Supplementary Information to:

A pair of readers of histone H3K4 methylation recruit Polycomb repressive complex 2 to regulate photoperiodic flowering

by

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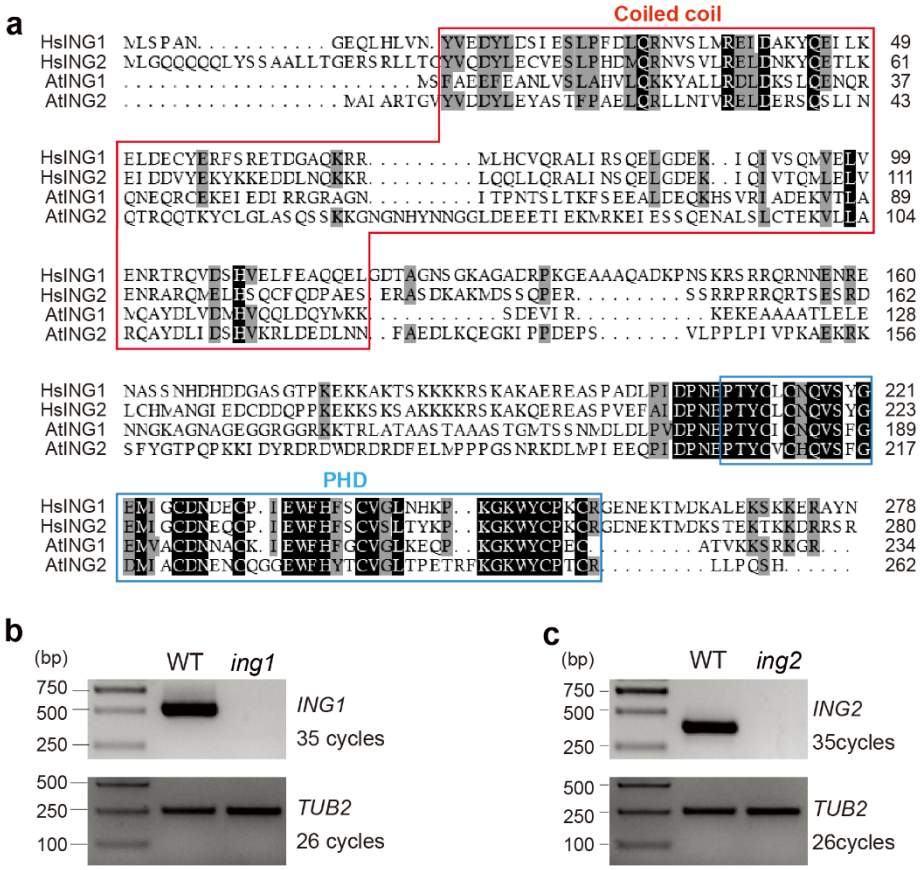
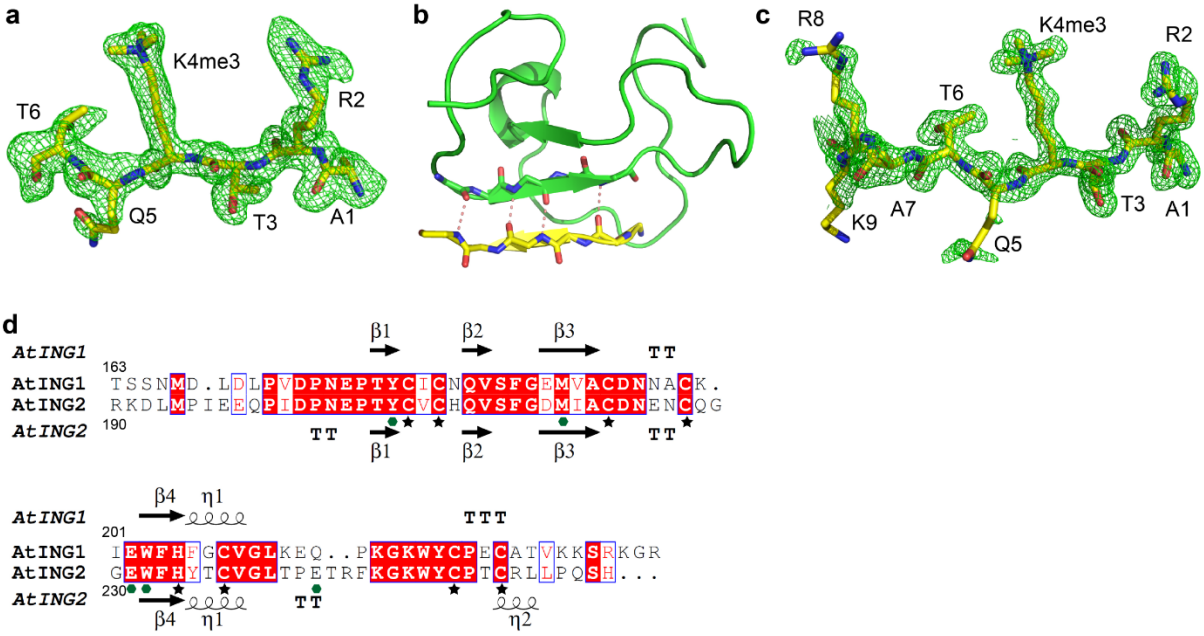
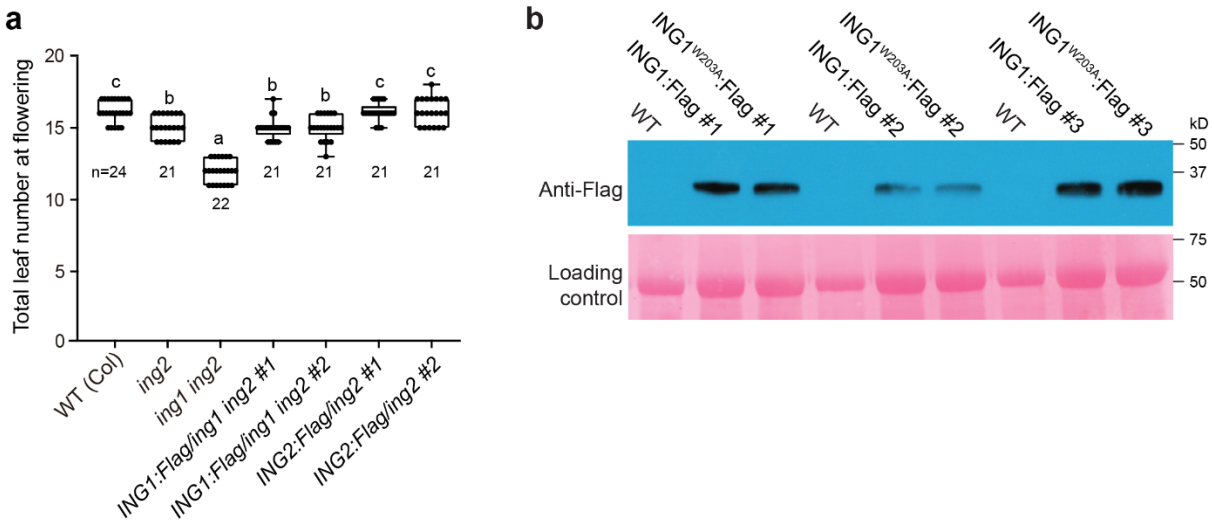


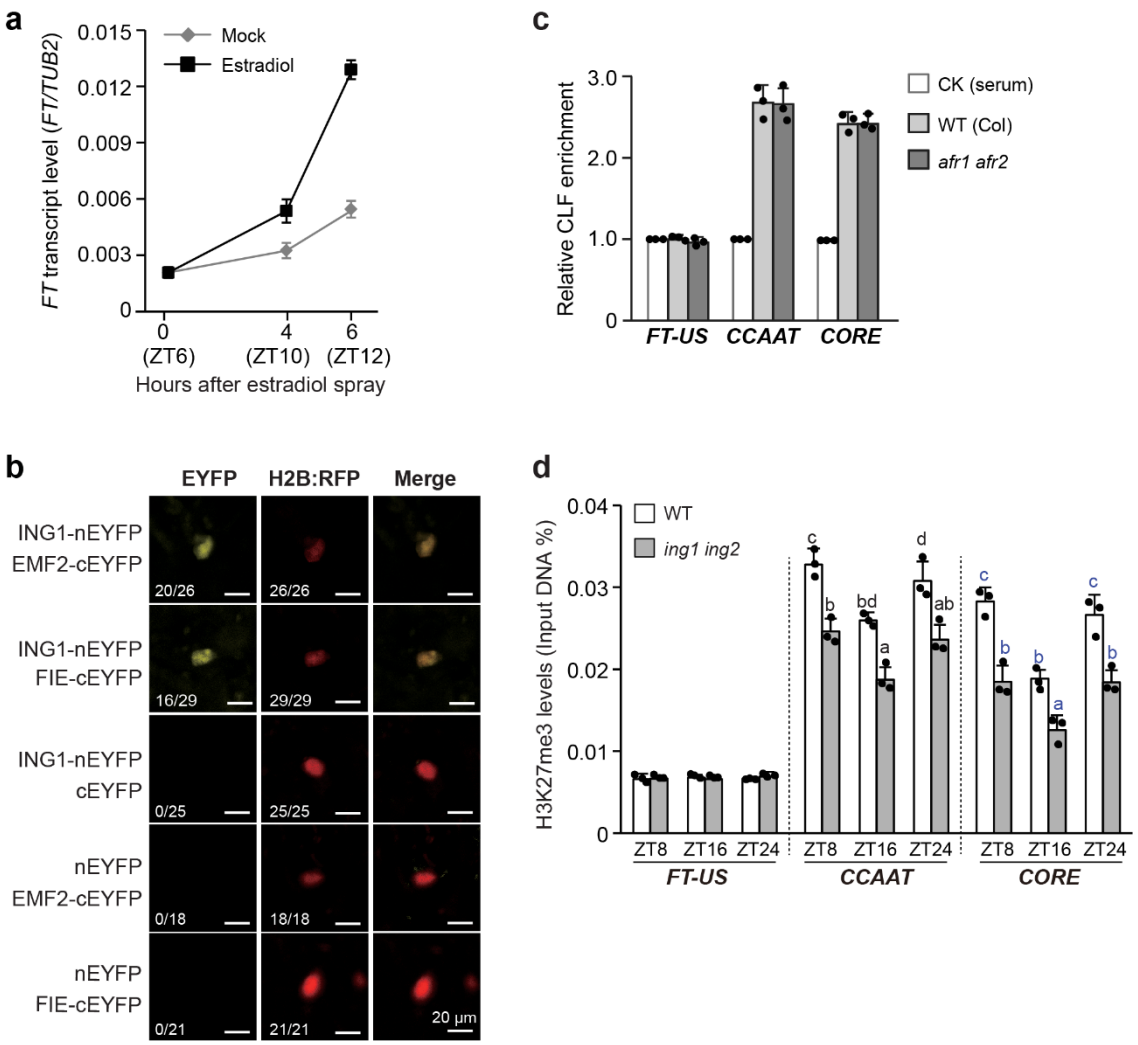
Supplementary Fig. 1: Amino acid sequence alignment of INGs and characterization of *ing1* and *ing2* mutants. a, Amino acid sequence alignment of *Homo sapiens* ING1 (accession: KJ891475.1), HsING2 (accession: KJ897059.1), AtING1 and AtING2. Identical residues are shaded in black, while similar residues are shaded in gray. Coiled-coil domains and PHD-finger domains are highlighted with red and blue boxes, respectively. **b**, **c**, RT-PCR analysis of *ING1* and *ING2* expression in WT, *ing1* (**b**), and *ing2* (**c**). The constitutively-expressed *TUB2* serves as an internal control. The experiments were performed twice with similar results.



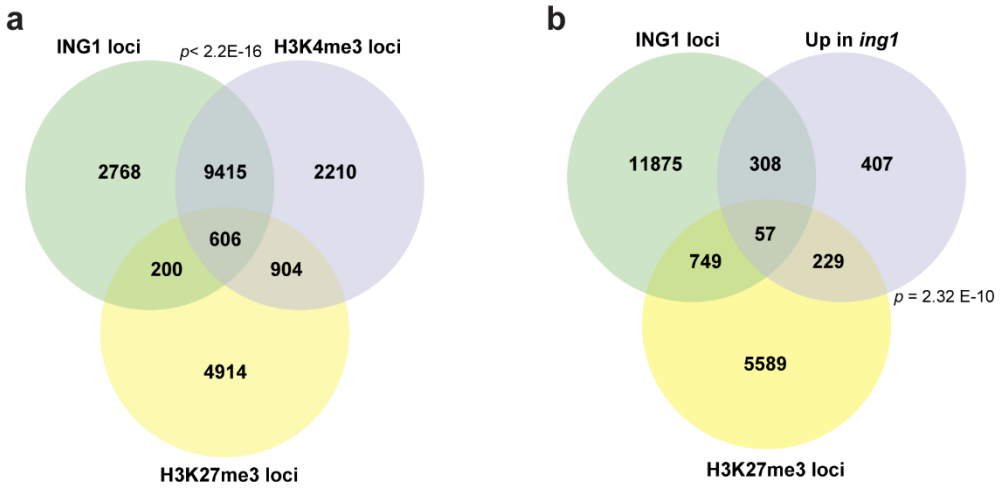
Supplementary Fig. 2: Structural analysis of the PHD-finger domains of AtING1 and AtING2 in H3K4me3 recognition. a, An Fo-Fc omit electron density map of the bounded H3K4me3 peptide from the AtING1 PHD-H3K4me3 complex at 1.7 Å resolution and 2.0 sigma level. **b**, Hydrogen bonding interaction between the main chain of the peptide Arg2 to Thr6 and the main chain the PHD finger Gly189 to Ala193 are of typical antiparallel β-sheet like manner. The main chains of the two segments are shown in stick model and the hydrogen bonds are highlighted in dashed red lines. **c**, An Fo-Fc omit electron density map of the H3K4me3 peptide from the AtING2 PHD-H3K4me3 complex at 1.6 Å resolution and 2.0 sigma level. **d**, Structure-based sequence alignment of AtING1 and AtING2 PHD fingers. The secondary structures of AtING1 and AtING2 PHD fingers are shown on the top and bottom of the alignment, respectively. The important conserved Cys and His residues to coordinate Zn^{2+} ions are marked by black stars. The conserved residues for H3K4me3 peptide binding are marked by green hexagons. β1-β4 for beta sheets, η1-η2: helices, and TT/TTT for turns.



Supplementary Fig. 3: Functional analysis of *ING1/2* and protein expression of Flag-tagged ING1 variants. a, Analysis of *ING1pro-ING1:FLAG* and *ING2pro-ING2:FLAG* functionality. Total number of leaves at flowering was scored for each line. Box plots display interquartile range, median (solid line), and whiskers extending from the boxes to the observed minimum and maximum values. Letters indicate statistically significant differences (one-way ANOVA using Tukey's multiple comparisons, with $\log_{10}$-transformed data, $p<0.01$). **b**, Western blot analysis to assess the abundances of ING1:FLAG and ING1^{W203A}:FLAG in the indicated seedlings grown in LDs. Ponceau S-stained blot served as a loading control.



Supplementary Fig. 4: Characterization of *FT* induction as well as *FT* regulation by CLF-PRC2. a, *FT* induction in the estradiol-treated seedlings of doubly hemizygous $LexA_{pro}$-*CO ING1:FLAG* grown in LDs. β-estradiol or mock was applied to the seedlings at ZT6; *FT* levels were measured at the indicated time points by RT-qPCR and directly normalized to *TUB2*. **b**, BiFC analysis of ING1 interactions with EMF2 and FIE in the epidermal cells of *Nicotiana benthamiana*. RFP (H2B:RFP) fluorescence indicates nuclei. The proportion of fluorescent nuclei in each experiment is presented as n/N (N for the total nuclei examined). Scale bars, 20 μm. **c**, ChIP analysis of CLF enrichment on *FT* promoter chromatin in WT and *afr1 afr2* seedlings at ZT16 in LDs. ChIP was conducted with rabbit polyclonal anti-CLF. Relative CLF fold enrichments in each examined region over a background control (WT IP with rabbit serum), are shown. **d**, ChIP analysis of H3K27me3 on *FT* promoter chromatin in WT and *ing1 ing2* seedlings at indicated time points over a LD cycle. Levels of the genomic fragments immunoprecipitated using anti-H3K27me3 were quantified by qPCR and normalized directly to 'input DNA'. **a**,**c-d**, Values are means ± s.d. of three biological replicates. Letters in the same color in (**d**) indicate statistically significant differences among a group of means (two-way ANOVA with Tukey's multiple comparisons, $p<0.05$).



Supplementary Fig. 5: Characterization of genome-wide regulation of *ING1* in Arabidopsis. a, Venn diagram showing overlapping of the list of ING-bound loci with H3K4me3- and/or H3K27me3-bearing loci in Arabidopsis seedlings. **b**, Venn diagram showing overlapping of the list of upregulated protein-coding genes with H3K27me3-bearing loci. The list of ING1-bound loci and list of the genes upregulated in *ing1* at a seedling stage were downloaded from a recent study[1], while the list of H3K4me3-bearing loci and list of H3K27me3-bearing loci (at a seedling stage) were extracted from GSE185121 and GSE185120 (ChIP-seq data at NCBI GEO), respectively. Hypergeometric test (one-sided) was conducted to analyze the statistical significance of overlapping (only *p* values <0.05 are indicated).

Supplementary Table 1: ITC data analysis

Protein	Peptide	Kd (μM)	*N* value
ING1 PHD	H3K4me3	0.86 ± 0.04	1.06 ± 0.00
ING1 PHD	H3K4me2	1.21 ± 0.13	1.02 ± 0.01
ING1 PHD	H3K4me1	5.65 ± 0.40	1.15 ± 0.01
ING1 PHD	H3K4me0	NDB	NA
ING2 PHD	H3K4me3	2.91 ± 0.23	1.06 ± 0.01
ING2 PHD	H3K4me2	4.37 ± 0.76	1.09 ± 0.02
ING2 PHD	H3K4me1	8.70 ± 1.15	0.86 ± 0.03
ING2 PHD	H3K4me0	NDB	NA
ING1 PHD Y180A	H3K4me3	6.85 ± 0.61	1.12 ± 0.01
ING1 PHD M191A	H3K4me3	29.7 ± 0.8	1.02 ± 0.01
ING1 PHD E202A	H3K4me3	3.45 ± 0.09	1.02 ± 0.00
ING1 PHD W203A	H3K4me3	NDB	NA
ING2 PHD E205A	H3K4me3	8.56 ± 1.34	1.17 ± 0.02
ING2 PHD Y208A	H3K4me3	59.4±5.6	1.10 ± 0.04
ING2 PHD M219A	H3K4me3	23.4 ± 3.6	0.97 ± 0.11
ING2 PHD W232A	H3K4me3	NDB	NA

Supplementary Table 2: Data collection and refinement statistics for crystal structure

	AtING1 PHD-H3K4me3	AtING2 PHD-H3K4me3
Data collection		
PDB code	9M4R	9M4S
Beamline	SSRF-BL19U1	SSRF-BL18U1
Space group	$P3_121$	$P6_1$
Wavelength (Å)	1.2824	0.9792
Cell dimensions		
a, *b*, *c* (Å)	41.8, 41.8, 69.4	50.7, 50.7, 55.2
α, β, γ (°)	90, 90, 120	90, 90, 120
Resolution (Å)	50.0-1.7 (1.76-1.70)*	50.0-1.6 (1.66-1.60)
R_{merge}	0.090 (0.220)	0.093 (0.339)
I / σI	68.5 (25.5)	38.9 (9.4)
Completeness (%)	99.9 (100.0)	99.8 (100.0)
Redundancy	18.2 (17.8)	8.4 (8.6)
Refinement		
No. reflections	14,947	10,700
R_{work} / R_{free}	0.157 / 0.200	0.185 / 0.225
No. atoms	507	637
Protein	393	480
Peptide / Zn^{2+}	51 / 2	76 / 2
Water	61	79
B-factors (Å^2)	29.3	27.4
Protein	27.1	25.7
Peptide / Zn^{2+}	33.5 / 26.6	33.5 / 18.3
Water	40.0	31.8
R.m.s. deviations		
Bond lengths (Å)	0.019	0.016
Bond angles (°)	1.712	1.501

*Highest-resolution shell is shown in parentheses.

Supplementary Table 3: Primers and peptides used in this study

Experiment	Amplified region /peptide	Sequence (5'to 3' or N to C)
RT-qPCR	*TUB2*	F: GCCTTGTACGATATTTGCTTCAGGAC
		R: CGGAGGTCAGAGTTGAGTTGAC
	FT	F: GACCTCAGGAACTTCTATACTTTGGTTATG
		R: CTGTTTGCCTGCCAAGCTG
	CO	F: CACTACAACGACAATGGTTCC
		R: GGTCAGGTTGTTGCTCTACTG
ChIP-qPCR	*TUB2*	F: ATCCGTGAAGAGTACCCAGAT
		R: AAGAACCATGCACTCATCAGC
	FT	F: ACGTTGATGATAGTGAAGTGA
	CCAAT region	R: ACGCAACCAAGTAGAGACGT
	FT	F: GTGTGGTGGGTTTGGAATACC
	CORE region	R: ACTCGGGTCGGTGAAATCAT
	FT-US	F: GCGGCATTGTACTAAACGAAA
		R: GCCACCAAATCTTATCACCCT
	LEC1	F: GTCGGGTCAAGATGAATCCAGTG
		R: CAACACTTTTCCTTAAAGAAGCAACAT
RT-PCR	*ING1*	F: GATAAAAGTTTGCAAGAAAACCAACG
		R: ACCACTCAATCTTGCACGCATTGT
	ING2	F: CGACAAGCGTATGATCTTATAGATAGTC
		R: ATTCACCTCCTTGGCAATTCTCAT
	TUB2	F: ACTCACTACCCCCAGCTTTGGTG
		R: ACATCATGTTCTTGGAGTCCCACAT
ITC	H3 (1-10)	ARTKQTARKS
	H3(1-10) K4me1	ARTK(me1)QTARKS
	H3(1-10) K4me2	ARTK(me2)QTARKS
	H3(1-10) K4me3	ARTK(me3)QTARKS

Reference

1. Wu, C.J. *et al.* Conserved and plant-specific histone acetyltransferase complexes cooperate to regulate gene transcription and plant development. *Nat Plants* **9**, 442-459 (2023). https://doi.org/10.1038/s41477-023-01359-3