

Supplementary materials and methods, Supplementary Figure Legends and Figures, and the corresponding full-length originals of western blot analysis for “Ultrafine silicon dioxide nanoparticles cause lung epithelial cells apoptosis via oxidative stress-activated PI3K/Akt-mediated mitochondria- and endoplasmic reticulum stress-dependent signaling pathways”

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Supplementary materials and methods

Animals. To establish the SiO₂NPs exposed mouse model, four-week-old C57BL/6 male mice was obtained from BioLASCO Taiwan Co., Ltd (Taipei, Taiwan). All protocols used were approved by the Institutional Animal Care and Use Committee (IACUC), and the care and use of laboratory animals conducted in accordance with the guidelines of the Animal Research Committee of China Medical University, Taiwan. Mice was housed in a room at a constant temperature of $22 \pm 2^{\circ}\text{C}$ with a 12 h light-dark cycle. The mice were randomly assigned to pretreatment groups, weighed, and administered with the indicated drugs or vehicle. The mice were randomly distributed into four groups: (a) vehicle control, (b) SiO₂NPs 6.2 mg/kg, (c) SiO₂NPs 12.5 mg/kg, (b) SiO₂NPs 31 mg/kg. Mice placed under deep anesthetized by intraperitoneal injection of zoletil 50 (40 mg/kg). The SiO₂NPs was diluted by PBS, and 50 μL of mixture exposed to mice via intranasal route. The mortality rate of mice was observed after treated with SiO₂NPs for 1 to 8 days.

Wet -to -dry weight ratio. At day 8 of the experiment completion, all lungs were dissected free of nonpulmonary tissue and weighed and then dried to a constant weight at 60°C . Wet-to-dry (W/D) ratios will be obtained by dividing the wet weight by the final dried weight⁵³.

Lipid peroxidation assay. Lung tissues were harvested from mice under zoletil 50 anesthesia (40 mg/kg i.p.). All samples were homogenized and centrifuged at 3000 g for 10 min at 4°C . The cell lysate were homogenized and centrifuged at 1000 rpm for 20 min at 4°C .

Collecting the supernatant and assays were carried out immediately using the lipid peroxidation assay kit (Calbiochem). Absorbance at 586 nm for malondialdehyde(MDA) and 405 nm for MPO was measured using an ELISA microplate reader⁵⁴.

Histological evaluation.The lung tissue was graded by Animal Disease Diagnostic Center College of Veterinary Medicine, National Chung Hsing University, Taiwan. The alteration of edema, hemorrhage, leukocyte infiltrate and necrosis in lung tissue was observed by hematoxylin and eosin (H&E) staining. Histological changes will be scored by counting the frequency of foci per field observed at 40X, using a 0-to 4-point scale with injury in 0, 25, 50, 75 or 100% of the investigated tissue. The scoring scale is as follows: 0 (absent), 1 (mild), 2 (moderate), 3 (severe) and 4 (overwhelming).

Figure legend of Supplementary Data

Supplementary Figure 1. Effects of SiO₂NPs on cells viability and caspase-3 activity in A549 human alveolar epithelial cells. (A) Cells were treated with SiO₂NPs (0 to 1000 µg/mL) for 24 hours. The cell viability was determined by MTT assay. (B) Cells were pretreated with NAC (1 mM) or LY294002 (2.5 µM) for 1 hour, and then treated with SiO₂NPs (400 µg/mL) for 48 hours. Caspase 3 activity was detected by Caspase 3 activity assay kit as described in the Materials and Methods. All data are presented as the means ± S.D. of four independent experiments with triplicate determination. * $p < 0.05$ as compared to vehicle control. # $p < 0.05$ as compared to SiO₂NPs groups. Con: control.

Supplementary Figure 2. Effects of SiO₂NPs on ROS generation and protein expression of phospho-AKT, cleaved caspase-3 in A549 human alveolar epithelial cells. Cells were pretreated with NAC (1 mM) or LY294002 (2.5 µM) for 1h, and then treated with SiO₂NPs (400 µg/mL) for 1.5 hours. (A) The intracellular ROS generation was monitored by flow cytometry using peroxide-sensitive fluorescent probe (2,7 -dichlorofluorescein diacetate; DCFH-DA). (B) The protein expression of phospho-AKT was determined by Western blot analysis. Data in (A), are presented as the means ± S.D. of four independent experiments with triplicate determination. * $p < 0.05$ as compared to vehicle control. Data in (B), are representative of three independent experiments performed in triplicate.

Supplementary Figure 3. The mortality rate of mice after SiO₂NPs treatment. C57BL/6 male mice were instilled via intranasal route with PBS, and 6.2 mg/kg, 12.5 mg/kg, 31 mg/kg of SiO₂NPs. (A) The mortality rate was determined after SiO₂NPs treatment for 1 to 8 days. (B) The wet -to -dry weight ratio was determined after SiO₂NPs treatment at day 8. (C) The alteration of MDA levels were determined after SiO₂NPs treatment at day 8. All data are presented as the means $\pm$ S.D.; n = 16 for all groups. * $p < 0.05$ as compared to vehicle control. Con: control.

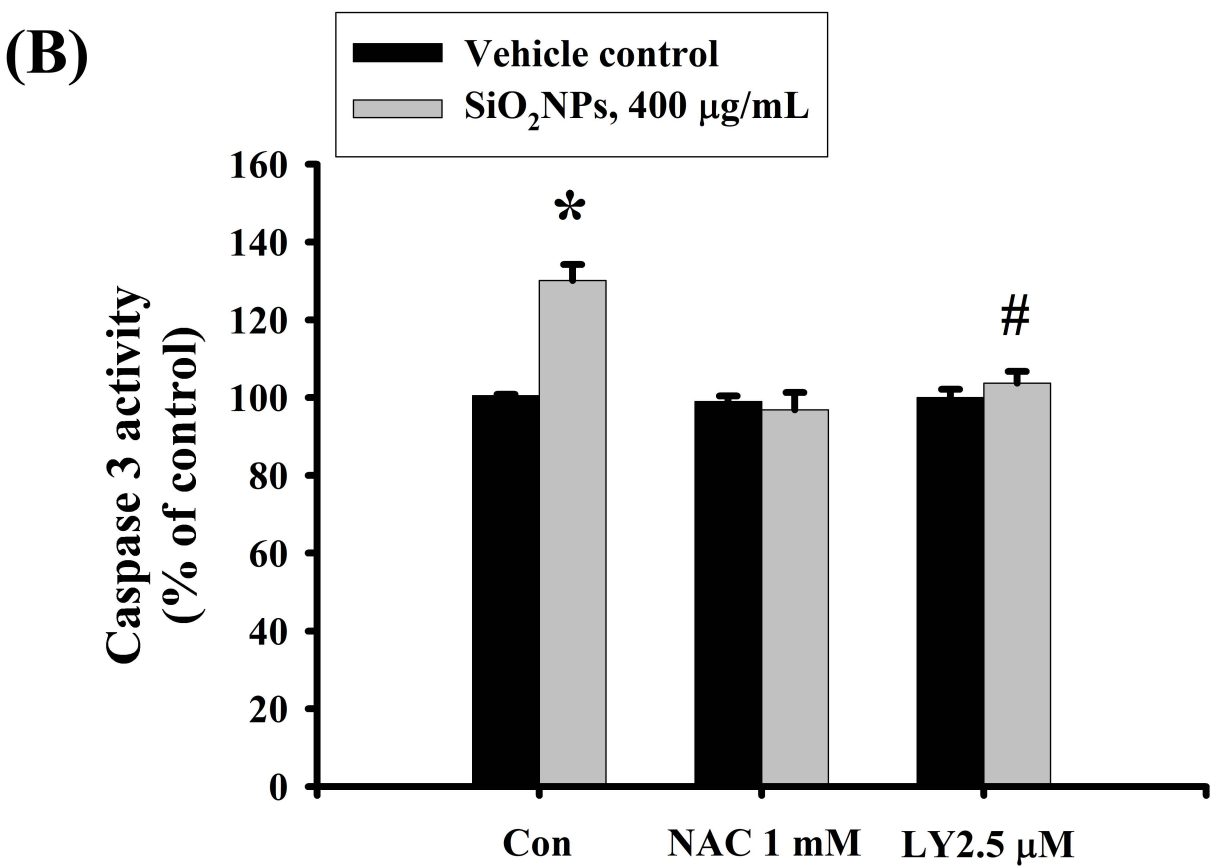
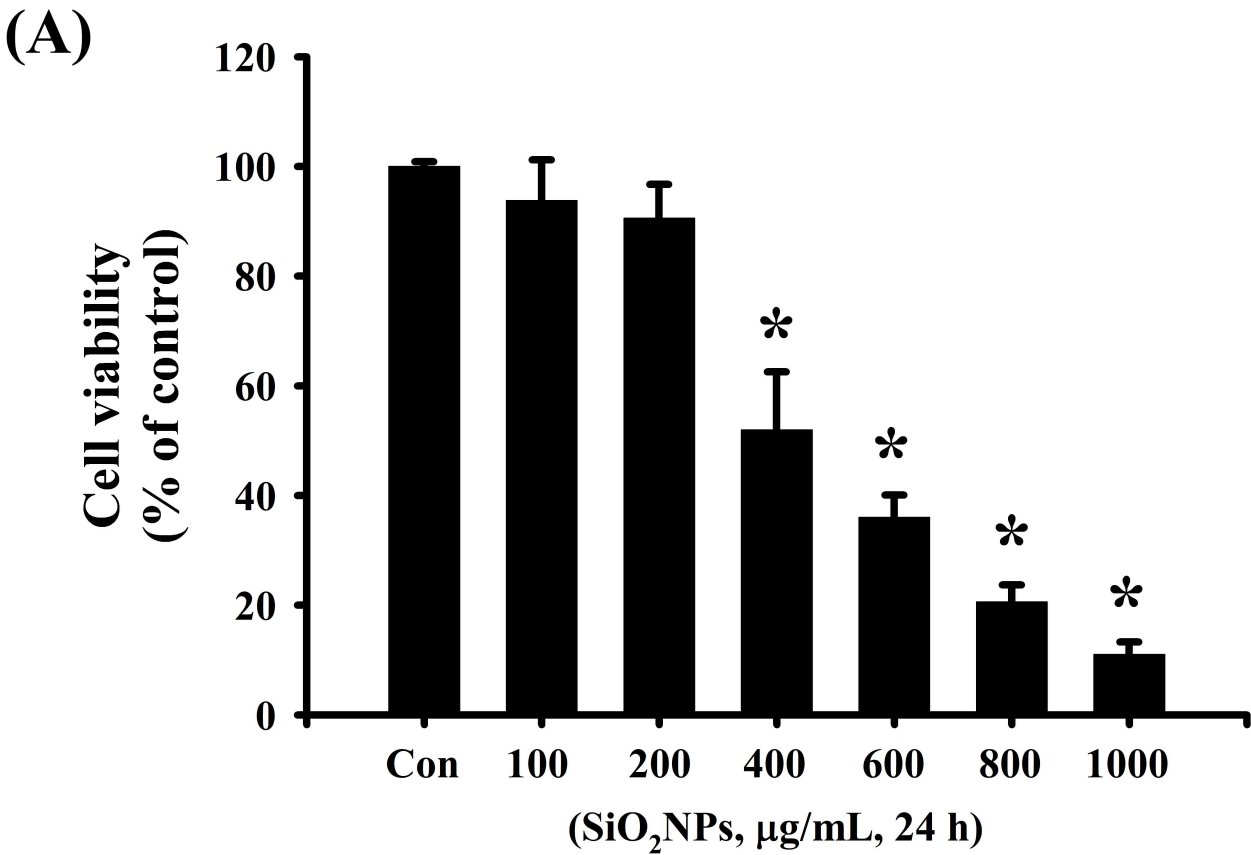
Supplementary Figure 4. Histopathology of lung tissues (H&E staining) after SiO₂NPs treatment at day 8 in mice. C57BL/6 male mice were instilled via intranasal route with PBS, and 6.2 mg/kg, 12.5 mg/kg, 31 mg/kg of SiO₂NPs. (A) 20x, (B) 100x, (C) 400x, show lung tissue of control, and 12.5 mg/kg, 31 mg/kg of SiO₂NPs group. Lung tissue 12.5 mg/kg, 31 mg/kg of SiO₂NPs group shows marked inflammation and focal hemorrhage in terminal bronchial (arrows).

Supplementary Figure 5. The mRNA expression of lung tissues in mice after SiO₂NPs treatment. C57BL/6 male mice were instilled via intranasal route with PBS, and 6.2 mg/kg, 12.5 mg/kg, 31 mg/kg of SiO₂NPs. The lung tissues were collected at day 8 and the mRNA

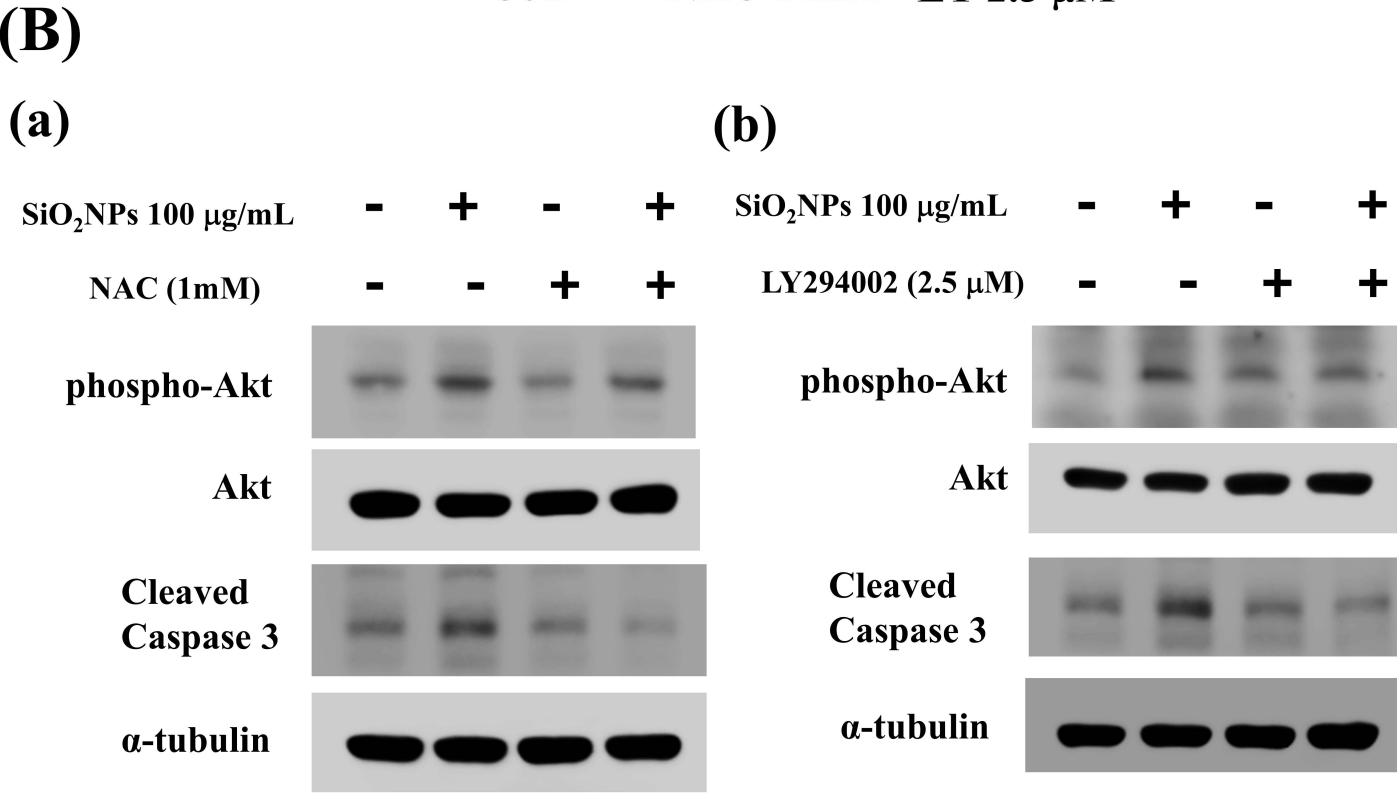
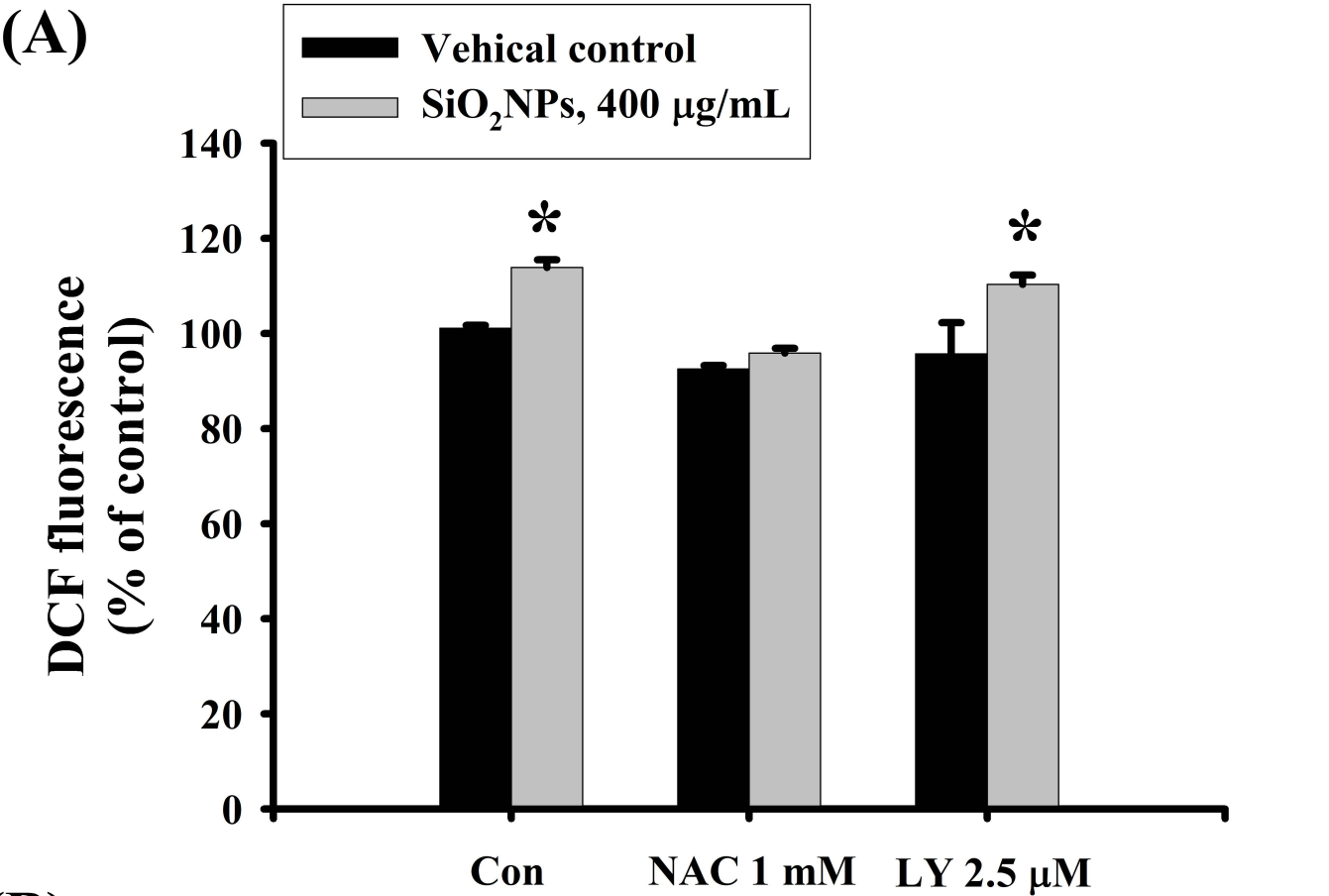
expression of caspase-3 (A), caspase-7 (B), and caspase-9 (C) was determined by quantitative real-time polymerase chain reaction (qPCR) analysis. All data are presented as the means $\pm$ S.D.; n = 16 for all groups. * $P < 0.05$ as compared to the vehicle control group. Con: control.

Supplementary Figure 6. The mRNA expression of lung tissues in mice after SiO₂NPs treatment. C57BL/6 male mice were instilled via intranasal route with PBS, and 6.2 mg/kg, 12.5 mg/kg, 31 mg/kg of SiO₂NPs. The lung tissues were collected at day 8 and the mRNA expression of CHOP (A), XBP-1 (B), Grp78 (C), Grp94 (D), and capase-12 (E) was determined by quantitative real-time polymerase chain reaction (qPCR) analysis. All data are presented as the means $\pm$ S.D.; n = 16 for all groups. * $P < 0.05$ as compared to the vehicle control group. Con: control.

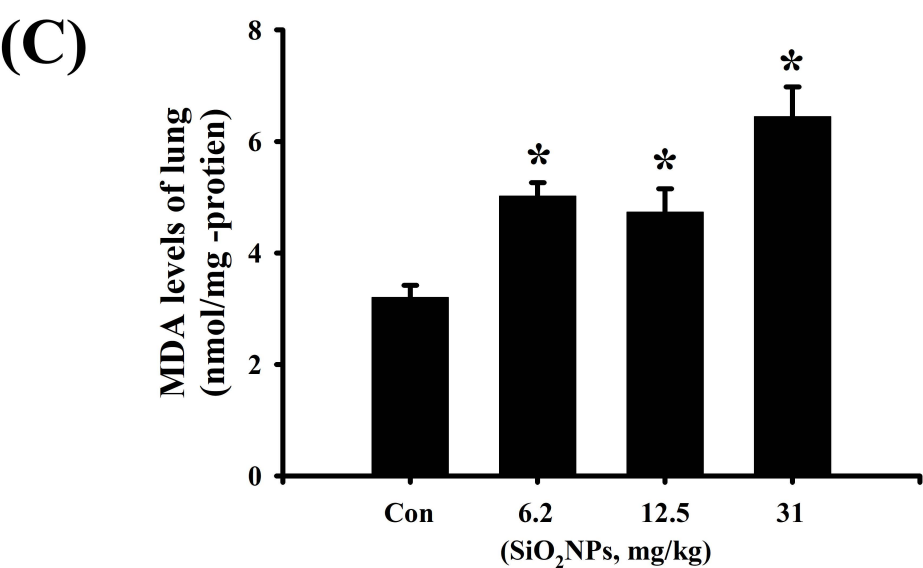
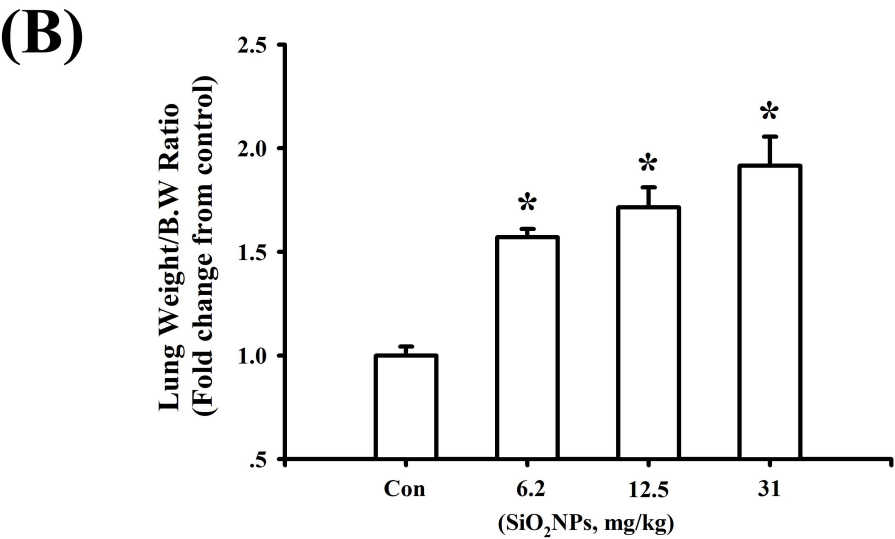
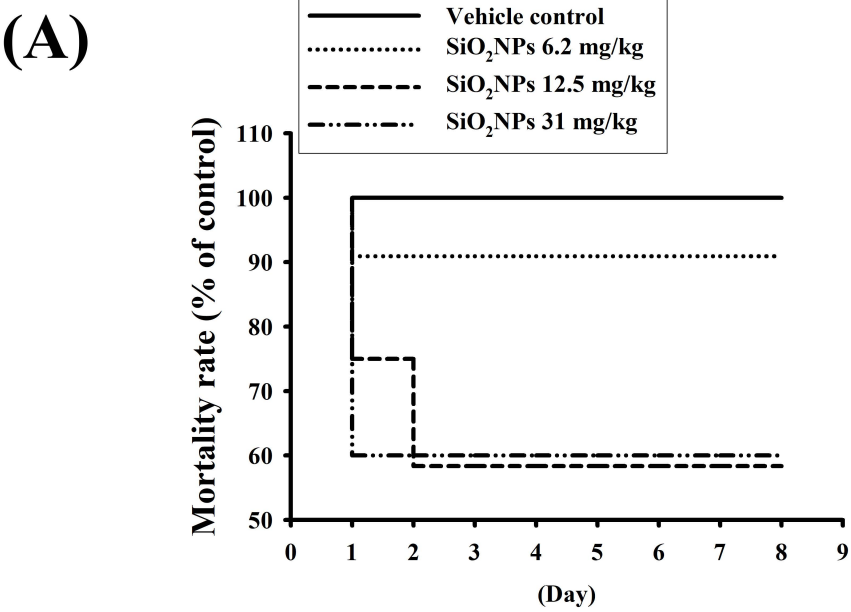
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

Control

12.5 mg/kg

31 mg/kg

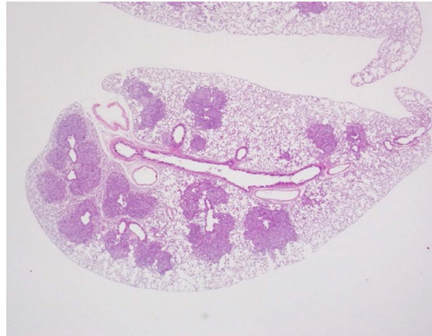
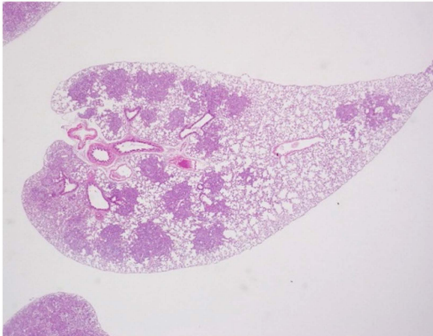
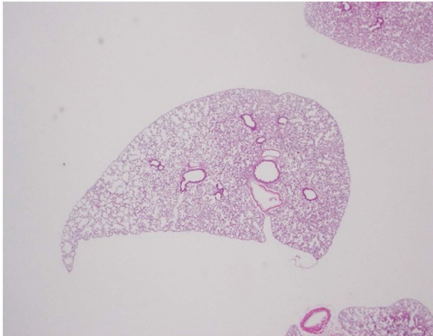
(A)

(20X)

(a)

(b)

(c)



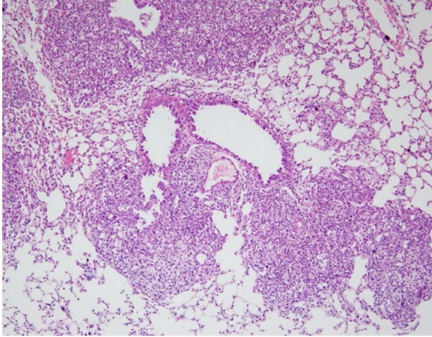
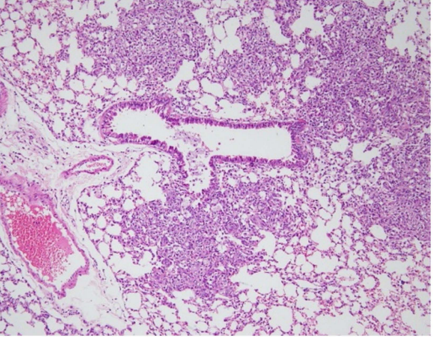
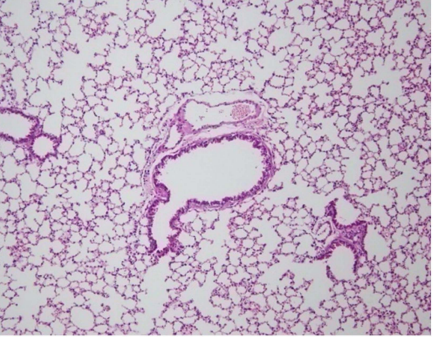
(B)

(100X)

(d)

(e)

(f)



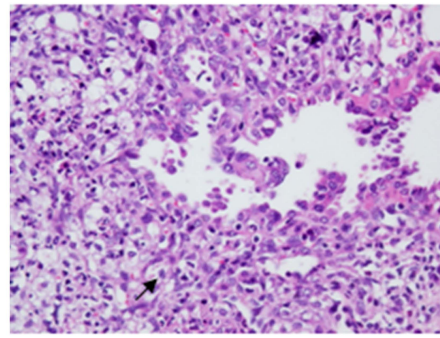
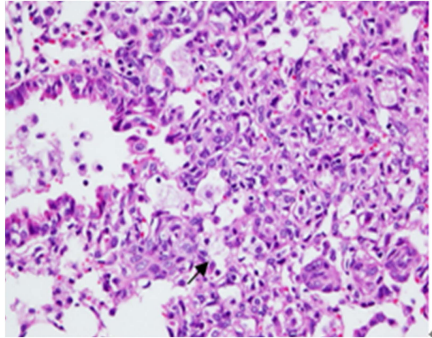
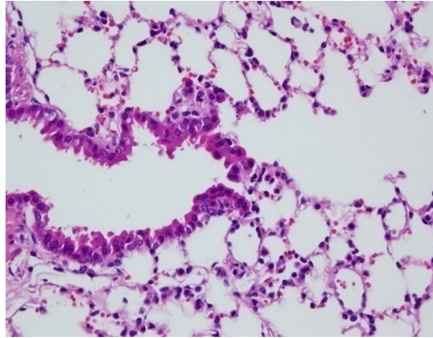
(C)

(400X)

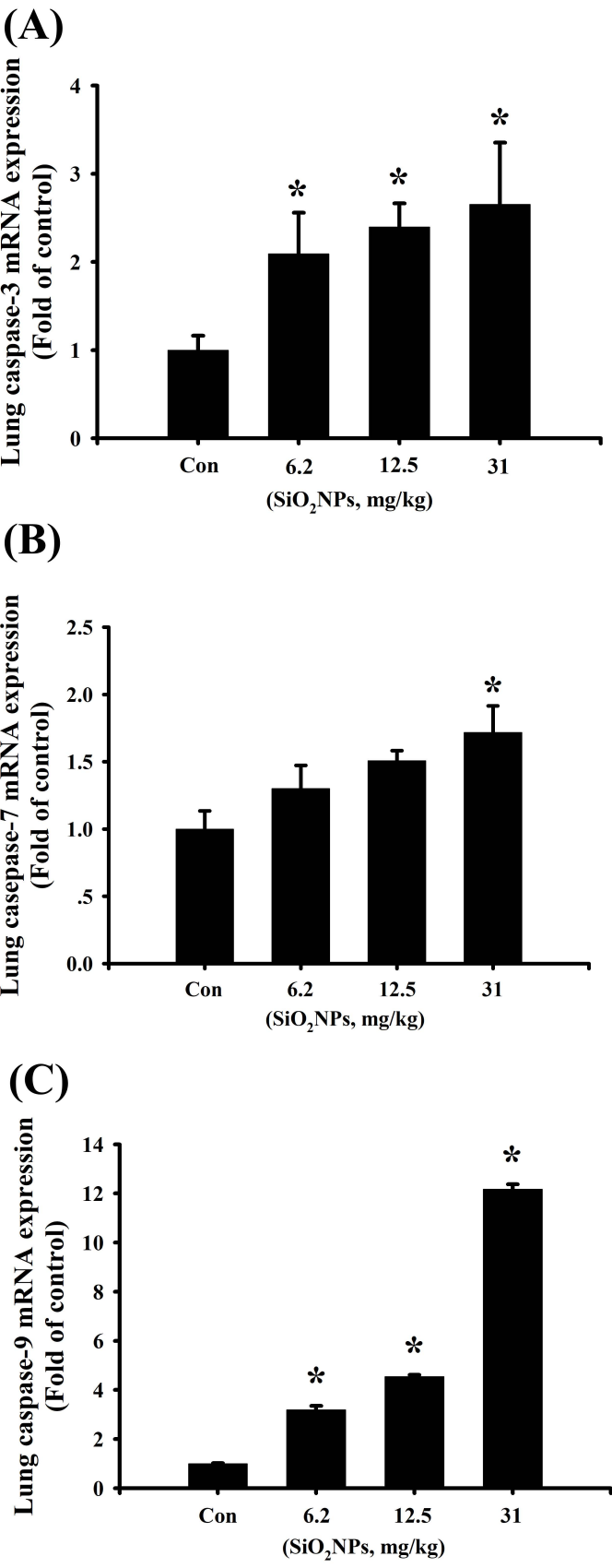
(g)

(h)

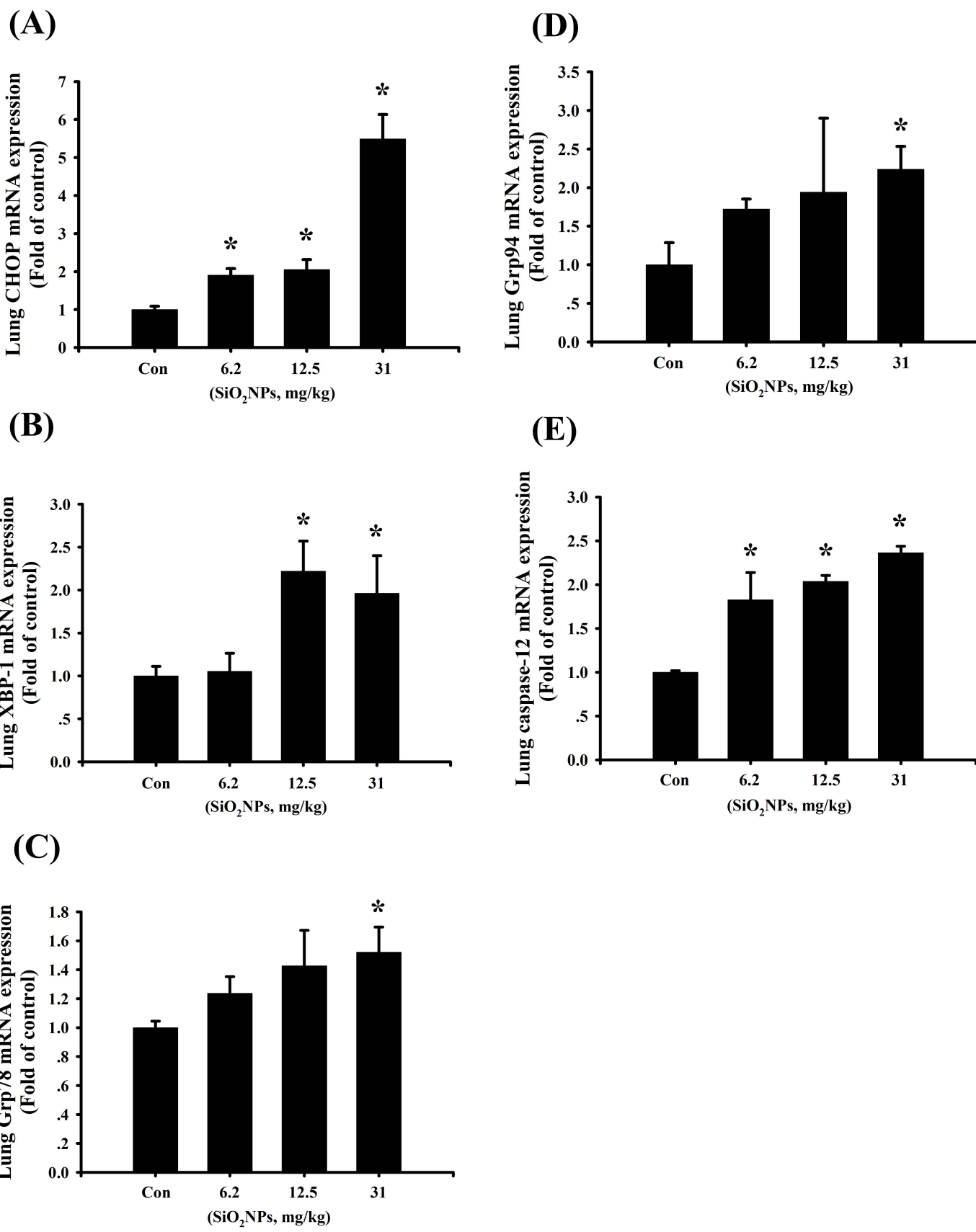
(i)



Supplementary Figure 5

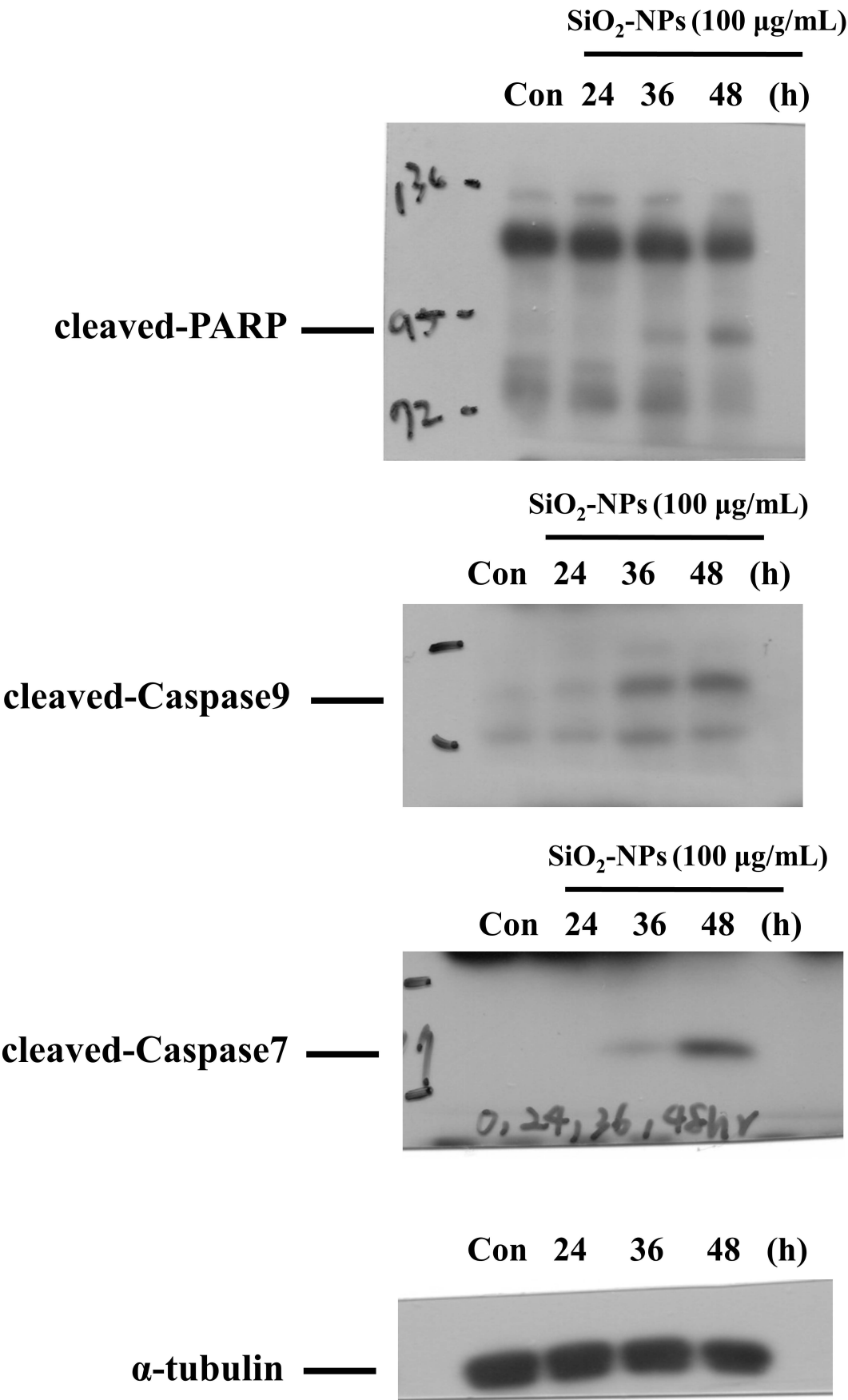


Supplementary Figure 6

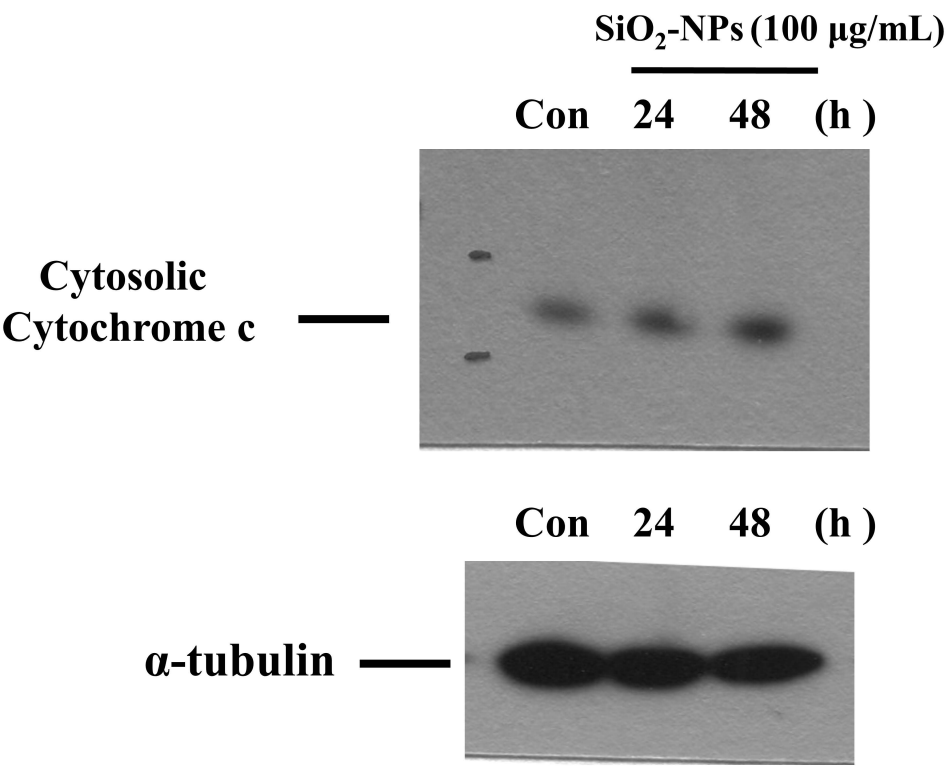


**The corresponding full-length originals
of
Western blot analysis**

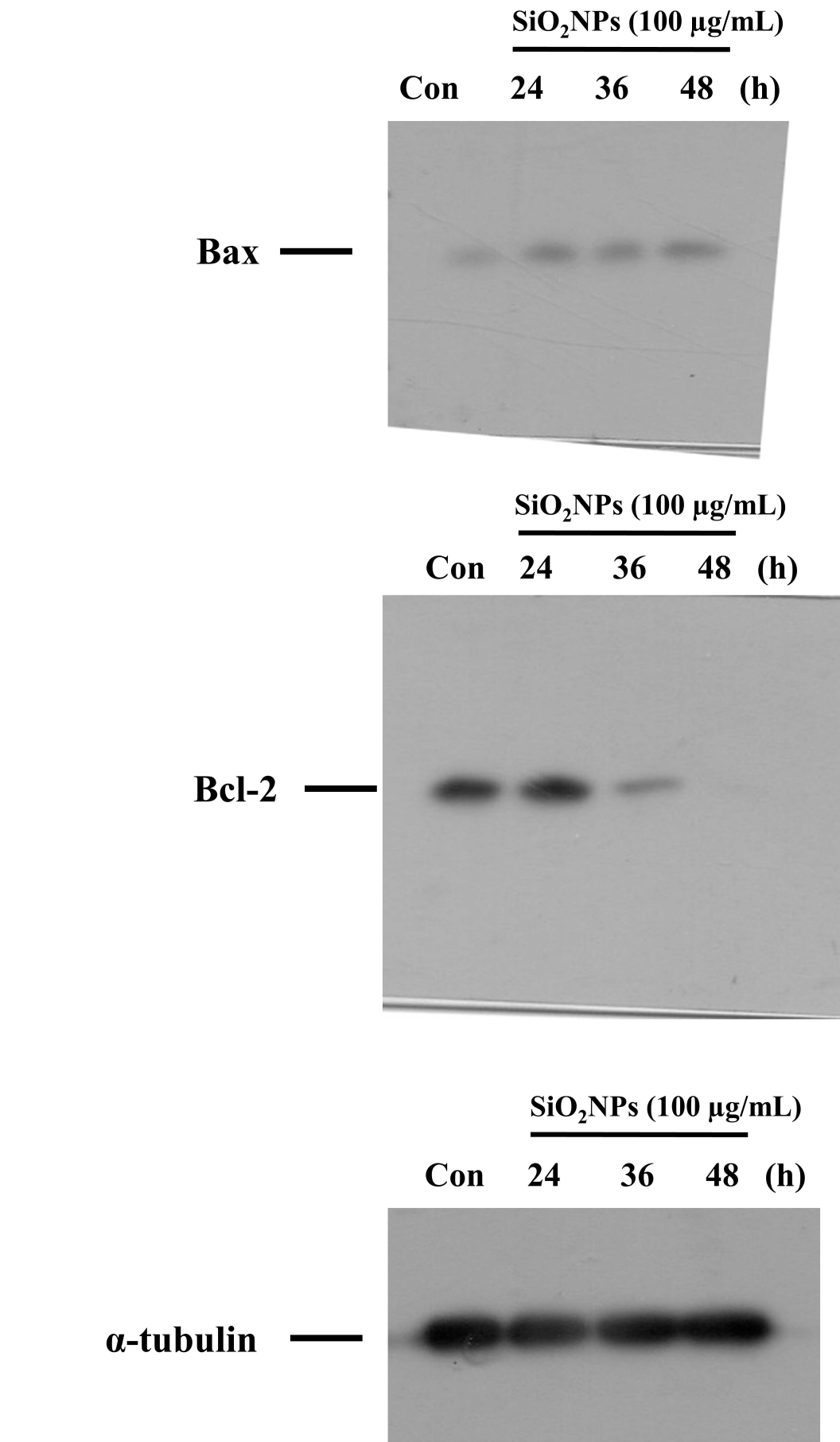
Full-length originals of western blot image for Figure 2C



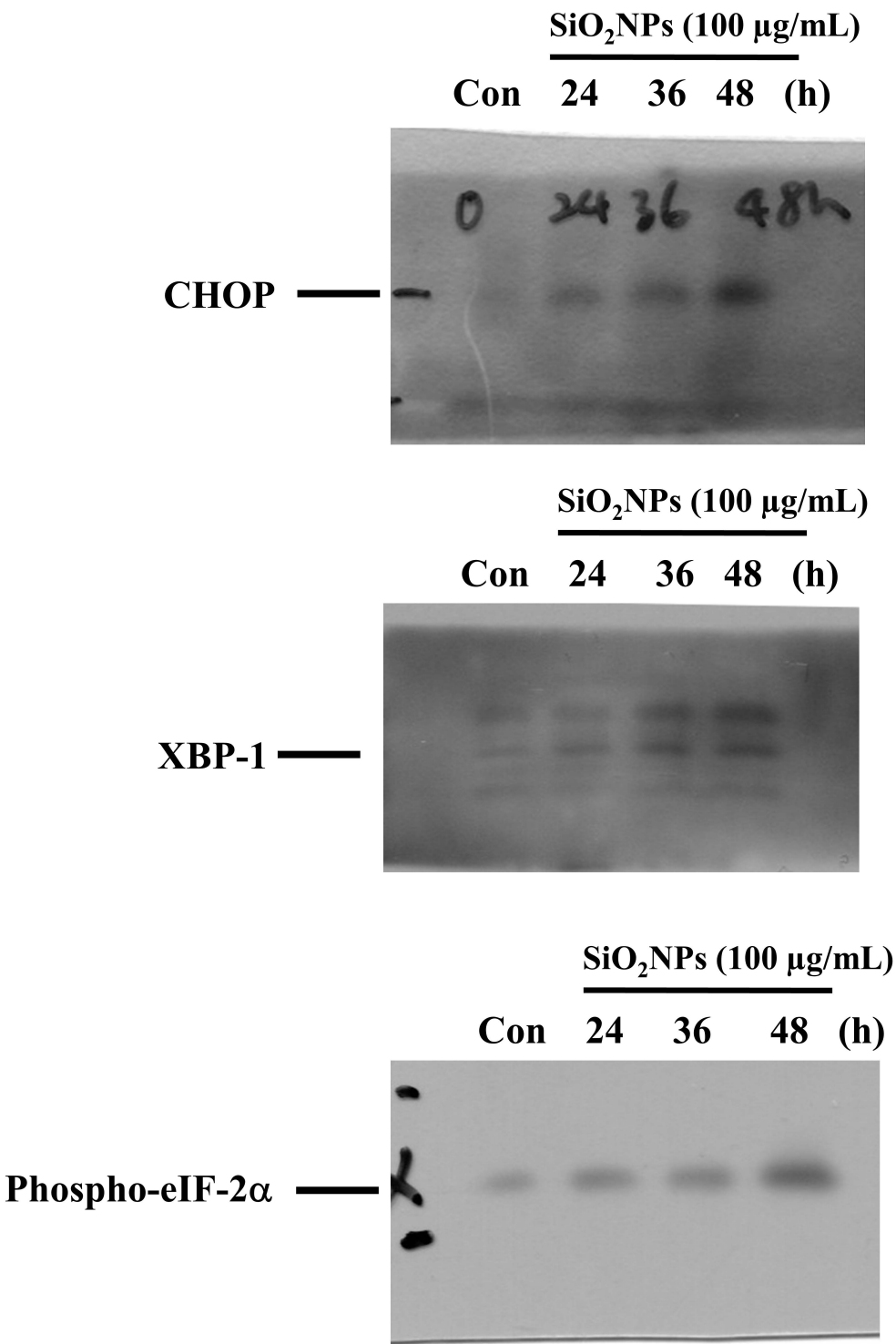
Full-length originals of western blot image for Figure 3C



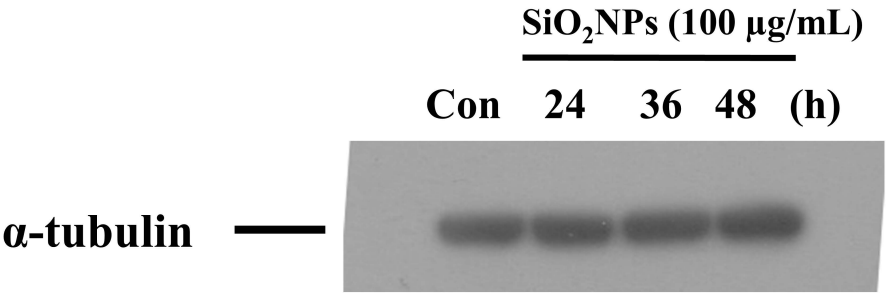
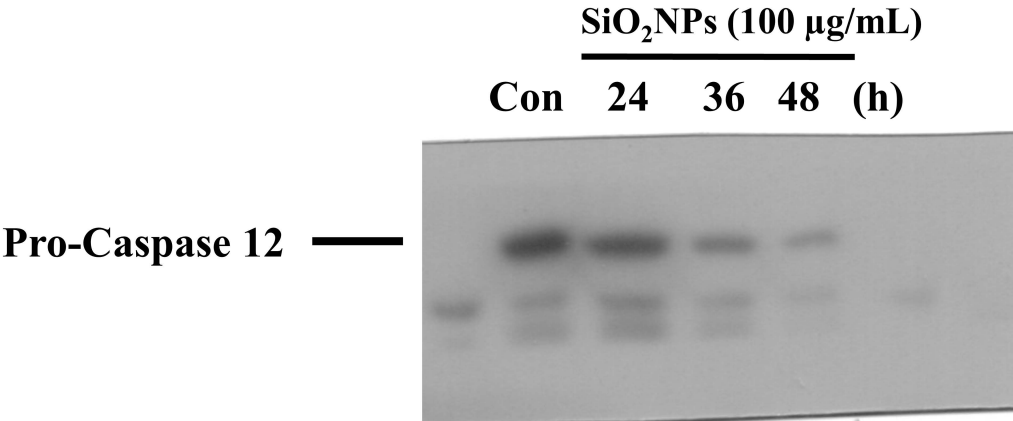
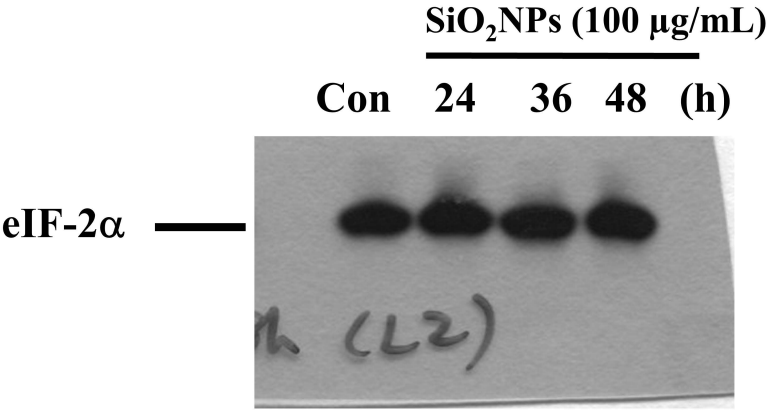
Full-length originals of western blot image for Figure 3D



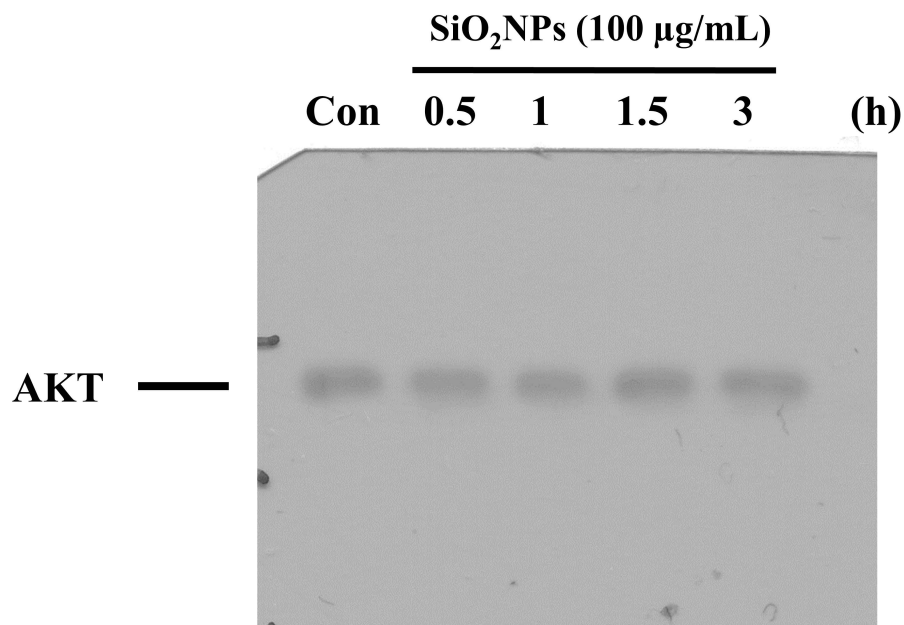
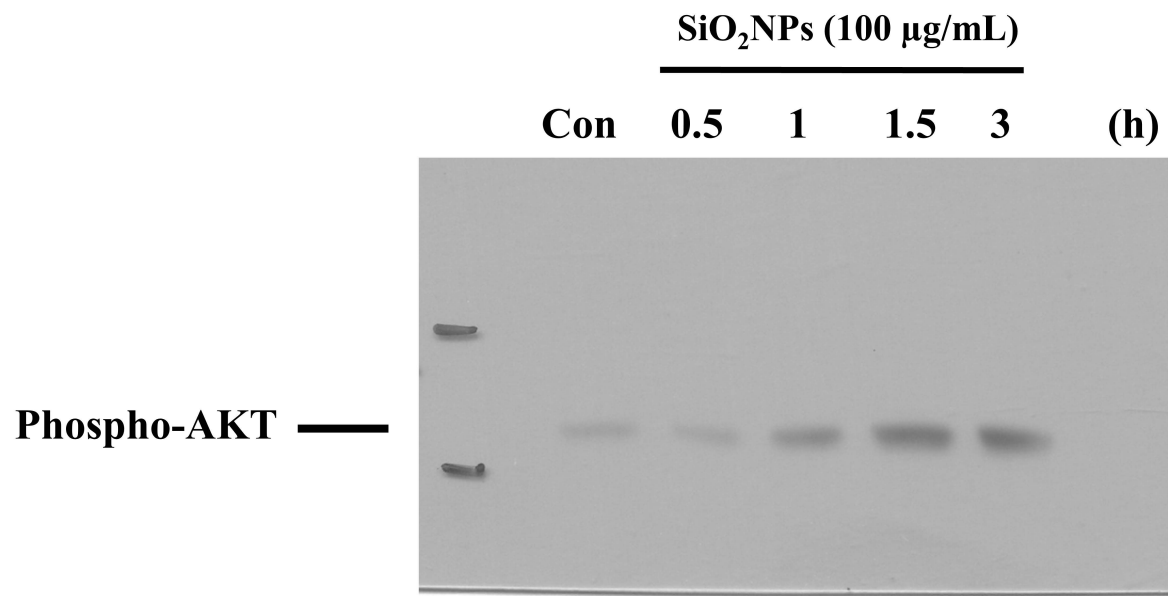
Full-length originals of western blot image for Figure 3F



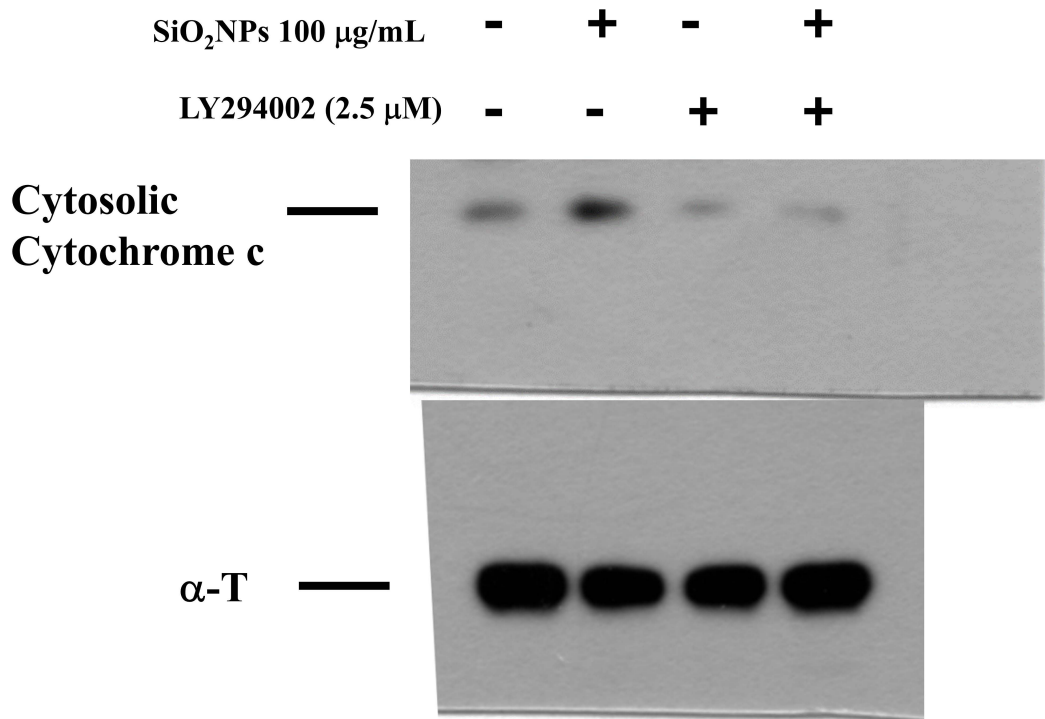
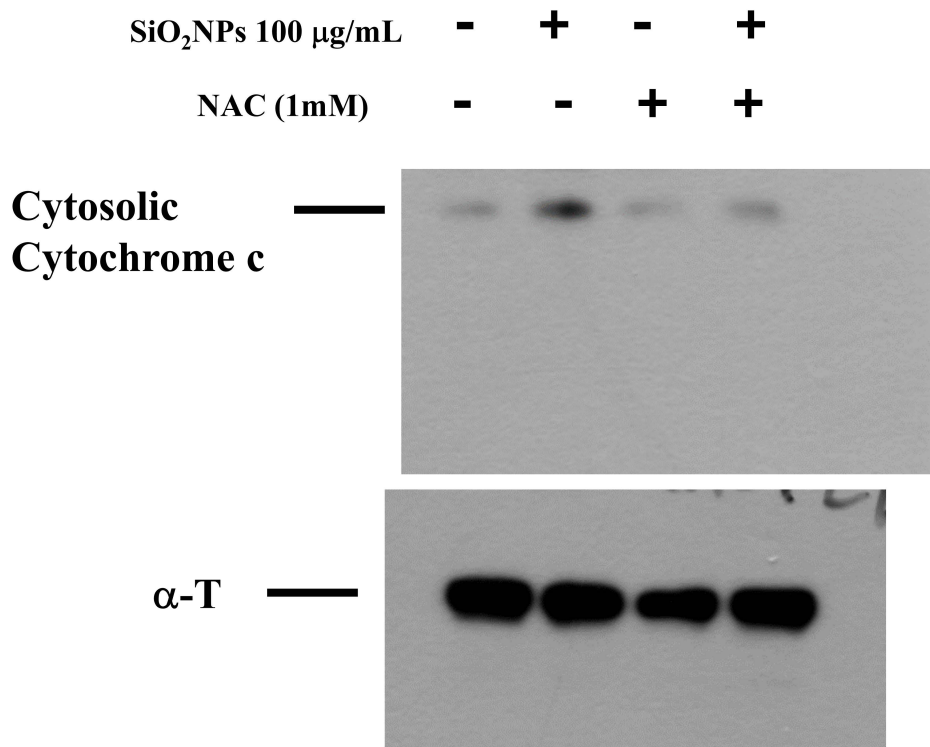
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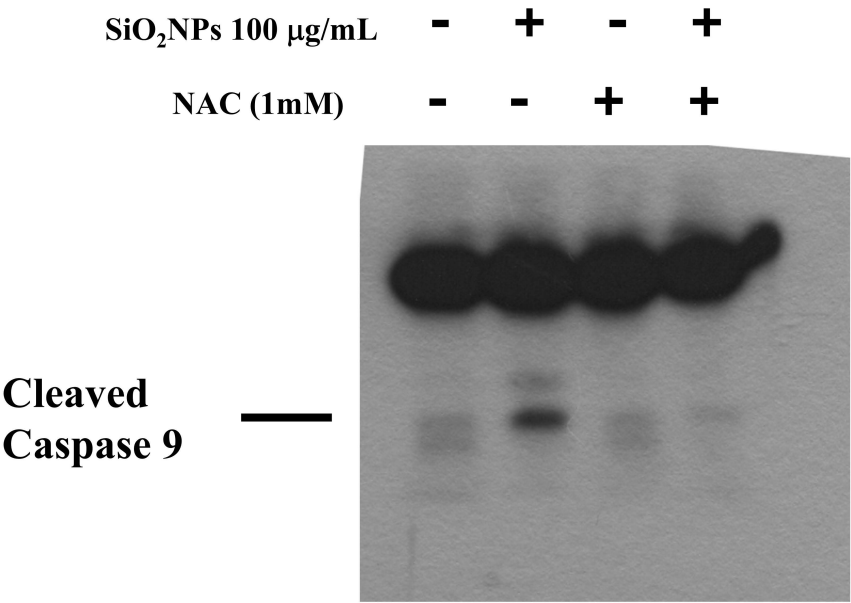
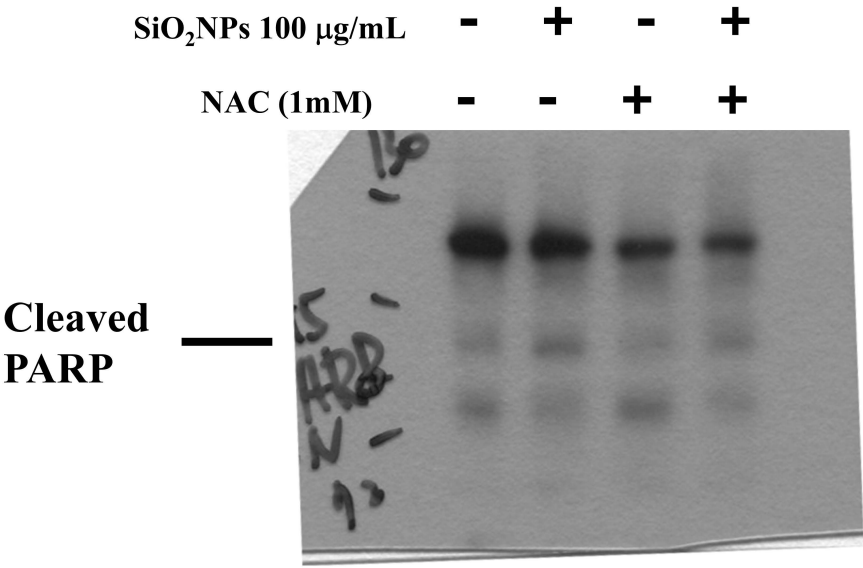
Full-length originals of western blot image for Figure 4B



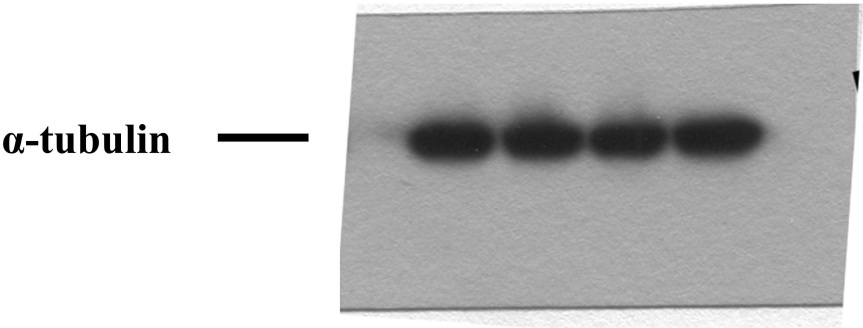
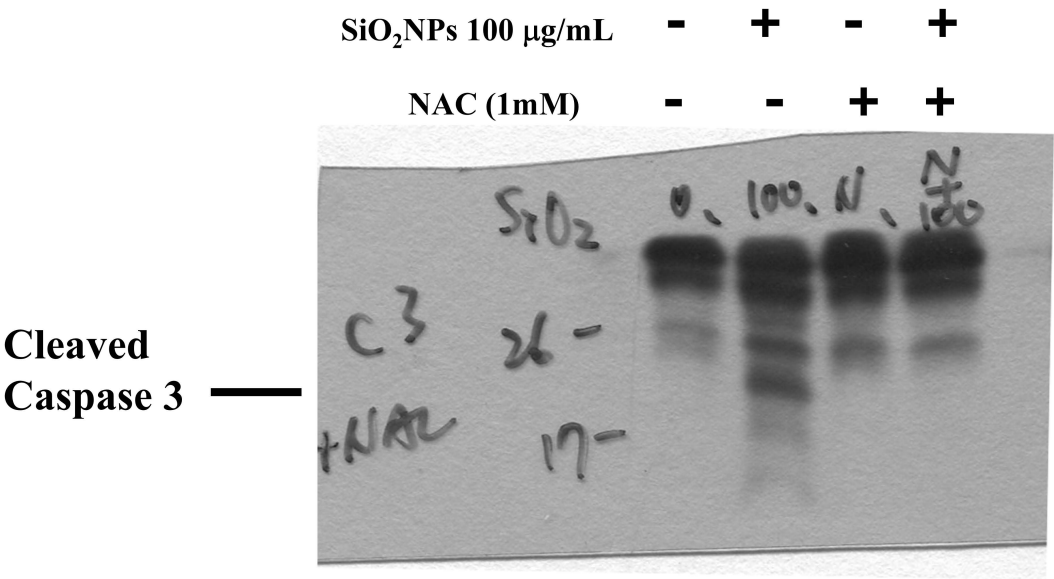
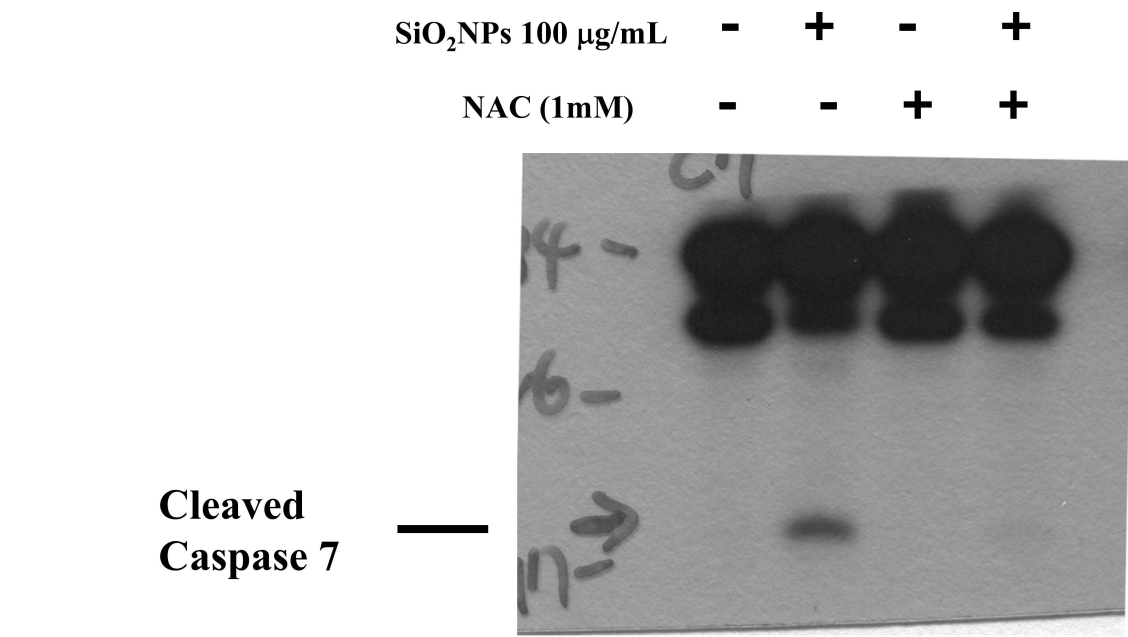
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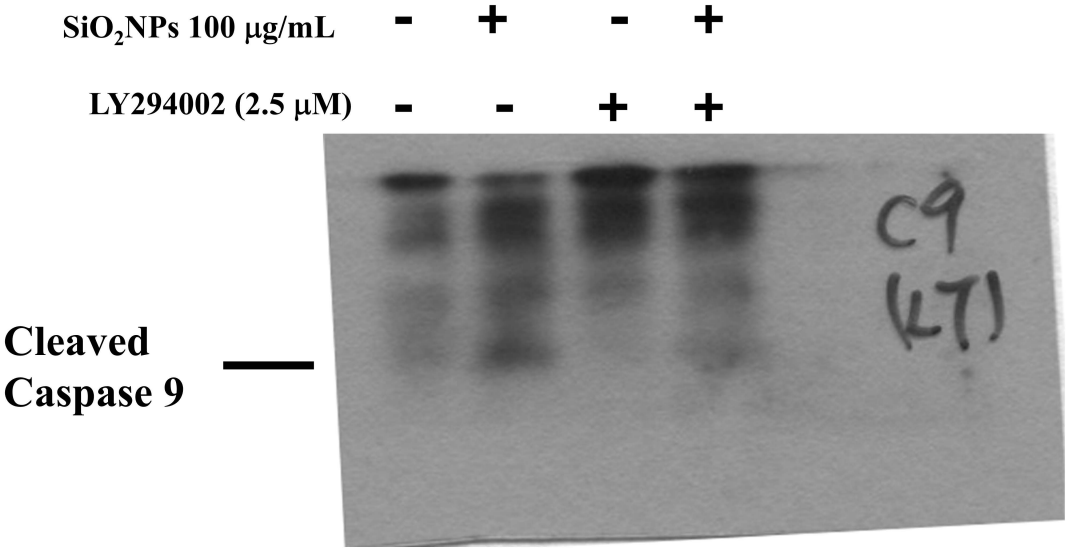
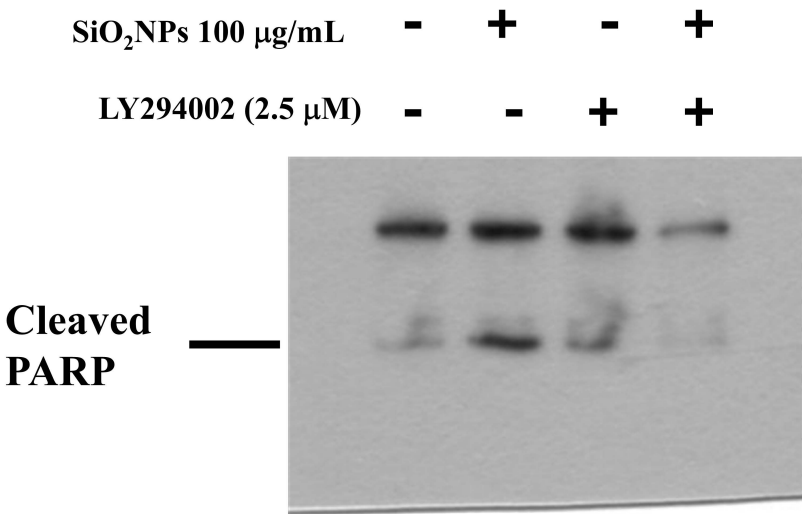
Full-length originals of western blot image for Figure 7A



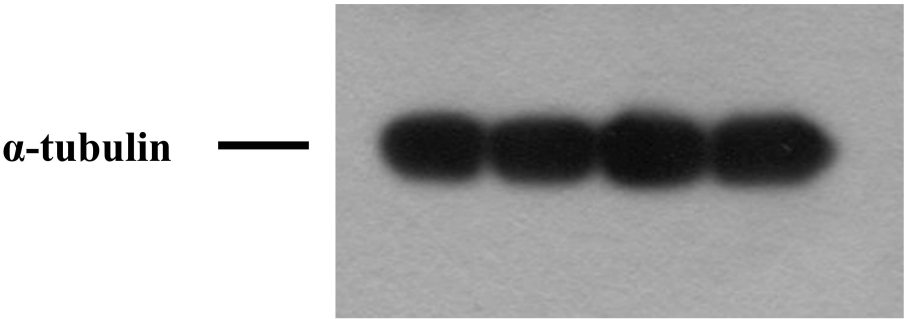
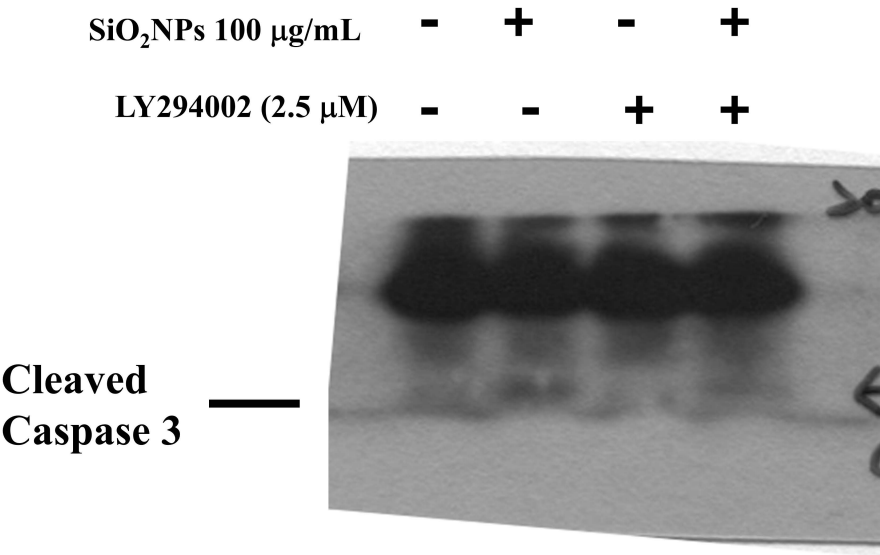
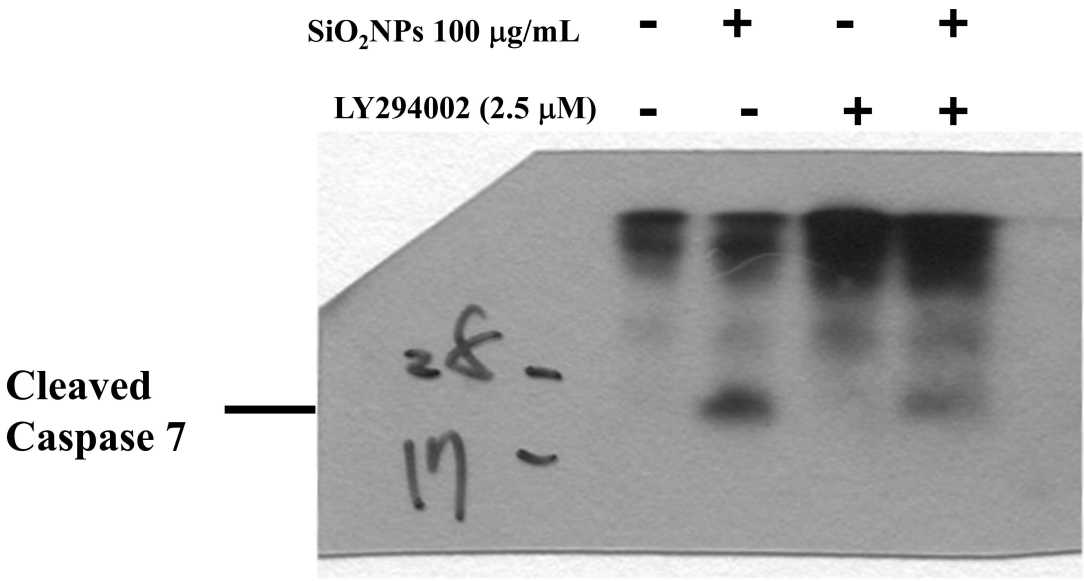
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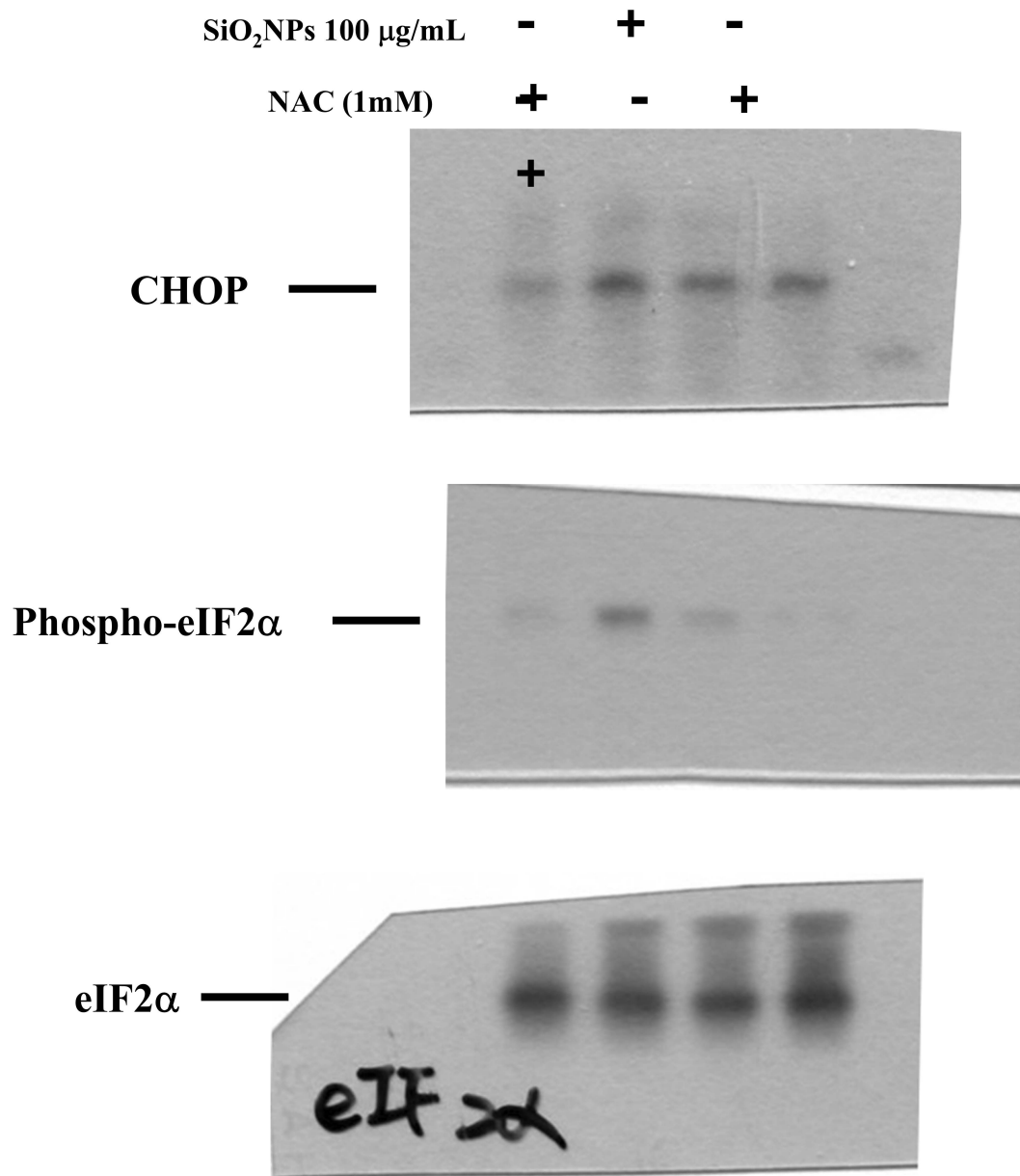
Full-length originals of western blot image for Figure 7B



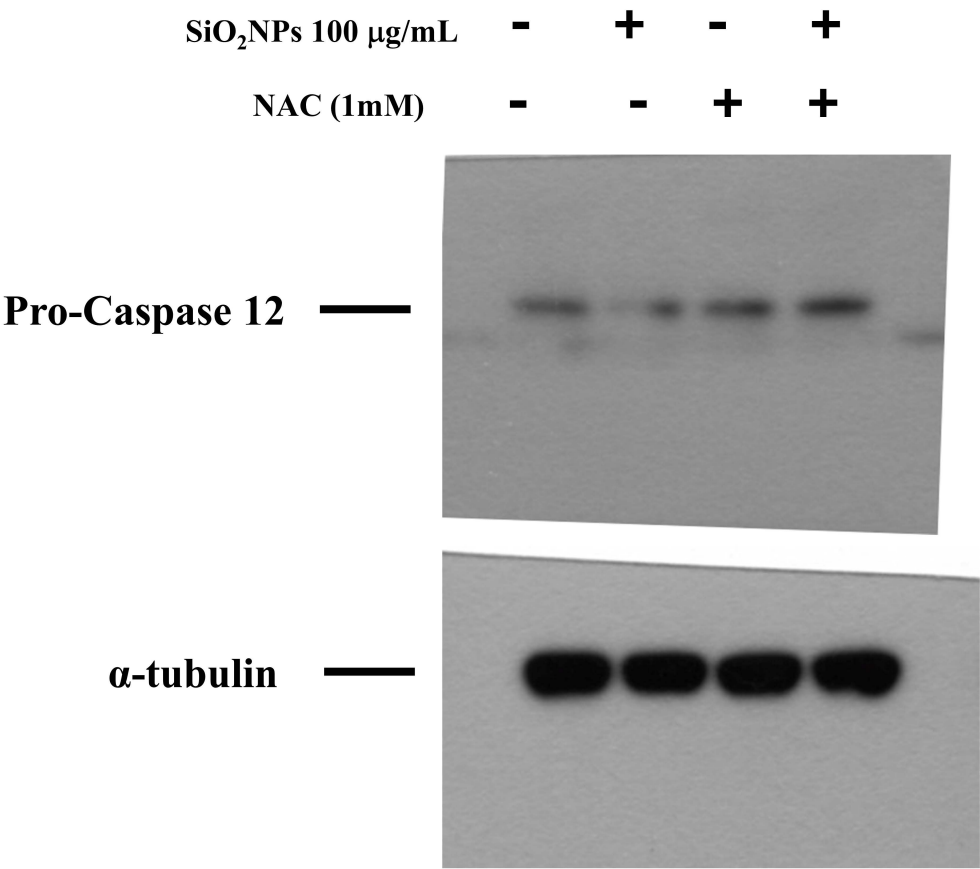
Full-length originals of western blot image for Figure 7B



Full-length originals of western blot image for Figure 7C



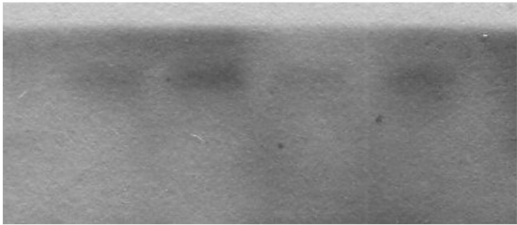
Full-length originals of western blot image for Figure 7C



Full-length originals of western blot image for Figure 7D

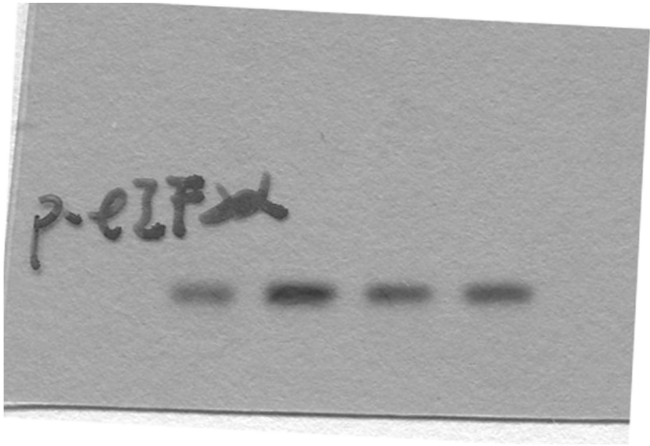
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LY294002 (2.5 µM)	-	-	+	+

CHOP —

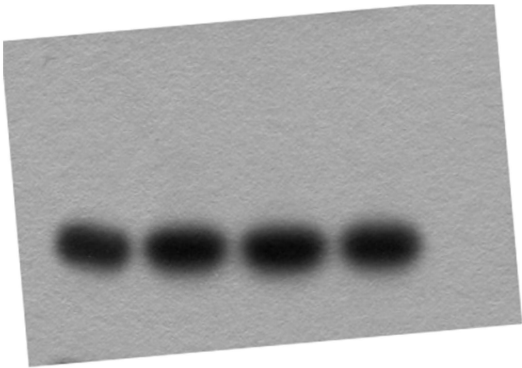


SiO ₂ NPs 100 µg/mL	-	+	-	+
LY294002 (2.5 µM)	-	-	+	+

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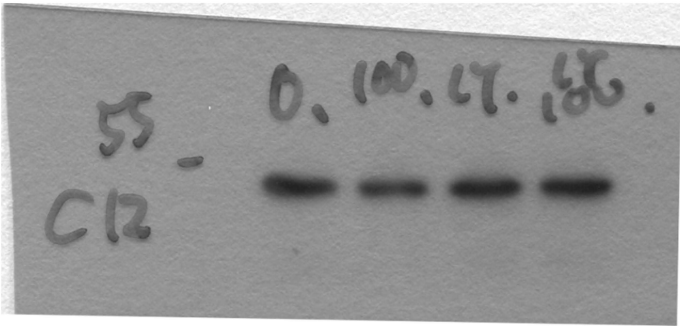
eIF2α —



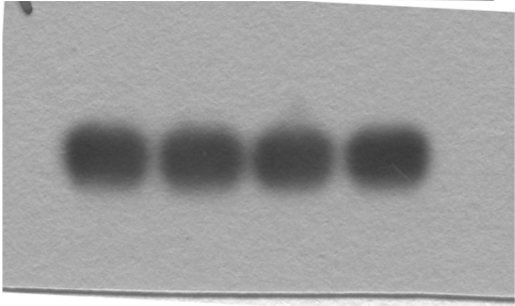
Full-length originals of western blot image for Figure 7D

SiO ₂ NPs 100 µg/mL	-	+	-	+
LY294002 (2.5 µM)	-	-	+	+

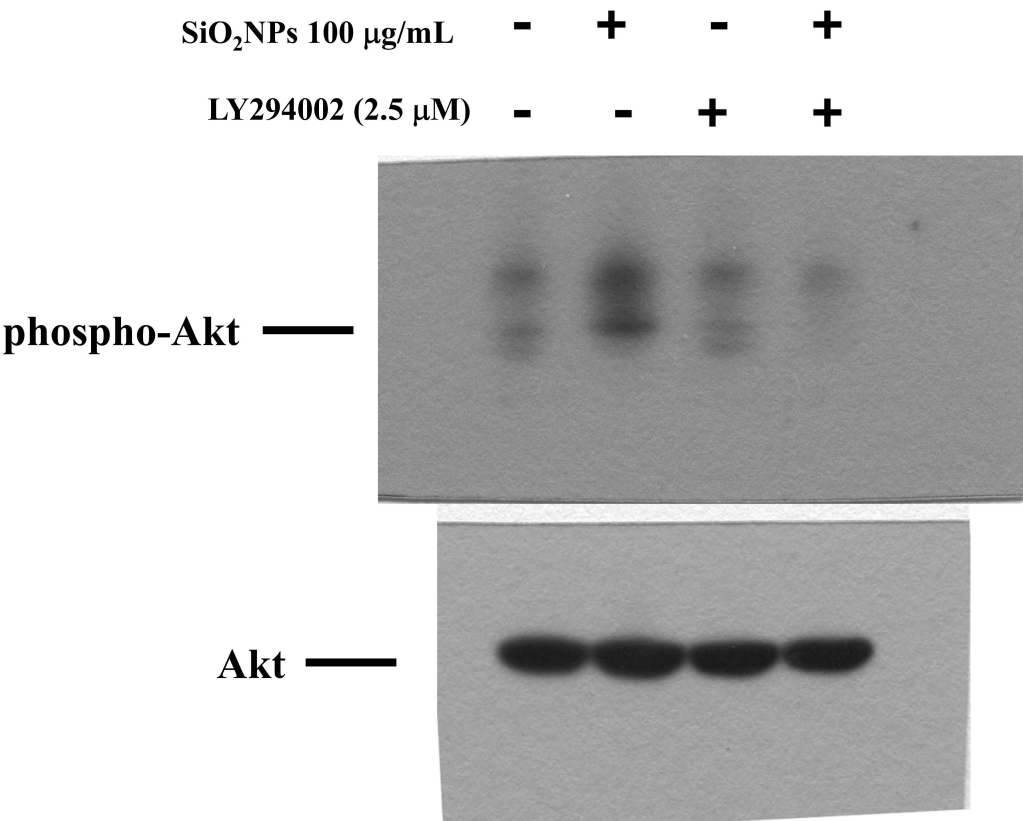
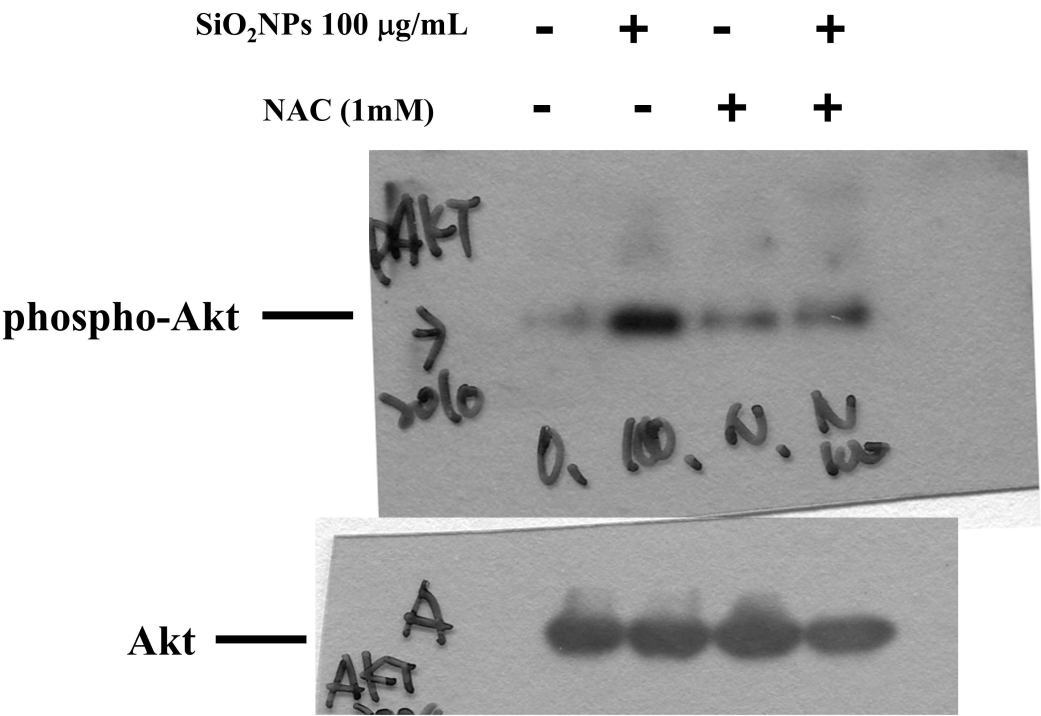
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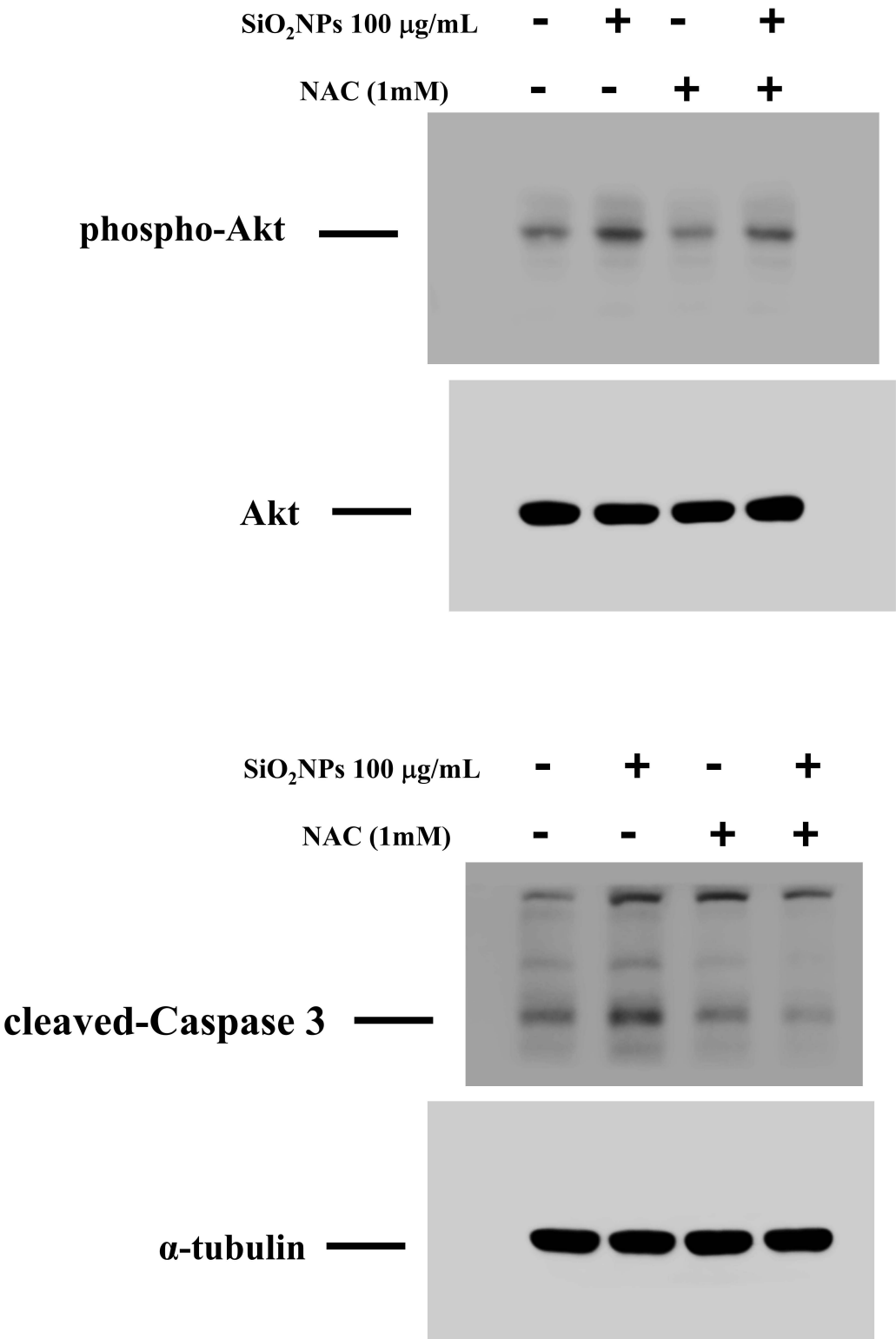
α-tubulin —



Full-length originals of western blot image for Figure 8C



Full-length originals of western blot image for Supplementary Figure 2B



Full-length originals of western blot image for Supplementary Figure 2B

