Supporting Information

Metabolomics reveals the mechanism of persistent toxicity of AgNPs at environmentally relevant concentrations to *Daphnia magna*

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Materials and Methods

Acute toxicity test

The acute toxicity experimental protocol for *D. magna* was conducted. According to the concentrations of AgNPs reported by Hou et al.,[1] acute toxicity experiments were set up with seven AgNPs concentration gradients: 0, 2, 4, 8, 16, 32, and 64 μg/L, and each concentration contained five parallels, and six experimental *D. magna* were placed in each beaker, and no feeding was performed during the experimental period. After 24 h of exposure, the death of *D. magna* was recorded and the LC_{50} values were calculated using the logistic curve combined with probit model. Meanwhile, the sensitivity of *D. magna* was tested using potassium dichromate compounds and this study found the immobility of *D. magna* was compliant with OECD (2004) Guidelines standards. In addition, in order to distinguish the acute toxicity of silver nanoparticles and their released silver ions, control groups of silver ion supernatant without nanoparticles (i.e., Ctrl-10 nm and Ctrl-70 nm groups) were set up in this study. The Ctrl-10 nm group was the silver ion supernatant after centrifugation of AgNPs-10 nm, and the Ctrl-70 nm group was the AgNPs-70 nm centrifuged silver ion supernatant. The method for obtaining silver ion supernatant is as follows: seven different concentrations of AgNPs (0, 2, 4, 8, 16, 32, and 64 μg/L, 10 or 70 nm) cultures were exposed in an incubator for 24 h, after which they were transferred to a regular centrifuge tube and ultracentrifuged (4 °C, 12000 rpm, 150 min). After centrifugation, all the supernatants were put into ultrafiltration tubes (Millipore Amicon Ultra-15, 10 kDa) for further ultrafiltration separation (4 °C, 3000 g, 30 min), and the filtered solution after centrifugation was the

silver ions supernatant.

References

1. J. Hou, Y. Zhou, C. J. Wang, S. G. Li and X. K. Wang. Toxic effects and molecular mechanism of different types of silver nanoparticles to the aquatic crustacean *Daphnia magna*. *Environ. Sci. Technol.*, 2017, **51**, 12868-12878.

Figures and Tables

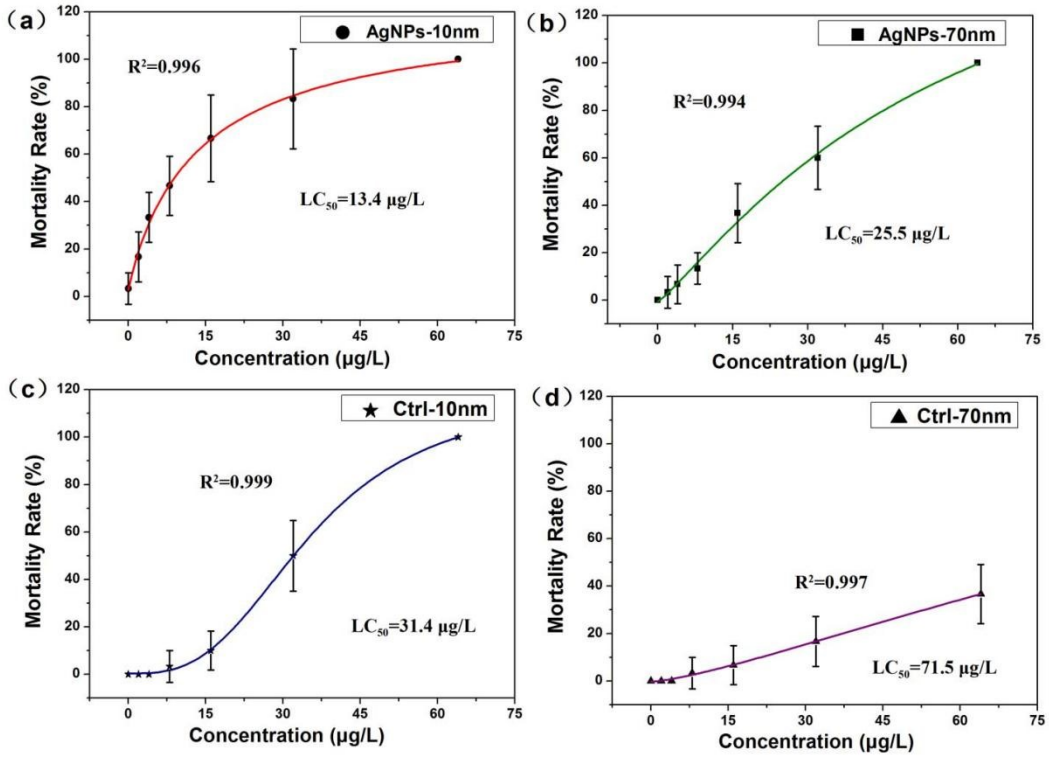

Fig S1. Mortality rate of *D. magna* exposed to different concentrations of AgNPs and Ag^+ supernatant for 24 h. (a) AgNPs-10nm. (b) AgNPs-70nm. (c) Ctrl-10nm. (d) Ctrl-70nm.

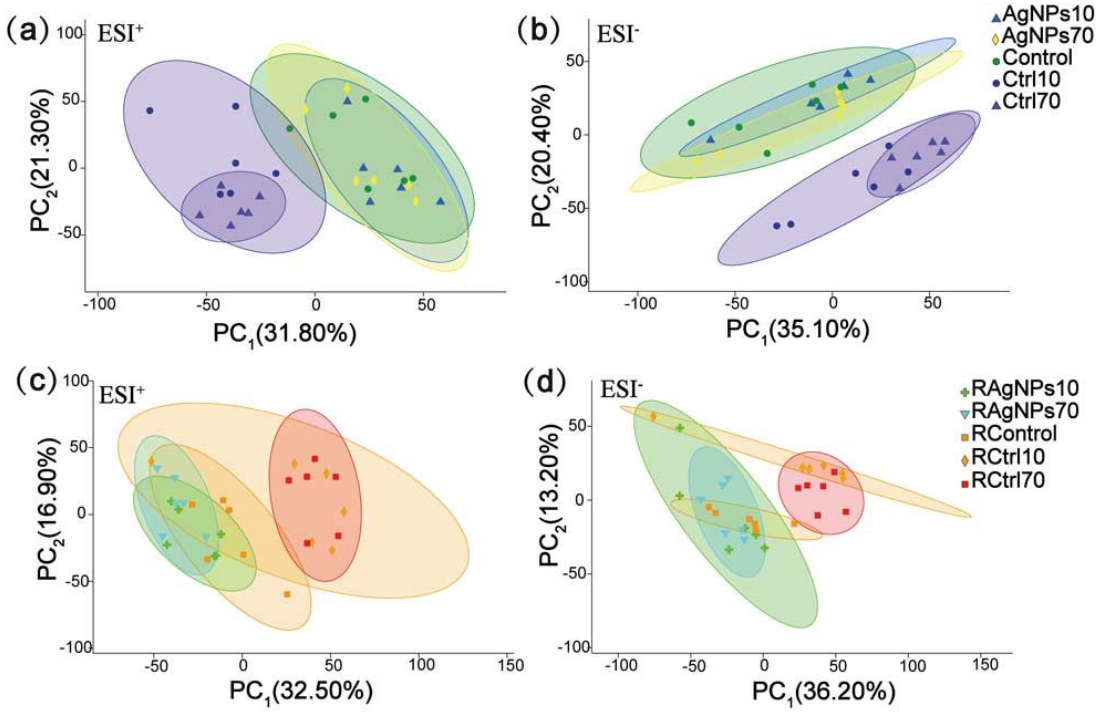

Fig S2. Scatter plots of scores for the principal component analysis of metabolites in *D. magna* after exposure and recovery of AgNPs with different sizes. (a,b) Exposure stage. (c,d) Recovery stage. (a,c) ESI positive model. (b,d) ESI negative model.

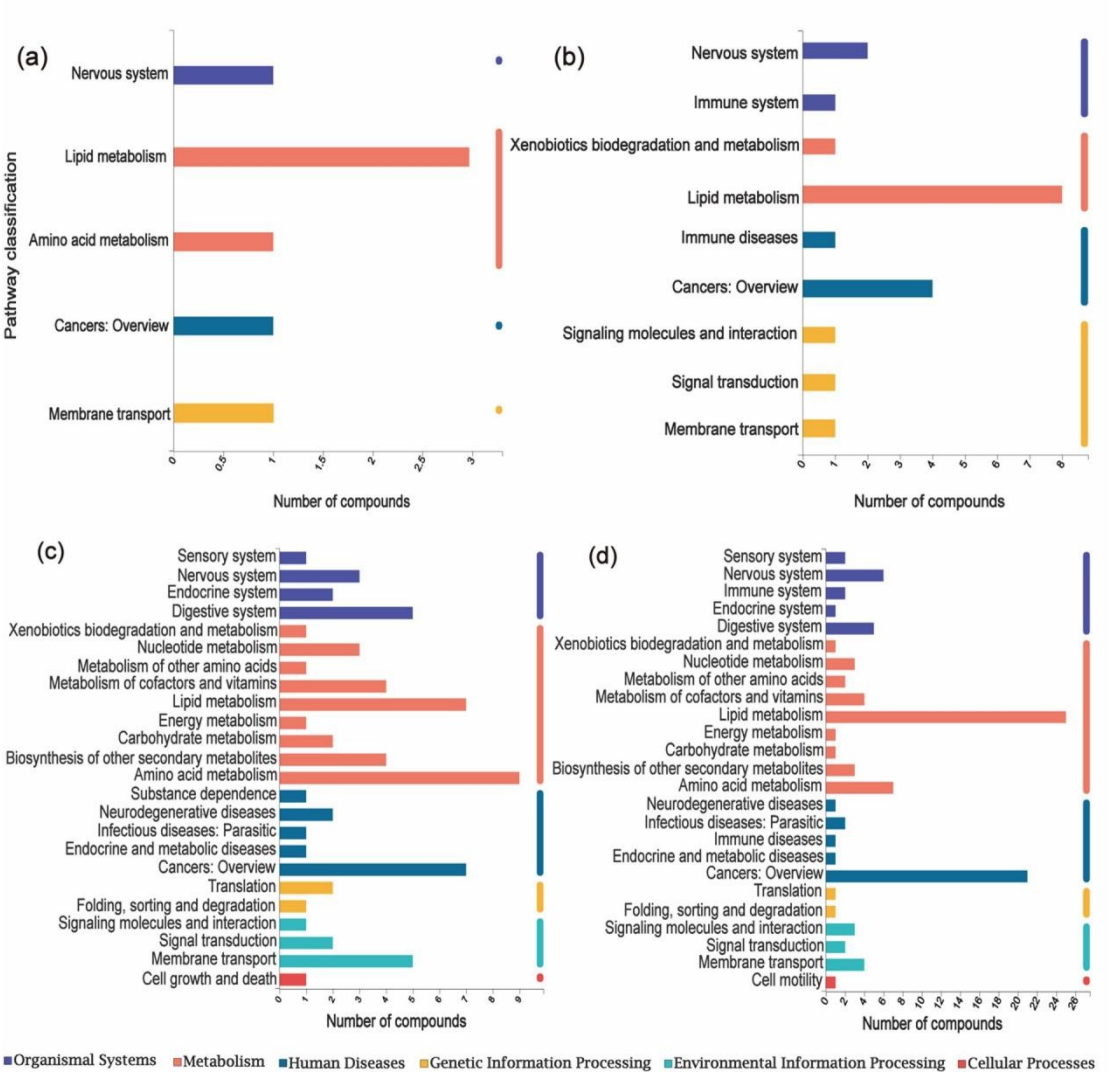

Fig S3. KEGG classification analysis of differential metabolites in *D. magna* during the exposure stage. (a) AgNPs-10nm. (b) AgNPs-70nm. (c) Ctrl-10nm. (d) Ctrl-70nm.

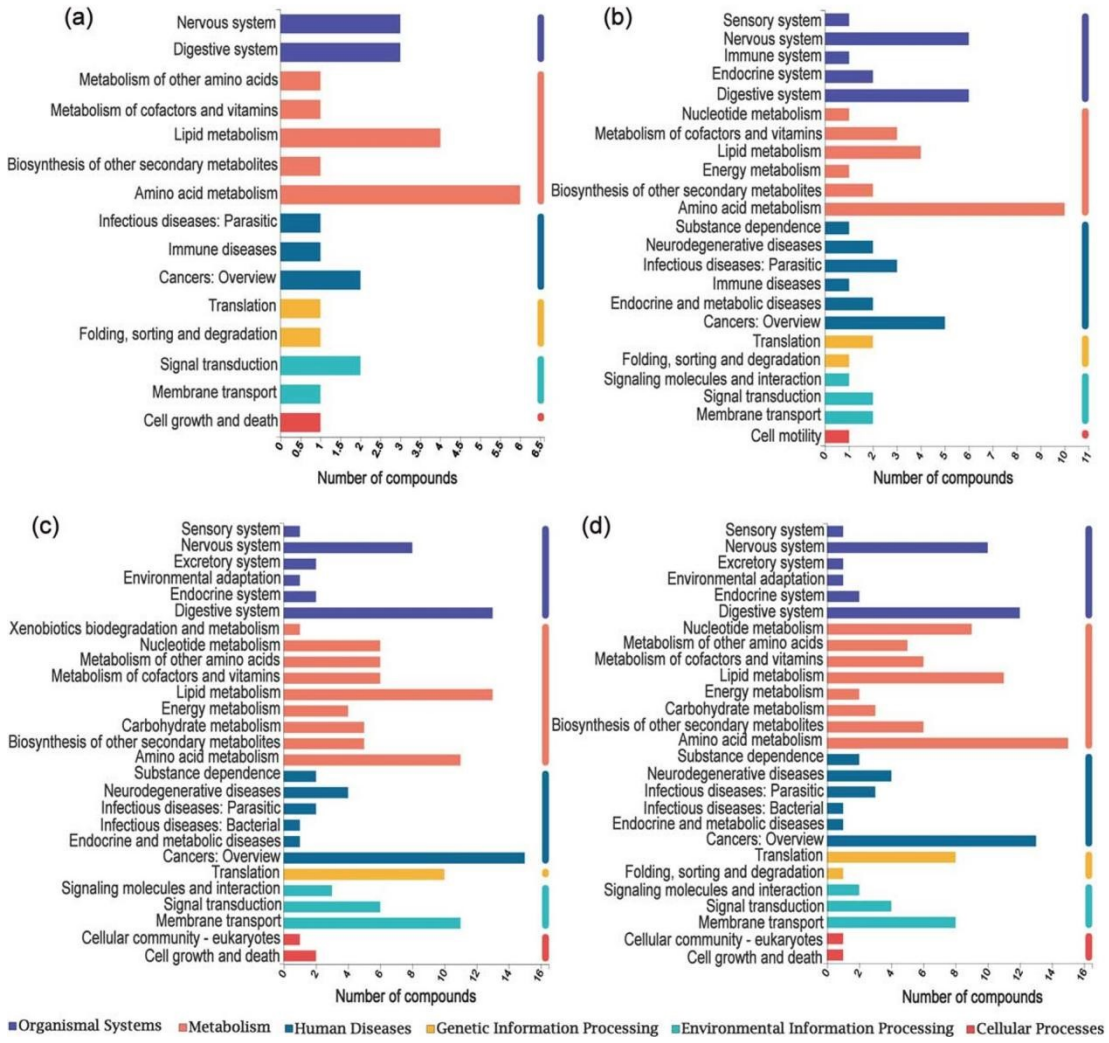

Fig S4. KEGG classification analysis of differential metabolites in *D. magna* during the recovery stage. (a) AgNPs-10nm. (b) AgNPs-70nm. (c) Ctrl-10nm. (d) Ctrl-70nm.

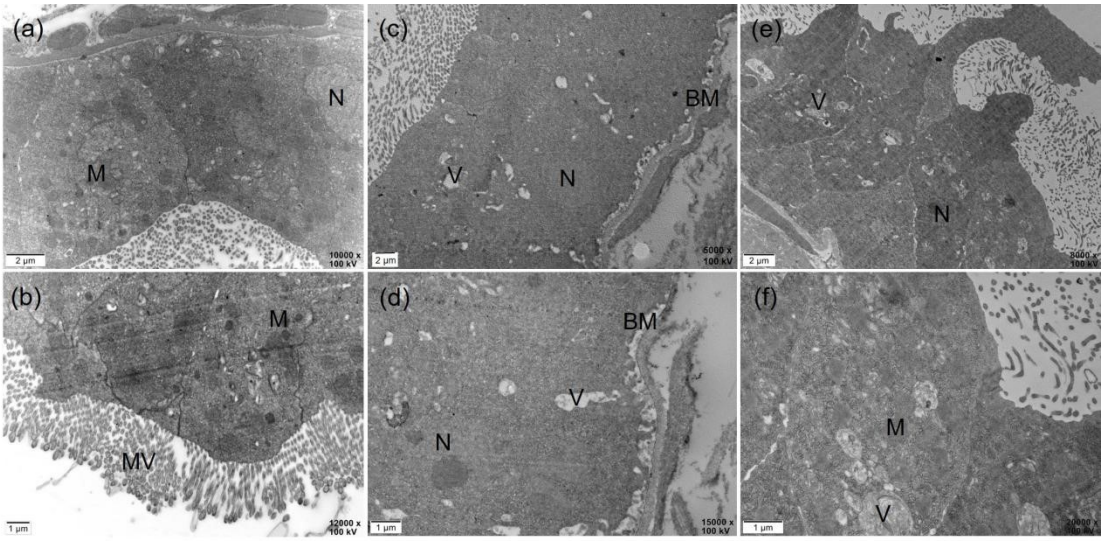

Fig S5. TEM images of the gut of *D. magna* after AgNPs exposure for 24 h. (a, b) Control group. (c, d) AgNP-10 nm group. (e, f) AgNP-70 nm group. MV: microvilli; M: mitochondrion; N: nucleus; V: vacuole; BM: basement membrane.

Table S1. Analysis of specific metabolic pathways during the exposure stage

No.	Pathway name	*P* values			
		AgNPs-10	AgNPs-70	Ctrl-10	Ctrl-70
1	Lysine degradation	0.0542	/	/	/
2	Arachidonic acid metabolism	0.0774	0.0192*	/	0.0816
3	Alpha-Linolenic acid metabolism	0.0439*	0.1163	/	0.2368
4	Lysine biosynthesis	0.0367*	/	0.194	0.2015
5	Linoleic acid metabolism	0.0295*	0.079	/	0.1646
6	Retrograde endocannabinoid signaling	0.0201*	0.0542	/	0.1148
7	Fatty acid degradation	0.0521	0.137	/	0.2754
8	Fc epsilon RI signaling pathway	/	0.0317*	/	0.0861

Note: * represent the significant difference at $p<0.05$ levels.