Supplemental Materials

Toxicological Profiling of Metal Oxide Nanoparticles in Liver Context reveals Pyroptosis in Kupffer Cells and Macrophages *Versus* Apoptosis in Hepatocytes

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Table S1. MOx nanoparticle library, depicting the commercial source, crystal structure, primary size, hydrodynamic size and zeta potential. The hydrodynamic size and zeta potential were determined in deionized water and complete cell culture medium (supplemented with 10% fetal bovine serum). While some of the data for primary particle size and crystallinity were obtained from our historical data bank (see previous publications as listed below the table), new measurements were obtained for newly introduced REOs.

Nanoparticles	Source	Crystal Structure	Primary Size (nm)	Hydrodynamic Size (nm)		z-Potential (mV)	
				DI Water	DMEM	DI Water	DMEM
Al_2O_3	Meliorum	Rhombohedral*	14.7 ± 5.2*	441.4 ± 22.4	428.4 ± 6.2	41.0 ± 0.7	-12.0 ± 1.0
CeO_2	Meliorum	Cubic*	18.3 ± 6.8*	541.9 ± 27.1	250.3 ± 3.2	21.9 ± 0.8	-15.3 ± 0.7
CoO	US-Nano	Cubic*	71.8 ± 16.2*	830.1 ± 38.4	585.6 ± 16.4	23.2 ± 1.9	-12.8± 1.5
Co_3O_4	Lutz Madler	Cubic*	10.0 ± 2.4*	336.7 ± 5.5	311.7 ± 3.7	23.9 ± 2.1	-10.7 ± 1.2
Cr_2O_3	US-Nano	Rhombohedral*	193.0 ± 90.0*	284.7 ± 3.7	298.8 ± 2.2	-17.9 ± 0.3	-9.5 ± 1.8
CuO	Lutz Madler	Monoclinic*	12.8 ± 3.4*	266.0 ± 9.8	284.7 ± 5.2	26.8 ± 1.5	-12.2 ± 1.0
Dy_2O_3	US-Nano	Cubic	37.5 ± 6.6*	818.4 ± 37.9	738.6 ± 40.1	9.7 ± 1.6	-9.1 ± 0.6
Er_2O_3	US-Nano	Cubic	113.8 ± 37.8*	390.6 ± 19.7	267.4 ± 3.6	34.0 ± 1.4	-16.4 ± 3.1
Eu_2O_3	US-Nano	Cubic	52.8 ± 11.7*	379.5 ± 7.3	588.1 ± 11.9	31.8 ± 1.2	-14.9 ± 1.8
Fe_2O_3	US-Nano	Rhombohedral*	12.3 ± 2.9*	296.5 ± 12.8	377.8 ± 15.9	20.7 ± 2.2	-15.2 ± 3.5
Fe_3O_4	Lutz Madler	Cubic*	12.0 ± 3.2*	146.2 ± 1.6	218.4 ± 3.4	-23.6 ± 0.2	-14.2 ± 2.2
Gd_2O_3	NanoAmor	Cubic*	43.8 ± 15.8*	655.3 ± 16.5	898.0 ± 13.2	34.6 ± 2.3	-10.7 ± 1.4
HfO_2	US-Nano	Monoclinic*	28.4 ± 7.3*	369.7 ± 3.1	237.8 ± 7.6	26.3 ± 1.4	-12.6 ± 2.2
In_2O_3	US-Nano	Cubic*	59.6 ± 19.0*	193.4 ± 5.4	241.8 ± 5.2	50.1 ± 1.4	-13.6 ± 2.7
La_2O_3	NanoAmor	Hexagonal*	24.6 ± 5.3*	997.1 ± 98.6	874.3 ± 32.8	13.4 ± 1.1	-11.3 ± 0.7
Mn_2O_3	NanoAmor	Tetragonal*	51.5 ± 7.3*	493.0 ± 21.4	426.7 ± 18.9	-48.5 ± 0.6	-14.0 ± 1.1
Nd_2O_3	NanoAmor	Cubic	133.8 ± 51.6*	448.4 ± 13.6	338.8 ± 3.2	18.5 ± 0.3	-14.6 ± 1.7
NiO	Sigma	Cubic*	13.1 ± 5.9*	425.6 ± 6.4	457.4 ± 3.0	43.0 ± 0.2	-12.8 ± 2.9
Ni_2O_3	US-Nano	Hexagonal*	140.6 ± 52.5*	482.8 ± 14.7	339.6 ± 12.9	18.5 ± 1.1	-11.3 ± 1.7
Sb_2O_3	Lutz Madler	Orthorhombic*	11.8 ± 3.3*	237.4 ± 16.4	235.2 ± 5.0	-34.6 ± 1.0	-13.6 ± 3.3
SiO_2	NanoAmor	N/A*	13.5 ± 4.2*	337.8 ± 20.8	277.2 ± 8.5	-37.3 ± 1.5	-15.4 ± 4.0
Sm_2O_3	US-Nano	Cubic	108.3 ± 47.4*	797.3 ± 57.0	797.6 ± 14.7	40.1 ± 1.8	-11.4 ± 2.6
SnO_2	US-Nano	Tetragonal*	62.4 ± 13.2*	493.3 ± 23.6	538.1 ± 13.4	-38.6 ± 0.6	-13.5 ± 0.9
TiO_2	Lutz Madler	Tetragonal*	12.6 ± 4.3*	229.5 ± 5.4	201.9 ± 3.5	4.0 ± 0.4	-11.9 ± 1.6
WO_3	Lutz Madler	Monoclinic*	16.6 ± 4.3*	119.1 ± 4.5	116.2 ± 8.4	-44.9 ± 1.3	-14.5 ± 1.8
Y_2O_3	Meliorum	Cubic*	32.7 ± 8.1*	1004.1± 166.8	500.1 ± 33.0	28.5 ± 1.6	-9.8 ± 1.1
Yb_2O_3	MK-Nano	Cubic*	61.7 ± 11.3*	601.8 ± 29.6	352.8 ± 53.3	7.0 ± 0.9	-13.1 ± 1.4
ZnO	Lutz Madler	Hexagonal*	22.6 ± 5.1*	369.0 ± 8.5	327.6 ± 55.5	18.3 ± 0.4	-12.3 ± 2.4
ZrO_2	US-Nano	Monoclinic*	40.1 ± 12.6*	404.9 ± 63.0	200.8 ± 10.8	-16.1 ± 0.8	-13.6 ± 2.3

*Zhang *et al.* ACS Nano 2012, 6, 4349-4368; Li *et al.* ACS Nano 2014, 8, 1771-1783.

Figure Legends:

Video S1. Time-lapse optical microscopy video to show the evolving features of pyroptosis in KUP5 cells treated with Gd_2O_3 particles. LPS-primed (1 µg/mL, 4 h) KUP5 cells were seeded into a glass bottom petri dish and exposed to 50 µg/mL Gd_2O_3 nanoparticles. The cellular features of swelling and membrane blebbing was monitored and captured by confocal microscopy over 6 h.

Figure S1. REO nanoparticles induce pyroptosis in KUP5 cells. LPS-primed (1 µg/mL, 4 h) KUP5 cells were exposed to 12.5 µg/mL MOx nanoparticles for 6 h. Optical microscopy images show cell swelling or membrane blebbing by REOs (except Yb_2O_3) in KUP5 cells. In contrast, ZnO nanoparticles did not induce cell swelling. The scale bar is 25 µm.

Figure S2. REO nanoparticles induce apoptotic cell death in Hepa 1-6 cells. LPS-primed (1 µg/mL, 4 h) Hepa 1-6 cells were exposed to 12.5 µg/mL MOx nanoparticles for 18 h. Optical microscopy images show no cell swelling or membrane blebbing for REO-treated Hepa 1-6 cells. The scale bar is 25 µm.

Figure S3. Western blotting to show cleaved and activated caspases 3 and 7. The activation of these caspases was performed in KUP5 (A) and Hepa 1-6 (B) cells following their incubation with 50 µg/mL of nanoparticle for 3 h. Immunoblotting was performed as described in Materials and Methods.

Figure S4. REOs induce NLRP3 inflammasome activation in KUP5 cells. siRNA knockdown of NLRP3 (A) and caspase-1 (B) ameliorated the REO nanoparticle-induced cell death in KUP5 cells. LPS-primed (1 µg/mL, 4 h) KUP5 cells were exposed to 200 µg/mL MOx nanoparticles for 3 h. Cell death was determined through the use of a LDH assay. IL-1β release in the

supernatant was quantified by ELISA (C,D). Nigericin (10 μM) was used as a positive control. $^{*}p<0.05$ compared to particle treated wild type KUP5 cells.

Figure S5. Dose-dependent IL-1β production in response to exposure to the MOx nanoparticles in LPS-primed J774A.1, RAW 264.7 cells and BMDMs. (A-C) LPS-primed J774A.1 (1 μg/mL, 4 h), RAW 264.7 cells (0.1 μg/mL, 4 h) and BMDMs (1 μg/mL, 4 h) were exposed to a wide dose range of particles (6.25-200 μg/mL for J774A.1 and RAW 264.7 cells, and 25-200 μg/mL for BMDMs) for 24 h. IL-1β production in the supernatant was quantified by ELISA.

Figure S6. REO nanoparticles induce non-pyroptotic cell death in primary hepatocytes. LPS-primed (1 μg/mL, 4 h) primary hepatocytes were exposed to 50 μg/mL MOx nanoparticles for 24 h. (A) Cell death was determined by the MTS assay. (B) IL-1β release in the supernatant was quantified by ELISA. Nigericin (10 μM) was used as a positive control. Optical microscopy images show no cell swelling or membrane blebbing for REO-treated primary hepatocytes (C). The scale bar is 25 μm. $^{*}p<0.05$ compared to untreated control cells.

Figure S7. TMOs reduce intracellular GSH levels in KUP5 and Hepa 1-6 cells. KUP5 and Hepa 1-6 cells were exposed to 50 μg/mL Co_3O_4 and ZnO nanoparticles for 24, and their intracellular GSH levels were measured by the GSH-Glo™ Glutathione Assay according to the manufacturer's instructions. $^{*}p<0.05$ compared to untreated control cells.

Figure S1

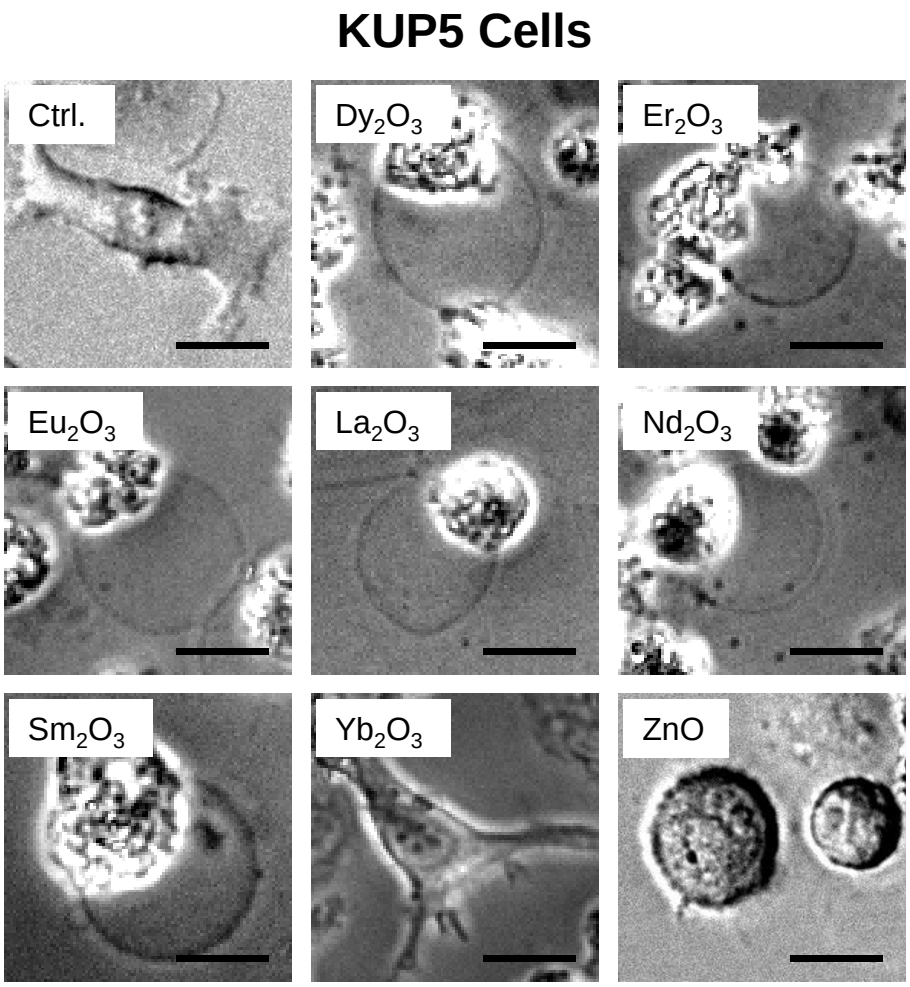

KUP5 Cells
Ctrl.
Dy_2O_3
Er_2O_3
Eu_2O_3
La_2O_3
Nd_2O_3
Sm_2O_3
Yb_2O_3
ZnO

Figure S2

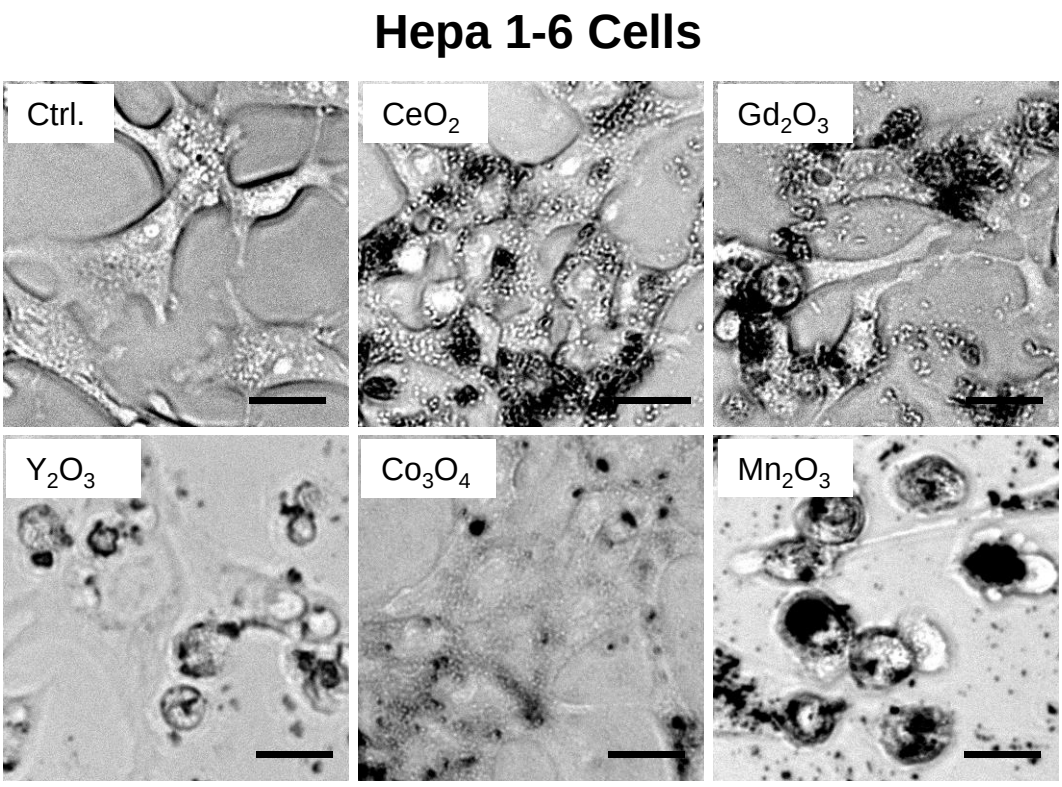


Figure S3

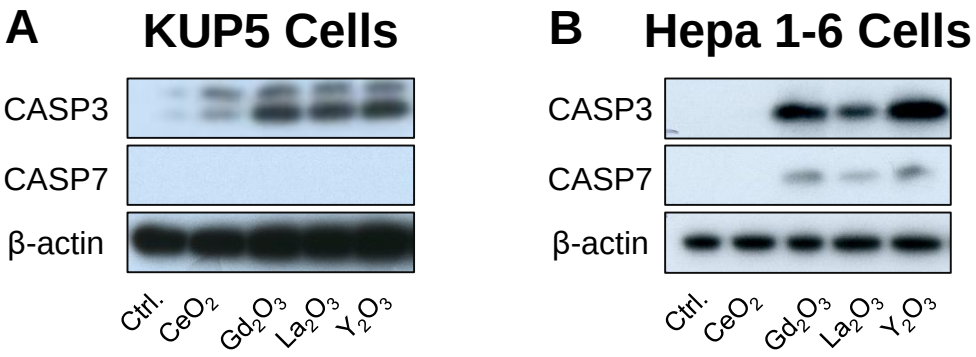

A
KUP5 Cells
CASP3
CASP7
β-actin
Ctrl.
CeO2
Gd2O3
La2O3
Y2O3
B
Hepa 1-6 Cells
CASP3
CASP7
β-actin
Ctrl.
CeO2
Gd2O3
La2O3
Y2O3

Figure S4

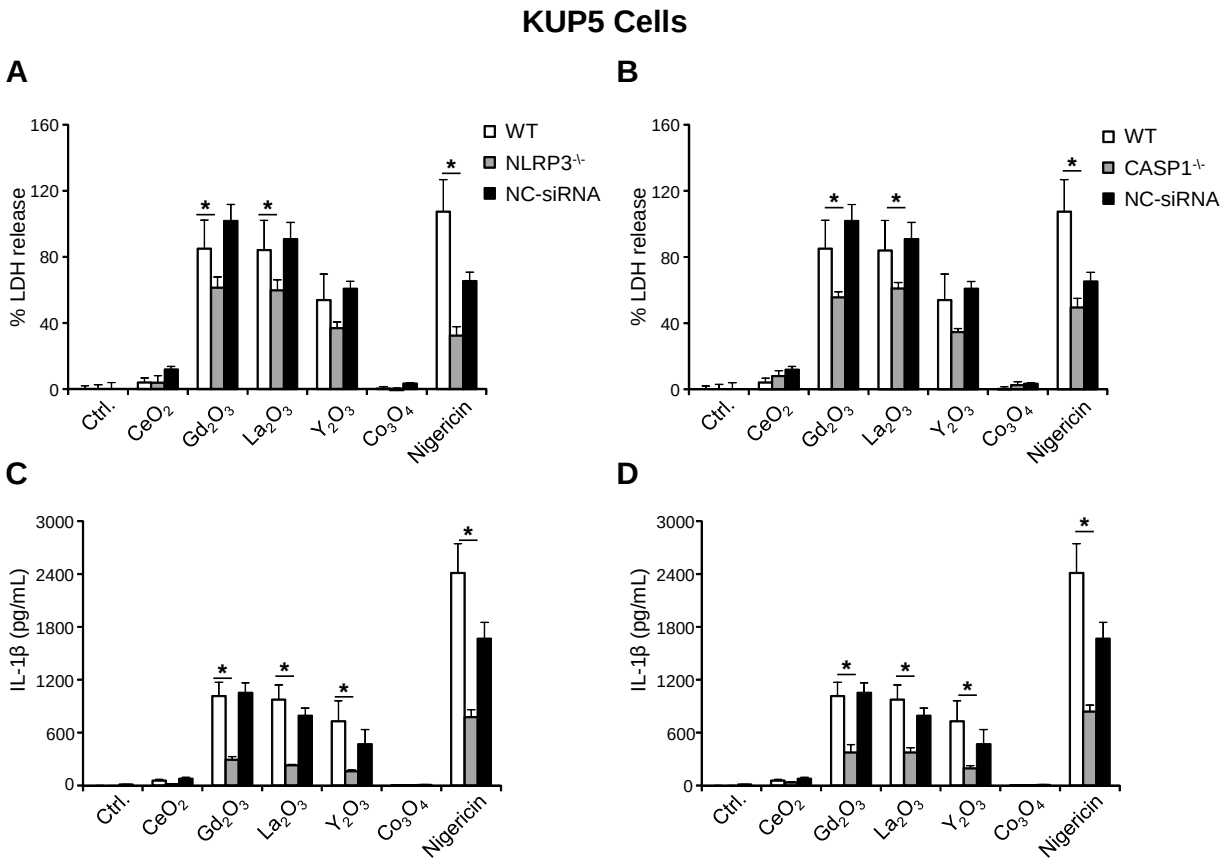
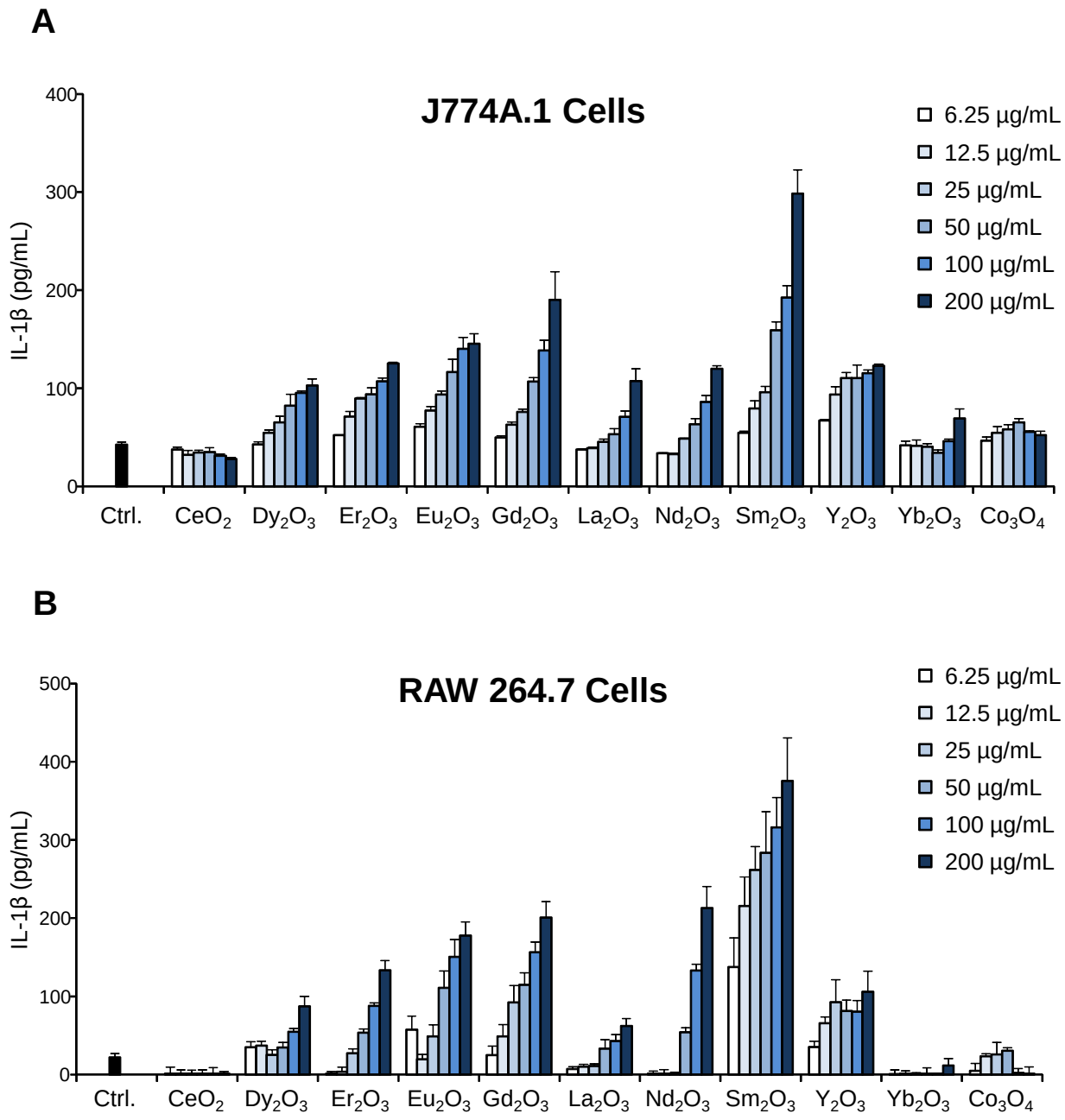

KUP5 Cells
A
B
C
D
% LDH release
IL-1β (pg/mL)
WT
NLRP3-/-
CASP1-/-
NC-siRNA
Ctrl.
CeO2
Gd2O3
La2O3
Y2O3
Co3O4
Nigericin
*

Figure S5


A
J774A.1 Cells
IL-1β (pg/mL)
400
300
200
100
0
Ctrl.
CeO2
Dy2O3
Er2O3
Eu2O3
Gd2O3
La2O3
Nd2O3
Sm2O3
Y2O3
Yb2O3
Co3O4
6.25 µg/mL
12.5 µg/mL
25 µg/mL
50 µg/mL
100 µg/mL
200 µg/mL
B
RAW 264.7 Cells
IL-1β (pg/mL)
500
400
300
200
100
0
Ctrl.
CeO2
Dy2O3
Er2O3
Eu2O3
Gd2O3
La2O3
Nd2O3
Sm2O3
Y2O3
Yb2O3
Co3O4
6.25 µg/mL
12.5 µg/mL
25 µg/mL
50 µg/mL
100 µg/mL
200 µg/mL

C

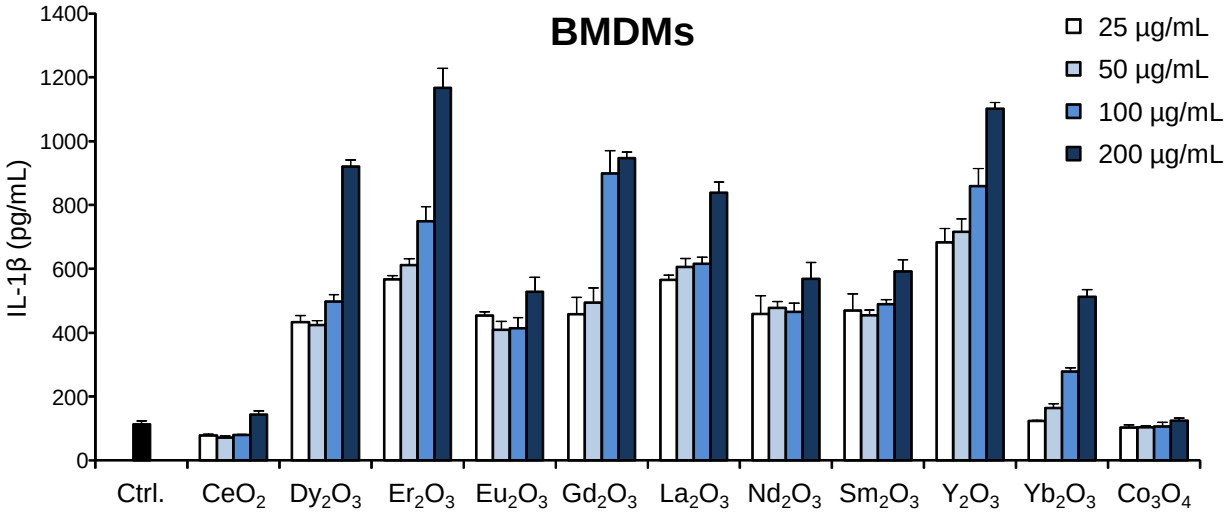

BMDMs
25 μg/mL
50 μg/mL
100 μg/mL
200 μg/mL
IL-1β (pg/mL)
1400
1200
1000
800
600
400
200
0
Ctrl.
CeO2
Dy2O3
Er2O3
Eu2O3
Gd2O3
La2O3
Nd2O3
Sm2O3
Y2O3
Yb2O3
Co3O4

Figure S6

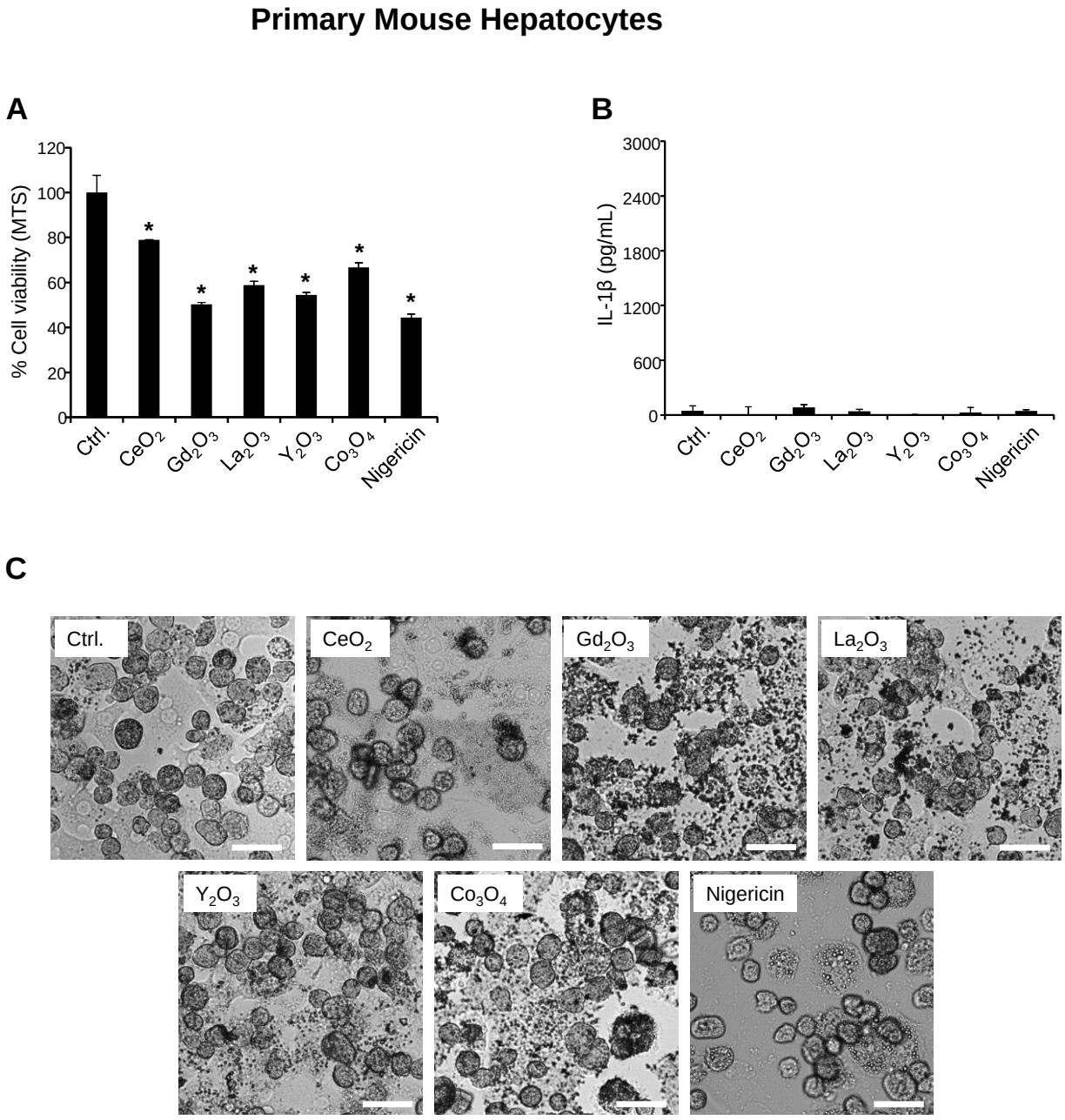
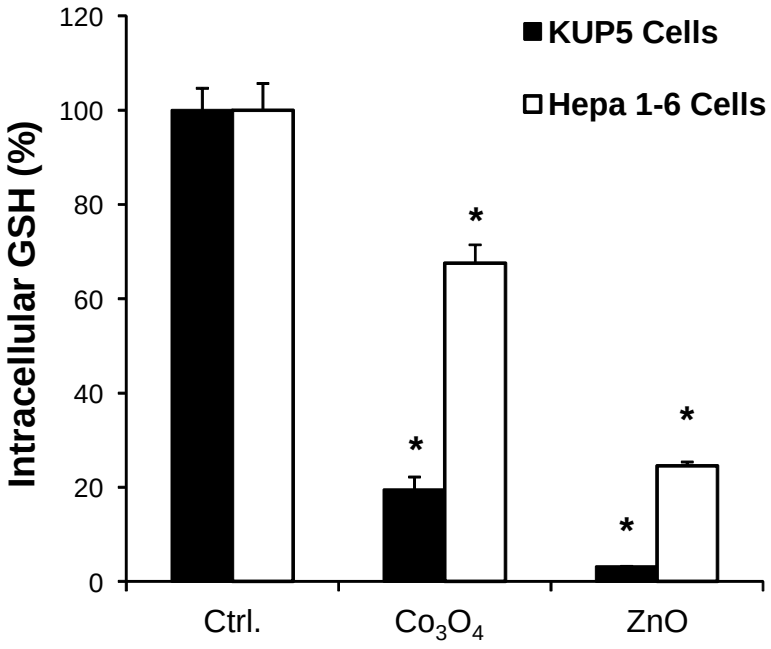

Primary Mouse Hepatocytes
A
% Cell viability (MTS)
120
100
80
60
40
20
0
Ctrl.
CeO2
Gd2O3
La2O3
Y2O3
Co3O4
Nigericin
B
IL-1β (pg/mL)
3000
2400
1800
1200
600
0
Ctrl.
CeO2
Gd2O3
La2O3
Y2O3
Co3O4
Nigericin
C
Ctrl.
CeO2
Gd2O3
La2O3
Y2O3
Co3O4
Nigericin

Figure S7


120
100
80
60
40
20
0
Intracellular GSH (%)
KUP5 Cells
Hepa 1-6 Cells
*
*
*
Ctrl.
Co3O4
ZnO