Supplementary Information (SI) for Environmental Science: Nano.

Supplementary material

Table S1 Physical and chemical properties of CuO-NPs and CuO-SMPs

Property	CuO-NPs	CuO-SMPs
Diameter (nm)	40	150-250
Purity (%)	99.5	99.0%
Specific surface area (m^2/g)	29.17	14.75
Zeta potential (Mv)	-13.9 ± 1.41	-7.5 ± 0.63
Hydraulic particle size in algal liquor (nm)	633.5 ± 72	814.3 ± 93

Table S2 Component of SE medium

#	Component	Dosage (mL/L)	Parent Solution (g/L dH_2O)
1	$NaNO_3$	1.00	2.50
2	K_2HPO_4	1.00	0.75
3	$MgSO_4 \cdot 7H_2O$	1.00	0.75
4	$CaCl_2 \cdot 2H_2O$	1.00	0.25
5	KH_2PO_4	1.00	1.75
6	NaCl	1.00	0.25
7	$FeCl_3 \cdot 6H_2O$	1.00	0.05
8	EDTA-Fe*	1.00	
9	A_5 (Trace mental solution)**	1.00	
10	Soil extract***	40.00	

*EDTA-Fe

1N HCl: 4.1 mL HCl was diluted to 50 mL with distilled water

0.1N EDTA-Na_2: 0.9306 g EDTA-Na_2 was dissolved in 50 mL distilled water

EDTA-Fe: 0.901 g $FeCl_3 \cdot 6H_2O$ was dissolved in 10 mL of 1 N HCl, then mixed with 10 mL of 0.1 N EDTA-Na_2 and diluted to 1000 mL with distilled water

**A_5 (Trace mental solution)

#	Component	Concentration (g/L dH_2O)
1	H_3BO_3	2.86
2	$MnCl_2 \cdot 4H_2O$	1.86
3	$ZnSO_4 \cdot 7H_2O$	0.22
4	$Na_2MoO_4 \cdot 2H_2O$	0.39
5	$CuSO_4 \cdot 5H_2O$	0.08
6	$Co(NO_3)_2 \cdot 6\ H_2O$	0.05

***Soil extract

200 g unfertilized soil was added to 1000 mL of distilled water, water bath at 100 for 3 h, cooled, and precipitated for 24 h. This process was carried out 3 times in a row, then filtered, and the supernatant was taken, sterilized (121 ºC, 30 min) and stored in a refrigerator at 4 ºC

Text S1 Calculation formula of microalgae growth inhibition rate

The growth inhibition rate (BIR) of *C. reinhardtii* was calculated by the following formula:

BIR (%) = $(1-N/N_0) \times 100\%$, where N was the cell density (cells/mL) in the CuO-NPs and CuO-MPs experimental group, and N_0 was the cell density (cells/mL) in the control group.

Figure S1 The effects of (a) CuO-NPs and (b) CuO-SMPs on the cell size and granularity of *Chlamydomonas reinhardtii*.

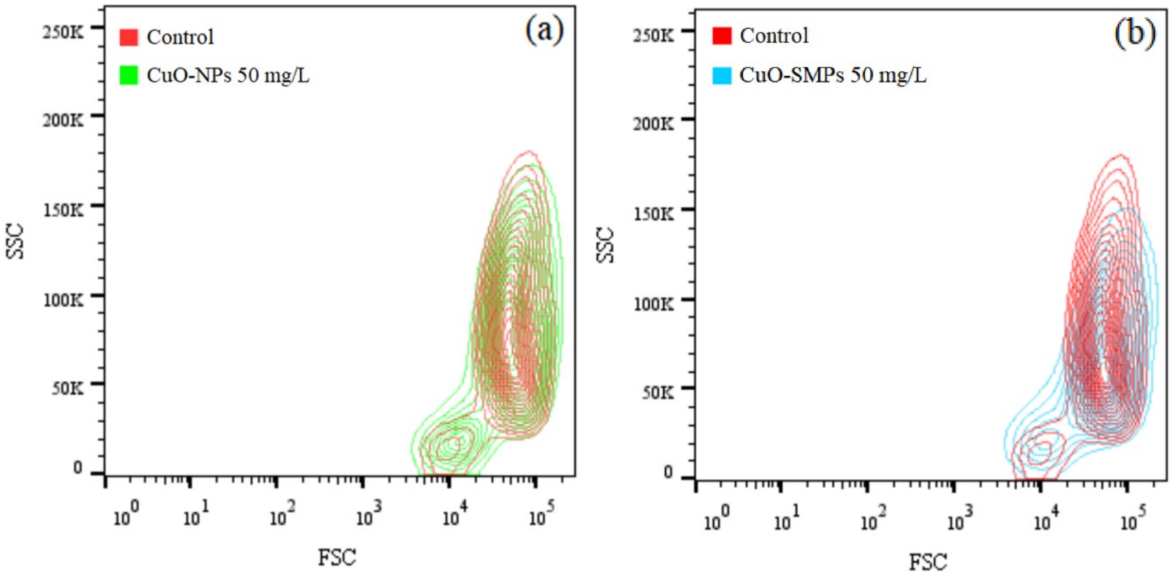


Fig.S1 The effects of (a) CuO-NPs and (b) CuO-SMPs on the cell size and granularity of *Chlamydomonas reinhardtii*.

Figure S2 The contour offset plot of cell membrane integrity of *Chlamydomonas reinhardtii* under stress of CuO-NPs and CuO-SMPs

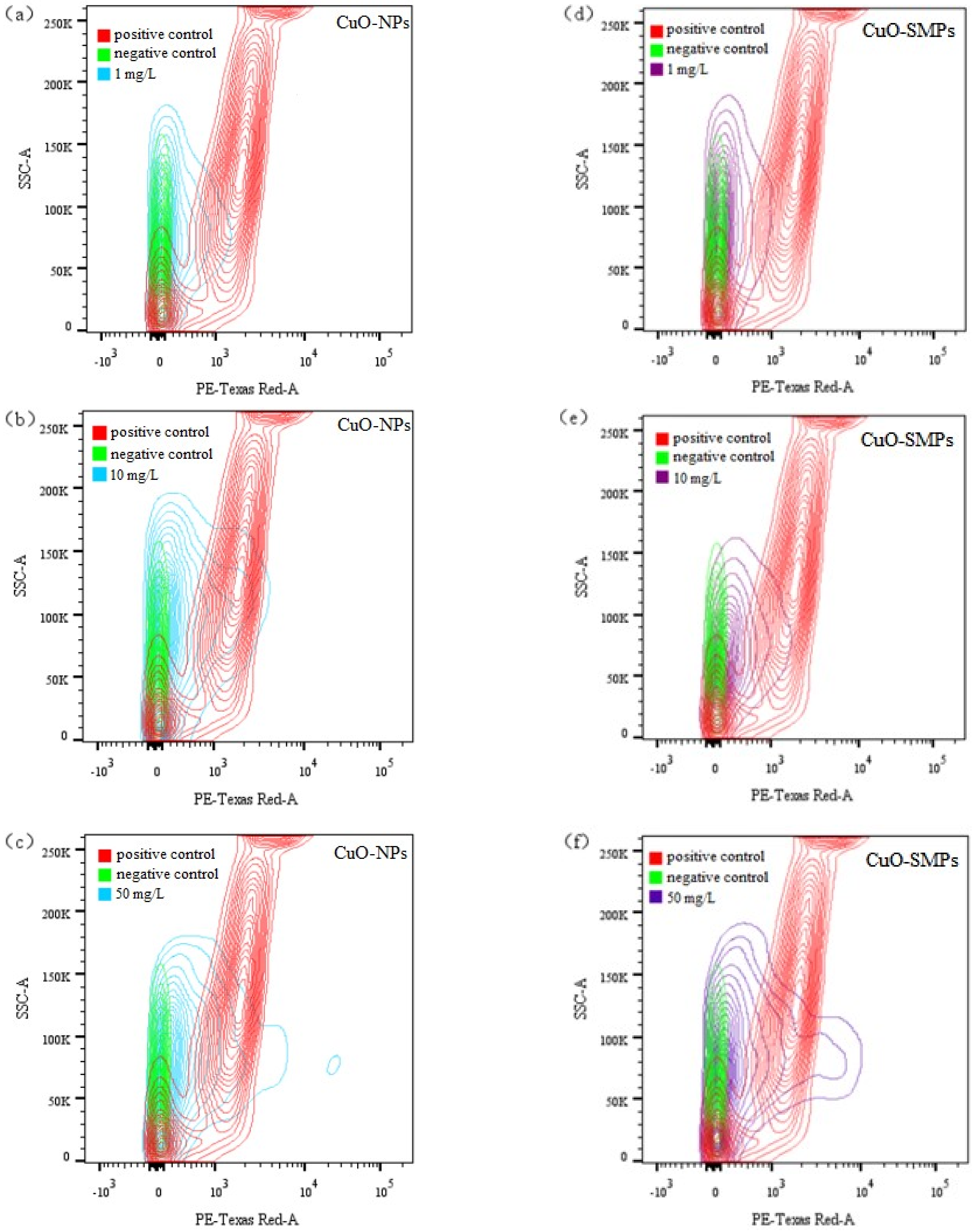


Fig. S2. The contour offset plot of cell membrane integrity of *Chlamydomonas reinhardtii* under stress of CuO-NPs and CuO-SMPs. CuO-NPs (a-c), CuO-SMPs(d-f).

Figure S3 The heat map of 77 differential genes associated with oxidoreductase activity

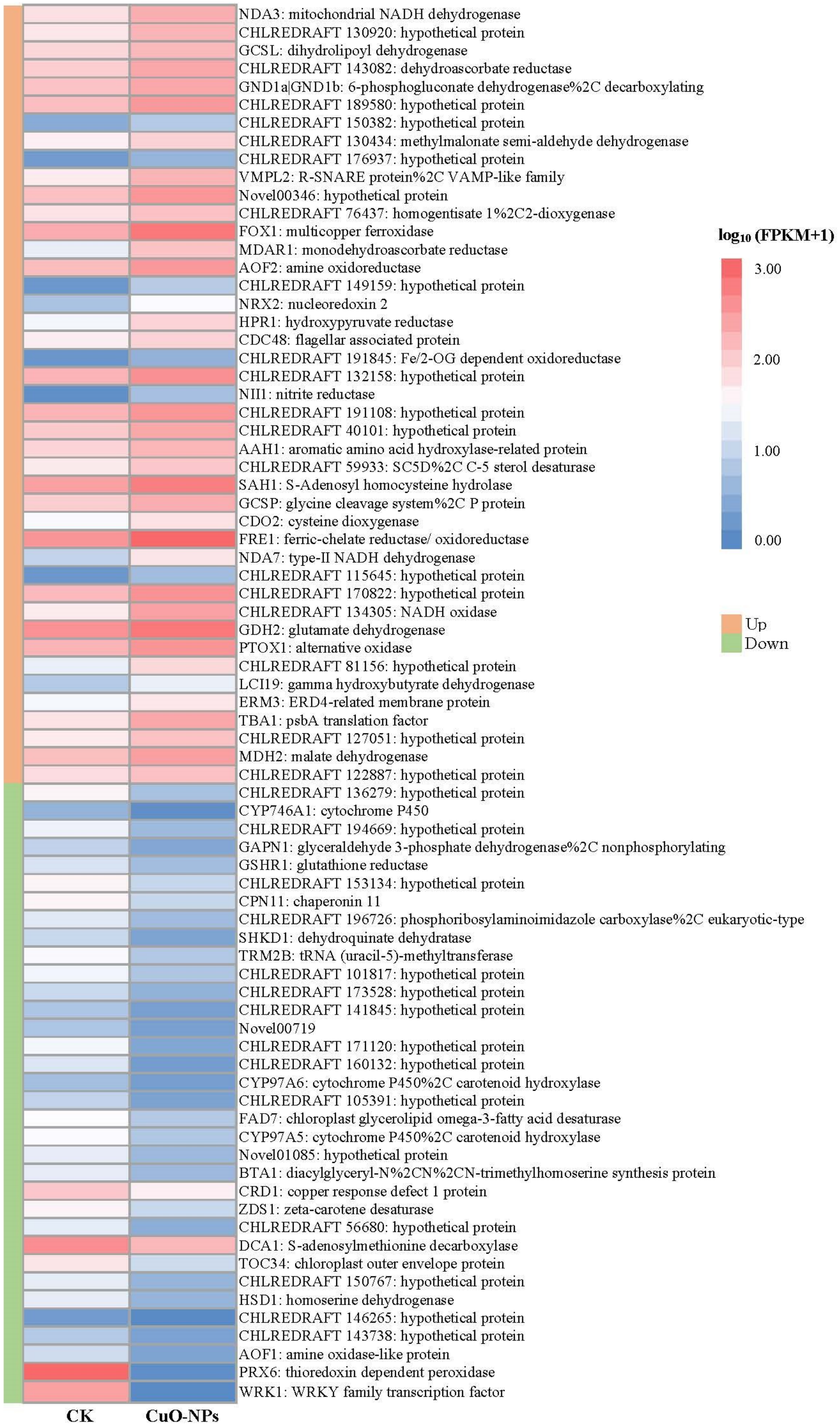


Fig. S3. The heat map of 77 differential genes associated with oxidoreductase activity

Figure S4 Photosynthesis pathway

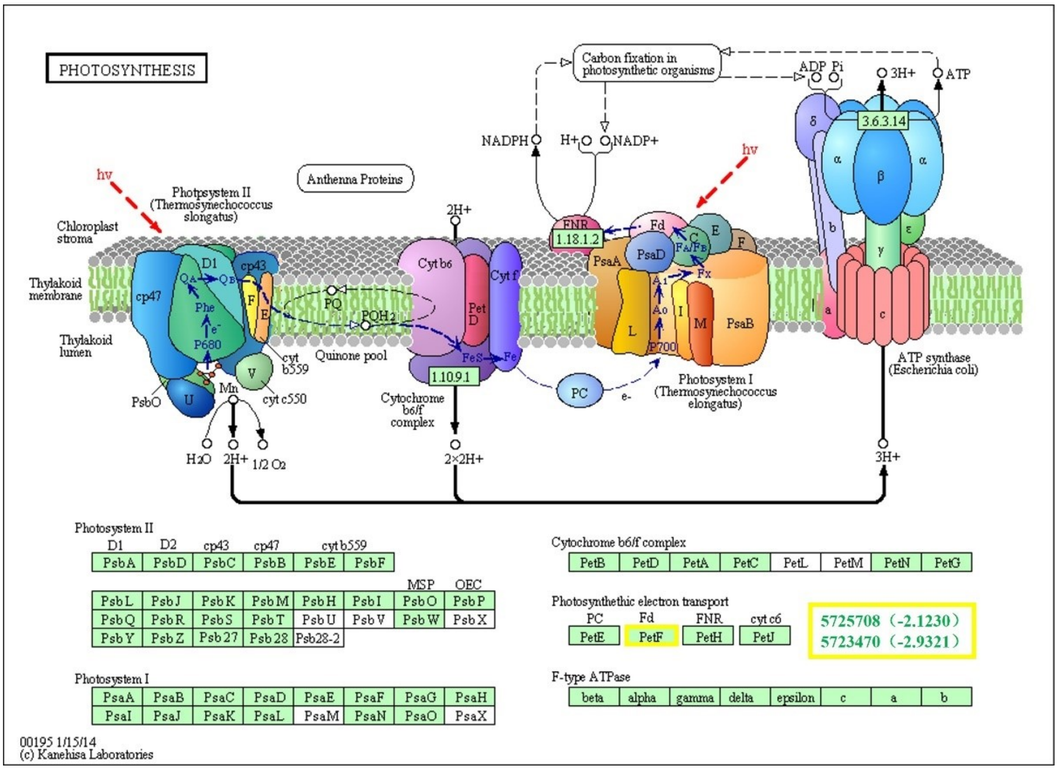


Fig. S4. Photosynthesis pathway. red represents the up-regulated gene, green represents the down-regulated gene, yellow represents both up-regulated and down-regulated genes, $\log_2$ (Fold change）in parentheses.

Figure S5 Photosynthesis-antenna protein pathway

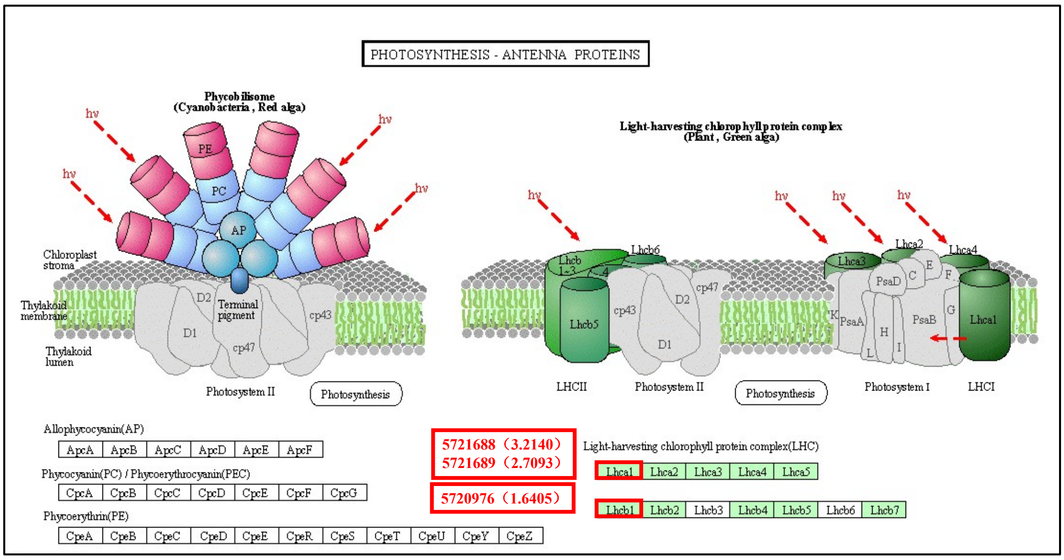


Fig. S5. Photosynthesis-antenna protein pathway. Red represents the up-regulated gene, green represents the down-regulated gene, yellow represents both up-regulated and down-regulated genes, $\log_2$ (Fold change）in parentheses.

Figure S6 Peroxisome pathway

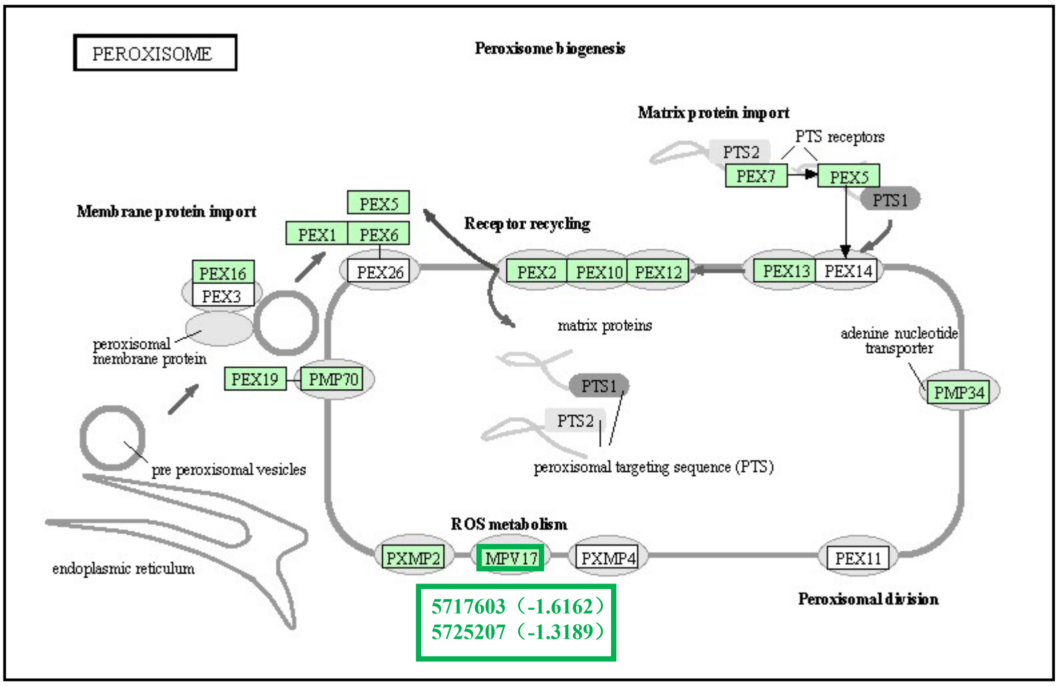


Fig. S6. Peroxisome pathway. Red represents the up-regulated gene, green represents the down-regulated gene, yellow represents both up-regulated and down-regulated genes, log$_2$ (Fold change）in parentheses.

Figure S7 Ascorbate pathway

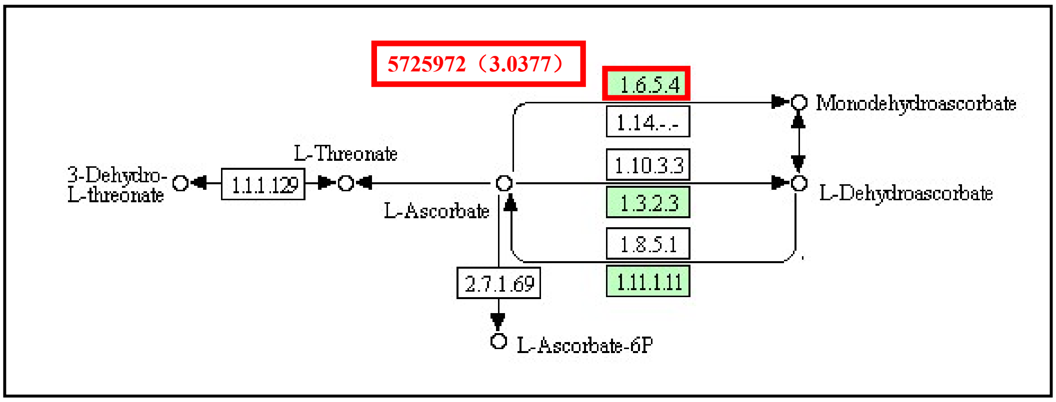
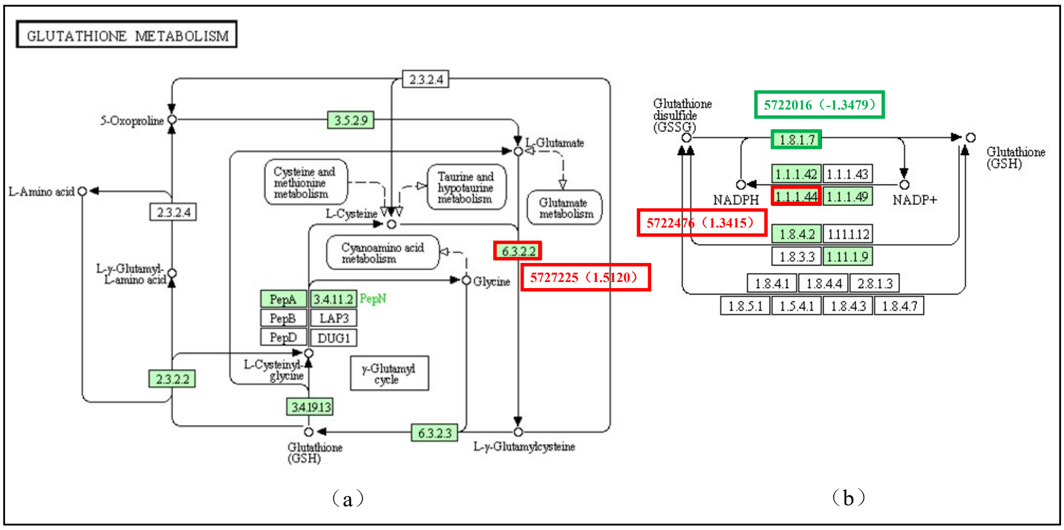


Fig. S7. Ascorbate pathway. Red represents the up-regulated gene, green represents the down-regulated gene, yellow represents both up-regulated and down-regulated genes, $\log_2$ (Fold change） in parentheses.

Figure S8 Glutathione metabolism pathway



（a） （b）

Fig. S8. Glutathione metabolism pathway. synthesis of GSH(a), redox process of GSH and GSSG (b). Red represents the up-regulated gene, green represents the down-regulated gene, yellow represents both up-regulated and down-regulated genes, log2（Fold change）in parentheses.

Fig. S9 ABC transporters pathway

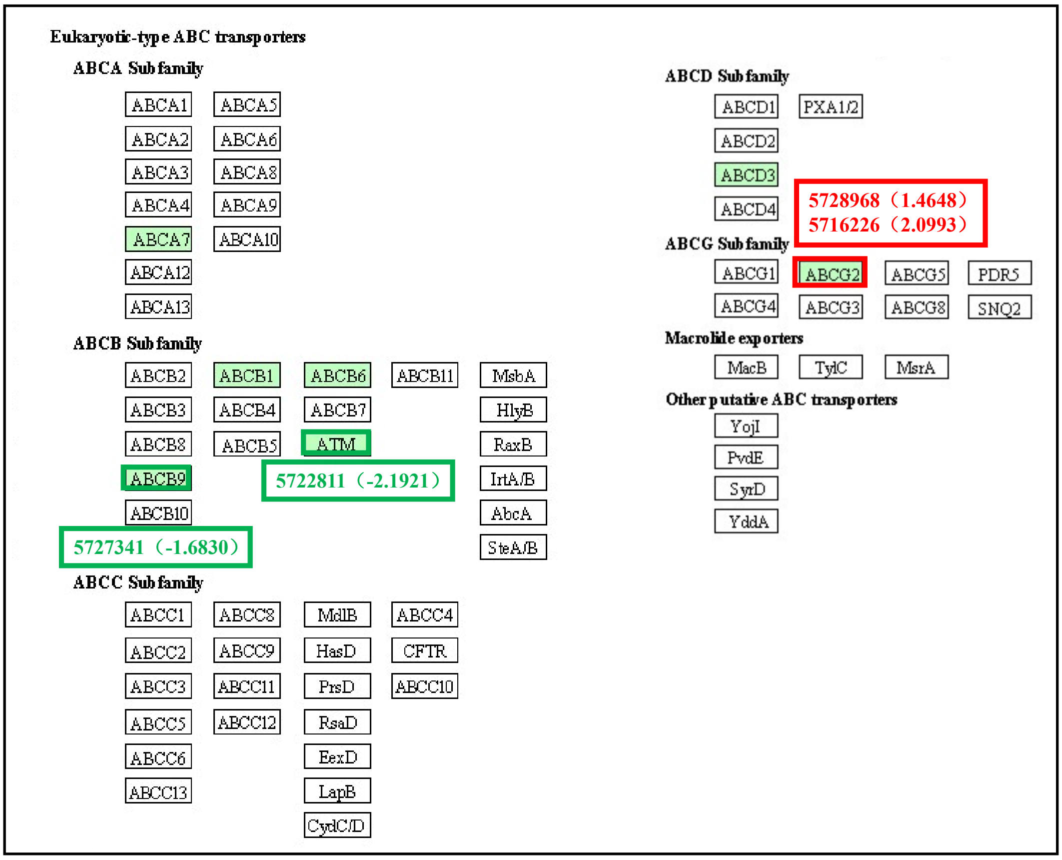


Fig. S9. ABC transporters pathway. Red represents the up-regulated gene, green represents the down-regulated gene, yellow represents both up-regulated and down-regulated genes, log2（Fold change）in parentheses.