Electronic Supplementary Material (ESI) for Toxicology Research.

Additional Material

Supplementary Table 1

sequence ID	gene name	sequence ID	gene name	sequence ID	gene name	sequence ID	gene name
AB040883	KIAA1450	BC073167	EDG2	NM_003897	IER3	NM_030583	MATN2
AB052918	CLCC1	BC073983	EGR1	NM_003955	SOCS3	NM_052815	IER3
AB084281	N/A	BC074742	OPCML	NM_004387	NKX2-5	NM_130845	SNTB2
AF107493	RBM5	BC101031	GALNT13	NM_005219	DIAPH1	NM_144999	LRRC45
AF177203	CEECAM1	BC101568	UNC93B1	NM_005252	FOS	NM_147152	ITSN2
AF281070	EIF4G1	BC105088	IGHMBP2	NM_005324	H3F3B	NM_152551	C6orf151
AK022533	POLR1B	BC111717	UNC84B	NM_005501	ITGA3	NM_153331	KCTD6
AK026767	HDAC7A	BX537635	FLJ31951	NM_005561	LAMP1	NM_170711	DAZAP1
AK027319	N/A	L07594	TGFBR3	NM_005607	PTK2	NM_173158	NR4A1
AK090554	KIRREL	NM_000268	NF2	NM_005736	ACTR1A	NM_181726	ANKRD37
AK097342	N/A	NM_000399	EGR2	NM_005985	SNAI1	NM_182492	LRP5L
AK128423	CIRBP	NM_000660	TGFB1	NM_006186	NR4A2	XM_928276	LOC643446
AK130627	BBX	NM_001002260	C9orf58	NM_006311	NCOR1	XM_928458	LOC645417
AL833934	LMBR1	NM_001008657	TCOF1	NM_006470	TRIM16	XM_934740	LOC285643
BC000844	IER3	NM_001020818	MYADM	NM_006732	FOSB	XM_934873	LOC653562
BC002347	TUBB	NM_001025356	TMEM16F	NM_006901	MYO9A	XM_934875	LOC653562
BC004490	FOS	NM_001037872	REV1L	NM_007238	PXMP4	XM_934877	LOC653562
BC009288	NR4A2	NM_001039667	ANGPTL4	NM_012201	GLG1		
BC015149	MAP4	NM_001102	ACTN1	NM_013264	DDX25		
BC017694	FLJ14346	NM_001964	EGR1	NM_013376	SERTAD1		
BC020658	TMEM40	NM_002135	NR4A1	NM_014982	PCNX		
BC025711	NKX2-5	NM_002204	ITGA3	NM_015064	RAB6IP2		
BC030541	WDR1	NM_002213	ITGB5	NM_015492	C15orf39		
BC032131	MT1X	NM_002228	JUN	NM_016084	RASD1		
BC035625	EGR2	NM_002318	LOXL2	NM_016339	RAPGEFL1		
BC036797	ADRBK2	NM_002632	PGF	NM_016836	RBMS1		
BC043917	ITGA3	NM_002928	RGS16	NM_018717	MAML3		
BC048768	PTPRF	NM_002950	RPN1	NM_020533	MCOLN1		
BC048769	TCF7	NM_003243	TGFBR3	NM_021629	GNB4		
BC053636	H19	NM_003407	ZFP36	NM_024871	MAP6D1		

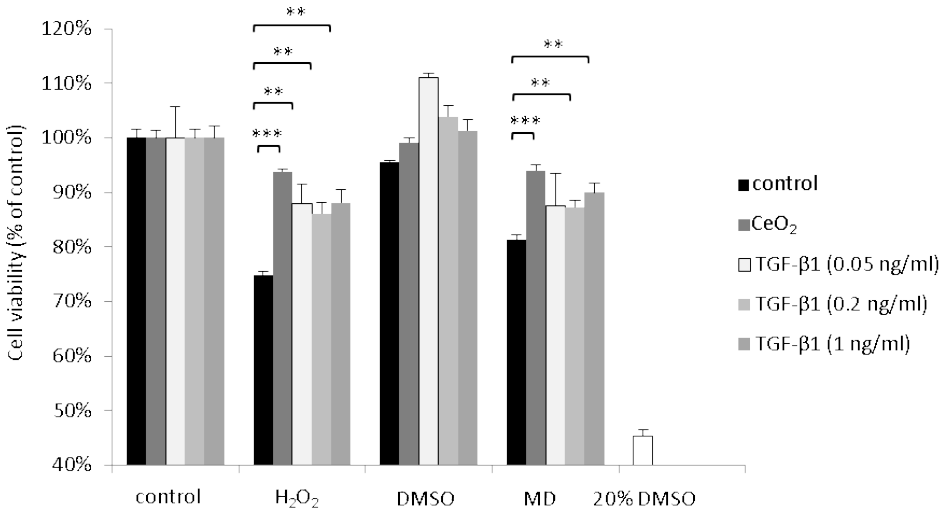
Supplementary Table 1. List of 107 genes identified as likely involved in the response to CeO_2 nanoparticle in protecting A549 cells from H_2O_2-induced oxidative stress. The gene lists were generated from Qlucore under p value 0.002, when the separation of the control & H_2O_2+CeO_2 groups with the closest distance could be observed on PCA plot. Genes identified with the TGF-β signaling pathway from IPA analysis are highlighted.

Supplementary Table 2

sequence ID	gene name	sequence ID	gene name	sequence ID	gene name	sequence ID	gene name	sequence ID	gene name
AB046778	SAPS3	BC007580	JUB	NM_000836	GRIN2D	NM_006185	NUMA1	NM_198506	FLJ44691
AB078026	ATF3	BC007948	FSCN1	NM_000872	HTR7	NM_006186	NR4A2	NM_198515	C10orf96
AB209701	SPOCK1	BC009288	NR4A2	NM_001008800	CCT3	NM_006302	GCS1	XM_212170	LOC286094
AF493931	RGS7	BC014261	RHOH	NM_001012971	C20orf106	NM_006917	RXRG	XM_926197	LOC642775
AJ535839	FUT10	BC017694	FLJ14346	NM_001017523	BTBD11	NM_007270	FKBP9	XM_927094	LOC643814
AK026968	RNF123	BC021978	FBXO46	NM_001017927	LOC130355	NM_013376	SERTAD1	XM_927258	LOC644005
AK027287	CIZ1	BC022483	ARHGAP29	NM_001018008	TPM1	NM_014663	JMJD2A	XM_928107	LOC645057
AK056120	CCNL2	BC027623	CPXM2	NM_001024847	TGFBR2	NM_014924	KIAA0831	XM_928212	LOC645177
AK057261	FBF1	BC028138	UBASH3A	NM_001025356	TMEM16F	NM_015053	PPFIA4	XM_928474	LOC645438
AK091168	GORASP1	BC032430	DGCR2	NM_001030287	ATF3	NM_015064	RAB6IP2	XM_929469	LOC646541
AK092698	N/A	BC032558	DLX2	NM_001031679	MSRB3	NM_015456	COBRA1	XM_929644	LOC646698
AK094008	PAEP	BC035625	EGR2	NM_001039577	CCNL2	NM_016830	VAMP1	XM_933427	LOC653706
AK127017	SPFH1	BC036399	ARPP-21	NM_001089	ABCA3	NM_017415	KLHL3	XM_933624	LOC643669
AK127062	TBC1D20	BC045660	HP1BP3	NM_001273	CHD4	NM_017623	CNNM3	XM_934088	LOC644372
AK127498	N/A	BC046109	PTK7	NM_001463	FRZB	NM_018717	MAML3	XM_934201	LOC132241
AK127723	N/A	BC046932	FAM3C	NM_001640	APEH	NM_021070	LTBP3		
AK127788	NCOR2	BC047504	EHMT1	NM_001802	CDR2	NM_022568	ALDH8A1		
AK127889	N/A	BC063491	RP3-473B4.1	NM_001964	EGR1	NM_025152	NUBPL		
AK131479	N/A	BC066890	NR4A2	NM_003119	SPG7	NM_032053	PCDHGA4		
AY101187	TDH	BC067473	GPR83	NM_003670	BHLHB2	NM_032637	SKP2		
AY138547	DAOA	BC071622	CCNL2	NM_003815	ADAM15	NM_033088	FAM40A		
AY262057	CYP4Z2P	BC073983	EGR1	NM_004302	ACVR1B	NM_033225	CSMD1		
AY358692	IGFL2	BC074876	GPA33	NM_004341	CAD	NM_052887	TIRAP		
AY599883	ITFG2	BC074949	IL20	NM_004405	DLX2	NM_133374	ZNF618		
AY823398	RAB40C	BC080541	ROR1	NM_004588	SCN2B	NM_133499	SYN1		
BC000844	IER3	BC101023	PANX2	NM_005189	CBX2	NM_138632	TRIOBP		
BC003067	PPP1R15A	BC103996	YIPF7	NM_005252	FOS	NM_139025	ADAMTS13		
BC003648	C4orf15	BC106925	ALDOC	NM_005505	SCARB1	NM_148910	TIRAP		
BC004371	APLP2	BX640827	WWC1	NM_005985	SNAI1	NM_153463	IL17RC		
BC007360	MGC16121	CR611490	RHBDD2	NM_006139	CD28	NM_182642	CTDSP1		

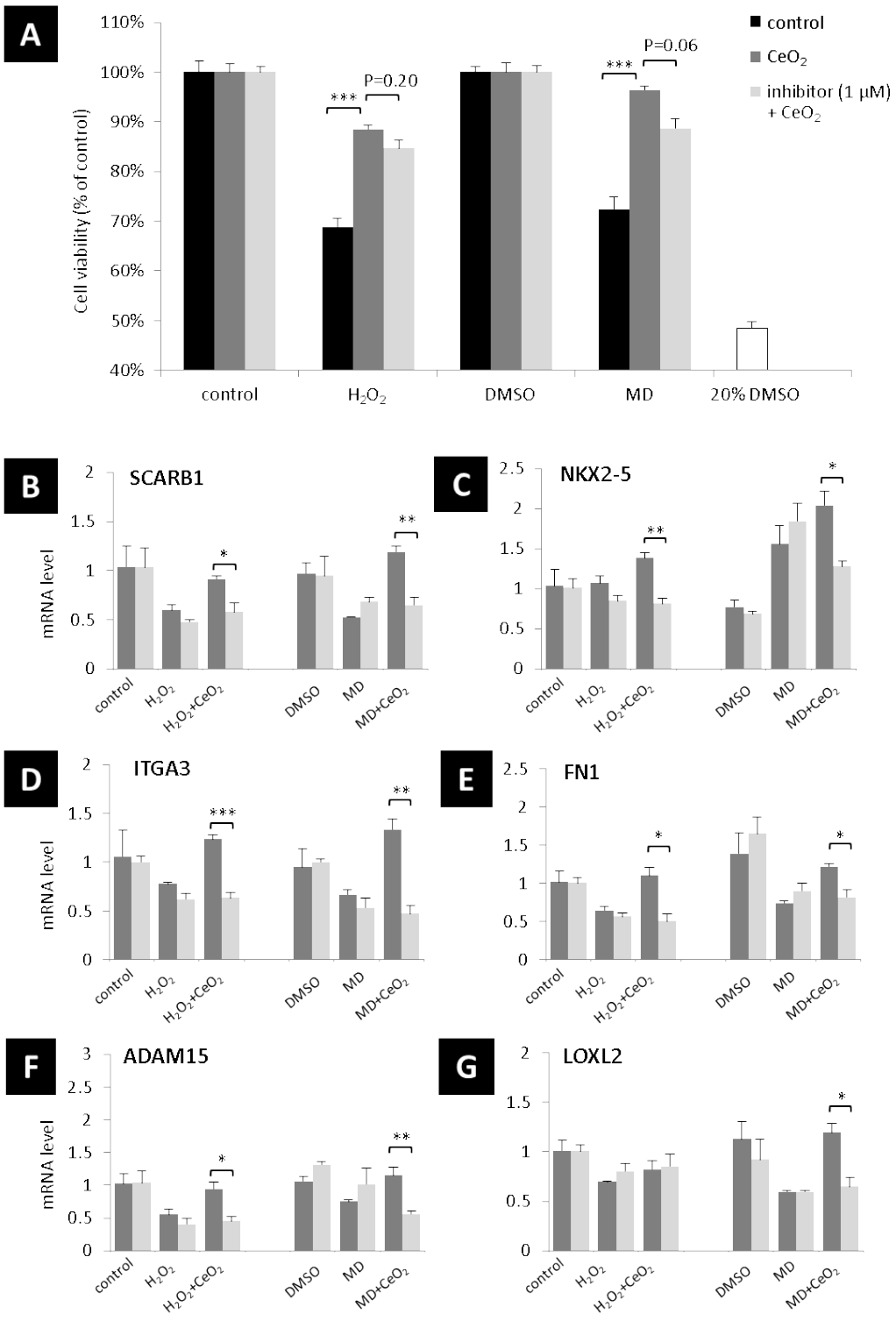
Supplementary Table 2. List of 135 genes identified as likely involved in the response to CeO_2 nanoparticle in protecting A549 cells from menadione (MD) -induced oxidative stress. The gene lists were generated from Qlucore under p value 0.002, when the separation of the DMSO & MD+CeO_2 groups with the closest distance could be observed on PCA plot. Genes identified with the TGF-β signaling pathway from IPA analysis are highlighted.

Supplementary Figure 1



Supplementary Figure 1. Pre-incubation of TGF-β1 at different concentrations (0.05 ng/ml. 0.2 ng/ml and 1 ng/ml) improved cell viability of A549 cells under oxidative stress triggered by H2O2 (200 µM) or MD (20 µM). Statistical significance was compared against H2O2 or MD without pre-incubation of CeO2 nanoparticles or TGF-β1 (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) (n=4).

Supplementary Figure 2



Supplementary Figure 2A. **Inhibition of TGF-β signalling attenuates the protective effect of CeO_2 nanoparticles against oxidant injury.** (A) By blocking TGF-β signalling using the inhibitor SB431542, the protective effects of CeO2 NPs in A549 cells against oxidative stress triggered by H_2O_2 (200 μM) or MD (20 μM) were investigated. Values were calculated as a percentage of individual control levels (Control, CeO_2 and TGF-β1) and p values between groups were calculated and displayed as individual values or as indicated (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) (n=4). (B-G) Gene regulation of SCARB1 (B), NKX2-5 (C), ITGA3 (D), FN1 (E), ADAM15 (F) and LOXL2 (G) upon blocking TGF-β signalling. ■ mRNA level without TGF-β receptor inhibitor and ■ mRNA level with TGF-β receptor inhibitor (1 μM). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ for conditions compared between with and without TGF-β receptor inhibitor under oxidative stress with the pre-incubation of CeO_2 NPs (H_2O_2+CeO_2 or MD+CeO_2) (n=3).

Additional Methods

Flow cytometry (FACS)

After treatment, cells were washed three times with ice-cold PBS in order to remove unbound CeO_2. Cells were then trypsinised and centrifuged at 1000 g for 5 minutes at 4 °C and the pellet was washed once with PBS. Cells were resuspended in PBS and fixed with 0.01% paraformaldehyde (PFA) in PBS for 10 minutes at RT. The cells were washed three times by centrifugation at 1000 g for 5 minutes with PBS and resuspended at 4 °C in PBS plus 3% (w/v) BSA. Cells were analyzed using a Guava EasyCyte HT (Millipore) analyser.

Cytotoxicity assay

A modified version of the lactate dehydrogenase (LDH) assay was used to assess cytotoxicity. Assays were performed in 96-well plates. After treatment, medium was removed and adherent cells were lysed in 100 µl of 1% v/v Triton X100 in media. After incubation for 60 minutes at 37 °C, the cell lysate was collected and centrifuged at 6000 g for 5 minutes before the supernatants were transferred to a new 96-well plate. 50 µl of reconstituted substrate solution was added and incubated for 15 minutes at room temperature in the dark. 50 µl of stop solution was added to each well and the absorbance (Ab) was measured at 492 nm. The amount of LDH detected represented the number of cells that survived the treatment.

Carbonylation assessment

After treatment in 6- well plates, cells were trypsinised and collected by centrifugation. The cell pellet was sonicated on ice in 1 ml of cold PBS followed by centrifugation at 10,000 g for 15 minutes at 4 ^{o}C. The supernatant was removed and stored on ice. 1% streptomycin sulfate was added to remove nucleic

acids. The protein containing solution was divided between two tubes at 200 µl each. 800 µl of 2,4-*dinitrophenylhydrazine* (DNPH) was added to the "sample" tube and 800 µl of 2.5 M HCl was added to the other "control" tube. Both tubes were incubated in the dark at RT for 1 hr, with a brief vortex every 15 minutes. 1 ml of 20% trichloroacetic acid (TCA) (w/v) solution was added to each tube. They were then vortexed and left on ice for 5 minutes, followed by centrifugation at 10,000 g for 10 minutes at 4 ^{o}C. The pellet was resuspended in 1 ml of (1:1) Ethanol/Ethyl Acetate mixture and then centrifuged at 10,000 g for 10 minutes at 4 ^{o}C. Then the protein pellet was washed twice more with 1 ml of (1:1) Ethanol/Ethyl Acetate mixture and resuspended in 500 µl of guanidine hydrochloride by vortexing. The samples were centrifuged at 10,000 g for 10 minutes at 4°C to remove debris. 220 µl of supernatant from each tube were transferred to a 96-well plate and absorbance (Ab) was measured at a wavelength between 360-385 nm using a plate reader. Protein carbonylation was calculated using [Ab (the average of each sample) – Ab (the average of each control)] divided by **[**Ab (the average of untreated sample) – Ab (the average of untreated control)].

Assessment of intracellular H_2O_2 levels

The level of intracellular H_2O_2 was measured using intracellular peroxide-dependent oxidation of 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) to form a fluorescent compound, 2',7'-dichlorodihydrofluorescein (H_2DCF). H_2DCF-DA was dissolved in DMSO and diluted in cell culture medium to a final concentration of 20 µM. The final concentration of DMSO in medium was 0.001%. Experiments were performed in 96-well plates. Cells were pre-incubated with CeO_2 nanoparticle suspension dilutions (50 µg/ml) for 24 hrs, followed by an incubation of H_2DCF-DA (20 µM) for 1 hr. Cells were washed three times with full media before oxidative stress treatment for 3 hrs. The plate was then placed immediately on the plate reader. Fluorescence was measured with an excitation wavelength of 485 nm and an emission wavelength of 515 nm.