

Supplementary Data

Table S1. Species information for cyFBPase from diverse photosynthetic eukaryotes

Species	Name
Land plants	<i>Arabidopsis thaliana</i>
	<i>Nicotiana tabacum</i>
Chlorophyta	<i>Chlamydomonas reinhardtii</i>
	<i>Nannochloropsis gaditana</i>
Bacillariophyta	<i>Fragilariopsis cylindrus</i>
	<i>Fistulifera solaris</i>
	<i>Phaeodactylum tricornutum</i>
	<i>Aureococcus anophagefferens</i>
Chrysophyta	<i>Emiliana huxleyi</i>
	<i>Pelagophyceae</i> sp.
Cyanobacteria	<i>Cyanobacterium</i> sp.
Euglenozoa	<i>Eutreptia viridis</i>
	<i>Discoplastis spathirhyncha</i>
	<i>Euglena gracilis</i>
Eumycophyta	<i>Saccharomyces cerevisiae</i>
Haptophyta	<i>Pavlova</i> sp.
	<i>Chrysochromulina tobinii</i>
	<i>Diacronema lutheri</i>
	<i>Globisporangium splendens</i>
	<i>Lagenidium giganteum</i>
Oomycota	<i>Phytophthora sojae</i>
	<i>Plasmopara halstedii</i>
	<i>Pythium brassicae</i>
	<i>Pythium insidiosum</i>
Pyrrophyta	<i>Pythium oligandrum</i>
	<i>Symbiodinium</i> sp.

Chlamydomonas/1-374 29YDEELIKTGVVASKGRILAMDESNAATCGRLDSIGVENTEENRRAYRELLVTAFLGQVYISGAILFEETLYQSTAS 107
Saccharomyces/1-348 32 KQFKNATGDFTLVLNALQFAKFFVSHITRRRELNVNIVGLAGASNFTGQQKKLDVLG..... 88
Arabidopsis/1-417 96 I....DAELTIVMSSISLACKQIASLVQRAGISNLT..GVQAINIOGEDOKKLDVLS..... 147
Nannochloropsis/1-356 27 K....DIQLTLLMTSIQMGCKSIARAVRKAGIAGLY..GLHGSENVSGDQVKKLDVLS..... 78
Phaeodactylum/1-328 16 R....DHDLVILLNATSKLITSAVQRAGVAKLY..GLAGEVNSTGDDQKKLDVLS..... 67

Chlamydomonas/1-374 108 GKKFVDVMKEQNI VPGIK...VDKGLVPCTPTMSWCMGLD...QWTSAAEYVKAARLAKWRSVVS.....I-PHPS..... 174
Saccharomyces/1-348 89 DEIFINAMRAAGIKNLVSEEDQLI..WFPNTISAWCCDFIDGSSNLDAGVSGTIFASIFRLLPD.....SSGTINDVLRCKEMVAA 172
Arabidopsis/1-417 148 NEVFSNCLRSSGRTGIIASEEDVPVAMEESYSGNVVVVDFPLDSSNIDAAVSTGSI FGIYSPNDECIVDDSDDISALGSEEQRCIVNVCPGNLLAA 247
Nannochloropsis/1-356 79 DEIFVNCLKESHCACVLYSEERDDPIIVEAAKAGKYCVAFDPLDSSNIDCNVSTGTIFAIYERISA.....SDQSPVTDILRAGTAIVAA 165
Phaeodactylum/1-328 68 NDMMINALVNSGVCCVLYSEENEEPIIVPFGKAGKYCVAFDPLDSSNIDCNVSTGTIFSVYERKPG.....SNG..SAEDLLRS GADCI CA 152

Chlamydomonas/1-374 175IMPLRAYGLARYAAIAQNAVLPIVEPEVL..LDGEHIDRCLVQEAIAETFKYMAENKV..... 235
Saccharomyces/1-348 173 CYAMYGSSTHIVLTL..GDQVDGFTL..DTNLGEFILTHPNLRIFPQ..KAIYSINEGNTLYNNETIRTFIEKVKQPQADNNNKTF SARVYVGSVMVADVHRTF 268
Arabidopsis/1-417 248 GYCMYSSSVIFVLT..GKGVFSFTL..DPMYGEFVLTQENIEIPKA..GRIYSFNEGNYQMDDKLLKYIDDLKDPGP..TGKPYSAARYIGSLVGDVHRTL 341
Nannochloropsis/1-356 166 GYCMYGSATDLVLT..GHGVHRTL..DPTLGEFIHTGGPVKLPKA..PKHIYSCNEGNYSLWDDAMRAAVDAFKHQ...DPFYAARYVGSVMVADVHRTL 257
Phaeodactylum/1-328 153 GYVYSSAVEMVFTFRGSDVHGFTL..DSTIGEFTVHTREKMFVFAEGGKRIYSCNEGNNANNWQPIKDACHFRDS...EQFYTARYVGSVMVADVHRTI 246

Chlamydomonas/1-374 236 MLOEILLKIAMVTPGADCKNKAPAKVAEYTLKMAARAPFVPEMFLSGQSEL.....ESTINTNAMNQSINPWIVSFSYARALTNTVLKTWQASP 328
Saccharomyces/1-348 269 LYGGIFAYT.....CDKNSPNGLKRL.....LYEAFPMFALMEQAGGKAVN...DRGERIIDL...VSHIHDK.....SSTWLGSS 334
Arabidopsis/1-417 342 LYGGIYGYF.....RDAKSENGKLR.....LYEAPMSFIVEQAGGKGSDDGH...SRVLDI...QTEIHQR.....VFLYIGST 406
Nannochloropsis/1-356 258 LYGGIFLYP.....ADKSKIKGLRV.....LYEGFPMAKIVVEDAGGIATTGLFGQKIQRVLDI...HPSNVHNR.....CPIILGTP 327
Phaeodactylum/1-328 247 LYGGIYMYT.....ADKSPKGLKRL.....LYEGIPMAMII EQAGGVASTGFFNGKIQRVLDI...VDETHAK.....CPIIMGK 316

Figure S1. Multi-sequence alignment analysis. Red boxes are active areas. Analysis using InterPro (InterPro (ebi.ac.uk)).

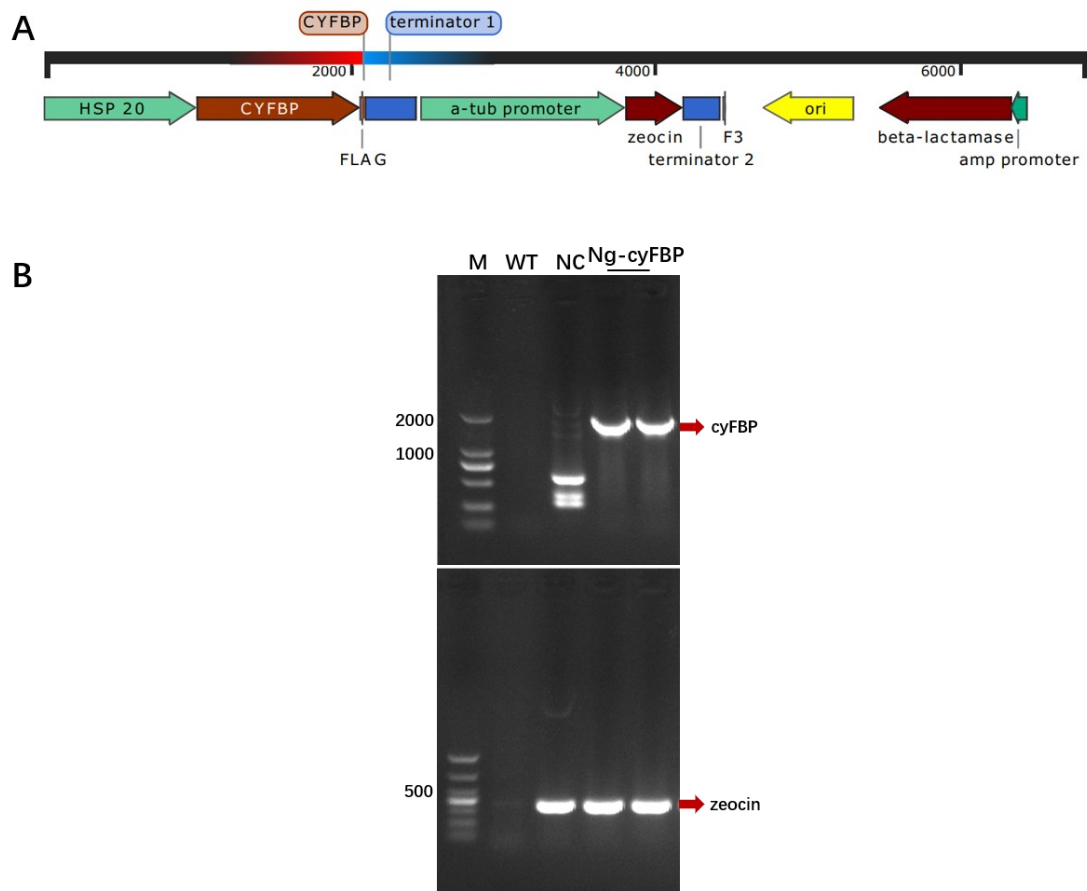


Figure S2. (A) overexpression vectors of Ng-cyFBP. (B) Electrophoretic verification results of the *cyFBP* and zeocin fragments derived from the transformed *N. gaditana* genome. NC: negative control (A vector lacking the target gene *cyFBPase*, containing only the zeocin resistance gene, to serve as a negative control)

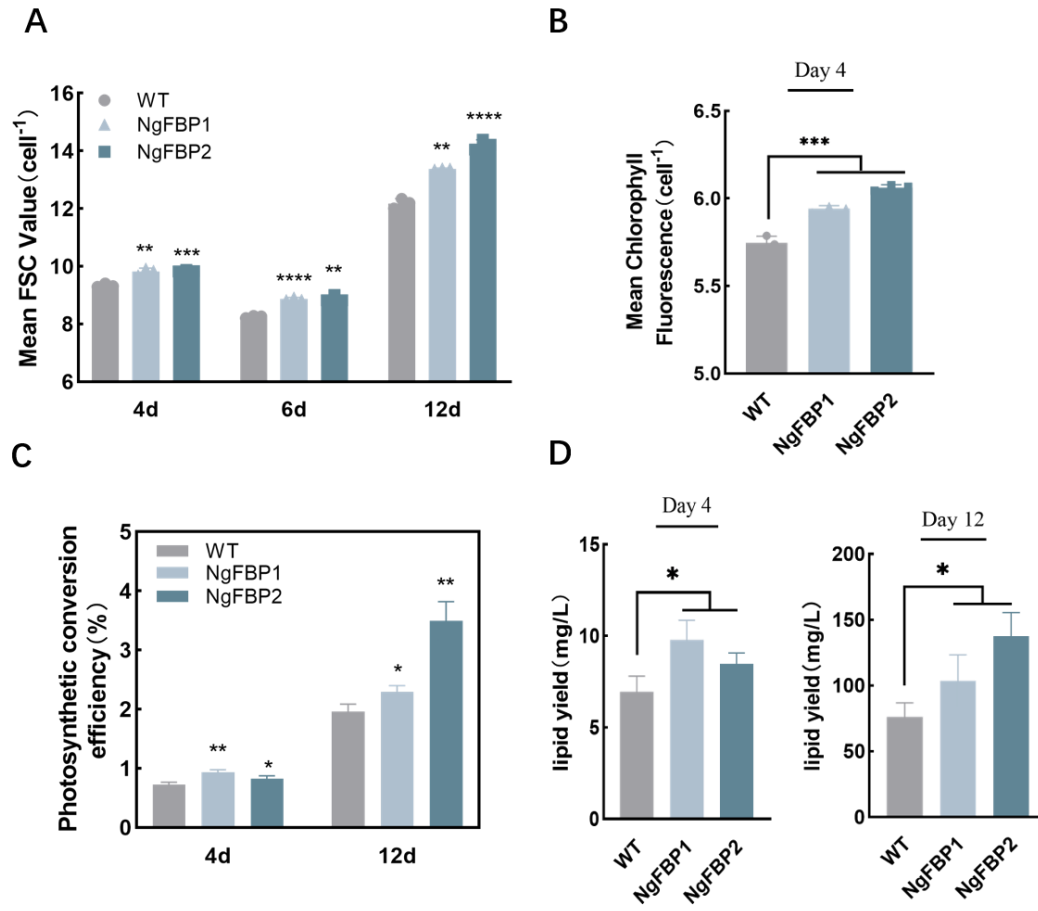


Figure S3. (A) FSC Value of the WT and NgFBP. (B) Chlorophyll fluorescence of WT and NgFBP. (C) Photosynthetic conversion efficiency of NgFBP. (D) Lipid yield on day 4 and day 12. ns indicates $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Error bars represent standard deviations calculated from three independent biological replicates. All data were analyzed through two-way ANOVA (GraphPad Prism).

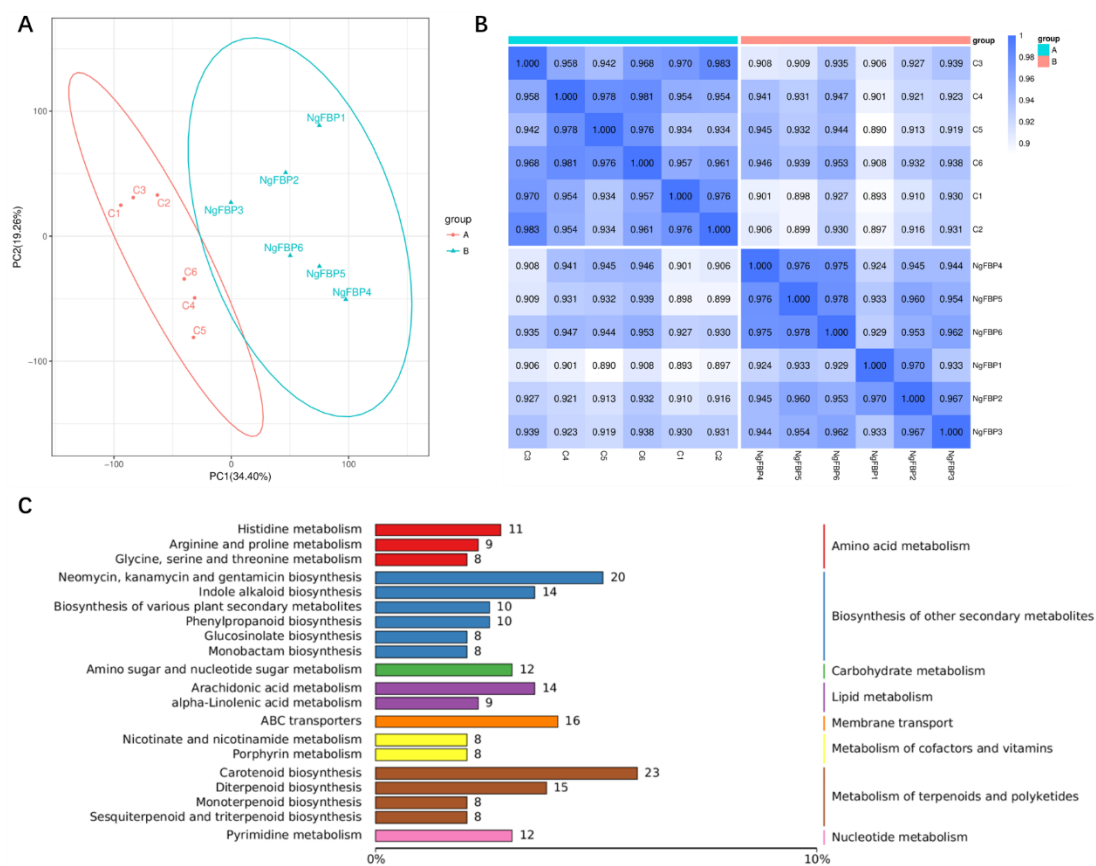


Figure S4. (A) Principal Component Analysis (PCA). (B) Correlation analysis of WT and NgFBP. (C) KEGG enrichment analysis of differentially metabolites in WT and NgFBP on day 4.