

Supplemental materials:

Table S1. Genotypes of *E. coli* mutant and parental strains.

Strains	Genotype
<i>ΔfabD</i> parental	<i>araC14, lacY1, tsx-57, glnX44(AS), gltA5, galK2(Oc), Rac-0, rfbC1, rpsL20(strR), xylA5, mtl-1, lldD1, thiE1, tfr-5</i>
<i>ΔfabD</i>	<i>F⁻, araC14, lacY1, tsx-57, glnX44(AS), gltA5, galK2(Oc), fabD89(ts), rpsL20(strR), xylA5, mtl-1, lldD1, thiE1, tfr-5</i>
<i>ΔtesAtesB</i> parental	<i>K-12 F⁻ λ⁻ ilvG⁻ rfb-50 rph-1</i>
<i>ΔtesAtesB</i>	<i>F⁻, ΔtesA::cat ΔtesB, λ⁻, λ⁻ ilvG⁻ rfb-50 rph-1</i>
<i>ΔfadD</i>	<i>F⁻ ompT gal dcm lon hsdSB(rB-mB-) λ(DE3 [lacI lacUV5-T7p07 ind1 sam7 nin5]) [malB+]K-12(λS) ΔfadD</i>
SHuffle	<i>F⁺ lac, pro, lacIQ / Δ(ara-leu)7697 araD139 fhuA2 lacZ::T7 gene1 Δ(phoA)</i> <i>PvuII phoR ahpC* galE (or U) galK λatt::pNEB3-r1-cDsbC (SpecR, lacIq) ΔtrxB rpsL150(StrR) Δgor Δ(malF)3</i>
BL21 (DE3)	Star <i>fhuA2 [lon] ompT gal [dcm] ΔhsdS</i>
Rosetta (DE3)	<i>F⁻ ompT hsdSB(rB- mB-) gal dcm (DE3) pRARE (CamR)</i>

Table S2. Plasmids used for AT-like domain cloning.

Plasmids	Relevant genotype	Resistance
pCDFDuet-1_ORF-B	T7 promoter, contained ORF-B of the PUFA synthase from <i>Thraustochytrium</i> sp. 26185	Streptomycin
pBAD	T7 promoter, inducible expression through glucose, glycerol and arabinose	Ampicillin
pET28a	T7 promoter. High recombinant proteins expression	Kanamycin

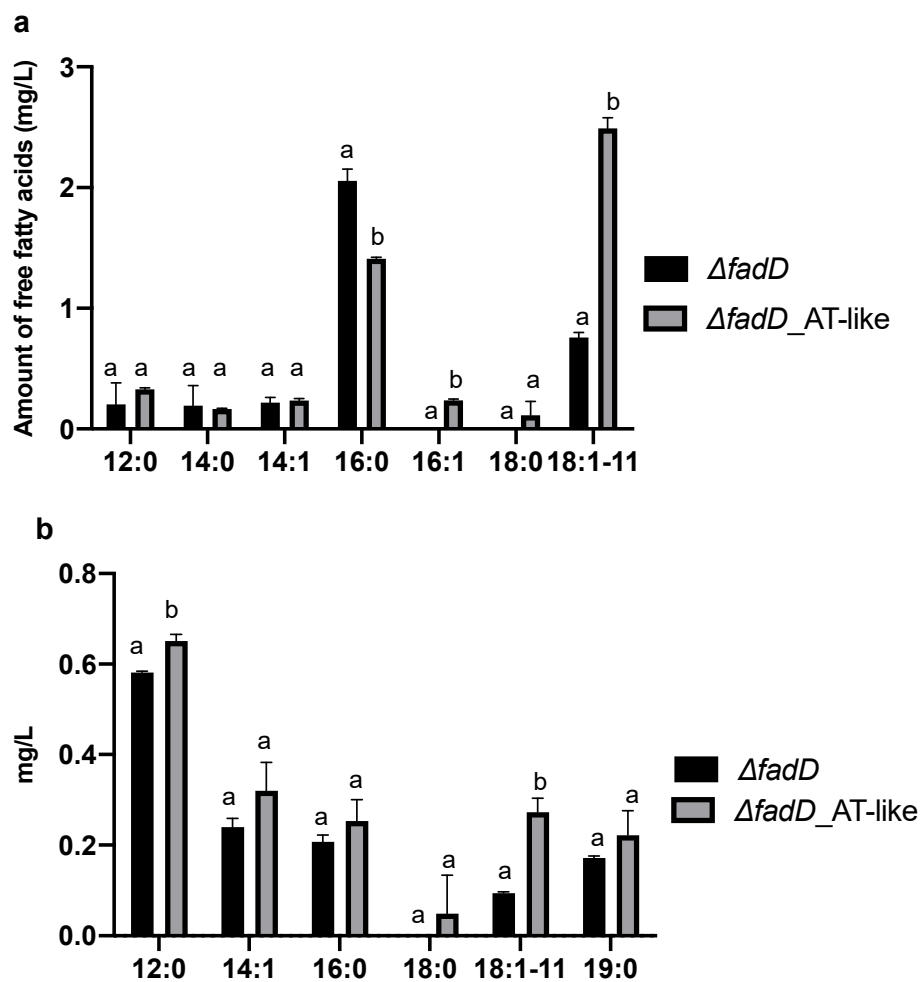


Figure S2. Free fatty acids (a) in cell pellets and (b) supernatant of the culture $\Delta fadD$ mutant with the AT-like domain. Values are reported as means \pm standard deviations for three biological replicates. Means with different letters are statistically different according to an unpaired t test ($p < 0.05$).

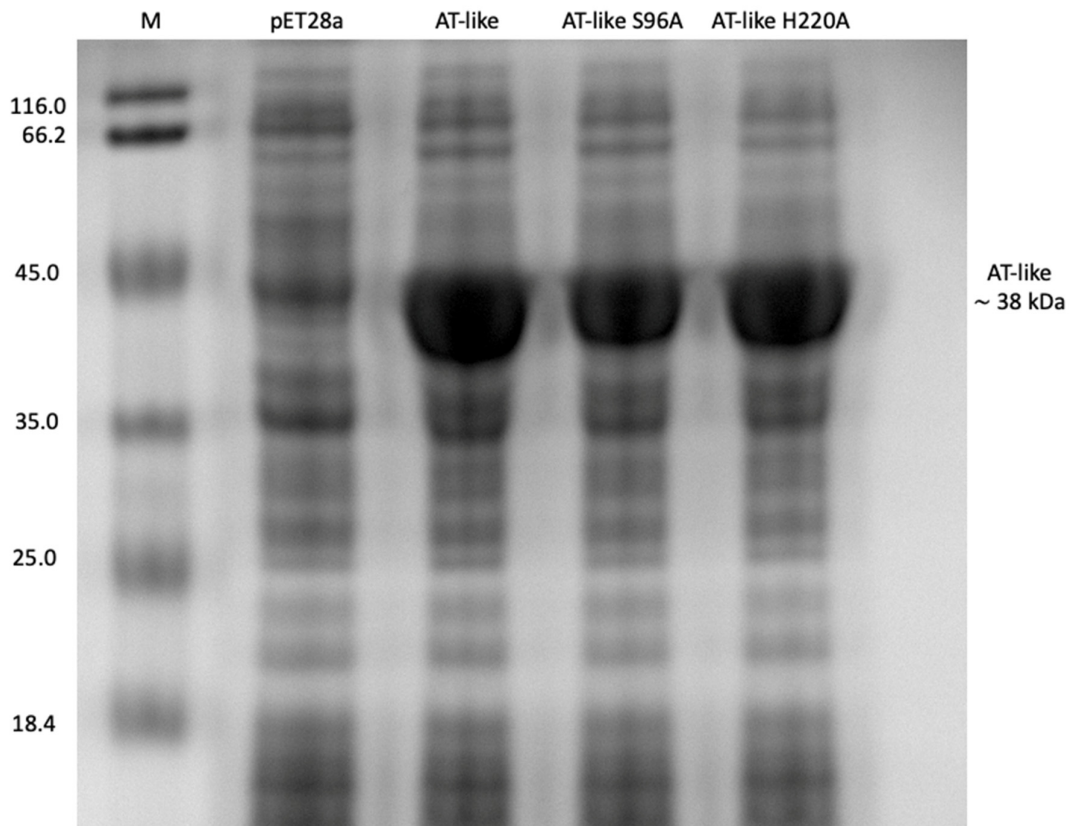


Figure S3. Expression of AT-like domain, AT-like domain S96A and AT-like domain H220A in *E. coli* Δ *fadD* mutant. M: Marker. pET28a: *E. coli* Δ *fadD* mutant with empty vector.

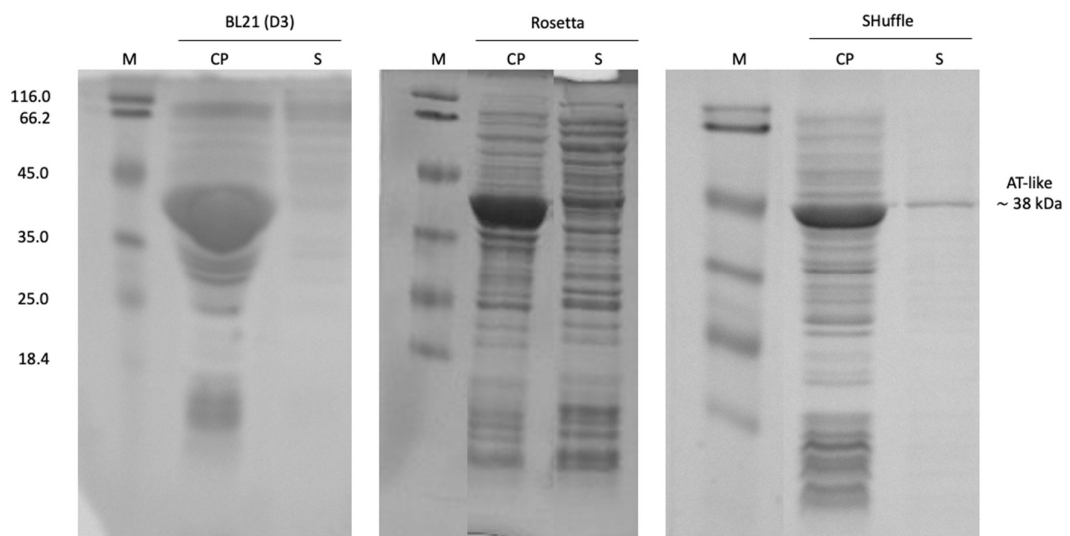


Figure S4. Expression of AT-like domain in *E. coli* BL21 (D3), Rosetta and Shuffle strains. M: Marker. CP: cell pellet (insoluble fraction). S: supernatant (soluble fraction).