

# Supplemental Figure 1. Heading Date Investigation of WT and *lvp1* Plants in SD and LD Conditions.

Plants were grown in growth chambers under artificial Long day (LD), 14 h light  $(30 \ C)/10$  h dark (28  $\ C$ ) photoperiod; and artificial short day (SD), 10 h light  $(30 \ C)/14$  h dark (28  $\ C$ ) photoperiod. WT, wild-type. Error bars show standard deviations (n=10).



Supplemental Figure 2. Rescue of *lvp1* Phenotype by Transformation with SDG724. (A) Gross morphology of wild-type (WT, left), long vegetative phase 1 (lvp1; middle) mutant, and lvp1complemented (*lvp1-C-1*, right) transgenic plants.

(B) PCR product to show positive complementation of transgenic lines. *HPT* primer pairs (Supplemental Table 1) for the hygromycin gene of the pCAMBIA1300 vector were used. (C) Heading dates of *lvp1* and complemented transgenic plants.





**Supplemental Figure 3. Phylogenetic Analysis of SDG724 Homologs in Plants.** (A) Phylogenic tree of SDG724 homologs in rice, Arabidopsis and maize. The tree was generated comparing amino acid sequences of the conserved protein domain using the program ClustalX and MEGA4. The length of tree branch denotes the genetic distance, numbers at the tree branch indicate bootstrap values, and the scale bar indicates sequence divergence.

(B) Protein alignment using Clustal X and GenDoc. Shades of black and gray indicate conserved amino acids, white letter with black background indicates 100% identity, white letter with gray background indicates 75% identity, and black letter with gray background indicates 50% identity. Protein accessions were used as described in <u>http://www.chromdb.org/</u>.



**Supplemental Figure 4. Quantification of Immunoblot Signals** 

Immunoblot chemiluminescence intensities were measured using the software Image J following a protocol available at http://imagej.nih.gov/ij/docs/guide. For each independent immunoblot experiment, the chemiluminescence intensity level of the wild-type was set as 1.0 (including H3 levels). The relative intensities of H3K36me1/2/3, H3K4me1/2/3, and H3K9me2 bands from *lvp1-1* and *lvp1-2* material were determined by dividing their band intensities by the intensity of corresponding H3 bands.



Supplemental Figure 5. Expression Levels of MADS50, Ehd1, Hd3a and RFT1 at Different Developmental Stages of **LD Grown Plants.** 

Leaves were collected from plants grown under Beijing natural LD conditions at the initiation of the light phase at 30, 60, 90 and 120 days after germination (DAG). Values are the means of three biological replicates. The value for wild type (WT) plants 30 DAG was set as 1.0. Error bars indicate standard deviations. Y-axis, relative transcript levels of genes normalized with those of rice *Ubiquitin*. Error bars show standard deviations (n = 3 or more).

A C 15 SD No. of plants 12 lvp1 se5 se5 9 6 3 0 40 43 Days to heading D 12 LD No. of plants 9 6 3 B SNP 0 wpl se5 sez 40 43 46 49 52 55 58 61 64 (DAG) Days to heading

### Supplemental Figure 6. Analysis of Double Mutant *lvp1 se5* Plants.

(A) Phenotypes of WT, se5, and lvp1 se5 plants.

(B) Identification of double mutant *lvp1 se5* among F<sub>2</sub> progeny plants. Among all F<sub>2</sub> progeny plants with etiolated leaves (the se5 mutant has a yellow leaf phonotype), PCR amplifications were used to detect plants homozygous for the mutated *lvp1* fragment (Figure 2C) to identify *lvp1* and *se5* double mutant progeny.

(C) and (D) Analysis of heading date in se5 and lvp1 se5 plants grown under SD (C) and LD (D) conditions. X-axis, days after germination (DAG), Y-axis, numbers of heading plants in groups of three days. Red arrows indicate the peak heading date fraction for each flowering time distribution.





Supplemental Figure 7. Expression Analysis of Flowering Genes in *se5* Single and *lvp1 se5* Double Mutant Plants Grown under SD Conditions.

Total RNA was isolated from plant leaves 30 days after germination (DAG) in SD conditions at the initiation of the light phase. Y-axis, relative transcript levels of genes normalized to those of rice *Ubiquitin*. Error bars show standard deviations (n = 3 or more). WT, wild-type; *ls*, double mutant *lvp1 se5*.



Supplemental Figure 8. Expression Analysis of Flowering Genes in *se5* Single and *lvp1 se5* Double Mutant Plants Grown under LD Conditions.

Total RNAs were isolated from plant leaves 30 days after germination (DAG) in LD conditions at the initiation of the light phase. Y-axis, relative transcript levels of genes normalized to those of rice *Ubiquitin*. Error bars show standard deviations (n = 3 or more). WT, wild-type; *ls*, double mutant *lvp1 se5*.

Supplemental Data. Sun et al. (2012). Plant Cell 10.1105/tpc.111.101436



Supplemental Figure 9. H3K36me2/3 Levels are not Affected at the MADS51 and Hd3a Loci in lvp1 Mutant **Plants Grown under SD Conditions.** 

Chromatin was isolated from leaves of 25-day-old plants grown under SD conditions and immunoprecipitated with antibodies against specific histone H3K methylation tags. Chromatin abundance was determined by quantitative PCR to amplify five different regions of MADS51 (B) and six different regions of Hd3a (C). Regions assayed in (B and C) are indicated by lines and numbered (A and Figure 7B). The rice Actin1 gene was used as an internal control. Relative histone lysine methylation levels were calculated as the ratio of specific H3K methylation tags over total H3 and normalized as the ratio over total H3 at *Actin1*; error bars indicate standard deviations (n = 3 or more). All the primers are listed in Supplemental Table 1.



## Supplemental Figure 10. Expression Pattern Analysis of *SDG724* in Different Tissues by Quantitative **RT-PCR**.

Total RNA was isolated from root, stem, old leaf (Oleaf), young leaf (Yleaf), panicle, and callus. Y-axis, relative transcript levels of *SDG724* normalized with those of rice *Ubiquitin*. Error bars show standard deviations (n = 3 or more).

# Supplemental Table 1. Primers used in this study.

Markers used in mapping				
Primer Name	Loci (bp)	Forward $(5' \rightarrow 3')$	Reverse $(5' \rightarrow 3')$	
<b>S</b> 1	5,581,050	GTGCCGTCGCCTCAACTA	GCCTCTATCACCCACCTTT	
<b>S</b> 2	11,695,191	TGCATGCTTCGTTCAGCTAG	GTCTCCGAGCTCCTCAGGTC	
<b>S</b> 3	7,202,979	AACCTAAAGGGCAGTTTCC	GCGATAAGTTTCTTGTTGATG	
<b>S</b> 4	8,189,574	CTACTTCCTTCATACCCTAC	AACATACTCCCTCCATTT	
S5	7,961,717	AACATCAACTCGAAGGAC	GACAATTTGTGAACGCTA	
<b>S</b> 6	8,100,151	TTGAAGAAAGGCACAGCA	AGTGTATGCCAGTCAGACC	
Probes for quantitative RT-PCR in flowering expression detected				
Gene Name		Forward $(5' \rightarrow 3')$	Reverse $(5' \rightarrow 3')$	Accession Numbers
SDG724		AAAATGAAGAGGCAGGGTG	CTCCCTCAACAGTCCATTTCTG	Os09g0307800
Ehd2/ID1/RID1		CGACGACAATAGCTCGATCGC	GTGCATGGTCACGGAGCCTT	Os10g0419200
MADS50		AAAGCTGACGCTGATGGTTTG	GTTTCGACATCCATGTTGTC	Os03g0122600

MADS50	AAAGCTGACGCTGATGGTTTG	GTTTCGACATCCATGTTGTC	Os03g0122600
MADS51	GTTTGCTCTGCTCCTACTC	ACTCCTCCTCCAGCATTGAA	Os01g0922800
Ehd1	GAGCAAGTTGCCAGTC	CATGCACTCTGAGCCA	Os10g0463400
SE5	TCGTGGAGGAGATGAGGG	ATGTAGTGCCGGGAGCAG	Os06g0603000
Ghd7	AGGTGCTACGAGAAGCAAATCC	GGGCCTCATCTCGGCATAG	Os07g0261200
Hd3a	AGCCCAAGTGACCCTAACCT	GTTGTAGAGCTCGGCGAAGT	Os06g0157700
RFT1	ACCCTAACCTTAGGGAGTATCTACAC	GCCTGCATGCATATACAGCTAGGCAG	Os06g0157500
GI	GTGGATGCGCTTTGTGACAT	GGCCTGCAGAACGATAGCA	Os01g0182600
Hd1	GAGTACTTTGATCTTGTCGGGTACA	ACATCTGATCTCTTGGCGAAAC	Os06g0275000
Ubq	AACCAGCTGAGGCCCAAGA	ACGATTGATTTAACCAGTCCATGA	

Primers for quantitative PCR analysis of the production of ChIP

Gene Name	ID	Forward $(5' \rightarrow 3')$	Reverse $(5' \rightarrow 3')$
MADS50	1	TCTCTGAAAGTCCACTAGGA	CCCTTATATTATGGGACGGA
	2	CGCCTCCACCACCACTCCTT	CGCCGATGAACCAACCAACC
	3	GTATTTCTTCGTCCTCGTT	GACATGAATCGTCGCACT
	4	ACGGTCATCCCTACCTTGG	CGCACAACCGCTACCAC
	5	TACGAAGTTGGTGAGGTA	GTGAAACTTTTACTGCCG
	6	CCAGGAATAAGCTGGATC	AAGGGTTTCTGCCTGGTG
Ehd1	7	ATCTACCCACCAACTCCA	CTCCTCTACCCTCAAGCA
	8	AGCACATATTCCGAAAGC	TCGCCATTGTAGTTGACC
	9	TACGCACTGCTGCTCATG	ATTTCAAACCCTGCCTCT
	10	GTAGAGAATATAATGAT	GTAGACAACCTATGTAC
	11	CACATGCAAAGATTATG	GTAAAATCCAGAAAAT
MADS51	1	ACCAACTTGCGTAAACAG	ACATCTACACTACCACCAC
	2	TGACACCGAAATGATGCCAACG	CGCAAAAGCCTCCCTCTCAT
	3	ACTCTCAAGGAGAACCG	GGGGGTGCAAAACTAGAA
	4	TGGTGTCGTAGGAAGAGA	ATGCCAAGAATGGAAGGT
	5	AGATGGTTACGTTGCTGTAT	TACTCCAGTTTGTACTAGTA
RFT1	1	GCATTAAATAAGCTGCTAC	AACCCAAGACACTACACG
	2	GGATTGAACGGCAGGAGA	GCTAACCCACTAACTTATGAA
	3	AATGACATGAGGACGTTCTACAC	ATTAAATAACTTCTGGTGGGTCT
	4	GCCCAAGCAACCCTAACC	CCAGCCTACAGACAGACAAAGA
	5	CAAGAGGTGATGTGCTAC	GCGAGCCGAGGTTGTAGAG
	6	ATGAGAAGACTGTCATGTG	GAGGCCGCAGCCATTGGTT
Hd3a	7	ACTAACGGTACGGAAATG	AAATCGCTAAGAAGACAC
	0	TTGTGGTTGGTAGGGTTG	AAGGGTGTAGAATGTCCTCATG

Actin1		ACCATTGGTGCTGAGCGTTT	CGCAGCTTCCATTCCTATGAA
_	12	CGATGATCCCGATCGATCTGC	TGAGAGACCTTAGCCTTGCTC
	11	GCTGGTGTTCGTGCTGTT	ATGCTGGATGATGATAGTGA
	10	AATAAGAAGAATGATCGTCAA	ACACATGAAGAAGAAATGTTC
	9	GATATGTCGGCTTGGGCTTGG	CGAATCATCCCGTCACTATCTT
	-		

Other primers used in this study

Primers	Forward $(5' \rightarrow 3')$	Reverse $(5' \rightarrow 3')$
HPT	GCTGGGGCGTCGGTTTCCACTATCGG	CGCTCCAGTCAATGACCGCTGTTATGCG
SDG724-C	AACTTGTATGGGAAGAGGCA	AACCGAACCTTATCATTTGC
SDG724-MBP	GGAATTC ATGCCTCGGCCGGCGAAAAT	CCTCGAG CACTTCCTCAGGACCATCTG
FR	ATGCCTCGGCCGGCGAAAAT	CACTTCCTCAGGACCATCTG
SNP	AGCTGACCTATGATTACAAG	CGAGTTTATCAATCCAGTAG