Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Transcriptomic and microRNAomic profiling reveals molecular mechanisms to cope with silver nanoparticle exposure in *Euplotes vannus*

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Supplementary Information

Summary

The supplementary information includes materials and methods, five figures and nine tables.

Materials and Methods

The *E. vannus* were treatment with 15 mg/L AgNP (1/2 12h-LC_{50}) for 0, 1, 3, 6, 12 and 24 hours, samples were harvested at each time point by filtered with a 20 μm pore-size sieve and washed with Oshima's artificial seawater for five times in order to mechanically remove nanoparticles and bacteria. Then the samples were used for detected various parameters.

(1) Detected the bioaccumulation. The *E. vannus* were dried at 80 °C for two days to obtain a constant weight, and transferred to a digestion tube. Then, 1 mL of 65% nitric acid were added to the tube, and the *E. vannus* individuals were digested at room temperature for 8 hours and 100 °C for 8 hours. The digestion samples were dilution with 3% nitric acid and analysis by ICP-MS (Agilent 7700X, USA). Ag bioaccumulation was quantified on the basis of the dry weight (dry wt) of the *E. vannus* (μg g^{-1} dry wt).

(2) Determination of ROS content. H2DCFDA was used to determine the amount of ROS generated in the *E. vannus*. All of the samples were centrifuged first and then re-suspended in PBS buffer (pH 7.2). H2DCFDA was added to this suspension to a final concentration of 0.1 mM and mixed well. The mixture was incubated at 25 °C in the dark for 30 min. After being stained, the cells were rinsed and their fluorescence intensity was detected by flow cytometer in the FL1 channel (BD C6, USA) and Olympus BX51 microscope (Olympus Optical, Tokyo, Japan).

(3) Lipid peroxidation assay. Lipid peroxidation was monitored by measuring malondiadehyde (MDA), a stable end product of lipid peroxidation cascades using an MDA assay kit (Beyotime Co., China). *E. vannus* were lysis with Western and IP lysis buffer (in 1 % PMSF; Beyotime Co., China), then cell homogenates were centrifuged at 16000 x g at 4˚C for 10 min. The supernatant was used for MDA assay and protein determination. The total protein concentrations were measured using BCA Protein assay kit (Beyotime Co., China) according to the manufacturer's instructions. For MDA measurement, 100 μl samples were added into a 15-ml tube followed by addition of 200 μl MDA working solution. The mixture was heated at 100˚C for 15 min, chilled to room temperature, and centrifuged at 1,000 x g for 10 min. Supernatants of 200 μl were transferred to 96-well plates, and the absorbance of each group was read with an Infinite M200 Pro plate reader (Tecan, Switzerland) at 532 nm.

(4) Determination of ATP levels. Intracellular ATP levels were determined using the luciferin-luciferase-based ATP luminescence assay kit (Beyotime Co., China) as instructed by the manufacturer.

(5) Determination of GPx and Gr activity. The enzyme activities of GPx and Gr were determined by specific assay kits purchased from Beyotime Co., China and carried out according to the manufacturer's instructions. The enzyme activities were expressed as U/ mg protein.

Figures

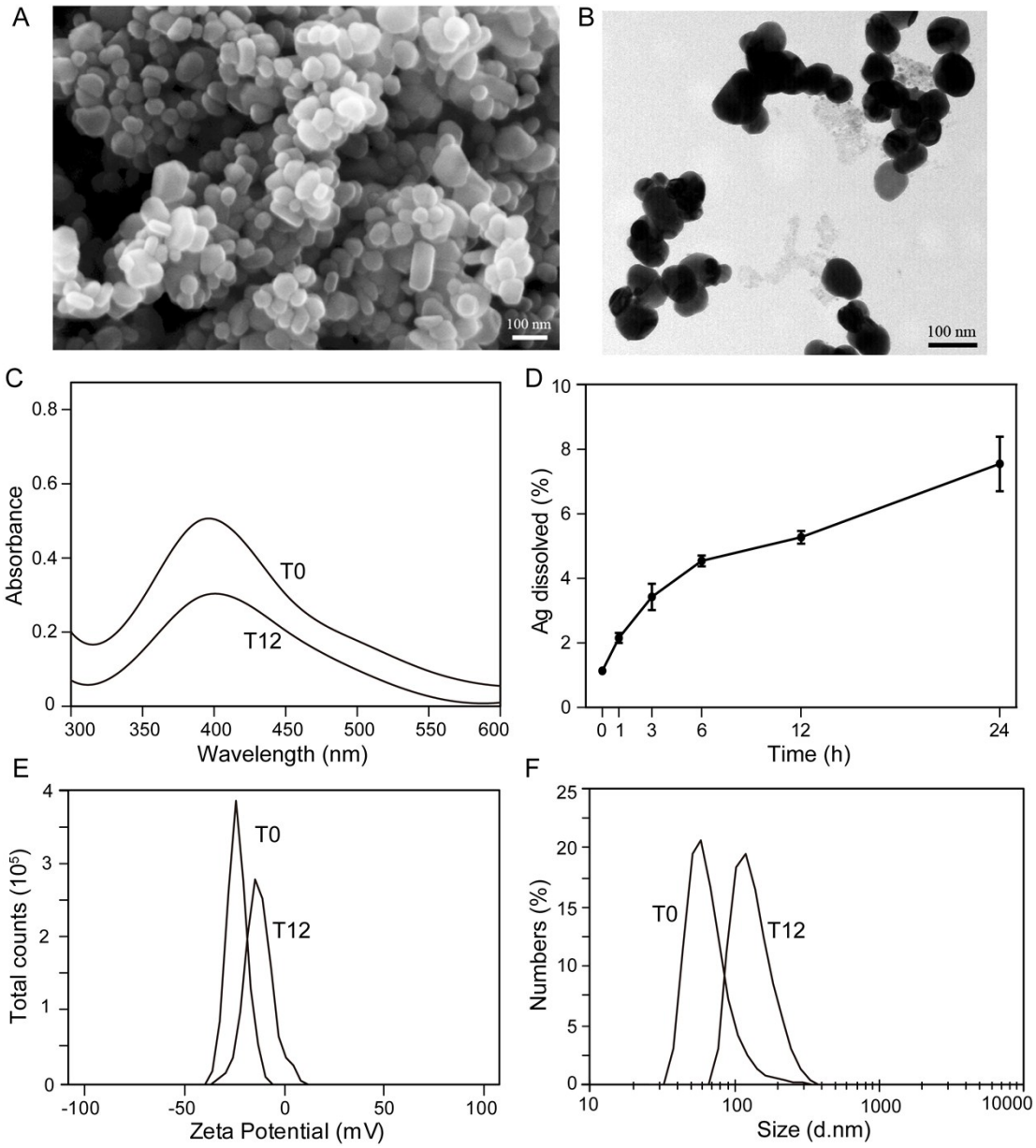

Figure S1. Characterization of AgNPs. (A) Scanning electron microscopy image of AgNPs. (B) Transmission electron microscopy image of AgNPs. (C) UV-Vis absorption spectra. (D) Ag^{+} dissolution rate. (E) Zeta potential. (F) The size distribution. (C)-(F) were performed in exposure medium at 15 mg/L AgNPs. Scale bars represent 100 nm. T0: 0 hour; T12: 12 hour.

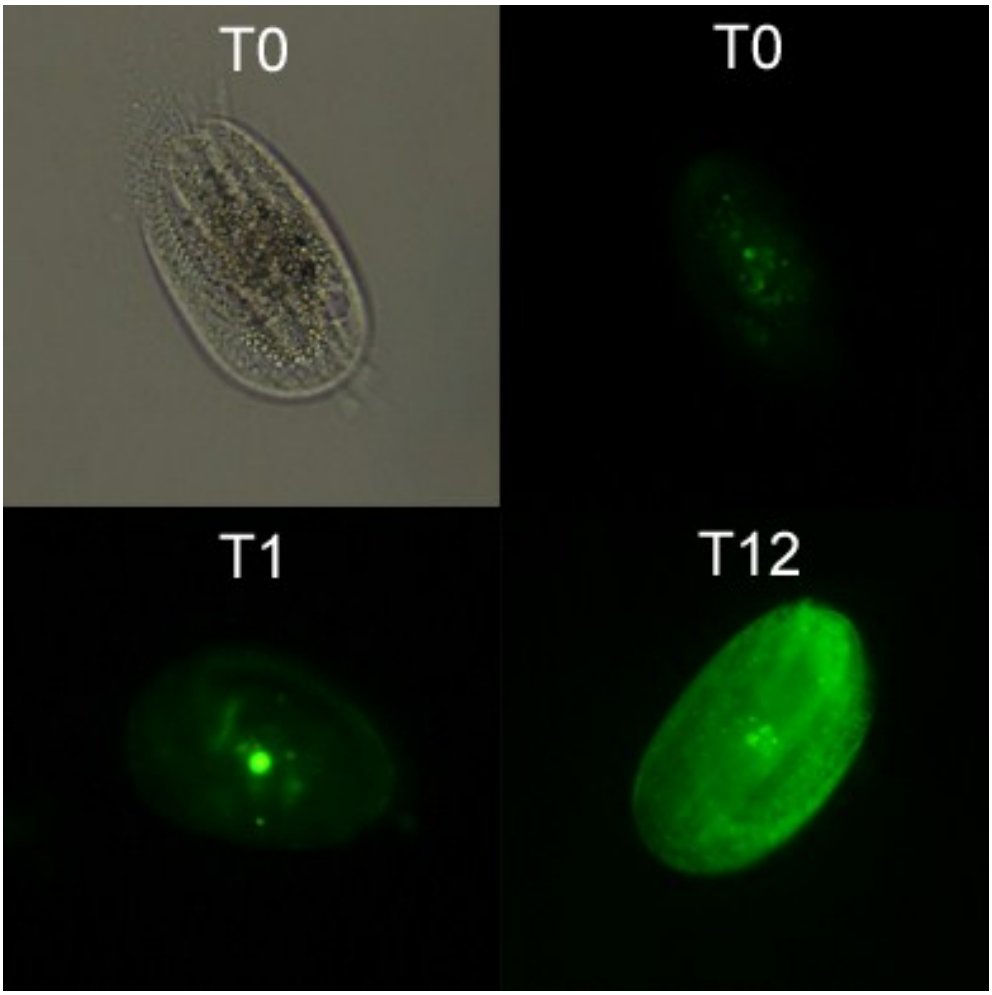

Figure S2. ROS production in *E. vannus* induced by AgNPs. *E. vannus* were treated with increasing time by 15 mg/L AgNPs. Images were taken on an Olympus BX51 microscope. T0: control; T1: treated with 1 hour; T12: treated with 12 hours.

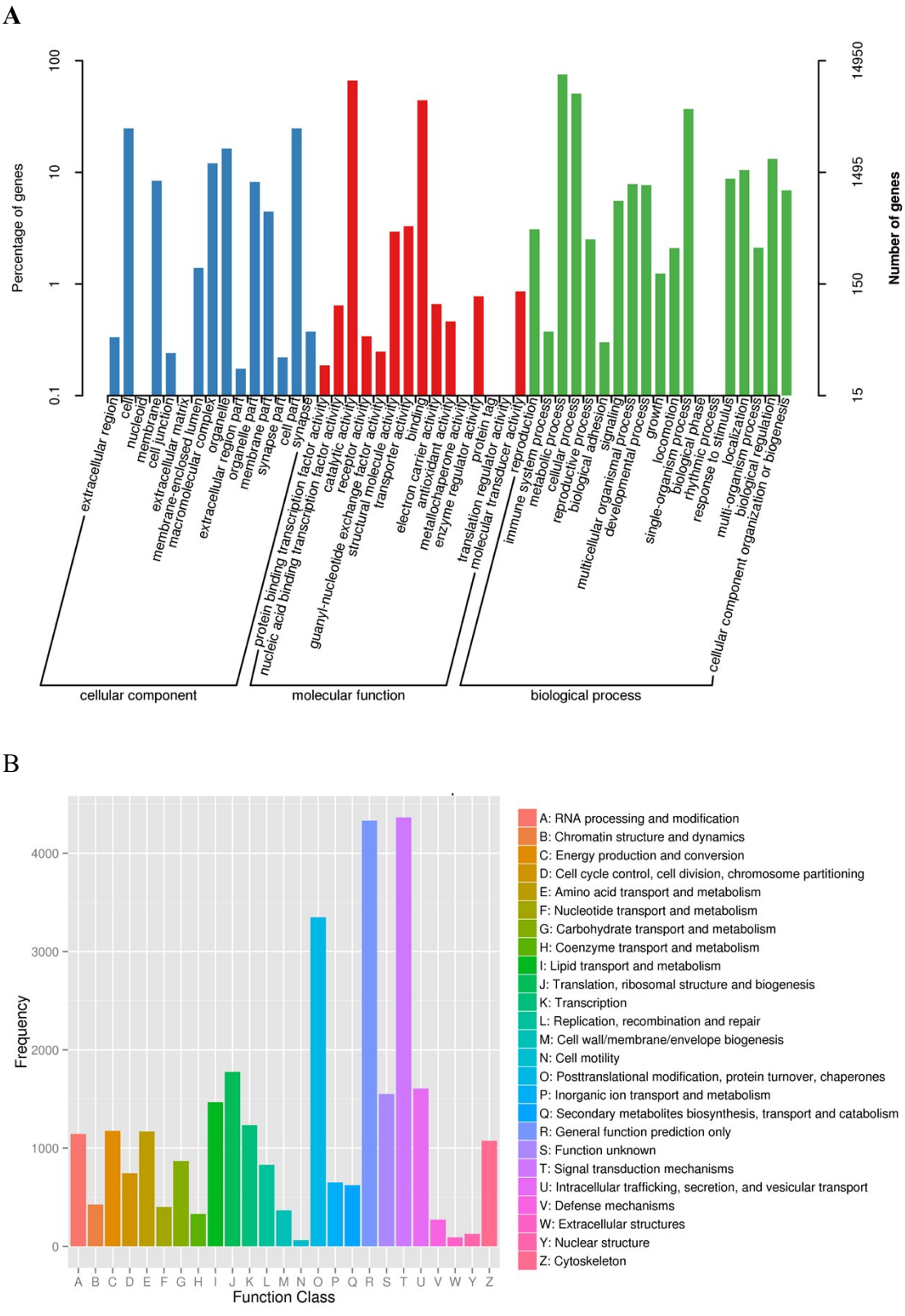

Figure S3. Gene annotation of *E. vannus* transcripts using gene ontology (GO) and KOG database. (A) Gene Ontology analysis of unigenes; (B) KOG-based functional classification.

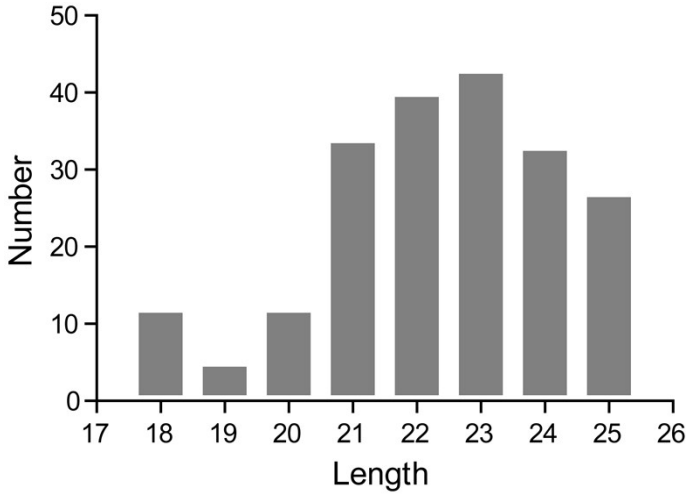

Figure S4. Length and number distribution of microRNA detected from *E. vannus*.

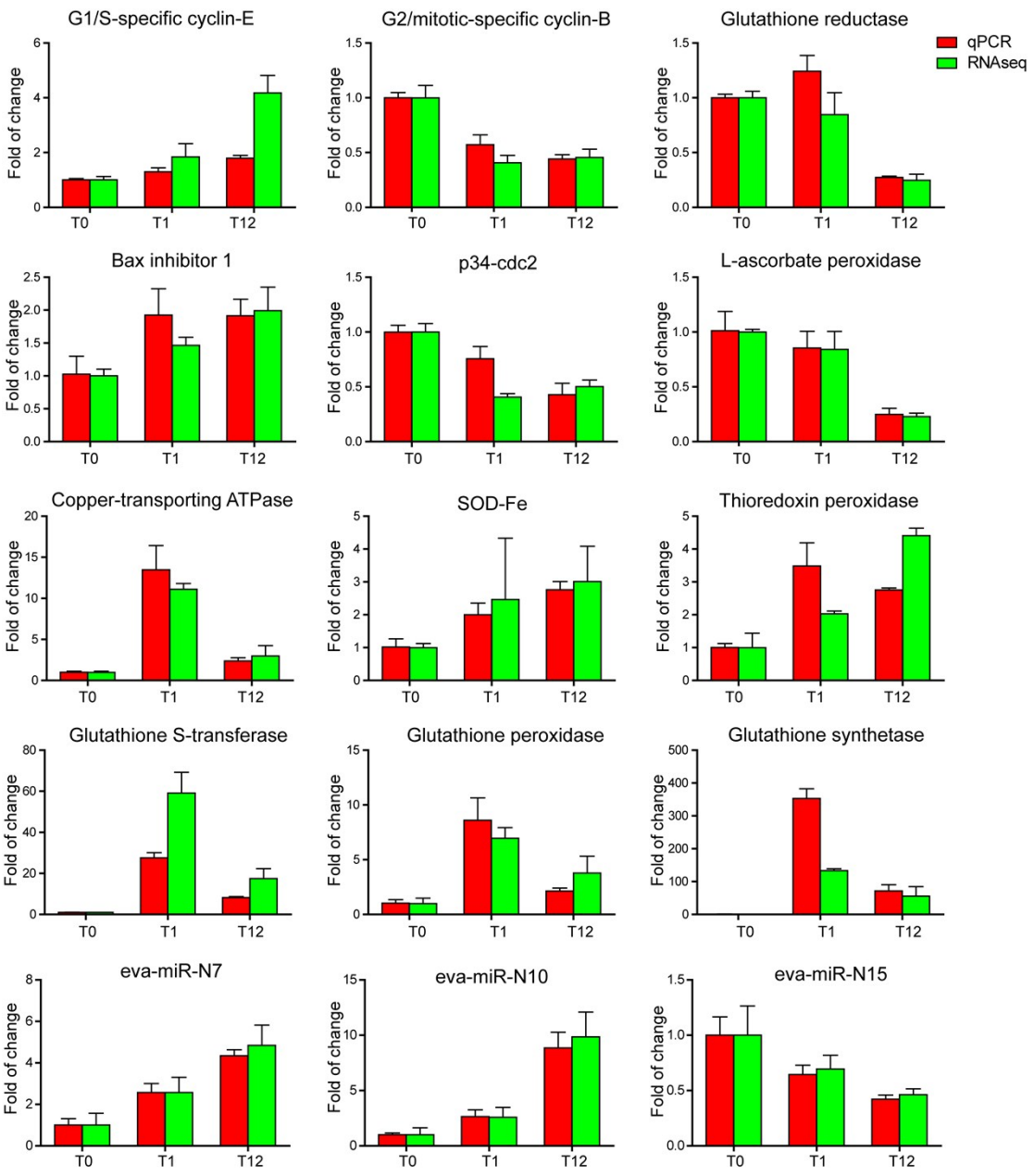

Figure S5. The mRNA and microRNA relative expression levels detected by qPCR and RNA-seq. Error bars indicate ± s.d. of biological triplicates.

Tables

Table S1. Illumina sequencing statistics of mRNA dataset.

Sample	Base Number (Gb)	Raw reads	Clean reads	Mapped reads	%≥Q30
T0-01	6.31	21,972,106	21,204,281	17,995,730	90.66
T0-02	7.64	27,033,067	25,606,570	21,529,026	90.71
T0-03	7.09	26,281,530	23,776,464	20,214,840	90.80
T1-01	7.07	25,818,673	23,739,691	20,129,465	90.97
T1-02	6.00	21,410,674	20,070,546	16,850,689	90.54
T1-03	9.42	33,814,356	31,475,470	26,550,135	89.79
T12-01	7.77	27,175,226	26,097,176	22,077,648	90.91
T12-02	8.11	28,980,661	27,153,056	22,916,490	90.07
T12-03	8.66	30,544,186	29,030,314	24,809,499	90.47

Table S2. Overview of the transcript annotation.

Annotation database	Number of Unigenes	Percentage (%)
Annotated in COG	19069	20.88
Annotated in GO	14950	16.37
Annotated in KEGG	19539	21.39
Annotated in KOG	26766	29.30
Annotated in PFAM	33081	36.22
Annotated in SwissProt	21192	23.20
Annotated in eggNOG	37920	41.51
Annotated in NR	37555	41.11
Shared annotated in all Databases	6259	6.85
Annotated in at least one Database	46917	51.36
Total Unigenes	91343	100

Table S3. Illumina sequencing statistics of microRNA dataset.

Sample	Raw reads	Clean reads	Mapped reads	%≥Q30
T0-01	21,574,969	14,907,124	5,996,255	98.91
T0-02	18,706,046	12,389,359	5,598,748	98.66
T0-03	16,840,337	12,128,375	5,509,306	98.92
T1-01	27,690,617	19,267,428	7,338,891	98.59
T1-02	23,013,322	16,427,038	7,485,366	98.86
T1-03	20,503,595	14,996,338	6,903,114	98.69
T12-01	19,834,473	13,551,290	4,994,575	98.77
T12-02	19,318,577	13,256,688	5,235,088	98.90
T12-03	18,994,517	11,991,804	4,580,841	98.81

Table S4. Differentially expressed of microRNA and their target gene number at each comparison groups.

microRNA	T1 *vs.* T0				T12 *vs.* T0				T12 *vs.* T1				Target Gene number	DEG number
	log2FC	*p* value	FDR	regulated	log2FC	*p* value	FDR	regulated	log2FC	*p* value	FDR	regulated		
eva-miR-N1	1.35	0.018	0.583	up	—	—	—	—	-1.11	0.050	0.791	down	167	38
eva-miR-N2	6.16	<0.001	**0.003**	up	—	—	—	—	-2.04	0.031	0.733	down	367	113
eva-miR-N3	1.95	0.013	0.491	up	—	—	—	—	-2.46	0.005	0.169	down	499	111
eva-miR-N4	1.28	0.011	0.491	up	—	—	—	—	-1.17	0.005	0.169	down	95	17
eva-miR-N5	5.38	0.001	0.074	up	—	—	—	—	—	—	—	—	117	27
eva-miR-N6	1.44	0.031	0.843	up	1.11	0.047	0.813	up	—	—	—	—	1038	248
eva-miR-N7	1.16	0.006	0.383	up	2.12	<0.001	**<0.001**	up	—	—	—	—	417	127
eva-miR-N8	4.49	0.041	0.957	up	4.72	0.019	0.505	up	—	—	—	—	540	151
eva-miR-N9	—	—	—	—	4.41	0.039	0.745	up	—	—	—	—	220	71
eva-miR-N10	—	—	—	—	3.33	<0.001	**<0.001**	up	2.33	0.03	**<0.001**	up	303	108
eva-miR-N11	—	—	—	—	3.92	<0.001	**<0.001**	up	1.91	0.003	0.152	up	194	56
eva-miR-N12	—	—	—	—	1.68	0.03	0.706	up	1.55	0.036	0.733	up	175	51
eva-miR-N13	—	—	—	—	—	—	—	—	2.10	0.036	0.733	up	19	4
eva-miR-N14	—	—	—	—	—	—	—	—	2.65	0.039	0.733	up	205	47
eva-miR-N15	—	—	—	—	-1.36	<0.001	**0.002**	down	—	—	—	—	653	121
eva-miR-N16	—	—	—	—	-1.45	0.003	0.086	down	—	—	—	—	162	18

Table S5. Biological processes (BP), Cellular components (CC) and Molecular function (MF) enriched in all DEGs in each comparison groups with corrected Benjamini *p*-value < 0.05.

Group		Term name	Gene count	Fold Enrichment	Benjamini
T12 vs. T0	BP	macropinocytosis	240	1.6	5.50E-15
		translation	86	1.5	3.90E-02
	CC	phagocytic vesicle	71	2.1	6.50E-08
		ribosome	54	1.8	3.70E-03
T12 vs. T1	BP	DNA replication	30	3.5	1.20E-06
		DNA-dependent DNA replication	9	7.1	2.20E-03
		phosphorylation	58	1.7	1.70E-02
		DNA replication initiation	10	4.8	1.90E-02
		macropinocytosis	116	1.4	2.00E-02
		protein phosphorylation	54	1.7	3.40E-02
	CC	nucleus	177	1.3	9.90E-03
		microtubule associated complex	25	2.2	4.10E-02
		proteasome complex	17	2.6	4.00E-02
	MF	transferase activity	108	1.5	9.40E-04
		ATP binding	166	1.3	2.70E-03
		kinase activity	61	1.7	6.50E-03
		DNA binding	64	1.6	2.10E-02
		3'-5' DNA helicase activity	7	6.4	2.10E-02
		protein serine/threonine kinase activity	53	1.6	2.50E-02
		protein kinase activity	49	1.6	3.20E-02

Table S6. KEGG enrichment analysis of the target genes of the up-regulated and down-regulated microRNA in each comparison groups. Shown are significantly enriched pathways (corrected Benjamini *p*-value < 0.05).

Group	Term name	Gene count	Fold Enrichment	Benjamini
T1 vs. T0	FoxO signaling pathway	38	2.3	1.18E-04
Up-miRNA	cAMP signaling pathway	44	2.1	1.98E-04
	mTOR signaling pathway	38	1.9	2.36E-03
	Autophagy	39	1.5	2.98E-02
	RNA degradation	19	1.8	4.30E-02
	Calcium signaling pathway	22	1.7	4.67E-02
T12 vs. T0	cAMP signaling pathway	40	2.2	6.64E-05
Up-miRNA	Calcium signaling pathway	26	2.4	4.67E-04
	Autophagy	38	1.8	4.15E-03
	cGMP-PKG signaling pathway	24	2.0	6.79E-03
	mTOR signaling pathway	31	1.8	8.61E-03
	FoxO signaling pathway	25	1.8	2.34E-02
T12 vs. T1	Apoptosis	12	3.0	2.07E-02
Up-miRNA	cGMP-PKG signaling pathway	11	2.9	3.17E-02
T12 vs. T1 Down-miRNA	FoxO signaling pathway	17	2.8	4.03E-02

Table S7. Biological processes (BP), Cellular components (CC) and Molecular function (MF) enriched of the microRNA target genes in each comparison groups with corrected Benjamini *p*-value < 0.05.

Group		Term name	Gene count	Fold Enrichment	Benjamini
T1 vs. T0	BP	phosphorus metabolic process	91	1.8	1.89E-06
Up-miRNA		phosphate-containing compound metabolic process	86	1.8	1.90E-05
		phosphorylation	61	2.0	3.37E-05
		cellular protein modification process	50	1.9	2.10E-03
		protein modification process	50	1.9	2.10E-03
		macromolecule modification	53	1.8	2.24E-03
		protein phosphorylation	29	2.4	2.68E-03
	MF	protein kinase activity	93	2.4	5.97E-14
		phosphotransferase activity, alcohol group as acceptor	98	2.3	1.45E-13
		transferase activity	244	1.5	2.50E-10
		catalytic activity, acting on a protein	125	1.7	2.01E-08
		protein serine/threonine kinase activity	38	2.4	2.30E-05
		catalytic activity	485	1.1	4.47E-05
T12 vs. T0	BP	phosphorus metabolic process	74	1.9	5.20E-06
Up-miRNA		phosphorylation	51	2.2	2.70E-05
		phosphate-containing compound metabolic process	70	1.9	3.61E-05
		protein phosphorylation	23	2.5	1.41E-02
	MF	transferase activity, transferring phosphorus-containing groups	149	1.9	7.84E-14
		kinase activity	111	2.0	1.23E-11
		transferase activity	204	1.5	2.48E-10
		protein kinase activity	73	2.4	3.04E-10
		phosphotransferase activity, alcohol group as acceptor	76	2.2	1.50E-09
		catalytic activity	399	1.1	2.17E-06
		protein serine/threonine kinase activity	32	2.5	6.56E-05
		catalytic activity, acting on a protein	93	1.6	1.05E-04
T12 vs. T1	MF	phosphotransferase activity, alcohol group as acceptor	39	2.4	3.56E-05
Down-miRNA		transferase activity, transferring phosphorus-containing groups	66	1.8	6.20E-05
		protein kinase activity	35	2.4	9.23E-05
		kinase activity	49	1.9	2.51E-04
		transferase activity	91	1.4	1.19E-03
		catalytic activity, acting on a protein	49	1.8	1.22E-03

Table S8. Oligonucleotide primers used in this work.

Sequence ID	Gene name	Forward Primer	Reverse Primer
mRNA			
MG999513	18S	ACAATTGGAGGGCAAGTCTG	CCAGAAATCCAACTACGAGCA
c22466.graph_c0	G1/S-specific cyclin-E	TGGCTCCAAGAGTGTTGTGAA	TGAAGGCAATCAGAAGTCACG
c46962.graph_c0	G2/mitotic-specific cyclin-B	TCTCTGGAGGAGATGCTGGA	AATGGATGGTCTGCTGGTGA
c41561.graph_c0	Glutathione reductase	GAGCCGTCAGCATTCTTGTC	TCTTGGCAGGACTTGGATCA
c54336.graph_c0	BAX inhibitor 1	GGTCAATCTCAGCGGCTCTT	TGGATTCCTCCTCCTCTCTATCA
c55100.graph_c0	p34-cdc2	AAGTCATGCAGGTGGCTCTG	GCCAGGAGTCAGCGAACTT
c55459.graph_c0	L-ascorbate peroxidase	GCGCTTATGCTGCTCTTGAA	GCGACAATCTCATCGTCTGAA
c37802.graph_c0	Copper transporting ATPase 2	GGCAAGGCAAGATTCACCAG	TCAGCGGTAAGGAAGAGGTCA
c23622.graph_c0	SOD-Fe	GGCTGTTGCGGTAGTCAATG	GGCCACTTCGGTTCTGGTT
c18811.graph_c1	Thioredoxin peroxidase	CTCCAAGGCTTACGGATGCT	GGCACACTTCTCCATTCTCATC
c46357.graph_c0	Glutathione s-transferase	TCTGAAGAACGGCAGGATCA	CCATCCAACTGTGGTTCCAA
c20873.graph_c0	Glutathione peroxidase	CCTGGTAATGGTCAGGTATGGA	ACATTCTGCAAGCGCAATTC
c30192.graph_c0	Glutathione synthetase	AGTCCTTCACAGTACCTCGATCA	AATTGCAGCATCCGATCATT
microRNA			
eva-miR-N7		CTGAAGGTGCTCACTGACA	
eva-miR-N10		TCCTAGCCCTGTCACTACAA	
eva-miR-N15		TTTGGTGTGATTTTGGCTCGG	

Table S9. Summarize the differentially expressed of ABC transporter family genes.

Sequence ID	Swissprot annotation	Regulated		
		T1 vs. T0	T12 vs. T0	T12 vs. T1
c51365.graph_c0	ABC transporter A family member 1	normal	down	down
c34769.graph_c0	ABC transporter A family member 10	normal	up	normal
c51764.graph_c0	ABC transporter A family member 2	down	down	normal
c36462.graph_c0	ABC transporter A family member 2	up	normal	normal
c43331.graph_c0	ABC transporter A family member 2	normal	down	normal
c27769.graph_c0	ABC transporter A family member 2	up	up	normal
c51585.graph_c0	ABC transporter A family member 3	normal	normal	down
c50870.graph_c0	ABC transporter A family member 5	up	normal	down
c35730.graph_c1	ABC transporter A family member 5	normal	down	down
c35730.graph_c0	ABC transporter A family member 5	normal	down	down
c35511.graph_c0	ABC transporter A family member 7	normal	down	normal
c49503.graph_c1	ABC transporter B family member 2	normal	down	down
c50197.graph_c0	ABC transporter B family member 3	normal	up	normal
c49503.graph_c0	ABC transporter B family member 3	normal	normal	down
c51489.graph_c0	ABC transporter B family member 3	normal	down	down
c44749.graph_c0	ABC transporter B family member 5	up	normal	down
c19326.graph_c1	ABC transporter B family member 5	normal	normal	down
c53881.graph_c0	ABC transporter B family member 5	up	up	normal
c19326.graph_c0	ABC transporter B family member 6	up	normal	normal
c44185.graph_c0	ABC transporter C family member 10	normal	up	normal
c25690.graph_c0	ABC transporter C family member 12	down	down	normal
c52069.graph_c0	ABC transporter C family member 12	normal	down	down
c49354.graph_c0	ABC transporter C family member 3	normal	up	up
c30574.graph_c0	ABC transporter C family member 3	normal	up	up
c42211.graph_c0	ABC transporter C family member 6	normal	normal	up
c39151.graph_c1	ABC transporter C family member 6	normal	down	normal
c48079.graph_c0	ABC transporter F family member 2	normal	up	normal
c36976.graph_c0	ABC transporter G family member 18	normal	normal	up
c43032.graph_c0	ABC transporter G family member 18	normal	up	up
c46036.graph_c0	ABC transporter G family member 18	normal	up	normal