Electronic supplementary information

Understanding the toxicity mechanism of CuO nanoparticles: the intracellular view of exposed earthworm cells

Authors

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A	Primary particle size				Shape	
CuO NPs	**5-15 nm**				**Spherical/rod**	
B	**Z-Avg. (nm)[a]**		**ζ (mV)[b]**		**UV/Vis (nm)**	
	2 h	24 h	2 h	24 h	2 h	24 h
Distilled water	1050±74.9	934±343	26.1±1.92	-15.7±1.82	400-500	400-500
R-RPMI 1640 medium	316±8.08	351±51.7	-15.9±0.675	-15.7 ±0.581	<230	<230

Table S1. Physico-chemical characterization of CuO NPs.

A) Primary particle characterization. Average obtained by transmission electron microscope.

B) Characterization of 100 µg/mL CuO NPs suspensions in miliQ water water and R-RPMI 1640 medium.

[a]Hydrodynamic size of 100 µg/mL CuO NPs suspensions determined by Dynamic Light Scattering (DLS). Mean of 3 measurements ± SD.

[b]ζ = zeta potential. Mean of 3 measurements ± SD.

Gene	Direction	Sequence	Size (bp)	GenBank No.
Metallothionein	For	5'-AAA AAG CTT TGC TGT GCT GAT GCT-3'	154	KP770991
	Rev	5'-CGT ATT TCA ATG CCT TGG CTC TCA-3'		
Phytochelatin	For	5'-CTG GAA GGG ACC GTG GAG ATG-3'	202	KP770990
	Rev	5'-ACC CTT CGA CAC CCG TTT CAC AA-3'		
Mn-SOD	For	5'-GAA GCT CAG ACC AAA GGA GAC-3'	91	KU057379
	Rev	5'-TGA TTG ATA TGT CCT CCG CC-3'		
CuZn-SOD	For	5'-ATG AGT TTA GCA AGA CCA CTG-3'	103	KR106132
	Rev	5'-GTC CAA GCC AAC CAT ATC AC-3'		
Catalase	For	5'-TAC AAA CTG GTG AAC GCC GA-3'	139	DQ286713
	Rev	5'-AAA GGT CAC GGG TCG CAT AG-3'		
EMAPII	For	5'-CAT CCC GAT GCG GAC AGT CTG TA-3'	244	AEB92227
	Rev	5'-TCC CCA ATG GCA GCA CCA ATT-3'		
Fet/Lys	For	5’-TGG CCA GCT GCA ACT CTT-3’	176	U02710 D85846 D85848 D85847 DQ144453
	Rev	5’-CCA GCG CTG TTT CGG ATT AT-3’		
Lumbricin	For	5'-AGG CCA TAC TCG GAA CGC AAG AA-3'	213	KX816866
	Rev	5'-CAC ACG CTC CAT CGA AAT CAA CTC-3'		
MEK Kinase 1	For	5'-CAA GGA ACG ATC CCA TTC AT-3'	147	EH672240
	Rev	5'-GTA TCA TGG TGC AAC CAA CG-3'		
PKC 1	For	5'-TTT TAT GCG GCC GAA GTC A-3'	120	DQ286716
	Rev	5'-GTC GGC GAT TTT GCA GTG A-3'		
RPL 17	For	5’-CAT CAC ACC CTA CAT GAG CA-3’	179	BB998250
	Rev	5’-TAA CGG AAG AAG GGG TTA GC-3’		
RPL 13	For	5’-CAC AAT TGG AAT TGC TGT CG-3’	144	BB998075
	Rev	5’-GTG GCA TCA CCC TTG TTA GG-3’		

Table S2. Primer sequences used for RT-PCR. Mn-SOD: *manganese superoxide dismutase; CuZn-SOD:* copper-zinc superoxide dismutase; EMAP II: endothelial monocyte-activating polypeptide-II; Fet/Lys: Fetidin/Lysenin; PKC1: protein kinase C 1; RPL 17 – ribosomal protein L17; RPL 13 – ribosomal protein L13.

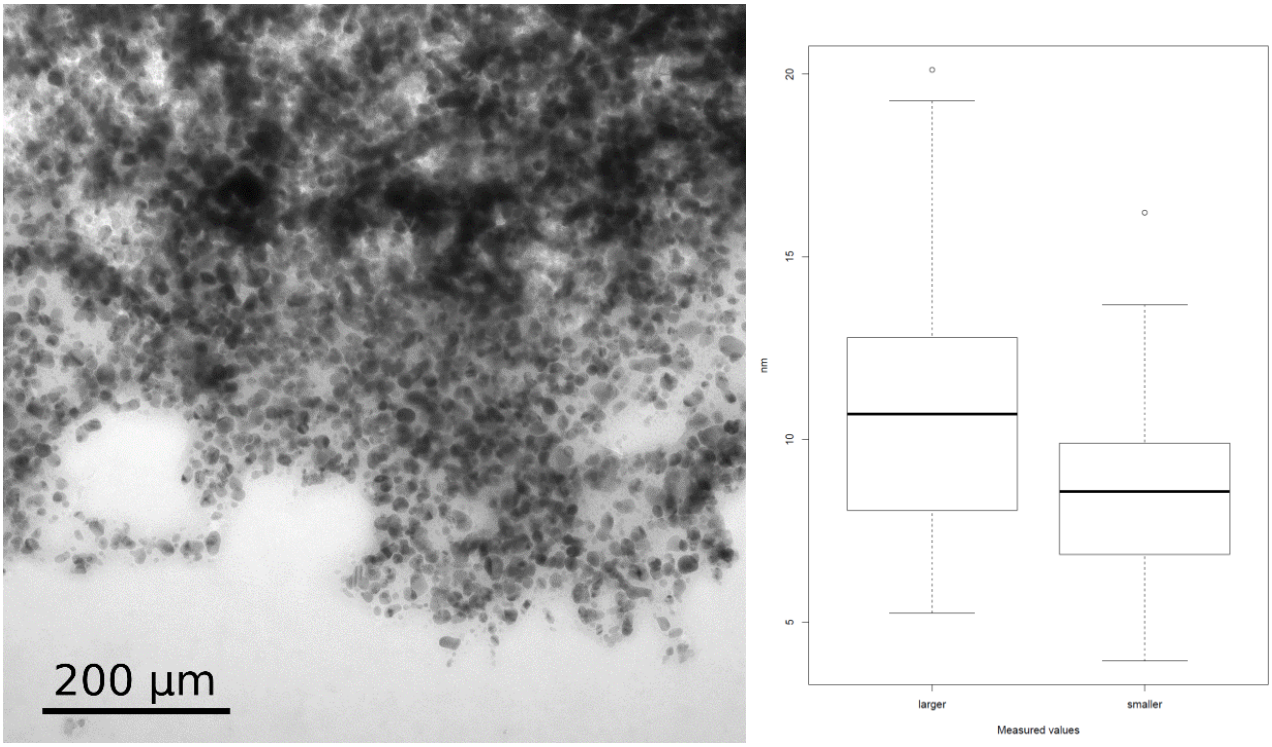

Fig. S1. TEM image of CuO NPs at the 100 µg/mL concentration, and boxplot of primary size particles of 100 µg/mL CuO NPs.The boxplot shows the measured values of CuO NPs and its sizes (nm), larger - max. diameter; smaller - min. diameter.

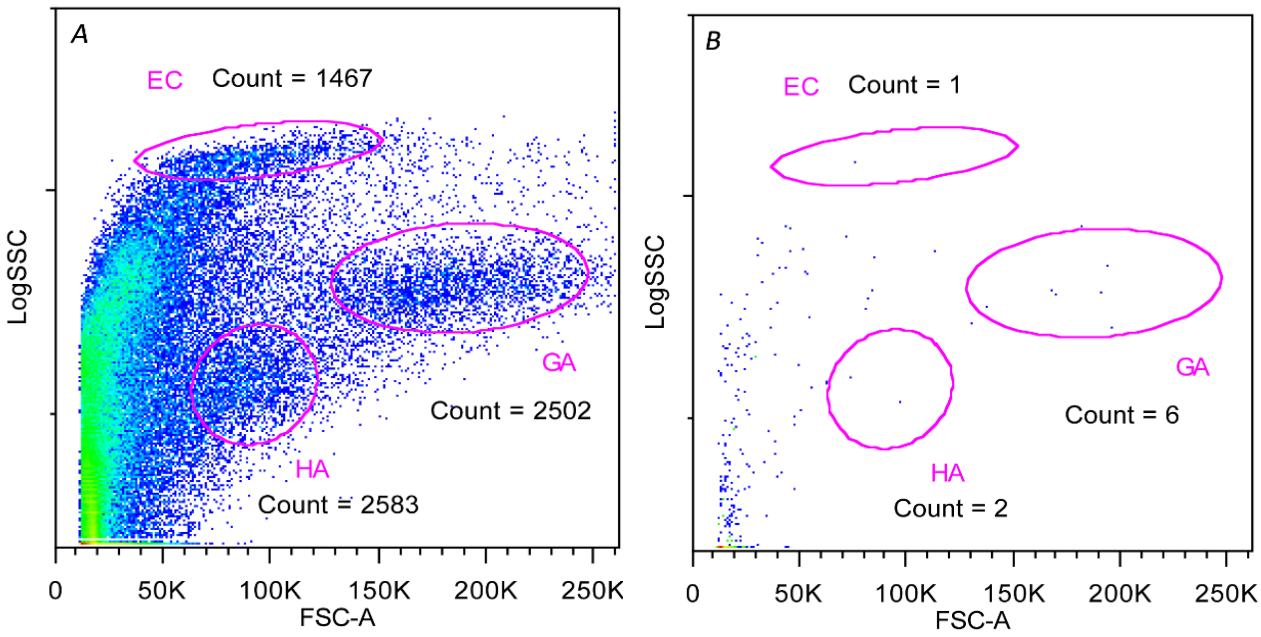

Fig. S2. Flow cytometer analyses of non-treated coelomocytes. Three cell populations were detected; hyaline amoebocytes (HA), granular amoebocytes (GA), and eleocytes (EC). A) control cells in R-RPMI 1640 cultivation medium, B) R-RPMI 1640 cultivation medium without cells.

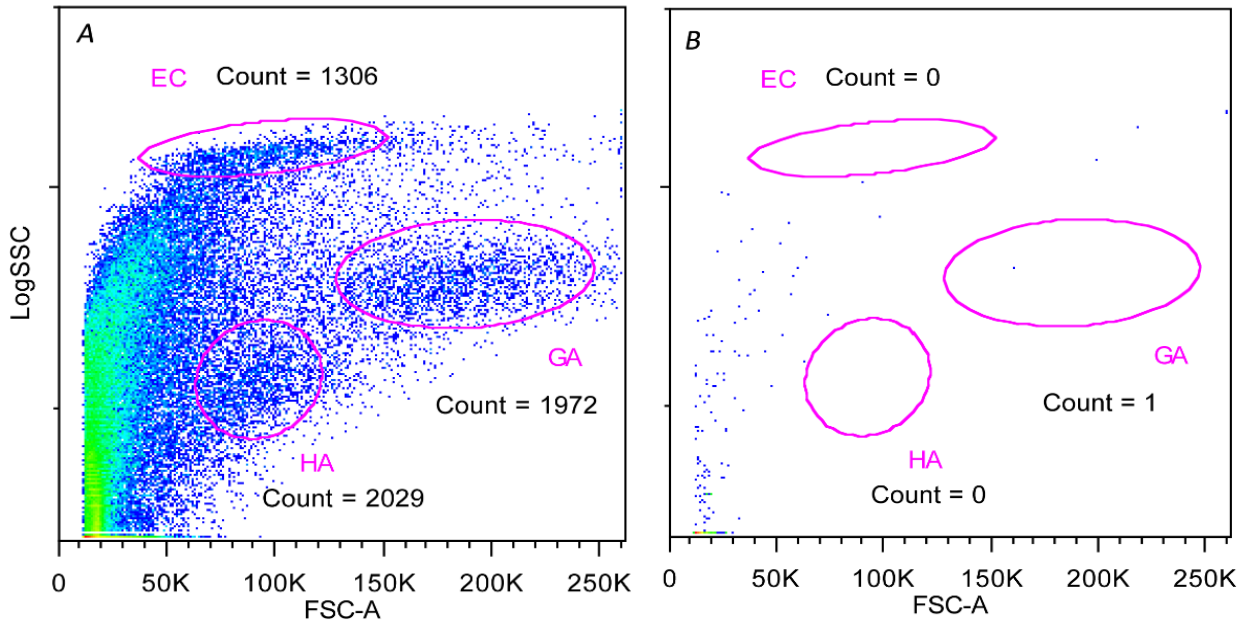

Fig. S3. Flow cytometer analyses of coelomocytes treated with CuO NPs. Three populations were detected; hyaline amoebocytes (HA), granular amoebocytes (GA), and eleocytes (EC); A) cells exposed to 100 µg/mL of Cu in the form of CuO NPs in R-RPMI 1640 cultivation medium, B) R-RPMI 1640 cultivation medium without cells with 100 µg/mL of Cu in the form of CuO NPs.

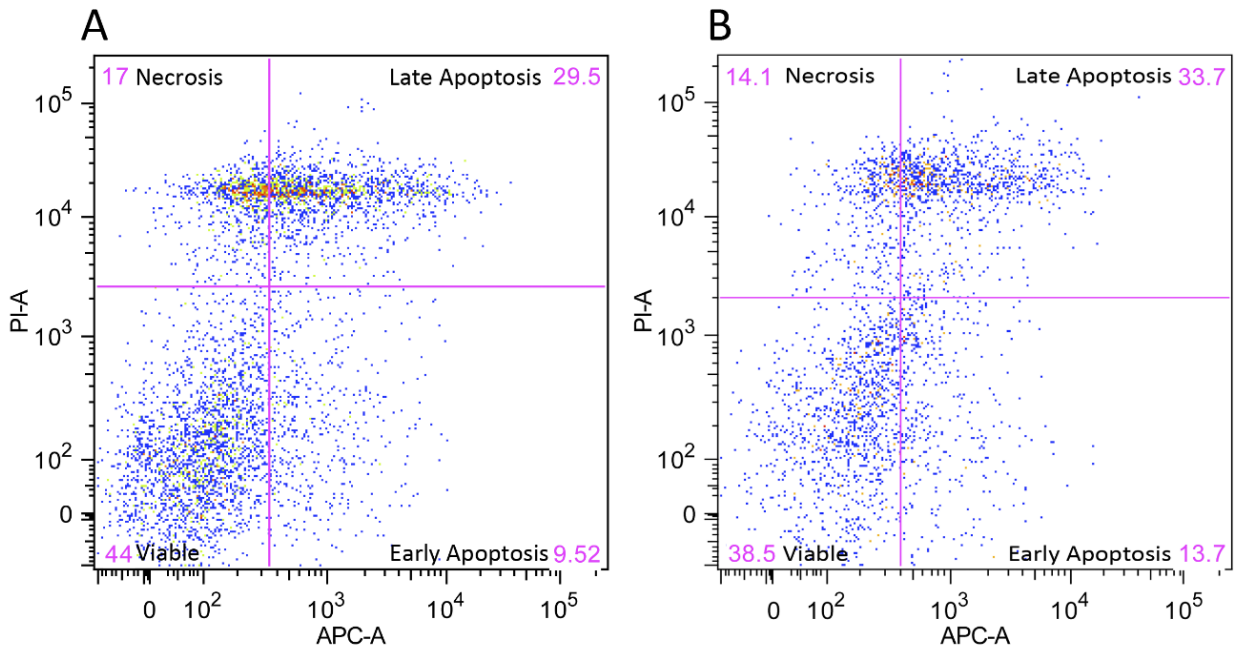

Fig. S4. Apoptosis of amoebocytes. Apoptosis of A) hyaline and B) granular amoebocytes incubated with 100 µg/mL of Cu in the form of CuO NPs after 24 h.