



Supplementary Material

Transcriptomic and Physiological Responses of *Chlorella pyrenoidosa* during Exposure to 17 α -Ethinylestradiol

Annexed Table

Table Captions:	
Table S1.	Formulae and terms used in the analysis of the O-J-I-P fluorescence-induction dynamics curve.
Table S2.	Variance of ABS/RC, RC/CS ₀ , ET ₀ /ABS, ψ_0 , RC/CS ₀ , DI ₀ /RC, ET ₀ /RC, and TR ₀ /RC in response to exposure time under at different 17 α -EE ₂ concentrations.
Table S3.	The lists of primers used in real-time-quantitative PCR.
Table S4.	The Lists of DEGs belonging to different pathways in the LD, MD, and HD groups.
Figure captions:	
Fig. S1	The transcript abundance of genes related to <i>psaD</i> , <i>sodA</i> , and <i>por</i> in cultures of <i>C. pyrenoidosa</i> exposure to 17 α -EE ₂ .
Fig. S2	Scatter plots of KEGG enrichment pathway in the LD, MD, and HD groups.
Fig. S3	KEGG-annotated changes in gene expression for photosynthesis pathways in the LD group.
Fig. S4	KEGG-annotated changes in gene expression for oxidative phosphorylation pathways in the LD group.
Fig. S5	KEGG-annotated changes in gene expression for ribosome biogenesis in eukaryotes pathways in the LD group.
Fig. S6	KEGG-annotated changes in gene expression for ribosome biogenesis in eukaryotes pathways in the MD group.
Fig. S7	KEGG-annotated changes in gene expression for pyrimidine metabolism pathways in the MD group.
Fig. S8	KEGG-annotated changes in gene expression for porphyrin and chlorophyll metabolism pathways in the MD group.
Fig. S9	KEGG-annotated changes in gene expression for nitrogen metabolism pathways in the MD group.
Fig. S10	KEGG-annotated changes in gene expression for DNA replication pathways in the MD group.
Fig. S11	KEGG-annotated changes in gene expression for DNA replication pathways in the HD group.
Fig. S12	KEGG-annotated changes in gene expression for mismatch repair pathways in the HD group.
Fig. S13	KEGG-annotated changes in gene expression for homologous recombination pathways in the HD group.

Table S1. Formulae and terms used in the analysis of the O-J-I-P fluorescence-induction dynamics curve.

Formulae and Terms	Illustrations
O-step, K-step, J-step, I-step, P-step	Fluorescence intensity at t=50 μ s, 30 μ s, 2 ms, 30 ms, and the highest peak of fluorescence, respectively
ABS/RC	Light energy absorbed per unit reaction center
Fv/Fm	The maximum quantum yield for primary photochemistry
ET ₀ /ABS	Quantum yield for electron transport (at t=0)
ψ_0	Probability that a trapped exciton moves an electron into the electron transport chain beyond QA ⁻ (at t=0)
RC/CS ₀	Density of RCs (QA-reducing PSII reaction centers)
DI ₀ /RC	Dissipated energy flux per RC (at t=0)
ET ₀ /RC	Electron transport flux per RC (at t=0)
TR ₀ /RC	Trapped energy flux per RC (at t=0)

Table S2. Variance of ABS/RC, TR₀/RC, ET₀/RC, DI₀/RC, Fv/Fm, RC/CS₀, ψ_0 , and ET₀/ABS in response to exposure time under different 17 α -EE₂ concentrations. Values represent mean \pm standard error. The letters (a–f) indicate homogeneity of different treatments (P < 0.05).

Photosynthetic Parameters	Time (h)	CK	LD	MD	HD
ABS/RC	24	2.52 \pm 0.022a	2.64 \pm 0.054c	2.55 \pm 0.022ab	2.60 \pm 0.021bc
	48	3.63 \pm 0.601a	3.37 \pm 0.036a	3.36 \pm 0.024a	3.46 \pm 0.055a
	72	2.99 \pm 0.037a	2.92 \pm 0.023a	2.94 \pm 0.041a	3.00 \pm 0.075a
	96	3.14 \pm 0.008a	3.07 \pm 0.018b	3.07 \pm 0.016b	3.14 \pm 0.040a
TR ₀ /RC	24	1.59 \pm 0.003a	1.66 \pm 0.043b	1.64 \pm 0.015b	1.59 \pm 0.020a
	48	2.33 \pm 0.052a	2.35 \pm 0.014a	2.33 \pm 0.024a	2.37 \pm 0.041a
	72	2.20 \pm 0.029a	2.16 \pm 0.013a	2.15 \pm 0.028a	2.14 \pm 0.036a
	96	1.59 \pm 0.022a	1.66 \pm 0.011b	1.64 \pm 0.010bb	1.59 \pm 0.033b
ET ₀ /RC	24	0.70 \pm 0.007a	0.72 \pm 0.016a	0.69 \pm 0.020a	0.62 \pm 0.005b
	48	1.14 \pm 0.044a	1.11 \pm 0.005a	1.03 \pm 0.055b	0.96 \pm 0.026c
	72	0.95 \pm 0.009a	1.02 \pm 0.014b	0.99 \pm 0.029ab	1.01 \pm 0.053b
	96	0.70 \pm 0.009a	0.72 \pm 0.017b	0.69 \pm 0.050b	0.62 \pm 0.067a
DI ₀ /RC	24	0.92 \pm 0.018a	0.97 \pm 0.018b	0.91 \pm 0.020a	1.01 \pm 0.010c
	48	1.30 \pm 0.566a	1.02 \pm 0.052a	1.02 \pm 0.040a	1.10 \pm 0.209a
	72	0.79 \pm 0.027a	0.77 \pm 0.013a	0.79 \pm 0.029a	0.86 \pm 0.039b
	96	0.92 \pm 0.022a	0.97 \pm 0.013a	0.91 \pm 0.023a	1.01 \pm 0.015b
Fv/Fm	24	0.63 \pm 0.004a	0.63 \pm 0.006a	0.64 \pm 0.006b	0.61 \pm 0.004c
	48	0.64 \pm 0.104a	0.70 \pm 0.006a	0.70 \pm 0.005a	0.68 \pm 0.007a
	72	0.74 \pm 0.007a	0.74 \pm 0.003a	0.73 \pm 0.007a	0.71 \pm 0.006b
	96	0.73 \pm 0.007a	0.72 \pm 0.003ab	0.72 \pm 0.006b	0.70 \pm 0.004c
RC/Cso	24	62.42 \pm 3.551a	58.03 \pm 2.610a	52.09 \pm 1.816b	48.523 \pm 2.099b
	48	128.05 \pm 9.343a	119.21 \pm 2.355a	98.54 \pm 2.978b	78.57 \pm 1.616c
	72	84.67 \pm 1.526a	86.13 \pm 2.466a	73.44 \pm 2.723b	58.65 \pm 2.972c
	96	102.65 \pm 4.231a	103.40 \pm 4.691a	89.15 \pm 5.479b	70.67 \pm 4.966c
ψ_0	24	0.42 \pm 0.005a	0.43 \pm 0.004ab	0.42 \pm 0.015b	0.39 \pm 0.007c
	48	0.49 \pm 0.011a	0.47 \pm 0.004a	0.44 \pm 0.026b	0.40 \pm 0.016c
	72	0.46 \pm 0.009a	0.52 \pm 0.006b	0.51 \pm 0.017b	0.47 \pm 0.018b
	96	0.43 \pm 0.008a	0.47 \pm 0.007b	0.46 \pm 0.024b	0.47 \pm 0.034a
ET ₀ /ABS	24	0.32 \pm 0.004a	0.35 \pm 0.001a	0.34 \pm 0.010a	0.34 \pm 0.003b
	48	0.33 \pm 0.049a	0.38 \pm 0.004a	0.37 \pm 0.019a	0.33 \pm 0.012a
	72	0.33 \pm 0.005a	0.38 \pm 0.005b	0.37 \pm 0.010b	0.33 \pm 0.011b
	96	0.32 \pm 0.003a	0.35 \pm 0.004b	0.34 \pm 0.015b	0.34 \pm 0.022a

Table S3. The lists of primers used in real-time-quantitative PCR.

Gene Name	Forward Primers (5'-3')	Reverse Primers (5'-3')
<i>psaD</i>	GATTTTACGGCAGCAAGA-GAGC	ACAGAACGAGGGCGACCAT
<i>sodA</i>	AAGGTCTACACCAC-CGAGCG	GTGCCTTTCGGATTTCGTTG
<i>por</i>	CTTCTTT-GCCCTCTCTGGTGTG	TACGACCTTCTCGCCCTTCTT
<i>18s</i>	CAGCCTGCTAAA-TAGTCACGG	CAGAACATCTAAGGGCATCACA

Abbreviation: *psaD*, Photosystem I P700 chlorophyll a apoprotein A2; *sodA*, manganese superoxide dismutase; *por*, pyruvate: ferredoxin (flavodoxin) oxidoreductase.

Table S4. The lists of DEGs belonging to different pathways in the LD, MD, and HD groups.

Pathway	Gene Name	KO Entry	log2FC	P-Value	Description
Photosynthesis	<i>psbA</i>	K02703	-3.35	0.00039	Photosystem II protein D1
	<i>psbB</i>	K02704	-2.99	0.00060	Photosystem II 47 kDa protein
	<i>psbC</i>	K02705	-3.06	0.00033	Photosystem II protein
	<i>psbE</i>	K02707	-2.57	0.00051	Cytochrome b559 alpha subunit of photosystem II
	<i>psbI</i>	K02710	-2.38	0.00049	Photosystem II reaction center protein
	<i>Psb28</i>	K08903	0.55	0.00040	Photosystem II reaction center psb28 chloroplastic
	<i>psaD</i>	K02692	2.03	0.00041	Photosystem I reaction center subunit chloroplastic
	<i>petJ</i>	K08906	-0.21	0.00042	Cytochrome c6
	<i>petB</i>	K02635	-3.02	0.00117	Cytochrome b6 (chloroplast)
	<i>petF</i>	K02639	-0.25	0.00003	Chloroplast precursor
Oxidative phosphorylation	<i>crtz</i>	K15746	-0.29	0.00022	Beta-carotene hydroxylase
	<i>atpA</i>	K02111	-3.14	0.00020	ATP synthase CF1 alpha subunit
	<i>atpG</i>	K02109	-0.03	0.00091	CF0 ATP synthase subunit II precursor
	<i>coxI</i>	K02256	-2.48	0.00044	Cytochrome c oxidase subunit I
	<i>sodA</i>	K04564	2.13	0.00889	Manganese superoxide dismutase
	<i>por</i>	K02956	1.17	0.00004	Pyruvate:ferredoxin (flavodoxin) oxidoreductase
Ribosome biogenesis in eukaryotes	<i>Kin14n</i>	K14565	-1.45	0.00067	P-loop containing nucleoside triphosphate hydrolase
	<i>nita</i>	K10534	-2.96	0.00001	Nitrate reductase
Nitrogen metabolism	<i>gdh2</i>	K00261	-2.11	0.00045	Glutamate dehydrogenase
DNA replication	<i>mcm4</i>	K02212	-2.00	0.00032	DNA replication licensing factor
	<i>rpa1D</i>	K07466	-1.99	0.00011	Replication A 70 kDa DNA-binding subunit B
Mismatch repair	<i>msh1</i>	K08737	1.11	0.00001	DNA mismatch repair protein MSH1, mitochondrial
	<i>exo1</i>	K10746	-2.28	0.00003	Exonuclease 1
	<i>mus1</i>	K08735	-1.56	0.00007	DNA mismatch repair

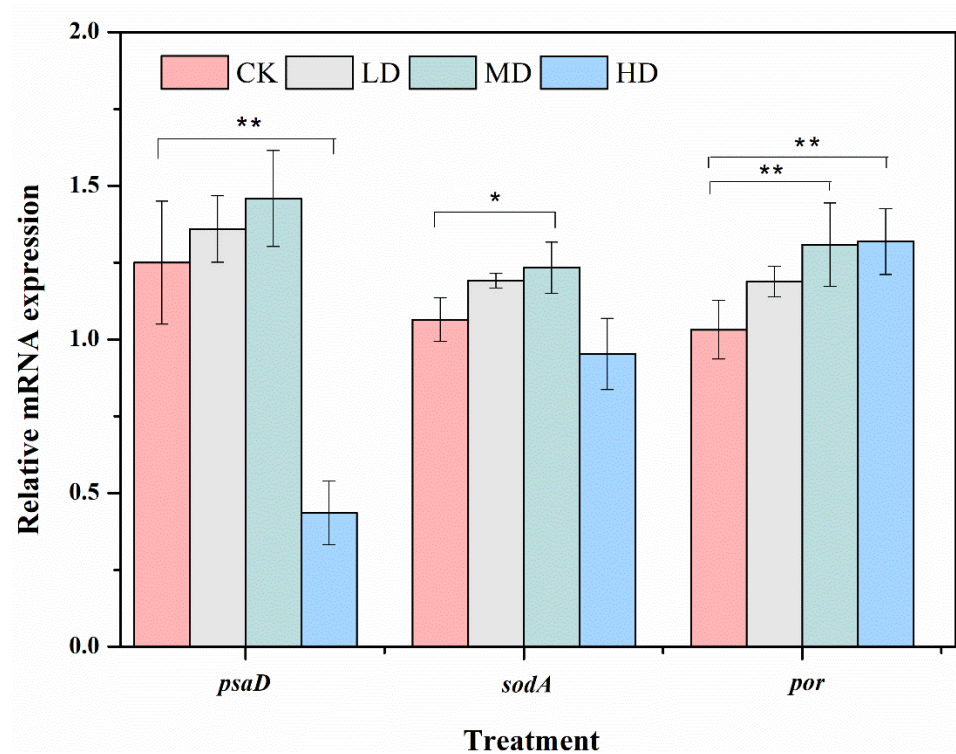


Figure S1. The transcript abundance of genes related to *psaD*, *sodA*, and *por* in cultures of *C. pyrenoidosa* exposed to 17 α -EE₂. Error bars indicate mean \pm SE. (*) and (**) represent statistically significant differences with respect to the values of control cultures at p < 0.05 and at p < 0.01 levels, respectively.

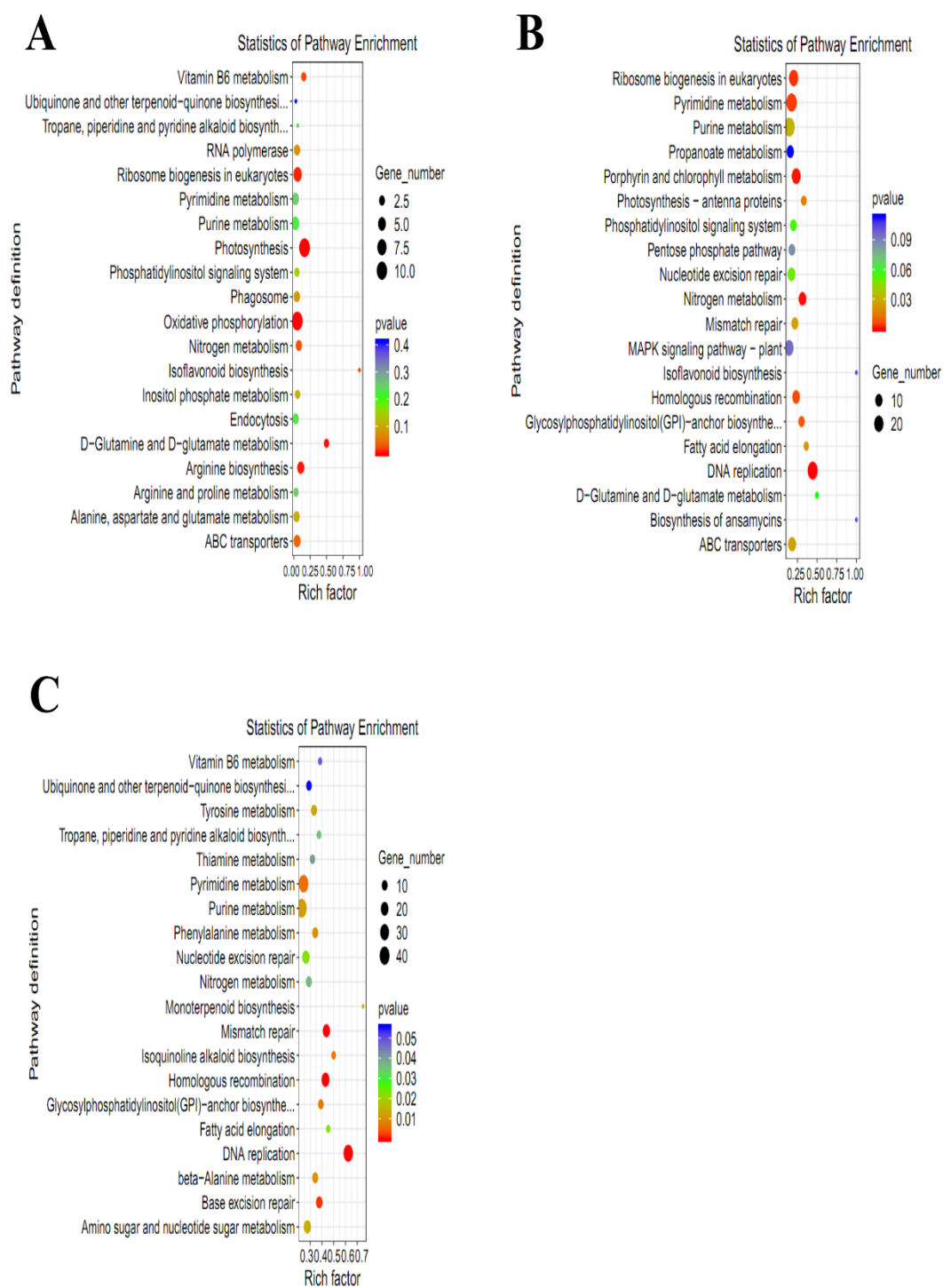


Figure S2. Scatter plots of KEGG enrichment pathway in the LD (A), MD (B), and HD (C) groups.

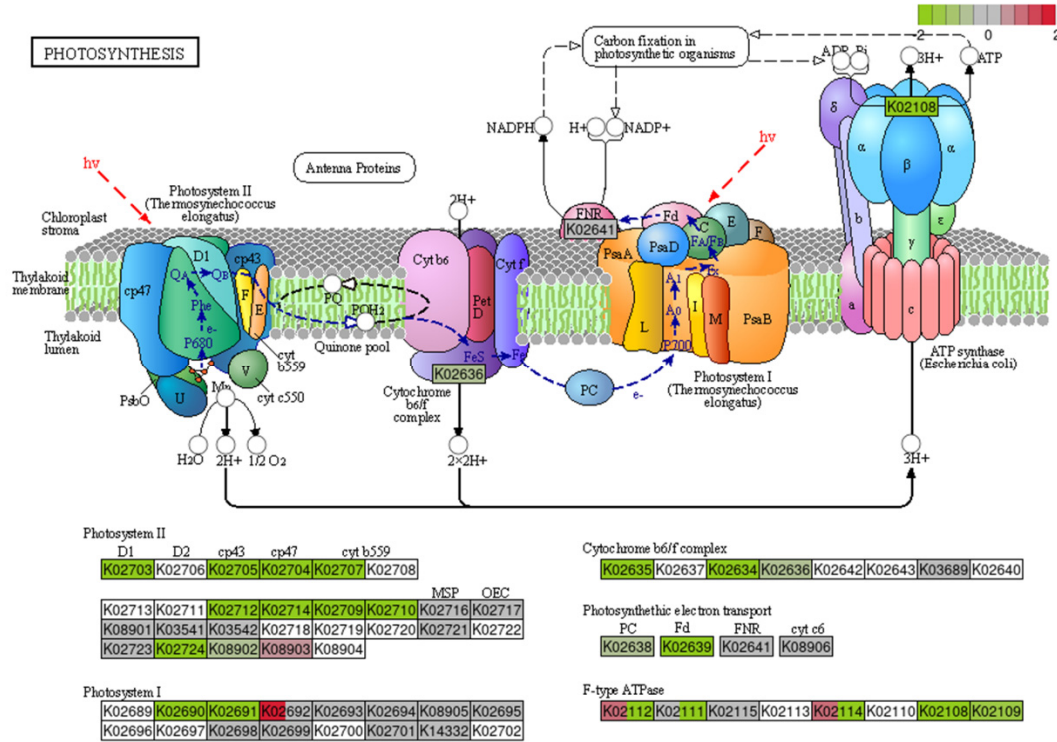
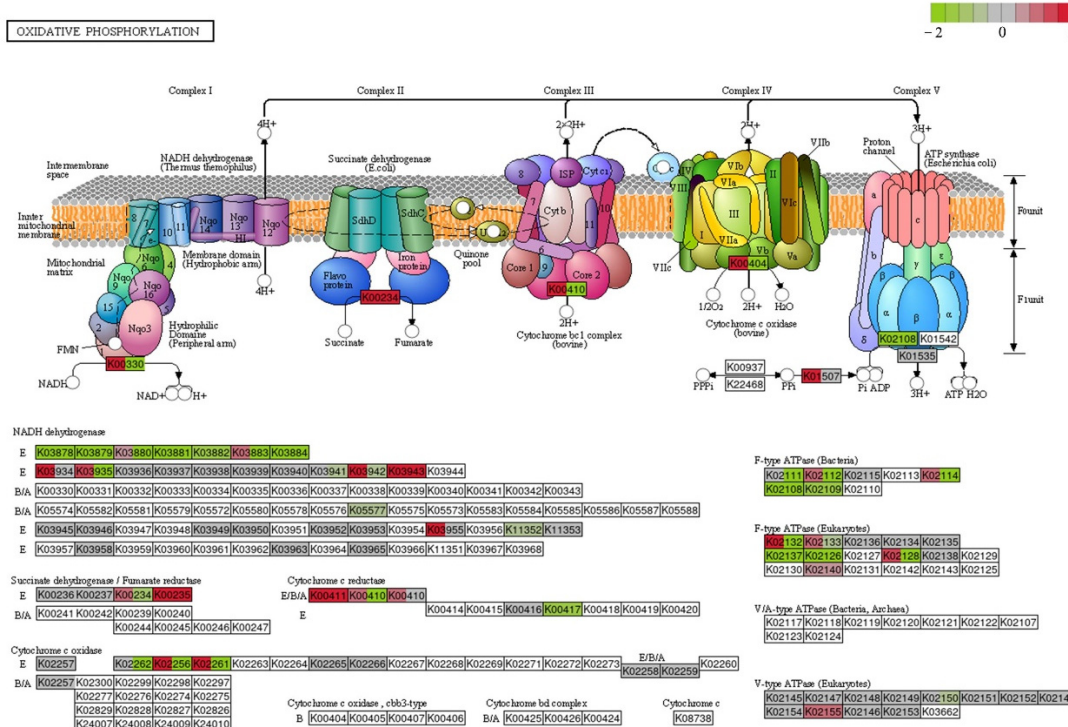


Figure S3. KEGG-annotated changes in gene expression for photosynthesis pathways in the LD group (red square: compared with CK, significantly up-regulated in LD; green square: compared with CK, significantly down-regulated in LD).



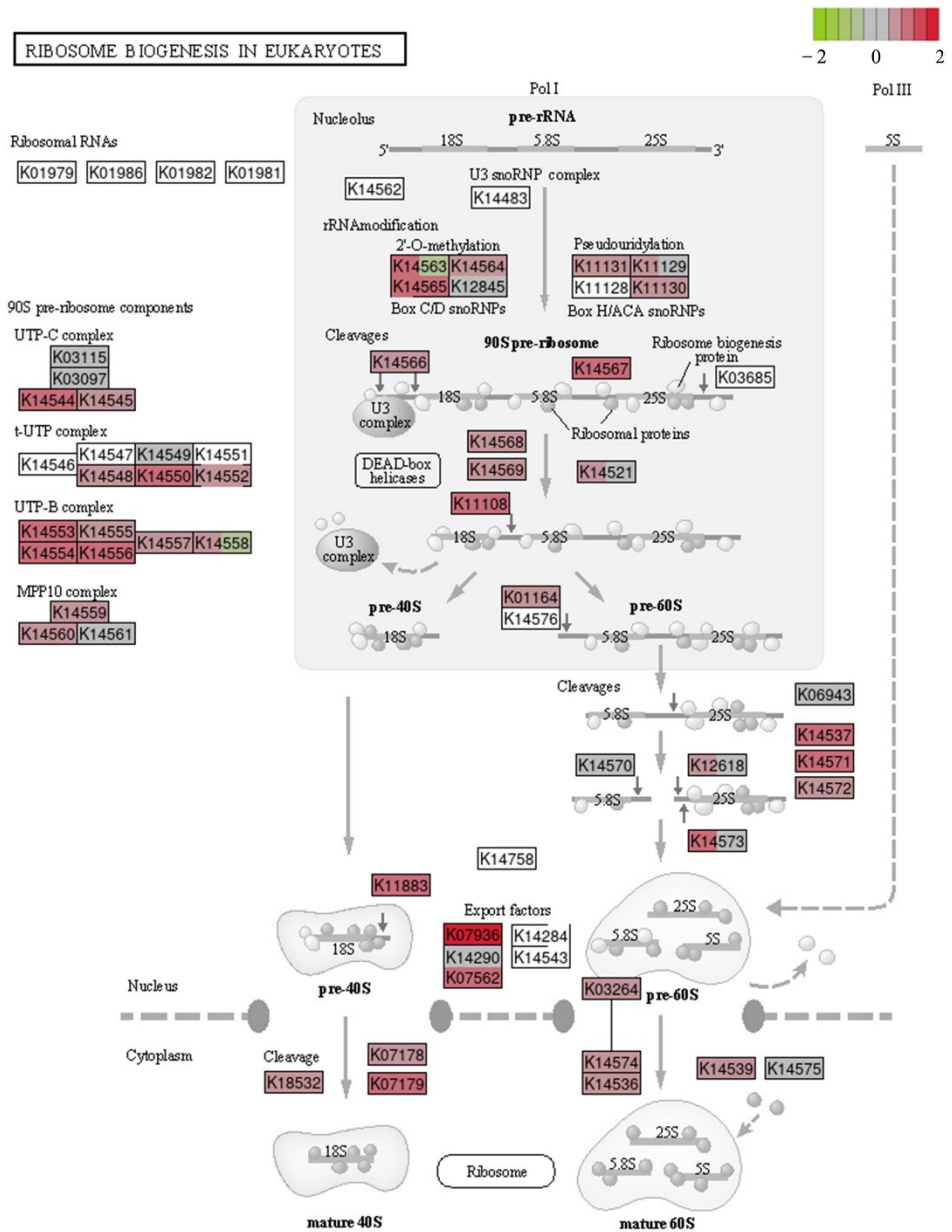


Figure S5. KEGG-annotated changes in gene expression for ribosome biogenesis in eukaryotes pathways in the LD group (red square: compared with CK, significantly up-regulated in LD; green square: compared with CK, significantly down-regulated in LD).

Metabolic map of chlorophyll metabolism in *Arabidopsis thaliana*. The map illustrates the biosynthetic pathways for various chlorophylls, including Chlorophyll a, Chlorophyll b, and Chlorophyll c. Key intermediates include Protochlorophyllide, Prochlorophyllide, and various chlorophyll precursors. The map is color-coded by gene family, with a legend at the top right showing a color scale from -2 (green) to 2 (red).

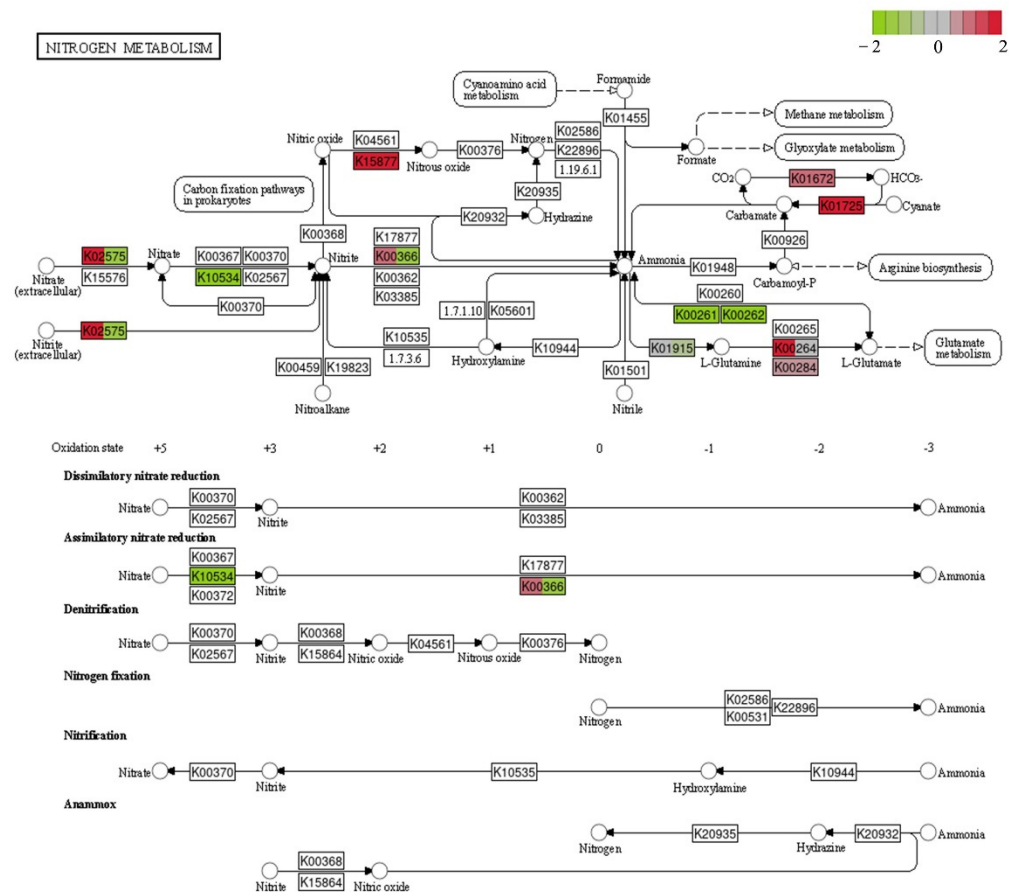


Figure S9. KEGG-annotated changes in gene expression for nitrogen metabolism pathways in the MD group (red square: compared with CK, significantly up-regulated in MD; green square: compared with CK, significantly down-regulated in MD).

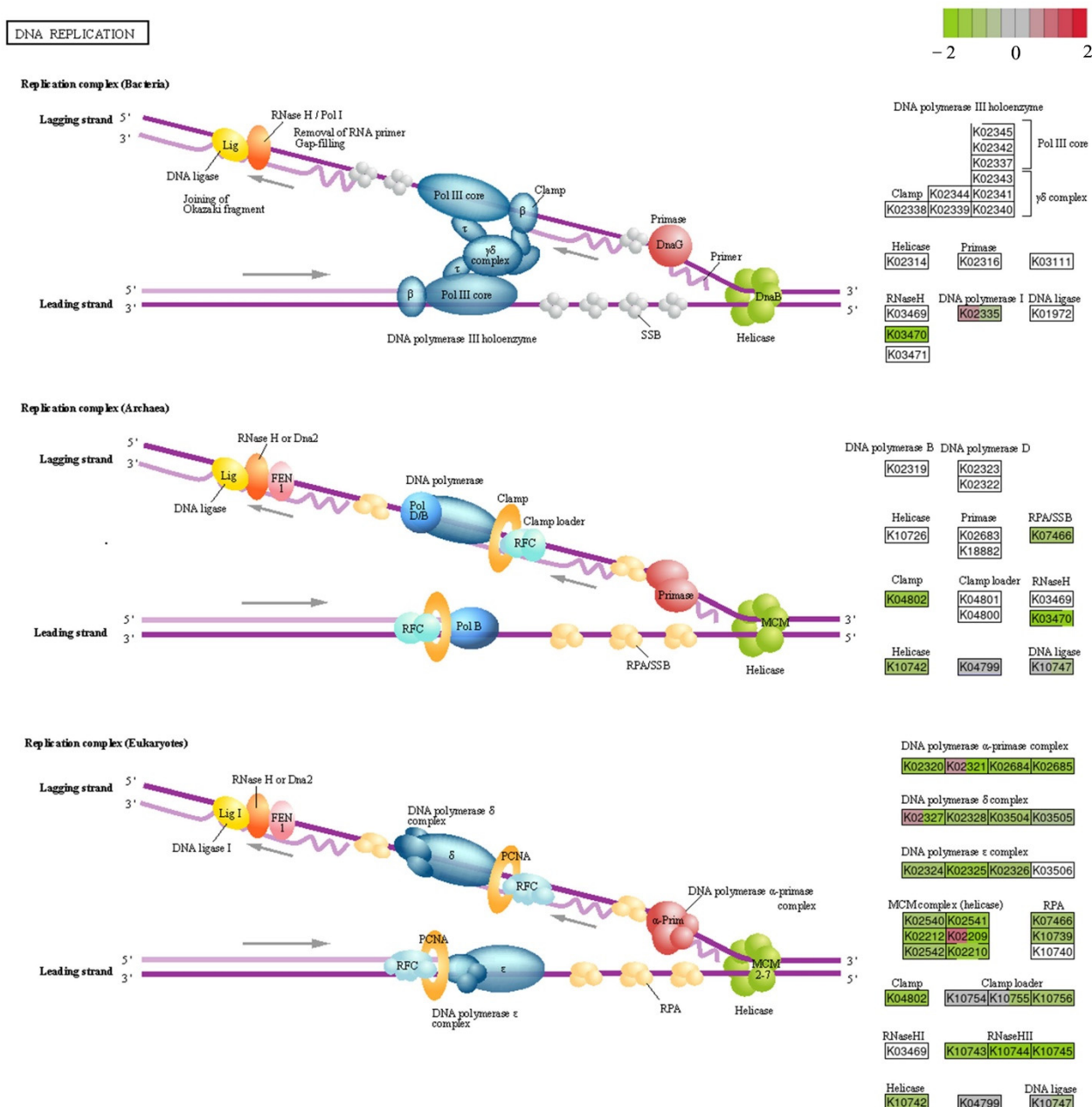


Figure S10. KEGG-annotated changes in gene expression for DNA replication pathways in the MD group (red square: compared with CK, significantly up-regulated in MD; green square: compared with CK, significantly down-regulated in MD).

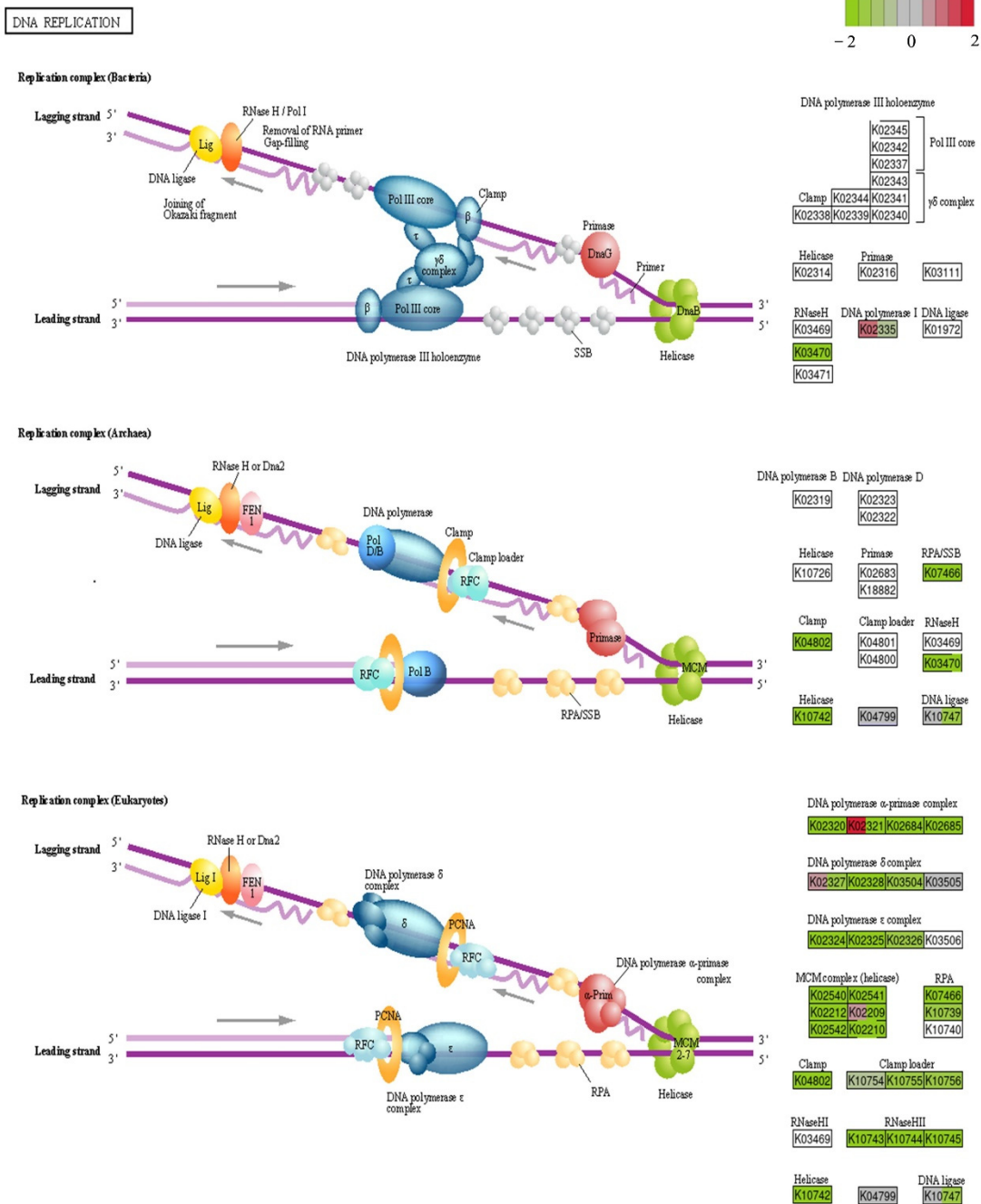


Figure S11. KEGG-annotated changes in gene expression for DNA replication pathways in the HD group (red square: compared with CK, significantly up-regulated in HD; green square: compared with CK, significantly down-regulated in HD).

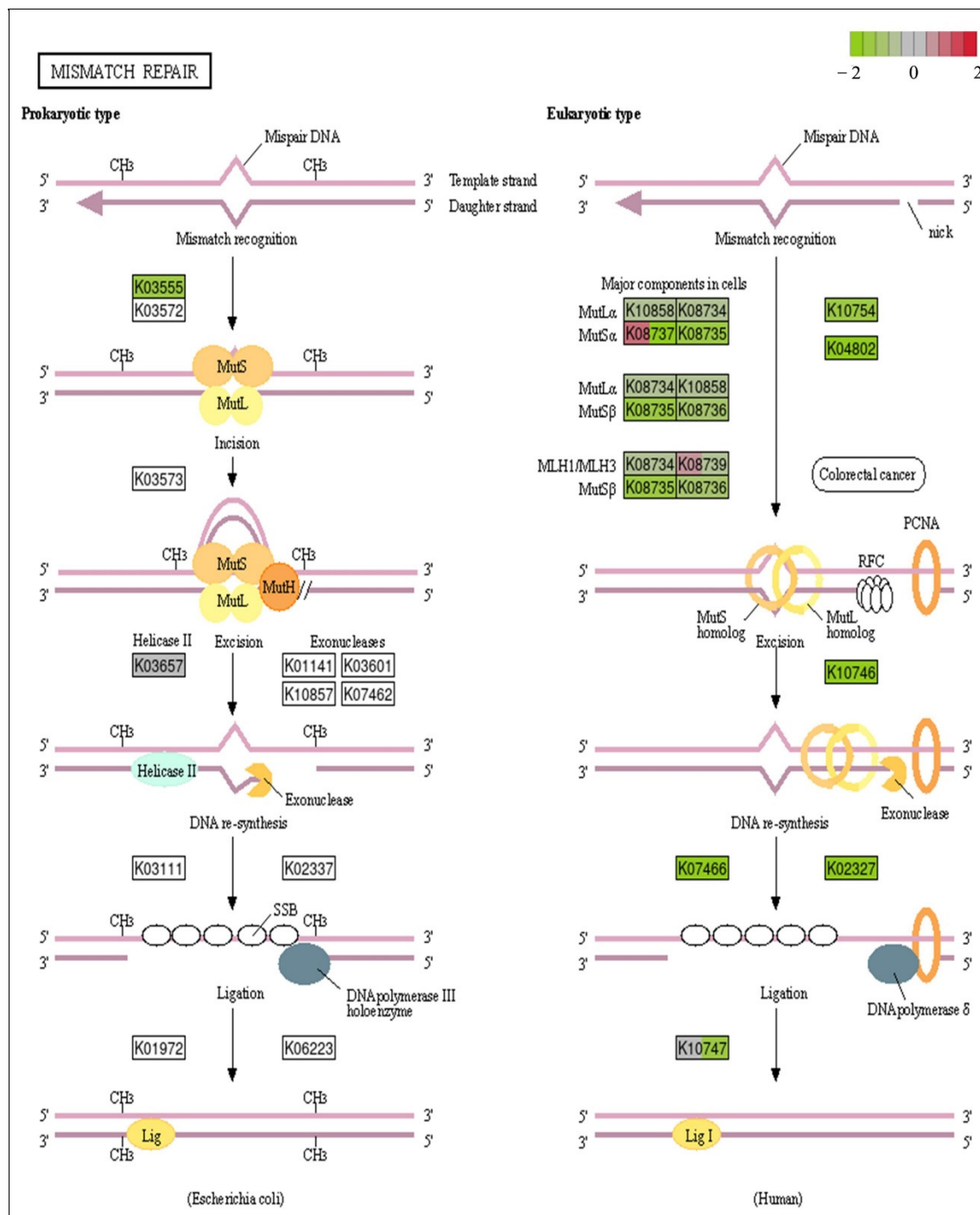


Figure S12. KEGG-annotated changes in gene expression for mismatch repair pathways in the HD group (red square: compared with CK, significantly up-regulated in HD; green square: compared with CK, significantly down-regulated in HD).

