Supporting Information

Reduction of Acute Inflammatory Effects of Fumed Silica Nanoparticles in the Lung by Adjusting Silanol Display through Calcination and Metal Doping

Bingbing Sun,[†] Suman Pokhrel,[‡] Darren R. Dunphy,[§] Haiyuan Zhang,[∥] Zhaoxia Ji,[⊥] Xiang Wang,[⊥] Meiying Wang,[†] Yu-Pei Liao,[†] Chong Hyun Chang,[⊥] Juyao Dong,[#] Ruibin Li,[†] Lutz Mädler,[‡] C. Jeffrey Brinker,[§,¶,▲] André E. Nel,[*,†,⊥] and Tian Xia[*,†,⊥]

[†]Division of NanoMedicine, Department of Medicine, [⊥]California NanoSystems Institute, and [#]Department of Chemistry, University of California, Los Angeles, California 90095, United States, [‡]Foundation Institute of Materials Science (IWT), Department of Production Engineering, University of Bremen, 28359 Bremen, Germany, [§]Department of Chemical and Nuclear Engineering, and [¶]Department of Molecular Genetics and Microbiology, University of New Mexico, Albuquerque, New Mexico 87131, United States, [∥]Laboratory of Chemical Biology, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, Jilin, China, and [▲]Self-Assembled Materials Department, Sandia National Laboratories, P.O. Box 5800 MS1349, Albuquerque, New Mexico 87185, United States

[*]Address correspondence to
txia@ucla.edu
anel@mednet.ucla.edu

Figure S1

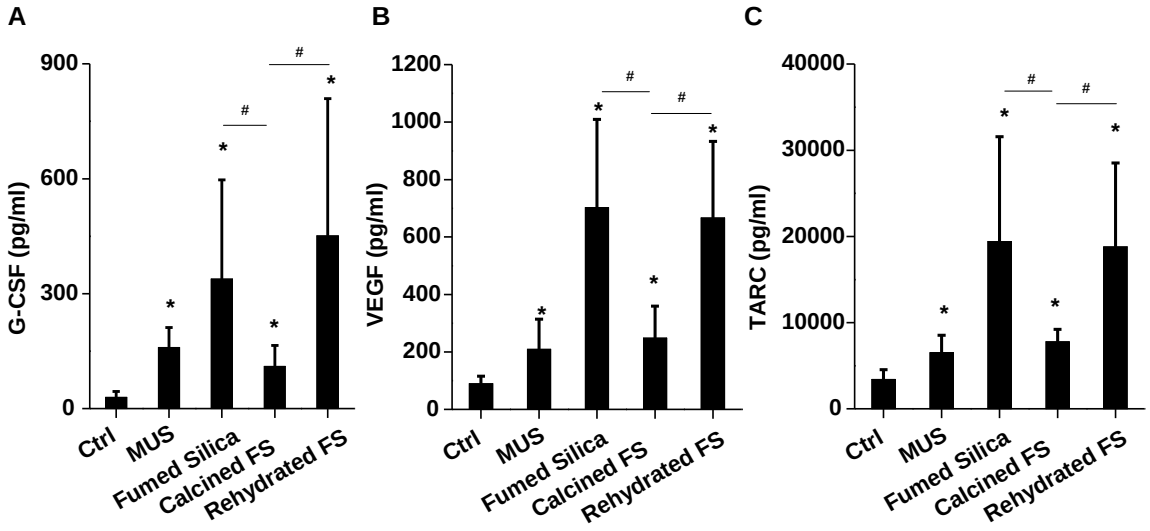

Figure S1. Reduced silanol display by calcination decreased fumed silica-induced inflammation in mice lung, which was exacerbated by rehydration. *wt* C57BL/6 (n=6) mice were exposed to 1.6 mg/kg of different fumed silica nanoparticles by oropharyngeal aspiration. BAL fluid was collected to determine (A) G-CSF, (B) VEGF, and (C) TARC level at 40 h. $^{*}p<0.05$ compared to control mice. $^{\#}p<0.05$ compared to calcined fumed silica-treated mice.

Figure S2

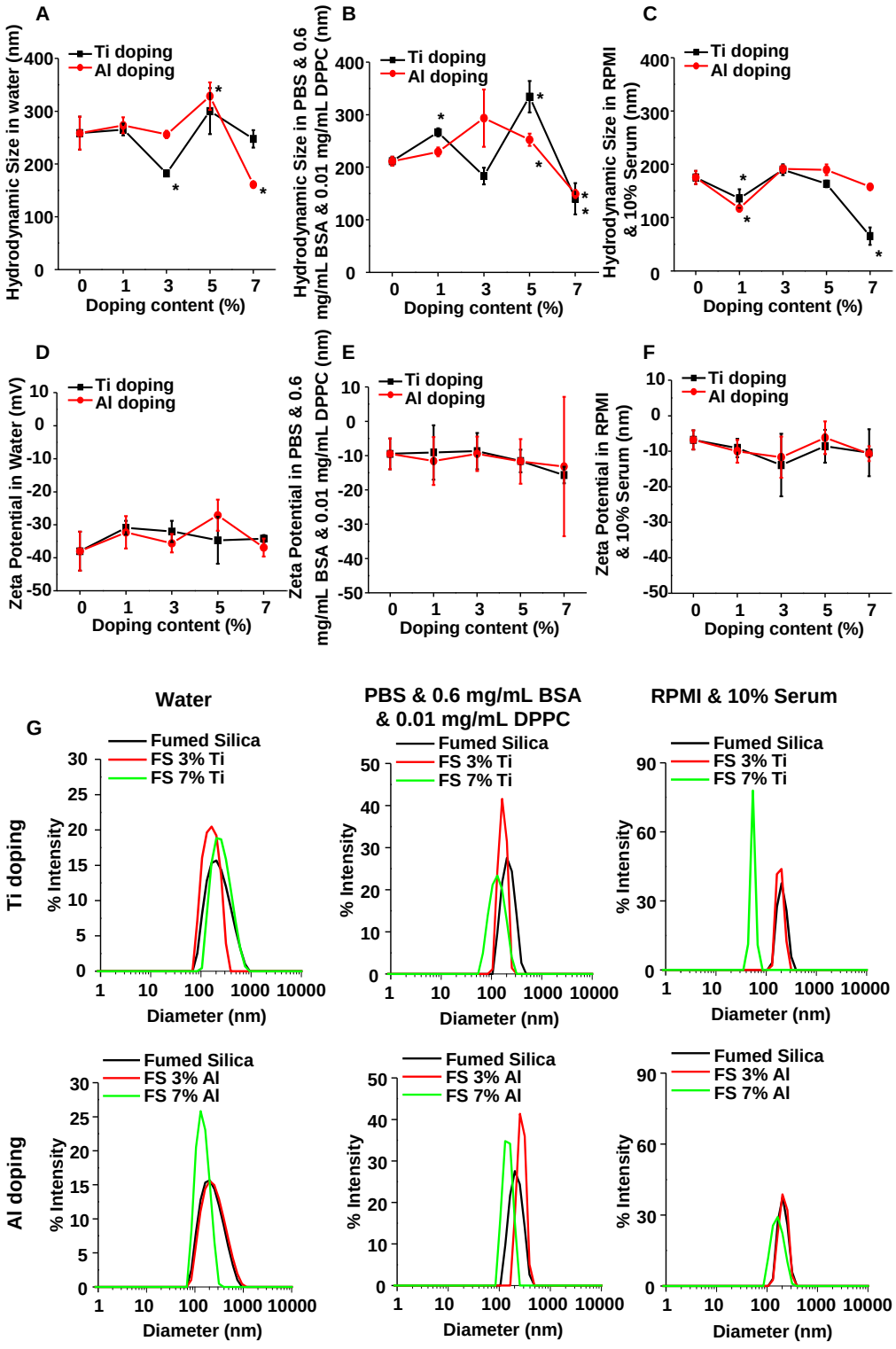
A
Hydrodynamic Size in water (nm)
Ti doping
Al doping
Doping content (%)
B
Hydrodynamic Size in PBS & 0.6 mg/mL BSA & 0.01 mg/mL DPPC (nm)
C
Hydrodynamic Size in RPMI & 10% Serum (nm)
D
Zeta Potential in Water (mV)
E
Zeta Potential in PBS & 0.6 mg/mL BSA & 0.01 mg/mL DPPC (nm)
F
Zeta Potential in RPMI & 10% Serum (nm)
G
Water
PBS & 0.6 mg/mL BSA & 0.01 mg/mL DPPC
RPMI & 10% Serum
Ti doping
Al doping
% Intensity
Diameter (nm)
Fumed Silica
FS 3% Ti
FS 7% Ti
FS 3% Al
FS 7% Al

Figure S2. Hydrodynamic size and ζ-potential of pristine and doped fumed silica. Hydrodynamic size of pristine and doped fumed silica in (A) water, (B) PBS&0.6 mg/mL BSA&0.01 mg/ml DPPC, and (C) RPMI&10% serum. Zeta potential of pristine and doped fumed silica in (D) water, (E) PBS&0.6 mg/mL BSA&0.01 mg/ml DPPC, and (F) RPMI&10% serum. (G) Size distribution of pristine and doped fumed silica in various exposure media.

Figure S3

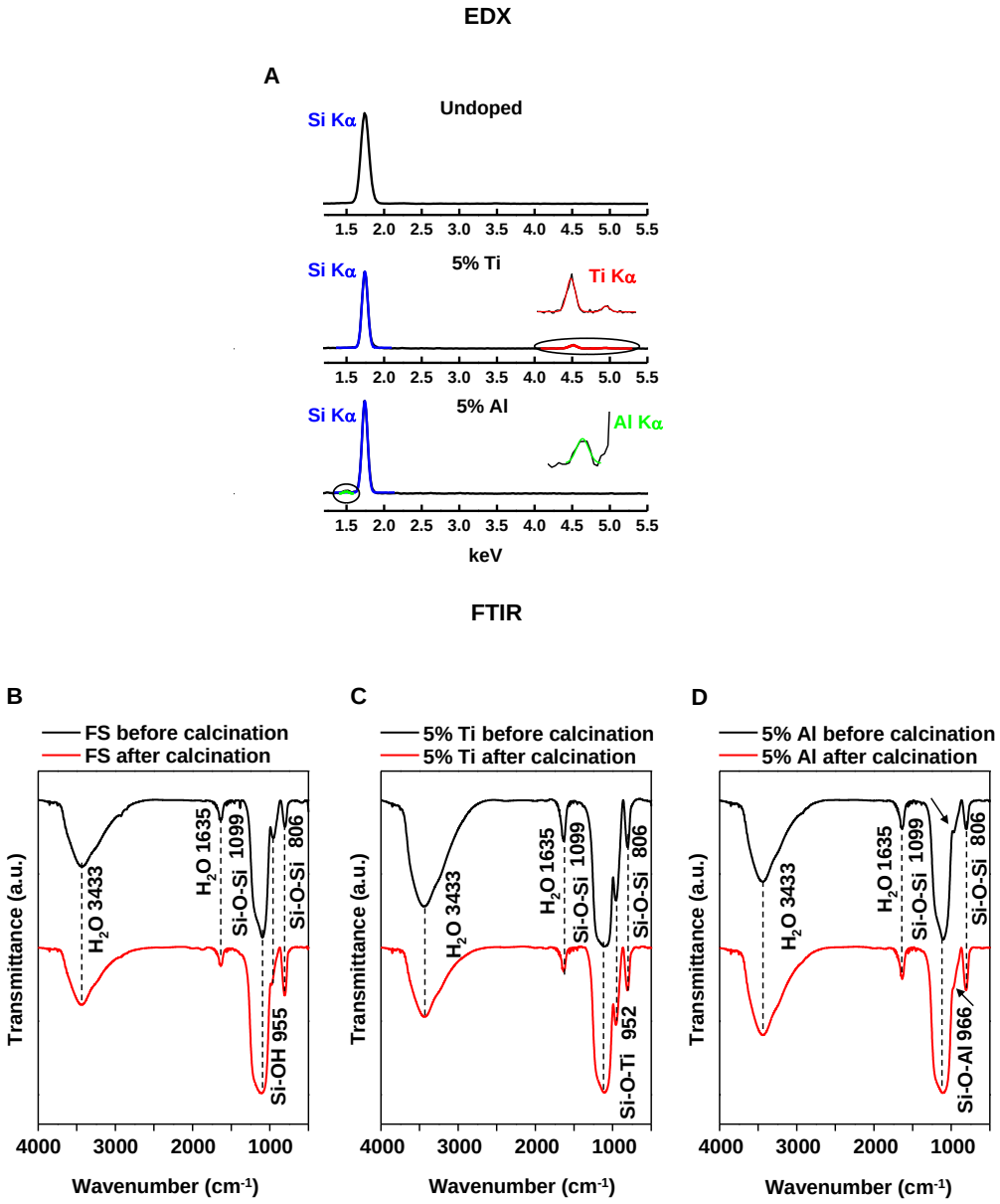

Figure S3. Physicochemical characterization of fumed silica nanoparticles. (A) EDX and (B-D) FTIR analysis of non-doped and 5% Ti- or Al-doped fumed silica nanoparticles before and after calcination at 800 ºC for 6 h.

Figure S4

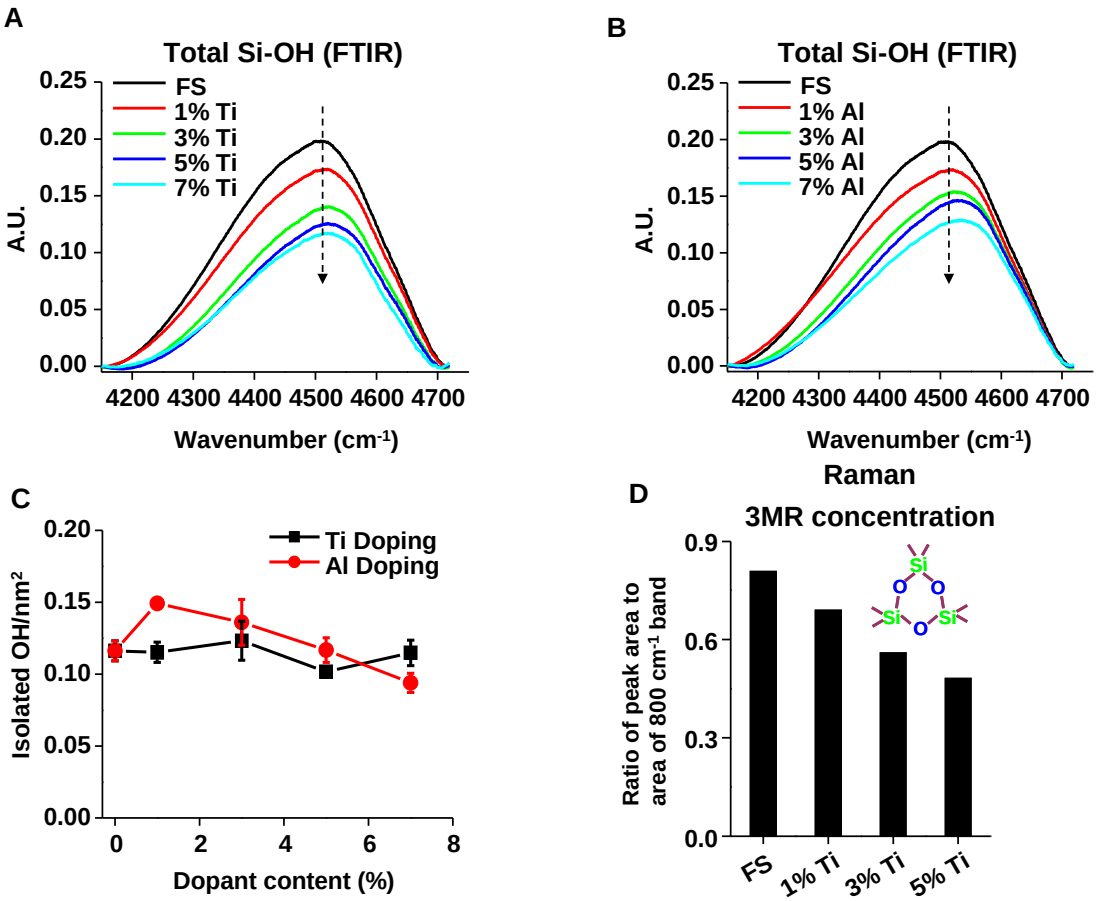

Figure S4. Decrease in total silanol density and concentration of strained three-membered rings (3MR) in doped fumed silica nanoparticles. (A-B) FTIR spectra showing the total silanol density in doped fumed silica nanoparticles. (C) Isolated silanol density in fumed silica nanoparticles. (D) 3MR concentration in Ti-doped fumed silica nanoparticles obtained from peak fitting of Raman data and normalization to the 800 cm^{-1} band attributable to the total siloxane content.

Figure S5

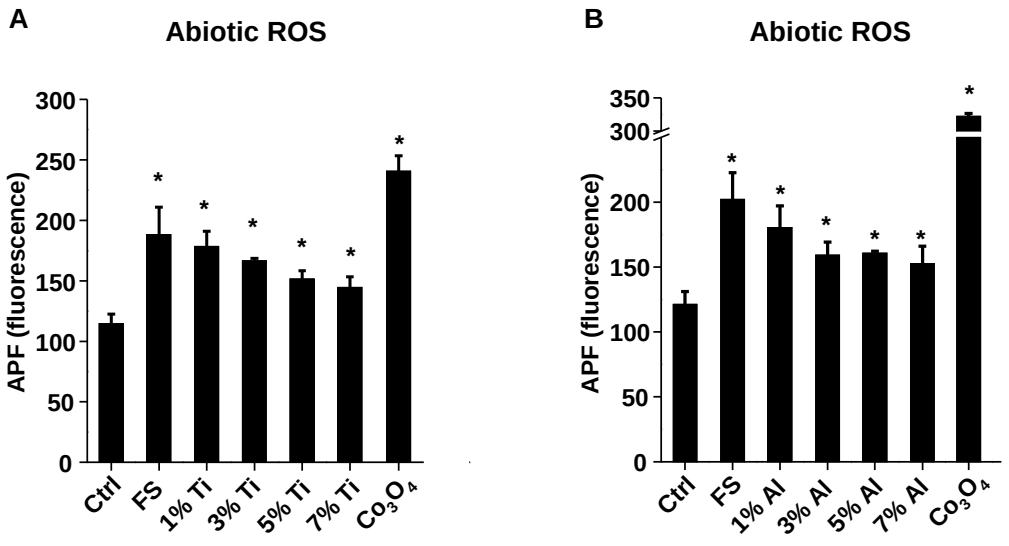

Figure S5. Abiotic ROS generation by fumed silica nanoparticles. Hydroxyl radical generation by (A) Ti- and (B) Al-doped fumed silica nanoparticles was determined by the APF. 100 μg/ml of non-doped and doped fumed silica nanoparticles were incubated with 10 μmol/L of APF (in PBS) in a volume of 100 μL in a 96-well plate at room temperature for 6 h. Fluorescence was collected at 514 nm with an excitation wavelength of 455 nm in a microplate reader. $^{*}p<0.05$ compared to particle-free control.

Figure S6

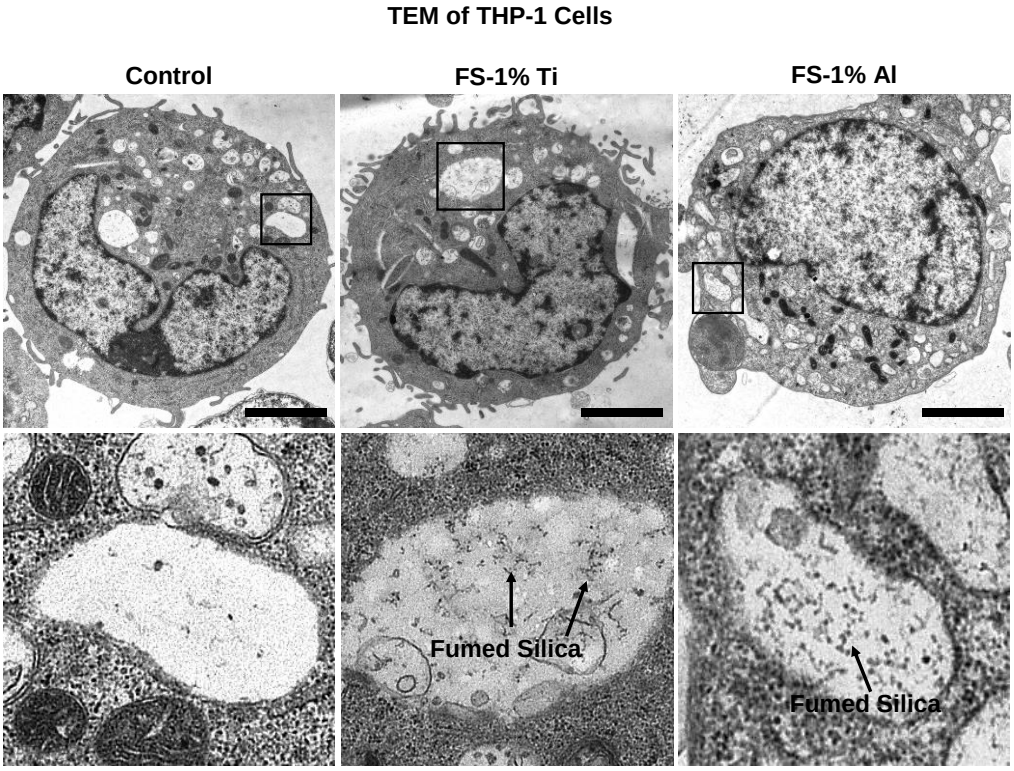

Figure S6. Cellular uptake and intracellular distribution of doped fumed silica in THP-1 cells. TEM analysis of THP-1 cells exposed to doped fumed silica nanoparticles. THP-1 cells were exposed to doped fumed silica for 12 h. The images were taken with a JEOL 100CX electron microscope at 80 kV. The scale bar is 5 µm.

Figure S7

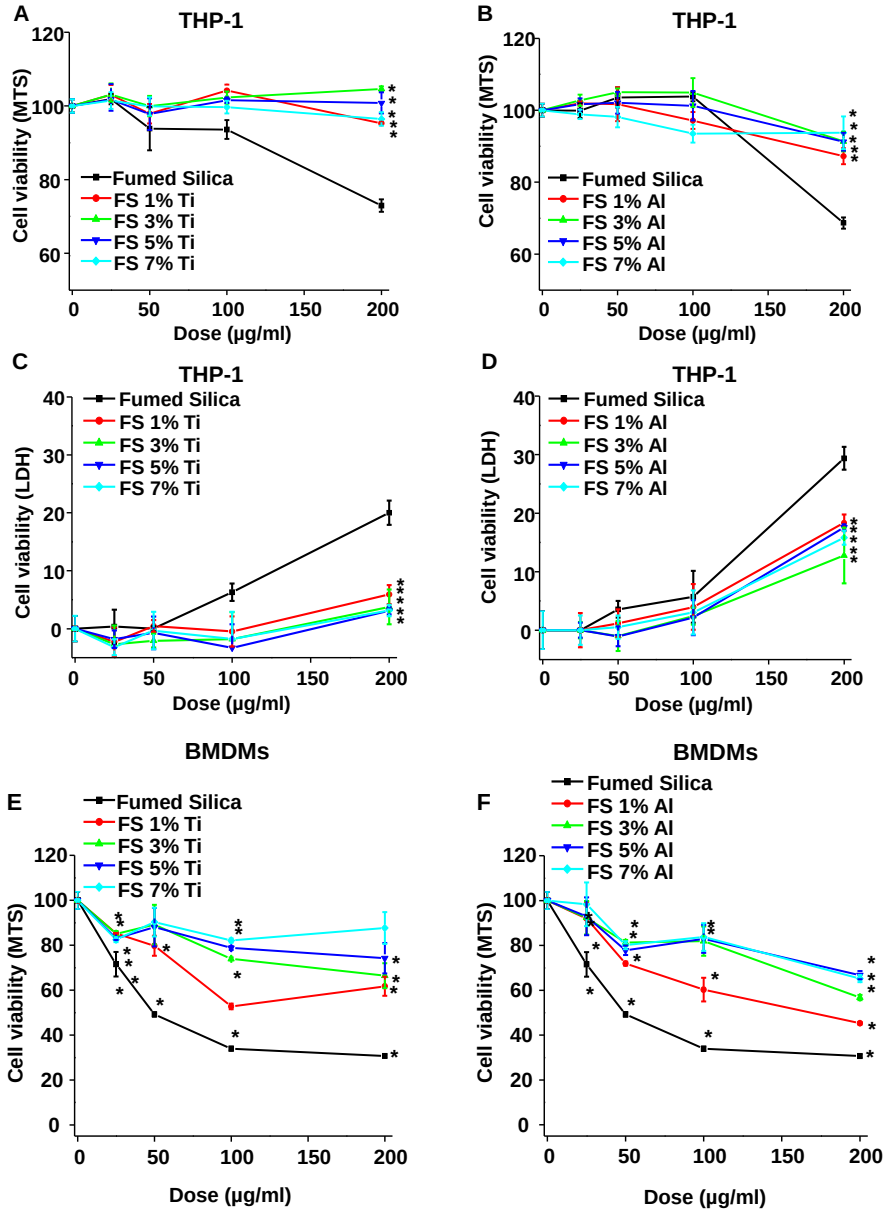

Figure S7. Doping attenuates fumed silica induced cell death of THP-1 cells and BMDMs. (A-B) Cell viability of THP-1 cells after exposure to fumed silica nanoparticles for 24 h was determined using a MTS assay. The cell viability of the fumed silica-treated cells was normalized to the value of non-treated control cells, for which the viability was regarded as 100%. $^{*}p<0.05$ compared to non-doped fumed silica-treated cells. (C-D) Cell

death of THP-1 cells after exposure to fumed silica nanoparticles for 24 h was determined using a LDH assay. The cell death of the fumed silica-treated cells was normalized to the value of non-treated control cells, for which the LDH level was regarded as zero. $^{*}p<0.05$ compared to non-doped fumed silica-treated cells. (E-F) Cell viability of BMDMs after exposure to fumed silica nanoparticles for 24 h was determined using a MTS assay. The cell viability of the fumed silica-treated cells was normalized to the value of non-treated control cells, for which the viability was regarded as 100%. $^{*}p<0.05$ compared to non-doped fumed silica-treated cells.

Figure S8

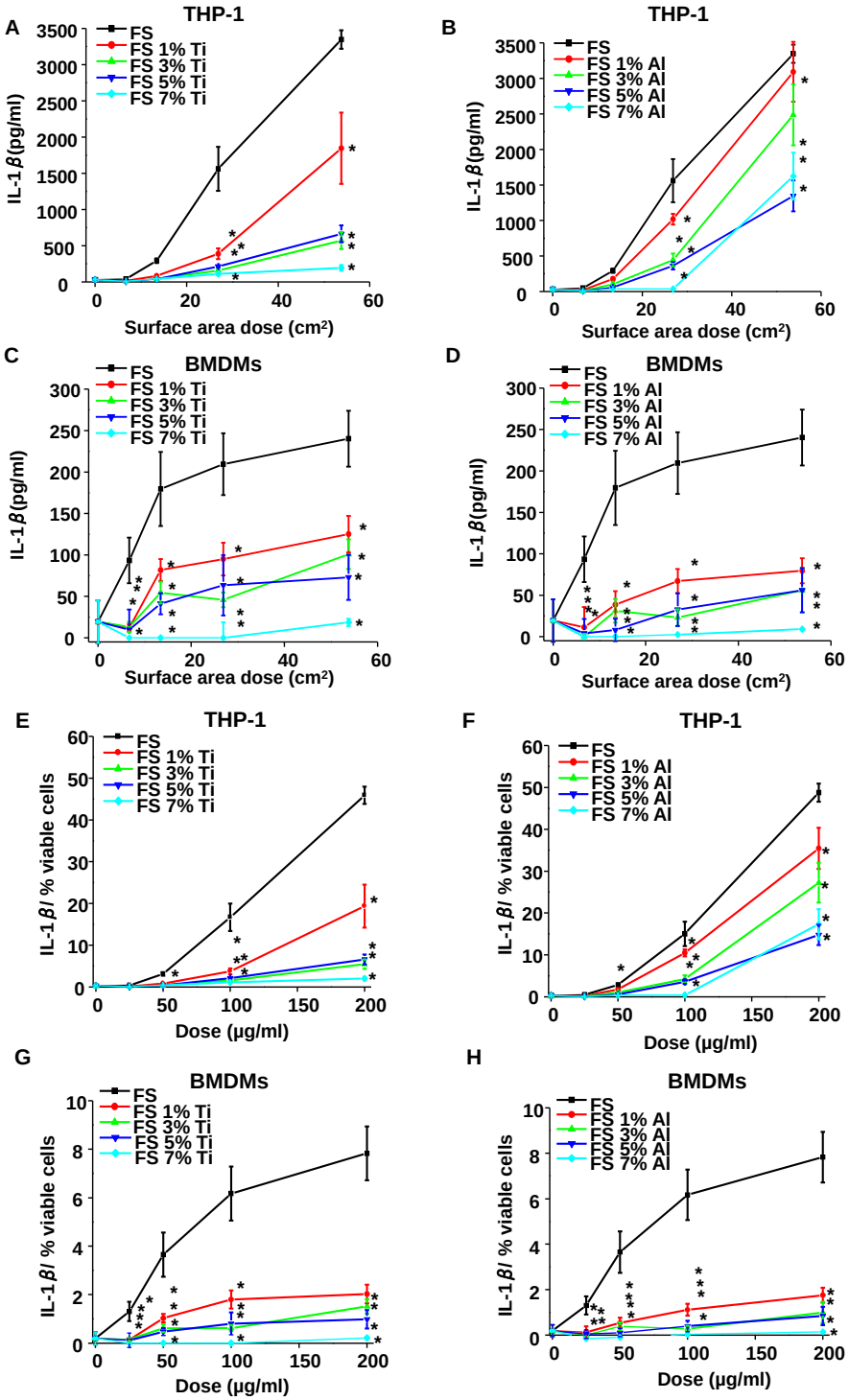
A
THP-1
FS
FS 1% Ti
FS 3% Ti
FS 5% Ti
FS 7% Ti
IL-1β(pg/ml)
Surface area dose (cm²)
B
THP-1
FS
FS 1% Al
FS 3% Al
FS 5% Al
FS 7% Al
IL-1β(pg/ml)
Surface area dose (cm²)
C
BMDMs
FS
FS 1% Ti
FS 3% Ti
FS 5% Ti
FS 7% Ti
IL-1β(pg/ml)
Surface area dose (cm²)
D
BMDMs
FS
FS 1% Al
FS 3% Al
FS 5% Al
FS 7% Al
IL-1β(pg/ml)
Surface area dose (cm²)
E
THP-1
FS
FS 1% Ti
FS 3% Ti
FS 5% Ti
FS 7% Ti
IL-1β/ % viable cells
Dose (µg/ml)
F
THP-1
FS
FS 1% Al
FS 3% Al
FS 5% Al
FS 7% Al
IL-1β/ % viable cells
Dose (µg/ml)
G
BMDMs
FS
FS 1% Ti
FS 3% Ti
FS 5% Ti
FS 7% Ti
IL-1β/ % viable cells
Dose (µg/ml)
H
BMDMs
FS
FS 1% Al
FS 3% Al
FS 5% Al
FS 7% Al
IL-1β/ % viable cells
Dose (µg/ml)

Figure S8. Reduction in IL-1β production by Ti and Al doping of fumed silica nanoparticles. IL-1β production induced by doped fumed silica in (A-B) THP-1 cells and (C-D) BMDMs is plotted as a function of dose expressed as the surface area of the respective fumed silica samples. Naive THP-1 cells were treated with PMA (1 μg/mL) for 16 h. Then PMA-differentiated THP-1 cells were exposed to 6.7-53.8 cm^2 of (A) Ti-doped and (B) Al-doped fumed silica nanoparticles for 24 h in the presence of LPS (10 ng/mL). (C-D) IL-1β production induced by doped fumed silica in bone marrow-derived macrophages (BMDMs). BMDMs obtained from wild type C57BL/6 mice were exposed to 6.7-53.8 cm^2 of (C) Ti-doped and (D) Al-doped fumed silica nanoparticles for 24 h in the presence of LPS (500 ng/mL). IL-1β production was quantified by ELISA. $^{*}p<0.05$ compared to non-doped fumed silica. (E-H) IL-1β production was normalized according to the percentage of viable cells (MTS assay). (E-F) IL-1β production in THP-1 cells after (E) Ti doping or (F) Al doping. (G-H) IL-1β production in BMDMs after (G) Ti doping or (H) Al doping. IL-1β production was quantified by ELISA, cell viability was determined by MTS. $^{*}p<0.05$ compared to non-doped fumed silica.

Figure S9

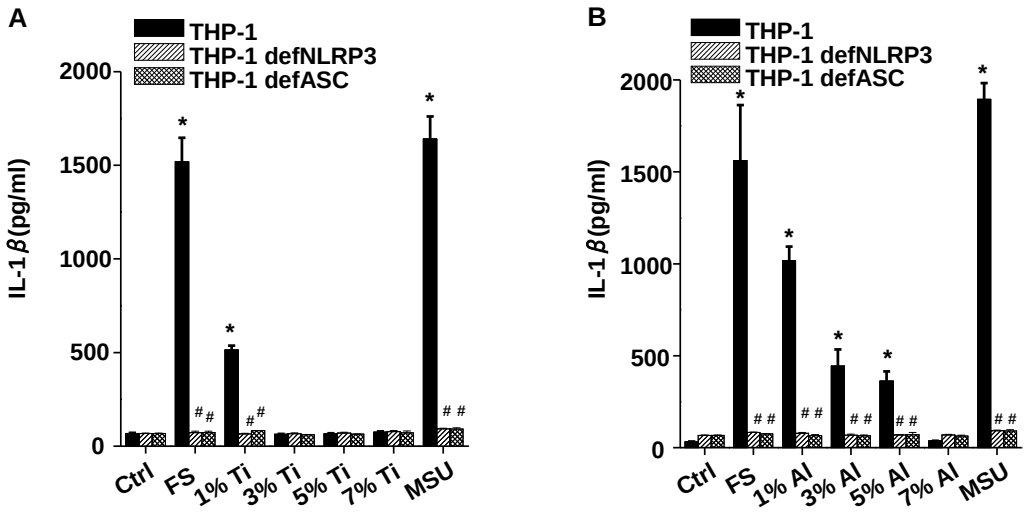

Figure S9. Fumed silica induced IL-β production is NLRP3 inflammasome dependent and is attenuated by Ti and Al doping. Naive THP-1, THP-1 defNLRP3 and THP-1 defASC cells were treated with PMA (1 μg/mL) for 16 h to induce differentiation. PMA-differentiated cells were exposed to (A) Ti-doped and (B) Al-doped fumed silica nanoparticles at 100 μg/mL for 24 h in the presence of LPS (10 ng/mL). IL-1β production was quantified by ELISA. $^{*}p<0.05$ compared to control cells (PMA-differentiated, in the presence of LPS). $^{\#}p<0.05$ compared to same particle-treated wild type THP-1 cells (PMA-differentiated, in the presence of LPS).

Figure S10

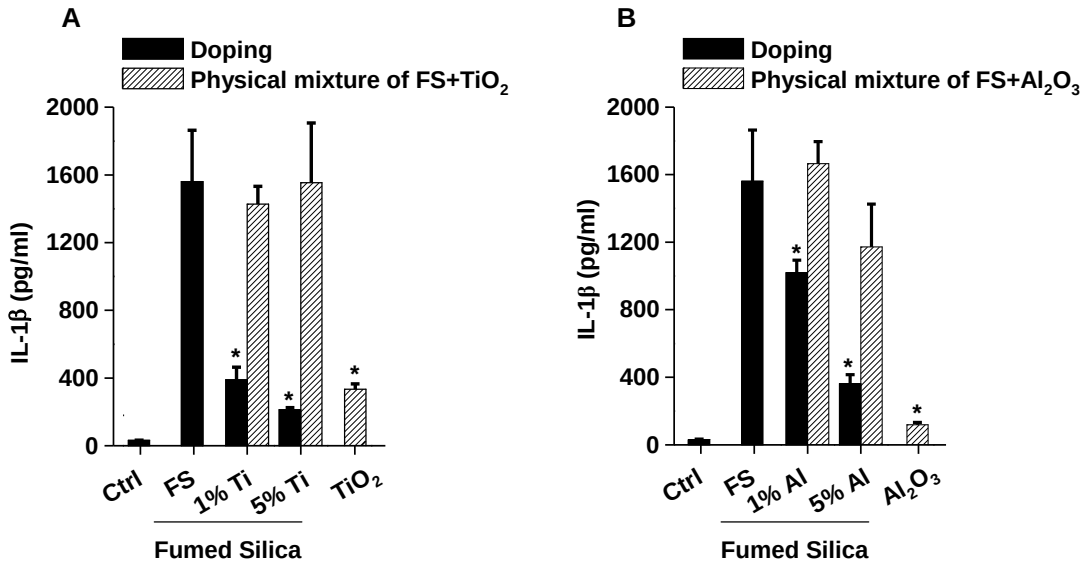

Figure S10. Physical mixing of fumed silica with TiO_2 or Al_2O_3 nanoparticles has no effect on IL-1β production. (A-B) THP-1 cells were treated by (A) Ti-doped and (B) Al-doped fumed silica nanoparticles (100 μg/ml) and the results were compared to cells treated by physical mixtures of fumed silica with (A) TiO_2 or (B) Al_2O_3 nanoparticles for 24 h. IL-1β production was quantified by ELISA. $^{*}p<0.05$ compared to non-doped fumed silica.

Figure S11

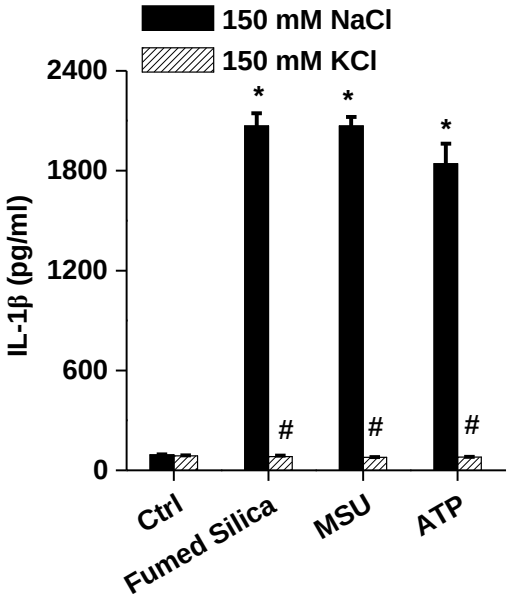

Figure S11. Fumed silica-induced IL-1β production is dependent on potassium efflux. THP-1 cells were treated with fumed silica (100 μg/ml), MSU (100 μg/ml) and ATP (5 mM) in serum-free buffer containing either 150 mM NaCl or 150mM KCl. IL-1β production was quantified by ELISA. $^{*}p<0.05$ compared to control cells without particle treatment; $^{\#}p<0.05$ compared to THP-1 cells of same particle treatment.

Table S1 Hydrodynamic Size and ζ-Potential of Nanoparticles Used in This Study.

	hydrodynamic size (nm)			ζ-potential (mV)		
	Water	PBS&0.6 mg/mL BSA&0.01 mg/ml DPPC	RPMI 1640&10% serum	Water	PBS&0.6 mg/mL BSA&0.01 mg/ml DPPC	RPMI 1640&10% serum
TiO_2	601.9±26.2	985.8±55.1	651.0±42.7	12.8±3.6	-14.5±4.8	-8.8±5.7
Al_2O_3	322.9±14.3	801.4±66.8	369.7±30.7	38.0±0.8	-4.0±10.3	-10.5±5.3

Table S2 Dosimetry Calculations

Calculated fumed silica deposition (mass) during occupational exposure to 10.5 [mg/m^3] in a working facility[1]

1. Calculated monthly SiO_2 deposition (mass) at peak exposure

Assumptions:

Ventilation rate of a healthy human adult: [20 L/min]

Deposition fraction: 30%

Monthly exposure period: 8 [h/day], 5 [day/week], 4 weeks

Calculation of monthly deposition:

$$\frac{10.5mg}{m^3}\times\frac{20L}{\min\cdot person}\times 30\%\times\frac{60\min}{hour}\times\frac{8hour}{day}\times\frac{5day}{week}\times\frac{4weeks}{month}\times\frac{m^3}{1000L}=604.8\,{}^{mg}\!/_{person}$$

2. Monthly deposition level (mass/surface area) in a human worker

Assumptions:

Human alveolar surface area: 102 [m^2/person]

Calculation:

$$\frac{604.8mg}{person}\times\frac{person}{102m^2}\times\frac{1000\mu g}{mg}=5929.4\,{}^{\mu g}\!/_{m^2}$$

3. Comparable deposition level in a mouse receiving a one-time installation

Assumptions:

Alveolar epithelium surface area of a mouse: [0.05 m^2/mouse]

Weight of a mouse: 25 [g]

Calculation:

$$\frac{5929.4\mu g}{m^2}\times\frac{0.05m^2}{mouse}\times\frac{1mg}{1000\mu g}\times\frac{mouse}{25g}\times\frac{1000g}{kg}=11.9\,{}^{mg}\!/_{kg}$$

EXPERIMENTAL SECTION

Cell Culture. Human THP-1 cells were grown in RPMI-1640 media supplemented with 10% (vol/vol) of fetal bovine serum (FBS), 100 U/mL-100 µg/mL of Penicillin-Streptomycin and 50 µM of beta-mercaptoethanol. The passage number of THP-1 cells was maintained between 3 and 10. BMDMs were prepared from the bone marrow of female *wt* C57BL/6 mice. Briefly, femurs and tibia were cut at both ends and the marrow cavity was flushed with DMEM medium using a 5-mL syringe with a 25-G needle. The cell suspension was repeatedly aspirated with a 10-mL pipet to disperse the clumps and then passed through a 70-µm cell strainer. Cells were spin down at 400 g for 10 min at 4 °C, and resuspended in 1 mL of ice-cold 25% LADMAC conditioned medium. The cell concentration was adjusted to 10^6 cells/mL in 25% LADMAC conditioned media, and cells were plated in 100 mm petri dish. Cells were maintained for seven days at 37 °C. The media was replaced with fresh 25% LADMAC conditioned media every two days. After seven days, cells were dissociated from the plate using trypsin and re-plated at 5×10^4 cells/well in complete DMEM medium in a 96-well flat-bottom tissue culture plate. The bone marrow-derived macrophages (BMDMs) were treated with 10 ng/mL of recombinant murine IFN-γ for 48 h prior to use.

Determination of the Abiotic Hydroxyl Radical Generation in Fumed Silica Nanoparticles. Abiotic hydroxyl radical generation by fumed silica nanoparticles was determined by the increased fluorescence of 3'-(p-aminophenyl) fluorescein (APF), which predominantly reacts with hydroxyl radicals. 96 µL of a 10 µmol/L APF suspension in PBS was added to each well of a black 96-well plate (Costar, Corning, NY). 2 µL of 5 mg/mL nanoparticle suspension was subsequently added to each well and

mixed well. Following 6 h incubation, the emission of APF fluorescence was collected at 480-600 nm with an excitation wavelength of 455 nm in a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA).

Determination of Cytotoxicity of Fumed Silica Nanoparticles in THP-1 cells. The cytotoxicity of fumed silica nanoparticles in THP-1 cells was determined by a MTS assay using CellTiter 96 AQueous (Promega Corporation, WI). After 24 h exposure to fumed silica nanoparticles in a 96-well plate, the cell culture medium was removed and replenished with 120 μL of complete cell culture media containing 16.7% of MTS stock solution for an one hour at 37 °C. The plate was centrifuged at 2000*g* for 10 min in an Eppendorf 5430 microcentrifuge with microplate rotor to spin down the cell debris and nanoparticles. 100 μL of the supernatant was removed from each well and transferred into a new 96-well plate. The absorbance of formed formazan was read at 490 nm on a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA). The cytotoxicity of fumed silica nanoparticles in THP-1 cells was also assessed by the LDH assay using CytoTox 96 Non-Radioactive Cytotoxicity Assay (Promega Corporation, WI). After 24 h exposure to fumed silica nanoparticles in a 96-well plate, 10 μL of Lysis Solution was added to each well. The plate was subsequently incubated for one hour at 37 °C and 5% CO_2. Following centrifugation at 250 g for 4 min, 50 μL of cell lysate was mixed with 50 μL of reconstituted Substrate Mix, and was incubated at room temperature for 30 min. 50 μL of Stop Solution was added to each well and the absorbance was read at 490 nm on a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA).

Cellular Uptake Determined by Transmission Electron Microscopy (TEM). Differentiated THP-1 cells were treated with fumed silica nanoparticles for 12 h. The

cells was collected and washed with PBS. The cells were treated with 2.5 % of glutaraldehyde (in PBS) for 2 h at room temperature. After fixation in 1% of OsO4 in PBS for 1 h, the cells were dehydrated in a graded ethanol series, treated with propylene oxide, and embedded in Epon. Approximately 60–70 nm thick sections were prepared on a Reichert-Jung Ultracut E ultramicrotome and placed on Formvar-coated copper grids. The sections were stained with uranyl acetate and Reynolds lead citrate and examined on a JEOL 100CX electron microscope at 80 kV in the UCLA BRI Electron Microscopy Core.

Determination of Cell Membrane Potential. Cell membrane potential in THP-1 cells after fumed silica exposure was determined using the FLIPR assay kit (Molecular Devices, Sunnyvale, CA). Briefly, differentiated THP-1 cells were exposed to fumed silica for 1h, following which the cells were loaded with FLIPR reagent (100 µl/well, red) for 30 min at 37 °C. The fluorescence was measured at Excitation530/Emission565 using a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA).

Determination of Intracellular GSH Content. A GSH-Glo assay kit (Promega, Madison, WI) was used to determine the intracellular GSH levels after fumed silica exposure. The THP-1 cells were exposed to fumed silica (100 µg/mL) in a 96-well plate at 37 °C and 5% CO_2 for the indicated time. After exposure, the cellular supernatant was removed and 100 µL of GSH-Glo reaction buffer containing Luciferin-NT and glutathione S-transferase was added to each well and incubated at room temperature with constant shaking for 30 min. Subsequently, 100 uL of Luciferin D detection reagent was added to each well and the plate was incubated at room temperature with constant

shaking for another 15 min. The luminescent signal was quantified using a SpectraMax M5 microplate reader (Molecular Devices; Sunnyvale, CA).

REFERENCES AND NOTES

1. Choudat, D.; Frisch, C.; Barrat, G.; el Kholti, A.; Conso, F., Occupational Exposure to Amorphous Silica Dust and Pulmonary Function. *Br. J. Ind. Med.* **1990**, 47, 763-766.