**Supporting Information**

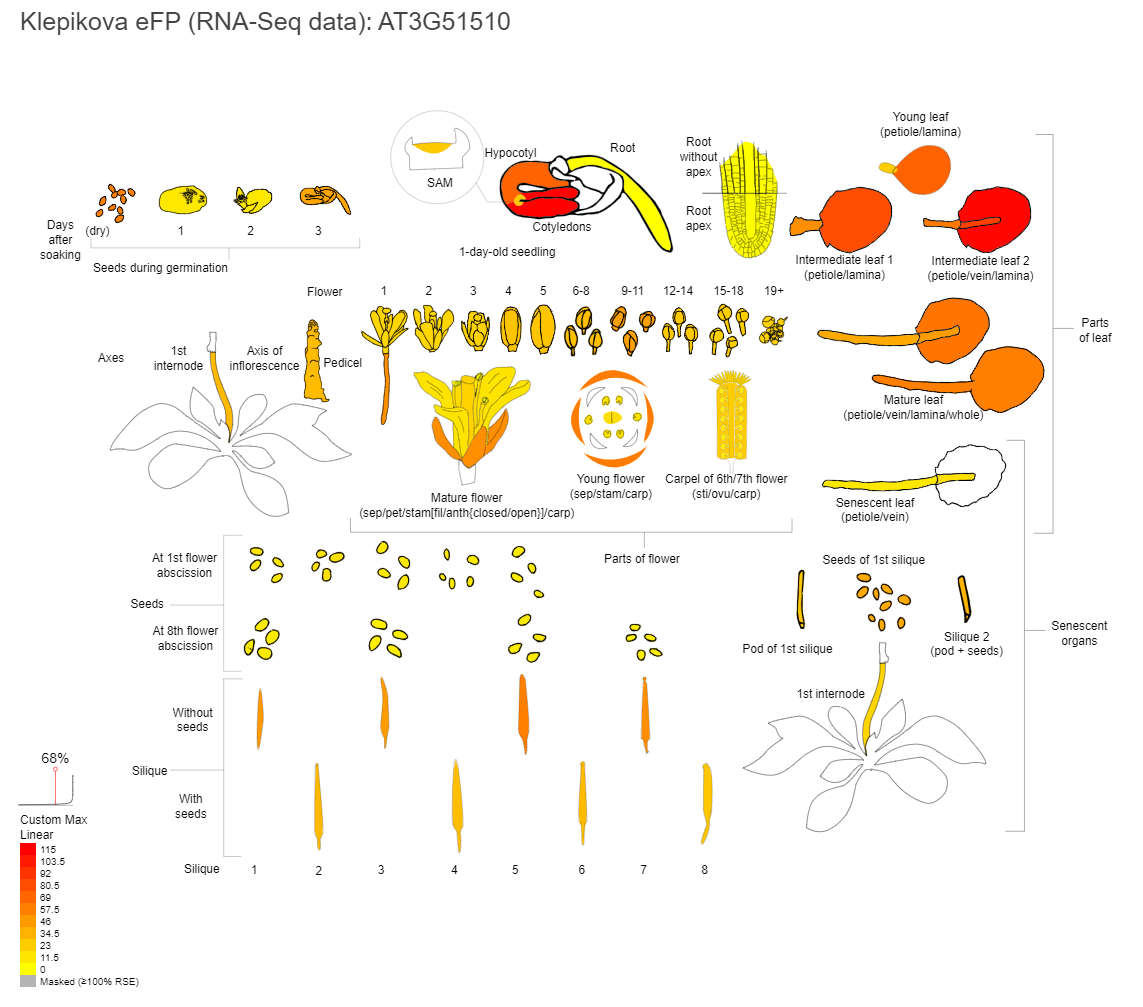
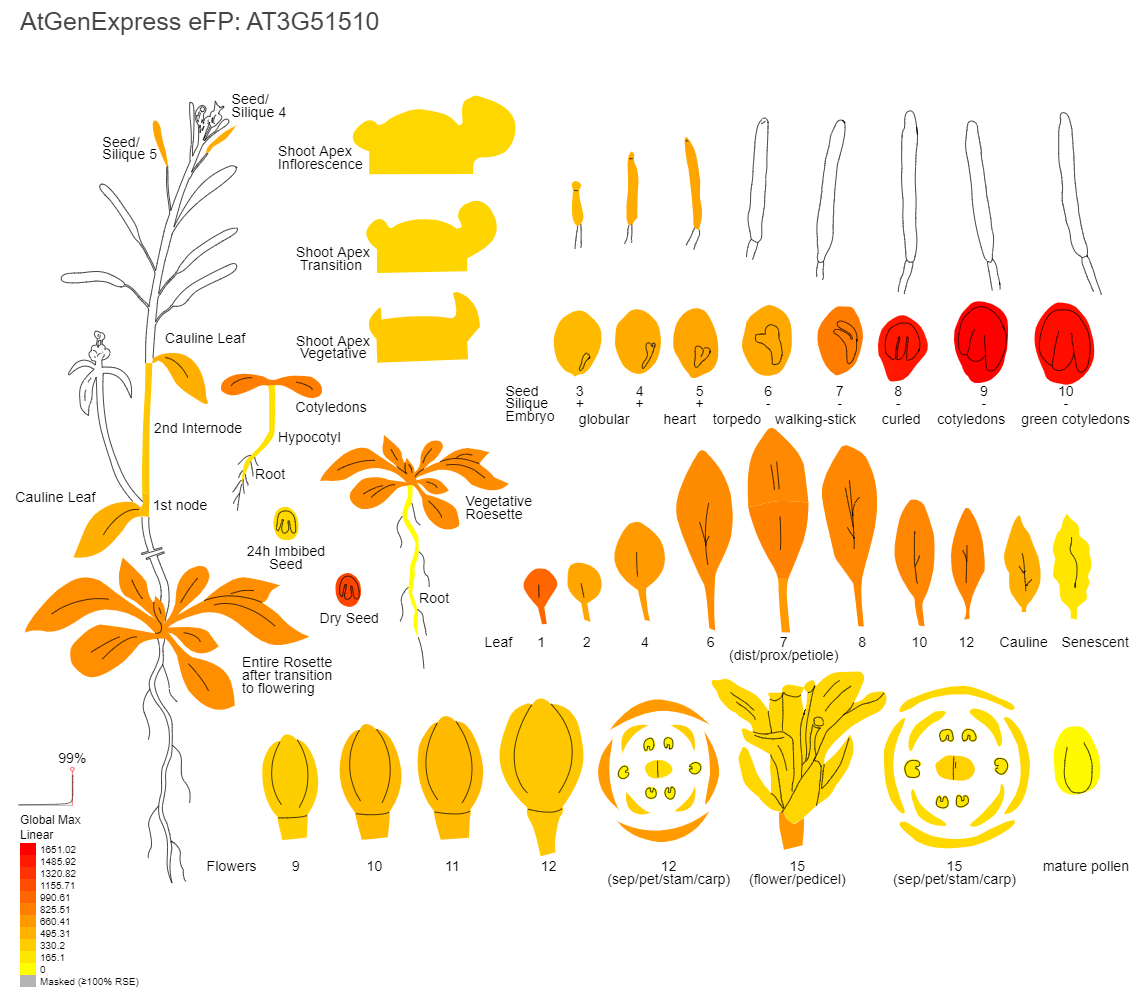
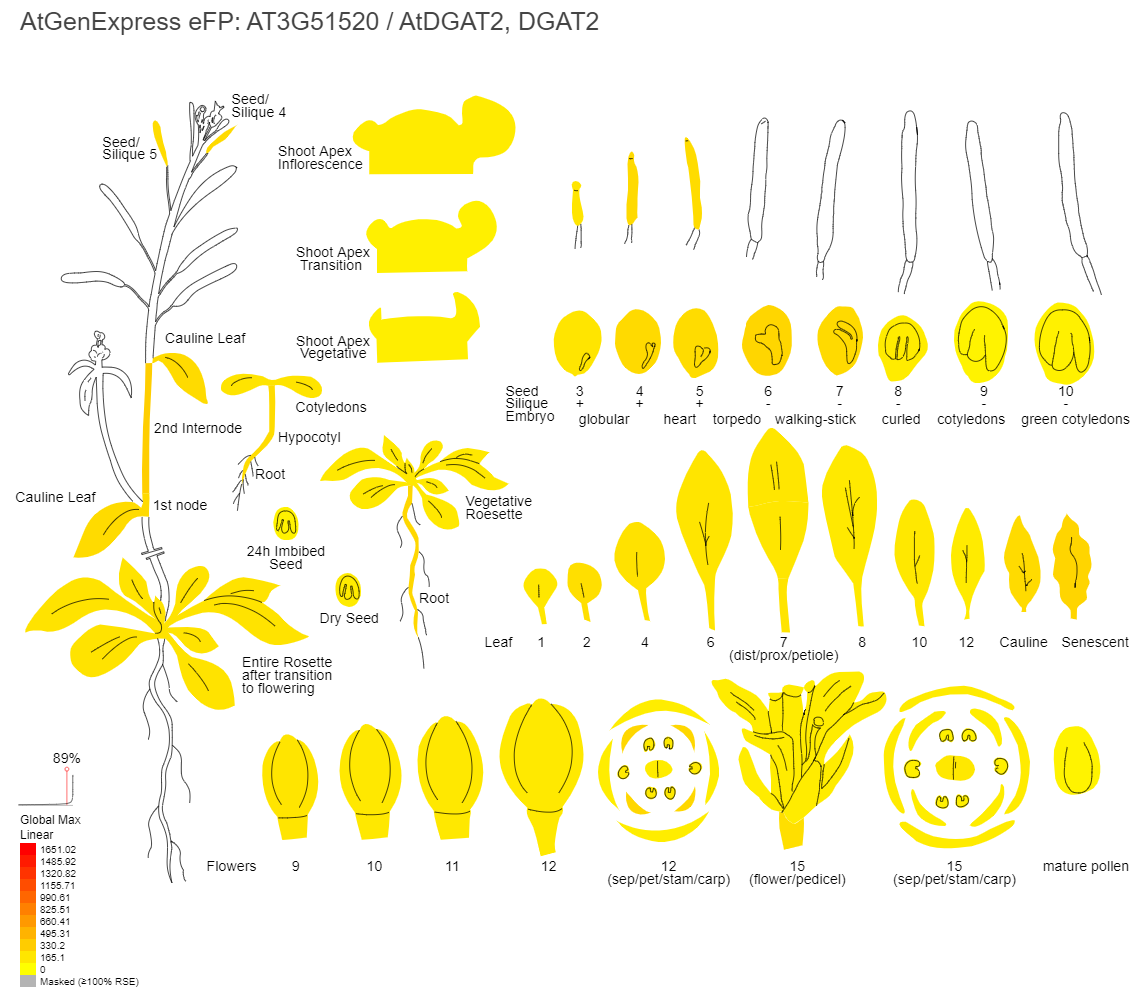
A native promoter–gene fusion created by CRISPR/Cas9-mediated genomic deletion offers a transgene-free method to drive oil accumulation in leaves

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**Fig. S1.** Developmental expression patterns of *DGAT2* and *DUG1*. Heat maps for *DGAT2* (At3g51520) and *DUG1* (At3g51510) gene expression derived from Affymetrix microarray data (Developmental Map) and RNA-Seq data (Klepikova Atlas) were generated by AtGenExpress eFP (bar.utoronto.ca/eplant/). Global Max Colour gradient setting was selected to allow quantitative comparison of gene expression between the two genes.



**Fig. S2.** gRNAs design. Untranslated sequences 5’ of the start codon were screened using CRISPR-PLANT. gRNA sites are underlined and PAMs are in bold. Double underlined nucleotides mark the putative start of transcription based on paired-end analysis.

AT3G51510 5’UTR

>Chr3:19108990..19109117 (+ strand) length=128

ACAGAATGATGATGGATTAGATATTTCTATTCAAAAACTATAACGTGTGGCTGCAAATCGATTCA**CCG**CTTCAGACTCTGTTTTAGA**CCA**AAGTCGAGTGAGTGCTTTCATCTTCTTCTTAAGCATCT

Class1.0 gRNA

SeqID minMM\_GG minMM\_AG Spacer seq (5'->3') PAM (5'->3')

Chr3:19109057-19109077:c 3 3 GTCTAAAACAGAGTCTGAAG **CGG**TGAATCG (gRNA1)

Chr3:19109079-19109099:c 4 4 TGAAAGCACTCACTCGACTT **TGG**TCTAAAA (gRNA2)

AT3G51520 5’UTR

>Chr3:19110596..19110738 (+ strand) length=143

AGTTAAAAGATTGGTTATTTGGGCTCTGCACTCAAGTGAGAGAGAAGATAGATAGATCTGAGTAGAATCTTCGATTCATTATTCGTTGTCGTCGTTCATCTGTGAGAAG**CGG**ACAAA**CCA**AAGAATCCACCGGAGCTAGTGAT

Class1.0 gRNA

SeqID minMM\_GG minMM\_AG Spacer seq (5'->3') PAM (5'->3')

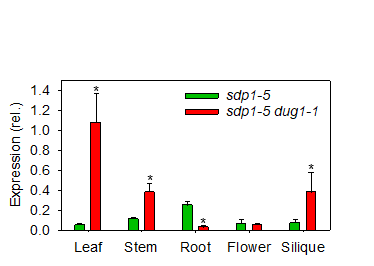
Chr3:19110586-19110606 4 4 AAGTTGGGTAGTTAAAAGAT TGGTTATTTG

Chr3:19110684-19110704 5 4 CGTCGTTCATCTGTGAGAAG **CGG**ACAAACC (gRNA3)

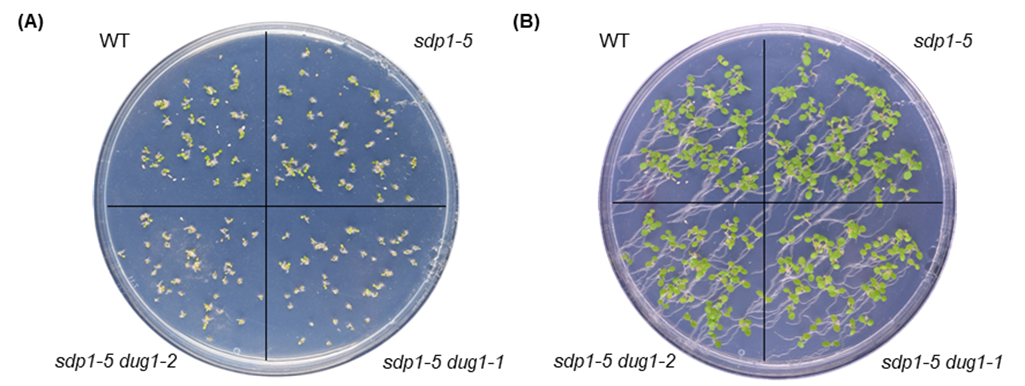
Chr3:19110715-19110735:c 3 3 ACTAGCTCCGGTGGATTCTT **TGG**TTTGTCC (gRNA4)

Chr3:19110724-19110744:c 5 5 ACCCATATCACTAGCTCCGG TGGATTCTTT

Chr3:19110727-19110747:c 4 4 ACCACCCATATCACTAGCTC CGGTGGATTC

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**Fig. S3.** *DGAT2* expression in various tissues of *sdp1-5 dug1-1*. Measurements were performed using quantitative RT-PCR. Values are presented as mean ± SE (n=3) and are expressed relative to the geometric mean of three reference genes. Asterisks denote values significantly (P < 0.05) different from *sdp1-5* (ANOVA + Tukey HSD test).

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**Fig. S4.** Seedling establishment. Images of seedlings after (A) three days and (B) six days. The seedlings were grown on agar plates (9 cm diameter), containing half-strength Murashige and Skoog salts (pH 5.7) plus 1% (w/v) sucrose, as described in the Material and Methods section.

**Table S1.** Primers used in study.

**Genotyping**

DUG1P-F: 5’-TGTCGTTTATTTGCACCACG-3’

DGAT2G-R: 5’-AACAGAGAACAAGAGCGACG-3’

**Q-PCR**

QDUG1-F: 5’-TTCCTCATCCGCTCCG-3’

QDUG1-R: 5’-CAATGACTCCTGCGGC-3’

QDGAT2-F: 5’-TGGTGGAAGCCGGATT-3’

QDGAT2-R: 5’-CGGGACTTGTGCCTCT-3’

QACT8-F: 5’-GAATTACCCGACGGACA-3’

QACT8-R: 5’-ACGGTCTGCAATACCT-3’

QUBI5-F: 5’-GACGCTTCATCTCGTCC-3’

QUBI5-R: 5’-CCACAGGTTGCGTTAG-3’

QEF1α-F: 5’-TCCAGCTAAGGGTGCC-3’

QEF1α-R: 5’-GGTGGGTACTCGGAGA-3

**5’-RACE**

GSP1: 5’-CCAGGTACAAGAACACAACT-3’

GSP2: 5’-GAGCAACAACTCCAATCGGTAGCAC-3’

**Table S2.** Total lipid content of seeds.

|  |  |
| --- | --- |
| Genotype | Lipid content (% of CDW) |
| WT | 27.52 ±1.12 |
| *sdp1-5* | 28.15 ±0.58 |
| *sdp1-5 dug1-1* | 27.41 ±1.12 |
| *sdp1-5 dug1-2* | 28.25 ±1.67 |

Values are shown as a percentage of cell dry weight (CDW) and are the mean ± SE (n=3) of measurements on seed batches from plants of each genotype. The values are not significantly different (P > 0.05, ANOVA).