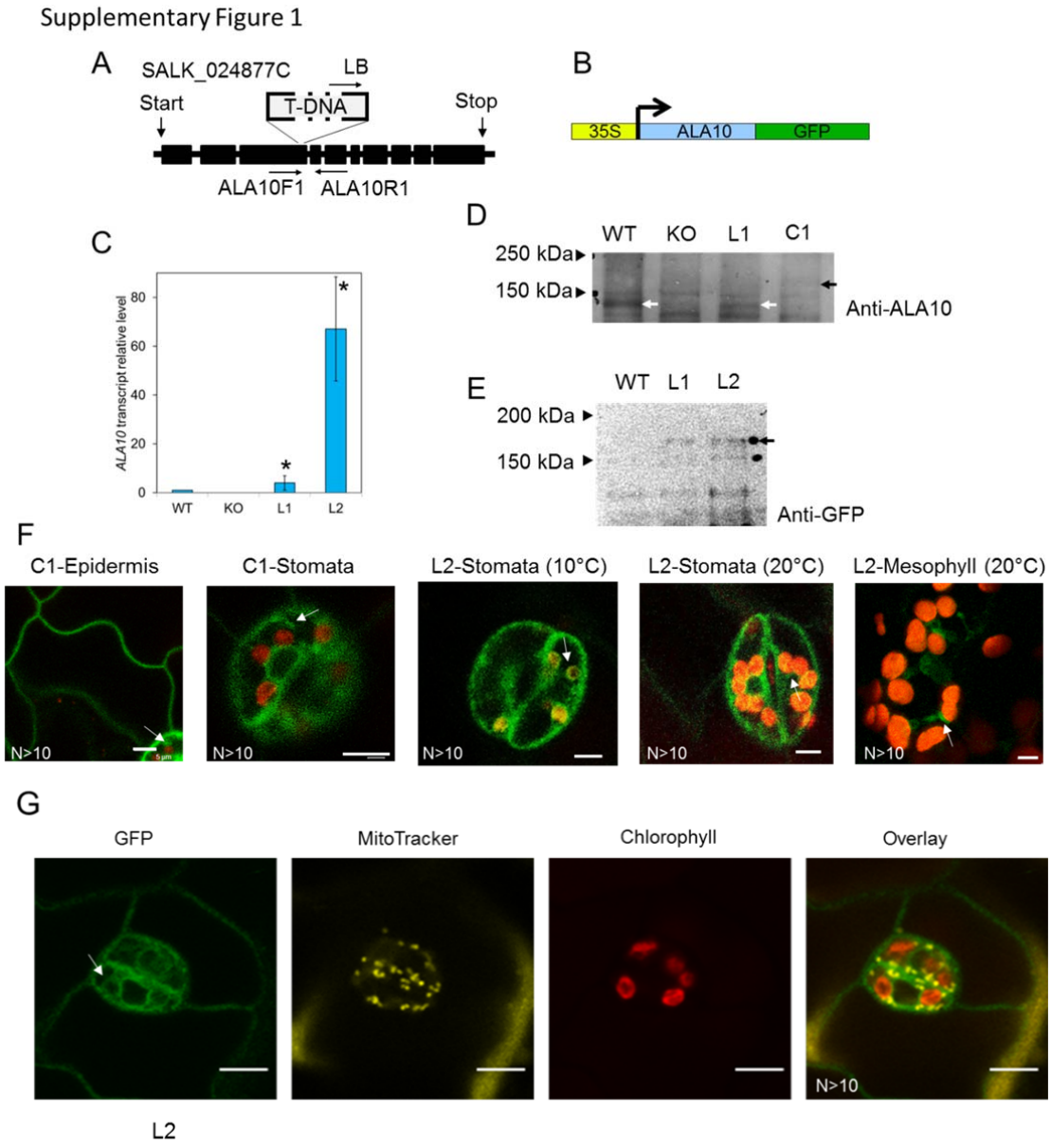
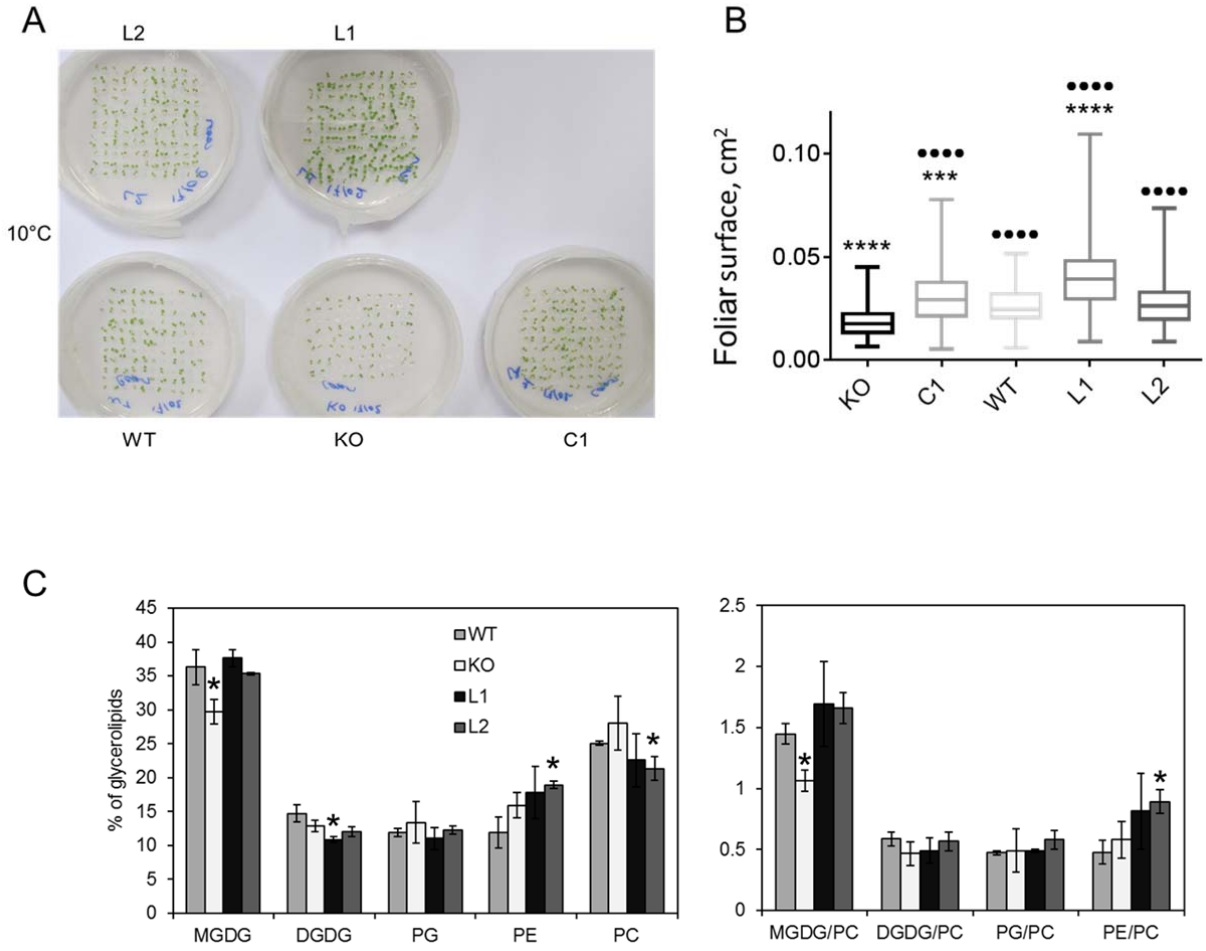


Supplementary Figure 1: Description of the ALA10 lines analyzed in the study. A- Position of the T-DNA insertion in the ALA10 gene of the SALK_024877C mutant line (KO). Arrows indicate positions of primers. For genotyping of WT and KO lines we used ALA10F1 and ALA10R1 primers in 1 and 3 or LB and ALA10R1 primers in 2 and 4. B- DNA construct used for expression of ALA10-GFP in L1, L2, and C1. C- RT-qPCR analysis of relative level of ALA10 transcripts in the WT, KO and the L1 and L2 ALA10-GFP expressing lines normalized over the WT level. Results are mean values +- SD of 6 biological samples of WT, KO and L2 and 3 of L1 collected over 3 different trials. * indicates a statistically significant difference relative to WT (Student's t-test with $P < 0.05$). D and E- Western blot immunodetection on leaf proteins from the different lines with antibodies dressed against ALA10 (D), or against GFP (E). In (D and E) black and white arrows indicate position of ALA10-

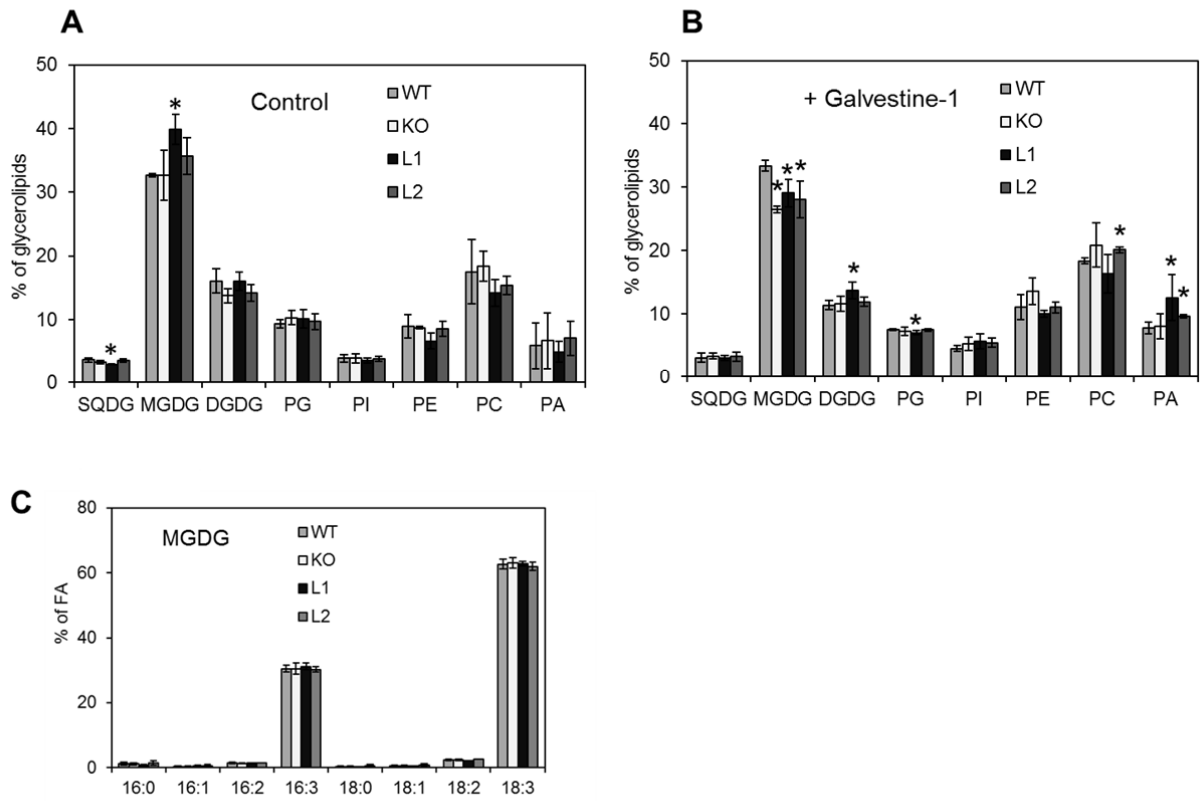
GFP and native ALA10 respectively. F- Confocal imaging of GFP (green) and chlorophyll (red) on leaves of C1 and of L2 plants grown at 20°C or 10°C. Overlays are presented and allow detection of GFP in the vicinity of chloroplasts in stomata and mesophyll cells (white arrows). G- Confocal imaging of GFP (green), Mitotracker (yellow) and chlorophyll (red) on leaves of L2 plants grown at 20°C. Chlorophyll and Mitotracker allow location of chloroplasts and mitochondria, respectively. Overlay indicates location of the GFP signal in the vicinity of or in the plasma membrane in the epidermal cells and, in stomata cells, in the ER around chloroplasts (white arrows). We observed no specific enrichment of GFP signal within mitochondria. Scale bar: 5 µm. L1: overexpressing line 1; L2: overexpressing line 2; C1: complemented line 1. N: Number of similar images obtained independently.

Supplementary Figure 2



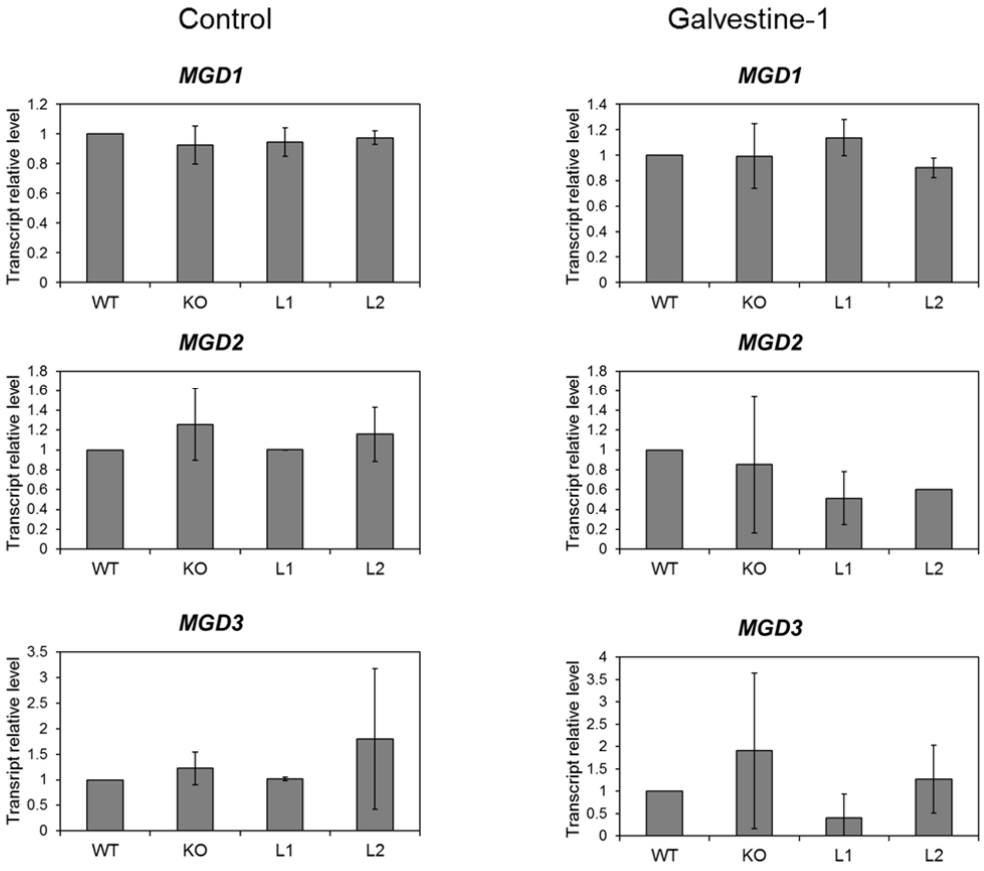
Supplementary Figure 2: Growth phenotype and lipid composition of ALA10 plant lines cultured at 10°C. A- Plants were grown for 1 week at 10°C. B- Statistical analysis of foliar surface of plants shown in A. Foliar area was measured with ImageJ software. Distribution of data obtained for each line is presented in a box plot with the median as the solid line inside the box, the first (Q1) and third quartile (Q3) as the lower and upper line of the box, and the minimum and maximum value as the bottom and upper end, respectively. Number of samples are 76 KO, 104 C1, 85 WT, 105 L1, 102 L2. Data were compared to WT (*) or to KO (●). Symbols indicate a statistically significant difference (Student's t-test with $P < 0.01$). Four symbols indicate a p-value < 0.0001, 3 symbols a p-value between 0.001 and 0.0001. C- Lipid composition of leaves from ALA10 mutants grown at 10°C for 7 weeks. Each result is the mean value +- SD of 3 biological samples for WT and KO and 2 for L1 and L2. Asterisk indicates a statistically significant difference with WT (Student's t-test with $P < 0.05$).

Supplementary Figure 3



Supplementary Figure 3: Lipid composition of leaves in ALA10 lines under Galvestine-1 treatment. Plants were grown at 20°C without (control) or with 100 µM Galvestine-1 as in Figure 4 and 5. A- Lipid composition of control plants. B- Lipid composition of plants treated with Galvestine-1. C- Fatty acid (FA) profile of MGDG in control plants. Results are mean values +- SD of 3 biological replicates. (*) indicates a statistically significant difference compared with WT (Student's t-test with $P < 0.05$). SQDG: sulfoquinovosyldiacylglycerol, MGDG: monogalactosyldiacylglycerol, DGDG: digalactosyldiacylglycerol, PG: phosphatidylglycerol, PI: phosphatidylinositol, PE: phosphatidylethanolamine, PC: phosphatidylcholine, PA: phosphatidic acid.

Supplementary Figure 4



Supplementary Figure 4: Comparison of the MGD expression in the ALA10 lines. RT-qPCR analysis of relative level of MGD transcripts in the WT, KO and the L1 and L2 ALA10-GFP expressing lines on leaves either from plants grown with 100 µM Galvestine-1 or from controls without Galvestine-1. Each data is the mean value +-SD of 6 samples over 2 trials.

Supplementary Table 1: List of primers used for BiFC constructs and qPCR analysis.

Primer	Sequence
ALA10 BiFC Fw	CACCATGGCTGGTCCAAGTCGGAG
ALA10 BiFC Rv	GACACCGACAAGATCCTTATAGATCTGATC
ALIS1 BiFC Fw	CACCATGTCTTCTTCTAACACGCCATCTTCT
ALIS1 BiFC Rv	ACGACCTCCAGGAATTCTGTTCC
ALIS2 BiFC Fw	CACCATGGAAGTGGAAGGATCGATGAATCG
ALIS2 BiFC Rv	ATTCTTGGAACAAGAAAAGCCTTTCAAGT
ALIS3 BiFC Fw	CACCATGAGTTCCAATACGGCGTCGT
ALIS3 BiFC Rv	CCGACCTCCAGGATTTCTATTCCA
ALIS5 BiFC Fw	CACCATGAGTTCCACCGCGGC
ALIS5 BiFC Rv	CTGTAAACCTCCAGCACTTCTGTTC
FAD2 BiFC Fw	CACCATGGGTGCAGGTGGAAGAATG
FAD2 BiFC Rv	TAACTTATTGTTGTACCAGTACACACCTTTCT
FAD3 BIFC Fw	CCACATGGTTGTTGCTATGGACCAACG
FAD3 BiFC Rv	ATTGATTTTAGATTTGTCAGAAGCGTAAACGT
ALA10Fw	CGCGGGTAAGGCTTATGGACGCGG
ALA10Rv	TCTGCAACACGGCAGCCTCAGGC
FAD2Fw	CAATGACCGAGAACGCCTCC
FAD2Rv	GGCAACGAGGGATGAGTGTG
FAD3Fw	CCATCGCTGCCGTGTATGTT
FAD3Rv	TGGTGTGTCCGGTGGCTTAT
MGD1Fw	GCCCTGGCCGTTCAACCAGC
MGD1Rv	CCCTTGGGCGATTTCCCTGGCA
MGD2Fw	TCCTACTTGGTTTCATCCGGGGGT
MGD2Rv	CCTTCACCAAAACCGCTCGTGC
MGD3Fw	ATGCGGCCGGAACAAAGTCCT
MGD3Rv	TCGTACCCGGACCAGCCTTAGTG
ACT8Fw	CGCCGATGGTCGTACAACCGGTAT
ACT8Rv	TCCCGTTCTGCTGTTGTGGTGAACA
UBQ10Fw	GGCCTTGTATAATCCCTGATGAATAAG
UBQ10Rv	AAAGAGATAACAGGAACGGAAACATAGT
TIP4Fw	GTGAAAACTGTTGGAGAGAAGCAA
TIP4Rv	TCAACTGGATACCCTTTCGCA