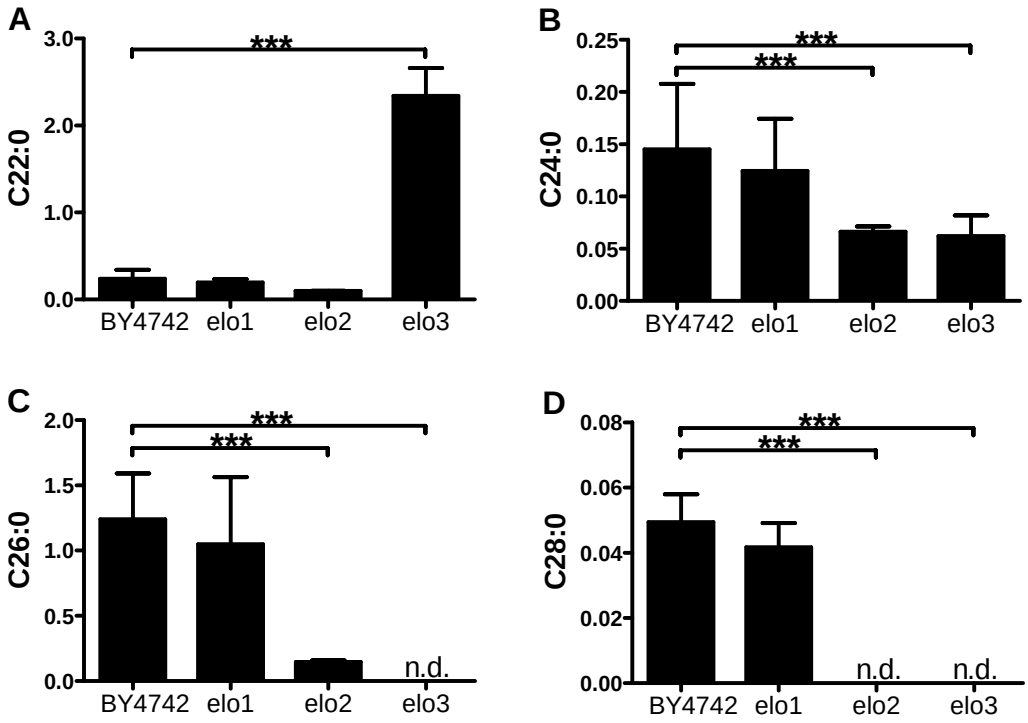
Supplemental data

Fig S1: Disruption of Elo3p in *Saccharomyces cerevisiae* blocks synthesis of VLCFA



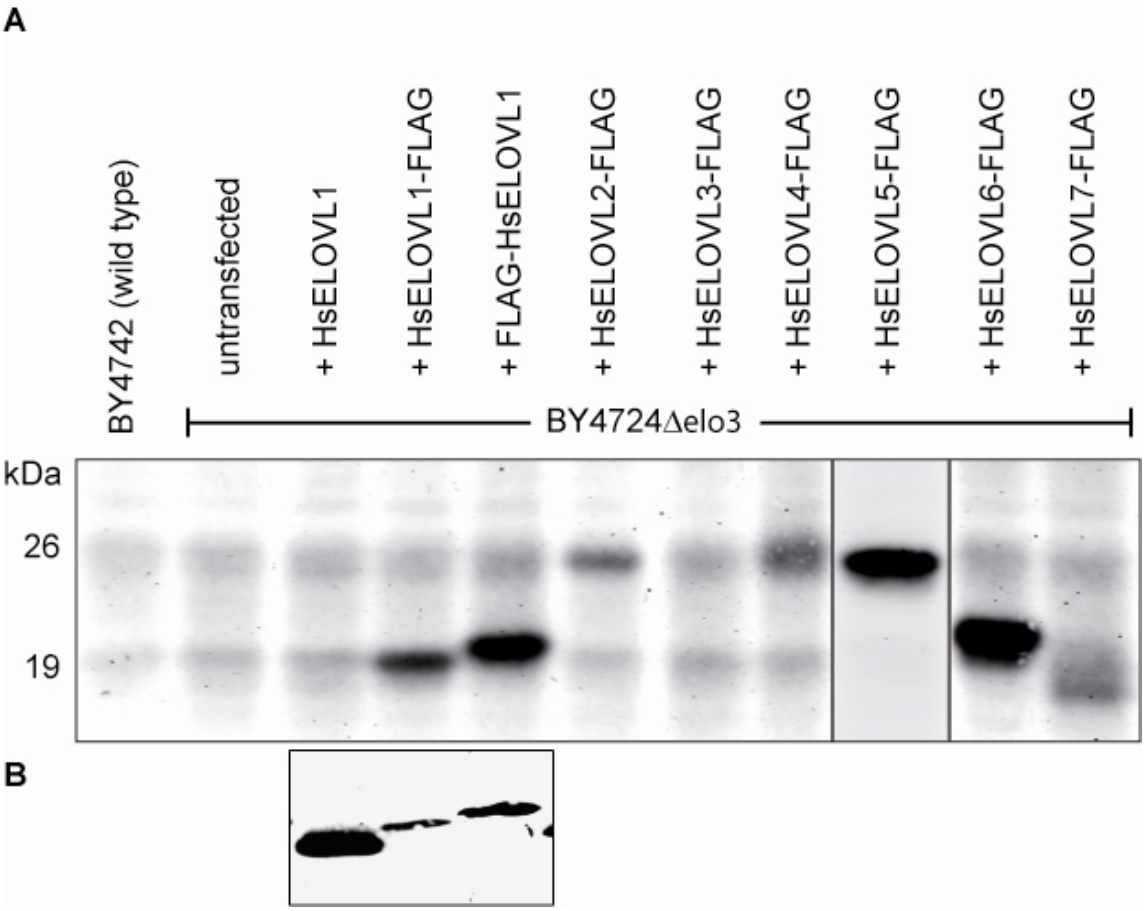
VLCFA analysis in wild type yeast and elongase deletion mutants. VLCFA analysis of wild type (BY4742) yeast and BY4742Δ*elo1*, BY4742Δ*elo2*, BY4742Δ*elo3* strains. (A) C22:0, (B) C24:0, (C) C26:0 and (D) C28:0. Fatty acid concentrations are in nmol/OD600 = 1. Data are mean ± SD. n.d. = not detectable. *** = $P < 0.001$ by ANOVA followed by Dunnett's multiple comparison test compared with wild type.

In S. cerevisiae, three elongases (Elo1p, Elo2p and Elo3p) have been identified and characterized (Oh et al., 1997; Toke and Martin, 1996). Fatty acid analysis of the wild type strain (BY4742) and the Elo1p, Elo2p and Elo3p deletion strains (BY4742Δ*elo1*, BY4742Δ*elo2* and BY4742Δ*elo3*, respectively) confirmed previous reports regarding the involvement of the Elo1p, Elo2p and Elo3p in fatty acid synthesis in yeast (Supplemental Figure 1). The BY4742Δ*elo1* deletion strain had a 3-fold higher level of C14:0 and a 25% lower amount of C16:0 compared to wild type yeast (data not shown), which is in line with the involvement of Elo1p in the elongation of C14:0 to C16:0 fatty acids (Toke and Martin, 1996). The BY4742Δ*elo2* cells had reduced levels of C24:0, C26:0 and C28:0 (Figures B-D) confirming the role of Elo2p in the elongation of fatty acids up to C24:0 (Oh et al., 1997). However, only the BY4742Δ*elo3* knockout strain had no detectable C26:0 (Figure 3C) and C28:0 (Figure D) and a 10-fold increase in C22:0 levels (Figure A) confirming that Elo3p is the elongase primarily involved in the synthesis of C26:0 from C22:0 in yeast (Oh et al., 1997).

References:

Oh CS, Toke DA, Mandala S, Martin CE. 1997. J Biol Chem 272:17376-17384.
Toke DA, Martin CE. 1996. J Biol Chem 271:18413-18422.

Fig S2: Protein expression analysis of BY4742Δ*elo3* yeasts expressing FLAG-tagged ELOVL proteins.



Protein expression analysis of BY4742Δ*elo3* yeasts expressing FLAG-tagged ELOVL proteins. The blot was probed with anti-FLAG M2 antibody (A), or human anti-ELOVL1 antibody (B). For each sample, 50 µg yeast homogenate was loaded, except for BY4742Δ*elo3* expressing FLAG-tagged ELOVL5 for which 10 µg was loaded (insert supplemental Figure 1A).

A C-terminal FLAG-tag was introduced to the seven human ELOVL proteins in order to confirm their expression at the protein level by Western blot analysis using anti-FLAG M2 antibody (Figure A). This indirect approach was chosen due to the lack of commercially available antibodies targeting human elongase 2-7. However, an antibody targeting human ELOVL1 enabled direct assessment of ELOVL1 protein expression. Interestingly, introduction of a FLAG-tag was shown to markedly reduce ELOVL1 protein expression (Figure B). The above-mentioned indirect method may thus have resulted in an underestimation of the expression of mammalian elongases in transfected BY4742Δ*elo3* yeast strains. This could explain why expression of FLAG-tagged ELOVL3 was not confirmed on Western blot analysis. The latter finding was unexpected as transformants expressing ELOVL3 had increased C22:1 and C24:1 levels when grown on galactose medium (Table I).

The apparent molecular weights of FLAG-tagged ELOVL1, 6 and 7 were approximately 20 kDa and thus significantly lower than the predicted molecular weights based on their primary amino acid sequences which are 32.6, 31.4 and 33.3 kDa, respectively. To

investigate whether this could be the result of proteolytic cleavage, we also expressed ELOVL1 with a N-terminal FLAG-tag. Based on the results of the Western blot analysis as shown in Supplemental Figure 1B, we conclude that as both N-terminal and C-terminal FLAG tags were present, ELOVL1 is expressed as a full-length protein without proteolytic degradation. It remains to be investigated whether this low apparent molecular weight by SDS-PAGE analysis is due to post-translational modification.

Supplemental Table SI

Sequences of the primers used for cloning of the seven human elongases, yeast ELO3 and for quantitative RT-PCR analysis.

Primer	**Sequence 5' → 3'**
ELOVL1F	AAGCTT**ATG**GAGGCTGTTGTGAAC
ELOVL1F-FLAG	AAGCTT**ATG***GATTACAAGGATGACGACGATAAG*GAGGCTGTTGTGAACTTG
ELOVL1R	GAATTC**TCA**GTTGGCCTTGACCTTGG
ELOVL1R-FLAG	GAATTC**CTA***CTTATCGTCGTCATCCTTGTAATC*GTTGGCCTTGACCTTGGC
ELOVL2F	AAGCTT**ATG**GAACATCTAAAGGC
ELOVL2R	GAATTC**TTA**TTGTGCTTTCTTGTTC
ELOVL2R-FLAG	GAATTC**CTA***CTTATCGTCGTCATCCTTGTAATC*TTGTGCTTTCTTGTTC
ELOVL3F	GGATCC**ATG**GTCACAGCCATGAATG
ELOVL3R	GAATTC**TCA**CTGGCTCTTGGTCTTGG
ELOVL3R-FLAG	GAATTC**CTA***CTTATCGTCGTCATCCTTGTAATC*CTGGCTCTTGGTCTTGG
ELOVL4F	AAGCTT**ATG**GGGCTCCTGGACTCG
ELOVL4R	GGATCC**TTA**ATCTCCTTTTGCTTTTCC
ELOVL4R-FLAG	GGATCC**CTA***CTTATCGTCGTCATCCTTGTAATC*ATCTCCTTTTGCTTTTCC
ELOVL5F	AAGCTT**ATG**GAACATTTTGATGCATC
ELOVL5R	GAATTC**TCA**ATCCTTCCGCAGCTTC
ELOVL5R-FLAG	GAATTC**CTA***CTTATCGTCGTCATCCTTGTAATC*ATCCTTCCGCAGCTTC
ELOVL6F	AAGCTT**ATG**AACATGTCAGTGTTGAC
ELOVL6R	GGATCC**CTA**TTCAGCTTTCGTTGTTTTC
ELOVL6R-FLAG	GGATCC**CTA***CTTATCGTCGTCATCCTTGTAATC*TTCAGCTTTCGTTGTTTTC
ELOVL7F	AAGCTT**ATG**GAAAAGCCCATTAATATTC
ELOVL7R	GGATCC**TCA**ATTATCTTTGTTTTTGC
ELOVL7R-FLAG	GGATCC**CTA***CTTATCGTCGTCATCCTTGTAATC*ATTATCTTTGTTTTTGC
ELOVL1-31F	GTGATGAAGCACGCAGATCC
ELOVL1-330R	CACCCGAACCATCCTAAGTG
ScELO3F	GGATCC**ATG**AACACTACCACATCTACT
ScELO3R	GAATTC**TTA**AGCTTTCCTGGAAGAGAC
ScELO3R-FLAG	GAATTC**CTA***CTTATCGTCGTCATCCTTGTAATC*AGCTTTCCTGGAAGAGAC
36B4-F	ACGGGTACAAACGAGTCCTG
36B4-R	GCCTTGACCTTTTCAGCAAG

The initiator (ATG) methionine codon and the stop codons are presented by bold nucleotides; the FLAG tag (DYKDDDDK) is presented by underlined/italic nucleotides and the restriction sites used for cloning are presented by double underlined nucleotides. The forward primer introduced a restriction site immediately upstream of the ATG initiator codon and the reverse primer immediately downstream of the stop codon.