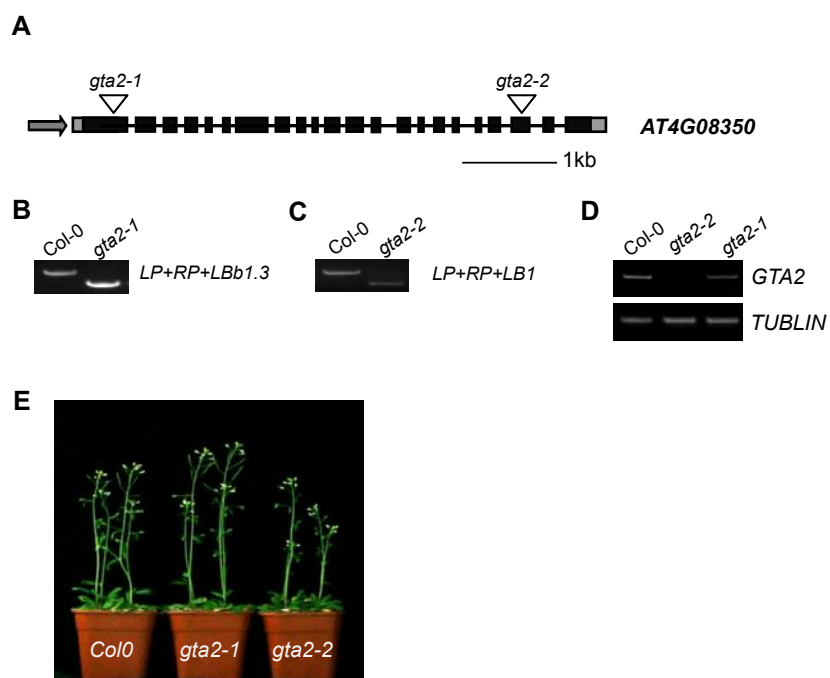


Supplemental Figure 1

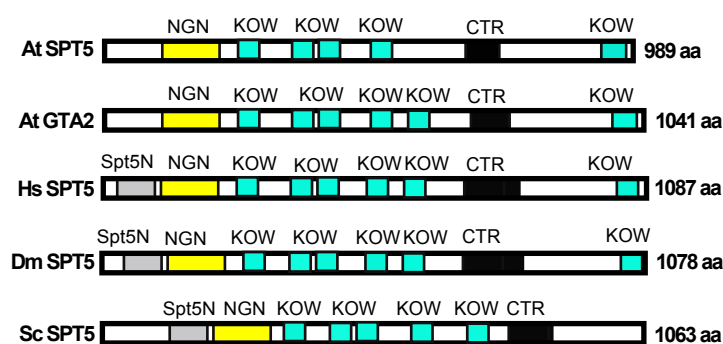


Supplemental Figure 1. Phenotypic analysis of *gta2* mutants.

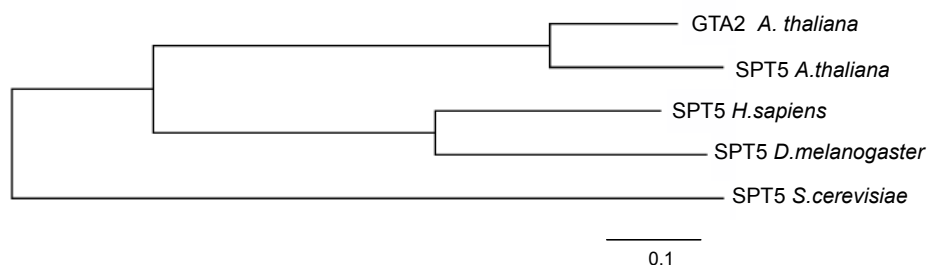
- (A) Gene structure of *GTA2*. Exons are shown as boxes and introns are indicated as lines.
- (B) and (C) Genotypic analysis of *gta2* mutants with left genomic primer (LP), right genomic primer (RP), and the vector primer (LBb1.3 or LB1)
- (D) The transcripts of *GTA2* were tested in Col-0 and *gta2* mutants.
- (E) The flowering time of Col-0 and *gta2* mutants.

Supplemental Figure 2

A



B



Supplemental Figure 2. Phylogenetic analysis of SPT5 proteins from Arabidopsis, humans, *Drosophila*, and yeast.

(A) Diagram of the domain structures of SPT5 proteins from Arabidopsis, humans, *Drosophila*, and yeast. Proteins all contain NGN (yellow boxes), KOW (blue boxes), and CTR (black boxes) domains. aa = amino acids.

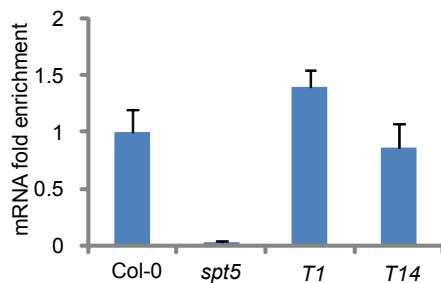
(B) The SPT5 proteins from Arabidopsis, human, *Drosophila*, and yeast were aligned with ClustalW. The relationships of those sequences were examined with MEGA5. The evolutionary scar bar was indicated in bottom.

Supplemental Figure 3

A



B

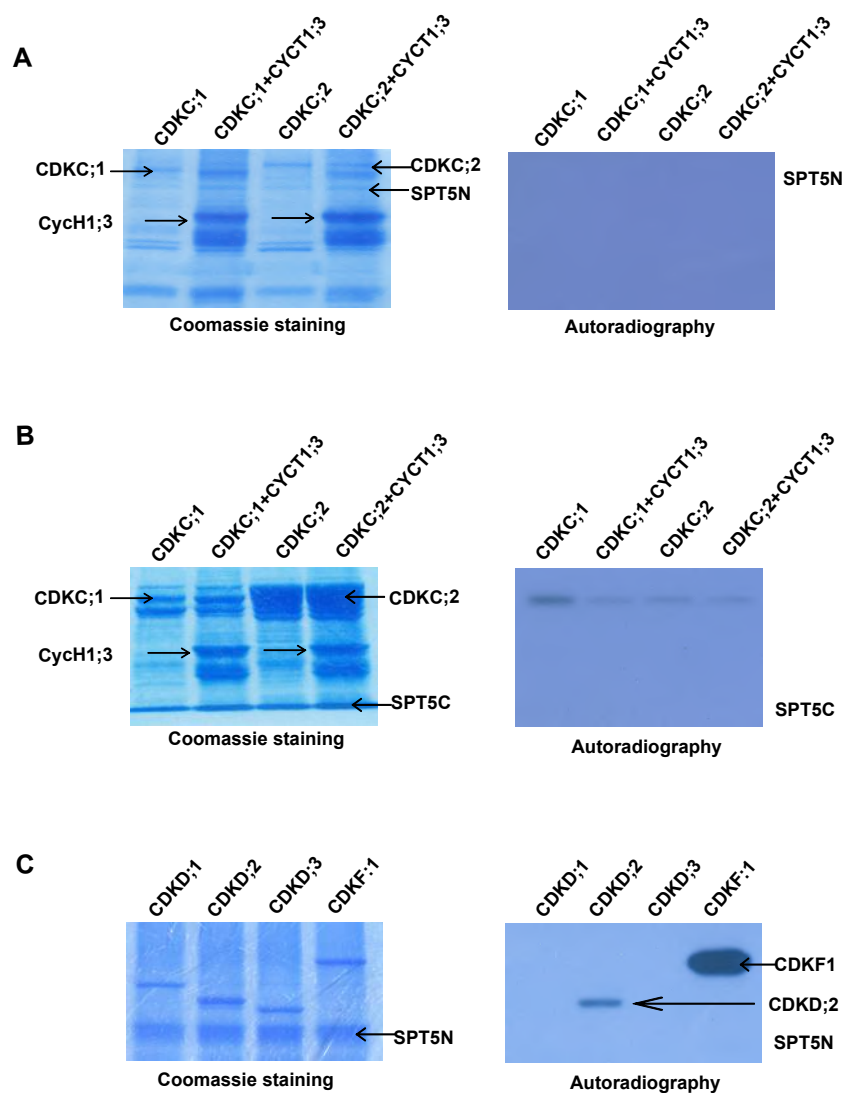


Supplemental Figure 3. Complementation of *spt5* with *ProSPT5::FLAG-SPT5*.

(A) Phenotypic analysis of flowering time in 4-week-old plants. Over 40 independent transgenic lines were generated, and two representative transgenic plants are shown.

(B) Transcript analysis of *SPT5* in the Col-0, *spt5* mutant, and two transgenic lines (*T1* and *T14*). Experiments were repeated at least three times, and each experiment included three replicates, the representative experiments shown indicate the mean \pm SE, n = 3 replicates.

Supplemental Figure 4

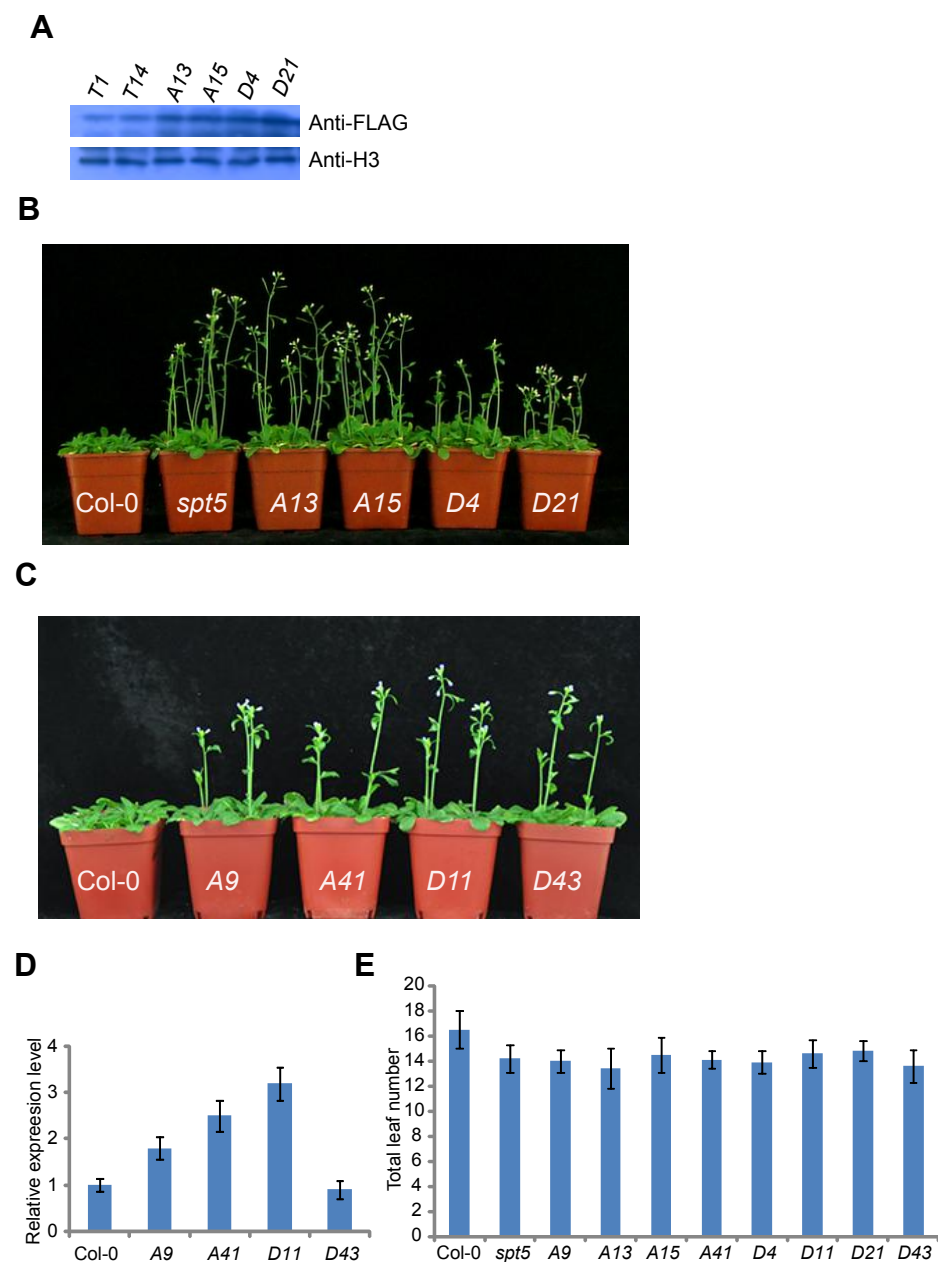


Supplemental Figure 4. The phosphorylation activity of CDKC and CDK proteins

(A) and (B) CDKC;1 and CDKC;2 were tested for their ability to phosphorylate the N-terminus of SPT5 (SPT5N) or C-terminus of SPT5 (SPT5C). Cyclin T1;3 was used to enhance the CDKC;1 and CDKC;2 phosphorylation activity. The left panel shows the Coomassie blue-stained gel. The positions of different proteins are indicated on the right. Autoradiography in the right panel shows the activity and specificity of the kinase. The positions of the proteins are indicated by arrows.

(C) CDKD;1, CDKD;2, CDKD;3, and CDKF;1 were tested for their ability to phosphorylate the N-terminus of SPT5 (SPTN). The left panel shows the Coomassie blue-stained gel. The positions of different CDK proteins and SPT5 are indicated on the right. Autoradiography in the right panel shows the activity and specificity of the kinase.

Supplemental Figure 5

**Supplemental Figure 5. Complementation of *spt5* with mutated *SPT5***

(A) *SPT5* accumulation in different transgenic plants. The *spt5* mutant was complemented by genomic *SPT5* with mutations that encoded nonphosphorylatable ($SPT5^{TA}$; transgenic lines *A13* and *A15*) or phospho-mimic ($SPT5^{TD}$; transgenic lines *D4* and *D21*) forms of *SPT5*. *T1* and *T14* are indicated in Supplemental Figure 3. The amount of *SPT5* was determined by immunoblot analysis with a FLAG antibody (anti-FLAG). H3 was used as the internal control (anti-H3).

(B) The flowering time of Col-0, *spt5*, and transgenic plants (transgenic lines *A13*, *A15*, *D4* and *D21*) in a long-day photoperiod.

(C) The flowering time of Col-0 and nonphosphorylatable (transgenic lines *A9* and *A41*) or phospho-mimic (transgenic lines *D11* and *D43*) forms of *SPT5* in a long-day photoperiod.

(D) The transcripts of *SPT5* in transgenic plants (*A9*, *A41*, *D11*, and *D43*). Experiments were repeated at least three times, and each experiment included three replicates, and the representative experiments shown indicate the mean \pm SE, $n = 3$ replicates.

(E) Flowering time was assessed by counting the total leaf number at bolting under long-day photoperiod.

Supplemental Figure 6

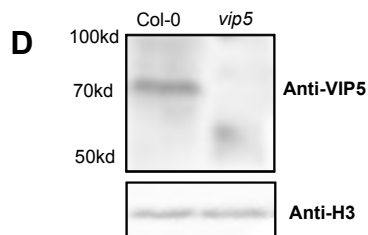
A

Hs RTF1 VHDFDVKIDLQVPSSSESKALA-ITSKAPPAKDGAPRRSLNL--EDYKKRRGLI
At VIP5 LQKYGGPQGVQKAFMARKQLTEATVGC RVAENDGKRHGLTLTVSDYKRRRGLL
 + + +Q K L T A++ R L L DYK+RRGL+

B



C



Supplemental Figure 6. Commercial antibody to mammalian RTF1 recognizes Arabidopsis VIP5.

(A) The amino acid sequences of human RTF1 (660-710 amino acids) and Arabidopsis VIP5 (590-643 amino acids) were aligned by ClustalW2, with the sequence of the human antigen peptide used to make antibodies shown in red.

(B) The cDNA for the C-terminus of VIP5 was expressed in *E. coli* as a N-terminal fusion to His. The His-purified fusion proteins (His-VIP5C) produced were visualized on a Coomassie-stained protein gel.

(C) The same purified samples were analyzed by immunoblot using a commercially available antibody to human RTF1 (Abcam ab52887). The antibody is specific to the C-terminus of human RTF1 and recognizes Arabidopsis VIP5.

(D) The cell extracts from wild-type and *vip5* plants were blotted with anti-RTF1 antibody (top panel) using H3 as a control (anti-H3; bottom panel).

Supplemental Table 1

GST-CDKC;1

To generate *GST-CDKC;1*, the *CDKC;1* was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'- CGGAATTCATGGCGATGGCATCATTCGG-3') and reverse primer (5'- GCGTCGACCTGTTGCCATCCGTATTGCT-3'), and cloned into pGEX-6p-1 with EcoRI/ SalI

GST-CDKC;2

To generate *GST-CDKC2*, the *CDKC;2* was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'- CGGAATTCATGGCGGCTGCGGCTTTTGG-3') and reverse primer (5'- GCGTCGACCGGTTGCCATCCATATTGTT-3'), and cloned into pGEX-6p-1 with EcoRI/ SalI

GST- CYCT1;3

To generate *GST- CYCT1;3*, the *CYCT1;3* was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'- GGAATTCATGGGAGAGGAGCATCCGAGAAAG -3') and reverse primer (5'- GACGTCGACCCAGATGCCAGCCTGTCTATAGGA -3'), and cloned into pGEX-6p-1 with EcoRI/ SalI

GST-CDKF;1

To generate *GST-CDKF;1*, the *CDKF;1* was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-TCCCCCGGG TTAAAGGAGATGGATAAACAA-3') and reverse primer (5'- ACGCGTCGAC ATGAAAAATAGGGTAAAGAATGGC-3'), and cloned into pGEX-6p-1 with SmaI/ SalI

GST-CDKD;1

To generate *GST-CDKD;1*, the *CDKD;1* was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'- "CGGGATCC CTAAAAATGTGCGATTCTTCTGTGTA-3') and reverse primer (5'- "CGGAATTCTCAAGAAGAAGCCTGTTACGCGAT-3'), and cloned into pGEX-6p-1 with BamHI/EcoRI

GST-CDKD;2

To generate *GST-CDKD;2*, the *CDKD;2* was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'- "CGGGATCC GCCCTCGTACTTCCTCGAAGTGTA-3') and reverse primer (5'- TCCCCCGGG AATCCTTCAGGACCCATCACTCTC-3'), and cloned into pGEX-6p-1 with BamHI/ SmaI

GST-CDKD;3

To generate *GST-CDKD;3*, the *CDKD;3* was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'- "CGGGATCC ATTATTGAGTGGGTTCTGAAAAAC-3') and reverse primer (5'- ACGCGTCGAC ATAACCAACAGTCTTACTGGAAC-3'), and cloned into pGEX-6p-1 with BamHI/ SalI

pGEX6p-1-SPT5 N

To generate *pGEX6p-1-SPT5 N*, the *SPT5 N* fragment was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-CGCGGATCCATGCTTTTCAAAGATGGTTTTTC-3') and reverse primer (5'-CGGAATTCTTGCTTCCCATATTATACTGCGG-3'), and cloned into pGEX-6p-1 with BamHI/EcoRI.

GST-SPT5 C

To generate *GST-SPT5 C*, the *C* fragment from *SPT5* cDNA was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-CGGGATCCGTCGCAACCCCGCAGTATAATAT-3') and reverse primer (5'-CGGAATTC TCACTCATGAACTAACTTGGCTAA-3'), and cloned into pGEX-6p-1 with BamHI/EcoR I

pGEX6p-1-SPT5 CTR

To generate *pGEX6p-1-SPT5 CTR*, the *SPT5 CTR* fragment was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-CGGGATCCGTCGCAACCCCGCAGTATAATAT-3') and reverse primer (5'-CGGAATTCGAGACATGACATCGAGATCGGTTTC-3'), and cloned into pGEX-6p-1 with BamHI/EcoRI.

PET30a-SPT5 KOW

To generate *pGBKT7-SPT5 KOW6*, the *KOW6* fragment was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-CGGGATCCAACAAAGTGAGCCTAGTTTGTC-3') and reverse primer (5'-CGGAATTC TCACTCATGAACTAACTTGGCTAA-3'), and cloned into PET30a with BamHI/EcoRI.

pGEX6p-1-SPT5 KOW

To generate *pGEX6p-1-SPT5 KOW6*, the *KOW6* fragment was digested from *PET30a-SPT5 KOW6* by BamHI/EcoRI, cloned into pGEX6p-1.

PET- SPT5 CTR

To generate *PET- SPT5 CTR4* repeat, the *CTR4* repeat was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-CGGGATCCGGAAGCCAAACTCCTATGCATCCTT-3') and reverse primer (5'-CGGAATTCGATAGGTGTAGCTCCAGAATGCCGC-3'), and cloned into PET-30a with BamHI/EcoRI

PET- SPT5 CTR^{SA}

To generate *PET- SPT5 CTR^{SA}*, the *SPT5 CTR^{SA}* fragment was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-CCGGAATTC GAAACACCTATGCATCCTG-3') and reverse primer (5'-CCGCTCGAG CCTCATTCATCATGGATTGGTGTAGCTCCAGC-3'), and cloned into PET-30a with EcoRI/XhoI

PET-SPT5 CTR^{TA}

To generate *PET-SPT5 CTR^{TA}*, the *CTR^{TA}* fragment was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-

CGGGATCCGGAAGCCAAGCTCCTATGCATCCTTCCCGGGCTCCACTT-3') and reverse primer (5'- CGGAATTCGATAGGTGCAGCTCCAGAATGCCGCATTGGAGCCATACA -3'), and cloned into PET-30a with EcoRI/BamHI

PET-SPT5 CTR^{TD}

To generate *PET-SPT5 CTR^{TD}*, The *CTR^{TD}* fragment was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'- CATGCCATGG GGAAGCCAAGATCCTATGCATCCTTCCCGGGATCCACTT-3') and reverse primer (5'- CGGAATTC GATAGGATCAGCTCCAGAATGCCGCATTGGATCCATACA -3'), and cloned into PET-30a with NcoI/BamHI

PET- SPT5 CTR^{TE}

To generate *PET- SPT5 CTR^{TE}*, the *CTR^{TE}* fragment was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'- CGGGATCC GGAAGCCAAGAACCTATGCATCCTTCCCGGGAACCACTT-3') and reverse primer (5'- CGGAATTC GATAGGTTTCAGCTCCAGAATGCCGCATTGGTTCCATACA -3'), and cloned into PET-30a with BamHI/EcoRI

ProSPT5::SPT5-1300FLAG/N

To generate *ProSPT5::SPT5-1300Flag/N*. The *SPT5* promoter was amplified from an Arabidopsis genomic using forward primer (5'- TGCCTGCAGG AGAGACACTCACCAACAGAGACCT-3') and reverse primer (5'-CGGGGTACC TGGTTCGATTACAGAGAACTAGG-3'), and cloned into pCAMBIA1300Flag/N with SbfI/KpnI. The *SPT5* was amplified from an Arabidopsis genomic using forward primer (5'- TCAGC AGTCGAAGAGC ATGTCTCAGTACTCAGACGACGAT-3') and reverse primer (5'- TTAGCGTGTGAAGA GC CTCCTGAACCTAAGTGGCTAA-3'), cloned into *ProSPT5::* pCAMBIA1300FLAG/N by Gateway.

ProSPT5::SPT5-1300FLAG/N CTR^{TA}

To generate *ProSPT5::SPT5-1300FLAG/N CTR^{TA}*. The *CTR* T mutant to A was amplified from *ProSPT5::SPT5-1300Flag/N* using forward primer 1 (5'- GCTCCTATGCATCCTTCCCGGGCTCCACTTCATCCCTGTATGGCTCCAATGCGGCATTCT G-3'), reverse 1 primer (5'- CCATACAGGGATGAAGTGGAGCCCGGGAAGGATGCATAGGAGCTTGGCTTCCCATAT TA -3'), forward primer 2 (5'- GCACCTATCCATGATGGAATGAGGGCACCTATGCGTGGt-3'), reverse primer 2 (5'- TGCCCTCATTCCATCATGGATAGGTGCAGCTGAATTTTT-3'), forward primer 3 (5'- AGATTGGGGTAGTAGTGCTCCTGGTTCGTA-3'), and reverse primer 3 (5'- AGCACTACTACCCCAATCTGATCCAGGCG-3')

ProSPT5::SPT5-1300FLAG/N CTR^{TD}

To generate *ProSPT5::SPT5-1300FLAG/N CTR^{TD}*. The *CTR* T mutant to D was amplified from *ProSPT5::SPT5-1300FLAG/N* using forward primer 1 (5'- GATCCTATGCATCCTTCCCGGGATCCACTTCATCCCTGTATGGATCCAATGCGGCATTC

-3'), reverse 1 primer (5'-ATCCATACAGGGATGAAGTGGATCCCGGGAAGGATGCATAGGATCTTGGCTTCCCATA-3'), forward primer 2 (5'-GATCCTATCCATGATGGAATGAGGGATCCTATGCGTGG-3'), reverse primer 2 (5'-ATCCCTCATTCCATCATGGATAGGATCAGCTGAATTTTT-3'), forward primer 3 (5'-AGATTGGGGTAGTAGTGATCCTGGTCGTA-3'), and reverse primer 3 (5'-ATCACTACTACCCCAATCTGATCCAGGCG-3')

pGBKT7-SPT5 N

To generate *pGBKT7-SPT5 N*, the *SPT5 N* fragment was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-CATGCCATGGAGGATGAGGACGAAGAAGAC-3') and reverse primer (5'-CGGAATTCTAACCCACGAGTCACGGGATAAAT-3'), and cloned into pGBKT7 with NcoI/EcoR I.

pGBKT7-SPT5 KOW

To generate *pGBKT7-SPT5 KOW*, the *KOW* fragment was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-CATGCCATGGCTGCTGTTCTTTCTGTGCGAG-3') and reverse primer (5'-CGGAATTCTTGGCTTCCCATATTATACTGCGG-3'), and cloned into pGBKT7 with NcoI/EcoR I.

PET-SPT5 C

To generate *PET-SPT5 C*, the *C* fragment from *SPT5* cDNA was digested from *pGEX6p-1-SPT5 C* by BamHI/EcoRI, and cloned into PET30a.

pGBKT7-SPT5 C

To generate *pGBKT7-SPT5 C*, the *SPT5 C* fragment was digested from *PET30a-SPT5 C* by NcoI/EcoR I, and cloned into pGBKT7.

PET-SPT5 CTR

To generate *PET-SPT5 CTR*, the *SPT5 CTR* fragment was digested from pGEX6p-1-SPT5 CTR by BamHI/EcoRI, and cloned into PET30a.

pGBKT7-SPT5 CTR

To generate *pGBKT7-SPT5 CTR*, the *CTR* fragment was digested from *PET30a-SPT5 CTR* by NcoI/EcoR I, and cloned into pGBKT7.

pGBKT7-SPT5 KOW6

To generate *pGBKT7-SPT5 KOW6*, the *KOW6* fragment was digested from *PET30a-SPT5 KOW6* by NcoI/EcoR I, and cloned into pGBKT7.

PET-VIP5

To generate *PET-VIP5*, the *VIP5* was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-CGCGGATCC ATGGGTGATTTAGAGA AACTTGCTT-3') and reverse primer (5'-CCGGAATTC AATGCAAATAAATCCGAGAAGA-3'), and cloned into PET-30a with BamHI/EcoRI

pGADT7-VIP5

To generate *pGADT7-VIP5*, the *VIP5* cDNA was digested from *PET30a-VIP5* by *NcoI*/ *EcoRI*, and cloned into *pGADT7*

pGADT7-VIP5 PLUS3

To generate *pGADT7-VIP5 plus3*, the *plus3* fragment was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-CATGCCATGGTTGAGTAGTTCCAGCCAAAGTGACA-3') and reverse primer (5'-CCGCTCGAGAACATTCATTGGCCTGACTGACGCA-3'), and cloned into *pGADT7* with *NcoI*/*XhoI*

PUC-spYNE-VIP5

To generate *PUC-spYNE-VIP5*. The *VIP5* cDNA was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-CGCGGATCC ATGGGTGATTTAGAGAACTTGCTT-3') and reverse primer (5'-TCCCCCGGG AATGCAAATAAATCCGAGAAGA-3'), and cloned into *PUC-spYNE* with *BamHI*/ *SmaI*

PUC-spYNE-CDKD2

To generate *PUC-spYNE-CDKD2*, the *CDKD2* cDNA was digested from *pGEX6p-1-CDKD2* by *BamHI*/*SmaI*, and cloned into *PUC-spYNE*.

PUC-spYCE-SPT5 C

To generate *PUC-spYCE-SPT5 C*, the *SPT5 C* fragment was digested from *pGEX6p-1-SPT5 C* by *BamHI*/*XhoI*, and cloned into *PUC-spYCE*.

PET-VIP5 C

To generate *PET-VIP5 C*, the *VIP5 C* fragment was amplified from an Arabidopsis first strand cDNA pool using forward primer (5'-CCGGAATTC GAAGCAGCTTTAGAAAGCAGCTG-3') and reverse primer (5'-TCCCCCGGG AATGCAAATAAATCCGAGAAGA-3'), and cloned into *PET-30a* with *BamHI*/*SmaI*.

Genotyping primers

SALK_062223

Forward primer: GTCTCTTGCCATCGTTCTCTG

Reverse primer: ATGTGCCTACGTTTGAGGATG

SALKseq_9711

Forward primer: ATTCAACATTCAGCAACCAGC

Reverse primer: AGGGTAATATAACGCATGGGG

SALK_065163

Forward primer: GATGGTTTGTGTTTTGGTGTGG

Reverse primer: CTCAGCAAAAATACAGCCTGC

SALK_063929

Forward primer: TTTCCGGATAGCATTTTGTG

Reverse primer: AAGCTGGAATCTGAAGCTTCC

SALK_126891

Forward primer: GGAATTCTGAAACCTCCGAAG

Reverse primer: AAGGTCCGTTCCATTATCCAC

CS813305

Forward primer: AGGAAATCAAGCCAAACCATG

Reverse primer: TGGTGGTAGTTACTCGGATGC

flc-3

Forward primer: TATCGCCGGAGGAGAAGC

Reverse primer: TAGAAAGAAATAAAGCGAGAAAAGGA

FRIGIDA

Forward primer: GGGGTACCATGGTGAGCAAGGGCGAGGAGCTGTT

Reverse primer: GGGGTACCACTTGTACAGCTCGTCCATGCCGAGA

SALK_126891

Forward primer: GGAATTCTGAAACCTCCGAAG

Reverse primer: AAGGTCCGTTCCATTATCCAC

CS 813305

Forward primer: AGGAAATCAAGCCAAACCATG

Reverse primer: TGGTGGTAGTTACTCGGATGC

RT-PCR primer

SPT5

Forward primer: CGGTGAAAGATGTTGTCAGG

Reverse primer: AAGGTTATGCCGGTCATGTAT

VIP5

Forward primer: GAAATGAAACCTCGGCGGCT

Reverse primer: TCATTGGCCTGACTGACGCA

CDKD;2

Forward primer: GATTAGTCTTGTTCTTGATG

Reverse primer: CTACAGAACTAATCAATTGC

FLC

Forward primer: CGGTCTCATCGAGAAAGCTC

Reverse primer: CCACAAGCTTGCTATCCACA

GTA2

Forward primer: GAGTCCTCAATATCAGCCGG

Reverse primer: TCACGGTTGCACAACTTGG

UBIQUITIN 10

Forward primer: AGGATGGCAGAACTCTTGCT

Reverse primer: TCCCAGTCAACGTCTTAACG

Primers for ChIP-PCR

FLC

Region 1

Forward primer: TGGAGGGAACAACCTAATGC

Reverse primer: TCATTGGACCAAACCAAACC

Region 2

Forward primer: CGACAAGTCACCTTCTCCAAA

Reverse primer: AGGGGGAACAAATGAAAACC

Region 3

Forward primer: GGCGGATCTCTTGTTGTTTC

Reverse primer: CTTCTTCACGACATTGTTCTTCC

Region 4

Forward primer: GGGGCTGCGTTTACATTTTA

Reverse primer: GTGATAGCGCTGGCTTTGAT

Region 5

Forward primer: CTTTTTCATGGGCAGGATCA

Reverse primer: TGACATTTGATCCCACAAGC

Region 6

Forward primer: CTTTTTCATGGGCAGGATCA

Reverse primer: TGACATTTGATCCCACAAGC

Region 6

Forward primer: CGTGTGAGAATTGCATCGAG

Reverse primer: AAAAACGCGCAGAGAGAGAG

UBIQUITIN 10

Forward primer: AGGATGGCAGAACTCTTGCT

Reverse primer: TCCAGTCAACGTCTTAACG