



Supplementary Material

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

Annexed Table

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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/CS ₀	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
Ribosome biogenesis in eukaryotes	<i>por</i>	K02956	1.17	0.00004	Pyruvate:ferredoxin (flavodoxin) oxidoreductase
	<i>Kin14n</i>	K14565	-1.45	0.00067	P-loop containing nucleoside triphosphate hydrolase
Nitrogen metabolism	<i>nita</i>	K10534	-2.96	0.00001	Nitrate reductase
	<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

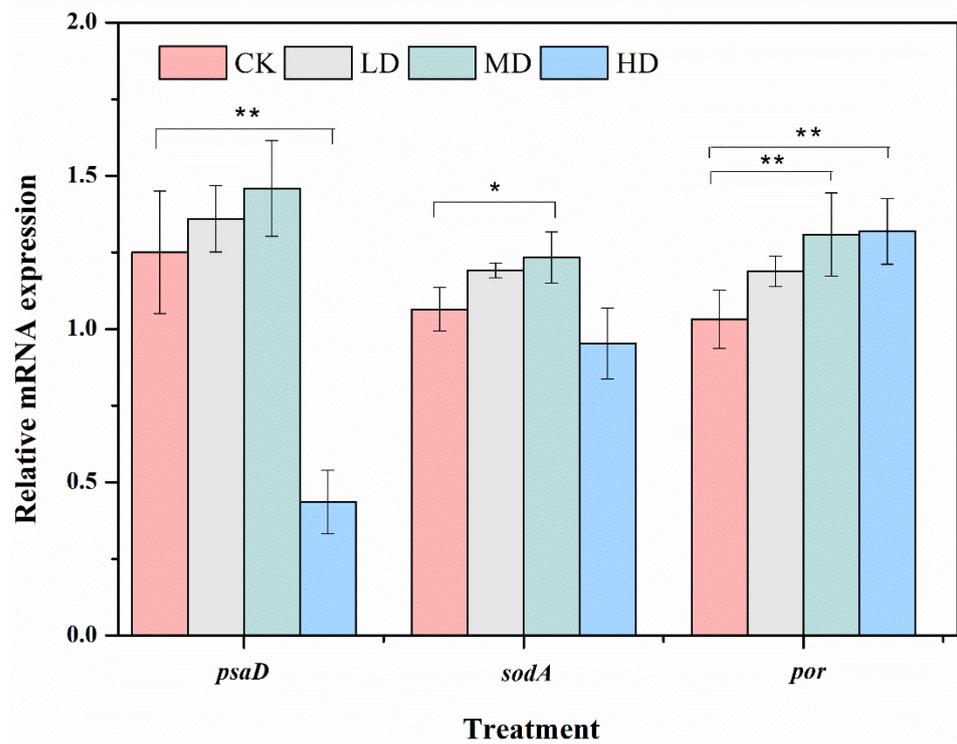


Figure S1. The transcript abundance of genes related to *psaD*, *sodA*, and *por* in cultures of *C. pyrenoidosa* exposed to 17 α -EE₂. Error bars indicate mean \pm SE. (*) and (**) represent statistically significant differences with respect to the values of control cultures at $p < 0.05$ and at $p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

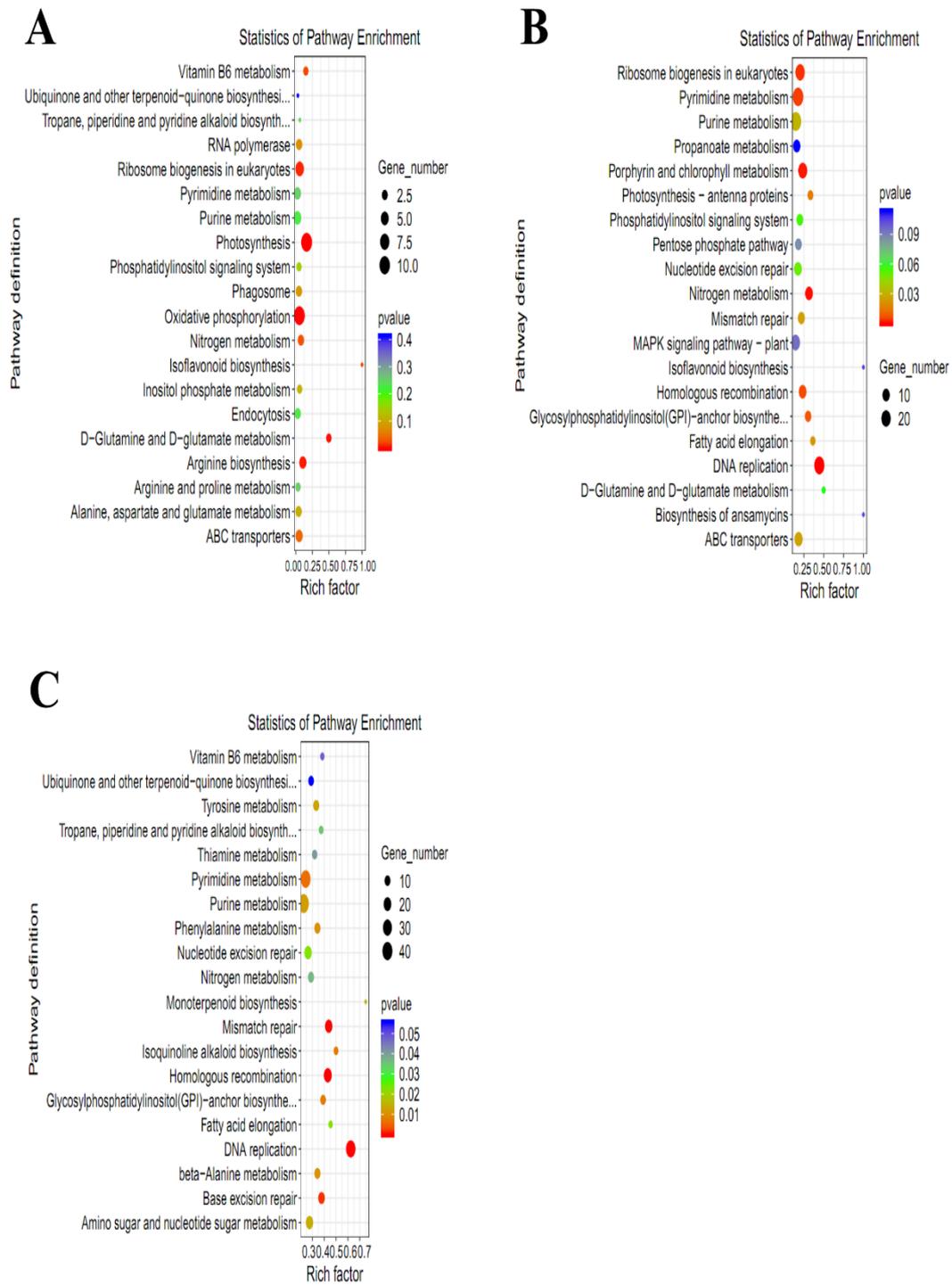


Figure S2. Scatter plots of KEGG enrichment pathway in the LD (A), MD (B), and HD (C) groups.

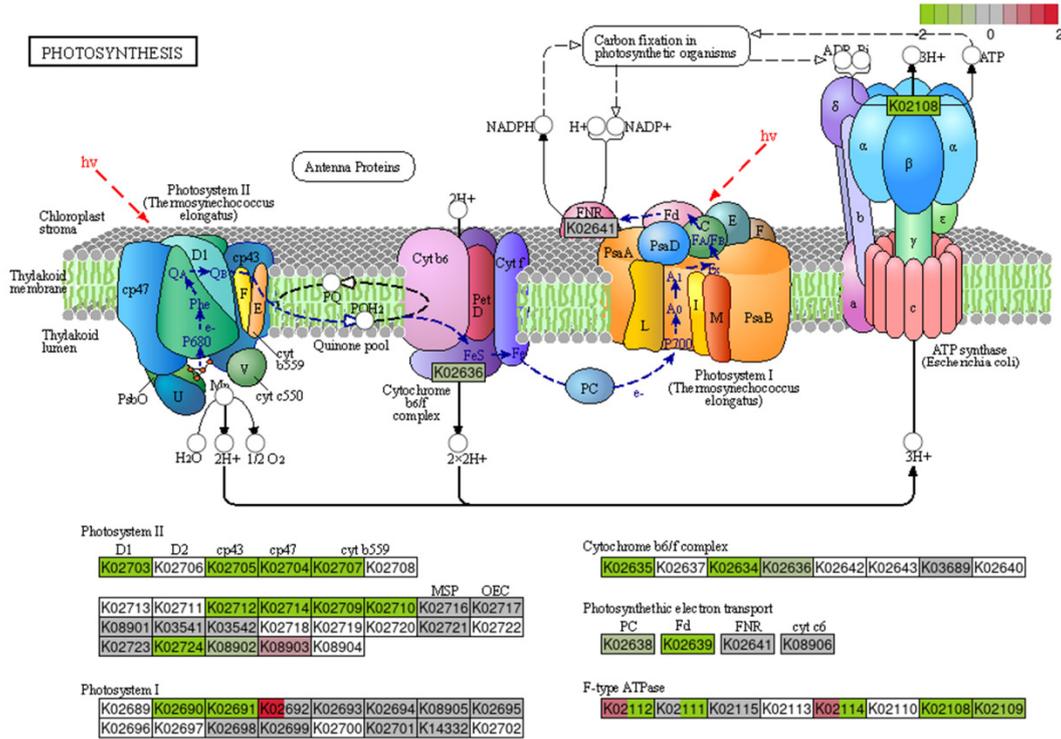


Figure S3. KEGG-annotated changes in gene expression for photosynthesis pathways in the LD group (red square: compared with CK, significantly up-regulated in LD; green square: compared with CK, significantly down-regulated in LD).

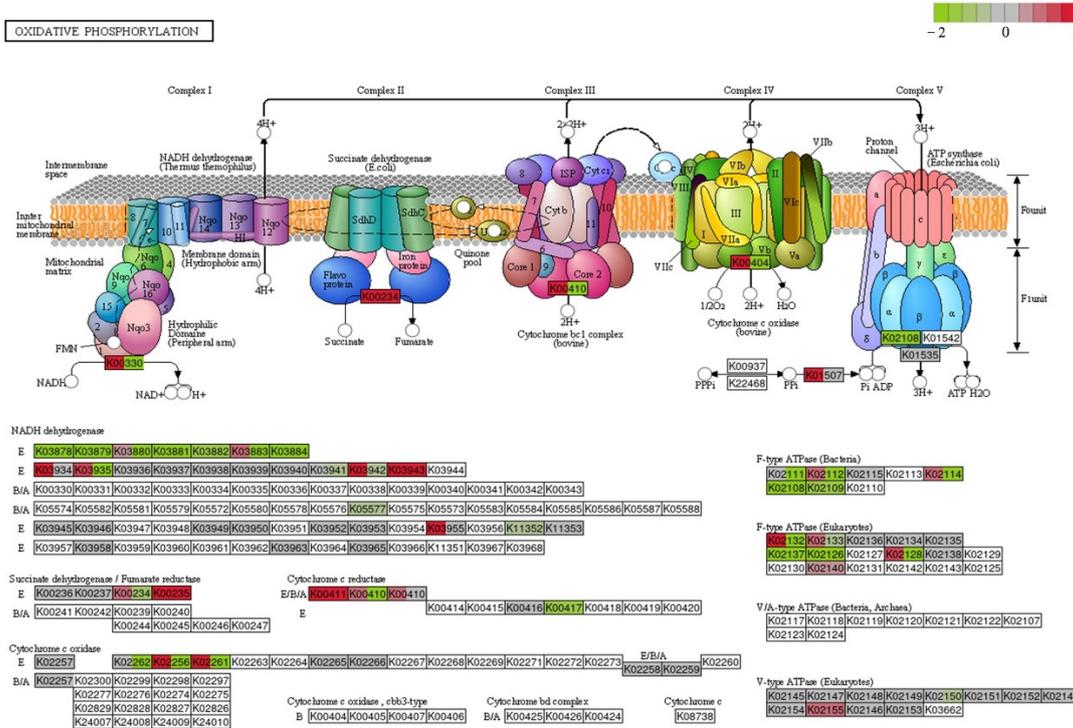


Figure S4. KEGG-annotated changes in gene expression for oxidative phosphorylation pathways in the LD group (red square: compared with CK, significantly up-regulated in LD; green square: compared with CK, significantly down-regulated in LD).

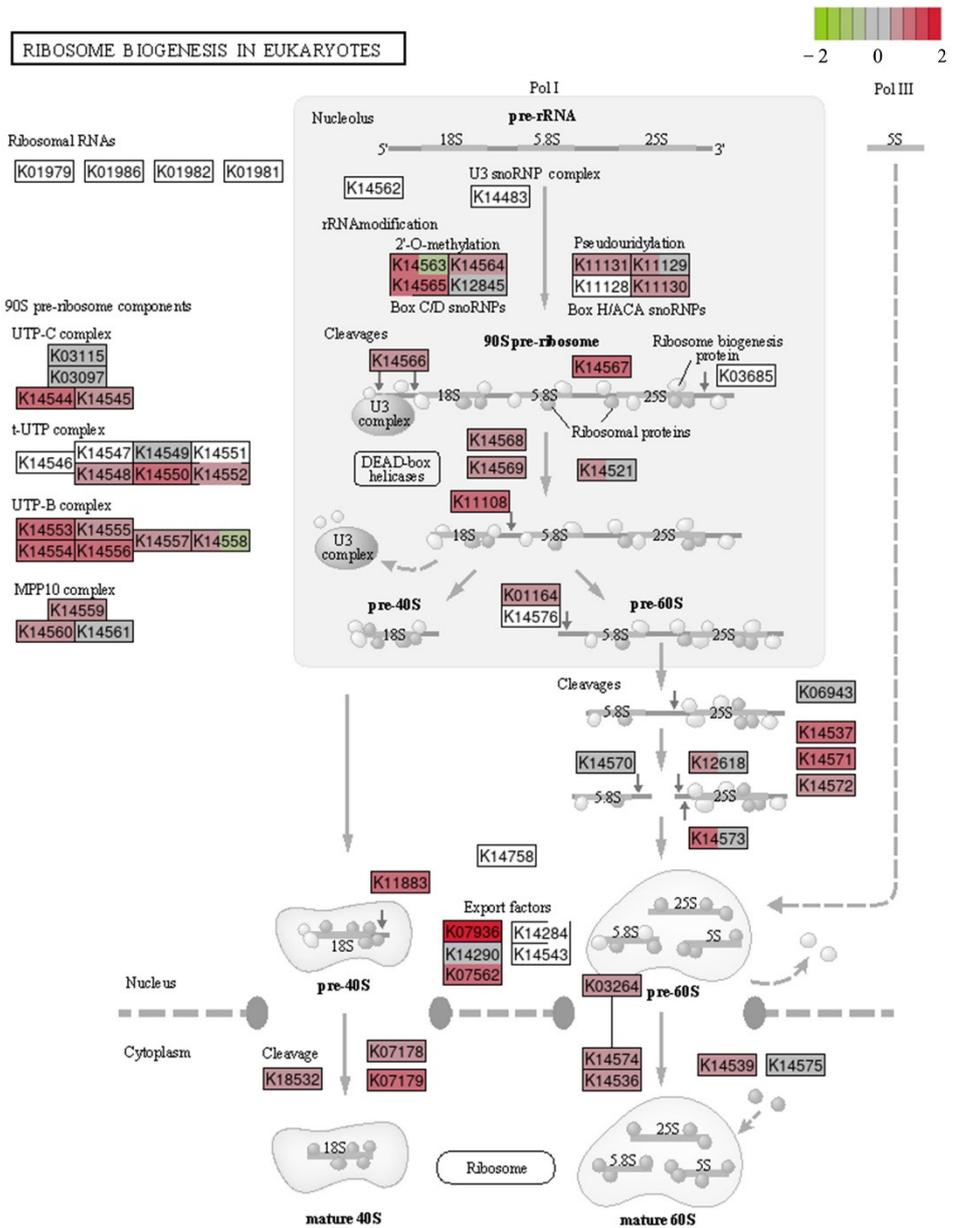
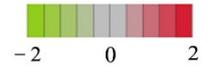
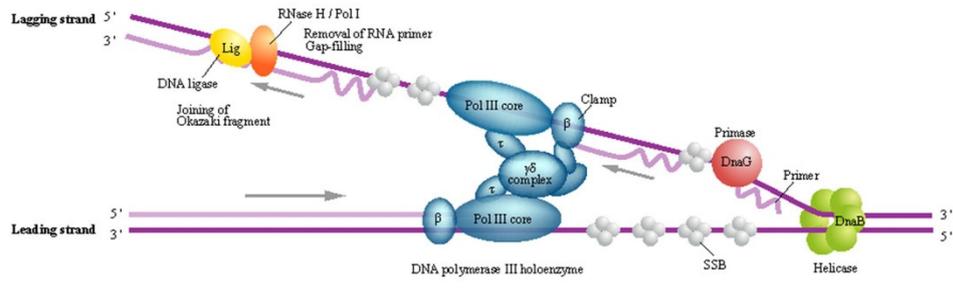


Figure S5. KEGG-annotated changes in gene expression for ribosome biogenesis in eukaryotes pathways in the LD group (red square: compared with CK, significantly up-regulated in LD; green square: compared with CK, significantly down-regulated in LD).

DNA REPLICATION

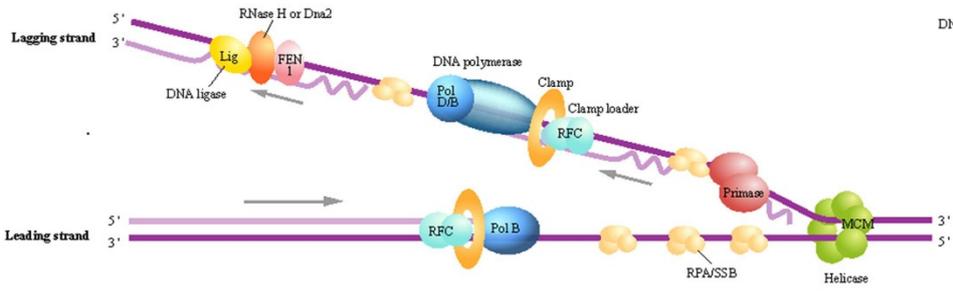


Replication complex (Bacteria)



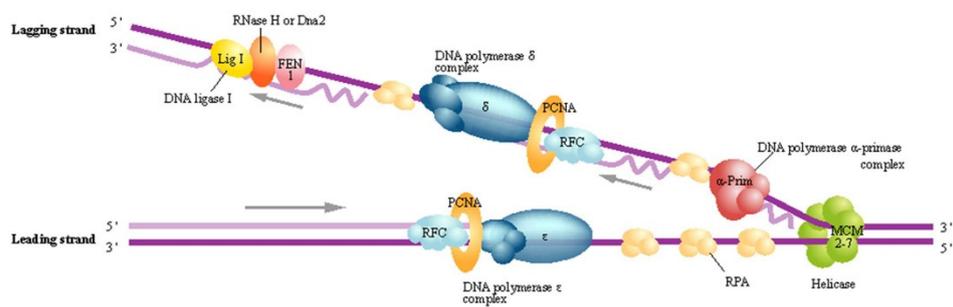
DNA polymerase III holoenzyme					
	K02345	Pol III core			
	K02342				
	K02337				
	K02343				
Clamp	K02344	K02341	γδ complex		
	K02338	K02339		K02340	
Helicase	K02314	Primase	K02316	K03111	
RNaseH	K03469	DNA polymerase I	K02335	DNA ligase	K01972
	K03470				
	K03471				

Replication complex (Archaea)



DNA polymerase B		DNA polymerase D			
	K02319	K02323			
		K02322			
Helicase	K10726	Primase	K02683	RPA/SSB	K07466
		K18882			
Clamp	K04802	Clamp loader	K04801	RNaseH	K03469
			K04800		K03470
Helicase	K10742		K04799	DNA ligase	K10747

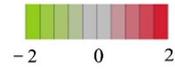
Replication complex (Eukaryotes)



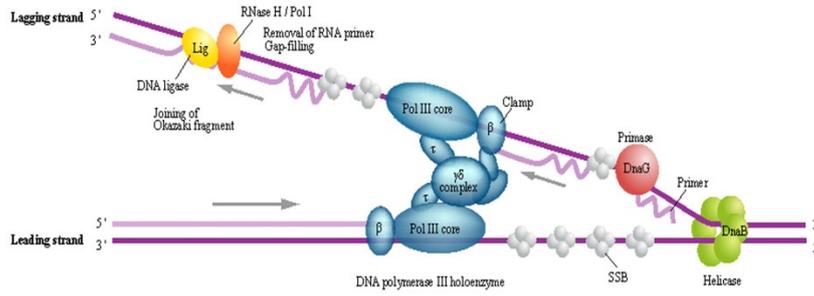
DNA polymerase α-primase complex					
	K02320	K02321	K02684	K02685	
DNA polymerase δ complex					
	K02327	K02328	K03504	K03505	
DNA polymerase ε complex					
	K02324	K02325	K02326	K03506	
MCM complex (helicase)		RPA			
	K02540	K02541	K07466		
	K02212	K02209	K10739		
	K02542	K02210	K10740		
Clamp	K04802	Clamp loader			
		K10754	K10755	K10756	
RNaseHI	K03469	RNaseHII			
		K10743	K10744	K10745	
Helicase	K10742		K04799	DNA ligase	K10747

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).

DNA REPLICATION

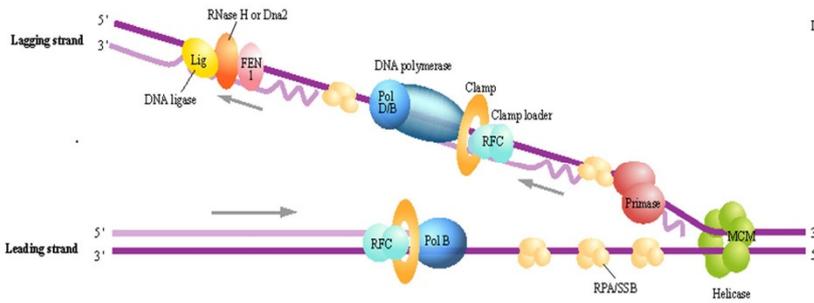


Replication complex (Bacteria)



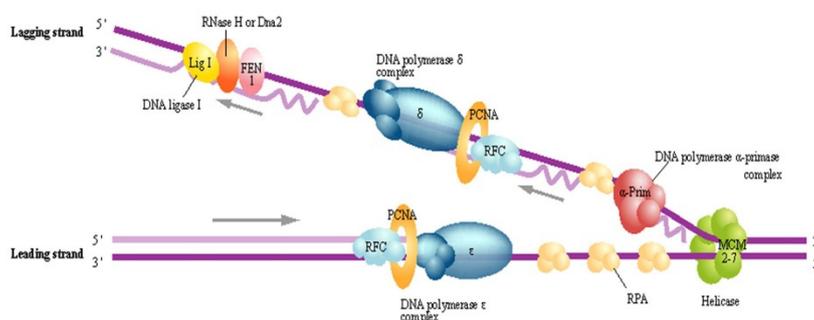
DNA polymerase III holoenzyme			
	K02345	Pol III core	
	K02342		
	K02337		
	K02343		
Clamp	K02344	K02341	β ₈ complex
	K02338	K02339	
Helicase	K02314	Primase	K03111
	K02316		
RNaseH	K03469	DNA polymerase I	K01972
	K02335	DNA ligase	
	K03470		
	K03471		

Replication complex (Archaea)



DNA polymerase B		DNA polymerase D	
	K02319	K02323	
		K02322	
Helicase	K10726	Primase	RPA/SSB
		K02683	K07466
		K18882	
Clamp	K04802	Clamp loader	RNaseH
		K04801	K03469
		K04800	K03470
Helicase	K10742		DNA ligase
		K04799	K10747

Replication complex (Eukaryotes)



DNA polymerase α-primase complex			
	K02320	K02321	K02684
			K02685
DNA polymerase δ complex			
	K02327	K02328	K03504
			K03505
DNA polymerase ε complex			
	K02324	K02325	K02326
			K03506
MCM complex (helicase)		RPA	
	K02540	K02541	K07466
	K02212	K02209	K10739
	K02542	K02210	K10740
Clamp		Clamp loader	
	K04802	K10754	K10755
		K10756	K10756
RNaseHI		RNaseHII	
	K03469	K10743	K10744
		K10745	K10745
Helicase		DNA ligase	
	K10742	K04799	K10747

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).

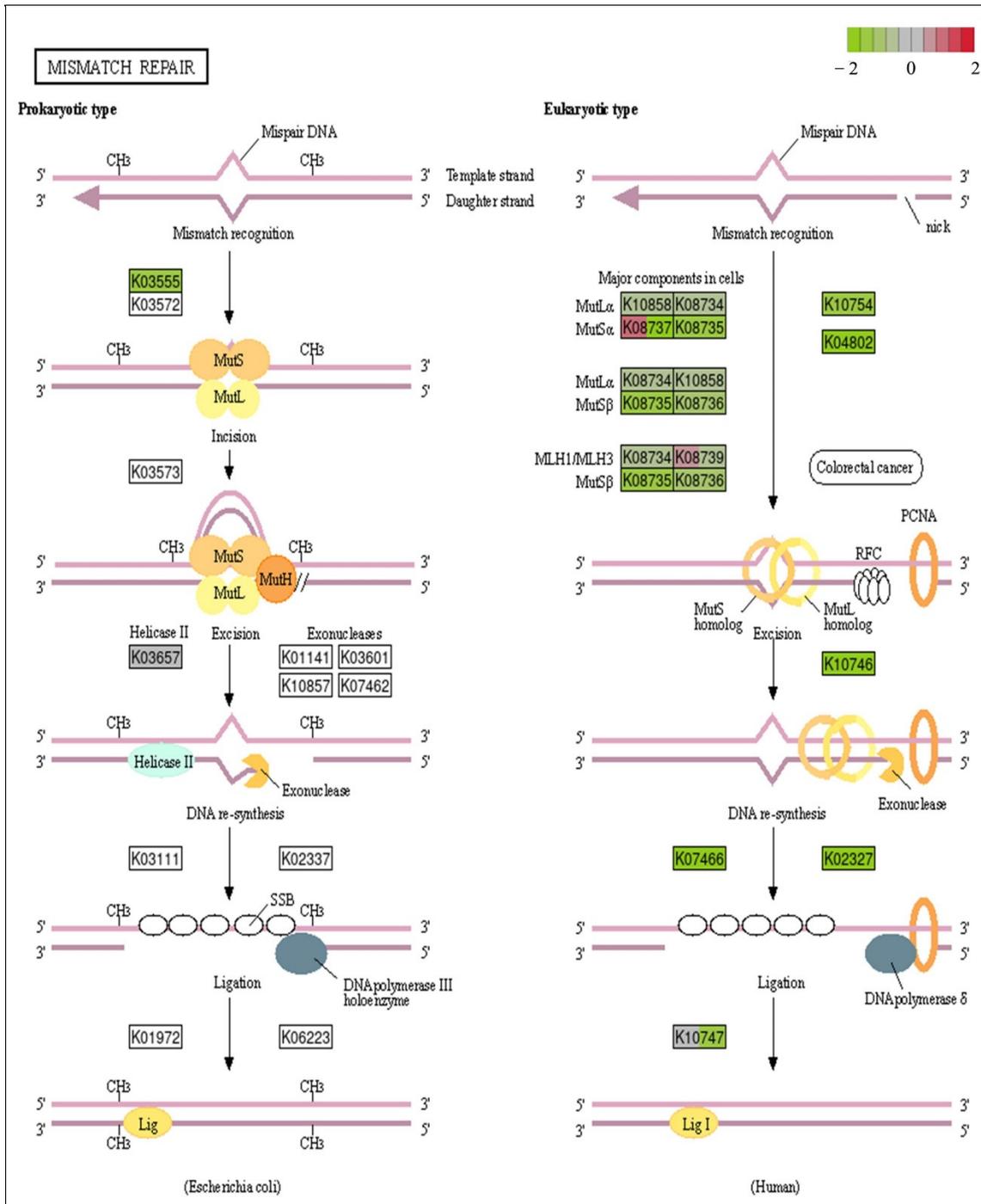


Figure S12. KEGG-annotated changes in gene expression for mismatch repair pathways in the HD group (red square: compared with CK, significantly up-regulated in HD; green square: compared with CK, significantly down-regulated in HD).

