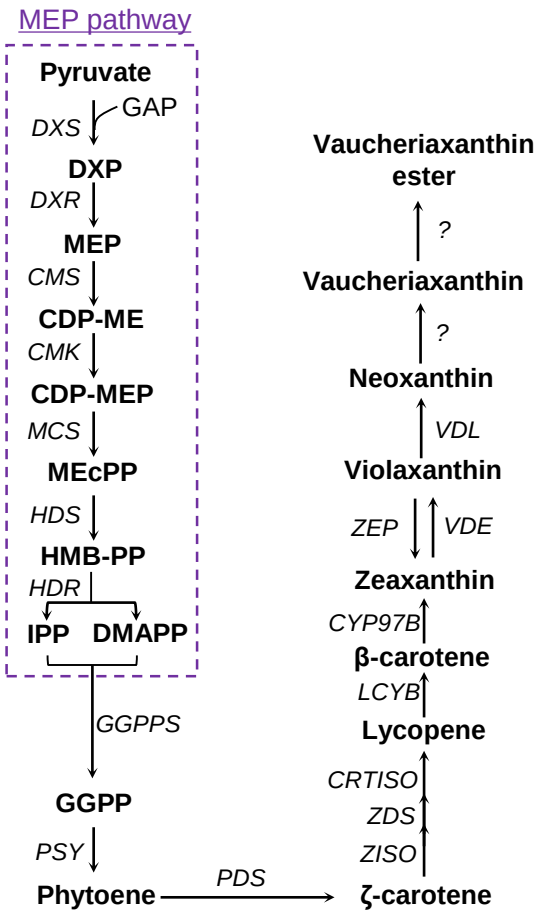


Fig. S1 Proposed carotenoid biosynthetic pathways in *N. ocenica*. CDP-ME, 4-diphosphocytidyl-2-C-methylerythritol; CDP-MEP, 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate; CMK, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase; CMS, 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase; CRTISO, carotenoid isomerase; CYP97, cytochrome P450 beta-hydroxylase; DMAPP, dimethylallyl pyrophosphate; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; DXP, 1-deoxy-D-xylulose 5-phosphate; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; GAP, glyceraldehyde 3-phosphate; GGPP, geranylgeranyl diphosphate; GGPPS, geranylgeranyl diphosphate synthase; HDR, 4-hydroxy-3-methylbut-2-en-1-yl diphosphate reductase; HDS, 4-hydroxy-3-methyl-but-2-en-1-yl diphosphate synthase; HMB-PP, (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate; IPP, isopentenyl pyrophosphate; IPPI, isopentenyl-diphosphate Delta-isomerase; LCYB, lycopene beta cyclase; MCS, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; MEcPP, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate; MEP, 2-C-methylerythritol 4-phosphate; PDS, phytoene desaturase; PSY, phytoene synthase; VDE, violaxanthin de-epoxidase; VDL, violaxanthin de-epoxidase like; ZDS, zeta-carotene desaturase; ZEP, zeaxanthin epoxidase; ZISO, zeta-carotene isomerase

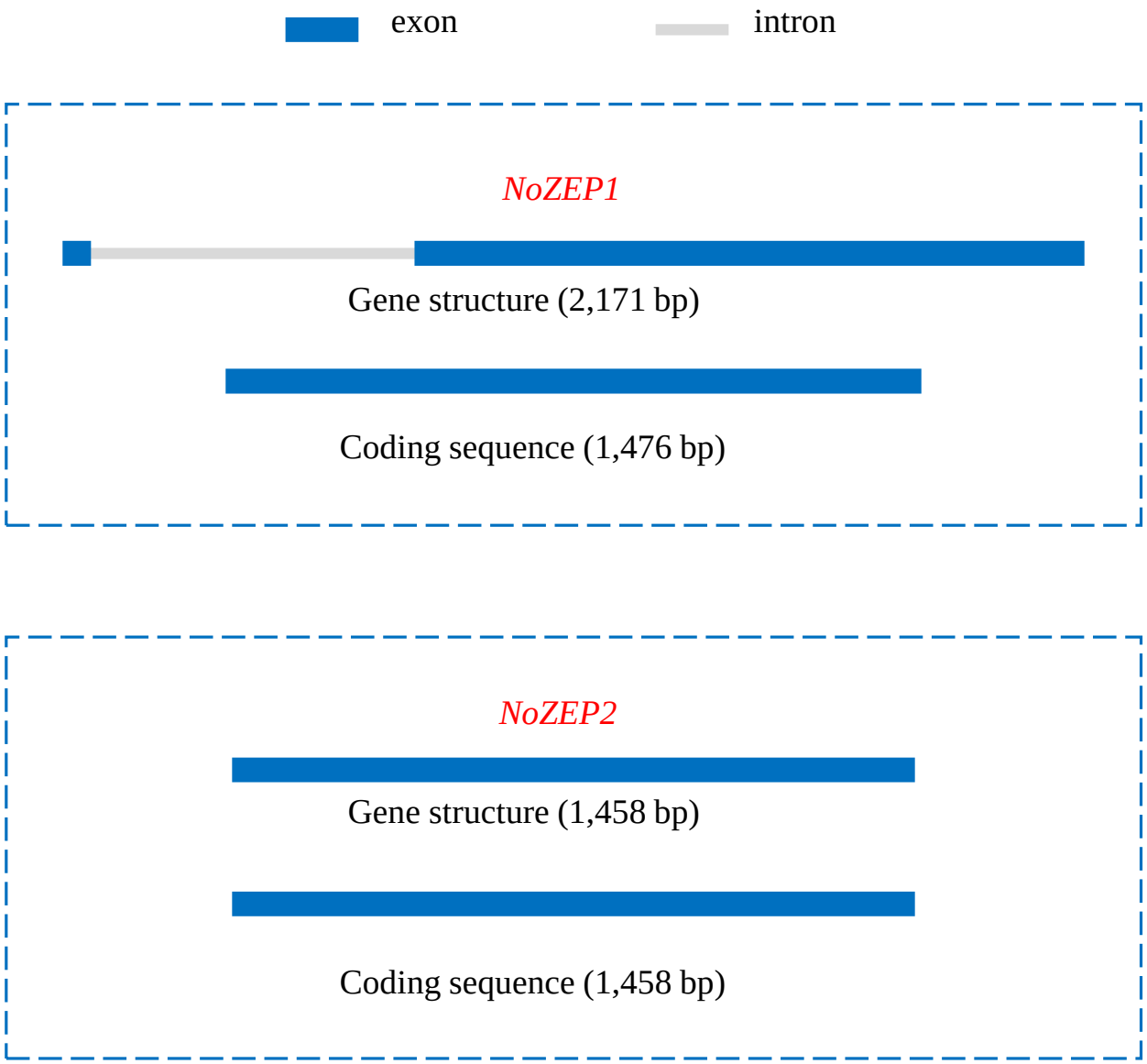


Fig. S2 Gene structures of *NoZEP1* and *NoZEP2*

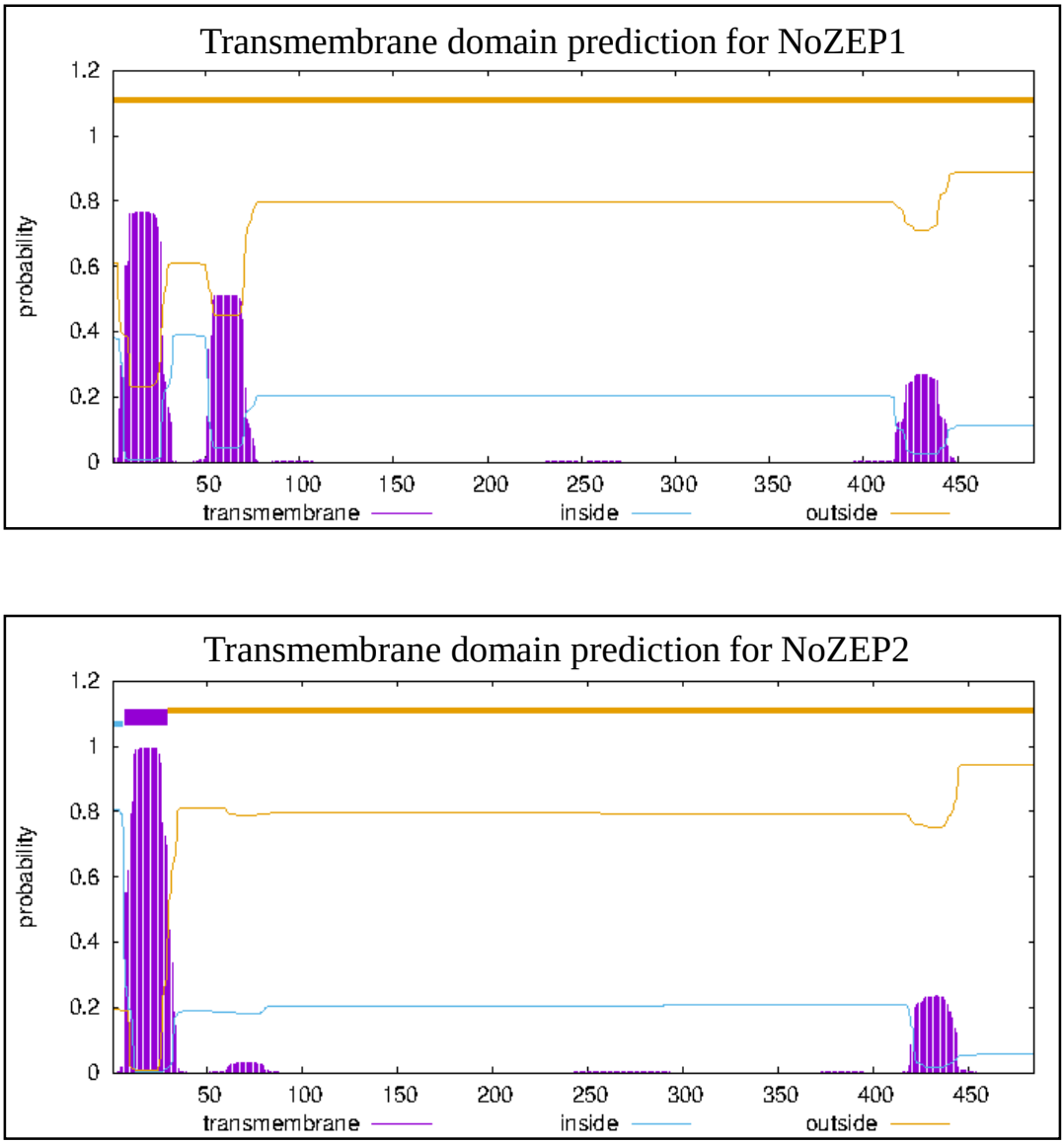


Fig. S3 Transmembrane domains predication for NoZEP1 and NoZEP2 by TMHMM (V2.0, http://www.cbs.dtu.dk/services/TMHMM/)

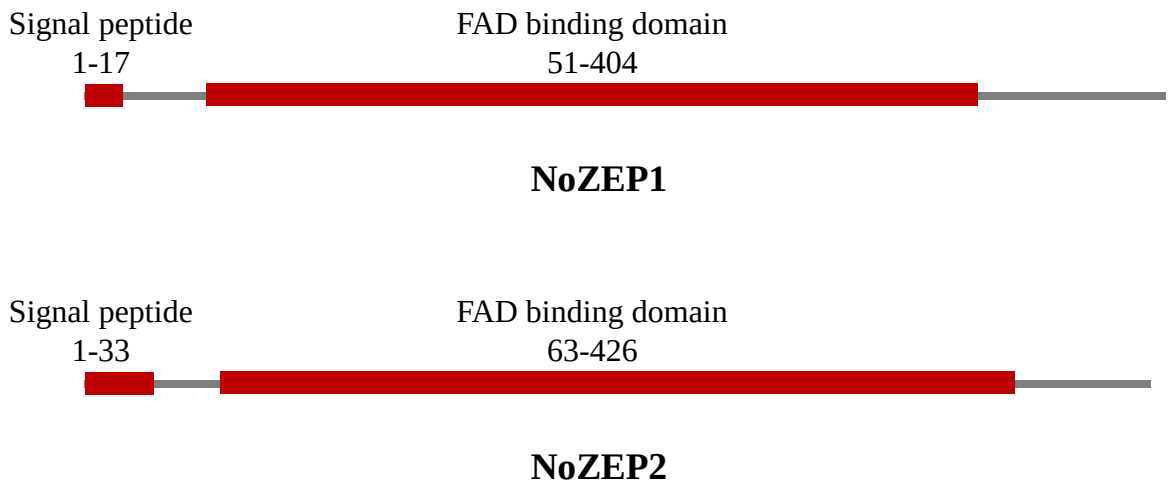


Fig. S4 Predicted domains of NoZEP1 and NoZEP2 via SMART analysis (http://smart.embl-heidelberg.de/)

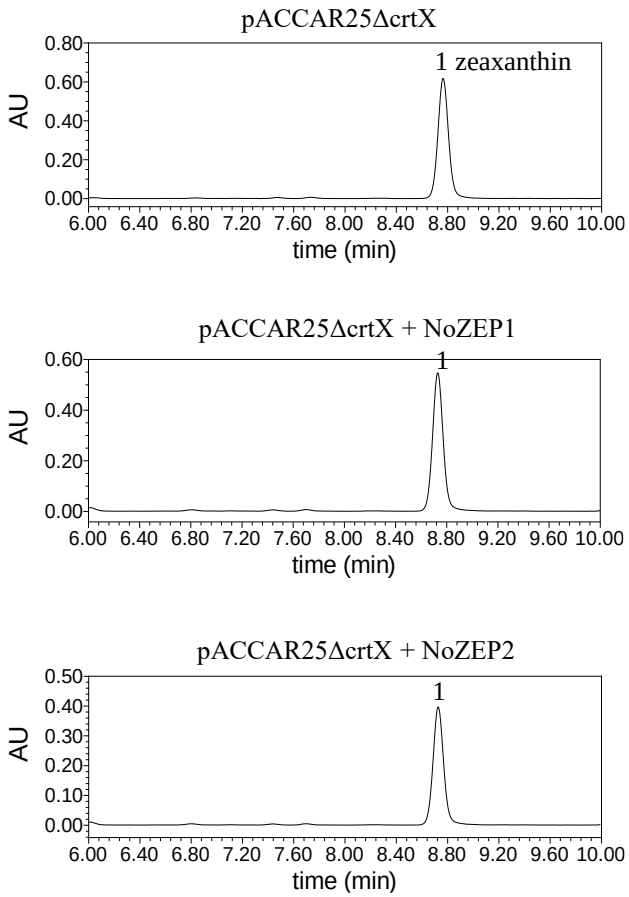


Fig. S5 HPLC elution profiles of carotenoids produced in the zeaxanthin-producing *E. coli* strain without (top) or with *NoZEP*s (middle and bottom). The absorbance was recorded at 450 nm.

HECTAR v1.3 (https://webtools.sb-roscoff.fr/)

Protein id	Predicted targeting category	Signal peptide score	Signal peptide cleavage site	Chloroplast score	Mitochondrion score
NoZEP1	Chloroplast	0.8244	18	0.7578	-
NoZEP2	Signal peptide	0.8271	34	0.3599	-

Cell-Ploc 2.0 (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/)

Protein id	Predicted location
NoZEP1	Chloroplast
NoZEP2	Chloroplast

Fig. S6 Subcellular localization predication for NoZEP1 and NoZEP2

Fig. S7 Cultures of WT and *NoZEP*-knockdown lines from two days of HL in Fig. 4

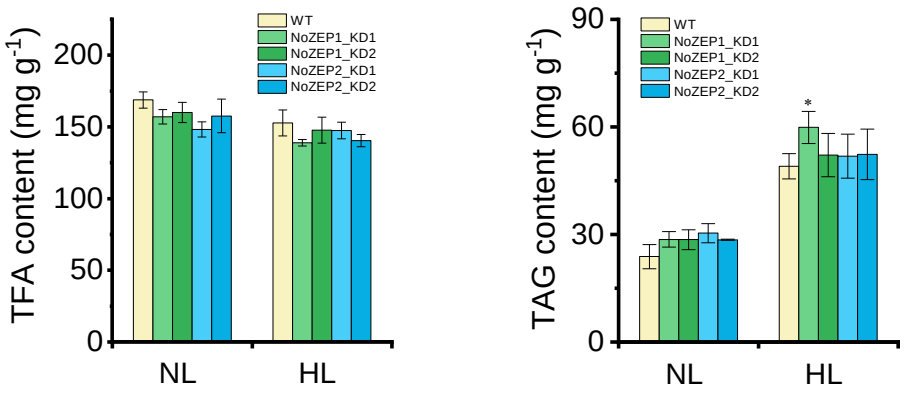


Fig. S8 The content of TFA and TAG as affected by *NoZEP1* or *NoZEP2* knockdown in *N. oceanica* under NL and HL conditions. The NL and HL samples were from day 4 and day 2, respectively. Data represent mean values ± SD (n = 3). The asterisk indicates the significant difference (Student's t-test, P < 0.05*) between WT and knockdown lines.

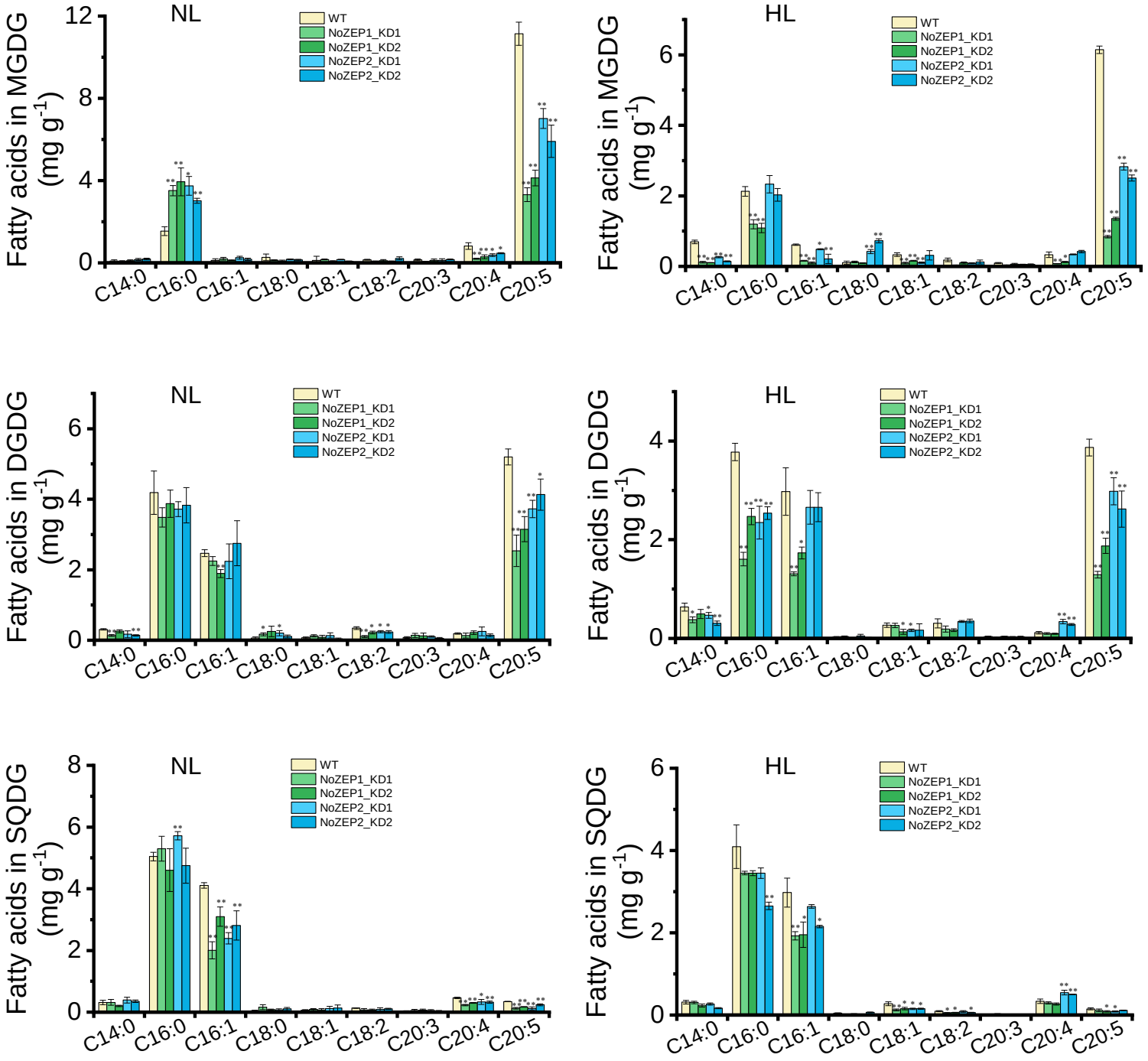


Fig. S9 The content of individual fatty acids of MGDG, DGDG, and SQDG as affected by *NoZEP1* or *NoZEP2* knockdown in *N. oceanica* under NL and HL conditions. The NL and HL samples were from day 4 and day 2, respectively. Data represent mean values ± SD (n = 3). The asterisk indicates the significant difference (Student's t-test, P < 0.05* or P < 0.01**) between WT and knockdown lines.

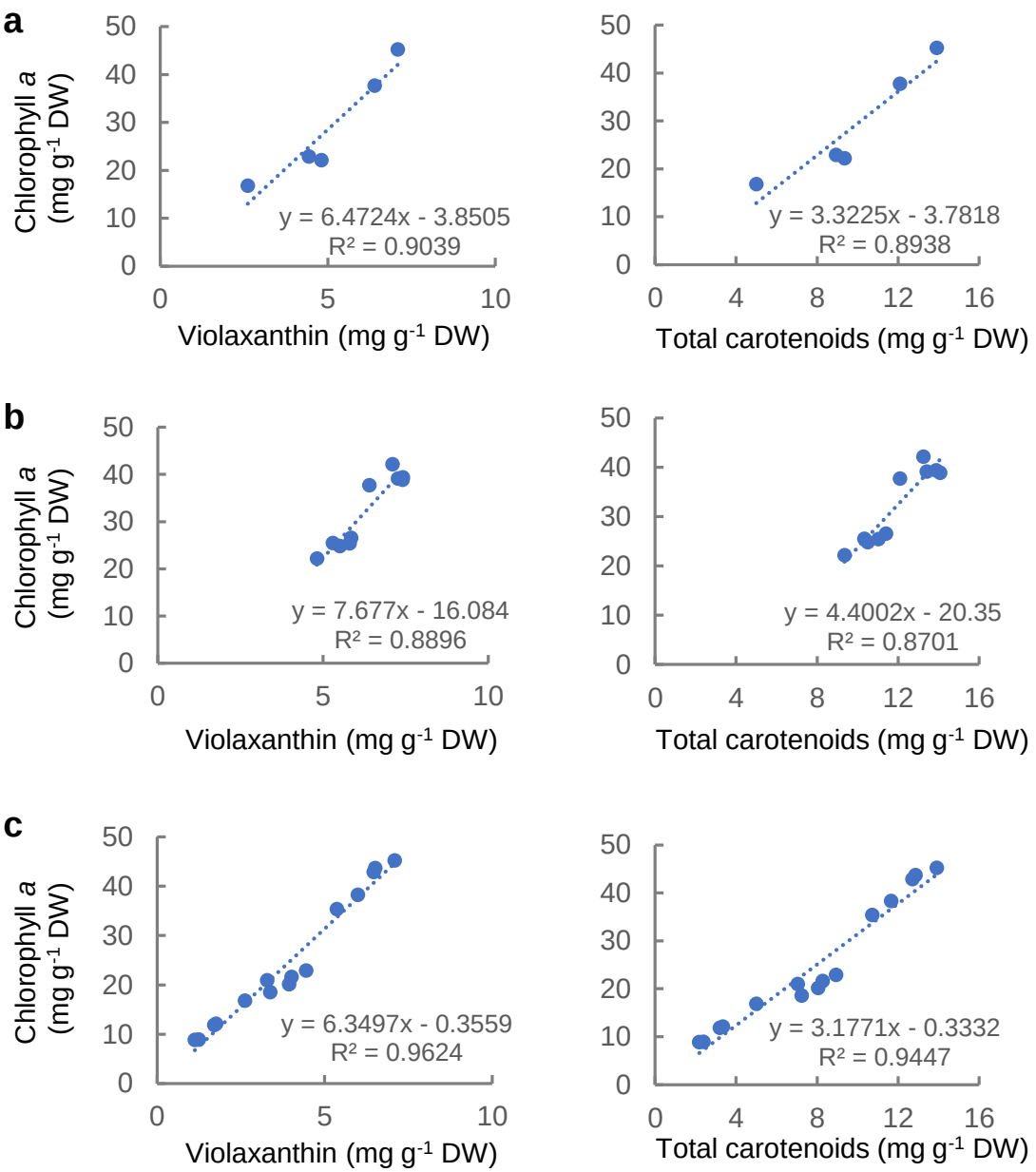


Fig. S10 Correlation between chlorophyll *a* and violaxanthin and between chlorophyll *a* and total carotenoids in *N. oceanica*. (a) WT under different light conditions. Data were retrieved from Figs 3-5. (b) WT and overexpression lines. Data were retrieved from Fig. 3. (c) WT and knockdown lines. Data were retrieved from Figs 4 and 5.

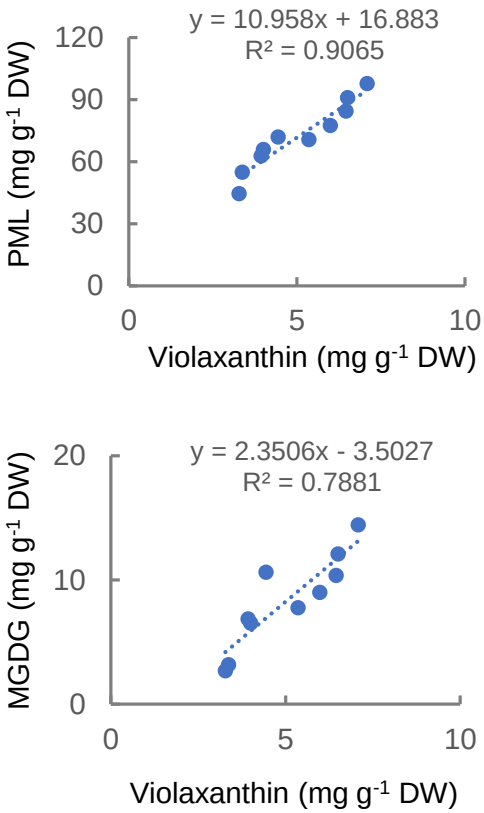
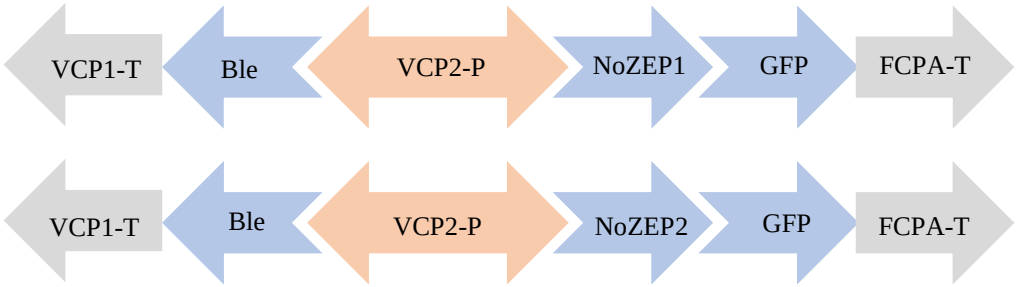


Fig. S11 Correlation between membrane lipids and violaxanthin in *N. oceanica*. Data were retrieved from Figs 4 and 6. PML, polar membrane lipids.

Vectors for *NoZEP1* and *NoZEP2* subcellular localization and overexpression



Vectors for *NoZEP1* and *NoZEP2* knockdown

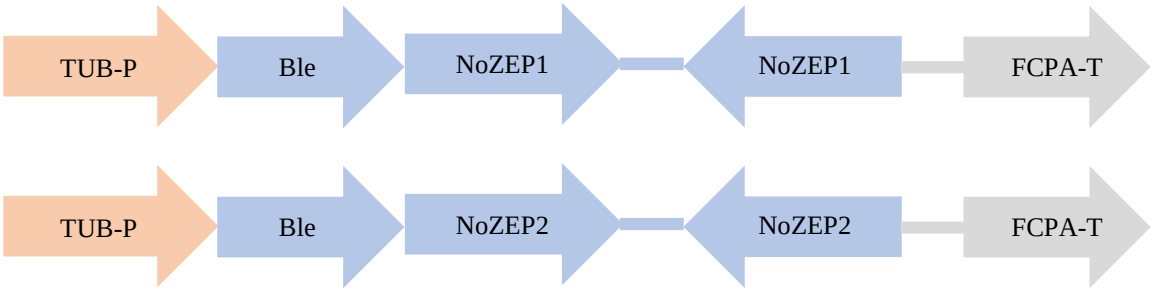


Fig. S12 Illustration of vectors used in this study for *N. oceanica* transformation

Table S1. Primers used in the present study

Primer name	Sequence (5' to 3')	Purpose
NoZEP1-F1 (*BamHI*)	taggatccATGGTTCAGCTGGGGACTTTG	Cloning NoZEP1 CDS into pUC19 vector
NoZEP1-R1 (*EcoRI*)	cggaattcCTAGACAGCAACCGTCTCTTT	
NoZEP2-F1 (*BamHI*)	taggatccATGACGGGTAGGAGAAGTGGT	Cloning NoZEP2 CDS into pUC19 vector
NoZEP2-R1 (*XhoI*)	gactcgagCATGAGGGGAAACATACGCCT	
NoZEP1-F2 (*KpnI*)	accactctctcaagtggtaccATGGTTCAGCTGGGGACTTTG	Cloning NoZEP1 CDS into the overexpression vector and PCR verification
NoZEP1-R2 (*EcoNI*)	accattcctcctcctcctcccctaggGACAGCAACCGTCTCTTT	
NoZEP1-F3	GGGGGTCCGATTCAAGTGCA	
NoZEP1-R3	TATAGGCAACCTCCTTCACG	
NoZEP2-F2 (*KpnI*)	accactctctcaagtggtaccATGACGGGTAGGAGAAGTGG	Cloning NoZEP2 CDS into the overexpression vector and PCR verification
NoZEP2-R2 (*EcoNI*)	accattcctcctcctcctcccctaggCATGAGGGGAAACATACG	
NoZEP2-F3	CGGACCTGGAGCGTCGAGGC	
NoZEP2-R3	ACACCACGAATGGCCTGATA	
NoZEP1-F4 (*BamHI*)	cgggatccTCGACCAGAGTCGTCTGCACAC	Constructing the knockdown vector of NoZEP1
NoZEP1-R4 (*XbaI*)	gctctagaCGGTCGTACAAATCCCTCTGGT	
NoZEP1-F5 (*XbaI*)	gctctagaTGCATTCGACCCTTCCCCACAT	
NoZEP1-R5 (*EcoRI*)	cggaattcTCGACCAGAGTCGTCTGCACAC	
NoZEP2-F4 (*BamHI*)	cgggatccATGCGTAGCGACCTGTTACGCC	Constructing the knockdown vector of NoZEP2
NoZEP2-R4 (*XbaI*)	gctctagaGCAGGCTTCGAACTTTCTGCAG	
NoZEP2-F5 (*XbaI*)	gctctagaGCCTGATAACCGGCATAGTTGG	
NoZEP2-R5 (*EcoRI*)	cggaattcATGCGTAGCGACCTGTTACGCC	
NoZEP1-F6	GGATTGCGTCTGACGTGTTG	RT-qPCR for NoZEP1
NoZEP1-R6	CAGAACCCCAGTCGCTCTCA	
NoZEP2-F6	GCCACATGAATTCCGGTACAT	RT-qPCR for NoZEP2
NoZEP2-R6	CCGCAAGACAACTCGGCTAT	
NoActin-F	GCCGTTATTGGATGGATATG	RT-qPCR for NoActin
NoActin-R	ACAACAACTCTCCTTCACA	