

Supplementary Material

Porphyrin metabolism and carbon fixation responding to mixed emerging PFASs with environmental concentrations in *Skeletonema costatum* at different growth phases

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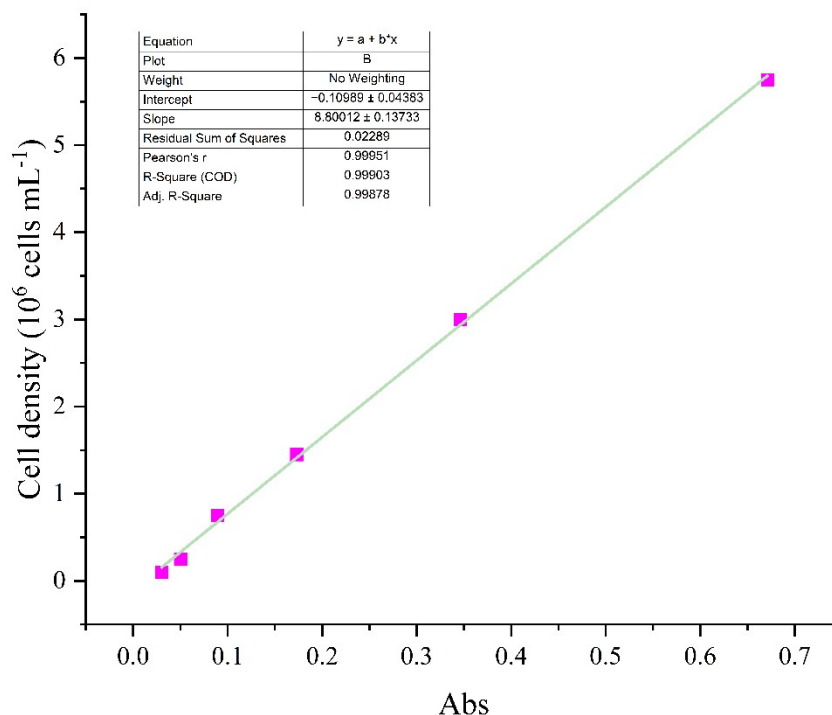
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2. Materials and methods

TEXT S1: ANALYSIS OF POPULATION AND CELLULAR LEVELS

Linear relation between the cell density and the OD



$$Y = 8.8x - 0.11$$

Determination of enzymatic activities

The algal suspensions (20 mL) from different samples were centrifuged at 5000 rpm for 10 min at 4°C. The cellular precipitates were resuspended with 2.5 mL PBS (0.1 M, pH 7.4) and freeze-thawed for three times. The samples were broken with ultrasonic cell disruptor (FS-900N, Shanghai Shengyang Ultrasonic Instrument Co., Ltd, China) in 270W for 20 min (work 5s, stop 5s) at ice-bath condition. The mixtures were centrifuged at 12000 rpm for 10 min at 4°C. The extractive was used for determination, stored at 4°C.

Mensuration of the content of total proteins: The stock solution of Coomassie brilliant blue (CBB) was diluted in a ratio of 1:4. The 50 µL of distilled water, protein standard, and samples were added to the Coomassie bright blue color solution (3 mL), respectively. The mixtures were placed for 10 min and measured in absorbance value (A_0) with ultraviolet-visible spectrophotometer (Hash DR-6000, Hash Corporation, USA) at 595 nm. The concentrations of samples (C_{pr}) were calculated as follow:

$$C_{pr} (g L^{-1}) = (A_{0\ sample} - A_{0\ blank}) / (A_{0\ standard} - A_{0\ blank}) \times C_{st1} \times N \quad (1)$$

where C_{st1} ($C_{standard}$) is $0.524\ g\ L^{-1}$, the N represent dilution multiple of the sample before testing.

Mensuration of T-SOD activity: The solution was prepared and added in turn according to the instruction. The final mixtures were placed for 10 min at room temperature and determined in absorbance value (A_2) with ultraviolet-visible spectrophotometer at 550 nm. The vitalities (VI) of samples were calculated as follow:

$$VI_{sample} (U\ mgprot^{-1}) = (A_{2\ control} - A_{2\ sample}) / A_{2\ control} / 50\% \times (V_1 / V_2) / C_{pr} \quad (3)$$

where the V_1 , V_2 and C_{pr} represent total volumes of solution, volumes of sample, and the corresponding to concentration of proteins. The “mgprot” is the abbreviation of “milligrams of protein”.

Mensuration of the content of GSH: The solution was prepared and added in turn according to the instruction. The final mixtures were measured in absorbance value (A_3) with ultraviolet-visible spectrophotometer at 420 nm. The contents of GSH (CG) in samples were calculated as follow:

$$CG_{sample} (gGSH\ prot^{-1}) = (A_{3\ sample} - A_{3\ blank}) / (A_{3\ standard} - A_{3\ blank}) \times C_{st3} / C_{pr} \quad (4)$$

where C_{st3} is $20 \times 10^{-6}\ mol\ L^{-1}$ and Mr (GSH) is $307\ g\ mol^{-1}$.

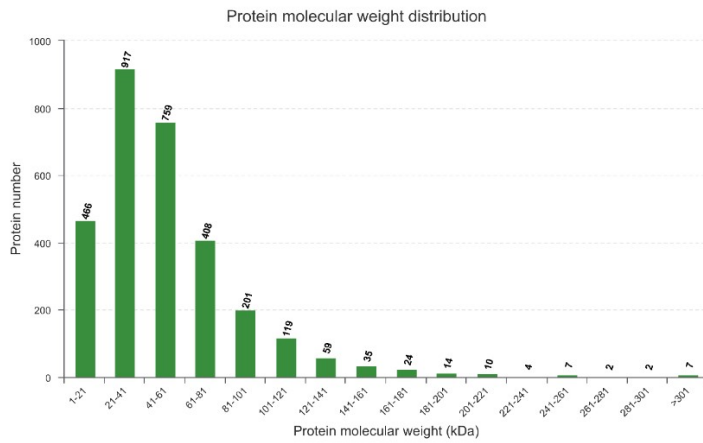
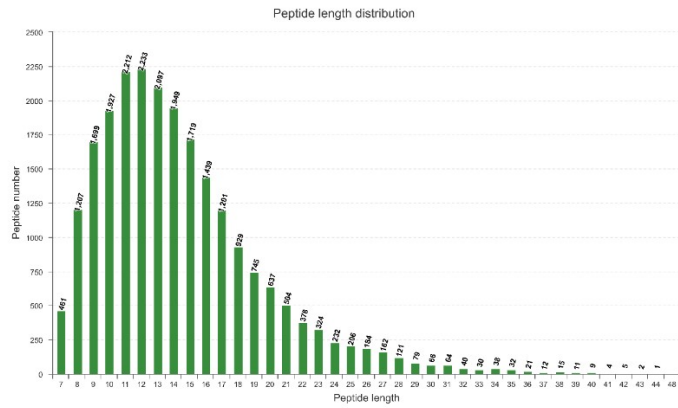
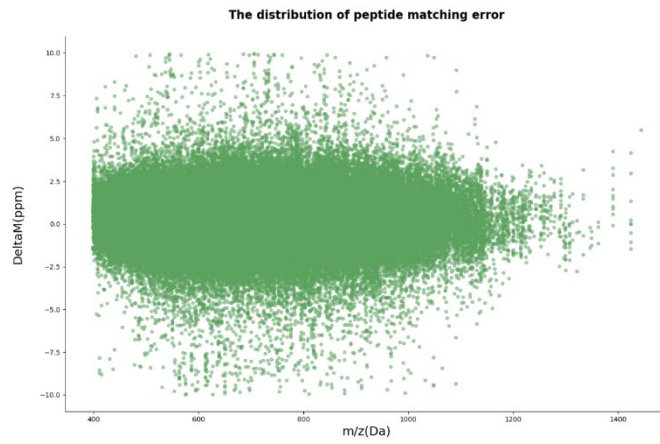
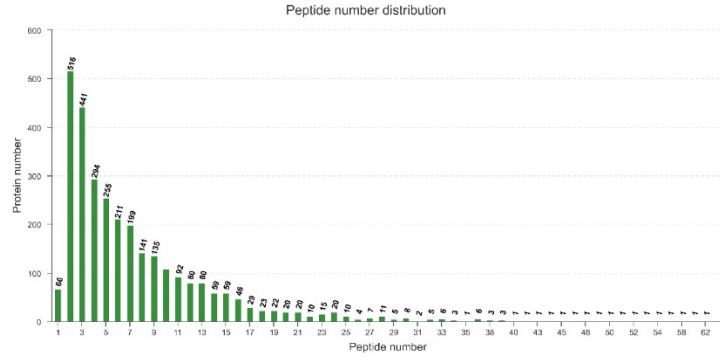
TEXT S2: ANALYSIS OF MULTI-OMICS

The data background of transcriptome

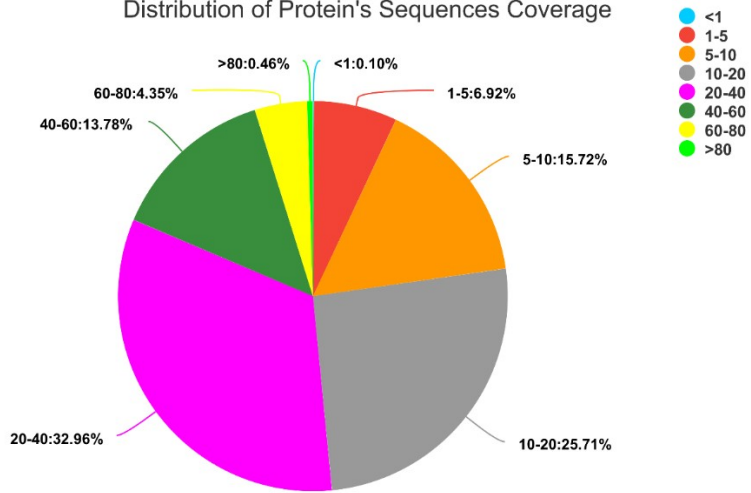
The quality control of sequencing data statistic. The clean data of each sample reached 6.29 Gb. The Q30 base percentages were above 94.9%.

The data background of proteome

The quality control of sequencing data statistic as follows:



Distribution of Protein's Sequences Coverage



Protein information

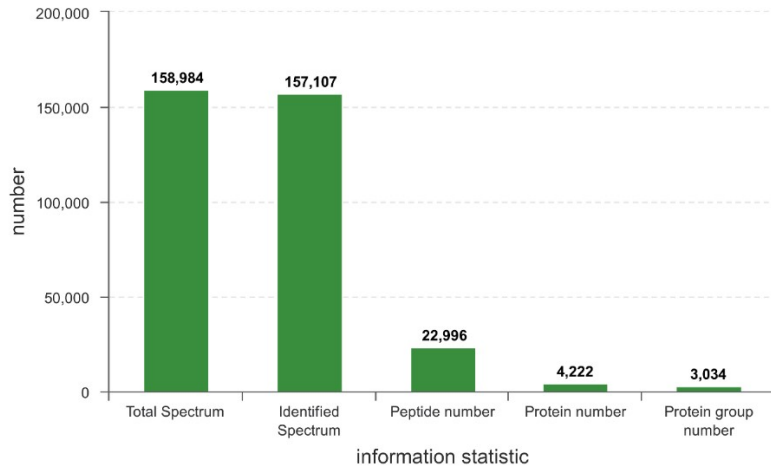
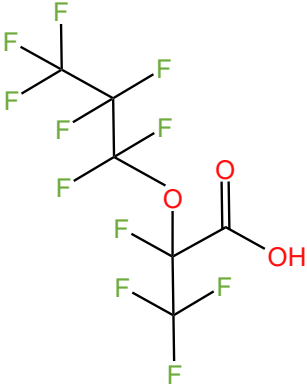
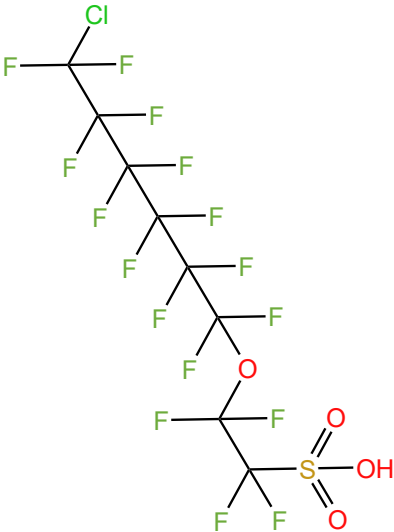
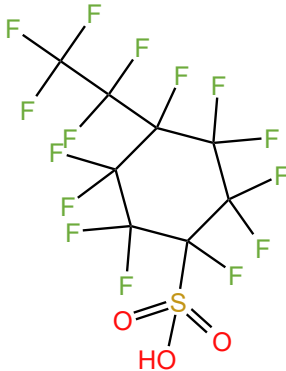


Table S1

The physiochemical characteristics of hexafluoropropylene oxide dimer acid (HFPO-DA), 6:2 chlorinated polyfluorinated ether sulphonate (6:2 Cl-PFAES), and perfluoroethylcyclohexane sulphonate (PFECHS).

Compound	HFPO-DA	6:2 Cl-PFAES	PFECHS	References
CAS #	13252-13-6	756428-58-1	646-83-3	(Mahoney et al., 2022)
Structure				
Molecular mass (g mol ⁻¹)	330.04	300.10	461.13	(Mahoney et al., 2022)
Water solubility (mol L ⁻¹)	>2.61	1.15e-3	9.68e-6 to 1.35e-3 ^a	(Mahoney et al., 2022)
Concentrations range ^b	3.9-40000 ng L ⁻¹	7.9-7600 ng L ⁻¹	0.14-195 ng L ⁻¹	(Mahoney et al., 2022; Xiao, 2017)

^a Prediction.

^b Concentrations range from the wastewater's (high values) to the marine's (low values).

Table S2

Culture condition of *S. costatum* (GY-11). The level of composition of culture medium is g L⁻¹.

Items	Contents	Concentrations of stock solution (g L ⁻¹)
Culture medium	F/2 medium	
Composition of culture medium	NaNO ₃	75.00
	NaH ₂ PO ₄ • H ₂ O	5.00
	Na ₂ SiO ₃ • 9H ₂ O	30
	Na ₂ EDTA	4.36
	FeCl ₃ • 6H ₂ O	3.15
	CoCl ₂ • 6H ₂ O	10
	Na ₂ MoO ₄ • 5H ₂ O	6.3
	MnCl ₂ • 4H ₂ O	180
	ZnSO ₄ • 7H ₂ O	23
	CuSO ₄ • 5H ₂ O	9.8
	Vitamin B1	0.1
	Vitamin B12	0.0005
Vitamin H	0.0005	
Culture device	Intelligent artificial climate incubator (RXM-168B, Ningbo Jiangnan Instrument Factory Co., Ltd, China)	
Culture light source	white fluorescent light, 80 μmol·photons m ⁻² ·s ⁻¹ light intensity	
Other materials	Pinhole filter	

Table S3

The results of the one-way ANOVA analysis of variance for the comparison of OD, cell viability, Chl-*a* content, T-SOD activity and GSH content between solvent control (SC) and treatment with 300 ng/L of mixed PFAS (TF). The paired sample T test analysis for comparison between TF 14 and TF 8.

Group	<i>p</i> -value				
	OD	Cell Viability	Chl- <i>a</i> Content	T-SOD Activity	GSH Content
TF_8_vs_SC_8 (G1)	--	--	--	--	--
TF_14_vs_SC_14 (G2)	--	--	--	--	< 0.041
TF_14_vs_TF_8 (G3)	< 0.001	< 0.006	< 0.031	--	< 0.001

The "--" represents no significant differences compared to the solvent control, namely $p > 0.05$.

Table S4

The GO function enrichment (top 5 in DEPs) of correlation analysis between transcriptome and proteome.

Comparison group	Upregulated term	Downregulated term
(TF_8_vs_SC_8)	1. chloroplast organization	1. methyltransferase activity
	2. plastid organization	2. nucleotide-excision repair
	3. response to nitrogen compound	3. pyruvate, phosphate dikinase activity
	4. negative gravitropism	4. phosphotransferase activity, paired acceptors
	5. response to ammonium ion	5. spliceosomal complex
(TF_14_vs_SC_14)	1. response to ammonium ion	1. secretion by cell
	2. response to ethylene	2. secretion
	3. negative gravitropism	3. exocyst localization
	4. response to nitrogen compound	4. DNA replication checkpoint
	5. response to cation stress	5. mitotic DNA replication checkpoint
TF_14_vs_TF_8	1. pyruvate, phosphate dikinase activity	1. translation
	2. phosphotransferase activity, paired acceptors	2. amide biosynthetic process
	3. oxidoreductase activity	3. peptide biosynthetic process
	4. tricarboxylic acid cycle	4. structural molecule activity
	5. macromolecule biosynthetic process	5. structural constituent of ribosome

References:

- Mahoney, H., Xie, Y., Brinkmann, M., Giesy, J.P., 2022. Next generation per- and poly-fluoroalkyl substances: Status and trends, aquatic toxicity, and risk assessment. *Eco-Environment & Health* 1(2), 117-131.
- Xiao, F., 2017. Emerging poly- and perfluoroalkyl substances in the aquatic environment: A review of current literature. *Water Res.* 124, 482-495.

3. Transcriptomic and proteomic characteristics and function enrichment analysis of DEGs and DEPs of *S. costatum*

Fig. S1. The expressed genes between SC and TF on the 8th and 14th days in the 300 ng L⁻¹.

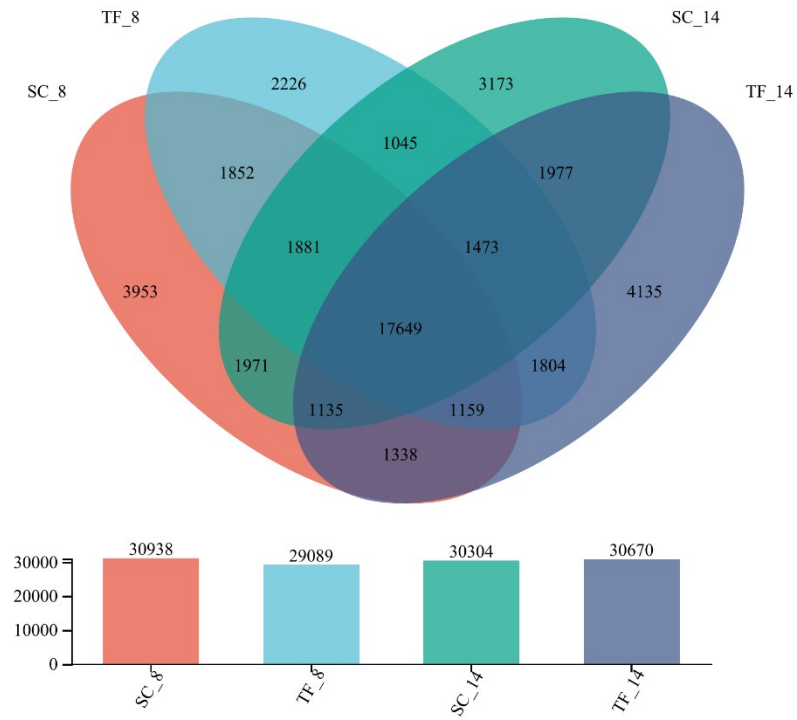
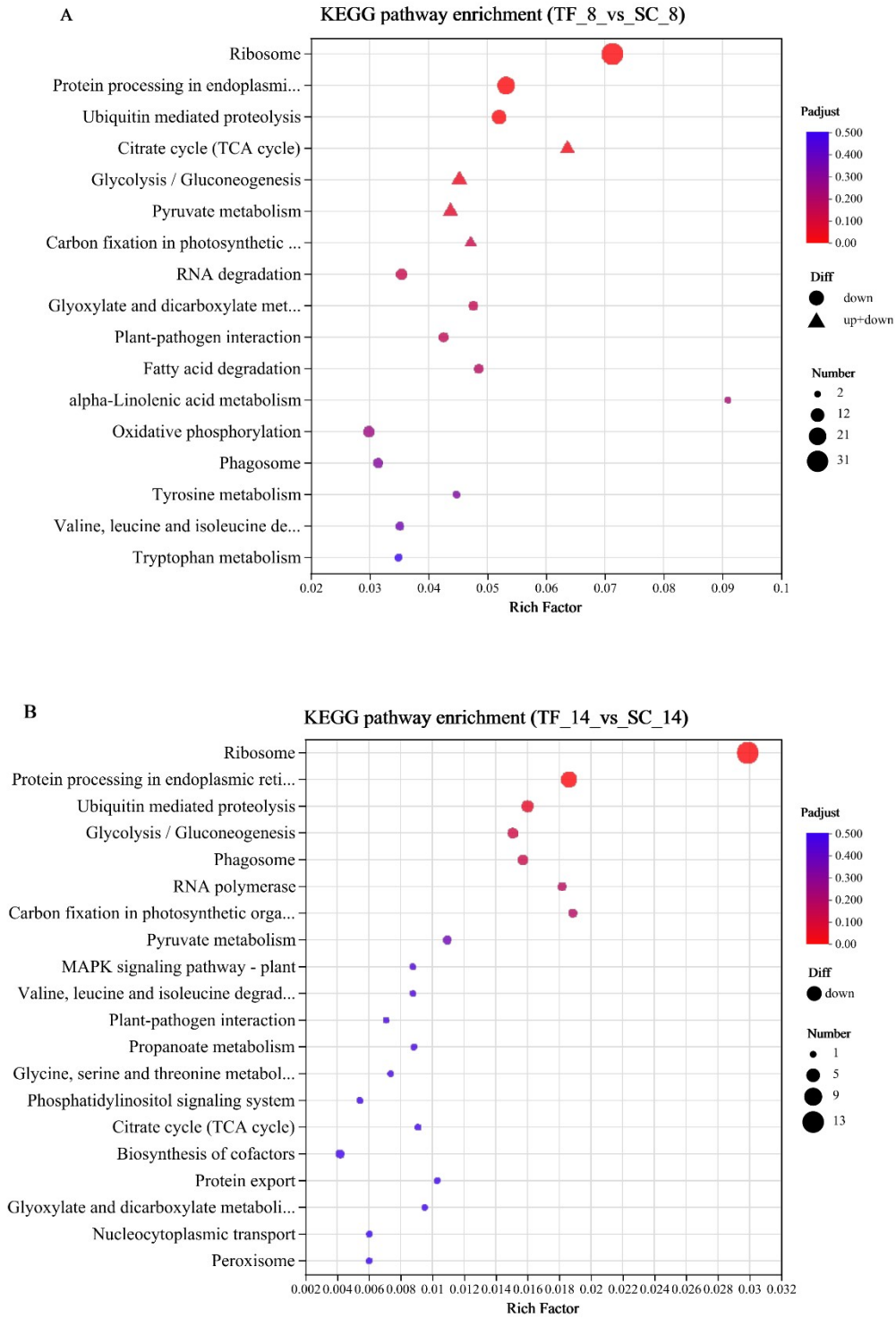


Fig. S2 KEGG pathways enrichment analysis of DEGs in G1, G2, and G3 (Top 20).



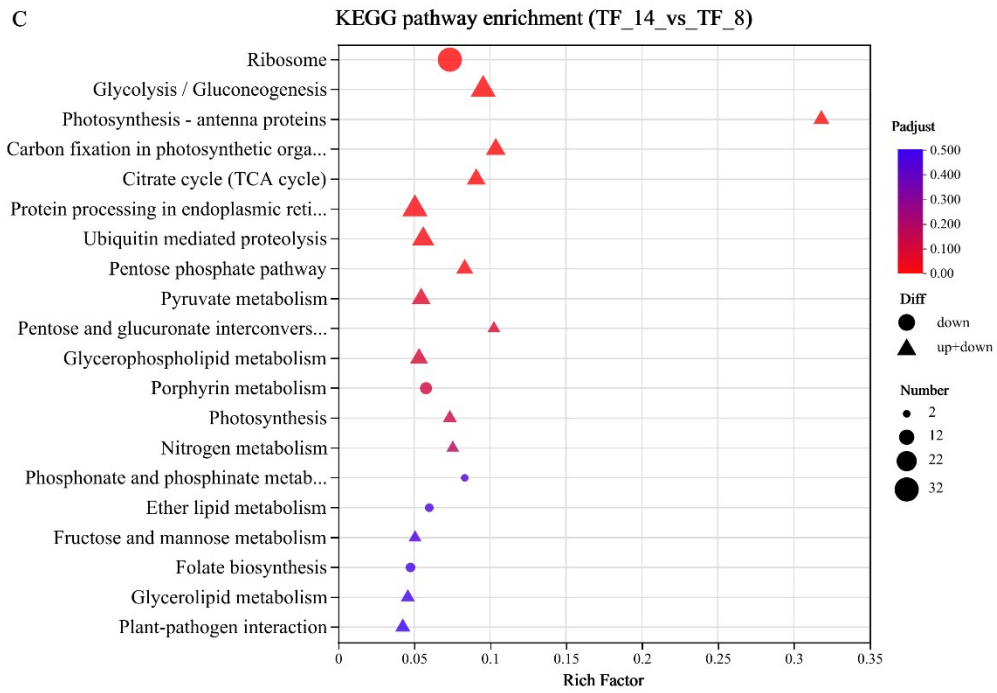


Fig. S3. The expressed proteins between SC and TF on the 8th and 14th days in the 300 ng L⁻¹.

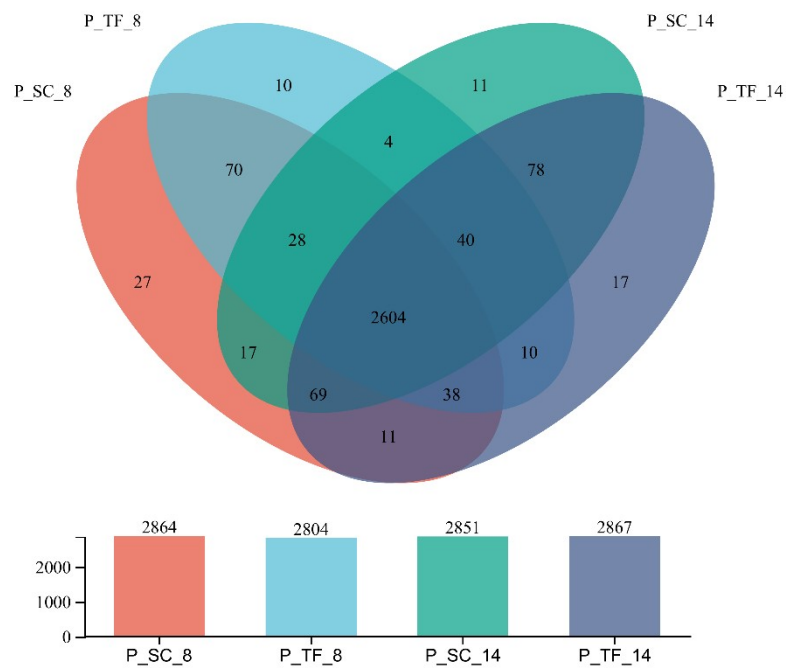
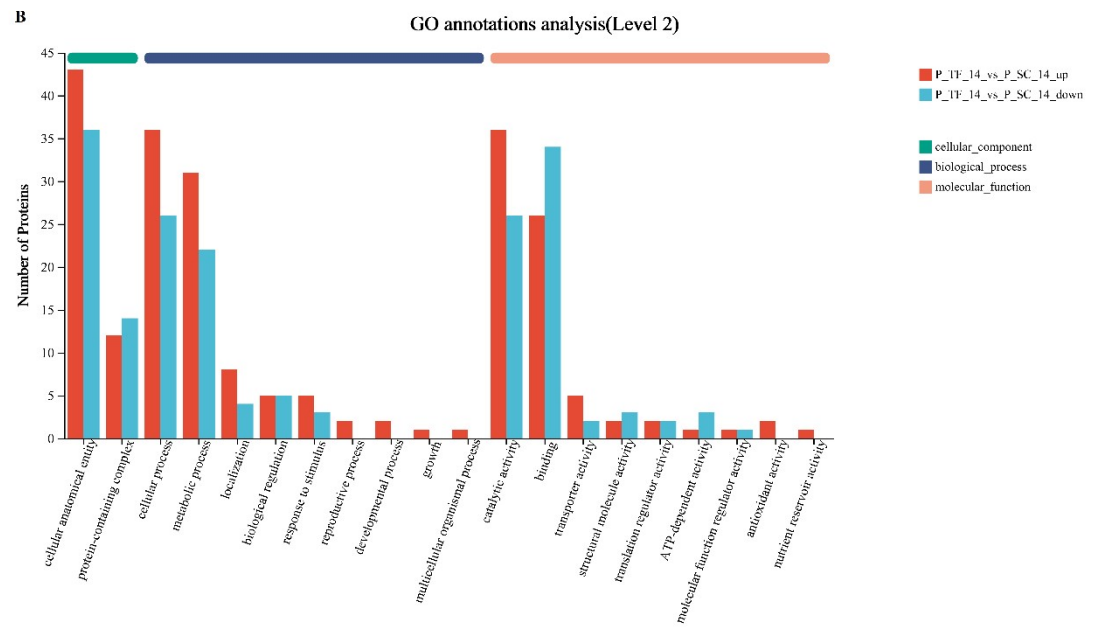
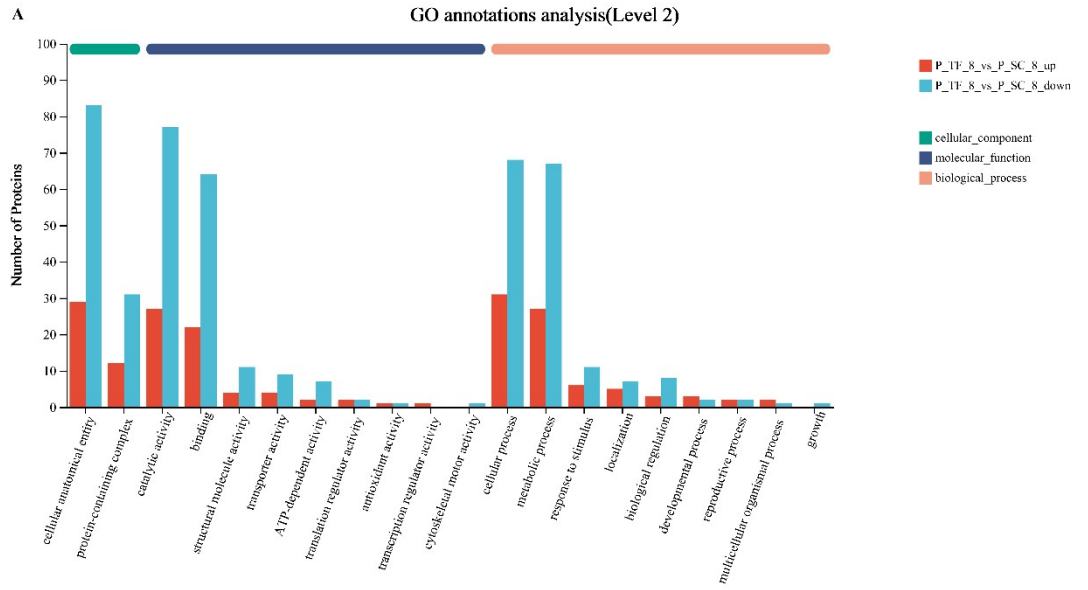


Fig. S4 GO annotations analysis of DEPs in G1, G2, and G3.



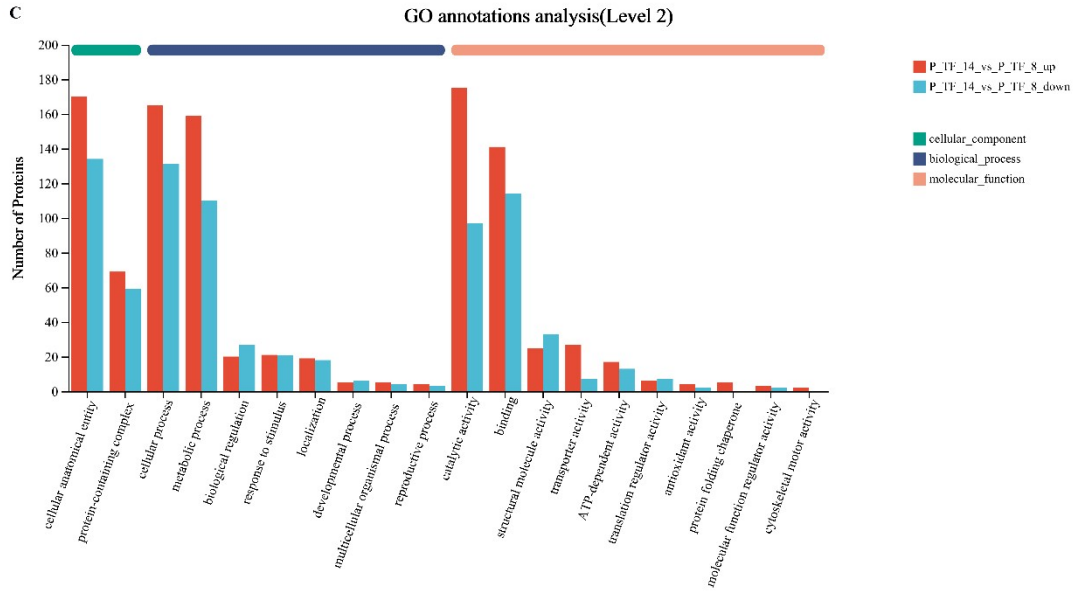
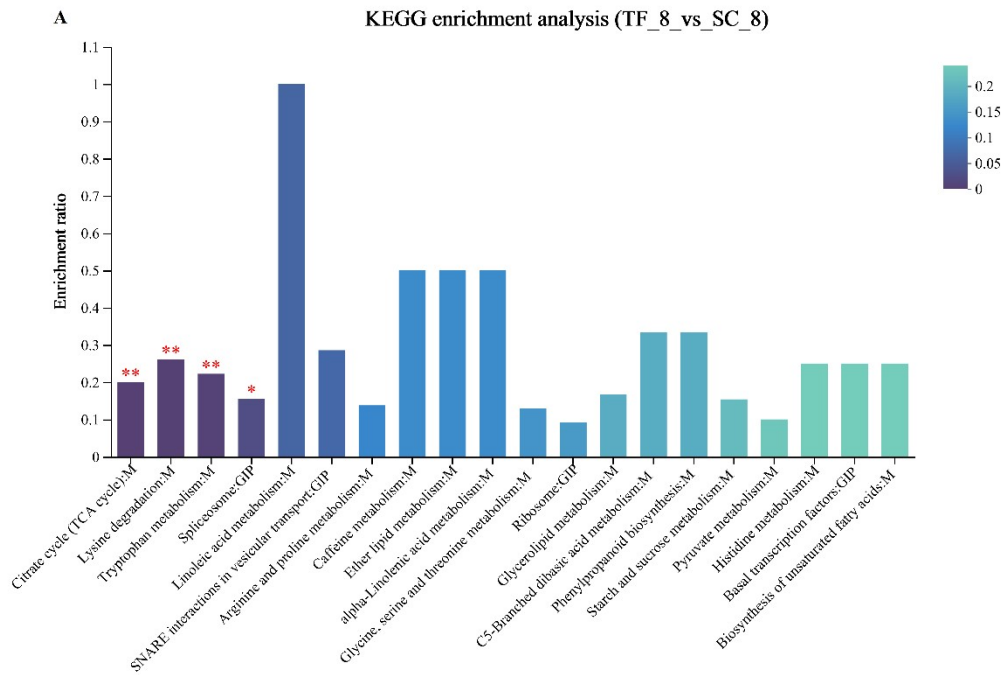


Fig. S5 KEGG pathways enrichment analysis of DEPs in G1, G2, and G3.



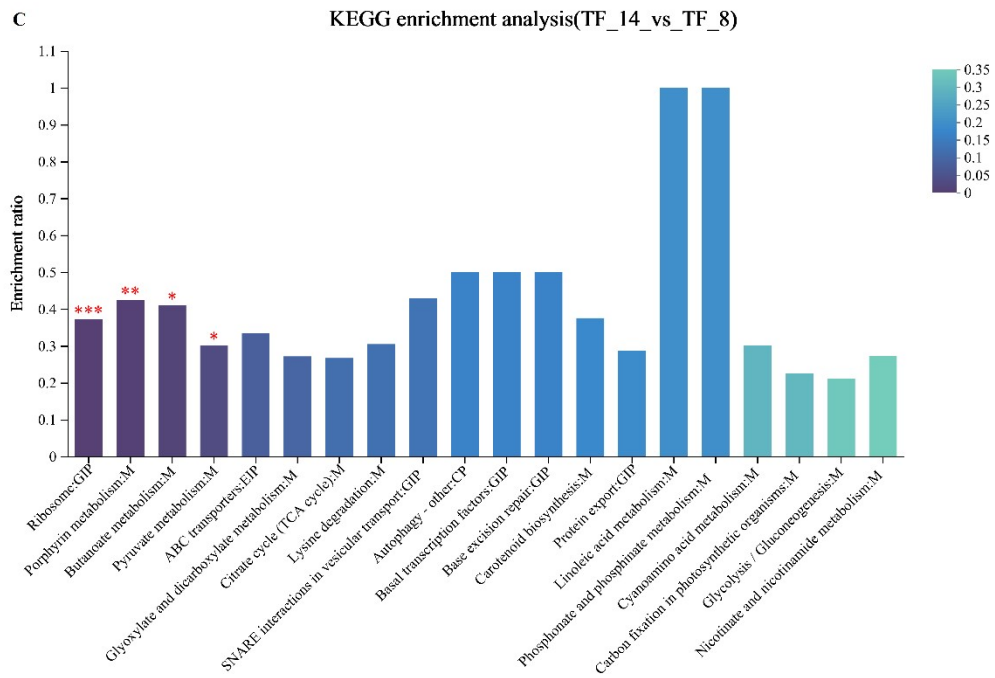
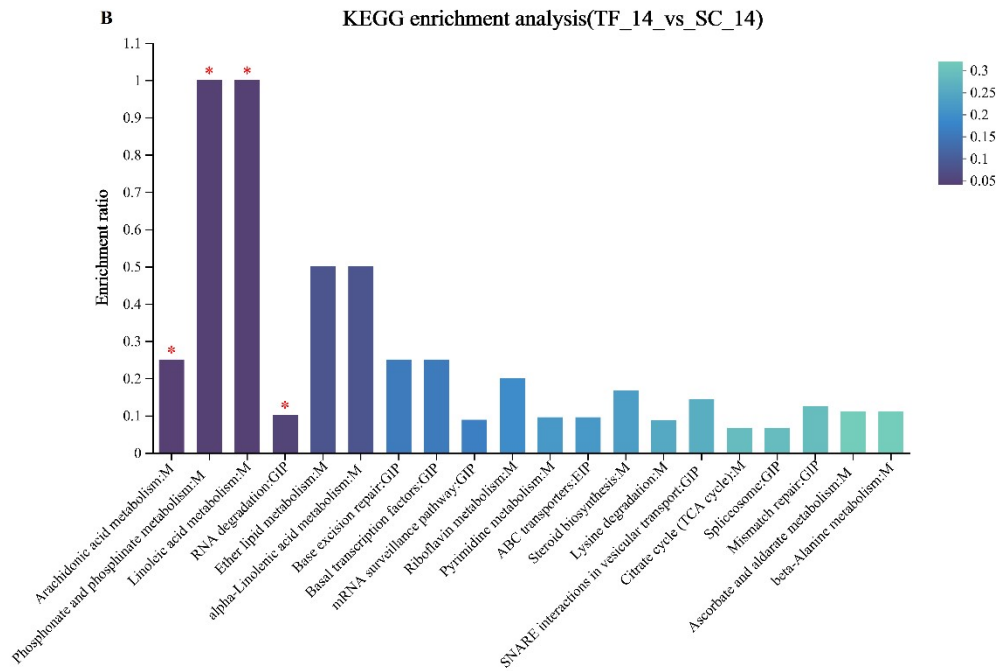


Fig. S6 Relationship between expressed genes and proteins.

Relationship between gene and protein

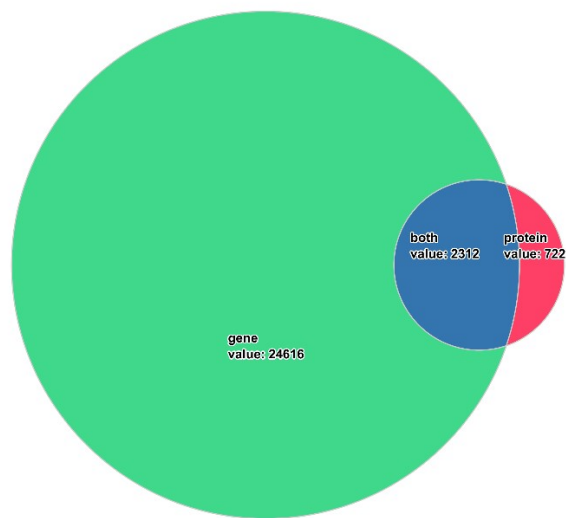
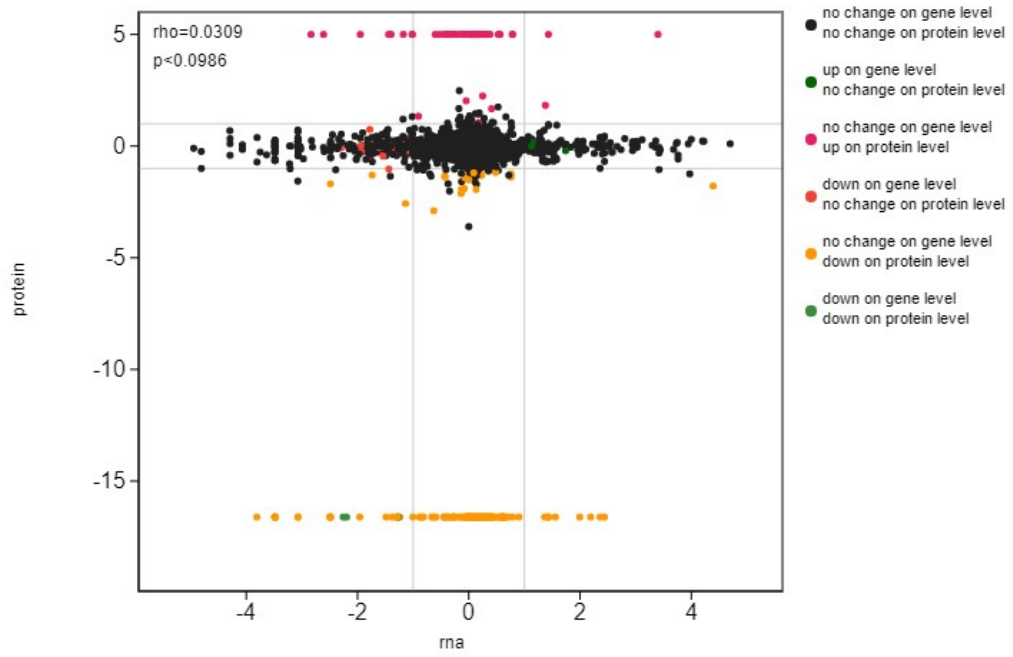


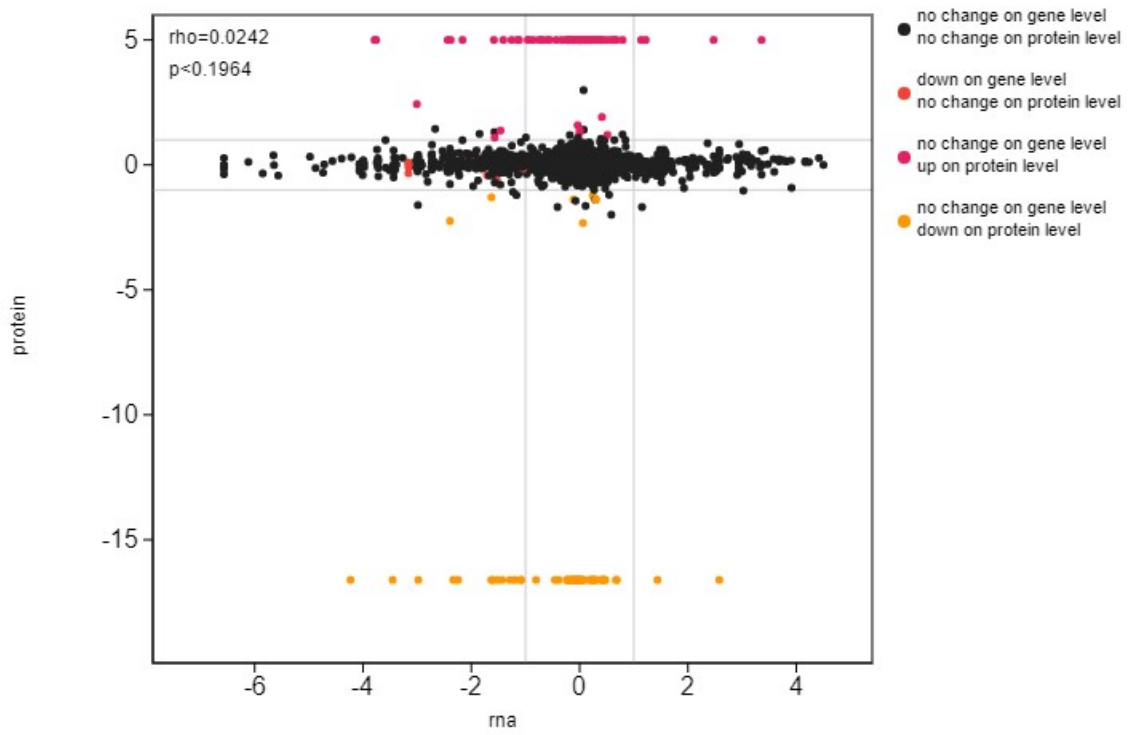
Fig. S7 Differential expression analysis between transcriptome and proteome of G1, G2, and G3 (nine-quadrant diagram).

A

TF_8_vs_SC_8

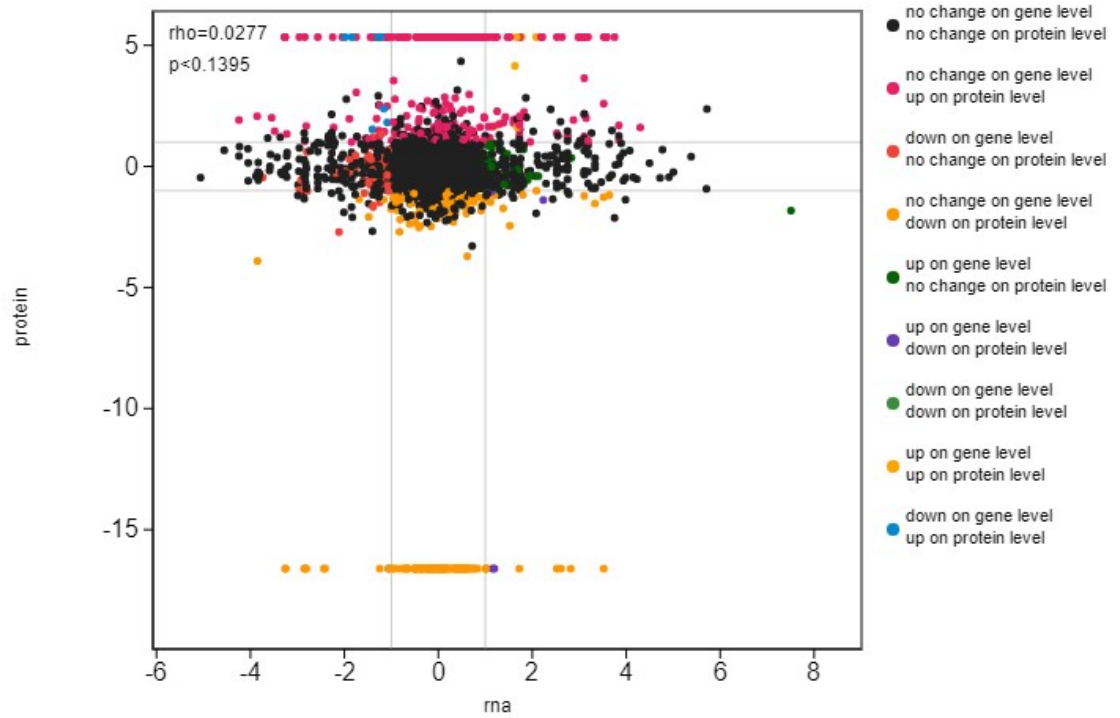
**B**

TF_14_vs_SC_14



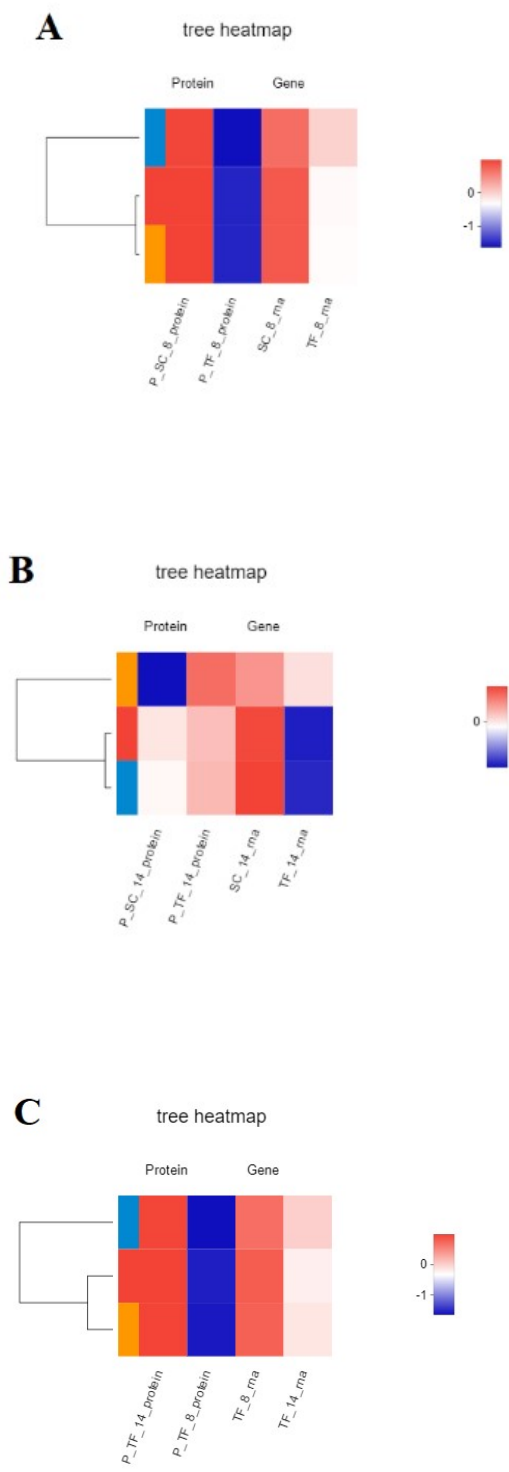
C

TF_14_vs_TF_8



The vertical coordinate represents $\log_2(\text{ratio of protein})$, and the horizontal coordinate represents $\log_2(\text{ratio of gene})$, the expression ratio of the corresponding transcript in the comparison group. The rho represents a person relationship between transcriptome and proteome; the p represents the correlation test p value; the greater the absolute value of rho, the greater the correlation between the two omics.

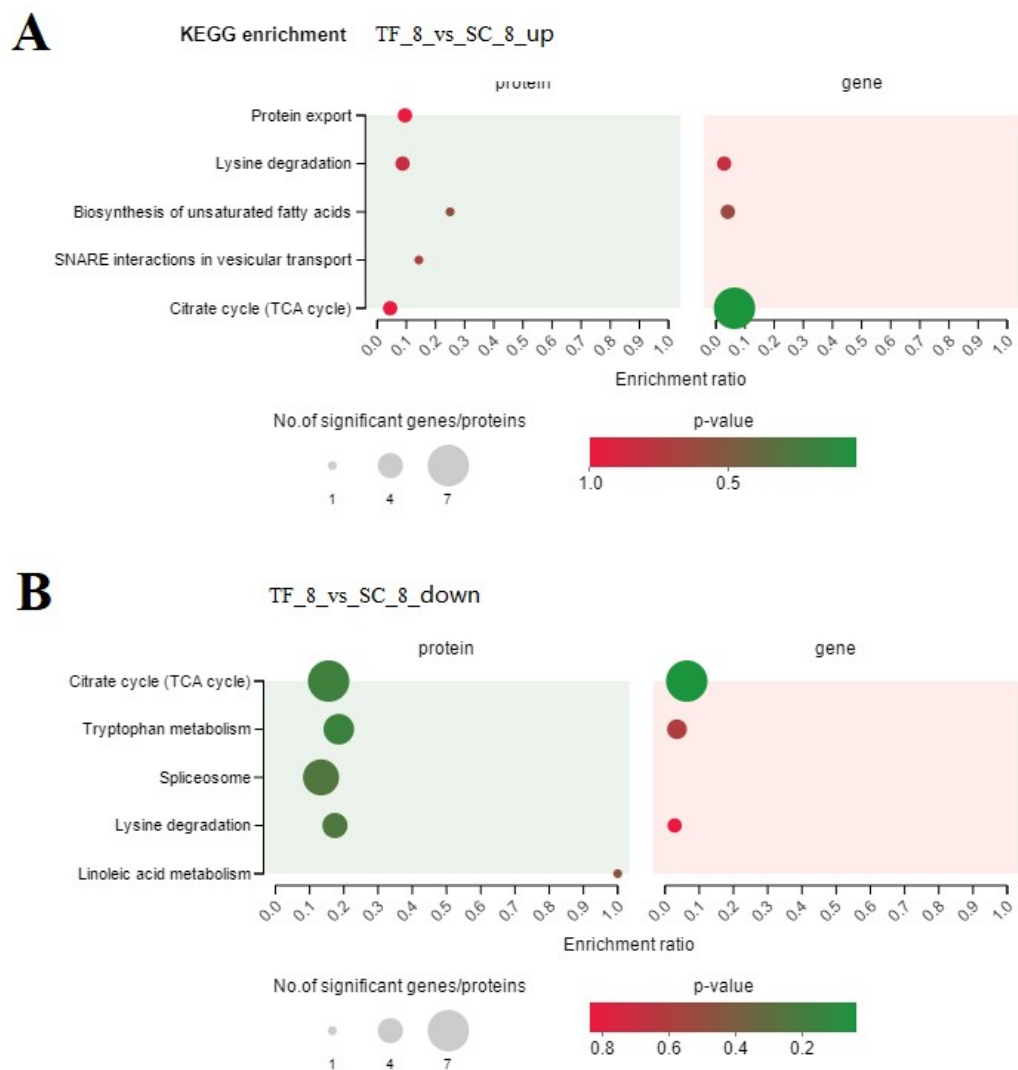
Fig. S8 Differential expression analysis between transcriptome and proteome of G1, G2, and G3 (tree heatmap).

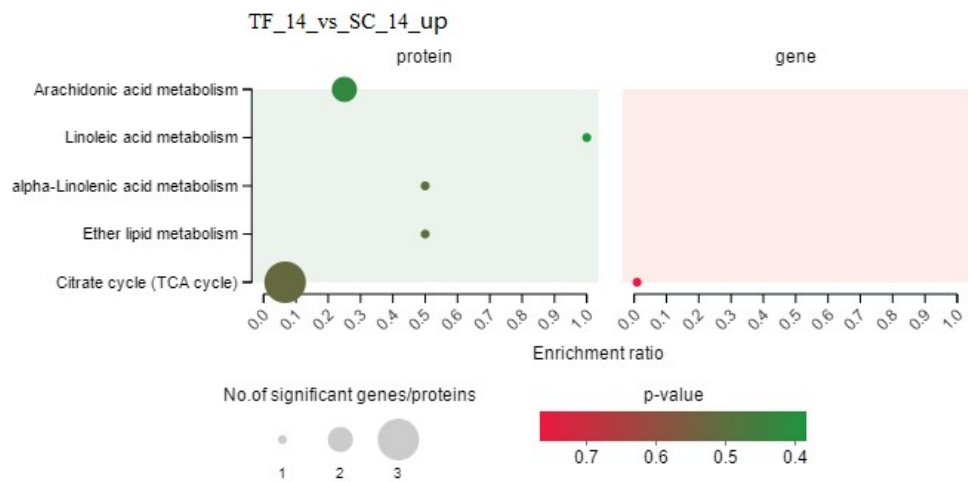
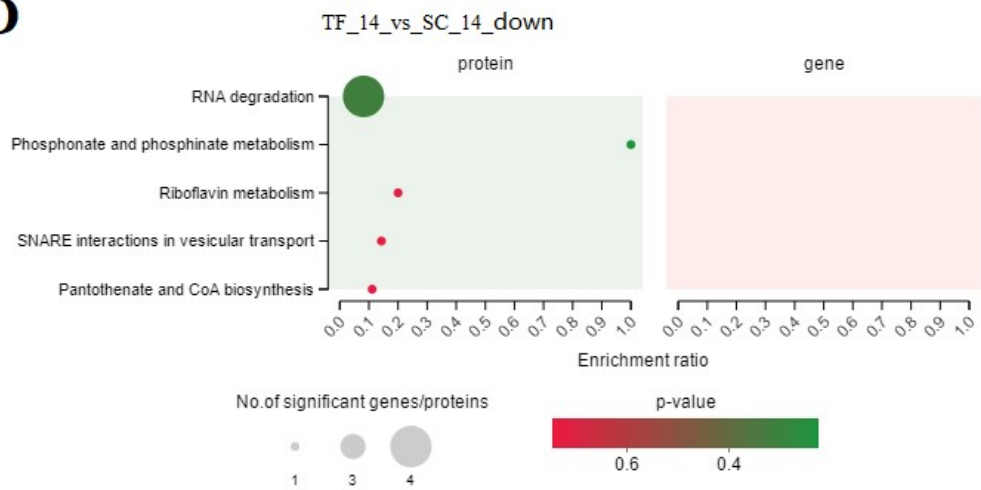
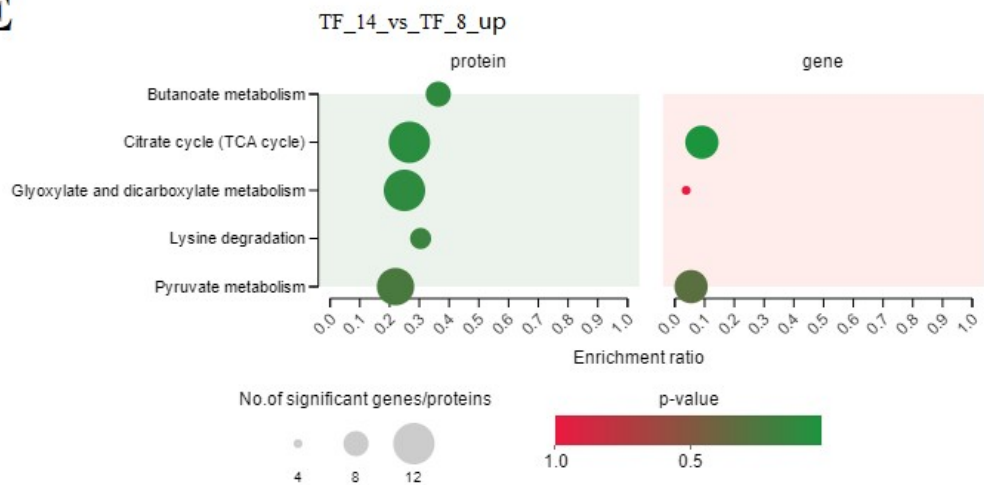


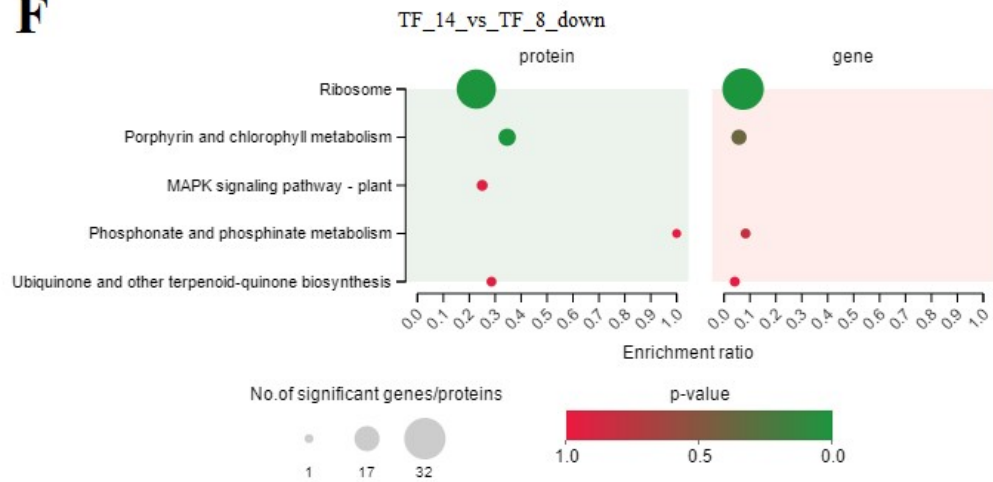
Each column in the figure represents a group or sample, each row represents a protein/gene, and the color in the figure represents the relative expression level of the protein/gene in the group of

samples. For the specific change trend of the expression level, please see the digital label under the color bar on the upper left. On the left is the tree diagram of protein/gene clustering, and on the right is the name of protein/gene. The closer the two protein/gene branches are, the closer their expression levels are. The top is the tree diagram of sample clustering, and the bottom is the name of the sample. The closer the branches of the two samples are, the closer the expression patterns of all proteins/genes in the two samples are, that is, the closer the change trend of expression quantity is.

Fig. S9 KEGG pathways enrichment of association analysis between transcriptome and proteome of G1, G2, and G3 (top 5 in DEPs).



C**D****E**

F

The KEGG pathways enrichment (top 5) of correlation analysis between transcriptome and proteome. The enrichment ratio indicates the ratio of DEGs/DEPs to the total number of annotated genes/proteins in this KEGG pathway. Each bubble represents a KEGG pathway. The different colors of the bubbles represent p-value.

PORPHYRIN METABOLISM

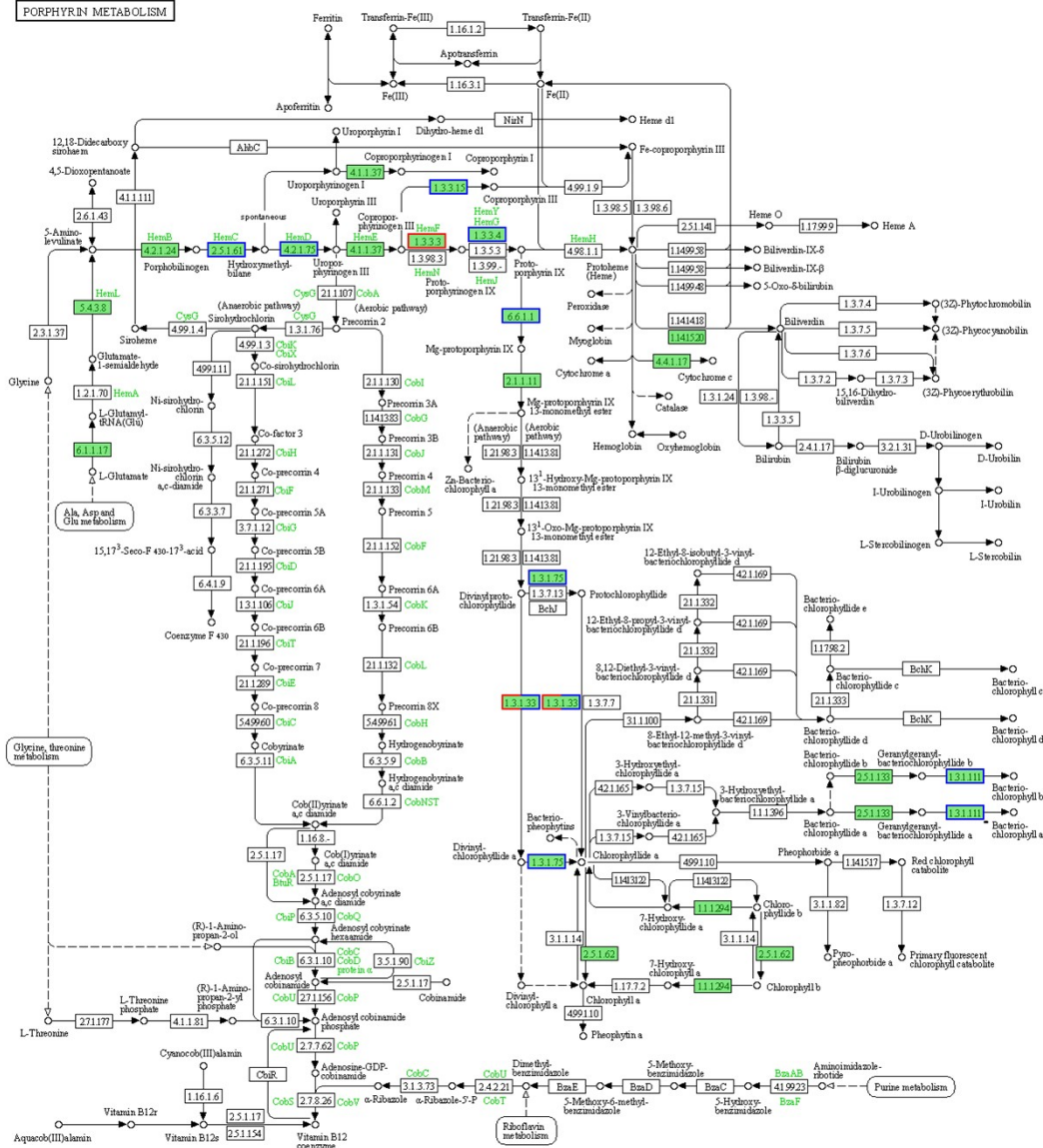
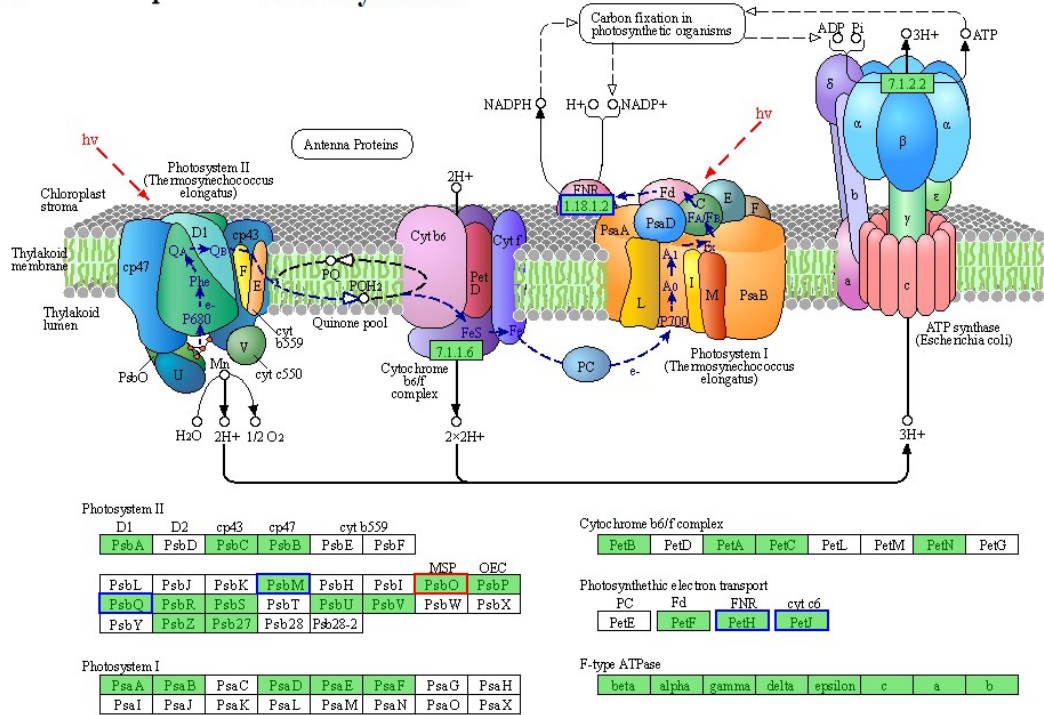


Fig. S11 KEGG pathways enrichment analysis in electron transport chain of photosynthesis of DEGs and DEPs in G3 (TF_14_vs_TF_8).

A Transcriptome Photosynthesis



B Proteome Photosynthesis

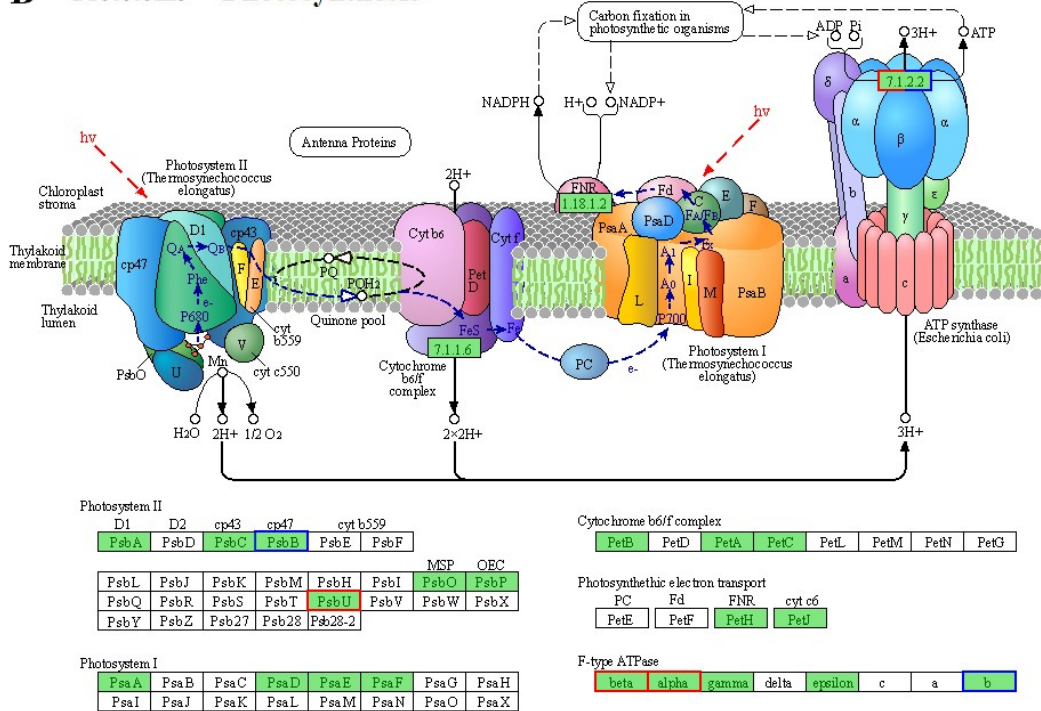
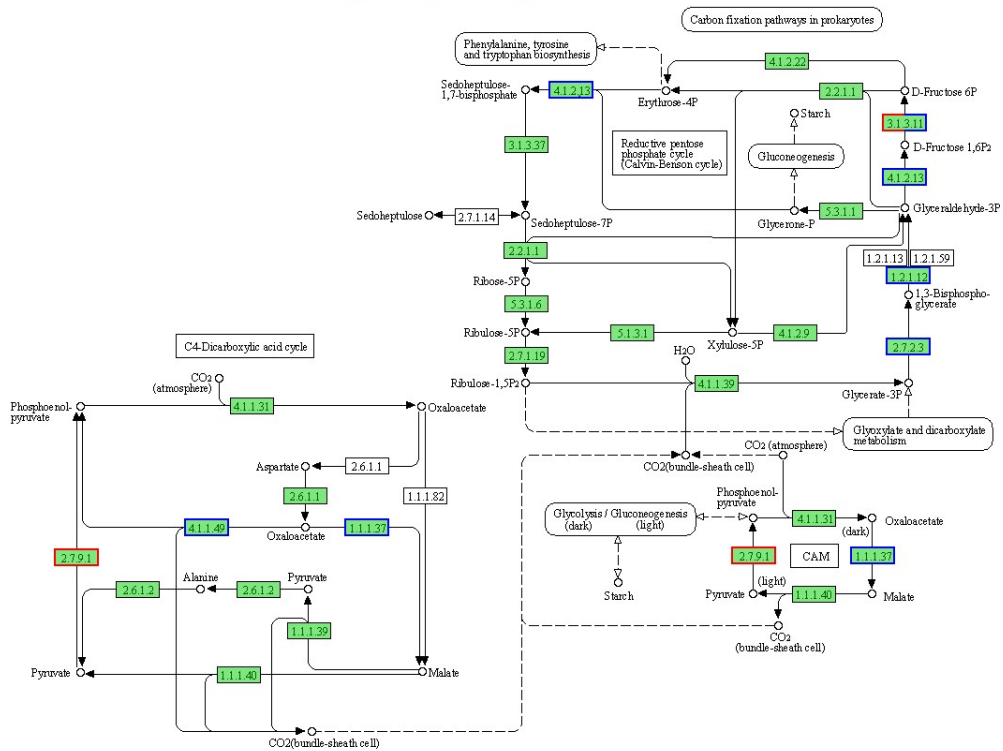


Fig. S12 KEGG pathways enrichment analysis in carbon fixation in photosynthetic organisms of DEGs and DEPs in G3 (TF_14_vs_TF_8).

A Transcriptome Carbon fixation in photosynthetic organism



B Proteome Carbon fixation in photosynthetic organism

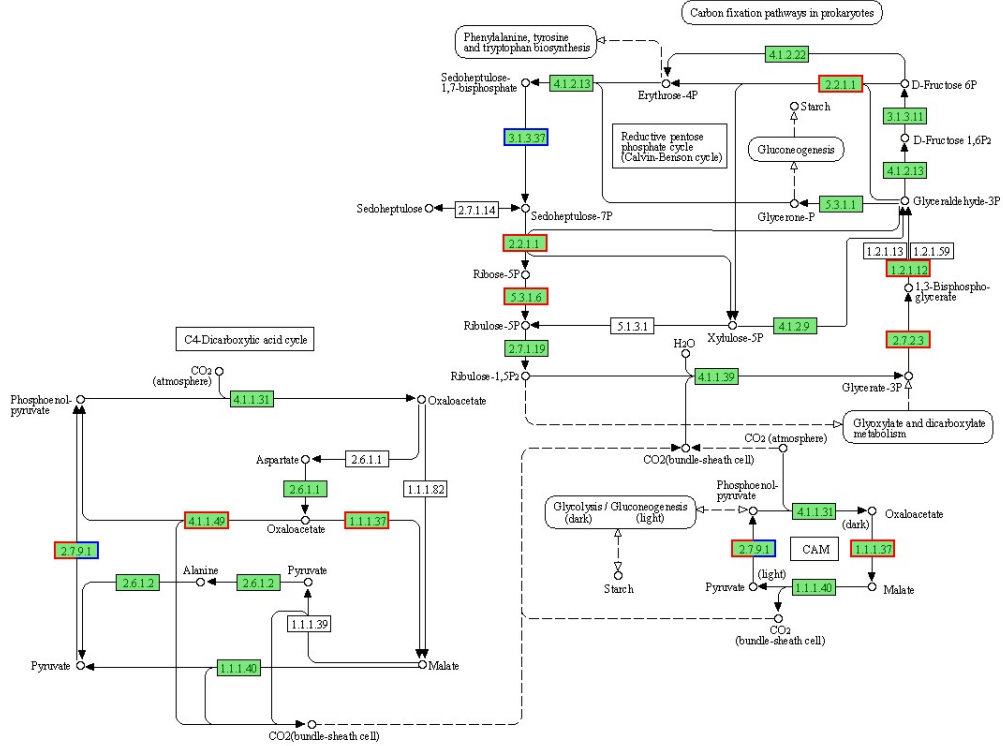
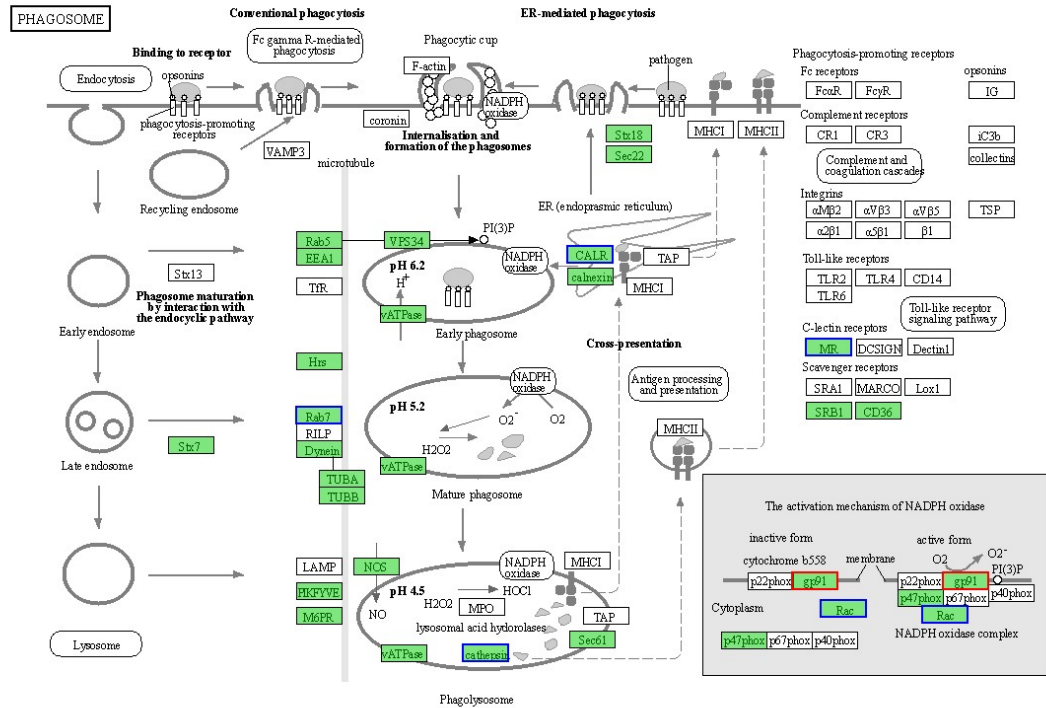


Fig. S13 KEGG pathways enrichment analysis in peroxisome and glutathione metabolism of DEGs and DEPs in G3 (TF_14_vs_TF_8).

Phagosome

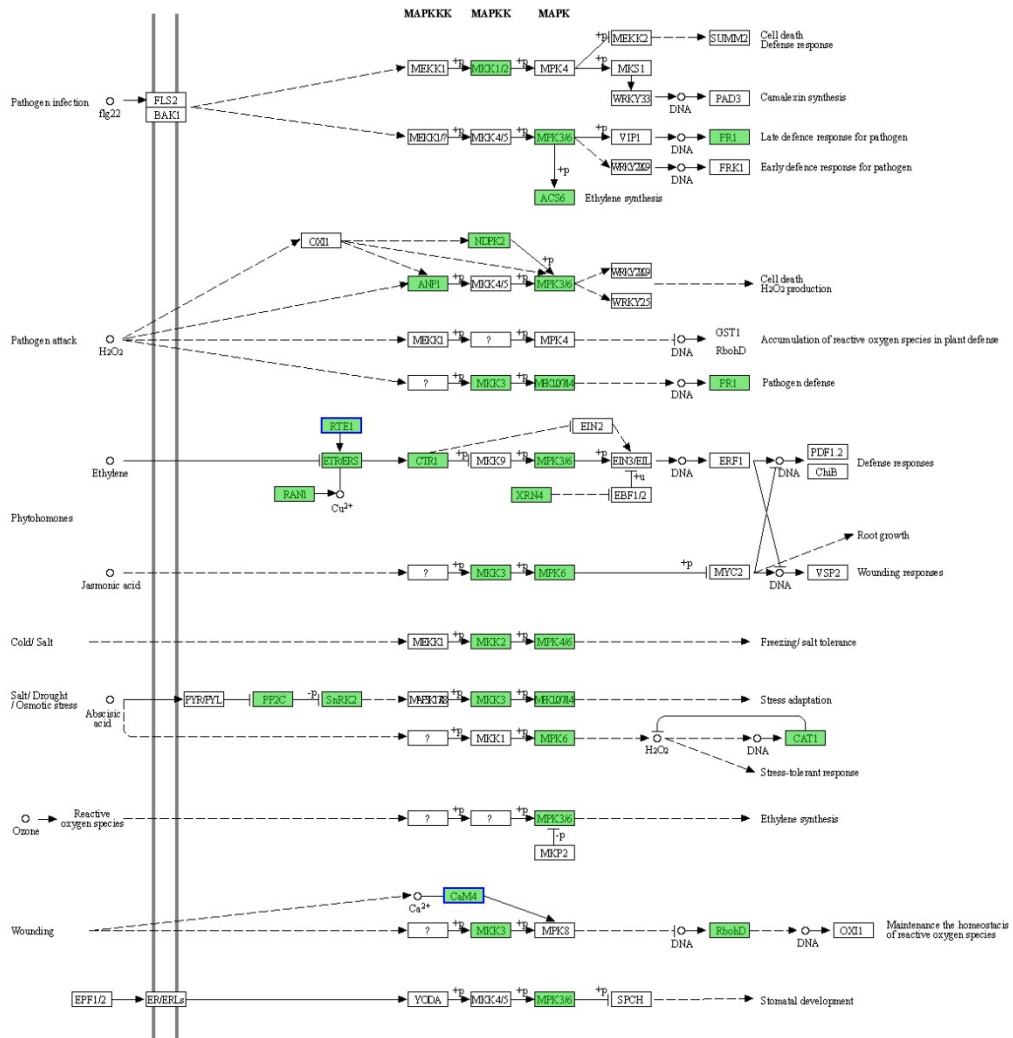
Transcriptome



MAPK signaling pathway - plant

Transcriptome

MAPK SIGNALING PATHWAY - PLANT



Proteome

