Electronic Supplementary Material (ESI) for Molecular Omics.

Supporting Information

Lipidomics reveals insights on the biological effects of copper oxide nanoparticles on a human colon carcinoma cell line

N. G. Chavez Soria, D. S. Aga*, G. E. Atilla-Gokcumen*

Department of Chemistry, University at Buffalo, The State University of New York (SUNY), Buffalo, NY 14260 USA

*Corresponding authors
Diana S. Aga (dianaaga@buffalo.edu)
G. Ekin Atilla-Gokcumen (ekinatil@buffalo.edu)

Figure S1. Characterization of CuO NPs. Particle size distribution of CuO NPs by **A**) dynamic light scattering and **B**) transmission electron microscopy shows agglomeration of CuO NP in the growth medium.

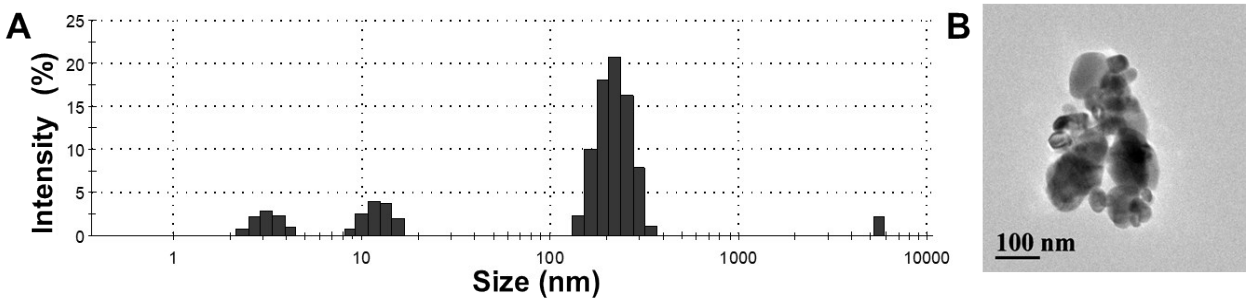

Figure S2. Effect of $CuSO_4$ on cell viability. Viability of HCT-116 (human colon carcinoma) cell lines exposed to $CuSO_4$ for 24 hours.

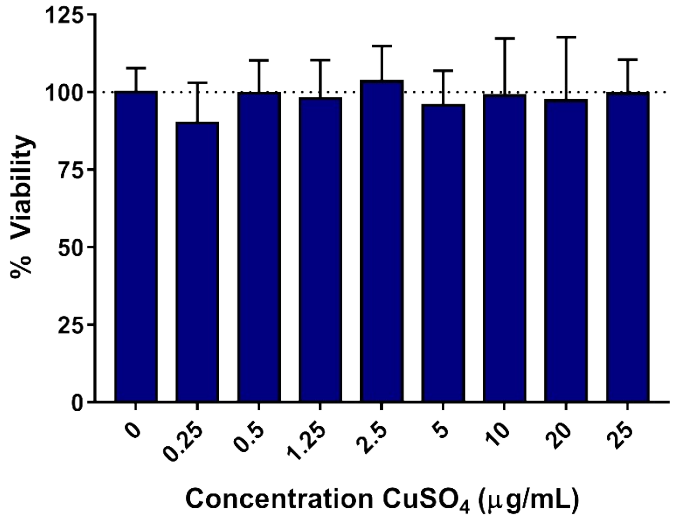

Figure S3: Dose-dependent upregulation of p62 and LC3B-II in HCT-116. Western blotting quantitative results showing levels of **A)** p62 (n=3) and **B)** LC3B-II (n=3) as a normalized ratio of α-tubulin for control and CuO NP expose cells (1.25-20 μg/ml) control. (p-value: *: p≤0.05, **: p≤ 0.01, ***:p ≤0.001, ****:p ≤0.0001, no sign: not significant).

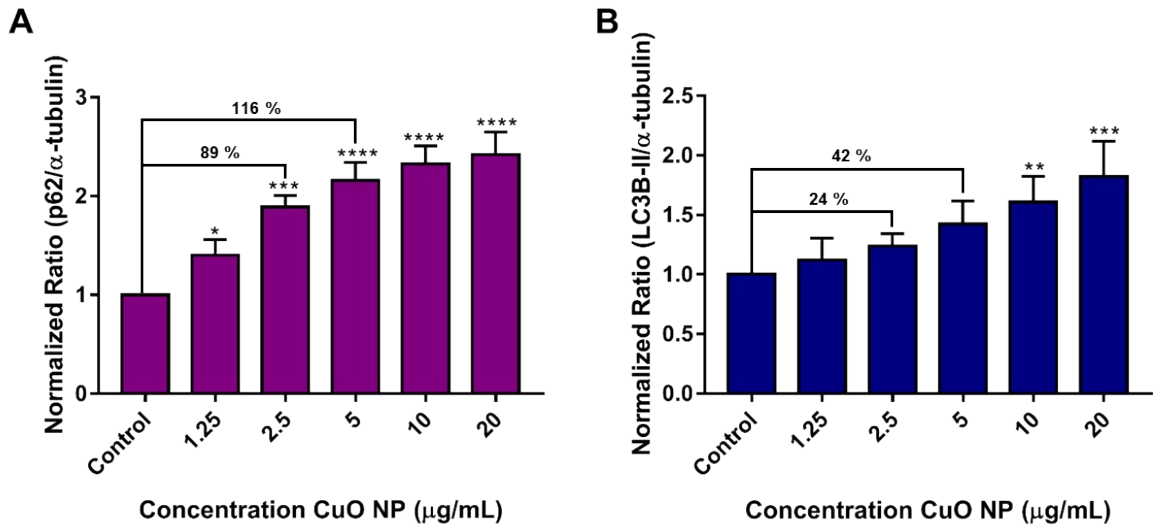

Figure S4: A) Western blotting results showing levels of PARP, cleaved PARP (C-PARP), Caspase 3, p21, p16(arrow denotes expected p16 band) and α-tubulin for control and CuO NP expose cells (1.2-20 µg/ml) control. A positive control for apoptotic cells are run in the same gel to confirm the lack of cleaved PARP (C-PARP) **B**) Viability HCT-116 cell lines that are exposed to CuO NP and pan caspase inhibitor zVAD-fmk.

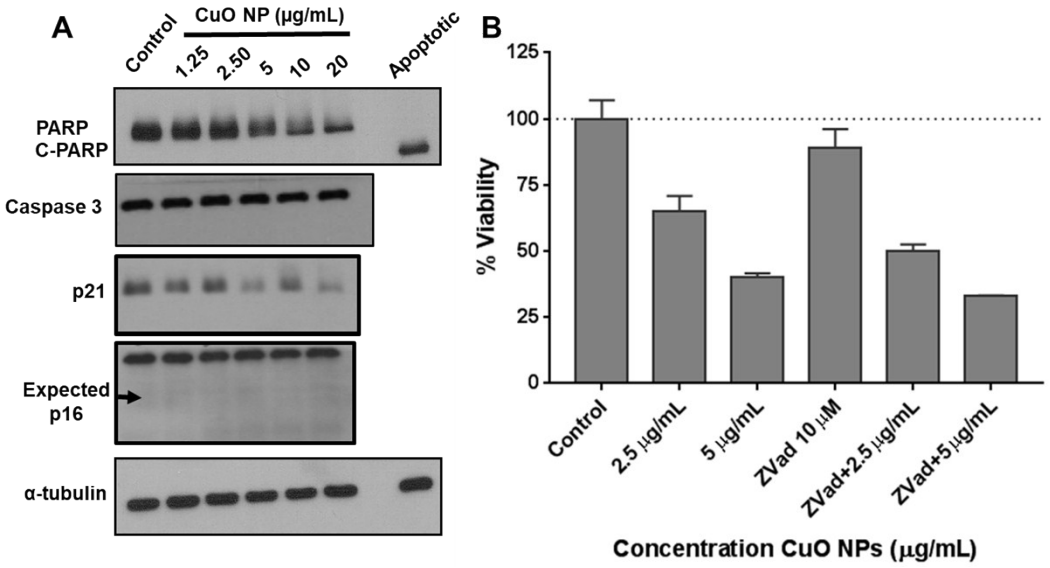

Figure S5. Metabolomics collection, preparation, and analysis workflow A) Sample treatment, collection, biphasic extraction, the addition of internal standards (TAG (13:0/13:0/13:0), ceramide (17:0) and d_9-oleic acid) and data acquisition for metabolomics of human colon carcinoma cell lines exposed to CuO NPs. **B)** Data acquisition and analysis parameters for the identification of up and down-regulated species after treatment.

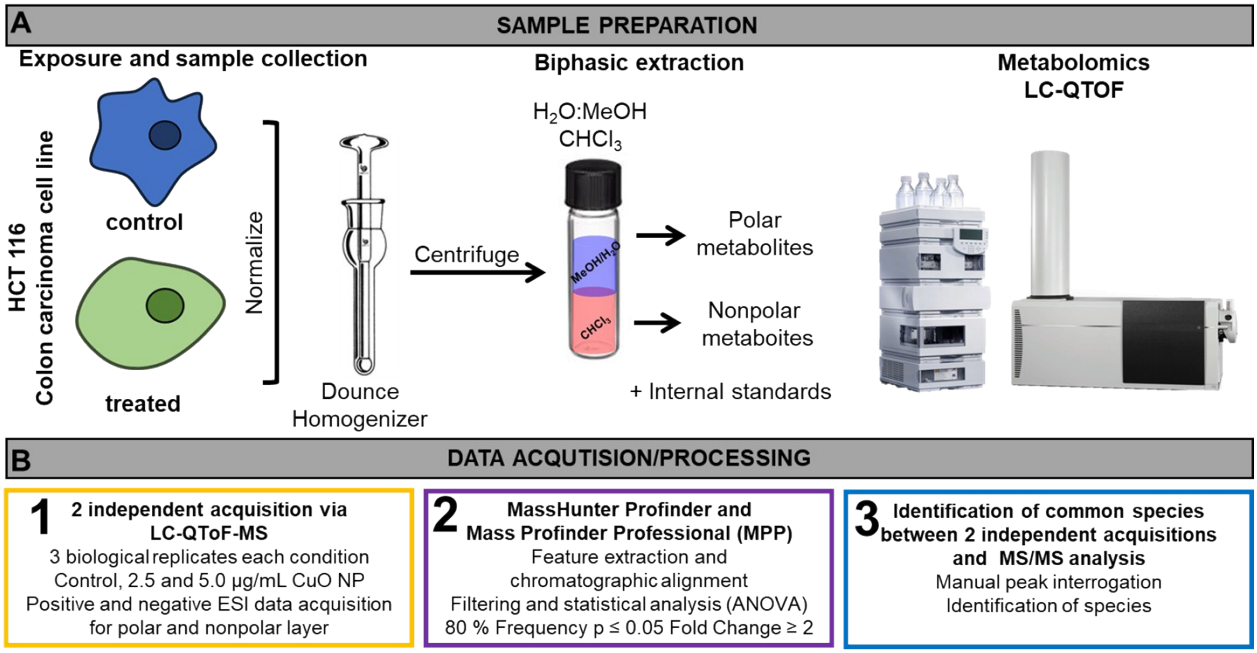

Figure S6. Total copper concentration in HEK 293T cells and growth medium. A) Total copper concentration (^{65}Cu) in growth cell medium in µg of Cu per mL (mean ± std dev) of growth medium. **B**) Total copper concentration (^{65}Cu) in 293T HEK cells in ng of Cu per mg of protein after 24-hour treatment. **C)** Western blotting results showing levels of **D)** p62 (n=3) and **E)** LC3B-II (n=3) as a normalize ratio of α-tubulin for control and CuO NP expose cells (1.25-10 µg/ml) control. (p-value: *: p≤0.05, **: p≤ 0.01, ***:p ≤0.001, ****:p ≤0.0001, no sign: not significant).

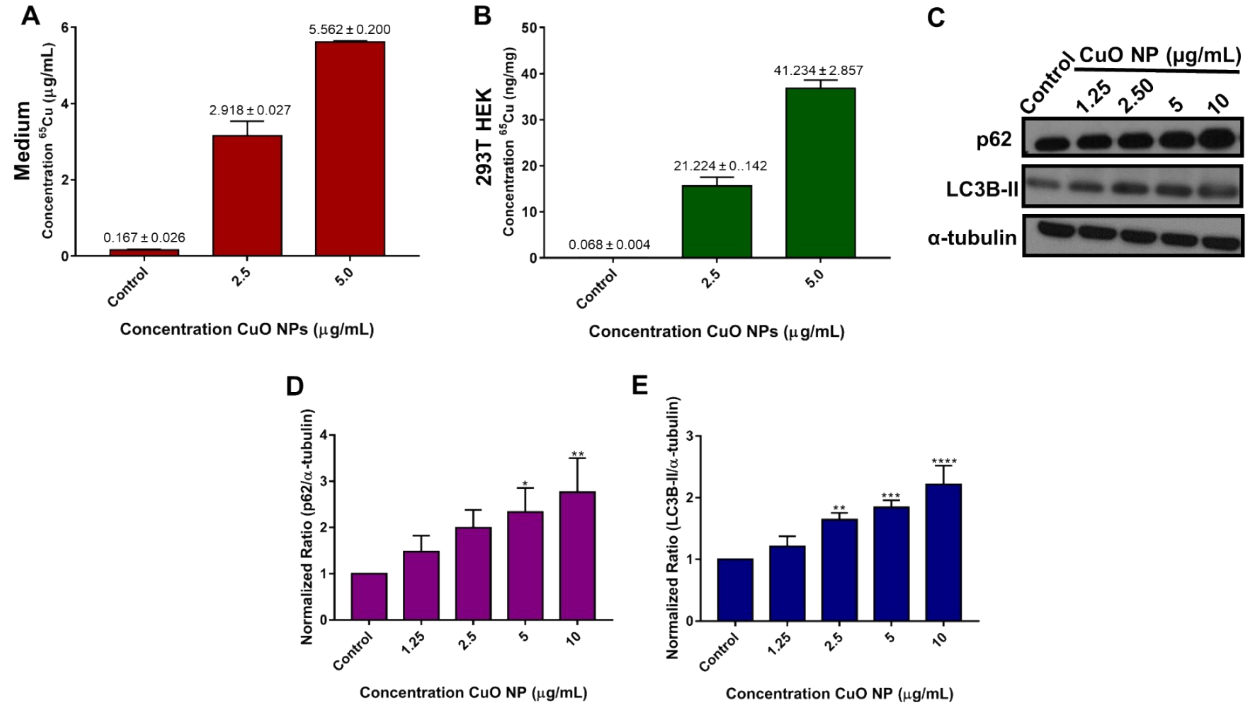

Table S1. Species that change after CuO NP treatment. Untargeted lipidomics results showing species accumulating or depleting species in the non-polar layer. (p-value: *: p≤0.05, **: p≤ 0.01, ***:p ≤0.001, no sign: not significant). Species that are shown in bold remain unidentified (we were not able to assign structures based on the fragmentation information).

Retention time (min)	**m/z**	***Adduct***	**Fold Change [2.5 µg/mL /Control]**	**Fold Change [5 µg/mL /Control]**	**Identification (MS/MS)**
63.83	564.5325	$[M-H]^-$	6.05***	8.52**	Ceramide (18:1,18:0)
65.61	618.5807	$[M-H]^-$	1.84***	2.18**	Ceramide (18:1, 22:1)
65.94	606.5792	$[M-H]^-$	2.33***	3.06**	Ceramide (16:1, 23:0)
66.45	620.5957	$[M-H]^-$	1.94***	2.30**	Ceramide (18:1, 22:0)
66.75	622.6063	$[M-H]^-$	1.98**	2.55**	Dihydroceramide (18:0, 22:0)
7.87	**485.3569**	**-**	**1.60**	**2.19****	**No match**
38.97	**416.3376**	**-**	**3.42**	**6.23****	**No match**
41.52	468.3114	$[M+H]^+$	2.29**	2.75**	Lyso PC (14:0)
43.39	568.3429	$[M+H]^+$	1.96**	2.84**	Lyso PC (22:6)
43.41	544.3445	$[M+H]^+$	1.80**	2.90**	Lyso PC (20:4)
43.86	**596.3352**	**$[M+H]^+$**	**1.22**	**2.62*****	**Phosphocholine containing lipid**
43.85	**572.3360**	**$[M+H]^+$**	**1.42**	**3.58*****	**Phosphocholine containing lipid**
44.44	496.3419	$[M+H]^+$	2.22**	2.76**	Lyso PC (16:0)
44.59	**598.3516**	**$[M+H]^+$**	**1.10**	**2.51*****	**Phosphocholine containing lipid**
45.65	482.3609	$[M+H]^+$	2.80***	3.82**	Lyso PC (O-16:0)
46.33	508.3752	$[M+H]^+$	2.31**	2.89**	Lyso PC (O-18:1)
47.14	**524.3714**	**$[M+H]^+$**	**2.18*****	**2.77****	**Phosphocholine containing lipid**
60.61	627.5369	$[M+H-H_2O]^+$	1.77**	2.08*	DAG 38:4 (18:0,20:4)
65.80	868.7379	$[M + NH_4]^+$	1.82**	2.82**	TAG 52:6 (14:0, 22:6, 16:0)
66.06	894.7598	$[M + NH_4]^+$	1.85**	2.53**	TAG 54:7 (16:1, 22:6, 16:0)
66.31	896.7717	$[M + NH_4]^+$	1.73*	2.14***	TAG 54:6 (18:2, 20:4, 16:0)
66.56	922.7879	$[M + NH_4]^+$	1.96**	2.62**	TAG 56:7 (18:1, 22:5, 16:1)
66.76	924.8021	$[M + NH_4]^+$	1.99**	2.89**	TAG 56:6 (18:1, 22:5,16:0)
67.37	978.8565	$[M + NH_4]^+$	1.95***	2.99**	TAG 60:7 (18:1, 18:1,24:5)

Table S2. This is provided as a separate Excel document. Abundances of lipids studied via untargeted lipidomics. Abundances, *m/z's*, adducts observed, specific fragments and retention times in each sample for lipids identified in the untargeted and targeted analysis are provided. **Sheet 1** is the species identified by untargeted lipidomics. **Sheet 2** is the lipid species analyzed by targeted analysis.

Table S3. Operational parameters for ICP-MS analysis of copper

Nebulizer and spray chamber	Glass concentric nebulizer in Peltier cooled cyclonic spray chamber (@ 2 ^{o}C)
Plasma forward Power, W	1400
Cooling gas flow (argon), L/min	13
Auxiliary gas flow (argon), L/min	0.70
Nebulizer gas flow (argon), L/min	0.94
Pole bias, V	-1.50
Hexapole bias, V	-4.00
Scanning modes	Survey and peak jumping
Dwell time (ms)	30 for Cu