

Supplementary Information for

Robust control of floral meristem determinacy by position-specific multifunctions of KNUCKLES

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SI Materials and Methods

Plant Materials and Growth Condition

All *Arabidopsis thaliana* plants are Landsberg *erecta* (L*er*) background, except for *pWUS:WUS-linker-GFP wus-101* (GABI-Kat line GK870H12, Columbia background). All plants are grown at 22°C under continuous light.

DEX (D4902, Sigma-Aldrich) and CHX (01810, Sigma-Aldrich) treatments were performed as described previously (1). 1 μM DEX solution was used for for *ap1 cal 35S:AP1-GR*, 10 μM DEX for *ap1 cal 35S:KNU-GR-myc*, and 10 μM CHX for *ap1 cal 35S:KNU-GR-myc*. The initial time of DEX or CHX treatment was defined as Day 0 or 0 h.

Vector Construction

For *pCLV3:KNU* vector*,* construction process was as follows: The 3156-bp promoter fragment upstream of the start codon of *CLV3* was amplified and cloned into pCR8 TOPO cloning vector (Invitrogen). Then 2208-bp fragment downstream of *CLV3* stop codon was introduced after the promoter fragment. KNU full-length coding sequence was later inserted into the Sac I site created at the 3' end of *CLV3* promoter fragment. Finally, the cloned fragment in pCR8 was recombined into CD3-694 vector by LR recombinase (Invitrogen).

To generate *pCLV3:KNU-GFP, pCLV3:KNU-NLS* and *pCLV3:KNU-GFP-NLS,* the coding sequences of GFP, SV40 NLS, and GFP-NLS were introduced at the 3' end of *KNU* coding sequence in pCR8*-pCLV3:KNU* vector respectively and recombined into CD3-694 vector.

To generate *pWUS:WUS-myc*, 4058-bp *WUS* genomic fragment including 2489-bp *WUS* promoter sequence upstream of start codon was cloned into pENTR-D (Invitrogen). Then, SfoI site was introduced before the stop codon. Subsequently, the 1220-bp sequence downstream of *WUS* stop codon was added to generate the pENTR-D-*pWUS:WUS*. Later, *myc* fragment was introduced into the SfoI site. Finally, the pENTR-D based construct was recombined into the binary vector pKGW by LR recombinase (Invitrogen).

In order to obtain *pWUS:KNU*, the intermediate pENTR-D vector containing 2489-bp *WUS* promoter sequence followed by 1220-bp sequence downstream of *WUS* stop codon was first created. Then *KNU* CDS was cloned after the 2489-bp *WUS* promoter sequence to generate pENTR-D-*pWUS:KNU*. Finally, pENTR-D based constructs were recombined into the binary pBGW vector.

For the construction of *pGIR1:KNU* or *pGIR1:KNU-eGFP*, the 2242-bp promoter fragment upstream of *GIR1* start codon was cloned into pCR8 vector. Then *KNU* or *KNU*-*eGFP* coding sequences were added after the *GIR1* promoter fragment. To generate *pAtML1:KNU* or *pAtML1:KNU-eGFP*, the 3616-bp promoter fragment upstream of *AtML1* start codon followed by 957-bp fragment downstream of *AtML1* stop codon was cloned into pCR8 vector. Subsequently, *KNU* or *KNU-eGFP* coding sequences were inserted into the SfoI site at the end of the *AtML1* promoter fragment. For *pMCT1:KNU* or *pMCT1:KNU-eGFP*, 2396-bp *MCT1* promoter fragment upstream of start codon was cloned into pCR8 vector, and *KNU* or *KNU-eGFP* coding sequences were added after *MCT1* promoter fragment. To generate the *pCLV1:KNU* or *pCLV1:KNU-eGFP*, 5704-bp *CLV1* promoter upstream of start codon and 1063-bp fragment downstream of stop codon was introduced to pCR8 vector. Then *KNU* or *KNU-eGFP* were cloned to the 3' end of *CLV1* promoter fragment. Finally, the fused fragments in pCR8 were introduced to pBGW.

To generate amiRNA for silencing *KNU*, the amiRNAs (21-nt) were designed by the Web MicroRNA Designer (WMD3, [http://wmd3.weigelworld.org./cgi-bin/webapp.cgi\)](http://wmd3.weigelworld.org./cgi-bin/webapp.cgi). Pre-amiRNA was assembled by several rounds of PCR using primers listed in *SI Appendix,* Table S2. The final PCR fragments were driven under aforementioned *CLV3* or *WUS* promoters in pCR8 or pENTR-D, and later recombined into the binary CD3-694 or pBGW vectors respectively.

RNA Extraction and Expression Analyses

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The total RNA was isolated from inflorescence tissues using RNA isolater Total RNA Extraction Reagent (Vazyme). The HiScript II Q RT SuperMix for qPCR kit (Vazyme) was used to synthesize cDNA. ChamQ Universal SYBR qPCR Master Mix was used for quantitative real-time PCR (Vazyme), and the assays were performed in triplicate using the Step One Plus real-time PCR system (Applied Biosystems). The gene *Tip41-like* (AT4G34270) was used as the internal reference gene. Except for expression assays of *MIR394B* and *LCR* (performed with two biological replicates), all gene expression assays were carried out for three biological replicates.

Chromatin Immunoprecipitation Assay

Chromatin immunoprecipitation (ChIP) experiments were performed according to previous description (1) using a ChIP Assay Kit (17-295, Millipore). To estimate the H3K27me3 enrichment, cleared chromatin solution was incubated with anti-H3K27me3 antibody (07-449, Millipore) in a ratio of 1:200 (v/v). To monitor the Pol II enrichment on *CLV3* locus, Pol II antibody (ab817, Abcam) was used for immunoprecipitation in a ratio of 1:200 (v/v). To check the enrichment of KNU on *CLV3* and *CLV1*, the chromatin complexes from *ap1 cal 35S:KNU-GR-myc* inflorescence tissues was pre-cleared with protein-A agarose beads (16-157, Millipore), and then incubated with anti–c-Myc-Agarosebeads (A7470, Sigma-Aldrich) in a ratio of 1:20 (v/v). To estimate the enrichment of KNU on *CLV3* locus, the chromatin complexes from *pCLV3:KNU-GFP* and *pCLV3:KNU-GFP-NLS* inflorescence tissues was pre-cleared with protein-G agarose beads (16-201, Millipore), and incubated with GFP-Trap Agarose (gta-20, ChromoTek) in a ratio of 1:20 (v/v) overnight. Agarose beads without antibody were added to same solution as control. DNA fragments was purified using PCR Purification Kit (#K0702, Thermo). On *CLV3* locus, nine primer pairs for ChIP assays were designed and eventually six pairs were chosen as representatives. The other three pairs with negative KNU enrichment were only presented in supplemental figures. The enrichment rate for H3K27me3 level on *CLV3* locus was presented as the ratio of bound DNA after IP to the input DNA before IP. The enrichment rate of other ChIP experiments were shown as antibody-bound

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DNA to no antibody control. *Mu-like* transposons was taken for the negative control locus and the relative enrichment rate was set to 1 for all ChIP experiments. Except for KNU binding on *BAM* genes (performed with two biological replicates), three independent biological replicates were performed and each ran in triplicate (technical replicates) for all ChIP assays.

Electrophoretic Mobility Shift Assay

The electrophoretic mobility shift assays were carried out according to previous procedures (1). The CDS of *WUS* and *KNU* were individually cloned into the multiple cloning sites and fused with *MBP* in pMAL-c5G vector (NEB). The MBP-tagged proteins were expressed in Transetta (DE3) Chemically Competent Cells (TransGen Biotech) and purified using Amylose Resin (#E8021V, NEB). The DNA-protein binding reactions, and detection of biotin-labeled probes were performed by using the EMSA kit (20148, Thermo).

Size-Exclusion Chromatography (SEC)

Full-length *KNU* CDS was cloned into the expression vector pMAT9s containing an N-terminal 6×His-tag followed by maltose binding protein (MBP) and a SARS-Mpro cleavage site. The recombinant *KNU* was expressed in BL21 CodonPlus (DE3)-RIL *Escherichia coli* cells (Stratagene) and induced with 0.3 mM IPTG at 18°C for 20 h. The resulting MBP-fusion protein was purified by an MBPtrap HP affinity column (GE Healthcare), followed by a HitrapQ anion exchange column (GE Heathcare) in 50 mM Tris-HCl (pH 8.0) using a 50-300 mM NaCl gradient elution. MBP-KNU was further purified using a Superdex 200 Increase 10/300 GL column (GE Heathcare) equilibrated in 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1 mM Tris (2-carboxyethyl) phosphine (TCEP) and the protein purity was examined by SDS-PAGE. Duplex DNAs were annealed using complementary single-strand HPLC purified DNAs (GenScript). Lyophilized oligonucleotides were dissolved in 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1 mM TCEP and mixed 1:1 with the complementary strand at a final concentration of 500 µM. The reaction mixture was heated to 95 °C for 5 min and naturally cool to

room temperature.The SEC was performed at 4 °C using a Superdex 200 Increase 10/300 GL column (GE Healthcare) pre-equilibrated in 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1 mM TCEP. The KNU protein (0.8 mg/mL) was mixed with a solution of annealed, blunt-ended P3-2 dsDNA (55-bp) at a molar ratio of 1:0.6 (protein:dsDNA) and incubated on ice for about 30 min. The \sim 100 μ L volume of mixture was then injected and eluted one column volume at a flow rate of 0.5 mL/min.

Sectioning and Confocal Microscopy Imaging

For floral section, inflorescence tissues were embedded in 5% liquid LM agarose (CA1351, Coolaber) at 40°C and then placed on ice for 10 min. Sections of 80 μm thickness were produced using a vibratome (VT1000S, Leica). Then the obtained floral sections were placed onto microscope slides mounted with 0.1% MES or 1 μg/mL FM4-64 (T13320, Thermo) solution. Floral sections were snapped with Zeiss LSM880 confocal microscope. For GFP-only floral tissues, the excitation wavelength was 488 nm, and the detection wavelength was between 493 and 594 nm. For the observation of floral tissues expressing both GFP and VENUS, GFP was stimulated at 488 nm and detected with a spectrum from 493 to 518 nm, and VENUS was stimulated at 514 nm and detected with a spectrum from 519 to 620 nm. For FM4-64 observation, it was excited at 514 nm and the detection wavelength was between 592 and 758 nm.

Co-IP Assays

The floral buds at stage 6 from transgenic line *ap1 cal 35S:AP1-GR pKNU:KNU-VENUS pWUS:WUS-myc* were collected for protein extraction and *ap1 cal 35S:AP1-GR* or *ap1 cal 35S:AP1-GR pKNU:KNU-VENUS* was used as a negative control. Co-IP experiments was operated as described previously (1). In Western blot analysis, KNU-VENUS and WUS-myc were immunoblotted with anti-GFP antibody (2856, Cell Signaling Technology) and anti-myc antibody (A00173, GenScript) respectively. Three independent experiments were performed, and similar results were obtained, and representative data were exhibited.

Yeast One-Hybrid Assays

The experimental procedures was conducted followed the manufacturer's instructions (Clontech). Three tandem repeated CLV3-P3 fragments were cloned into the pABAi vector (Clontech). The resulting constructs were linearized with BstBI and transformed into the Y1H Gold yeast strain (Weidi Biotech). The full coding sequence of *KNU* was fused with the GAL4 activation domain in vector pGADT7, forming AD-KNU. The plasmid containing AD-KNU was subsequently transformed into the Y1HGold strain containing the pABAi-CLV3-P3 constructs. The validated interaction between WUS-W2 and KNU was used as a positive control (1), and the empty pGADT7 was used as a negative control. The DNA-protein interaction in yeast was selected by SD/-Ura agar plates containing 150 ng/mL aureobasidin A (AbA).

Yeast Two-Hybrid Assays

To obtain yeast two-hybrid assays vectors, the full length KNU and series truncated KNU were individually cloned into pGADT7 (Clontech). The full length WUS and series truncated WUS were individually cloned into pGBKT7 (Clontech). The full length CLV3 was also cloned into pGBKT7. The yeast two hybrid assays were performed using the Yeastmaker Yeast Transformation System 2 (Clonetech) according to the manufacturer's instructions.

Yeast Three-Hybrid Assays

The vectors of pBridge (Clontech) and pGADT7 (Clontech) were used for yeast three-hybrid assays. The full length WUS and KNU were cloned into MCS I and MCS II of pBridge respectively. The full length WUS, STM and HAM1 were cloned into pGADT7 vector respectively. The pGADT7 vectors were co-transformed with pBridge vectors into the yeast strain AH109 in designed combinations, and screened on SD/-Leu/-Trp/-Met agar plates. The interactions among three proteins were evaluated on SD/-Leu/-Trp/-Met/-His agar plates complemented with 3-AT .

Protein Extraction and Expression Analyses in Yeast

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Protein extraction experiments were performed according to previous method (2). Yeast cells were collected at OD600 = 1.0 and then treated with 2.0 M LiAC solution for 1 min, then replacing with 0.4 M NaOH for 5 min. Subsequently, cells were resuspended in SDS-PAGE loading buffer and boiled for 10 min. For relative level of protein expression, BD-WUS was immunoblotted with anti-BD antibody (ABP57232, Abbkine), and AD-HA-STM or HA-KNU was immunoblotted with anti-HA antibody (26D11, Abmart) by Western blot.

Bimolecular Fluorescence Complementation Assay

The full-length coding sequences of KNU and WUS were cloned into pGreen vectors and transformed into Agrobacterium. The Agrobacteria were co-infiltrated into tobacco (*Nicotiana benthamiana*) leaves of 4-week-old plants as described previously (3). The infected tobacco leaves were cultured for 48-72 h before observation. For DAPI staining, leaves were immersed in 5 μg/mL DAPI solution. Leica TCS SP8-MaiTai M confocal microscopy was used for imaging.

Bimolecular Luciferase Complementation Assays

Bimolecular luciferase complementation (BiLC) assays were performed as previous description (4). The full length *WUS* CDS was cloned into JW771. The full length CDS of *WUS, HAM1* and *STM* were cloned into JW772 respectively. The full length *KNU* CDS was cloned into pGreen vector. The constructs were transformed into Agrobacterium subsequently. To test the WUS-WUS interaction, WUS-HAM1 interaction or WUS-STM interaction, Agrobacterium cells were resuspend at $OD₆₀₀=0.8$ and different combinations were co-infiltrated into tobacco leaves of 4-week-old plants. To monitor the disruptive effects of KNU for WUS-WUS, WUS-HAM1 or WUS-STM interactions, increasing amounts of Agrobacterium cells transformed with 35S:KNU (OD₆₀₀=0.2 and 0.4) were incorporated and Agrobacterium cells transformed with empty pGreen vector served as control (OD600=0.4). The infected tobacco leaves were cultured for 72 h before observation. The LUC activity was monitored with an imaging system (ChemiDOC™ XRS+, Bio-Rad)

after luciferin was infiltrated for 10 min.

Statistical Analyses

The statistical difference between two groups were evaluated by Student's t-tests using SPSS software (version 21, IBM). The *P*-value < 0.05 represents significant difference. *P*-values < 0.05 are represented by an asterisk (*) and *P*-values < 0.01 are represented by two asterisk (**). Detailed results of statistical analyses are available in *SI Appendix,* Table S3.

Accession Numbers

All genes information in this study were obtained in the *Arabidopsis* Genome Initiative according to accession numbers as follows: *KNU* (AT5G14010), *WUS* (AT2G17950), *CLV1* (AT1G75820), *CLV2* (AT1G65380), *CLV3* (AT2G27250), *GIR1* (AT5G06270), *AtML1* (AT4G21750), *MCT1* (AT1G37140), *STM* (AT1G62360), *HAM1* (AT2G45160), *CRN* (AT5G13290), *RPK2* (AT3G02130), *BAM1* (AT5G65700), *BAM2* (AT3G49670), *BAM3* (AT4G20270), *CIK1* (AT1G60800), *CIK2* (AT2G23950), *CIK3* (AT4G30520), *CIK4* (AT5G45780), *CLE6* (AT2G31085), *CLE9* (AT1G26600), *CLE10* (AT1G69320), *CLE11* (AT1G49005), *CLE12* (AT1G68795), *CLE13* (AT1G73965), *CLE18* (AT1G66145), *CLE19* (AT3G24225), *CLE20* (AT1G05065), *CLE21* (AT5G64800), *CLE22* (AT5G12235), *CLE45* (AT1G69588), *AP1* (AT1G69120), *CAL* (AT1G26310), *MIR394B* (AT1G76135), *LCR* (AT1G27340).

Fig. S1. Expression patterns of *KNU*, *CLV3* and *WUS* in floral buds. (*A*-*M*) Expression patterns of *KNU* and *CLV3* in floral buds from plant doubly transgenic for *pKNU:KNU-VENUS* (red) and *pCLV3:GFP-ER* (green). (*A*–*D*) stage 6 floral bud, (*E*-*I*) stage 7 floral bud, and (*J*–*M*) stage 8 floral bud. (*N*-*S*) *WUS* expressions in early floral buds. (*N*-*P*) WUS activity in flower buds in stage 6 (*N*), stage 7 (*O*) and stage 8 (*P*) of *pWUS:WUS-linker-GFP*. (*Q*-*S*) WUS activity in flower buds in stage 6 (*Q*), stage 7 (*R*) and stage 8 (*S*) of *knu-2 pWUS:WUS-linker-GFP*. (Scale bars: 50 µm).

Fig. S2. The indeterminate phenotype of *knu-2* is rescued by induced KNU in *35S:KNU-GR knu-2* lines. (*A*) Flower phenotype of *knu-2*. (*B-C*) Flower phenotypes of *35S:KNU-GR knu-2* with one time (*B*) and 3 times (*C*) of DEX treatments. (Scale bars: 1 mm). Numbers indicate the ratio of flowers with little rescue (*B*) and full rescue (*C*).

Fig. S3. KNU directly binds to *CLV3* promoter. (*A*) Schematic diagram of *CLV3* locus and originally designed 9 primer sets used for ChIP assays. (*B*) ChIP assay using *ap1 cal 35S:KNU-GR-myc* inflorescences. Nuclear proteins were immuno-precipitated with anti-c-Myc agarose beads, and the enriched DNA was used for q-PCR assays. The y-axis shows relative enrichment compared with no antibody (negative control). MU served as a negative control locus, and the values of MU were calibrated to 1. Error bars represent SD of three biological replicates. Asterisks indicate significant differences between MU and different primer sets on *CLV3* (**P* < 0.05 and ***P* < 0.01, Student's *t*-test). N1-N3 primer sets showing no enrichment and were omitted in main figures, and P1-P6 were used as representative primer sets in the main figures. (*C*) Sequences of WT and mutated competitors of P3-2 oligonucleotides (from –210-bp to –156bp upstream of ATG start codon) used for the EMSAs. The red letters represent mutated oligonucleotides. (*D*) The binding of KNU to WT P3-2 is not affect by M4, and is slightly weakened by single nucleotide mutant competitor probes including M4-1, M4-2, M4-3, M4-4 and M4-6. The black arrow indicates DNA-protein complex. Non-labeled oligo-nucleotides were used as competitors. (*E* and *F*) Interaction of KNU with P3-2 dsDNA in vitro. (*E*) The purity of recombinant MBP-tagged KNU (MBP-KNU) as demonstrated by SDS-PAGE (left) and analytical size-exclusion chromatography (right). MBP-KNU (100 μL) at 1 mg/ml was applied to a Superdex 200 Increase 10/300 GL column. (*F*) Size-exclusion chromatographic profiles of individual MBP-KNU (150 μM) (dotted line), dsDNA (75 μM) (dashed line), and their mixture (solid line). Samples were detected by examining the absorbance at 280 nm (blue lines) and 260 nm (red lines).

Fig. S4. H3K27me3 deposition on *CLV3* is KNU-dependent. (*A*) Schematic diagram of *CLV3* locus and primer sets used for ChIP assays. (*B* and *C*) Analysis of H3K27me3 enrichment on *CLV3* by ChIP assays using *ap1 cal 35S:AP1-GR* inflorescences (*B*) and *knu-2 ap1 cal 35S:AP1-GR* (*C*) in day 0 (D0) and day 4 (D4) after treatment with DEX. MU served as negative control locus and the values of MU were calibrated to 1. Error bars represent SD of three biological replicates. Asterisks indicate significant differences between D0 and D4 at certain primer sets on *CLV3* (**P* < 0.05 and ***P* < 0.01, Student's *t* test).

Fig. S5. KNU represses *CLV3* in *pCLV3:KNU* and *pCLV3:KNU-NLS*. (*A*) Phenotypic analysis of L*er*-0 (*n* = 100), *pCLV3:KNU* (*n* = 113) and *pCLV3:KNU-NLS* (*n* = 228) T1 plants. (*B* and *C*) Phenotypes of L*er*-0 (*B*) and *pCLV3:KNU* lines of moderate phenotype (*C*). (*D*) Average numbers of floral organs in *Ler-0*, *pCLV3:KNU* lines of moderate phenotype and *pCLV3:KNU-NLS* enhanced FM lines. Floral organs from 100 random flowers were used for analysis. (*E* and *F*) The seedling phenotypes of L*er*-0 (*E*) and *pCLV3:KNU* (*wus*-like)lines (*F*)*.* (*G*) Levels of *CLV3* and *WUS* mRNA in inflorescence samples from L*er*-0, *pCLV3:KNU* moderate lines and *pCLV3:KNU-NLS* (*clv3*-like)lines. (*H*) The activity of KNU was not detected in stage 3 bud of *pKNU:KNU-VENUS* plants. (*I*) Levels of endogenous *KNU* mRNA in inflorescence samples (with floral buds no later than stage 7) from L*er*-0 and *pCLV3:KNU-NLS* (*clv3*-like) lines. Transcript levels are quantified by q-PCR in (*G* and *I*). For q-PCR, *Tip41-like* served as the internal control. Error bars represent SD of three biological replicates. Asterisks indicate significant differences between L*er*-0 and transgenic lines (**P* < 0.05 and ***P* < 0.01, Student's *t* test). (*J* and *K*) The inflorescence phenotypes of *pCLV3:KNU-NLS* (*clv3*-like)lines (*J*) and *clv3-2* (*K*). (*L* and *M*) The silique phenotypes of *pCLV3:KNU-NLS* (*clv3*-like)lines (*L*) and *clv3-2* (*M*). (Scale bars: 2 cm in *B* and *C*; 1 cm in *E* and *F*; 50 μm in *H*; 1 mm in *J*–*M*).

Fig. S6. KNU represses *CLV3* through *CLV-WUS* pathway. (*A*-*C*) Plant phenotypes of *wus-7 pCLV3:KNU*-*NLS* (*clv3*-like) (*A*), *wus-7* (*B*) and *pCLV3:KNU*-*NLS* (*clv3*-like)(*C*). (*D*-*F*) Flower phenotypes of *wus-7 pCLV3:KNU*-*NLS* (*clv3*-like)(*D*), *wus-7* (*E*) and *pCLV3:KNU*-*NLS* (*clv3*-like)(*F*). (Scale bars: 2 cm in *A*-*C*; 1 mm in *D*–*F*).

Fig. S7. KNU represses *CLV3* in FM. (*A*-*I*) GFP signals in SAM (*A*-*F*) and stage 3-4 floral bud (*G*-*I*) of *pCLV3:GFP-ER* plants. (*J*-*L*) GFP signals in stage 3-4 floral bud of *pCLV3:KNU-GFP* plants. (*M*) Flower phenotype of *pCLV3:KNU-GFP*. (*N*-*P*) GFP signals in stage 3-4 floral bud of *pCLV3:KNU-GFP-NLS* (enhanced FM). (*Q*) Flower phenotype of *pCLV3:KNU-GFP-NLS* (enhanced FM). (Scale bars: 50 μm in *A*-*L*, *N*-*P*; 1 mm in *M* and *Q*).

Fig. S8. KNU represses of *CLV3* promoter activity. (*A*-*C*) Siliques of *pCLV3:KNU-GFP-NLS* showing different extent of FM indeterminacy. (*D*-*F*) GFP signals in stage 3-4 floral buds corresponding to siliques in (*A*-*C*). (*G* and *H*) ChIP assays for KNU (*G*) and Pol II (*H*) binding on *CLV3* by using inflorescences from *pCLV3:KNU-GFP* moderate lines and *pCLV3:KNU-GFP-NLS* (*clv3*-like) lines. Nuclear proteins were immuno-precipitated with GFP-trap in (*G*) and anti-pol II antibody in (*H*). MU served as negative control locus and the values of MU were calibrated to 1. Error bars represent SD of three biological replicates. Asterisks indicate significant differences between the two different lines at certain primer sets on *CLV3* (**P* < 0.05 and ** *P* < 0.01, Student's *t* test). (Scale bars: 1 mm in *A*-*C*; 50 μm in *D*-*F*).

Fig. S9. KNU represses *CLV3* and *WUS* in FM synergistically. (*A*) Phenotypic analysis of L*er*-0 (*n* = 100) and *pWUS:KNU* (*n* = 138). (*B*) Plant of *pWUS:KNU* with moderate phenotype. (*C*) Average number of floral organs in *Ler-0* (*n* = 100), *pCLV3:KNU* (*n* = 100) with moderate phenotype, *pWUS:KNU* (*n* = 100) with moderate phenotype and *pCLV3:KNU pWUS:KNU* (*n* = 100). (*D*) Phenotype of *pWUS:KNU* (*wus-*like) seedling . (*E*) *CLV3* and *WUS* mRNA levels in inflorescences of L*er*-0 and *pWUS:KNU* with moderate phenotype. Transcript levels are quantified by q-PCR. *Tip41-like* served as the internal control. Error bars represent SD of three biological replicates. Asterisks indicate significant differences between L*er*-0 and *pWUS:KNU* (***P* < 0.01, Student's *t* test). (*F* and *H*) Phenotypes of *pCLV3:KNU pWUS:KNU* plants. (*G*) Flower of *pCLV3:KNU pWUS:KNU*. (Scale bars: 2 cm in *B* and *F*; 1 cm in *D* and *H* and 1 mm in *G*).

Fig. S10. Silique phenotypes of *pCLV3:amiR-KNU* (*A* and *E*), *pWUS:amiR-KNU* (*B* and *F*), *knu-2* (*C* and *G*) and L*er*-0 (*D* and *H*). (Scale bars: 1 cm).

Fig. S11. KNU regulates FM determinacy in different stem cell layers independent of *miR394* signaling. (*A*) Phenotypic analysis of L*er*-0 (*n* = 100), *pGIR1:KNU* (*n* = 101), *pAtML1:KNU* (*n* = 87), *pMCT1:KNU* (*n* = 122) and *pCLV3:KNU* (*n* = 113). (*B*) Average number of floral organs in *Ler-0*, *pGIR1:KNU* lines of mild phenotype, *pAtML1:KNU* lines of mild phenotype, *pMCT1:KNU* lines of mild phenotype, *pMCT1:KNU* lines of moderate phenotype and *pCLV3:KNU* lines of moderate phenotype. Random 100 flowers each were used for analysis. (*C* and *D*) Flower phenotypes of *pAtML1:KNU* (*C*) and *pMCT1:KNU* (*D*). (*E*) *CLV3* and *WUS* mRNA levels in inflorescence from L*er*-0, *pGIR1:KNU*, *pAtML1:KNU* and *pMCT1:KNU*. Transcript levels are quantified by q-PCR.. Asterisks indicate significant differences between L*er*-0 and different transgenic lines (**P* < 0.05 and ***P* < 0.01, Student's *t* test). (*F*) *MIR394B* and *LCR* mRNA levels in *ap1 cal 35S:KNU-GR-myc* inflorescences after single DEX treatment. Gene transcript levels are quantified by q-PCR. For q-PCR analysis (*E* and *F*), *Tip41-like* served as the internal control. Error bars represent SD of three biological replicates for (*E*) and two biological replicates for (*F*). (Scale Bars: 1 mm).

Fig. S12. KNU physically interacts with WUS but not CLV3. (*A*-*D*) Expression patterns of *KNU* and *WUS* in stage 6 floral bud from plant doubly transgenic for *pKNU:KNU-VENUS* (red) and *pWUS:WUS-linker-GFP* (green). (Scale bars: 50 μm). (*E*) Y2H assay using full-length KNU and CLV3. Transformed yeast cells were grown on SD/-Leu/-Trp and SD/-Leu/-Trp/-His supplemented with 2 mM 3-AT. AD-KNU/BD-WUS serves as positive control. AD or BD refers to empty vector only. (*F*) Co-IP assay. Nuclear extracts were incubated with anti-c-Myc agarose beads. In Western blot analysis, KNU-VENUS and WUS-cMyc was immunoblotted with anti-GFP and anti-cMyc, respectively. Test and control represent samples from stage 6-7 flower buds of *ap1 cal 35S:AP1-GR pKNU:KNU-VENUS pWUS:WUS-myc* (*KNU-VENUS WUS-cMyc*) and *ap1 cal 35S:AP1-GR pKNU:KNU-VENUS* (*KNU-VENUS*), respectively. IP represents immunoprecipitation.

Fig. S13. Y2H assay for different domains of KNU and WUS. (*A*) Schematic diagrams of full length and truncated forms of protein KNU and WUS. For KNU, C2H2 and EARL indicate C2H2 domain (amino acids 40-60) and EAR-like motif (amino acids 155-161) respectively. For WUS, HD indicates homeodomain (amino acids 34-99), HOD2 indicates the second homodimerization interaction domains (amino acids 134-208), HBD indicates HAM binding domain (amino acids 203-236), Acidic indicates acidic domain, Wbox indicates WUS box and EARL indicates EAR-like motif. (*B*) Y2H assays. Transformed yeast cells were grown on SD/–Leu/–Trp (top panel) and SD/-Leu/-Trp/-His/-Ade/ (bottom panel) media. Empty refers to the AD or BD only.

Fig. S14. KNU does not affect WUS-STM interaction. (*A* and *B*) Y3H assay. Transformed yeast cells were grown on SD/-Leu/-Trp/-Met and SD/-Leu/-Trp/-Met/-His medium supplemented with 20 mM 3-AT. (*C*) Protein expression of WUS, STM and KNU in yeast cells for Y3H assay. Protein for Western blot analysis were extracted from AH109 (L1), AH109 transformed with AD-STM/BD-WUS/pMet-Empty (L2), and AH109 transformed with AD-STM/BD-WUS/pMet-KNU (L3). α-tubulin serves as a loading control. (*D*) BiLC assays. nLUC and cLUC refer to the N-terminal and C-terminal of luciferase. WUS-nLUC indicates WUS-nLUC fusion and STM-cLUC indicates STM-cLUC fusion. The color column on the right presents the range of luminescence intensity.

Fig. S15. KNU directly represses *CLV1*. (*A*) EMSAs confirm that KNU binds to the P4-1 and P4-2 fragments. The black arrow indicates DNA-protein complex. Non-labeled oligo-nucleotides were used as competitors. MBP was used as a negative control. (*B*) GFP signal in the stage 3-4 floral buds of *pCLV1:KNU-GFP*. (*C*-*E*) Phenotypes of plant (*C*), flower (*D*) and silique (*E*) of *pCLV1:KNU* (*clv1*-like). (*F* and *G*) Phenotypes of flower (*F*) and silique (*G*) of *clv1-1*. (*H*) *CLV1*, *CLV3* and *WUS* mRNA levels in inflorescences of L*er*-0 and *pCLV1:KNU*. Transcript levels are quantified by q-PCR. *Tip41-like* served as the internal control. Error bars represent SD of three biological replicates. Asterisks indicate significant differences between L*er*-0 and *pCLV1:KNU* (* *P* < 0.05 and ***P* < 0.01, Student's *t* test). (*I* and *J*) Phenotypes of plant (*I*) and flower (*J*) from *pCLV1:KNU* with moderate phenotype. (*K*) Phenotype of *pCLV1:KNU* (*wus-*like). (Scale bars: 50 μm in *B*; 2 cm in *C* and *I*; 1 mm in *D*-*G* and *J*; 1 cm in *K*).

Fig. S16. The compensation mechanisms in *pCLV1:KNU* and *ap1 cal 35S:KNU-GR-myc*. (*A*) Transcript levels of *BAMs* in inflorescences of L*er*-0 and *pCLV1:KNU*. Gene transcript levels are quantified by q-PCR. (*B*-*G*) KNU does not directly binds to *BAMs* locus in plants. (*B*, *D* and *F*) Schematic diagrams of *BAM1* (*B*)*, BAM2* (*D*) and *BAM3* (*F*) loci, and primer sets used for ChIP assays. (*C*, *E* and *G*) ChIP assays for KNU binding to *BAM1* (*C*)*, BAM2* (*E*) and *BAM3* (*G*) using *ap1 cal 35S:KNU-GR-myc* inflorescences. Nuclear proteins were immuno-precipitated with anti-c-Myc agarose beads, and the enriched DNA was used for q-PCR assays. The y-axis shows relative enrichment compared with no antibody (negative control). MU served as a negative control locus, and the values of MU were calibrated to 1. Error bars represent SD of two biological replicates. (*H* and *I*) Transcript levels of compensatory *CLV-*like signaling components (*H*) and *CLEs* (*I*) in *ap1 cal 35S:KNU-GR-myc* inflorescences after single DEX treatment. Gene transcript levels are quantified by q-PCR. ND indicates none detected. For q-PCR analysis in (*A*, *H* and *I*), *Tip41-like* served as the internal control. Error bars represent SD of three biological replicates. Asterisks mean significant differences between different time points (**P* < 0.05 and ***P* < 0.01, Student's *t* test).

Table S1. Phenotypic statistics.

Figure S5A. Phenotypic analysis of L*er*-0, *pCLV3:KNU, pCLV3:KNU-NLS*, *pCLV3:KNU-GFP* and *pCLV3:KNU-GFP-NLS* T1 plants.

Figure S5D. Average numbers of floral organs in L*er*-0, *pCLV3:KNU* lines of moderate repression phenotype and *pCLV3:KNU-NLS* enhanced FM lines.

Figure S9C. Average numbers of floral organs in L*er*-0, *pCLV3:KNU* with moderate repression *, pWUS:KNU* with moderate repression and *pCLV3:KNU pWUS:KNU* lines.

Figure S11B. Average numbers of floral organs in L*er*-0, *pGIR1:KNU* (mild repression), *pAtML1:KNU* (mild repression), *pMCT1:KNU* (mild repression), *pMCT1:KNU* (moderate repression) and *pCLV3:KNU* lines of moderate repression.

Table S2. Primer sequences.

Table S3. Statistical Analysis.

SI References

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