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Supplementary Materials for

LEAFY Controls Auxin Response Pathways in Floral Primordium Formation

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The PDF file includes:

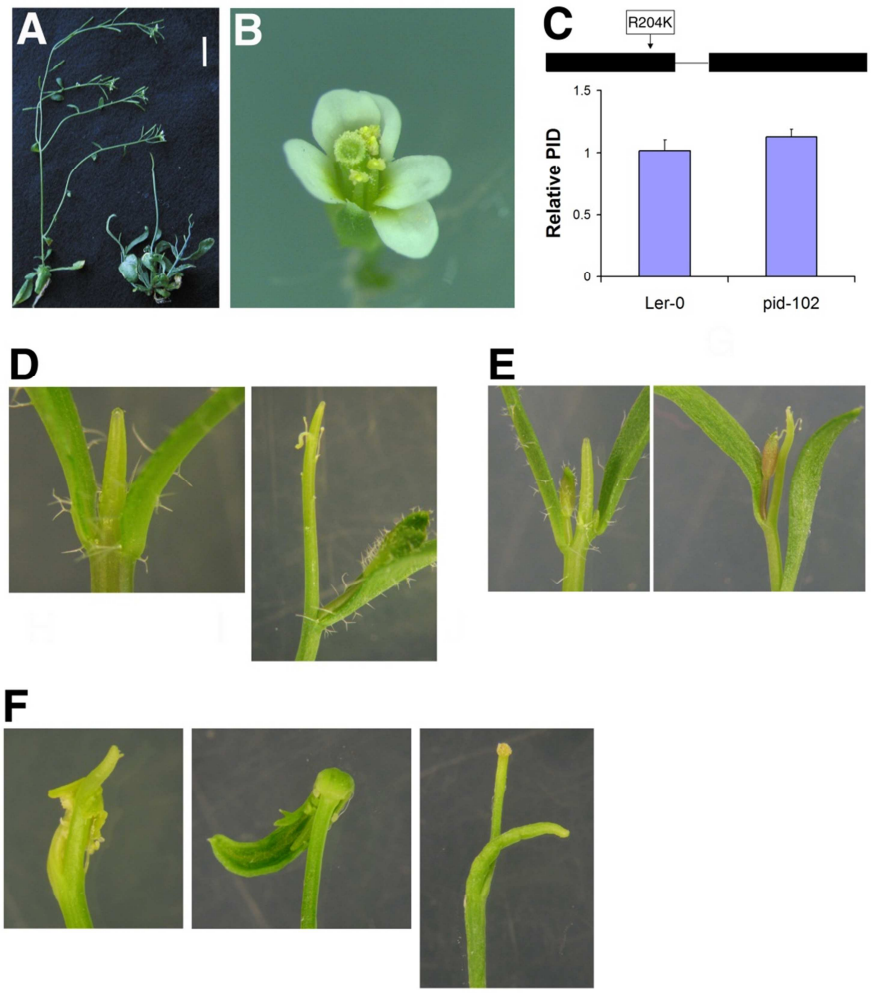


Fig S1. Phenotypic analysis of the *pid-102* allele, *lfy pid* double mutant plants, and response of *lfy-5 pid-8* shoot apex pin structures to exogenous auxin treatment. (**A**) A *pid-102 lfy5* double mutant plant (right) and a wild type Ler-0 plant (left). (**B**) A typical flower of the *pid-102* mutant showing increased number of petals. *pid-102* flowers have 4.9 ± 0.2 petals (n=68) instead of 4 commonly found in wild type flowers. (**C**) The *pid-102* allele maps to an R204K mutation and this mutation does not regulate endogenous PID promoter activity as the abundance of the *PID* transcript was similar in wild-type and *pid-102* shoot apices (primers were designed to detect the 975-1116 bp region in the coding sequence, and results are shown as average and standard error of three samples). (**D**) Shoot apices of two representative *lfy-5 pid-8* double mutant plants, showing the pin structures. (**E**) Shoot apices of two representative *lfy-6 pid-8* double mutant plants. (**F**) The lateral organ production stimulated by exogenous auxin treatment of three representative *lfy-5 pid-8* double mutant plants. Images show lateral organs produced 2 weeks after treatment with exogenous 10 µM IAA in lanolin paste.

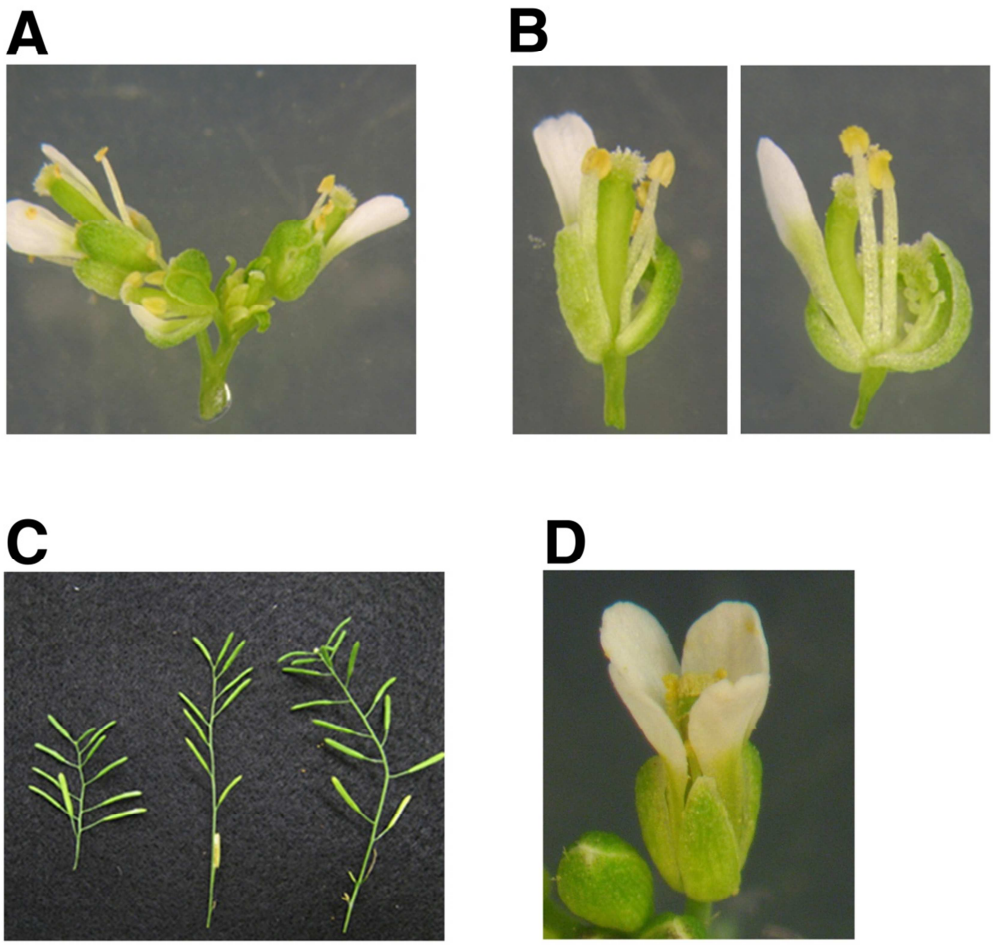
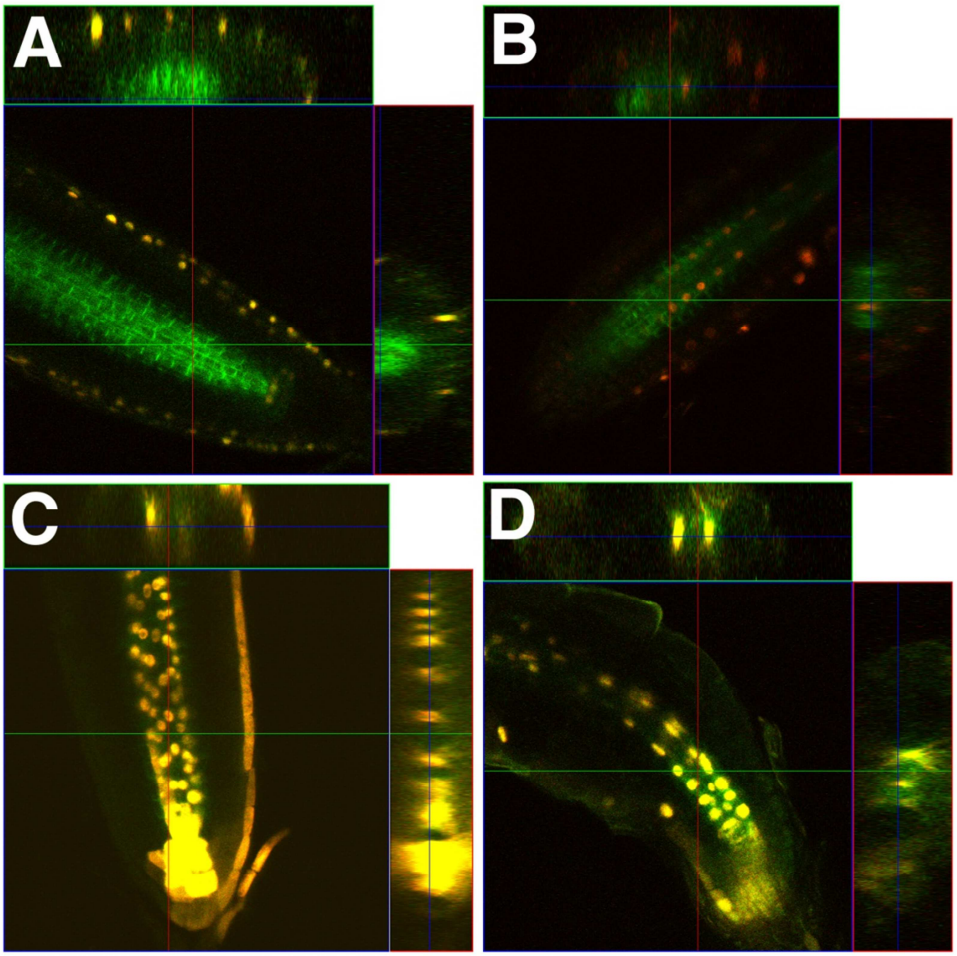


Fig S2. Effect of inducible overexpression of LFY on flower development. Transgenic plants expressing *p35S::GVG-6XUAS::LFY*, which is induced by dexamethasone. (**A**) Abnormal inflorescence structures similar to those of the weak *lfy-5* allele. (**B**) Two representative plants showing defective floral organ development. (**C**) Three representative plants showing altered phyllotaxy. Plants in A-C were exposed to 20 µM dexamethasone for 24 hours and then imaged after 2 weeks. (**D**) Flower development of plants that were exposed to DMSO.



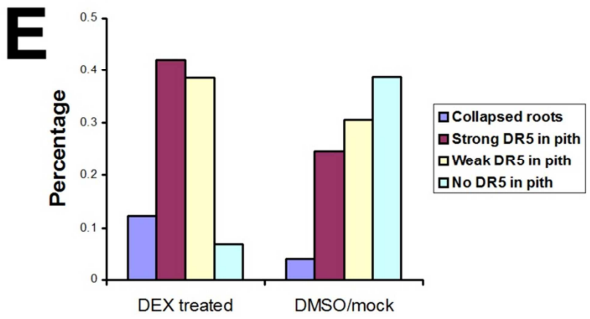


Fig S3. Effect of LFY overexpression on the Venus-N7 reporter of auxin signaling in roots. 7-d old seedlings of *p35S::GVG-6XUAS::LFY, pPIN1::PIN1-GFP, pDR5rev::3XVenus-N7* plants grown in the presence of dexamethasone were imaged for PIN1-GFP (green) and Venus-N7 (red). The seedlings were grouped into 4 categories on the basis of the domain size and signal intensity of Venus-N7, which is referred to as DR5 in the classification. (**A**) No DR5 in pith: DR5 signal present only in the quiescent center with no detectable signal in pith cells; (**B**) Weak DR5 in pith: low to moderate DR5 signal detectable in root pith cells; (**C**) Strong DR5 in pith: strong DR5 signal broadly present in root pith cells; and (**D**) Collapsed roots: roots with broad and strong DR5 signals and with signs of being collapsed. (**E**) Quantitative analysis of the DR5 signal distribution in roots overexpressing LFY. 57 DEX-treated seedlings and 48 Mock (DMSO)-treated seedlings were scored. The distribution of DR5 in the DEX-treated group is significantly different from the untreated one (p=1.290 × 10^{-8}, chi-square test).

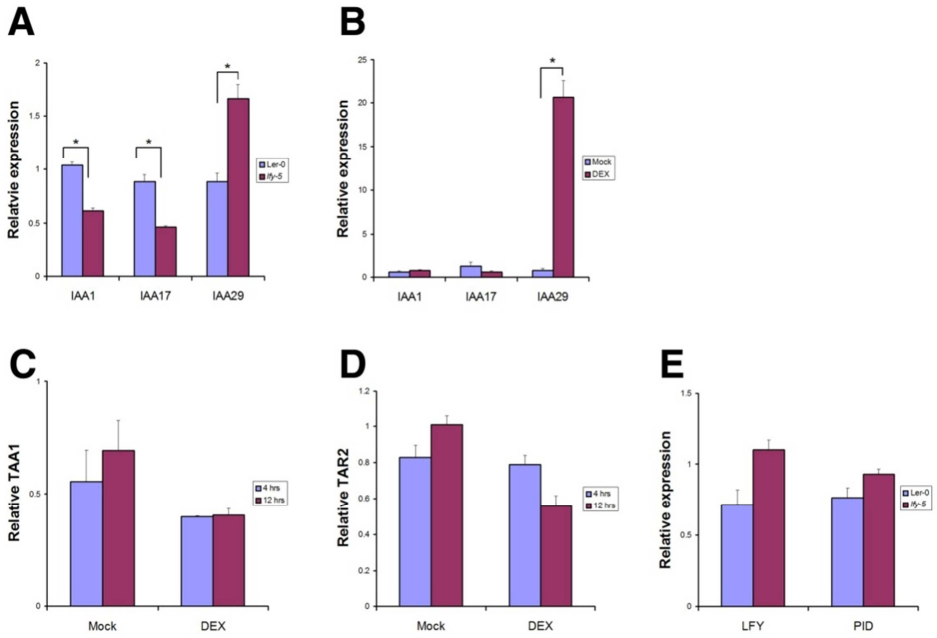


Fig S4. Relative expression of *LFY*, *PID*, *IAA1*, *IAA17*, *IAA29*, *TAA1*, and *TAR2* genes in *lfy-5* and dexamethasone-treated *p35S::GVG-6XUAS::LFY* plants. (**A**) Relative expression of the *IAA1*, *IAA17*, and *IAA29* genes in shoot apices of the indicated genotypes. Plants were exposed to DEX for 4 hours. p = 0.0286, Wilcoxon test. (**B**) The relative expression of *IAA1*, *IAA17*, and *IAA29* in *p35S::GVG-6XUAS::LFY* shoot apices exposed to DEX to induce LFY overexpression. Plants were exposed to DEX for 4 hours or 12 hours. p = 0.0058, Wilcoxon test. (**C**) The relative expression of *TAA1* in *p35S::GVG-6XUAS::LFY* shoot apices exposed to DEX to induce LFY overexpression. Plants were exposed to DEX for 4 hours or 12 hours. (**D**) The relative expression of *TAR2* in *p35S::GVG-6XUAS::LFY* shoot apices exposed to DEX to induce LFY overexpression. Plants were exposed to DEX for 4 hours or 12 hours. Materials used for panels B, C, and D were the T3 generation plants from the homozygous T2 transgenic line #32. (**E**) Relative expression of the *LFY* and *PID* genes in plants of the indicated genotypes. All data are shown as the average and standard error of three experiments. Data are normalized to *ACTIN2* expression.

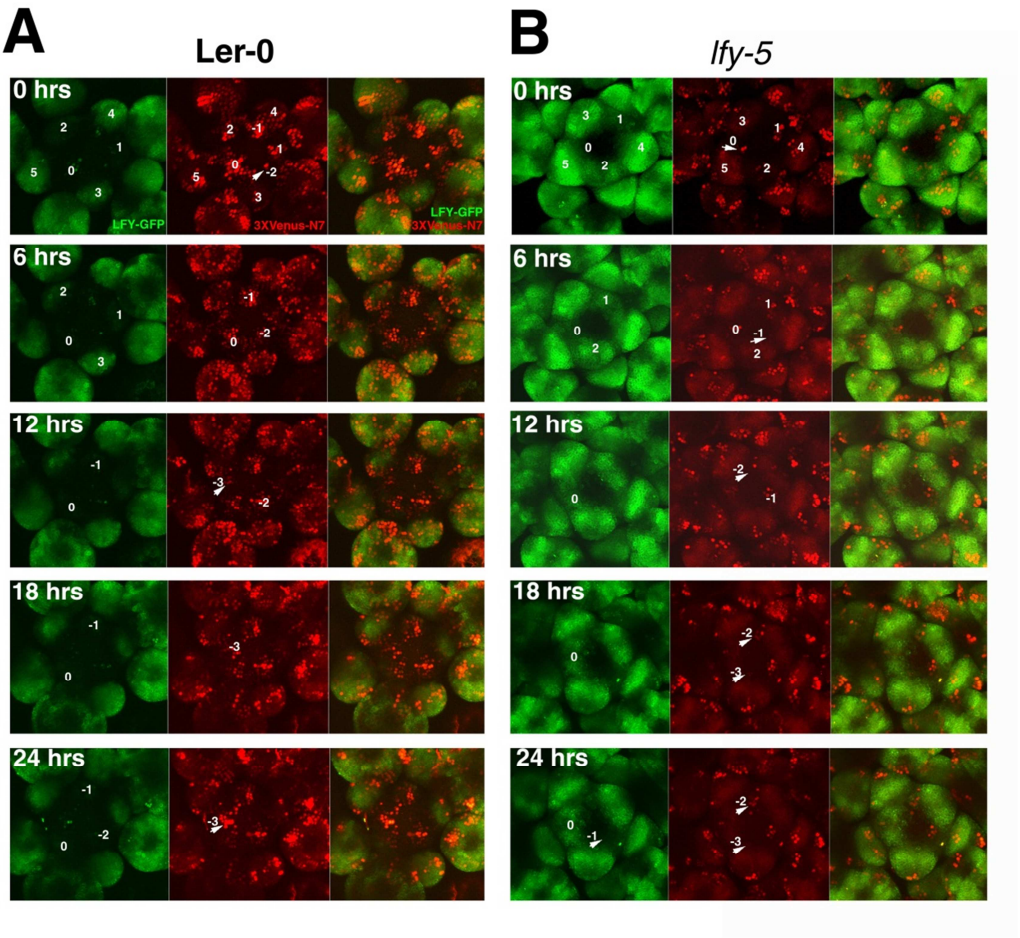


Fig S5. Live imaging of the LFY reporter and the auxin signaling reporter DR5 in SAMs of wild-type and *lfy-5* plants. All plants express *pLFY::GFP-ER* and *pDR5rev::3XVenus-N7*. The LFY reporter is green and the auxin signaling reporter is red. (**A**) Ler-0 plants imaged at a starting time (0) and showing images taken at the indicated times from the same SAM. (**B**) *lfy-5* plants imaged at a starting time (0) and showing images taken at the indicated times from the same plant. The right panels are the overlay of the left and middle panels. Numbers in panels A and B indicate the sites of primordia with the larger numbers represent older (in age) primordia. Number 0 in the top panels indicates the sites of percipient primordia with the background intensity of LFY-GFP signal. Numbers are at the primordia regions unless indicated with arrowheads in which cases the designated primordia sites are pointed at with the arrowheads.

	Mean	s.d.	%95 CI
wild type	2718	205	2315-3121
lfy-5	1476	164	1155-1798

Table S1. Venus-N7 signal intensity in primordial cell nuclei in wild-type and *lfy-5* plants. Representative images of 5 wild-type SAMs and 6 *lfy-5* SAMs were analyzed. All cells of the first four clusters of primordial regions were measured (~20-30 cells per SAM). For wild type, 20-30 cells were picked in two representative clusters among the first four primordial clusters. Two-tailed student's t-test assuming unequal variances showed that p= 0.0028.

Gene	Forward primer	Reverse primer	
qRT-PCR primers			
Actin2	CCTTCGTCTTGATCTTGCGG	AGCGATGGCTGGAACAGAAC	At3g18780
IAA1	TCACAATCCCAAGAAGAGCA	AAGGTTAAGCCCATTGGTGA	At4g14560
IAA17	TAATGATGGGCAGTGTCGAG	CCGTCTCTGAGAACCCTCTCT	At1g04250
IAA29	CAAAGACGAAGCTGCCTTAGA	GCACAATGCTTGTTGAGGTC	At4g32280
LFY	CTTTCGTTGGGAGCTTCTTG	GGCGGAGAGTAGCAAATGAC	At5G61850
LFY 3'-UTR	CTTTGCCATTTGGAGCGGAACAAT	ACGACTAAACAAACCACAACTACCCA	At5g61850
PID	ATATGGCAAGACTCCGTTCG	GTTAAGCAACCCGGAAATCA	At2g34650
TAA1	GATGAAGAATCGGTGGGAGAAGC	CGTCCCTAGCCACGCAAACGCAGG	At1g70560
TAR2	CATGATTTGGCTTACTATTGGCCACAG	GTCTTTCACCAAAGCCCATCCAATC	At4g24670
YUC1	ATGGAGTCTCATCCTCACAACAAAACTG	AAGGGACTCCACGGCTCGAG	At4g32540
YUC4	ATGGGCACTTGTAGAGAATCAGAACC	TAACCGATGGTACGCCTCGGTTT	At5g11320
LFY overexpressor cds for pTA7002	GGGGACAAGTTTGTACAAAAAAGCAGGCT CC ATGGATCCTGAAGGTTTCACGA	GGGGACCACTTTGTACAAGAAAGCTGG GTA CTAGAAACGCAAGTCGTCGCCG	
pLFY::LFY-eGFP:tLFY construct			
pLFY	AGCCCGGGACTAGTTTAATTAA CCTAGG ACGTATACCGTACGGACCGAA	AGAAGTTGCTCGAGTGGTCA	
cLFY	AGCCTAGGGAAACGCAAGTCGTCGCCGC	AGCCTAGGGCTGCGGCTGCTGCCGCTG CGGCAGCC ATGGTGAGCAAGGGCGAGGA	
eGFP	GATTAATTAACTATTACTTGTACAGCTCGT CCATGC	GATTAATTAA T TAGTTTGGTTTGGGTAGTTGTGG	
tLFY, 3'UTR	GAACTAGTCCCGGGTGATCAATCAAAGGG ATCGCCAGAGA	GACATATGAGGACCTTTCATCTCAGGCT	
lfy-5* dCAPS	GGACAGAGAGACAGAGGGAGGATC	TCGTACAAGTGGAACAGATAATCTAAG C	
lfy-6* dCAPS*	AGCAATCTGCTCAAAAGAGTAAAGA	AGAGAGAGAGAGAGAGAGAGAGAC CAAGTCGCATCCCAAAACCAGC	

Table S2. List of primers used in this study. Sequences are listed 5'-3'.
*For *lfy-5* genotyping, dCAPS primers were used to amplify a 100 bp fragment followed with BamHI digestion. Product from Ler-0 DNA will be cut into a 20bp and a 80 bp fragments whereas *lfy-5* product will not be cut.
**For *lfy-6* genotyping, amplification with the dCAPS primers will generate a 234 bp fragment. When cut with BbvI, Ler-0 product will produce a 28bp, a 65 bp, and a 143bp fragments; while *lfy-6* products will produce a 93bp and a 143bp fragments.