Mechanistic Differences in Cell Death Responses to Metal-based Engineered Nanomaterials in Kupffer Cells and Hepatocytes

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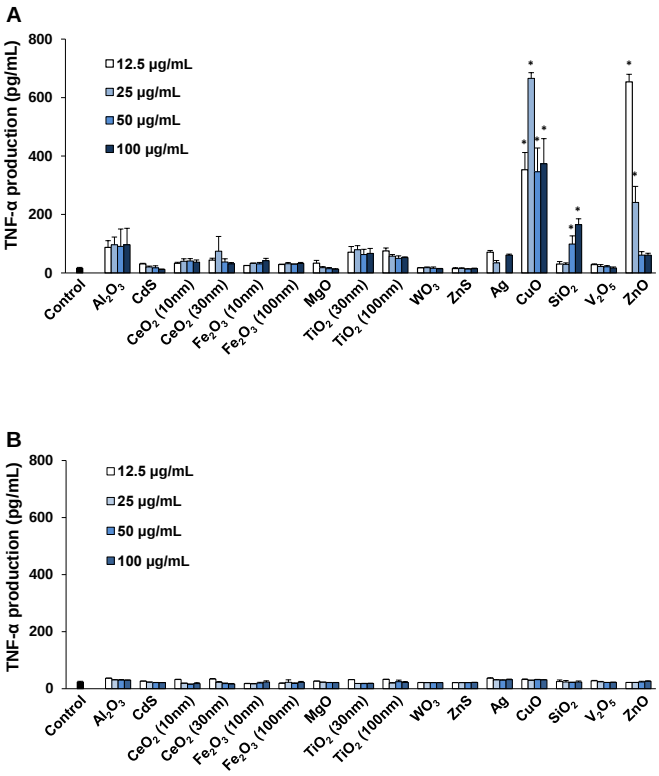

Figure S1. **Assessment of nanoparticles induction of TNF-α release in KUP5 and Hepa 1-6 cells**. (A) Dose-dependent TNF-α release by (A) KUP5 and (B) Hepa 1−6 cells after exposure to nanoparticles for 24 h over a dose range of 12.5−100 μg/mL. Supernatants were collected to measure TNF-α production by ELISA. *$p < 0.05$ compared to control cells.

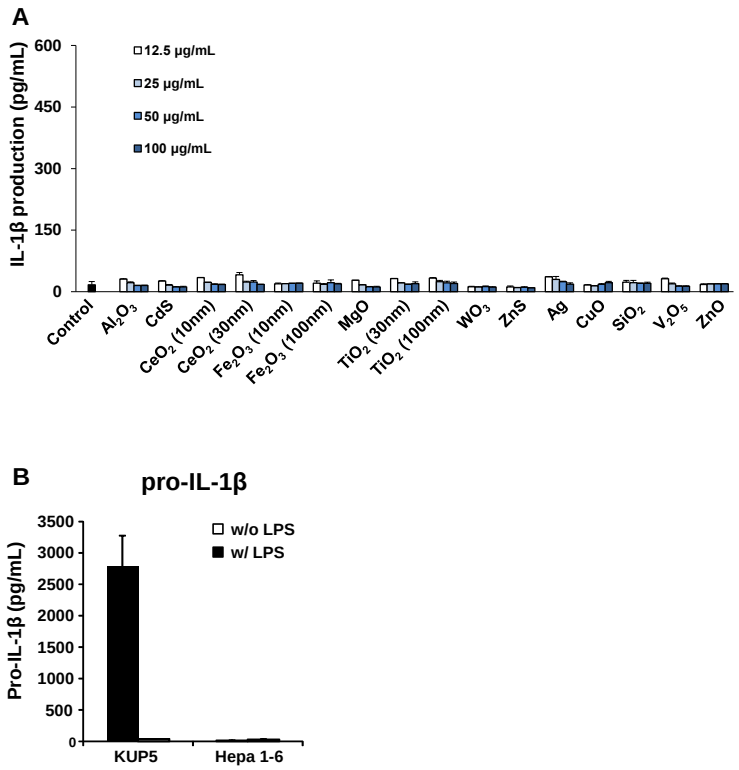

Figure S2. **Assessment of IL-1β levels in Hepa 1-6 cells and pro-IL-1β level in KUP5 and Hepa 1-6 cells**. (A) KUP5 and Hepa 1−6 cells were treated with 1 μg/mL of LPS for 4 h. Cells were lysed to release intracellular proteins. Pro-IL-1β levels were quantified by ELISA; (B) Dose-dependent IL-1β release by 16 NPs in Hepa 1−6 cells. Hepa 1−6 cells were exposed to NPs (12.5−100 μg/mL) for 24 h. Supernatants were collected to measure IL-1β production by ELISA; *$p < 0.05$ compared to control cells.

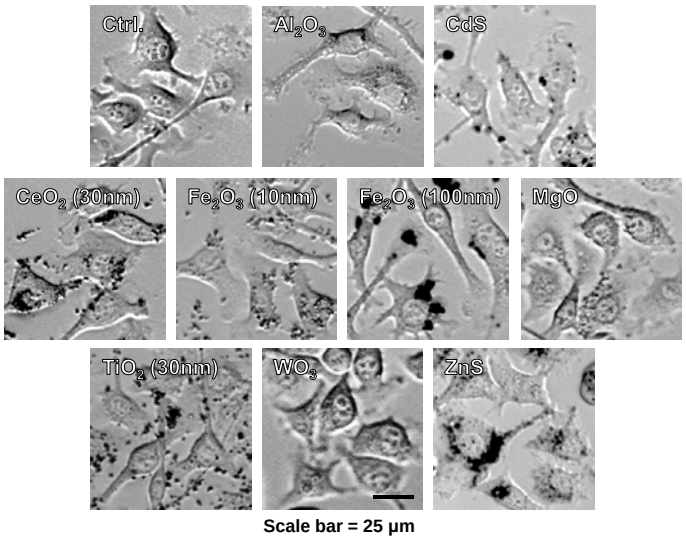

Figure S3. **Optical microscopy images of NP-treated KUP5 cells.** Optical microscopy images showing the morphology of KUP5 cells exposed to the other eleven NPs in addition to the seven in Figure 4A at 12.5 µg/mL for 6 h. The scale bar is 25 µm.

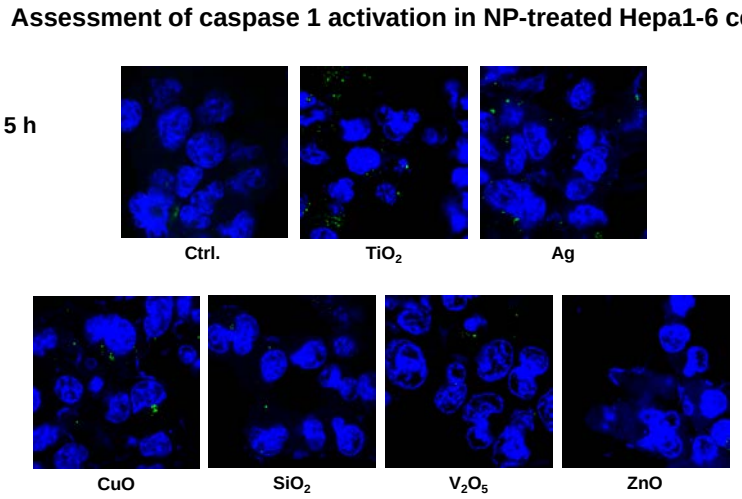

Figure S4. **Assessment of caspase 1 activation in NP-treated Hepa 1−6 cells**. Hepa 1−6 cells were incubated with nanoparticles at 25 μg/mL for 5 h. Cells were washed with PBS and stained with FAM-FLICA Caspase-1 substrate for 1 h at 37 °C following the manufacturer's procedure. Cells were then stained with Hoechst 33342.

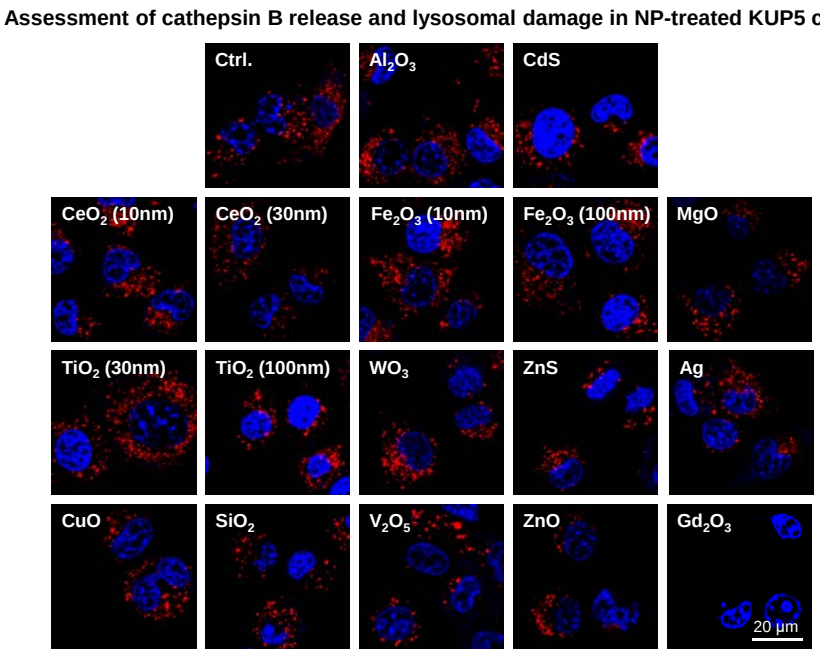

Figure S5. **Confocal microscopy to assess lysosome damage in KUP5**. LPS-primed (1 µg/mL, 4 h) KUP5 cells were exposed to 25 µg/mL of nanoparticles for 3 h. Cells were stained with Magic Red-labeled cathepsin B substrate for 30 min as well as Hoechst 33342 to reveal nuclear localization. Confocal images were acquired with a Confocal SP8-SMD microscope using DAPI and Magic Red filters. The scale bar is 20 µm. Gd_2O_3 NPs was used as a positive control.

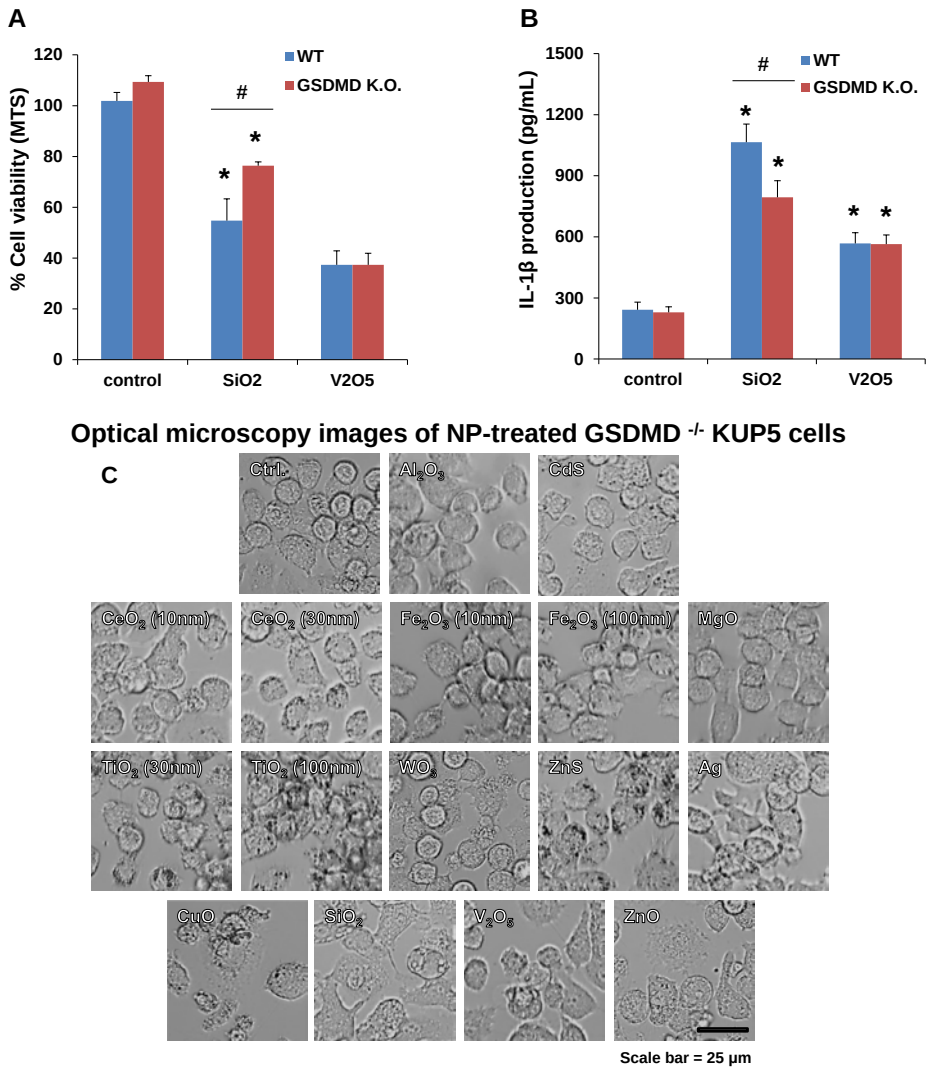

Figure S6. **Comparison of the NP-induced IL-1β release in wildtype and GSDMD$^{-/-}$ KUP5 cells**. (A) Cell viability screening of wildtype and GSDMD$^{-/-}$ KUP5 exposed to SiO_2 and V_2O_5, NPs. Use of MTS assay to assess the viability of LPS-primed (1 μg/mL for 4 h) wildtype and GSDMD$^{-/-}$ KUP5 cells post-exposed to 50 μg/mL of the NPs for 18 h. The viability of nontreated control cells was regarded as 100%; (B) IL-1β release by SiO_2 and V_2O_5 NPs in wildtype and GSDMD$^{-/-}$ KUP5 cells. LPS-primed (1 μg/mL for 4 h) KUP5 cells were exposed to nanoparticles at 50 μg/mL for 24 h. Supernatants were collected to measure IL-1β production by ELISA. *$p < 0.05$ compared to control cells; #$p < 0.05$ between wildtype and GSDMD$^{-/-}$ KUP5 cells; (C) Optical microscopy images showing the morphology of GSDMD$^{-/-}$ cells exposed to the 16 NPs (12.5 μg/mL) for 6 h. The scale bar is 25 μm.

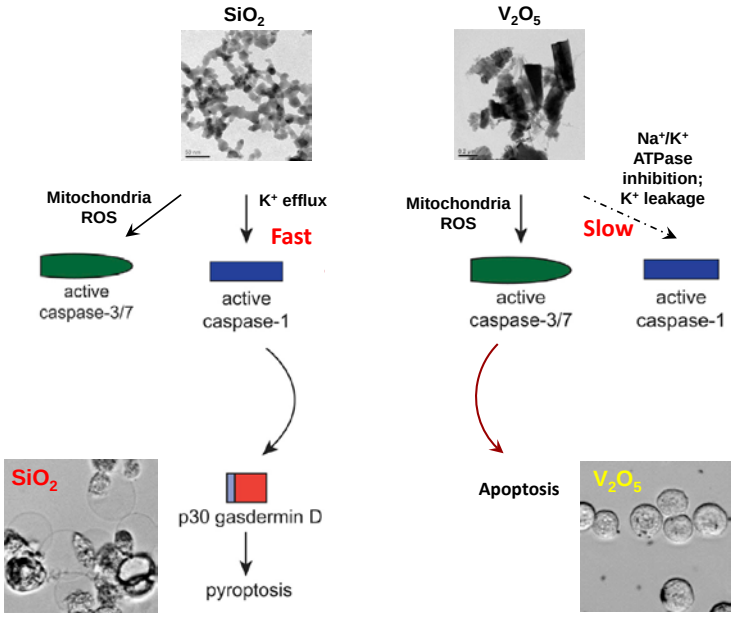

Figure S7. A schematic to explain contrasting mechanisms in cell death between SiO_2 and V_2O_5 NPs in KUP5 cells.

Supporting Experimental Section

Determination of TNF-α Production: KUP5 and Hepa 1−6 cells in 100 μL of tissue culture medium were plated overnight at a density of 2×10^4 cells per well in a 96-well plate. The cells were primed by replacing the tissue culture medium with fresh medium containing 1 μg/mL LPS to treat for 4 h. The primed cells were exposed to nanoparticles at the indicated concentrations and time periods as shown in each figure legend. The cellular supernatants were collected for TNF-α quantification by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

Determination of Pro-IL-1β Activity in LPS-Primed Cells: Hepa 1−6 cells were plated overnight in 96-well plates at 2×10^4 cells per well. Following priming of the cells with 1 μg/mL LPS for 4 h, cells were lysed by using three freeze−thaw cycles in 100 μL of lysis buffer (10 μM 2-ME, 9 mM MgCl2, and 0.1% triton X-100 in PBS). The cellular lysates were obtained after centrifugation and used for the quantification of pro-IL-1β by an ELISA (Thermo Fisher Scientific).

Determination of Caspases 1 Activity by Confocal Microscopy: Hepa 1-6 cells were cultured in an 8-well Lab-Tek chamber slide at 5×10^4 cells/400 μL medium at 37 °C and 5% CO_2. The cells were incubated with nanoparticles at 50 μg/mL for 3 h. The treated cells were washed with PBS and stained with FAM-FLICA Caspase-1 or Caspase 3/7 substrates for 1 h at 37 °C following the manufacturer's procedure. Finally, the cells were fixed with 4 % paraformaldehyde in PBS, stained with Hoechst 33342 (5 μg/mL), and imaged using Leica Confocal SP8-SMD microscope.

Assessment of Lysosomal Damage by Cathepsin B Staining: After KUP5 cells were cultured overnight in an 8-well Lab-Tek chamber slide at 5×10^4 cells per well, the cells were primed with LPS (1 μg/mL) for 4 h and exposed to particles (50 μg/mL) for 1 h at 37 °C. The cells were washed with PBS and incubated with Magic Red working solution for 30 min at 37 °C. Cells were washed with PBS and fixed with 4% paraformaldehyde in PBS. Finally, the cells were stained with Hoechst 33342 (5 μg/mL), washed with PBS, and imaged under a Leica Confocal SP8-SMD microscope.

siRNA Knockdown in KUP5 Cells: Knockdown of GSDMD gene was performed in KUP5 cells using electroporation at the Integrated Molecular Technologies Core Facility (University of California, Los Angeles). Briefly, 6 μg of siRNA in 100 μL of media was electroporated into 1×10^6 KUP5 cells. After electroporation, cells were maintained in complete media for another 48 h before cytotoxicity and IL-1β production analysis.

Assessment of Pyroptotic Cell Death under Optical Microscopy: Optical microscopy was used to monitor the morphology of GSDMD knockdown KUP5 cells, pretreated with 1 μg/mL LPS for 4 h, during exposure to 12.5 μg/mL nanoparticles for 6 h at 37 °C and 5% CO_2 in an 8-well Lab-Tek chamber slide. The cells were examined using a Zeiss Optical Microscope. In order to monitor the time kinetics of the pyroptosis event in LPS pretreated cells, 10×10^4 KUP5 cells/mL were exposed to 50 μg/mL nanoparticles for 6 h in a 35 mm glass bottom dish (In Vitro Scientific) at 37 °C and 5% CO_2. Light optic images were obtained using a Leica Confocal SP5 Blue microscope.

Assessment of Nanoparticle Cytotoxicity: The viability of KUP5 cells was assessed by the MTS assay. Cells were exposed to V_2O_5 nanoparticles and V^{5+} ions at the indicated concentrations for 18 h in a 96-well plate, followed by removal of the medium and replacement with 120 μL of complete cell culture media containing 16.7% of an MTS stock solution for 0.5 h at 37 °C. The plates were centrifuged at 2000g for 10 min in an Eppendorf 5430 microcentrifuge, and then 100 μL of supernatant was collected from each well and transferred into a new 96-well plate. The absorbance was read at 490 nm on a SpectraMax M5e microplate reader (Molecular Devices, Sunnyvale, CA).