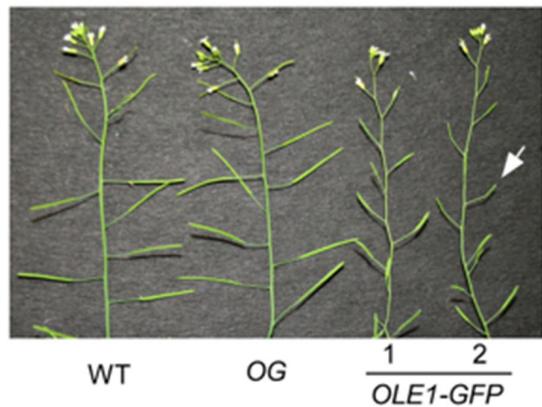
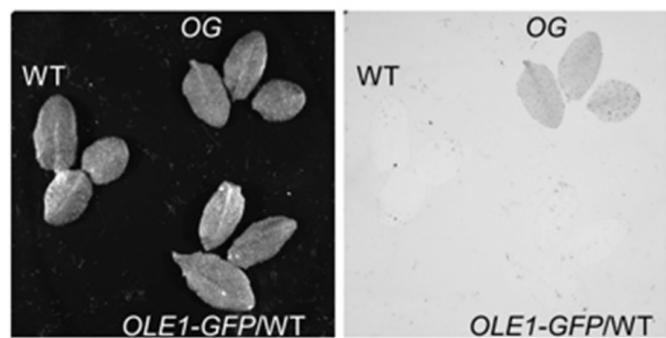


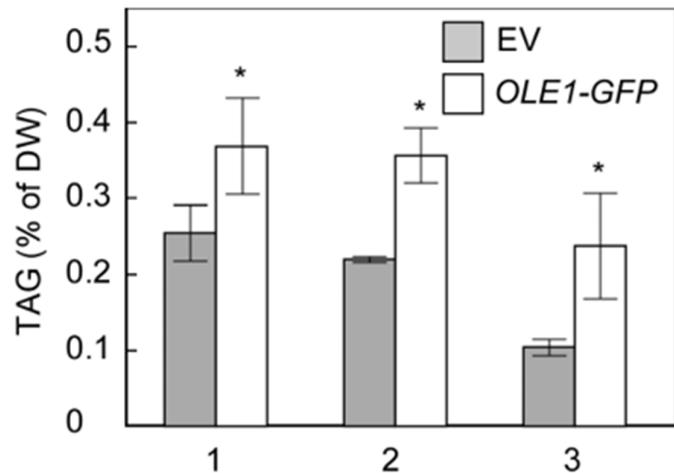
A



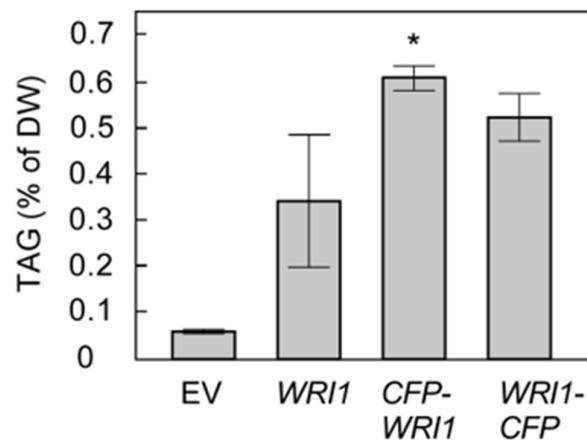
B



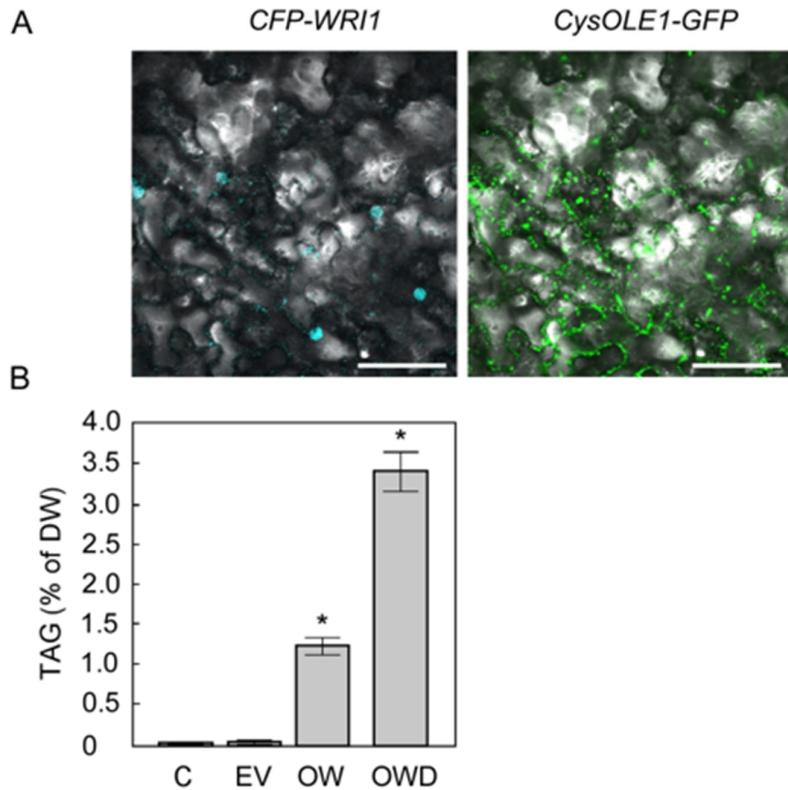
Supplemental Figure 1. Characterization of WT *OLE1-GFP* transgenic (*OLE1-GFP/WT*) plants from independent transformation with the same genetic construct that was described to create the OG line. (A) *OLE1-GFP/WT* lines showed shorter siliques than OG. Arrows point to abnormal siliques of *Ole1-GFP* transgenic plants. (B) GFP fluorescence signal of *OLE1-GFP/WT* is significantly lower than OG. Bright light (above) and GFP fluorescence (below) of WT, OG, and a representative *OLE1-GFP/WT* line by fluorescence image analyzer (ImageQuant LAS4000).



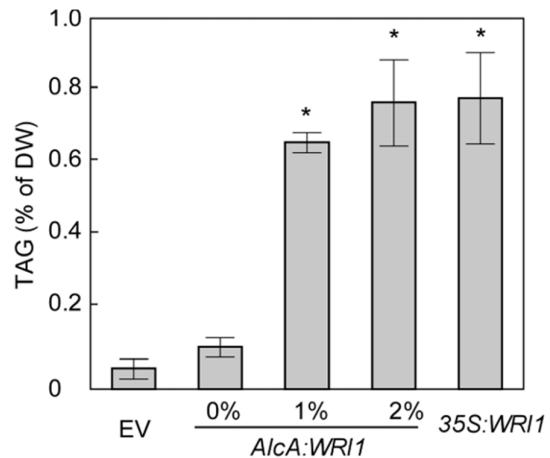
Supplemental Figure 2. Oil accumulation in *N. benthamiana* leaves transiently expressing *OLE1-GFP*. EV is empty vector, and 1, 2, and 3 stand for three independent transient assay experiments. Values are means \pm SE of measurements on 8 leaves from 4 5-week-old *N. benthamiana* plants infiltrated with *Agrobacterium* for 4 days. Asterisks denote statistically significant differences compared with EV (Student's *t* test, $p < 0.01$).



Supplemental Figure S3 TAG levels in *N. benthamiana* leaves that were transiently transformed with EV (empty vector), *WRI1*, CFP-*WRI1* (CFP fused to the N terminus of *WRI1*), or *WRI1-CFP* (CFP fused to C terminus of *WRI1*). Values in this figure are means \pm SE of measurements on 8 leaves from 4 5-week-old *N. Benthamiana* plants infiltrated with *agrobacterium* for 4 days. Asterisks denote statistically significant differences compared with *WRI1* (Student's *t* test, $p < 0.01$)



Supplemental Figure S4. Laser scanning confocal images showed the co-expression of WRI1 and OLE1 in *N. benthamiana* epidermis cells transiently transformed with OWD (with *Cys-OLE1-GFP*, *CFP-WRI1*, and *DGAT1* contained in one engineered T-DNA). Bar = 50 μ m. Values in this figure are means \pm SE of measurements on 8 leaves from 4 5-week-old *N. Benthamiana* plants infiltrated with *agrobacterium* for 4 days.



Supplemental Figure 5. TAG levels in *N. benthamiana* leaves that were transiently transformed with *AICa:WRI1* (ethanol inducible) or *35S:WRI1* (constitutive). Expression of *WRI1* was induced by irrigating with 0%, 1%, or 2% of ethanol solution for 4 days. Asterisks denote statistically significant differences compared with 0% of ethanol induction (Student's *t* test, $p < 0.01$). Values in this figure are means \pm SE ($n = 8$).

Supplemental Table 1. Primer sequences used in this study.

| Gene | Primer pair sequences | Purpose |
|---|--|----------------------|
| <i>OLE1</i> | GGGGACAAGTTGTACAAAAAAGCAGG CTTCATGGCGGATACAGCTAGAGG and GGGGACCACTTGTACAAGAAAGCTGG GTCAGTAGTGTGCTGGCCACCA | pGKPGWG |
| <i>WR11</i> | GGGGACAAGTTGTACAAAAAAGCAGG CTTCATGAAGAACGCCTAACACTTC and GGGGACCACTTGTACAAGAAAGCTGG GTCTTATTCAAGAACCAACGAACAAGCC | pGWB45 |
| <i>WR11</i> | GGGGACAAGTTGTACAAAAAAGCAGG CTTCATGAAGAACGCCTAACACTTC and GGGGACCACTTGTACAAGAAAGCTGG GTCGGACCAAATAGTTACAAGAAACCG AGG | pMDC85 |
| <i>DGAT1</i> | GGGGACAAGTTGTACAAAAAAGCAGG CTTCATGGCGATTGGATTCTGCTG and GGGGACCACTTGTACAAGAAAGCTGG GTCTCATGACATCGATCCTTTCGG | pGWB414 |
| <i>Cys-OLE expression module</i> | GGCCAGTGCCAAGCTTGTGGAGCACGAC ACACTTGTCT and GCAGGCATGCAAGCTTGCCAAGCTAGC TTGATGCATG | pGWB45 |
| <i>DAGA1 expression module</i> | AAACACTGATAGTTAACGCCAGGTCCC CAGATTAGCCTTTTC and TCCCGCCTTCAGTTAACAGTTAGCTC ACTCATTAGGCACCC | pGWB45 |
| <i>Cys-OLE1</i> | CTTGGGTACCATGGCGTGTCAATTGGT CAACAAAC and GCTATCTAGA GCTGGTCTGGCTACCTGC | pCHF3 and pBJ36_AlcA |
| <i>GBSS1</i> | ACCAGGTCTCAGGAGTCAGGCTGCTGTT GGACTTCC and ACCAGGTCTCATCGT GCCTTCCCTGGAACTTCTCTT | pRNAi-GG |
| <i>DGAT1</i> | CTTGGGTACCATGGCGATTGGATTCT GCTG and GCTATCTAGATGACATCGATCCTTTCG GTTCATC | pBJ36_AlcA |
| <i>WR11</i> ethanol inducible expression module | GACCTGCAGGCCGCCGCATATGCGGGAT AGT and ATCACTAGTGCGCCCTCTGCTGAGCCT CGACATGTTGTCGC | <u>pMBLART_AlcR</u> |
| <i>Cys-OLE1</i> ethanol inducible expression module | GACCTGCAGGCCGCCGCATATGCGGGAT AGT and CCCGCATATGCCCTCTGCTGAGCCT CGACATGT | <u>pMBLART_AlcR</u> |
| <i>DGAT1</i> Ethanol inducible expression module | GACCTGCAGGCCGCCGCATATGCGGGAT AGT and CCCGCATATGCCCTCTGCTGAGCCT CGACATGT | <u>pMBLART_AlcR</u> |
| <i>GBSS1</i> | CATGGACCAAGACTCTCTCTG and TCACTTCCGCCACATTAAG | qRT-PCR |
| <i>F-box</i> | TTTCGGCTGAGAGGTTGGAGT and GATTCCAAGACGTAAAGCAGATCAA | qRT-PCR |
| LBa1 | GGTCGGACTCTAGCTAGAGTCAG | |
| LBb1 | GATTGAATCCTGTTGCCGGTCTTG | |
| LSA1 | GTAATACGACTCACTTAGGCCACGCC GTCGACGCCGGCTGC | |
| SSAHind | AGCTGCAGGCCGGGCC | |
| SSAEco | AATTGCAGGCCGGGCC | |
| AP1 | GTAATACGACTCACTATAGGGC | |
| AP2 | TGGTCGACGGCCCGGGCTGC | |

| | |
|--------|--|
| LAD1-1 | ACGATGGACTCCAGAGCGGCCGC |
| AC1 | ACGATGGACTCCAGAG |
| RB-0b | CTTGACGAGTTCTTCTGAGCGGGACTC |
| RB-1b | ACGATGGACTCCAGTCCGGCCGAGTCAA GCAGATCGTCAAACATTGGC |
| RB-2b | ATCCTGTTGCCGGTCTTGCATG |
