

Supplementary Information

Metatranscriptomic Analysis of corals inoculated with tolerant and non-tolerant symbiont exposed to high temperature and light stress

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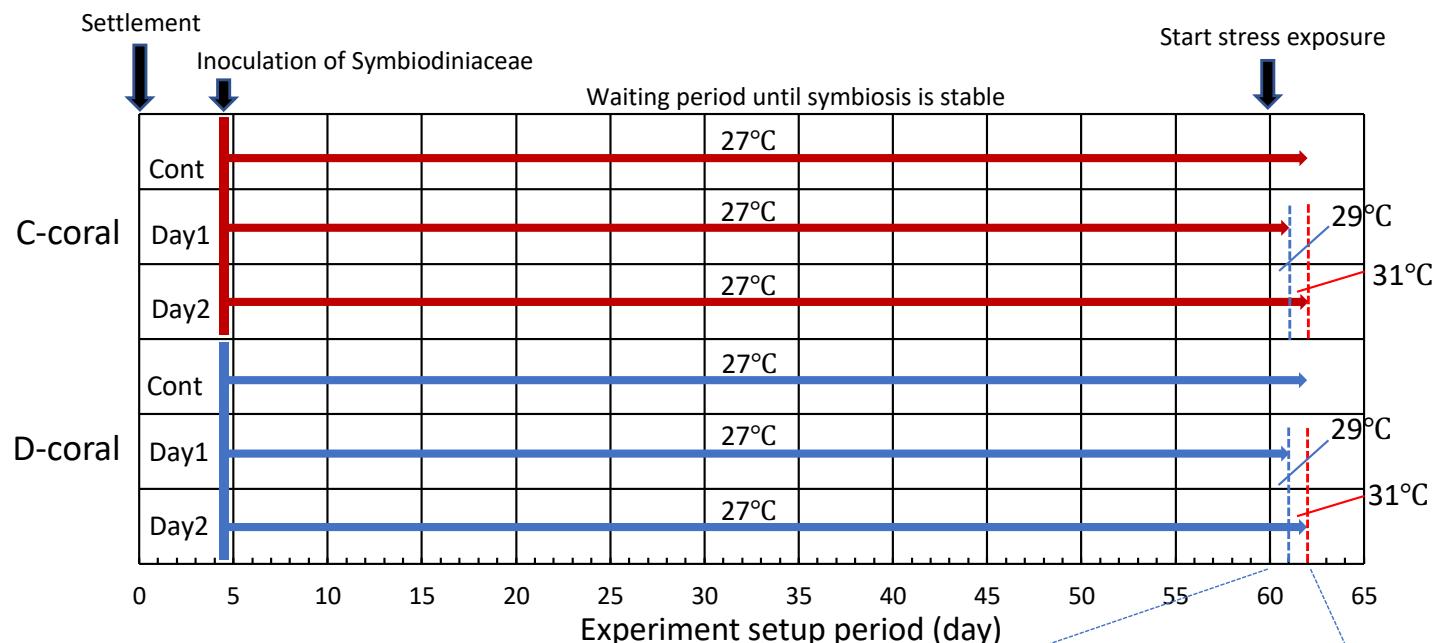
Supplementary Table

Table S1. The number of aligned sequences to the database. 17

Table S2. Statistics of obtained Symbiodiniaceae (Cladocopium and Durusdinium) derived contigs and *Acropora solitaryensis* derived contigs. 17

Fig. S1. Schematic diagram of the incubation experiments. (A) Corals were incubated approximately two months after inoculation with *Cladocopium* or *Durusdinium*. To examine the bleaching response, some corals were exposed to stress for 1 or 2 days. (B) In the stress treatment groups, the temperature was gradually increased to 31 °C. For the control populations, corals were maintained at 27°C for approximately 2 months and then fixed at the same time as the stress day 2 corals.

(A)



(B)

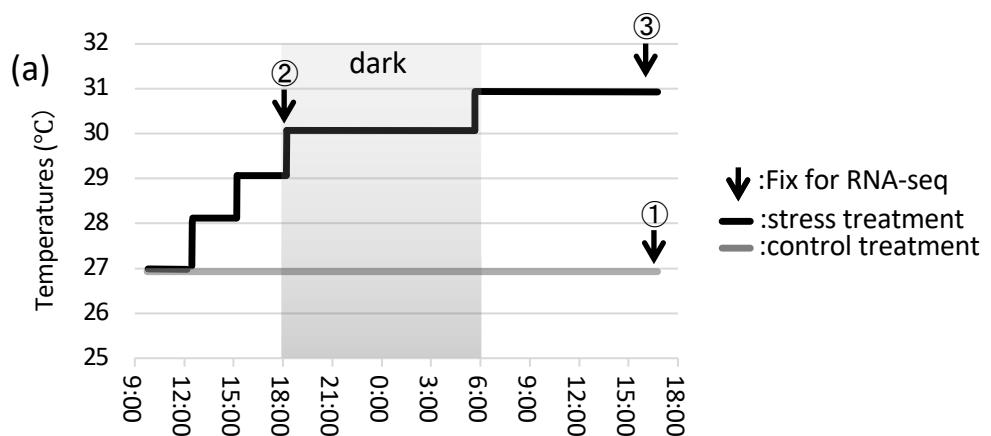
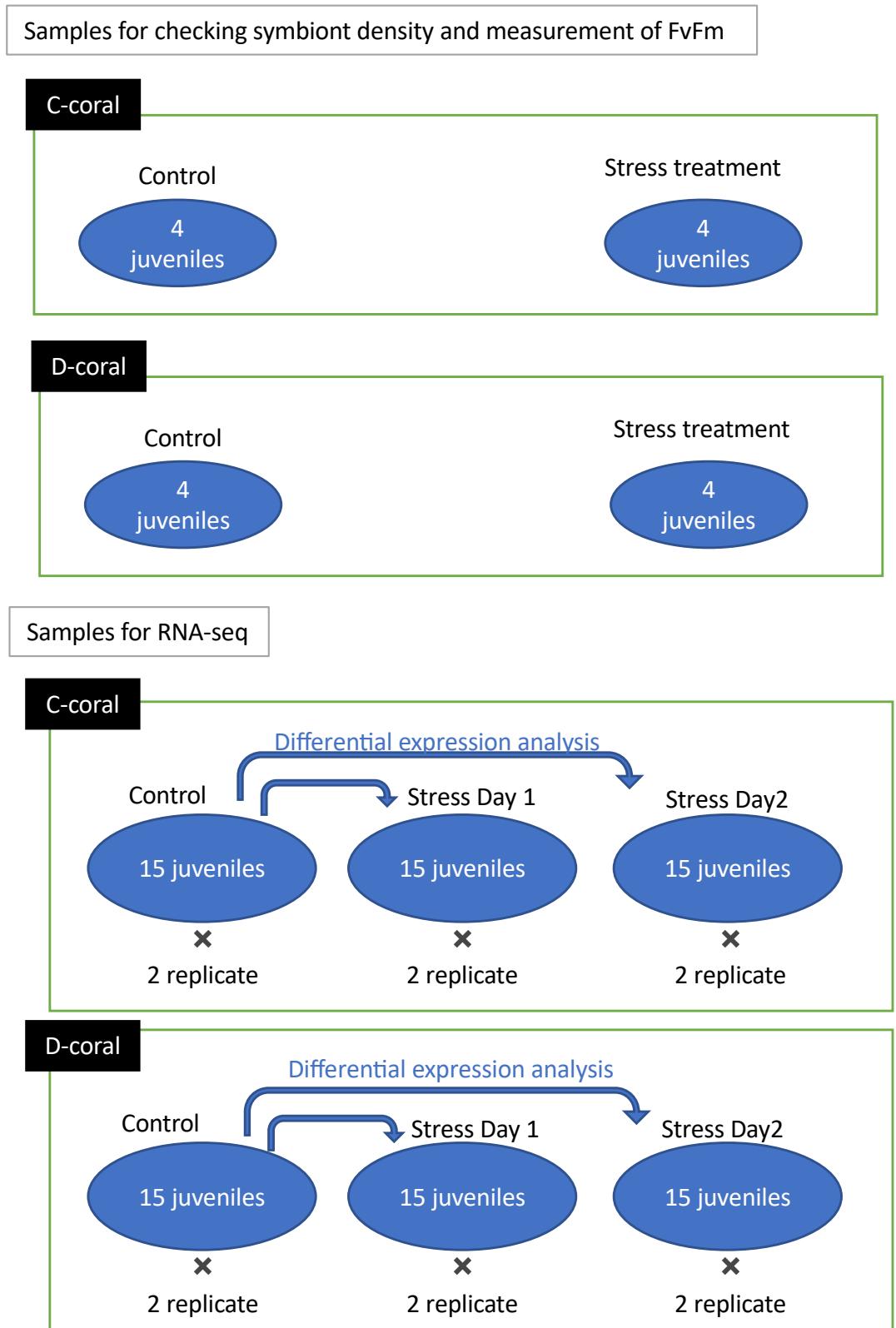
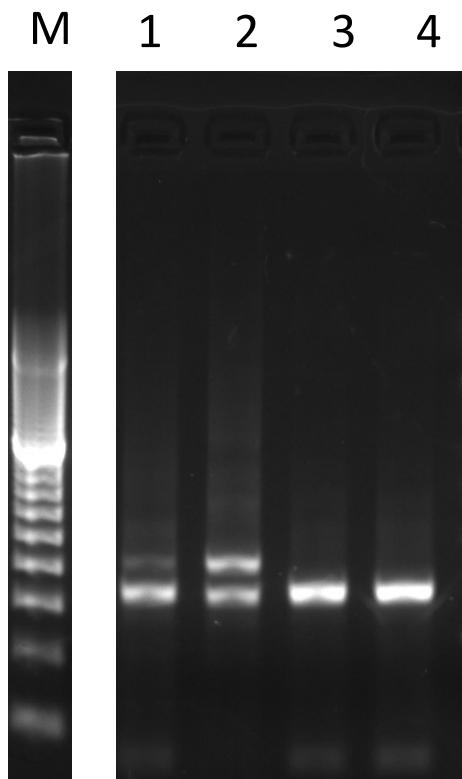


Fig. S2. Numbers of corals used for each analysis. Approximately 200 corals (100 *Cladcopium*-associated and 100 *Durusdinium*-associated corals) were used. These corals were used in stress exposure experiments to investigate symbiont densities and Fv/Fm, and for RNA-Seq analysis, respectively.



Differential expression analysis were conducted between Stress Day 2 sample and control, and between stress Day 1 sample and control.

Fig.S3. Restriction fragment length polymorphism (RFLP) (28S) band patterns of Symbiodiniaceae in juvenile corals inoculated with *Cladocopium* and *Durusdinum*. RFLP analysis was performed to verify the genotype of Symbiodiniaceae colonizing corals before the start of the stress exposure experiments. In RFLP analysis, *Cladocopium* is associated with two bands and *Durusdinum* with one band (Yuyama et al., 2014). Electrophoresis bands specific to *Cladocopium* and *Durusdinum* were detected in *A. solitaryensis* inoculated with either Symbiodiniaceae.



M: 100bp DNA ladder

- 1 : *Acropora tenuis* juveniles inoculated with clade C
- 2 : *Acropora solitaryensis* juveniles inoculated with clade C
- 3 : *Acropora tenuis* juveniles with clade D
- 4 : *Acropora solitaryensis* juveniles inoculated with clade D

**A. tenuis* is not used in this study.

Fig. S4. Symbiont density in corals associated with *Cladocopium* (C-corals) and those associated with *Durusdinium* (D-corals) and photographs of C-corals and D-corals exposed to heat and light stress. The density of the symbiotic algae (*Durusdinium* and *Cladocopium*) in the corals for the control populations and for the 2-day stress treatment are shown. Cell density was calculated based on the surface area of the photographed coral. As the temperature increased, the symbiont number inside the coral body decreased, causing the coral to appear whiter. Scale bars = 0.5mm.

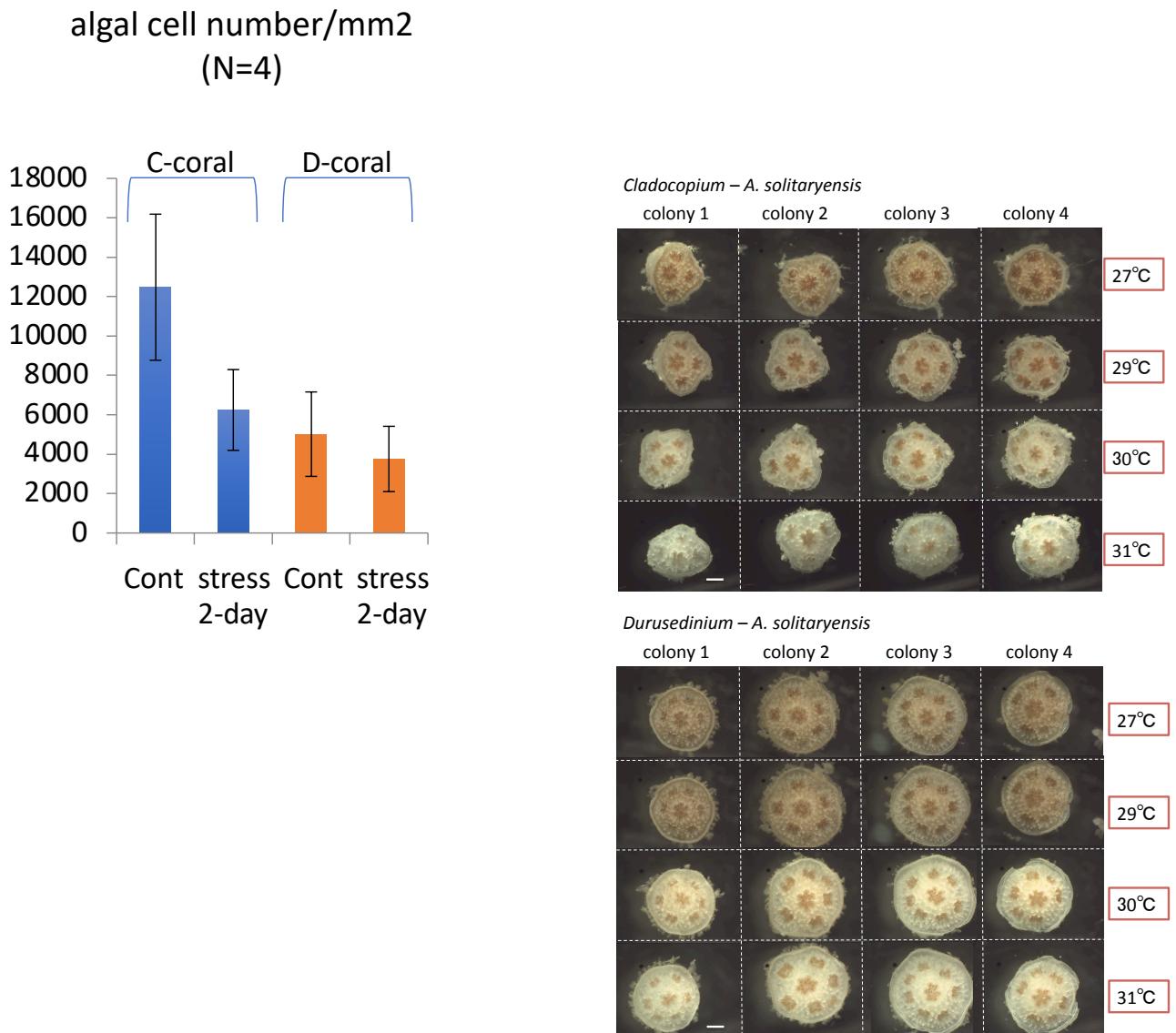
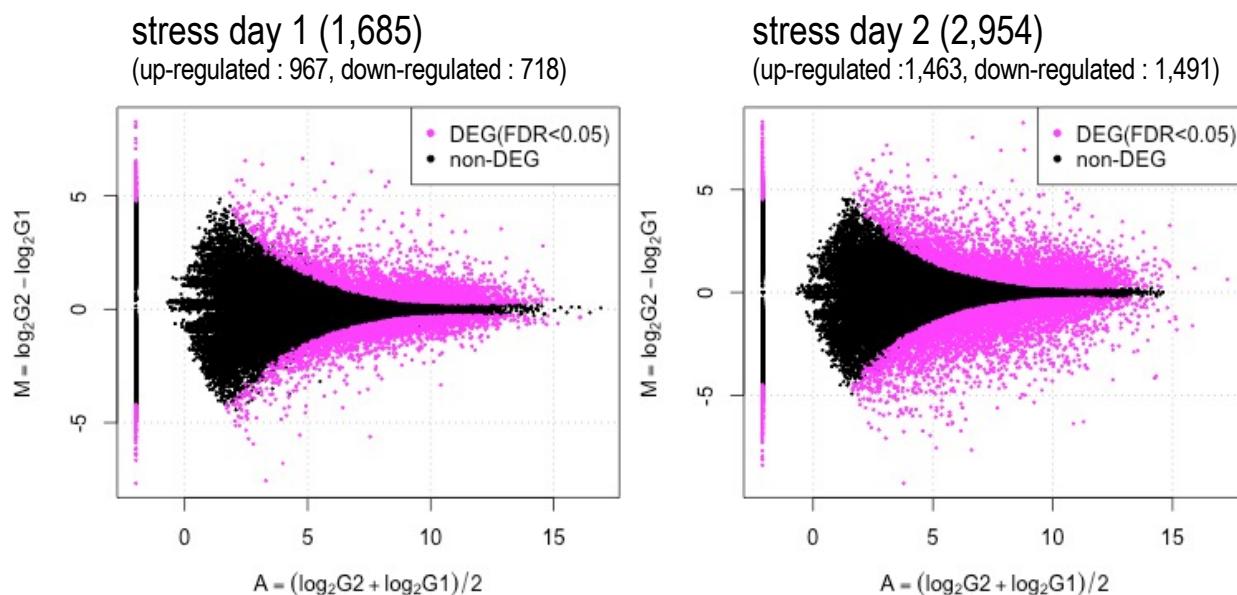


Figure S5, MA plot for edgeR for differentially expression analysis on *Acropora solitaryensis* associated with *Cladocopium* or *Durusdinium*. The log₂ fold change in stress conditions compared with the control conditions is plotted on the y-axis and the average log₂ counts across samples are shown on the x-axis. Isoforms that are significantly different among two conditions less than 0.05 are colored in red, others are colored in gray. The number of detected differentially expressed genes are shown in parentheses.

Corals associated with Cladocopium (C-coral)



Corals associated with Durusdinium (D-coral)

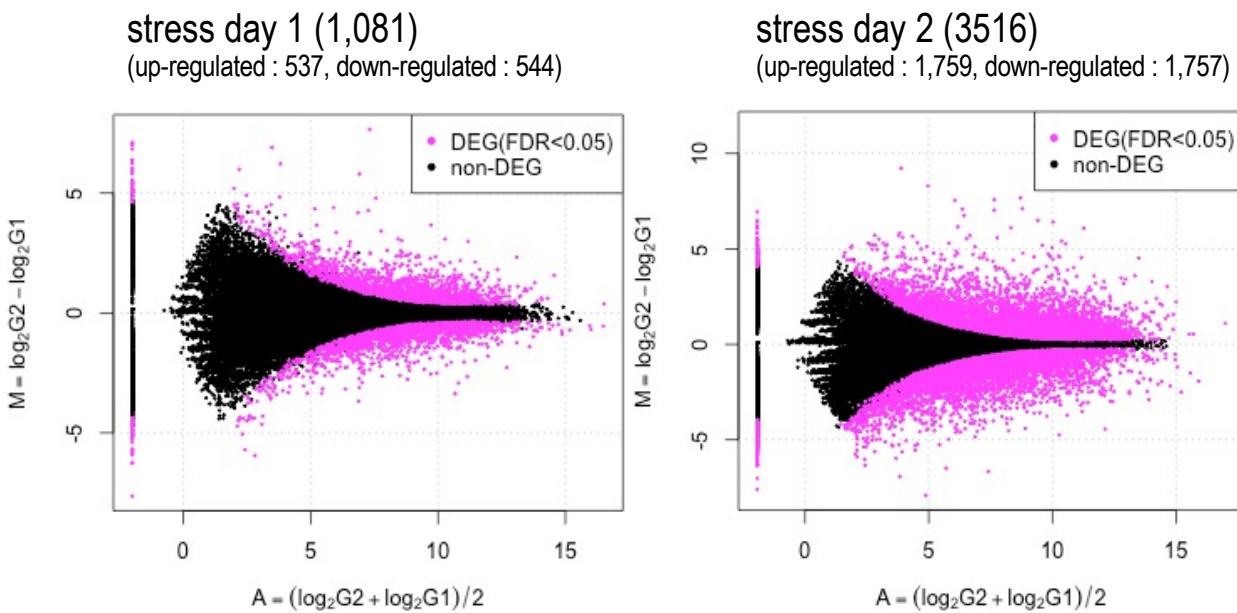
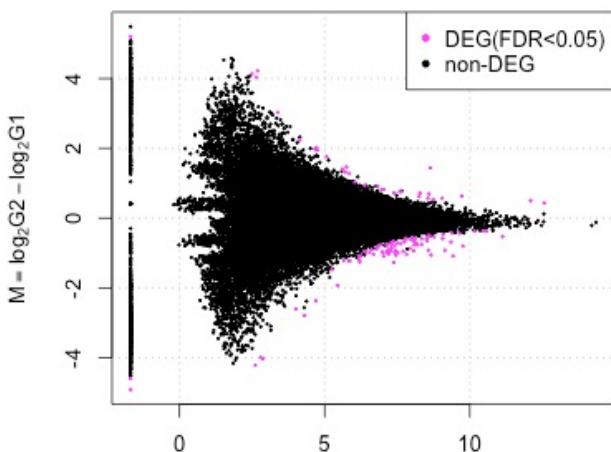


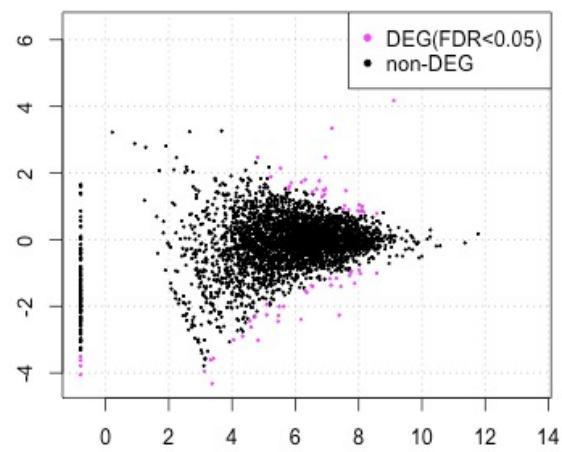
Figure S6, MA plot for edgeR for differentially expression analysis on Cladocopium and Durusdinium. The log₂ fold change in stress conditions compared with the control conditions is plotted on the y-axis and the average log₂ counts across samples are shown on the x-axis. Isoforms that are significantly different among two conditions less than 0.05 are colored in red, others are colored in gray. The number of detected differentially expressed genes are shown in parentheses.

Cladocopium (C-type)

stress day 1 (117)
(up-regulated :33, down-regulated : 84)

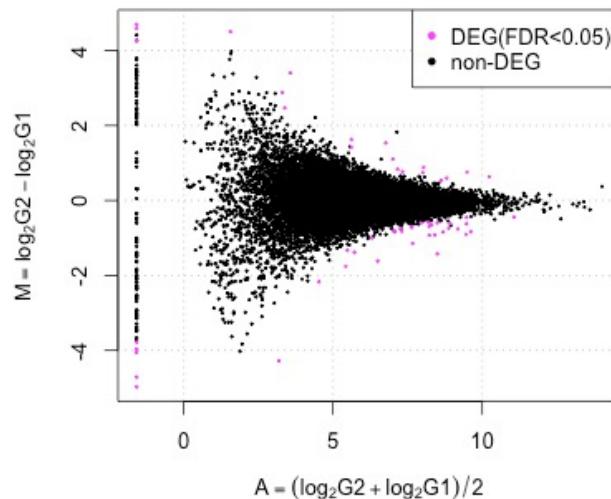


stress day 2 (109)
(up-regulated :30, down-regulated : 79)



Durusdinium (D-type)

stress day 1 (56)
(up-regulated :20, down-regulated : 36)



stress day 2 (296)
(up-regulated : 127, down-regulated : 169)

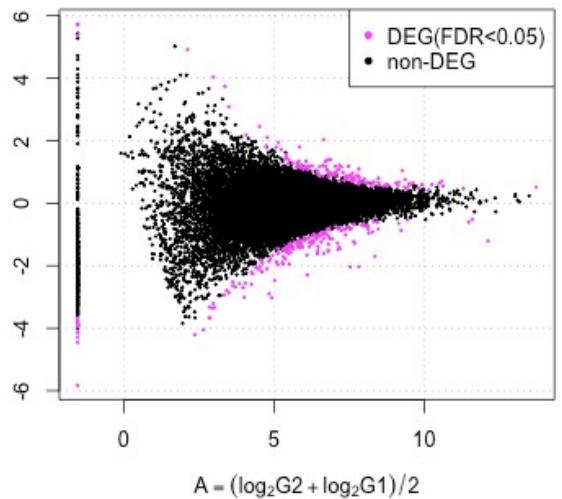
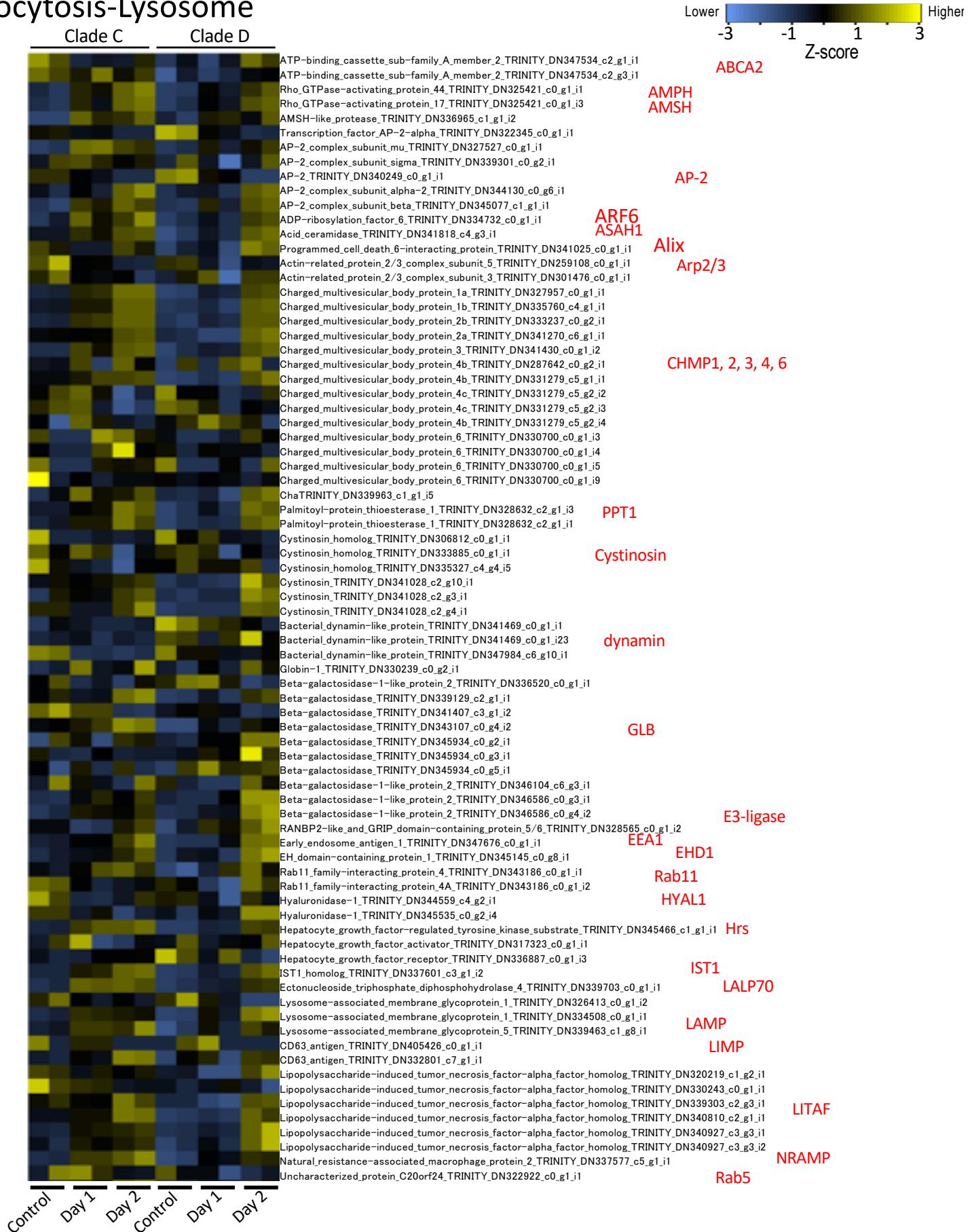
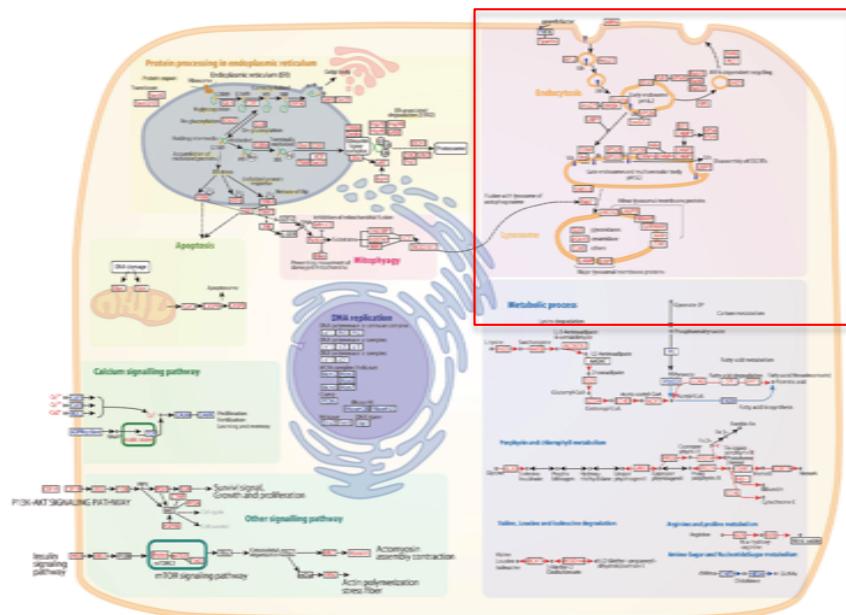
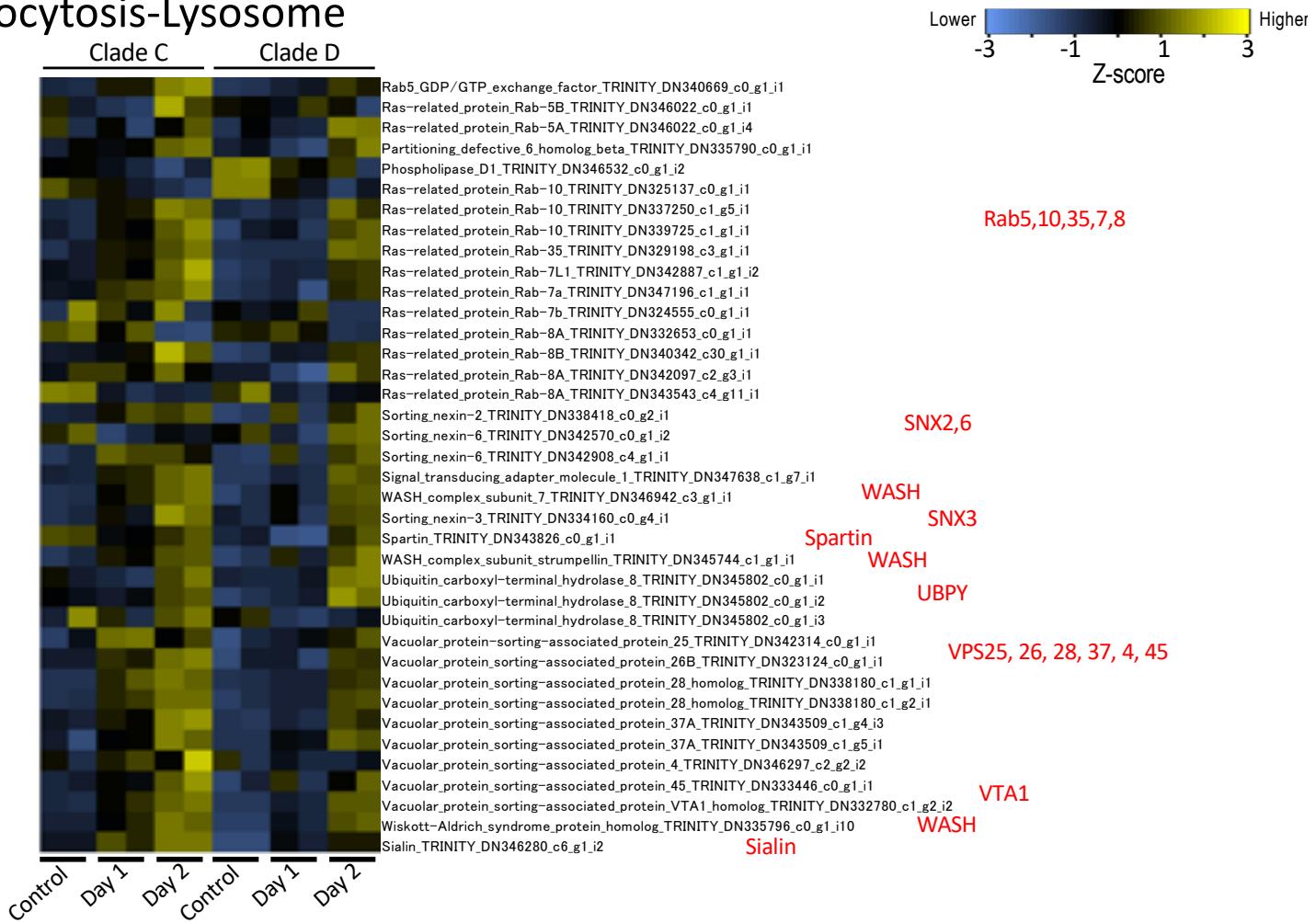


Fig. S7. The heatmap shows the expression level of genes related to each pathway described in Fig. 5.

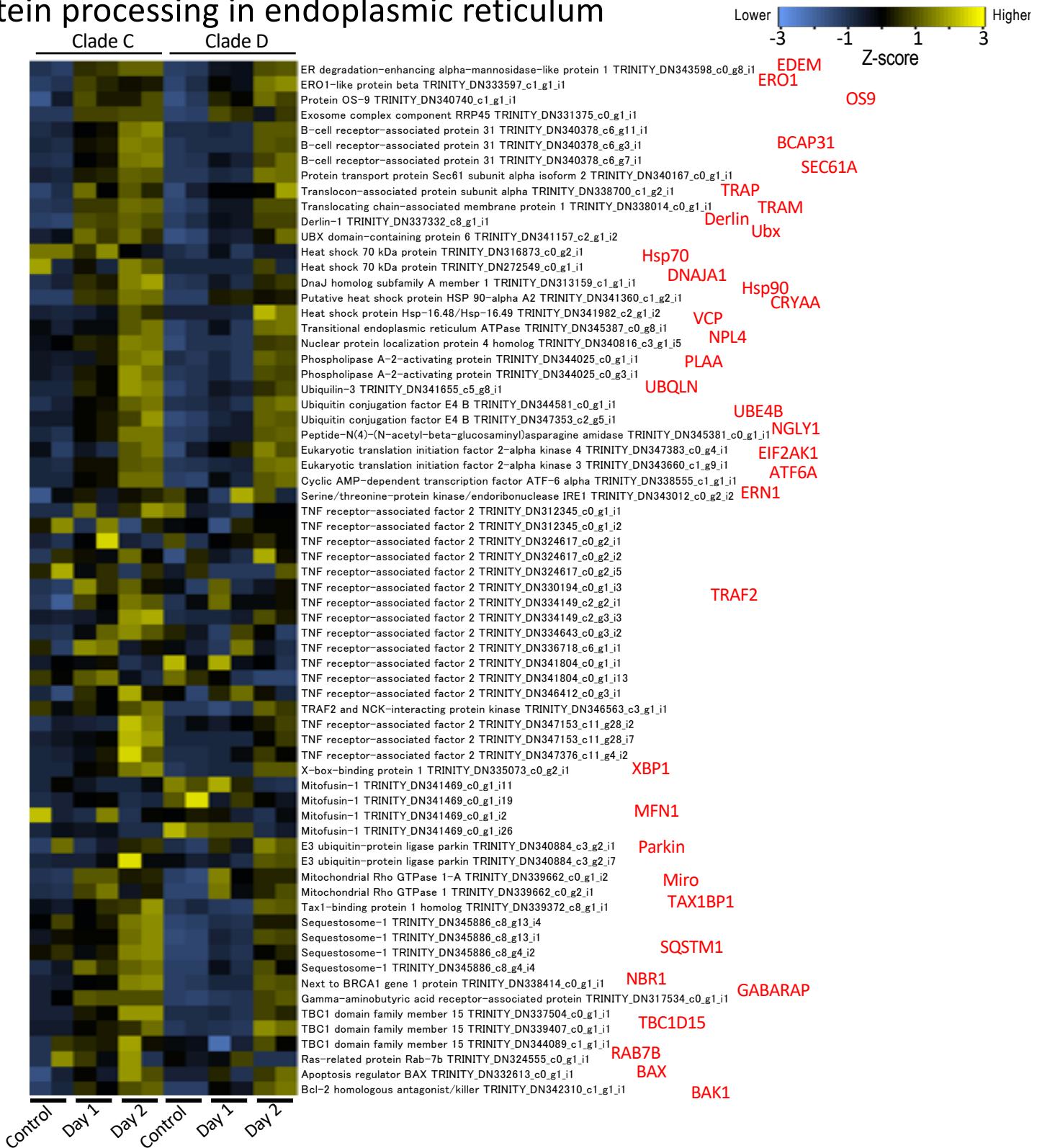
Endocytosis-Lysosome



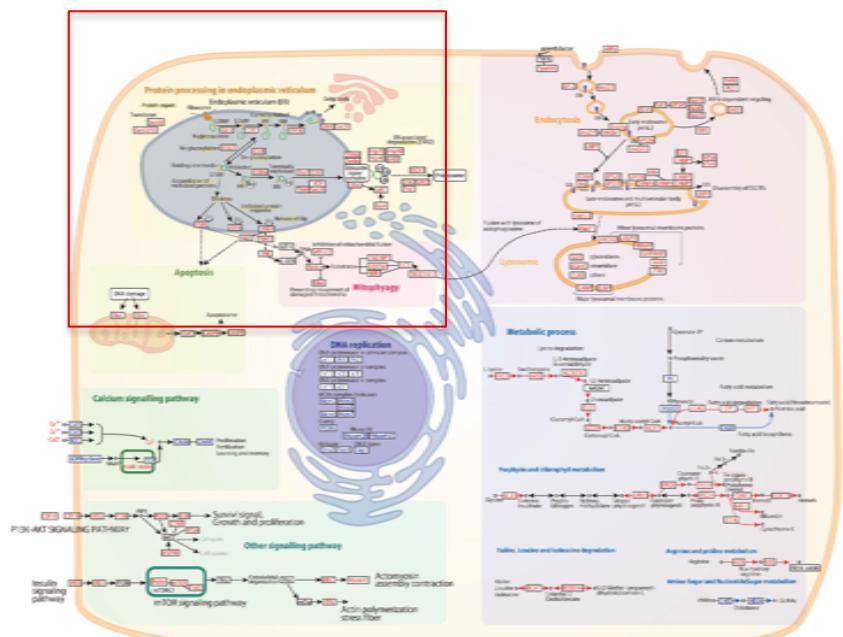
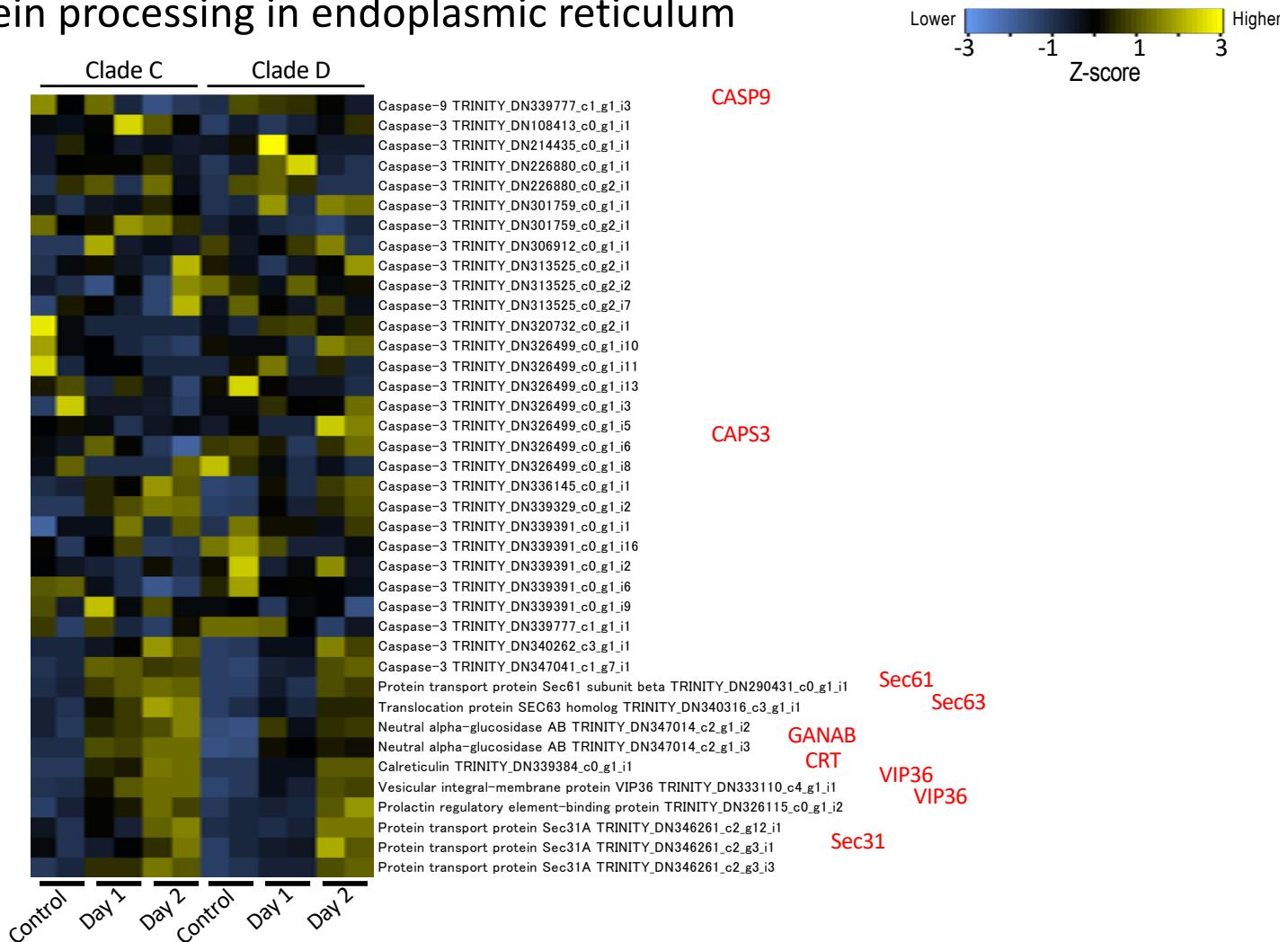
Endocytosis-Lysosome



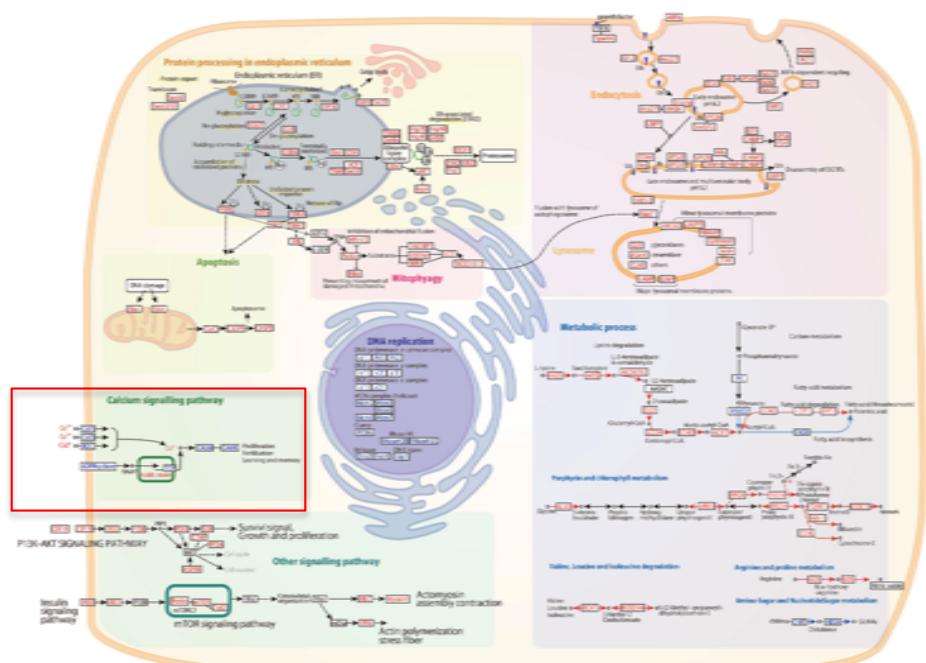
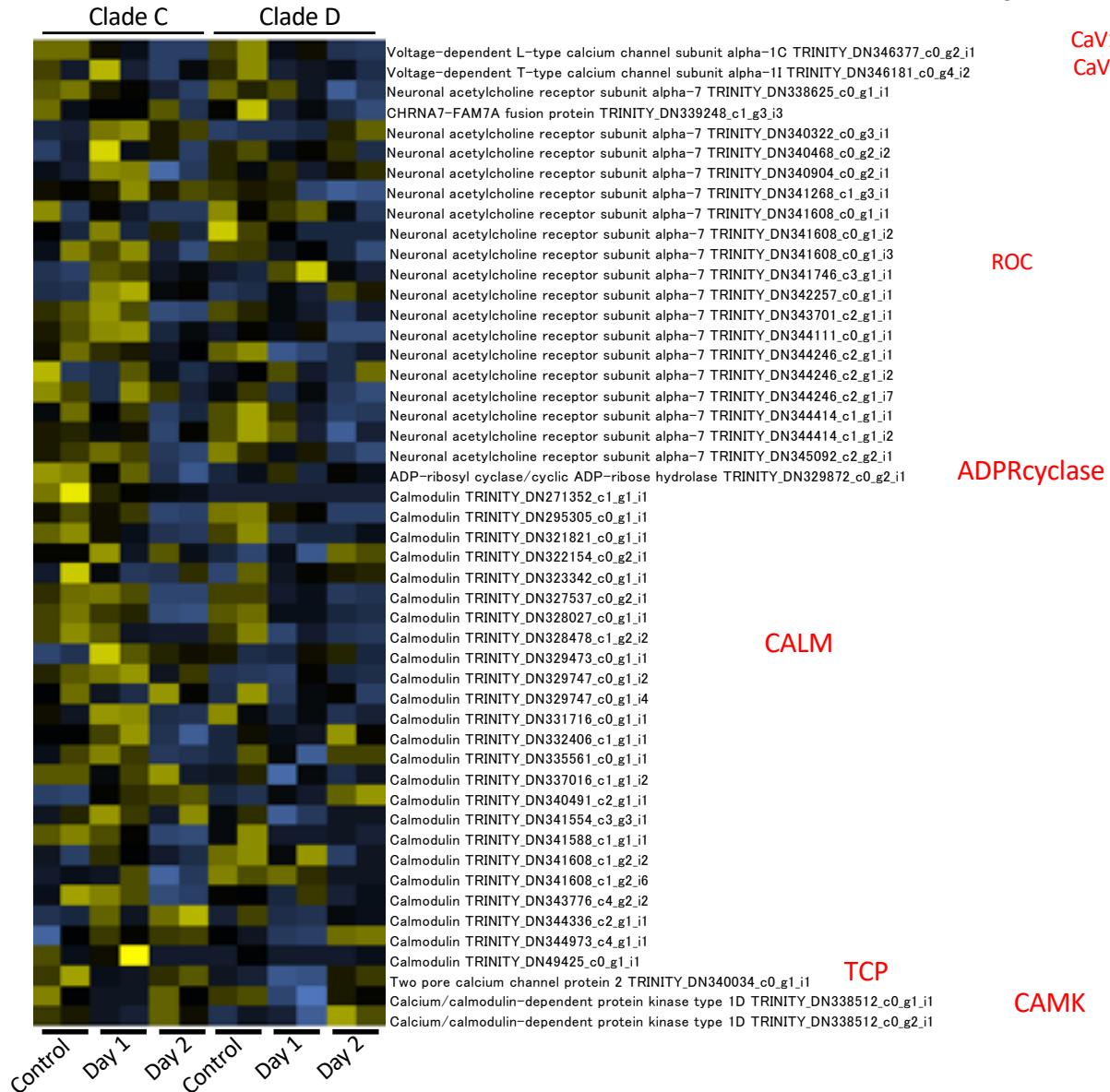
Protein processing in endoplasmic reticulum



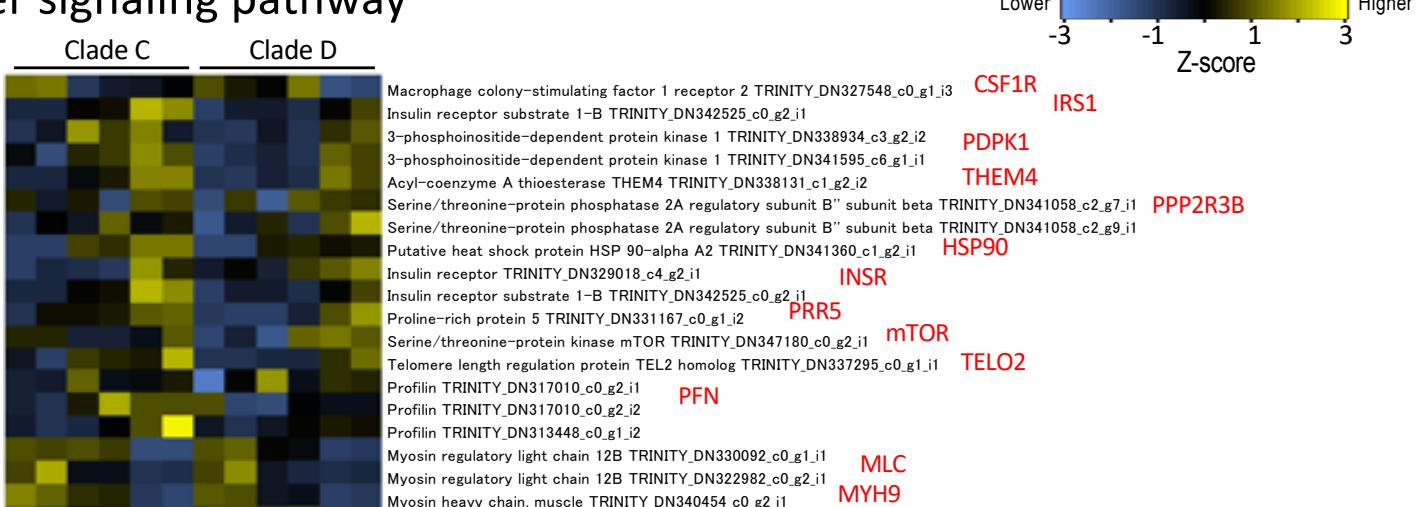
Protein processing in endoplasmic reticulum



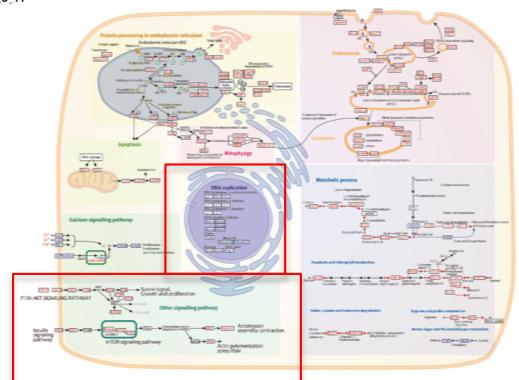
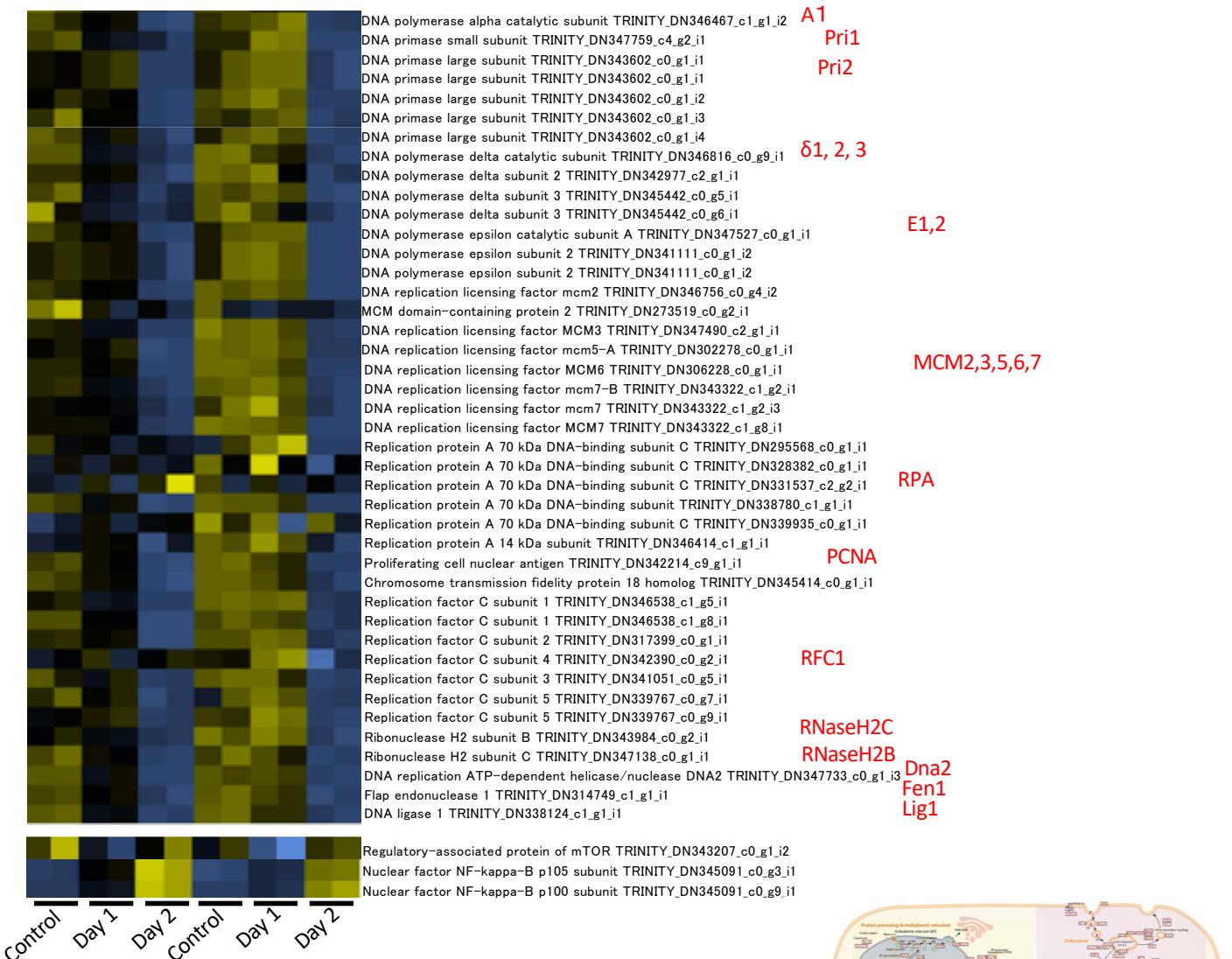
Calcium signaling pathway



Other signaling pathway



DNA replication



Metabolic process

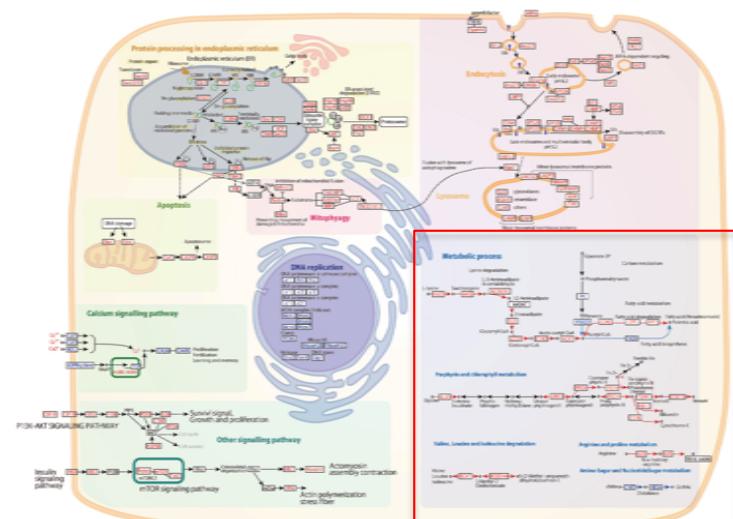
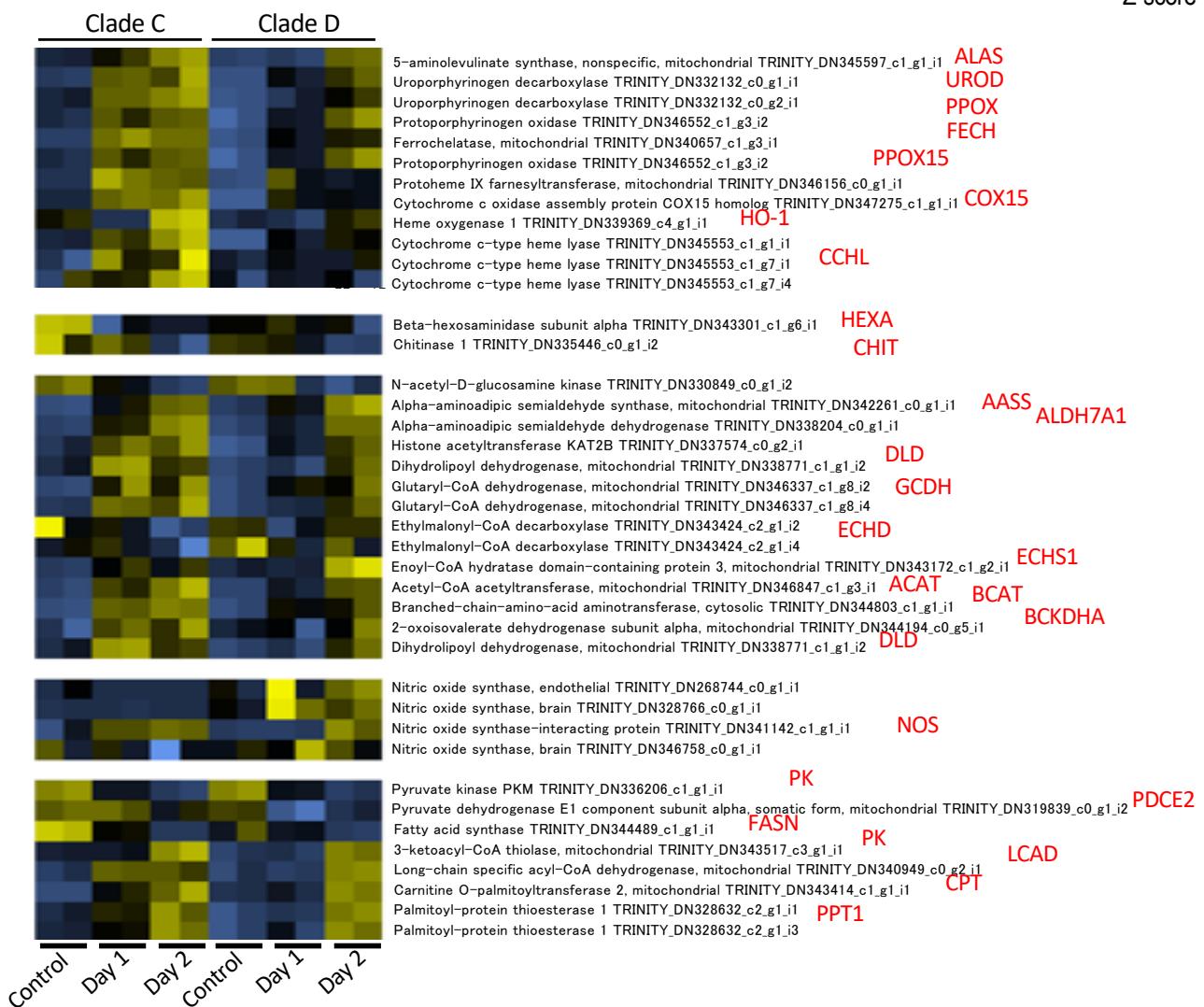
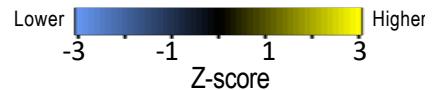
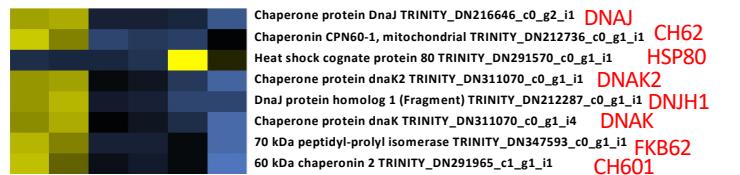


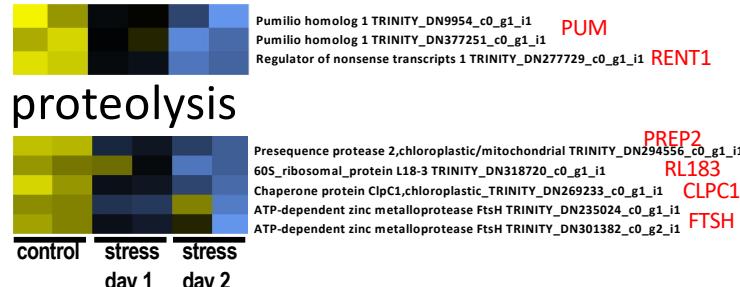
Figure S8. Expression pattern of algal DEG (differentially expressed genes) related to GO term in figure 6. Heat map representation of each gene expression pattern between control conditions and stress conditions: yellow highlight being higher expressed and blue highlight being lower expressed. Each expression values were converted into z-score and plotted by heatmap. 2 in R. Only major GO terms are listed. The abbreviations for the genes listed in 6 are shown in red near their gene expression data.

Cladocopium (C-type)

protein folding



Regulation of transcription



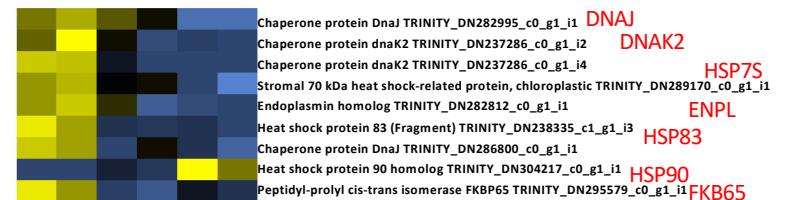
Color Key

-1 1

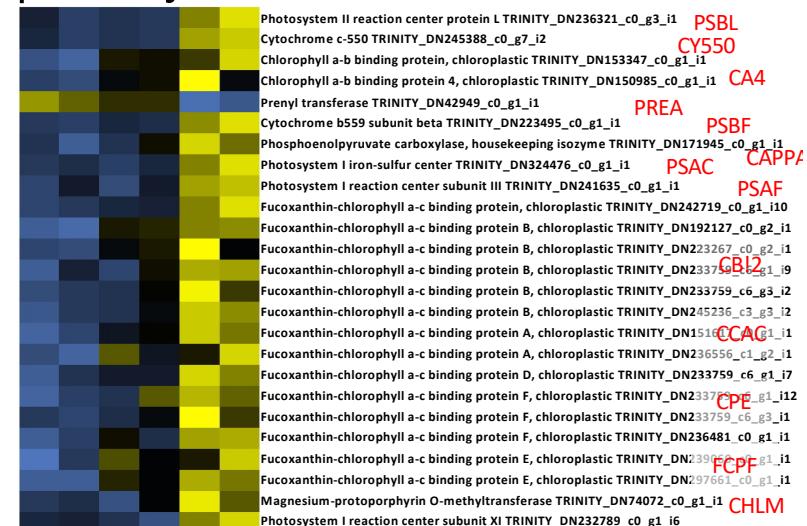
Z-score

Durusdinium (D-type)

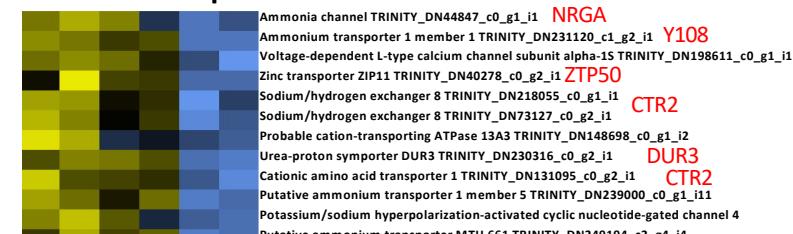
protein folding



photosynthesis



ion transport



Oxidation reduction

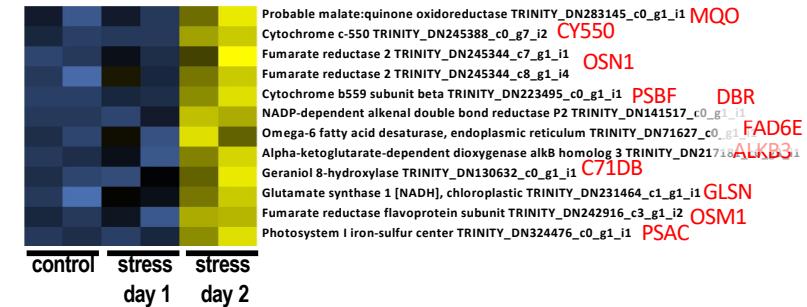


Table S1. The number of aligned sequences to the database.

Sequences aligned only with the coral database were designated as *A. solitaryensis* (coral) derived contigs (A). Sequences aligned only with the Symbiodiniaceae database were designated as Symbiodiniaceae (algal) derived contigs (B). The coral derived contigs and algal derived contigs were used as reference gene sets for differentially expression analysis.

(A)

	contigs from all samples
aligned with symbiodiniaceae sequences	45,209
aligned with coral sequences	41,343
aligned with both (coral and Symbiodiniaceae) sequences	1,307
aligned only with coral sequences	40,036

(B)

	contigs of Cladocopium-symbiosis	contigs of Durusdinium-symbiosis
aligned with symbiodiniaceae database	44,822	20,709
aligned with coral database	31,723	31,657
aligned with both (coral and Symbiodiniaceae) database	376	341
aligned only with symbiodiniaceae database	44,446	20,368

Table S2. Statistics of obtained Symbiodiniaceae (Cladocopium and Durusdinium) derived contigs and *Acropora solitaryensis* derived contigs

Statistic	Cladocopium derived contigs	Durusdinium derived contigs	<i>A. solitaryensis</i> derived contigs
Total length (bp)	51,905,796	30,472,320	35,974,968
Number of contigs	44,446	20,368	40,036
Average length	1,168	1,496	899
Median length	927	1,230	558
Max length	14,022	14,082	19,335
Min length	297	297	297
N50	1,509	1,836	1,221
GC content	55.00%	56.00%	43.92%