1. Supplemental Tables

Primer name	Restriction enzyme	Oligo nucleotide sequence(5'-3') ^a	Function				
Fad-F	EcoRI	CCG <u>GAATTC</u> ATGGCTTCGTCCACCGTTG	FADS15 amplification				
Fad-R	XhoI	for expression in <i>S. cerevisiae</i>					
Rid-F	EcoRI	CCG <u>GAATTC</u> ATGTCGCCCTTGGAGC	oRiFADS17 amplification for expression in <i>S. cerevisiae</i>				
Rid-R	XhoI	CCG <u>CTCGAG</u> TTACTGGTTCTTCTCCTTCTGG					
T7	- TAATACGACTCACTATAGGG		Target genes detection for				
pYES2.R	_	- TCGGTTAGAGCGGATGTG					
FA	-	AAGTCCCTCCAGTACGTCGTCAAGGATCTGG					
FB	_	AAGTCGATCCTGCATGTCCTGTGGGACCTC					
FC	-	CACGACTGCGGCCAC <u>GGAGCGTTCTCGGAC</u>					
FD	-	CACGAGTGCGGCCAT <u>GGTTCGTTCTCCCG</u>					
FE	-	CACCGCCACCACCACAAGGGCACTGGATCC					
FF	-	CATTCCAAGCACCACAAGAACACCGGAAACATCG					
FG	-	GTTCCTGAAGAACCGT <u>CGCAAGAACATTTTC</u>	Overlap				
FH	-	GTATGAGCCTCACCAG <u>CTCGGTGCCATCATCTCG</u>	extension PCR for 12 fusion				
FI	-	GCTCGTGGCTGGTCATCACCTATCTCCAGC	genes				
FJ	-	GCTTGGATCGTCTGC <u>ACCACCTTCCTCCACC</u>					
RA	-	CCAGATCCTTGACGACGTACTGGAGGGACT T					
RB	-	GAGGTCCCACAGGACATGCAGGATCGACTT					
RC	-	GTCCGAGAACGCTCCGTGGCCGCAGTCGTG					
RD	-	CGGGAGAACGAACCATGGCCGCACTCGTG					
RE	-	<u>GGATCCAGTGCCCT</u> TGTGGTGGTGGCGGTG					

Table S1. Primers used in this study

RF	-	CGATGTTTCCGGTGTTCTTGTGGTGCTTGGAATG						
RG	-	GAAAATGTTCTTGCGACGGTTCTTCAGGAAC						
RH	-	CGAGATGATGGCACCGAGCTGGTGAGGCTCATAC						
RI	-	GCTGGAGATAGGTGATGATGACCAGCCACGAGC						
RJ	-	<u>GGTGGAGGAAGGTGGT</u> GCAGACGATCCAAGC						
W129T		CACCATCTTTGGA <u>ACG</u> GTCCTTCACTCTGC	Targetgeted					
V137T		CACTCTGCTCTTTTG <u>ACG</u> CCCTACCAGGCTTG	mutagenesis for					
Y139F		GCTCTTTTGGTGCCC <u>TTC</u> CAGGCTTGGGCC						
S145T		GGCTTGGGCCATG <u>ACG</u> CATTCCAAGCACCAC						
T144W		CGACATCATCGGC <u>TGG</u> TTGCTGCACACCTTC	Targetgeted					
V152T		CACCTTCATCTTG <u>ACC</u> CCCTACACCACCTGG	mutagenesis					
Y154F		CATCTTGGTCCCC <u>TTC</u> ACCACCTGGAAGCTG	constructing oRiFADS17					
S160T		CCACCTGGAAGCTG <u>ACC</u> CACCGCCACCACCAC	mutants					

^aUnderlined sequences indicate the additional restriction sites, fragments in FADS15 sequence or the mutant sites.

Table S2. Literature summary of catalytic efficiency of ω -3 desaturases from various species with LA, GLA, DGLA and AA substrates.

Rank ª	Preference Index ^b	Strain name	The catalytic efficienc y of w3Des on LA (%) ^{c,d}	The catalytic efficienc y of ω3Des on GLA (%)	The catalytic efficienc y of w3Des on DGLA (%)	The catalytic efficienc y of w3Des on AA (%)	Locus	Reference or source
+9	>>56	Pythium aphanidermatum	-	5.97	28.85	56.46	FW362186.1	15
+8	>>49	Phytophthora sojae	-	6.18	35.45	48.79	FW362213.1	15
+7	>>37	Phytophthora ramorum	-	4.70	31.02	37.12	FW362214.1	15
+6	>>31	Phytophthora infestans	-	-	-	30.94	CAJ30870.1	14
+5	>>26	Saprolegnia diclina	-	-	4.98	25.9	AY373823	13
+4	5	Phytophthora parasitica	7.11	5.63	25.34	49.70	KT372001	16
+3	5.1	Octopus bimaculoides	4.4	4.3	23.5	22.6	MH028785	Our lab
+2	5.1	Caenorhabditis elegans	11	-	0	≈ 56	CELE_Y67H2A.8	34
+1	1.7	Rhizophagus irregularis	34.2	41.8	61.8	58.5	MH028784	This study
0	1	Pichia pastoris	36.5	33.8	35.1	35.3	EF116884	33
-1	-1.7	Mortierella alpina 1S-4	11.50	8.90	3.60	6.70	AB182163	11
-2	-2.6	Mortierella alpina ATCC32222	62.37	63.99	41.82	23.67	AGZ84120.1	This study
-3	-4	Magnaporthe grisea	18.6	3.40	4.6	4.7	XP 362963	9
-4	-5	Fusarium moniliforme	49.30	15.70	17.50	9.80	DQ272516	9
-5	-6.2	Fusarium graminearum	17.40	3.10	5.80	2.80	EAA75859	9
-6	-13.8	Saccharomyces kluyveri	22.0	10.0	3.9	1.60	AB118663	7,8
-7	<<-10.6	Perilla frutescens	10.6	-	-	-	KX880389	12
-8	<<-16.9	Salvia hispanica	16.9	-	-	-	KX610653	12

^a The "Rank" numbers represent the substrate preference level of corresponding ω 3Des. E.g.: "+9" represents the strongest AA substrate preference; "-8" represents the strongest LA substrate preference; "0" represents no substrate preference.

^b Preference indexes = [Catalytic efficiency EPA / Catalytic efficiency LA] or - [Catalytic efficiency LA/ Catalytic efficiency EPA].

^c These data indicated catalytic efficiency of LA, GLA, DGLA and AA, respectively.

^d The "-" represents catalytic efficiency not mentioned in corresponding reference, but its preference is known.

2. Supplemental Figures



Figure S1. Schematic diagram of the fatty acid pathways for EPA and DHA synthesis.