



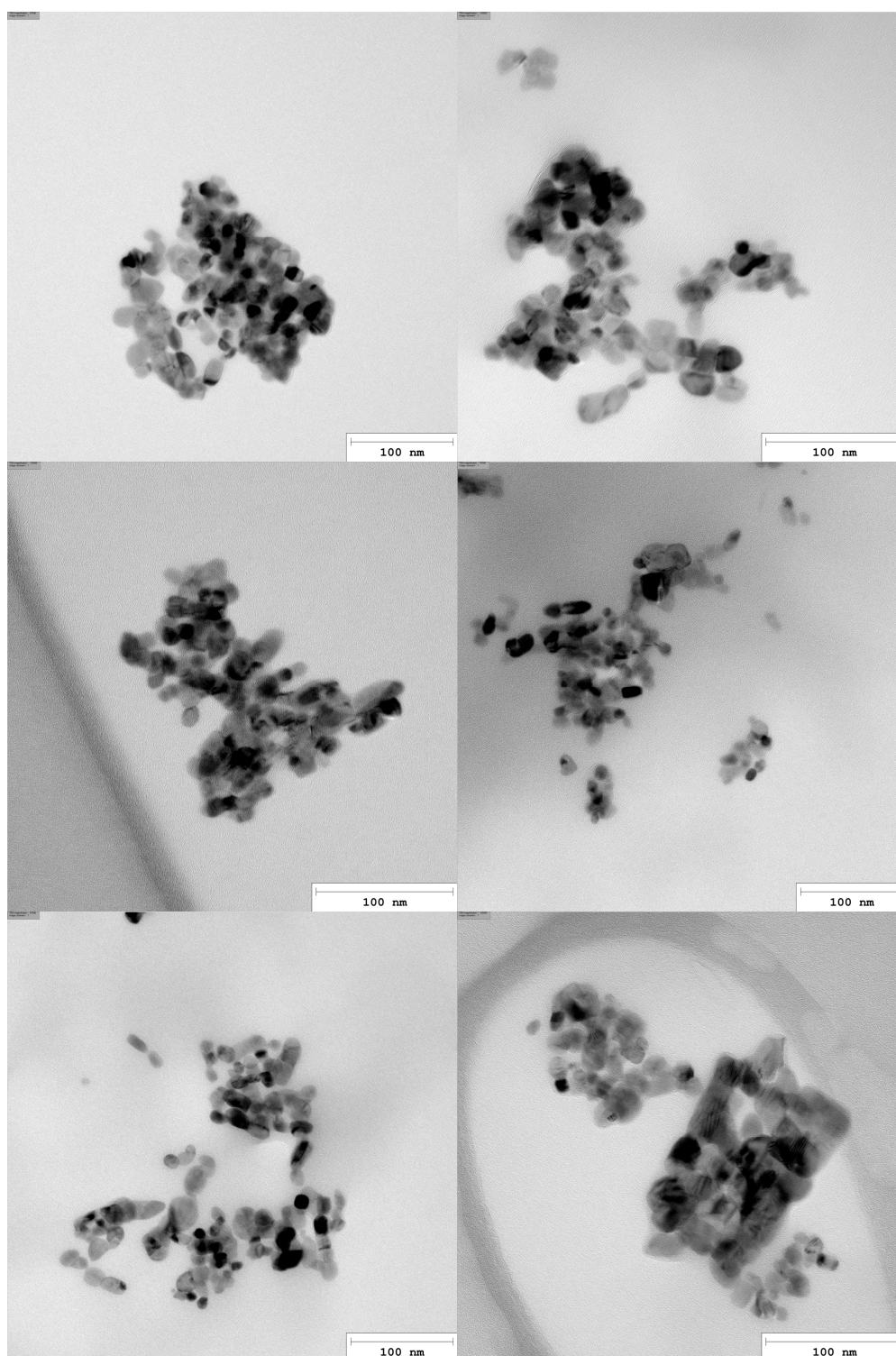
Supplementary files

Impact of differentiated macrophage-like cells on the transcriptional toxicity profile of CuO nanoparticles in co-cultured lung epithelial cells

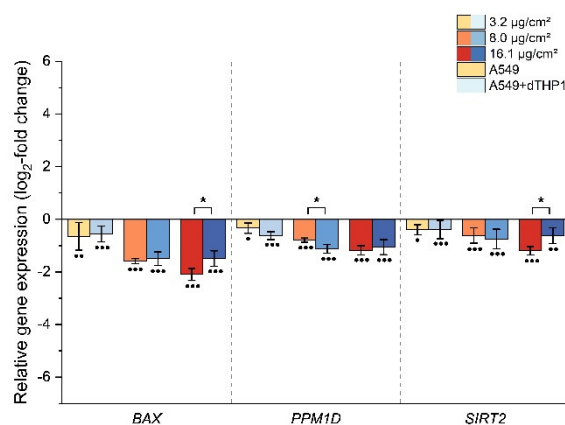
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Supplementary Figure S1. Representative TEM images of CuO NP at 2.56 mg/mL in 0.05 % BSA after dispersion according to the described dispersion protocol.



Supplementary Figure S2. Impact of CuO NP on genes related to apoptosis in A549 monoculture (A549) and co-culture (A549+dTHP-1) after 24 h incubation. Depicted is the log₂-fold change of at least three independently conducted experiments \pm SD. Significantly different from negative controls: • ≤ 0.05 (ANOVA-Dunnett's *t*-test); significantly different between cell models: * ≤ 0.05 (unpaired *t*-test).

Supplementary Table S1. Extended primer set for inflammatory response and fibrosis.

Gene	Name of the coding protein	Gene ID	RefSeq		Sequence (5' → 3')	Position RefSeq (nt)	Position exon	Amplicon-size [bp]	T _m Amplicon [°C]	Isoform hits
<i>CCL22</i>	C-C motif chemokine 22 (CCL22)	6367	NM_002990.5	fwd:	CGTCTGCTGCCGTGATTAC	136	2	191	81	NM_002990.5
				rev:	AGTAGGCTCTTCATTGGCTCAG	326	3			
<i>PTGS2</i>	Prostaglandin G/H synthase 2 (PGH2)	5743	NM_000963.4	fwd:	CCGAGGTGTATGTATGAGTGTGG	85	2	136	76	NM_000963.4
				rev:	TGTGTTTGGAGTGGGTTTCAGA	220	3			
<i>IL-1a</i>	Interleukin-1 alpha (IL-1 alpha)	3552	NM_000575.5	fwd:	GAATGACGCCCTCAATCAAAGT	467	5	203	79	NM_000575.5;
				rev:	GCAGCACTGGTTGGTCTTC	669	6			NM_001371554.1
<i>IL-1b</i>	Interleukin-1 beta (IL-1 beta)	3553	NM_000576.2	fwd:	GAGGCACAAGGCACAACA	16	3	186	82	NM_000576.2
				rev:	CCTGGAAGGAGCACTTCATCT	199	4			
<i>IL-6</i>	Interleukin-6 (IL-6)	3569	NM_000600.4	fwd:	CCAATCTGGATTCAATGAGGAGAC	427	3-4	158	80	NM_001318095.2
				rev:	GCAGGAAGTGGATCAGGACTT	584	4			NM_001371096.1
<i>TNF</i>	Tumor necrosis factor (TNF-a)	7124	NM_000594.4	fwd:	ACACCATGAGCACTGAAAGC	173	1	225	87	NM_000594.4
				rev:	CCAGAGGGCTGATTAGAGAGA	397	2			
<i>ACTA2</i>	actin, aortic smooth muscle (ACTA2)	59	NM_001141945.2	fwd:	GACCTTTGGCTTGGCTTGT	59	1	117	84	NM_001141945.2;
				rev:	GGTGCGGACAGGAATTGAAG	175	1			NM_001320855.1;
										NM_001613.4
<i>COL1A1</i>	collagen alpha-1(I) chain (COL1A1)	1277	NM_000088.3	fwd:	GCCAAGACGAAGACATCCC	215	1-2	235	88	NM_000088.3
				rev:	GTGGTTTCTTGGTCGGTGG	499	3			
<i>CTNNB1</i>	catenin beta-1 (CTNNB1)	1499	NM_001098209.2	fwd:	CTGAGGAAGAGGATGTGGATACC	369	3	199	81	NM_001098209.2;
				rev:	TGAGCAGCATCAAACCTGTGTAGA	567	4			NM_001098210.2;
										NM_001330729.2;
										NM_001904.4
<i>FN1</i>	Fibronectin (FN)	2335	NM_001306129.1	fwd:	CGGTGGCTGTCAGTCAAAG	393	1	128	81	NM_001306129.1;
				rev:	ACCTCGGCTTCCTCCATAAC	520	2			NM_001306130.2;
										NM_001306131.2;
										NM_001306132.2;
										NM_001365517.2;

									NM_001365518.2; NM_001365519.2; NM_001365520.2; NM_001365521.2; NM_001365522.2; NM_001365523.2; NM_001365524.2; NM_002026.4; NM_054034.3; NM_212474.3; NM_212476.3; NM_212478.3; NM_212482.4
<i>SPP1</i>	Osteopontin (OPN)	6696	NM_000582.2	fwd: TGAATCTGATGAACTGGTCACTGA rev: TGATGTCCTCGTCTGTAGCA	504 691	5 6	188	82	NM_000582.2; NM_001040058.2; NM_001040060.2; NM_001251829.2; NM_001251830.2
<i>PDGFA</i>	Platelet-derived growth factor subunit A (PDGFA)	5154	NM_002607.5	fwd: CGATGAGGACCTTGGCTTG rev: CTTCTCGGGCACATGCTTAG	842 1074	1 6	233	87	NM_002607.5; NM_033023.5
<i>TGFB1</i>	Transforming growth factor beta-1 (TGFB1)	7040	NM_000660.5	fwd: GGAAACCCACAACGAAATCTATGA rev: GCTGAGGTATCGCCAGGAAT	1181 1388	1-2 3	208	84	NM_000660.5
<i>TIMP1</i>	Metalloproteinase inhibitor 1 (TIMP1)	7076	NM_003254.3	fwd: GATACTTCCACAGGTCCCAACAAC rev: GGTCCGTCCACAAGCAATGA	328 564	4 6	237	85	NM_003254.3
<i>VIM</i>	Vimentin (VIME)	7431	NM_003380.5	fwd: AAGACGGTTGAACTAGAGATGGA rev: TGTAGGAGTGTTCGGTTGTTAAGAA	1764 1995	9 10	232	79	NM_003380.5

All other used primers of the used gene set were describe in [22], with exception of *ABCB1*, *ABCC1*, *ADH1B*, *ALDH1A1*, *GSTP1*, *NAT1*, *SULT1A1*, *UGT1A*, *SEPP1*, *TP53*, *AXIN2*, *BCL2L1*, *ERCC2*, *POLB*, *OGG1*, and *XRCC5* which were removed from the gene set to open space for inflammatory and fibrotic marker genes.

Supplementary Table S2. Log₂-fold changes in gene expression and p-values of untreated co-culture controls compared to A549 monoculture.

Gene name	Log ₂ -fold change	p-value	Gene name	Log ₂ -fold change	p-value
<i>CYP1A1</i>	-0.38	0.001	<i>BCL2</i>	0.00	0.992
<i>AHR</i>	-0.33	0.178	<i>BTRC</i>	-0.26	0.002
<i>NQO1</i>	-0.48	0.000	<i>CCND1</i>	-0.50	0.000
<i>CCL22</i>	5.00	0.000	<i>CDKN1A</i>	0.49	0.000
<i>PTGS/COX2</i>	-0.22	0.223	<i>CDKN1B</i>	-0.29	0.000
<i>IL-1b</i>	4.18	0.000	<i>E2F1</i>	-0.80	0.000
<i>IL-6</i>	0.54	0.262	<i>EGFR</i>	-0.12	0.055
<i>IL-8</i>	0.15	0.455	<i>JUN</i>	0.27	0.022
<i>ACTA2</i>	0.33	0.019	<i>MDM2</i>	-0.25	0.004
<i>CTNNB1</i>	-0.10	0.043	<i>MYC</i>	-0.18	0.027
<i>FN1</i>	0.17	0.237	<i>PLK3</i>	0.23	0.173
<i>SPP1</i>	0.36	0.025	<i>PMAIP1</i>	-0.11	0.025
<i>PDGFA</i>	0.12	0.209	<i>PPM1D</i>	-0.12	0.111
<i>TGFB1</i>	0.07	0.284	<i>SIRT2</i>	-0.13	0.579
<i>TIMP1</i>	-0.23	0.010	<i>TNFRSF10B</i>	-0.32	0.000
<i>VIM</i>	-0.06	0.506	<i>VEGFA</i>	0.03	0.864
<i>FTH1</i>	0.21	0.309	<i>XIAP</i>	-0.11	0.255
<i>MT1X</i>	-0.20	0.016	<i>APEX1</i>	-0.19	0.049
<i>MT2A</i>	-0.43	0.001	<i>ATM</i>	-0.24	0.000
<i>SLC30A1</i>	-0.14	0.150	<i>ATR</i>	-0.48	0.000
<i>TFRC</i>	-0.19	0.138	<i>BRCA1</i>	-0.31	0.004
<i>CAT</i>	-0.26	0.000	<i>BRCA2</i>	-0.41	0.000
<i>G6PD</i>	-0.59	0.000	<i>DDB1</i>	-0.35	0.008
<i>GCLC</i>	-0.30	0.000	<i>DDB2</i>	0.03	0.840
<i>GPX1</i>	0.30	0.026	<i>DDIT3</i>	0.66	0.002
<i>GPX2</i>	-0.21	0.031	<i>ERCC1</i>	-0.24	0.013
<i>GSR</i>	-0.51	0.000	<i>ERCC4/XPF</i>	-0.42	0.000
<i>HMOX1</i>	0.61	0.000	<i>ERCC5/XPG</i>	-0.33	0.000
<i>HSPA1A</i>	-0.54	0.001	<i>GADD45A</i>	0.43	0.003
<i>KEAP1</i>	-0.29	0.004	<i>LIG1</i>	-0.50	0.000
<i>MAP3K5</i>	-0.15	0.057	<i>LIG3</i>	-0.51	0.000
<i>NFE2L2</i>	-0.17	0.071	<i>MGMT</i>	-0.11	0.417
<i>NFKB1</i>	-0.06	0.283	<i>MLH1</i>	-0.49	0.000
<i>NFKB2</i>	0.49	0.005	<i>MSH2</i>	-0.27	0.000
<i>NFKBIA</i>	0.25	0.275	<i>PARP1</i>	-0.40	0.001
<i>PRDX1</i>	-0.33	0.012	<i>PCNA</i>	-0.24	0.014
<i>SOD1</i>	-0.31	0.010	<i>POLD1</i>	-0.24	0.071
<i>SOD2</i>	0.15	0.196	<i>POLQ</i>	-0.49	0.000
<i>TXN</i>	-0.45	0.000	<i>RAD50</i>	0.04	0.674
<i>TXNRD1</i>	-0.57	0.000	<i>RAD51</i>	-0.41	0.000
<i>APAF1</i>	-0.07	0.467	<i>RRM2B</i>	-0.20	0.044
<i>BAX</i>	0.02	0.707	<i>XPC</i>	-0.19	0.024

BBC3	0.35	0.089
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Supplementary Table S3. X-fold changes in gene expression treated mono- and co-cultures compared to untreated controls.
N.q.: not quantifiable.

Dose (µg/mL)	A549			A549+dTHP-1		
	3.2	8.0	16.1	3.2	8.0	16.1
<i>AHR</i>	0.2	0.1	0.1	0.4	0.1	0.1
<i>CYP1A1</i>	4.5	6.0	6.2	3.9	7.0	4.9
<i>NQO1</i>	0.5	0.5	0.6	0.6	0.5	0.6
<i>CCL22</i>	1.0	1.8	2.6	0.2	0.1	0.0
<i>PTGS/COX2</i>	0.1	0.1	0.1	0.4	0.1	0.2
<i>IL-1a</i>	n.q.	n.q.	n.q.	7.4	5.6	2.0
<i>IL-1b</i>	0.5	0.6	0.5	0.2	0.2	0.1
<i>IL-6</i>	0.5	0.7	0.6	6.1	6.2	1.7
<i>IL-8</i>	2.6	19.9	50.3	4.0	14.4	37.8
<i>TNF-α</i>	n.q.	n.q.	n.q.	0.5	0.4	0.3
<i>ACTA2</i>	0.2	0.1	0.2	0.5	0.1	0.2
<i>COL1A1</i>	n.q.	n.q.	n.q.	1.1	0.6	1.2
<i>CTNNA1</i>	0.7	0.4	0.3	0.8	0.5	0.4
<i>FN1</i>	0.4	0.5	0.6	0.5	0.4	0.6
<i>SPP1</i>	0.8	0.6	0.5	0.6	0.6	0.7
<i>PDGFA</i>	0.3	0.1	0.0	0.4	0.1	0.1
<i>TGFB1</i>	0.5	0.5	0.5	0.7	0.5	0.5
<i>TIMP1</i>	0.7	1.1	1.2	0.8	1.2	1.6
<i>VIM</i>	0.5	0.4	0.7	0.6	0.5	0.6
<i>FTH1</i>	1.5	1.2	0.8	1.7	1.3	0.9
<i>MT1X</i>	2.3	8.5	8.8	34.0	8.0	7.6
<i>MT2A</i>	1.3	13.2	12.6	25.6	14.7	14.3
<i>SLC30A1</i>	2.1	0.6	0.6	2.4	0.7	0.7
<i>TFRC</i>	0.9	0.2	0.2	1.5	0.4	0.2
<i>CAT</i>	0.5	0.3	0.3	0.7	0.4	0.4
<i>G6PD</i>	0.4	0.5	0.5	0.6	0.5	0.5
<i>GCLC</i>	0.4	0.2	0.1	0.5	0.2	0.2
<i>GPX1</i>	0.7	0.8	0.8	0.5	0.5	0.8
<i>GPX2</i>	0.3	0.2	0.2	0.4	0.2	0.4
<i>GSR</i>	0.9	0.8	0.8	1.1	1.1	0.8
<i>HMOX1</i>	22.6	28.4	23.6	12.1	21.9	21.7
<i>HSPA1A</i>	16.7	57.6	58.5	16.0	68.2	69.8
<i>KEAP1</i>	0.3	0.1	0.1	0.5	0.1	0.1
<i>MAP3K5</i>	0.4	0.1	0.1	0.4	0.1	0.1
<i>NFE2L2</i>	0.5	0.1	0.1	0.6	0.2	0.1
<i>NFKB1</i>	0.8	0.3	0.2	1.0	0.5	0.3

<i>NFKB2</i>	3.0	1.0	0.5	2.8	0.9	0.5
<i>NFKBIA</i>	0.9	0.2	0.0	1.3	0.2	0.0
<i>PRDX1</i>	1.0	1.2	1.3	1.1	1.6	1.4
<i>SOD1</i>	1.0	1.1	1.2	1.1	1.6	1.4
<i>SOD2</i>	1.1	1.3	1.1	2.6	2.7	1.9
<i>TXN</i>	0.8	1.0	1.3	1.0	1.6	1.6
<i>TXNRD1</i>	0.8	0.8	1.0	1.1	1.0	1.2
<i>APAF1</i>	0.7	0.3	0.1	0.7	0.3	0.2
<i>BAX</i>	0.6	0.3	0.2	0.7	0.4	0.4
<i>BBC3</i>	0.2	0.1	0.1	0.4	0.1	0.1
<i>BCL2</i>	0.5	0.2	0.3	0.4	0.2	0.2
<i>BTRC</i>	0.8	0.7	0.6	0.8	0.8	0.7
<i>CCND1</i>	0.5	0.4	0.4	0.6	0.4	0.3
<i>CDKN1A</i>	1.0	0.8	0.5	1.0	0.7	0.5
<i>CDKN1B</i>	0.2	0.1	0.0	0.3	0.1	0.1
<i>CDKN2B</i>	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
<i>E2F1</i>	0.9	0.5	0.6	1.3	0.5	0.6
<i>EGFR</i>	0.6	0.4	0.4	0.9	0.4	0.4
<i>JUN</i>	1.2	2.3	3.5	1.0	1.4	2.6
<i>MDM2</i>	0.5	0.3	0.3	0.6	0.4	0.3
<i>MYC</i>	0.3	0.1	0.0	0.4	0.1	0.0
<i>PLK3</i>	0.6	0.2	0.1	0.7	0.2	0.2
<i>PMAIP1</i>	0.9	0.8	0.7	1.1	0.9	0.9
<i>PPM1D</i>	0.8	0.6	0.4	0.6	0.5	0.5
<i>SIRT2</i>	0.8	0.7	0.4	0.8	0.6	0.6
<i>TNFRSF10B</i>	1.1	0.7	0.6	1.4	0.9	0.9
<i>VEGFA</i>	1.4	0.6	0.4	1.5	0.6	0.5
<i>XIAP</i>	0.7	0.5	0.6	0.7	0.7	0.6
<i>APEX1</i>	0.7	0.3	0.4	0.8	0.4	0.5
<i>ATM</i>	0.5	0.3	0.3	0.7	0.3	0.4
<i>ATR</i>	0.6	0.2	0.1	0.7	0.3	0.1
<i>BRCA1</i>	0.8	0.2	0.1	1.0	0.3	0.2
<i>BRCA2</i>	0.9	0.2	0.1	1.2	0.4	0.2
<i>DDB1</i>	0.6	0.5	0.5	0.8	0.6	0.5
<i>DDB2</i>	0.5	0.1	0.1	0.7	0.1	0.1
<i>DDIT3</i>	6.0	14.9	18.2	4.6	7.2	22.5
<i>ERCC1</i>	0.5	0.5	0.6	0.6	0.5	0.7
<i>ERCC4/XPF</i>	0.3	0.1	0.0	0.4	0.1	0.1
<i>ERCC5/XPG</i>	0.3	0.2	0.1	0.4	0.2	0.1
<i>GADD45A</i>	2.5	3.3	3.1	2.3	3.5	5.3
<i>LIG1</i>	0.6	0.3	0.2	0.8	0.3	0.3
<i>LIG3</i>	0.4	0.2	0.2	0.5	0.3	0.2
<i>MGMT</i>	0.6	0.9	1.0	0.6	0.7	1.1
<i>MLH1</i>	0.7	0.3	0.2	10	0.4	0.3
<i>MSH2</i>	0.7	0.2	0.1	0.8	0.2	0.2
<i>PARP1</i>	0.7	0.4	0.4	0.8	0.4	0.4
<i>PCNA</i>	0.8	0.1	0.0	0.9	0.2	0.1

<i>POLD1</i>	0.9	0.3	0.2	1.0	0.4	0.3
<i>POLQ</i>	0.6	0.3	0.3	0.9	0.3	0.4
<i>RAD50</i>	0.4	0.2	0.2	0.5	0.2	0.3
<i>RAD51</i>	1.4	0.2	0.1	1.7	0.4	0.1
<i>RRM2B</i>	0.5	0.3	0.3	0.7	0.4	0.3
<i>XPA</i>	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.
<i>XPC</i>	0.3	0.1	0.1	0.5	0.1	0.1
