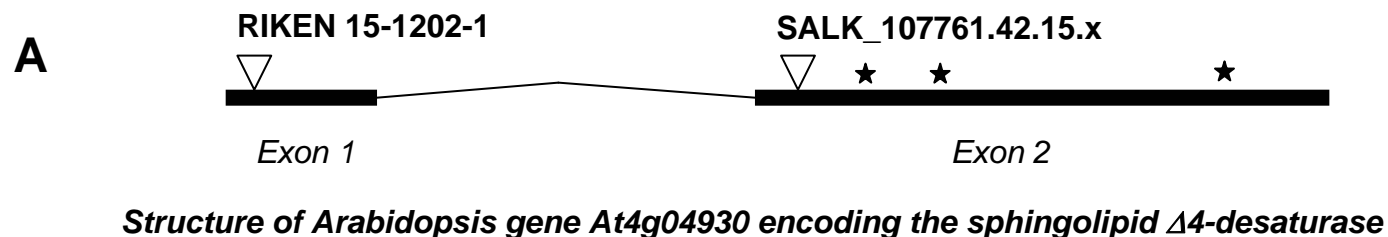


Fig. S1



B



C

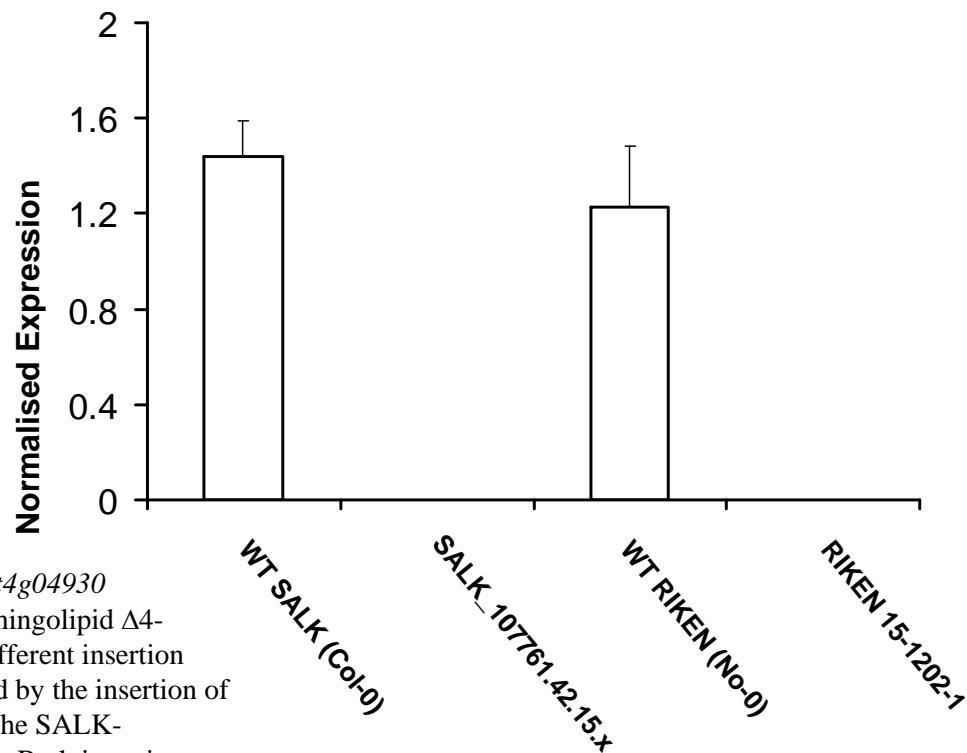


Figure S2 Identification of insertion mutants for Arabidopsis gene At4g04930

A. Genomic structure of Arabidopsis gene At4g04930 encoding the sphingolipid Δ 4-desaturase. The position of the single intron is shown, as are the two different insertion alleles described in the text. The RIKEN 15-1202-1 mutant is generated by the insertion of modified *Ds*-transposon shortly after the start of the coding sequence. The SALK-107761.42.15.x mutant results from the insertion of a modified T-DNA. Both insertions are predicted to disrupt the ORF of this gene, not least of all with respect to the critical histidine boxes required from desaturase function (indicated by solid stars).

B. Stage of floral development at which RNA was isolated for detection of transcripts derived from At4g04930.

C. Q-PCR was used to detect transcripts from WT (Col-0, No-0) and insertion mutants. Expression was normalised as described in Griffiths et al. (2006).

Fig. S2

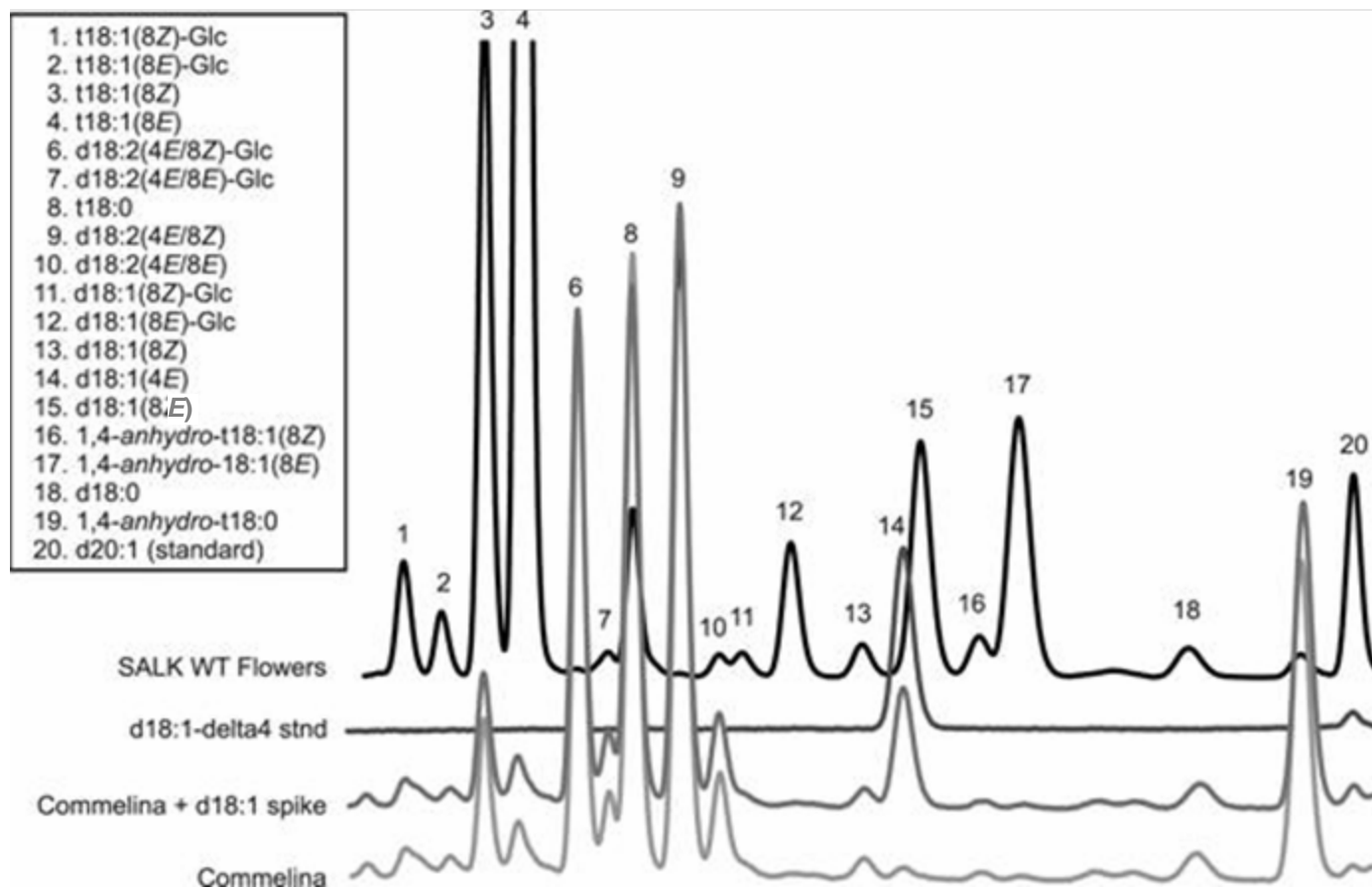
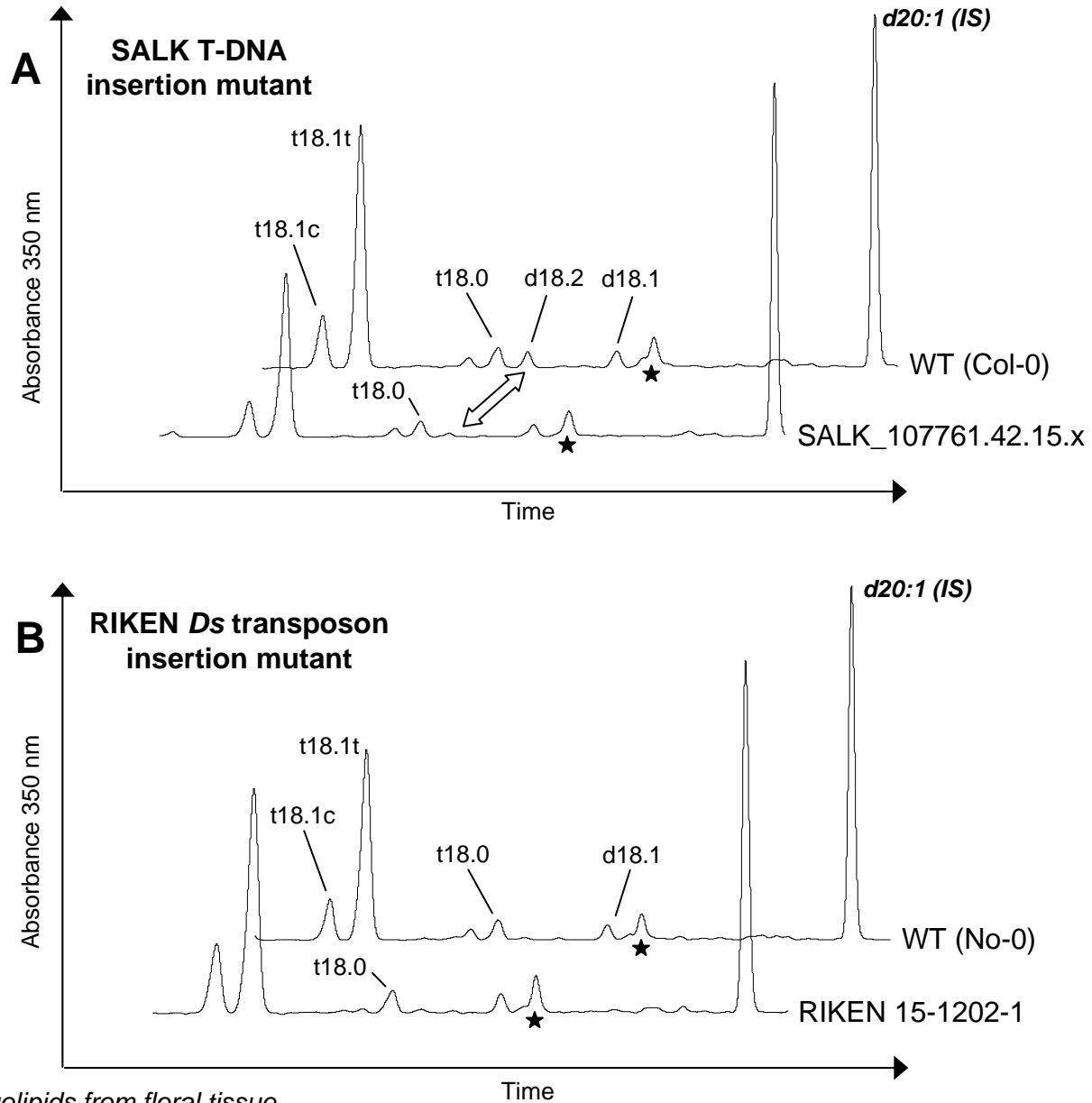


Fig. S2 HPLC separation and identification of plant LCBs.

LCBs were prepared by hydrolysis and *o*-phthalaldehyde derivitization as previously described (Markham et al. 2006). Identification of the d18:1(4*E*) LCB was confirmed in extracts from *Commelina* by spiking with synthetic d18:1(4*E*) and comparing retention times with the synthetic standard alone. The *Arabidopsis* sample contained no detectable amounts of d18:1(4*E*) and only minor amounts of d18:2(4*E*/8*Z*). LCB nomenclature and peak identification labels are described in Markham et al. 2006.

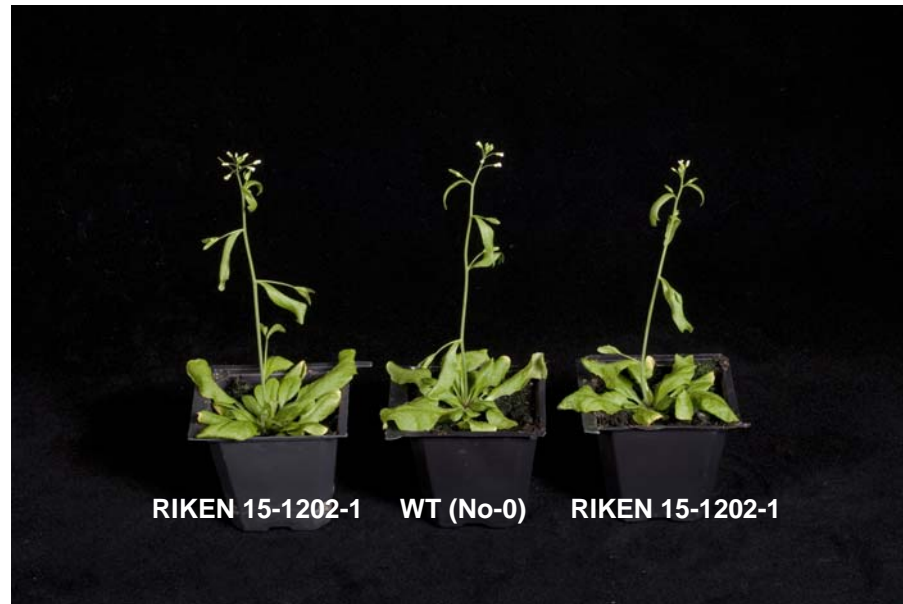
Fig S3



LCB analysis of sphingolipids from floral tissue.

Total LCBs were extracted from developing flowers (Fig 3B) and analysed by highly resolving HPLC). T-DNA insertion mutant SALK_107761.42.15 lacked any detectable Δ^4 -unsaturated LCBs compared with the WT Col-0 (the position of Δ^4 -unsaturated sphinga-4,8-diene is indicated with an open arrow). The No-0 ecotype naturally lacks this LCB (compare WT No-0 with insertion mutant RIKEN 15-1202-1). No d18:1 Δ^4 LCBs were identified in any samples, though low levels of d18:1 Δ^8 are detectable (cf. Table S2). Anhydro-LCBs are indicated with a star (see also Fig S2).

Fig. S4



RIKEN *Ds*
transposon mutant



SALK T-DNA
insertion mutant

Insertional mutagenesis of sphingolipid Δ 4-desaturase At4g04930 does not result in any gross phenotypic alteration

Table S1 *Microarray-derived tissue-specific expression of At4g04930*

| Tissue | Expression Level | Standard Deviation |
|--|-------------------------|---------------------------|
| Dry seed | 4.68 | 3.15 |
| Imbibed seed, 24 h | 3.7 | 3.18 |
| 1st Node | 3.7 | 1.81 |
| Flower Stage 12, Stamens | 1018.9 | 23.36 |
| Cauline Leaf | 5.71 | 1.39 |
| Cotyledon | 7.5 | 0.42 |
| Root | 4.66 | 1.05 |
| Entire Rosette After Transition to Flowering | 6.36 | 2 |
| Flower Stage 9 | 5.58 | 1.64 |
| Flower Stage 10/11 | 8.68 | 2.51 |
| Flower Stage 12 | 120.35 | 7.43 |
| Flower Stage 15 | 227.5 | 3.37 |
| Flower Stage 12, Carpels | 6.18 | 1.36 |
| Flower Stage 12, Petals | 40.38 | 4.63 |
| Flower Stage 12, Sepals | 26.13 | 0.94 |
| Flower Stage 15, Carpels | 55.68 | 8.92 |
| Flower Stage 15, Petals | 55.61 | 4.78 |
| Flower Stage 15, Sepals | 49.66 | 4.9 |
| Flower Stage 15, Stamen | 1225.5 | 34.03 |
| Flowers Stage 15, Pedicels | 9.35 | 1.61 |
| Leaf 1 + 2 | 6.33 | 1.5 |
| Leaf 7, Petiole | 6.81 | 4 |
| Leaf 7, Distal Half | 7.4 | 2.19 |
| Leaf 7, Proximal Half | 8.73 | 2.48 |
| Hypocotyl | 4.03 | 0.45 |
| Root | 5.33 | 1.69 |
| Rosette Leaf 2 | 7.06 | 0.61 |
| Rosette Leaf 4 | 6.25 | 0.56 |
| Rosette Leaf 6 | 11.45 | 1.47 |
| Rosette Leaf 8 | 6.65 | 0.31 |
| Rosette Leaf 10 | 9.25 | 1.24 |
| Rosette Leaf 12 | 6.08 | 1.55 |
| Senescing Leaf | 5.05 | 1.76 |
| Shoot Apex, Inflorescence | 4.9 | 1.82 |
| Shoot Apex, Transition | 3.28 | 0.42 |
| Shoot Apex, Vegetative | 3.01 | 3.45 |
| Stem, 2nd Internode | 7.26 | 3.24 |
| Mature Pollen | 6802.68 | 239.51 |
| Seeds Stage 3 w/ Siliques | 256.36 | 0.51 |
| Seeds Stage 4 w/ Siliques | 25.4 | 2.02 |
| Seeds Stage 5 w/ Siliques | 24.06 | 1.65 |
| Seeds Stage 6 w/o Siliques | 9.21 | 2.21 |
| Seeds Stage 7 w/o Siliques | 10.91 | 1.88 |
| Seeds Stage 8 w/o Siliques | 11.98 | 3.03 |
| Seeds Stage 9 w/o Siliques | 13.73 | 2 |
| Seeds Stage 10 w/o Siliques | 8.6 | 2.19 |
| Vegetative Rosette | 5.01 | 3.13 |

Data extracted from Arabidopsis eFP browser at bar.utoronto.ca

Table S2

| nmol g dw ⁻¹ | Col-0 Floral | SALK_107781 Floral | Col-0 Leaf | SALK_107781 Leaf | No-0 Floral | RIKEN 15- 1202-1 Floral | No-0 Leaf | RIKEN 15- 1202-1 Leaf |
|-------------------------|-----------------|-----------------------|---------------|---------------------|----------------|-------------------------------|--------------|-----------------------------|
| t18:1(Z) | 292 | 309 | 204 | 216 | 290 | 283 | 237 | 254 |
| t18:1(E) | 970 | 978 | 601 | 639 | 919 | 888 | 743 | 829 |
| t18:0 | 77 | 81 | 121 | 115 | 95 | 88 | 104 | 127 |
| d18:2(E/Z) | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| d18:2(E/E) | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| d18:1(Z) | 26 | 26 | 18 | 17 | 28 | 22 | 11 | 16 |
| d18:1(E) | 186 | 200 | 111 | 110 | 172 | 159 | 72 | 92 |
| d18:0 | 19 | 22 | 31 | 26 | 23 | 21 | 13 | 19 |
| TOTAL | 1594 | 1615 | 1087 | 1124 | 1527 | 1461 | 1180 | 1337 |
| mol% | | | | | | | | |
| t18:1(Z) | 18 | 19 | 19 | 19 | 19 | 19 | 20 | 19 |
| t18:1(E) | 61 | 61 | 55 | 57 | 60 | 61 | 63 | 62 |
| t18:0 | 5 | 5 | 11 | 10 | 6 | 6 | 9 | 9 |
| d18:2(E/Z) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| d18:2(E/E) | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| d18:1(Z) | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 1 |
| d18:1(E) | 12 | 12 | 10 | 10 | 11 | 11 | 6 | 7 |
| d18:0 | 1 | 1 | 3 | 2 | 2 | 1 | 1 | 1 |
| TOTAL | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% |

Table S2. LCB analysis of wild-type and sphingolipid $\Delta 4$ -desaturase mutants.

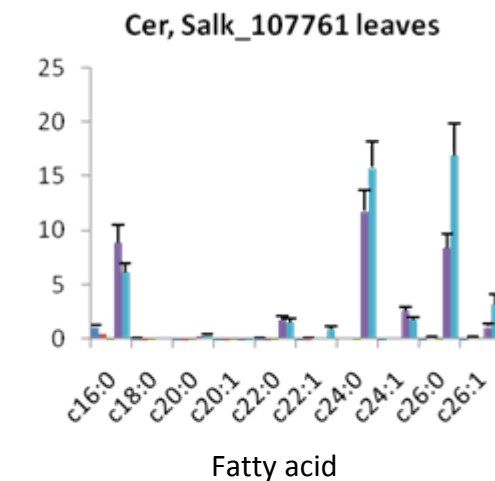
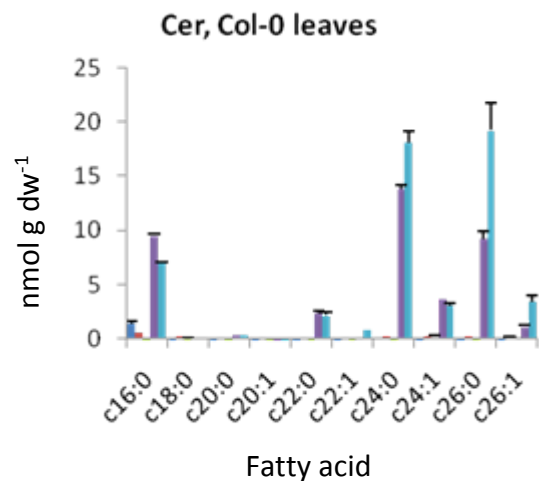
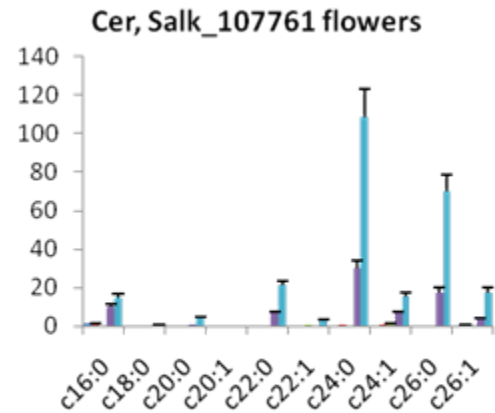
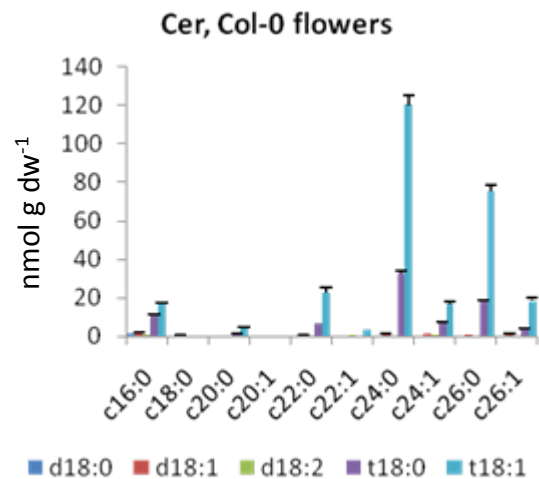
Tissue from each genotype was hydrolyzed in the presence of 1nmol synthetic d20:1 internal standard and the LCB content measured by *o*-phthalaldehyde derivatization, HPLC separation and fluorescence detection as previously described (Markham et al. 2006). $\Delta 4$ -desaturated LCBs were only detected in Col-0 sample derived from floral tissues, and then only as d18:2 species. No d18:2 was detected in samples from the SALK mutant line and no d18:2 was detectable in leaf tissue or any of the samples in the No-0 background.

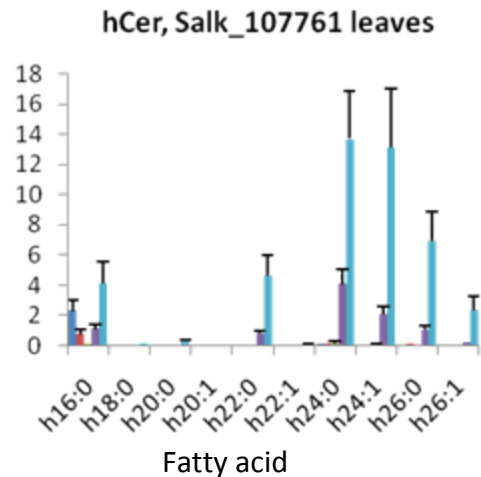
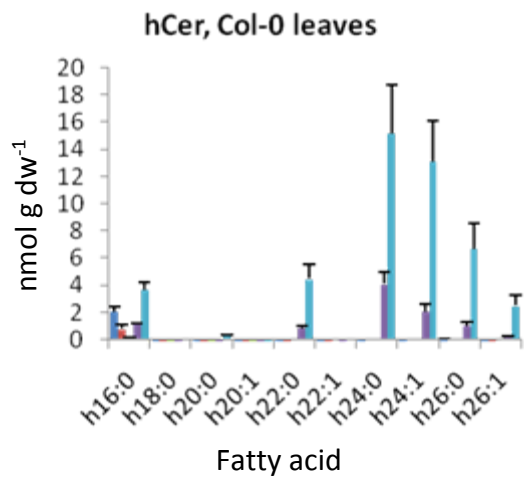
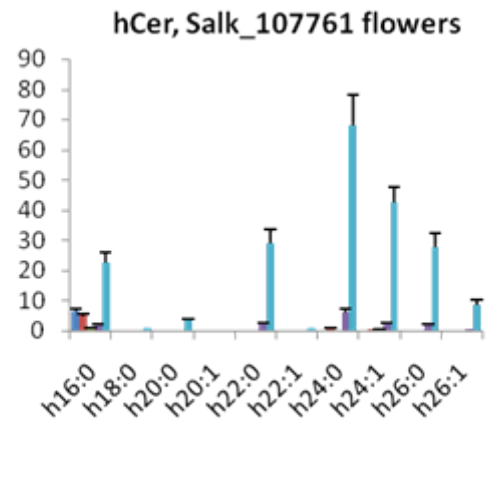
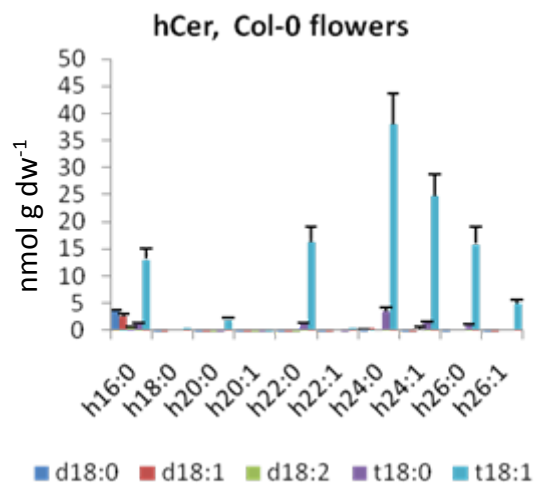
Table S3

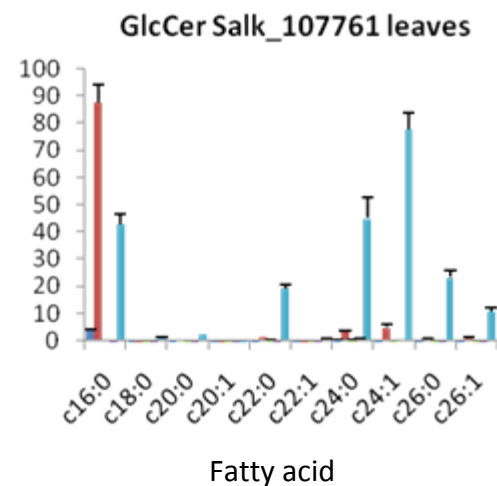
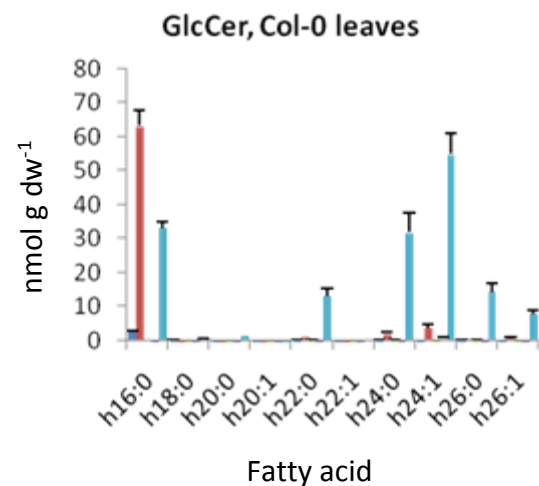
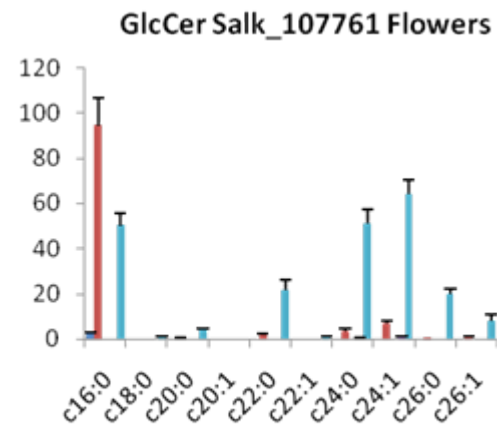
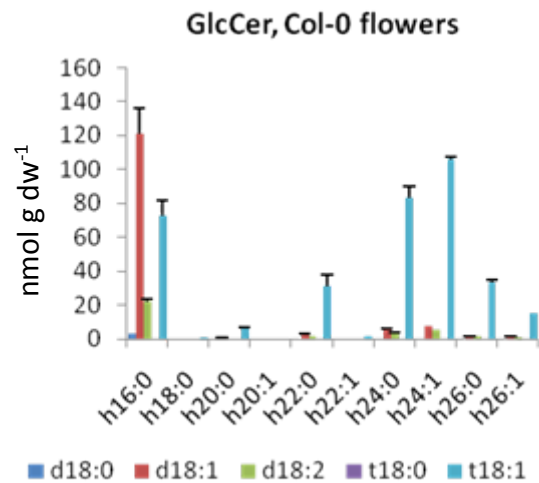
PCR Primers used to generate the P. pastoris sphingolipid $\Delta 4$ -desaturase knockout strain and to express the sphingolipid $\Delta 4$ -desaturase gene of A. thaliana in P. pastoris.

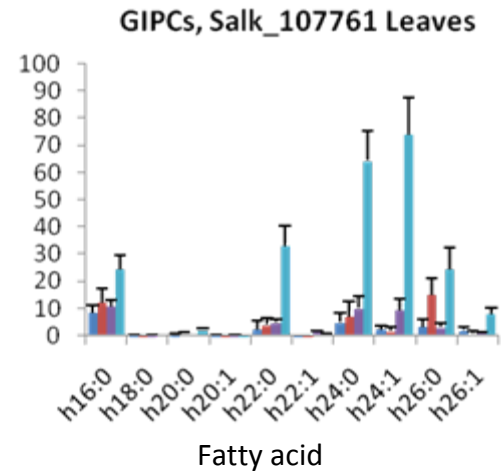
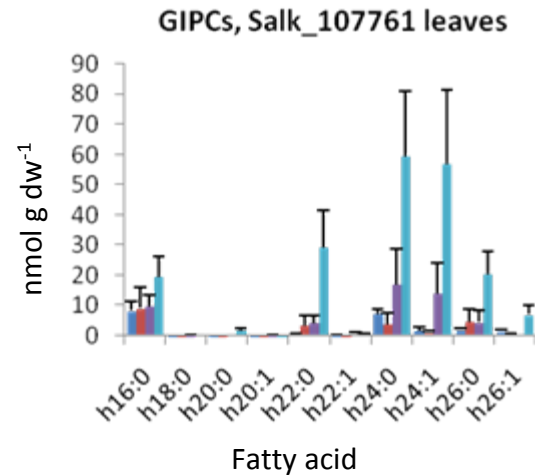
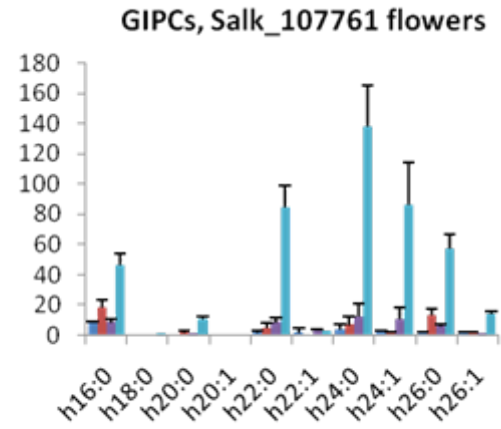
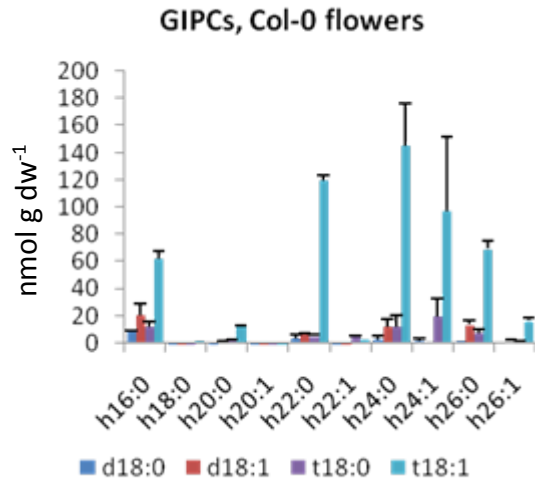
| Primer Name | Sequence (5' → 3') |
|------------------|--|
| Delta4-F-EcoRI | <u>GGAATTC</u> CAGTTCAGATCTTAGATACACATG |
| Delta4-R-HindIII | CCATGGGA <u>AAGCTT</u> GTACTCTTCACCATACTGCCC |
| Delta4-Test-F | ATCTCCTGCGACGTCTTAATG |
| Delta4-Test-R | GTCGCCTTTGGTAACCTGAAG |
| Zeo-F-XbaI | GCTCTAGACACACACCATAGCTTCAAAATG |
| Zeo-R-XmnI | <u>GAACAAATTC</u> CCAGCTTGCAAATTAAGCCTTC |
| Zeo-int-F | CGGCATAGTATATCGGCAT |
| Zeo-int-R | ATGCCGATATACTATGCCG |
| d4At-F | <u>GAATTC</u> ATTATGGGGAAAGGAGGACGTGAG |
| d4At-R | <u>GCGGCCGCTT</u> AGTCAGACTTTGAGAGCTTCC |

Supplementary Data 1: Spingolipid analysis

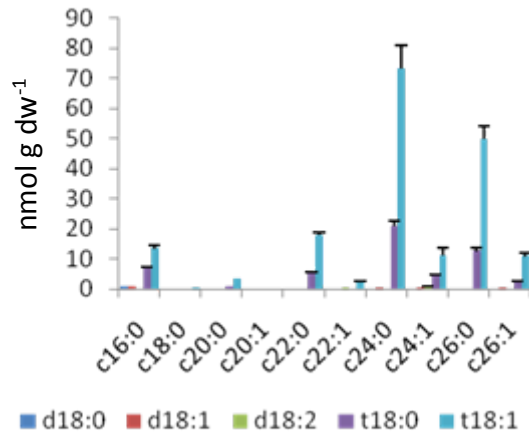




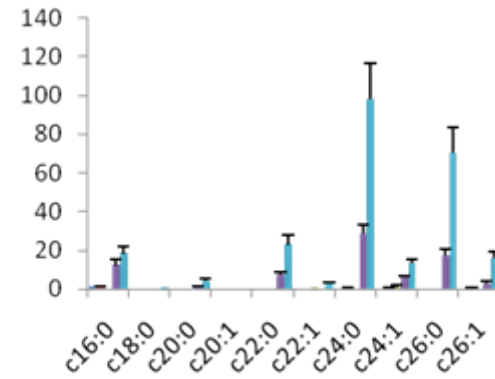




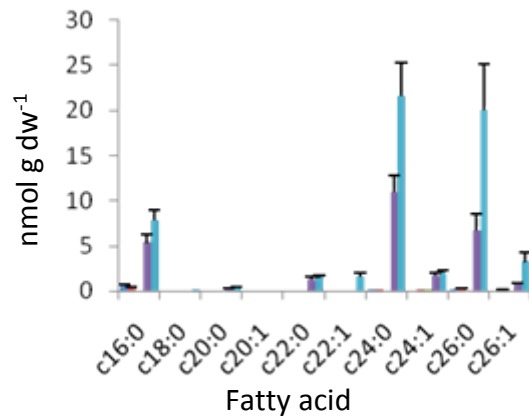
Cer, No-0 flowers



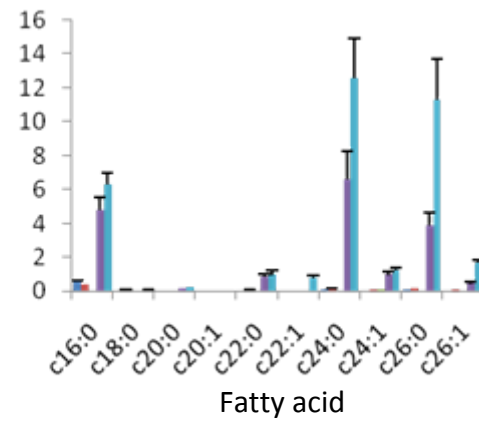
Cer, Riken 15-1202-1 flowers

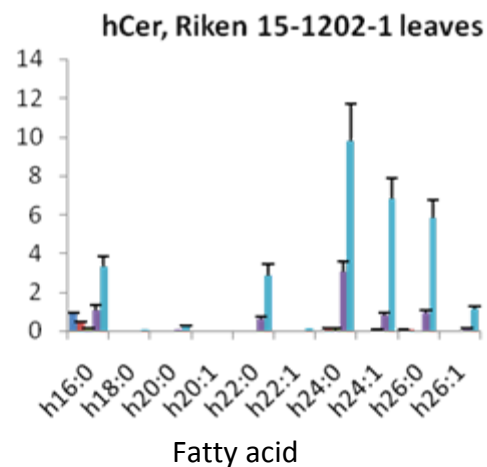
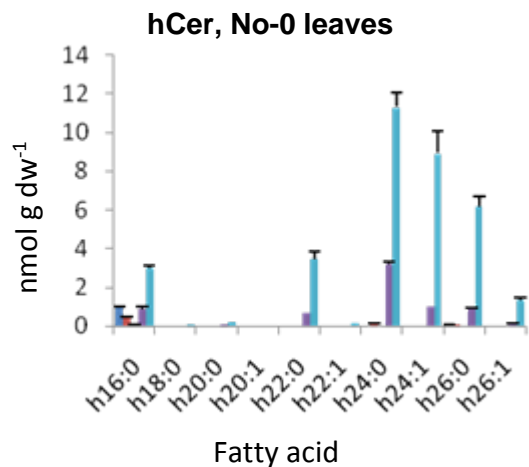
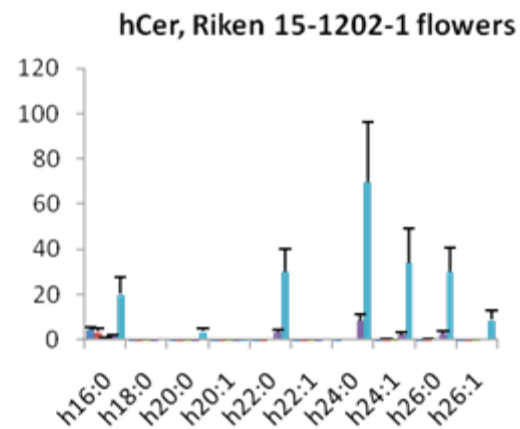
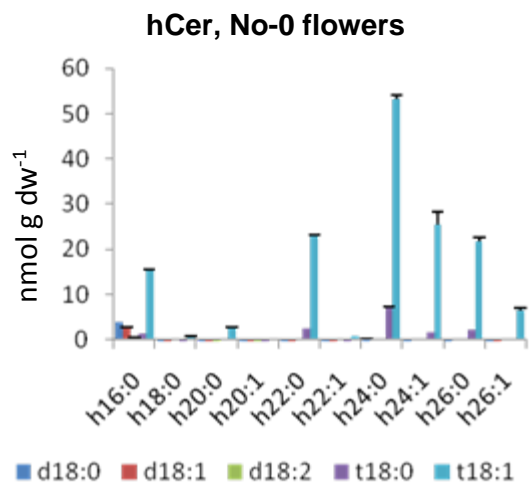


Cer, No-0 leaves

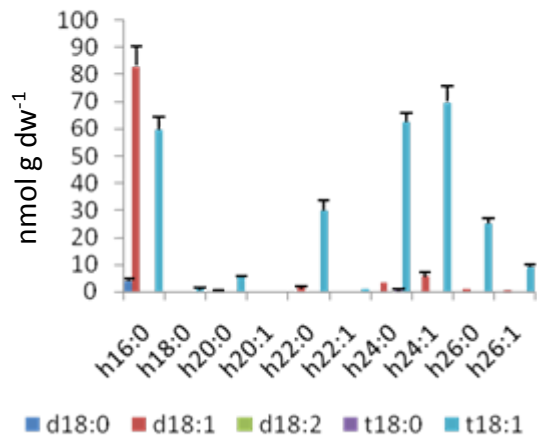


Cer, Riken 15-1202-1 leaves

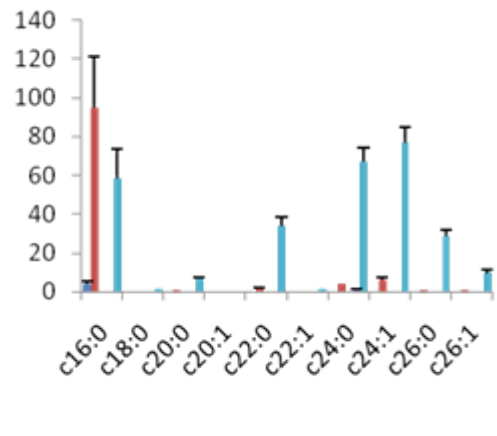




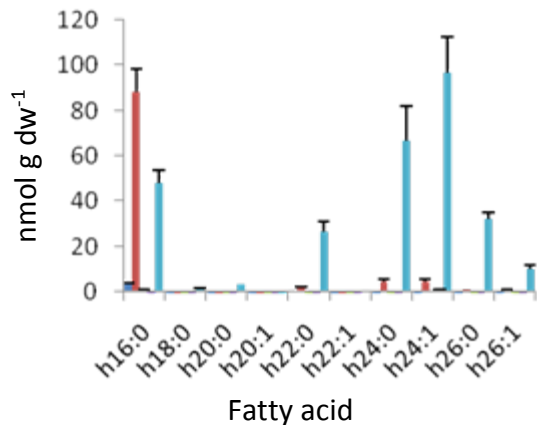
GlcCer, No-0 flowers



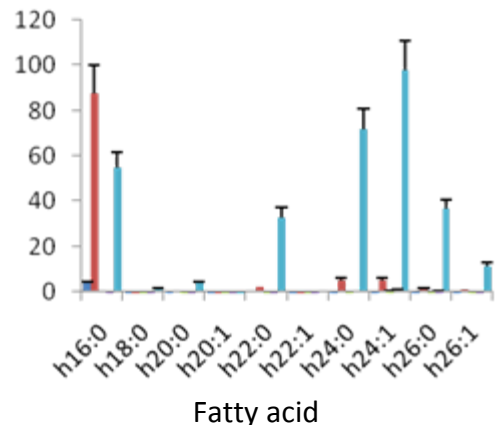
GlcCer, Riken 15-1202-1 flowers



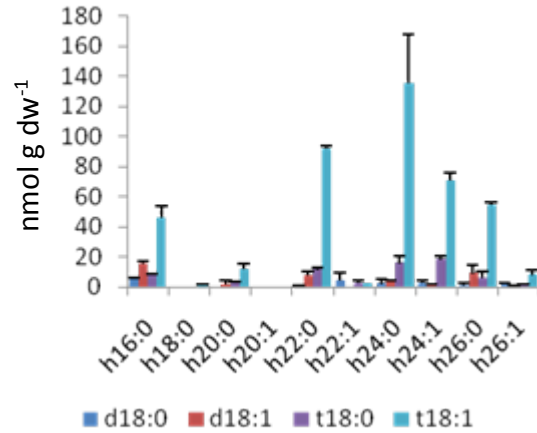
GlcCer, No-0 leaves



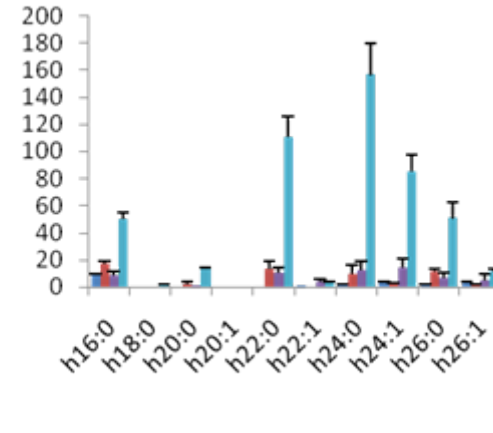
GluCer, Riken 15-1202-1 leaves



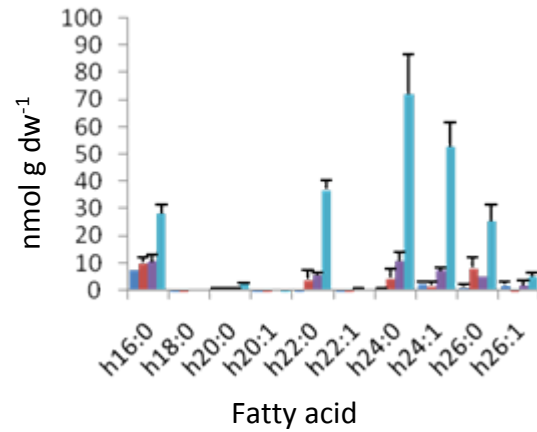
GIPCs, No-0 flowers



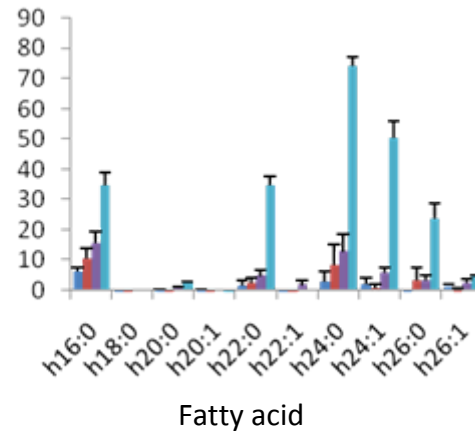
GIPCs, Riken 15-1202-1 flowers



GIPCs, No-0 leaves



GIPCs, Riken 15-1202-1 leaves



Supplementary Data 2: Free LCB and LCB-1-P analysis

