Supplementary Information

Sensitive detection of caspase-3 enzymatic activities and inhibitor screening by mass spectrometry with dual maleimides labelling quantitation

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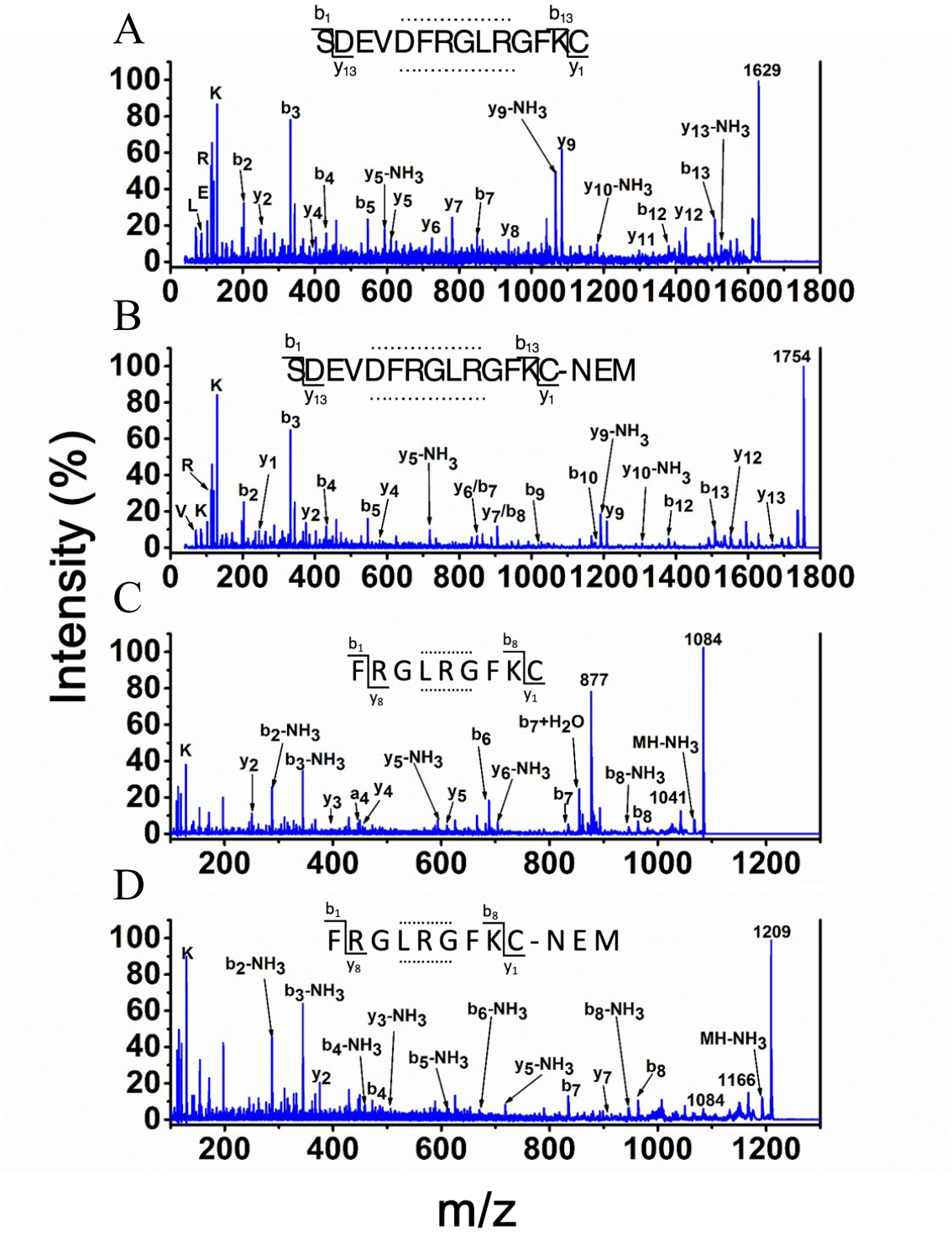

Figure S1. MS/MS spectra of (A) unlabeled P1 at m/z 1629, (B) NEM-P1 at m/z 1754, (C) fragment of caspase-3 cle*a*ved P1 at m/z 1084 and (D) fragment of caspase-3 cleaved NEM-P1 at m/z 1209.

Table S1. List of b- and y-type of fragments generated from the parent ion peak at m/z of 1629.

Name of fragment	Theoretical m/z	Name of fragment	Theoretical m/z
b_2	203.06	y_2	250.12
b_3	332.11	y_4	454.21
b_4	431.18	y_5	610.31
b_5	546.20	y_6	723.40
b_7	849.37	y_7	780.42
b_{12}	1379.67	y_8	936.52
b_{13}	1507.77	y_9	1083.59

Table S2. List of b- and y-type of fragments generated from the parent ion peak at m/z of 1754.

Name of fragment	Theoretical m/z	Name of fragment	Theoretical m/z
b_2	203.06	y_1	247.16
b_3	332.11	y_2	375.25
b_4	431.18	y_4	579.34
b_5	546.20	y_6	848.53
b_7	849.37	y_7	905.55
b_{12}	1379.67	y_9	1208.72
b_{13}	1507.77	y_{12}	1551.86

Table S3. List of b- and y-type of fragments generated from the parent ion peak at m/z of 1084.

Name of fragment	Theoretical m/z	Name of fragment	Theoretical m/z
b_2-NH_3	287.15	y_2	250.12
b_3-NH_3	344.17	y_3	397.19
b_6	687.40	y_4	454.21
b_7	834.47	y_5-NH_3	593.29
b_7+H_2O	852.47	y_5	610.31
b_8-NH_3	945.54	y_6-NH_3	706.37
b_8	962.57	K	126.09

Table S4. List of b- and y-type of fragments generated from the parent ion peak at m/z of 1209.

Name of fragment	Theoretical m/z	Name of fragment	Theoretical m/z
b_2-NH_3	287.15	y_2	375.25
b_3-NH_3	344.17	y_3-NH_3	505.29
b_4	474.28	y_5-NH_3	718.42
b_5-NH_3	613.36	y_7	905.55
b_6-NH_3	670.38	b_8-NH_3	945.54
b_7	834.47	MH-NH_3	1066.56
b_8	962.57	K	126.09

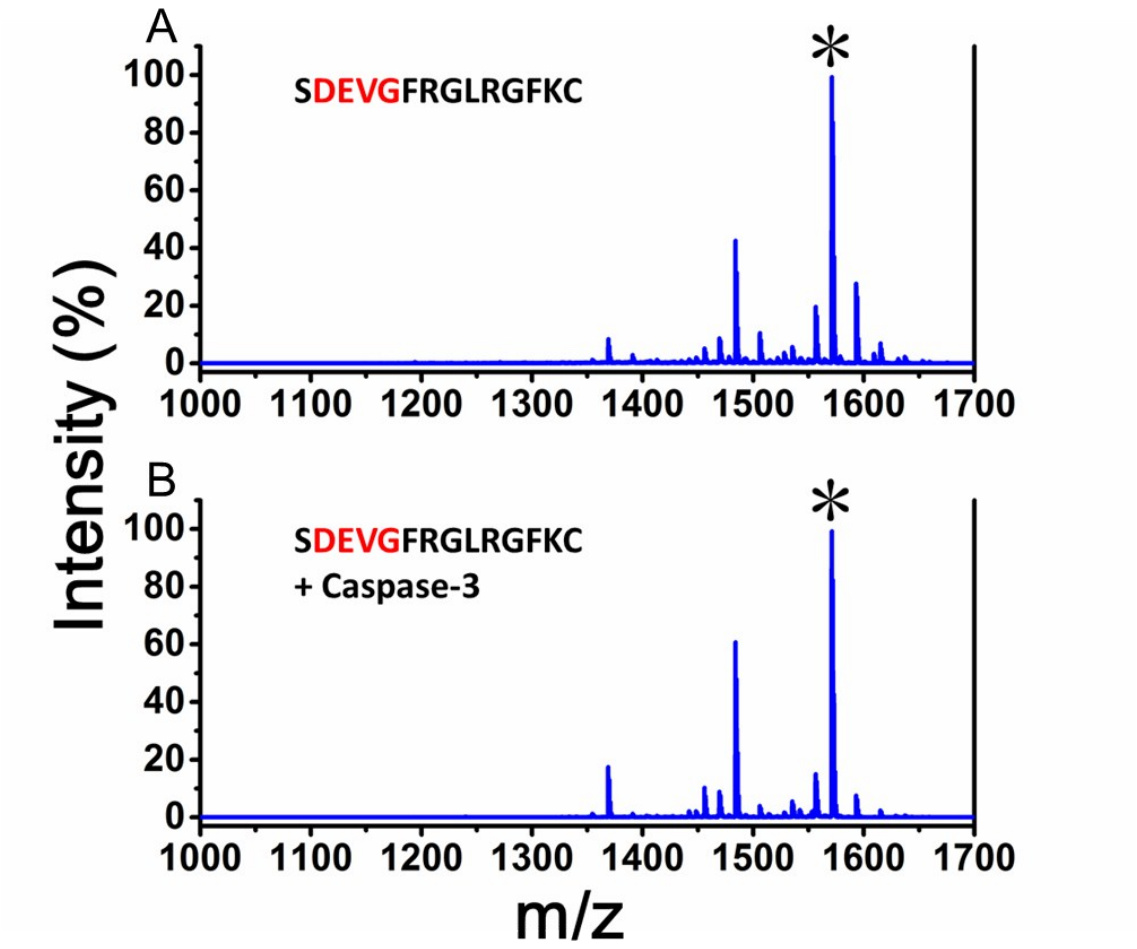

Figure S2. Mass spectra of control peptide P3 before (A) and after (B) incubation with 10 µL 2 µg/mL of caspase-3at 37 °C for 1 h. The result suggests that P3 cannot be cleaved by caspase-3.

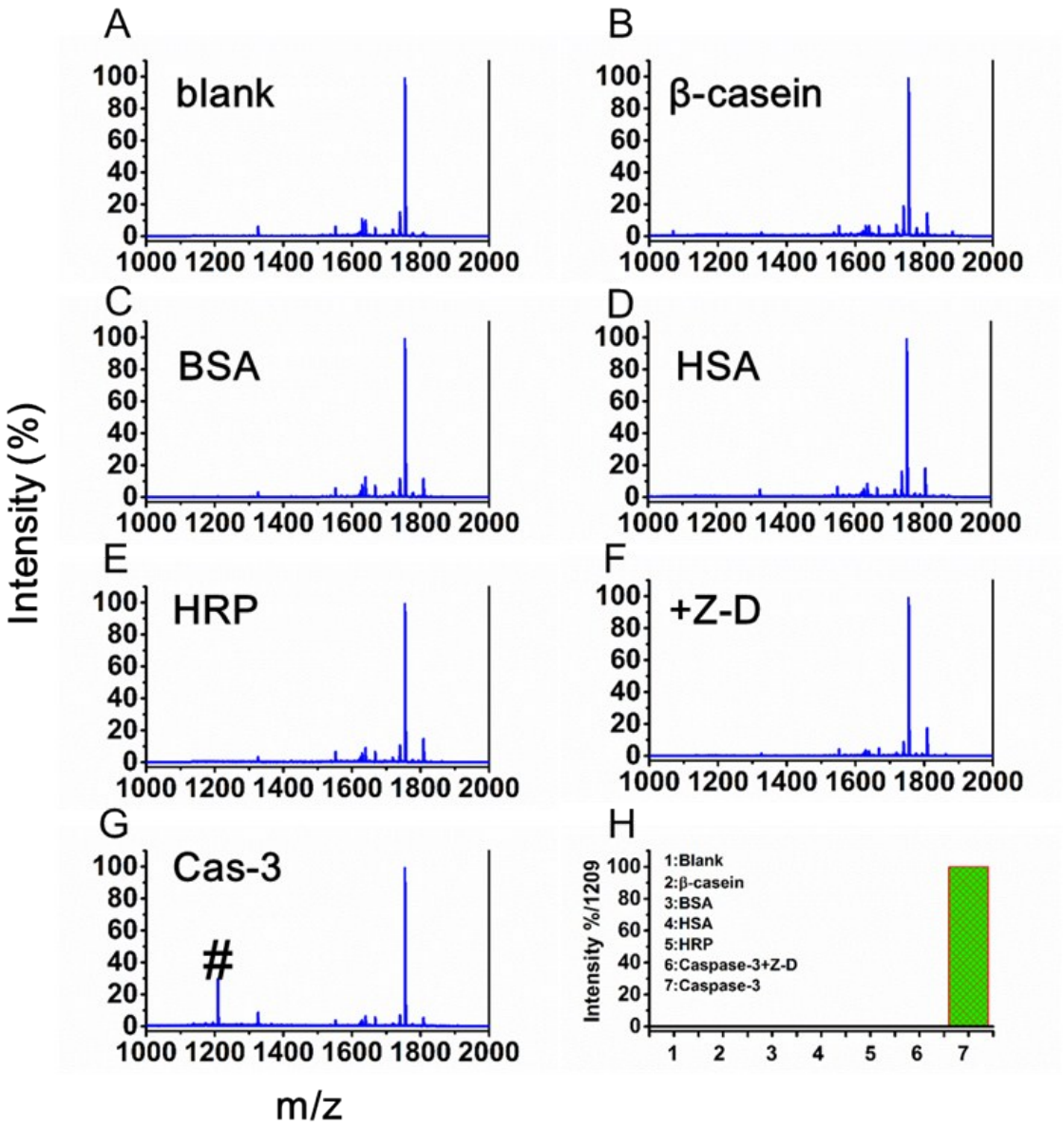

Figure S3. Mass spectra of 50 μM NEM-P1 incubated with 200 ng/ml different proteins or enzymes at 37 ^{o}C for 1 h .(A)blank, (B) β-casein, (C) BSA, (D) HSA, (E) HRP, (F) caspase-3 with inhibitor Z-DEVD-FMK, (G) caspase-3 alone. (H) Intensity of enzyme cleaved fragment at m/z of 1209 for different samples. The result suggests NEM-P1 can be specifically cleaved by caspase-3.

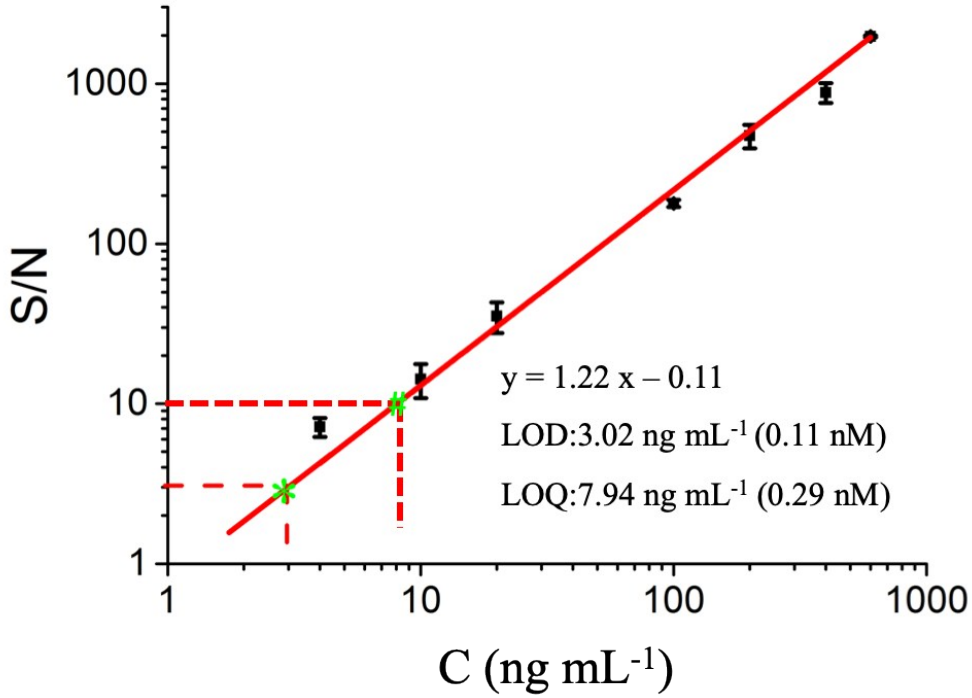

Figure S4. Limit of detection (LOD) and quantitation (LOQ) for quantitative detection of caspae-3 activity *in vitro*. The LOD was calculated by extrapolating the calibration curve till S/N = 3, and S/N=10 for LOQ. Error bar: standard deviation (n=3).

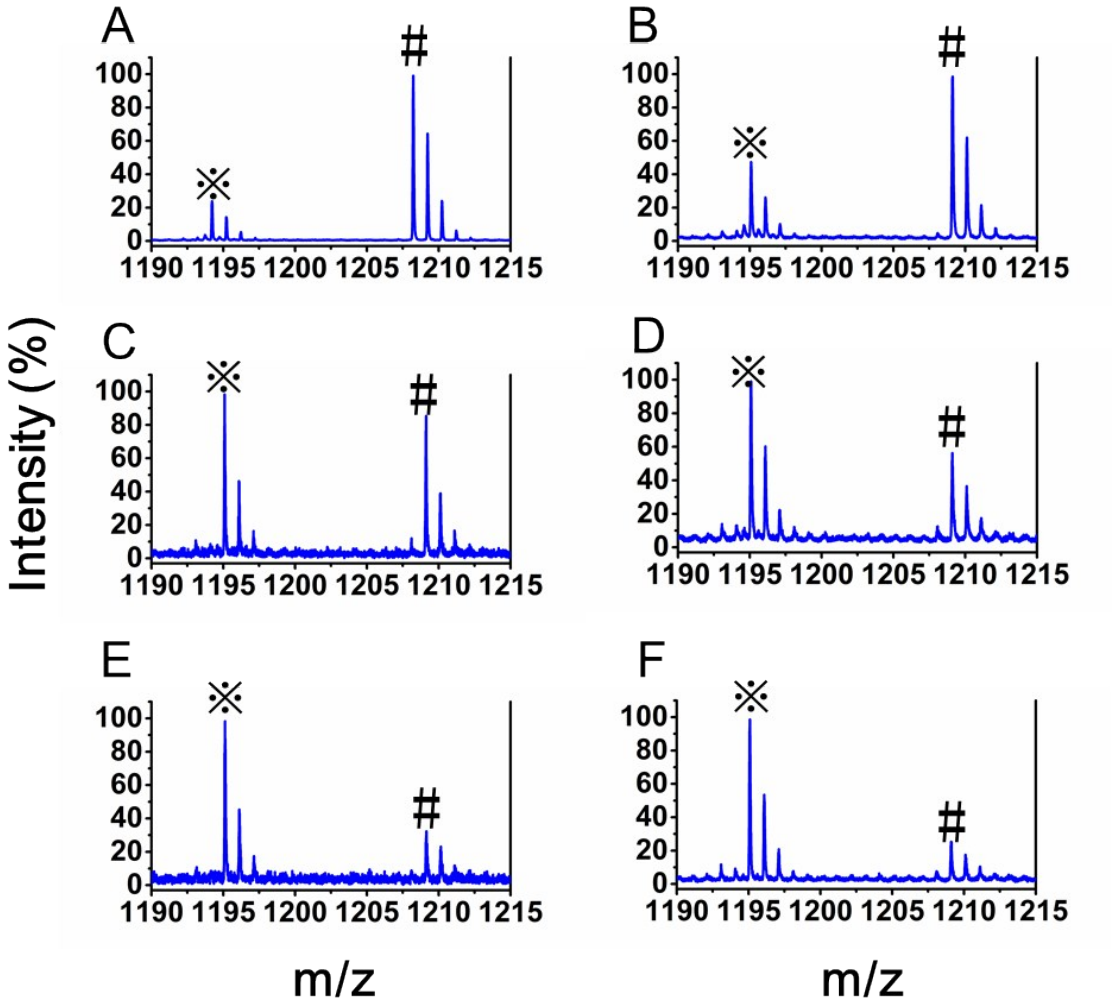

Figure S5. Mass spectra of 50 μM NEM-P1 incubated with 200 ng/mL caspase-3 and different concentrations of its inhibitor Emricasan at 37 °C for 120 min. The concentration from (A) to (F) is 0, 10, 50, 100, 500 and 10000 nM, respectively. ※ protonated internal standard NMM-P2 at m/z 1195, # protonated enzyme cleaved fragment from NEM-P1 at m/z 1209

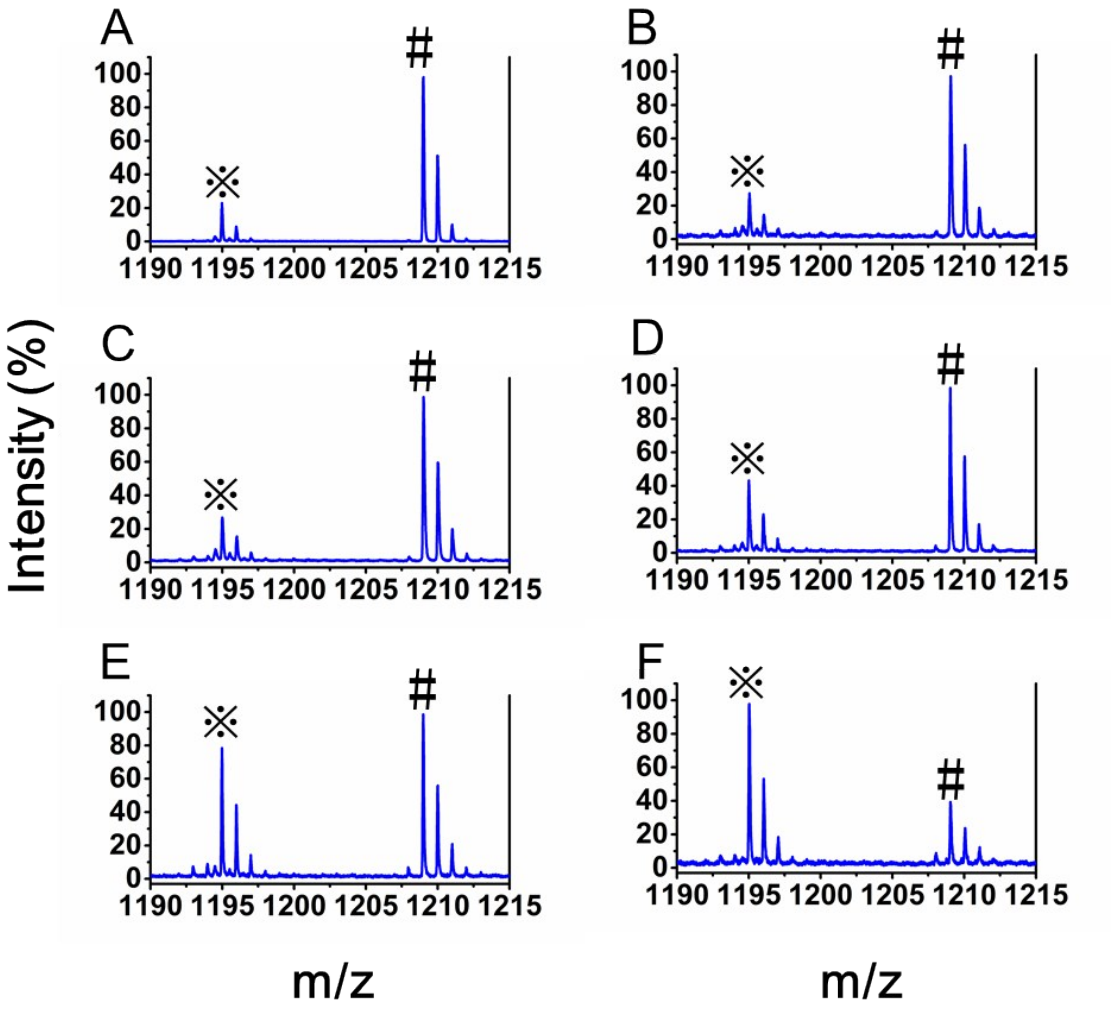

Figure S6. Mass spectra of 50 μM NEM-P1 incubated with 200 ng/mL caspase-3 and different concentrations of its inhibitor Z-DEVD-FMK at 37°C for 120 min. The concentration from (A) to (F) is 0, 10, 100, 500, 1000 and 10000 nM, respectively. ※ protonated internal standard NMM-P2 at m/z 1195, # protonated enzyme cleaved fragment from NEM-P1 at m/z 1209.

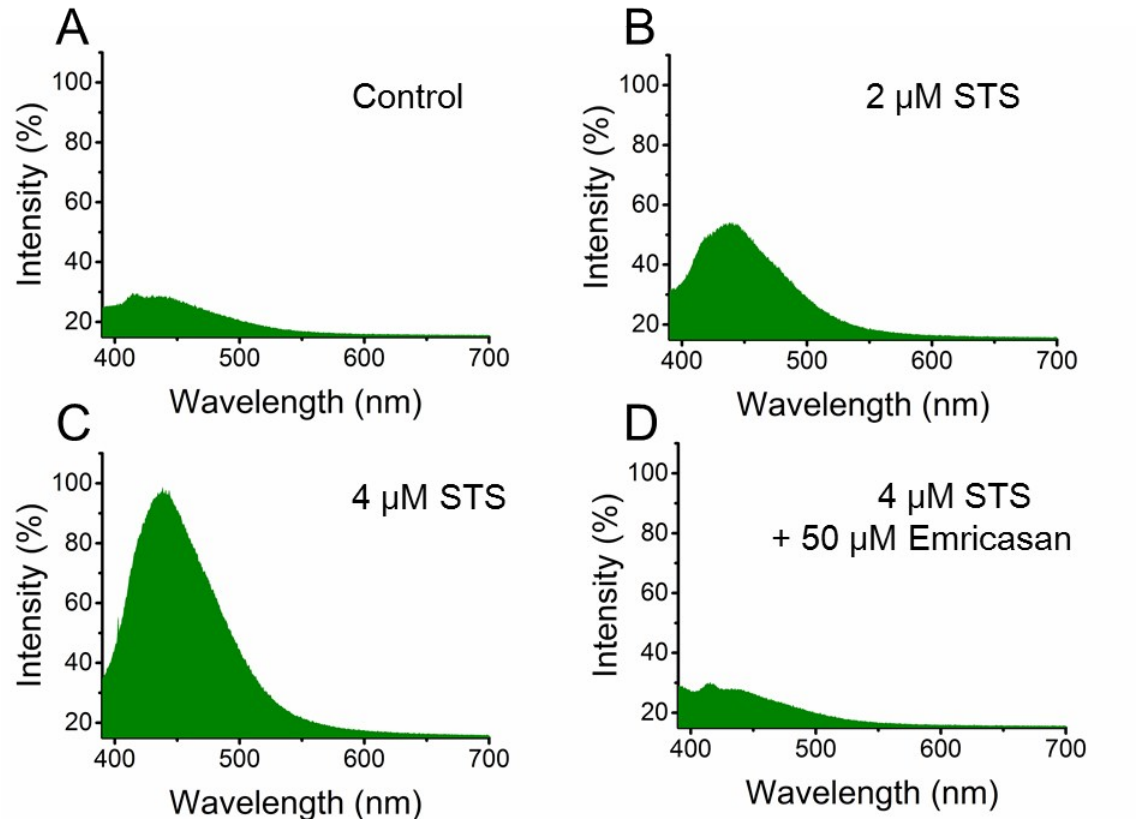

Figure S7. Fluorescence images of caspase-3 activity detection in PC12 cell lysates treated with (A) 0, (B) 2, (C) 4 μM STS, and (D) 4 μM STS and 50 μM inhibitor Emricasan. The cell density was 900,000 cells/dish, and the commercial fluorescence probe was 5 μM AC-DEVD-AMC.