Electronic Supplementary Information

**Aptamer-mediated rolling circle amplification for label-free and sensitive detection of histone acetyltransferase activity**

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## MATERIALS AND METHODS

**Materials.** The human recombinant Tip60 (residues 2-513) was purchased from Cayman Chemical (Ann Arbor, MI, USA). Acetyl-coenzyme A sodium salt (Ac-CoA), 5-(1,2-Thiazol-5-yldisulfanyl)-1,2-thiazole (NU9056), bovine serum albumin (BSA), dimethyl sulfoxide (DMSO)，potassium chloride (KCl)，sodium chloride (NaCl)，di-Sodium hydrogen phosphate（$Na_2HPO_4$），monobasic potassium phosphate（$KH_2PO_4$），magnesium chloride（$MgCl_2$）and 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid sodium salt (HEPES) were bought from Sigma-Aldrich (St. Louis, MO, USA). Prior to use, acetyl-coenzyme A sodium salt was diluted to 5 mM with ultra-pure water. NU9056 (20 mM) stock solution was prepared with DMSO. Acetyl-coenzyme A sodium salt and NU9056 were stored at -20°C in the dark. The 10000 × SYBR Gold was purchased from Invitrogen (Carlsbad, CA, USA). The streptavidin-coated magnetic beads (4 mg/mL), phi29 DNA polymerase (10000 units/mL), 10× phi29 DNA polymerase reaction buffer, deoxyribonucleoside 5'-triphosphate mixture (dNTPs, 10 mM), Dam methyltransferase (Dam, 8000 units/mL), CpG methyltransferase (M.SssI, 4000 units/mL), protein kinase A (PKA, 2500000 units/mL), and T4 polynucleotide kinase (PNK，10000 units/mL) were purchased from New England BioLabs (Beverly, MA, USA). Circle template and DNA probe (Table S1) were obtained from Takara Biotechnology Co. Ltd. (Dalian, China). H4 peptide substrate (GGK GLG KGG AKR HRK-biotin) was synthesized and purified by Ontores Biotechnologies (Hangzhou, Zhejiang, China). Prior to use, H4 peptide substrate was diluted to 1 mM with ultra-pure water and stored at -20°C. The ultra-pure water obtained from a Millipore filtration system (Millipore, Billerica, MA).

**Table S1. Sequences of the Oligonucleotides**[a]

| oligonucleotide | sequence |
|---|---|
| circular RCA template | <u>CTC AGC TGT GT</u>A ACA ACA TGA AGA TTG TAG GTC AGA ACT CAC CTG TTA GAA ACT GTG AAG ATC GCT TAT T<u>AT GTC CTA TC</u> |
| DNA probe | 5'-**GTA AGT TAA TTG GAC TTG GTC GTG TGC GGC ACA GCG AT**A AAA AAA AAA AAA A<u>AC ACA GCT GAG GAT AGG ACA T</u>-3' |

[a] The circular RCA template was synthesized and purified by Takara Biotechnology Co. Ltd. (Dalian, China). The underlined bases indicate the complementary regions for binding primer sequence of DNA probe to the RCA template, and the boldface bases represent the aptamer sequence of DNA probe.

**Tip60 detection.** The 10 μL of reaction mixture containing Tip60 with indicated concentration, 60 μM peptide substrate, 500 μM AcCoA, 1 × reaction buffer (100 mM HEPES, pH 7.5) was incubated at 37 °C for 40 min. Then 500 nM DNA probe was added and incubated at 24 °C for 60 min in the 1× binding buffer (3 mM KCl, 10 mM $Na_2HPO_4$, 2 mM $KH_2PO_4$, 10 mM NaCl, and 5 mM $MgCl_2$, pH 7.4). Subsequently, 20 μL of magnetic beads was added and incubated for 30 min at room temperature. After the magnetic beads were washed for 3 times, the remaining DNA probes were dissociated by 40 μL of deionized water, followed by incubation at 95 ºC for 10 min. For RCA reaction, 50 μL of reaction mixture containing 5 μL of 10× phi29 DNA polymerase reaction buffer (500 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM $(NH_4)_2SO_4$, 40 mM DTT, pH 7.5),

0.4 nM RCA template, 200 μM dNTP, 0.6 U of phi29 DNA polymerase, and 40 μL of eluted DNA probe was incubated at 37 °C for 60 min. The RCA products were stained with 1 × SYBR Gold and measured by a Hitachi F-7000 fluorometer (Tokyo, Japan). A final volume of 60 μL containing 50 μL of RCA products, 6 μL of 10× SYBR Gold, and 4 μL of deionized water was measured using a 1-cm path length quartz cuvette. Both the excitation and emission slits were set for 5.0 nm. The excitation wavelength is 495 nm. The emission spectra in the range of 520 – 750 nm were recorded for data analysis.

**Kinetic Analysis**

To measure the initial velocity of Tip60 reaction, 10 μL of reaction mixture containing 200 nM Tip60, 60 μM peptide substrate, Ac-CoA with indicated concentrations, and 1× reaction buffer (100 mM HEPES, pH 7.5) was incubated at 37 °C for 10 min. The reaction mixture was subjected to RCA reaction and subsequent fluorescence measurement described above, with the fluorescence intensity reflecting the enzymatic activity of Tip60. The velocity ($V$, s$^{-1}$) is calculated according to $V = F/360$, where $F$ is the fluorescence intensity of SYBR Gold. The $V$ value is fitted against the concentration of Ac-CoA according to Michaelis–Menten equation:

$$V = \frac{V_{max}[S]}{K_m + [S]}$$

where $V_{max}$ is the maximum initial velocity, [S] is the concentration of Ac-CoA substrate, and $K_m$ is the Michaelis−Menten constant.

**Gel Electrophoresis Analysis**. The 20 μL of final RCA products was analyzed by 1% agarose gel (1.4 g of agarose, 2.8 mL of 50 × TAE buffer, 137.2 mL of deionized water) electrophoresis at room temperature at 110 V constant voltages for 50 min in 1× TAE (40 mM Tris-ethylic acid, 2 mM EDTA). The mixture containing 20 μL of final RCA products, 1× loading buffer, 1× SYBR

Gold was loaded onto the gel to run. The gels were stained by SYBR Gold dye and imaged by Bio-Rad ChemiDoc MP imaging system (California, U.S.A.).

**Inhibition Assay.** For the Tip 60 inhibition assay, 9 μL of reaction mixture containing NU9056 with indicated concentrations, 0.54 μL of 3.71 μM Tip 60，0.6 μL of 1 mM peptide substrate, 1 μL 10× reaction buffer (1 M HEPES, pH 7.5) was incubated at room temperature for 10 min. Subsequently, 1 μL of 5 mM Ac-CoA was added into the mixture and incubated at 37 °C for 40 min. The Tip60 activity assay follows the same procedures described above. The relative activity (RA) of Tip60 is calculated according to following equation.

$$RA\ (\%) = \frac{F_i - F_0}{F_t - F_0} \times 100\%$$

where $F_0$ represents the fluorescence intensity in the absence of Tip60, $F_t$ represents the fluorescence intensity in the presence of 200 nM Tip60, and $F_i$ represents the fluorescence intensity in the presence of 200 nM Tip60 and inhibitor NU905.

**Cell Culture and Preparation of Cell Extracts.** HeLa cells were cultured in Dulbecco's modified Eagles medium (DMEM) with 10% fetal bovine serum (FBS) and 50 U/mL penicillin plus 50 μg/mL streptomycin in a 100% humidified chamber containing 5% $CO_2$ at 37 °C. The whole-cell extracts were prepared using a Nuclear Extract Kit (ActiveMotif, Carlsbad, CA) according to the manufacturer's instructions. Briefly, the cells were washed twice with 8 mL of ice-cold PBS/phosphatase inhibitors buffer (0.8 mL of 10× PBS, 6.8 mL of deionized water, 0.4 mL of phosphatase inhibitors), followed by removing from dish by gently scraping with cell lifter and transferring to a pre-chilled 2-mL tube. Cell suspension was centrifuged for 5 min at 200 g in a centrifuge pre-cooled at 4 °C. After the supernatant was discarded, the cell pellet was resuspended in 300 μL of lysis buffer (30 μL of 10 mM DTT, 267 μL of lysis buffer AMI, 3.0 μL

of protease inhibitor cocktail). The suspension was incubated for 30 minutes on ice and then mixed for 3 min by pipetting up and down at a time interval of 2 min. The suspension was vortexed for 30 sec and centrifuged for 20 min at 14000 g in a micro centrifuge pre-cooled at 4 °C. The supernatant containing nuclear protein extracts was transferred into a pre-chilled microcentrifuge tube and stored at -80 °C.
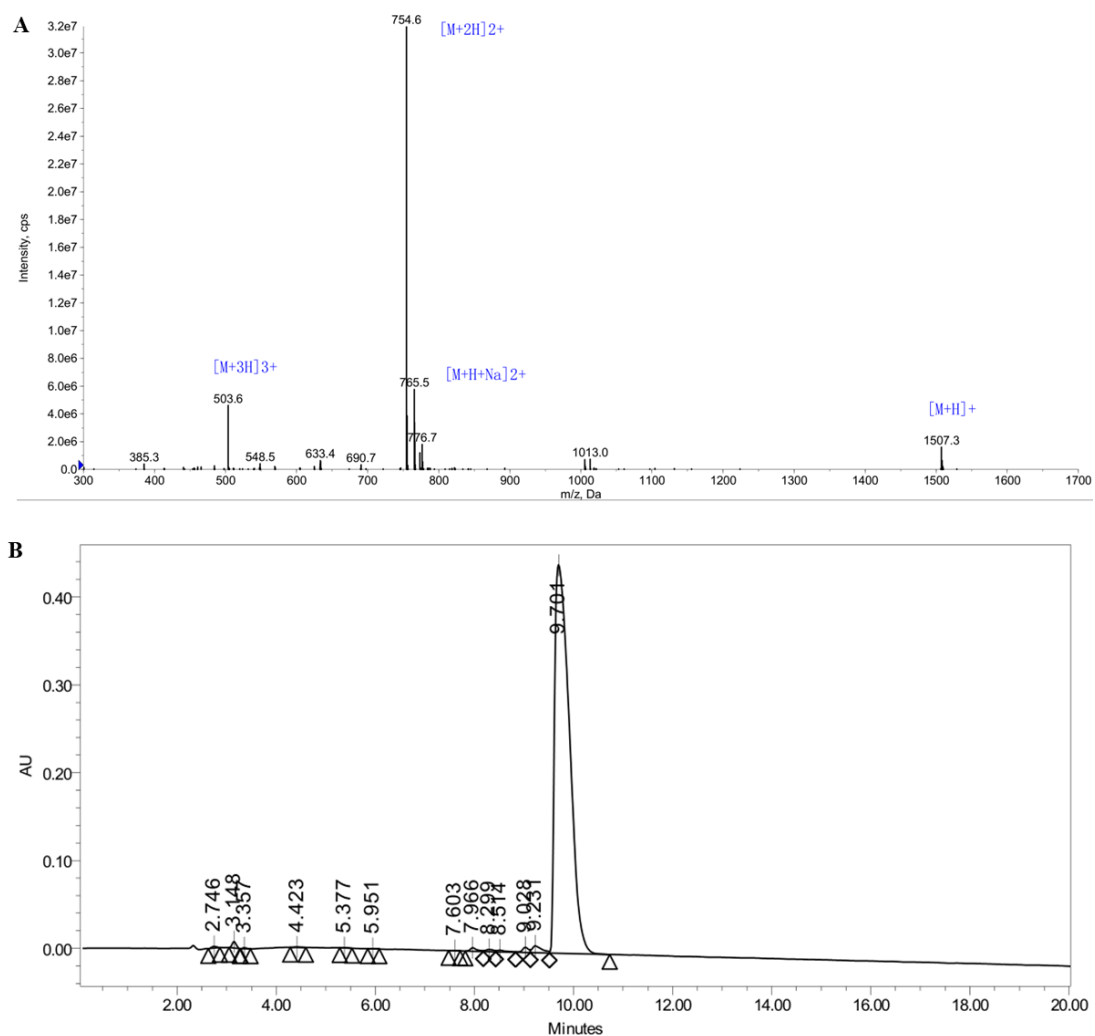
**SUPPLEMENTARY RESULTS**



**Fig. S1** Mass spectral (A) and HPLC (B) analysis of the peptide substrate. The calculated

molecular weight of the peptide substrate is 1506.77 with a peptide purity of 96.62%.
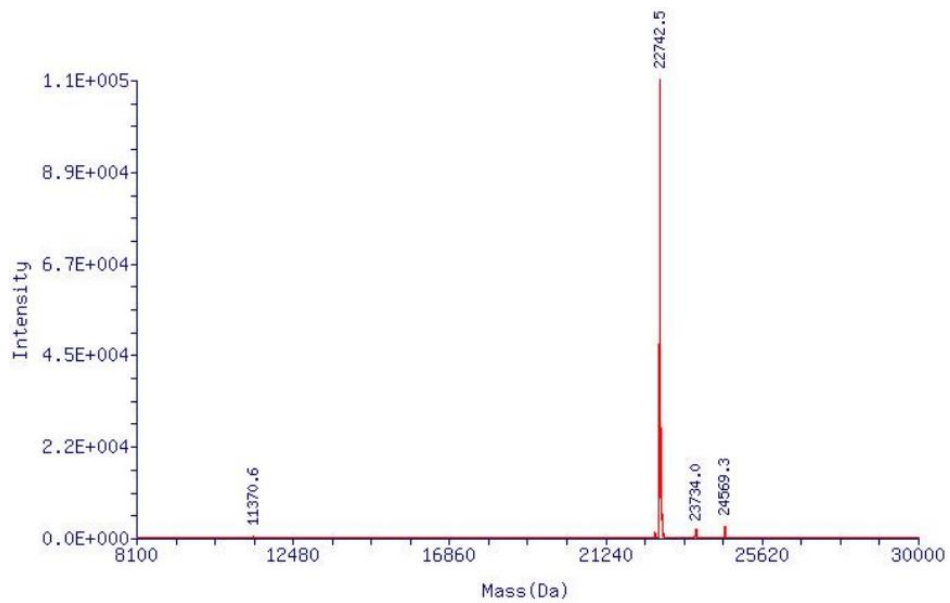
**Fig. S2** Mass spectral analysis of the DNA probe. The calculated molecular weight of DNA probe
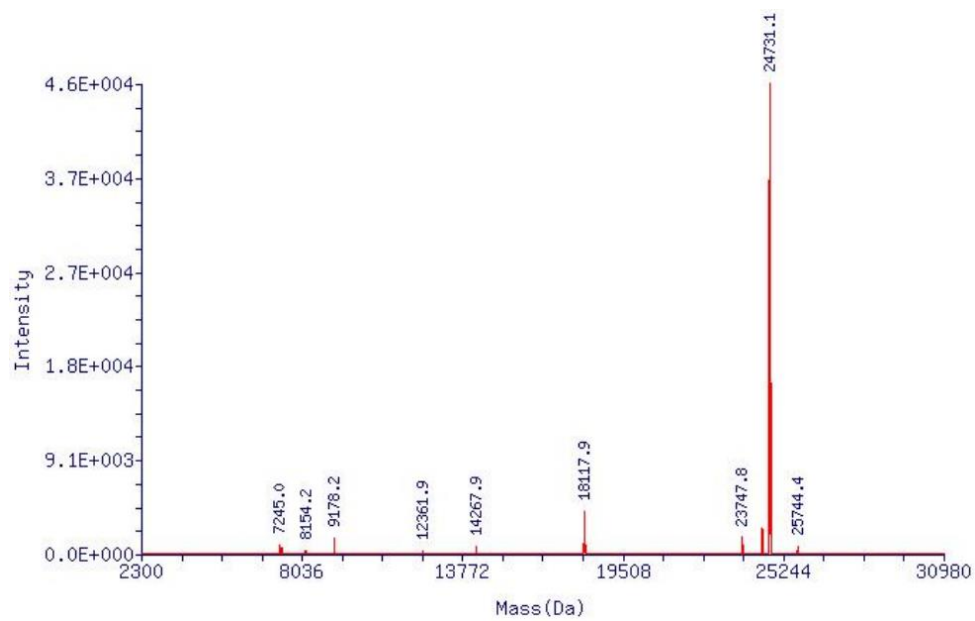
is 22739.8.

**Fig. S3** Mass spectral analysis of the RCA template. The calculated molecular weight of RCA

template is 24730.

**A** 5' <span style="color:red">CTCAGCTGTGT</span>AACAACATGAAGATTGTAGGTCAGAACTCACCTGTTAGAAACTGTGAAGATCGCTTATT<span style="color:red">ATGTCCTATC</span> 3'

Linear RCA template

Circularization

Circular RCA template

Primer binding site

**B**

aptamer        spacer   Primer

5' <span style="color:blue">GTAAGTTAATTGGACTTGGTCGTGTGCGGCACAGCGAT</span><span style="color:magenta">AAAAAAAAAAAAAAA</span><span style="color:green">ACACAGCTGAGGATAGGACAT</span> 3'

DNA probe

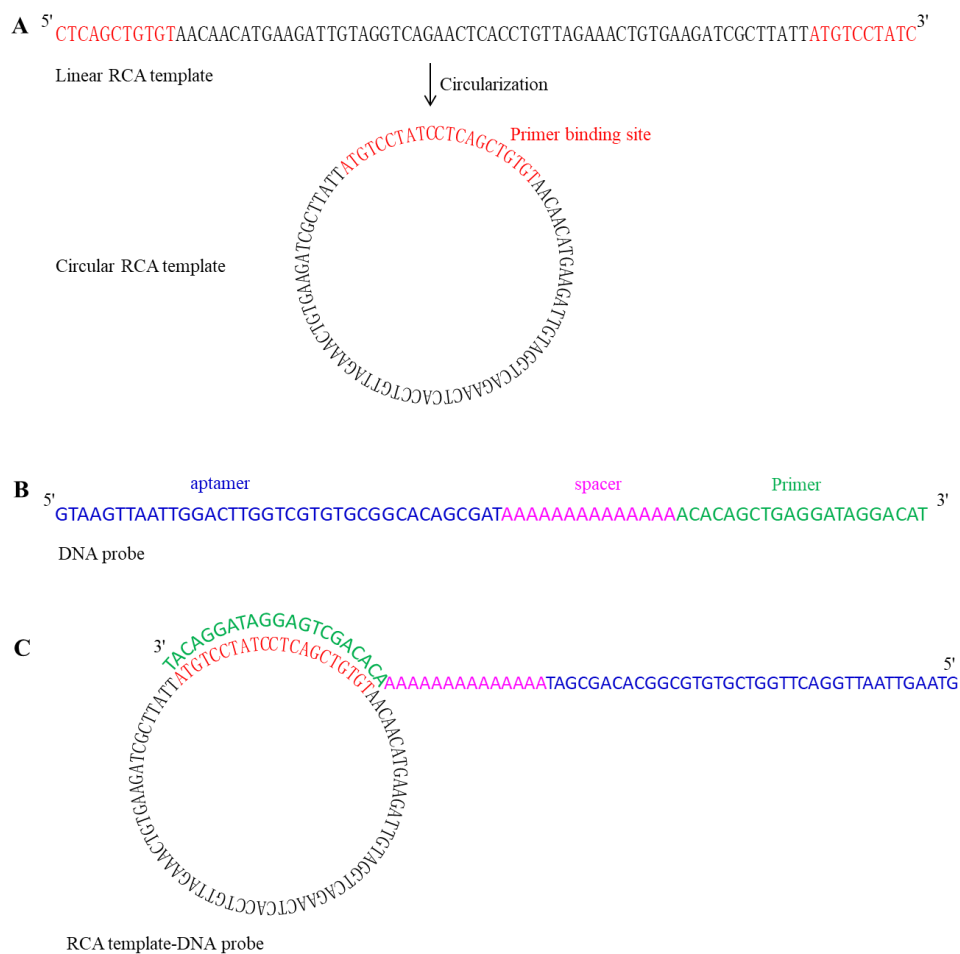**C**

RCA template-DNA probe

**Fig. S4** (A) Structure of RCA template. The circular template was prepared through the circularization of linear RCA template by Takara Biotechnology Co. Ltd. (Dalian, China). Red sequence indicates the primer binding site. (B) Structure of DNA probe. From 3' to 5' end, primer sequence is marked by green color, spacer sequence is marked by pink color, and aptamer sequence is marked by blue color. (C) The binding of RCA template to DNA probe.
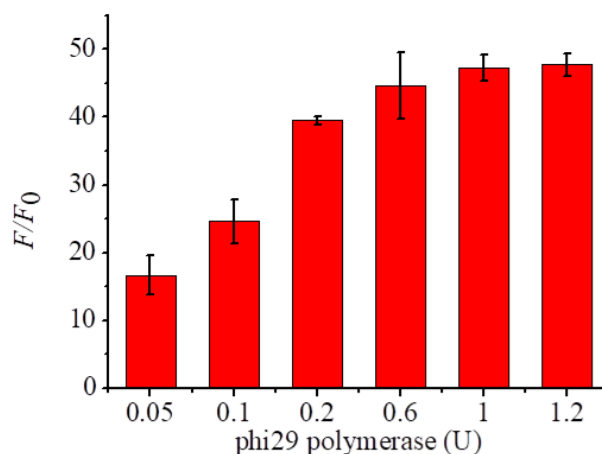
**Fig. S5** Variance of $F/F_0$ value as a function of the amount of phi29 polymerase. Error bars show the standard deviation of three experiments.

To obtain the best signal amplification performance, we optimized the experimental conditions of RCA reaction including the amount of phi29 polymerase, the concentration of dNTP, the concentration of circular template, and the reaction temperature. The $F/F_0$ value is used for quantitative analysis of the assay performance, where $F$ and $F_0$ represent the fluorescence intensity in the presence and absence of DNA probe, respectively. In this assay, the phi29 polymerase is employed for carrying out RCA reaction due to its excellent strand displacement and processive synthesis properties. As shown in Fig. S5, the $F/F_0$ value enhances with the increasing amount of phi29 polymerase from 0.05 to 0.6 U, and levels off beyond the amount of 0.6 U. This can be explained by the fact that both the signals of experimental and control groups are enhanced with the increase of polymerase amount[1] and the $F/F_0$ value reaches the saturation at 0.6 U of polymerase. Therefore, 0.6 U of phi29 polymerase is used in the subsequent experiments.
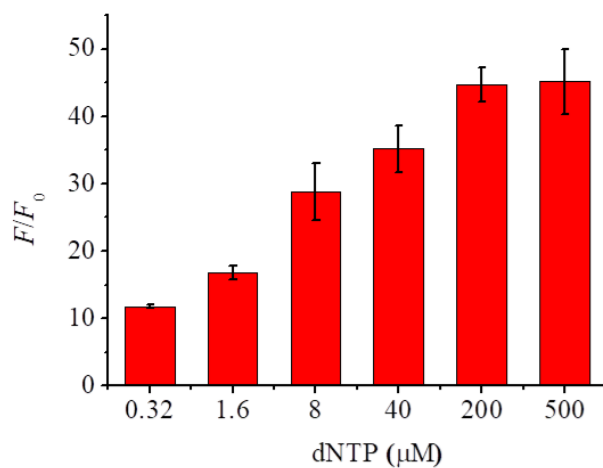
**Fig. S6** Variance of $F/F_0$ value as a function of dNTP concentration. Error bars show the standard deviation of three experiments.

The deoxynucleotide (dNTP) is an equimolar mixture of dATP, dCTP, dGTP and dTTP, and it serves as raw material of RCA reaction. As shown in Fig. S6, the $F/F_0$ value enhances with the increasing concentration of dNTP from 0.32 to 200 µM, and reaches a plateau beyond the concentration of 200 µM. This can be explained by the fact that both the signals of experimental and control groups are enhanced with the increase of dNTP concentration[1] and $F/F_0$ value reaches the saturation at 200 µM dNTP. Therefore, 200 µM is used as the optimum dNTP concentration.
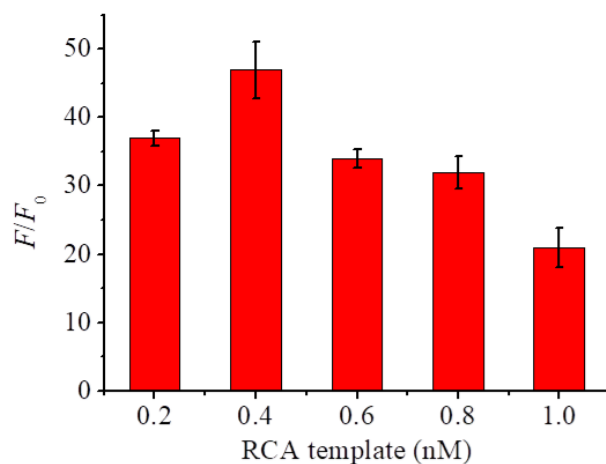
**Fig. S7** Variance of $F/F_0$ value as a function of circular template concentration. Error bars show the standard deviation of three experiments.

The RCA template is a single-stranded circular DNA which is partially complementary to the primer region of DNA probe. As shown in Fig. S7, a maximum value of $F/F_0$ is obtained at the RCA template concentration of 0.4 nM. This can be explained by the fact that the amplification efficiency of background is higher than that of experimental group when RCA template is more than 0.4 nM, consistent with previous research that high circular template concentration leads to the decrease of signal-to-noise ratio.[2] Therefore, 0.4 nM RCA template is used in the subsequent experiments.
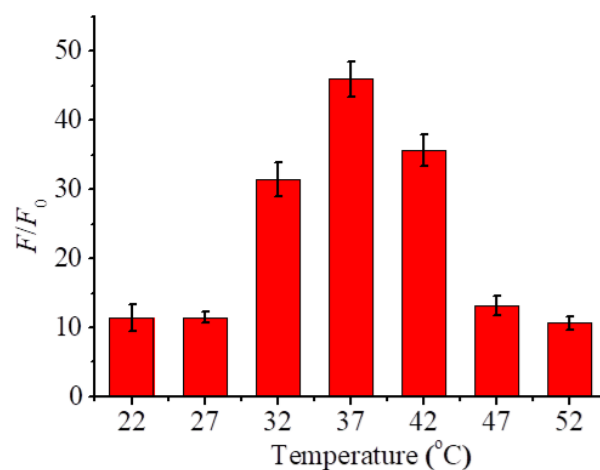
**Fig. S8** Variance of $F/F_0$ value as a function of reaction temperature. Error bars show the standard deviation of three experiments.

We further optimized the reaction temperature. As shown in Fig. S8, the $F/F_0$ value enhances with the increase of temperature from 22 to 37 $^{\circ}$C, followed by the decrease beyond the temperature of 37 $^{\circ}$C. This can be explained by the fact that the high reaction temperature of > 37 $^{\circ}$C may induce the decrease of polymerase activity and disrupt the probe – template binding.[3] Therefore, 37 $^{\circ}$C is used as optimum reaction temperature for RCA reaction.
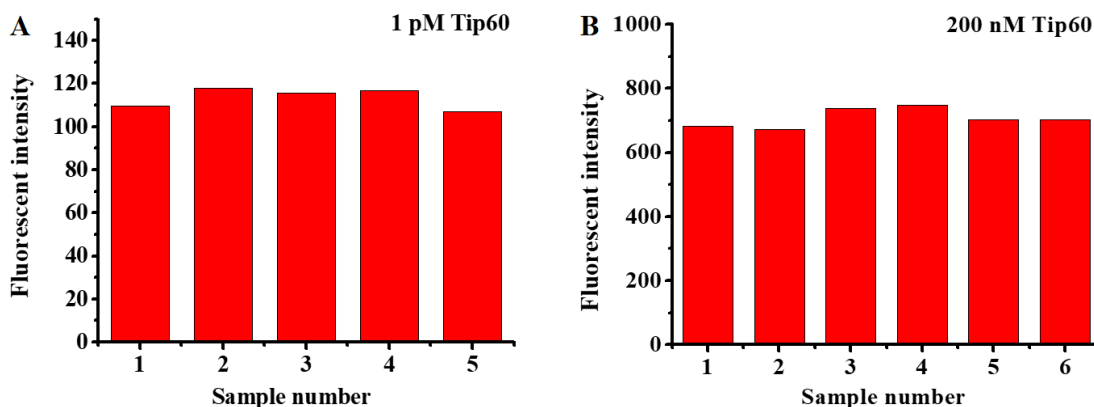
**Fig. S9** Resproducibilty assessment of the proposed method by measuring 1 pM (A) and 200 nM (B) Tip60, respectively

We further evaluated the reproducibility of the proposed method. The relative standard deviation (RSD) of the proposed method in response to 1 pM Tip60 (Fig. S9A) and 200 nM Tip60 (Fig. S9B) is measured to be 4.2% and 4.1%, respectively, suggesting the good reproducibility of the proposed method.
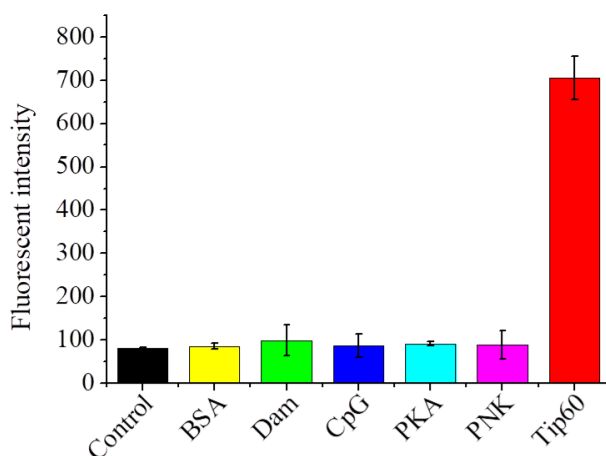


**Fig. S10** Fluorescence intensity in response to BSA (10 μg/mL), Dam (10 U/mL), CpG (10 U/mL), PKA (10 U/mL), PNK (10 U/mL), TIP60 (200 nM), and the control group without any proteins / enzymes. Error bars represent the standard derivation from three independent measurments.

We further invesitigated the specificity of the proposed biosesnor by using bovine serum albumin (BSA), DNA adenine methyltransferase (Dam), CpG methyltransferase (CpG), cyclic-AMP dependent protein kinase A (PKA), T4 polynucleotide kinase (PNK) as the negative controls. BSA is a frequently used irrelevant protein.[4] Dam and CpG are methyltransferases that catalyze the methylation of adenine residue in the 5'…GATC…3' sequence and cytosine residues in the 5'...CG...3' sequence,[5] respectively. PKA is responsible for phosphorylation of serine/threonine residues.[6] PNK can catalyze the transfer of phosphate group from ATP to 5'-hydroxyl terminus of DNA/RNA.[7] Theoretically, non of these protein/enzymes enables the acetylation of peptide substatre, and thus no DNA probe can be captured by the magnetic bead for RCA reaction and no signal is detected. As expected, a high fluorescent signal is observed only in response to target Tip60 (Fig. S10, red column), but a low signal similar to the control group (Fig. S10, black column) is observed in response to BSA (Fig. S10, yellow column), Dam (Fig. S10, green column), CpG (Fig. S10, blue column), PKA (Fig. S10, cyan column), and PNK (Fig. S10, red column), respectively. These results demonstrate the excellent specificity of the proposed aptasensor toward Tip60.
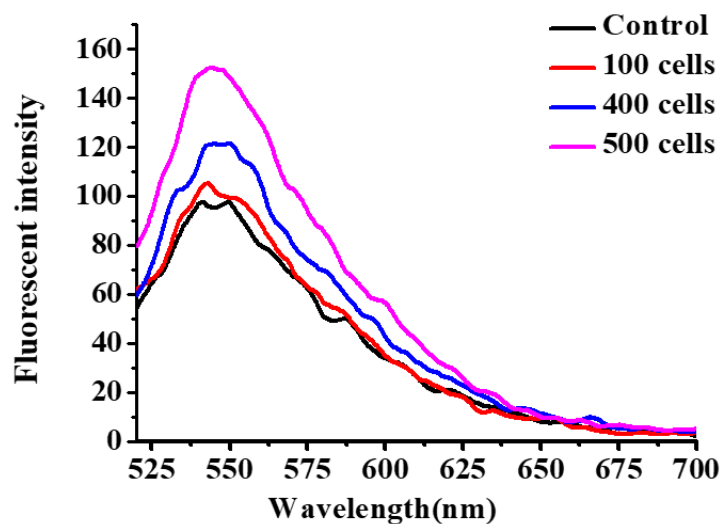
**Fig. S11** Fluorescence emission spectra in response to different numbers of HeLa cells from 0 (control) to 500 cells.

**Table S2.** Comparison of the proposed method with the reported methods for histone acetyltransferase assay

| Method | Target | Label | LOD | Sample | Ref. |
|---|---|---|---|---|---|
| Fluorescent assay based on acetylation protection and magnetic graphitic nanocapsules | P300 | FITC | 0.1 nM | No | 8 |
| Fluorescent assay based on antibody and single-molecule detection | Gcn5 | Alexa Fluor 488 | 21 pM | HeLa cell | 9 |
| Fluorescent assay based on antibody and quantum dot | p300 | Alexa Fluor 647 | 0.5 nM | no | 10 |
| Fluorescent assay based on coordination polymer | p300 | no | 0.2 nM | no | 11 |

| Assay | Target | Label | LOD | Sample | Ref |
|---|---|---|---|---|---|
| Luminescent assay based on DNA-sensitized Tb (III) probes | p300 | no | 50 pM | HeLa cell | 12 |
| Fluorescent assay based on G-quadruplex | p300 | no | 50 pM | HeLa cell | 13 |
| Photoelectrochemical assay based on polydopamine sensitized layered $WS_2$ | p300 | no | 47 pM | no | 14 |
| Electrochemical assay based on coordination polymer | p300 | no | 67 pM | no | 15 |
| Electrochemical assay based on antibody and graphene oxide | p300 | methylene blue | 3.6 pM | HeLa cell, serum, urine | 16 |
| Chemiluminescent assay based on hybridization chain reaction and the silver clusters | p300 | no | 490 pM | HeLa cell, serum | 17 |
| Chemiluminescence assay based on tannic acid assembled nanoprobes | p300 | no | 74 pM | MCF-7 cell | 18 |
| Colorimetric assay based on gold nanoparticles | PCAF HAT | no | 0.5 nM | No | 19 |
| Fluorescent assay based on aptamer and rolling circle amplification | Tip60 | no | 220 fM | HeLa cell | this work |

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