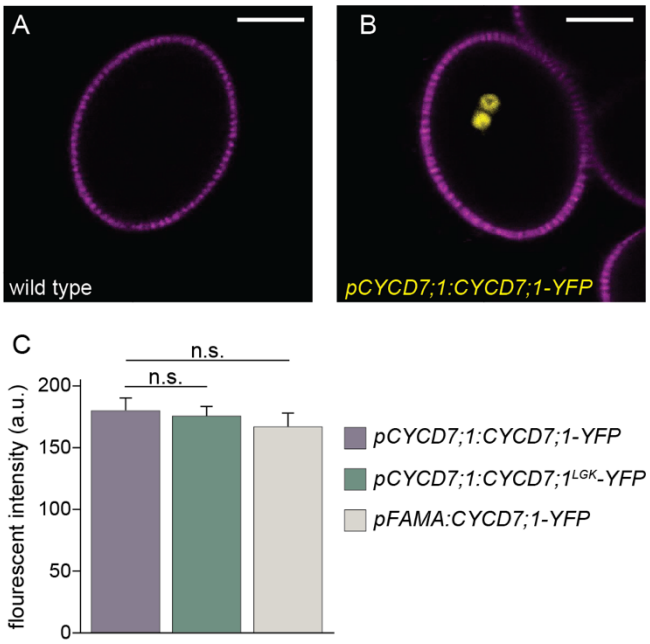
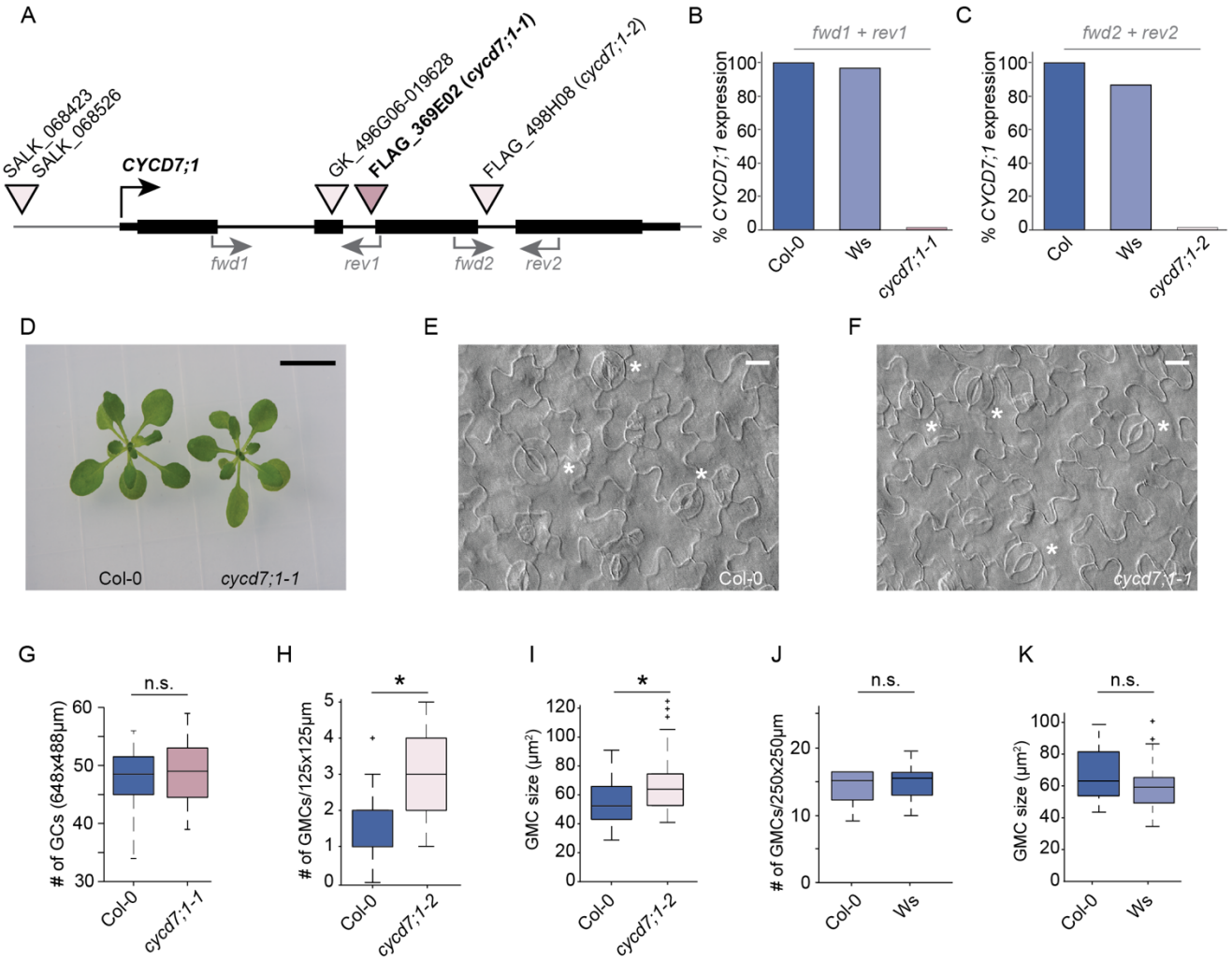
Supplementary Data

Figure S1: Additional CYCD7;1 expression patterns outside of the stomatal lineage



(A, B) CYCD7;1 (yellow) is expressed in sperm cells during pollen anthesis. **(C)** Intensity measurements of fluorescent nuclei were 179 a.u. +/-10 S.E.M. for *proCYCD7;1:CYCD7;1-YFP* vs 176 a.u. +/-8 S.E.M. for *proCYCD7;1:CYCD7;1^{LGK}-YFP* (N=15 nuclei/line; p> 0.05; Student's t-test) and 166 a.u. +/-11 S.E.M. for *proFAMA:CYCD7;1-YFP* (N=15 nuclei/line; p> 0.05; Student's t-test). Error bars show standard error. a.u., arbitrary units; n.s. non-significant; S.E.M. standard error of measurement.

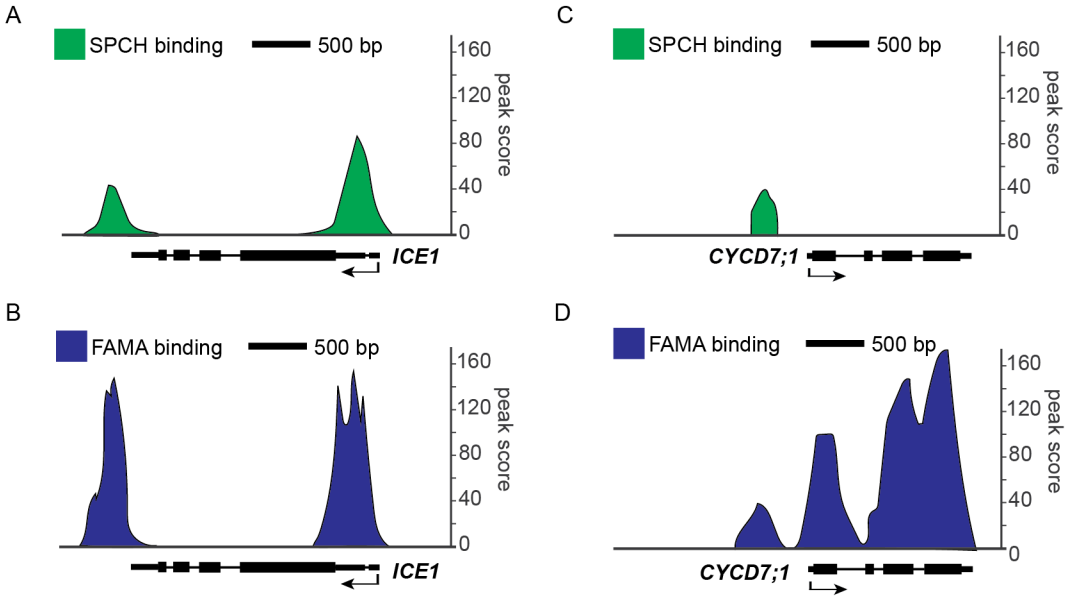
Figure S2: Additional *CYCD7;1* T-DNA insertional alleles and mutant phenotypes



(A) Schematic drawing of *CYCD7;1* gene structure with available T-DNA insertion lines and their insertion sites. Black boxes indicate exons. Gray arrowheads marked with *fwd* and *rev* show primer binding sites for qPCR. **(B)** qPCR of *CYCD7;1* expression in wild type (Col-0 and Ws) and the *cycd7;1-1* mutant. Primer binding sites are shown in (A). **(C)** qPCR of *CYCD7;1* expression in wild type (Col-0 and Ws) and the *cycd7;1-2* mutant. Primer binding sites are shown in (A). **(D)** Wild type and *cycd7;1-1* mutant seedlings at 14 dag. **(E)** Wild type cotyledon with mature GCs, labeled with black asterisks at 7 dag. **(F)** Cotyledon of *cycd7;1-1* mutant with mature GCs, labeled with black asterisks, images were taken at 7 dag. **(G)** Quantification of GCs in wild type and *cycd7;1-1* mutants at 5 dag on the abaxial side of cotyledons (N =12 cotyledons for each genotype). Difference between the wild type and *cycd7;1-1* is not significant (p-value = 0.8169; Mann-Whitney U test). **(H)** Quantification of the number of GMCs in wild type and *cycd7;1-2* cotyledons at 4 dag. Asterisk indicates significant difference (p-value = 0.0031; Mann-Whitney U test). **(I)** Quantification of GMC area in wild type (N=29) and *cycd7;1-2* (N=46) cotyledons at 4 dag. Asterisk indicates significant difference (p-value = 0.0053; Mann-Whitney U test). **(J)** Quantification of the number of GMCs in Col-0 wild type and Ws wild type cotyledons at 4 dag. Difference is not significant (p-value = 0.6970; Mann-Whitney U test). **(K)** Quantification of GMC area in Col-0 wild type (N=22) and Ws wild type (N=45) cotyledons at 4 dag. Difference is not significant (p-value = 0.2295; Mann-Whitney U test).

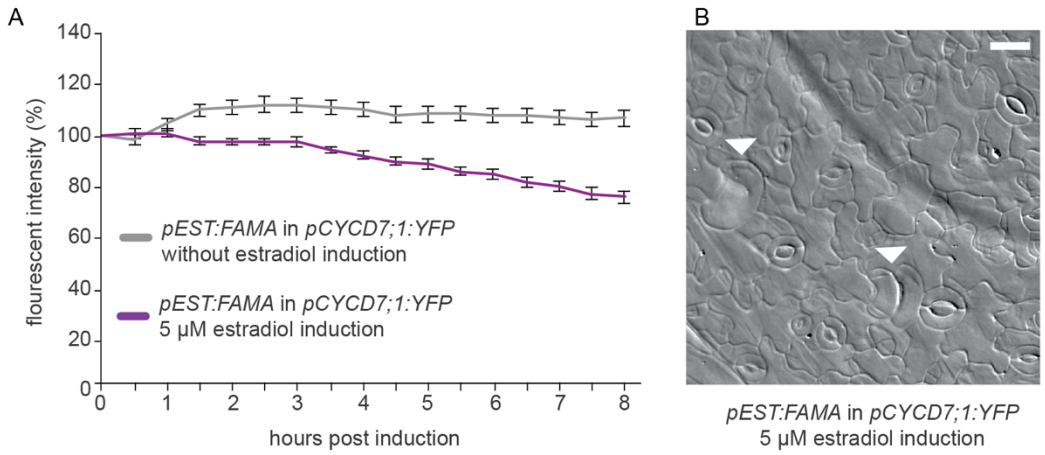
Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 2.5 times the interquartile range from the 97.5th percentile. Scale bar 1 cm in (C) and 20 µM in (E and F). Note that stomatal production is dynamic and is sensitive to exact age and growth conditions (e.g. media, light, temperature). Therefore, all quantitative measurements were performed with wildtype controls grown side-by-side with mutants under the exact same conditions to enable comparisons.

Figure S3: ChIP-seq profiles of FAMA and SPCH on selected loci



ChIP-Seq profile of SPCH (green) and FAMA (blue) binding to the promoter and gene body of ICE1 and CYCD7;1, respectively. The y-axis is the output peak score from MACS2 (in arbitrary units). ICE1 was previously demonstrated to be a direct SPCH target (Lau et al., 2014) and serves as a reference to provide intuition about the meaning of these peak score values. Black arrow indicates gene orientation and transcriptional start sites. The profile in (D) is replicated from Fig. 5G to enable a more convenient comparison among transcription factors and targets.

Figure S4: Evidence that FAMA represses CYCD7;1 in GMCs



(A) Transgenic plants expressing *proCYCD7;1:YFP* were subjected to FAMA induction and the fluorescence intensity of YFP was monitored over time in cotyledon GMCs. Four-day old plants treated with 5µM β-estradiol show ~25% reduction in YFP fluorescence as compared to the control treatment in an 8-hour imaging period suggesting that FAMA represses *CYCD7;1*. Error bars represent standard error. **(B)** DIC image of the epidermis of a 10 dag cotyledon from genotypically identical siblings of the plants monitored in (A) grown on media supplemented with 50µM β-estradiol. The plant demonstrates the typical FAMA overexpression phenotype of ectopic unpaired (kidney shaped) GCs. Scale bar is 25µm.

Table S1: Primers used in this study.

	Forward primer (5'-3')	Reverse primer
CYCD7 genomic region (promoter + CDS)	CACCGAGAAACTATAGTAGAAGGAAAC	AATGTAATTTGACATTTCAATTG
CYCD7;1^{LGK} genomic	TAATCTACTCGGAGAAAAATCTTGGCCCGCGAGTCC	CTCGCGGGCCAAGATTTTTCTCCGAGTAG ATTATCC
CYCD7;1 promoter	CACCGAGAAACTATAGTAGAAGGAAAC	GCGGCCGCTTGGAAACTGAACCGGTTT
CYCD7;1 genomic	CACCATGGATAATCTACTCTGCGAAG	AATGTAATTTGACATTTCAATTG
CYCD7;1^{LGK} genomic	CACCATGGATAATCTACTCTGCGAAG	AATGTAATTTGACATTTCAATTG
CYCD7;1 qPCR (fwd1 and rev1)	TCCATGCGTTTCAATGGCTAATCC	TCCACCATCCAATTCGTCCATTCG
CYCD7;1 qPCR (fwd2 and rev2)	GTGTGAACGCGGTTACGAG	TGAAGCATTTTTAAATCGCATATAACA
ACTIN qPCR	CAAGGCCGAGTATG	GAAACGCAGACGTA
cycd7;1-1 RB T-DNA	CCAGACTGAATGCCCACAGGCCGTC	
CYCD7;1	ATGGATAATCTACTCTGCGA	AATGTAATTTGACATTTCAATTG

Supplemental Material and Methods

Time-lapse of estradiol inducible constructs

Estradiol inducible FAMA (*proEST:FAMA*) was transformed into plants harboring *proCYCD7;1:YFP* and 4 dag T3 plants were used for time-lapse experiments following general protocols described in (Davies and Bergmann, 2014), except that that normal media (1/4 strengths MS, 0.75% Sucrose) was supplemented with 5µM β-estradiol (25µL of 10mM β-estradiol dissolved in 95% ethanol for 50mL media) or ethanol alone (25µL 95% ethanol for 50mL media), and pumped through the chamber with a constant flow at 2mL/hour using a syringe pump. Z-stacks through the epidermis were captured on a confocal microscope with Leica software every 30 min for 8 hours. Fiji software was used to measure Integrated Density (total fluorescence) of 36 (control) or 28 (estradiol-treated) GMC nuclei. % fluorescence per nucleus was calculated with respect to "initiation" time point (T0). Three independent replicates of the time course were performed, each on a separate plant, for both control and induced lines, and the averaged data plotted in Fig S4.