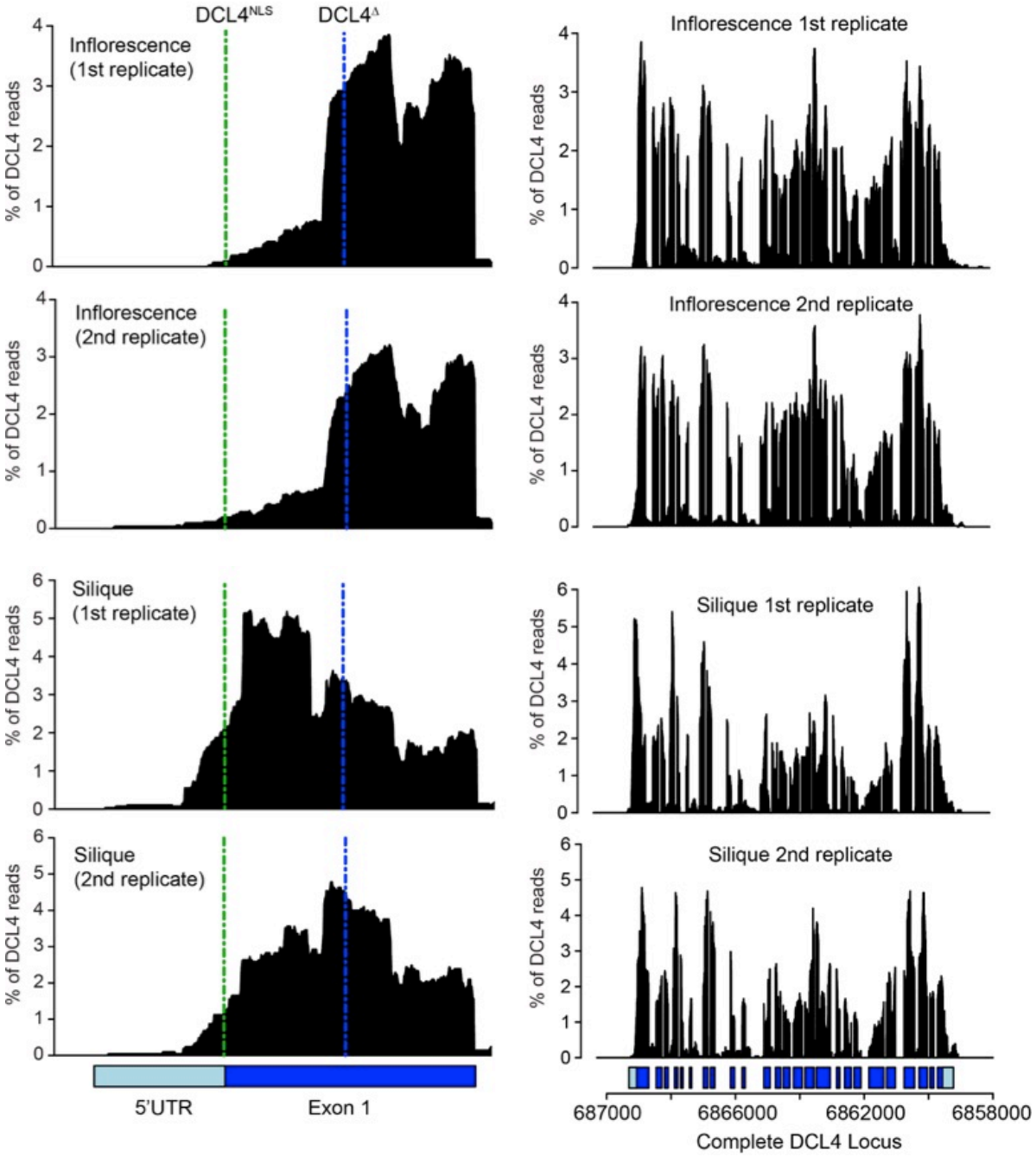
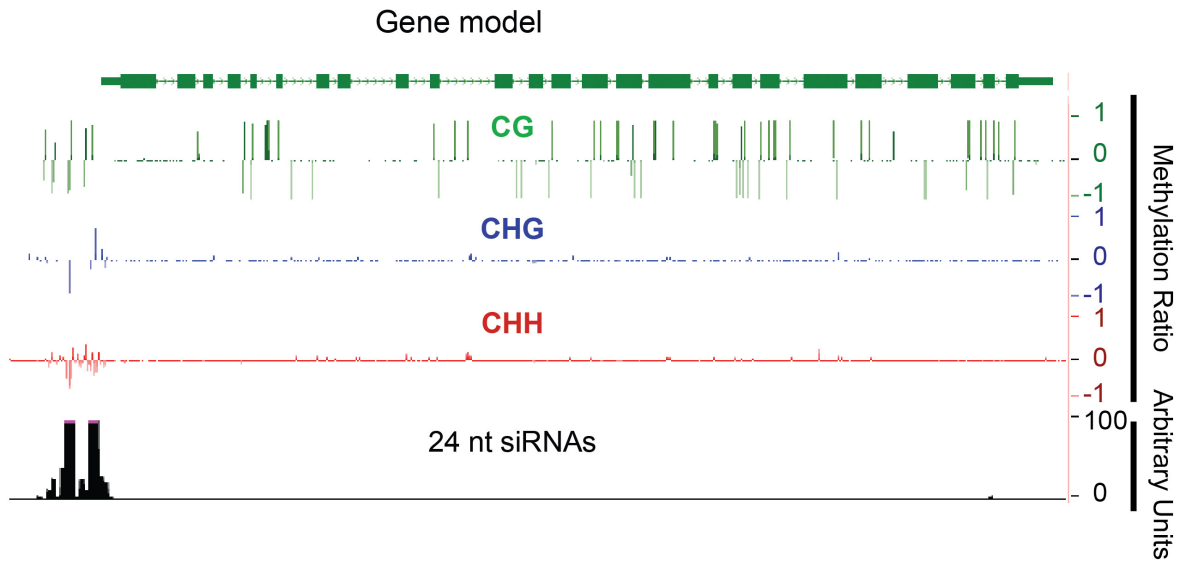
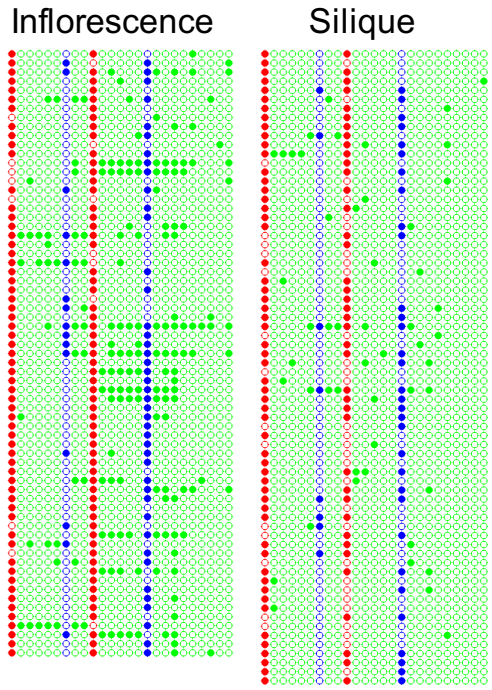


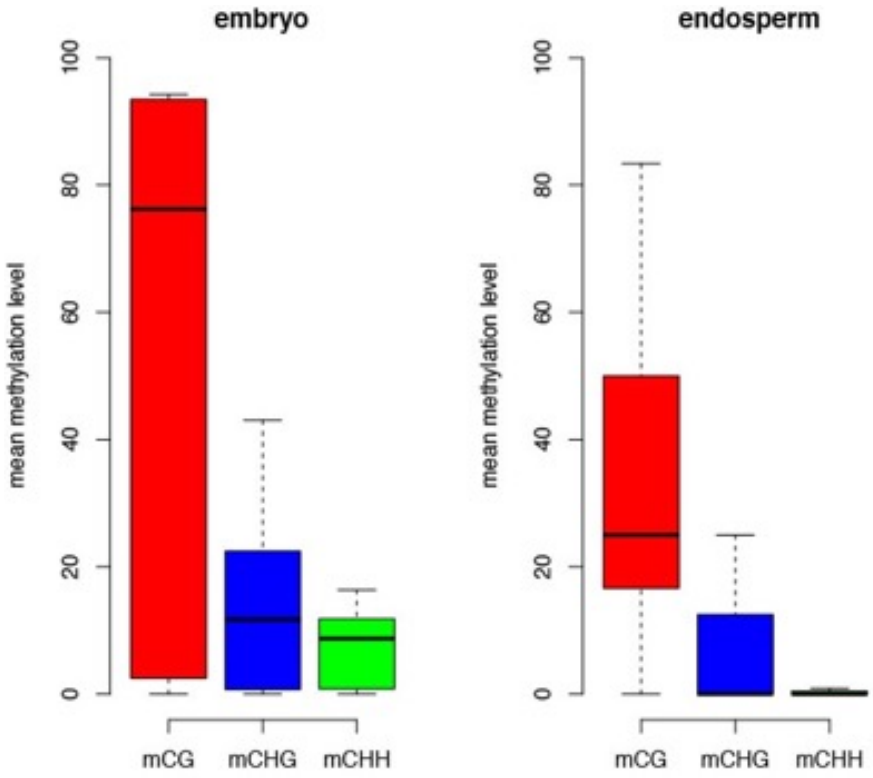
Supplemental Figure 1: RNA-sequencing reads of DCL4
RNA sequencing reads from inflorescence and silique tissues mapped onto the DCL4 locus. Reads are graphed as percent of library size-normalized reads mapping over the locus. Entire locus (right panels) and magnification of exon 1 (left panels) are shown. The magnified illustration from replicates of inflorescence and silique tissues are shown in Figure 1A.



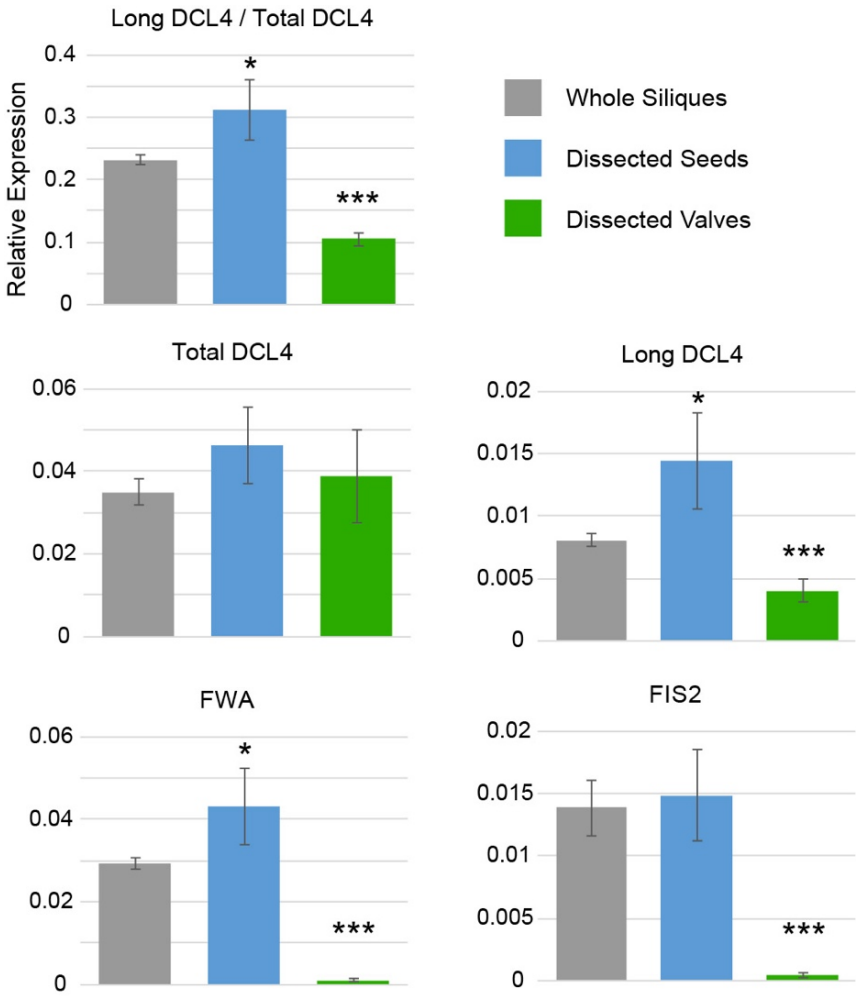
Supplemental Figure 2: DCL4 methylation and small RNA accumulation
Illustration of the entire DCL4 genomic locus from the UCSC genome browser. Exons (blocks) and introns (lines) are depicted above methylation state and 24 nt siRNAs. Methylation and small RNAs overlap specifically the region upstream of the coding sequence.



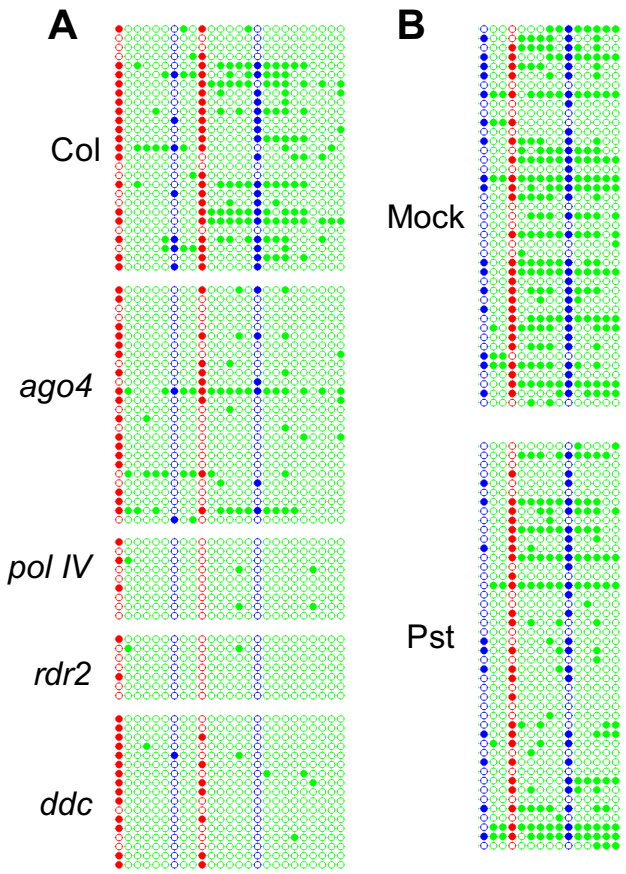
Supplemental Figure 3: DCL4 promoter methylation bisulfite data
Dot-plot representation comparing DCL4 promoter methylation state of DNA extracted from inflorescence tissue and silique tissue. Sanger-sequencing results were analyzed with Kismeth webtool. Each row corresponds to one individual cloned sequence. Open circles represent unmethylated cytosines, closed circles represent methylated cytosines. Red = CG, blue = CHG, green = CHH.



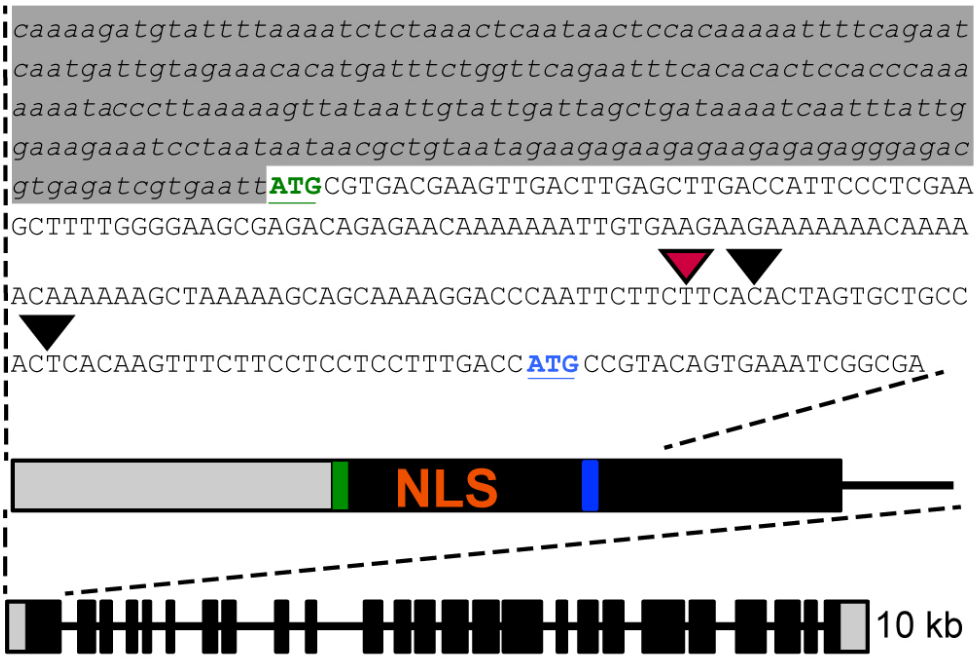
Supplemental Figure 4: DCL4 promoter methylation in endosperm and embryo
Box-plot representation of the DCL4 promoter region DNA methylation level from bisulfite-sequencing data. Representations were produced using processed data (embryo: GSM1276483, endosperm: GSM1276484) published in {Pignatta et al., 2014, #25083}. Filled bars indicate median percent methylation as provided in the processed data, boxes indicate data between the first and third quartiles (Q1 and Q3, respectively) defining the interquartile range (IQR). Whiskers correspond to data between Q1 - 1.5 x IQR or Q3 + 1.5 IQR. Outliers are not shown. Only values from 300bp windows corresponding to the DCL4 promoter region (Chr3:6868600..6869966, TAIR8 annotation) were considered.



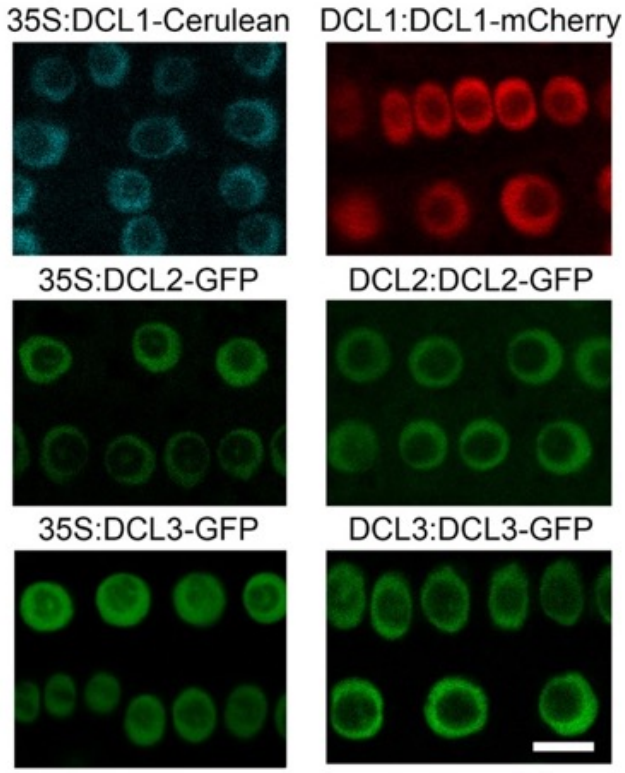
Supplemental Figure 5: DCL4 isoform expression in dissected seeds and valves
RNA was extracted from intact siliques approximately 3-4 days after pollination (Whole Siliques, grey bars), and following microscopic dissection to separate valves (Dissected Valves, green bars) and seeds (Dissected Seeds, blue bars), the latter of which included some septum tissue. Relative expression was measured by qPCR of Long DCL4 normalized to Total DCL4, and each primer set normalized to GAPC. Expression of endosperm-specific FWA and FIS2 normalized to GAPC serve as controls. Mean +/- standard deviation of biological replicates (siliques n=3, seeds n=5, valves n=4) shown. * denotes p-value <0.05, *** $p < 0.005$ in a pair-wise two-tailed t-test compared with Whole Siliques.



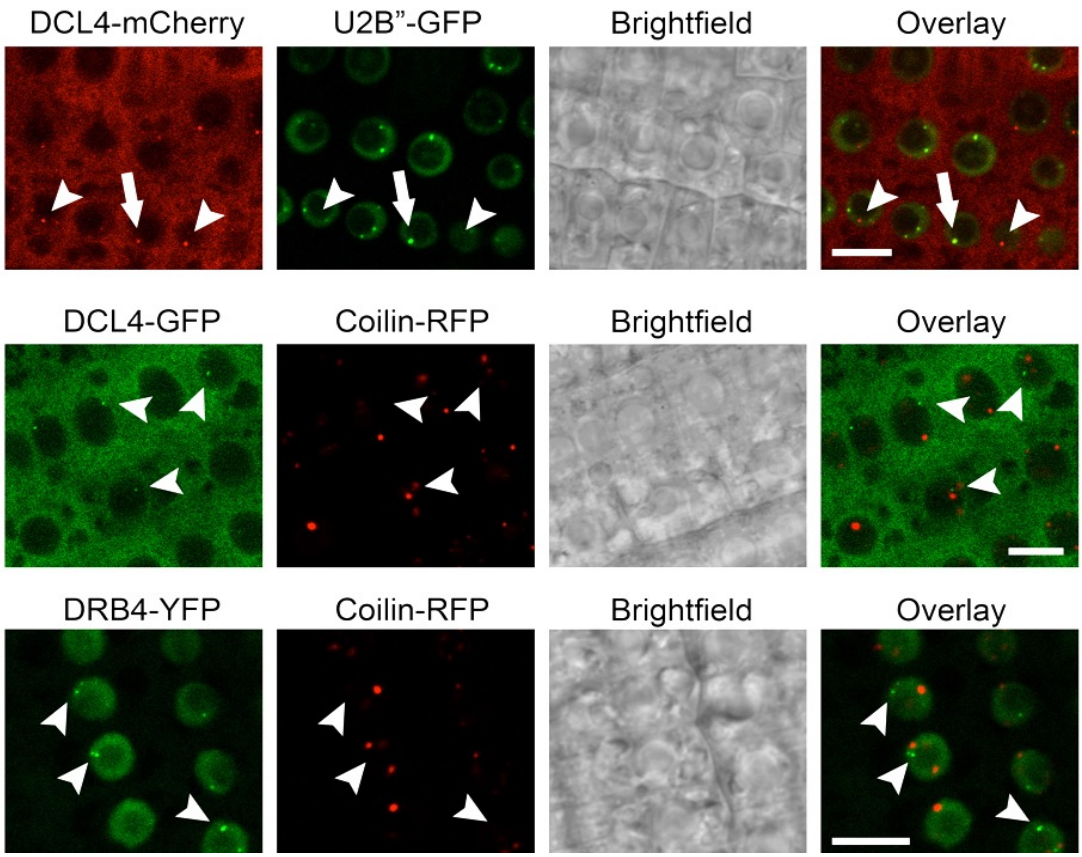
Supplemental Figure 6: DCL4 promoter methylation bisulfite data
(A) Dot-plot representation depicting methylation on the DCL4 promoter region. DCL4 promoter region was amplified from bisulfite-converted DNA extracted from Col (WT), ago4-5, polIV, rdr2 and drm1 drm2 cmt3 (ddc) triple mutant seedlings. Sanger-sequencing results were analyzed with Kismeth webtool. Each row corresponds to one individual cloned sequence. Open circles represent unmethylated cytosines, closed circles represent methylated cytosines. Red = CG, blue = CHG, green = CHH.
(B) Similar analysis as A) with mock or Pseudomonas syringae infected leaves harvested 5 days after infection.



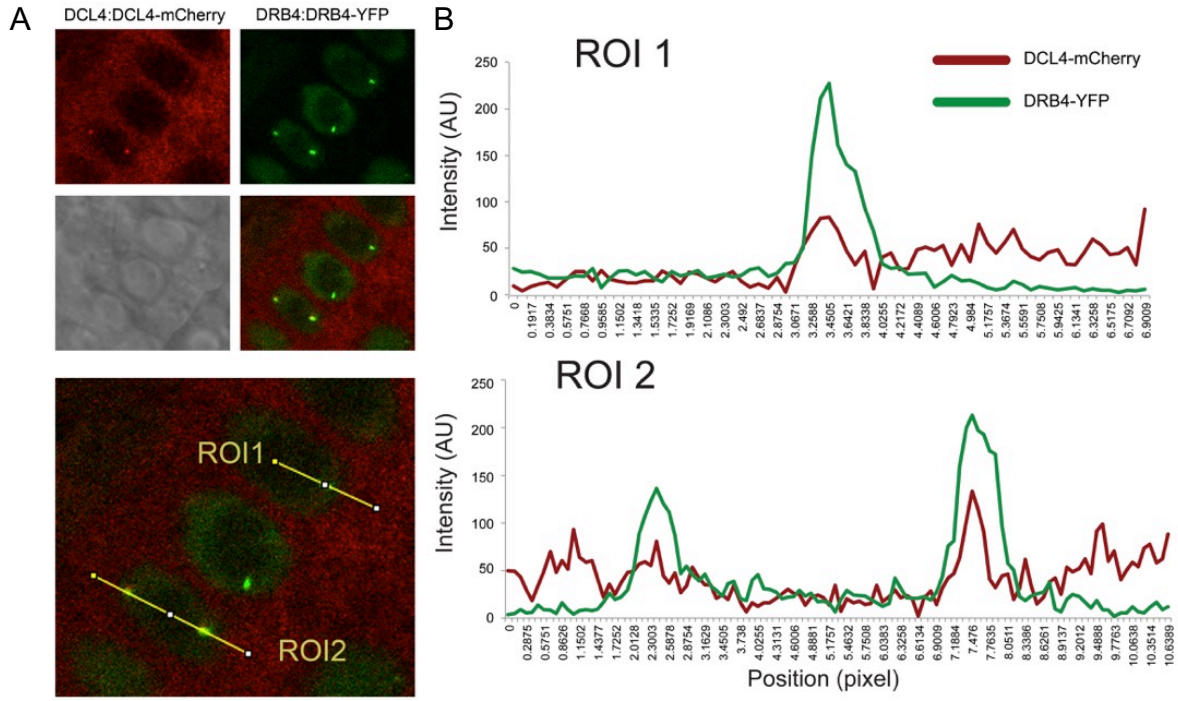
Supplemental Figure 7: DCL4 TSS determined by 5' RACE sequencing
TSSs determined by direct sequencing of bulk PCR products from nested 5' RACE PCR are depicted with arrowheads superimposed on the DCL4 exon 1 sequence: Col WT (black), DCL4:DCL4-mCherry.



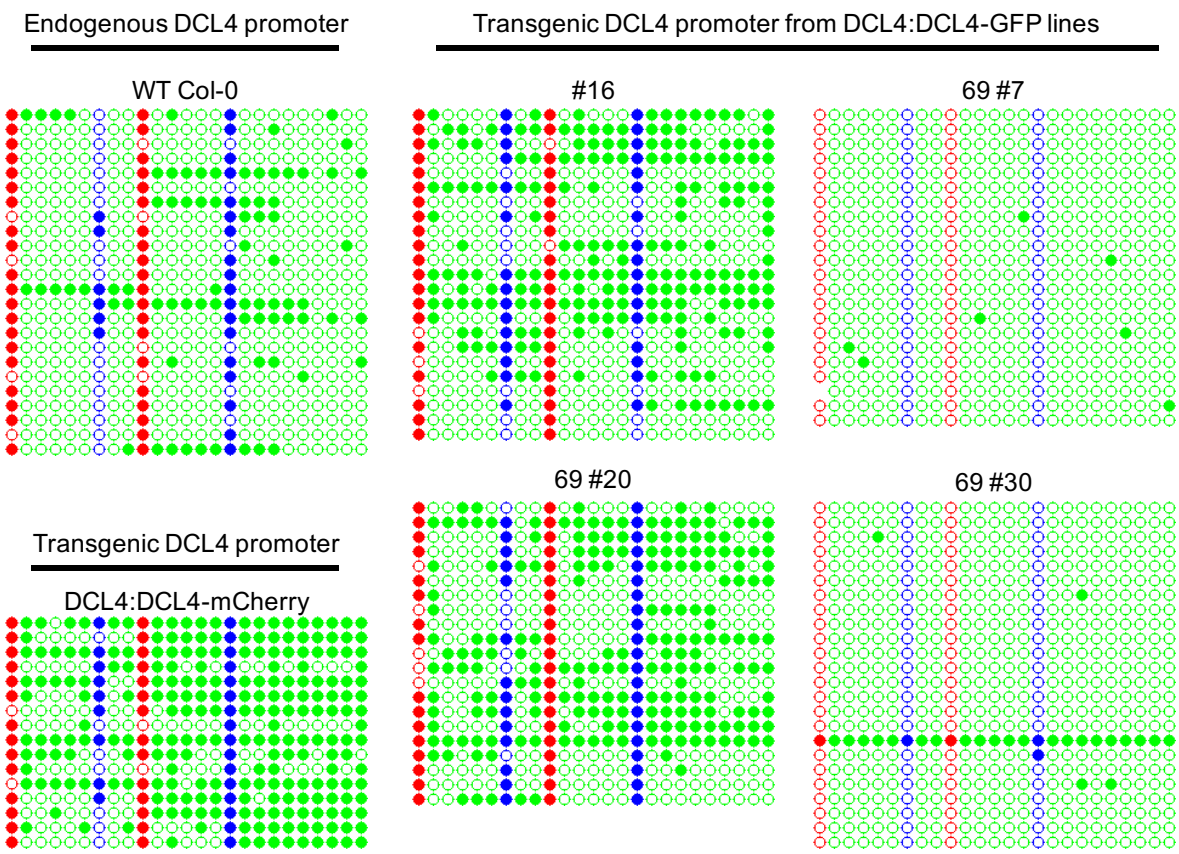
Supplemental Figure 8: Localization of DCL proteins
Confocal images of DCL1, DCL2 and DCL3 under 35S (Left) or respective native promoters (Right) in live roots of stably-transformed Arabidopsis plants.
Scale bar = 10 μm.



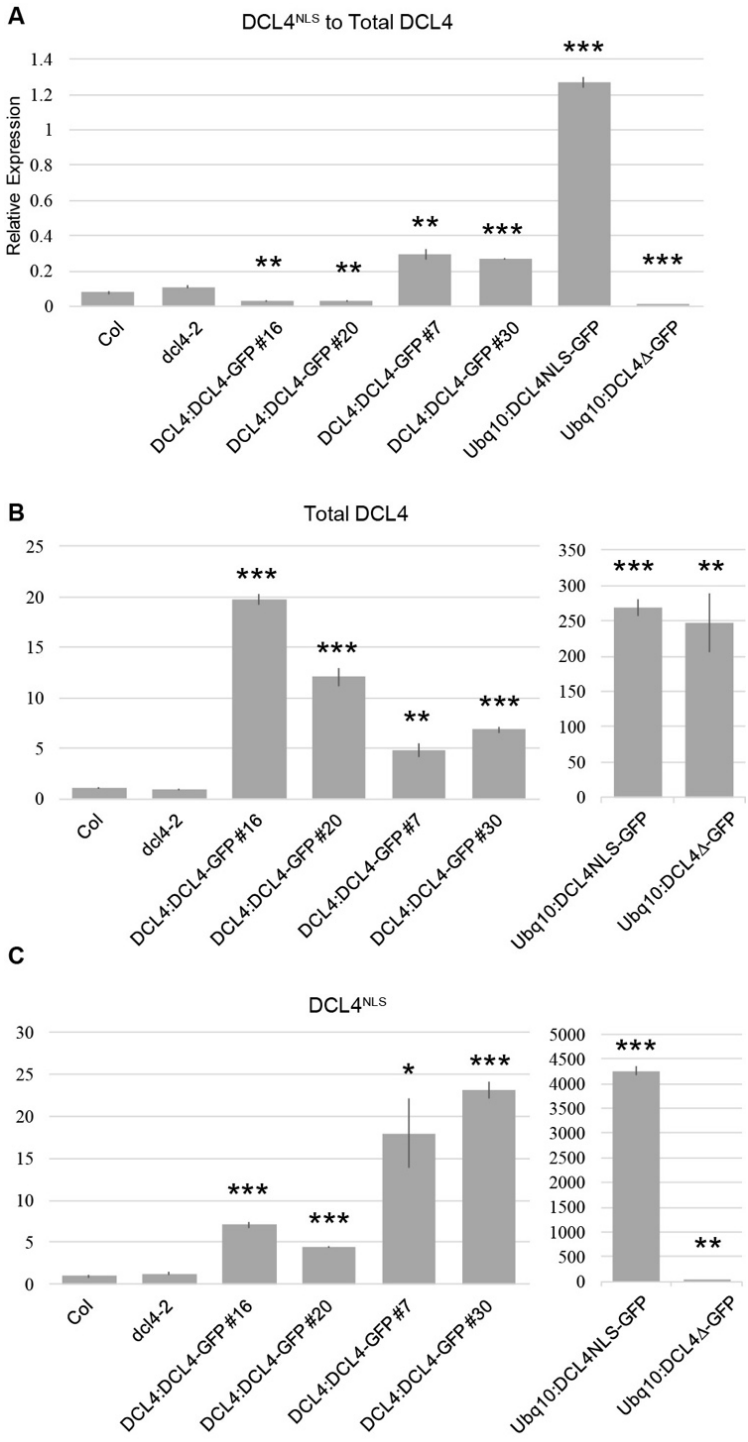
Supplemental Figure 9: Colocalization analysis with Cajal body markers
Localization of DCL4:DCL4-GFP/mCherry with Cajal body markers 35S:U2b"-GFP (top), 35S:Coilin-RFP (middle) and DRB4:DRB4-YFP with 35S:Coilin-RFP (bottom). Dicing bodies indicated with arrowhead; colocalized DCL4 and U2b" indicated with arrow. Scale bars = 10 μm.



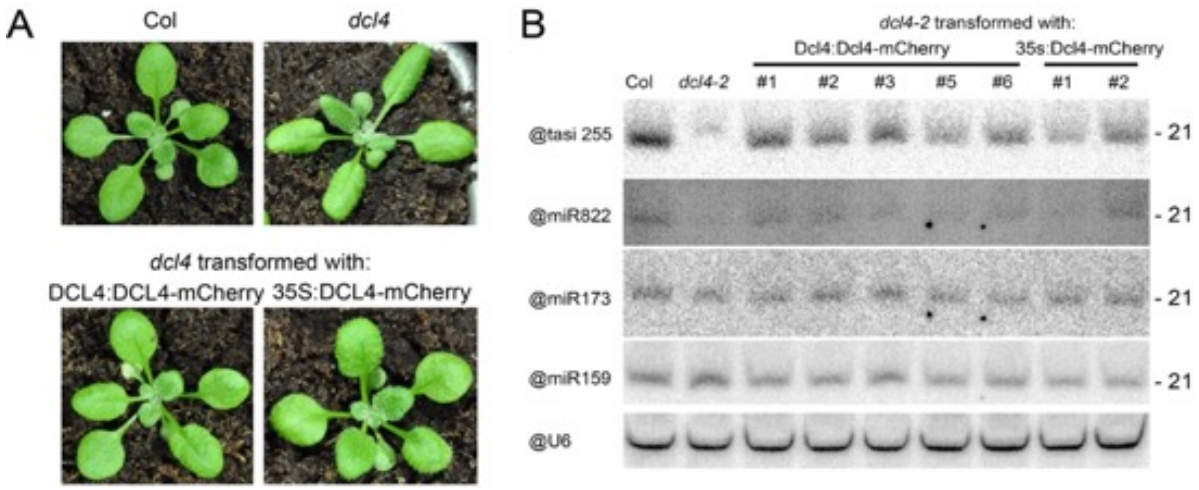
Supplemental Figure 10: Colocalization of DCL4 and DRB4 in nuclear bodies
(A) Colocalization of DCL4:DCL4-mCherry with DRB4:DRB4-YFP in nuclear dicing bodies of Arabidopsis roots. Confocal images of a single plane of the Z axis shows colocalizing fluorescence patterns. Regions of Interest (ROI) were drawn in FIJI to measure intensity.
(B) Plot Profile function in FIJI was used to quantify signal intensity for both channels across two regions of interest (ROI1 & 2). Intensity (in arbitrary units) is plotted against position across the region, showing overlapping peaks of intensity in nuclear bodies.



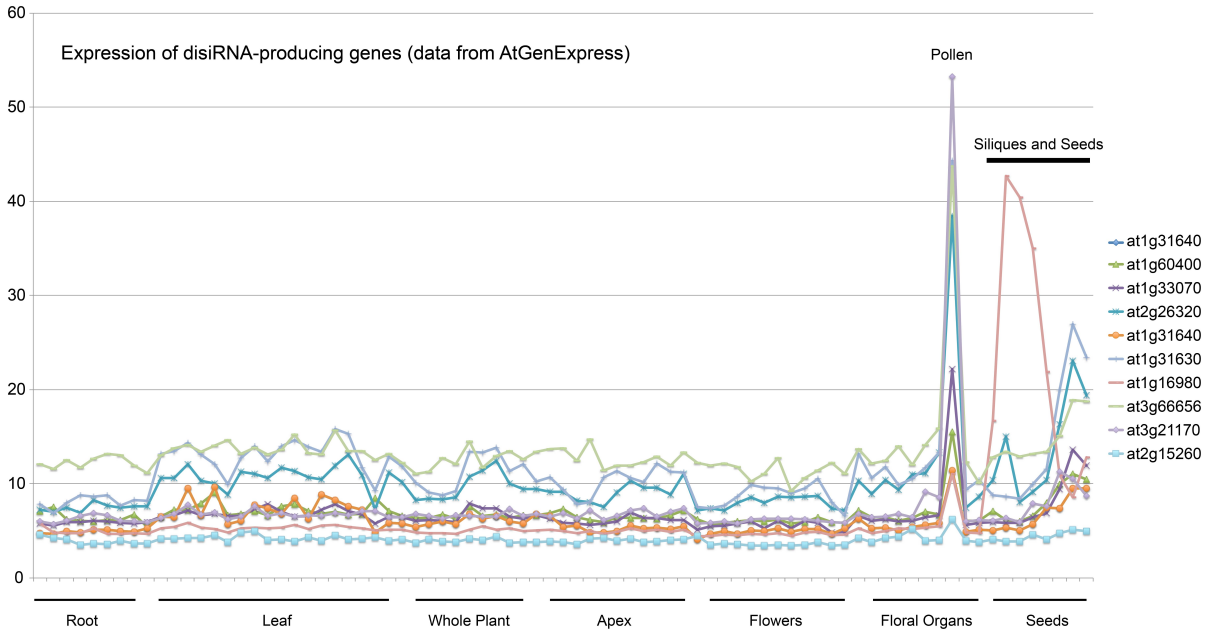
Supplemental Figure 11: DCL4 transgenic promoter methylation bisulfite data
Dot-plot representation depicting methylation on the DCL4 promoter region in transgenic lines. Endogenous DCL4 promoter region was amplified from bisulfite-converted DNA extracted from Col (WT). Transgenic DCL4 promoter was amplified from four independent transgenic lines using a primer that recognizes the cloning junction between promoter and coding sequence. Sanger-sequencing results were analyzed with Kismeth webtool. Each row corresponds to one individual cloned sequence. Open circles represent unmethylated cytosines, closed circles represent methylated cytosines. Red = CG, blue = CHG, green = CHH.



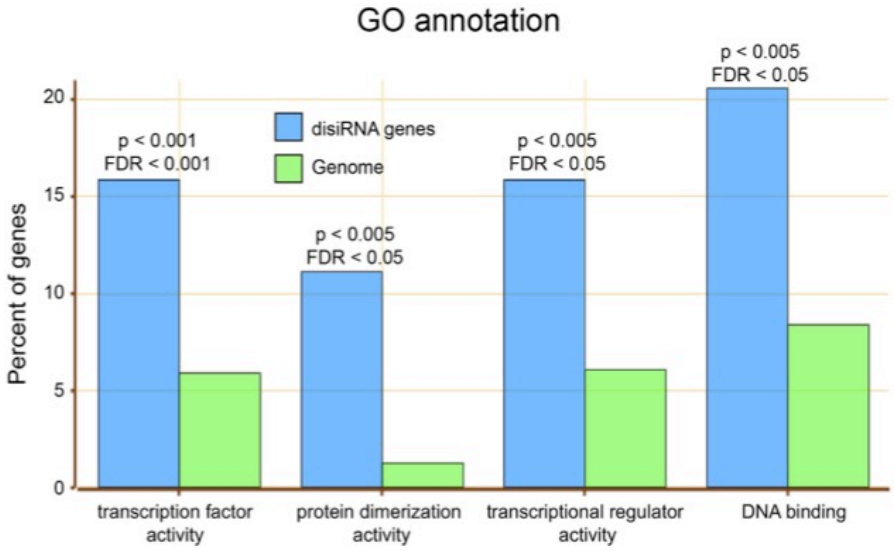
Supplemental Figure 12: DCL4 expression in independent transgenic lines
(A) Relative expression by qPCR of DCL4NLS normalized to total DCL4 in Col, dcl4-2 mutants and transgenic seedlings including Ubq10 promoter fusions as controls.
(B) Relative expression of total DCL4, or
(C) DCL4NLS, normalized to Actin2. Mean +/- SEM of three biological replicates shown. * denotes p-value <0.05, ** denotes p-value <0.01, *** denotes p-value <0.001, as determined by a pairwise two-tailed t-test compared with Col.



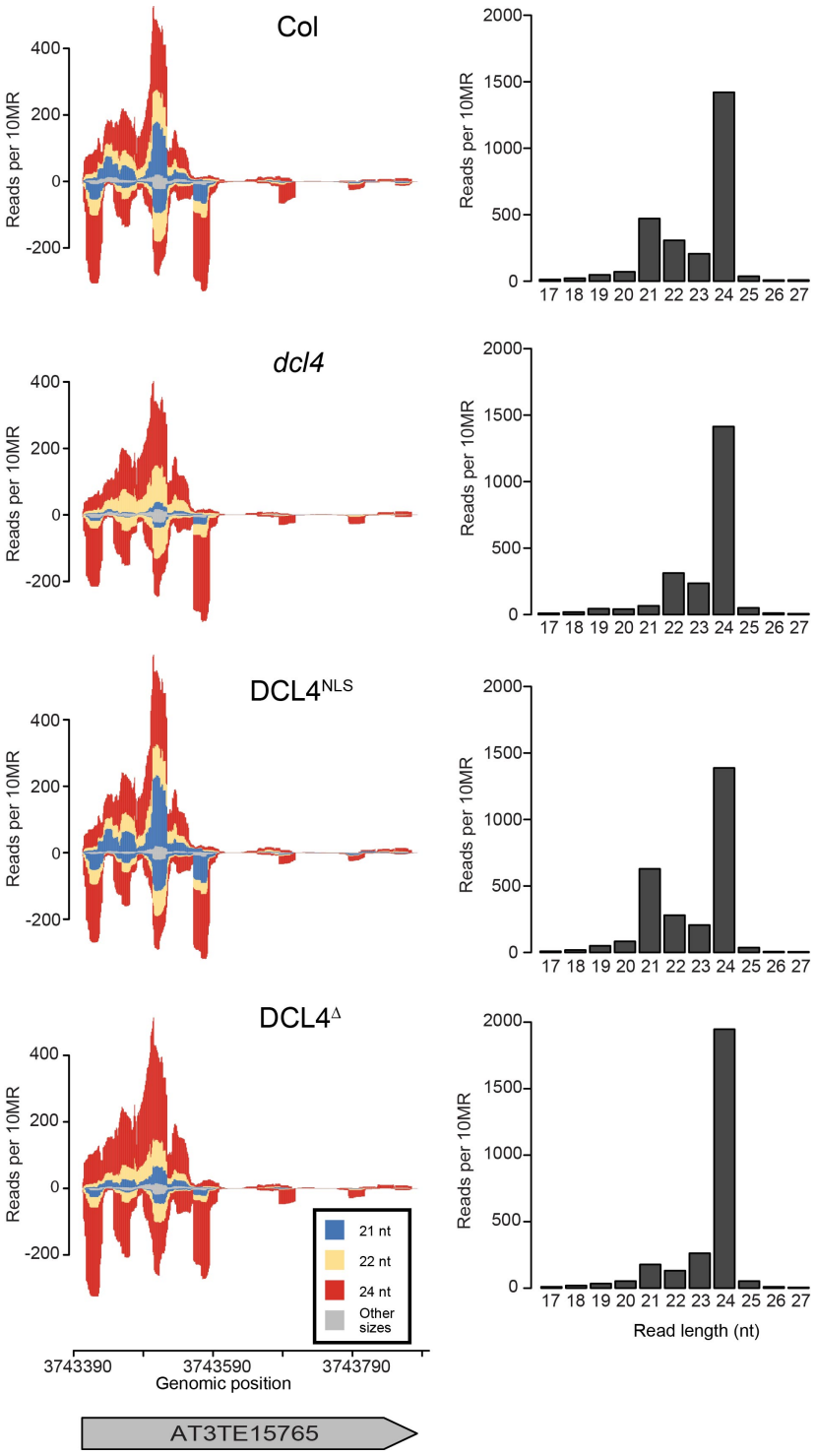
Supplemental Figure 13: DCL4 isoform complementation analysis
(A) Morphology of Col, dcl4-2 mutants, and complementation of the dcl4 leaf phenotype by DCL4:DCL4-mCherry and 35S:DCL4-mCherry.
(B) RNA gel blot analysis showing molecular complementation of DCL4-dependent small RNAs. @ indicates probes hybridized. tasiRNA255 and miR822 are DCL4-dependent. U6, and DCL1-dependent miR173 and miR159 are shown as controls. Analysis performed by stripping and probing the same membrane.



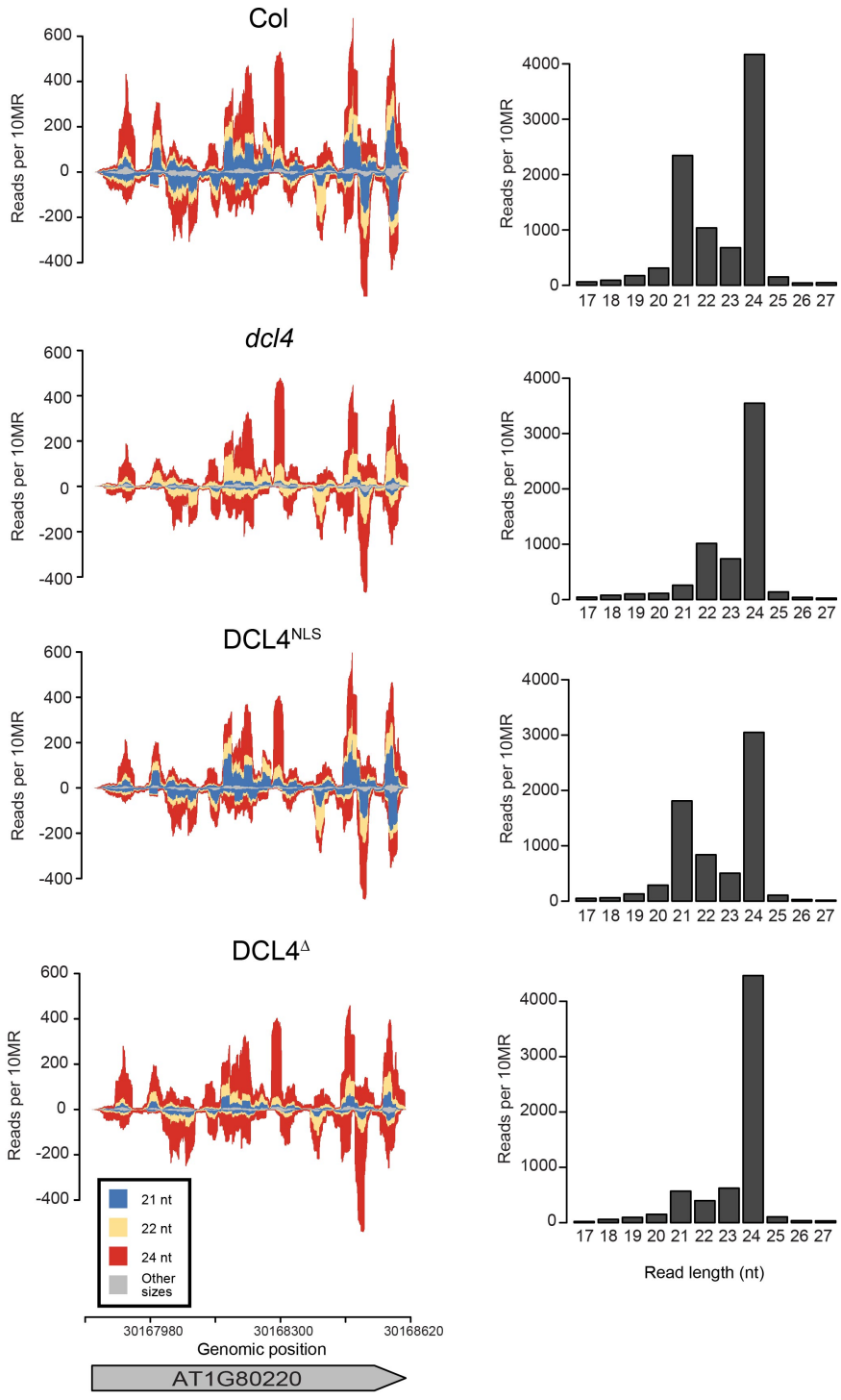
Supplemental Figure 14: Expression pattern of disiRNA-producing genes
Expression analysis of disiRNA-producing genes in the AtGenExpress dataset (http://jsp.weigelworld.org/expviz/expviz.jsp), revealing expression generally restricted to pollen and developing siliques/seeds.



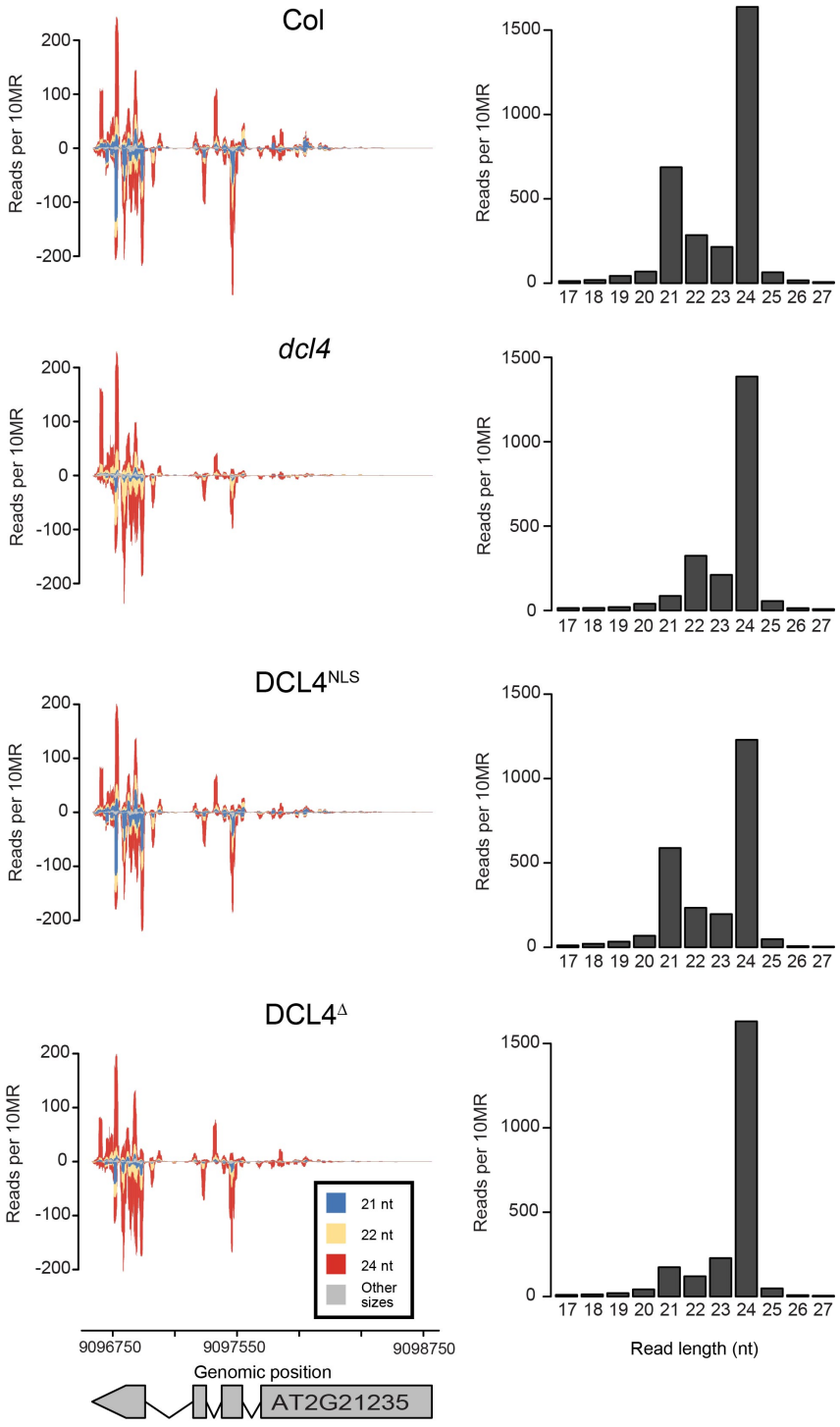
Supplemental Figure 15: GO annotation of disiRNA-producing genes
GO annotation performed by agriGO to identify enriched gene annotations producing disiRNAs. P- and FDR-values noted for each category analysis.



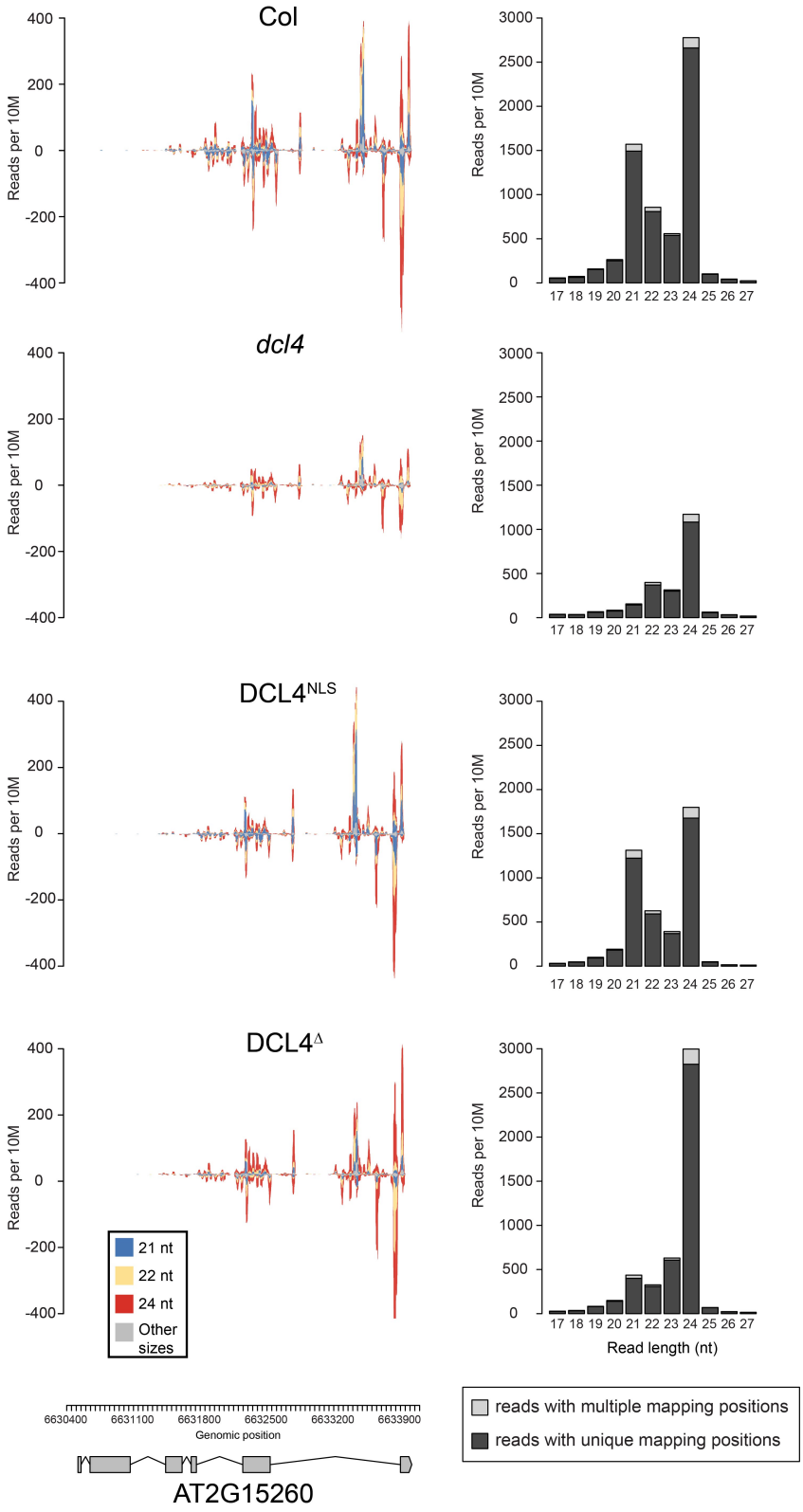
Supplemental Figure 16: sRNA accumulation in siliques over AT3TE15765
small RNA sequencing reads mapped onto AT3TE15765 as an additional example to compare with Figure 7E. 24nt in red, 22nt in green, and 21nt siRNAs in blue. Histogram of small RNAs mapping to the entire locus shown on right.



Supplemental Figure 17: sRNA accumulation in siliques over AT1G80220
small RNA sequencing reads mapped onto AT1G80220 as an additional example to compare with Figure 7E. 24nt in red, 22nt in green, and 21nt siRNAs in blue. Histogram of small RNAs mapping to the entire locus shown on right.



Supplemental Figure 18: sRNA accumulation in siliques over AT2G21235
small RNA sequencing reads mapped onto AT2G21235 as an additional example to compare with Figure 7E. 24nt in red, 22nt in green, and 21nt siRNAs in blue. Histogram of small RNAs mapping to the entire locus shown on right.



Supplemental Figure 19: sRNA accumulation in siliques over AT2G15260
small RNA sequencing reads mapped onto AT2G15260 as an additional example to compare with Figure 7E. 24nt in red, 22nt in green, and 21nt siRNAs in blue. Histogram of small RNAs mapping to the entire locus shown on right.