

1 **Figure S1. Optimization of *EaDacT* activity in vitro.**

2 (A) Quantification of acetyl-TAG produced in vitro after incubation of microsomes  
3 containing *EaDacT* with [1-<sup>14</sup>C] acetyl-CoA for 30 minutes at different temperatures. (B)  
4 Quantification of acetyl-TAG produced in vitro after incubation of *EaDacT* containing  
5 microsomes at different pH. (C) Quantification of acetyl-TAG produced in vitro after  
6 incubation of different amounts of *EaDacT* microsomes with [1-<sup>14</sup>C] acetyl-CoA. The  
7 trend line was fitted using linear regression. (D) Quantification of acetyl-TAG produced  
8 in vitro after *EaDacT* microsomes were incubated with [1-<sup>14</sup>C] acetyl-CoA for different  
9 amounts of time. The trend line was fitted using non-linear regression (GraphPad  
10 Prism).

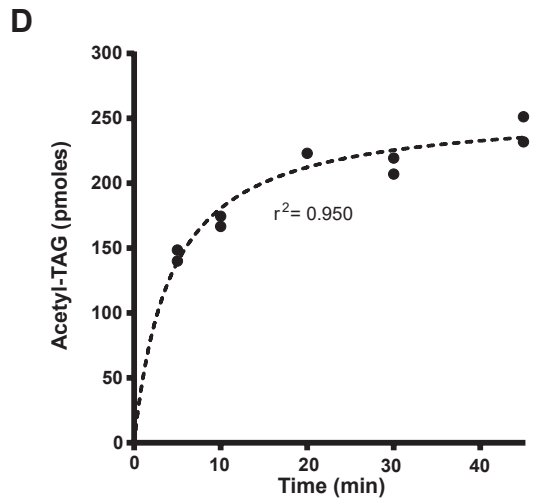
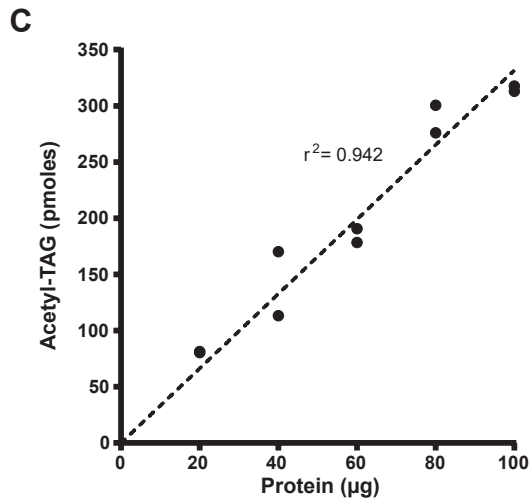
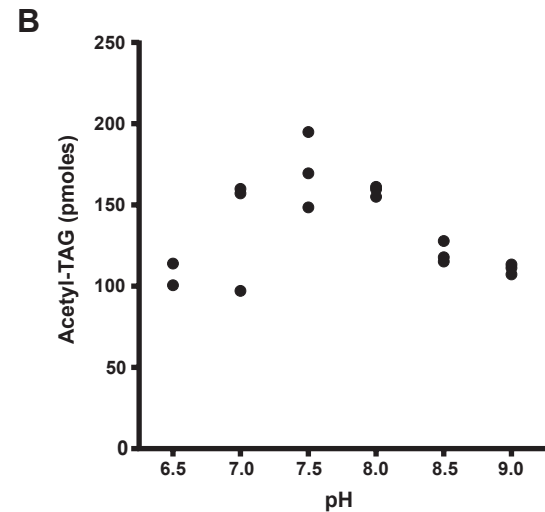
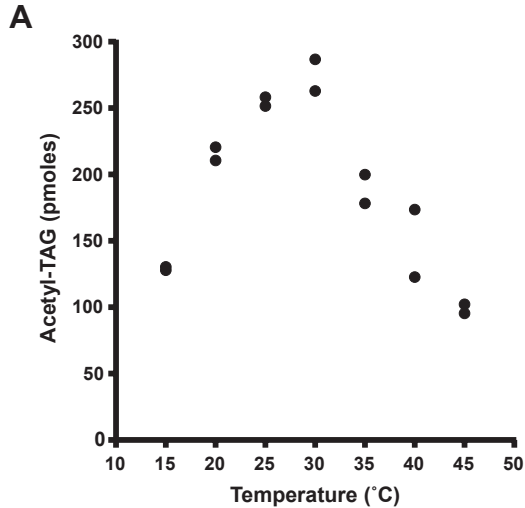
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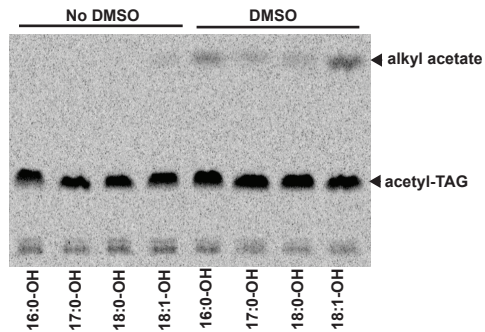
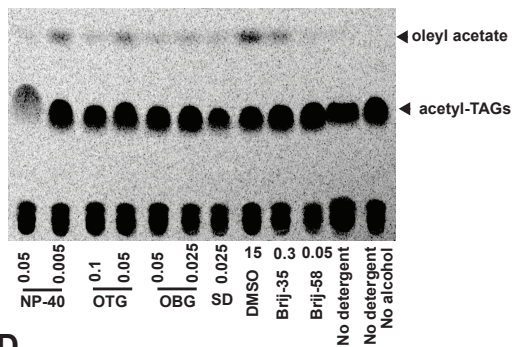
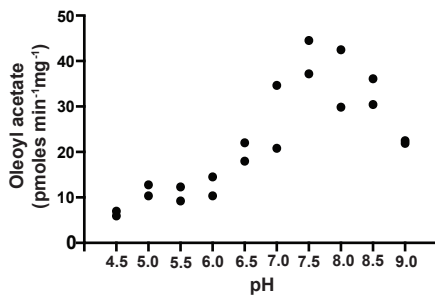
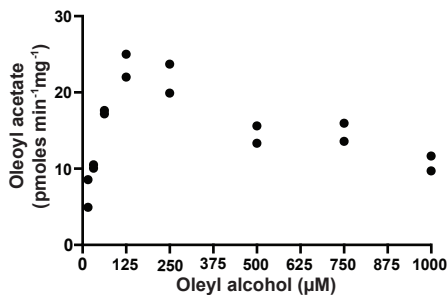
12 **Figure S2. Optimization of in vitro wax synthase assay conditions for *EaDacT*.**

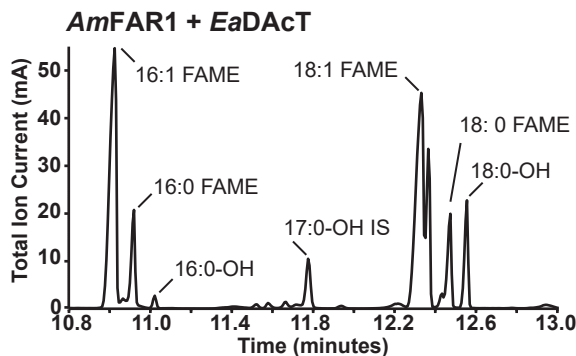
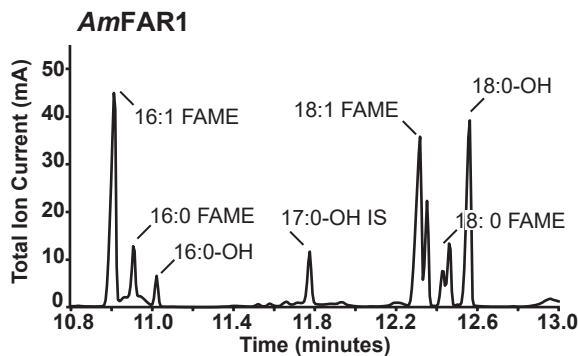
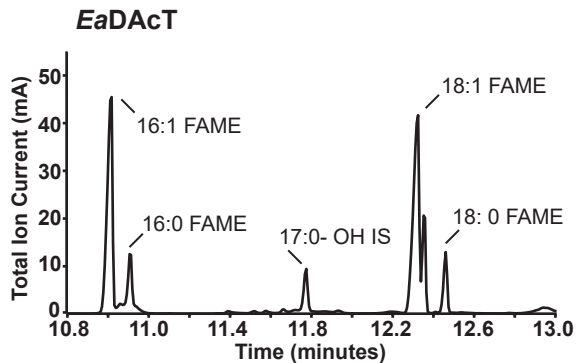
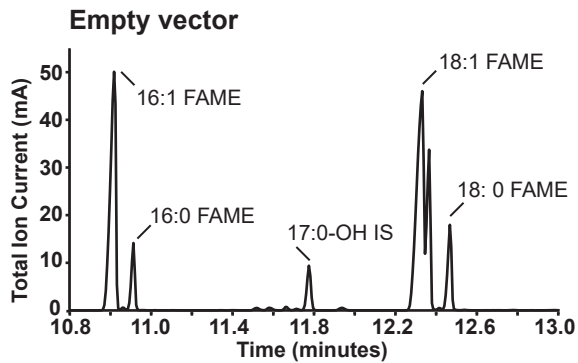
13 (A) Autoradiogram of TLC separation of total lipids extracted from microsomes  
14 containing *EaDacT* incubated with [1-<sup>14</sup>C] acetyl-CoA, different fatty alcohols (250 μM  
15 each) and with or without DMSO. Fatty alcohols are denoted using x:y where x indicates  
16 the number of carbons and y the number of double bonds. (B) Autoradiogram of TLC  
17 separation of total lipids extracted microsomes containing *EaDacT* incubated with [1-  
18 <sup>14</sup>C] acetyl-CoA, 125 μM oleyl alcohol and different detergents. NP-40,  
19 nonyl phenoxypolyethoxyethanol-40; OTG, octyl beta-thio-glucoside; OBG, octyl-beta-  
20 glucoside; SD, sodium deoxycholate. (C) Quantification of alkyl acetate production from  
21 microsomes containing *EaDacT* incubated with [1-<sup>14</sup>C] acetyl-CoA and oleyl alcohol  
22 under different pH conditions. (D) Quantification of alkyl acetate production from  
23 microsomes containing *EaDacT* incubated with [1-<sup>14</sup>C] acetyl-CoA and with different  
24 concentrations of oleyl alcohol.

25

26 **Figure S3. Yeast expressing *AmFAR1* produce fatty alcohols in vivo.** (A) GC-MS  
27 chromatograms of fatty alcohols purified from H1246 yeast expressing the empty vector  
28 pESC-URA or combinations of *AmFAR1* and *EaDacT*. (B) Quantification of fatty alcohols  
29 from (A). Values represent mean ± SD for three biological replicates.



**A****B****C****D**

**A****B**