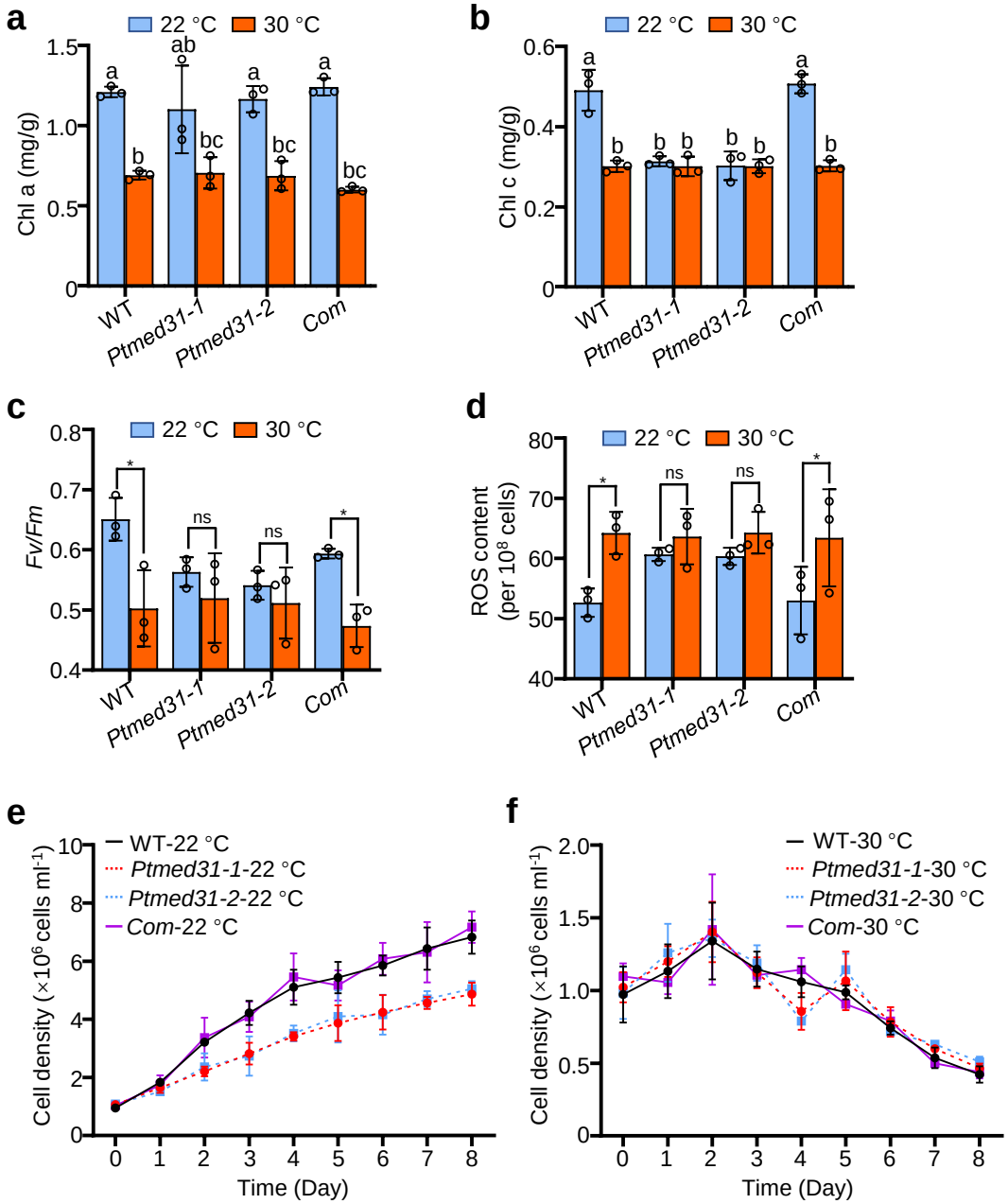
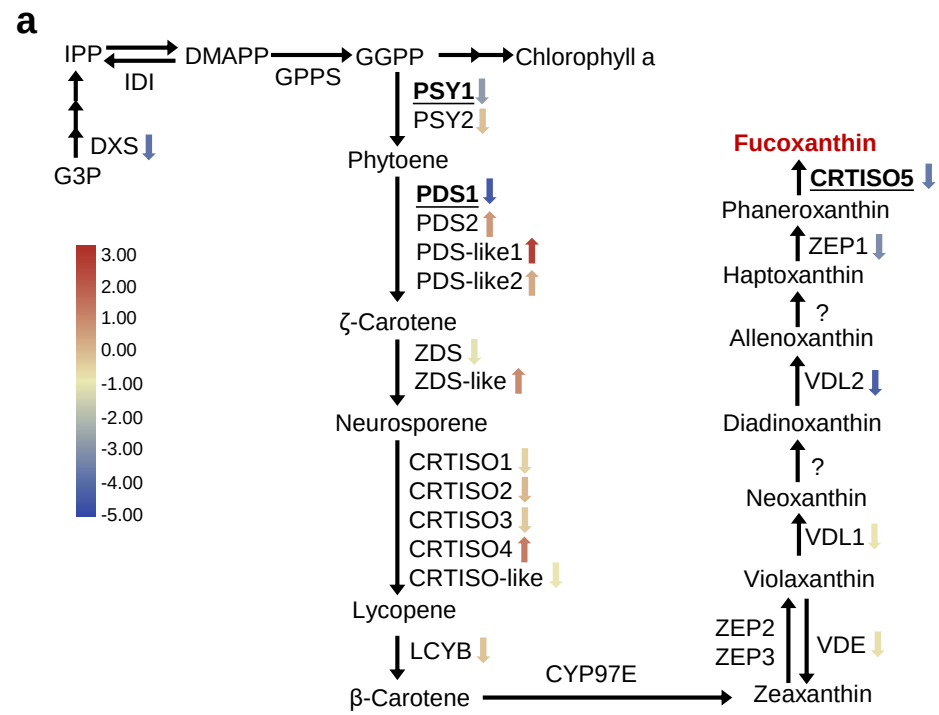
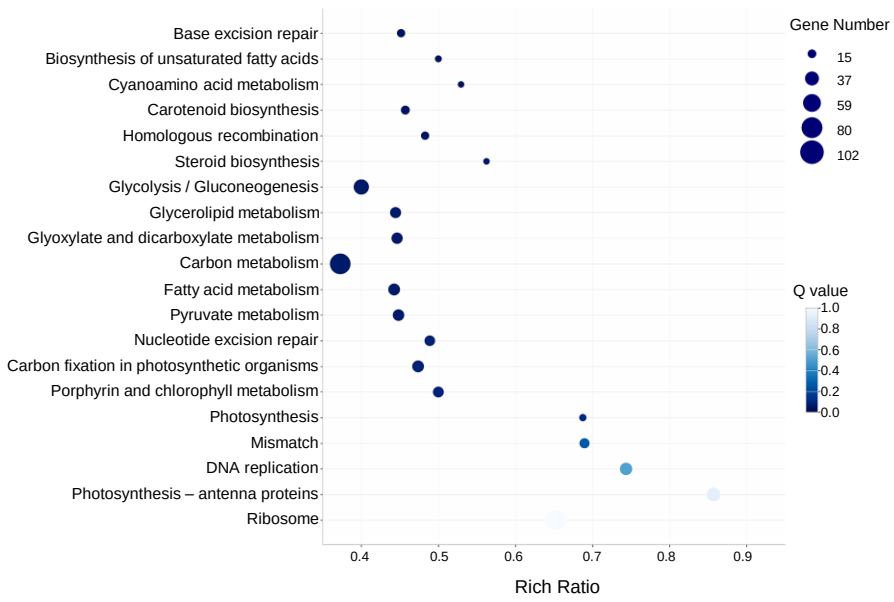
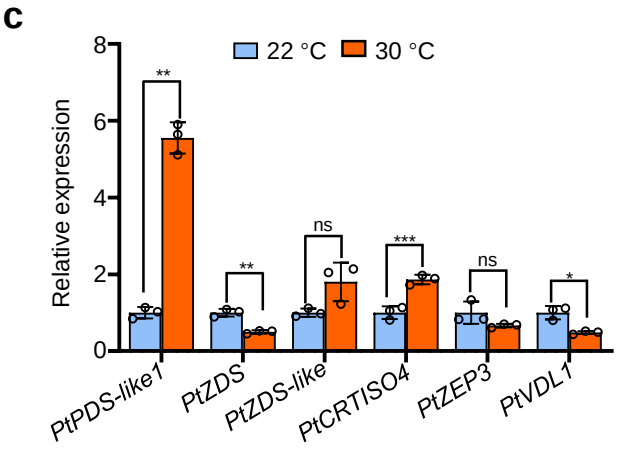
A proteolysis-dependent Mediator switch controls heat-responsive fucoxanthin biosynthesis in diatoms


a
22 °C
30 °C
Chl a (mg/g)
1.5
1.0
0.5
0
a
ab
a
a
b
bc
bc
bc
WT
Ptmed31-1
Ptmed31-2
Com
b
Chl c (mg/g)
0.6
0.4
0.2
0
a
b
b
b
b
b
a
b
c
Fv/Fm
0.8
0.7
0.6
0.5
0.4
*
ns
ns
*
d
ROS content
(per 10^8 cells)
80
70
60
50
40
*
ns
ns
*
e
WT-22 °C
Ptmed31-1-22 °C
Ptmed31-2-22 °C
Com-22 °C
Cell density (×10^6 cells ml^-1)
10
8
6
4
2
0
0 1 2 3 4 5 6 7 8
Time (Day)
f
WT-30 °C
Ptmed31-1-30 °C
Ptmed31-2-30 °C
Com-30 °C
Cell density (×10^6 cells ml^-1)
2.0
1.5
1.0
0.5
0
0 1 2 3 4 5 6 7 8
Time (Day)

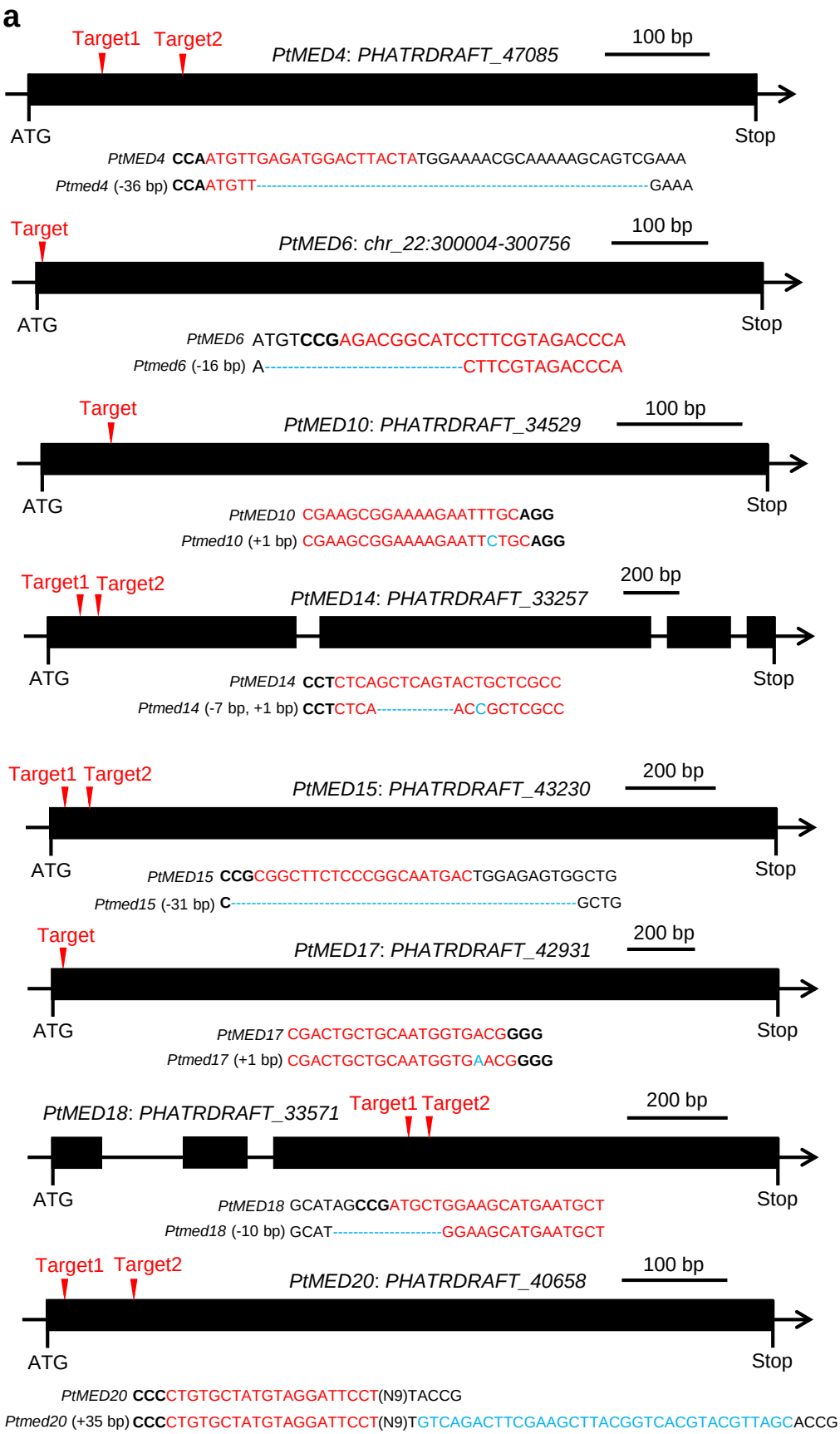
Supplementary Fig. 1 | PtMED31 contributes to photosynthetic pigment accumulation and photophysiological performance under normal and heat stress conditions. a,**b** Chlorophyll a (**a**) and chlorophyll c (**b**) contents of indicated lines at 22 °C and 30 °C. **c** Maximum quantum yield of photosystem II (PSII), measured as *Fv/Fm*, in the indicated lines at 22 °C and 30 °C. **d** Reactive oxygen species (ROS) levels in the indicated lines at 22 °C and 30 °C. **e**,**f** Time-course analysis of cell density in the indicated cell lines at 22 °C (**e**) and 30 °C (**f**). Data are presented as mean ± SD from three independent biological replicates. In (**a**, **b**), different letters indicate significant differences among groups at $P < 0.05$. In (**c**, **d**), statistical significance was determined using Student's *t*-test between 22 °C and 30 °C within each line. *$P < 0.05$; ns, not significant.

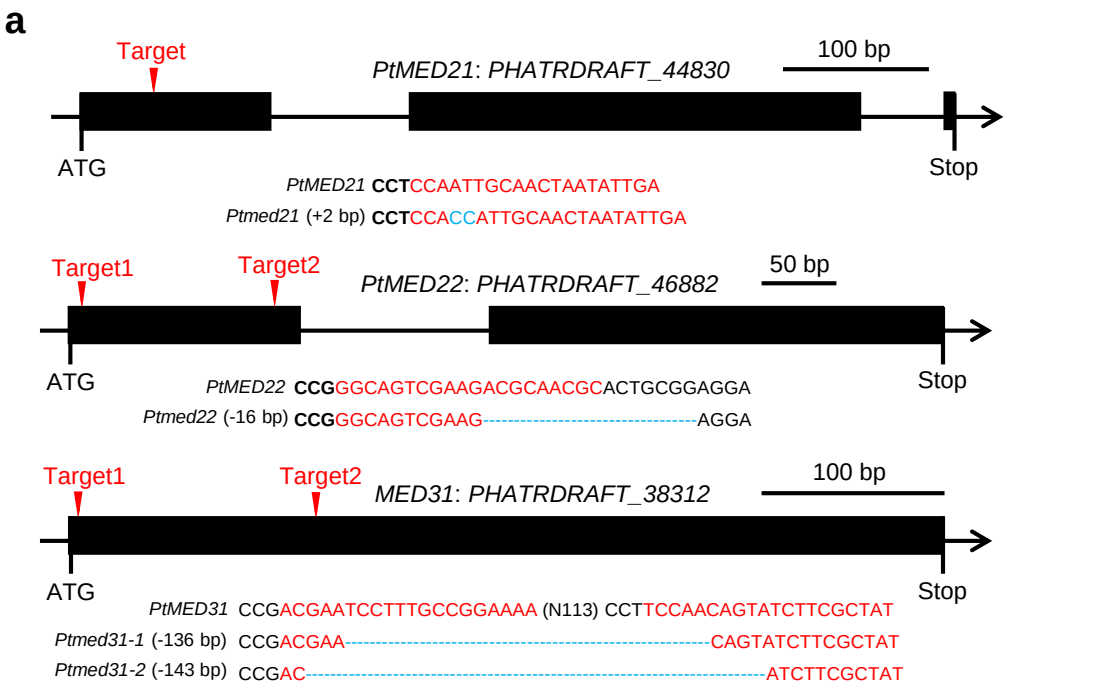

a
IPP
IDI
DMAPP
GPPS
GGPP
Chlorophyll a
DXS
G3P
PSY1
PSY2
Phytoene
PDS1
PDS2
PDS-like1
PDS-like2
ζ-Carotene
ZDS
ZDS-like
Neurosporene
CRTISO1
CRTISO2
CRTISO3
CRTISO4
CRTISO-like
Lycopene
LCYB
β-Carotene
CYP97E
Zeaxanthin
ZEP2
ZEP3
VDE
Violaxanthin
VDL1
Neoxanthin
?
Diadinoxanthin
VDL2
Allenoxanthin
?
Haptoxanthin
ZEP1
Phaneroxanthin
CRTISO5
Fucoxanthin
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00

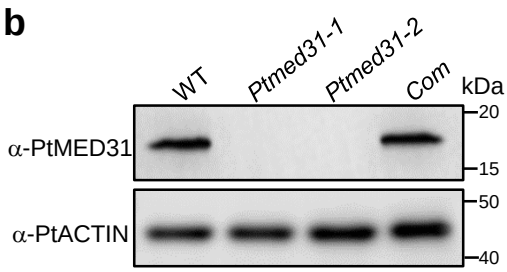

b
Base excision repair
Biosynthesis of unsaturated fatty acids
Cyanoamino acid metabolism
Carotenoid biosynthesis
Homologous recombination
Steroid biosynthesis
Glycolysis / Gluconeogenesis
Glycerolipid metabolism
Glyoxylate and dicarboxylate metabolism
Carbon metabolism
Fatty acid metabolism
Pyruvate metabolism
Nucleotide excision repair
Carbon fixation in photosynthetic organisms
Porphyrin and chlorophyll metabolism
Photosynthesis
Mismatch
DNA replication
Photosynthesis – antenna proteins
Ribosome
0.4
0.5
0.6
0.7
0.8
0.9
Rich Ratio
Gene Number
15
37
59
80
102
Q value
1.0
0.8
0.6
0.4
0.2
0.0

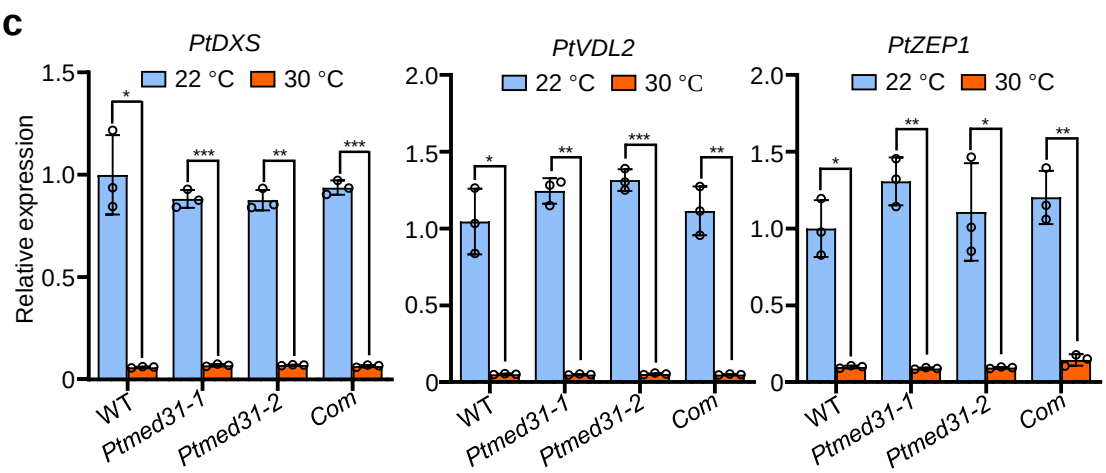

c
22 °C
30 °C
Relative expression
0
2
4
6
8
**
**
ns
***
ns
*
PtPDS-like1
PtZDS
PtZDS-like
PtCRTISO4
PtZEP3
PtVDL1

Supplementary Fig. 2 | Transcriptomic profiling of heat-repressed fucoxanthin biosynthesis and validation of selected genes by RT-qPCR. a Fucoxanthin biosynthetic pathway and transcriptomic changes in pathway-related genes in WT *P. tricornutum* under heat stress. The $\log_2$ fold changes in gene expression (30 °C vs 22 °C) in wild-type *P. tricornutum* are depicted by colored arrows positioned adjacent to each gene name. Genes exhibiting upregulation and downregulation are denoted by upward and downward arrows, respectively. **b** Top 20 enriched KEGG pathways identified from differentially expressed genes under heat stress. The y-axis denotes KEGG pathway terms, and the x-axis represents the rich ratio. Bubble size indicates the number of enriched genes, and bubble color represents the Q value, with lower Q values indicating greater statistical significance. **c** RT-qPCR validation of selected fucoxanthin-biosynthetic genes, including *PtPDS-like1*, *PtZDS*, *PtZDS-like*, *PtCRTISO4*, *PtZEP3*, and *PtVDL1*, in WT *P. tricornutum* cells incubated at 22 °C or 30 °C for 24 h before RNA extraction. Data are presented as mean ± SD from three independent biological replicates, **P* < 0.05, ***P* < 0.01, ****P* < 0.001 (Student's *t* test).

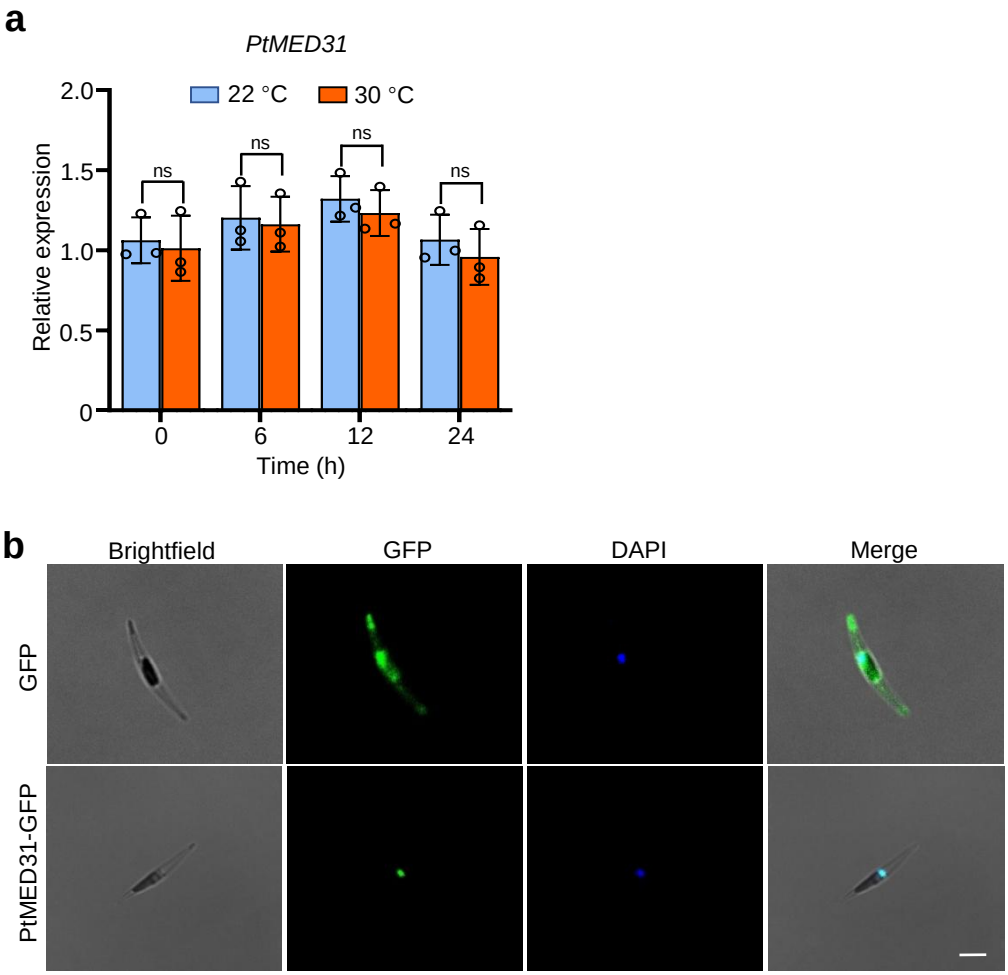

a
Target1
Target2
PtMED4: PHATRDRAFT_47085
100 bp
ATG
Stop
PtMED4 CCAATGTTGAGATGGACTTACTATGGAAAACGCAAAAAGCAGTCGAAA
Ptmed4 (-36 bp) CCAATGTT----------GAAA
Target
PtMED6: chr_22:300004-300756
100 bp
ATG
Stop
PtMED6 ATGTCCGAGACGGCATCCTTCGTAGACCCA
Ptmed6 (-16 bp) A----------CTTCGTAGACCCA
Target
PtMED10: PHATRDRAFT_34529
100 bp
ATG
Stop
PtMED10 CGAAGCGGAAAAGAATTTGCAGG
Ptmed10 (+1 bp) CGAAGCGGAAAAGAATTCTGCAGG
Target1
Target2
PtMED14: PHATRDRAFT_33257
200 bp
ATG
Stop
PtMED14 CCTCTCAGCTCAGTACTGCTCGCC
Ptmed14 (-7 bp, +1 bp) CCTCTCA----------ACCGCTCGCC
Target1
Target2
PtMED15: PHATRDRAFT_43230
200 bp
ATG
Stop
PtMED15 CCGCGGCTTCTCCCGGCAATGACTGGAGAGTGGCTG
Ptmed15 (-31 bp) C----------GCTG
Target
PtMED17: PHATRDRAFT_42931
200 bp
ATG
Stop
PtMED17 CGACTGCTGCAATGGTGACGGGG
Ptmed17 (+1 bp) CGACTGCTGCAATGGTGAACGGGG
PtMED18: PHATRDRAFT_33571
Target1
Target2
200 bp
ATG
Stop
PtMED18 GCATAGCCGATGCTGGAAGCATGAATGCT
Ptmed18 (-10 bp) GCAT----------GGAAGCATGAATGCT
Target1
Target2
PtMED20: PHATRDRAFT_40658
100 bp
ATG
Stop
PtMED20 CCCCTGTGCTATGTAGGATTCCT(N9)TACCG
Ptmed20 (+35 bp) CCCCTGTGCTATGTAGGATTCCT(N9)TGTCAGACTTCGAAGCTTACGGTCACGTACGTTAGCACCG


a
Target
PtMED21: PHATRDRAFT_44830
100 bp
ATG
Stop
PtMED21 CCTCCAATTGCAACTAATATTGA
Ptmed21 (+2 bp) CCTCCACCATTGCAACTAATATTGA
Target1
Target2
PtMED22: PHATRDRAFT_46882
50 bp
ATG
Stop
PtMED22 CCGGGCAGTCGAAGACGCAACGCACTGCGGAGGA
Ptmed22 (-16 bp) CCGGGCAGTCGAAG----------AGGA
Target1
Target2
MED31: PHATRDRAFT_38312
100 bp
ATG
Stop
PtMED31 CCGACGAATCCTTTGCCGGAAAA (N113) CCTTCCAACAGTATCTTCGCTAT
Ptmed31-1 (-136 bp) CCGACGAA----------CAGTATCTTCGCTAT
Ptmed31-2 (-143 bp) CCGAC----------ATCTTCGCTAT

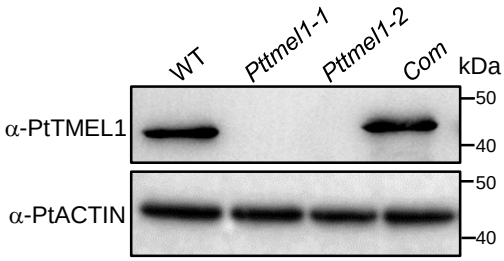

b
WT
Ptmed31-1
Ptmed31-2
Com
kDa
α-PtMED31
α-PtACTIN
20
15
50
40


c
PtDXS
PtVDL2
PtZEP1
22 °C
30 °C
Relative expression
1.5
1.0
0.5
0
2.0
WT
Ptmed31-1
Ptmed31-2
Com

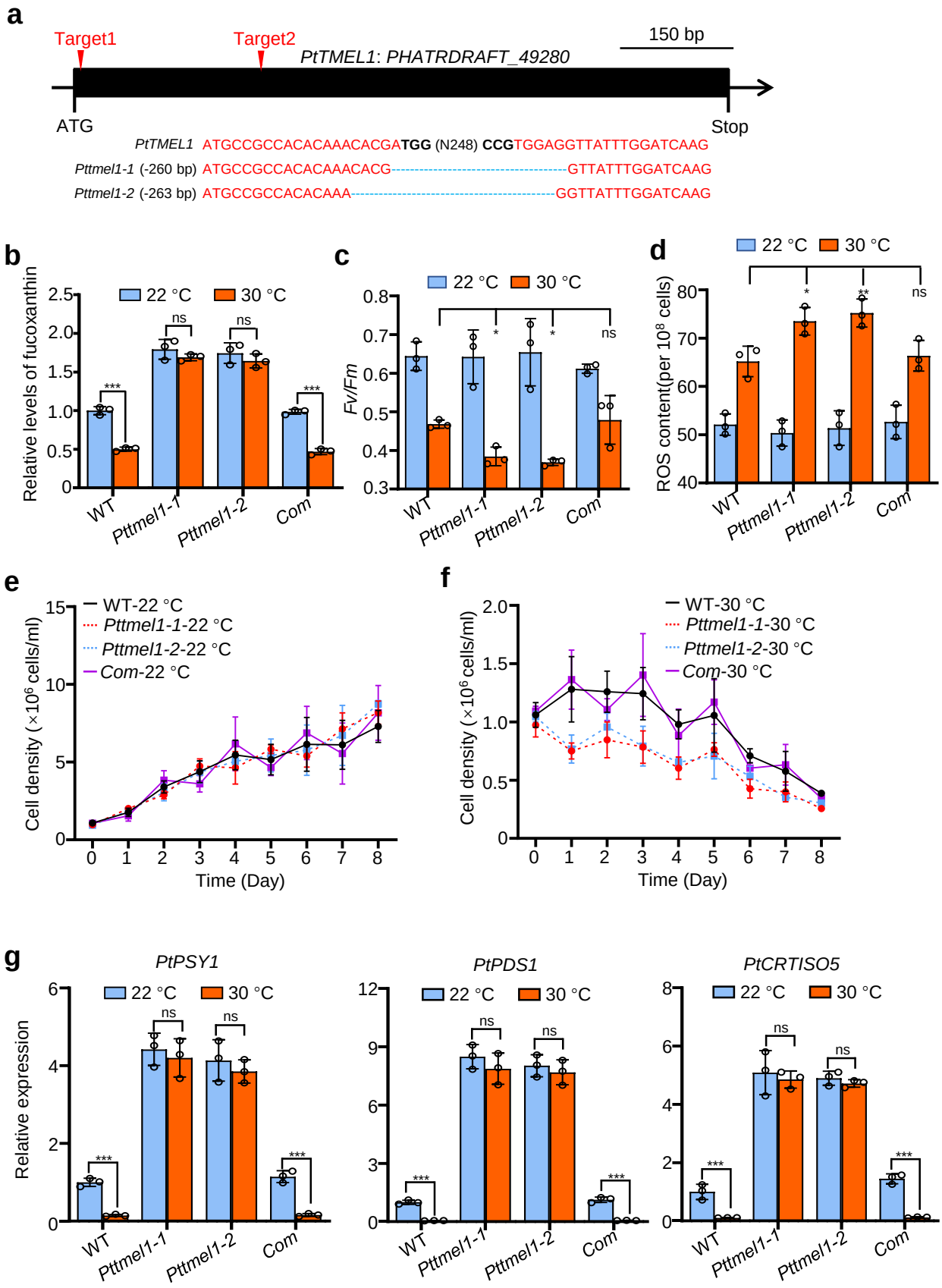
Supplementary Fig. 3 | Generation and molecular characterization of CRISPR/Cas9-edited *Ptmed* mutant lines. a Schematic diagrams showing CRISPR/Cas9 target sites and representative edited alleles of the indicated *PtMED* genes. Coding regions were targeted using specific sgRNAs. sgRNA target sequences are shown in red, and PAM sequences are shown in bold. Blue dashes indicate deletions, and blue letters indicate insertions. Numbers in parentheses indicate the sizes of insertions or deletions; +, insertion; −, deletion. **b** Immunoblotting analysis of PtMED31 protein abundance in WT, two independent *Ptmed31* mutant lines, and the *PtMED31*-complemented line. PtMED31 was detected using an anti-PtMED31 antibody, and PtACTIN was used as a loading control. **c** RT-qPCR analysis of the relative transcript levels of *PtDXS* (left), *PtVDL2* (middle), and *PtZEP1* (right) in the indicated lines incubated at 22 °C and 30 °C for 24 h before RNA extraction. In **c**, data are presented as mean ± SD from three independent biological replicates. Statistical significance was determined using Student's *t*-test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.



Supplementary Fig. 4 | Transcript abundance and subcellular localization of PtMED31. a RT-qPCR showing the *PtMED31* mRNA levels in WT at 22 °C and 30 °C for the indicated times. Data are presented as mean ± SD from three independent biological replicates. Statistical significance was determined using Student's *t*-test between the 22 °C and 30 °C treatments at each time point; ns, not significant. **b** Representative fluorescence microscopy images showing the subcellular localization of PtMED31-GFP. DAPI was used to stain nuclei. Scale bar, 5 μm.

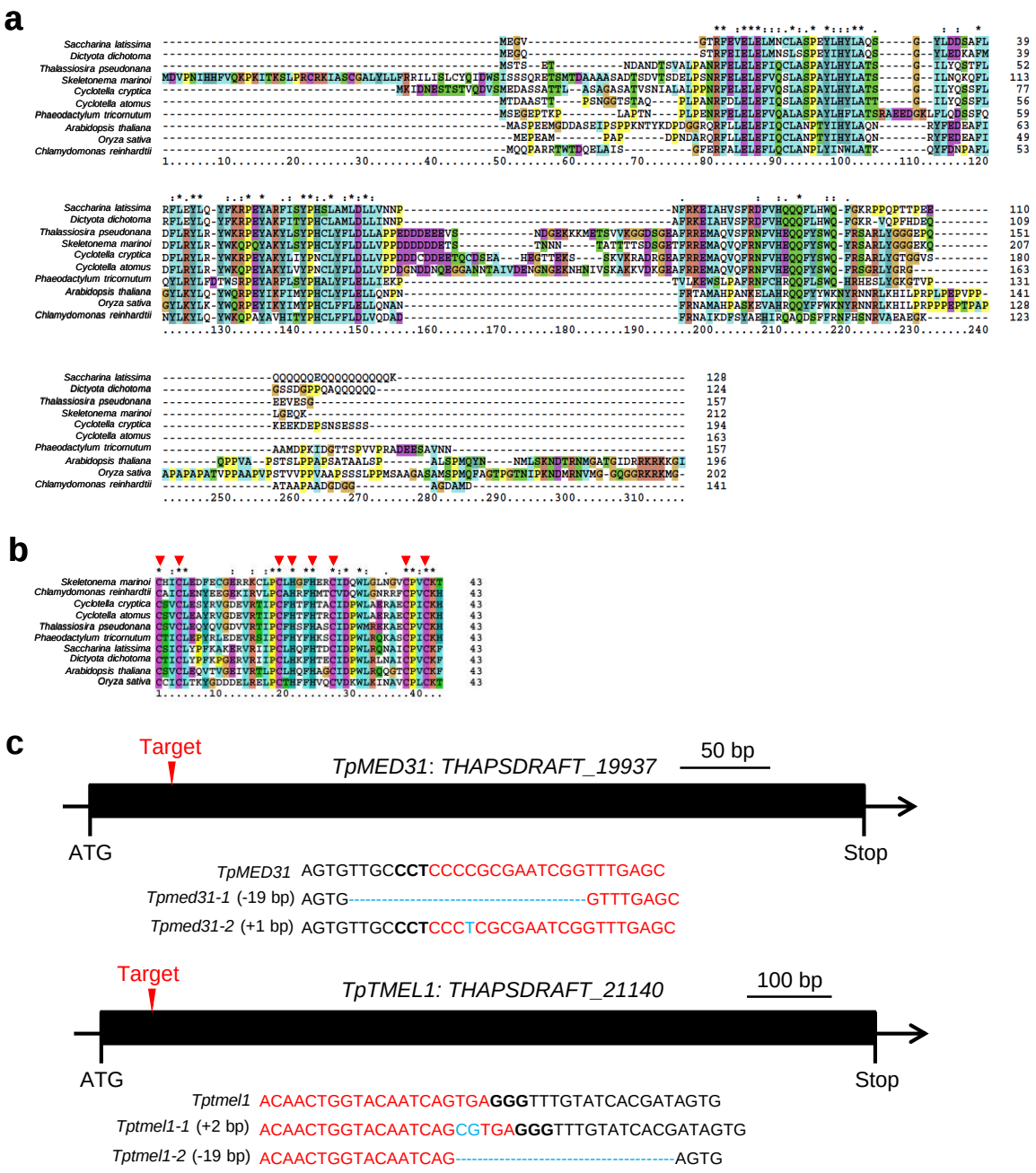


Supplementary Fig. 5 | Specificity validation of the anti-PtTMEL1 antibody. Immunoblotting analysis of PtTMEL1 protein abundance in WT, the *Pttmel1* mutant, and the *PtTMEL1*-complemented line (*Com*). Endogenous PtTMEL1 was detected using an anti-PtTMEL1 antibody, and PtACTIN was used as a loading control. The immunoblot is representative of three independent experiments.


a
Target1
Target2
150 bp
PtTMEL1: PHATRDRAFT_49280
ATG
Stop
PtTMEL1 ATGCCGCCACACAAACACGATGG (N248) CCGTGGAGGTTATTTGGATCAAG
Pttmel1-1 (-260 bp) ATGCCGCCACACAAACACG----------GTTATTTGGATCAAG
Pttmel1-2 (-263 bp) ATGCCGCCACACAAA----------GGTTATTTGGATCAAG
b
Relative levels of fucoxanthin
22 °C
30 °C
WT
Pttmel1-1
Pttmel1-2
Com
c
Fv/Fm
d
ROS content(per 10^8 cells)
e
Cell density (×10^6 cells/ml)
WT-22 °C
Pttmel1-1-22 °C
Pttmel1-2-22 °C
Com-22 °C
Time (Day)
f
WT-30 °C
Pttmel1-1-30 °C
Pttmel1-2-30 °C
Com-30 °C
g
PtPSY1
PtPDS1
PtCRTISO5
Relative expression

Supplementary Fig. 6 | Generation and characterization of *Pttmel1* mutant and complemented lines. **a** Schematic diagram showing CRISPR/Cas9-mediated editing of the *PtTMEL1* locus. Two sgRNAs were designed to target the coding region of *PtTMEL1*. sgRNA target sequences are shown in red, and PAM sequences are shown in bold. Blue dashes indicate deletions. Numbers in parentheses indicate deletion sizes. **b** Relative fucoxanthin levels in WT, two independent *Pttmel1* mutant lines, and the *PtTMEL1*-complemented line at 22 °C and 30 °C. **c** Maximum quantum yield of PSII, measured as *Fv/Fm*, in the indicated lines at 22 °C and 30 °C. **d** ROS levels in the indicated lines at 22 °C and 30 °C. **e**,**f** Time-course analysis of cell density in the indicated lines at 22 °C (**e**) and 30 °C (**f**). **g** RT-qPCR analysis of the relative transcript levels of *PtPSY1* (left), *PtPDS1* (middle), and *PtCRTISO5* (right) in the indicated lines at 22 °C and 30 °C for 24 h before RNA extraction. In (**b**-**g**), data are presented as mean ± SD from three independent biological replicates. **P* < 0.05, ****P* < 0.001 (Student's *t* test); ns, not significant**.**

Supplementary Fig. 7 | Generation and genotyping of *Ptmed31-1 Pttmel1* double-mutant lines. Schematic diagram showing CRISPR/Cas9-edited alleles at the *PtMED31* and *PtTMEL1* loci. The *Ptmed31-1 Pttmel1* double-mutant lines were generated in the *Ptmed31-1* mutant background by further editing the *PtTMEL1* coding region. The *Ptmed31-1* allele carried the original deletion at the *PtMED31* locus, whereas the *PtTMEL1* coding region was targeted using a single sgRNA. sgRNA target sequences are shown in red, and PAM sequences are shown in bold. Blue dashes indicate deletions, and blue letters indicate insertions. Numbers in parentheses indicate the sizes of insertions or deletions; +, insertion; −, deletion.


a
Saccharina latissima
Dictyota dichotoma
Thalassiosira pseudonana
Skeletonema marinoi
Cyclotella cryptica
Cyclotella atomus
Phaeodactylum tricornutum
Arabidopsis thaliana
Oryza sativa
Chlamydomonas reinhardtii
b
Skeletonema marinoi
Chlamydomonas reinhardtii
Cyclotella cryptica
Cyclotella atomus
Thalassiosira pseudonana
Phaeodactylum tricornutum
Saccharina latissima
Dictyota dichotoma
Arabidopsis thaliana
Oryza sativa
c
Target
TpMED31: THAPSDRAFT_19937
50 bp
ATG
Stop
TpMED31 AGTGTTGCCCTCCCCGCGAATCGGTTTGAGC
Tpmed31-1 (-19 bp) AGTG----------GTTTGAGC
Tpmed31-2 (+1 bp) AGTGTTGCCCTCCCTCGCGAATCGGTTTGAGC
Target
TpTMEL1: THAPSDRAFT_21140
100 bp
ATG
Stop
Tptmel1 ACAACTGGTACAATCAGTGAGGGTTTGTATCACGATAGTG
Tptmel1-1 (+2 bp) ACAACTGGTACAATCAGCGTGAGGGTTTGTATCACGATAGTG
Tptmel1-2 (-19 bp) ACAACTGGTACAATCAG----------AGTG
d
Thalassiosira pseudonana
WT
Tpmed31-1
Tpmed31-2
Com
kDa
α-PtMED31
α-PtACTIN
20
15
50
40
e
Thalassiosira pseudonana
WT
Tptmel1-1
Tptmel1-2
Com
kDa
α-PtTMEL1
α-PtACTIN
50
40
50
40

Supplementary Fig. 8 | Conservation of MED31 and the TMEL1 RING domain and generation of *Tpmed31* and *Tptmel1* mutant lines. a Alignment of MED31 proteins among different species using the ClustalX2 software (version, 2.1). Conserved residues are highlighted according to the ClustalX color scheme. **b** Alignment of RING domain of TMEL1 among different species using the ClustalX2 software (version, 2.1). Arrowheads indicate conserved cysteine and histidine in C3H2C3-type RING-H2 finger consensus motif (C-X2-C-X(9-39)-C-X(1-3)-H-X(2-3)-H-X2-C-X(4-48)-C-X2-C). **c** Schematic diagrams showing CRISPR/Cas9-mediated editing of the *TpMED31* and *TpTMEL1* loci. The coding regions of *TpMED31* and *TpTMEL1* were each targeted using a single sgRNA. sgRNA target sequences are shown in red, and PAM sequences are shown in bold. Blue dashes indicate deletions, and blue letters indicate insertions. Numbers in parentheses indicate the sizes of insertions or deletions; +, insertion; −, deletion. **d** Immunoblotting analysis of TpMED31 protein abundance in WT, two independent *Tpmed31* mutant lines, and the *TpMED31*-complemented line (*Com*). TpMED31 was detected using an anti-PtMED31 antibody that cross-reacts with TpMED31. **e** Immunoblotting analysis of TpTMEL1 protein abundance in WT, two independent *Tptmel1* mutant lines, and the *TpTMEL1*-complemented line (*Com*). TpTMEL1 was detected using an anti-PtTMEL1 antibody that cross-reacts with TpTMEL1. In (**d**,**e**), ACTIN was used as a loading control.