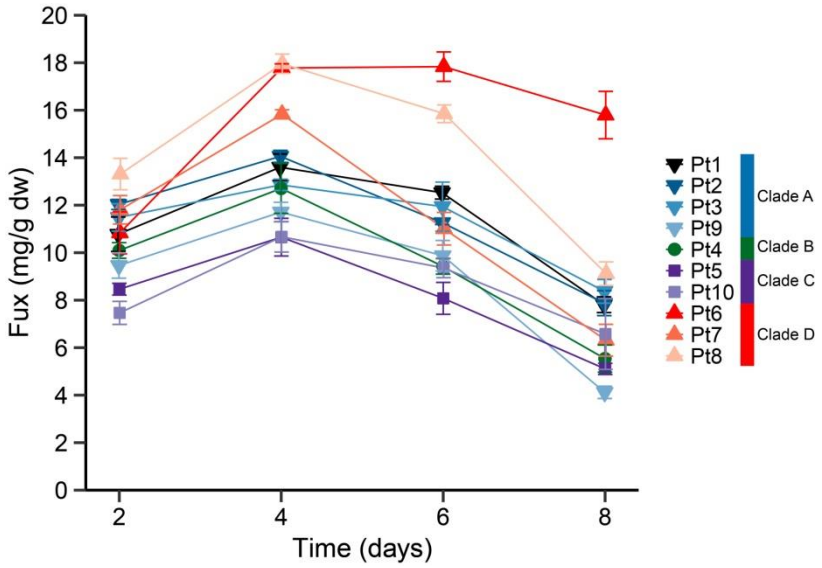


Fig. S1 Fucoxanthin content in different accessions of *P. tricornutum*.

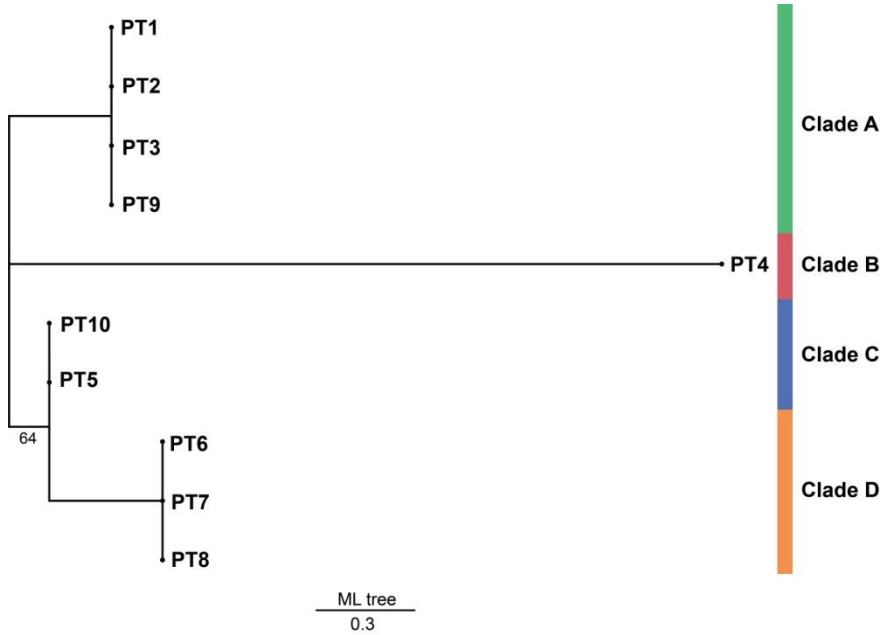
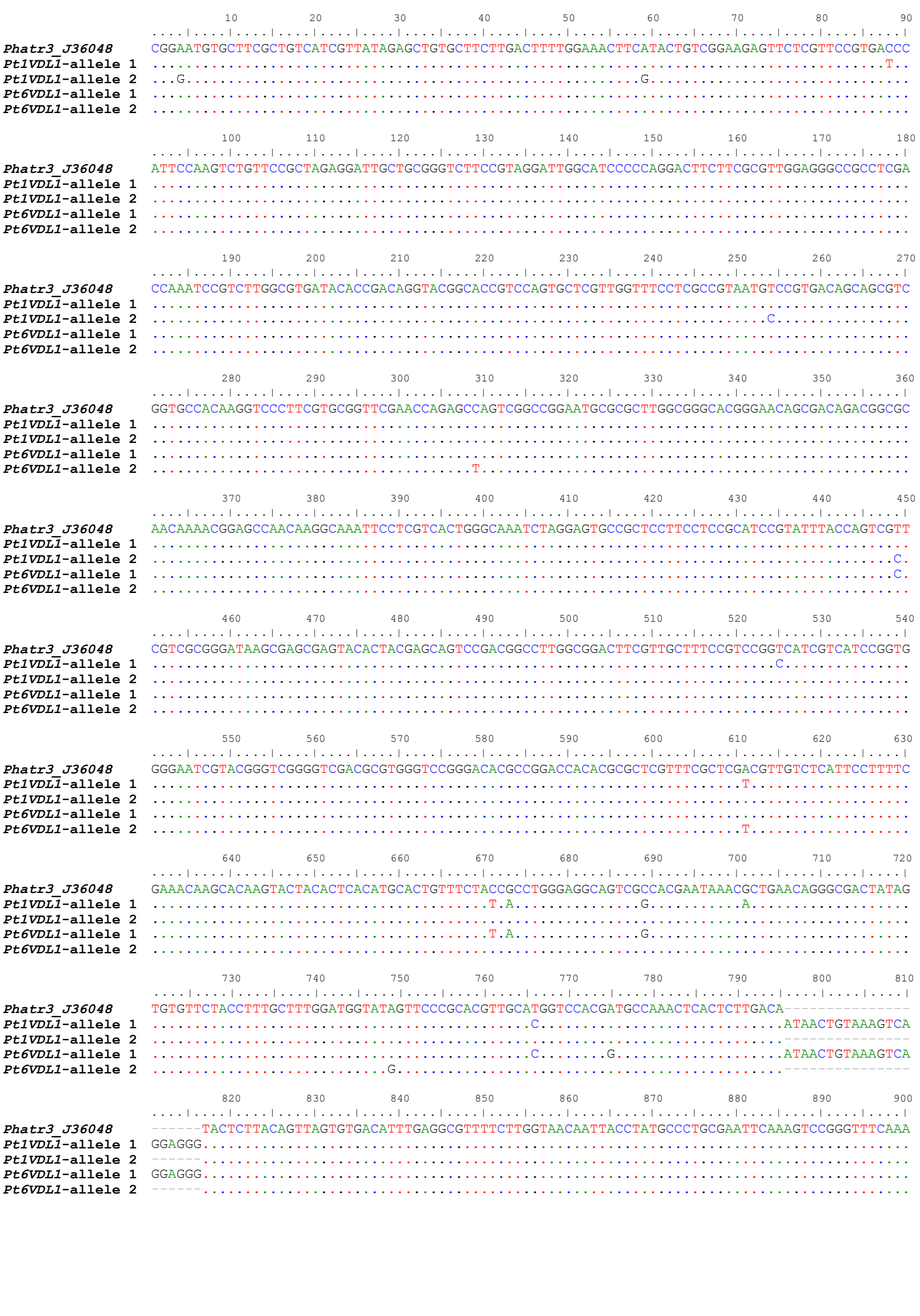


Fig. S2 Phylogenetic association of *VDL1* gene in different accessions of *P. tricornutum* based on polymorphic sites (including SNP and INDELS) using a maximum likelihood approach. The four genetic clades (Genotype A–D) of *P. tricornutum* have been described in Rastogi et al. (2020).


10 20 30 40 50 60 70 80 90
Phatr3_J36048 CGGAATGTGCTTCGCTGTCATCGTTATAGAGCTGTGCTTCTTGACTTTTGGAAACTTCATACTGTCGGAAGAGTTCTCGTTCCGTGACCC
Pt1VDL1-allele 1 .....T..
Pt1VDL1-allele 2 ...G.....G.....
Pt6VDL1-allele 1 .....
Pt6VDL1-allele 2 .....
100 110 120 130 140 150 160 170 180
Phatr3_J36048 ATTCCAAGTCTGTTCCGCTAGAGGATTGCTGCGGGTCTTCCGTAGGATTGGCATCCCCCAGGACTTCTTCGCGTTGGAGGGCCGCCTCGA
Pt1VDL1-allele 1 .....
Pt1VDL1-allele 2 .....
Pt6VDL1-allele 1 .....
Pt6VDL1-allele 2 .....
190 200 210 220 230 240 250 260 270
Phatr3_J36048 CCAAATCCGTCTTGGCGTGATACACCGACAGGTACGGCACCGTCCAGTGCTCGTTGGTTTCCTCGCCGTAATGTCCGTGACAGCAGCGTC
Pt1VDL1-allele 1 .....
Pt1VDL1-allele 2 .....C.....
Pt6VDL1-allele 1 .....
Pt6VDL1-allele 2 .....
280 290 300 310 320 330 340 350 360
Phatr3_J36048 GGTGCCACAAGGTCCCTTCGTGCGGTTCGAACCAGAGCCAGTCGGCCGGAATGCGCGCTTGGCGGGCACGGGAACAGCGACAGACGGCGC
Pt1VDL1-allele 1 .....
Pt1VDL1-allele 2 .....
Pt6VDL1-allele 1 .....
Pt6VDL1-allele 2 .....T.....
370 380 390 400 410 420 430 440 450
Phatr3_J36048 AACAAAACGGAGCCAACAAGGCAAATTCCTCGTCACTGGGCAAATCTAGGAGTGCCGCTCCTTCCTCCGCATCCGTATTTACCAGTCGTT
Pt1VDL1-allele 1 .....
Pt1VDL1-allele 2 .....C.
Pt6VDL1-allele 1 .....C.
Pt6VDL1-allele 2 .....
460 470 480 490 500 510 520 530 540
Phatr3_J36048 CGTCGCGGGATAAGCGAGCGAGTACACTACGAGCAGTCCGACGGCCTTGGCGGACTTCGTTGCTTTCCGTCCGGTCATCGTCATCCGGTG
Pt1VDL1-allele 1 .....C.....
Pt1VDL1-allele 2 .....
Pt6VDL1-allele 1 .....
Pt6VDL1-allele 2 .....
550 560 570 580 590 600 610 620 630
Phatr3_J36048 GGGAATCGTACGGGTCGGGGTCGACGCGTGGGTCCGGGACACGCCGGACCACACGCGCTCGTTTCGCTCGACGTTGTCTCATTCCTTTTC
Pt1VDL1-allele 1 .....T.....
Pt1VDL1-allele 2 .....
Pt6VDL1-allele 1 .....
Pt6VDL1-allele 2 .....T.....
640 650 660 670 680 690 700 710 720
Phatr3_J36048 GAAACAAGCACAAGTACTACACTCACATGCACTGTTTCTACCGCCTGGGAGGCAGTCGCCACGAATAAACGCTGAACAGGGCGACTATAG
Pt1VDL1-allele 1 .....T.A.....G.....A.....
Pt1VDL1-allele 2 .....
Pt6VDL1-allele 1 .....T.A.....G.....
Pt6VDL1-allele 2 .....
730 740 750 760 770 780 790 800 810
Phatr3_J36048 TGTGTTCTACCTTTGCTTTGGATGGTATAGTTCCCGCACGTTGCATGGTCCACGATGCCAAACTCACTCTTGACA----------
Pt1VDL1-allele 1 .....C.....ATAACTGTAAAGTCA
Pt1VDL1-allele 2 .....----------
Pt6VDL1-allele 1 .....C.....G.....ATAACTGTAAAGTCA
Pt6VDL1-allele 2 .....G.....----------
820 830 840 850 860 870 880 890 900
Phatr3_J36048 ------TACTCTTACAGTTAGTGTGACATTTGAGGCGTTTTCTTGGTAACAATTACCTATGCCCTGCGAATTCAAAGTCCGGGTTTCAAA
Pt1VDL1-allele 1 GGAGGG.....
Pt1VDL1-allele 2 ------.....
Pt6VDL1-allele 1 GGAGGG.....
Pt6VDL1-allele 2 ------.....


910 920 930 940 950 960 970 980 990
Phatr3_J36048 TGGTCACAATCGGTTCAAATTACAGCTTGGACGTGTTGACTGTGATCACAGATACGTTAACGTACCGTTAAAACTATGCCTGGAGCCGAG
Pt1VDL1-allele 1 .....G.....
Pt1VDL1-allele 2 .A.....
Pt6VDL1-allele 1 C..G....T.....
Pt6VDL1-allele 2 .A.....
1000 1010 1020 1030 1040 1050 1060 1070 1080
Phatr3_J36048 CCCTATGTGACCACGGTCTATAGATGTCGGGTTCAACTCGGCGTCTGTCTCCCGGACTGTGACGGGGATAAACCGAAAATCCTCCGTCGT
Pt1VDL1-allele 1 .....
Pt1VDL1-allele 2 .....A.....---.....G.....
Pt6VDL1-allele 1 .....A.....---.....G.....
Pt6VDL1-allele 2 .....A.....---.....G.....
1090 1100 1110 1120 1130 1140 1150 1160 1170
Phatr3_J36048 GCCATCACCATATCGCATCGCGTCAATCCAATCCTAACACTGGCGTTTCTACTAGGAGGGACCCCGTTACACAACGTCAAAGCGTATCCA
Pt1VDL1-allele 1 .....
Pt1VDL1-allele 2 .....A.....G.....
Pt6VDL1-allele 1 .....G.....
Pt6VDL1-allele 2 .....A.....G.....
1180 1190 1200 1210 1220 1230 1240 1250 1260
Phatr3_J36048 CGGGGGAAAATGCGATTCGCTTGGGTGGTAGCAGCCGGAGTCGTCTTGACGACGACGACACAAGCCTTGGTGCCTTTAGATTGCACGGGT
Pt1VDL1-allele 1 .....
Pt1VDL1-allele 2 .....T.....
Pt6VDL1-allele 1 .....
Pt6VDL1-allele 2 .....
1270 1280 1290 1300 1310 1320 1330 1340 1350
Phatr3_J36048 ATGGGTGAAACAAGAACGAGCGGCATCCGTCCGATCCGGGGGTCTGGAAAGCAACATGGCCCGGTACGCTACTGTACGCCACGGCACCGAC
Pt1VDL1-allele 1 .....A.....
Pt1VDL1-allele 2 .....
Pt6VDL1-allele 1 .....C.....
Pt6VDL1-allele 2 .....
1360 1370 1380 1390 1400 1410 1420 1430 1440
Phatr3_J36048 CAGACGAACCACGGCATAACTTCCTCTTCTGAAAGACAGTGGCCTTTTCCACGCGGGGGTTCTTCTCCCCAGAGCGGTAGCGAGGTCCGTG
Pt1VDL1-allele 1 .....
Pt1VDL1-allele 2 .....GTG.....
Pt6VDL1-allele 1 .....
Pt6VDL1-allele 2 .....GTG.....
1450 1460 1470 1480 1490 1500 1510 1520 1530
Phatr3_J36048 GCGACGTTCGGACTCGGTTTCTCCATCGCACTGGCGAGTGTTTTCGGAGTCGCGGCGGCTCCCGTCGGGGCCGACACGACTCCCGCCGTC
Pt1VDL1-allele 1 .....G.....C.....
Pt1VDL1-allele 2 .....
Pt6VDL1-allele 1 .....T.....
Pt6VDL1-allele 2 .....
1540 1550 1560 1570 1580 1590 1600 1610 1620
Phatr3_J36048 AAGTACGACGGATTCGCAGAATACGCTCAAGATAACCAAATGGAGCAATCTGACGTCGGATGCTTTATCAATAAATGTGGTGATCAAACC
Pt1VDL1-allele 1 .....T.....
Pt1VDL1-allele 2 .....C.....
Pt6VDL1-allele 1 .....T.....C.....
Pt6VDL1-allele 2 .....C.....
1630 1640 1650 1660 1670 1680 1690 1700 1710
Phatr3_J36048 AAGGCTCTCTTTTAGCAATCCGCGTGGAATCAAAGGTGTCTCCTGTTTGGGACGCTGCAAGGGCGAGCAATCGTGTGCGACGCGGTGTTTT
Pt1VDL1-allele 1 .....A.....
Pt1VDL1-allele 2 .....
Pt6VDL1-allele 1 .....A.....
Pt6VDL1-allele 2 .....
1720 1730 1740 1750 1760 1770 1780 1790 1800
Phatr3_J36048 GCCGAATTCGGAAGCGAGAGCTTAAACGCATGGTTGTCCTGTACTATCGAGGAAAATGAGTGCGTCAAGGTCCCGAAAAATGTCGACAAC
Pt1VDL1-allele 1 .....C.....
Pt1VDL1-allele 2 .....G.....
Pt6VDL1-allele 1 .....G.....
Pt6VDL1-allele 2 .....G.....

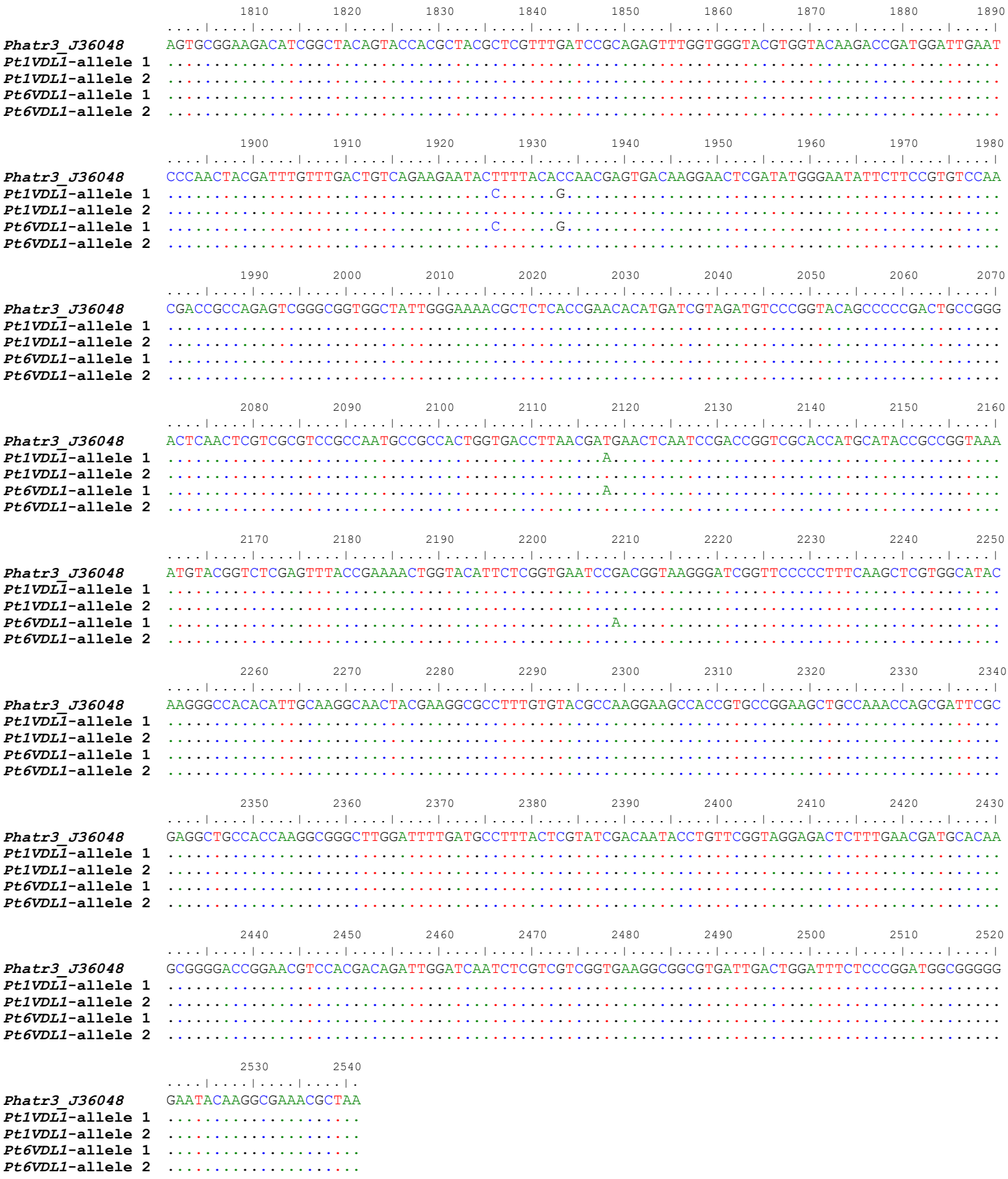


Fig. S3 Single nucleotide variants (SNVs) of the *VDL1* gene in *P. tricornutum* strains CCMP2561 (Pt1) and CCMP631 (Pt6). Allele 1 and allele 2 represent the *VDL1* alleles 1 and 2, respectively. 4-bp deletion is indicated in the red box and translation start site in the blue box. *Phatr3_J36048* is the sequence obtained from Ensembl.

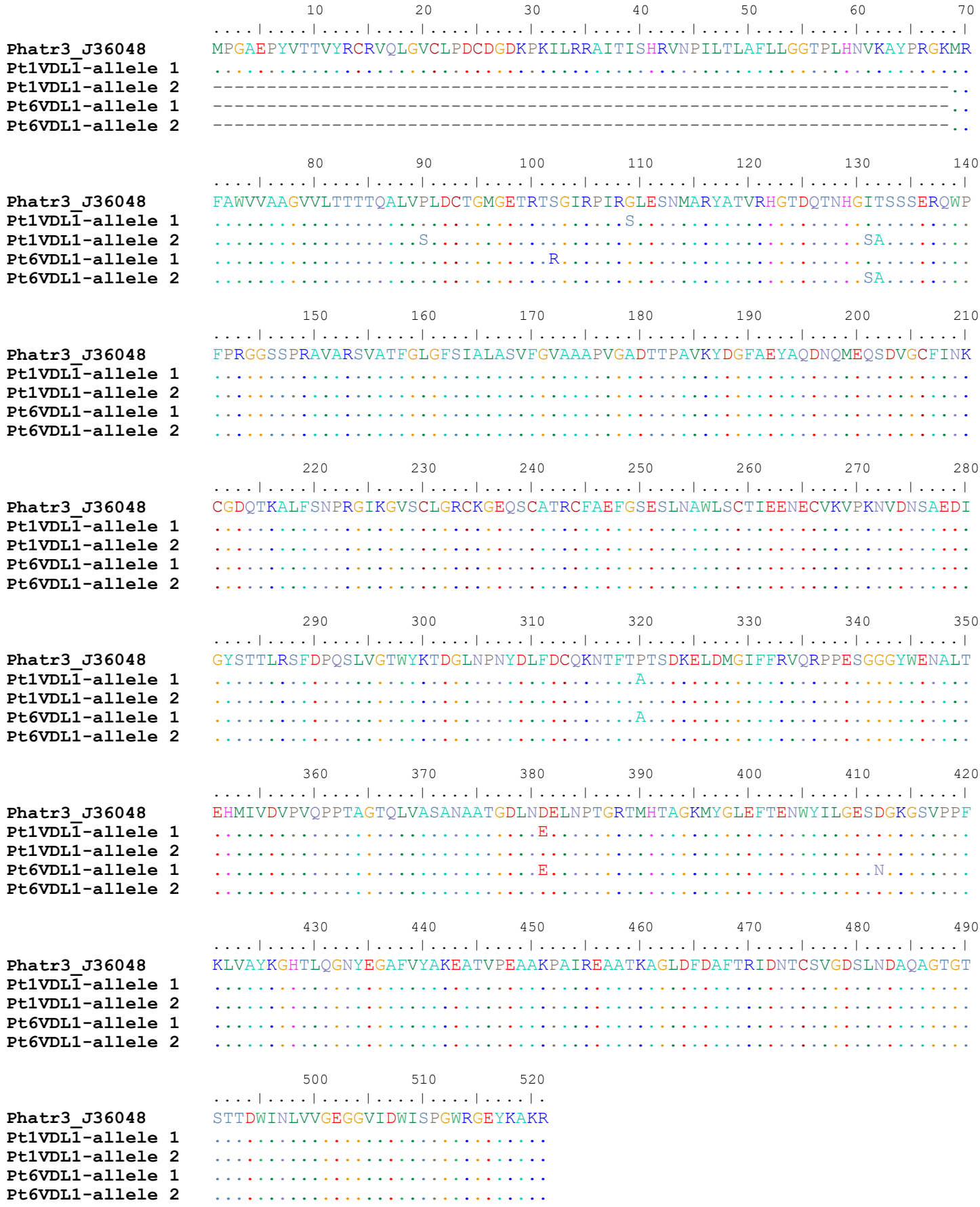


Fig. S4 Sequence alignment of the amino acids of VDL1 in *P. tricornutum* strains CCMP2561 (Pt1) and CCMP631 (Pt6). Allele 1 and allele 2 represent the *VDL1* alleles 1 and 2 encoding amino acid sequences respectively, and Phatr3_J36048 is the sequence obtained from Ensembl.

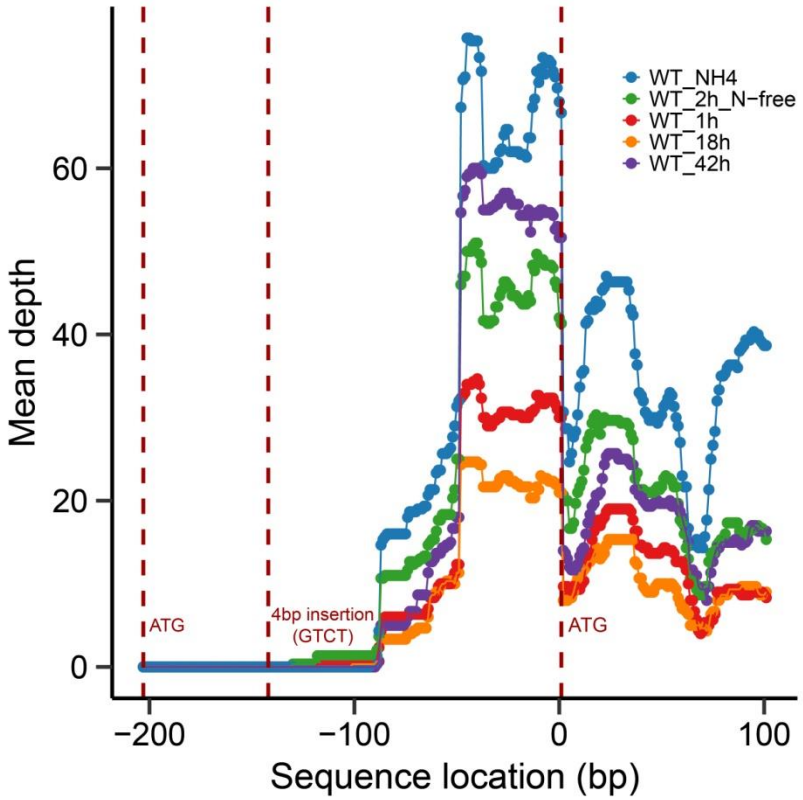


Fig. S5 Depth of base calls at each nucleotide position of the 5' end of *VDL1* gene based on the published RNA sequencing data (Mccarthy et al., 2017) of *P. tricornutum* strain CCMP2561 (Pt1). WT_NH4 stands for mid-exponential phase cells with 880 μM NH_4^+; WT_2h N-free stands for 2 h of incubation in N-free media; and WT_1h, 18h, 42h stand for 1~42 h after the transfer of cultures into 300 μM NO_3^- media.

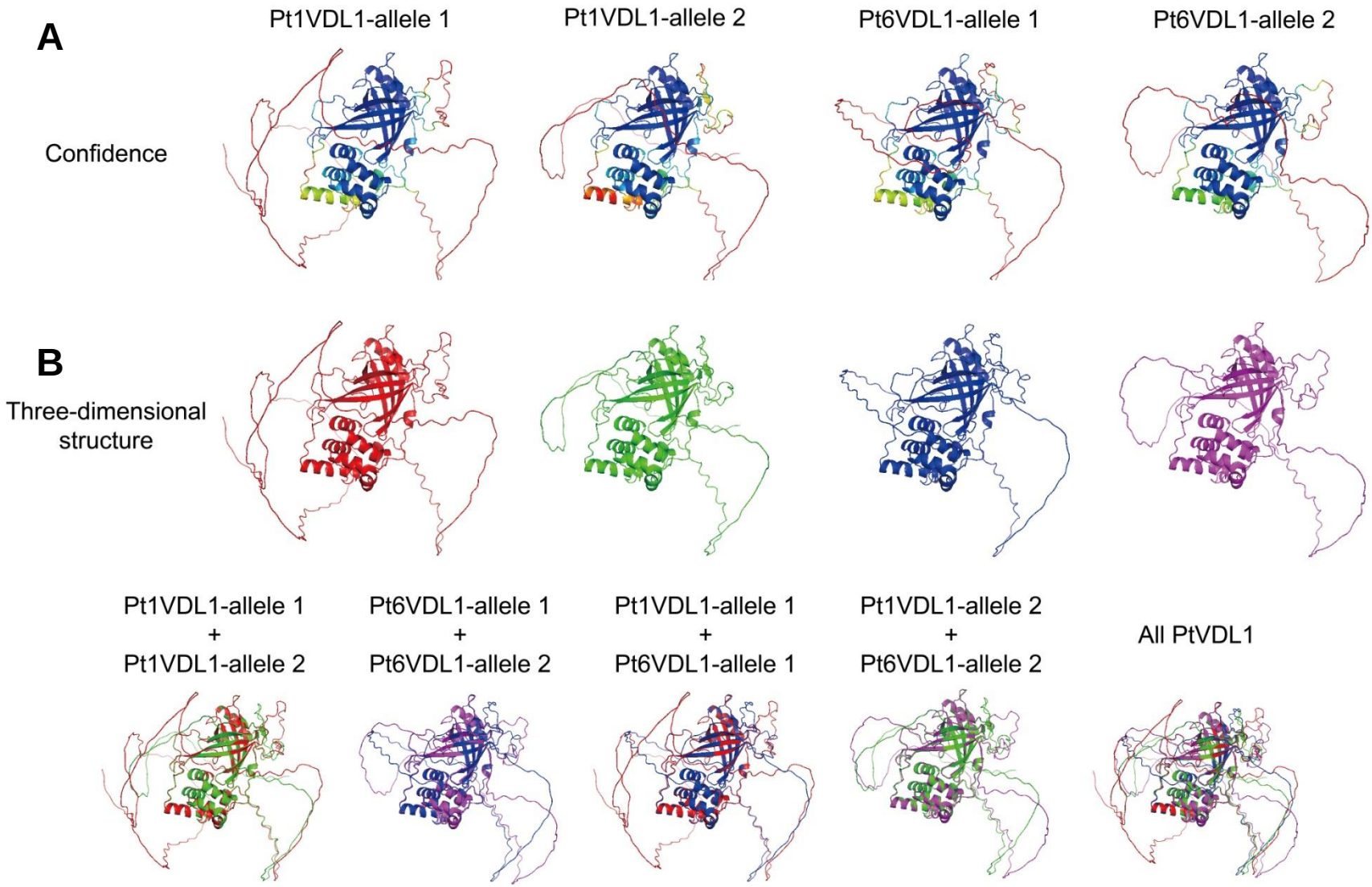


Fig. S6 Predicted structure of VDL1 by AlphaFold. **A.** Predicted structure of VDL1s coloured according to the per-residue confidence score (predicted local distance difference test, pLDDT), very low: red (pLDDT < 50), low: yellow (70 > pLDDT > 50), confident: cyan (90 > pLDDT > 70), very high: blue (pLDDT > 90). **B.** Comparison of predicted structure of VDL1s. VDL1-allele 1 and VDL1-allele 2: VDL1 proteins encoded by alleles 1 and 2. Pt1, *P. tricornutum* strain CCMP2561; Pt6, *P. tricornutum* strain CCMP631.

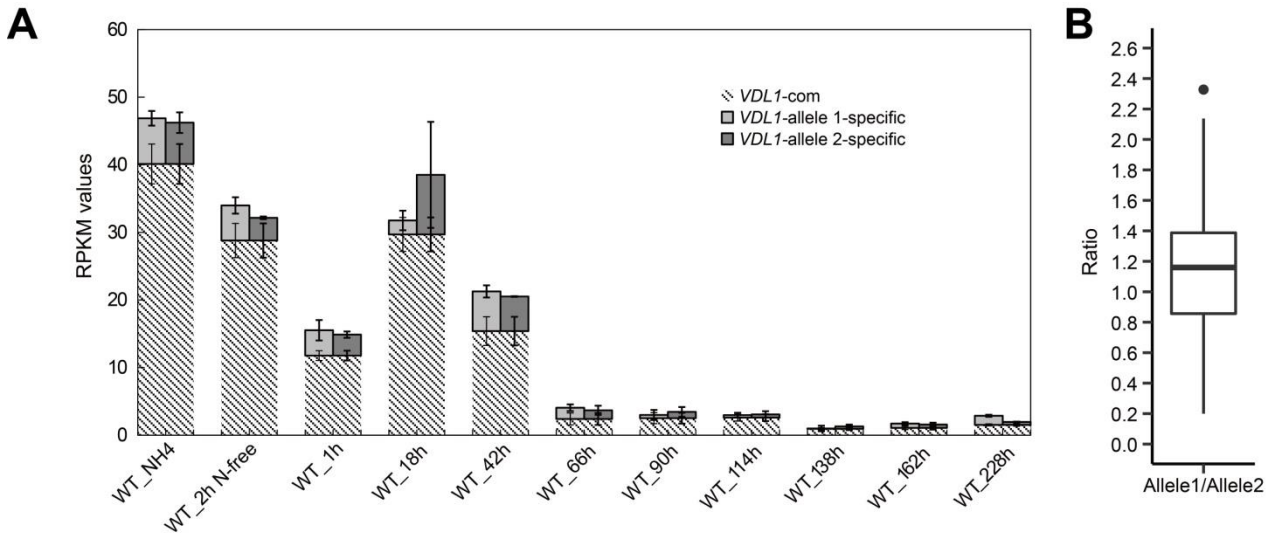


Fig. S7 The analysis of allele-specific expression (ASE) of *VDL1* based on the published RNA sequencing data (Mccarthy et al., 2017) of *P. tricornutum* strain CCMP2561 (Pt1). **A.** RPKM values of two alleles in response to nitrogen stress and culture time. **B.** Average ratio of RPKM values of allele 1 versus those of allele 2. *VDL1*-com refers to the reads mapped to the regions with no difference between *VDL1* alleles; *VDL1*-allele 1-specific and *VDL1*-allele 2-specific refer to the reads specifically mapped to the two *VDL1* alleles 1 and 2, respectively. WT_NH4 stands for mid-exponential phase cells with 880 μM NH_4^+; WT_2h N-free stands for 2 h of incubation in N-free media; and WT_1h, 18h, 42h, 66h, 90h, 114h, 138h, 162h, 228h stand for 1~228 h after the transfer of cultures into 300 μM NO_3^- media.

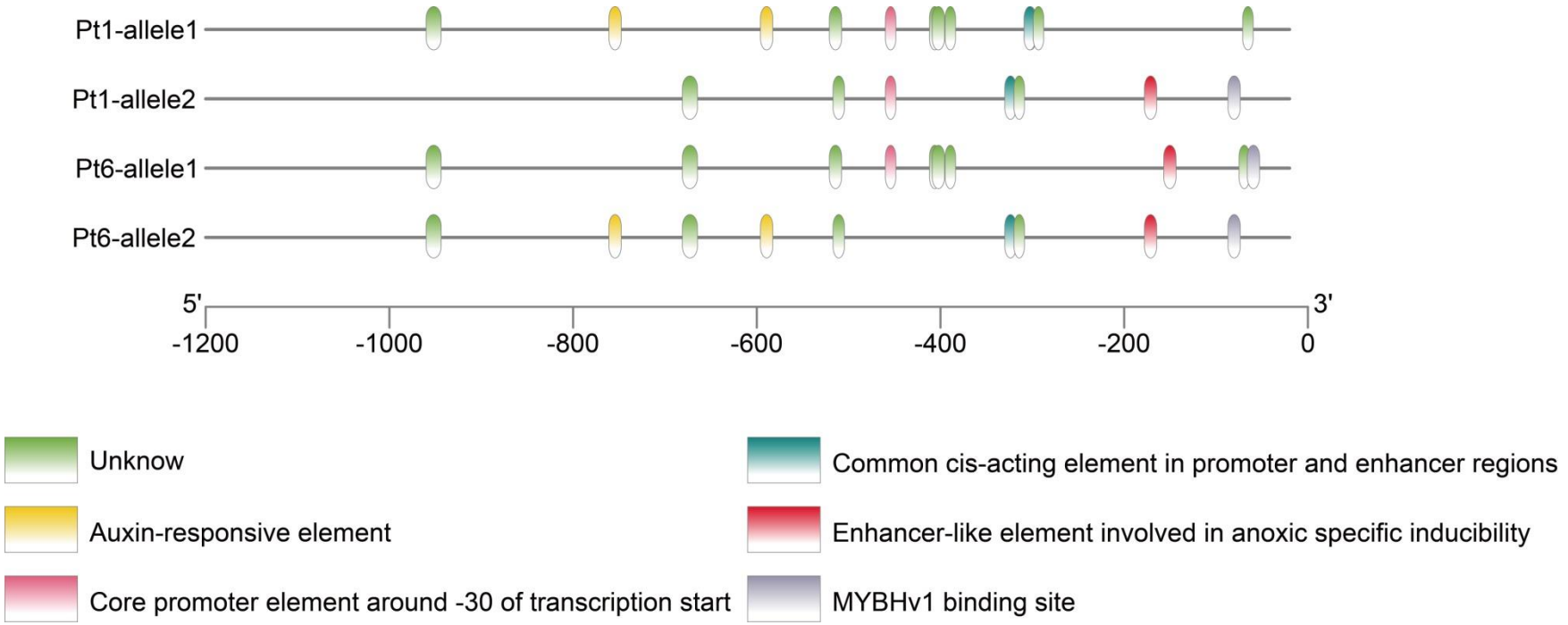


Fig. S8 Sequence analysis of *VDL1* promoters from *P. tricornutum* strains Pt1 and Pt6 by PlantCare. Different potentially functional elements between the two strains were shown. Pt1-allele1 and Pt1-allele2, Pt6-allele1 and Pt6-allele2: promoters of *VDL1* alleles from Pt1 and Pt6, respectively.

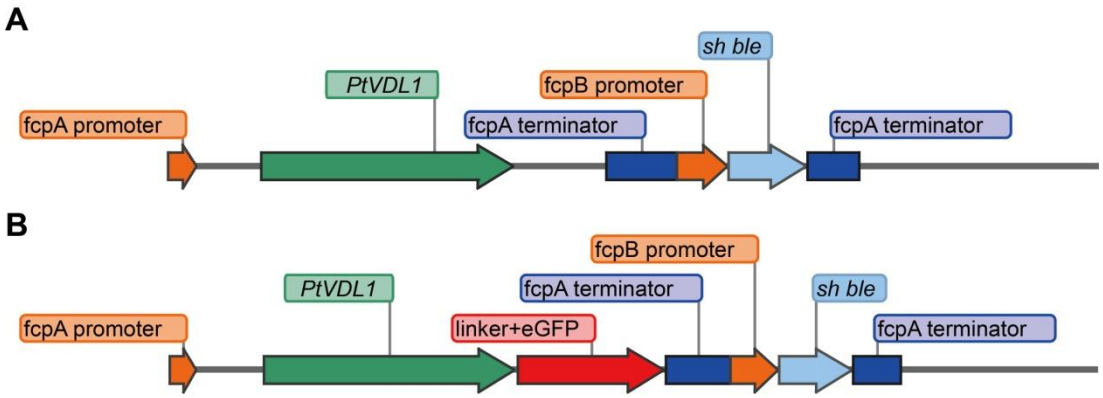


Fig. S9 Schematic representations of the expression constructs used in this study. **A.** Overexpression vector of *PtVDL1*. **B.** *eGFP* fusion vector of *PtVDL1*.