Supplementary materials to article

Divergent proteomic responses offer insights into resistant physiological responses of a reef-foraminifera to climate change scenarios

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Supplementary Materials:

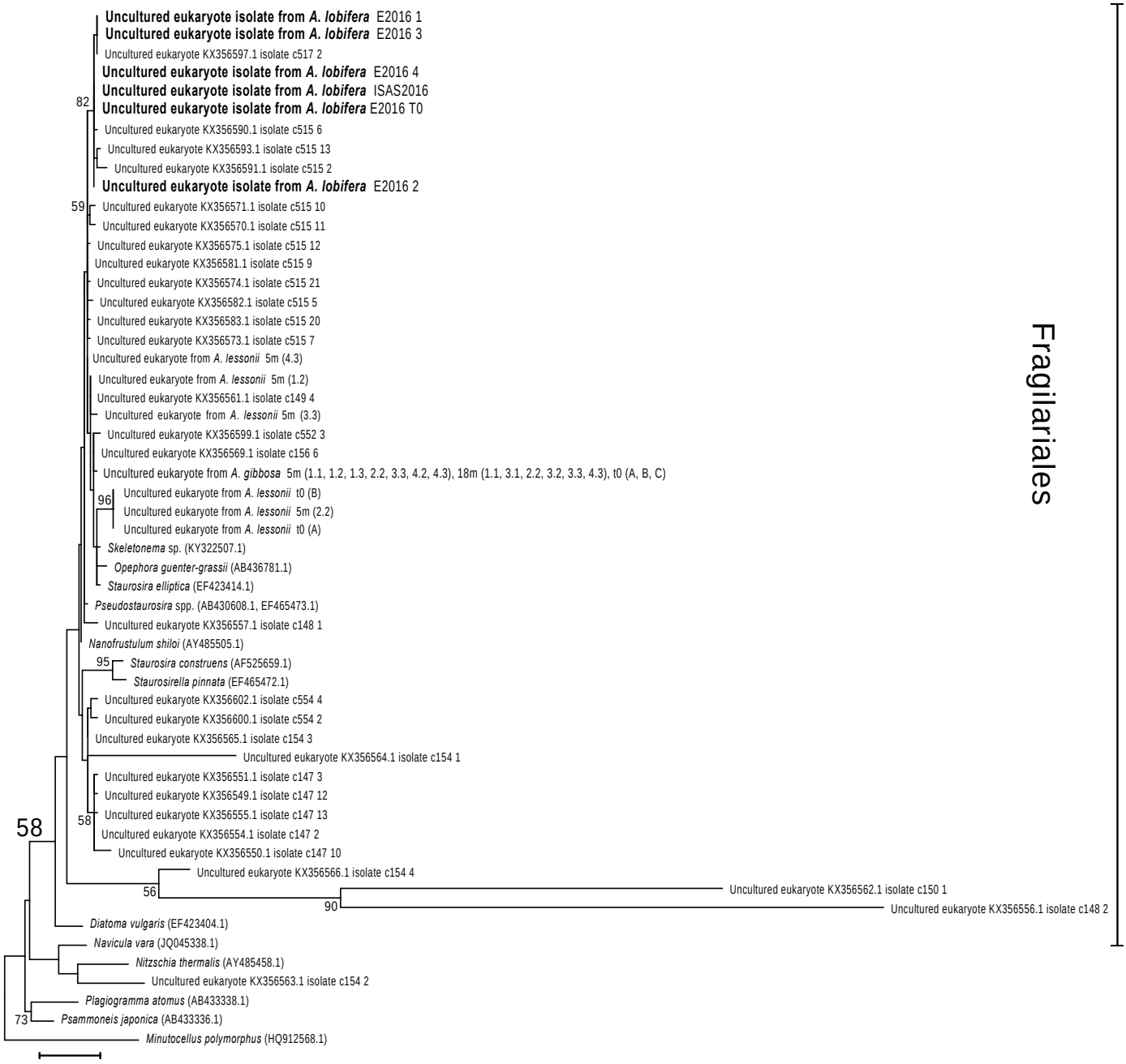

Figure S1. Phylogenetic assignment of photosymbionts from *Amphistegina lobifera* from the Red Sea, sampled at the Inter-University Institute (IUI) for Marine Sciences in Eilat, Israel, at about 3 m depth, as well as reference symbiont sequences from *A. lobifera* from Schmidt et al. (2016, labelled 'Uncultured eukaryote KX3565x'). Additionally, *A. gibbosa* and *A. lessonii* photosymbiont sequences from Stuhr et al. (2018) 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 Fragilariales with a bootstrap support of 58%.

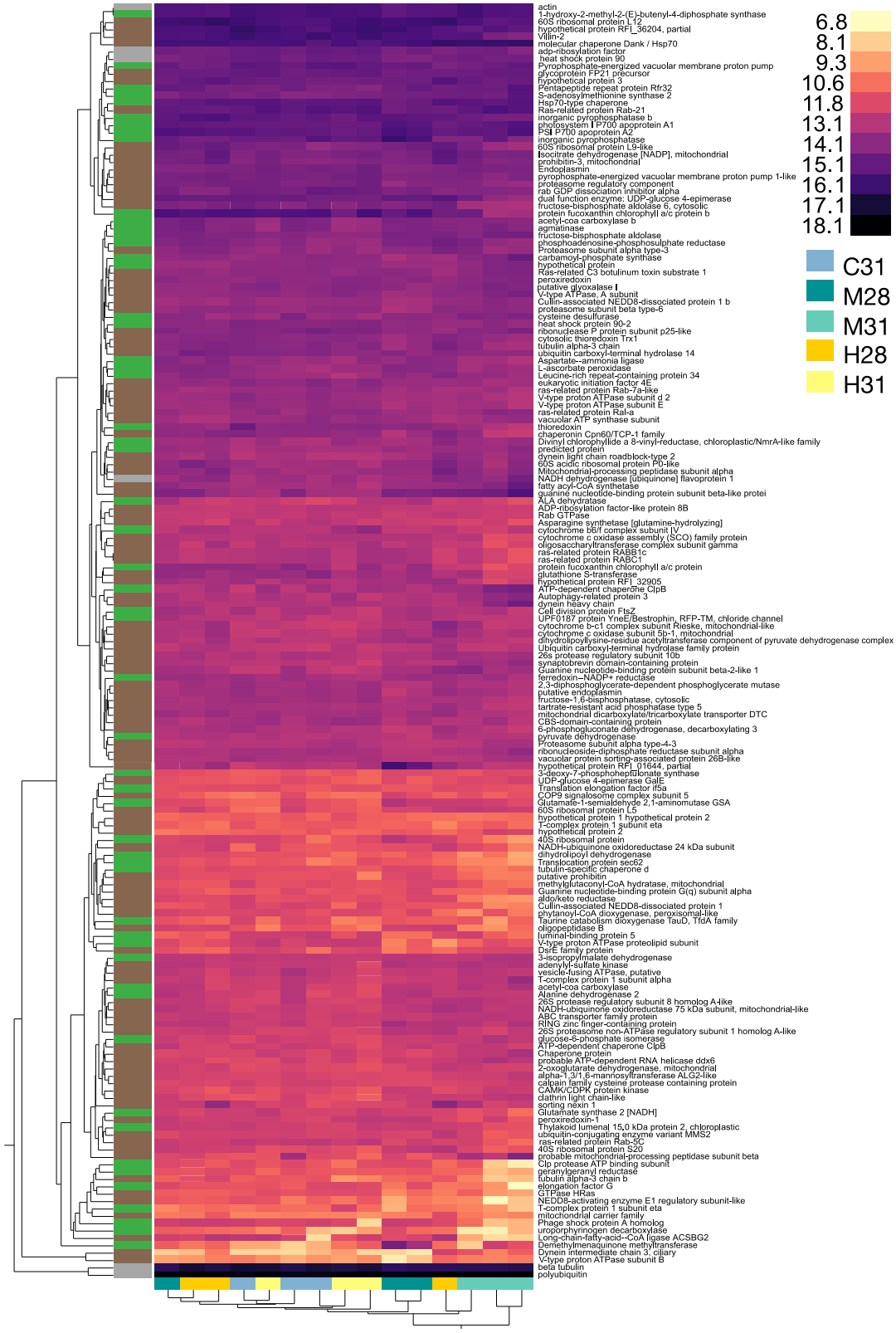

Figure S2. Heatmap of differentially abundant proteins in *Amphistegina lobifera* and their photosymbionts in response to treatments of elevated pCO_2 (C = 492 ppm, M = 963 ppm, H = 3182 ppm) at 28°C or 31°C, where rows represent differentially abundant protein (n = 173) and columns represent biological 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 pCO_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. 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 $\log_2 FC \geq |1|$).

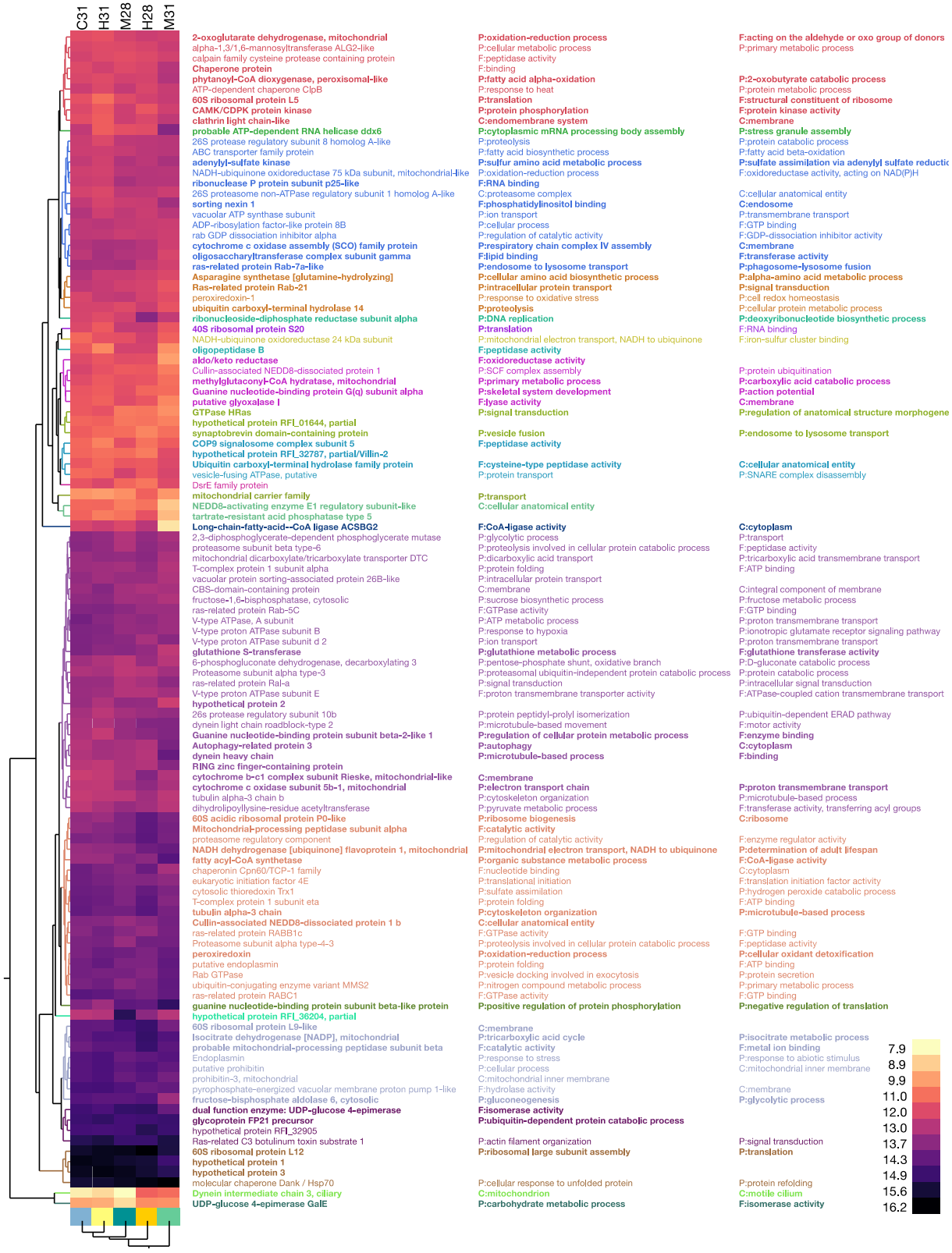

Figure S4. Heatmap of differentially abundant proteins of the host foraminifera *Amphistegina lobifera* in response to treatments of elevated pCO_2 (C = 492 ppm, M = 963 ppm, H = 3182 ppm) at 28°C or 31°C, where rows represent differentially abundant proteins, for which the first two GO terms are given. 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 $\log_2 FC \geq |1|$).

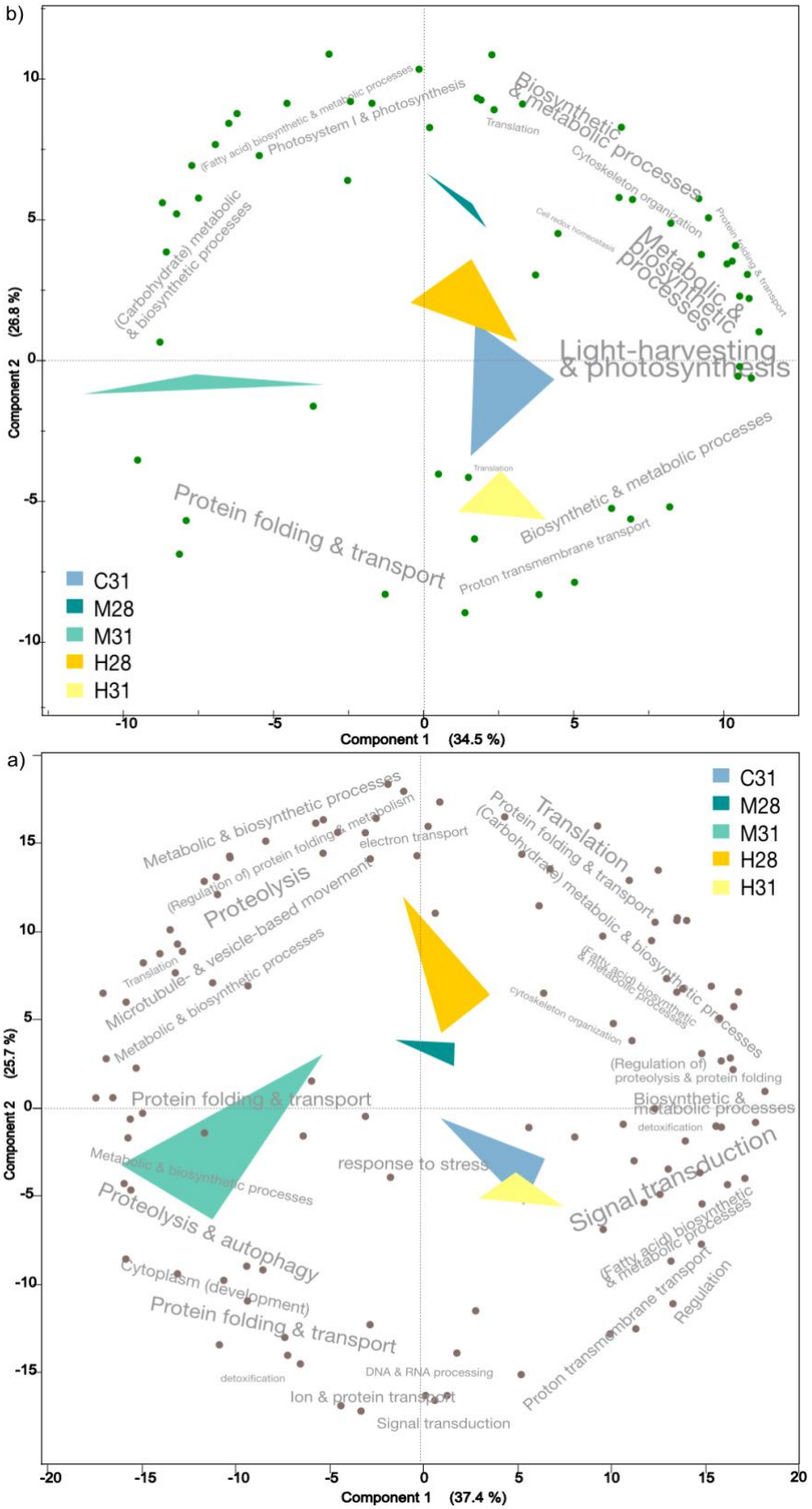

Figure S5. Principal component analysis biplot of differentially abundant proteins in (**a**) photosymbionts and (**b**) foraminiferal host of *Amphistegina lobifera* in response to treatments of elevated pCO_2 (C = 492 ppm, M = 963 ppm, H = 3182 ppm) at 28°C or 31°C, indicating associated groups of protein functions or processes. Corners of triangles indicate triplicate samples of the treatments, dots indicate proteins as variables. The respective protein names are given in Figure S6.

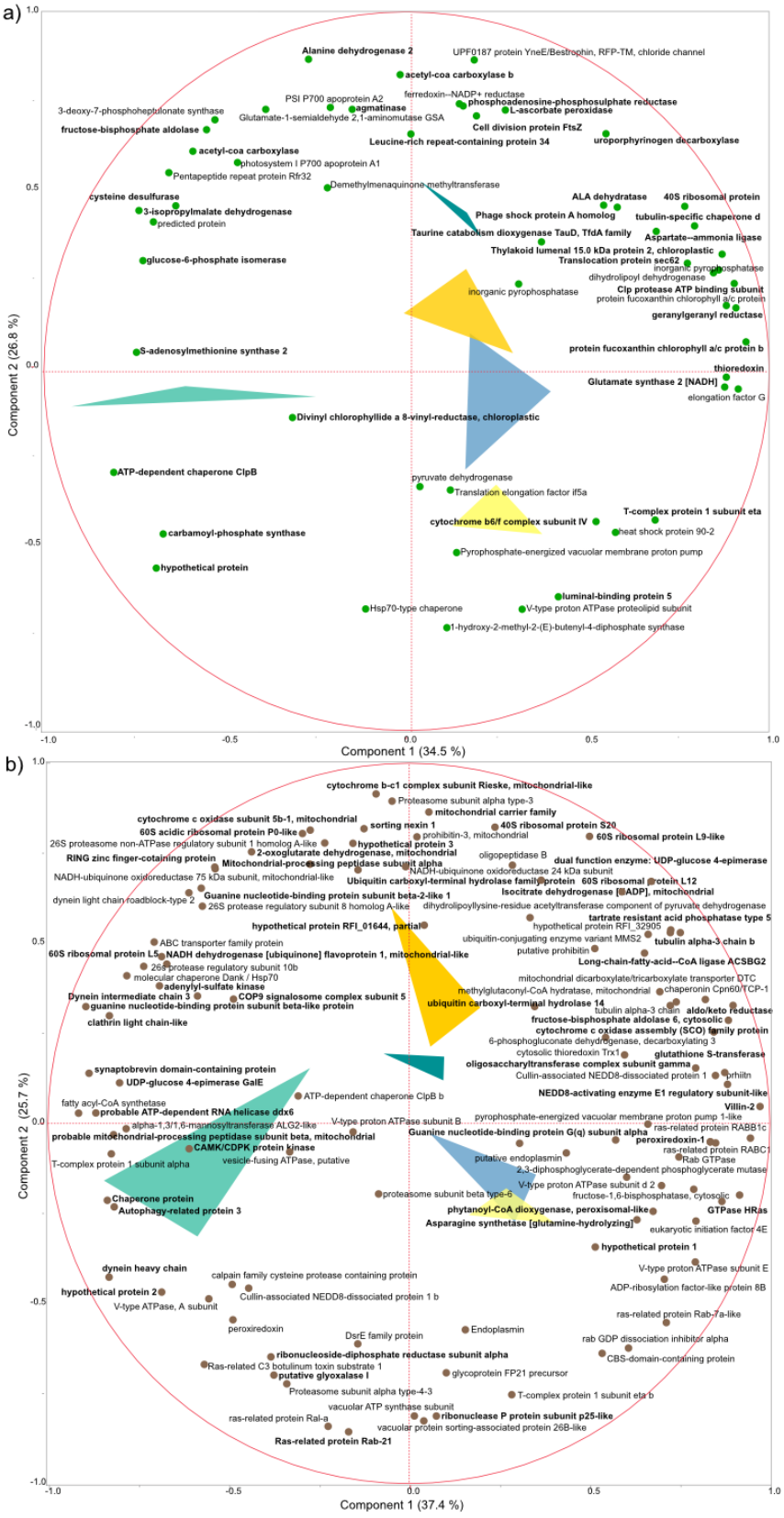

Figure S6. Principal component analysis biplot of differentially abundant proteins in (**a**) photosymbionts and (**b**) foraminiferal host of *Amphistegina lobifera* in response to treatments of elevated pCO_2 (C = 492 ppm, M = 963 ppm, H = 3182 ppm) at 28°C or 31°C (legend as in Figure S5). Corners 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 $\log_2 FC \geq |1|$).

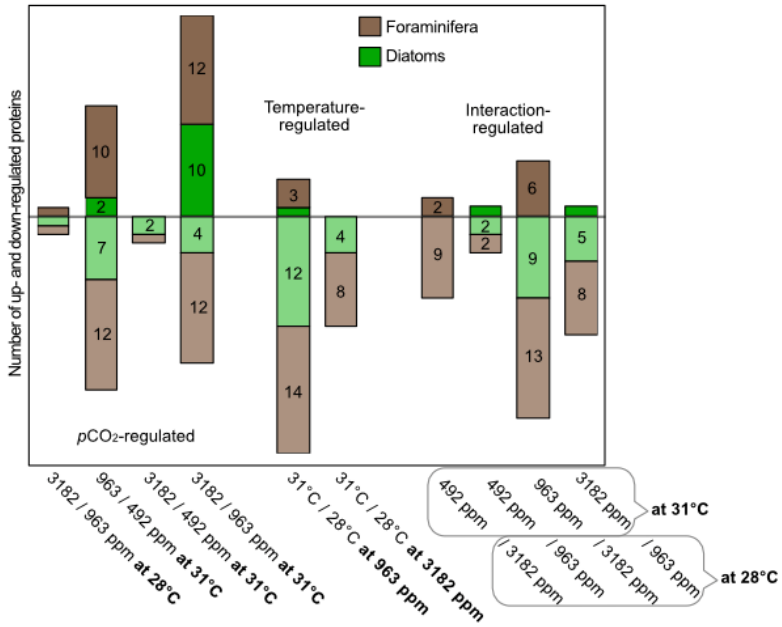

Figure S7. Number of regulated proteins (i.e. DAP with Tukeys' $p \leq 0.05$ and $\log_2 FC \geq |1|$) in *A. lobifera* hosts (brown) and symbionts (green), which were either significantly up- or downregulated (Tables 3, 4 and S6), comparing all treatments with each other. Contrasts are shown by groups of main effects of pCO_2, temperature or both.

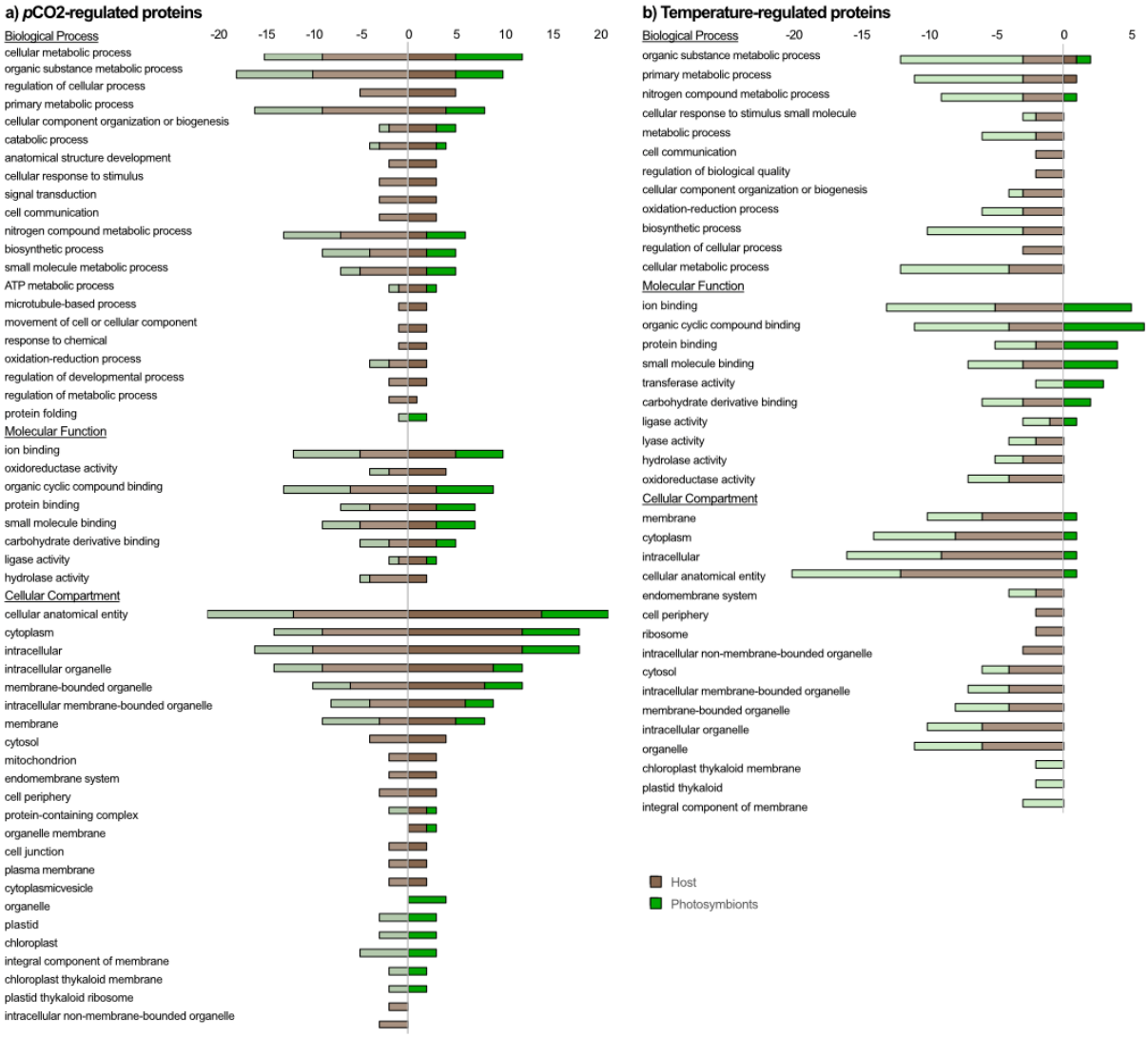

Figure S8. Bar plots showing the number of gene ontology term hits of solely (**a**) pCO_2- and (**b**) temperature-regulated proteins (Figure S7) in *Amphistegina lobifera* host foraminifera (red) and photosymbiotic diatoms (purple) grouped by 3rd level GO terms for biological process, molecular functions 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_2 (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_{NBS}) in each of the replicate tanks (n = 4). Carbonate system parameters (carbonate ion concentration = $[CO_3^{2-}]$, bicarbonate ion concentration = $[HCO_3^-]$, dissolved CO_2 = $[CO_2]_{SW}$) were calculated from weekly measurement of TA, DIC, salinity, and temperature (n = 8).

Treatment	Sal (psu)	pH_{NBS}	$[CO_3^{2-}]$ (µM)	$[HCO_3^-]$ (µM)	$[CO_2]_{SW}$ (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 pCO_2 (C = 492 ppm, M = 963 ppm, H = 3182 ppm) crossed with temperature (T) 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	χ^2	*p*-value
Mortality	T	1	0.503	0.478
	pCO_2	2	0.606	0.739
	$pCO_2 \times T$	2	3.982	0.136
Breakage	T	1	0.557	0.456
	pCO_2	2	0.988	0.610
	$pCO_2 \times T$	2	5.575	**0.062**

Table S3. Permutational multivariate anaylsis results for $\Delta[H^+]$ between seawater and the test surface of *Amphistegina lobifera* for the fixed effects of pCO_2 (C = 492 ppm, M = 963 ppm, H = 3182 ppm) and temperature (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 $\Delta[H^+]$ at and FDR of 0.1.

Effect	DF	MS	F	Pr(>F)
pCO_2	2	5.033e31	6.952	**0.003**
T	1	1.339e30	0.185	0.664
$pCO_2 \times T$	1	-5.211e29	-0.072	0.422
Light/dark	1	2.82e32	5.207	**0.021**

Contrasting treatment combinations		*p*-adj.
H31 light	M31 dark	0.0017
H31 light	C31 dark	0.0017
H31 light	C28 dark	0.0017
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 pCO_2 (C = 492 ppm, M = 963 ppm, H = 3182 ppm) crossed with temperature (T) 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*-value	post-hoc test summary
Chl *a*	T	1	0.017	0.630	0.438	
	pCO_2	2	0.001	0.050	0.951	
	$pCO_2 \times$ T	2	0.000	0.015	0.985	
*L**	T	1	3.896	0.094	0.763	
	pCO_2	2	2.443	0.059	0.943	
	$pCO_2 \times$ T	2	49.191	1.183	0.329	
*a**	T	1	5.802	0.542	0.471	
	pCO_2	2	4.555	0.425	0.660	
	$pCO_2 \times$ T	2	2.738	0.256	0.777	
*b**	T	1	0.683	0.096	0.760	
	pCO_2	2	27.530	3.867	**0.040**	C > H (0.053)
	$pCO_2 \times$ T	2	9.043	1.270	0.305	
Growth rate	T	1	0.008	5.029	**0.038**	28 > 31 (0.038)
	pCO_2	2	0.002	1.200	0.324	
	$pCO_2 \times$ T	2	0.003	1.928	0.174	
Pore size	T	1	0.951	0.375	0.542	
	pCO_2	2	5.820	2.295	0.108	
	$pCO_2 \times$ T	2	11.396	4.493	**0.015**	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 pCO_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 $^{+x}$ 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^{+1}		28	8	15
M31	11^{+1}	19^{+1}		30	32
H28	5^{+1}	3^{+1}	11^{+1}		20
H31	5^{+1}	14^{+2}	18^{+1}	6^{+0}	