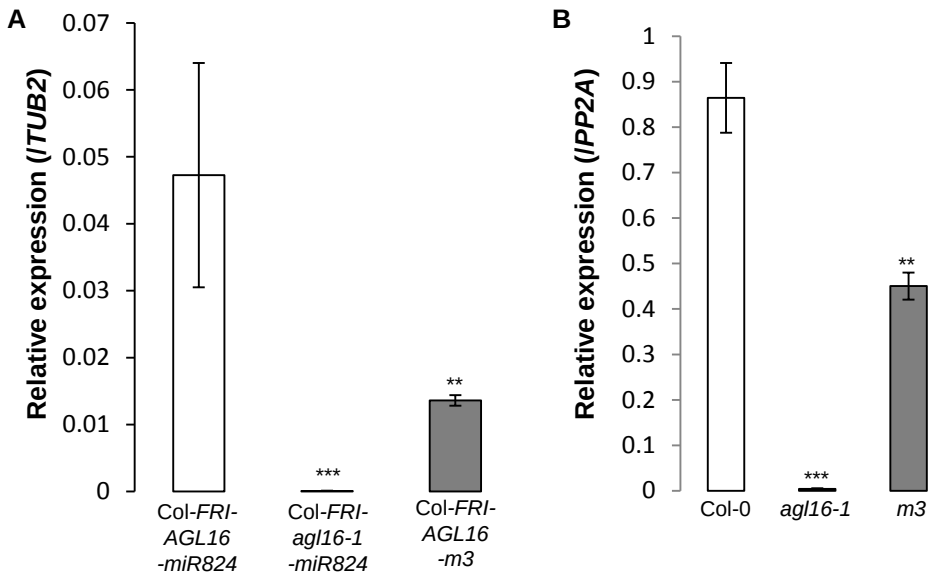
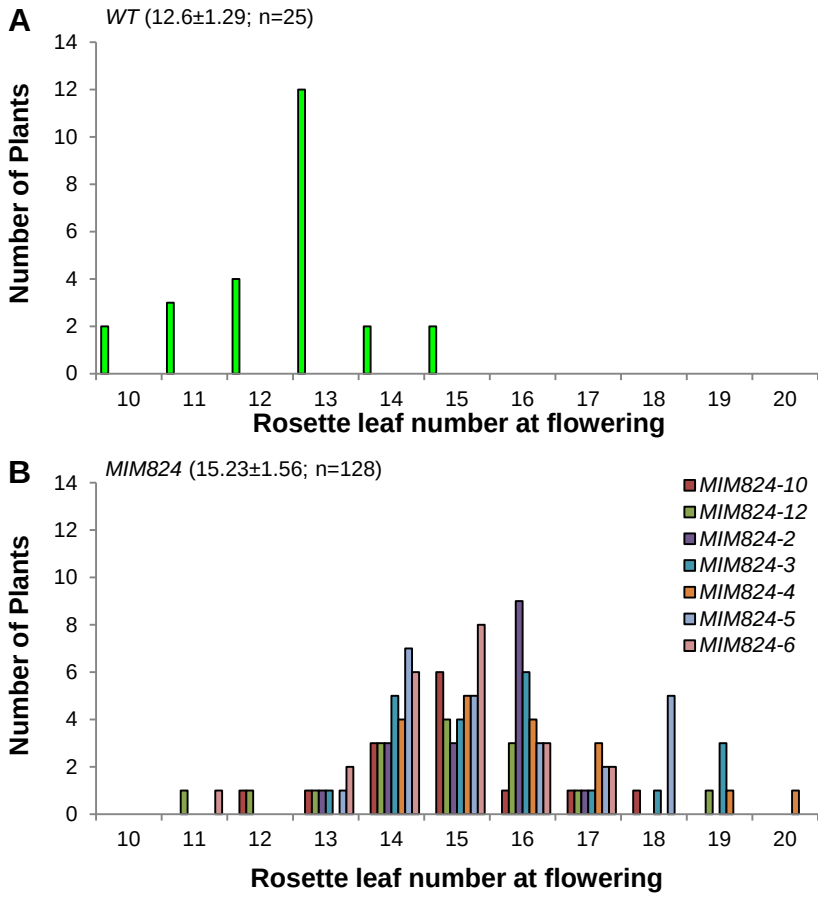


Supplemental Figure 1. *AGL16* expression was repressed in *m3*.

Relative accumulation of *AGL16* transcripts was quantified with real-time RT-PCR and normalized to the transcript level of *Tubulin2* (**A**) or *PP2A* (**B**) in wild type (white bars), *agl16-1* (black bars) and *m3* (gray bars) seedlings at DAG9 grown under long-day conditions. **A** in Col-*FRI* and **B** in Col-0 backgrounds. Bars are standard deviation of three biological replicates. ** and *** indicate the significant difference comparing to wild types (Students' t-test; **, $p<0.01$; ***, $p<0.001$).

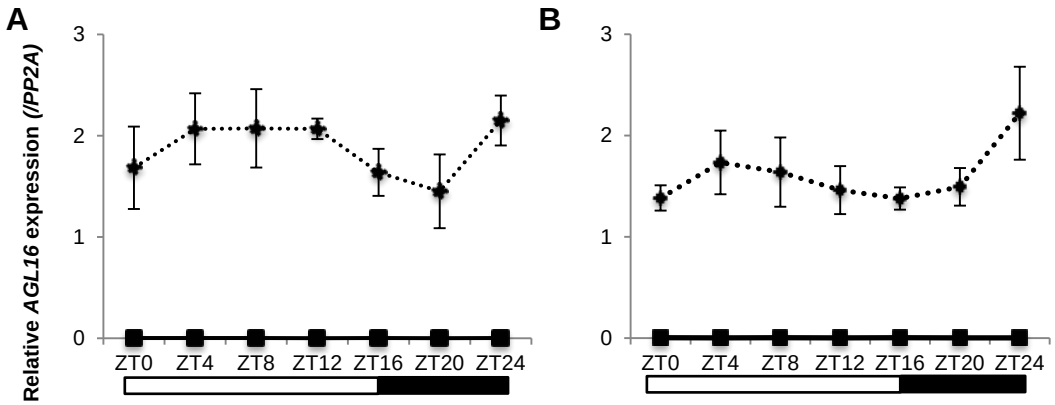


Supplemental Figure 2. *miR824* target mimicries flowered later under long-day conditions (supplementary to Figure 4).

Histograms with the y-axis indicating the number of individual plants as a function of the number of rosette leaves upon flowering (shown on x-axis). **A** for wild type Col-0 transformed with an empty binary vector and **B** for seven independent *MIM824* lines. In brackets: mean rosette leaf numbers, standard deviation and the total number of plant individuals tested (see Supplemental Dataset 1).

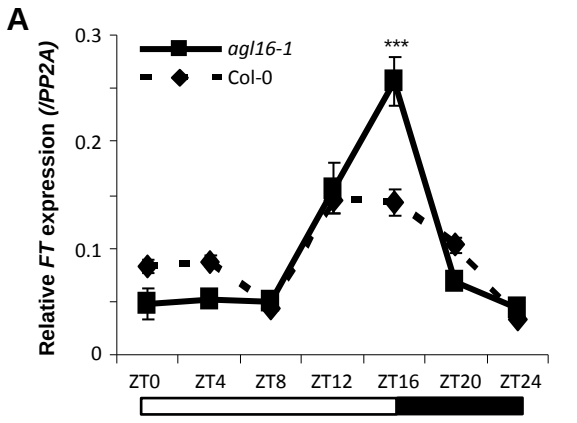
Supplemental Figure 3. *Promoter-miR824:GUS* staining reveals the expression of *miR824* in guard cells.

Promoter-miR824:GUS activity in guard cells of two stomata (taken from **Figure 5B**) is shown.

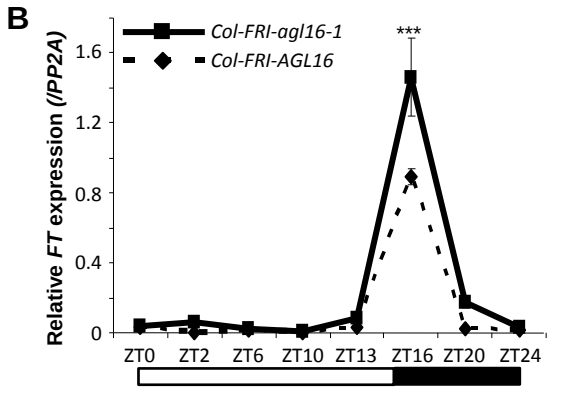


Supplemental Figure 4. The expression of *AGL16* in wild type and mutants in both Col-0 (A) and Col-*FRI* (B) backgrounds.

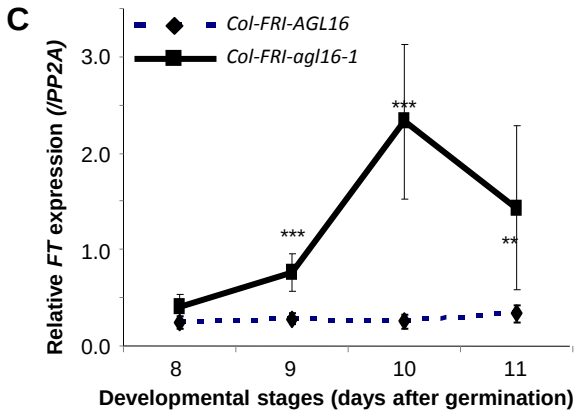
Real-time PCR following reverse transcription was used to quantify the expression of *AGL16*, which was normalized to the expression level of *PP2A*, as shown on the y-axis. Aerial parts of nine-day-old seedlings grown under LD conditions was used to quantify the expression at each zeitgeber time points (from ZT0 to ZT24). From ZT0 to ZT16 is day time, while from ZT16 till ZT24 is night time as shown below the x-axis with open (day) and filled (night) bars. Bars indicated standard deviation of three technical replicates.



Supplemental Figure for Figure 5D.

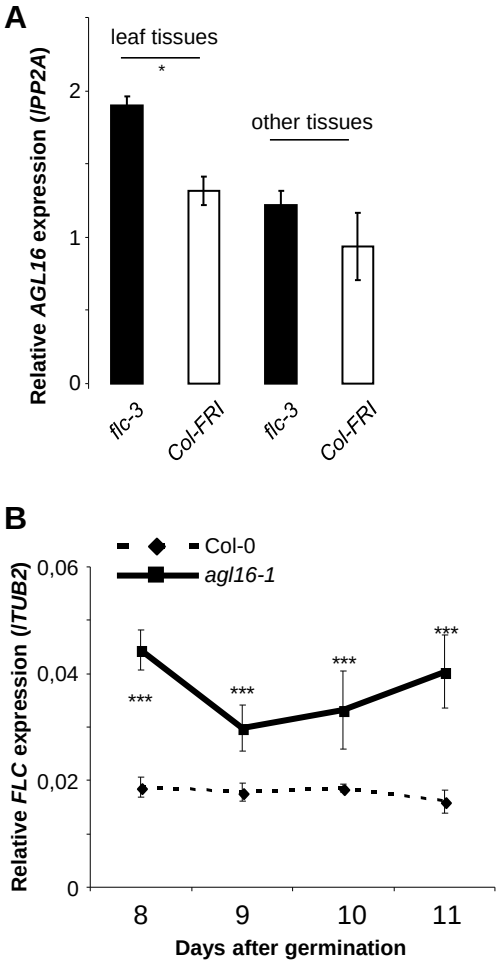


Supplemental Figure for Figure 5E.

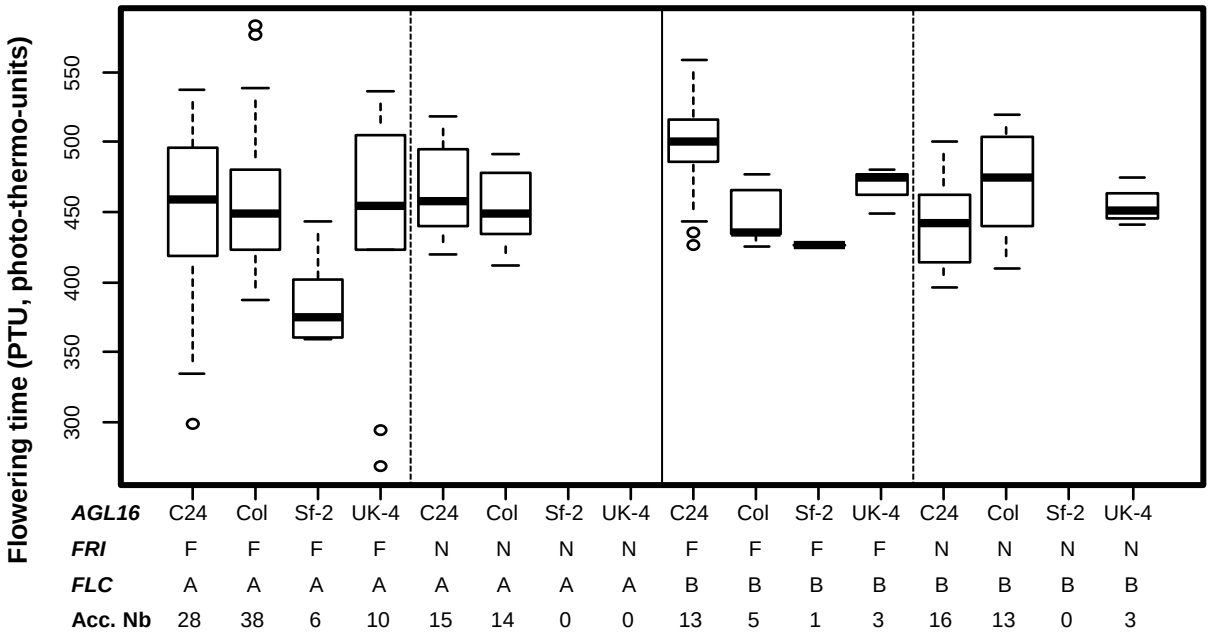


Supplemental Figure for Figure 5F.

Supplemental Figure 5. **The relative *FT* expression in a second independent trial confirming the pattern shown in Figure 5**. **A** for Figure 5D, **B** for Figure 5E, and **C** for Figure 5F. See Figure 5 for detailed information.



Supplemental Figure 6. The relative expression in an indepdent trial confirming the expression pattern for *AGL16* observed in Figure 6A (A) in the *flc-3* mutant and for *FLC* (B) in the Col-0 background (supplemental for Figure 6B).



Supplemental Figure 7. Polymorphisms at *AGL16* are associated with flowering time variation in common garden conditions.

Flowering time (measured in photo-thermo-units (PTU) accumulated until flowering by Brachi et al. (2011); Supplemental Dataset 3), as a function of allelic combinations at *AGL16*, *FRI*, and *FLC*. Each box encloses the 25-75% quantiles of the distribution, with the horizontal line marking the median. The lines extending from each box mark the minimum (5%) and maximum (95%) of the distribution. Circles mark the outliers (outside of the 5-95% distribution). The number of accessions included is given for each allelic combination. Some allelic combinations are rare or absent from the set of accessions used by Brachi et al. (2011). *AGL16* genotypes were grouped in four haplotypes: C24 (77 accessions), Col-0 (71 accessions), SF-2 (7 accessions) and UK-4 (16 accessions); *FRI* alleles are grouped into F (functional) and N (non-functional); and *FLC* alleles are group in A and B alleles (see supplemental tables 3 and 4 for statistical analysis).

Brachi, B., Faure, N., Horton, M., Flahauw, E., Vazquez, A., Nordborg, M., Bergelson, J., Cuguen, J., and Roux, F. (2010). Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. PLoS Genet **6,** e1000940.

Supplemental Table 1. Statistical significance for pairwise mean flowering time differences among lines presented in Figure 3B.

comparisons	t-test p-value	Bofferroni corrected
agl16/fri/FLC/miR824 (agl16-1) vs. AGL16/fri/FLC/miR824 (WT)	0.000168608	0.001854692
AGL16/fri/FLC/miR824-OX (m3) vs. AGL16/fri/FLC/miR824 (WT)	0.004062761	0.044690368
AGL16/fri/flc/miR824 vs. AGL16/fri/FLC/miR824 (WT)	0.00191289	0.021041793
agl16/FRI/FLC/miR824 vs. AGL16/FRI/FLC/miR824 (Col-FRI)	1.87326E-09	2.06058E-08
AGL16/FRI/FLC/miR824-OX vs. AGL16/FRI/FLC/miR824 (Col-FRI)	7.12298E-06	7.83528E-05
agl16/fri/flc/miR824 vs. AGL16/fri/FLC/miR824 (WT)	3.75172E-09	4.12689E-08
agl16/fri/flc/miR824 vs. agl16/fri/FLC/miR824 (agl16-1)	0.000720086	0.007920949
agl16/fri/flc/miR824 vs. AGL16/fri/flc/miR824	4.74972E-06	5.2247E-05
AGL16/FRI/flc/miR824 (flc-3) vs. agl16/FRI/flc/miR824	1.16049E-05	0.000127654
agl16/FRI/flc/miR824 vs. agl16/FRI/FLC/miR824	4.00558E-14	4.40613E-13
AGL16/FRI/FLC/miR824 (Col-FRI) vs. AGL16/FRI/flc/miR824 (flc-3)	2.74129E-13	3.01542E-12

Supplemental Table 2. Primers used in this study. * for expression; ** for genotyping; *** for cloning.

Note that sequences underlined indicate the attB adaptor sequences.

primer	gene	sequence (5' to 3')
m151*	*AGL16*	ACCTCCACAAGAAAGTAAACCTAATGC
m152*	*AGL16*	TGGCTGAGCTGAAGATGGACATG
m177**	*AGL16*	CCGAGAGGTGGGACTATGGTT
m178**	*AGL16*	TCTCCATGCATTTTCGGTTTT
m698***	*AGL16*	ggggacaagtttgtacaaaaaagcaggcttcatgggaaggggcaagatcgcga
m915***	*AGL16*	ggggaccactttgtacaagaaagctgggtcttatgcaatgaaggaaaaatagttgagttgg
m169*	*FLC*	TTCAACTGGAGGAACACCTTGA
m170*	*FLC*	CATGAGTTCGGTCTTCTTGGC
m185**	*FLC*	TCATGCGGTACACGTGGCAA
m186**	*FLC*	TCGCCGGAGGAGAAGCTGTA
m1068***	*FLC*	ggggacaagtttgtacaaaaaagcaggcttcATGGGAAGAAAAAAACTAGAAATCAAGCG
m1069***	*FLC*	ggggaccactttgtacaagaaagctgggtcCTAATTAAGTAGTGGGAGAGTCACCGG
m187**	*FRI*	TTGATAAGGATGAGTGGTTCGA
m188**	*FRI*	TGTCAACAAAAGGAACCACCTT
m141*	*FT*	CTTGGCAGGCAAACAGTGTATGCAC
m142*	*FT*	GCCACTCTCCCTCTGACAATTGTAGA
m179**	*miR824*	TGATCCGTGTGGTCCTTCAA
m180**	*miR824*	GTCGGAAAAAGCCGTGATGTG
m165*	*PP2A*	TAACGTGGCCAAAATGATGC
m166*	*PP2A*	GTTCTCCACAACCGCTTGGT
P004**	*T-DNA*	TGGTTCACGTAGTGGGCCATCG
m149*	*TUBLIN2*	GAGAATGCTGATGAGTGCATGG
m150*	*TUBLIN2*	AGAGTTGAGTTGACCAGGGAACC
FTRe**	*FT*	TGGAGATATTCTCGGAGGTG
FTFw**	*FT*	TGTTCCTCCTACCTAATAAT
LHP1F***	*LHP1*	ggggacaagtttgtacaaaaaagcaggcttcATGAAAGGGGCAAGTGGTGCTG
LHP1R***	*LHP1*	ggggaccactttgtacaagaaagctgggtcAGGCGTTCGATTGTACTTGAGATG
m147*	*SVP*	CAAGGACTTGACATTGAAGAGCTTCA
m148*	*SVP*	CTGATCTCACTCATAATCTTGTCAC
m189**	*SVP*	acccactagttatcagctcagttcctatc
m190**	*SVP*	Ccataatgatctaaagctcaactctctacac

Supplemental Table 3. Statistical test of *AGL16* allele association with flowering time in field conditions (Data from Brachi et al. 2011). Final model used for the *glm* analysis in R is (FT ~ AGL16 + FRI + FLC + PC1 + PC2 + AGL16:FLC + FRI:FLC + AGL16:FRI:FLC), following the recommendations by Crawley (2005). The flowering time data PTU was considered as count data. As a poisson fit gave signs of over-dispersion, quasipoisson correction was applied.

	Estimate	Std. Error	t_value	Pr(>|t|)	
(Intercept)	6.304433	0.216149	29.167	< 2e-16	***
AGL16_COL	0.008211	0.025385	0.323	0.74681	
AGL16_SF-2	**-0.160863**	**0.049302**	**-3.263**	**0.00137**	**
AGL16_UK-4	-0.032530	0.038131	-0.853	0.39495	
FRI_N	0.029749	0.032379	0.919	0.35969	
FLC_B	**0.092755**	**0.033414**	**2.776**	**0.00621**	**
PC1	-2.490262	2.854973	-0.872	0.38446	
PC2	0.015051	0.111857	0.135	0.89315	
AGL16_COL:FLC_B	-0.109686	0.058954	-1.861	0.06477	.
AGL16_SF-2:FLC_B	0.001335	0.120107	0.011	0.99114	
AGL16_UK-4:FLC_B	-0.020025	0.074685	-0.268	0.78897	
FRI_N:FLC_B	**-0.140403**	**0.049272**	**-2.850**	**0.00499**	**
AGL16_COL:FRI_N:FLC_A	-0.037258	0.045272	-0.823	0.41182	
AGL16_COL:FRI_N:FLC_B	**0.163533**	**0.065350**	**2.502**	**0.01341**	*
AGL16_UK-4:FRI_N:FLC_B	0.079913	0.090797	0.880	0.38019	

Supplemental Table 4. Wilcoxon rank sum test of pairwise mean flowering comparison for various allelic comparisons. Genotypes at *AGL16* (first position), *FRI* (middle position) and *FLC* (last position) are given.

Comparison	Wilcoxon rank sum test W	Wilcoxon rank sum p-value
SF-2 and C24+Col+UK4	1026.5	**0.0004**
Col;F;A and SF-2;F;A	209	**0.0012**
C24;F;B and C24;N;B	175	**0.002**
C24;F;A and SF-2;F;A	145	**0.0063**
C24;F;B and Col;F;B	56.5	**0.0205**
C24;F;A and C24;F;B	101	**0.0241**
C24;N;B and Col;N;B	59.5	0.0536
UK4;F;A and SF-2;F;A	46	0.093
Col;F;B and Col;N;B	19.5	0.2177
C24;N;A and Col;N;A	133	0.2297
Col;N;A and Col;N;B	66	0.2343
Col;F;A and Col;F;B	96.5	0.97