Supplementary information, Data S1

Scheme of the thermocouple structure

The thermocouple consists of tungsten (W) probe with a tip curvature radius smaller than 100 nm as a substrate, an insulating layer (polyurethane PU) as an outer layer(except at the tip), and platinum (Pt) thin film as an outermost layer (Figure S1).

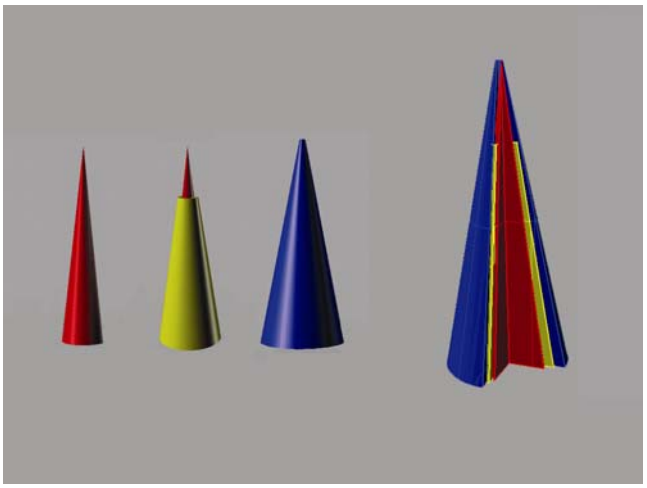

Figure S1 The scheme of the thermocouple with sandwich structure. Red, yellow, blue represent tungsten, polyurethane, platinum, respectively.

Equipment: Navitar Zoom 6000 UltraZoom system, PI Nanoposition controller , Ion sputtering coater JGP350A, Agilent 34410A multimeter, ITech 6120 High Resolution Programmable Power Supply.

Fabrication steps

1. Tungsten (W) probe: Electrochemical etching technique was used to manufacture W probe. At the air/electrolyte interface, the sharp tip formed at the very end of the tip when the fracture occurred. It was crucial to cut off the power as soon as the fracture occurs, for the residual current could etch the tip from sharp to blunt. In this work, the cutoff time was approximation 20us which made tip sharp enough (tip curvature radius below 100nm).

2. Insulating layer： The tungsten probe with a tip curvature radius smaller than 100 nm was chosen as the substrate. The tungsten probe was firstly immersed in absolute ethanol solution for 30min to achieve a better encapsulation with PU. The PU chloroform dissolvent (16g.L^{-1}) was filled into a high light transmissivity quartz container. Both the PU chloroform dissolvent level and the tungsten (W) probe tip could be seen through microscope. Firstly, the tungsten probe tip was observed by the microscope, which was not merged in the PU chloroform dissolvent. Then, the PU chloroform dissolvent level moved upwards as the quartz container moved upwards, driving by the moving system (including a three axis micro position system, and PI nanopositioncontroller). When the length of the probe tip not merged reached to about one micrometer, the moving system ceased. After that, PU chloroform dissolvent level moved downwards because of the dissolvent evaporation. Meanwhile, the PU insulating layer formed on the W probe (Figure 1 A).

3.Pt coating: Finally, the PU coated probes were placed in the ion sputtering coater JGP350A (60W/10min/<3×10-3Pa). Pt film thickness about 100nm was sputtered on

the probe (Figure S2). Thus the Pt-W junction of the thermocouple had been done (Figure 1 B).

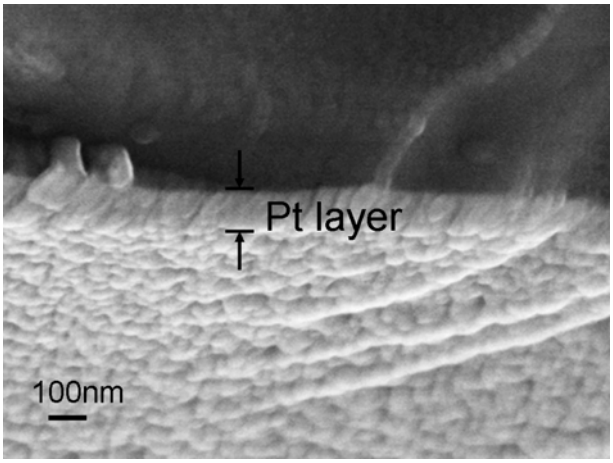

Figure S2 The SEM image of Pt layer with 100nm thickness.

Thermocouple calibration

The thermoelectric power and seebeck coefficient are important for the TCs. In this calibration, the hot junction immersed in water bath temperature T1 varied over the range 0°C to 90°C, and with the cold junction immersed in ice water mixture maintained at a constant temperature T2=0°C, taken as the reference temperature.

Red curve is standard curve of the bulk Pt-W thermocouple.

$$V = \left(-3.12181 + 4.63102\mathrm{T} + 0.0309219\mathrm{T}^{2}\right) \times 10^{-6} \quad \textbf{(Formula 1)}$$

$$S = \left(4.63102 + 0.0618438\mathrm{T}\right) \times 10^{-6} \quad \textbf{(Formula 2)}$$

The Seebeck coefficient is from 4.6μV/°C to 10.2μV/°C (from 0°C to 90°C). After incorporating the resolution limit of the digital multimeter (Agilent 34410A, 0.1μV), we determined that the temperature resolution of our measurement was from 0.02°C to 0.01°C (The resolution limit of the digital multimeter was divided by the Seebeck coefficient).

Simulation

To obtain the information of thermal response time of the TC, herein, we simulated the TC response process in favor of computer. We hypothesized a single cell model, in which the temperature was instantaneously higher than environment and produce heat dissipating very fast. The TC was inserted into the cell and recorded this process. The simulated results showed that when the time reaches 400ns, the thermocouple tip was almost same temperature as the cell. Hence, it could be considered that response time of the thermocouple was as fast as 400ns (Figure S3).

The simulation was based on the general heat transfer equation (formula 3) and COMSOL Multiphysics 3.5a software was used.

$$\rho C\frac{\partial T}{\partial \tau}=\nabla(K\nabla T)+Q+q_m T \qquad \textbf{(formula 3)}$$

where ρ is density of the tissue (kg/m^3), C is heat specific $(J/kg°C)$, T is temperature, τ is time (s) K is thermal conductivity $(W/m°C)$, Q is the heat source (W/m^3), q_m is the absorption coefficient $(W/m^3°C)$

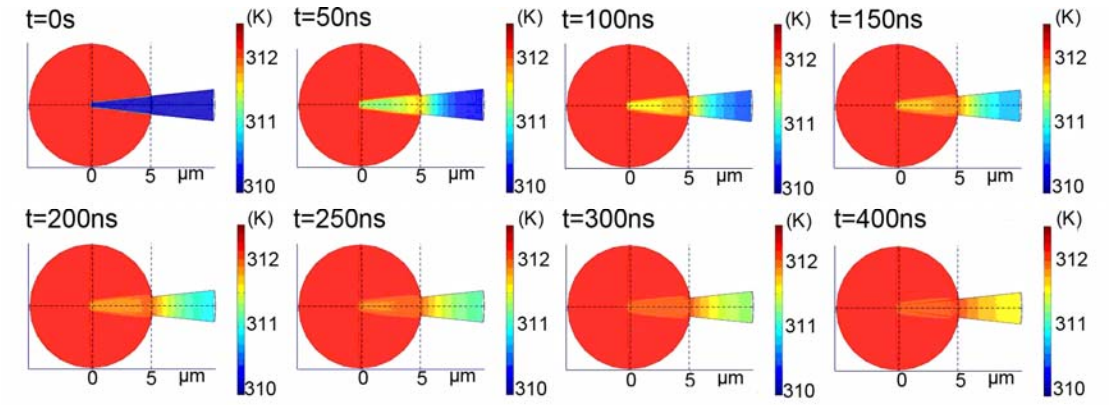

Figure S3 The simulated results of the TC probe response to a cell 2°C **higher than the environment. a, b, c, d, e, f, g, h represent 0ns, 50ns,100ns,150ns,200ns,250ns,300ns,400ns in separate.**

We detected temperature rise by the TC in single U251 cell in ten groups, respectively. Five groups were added camptothecin, the average of temperature rise after 30 minutes was 0.6±0.2°C. Five groups were added doxorubicin, the average of temperature rise was 0.1±0.1°C.

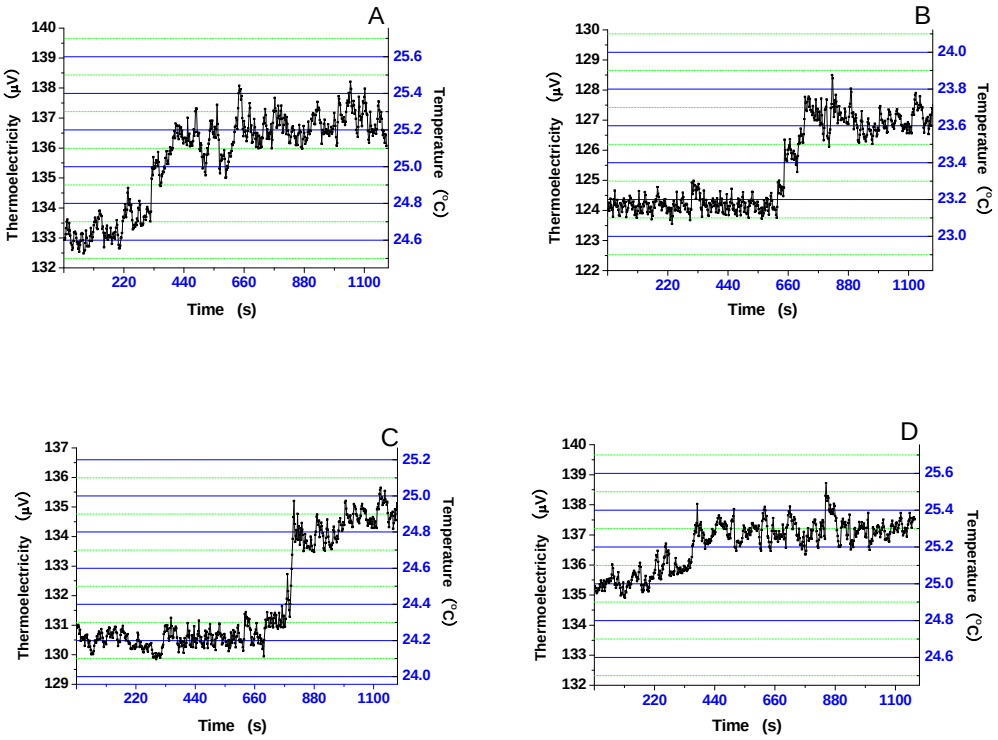

Figure S4 The curves of the intracellular temperature fluctuation after cells exposed to camptothecin (A-D) in four groups.

Figure S5 The photo of the micromanipulation system.