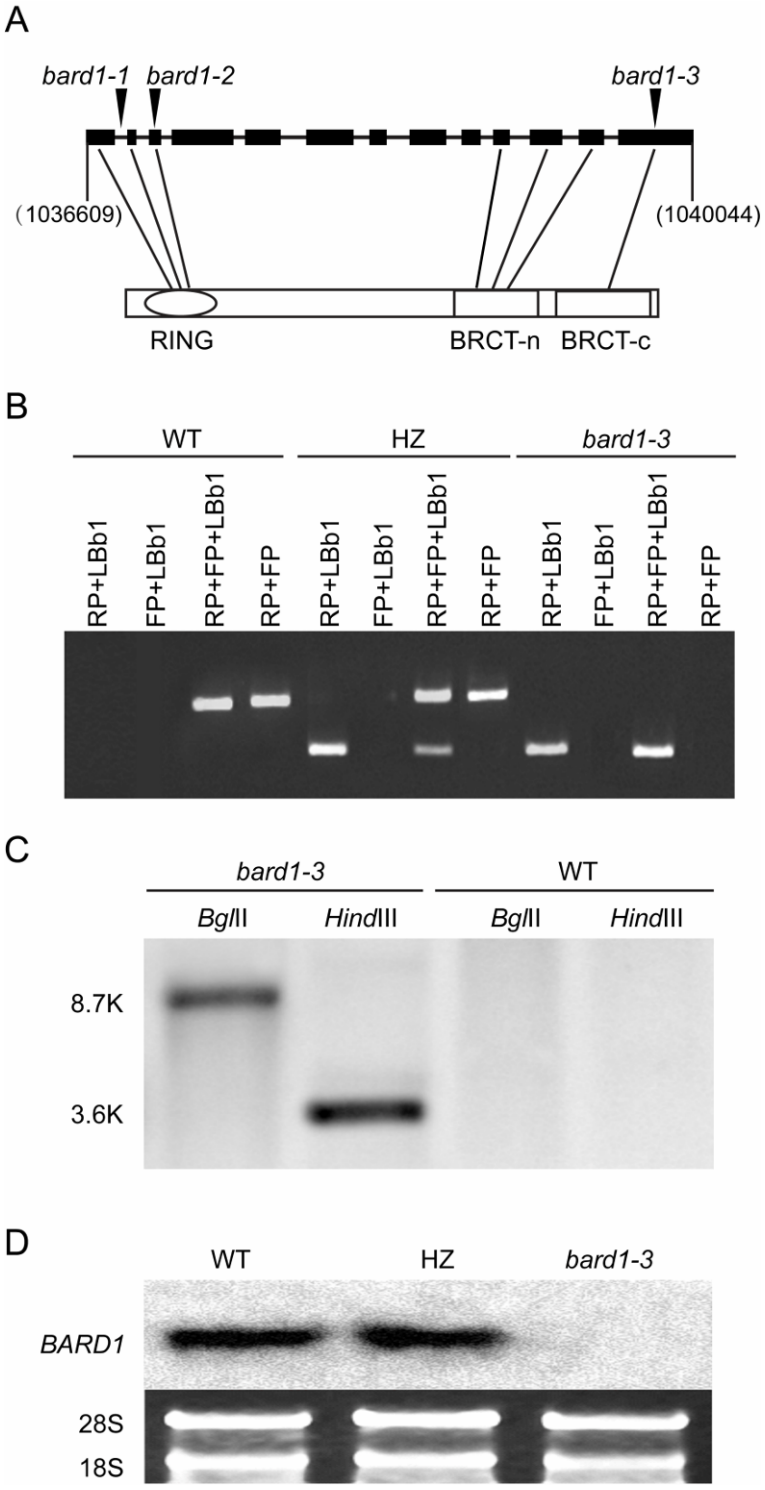
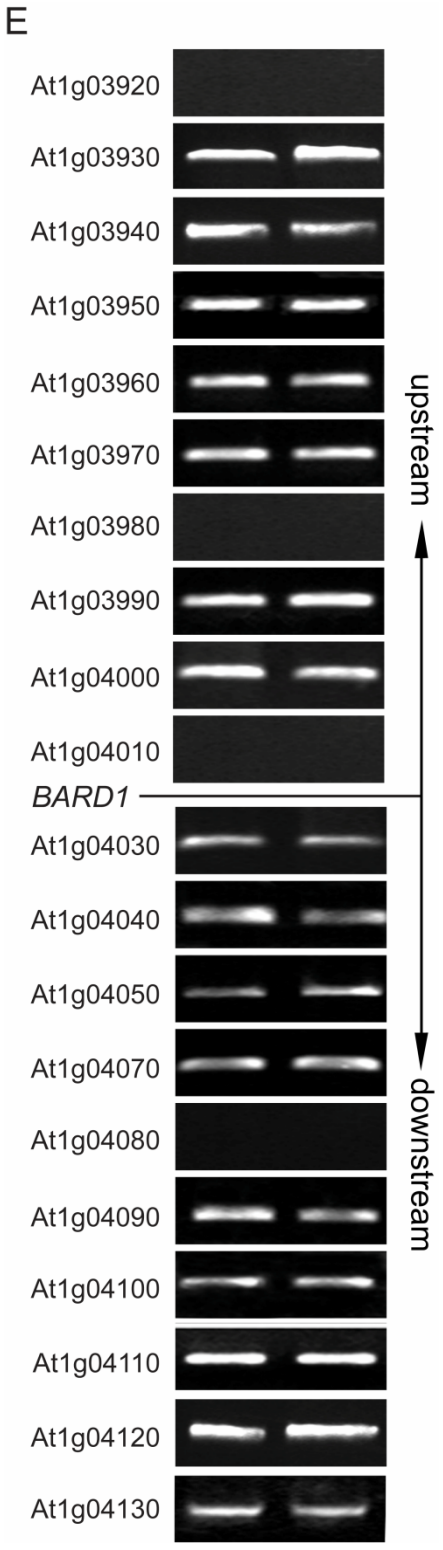
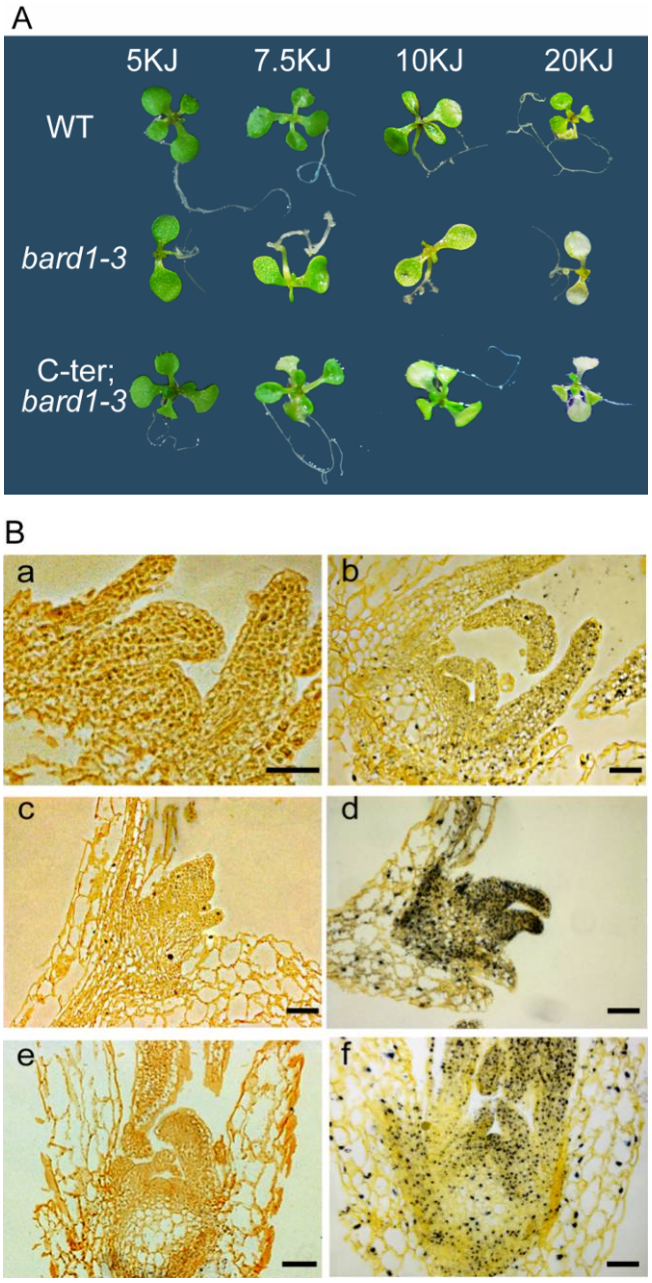
Supplemental Data. Han et al. (2008). Mutation of *Arabidopsis BARD1* Causes Meristem Defects by Failing to Confine *WUSCHEL* Expression to the Organizing Center



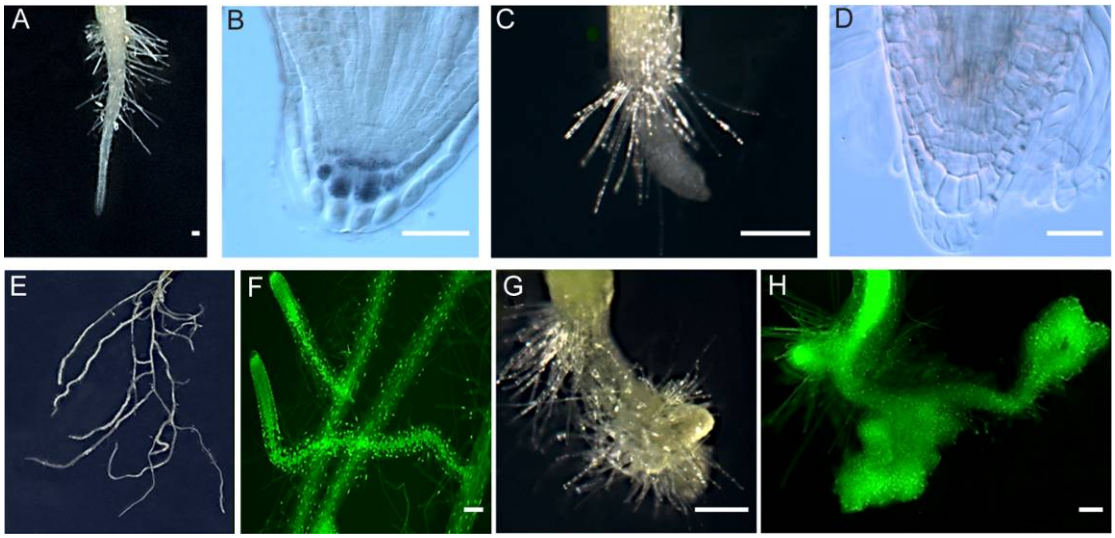


Supplemental Figure 1. Characterization of Different *bard1* Mutant Alleles.
(A) Schematic drawing of the genomic organization of *BARD1* and positions of T-DNA insertions. The *BARD1* coding region is 2142 bp long and is comprised of 13 exons (black boxes). T-DNAs were found in the first intron (*bard1-1*), and the third

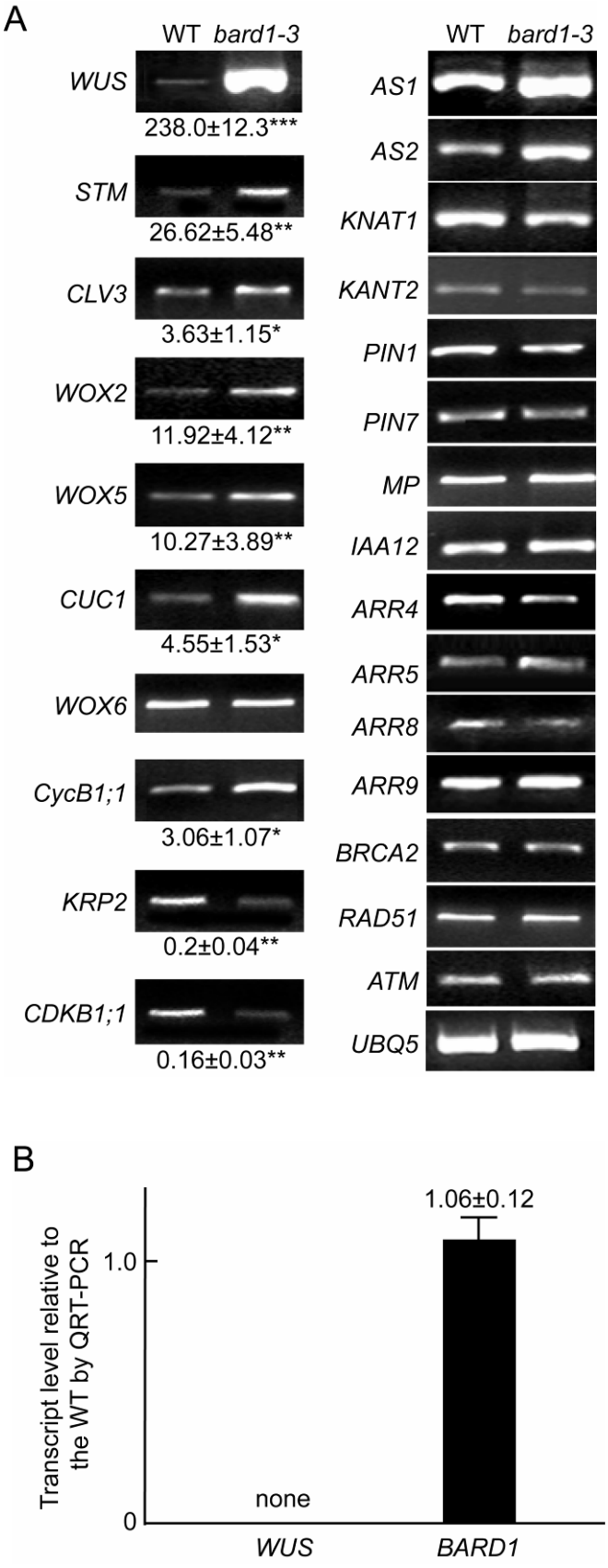
(*bard1-2*) and last (*bard1-3*) exons. The most conserved, and presumably most important, regions of the predicted protein are indicated below the schematic. The numbers in parentheses beneath the drawing indicate the precise location of the *BARD1* coding region on chromosome 1. The T-DNA in *BARD1-3* was inserted between nucleotides 1039950 and 1039951 as determined by sequencing of a genomic clone amplified from the mutant using the primer pair RP' and FP' (Supplemental Table 1 online).
(B) Genomic PCR analysis of wild-type (WT), heterozygous (HZ, *BARD1*/*bard1-3*) and homozygous (*bard1-3*) mutant lines. RP, reverse primer, complementary to the region downstream of the T-DNA; FP, forward primer, complementary to the region upstream of the T-DNA; LBb1, complementary to the left-most region of the T-DNA (Supplemental Table 1 online).
(C) Southern blot analysis. Genomic DNA was isolated using the DNAeasy Plant kit (Qiagen, Germany), and 10 μg was digested with *Hind*III or *Bgl*II (Promega, Madison, WI) and blotted for hybridization using a [α-^{32}P]dCTP-labeled (Takara, Japan) NPTII probe with the primers described in Supplemental Table 1 online.
(D) Northern blot. Total RNA (20 μg) isolated from each of the three *Arabidopsis* lines was loaded in each lane and hybridized with a 696-bp BARD1 C-terminal fragment using the primers described in Supplemental Table 1 online and ^{32}P-labeled probes.
(E) RT-PCR analyses of ten open reading frames up- and down-stream of the *BARD1* locus using primers described in Supplemental Table 1 online.



Supplemental Figure 2. *bard1-3* is Hypersensitive to DNA Damage.
(A) UVC recovery assay. Ten-day-old wild-type (WT), *bard1-3*, and BARD1:C-ter*;bard1-3* (C-ter*;bard1-3*) seedlings were treated with various doses of UVC as indicated and photographed after 48 h of recovery under continuous light in the growth chamber.
(B) TUNEL-based *in situ* cell death analyses using sections prepared from apical tissues of 3-week-old wild-type (a, b), *bard1-3* (c, d), and BARD1:C-ter*;bard1-3* (e, f) *Arabidopsis* seedlings. In a, c and e, no UVC treatment was given. In b, d and f, seedlings received 10 kJ/m^2 of UVC and were allowed 48 h to recover in the growth chamber before fixation for the assay. Scale bars, 50 μm.

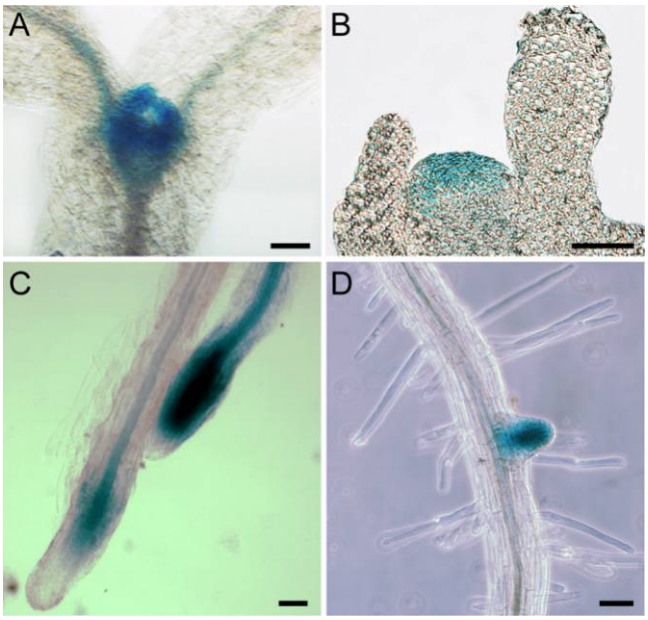


Supplemental Figure 3. Phenotypic Characterization of the *bard1-3* Root System.
(A), (B), One-week-old wild-type Col-0 roots; (C), (D), one-week-old *bard1-3* roots; (E), (F) 3-week-old wild-type roots; (G), (H), 3-week-old *bard1-3* roots. The images shown in (A), (C), (E) and (G) were taken through dissection microscopes. In (B) and (D), root tips of wild-type and the mutant, respectively, were observed under a differential interference contrast microscope after Lugol's staining. Note the absence of starch granules in the mutant. In (F) and (H), 3-week-old wild-type and mutant roots, respectively, were visualized with a microscope equipped with a UV fluorescence filter set after DAPI staining. Note the large aggregates of cells at the primary root tip as well as in the presumed lateral root initiation sites. Bars in B and D, 10 μm; bars in all other panels, 200 μm.



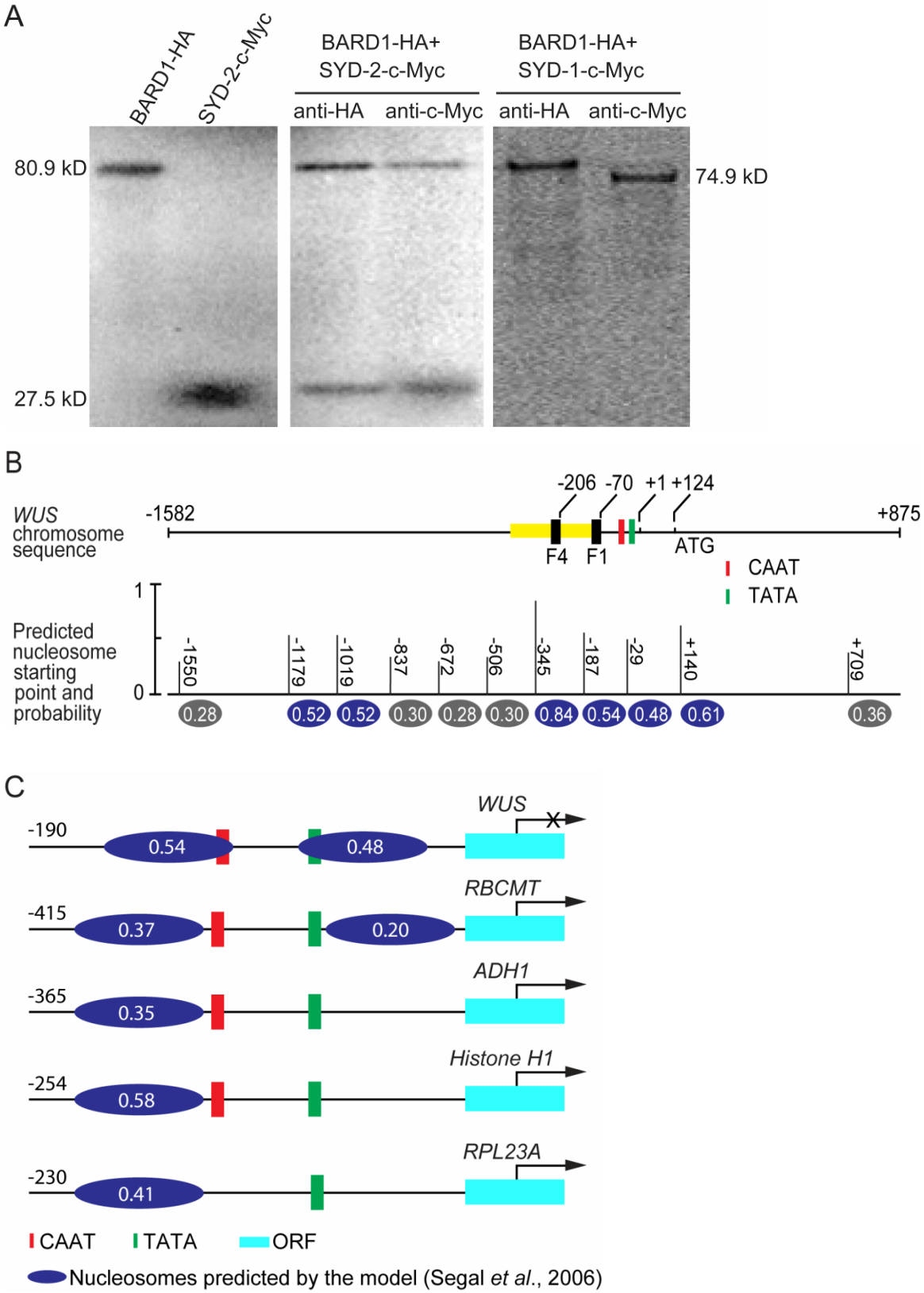
Supplemental Figure 4. RT- and QRT-PCR Analyses of Potential Target Gene Expression.

(A) RT-PCR analysis of 25 candidate genes obtained from a search of https://www.genevestigator.ethz.ch/. QRT-PCR results, as percentages of the wild-type (WT) level, are shown underneath the RT-PCR data when the transcripts of a particular gene were significantly changed (as assessed by visual inspection) in the *bard1-3* mutant. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.
(B) QRT-PCR analysis of *WUS* and *BARD1* expression in the *wus-1* mutant. The amount of *WUS* and *BARD1* transcripts in wild-type *Arabidopsis* shoots was arbitrarily set to 1.0 for comparison purposes. 'None' denotes less than 0.003% of WT levels.



Supplemental Figure 5. GUS Expression in Transgenic *Arabidopsis* Plants Carrying the *BARD1:GUS* Construct.

A 1.91-kb 5' upstream region of *BARD1* was fused to the coding sequence of *GUS* in pBI121 for genetic transformation of *Arabidopsis* plants. A cleared shoot (A), a longitudinal section (B), a root branch site (C) and root tips (D) of 10-day-old transgenic seedlings are shown after GUS staining. Scale bars in (A) and (B), 50 μm; (C) and (D), 200 μm.



Supplemental Figure 6. *BARD1* May Repress *WUS* Expression by Inhibiting the Chromatin Remodeling Process that is Essential for *WUS* Promoter Function.

(A) BARD1 co-immunoprecipitates the SYD ATPase functional domain. Left panel: *In vitro* translated BARD1-HA (80.9 kDa) and SYD-2-c-Myc (27.5 kDa) were co-immunoprecipitated by anti-HA and anti-c-Myc, respectively. Middle panel: Both BARD1-HA and SYD-2-c-Myc were co-immunoprecipitated by either anti-HA or anti-c-Myc. Right panel: No interaction between BARD1-HA and SYD-1-c-Myc (74.9 kDa) was observed after co-immunoprecipitation.
(B) Predictions of intrinsic nucleosome organization of *WUS* upstream chromosomal DNA using a computational model (Segal et. al., 2006). Upper schematic: Part of the *WUS* chromosomal sequence from nucleotides –1582 to +875; the translation start site (ATG), transcriptional initiation site (+1), F1, and F4 are indicated. Lower schematic: The probability of a particular base pair to initiate a nucleosome is reported beneath the schematic using a scale of 0.0 to 1.0 (vertical axis). The exact positions of individual base pairs having a probability >0.2 of initiating a nucleosome are given on the vertical lines, with the probability in each case reported in an ovoid below/right of each vertical line.
(C) The CAAT and TATA boxes in the *WUS* promoter are located in a predicted nucleosome region (blue ovoids), whereas they are found in the inter-nucleosome regions for more ubiquitously expressed housekeeping genes, such as *RBCMT* (ribulose-1,5 bisphosphate carboxylase oxygenase, RUBISCO large subunit N-methyltransferase, At1g14030), *ADH1* (alcohol dehydrogenase, At1g77120), *Histone H1* (At1g06760), and *RPL23A* (60S ribosomal protein L23, At1g04480). Note that only the relevant region for a particular gene is reported in the figure. The calculated probability of nucleosome formation is indicated inside each nucleosome/ovoid.