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Interaction of antibacterial silver nanoparticles and microbiota-dependent holobiont revealed by meta-transcriptomic analysis

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Running Title: Gut microbe-dependent detoxification of silver nanoparticles in zooplankton

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Method for measuring AgNPs related dissolution process

The release kinetics of soluble Ag^+ from AgNPs were determined by conventional ultracentrifugation followed with ICP-MS detection. At different time points, the exposure medium was mixed, collected and the dissolved Ag fraction was separated from the AgNPs by ultracentrifugation through 3 kDa membranes (Millipore). Afterwards, the filtrate (about 2 mL) containing the soluble Ag was sampled followed by acid digestion at 80 °C. For full digestion of the samples, 289 μL of 69% nitric acid (trace metal grade) was added to each sample and digested for 12 h. After conducting full optimization of the ICP-MS, a blank solution and five standard solutions were measured to obtain a calibration curve. Samples were then analyzed to obtain Ag concentration of the digested samples. To confirm accuracy of the ultracentrifugation coupled with ICP-MS method, the standard addition method was conducted, and the recovery was within 82.3-95.8 %, indicting the reliability of the adopted method for the separation of AgNPs and Ag+ and the detection of Ag concentration.

Table S1 Primers for qPCR detection

Gene	Forward (5'-3')	Reverse (5'-3')	Target
metK (S-adenosylmethionine synthetase)	ATCGCTCTGCTGCTTATG	TTGGATGTGCCGTAATCG	*D. magna*
ahcY (adenosylhomocysteinase)	GCGTCAGGAATATCAACCA	CAGGCGAAGATGGAGAAC	*D. magna*
CTH (cystathionine gamma-lyase)	CTACCGTCTTCAATCTGCTA	GCCGAGTTCATCACAATATC	*D. magna*
HSP90B (heat shock protein 90kDa beta)	CCGAGGCTGTTGATGAATA	AGGATGGCGAGGATTGAT	*D. magna*
DNAJC3 (DnaJ homolog subfamily C member 3)	GCATCCAGACAACTTCCA	TCCTCGCCATTATCATACTT	*D. magna*
DNAJB11 (DnaJ homolog subfamily B member 11)	TTGAACACTTGGATGGACAT	TTGATTGAATCTTGCGACAG	*D. magna*
CALR (calreticulin)	TGGAGGTGGCTATGTCAA	TCTGGCTTAACGATGAGTG	*D. magna*
BIP (heat shock 70kDa protein 5)	CTAAGGTTCAGCAGTTGGT	TGGTCGTTGGTAATGGTAAT	*D. magna*
SEC61A (protein transport protein SEC61 subunit alpha)	TGCCGTCGTCATATACTTC	TGCCGTCGTCATATACTTC	*D. magna*
WBP1 (oligosaccharyltransferase complex subunit beta)	ATCCTCTGGTCCTTGAAGT	GACTGGCGATGAGAAGAAT	*D. magna*
18S rRNA	CCTGCCAAGTATGATGACATCAA	AGCCCAGGATGCCCTTTAGT	*D. magna*
dsrA (dissimilatory sulfite reductase alpha subunit)	ACSCACTGGAAGCACGCCGG	GTGGMRCCGTGCAKRTTGG	Gut microbiota
butyryl-CoA CoA transferase	GCIGAICATTTCACITGGAAYWSITGGCAYATG	CCTGCCTTTGCAATRTCIACRAANGC	Gut microbiota
narG (nitrate reductase / nitrite oxidoreductase, alpha subunit)	TAYGTSGGGCAGGARAAACTG	CGTAGAAGAAGCTGGTGCTGTT	Gut microbiota
nirK (nitrite reductase)	TCATGGTGCTGCCGCGKGACGG	GAACTTGCCGGTKGCCCAGAC	Gut microbiota
SoxB (sarcosine oxidase, subunit beta)	GGATACTTGGCAGGGTTCG	GTAATAGGGGTCGGCGTTG	Gut microbiota
fliC (flagellin)	CCACGACAGGTCTTTATGATCTGA	CAACTGTGACTTTATCGCCATTCC	Gut microbiota
16S rRNA	TCCTACGGGAGGCAGCAGT	GGACTACCAGGGTATCTAATCCTGTT	Gut microbiota

Table S2 Illumina sequencing statistics of mRNA dataset

Sample	Base number (Gb)	Raw reads	Clear reads	%>Q30
Normal-1	37.42	113,581,610	113,578,438	93.41
Normal-2	35.66	102,373,718	102,371,140	92.31
Normal-3	36.28	109,114,324	103,112,170	91.18
Low AgNPs-1	38.64	112,155,218	112,153,468	93.31
Low AgNPs-2	42.82	114,113,649	114,108,630	91.72
Low AgNPs-3	41.54	113,471,381	113,467,344	91.87
High AgNPs-1	36.78	111,805,444	111,803,212	91.04
High AgNPs-2	39.48	111,876,354	111873,642	91.38
High AgNPs-3	39.62	111,881,432	111,879,324	90.73
TLAgNPs-1	35.08	82,027,502	82,024,144	92.76
TLAgNPs-2	37.74	114,113,322	114,110,242	91.68
TLAgNPs-3	37.46	113,821,422	113,816,388	91.67
FLAgNPs-1	66.26	189,911,428	189,907,354	90.34
FLAgNPs -2	65.34	187,409,496	187,407,788	91.51
FLAgNPs -3	36.13	118,322,114	188,318,946	92.43

Table S3 Summary of the mRNA assembly and coding regions

Sample	Assembled contigs >200 (*Daphnia*)	N50 (*Daphnia*)	Assembled contigs (Gut bacteria)	N50 (Gut bacteria)	Coding regions (*Daphnia*)	Coding regions (Gut bacteria)
Normal-1	217,496	1,842	45,711	1174	95897	17,712
Normal-2	204,417	1,733	47,146	1038	93987	15,573
Normal-3	215,331	1,697	47,044	974	94113	16,635
Low AgNPs-1	238,221	1,831	73,086	1247	87924	34,738
Low AgNPs-2	243,877	1,763	79,347	1311	88595	36,329
Low AgNPs-3	239,447	1,943	76,422	1287	88192	35,409
High AgNPs-1	214,183	1,587	21,344	773	92402	7,896
High AgNPs-2	216,357	1,677	20,745	794	94651	8,544
High AgNPs-3	211,853	1,718	22,664	787	94718	8,648
TLAgNPs-1	234,125	1,811	67,414	1194	98941	20,834
TLAgNPs-2	225,495	1,742	62,322	1231	99987	19,779
TLAgNPs-3	227,487	1,682	65,447	934	99471	19,933
FLAgNPs-1	283,904	2,318	232	-	100167	92
FLAgNPs -2	282,012	2,274	314	-	111235	71
FLAgNPs -3	215,765	1,714	149	-	94311	17

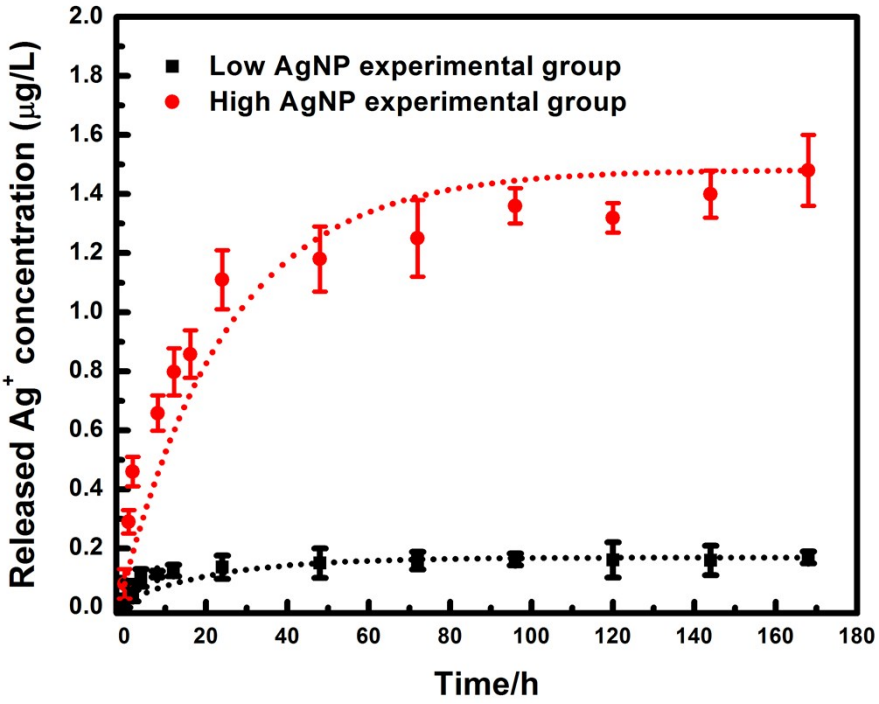

Figure S1. the concentration of released silver ion from the dissolution process of AgNPs at different time point.

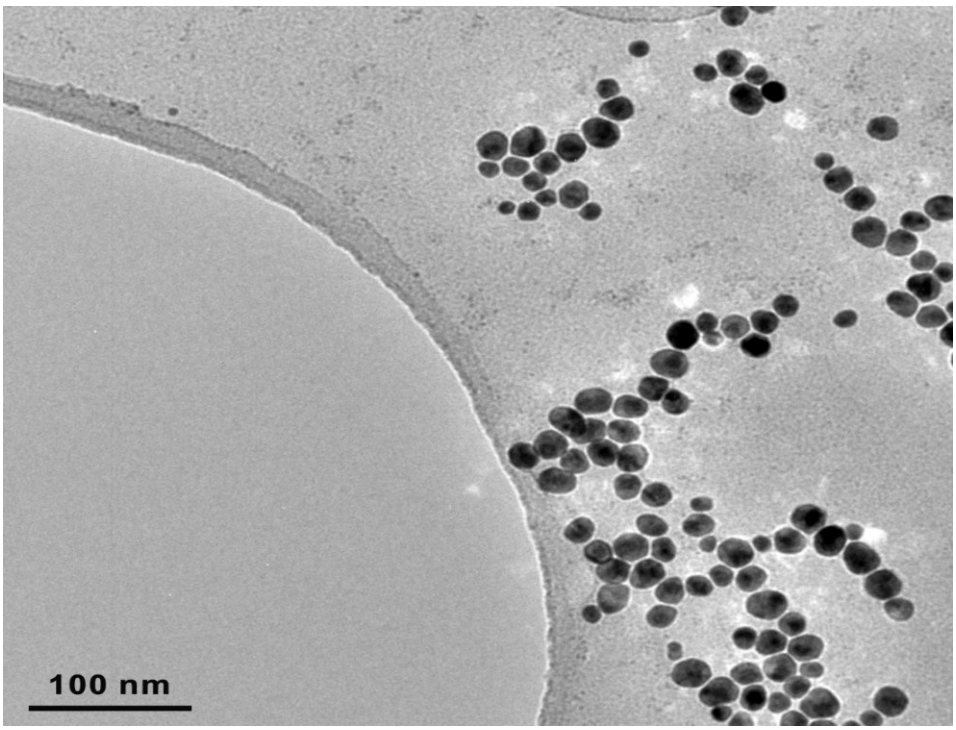

Figure S2. TEM image of self-prepared citrate-stabilized 20nm AgNPs

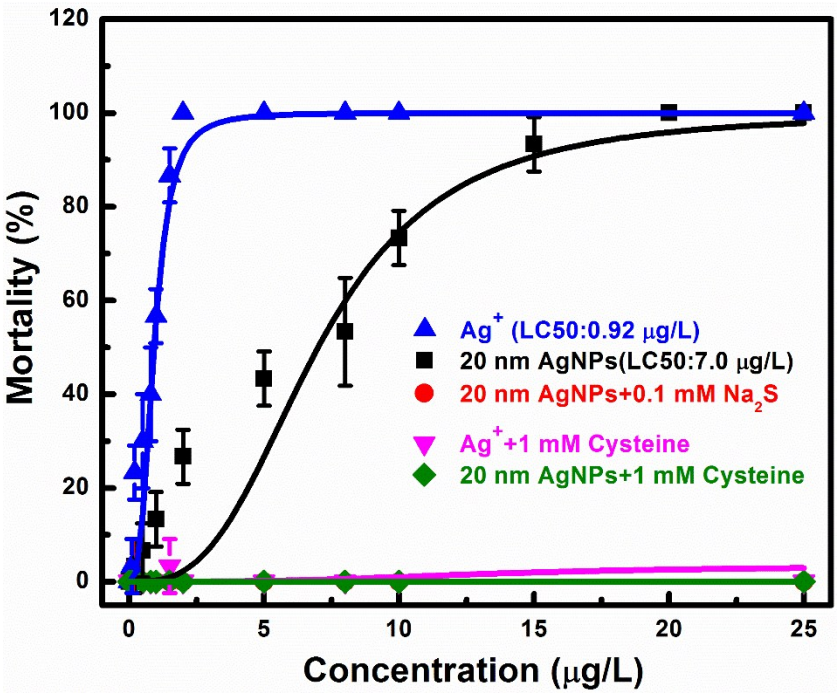

Figure S3. A 48-h mortality test of *D. magna* following exposure to 20 nm AgNPs and Ag+ under different exposure condition. (▲) Different concentration of Ag^+, (■) different concentration of 20 nm AgNPs, (●) different concentration of 20 nm AgNPs mixed with 0.1 mM Na_2S, (▼) different concentration of Ag^+ mixed with 1 mM cysteine, (◆) different concentration of 20 nm AgNPs mixed with 1 mM cysteine.

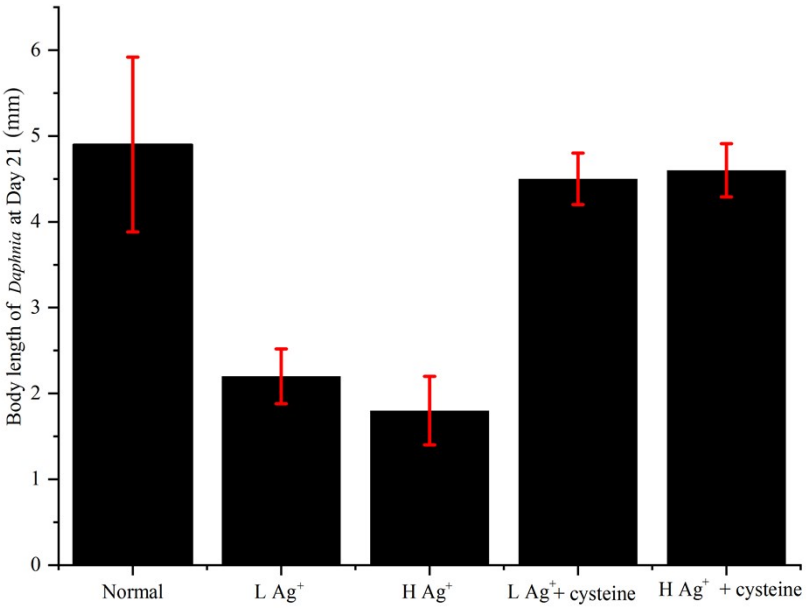

Figure S4. Body length of *D. magna* over a period of 21 days after exposed under normal condition as control, high Ag+ concentration (H Ag^+), low Ag+ concentration (L Ag^+), high Ag+ concentration mixed with 1 mM cysteine (H Ag^++ cysteine) and low Ag^+ concentration mixed with 1 mM cysteine (L Ag^++ cysteine), respectively.

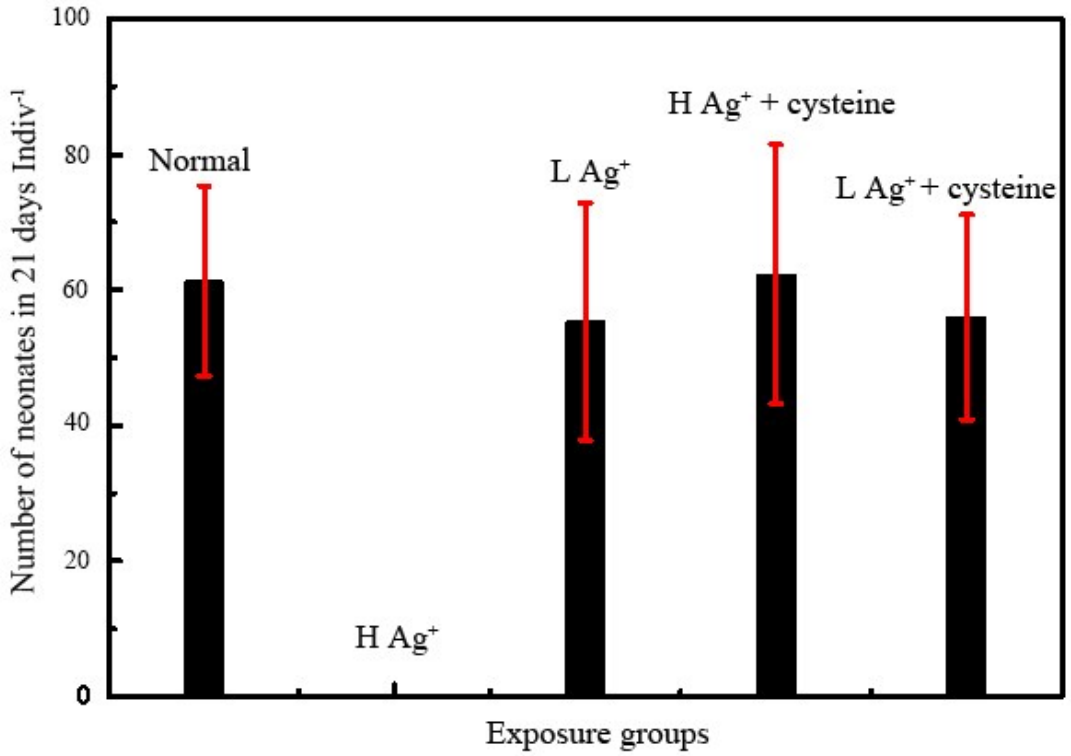

Figure S5. Number of neonates generated by each *D. magna* over a period of 21 days after exposed under normal condition as control, high Ag^+ concentration (H Ag^+), low Ag^+ concentration (L Ag^+), high Ag+ concentration mixed with 1 mM cysteine (H Ag^++ cysteine) and low Ag+ concentration mixed with 1 mM cysteine (L Ag^++ cysteine), respectively.

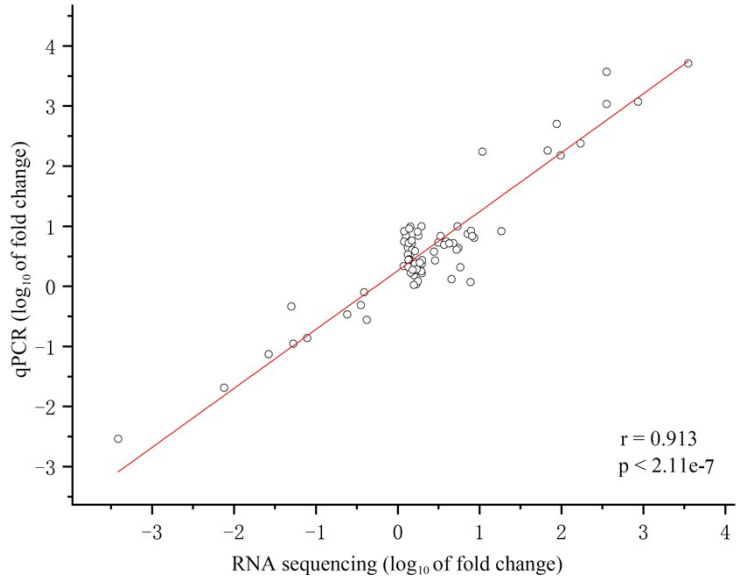

Fig. S6 Spearman's correlation between qPCR and RNA sequencing results for the sixteen selected genes. Each point represents a value of fold change. Fold change values were log10

transformed.

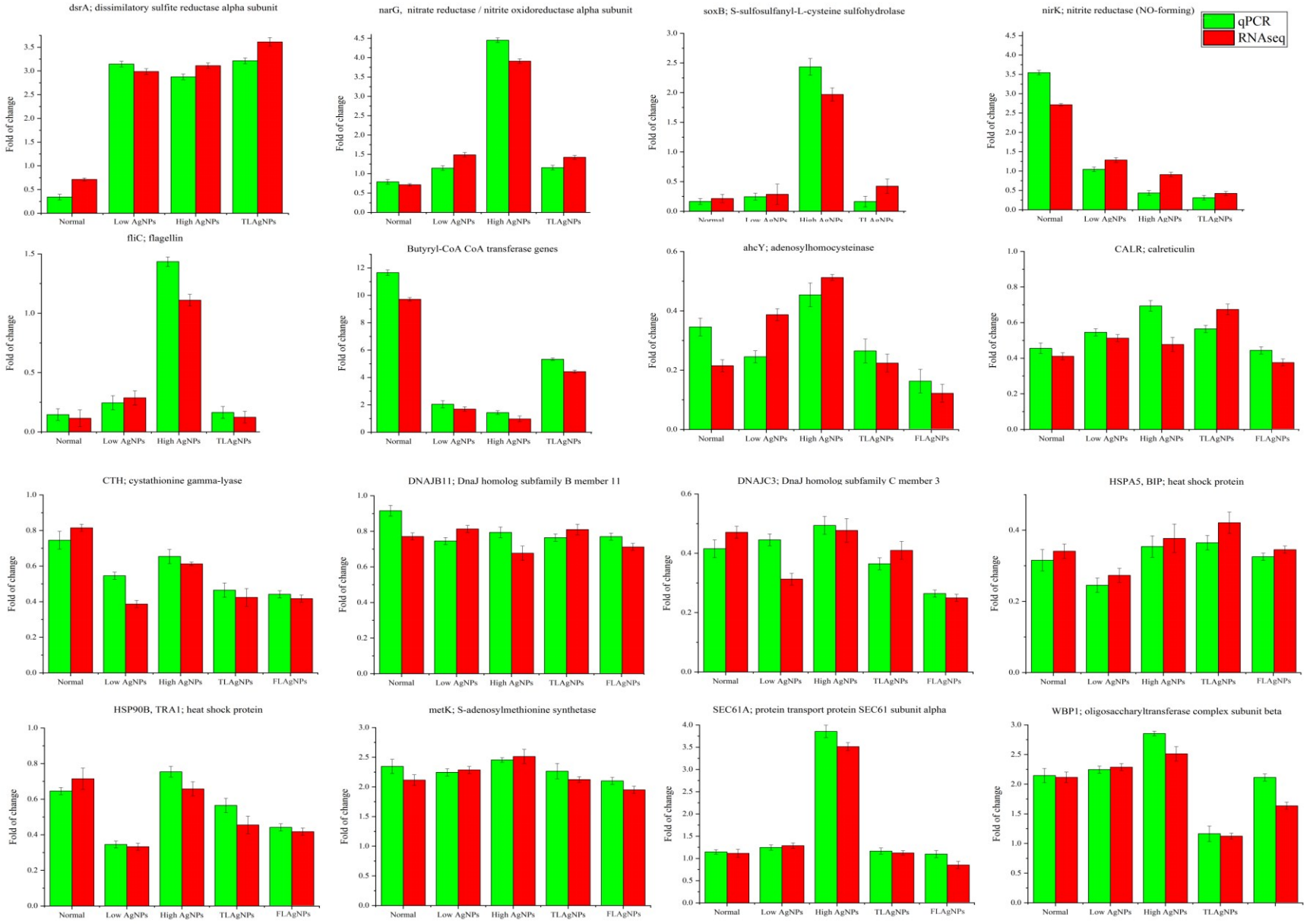
dsrA; dissimilatory sulfite reductase alpha subunit
narG, nitrate reductase / nitrite oxidoreductase alpha subunit
soxB; S-sulfosulfanyl-L-cysteine sulfohydrolase
nirK; nitrite reductase (NO-forming)
qPCR
RNAseq
fliC; flagellin
Butyryl-CoA CoA transferase genes
ahcY; adenosylhomocysteinase
CALR; calreticulin
CTH; cystathionine gamma-lyase
DNAJB11; DnaJ homolog subfamily B member 11
DNAJC3; DnaJ homolog subfamily C member 3
HSPA5, BIP; heat shock protein
HSP90B, TRA1; heat shock protein
metK; S-adenosylmethionine synthetase
SEC61A; protein transport protein SEC61 subunit alpha
WBP1; oligosaccharyltransferase complex subunit beta
Fold of change
Normal
Low AgNPs
High AgNPs
TLAgNPs
FLAgNPs

Figure S7. The mRNA and microRNA relative expression levels detected by qPCR and RNA-seq. Error bars indicate ± s.d. of biological triplicates.

Commands used in R with edger package

```
library(edgeR)

x <- read.delim("TableOfCounts.txt", row.names="Symbol")

group <- factor(c(1,1,1,2,2,2,3,3,3,4,4,4,5,5,5))

y <- DGEList(counts=x,group=group)

y<- calcNormFactors(y)

design <- model.matrix(+0~group)

y <- estimateDisp(y,design)

fit <- glmQLFit(y,design)

qlf_normal_vs_LowAgNPs <- glmQLFTest(fit, contrast=(-1,1,0,0,0))

qlf_normal_vs_HighAgNPs <- glmQLFTest(fit, contrast=(-1,0,1,0,0))

qlf_normal_vs_FLAgNPs <- glmQLFTest(fit, contrast=(-1,0,0,1,0))

qlf_normal_vs_TLAgNPs <- glmQLFTest(fit, contrast=(-1,0,0,0,1))
```