Supplementary Materials and Methods

Plasmid construction

Epitope tagged versions of PKL were both generated from a PKL expression construct in which PKL cDNA is flanked by the genomic sequences that flank PKL and which has previously been shown to rescue *pkl*-associated phenotypes (Li et al., 2005). In pJO981 (Li et al., 2005), the termination codon of the PKL cDNA is flanked by *Cla*I and *Sph*I sites. A vector containing 6 copies of c-Myc (6xc-Myc) from ABRC (stock number CD3-128) was modified with linkers such that the 6xc-Myc fragment was flanked by *Cla*I and *Sph*I, which was then cloned into pJO981 to generate the *PKL-c-Myc* construct pJO984. Similarly, an adaptor cassette was designed containing 3 copies of FLAG flanked by *Cla*I and *Sph*I and was then cloned into pJO981 to generate the *PKL-FLAG* construct pJO1048.

ChIP protocol

Tissue was immersed in 0.1% Triton X-100 with 1% formaldehyde under house vacuum. After 10 min 1/15 volume of 2 M glycine was added and the tissue kept under vacuum for another 5 min. The tissue was washed and either flash-frozen in liquid nitrogen or ground fresh. If the tissue was flashed frozen, it was then ground into fine powder and resuspended in 20 ml of cold Extraction Buffer 1 (0.4 M sucrose, 10 mM Tris•Cl pH 8.0, 10 mM MgCl2) with protease inhibitors (1x protease inhibitor cocktail (Sigma P9599)). The following steps were performed at 4ºC or on ice unless otherwise indicated. The lysate was filtered through one layer of miracloth (Calbiochem 475855) and centrifuged at 3,000x g for 20 min. The pellet was resuspended in 1.5 ml of Extraction Buffer 2 (0.25 M sucrose, 10 mM Tris•Cl pH8.0, 10 mM MgCl2, 1% Triton X-100) with protease inhibitors and centrifuged at 12,000x g for 10 min. Each pellet was resuspended in another 1 mL of extraction buffer 2 with protease inhibitors and centrifuged at 12,000x g. Each pellet was then resuspended in 600 µl of Extraction Buffer 3 (1.7 M sucrose, 10 mM Tris•Cl pH8.0, 2 mM MgCl2, 0.15% Triton X-100) with protease inhibitors, layered on top of 600 µl of EB3 and centrifuged (16,000x g, 1 hour). Pellet from each tube was resuspended in 750 µl of HEPES buffer (50 mM Hepes•KOH pH7.5, 140 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1mM EDTA) with protease inhibitors and the chromatin was sheared into ~250-750 bp fragments by sonication. The tubes were then centrifuged (16,000x g, 10 min) and the supernatant was transferred to a new tube. ~200 µl of the supernatant was used for each immunoprecipitation reaction. Before adding the antibody, the reaction was precleared by adding 20 µl of Dynabeads Protein G (Invitrogen, 100.04D; equilibrated with HEPES buffer, 50%

slurry) and rocked at 4^0C for 1 hour. Appropriate antibodies (anti-H3, Abcam ab1791; anti-H3K27me3, Millipore 07-449; anti-Flag M2, Sigma F1804, anti-Myc, Millipore 05-724) were added at 1:100 dilution and the tubes were rotated at 4ºC for ~15 hours. Then 20 µl of Dynabeads Protein G (Invitrogen, 100.04D; equilibrated with HEPES buffer, 50% slurry) was added to each tube and the incubation continued for another 1 hour. The beads were washed sequentially with 1 ml of each of the following: HEPES buffer (50 mM Hepes•KOH pH7.5, 140 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 1mM EDTA), HEPES HS (as HEPES buffer, but with 500 mM NaCl), LNDET (0.25 M LiCl, 0.5% NP40, 0.5% sodium deoxycholate, 1 mM EDTA, 10 mM Tris•Cl pH8.0), and TE (10 mM Tris•Cl pH8.0, 1 mM EDTA). The immunocomplexes were eluted using 300ul of 10% (w/v) Chelex 100 (Biorad, 142-1253). After addition of Chelex 100, the tubes were incubated at 100^oC for 15 minutes. The samples were cooled at room temperature then treated with proteinase K for 1 hour at 42^oC. Incubating the tubes at 75oC for 10 minutes inactivated the Proteinase K. The tubes were then spun at 12,000x g for 1 minute and the supernatant removed to a new tube. 4 µl of each sample was used in each qPCR reaction.

REFERENCES

Charron JB, He H, Elling AA, Deng XW (2009) Dynamic landscapes of four histone modifications during deetiolation in Arabidopsis. Plant Cell **21:** 3732-3748

Li HC, Chuang K, Henderson JT, Rider SD, Jr., Bai Y, Zhang H, Fountain M, Gerber J, Ogas J (2005) PICKLE acts during germination to repress expression of embryonic traits. Plant J **44:** 1010-1022

Tian L, Fong MP, Wang JJ, Wei NE, Jiang H, Doerge RW, Chen ZJ (2005) Reversible histone acetylation and deacetylation mediate genome-wide, promoter-dependent and locus-specific changes in gene expression during plant development. Genetics **169:** 337-345

Turck F, Roudier F, Farrona S, Martin-Magniette ML, Guillaume E, Buisine N, Gagnot S, Martienssen RA, Coupland G, Colot V (2007) Arabidopsis TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 lysine 27. PLoS Genet **3:** e86

Vlachonasios KE, Thomashow MF, Triezenberg SJ (2003) Disruption mutations of ADA2b and GCN5 transcriptional adaptor genes dramatically affect Arabidopsis growth, development, and gene expression. Plant Cell **15:** 626-638

Zhang X, Yazaki J, Sundaresan A, Cokus S, Chan SW, Chen H, Henderson IR, Shinn P, Pellegrini M, Jacobsen SE, Ecker JR (2006) Genome-wide high-resolution mapping and functional analysis of DNA methylation in arabidopsis. Cell **126:** 1189-1201

Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S (2007) Genome-wide analysis of Arabidopsis thaliana DNA methylation uncovers an interdependence between methylation and transcription. Nat Genet **39:** 61-69

Figure S1

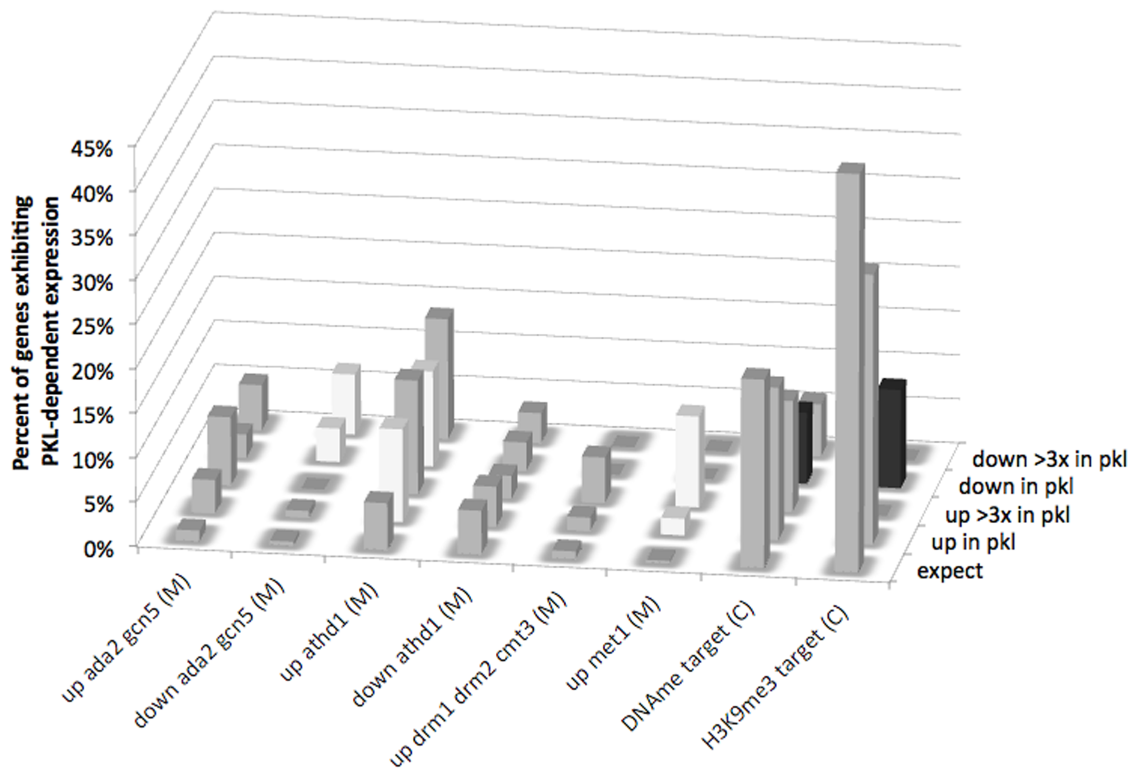

Figure S1. Analysis of the intersection of *PKL*-dependent genes in 14-day-old plants with other epigenomic data sets is consistent with the hypothesis that PKL does not function in the plant equivalent of a Mi-2/NuRD complex. A, Fisher's exact test (see "Materials and Methods") was used to examine the intersection of genes linked to an epigenetic pathway (*x*-axis) and genes that exhibit altered expression in response to *pkl* according to various selection criteria (*y*-axis). The percent of genes that exhibit altered expression in *pkl* plants that fall within each intersection is represented on the *z*-axis. The *expect* category on the *y*-axis indicates the percentage of *PKL*-dependent genes expected to found in the intersection of the compared sets of genes. A *white bar* denotes more genes observed in common between the two sets than expected (at $p < 1 \times 10^{-3}$), whereas a *dark bar* denotes fewer genes observed than expected (at $p < 1 \times 10^{-3}$). In *x*-axis categories, *C* denotes ChIP-chip data, and *M* denotes microarray-derived expression data. Microarray analyses in *ada2 gcn5* plants (Vlachonasios et al., 2003) and *athd1* plants (Tian et al., 2005) examine the effect of impaired histone deacetylases on the transcriptome. Microarray analyses in *drm1 drm2 cmt3* plants and *met1* plants examine the effect of impaired cytosine methyltransferases on the transcriptome (Zhang et al., 2006). DNAme refers to genes identified

as enriched for DNA methylation by ChIP-chip analysis (Zilberman et al., 2007). H3K9me3 refers to genes identified as enriched for H3K9me3 by ChIP-chip analysis (Turck et al., 2007).

Figure S2

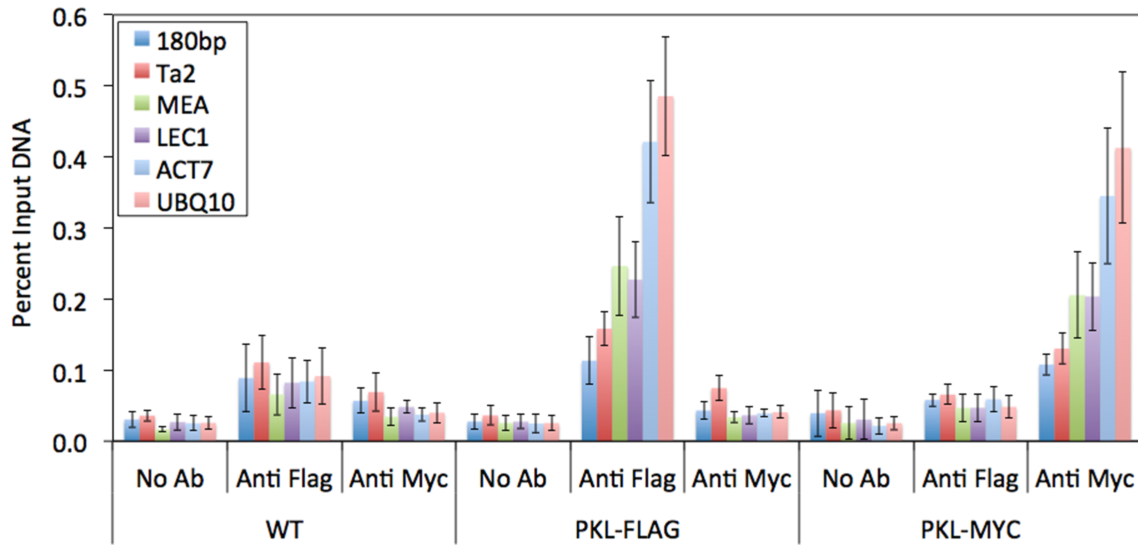

Figure S2. ChIP using *PKL-FLAG* plants reproduce data obtained from *PKL-c-Myc* plants. ChIP was used to examine recruitment of PKL in 5-day-old plants. ChIP was carried out using no antibody (No Ab), anti-FLAG (Anti Flag), or anti-c-MYC (Anti Myc) using cross-linked DNA from wild-type (WT), *PKL-FLAG* (PKL-FLAG), and *PKL-c-Myc* (PKL-MYC) plants and the indicated loci were examined. The *y*-axis denotes percent of input DNA brought down for a given immunoprecipitation. All data are the average of four biological replicates. Error bars denote standard deviation.