

Supplementary Information

Transcriptomic changes with increasing algal symbiont reveal the detailed process underlying establishment of coral-algal symbiosis

Ikuko Yuyama, Masakazu Ishikawa, Masafumi Nozawa, Masa-aki Yoshida, Kazuho Ikeo

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Electronic supplementary material

- Table. S2. List of top 100 positive slope genes in the group with highly correlation coefficient ($R>0.90$) on relationship between algal density (cells/polyp) and gene expression (FPKM) of D-corals (apo, 10 days, 20 days).
- Table. S3. List of top 100 negative slope genes in the group with highly correlation coefficient ($R<-0.90$) on relationship between algal density (cells/polyp) and gene expression (FPKM) of clade D symbiosis polyp (apo, 10 days, 20 days).
- supplementary_csv. The results of blastx search of each contig against UniProtKB, and FPKM values of each contigs for all 12 samples
 (apo_10d_1, apo_10d_2: aposymbiotic corals replicate 1 and 2 at day 10,
 apo_20d_1, apo_20d_1: aposymbiotic corals replicate 1 and 2 at day 20,
 c_10d_1, c_10d_2: C-corals replicate 1 and 2 at day 10,
 c_20d_1, c_20d_2: C-corals replicate 1 and 2 at day 20,
 d_10d_1, d_10d_2: D-corals replicate 1 and 2 at day 10,
 d_20d_1, d_20d_2: D-corals replicate 1 and 2 at day 20).

I. SI Materials and Methods

1.1 Blastx search, GO enrichment analysis, and KEGG pathway analysis of DEGs

The contigs containing candidate coding sequences were BLASTX aligned against the NCBI nr protein database to evaluate sequence similarity to genes in other species with an E-value cutoff of 1e-04. To identify enriched functional gene groups and pathways, GO enrichment analysis was performed using the database for annotation, visualization, and integrated discovery (DAVID). Significant processes were selected based on a corrected P-value using the method of Benjamini with a cutoff of 0.05. Pathway enrichment analysis was also conducted using KEGG.

1.2 Quantitative PCR

To verify the quantitative accuracy of RNA-seq data, we investigated the expression levels of selected DEGs using qPCR. Total RNA samples extracted from clade D-colonized corals and aposymbiotic corals after 20 days were used for qPCR (same RNA samples with RNA-seq). cDNA were synthesized from the 50 ng total RNA with SuperScript VILO MasterMix (Invitrogen, Carlsbad, CA). Primer were designed using primer3plus (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/>) for contigs which showed up-regulated in clade D infected polyp. The quantitateive qPCR was performed in triplicate using SYBR Premix Ex Taq II (Takara) and the mean values were calculated. The data were analyzed using an Chromo 4™ System (Bio-Rad, Hercules, CS, USA) with reaction conditions as follows: 95°C for 10 min, 40 cycles of 95°C for 15 sec and 60°C for 60 sec. A ten-fold serial dilution (1 to 0.0001 ng) was prepared and used to generate a standard curve. Each standard curve was generated by linear regression of the plotted points. Three putative housekeeping genes were tested for the stability of their expressions using Normfinder software (<http://moma.dk/normfinder-software>) (Andersen et el., 2004). Between aspoymbiotic corals and symbiotic corals, the most stable expression was noted for β-tubulin. The most stable gene, β-tubulin, was used to calculate the normalization factor for each of the cDNA samples.

Reference for SI Materials and Methods:

Andersen CL, Ledet-Jensen J, Ørntoft T (2004) Normalization of real-time quantitative RT-PCR data: a model based variance estimation approach to identify genes suited for normalization - applied to bladder- and colon-cancer datasets. Cancer Res. 64:5245-5250

II. Supplementary Figures

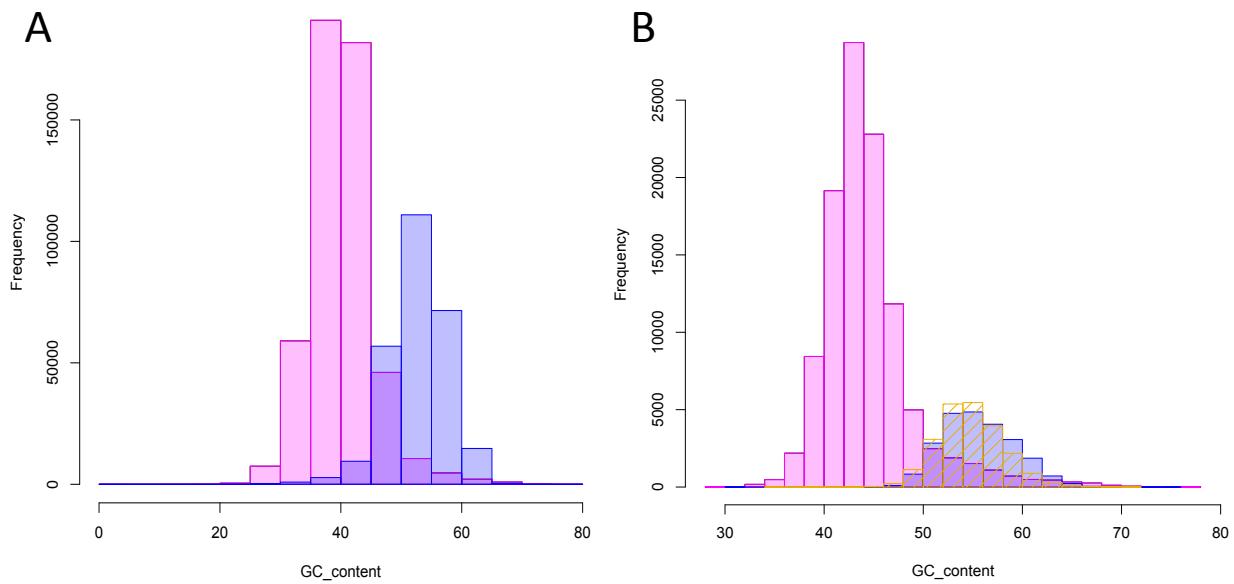
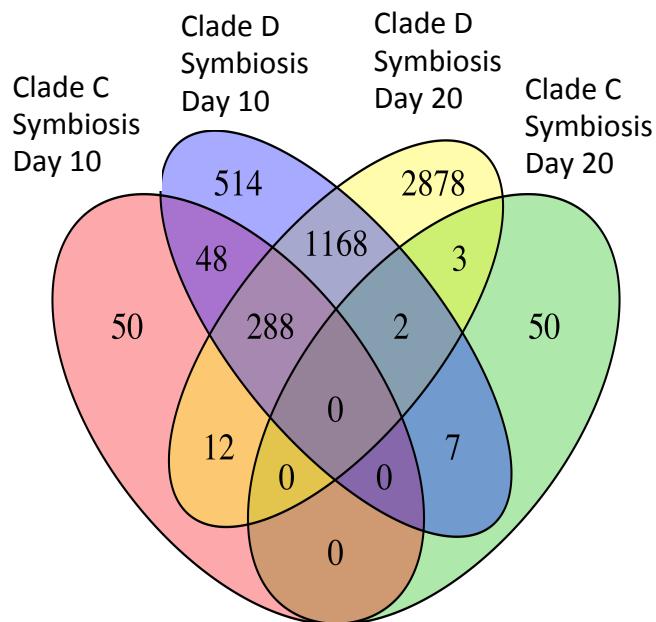


Fig. S1. The distribution of GC contents of *Acropora*, *Symbiodinium* gene dataset.

The distribution of GC contents of *Acropora*, *Symbiodinium* reference gene dataset (A), and GC contents of our assembled contigs from Illumina reads (B). Pink bars shows GC contents of *Acropora* data and blue r bars shows GC contents of *Symbiodinium* reference data. *Symbiodinium* dataset have a higher GC-contents in contrast to the *Acropora* dataset.

Up-regulated genes in symbiotic corals



Down-regulated genes in symbiotic corals

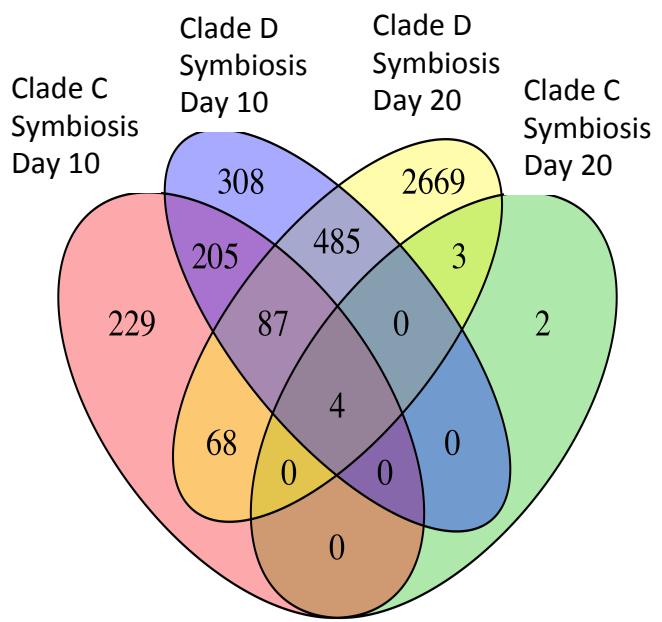


Fig. S2. Venn diagram showing overlap of differentially expressed coral genes as identified with RNA-seq.

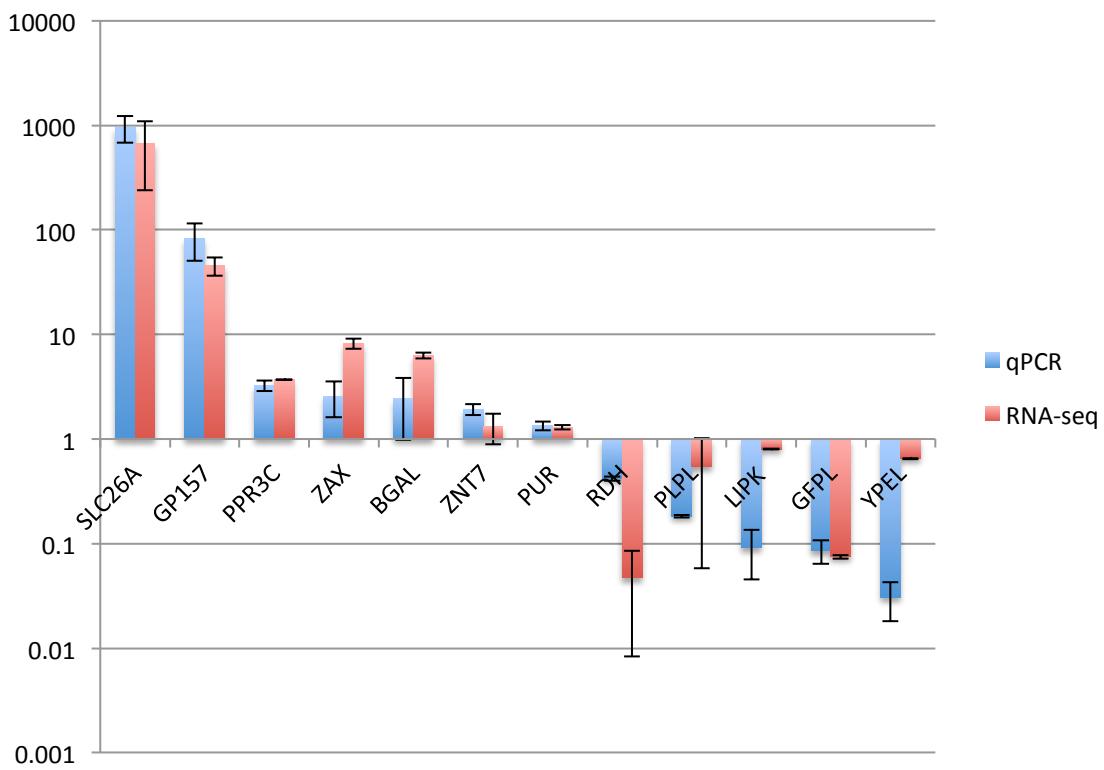


Fig. S3. Comparison of expression profiles obtained from RNA-Seq and qPCR analysis of the same samples.

Expression levels of 13 selected differentially expressed gene (DEG) identified by RNA-Seq was measured by qPCR . The average fold difference of each gene expression in corals associated with clade D as compared with aposymbiotic is presented for the average of 2 biological replicates in RNA-seq and three technical replicates in qPCR.

Regulation of immune system process (coral)

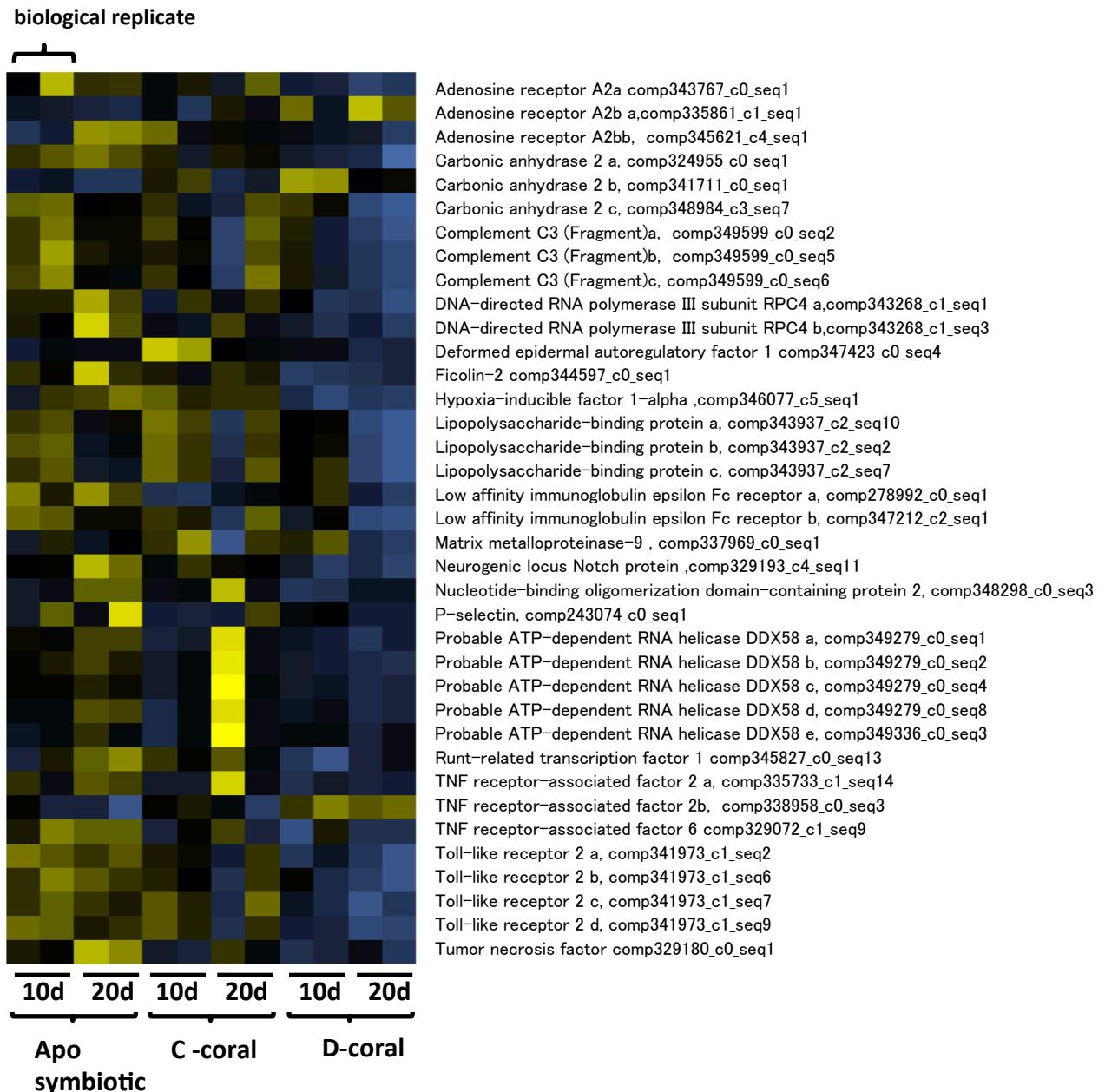
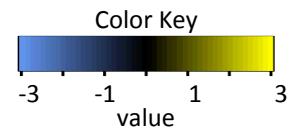


Fig. S4 . Heat map of the expression patterns of genes related to Regulation of immune system process.

Heat map shows the expression patterns of genes related to Regulation of immune system process in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. There are selected notable treatment-specific genes (FDR P < 0.05).

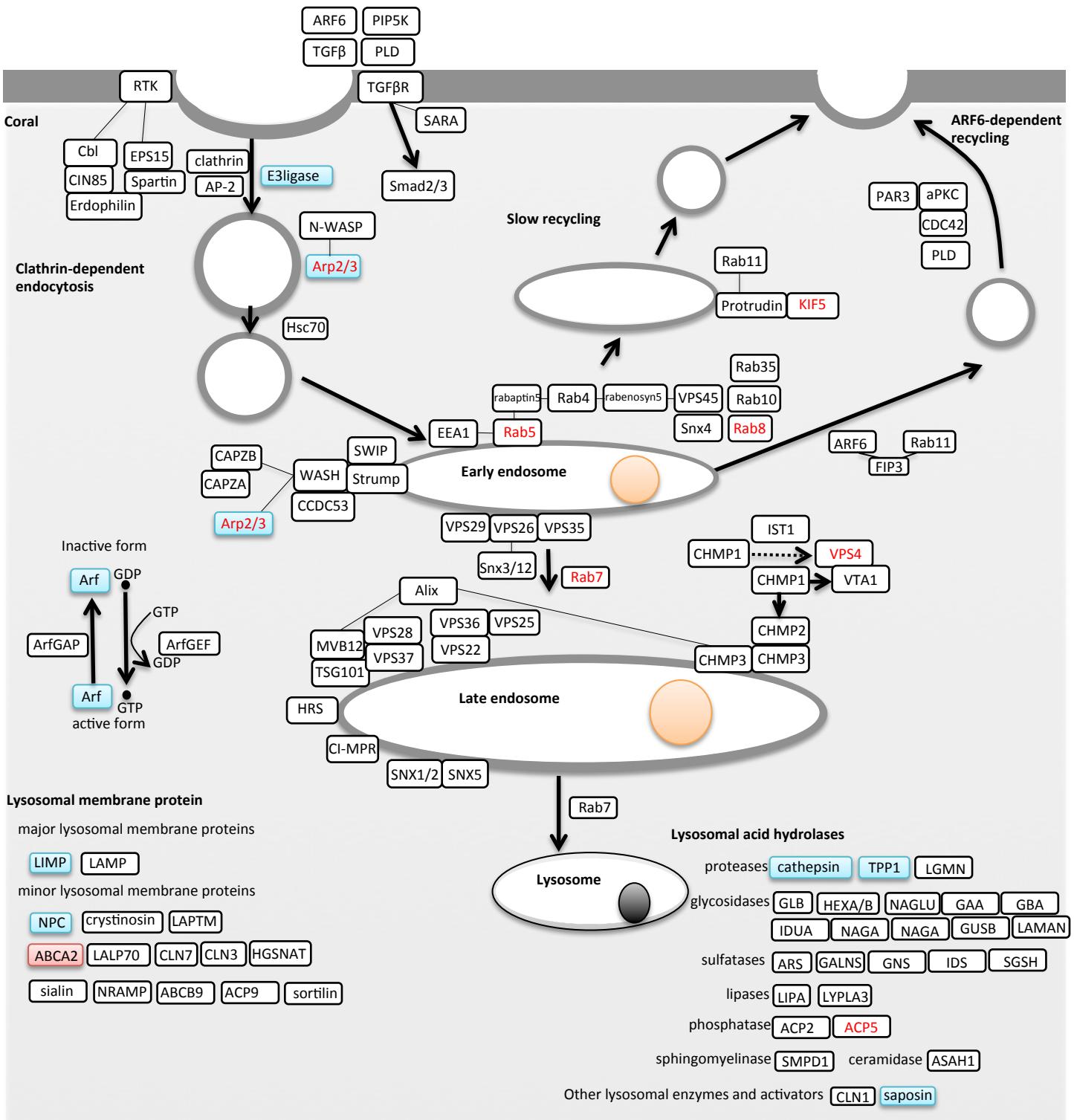


Fig. S5a. Gene expression patterns of endocytosis and lysosomal related genes in corals associated with clade C on 10days.

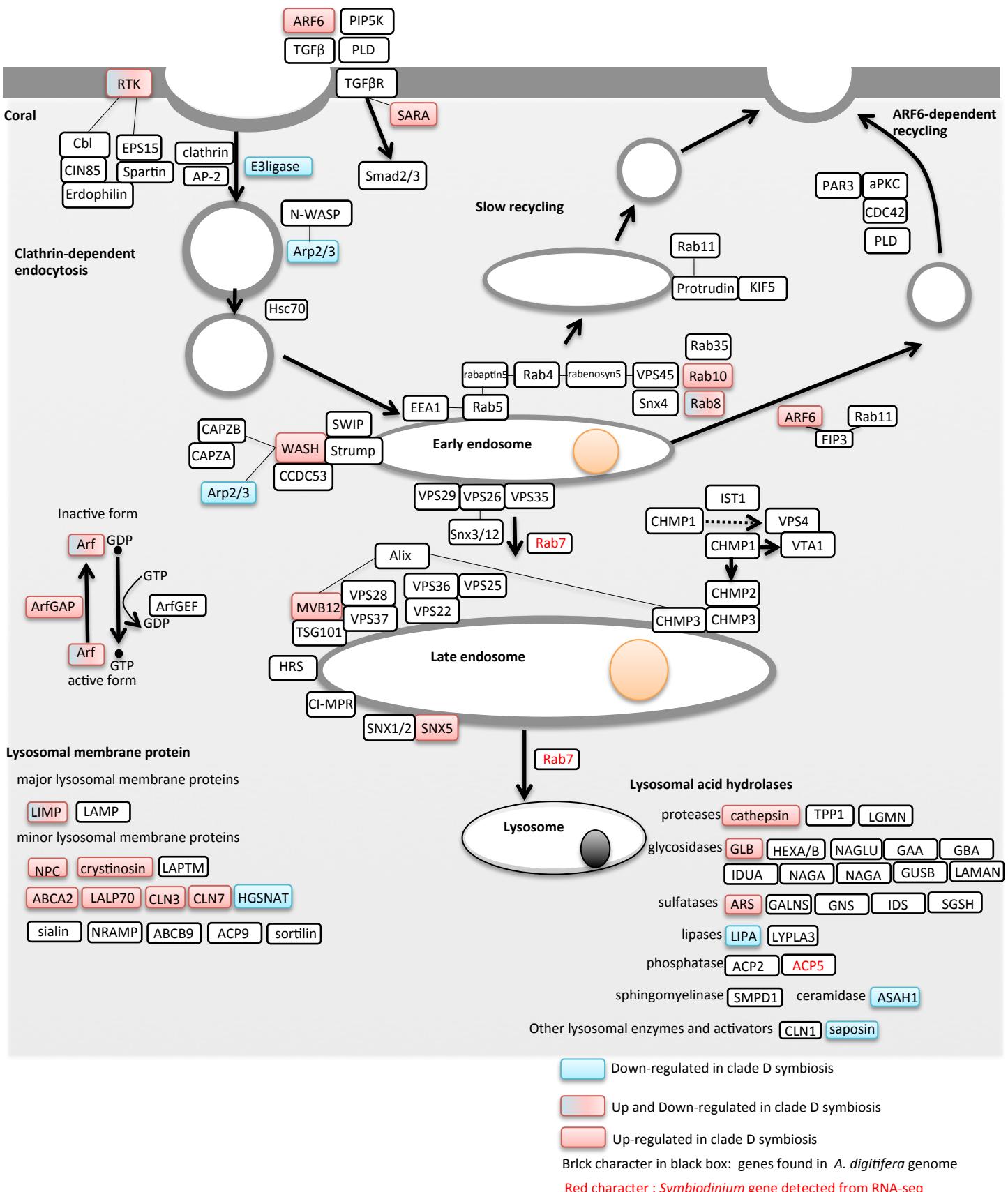


Fig. S5b. Gene expression patterns of endocytosis and lysosomal related genes in corals associated with clade D on 10days.

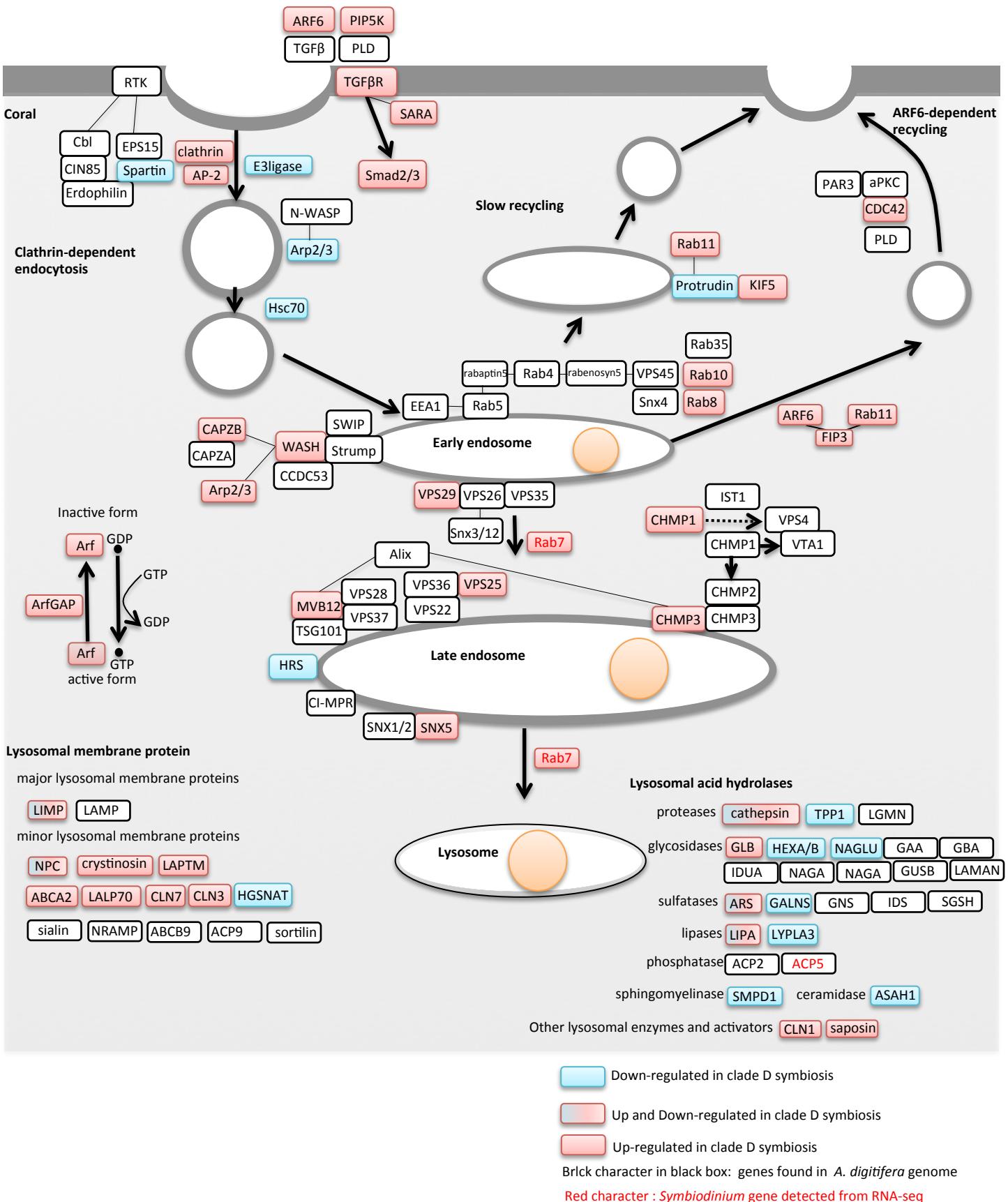


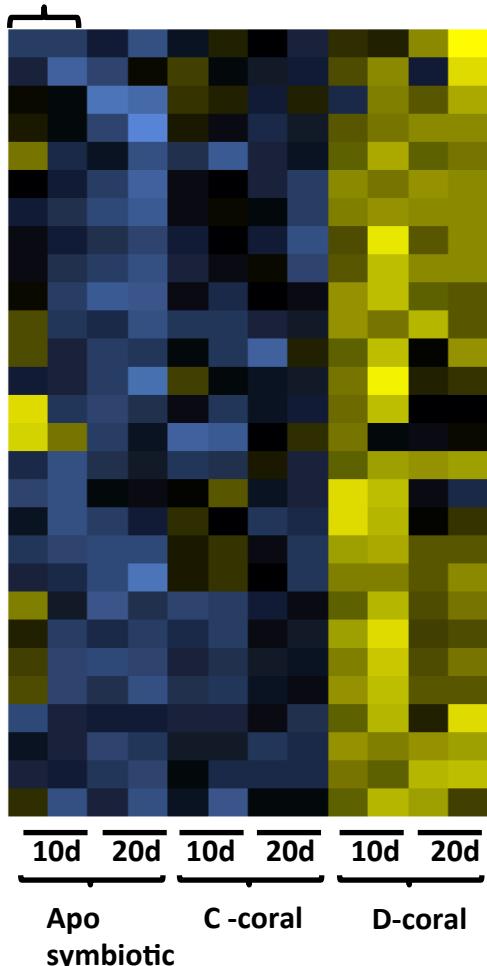
Fig. S5c. Gene expression patterns of endocytosis and lysosomal related genes in corals associated with clade D on 20days.

Endocytosis (coral/*Symbiodinium*)

coral



biological replicate



Rab-like protein 2A, comp348940_c0_seq1
 Ras-related protein Rab-10 a, comp349370_c0_seq5
 Ras-related protein Rab-10b, comp350830_c0_seq2
 Ras-related protein Rab-11A comp343064_c0_seq1
 Ras-related protein Rab-13 comp332502_c1_seq1
 Ras-related protein Rab-1A a, comp337990_c2_seq1
 Ras-related protein Rab-1A b, comp337990_c2_seq2
 Ras-related protein Rab-21 a, comp342289_c0_seq1
 Ras-related protein Rab-21 b, comp342289_c0_seq2
 Ras-related protein Rab-27A a, comp336390_c0_seq1
 Ras-related protein Rab-27A b, comp336390_c0_seq11
 Ras-related protein Rab-27A c, comp336390_c0_seq2
 Ras-related protein Rab-28 comp335784_c0_seq2
 Ras-related protein Rab-3 comp334754_c1_seq1
 Ras-related protein Rab-32B comp344147_c0_seq4
 Ras-related protein Rab-37 comp349925_c1_seq2
 Ras-related protein Rab-38 a, comp337445_c0_seq13
 Ras-related protein Rab-38 b, comp337445_c0_seq7
 Ras-related protein Rab-3A comp348880_c1_seq5
 Ras-related protein Rab-5C comp341905_c1_seq1
 Ras-related protein Rab-6A comp337469_c1_seq5
 Ras-related protein Rab-7a a, comp340746_c1_seq2
 Ras-related protein Rab-7a b, comp340746_c1_seq3
 Ras-related protein Rab-7a c, comp340746_c1_seq4
 Ras-related protein Rab-8A a, comp350121_c1_seq11
 Ras-related protein Rab-8A b, comp350121_c1_seq5
 Ras-related protein Rab-8A c, comp350121_c1_seq8
 Ras-related protein Rab-9A d, comp329377_c0_seq1

Fig. S6a. Heat map of the expression patterns of genes related to endocytosis.

Heat map shows the expression patterns of genes related to endocytosis in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. Genes whose expression were down-regulated were shown. There are selected notable treatment-specific genes (FDR P < 0.05).

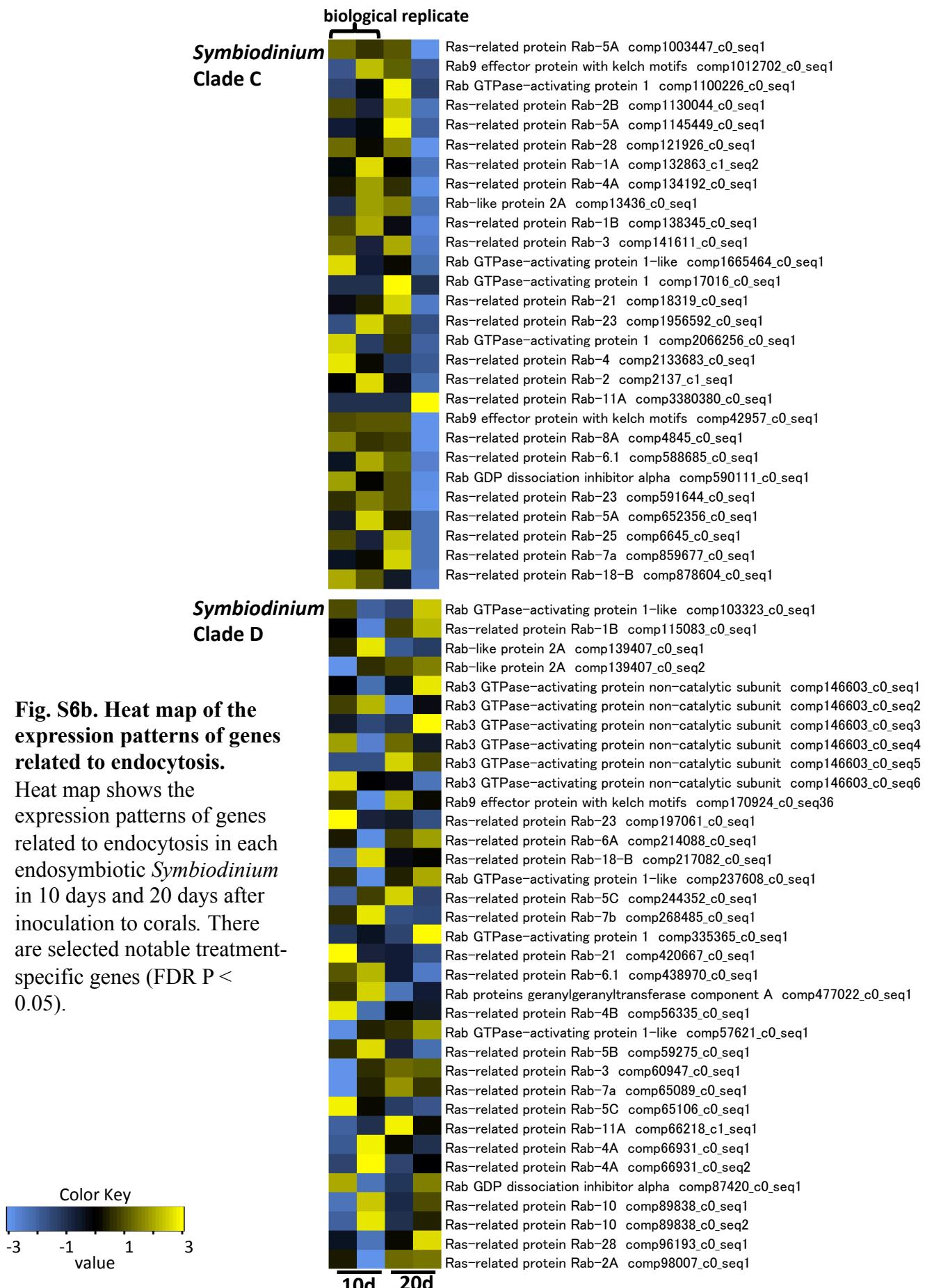


Fig. S6b. Heat map of the expression patterns of genes related to endocytosis.

Heat map shows the expression patterns of genes related to endocytosis in each endosymbiotic *Symbiodinium* in 10 days and 20 days after inoculation to corals. There are selected notable treatment-specific genes (FDR P < 0.05).

Lysosomal acid hydrolases (coral)



biological replicate

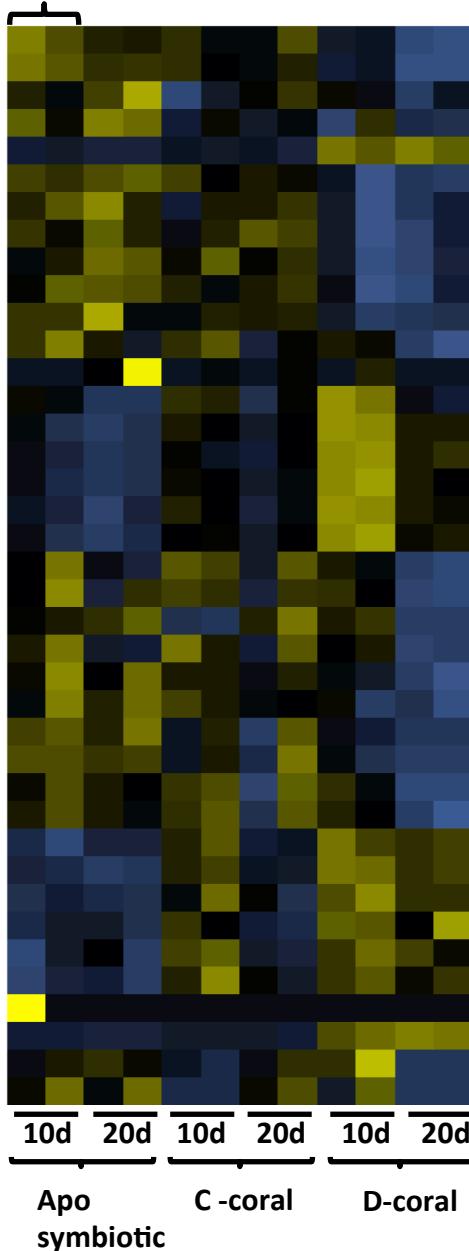


Fig. S7. Heat map of the expression patterns of genes related to lysosomal acid hydrolases.

Heat map shows the expression patterns of genes related to lysosomal acid hydrolases in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. Genes whose expression were down-regulated were shown. There are selected notable treatment-specific genes (FDR P < 0.05).



RNAi (*Symbiodinium*)

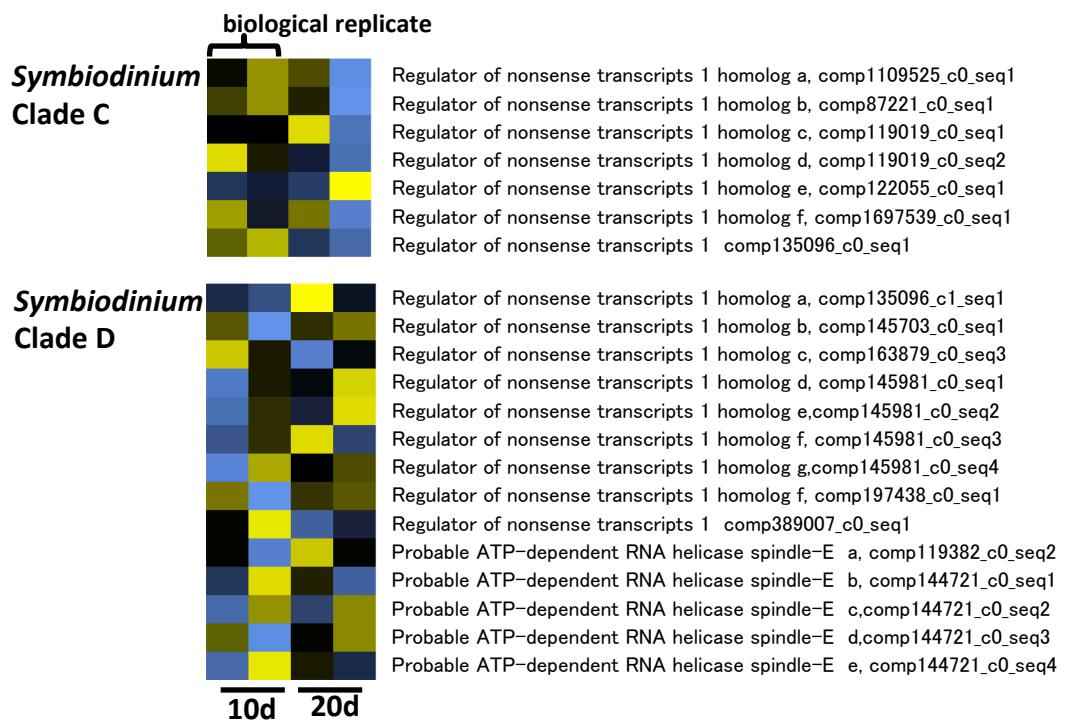


Fig. S8. Heat map of the expression patterns of genes related to RNAi.

Heat map shows the expression patterns of genes related to RNAi in each endosymbiotic *Symbiodinium* in 10 days and 20 days after inoculation to corals. There are selected notable treatment-specific genes (FDR P < 0.05).

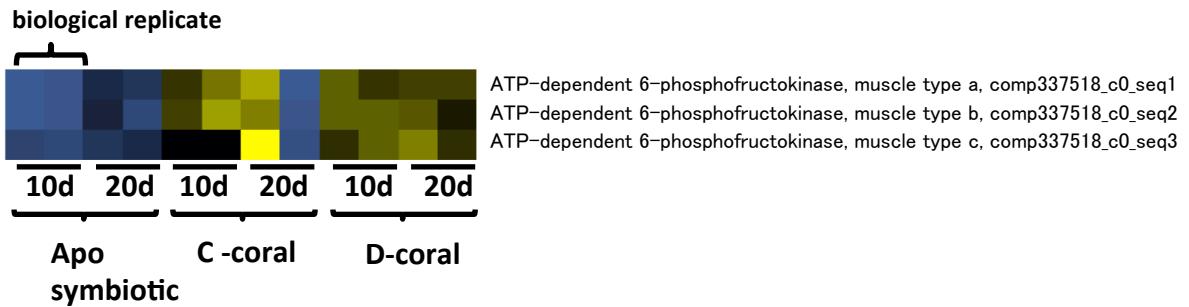


Fig. S9. Heat map of the expression patterns genes related to Glucose, Fluctose metabolic process.
 Heat map shows the expression patterns genes in in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. There are selected notable treatment-specific genes (FDR P < 0.05).

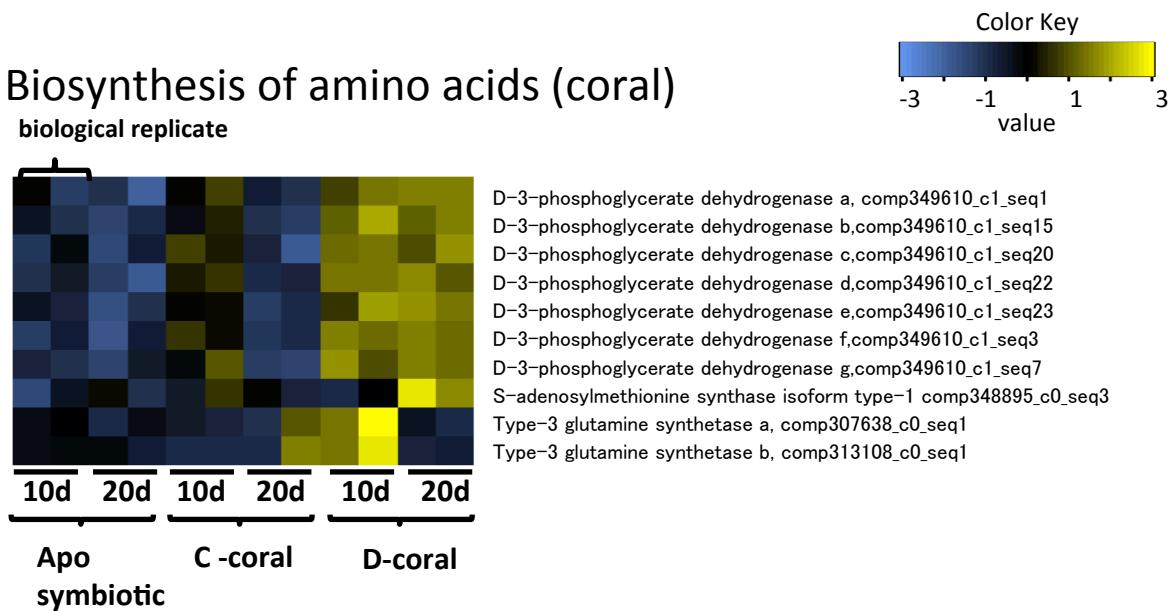


Fig. S10. Heat map of the expression patterns genes related to biosynthesis of amino acids.

Heat map shows the expression patterns genes related to biosynthesis of amino acids in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. There are selected notable treatment-specific genes (FDR P < 0.05).

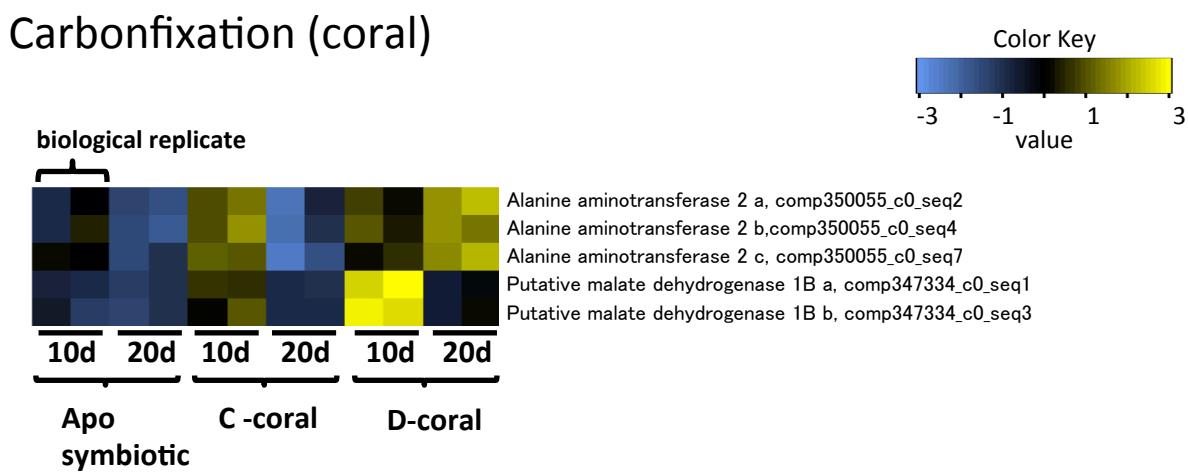


Fig. S11. Heat map of the expression patterns genes related to carbon fixation.

Heat map shows the expression patterns genes related to carbon fixation in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. There are selected notable treatment-specific genes (FDR P < 0.05).

Pentose phosphate pathway



biological replicate

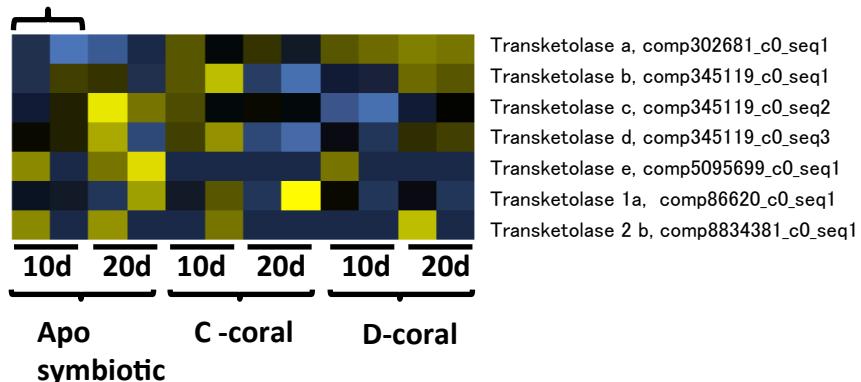


Fig. S12. Heat map of the expression patterns genes related to Pentose phosphate pathway.

Heat map shows the expression patterns genes related to pentose phosphate pathway in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. There are selected notable treatment-specific genes (FDR P < 0.05).

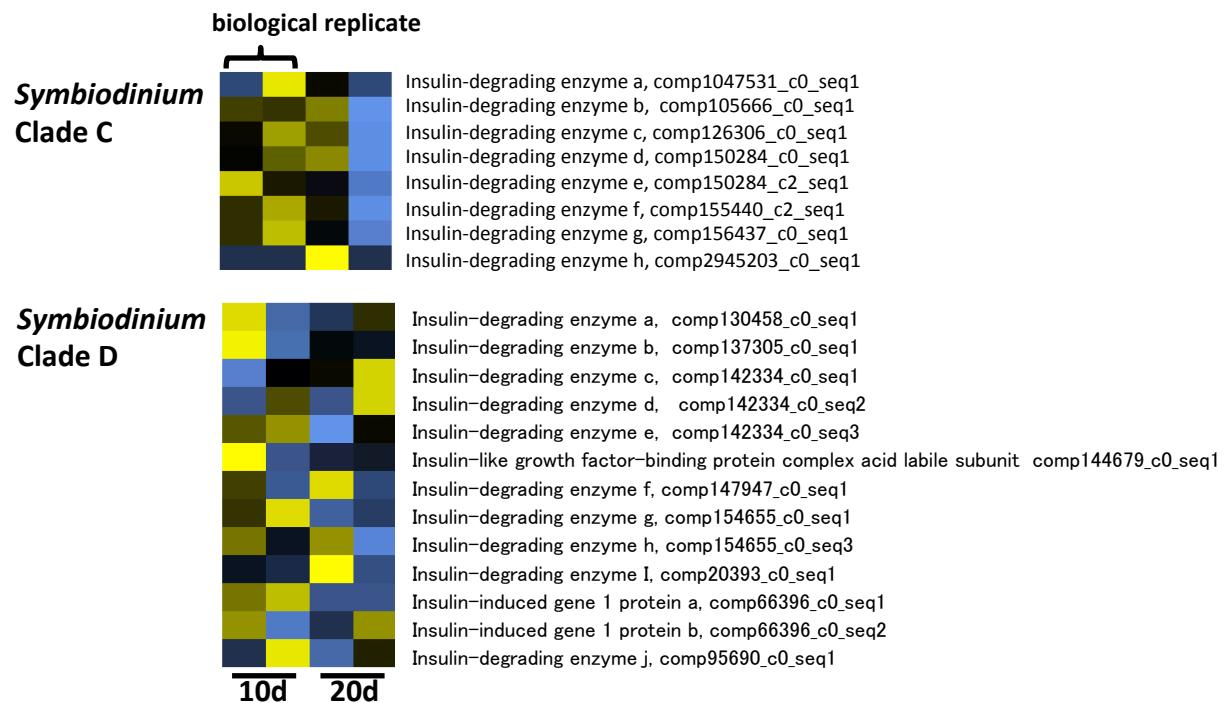
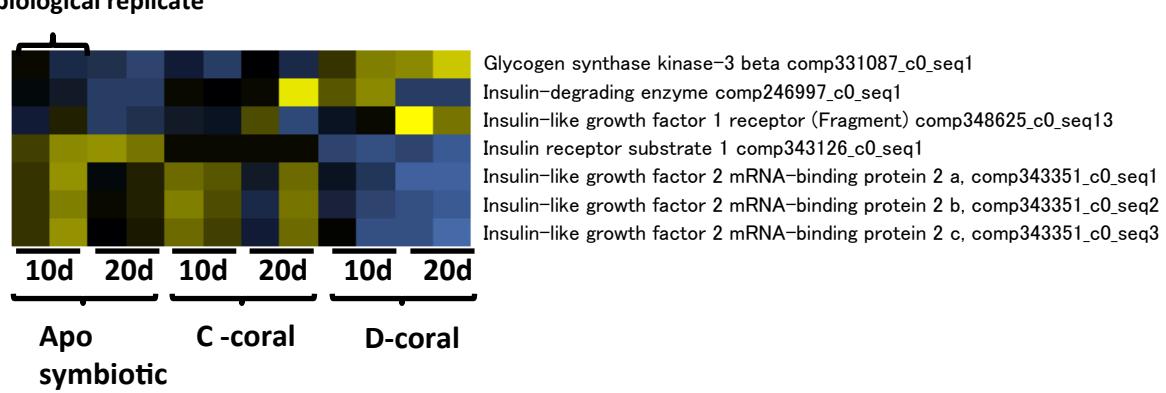
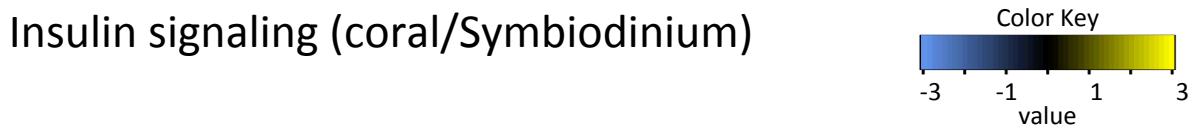


Fig. S13. Heat map of the expression patterns genes related to Insulin signaling.

Insulin signaling in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. And expression data of Insulin-related genes of *Symbiodinium* are also shown. There are selected notable treatment-specific genes (FDR P < 0.05).

Nitrate Assimilation (*Symbiodinium*)



Symbiodinium

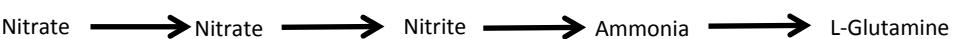
Clade C

biological replicate

nitrate_transporter

Nitrate_reductase

Glutamine synthetase



High affinity nitrate transporter 2.5 a, comp1013697_c0_seq1
High affinity nitrate transporter 2.5 b, comp1017338_c0_seq1
High affinity nitrate transporter 2.5 c, comp1131097_c0_seq1
Probable peptide/nitrate transporter At3g43790 a, comp1268518_c0_seq1
Probable peptide/nitrate transporter At3g43790 b, comp157416_c0_seq1
High affinity nitrate transporter 2.5 d, comp191062_c0_seq3
High affinity nitrate transporter 2.5 e, comp191062_c0_seq8
Nitrate reductase [NADH] a, comp191062_c0_seq4
Nitrate reductase [NADH] b, comp191062_c0_seq6
Nitrate reductase [NADH] c, comp191062_c0_seq7
Glutamine synthetase 3 a, comp126765_c0_seq1
Glutamine synthetase 3 b, comp126765_c1_seq1
Glutamine synthetase cytosolic isozyme, comp139640_c0_seq1
Glutamine synthetase, comp173628_c0_seq3
Glutamine synthetase cytosolic isozyme, comp190065_c0_seq1
Glutamine synthetase a, comp58353_c0_seq1
Glutamine synthetase b, comp764286_c0_seq1

10d 20d

Symbiodinium

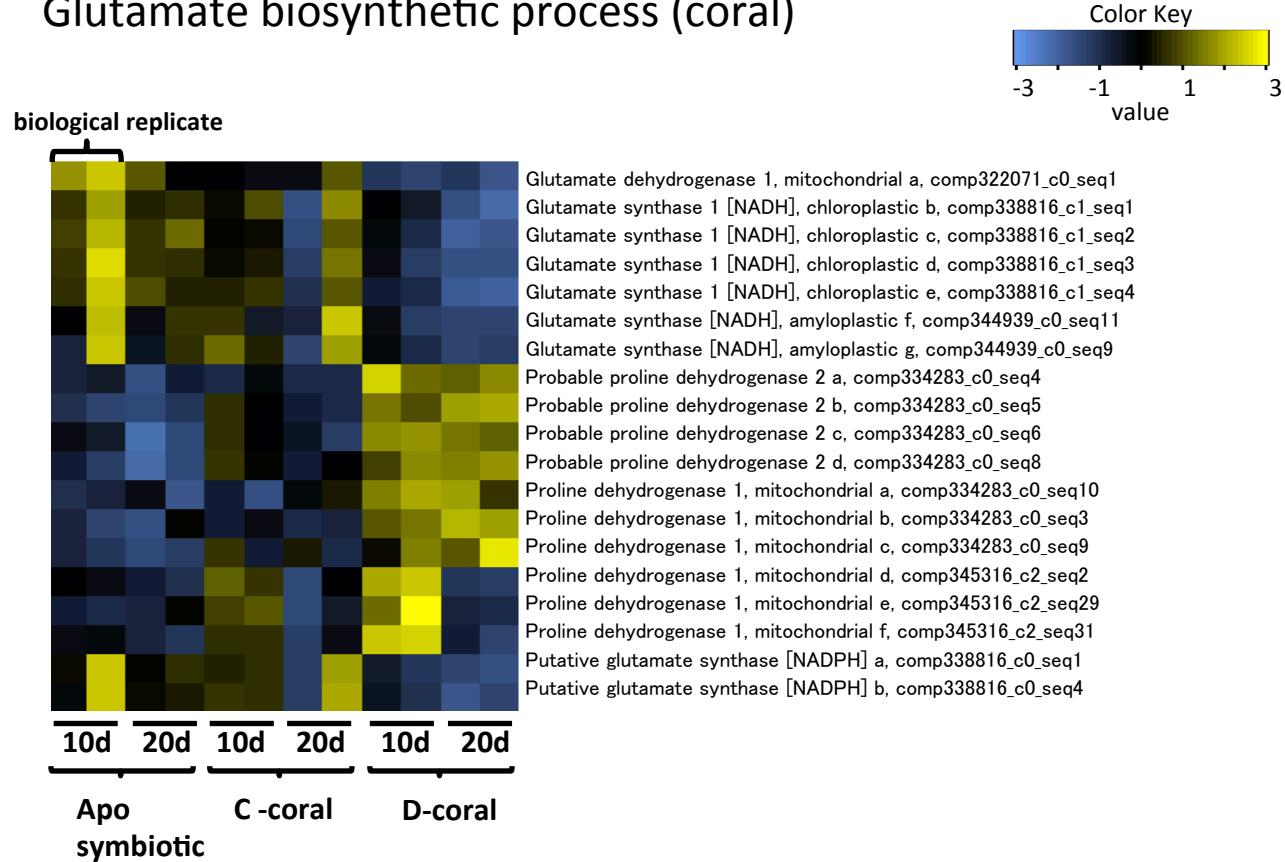
Clade D

Glutamine synthetase a, comp137644_c0_seq1
Glutamine synthetase b, comp137644_c3_seq1
Glutamine synthetase c, comp169920_c0_seq1
Glutamine synthetase d, comp169920_c0_seq2
Glutamine synthetase e, comp169920_c0_seq4
Glutamine synthetase f, comp169920_c0_seq5
Glutamine synthetase g, comp75337_c0_seq1
Glutamine synthetase h, comp93923_c0_seq1
Glutamine synthetase I, comp96317_c0_seq1
Glutamine synthetase 3 comp73896_c0_seq1
Glutamine synthetase cytosolic isozyme comp197144_c0_seq1
High affinity nitrate transporter 2.5 a, comp135939_c0_seq1
High affinity nitrate transporter 2.5 b, comp159493_c0_seq1
High affinity nitrate transporter 2.5 c, comp159493_c2_seq2
High affinity nitrate transporter 2.5 d, comp171796_c0_seq3
High affinity nitrate transporter 2.5 e, comp171796_c0_seq9
High affinity nitrate transporter 2.5 f, comp179283_c0_seq1
Nitrate reductase [NADH] a, comp177584_c0_seq1
Nitrate reductase [NADH] b, comp177584_c0_seq10
Nitrate reductase [NADH] c, comp177584_c0_seq11
Nitrate reductase [NADH] d, comp177584_c0_seq12
Nitrate reductase [NADH] e, comp177584_c0_seq13
Nitrate reductase [NADH] f, comp177584_c0_seq14
Nitrate reductase [NADH] g, comp177584_c0_seq15
Nitrate reductase [NADH] h, comp177584_c0_seq16
Nitrate reductase [NADH] i, comp177584_c0_seq17
Nitrate reductase [NADH] j, comp177584_c0_seq18
Nitrate reductase [NADH] k, comp177584_c0_seq19
Nitrate reductase [NADH] l, comp177584_c0_seq2
Nitrate reductase [NADH] m, comp177584_c0_seq20
Nitrate reductase [NADH] n, comp177584_c0_seq3
Nitrate reductase [NADH] o, comp177584_c0_seq4
Nitrate reductase [NADH] p, comp177584_c0_seq5
Nitrate reductase [NADH] q, comp177584_c0_seq6
Nitrate reductase [NADH] r, comp177584_c0_seq7
Nitrate reductase [NADH] s, comp177584_c0_seq8
Nitrate reductase [NADH] t, comp177584_c0_seq9
Nitrate reductase [NADH] u, comp322701_c0_seq1
Nitrate reductase [NADH] 1 comp2343132_c0_seq1
Probable peptide/nitrate transporter At3g43790 a, comp92880_c0_seq1
Probable peptide/nitrate transporter At3g43790 b, comp99674_c0_seq1

Fig. S14. Heat map of the expression patterns of genes involved in nitrate assimilation.

Heat map shows the expression patterns genes related to nitrate assimilation of *Symbiodinium*. There are selected notable treatment-specific genes (FDR P < 0.05).

Glutamate biosynthetic process (coral)



Osmoregulated proline transporter (*Symbiodinium*)

Symbiodinium

Clade C

Not detect

Symbiodinium

Clade D

biological replicate



10d 20d

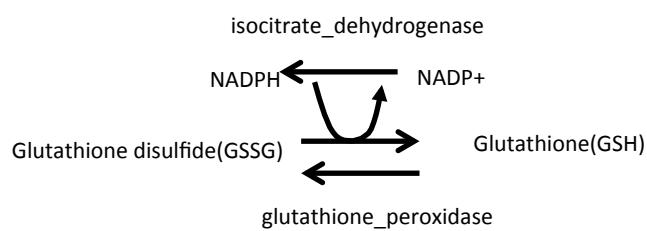
Osmoregulated proline transporter a, comp223048_c0_seq1

Osmoregulated proline transporter b, comp97111_c0_seq1

Fig. S15. Heat map of the expression patterns of genes related to glutamate biosynthetic process.

Heat map shows the expression patterns of genes related to glutamate biosynthetic process in aposymbiotic corals, corals associated with clade C (C-corals) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. And expression data of genes coding Osmoregulated proline transporter of *Symbiodinium* are also shown. There are selected notable treatment-specific genes (FDR P < 0.05).

Glutathione metabolic process (coral)



biological replicate

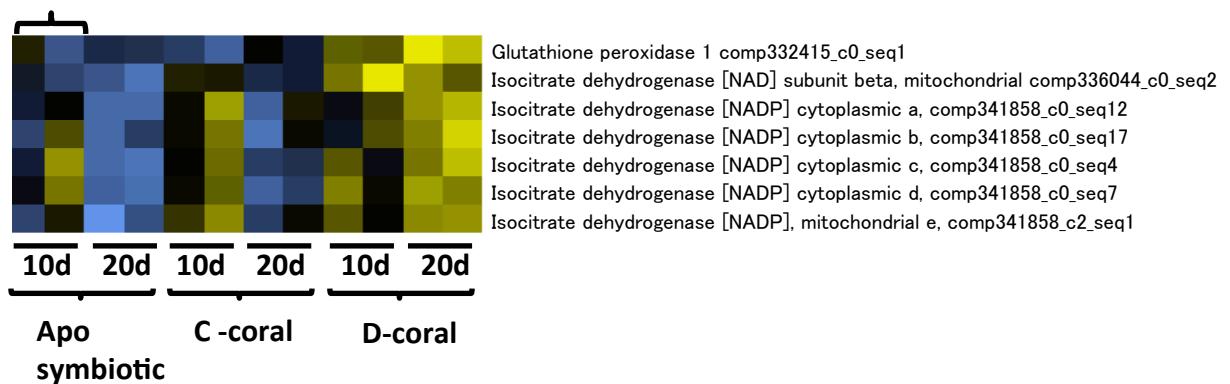


Fig. S16. Heat map of the expression patterns of genes related to glutathione metabolic process.

Heat map shows the expression patterns of genes related to glutathione metabolic process in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. There are selected notable treatment-specific genes (FDR P < 0.05).

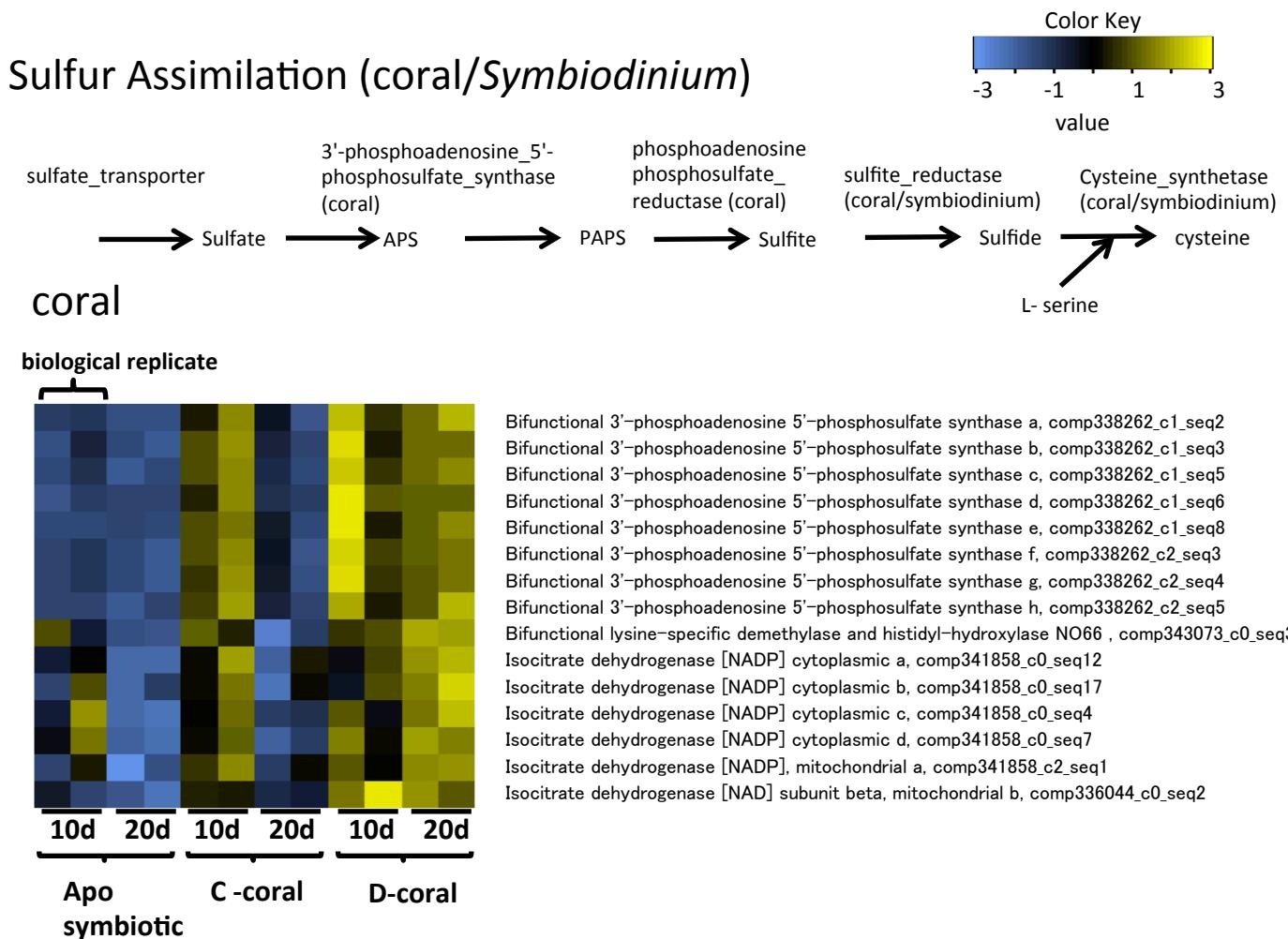


Fig. S17a. Heat map of the expression patterns of genes related sulfur assimilation. Heat map shows the expression patterns of genes related sulfur assimilation in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. There are selected notable treatment-specific genes (FDR P < 0.05).

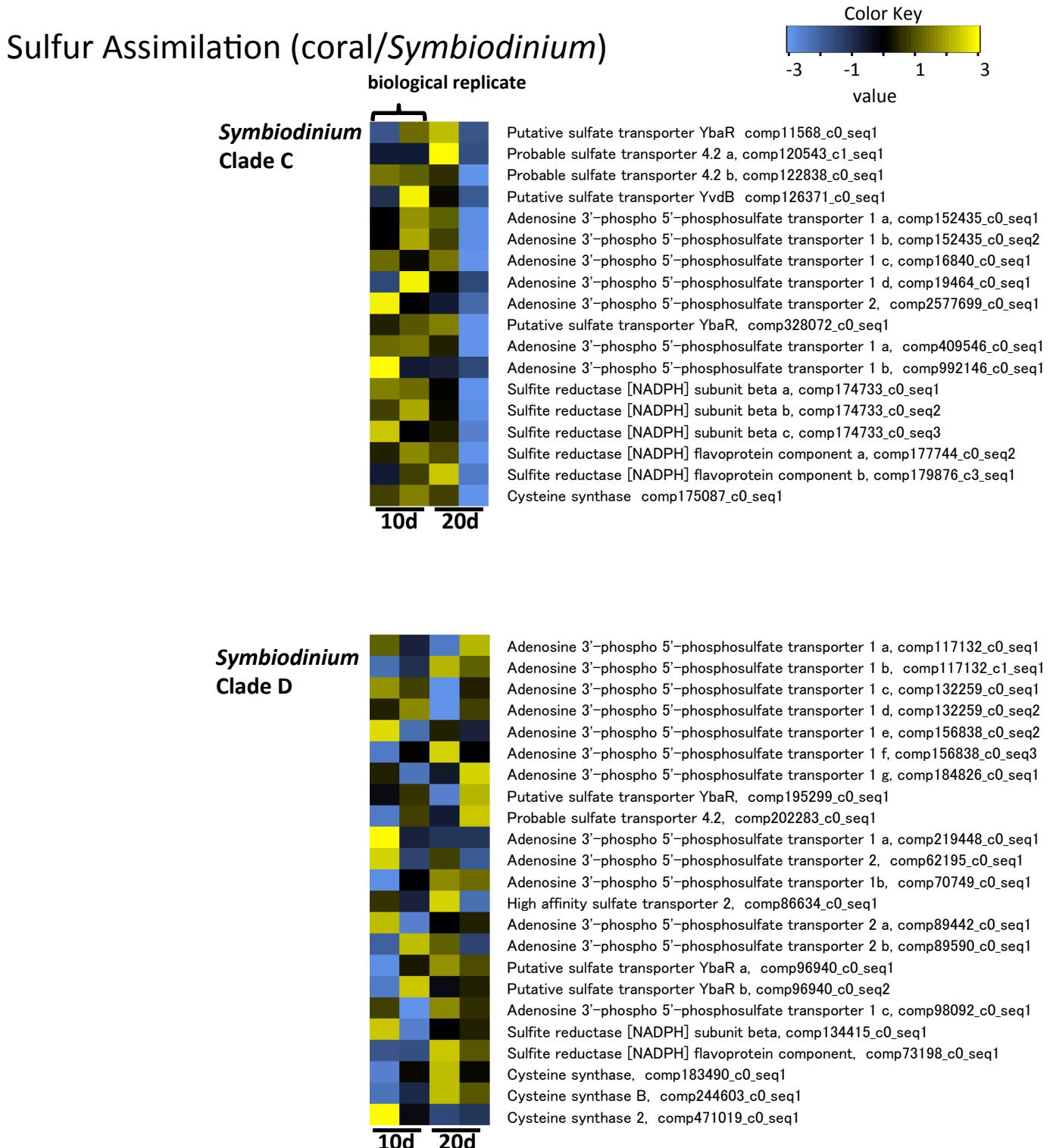


Fig. S17b. Heat map of the expression patterns of genes related to endocytosis.

Heat map shows the expression patterns of genes related to sulfur assimilation in each endosymbiotic *Symbiodinium* in 10 days and 20 days after inoculation to corals. There are selected notable treatment-specific genes (FDR P < 0.05).

Cyanoamino acid metabolism (coral)

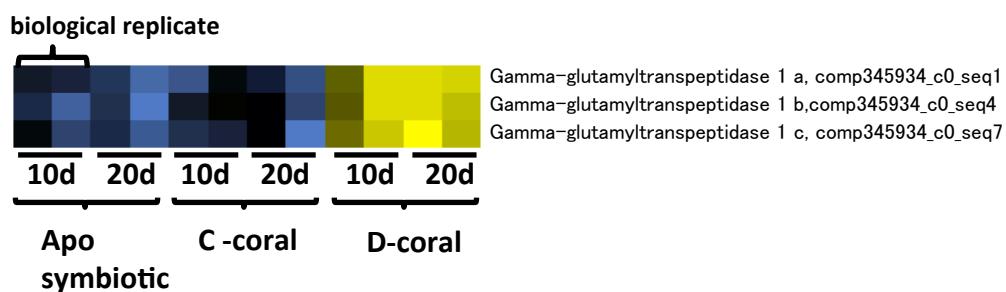
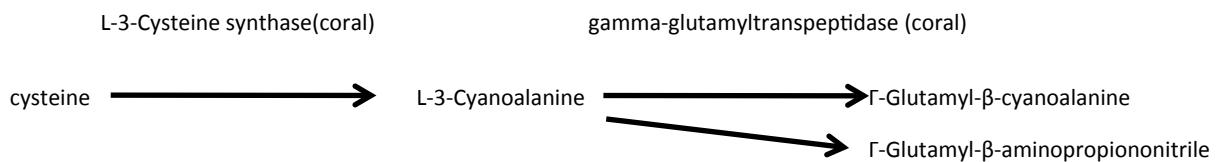
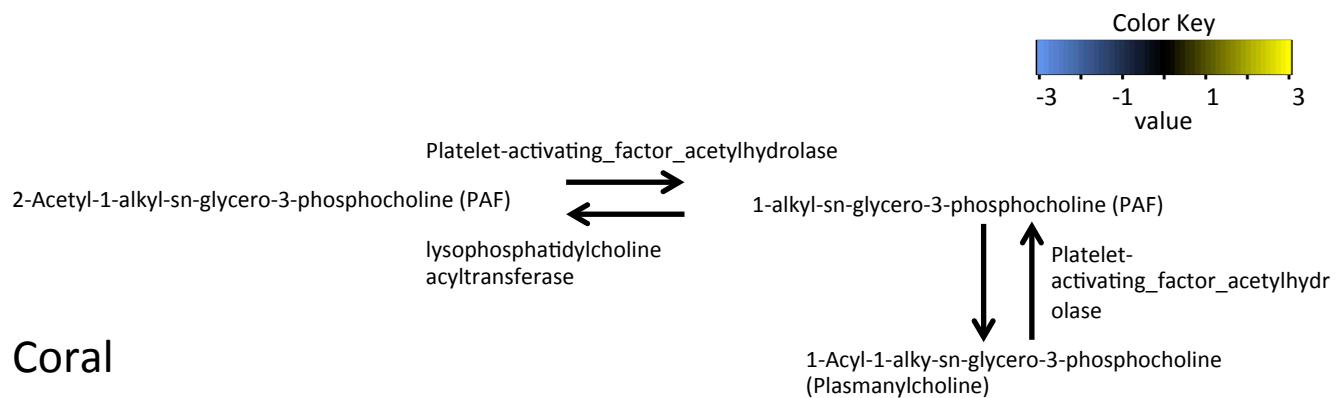


Fig. S18. Heat map of the expression patterns of genes related to cyanoamino acid metabolism.

Heat map show of the expression patterns of genes related to cyanoamino acid metabolism in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. There are selected notable treatment-specific genes (FDR P < 0.05).

Biosynthesis of Plasmanylcholine (coral)



Coral

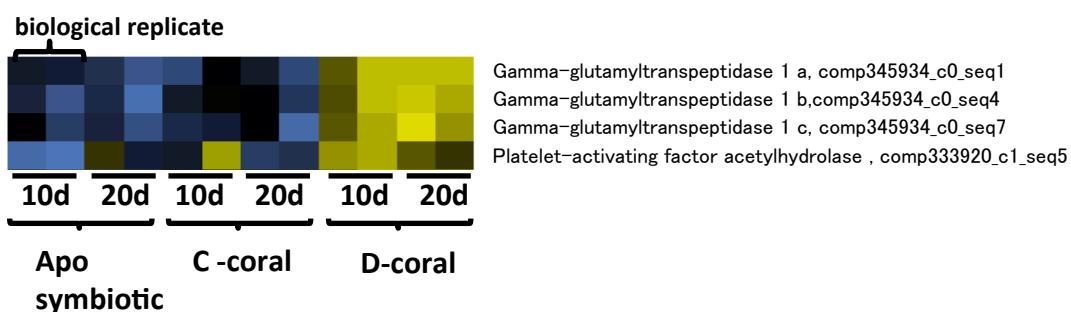
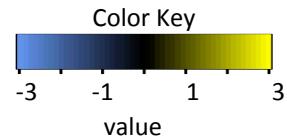


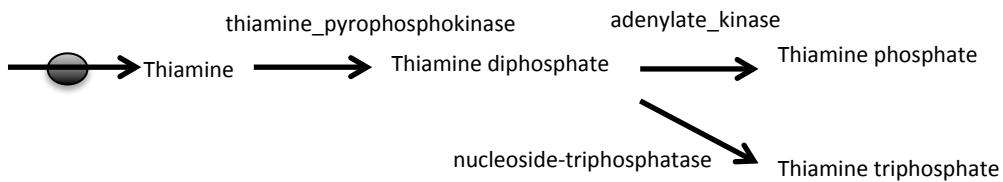
Fig. S19. Heat map of the expression patterns of genes related to biosynthesis of plasmanylcholine.

Heat map shows the expression patterns of genes related to biosynthesis of plasmanylcholine in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. There are selected notable treatment-specific genes (FDR P < 0.05).

Thiamine metabolism (coral)



riboflavin transporter 2



coral

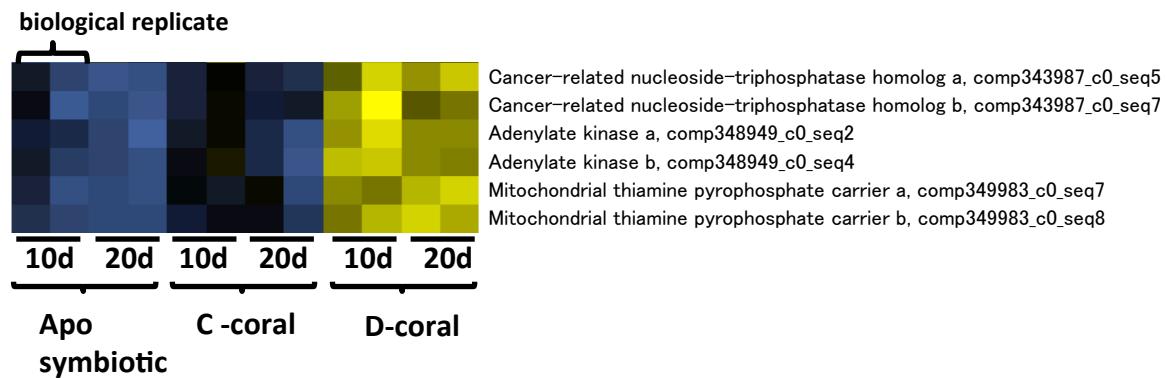


Fig. S20. Heat map of the expression patterns of genes related to thiamine (Vitamin B) metabolism.

Heat map shows the expression patterns of genes related to thiamine (Vitamin B) metabolism in aposymbiotic corals, corals associated with clade C (C-coral) or clade D (D-corals) in 10 days and 20 days after inoculation of each *Symbiodinium*. There are selected notable treatment-specific genes (FDR P < 0.05).

Gene expression pattern from the result of RNA-seq					The result of blastx			
gene_id	log2FoldChange in clade C-symbiosis on 10day	log2FoldChange in clade C-symbiosis on 20day	log2FoldChange in clade D-symbiosis on 10day	log2FoldChange in clade D-symbiosis on 20day	UniPlot KB accession	Protein name	Organism Name	E- value
comp343541_c0_seq2	-1.539859679	-1.957416973	-2.01262258	-4.10760851Q9U6Y6	GFP-like fluorescent chromoprotein amFP486	Anemonea manjano	7.00E-112	
comp343541_c0_seq6	-1.304746951	-1.870523076	-1.396355143	-3.306085Q9U6Y6	GFP-like fluorescent chromoprotein amFP486	Anemonea manjano	2.00E-96	
comp343541_c0_seq7	-1.60733518	-1.877147263	-2.048693528	-4.161228583Q9U6Y6	GFP-like fluorescent chromoprotein amFP486	Anemonea manjano	7.00E-112	
comp343546_c2_seq4	-1.065026917	-1.599162044	-1.415382344	-3.908150632				

Table. S1. Common differentially expressed genes (DEGs) in D-coral and C-coral on day10, day20.