The fluorescence and 5' phosphate-modified DNA strands were purified via high-performance liquid chromatography (HPLC). Their sequences were listed in Table S1. The sequences of the DNA oligos were designed by NUPACK (http://www.nupack.org) to prevent undesired structures. The EXO λ and the corresponding reaction buffer (67 mM Glycine-KOH, 2.5 mM MgCl2, 50 μg/mL BSA, pH 9.4 @ 25°C) were obtained from New England Biolabs (Beijing, China). All DNA oligos were diluted with ultra-pure water (18.2 MΩ·cm) to 50 μM and stored at 4 °C. During the experiments, the concentrations of the DNA oligos were measured using a Nanodrop 2000 spectrophotometer (Thermo-Fisher Scientific, USA) at 260 nm.

DNA assembly:

The substrates corresponding to all of the reaction pathways were annealed by the two DNA strands shown in the correlation diagram Fig. 1B (e.g. Sub1-2). The DNA oligos were first diluted with ultra-pure water and determined with Nanodrop 2000 spectrophotometer. Adjust the concentration to 50 μM and set aside to be used. When the experiment was conducted, the two DNA strands that form the Sub reaction substrate were mixed together and diluted to 10 μM with 10× EXO λ reaction buffer and water purchased in NEB. It was then placed into the C1000 PCR (Bio-Rad, USA) to reduce the temperature from 95 °C to 25 °C at a rate of 1°C per minute. The reaction substrate obtained was refrigerated at 4 °C for later use.

Polyacrylamide gel electrophoresis experiment:

The construction of the relevant reaction pathways was verified by native polyacrylamide gel electrophoresis (PAGE) experiments. The obtained 10 μM Sub1-2F reaction substrate was mixed with 2 μL 20 U EXO λ and 2 μL 10× EXO λ reaction buffers, and then different concentrations of In1 were added. The reaction volume was supplemented with ultra-pure water to 20 μL. The reaction temperature was controlled at 28 ℃ and the reaction time was 2 h. Briefly, 12% gel was prepared with 1× PBS buffer. All samples were electrophoresed at room temperature for 100 min at 75 V. The instrument used for electrophoresis was the DYY-6D (LIUYI, China). The PAGE gels were illuminated by ultraviolet lamp and stained with Stain-All, then imaged on a Canon scanner.

Fluorescence resonance energy transfer experiment:

The reaction substrate corresponding to each pathway, input DNA strand, EXO λ and reporters modified with FAM-BHQ1, ROX-BHQ2, and Cy5-BHQ3 fluorescence-

quenching pairs were added to 1× EXO lambda buffer according to specific predetermined concentrations described later in this document. The reaction volume was supplemented with ultra-pure water to 50 μL. The excitation-emission wavelengths of the microplate reader (Tecan, Switzerland) are adjusted to 457-520, 558-606, and 620-665 nm. The temperature and detection interval are 28 °C and 2 min.

Oligonucleotides	Sequences (5 '-3 ')
D*1-T*1-D1-D*2-T*2	PO_4-CGTAATATTC GCTAGACTCCATACT GAATATTACG TCATCACGGA GTAGCAAGTCACATA
T2-D2	TATGTGACTTGCTAC TCCGTGATGA
D*1-T*1-D1-D*2-T*2-BHQ1	PO_4-CGTAATATTC GCTAGACTCCATACT GAATATTACG TCATCACGGA GTAGCAAGTCACATA-BHQ1
FAM-T2-D2	FAM-TATGTGACTTGCTAC TCCGTGATGA
T-T1-D1	TTTTT AGTATGGAGTCTAGC GAATATTACG
D*2-T*2-D2-D*1-T*1	PO_4-TCATCACGGA GTAGCAAGTCACATA TCCGTGATGA CGTAATATTC GCTAGACTCCATACT
T-T2-D2	TTTTT TATGTGACTTGCTAC TCCGTGATGA
D*2-T*2-D2-D*1-T*1-BHQ1	PO_4-TCATCACGGA GTAGCAAGTCACATA TCCGTGATGA CGTAATATTC GCTAGACTCCATACT-BHQ1
T-FAM-T1-D1	TTTTT-FAM-AGTATGGAGTCTAGC GAATATTACG
T-FAM-T2-D2	TTTTT-FAM-TATGTGACTTGCTAC TCCGTGATGA
D*2-T*2-D2-D*3-T*3	PO_4-TCATCACGGA GTAGCAAGTCACATA TCCGTGATGA CCAGAGATCC GAATGCAGCTAACAC
D*3-T*3-D3-D*1-T*1	PO_4-CCAGAGATCC GAATGCAGCTAACAC GGATCTCTGG CGTAATATTC GCTAGACTCCATACT
D*2-T*2-D2-D*3-T*3-BHQ1	PO_4-TCATCACGGA GTAGCAAGTCACATA TCCGTGATGA CCAGAGATCC GAATGCAGCTAACAC-BHQ1
D*3-T*3-D3-D*1-T*1-BHQ1	PO_4-CCAGAGATCC GAATGCAGCTAACAC GGATCTCTGG CGTAATATTC GCTAGACTCCATACT-BHQ1
T-T3-D3	TTTTT GTGTTAGCTGCATTC GGATCTCTGG
T3-D3	GTGTTAGCTGCATTC GGATCTCTGG
T-FAM-T3-D3	TTTTT-FAM-GTGTTAGCTGCATTC GGATCTCTGG
T0-D0	TTATGCAATTCGAAC TACGATCTTA
D*0-T*0-D0-D*1-T*1	PO_4-TAAGATCGTA GTTCGAATTGCATAA TACGATCTTA CGTAATATTC GCTAGACTCCATACT

D*0-T*0-D0-D*2-T*2	PO_4-TAAGATCGTA GTTCGAATTGCATAA TACGATCTTA TCATCACGGA GTAGCAAGTCACATA
D*0-T*0-D0-D*3-T*3	PO_4-TAAGATCGTA GTTCGAATTGCATAA TACGATCTTA CCAGAGATCC GAATGCAGCTAACAC
FAM-T0	FAM-TTATGCAATTCGAAC
FAM-T1	FAM-AGTATGGAGTCTAGC
ROX-T2	ROX-TATGTGACTTGCTAC
Cy5-T3	Cy5-GTGTTAGCTGCATTC
D*0-T*0-BHQ1	TAAGATCGTA GTTCGAATTGCATAA-BHQ1
D*1-T*1-BHQ1	CGTAATATTC GCTAGACTCCATACT-BHQ1
D*2-T*2-BHQ2	TCATCACGGA GTAGCAAGTCACATA-BHQ2
D*3-T*3-BHQ3	CCAGAGATCC GAATGCAGCTAACAC-BHQ3
D*1-T*1-D1-D*0-T*0	PO_4-CGTAATATTC GCTAGACTCCATACT GAATATTACG TAAGATCGTA GTTCGAATTGCATAA
D*2-T*2-D2-D*0-T*0	PO_4-TCATCACGGA GTAGCAAGTCACATA TCCGTGATGA TAAGATCGTA GTTCGAATTGCATAA
D*3-T*3-D3-D*0-T*0	PO_4- CCAGAGATCC GAATGCAGCTAACAC GGATCTCTGG TAAGATCGTA GTTCGAATTGCATAA
D*1-T*1-3T-D1-D*2-T*2	PO_4- CGTAATATTC GCTAGACTCCATACT **TTT** GAATATTACG TCATCACGGA GTAGCAAGTCACATA
D*1-T*1-6T-D1-D*2-T*2	PO_4- CGTAATATTC GCTAGACTCCATACT **TTTTTT** GAATATTACG TCATCACGGA GTAGCAAGTCACATA
D*3-T*3-D3-D*4-T*4	PO_4- CCAGAGATCC GAATGCAGCTAACAC GGATCTCTGG TCTGATAAGCTATTA TCATCACGGA
T4-D4	TCCGTGATGA TAATAGCTTATCAGA
FAM-T2	FAM-TATGTGACTTGCTAC
T4-ROX	TAATAGCTTATCAGA-ROX
BHQ2-T4*D2*T2*-BHQ1	BHQ2-TCTGATAAGCTATTA TCATCACGGA GTAGCAAGTCACATA-BHQ1

Table S1: The DNA oligos used in this study for the electrophoresis and FRET experiments were obtained from Sangon Biotech, and DNA sequences are shown above. t

“PO_4” represent 5' -terminal phosphate modification, “TTTTT”is 5nt poly-T to improve the discrimination of gel bands for reaction substrates. “FAM”, “ROX”, “Cy5” represent different kinds of fluorescent group modification which can be quenched by “BHQ-1”, “BHQ-2”, “BHQ-3”.

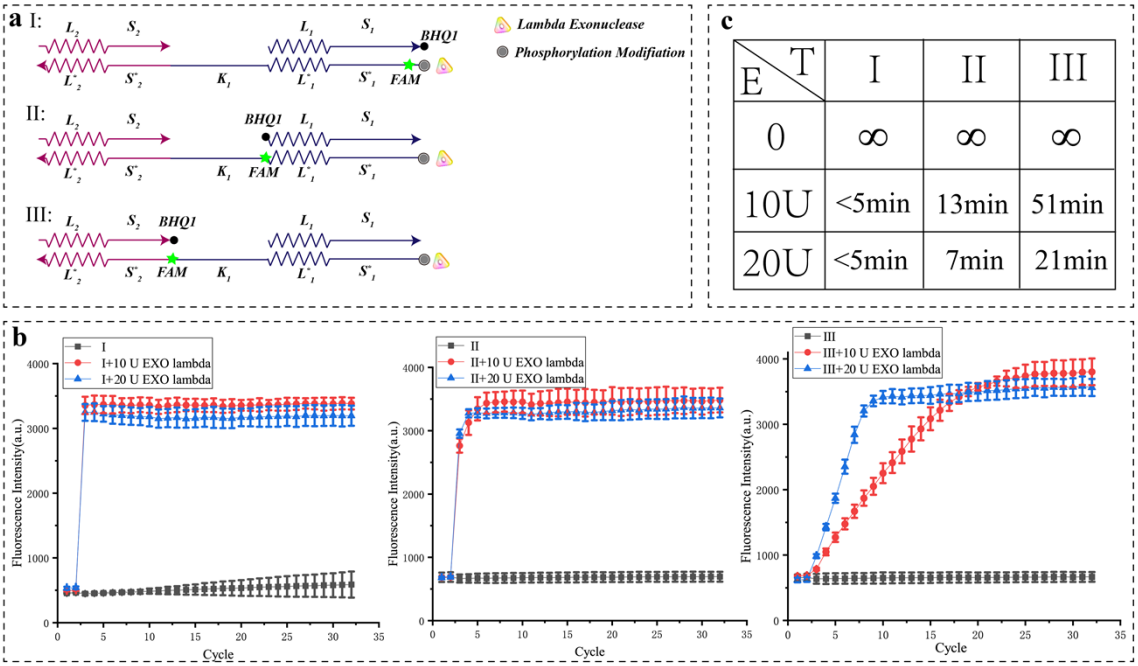

Figure S1: a. Three different fluorescent modification sites on the H-EAST architecture. b. In a 50 μl reaction volume, with the reaction substrate (I, II, III) concentration controlled at 400 nM, add 0, 10, and 20 U of EXO lambda respectively, and detect the changes in fluorescence intensity. Due to the sample addition operation, it takes 5 minutes for the second to the third cycle, and the detection interval for each other cycle is 2 minutes. c. Record the time taken for the reaction when the fluorescence reaches its maximum value. It can be observed that the rate of EXO lambda in hydrolyzing single-stranded DNA is significantly slower than that of double-stranded DNA.

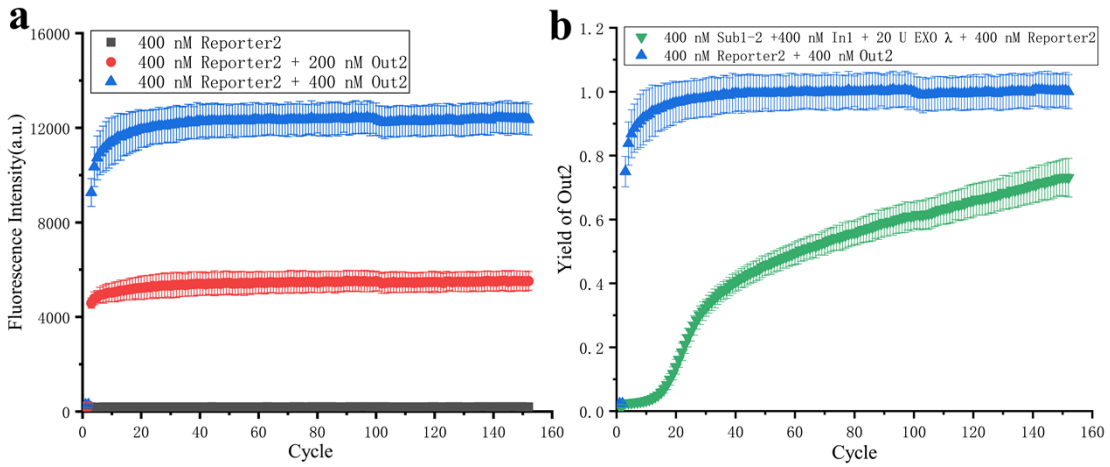

Figure S2. Taking Sub1-2 as an example, we studied the yield of the reaction substrate. We detected the produced Out2 by adding Reporter2 to the reaction system. First, 0, 200, 400 nM of the Out2 strands were added to 400 nM of Reporter2, and the fluorescence end values were measured, as shown in Figure. S2(a). Then, the fluorescence end values were used to normalized the fluorescence values during the Sub1-2 reaction process, obtaining the yield curve data, as shown in Figure. S2(b). Every detection cycle takes 2 min.

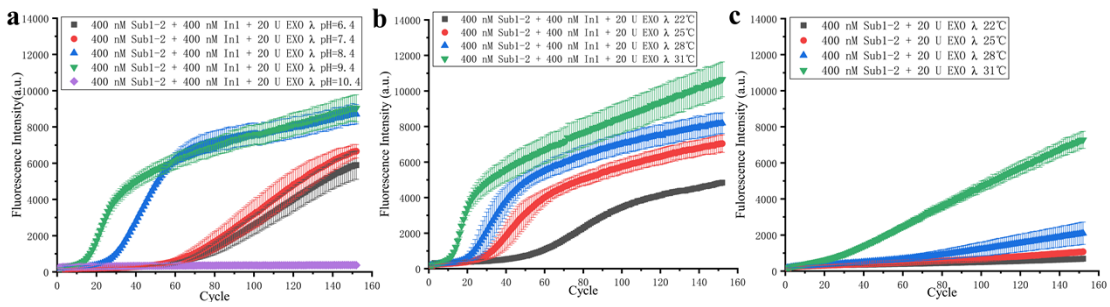

Figure S3. Taking Sub1-2 as an example, after adding In1 to the reaction, we adjusting the pH from 6.4 to 10.4, it can be observed that more Out2 is produced at pH=9.4, as shown in Figure. S3(a). By adjusting the reaction temperature from 22℃ to 31℃, although the higher the temperature, the higher the yield of Out2, as shown in Figure. S3(b). However, when In1 is not added to the reaction system, using a higher reaction temperature would result in greater molecular signal leakage (false positive), as shown in Figure. S3(c). Therefore, based on the above observation, we have selected 28℃ and pH 9.4 as the reaction conditions for the experiment.

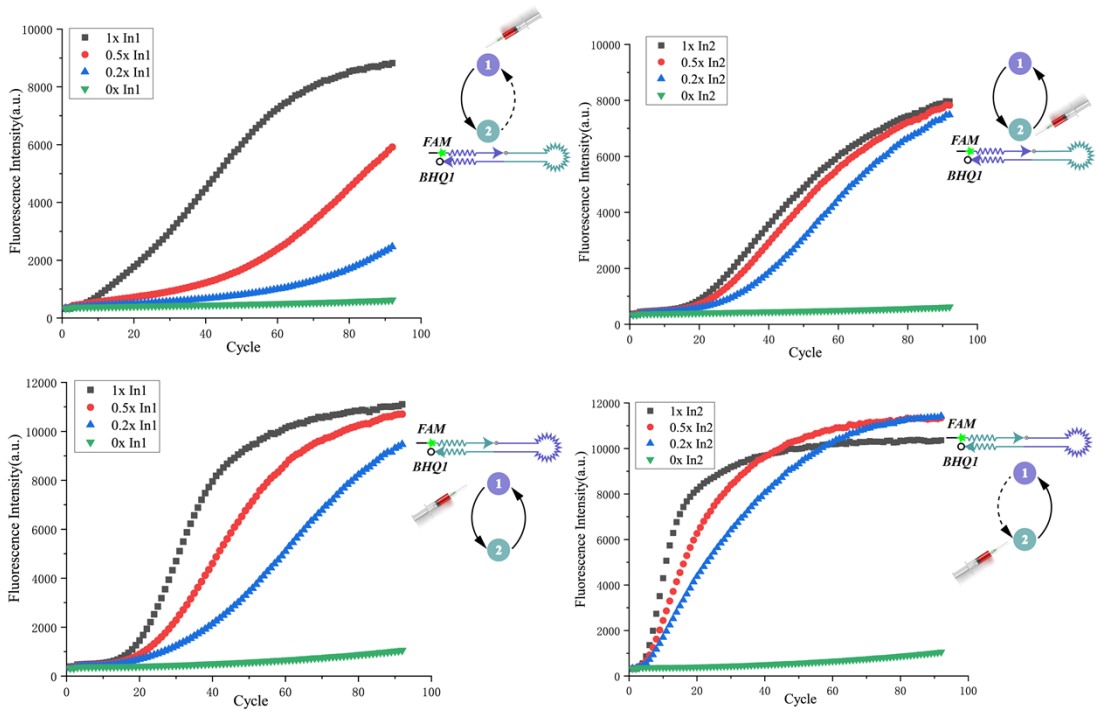

Figure S4. Based on the real-time fluorescence diagram of cross-catalytic reaction using the H-EAST architecture, we can replace TSub1-2 and TSub2-1 in the reactions substrates with TSub1-2F and TSub2-1F, respectively, and input TIn1 and TIn2, continuously monitoring the changes in fluorescence intensity.

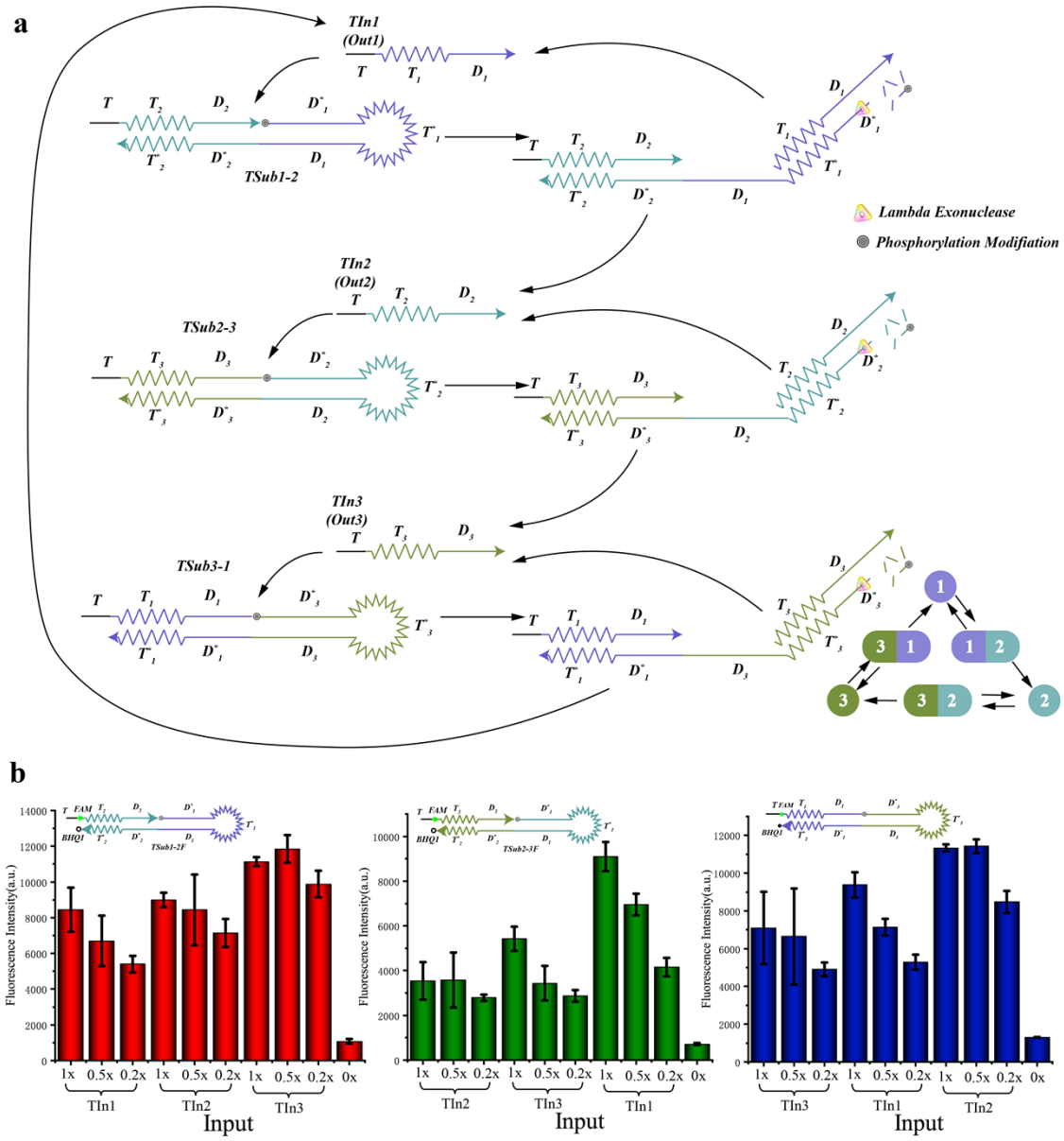

Figure S5. a. Schematic of a cyclic catalytic reaction with three nodes. b. FRET experiment for the catalytic reaction with input strands. Monitoring the fluorescence output from nodes 1, 2, and 3 after 4 hours in relation to the input strand concentration ranging from 0 to 1x, using 20 U EXO lambda. The substrate concentration represented by each node is 400 nM.

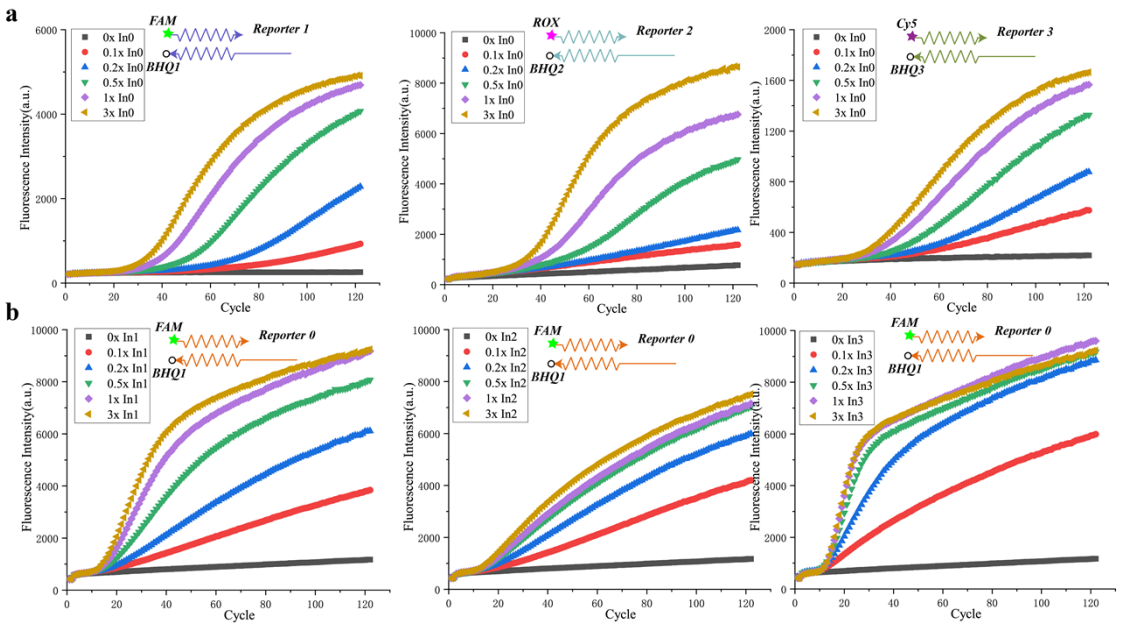

Figure S6. a. Real-time fluorescence data graph of a Fan-out circuit based on the H-EAST architecture, with input In0 detecting the fluorescence signal intensity of the FAM, ROX, and Cy5 channels. b. Real-time fluorescence data graph of a Fan-in circuit based on the H-EAST architecture, detecting the changes in fluorescence signal intensity caused by inputs In1, In2, and In3 using Reporter0, with each detection cycle being 2 minutes.

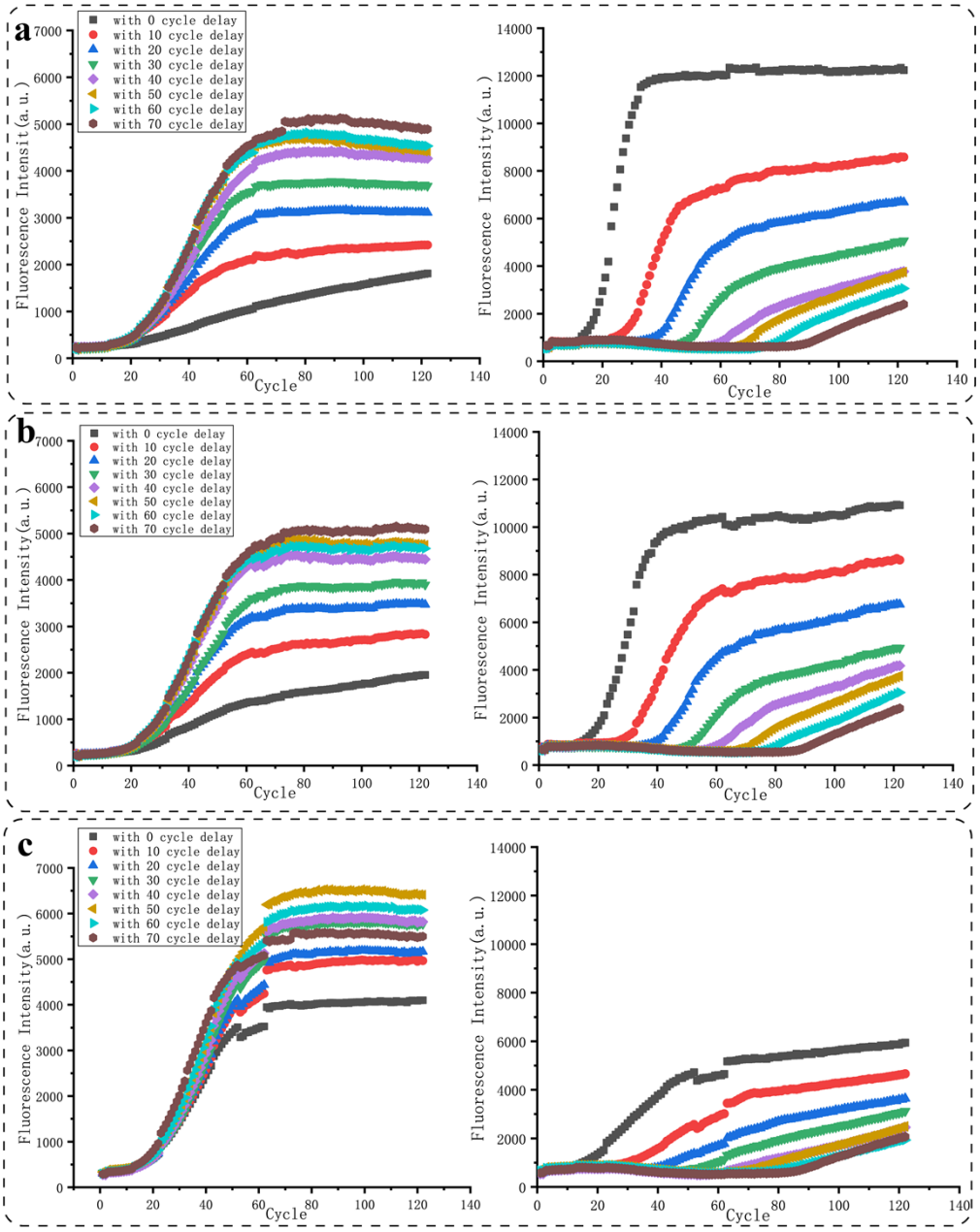

Figure S7. Modify the input concentrations and timing of the temporal OR logic gate and record the fluorescence intensity change curve. Control $[Sub1\text{-}2]_0$=$[Sub3\text{-}4]_0$=400 nM, $[Reporter2\text{-}4]_0$=400 nM, under the effect of 20 U EXO lambda, add the following relactents to a 50 μl reaction system: a. $[In1]_0$=400 nM, $[In3]_0$=400 nM, b. $[In1]_0$=400 nM, $[In3]_0$=200 nM, c. $[In1]_0$=400 nM, $[In3]_0$=80 nM. In1 was added in the second cycle, while In3 is added in the 2nd, 12th, 22nd, 32nd, 42nd, 52nd, 62nd, and 72nd cycles.