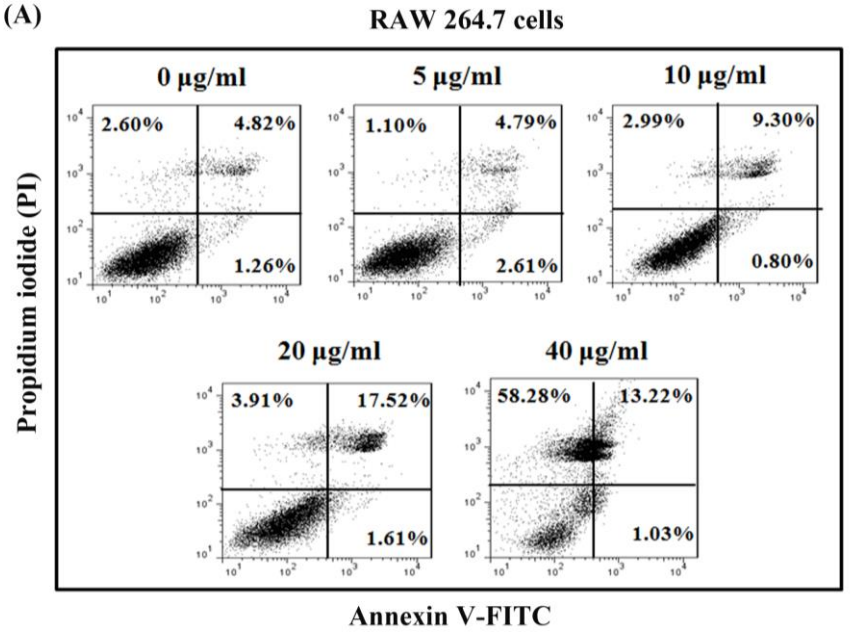
Supporting Information

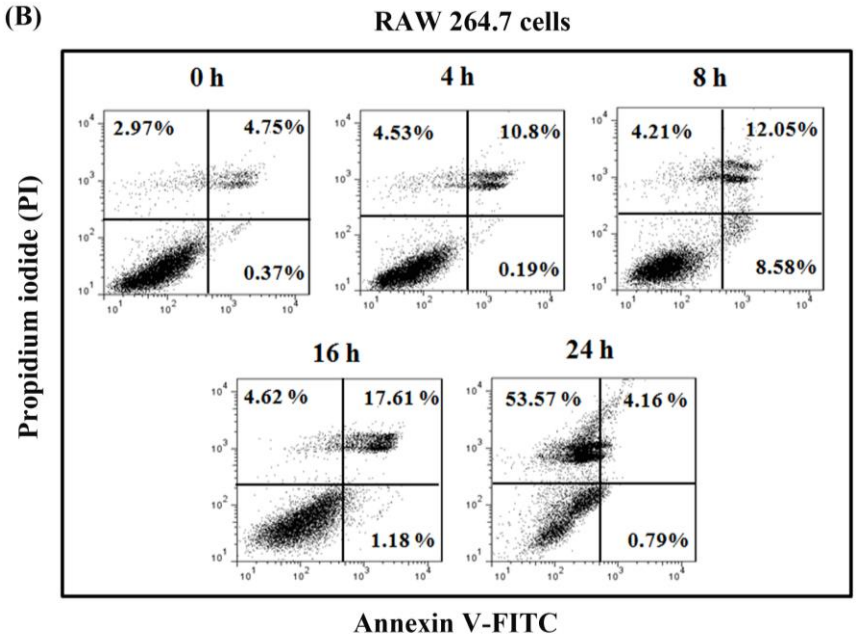
1. Experimental Section

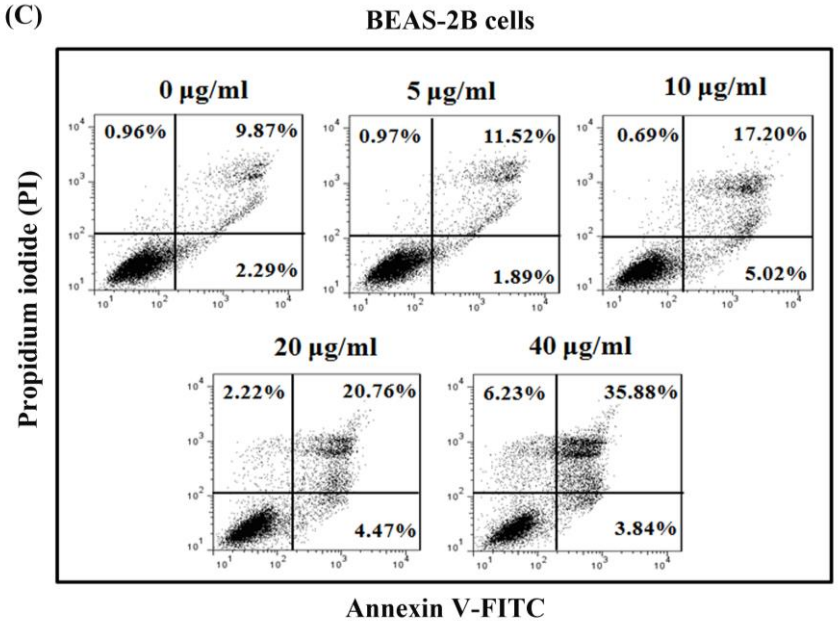
Annexin V and propidium iodide (PI) staining assay

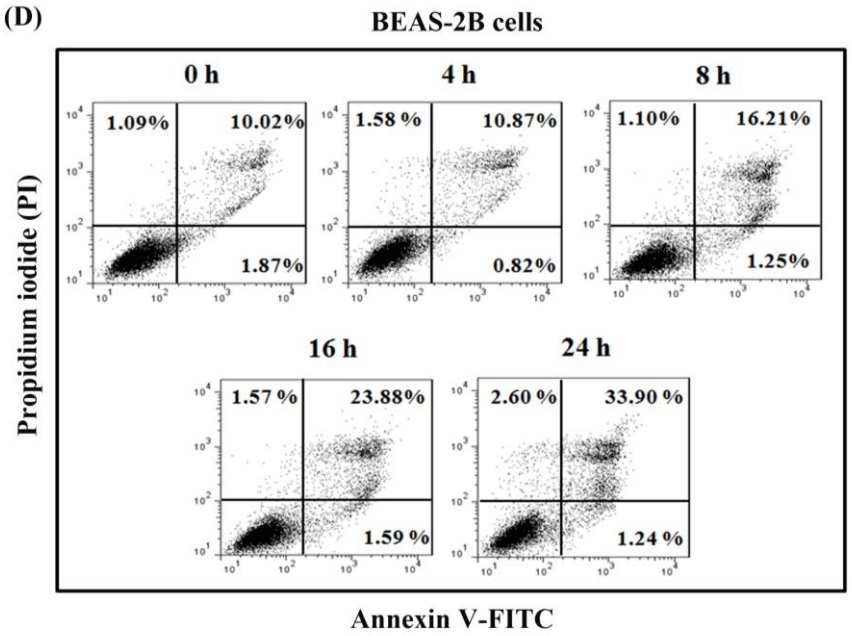
Cells were trypsinised, washed with PBS and centrifuged at 2000 rpm for 5 min. Cells were resuspended in 100 μl of 1×Annexin V-binding buffer (10 mM HEPES (pH 7.4), 0.14 M NaCl and 2.5 mM $CaCl_2$) that contained 2 μl of Annexin V-FITC (Calbiochem, CA, USA) alone or in combination with 10 μl of PI (50 μg/ml) and were incubated in the dark at room temperature for 15 min. The 1× binding buffer (400 μl) was added to stop the reaction, and the staining was analysed by FACScan flow cytometry (Becton Dickinson, USA). Cells were pretreated with the pan-caspase inhibitor, Z-V-A-D(OMe)-FMK (R&D systems, MN, USA) for 1 h before NH_2-PS treatment.

2. Supplementary Results


(A)
RAW 264.7 cells
0 µg/ml
5 µg/ml
10 µg/ml
20 µg/ml
40 µg/ml
2.60%
4.82%
1.26%
1.10%
4.79%
2.61%
2.99%
9.30%
0.80%
3.91%
17.52%
1.61%
58.28%
13.22%
1.03%
Propidium iodide (PI)
Annexin V-FITC


(B)
RAW 264.7 cells
0 h
4 h
8 h
16 h
24 h
2.97%
4.75%
0.37%
4.53%
10.8%
0.19%
4.21%
12.05%
8.58%
4.62 %
17.61 %
1.18 %
53.57 %
4.16 %
0.79%
Propidium iodide (PI)
Annexin V-FITC


(C)
BEAS-2B cells
0 µg/ml
5 µg/ml
10 µg/ml
0.96%
9.87%
2.29%
0.97%
11.52%
1.89%
0.69%
17.20%
5.02%
20 µg/ml
40 µg/ml
2.22%
20.76%
4.47%
6.23%
35.88%
3.84%
Propidium iodide (PI)
Annexin V-FITC


(D)
BEAS-2B cells
0 h
4 h
8 h
1.09%
10.02%
1.87%
1.58 %
10.87%
0.82%
1.10%
16.21%
1.25%
16 h
24 h
1.57 %
23.88%
1.59 %
2.60 %
33.90 %
1.24 %
Propidium iodide (PI)
Annexin V-FITC

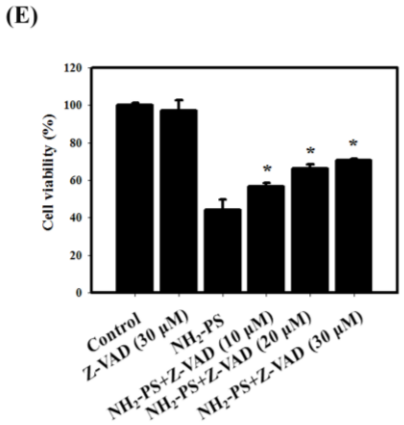


Figure S1.

Annexin V/PI staining in RAW 264.7 and BEAS-2B cells treated with NH_2-PS. The induction of apoptosis and necrosis was determined by flow cytometric analysis of Annexin V and PI-staining. Cells in the lower right quadrant indicate Annexin V-positive, early apoptotic cells. The cells in the upper right quadrant indicate Annexin-positive/PI-positive, late apoptotic cells or necrosis. Cells in the lower left quadrant indicate Annexin-negative/PI-negative, viable cells. The cells in the upper left quadrant indicate Annexin-negative/PI-positive, necrosis. (A) (C) RAW and BEAS-2B cells treated with different concentrations of NH_2-PS were assessed using Annexin V/PI staining. Cells were incubated with 0-40 μg/ml NH_2-PS for 16 hrs. (B) (D) RAW and BEAS-2B cells treated with different time of NH_2-PS were assessed using Annexin V/PI staining. Cells were incubated with 20 μg/ml NH_2-PS for 0-24 hrs. (E) Cytotoxic effects in the absence or presence of Z-VAD in BEAS-2B cells. The cells were pretreated with Z-VAD for 1 hr before NH_2-PS treatment for 16 hrs. *,

p<0.05, NH_2-PS versus NH_2-PS+ Z-VAD.

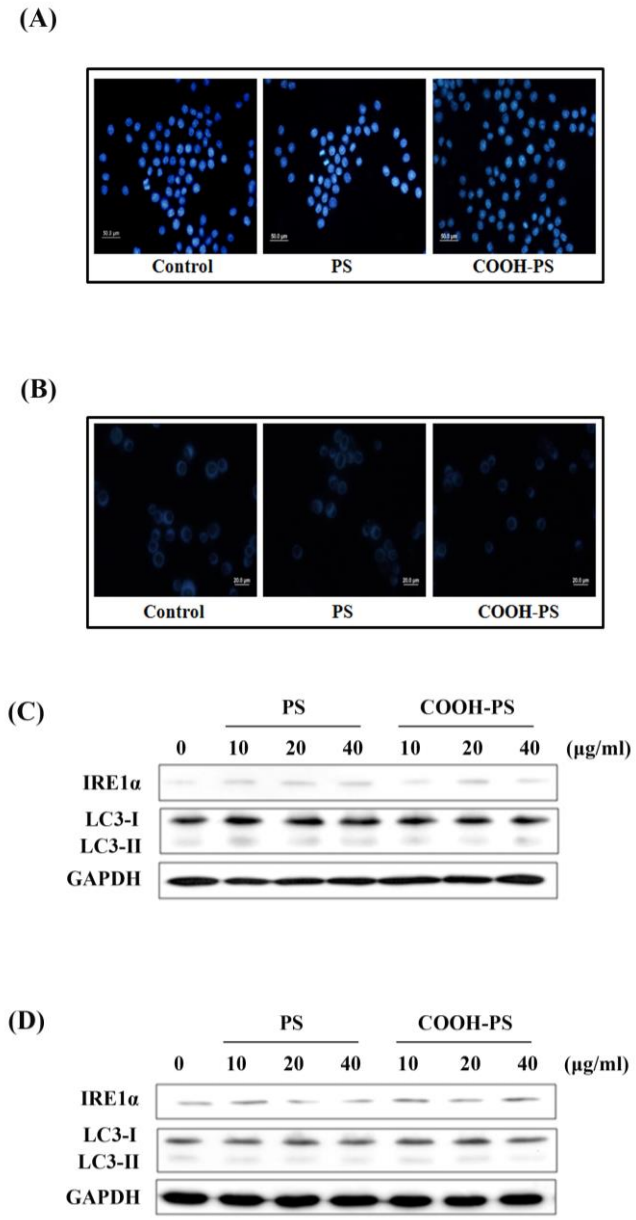


Figure S2.

(A) The cells were treated with PS and COOH-PS at 20 μg/ml for 16 hrs and stained with Hoechst 33342 and then with a ProteoStat Aggresome Detection Kit in RAW 264.7 cells. The red color and the blue color indicate the fluorescence of detected

aggregates and stained nuclei, respectively. (B) The cells were treated with PS and COOH-PS at 20 μg/ml for 16 hrs and were treated with ER Tracker Blue-White DPX probe for ER staining in RAW 264.7 cells. Western blotting for IRE1α, LC3-I and LC3-II in RAW 264.7 (C) and BEAS-2B (D) cells. The cells were treated with 0-40 μg/ml PS or COOH-PS for 16 hrs.

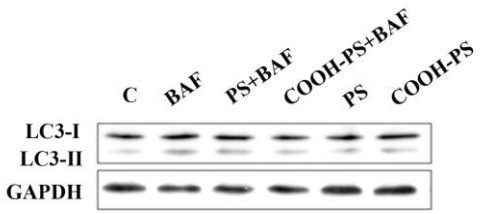


Figure S3.

Autophagic flux was determined by western blotting with an anti-LC3 antibody in BEAS-2B cells. The cells were pretreated with BAF (10 nM) for 1 hr before NH_2-PS treatment (20 μg/ml) for 16 hrs.

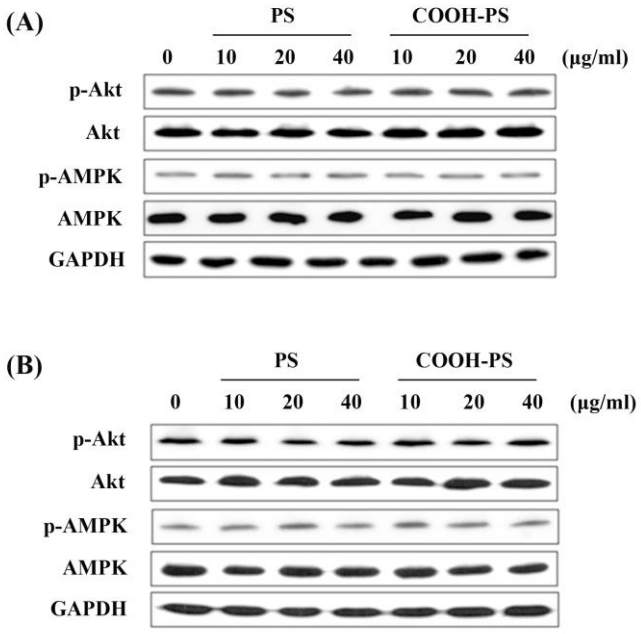


Figure S4.

Western blotting for Akt phosphorylation, AMPK phosphorylation, Akt and AMPK in RAW 264.7 (A) and BEAS-2B (B) cells. The cells were treated with 0-40 μg/ml PS or COOH-PS for 16 hrs.