Figure 1. SMXL7 regulates plant development and flowering time

Figure 2. SMXL7 Forms Phase-separated Condensates

Figure 3. D14 colocalizes with SMXL7 nuclear condensates in a SL-Dependent Manner

Figure4. SMXL7 is directly associated with the H3K27me3 heterochromatin

Figure 5. SMXL7 nuclear condensates are enriched with JMJ30 to regulate flower time

Figure 6 SMXL7 is required for chromatin architecture regulation

Figure 1. SMXL7 regulates plant development and flowering time

a, Leaf morphology of 3-week-old plants. The fifth leaves are marked by white arrows. Scale bars, 1 cm.

b, The flowering phenotypes in selected plants during LD and SD photoperiods. Scale bars, 2 cm.

c, The morphological phenotypes of adult whole plants, Scale bars, 5 cm.

d, The morphological phenotypes of inflorescence tissues, siliques, rosette, and cauline leaves from adult plants. Scale bars, 2 cm.

e, Ratio of leaf length to width for the fifth leaves of plants after growth for 3 weeks

f, numbers of rosette leaves at flowering and days to bolting in selected plants during LD and SD photoperiods

g, Quantitative analysis of shoot branching in the adult plants shown in c. We counted the number of primary branches grown from the rosette leaf axil of at least 0.5 cm.

Values are the mean ± *SD* from at least 20 plants. *P* values were determined by two-tailed Student’s *t*-test and are indicated above columns

Figure 2. SMXL7 Forms Phase-separated Condensates

**a,** Representative confocal microscopic images of transiently expressed SMXL7-YFP/GFP in *Nicotiana benthamiana* leaf epidermal cells under the control of the *35S* promoter. Scale bars, 5 μm.

**b,** FRAP of SMXL7 nuclear condensates formed in *N. benthamiana* leaf epidermal cells. The white arrows indicate that the condensate is bleached. Time 0 s indicates the time of the photobleaching pulse; Data are representative of 11 independent experiments. Scale bars, 2 μm.

**c,** Plot showing the time course of the recovery after photobleaching SMXL7 nuclear condensates. Data are presented as mean ± s.d. (n = 11**)**.

**d,** TopImages showing the fusion of two SMXL7-YFP nuclear condensates in *N. benthamiana* leaf epidermal cells. Images are representative of three independent experiments. White arrows indicate the nuclear condensates that undergo fusion.

bottom Fluorescence time-lapse microscopy of *N. benthamiana* leaf epidermal cell nuclei that express SMXL7-GFP. Images are representative of three independent experiments. White arrows indicate the nuclear condensates that disrupt or shape. Scale bars, 2 μm

**e,** Model of the three-dimensional structure of the SMXL7 protein predicted by Phyre2. Major SMXL7 domains are indicated: Double Clp-N domain (N, purple), putative ATPase domain 1 (D1, green), middle domain (M, yellow), ATPase domain 2 (D2, blue).

D2 is divided into D2a (dark blue) and D2b (light blue) subdomains. RGKT motif and EAR motif are highlighted in red and green.

**f**, Representative confocal microscopic images of SMXL7 different domains and other SMXLs fused to GFP after transient expression in *N. benthamiana* epidermal cells. Scale bars, 5 μm.

g, In vitro phase separation assay of 10 μM GFP–SMXL7-MD2 proteins. Scale bars, 10 μm. Data are representative of three independent experiments

h, In vitro phase separation assay of GFP–SMXL7-MD2 at various protein concentrations. Scale bar, 10 μm. Data are representative of three independent experiments

i, FRAP of GFP–SMXL7-MD2 droplets. Time 0 indicates the time of the photobleaching pulse. Scale bar, 2 μm. Data are representative of 13 independent experiments.

j, Plot showing the time course of the recovery after photobleaching GFP–SMXL7-MD2 droplets. Data are presented as mean ± s.d. (n = 13).

k, Fusion of GFP–SMXL7-MD2 droplets. Scale bar, 5 μm. Data are representative of three independent experiments.

Figure 3. D14 colocalizes with SMXL7 nuclear condensates in a SL-Dependent Manner

**a**, Representative confocal microscopic images of *d14* tobacco leaf nuclei that express the indicated proteins. Scale bars, 5 μm.

**b**, Colocalization of free mCherry, FCA-meCherry, D14–mCherry, with SMXL7–GFP in *d14* tobacco leaf nuclei. Images are representative of three independent experiments. Scale bars, 5 μm.

Figure4. SMXL7 is directly associated with the H3K27me3 heterochromatin

**a**, Relative frequencies of decondensed, partially decondensed (Intermediate), or wild-type (Highly condensed) chromocenters in DAPI-stained nuclei of *smxl678, max2*, *d14*, Col-0, *35Spro:GFP*/Col-0, *35Spro:SMXL7d53-YFP*/Col-0(*SMXL7d53-YFP*/Col-0) plants. Representative nuclear condensation status stained with DAPI is showed at the bottom. Three biological replicates were performed. Scale bars, 2 μm.

**b**, H3K27me3 immunostaining in Col-0, *smxl678* mutants and *35Spro:SMXL7d53-YFP*/Col-0(*SMXL7d53*) overexpression plants. Three biological replicates were performed. Scale bars, 2 μm.

**c,** Quantification of H3K27me3 immunostaining and DAPI staining show similar chromocenter condensation patterns in nuclei. Y represents perfectly co-localized or similar patterns, N represents not. Three biological replicates were performed.

**d,** Immunostaining of interphase nuclei *35Spro:SMXL7d53-YFP*/Col-0 transgenic plants. Colors indicated the DNA counterstained with DAPI (blue), SMXL7-YFP (green), and H3K27me3 (red). Three biological replicates were performed. Scale bars, 2 μm.

**e,** Distribution of SMXL7 (black) and H3K27me3 (red) in the 5 Arabidopsis chromosomes. The gray boxes indicate the pericentromeric region of each chromosome. The left y axis means log2 ratio of SMXL7-GFP DAP-seq signals to 35S:GFP ChIP-seq and the right y axis means log2 ratio of H3K27me3 to H3 ChIP-seq signals in Col-0.The data were plotted with the mean of two biological replicates and smoothed using LOESS method by GraphPad Prism.

f, Snapshots of SMXL7 and H3K27me3 signals in selected chromosome arm. H3K27me3 ChIP-seq and SMXL7-GFP DAP-seq signals are showed as reads per kilobase per million mapped reads (RPKM). GFP ChIP-seq in 35S:GFP is shown as the negative control.

g Metaplots of SMXL7 DAP-seq signal in H3K27me3-marked regions. Metaplots of H3K27me3 level in SMXL7-enriched peaks. Metaplots of SMXL7 ChIP-seq signals in the protein coding genes

h Heat map of H3K27me3 enrichment in WT, mutant and overexpression seedlings. The colour scale indicates reference-adjusted reads per million (RRPM) surrounding peak summit from the ChIP-Rx–seq data

i, H3K27me3 western blot in Col-0, *smxl678* mutants and *35Spro:SMXL7d53-YFP*/Col-0(*SMXL7d53*) overexpression plants.

Figure 5. SMXL7 nuclear condensates are enriched with JMJ30 to regulate flower time

a,Colocalization of ADCP1-meCherry, LHP1–mCherry, JMJD5–mCherry with SMXL7–GFP in *d14* tobacco leaf nuclei. Images are representative of three independent experiments. Scale bars, 5 μm.

b The split luciferase complementation assays show that SMXL7 associates with JMJ30 in *N. benthamiana*. SMXL7-nLUC and cLUC-JMJ30 were co-expressed in *N. benthamiana* leaves. Luciferase activity was detected 36 hours after infiltration. The pseudocolor bar represents the range of luminescence intensity.

c, Images showing the incorporation of JMJ30-mCherry into droplets formed by SMXL7-MD2–GFP. Free mCherry is unable to form droplets.

d, Transcriptional activity assayin tobacco, showing that D53 represses the transcriptional activation activity of JMJ30 on FLC.

e, The IGV browser view of H3K27me3 occupancy at JMJ30 target genes. ACT2, a non-JMJ30-target gene, serves as a negative control.

f, Transcriptome reprogramming in different seedlings. The heat map shows transcriptomic changes of *s678* (versus WT) and S7d53 (versus WT) from RNA-seq analyses. FC, fold change.

g, Comparative expression analyses of key transcription factor genes in diverse developmental programmes. Heat map of RNA-seq data from triplicate biological samples prepared from WT, s678, S7d53 plants. The RNA expression data were normalized to the value in WT plants.

h, The morphological phenotypes of *s678jmj30*

Figure 6 SMXL7 is required for chromatin architecture regulation

ATAC-seq levels are changed in s678 mutants over SMXL7 peaks

Genome-wide interaction frequency fold change heatmap

Compartments switches and compartmentalization strength change

Analysis of compartment switching types

Differentially expressed genes within compartment switching regions are associated with plant development.

SMXL7 function to promote H3K27me3 and prevent compartment switching