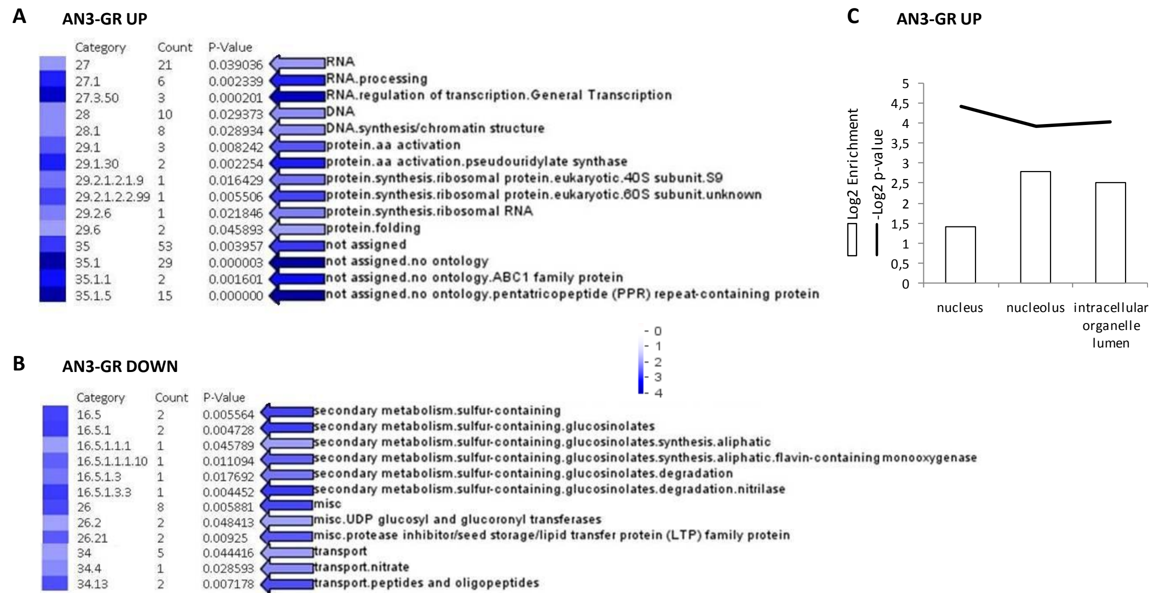
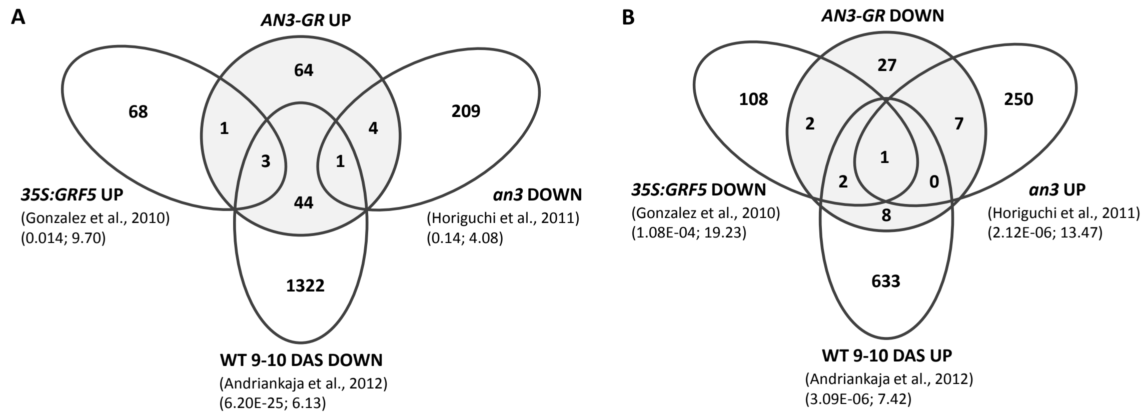


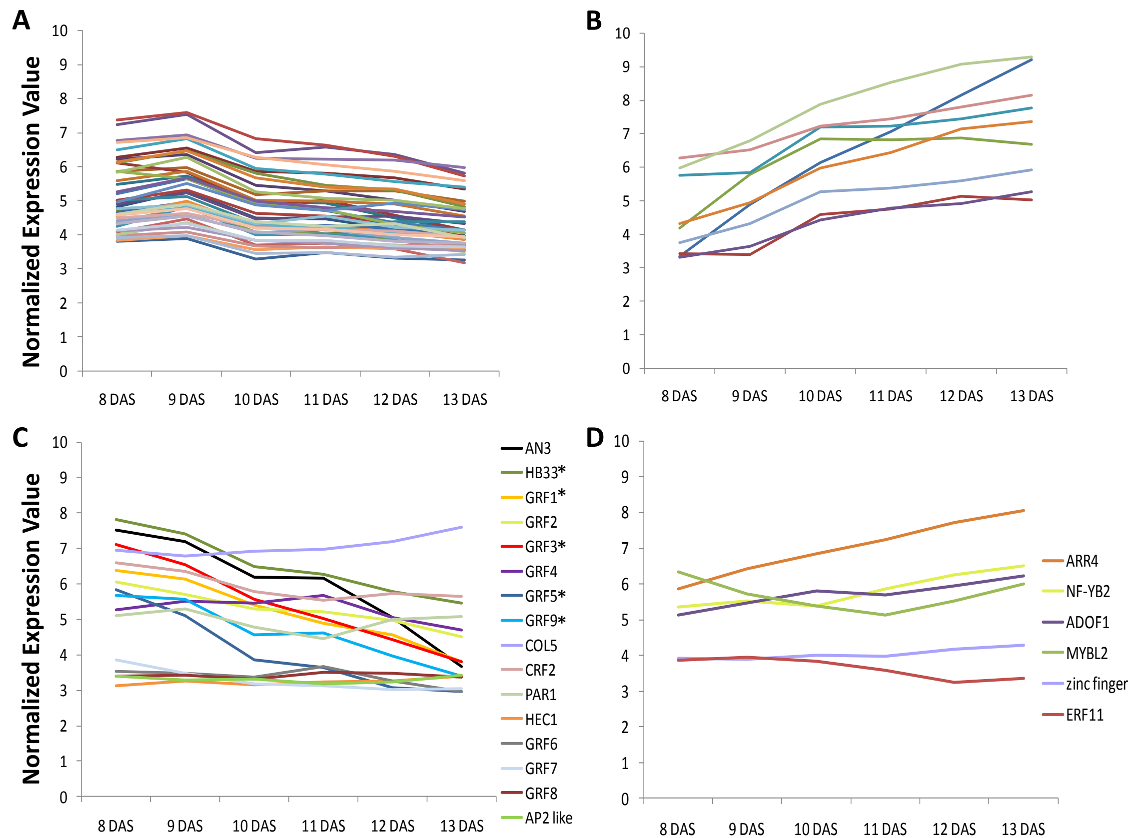
Supplemental Figure 1. Overrepresentation Analysis of Differentially Expressed Genes after AN3 Induction.

(A) and **(B)** PageMan (Usadel et al. 2006) enrichments of functional categories amongst genes upregulated in *AN3-GR* **(A)** and genes downregulated in *AN3-GR* **(B)** 8 h after DEX treatment compared to DEX-treated wild-type controls. The blue color intensities indicate the z-scores, calculated from the corresponding P-values, with a z-score of 4 representing a P-value of 0.0001.
(C) GO enrichment of subcellular localization of proteins encoded by the genes upregulated in *AN3-GR* using PLAZA (Van Bel et al. 2012).



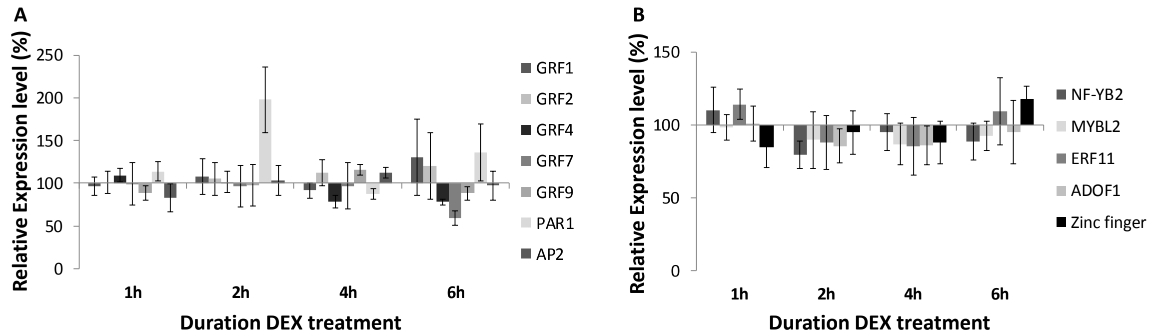
Supplemental Figure 2. Comparison of the Differentially Expressed Genes after AN3 Induction with Publicly Available Microarray Datasets.

(A) Intersection of *AN3-GR* upregulated genes with genes upregulated in *35S:GRF5* and downregulated in *an3* and between days 9 and 10 during wild-type leaf 3 development.
(B) Intersection of *AN3-GR* downregulated genes with genes going down in *35S:GRF5* and up in *an3* and during wild-type leaf 3 development between days 9 and 10. The numbers between brackets indicate P-value (Fisher exact test) and fold enrichment, respectively, for each overlap.



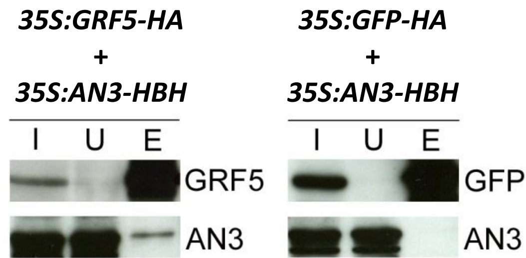
Supplemental Figure 3. Expression Profiles of Selected Genes during Leaf 3 Development from Proliferation to Expansion.

(A) to **(D)** Normalized expression values were calculated from AGRONOMICS1 tiling arrays by Andriankaja et al. (2012).
(A) Genes from the intersection between *AN3-GR* UP and WT 9-10 DAS DOWN, as shown in Supplemental Figure 2A.
(B) Genes from the intersection between *AN3-GR* DOWN and WT 9-10 DAS UP, as shown in Supplemental Figure 2B.
(C) Transcription factors upregulated in *AN3-GR*, including *AN3* and the remaining *GRF*s.
(D) Transcription factors downregulated in *AN3-GR*. * Significantly differentially expressed between days 9 and 10 (P-value < 0.05).



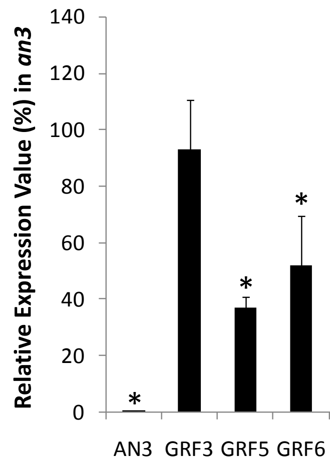
Supplemental Figure 4. Transcription Factors Not Differentially Expressed 1, 2, 4, or 6 h after AN3 Induction.

(A) and **(B)** Wild-type and *AN3-GR* plants were grown for 8 days and transferred to medium supplemented with 5 µM DEX for 1, 2, 4, or 6 h. qRT-PCR expression levels were normalized to DEX-treated wild-type expression levels which are set at 100% for each time point. Error bars are SE (n = 3).
(A) Transcript levels of the remaining *GRFs* and transcription factors identified as upregulated 8 h after DEX treatment of *AN3-GR* plants, but not shown in Figure 2B.
(B) Expression levels of the transcription factors identified as downregulated 8 h after DEX treatment of *AN3-GR* plants.



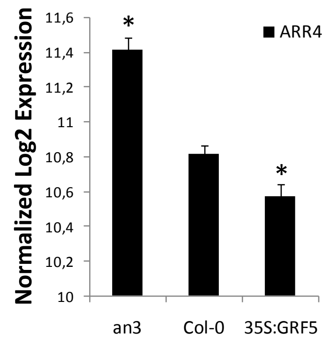
Supplemental Figure 5. Co-Immunoprecipitation of AN3 and GRF5.

GRF5-HA (left) or GFP-HA (right) complexes were immunoprecipitated with anti-HA antibodies, from total protein extracts from cell suspension cultures that were co-transformed with *35S:GRF5-HA* and *35S:AN3-HBH* (left) or *35S:GFP-HA* and *35S:AN3-HBH* (right). The purified complexes were subjected to immunoblot analysis with anti-HA (upper lane), and anti-His (lower lane) antibodies. I: 1/10th of the total input protein extract. U: 1/10th of the unbound fraction. E: the total bead bound fraction.



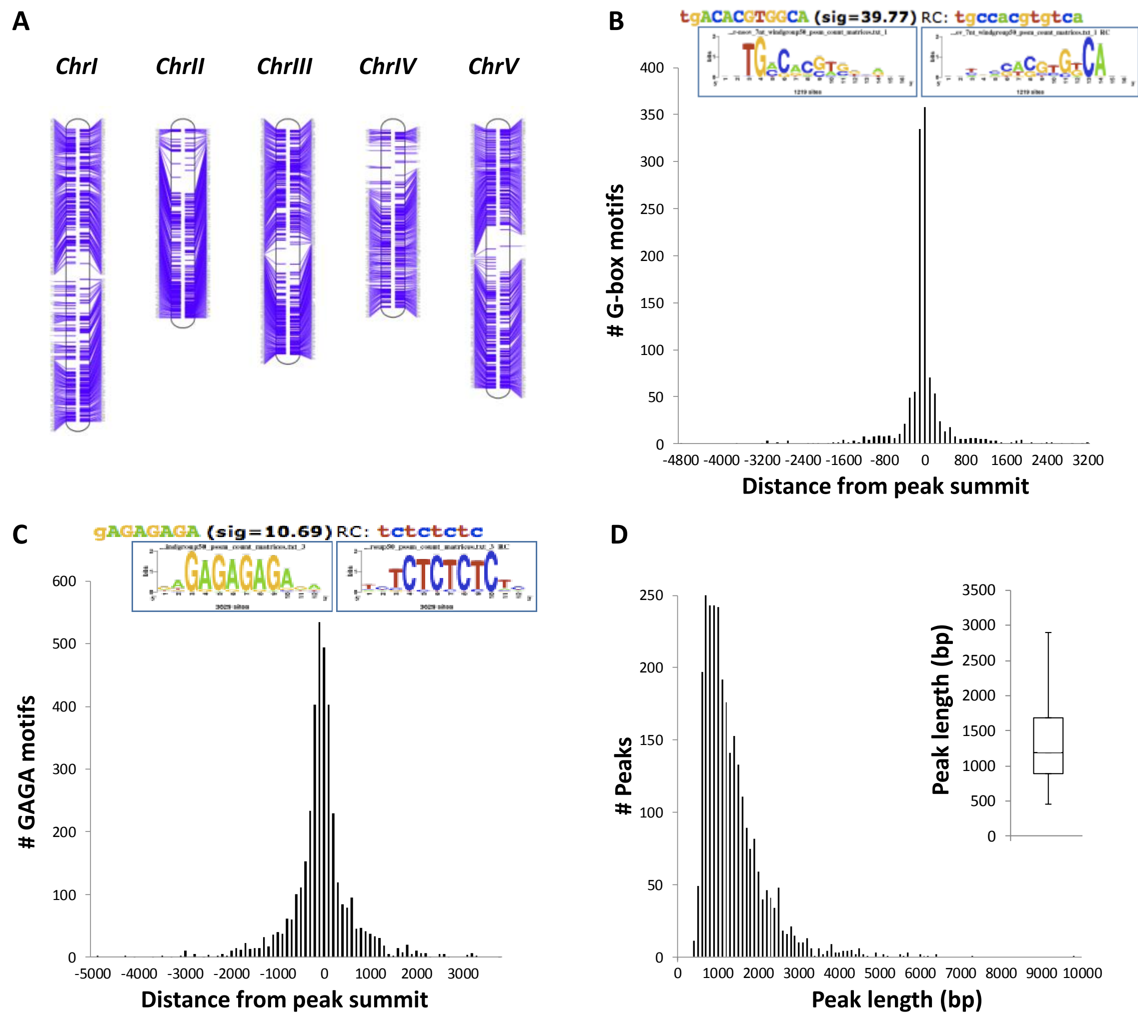
Supplemental Figure 6. *AN3* and *GRF* Expression in *an3* Plants.

Relative *AN3*, *GRF3*, *GRF5*, and *GRF6* expression levels in 12-d-old *an3* shoots, normalized to wild-type expression level for each gene. Error bars are SE (n = 3). * Significantly different from wild-type plants (P < 0.075, Student's *t* test).



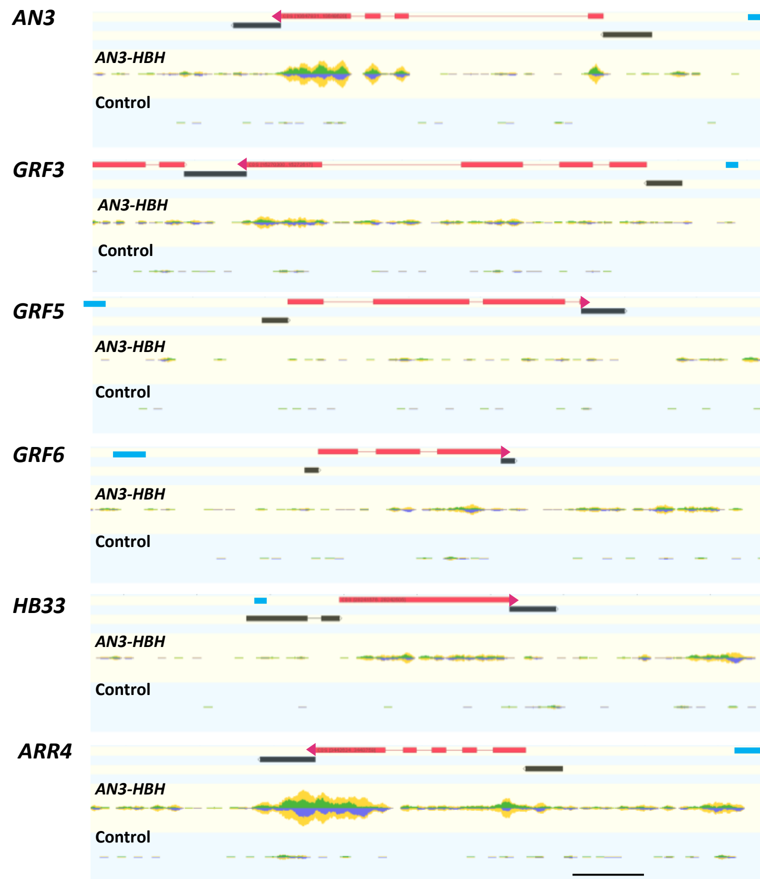
Supplemental Figure 7. *ARR4* Expression in *an3* and *35S:GRF5* Leaves.

Transcript levels were determined in 6-d-old microdissected leaves 1&2 by the nCounter nanostring technology (see Methods). Error bars are SE ($n \geq 3$). * Significantly different from Col-0 plants ($P < 0.01$, Student's *t* test).



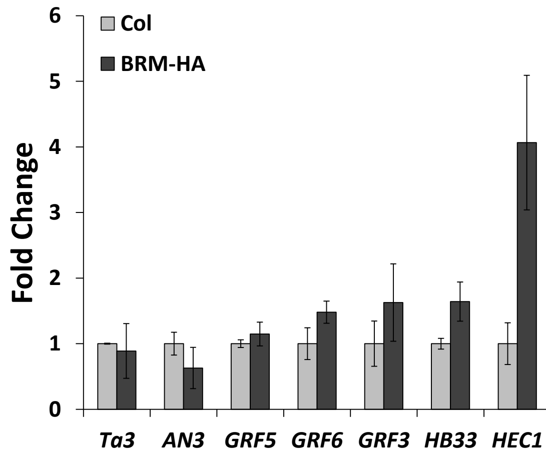
Supplemental Figure 8. Distribution, Length, and Motif Analysis of AN3 Binding Sites Determined by TChAP-Sequencing.

(A) Distribution of AN3 binding sites across the five *Arabidopsis* chromosomes.
(B) The G-box-derived motif with sequence tgaCACGTGgca, identified by RSAT peak-motifs (Thomas-Chollier et al., 2012), and the distribution in relation to the peak summits.
(C) The GAGA motif, GAGAGAGA, identified by RSAT peak-motifs (Thomas-Chollier et al., 2012), and the distribution in relation to the peak summits.
(D) Distribution of the peak lengths in base pairs (bp) of all 2836 peaks. The boxplot in the inset shows the median peak length.



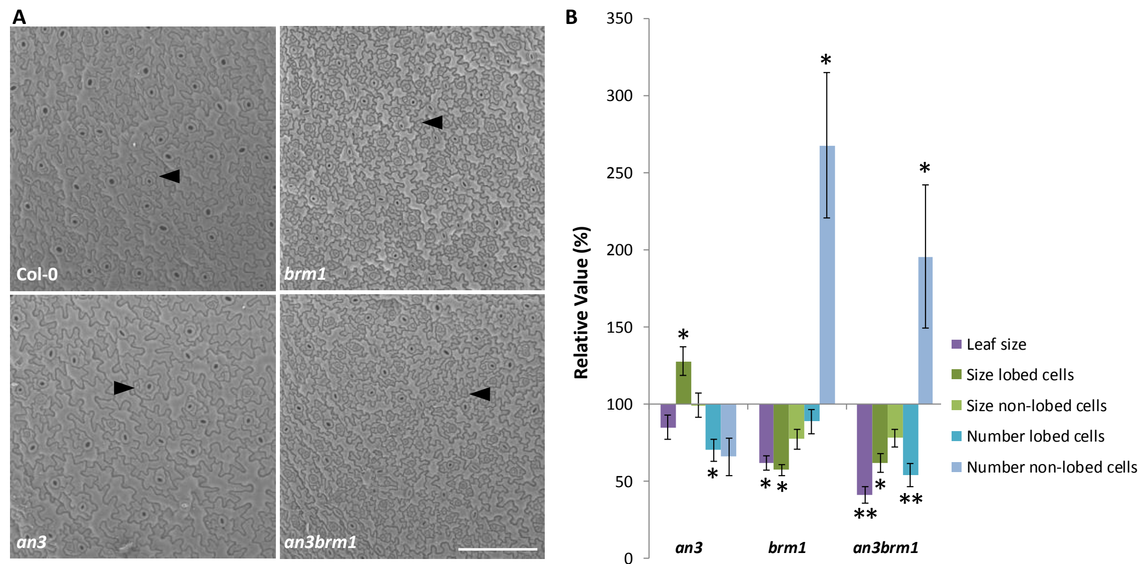
Supplemental Figure 9. TChAP-Sequencing Results for *AN3*, *GRF*, *HB33*, and *ARR4* Loci.

GenomeView representation (Abeel et al., 2012) of the TChAP-seq results for *AN3*, *GRF3*, *GRF5*, *GRF6*, *HB33*, and *ARR4* loci, showing read coverage in the TChAP-purified *AN3-HBH* versus the wild-type control samples. The reads are piled up with forward reads above the axis displayed in green and reverse reads below the axis in blue. Total coverage is indicated in yellow, but no significant peaks were detected. Scaling was done relative to the maximum number of reads displayed in Figure 3C. The coding regions are indicated as pink boxes, and the UTRs as black boxes. The regions amplified with AN3-GFP, SWP73B-CFP, and BRM-HA ChIP-qPCR are indicated in light blue. Scale bar: 0.4 kb.



Supplemental Figure 10. BRM binds the *HEC1* promoter.

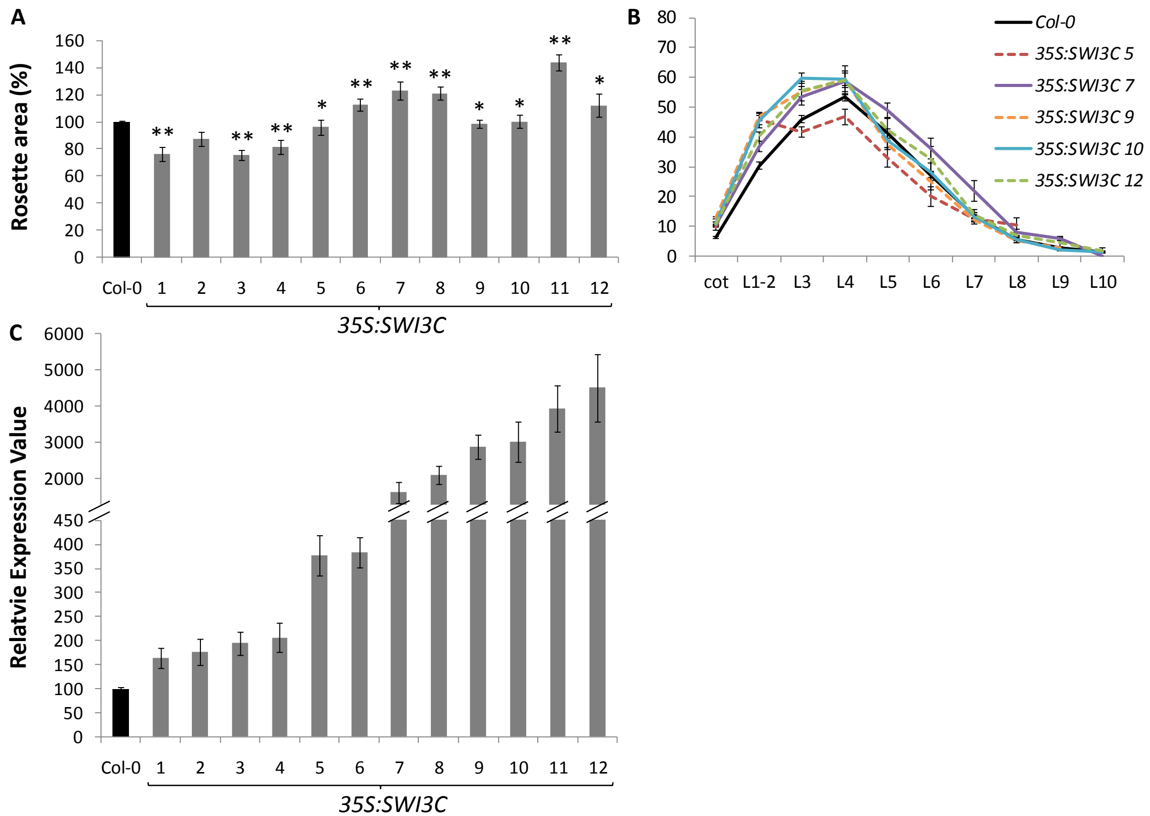
ChIP with anti-HA antibody on plants expressing HA-tagged *BRM* and wild-type (Col) plants. Enrichment was determined with qPCR and for each locus normalized against the wild type. *Ta3*: heterochromatic control region (Johnson et al., 2002). BRM binding to the *CRF2*, *COL5*, and *ARR4* loci was not tested. For diagrams of the loci including the amplified regions see Figure 3D and Supplemental Figure 9 online. Error bars are SE (n = 2).



Supplemental Figure 11. Cellular analysis of *an3brm1* leaves at 14 DAS.

(A) SEM pictures of the abaxial epidermis of 14-d-old leaves 1&2 of wild-type, *an3*, *brm1*, and *an3brm1* plants. Examples of small non-lobed cells are indicated by arrowheads. Scale bar: 150 µm.

(B) Leaf size, pavement cell sizes, and pavement cell numbers of 14-d-old leaves 1&2. Non-lobed and lobed cells are defined as follows: non-lobed cells < 17.5 μm^2 < lobed cells. Normalization was done relative to the wild type (Col-0), which is set at 100% for each measurement. Error bars are SE (n = 5). * Significantly different from wild-type plants and ** significantly different from *brm1* plants (P < 0.05, Student's *t* test).



Supplemental Figure 12. *35S:SWI3C* lines.

(A) Total rosette areas calculated from individual leaf sizes from 21-day-old plants. Error bars are SE (n ≥ 10). ** Significanlty different from the wild type (Col-0) (P < 0.05, Student's *t* test). * Rosette area is not significantly different from Col-0, but leaves 1&2 are increased in size.
(B) Individual cotyledon (Cot) and leaf areas (L1-L10) measured from leaf series made at 21 DAS from plants with increased leaf growth, as indicated in **(A)**.
(C) Relative expression levels of *SWI3C* in different independent transgenic lines at 15 DAS, determined by qRT-PCR and normalized to wild-type (Col-0) expression levels. Error bars are SE (n ≥ 2).

Supplemental Table 1. Phenotype of the F3 progeny of Different Self-Pollinated Parent Plants Carrying the *an3* and/or *brm1* Allele(s).

Genotype Parent		**Phenotype Progeny**						
		Col-0 %	expected %	***brm1* %**	expected %	**an3 %**	expected %	n
brm1/BRM1	AABb	**83.00** ±6.00	**75.00**	**17.00** ±6.00	**25.00**	0	0	184
AN3brm1/AN3BRM1	AABb	**84.43 ±2.99**	**75.00**	**15.57 ±2.99**	**25.00**	0	0	372
an3BRM1/AN3BRM1	AaBB	**56.04** ±4.72	**75.00**	0	0	**43.96** ±4.72	**25.00**	189
an3brm1/AN3BRM1	AaBb	**61.65** ±5.31	**56.25**	**9.31** ±3.27	**25.00**	**29.04** ±6.49	**18.75**	195
an3BRM1/an3BRM1	aaBB	0	0	0	0	**100**	**100**	162
an3brm1/an3BRM1	aaBb	0	0	**16.00** ±2.28	**25.00**	**84.00** ±2.28	**75.00**	106

The percentages ± SE of plants that have the wild-type Col-0, *brm1*, or *an3* phenotype are shown. Expected % represents the percentage of plants that are expected to have a phenotype in case of a Mendelian segregation and *an3brm1*/*an3brm1* double homozygous plants having the *brm1* phenotype. N indicates the total number of plants that was analyzed.

Supplemental Table 2. qPCR and ChIP Primer Sequences.

AGI code	Annotation		qPCR primers	ChIP primers
AT5G28640	AN3	Forward	CAGGGAGAAGGAGGGTCACAC	TGAGACGAGACGAGTCATGC
		Reverse	ATTGAAGATCGAGCCGCCATTAG	TGCGGATTAGATTTGGCATA
AT2G22840	GRF1	Forward	TGGCTCCATCTTCCCCTGTTC	
		Reverse	CTCGGTGTTGACGCCTAATCC	
AT4G37740	GRF2	Forward	ATCCTTCCACCGTCAACTTACAAC	
		Reverse	CGGGTTGAGTAACGAATCAGAGG	
AT2G36400	GRF3	Forward	TCCACCTTAGTCAAAGTTGTTCGG	TGGTATTTCCAAAGCAGCAA
		Reverse	GGACTCGTATGGCCTCTTGTTG	CCCAGAAAGAACCACTCTGC
AT3G52910	GRF4	Forward	TTGGTATTGGGGAAGAGGAG	
		Reverse	GGTTTCCACAGGCTTTCTTG	
AT3G13960	GRF5	Forward	TCAGTTCAATGTCTTAGCCTCTGC	GAAAGATCTAGGGGGCTTCAA
		Reverse	CCCAACTCCTCCAACTCTCTCC	GGGTTGGGTTCATTAAGTGC
AT2G06200	GRF6	Forward	CCTCAAGAAAGCCTCCTCCT	GAGTCGCAGAGAGTGCAGAA
		Reverse	TTGACCCGGAAGGTTCTATG	TCGTAGCTCCAACACATTCG
AT5G53660	GRF7	Forward	GAGACTCCAGAGGAAGTGAGAAA	
		Reverse	TGCCATTGGAAGAAGAAACA	
AT4G24150	GRF8	Forward	CCACAGAGGACTGAGGTGGT	
		Reverse	TAATCGAATCCCCGTTTGAG	
AT2G45480	GRF9	Forward	CAAGAACGTGGTTGTTTTCG	
		Reverse	CATCTTTGCTGCATCTCCAC	
AT1G75240	HB33	Forward	TGGACTTTGCGGAGAAGCTAGG	CTTTCTCTCATGGCCTCACC
		Reverse	TCGCCGCAGAATCTCTTAAGCTC	ATGTCTCAGTGCTCCCAACA
AT4G23750	CRF2	Forward	CGTTGTAGTCAAGGAAGAGCCATC	CGCACGGAGGTTATGAGATT
		Reverse	CGTACACATATCCGCCGTGAATAG	GGGCAGCTGACAAAGAGAAA
AT5G57660	COL5	Forward	CAGTCAGCCACAGTGTTTCG	CGATATCAAGGACCAGCCTAA
		Reverse	TCGTCGAGCTAGTGATCGTG	CACGTGTAAACATGACATCCA
AT5G67060	HEC1	Forward	GAGGAGGAGGAAGGGTTTTG	TCATGAACCTTGCTTTGTCG
		Reverse	ATGAGTCCCCACTGTTCCAC	ATCGACCGTCCAGATCATGT
AT2G42870	PAR1	Forward	TAACGCAAGCGTACGTGAAG	
		Reverse	CTCTGCAACGCCTCAATCTT	
AT4G39780	AP2	Forward	AATCTTCTTCGCCGGAATCT	
		Reverse	ATCGCATCCCAATCAATCTC	
AT5G47640	NF-YB2	Forward	GGGAGAGGACTGGACTAGGG	
		Reverse	TGGTTCTGCTGGTGAAGAAA	
AT1G71030	MYBL2	Forward	GCACTTCTTGGCAATAGATGG	
		Reverse	GTGTGATGGTGGAGACGATG	
AT1G28370	ERF11	Forward	ACATCAACGCCCACTGCTTGAG	
		Reverse	ATGATTCCACGGTGCTGCTCTG	
AT1G51700	ADOF1	Forward	AGTTGGAGATCCGAATGGTG	
		Reverse	CGTGTCATCACTCCTCCGTA	
AT5G66070	zinc finger	Forward	AAAGGGTCTCACTGGGGACT	
		Reverse	GTCCTGAAGGCAGACAGAGC	
AT1G10470	ARR4	Forward	GAATCGACAGATGCCTTGAG	GGCCCATATCGTTTGAAAAA
		Reverse	CGACGAGAAGACATGGAAATC	ATCGATCACAGTCGTGGATG
AT2G46020	BRM	Forward	CCCACTCATCCAAACAACAG	
		Reverse	GCTAGGCCGTCTTTTACCAG	
AT1G21700	SWI3C	Forward	TCAACAGCAAGATGGAGCAC	
		Reverse	ACCTTATCCTGTGGCAGTGG	

Supplemental Method 1. Tandem Chromatin Affinity Purification (TChAP)

Two-day-old exponentially growing cell cultures were treated with 0.75% formaldehyde for 10 min. Crosslinking was stopped by addition of 0.25 M glycine during 10 min. Cells were filtered and washed with PBS pH 7.2 (0.14 M NaCl, 2.7 mM KCl, 10 mM PO43-). The harvested material was ground in liquid nitrogen and solubilized in NLB buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 1 µg/ml pepstatin A, 1 µg/ml aprotinin, 1 µg/ml leupeptin, 1 mM PMSF) with an Ultra-Turrax T25 mixer. Chromatin was fragmented on ice with a probe sonicator to obtain ~200–800-bp fragments. After sonication, the suspension was centrifuged twice for 20 minutes at 16000 rpm, the protein concentration was determined by using the Bio-Rad protein assay kit, and the extract was used for chromatin precipitation.

Extract containing 200 mg protein was adjusted to 10 mM imidazole and incubated overnight on a rotating wheel with 300 µl Ni-NTA Superflow (Qiagen). The NiNTA resin was washed three times with 10 ml NLB + 10 mM imidazole and complexes were eluted with two times 1.5 ml NLB + 150 mM imidazole. Next, the eluates from three NiNTA purifications were pooled on 100 µl Streptavidin Sepharose. After 3-h incubation on a rotating wheel, the Streptavidin Sepharose beads were washed with 10 ml NLB + 10 mM imidazol and three times with 1ml NLB containing 750 mM NaCl and increasing SDS concentrations (0.1%, 0.5% and 1%).

Finally, the beads were washed with 15 ml TE buffer (10 mM TrisCl pH8, 500 mM NaCl, 1 mM EDTA) and the bound AN3-DNA complexes eluted and reverse crosslinked by incubation overnight at 65ºC on a rotating wheel in 1 ml 10 mM TrisCl pH8, 1 mM EDTA, 0.5 M NaCl, 1% SDS + 0.5 µl RNAse A (100 mg/ml). Next, the eluates were incubated with 100 µg Proteinase K for 2 h at 42ºC on a rotating wheel and the DNA extracted by phenol/chloroform/IAA followed by purification using Qiaquick PCR purification kit and DNA quantification by the Quant-iT dsDNA High Sensitivity kit (Invitrogen).

Supplemental Method 2. LC-MS/MS Analysis

Proteins were separated in a short run of 7 minutes on a 4-12% gradient NuPAGE gel (Invitrogen) and visualized with colloidal Coomassie Brilliant Blue staining. The protein gel was washed for 1 h in H_2O, polypeptide disulfide bridges were reduced for 40 min in 25 mL of 6,66 mM DTT in 50 mM NH_4HCO_3, and sequentially the thiol groups were alkylated for 30 min in 25 mL 55 mM IAM in 50 mM NH_4HCO_3. After washing with H_2O, a broad zone containing the proteins was cut from the protein gel, sliced into 24 gel plugs, and collected together in a single Eppendorf. Gel plugs were washed twice with H_2O, dehydrated with 95% CH_3CN (v/v), rehydrated with H_2O, and dehydrated again with 95% CH_3CN (v/v). Dehydrated gel particles were rehydrated in 60 µL digest buffer containing 750 ng trypsin (MS Gold; Promega, Madison, WI), 50 mM NH_4HCO_3, and 10% CH_3CN (v/v) for 30 min at 4° C. Proteins were digested at 37° C for 3.5 hours. The obtained peptide mixtures were introduced into an LC-MS/MS system, the Ultimate 3000 RSLC nano (Dionex, Amsterdam, The Netherlands) in-line connected to an LTQ Orbitrap Velos (Thermo Fisher Scientific, Bremen, Germany). The sample mixture was loaded on a trapping column (made in-house, 100 µm internal diameter (I.D.) x 20 mm (length), 5 µm C18 Reprosil-HD beads, Dr. Maisch GmbH, Ammerbuch-Entringen, Germany).

After back-flushing from the trapping column, the sample was loaded on a reverse-phase column (made in-house, 75 µm I.D. x 150 mm , 5 µm C18 Reprosil-HD beads, Dr. Maisch). Peptides were loaded with solvent A (0.1% trifluoroacetic acid, 2% acetonitrile), and separated with a linear gradient from 2% solvent A' (0.1% formic acid) to 50% solvent B' (0.1% formic acid and 80% acetonitrile) at a flow rate of 300 nl/min, followed by a washing step reaching 100% solvent B'.

The mass spectrometer was operated in data-dependent mode, automatically switching between MS and MS/MS acquisition for the ten most abundant peaks in a given MS spectrum. In the LTQ-Orbitrap Velos, full scan MS spectra were acquired in the Orbitrap at a target value of 1E6 with a resolution of 60,000. The ten most intense ions were then isolated for fragmentation in the linear ion trap, with a dynamic exclusion of 20 seconds. Peptides were fragmented after filling the ion trap at a target value of 1E4 ion counts.

From the MS/MS data in each LC run, Mascot Generic Files were created using the Mascot Distiller software (version 2.4.1.0, Matrix Science, www.matrixscience.com/Distiller.html). When generating these peak lists, grouping of spectra was allowed with a maximum intermediate retention time of 30 s and a maximum intermediate scan count of 5 was used where possible. Grouping was done with 0.005 Da precursor tolerance. A peak list was only generated when the MS/MS spectrum contained more than 10 peaks. There was no de-isotoping and the relative signal-to-noise limit was set to 2. These peak lists were then searched with the Mascot search engine (version 2.3, MatrixScience, www.matrixscience.com) using the Mascot Daemon interface (Matrix Science). Spectra were searched against the TAIR10 database containing 35,386 sequence entries. Variable modifications were set to methionine oxidation and methylation of aspartic acid and glutamic acid. Fixed modifications were set to carbamidomethylation of cysteines. Mass tolerance on MS was set to 10 ppm (with Mascot's C13 option set to 1) and the MS/MS tolerance at 0.5 Da. The peptide charge was set to 1+, 2+ and 3+ and the instrument setting was set to ESI-TRAP. Trypsin was set as the protease used, allowing for 1 missed cleavage, and also cleavage was allowed when arginine or lysine is followed by proline. Only high confident peptides, ranked one and with scores above the threshold score, set at 99% confidence, were withheld. Of these, only proteins with at least two matched high confident peptides were retained.