

Efficient co-production of EPA and DHA by *Schizochytrium* sp. via regulation of the polyketide synthase pathway

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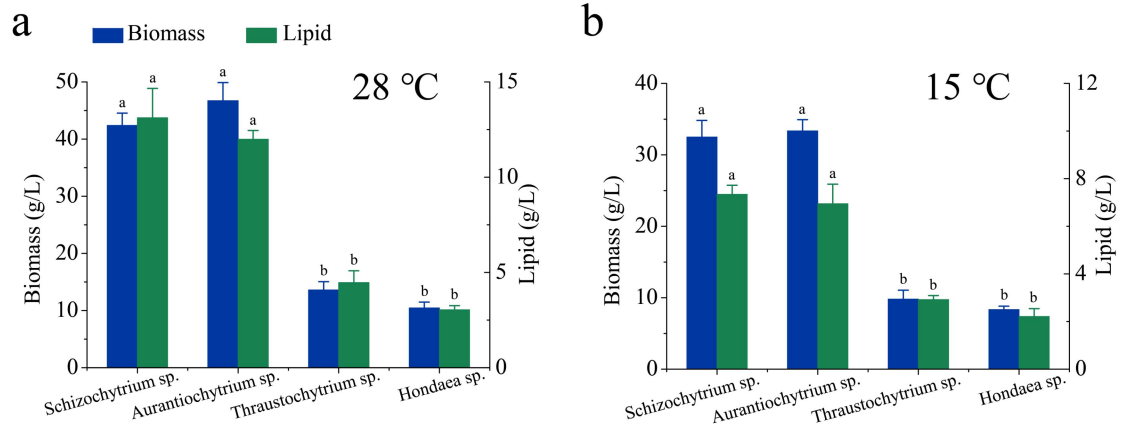
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Tables S1

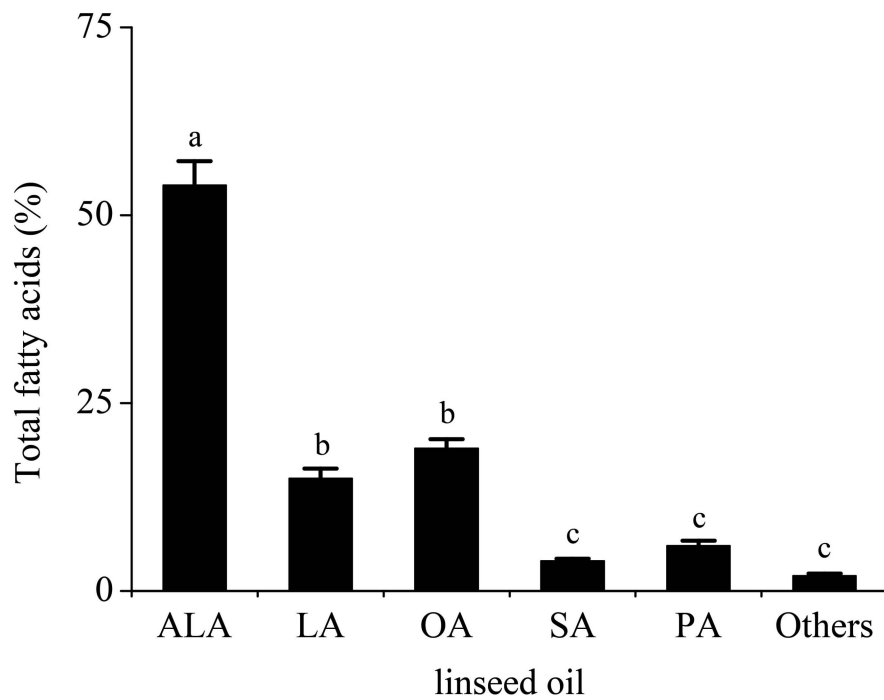
Figures S1 to S11

Supplementary Table1. Primers used in this study.

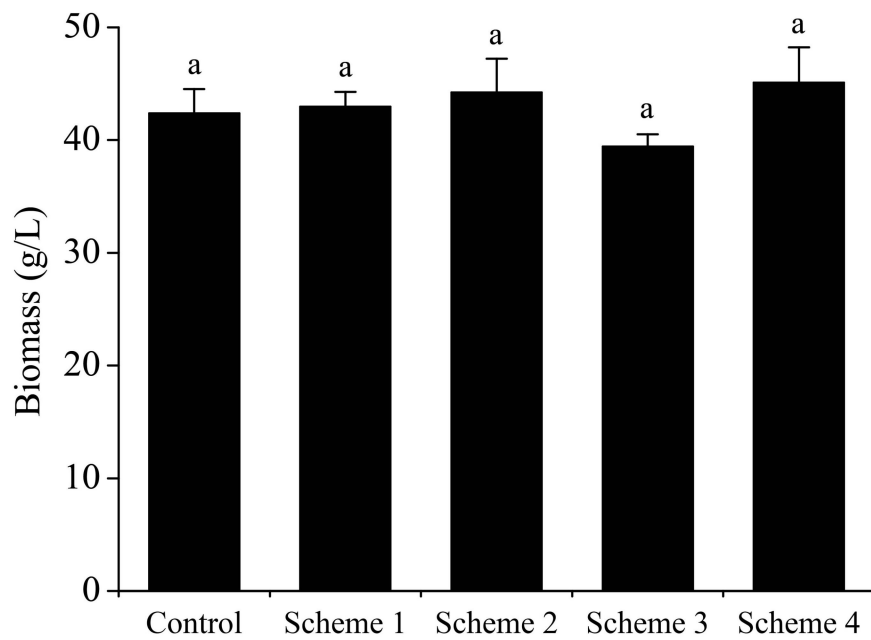
Target gene	Primer	Sequence (5'-3')
<i>β-actin</i>	F	TGTCCTCACGCTCAAGTA
	R	GAAGGTGTGGTGCCAGAT
<i>Δ9 elongase</i>	F	TCCTCTCATTACGCAGTTG
	R	TTCACTATGACTGCGAGC
<i>Δ6 desaturase</i>	F	CGAGCATAACAGTTGACAT
	R	AATTGCCAGGTGATTCAT
<i>Δ8 desaturase</i>	F	CGCATTCCCTTATATTGTTCT
	R	AACACTGACAACCTGGATT
<i>Δ12 desaturase</i>	F	ACTTTCTCCCTTACCTTGT
	R	CATACTCACCAAGATGTCC
<i>MetE-like</i>	F	TACATTGACTACATTAACCTTCGT
	R	GTTCTTGCTGTTGTGAGT
<i>orf A</i>	F	CATCAAGGAGAACGAGAC
	R	AGGGAATTTGAACTGCTC
<i>orf B</i>	F	GAAGAAGAAGAAGAAGAAGAA
	R	AGATCCGATCCTCAACAAG
<i>orf C</i>	F	TCTTGAGACCGTTAATGTAA
	R	CATCGAGAAGCGTATCTT
<i>GPAT1</i>	F	ACCGACAAGAATGGAGAA
	R	AGATGAAGATGCTGTTGC
<i>GPAT2</i>	F	AACAAGAAGTCAAGAACAT
	R	CGTAGATAGACTGCGTAT
<i>LPAAT1</i>	F	ATTGATTACGAAGAGTTCT
	R	CTCAGGAACCATTAACCTT
<i>LPAAT2</i>	F	TTACCTTGATCTGATTGA
	R	CTTAACGAGAATATGCTC
<i>LPAAT3</i>	F	AAACCCACACCGACAAGA
	R	CAATCCAACCTCCTCATCTTCATA
<i>PAP1</i>	F	CTTGGAGTATCACAGTGT
	R	GGATGGTTCTTCGTATGG
<i>PAP2</i>	F	ATTTCCAGAACCCGATAT
	R	AATCAAGTTATCCTCGGT
<i>PAP3</i>	F	CAATTTGACCGCTACTAT
	R	CCATTATCCTTCTCTTGC
<i>DGAT1</i>	F	TTCCTCTCGCCAACATAA
	R	AGAAATGGAAATCACTTGGTT
<i>DGAT2</i>	F	CAAAGACCACAGCACATC
	R	ATTGTGCTAGGTGTTCTCT
<i>DGAT3</i>	F	GTGATCCGTACATTGCTA
	R	GTTAATACGATGACCTCTTG



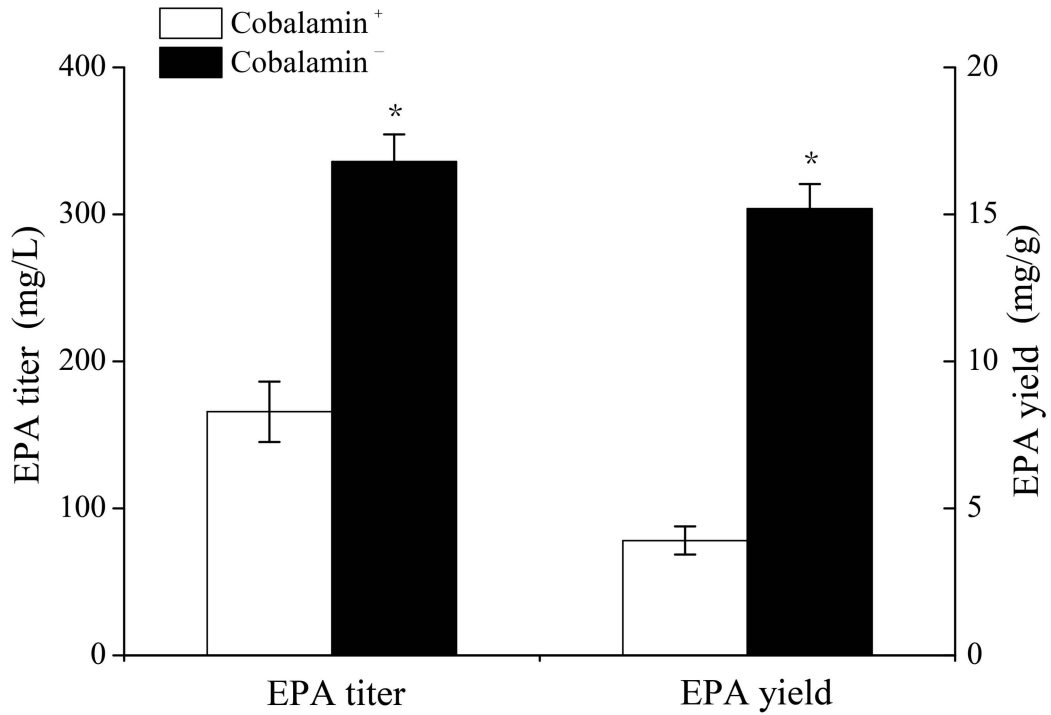
Supplementary Figure 1. Biomass and lipid accumulation of different thraustochytrid genera under (a) 28°C fermentation conditions and (b) 15 °C fermentation conditions. Letters above the bars indicate significant difference ($p \leq 0.05$), based on one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test. The data shown are the mean \pm S.D. (n=3).



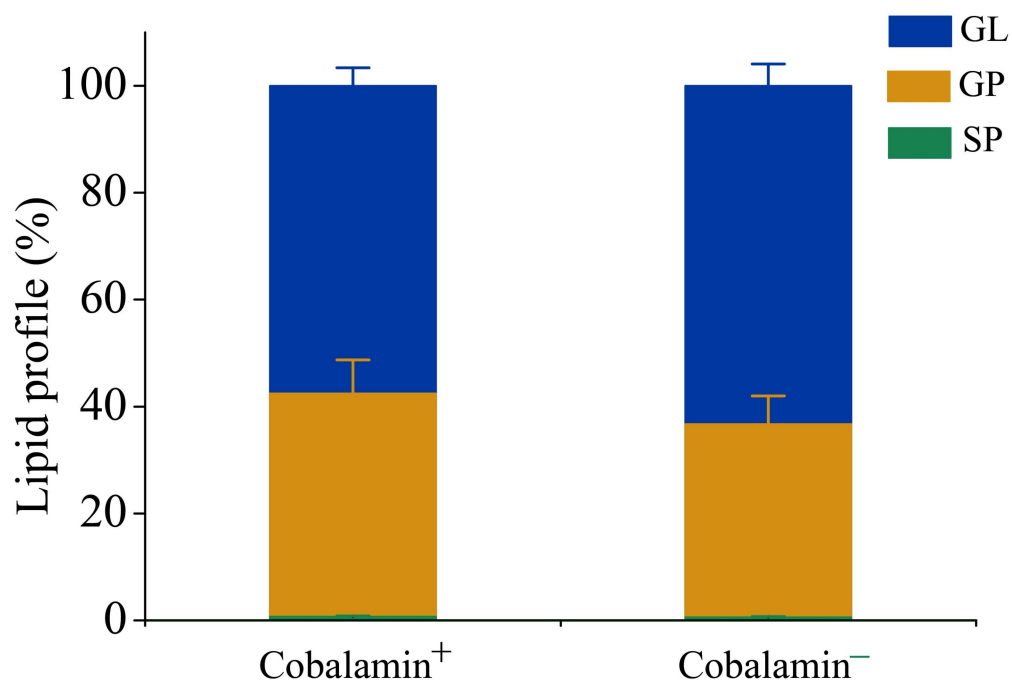
Supplementary Figure 2. Fatty acid composition of linseed oil. ALA α -linolenic acid, LA linoleic acid, OA oleic acid, SA stearic acid, PA palmitic acid. Letters above the bars indicate significant difference ($p \leq 0.05$), based on one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test. The data shown are the mean \pm S.D. (n=3).



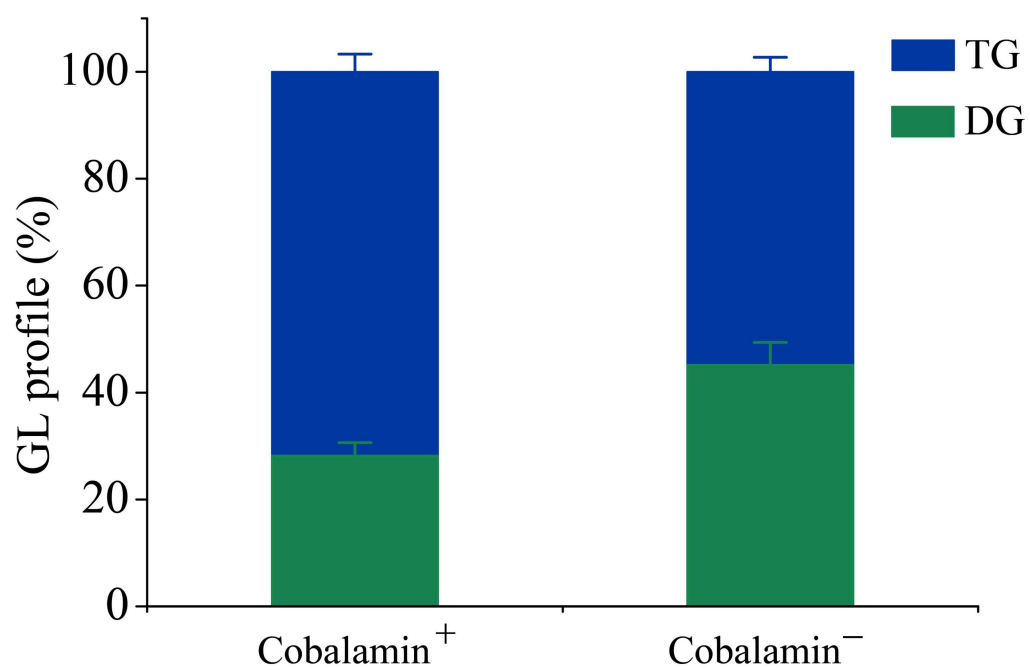
Supplementary Figure 3. Biomass of Schizochytrium under different adding schemes. Letters above the bars indicate significant difference ($p \leq 0.05$), based on one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test. The data shown are the mean \pm S.D. (n=3).



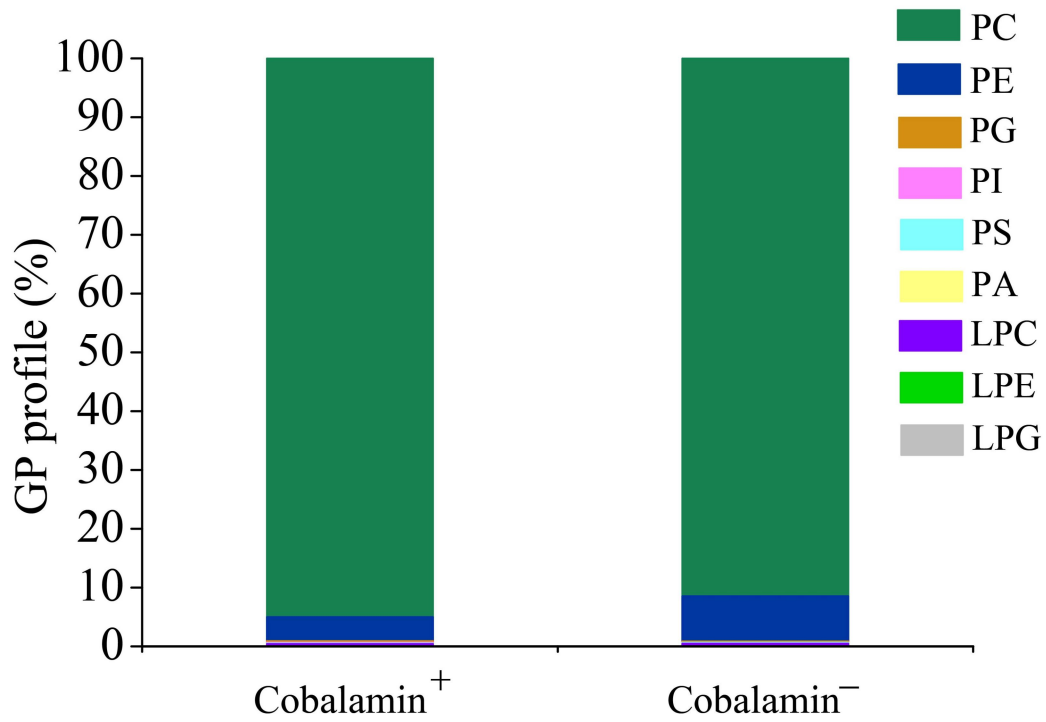
Supplementary Figure 4. Effects of EPA yield and EPA titer between cobalamin⁺ and cobalamin⁻ of *Schizochytrium* sp.. The statistical significances of the final results were analyzed by t-test, * $p < 0.05$. The data shown are the mean \pm S.D. (n=3).



Supplementary Figure 5. Distribution of fatty acid storage in *Schizochytrium* sp. under different fermentation conditions. GL Glycerolipids, GP Glycerophospholipids, SP Sphingolipids. The data shown are the mean \pm S.D. (n=3).

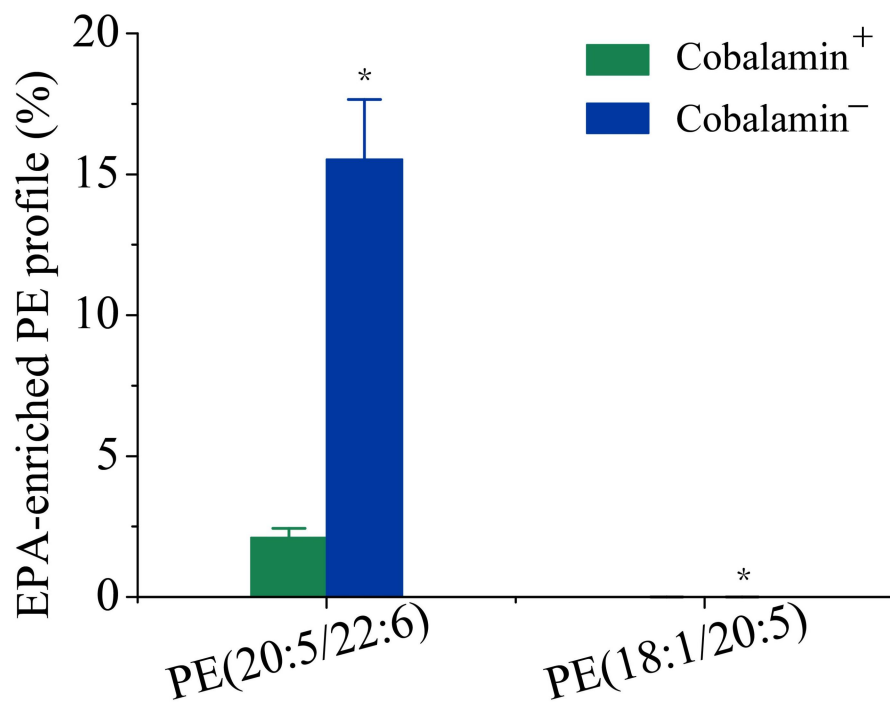


Supplementary Figure 6. Glycerolipid composition of *Schizochytrium* sp.. TG Triradylglycerols, DG Diradylglycerols. The data shown are the mean \pm S.D. (n=3).



Supplementary Figure 7. Glycerophospholipid composition of *Schizochytrium* sp..

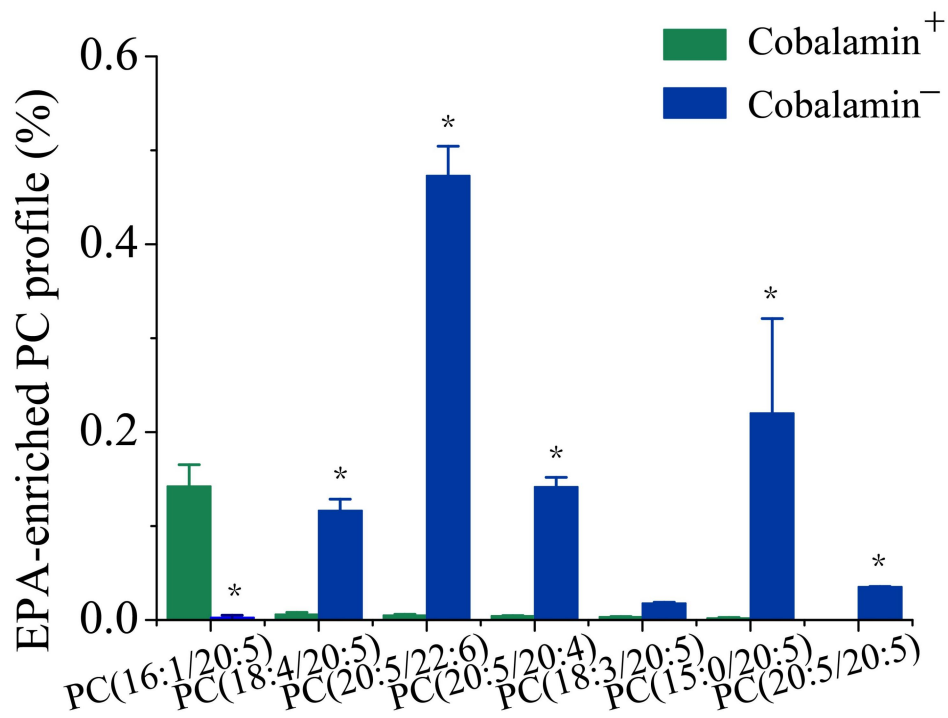
PC phosphocholines, PE phosphoethanolamines, PG phosphoglycerols, PI phosphoinositols, PS phosphoserines, PA phosphates, LPC lysophosphatidylcholine, LPE lysophosphatidylethanolamine, LPG lysophosphatidylglycerol. The statistical significances of the final results were analyzed by t-test, * $p < 0.05$.



Supplementary Figure 8. EPA-enriched PE profile in *Schizochytrium* sp.(60 h).

The statistical significances of the final results were analyzed by t-test, $*p < 0.05$. The

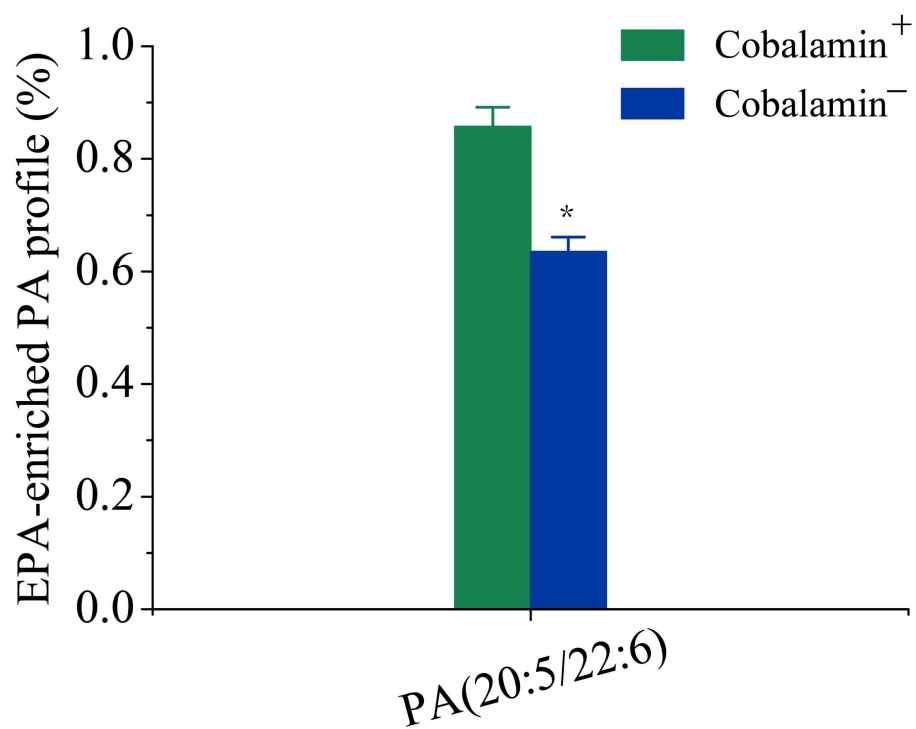
data shown are the mean \pm S.D. (n=3).



Supplementary Figure 9. EPA-enriched PC profile in *Schizochytrium* sp.(60 h).

The statistical significances of the final results were analyzed by t-test, * $p < 0.05$. The

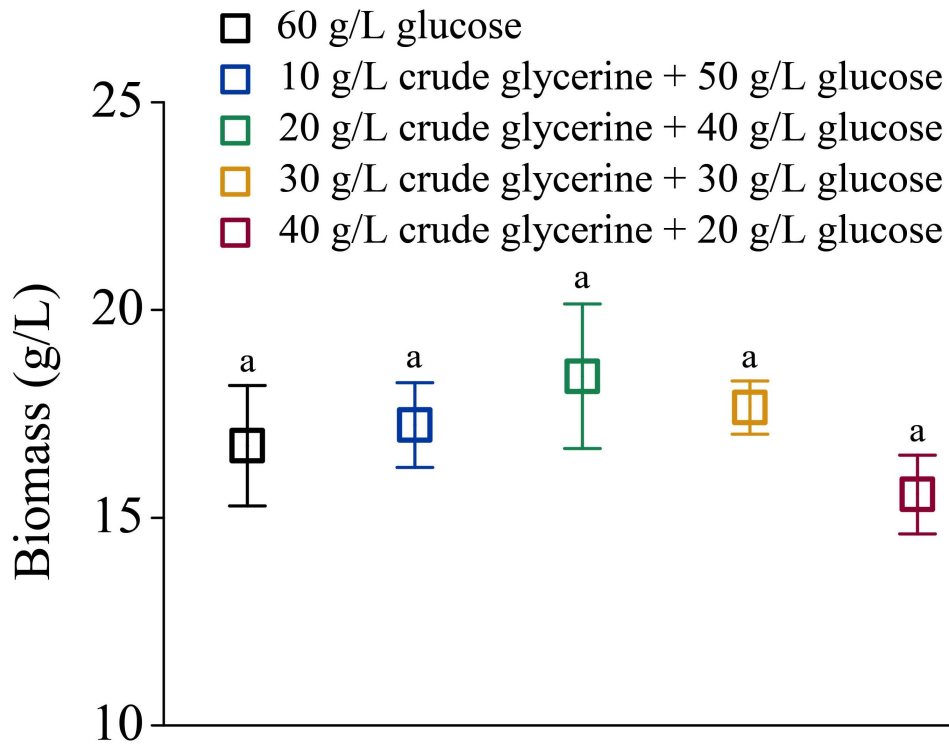
data shown are the mean \pm S.D. (n=3).



Supplementary Figure 10. EPA-enriched PA profile in *Schizochytrium* sp.(60 h).

The statistical significances of the final results were analyzed by t-test, * $p < 0.05$. The

data shown are the mean \pm S.D. (n=3).



Supplementary Figure 11. Effects of mixed carbon sources of different proportions of crude glycerol and glucose on the Biomass of *Schizochytrium* sp.. Letters above the bars indicate significant difference ($p \leq 0.05$), based on one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test. The data shown are the mean \pm S.D. (n=3).