Electronic Supplementary Material (ESI) for Analytical Methods.
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Supporting Information for

A fluorescent biosensor for highly specific and ultrasensitive detection of adenosine triphosphate based on ligation-triggered branched rolling circle amplification

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1. The oligonucleotides used in this work

Table S1: The oligonucleotides used in this work

Name	Sequence (5′−3′)
LT	CTCACACGAATTCATCTGAC
PadLock	P-ATTCGTGTGAGAAAACGGAACTGCGTCTAGGCAAAAGTCAGATGA
Primer	TCATCTGAC
Reverse Primer	AGGCAAAAGT

“P” in PadLock indicate 5′-phosphorylation modification.

2. Optimization of experimental conditions for the sensing system

The experiment conditions, including digestion reaction time, ligation reaction time, BRCA reaction time and the amounts of SG I and phi29 DNA polymerase were investigated. **Figure S1** shown the optimization results, the optimal digestion reaction time, ligation reaction time and RCA reaction time were 1.0 h, 1.5 h and 3.0 h, respectively. The optimized amounts of SG I and phi29 DNA polymerase were 2 × and 5 U respectively.

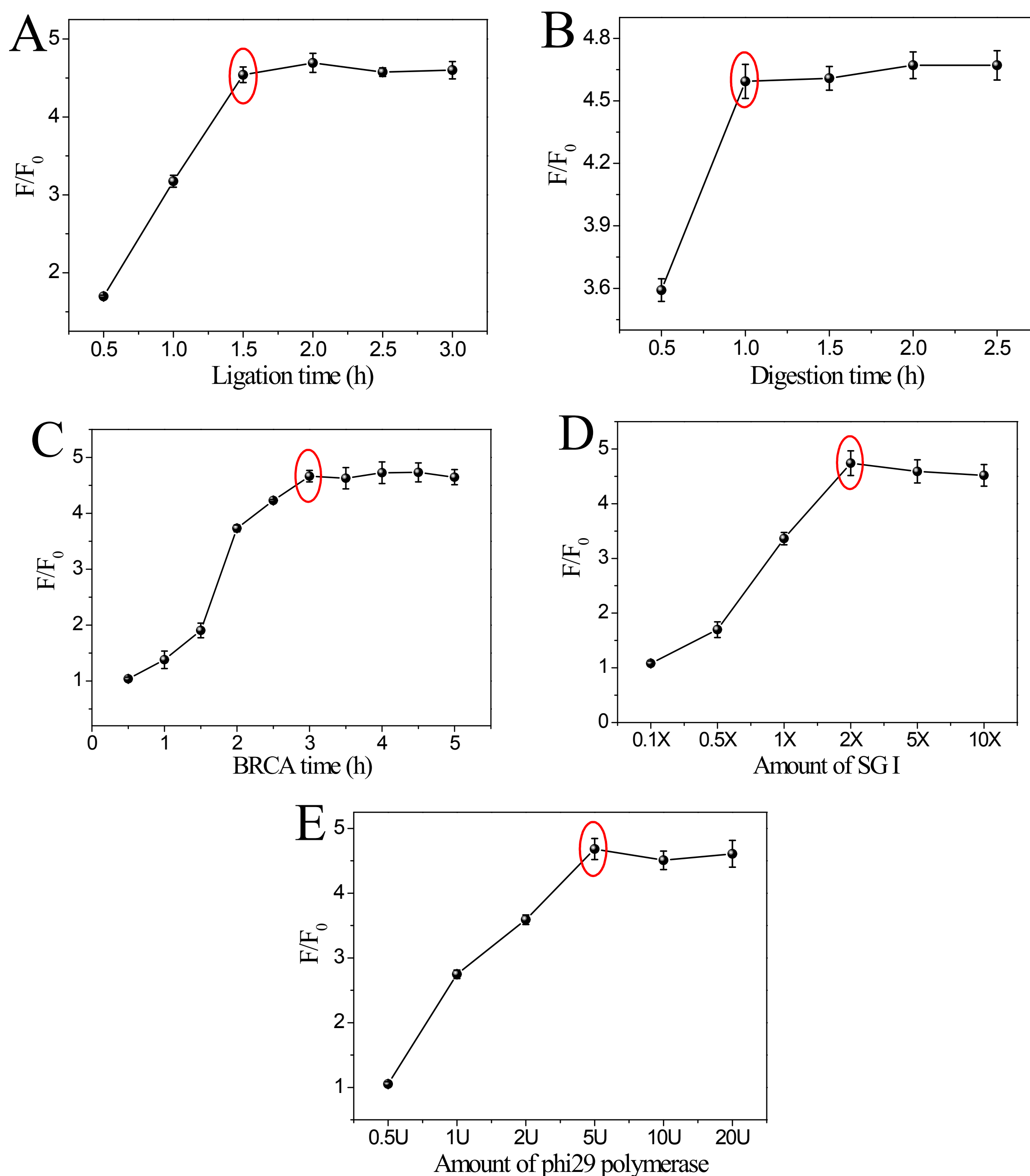

Figure S1. Optimization of ligation reaction time (a), digestion reaction time (b), BRCA reaction time (c), amounts of SG I (d) and phi29 DNA polymerase (e) in BRCA-based ATP-sensing system. F and F_0 are the fluorescence intensities of SG I at 525 nm in the presence and absence of 1μM ATP, respectively.

3. Specificity of the proposed ATP sensor

In order to evaluate the selectivity of this strategy for ATP detection, the fluorescence responses of the sensing platform to several ATP analogs were tested and compared with that to ATP under the same conditions (**Figure S2**). When 1μM ADP, AMP, UTP, CTP or GTP was added in the sensing system instead of ATP, no obvious fluorescence enhancement could be observed compared to the blank control without ATP. However, the fluorescence intensity was obviously enhanced when the same concentration (1μM) of ATP was added. The experimental results demonstrated that this sensing system shows high selectively toward ATP detection and has good capability to discriminate ATP from its analogs.

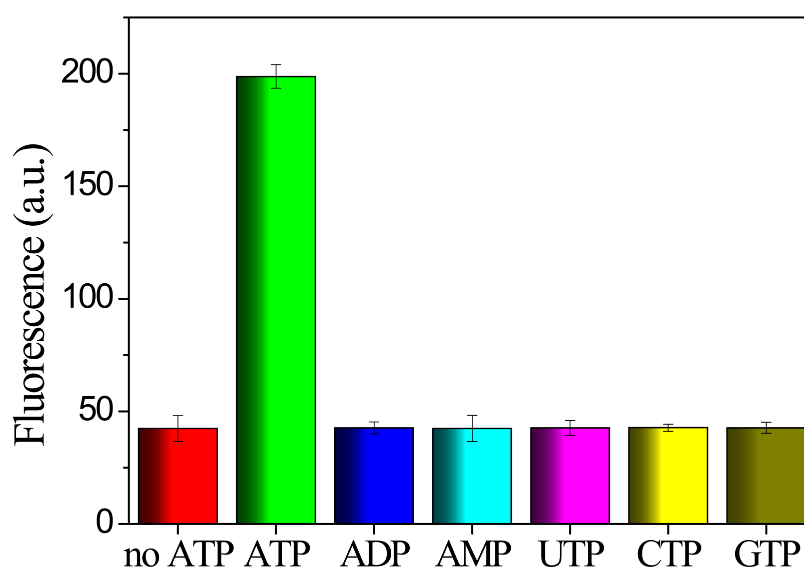

Figure S2. The concentration of ATP and those of its analogues (UTP, CTP, GTP and AMP) are all 1 μM. The error bars show the standard deviation of three independent experiments.

4. Application of the proposed method in real human serum human serum and HeLa cell lysates nucleoprotein samples

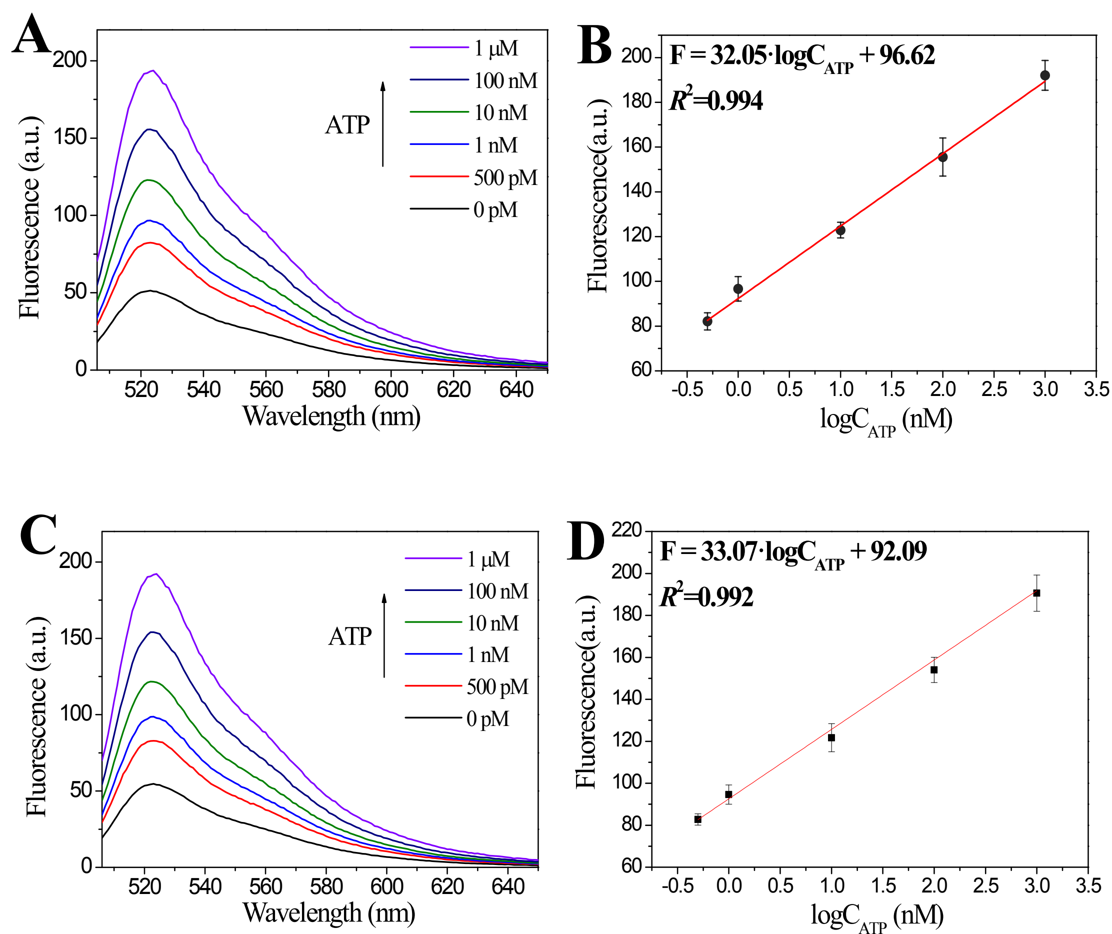

Figure S3. The fluorescence signal change as a function of ATP concentration in (A) (B) human serum, and (C) (D) HeLa cell lysates. The error bars show the standard deviation of three independent experiments.

5. ATP detection in serum samples and HeLa cell lysates nucleoprotein samples

Table S2. Recoveries of added ATP in 20-fold diluted human serum and 10-fold diluted HeLa cell lysates nucleoprotein samples

Real sample	Added (nM)	Found (nM)	Recovery (%)	RSD (%, n=3)
20-fold diluted Human Serum	0.5	0.522	104.4	5.8
	1	0.952	95.2	3.9
	10	9.59	95.9	6.7
	100	97.8	97.8	4.2
	1000	9986	98.6	5.4
10-fold diluted Hela cell lysates nucleoprotein	0.5	0.482	96.4	4.2
	1	1.02	102	3.9
	10	9.86	98.6	5.1
	100	99.5	99.5	2.1
	1000	1025	103	3.2