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Regulatory networks of AtBMI1 proteins

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The Arabidopsis Polycomb Repressive Complex 1 (PRC1) components AtBMI1A, B and C impact gene networks throughout all stages of plant development

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Author contributions

W.M., A.G.Z. and Y.Z performed the experiments and W.M. and F.J.R.C. analyzed the data. M.C. conceived and designed the study and M.C. and F.T. prepared the manuscript.

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One-sentence Summary

Genome wide transcriptomic data in combination with H3K27me3 and protein localization data unveiled the roles of the AtBMI1s and their possible relationship with other PRC1 components

Abstract

PcG regulation in Arabidopsis is required to maintain cell differentiation and to allow developmental phase transitions. This is achieved by the activity of three PRC2s and the participation of a yet poorly defined PRC1. Previous results showed that apparent PRC1 components perform discrete roles during plant development, suggesting the existence of PRC1 variants; however, it is not clear in how many processes these components participate. We show that AtBMI1 proteins are required to promote all developmental phase transitions and to control cell proliferation during organ growth and development, expanding their proposed range of action. While AtBMI1 function during germination is closely linked to B3 domain transcription factors VAL1/2 possibly in combination with *GT-box* binding factors, other AtBMI1 regulatory networks require participation of different factor combinations. Conversely, EMF1 and LHP1 bind many H3K27me3 positive genes upregulated in *atbmi1a/b/c* mutants; however, loss of their function affects expression of a different subset, suggesting that even if EMF1, LHP1 and AtBMI1 exist in a common PRC1 variant, their role in repression depends on the functional context.

Introduction

The evolutionary conserved Polycomb Group (PcG) machinery plays a crucial role in maintaining repression of genes that are not required during a specific cell fate (Ringrose and Paro, 2004). PcG proteins form multiprotein complexes with different histone modifying activities, including PcG repressive complex 2 (PRC2), which possesses histone H3 lysine 27 (H3K27) tri-methyltransferase activity (Müller et al., 2002), and PRC1, which has histone H2A lysine 119 (H2AK119) E3 ubiquitin ligase activity (Cao et al., 2005) as well as other non-enzymatic functions critical for chromatin compaction (Francis et al., 2004). The combined activity of both complexes is required for stable repression of target genes.

In Drosophila, single-copy genes encode the four core subunits of PRC2: Suppressor of Zeste 12 [Su(z)12], Extra sex combs (Esc),p55, and the catalytic subunit Enhancer of Zeste [E(z)] (Simon and Kingston, 2013). *Arabidopsis thaliana* (Arabidopsis) has three E(z) homologs, CURLY LEAF (CLF), MEDEA (MEA) and

SWINGER (SWN) (Goodrich et al., 1997; Grossniklaus et al., 1998; Chanvivattana et al., 2004) and three Su(z)12 homologs, EMBRYONIC FLOWER 2 (EMF2), VERNALISATION 2 (VRN2) and FERTILISATION INDEPENDENT SEED 2 (FIS2) (Luo et al., 1999; Gendall et al., 2001; Yoshida et al., 2001); while MULTIPLE SUPPRESSOR OF IRA 1 (MSI1), which is one of the five p55 homologs in Arabidopsis (Hennig et al., 2005), and the Esc homolog FERTILIZATION INDEPENDENT ENDOSPERM (FIE) (Ohad et al., 1999) are common subunits to the different possible PRC2s (Mozgova et al., 2015).

Drosophila PRC1 contains Polycomb (Pc), Polyhomeotic (Ph), Posterior sex comb (Psc), and dRing1 (Shao et al., 1999; Peterson et al., 2004), each with multiple homologs in vertebrates (Schwartz and Pirrotta, 2013). Furthermore, vertebrate PRC1 complexes exist in canonical or non-canonical forms. Canonical variants harbor homologs to the four Drosophila core subunits (Schwartz and Pirrotta, 2013), while non-canonical PRC1 complexes contain RING1A or RING1B and one of the six different homologs of Drosophila Psc (PCGF) to form a H2A mono-ubiquitination (H2Aub) module, along with additional subunits that further add specific biochemical properties and genomic localization to the different variants (Schwartz and Pirrotta, 2013). In Arabidopsis, several pieces of evidence suggest a similar high degree of complexity (Förderer et al., 2016). Two RING1 homologs, AtRING1A and AtRING1B, and three Psc/PCGF homologs, AtBMI1A, AtBMI1B and AtBMI1C have been characterized (Sanchez-Pulido et al., 2008; Xu and Shen, 2008; Bratzel et al., 2010; Chen et al., 2010; Bratzel et al., 2012; Yang et al., 2013; Calonje, 2014). Plants with mutations in these genes suggest a high degree of functional redundancy between AtRING1 or AtBMI1 proteins, thus, it is not clear whether each paralog can regulate a different subset of targets (Bratzel et al., 2010; Chen et al., 2010; Yang et al., 2013). The analysis is complicated by the observation that several mutant alleles are knock-downs rather than null alleles and that phenotypes show a wide range of stochastic variation among segregating siblings with “weak” and “strong” phenotypes (Bratzel et al., 2010; Chen et al., 2010).

Two other plant-specific proteins have been related to PRC1, EMBRYONIC FLOWER 1 (EMF1) mediating chromatin compaction (Calonje et al., 2008; Beh et al., 2012), and LIKE-HETEROCHROMATIN PROTEIN 1 (LHP1), which, as Drosphila Pc, binds H3K27me3 marks through its chromodomain (Turck et al., 2007). Although

both proteins can interact with either AtRING1 or AtBMI1 (Bratzel et al., 2010; Chen et al., 2010), recent reports showed that they also co-purify with PRC2 components (Derkacheva et al., 2013; Liang et al., 2015); thus, it is not clear in which context they carry out their functions. Additional proteins with chromatin related functions have been shown to participate in PRC1 mediated repression of specific target genes, such as the VIVIPAROUS 1 (VP1)/ ABCISIC ACID INSENSTIVE 3 (ABI3)-Like 1 and 2 proteins (VAL1/2) (Yang et al., 2013), ALFIN1-like proteins (ALs) (Molitor et al., 2014) and JMJ14 (Wang et al., 2014).

In plants, PcG repression maintains the differentiated state of the cells but also orchestrates developmental phase transitions by controlling the establishment of new cell identities. This likely requires different PRC1s but little is known about their subunit composition. The repression of several seed maturation genes after germination requires the AtBMI1 and AtRING1 proteins (Bratzel et al., 2010; Chen et al., 2010; Yang et al., 2013) and a recent genome wide study showed gene networks regulated by AtBMI1s and AtRING1s during the suppression of seed development in seedlings (Wang et al., 2016). As these results were derived from the analysis of *atring1a/b* and *atbmi1a/b* mutants developing a weak phenotype (Bratzel et al., 2010; Chen et al., 2010), their possible implication in other developmental processes or stages was not unveiled. Conversely, the repression of flower homeotic genes in seedlings requires EMF1 (Kim et al., 2012) and LHP1 (Gaudin et al., 2001) but their role in regulating other processes is not clear.

In this work, by analyzing the transcriptome of single, strong double and triple *atbmi1* mutants we have identified a more comprehensive set of candidate genes regulated by AtBMI1 proteins. Our results indicate that in addition to switching off the seed maturation program after germination, AtBMI1s promote the transition from each developmental phase to the next throughout development and furthermore control cell proliferation during organ growth and development. By integrating transcriptomics datasets with previously published data, we show that AtBMI1 and VAL1/2 act together only in the regulation of seed maturation genes. Enrichment of cis-regulatory elements at VAL1/2-dependent and -independent genes suggests that AtBMI1-mediated gene repression requires different combinational modules always involving VAL related B3 domain factors. Conversely, while EMF1 and LHP1 occupy a considerable number of genes upregulated in *atbmi1a/b/c* mutants, loss of their function does not impact the

expression of most but affects the expression of a different subset of genes. Together these results suggest that the different PRC1 variants may differ in subunit composition but also in the role that single components play all depending on the cis-regulatory context.

Results

Genome-wide transcriptomic data analysis of *atbmi1* mutants

Previous data have suggested that *AtBMI1A* and *AtBMI1B* are ubiquitously expressed and act mostly redundantly throughout development (Bratzel et al., 2010), whereas *AtBMI1C*, which is expressed in roots, endosperm and stamen, may have functionally diverged since it cannot fully rescue *atbmi1a/b* defects when overexpressed (Yang et al., 2013; Merini and Calonje, 2015); nevertheless, *atbmi1a/c* and *atbmi1b/c* do not show phenotypic alterations (Yang et al., 2013), suggesting that loss of AtBMI1C function is compensated by the other two AtBMI1s. Therefore, to gain insight into the regulatory roles of AtBMI1s, we performed genome-wide transcriptome analysis using RNA sequencing (RNA-seq) of wild type Col-0 (WT), *atbmi1a*, *atbmi1b*, *atbmi1a/b* and *atbmi1a/b/c* mutants at 10 days after germination (DAG). Since individual *atbmi1a/b* double mutants display a wide range of phenotypes (Bratzel et al., 2010), we chose to select the strong *atbmi1a/b* mutant phenotype for the analysis, which differs from the *atbmi1a/b/c* phenotype mainly in the root [(Yang et al., 2013); Supplemental Fig. S1]. The Tuxedo protocol (Trapnell et al., 2012) was used for transcript assembly and differential expression analysis. All sequencing samples were of high quality (Supplemental Fig. S2; Supplemental Table S1). Differentially expressed genes were determined using stringent criteria consisting of a combination of fold change >4 and a p-value <0.05. The number of genes scored as present in at least one of our samples was 24,503, representing 72.96% of the entire Arabidopsis transcriptome. We found less than 3-4% of the surveyed transcriptome affected in single mutants and around 15% and 20% differentially expressed in strong *atbmi1a/b* double and *atbmi1a/b/c* triple mutants, respectively (Fig. 1A; Supplemental Fig. S3). Principal components analysis showed that the transcriptomes of WT, *atbmi1a* and *atbmi1b* mutants clustered together, whereas the transcriptomes of *atbmi1a/b* and *atbmi1a/b/c* mutants constituted two distant and distinct clusters, indicating not only differences to the WT and single mutant group but also in between (Fig. 1B). In any case, we found a

185 considerable number of genes misregulated in the single mutants (Fig. 1C;
186 Supplemental Table S2) of which a majority were a subset of those affected in double
187 and triple mutants (Supplemental Fig. S4A,B). The number of up-regulated genes for
188 *atbmi1a*, *atbmi1b*, and *atbmi1a/b* was higher than down-regulated (Fig. 1C), which
189 might confirm the role of AtBMI1 proteins in transcriptional repression. However,
190 *atbmi1a/b/c* mutant showed higher number of down-regulated genes than upregulated
191 genes. This may be a consequence of the developmental stage of these mutants, in
192 which all organs are stuck in a seed maturation phase. Upregulation of some genes
193 within this context may have a stronger negative impact on gene expression.

194 Globally, the upregulated genes in the strong *atbmi1a/b* and *atbmi1a/b/c* mutants
195 (Supplemental Fig. S5A and Supplemental Fig. S6A) showed over-representation of
196 Gene Ontology (GO) terms associated with response to different stimuli (e.g. water
197 stress, temperature, hormones) and lipid metabolism (e.g. transport, biosynthesis,
198 storage); whereas the downregulated genes were enriched for GO terms related to
199 photosynthesis and metabolic processes (Supplemental Fig. S5B and Supplemental Fig.
200 S6B). This is consistent with the developmental fate of the mutants, which are trapped
201 in the seed maturation phase (Yang et al., 2013). During this phase, seeds acquire
202 desiccation tolerance and accumulate storage reserves, prevailing in the form of lipids
203 (Vicente-Carbajosa and Carbonero, 2005), while chloroplast structure is disrupted
204 (Delmas et al., 2013).

205 As PcG function is involved in the repression of master regulatory genes (Xiao
206 and Wagner, 2015), misregulation in the different *atbmi1* mutants may be an indirect or
207 direct consequence of the loss of AtBMI1 function, or a mix of both. Conversely, a
208 considerable number of AtBMI1 direct target genes may not display altered expression
209 in absence of their upstream transcriptional activators, as has been reported for other
210 PcG loss of function mutants (Bouyer et al., 2011; Kim et al., 2012; Derkacheva et al.,
211 2013). In any case, although the interrelationship between PRC1 and PRC2 is not clear
212 yet, the activity of both complexes is required for stable PcG-mediated repression;
213 therefore, selecting genes upregulated in *atbmi1* mutants and H3K27me3 marked in WT
214 seedlings should enrich for a subset of candidate genes directly controlled by AtBMI1s.
215 Accordingly, we intersected genes upregulated in the different mutants with a set of
216 5360 H3K27me3 target genes previously identified in two independent analyses in
217 seedlings [Supplemental Table S3; (Bouyer et al., 2011; Kim et al., 2012)] to selected

upregulated H3K27me3 positive (up_K27) genes (Fig. 1D). The analysis showed significant overlaps between H3K27me3 marked genes and upregulated genes in the different mutants except for *atbmi1b* probably because it is a knock-down mutant (Bratzel et al., 2010). The same analysis using downregulated genes showed non-significant overlaps in all cases excluding *atbmi1a/b/c* due to the high number of downregulated genes in this mutant (Supplemental Fig. S7; Supplemental Table S3).

To determine whether there were AtBMI1A and AtBMI1B specific candidate targets, we compared up_K27 genes in the single and double mutants (Fig. 2A). Their number in the double mutant was considerably higher than in the single mutants, illustrating a high degree of functional redundancy. Also, most of the up_K27 genes in single mutants were included in the double mutants set of up_K27 genes; however, a group of genes seemed to be exclusively upregulated in *atbmi1a* and *atbmi1a/b* or in *atbmi1b* and *atbmi1a/b* (104 and 27 genes, respectively). Up_K27 genes in *atbmi1a* and *atbmi1a/b* were expressed at very low levels in both single compared to the double mutants (Fig. 2B), indicating redundant regulation by AtBMI1A and B. The *atbmi1b* mutant shows some remnant expression of *AtBMI1B* possibly explaining higher expression in *atbmi1a* vs *atbmi1b* and the greater number of affected genes in the *atbmi1a* single mutant (Bratzel et al., 2010). Nevertheless, some genes were indeed specifically sensitive to AtBMI1B being more affected in *atbmi1b* than *atbmi1a* and not further increased in double mutants (Fig. 2B).

We next investigated the degree of redundancy between AtBMI1A/B and AtBMI1C by comparing the genes up_K27 in *atbmi1a/b* and *atbmi1a/b/c* (Fig. 3A). Clustering analysis showed that *atbmi1a/b* and *atbmi1a/b/c* shared 2/3 of the up_K27 genes (Cluster I, Supplemental Table S3) but the remaining 1/3 was genotype-specific (Cluster II, *atbmi1a/b/c* specific and Cluster III, *atbmi1a/b* specific). The expression pattern of genes in Cluster I fell into two distinct sub-groups. Cluster Ia included genes that displayed a gradual increase of expression in double and triple mutants, suggesting redundant regulation by AtBMI1A/B and by AtBMI1C (Fig. 3B and Supplemental Fig. S8A). Cluster Ib contained genes whose regulation may depend exclusively on AtBMI1A/B, as the loss of AtBMI1C function did not affect significantly their overall expression levels (Fig. 3B and Supplemental Fig. S8A). Cluster II (Supplemental Table S3) included genes exclusively upregulated in *atbmi1a/b/c*, indicating that these are AtBMI1C specific targets or, alternatively, that AtBMI1C fully compensates the loss of

AtBMI1A/B function in regulating these genes (Fig. 3B and Supplemental Fig. S8A). To discern between these two possibilities, we measured the levels of a subset of cluster II genes in WT, *atbmi1c* single and *atbmi1a/b/c* mutants in whole seedlings and roots at 10 days after germination (DAG) by quantitative RT-PCR (qRT-PCR). As they were not misexpressed in *atbmi1c* single mutants (Supplemental Fig. S8B), we concluded that AtBMI1C compensates for the loss of AtBMI1A/B function in the regulation of these genes. Finally, genes in Cluster III (Supplemental Table S3) were exclusively upregulated in *atbmi1a/b* mutants, but not in *atbmi1a/b/c* (Fig. 3B and Supplemental Fig. S8A). Although *a priori* unexpected, the result can be explained if the activation of these genes requires a developmental stage that is not reached in *atbmi1a/b/c*.

All together these data indicated that AtBMI1A and B regulate genes predominantly redundantly, whereas AtBMI1C affects only a subset of AtBMI1A/B possible targets.

Deregulated developmental programs in *atbmi1* mutants

AtBMI1 proteins were previously shown to participate in the regulation of several seed maturation (Bratzel et al., 2010; Chen et al., 2010; Yang et al., 2013) and germination related genes (Molitor et al., 2014). In addition, a recent transcriptome analysis of *atbmi1a/b* weak phenotype confirmed the role of AtBMI1 function in regulating seed development (Wang et al., 2016). When we compared the H3K27me3 upregulated genes in the *atbmi1a/b* weak (fold change ≥2, according to Wang et al. 2016; Supplemental Table S1) to those in *atbmi1a/b* strong phenotype mutants, we found significantly more genes in the stronger mutant (Fig. 4A). Among the genes upregulated in both datasets there were genes previously identified as AtBMI1 target genes, like *ABI3*, and *DELAY OF GERMINATION 1* (*DOG1*); however, other well-known AtBMI1 targets, such as *FUSCA 3* (*FUS3*) or *BABYBOOM* (*BBM*) (Yang et al., 2013), were included only in *atbmi1a/b* strong dataset. A similar picture was obtained comparing *atbmi1a/b* weak and *atbmi1a/b/c* datasets (Supplemental Fig. S9). Therefore, to obtain a more comprehensive picture of the developmental processes regulated by AtBMI1s, we examined the annotated developmental functions of up_K27 genes in *atbmi1a/b/c* mutants, as they displayed the strongest developmental alterations.

Seed maturation and dormancy

Changes in the triple *atbmi1a/b/c* mutant uncovered additional genes involved in seed maturation and abscisic acid (ABA) response, such as *FUS3* and *ABI4*, and in seed dormancy, like *SOMNUS* (*SOM*). Also, there were genes involved in regulating carbohydrate and lipid metabolism, like *WRINKLED 1* (*WRI1*) (Supplemental Fig. S9; Supplemental Table S3). Most of these genes are switched off after germination in WT; however, the *ABIs* are required for plant responses to various biotic and abiotic stresses (Cutler et al., 2010), suggesting involvement of AtBMI1s in regulating responses to environmental conditions.

Endosperm specific genes

Maturation genes were not the only seed genes upregulated in *atbmi1a/b/c* mutants. We found upregulation of genes that are predominantly expressed in endosperm and but not in the seed coat and vegetative tissues (Wolff et al., 2011). Interestingly, among these were genes displaying a maternal [*FLOWERING WAGENINGEN* (*FWA*), *HOMEODOMAIN GLABROUS 8* (*HDG8*), and *AtBMI1C*] or paternal [*PICKLE RELATED 2* (*PKR2*), *VARIANT IN METHYLATION 5* (*VIM5*), *AT2G21930* and *AT3G49770*] preferred expression in the endosperm (Supplemental Fig. S9; Supplemental Table S3).

Meristem maintenance and cell proliferation related genes

The *atbmi1a/b/c* mutant also upregulated genes involved in meristem maintenance and cell proliferation throughout plant life. Remarkably, two gene families with crucial roles in these processes were upregulated in the mutants. The first encompassed the *PLETHORA* (*PLT*) or *AINTEGUMENTA-LIKE* (*AIL*) genes. Six out of eight members of this family were up_K27 in *atbmi1a/b/c* mutants (*PLT1/2/3/5/7* and *BBM*) (Supplemental Fig. S9 and Supplemental Fig. S10; Supplemental Table S3). Some of these *PLT* genes have overlapping roles in regulating embryo patterning, shoot and root apical meristem maintenance and organ primordia initiation (Horstman et al., 2014). The second was the *WUS homeobox-containing* (*WOX*) gene family, which comprises fourteen members (van der Graaff et al., 2009), among which *WUS* and *WOX2/3/4/5/8/9/11/12* were upregulated in *atbmi1a/b/c* mutants (Supplemental Fig. S9 and Supplemental Fig. S10; Supplemental Table S3). These factors promote cell division and prevent premature cell differentiation, which are crucial processes required for stem-cell maintenance and organ formation. In addition, we found upregulation of other genes with related functions, for instance *CUP SHAPED COTYLEDON 3* (*CUC3)*

and *ENHANCER OF SHOOT REGENERATION 1* (*ESR1*) and the *GROWTH REGULATING FACTOR 5 (GRF5).*

Root development specific genes

Apart from the genes involved in root meristem maintenance, we found in *atbmi1a/b/c* upregulation of genes that play a crucial role in postembryonic root development, as *CEGENDUO* (*CEG*), *MAGPIE* (*MGP*), *INDOLE-3-ACETIC ACID INDUCIBLE 30* (*IAA30*), the *ROOT MERISTEM GROWTH FACTOR 2* (*RGF2)*, and the Class IIB NAC transcription factor *SOMBRERO* (*SMB*), underpinning the importance of AtBMI1 function for root development (Supplemental Fig. S9; Supplemental Table S3).

Other developmental genes

Among the up_K27 genes in *atbmi1a/b/c* mutants were genes involved in regulating other developmental processes, such as gametophyte development, leaf development and the flowering transition [e.g. *KANADI 2* (*KAN2), KNUCKLES* (*KNU*), *DEVELOPMENT-RELATED PcG TARGET IN THE APEX 4* (*DPA4), SEPALLATA 2 (SEP2), FLOWERING LOCUS C* (*FLC), MADS AFFECTING FLOWERING 4* (*MAF4), MAF5 and FACTOR PROMOTING FLOWERING 1* (*FPF1*)] (Supplemental Fig. S9; Supplemental Table S3).

Secondary metabolic processes

In addition, *atbmi1a/b/c* mutants upregulated genes involved in secondary metabolic processes like those involved in phenylpropanoid metabolism. Upregulated genes involved in this pathway were *CHALCONE SYNTHASE (CHS, TRANSPARENT TESTA 4 (TT4), CHALCONE ISOMERASE (CHI, TT5), FLAVONOID 3'-HYDROXYLASE (F3'H, TT7), DIHYDROFLAVONOL 4-REDUCTASE (DFR)*, and transcription factors (TFs) such as *AtMYB90 (PRODUCTION OF ANTTHOCYANIN PIGMENT 2 (PAP2)), AtMYB111 and AtMYB11* (Supplemental Fig. S9; Supplemental Table S3).

In summary, AtBMI1 function in Arabidopsis is required to regulate more developmental processes than previously thought.

Regulatory cross-talk between chromatin complexes

RNA-seq data revealed upregulation of several PcG or PcG-related genes in *atbmi1a/b/c* mutants, like *AtRING1A*, *AtRING1B*, *VAL1*, *VAL2,* and *VIN3*. Conversely, we did not find a significant change in the expression of *CLF*, *SWN*, *MEA*, *EMF2*, *VRN2*, *FIS2*, *MSI1*, *FIE*, *EMF1* and *LHP1* (Supplemental Fig. S10). On the other hand, the Trithorax Group (TrxG) genes *ULTRAPETALA 1* (*ULT1*), *ULT2* and *PKR2* that act antagonistically to PcG complexes were upregulated in *atbmi1a/b/c* mutants (Supplemental Fig. S10). Misregulation of some of these chromatin factors could contribute to the strongly altered expression pattern of *atbmi1a/b/c* mutants.

Several master regulators of the flowering program are downregulated in *atbmi1a/b/c* mutants

Several MADS-box transcription factors required to specify floral meristem identity or involved in floral organ development were downregulated in *atbmi1a/b/c* mutants (Fig. 4B; Supplemental Table S2) [e.g. *AGL42*, *SUPRESSOR OF CONSTANS 1* (*SOC1*), *SEP3*, *SEP4*, *AGL24*, *SHORT VEGETATIVE PHASE* (*SVP*)]; but also other key regulatory flowering genes, such as, *TEMPRANILLO 1* (*TEM1*) and several *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPLs*) (e.g. *SPL2,3,4,8,12)*. In addition, we found that some flowering factors that have basal expression levels in WT seedlings at 10 DAG expressed at lower levels in *atbmi1a/b/c* [e.g. *AGAMOUS* (*AG*), *APETALA 3* (*AP3*), *FLOWERING LOCUS T (FT*); Fig. 4B]. The fact that the flowering program seems to be more repressed in *atbmi1a/b/c* mutants than in WT seedlings points to a requirement of AtBMI1 function for proper regulation of flower development.

VAL1/2 and the AtBMI1s co-regulate a subset of potential AtBMI1 targets

VAL1/2 and AtBMI1 proteins are required for the initial repression of several seed maturation genes after germination, such as *FUS3*, *LEC1* and *ABI3*. Furthermore, we previously showed that the VAL1/2 recruit AtBMI1 proteins to these genes; accordingly, *val1/2* and *atbmi1a/b/c* mutants display a very similar phenotype (Yang et al., 2013). However, *WUS* is an AtBMI1 but not a VAL1/2 regulated gene, indicating that there are also differences between those mutants (Yang et al., 2013). To determine to which extent the VAL1/2 and AtBMI1 proteins act together in regulating gene expression, we compared genes upregulated in *val1/2* [(Suzuki et al., 2007); Supplemental Table S2] and H3K27me3 marked in WT according to our dataset

(Supplemental Table S3) with up_K27 genes in *atbmi1a/b/c* (Fig. 5A). We found that 70% of *val1/2* up_K27 genes were included in the up_K27 *atbmi1a/b/c* dataset; these genes represented 1/3 of the genes up_K27 in *atbmi1a/b/c*, indicating that, despite the fact that they co-regulate a considerable number of genes, AtBMI1 proteins clearly perform functions independently of VAL1/2.

The VAL proteins (VAL1, 2 and 3) belong to a subfamily of plant-specific B3 domain containing proteins (Swaminathan et al., 2008) that is predicted to bind to *LEC2/ABI3/VP1* elements [also known as *RY* elements (CATGCA); (Suzuki et al., 2007)]; in fact, a recent report showed that a point mutation in a *LEC2/ABI3/VP1* element located at the first intron of *FLC* prevents the epigenetic silencing of the gene during vernalization (Qüesta et al., 2016). *FLC* is upregulated in *val1/2* and *atbmi1* mutants (Supplemental Table S2, S3). Therefore, we investigated whether this or other cis-regulatory motifs were enriched at the promoter of AtBMI1/VAL1/2 co-regulated genes. Indeed, we found enrichment of *LEC2/ABI3/VP1* motifs but also of ABA responsive elements (*ABRE*) [ACGT or *G-box* (Choi et al., 2000)] (Fig. 5A). *ABRE/G-box* elements are recognized by bZIP transcription factors such as ABI5 (Carles et al., 2002). *LEC2/ABI3/VP1* and *ABRE* elements are clustered in the 5′ upstream regions of genes regulated by ABI3/VP1 factors and ABA (Suzuki et al., 2005), and are required for the correct expression of seed maturation genes (Santos-Mendoza et al., 2008). On the other hand, the plant-specific trihelix DNA binding protein ARABIDOPSIS 6B-INTERACTING PROTEIN 1-LIKE 1 (ASIL1) that is involved in the repression of seed maturation genes after germination binds *GT-box* elements (GTGATT and variations of this) (Gao et al., 2009). These elements are closely associated with *ABRE/G-box* and *LEC2/ABI3/VP1* elements at the promoter of several seed maturation genes. Furthermore, *GT-box* elements frequently overlap with *ABRE/G-box* elements, leading to the proposal that ASIL1 represses embryonic genes by competing with the binding of transcriptional activators (Gao et al., 2009). Therefore, we looked for co-occurrence of both elements at the promoter of AtBMI1/VAL1/2 co-regulated genes. Co-occurrence was indeed significant (Fig. 5B); moreover, both elements significantly overlapped at the promoter of these genes (Fig. 5B). Therefore, the combination of *LEC2/ABI3/VP1 and GT-box* co-occurring with *ABRE/G-box* elements represents a landmark for the subset of AtBMI1/VAL1/2 co-regulated genes.

Surprisingly, the *LEC2/ABI3/VP1* elements were as highly over-represented at promoter regions of genes exclusively up_K27 in *atbmi1a/b/c*, which suggests that their repression may be functionally connected to other B3 domain transcription factors. The specific combination of *LEC2/ABI3/VP1* and *ABRE/GT-box* elements was not detected in this group. Conversely, other motifs were enriched in the VAL1/2-independent up_K27 subset, such as *SQUAMOSA BINDING PROTEIN* (*SBP*)-, *ZAP1* (WRKY)-, *ALFIN1*- and *MYB*-binding sites and a frequent Z-box promoter motif that is bound by a new class of transcription factors, the Z-box BINDING FACTORS (ZBFs), whose roles in regulating plant development have just started to be unraveled (Gangappa et al., 2013) (Fig. 5A). ALFIN1 elements are bound by plant-specific ALFIN1-like proteins [AL1-7; (Lee et al., 2009)], which mediate gene repression (Wei et al., 2015) and interact with AtRING1 and AtBMI1 (Molitor et al., 2014), supporting the existence of other combinatorial modules involving B3 domain factors and diverse partners for AtBMI1-mediated gene repression.

Regulatory networks of AtBMI1, EMF1 and LHP1

To investigate the functional relationship between AtBMI1 proteins and EMF1, we compared direct EMF1 targets as previously determined through genome-wide ChIP-chip analysis (Kim et al., 2012) with our WT_K27 gene dataset and with genes with altered expression (up and downregulated) in *atbmi1a/b/c* mutants (Supplemental Fig. S11A; Supplemental Table S4). Clustering analysis showed a subgroup of 786 overlapping genes, indicating that among the misexpressed genes in *atbmi1a/b/c* there is a significant amount of EMF1 targets. Then, we determined the number of up_K27 genes in *atbmi1a/b/c* that were included in this subgroup (Fig. 6A). We found that half of *atbmi1a/b/c* up_K27 genes were EMF1 targets, suggesting interplay of EMF1 and AtBMI1 proteins in the regulation of a considerable number of genes.

There was little overlap between genes up_K27 in *atbmi1a/b/c* and *emf1-2* [Fig. 6B; Supplemental Table S4; (Kim et al., 2010)]; furthermore, the majority of EMF1 target genes up_K27 in *atbmi1a/b/c* were not upregulated in *emf1-2* mutants, which is consistent with the previous observation that expression of only a small percentage of EMF1 target genes is increased in *emf1-2* mutants [(Kim et al., 2012); Fig. 6C]. LHP1 has been shown to co-localize with 85-90% of H3K27me3 marked sites in Arabidopsis (Turck et al., 2007; Zhang et al., 2007; Engelhorn et al., 2012); consistent with this, 92.3% of our list of H3K27me3 marked genes (4949 out of 5360) were occupied by

LHP1 according to a recently published data set of LHP1 targets (Veluchamy et al., 2016); of these genes, 1406 significantly overlap with the genes misexpressed (up and downregulated) in *atbmi1a/b/c* mutants (Supplemental Table 4; Supplemental Fig. 11B). Furthermore, we found that 93.9% of *atbmi1a/b/c* up_K27 genes were LHP1 targets (Fig. 6C), suggesting that AtBMI1 and LHP1 co-regulate a high number of genes. However, when we compared H3K27me3 marked genes upregulated in *lhp1* (fold change ≥2, according to Wang et al. 2016; Supplemental Table S3) with up_K27 *atbmi1a/b/c* genes (Fig. 6D) we found very little overlap, indicating that loss of LHP1 function has also little impact on the expression of AtBMI1 regulated genes. Loss of LHP1 function, as loss of EMF1 function, mostly impacts the expression of genes involved in reproductive development. These genes were not upregulated in *atbmi1a/b/c* mutants and some were even repressed, suggesting that LHP1 and EMF1 play different roles in their regulation. In conclusion, regulation is not correlated to the co-distribution of EMF1 and LHP1 and likely also AtBMI1 proteins, at target genes.

Discussion

PcG regulation in Arabidopsis requires the activity of three different PRC2s, which regulate different developmental stages and display partial target specificity, and PRC1, whose identity and function is not yet well defined. Although several putative subunits have been identified (Merini and Calonje, 2015), and some evidence suggested the existence of different functional PRC1 variants (Yang et al., 2013; Calonje, 2014; Wang et al., 2014; Merini and Calonje, 2015), little is known about their composition and function. In this work, we integrated genome wide transcriptome data with H3K27me3 and protein localization data in order to shed some light on the role of different PRC1 components and their possible relationship throughout plant development.

Functional redundancy among the AtBMI1s

The identification of three AtBMI1 paralogs in Arabidopsis raised the question of whether they display functional divergence (Sanchez-Pulido et al., 2008). We found that AtBMI1A and B display mainly redundant functions throughout development, although a small number of genes were specifically sensitive to AtBMI1B. A splice variant is annotated at the *AtBMI1B* locus [the Arabidopsis information resource (TAIR)], which encodes a variant isoform without the amino-terminal RING finger domain

475 (Supplemental Fig. S12). It is possible that alternative roles of the variant protein
476 explain the observed differences in gene expression between *atbmi1a* and *atbmi1b*
477 mutants. Conversely, AtBMI1C regulates a subset of AtBMI1A/B targets. The fact that
478 ectopic expression of *AtBMI1C* in double mutants [(Yang et al., 2013); Supplemental
479 Table S2] cannot rescue *atbmi1a/b* defects in the aerial part of the seedling points to a
480 requirement of tissue specific factors for AtBMI1C mediated repression. Accordingly,
481 AtBMI1C acts redundantly to AtBMI1A/B in the regulation of a considerable number
482 of genes involved in root development. Differences in protein sequence between
483 AtBMI1C and AtBMI1A/B (Bratzel et al., 2010; Chen et al., 2010; Bratzel et al., 2012)
484 may have restricted the possibilities of AtBMI1C to interact with some factors and/or
485 favored interaction with others. Likewise, MEA cannot compensate the loss of CLF and
486 SWN function despite its ectopic expression in *clf/swn* double mutants (Farrona et al.,
487 2011). In any case, AtBMI1A, AtBMI1B and in part AtBMI1C display functional
488 redundancy, indicating how important it is to ensure AtBMI1 function throughout
489 development.

490 **Role of AtBMI1 function in plant development**

491 Transcriptome analysis revealed that 20% of the surveyed transcriptome was
492 misregulated in *atbmi1a/b/c* mutants, a much higher percentage than the one reported
493 for other PcG mutants, including *clf/swn* (Bouyer et al., 2011; Kim et al., 2012; Wang et
494 al., 2016) thereby underlining the central role of AtBMI1s in gene regulation. To
495 determine AtBMI1 regulatory gene network, we focused on genes that were upregulated
496 in *atbmi1* mutants and H3K27me3 marked in WT seedlings of the same age, even
497 though these genes may represent a subset of candidate AtBMI1 targets. Our analysis
498 supported a requirement of AtBMI1 function for the repression of the seed
499 maturation/dormancy program after germination (Bratzel et al., 2010; Chen et al., 2010;
500 Molitor et al., 2014; Wang et al., 2016); however, it also unveiled the crucial role of
501 these proteins in promoting the transition from one developmental phase to the next
502 throughout development (Fig. 7A). After embryogenesis, plants undergo the transition
503 from seed dormancy to germination that is antagonistically regulated by two hormones,
504 ABA and Gibberelins (GA) (Shu et al., 2016). During seed maturation, endogenous
505 ABA accumulates in the seed, inducing and maintaining seed dormancy. In contrast,
506 before the onset of germination endogenous ABA levels in the seed are down-regulated,
507 while the GA content is up-regulated. Among the upregulated genes in *atbmi1a/b/c*

508 mutants were genes involved in inducing ABA and/or inhibiting GA signaling (e.g.
509 *ABI3*, *ABI4*, *DOG1*, *PLT5*, *SOM*) (Fig. 6A), indicating that AtBMI1 mediated
510 repression of these genes promotes this developmental transition. Following
511 germination, plants pass through a phase of vegetative growth that can be further
512 divided into a juvenile and an adult vegetative phase. The *microRNA 156* (*miR156*)
513 regulates a subset of *SPL* transcription factors that have been shown to promote the
514 transition from juvenile to adult phase (Wu and Poethig, 2006); therefore, to allow
515 phase transition, *miR156* levels need to decrease. Although our transcriptome analysis
516 could not detect mature *miRNAs*, it has been previously shown that *pri-miR156* was
517 upregulated in *atbmi1a/b* mutants of all phenotypic severity (Pico et al., 2015);
518 accordingly, we found downregulation of several *SPLs* (e.g. *SPL2/3/4/8/12*) (Fig. 6A),
519 supporting that AtBMI1 function is required to allow this transition. Eventually, plants
520 experience the transition from vegetative to reproductive development. This transition
521 requires the repression of several flowering repressors such as *FLC*, *MAF4/5* (Gu et al.,
522 2013) and *AGL15* (Fernandez et al., 2014), which are upregulated in double and triple
523 *atbmi1* mutants (Fig. 7A). Consequently, flowering genes like *FT*, *SOC1* and *AGL24*
524 were downregulated in *atbmi1* mutants; therefore, AtBMI1 activity is also required to
525 switch from vegetative to reproductive development.

526 Furthermore, our data revealed the key role of AtBMI1 activity in controlling
527 stem cell niche specification and cell proliferation for a proper organ growth and
528 development via the repression of several master regulators (e.g. *PLT* and *WOX* genes)
529 (Fig. 7B), which is consistent with the wide spread acquisition of proliferating capacity
530 of *atbmi1* strong mutants and the alterations in root, leaf and flower development
531 observed in different *atbmi1* mutants (Bratzel et al., 2010; Yang et al., 2013).

532 **Interplay of AtBMI1 with other PcG related factors**

533 The function of AtBMI1 has been linked to the function of VAL1/2 proteins for
534 the regulation of several seed maturation genes (Yang et al., 2013). Here, we show that
535 VAL1/2 and AtBMI1s act together in the regulation of the seed maturation/dormancy
536 program; however, they do not seem to collaborate in the regulation of other
537 developmental processes. We found a specific enrichment of *LEC2/ABI3/VP1* and
538 *ABRE/G-box* overlapping with *GT-box* cis-regulatory elements at the promoters of
539 genes co-regulated by AtBMI1 and VAL1/2 proteins. An enrichment of
540 *LEC2/ABI3/VP1* and *ABRE BINDING FACTOR 1* (*ABF1*) elements has been previously

541 reported at the promoter of genes upregulated in *atbmi1a/b* weak phenotype (Wang et
542 al., 2016). Genes co-regulated by ABI3/VP1-like proteins and ABA contain these
543 motifs at their promoters (Suzuki et al., 2005). Accordingly, ABI3 and ABI5 regulate
544 gene expression synergistically. Moreover, ABI3 interacts physically with ABI5,
545 thereby ABI3 is also recruited to the promoters of the target genes via protein-protein
546 interaction (Nakamura et al., 2001). A similar mechanism could be assumed for
547 repression in which the VAL1/2 proteins bind to *LEC2/ABI3/VP1* and ASIL1 to the *GT-*
548 *box* element, resulting in a direct competition with the transcriptional activators. The
549 binding of VAL1/2 and possibly ASIL1 proteins could recruit the AtBMI1s and the
550 other PcG proteins to establish chromatin modifications that maintain gene repression.
551 Whether ASIL1-mediated repression involves *in vivo* interaction with VAL and/or PcG
552 proteins remains to be investigated; however, in support of this, it has been shown that
553 EMF1 interacts with ASIL1 (named EIP7) in yeast two hybrid experiments (Park et al.,
554 2011).

555 We also found an enrichment of *LEC2/ABI3/VP1* elements, but not *ABRE* or *GT-*
556 *box* elements, at the promoter of genes exclusively up_K27 in *atbmi1a/b/c* mutants,
557 suggesting an implication of B3 factors in the regulation of these genes as well.
558 Interestingly, two *VAL1* splice variants have been identified through RNA sequencing
559 analysis: a full-length form and a truncated form lacking the plant homeodomain-like
560 domain (PHD-L) similar to VAL3, which also lacks the PHD-L domain (Schneider et
561 al., 2016). It is possible that truncated VAL1 and VAL3 target this group of genes,
562 explaining their lack of upregulation in *val1/2* mutants. Alternatively, since the B3
563 superfamily encompasses other subfamilies, such as the AUXIN RESPONSE
564 FACTORS (ARF), the RELATED ABI3/VP1 (RAV) and the REPRODUCTIVE
565 MERISTEM (REM) subfamilies (Swaminathan et al., 2008), some uncharacterized
566 members of these might bind the *LEC2/ABI3/VP1* element or a variation of it. In any
567 case, the promoters of the VAL1/2-independent genes are also enriched in other cis-
568 regulatory elements such as ALFIN1 motifs that are recognized in Arabidopsis by the
569 ALs. Since the AL proteins interact with AtBMI1 proteins (Molitor et al., 2014), it is
570 likely that a combination of B3 and AL factors participates in the regulation of a subset
571 of these genes.

572 The relationship between AtBMI1 and EMF1 has been controversial. On one side,
573 mutants in both display a very different phenotype and misexpress different subsets of

PRC2 targets (Kim et al., 2010; Pu et al., 2013; Yang et al., 2013), which has led to propose the existence of PRC1 variants (Calonje, 2014; Merini and Calonje, 2015); however, they also co-regulated a subset of targets (e.g. *ABI3*, *ABI4*, *FLC*) and *in vitro* they interact. Recent reports have shown that EMF1 co-purifies with PRC2 components (Liang et al., 2015), questioning its exclusive association with PRC1. However, EMF1 co-localizes with only 45% of H3K27me3 marked genes showing a more narrow distribution at target genes than H3K27me3 marks (Kim et al., 2012). Another putative PRC1 component, LHP1, which broadly distributes across H3K27me3 marked sites (Turck et al., 2007; Zhang et al., 2007; Engelhorn et al., 2012), also co-purifies with PRC2 (Derkacheva et al., 2013; Liang et al., 2015) and interacts with AtBMI1 and AtRING1 proteins *in vitro* (Xu and Shen, 2008; Bratzel et al., 2010). However, neither EMF1 nor LHP1 seem to be PRC2 core components since they are required for H3K27me3 marking of only a subset of PRC2 targets (Kim et al., 2012; Wang et al., 2016).

Interestingly, when we compared the H3K27me3 marked genes that were upregulated in *atbmi1a/b/c* with K27_EMF1 direct targets, we found that 50% of the upregulated genes in *atbmi1* mutants were also EMF1 targets, suggesting that AtBMI1 and EMF1 could be in a complex and potentially both impact the expression of these genes. Since LHP1 is at 93.9% of genes up_K27 in *atbmi1a/b/c* mutants, the same holds true also for this PRC1 component. However, the little overlap between the genes upregulated in *atbmi1a/b/c* and *emf1-2* or *lhp1* suggests a decisive role of AtBMI1 function in maintaining their repression. There were also genes exclusively upregulated in *emf1-2* or *lhp1*, the majority of which are involved in flower development and these genes were not upregulated in *atbmi1a/b/c* mutants. An interesting possibility could be that a PcG mechanism dependent on EMF1, LHP1 and PRC2 activities has evolved to specifically regulate the flower developmental program, which is consistent with the finding of these proteins co-purifying with PRC2 (Liang et al., 2015).

Conclusions

In summary, our data point to different PRC1 functional networks in which genes may be regulated by AtBMI1 and/or EMF1 together with LHP1 and PRC2, and that additional proteins are required to regulate distinct subsets of genes. This is the case of VAL1/2 proteins in the seed development program, which built a network that

apparently also includes *ABRE/GT-box* binding factors (Fig. 7C). Furthermore, it seems highly likely that other B3 domain transcription factors and ALs are part of AtBMI1-repressive circuits. In contrast, there seems little or no overlap in gene regulation by AtBMI1 on the one side and EMF1 and LHP1 on the other, although these factors may physically interact and be simultaneously present at target genes.

Materials and Methods

Plant material and growth conditions

Arabidopsis *atbmi1a* (N645041 line), *atbmi1b* (CS855837 line) *atbmi1a/b* and *atbmi1a/b/c (atbmi1c* is a GT21221.Ds5.09.01.2006.jz07.348 line) mutants were described previously (Bratzel et al., 2010; Yang et al., 2013). Segregation of "weak" and "strong" *atbmi1a/b* phenotypes has been previously shown (Bratzel et al., 2010; Pico et al., 2015). Plants were grown under long-day conditions at 21 °C on MS agar plates containing 1.5% sucrose and 0.8% agar. Seedling samples were collected at zeitgeber time 2.

Transcriptomic Analysis by RNA sequencing

The experimental design in our study consisted of two replicates for each genotype (WT Col-0, *atbmi1a*, *atbmi1b*, *atbmi1a/b* and *atbmi1a/b/c*). RNA extraction was performed using Qiagen-RNAesy mini-kit, following the manufacturer's instruction. RNA concentration and purity was tested using nanodrop-photometric quantification (Thermo Scientific). Library preparation was carried out following the manufacturer's recommendations (TruSeq RNA Sample Prep Kit v2, Illumina). Sequencing of RNA libraries was performed with the Illumina HiSeq 2000 sequencer, yielding an average of approximately 15 million 100 bp long paired-end reads for each sample. The software package FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used for quality control. All sequencing samples were of high quality, and no preprocessing of the reads was required to remove low-quality reads or read fragments (Supplemental Fig. S2). The *Arabidopsis thaliana* Col-0 reference genome and annotation were downloaded from the Phytozome database (TAIR10) (Goodstein et al., 2012). Mapping of reads to the reference genome, transcript assembly, and differential expression were performed with the software tools Bowtie, TopHat, and Cufflinks (Trapnell et al., 2012) using default parameters producing a high percentage of concordant pair aligmnet rate

(Supplemental Table S1). The R package from Bioconductor CummeRbund (http://www.bioconductor.org/) was used for subsequent analysis and graphical representation of the results. Differentially expressed genes were selected as those exhibiting an expression fold change greater than four when compared with the WT and a p-value < 0.05. Venn diagrams comparing the different sets of differentially expressed genes were generated with Venny 2.0.2 (http://bioinfogp.cnb.csic.es/tools/venny/index.html) and the significance of their intersections with H3K27me3 marked genes was performed using Fisher's exact test. Gene ontology term enrichment was performed over the sets of differentially expressed genes with the web-based tools AgriGO and ReViGO (Supek et al., 2011; Yu et al., 2012) and the R bioconductor package ClusterProfiler (Du et al., 2010) using Singular Enrichment Analysis.

The clustering analysis was performed using the hierarchical algorithm implemented in the R package cluster over normalized expression levels measured using FPKM.

Quantitative Real Time-PCR (qRT-PCR)

For qRT-PCR analysis, cDNAs were reverse-transcribed from total RNAs with QuantiTect reverse transcription kit (Qiagen). qRT-PCRs were performed using Sensi FAST SYBR & Fluorescein kit (Bioline) and an iQ5 Biorad system. Expression was calculated relative to *ACTIN*. Primers used were as follow:
WOX9-RT-Fw (5´ACTGTCGGAGGGTTTGAAGGTATC 3´); WOX9-RT-Rev (5´AGTGGTAGCGTAACAAATCTGAGTCT 3´);
WOX2-RT-Fw (5´GCTTACTTCAATCGCCTCCTCCACAA 3´); WOX2-RT-Rev (5´GTCCGTTTCTCGTAGCCACCACTTG 3´);
SMB-RT-Fw (5´ACGAATATCGCTTGGACGATAG 3´); SMB-RT-Rev (5´GCTCTTGTTCTTGGTGAAATCC 3´);
ACT2-RT-Fw (5´CACTTGCACCAAGCAGCATGAAGA 3´); ACT2-RT-Rev (5´ AATGGAACCACCGATCCAGACACT 3´).

Motif and Transcription factor binding site enrichment analysis

Transcription Factor Binding Sites (TFBS) enrichment analysis was performed using HOMER (Heinz et al. 2010) and the known TFBS sequences in plants from the databases AGRIS (Davuluri et al., 2003), JASPAR (Sandelin et al., 2004) and AthaMap (Steffens et al., 2004). The findMotifs.pl script was used with default parameters to

perform known and de-novo motif over-representation analysis for DNA sequences of 6, 7, 8 and 9 bp lengths. The target set consisted of all the gene promoters of interest. The background used for the over-representation analysis consisted of all the gene promoters annotated in the Arabidopsis TAIR10 genome. For the co-occurrence of the *ABRE* and *GT-box* motifs, we first identify the locations of the *ABRE* motif at the promoters and then extracted the DNA sequences 100bp upstream and downstream from the center of the ABRE motif. We performed an enrichment analysis of the *GT-box* motif in these DNA sequences using the findMotifsGenome.pl HOMER script with default parameters. The significance of the overlapping between motifs was performed as an enrichment analysis of the DNA sequence resulting from the combination of both motifs. DNA sequences used in these analyses were downloaded using the BioMart functionality associated with Phytozome (Goodstein et al., 2012). Gene promoters were defined as the 1000 bp DNA sequence upstream of the start codon of the corresponding gene.

Data availability

The RNA-seq raw data generated in this study are publicly available from the GEO database identified with accession number GSE83568 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?&acc=GSE83568).

Supplemental materials

Figure S1. Phenotypes of *atbmi1a/b* and *atbmi1a/b/c* mutants.

Figure S2. Boxplots representing the read quality scores (Illumina 1.5 encoding) per base for the first replicate of all samples.

Figure S3. Correlation among differentially expressed genes in WT and the different genotypes.

Figure S4. Altered gene expression in *atbmi1* mutants.

Figure S5. Gene ontology (GO) enrichment analysis of up- and downregulated genes in *atbmi1a/b* mutants.

Figure S6. Gene ontology (GO) enrichment analysis of up- and downregulated genes in *atbmi1a/b/c* mutants.

Figure S7. Putative AtBMI1direct target genes.

Figure S8. Genes differentially expressed in *atbmi1a/b* and *atbmi1a/b/c*.

Figure S9. Different gene expression patterns of *atbmi1a/b* weak and *atbmi1a/b/c* mutants.

Figure S10. Expression levels of different important developmental genes in WT and *atbmi1a/b/c* mutants.

Figure S11. AtBMI1, EMF1 and LHP1 functional relationship.

Figure S12. *AtBMI1B* (*At1g06770*) splice variants.

Table S1. Number of reads and concurrent pair alignment rate per sequencing sample

Table S2. Up- and downregulated genes in *atbmi1* mutants.

Table S3. Upregulated genes in *atbmi1* and *val1/2* mutants that are marked with H3K27me3 marks in WT, and genes in cluster I, II and III after comparing genes up_K27 in *atbmi1a/b* and *atbmi1a/b/c*.

Table S4. Upregulated genes in *emf1-2* and *lhp1* mutants that are marked with H3K27me3 marks in WT.

Figure legends

Figure 1. Transcriptome analysis of WT and selected *atbmi1* mutants at 10 DAG. (A) Volcano plots representing differentially expressed genes in *atbmi1* mutants compared to WT according to a 4-fold change and a p-value of 0.05. Green color indicates significantly upregulated genes and red color significantly downregulated genes. **(B)** Principal Component Analysis of the transcriptomes showing that WT, *atbmi1a* and *atbmi1b* cluster together, whereas *atbmi1a/b* and *atbmi1a/b/c* constitute two distinct clusters. **(C)** Differentially expressed genes in the different genotypes, where the number of up and down regulated genes is indicated. **(D)** Number of genes that were upregulated in the different mutants and H3K27me3 marked in WT seedlings of the same age (up_K27).

Figure 2. Genes regulated by AtBMI1A and AtBMI1B. (A) Venn diagram showing the number of up_K27 genes that overlap among *atbmi1a*, *atbmi1b* and *atbmi1a/b* mutants. All overlaps are significant with p-values lower than 2.2×10^{-16} and odds ratios greater than 17 according to Fisher's Exact test **(B)** Expression of levels of genes that were apparently specifically upregulated in *atbmi1a* or *atbmi1b* mutants in the different genotypes.

Figure 3. Functional redundancy between AtBMI1A/B and AtBMI1C. (A) Clustering analysis of genes up_K27 in *atbmi1a/b* and *atbmi1a/b/c* mutants. This is a significant overlap with a p-value lower than $2.2x10^{-16}$ and an odds ratio greater than 21 according to Fisher's Exact test. **(B)** Expression levels in WT, *atbmi1a/b* and *atbmi1a/b/c* of genes from the different clusters. The color code represents normalized expression values measured in FPKM.

Figure 4. Different gene expression patterns of *atbmi1a/b* weak and *strong* mutants. (A) Venn diagram showing overlap between the genes up_K27 in *atbmi1a/b* weak and strong mutants. The overlap is significant with a p-value lower than $2.2x10^{-16}$ and an odds ratio greater than 15 according to Fisher's Exact test. Some representative transcription factors (TFs) in each dataset are indicated. TFs found in the two data sets are highlighter in red. **(B)** Key flowering genes are downregulated in *atbmi1a/b/c* mutants. The color code in upper panel represents normalized expression values measured in FPKM.

Figure 5. Interplay of AtBMI1 proteins with VAL1/2 proteins. (A) Venn diagram showing overlap between the genes up_K27 in *atbmi1a/b/c* and *val1/2* mutants. Sequence LOGOs of cis-regulatory elements enriched only in up_K27 *atbmi1a/b/c* and in *atbmi1a/b/c* and *val1/2* overlapping genes. **(B)** Co-occurrence and overlapping of *ABRE/G-bo*x and *GT-box* at the promoter of AtBMI1/VAL1/2 co-regulated genes. P-values and percentage in targets and background are indicated.

Figure 6. AtBMI1, EMF1 and LHP1 regulatory networks. (A) Comparison of genes H3K27me3 marked bound by EMF1 and misexpressed in *atbmi1a/b/c* and with genes up_K27 in *atbmi1a/b/c*. **(B)** Venn diagram showing up_K27 genes in *atbmi1a/b/c* and *emf1-2*. **(C)** Comparison of genes H3K27me3 marked bound by LHP1 and misexpressed in *atbmi1a/b/c* and with genes up_K27 in *atbmi1a/b/c*. **(D)** Venn diagram showing up_K27 genes in *atbmi1a/b/c* and *lhp1*. Some overlapping and non-overlapping representative genes are indicated. All these overlaps are significant (p-values and Fisher's Exact test results are indicated).

Figure 7. Role of AtBMI1 proteins in regulating plant development. (A) AtBMI1 proteins and PRC2 promote developmental phase transitions by the repression of key regulatory genes. **(B)** AtBMI1 and PRC2 are required to control cell proliferation and differentiation during organ growth and development through the repression of master

regulators. **(C)** PRC1 variants differing in component composition and biochemical properties may collaborate with PRC2 activity in regulating phase transitions and different developmental processes throughout plant development. VAL and ASIL1/2 or AL1-7 proteins may recruit AtBMI1-containing complexes to target gene promoters by binding the appropriate combination of cis-regulatory elements.

References

Beh LY, Colwell LJ, Francis NJ (2012) A core subunit of Polycomb repressive complex 1 is broadly conserved in function but not primary sequence. Proc Natl Acad Sci U S A **109**: E1063-1071

Bouyer D, Roudier F, Heese M, Andersen ED, Gey D, Nowack MK, Goodrich J, Renou J-P, Grini PE, Colot V, et al (2011) Polycomb repressive complex 2 controls the embryo-to-seedling phase transition. PLoS Genet **7**: e1002014

Bratzel F, López-Torrejón G, Koch M, Del Pozo JC, Calonje M (2010) Keeping cell identity in Arabidopsis requires PRC1 RING-finger homologs that catalyze H2A monoubiquitination. Curr Biol CB **20**: 1853–1859

Bratzel F, Yang C, Angelova A, López-Torrejón G, Koch M, del Pozo JC, Calonje M (2012) Regulation of the new Arabidopsis imprinted gene AtBMI1C requires the interplay of different epigenetic mechanisms. Mol Plant **5**: 260–269

Calonje M (2014) PRC1 marks the difference in plant PcG repression. Mol Plant **7**: 459–471

Calonje M, Sanchez R, Chen L, Sung ZR (2008) EMBRYONIC FLOWER1 participates in polycomb group-mediated AG gene silencing in Arabidopsis. Plant Cell **20**: 277–291

Cao R, Tsukada Y-I, Zhang Y (2005) Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene silencing. Mol Cell **20**: 845–854

Carles C, Bies-Etheve N, Aspart L, Léon-Kloosterziel KM, Koornneef M, Echeverria M, Delseny M (2002) Regulation of Arabidopsis thaliana Em genes: role of ABI5. Plant J Cell Mol Biol **30**: 373–383

Chanvivattana Y, Bishopp A, Schubert D, Stock C, Moon Y-H, Sung ZR, Goodrich J (2004) Interaction of Polycomb-group proteins controlling flowering in Arabidopsis. Dev Camb Engl **131**: 5263–5276

Chen D, Molitor A, Liu C, Shen W-H (2010) The Arabidopsis PRC1-like ring-finger proteins are necessary for repression of embryonic traits during vegetative growth. Cell Res **20**: 1332–1344

Choi H, Hong J, Ha J, Kang J, Kim SY (2000) ABFs, a family of ABA-responsive element binding factors. J Biol Chem **275**: 1723–1730

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol **61**: 651–679

Davuluri RV, Sun H, Palaniswamy SK, Matthews N, Molina C, Kurtz M, Grotewold E (2003) AGRIS: Arabidopsis gene regulatory information server, an information resource of Arabidopsis cis-regulatory elements and transcription factors. BMC Bioinformatics **4**: 25

Delmas F, Sankaranarayanan S, Deb S, Widdup E, Bournonville C, Bollier N, Northey JGB, McCourt P, Samuel MA (2013) ABI3 controls embryo degreening through Mendel's I locus. Proc Natl Acad Sci U S A **110**: E3888-3894

Derkacheva M, Steinbach Y, Wildhaber T, Mozgová I, Mahrez W, Nanni P, Bischof S, Gruissem W, Hennig L (2013) Arabidopsis MSI1 connects LHP1 to PRC2 complexes. EMBO J **32**: 2073–2085

Du Z, Zhou X, Ling Y, Zhang Z, Su Z (2010) agriGO: a GO analysis toolkit for the agricultural community. Nucleic Acids Res **38**: W64-70

Engelhorn J, Reimer JJ, Leuz I, Göbel U, Huettel B, Farrona S, Turck F (2012) Development-related PcG target in the apex 4 controls leaf margin architecture in Arabidopsis thaliana. Dev Camb Engl **139**: 2566–2575

Farrona S, Thorpe FL, Engelhorn J, Adrian J, Dong X, Sarid-Krebs L, Goodrich J, Turck F (2011) Tissue-specific expression of FLOWERING LOCUS T in Arabidopsis is maintained independently of polycomb group protein repression. Plant Cell **23**: 3204–3214

Fernandez DE, Wang C-T, Zheng Y, Adamczyk BJ, Singhal R, Hall PK, Perry SE (2014) The MADS-Domain Factors AGAMOUS-LIKE15 and AGAMOUS-LIKE18, along with SHORT VEGETATIVE PHASE and AGAMOUS-LIKE24, Are Necessary to Block Floral Gene Expression during the Vegetative Phase. Plant Physiol **165**: 1591–1603

Förderer A, Zhou Y, Turck F (2016) The age of multiplexity: recruitment and interactions of Polycomb complexes in plants. Curr Opin Plant Biol **29**: 169–178

Francis NJ, Kingston RE, Woodcock CL (2004) Chromatin compaction by a polycomb group protein complex. Science **306**: 1574–1577

Gangappa SN, Srivastava AK, Maurya JP, Ram H, Chattopadhyay S (2013) Z-box binding transcription factors (ZBFs): a new class of transcription factors in Arabidopsis seedling development. Mol Plant **6**: 1758–1768

Gao M-J, Lydiate DJ, Li X, Lui H, Gjetvaj B, Hegedus DD, Rozwadowski K (2009) Repression of Seed Maturation Genes by a Trihelix Transcriptional Repressor in Arabidopsis Seedlings. Plant Cell **21**: 54–71

Gaudin V, Libault M, Pouteau S, Juul T, Zhao G, Lefebvre D, Grandjean O (2001) Mutations in LIKE HETEROCHROMATIN PROTEIN 1 affect flowering time and plant architecture in Arabidopsis. Dev Camb Engl **128**: 4847–4858

Gendall AR, Levy YY, Wilson A, Dean C (2001) The VERNALIZATION 2 gene mediates the epigenetic regulation of vernalization in Arabidopsis. Cell **107**: 525–535

Goodrich J, Puangsomlee P, Martin M, Long D, Meyerowitz EM, Coupland G (1997) A Polycomb-group gene regulates homeotic gene expression in Arabidopsis. Nature **386**: 44–51

Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, et al (2012) Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res **40**: D1178-1186

van der Graaff E, Laux T, Rensing SA (2009) The WUS homeobox-containing (WOX) protein family. Genome Biol **10**: 248

Grossniklaus U, Vielle-Calzada JP, Hoeppner MA, Gagliano WB (1998) Maternal control of embryogenesis by MEDEA, a polycomb group gene in Arabidopsis. Science **280**: 446–450

Gu X, Le C, Wang Y, Li Z, Jiang D, Wang Y, He Y (2013) Arabidopsis FLC clade members form flowering-repressor complexes coordinating responses to endogenous and environmental cues. Nat Commun **4**: 1947

Hennig L, Bouveret R, Gruissem W (2005) MSI1-like proteins: an escort service for chromatin assembly and remodeling complexes. Trends Cell Biol **15**: 295–302

Horstman A, Willemsen V, Boutilier K, Heidstra R (2014) AINTEGUMENTA-LIKE proteins: hubs in a plethora of networks. Trends Plant Sci **19**: 146–157

Kim SY, Lee J, Eshed-Williams L, Zilberman D, Sung ZR (2012) EMF1 and PRC2 cooperate to repress key regulators of Arabidopsis development. PLoS Genet **8**: e1002512

Kim SY, Zhu T, Sung ZR (2010) Epigenetic regulation of gene programs by EMF1 and EMF2 in Arabidopsis. Plant Physiol **152**: 516–528

Lee WY, Lee D, Chung W-I, Kwon CS (2009) Arabidopsis ING and Alfin1-like protein families localize to the nucleus and bind to H3K4me3/2 via plant homeodomain fingers. Plant J Cell Mol Biol **58**: 511–524

Liang SC, Hartwig B, Perera P, Mora-García S, de Leau E, Thornton H, de Alves FL, Rapsilber J, Yang S, James GV, et al (2015) Kicking against the PRCs -

A Domesticated Transposase Antagonises Silencing Mediated by Polycomb Group Proteins and Is an Accessory Component of Polycomb Repressive Complex 2. PLoS Genet **11**: e1005660

Luo M, Bilodeau P, Koltunow A, Dennis ES, Peacock WJ, Chaudhury AM (1999) Genes controlling fertilization-independent seed development in Arabidopsis thaliana. Proc Natl Acad Sci U S A **96**: 296–301

Merini W, Calonje M (2015) PRC1 is taking the lead in PcG repression. Plant J Cell Mol Biol. doi: 10.1111/tpj.12818

Molitor AM, Bu Z, Yu Y, Shen W-H (2014) Arabidopsis AL PHD-PRC1 complexes promote seed germination through H3K4me3-to-H3K27me3 chromatin state switch in repression of seed developmental genes. PLoS Genet **10**: e1004091

Mozgova I, Köhler C, Hennig L (2015) Keeping the gate closed: functions of the polycomb repressive complex PRC2 in development. Plant J Cell Mol Biol **83**: 121–132

Müller J, Hart CM, Francis NJ, Vargas ML, Sengupta A, Wild B, Miller EL, O'Connor MB, Kingston RE, Simon JA (2002) Histone methyltransferase activity of a Drosophila Polycomb group repressor complex. Cell **111**: 197–208

Nakamura S, Lynch TJ, Finkelstein RR (2001) Physical interactions between ABA response loci of Arabidopsis. Plant J Cell Mol Biol **26**: 627–635

Ohad N, Yadegari R, Margossian L, Hannon M, Michaeli D, Harada JJ, Goldberg RB, Fischer RL (1999) Mutations in FIE, a WD polycomb group gene, allow endosperm development without fertilization. Plant Cell **11**: 407–416

Park H-Y, Lee S-Y, Seok H-Y, Kim S-H, Sung ZR, Moon Y-H (2011) EMF1 interacts with EIP1, EIP6 or EIP9 involved in the regulation of flowering time in Arabidopsis. Plant Cell Physiol **52**: 1376–1388

Peterson AJ, Mallin DR, Francis NJ, Ketel CS, Stamm J, Voeller RK, Kingston RE, Simon JA (2004) Requirement for sex comb on midleg protein interactions in Drosophila polycomb group repression. Genetics **167**: 1225–1239

Pico S, Ortiz-Marchena MI, Merini W, Calonje M (2015) Deciphering the role of Polycomb Repressive Complex 1 (PRC1) variants in regulating the acquisition of flowering competence in Arabidopsis. Plant Physiol. doi: 10.1104/pp.15.00073

Pu L, Liu M-S, Kim SY, Chen L-FO, Fletcher JC, Sung ZR (2013) EMBRYONIC FLOWER1 and ULTRAPETALA1 Act Antagonistically on Arabidopsis Development and Stress Response. Plant Physiol **162**: 812–830

Qüesta JI, Song J, Geraldo N, An H, Dean C (2016) Arabidopsis transcriptional repressor VAL1 triggers Polycomb silencing at FLC during vernalization. Science **353**: 485–488

Ringrose L, Paro R (2004) Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. Annu Rev Genet **38**: 413–443

Sanchez-Pulido L, Devos D, Sung ZR, Calonje M (2008) RAWUL: a new ubiquitin-like domain in PRC1 ring finger proteins that unveils putative plant and worm PRC1 orthologs. BMC Genomics **9**: 308

Sandelin A, Alkema W, Engström P, Wasserman WW, Lenhard B (2004) JASPAR: an open-access database for eukaryotic transcription factor binding profiles. Nucleic Acids Res **32**: D91-94

Santos-Mendoza M, Dubreucq B, Baud S, Parcy F, Caboche M, Lepiniec L (2008) Deciphering gene regulatory networks that control seed development and maturation in Arabidopsis. Plant J Cell Mol Biol **54**: 608–620

Schneider A, Aghamirzaie D, Elmarakeby H, Poudel AN, Koo AJ, Heath LS, Grene R, Collakova E (2016) Potential targets of VIVIPAROUS1/ABI3-LIKE1 (VAL1) repression in developing Arabidopsis thaliana embryos. Plant J Cell Mol Biol **85**: 305–319

Schwartz YB, Pirrotta V (2013) A new world of Polycombs: unexpected partnerships and emerging functions. Nat Rev Genet **14**: 853–864

929 **Shao Z, Raible F, Mollaaghababa R, Guyon JR, Wu CT, Bender W, Kingston RE**
930 (1999) Stabilization of chromatin structure by PRC1, a Polycomb complex. Cell
931 **98**: 37–46

932 **Shu K, Liu X, Xie Q, He Z** (2016) Two Faces of One Seed: Hormonal Regulation of
933 Dormancy and Germination. Mol Plant **9**: 34–45

934 **Simon JA, Kingston RE** (2013) Occupying chromatin: Polycomb mechanisms for
935 getting to genomic targets, stopping transcriptional traffic, and staying put. Mol
936 Cell **49**: 808–824

937 **Steffens NO, Galuschka C, Schindler M, Bülow L, Hehl R** (2004) AthaMap: an
938 online resource for in silico transcription factor binding sites in the Arabidopsis
939 thaliana genome. Nucleic Acids Res **32**: D368-372

940 **Supek F, Bošnjak M, Škunca N, Šmuc T** (2011) REVIGO summarizes and visualizes
941 long lists of gene ontology terms. PloS One **6**: e21800

942 **Suzuki M, Ketterling MG, McCarty DR** (2005) Quantitative statistical analysis of
943 cis-regulatory sequences in ABA/VP1- and CBF/DREB1-regulated genes of
944 Arabidopsis. Plant Physiol **139**: 437–447

945 **Suzuki M, Wang HH-Y, McCarty DR** (2007) Repression of the LEAFY
946 COTYLEDON 1/B3 regulatory network in plant embryo development by
947 VP1/ABSCISIC ACID INSENSITIVE 3-LIKE B3 genes. Plant Physiol **143**:
948 902–911

949 **Swaminathan K, Peterson K, Jack T** (2008) The plant B3 superfamily. Trends Plant
950 Sci **13**: 647–655

951 **Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg**
952 **SL, Rinn JL, Pachter L** (2012) Differential gene and transcript expression
953 analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc **7**:
954 562–578

955 **Turck F, Roudier F, Farrona S, Martin-Magniette M-L, Guillaume E, Buisine N,**
956 **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

Veluchamy A, Jégu T, Ariel F, Latrasse D, Mariappan KG, Kim S-K, Crespi M, Hirt H, Bergounioux C, Raynaud C, et al (2016) LHP1 Regulates H3K27me3 Spreading and Shapes the Three-Dimensional Conformation of the Arabidopsis Genome. PloS One **11**: e0158936

Vicente-Carbajosa J, Carbonero P (2005) Seed maturation: developing an intrusive phase to accomplish a quiescent state. Int J Dev Biol **49**: 645–651

Wang H, Liu C, Cheng J, Liu J, Zhang L, He C, Shen W-H, Jin H, Xu L, Zhang Y (2016) Arabidopsis Flower and Embryo Developmental Genes are Repressed in Seedlings by Different Combinations of Polycomb Group Proteins in Association with Distinct Sets of Cis-regulatory Elements. PLoS Genet **12**: e1005771

Wang Y, Gu X, Yuan W, Schmitz RJ, He Y (2014) Photoperiodic control of the floral transition through a distinct polycomb repressive complex. Dev Cell **28**: 727–736

Wei W, Zhang Y-Q, Tao J-J, Chen H-W, Li Q-T, Zhang W-K, Ma B, Lin Q, Zhang J-S, Chen S-Y (2015) The Alfin-like homeodomain finger protein AL5 suppresses multiple negative factors to confer abiotic stress tolerance in Arabidopsis. Plant J Cell Mol Biol **81**: 871–883

Wolff P, Weinhofer I, Seguin J, Roszak P, Beisel C, Donoghue MTA, Spillane C, Nordborg M, Rehmsmeier M, Köhler C (2011) High-resolution analysis of parent-of-origin allelic expression in the Arabidopsis Endosperm. PLoS Genet **7**: e1002126

Wu G, Poethig RS (2006) Temporal regulation of shoot development in Arabidopsis thaliana by miR156 and its target SPL3. Dev Camb Engl **133**: 3539–3547

Xiao J, Wagner D (2015) Polycomb repression in the regulation of growth and development in Arabidopsis. Curr Opin Plant Biol **23**: 15–24

Xu L, Shen W-H (2008) Polycomb silencing of KNOX genes confines shoot stem cell niches in Arabidopsis. Curr Biol CB **18**: 1966–1971

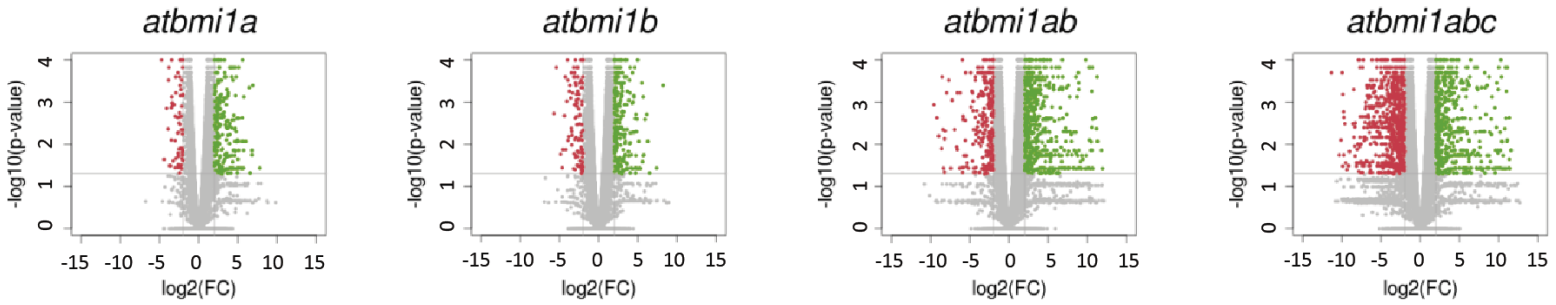
Yang C, Bratzel F, Hohmann N, Koch M, Turck F, Calonje M (2013) VAL- and AtBMI1-mediated H2Aub initiate the switch from embryonic to postgerminative growth in Arabidopsis. Curr Biol CB **23**: 1324–1329

Yoshida N, Yanai Y, Chen L, Kato Y, Hiratsuka J, Miwa T, Sung ZR, Takahashi S (2001) EMBRYONIC FLOWER2, a novel polycomb group protein homolog, mediates shoot development and flowering in Arabidopsis. Plant Cell **13**: 2471–2481

Yu G, Wang L-G, Han Y, He Q-Y (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. Omics J Integr Biol **16**: 284–287

Zhang X, Germann S, Blus BJ, Khorasanizadeh S, Gaudin V, Jacobsen SE (2007) The Arabidopsis LHP1 protein colocalizes with histone H3 Lys27 trimethylation. Nat Struct Mol Biol **14**: 869–871

A



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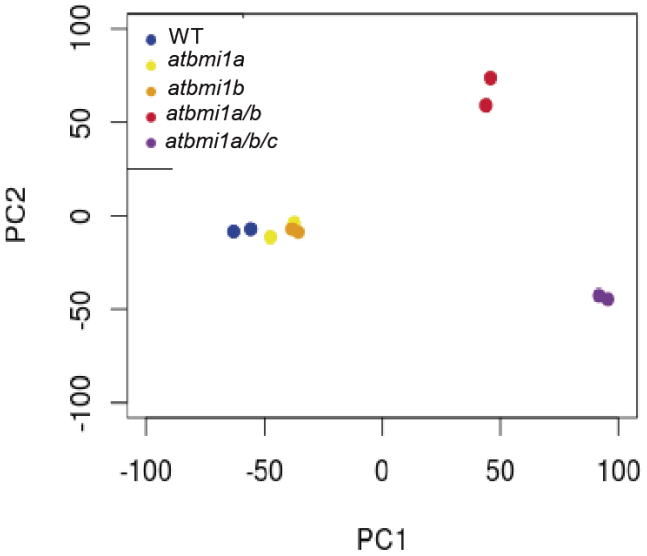
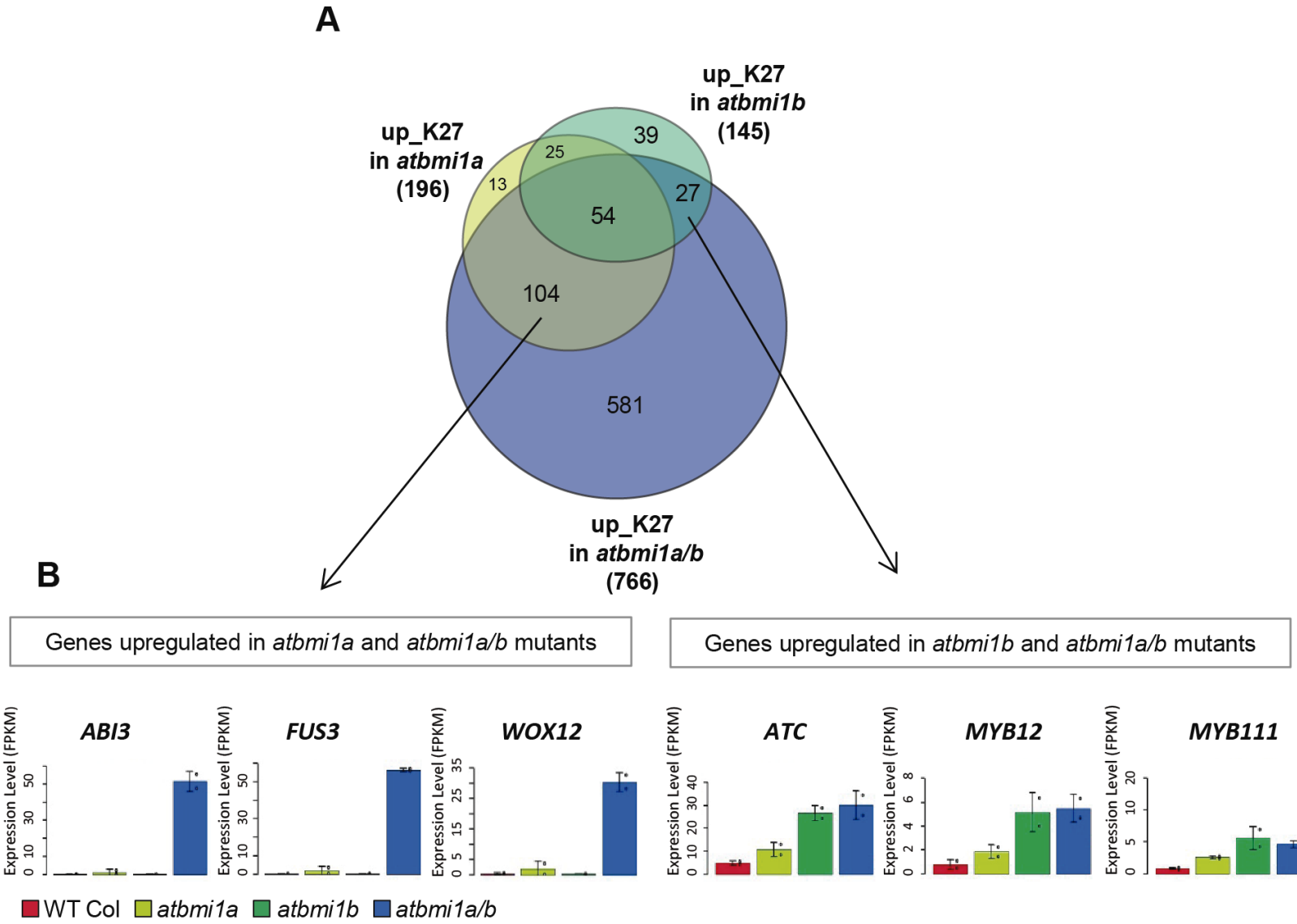


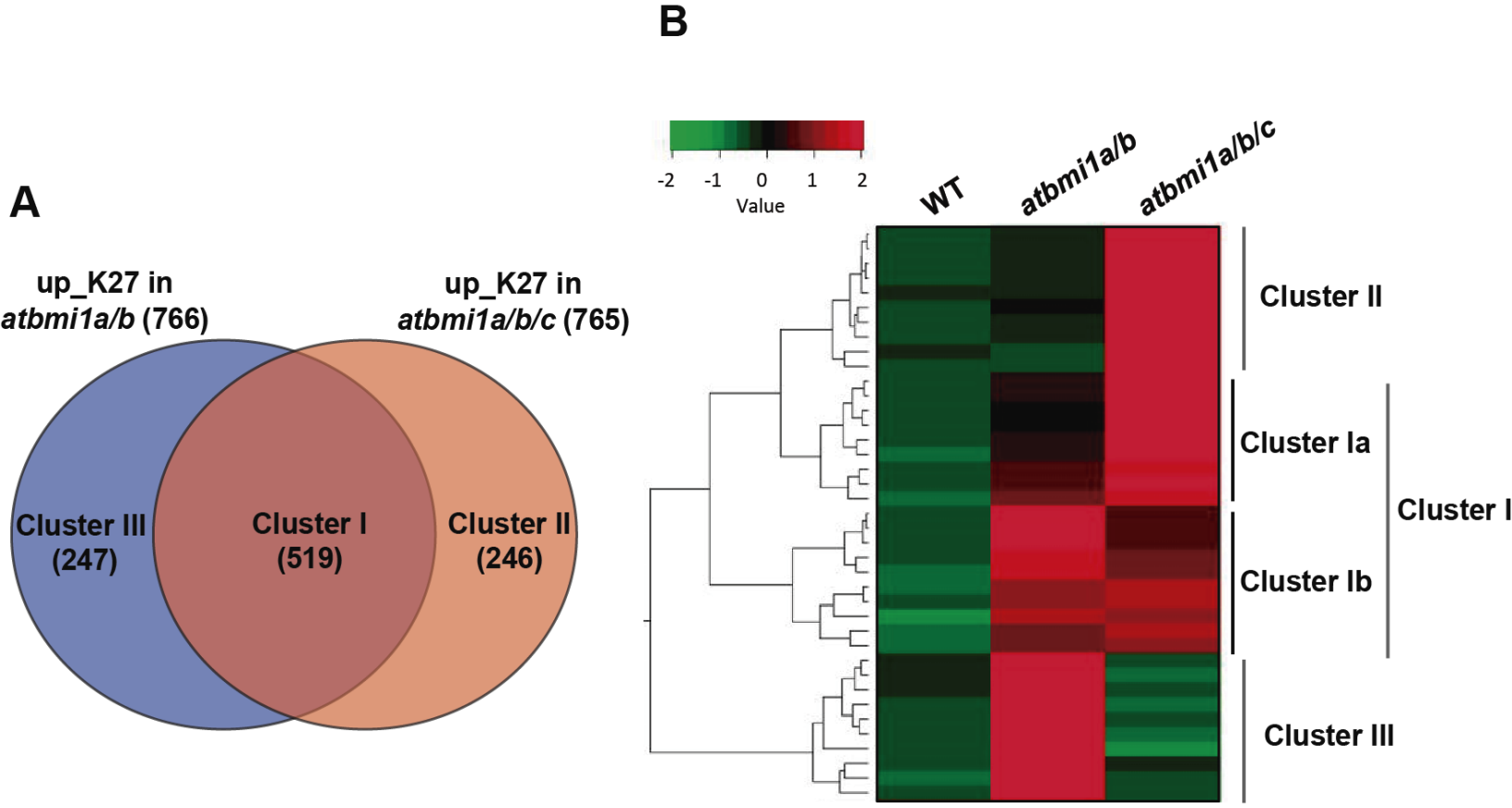
C

Gene expression	*atbml1a*	*atbmi1b*	*atbmi1a/b strong*	*atbmi1a/b/c*
Upregulated genes	594	671	2153	2162
Downregulated genes	197	393	1489	2935

D

Gene expression	*atbml1a*	*atbmi1b*	*atbmi1a/b strong*	*atbmi1a/b/c*
Up_K27 genes	196	145	766	765





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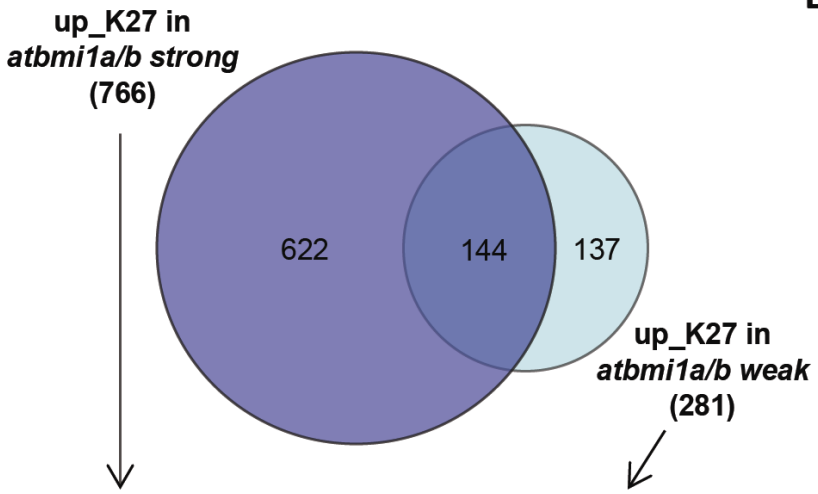
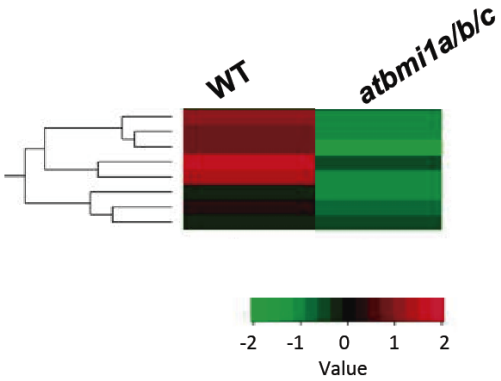


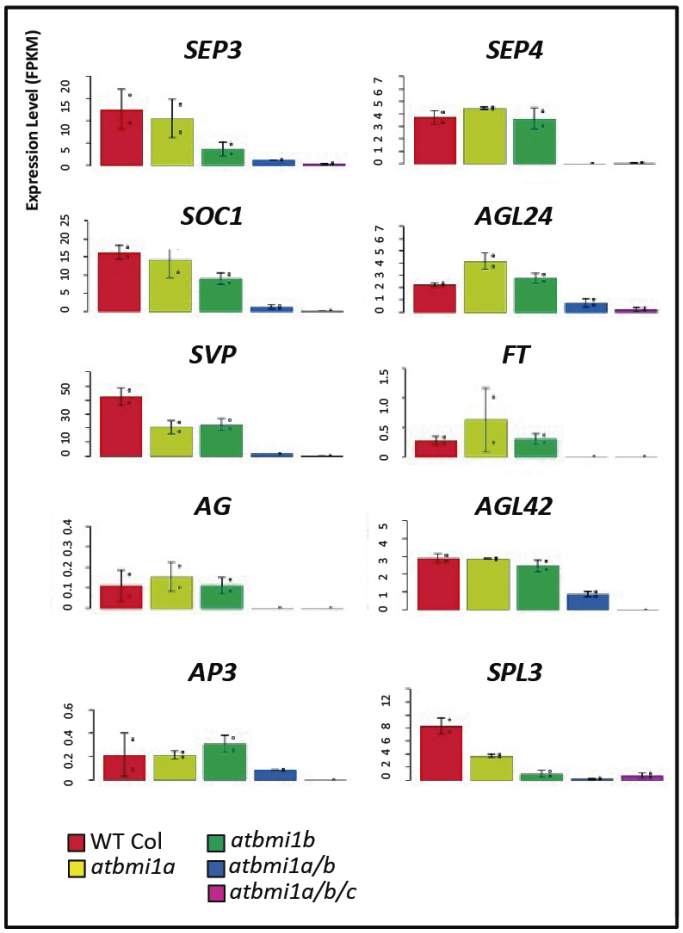
TF Family Name	Representative Gene
ABI3VP1	*ABI3*, *FUS3*
AP2-EREBP	*ESR1*, *ABI4*, *AIL2/BBM*, *AIL5/CHO1*, *AIL6/PLT3*, *AIL7*, *WRI1*
bHLH	*MEE8*, *FT1*, *GL3*
bZIP	*DPBF2*
Homeobox	*WOX4*, *WOX3*, *WOX12*, *WOX5*, *WOX8*
HSF	*HSFA6B*
MADS	*FLC*, *AGL15*, *MAF4*, *MAF5*
MYB	*PAP2*, *MYB47*, *MYB113*, *MYB12*, *MYB78*, *MYB84*, *PFG2*, *MYB41*, *MYB111*
NAC	*CUC3*
WRKY	*WRKY23*, *WRKY29*, *WRKY38*, *WRKY8*, *WRKY48*
ZF-HD	*AtHB28*, *ZFHD1*

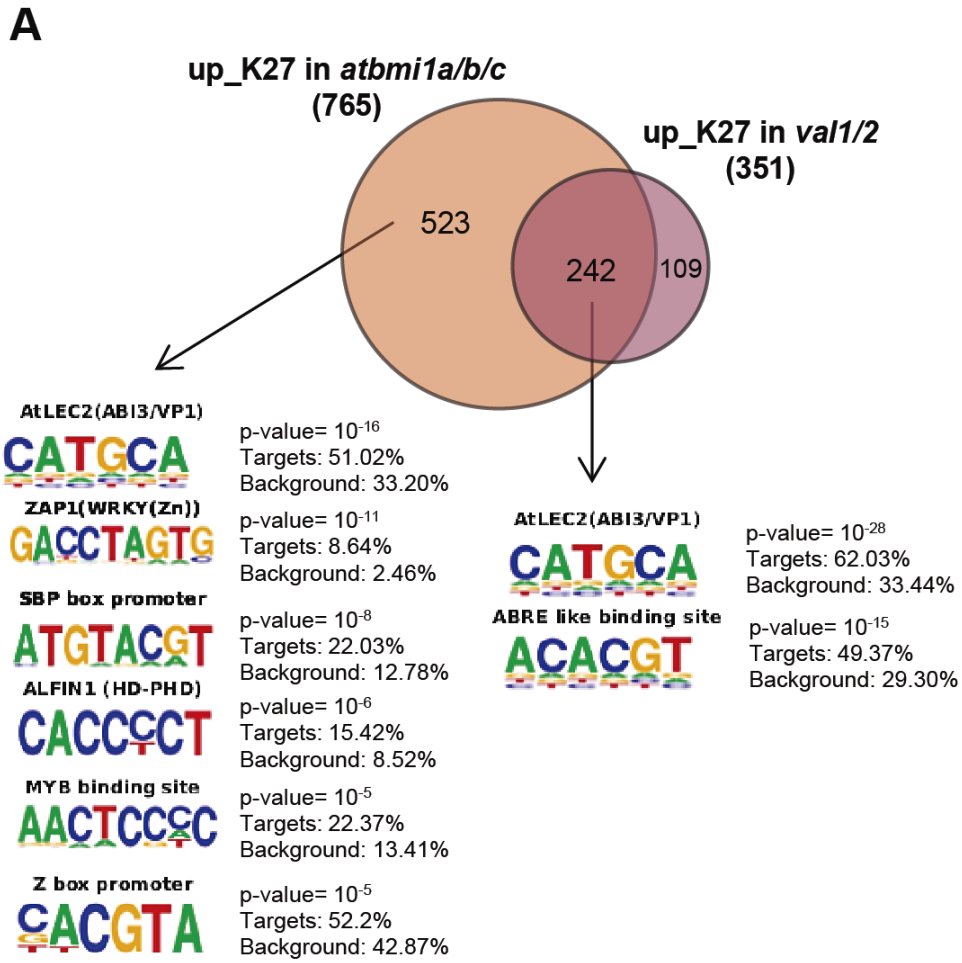
TF Family Name	Representative Gene
ABI3VP1	*ABI3*
AP2-EREBP	*SNZ*, *SHINE2*
C2H2	*SRS4*, *SRS2*
Homeobox	*WOX5*, *HDG4*
MADS	*PHE1*, *AGL15*, *MAF4*
MYB	*MYB47*
NAC	*NARS1*
REM	*REM1*
WRKY	*WRKY28*

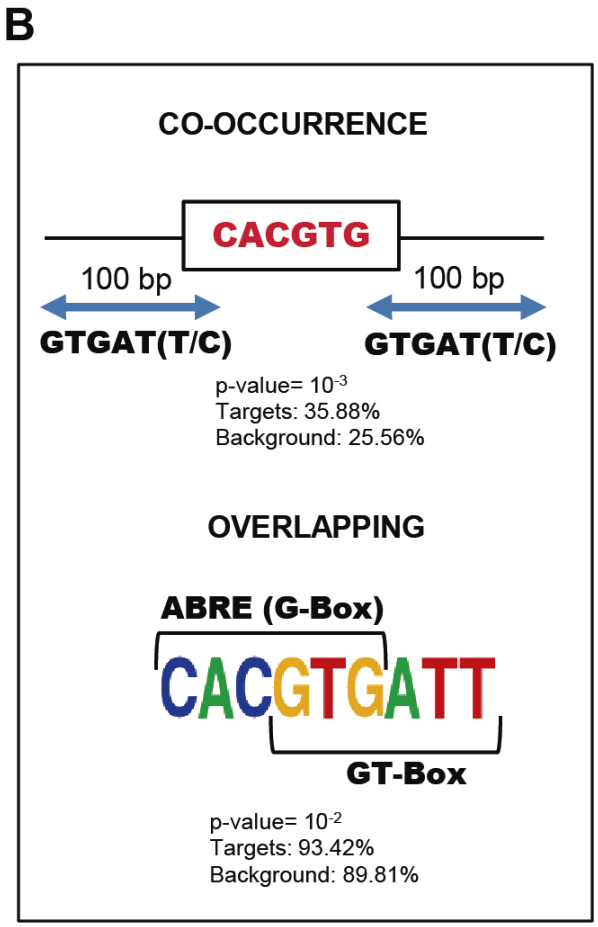
B

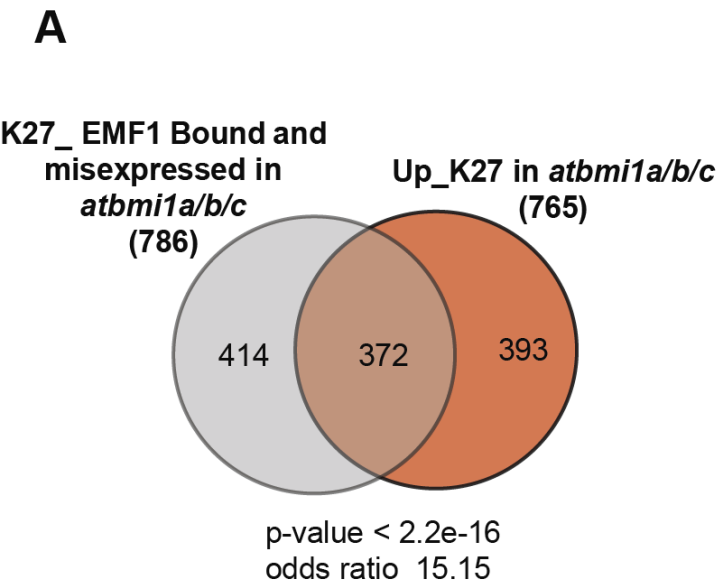
Downregulated genes in *atbmi1a/b/c*

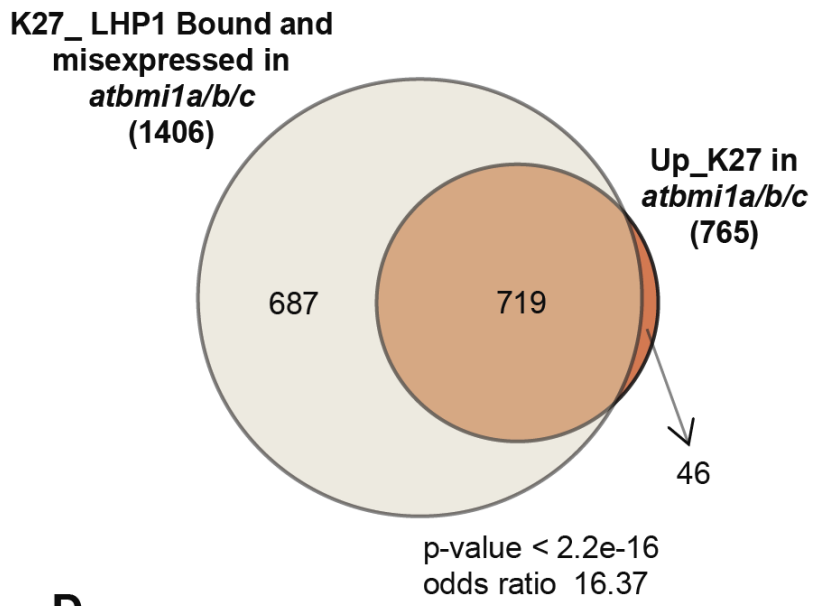


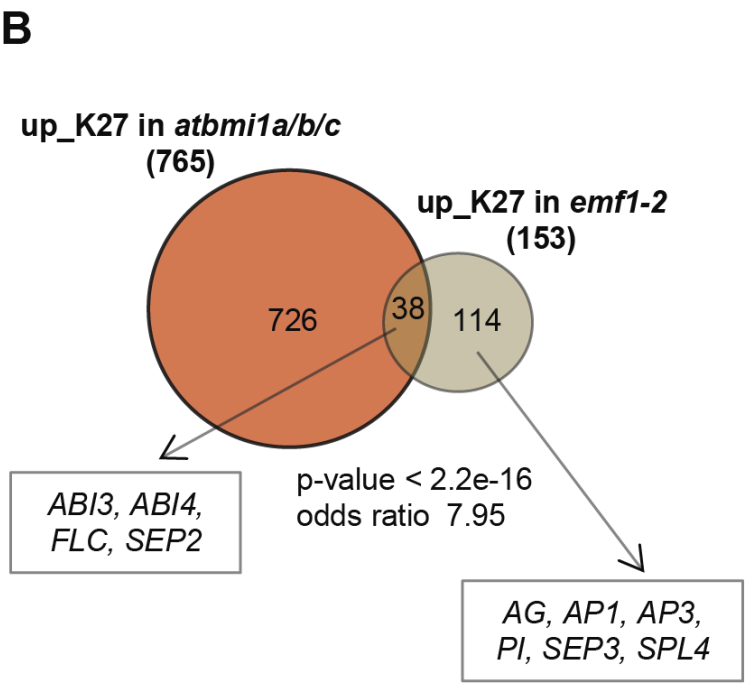


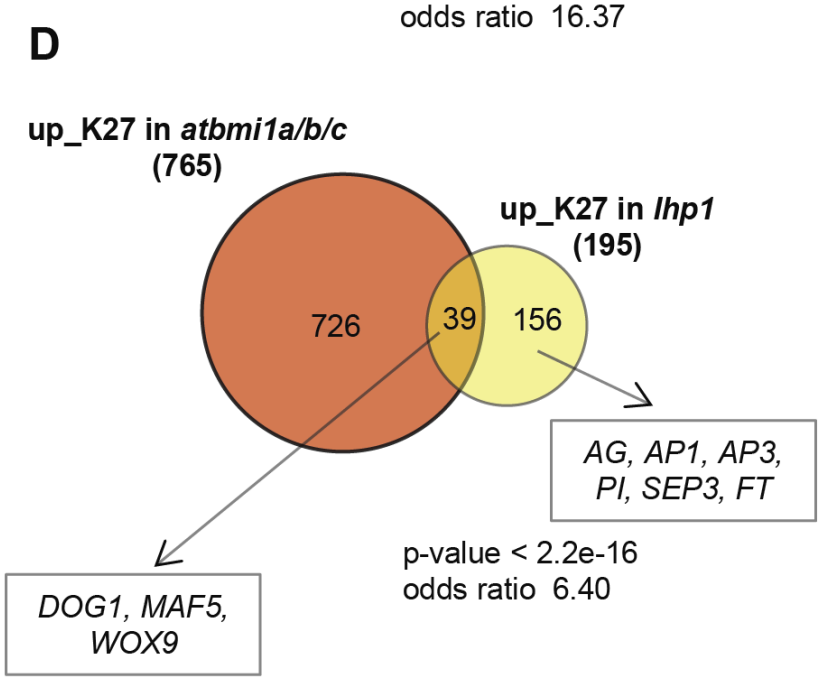


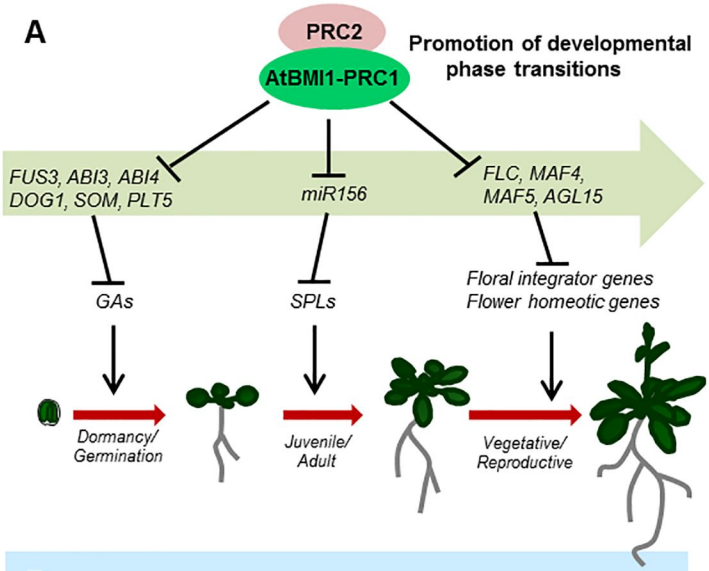


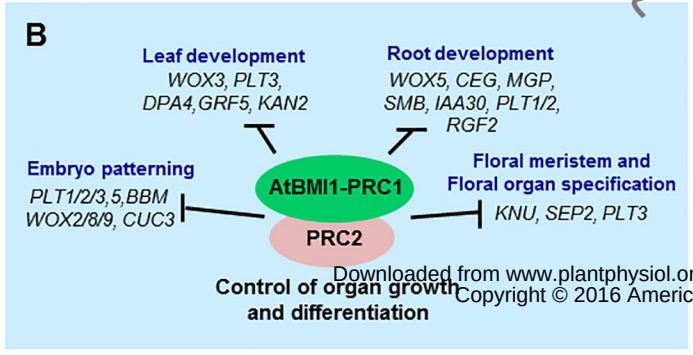


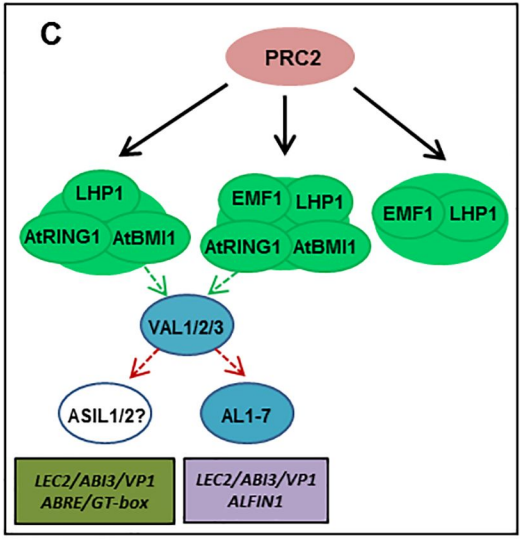












Parsed Citations

Beh LY, Colwell LJ, Francis NJ (2012) A core subunit of Polycomb repressive complex 1 is broadly conserved in function but not primary sequence. Proc Natl Acad Sci U S A 109: E1063-1071
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CrossRef: Author and Title
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Bouyer D, Roudier F, Heese M, Andersen ED, Gey D, Nowack MK, Goodrich J, Renou J-P, Grini PE, Colot V, et al (2011) Polycomb repressive complex 2 controls the embryo-to-seedling phase transition. PLoS Genet 7: e1002014
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CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Bratzel F, López-Torrejón G, Koch M, Del Pozo JC, Calonje M (2010) Keeping cell identity in Arabidopsis requires PRC1 RING-finger homologs that catalyze H2A monoubiquitination. Curr Biol CB 20: 1853-1859
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Google Scholar: Author Only Title Only Author and Title

Bratzel F, Yang C, Angelova A, López-Torrejón G, Koch M, del Pozo JC, Calonje M (2012) Regulation of the new Arabidopsis imprinted gene AtBMI1C requires the interplay of different epigenetic mechanisms. Mol Plant 5: 260-269
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CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

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Google Scholar: Author Only Title Only Author and Title

Calonje M, Sanchez R, Chen L, Sung ZR (2008) EMBRYONIC FLOWER1 participates in polycomb group-mediated AG gene silencing in Arabidopsis. Plant Cell 20: 277-291
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Cao R, Tsukada Y-I, Zhang Y (2005) Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene silencing. Mol Cell 20: 845-854
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Carles C, Bies-Etheve N, Aspart L, Léon-Kloosterziel KM, Koornneef M, Echeverria M, Delseny M (2002) Regulation of Arabidopsis thaliana Em genes: role of ABI5. Plant J Cell Mol Biol 30: 373-383
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chanvivattana Y, Bishopp A, Schubert D, Stock C, Moon Y-H, Sung ZR, Goodrich J (2004) Interaction of Polycomb-group proteins controlling flowering in Arabidopsis. Dev Camb Engl 131: 5263-5276
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CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Chen D, Molitor A, Liu C, Shen W-H (2010) The Arabidopsis PRC1-like ring-finger proteins are necessary for repression of embryonic traits during vegetative growth. Cell Res 20: 1332-1344
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Choi H, Hong J, Ha J, Kang J, Kim SY (2000) ABFs, a family of ABA-responsive element binding factors. J Biol Chem 275: 1723-1730
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol 61: 651-679
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Davuluri RV, Sun H, Palaniswamy SK, Matthews N, Molina C, Kurtz M, Grotewold E (2003) AGRIS: Arabidopsis gene regulatory information server, an information resource of Arabidopsis cis-regulatory elements and transcription factors. BMC Bioinformatics 4: 25
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Delmas F, Sankaranarayanan S, Deb S, Widdup E, Bournonville C, Bollier N, Northey JGB, McCourt P, Samuel MA (2013) ABI3 controls embryo degreening through Mendel's I locus. Proc Natl Acad Sci U S A 110: E3888-3894

Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Derkacheva M, Steinbach Y, Wildhaber T, Mozgová I, Mahrez W, Nanni P, Bischof S, Gruissem W, Hennig L (2013) Arabidopsis MSI1 connects LHP1 to PRC2 complexes. EMBO J 32: 2073-2085
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Du Z, Zhou X, Ling Y, Zhang Z, Su Z (2010) agriGO: a GO analysis toolkit for the agricultural community. Nucleic Acids Res 38: W64-70
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Engelhorn J, Reimer JJ, Leuz I, Göbel U, Huettel B, Farrona S, Turck F (2012) Development-related PcG target in the apex 4 controls leaf margin architecture in Arabidopsis thaliana. Dev Camb Engl 139: 2566-2575
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Farrona S, Thorpe FL, Engelhorn J, Adrian J, Dong X, Sarid-Krebs L, Goodrich J, Turck F (2011) Tissue-specific expression of FLOWERING LOCUS T in Arabidopsis is maintained independently of polycomb group protein repression. Plant Cell 23: 3204-3214
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Fernandez DE, Wang C-T, Zheng Y, Adamczyk BJ, Singhal R, Hall PK, Perry SE (2014) The MADS-Domain Factors AGAMOUS-LIKE15 and AGAMOUS-LIKE18, along with SHORT VEGETATIVE PHASE and AGAMOUS-LIKE24, Are Necessary to Block Floral Gene Expression during the Vegetative Phase. Plant Physiol 165: 1591-1603
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Förderer A, Zhou Y, Turck F (2016) The age of multiplexity: recruitment and interactions of Polycomb complexes in plants. Curr Opin Plant Biol 29: 169-178
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Francis NJ, Kingston RE, Woodcock CL (2004) Chromatin compaction by a polycomb group protein complex. Science 306: 1574-1577
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gangappa SN, Srivastava AK, Maurya JP, Ram H, Chattopadhyay S (2013) Z-box binding transcription factors (ZBFs): a new class of transcription factors in Arabidopsis seedling development. Mol Plant 6: 1758-1768
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gao M-J, Lydiate DJ, Li X, Lui H, Gjetvaj B, Hegedus DD, Rozwadowski K (2009) Repression of Seed Maturation Genes by a Trihelix Transcriptional Repressor in Arabidopsis Seedlings. Plant Cell 21: 54-71
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gaudin V, Libault M, Pouteau S, Juul T, Zhao G, Lefebvre D, Grandjean O (2001) Mutations in LIKE HETEROCHROMATIN PROTEIN 1 affect flowering time and plant architecture in Arabidopsis. Dev Camb Engl 128: 4847-4858
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gendall AR, Levy YY, Wilson A, Dean C (2001) The VERNALIZATION 2 gene mediates the epigenetic regulation of vernalization in Arabidopsis. Cell 107: 525-535
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Goodrich J, Puangsomlee P, Martin M, Long D, Meyerowitz EM, Coupland G (1997) A Polycomb-group gene regulates homeotic gene expression in Arabidopsis. Nature 386: 44-51
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, et al (2012) Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res 40: D1178-1186
Pubmed: Author and Title

CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

van der Graaff E, Laux T, Rensing SA (2009) The WUS homeobox-containing (WOX) protein family. Genome Biol 10: 248
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Grossniklaus U, Vielle-Calzada JP, Hoeppner MA, Gagliano WB (1998) Maternal control of embryogenesis by MEDEA, a polycomb group gene in Arabidopsis. Science 280: 446-450
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Gu X, Le C, Wang Y, Li Z, Jiang D, Wang Y, He Y (2013) Arabidopsis FLC clade members form flowering-repressor complexes coordinating responses to endogenous and environmental cues. Nat Commun 4: 1947
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Hennig L, Bouveret R, Gruissem W (2005) MSI1-like proteins: an escort service for chromatin assembly and remodeling complexes. Trends Cell Biol 15: 295-302
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Horstman A, Willemsen V, Boutilier K, Heidstra R (2014) AINTEGUMENTA-LIKE proteins: hubs in a plethora of networks. Trends Plant Sci 19: 146-157
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kim SY, Lee J, Eshed-Williams L, Zilberman D, Sung ZR (2012) EMF1 and PRC2 cooperate to repress key regulators of Arabidopsis development. PLoS Genet 8: e1002512
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Kim SY, Zhu T, Sung ZR (2010) Epigenetic regulation of gene programs by EMF1 and EMF2 in Arabidopsis. Plant Physiol 152: 516-528
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Lee WY, Lee D, Chung W-I, Kwon CS (2009) Arabidopsis ING and Alfin1-like protein families localize to the nucleus and bind to H3K4me3/2 via plant homeodomain fingers. Plant J Cell Mol Biol 58: 511-524
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Liang SC, Hartwig B, Perera P, Mora-García S, de Leau E, Thornton H, de Alves FL, Rapsilber J, Yang S, James GV, et al (2015) Kicking against the PRCs - A Domesticated Transposase Antagonises Silencing Mediated by Polycomb Group Proteins and Is an Accessory Component of Polycomb Repressive Complex 2. PLoS Genet 11: e1005660
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Luo M, Bilodeau P, Koltunow A, Dennis ES, Peacock WJ, Chaudhury AM (1999) Genes controlling fertilization-independent seed development in Arabidopsis thaliana. Proc Natl Acad Sci U S A 96: 296-301
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Merini W, Calonje M (2015) PRC1 is taking the lead in PcG repression. Plant J Cell Mol Biol. doi: 10.1111/tpj.12818
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Molitor AM, Bu Z, Yu Y, Shen W-H (2014) Arabidopsis AL PHD-PRC1 complexes promote seed germination through H3K4me3-to-H3K27me3 chromatin state switch in repression of seed developmental genes. PLoS Genet 10: e1004091
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Mozgova I, Köhler C, Hennig L (2015) Keeping the gate closed: functions of the polycomb repressive complex PRC2 in development. Plant J Cell Mol Biol 83: 121-132
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Müller J, Hart CM, Francis NJ, Vargas ML, Sengupta A, Wild B, Miller EL, O'Connor MB, Kingston RE, Simon JA (2002) Histone

methyltransferase activity of a Drosophila Polycomb group repressor complex. Cell 111: 197-208
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Nakamura S, Lynch TJ, Finkelstein RR (2001) Physical interactions between ABA response loci of Arabidopsis. Plant J Cell Mol Biol 26: 627-635
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ohad N, Yadegari R, Margossian L, Hannon M, Michaeli D, Harada JJ, Goldberg RB, Fischer RL (1999) Mutations in FIE, a WD polycomb group gene, allow endosperm development without fertilization. Plant Cell 11: 407-416
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Park H-Y, Lee S-Y, Seok H-Y, Kim S-H, Sung ZR, Moon Y-H (2011) EMF1 interacts with EIP1, EIP6 or EIP9 involved in the regulation of flowering time in Arabidopsis. Plant Cell Physiol 52: 1376-1388
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Peterson AJ, Mallin DR, Francis NJ, Ketel CS, Stamm J, Voeller RK, Kingston RE, Simon JA (2004) Requirement for sex comb on midleg protein interactions in Drosophila polycomb group repression. Genetics 167: 1225-1239
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Pico S, Ortiz-Marchena MI, Merini W, Calonje M (2015) Deciphering the role of Polycomb Repressive Complex 1 (PRC1) variants in regulating the acquisition of flowering competence in Arabidopsis. Plant Physiol. doi: 10.1104/pp.15.00073
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Pu L, Liu M-S, Kim SY, Chen L-FO, Fletcher JC, Sung ZR (2013) EMBRYONIC FLOWER1 and ULTRAPETALA1 Act Antagonistically on Arabidopsis Development and Stress Response. Plant Physiol 162: 812-830
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Qüesta JI, Song J, Geraldo N, An H, Dean C (2016) Arabidopsis transcriptional repressor VAL1 triggers Polycomb silencing at FLC during vernalization. Science 353: 485-488
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Ringrose L, Paro R (2004) Epigenetic regulation of cellular memory by the Polycomb and Trithorax group proteins. Annu Rev Genet 38: 413-443
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sanchez-Pulido L, Devos D, Sung ZR, Calonje M (2008) RAWUL: a new ubiquitin-like domain in PRC1 ring finger proteins that unveils putative plant and worm PRC1 orthologs. BMC Genomics 9: 308
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Sandelin A, Alkema W, Engström P, Wasserman WW, Lenhard B (2004) JASPAR: an open-access database for eukaryotic transcription factor binding profiles. Nucleic Acids Res 32: D91-94
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Santos-Mendoza M, Dubreucq B, Baud S, Parcy F, Caboche M, Lepiniec L (2008) Deciphering gene regulatory networks that control seed development and maturation in Arabidopsis. Plant J Cell Mol Biol 54: 608-620
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Schneider A, Aghamirzaie D, Elmarakeby H, Poudel AN, Koo AJ, Heath LS, Grene R, Collakova E (2016) Potential targets of VIVIPAROUS1/ABI3-LIKE1 (VAL1) repression in developing Arabidopsis thaliana embryos. Plant J Cell Mol Biol 85: 305-319
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Schwartz YB, Pirrotta V (2013) A new world of Polycombs: unexpected partnerships and emerging functions. Nat Rev Genet 14: 853-864
Pubmed: Author and Title

CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Shao Z, Raible F, Mollaaghababa R, Guyon JR, Wu CT, Bender W, Kingston RE (1999) Stabilization of chromatin structure by PRC1, a Polycomb complex. Cell 98: 37-46
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Shu K, Liu X, Xie Q, He Z (2016) Two Faces of One Seed: Hormonal Regulation of Dormancy and Germination. Mol Plant 9: 34-45
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Simon JA, Kingston RE (2013) Occupying chromatin: Polycomb mechanisms for getting to genomic targets, stopping transcriptional traffic, and staying put. Mol Cell 49: 808-824
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Steffens NO, Galuschka C, Schindler M, Bülow L, Hehl R (2004) AthaMap: an online resource for in silico transcription factor binding sites in the Arabidopsis thaliana genome. Nucleic Acids Res 32: D368-372
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Supek F, Bošnjak M, Škunca N, Šmuc T (2011) REVIGO summarizes and visualizes long lists of gene ontology terms. PloS One 6: e21800
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Suzuki M, Ketterling MG, McCarty DR (2005) Quantitative statistical analysis of cis-regulatory sequences in ABA/VP1- and CBF/DREB1-regulated genes of Arabidopsis. Plant Physiol 139: 437-447
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Suzuki M, Wang HH-Y, McCarty DR (2007) Repression of the LEAFY COTYLEDON 1/B3 regulatory network in plant embryo development by VP1/ABSCISIC ACID INSENSITIVE 3-LIKE B3 genes. Plant Physiol 143: 902-911
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Swaminathan K, Peterson K, Jack T (2008) The plant B3 superfamily. Trends Plant Sci 13: 647-655
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L (2012) Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc 7: 562-578
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Turck F, Roudier F, Farrona S, Martin-Magniette M-L, 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
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Veluchamy A, Jégu T, Ariel F, Latrasse D, Mariappan KG, Kim S-K, Crespi M, Hirt H, Bergounioux C, Raynaud C, et al (2016) LHP1 Regulates H3K27me3 Spreading and Shapes the Three-Dimensional Conformation of the Arabidopsis Genome. PloS One 11: e0158936
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Vicente-Carbajosa J, Carbonero P (2005) Seed maturation: developing an intrusive phase to accomplish a quiescent state. Int J Dev Biol 49: 645-651
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang H, Liu C, Cheng J, Liu J, Zhang L, He C, Shen W-H, Jin H, Xu L, Zhang Y (2016) Arabidopsis Flower and Embryo Developmental Genes are Repressed in Seedlings by Different Combinations of Polycomb Group Proteins in Association with Distinct Sets of Cis-regulatory Elements. PLoS Genet 12: e1005771
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wang Y, Gu X, Yuan W, Schmitz RJ, He Y (2014) Photoperiodic control of the floral transition through a distinct polycomb repressive complex. Dev Cell 28: 727-736
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wei W, Zhang Y-Q, Tao J-J, Chen H-W, Li Q-T, Zhang W-K, Ma B, Lin Q, Zhang J-S, Chen S-Y (2015) The Alfin-like homeodomain finger protein AL5 suppresses multiple negative factors to confer abiotic stress tolerance in Arabidopsis. Plant J Cell Mol Biol 81: 871-883
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wolff P, Weinhofer I, Seguin J, Roszak P, Beisel C, Donoghue MTA, Spillane C, Nordborg M, Rehmsmeier M, Köhler C (2011) High-resolution analysis of parent-of-origin allelic expression in the Arabidopsis Endosperm. PLoS Genet 7: e1002126
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Wu G, Poethig RS (2006) Temporal regulation of shoot development in Arabidopsis thaliana by miR156 and its target SPL3. Dev Camb Engl 133: 3539-3547
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Xiao J, Wagner D (2015) Polycomb repression in the regulation of growth and development in Arabidopsis. Curr Opin Plant Biol 23: 15-24
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Xu L, Shen W-H (2008) Polycomb silencing of KNOX genes confines shoot stem cell niches in Arabidopsis. Curr Biol CB 18: 1966-1971
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yang C, Bratzel F, Hohmann N, Koch M, Turck F, Calonje M (2013) VAL- and AtBMI1-mediated H2Aub initiate the switch from embryonic to postgerminative growth in Arabidopsis. Curr Biol CB 23: 1324-1329
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yoshida N, Yanai Y, Chen L, Kato Y, Hiratsuka J, Miwa T, Sung ZR, Takahashi S (2001) EMBRYONIC FLOWER2, a novel polycomb group protein homolog, mediates shoot development and flowering in Arabidopsis. Plant Cell 13: 2471-2481
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Yu G, Wang L-G, Han Y, He Q-Y (2012) clusterProfiler: an R package for comparing biological themes among gene clusters. Omics J Integr Biol 16: 284-287
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

Zhang X, Germann S, Blus BJ, Khorasanizadeh S, Gaudin V, Jacobsen SE (2007) The Arabidopsis LHP1 protein colocalizes with histone H3 Lys27 trimethylation. Nat Struct Mol Biol 14: 869-871
Pubmed: Author and Title
CrossRef: Author and Title
Google Scholar: Author Only Title Only Author and Title

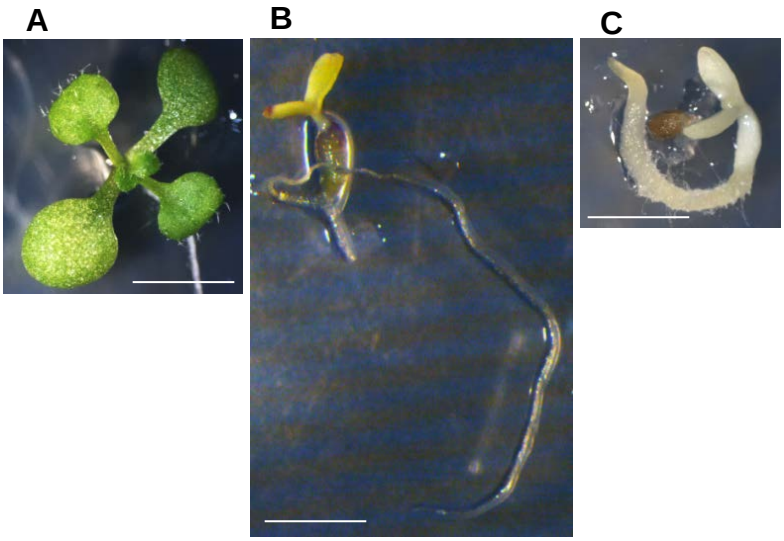


Figure S1. Phenotypes of *atbmi1a/b* and *atbmi1a/b/c* mutants. (A) WT seedling at 10 DAG. **(B)** *atbmi1a/b* mutants at 10 DAG. **(C)** *atbmi1a/b/c* mutants at 10 DAG. Bars, 2 mm.

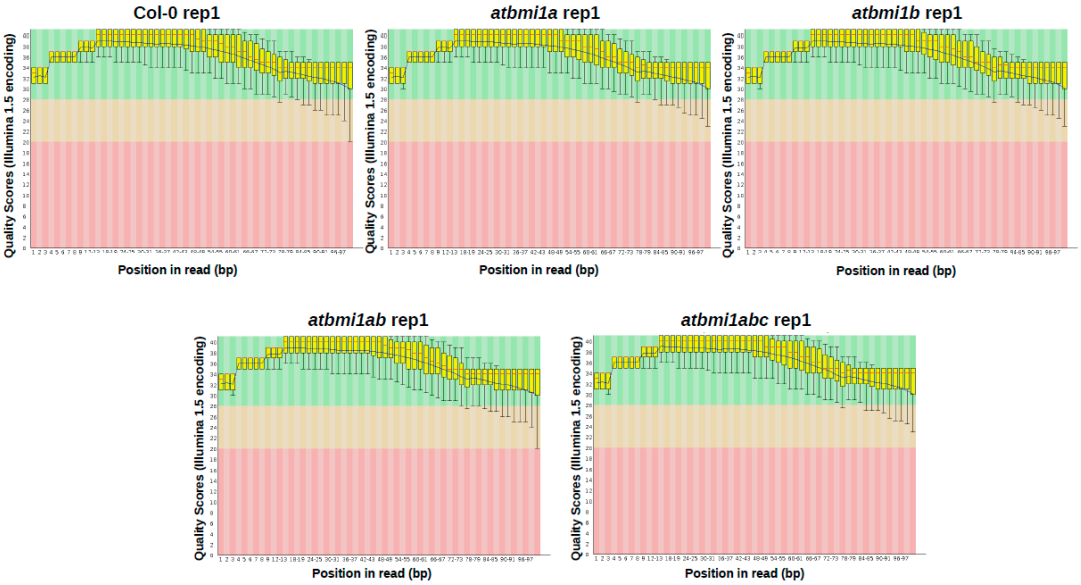


Figure S2. Boxplots representing the read quality scores (Illumina 1.5 encoding) per base for the first replicate of all samples. The quality scores for each base in the reads remained within the green area indicating a high sequencing quality. The common decrease in quality at the end of the reads is observed. Nevertheless, the quality never enters the problematic red area.

SAMPLE	NUMBER OF READS	CONCURRENT PAIR ALIGNMENT RATE
WT Col0 rep1	14578745	96.5%
WT Col0 rep2	16253159	96.6%
atbmi1 a rep1	14982986	94.5%
atbmi1 a rep2	18324516	96.1%
atbmi1 b rep1	15714678	95.8%
atbmi1 b rep2	18412180	95.3%
atbmi1 ab rep1	14885215	94.9%
atbmi1 ab rep2	14547885	95.1%
atbmi1 abc rep1	14832480	94.5%
atbmi1 abc rep2	12349849	94.9%

Table S1. Number of reads and concurrent pair alignment rate per sequencing sample. On average the number of reads per sample is approximately 15 million and the average concurrent pair alignment rate is greater than 95.%. This indicates a high read sequencing quality and the lack of sample contamination.

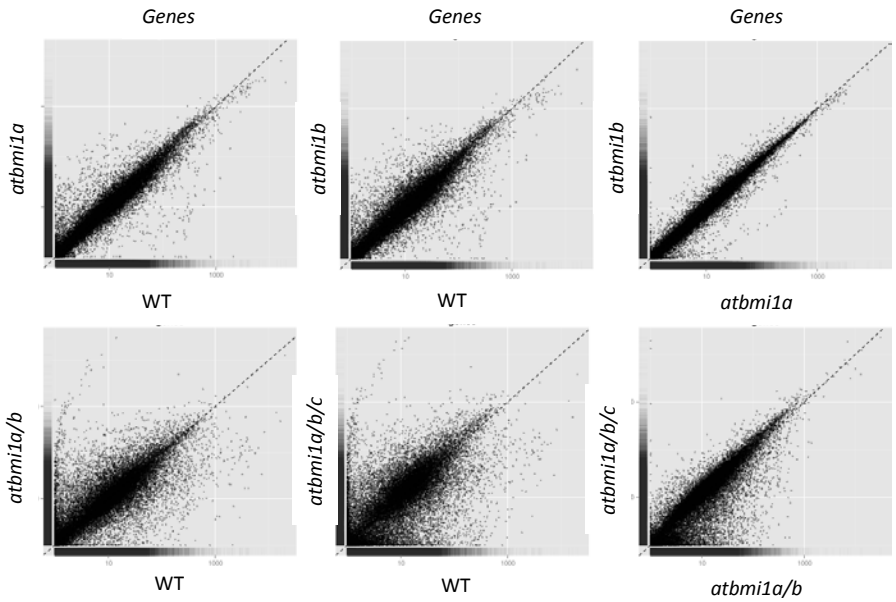


Figure S3. Correlation among differentially expressed genes in WT and the different genotypes. Scatter plots comparing gene expression levels in the different mutants against WT, single *atbmi1a* against *atbmi1b*, and *atbmi1a/b* against *atbmi1a/b/c*.

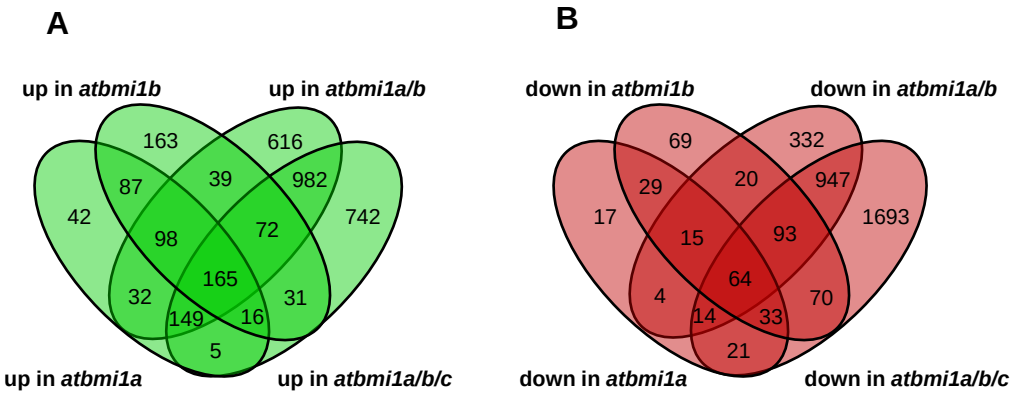
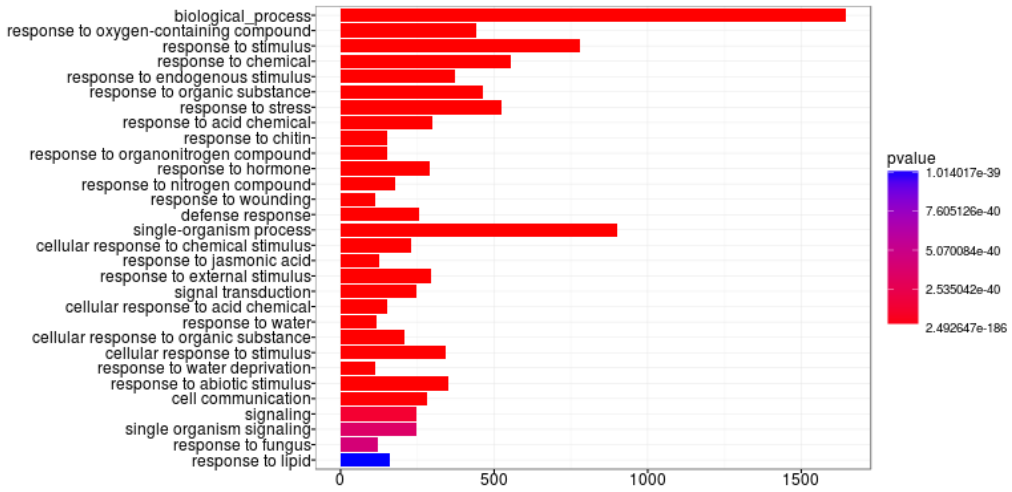


Figure S4. Altered gene expression in *atbmi1* mutants. (A) Venn diagram showing the number of genes up- and **(B)** downregulated that overlap among the different genotypes.

A

GO of genes upregulated in *atbmi1a/b*



B

GO of genes downregulated in *atbmi1a/b*

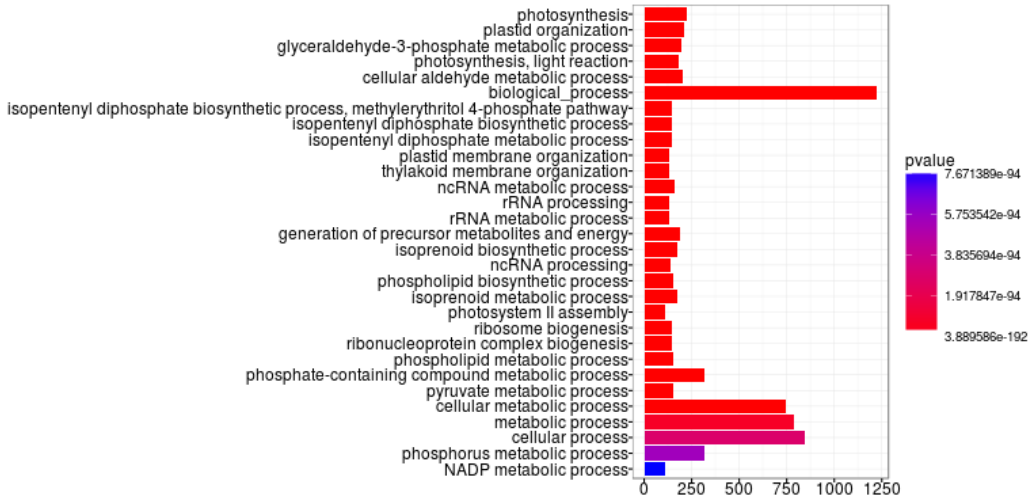
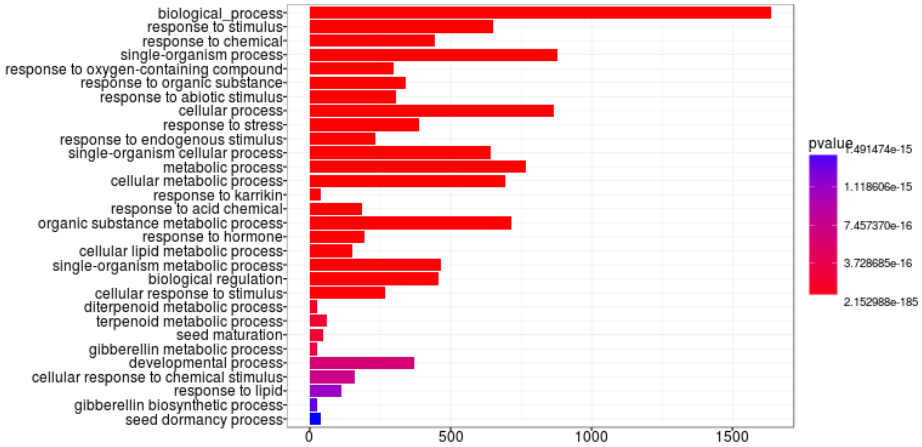


Figure S5. Gene ontology (GO) enrichment analysis of up- and downregulated genes in *atbmi1a/b* mutants. Distribution of enriched GO terms into the different "biological process" categories as defined by TAIR. p-values are indicated.

A

GO of genes upregulated in *atbmi1a/b/c*



B

GO of genes downregulated in *atbmi1a/b/c*

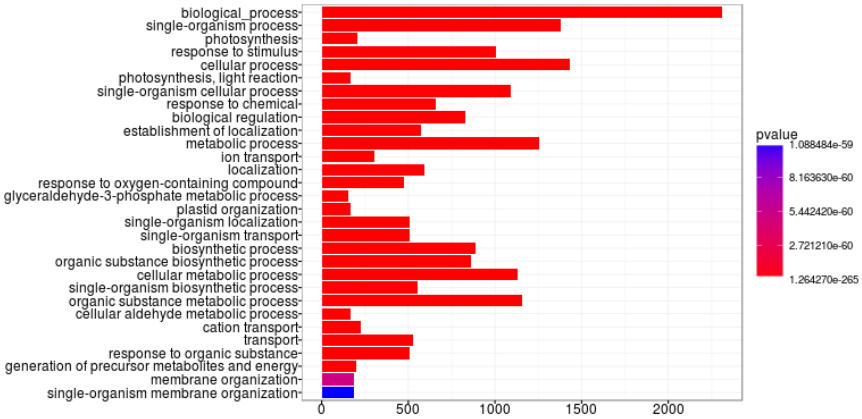


Figure S6. Gene ontology (GO) enrichment analysis of up- and downregulated genes in *atbmi1a/b/c* mutants. Distribution of enriched GO terms into the different "biological process" categories as defined by TAIR. p-values are indicated.

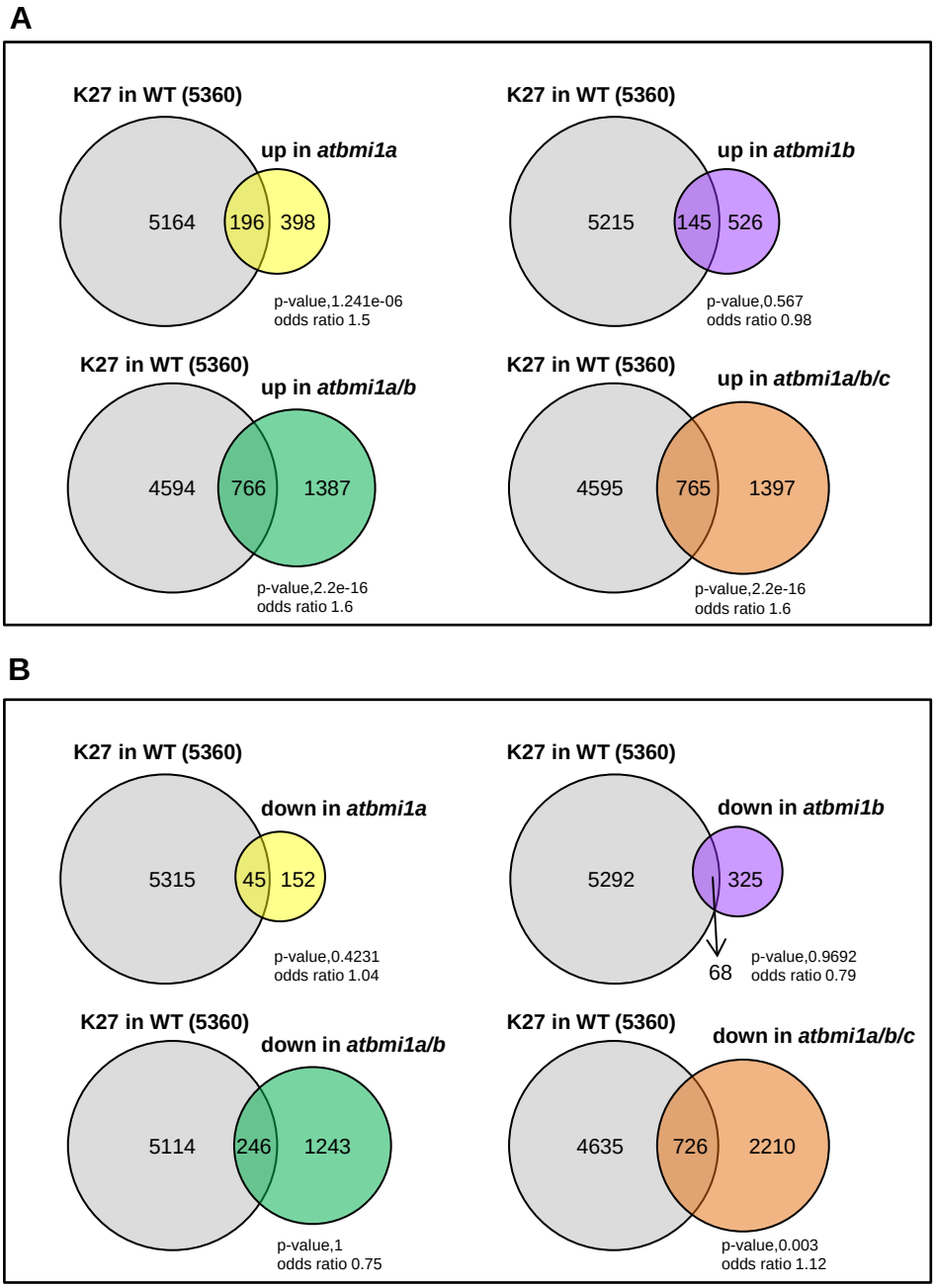
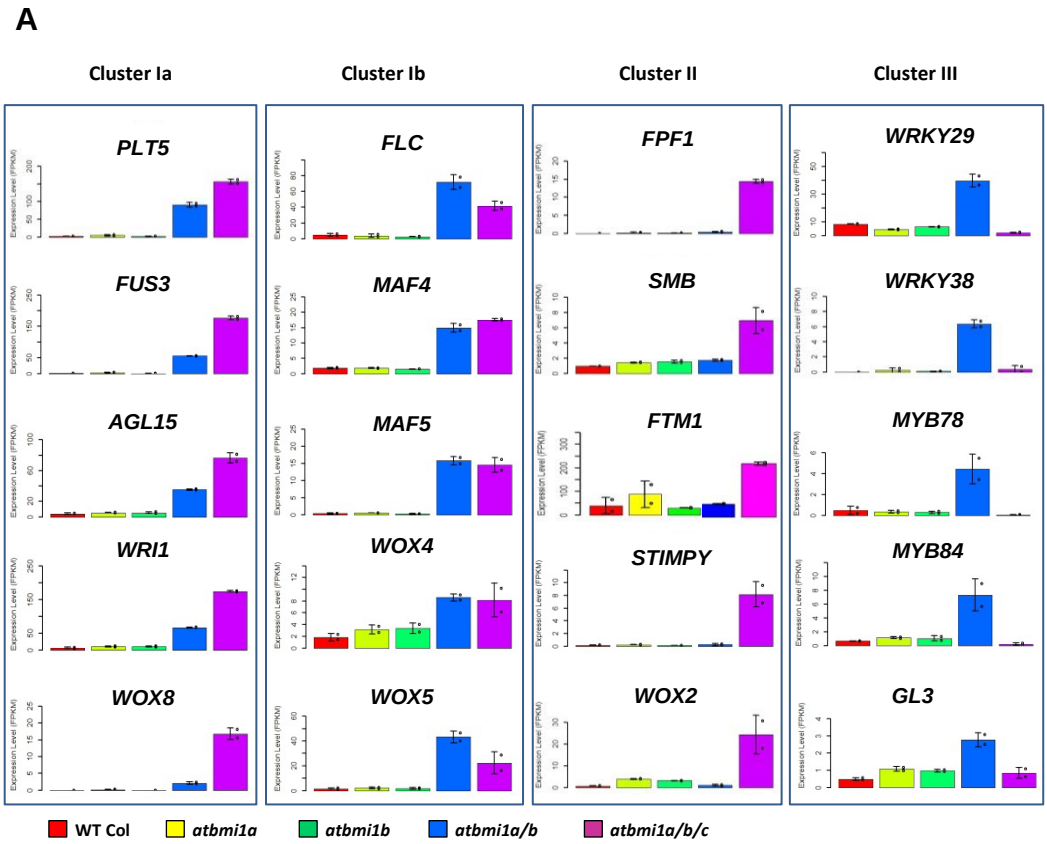


Figure S7. Putative AtBMI1direct target genes. (A) Venn diagrams showing the number of genes that were upregulated (up) in the different mutants and H3K27me3 marked (K27) in WT seedlings of the same age. All these overlaps are significant with p-values lower than 1.2 x10^{-6} and odds ratios greater 1.5 according to Fisher's Exact test except in the case of the *atbmi1b* mutant, which is probably because it is a knock-down mutant. **(B)** Venn diagrams showing the number of genes that were downregulated (down) in the different mutants and H3K27me3 marked (K27) in WT seedlings of the same age. All these overlaps are non-significant with p-values higher than 0.4231 and odds ratios lower than 1.044 according to Fisher's Exact test except in the case of the *atbmi1abc* mutant, which is probably because the developmental stage of the mutant.



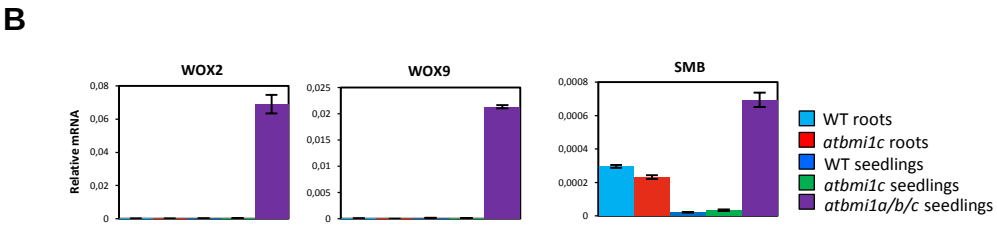
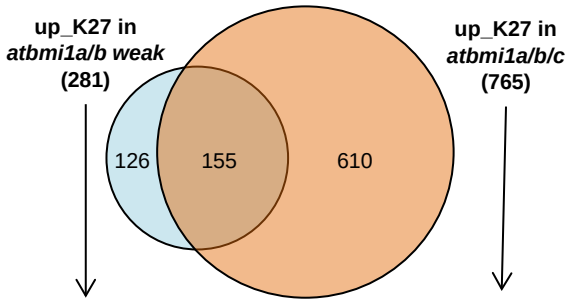


Figure S8. Genes differentially expressed in *atbmi1a/b* and *atbmi1a/b/c*. (A) Expression levels of several genes from the different clusters in WT seedlings and the different mutants. **(B)** qRT-PCR analysis of *WOX2*, *WOX9* and *SMB* expression levels y whole seedlings and roots of WT, *atbmi1c* and *atbmi1a/b/c* mutants. Quantifications are relative to *ACTIN* levels. Error bars of three independent measurements are indicated.

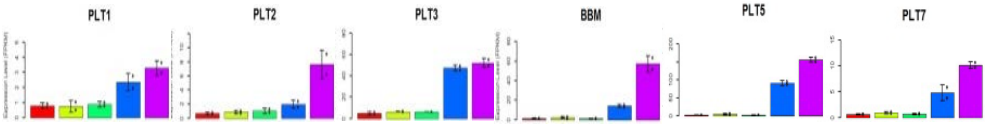


TF Family Name	Representative Gene
ABI3VP1	*ABI3*
AP2-EREBP	*SNZ, SHINE2*
C2H2	*SRS4, SRS2*
Homeobox	*WOX5, HDG4*
MADS	*PHE1, AGL15, MAF4*
MYB	*MYB47*
NAC	*NARS1*
REM	*REM1*
WRKY	*WRKY28*

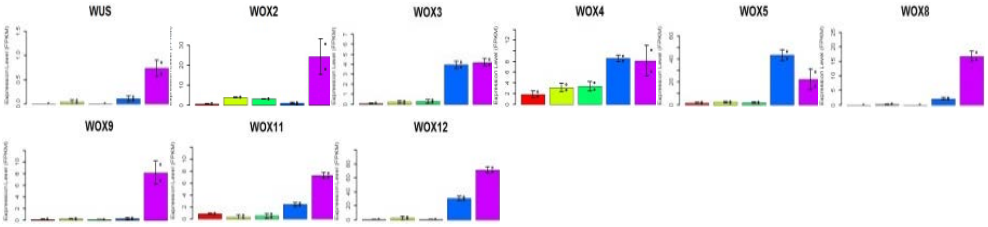
TF Family Name	Representative Gene
ABI3VP1	*ABI3, FUS3*
AP2-EREBP	*ABI4, AIL2/BBM, AIL3/PLT1, AIL4/PLT2, AIL5/CHO1, AIL6/PLT3, AIL7, ESR1, Rap2.6L, WRI1*
bHLH	*MEE8*
bZIP	*DPBF2*
C2C2-Gata	*MNP, GATA-19*
C2H2	*MGP, SRS4, ZFP10, GIS, RBE, KNU*
G2-like	*KAN2*
GRF	*GRF5*
Homeobox	*WOX4, WOX3, FWA, HDG8, WOX11, WOX12, WOX2, WOX5, WOX8, WOX9, WUS*
HSF	*HSFB4*
MADS	*SEP2, AGL13, FLC, AGL15, MAF4, MAF5*
MYB	*PAP2, MYB62, MYB101, MYB26, MYB15, PFG2, MYB40, MYB111, MYB53, MYB90, MYB15*
NAC	*ANAC015, CUC3, SMB*
RAV	*DPA4*
WRKY	*WRKY23, WRKY8, WRKY48*
ZF-HD	*AtHB28*

Figure S9. Different gene expression patterns of *atbmi1a/b* weak and *atbmi1a/b/c* mutants. Venn diagram showing overlapping between the genes up_K27 in *atbmi1a/b* weak and *atbmi1a/b/c* mutants. The overlap is significant with a p-value lower than 2.2×10^{-16} and an odds ratio greater than 17 according to Fisher's Exact test. Some representative transcription factors (TFs) in each dataset are indicated. TFs found in the two data sets are highlighter in red.

***PLT* gene family**

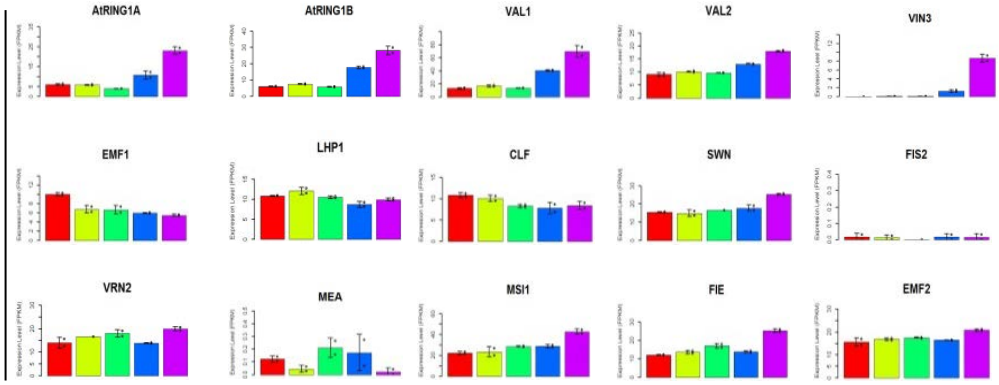


***WOX* gene family**



CHROMATIN Factors

PcG- related



TrxG- related

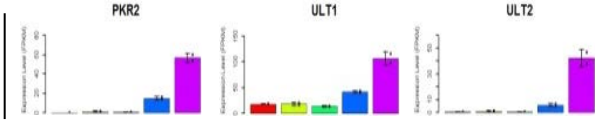


Figure S10. Expression levels of different important developmental genes in WT and *atbmi1a/b/c* mutants. Transcript levels of genes from *PLT* and *WOX* gene families and chromatin related factors belonging to the PcG and TrxG families.

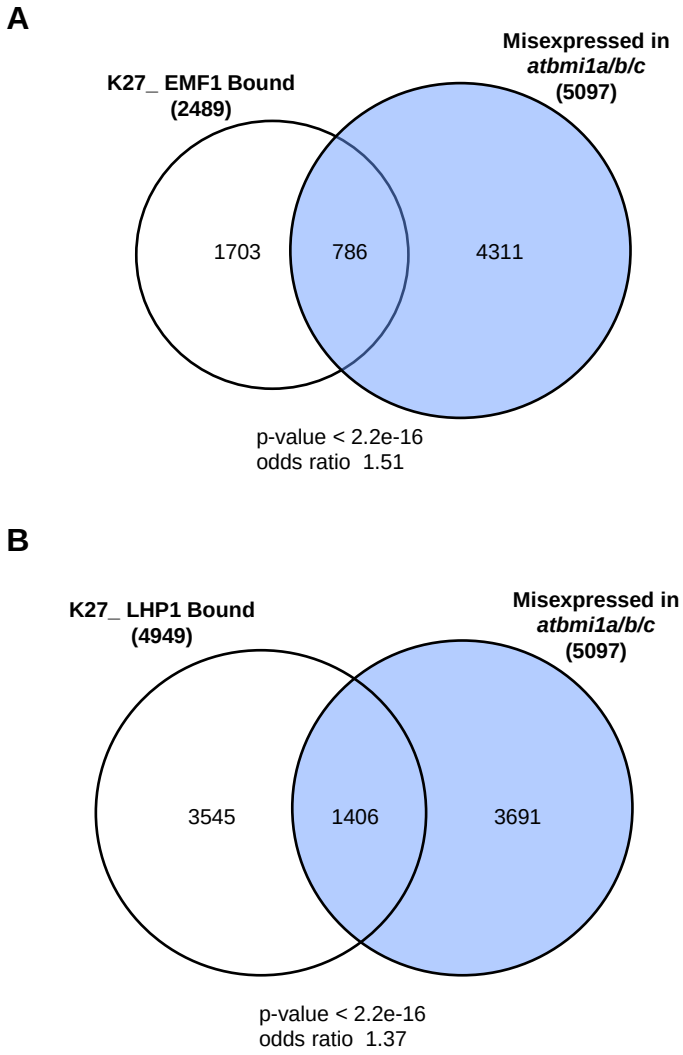
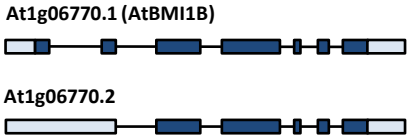


Figure S11. AtBMI1, EMF1 and LHP1 functional relationship. (A) Clustering analysis of genes misexpressed (up and downregulated) in *atbmi1a/b/c* and H3K27me3 marked genes bound by EMF1. **(B)** Clustering analysis of genes misexpressed (up and downregulated) in *atbmi1a/b/c* and H3K27me3 marked genes bound by LHP1. These overlaps are significant (p-values and Fisher's Exact test results are indicated).




```
At1g06770.1     MMIKVKKETMRACLSCSICDNILRDATTISECLHTFCRKCIYEKITEDEI
At1g06770.2     --------------------------------------------------

At1g06770.1     ETCPVCNIDLGSTPLEKLRPDHNLQDLRAKIFALKRRKVKAPGIVSLPGK
At1g06770.2     --------------------------------------------------

At1g06770.1     RKERSISSLVVSTPMVSAQAGTTRRRTKAPTRKELRNGSLAERTVKKEES
At1g06770.2     --------------MVSAQAGTTRRRTKAPTRKELRNGSLAERTVKKEES
                                ************************************
At1g06770.1     SGDELLESTSSPDTLNKFTQNKRQSKKSCKESISNKENKDGDEPWDSKMD
At1g06770.2     SGDELLESTSSPDTLNKFTQNKRQSKKSCKESISNKENKDGDEPWDSKMD
                **************************************************
At1g06770.1     WKPLNFLVEVANGTKPLKSSASQGSGSKSEHANVSRNQFQGSKTKTKNKK
At1g06770.2     WKPLNFLVEVANGTKPLKSSASQGSGSKSEHANVSRNQFQGSKTKTKNKK
                **************************************************
At1g06770.1     RKCKREDDKSNNGDPTTSETVTPKRMRTTQRKRSATTLGDSRNLPQPDES
At1g06770.2     RKCKREDDKSNNGDPTTSETVTPKRMRTTQRKRSATTLGDSRNLPQPDES
                **************************************************
At1g06770.1     SAKQERRNGPVWFSLVASNDQEGGTSLPQIPANFLRIRDGNTTVSFIQKY
At1g06770.2     SAKQERRNGPVWFSLVASNDQEGGTSLPQIPANFLRIRDGNTTVSFIQKY
                **************************************************
At1g06770.1     LMRKLDLESENEIEIKCMGEAVIPTLTLYNLVDLWLQKSSNHQRFAALVG
At1g06770.2     LMRKLDLESENEIEIKCMGEAVIPTLTLYNLVDLWLQKSSNHQRFAALVG
                **************************************************
At1g06770.1     SSAKDFTMVLVYARKLPECNM
At1g06770.2     SSAKDFTMVLVYARKLPECNM
                *********************
```

Figure S12. Schematic representation of *AtBMI1B* (*At1g06770*) splice variants (left) and predicted protein sequence comparison (right). Light boxes indicate untranslated regions, blue boxes exons, and black lines introns.