Supporting Information

A rapid and highly sensitive ctDNA detection platform based on locked nucleic acids-assisted CHA circuits

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Table S1 Sequences of the oligonucleotides for ctDNA-initiated catalytic hairpin assembly (CHA) system.

Name	Sequence(5'-3')
PIK3CA E542K (Target ctDNA)	CTCAGTGA(3*) TTTTAGA(2*) GAGAGGAT(1*)
DNA-H_1	ATCCTCTC(1) TCTAAAA(2) TCACTGAG(3) CCATGTGTAGA(4*) CTCAGTGA(3*) TTTTAGA(2*) CCTTGTCA(5*) TAGAGCAC(6*)
H_1	ATCCTCTC TCTAAAA TCACTGAG CCATGTGTAGA CTCAGTGA TTTTAGA CCTTGTCA TAGAGCAC
$H_{1\text{-}S}$	ATCCTCTC TCTAAAA TCACTGAG CCATGTGTAGA CTCAGTGA TTTTAGA CCTTGTCA TAGAGCAC
$H_{1\text{-}R}$	ATCCTCTC TCTAAAA TCACTGAG CCATGTGTAGA CTCAGTGA TTTTAGA CCTTGTCA TAGAGCAC
DNA-H_2	TCACTGAG(3) TCTACACATGG(4) CTCAGTGA(3*) TTTTAGA(2*) CCATGTGTAGA(4*)
H_2	TCACTGAG TCTACACATGG CTCAGTGA TTTTAGA CCATGTGTAGA
Reporter-F (Rep-F)	FAM-CGA GTGCTCTA TGACAAGG TCTAAAA
Regular Rep-F	FAM-CGA GTGCTCTA TGACAAGG TCTAAAA
Reporter-Q (Rep-Q)	CCTTGTCA TAGAGCAC TCG-BHQ-1
KRAS G12DM	ACT CTT GCC TAC GCC ATC AGC TCC AAC TAC CAC AAG TTT
EGFR L858R	CAG CAG TTT GGC CAG CCC AAA ATC TGT GAT CTT GAC ATG
BRAF V600E	GAT TTT GGT CTA GCT ACA GAG AAA TCT CGA TGG AGT GGG
1-Mut	CTC AGT GAT TTA AGA GAG AGG AT
2-Mut	CTC AGT GAT TTA AGA GTG AGG AT
3-Mut	CTC AGA GAT TTA AGA GTG AGG AT
Molecular Beacon (H_3)	FAM-GCT AGC ATC CTC TCT CTA AAA TCA CTG AGG CTA GC-BHQ-1

Red letters denote locked nucleic acid bases.

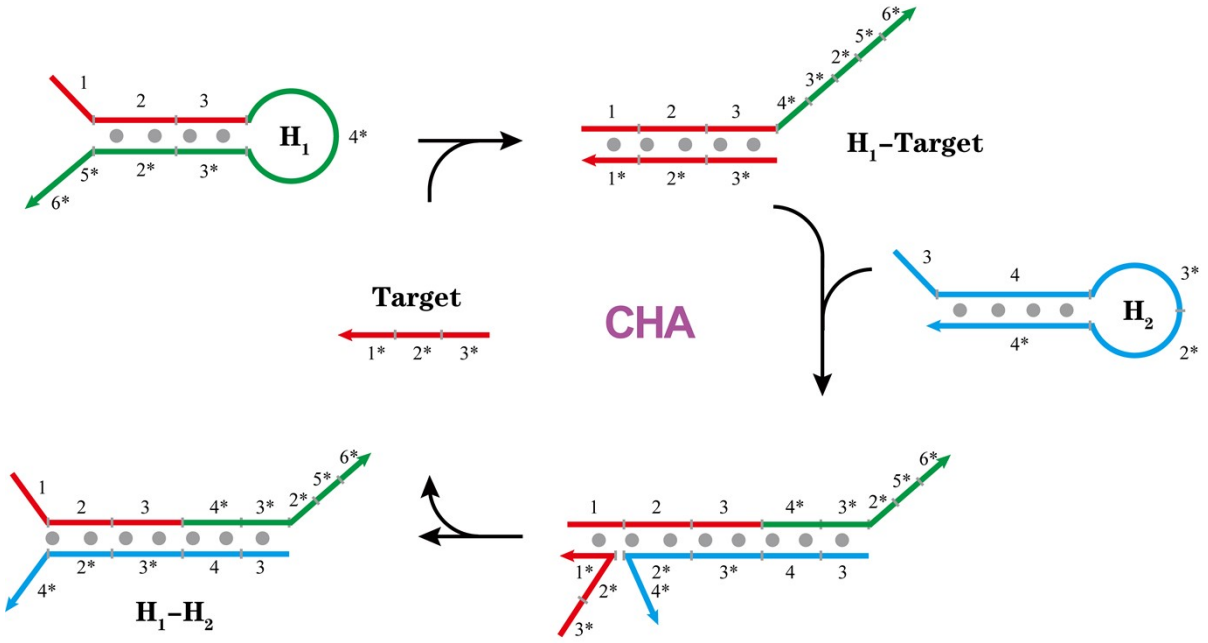

Fig. S1 Proposed scheme of updated LNA-assisted catalyzed hairpin assembly (CHA) system triggered by target ctDNA. Two pairs of LNAs are respectively incorporated near the stem-end of hairpin H_1 and H_2 (detailed site, see **Table S1**). Arrows of DNA strands denote 3'-termini. Gray circles denote base-pairing. The numbers of domains are complementary to the corresponding asterisks. Short gray dashes denote the junctions between domains.

The CHA reaction is consisted of the two hairpins (H_1 and H_2). The sequence 1-2-3 of hairpin H_1 is complementary to the sequence 1*-2*-3* of target ctDNA. And the stem domain 3* of hairpin H_1 is complementary to the toehold region 3 of hairpin H_2. Hairpin H_2 is encoded with a partially complementary sequence 3*-4*-3-2 to hairpin H_1. Target ctDNA firstly opens hairpin H_1 via a toehold-mediated strand displacement, leading to the formation of H_1-target hybrid. And the newly exposed sticky sequence 3* of hairpin H_1 docks to the toehold 3 of hairpin H_2, and then opens hairpin H_2 via branch-migration, leading to the assembly of H_1-H_2 duplex DNA and the regeneration of target ctDNA for next CHA reaction. Thus, target ctDNA stimulates the efficient CHA reaction, leading to the successive hybridizations between hairpins H_1 and H_2, and continuously generating H_1-H_2 product.

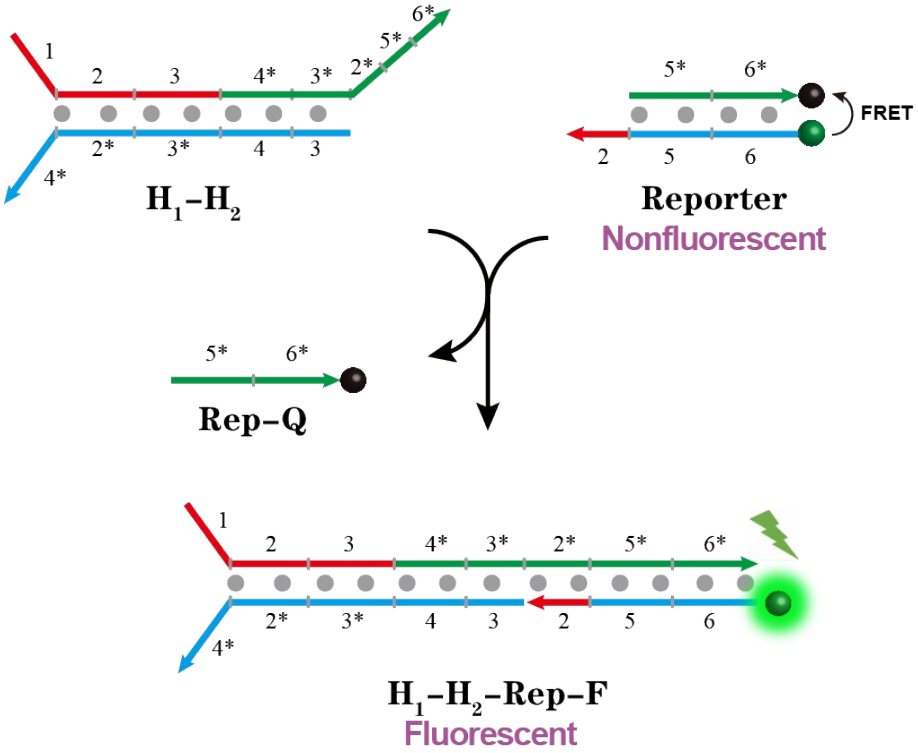

Fig. S2 Design diagram of the fluorescent reporter in updated CHA system. The reporter is four LNA nucleotides-containing hybridized DNA duplex (detailed site, see **Table S1**) with a FAM-labeled strand Rep-F and a BHQ-1-labeled strand Rep-Q (FAM denoted as 6-carboxy fluorescein; BHQ-1 denoted as Black Hole Quencher-1), resulting in the fluorescence quenching via the fluorescence resonance energy transfer (FRET) mechanism. Hybridization of hairpin H_1 and hairpin H_2 exposes the domain 2* of hairpin H_1, which docks to the toehold 2 of Rep-F and leads to the separation of Rep-Q via toehold-mediated strand displacement, thus generating high fluorescence readout.

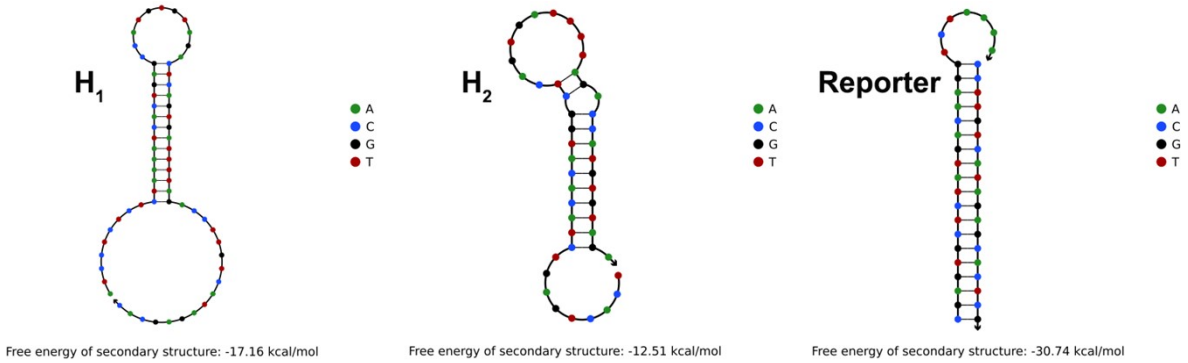

Fig. S3 Optimized structures of the updated CHA constructs H_1, H_2 and reporter by theoretical calculations.

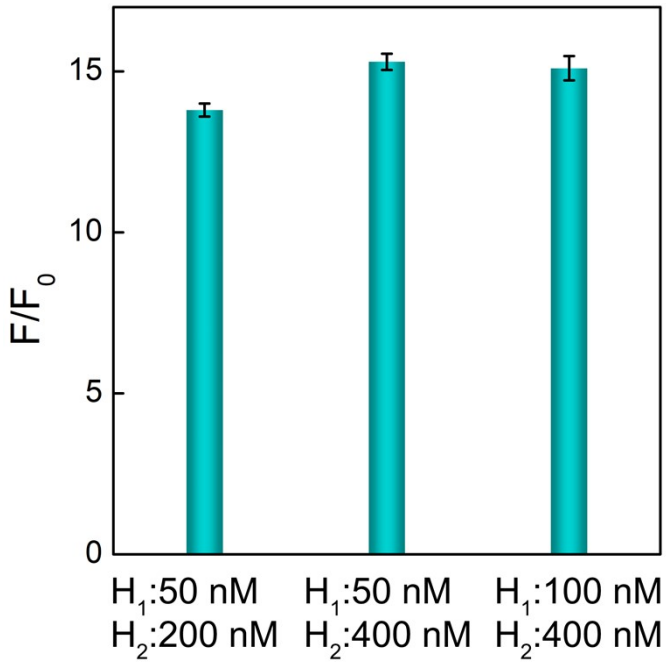

Fig. S4 Optimized concentration of metastable LNA-incorporated hairpins in the updated CHA system. H_1 (50 nM, 100 nM), H_2 (200 nM, 400 nM), reporter (50 nM) and target ctDNA (5 nM). F and F_0 refer to the fluorescence intensity with and without target ctDNA, respectively. The reaction time is set as 40 min, and the reaction temperature is set as 37℃. Results are presented as means ± standard deviation (SD) (n=3).

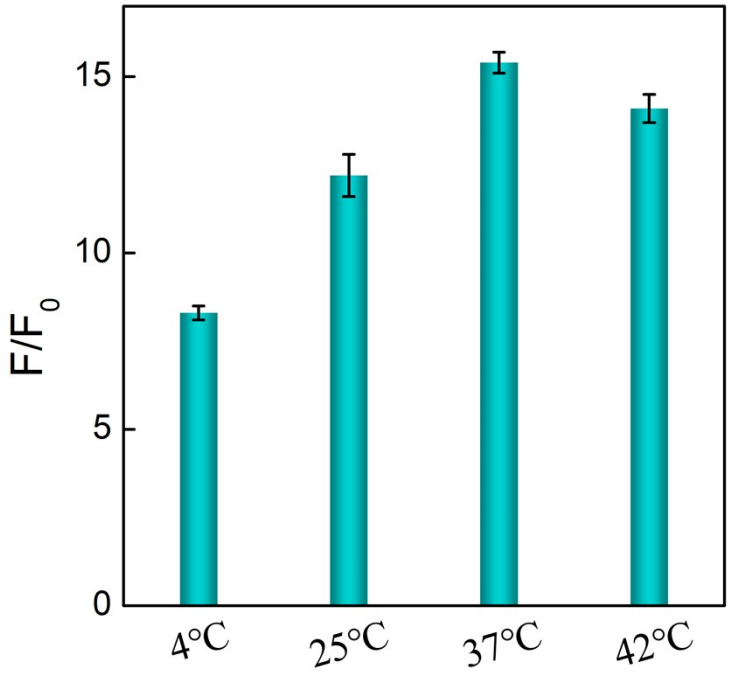

Fig. S5 Optimized reaction temperature of metastable LNA-incorporated hairpin in the updated CHA system. H_1 (50 nM), H_2 (400 nM), reporter (50 nM) and target ctDNA (5 nM). F and F_0 refer to the fluorescence intensity with and without target ctDNA, respectively. The reaction time is set as 40 min. Results are presented as means ± standard deviation (SD) (n=3).

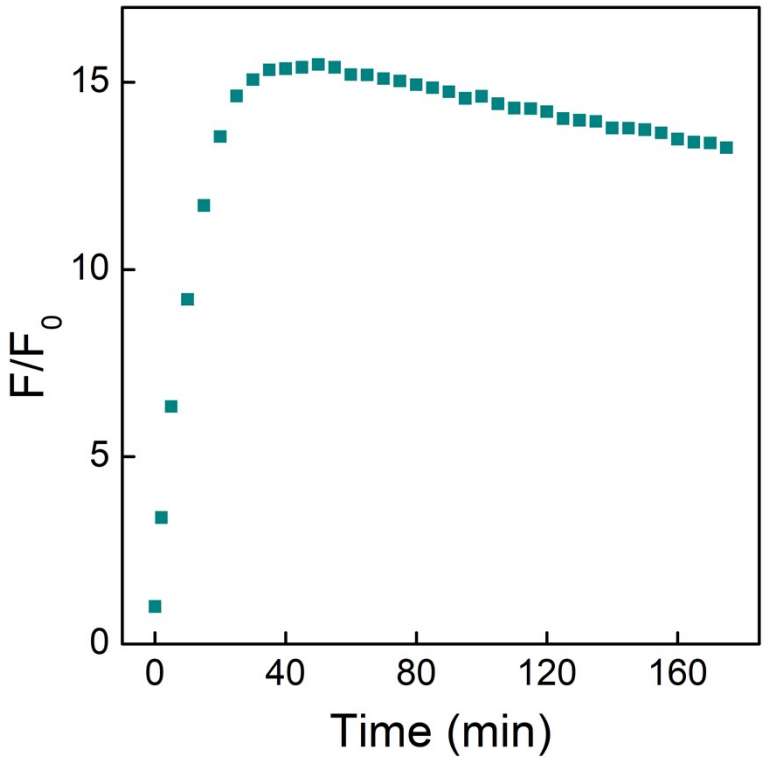

Fig. S6 Fluorescence kinetics of the updated CHA system in the presence of target ctDNA (5 nM). The used concentrations of constituents are hairpin H_1 (50 nM), H_2 (400 nM) and reporter (50 nM). F and F_0 refer to the fluorescence intensity with and without target ctDNA, respectively. The reaction temperature is set as 37℃.

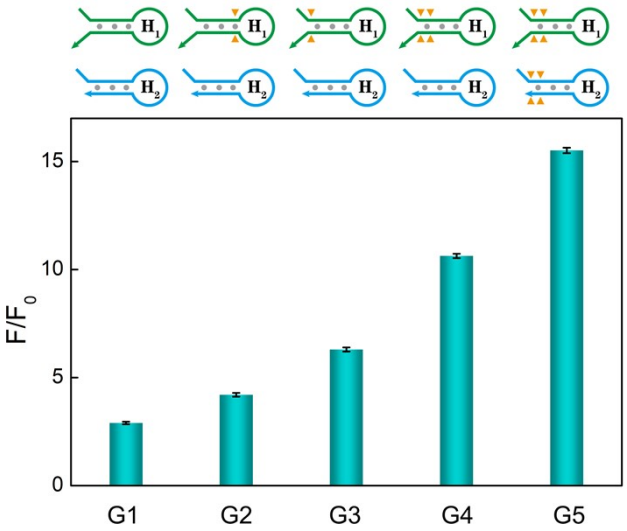

Fig. S7 Fluorescence response of the CHA system to target ctDNA introducing different LNAs number and location within hairpins. The component concentration is H_1 (50 nM), H_2 (400 nM) and reporter (50 nM). The reaction time is set as 40 min, and the reaction temperature is set as 37℃. F and F_0 refer to the fluorescence intensity with and without target ctDNA (5 nM), respectively. Results are presented as means ± standard deviation (SD) (n=3).

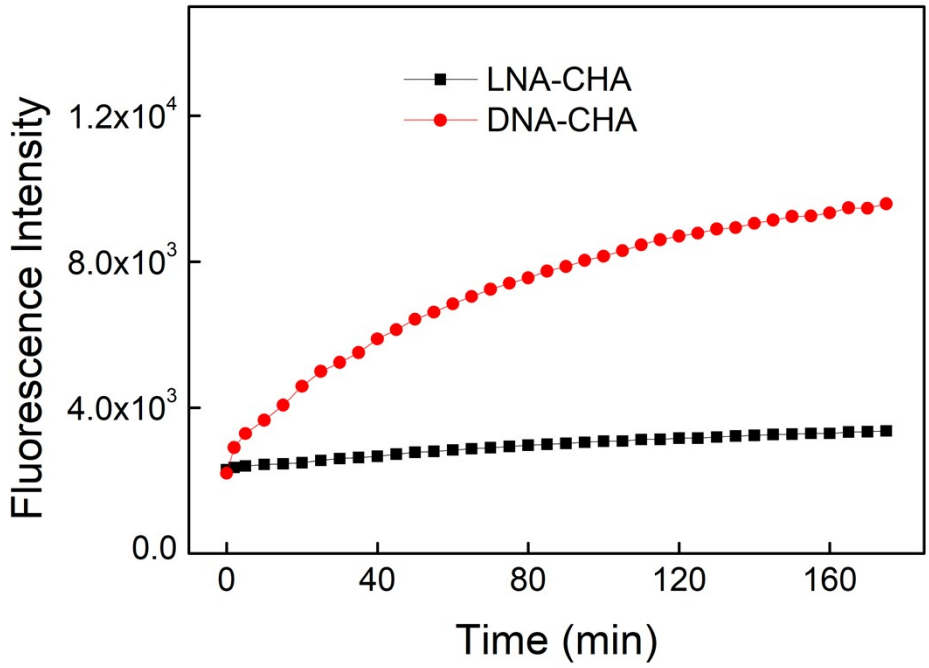

Fig. S8 Fluorescence kinetics of locked nucleic acid-modified CHA system (LNA-CHA) and regular CHA system (DNA-CHA) in MCF-7 cell lysate. The used concentrations of constituents are hairpin H_1 (50 nM), H_2 (400 nM) and reporter (50 nM). The reaction temperature is set as 37℃.

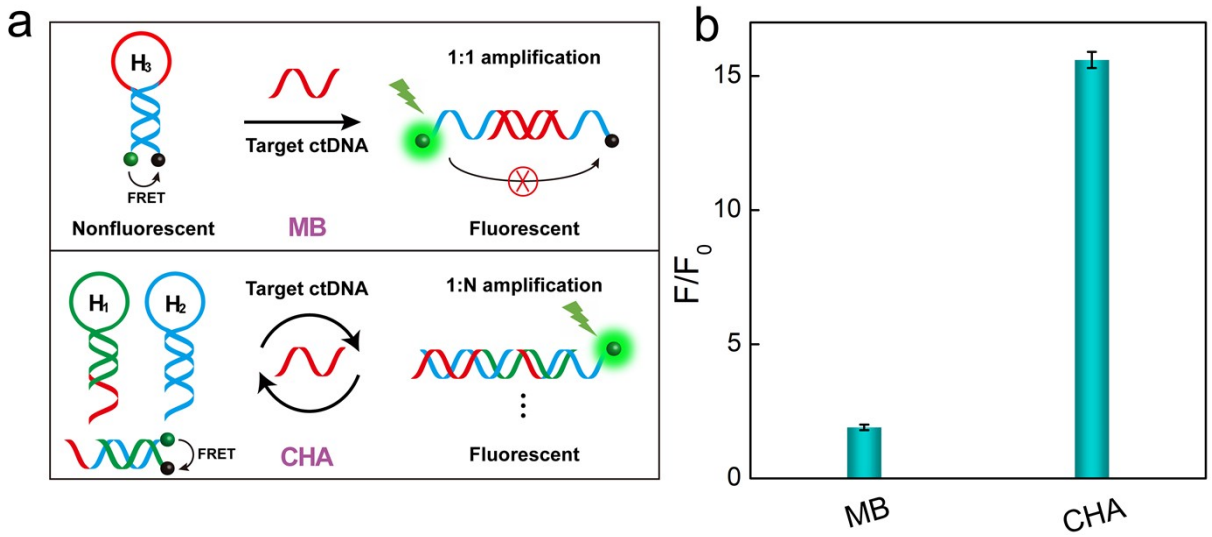

Fig. S9 (a) Illustration of updated CHA system and molecular beacon (MB) system for catalytic signal enhancement of target ctDNA. (b) Fluorescence amplification capability of CHA system is compared with MB system in the presence of target ctDNA (5 nM). F and F_0 refer to the fluorescence intensity with and without target ctDNA, respectively. H_1 (50 nM), H_2 (400 nM), reporter (50 nM), and H_3 (50 nM). The reaction time is set as 40 min, and the reaction temperature is set as 37°C. Results are presented as means ± standard deviation (SD) (n=3).

Table S2. Comparison of analytical performances of different ctDNA biosensors.

Method	Detection limit	Linear range	Enzyme	Convenience	Ref.
Surface-enhanced Raman scattering	0.3 fM	10-10^6 fM	Required	Multiple steps 90 min	S1
Localized surface plasmon resonance	200 fM	50-3.2×10^3 fM	Not required	Multiple steps >2 h	S2
Colorimetry	100 fM	500-5×10^5 fM	Not required	Multiple steps 4 h	S3
Electrochemistry	0.5 nM	0.5-50 nM	Not required	Multiple steps >2 h	S4
Electrochemistry	8.3 fM	10-5×10^6 fM	Not required	Multiple steps >2 h	S5
Electrochemistry	5 fM	0.2-20×10^6 fM	Not required	Multiple steps 20 min	S6
Fluorescence	0.22 fM	1-1×10^5 fM	Required	One step 4 h	S7
Fluorescence	0.16 fM	0.2-2×10^8 fM	Required	Multiple steps 120 min	S8
Fluorescence	3.3 pM	10-1×10^3 pM	Not required	One step 40 min	This work

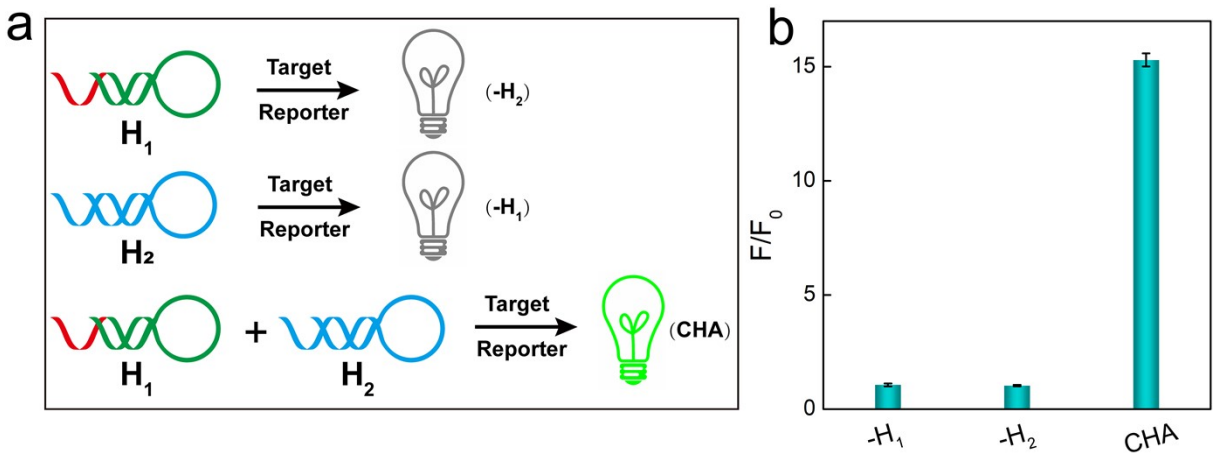

Fig. S10 (a) Schematic illustration and (b) fluorescence response of the updated CHA system that was subtracted by H_1 alone, or by H_2 alone, and the intact CHA system. H_1 (50 nM), H_2 (400 nM) and reporter (50 nM) in the presence of target ctDNA (5 nM). The reaction time is set as 40 min, and the reaction temperature is set as 37℃. F and F_0 refer to the fluorescence intensity with and without target ctDNA, respectively. Results are presented as means ± standard deviation (SD) (n=3).

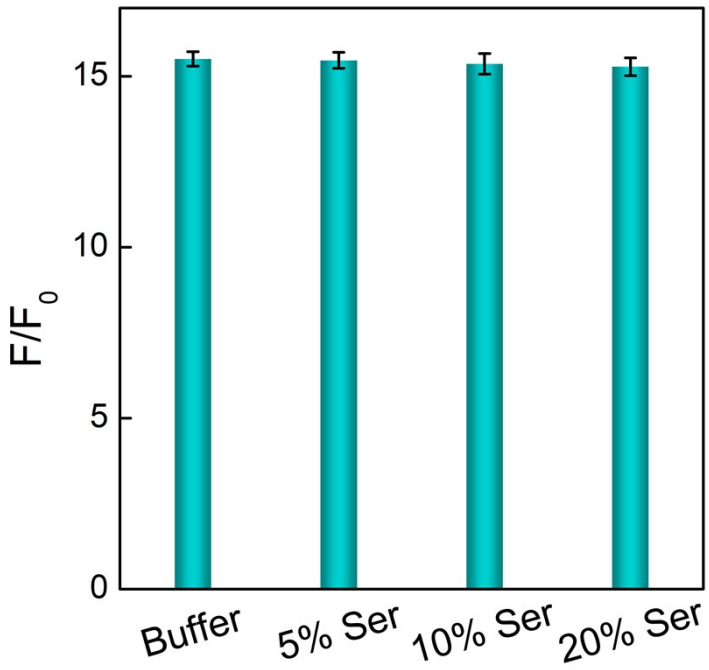

Fig. S11 Fluorescence response of the updated CHA system to target ctDNA in different ratios of fetal bovine serums: buffer, 5% serum, 10% serum, and 20% serum. H_1 (50 nM), H_2 (400 nM) and reporter (50 nM). The reaction time is set as 40 min, and the reaction temperature is set as 37℃. F and F_0 refer to the fluorescence intensity with and without target ctDNA (5 nM), respectively. Results are presented as means ± standard deviation (SD) (n=3).

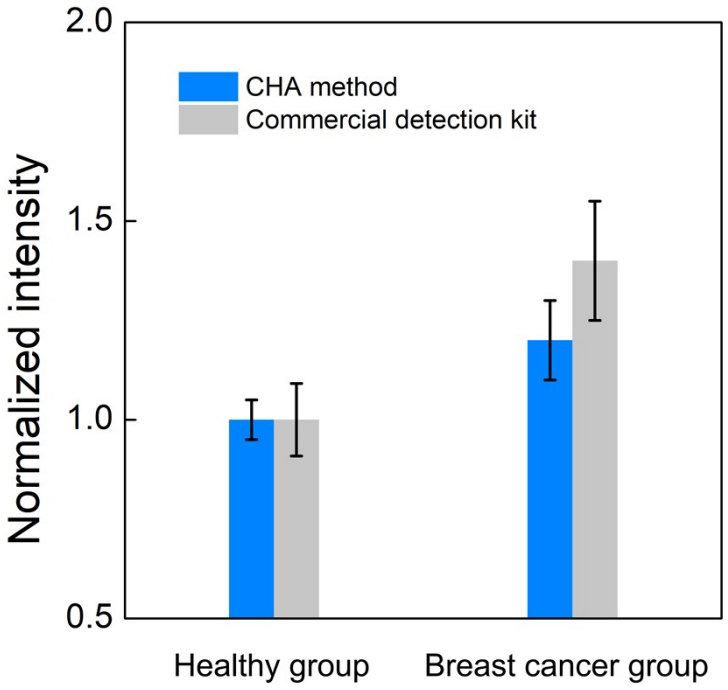

Fig. S12 Clinical samples analysis of ctDNA through CHA method and commercial detection kit. Normalization is performed against healthy group. Results are presented as means ± standard deviation (SD) (n=3).

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