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Interleukin-1β released from macrophages stimulated with indium tin oxide nanoparticles induces epithelial-mesenchymal transition in A549 cells

Yosuke Tabei[1,*], Kazumichi Yokota[1], Yoshihiro Nakajima[1]

[1]Health and Medical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 2217-14 Hayashi-cho, Takamatsu, Kagawa 761-0395, Japan

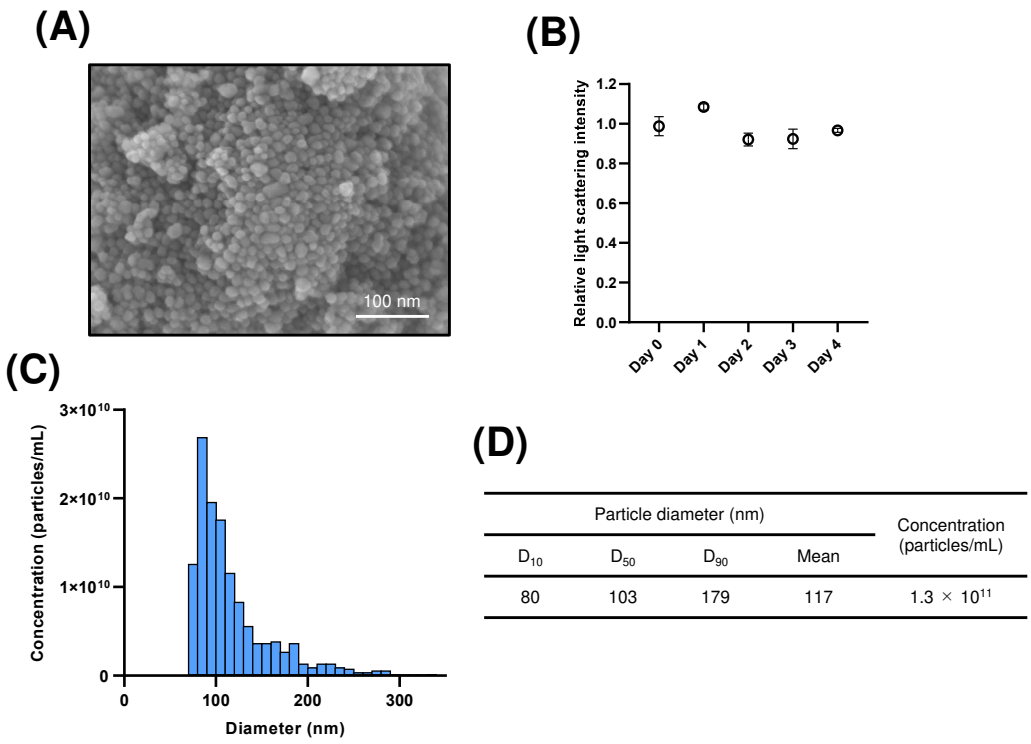
[*]Corresponding author

Address: Health and Medical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 2217-14 Hayashi-cho, Takamatsu, Kagawa 761-0395, Japan.

E-mail: y-tabei@aist.go.jp.

Tel: +81-87-869-3598

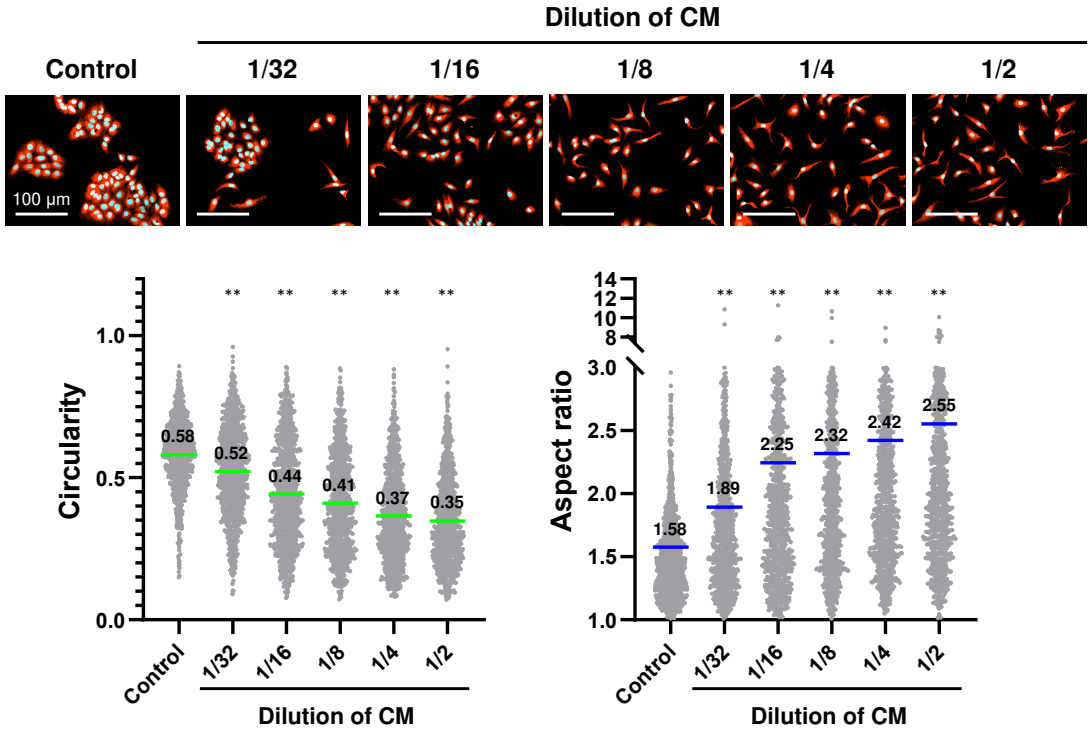
Fax: +81-87-869-3553



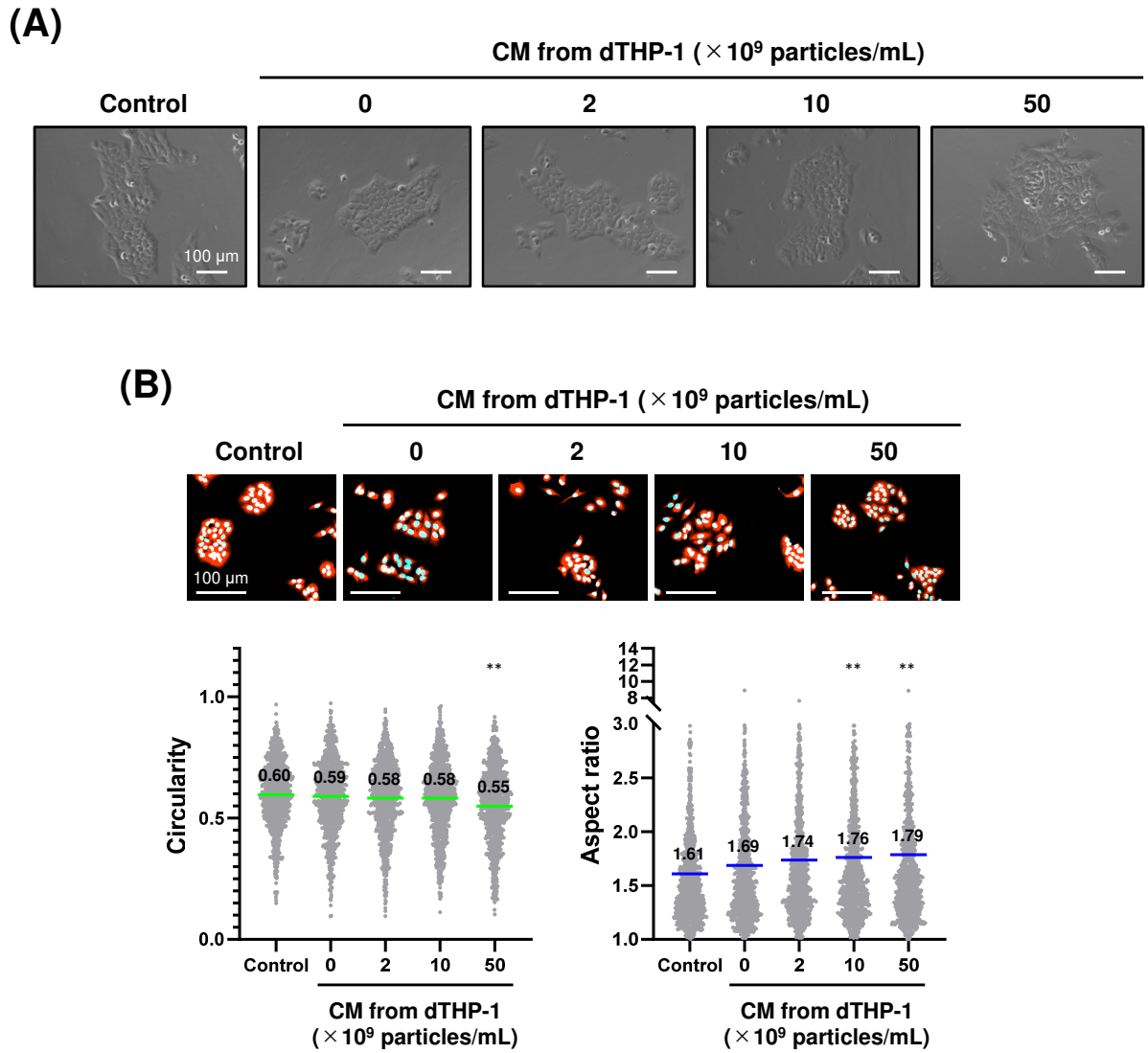
(D)

Particle diameter (nm)				Concentration (particles/mL)
D_{10}	D_{50}	D_{90}	Mean	
80	103	179	117	1.3×10^{11}

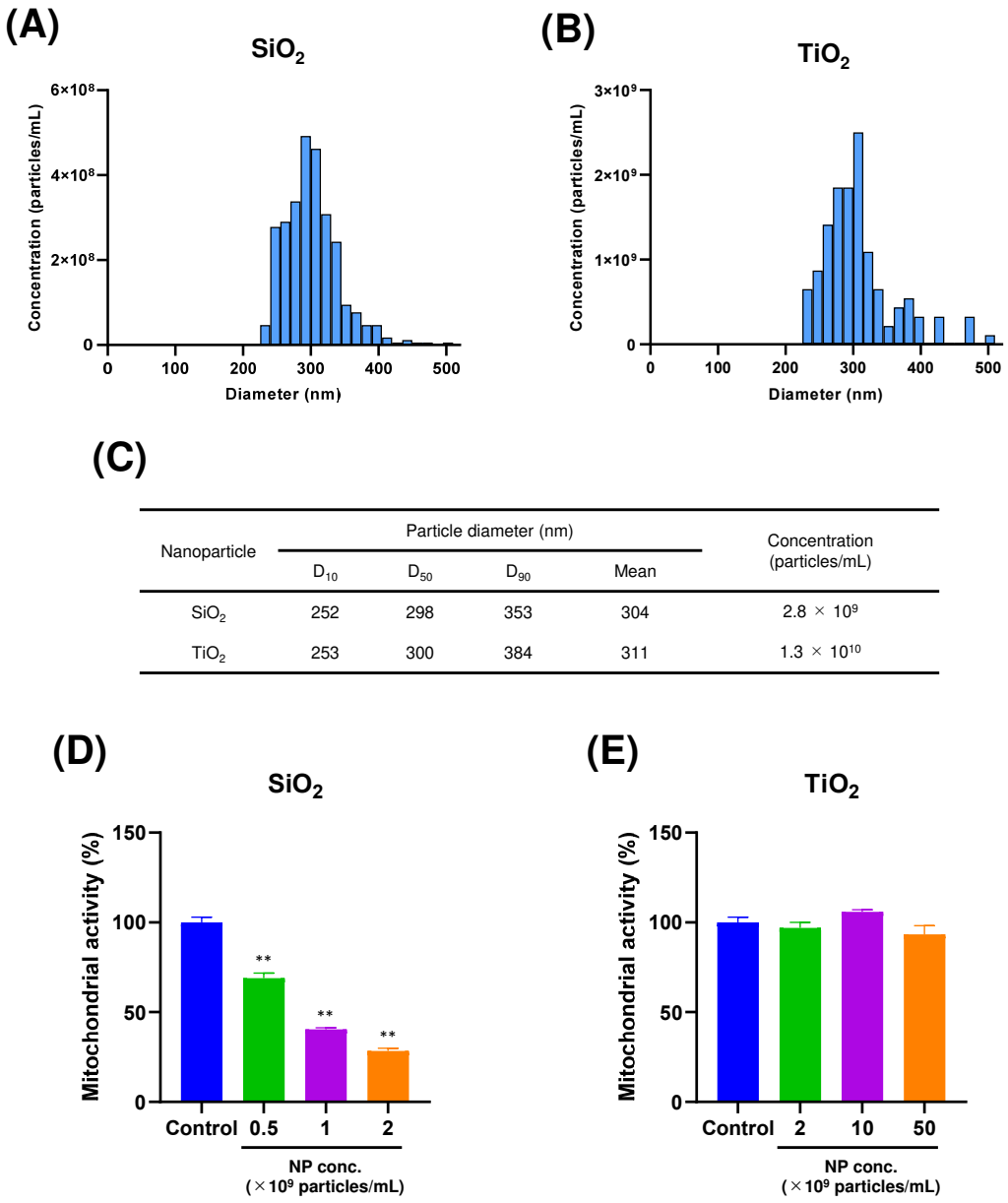
Supplementary Figure S1. Characteristics of ITO NPs used in this study. (A) FE-SEM analysis of ITO NPs prepared in culture medium. (B) Stability of ITO NPs in the medium dispersions. Light scattering intensities were measured by DLS. (C) Size distribution of ITO NPs measured using the qNano particle counter. (D) Summary of diameter (D_{10}, D_{50}, D_{90}, and mean) and concentration of ITO NPs prepared in this study as described in Materials and Methods.



Supplementary Figure S2. Effects of CM obtained from dTHP-1 cells treated with ITO NPs for 72 h on the morphology of A549 cells. The cells were treated with control medium or various dilutions of CM obtained from dTHP-1 cells treated with 50 × 10^9/mL ITO NPs for 72 h. The cells were stained with Hoechst 33342 and CellTracker Red CMPTX dye and visualized under a CQ1 confocal quantitative image cytometer (upper panels). Cell circularity and aspect ratio were analyzed as described in Materials and Methods. Values are means, n = 1000, one-way ANOVA followed by Tukey's multiple comparison test. $^{**}P < 0.01$, compared with untreated control.



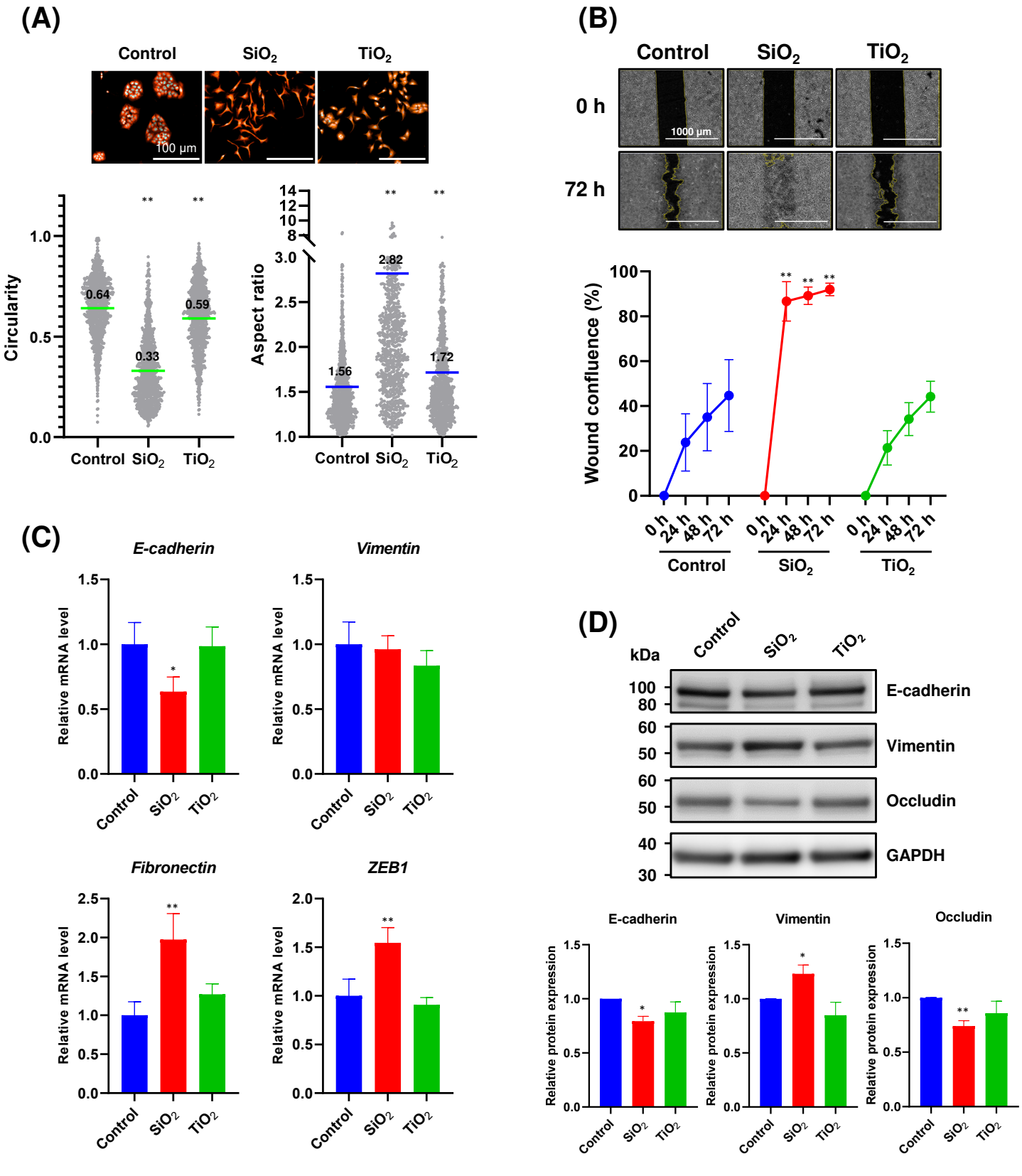
Supplementary Figure S3. Effects of CM obtained from dTHP-1 cells treated with ITO NPs for 24 h on the morphology of A549 cells. (A) Microscopic images of A549 cells. A549 cells were exposed to CM from ITO NP-treated dTHP-1 cells for 72 h. (B) Quantitative analysis of cell morphologies altered by EMT induction. A549 cells were treated with CM from ITO NP-treated dTHP-1 cells for 72 h and stained with Hoechst 33342 and CellTracker Red CMPTX dye. The cells were visualized under a CQ1 confocal quantitative image cytometer (upper panels). Cell circularity and aspect ratio were analyzed as described in Materials and Methods. Values are means, n = 1000, one-way ANOVA followed by Tukey's multiple comparison test. $^{**}P < 0.01$, compared with untreated control.



(C)

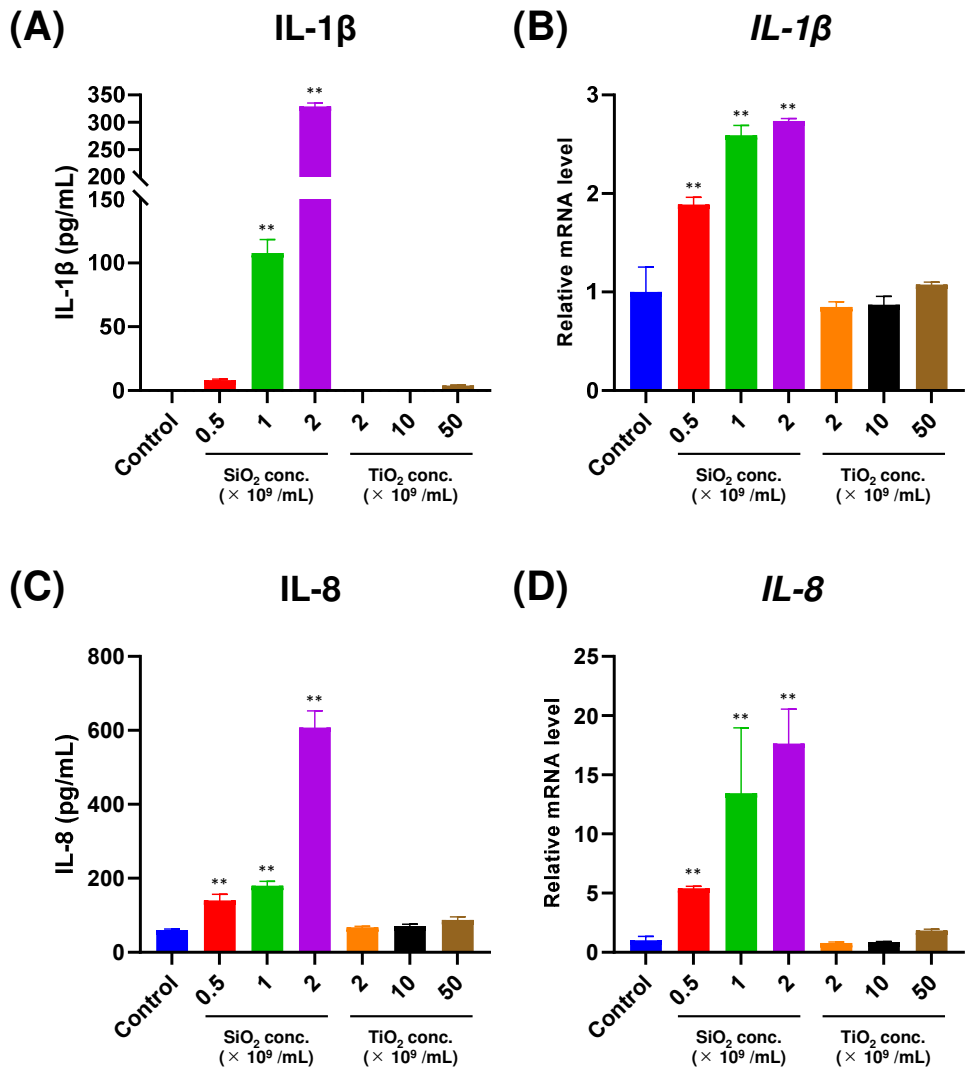
Nanoparticle	Particle diameter (nm)				Concentration (particles/mL)
	D_{10}	D_{50}	D_{90}	Mean	
SiO_2	252	298	353	304	2.8×10^9
TiO_2	253	300	384	311	1.3×10^{10}

Supplementary Figure S4. Cytotoxicity of SiO_2 NPs and TiO_2 NPs toward dTHP-1 cells. (A and B) Size distributions of SiO_2 NPs and TiO_2 NPs measured using the qNano particle counter. (C) Summary of diameters (D_{10}, D_{50}, D_{90}, and mean) and concentrations of SiO_2 NPs and TiO_2 NPs prepared in this study as described in Materials and Methods. (D and E) Effects of SiO_2 NPs and TiO_2 NPs on mitochondrial activity. dTHP-1 cells were exposed to SiO_2 NPs and TiO_2 NPs for 72 h. Mitochondrial activity was measured by conducting the WST-1 assay, and the results are expressed as percentage of untreated controls. Values are means ± SD, n = 3, one-way ANOVA followed by Tukey's multiple comparison test. $^{**}P < 0.01$, compared with untreated control.

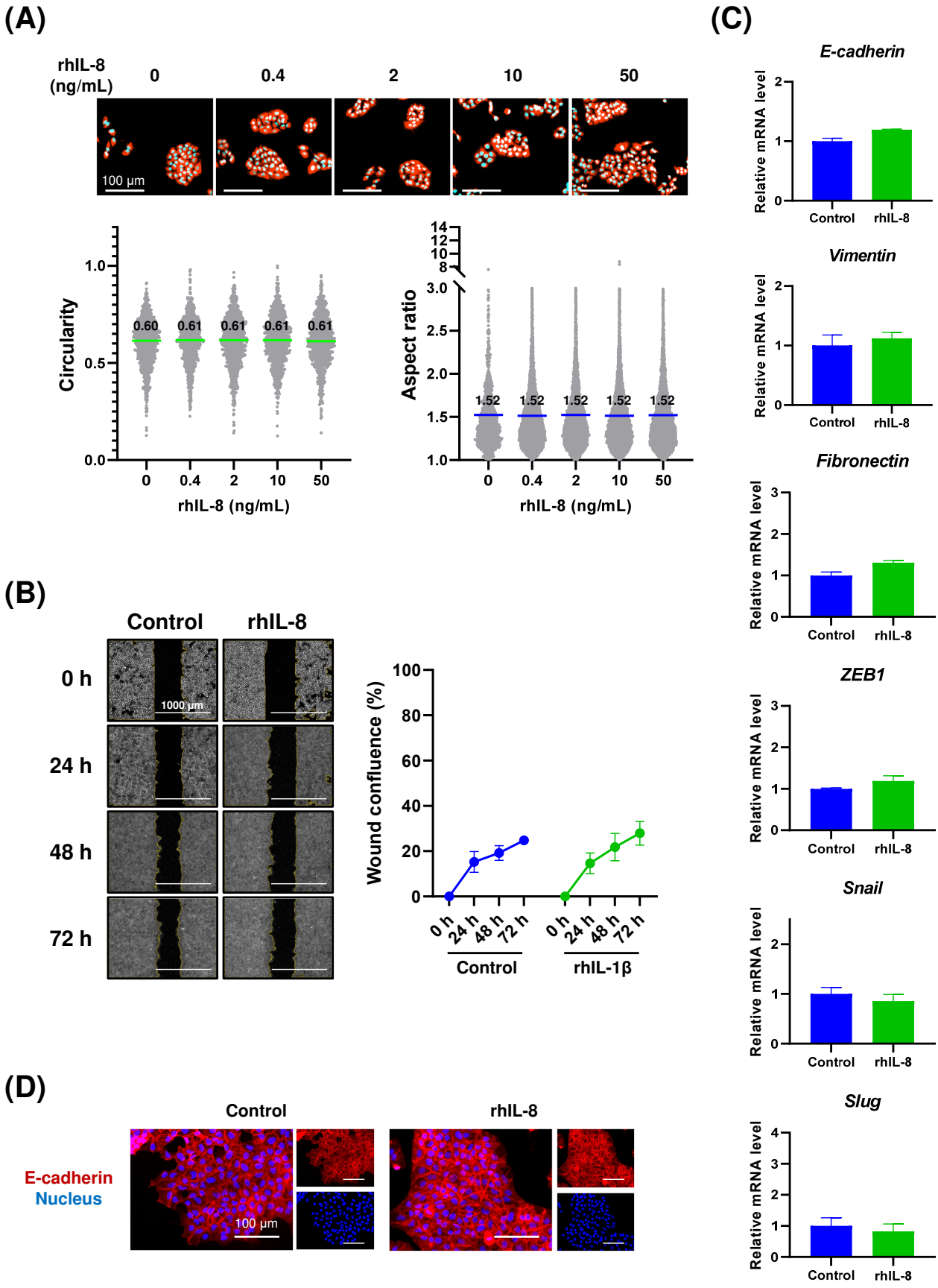


Supplementary Figure S5

Supplementary Figure S5. Effects of CM from SiO2 NP- or TiO2 NP-treated dTHP-1 cells on the induction of EMT in A549 cells. (A) Quantitative analysis of A549 cell morphology. The cells were exposed to CM from SiO_2 NP- (2×10^9/mL) or TiO_2 NP- (50×10^9/mL) treated dTHP-1 cells for 72 h and stained with Hoechst 33342 and CellTracker Red CMPTX dye. The cells were visualized under a CQ1 confocal quantitative image cytometer (upper panels). Cell circularity and aspect ratio were analyzed as described in Materials and Methods. Values are means, n = 1000, one-way ANOVA followed by Tukey's multiple comparison test. $^{**}P < 0.01$, compared with untreated control. (B) Wound healing assay of A549 cells. The cells were exposed to CM from SiO_2 NP- (2×10^9/mL) or TiO_2 NP- (50×10^9/mL) treated dTHP-1 cells for 72 h. Images and wound confluence values were obtained using a LionHeart FX Automated Live Cell Imager and Gen5 software, respectively. Values are means ± SD, n = 3, two-way ANOVA followed by Tukey's multiple comparison test. $^{**}P < 0.01$, compared with the corresponding time point of untreated control. (C) mRNA expression levels of *E-cadherin*, *vimentin*, *fibronectin*, and *ZEB1* in A549 cells. The cells were exposed to CM from SiO_2 NP- (2×10^9/mL) or TiO_2 NP- (50×10^9/mL) treated dTHP-1 cells for 72 h. Each mRNA expression level was normalized to the corresponding *β-actin* value and is presented as relative units compared with untreated control. Values are means ± SD, n = 3, one-way ANOVA followed by Tukey's multiple comparison test. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with untreated control. (D) Western blot analysis and quantification of E-cadherin, vimentin, and occludin in A549 cells. The cells were exposed to CM from SiO_2 NP- (2×10^9/mL) or TiO_2 NP- (50×10^9/mL) treated dTHP-1 cells for 72 h. Images are representative of three independent experiments. GAPDH was used as internal control. Values are means ± SD, n = 3, one-way ANOVA followed by Tukey's multiple comparison test. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with untreated control. (E) Immunofluorescence analysis of E-cadherin in A549 cells. The cells were exposed to CM from SiO_2 NP- (2×10^9/mL) or TiO_2 NP- (50×10^9/mL) treated dTHP-1 cells for 72 h. Nuclei were stained with NucBlue. Fluorescence images were obtained by using a BZ-X710 fluorescence microscope.

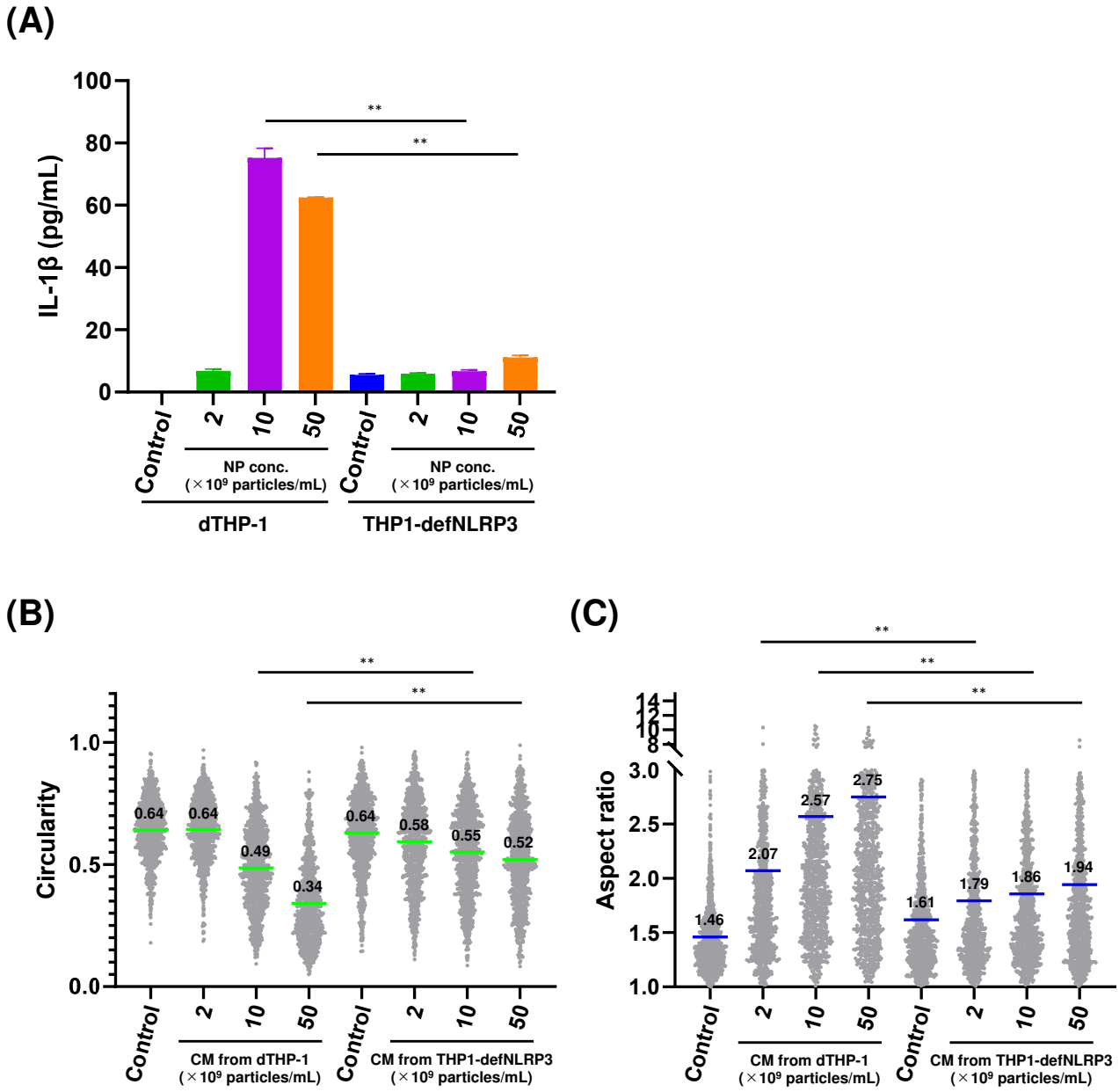


Supplementary Figure S6. Effects of SiO2 NPs and TiO2 NPs on protein release and transcript levels of IL-1β and IL-8 in dTHP-1 cells. (A) Protein release and (B) transcript levels of IL-1β in dTHP-1 cells treated with SiO_2 NPs and TiO_2 NPs. (C) Protein release and (D) transcript levels of IL-8 in dTHP-1 cells treated with SiO_2 NPs and TiO_2 NPs. The dTHP-1 cells were exposed to SiO_2 NPs and TiO_2 NPs for 72 h. IL-1β and IL-8 released into culture medium were determined using ELISA. The mRNA levels of *IL-1β* and *IL-8* were determined by quantitative real-time PCR analysis. Each mRNA expression level was normalized to the corresponding *β-actin* value and is presented as relative units compared with untreated control. Values are means ± SD, n = 3, one-way ANOVA followed by Tukey's multiple comparison test. $^{**}P < 0.01$, compared with untreated control.

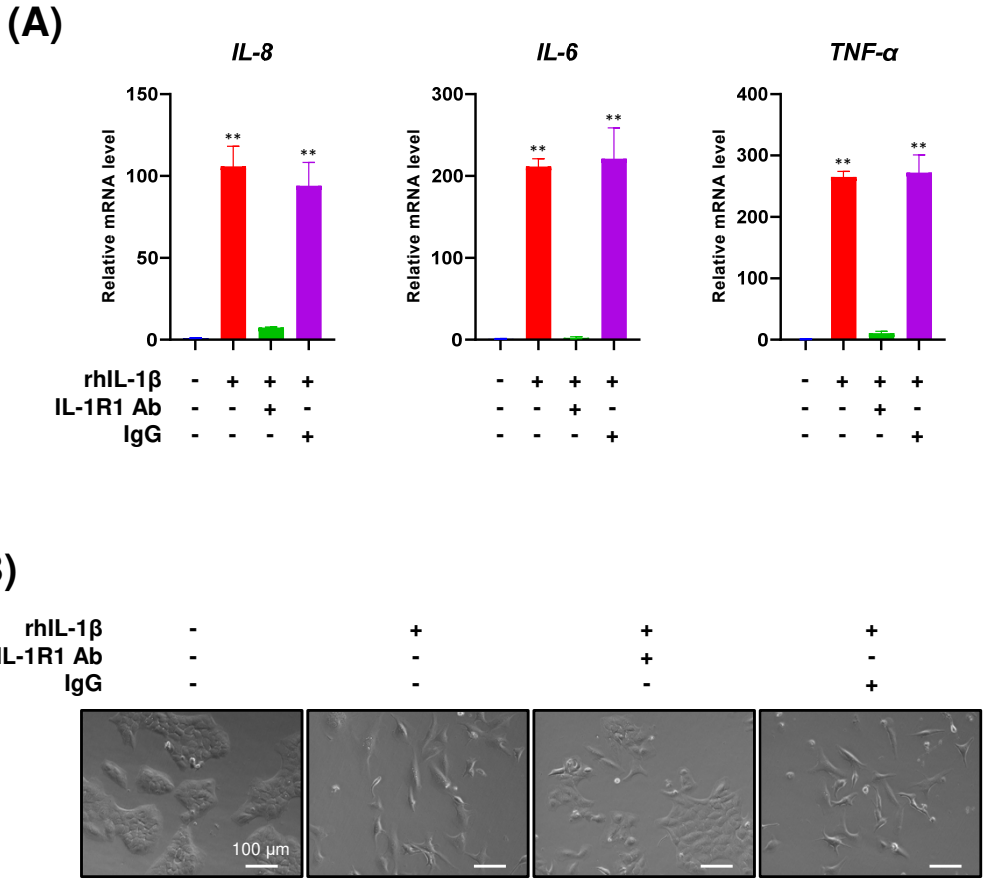


Supplementary Figure S7

Supplementary Figure S7. Effects of rhIL-8 on the induction of EMT in A549 cells. (A) Quantitative analysis of morphological changes induced by rhIL-8 treatment. The cells were treated with various concentrations of rhIL-8 for 72 h and stained with Hoechst 33342 and CellTracker Red CMPTX dye. The cells were visualized under a CQ1 confocal quantitative image cytometer (upper panels). Cell circularity and aspect ratio were analyzed as described in Materials and Methods. Values are means, n = 1000, one-way ANOVA followed by Tukey's multiple comparison test. **P* < 0.05, compared with untreated control. (B) Wound healing assay of A549 cells treated with rhIL-8. The cells were exposed to 1 ng/mL rhIL-8 for 72 h. Images and wound confluence values were obtained using a LionHeart FX Automated Live Cell Imager and Gen5 software, respectively. Values are means ± SD, n = 3, two-way ANOVA followed by Tukey's multiple comparison test. **P* < 0.05, compared with the corresponding time point of untreated control. (C) mRNA expression levels of *E-cadherin*, *vimentin*, *fibronectin*, *ZEB1*, *Snail*, and *Slug* in A549 cells treated with 1 ng/mL rhIL-8. Each mRNA expression level was normalized to the corresponding *β-actin* value and is presented as relative units compared with untreated control. Values are means ± SD, n = 3, unpaired two-tailed Student's *t*-test. (D) Immunofluorescence analysis of E-cadherin in A549 cells treated with 1 ng/mL rhIL-8. Nuclei were stained with NucBlue. Fluorescence images were obtained by using a BZ-X710 fluorescence microscope.



Supplementary Figure S8. Involvement of NLRP3 inflammasome activation in IL-1β release from ITO NP-treated dTHP-1 cells. (A) IL-1β release from dTHP-1 cells or THP1-defNLRP3 cells treated with ITO NPs. The cells were treated with ITO NPs for 72 h, and IL-1β released into culture medium was determined using ELISA. Values are means ± SD, n = 3, one-way ANOVA followed by Tukey's multiple comparison test. $^{**}P < 0.01$, compared with cells treated with ITO NPs at the corresponding concentrations. (B and C) Quantitative analysis of A549 cell morphology. The cells were exposed to CM from ITO NP-treated dTHP-1 cells or THP1-defNLRP3 cells for 72 h and stained with Hoechst 33342 and CellTracker Red CMPTX dye. The cells were visualized under a CQ1 confocal quantitative image cytometer. Cell circularity and aspect ratio were analyzed as described in Materials and Methods. Values are means, n = 1000, one-way ANOVA followed by Tukey's multiple comparison test. $^{**}P < 0.01$, compared with cells treated with CM from dTHP-1 cells or THP1-defNLRP3 cells exposed to ITO NPs at the corresponding concentrations.



Supplementary Figure S9. Effects of anti-IL-1 receptor antibody (IL-1R1 Ab) on rhIL-1β-treated A549 cells. (A) Effects of IL-1R1 Ab on the mRNA levels of *IL-8*, *IL-6*, and *TNF-α* in rhIL-1β-treated A549 cells. The A549 cells were pretreated with 5 μg/mL IL-1R1 Ab or 5 μg/mL control IgG for 16 h and then exposed to 100 pg/mL rhIL-1β for 72 h. Each mRNA expression level was normalized to the corresponding *β-actin* value and is presented as relative units compared with untreated control. Values are means ± SD, n = 3, one-way ANOVA followed by Tukey's multiple comparison test. $^{**}P < 0.01$, compared with untreated control. (B) Microscopic images of A549 cells treated with 100 pg/mL rhIL-1β in the presence or absence of 5 μg/mL IL-1R1 Ab for 72 h.