## **Supplemental Figure Legends**

Fig. S1. GC-MS analysis of DMOX derivatives of the fatty acid (peak 6 in Fig. 3E) produced by the mutant D6d-mut8. D6d-mut8 was expressed in yeast in the presence of DGLA, and total cellular lipids were transmethylated and derivatized with DMOX for GC-MS analysis. Predicted molecular mass numbers of daughter ions of ARA are shown on the structural formula.

Fig. S2. Fatty acid analysis of desaturation products of mutant D6d-25m. Null vector pYES2, intact D6d, intact D5d and D6d-25m were expressed in yeast in the presence of LA (peak 5, panels A-D) or DGLA (peak 7, panels E-H), and the fatty acid composition of total cellular lipids was analyzed by GC. Panel A, null vector + LA; B, intact D6d + LA; C, intact D5d + LA; D, D6d-25m + LA; E, null vector + DGLA; F, intact D6d + DGLA; G, intact D5d + DGLA; H, D6d-25m + DGLA. Other peaks in panels A–H are 16:0 (peak 1), 16:1  $\Delta$ 9 (peak 2), 18:0 (peak 3), 18:1  $\Delta$ 9 (peak 4), 18:3  $\Delta$ 6,9,12 (peak 6), and 20:4  $\Delta$ 5,8,11,14 (peak 8). Mass spectra of DMOX derivatives of fatty acids generated by D6d-25m expressed in yeast in the presence of LA (panel I; peak 6 in panel D) or DGLA (panel J; peak 8 in panel H). Predicted molecular masses of daughter ions of GLA and ARA are shown on the respective structural formulas in panels I and J.

Fig. S3. GC-MS analysis of DMOX derivative of the fatty acid (peak 2 in Fig. 5B) produced by mutant D6d-L323F. D6d-L323F was expressed in yeast in the presence of DGLA, and total cellular lipids were transmethylated and derivatized with DMOX for GC-MS analysis. Predicted molecular mass numbers of daughter ions of ARA are shown on the structural formula.



FID response



