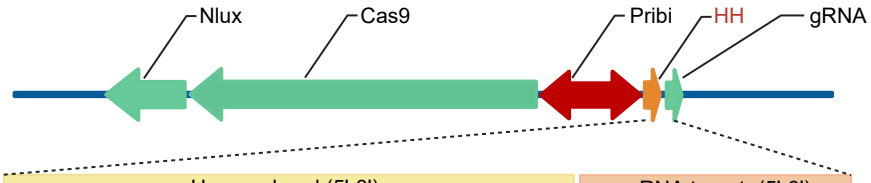
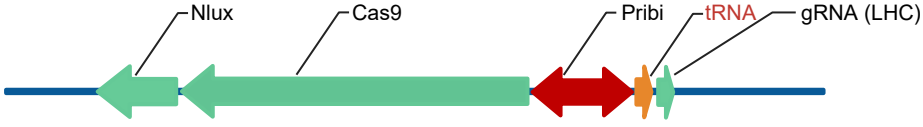
Figure S1

A



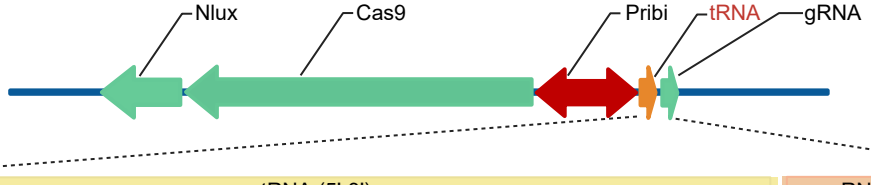
	Hammerhead (5'-3')		gRNA target (5'-3')	
Plamid-1	CCCTCC	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	GGAGCG	GTTAGCAAGCACGG
Plamid-2	TTCCCC	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	CGAGAA	GACAATATGCCCGG
Plamid-3	ACGACT	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	CGAGTA	CCAAGACAAGACAG
Plamid-4	GGTCGG	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	ACACGT	TTGCACGACCCCTG
Plamid-5	GAACCG	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	CATGTC	GGAACACTGCGCCG
Plamid-6	GGATGT	CTGATGAGTCCGTGAGGACGAA-CGAGTAAGCTCGTC	ACATCC	ATGTACAAACACAG
Plamid-7	AGCAAC	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	GTTTGT	CCGAAATGTGCCGA
Plamid-8	AGGATC	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	ACCGCT	AATCCTTTACCATG
Plamid-9	GGCCTT	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	CCCGTC	CCAAGTCATGCCGG
Plamid-10	GTGGAA	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	TTCCAC	CCAAGCCCTCCACG
Plamid-11	TCTATT	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	TCTATA	CTTCTTAAACGACA
Plamid-12	GACGAC	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	GACATC	CATGAAGTACCATG
Plamid-13	ATGTCC	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	AAACGT	TAAGGGCTTTCCTG
Plamid-14	TGGGGC	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	GCTGCC	GTTCGAGAACATCG
Plamid-15	GTCTCC	CTGATGAGTCCGTGAGGACGAAACGAGTAAGCTCGTC	GGATAC	GAGGGGAAAGACGT

B



	gRNA target (5'-3')	Frequency
WT	GAACTCAGGGATCTCAA-CATCGG	
Mutation-1	GAACTCAGGGATCTCAAACATCGG	10/12
Mutation-2	GAACTCAGGGATCTCAA--ATCGG	1/12
Mutation-3	GAACTCAGGGATCTCA----TCGG	1/12

C



	tRNA (5'-3')	gRNA target (5'-3')
Plamid-1	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	CGACGGGGATGATGTCACCG
Plamid-2	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	TTTTTCTCCAGGGAAAAGGG
Plamid-3	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	CTAAGACATCAATGCTACCC
Plamid-4	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	AACCGGCACAGGACGAACAG
Plamid-5	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	CGACGAGGACCAGCGCATCG
Plamid-6	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	TGAGCTATAGACAGCAGCGG
Plamid-7	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	CAAAGAGATCAGCAGCACGA
Plamid-8	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	TGTGTGGTCTTAGATAGAGG
Plamid-9	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	GTATGCATGCGTGGTCGAGG
Plamid-10	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	GCTCGTAGTTGAGTACCTAG
Plamid-11	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	GTCAATTGGTGCATCGTGG-
Plamid-12	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	TCAAGGCCTTCTACACAGTG
Plamid-13	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	GGGGGATTCAGCACACCATG
Plamid-14	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	AGGAAGGTTTAATTACCCCG
Plamid-15	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	GAGGACCACGTCACCCACAG
Plamid-16	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	CATATGTCAGGGTCAGAAAG
Plamid-17	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	ACTGGCTATTGACATATGAG
Plamid-18	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	GCCGTAGAGAGGCATTTGAG
Plamid-19	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	GATCCGGGTCAAATGCAACG
Plamid-20	GCAGGCATAGTTTAGTGGTAGAATTGGTCGTTGCCATCGACCAGGCCCGGGTCCGATTCCCGGTGCCTGCA	CAGGATGCGAGATTCTTGGG

Figure S1. Enhanced ligation accuracy for the gRNA library ligation to the plasmid with CRISPR/Cas Systems via Hammerhead (HH) replacement with tRNA. The Sanger sequencing results for the plasmids after infusion ligation to the HH-based (**A**) and tRNA-based (**C**) CRISPR/Cas systems are presented, respectively. The first six bases of the HH and gRNA sequences are highlighted in purple. The correctly ligated plasmids to the HH-based CRISPR/Cas system are marked in green, while the correctly ligated plasmids to the tRNA-based CRISPR/Cas system are marked in blue. (**B**) The mutation of light-harvesting complex protein (LHC) by the CRISPR/Cas system with tRNA at the 5' end of gRNA. The LHC was mutated by the tRNA-based CRISPR/Cas system with high efficiency.

Figure S2

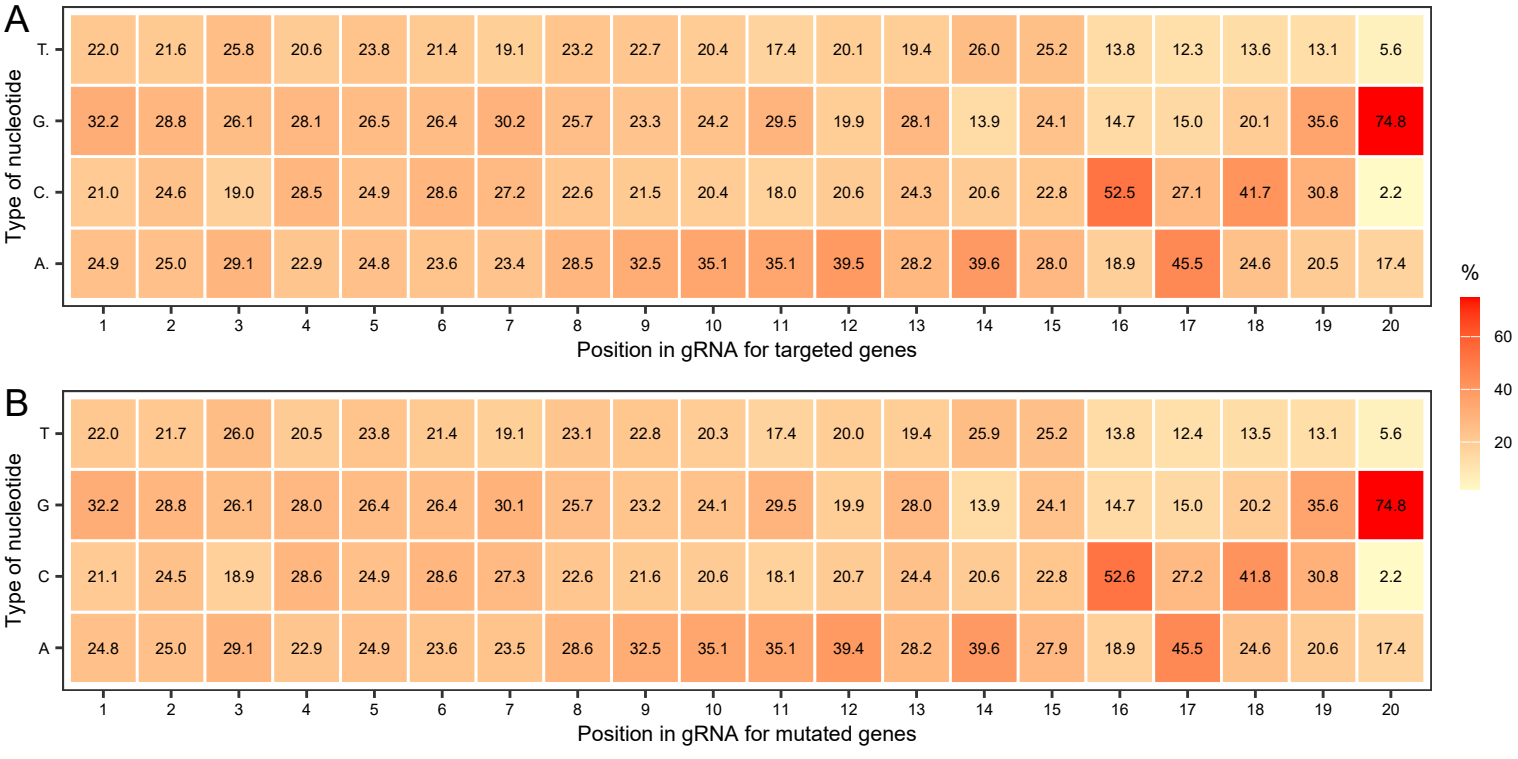
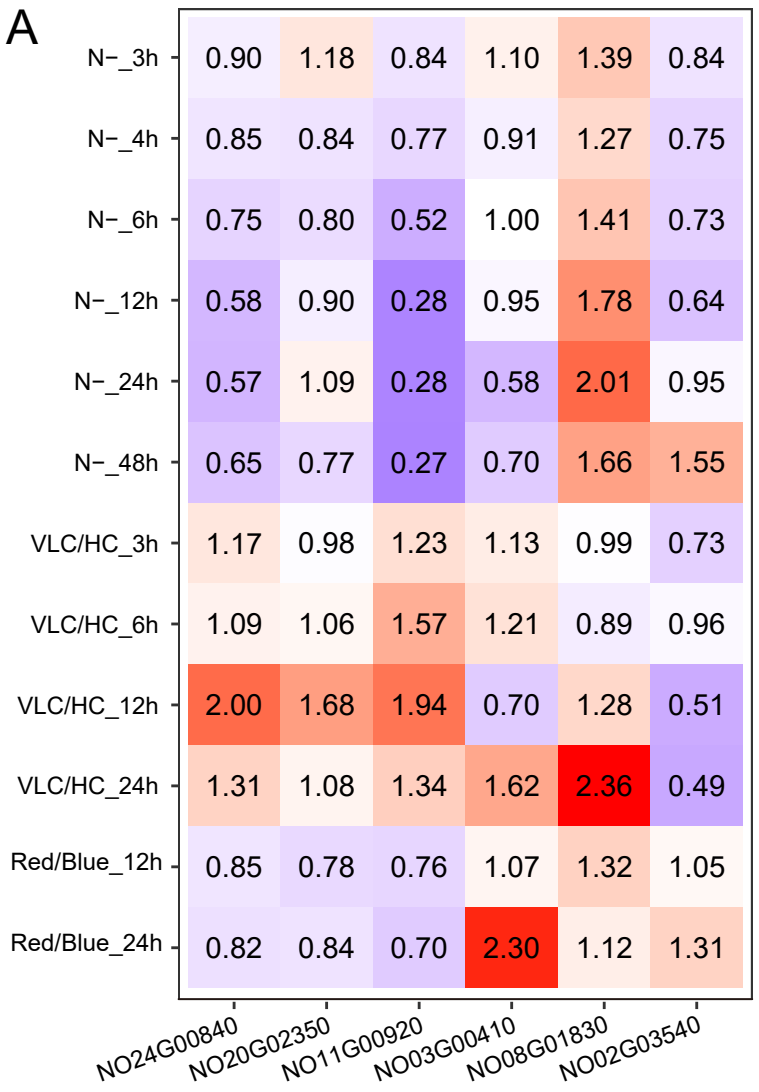


Figure S2. The positional base preferences within the gRNA sequences for the designed gRNA library (A) and the valid gRNA library (B). A gRNA is considered valid if it successfully targets the designated gene and yields at least one mutant.

Figure S3

A

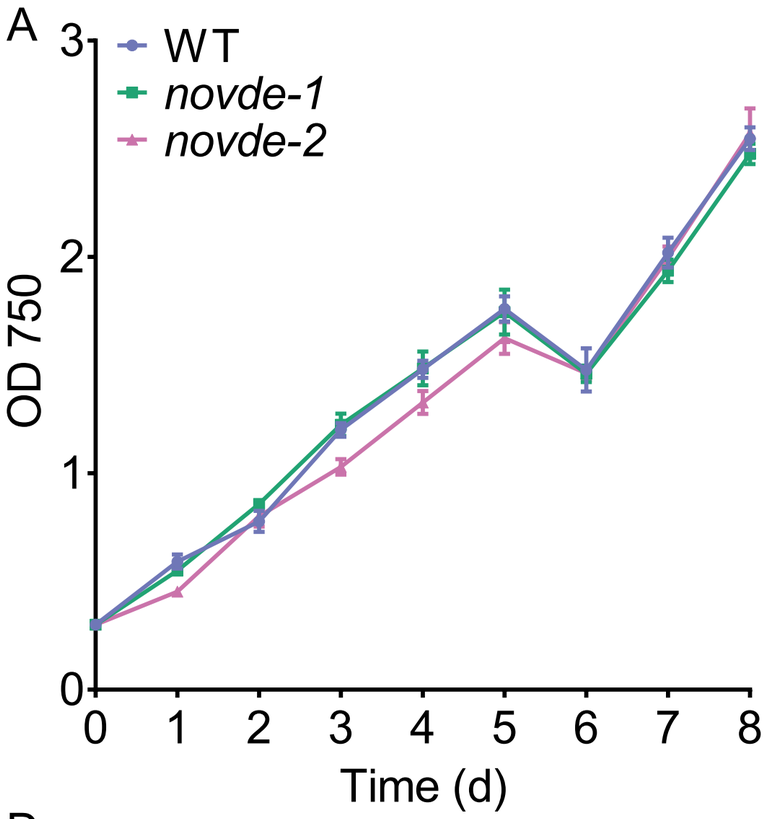


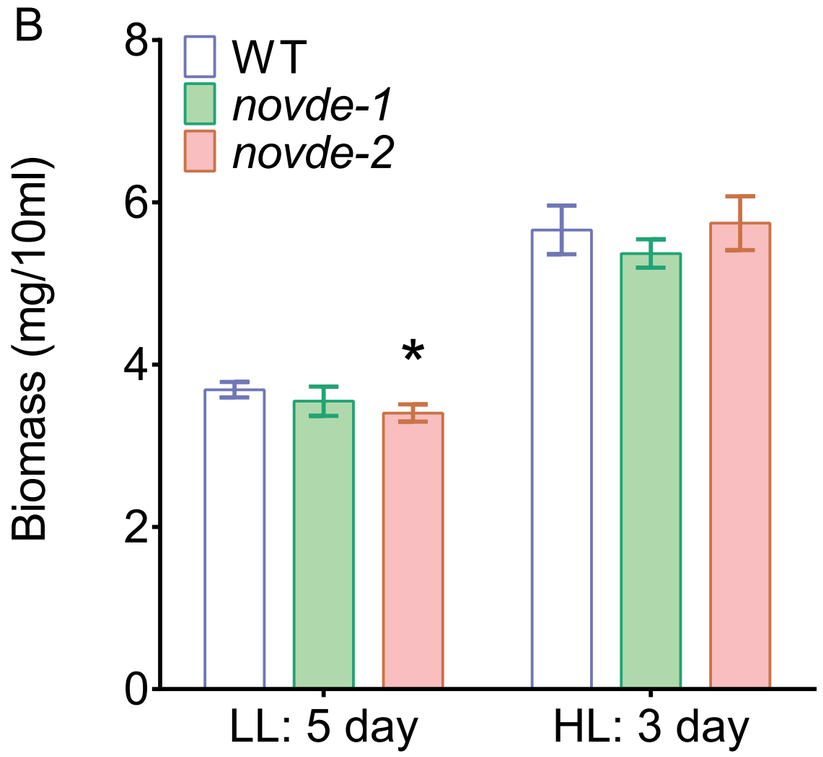
B

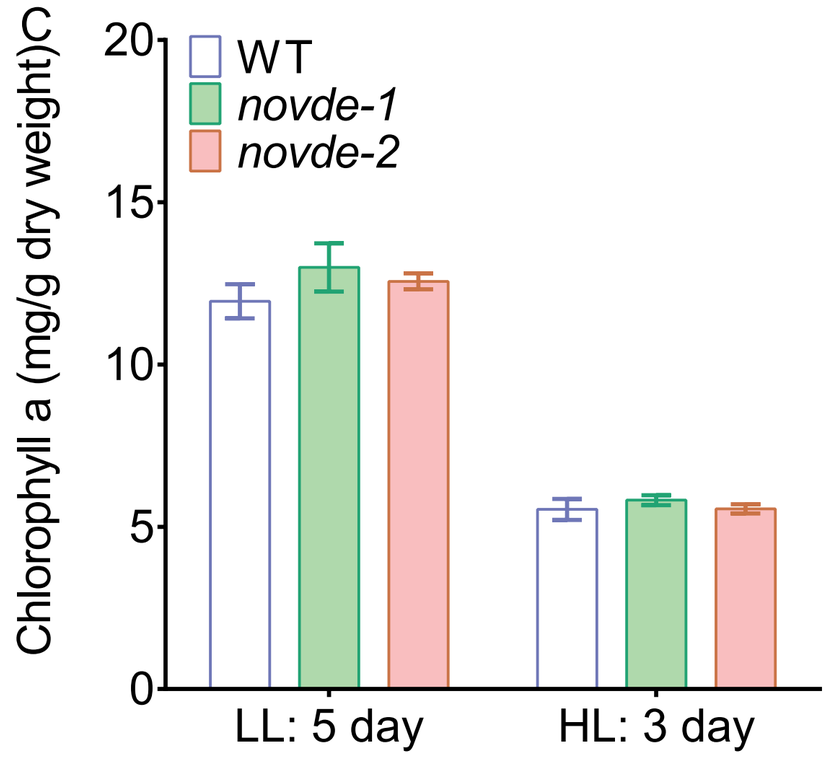
	H3	H6	H12	H24	r	Gene description
NO11G02410	−0.2	−0.0	−0.6	−1.1	0.94	Proteasome subunit beta type
NO05G01210	−0.2	−0.0	−0.7	−1.1	0.95	26s proteasome non−atpase regulatory subunit rpn12a
NO29G01120	−0.2	−0.1	−0.7	−1.0	0.92	26s proteasome non−atpase regulatory subunit 13
NO03G00430	−0.3	−0.1	−0.8	−1.2	0.96	26S proteasome non−ATPase regulatory subunit 7 homolog B
NO09G00980	0.0	−0.0	−0.7	−1.0	0.90	26s proteasome subunit 4
NO06G02900	0.1	−0.1	−0.8	−1.1	0.84	26s proteasome non−atpase regulatory
NO04G01770	0.1	−0.2	−0.6	−1.1	0.81	26s proteasome regulatory subunit n7
NO15G02030	−0.2	−0.0	−0.5	−1.2	0.88	Proteasome endopeptidase complex
NO09G00780	−0.2	−0.1	−0.6	−1.2	0.89	26S proteasome regulatory subunit S2 1B
NO22G00570	−0.3	−0.0	−0.7	−1.4	0.93	20S proteasome alpha subunit E2
NO16G00520	−0.2	−0.1	−0.7	−1.4	0.88	Proteasome subunit beta type
NO21G01470	−0.2	0.2	−0.6	−1.2	0.97	Proteasome subunit beta type
NO11G01810	0.1	0.2	−0.6	−1.2	0.93	Proteasome maturation factor ump1 family protein
NO07G03800	−0.2	0.0	−0.8	−1.3	0.95	26s proteasome non−atpase regulatory subunit 11
NO01G00750	−0.2	−0.1	−0.8	−1.4	0.94	Proteasome subunit alpha type
NO18G01720	−0.3	−0.1	−1.0	−1.3	0.94	Proteasome subunit beta type
NO01G03780	−0.2	−0.0	−1.0	−1.4	0.96	Proteasome subunit beta type
NO17G00810	−0.1	−0.1	−1.0	−1.2	0.91	26s proteasome non−atpase regulatory subunit 12
NO13G00360	−0.1	−0.1	−0.9	−1.3	0.90	26S protease regulatory subunit 7
NO06G00810	0.0	−0.1	−0.7	−1.4	0.86	Proteasome subunit alpha type
NO02G04170	0.1	−0.1	−0.8	−1.3	0.86	26s protease regulatory subunit 6a−a
NO12G00630	−0.0	−0.0	−0.9	−1.5	0.90	Proteasome subunit alpha type
NO08G00580	−0.3	0.0	−0.9	−1.9	0.94	proteasome subunit PAB1
NO01G03010	−0.2	0.1	−1.5	−1.6	0.94	Proteasome activator subunit 1
NO24G02110	−0.2	−0.0	−0.8	−0.3	0.69	Proteasome subunit
NO11G03130	−0.3	−0.0	−0.9	−0.5	0.78	26S proteasome regulatory subunit T3
NO14G03240	−0.1	−0.2	−0.7	−0.6	0.86	Proteasome subunit alpha type
NO28G00670	−0.2	0.1	−0.7	−0.8	0.99	26s proteasome regulatory atpase rpt4
NO11G01440	−0.3	0.2	−0.7	−0.7	0.98	26s proteasome non−atpase regulatory subunit 14
NO16G01480	−0.1	0.1	−0.7	−0.6	0.95	deletion of SUV3 suppressor 1(I)
NO24G00350	−0.4	−0.1	−0.8	−1.0	1.00	26s proteasome non−atpase regulatory subunit 3
NO12G02270	−0.5	−0.3	−0.7	−0.9	0.98	26S proteasome regulatory complex, Rpn2/Psmd1 subunit
NO02G01940	−0.0	0.1	−0.1	−0.8	0.80	GTP−binding nuclear protein
NO01G03930	0.0	−0.0	−0.4	−0.9	0.83	Adhesion regulating molecule 1
NO26G01150	−0.0	0.1	−0.3	−0.5	0.99	Proteasome subunit beta type
NO16G01700	−0.3	−0.3	−0.0	−0.8	0.34	Proteasome a type
NO01G00310	−0.4	−0.2	−0.4	−0.8	0.91	Atp binding protein
NO06G02060	−0.1	−0.1	0.4	0.0	−0.56	Proteasome alpha5 isoform a
NO02G02680	−0.4	−0.2	0.3	0.1	−0.73	Proteasome subunit alpha
NO24G02020	−0.4	−0.1	0.2	−0.2	−0.25	Proteasome subunit beta type−7
NO21G01510	0.0	−0.2	0.5	−0.3	−0.18	Proteasome, beta−type subunit, conserved site
NO02G02900	−0.2	0.5	0.5	0.1	0.26	Proteasome subunit beta type
NO03G01250	−0.1	0.1	0.0	0.5	−0.63	Proteasome, subunit alpha/beta
NO02G04070	−0.2	0.0	−0.1	0.3	−0.42	Subunit of proteaseome activator complex
NO15G01020	0.1	0.0	−0.6	0.3	0.03	26s protease regulatory subunit 8
NO06G01130	0.3	0.1	0.2	0.1	−0.11	Wd repeat−containing protein 70
NO03G04380	0.3	−0.1	0.3	0.1	−0.38	Proteasome subunit alpha
NO29G01510	0.1	−0.1	0.2	0.2	−0.97	Proteasome subunit beta type−6
NO01G00880	0.5	0.1	0.7	0.5	−0.78	Rrna processing protein
NO22G01880	−0.2	0.0	0.4	0.3	−0.66	Proteasome subunit beta type−2
NO13G00910	−0.0	−0.1	0.6	0.3	−0.81	26s proteasome non−atpase regulatory subunit 9
NO15G00610	−0.1	−0.1	0.5	0.6	−0.90	Proteasome activator complex subunit 4A

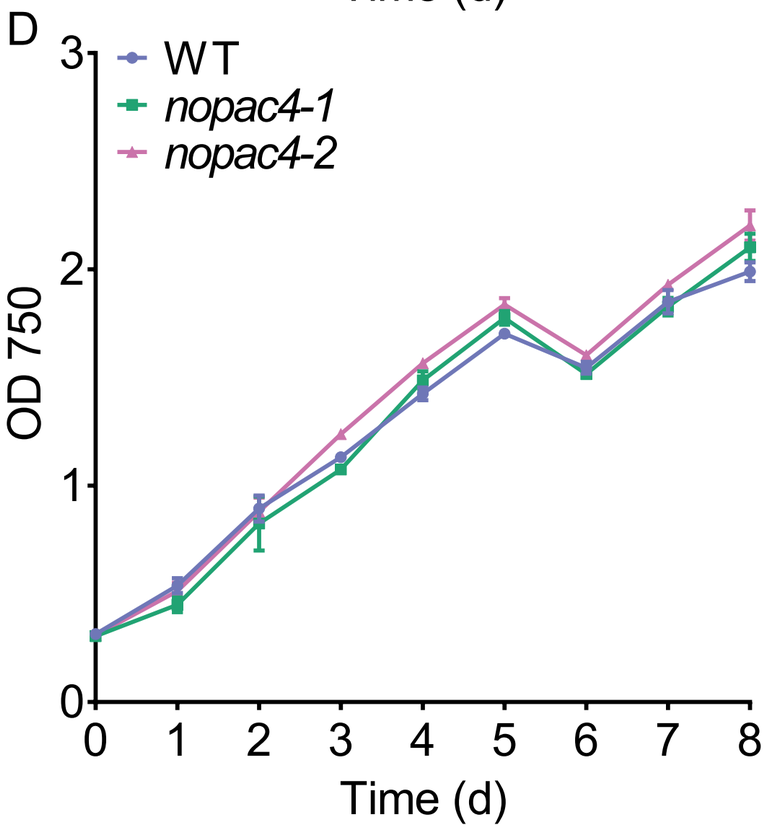
Figure S3. Transcriptomic analyses of NO02G03540 and the 26S proteasome genes of *N. oceanica* under various conditions. (**A**) The fold change in transcriptomic levels of *noVDE*s and *noPAC4* under various conditions, including very low carbon (VLC, 100 ppm CO_2) versus high carbon (HC, 5% CO_2) levels, nitrogen-depleted (N-) versus nitrogen-replete (N+) conditions, and exposure to red light versus blue light. (**B**) Comparative heatmap analysis of log2-transformed fold changes (VLC vs. HC) for proteasome-associated genes across experimental conditions. A bidirectional color gradient illustrates the differential expression (red for upregulation and green for downregulation) relative to the HC baseline. Pairwise Pearson correlation coefficients (*r*-values) between the transcriptional dynamics of NO02G03540 and individual 26S proteasome genes are displayed in a column following the heatmap.

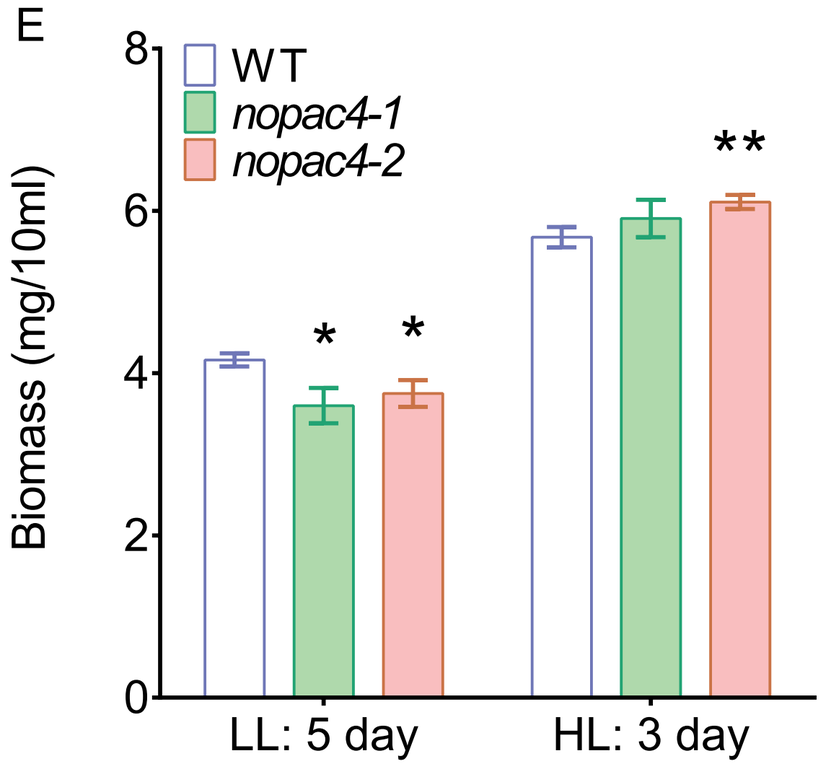
Figure S4











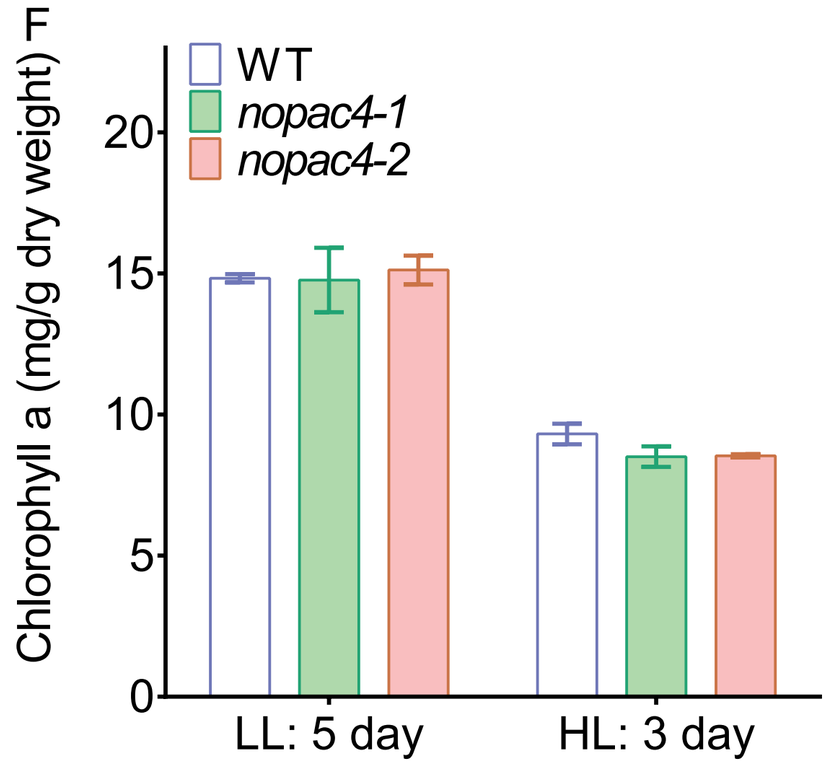


Figure S4. The phenotype identification of growth rate, biomass and chlorophyll a content in the mutants and WT. (A-C) The growth rate, biomass and chlorophyll a in *noVDE*s mutants and the WT. **(D-F)** The growth rate, biomass and chlorophyll a content in *noPAC4* mutants and the WT.

Figure S5

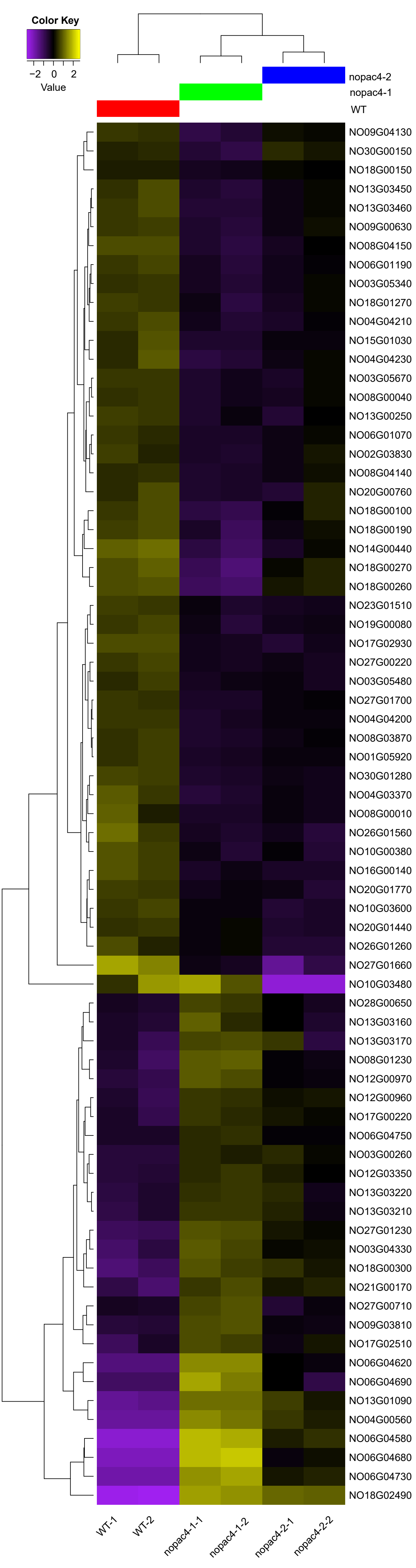


Figure S5. The heatmap demonstrates the high reproducibility of transcriptome profiles among biological replicates for each group. Differentially expressed genes are utilized to represent the transcriptional profiles of each sample. The cells in the heatmap are colored according to the expression levels of these genes on a log2 scale, with data centered.

Figure S6

NO06G01130	51.0	55.9	55.5	Wd repeat−containing protein 70
NO05G01210	47.2	52.6	51.2	26s proteasome non−atpase regulatory subunit rpn12a
NO24G02020	31.1	35.1	34.9	Proteasome subunit beta type−7
NO11G03130	161.5	181.3	169.3	26S proteasome regulatory subunit T3
NO15G01020	41.4	43.7	42.2	26s protease regulatory subunit 8
NO03G04380	46.4	50.0	47.8	Proteasome subunit alpha
NO15G00610	39.4	42.2	42.3	Proteasome activator complex subunit 4A
NO14G03240	147.4	141.8	148.6	Proteasome subunit alpha type
NO02G02900	48.7	46.4	48.7	Proteasome subunit beta type
NO03G01250	95.5	93.0	95.9	Proteasome, subunit alpha/beta
NO01G00310	46.9	47.9	47.1	Atp binding protein
NO24G02110	40.3	40.7	43.5	Proteasome subunit
NO16G01700	18.1	17.3	19.5	Proteasome a type
NO16G01480	57.5	67.0	57.0	deletion of SUV3 suppressor 1(I)
NO06G02060	38.6	41.0	35.5	Proteasome alpha5 isoform a
NO21G01510	44.0	37.6	41.8	Proteasome, beta−type subunit, conserved site
NO22G01880	17.7	10.4	16.8	Proteasome subunit beta type−2
NO26G01150	52.3	62.0	56.8	Proteasome subunit beta type
NO06G02900	37.1	43.8	40.8	26s proteasome non−atpase regulatory
NO02G04170	16.7	20.1	19.2	26s protease regulatory subunit 6a−a
NO01G00750	156.6	186.3	178.8	Proteasome subunit alpha type
NO28G00670	52.8	65.5	56.6	26s proteasome regulatory atpase rpt4
NO22G00570	94.7	116.7	103.3	20S proteasome alpha subunit E2
NO11G01440	66.2	80.0	71.0	26s proteasome non−atpase regulatory subunit 14
NO17G00810	69.1	93.7	75.2	26s proteasome non−atpase regulatory subunit 12
NO12G02270	52.8	71.0	56.5	26S proteasome non−atpase regulatory subunit, Rpn2/Psmd1
NO29G01510	80.2	105.5	85.8	Proteasome subunit beta type−6
NO18G01720	35.1	45.3	39.4	Proteasome subunit beta type
NO09G00980	108.7	140.3	121.3	26s proteasome subunit 4
NO09G00780	88.0	120.9	98.6	26S proteasome regulatory subunit S2 1B
NO15G02030	77.6	103.0	92.3	Proteasome endopeptidase complex
NO01G03010	57.4	76.6	68.1	Proteasome activator subunit 1
NO02G04070	49.0	65.8	59.0	Subunit of proteaseome activator complex
NO13G00360	73.1	100.7	87.6	26S protease regulatory subunit 7
NO06G00810	164.6	219.8	204.8	Proteasome subunit alpha type
NO03G00430	73.7	100.8	90.6	26S proteasome non−ATPase regulatory subunit 7 homolog B
NO16G00520	67.1	85.4	80.7	Proteasome subunit beta type
NO01G03930	49.9	63.0	62.1	Adhesion regulating molecule 1
NO11G02410	75.3	103.3	95.9	Proteasome subunit beta type
NO11G01810	44.1	60.0	57.1	Proteasome maturation factor ump1 family protein
NO12G00630	126.3	175.8	164.5	Proteasome subunit alpha type
NO01G03780	163.4	226.4	214.9	Proteasome subunit beta type
NO21G01470	58.6	82.5	74.4	Proteasome subunit beta type
NO08G00580	50.8	73.0	64.7	proteasome subunit PAB1
NO13G00910	168.7	195.4	208.1	26s proteasome non−atpase regulatory subunit 9
NO02G01940	568.1	640.5	692.3	GTP−binding nuclear protein
NO01G00880	25.4	28.0	32.6	Rrna processing protein
NO02G02680	37.8	40.7	44.2	Proteasome subunit alpha
NO29G01120	52.1	79.4	66.5	26s proteasome non−atpase regulatory subunit 13
NO24G00350	46.3	71.2	59.9	26s proteasome non−atpase regulatory subunit 3
NO04G01770	115.1	169.4	144.9	26s proteasome regulatory subunit n7
NO07G03800	22.8	38.1	28.7	26s proteasome non−atpase regulatory subunit 11
NO11G02570	69.7	116.1	71.3	20S proteasome beta subunit D1
	WT	*nopac4-1*	*nopac4-2*	Gene description

Figure S6. The heatmap illustrates the average transcriptional levels (in TPM) of genes associated with the proteasome pathway for both WT and mutant samples. Gene set enrichment analysis of KEGG pathways revealed that the proteasome pathway was significantly upregulated in the *noPAC4*-knockout mutants. In the heatmap, Cells are color-coded based on the fold change in gene expression for the corresponding genes, with red indicating up-regulation and green denoting down-regulation.