

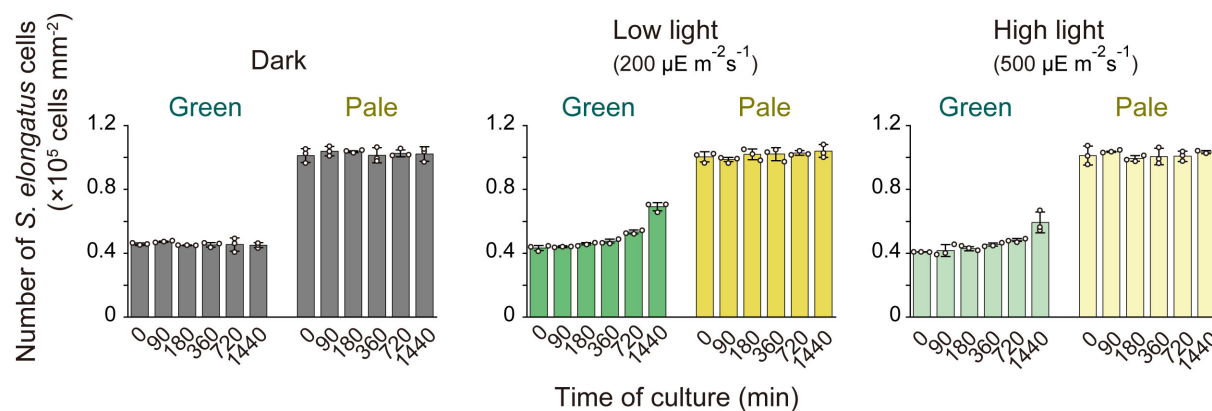
Supplementary Information

Responses of unicellular predators to cope with the phototoxicity of photosynthetic prey

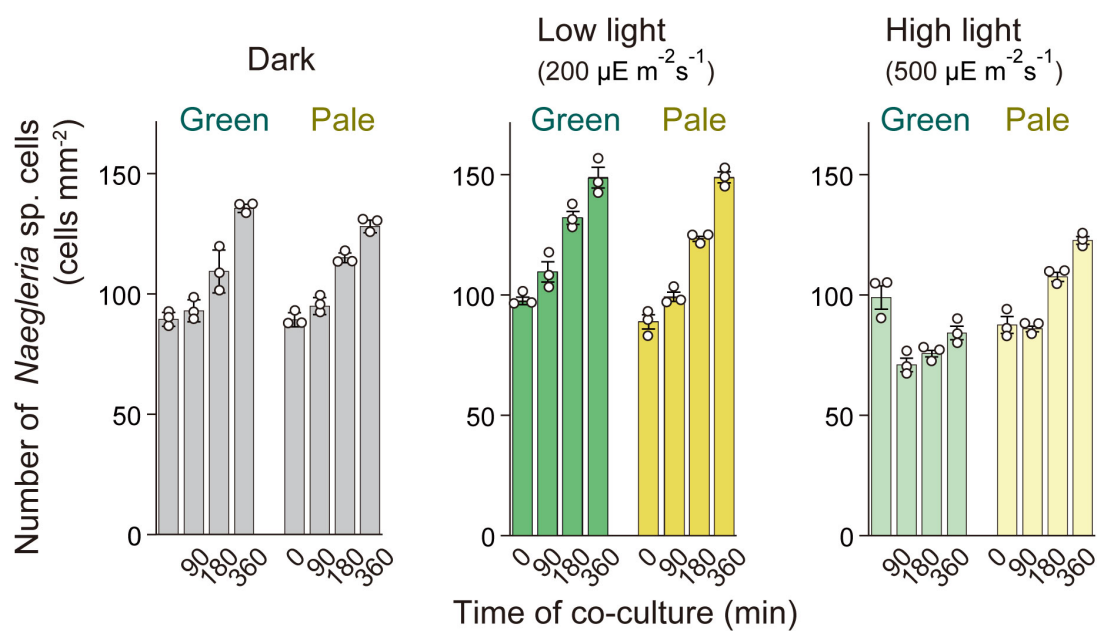
Uzuka, Kobayashi, Onuma et al.

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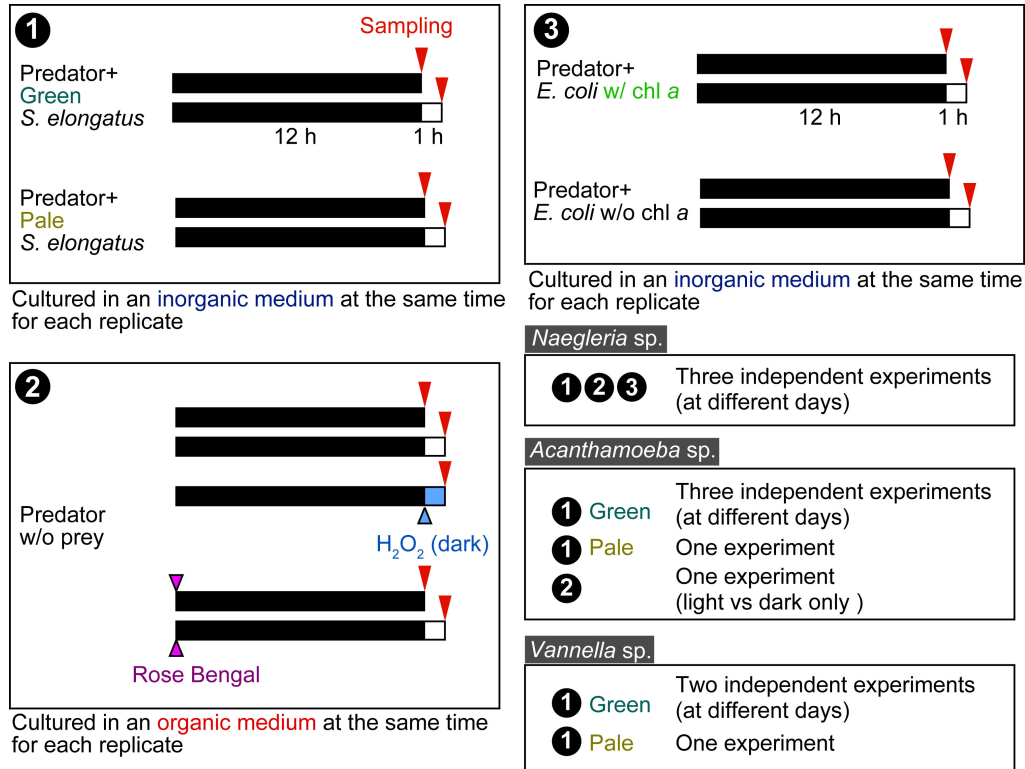
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Supplementary Fig. 1. The changes in the cell number of green or pale *S. elongatus* under dark, low-light, or high-light conditions. Green or pale *S. elongatus* without *Naegleria* sp. was cultured in inorganic medium on Petri dishes as in Fig. 2a for 24 h and the change in cell number was determined. The error bar represents the standard deviation of three independent cultures (cultured at the same time). The details are described in Fig. 2. Source data are provided as a Source Data file.



Supplementary Fig. 2. The changes in the cell number of *Naegleria* sp. feeding on green or pale *S. elongatus* under dark, low-light, or high-light conditions. The details are described in Fig. 2. The error bar represents the standard deviation of three independent cultures (cultured at the same time). Source data are provided as a Source Data file.



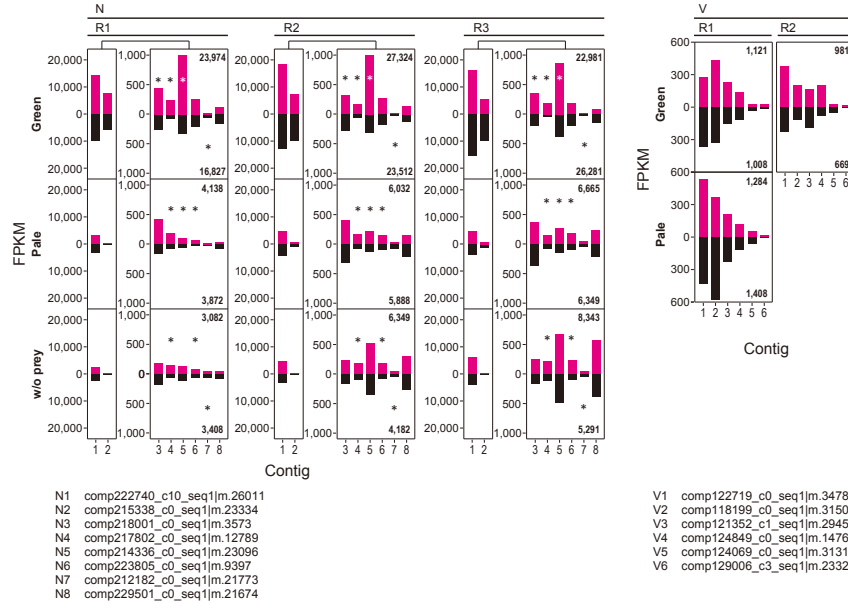
Supplementary Fig. 3. Schematic diagram of the culture conditions for transcriptome analyses. The excavate *Naegleria* sp. and the amoebozoans *Acanthamoeba* sp. and *Vannella* sp. were used as predators. Four types of bacteria, green and pale *S. elongatus* and *E. coli* with or without chlorophyll *a* staining, were used as prey. The co-culture in inorganic medium was incubated at 20 °C in the dark for 12 h and then illuminated ($200 \mu\text{E m}^{-2} \text{s}^{-1}$) for 1 h (group 1 for the three species and group 3 for *Naegleria* sp. only).

Cells were also cultured without bacterial prey in organic medium (group 2; only for *Naegleria* sp. and *Acanthamoeba* sp. because *Vannella* sp. did not grow under this condition). The cells were cultured in the dark for 12 h and were either illuminated ($200 \mu\text{E m}^{-2} \text{s}^{-1}$) or incubated in the dark with 0.1 mM H₂O₂ (*Naegleria* sp. only) for 1 h. For the RB treatment (*Naegleria* sp. only), RB was added to the culture at the onset of the incubation for 12 h in the dark, followed by illumination for 1 h.

As shown in the figure, cultures of each replicate of group 1 (co-culture of the cells and green or pale *S. elongatus* prey), group 2 (culture of the cells without bacterial prey), and group 3 (co-culture of the cells and *E. coli* with or without chlorophyll *a* staining) were performed at the same time for RNA extraction.

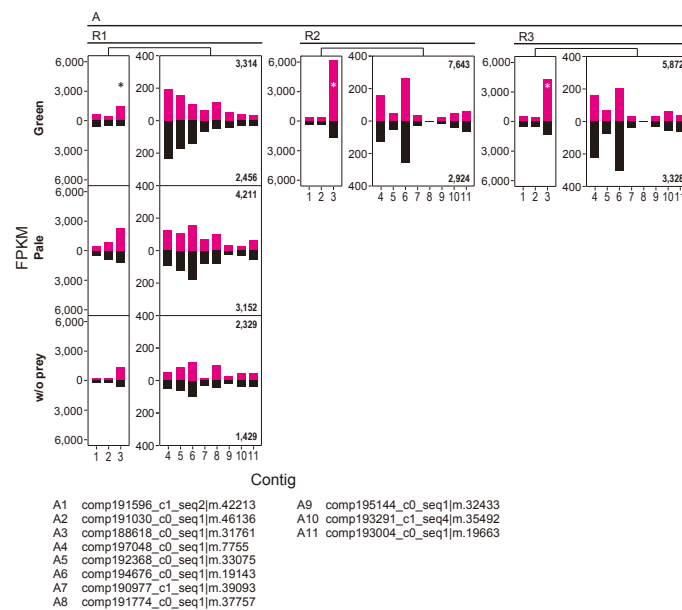
Cultures of *Naegleria* sp. in all of the conditions described above were performed three times on different days with *Naegleria* sp. cells newly prepared from the original frozen stock and bacterial prey prepared at the time of use. Because *Acanthamoeba* sp. and *Vannella* sp. growth became unstable after long-term storage, the number of experimental replicates became limited. Cultures of *Acanthamoeba* sp. with green *S. elongatus* prey were performed three times, while those of *Vannella* sp. were performed two times on different days with cells newly prepared from the original frozen stock and bacterial prey prepared at the time of use. Cultures in other conditions were performed once.

Oxidation and reduction
Oxidative stress response 1
Thioredoxin



Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp. Respective cells were co-cultured with green or pale *S. elongatus* prey in the dark for 12 h and then transferred to light ($200 \mu\text{E m}^{-2} \text{s}^{-1}$) conditions for 1 h. The cultures with green prey were performed three times (R1, R2, and R3) for *Naegleria* sp. and *Acanthamoeba* sp., while those for *Vannella* sp. were performed two times (R1 and R2) independently on different days. To examine the effect of illumination that is independent of prey, respective species were cultured in organic growth medium without bacterial prey in the dark for 12 h and then transferred to light conditions for 1 h. *Naegleria* sp. cultures were also performed three times independently on different days (R1, R2, and R3), while cultures of *Acanthamoeba* sp. and *Vannella* sp. were performed once. Graphs show the mRNA levels (FPKM values) of contigs (genes) that are related to oxidation and reduction/oxidative stress responses, carotenoid synthesis, DNA repair, respiration and oxygen-consuming metabolism or encoding myosin, actin and PAO-like proteins under dark (black bar) and light (magenta bar) conditions. Each bar corresponds to one contig. For example, 8, 11, and 6 contigs (genes) encoded thioredoxin in *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp., respectively. Numbers in the graph indicate total FPKM values in respective conditions. The contig IDs are shown beside each graph. *, FDR < 0.01 (edgeR) only when datasets of three independent replicates were obtained. The details of the culture conditions are described in Supplementary Fig. 3.

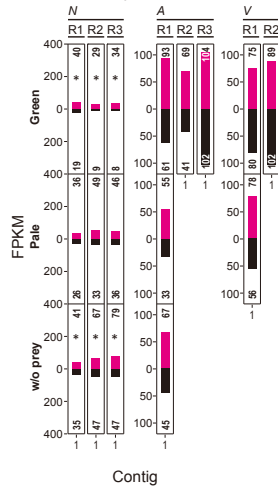
Oxidation and reduction
Oxidative stress response 1
Thioredoxin



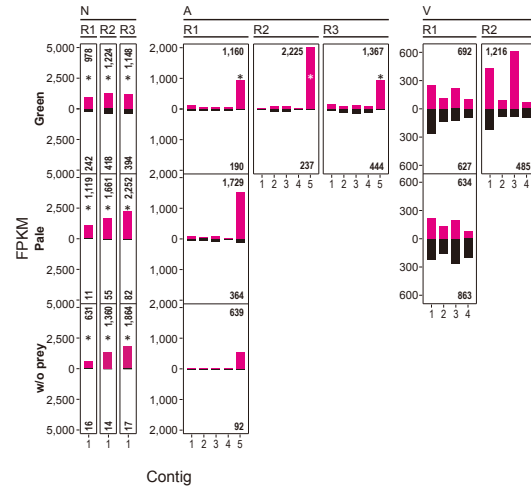
Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp. (continued)

Oxidation and reduction
Oxidative stress response 2

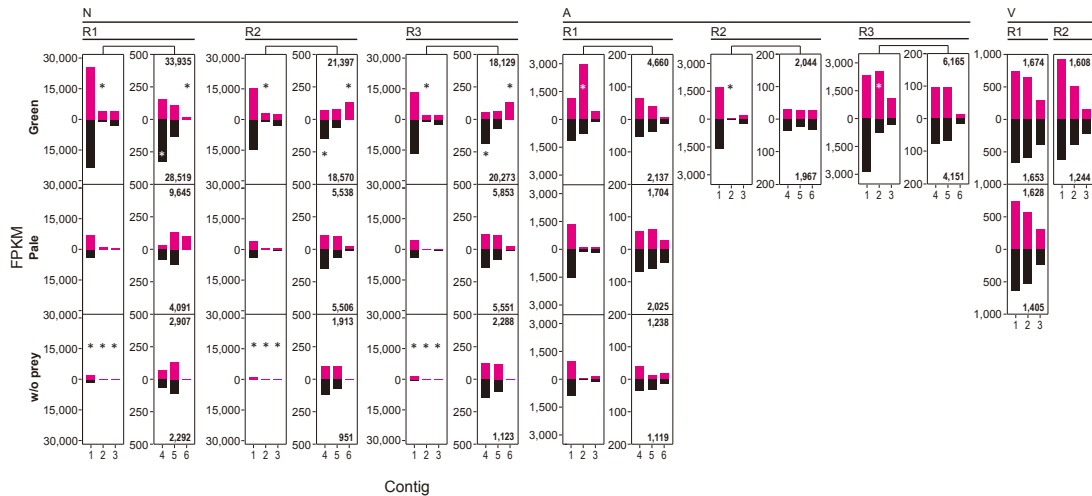
Glutathione synthetase



Carbonyl reductase



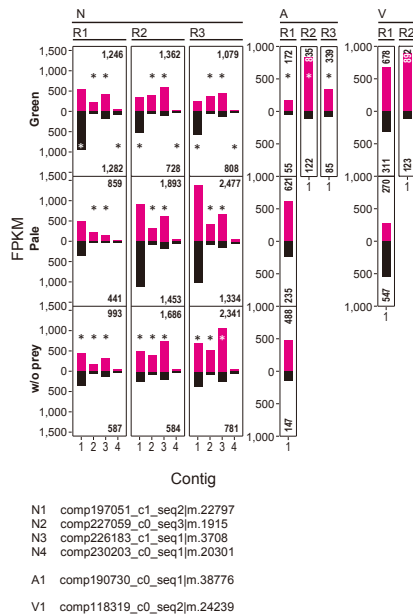
Glutathion S-transferase



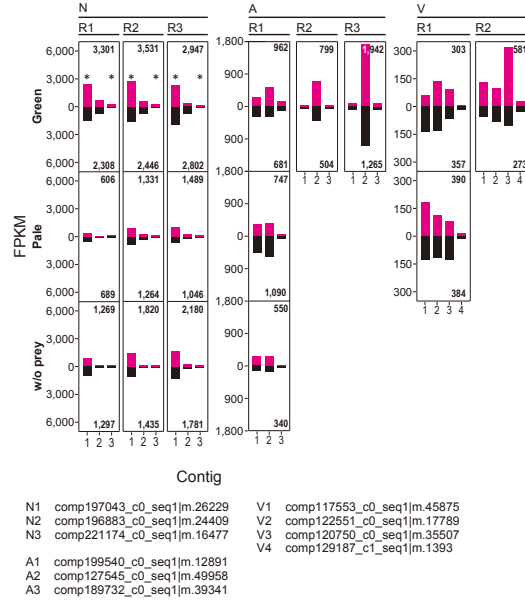
Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp.
(continued)

Oxidation and reduction Oxidative stress response 3

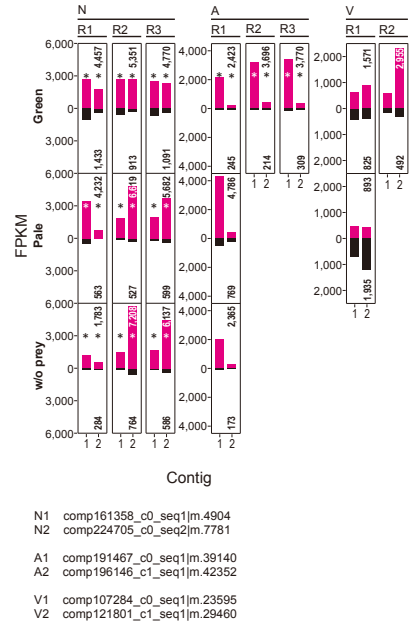
Glutathione peroxidase



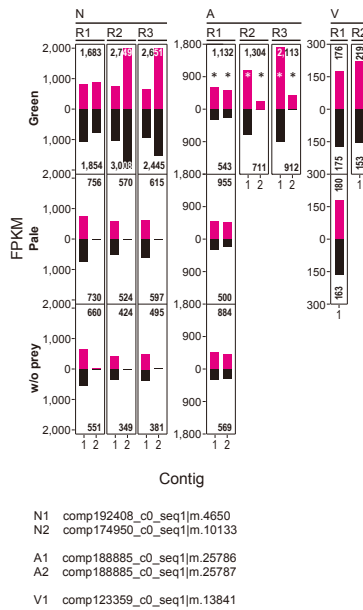
Glutaredoxin



Peptide methionine sulfoxide reductase



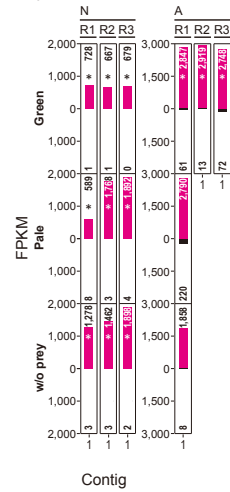
Catalase



Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp. (continued)

Carotenoid synthesis

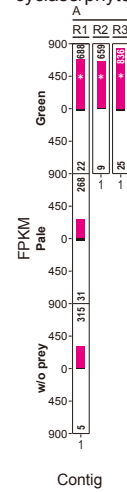
Phytoene desaturase



N1 comp217346_c0_seq1|m.11870

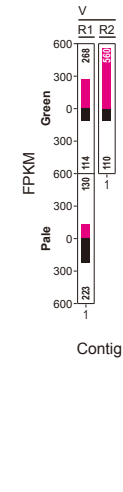
A1 comp194869_c0_seq1|m.28844

Bifunctional lycopene cyclase/phytoene synthase



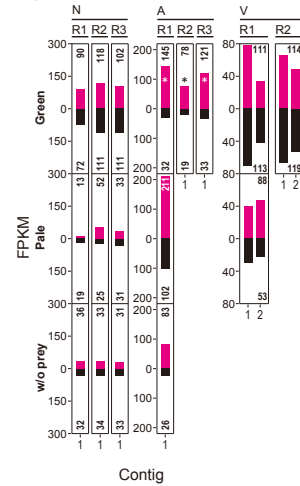
A1 comp195533_c0_seq1|m.19074

Zeaxanthin epoxidase



V1 comp124759_c0_seq1|m.21868

Geranylgeranyl pyrophosphate synthase



N1 comp213578_c0_seq1|m.14664

A1 comp194723_c0_seq1|m.21777

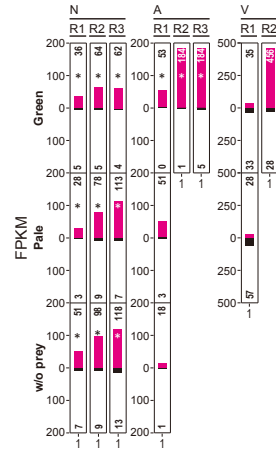
V1 comp124726_c1_seq1|m.28171

V2 comp124726_c0_seq1|m.31417

Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp.
(continued)

DNA repair

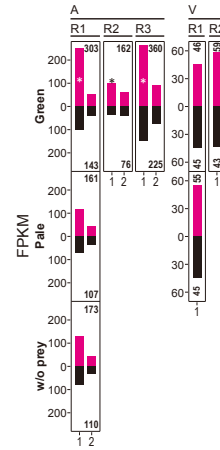
DNA-(apurinic or apyrimidinic site) lyase



Contig

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A1 comp199388_c0_seq1|m.9331
V1 comp126066_c0_seq2|m.29588

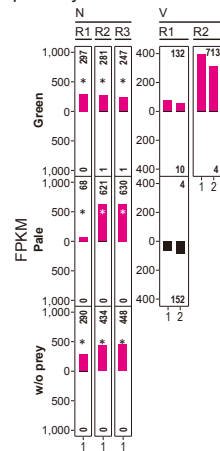
RAD23



Contig

A1 comp191210_c0_seq1|m.31519
A2 comp193102_c0_seq1|m.31176
V1 comp123008_c0_seq1|m.15170

Deoxyribodipyrimidine photo-lyase

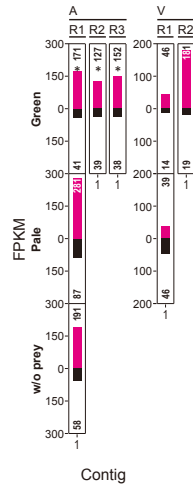


Contig

N1 comp222533_c0_seq1|m.15479
V1 comp125035_c0_seq1|m.12561
V2 comp127813_c1_seq1|m.15993

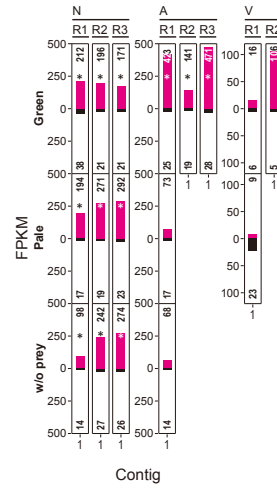
Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp. (continued)

Respiration 1 COX11



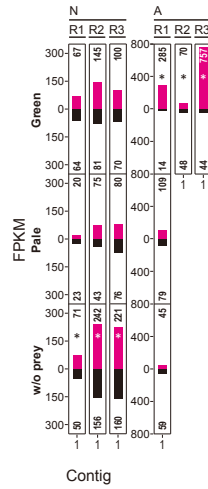
A1 comp193195_c0_seq1|m.37792
V1 comp122488_c0_seq2|m.24697

COX15



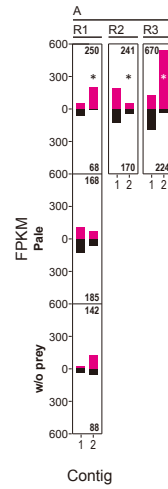
N1 comp228272_c0_seq1|m.12304
A1 comp192893_c0_seq1|m.25498
V1 comp125185_c0_seq2|m.16544

TIM14



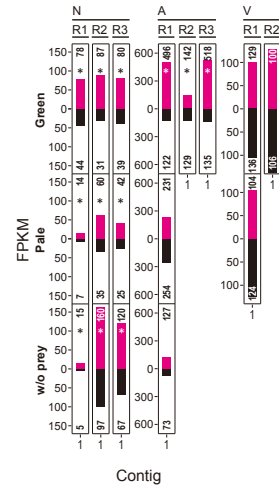
N1 comp220797_c0_seq1|m.5849
A1 comp192514_c0_seq1|m.44559

TIM16



A1 comp181094_c0_seq2|m.48439
A2 comp184602_c0_seq1|m.48738

TIM17

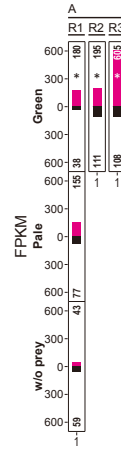


N1 comp186302_c0_seq1|m.19129
A1 comp178054_c1_seq1|m.47330
V1 comp120032_c0_seq1|m.21088

Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp. (continued)

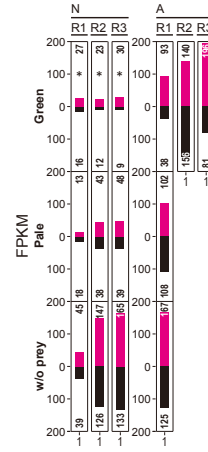
Respiration 2

TIM23



A1 comp190408_c0_seq1|m.49523

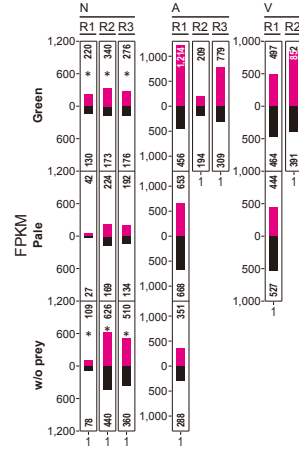
TIM44



N1 comp182943_c0_seq1|m.12522

A1 comp192049_c0_seq1|m.28999

Prohibitin 1

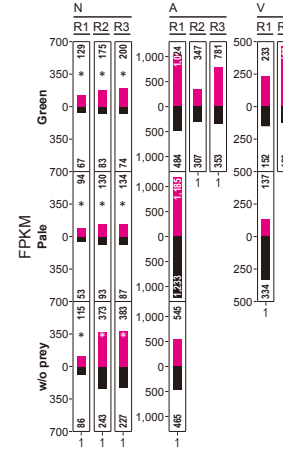


N1 comp228209_c0_seq1|m.19680

A1 comp189959_c0_seq1|m.42468

V1 comp122032_c0_seq1|m.30636

Prohibitin 2



N1 comp227900_c0_seq1|m.15768

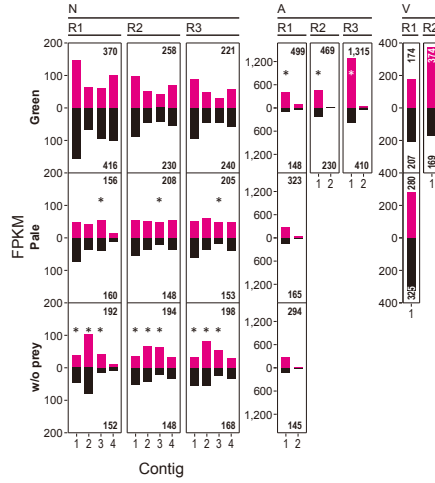
A1 comp192167_c0_seq1|m.37994

V1 comp122381_c0_seq1|m.21229

Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp. (continued)

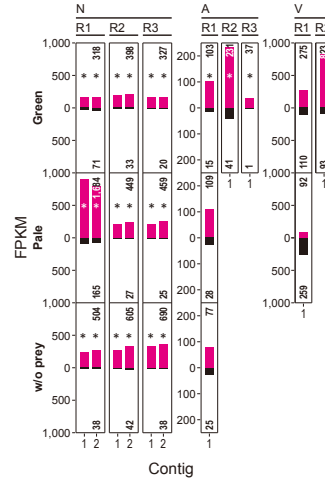
Oxygen consuming metabolisms

Methylsterol monooxygenase



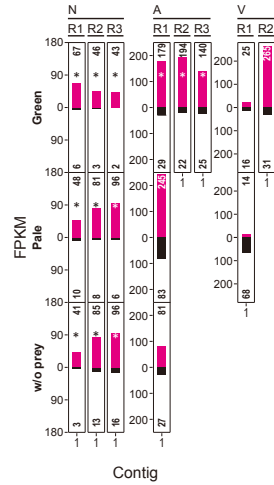
N1 comp228408_c0_seq1|m.17822
 N2 comp224022_c0_seq1|m.7534
 N3 comp228514_c0_seq1|m.11837
 N4 comp218051_c0_seq1|m.11145
 A1 comp193498_c0_seq1|m.19446
 A2 comp198885_c0_seq1|m.33502
 V1 comp121846_c0_seq1|m.24109

Squalene monooxygenase



N1 comp216080_c1_seq1|m.5734
 N2 comp216080_c0_seq1|m.19550
 A1 comp195623_c0_seq1|m.11809
 V1 comp122050_c0_seq1|m.9848

L-gulonolactone oxidase

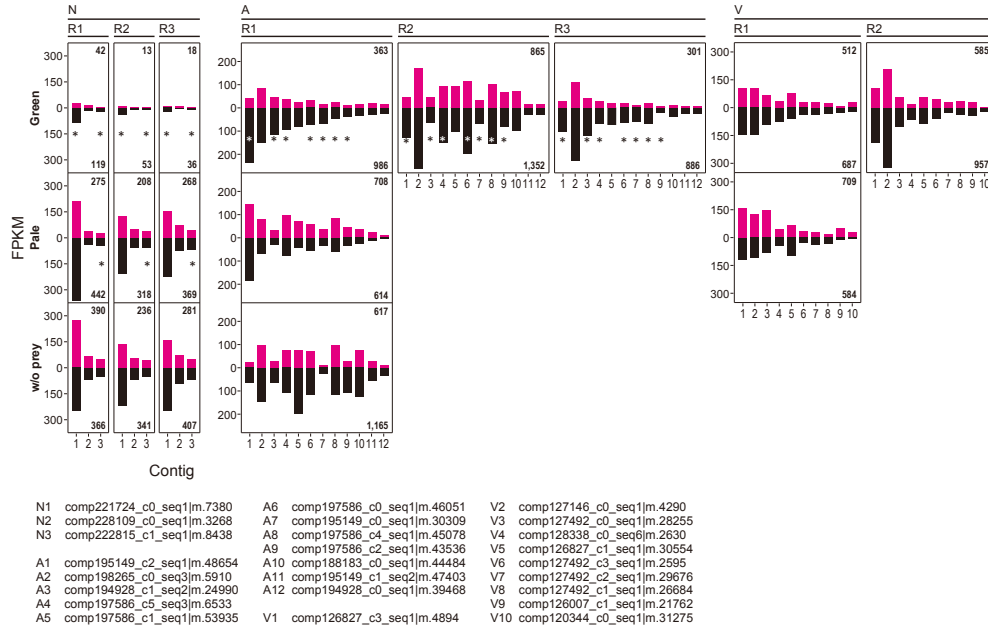


N1 comp220489_c0_seq1|m.13475
 A1 comp194339_c0_seq2|m.27513
 V1 comp128213_c0_seq2|m.16856

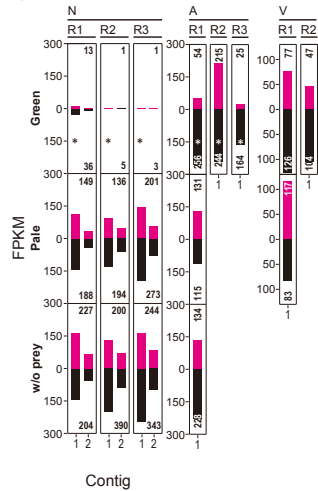
Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp. (continued)

Myosin 1

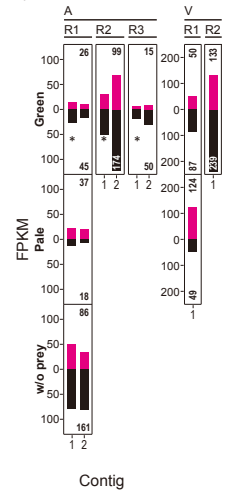
Type I



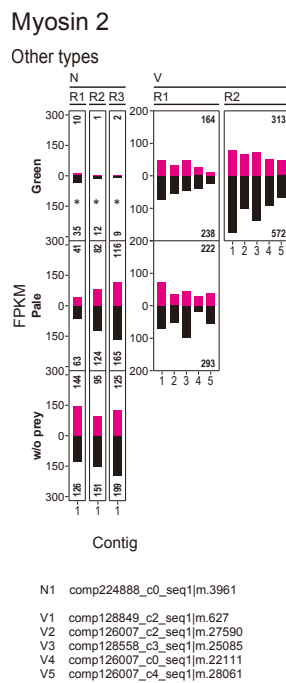
Type II



Type VII

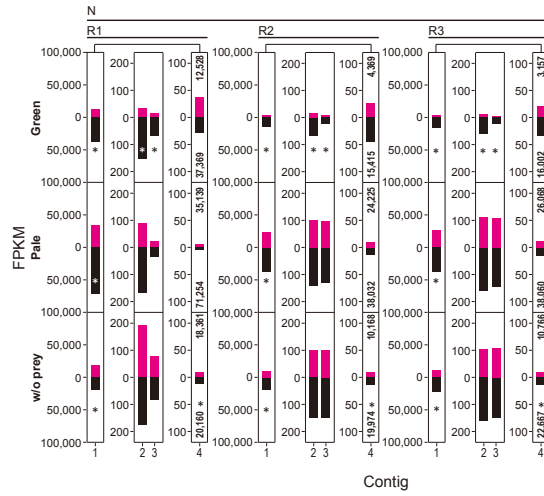


Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp. (continued)

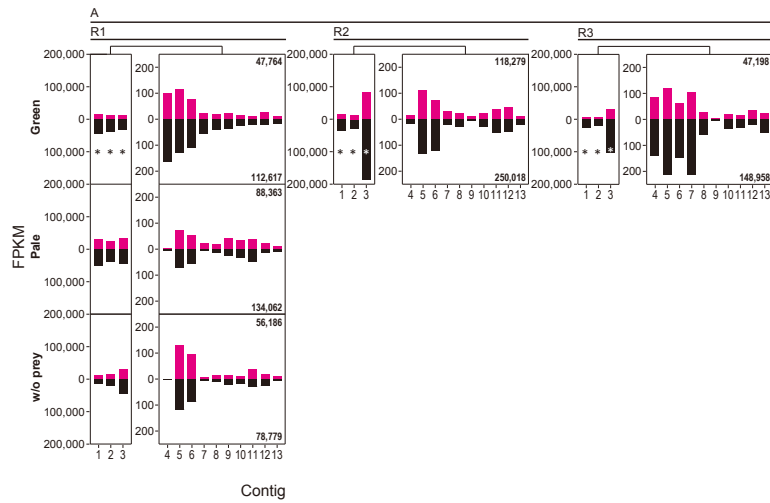


Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp.
(continued)

Actin



N1 comp226268_c0_seq1|m.15372
 N2 comp218105_c0_seq1|m.17135
 N3 comp212496_c0_seq1|m.10790
 N4 comp225048_c0_seq1|m.5103



A1 comp197233_c0_seq1|m.48321
 A2 comp197233_c3_seq1|m.51575
 A3 comp197233_c1_seq1|m.54766
 A4 comp192995_c0_seq1|m.38899
 A5 comp193353_c0_seq1|m.5798
 A6 comp189645_c0_seq1|m.55912
 A7 comp191378_c0_seq3|m.30974
 A8 comp197190_c1_seq1|m.24458
 A9 comp195956_c0_seq1|m.9932
 A10 comp196783_c0_seq1|m.13041
 A11 comp197962_c1_seq1|m.12530
 A12 comp198043_c0_seq5|m.9355
 A13 comp195269_c1_seq1|m.31367

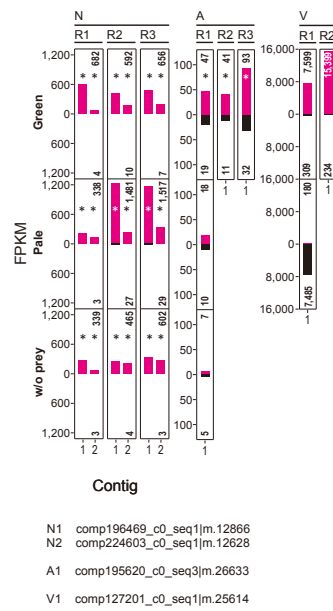
Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp. (continued)

Figure 1: Heatmap of FPKM values for 12 contigs across 12 samples. The heatmap is divided into two panels, R1 and R2. The y-axis is labeled 'FPKM' and ranges from 0 to 150,000. The x-axis is labeled 'Contig' and lists 12 contigs. The top row of the heatmap is labeled 'Green' and the bottom row is labeled 'Pale'. The heatmap shows a clear pattern of high FPKM values for contigs 1, 2, and 3 in the 'Green' row, and low FPKM values for contigs 4 through 12. The 'Pale' row shows a similar pattern but with lower overall FPKM values. The heatmap is color-coded by FPKM value, with red indicating high values and blue indicating low values. The heatmap is also annotated with sample IDs and their corresponding FPKM values for each contig.

Sample	Contig 1	Contig 2	Contig 3	Contig 4	Contig 5	Contig 6	Contig 7	Contig 8	Contig 9	Contig 10	Contig 11	Contig 12
V1	127126_c1_seq1	127126_c3_seq1	121056_c0_seq1	126422_c0_seq1	126520_c0_seq1	124568_c0_seq2	126591_c0_seq1	125992_c0_seq2	123034_c0_seq2	128124_c0_seq5	124299_c0_seq1	126794_c0_seq2
V2	127126_c1_seq1	127126_c3_seq1	121056_c0_seq1	126422_c0_seq1	126520_c0_seq1	124568_c0_seq2	126591_c0_seq1	125992_c0_seq2	123034_c0_seq2	128124_c0_seq5	124299_c0_seq1	126794_c0_seq2
V3	127126_c1_seq1	127126_c3_seq1	121056_c0_seq1	126422_c0_seq1	126520_c0_seq1	124568_c0_seq2	126591_c0_seq1	125992_c0_seq2	123034_c0_seq2	128124_c0_seq5	124299_c0_seq1	126794_c0_seq2
V4	127126_c1_seq1	127126_c3_seq1	121056_c0_seq1	126422_c0_seq1	126520_c0_seq1	124568_c0_seq2	126591_c0_seq1	125992_c0_seq2	123034_c0_seq2	128124_c0_seq5	124299_c0_seq1	126794_c0_seq2
V5	127126_c1_seq1	127126_c3_seq1	121056_c0_seq1	126422_c0_seq1	126520_c0_seq1	124568_c0_seq2	126591_c0_seq1	125992_c0_seq2	123034_c0_seq2	128124_c0_seq5	124299_c0_seq1	126794_c0_seq2
V6	127126_c1_seq1	127126_c3_seq1	121056_c0_seq1	126422_c0_seq1	126520_c0_seq1	124568_c0_seq2	126591_c0_seq1	125992_c0_seq2	123034_c0_seq2	128124_c0_seq5	124299_c0_seq1	126794_c0_seq2
V7	127126_c1_seq1	127126_c3_seq1	121056_c0_seq1	126422_c0_seq1	126520_c0_seq1	124568_c0_seq2	126591_c0_seq1	125992_c0_seq2	123034_c0_seq2	128124_c0_seq5	124299_c0_seq1	126794_c0_seq2
V8	127126_c1_seq1	127126_c3_seq1	121056_c0_seq1	126422_c0_seq1	126520_c0_seq1	124568_c0_seq2	126591_c0_seq1	125992_c0_seq2	123034_c0_seq2	128124_c0_seq5	124299_c0_seq1	126794_c0_seq2
V9	127126_c1_seq1	127126_c3_seq1	121056_c0_seq1	126422_c0_seq1	126520_c0_seq1	124568_c0_seq2	126591_c0_seq1	125992_c0_seq2	123034_c0_seq2	128124_c0_seq5	124299_c0_seq1	126794_c0_seq2
V10	127126_c1_seq1	127126_c3_seq1	121056_c0_seq1	126422_c0_seq1	126520_c0_seq1	124568_c0_seq2	126591_c0_seq1	125992_c0_seq2	123034_c0_seq2	128124_c0_seq5	124299_c0_seq1	126794_c0_seq2
V11	127126_c1_seq1	127126_c3_seq1	121056_c0_seq1	126422_c0_seq1	126520_c0_seq1	124568_c0_seq2	126591_c0_seq1	125992_c0_seq2	123034_c0_seq2	128124_c0_seq5	124299_c0_seq1	126794_c0_seq2
V12	127126_c1_seq1	127126_c3_seq1	121056_c0_seq1	126422_c0_seq1	126520_c0_seq1	124568_c0_seq2	126591_c0_seq1	125992_c0_seq2	123034_c0_seq2	128124_c0_seq5	124299_c0_seq1	126794_c0_seq2

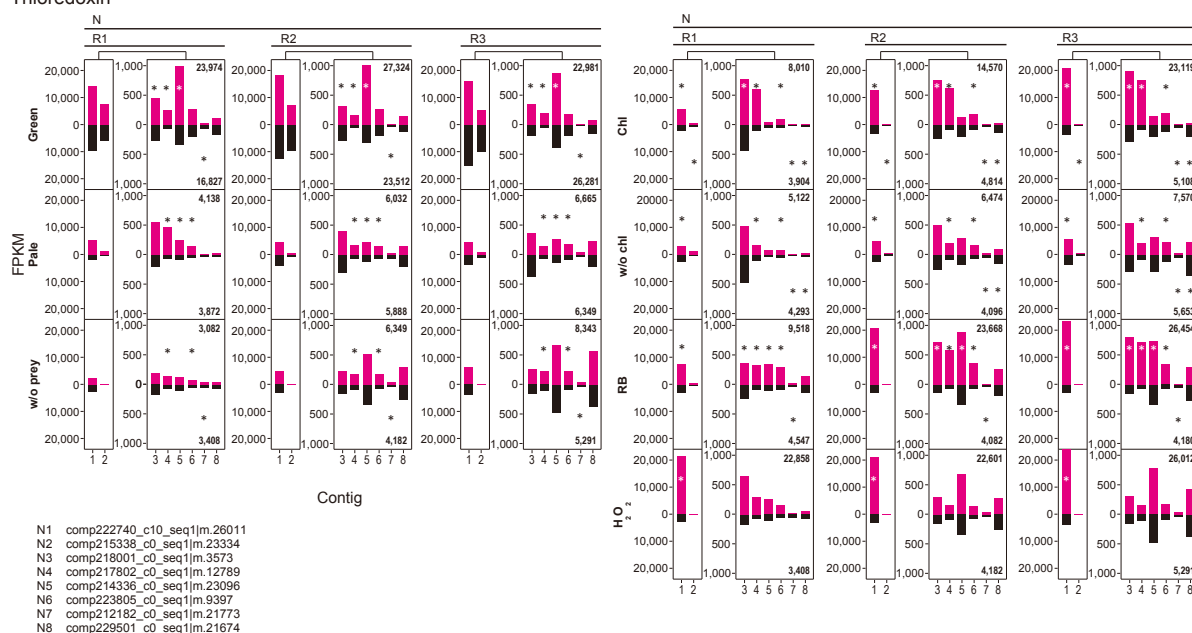
17

PAO-like protein



Supplementary Fig. 4. Comparison of the effect of the photosynthetic trait of bacterial prey on mRNA levels of selected genes of *Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp.
(continued)

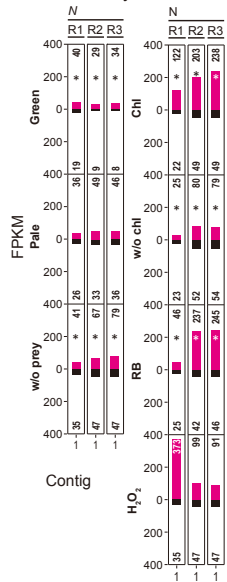
Oxidation and reduction
Oxidative stress response 1
Thioredoxin



Supplementary Fig. 5. Effect of ROS and chlorophyll on mRNA levels of selected *Naegleria* sp. genes. To examine the effect of chlorophyll *a*, *Naegleria* sp. was cultured with *E. coli* stained with/without chlorophyll *a* in the dark in inorganic medium for 12 h and then transferred to light ($200 \mu\text{E m}^{-2} \text{s}^{-1}$) conditions for 1 h. To examine the effect of singlet oxygen, *Naegleria* sp. was cultured without bacterial prey in organic medium with 30 nM RB, which produces singlet oxygen by illumination, in the dark for 12 h and then transferred to light conditions for 1 h. To examine the effects of H_2O_2 , *Naegleria* sp. was cultured without bacterial prey in organic medium for 12 h in the dark and treated with 0.1 mM H_2O_2 in the dark for 1 h. Cultures under respective conditions were performed three times independently on different days (R1, R2, and R3). Graphs show the levels of mRNA (FPKM values) that are related to oxidation and reduction/oxidative stress responses, carotenoid synthesis, DNA repair, respiration and oxygen-consuming metabolism or encoding myosin, actin and PAO-like proteins under dark (black bar) and light (magenta bar) conditions, except for H_2O_2 . In the case of H_2O_2 treatment, the black bar indicates the value just before the addition of H_2O_2 and the magenta bar indicates the value 1 h after its addition in the dark. Each bar corresponds to one contig (gene). For example, four contigs (genes) encoded glutathione peroxidase in *Naegleria* sp. Numbers in the graph indicate the total FPKM values in respective conditions. The contig IDs are shown below each graph. For comparison, the values in the cultures with green or pale *S. elongatus* prey and illumination alone (w/o prey) (shown in Supplementary Fig. 4) are redisplayed. *, FDR < 0.01 (edgeR; three biological replicates). The details of the culture conditions are described in Supplementary Fig. 3.

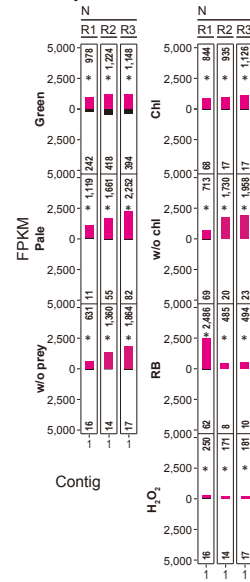
Oxidation and reduction
Oxidative stress response 2

Glutathione synthetase



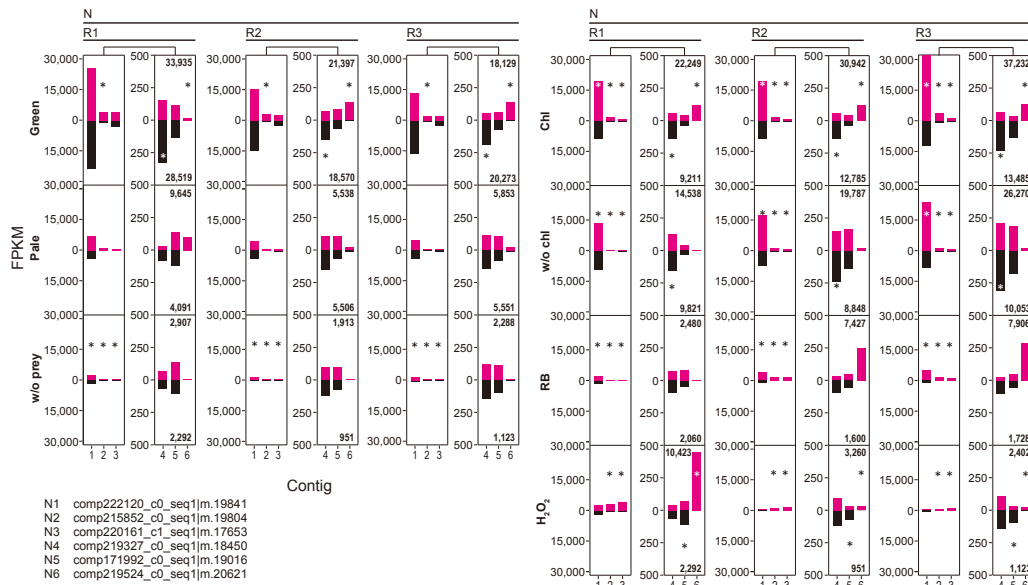
N1 comp170906_c0_seq1|m.12558

Carbonyl reductase



N1 comp222757_c0_seq1|m.17440

Glutathione S-transferase

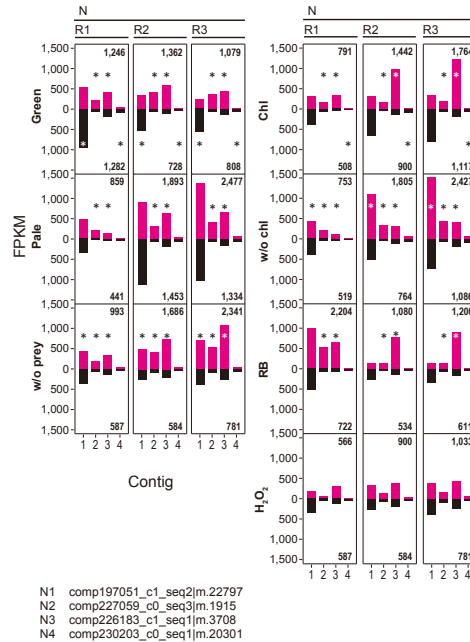


N1 comp222120_c0_seq1|m.19841
N2 comp215852_c0_seq1|m.19804
N3 comp220161_c1_seq1|m.17653
N4 comp219327_c0_seq1|m.18450
N5 comp171992_c0_seq1|m.19016
N6 comp219524_c0_seq1|m.20621

Supplementary Fig. 5. Effect of ROS and chlorophyll on mRNA levels of selected *Naegleria* sp. genes. (continued)

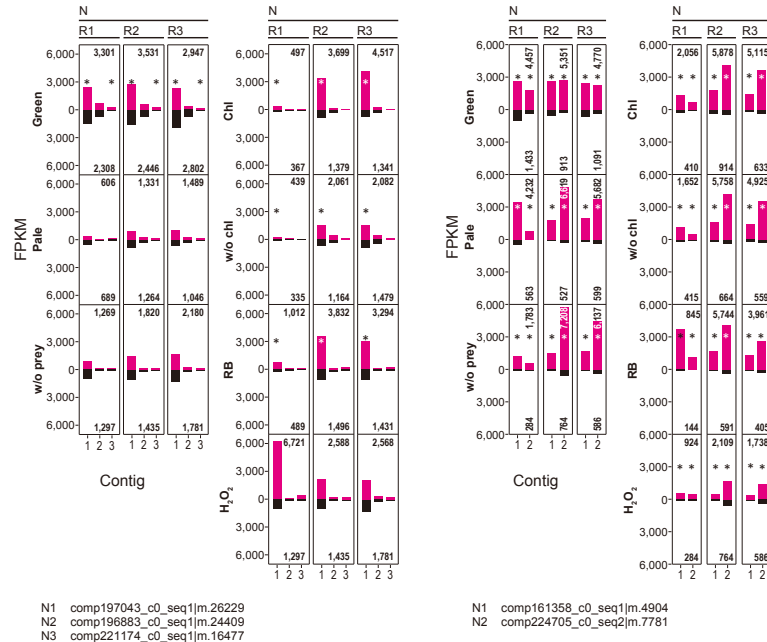
Oxidation and reduction
Oxidative stress response 3

Glutathione peroxidase



Peptide methionine sulfoxide
reductase

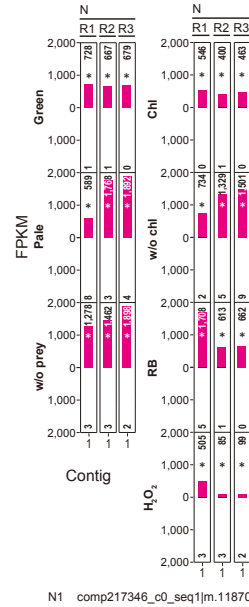
Glutaredoxin



Supplementary Fig. 5. Effect of ROS and chlorophyll on mRNA levels of selected *Naegleria* sp. genes. (continued)

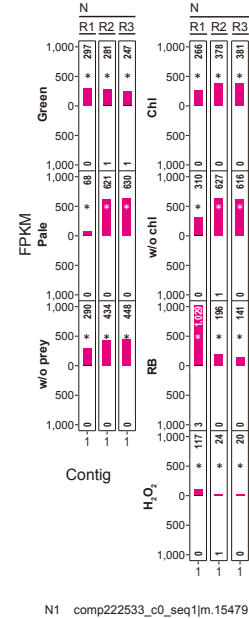
Carotenoid synthesis

Phytoene desaturase

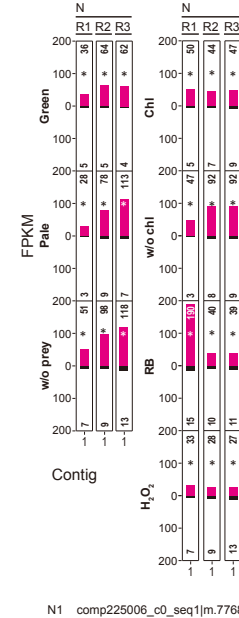


DNA repair

Deoxyribodipyrimidine photo-lyase



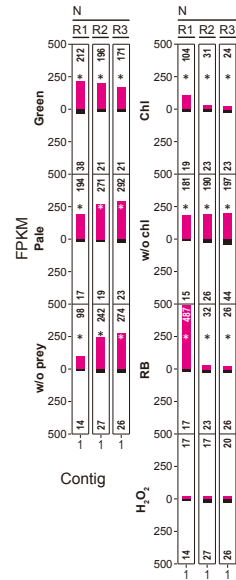
DNA-(apurinic or apyrimidinic site) lyase



Supplementary Fig. 5. Effect of ROS and chlorophyll on mRNA levels of selected *Naegleria* sp. genes. (continued)

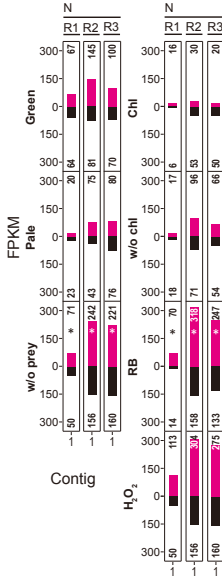
Respiration

COX15



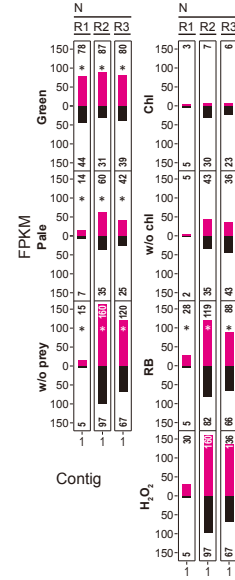
N1 comp228272_c0_seq1|m.12304

TIM14



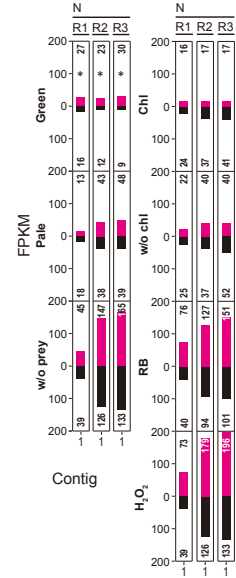
N1 comp220797_c0_seq1|m.5849

TIM17



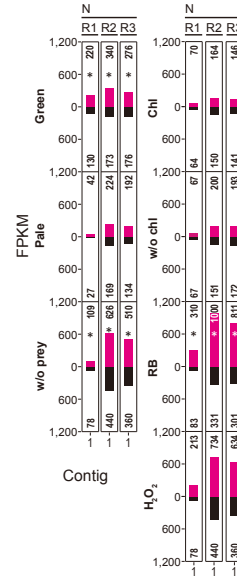
N1 comp186302_c0_seq1|m.19129

TIM44



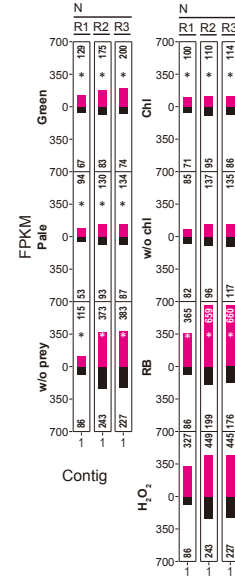
N1 comp182943_c0_seq1|m.12522

Prohibitin 1



N1 comp228209_c0_seq1|m.19680

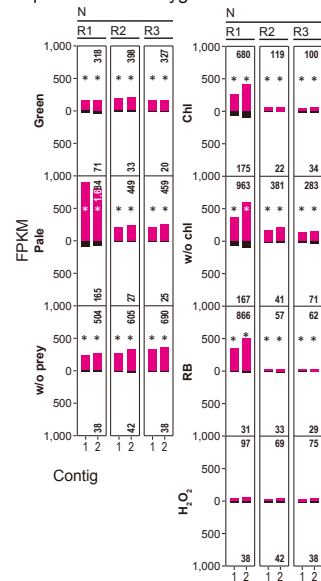
Prohibitin 2



N1 comp227900_c0_seq1|m.15768

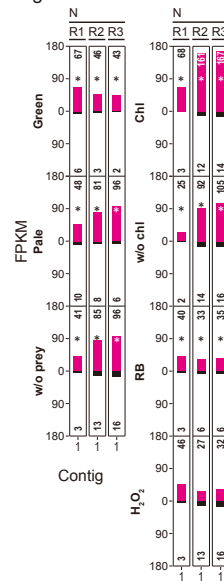
Supplementary Fig. 5. Effect of ROS and chlorophyll on mRNA levels of selected *Naegleria* sp. genes. (continued)

Oxygen consuming metabolism
Squalene monooxygenase



N1 comp216080_c1_seq1[m.5734
N2 comp216080_c0_seq1[m.19550

L-gulonolactone oxidase

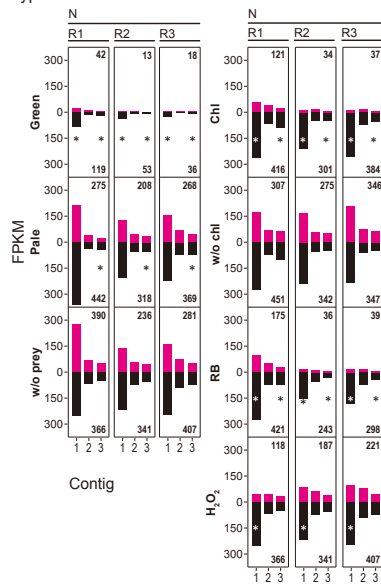


N1 comp220489_c0_seq1[m.13475

Supplementary Fig. 5. Effect of ROS and chlorophyll on mRNA levels of selected *Naegleria* sp. genes. (continued)

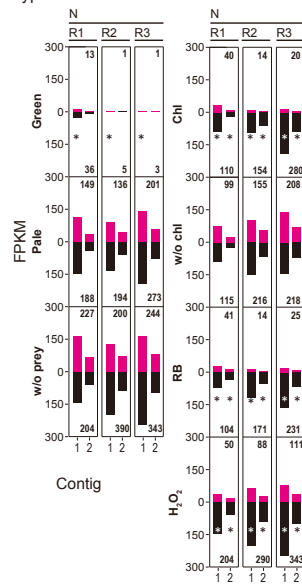
Myosin

Type I



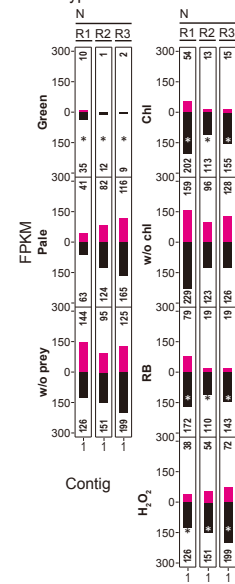
N1 comp221724_c0_seq1|m.7380
N2 comp228109_c0_seq1|m.3268
N3 comp222815_c1_seq1|m.8438

Type II



N1 comp226013_c0_seq2|m.5781
N2 comp152034_c0_seq1|m.18471

Other types



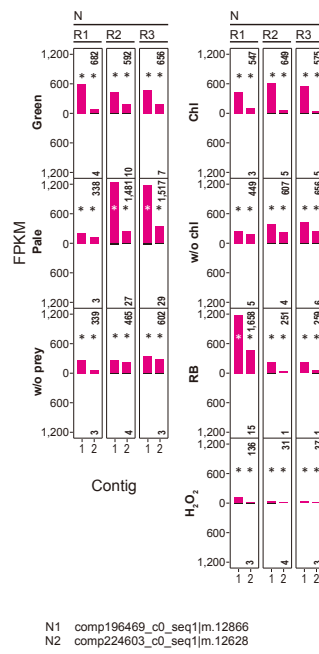
N1 comp224888_c0_seq1|m.3961

Supplementary Fig. 5. Effect of ROS and chlorophyll on mRNA levels of selected *Naegleria* sp. genes. (continued)

Figure 10 displays 18 bar charts showing the expression of 18 genes (N1 to N18) across three conditions: Green, Pale, and w/o prey. The genes are grouped into three categories: Green (N1, N2, N3, N4), Chl (N5, N6, N7, N8), and w/o chl (N9, N10, N11, N12). The charts are arranged in a 6x3 grid. Each chart shows the expression level (FPKM) for each gene across three conditions: Green, Pale, and w/o prey. The y-axis represents FPKM, and the x-axis represents the condition. The charts are labeled with the gene ID (N1 to N18) and the condition (Green, Pale, w/o prey). The charts show that the expression of most genes is significantly higher in the Green condition compared to the Pale and w/o prey conditions. The expression of some genes (e.g., N1, N2, N3, N4) is also significantly higher in the Pale condition compared to the w/o prey condition. The charts are labeled with the gene ID (N1 to N18) and the condition (Green, Pale, w/o prey). The charts show that the expression of most genes is significantly higher in the Green condition compared to the Pale and w/o prey conditions. The expression of some genes (e.g., N1, N2, N3, N4) is also significantly higher in the Pale condition compared to the w/o prey condition.

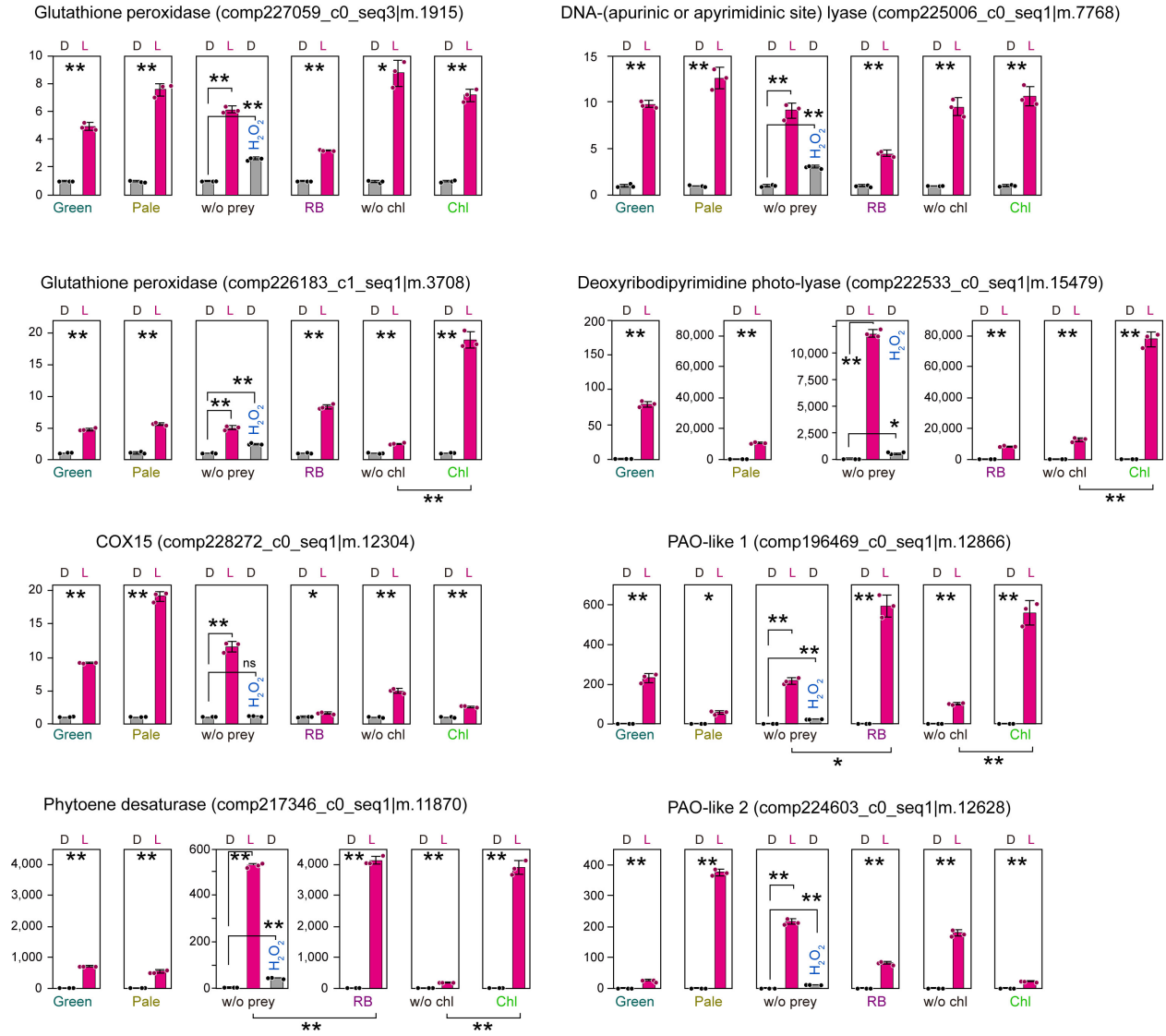
26

PAO-like protein



Supplementary Fig. 5. Effect of ROS and chlorophyll on mRNA levels of selected *Naegleria* sp. genes. (continued)

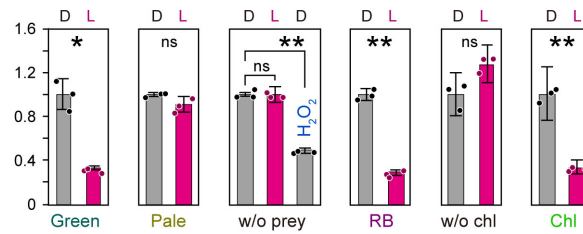
Naegleria sp. qRT-PCR



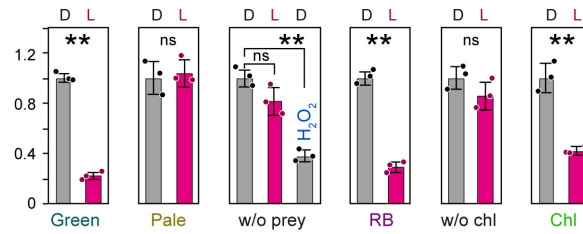
Supplementary Fig. 6. Quantitative RT-PCR analyses comparing mRNA levels of respective genes in *Naegleria* sp. in respective culture conditions. The corresponding RNA-seq results are shown in Fig. 4b and Supplementary Fig. 5. The details of the culture conditions are described in Supplementary Fig. 3. *EF1α* (comp225284_c0_seq1|m.13820) was used as an internal control. The level in the dark conditions (or before H₂O₂ addition) was defined as 1.0. The error bar represents the standard deviation of three replicates (three sets of RNA samples prepared from three sets of *Naegleria* sp. cultures that were performed at the same time). **p* < 0.05; ***p* < 0.005; ns, not statistically significant (*t*-test). Source data are provided as a Source Data file.

Naegleria sp. qRT-PCR

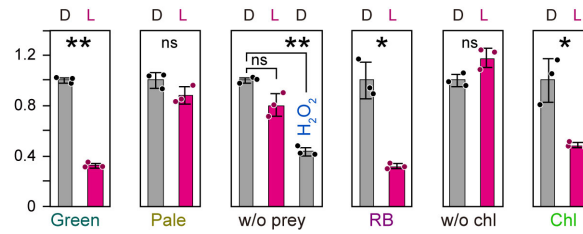
Myosin type I (comp221724_c0_seq1|m.7380)



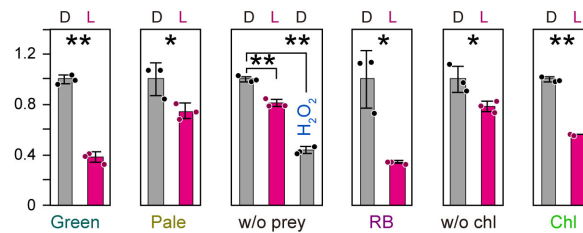
Myosin type II (comp226013_c0_seq2|m.5781)



Myosin family protein (comp224888_c0_seq1|m.3961)



Actin (comp226268_c0_seq1|m.15372)



Supplementary Fig. 6. Quantitative RT-PCR analyses comparing mRNA levels of respective genes in *Naegleria* sp. in respective culture conditions. (continued)

Supplementary Table 1. BLAST top hits of 18S rDNA sequences of the three species of amoeboid organisms isolated and used in this study.

Species	Accession number	Classification	E-value
<i>Naegleria</i> sp. KDN1 (query; this study)	LC368140		
Top 10 hits			
<i>Naegleria jejuensis</i> JJ42108A	MF113400.1	Heterolobosea; Schizopyrenida	0
<i>Naegleria pagei</i> A22/I	DQ768714.1	Heterolobosea; Schizopyrenida	0
<i>Naegleria pagei</i> 4830/I	DQ768721.1	Heterolobosea; Schizopyrenida	0
<i>Naegleria gruberi</i> EGB	MG699123.1	Heterolobosea; Schizopyrenida	0
<i>Naegleria gruberi</i> NEG-M	AB298288.1	Heterolobosea; Schizopyrenida	0
<i>Naegleria gruberi</i>	M18732.1	Heterolobosea; Schizopyrenida	0
<i>Naegleria</i> sp. 62K4	JQ271695.1	Heterolobosea; Schizopyrenida	0
<i>Naegleria</i> sp. COHH 64	GU320598.1	Heterolobosea; Schizopyrenida	0
<i>Naegleria australiensis</i> BCHV5	JQ271699.1	Heterolobosea; Schizopyrenida	0
<i>Naegleria fultoni</i> 4796/I	DQ768719.1	Heterolobosea; Schizopyrenida	0
Top 10 hits other than <i>Naegleria</i> spp.			
<i>Heterolobosea</i> sp. EV10A	FM244741.1	Heterolobosea	0
<i>Acrasis helenhemmesae</i> BM07-A1-1	GU437220.1	Heterolobosea; Acrasida	3E-144
<i>Tulamoeba peronaphora</i> A1	FJ222603.1	Heterolobosea; Tulamoebidae	5E-132
<i>Allovahlkampfia</i> sp. SG-2014b	KF547919.1	Heterolobosea; Schizopyrenida	8E-130
<i>Allovahlkampfia</i> sp. SG-2014d	KF547918.1	Heterolobosea; Schizopyrenida	3E-129
<i>Allovahlkampfia</i> sp. SG-2014c	KF547915.1	Heterolobosea; Schizopyrenida	4E-128
<i>Allovahlkampfia</i> sp. SO/1P	EU266548.2	Heterolobosea; Schizopyrenida	4E-128
<i>Allovahlkampfia spelaea</i> SK1	EU696948.1	Heterolobosea; Schizopyrenida	4E-128
<i>Tetramitus thermacidophilus</i>	AJ621575.1	Heterolobosea; Schizopyrenida	1E-127
<i>Tulamoeba</i> sp. H3 clone 4/7	KT210085.1	Heterolobosea; Tulamoebidae	3E-127
<i>Acanthamoeba</i> sp. KDN1 (query; this study)	LC368141		
Top 10 hits			
<i>Acanthamoeba</i> sp. KA/E5	AY148954.1	Amoebozoa; Discosea; Longamoebia	0
<i>Acanthamoeba</i> sp. KA/MSS7	AY173015.1	Amoebozoa; Discosea; Longamoebia	0
<i>Acanthamoeba</i> sp. KA/MSS2	AY173013.1	Amoebozoa; Discosea; Longamoebia	0
<i>Acanthamoeba</i> sp. KA/MSS6	AY173014.1	Amoebozoa; Discosea; Longamoebia	0
<i>Acanthamoeba</i> sp. KA/E21	EF140633.1	Amoebozoa; Discosea; Longamoebia	0
<i>Acanthamoeba</i> sp. KA/E4	AF349045.1	Amoebozoa; Discosea; Longamoebia	0
<i>Acanthamoeba</i> sp. AcaVN14	GQ397476.1	Amoebozoa; Discosea; Longamoebia	0
<i>Acanthamoeba griffini</i> H37	S81337.1	Amoebozoa; Discosea; Longamoebia	0
<i>Acanthamoeba</i> sp. Egy4	MF350347.1	Amoebozoa; Discosea; Longamoebia	0
<i>Acanthamoeba griffini</i> S-7 ATCC 30731	U07412.1	Amoebozoa; Discosea; Longamoebia	0
Top 10 hits other than <i>Acanthamoeba</i> spp.			
<i>Comandonia operculata</i>	AY033896.1	Amoebozoa; Discosea; Longamoebia	0
<i>Comandonia</i> sp. E_5F	AB425954.1	Amoebozoa; Discosea; Longamoebia	0
<i>Comandonia</i> sp. E_5E	AB425956.1	Amoebozoa; Discosea; Longamoebia	0
<i>Acanthamoebidae</i> sp. EO-2012	JQ361081.1	Amoebozoa; Discosea; Longamoebia	0
<i>Balamuthia mandrillaris</i> V039	AF477019.1	Amoebozoa; Discosea; Longamoebia	0
<i>Balamuthia mandrillaris</i> Itson-1	KF874819.1	Amoebozoa; Discosea; Longamoebia	0
<i>Balamuthia mandrillaris</i> CDC:V630	JX524850.1	Amoebozoa; Discosea; Longamoebia	0
<i>Balamuthia mandrillaris</i> CDC:V039	AF019071.1	Amoebozoa; Discosea; Longamoebia	4E-178
<i>Mycamoeba gemmipara</i>	KX687875.1	Amoebozoa; Mycamoeba	7E-136
<i>Amoebozoa</i> sp. amMP3	JX312795.1	Amoebozoa; unclassified Amoebozoa	7E-136

Supplementary Table 1. BLAST top hits of 18S rDNA sequences of the three species of amoeba-like organisms isolated and used in this study (continued).

Species	Accession number	Classification	E-value
<i>Vannella</i> sp. KDN1 (query; this study)	LC368140		
Top 10 hits			
<i>Vannella</i> sp. GERL34	HM363631.1	Amoebozoa; Discosea; Flabellinia; Vannellidae	0
<i>Vannella</i> sp. BEN3V	JQ271727.1	Amoebozoa; Discosea; Flabellinia; Vannellidae	0
<i>Ripella tribonemae</i> 70.1.3	MF683568.1	Amoebozoa; Discosea; Flabellinia; Vannellidae	0
<i>Platymoeba</i> sp. SUM1S/I	AY929921.1	Amoebozoa; Discosea; Flabellinia; Vannellidae	0
<i>Vannella</i> sp. GERL14	HM363630.1	Amoebozoa; Discosea; Flabellinia; Vannellidae	0
<i>Vannella</i> sp. GERB	HM363624.1	Amoebozoa; Discosea; Flabellinia; Vannellidae	0
<i>Ripella decalvata</i> 26.2.18	MF683561.1	Amoebozoa; Discosea; Flabellinia; Vannellidae	0
<i>Ripella platypodia</i> 26.9.33	MF683501.1	Amoebozoa; Discosea; Flabellinia; Vannellidae	0
<i>Ripella</i> sp. DP13	JQ271714.1	Amoebozoa; Discosea; Flabellinia; Vannellidae	0
<i>Vannella</i> sp. CAZ6/I	AY929914.1	Amoebozoa; Discosea; Flabellinia; Vannellidae	0
Top 10 hits other than Vannellidae			
<i>Balamuthia mandrillaris</i> Itson-1	KF874819.1	Amoebozoa; Discosea; Longamoebia	9E-108
<i>Amoebozoa</i> sp. C3	KT892701.1	Amoebozoa; unclassified Amoebozoa	1E-106
<i>Dictyamoeba vorax</i> WalEn	KP864096.1	Amoebozoa; Dictyamoeba	2E-105
<i>Paramoeba eilhardi</i> CCAP1560/2	JN202438.1	Amoebozoa; Discosea; Flabellinia	6E-105
<i>Paramoeba</i> sp. EkV-2019b	MK168790.1	Amoebozoa; Discosea; Flabellinia	2E-104
<i>Paramoeba branchiphila</i> KPF3	KY465831.1	Amoebozoa; Discosea; Flabellinia	7E-104
<i>Neoparamoeba</i> sp. SM53	AY193726.1	Amoebozoa; Discosea; Flabellinia	7E-104
<i>Neoparamoeba longipodia</i> DIVA3	MF197371.1	Amoebozoa; Discosea; Flabellinia	3E-102
<i>Neoparamoeba longipodia</i> DIVA3 574/3	MF197370.1	Amoebozoa; Discosea; Flabellinia	3E-102
<i>Paramoeba pemaquidensis</i> FHL	KY465849.1	Amoebozoa; Discosea; Flabellinia	3E-102

Supplementary Table 2. Numbers of HiSeq reads that were assigned to mRNA contigs of respective species under respective culture conditions. The details of the culture conditions for RNA-seq analyses are summarized in Supplementary Fig. 3.

Naegleria sp.			
Green prey	Rep. 1	Rep. 2	Rep. 3
Dark	15,406,680	15,852,082	21,460,963
Light	17,698,525	14,203,796	13,370,422
Pale prey	Rep. 1	Rep. 2	Rep. 3
Dark	14,864,745	13,907,775	12,962,365
Light	11,288,270	16,585,657	14,293,262
w/o prey	Rep. 1	Rep. 2	Rep. 3
Dark	11,393,374	15,978,300	15,696,333
Light	11,762,434	16,540,194	14,695,015
Dark+H ₂ O ₂	12,955,437	17,445,128	14,132,034
Chl	Rep. 1	Rep. 2	Rep. 3
Dark	4,553,864	17,601,604	15,519,770
Light	6,081,370	13,064,417	13,211,099
w/o chl	Rep. 1	Rep. 2	Rep. 3
Dark	2,906,034	15,726,985	13,801,854
Light	3,081,746	16,191,173	14,001,802
RB	Rep. 1	Rep. 2	Rep. 3
Dark	8,669,676	16,586,333	13,827,326
Light	7,443,787	16,416,071	14,695,762

Acanthamoeba sp.			
Green prey	Rep. 1	Rep. 2	Rep. 3
Dark	4,057,426	11,893,151	10,441,191
Light	4,679,008	12,139,051	12,116,824
Pale prey	Exp. 1		
Dark	5,523,730		
Light	9,998,379		
w/o prey	Exp. 1		
Dark	9,304,498		
Light	6,421,569		

Vannella sp.		
Green prey	Rep. 1	Rep. 2
Dark	8,422,189	3,002,741
Light	7,394,133	4,642,215
Pale prey	Exp. 1	
Dark	3,246,243	
Light	8,372,368	

Supplementary Table 3. GO terms enriched in upregulated genes (FDR < 0.01; edgeR; three biological replicates) when *Naegleria* sp. cultured with green *S. elongatus* prey was transferred from dark to light conditions ($p < 0.05$; GSeq)

Category	GO term	GO ID	P-value
-	MF ATPase activity	GO:0016887	1.33E-06
-	MF ATPase activity, coupled to transmembrane movement of substances	GO:0042626	9.73E-06
-	MF zinc ion binding	GO:0008270	1.19E-05
-	MF DNA-binding transcription factor activity, RNA polymerase II-specific	GO:0000981	1.49E-05
oxidation and reduction	BP oxidation-reduction process	GO:0055114	1.94E-05
-	CC Nucleus	GO:0005634	4.66E-05
-	BP ubiquitin-dependent protein catabolic process	GO:0006511	1.20E-04
respiration	CC Mitochondrion	GO:0005739	2.14E-04
-	BP Transport	GO:0006810	3.19E-04
-	BP regulation of transcription, DNA-templated	GO:0006355	3.33E-04
oxidation and reduction	MF oxidoreductase activity	GO:0016491	3.98E-04
DNA repair	BP DNA repair	GO:0006281	5.57E-04
-	BP protein ubiquitination	GO:0016567	6.08E-04
-	MF ATP binding	GO:0005524	1.03E-03
-	CC proteasome complex	GO:0000502	1.47E-03
respiration	CC mitochondrial inner membrane	GO:0005743	2.34E-03
-	MF helicase activity	GO:0004386	2.67E-03
respiration	CC mitochondrial matrix	GO:0005759	3.33E-03
oxidation and reduction	MF oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	GO:0016705	4.12E-03
-	BP iron-sulfur cluster assembly	GO:0016226	4.80E-03
-	CC chloroplast inner membrane	GO:0009706	4.83E-03
oxidative stress response	BP response to oxidative stress	GO:0006979	5.19E-03
-	MF heme binding	GO:0020037	5.95E-03
-	MF ubiquitin-protein transferase activity	GO:0004842	8.41E-03
DNA repair	BP nucleotide-excision repair	GO:0006289	8.71E-03
-	BP protein folding	GO:0006457	9.06E-03
-	MF serine-type endopeptidase activity	GO:0004252	0.013
-	MF iron ion binding	GO:0005506	0.014
-	MF aromatase activity	GO:0070330	0.015
-	BP Proteolysis	GO:0006508	0.015
-	MF ATP-dependent helicase activity	GO:0008026	0.016
-	MF metal ion binding	GO:0046872	0.020
oxidation and reduction	MF flavin adenine dinucleotide binding	GO:0050660	0.022
respiration	CC mitochondrial intermembrane space	GO:0005758	0.024
oxidation and reduction	MF FMN binding	GO:0010181	0.024
-	CC integral component of membrane	GO:0016021	0.026
DNA repair	BP base-excision repair	GO:0006284	0.029
DNA repair	MF damaged DNA binding	GO:0003684	0.029
-	CC melanosome	GO:0042470	0.029
-	BP cholesterol metabolic process	GO:0008203	0.029
oxidation and reduction	MF oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, NAD(P)H as one donor, and incorporation of one atom of oxygen	GO:0016709	0.029
-	MF translation initiation factor activity	GO:0003743	0.029
-	BP response to heat	GO:0009408	0.030

Some GO terms were categorized into 6 categories: cytoskeleton and motors, DNA repair, oxidation and reduction, oxidative stress responses, phagocytosis, respiration. GO, gene ontology; MF, molecular function; CC, cellular component; and BP, biological process. The results shown are based on three independent culture sets that were performed at different days.

Supplementary Table 3. GO terms enriched in upregulated genes (FDR < 0.01; edgeR; three biological replicates) when *Naegleria* sp. cultured with green *S. elongatus* prey was transferred from dark to light conditions ($p < 0.05$; GSeq) (continued)

Category	GO term	GO ID	P-value
DNA repair	MF ligase activity	GO:0016874	0.033
-	BP tRNA processing	GO:0008033	0.040
-	MF metalloendopeptidase activity	GO:0004222	0.040
-	BP transmembrane transport	GO:0055085	0.043
-	BP response to drug	GO:0042493	0.044
-	CC chloroplast envelope	GO:0009941	0.044
-	BP mRNA processing	GO:0006397	0.044
-	MF hydrolase activity	GO:0016787	0.049

Supplementary Table 4. GO terms enriched in downregulated genes (FDR < 0.01; edgeR; three biological replicates) when *Naegleria* sp. cultured with green *S. elongatus* prey was transferred from dark to light conditions ($p < 0.05$; GSeq)

Category	GO term	GO ID	P-value
cytoskeleton and motors	MF actin filament binding	GO:0051015	4.74E-07
cytoskeleton and motors	BP actin cytoskeleton organization	GO:0030036	5.40E-07
-	MF calcium ion binding	GO:0005509	4.57E-06
cytoskeleton and motors	MF actin binding	GO:0003779	5.16E-06
-	BP cell adhesion	GO:0007155	3.05E-05
-	CC intracellular	GO:0005622	4.87E-05
-	CC neuron projection	GO:0043005	5.07E-05
-	MF heparin binding	GO:0008201	6.23E-05
cytoskeleton and motors	CC actin cytoskeleton	GO:0015629	1.36E-04
-	BP aggregation involved in sorocarp development	GO:0031152	1.39E-04
-	BP collagen fibril organization	GO:0030199	2.53E-04
-	CC extracellular region	GO:0005576	2.84E-04
cytoskeleton and motors	BP cytoskeleton organization	GO:0007010	3.11E-04
cytoskeleton and motors	CC cytoskeleton	GO:0005856	3.44E-04
-	BP small GTPase mediated signal transduction	GO:0007264	4.32E-04
-	BP cell-matrix adhesion	GO:0007160	4.65E-04
-	MF integrin binding	GO:0005178	5.06E-04
-	BP collagen metabolic process	GO:0032963	5.06E-04
-	BP regulation of JUN kinase activity	GO:0043506	5.06E-04
-	BP elastic fiber assembly	GO:0048251	5.06E-04
cell motion	BP movement of cell or subcellular component	GO:0006928	8.76E-04
cytoskeleton and motors	CC cortical actin cytoskeleton	GO:0030864	1.57E-03
-	BP adenylate cyclase-modulating G protein-coupled receptor signaling pathway	GO:0007188	1.57E-03
-	MF guanyl nucleotide binding	GO:0019001	1.58E-03
-	MF G-protein beta/gamma-subunit complex binding	GO:0031683	1.58E-03
cytoskeleton and motors	MF motor activity	GO:0003774	1.58E-03
-	BP triglyceride metabolic process	GO:0006641	1.69E-03
-	CC extracellular space	GO:0005615	2.89E-03
cell motion	CC pseudopodium	GO:0031143	3.49E-03
cell motion	CC Filopodium	GO:0030175	3.51E-03
cytoskeleton and motors	CC myosin complex	GO:0016459	3.54E-03
-	BP mitotic cytokinesis	GO:0000281	3.82E-03
-	MF protein heterodimerization activity	GO:0046982	4.21E-03
-	BP fatty acid metabolic process	GO:0006631	4.27E-03
-	CC cell cortex	GO:0005938	5.79E-03
-	BP G protein-coupled receptor signaling pathway	GO:0007186	7.34E-03
cytoskeleton and motors	CC Arp2/3 protein complex	GO:0005885	7.86E-03
-	MF protein kinase C activity	GO:0004697	7.90E-03
-	BP regulation of Rho protein signal transduction	GO:0035023	7.93E-03
-	BP self proteolysis	GO:0097264	7.94E-03
-	BP regulation of small GTPase mediated signal transduction	GO:0051056	7.94E-03
cell motion	CC cell leading edge	GO:0031252	8.47E-03
cytoskeleton and motors	BP actin filament polymerization	GO:0030041	8.50E-03

Some GO terms were categorized into 6 categories: cytoskeleton and motors, DNA repair, oxidation and reduction, oxidative stress responses, phagocytosis, respiration. GO, gene ontology; MF, molecular function; CC, cellular component; and BP, biological process. The results shown are based on three independent culture sets that were performed at different days.

Supplementary Table 4. GO terms enriched in downregulated genes (FDR < 0.01; edgeR; three biological replicates) when *Naegleria* sp. cultured with green *S. elongatus* prey was transferred from dark to light conditions ($p < 0.05$; GSeq) (continued)

Category	GO term	GO ID	P-value
cell motion	BP chemotaxis to cAMP	GO:0043327	8.56E-03
-	BP intracellular signal transduction	GO:0035556	9.96E-03
-	CC Ribosome	GO:0005840	0.011
-	MF structural constituent of ribosome	GO:0003735	0.015
-	MF lipid binding	GO:0008289	0.016
phagocytosis	CC phagocytic vesicle	GO:0045335	0.016
-	BP Translation	GO:0006412	0.017
phagocytosis	BP phagocytosis	GO:0006909	0.017
-	BP ER to Golgi vesicle-mediated transport	GO:0006888	0.017
-	BP cell morphogenesis	GO:0000902	0.017
-	CC cell-cell junction	GO:0005911	0.017
-	MF Rac GTPase binding	GO:0048365	0.017
cytoskeleton and motors	BP Arp2/3 complex-mediated actin nucleation	GO:0034314	0.018
-	MF phospholipase activity	GO:0004620	0.018
-	CC extracellular exosome	GO:0070062	0.018
cell motion	BP cell migration	GO:0016477	0.018
cytoskeleton and motors	BP actin filament bundle assembly	GO:0051017	0.018
-	CC intrinsic component of the cytoplasmic side of the plasma membrane	GO:0031235	0.018
-	BP negative regulation of Ras protein signal transduction	GO:0046580	0.018
-	MF transaminase activity	GO:0008483	0.018
-	BP regulation of cell shape	GO:0008360	0.018
-	MF G protein-coupled receptor binding	GO:0001664	0.018
-	CC heterotrimeric G-protein complex	GO:0005834	0.018
-	BP negative regulation of transcription by RNA polymerase II	GO:0000122	0.024
-	MF signaling receptor binding	GO:0005102	0.026
-	BP lipid catabolic process	GO:0016042	0.028
cytoskeleton and motors	BP regulation of actin filament polymerization	GO:0030833	0.034
-	MF Rho guanyl-nucleotide exchange factor activity	GO:0005089	0.034
phagocytosis	CC phagocytic cup	GO:0001891	0.034
-	BP phosphatidylinositol catabolic process	GO:0031161	0.040
-	BP phosphatidylethanolamine catabolic process	GO:0046338	0.040
cell motion	CC filopodium tip	GO:0032433	0.040
-	BP cellular response to insulin stimulus	GO:0032869	0.040
cell motion	BP Thermotaxis	GO:0043052	0.040
-	MF hydrolase activity, acting on glycosyl bonds	GO:0016798	0.040
cell motion	BP positive regulation of filopodium assembly	GO:0051491	0.040
-	CC chromosome, centromeric region	GO:0000775	0.040
-	MF calcium-dependent protein kinase C activity	GO:0004698	0.040
cytoskeleton and motors	BP actin filament capping	GO:0051693	0.040
-	CC cell projection	GO:0042995	0.040
-	BP negative regulation of cell adhesion	GO:0007162	0.040
-	BP calcium-mediated signaling	GO:0019722	0.040
cytoskeleton and motors	MF microfilament motor activity	GO:0000146	0.040
-	BP extracellular matrix organization	GO:0030198	0.040
cell motion	BP Chemotaxis	GO:0006935	0.040
-	CC integral component of plasma membrane	GO:0005887	0.040
phagocytosis	CC phagocytic vesicle membrane	GO:0030670	0.047

Supplementary Table 5. GO terms enriched in upregulated genes (FDR < 0.01; edgeR; three biological replicates) when *Acanthamoeba* sp. cultured with green *S. elongatus* prey was transferred from dark to light conditions ($p < 0.05$; GOseq)

Category	GO term	GO ID	P-value
oxidation and reduction	BP oxidation-reduction process	GO:0055114	1.66E-10
respiration	CC Mitochondrion	GO:0005739	2.33E-10
oxidation and reduction	MF oxidoreductase activity	GO:0016491	2.91E-06
-	BP Proteolysis	GO:0006508	3.51E-06
-	BP metabolic process	GO:0008152	4.39E-06
oxidation and reduction	MF flavin adenine dinucleotide binding	GO:0050660	6.01E-06
DNA repair	BP DNA repair	GO:0006281	6.44E-05
-	MF ATPase activity	GO:0016887	6.90E-05
-	MF metal ion binding	GO:0046872	1.03E-04
-	CC proteasome complex	GO:0000502	1.09E-04
respiration	CC mitochondrial inner membrane	GO:0005743	1.47E-04
-	BP proteolysis involved in cellular protein catabolic process	GO:0051603	1.84E-04
-	CC integral component of membrane	GO:0016021	2.43E-04
-	MF threonine-type endopeptidase activity	GO:0004298	3.15E-04
-	CC proteasome core complex	GO:0005839	3.15E-04
DNA repair	MF ligase activity	GO:0016874	3.20E-04
respiration	CC mitochondrial matrix	GO:0005759	3.32E-04
-	MF pyridoxal phosphate binding	GO:0030170	9.45E-04
-	CC Nucleus	GO:0005634	1.12E-03
-	BP biosynthetic process	GO:0009058	1.63E-03
-	BP ubiquitin-dependent protein catabolic process	GO:0006511	2.32E-03
-	BP protein catabolic process	GO:0030163	2.75E-03
oxidation and reduction	MF oxidoreductase activity, acting on the CH-CH group of donors	GO:0016627	2.78E-03
-	BP Transport	GO:0006810	4.29E-03
-	BP sorocarp morphogenesis	GO:0031288	4.76E-03
-	MF iron ion binding	GO:0005506	7.73E-03
oxidation and reduction	MF iron-sulfur cluster binding	GO:0051536	8.02E-03
oxidation and reduction	MF 4 iron, 4 sulfur cluster binding	GO:0051539	8.08E-03
-	MF acyl-CoA dehydrogenase activity	GO:0003995	8.17E-03
-	MF endopeptidase activity	GO:0004175	0.014
-	BP DNA replication	GO:0006260	0.014
-	BP glycolytic process	GO:0006096	0.014
-	MF metalloendopeptidase activity	GO:0004222	0.014
-	CC proteasome accessory complex	GO:0022624	0.014
oxidation and reduction	MF 2 iron, 2 sulfur cluster binding	GO:0051537	0.014
-	MF ubiquitin-protein transferase activity	GO:0004842	0.015
-	CC Peroxisome	GO:0005777	0.015
-	MF hydrolase activity	GO:0016787	0.017
-	CC endoplasmic reticulum membrane	GO:0005789	0.017
-	BP transmembrane transport	GO:0055085	0.022
oxidation and reduction	MF electron transfer activity	GO:0009055	0.023
-	CC proteasome core complex, alpha-subunit complex	GO:0019773	0.024
oxidation and reduction	BP fatty acid beta-oxidation	GO:0006635	0.024
-	BP steroid biosynthetic process	GO:0006694	0.024
-	BP protein folding	GO:0006457	0.024
-	MF ATPase activity, coupled to transmembrane movement of substances	GO:0042626	0.025
-	MF catalytic activity	GO:0003824	0.030

Some GO terms were categorized into 6 categories: cytoskeleton and motors, DNA repair, oxidation and reduction, oxidative stress responses, phagocytosis, respiration. GO, gene ontology; MF, molecular function; CC, cellular component; and BP, biological process. The results shown are based on three independent culture sets that were performed at different days.

Supplementary Table 5. GO terms enriched in upregulated genes (FDR < 0.01; edgeR; three biological replicates) when *Acanthamoeba* sp. cultured with green *S. elongatus* prey was transferred from dark to light conditions ($p < 0.05$; GOseq) (continued)

Category	GO term	GO ID	P-value
-	BP protein transport	GO:0015031	0.038
-	BP cell differentiation	GO:0030154	0.038
respiration	MF NADH dehydrogenase (ubiquinone) activity	GO:0008137	0.040
oxidation and reduction	MF oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	GO:0016616	0.041
-	BP cholesterol biosynthetic process	GO:0006695	0.041
-	MF metalloproteinase activity	GO:0008237	0.041

Supplementary Table 6. GO terms enriched in downregulated genes (FDR < 0.01; edgeR; three biological replicates) when *Acanthamoeba* sp. cultured with green *S. elongatus* prey was transferred from dark to light conditions ($p < 0.05$; GOseq)

Category	GO term	GO ID	P-value
-	MF protein serine/threonine kinase activity	GO:0004674	4.10E-09
-	MF Rho guanyl-nucleotide exchange factor activity	GO:0005089	4.24E-08
-	BP intracellular signal transduction	GO:0035556	6.39E-08
cytoskeleton and motors	CC myosin complex	GO:0016459	1.04E-07
-	BP regulation of Rho protein signal transduction	GO:0035023	2.35E-07
cytoskeleton and motors	MF motor activity	GO:0003774	2.56E-07
-	CC cell cortex	GO:0005938	3.03E-07
-	BP signal transduction	GO:0007165	5.09E-07
-	BP regulation of small GTPase mediated signal transduction	GO:0051056	1.29E-06
-	MF GTPase activator activity	GO:0005096	1.59E-06
-	BP protein phosphorylation	GO:0006468	2.47E-06
cytoskeleton and motors	CC cytoskeleton	GO:0005856	2.88E-06
-	BP small GTPase mediated signal transduction	GO:0007264	8.51E-06
-	MF protein kinase activity	GO:0004672	8.69E-06
-	BP cyclic nucleotide biosynthetic process	GO:0009190	1.01E-05
-	MF phosphorus-oxygen lyase activity	GO:0016849	1.01E-05
cell motion	CC cell leading edge	GO:0031252	2.27E-05
cytoskeleton and motors	MF actin filament binding	GO:0051015	3.66E-05
cytoskeleton and motors	MF actin binding	GO:0003779	5.68E-05
-	CC intracellular	GO:0005622	7.13E-05
-	MF Rac GTPase binding	GO:0048365	1.42E-04
-	BP mitotic cytokinesis	GO:0000281	3.12E-04
-	MF guanyl-nucleotide exchange factor activity	GO:0005085	3.14E-04
cell motion	CC pseudopodium	GO:0031143	3.29E-04
cell motion	CC lamellipodium	GO:0030027	3.36E-04
phagocytosis	CC early phagosome	GO:0032009	3.42E-04
cell motion	CC filopodium	GO:0030175	8.09E-04
cell motion	BP chemotaxis	GO:0006935	8.19E-04
phagocytosis	CC phagocytic cup	GO:0001891	8.23E-04
-	NA NA	GO:0032320	8.40E-04
-	MF calcium ion binding	GO:0005509	8.96E-04
-	BP protein dephosphorylation	GO:0006470	9.16E-04
cytoskeleton and motors	CC cortical actin cytoskeleton	GO:0030864	1.97E-03
cytoskeleton and motors	BP regulation of actin filament polymerization	GO:0030833	1.99E-03
-	MF protein binding	GO:0005515	3.57E-03
-	BP Ras protein signal transduction	GO:0007265	4.73E-03
-	BP cell morphogenesis	GO:0000902	4.87E-03
cell motion	CC ruffle	GO:0001726	4.91E-03
phagocytosis	BP phagocytosis	GO:0006909	5.04E-03
actomyosin	BP actin cytoskeleton organization	GO:0030036	5.12E-03
-	BP sorocarp development	GO:0030587	5.18E-03
phagocytosis	CC phagocytic vesicle membrane	GO:0030670	9.96E-03
-	BP cell-substrate adhesion	GO:0031589	0.012
cytoskeleton and motors	BP actin filament-based movement	GO:0030048	0.012
-	MF protein serine/threonine phosphatase activity	GO:0004722	0.012
-	BP exocytosis	GO:0006887	0.015

Some GO terms were categorized into 6 categories: cytoskeleton and motors, DNA repair, oxidation and reduction, oxidative stress responses, phagocytosis, respiration. GO, gene ontology; MF, molecular function; CC, cellular component; and BP, biological process. The results shown are based on three independent culture sets that were performed at different days.

Supplementary Table 6. GO terms enriched in downregulated genes (FDR < 0.01; edgeR; three biological replicates) when *Acanthamoeba* sp. cultured with green *S. elongatus* prey was transferred from dark to light conditions ($p < 0.05$; Goseq) (continued)

Category	GO term	GO ID	P-value
-	MF phosphoprotein phosphatase activity	GO:0004721	0.015
-	NA NA	GO:0005099	0.015
-	CC intrinsic component of the cytoplasmic side of the plasma membrane	GO:0031235	0.015
-	MF protein tyrosine phosphatase activity	GO:0004725	0.019
-	NA NA	GO:0032318	0.022
cytoskeleton and motors	CC Actomyosin	GO:0042641	0.022
cell motion	CC cell projection	GO:0042995	0.022
-	BP aggregation involved in sorocarp development	GO:0031152	0.026
phagocytosis	CC phagocytic cup lip	GO:0097203	0.029
cytoskeleton and motors	CC Arp2/3 protein complex	GO:0005885	0.029
cytoskeleton and motors	BP Arp2/3 complex-mediated actin nucleation	GO:0034314	0.029
cytoskeleton and motors	BP actin filament polymerization	GO:0030041	0.029
-	BP positive regulation of protein kinase B signaling	GO:0051897	0.029
cytoskeleton and motors	MF actin-dependent ATPase activity	GO:0030898	0.029
cell motion	BP filopodium assembly	GO:0046847	0.029
-	BP establishment or maintenance of cell polarity	GO:0007163	0.029
-	BP cell-cell junction organization	GO:0045216	0.029
cytoskeleton and motors	BP actin filament bundle assembly	GO:0051017	0.029
cell motion	BP axon guidance	GO:0007411	0.029
-	MF phosphatidylinositol-3-phosphatase activity	GO:0004438	0.029
-	MF Ras guanyl-nucleotide exchange factor activity	GO:0005088	0.029
-	BP regulation of cell shape	GO:0008360	0.029
-	MF Rac guanyl-nucleotide exchange factor activity	GO:0030676	0.029
-	BP negative regulation of Ras protein signal transduction	GO:0046580	0.030
cytoskeleton and motors	BP actin filament organization	GO:0007015	0.047
cytoskeleton and motors	BP actin crosslink formation	GO:0051764	0.047
-	BP phosphatidylinositol dephosphorylation	GO:0046856	0.047
-	CC Centrosome	GO:0005813	0.047

Supplementary Table 7. Primers used for quantitative RT-PCR in this study (Fig. 4b and Supplementary Fig. 6)

Primer name	Sequence (5'-3')
EF1-alpha-F	AACGTTGTCGTCATTGGTCA
EF1-alpha-R	CTTGTCCAAAACCCAAGCAT
Phytoene desaturase-F	GATGCTGGAAGGATTCTGC
Phytoene desaturase-R	TCAACCCATTCTCGTAACC
DNA-(apurinic or apyrimidinic site) lyase-F	ATCGATTGGAGTGGGTGAAA
DNA-(apurinic or apyrimidinic site) lyase-R	GGGGATGGCTTTGAAGATTT
Deoxyribodipyrimidine photo-lyase-F	TGGGAAATACCAACAGACCA
Deoxyribodipyrimidine photo-lyase-R	CAGCACCCATTCTCCAATCT
Myosin type I-F	CAGCCAGCTCTCAACCAAAG
Myosin type I-R	TCGTCATCTGCTGATGCTTG
Myosin type II-F	AGCTGGTCTCGTTCGTGAAA
Myosin type II-R	GCCTTGACGAATGGAATTGA
Myosin family-F	CACAAGCTGCATGTCAAGGT
Myosin family-R	AACGATCAGCCTCAACCAAT
Actin-F	GCCGAAAGAGAAATCGTCAG
Actin-R	CCAGCAGCTTCCATACCAAT
COX15-F	TGCTGCAAAGGGATTCTTCT
COX15-R	GCCAAATCGAAACTCTTCCA
Glutathione peroxidase (comp227059)-F	CATCAGCATGAGCGAAATGA
Glutathione peroxidase (comp227059)-R	GGTCTTTGGTGAAGGCACAA
Glutathione peroxidase (comp226183)-F	TGATGCACAAGGAAACGATG
Glutathione peroxidase (comp226183)-R	TATCTCTGGCGAATGTGCAA
PAO-like 1-F	GATTCCAACCTGGTCAAAACGA
PAO-like 1-R	CCCTTAATTGGAGCATCAGC
PAO-like 2-F	TCCAAACGAAACCTCATTCC
PAO-like 2-R	ACGAAACATGGAGGGACAAA