Supporting Information for

Metabolomics to Detect Response of Lettuce (*Lactuca sativa*) to $Cu(OH)_2$ Nano-pesticides: Oxidative Stress Response and Detoxification Mechanisms

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Table S1 T-test results from 159 identified compounds

Leaf	p-value	Root	p-value
xylonic.acid	0.000535387	xylonic.acid	0.726538683
uridine	0.01977109	uridine	0.254120358
tocopherol.gamma.	0.595673826	tocopherol.gamma.	0.039125425
threonic.acid	1.37E-07	threonic.acid	0.027726816
sulfuric.acid	4.75E-05	sulfuric.acid	0.031817052
ribonic.acid	0.03293103	ribonic.acid	0.962181006
ribitol	0.034561787	ribitol	0.023493418
pyruvic.acid	0.007680636	pyruvic.acid	0.311996184
putrescine	0.010792429	putrescine	0.491751098
propane.1.3.diol.NIST	0.058200954	propane.1.3.diol.NIST	0.735293829
proline	0.018971595	proline	0.034780617
pipecolinic.acid	0.400334925	pipecolinic.acid	0.009303557
phytol	0.001538707	phytol	0.688019717
palatinitol	0.212951705	palatinitol	0.037701352
oxoproline	0.00051673	oxoproline	0.308851978
oxalic.acid	5.00E-06	oxalic.acid	0.351319573
nicotinamide	0.003546965	nicotinamide	0.440866798
nicotianamine	4.26E-07	nicotianamine	0.675310674
N.acetylmannosamine	0.008189876	N.acetylmannosamine	0.208323157
methanolphosphate	0.011674693	methanolphosphate	0.127549923
mannonic.acid.NIST	0.01442851	mannonic.acid.NIST	0.02330381
maleic.acid	0.008129262	maleic.acid	0.495035803
lyxitol	0.04241404	lyxitol	0.083642767
lysine	0.000100152	lysine	0.135304369
levoglucosan	1.71E-05	levoglucosan	0.29520042
leucrose	0.013255928	leucrose	0.129918902
leucine	0.004698212	leucine	0.015617482
isocitric.acid	0.000745753	isocitric.acid	0.042642595
hydroxylamine	1.72E-06	hydroxylamine	0.199086145
hexadecylglycerol.NIST	0.039212292	hexadecylglycerol.NIST	0.016581344
glycerol.3.galactoside	0.008180436	glycerol.3.galactoside	0.30990132
glycerol	0.00021766	glycerol	0.887669031
gluconic.acid	0.03135982	gluconic.acid	1.12E-06
glucoheptulose	0.003618541	glucoheptulose	0.106661909
ethanolamine	0.000174052	ethanolamine	0.367300112
erythronic.acid.lactone	0.00638892	erythronic.acid.lactone	0.542518509
dehydroascorbic.acid	8.47E-07	dehydroascorbic.acid	0.344595168
conduritol.beta.epoxide	0.011067076	conduritol.beta.epoxide	0.010427284
citrulline	0.1690631	citrulline	0.027123157
citric.acid	0.000571885	citric.acid	0.023217627
cis.caffeic.acid	0.016612361	cis.caffeic.acid	0.070663673
chlorogenic.acid	0.000482381	chlorogenic.acid	0.000474277
cerotinic.acid	1.16E-05	cerotinic.acid	0.537721147

butyrolactam.NIST	0.049373282	butyrolactam.NIST	0.238846179
beta.mannosylglycerate	9.11E-05	beta.mannosylglycerate	0.023385433
behenic.acid	0.000596282	behenic.acid	0.474966974
aspartic.acid	0.009633439	aspartic.acid	0.441889342
alpha.ketoglutarate	0.000431559	alpha.ketoglutarate	0.483482045
adenosine	0.817993524	adenosine	0.006208763
aconitic.acid	0.000379211	aconitic.acid	0.000111128
X4.hydroxybutyric.acid	0.006421733	X4.hydroxybutyric.acid	0.850480406
X4.aminobutyric.acid	0.015225612	X4.aminobutyric.acid	0.235176687
X3.4.dihydroxycinnamic.acid	0.00336161	X3.4.dihydroxycinnamic.acid	0.005815868
X2.5.dihydroxypyrazine.NIST	0.000180332	X2.5.dihydroxypyrazine.NIST	0.84550523
X1.methylgalactose.NIST	4.49E-12	X1.methylgalactose.NIST	0.905950642
X1.2.anhydro.myo.inositol.NIST	0.780458156	X1.2.anhydro.myo.inositol.NIST	0.017922338

The comparison is between control and all nanopesticides treated groups; compounds labeled with red color mean statistically significant ($p < 0.05$)

Table S2. Effect of $Cu(OH)_2$ nanopesticides on inorganic ion homeostasis

	Leaf											
	Mg	SD	P	SD	K*	SD	Ca	SD	Fe	SD	Zn	SD
Control	5685	198	6116	402	50208	1666	7787	595	88.5	64.6	21.8	0.2
uncovered low	5842	357	5782	443	55774	1866	7238	288	64.8	10.5	22.8	2.1
uncovered medium	6326	1022	6590	877	64647	11527	7498	419	93.5	34.1	25.9	4.7
uncovered high	6018	661	6099	463	58441	5583	7267	618	80.2	23.5	24.3	3.8
covered low	6051	348	6759	639	64344	4907	7583	368	75.7	11.8	27.9	3.1
covered medium	5676	329	6328	383	60868	3438	7046	458	70.7	10.2	25.5	2.4
covered high	5609	809	6490	648	56721	4422	6883	889	77.8	12.7	23.4	4.6
	Root											
	Mg	SD	P	SD	K	SD	Ca	SD	Fe	SD	Zn	SD
Control	4247	1110	2364	509	29961	6109	2227	32	2974	2255	12	2
uncovered low	3612	1294	3199	584	38924	11993	2566	613	953	784	10	3
uncovered medium	5246	2892	2409	373	35029	5841	2310	207	2632	2976	10	3
uncovered high	7325	5566	3228	448	48381	6714	2788	278	1697	1784	12	3
covered low	3622	910	2583	195	33959	2014	2294	281	733	369	8	1
covered medium	3805	892	2432	621	40765	10706	2432	313	578	138	9	2
covered high	3838	848	2546	912	42035	12757	2546	358	1459	1044	11	3

Data are average of five replicate, * means statistically significant compared to control. SD = standard deviation

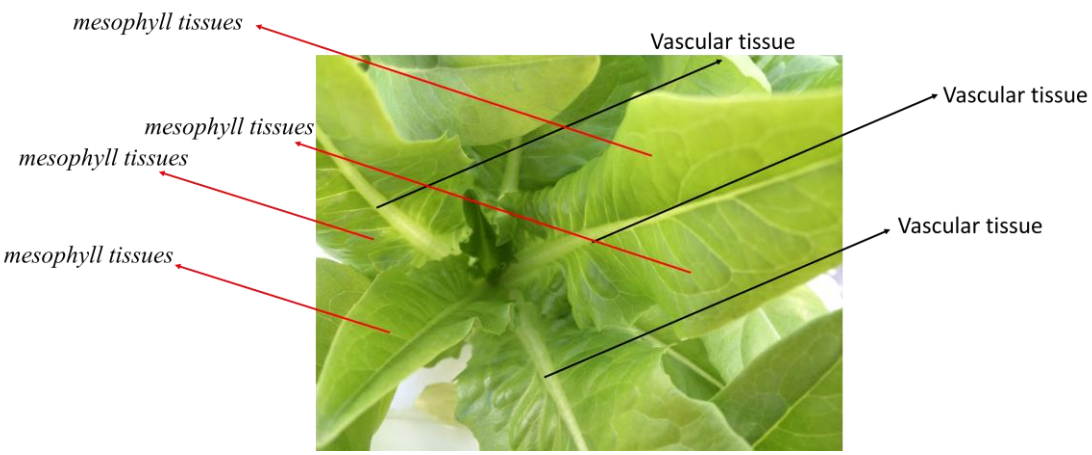

Figure S1. Vascular and mesophyll tissues

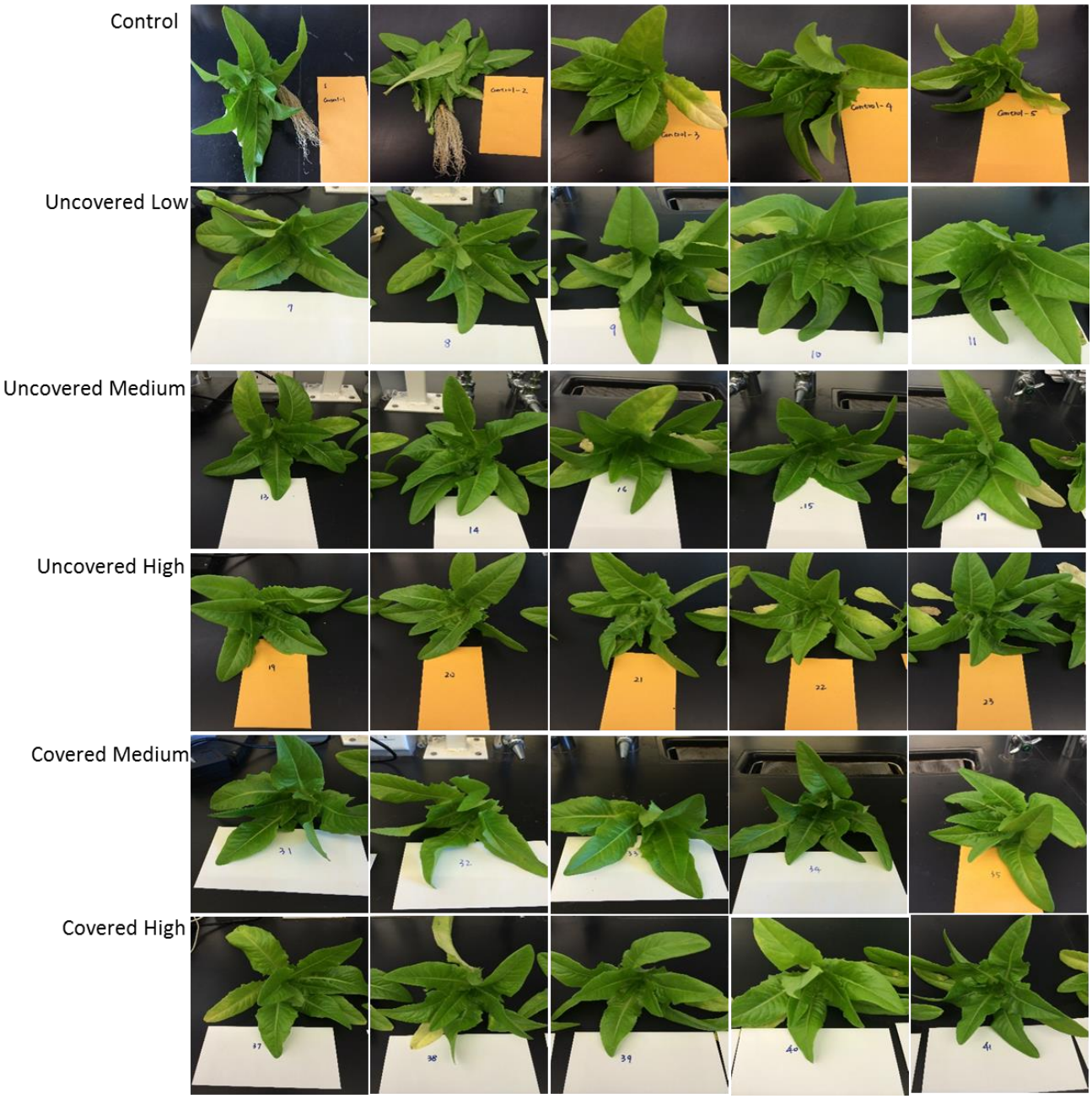

Figure S2. Photograph of lettuces at harvest (group of covered low were missing)

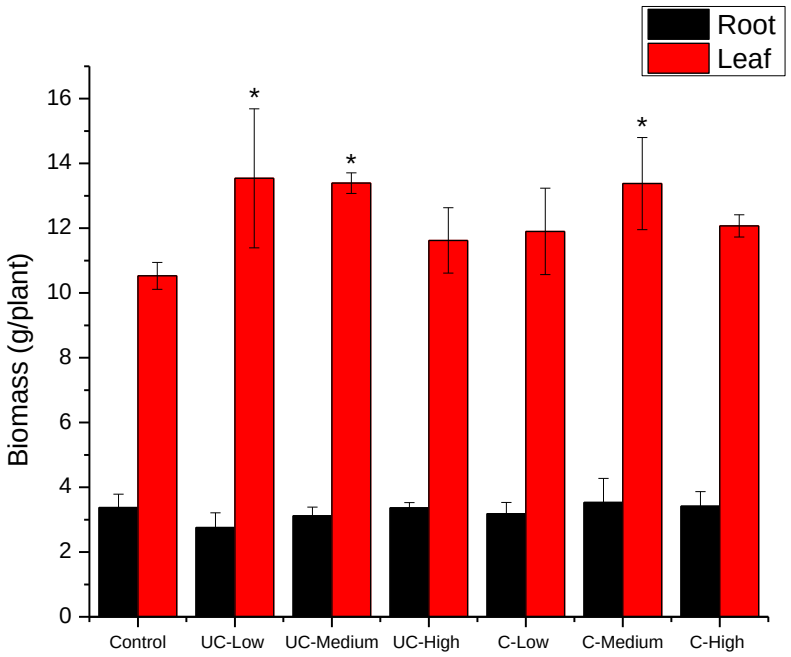

Figure S3. Biomass accumulation after 24-day-old lettuces were exposed to different doses (0, Low, Medium, High) of $Cu(OH)_2$ nanopesticides. Error bars represents the standard deviation of five replicates. Asterisk above a bar means significant difference between $Cu(OH)_2$ nanopesticides and control ($p < 0.05$).

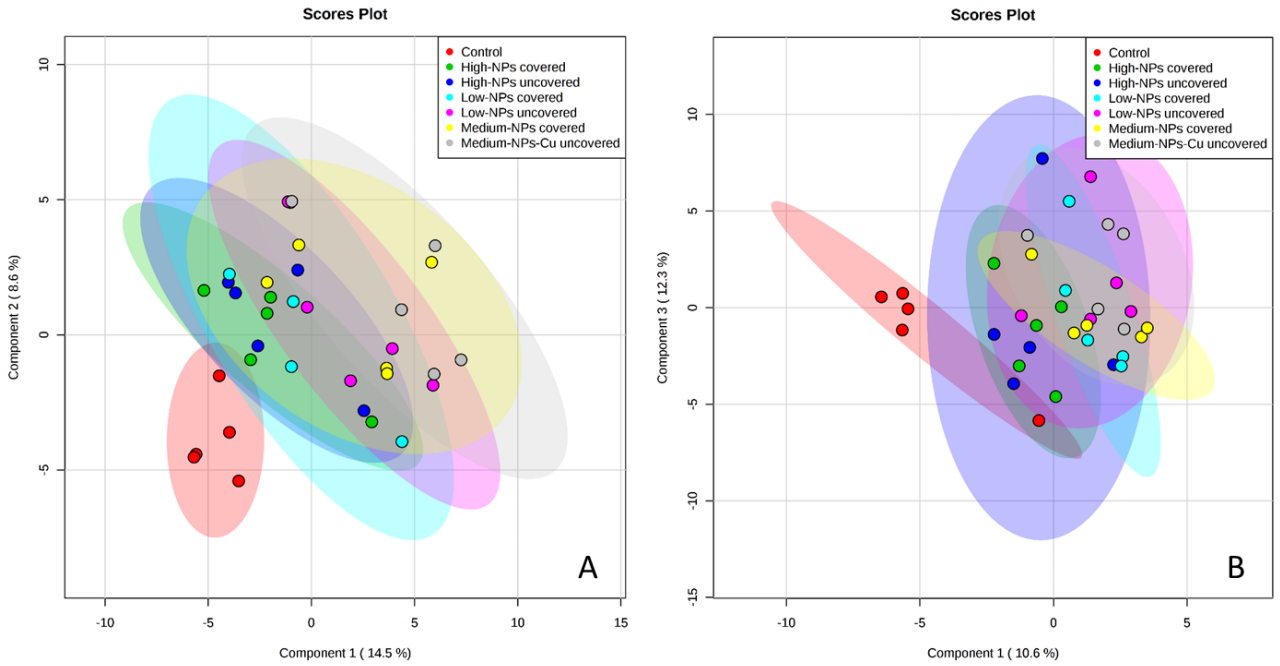

Figure S4. Partial least square (PLS) analysis of metabolites in lettuce (**A**) leaves and (**B**) roots as affected by different concentrations of copper hydroxide NPs. The data are from 159 metabolites identified via GC-TOF-MS.

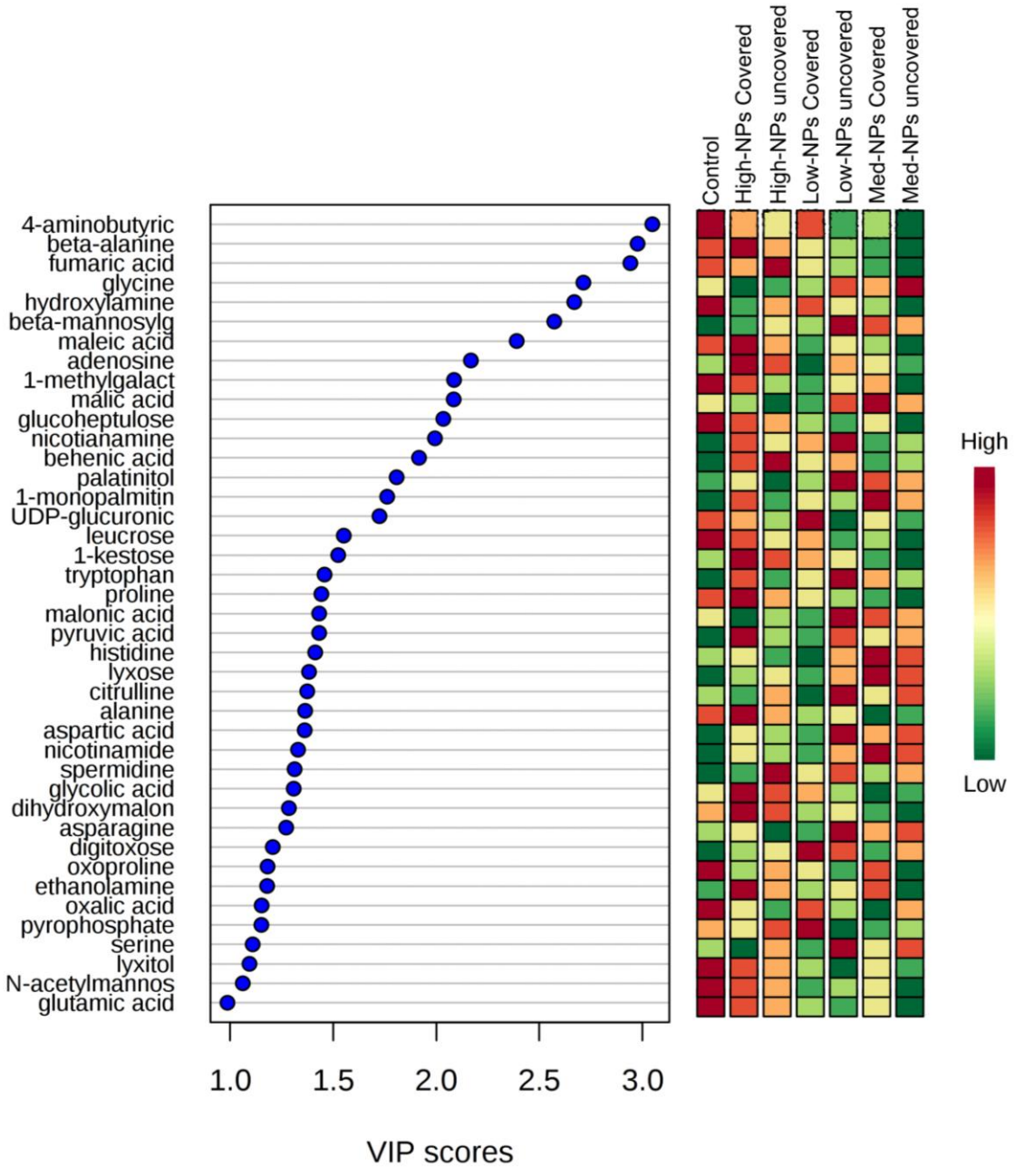

Figure S5. VIP scores from PLS-DA analysis of leaf metabolites showing how the discriminating metabolites induce group separation. The sample names in the right corner are: Control, Hight-NPs-covered, High-NPs-uncovered, Low-NPs-covered, Low-NPs-uncovered, Medium-NPs-covered, Medium-NPs-uncovered.

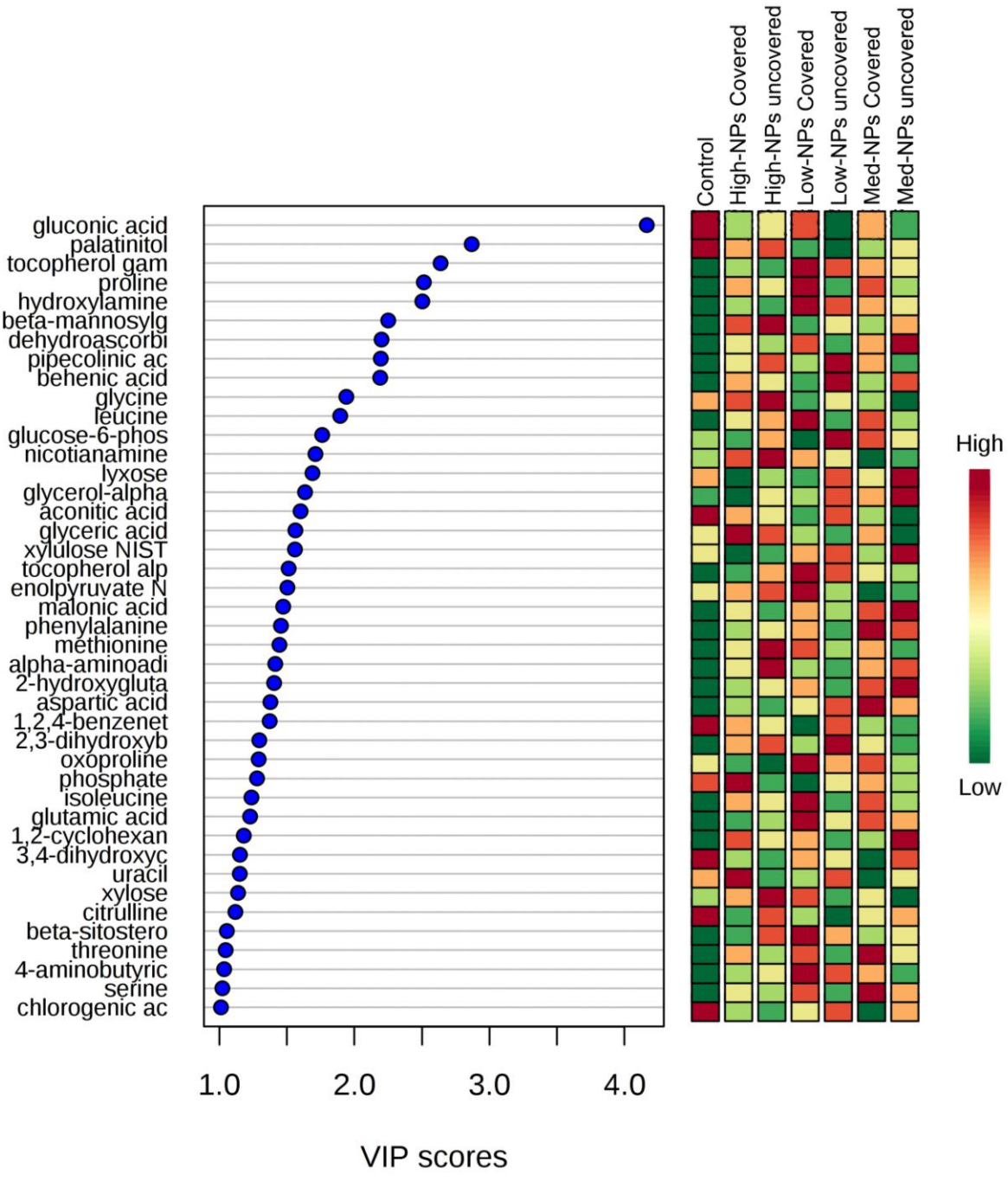

Figure S6. VIP scores from PLS-DA analysis of root metabolites showing how the discriminating metabolites induce group separation. The sample names in the right corner are: Control, Hight-NPs-covered, High-NPs-uncovered, Low-NPs.

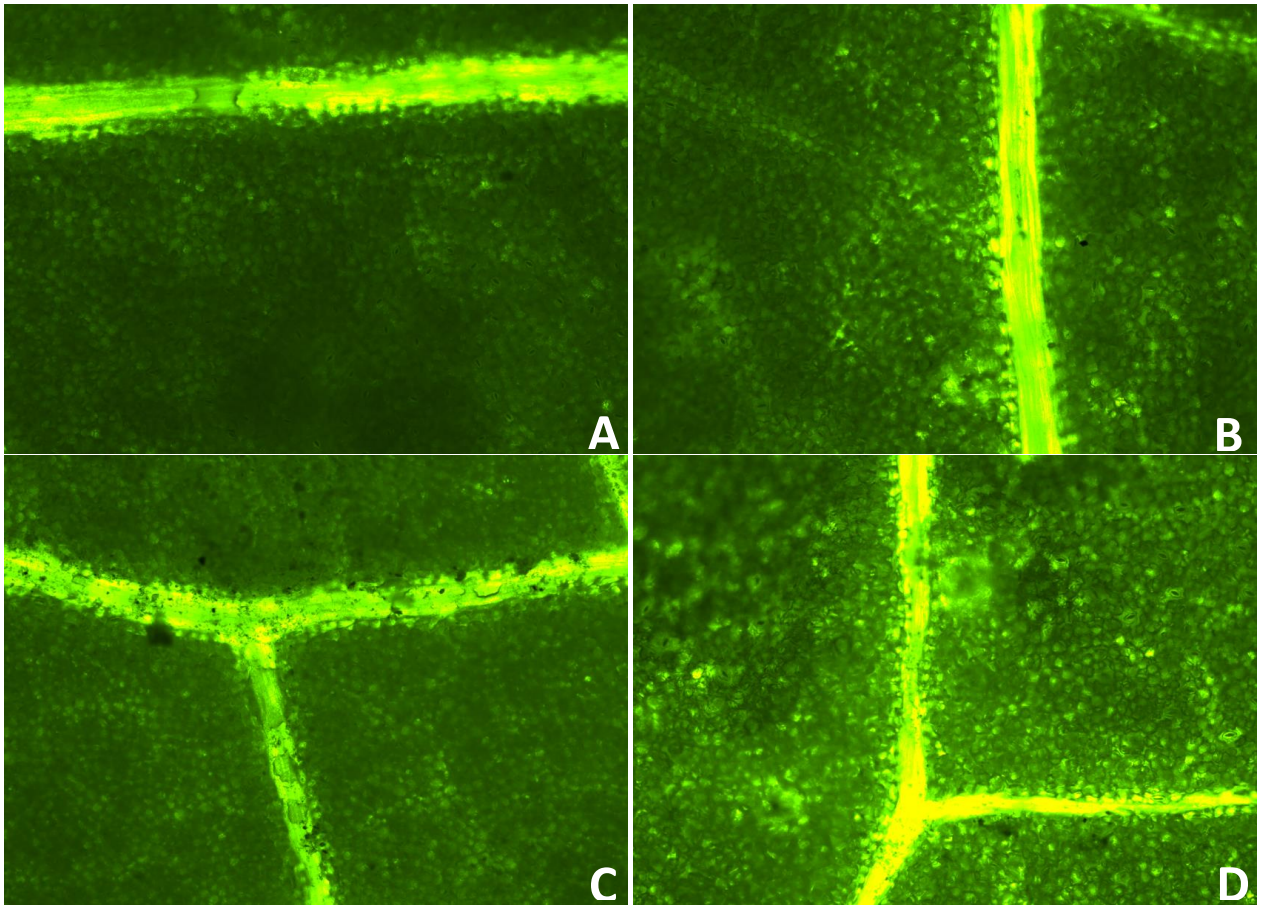

Figure S7. Fluorescence micrographs of the abaxial surface of lettuce leaves after foliar spray of copper hydroxide particles for 24 hours. Representative patterns of ROS production in lettuce leaves as indicated by the fluorescence of 2,7-dichlorodihydrofluorescein diacetate (DCF-DA), in the absence or presence of copper hydroxide NPs. A and C are control and NPs treated lettuce leaves without DCF-DA, the fluorescence indicate auto fluorescence of leave tissue; B and D are control and NPs treated lettuce leaves with DCF-DA, the intensity of fluorescence represent the ROS production. The fluorescence intensity of D (NPs treated) is much stronger than that in B (Control).