New Phytologist

***New Phytologist* Supporting Information**

Article title: **Hypometabolism to survive the long polar night and subsequent successful return to light in the diatom *Fragilariopsis cylindrus***

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The following Supporting Information is available for this article:

Figure S1. Global contribution of *Fragilariopsis* barcodes (V9) to diatom populations across the *Tara* Oceans expedition.

Figure S2. Results from the physiological, biochemical, cytometry and protein analysis in *Fragilariopsis cylindrus*.

Figure S3. SEM images and TEM image analysis of *Fragilariopsis cylindrus* in light and dark conditions

Figure S4. 3D reconstruction of cells of *Fragilariopsis cylindrus* using Focused Ion Beam-Scanning Electron microscopy (FIB-SEM).

Figure S5. Fluorescence micrograms of *Fragilariopsis cylindrus* stained with DAPI and NileRed.

Figure S6. Tracking genes significantly differentially expressed at single time points in *Fragilariopsis cylindrus*.

Figure S7. Western blot targeting the active form of RNA Polymerase II (RPB1 CTD Ser2p) in *Fragilariopsis cylindrus*.

Figure S8. Principal component analysis of a total 17 light and dark transcriptomes of *Fragilariopsis cylindrus* from cultures in the acclimation phase in full light (T0LIGHT), in prolonged darkness (1 month), and upon return to light (up to 7 days).

Excel file (submitted separately):

Table S1. Summary and timing of the parameters measured from the cultures of *Fragilariopsis cylindrus*.

Table S2: Global contribution of Fragilariopsis barcodes (V9) to diatom populations across Tara Oceans.

Table S3. ID of genes of *Fragilariopsis cylindrus* included within each DREM subclusters and associated enriched GO categories.

Table S4. Complete list of genes of *Fragilariopsis cylindrus* that are significantly differentially expressed at single time points as compared to T0LIGHT.

Table S5. Correspondence of genes names in *Fragilariopsis cylindrus* with acronyms, the number of genes considered and the subcellular predictions from Figure 5.

Table S6. Complete list of genes explored in *Fragilariopsis cylindrus*.

Annotation files (submitted separately) (gff files can be opened as text files) :

Dataset S1 Annotation file for gene

Dataset S1 Annotation file for transposable elements

Videos 1-6 available for download at

https://zenodo.org/record/7766698#.ZB2EChWZNAd:

Video 1 FIB-SEM-Light-1: 3D reconstruction of a full light-acclimated cell allowed with focused ion beam/scanning electron microscopy [FIB-SEM-Light-1.avi].

Video 2 FIB-SEM-Light-2: 3D reconstruction of a full light-acclimated cell allowed with focused ion beam/scanning electron microscopy [FIB-SEM-Light-2.avi].

Video 3 FIB-SEM-Light-3: 3D reconstruction of a full light-acclimated cell allowed with focused ion beam/scanning electron microscopy [FIB-SEM-Light-3.avi].

Video 4 FIB-SEM-Dark-1: 3D reconstruction of a dark-acclimated cell allowed with focused ion beam/scanning electron microscopy [FIB-SEM-Dark-1.avi].

Video 5 FIB-SEM-Dark-2: 3D reconstruction of a dark-acclimated cell allowed with focused ion beam/scanning electron microscopy [FIB-SEM-Dark-2.avi].

Video 6 FIB-SEM-Dark-3: 3D reconstruction of a dark-acclimated cell allowed with focused ion beam/scanning electron microscopy [FIB-SEM-Dark-3.avi].

Fig. S1 Global contribution of *Fragilariopsis* barcodes (V9) to diatom populations across the *Tara* Oceans expedition after pooling the four size fractions (0.8-5 µm; 5-20 µm; 20-180 µm and 180-2000 µm) and the two depths (surface and deep chlorophyll maximum), with longitude and latitude details of the samples given in **Table S2**.

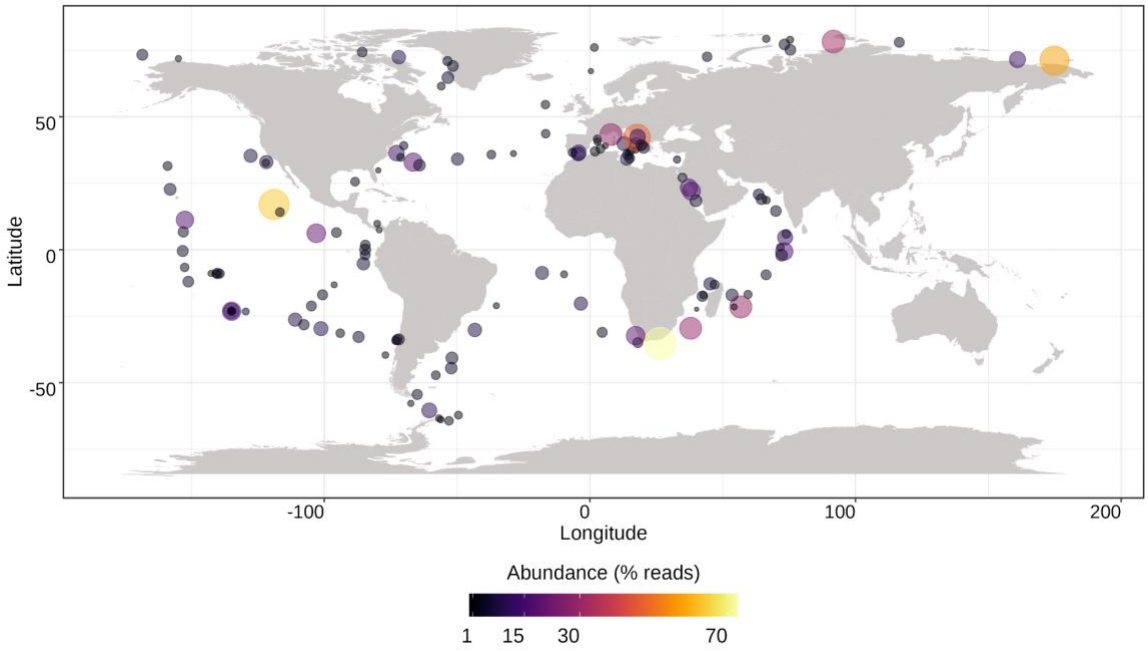
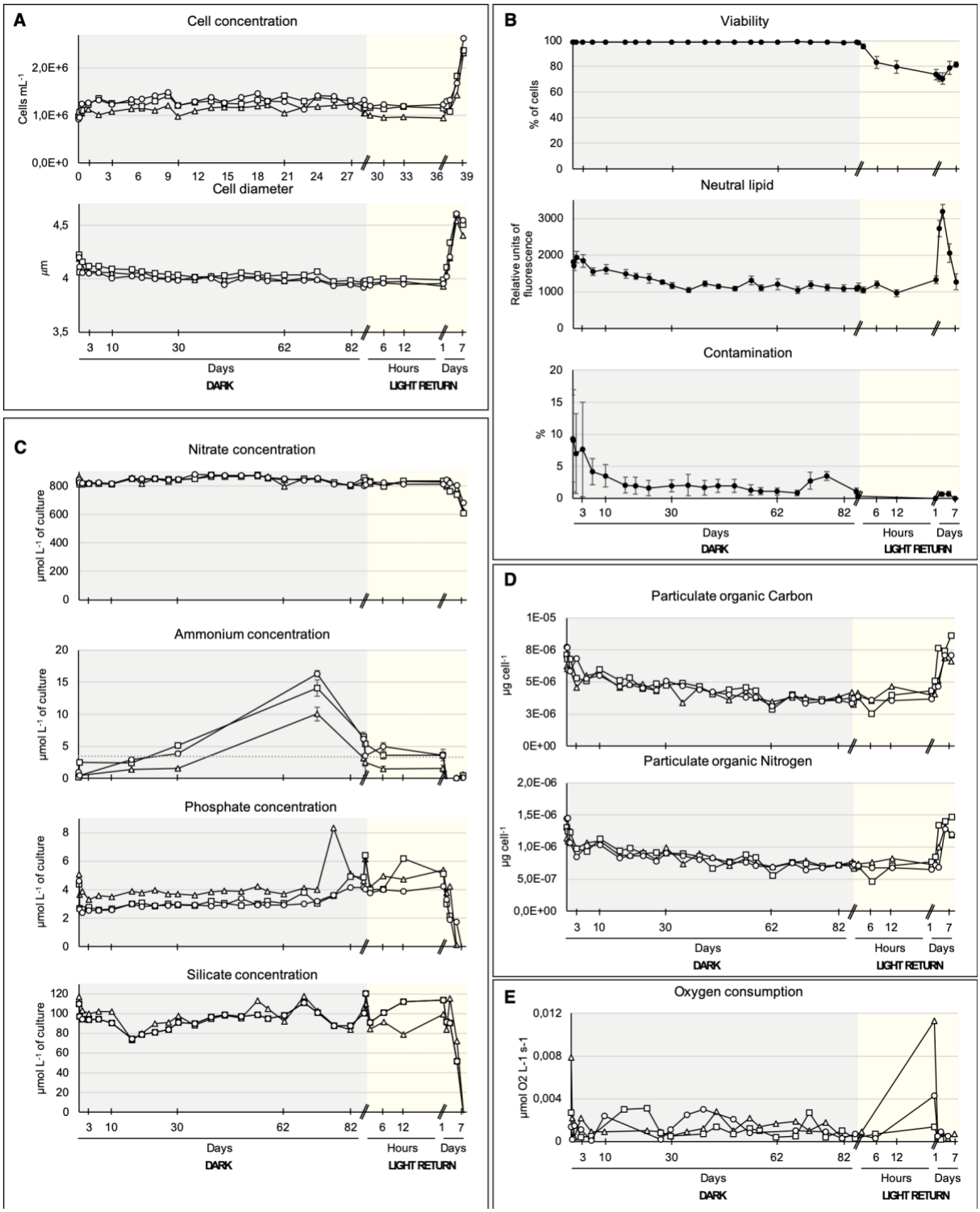
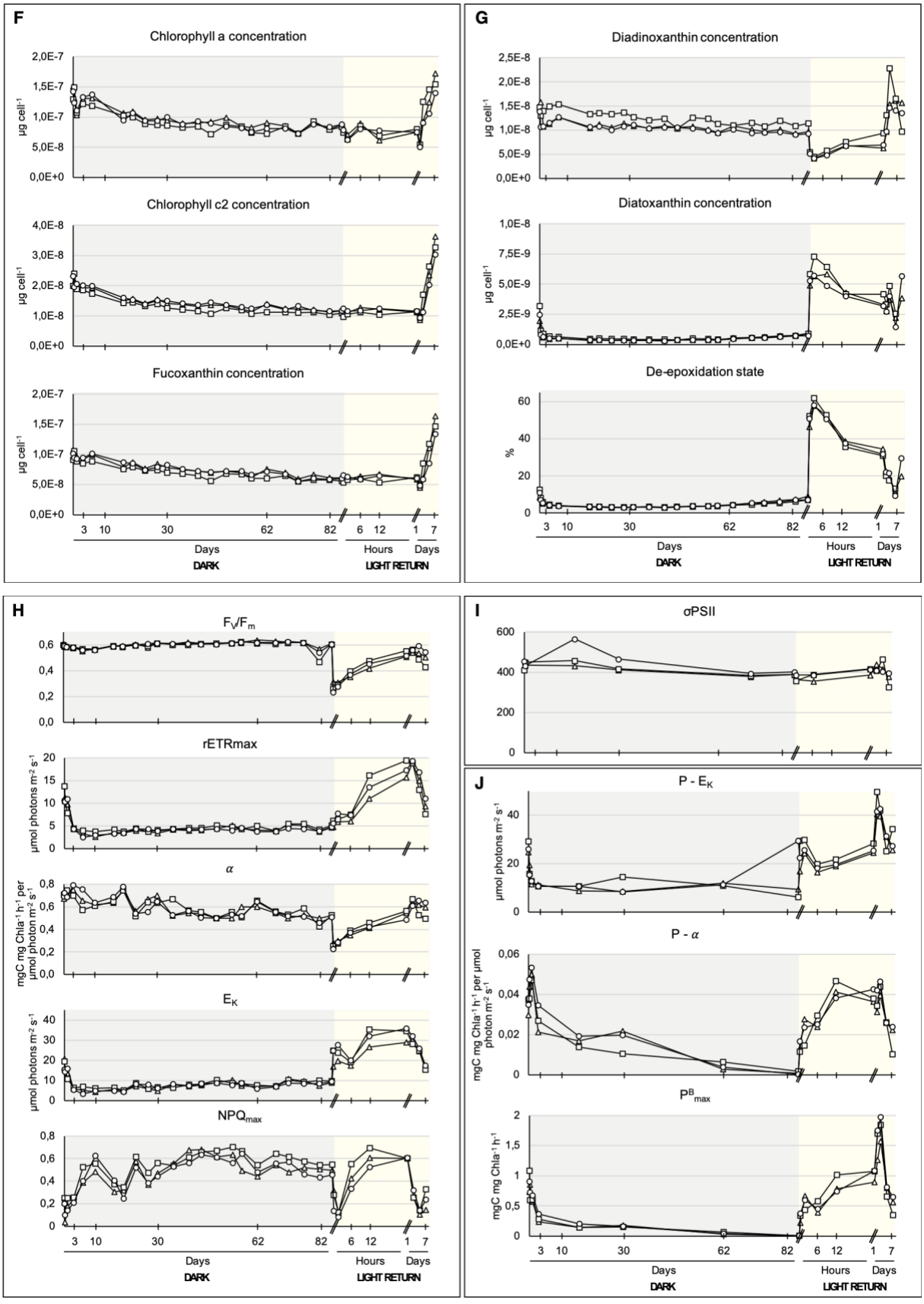


Fig. S2 Results from the physiological, biochemical, cytometry and protein analysis in *Fragilariopsis cylindrus*. The triangles represent cylinder 1, squares for Cylinder 2 and circles for Cylinder 3. **(A)** Cell abundance and cell diameter. **(B)** Average and standard deviation of parameters measured by cytometry: percentage (%) of viability of cell (SYTOX), neutral lipid content expressed as relative units (BODIPY) and % of bacterial contamination (SYBR GREEN). **(C)** Nitrate, Phosphate and Silicate concentrations in µmol L^{-1} of culture measured with autoanalyzer. Ammonium concentration in µmol L^{-1} of culture (and standard deviation) measured by incubation and fluorescence. **(D)** Carbon and Nitrogen concentrations in µg $cell^{-1}$. **(E)** Oxygen consumption in the culture in µmol O_2 L^{-1} s^{-1}. **(F)** Photosynthetic pigments (Chl a, Chl c2 and Fucoxanthin) concentrations in µg $cell^{-1}$. **(G)** Photoprotective pigments (DD and DT) concentrations in µg $cell^{-1}$. The de-epoxidation state of xanthophyll cycle pigments in %. **(H)** Parameters from Phyto-PAM Rapid Light Curves protocol; maximum quantum yield efficiency of PSII (F_V/F_m), maximum relative photosynthetic electron transport rate in µmol photons m^{-2} s^{-1} ($rETR_{max}$), chlorophyll specific photosynthetic efficiency coefficient in mgC mg $Chla^{-1}$ h^{-1} per µmol photon m^{-2} s^{-1} (α), light saturation coefficient in µmol photons m^{-2} s^{-1} (E_K), and non-photochemical quenching (NPQ_{max}). **(I)** Average absorption cross-section σPSII measured by a mini fluorescence induction and relaxation (FIRe) in $angstrom^2$. **(J)** Parameters from PvsE curves (^{14}C incubation); Light saturation coefficient in µmol photons m^{-2} s^{-1} (P - E_K), chlorophyll specific photosynthetic efficiency coefficient in mgC mg $Chla^{-1}$ h^{-1} per µmol photon m^{-2} s^{-1} (P - α) and maximum specific carbon fixation rate in mgC mg $Chla^{-1}$ h^{-1} (P^B_{max}). **(K)** Total protein measured in µg $cell^{-1}$. The photosynthetic proteins RbcL and PsbA concentrations are given in µg. Samples in red fall below the quantification limit.


A
Cell concentration
Cells mL-1
2,0E+6
1,0E+6
0,0E+0
0 3 6 9 12 15 18 21 24 27 30 33 36 39
Cell diameter
µm
4,5
4
3,5
3 10 30 62 82 6 12 1 7
Days
Hours
Days
DARK
LIGHT RETURN
B
Viability
% of cells
100
80
60
40
20
0
Neutral lipid
Relative units of fluorescence
3000
2000
1000
0
Contamination
%
20
15
10
5
0
3 10 30 62 82 6 12 1 7
Days
Hours
Days
DARK
LIGHT RETURN
C
Nitrate concentration
µmol L-1 of culture
800
600
400
200
0
Ammonium concentration
µmol L-1 of culture
20
15
10
5
0
Phosphate concentration
µmol L-1 of culture
8
6
4
2
0
Silicate concentration
µmol L-1 of culture
120
100
80
60
40
20
0
3 10 30 62 82 6 12 1 7
Days
Hours
Days
DARK
LIGHT RETURN
D
Particulate organic Carbon
µg cell-1
1E-05
8E-06
5E-06
3E-06
0E+00
Particulate organic Nitrogen
µg cell-1
2,0E-06
1,5E-06
1,0E-06
5,0E-07
0,0E+00
3 10 30 62 82 6 12 1 7
Days
Hours
Days
DARK
LIGHT RETURN
E
Oxygen consumption
µmol O2 L-1 s-1
0,012
0,008
0,004
0
3 10 30 62 82 6 12 1 7
Days
Hours
Days
DARK
LIGHT RETURN


F
Chlorophyll a concentration
µg cell-1
2,0E-7
1,5E-7
1,0E-7
5,0E-8
0,0E+0
Chlorophyll c2 concentration
4,0E-8
3,0E-8
2,0E-8
1,0E-8
0,0E+0
Fucoxanthin concentration
3 10 30 62 82 6 12 1 7
Days Hours Days
DARK LIGHT RETURN
G
Diadinoxanthin concentration
2,5E-8
2,0E-8
1,5E-8
1,0E-8
5,0E-9
0,0E+0
Diatoxanthin concentration
1,0E-8
7,5E-9
5,0E-9
2,5E-9
0,0E+0
De-epoxidation state
%
60
40
20
0
3 10 30 62 82 6 12 1 7
Days Hours Days
DARK LIGHT RETURN
H
FV/Fm
0,6
0,4
0,2
0,0
rETRmax
µmol photons m-2 s-1
20
15
10
5
0
α
mgC mg Chla-1 h-1 per µmol photon m-2 s-1
0,8
0,6
0,4
0,2
0,0
EK
µmol photons m-2 s-1
40
30
20
10
0
NPQmax
0,8
0,6
0,4
0,2
0
3 10 30 62 82 6 12 1 7
Days Hours Days
DARK LIGHT RETURN
I
σPSII
600
400
200
0
J
P - EK
µmol photons m-2 s-1
40
20
0
P - α
mgC mg Chla-1 h-1 per µmol photon m-2 s-1
0,06
0,04
0,02
0
PBmax
mgC mg Chla-1 h-1
2
1,5
1
0,5
0
3 10 30 62 82 6 12 1 7
Days Hours Days
DARK LIGHT RETURN

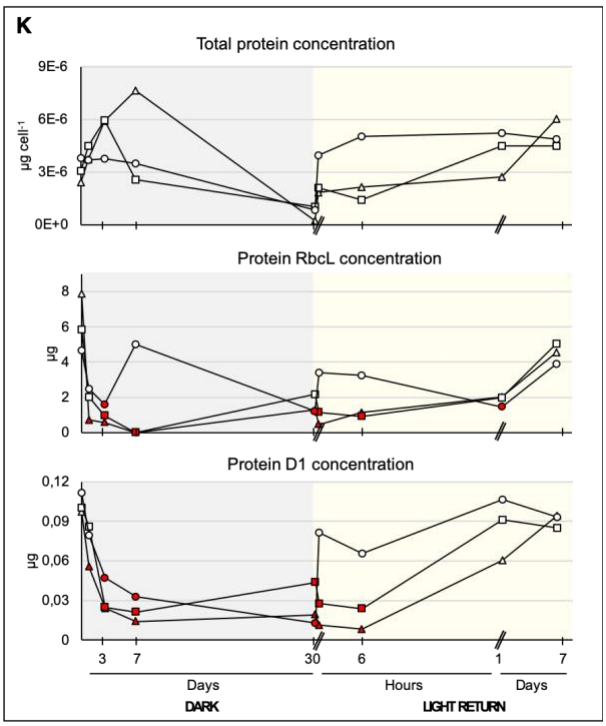

K
Total protein concentration
9E-6
6E-6
3E-6
0E+0
µg cell-1
Protein RbcL concentration
8
6
4
2
0
µg
Protein D1 concentration
0,12
0,09
0,06
0,03
0
µg
3
7
30
6
1
7
Days
Hours
Days
DARK
LIGHT RETURN

Fig. S3 SEM images and TEM image analysis of *Fragilariopsis cylindrus* in light and dark conditions. **(A)** External morphology of light-adapted cells observed by SEM. Scale bar represents 10µm. **(B)** External morphology of dark-adapted cells observed by SEM. Scale bar represents 10µm. **(C)** Image of a dark cell illustrating the identification of the major organelle. The droplets filled with a whitish material were different from the content of vacuoles and from artifacts created during cell cutting and could be chrysolaminarin (CHY). Scale bar represents 1µm. **(D)** Images of the only two dark cells illustrating the presence of bodies inside the vacuoles. Scale bar represents 1µm. **(E)** Diagram comparing the proportion of non-dividing light/dark cells (blue) to the dividing ones (red). **(F)** Violin plot comparing the surface area (in μm^2) of the non-dividing cell cross-section in light/dark conditions. **(G)** Diagram comparing the proportion of light/dark cells that do not contain a nucleoli (blue) to one that do (red). **(H)** Violin plot comparing the surface area (in μm^2) of the cell cross-section occupied by the nucleoli in light/dark conditions. For violins plots, mean is shown by the bold horizontal bar, its 95% confidence interval is between the two error bars. Result of mean comparison is represented by its p-value above the plots. N is the number of images used for each plot.

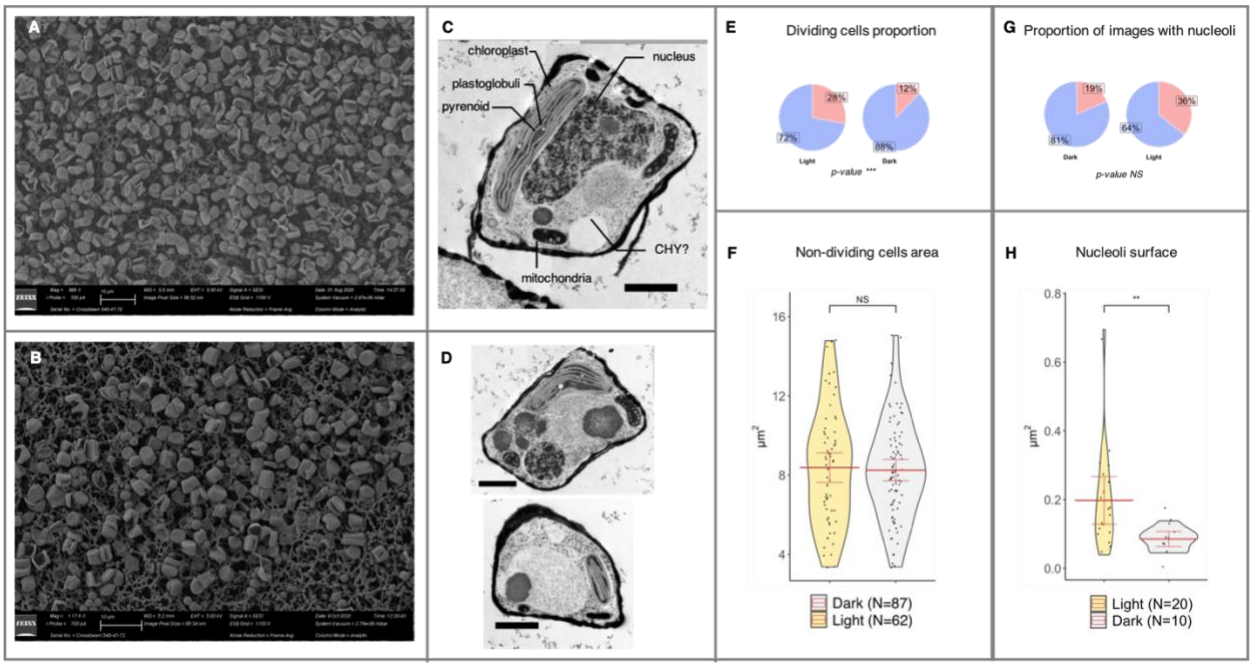


Fig. S4 3D reconstruction of cells of *Fragilariopsis cylindrus* using Focused Ion Beam-Scanning Electron microscopy (FIB-SEM). Videos illustrating the 3D cell reconstruction from FIB-SEM are available at https://zenodo.org/record/7766698#.ZB2EChWZNAd. **(A)** Example of two light-grown cells. **(B)** Example of two dark-adapted cells. **(C)** Average cell volume for light (n=5) and dark (n=3) cells and average proportions of the chloroplasts (in green), nucleus (in blue) and vacuole (in pink) expressed in percentage (%) of the cell volume.

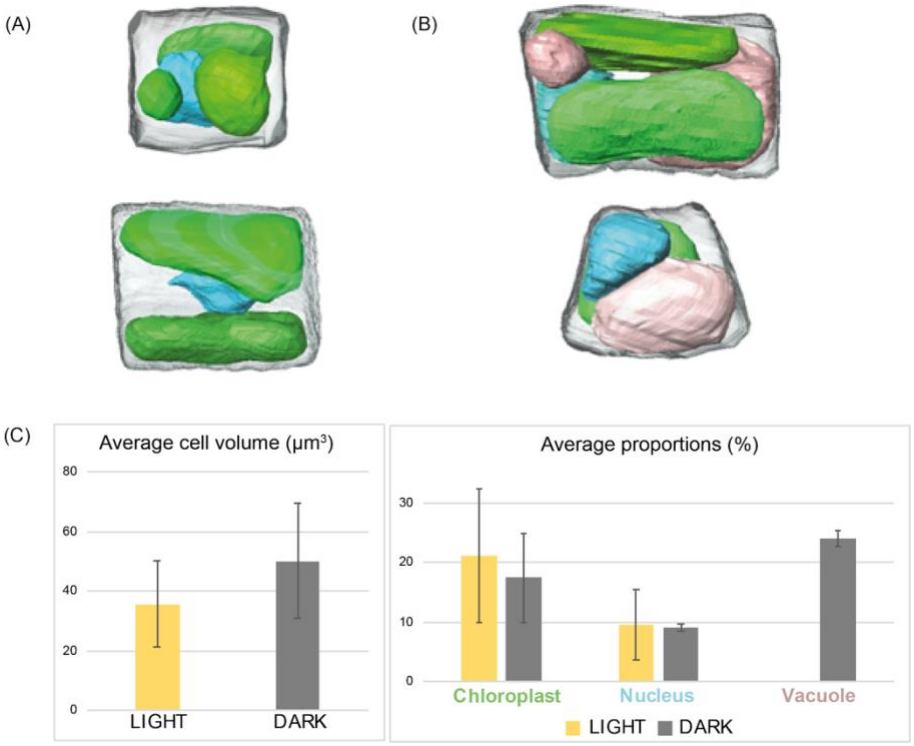


Fig. S5 Fluorescence micrograms of *Fragilariopsis cylindrus* stained with DAPI and NileRed. Scale bar = 2 µm. Chlorophyll autofluorescence, fluorescence of DAPI, and fluorescence of NileRed are visualized in green, blue, and red colors, respectively.

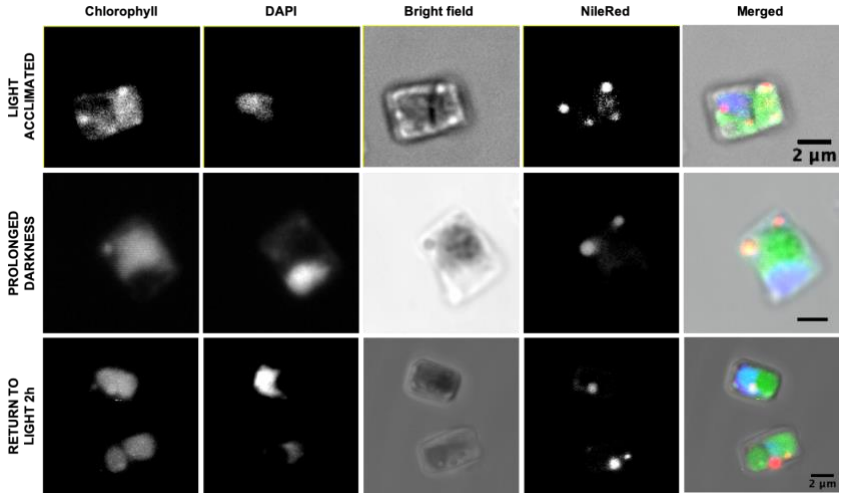
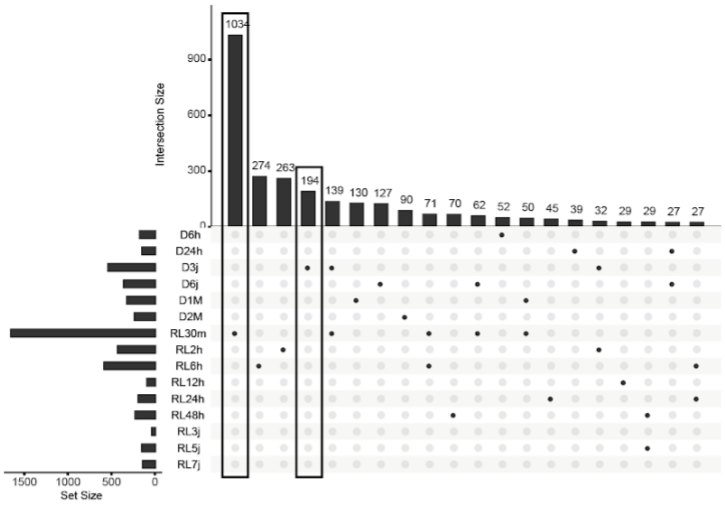
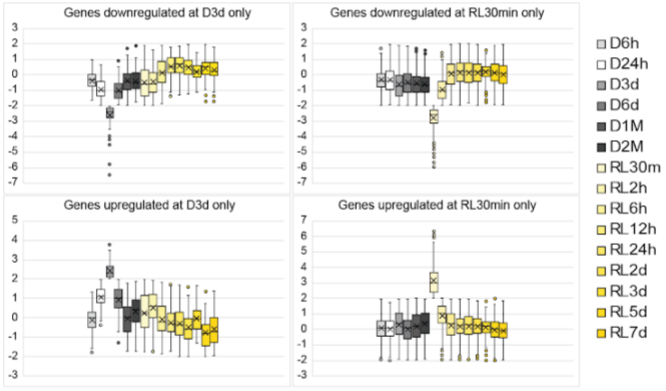


Fig. S6 Tracking genes significantly differentially expressed at single time points in *Fragilariopsis cylindrus*. **(A)** Upset plot of the 3619 genes not considered in the DREM analysis, that are significantly differentially regulated (|log2FC| > 2) in non-adjacent time points. **(B)** Boxplot of the genes differentially expressed (|log2FC| > 2) only after 3 days of darkness (left) or after 30 minutes of light return (right). The genes had been divided in two groups based on the positive (upper panels) or negative (lower panels) log2FC compared to T0LIGHT. **(C)** Heatmaps of selected examples of genes differentially expressed only after 3 days of darkness (upper panel) or after 30 minutes of light return (lower panel) and their biological function.

A)



B)



C)

Gene ID	D6h	D24h	D3d	D6d	D1M	D2M	RL30m	RL2h	RL6h	RL12h	RL24h	RL2d	RL3d	RL5d	RL7d	Function
179014																Histone variant H3.1
169745																Heat shock transcription factor
171517																Cytosolic Ca2+-dependent cysteine protease (calpain), large subunit
207857																Predicted seven transmembrane receptor - rhodopsin family
244363																Serine/threonine protein kinase (role in apoptosis)
264097																VAMP-associated protein (required for autophagic degradation)
264380																Beta tubulin
247148																Nucleolar GTPase/ATPase p130 / Peroxisomal biogenesis factor 11
271239																Ubiquinol cytochrome c reductase, subunit RIP1

Gene ID	D6h	D24h	D3d	D6d	D1M	D2M	RL30m	RL2h	RL6h	RL12h	RL24h	RL2d	RL3d	RL5d	RL7d	Function
234892																Basic-leucine zipper transcription factor
272712																Mitochondrial/chloroplast ribosomal protein S16
219511																Mitochondrial/chloroplast ribosomal protein S16
223086																Ubiquinol cytochrome c reductse, subunit RIP1
216240																Ubiquinol cytochrome c reductase, subunit RIP1
265576																Mitochondrial protein Surfeit 1/SURF1/SHY1 (required for expression of COX)
261886																Cytochrome b5
270097																Putative cytochrome C oxidase assembly protein
225175																Pyruvate dehydrogenase E1, beta subunit

Fig. S7 Western blot targeting the active form of RNA Polymerase II (RPB1 CTD Ser2p) in *Fragilariopsis cylindrus*. Histone H4 was used as a loading control. On the left the gel in which the first three columns represent light samples and the last three represent dark samples. On the right the quantification of the blot, taking into account the normalization using the H4 protein.

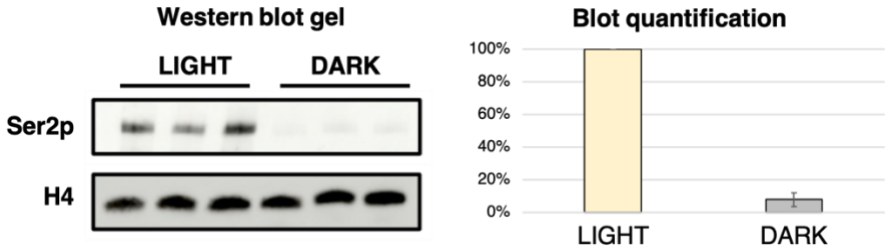


Fig. S8 Principal component analysis of a total 17 light and dark transcriptomes of *Fragilariopsis cylindrus* from cultures in the acclimation phase in full light (T0LIGHT), in prolonged darkness (1 month (M)), and upon return to light (from ½ hour (h) up to 7 days (d)). Each dot represents one transcriptome and the shapes refer to one of the three biological replicates.