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Supplementary Materials for

Phase separation of HRLP regulates flowering time in Arabidopsis

Yu Zhang *et al.*

Corresponding author: Lisha Shen, lisha@tll.org.sg; Hao Yu, dbsyuhao@nus.edu.sg

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Supplementary Materials

abn5488 " Phase separation of HRLP regulates flowering time in Arabidopsis"

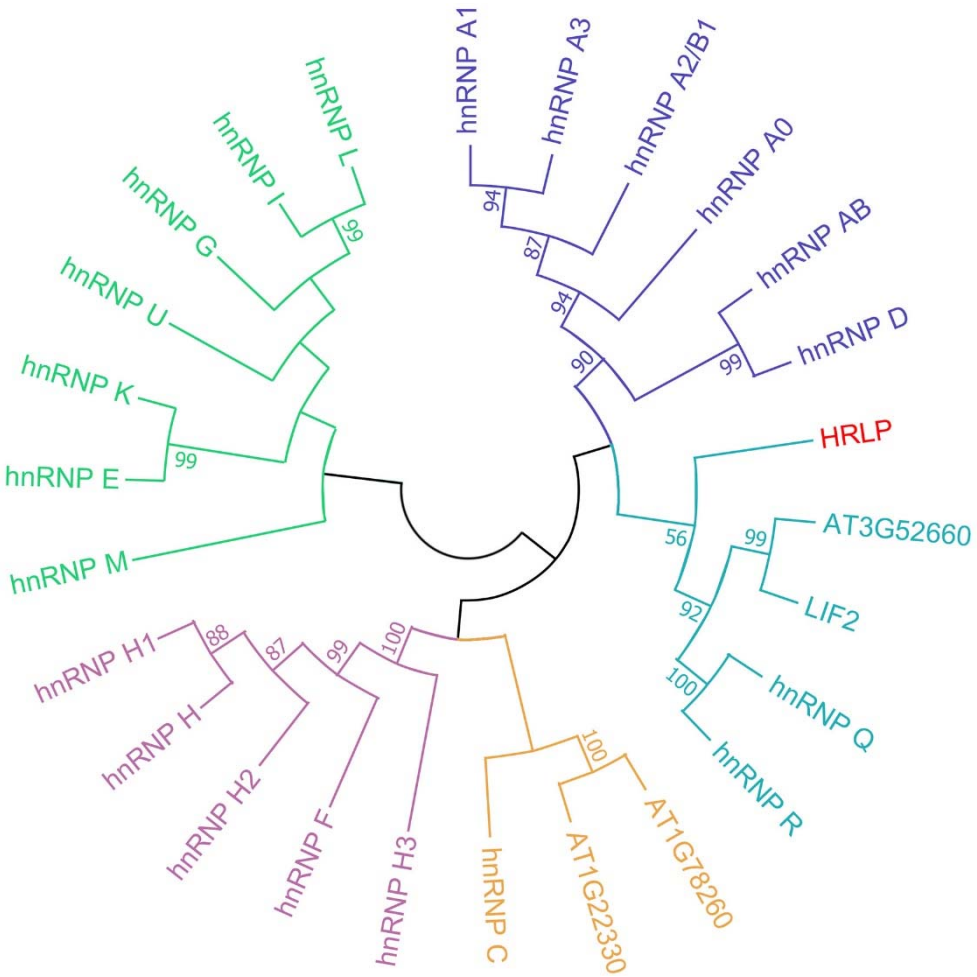

Fig. S1. Phylogenetic analysis of HRLP, its closest homologs in *Arabidopsis*, and human hnRNPs. The protein sequences were obtained from TAIR or NCBI. ClustalW was used to perform the multiple sequence alignment. The phylogenetic tree was constructed by MEGA X using the Maximum Likelihood method. Bootstrap values (> 50%) in 1,000 replicates are shown at the nodes.

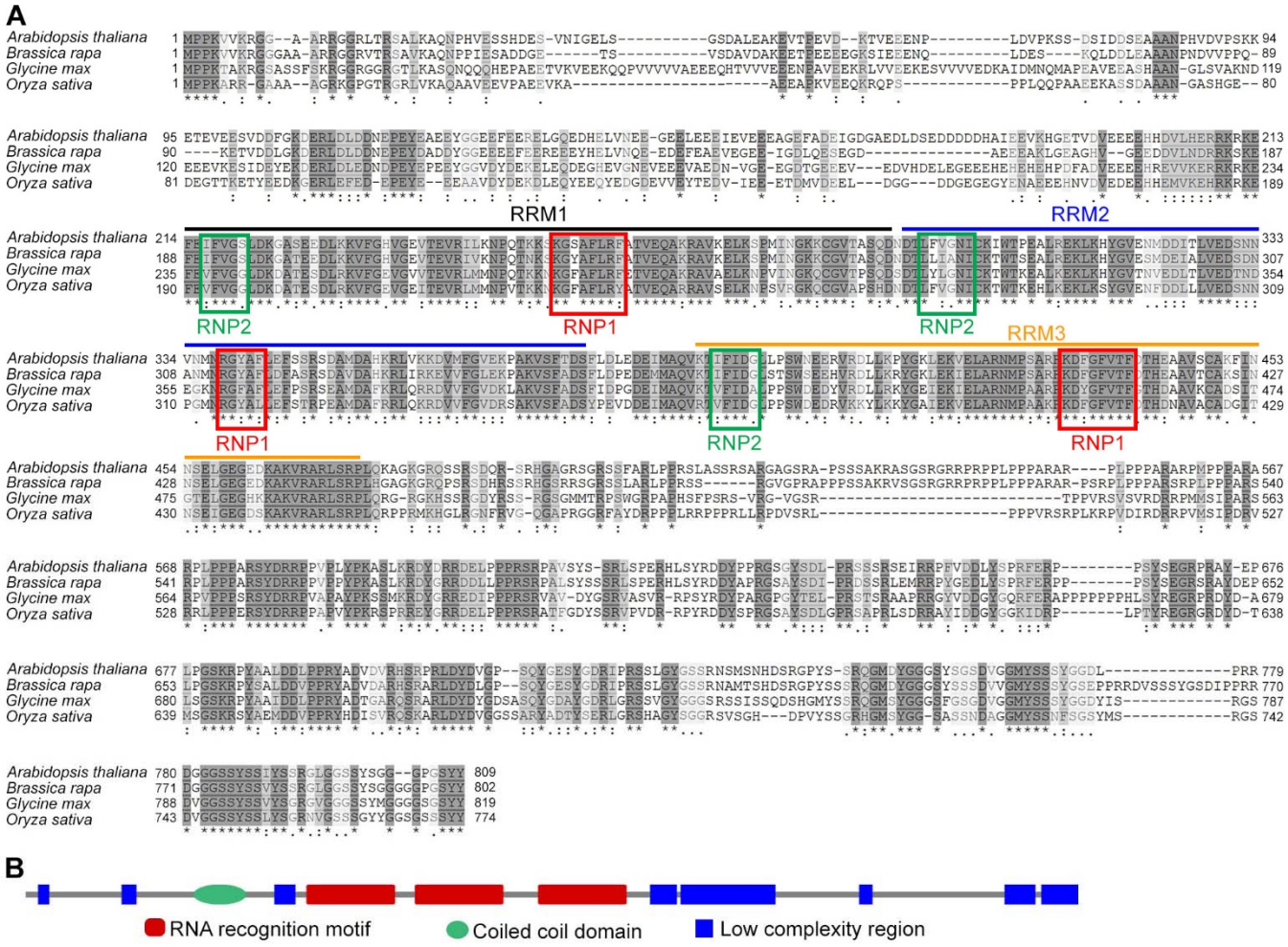

Fig. S2. Protein sequence alignment of HRLP and its closest homologs in other plant species. (**A**) Amino acid sequence alignment of HRLP in *Arabidopsis* and its closest homologs in *Brassica rapa* (Bra004858), *Glycine max* (Glyma.07G119000), and *Oryza sativa* (Os04g45930). These sequences were retrieved from TAIR or NCBI. Three RNA recognition motifs (RRMs) are indicated. Two types of the conserved sequences within the RRMs, RNP1 and RNP2, are shown in red and green boxes, respectively. (**B**) Schematic diagram shows various protein domains of HRLP, including three RRMs, one coiled-coil domain and multiple low complexity regions.

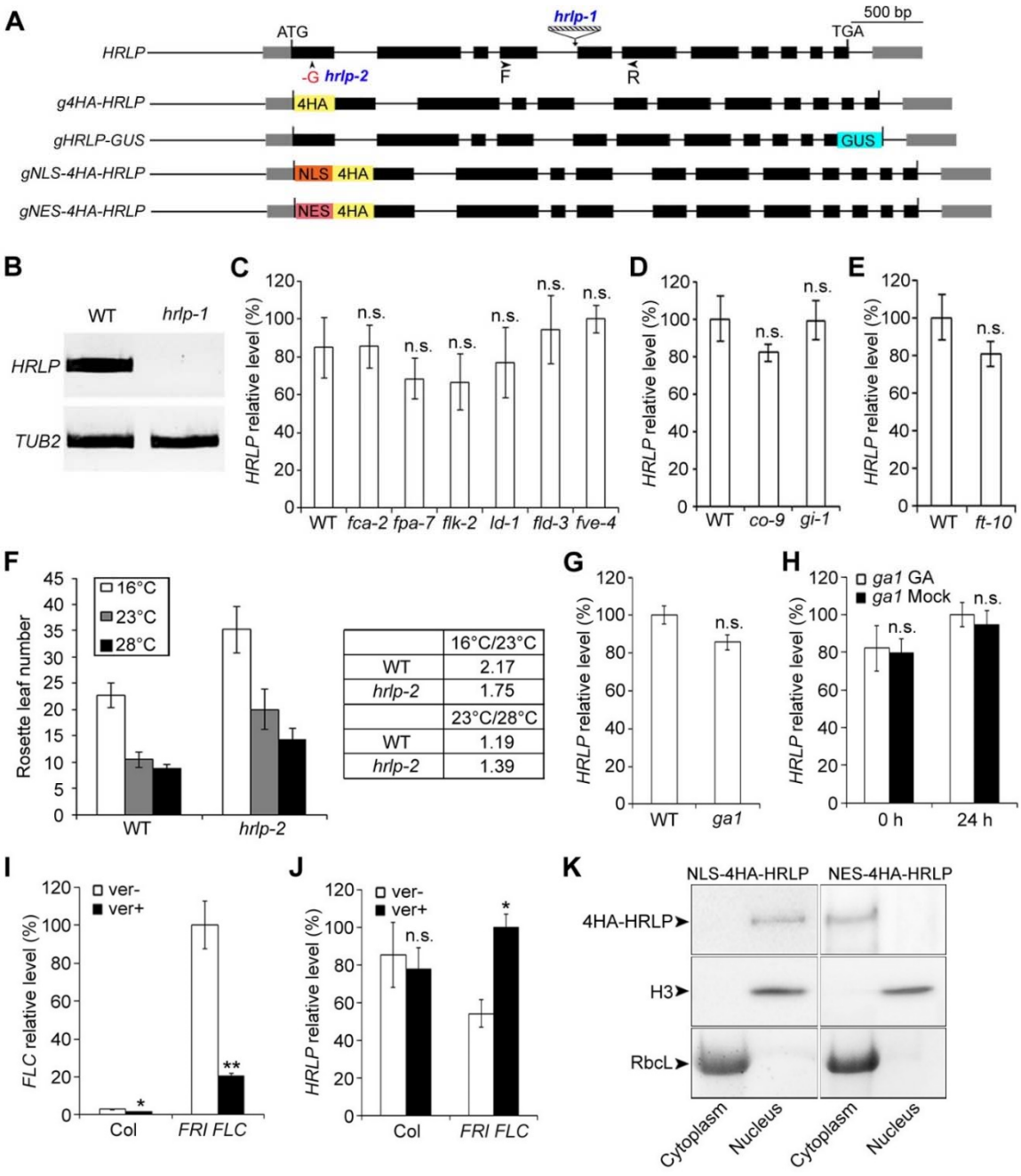

Fig. S3. *HRLP* expression in various flowering pathway mutants. (**A**) Schematic diagrams of *g4HA-HRLP*, *gHRLP-GUS*, *gNLS-4HA-HRLP*, and *gNES-4HA-HRLP* constructs. Exons in the coding regions and UTRs are indicated by black and grey boxes, respectively, while the 5' upstream regions and introns are indicated by black line. The start codon (ATG), stop codon (TGA), as well as the positions of T-DNA insertion site (*hrlp-1*), the 1-bp G deletion (*hrlp-2*) and primers used in semi-quantitative RT-PCR (B) are indicated. (**B**) Semi-quantitative RT-PCR analysis shows *HRLP* expression in wild-type plants, but not in *hrlp-1*. *TUB2* was amplified as an internal control. (**C**) qRT-PCR analysis of *HRLP* expression in 9-day-old wild-type plants and various mutants in the autonomous pathway. Error bars, means ± SD; $n = 3$. n.s. indicate no significant difference between mutants and wild-type plants (two-tailed paired Student's *t*-test;

$P > 0.05$). (**D**) qRT-PCR analysis of *HRLP* expression in 9-day-old wild-type, *co-9* and *gi-1* plants. Error bars, means ± SD; $n = 3$. n.s. indicate no significant difference between mutants and wild-type plants (two-tailed paired Student's *t*-test; $P > 0.05$). (**E**) qRT-PCR analysis of *HRLP* expression in 9-day-old wild-type and *ft-10* plants. Error bars, means ± SD; $n = 3$. n.s. indicate no significant difference between mutants and wild-type plants (two-tailed paired Student's *t*-test; $P > 0.05$). (**F**) Flowering time of *hrlp-2* and wild-type plants grown at 16°C, 23°C, and 28°C under LDs. The ratios of flowering time between 16°C and 23°C, and between 23°C and 28°C are indicated in the attached table. Error bars, means ± SD; $n = 15$. (**G**) qRT-PCR analysis of *HRLP* expression in 3-week-old wild-type and *ga1-3* plants grown under SDs. Error bars, means ± SD; $n = 3$. n.s. indicate no significant difference between mutants and wild-type plants (two-tailed paired Student's *t*-test; $P > 0.05$). (**H**) qRT-PCR analysis of *HRLP* expression in wild-type plants mock-treated (Mock) or treated with 100 μM GA_3 (GA). Error bars, means ± SD; $n = 3$. n.s. indicate no significant difference between mock- and GA-treated plants (two-tailed paired Student's *t*-test; $P > 0.05$). (**I** and **J**) qRT-PCR analysis of *FLC* (I) and *HRLP* (J) expression in 9-day-old wild-type (Col) or *FRI FLC* plants grown under LDs. For vernalization treatment, seedlings were vernalized at 4°C for 8 weeks. Error bars, means ± SD; $n = 3$. Asterisks or n.s. indicate significant or no significant differences, respectively, between treated and non-treated plants (two-tailed paired Student's *t*-test, $*P < 0.05$; $**P < 0.01$; n.s., no statistically significant differences, $P > 0.05$). (**K**) NLS-4HA-HRLP and NES-4HA-HRLP are present exclusively in the nucleus and cytoplasm, respectively. Cytoplasmic and nuclear proteins were extracted from 9-day-old *hrlp-2 gNLS-4HA-HRLP* and *hrlp-2 gNES-4HA-HRLP* seedlings and detected using anti-HA antibody. Histone 3 (H3) examined by anti-H3 antibody and Rubisco large subunit (RbcL) stained with Ponceau S served as the internal controls for nuclear and cytosol fractions, respectively.

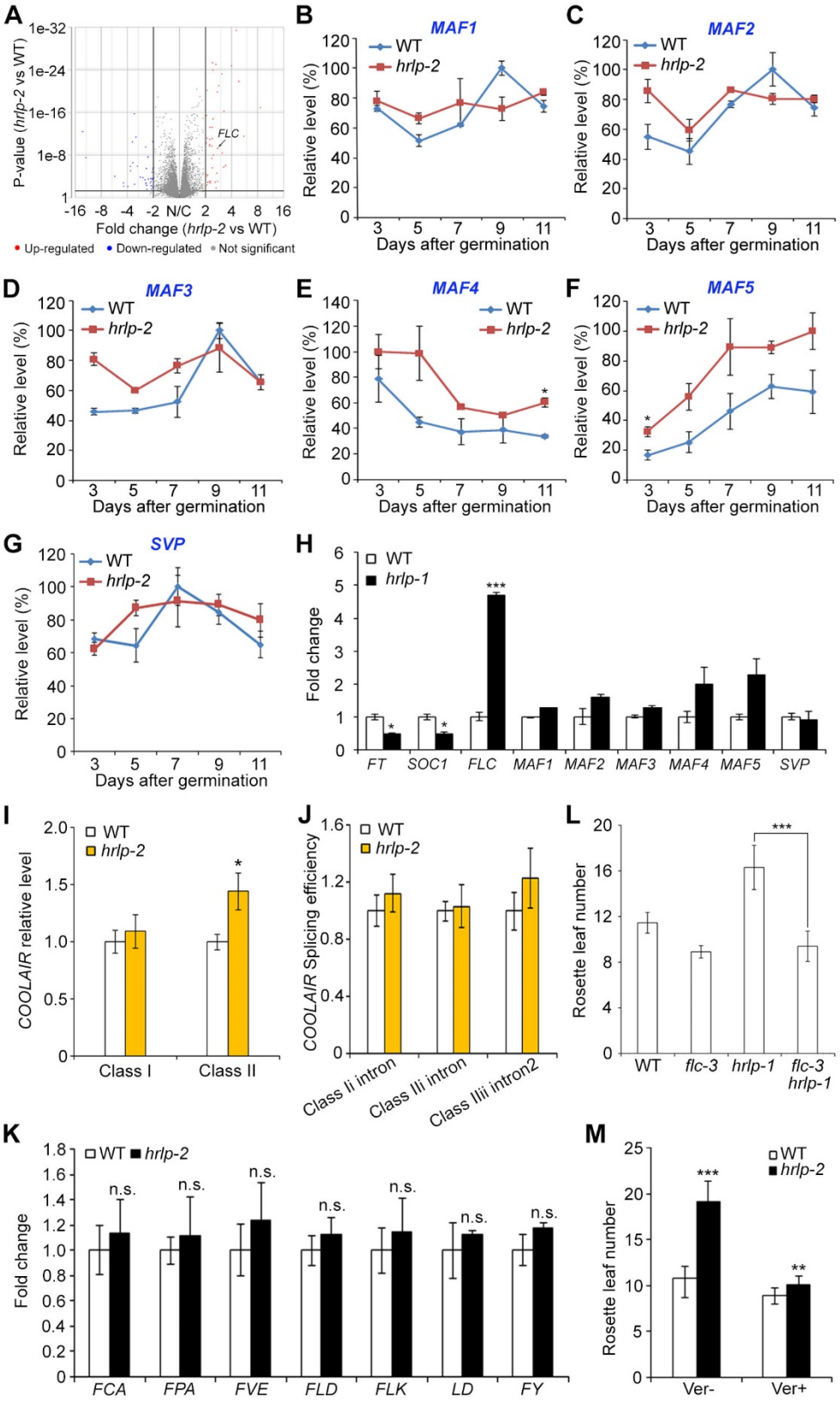

Fig. S4. Expression of various flowering time genes in *hrlp* mutants. (**A**) Volcano plot shows differentially expressed genes in 9-day-old wild-type and *hrlp-2* seedlings. Black lines represent

the significance thresholds of $P < 0.05$ and fold change > 2.0. The flowering time gene *FLC* is indicated with an arrow. (**B** to **G**) Temporal expression of *MAF1* (B), *MAF2* (C), *MAF3* (D), *MAF4* (E), *MAF5* (F), and *SVP* (G) in wild-type and *hrlp-2* seedlings under LDs. Levels of gene expression normalized to *TUB2* expression are shown relative to the maximal expression level set at 100%. Error bars, means ± SD; $n = 3$. Asterisk indicates a statistically significant difference between wild-type and *hrlp-2* plants (two-tailed paired Student's *t*-test, $*P < 0.05$). (**H**) Comparison of expression levels of several flowering genes in 9-day-old WT and *hrlp-1* seedlings. Error bars, means ± SD; $n = 3$. Asterisks indicate statistically significant differences between wild-type and *hrlp-1* seedlings (two-tailed paired Student's *t*-test, $*P < 0.05$, $***P < 0.001$). (**I**) Expression of class I and class II *COOLAIR* in 9-day-old wild-type and *hrlp-2* seedlings. Error bars, means ± SD; $n = 3$. Asterisk indicates a statistically significant difference between wild-type and *hrlp-2* seedlings (two-tailed paired Student's *t*-test, $*P < 0.05$). (**J**) Splicing efficiency of *COOLAIR* class Ii intron, class IIi intron and class IIii intron2 in 9-day-old wild-type and *hrlp-2* seedlings. The splicing efficiency was determined by normalizing the expression levels of the featured exon against the corresponding unspliced alternative intron. The levels in wild-type plants are set as 1.0. Error bars, means ± SD. (**K**) Comparison of expression levels of autonomous pathway genes in 9-day-old wild-type and *hrlp-2* seedlings. Error bars, means ± SD; $n = 3$. n.s. indicates no statistically significant differences between wild-type and *hrlp-2* (two-tailed paired Student's *t*-test, n.s., $P > 0.05$). (**L**) Flowering time of wild-type, *flc-3*, *hrlp-1*, and *flc-3 hrlp-1* under LDs. Error bars, means ± SD; $n = 20$. Asterisks indicate a significant difference between the specified genotypes (two-tailed paired Student's *t*-test, $***P < 0.001$). (**M**) Flowering time of *hrlp-2* and wild-type plants with and without vernalization under LDs. Seedlings were vernalized at 4°C for 8 weeks before they were moved to LDs. Error bars, means ± SD; $n = 20$. Asterisks indicate statistically significant differences between wild-type and *hrlp-2* mutants with or without vernalization (two-tailed paired Student's *t*-test, $**P < 0.01$, $***P < 0.001$).

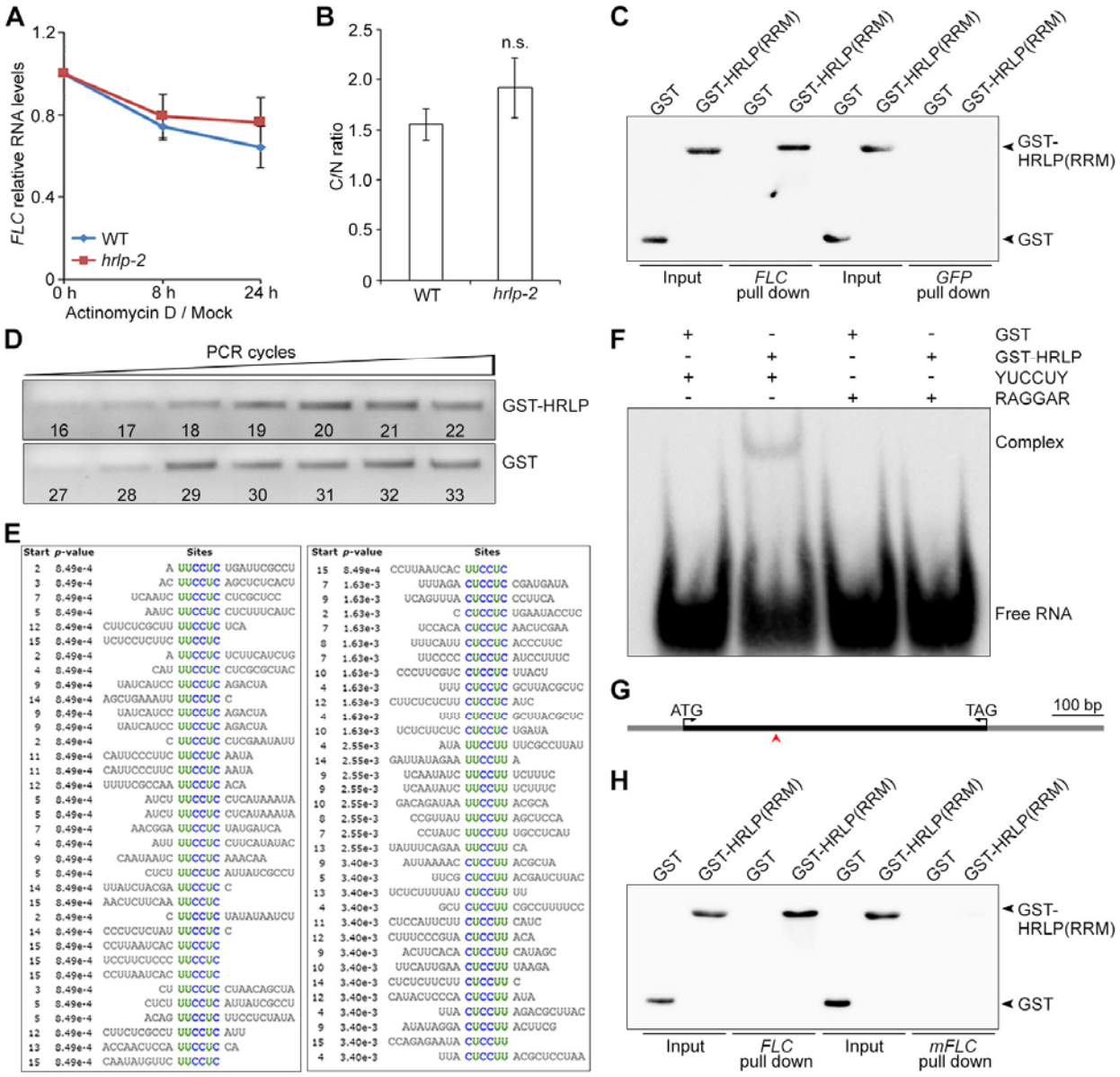

Fig. S5. Examination of HRLP effect on other aspects of *FLC* RNA metabolism. (**A**) qRT-PCR analysis of transcript levels of *FLC* in 5-day-old wild-type and *hrlp-2* seedlings treated by actinomycin D versus mock treatment for 0, 8, and 24 h. *FLC* relative levels were calculated by normalizing the expression in actinomycin D-treated seedlings against that in mock-treated seedlings. Error bars, means ± SD; $n = 3$. (**B**) *FLC* expression levels in cytoplasm (C) versus nucleus (N) in 9-day-old wild-type and *hrlp-2* seedlings. Error bars, means ± SD; $n = 3$. n.s. indicates no statistical differences between *hrlp-2* and wild-type (two-tailed paired Student's *t*-test, $P > 0.05$). (**C**) *In vitro* binding of GST-HRLP(RRM) to the *FLC* mRNA probe. GST protein and *GFP* mRNA probe are included as negative controls. (**D**) Gel electrophoresis of enriched fragments after seven rounds of selection in SELEX experiments. The numbers indicate PCR cycles. DNA fragments amplified from 17 and 28 cycles of GST-HRLP and GST selection, respectively, were selected for sequencing. (**E**) Sequence alignment of SELEX results of GST-HRLP selection. MEME suite (http://meme-suite.org/index.html) was used to align the

sequences. (**F**) EMSA assay shows the specific *in vitro* binding of GST-HRLP to the YUCCUY motif. Biotin labeled RNA oligos containing four repeats of either 5'-YUCCUY-3' or 5'-RAGGAR-3' were used as probes. (**G**) Location of the YUCCUY motif (indicated by the red arrowhead) in the *FLC* mRNA. The UTRs and CDS are indicated by grey and black boxes, respectively. (**H**) Deletion of the YUCCUY sequence from *FLC* mRNA (*mFLC*) abolishes its *in vitro* binding with GST-HRLP(RRM).

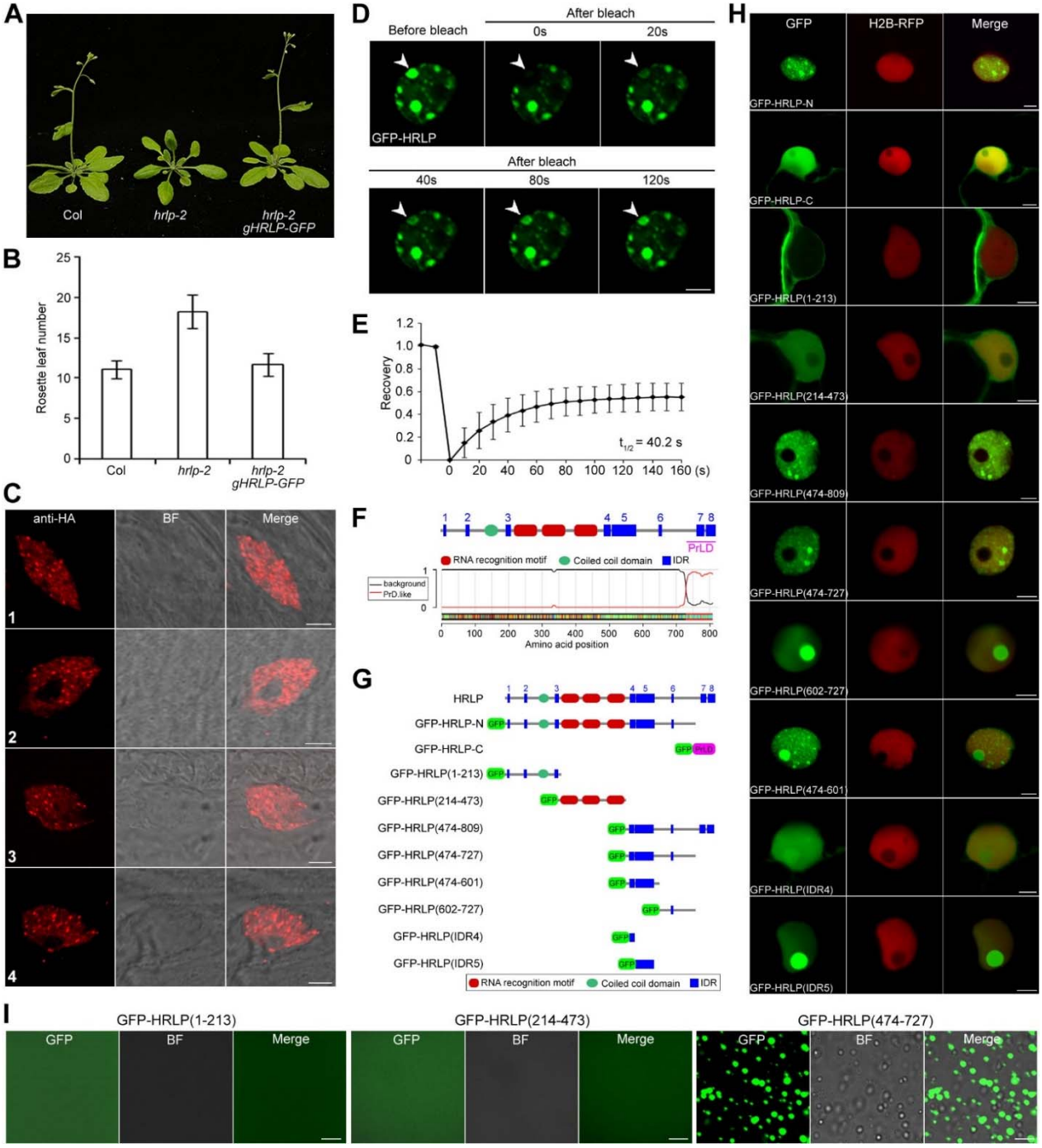

Fig. S6. The IDR (4&5) is required for the formation of HRLP nuclear bodies. (**A**) A representative line of *hrlp-2 gHRLP-GFP* shows comparable flowering time to a wild-type plant. (**B**) Flowering time of *hrlp-2 gHRLP-GFP* under LDs. Error bars, means ± SD; $n = 20$. (**C**) Immunolocalization of 4HA-HRLP in nuclear bodies of four *hrlp-2 g4HA-HRLP* root tip cells. BF, bright field; Merge, merge of anti-HA and BF images. Bars = 5 μm. (**D**) FRAP assay of a GFP-HRLP nuclear body in *N. benthamiana* leaf epidermal cells infiltrated with *35S:GFP-HRLP*. Time (0s to 120s) indicates the duration of the photobleaching pulse. Arrowheads indicate the photobleached GFP-HRLP nuclear bodies. Bar = 5 μm. (**E**) FRAP recovery plot of

HRLP-GFP nuclear bodies in *N. benthamiana* leaf epidermal cells. The value at the beginning of photobleaching is set as 0. Error bars, means ± SD; n = 15. The half-time of recovery ($t_{1/2}$) was calculated from the logarithmic curve and the best-fit values were generated by GraphPad. (**F**) Protein domain structure of HRLP. The bottom panel shows the prediction of the PrLD domain by "Prion-like Amino Acid Composition" (PLAAC; http://plaac.wi.mit.edu/). (**G**) The schematic diagrams of truncated HRLP proteins fused with GFP at the N-terminus. (**H**) Subcellular localization of truncated HRLP proteins in *N. benthamiana* leaf epidermal cells. GFP, GFP fluorescence; H2B-RFP, RFP fluorescence of H2B-RFP; Merge, merge of GFP and H2B-RFP images. Bars = 5 μm. (**I**) *In vitro* phase separation of GFP-HRLP(1-213), GFP-HRLP(214-473), GFP-HRLP(474-727) proteins (10 μM) in the presence of PEG8000. GFP, GFP fluorescence; BF, bright field; Merge, merge of GFP and BF images. Bar = 10 μm.

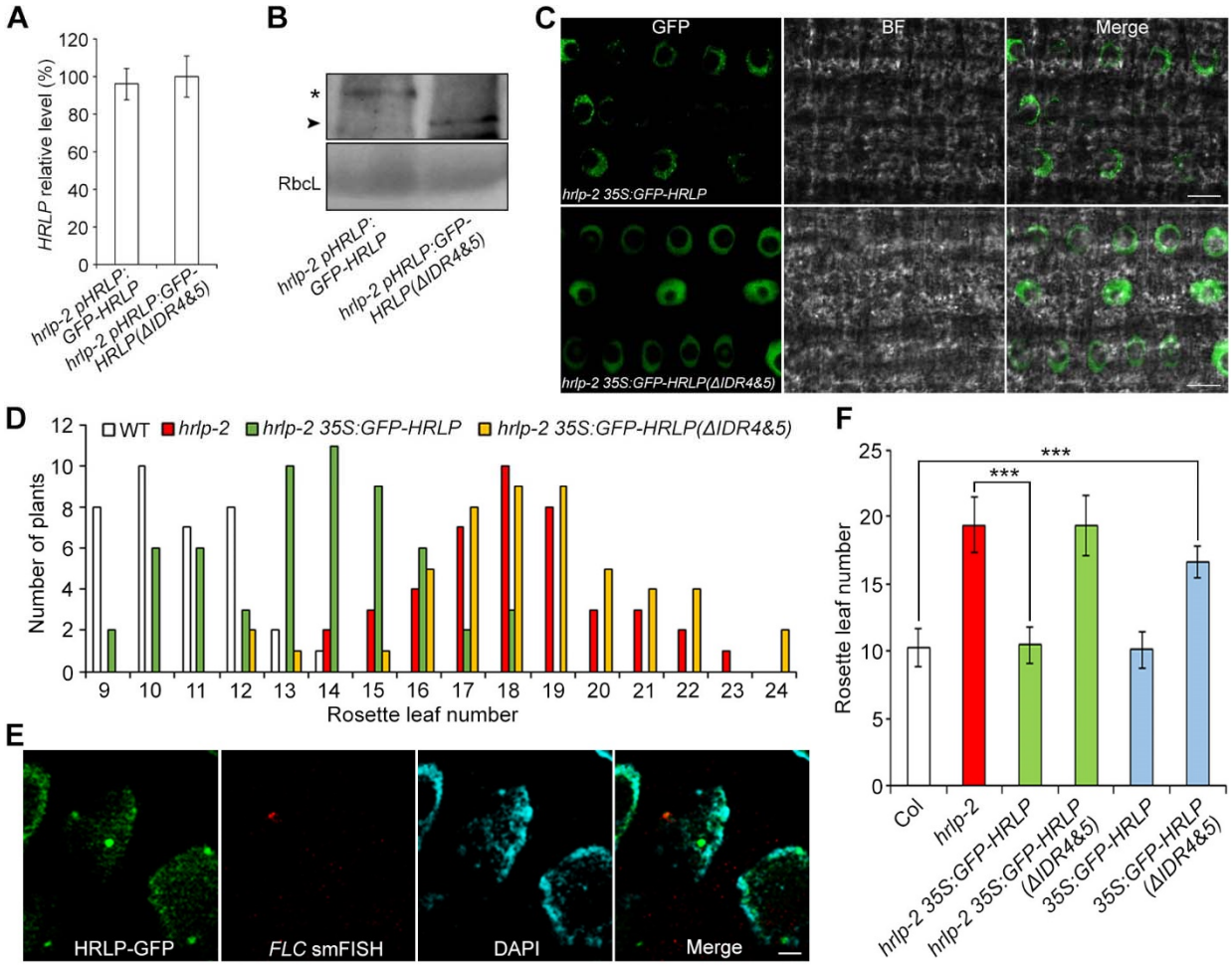

Fig. S7. Phase separation of HRLP is required for its function in flowering time control. (**A**) *HRLP* expression in the representative *hrlp-2 pHRLP:GFP-HRLP* and *hrlp-2 pHRLP:GFP-HRLP(ΔIDR4&5)* transgenic plants. Levels of gene expression normalized to *TUB2* expression are shown relative to the maximal expression level set at 100%. Error bars, means ± SD; $n = 3$. (**B**) Western blot analysis of GFP-HRLP and GFP-HRLP(ΔIDR4&5) expression in the representative *hrlp-2 pHRLP:GFP-HRLP* and *hrlp-2 pHRLP:GFP-HRLP(ΔIDR4&5)* transgenic plants. RbcL stained with Ponceau S served as the internal control. Asterisk and arrowhead indicate the position of GFP-HRLP and GFP-HRLP(ΔIDR4&5), respectively. (**C**) Subcellular localization of GFP-HRLP and GFP-HRLP(ΔIDR4&5) in root tip cells of 5-day-old *hrlp-2 35S:GFP-HRLP* and *hrlp-2 35S:GFP-HRLP(ΔIDR4&5)* transgenic lines, respectively. BF, bright field; Merge, merge of GFP and BF images. Bar = 10 μm. (**D**) Flowering time distribution of T1 transgenic plants of *hrlp-2 35S:GFP-HRLP* and *hrlp-2 35S:GFP-HRLP(ΔIDR4&5)*. (**E**) Representative images showing *hrlp-2 gHRLP-GFP* root tip cells hybridized with smFISH probes against *FLC* intron 1. HRLP-GFP, GFP fluorescence of HRLP-GFP; *FLC* smFISH, signals of nascent *FLC* transcripts; DAPI, DAPI staining indicating nuclei; Merge, merge of all channels. Bar = 2 μm. (**F**) Flowering time of various transgenic plants. Error bars, means ± SD; $n = 20$. Asterisks indicate significant differences between the specified genotypes (two-tailed paired Student's *t*-test, $***P < 0.001$).

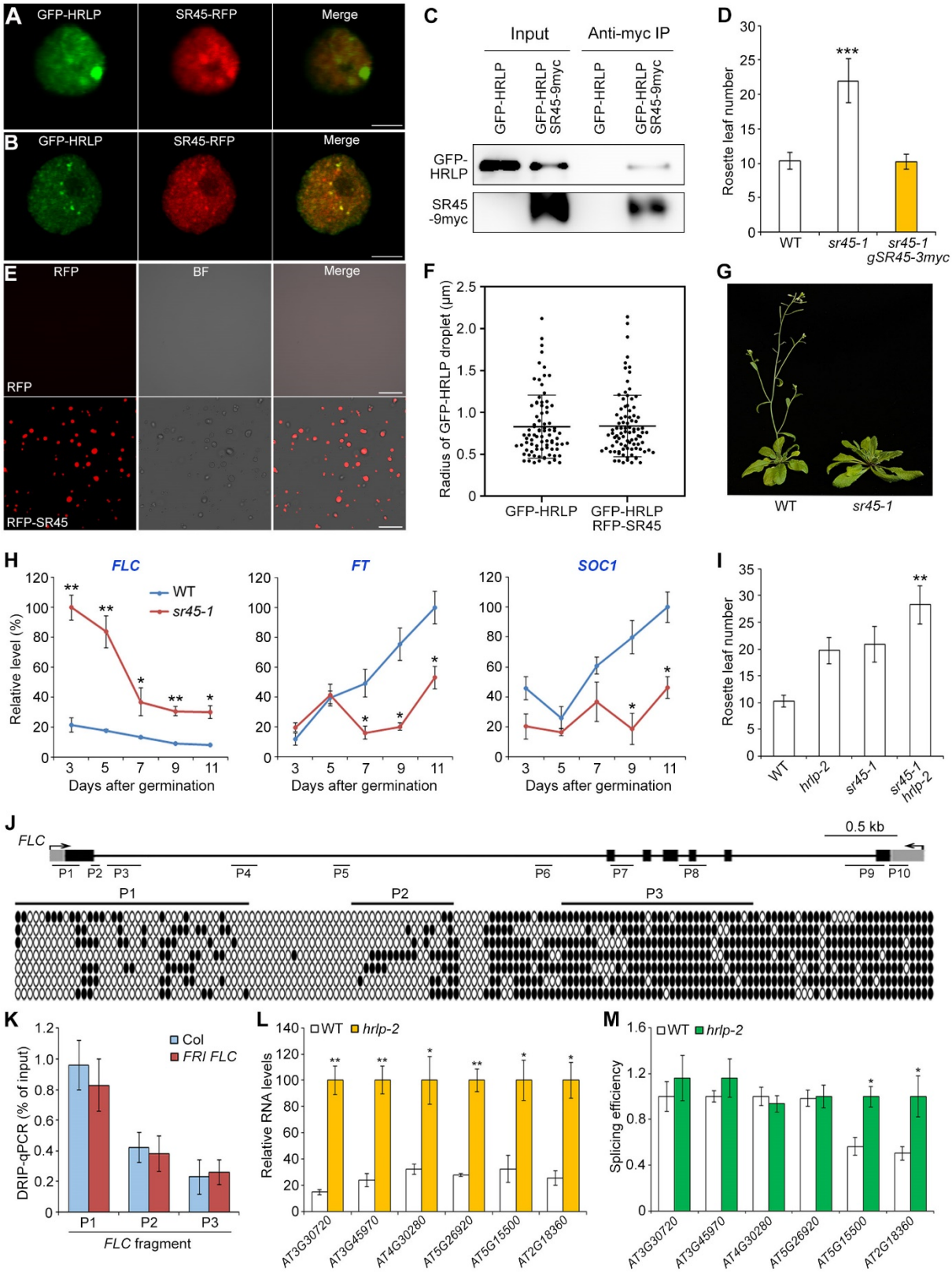

Fig. S8. SR45 controls flowering time in *Arabidopsis*. (**A and B**) Colocalization of GFP-HRLP and SR45-RFP in an *Arabidopsis* mesophyll protoplast (A) and a *N. benthamiana* leaf epidermal

cell (B). Merge, merge of GFP-HRLP and SR45-RFP images. Bar = 5 μm. (**C**) CoIP experiment shows the interaction between HRLP and SR45. *35S:GFP-HRLP* and *35S:SR45-9myc* were co-expressed in *N. benthamiana* leaf epidermal cells. Nuclear proteins were extracted and immunoprecipitated with anti-myc antibody. Protein extracts as the input and co-immunoprecipitated proteins were detected by either anti-GFP (upper panel) or anti-myc (lower panel) antibody. (**D**) Flowering time of *sr45-1* and *sr45-1 gSR45-3myc* under LDs. Error bars, means ± SD; $n = 20$. Asterisk indicates a statistically significant difference between wild-type and *sr45-1* plants (two-tailed paired Student's *t*-test, ***$P < 0.001$). **(E)** *In vitro* phase separation of RFP and RFP-SR45 proteins (10 μM) with the addition of PEG8000. RFP, RFP fluorescence; BF, bright-field image; Merge, merge of RFP and BF images. Bars = 10 μm. (**F**) Comparison of the size of droplets formed by GFP-HRLP without or with RFP-SR45 in *in vitro* phase separation assays using Image J. There is no significant difference between these two groups (two-tailed paired Student's *t*-test, $P > 0.05$). (**G**) *sr45-1* exhibits late flowering under LDs. (**H**) Temporal expression of *FLC*, *FT* and *SOC1* in developing wild-type and *sr45-1* seedlings under LDs. Levels of gene expression normalized to *TUB2* expression are shown relative to the maximal expression level set at 100%. Error bars, means ± SD; $n = 3$. Asterisks indicate significant differences between wild-type and *sr45-1* plants (two-tailed paired Student's *t*-test, *$P < 0.05$, **$P < 0.01$). (**I**) Comparison of flowering time of wild-type, *hrlp-2*, *sr45-1*, and *sr45-1 hrlp-2* plants under LDs. Error bars, means ± SD; $n = 20$. Asterisk indicates a significant difference between *sr45-1 hrlp-2* and their single mutants (two-tailed paired Student's *t*-test, **$P < 0.01$). (**J**) Sequence analysis of R-loop foot-printing assay. An empty circle indicates a T nucleotide converted from an unpaired C nucleotide under sodium bisulfite treatment, whereas a solid circle indicates an unconverted and paired C nucleotide. (**K**) DRIP analysis of R-loop at the *FLC* locus in 9-day-old wild-type (Col) and *FRI FLC* seedlings. Primer pairs (P1 to P3) are designed as shown in Fig. 7H. Error bars, means ± SD; $n = 3$. (**L**) Expression of several differentially expressed genes selected from fig. S4A in 9-day-old wild-type and *hrlp-2* seedlings. Levels of gene expression normalized to *TUB2* expression are shown relative to the maximal expression level set at 100%. Error bars, means ± SD; $n = 3$. Asterisks indicate significant differences between wild-type and *hrlp-2* seedlings (two-tailed paired Student's *t*-test, **$P < 0.01$). (**M**) Splicing efficiency of introns of the genes examined in (L) in 9-day-old wild-type and *hrlp-2* seedlings. The levels in wild-type plants are set as 1.0. Error bars, means ± SD; $n = 3$.

Table S1. Potential interacting partners of HRLP isolated from CoIP followed by LC-MS/MS analysis.

Gene Locus	Unique peptide numbers	Confidence (%)	Peptides' sequences	Annotations
AT2G44710 (bait)	8	99	ETEVEESVDDFGKDER, EVTPEVDKTVEEENPLDVPK, FINNSELGEGEDKAK, RPFVDDLYSPR, SSDSIDDSEAAANPHVDVPSK, SSDSIDDSEAAANPHVDVPSKK, VFGHVGEVTEVR, RPPVPLYPK	HRLP (this study)
AT5G26742	6	98.79-99	AVLVPALQGR, GVDVVVGTPGR, IFLIADDR, IIDLIEGR, SFGGSCFICGK, FLVLAPTR	DEAD box RNA helicase (RH3)
AT1G10270	5	99	GQAADSTVYNNLIR, IGDAASLLR, LPDSTSALVGQR, LTESAEVLTK, TMAFSSAEEAAAER	glutamine-rich protein 23
AT5G40490	2	99	IELSGTQVEIK, YGEITDSVIMK	RNA-binding (RRM/RBD/RNP motifs) family protein
AT3G49430	2	99	KAGDVCFAEVTR, SIYVGNLPGDIR	SER/ARG-rich protein 34A
AT4G38780	2	99	SNPALYVLR, TAEEVAALVR	pre-mRNA-processing-splicing factor-like protein
AT1G76010	2	99	FLAPVNPFAK, LADCSIGFGR	Alba DNA/RNA-binding protein
AT1G80070	2	99	SNPALYVLR, TAEEVAALVR	Pre-mRNA-processing-splicing factor
AT1G16610	2	98.87-99	AQLYMDGAQIDGK, ATFTLPPR	arginine/serine-rich 45
AT5G64200	1	99	VVDVFIPR	serine/arginine-rich splicing factor-like protein
AT1G20220	1	99	ADTPIAENEIR	Alba DNA/RNA-binding protein
AT4G35785	1	97.91	YLNQSVLEGR	RNA-binding (RRM/RBD/RNP motifs) family protein

Table S2. List of primers used in this study.

Primers used for plasmid construction

Plasmid	Primer name	Primer sequence
g4HA-HRLP	gHRLP-F	5'-CACCAACATGTCTAGTACTACTTTCTTTG-3'
	gHRLP-R	5'-AAGCTGAGAAGACGGTGAAAGC-3'
	4HA-F-*Spe*I	5'-GGACTAGTTATCCATATGACGTTCCAGA-3'
	4HA-R-*Spe*I	5'-GGACTAGTTCTAGTAGCGTAATCTG-3'
	gHRLP-N-*Spe*I-F	5'-TGGAAAGCGATGACTAGTCCACCGAAGGTTG-3'
	gHRLP-N-*Spe*I-R	5'-CAACCTTCGGTGGACTAGTCATCGCTTTCCA-3'
gNLS-4HA-HRLP	gNLS-4HA-F-*Spe*I	5'-CCCACTAGTCCTAAGAAGAAGAGAAAGGTTT ATCCATATGACGTTCCAGA-3'
	4HA-R-*Spe*I	5'-GGACTAGTTCTAGTAGCGTAATCTG-3'
gNES-4HA-HRLP	gNES-4HA-F-*Spe*I	5'-CCCACTAGTCTTGCTCTTAAGTTGGCTGGACTT GATATTTATCCATATGACGTTCCAGA -3'
	4HA-R-*Spe*I	5'-GGACTAGTTCTAGTAGCGTAATCTG-3'
gHRLP-GFP	gHRLP-C-*Spe*I-F	5'-AGGATCATACTACACTAGTTGAATGTGAGTGT GTT-3'
	gHRLP-C-*Spe*I-R	5'-AACACACTCACATTCAACTAGTGTAGTATGAT CCT-3'
	GFP-F-*Spe*I	5'-GGACTAGTATGAGTAAAGGAGAAGAACT-3'
	GFP-R-*Spe*I	5'-GGACTAGTTTTGTATAGTTCATCCATGC-3'
gHRLP-GUS	GUS-F-*Spe*I	5'-GGACTAGTATGTTACGTCCTGTAGAAAC-3'
	GUS-R-*Spe*I	5'-GGACTAGTTCATTGTTTGCCTCCCTGCT-3'
35S:GFP-HRLP; *35S:HRLP-GFP*	HRLP-F-*BamH*I	5'-CGGGATCCATGCCACCGAAGGTTGTGAAG-3'
	HRLP-R-*Spe*1	5'-GGACTAGTTCAGTAGTATGATCCTGGAC-3'
GST-HRLP	HRLP-F-*BamH*I	5'-CGGGATCCATGCCACCGAAGGTTGTGAAG-3'
	HRLP-R-*Xho*I	5'-CCGCTCGAGTCAGTAGTATGATCCTGGAC-3'
GST-HRLP(RRM)	RRM-F-*Xma*I	5'-CCCCCCGGGATGCATCATGATGTTCTCCATGA-3'
	RRM-R-*Xho*I	5'-CCGCTCGAGTCAACTCCTTCCAGACCGTCCAG-3'
35S:GFP-HRLP-N	HRLP-F-*BamH*I	5'-CGGGATCCATGCCACCGAAGGTTGTGAAG-3'
	HRLP(N)-R-*Spe*I	5'-GGACTAGTACTAGACCTAGGAATCCGGT-3'
35S:GFP-HRLP-C	PrLD-F-*BamH*I	5'-CGGGATCCCTAGGATATGGAAGCAGCCG-3'
	HRLP-R-*Spe*I	5'-GGACTAGTTCAGTAGTATGATCCTGGAC-3'
35S:GFP-HRLP(1-213)	HRLP-F-*BamH*I	5'-CGGGATCCATGCCACCGAAGGTTGTGAAG-3'
	213-R-*Spe*I	5'-GGACTAGTCTCCTTACGCTTACGCCTCT-3'
35S:GFP-HRLP(214-473)	214-F-*BamH*I	5'-CGGGATCCTTCGAAATATTTGTTGGGAG-3'
	473-R-*Spe*I	5'-GGACTAGTTGGTCTAGACAGGCGTGCTC-3'
35S:GFP-HRLP(474-809)	474-F-*BamH*I	5'-CGGGATCCCTTCAGAAAGCAGGTAAAGG-3'
	HRLP-R-*Spe*I	5'-GGACTAGTTCAGTAGTATGATCCTGGAC-3'
35S:GFP-HRLP(474-727)	474-F-*BamH*I	5'-CGGGATCCCTTCAGAAAGCAGGTAAAGG-3'
	HRLP(N)-R-*Spe*I	5'-GGACTAGTACTAGACCTAGGAATCCGGT-3'
35S:GFP-HRLP(IDR4&5)	474-F-*BamH*I	5'-CGGGATCCCTTCAGAAAGCAGGTAAAGG-3'
	601-R-*Spe*I	5'-GGACTAGTAAGTTCATCACGCCGATCAT-3'
35S:GFP-HRLP(ΔIDR4&5)	HRLP-F-*BamH*I	5'-CGGGATCCATGCCACCGAAGGTTGTGAAG-3'
	473-R-*Spe*I	5'-GGACTAGTTGGTCTAGACAGGCGTGCTC-3'
	602-F-*Spe*I	5'-GGACTAGTCCTCCTCCAAGAAGCAGACC-3'
	HRLP-R-*Spe*I	5'-GGACTAGTTCAGTAGTATGATCCTGGAC-3'
GST-GFP	GST-GFP-F-*BamH*I	5'-CGCGGATCCATGAGTAAAGGAGAAGAACT-3'

	GST-GFP-R-*EcoR*I	5'-CCGGAATTCTTTGTATAGTTCATCCATGC-3'
GST-GFP-HRLP; *GST-GFP-HRLP(Δ IDR4&5)*	HRLP-F-*Sal*I HRLP-R-*Not*I	5'-ACGCGTCGACTCATGCCACCGAAGGTTGTGAAG-3' 5'-AAGGAAAAAAGCGGCCGCTCAGTAGTATGATCC TGGAC-3'
GST-GFP-HRLP(IDR4&5)	474-F-*Sal*I 601-R-*Not*I	5'-ACGCGTCGACTCCTTCAGAAAGCAGGTAAAGG-3' 5'-AAGGAAAAAAGCGGCCGCAAGTTCATCACGCCG ATCAT-3'
GST-RFP-SR45	GST-RFP-F(*BamH*1) GST-RFP-R(*Xma*I) SR45-F(*Xma*1) SR45-R(*Not*1)	5'-GGGGCCCCTGGGATCCATGGCCTCCTCCGAGGAC G-3' 5'-CGCTCGAGTCGACCCGGGGGCGCCGGTGGAGTGG CG-3' 5-CCCCCCGGGATGGCGAAACCAAGTCGTGG-3' 5'-AAGGAAAAAAGCGGCCGCAGTTTTACGAGGTGGA GGTGG-3'
35S:SR45-9myc; *35S:SR45-RFP*	SR45-F-*Xma*I SR45-R-*Spe*I	5'-CCCCCCGGGATGGCGAAACCAAGTCGTGG-3' 5'-GGACTAGTAGTTTTACGAGGTGGAGGTG-3'
gSR45-3myc	gSR45-F(Pst1) gSR45-R(Xma1)	5'-AACTGCAGGCCACTGCAATCATTTCTCT-3' 5'-CCCCCCGGGAGTTTTACGAGGTGGAGGTG-3'

Primers used for semi-quantitative PCR

Gene Name	Primer
HRLP	5'-ACATATGCAAGATATGGACCCCA-3'
	5'-AGGCGTGCTCTCACTTTTGC-3'
TUB2	5'-ATCCGTGAAGAGTACCCAGAT-3'
	5'-TCACCTTCTTCATCCGCAGTT-3'

Primers used for quantitative real-time PCR

Gene name	Primer
FLC	5'-AGCCAAGAAGACCGAACTCA-3'
	5'-TTTGTCCAGCAGGTGACATC-3'
SOC1	5'-AGCTGCAGAAAACGAGAAGCTCTCTG-3'
	5'-GGGCTACTCTCTTCATCACCTCTTCC-3'
CO	5'-TCAGGGACTCACTACAACGACAATGG-3'
	5'-TTGGGTGTGAAGCTGTTGTGACACAT-3'
MAF4	5'-AACCCCGGTAGATTTCATGG-3'
	5'-TCTTGAGCTGCTCTTCCAGG-3'
FT	5'-CTTGGCAGGCAAACAGTGTATGCAC-3'
	5'-GCCACTCTCCCTCTGACAATTGTAGA-3'
MAF1	5'-CATGCTGATGAACTTAGAGCCTTAGATC-3'
	5'-CAGCAACGTATTCTTTCCCAT-3'
MAF2	5'-AACTCGGAATTATCTGCCACTCAAAG-3'
	5'-CTTCCCCCATCATTAGTTCTGTCTTC-3'
MAF5	5'-GTTCTAGCTAGCGAGGTGGG-3'
	5'-GCCGTTGATGATTGGTGGTT-3'
FVE	5'-GGCCTTCACTCTCTTGCAGATG-3'
	5'-AGACGCTGGCGATTCTTGTAGG-3'
MAF3	5'-GAAAGGGAGAAGTTGCTGATAGAAGAG-3'
	5'-AGCACAAGAACTCTGATATTTGTCTAC-3'
LD	5'-TTGCGCAAGAAATGGCTGCTG-3'

	5'-ACAAGTCTCTTGGCCGCTTTCC-3'
FLD	5'-GGAAAGCAAGTCTTTGAGCACAGG-3'
	5'-CACCAACATGTAAGGAACCACCAG-3'
SVP	5'-CAAGGACTTGACATTGAAGAGCTTCA-3'
	5'-CTGATCTCACTCATAATCTTGTCAC-3'
FPA	5'-GGTCACAAGCGCCAAAAAAAGAAC-3'
	5'-GTCTTCATATGAGTGAGGATACTGCGG-3'
FCA	5'-TGGTCTAACGGGTGAAAGCAAGTG-3'
	5'-TGCTGTTTCTGTTGCTCTCGTTC-3'
FY	5'-TCATCAGATGCCTGGATCAATGGG-3'
	5'-TTGAAACCACCACGTCCTTGGG-3'
FLK	5'-TGCCGTGCAGCTTATTCAGAAC-3'
	5'-TACAGTTTGAGGCTGTGCTGGTG-3'
COOLAIR I	5'-CTCGATGCAATTCTCACACGA-3'
	5'-TCCTTGGATAGAAGACAAAAAGAGA-3'
COOLAIR II	5'-CTCGATGCAATTCTCACACGA-3'
	5'-TTCTCCTCCGGCGATAAGTAC-3'
TUB2	5'-ATCCGTGAAGAGTACCCAGAT-3'
	5'-AAGAACCATGCACTCATCAGC-3'

Primers used for detecting nascent *FLC* expression

Fragment	Primer	Location	sequence
a	FLC 215F	5'UTR	5'-CTGTTCTCTGTGACGCATCC-3'
	FLC 333R	Intron 1	5'-AGGGGGAACAAATGAAAACC-3'
b	FLC-23 unspliced-F	Intron 2	5'-CGCAATTTTCATAGCCCTTG-3'
	FLC-23 unspliced-R	Intron 3	5'-CTTTGTAATCAAAGGTGGAGAGC-3'
c	FLC-456 spliced-F	Exon 4	5'-AGCCAAGAAGACCGAACTCA-3'
	FLC-456 spliced-R	Exon 7	5'-TTTGTCCAGCAGGTGACATC-3'
d	FLC_67F	Exon 1	5'-TGAGGATCAAATTAGGGCACA-3'
	FLC_234R	Exon 1	5'-GGATGCGTCACAGAGAACAG-3'
e	FLC exon7 F	Exon 7	5'-GATATGTAATTATTCCGCTGATAAGG-3'
	FLC exon7 R	3'UTR	5'-TCTTGGCCAAAGAGAGAGTATT-3'
EF1A	EF1A-F	*EF1A* intron 3	5'-ATGGTGACGCTGGTATGGTT-3'
	EF1A-R	*EF1A* intron 3	5'-TCCTTCTTGTCCACGCTCTT-3'

Primers used for SELEX assay

Primers	Sequence
JK251	5'-GCGTCTCTGCAGTAGTTA(N)20AGTCGGCATCTTGGTACCCTTTAGTGAGGG TTAATTTC-3'
JK252	5'-GAAATTAACCCTCACTAAAGGGTACCAAGATGCCGACT-3'
JK253	5'-GCGTCTCTGCAGTAGTTA-3'

Primers used for examination of splicing efficiency

Primers	Sequence
FLC.1-real-F	5'-GGCTAGCCAGATGGAGAATAA-3'
FLC.1-real-R	5'-TCAACCGCCGATTTAAGGT-3'
FLC.2-real-F	5'-AAAATGCTGAAAGAAGAGAACCA-3'
FLC.2-real-R	5'-TGTACGATAATCATAGGTCAAAATCA-3'
FLC.3-real-F	5'-AAATGCTGAAAGAAGAGAACCAG-3'
FLC.3-real-R	5'-ACTTCTAGACACTTGGAGTTGGA-3'
FLC.4-real-F	5'-TGTTGAGAATCTTAAAGAAAAGATGG-3'

FLC.4-real-R	5'-GGAGAGTCACCGGAAGATTG-3'
FLC.1-real-UF	5'-TGGTTGTTATTTGGTGGTGTG-3'
FLC.1-real-UR	5'-GGAGAGTCACCGGAAGATTG-3'
FLC.2-real-UF	5'-TGGTTGTTATTTGGTGGTGTG-3'
FLC.2-real-UR	5'-TCTCCATCTCAGCTTCTGCTC-3'
FLC.3-real-UF	5'-TTGGTTTCCTTGAAGGTTGTG-3'
FLC.3-real-UR	5'-ACTTCTAGACACTTGGAGTTGGA-3'
FLC intron1 SF	5'ATTAGGGCACAAAGCCCTCT-3'
FLC intron1 SR	5'-AAGGATCTTGACCAGGTTATCG-3'
FLC intron1 UF	5'-AGTTTCCAGTGGCCTTTTCA-3'
FLC intron1 UR	5'-AGCATGCTGTTTCCCATATC-3'
FLC intron1 RT	5'-CAAGGCTTTAAGATCATCAGCA-3'
AT3G30720 SF	5'-TGAAGACCAATAGAGAGCAGGA-3'
AT3G30720 S/U R	5'-GTAGAACTGAAGCCCGACCC-3'
AT3G30720 UF	5'-TTAAATCTTTCGTTTTGTAGCAGGA-3'
AT3G45970 SF	5'-TGCTCTCTCTTCTGGAGCTTG-3'
AT3G45970 SR	5'-AGTGCTACACAGCTTAGGGTTC-3'
AT3G45970 UF	5'-TTTGCAGCTGGAGCTTGTGC-3'
AT3G45970 UR	5'-CTTTGTAGATAGAAGGGATAGCTGC-3'
AT4G30280 SF	5'-CATCTATGGTTCGACCCAACTGT-3'
AT4G30280 SR	5'-CGTAAAGCCTCATTGGTTGCTT-3'
AT4G30280 UF	5'-GGCTGTGAGCTCATGAACCG-3'
AT4G30280 UR	5'-CGTTGGGAACGGAACTCCAA-3'
AT5G26920 SF	5'-CCGGGCGTAACACTTCTCTT-3'
AT5G26920 SR	5'-GGATCCAAACTTCCTTGAAAGTCG-3'
AT5G26920 UF	5'-AAAGGTCCTTACAGTGCCCG-3'
AT5G26920 UR	5'-TCAAAAGACTTAACACCATGACCA-3'
AT5G15500 F	5'-AGGGAGACATGGTATGACGC-3'
AT5G15500 R	5'-TCCACAATGCTTTCGGGACA-3'
AT5G15500 UF	5'-AGTATTGAAGTAGGACGAACAGTC-3'
AT5G15500 UR	5'-AATGCTTTCGGGACAACCCA-3'
AT2G18360 F	5'-CCGAAACGGCTCTTCAAGGA-3'
AT2G18360 R	5'-CGCCTAGTTGCTCTTTCATGC-3'
AT2G18360 UF	5'-AAGTCGTCGGCGGATCTTTT-3'
AT2G18360 UR	5'-GAGAGCCAGTGGCAGAGTTT-3'
COOLAIR I SF	5'-GACAAATCTCCGACAATCTTCC-3'
COOLAIR I SR	5'-CTCACACGAATAAGGTGGCTAAT-3'
COOLAIR I UF	5'-CGACAATCTTCCGGTGACTCT-3'
COOLAIR I UR	5'-TACAAACGCTCGCCCTTATC-3'
COOLAIR IIi SF	5'-CTCCTCCGGCGATAAGTA-3'
COOLAIR IIi SR	5'-CTCACACGAATAAGAAAAGTAAAA-3'
COOLAIR IIi&ii UF	5'-TCGCTCTTCTCGTCGTCTC-3'
COOLAIR IIi&ii UR	5'-AAAACACAAACAAACACAGAACC-3'
COOLAIR IIii SF	5'-CTCCTCCGGCGATAAGTA-3'
COOLAIR IIii SR	5'-ACGATAATCATAGAAAAGTAAAAGAGC-3'

Primers used for RIP

Fragment	Primers
RIP1	5'-CTGTTCTCTGTGACGCATCC-3'
	5'-AGGGGGAACAAATGAAAACC-3'
RIP2	5'-TTCTGCATGGATTTCATTATTTCCT-3'

	5'-CAACATCGAGCACGCATCAG-3'
RIP3	5'-TGAAGTTTCAAGCCATCTTTGA-3'
	5'-TCACTCTGAAAAGAGACATTAATCA-3'
RIP4	5'-AGCCACATTAATTGGGAAACTATGA-3'
	5'-TGTGTAACTGCAAGAGTGGGA-3'
RIP5	5'-ATCTGTCTTAGTCGCTTCCTTCT-3'
	5'-AAGAGTTTTCTGCCCTTGAAGTT-3'
RIP6	5'-GAGACTGCCCTCTCCGTGACTAGA-3'
	5'-AGCAAATATCTGACCTTTTCTTTAAGA-3'
RIP7	5'-TGGTTTCCTTGAAGGTTGTGTA-3'
	5'-TGGAGTTGGAACTGAGCCAC-3'
RIP8	5'-ATCCGAGTGGCTCAGTTCC-3'
	5'-CTATTCACTTTCTCTTTTTGTCTTC-3'
RIP9	5'-TCGTGTGAGAATTGCATCGAGA-3'
	5'-GAGAGCCACGTCCCTGTTG-3'
TUB2	5'-ATCCGTGAAGAGTACCCAGAT-3'
	5'-AAGAACCATGCACTCATCAGC-3'

Primers used for ChIP and DRIP

Fragment	Primers
P1	5'-CGACTTGAACCCAAACCTGA-3'
	5'-GGATGCGTCACAGAGAACAG-3'
P2	5'-CTTCTCCTCCGGCGATAAGT-3'
	5'-ACCCAGGTAAGGAAAAGGCG -3'
P3	5'-ATCTCTTGTTGTTTCTCGGTTCTG-3'
	5'-AACAAATCGTGAATGACATGC-3'
P4	5'-AGCCACATTAATTGGGAAACTATGA -3'
	5'-TGTGTAACTGCAAGAGTGGGA-3'
P5	5'-AGCCTTGGAATTGTCGAG-3'
	5'-TAACTGAGTAACTAAGGGTTCCACG-3'
P6	5'-GAGGCTTATGTTTAGGGTTCTT-3'
	5'-GAAAAGTCATACAAAGGCATACAGAT-3'
P7	5'-ATCTCTCCAGCCTGGTCAAGATC-3'
	5'-ATCACTCGGCAAACTAATAGTAAATT-3'
P8	5'-GAGACTGCCCTCTCCGTGACTAGA-3'
	5'-AGCAAATATCTGACCTTTTCTTTAAGA-3'
P9	5'-ATCCGAGTGGCTCAGTTCC-3'
	5'-CTATTCACTTTCTCTTTTTGTCTTC-3'
P10	5'-CACCTTAAATCGGCGGTTG-3'
	5'-TACAAACGCTCGCCCTTATC-3'
TUB2	5'-ATCCGTGAAGAGTACCCAGAT-3'
	5'-AAGAACCATGCACTCATCAGC-3'

Primers used for R-loop foot-printing

Primers	Sequence
Footp-C-F	5'-TTTTGGTGATTTGAATTTAAATT-3'
Footp-R	5'-ATAGATCTCCCGTAAGTGCATTG-3'