

Supplementary materials

# Divergent Proteomic Responses Offer Insights into Resistant Physiological Responses of a Reef-Foraminifera to Climate Change Scenarios

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## Supplementary Materials:

Figure S1: Phylogenetic assignment of photosymbionts

Figure S2: Heatmap of differentially abundant proteins per sample

Figure S3: Heatmap of differentially abundant proteins in photosymbionts

Figure S4: Heatmap of differentially abundant proteins of the host foraminifera

Figure S5: Principal component analysis of differentially abundant proteins in a) photosymbionts and b) foraminiferal host including GO term summaries

Figure S6: Principal component analysis of differentially abundant proteins in a) photosymbionts and b) foraminiferal host including protein descriptions

Figure S7: Number of up- and down-regulated proteins comparing all treatments

Figure S8: Bar plots showing the number of gene ontology term hits of (a)  $p\text{CO}_2$ - and (b) temperature-regulated proteins

Table S1: Additional data for mean chemical conditions

Table S2: Results of nominal logistic models for the frequencies of test breakage and mortality

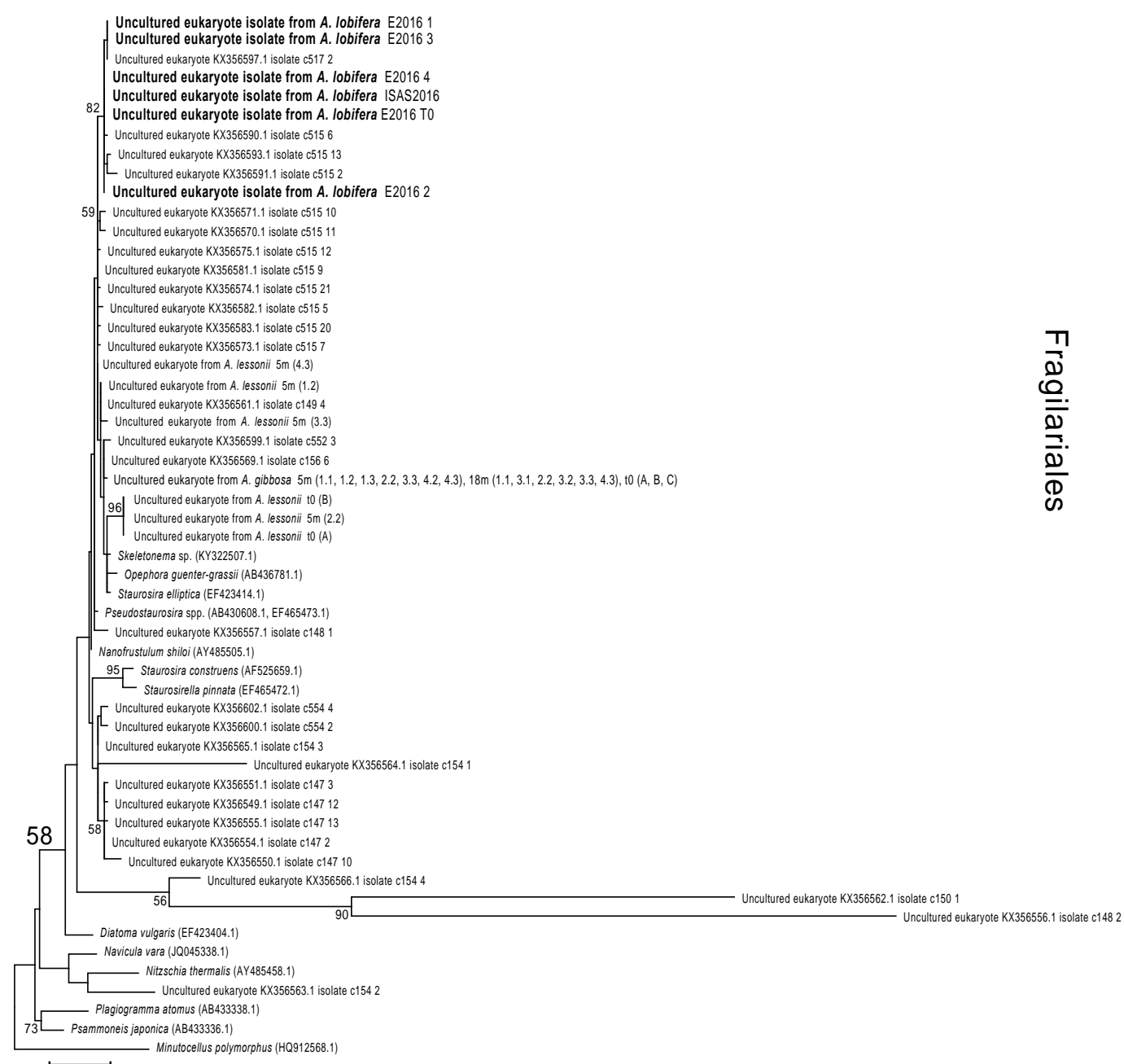
Table S3: Kruskal-Wallis non-parametric results for DBL  $\Delta[\text{H}^+]$

Table S4: Results of two-factorial analysis of variance (ANOVA) of the measured physiological parameters

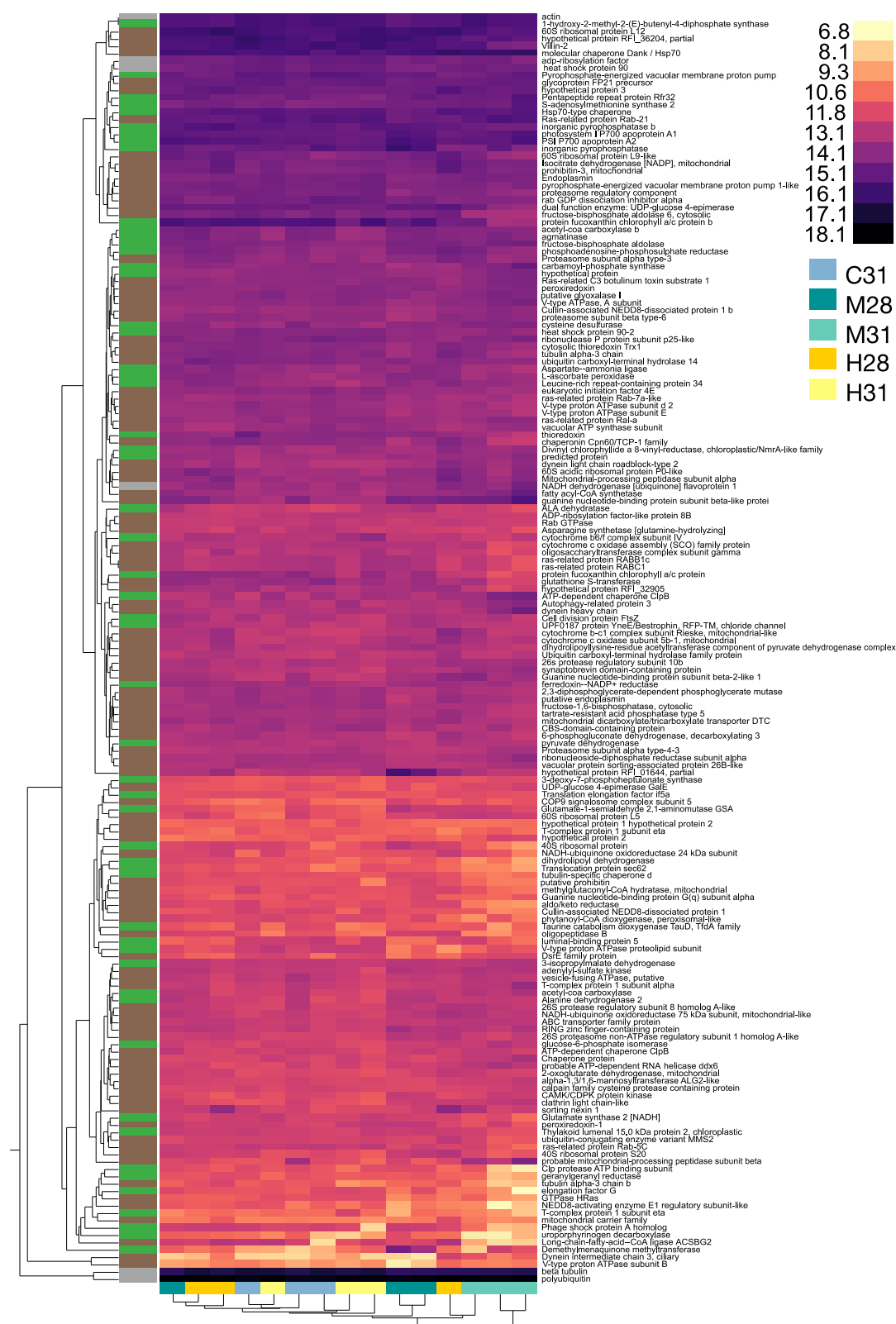
Table S5: List of all proteins detected

Table S6: List of normalized abundances values and  $\log_2\text{FC}$  of all differentially abundant proteins

Table S7: Number of DAPs in between treatments



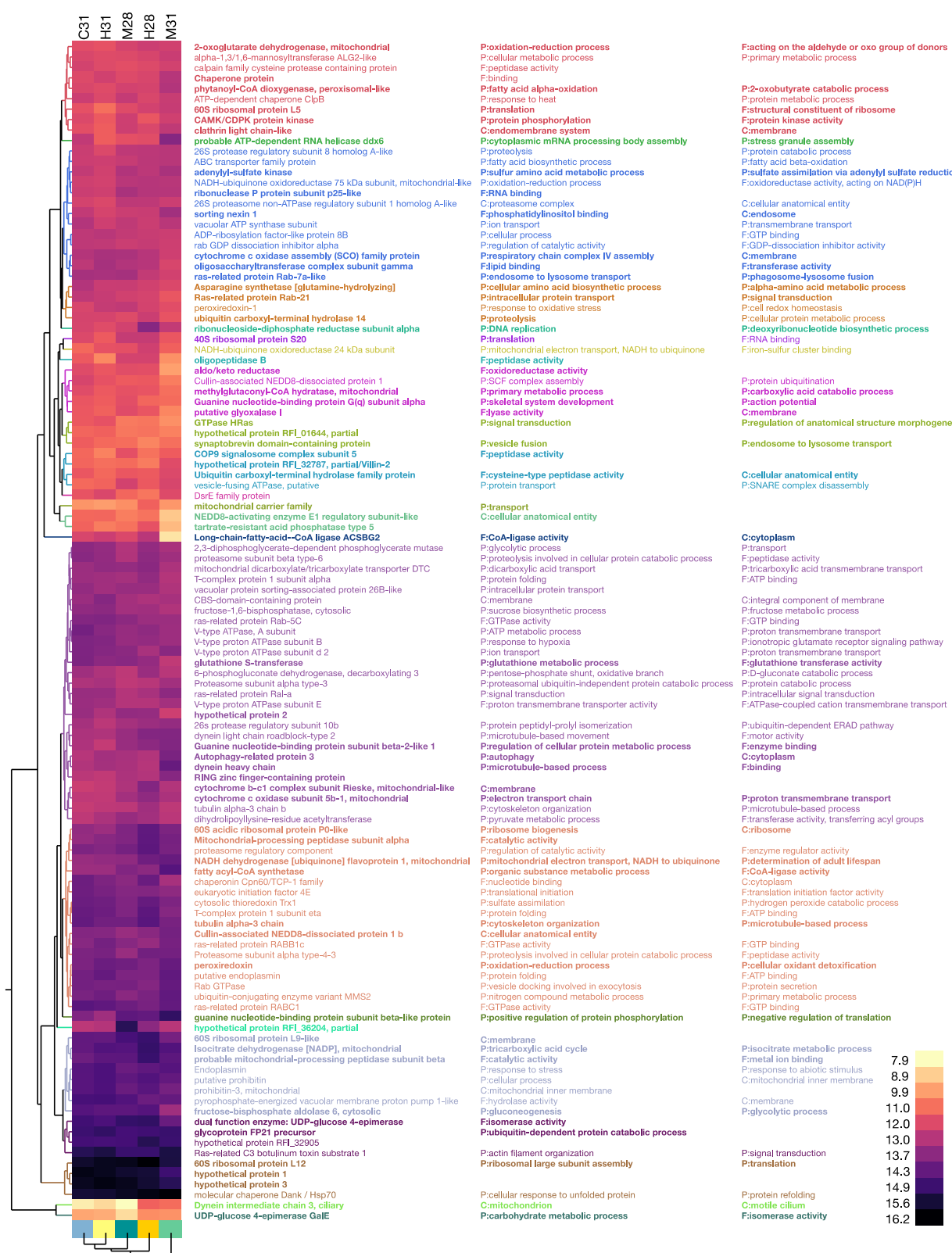
**Figure S1.** Phylogenetic assignment of photosymbionts from *Amphistegina lobifera* from the Red Sea, sampled at the Inter-University Institute (IUI) for Marine Sciences in Eilat, Israel, at about 3 m depth, as well as reference symbiont sequences from *A. lobifera* from Schmidt et al. (2016, labelled 'Uncultured eukaryote KX3565x', [1]). Additionally, *A. gibbosa* and *A. lessonii* photosymbiont sequences from Stuhr et al. (2018) [2] and sequences of diatoms formerly described from large benthic foraminifera together with the other closely related algae as identified by BLAST searches completed the dataset. Bootstrap support above 50% is given at the respective nodes of the maximum-likelihood tree. All recovered symbiont sequences were found within the Fragilariiales with a bootstrap support of 58%.



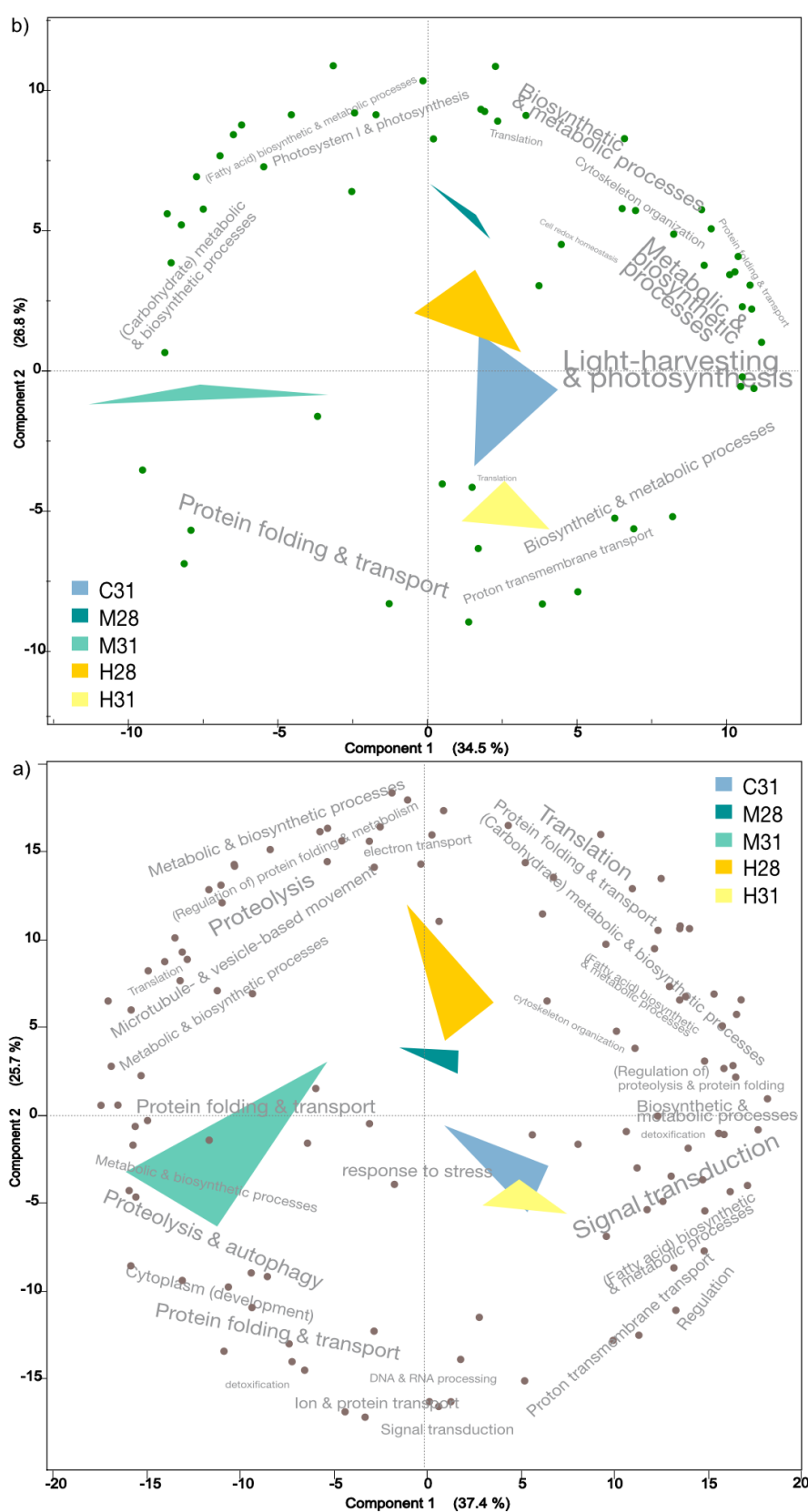
**Figure S2.** Heatmap of differentially abundant proteins in *Amphistegina lobifera* and their photosymbionts in response to treatments of elevated  $p\text{CO}_2$  (C = 492 ppm, M = 963 ppm, H = 3182 ppm) at 28 °C or 31 °C, where rows represent differentially abundant protein (n = 173) and columns represent biological replicates (n = 3 per treatment). Proteins and replicates are clustered using average linkage of log-transformed protein abundance values. Bars on the left show whether the protein was associated to: host = brown, photosymbionts = green, or both = grey.



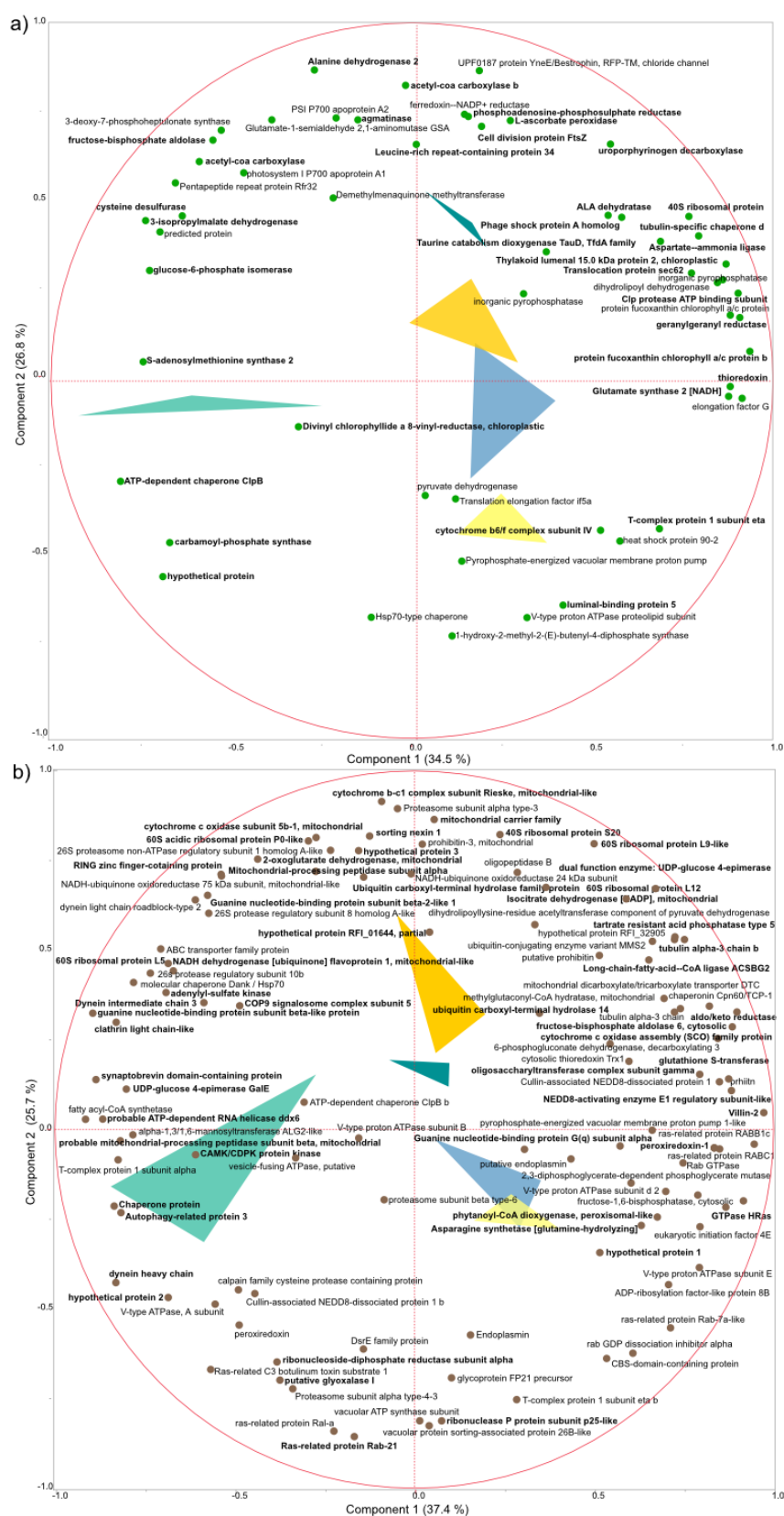
**Figure S3.** Heatmap of differentially abundant proteins in photosymbionts of *Amphistegina lobifera* in response to treatments of elevated  $p\text{CO}_2$  (C = 492 ppm, M = 963 ppm, H = 3182 ppm) at 28 °C or 31 °C, where rows represent differentially abundant proteins, for which the first two GO terms given, and columns represent the log-transformed average of three replicates per treatments. Average protein abundances were log-transformed and clustered using average linkage. Bold proteins were strongest regulated (GLM,  $q \leq 0.05$ , Tukeys'  $p \leq 0.05$ , and  $\log_2\text{FC} \geq 11$ ).



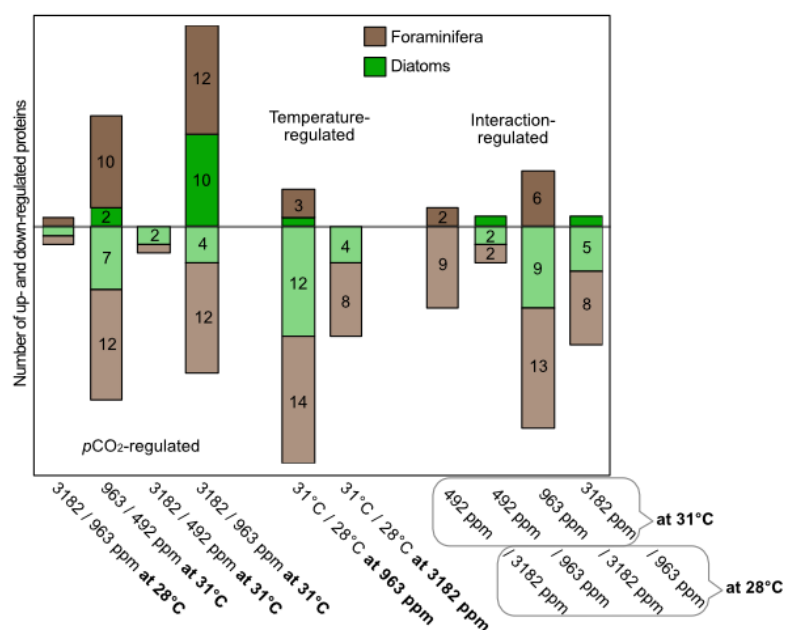
**Figure S4.** Heatmap of differentially abundant proteins of the host foraminifera *Amphistegina lobifera* in response to treatments of elevated  $p\text{CO}_2$  (C = 492 ppm, M = 963 ppm, H = 3182 ppm) at 28 °C or 31 °C, where rows represent differentially abundant proteins, for which the first two GO terms are given, and columns represent the log-transformed average of three replicates per treatments. Average protein abundances were log-transformed and clustered using average linkage. Bold proteins were strongest regulated (GLM,  $q \leq 0.05$ , Tukeys'  $p \leq 0.05$ , and  $\log_2\text{FC} \geq 11$ ).





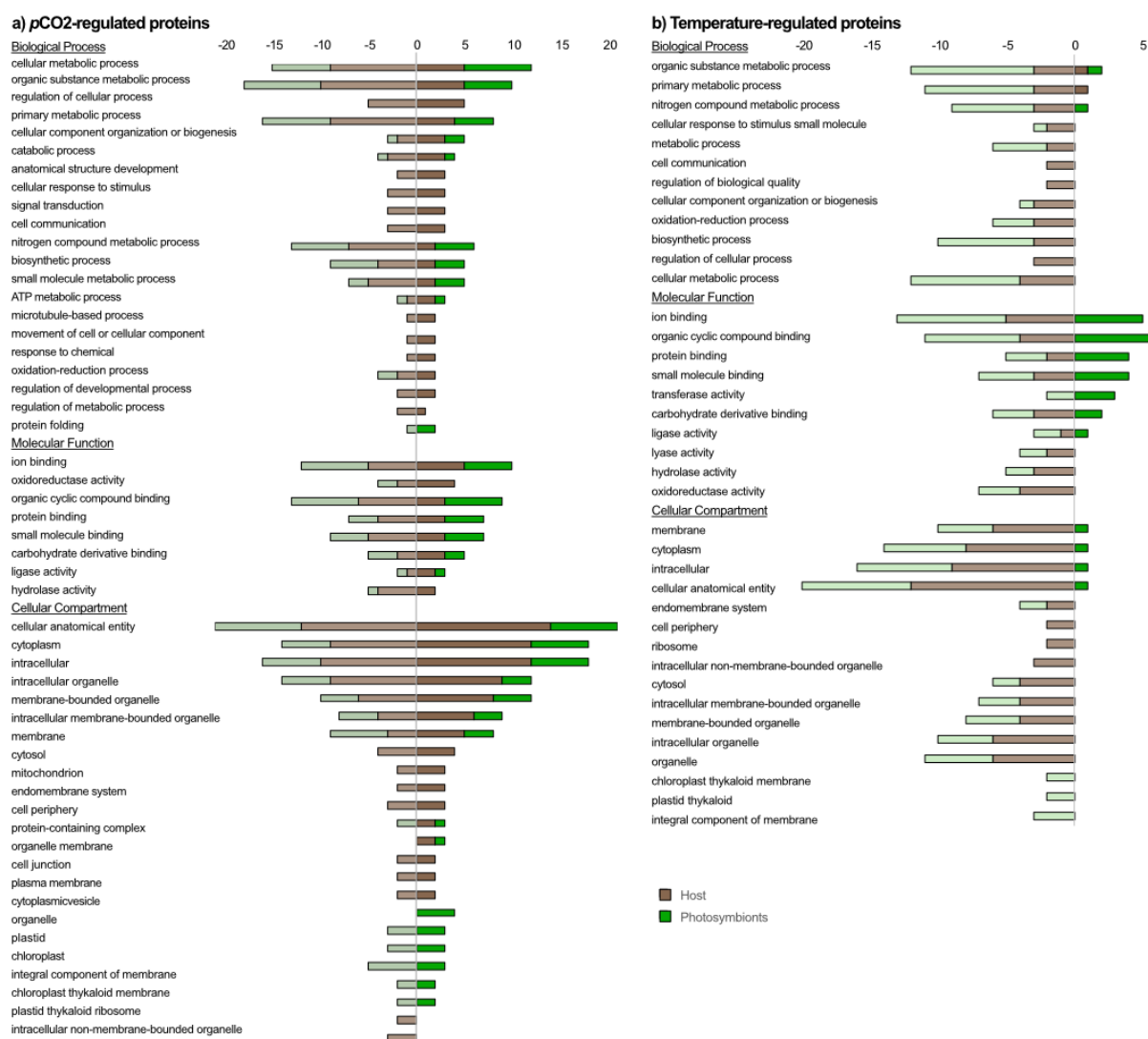


**Figure S6.** Principal component analysis biplot of differentially abundant proteins in (a) photosymbionts and (b) foraminiferal host of *Amphistegina lobifera* in response to treatments of elevated  $p\text{CO}_2$  (C = 492 ppm, M = 963 ppm, H = 3182 ppm) at 28 °C or 31 °C (legend as in Figure S5). Corners of triangles indicate triplicate samples of the treatments, dots indicate proteins with descriptions as variables. Bold proteins were strongest regulated (GLM,  $q \leq 0.05$ , Tukeys'  $p \leq 0.05$ , and  $\log_2\text{FC} \geq 1$ ).



**Figure S7.** Number of regulated proteins (i.e. DAP with Tukeys'  $p \leq 0.05$  and  $\log_2FC \geq |1|$ ) in *A. lobifera* hosts (brown) and symbionts (green), which were either significantly up- or downregulated (Tables 3, 4 and S6), comparing all treatments with each other. Contrasts are shown by groups of main effects of pCO<sub>2</sub>, temperature or both.





**Figure S8.** Bar plots showing the number of gene ontology term hits of solely (a) pCO<sub>2</sub>- and (b) temperature-regulated proteins (Figure S7) in *Amphistegina lobifera* host foraminifera (red) and photosymbiotic diatoms (purple) grouped by 3rd level GO terms for biological process, molecular functions and cellular components for selected categories with ≥2 hits (Tables 3, 4 and S6).

**Table S1.** Additional data for mean chemical conditions with standard deviations of treatments of elevated pCO<sub>2</sub> (C = 492 ppm, M = 963 ppm, H = 3182 ppm) crossed with 28 °C and 31 °C over the eight-week experiment. Salinity (Sal) and pH on NBS scale (pH<sub>NBS</sub>) in each of the replicate tanks (n = 4). Carbonate system parameters (carbonate ion concentration = [CO<sub>3</sub><sup>2-</sup>], bicarbonate ion concentration = [HCO<sub>3</sub><sup>-</sup>], dissolved CO<sub>2</sub> = [CO<sub>2</sub>]<sub>sw</sub>) were calculated from weekly measurement of TA, DIC, salinity, and temperature (n = 8).

Treatment	Sal (psu)	pH <sub>NBS</sub>	[CO <sub>3</sub> <sup>2-</sup> ] (μM)	[HCO <sub>3</sub> <sup>-</sup> ] (μM)	[CO <sub>2</sub> ] <sub>sw</sub> (μM)
C28	35.05 ± 0.69	8.27 ± 0.10	340 ± 72	2133 ± 179	13 ± 2
C31	35.75 ± 0.73	8.24 ± 0.08	294 ± 71	2042 ± 231	12 ± 1
M28	35.54 ± 0.56	8.04 ± 0.10	232 ± 120	2394 ± 291	26 ± 4
M31	35.98 ± 0.59	8.12 ± 0.10	262 ± 39	2480 ± 170	23 ± 3
H28	35.79 ± 0.52	7.63 ± 0.11	88 ± 22	2703 ± 426	80 ± 21
H31	35.76 ± 0.6	7.69 ± 0.10	112 ± 29	3040 ± 280	85 ± 21

**Table S2.** Results of nominal logistic models for the frequencies of test breakage and mortality (empty tests) on *Amphistegina lobifera* exposed to elevated  $p\text{CO}_2$  (C = 492 ppm, M = 963 ppm, H = 3182 ppm) crossed with temperature (Temp.) of 28 °C and 31 °C for eight weeks. Bold values indicate significant effects ( $p \leq 0.05$ ). Marginally significant variations between (groups of) treatments are indicated in grey ( $0.07 > p\text{-value} > 0.05$ ).

Parameter	Effect	DF	$\chi^2$	$p\text{-value}$
Mortality	Temp.	1	0.503	0.478
	$p\text{CO}_2$	2	0.606	0.739
	$p\text{CO}_2 \times \text{Temp.}$	2	3.982	0.136
Breakage	Temp.	1	0.557	0.456
	$p\text{CO}_2$	2	0.988	0.610
	$p\text{CO}_2 \times \text{Temp.}$	2	5.575	<b>0.062</b>

**Table S3.** Permutational multivariate analysis results for  $\Delta[\text{H}^+]$  between seawater and the test surface of *Amphistegina lobifera* for the fixed effects of  $p\text{CO}_2$  (C = 492 ppm, M = 963 ppm, H = 3182 ppm) and temperature (Temp., 28 °C, 31 °C), or light/dark condition (left side), with bold values indicating significant effects ( $p \leq 0.05$ ); and significant adjusted  $p$ -values of pairwise comparison of all  $\Delta[\text{H}^+]$  at and FDR of 0.1.

Effect	DF	MS	F	Pr(>F)	Contrasting treatment combinations		$p\text{-adj.}$
$p\text{CO}_2$	2	5.033e31	6.952	<b>0.003</b>	H31 light	M31 dark	0.0017
Temp.	1	1.339e30	0.185	0.664	H31 light	C31 dark	0.0017
$p\text{CO}_2 \times \text{Temp.}$	1	-5.211e29	-0.072	0.422	H31 light	C28 dark	0.0017
Light/dark	1	2.82e32	5.207	<b>0.021</b>	H31 light	C31 light	0.0017
					H31 light	M31 light	0.0066
					H31 light	C28 light	0.0066
					H31 light	H28 dark	0.0066
					H31 light	M28 light	0.0066
					H31 light	H31 dark	0.0117
					H31 light	M28 dark	0.0165
					H31 light	H28 light	0.0618
					H28 light	M31 dark	0.0957

**Table S4.** Results of two-factorial analysis of variance (ANOVA) of the measured physiological parameters of *Amphistegina lobifera* and its photosymbionts exposed to elevated  $p\text{CO}_2$  (C = 492 ppm, M = 963 ppm, H = 3182 ppm) crossed with temperature (Temp.) of 28 °C and 31 °C for eight weeks. Bold values indicate significant effects ( $p \leq 0.05$ ). For significant effects, the analysis was followed by Tukeys' HSD post-hoc test for comparison of differences. Respective  $p$ -values of significant post-hoc results are given in brackets. Only marginally significant variations between (groups of) treatments are indicated in grey ( $0.07 > p\text{-value} > 0.05$ ).

Parameter	Effect	DF	MS	F-ratio	$p\text{-value}$	post-hoc test summary
Chl <i>a</i>	Temp.	1	0.017	0.630	0.438	
	$p\text{CO}_2$	2	0.001	0.050	0.951	
	$p\text{CO}_2 \times \text{Temp.}$	2	0.000	0.015	0.985	
<i>L</i> *	Temp.	1	3.896	0.094	0.763	
	$p\text{CO}_2$	2	2.443	0.059	0.943	
	$p\text{CO}_2 \times \text{Temp.}$	2	49.191	1.183	0.329	
<i>a</i> *	Temp.	1	5.802	0.542	0.471	
	$p\text{CO}_2$	2	4.555	0.425	0.660	
	$p\text{CO}_2 \times \text{Temp.}$	2	2.738	0.256	0.777	
<i>b</i> *	Temp.	1	0.683	0.096	0.760	
	$p\text{CO}_2$	2	27.530	3.867	<b>0.040</b>	C > H (0.053)
	$p\text{CO}_2 \times \text{Temp.}$	2	9.043	1.270	0.305	
Growth rate	Temp.	1	0.008	5.029	<b>0.038</b>	28 > 31 (0.038)
	$p\text{CO}_2$	2	0.002	1.200	0.324	
	$p\text{CO}_2 \times \text{Temp.}$	2	0.003	1.928	0.174	

Pore size	Temp.	1	0.951	0.375	0.542	
	$p\text{CO}_2$	2	5.820	2.295	0.108	
	$p\text{CO}_2 \times \text{Temp.}$	2	11.396	4.493	<b>0.015</b>	C31 > M31 (0.030), H31 (0.040)

**Tables S5** and **S6** are provided as Excel file in the electronic supplementary materials.

**Table S7.** Number of DAPs in *Amphistegina lobifera* between two treatments of elevated  $p\text{CO}_2$  (C = 492 ppm, M = 963 ppm, H = 3182 ppm) at 28°C and 31°C, without the control treatment C28. Values in the upper right half (white, 193 in total) represent host-associated proteins and values in the lower left half (grey, 97 in total) represent symbiont-associated proteins. Nine DAPs were assigned to both compartments and are indicated as subscript <sup>+</sup>x next to photosymbiont DAPs. Respective protein descriptions with annotated gene ontologies are provided in Table S6.

	C31	M28	M31	H28	H31
C31		10	32	17	1
M28	5 <sup>+1</sup>		28	8	15
M31	11 <sup>+1</sup>	19 <sup>+1</sup>		30	32
H28	5 <sup>+1</sup>	3 <sup>+1</sup>	11 <sup>+1</sup>		20
H31	5 <sup>+1</sup>	14 <sup>+2</sup>	18 <sup>+1</sup>	6 <sup>+0</sup>	

## Reference

- Schmidt, C.; Morard, R.; Prazeres, M.; Barak, H.; Kucera, M. Retention of high thermal tolerance in the invasive foraminifera *Amphistegina lobifera* from the Eastern Mediterranean and the Gulf of Aqaba. *Mar. Biol.* **2016**, *163*, 228.
- Stuhr, M.; Meyer, A.; Reymond, C.E.; Narayan, G.R.; Rieder, V.; Rahnenführer, J.; Kucera, M.; Westphal, H.; Muhando, C.A.; Hallock, P. Variable thermal stress tolerance of the reef-associated symbiont-bearing foraminifera *Amphistegina* linked to differences in symbiont type. *Coral Reefs* **2018**, *37*, 811–824.