Supplementary Materials

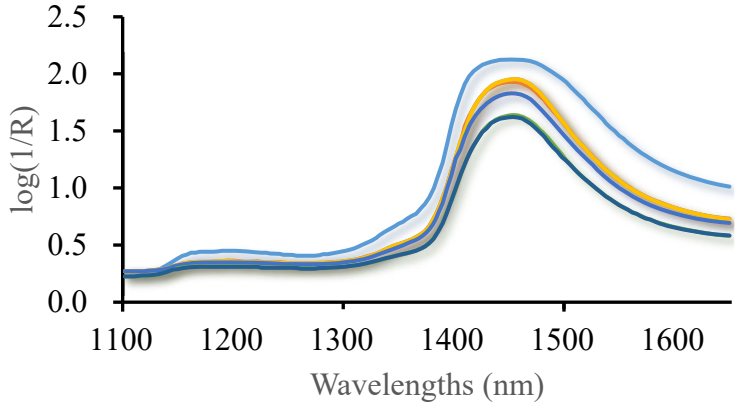

Fig. S1 Near-infrared spectroscopy (NIRS) of *Schizochytrium* sp. cultivation broth from different mutants.

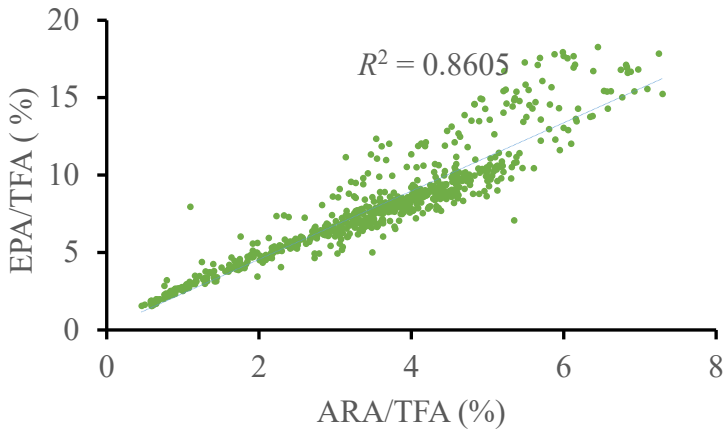

Fig. S2 Correlation between eicosapentaenoic acid (EPA)/total fatty acid (TFA) and arachidonic acid (ARA)/TFA of the mutants of all mutants cultured in a 48-deep-well plate.

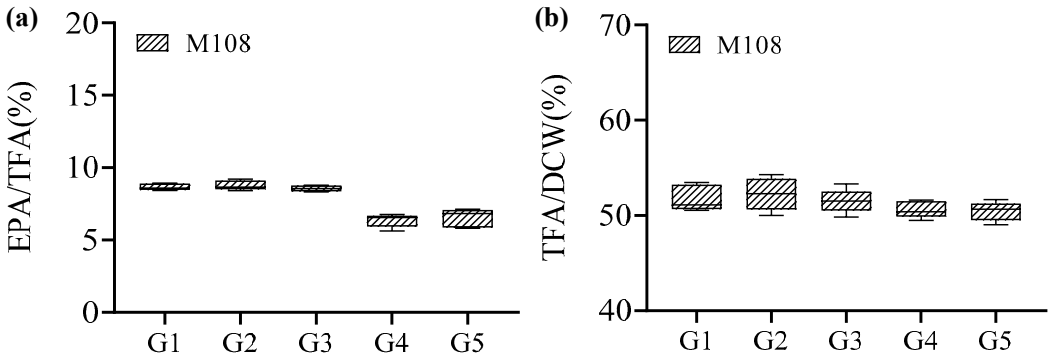

Fig. S3 Stability test of (a) eicosapentaenoic acid (EPA)/total fatty acid (TFA) and (b) TFA/dry cell weigh (DCW) of the mutants M108 grown across 5 generations through shake flask cultivation.

Fig. S4 The refined eicosapentaenoic acid (EPA) oil from four batches of fermentation produced by different mutants.

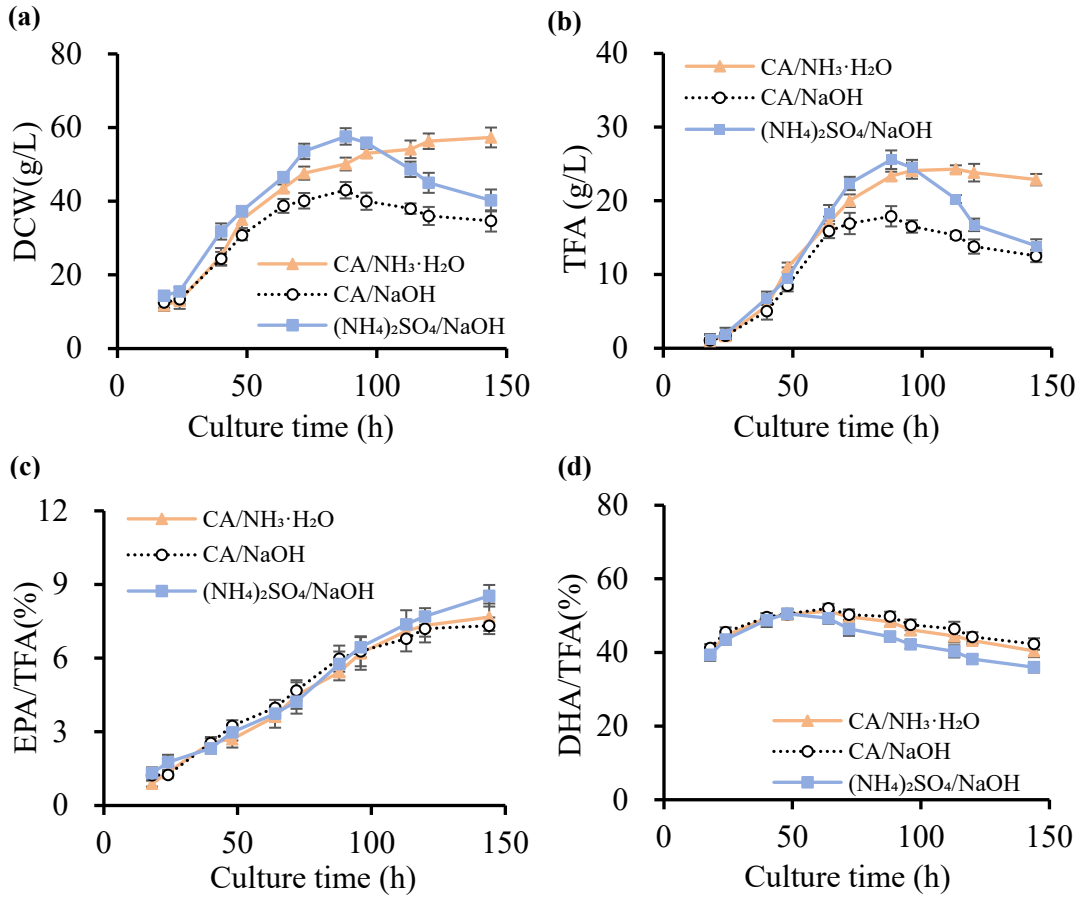

Fig. S5 Effects of different concentrations of acid-base regulators on (a) dry cell weigh (DCW); (b) total fatty acid (TFA); (c) eicosapentaenoic acid (EPA)/TFA and (d) docosahexaenoic acid (DHA)/TFA in 7 L fermentation of M7-25, which combinations including:(1) citric acid (CA)+ammonia water, (2) CA+sodium hydroxide, and (3) sodium hydroxide+ammonium sulfate.

Table S1 Time cost of fatty acids composition analysis by near-infrared spectroscopy (NIRS) compared to gas chromatography (GC) analysis. Sample pretreatment includes centrifugation, freeze-drying of wet biomass, and destruction of cell walls

Procedure	Time cost of GC (min)	Time cost of NIRS (min)
Sample pretreatment	5	0
Reaction	180	0
Detection	60	2