# **Supporting Information**

# Protease-Responsive Mass Barcoded Nanotranslaters for Simultaneously Quantifying the Intracellular Activity of Cascaded Caspases in Apoptosis Pathways

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### **Experimental section**

Reagents and Materials. Recombinant human active caspase-3, -8 and -9 proteins were all purchased from Abcam. The substrate peptides of caspase-3 (biotin-GGDEVDGKK, Mw=1130.25), caspase-8 (biotin-GGIETDGKK, Mw=1130.29) and caspase-9 (biotin-GGLEHDGKK, Mw=1166.33) were all synthesized by GL Biochem Ltd (Shanghai, China). HS-PEG-NHS (Mw=2000) and streptavidin were supplied by Aladdin. Mass tag 1  $[HS-(CH_2)_{11}(OCH_2CH_2)_3OH],$ mass tag 2 [HS-(CH<sub>2</sub>)<sub>11</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>4</sub>OH], mass tag 3 [HS-(CH<sub>2</sub>)<sub>11</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>6</sub>OH], IS [HS-(CH<sub>2</sub>)<sub>15</sub>-COOH], trifluoroacetic acid (TFA), 2, 5-dihydroxybenzoic acid (DHB) and sodium cyanoborohydride (NaBH<sub>3</sub>CN) were all purchased from Sigma. Staurosporine (STS), Ac-DEVD-CHO (caspase-3 inhibitor), Z-IETD-FMK (capase-8 inhibitor), Z-LEHD-FMK (caspase-9 inhibitor) were supplied by Keygen Bintech. Diosgenin, caspase-3 activity assay kit, caspase-8 activity assay kit and caspase-9 activity assay kit were purchased from Beyotime Biotechnology. All other reagents were of analytical grade and used without purification.

**Preparation of streptavidin coated Fe<sub>3</sub>O<sub>4</sub> nanospheres (Fe<sub>3</sub>O<sub>4</sub>@strep).** Fe<sub>3</sub>O<sub>4</sub> nanospheres were synthesized according to the literature.<sup>1</sup> Briefly, 1.0 g of FeCl<sub>3</sub>·6H<sub>2</sub>O was dissolved in 30 mL of ethylene glycol, and 2.0 g of sodium acetate and 7.0 g 1,6-hexadiamine were subsequently added into solution and stirred at 50 °C for 30 min. Finally, the mixture was transferred to Teflon-lined stainless-steel autoclave and heated at 200 °C for 6 h. The product was separated with a magnet and washed several times with ethanol and water.

Fe<sub>3</sub>O<sub>4</sub>@strep was synthesized as follows: 1 mg of amine-functionalized Fe<sub>3</sub>O<sub>4</sub> nanospheres were dispersed in 500  $\mu$ L of 10 mM PBS buffer containing 50  $\mu$ L of 25% glutaraldehyde and 2 mg of sodium cyanoborohydride and shaken at room temperature for 2 h. After being washed for three times, Fe<sub>3</sub>O<sub>4</sub> nanospheres were mixed with 0.07 mg of streptavidin and kept shaking for 12 h. Finally, the streptavidin coated magnetic cores were mixed with 1 mL of quenching buffer (50 mM Tris, pH 7.4) for 0.5 h, and then washed and stored at 4 °C in PBS buffer with a final concentration of 2 mg/mL.

**Preparation of the satellite AuNPs.** First, 13 nm AuNPs were synthesized as follows: 30 mL of trisodium citrate (38.8 mM) was injected into 1% HAuCl<sub>4</sub> solution under vigorous stirring. After boiling for 15 min, the solution was naturally cooled to room temperature and the color was converted from pale yellow into deep red. The concentration of AuNPs was calculated to be 16 nM and the average diameter was determined to be 13 nm. Next, bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP)-stabilized AuNPs was obtained by addition of 8 mg BSPP into 15 mL AuNPs solution (16 nM). After stirring for 12 h and discarding the unbound BSPP, the AuNPs solution was placed at 4 °C for further use.

AuNPs@Con-MT were synthesized according to the following procedures. For AuNPs@Con1-MT1, the substrate peptide (16 nmol) of caspase-3 was conjugated with HS-PEG-NHS (MW: 2000 Da, 16 nmol) via room temperature reaction in dimethyl sulfoxide (DMSO) solution containing 1% triethylamine for 6 h. Next, 200  $\mu$ L of BSPP-stabilized AuNPs (16 nM) were modified by PEG-peptide conjugate (80  $\mu$ M) and MT1 (24  $\mu$ M) for 12 h. The decorated AuNPs were washed with 400  $\mu$ L of deionized water for three times, followed by centrifugation for 20 min at 13000 rpm. For the preparation of AuNPs@PEG-IS, similar procedures were conducted except that the mixture was replaced by biotinylated thiol-PEG (Mw: 3400 Da, 8  $\mu$ M) and internal standard mass tag (80  $\mu$ M). For multiplex quantification of the activity of caspases, the same amount of PEG-substrate conjugate responsive to caspase-8 and MT2 were co-attached onto AuNPs to form AuNPs@Con2-MT2, and the PEG-substrate conjugate of caspase-9 and mass tag 3 (MT3) were applied for the construction of AuNPs@Con3-MT3. In final, the satellite AuNPs was suspended in 200  $\mu$ L of water and stored at 4 °C for further use.

**Preparation of the core-satellite PRMNTs.** The core-satellite nanotranslaters were prepared by mixing 20  $\mu$ L of Fe<sub>3</sub>O<sub>4</sub>@strep (2 mg/mL) with 12  $\mu$ L of AuNPs@Con1-MT1 (16 nM), 7  $\mu$ L of AuNPs@Con2-MT2 (16 nM), 6  $\mu$ L of AuNPs@Con3-MT3 (16 nM) and 10  $\mu$ L of AuNPs@PEG-IS (16 nM). After shaking for 30 min, the as-prepared PRMNTs were separated from the redundant satellite AuNPs with a magnet and then washed with PBS for three times. The PRMNTs specific for caspase-3 (denoted as

PRMNTs-Casp-3) were synthesized by only assembling AuNPs@PEG-IS and AuNPs@Con1-MT1 onto Fe<sub>3</sub>O<sub>4</sub>@strep. After each wash, MALDI MS analysis was performed to check the peak intensity of MTs and IS.

Quantification of the activity of caspases. According to the ICP-MS results, 40  $\mu$ L of PRMNTs-Casp-3 (2 mg/mL) was catalyzed by different amount of caspase-3 in 20  $\mu$ L of 40 mM pH 7.4 HEPES-CHAPS buffer containing 100 mM NaCl, 10 % sucrose and 0.1 % CHAPS at 37 °C. After kept for 1.5 h and rinsed by PBS buffer, this probe was deposited on a steel MALDI plate and mixed with 2, 5-dihydroxybenzoic acid (DHB) solution (10 mg/mL in 70 % ACN, 0.1 % TFA) for MALDI MS analysis. For the triplex detection of caspase-3, 8 and 9, the only modification was that the PRMNTs was firstly decorated with AuNPs@Con1-MT1, AuNPs@Con2-MT2, AuNPs@Con3-MT3 and AuNPs@PEG-IS and subsequently catalyzed by three caspases.

Quantification of intracellular activity of cascaded caspases in HeLa cells. HeLa cells ( $1.6 \times 10^6$  cells) were cultured in DMEM medium containing 10 % fetal bovine serum in a chamber with 5 % CO<sub>2</sub> at 37 °C. After being washed by sterile PBS three times, the cells were incubated with the PRMNTs (40 µg/mL) in 4 mL antibiotic-free MEM medium for 12 h at 37 °C. After washing the remaining PRMNTs by sterile PBS for three times, the cells were incubated with stimuli or inhibitors for 1.5 h. Thereafter, the core-satellite PRMNTs were extracted by breaking cells with lysis buffer under sonication for 30 s three times and then collected with a magnet. Finally, the PRMNTs were subjected to MALDI MS analysis.

**Cytotoxicity assay.** To access the cell viability, the HeLa cells were seeded in 96 -well plates at a density of  $1 \times 10^4$  cells per well. Different amount of core-satellite PRMNTs and satellite AuNPs was added and incubated for 12 h. After washing steps, 100 µL of MTT solution (0.5 mg/mL) were added into each well and kept for another 4 h. The supernatant was removed and the precipitate was dissolved with 100 µL of DMSO. The absorbance at 570 nm was finally recorded by microplate reader. The cell viability was calculated as: cell viability (%) = (average optical density of treatment group/average optical density of control) × 100%.

MALDI MS analysis. MALDI mass spectra of the PRMNTs were obtained in a

positive reflection mode on a 4800 Plus MALDI-TOF/TOF Mass Spectrometer (AB Sciex) that is equipped with a 355 nm Nd: YAG laser. DHB solution (10 mg/mL in 70 % ACN, 0.1 % TFA) and 1,5-DAN solution (0.5 mg/mL in 50% ACN, 0.1% TFA) were prepared as matrix. All samples were prepared on 384-well stainless steel target plate. For all MALDI MS experiments, 1  $\mu$ L of analyte was mixed with 0.5  $\mu$ L matrix solution, and then 1  $\mu$ L of this mixture was deposited onto the plate. MS spectrum was the average of 16 sub-spectra obtained from the edge bias of matrix spot with sum of 25 laser shots per sub-spectrum.

Characterization of Fe<sub>3</sub>O<sub>4</sub> nanospheres and Fe<sub>3</sub>O<sub>4</sub>@strep.



Figure S1. (a) TEM and (b) SEM images of  $Fe_3O_4$  nanospheres. (c) TEM and (d) SEM images of streptavidin capped  $Fe_3O_4$  nanospheres.



Verification of the successful conjugation between HS-PEG-NHS and the substrate of caspases.

Figure S2. MALDI mass spectra of (a) HS-PEG-NHS (Mw: ~2000) and HS-PEGsubstrate conjugates respectively responsive to (b) caspase-3, (c) caspase-8 and (d) caspase-9. The substrate peptides of caspase-3: biotin-GGDEVDGKK, Mw=1130.25, caspase-8: biotin-GGIETDGKK, Mw=1130.29, and caspase-9: biotin-GGLEHDGKK, Mw=1166.33. The mass shift after conjugation indicates the successful coupling between HS-PEG-NHS and biotinylated substrate peptides.

### Characterization of the satellite AuNPs.



Figure S3. Characterization of the satellite AuNPs. (a) TEM image of AuNPs@Con1-MT1. (b) The hydrodynamic size distribution of AuNPs and AuNPs@Con1-MT1. UVvis spectra of various satellite AuNPs in (c) water and (d) 10 mM PBS buffer (pH 7.4, 0.1M NaCl). Photographs show their stability in water and PBS buffer. 1. AuNPs; 2. AuNPs@PEG-IS; 3. AuNPs@Con1-MT1; 4. AuNPs@Con2-MT2; 5. AuNPs@Con3-MT3.



Comparison of the matrixes for MALDI MS analysis of four satellite AuNPs

Figure S4. The MALDI mass spectra of (a, b) blank solution, (c, d) 8 fmol AuNPs@Con1-MT1, (e, f) 8 fmol AuNPs@Con2-MT2, (g, h) 8 fmol AuNPs@Con3-MT3 and (i, j) 8 fmol AuNPs@PEG-IS using1,5-DAN (left panel) and DHB (right panel) as the matrix. Asterisk (\*) and pound sign (#) represent the matrix ion signals from 1,5-DAN and DHB, respectively.

Mass spectra of four satellite AuNPs.



Figure S5. MALDI mass spectra of (a) AuNPs@PEG-IS, (b) AuNPs@Con1-MT1, (c) AuNPs@Con2-MT2 and (d) AuNPs@Con3-MT3. (m/z 597: [2IS-H<sub>2</sub>+Na]<sup>+</sup>; 565 [2IS-H<sub>2</sub>S+Na]<sup>+</sup>; 613 [2IS-H<sub>2</sub>+K]<sup>+</sup>; 693 [2MT1-H<sub>2</sub>+Na]<sup>+</sup>; 661 [2MT1-H<sub>2</sub>S+Na]<sup>+</sup>; 709 [2MT1-H<sub>2</sub>+K]<sup>+</sup>; 781 [2MT2-H<sub>2</sub>+Na]<sup>+</sup>; 749 [2MT2-H<sub>2</sub>S+Na]<sup>+</sup>; 797 [2MT2-H<sub>2</sub>+K]<sup>+</sup>; 957 [2MT3-H<sub>2</sub>+Na]<sup>+</sup>; 925 [2MT3-H<sub>2</sub>S+Na]<sup>+</sup>; 973 [2MT3-H<sub>2</sub>+K]<sup>+</sup>)

### Reproducibility of the synthesis of the PRMNTs



Figure S6. The MALDI mass spectra of the PRMNTs in different batches.



Verification of the specific interaction between Fe<sub>3</sub>O<sub>4</sub>@strep and satellite AuNPs.

Figure S7. MALDI mass spectra of Fe<sub>3</sub>O<sub>4</sub>@strep incubated with (a) the mixture of AuNPs@Con1-MT1, AuNPs@Con2-MT2 and AuNPs@Con3-MT3, or (b) the mixture of AuNPs@MT1, AuNPs@MT2 and AuNPs@MT3. Insets show the remaining AuNPs in the supernatant after magnetic separation.

## Verification of the stability of PRMNTs-Casp-3 in PBS



Figure S8. (a) The MALDI mass spectra of PRMNTs-Casp-3 during one-week incubation in PBS. AuNPs@Con2-MT2 was deposited on the MALDI plate to produce MT2 ion signal as internal standard. (b) The variation of MT1 and IS ion intensity and their intensity ratio in the PRMNTs-Casp-3.



Mass spectra of satellite AuNPs and PRMNTs-Casp-3 after washing steps

Figure S9. The MALDI mass spectra of the AuNPs@Con1-MT1 (a) and the supernatants (b) after washing steps. The MALDI mass spectra of the PRMNTs-Casp-3 (c) and the supernatants (d) after washing steps.

### Optimization of the concentration of streptavidin.



Figure S10. The loading amount of streptavidin on  $Fe_3O_4$  nanospheres varied with concentrations of streptavidin, which was calculated by quantifying the remaining amount in the supernatant using BCA protein quantification kit.

# Optimization of the concentration ratio of MT1 to Con1 in the construction of AuNPs@Con1-MT1.

Different concentration of MT1 was combined with a fixed amount of HS-PEGsubstrate conjugate (Con1, 80  $\mu$ M) for assembly satellite AuNPs, which was further immobilized onto Fe<sub>3</sub>O<sub>4</sub>@strep together with AuNPs@PEG-IS. The prepared PRMNTs-Casp-3 was incubated with 20 U/mL of caspase-3 at 37 °C for 1.5 h, and the variation of MS peak intensity ratio of MT1 to IS was monitored.



Figure S11. The relationship between the relative variation in intensity ratio of MT1 to IS with different concentration ratios of MT1 to Con1 for assembling AuNPs@Con1-MT1.  $R_{caspase-3}$  was the MS intensity ratio of MT1 to IS in the PRMNTs-Casp-3 under incubation with 20 U/mL caspase-3, and  $R_{control}$  was the MS intensity ratio obtained from the PRMNTs-Casp-3 incubated with blank solution without caspase-3.

# Optimization of the modification density of MT1 and Con1 on AuNPs@Con1-MT1 and the size of AuNPs

The AuNPs with different sizes (5 nm, 13 nm and 20 nm) were selected as the satellite AuNPs, which were further decorated with the same amount of MT1 (80  $\mu$ M) and Con1 (24  $\mu$ M) and employed to fabricate the PRMNTs-Casp-3. To optimize the modification density of MT1 and Con1 on AuNPs@Con1-MT1, the dosage of MT1 and Con1 was increased or decreased proportionately ([MT1]/[Con1]=0.3). As-prepared satellite AuNPs and a fixed amount of AuNPs@PEG-IS were immobilized onto Fe<sub>3</sub>O<sub>4</sub>@strep to construct the PRMNTs-Casp-3. The fabricated PRMNTs-Casp-3 was incubated with 25 U/mL of caspase-3 at 37 °C for 1.5 h, and the variation of MS peak intensity ratio of MT1 to IS was monitored.



Figure S12. TEM images of (a) 5 nm and (b) 20 nm AuNPs. The relationship between the relative variation in intensity ratio of MT1 to IS with dependence on (c) the dosage of MT1 and Con1 for AuNPs decoration and (d) the size of AuNPs.

# Quantification of the loading amount of Con1 and MT1 on the surface of AuNPs@Con1-MT1.

To accurately quantify the amount of coupled substrate peptides onto the AuNPs@Con1-MT1, the FAM labeled peptide was selected as the substitute of Con1 for the preparation of AuNPs@Con1-MT1, and the loading amount was obtained by measuring the fluorescence of the peptides remaining in the supernatant.

The loading amount of MT1 on AuNPs@Con1-MT1 was calculated by using the internal standard method. First, a standard curve was plotted by recording the various intensity ratio of MT1 to IS with different concentration ratios of MT1 on AuNPs@MT1 to IS on AuNPs@IS (Figure S8). The actual concentration of MT1 or IS molecules modified on AuNPs were then determined by measuring the thiol-terminated MT1 or IS in the supernatant using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) colorimetric assay.<sup>2,3</sup> Thereafter, a known amount of AuNPs@Con1-MT1 and AuNPs@IS were mixed and subjected to MALDI MS analysis. According to the obtained MS signal intensity ratio, the concentration of MT1 anchored on AuNPs@Con1-MT1 could be calculated by referring to the fitted standard curve.

The number of Con1 and MT1 molecules on a single AuNP@Con1-MT1 was calculated by dividing the as-measured concentration of Con1 and MT1 by the molar concentration of AuNPs.



Figure S13. The relationship between the intensity ratios of MT1 to IS and the concentration ratios of MT1 on AuNPs@MT1 to IS on AuNPs@IS.

Optimization of the concentration ratio of AuNPs@Con1-MT1 to AuNPs@PEG-IS for the assembly of core-satellite PRMNTs-Casp-3.



Figure S14. Optimization of concentration ratio of AuNPs@Con1-MT1 to AuNPs@PEG-IS for assembling core-satellite PRMNTs-Casp-3. The intensity ratio of MT1 to IS on the PRMNTs-Casp-3 was assessed by MALDI MS after the treatment with 20 U/mL caspase-3 at 37 °C for 1.5 h.

Characterization of the PRMNTs-Casp-3 incubated with caspase-3.



Figure S15. (a) TEM, (b) SEM images and (c) photographs of PRMNTs-Casp-3 incubated with 20 U/mL caspase-3 at 37 °C for 1.5 h. (d) TEM, (e) SEM images and (f) photographs of PRMNTs-Casp-3 without treatment.



Specific cleavage of the substrate peptide decorated on satellite AuNPs

Figure S16. (a) Schematic cleavage of the PEG-substrate conjugate decorated on the AuNPs@Con1-MT1. (b) MALDI mass spectrum of the supernatant containing peptide segments from AuNPs@Con1-MT1 after incubation with 20 U/mL caspase-3. (c) MALDI mass spectrum of the supernatant of AuNPs@Con1-MT1 without caspase treatment. (d) MALDI mass spectrum of CHCA. BG: unidentified species from background, Mtr: the matrix ion signals from CHCA.

## Cytotoxicity of the PRMNTs and satellite AuNPs.



Figure S17. Cell viability of HeLa cells incubated with different concentrations of the PRMNTs and satellite AuNPs@Con1-MT1 for 12 h.

#### ICP-MS quantification of cellular uptake of the PRMNTs.

The cells ( $1.6 \times 10^6$  cells) were incubated with PRMNTs in 4 mL antibiotic-free MEM medium at 37 °C for 12 h, and then washed by sterile PBS three times. After scrape and centrifugation, the cell pellet was then dissolved by nitrohydrochloric acid, and the Fe content was analyzed by ICP-MS. The same amount of PRMNTs without treatment was also dissolved in acid solution and quantified by ICP-MS. Based on the average value of three experiments, the cellular uptake of PRMNTs was determined, and the dosage of PRMNTs were accordingly chosen for the activity quantification of caspases within cells.



Figure S18. The Fe content in the PRMNTs-treated HeLa cells determined by ICP-MS. The cellular uptake was calculated to be 51.1%. Since 80  $\mu$ g of PRMNTs were used in the establishment of calibration curve for *in vitro* protease activity assay, the dosage of PRMNTs should be adjusted to 160  $\mu$ g for cell incubation. In 4 mL of incubation solution, the concentration of PRMNTs was fixed at 40  $\mu$ g/mL.

### Quantification of the activity of caspase-3 in MCF-7 cells



Figure S19. (a) The MALDI mass spectra of PRMNTs-Casp-3 internalized by MCF-7 cells with different stimulation. (i) Without stimulation; (ii) 2  $\mu$ M STS; (iii) 2  $\mu$ M STS and 100  $\mu$ M Ac-DEVD-CHO. (b) Quantification of the activity of caspase-3 in MCF-7 cells with different stimulation. Statistical analysis: *t*-test (\*\*p<0.01,\*\*\*p<0.001).

Specificity of the PRMNTs to individual caspase.



Figure S20. The relative variation of intensity ratio of MT to IS in the PRMNTs incubated with 20 U/mL of caspase-3, 25 U/mL of caspase-8 or 25 U/mL of caspase-9 at 37 °C for 1.5 h, respectively.

Influence of the intracellular matrices on the specificity of the PRMNTs.



Figure S21. The relative variation of MS peak intensity ratio in the PRMNTs incubated with 20 U/mL of caspase mixture (caspase-3, caspase-8 and caspase-9) for 1.5 h or 4 mM GSH, 50  $\mu$ g/mL BSA, 10  $\mu$ g/mL of chymotrypsin, 10  $\mu$ g/mL of trypsin, MEM buffer for 12 h.

Sample No.	<i>I</i> (MT1) / <i>I</i> (IS)	<i>I</i> (MT2) / <i>I</i> (IS)	<i>I</i> (MT3) / <i>I</i> (IS)
1#	1.72±0.04	1.33±0.03	1.28±0.06
2#	1.49±0.05	1.27±0.02	1.06±0.08
3#	1.65±0.06	1.47±0.03	1.22±0.05
RSD	7.21%	7.62%	9.55%

Table S1 The varied peak intensity ratio of MTs to IS in the mass spectra of the PRMNTs in different batches.

Table S2. The relative ion intensity of MT1 in the mass spectra of AuNPs@Con1-MT1 after each wash.

Washing time	1#	2#	3#
<i>I</i> (MT1) / <i>I</i> (IS <sub>0</sub> )	0.72±0.01	0.63±0.04	0.62±0.04

Table S3. The relative ion intensity of MT1 and IS in the mass spectra of PRMNTs-Casp-3 after each wash.

Washing time	<i>I</i> (MT1) / <i>I</i> (IS <sub>0</sub> )	<i>I</i> (IS) / <i>I</i> (IS <sub>0</sub> )	<i>I</i> (MT1) / <i>I</i> (IS)
1#	0.61±0.03	0.48±0.03	1.21±0.04
2#	0.38±0.02	0.35±0.03	1.05±0.05
3#	0.38±0.01	0.39±0.05	1.02±0.03

	<i>I</i> (MT1) / <i>I</i> (IS)	<i>I</i> (MT1) / <i>I</i> (IS <sub>0</sub> )	<i>I</i> (IS) / <i>I</i> (IS <sub>0</sub> )
4 mM GSH	1.26±0.11	1.04±0.06	0.82±0.09
<b>Blank solution</b>	1.31±0.07	1.59±1.12	1.19±0.10
<b>Relative deviation</b>	3.81%	34.7%	31.1%

Table S4. The relative ion intensity of MT1 and IS in the mass spectra of PRMNTs-Casp-3 under different treatments.

After incubation in GSH containing or free media, the PRMNTs-Casp-3 were recovered and combined with AuNPs@MT2 for MALDI MS analysis. The reference ion signal generating from AuNPs@MT2 was denoted as IS<sub>0</sub>.

Analyte	Commercial activity assay kit (U/mL)	PRMNTs-based activity assay (U/mL)	Relative deviation
Caspase-3	10.76±0.83	9.38±0.56	12.82%

Table S5. Determination of the activity of caspase-3 in cell lysates by our method and commercial activity assay kit (Mean $\pm$ S.D., *n* =3).

Table S6. Determination of the activity of caspases in cell lysates by our method and commercial activity assay kit (Mean $\pm$ S.D., *n* =3).

	Commercial	PRMNTs-based	
Analyte	activity assay kit	activity assay	Relative deviation
	(U/mL)	(U/mL)	
Caspase-3	10.19±1.24	9.32±0.36	8.57%
Caspase-8	12.65±1.13	11.65±1.41	7.91%
Caspase-9	19.28±1.70	16.61±1.13	13.85%

**Protocols:** Quantification of the activity of caspases in cell extracts was performed according to the protocol provided by manufacturer. The assay principle is based on the formation of the chromophore p-nitroaniline (p-NA) derived from the cleavage of the labeled substrate (DEVD-pNA for caspase-3, IETD-pNA for caspase-8, LEHD-pNA for caspase-9). The p-NA can be quantified using a spectrophotometer or a microtiter plate reader reading absorbance at 405 nm. Briefly, HeLa cells ( $1.6 \times 10^6$  cells) were cultured overnight and then incubated with 2  $\mu$ M of STS for 1.5 h. After being washed with cold PBS three times and collected by centrifugation at 1200 rpm for 5 min, the cell pellet was lysed by adding lysis buffer (200  $\mu$ L) and incubated on ice for 15 min. After centrifuged at 12500 rpm for 15 min, the supernatant was collected and kept at -80 °C. The colorimetric activity assay was performed for three times in PCR tubes, which contained 40  $\mu$ L of reaction buffer, 50  $\mu$ L of cell lysates and 10  $\mu$ L of substrate (Ac-DEVD-pNA, Ac-IETD-pNA, or Ac-LEHD-pNA, 2 mM). The mixture was

incubated at 37 °C for 1 h and the absorbance at 405 nm was detected by a microplate reader. The procedures of quantifying the activity of caspase in cell extracts by our method was similar to the *in vitro* caspase activity assay except that the caspase standard solution was replaced by the 10  $\mu$ L of cell extracts.

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