

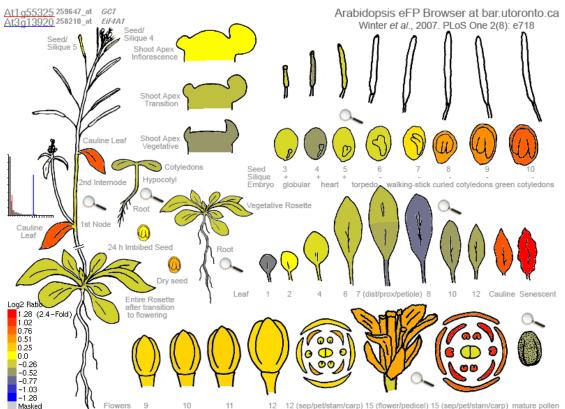
## Figure S1. Semi-quantitative RT-PCR analysis of *GCT* and *CCT* transcripts in all *gct* and *cct* alleles, and location of all *gct* and *cct* mutations.

(A) <u>Above left</u>, Semi-quantitative RT-PCR analysis of all *gct* alleles detected no *GCT* transcript in *gct-5*, as previously demonstrated by Ito et al. (2011). A primer pair at the 5' end of the transcript (GCT 5' sqPCR, shown in red below) was used to amplify from cDNA generated with a *GCT*-specific RT primer (GCT RT, shown in red below). The *GCT*-specific primer was used for RT because the 5' *GCT* primer pair did not amplify transcript from any wt or *gct* allele using cDNA transcribed with oligo dT, probably due to the 6 kb length of the *GCT* transcript (data not shown). Since the cDNA pool was specific to *GCT*, an eIF4a positive control could not be performed. <u>Above right</u>, semi-quantitative RT-PCR analysis of all *gct* alleles using a primer pair in the 3' region of the *GCT* transcript (GCT 3' sqPCR, shown in red below), as well as a primer pair for eIF4a as a control, to amplify from oligo dT cDNA pools. The 5' and 3' *GCT* primer pairs amplify both At1g55325.1 and At1g55325.2 splice variants. <u>Below</u>, gene model for *GCT* transcript At1g55325.2. The codon location for all *gct* mutations from Gillmor et al. (2010) are indicated in parentheses, with the exception of *gct-1*, which consists of a G>A change 14bp upstream of the splice acceptor for exon 22, creating an early splice acceptor that results in a downstream frameshift. *gct-5* corresponds to the T-DNA allele SAIL\_1169\_H11, and also to *mab2-2* (Ito et al., 2011). All other *gct* alleles were generated by EMS mutagenesis (Gillmor et al., 2010).

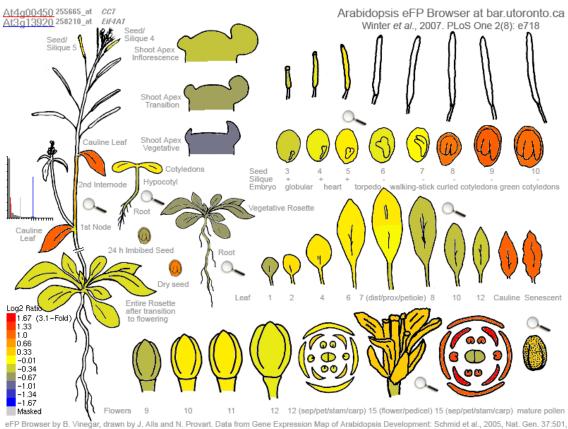
**(B)** <u>Above</u>, Semi-quantitative RT-PCR analysis detected no *CCT* transcript in *cct-2*, as previously demonstrated by Imura et al. (2012), and greatly reduced transcript levels in *cct-3*. A primer pair in the 3' region of the CCT transcript (*CCT* 3' sqPCR shown in red below) was used to amplify from oligo dT-primed cDNA pools. <u>Below</u>, gene model for *CCT* transcript At1g00450.1. The locations of mutations or insertions in all *cct* alleles reported in Gillmor et al. (2010) are shown. The codon locations differ significantly from those in the computationally predicted *CCT* gene model used as a reference in Gillmor et al. (2010). The gene model shown here is based on a cloned *CCT/CRP* cDNA, published by Imura et al. (2012). Importantly, the experimentally determined cDNA sequence demonstrates that the *cct-1* allele causes a translational stop at codon 316, not a Gly>Asp change as predicted by the non-experimentally verified *CCT* transcript model used in Gillmor et al. (2010). *cct-2* corresponds to T-DNA allele SALK\_108241, and also to *crp-3* (Imura et al., 2012), while *cct-3* corresponds to T-DNA allele SALK\_124276, and also to *crp-4* (Imura et al., 2012).

*GCT* and *CCT* gene models were drawn based on annotations at <u>www.arabidopsis.org</u>. For all RT-PCR experiments, representative results for one of three biological replicates are shown. RNA was extracted from pools of at least 5 four-week-old plants.

## Development 141: doi:10.1242/dev.111229: Supplementary Material

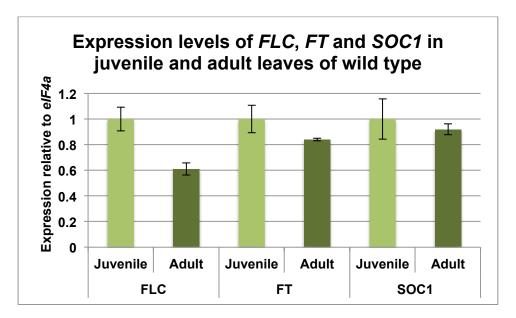


Masked Flowers 9 10 11 12 12 (sep/pet/stam/carp) 15 (flower/pedicel) 15 (sep/pet/stam/carp) mature pollen eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501, and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.



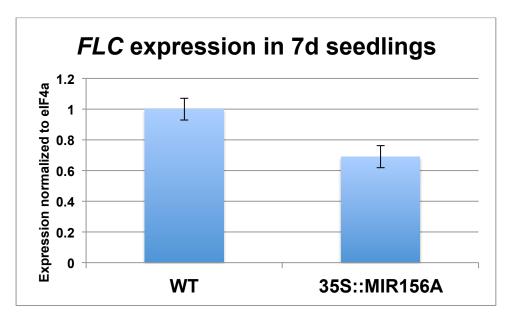
and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

**Figure S2.** Expression of *GCT* (top) and *CCT* (bottom) relative to *EIF4A*, throughout development. Analysis done with the BAR eFP expression browser (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi).



## Figure S3. Expression of *FLC, FT* and *SOC1* in juvenile and adult leaves of wild type.

Quantitative RT-PCR analysis of *FLC*, *FT*, and *SOC1* expression in juvenile and adult leaves of 20d old plants grown in long day conditions (see Methods). RNA was extracted from pools of the first three leaves (Juvenile) or the fifth to seventh leaves (Adult) of at least four plants for each biological replicate. Data are the average of three biological replicates, with three technical replicates for each biological replicate. Standard deviation for each average is shown with bars. *eIF4a* was used as a control for expression levels.



## Figure S4. Overexpression of miR156 causes a decrease in *FLC* levels.

Quantitative RT-PCR analysis of *FLC* expression in 7d wild type and 35S::MIR156A seedlings. Seedlings were grown in long day conditions (see Methods). The 35S::MIR156A transgenic line is described in Wu and Poethig (2006). Data are the average of three biological replicates, with three technical replicates for each biological replicate. Standard deviation is shown.

Name	Sequence (5' to 3')	Purpose	Notes
gct-2F	actggagatggcttgtaagcatccg	genotype gct-2 allele	amplification produces
gct-2R	tcgaagaaattcccaatgcg		a 200bp product; the wt Col allele is cut at bp 175 by HpaII; the gct-2 allele does not cut. 50 <sup>o</sup> C annealing temperature; 3mM MgCl <sub>2</sub> .
cct-1F	agtccagcatcaacaagcc	genotype <i>cct-1</i> allele	amplification produces
cct-1R	actgtagaagacgcaccagata		a 125bp product; <i>cct-1</i> allele is cut at bp 105 by EcoRV; wt Col allele does not cut. $50^{\circ}$ C annealing temperature; 3mM MgCl <sub>2</sub> .
flc-3F	gctcgtcatgcggtacacgtg	genotype <i>flc-3</i> allele	Amplify a 460bp
flc-3R	ggcgtacttatcgccggagg		fragment from wt Col, and a 356 bp fragment from <i>flc-3</i> .
	1		
GCT 5' sqPCR F	AATTCCGAGCGCTTCAAGAC	detect transcript in gct alleles at 5' region	
GCT 5' sqPCR R	TGTAGACCACCAATTCTGAAAAC		
GCT 3' sqPCR F	GGTACTCCAAGGGGATTGTTTCAG	detect transcript in gct alleles at 3' region	
GCT 3' sqPCR R	ACCAGCGGATACCAACTCA		
GCT RT	GCATCAGCGTCAAGATCACCAGTT	Reverse transcription with GCT-specific primer	
CCT 3' sqPCR F	GAGCTTACAGAATGAGCTTTCGC	detect transcript in cct alleles at 3' region	
CCT 3' sqPCR R	GAACAGAATGAGGCTGGCATGAGA		
miR156	GTGCTCACTCTCTTCTGTCA	miRNA Northern blot	
miR172	ATGCAGCATCATCAAGATTCT	miRNA Northern blot	
miR159	TAGAGCTCCCTTCAATCCAAA	miRNA Northern blot	
miR161	ACCCCGATGTAGTCACTTTCA	miRNA Northern blot	
miR168	TTCCCGACCTGCACCAAGCGA	miRNA Northern blot	
<i>U6</i>	AGGGGCCATGCTAATCTTCTC	<i>U6</i> miRNA Northern blot	

SPL3-F	CTTAGCTGGACACAACGAGAGAAGG	qRT-PCR
	С	
SPL3-R	GAGAAACAGACAGAGAGACACAGAGGA	qRT-PCR
SPL9-F	CAAGGTTCAGTTGGTGGAGGA	qRT-PCR
SPL9-R	TGAAGAAGCTCGCCATGTATTG	qRT-PCR
FLC-F	GCCAAGAAGACCGAACTCATGTTGA	qRT-PCR
FLC-R	TCCAGCAGGTGACATCTCCATCTC	qRT-PCR
FT-F	TGGTGGATCCAGATGTTCCAAGTC	qRT-PCR
FT-R	CTCATTGCCAAAGGTTGTTCCAGTT	qRT-PCR
SOC1-F	GGAGCTGCAACAGATTGAGCAACAG	qRT-PCR
SOC1-R	CGCTTTCATGAGATCCCCACTTTTC	qRT-PCR
At2S2-F	ACCCCATGGGCCCAAGACAGA	qRT-PCR
At2S2-R	GCTGGGGGCCTTGTGGGTTC	qRT-PCR
CRU1-F	CTCCTTTGCACGTTACATCATCGAG	qRT-PCR
CRU1-R	CGCATCCAGGGATCACTTTTCC	qRT-PCR
OLEO1-F	CATCACAGTTGCACTCCTCATCACC	qRT-PCR
OLEO1-R	TTGCACTGTCCAACTTGTCTGATCC	qRT-PCR
ATTI7-F	GCCATCTTTTTCATCCTCGTTTTGG	qRT-PCR
ATTI7-R	CGGGAATATTCGAGGTGCACAGTAG	qRT-PCR
EIF4aF	AAACTCAATGAAGTACTTGAGGGAC	qRT-PCR
EIF4aR	TCTCAAAACCATAAGCATAAATACCC	qRT-PCR

Table S2

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Table S3

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Table S4

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Table S5

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