

## Electronic Supplementary Information (ESI)

# NanoCluster Beacons as Reporter Probes in Rolling Circle Enhanced Enzyme Activity Detection

Sissel Juul,<sup>‡a</sup> Judy M. Obliosca,<sup>‡b</sup> Cong Liu,<sup>b</sup> Yen-Liang Liu,<sup>b</sup> Yu-An Chen,<sup>b</sup> Darren M. Imphean,<sup>b</sup> Birgitta R. Knudsen,<sup>c,d</sup> Yi-Ping Ho,<sup>d</sup> Kam W. Leong,<sup>\*e</sup> and Hsin-Chih Yeh<sup>\*b</sup>

<sup>a</sup>*Department of Biomedical Engineering, Pratt School of Engineering, Duke University, Durham, NC 27708, USA*

<sup>b</sup>*Department of Biomedical Engineering, Cockrell School of Engineering, University of Texas at Austin, Austin, TX 78712, USA*

<sup>c</sup>*Department of Molecular Biology and Genetics, Aarhus University, 8000 Aarhus C, Denmark*

<sup>d</sup>*Interdisciplinary Nanoscience Center (iNANO), Aarhus University, 8000 Aarhus C, Denmark*

<sup>e</sup>*Department of Biomedical Engineering, Columbia University, New York, NY 10027, USA*

<sup>‡</sup>These authors contributed equally.

### Contents:

<b>I.</b>	<b>Materials and Methods</b> .....	2
<b>II.</b>	<b>NanoCluster Beacons in Homogeneous Assay</b> .....	5
	<b>Figure S1</b> Fluorescence spectra and enhancement ratio of sNCB .....	5
	<b>Figure S2</b> Comparison between sNCB and molecular beacon .....	6
<b>III.</b>	<b>NanoCluster Beacons in REEAD Assay</b> .....	7
	<b>Table S1</b> hTopI substrate, primer and Rh-tagged probes in REEAD .....	7
	<b>Figure S3</b> Rolling circle amplification in REEAD assay .....	7
	<b>Figure S4</b> STEM images of sNCB/REEAD and Rh/REEAD .....	8
	<b>Figure S5</b> Human cell extract analysis using sNCB and Rh in REEAD .....	8
	<b>References</b> .....	9

## I. Materials and Methods

Sodium phosphate dibasic anhydrous ( $\text{Na}_2\text{HPO}_4$ ; F.W. 141.96), sodium phosphate monobasic monohydrate ( $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ ; F.W. 137.99) and sodium borohydride ( $\text{NaBH}_4$ ) were purchased from Fisher Scientific, whereas silver nitrate ( $\text{AgNO}_3$ ) was acquired from Sigma-Aldrich. All oligonucleotides were purchased from Integrated DNA Technologies and were purified by desalting. De-ionized (DI) water (18  $\text{M}\Omega \cdot \text{cm}$ ) was used for all solution preparations.

### 1. Preparation of NanoCluster Beacons (NCBs)

#### A. Preparation of Nanocluster Probes (NC Probes)

In a typical preparation,<sup>1-3</sup> a 15  $\mu\text{M}$  (final concentration) NC probe solution with a volume of 1  $\mu\text{l}$  was prepared by adding 12.5  $\mu\text{l}$  of 1.2 mM NC probe (see DNA sequence in Fig. S1A) to 940  $\mu\text{l}$  of 20 mM sodium phosphate buffer (pH 6.7). The solution was vortexed for 2 s and centrifuged at 14,000 rpm for 30 s. 45  $\mu\text{l}$  of 4 mM silver nitrate solution was then added and the mixture was again vortexed and centrifuged. The solution was allowed to sit in the dark for 10 min at room temperature. For silver cluster formation, 7  $\mu\text{l}$  of freshly prepared 13.2  $\mu\text{M}$   $\text{NaBH}_4$  solution was added, resulting in a pale yellow mixture which was then stored in the dark overnight. The resulting NC probe solution had the NC probe: $\text{Ag}^+$ : $\text{NaBH}_4$  molar ratio of 1:12:6.

#### B. Activation of NanoCluster Beacons through Guanine Proximity in Solution

An NCB consists of an NC probe (originally carrying dark silver clusters) and an enhancer probe (guanine-rich). When the enhancer probe is brought close to the NC probe through hybridization, the dark silver clusters are activated and become highly emissive through the interactions with the nearby guanine-rich enhancer sequence. We call this process “the guanine-proximity-induced activation of silver clusters” or, in short, “the activation of NCBs”. To activate sNCBs, 1.5  $\mu\text{l}$  of 1.2 mM synthetic DNA target (with enhancer sequence embedded) was added to a 120  $\mu\text{l}$  aliquot of the previously prepared 15  $\mu\text{M}$  NC probe solution. The mixture was vortexed, centrifuged, and immersed in a hot water bath (90-95  $^\circ\text{C}$ ) for 1 min, followed by gradual cooling to room temperature for 1 hr. The activated sNCB had the NC probe:target molar ratio of 1:1.

#### C. Fluorescence Measurements

Fluorescence emission and excitation scans were performed on a FluoroMax-4 spectrofluorometer from Horiba Scientific. 120  $\mu\text{l}$  NCB sample was placed in a 100  $\mu\text{l}$  quartz cuvette (16.100F-Q-10/Z15, Starna Cells) for fluorometer measurements. For 1D emission scan, the excitation wavelength was set to 580 nm and the emission wavelength was scanned from 595 nm to 800 nm using 5 nm slit size, 1 nm increment step, 1000 detector gain, and 0.5 s integration time. A blank sample, 20 mM sodium phosphate buffer (pH6.7), was also measured.

For the 2D measurement, both emission and excitation were scanned from 400 nm to 800 nm using 5 nm slit size, 5 nm increment step, and 0.1 s integration time. Color photos of inactivated (NC probe only) and activated (the duplex) NCBs were acquired by a digital camera (Canon PowerShot SX 500 IS) on a Syngene gel imager (365 nm excitation).

## 2. Rolling Circle Amplification

Our RCA process<sup>4,5</sup> is summarized below:

### A. Primer Printing

A commercially available amine-binding CodeLink<sup>®</sup> glass slide (~ 6 x 10 mm<sup>2</sup>) was diced and mounted on a typical microscope slide (1" x 3"). 5  $\mu$ l of a primer mix (containing 1, 2, and 3 parts of 6 $\times$  printing buffer, DI water, and primer, respectively) was added onto the slide. Thereafter, the slide was incubated inside the dehydration chamber containing saturated 5 M NaCl solution overnight.

### B. Primer Blocking and Washing

Primer blocking was carried out in a Coplin jar containing a pre-heated (50 °C) blocking buffer (0.1 M Tris, 50 mM ethanolamine, pH 9). Previously printed primer slide was immersed in this solution and incubated at 50 °C for 30 min. The blocking buffer was discarded and the slide was washed twice with DI water for 1 min. These steps were repeated using the pre-heated wash buffer 1 (4x SSC, 0.1 % SDS). The slide was then air-dried and labeled as “**A chip**”.

### C. Primer Hybridization with Human Topoisomerase I (hTopI) Circularized DNA Substrate

5  $\mu$ l 100 nM circularized hTopI substrate (in a buffer of 10 mM Tris-HCl pH 7.5, 1mM EDTA, 5 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub> and 100 mM NaCl) was added onto the **A chip**. This slide was incubated in a humidity chamber at room temperature for 30 min, and subsequently washed with wash buffer 2 (100 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.3 % SDS) for 1 min, washed with wash buffer 3 (100 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05 % Tween 20) for 1 min, rinsed with absolute ethanol and air dried.

### D. Rolling Circle Amplification (RCA)

3  $\mu$ l of the freshly prepared cold RCA reaction mix (containing 1 $\times$   $\phi_{29}$  buffer, 1  $\mu$ g/ $\mu$ l BSA, 250  $\mu$ M dNTP, and 1 U/ $\mu$ l  $\phi_{29}$  polymerase) was added onto the slide prepared from step C. The slide was incubated at 37 °C in the humidity chamber for 1 hr, washed with wash buffer 2 and wash buffer 3 and absolute ethanol for 1, 1 and 0.5 min, respectively. The slide was air dried and labeled as “**B chip**”.

## 3. Protein Purification and Cell Extract Preparation

### A. Protein Purification and Substrate Circularization

The plasmids expressing hTopI was transformed into the yeast *S. cerevisiae* strain RS190. The proteins were expressed, and purified enzyme was prepared as described previously.<sup>6</sup> 100 nM or 200 nM hTopI REEAD substrates were incubated with 200 nM purified enzyme (approximately 100 ng enzyme per 5  $\mu$ l reaction volume) in a buffer containing 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 5 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub> and 100 mM NaCl) for one hour at 37 °C. The circularization reaction was heat inactivated at 70 °C for 10 min before the samples were used directly or stored at -20 °C.

### B. Cell Culture

Human embryonic kidney HEK293 cells were cultured in GIBCO's Minimal Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) (Gemini Bio-Products), 100 units/ml penicillin and 100 mg/ml streptomycin (Invitrogen) in a humidified incubator (5% CO<sub>2</sub>/95% air atmosphere at 37°C). Cells were harvested with 0.25% Trypsin-EDTA (GIBCO) and resuspended in

Phosphate-buffered Saline (1xPBS, Cellgro), 1% Pluronic F-68 (Sigma-Aldrich), 0.1% BSA (Invitrogen). The cell densities were adjusted to 1 million cells/ml.

### *C. Cell Lysis and Substrate Circularization*

For generation of crude cell extracts with osmotic lysis, 0.5 million HEK293 cells were centrifuged at 3000 rpm for 5 min and the supernatant aspirated. The cell pellet was resuspended in cold lysis buffer (20 mM Tris-HCl pH 7.5, 2 mM EDTA, 1 mM DTT, 1 mM PMSF, 0.2% Tween 20) in a final concentration of 1 million cells/ $\mu$ l and incubated for 15 min with regular mixing. Volumes corresponding to the appropriate number of cells (1,000, 100, 10) were taken from this crude cell extract and incubated with 100 nM of the hTopI REEAD DNA substrate in buffer containing 20 mM Tris-HCl pH 7.5, 2 mM EDTA, 50 mM NaCl. This circularization reaction was incubated for one hour at 37°C. The circularization reaction was heat inactivated at 70 °C for 10 min before the samples were used.

## **4. Quantification of Endogenous hTopI Activity in Crude Human Cell Extract Using Rhodamine (Rh)-tagged Probe and sNCB**

Enzymatic activity experiment was carried out using three replicates of 10, 100, and 1000 cells (sample preparation described in *Section 3C*) in REEAD assays. These assays were then labeled with rhodamine-tagged probe and sNCB.

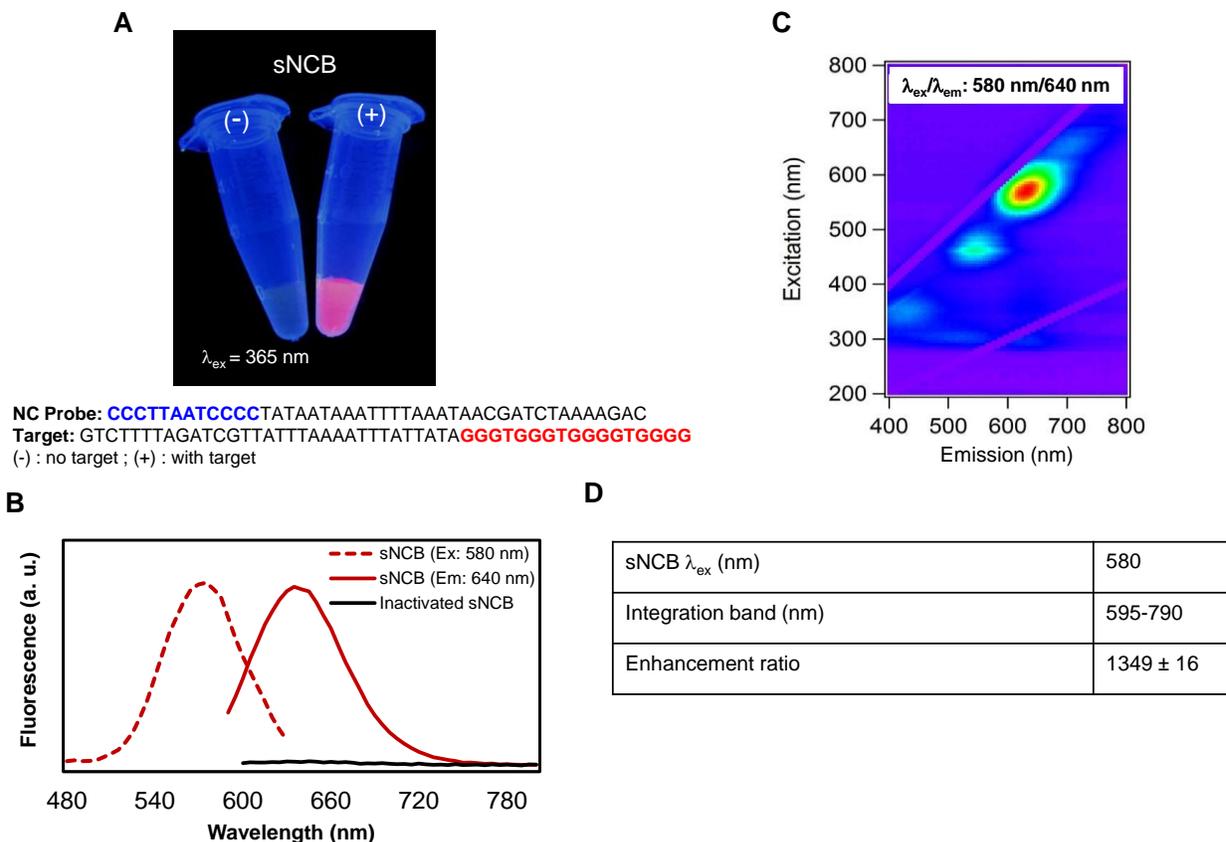
### *A. Rhodamine-tagged Probe Labeling*

0.1  $\mu$ l 10  $\mu$ M rhodamine-tagged probe was added to 2.5  $\mu$ l 2 $\times$  hybridization buffer (containing 40 % formamide, 10 % glycerol and 4x SSC). The solution was diluted to 5  $\mu$ l with DI water and added onto the **B chip** which was then incubated at 37 °C in the humidity chamber for 30 min. The slide was washed with wash buffer 2, wash buffer 3 and absolute ethanol for 1, 1 and 0.5 min, respectively. The resulting chip was dried and imaged under a fluorescence microscope (IX-71, Olympus; HCImage Live software, version 4.0.6.3, Hamamatsu Corporation, USA) using a water-immersion 60 $\times$  objective (UPlan SApo, Hamamatsu ORCA-Flash 4.0). To prevent rhodamine from rapid photobleaching, 2  $\mu$ l anti-fading mounting medium (Vectashield<sup>®</sup>) was added onto the REEAD assay before taking images under the microscope. For each sample replicate, ten background-corrected images were taken. hTopI signals (dots) were then counted using Image J (version 1.48v, National Institutes of Health, USA).

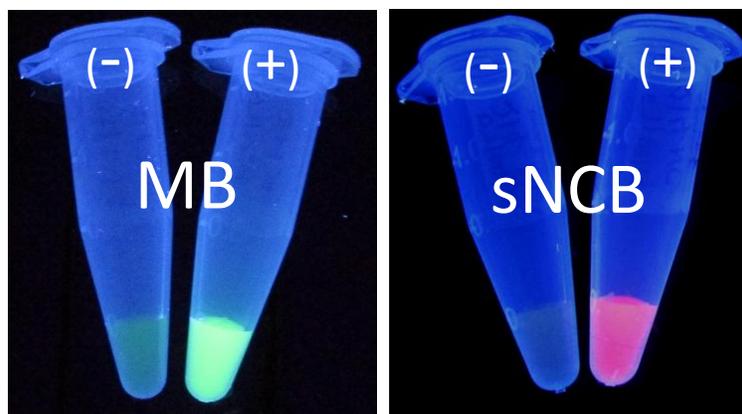
### *B. sNCB Labeling*

50  $\mu$ l of sNCB was first prepared by mixing 2  $\mu$ l 15  $\mu$ M NC probe (with dark Ag NCs), 25  $\mu$ l 100 mM sodium phosphate buffer, and 23  $\mu$ l DI water. 25  $\mu$ l of this mixture was added onto the previously prepared **B chip** which was then incubated at 85-90 °C in the humidity chamber for 1 min and gradually cooled down to room temperature for 70 min. The resulting slide was washed with 20 mM sodium phosphate buffer (pH 6.7) for 2 min, air dried and imaged under similar fluorescence microscope. For each sample replicate, ten background-corrected images were taken and hTopI signals (dots) were counted using Image J.

## II. NanoCluster Beacons in Homogeneous Assay



**Fig. S1 (A, top)** Color photos of the inactivated sNCB (i.e. NC probe only) and activated sNCB (i.e. the duplex).  $\lambda_{ex} = 365 \text{ nm}$ . sNCB was prepared at  $15 \mu\text{M}$  concentration. **(A, bottom)** Oligonucleotide sequences used in the experiment. Here, the nanocluster nucleation site of NC probe is shown in blue, the hybridization sequence in black, and the G-rich enhancer sequence in the target in red. Here the synthetic target mimics the truncated rolling circle amplification product (RCP) from REEAD assay. **(B)** Excitation/emission spectra of inactivated and activated sNCBs. **(C)** Normalized 2D fluorescence contour plots of sNCB. **(D)** The enhancement ratio of sNCB was estimated based on the 1D scans using a fixed excitation wavelength of 580 nm and calculated as  $(I_{activated} - I_{buffer}) / (I_{inactivated} - I_{buffer})$ , where  $I_{activated}$ ,  $I_{buffer}$  and  $I_{inactivated}$  are the integrated fluorescence intensities of the activated NCB (i.e. the duplex), the sodium phosphate buffer and the inactivated NCB (i.e. NC probe only), respectively. Fluorescence intensities before and after activation were integrated from 595 nm to 790 nm.



**Fig. S2** Color photos of molecular beacon (MB) and sNCB with (+) and without (-) a target. Both MB and sNCB were prepared at 15  $\mu\text{M}$  concentration. The buffer condition for MB is 1 mM  $\text{MgCl}_2$ , 20 mM Tris-HCl buffer (pH 8)<sup>7</sup> while for sNCB is 20 mM sodium phosphate buffer (pH 6.7).

DNA Sequence (5'  $\rightarrow$  3'):

**MB:** /56-FAM/CGCGATCCACCCCACCCTATAATAAATTATCGCG/3DAb/

**NC Probe of sNCB:** CCCTTAATCCCCTATAATAAATTTTAAATAACGATCTAAAAGAC

**RCP-mimicking synthetic Target:** GTCTTTTAGATCGTTATTTAAAATTTATTATA GGGTGGGGTG  
GGGTGGGG

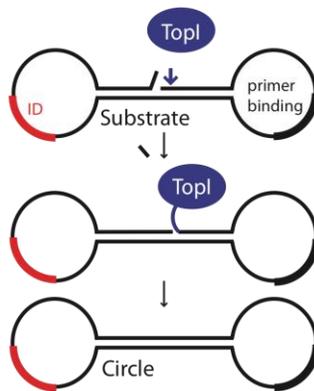
### III. NanoCluster Beacons in REEAD Assay

**Table S1.** hTopI substrate, primer and rhodamine-tagged probe sequences used in this study.

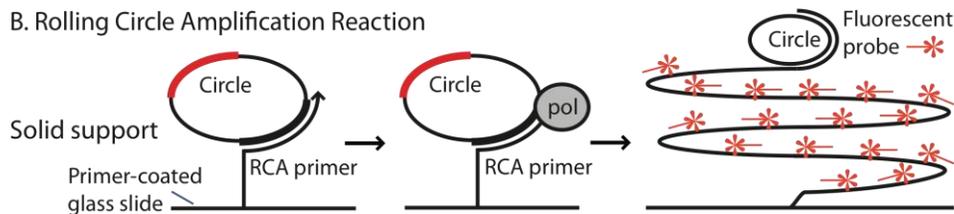
REEAD Design	DNA Sequence (5' → 3')
Substrate for hTopI	AGAAAAATTTTAAAAAACTGTGAAGATCGCTTATCCTCAATGCTGCTGCTGTACTACTTTTT TAAAAATTTTCTAAGTCTTTAGATCGTCCCCACCCACCCACCCTATAATAAATTTTAAATA ACGATCTAAAAGACTTAGA
Primer	Amine-CCAACCAACCAACCAATAAGCGATCTTCACAGT
Probe for RCP	Rhodamine-CCTCAATGCTGCTGCTGTACTAC

#### REEAD assay

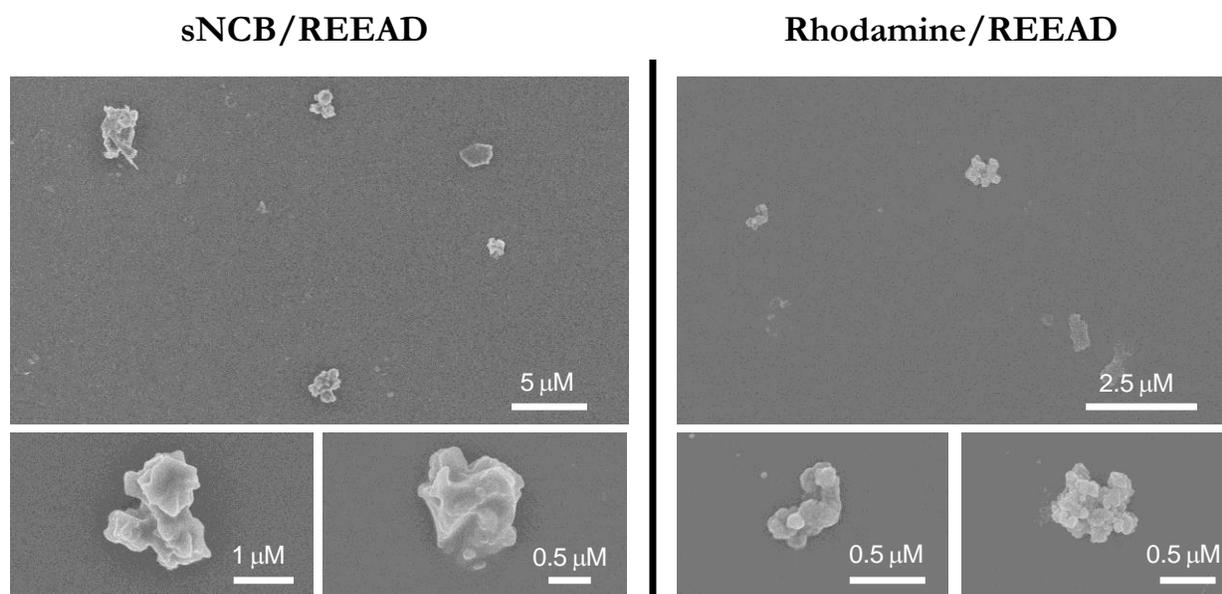
##### A. hTopI Reaction



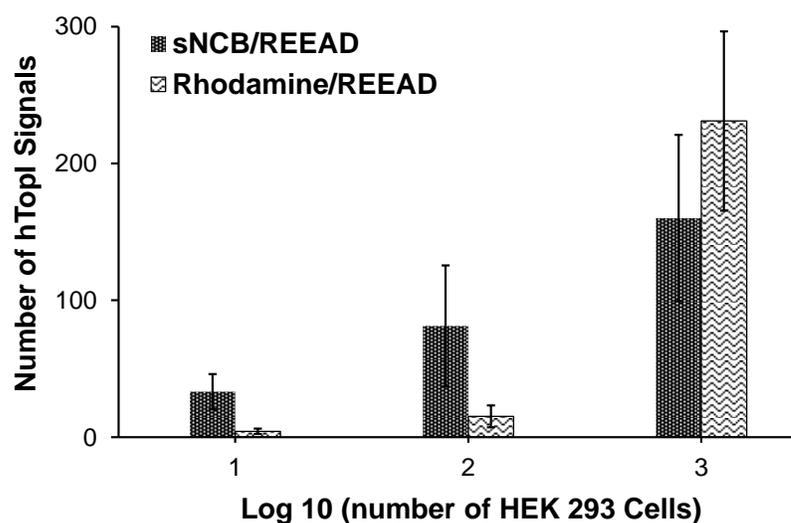
##### B. Rolling Circle Amplification Reaction



**Fig. S3** Detailed steps in the detection of human topoisomerase I (hTopI) activity. **(A)** The hTopI DNA substrate contains a primer-binding domain (thick black line) and a probe-annealing domain (thick red line). The substrate folds onto itself and forms a dumbbell structure, which can be circularized by the cleavage and ligation activity of hTopI. **(B)** The resulting circle allows for RCA, generating a rolling circle amplification product (RCP) with  $\sim 10^3$  tandem repeats. The RCP is then labeled with multiple fluorescent probes for visualization under a fluorescence microscope. As a result, each bright dot observed represents a “single” catalytic event of hTopI.



**Fig. S4** Low- and high-magnification STEM images of an sNCB/REEAD chip and a Rh/REEAD chip showing three-dimensional plate-like wavy structures. Samples were first coated with Au/Pd for 1 min at 40 mA power and analyzed under scanning transmission electron microscope (STEM S-5500) operated at 5 kV.



**Fig. S5** Quantification of endogenous hTopI enzyme activity in crude human cell extract using rhodamine probe and sNCB in REEAD assays. Three replicates for each concentration (10, 100 and 1000 cells) were carried out. Each error bar shows standard deviation from thirty background-corrected images collected on three replicates (i.e. 10 images/replicate).

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