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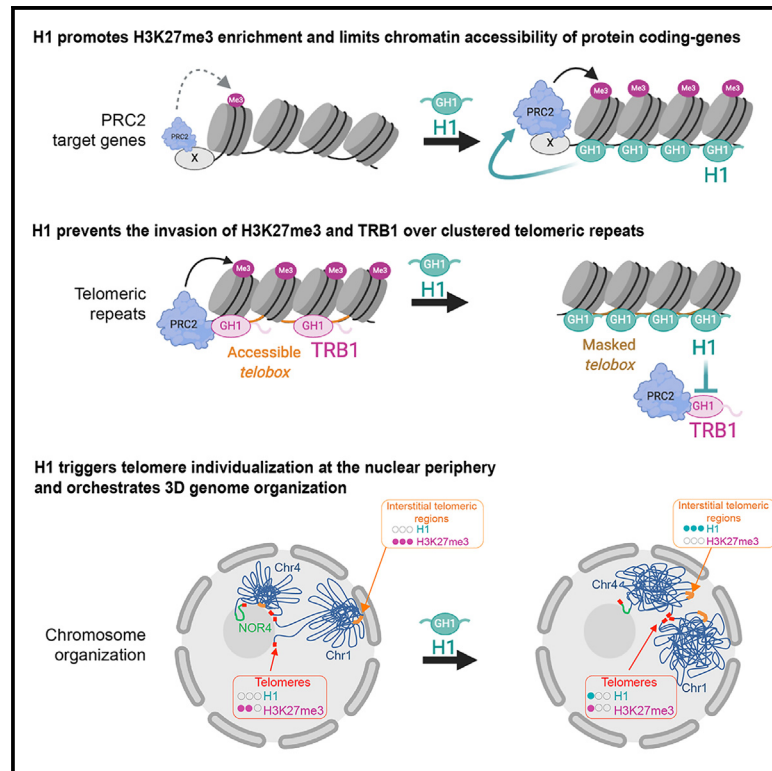
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Histone H1 protects telomeric repeats from H3K27me3 invasion in *Arabidopsis*

Graphical abstract



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In brief

Teano et al. report that linker histone H1 and a group of H1-related telomeric proteins interplay to selectively influence the *Polycomb* repressive landscape at telomeric repeats in *Arabidopsis*. These findings provide a mechanistic framework by which H1 affects the epigenome and nuclear organization in a sequence-specific manner.

Highlights

- H1 promotes PRC2 activity at a majority of genes
- H1 limits PRC2 activity at telomeres and interstitial telomeric repeats (ITRs)
- H1 orchestrates the spatial organization of telomeres and ITRs
- PRC2 repression is achieved by restricting accessibility to TRB proteins



Article

Histone H1 protects telomeric repeats from H3K27me3 invasion in *Arabidopsis*

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SUMMARY

While the pivotal role of linker histone H1 in shaping nucleosome organization is well established, its functional interplays with chromatin factors along the epigenome are just starting to emerge. Here we show that, in *Arabidopsis*, as in mammals, H1 occupies Polycomb Repressive Complex 2 (PRC2) target genes where it favors chromatin condensation and H3K27me3 deposition. We further show that, contrasting with its conserved function in PRC2 activation at genes, H1 selectively prevents H3K27me3 accumulation at telomeres and large pericentromeric interstitial telomeric repeat (ITR) domains by restricting DNA accessibility to Telomere Repeat Binding (TRB) proteins, a group of H1-related Myb factors mediating PRC2 *cis* recruitment. This study provides a mechanistic framework by which H1 avoids the formation of gigantic H3K27me3-rich domains at telomeric sequences and contributes to safeguard nucleus architecture.

INTRODUCTION

Both local and higher-order chromatin architecture rely to a large extent on the regulation of nucleosome density and accessibility, in which linker histone H1 and Polycomb Repressive complexes 1 and 2 (PRC1/2) play distinct roles. H1 modulates nucleosome distribution by contacting the nucleosome dyad with its central globular (GH1) domain and by binding linker DNA at the nucleosome entry and exit sites with its disordered carboxy-terminal domain. This indirectly contributes to dampen transcriptional activity by affecting the accessibility of transcription factors and RNA polymerases to DNA but also through interactions with histone and DNA modifiers (reviewed elsewhere^{1–3}).

Polycomb group activity is another determinant of chromatin organization that extensively regulates transcriptional activity, cell identity, and differentiation in metazoans,^{4,5} plants,⁶ and unicellular eukaryotes.⁷ While H1 incorporation directly influences the physicochemical properties of the chromatin fiber, PRC1 and PRC2 display enzymatic activities mediating histone H2A Lysine monoubiquitination (H2Aub) and histone H3 lysine 27 trimethylation (H3K27me3), respectively.^{4,5} In metazoans, chro-

matin of PRC target genes is highly compacted,^{8–10} a feature thought to hinder transcription (reviewed in Schuettengruber et al. and Illingworth^{5,11}). PRC2 can favor chromatin compaction either by promoting PRC1 recruitment or through its subunit Enhancer of Zeste Homolog 1 (Ezh1) in a mechanism not necessarily relying on the H3K27me3 mark itself.¹²

Mutual interplay between H1 and PRC2 activity first emerged *in vitro*. Human H1.2 preferentially binds to H3K27me3-containing nucleosomes¹³ while, vice versa, human and mouse PRC2 complexes display substrate preferences for H1-enriched chromatin fragments. The latter activity is stimulated more on dinucleosomes than on mono- or dispersed nucleosomes.^{14,15} *In vivo*, recent studies unveiled that H1 is a critical regulator of H3K27me3 enrichment over hundreds of PRC2 target genes in mouse cells.^{16,17} Chromosome conformation capture (Hi-C) analysis of hematopoietic cells,¹⁶ germinal center B cells,¹⁷ and embryonic stem cells¹⁸ showed that H1 triggers distinct genome folding during differentiation in mammals. These major advances raise the question of the mechanisms enabling H1 sequence-specific interplays with PRC2 activity in chromatin regulation and their evolution in distinct eukaryotes.



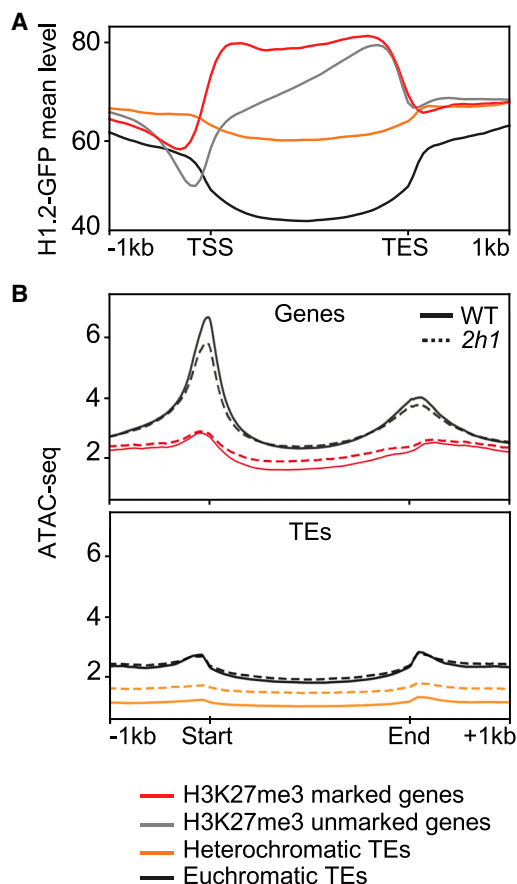


Figure 1. H1.2-GFP is enriched at PRC2-target genes where it contributes to restrain DNA accessibility

(A) H1.2-GFP mean read coverage at protein-coding genes and TEs. (B) ATAC-seq analysis of chromatin accessibility of genes and TEs described in (A) in WT (plain lines) and *2h1* (dashed lines) nuclei. Chromatin accessibility is estimated as read coverage. TSS, transcription start site; TES, transcription end site. In (A) and (B), H3K27me3-marked genes ($n = 7,542$) are compared to all other annotated protein-coding genes. Heterochromatic versus euchromatic TEs were defined previously.⁴³ The plots represent the mean of three (H1.2-GFP) or two (ATAC-seq) independent biological replicates.

In *Arabidopsis thaliana*, two canonical linker histone variants, H1.1 and H1.2, represent the full H1 complement in most somatic cells.^{19–21} These two linker histones, hereafter referred to as H1, are enriched over heterochromatic transposable elements (TEs) displaying high nucleosome occupancy; CG, CHG, and CHH methylation; as well as H3K9 dimethylation.^{22,23} H1 also contributes to CG methylation-mediated gene silencing²⁴ and is less abundant over expressed genes.^{22,23} As in mammals, *Arabidopsis* H1 incorporation is thought to dampen RNA Pol II transcription, an effect that also applies in plants to RNA polymerase Pol IV, which produces short interfering RNAs (siRNAs).²⁵ *Arabidopsis* H1 also restricts accessibility to DNA methyltransferases and demethylases, which mediates gene or TE silencing.^{26–30} This process is counter-balanced by incorporation of the H2A.W histone variant, presumably competing with H1 for DNA binding through its extended C-terminal tail.³¹

Besides TE silencing, recent studies suggested that H1 dynamics may affect PRC2 activity during *Arabidopsis* development. The first piece of evidence is that H1 is largely absent from the vegetative cell nucleus of pollen grain and is degraded during spore mother cell (SMC) differentiation at the onset of heterochromatin loosening and H3K27me3 reduction.^{26,32–34} Further evidence comes from the observation that *H1* loss-of-function mutant nuclei display a ~ 2 -fold lower H3K27me3 chromatin abundance, while a few discrete H3K27me3 sub-nuclear foci of undetermined nature displayed increased H3K27me3 signals.³⁵ Hence, despite evidence that variations in H1 abundance mediate epigenome reprogramming during plant development, there is no information on how H1 interplays with PRC2 activity and on the consequences of this interaction on the chromatin landscape and topology in these organisms.

Here, we profiled H3K27me3 in *h1* mutant plants and found that, while a majority of genes expectedly lost H3K27me3, telomeres and pericentromeric interstitial telomeric repeat (ITR) regions or interstitial telomeric sequences (ITSs) were massively enriched in this mark. We identified that H1 prevents PRC2 activity at these loci by hindering the binding of Telomere Repeat Binding (TRB) proteins, a group of H1-related proteins with extra-telomeric function in PRC2 recruitment.^{36,37} H1 safeguards telomeres and ITRs against excessive H3K27me3 deposition and preserves their topological organization. Collectively, our findings led us to propose a mechanism by which H1 orchestrates *Arabidopsis* chromosomal organization and contributes to the control of H3K27me3 homeostasis between structurally distinct genome domains.

RESULTS

H1 is abundant at H3K27me3-marked genes and reduces their chromatin accessibility

To assess the relationships between H1, PRC2 activity, and chromatin accessibility, we first compared the genomic distribution of H3K27me3 with that of H1.2, the most abundant canonical H1 variant in *Arabidopsis* seedlings.²² To maximize specificity, we used a GFP-tagged version of H1.2 expressed under the control of its endogenous promoter.²² In agreement with previous studies in several eukaryotes,^{22,23,38,39} H1.2 covers most of the *Arabidopsis* genome without displaying clear peaks. However, a closer examination revealed that, as compared to genes and to TEs that are not enriched in H3K27me3,^{40–42} H1 level was higher at coding genes marked by H3K27me3, especially toward their 5' regions (Figures 1A and S1A–S1C).

Having found that H1 is enriched at PRC2 marked genes, we tested whether H1 also contributes to regulate chromatin accessibility using assay for transposase-accessible chromatin followed by sequencing (ATAC-seq) in nuclei of wild-type (WT) and *h1.1h1.2* double-mutant plants (hereby named *2h1* for short). As previously reported in WT plants,⁴⁴ H3K27me3-marked genes tend to display low chromatin accessibility as compared to non-marked genes, which are usually expressed and typically display a sharp ATAC peak at their transcription start sites (TSSs) corresponding to the nucleosome-free region (Figure 1B). In *2h1* nuclei, gene body regions of H3K27me3-marked loci displayed a significant increase in accessibility (Figures 1B and S1D).

Hence, H1 tends to abundantly occupy PRC2 target gene bodies where it has a minor but detectable contribution in restricting chromatin accessibility.

H1 promotes H3K27me3 enrichment at a majority of PRC2 target genes while protecting a few genes displaying specific sequence signatures

To determine at which loci H1 influences PRC2 activity, we profiled the H3K27me3 landscape in WT and *2h1* seedlings. To enable absolute quantifications despite the general reduction of H3K27me3 in the mutant nuclei, we employed chromatin immunoprecipitation sequencing (ChIP-seq) with reference exogenous genome (ChIP-Rx) by spiking in equal amounts of *Drosophila* chromatin to each sample.⁴⁵ Among the ~7,500 genes significantly marked by H3K27me3 in WT plants, more than 4,300 were hypomethylated in *2h1* plants (Figures 2A–2C and S2A–S2D; Table S1). Hence, general loss of H3K27me3 in *2h1* seedlings identified by immunoblotting and cytology³⁵ results from a general effect at a majority of PRC2-regulated genes. It is noteworthy that ~85% of the genes marked by H3K27me3 in WT plants were still significantly marked in *2h1* plants (Figure S2D). Hence, H1 is required for efficient H3K27me3 maintenance or spreading rather than for PRC2 seeding. Our RNA sequencing (RNA-seq) analysis showed that genes encoding PRC1/PRC2 subunits are not downregulated in *2h1* plants, excluding indirect effects resulting from less-abundant PRC2 (Table S2). Unexpectedly, we also found that ~500 genes were hyper-marked or displayed *de novo* marking in *2h1* plants (Figures 2A–2C and S2A–S2D; Table S1).

To determine whether the hypo/hyper/unaffected gene sets had different functional properties, we inspected their transcript levels. Hyper-marked genes correspond to the least expressed gene category in WT plants, whereas many hypo-marked genes are significantly more expressed than unaffected genes (Figure 2D). Functional categorization of hypo-marked genes notably identified an over-representation of genes involved in transcriptional regulation and meristem maintenance (Figure S2H). These classifications are consistent with former reports of PRC2 repressing these biological processes.⁴⁶ In contrast, a feature of the hyper-marked gene set is the presence of TE or TE-gene annotations (Figure S2I; Table S1). Hence, H1-mediated PRC2 activation^{14–16} is most likely conserved in plants, but, in *Arabidopsis*, this property is contrasted by a heretofore-unsuspected negative effect at a minority of poorly expressed genes sometimes displaying TE features.

In vitro, PRC2 activity was proposed to be favored by local H1 abundance and/or at densely organized nucleosome arrays.¹⁴ Instead, we found that hypo-marked genes tend to display lower H1 level, to be more accessible and expressed than other genes marked by H3K27me3 (Figures 2D–2F and S2E). We therefore tested nucleosome density using ChIP-seq profiling of histone H3, confirming that hypo-marked genes display lower nucleosome occupancy than other marked gene categories (Figure S2G). Collectively, analysis of the hypo-marked loci suggests that chromatin of the corresponding genes is not sufficiently nucleosome dense to favor PRC2 local activity when H1 is absent.

We further explored whether the specific influence of H1 on H3K27me3 enrichment at genes could rely on a sequence-

dependent mechanism, especially at hyper-marked genes, since they do not incur H1-mediated PRC2 activation. In contrast to the promoter sequences of the hypo-marked genes in which no such motif is significantly over-represented, we identified three enriched motifs in the hyper-marked gene set (Figure S2J). A poly(A) motif is present in 84% of the gene promoters, and the AAACCCTA telomeric motif, referred to as *telobox*,^{47,48} which serves as *Polycomb Response Elements* (PREs) in plants,^{36,37} is found in 17% of them (Table S1). Based on these observations, we conclude that the capacity of H1 to counteract H3K27me3 enrichment at a small gene set presumably involves specific sequence features.

H1 contributes to define accessibility and expression of PRC2 target genes

To get insights into the functional consequences of H1 loss at genes where it either promotes or dampens H3K27me3 enrichment, we compared the chromatin accessibility and transcript levels of these gene sets in WT and *2h1* nuclei. ATAC-seq profiling showed that hypo-marked gene bodies were significantly more accessible in the mutant than in the WT line (Figures 2F and S2F), thereby correlating with reduced H3K27me3 levels. Accessibility of H3K27me3 hyper-marked genes was increased in *2h1* plants, still remaining at a very low level as compared to non-marked genes (Figures 2F and S2F). Conservation of this function in both hypo- and hyperH3K27me3 gene categories indicates that H1 incorporation reduces chromatin accessibility of *Arabidopsis* PRC2-target genes independently of its influence on H3K27me3 enrichment.

Confirming previous reports,^{23,35} our RNA-seq analysis showed that *H1* loss of function triggers minor gene expression changes (Table S2). However, we identified a significant tendency for increased transcript levels of the H3K27me3 hypo- and hyper-marked genes set in the *2h1* line (Figure 2G). Taken together, these analyses showed that, at a majority of PRC2 target genes, H1 depletion triggers H3K27me3 loss associated with a moderate increase in DNA accessibility and expression.

H1 prevents H3K27me3 invasion over a specific family of heterochromatic repeats

Considering the observed H3K27me3 enrichment at a few TE-related genes in *2h1* plants, we extended our analysis to TEs, which typically lack H3K27me3 in *Arabidopsis*.^{49,50} This revealed that 1,066 TEs are newly marked by H3K27me3 in *2h1* plants, most frequently over their entire length, thereby excluding *a priori* the possibility that H3K27me3 TE enrichment is due to spreading from neighboring genes (Figure 3A). We clustered H3K27me3-marked TEs into two groups, *TE cluster 1* and *TE cluster 2*, displaying high and low H3K27me3 enrichment, respectively (Figure 3A). While *TE cluster 2* (*n* = 850) is composed of a large variety of TE families, *TE cluster 1* (*n* = 216) mostly consists of *ATREP18* (189 elements) annotated in the TAIR10 genome as unassigned (Figure 3B). In total, *TE cluster 1* and *2* comprise 60% of all annotated *Arabidopsis ATREP18* elements, including many of the longest units (Figure S3A). A second distinguishing feature of *TE cluster 1* elements is their elevated H1 and H3 occupancy (Figures 3C and S3B–S3E). Accordingly, *TE cluster 1* and, more generally, *ATREP18* elements are strongly heterochromatic

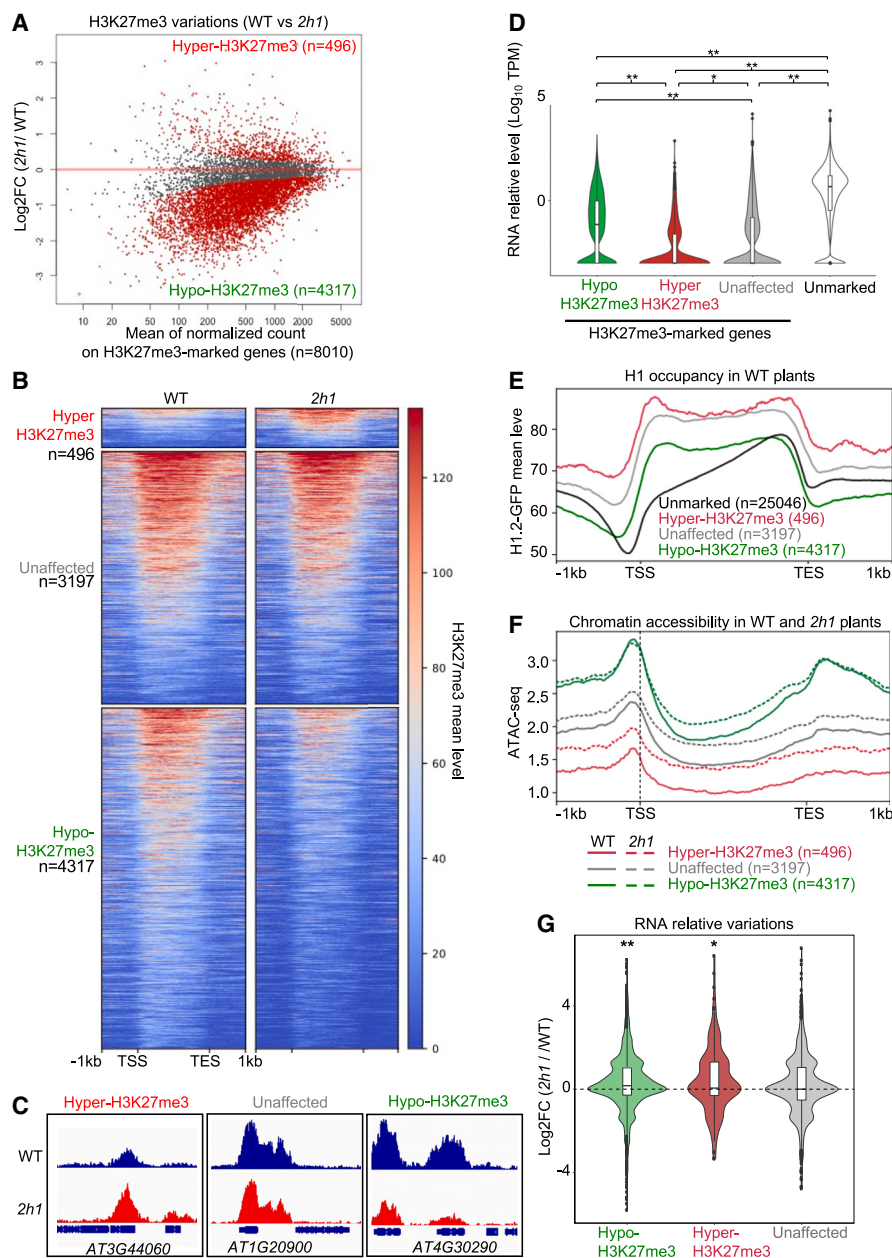


Figure 2. H1 influences H3K27me3 marking, chromatin accessibility, and expression of PRC2-target genes

(A) Identification of differentially marked genes using spike-in normalized DESeq2 ChIP-seq analysis identifies low H3K27me3 levels over a majority of the PRC2 target genes in *2h1* plants. All genes displaying an H3K27me3-enriched domain in WT or *2h1* plants (according to MACS2 peak detection; see STAR Methods) are individually shown as dots. Red dots, differentially marked genes (false discovery rate [FDR] <0.01).

(B) H3K27me3 profiles along all genes significantly marked in WT or *2h1* plants. Genes are grouped according to differential analysis in (A) and ranked within each group according to mean H3K27me3 levels.

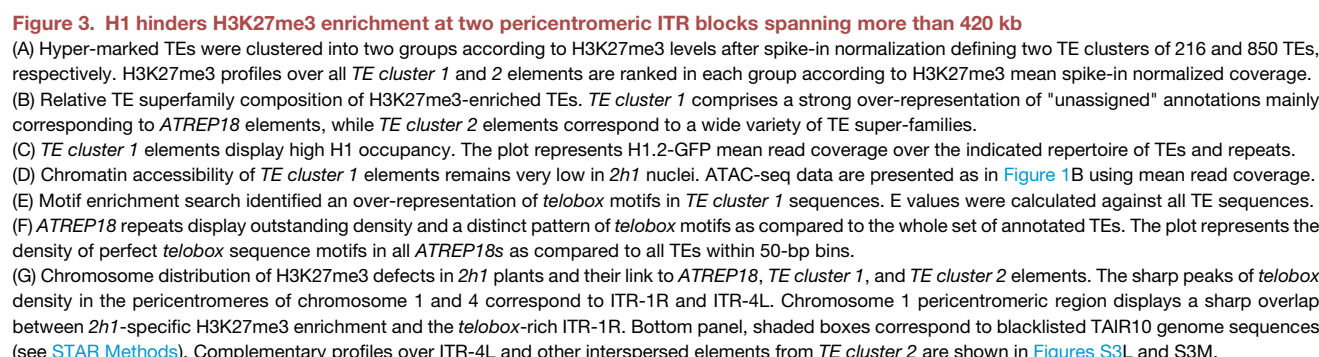
(C) H3K27me3 profile of representative genes of the three sets identified in (A) exemplifying the general tendency of PRC2-target genes to keep a weak H3K27me3 domain in *2h1* plants.

(D) Transcript levels in WT seedlings. The values represent RNA-seq log10 transcripts per million (TPM) values. The embedded boxplots display the median, while lower and upper hinges correspond to the first and third quartiles. * $p < 10^{-9}$ and ** $p < 10^{-15}$, Wilcoxon rank test.

(E) H1.2-GFP ChIP-seq profiling on the indicated gene sets (mean read coverage).

(F) ATAC-seq analysis of the indicated gene sets. ATAC-seq data are presented as in Figure 1B using mean read coverage.

(G) Transcript level variations between WT and *2h1* plants in the same three gene sets. The values represent mRNA log₂ fold changes. The embedded boxplots display the median, while lower and upper hinges correspond to the first and third quartiles. * $p < 5\%$ and ** $p < 1\%$, Student's *t* test. ChIP-Rx, ATAC-seq, and RNA-seq data correspond to two biological replicates each, and H1.2-GFP ChIP-seq correspond to three biological replicates.



Noteworthy, while MNase-seq analyses^{22,23} and our ATAC-seq data showed that heterochromatic TEs tend to be more accessible in *2h1* nuclei, the chromatin of *TE cluster 1* and *ATREP18* repeats remained very poorly accessible despite H1 loss (Figures 3D, S3C, and S3D). Hence, chromatin "inaccessibility" of *TE cluster 1* elements is either H1 independent or compensated by other mechanisms, possibly a local increase in PRC2 activity.

Aiming at determining the features potentially leading to a selective role of H1 at *TE cluster 1* elements, we first envisaged that H1 could locally prevent conversion of the H3K27me1 heterochromatic mark into H3K27me3. However, analysis of public datasets⁵¹ showed that, as compared to other TEs, H3K27me1 is not particularly abundant at *TE cluster 1* or at *ATREP18* elements, therefore ruling out this first hypothesis (Figure S3F). We then

Having not found evidence for indirect roles of H1 on H3K27me3 marking at *TE cluster 1*, we concluded that H1 hinders PRC2 recruitment or activity at these repeats, and this

despite a densely packed chromatin organization that theoretically constitutes an excellent substrate. As previously done for hyper-marked genes, we therefore tested whether *TE cluster 1* elements are distinguishable from other TEs by specific DNA motifs. MEME search identified a prominent sequence signature, the *telobox* motif (Figure 3E), which we had already identified in 17% of the hyper-marked genes (Figure S2J). As compared to all other TEs, *teloboxes* were found to be ~100-fold more densely represented in *ATREP18* elements as compared to all TEs (Figures 3E and 3F). With 7,328 *telobox* motifs, *TE cluster 1* contains ~53% of the whole TAIR10 *telobox* repertoire. Hence, if not considering proper telomeres that span 2 to 5 kb at the end of each chromosome,^{56,57} *TE cluster 1* repeats display the majority of telomeric motifs of *Arabidopsis* genome and the strongest propensity to attract PRC2 activity upon H1 loss.

Remarkably, these two properties can be seen at a chromosome scale by contrasting the genome distribution of *telobox* density and of H3K27me3 differential marking, since about 95% of *TE cluster 1* elements cluster within two outstandingly *telobox*-rich regions situated in the pericentromeres of chromosomes 1 and 4 (Figures 3G and S3L). Given this characteristic, we consider these domains as two of the nine *Arabidopsis* genome loci proposed to constitute ITRs,^{58,59} hereby referred to as ITR-1R and ITR-4L of ~355 kb and ~72 kb, respectively. In agreement with the description of ITRs in plants and vertebrates,^{60,61} *ATREP18* elements that constitute most of these domains display a high density in *telobox* motifs frequently organized as small clusters (Figures 3G, S3L, S3N, and S3O). Further supporting their telomeric evolutionary origin, *ATREP18s* encode no open reading frame or other TE features, are mostly oriented on the same DNA strand, and are tandemly organized (nearly 90% of them being positioned within 1 kb of each other; Figures S3P–S3R), hence they do not constitute *stricto sensu* TEs. Ectopic H3K27me3 deposition was also found at several interspersed elements of *TE cluster 2* located in all pericentromeric regions outside these two ITR blocks (Figure S3M), but our main conclusion is that H1 abundantly occupies two large blocks of pericentromeric ITRs where it prevents H3K27me3 marking.

H1 influences telomere chromatin composition and sub-nuclear positioning

Considering that telomeres display hundreds of perfect *telobox* motifs, the question arose whether, similarly to ITRs, H1 also prevents H3K27me3 deposition at chromosome ends. Because the perfect continuum of terminal telomeric motifs is not suited for quantitative NGS analyses, ChIPs were analyzed through hybridization with radioactively labeled concatenated telomeric probes.⁶² H3K27me3 ChIP dot blots led to the estimation that telomeres display an average ~4-fold more H3K27me3 enrichment in *2h1* as compared to WT plants, independently of detectable changes in nucleosome occupancy probed by anti-H3 ChIP dot blot (Figures 4A and S4A).

To assess whether H3K27me3 enrichment concerns a few telomeres or affects them all, we explored its occurrence in intact nuclei using H3K27me3 immunolabeling combined with telomere fluorescence *in situ* hybridization (DNA FISH). Consistent with our ChIP-blot analysis, most telomeric foci were enriched with H3K27me3 in *2h1* nuclei, with 2- to 4-telomere foci

frequently presenting outstandingly strong H3K27me3 signals (Figures 4B and 4C). We could not ascertain whether some of these strong signals corresponded to cross-hybridizing pericentromeric ITRs, but their frequent positioning near to the nuclear periphery may point to the latter hypothesis. Indeed, in *2h1* nuclei, telomeric foci were frequently re-distributed toward the nucleus periphery, thereby contrasting with the telomere rosette model proposed by Fransz et al.,⁶³ first establishing that telomeres cluster around the nucleolus (Figure 4D). In addition, the number of telomere foci was reduced in the mutant nuclei (Figures 4B and 4C), indicating that H1 not only prevents accumulation of H3K27me3 at ITRs and at most telomeres but is also required for the sub-nuclear organization and proper individualization of these domains.

H1 promotes heterochromatin packing but attenuates ITR insulation and telomere-telomere contact frequency

To better understand the altered telomere cytogenetic patterns of *2h1* nuclei and to extend our analysis to ITR topology, we employed *in situ* Hi-C of dissected cotyledons, composed of 80% mesophyll cells, which enabled us to reach high resolution. In agreement with previous reports,^{64–69} WT plants displayed frequent interactions within and between pericentromeric regions, which reflect packing of these domains within so-called chromocenters (Figures 5A, 5B, and S5E). Loosening of these heterochromatic structures in *2h1* mutant nuclei, formerly observed by microscopy,^{23,35} was expectedly identified here as a more steep decay with distance⁷⁰ and lower long-range interaction frequency within pericentromeric regions (Figures 5A, 5B, and S5H–S5J). However, this tendency appears to be a general trend in the mutant nuclei since it was also observed for chromosome arms. As also seen in *crwn* and *condensin* mutants,⁷¹ in a matrix of differential interaction frequency between WT and *2h1* nuclei these prominent defects are also visible as blue squares surrounding the centromeres, which are mirrored by increased interaction frequency between pericentromeric regions and their respective chromosome arms (i.e., red crosses along chromosome arms) (Figure 5C).

Having identified large-scale defects of chromosome organization in *2h1* mutant nuclei, we then focused on telomere-telomere interaction frequency. Because telomeres are not included in the TAIR10 reference genome, we used the most sub-telomeric 100-kb sequences of each chromosome end as a proxy to estimate telomere long-distance interactions, and these were controlled using an internal 100-kb region of each pericentromeric region as well as 100-kb regions randomly chosen in distal chromosomal arms. As previously noted, in WT plants, the telomere-proximal regions frequently interacted with each other through long-range interactions.^{64–68} We further observed that ITR-1R and ITR-4L do not particularly associate with each other or with telomeres (Figure S5H). In *2h1* nuclei, with the exception of the regions adjacent to the nucleolar organizer regions (NORs) of chromosome 2 and 4 (SubNOR2 and SubNOR4), which displayed atypical patterns (detailed in Figures S5H–S5J), interaction frequencies between all sub-telomeric regions were increased (Figure 5D). Furthermore, ITR-1R and 4L also showed increased ITR-ITR and ITR-telomere

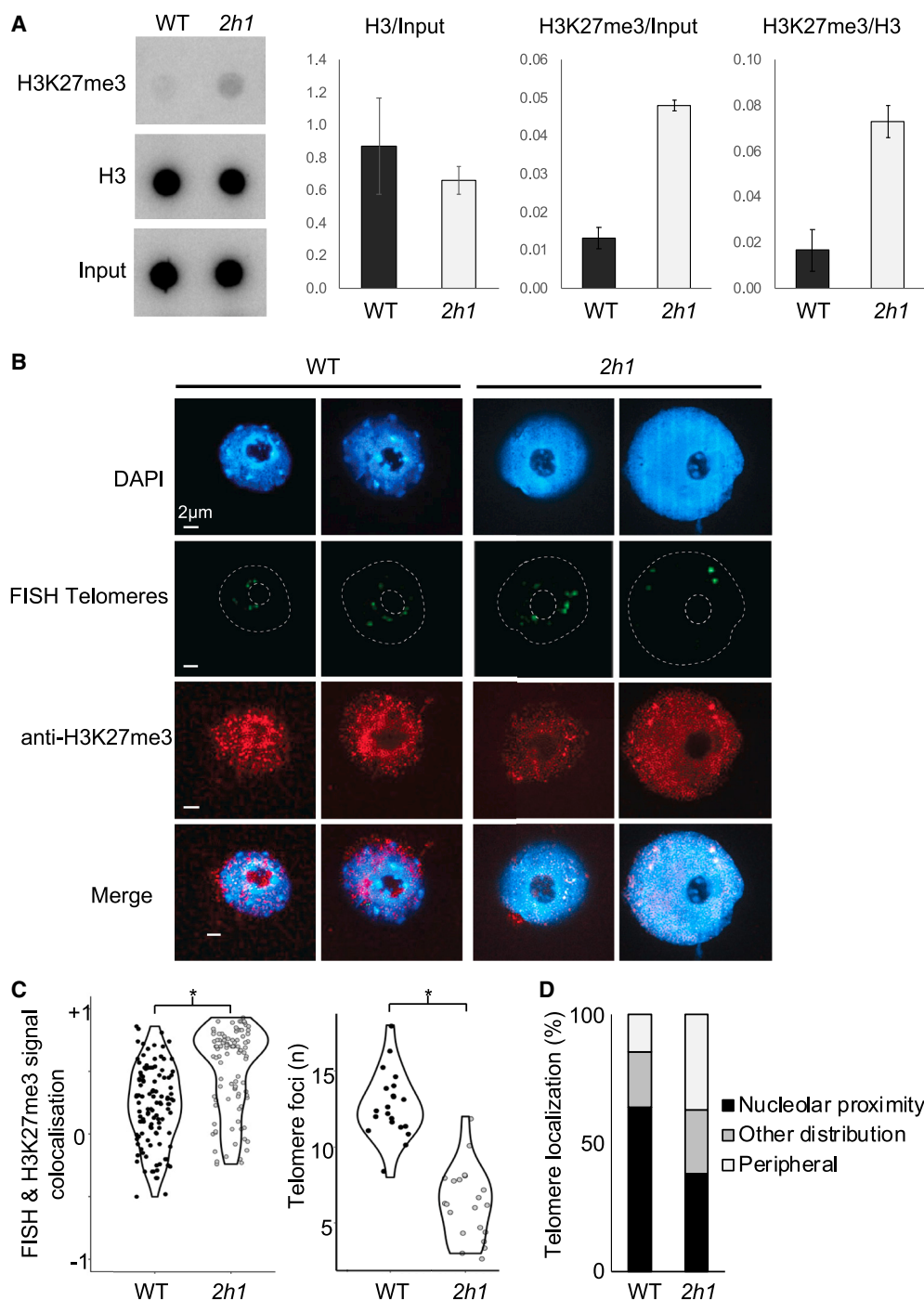


Figure 4. H1 influences both H3K27me3 enrichment and sub-nuclear organization of telomeres

(A) Increased H3K27me3 level at telomeres in *2h1* plants. H3 ChIP signal is used as a proxy of nucleosome occupancy. ChIPs were followed by dot-blot hybridization with a labeled telomeric probe. Data are the mean of two biologically and technically replicated experiments \pm SE. A second biological replicate is shown in Figure S5.

(B) Most telomeric loci are enriched in H3K27me3 and re-distributed toward the nucleus periphery in *2h1* plants. Representative collapsed z stack projections of cotyledon nuclei subjected to H3K27me3 immunolabeling and telomere DNA FISH are shown. Blue, DAPI DNA counterstaining; green, telomere FISH signals; red, H3K27me3 immunolabeling.

(C) Quantification of sub-nuclear telomeric signal properties. $*p < 1.6 \times 10^{-7}$, Wilcoxon signed-rank test.

(D) Quantification of nucleus classes displaying different patterns in telomere sub-nuclear localization. Number and position of telomeric foci were determined in two independent biological replicates ($n > 20$ each).

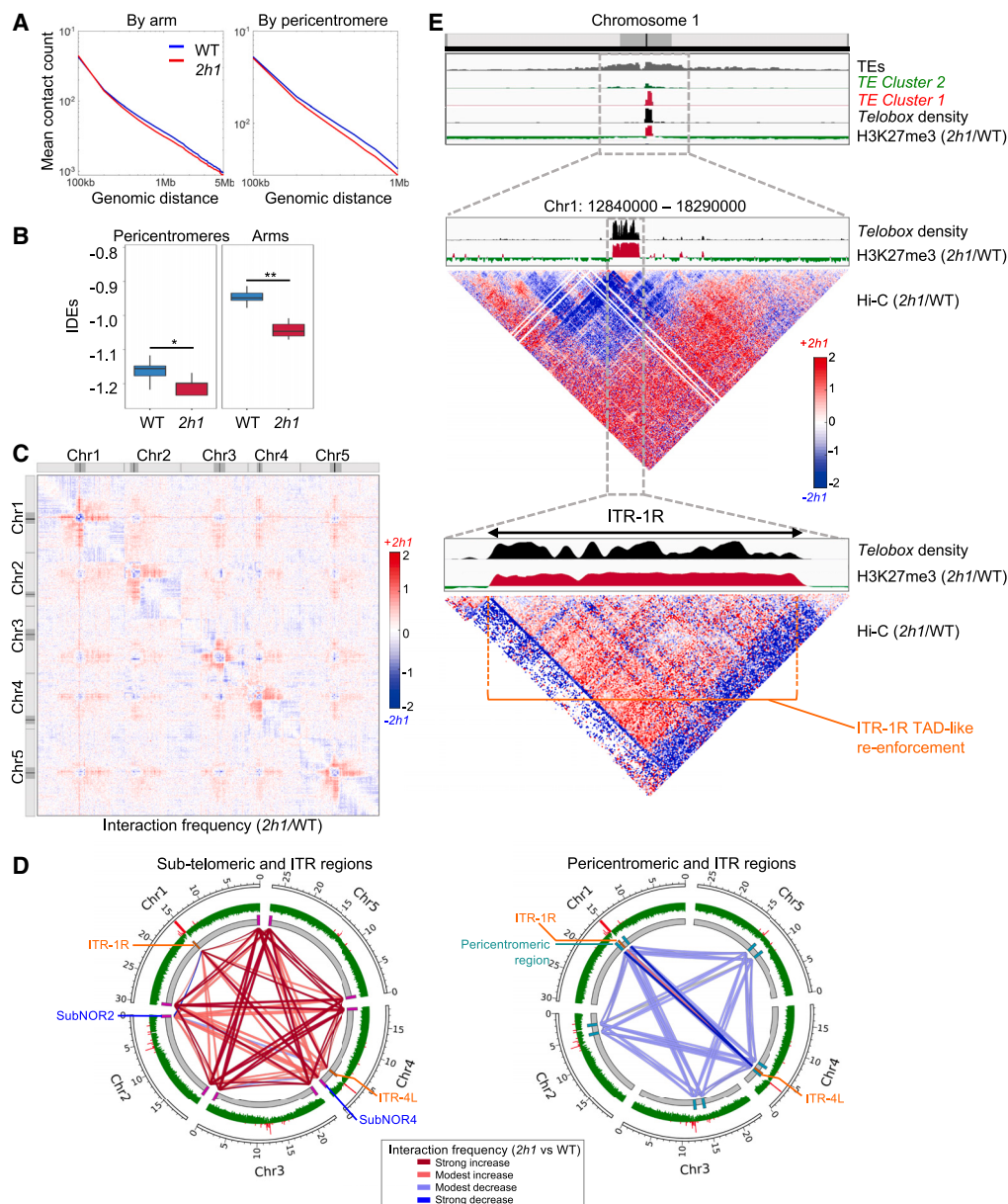


Figure 5. H3K27me3 accumulation at ITRs and at telomeres associates with ITR insulation and more frequent telomere-telomere interactions

(A) Mean contact count as a function of genomic distance for all chromosome arms at a 100-kb resolution.

(B) Distribution of interaction decay exponents (IDEs) determined at a 100-kb resolution for chromosome arms and pericentromeric regions of WT and 2h1 nuclei. Median IDE values of chromosome arms and pericentromeres were determined as $-0.95/-1.16$ in WT and $-1.05/-1.2$ in 2h1 nuclei, respectively. *p = 0.076 and **p = 0.001, pairwise Wilcoxon rank-sum test.

(C) Relative difference of interaction frequency between WT and 2h1 plants. The \log_2 values of observed/expected (O/E) interaction frequency along the five chromosomes in 2h1 versus WT are shown at a 100-kb resolution. Regions in red have more frequent contacts in 2h1 than in WT plants, while regions in blue have less. Pericentromeric regions are depicted in dark gray on the schematic chromosomes.

(D) H1 reduces the frequency of long-distance interactions between chromosome ends. Circos plots depict variations in inter-chromosomal interaction frequencies between telomere-proximal, pericentromeric, ITR-1R, and ITR-4L 100-kb domains. Yellow boxes, ITR regions. External green/red track, H3K27me3 variations in 2h1 versus WT plants (\log_2 ratio). Magenta boxes, telomere-proximal regions and SubNOR2 or SubNOR4.

(E) Reduced frequency of intra-pericentromeric O/E interactions in 2h1 mutant nuclei is contrasted by TAD re-enforcement of the H3K27me3-enriched ITR-1R 355 kb block. Top panel, location of ITR-1R in chromosome 1. Middle panel, magnification of the region surrounding chromosome 1 pericentromeres at a 10-kb resolution. Bottom panel, magnification of the pericentromere-embedded ITR-1R at a 2-kb resolution. Strong and modest increase correspond to $\log_2FC > 1$ and $\log_2FC 0.35-1$, respectively; modest and strong decrease correspond to $\log_2FC -0.33$ to -0.65 and $\log_2FC < -0.65$, respectively. Quantitative analyses are shown in the complementary Figures S4H–S4J. All Hi-C analyses combine three independent biological replicates.

interaction frequency (Figures 5D, S5G, and S5J). Consistent with a reduced number of telomere foci in intact *2h1* nuclei (Figure 4), this observation supports an organizational model in which telomeres tend to coalesce more frequently in the absence of H1.

Last, we examined chromosome topology at ITR loci. In WT plants, both of them formed large structures resembling topologically associating domains (TADs), which are themselves immersed within highly self-interacting pericentromeric regions. Interestingly, in *2h1* nuclei, intra-ITR interactions were strongly enhanced (i.e., TAD re-enforcement), while the surrounding pericentromeric environments expectedly showed an opposite trend linked to heterochromatin relaxation (Figures 5E and S5G). This observation was supported by comparing distal-to-local ratios (DLRs) of interaction frequency that showed clear local drops at each ITR in *2h1* nuclei, hence an increased tendency for interacting only with itself usually interpreted as increased domain compaction (Figure S5K).⁷² Altogether, these observations show that, in contrast to its general role in heterochromatin packing, H1 dampens the local insulation of ITRs from their neighboring environment. Remarkably, the boundaries of these compaction defects in *2h1* nuclei sharply correspond with H3K27me3 enrichment (Figures 5E and S5K).

H1 antagonizes TRB-mediated PRC2 activity at ITRs

With the aim to determine the molecular mechanisms by which H1 selectively represses PRC2 activity at *telobox*-rich elements, and more particularly at ITRs, we envisioned that TRB proteins might have a prominent role (Figure 6A). The TRB1, TRB2, and TRB3 founding members of these plant-specific single-Myb-histone proteins constitute part of the telomere nucleoprotein structure required to maintain telomere length⁷³ (Figure 6B). Their Myb domain has strong affinity to the G-rich strand of *telobox* DNA motifs^{73–75} and, combined with a coiled-coil domain that can associate with the CURLY-LEAF (CLF) and SWINGER (SWN) catalytic subunits of PRC2,^{36,37} TRBs act as transcriptional regulators of protein-coding genes bearing a *telobox* motif.^{76,77} Interestingly, despite their low protein sequence similarity to H1 (14% ± 2%; Figures S6A and S6B), TRBs display a typical GH1 domain.^{19,78} Hence, we hypothesized that antagonistic chromatin incorporation of the GH1 domains of TRB and H1 proteins might modulate PRC2 recruitment at ITRs.

To test this model, we first compared H1 and TRB1 genomic distribution. Analysis of available TRB1 ChIP-seq data⁷⁶ showed that TRB1 peak summits expectedly correlate with the position of *telobox* motifs located in protein-coding genes. However, despite the presence of numerous *telobox* sequences, TRB1 poorly occupies *TE cluster 1* elements (Figures 6C and S6C). Reciprocally, H1 average occupancy is low at TRB1 peaks over the genome (Figures 6D and S6D). These observations hint at an antagonistic *cis* enrichment of H1 and TRB1 at chromatin. To better resolve these general patterns and link them to linker DNA positioning, we examined the profiles of H1, TRB1, *telobox* motifs, and nucleosome occupancy around well-positioned nucleosome (WPN) coordinates defined using MNase-seq.²⁸ As expected, H1.2-GFP distribution was enriched at DNA linker regions. Surprisingly, this was also the case for *telobox* motif distribution, which sharply coincided with regions serving as linker DNA. While TRB1 peaks

appeared much broader, their summits were also more pronounced at regions corresponding to linker DNA coordinates (Figure 6E). Hence, if it exists, competitive binding between H1 and TRB proteins likely occurs at linker DNA.

These observations are all compatible with a mechanism by which high H1 occupancy at ITRs prevents TRB1 DNA binding. Vice versa, increased access to ITRs in *2h1* mutant plants would facilitate TRB1-mediated PRC2 recruitment. To functionally assess whether this model holds true, we first examined whether GFP-TRB1 accumulates at ITRs in *2h1* plants and then determined the H3K27me3 profile in mutant plants lacking both H1 and TRB1, TRB2, and TRB3. To undertake the first experiment, we crossed a *TRB1::GFP-TRB1* line^{76,77} with *2h1* and revealed GFP-TRB1 genome association by ChIP-seq and ChIP telomere dot blot. Comparison of GFP-TRB1 chromatin association in WT and *2h1* plants showed a significantly increased association at *TE cluster 1*, ITR-1R and ITR-4L, and at telomeres (Figures 7A, S4B, S7A, and S7B), thereby providing evidence that H1 restricts TRB1 binding to these loci *in vivo*.

We then determined whether abolishing simultaneously the expression of linker H1 and TRB1, TRB2, and TRB3 proteins affects PRC2 activity at ITRs. To probe H3K27me3 profiles in *h1.1h1.2trb1trb2trb3* quintuple-mutant plants (hereinafter referred to as *htrbQ* for short), we crossed *2h1* double-mutant plants to *trb1(+/-)trb2trb3* triple mutant plants propagated as a heterozygous state to accommodate the seedling lethality induced by *TRB123* combined loss of function.^{36,37} Homozygous *htrbQ* mutant seedlings exhibited an aggravated phenotype as compared to the *trb123* triple-mutant line (Figure 7B), a synergistic effect presumably reflecting a convergence of H1 and *TRB123* functions in the regulation of common genes.⁷⁹ Despite the dwarf morphology of the quintuple-mutant line, we conducted a ChIP-Rx profiling of H3K27me3 in homozygous WT, *2h1*, *trb123*, and *htrbQ* seedlings, all segregating from a single crossed individual. As compared to the *2h1* co-segregant siblings, in the quintuple-mutant seedlings, H3K27me3 enrichment was almost completely abolished at ITR-1R and more generally at *TE cluster 1* elements (Figures 7C, 7D, S7B, and S7C). Taken together, these analyses demonstrate that H1 occupancy at ITRs antagonizes TRB protein recruitment, thereby constituting a mechanism preventing invasion of these large chromosome blocks by H3K27me3.

DISCUSSION

H1 has a dual impact on H3K27me3 deposition in *Arabidopsis*

We report that *Arabidopsis* H1 is highly enriched at PRC2 target genes, where it typically promotes H3K27me3 enrichment and diminishes chromatin accessibility. Contrasting with this general tendency, we also identified an opposite role of H1 in limiting H3K27me3 deposition at interstitial and terminal telomeres as well as at a few genes. This unveiled that, in plants, H1 has a differential effect on H3K27me3 levels over thousands of protein-coding genes on the one hand and over loci characterized by repeated telomeric motifs on the other hand.

Considering that PRC2 activation is favored at chromatin made of closely neighboring nucleosomes,¹⁴ we postulate that

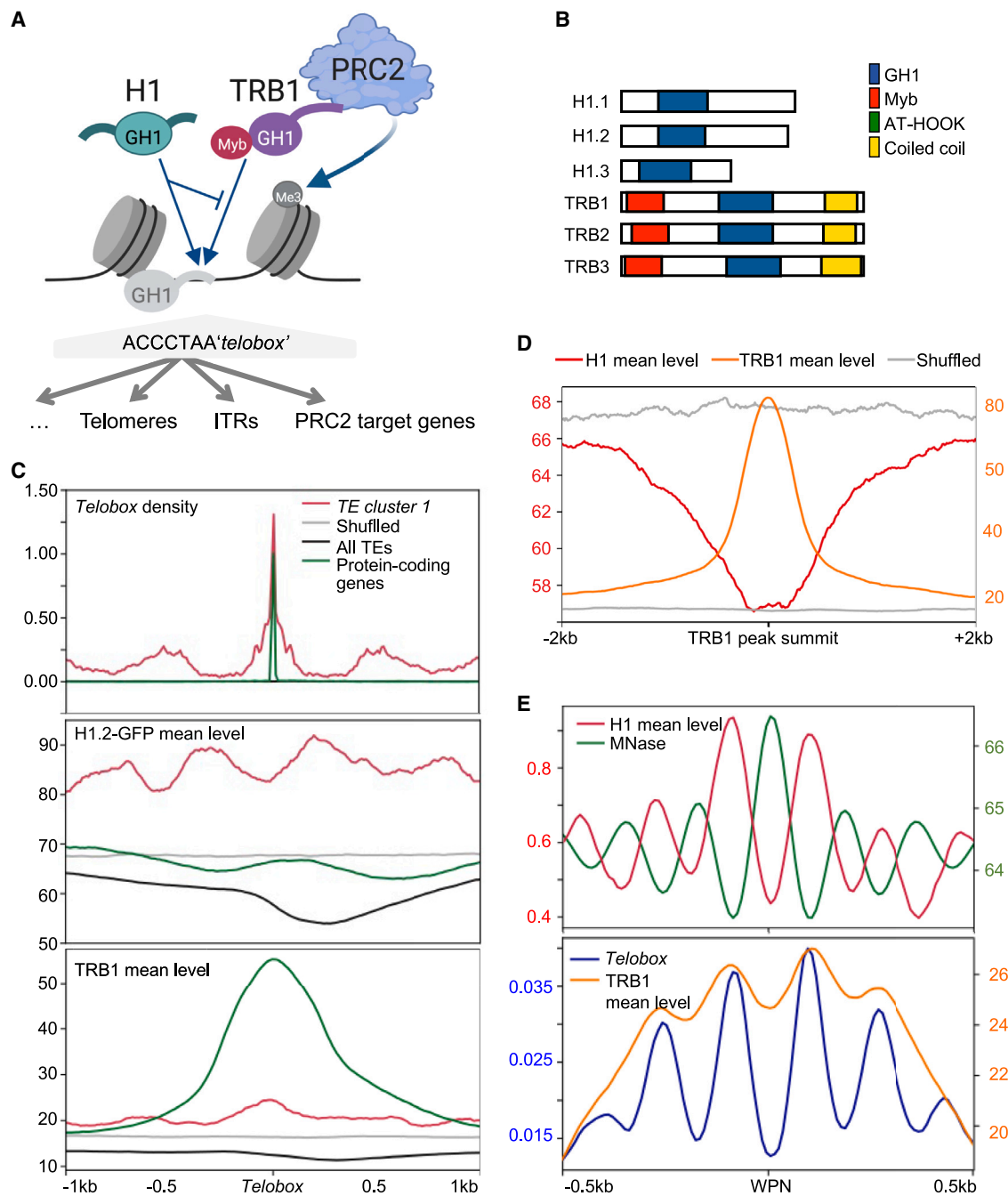


Figure 6. Antagonistic chromatin association of H1 and TRB1 over the genome

(A) Working model of H1/TRB1 antagonistic chromatin association at linker DNA-localized *telobox* motifs and its sequence-specific influence on PRC2 recruitment at distinct chromatin regions displaying telomeric repeats.

(B) TRB family members possess an amino-terminal single-Myb domain with sequence specificity for *telobox* motifs, a coiled-coil domain enabling their association with PRC2 subunits,^{36,37} and a central GH1 domain that may trigger competitive binding with H1.

(C) H1 and TRB1 patterns are both influenced by *telobox* positioning, and they display an opposite trend at *TE cluster 1 telobox* motifs. The plots display TRB1 and H1 mean read coverage at all TAIR10 genome *telobox* motifs.

(D) H1 occupancy is reduced at genome loci corresponding to TRB1 peak summits.

(E) H1, TRB1, and *telobox* motifs all tend to associate with DNA linker regions. Genome-wide profiles of H1, TRB1, and *telobox* sequence motifs were plotted over the coordinates of all *Arabidopsis* WPNs defined by Lyons and Zilberman.²⁸ In (C) and (D), shuffled controls were produced with random permutations of genomic position of the regions of interest.

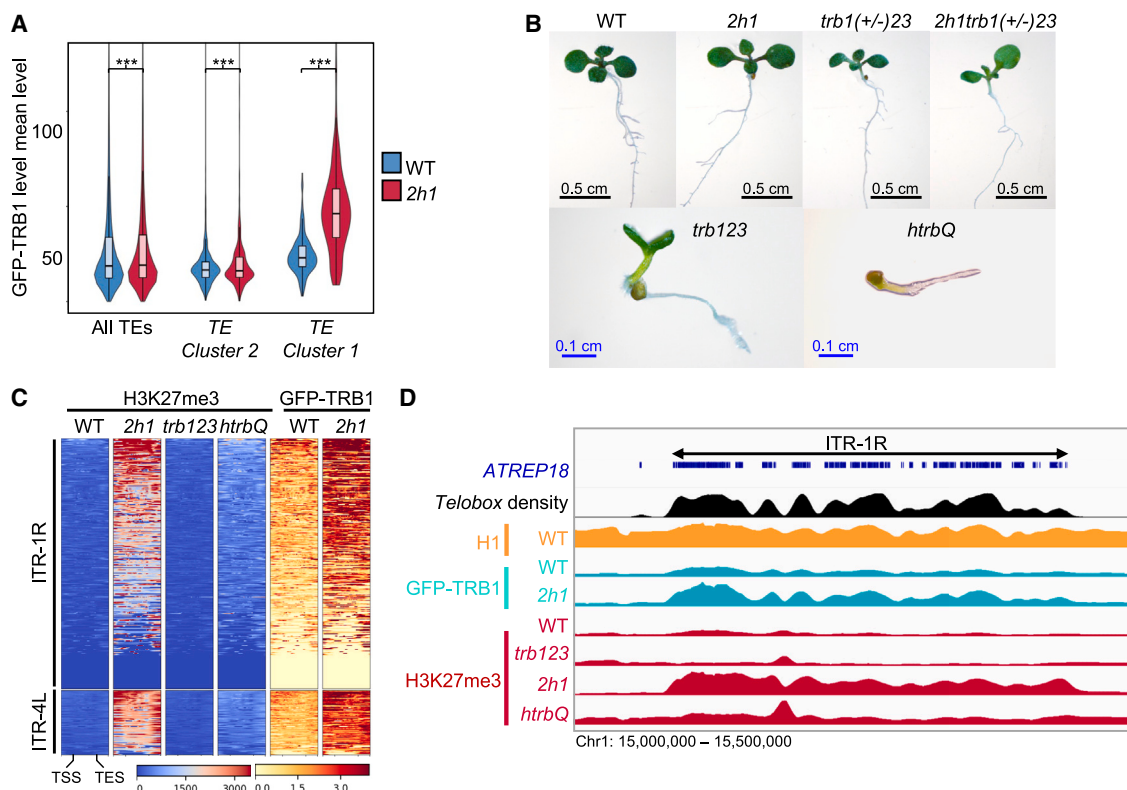


Figure 7. H1 antagonizes TRB1-mediated PRC2 activity at ITRs

(A) H1 restricts GFP-TRB1 protein association at *TE cluster 1* elements. The plots show GFP-TRB1 mean normalized coverage in WT and *2h1* seedlings at the indicated repeat categories. *** $p < 1.94 \times 10^{-5}$, Wilcoxon signed-rank test.

(B) Homozygous *h1.1h1.2trb1trb2trb3* (*htrbQ*) quintuple-mutant seedlings represented 25% of the segregating progeny and displayed strongly altered seedling phenotypes with deficient cotyledon development and slow root growth, indicating that morphogenesis is strongly affected upon combined *H1* and *TRB123* loss of function. WT, *2h1*, and *trb123* mutant lines have been selected as null F2 segregants from the same cross as the analyzed *htrbQ* plant line.

(C) H1 and TRB proteins are all required for H3K27me3 enrichment at ITR-1R and ITR-4L TEs. TAIR10 annotated repeats located within ITR-1R and ITR-4L coordinates were ranked similarly in all heatmaps. H3K27me3 levels were determined using spike-in normalized ChIP-seq analysis.

(D) Browser view showing that GFP-TRB1 and H3K27me3 enrichment at ITR-1R in *2h1* is lost in *htrbQ* mutant seedlings. Each ChIP series is shown as equally scaled profiles of the indicated genotypes. ChIP-seq and ChIP-Rx data represent the mean of two biological replicates each.

the repertoire of genes losing H3K27me3 upon H1 depletion are those where H1 is required to attain a compaction level enabling efficient PRC2 *cis* activity. Supporting this hypothesis, genes sensitive to H1 for efficient H3K27me3 marking tend to (1) display lower H1 and nucleosome occupancy, (2) be more accessible, and (3) be more expressed than genes unaffected by H1 depletion. In contrast, genes and TEs gaining H3K27me3 upon H1 loss tend to have an elevated nucleosome density and to be weakly accessible while exhibiting sequence signatures potentially triggering different mechanisms of PRC2 regulation, such as H1/TRB protein interplay. The large scale on which these antagonistic patterns are observed sheds light on the existence of prominent functional links between H1 and PRC2-based regulation, two main factors in the instruction of DNA accessibility.

Promoting H3K27me3 enrichment at genes: An evolutionarily conserved function of H1

We identified that H1 has a general role in H3K27me3 deposition at genes, yet most of the H3K27me3 peaks are still detectable in

2h1 plants. Hence, in agreement with the subtle phenotypes of *h1* mutant plants, H1 is likely not mandatory for the nucleation of PRC2 activity but rather for H3K27me3 maintenance or spreading in *Arabidopsis*. In term of chromatin function, H1 depletion results in a global increase in chromatin accessibility at gene bodies but its impact on expression was apparently more related to variations in H3K27me3 marking. Hence, consistent with the functional categories of the misregulated genes in *2h1* plants, part of the defects in gene expression resulting from H1 depletion might result from indirect consequences on PRC2 activity. The recent findings that depletion of H1 variants in mouse cells triggers widespread H3K27me3 loss and misregulation of PRC2-regulated genes, thereby phenocopying loss of EZH2,^{16,17} suggest that favoring PRC2 activity is an evolutionarily conserved function of H1.

H1 hinders PRC2 activity at telomeric repeats by preventing local association of TRB proteins

We provide evidence that H1 antagonizes TRB-mediated PRC2 activity at telomeric repeats. Waiting for an assessment

of their relative affinity for *telobox* elements in a chromatin context, H1/TRB1 proteins' antagonistic association along the genome plausibly results from competitive DNA binding of their respective GH1 protein domains. First, chromatin incorporation of H1 and TRB1 is negatively correlated at a genome-wide scale. Second, analysis of nucleosome positioning showed that *telobox* motifs are preferentially situated in linker DNA where TRB1 association is also pronounced; so that competition with H1 can occur on linker DNA. Third, profiling of TRB1 chromatin association in *2h1* plants showed that TRB1 ectopically invades ITRs and other *telobox*-rich elements upon H1 loss. These observations reveal that, in WT plants, elevated H1 incorporation limits TRB1 enrichment and/or accessibility on these loci despite the presence of repeated *telobox* motifs for which the TRB1 Myb domain has strong affinity.^{74,76}

H3K27me3 profiling in quintuple *2h1trb123* seedlings showed that H3K27me3 enrichment at ITRs in H1-depleted plants depends on TRB proteins, thereby demonstrating a functional framework in which repression of H3K27me3 deposition at telomeric repeats relies on H1 preventing local association of PRC2-associated TRB proteins. Future studies will determine whether other chromatin modifiers influencing H3K27me3 are implicated. The latter possibility cannot be discarded as, for example, the PRC1 subunit LIKE-HETEROCHROMATIN 1 (LHP1), acting as a chromatin reader of H3K27me3 in *Arabidopsis*,⁸⁰ prevents TRB1 enrichment at PRC2 target genes displaying *telobox* motifs.⁷⁷ The outstanding genome-wide pattern of *telobox* positioning in linker DNA also suggests a capacity of this sequence motif to influence chromatin organization, possibly by repelling nucleosomes.

H1 has a profound influence on the *Arabidopsis* 3D genome topology

Using Hi-C, we identified a reduced frequency of chromatin interactions within and among the pericentromeres in *2h1* nuclei. This is a typical feature of *Arabidopsis* mutants affecting chromocenter formation^{64,65,67} or when chromocenters get disrupted in response to environmental stress.⁶⁸ These analyses refine the recent observation that chromocenter formation is impaired in *2h1* nuclei,^{23,26,35} a defect that commonly reflects the spatial dispersion of pericentromeres within the nuclear space.⁶³ They also shed light on a complex picture in which ITR-1R and 4L embedded within the pericentromeres of chromosomes 1 and 4 escape the surrounding relaxation of heterochromatin induced by H1 depletion and organize themselves as TAD-like structures. In *2h1* nuclei, H3K27me3 invasion at ITRs might underlie the maintenance of compacted and poorly accessible chromatin, while neighboring heterochromatic regions tend to become more accessible. It is noteworthy that, in the absence of CTCF (CCCTC-binding factor) and of obvious related 3D structures, *Arabidopsis* is thought to lack proper TADs^{81–83}; hence, H1 regulation of ITR insulation represents a new regulatory function of *Arabidopsis* genome topology.

We also report that H1 depletion leads to a reduction in the number of telomeric foci and of their proportion near the nucleolus. This suggested that *2h1* mutants are impaired in telomere spatial individualization, which is indeed supported in our Hi-C

analyses by more frequent inter-chromosomal interactions between telomere-proximal regions. As the preferential positioning of telomeres around the nucleolus and centromeres near the nuclear periphery is an important organizing principle of *Arabidopsis* chromosome sub-nuclear positioning⁶³ and topology,⁶⁹ H1 therefore appears to be a crucial determinant of *Arabidopsis* nuclear organization.

Both PRC1 and PRC2 participate in defining *Arabidopsis* genome topology,^{64,84} and H3K27me3 is favored among long-distance-interacting gene promoters.⁶⁶ This led to the proposal that, as in animals, this mark could contribute to shape chromosomal organization in *Arabidopsis*, possibly through the formation of *Polycomb* sub-nuclear bodies.^{66,85} Here we mostly focused on large structural components of the genome, such as telomeres, pericentromeres, and ITR regions. In mammals, H1 depletion triggers not only higher-order changes in chromatin compartmentation^{16,17} but also extensive topological changes of gene-rich and transcribed regions.¹⁸ Future studies will determine to what extent the impact of H1 on the H3K27me3 landscape contributes to defining *Arabidopsis* genome topology.

H1 as a modulator of H3K27me3 epigenome homeostasis

In *Neurospora crassa*, artificial introduction of an (TTAGGG)₁₇ telomere repeats array at interstitial sites was shown to trigger the formation of a large H3K27me2/3-rich chromosome domain.⁸⁶ Followed by our study, this illustrates the intrinsic attractiveness of telomeric motifs for H3K27me3 deposition in multiple organisms. With several thousands of telomeric motifs altogether covering ~430 kb, ITRs represent at least twice the cumulated length of all telomeres in *Arabidopsis* diploid nuclei, thereby forming immense reservoirs of PRC2 targets. Our findings led us to hypothesize that H1-mediated repression of PRC2 activity at these scaffolding domains serves as a safeguard to avoid the formation of gigantic H3K27me3-rich blocks in both pericentromeric and telomeric regions, which not only can be detrimental for chromosome folding but could also be on a scale tethering PRC2 complexes away from protein-coding genes. In other terms, balancing PRC2 activity between protein-coding genes and telomeric repeats, H1 protein regulation may represent an important modulator of epigenome homeostasis during development.

Limitations of the study

Owing to their repetitive nature,^{87–92} chromatin composition and organization of plant telomeres has long remained enigmatic.^{93,94} Former studies indicated a dominance of H3K9me2 over H3K27me3 histone marks.^{62,89,95} Using ChIP dot blot and *in situ* immunolocalization with telomeric probes, here we showed that H1 moderates the accumulation of H3K27me3 at telomeres by 2- to 4-fold, yet this effect could be indirect and not homogeneous. Hence, two limitations of our study are that we could not assess the precise distribution of H3K27me3 enrichment along each telomere and whether H1 acts on PRC2 at telomeres *in cis*. In agreement with the mosaic chromatin status of telomeres in other organisms,⁹⁶ *Arabidopsis* telomeres are thought to be made of segments with distinct nucleosome repeat length (NRL) with average length of 150 bp,⁹⁷ a much

shorter size than the 189 bp estimated for H1-rich TEs.²³ Considering that H1 protects about 20 bp of DNA *in vitro*,⁹⁸ an NRL length of 150 bp is seemingly incompatible with H1 incorporation into telomere chromatin. For instance, H1 has been proposed to be under-represented at telomeres in plants^{97,99} as it is in mammals.^{93,100–102} This could explain the short NRL of *Arabidopsis* and human telomeres^{97,103} that, long after being suspected,¹⁰⁴ have recently been re-constructed as an H1-free state columnar organization.¹⁰⁵ In conclusion, the existence of distinct chromatin states at *Arabidopsis* telomeres needs to be explored in more detail to establish whether the H1-mediated repression of PRC2 activity is a global property of telomeres or rather affects a few segments through H1 *cis* association.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

G.T., L.W., I.B., and C. Bourbousse performed ChIP and ChIP-RX experiments. G.T. and L.W. performed ATAC-seq experiments. K.A. and M.F. performed telomere dot blots. I.B. performed phenotypic analyses. M.B. contributed to FACS nucleus sorting. S.A. performed cytological experiments. G.T. and S.G. generated the Hi-C datasets. A.K. and V.C. developed ATAC-seq bioinformatics tools. L. Concia, G.T., and C. Bourbousse performed RNA-seq, MNase-seq, ChIP-seq, and ATAC-seq bioinformatics analyses. L. Concia, L. Carron, and S.G. performed Hi-C bioinformatics. G.T., C. Bourbousse, L. Concia, and F.B. conceived the study. F.B., C. Bourbousse, A.C., C. Baroux, C. Bowler, A.C., S.A., A.P., U.G., P.P.S., J.F., and S.G. supervised research. F.B. wrote the manuscript and all authors edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit anti-H3K27me3	Merck	Cat# 07-449; RRID: AB_310624
Goat biotin anti Rabbit IgG	ThermoFisher	Cat# 65-6140; RRID: AB_2533969
Mouse anti-digoxigenin	Roche	Cat#11333062910; RRID: AB_514495
Rat anti-mouse FITC	Invitrogen	Cat# rmg101; RRID: AB_2556582
Mouse Cy3 anti-biotin antibody	Sigma	Cat# C5585; RRID: AB_258901
Anti-GFP	Thermo Fisher	Cat# A11122; RRID: AB_221569
Mouse Anti-H3	Abcam	Cat# Ab1791; RRID: AB_302613
Rabbit anti-H3K27me3	Diagenode	Cat# C15410069; RRID: AB_2814977
Chemicals, peptides, and recombinant proteins		
Percoll	Sigma-Aldrich	#P1644
Protein-A/G Dynabeads	Invitrogen	#10004D
Agencourt® AMPure® XP Beads	Beckman Coulter	#A63880
DpnII	New England Biolabs	#R0543T
DNA Polymerase I, Large (Klenow) Fragment	New England Biolabs	#M0210L
T4 ligase (HC)	Promega	#MI79A
Critical commercial assays		
Nextera DNA Library Prep Kit	Illumina	#FC-121-1030
MinElute® PCR Purification Kit	QIAGEN	#28004
RNeasy micro kit (Qiagen)	QIAGEN	#74004
KAPA LTP Library Preparation Kit	Roche	#KR0961
the Pierce™ BCA Protein Assay Kit	Thermo Scientific™	#23225
NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®	New England Biolabs	#E7645
Deposited data		
ATAC-seq of WT and <i>2h1</i> plants	This study	GEO: GSE160408
H3K27me3 ChIP-Rx of Wt and <i>2h1</i> plants	This study	GEO: GSE160410
H1-2-GFP ChIP-seq of WT plants	This study	GEO: GSE160411
Hi-C in WT and <i>2h1</i> plants	This study	GEO: GSE160412
RNA-seq of WT and <i>2h1</i> plants	This study	GEO: GSE160413
H3K4me3 ChIP-seq of WT plants	Fiorucci et al., ¹⁰⁶	GEO: GSE124318
H2Bub ChIP-seq of WT plants	Nassrallah et al., ⁴⁵	GEO: GSE112952
MNase-seq of WT and <i>2h1</i> plants	Lyons & Zilberman, ²⁸	GEO: GSE96994
WGBS of WT and <i>2h1</i> plants	Lyons & Zilberman., ²⁸	GEO: GSE96994
H3K9me2 ChIP-seq of WT plants	Ma et al., ⁵¹	GEO: GSE111814
H3K27me1 ChIP-seq of WT plants	Ma et al., ⁵¹	GEO: GSE111814
TRB1 ChIP-seq of WT plants	Schrumpfová et al., ⁷⁶	GEO: GSE69431
H3K27me3 ChIP-seq of WT and <i>ref. 6elf6jmj13</i> plants	Yan et al., ⁵²	GEO: GSE106942
Experimental models: Organisms/strains		
Arabidopsis: Col 0	Arabidopsis Biological Resource Center	CS22625
<i>h1.1h1.2</i>	Rutowicz et al.,	N/A
<i>TRB1::GFP-TRB1</i>	Schrumpfová, P.P. ⁷³	N/A
<i>trb1</i>	NASC	Salk_001540
<i>trb2</i>	FLAGdb/FST collection ¹⁰⁷	Flag_242F11
<i>trb3</i>	NASC	Salk_134641
<i>trb1trb2trb3</i>	This study	N/A

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
Trim Galore version: 0.6.4_dev Cutadapt version: 2.10	https://doi.org/10.5281/zenodo.5127898	N/A
STAR (version 2.7.3a)	Dobin et al., ¹⁰⁸	N/A
DESeq2 package	Love et al., ¹⁰⁹	N/A
HTSeq suite (version 0.11.3)	Anders et al., ¹¹⁰	N/A
R package dplyr	https://CRAN.R-project.org/package=dplyr	N/A
Hi-C Pro pipeline	Servant et al., ¹¹¹	N/A
Boost-HiC	Carron et al., ¹¹²	N/A
Juicebox toolsuite	Duran et al., ¹¹³	N/A
HOMER	Heinz et al., ⁷²	N/A
Bowtie2v.2.3.2	Langmead et al., ¹¹⁴	N/A
MACS2	Zhang et al., ¹¹⁵	N/A
sambamba v0.6.8.	Tarasov et al., ¹¹⁶	N/A
bedtools v2.29.2	Qinlan et al., ¹¹⁷	N/A
Genomics Viewer (IGV) version 2.8.0	Thorvaldsdóttir et al., ¹¹⁸	N/A
ASAP ATAC-Seq data Analysis Pipeline	https://zenodo.org/record/1466008	N/A
MEME version 5.1.1	Bailey et al., ¹¹⁹	N/A
REVIGO	Supek et al., ¹²⁰	N/A
T-Coffee	Notredame et al., ¹²¹	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Fredy Barneche (barneche@bio.ens.psl.eu).

Materials availability

Arabidopsis transgenic and mutant lines generated in this study will be made available upon request.

Data and code availability

All data reported in this paper has been deposited at Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo/>) public repository under the accession number GSE160414. This paper analyzes existing, publicly available datasets whose accession numbers are listed in the [key resources table](#). All original code is available in this paper's supplemental information. Publicly available code used in this paper is listed in the [key resources table](#). Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Arabidopsis thaliana

Seeds were surface-sterilized, plated on half strength Murashige and Skoog (MS) medium with 0.9% agar and 0.5% sugar, and cultivated under long-day (16h/8h) at 23/19°C light/dark photoperiod (100 $\mu\text{mol m}^{-2}\text{s}^{-1}$) for 5 days unless otherwise stated. Cotyledons, when used, were manually dissected under a stereomicroscope. The *h1.1h1.2 (2h1)* Arabidopsis mutant line and the transgenic *pH1.2::H1.2-GFP* line²² were kindly provided by Dr. Kinga Rutowicz (University of Zurich, Switzerland). The *TRB1::GFP-TRB1* line described in.⁷⁶ The *2h1/TRB1::GFP-TRB1* transgenic line was obtained upon manual crossing of the *2h1* and *TRB1::GFP-TRB1* line described previously in.⁷⁶ The *trb123* triple mutant line was produced by crossing a *trb1trb2* double homozygous plant (derived from a cross between *trb1* (Salk_001540) and *trb2* (Flag_242F11) mutant alleles) with a double homozygous *trb2trb3* mutant plant (derived from a cross between *trb2* (Flag_242F11) with *trb3* (Salk_134641).

METHOD DETAILS

Immuno-FISH

After fixation in 4% paraformaldehyde in 1X PME, cotyledons of 7-day-old seedlings were chopped directly in 1% cellulase, 1% pectolyase, and 0.5% cytohelicase in 1X PME, and incubated 15 min. Nucleus suspensions were transferred to poly-Lysine-coated

slides. One volume of 1% lipsol in 1X PME was added to the mixture and spread on the slide. Then, 1 volume of 4% PFA in 1X PME was added and slides were dried. Immunodetection and FISH were conducted as described previously⁷⁸ using the following antibodies: rabbit H3K27me3 (#07-449 - Merck) diluted 1:200, Goat biotin anti Rabbit IgG (#65-6140 - ThermoFisher) 1:500, mouse anti-digoxigenin (#11333062910 - ROCHE) 1:125, rat anti-mouse FITC (#rmg101 - Invitrogen) at 1:500 at 1:100, mouse Cy3 anti-biotin antibody (#C5585 - Sigma) at 1:1000. Acquisitions were performed on a structured illumination (pseudo-confocal) imaging system (ApoTome AxioImager M2; Zeiss) and processed using a deconvolution module (regularized inverse filter algorithm). The colocalization was analyzed via the colocalization module of the ZEN software using the uncollapsed z stack files. To test for signal colocalization, the range of Pearson correlation coefficient of H3K27m3 vs. telomeric FISH signals were calculated with the colocalization module of the ZEN software using z stack files. Foci with coefficients superior to 0.5 were considered as being colocalized.

ATAC-seq

Nuclei were isolated from 200 cotyledons of 5-day-old seedlings and purified using a two-layer Percoll gradient at 3000 g before staining with 0.5 μ M DAPI and sorting by FACS according to their ploidy levels using a MoFlo Astrios EQ Cell Sorter (Beckman Coulter) in PuraFlow sheath fluid (Beckman Coulter) at 25 psi (pounds per square inch), with a 100- μ m nozzle. We performed sorting with \sim 43 kHz drop drive frequency, plates voltage of 4000–4500 V and an amplitude of 30–50 V. Sorting was performed in purity mode. For each sample, 20000 sorted 4C nuclei were collected separately in PBS buffer and centrifuged at 3,000 g at 4°C for 5 min. The nuclei were re-suspended in 20 μ L of Tn5 transposase reaction buffer (Illumina). After tagmentation, DNA was purified using the MinElute PCR Purification Kit (Qiagen) and amplified with Nextera DNA Library Prep index oligonucleotides (Illumina). A size selection was performed with AMPure XP beads (Beckman Coulter) to collect library molecules longer than 150 bp. DNA libraries were sequenced by Beijing Genomics Institute (BGI Group, Hong-Kong) using the DNA Nanoballs (DNB) DNBseq in a 65 bp paired-end mode.

In situ Hi-C

Hi-C was performed as in Grob et al. (2014)⁶⁵ with downscaling using seedlings crosslinked in 10 mM potassium phosphate pH 7.0, 50 mM NaCl, 0.1 M sucrose with 4% (v/v) formaldehyde. Crosslinking was stopped by transferring seedlings to 30mL of 0.15 M glycine. After rinsing and dissection, 1000 cotyledons were flash-frozen in liquid nitrogen and ground using a Tissue Lyser (Qiagen). All sample were adjusted to 4 mL using NIB buffer (20 mM HEPES pH7.8, 0.25 M sucrose, 1 mM MgCl₂, 0.5 mM KCl, 40% v/v glycerol, 1% Triton X-100) and homogenized on ice using a Dounce homogenizer. Nuclei were pelleted by centrifugation and resuspended in the DpnII digestion buffer (10 mM MgCl₂, 1 mM DTT, 100 mM NaCl, 50 mM Bis-Tris-HCl, pH 6.0) before adding SDS to a final concentration of 0.5% (v/v). SDS was quenched by adding 2% Triton X-100. DpnII (200 u) was added to each sample for over-night digestion at 37°C dATP, dTTP, dGTP, biotinylated dCTP and 12 μ L DNA Polymerase I (Large Klenow fragment) were added before incubation for 45 min at 37°C. A total of 50 unit of T4 DNA ligase along with 7 μ L of 20 ng/ μ L of BSA (Biolabs) and 7 μ L of 100 mM ATP were added to reach a final volume of 700 μ L. Samples were incubated for 4h at 16°C with constant shaking at 300rpm. After over-night reverse crosslinking at 65°C and protein digestion with 5 μ L of 10 mg/ μ L proteinase K, DNA was extracted by phenol/chloroform purification and ethanol precipitation before resuspension in 100 μ L of 0.1X TE buffer. Biotin was removed from the unligated fragment using T4 DNA polymerase exonuclease activity. After biotin removal, the samples were purified using AMPure beads with a 1.6X ratio. DNA was fragmented using a Covaris M220 sonicator (peak power 75W, duty factor 20, cycles per burst 200, duration 150 s). Hi-C libraries were prepared using KAPA LTP Library Preparation Kit (Roche)⁶⁵ with 12 amplification cycles. PCR products were purified using AMPure beads (ratio 1.85X). Libraries were analyzed using a Qubit fluorometer (ThermoFisher) and a TAPE Station (Agilent) before sequencing in a 75 bp PE mode using a DNB-seq platform at the Beijing Genomics Institute (BGI Group; Honk Kong).

RNA-seq

Upon growth 5 days under long days conditions (16h light at 23°C, 8h dark at 19°C), seedlings were fixed in 100% cold acetone under vacuum for 10 min. Cotyledons from 100 plants were manually dissected and ground in 2 mL tubes using a Tissue Lyser (Qiagen) for 1 min 30 s at 30 Hz before RNA extraction using the RNeasy micro kit (Qiagen). RNA was sequenced using the DNBseq platform at the Beijing Genomics Institute (BGI Group) in a 100 bp paired-end mode. For raw data processing, sequencing adaptors were removed from raw reads with trim_galore! 0.6.4_dev Cutadapt version: 2.10. Reads were mapped onto TAIR10 genome using STAR version 2.7.3a¹⁰⁸ with the following parameters “–alignIntronMin 20 –alignIntronMax 100000 –outFilterMultimapNmax 20 –outMultimapperOrder Random –outFilterMismatchNmax 8 –outSAMtype BAM SortedByCoordinate –outSAMmultNmax 1 –alignMatesGapMax 100000”. Gene raw counts were scored using the htseq-count tool from the HTSeq suite version 0.11.3¹¹⁰ and analyzed with the DESeq2 package¹⁰⁹ to calculate Log₂-fold change and to identify differentially expressed genes (p value <0.01). TPM (Transcripts per Million) were retrieved by dividing the counts over each gene by its length in kb and the resulting RPK was divided by the total read counts in the sample (in millions). Mean TPM values between two biological replicates were used for subsequent analyses. To draw metagene plots, genes were grouped into expressed or not and expressed genes split into four quantiles of expression with the function ntile() of the R package dplyr (=https://CRAN.R-project.org/package=dplyr).

H1.2-GFP, GFP-TRB1 and H3 ChIP-seq experiments

H1.2-GFP and parallel H3 profiling were conducted as in Fiorucci et al. (2019)¹⁰⁶ after sonicating chromatin to mono/di-nucleosome fragment sizes. WT Col-0 or *pH1.2::H1.2-GFP* seedlings were crosslinked for 15 min using 1% formaldehyde. After dissection, 400 cotyledons were ground in 2 mL tubes using a Tissue Lyser (Qiagen) for 2 × 1 min at 30 Hz. After resuspension in 100 μ L Nuclei Lysis Buffer 0.1 %SDS, the samples were flash frozen in liquid nitrogen and chromatin was sheared using an S220 Focused-Ultrasonicator (Covaris) for 17 min at peak power 105 W, duty factor 5%, 200 cycles per burst, to get fragment sizes between 75 and 300 bp. Immunoprecipitation was performed on 150 μ g of chromatin quantified using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific) with 60 μ L of Protein-A/G Dynabeads and 3.5 μ L of anti-GFP (Thermo Fisher #A11122) for H1.2-GFP and mock (WT) sample or anti-H3 (Abcam #Ab1791) for H3 IPs. Immunoprecipitated DNA was subjected to library preparation using the TruSeq ChIP Sample Preparation Kit (Illumina) and sequenced using a NextSeq 500 system or DNBSEQ-G400 in a single-end 50 bp mode (Genewiz, USA; Fasteris, Switzerland and DNBseq BGI, Hong-Kong).

H3K27me3 ChIP-Rx

ChIP-Rx of WT and *2h1* plants corresponding to Figures 1, 2, 3, 4, 5, and 6 and of WT, *2h1*, *trb123* and *htrbQ* plants corresponding to Figure 7 were performed using anti-H3K27me3 #07–449 (Millipore) and #C15410069 (Diagenode), respectively. Both ChIP-Rx series were conducted as in Nassrallah et al. (2018)⁴⁵ using two biological replicates of 8-day-old WT and *2h1* seedlings. For each biological replicate, two independent IPs were carried out using 120 μ g of Arabidopsis chromatin mixed with 3% of Drosophila chromatin quantified using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). DNA samples eluted and purified from the two technical replicates were pooled before library preparation (Illumina TruSeq ChIP) and sequencing (Illumina NextSeq 500, 1x50bp or DNBSEQ-G400, 1x50bp) of all input and IP samples by Fasteris (Geneva, Switzerland) and BGI (Hong-Kong), respectively.

H3K27me3 and H3 ChIP-blot analyses

Anti-H3K27me3 (Millipore, #07–449 antibody) and anti-H3 (Abcam #Ab1791 antibody) ChIPs were conducted using 2 g of tissue. Pellets of both inputs (20%) and immunoprecipitated DNA were resuspended in 40 μ L of TE, pH 8.0 and analyzed through dot-blot hybridization using a radioactively labeled telomeric probe synthesized by non-template PCR.^{62,122} ITRs contribution to the hybridization signal was minimized using high stringency hybridization as detailed in.⁶²

Hi-C bioinformatics

Hi-C reads were mapped using the Hi-C Pro pipeline¹¹¹ with default pipeline parameters and merging data from three biological replicates at the end of the pipeline. Data were visualized using the Juicebox toolsuite¹¹³ and represented in Log10 scale after SCN normalization¹²³ with Boost-HiC¹¹² setting alpha parameter to 0.2. In Figure S4, we normalized the sequencing depth in each sample and scored the number of reads in each combination of genomic regions using HOMER.⁷² Read counts were further normalized for the bin size and the median value between the three biological replicates was reported. Distal-to-Local [log2] Ratios (DLR) were implemented as described in HOMER⁷² and adapted to define local interactions between a defined size window (k) and the two surrounding windows as distal regions at 10kb and 100kb for k = 2 to k = 150 bins and selected for each ITR a windows value corresponding of 3 ITR sizes (1050 kb for ITR-1R and 240 kb for ITR-4L).

ChIP-seq and ChIP-Rx bioinformatics

For H3K27me3 spike-in normalized ChIP-Rx, raw reads were pre-processed with Trimmomatic v0.36¹²⁴ to remove leftover Illumina sequencing adapters. 5' and 3' ends with a quality score below 5 (Phred+33) were trimmed and reads shorter than 20 bp after trimming were discarded (trimmomatic-0.36.jar SE -phred33 INPUT.fastq TRIMMED_OUTPUT.fastq ILLUMINACLIP:TruSeq3-SE.fa:2:30:10 LEADING:5 TRAILING:5 MINLEN:20). We aligned the trimmed reads against combined TAIR10 Arabidopsis thaliana and Drosophila melanogaster (dm6) genomes with Bowtie2v.2.3.2 using the “–very-sensitive” setting. Duplicated reads and reads mapping to regions with aberrant coverage or low sequence complexity defined in¹²⁵ were discarded with sambamba v0.6.8.¹¹⁶ Peaks of H3K27me3 read density were called using MACS2¹¹⁵ with the command “macs2 callpeak -f BAM –nomodel -q 0.01 -g 120e6 –bw 300 –verbose 3 –broad”. Only peaks found in both biological replicates and overlapping for at least 10% were retained for further analyses. Annotation of genes and TEs overlapping with peaks of histone marks H3K27me3, H3K4me3, and H2Bub were identified using bedtools v2.29.2 intersect as for H3K27me3. We scored the number of H3K27me3 reads overlapping with marked genes using bedtools v2.29.2 multicov and analyzed them with the DESeq2 package¹⁰⁹ in the R statistical environment v3.6.2 to identify the genes enriched or depleted in H3K27me3 in *2h1* plants (p value <0.01). To account for differences in sequencing depth we used the function SizeFactors in DESeq2, applying a scaling factor calculated as in Nassrallah et al. (2018).⁴⁵ For GFP-TRB1, H1.2-GFP and H3 ChIP-seq datasets, raw reads were processed as for H3K27me3. We counted the reads over genes and TEs using bedtools v2.29.2 multicov and converted them in median counts per million, dividing the counts over each gene or TE by its length and by the total counts in the sample and multiplying by 106 to obtain CPMs (Counts per Million reads). Mean read coverage was used in Figure 1A, while the ratio between median value between biological replicates in IP and median value in Input was used for violin-plot analysis of H1.2-GFP in Figures S3B and S6C. To include nucleosomes in close proximity to gene TSS, an upstream region of 250 bp was also considered for the overlap (minimum 150 bp) for H3K27me3, TRB1 and H3K4me3 (datasets detailed in the key resources table). H3K27me3 TE cluster 1 and TE cluster 2 were identified using Deeptools plotHeatmap using the –kmeans

option set at 2. Tracks were visualized using Integrative Genomics Viewer (IGV) version 2.8.0.¹¹⁸ Meta-gene plots and heatmaps were generated from depth-normalized read densities using Deeptools computeMatrix, plotHeatmap, and plotProfile. Violin-plots, histograms and box-plots were drawn using the package ggplot2 v3.2.1 (<https://cran.r-project.org/web/packages/ggplot2/>) in the R statistical environment. All scripts used will be made publicly available. Shuffled controls were produced with random permutations of genomic position of the regions of interest. The permutations were generated with bedtools v2.29.2 and the command "bedtools shuffle -chromFirst -seed 28776 -chrom".

MNase-seq bioinformatics

MNase read density²⁸ was obtained from NCBI GEO under the accession GSE96994. Genomic location of WPNs shared between WT and 2h1 plants were identified as overlapping WPN coordinates between the two genotypes calculated with bedtools v2.29.2 intersect.

ATAC-seq bioinformatics

Raw ATAC-seq data were treated using the custom-designed ASAP pipeline (ATAC-Seq data Analysis Pipeline; <https://zenodo.org/record/1466008>). Mapping was performed using Bowtie2 v.2.3.2¹¹⁴ with parameters -very-sensitive -X 2000. Mapped reads with MAPQ<10, duplicate pairs, and reads mapping to the mitochondrial genome as well as regions with aberrant coverage of low sequence complexity defined in¹²⁵ were filtered out. Concordant read pairs were selected and shifted as previously described by 4 bp.¹²⁶ Peak calling was performed using MACS2¹¹⁵ using broad mode and the following parameters: -nomodel -shift -50 -extsize 100. Heatmaps and metaplots were produced from depth-normalized read coverage (read per million) using the Deeptools suite.¹²⁷

DNA sequence analyses

Motifs enriched in gene promoters (-500 bp to +250 bp after the TSS) and in annotated units of TE cluster 1 elements were identified using MEME version 5.1.1.¹¹⁹ The following options were used for promoters: "-dna -mod anr -revcomp -nmotifs 10 -minw 5 -maxw 9" and for TEs: "-dna -mod anr -nmotifs 10 -minw 5 -maxw 9 -objfun de -neg Araport11_AllTEs.fasta -revcomp -markov_order 0 -maxsites 10000" where Araport11_AllTEs.fasta correspond to the fasta sequence of all TEs annotated in Araport11.

Telobox positioning was analyzed using the TAIR10 coordinates described in Zhou et al.³⁷ and obtained from https://gbrowse.mpg.de/cgi-bin/gbrowse/arabidopsis10_turck_public/?l=telobox;f=save+datafile. Telobox repeat numbers were scored over 10-bp non-overlapping bins, smoothed with a 50-bp sliding window and subsequently used to plot telobox density.

Gene ontology analysis

Gene ontology analysis of H3K27me3 differentially marked genes were retrieved using the GO-TermFinder software¹²⁸ via the Princeton GO-TermFinder interface (<http://go.princeton.edu/cgi-bin/GOTermFinder>). The REVIGO¹²⁰ platform was utilized to reduce the number of GO terms and redundant terms were further manually filtered. The Log10 p values of these unique GO terms were then plotted with pheatmap (<https://CRAN.R-project.org/package=pheatmap>) with no clustering.

Protein alignment

Protein sequences of H1.1, H1.2, H1.3, TRB1, TRB2 and TRB3 were aligned using T-Coffee¹²¹ (<http://tcoffee.crg.cat/apps/tcoffee/do:regular>) with default parameters. Pairwise comparison for similarity and identity score were calculated using Ident and Sim tool (https://www.bioinformatics.org/sms2/ident_sim.html).

QUANTIFICATION AND STATISTICAL ANALYSES

Unless stated otherwise, statistical tests were performed with the R package rstatix_0.7.1 (<https://CRAN.R-project.org/package=rstatix>) using the functions wilcox_test and wilcox_effsize. All pairwise comparisons between the read coverage in WT and 2h1 over a given set of gene or TEs were tested with Wilcoxon signed rank test for paired samples, using the wilcox_test function with the option "paired = TRUE". All other comparisons were tested with Wilcoxon rank-sum test for independent samples, setting the option "paired = FALSE".