Supplementary materials

Supplementary Table S1. Fatty acid composition (weight %) of yeast cells expressing genes when feeding with C16:1$^{\Delta 7}$..

Supplementary Table S2. Fatty acid composition (weight %) of yeast cells expressing genes when fed with C20:4$^{\Delta 8,11,14,17}$ or C20:4$^{\Delta 5,8,11,14}$.

Supplementary Table S3. Fatty acid composition (weight %) of yeast cells expressing CeFAT-1.

Supplementary Fig. S1. Mass spectra of products produced by CeFAT-2.
A-D are C16:2, C16:3, C18:2 and C18:3 respectively.

Supplementary Fig. S2. Mass spectra of minor FAME products or their DMOX adducts in yeast cells expressing CeFAT-2 when fed with C14:1$^{\Delta 9}$.
A. C14:2$^{\Delta 9,12}$; B. DMOX-C14:2$^{\Delta 9,12}$; C. DMOX-C16:1$^{\Delta 11}$; D. DMOX-C16:2$^{\Delta 11,14}$; E. DMOX-C18:1$^{\Delta 11}$; F. DMOX-C18:1$^{\Delta 13}$; G. DMOX-C18:2$^{\Delta 11,14}$.

Supplementary Fig. S3. Partial GC chromatogram of yeast feeding for CeFAT-2 with C15:0 and mass spectra of products.
A-C, FAMEs of yeast S288C expressing pYES2 vector only, AtFAD2 and CeFAT-2, respectively, fed with C15:0; D, DMOX-C15:1$^{\Delta 9}$; E, DMOX-C17:1$^{\Delta 9}$; F, DMOX-C15:2$^{\Delta 9,12}$; G, DMOX-C17:2$^{\Delta 9,12}$. New desaturated products from AtFAD2 or CeFAT-2 are indicated by arrows.

Supplementary Fig. S4. Mass spectra of new product of the feeding substrate C18:3$^{\Delta 6,9,12}$ by CeFAT-2.
A, Mass spectra of new peak C18:4 from CeFAT-2; B, Mass spectra of DMOX adduct of new product C18:4. The new desaturated products from AtFAD2 or CeFAT-2 are indicated by arrows.

Supplementary Fig. S5. Summary of CeFAT-2 substrates used by Δ12-desaturation and Δ15-desaturation activity. The symbol —● indicates no desaturation activity on this fatty acid, while ---> indicates low or trace activity on this fatty acid substrate.

Supplementary Fig. S6. Partial amino acid sequence alignment around His boxes between Δ12-desaturases, Δ15-desaturases and dual functional desaturases. The conserved His residues in His boxes are in bold. The GenBank accession numbers for the sequences used are, *Arabidopsis thaliana* Δ12-desaturase (AtFAD2, P46313), *Mortierella alpine* Δ12-desaturase (Ma Δ12, Q9Y8H5), *Umbelopsis isabellina* Δ12-desaturase (Ui Δ12, P59668), *Claviceps purpurea* Δ12-desaturase (Cp Δ12, ABS18716), *A. thaliana* Δ15-desaturase (AtFAD3, P48623), *Caenorhabditis elegans* Δ15-desaturase (CeFAT-1, L41807), *C. elegans* Δ12-desaturase (CeFAT-2, AF240777), *C. purpurea* Δ12/Δ15 bifunctional desaturase (Cp Δ12/15, ABS18717), *Fusarium moniliforme* Δ12/Δ15 bifunctional desaturase (Fm Δ12/15, DQ271516), *Magnaporthe grisea* Δ12/Δ15 bifunctional desaturase (Mg Δ12/15, XP_362963), *Aspergillus nidulans* Δ12/Δ15 bifunctional desaturase (An Δ12/15, XP_664808), Acanthamoeba castellanii Δ12/Δ15 bifunctional desaturase (Ac Δ12/15, ABK15557), *Sorghum bicolor* Δ12/Δ15 bifunctional desaturase (Sb Δ12/15, ABN49521). These sequences are grouped as Δ12-desaturase (Δ12), Δ15-desaturase (Δ15) and bifunctional desaturase (bi). The two critical residues of *C. purpurea* Δ12/Δ15 bifunctional desaturase, V152 and A206,

which were shown to contribute to regiospecificity of the enzyme (Meesapyodsuk *et al.* 2007, J. Biol. Chem. 282:20191-20199) are in red. The residues in CeFAT-2 that may potentially be important to the Δ15-desaturation in addition to Δ12-desaturation activity are in blue.