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Arabidopsis YAF9 histone readers modulate flowering time through NuA4-complex-dependent H4 and H2A.Z histone acetylation at *FLC* chromatin

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Summary

• Post-translational histone modifications and the dynamics of histone variant H2A.Z are key mechanisms underlying the floral transition. In yeast, SWR1-C and NuA4-C mediate the deposition of H2A.Z and the acetylation of histone H4, H2A and H2A.Z respectively. Yaf9 is a subunit shared by both chromatin remodeling complexes. The significance of the two Arabidopsis YAF9 homologs, YAF9A and YAF9B, is unknown.

• To get an insight on the role of Arabidopsis YAF9 proteins in plant developmental responses, we followed physiological, genetic, genomic, epigenetic, proteomics and cell biology approaches.

• Our data reveal that YAF9A and YAF9B are histone H3 readers with unequally redundant functions. Double mutant *yaf9a yaf9b* plants display pleiotropic developmental phenotypic alterations as well as misregulation of a wide variety of genes. We demonstrate that YAF9 proteins regulate flowering time by both FLC-dependent and independent mechanisms that work in parallel to SWR1-C. Interestingly, we show that YAF9A binds *FLC* chromatin and that YAF9 regulate *FLC* expression by modulating the acetylation levels of H2A.Z and H4 but not H2A.Z deposition.

• Our work highlights the key role exerted by YAF9 homologues in the post-translational modification of canonical histones and variants that regulate gene expression in plants to control development.

Keywords: YAF9; Arabidopsis; Flowering; SWR1-C, NuA4-C; H2A.Z, histone acetylation, histone reader

Introduction

The floral transition is controlled by a variety of environmental stimuli including temperature and light, which provide seasonal cues, and also by endogenous signals such as plant age, nutrient levels and phytohormones (Amasino & Michaels, 2010; Imaizumi, 2010; Srikanth & Schmid, 2011). The right timing of this developmental switch relative to optimal seasons for growth and reproduction is crucial to determine the reproductive success and

fitness of plant species (Amasino, 2010; Andres & Coupland, 2012). For that reason plants fine-tune the time of flowering initiation at multiple levels (Bloomer & Dean, 2017), ensuring that the production of flowers and fruits takes place under the most favourable conditions.

Arabidopsis thaliana is an annual facultative long-day (LD) species that perceives photoperiod cues in the leaves using circadian clock–associated mechanisms (Song *et al.*, 2015). In winter annuals, the vernalization pathway mediates the flowering induction by prolonged exposure to low non-freezing temperature, preventing early flowering in autumn (Bloomer & Dean, 2017; Whittaker & Dean, 2017). Vernalization requirement relies on dominant alleles at the *FRIGIDA* (*FRI*) (Johanson *et al.*, 2000) and *FLOWERING LOCUS C* (*FLC*) loci (Michaels & Amasino, 1999). FRI promotes the transcriptional activation of *FLC*, increasing its expression to levels that confer delayed flowering (Michaels & Amasino, 1999; Johanson *et al.*, 2000; Crevillen & Dean, 2011). The molecular mechanism underlying vernalization mainly involves the downregulation of *FLC* (Amasino, 2010; Bloomer & Dean, 2017), which encodes a MADS-box transcription factor that delays flowering (Michaels & Amasino, 1999) through repression of a set of floral activators, including *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (SOC1) (Lee *et al.*, 2000) among others.

The expression of *FLC* is simultaneously regulated by diverse intricate molecular mechanisms (Whittaker & Dean, 2017). For instance, FRI increases the transcription of *FLC* mRNA by interacting with the nuclear cap–binding complex (Geraldo *et al.*, 2009). FRI is present in a protein complex including a number of specific *FLC* regulators (Choi *et al.*, 2011). This FRI complex facilitates the recruitment of chromatin modifying factors to *FLC*, such as the ATP-dependent Swi2/Snf2 related chromatin remodeling complex SWR1-C that mediates the replacement of H2A/H2B by H2A.Z/H2B dimers (Morrison & Shen, 2009), and several histone methyltransferase complexes (Crevillen & Dean, 2011). When mutated, these chromatin-based non-specific *FLC* regulators confer pleiotropic phenotypes, in addition to early flowering, such as small size and developmental abnormalities (He *et al.*, 2004; Martin-Trillo *et al.*, 2006; Kim *et al.*, 2009).

H2A.Z is an evolutionary conserved histone variant of the H2A family (Zlatanova & Thakar, 2008) that roughly represents 15% of the total histone H2A cellular content (Jarillo & Pineiro, 2015). The Arabidopsis genome contains at least three genes encoding H2A.Z proteins (*HTA8*, *HTA9* and *HTA11*) (Yi *et al.*, 2006) and one or more homologous genes for most of the subunits of the yeast SWR1-C (March-Diaz & Reyes, 2009). Some of the orthologous components of the yeast SWR1-C, such as PHOTOPERIOD INDEPENDENT

EARLY FLOWERING 1 (PIE1), ACTIN-RELATED PROTEIN 6 (ARP6) and SWR1 COMPLEX 6 (SWC6), play key roles in the deposition of H2A.Z on *FLC* chromatin (Noh & Amasino, 2003; Martin-Trillo *et al.*, 2006; Choi *et al.*, 2007; Deal *et al.*, 2007; Lazaro *et al.*, 2008). Mutations in the corresponding genes cause an acceleration of flowering mainly due to low *FLC* expression (Jarillo & Pineiro, 2015), supporting a positive transcriptional role for H2A.Z deposition on *FLC* expression (Martin-Trillo *et al.*, 2006; Choi *et al.*, 2007; Deal *et al.*, 2007; March-Diaz *et al.*, 2007; Lazaro *et al.*, 2008). However, mutations affecting the same SWR1-C subunits result in the activation of a number of abiotic/biotic plant response genes, involved in drought, heat, phosphate starvation and disease resistance responses, as well as in the floral integrator gene *FT*, indicating a repressive role for H2A.Z in these loci (March-Diaz *et al.*, 2008; Kumar & Wigge, 2010; Smith *et al.*, 2010; Berriri *et al.*, 2016; Cortijo *et al.*, 2017; Sura *et al.*, 2017; Gomez-Zambrano *et al.*, 2018; Zahraeifard *et al.*, 2018). This dual transcriptional regulatory role of H2A.Z may be enlightened by the interplay of the SWR1-C with other chromatin modifiers that affect DNA methylation or post-translational modifications of histones and histone variants, and end up in different degrees of nucleosome stability (Deal & Henikoff, 2011; Billon & Cote, 2012; Coleman-Derr & Zilberman, 2012b; Gerhold & Gasser, 2014; Subramanian *et al.*, 2015; Dai *et al.*, 2017; Cai *et al.*, 2018; Carter *et al.*, 2018; Xu *et al.*, 2018). For example, differential stability of H2A.Z-containing nucleosomes by the acetylation of H2A.Z variant has been reported in yeast, vertebrates and humans (Millar *et al.*, 2006; Ishibashi *et al.*, 2009; Valdes-Mora *et al.*, 2017), but in plants, the consequences of H2A.Z acetylation on transcription remain to be explored.

YEAST ALL1-FUSED GENE FROM CHROMOSOME 9 (YAF9) homologs are characterized by harbouring the YEATS (Yaf9/GAS41-ENL-AF9-Taf14-Sas5) domain (Schulze *et al.*, 2009), a novel histone reader module with preferential recognition for acylated lysine residues (Zhao *et al.*, 2017). More than 100 proteins belonging to the YEATS domain family have been already described in eukaryotes. For instance, human YEATS domain family members, such as GLIOMA AMPLIFIED SEQUENCE 41 (GAS41), ELEVEN–NINETEEN LEUKEMIA (ENL) and AF9, are oncogenic proteins associated with cancer (Schulze *et al.*, 2009). Interestingly, AF9 YEATS domain links histone acetylation to DOT1L-mediated H3K79 methylation (Li *et al.*, 2014). In yeast, there are three YEATS domain-containing proteins, dubbed as Yaf9, Taf14 and Sas5 (Schulze *et al.*, 2009). A Yaf9 orthologue is present in the SWR1-C, and together with Swc4, Arp4 and Act-1 subunits constitute a module that is also shared by the Nucleosome Acetyl transferase of H4 complex (NuA4-C) (Lu *et al.*, 2009), indicating a possible functional link between these two

complexes. Arabidopsis SWC4 has been recently demonstrated to be required for locus-specific recruitment of the SWR1-C (Gomez-Zambrano *et al.*, 2018) and to associate with different SWR1-C subunits, including YAF9 proteins (Bieluszewski *et al.*, 2015; Gomez-Zambrano *et al.*, 2018), suggesting the conservation of the SWC4-YAF9 submodule also in plants. In addition, a role for YAF9A in the control of flowering time has been proposed either by regulation of *FLC* (Zacharaki *et al.*, 2012; Bieluszewski *et al.*, 2015) or *GIGANTEA (GI)* (Su *et al.*, 2017), although the precise molecular mechanism mediating this regulation and the relation between YAF9A and YAF9B, the two YAF9 homologues existing in Arabidopsis, is still far from being fully understood

To further understand the function of the plant SWR1-C and the interplay with other chromatin remodeling complexes, we have undertaken the genetic and molecular characterization of both Arabidopsis *YAF9* genes. Here we reveal that *YAF9A* and *YAF9B* have unequally redundant functions. In fact, double mutant plants *yaf9a yaf9b* showed pleiotropic developmental phenotypic alterations, including early flowering, and misregulation of a wide variety of genes. We demonstrate that *YAF9* genes regulate flowering time by both *FLC*-dependent and independent mechanisms. Besides, triple mutant combinations of *yaf9a, yaf9b* with *swc6* reveal a predominant role of SWR1-C independent pathways in the control of flowering time exerted by the YAF9A protein. Consistent with this, and in contrast to other *swr1-c* mutations (Martin-Trillo *et al.*, 2006), *yaf9a yaf9b* mutations do not suppress the late-flowering phenotype of *FRI* active alleles (Lee & Amasino, 1995). We also show that YAF9 proteins are novel histone readers that bind to unmodified and acetylated histone H3. Interestingly, YAF9A targets *FLC* chromatin and YAF9 proteins regulate the acetylation of H2A.Z and H4 but do not affect the deposition of H2A.Z at *FLC* locus. In agreement with that, we found that YAF9A interacts with HAM1, a putative catalytic subunit of NuA4-C. Altogether, our data unveil an additional layer of complexity in the regulation of *FLC* expression and show a novel regulatory mechanism of gene expression exerted by the YAF9 proteins through Nu4A-C-dependent H4 and H2A.Z histone acetylation in Arabidopsis.

Materials and Methods

Genetic stocks

All Arabidopsis mutant lines used in this study are in Columbia-0 *(*Col-0) background: *yaf9a-1* (SALK_106430) and *yaf9b-2* (SALK_046223) mutant seeds were obtained from the Nottingham Arabidopsis Stock Centre (NASC, UK); identification of

swc6-1 (Lazaro *et al.*, 2008), *arp6-1* (Deal *et al.*, 2005), *ft-10* (Yoo *et al.*, 2005) and *flc-3* mutants (Michaels & Amasino, 1999), plus Col *FRI Sant Feliu*-2 (*Sf-2*) plants (Lee & Amasino, 1995), together with transgenic Col *HTA11-GFP* (Kumar & Wigge, 2010) and *arp6-1 HTA11-GFP* lines (Choi *et al.*, 2013) were previously described.

RNA expression and microarray experiments

Total RNA was extracted from seedlings grown in petri dishes containing Murashige and Skoog medium supplemented with 1% (w/v) sucrose and 1% (w/v) plant agar. Semiquantitative RT-PCR and Real time quantitative PCR *(*Q-PCR) conditions were as described (Gomez-Zambrano *et al.*, 2018). The specific primers used are described in the Table S1.

Transcriptomic analyses were performed using Affymetrix Microarray (*Arabidopsis* ATH1 Genome Array) with RNA extracted from 20 day-old WT and *yaf9a yaf9b* seedlings grown on agar plates under SD at ZT8. Three independent biological replicates were hybridized (Dataset S1). We used Multiexperiment Viewer (MeV v4.8.1) (Saeed *et al.*, 2006) and Venny (http://bioinfogp.cnb.csic.es/tools/venny/index. html) software for microarray data analyses.

Chromatin Immunoprecipitation

Chromatin Immunoprecipitation (ChIP) experiments were performed as described (Song *et al.*, 2014) but supplementing all buffers with NaBu 5mM to detect acetylated histones, and starting from 1.5 g of 10 day-old seedlings grown on agar plates under LD conditions that were collected at ZT16. Immunoprecipitated DNA was quantified by Q-PCR using the oligonucleotides described in Table S1. DNA enrichment was estimated as the fraction of immunoprecipitated DNA relative to input (%INPUT). Relative histone modifications levels were determined as %INPUT of each region/%INPUT *ACTIN 2* (*ACT2*) fragment. We used the following antibodies: α-Myc (Merck-Millipore Clone 4A6), α-GFP (Roche A6455), α-H4ac (Merck-Millipore 06-598), α-HTA9 (Agrisera AS10718) and α-H2A.Zac (Diagenode C15410173, pAb-173-050, Lot No. A.406-001P). α-H2A.Zac specificity was thoroughly tested by western blot.

Generation of transgenic lines

Full-length *YAF9A* cDNA was obtained by standard PCR techniques and cloned into pGWB18 (Nakagawa *et al.*, 2007) and pC-TAPa plasmids (Rubio *et al.*, 2005), and transformed into *yaf9a-1* mutant to obtain *35S::YAF9A::myc*, and *2x35S::YAF9A::TAPa*

constructs respectively. Full-length *HAM1* cDNA clone was obtained by standard PCR techniques and cloned into pEarlygate201 (Earley *et al.*, 2006) to generate the *35S::HA::HAM1* construct. All cloning strategies were performed using Gateway technologies (Invitrogen). Transgenic plants for each gene construct (more than 10 independent lines) were generated following *Agrobacterium tumefaciens*-mediated transformation using the floral-dip method (Clough & Bent, 1998).

Statistical analyses

Box-plot graphic representations and statistical analyses (ANOVA, Student´s t-test) were performed with GraphPad Prism software. Box represents 25th to 75th percentiles of the data; a horizontal line indicates the median and dots are suspected outliers. Whiskers and outliers were determined according to Tukey test.

Data availability

Microarray data are available at GSE126020

See Supporting Information Methods S1 for the description of additional methods.

Results

Two Arabidopsis YEATS domain-containing proteins are the predicted homologs of yeast YAF9 protein.

We have determined that the Arabidopsis genome contains two genes encoding YEATS domain proteins, AT5G45600 and AT2G18000. Phylogenetic analysis showed that both proteins are homologs to yeast Yaf9 (Supplemental Fig. S1a) and were designated as YAF9A and YAF9B, respectively (Zacharaki *et al.*, 2012; Bieluszewski *et al.*, 2015). The deduced YAF9A and YAF9B polypeptides have predicted molecular weights about 30 KDa and share 56% amino acid sequence identity (Fig. S1b). We found that *YAF9A* was highly and widely expressed in different organs of the plant while *YAF9B* expression was only abundant in young flowers and roots (Fig. S2a). Both proteins were detected in the nucleus in transient expression assays performed in *N. benthamiana* (Fig. S2b,c), consistent with YAF9 proteins being present in chromatin remodeling complexes.

To address the role of Arabidopsis YAF9 proteins in developmental regulation, we characterized Knock-Out (KO) T-DNA insertions lines for both genes (*yaf9a-1* and *yaf9b-2*) (Bieluszewski *et al.*, 2015) (Fig. S3). We found that *yaf9a* mutants systematically showed a

moderate acceleration of flowering time in LD, as previously described (Zacharaki *et al.*, 2012; Bieluszewski *et al.*, 2015), and that also display a slight acceleration of flowering time under SD (Fig. 1a); however, *yaf9b* mutants were indistinguishable from WT plants (Figs 1a, S4a, S5a), suggesting a partially redundant function for the YAF9 proteins in the control of developmental responses in Arabidopsis. To further investigate this possible genetic interaction, we generated *yaf9a-1 yaf9b-2* double mutant lines, hereafter referred to as *yaf9a yaf9b* in the text. Double mutant plants *yaf9a yaf9b* showed pleiotropic developmental phenotypic alterations including conspicuous early flowering, accelerated senescence and chlorotic leaves with reduced chlorophyll content (Fig. 1a, S4a). In addition, *yaf9a yaf9b* double mutant plants displayed smaller leaves, flowers, fruits and roots and overall reduced plant size in comparison to WT plants (Figs 1b-f, S4), corroborating previous observations (Bieluszewski *et al.*, 2015). These developmental phenotypic alterations were complemented by expressing a *YAF9A::myc* gene construct under the control of a *35S* promoter (Fig. S5), indicating that the observed pleiotropic developmental defects were due to the concurrent loss of function of both *YAF9* genes**.** Altogether, these data indicate that the two Arabidopsis YAF9 proteins have unequally redundant functions in developmental responses.

Reduced size of *yaf9a yaf9b* plants is due to cell expansion and proliferation defects.

Arabidopsis *YAF9* defective plants displayed a reduction in plant and organ size (Figs 1, S4), defects commonly associated with impaired cellular proliferation and cell expansion processes (Gonzalez *et al.*, 2012). We performed scanning electron microscopy (SEM) to determine the cell size of adaxial leaf epidermis and the total number of cells per leaf of *yaf9a yaf9b* mutant plants in comparison to WT (Fig. 2a). We found that *yaf9a yaf9b* adaxial leaf epidermal cells were around half of the WT cell size, although *yaf9a yaf9b* contained approximately 1.5-fold more cells (Fig. 2b-d).

Reduced cell size could be an indication of altered ploidy in Arabidopsis leaves (Meagher *et al.*, 2007). We performed a detailed analysis of ploidy levels from mature rosette leaves of *yaf9a yaf9b* and WT 32 day-old plants grown under LD and SD conditions. Flow cytometry measurements in mature leaves 3 and 4 revealed that *yaf9a yaf9b* mutants displayed an increase of 4C and a reduction of 8C and 16C nuclei levels at LD and SD conditions (Fig. 2e). These data are consistent with the smaller cell size observed in *yaf9a yaf9b* mutants and support the participation of the YAF9 proteins in the regulation of leaf cell proliferation and expansion processes.

Loss of function of *YAF9* genes results in misregulation of a wide variety of genes

Arabidopsis plants defective in YAF9 functional proteins displayed several pleiotropic phenotypic alterations (Fig. 1), suggesting that *YAF9* genes are involved in the regulation of different developmental processes. To evaluate genes whose expression was misregulated in *yaf9a ya9b* plants, we performed a genome wide transcriptomic analysis of these seedlings by microarray approaches (*Arabidopsis* ATH1 Genome Array). We found 1014 genes downregulated and 1230 upregulated (log_2FC ratio <0.5 or >0.5; P< 0.5) in *yaf9a yaf9b* plants compared to the WT seedlings (Dataset S1). Q-PCR expression analyses of selected genes validated the data obtained with the microarray experiment (Fig. S6). Differentially expressed genes in *yaf9a yaf9b* plants show a moderate although statistically significant overlap with misregulated genes in *pie1*, *arp6*, *swc6*, *h2a.z* double (*hta9 hta11*) and triple (*hta8 hta9 hta11*) mutants (March-Diaz *et al.*, 2008; Kumar & Wigge, 2010; Coleman-Derr & Zilberman, 2012a; Berriri *et al.*, 2016) (Figs S7, S8). Strikingly, 33% of genes upregulated in *yaf9a yaf9b* double mutant were also upregulated in *swc4i* RNAi lines (Gomez-Zambrano *et al.*, 2018), which means a 7 fold over-enrichment compared to random expectations (Fig. S7). These data suggest that YAF9 proteins may have additional functions to the ones exerted by core SWR1-C subunits, but shared with SWC4.

Singular Enrichment Analysis (SEA) of Gene Ontology (GO) terms of misregulated genes show enrichments in stimuli and stress-related genes, post embryonic development and cell growth functions (Fig. S9a and Fig. S10). Among the misregulated genes in *yaf9a yaf9b* seedlings we found a number of loci related to cell size and growth regulation (Table S2), which may contribute to the altered cell size displayed by these mutants (Fig. 2a). Also genes related to the systemic acquired response (SAR) were found misregulated (Table S3) and may help to explain the accelerated senescence displayed by the double *yaf9a yaf9b* mutant seedlings (Fig. S11). Interestingly, the expression of a number of flowering time-related genes was also altered in the *yaf9a yaf9b* double mutants, including *FLC*, *FT*, *AGL24* and *CO*, whose misregulation might cause the early flowering phenotype observed in these plants (Fig. S9b).

***YAF9* genes regulate flowering time by both *FLC*-dependent and independent mechanisms.**

To assess the role of *YAF9* genes in the complex regulation of flowering time, we performed genetic analyses with *yaf9a* and *yaf9b* single and double mutant plants in combination with additional flowering time mutations under LD and SD. As described

previously, *yaf9a* mutants showed a modest acceleration of flowering time (Fig. 3a) whereas *yaf9b* mutants behaved as WT plants. However, *yaf9a yaf9b* double mutant plants flowered as early as representative *swr1-c* mutants (Martin-Trillo *et al.*, 2006; Lazaro *et al.*, 2008) (Fig. 3a), producing a similar number of leaves at bolting. These data indicate a partially redundant role between *YAF9A* and *YAF9B* loci in the control of flowering time.

The YAF9 homologue present in the yeast SWR1-C is required for H2A.Z deposition (Zhang *et al.*, 2004). Furthermore, in Arabidopsis the SWR1-C mediates the exchange of H2A by the histone variant H2A.Z in the chromatin of *FLC* locus and is necessary for the activation of this floral repressor gene (Deal *et al.*, 2007). In fact, our transcriptomic analysis showed that YAF9 function is necessary for full *FLC* expression (Dataset S1). For that reason, we decided to further explore how Arabidopsis *yaf9* mutations may affect *FLC* activation. Q-PCR experiments demonstrated that *FLC* transcript levels were clearly reduced in *yaf9a* and *yaf9a yaf9b*, but not in *yaf9b* mutants, in comparison to WT plants (Fig. 3b). This decrease in *FLC* expression may contribute to the acceleration of flowering time observed in *yaf9a* and *yaf9a yaf9b* mutants (Fig. 3a). To unveil the genetic relationship between *YAF9* and *FLC* genes we crossed *yaf9* mutants with the KO *flc-3* allele (Michaels & Amasino, 2001). We found that *flc-3* mutation was epistatic over *yaf9a* (Fig. 3a), indicating that the flowering time acceleration observed in *yaf9a* was completely dependent on *FLC* function. Interestingly, *flc-3* flowering phenotype was additive when combined with the concurrent loss of *YAF9A* and *YAF9B* activities (Fig. 3a), suggesting that *YAF9* genes control the floral initiation by both *FLC*-dependent and independent mechanisms.

We also combined *ft-10* (Yoo *et al.*, 2005) with *yaf9* mutations and found that triple *ft-10 yaf9a yaf9b* mutant plants displayed smaller size and flowered earlier than single *ft-10* plants (Fig. 3c,d). Altogether these data indicate that YAF9A regulates the floral transition mainly through *FLC* but, in addition, *YAF9A* and *YAF9B* genes might also target *FT* and extra flowering time genes to fine tune this developmental response.

YAF9 genes regulate flowering time through SWR1-C independent pathways.

FLC expression is regulated by SWR1-C (Deal *et al.*, 2007) and YAF9A protein interacts with several subunits of this complex, including SWC4 and SWC6 (Gomez-Zambrano *et al.*, 2018) To functionally test its genetic relationship, we crossed *swc6* with *yaf9a yaf9b* in order to obtain the *swc6-1 yaf9a-1 yaf9b-2* triple mutant and compare their flowering time with those of the double and single parental mutants. When flowering time was assayed under SD conditions, *swc6 yaf9a* and *swc6 yaf9a yaf9b* plants displayed an

extremely early flowering phenotype (Fig. 4c,d), earlier than any of the single parental mutants. Due to the conspicuous early flowering phenotype of *swc6* mutant, this additive effect was less noticeable under inductive LD conditions (Fig. 4a,b). Thus, the *YAF9A* locus is regulating flowering time independently of SWR1-C. In addition, *swc6 yaf9a* and *swc6 yaf9a yaf9b* mutants displayed a strong reduction in overall plant size (Fig. 4b,d). Altogether, these data indicate that although *YAF9* and *SWC6* genes may share common functions they also perform some independent roles in the control of plant development.

***yaf9* mutations partially suppress the late-flowering phenotype of *FRIGIDA*.**

Mutations in components of the Arabidopsis SWR1-C strongly suppress the function of *FRI*, a potent activator of *FLC* expression (Choi *et al.*, 2005). In addition, YAF9 proteins were found to co-purify with FRI protein in pulldown proteomic approaches (Choi *et al.*, 2011). To ascertain the genetic relationship between *YAF9* and *FRI* genes, we introgressed an active *FRI* allele from the *Sf-2* ecotype (Lee & Amasino, 1995) into *yaf9a, yaf9b* and the *yaf9a yaf9b* double mutant. Both *yaf9a FRI* and *yaf9b FRI* lines flowered late as *FRI* plants (Fig. 5a). However, *yaf9a yaf9b FRI* flowered earlier than Col *FRI* plants (Fig. 5a,c), due to a significant reduction of *FLC* levels (Fig 6b), although they were still late flowering when compared to Col or *arp6 FRI* (Fig. 5a). These data are consistent with the role of YAF9 proteins in regulating *FLC* expression. However, the effect of the loss of function of *YAF9* genes in the expression of *FLC* was not comparable to that previously described for *swr1*-c mutants (Martin-Trillo *et al.*, 2006) or other *FRI* suppressor mutants (Choi *et al.*, 2005). Thus, FRI activity requires H2A.Z deposition by SWR1-C for regulating *FLC* expression but it is less dependent on YAF9 activity (Fig. 4). Our data are consistent again with a role for YAF9 in regulating *FLC* expression independently of SWR1-C function.

YAF9 proteins are required to maintain H2A.Z and H4 acetylation at *FLC* chromatin but do not affect the deposition of H2A.Z in this locus

Yeast Yaf9 protein, as part of the SWR1-C, is involved in H2A.Z deposition (Zhang *et al.*, 2004). It has been reported that mutations in Arabidopsis SWR1-C components such as *ARP6* result in early flowering and low *FLC* transcript levels (Martin-Trillo *et al.*, 2006) due to reduced H2A.Z incorporation at *FLC* chromatin (Deal *et al.*, 2007). We wondered if mutations in *YAF9* genes may also cause lower deposition of H2A.Z at different regions of *FLC* chromatin (Fig. 6a). To measure H2A.Z levels in the *yaf9a yaf9b* mutant, we introgressed an HTA11-GFP tagged H2A.Z gene construct previously reported to be

functional (Kumar & Wigge, 2010), and performed ChIP experiments using an antibody against GFP. Strikingly, the results showed that histone H2A.Z levels at *FLC* chromatin were not affected in *yaf9a yaf9b* mutants, whereas *arp6* mutant plants showed a strong reduction in H2A.Z incorporation (Fig. 6b). These results were corroborated using specific antibodies against *HTA9*-encoded Arabidopsis H2A.Z (March-Diaz & Reyes, 2009). In WT and *yaf9a yaf9b* seedlings, HTA9 was highly enriched around *FLC* nucleosome +1, whereas in the *arp6* mutants HTA9 showed a clear decrease at *FLC* chromatin (Fig. 6c). Altogether these data indicate that YAF9 proteins are not required for H2A.Z deposition at *FLC* chromatin (Fig. 6).

In yeast, Yaf9 is also present in the NuA4-C (Doyon & Cote, 2004; Lu *et al.*, 2009; Wang *et al.*, 2018). In Arabidopsis, two putative homologs of the NuA4-C histone acetyl transferase (HAT) catalytic subunit are the MYST (for MOZ, Ybf2 (Sas3), Sas2, and Tip60) HAM1 and HAM2 proteins (Latrasse *et al.*, 2008). The main activity carried out by these proteins is the acetylation of histones H4, H2A and H2A.Z (Earley *et al.*, 2007). To assess the possible role of YAF9 proteins in mediating histone acetylation processes in the chromatin of *FLC* we pursued ChIP approaches using specific antibodies that recognize either H4 acetylation (H4ac) or H2A.Z acetylation (H2A.Zac) marks (Fig. S12). We found a strong reduction in histone H4ac levels at the nucleosome +1 of *FLC* chromatin in the *yaf9a yaf9b* double mutant compared to WT (Fig. 6d,e). Similar to *yaf9a yaf9b*, single *yaf9a* but not *yaf9b* mutant also showed reduced H4 ac levels (Fig. S13). Interestingly, in *yaf9a yaf9b* H2A.Zac level was also reduced at nucleosome +1 of *FLC* chromatin, where H2A.Z preferentially accumulates. The reduction in H2A.Zac in *yaf9a yaf9b* was not due to decreased H2A.Z levels as observed in the *arp6* mutant (Fig. 6a,b) but due to specific defects in the acetylation of histones (Fig. 6d). Our data indicate that YAF9 proteins regulate *FLC* expression by altering histone H2A.Z and H4 acetylation levels but not H2A.Z incorporation at this locus.

To determine if YAF9 proteins regulate other targets in a similar way, we focused on *FT*, an upregulated gene in *yaf9a yaf9b* seedlings (Fig. S9b). H2A.Z levels were reduced at *FT* nucleosome +1 (Fig. S14 a-b), the same chromatin region that is bound by YAF9A protein (Fig. S14d). However, no relevant changes in H2A.Zac levels were observed in *yaf9a yaf9b* compared with WT seedlings (Fig. S14c), revealing that YAF9 proteins modulate H2A.Z deposition at *FT*. Altogether, these data indicate that YAF9 proteins, as dual components of putative plant SWR1 and NuA4 complexes, can regulate gene expression by altering H2A.Z levels, histone acetylation and possibly both chromatin signatures at different target loci.

YAF9A is a histone binding protein that targets *FLC* chromatin and preferentially associates with NuA4-C components *in vivo*

To test if *FLC* is a direct target of YAF9 proteins, we generated *2x35S::YAF9A::TAPa* transgenic lines in *yaf9a-1* mutant background. The *YAF9A-TAPa* gene construct fully complements the early flowering time and the *FLC* downregulation showed by *yaf9a* mutant (Fig. S15). The TAPa tag contains a c-Myc epitope (Rubio *et al.*, 2005) that allowed us to test YAF9A binding to target loci following ChIP approaches. We used two independent functional transgenic lines and assayed a number of chromatin regions along the *FLC* locus (Fig. 6a). We found YAF9A enrichment in both lines around the TSS of the gene (Fig. 6f), but mainly at nucleosome +1 (+232 region), where H2A.Z and H4 acetylation accumulates (Fig. 6d,e). Thus, our data indicate that *FLC* is a direct target of this chromatin regulator.

Recent reports have defined the YEATS domain as a novel histone acetylation reader module (Zhao *et al.*, 2017). To test if YAF9A and B, the only two Arabidopsis YEATS domain-containing proteins, are able to bind histones, we performed pulldown assays using Arabidopsis histone extracts and found that recombinant YAF9A-GST and YAF9B-GST proteins were able to recognize unmodified and acetylated (K9/K14) histone H3 but not H2B (Fig. 7a). Histone peptide pulldown experiments also confirmed that, at least in our *in vitro* assays, YAF9A recognizes unmodified histone H3 and to a lesser extent H3K9ac and H3K27ac marks (Fig. 7b). Thus, we conclude that, as in other eukaryotes, Arabidopsis YAF9 proteins can be considered as histone H3 binding proteins.

Given that yeast SWR1-C and NuA4-C both contain the YAF9 protein we wondered if the Arabidopsis YAF9 homologues might be linked to any of these chromatin remodeling complexes. To address this, we performed a two-step TAPa-tag affinity purification followed by tandem mass spectrometry, using a YAF9A-TAPa line. Proteins co-purified with YAF9A-TAP in three independent pulldown experiments but not enriched in the control experiment are listed in Dataset S2. Among other proteins, we found shared components between SWR1-C and NuA4-C such as SWC4 and ARP4, and also specific components of NuA4-C co-purified with YAF9A, such as Arabidopsis Esa1-Associated factor 1 (AtEAF1) (Bieluszewski *et al.*, 2015) and HAM1 (Latrasse *et al.*, 2008)(Fig. 7c). In our experiments, there were also a number of peptides from RuvB-like protein 1, present in both SWR1 and INO80 complexes (Holt *et al.*, 2002), and components of the histone chaperone FACILITATES CHROMATIN TRANSCRIPTION (FACT) complex such as STRUCTURE-SPECIFIC RECOGNITION PROTEIN 1 (SSRP1) and SUPPRESSOR OF Ty16 (SPT16)

subunits (Zhou *et al.*, 2015; Pfab *et al.*, 2018). This complex is required for the progression of transcribing RNA polymerase on chromatin templates via nucleosome destabilization (Formosa, 2012).

Although our proteomics experiments are not fully comprehensive, it is worth emphasizing that we did not find peptides of any of the Arabidopsis SWR1-C core subunits: PIE1, ARP6 and SWC6. However, Arabidopsis HAM1 protein, one of the catalytic subunits of the putative plant NuA4-C (Latrasse *et al.*, 2008), appears in our interactors list. This YAF9A-HAM1 interaction was further confirmed *in vivo* by performing Co-IP experiments in *N. benthamiana* transient expression assays (Fig. 7d). Besides, recent independent proteomic data confirm the revealed physical interaction between YAF9A and HAM1 (Tan *et al.*, 2018). Altogether, these data indicate that YAF9A positively regulates the floral repressor *FLC* expression through HAM1-dependent histone acetylation, and concomitantly represses flowering time.

Discussion

YAF9 homologues are widely conserved from yeast to human and are integral components of SWR1-C and NuA4-C (Schulze *et al.*, 2009). They are involved in the control of a plethora of cellular and developmental processes. On this manner, Yaf9-defective yeast strains display defects in transcriptional regulation, histone acetylation, DNA repair and chromosome segregation (Le Masson *et al.*, 2003; Krogan *et al.*, 2004). On the other hand, human GAS41 protein levels are elevated in cancer cell lines and depletion of *GAS41* suppresses tumor growth (Hsu *et al.*, 2018). In this work, the relevance of Arabidopsis YAF9 proteins in controlling different developmental responses has been uncovered. We have demonstrated that *yaf9a yaf9b* double mutant plants showed pleiotropic phenotypic alterations of development in both vegetative and reproductive traits. In fact, more than 2000 genes were found misregulated in *yaf9a yaf9b* double mutants (Fig. S9 and Dataset S1). Among them, genes related to cell size and growth regulation, to the SAR response or involved in the control of flowering time that were deregulated in the *yaf9a yaf9b* double mutants (Fig. S9 and Tables S2, S3), may explain the conspicuous phenotypic alterations displayed by these plants (Fig. 1). Interestingly, GAS41 also regulates the expression of cell cycle-related genes (Hsu *et al.*, 2018). Thus, our observations indicate that YAF9 proteins contribute to the regulation of gene expression, possibly through modulation of H4 and H2A.Z acetylation levels.

Regarding the smaller size of *yaf9a yaf9b* mutants leaves, it has been proposed that cell size is associated with polyploidy in this organ (Meagher *et al.*, 2007). Our results revealed that depletion of *YAF9* activities in Arabidopsis led to lower endoreduplication level (Fig. 2), suggesting that YAF9 proteins may contribute to the regulation of the entry into the endocycle during leaf development. Similar results were recently observed in knock-down plants for *SWC4* (Gomez-Zambrano et al., 2018), encoding an interactor of YAF9A, supporting a shared role for these proteins in the regulation of leaf cell proliferation and expansion. Besides, a considerable overlap was observed in the transcriptomic profiles of *yaf9a yafa9* and *swc4i* plants (Fig. S7), reinforcing the link between YAF9 and SWC4 subunits.

We found that *yaf9a* and *yaf9a yaf9b* mutants display a conspicuous early flowering phenotype. Our genetic analyses indicate that YAF9 proteins regulate flowering time by both *FLC*-dependent and independent mechanisms (Fig. 3). We have revealed that the early flowering phenotype of *flc-3* mutant was almost completely epistatic over *yaf9a*, but that *flc-3* flowering phenotype was additive when combined in genetic backgrounds deficient in both *YAF9A* and *YAF9B* activities (Fig. 3a). The residual early flowering phenotype observed in *yaf9a yaf9b flc-3* triple mutants, especially under SD, indicates additional roles of *YAF9* genes in the repression of flowering that are independent of *FLC.*

The effects of *yaf9a yaf9b* mutations on flowering time are readily observed in the late-flowering *FRI-Sf-2* background (Fig. 5). When an active *FRI* allele was combined with the *yaf9a yaf9b* mutations, these plants showed an additive phenotype in which the *FRI* late-flowering phenotype was partially suppressed by *yaf9a yaf9b* (Fig. 5a). These non-epistatic effects of *yaf9a yaf9b* mutations over *FRI* flowering phenotype correlate well at the molecular level with a decrease in the steady state levels of *FLC* mRNA in *FRI* plants carrying *yaf9a yaf9b* mutant alleles (Fig. 5b). It has been recently proposed that the YAF9A partner HAM1 partly mediates FRI-dependent *FLC* upregulation (Li *et al.*, 2018), and this may help to explain the contribution of YAF9 proteins in *FLC* upregulation by FRI.

Histone acetylation dynamics are crucial to modulate flowering time (Wang *et al.*, 2014).The acetylation of histone H3 and H4 at *FLC* chromatin plays a key role in achieving high levels of *FLC* expression during early Arabidopsis development (Finnegan & Dennis, 2007). Results from this study and previous reports support the participation of HAM1 and HAM2, putative homologues of the Yeast Esa1 protein, the catalytic subunit of the NuA4-C, in the modulation of histone H4 and H2A.Z acetylation levels at *FLC* chromatin (Earley *et al.*, 2007; Latrasse *et al.*, 2008; Xiao *et al.*, 2013; Bieluszewski *et al.*, 2015). Besides, our

proteomics experiments revealed that Arabidopsis YAF9A interacts with a number of NuA4-C subunits, and *in vivo* Co-IP experiments from this work (Fig. 7d and Dataset S2) and independent pulldown assays involving HAM proteins (Tan *et al.*, 2018) corroborated the interaction with HAM1, consistent with YAF9A being part of this putative HAT complex in plants. Nevertheless, we cannot rule out that YAF9 proteins could be non-essential SWR1-C subunits or form part of additional chromatin remodeling complexes-(Zhang *et al.*, 2004). In fact, a possible link with the FACT complex has been revealed, based in the interaction of YAF9A with SSPR1 and SPT16 subunits (Fig. 7c and Dataset S2). Interestingly, plants depleted of SSRP1 and SPT16 subunits display various defects in vegetative and reproductive development, including early flowering due to reduced expression of *FLC* in these plants (Lolas *et al.*, 2010), resembling *yaf9a yaf9b* mutants.

The YEATS domain is a novel histone acetylation reader, although different YEATS proteins show unique binding preferences (Zhao *et al.*, 2017). Yeast Yaf9 was first described to bind unmodified histones (Wang *et al.*, 2009), but recently it has been proposed to preferentially recognizes H3K27ac (Klein *et al.*, 2018), whereas Taf14 binds to H3K9ac, and Sas5 interacts with unmodified histones (Shanle *et al.*, 2015). Interestingly, we have shown that, as its yeast or animal counterparts, Arabidopsis YAF9 proteins are able to recognize histone H3 and H3K9ac and H3K27ac marks (Fig. 7a-b), supporting that these proteins may function also as histone readers in plants, recognizing acetylated versions of histone H3.

We have also unveiled that both Arabidopsis YAF9 proteins mediate H2A.Z and H4 acetylation at *FLC* chromatin, and that YAF9A binds directly to this gene (Fig. 6). Yeast Yaf9 is required for H2A.Z incorporation into chromatin (Zhang *et al.*, 2004; Wu *et al.*, 2005; Wu *et al.*, 2009), and depletion of the Yaf9 human homolog *GAS41* affects gene expression by modulating H2A.Z occupancy in a high number of genes (Hsu et al., 2018). At the moment, we cannot exclude a similar role for Arabidopsis YAF9 proteins in H2A.Z exchange. In fact, we provide evidence indicating that they are required to deposit this histone variant at *FT* chromatin (Fig. S14), but our data clearly demonstrate that YAF9 is dispensable for H2A.Z incorporation at *FLC* chromatin (Fig. 6), a well-established target of the SWR1-C in Arabidopsis (Deal *et al.*, 2007). Further work will enlighten the contribution of these proteins to the global distribution of H2A.Z in plants.

Remarkably, in this work we have revealed for the first time in plants the occurrence of H2A.Z acetylation and demonstrated that the presence of this histone mark at *FLC* chromatin is required for its expression (Fig. 6). It has been proposed that NuA4-C dependent acetylation of H4 and H2A histones promotes the incorporation of H2A.Z by SWR1–C (Altaf

et al., 2010). A variety of data in yeast and avian cells support that the acetylated form of H2A.Z is augmented at active genes (Bruce *et al.*, 2005; Millar *et al.*, 2006). In humans, there are also a number of studies that correlate active gene expression with H2A.Zac, with consequences in cell differentiation and tumor progression (Ku *et al.*, 2012; Valdes-Mora *et al.*, 2012; Bellucci *et al.*, 2013; Dalvai *et al.*, 2013; Law & Cheung, 2015; Valdes-Mora *et al.*, 2017). Future studies will be necessary to conclude if the occurrence of H2A.Zac also correlates with gene activation in plant genomes.

Altogether, our data support that YAF9A and YAF9B proteins fine tune the floral transition through targeting *FLC*, and by the eventual modification of the H2A.Z and H4 acetylation levels. We have shown that this YAF9-mediated regulation occurs independently of SWR1-C function and is relevant to prevent precocious floral transition and fine-tune flowering time in Arabidopsis. It has been reported that YAF9A may also regulate flowering time acting at the level of *GI* gene by interacting with CIRCADIAN CLOCK ASSOCIATED 1 which recruits MUT9P-LIKE-KINASE 4 (MLK4), an enzyme responsible for the phosphorylation of histone H2A at Serine 95, which marks nucleosomes for H2A.Z deposition at *GI* chromatin (Su *et al.*, 2017). *yaf9a-1* mutant showed reduced expression of *GI* concomitantly with decreased H2A.Z and lower H4 acetylation levels in the promoter region of this gene compared to WT (Su *et al.*, 2017). Thus, MLK4 and YAF9A seem to regulate *GI* expression by modulating H2A.Z deposition and H4 acetylation of *GI*, revealing additional targets for YAF9A proteins in the control of flowering time. On this way, YAF9 may be linked to the photoperiod dependent pathway of flowering control (Su *et al.*, 2017) in addition to regulate *FLC*, and could assist to fine tune the recruitment of the SWR1-C mediated by SWC4 to specific target genes (Gomez-Zambrano *et al.*, 2018). Furthermore, YAF9A was proposed as a possible candidate flowering gene to be involved in the adaptation of *Arabidopsis thaliana* to the Yangtze River basin (Zou *et al.*, 2017), suggesting an important role for YAF9A in helping this species to fine tune local adaptation to climate.

In sum, the characterization of YAF9 proteins in Arabidopsis highlights the key role exerted by post-translational modification of canonical and histone variants in regulating flowering time. We have also shown that these two YEATS domain proteins, acting at chromatin level, are involved in the control of several plant developmental programs as well as cell expansion and proliferation processes. Further analyses of the molecular function of plant YAF9 proteins will contribute to unravel new mechanistic insights into the role of eukaryotic histone acetylation in controlling gene expression.

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Authors contributions

MP and JAJ designed the research. PC, AGZ and JAL performed research. JV contributed with new analytical tools. PC, MP and JAJ analysed all the data and wrote the paper.

References

Altaf M, Auger A, Monnet-Saksouk J, Brodeur J, Piquet S, Cramet M, Bouchard N, Lacoste N, Utley RT, Gaudreau L, et al. 2010. NuA4-dependent acetylation of nucleosomal histones H4 and H2A directly stimulates incorporation of H2A.Z by the SWR1 complex. *The Journal of Biological Chemistry* **285**: 15966-15977.

Amasino R. 2010. Seasonal and developmental timing of flowering. *Plant Journal* **61**: 1001-1013.

Amasino RM, Michaels SD. 2010. The timing of flowering. *Plant Physiology* **154**(2): 516-520.

Andres F, Coupland G. 2012. The genetic basis of flowering responses to seasonal cues. *Nature Review Genetic*s **13**: 627-639.

Bellucci L, Dalvai M, Kocanova S, Moutahir F, Bystricky K. 2013. Activation of p21 by HDAC inhibitors requires acetylation of H2A.Z. *PLOS One* **8**: e54102.

Berriri S, Gangappa SN, Kumar SV. 2016. SWR1 chromatin-remodeling complex subunits and H2A.Z have non-overlapping functions in immunity and gene regulation in *Arabidopsis*. *Molecular Plant* **9**: 1051-1065.

Bieluszewski T, Galganski L, Sura W, Bieluszewska A, Abram M, Ludwikow A, Ziolkowski PA, Sadowski J. 2015. AtEAF1 is a potential platform protein for Arabidopsis NuA4 acetyltransferase complex. *BMC Plant Biology* **15**: 75.

Billon P, Cote J. 2012. Precise deposition of histone H2A.Z in chromatin for genome expression and maintenance. *Biochimica et Biophysica Acta* **1819**: 290-302.

Bloomer RH, Dean C. 2017. Fine-tuning timing: natural variation informs the mechanistic basis of the switch to flowering in *Arabidopsis thaliana*. *Journal of Experimental*
Botany **68**: 5439-5452.

Bruce K, Myers FA, Mantouvalou E, Lefevre P, Greaves I, Bonifer C, Tremethick DJ, Thorne AW, Crane-Robinson C. 2005. The replacement histone H2A.Z in a hyperacetylated form is a feature of active genes in the chicken. *Nucleic Acids Research* **33**: 5633-5639.

Cai H, Zhang M, Chai M, He Q, Huang X, Zhao L, Qin Y. 2018. Epigenetic regulation of anthocyanin biosynthesis by an antagonistic interaction between H2A.Z and H3K4me3. *New Phytologist* **221**:295-308.

Carter B, Bishop B, Ho KK, Huang R, Jia W, Zhang H, Pascuzzi PE, Deal R, Ogas J. 2018. The Chromatin remodelers PKL and PIE1 act in an epigenetic pathway that determines H3K27me3 homeostasis in *Arabidopsis*. *Plant Cell* **30**:1337-135.

Clough SJ, Bent AF. 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant Journal* **16**: 735-743.

Coleman-Derr D, Zilberman D. 2012a. Deposition of histone variant H2A.Z within gene bodies regulates responsive genes. *PLOS Genetics:* **8**: e1002988.

Coleman-Derr D, Zilberman D. 2012b. DNA methylation, H2A.Z, and the regulation of constitutive expression. *Cold Spring Harbor Symposia on Quantitative Biology* **77**: 147-154.

Cortijo S, Charoensawan V, Brestovitsky A, Buning R, Ravarani C, Rhodes D, van Noort J, Jaeger KE, Wigge PA. 2017. Transcriptional regulation of the ambient temperature response by H2A.Z nucleosomes and HSF1 transcription factors in *Arabidopsis. Molecular Plant* **10**: 1258-1273.

Crevillen P, Dean C. 2011. Regulation of the floral repressor gene *FLC*: the complexity of transcription in a chromatin context. *Current Opinion in Plant Biology* **14**: 38-44.

Choi K, Kim J, Hwang HJ, Kim S, Park C, Kim SY, Lee I. 2011. The FRIGIDA complex activates transcription of *FLC*, a strong flowering repressor in *Arabidopsis*, by recruiting chromatin modification factors. *Plant Cell* **23**: 289-303.

Choi K, Kim S, Kim SY, Kim M, Hyun Y, Lee H, Choe S, Kim SG, Michaels S, Lee I. 2005. *SUPPRESSOR OF FRIGIDA3* encodes a nuclear ACTIN-RELATED PROTEIN6 required for floral repression in Arabidopsis. *Plant Cell* **17**: 2647-2660.

Choi K, Park C, Lee J, Oh M, Noh B, Lee I. 2007. *Arabidopsis* homologs of components of the SWR1 complex regulate flowering and plant development. *Development* **134**: 1931-1941.

Choi K, Zhao X, Kelly KA, Venn O, Higgins JD, Yelina NE, Hardcastle TJ, Ziolkowski PA, Copenhaver GP, Franklin FC, et al. 2013. *Arabidopsis* meiotic crossover hot spots overlap with H2A.Z nucleosomes at gene promoters. *Nature Genetics* **45**: 1327-1336.

Dai X, Bai Y, Zhao L, Dou X, Liu Y, Wang L, Li Y, Li W, Hui Y, Huang X, et al. 2017. H2A.Z represses gene expression by modulating promoter nucleosome structure and enhancer histone modifications in *Arabidopsis*. *Molecular Plant* **10**: 1274-1292.

Dalvai M, Fleury L, Bellucci L, Kocanova S, Bystricky K. 2013. TIP48/Reptin and H2A.Z requirement for initiating chromatin remodeling in estrogen-activated transcription. *PLOS Genetics* **9**: e1003387.

Deal RB, Henikoff S. 2011. Histone variants and modifications in plant gene regulation. *Current Opinion in Plant Biology* **14**: 116-122.

Deal RB, Kandasamy MK, McKinney EC, Meagher RB. 2005. The nuclear actin-related protein ARP6 is a pleiotropic developmental regulator required for the maintenance of *FLOWERING LOCUS C* expression and repression of flowering in *Arabidopsis*. *Plant Cell* **17**: 2633-2646.

Deal RB, Topp CN, McKinney EC, Meagher RB. 2007. Repression of flowering in *Arabidopsis* requires activation of *FLOWERING LOCUS C* expression by the histone variant H2A.Z. *Plant Cell* **19**: 74-83.

Doyon Y, Cote J. 2004. The highly conserved and multifunctional NuA4 HAT complex. *Current Opinion in Genetics &Development* **14**: 147-154.

Earley KW, Haag JR, Pontes O, Opper K, Juehne T, Song K, Pikaard CS. 2006. Gateway-compatible vectors for plant functional genomics and proteomics. *Plant Journal* **45**: 616-629.

Earley KW, Shook MS, Brower-Toland B, Hicks L, Pikaard CS. 2007. *In vitro* specificities of Arabidopsis co-activator histone acetyltransferases: implications for histone hyperacetylation in gene activation. *Plant Journal* **52**: 615-626.

Finnegan EJ, Dennis ES. 2007. Vernalization-induced trimethylation of histone H3 lysine 27 at *FLC* is not maintained in mitotically quiescent cells. *Current Biology* **17**: 1978-1983.

Formosa T. 2012. The role of FACT in making and breaking nucleosomes. *Biochimica et Biophysica Acta* **1819**: 247-255.

Geraldo N, Baurle I, Kidou S, Hu X, Dean C. 2009. FRIGIDA delays flowering in *Arabidopsis* via a cotranscriptional mechanism involving direct interaction with the nuclear cap-binding complex. *Plant Physiology*. **150**: 1611-1618.

Gerhold CB, Gasser SM. 2014. INO80 and SWR complexes: relating structure to function in chromatin remodeling. *Trends in Cell Biology* **24**: 619-631.

Gomez-Zambrano A, Crevillen P, Franco-Zorrilla JM, Lopez JA, Moreno-Romero J, Roszak P, Santos-Gonzalez J, Jurado S, Vazquez J, Kohler C, et al. 2018. *Arabidopsis* SWC4 binds DNA and recruits the SWR1 complex to modulate histone H2A.Z deposition at key regulatory genes. *Molecular Plant* **11**: 815-832.

Gonzalez N, Vanhaeren H, Inze D. 2012. Leaf size control: complex coordination of cell division and expansion. *Trends in Plant Science* **17**: 332-340.

He Y. 2012. Chromatin regulation of flowering. *Trends in Plant Science* **17**: 556-562.

He Y, Doyle MR, Amasino RM. 2004. PAF1-complex-mediated histone methylation of *FLOWERING LOCUS C* chromatin is required for the vernalization-responsive, winter-annual habit in *Arabidopsis*. *Genes &Development* **18**: 2774-2784.

Holt BF, 3rd, Boyes DC, Ellerstrom M, Siefers N, Wiig A, Kauffman S, Grant MR, Dangl JL. 2002. An evolutionarily conserved mediator of plant disease resistance gene function is required for normal *Arabidopsis* development. *Developmental Cell* **2**: 807-817.

Hsu CC, Shi J, Yuan C, Zhao D, Jiang S, Lyu J, Wang X, Li H, Wen H, Li W, et al. 2018. Recognition of histone acetylation by the GAS41 YEATS domain promotes H2A.Z deposition in non-small cell lung cancer. *Genes & Development* **32**: 58-69.

Huijser P, Schmid M. 2011. The control of developmental phase transitions in plants. *Development* **138**: 4117-4129.

Imaizumi T. 2010. *Arabidopsis* circadian clock and photoperiodism: time to think about location. *Current Opinion in Plant Biology* **13**: 83-89.

Ishibashi T, Dryhurst D, Rose KL, Shabanowitz J, Hunt DF, Ausio J. 2009. Acetylation of vertebrate H2A.Z and its effect on the structure of the nucleosome. *Biochemistry* **48**: 5007-5017.

Jarillo JA, Pineiro M. 2015. H2A.Z mediates different aspects of chromatin function and modulates flowering responses in Arabidopsis. *Plant Journal* **83**: 96-109.

Johanson U, West J, Lister C, Michaels S, Amasino R, Dean C. 2000. Molecular analysis of *FRIGIDA*, a major determinant of natural variation in *Arabidopsis* flowering time. *Science* **290**: 344-347.

Kim DH, Doyle MR, Sung S, Amasino RM. 2009. Vernalization: winter and the timing of flowering in plants. *Annual Review of Cell & Developmental Biology* **25**: 277-299.

Klein BJ, Ahmad S, Vann KR, Andrews FH, Mayo ZA, Bourriquen G, Bridgers JB, Zhang J, Strahl BD, Cote J, et al. 2018. Yaf9 subunit of the NuA4 and SWR1 complexes targets histone H3K27ac through its YEATS domain. *Nucleic Acids Research* **46**: 421-430.

Krogan NJ, Baetz K, Keogh MC, Datta N, Sawa C, Kwok TC, Thompson NJ, Davey MG, Pootoolal J, Hughes TR, et al. 2004. Regulation of chromosome stability by the histone H2A variant Htz1, the Swr1 chromatin remodeling complex, and the histone acetyltransferase NuA4. *Proceedings of the National Academy of Sciences, USA* **101**: 13513-13518.

Ku M, Jaffe JD, Koche RP, Rheinbay E, Endoh M, Koseki H, Carr SA, Bernstein BE. 2012. H2A.Z landscapes and dual modifications in pluripotent and multipotent stem cells underlie complex genome regulatory functions. *Genome Biology* **13**: R85.

Kumar SV, Wigge PA. 2010. H2A.Z-containing nucleosomes mediate the thermosensory response in *Arabidopsis*. *Cell* **140**: 136-147.

Latrasse D, Benhamed M, Henry Y, Domenichini S, Kim W, Zhou DX, Delarue M. 2008. The MYST histone acetyltransferases are essential for gametophyte development in *Arabidopsis*. *BMC Plant Biology* **8**: 121.

Law C, Cheung P. 2015. Expression of non-acetylatable H2A.Z in myoblast cells blocks myoblast differentiation through disruption of MyoD expression. *The Journal of Biological Chemistry* **290**: 13234-13249.

Lazaro A, Gomez-Zambrano A, Lopez-Gonzalez L, Pineiro M, Jarillo JA. 2008. Mutations in the *Arabidopsis SWC6* gene, encoding a component of the SWR1 chromatin remodelling complex, accelerate flowering time and alter leaf and flower development. J*ournal of Experimental Botany* : 653-666.

Le Masson I, Yu DY, Jensen K, Chevalier A, Courbeyrette R, Boulard Y, Smith MM, Mann C. 2003. Yaf9, a novel NuA4 histone acetyltransferase subunit, is required for the cellular response to spindle stress in yeast. *Molecular & Cellular Biology* **23**: 6086-6102.

Lee H, Suh SS, Park E, Cho E, Ahn JH, Kim SG, Lee JS, Kwon YM, Lee I. 2000. The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. *Genes &Development* **14**: 2366-2376.

Lee I, Amasino RM. 1995. Effect of vernalization, photoperiod, and light quality on the flowering phenotype of *Arabidopsis* plants containing the *FRIGIDA* gene. *Plant Physiology* **108**: 157-162.

Li Y, Wen H, Xi Y, Tanaka K, Wang H, Peng D, Ren Y, Jin Q, Dent SY, Li W, et al. 2014. AF9 YEATS domain links histone acetylation to DOT1L-mediated H3K79 methylation. *Cell* **159**: 558-571.

Li Z, Jiang D, He Y. 2018. *FRIGIDA* establishes a local chromosomal environment for *FLOWERING LOCUS C* mRNA production. *Nature Plants* **4**: 836-846.

Lolas IB, Himanen K, Gronlund JT, Lynggaard C, Houben A, Melzer M, Van Lijsebettens M, Grasser KD. 2010. The transcript elongation factor FACT affects *Arabidopsis* vegetative and reproductive development and genetically interacts with HUB1/2. *Plant Journal* **61**: 686-697.

Lu PY, Levesque N, Kobor MS. 2009. NuA4 and SWR1-C: two chromatin-modifying complexes with overlapping functions and components. *Biochemistry & Cell Biology* **87**: 799-815.

March-Diaz R, Garcia-Dominguez M, Florencio FJ, Reyes JC. 2007. SEF, a new protein required for flowering repression in *Arabidopsis*, interacts with PIE1 and ARP6. *Plant Physiology* **143**: 893-901.

March-Diaz R, Garcia-Dominguez M, Lozano-Juste J, Leon J, Florencio FJ, Reyes JC. 2008. Histone H2A.Z and homologues of components of the SWR1 complex are required to control immunity in *Arabidopsis*. *Plant Journal* **53**: 475-487.

March-Diaz R, Reyes JC. 2009. The beauty of being a variant: H2A.Z and the SWR1 complex in plants. *Molecular Plant* **2**: 565-577.

Martin-Trillo M, Lazaro A, Poethig RS, Gomez-Mena C, Pineiro MA, Martinez-Zapater JM, Jarillo JA. 2006. *EARLY IN SHORT DAYS 1* (*ESD1*) encodes ACTIN-RELATED PROTEIN 6 (AtARP6), a putative component of chromatin remodelling complexes that positively regulates *FLC* accumulation in *Arabidopsis*. *Development* **133**: 1241-1252.

Meagher RB, Kandasamy MK, Deal RB, McKinney EC. 2007. Actin-related proteins in chromatin-level control of the cell cycle and developmental transitions. *Trends in Cell Biology* **17**: 325-332.

Michaels SD, Amasino RM. 1999. *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* **11**: 949-956.

Michaels SD, Amasino RM. 2001. Loss of *FLOWERING LOCUS C* activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell* **13**: 935-941.

Millar CB, Xu F, Zhang K, Grunstein M. 2006. Acetylation of H2AZ Lys 14 is associated with genome-wide gene activity in yeast. *Genes & Development* **20**: 711-722.

Morrison AJ, Shen X. 2009. Chromatin remodelling beyond transcription: the INO80 and SWR1 complexes. *Nat Rev Mol Cell Biol* **10**(6): 373-384.

Nakagawa T, Kurose T, Hino T, Tanaka K, Kawamukai M, Niwa Y, Toyooka K, Matsuoka K, Jinbo T, Kimura T. 2007. Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. *Journal of Bioscience & Bioengineering* **104**: 34-41.

Noh YS, Amasino RM. 2003. PIE1, an ISWI family gene, is required for *FLC* activation and floral repression in *Arabidopsis*. *Plant Cell* **15**: 1671-1682.

Pfab A, Gronlund JT, Holzinger P, Langst G, Grasser KD. 2018. The Arabidopsis histone chaperone FACT: Role of the HMG-box domain of SSRP1. *Journal of Molecular Biology*. **430**: 2747–2759

Rubio V, Shen Y, Saijo Y, Liu Y, Gusmaroli G, Dinesh-Kumar SP, Deng XW. 2005. An alternative tandem affinity purification strategy applied to *Arabidopsis* protein complex isolation. *Plant Journal* **41**: 767-778.

Saeed AI, Bhagabati NK, Braisted JC, Liang W, Sharov V, Howe EA, Li J, Thiagarajan M, White JA, Quackenbush J. 2006. TM4 microarray software suite. *Methods in Enzymology* **411**: 134-193.

Schulze JM, Wang AY, Kobor MS. 2009. YEATS domain proteins: a diverse family with many links to chromatin modification and transcription. *Biochemistry & Cell Biology* **87**: 65-75.

Shanle EK, Andrews FH, Meriesh H, McDaniel SL, Dronamraju R, DiFiore JV, Jha D, Wozniak GG, Bridgers JB, Kerschner JL, et al. 2015. Association of Taf14 with acetylated histone H3 directs gene transcription and the DNA damage response. *Genes & Development* **29**: 1795-1800.

Smith AP, Jain A, Deal RB, Nagarajan VK, Poling MD, Raghothama KG, Meagher RB. 2010. Histone H2A.Z regulates the expression of several classes of phosphate starvation response genes but not as a transcriptional activator. *Plant Physiology* **152**: 217-225.

Song J, Rutjens B, Dean C. 2014. Detecting histone modifications in plants. *Methods in Molecular Biology* **1112**: 165-175.

Song YH, Shim JS, Kinmonth-Schultz HA, Imaizumi T. 2015. Photoperiodic flowering: time measurement mechanisms in leaves. *Annual Review in Plant Biology* **66**: 441-464.

Srikanth A, Schmid M. 2011. Regulation of flowering time: all roads lead to Rome. *Cellular & Molecular Life Sciences* **68**: 2013-2037.

Su Y, Wang S, Zhang F, Zheng H, Liu Y, Huang T, Ding Y. 2017. Phosphorylation of histone H2A at serine 95: a plant-specific mark involved in flowering time regulation and H2A.Z deposition. *Plant Cell* **29**: 2197-2213.

Subramanian V, Fields PA, Boyer LA. 2015. H2A.Z: a molecular rheostat for transcriptional control. *F1000Prime Rep* **7**: 01.

Sura W, Kabza M, Karlowski WM, Bieluszewski T, Kus-Slowinska M, Paweloszek L, Sadowski J, Ziolkowski PA. 2017. Dual role of the histone variant H2A.Z in transcriptional regulation of stress-response genes. *Plant Cell* **29**: 791-807.

Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, Doncheva NT, Roth A, Bork P, et al. 2017. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. *Nucleic Acids Research* **45**: D362-D368.

Tan LM, Zhang CJ, Hou XM, Shao CR, Lu YJ, Zhou JX, Li YQ, Li L, Chen S, He XJ. 2018. The PEAT protein complexes are required for histone deacetylation and heterochromatin silencing. The *EMBO Journal* **37**: e98770.

Valdes-Mora F, Gould CM, Colino-Sanguino Y, Qu W, Song JZ, Taylor KM, Buske FA, Statham AL, Nair SS, Armstrong NJ, et al. 2017. Acetylated histone variant H2A.Z is involved in the activation of neo-enhancers in prostate cancer. *Nature Communications* **8**: 1346.

Valdes-Mora F, Song JZ, Statham AL, Strbenac D, Robinson MD, Nair SS, Patterson KI, Tremethick DJ, Stirzaker C, Clark SJ. 2012. Acetylation of H2A.Z is a key epigenetic modification associated with gene deregulation and epigenetic remodeling in cancer. *Genome Research* **22**: 307-321.

Voinnet O, Rivas S, Mestre P, Baulcombe D. 2003. An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. *Plant Journal* **33**: 949-956.

Wang AY, Schulze JM, Skordalakes E, Gin JW, Berger JM, Rine J, Kobor MS. 2009. Asf1-like structure of the conserved Yaf9 YEATS domain and role in H2A.Z deposition and acetylation. *Proceedings of the National Academy of Sciences, USA* **106**: 21573-21578.

Wang X, Ahmad S, Zhang Z, Cote J, Cai G. 2018. Architecture of the *Saccharomyces cerevisiae* NuA4/TIP60 complex. *Nature Communications* **9**: 1147.

Wang Z, Cao H, Chen F, Liu Y. 2014. The roles of histone acetylation in seed performance and plant development. *Plant Physiology and Biochemistry* **84**: 125-133.

Whittaker C, Dean C. 2017. The *FLC* Locus: A Platform for Discoveries in Epigenetics and Adaptation. *Annual Review Of Cell & Developmental Biology* **33**: 555-575.

Wu WH, Alami S, Luk E, Wu CH, Sen S, Mizuguchi G, Wei D, Wu C. 2005. Swc2 is a widely conserved H2AZ-binding module essential for ATP-dependent histone exchange. *Nature Structural & Molecular Biology* **12**: 1064-1071.

Wu WH, Wu CH, Ladurner A, Mizuguchi G, Wei D, Xiao H, Luk E, Ranjan A, Wu C. 2009. N terminus of Swr1 binds to histone H2AZ and provides a platform for subunit assembly in the chromatin remodeling complex. *The Journal of Biological Chemistry* **284**: 6200-6207.

Xiao J, Zhang H, Xing L, Xu S, Liu H, Chong K, Xu Y. 2013. Requirement of histone acetyltransferases HAM1 and HAM2 for epigenetic modification of *FLC* in regulating flowering in *Arabidopsis*. *Journal of Plant Physiology* **170**: 444-451.

Xu M, Leichty AR, Hu T, Poethig RS. 2018. H2A.Z promotes the transcription of *MIR156A* and *MIR156C* in *Arabidopsis* by facilitating the deposition of H3K4me3. *Development* **145**:, dev152868.

Yi H, Sardesai N, Fujinuma T, Chan CW, Veena, Gelvin SB. 2006. Constitutive expression exposes functional redundancy between the *Arabidopsis* histone H2A gene HTA1 and other H2A gene family members. *Plant Cell* **18**: 1575-1589.

Yoo SK, Chung KS, Kim J, Lee JH, Hong SM, Yoo SJ, Yoo SY, Lee JS, Ahn JH. 2005. CONSTANS activates *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* through *FLOWERING LOCUS T* to promote flowering in *Arabidopsis*. *Plant Physiology.* **139**: 770-778.

Zacharaki V, Benhamed M, Poulios S, Latrasse D, Papoutsoglou P, Delarue M, Vlachonasios KE. 2012. The *Arabidopsis* ortholog of the YEATS domain containing protein YAF9a regulates flowering by controlling H4 acetylation levels at the *FLC* locus. *Plant Science* **196**: 44-52.

Zahraeifard S, Foroozani M, Sepehri A, Oh DH, Wang G, Mangu V, Chen B, Baisakh N, Dassanayake M, Smith AP. 2018. Rice H2A.Z negatively regulates genes responsive to nutrient starvation but promotes expression of key housekeeping genes. J*ournal of Experimental Botany* **69**: 4907-4919.

Zhang H, Richardson DO, Roberts DN, Utley R, Erdjument-Bromage H, Tempst P, Cote J, Cairns BR. 2004. The Yaf9 component of the SWR1 and NuA4 complexes is required for proper gene expression, histone H4 acetylation, and Htz1 replacement near telomeres. *Molecular & Cellular Biology* **24**: 9424-9436.

Zhao D, Li Y, Xiong X, Chen Z, Li H. 2017. YEATS Domain-A Histone Acylation Reader in Health and Disease. *Journal of Molecular Biology* **429**: 1994-2002.

Zhou W, Zhu Y, Dong A, Shen WH. 2015. Histone H2A/H2B chaperones: from molecules to chromatin-based functions in plant growth and development. *Plant Journal* **83**: 78-95.

Zlatanova J, Thakar A. 2008. H2A.Z: view from the top. *Structure* **16**: 166-179.

Zou YP, Hou XH, Wu Q, Chen JF, Li ZW, Han TS, Niu XM, Yang L, Xu YC, Zhang J, et al. 2017. Adaptation of *Arabidopsis thaliana* to the Yangtze River basin. *Genome Biology* **18**: 239.

Supporting information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Two Arabidopsis YEATS domain-containing proteins are the predicted homologs of yeast YAF9 protein.

Fig. S2 The two Arabidopsis YAF9 proteins are nuclear localized but differentially expressed.

Fig. S3 Isolation of Arabidopsis *YAF9* knock-out mutants.

Fig. S4 Double mutant *yaf9a yaf9b* plants display a broad range of pleiotropic developmental defects.

Fig S5 Complementation of the *yaf9a* mutant with a wild type copy of the *YAF9A* gene.

Fig. S6 Validation of microarray data obtained in the transcriptomic analysis of *yaf9ayaf9b* seedlings.

Fig. S7 Comparison of upregulated genes in *yaf9a yaf9b* seedlings and in selected *swr1-c* mutants.

Fig. S8 Comparison of downregulated genes in *yaf9a yaf9b* seedlings and in selected *swr1-c* mutants.

Fig. S9 Genome-wide transcriptomic analysis of *yaf9a yaf9b* mutant.

Fig. S10 Singular Enrichment Analysis (SEA) of Gene Ontology (GO) terms of genes with altered expression in *yaf9a yaf9b* seedlings.

Fig. S11 *yaf9 ayaf9b* plants display necrotic spots and paler colour in the leaves than Col. Pictures were taken at 30 day-old plants grown in SD.

Fig. S12 Determination of the specificity of α-H2A.Zac antibody used in the ChIP analysis.

Fig. S13 *yaf9a* but not *yaf9b* mutants showed reduced histone H4 acetylation levels at *FLC* chromatin.

Fig. S14 YAF9A protein directly binds *FT* chromatin and mediates in the deposition of H2A.Z in this locus.

Fig.S15 Characterization of the Arabidopsis YAF9A-TAPa 3 and 10 lines generated.

Table S1 List of primers used in this study.

Table S2 List of cell cycle-related genes misregulated in *yaf9yaf9b*.

Table S3 List of SAR-related genes deregulated in *yaf9yaf9b*.

Datasets:

Dataset S1 List of differentially expressed genes in *yaf9a yaf9b*.

Dataset S2 Arabidopsis YAF9A protein interactors.

Method S1 Supporting information for phenotypic analyses and growth conditions, microscopic analyses, Co-IP, SDS-PAGE, Western Blotting and histone preparations, pulldown assays with histone peptides and Arabidopsis histone extracts, protein affinity purification and tandem mass spectrometry analyses.

Fig. 1 Double mutant *yaf9a yaf9b* plants display pleiotropic developmental defects. (a, b) Pictures of Col, *yaf9a*, *yaf9b* and *yaf9a yaf9b* rosettes (a) and leaves (b). (c–f) Rosette diameter (n = 15) (c), leaf (n = 8) (d), flower (n = 21) (e) and silique length (n = 12) (f) of Col and *yaf9a*, *yaf9b* and *yaf9a yaf9b* mutant plants grown under long-day (LD) conditions. Error bars indicate ± SE of the mean; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ (Student´s *t*-test).

Fig. 2 Arabidopsis YAF9 proteins regulate leaf cell proliferation and expansion. (a) Cell size analysis of the adaxial leaf epidermal layers of wild-type (WT) and *yaf9a yaf9b* mutant plants 32 d after sowing (n > 35 cells); leaves 3 and 4 were analyzed by scanning electron microscopy (SEM) images (×200). Bars, 100 μm. (b–d) Cell area distribution (log μm^2). (b) The frequency (%) indicated on the *x*-axis corresponds to the number of cells of a defined size within each range. Measurements were carried out in the central region of at least six leaves in each case ($n \geq 400$ cells); (c) estimated cell number in the adaxial epidermis of leaves from plants grown under short-day (SD) conditions 32 d after sowing, and (d) average size of adaxial epidermal cells (leaf third and fourth) (n > 50 cells) from 32-d-old plants, Col and *yaf9a yaf9b* grown under SD conditions. Error bars indicate ± SE of the mean (n = 20); ****, $P < 0.0001$ (Student´s *t*-test). (e) Nuclear DNA ploidy distribution of 32-d-old Col and *yaf9a yaf9b* plants (leaf third and fourth) grown under SD and long-day (LD) conditions. The results shown are the average of three independent assays. Average values of each nuclear DNA content class (± standard deviation) are presented.

Fig. 3 Arabidopsis YAF9 proteins regulate flowering time by both *FLC*-dependent and independent mechanisms. (a) Flowering time of *yaf9a*, *yaf9b* and *yaf9a yaf9b* mutants in Col wild-type (WT) and *flc-3* mutant background under long-day (LD) (n = 10). Statistical

significance was calculated using one-way ANOVA (Tukey test correction for multiple comparisons). Different letters denote a significance level of $P < 0.05$; same letters indicate no significant differences. (b) Q-PCR analysis of *FLC* expression in 10-d-old Col and *yaf9a*, *yaf9b* and *yaf9a yaf9b* mutant seedlings grown under LD conditions. Error bars indicate ± SE of the mean ($n = 4$). Statistical significance compared to Col was calculated using Student´s *t*-test; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant. (c) Photograph of *yaf9a*, *yaf9b* and *ft-10* double and triple mutant plants grown under LD photoperiods. (d) Flowering time of *yaf9* and *ft-10* double and triple mutant combinations grown under LD conditions ($n = 15$). Whiskers were determined according to Tukey test. Statistical significance was calculated using one-way ANOVA (Tukey test correction for multiple comparisons). Different letters denote a significance level of $P < 0.05$; same letters indicate no significant differences.

Fig. 4 Arabidopsis YAF9 proteins regulate flowering time by SWR1-C independent pathways. (a–d) Flowering time of *yaf9a*, *yaf9b* and *swc6-1* double and triple mutant plants grown under (a) long-day (LD) ($n \geq 8$) and (c) short-day (SD) photoperiods ($n \geq 10$). Whiskers were determined according to Tukey test. Statistical significance was calculated using one-way ANOVA (Tukey test correction for multiple comparisons). Different letters denote a significance level of $P < 0.05$; same letters indicate no significant differences. Photographs of *yaf9a*, *yaf9b* and *swc6-1* double and triple mutant combination plants grown under (b) LD or (d) SD conditions.

Fig. 5 *yaf9a yaf9b* mutations partially suppress the late-flowering phenotype of *FRIGIDA*. (a) Flowering time of *yaf9a*, *yaf9b* and *yaf9a yaf9b* double mutant plants in *FRI-Sf-2* background grown under long-day (LD) (a) ($n \geq 8$) and short day (SD) conditions ($n = 10$). Whiskers were determined according to Tukey test. Statistical significance compared to Col *FRI*-Sf-2 (Col*FRI*) was calculated using Student´s t-test; *, $P < 0.05$; ***, $P < 0.001$; ns, not significant. (b) Q-PCR analysis of *FLC* expression in 10-d-old seedlings grown under LD conditions. Error bars indicate ± standard deviation ($n = 2$). Statistical significance compared to Col *FRI* was calculated using Student´s *t*-test; *, $P < 0.05$. (c) Photograph of representative *yaf9a yaf9b* double mutant plant in *FRI-Sf-2* background (*yaf9a yaf9b FRI*) and Col *FRI*-Sf-2 (Col*FRI*) plant.

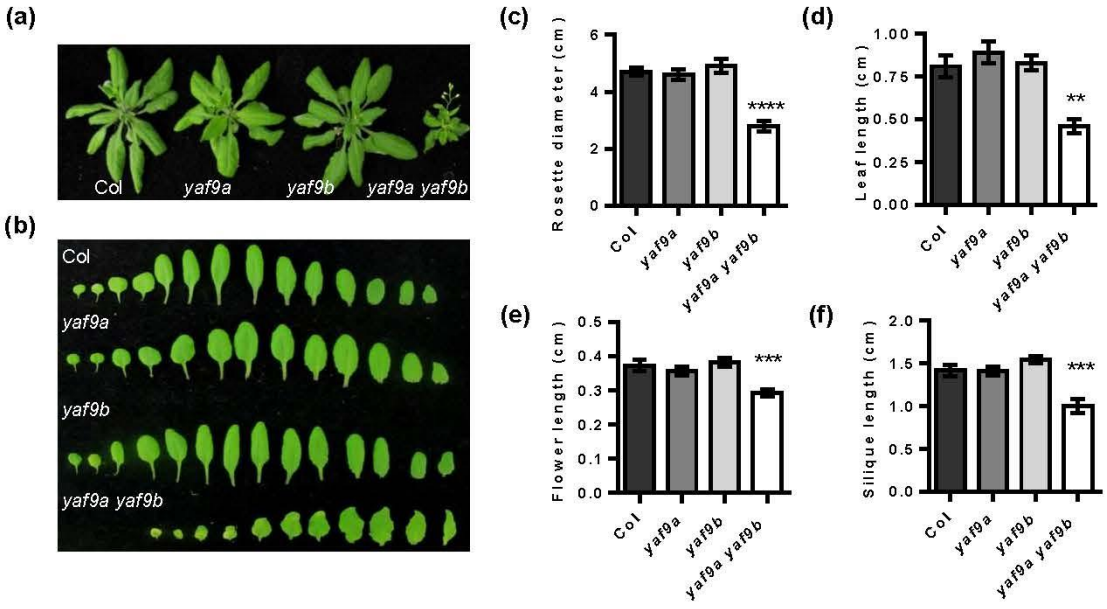
Fig. 6 Arabidopsis YAF9 proteins directly regulate H2A.Z and H4 acetylation levels but not H2A.Z deposition in the chromatin of *FLC* locus. (a) Schematic representation of the *FLC*

locus indicating the regions analyzed by chromatin immunoprecipitation (ChIP). (b) ChIP experiments using α-GFP to detect HTA11-GFP in Col, *yaf9a yaf9b* and *arp6* background. Col was included as a negative control (c–e) ChIP experiments using α-HTA9 (c), α-H2AZ.ac (d) and α-H4ac (e) in Col, *yaf9a yaf9b* and *arp6* mutant seedlings. Data are represented as the fraction of immunoprecipitated DNA normalized to an *ACT2* gene region. Graphs represent the average of three independent biological ChIP experiments quantified by Q-PCR. Error bars indicate ± SE of the mean. The statistical significance of the observed difference at region +232 of *FLC* chromatin was calculated using Student´s t-test (*, $P \leq 0.05$; **, $P \leq 0.01$, ***, $P \leq 0.001$). (f) ChIP experiments using α-Myc to detect YAF9-TAPa in two independent transgenic lines. Data are represented as the fraction of immunoprecipitated DNA of *FLC*/*ACT2* normalized to the values obtained in a control Col line. Graphs represent the average of two independent biological ChIP experiments for each transgenic line (YAF9A-TAP 3 and 10) analysed, quantified by Q-PCR. Error bars indicate ± standard deviation. The observed difference at region +232 of *FLC* chromatin was found statistically significant by Student´s *t*-test (*, $P \leq 0.05$).

Fig. 7 Arabidopsis YAF9 are histone binding proteins, and YAF9A interacts with the histone acetyltransferase HAM1 *in planta*. (a) YAF9A and YAF9B proteins bind histone H3 and H3Ac but not H2B in pulldown assays. Recombinant YAF9A-GST, YAF9B-GST and GST were incubated with purified Arabidopsis histones extracts and pulldown with glutathione sepharose beads. Recovered histones were detected by western blot using specific antibodies against histone H3, H3 acetylation (K9/K14) and H2B. (b) YAF9A protein binds unmodified histone H3, H3K9ac and H3K27ac peptides in peptide pulldown assays. Biotinylated histones peptides were incubated with recombinant YAF9A-GST or GST and pulldown with magnetic Dynabeads M-280 Streptavidin. Recovered proteins were detected by western blot using α-GST. (c) The list of proteins that co-purify with YAF9 identified by tandem affinity purification followed by tandem mass spectrometry were analyzed using the STRING database to identify groups of proteins that have predicted interactions (Szklarczyk *et al.*, 2017). The network connections between the submitted proteins were visualized by their confidence score, where a thicker line indicates a higher interaction score. Then, the network was subdivided into clusters; in the figure, a cluster of chromatin related proteins is shown. (d) Chromatin immunoprecipitation (ChIP) assays in agroinfiltrated *Nicotiana benthamiana* plants. HAM1-HA and YAF9A-GFP fusion proteins were produced alone or in combination

in WT *Nicotiana* plants; GFP fusion proteins were immunoprecipitated with GFP-Trap_A, and HAM1-HA was detected using anti-HA antibody. The arrow indicates the expected band for the calculated size of the HAM1-HA fusion protein.

FIGURE 1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yaf9b
yaf9a yaf9b
(b)
Col
yaf9a
yaf9b
yaf9a yaf9b
(c)
Rosette diameter (cm)
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(e)
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(f)
Silique length (cm)
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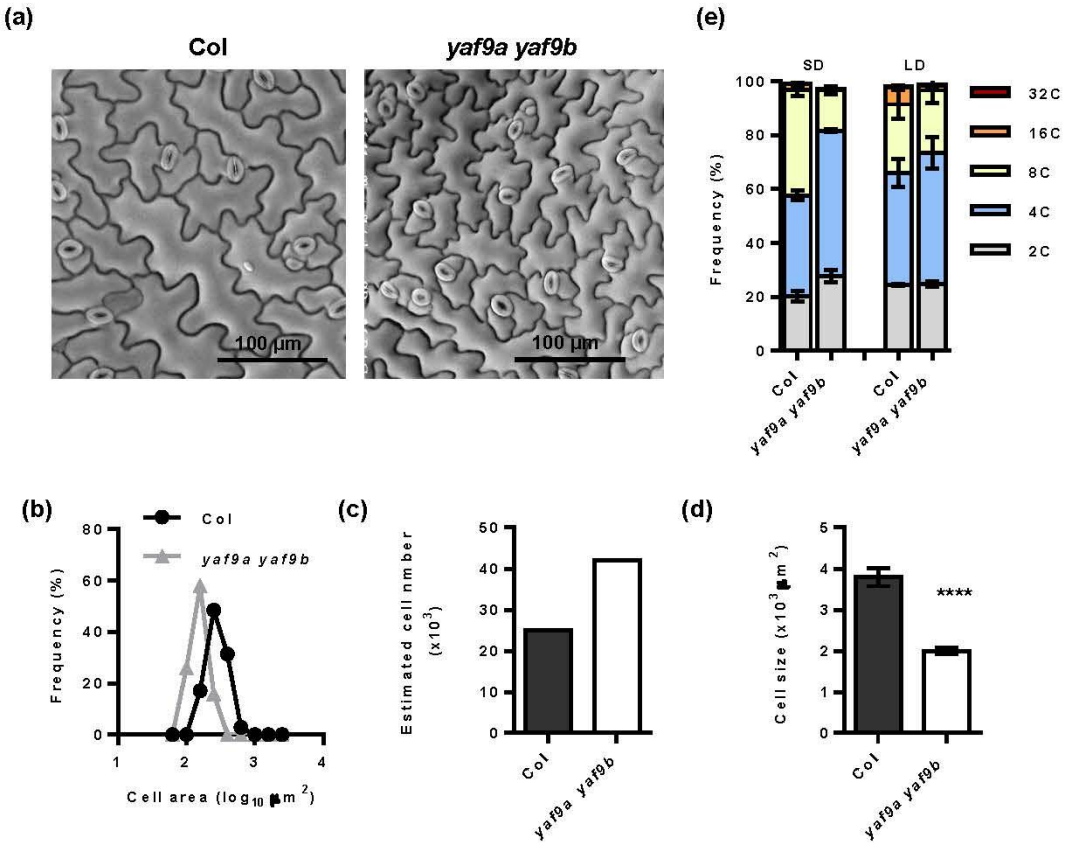
FIGURE 2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8C
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(b)
Col
yaf9a yaf9b
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(c)
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(d)
Cell size (x10^3 µm2)
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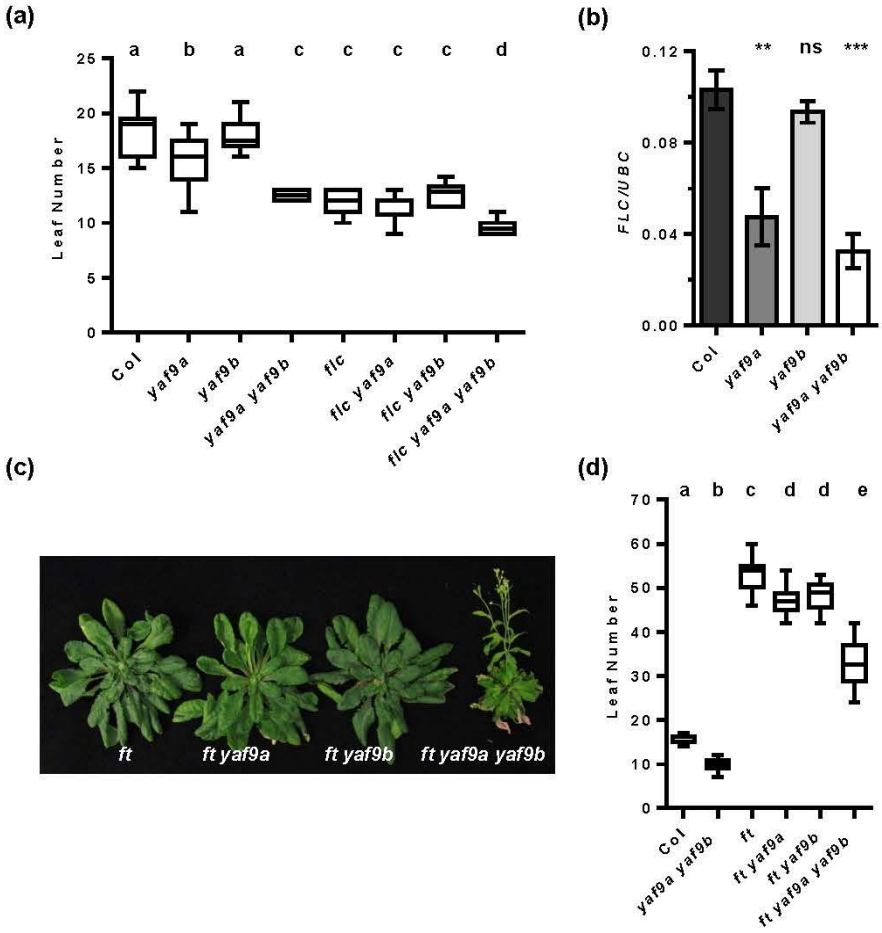
FIGURE 3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flc yaf9b
flc yaf9a yaf9b
(b)
** ns ***
FLC/UBC
0.12
0.08
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0.00
Col
yaf9a
yaf9b
yaf9a yaf9b
(c)
ft
ft yaf9a
ft yaf9b
ft yaf9a yaf9b
(d)
a b c d d e
Leaf Number
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50
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FIGURE 4

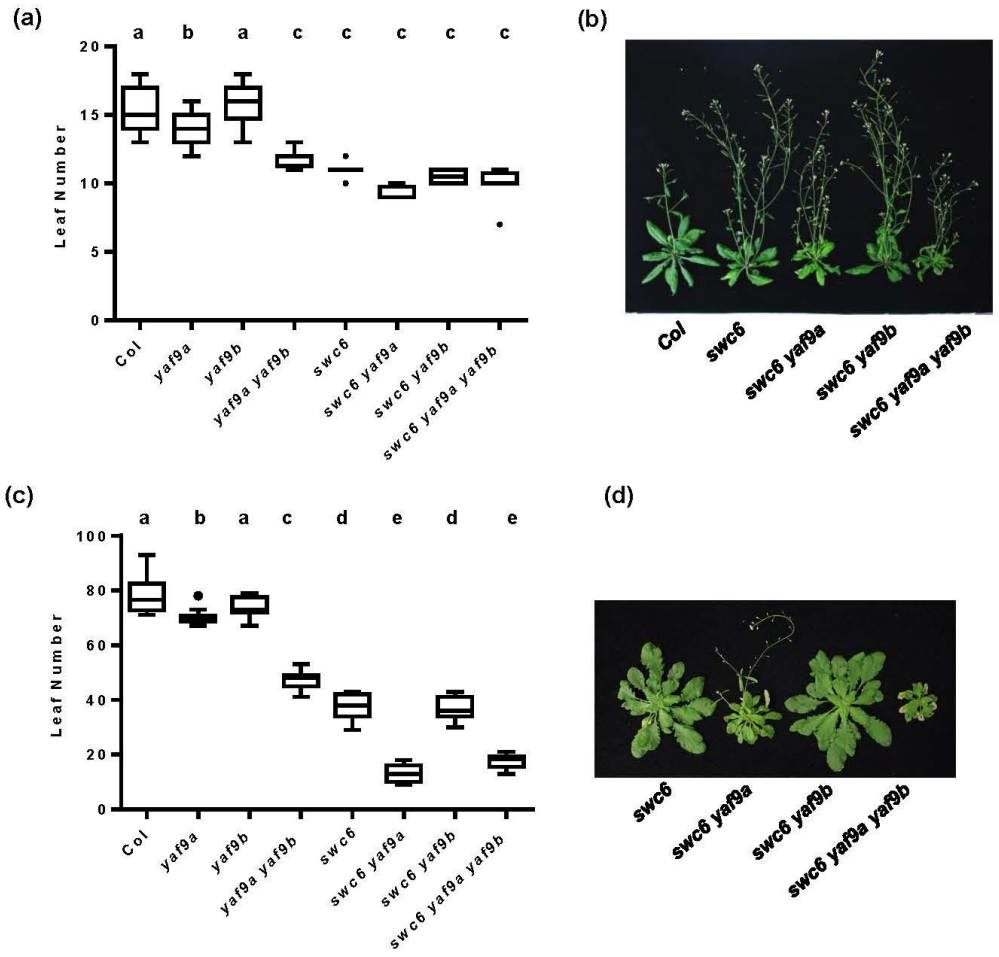
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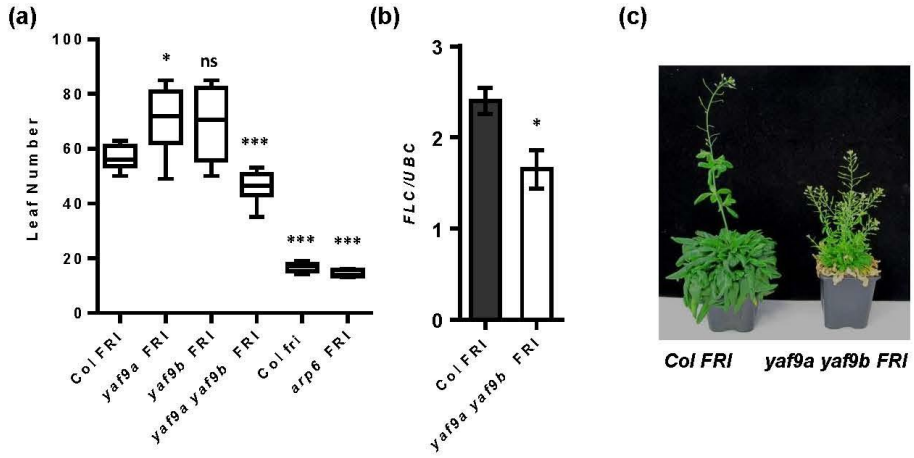
FIGURE 5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yaf9a yaf9b FRI
Col fri
arp6 FRI
(b)
FLC/UBC
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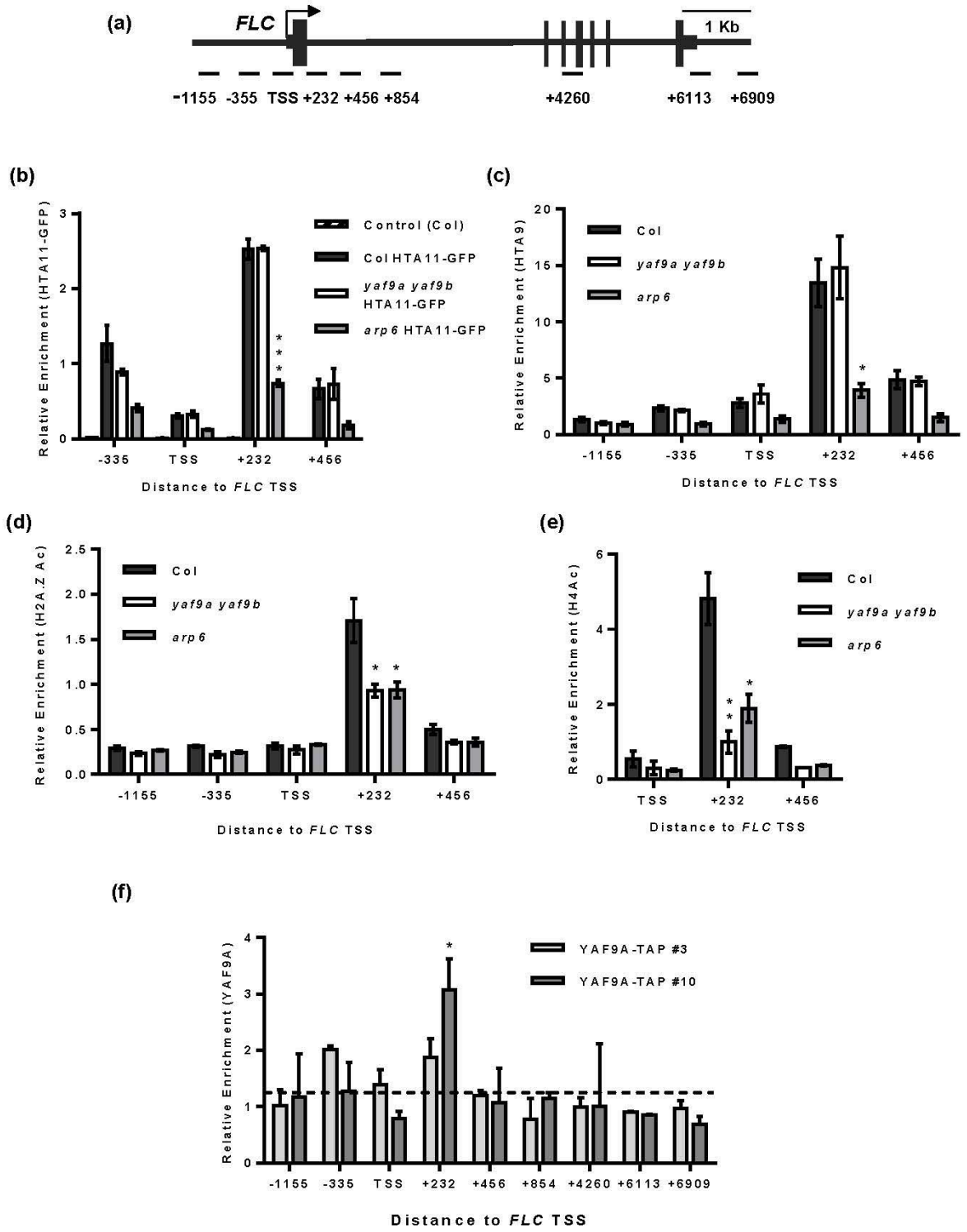
(c)
Col FRI
yaf9a yaf9b FRI

FIGURE 6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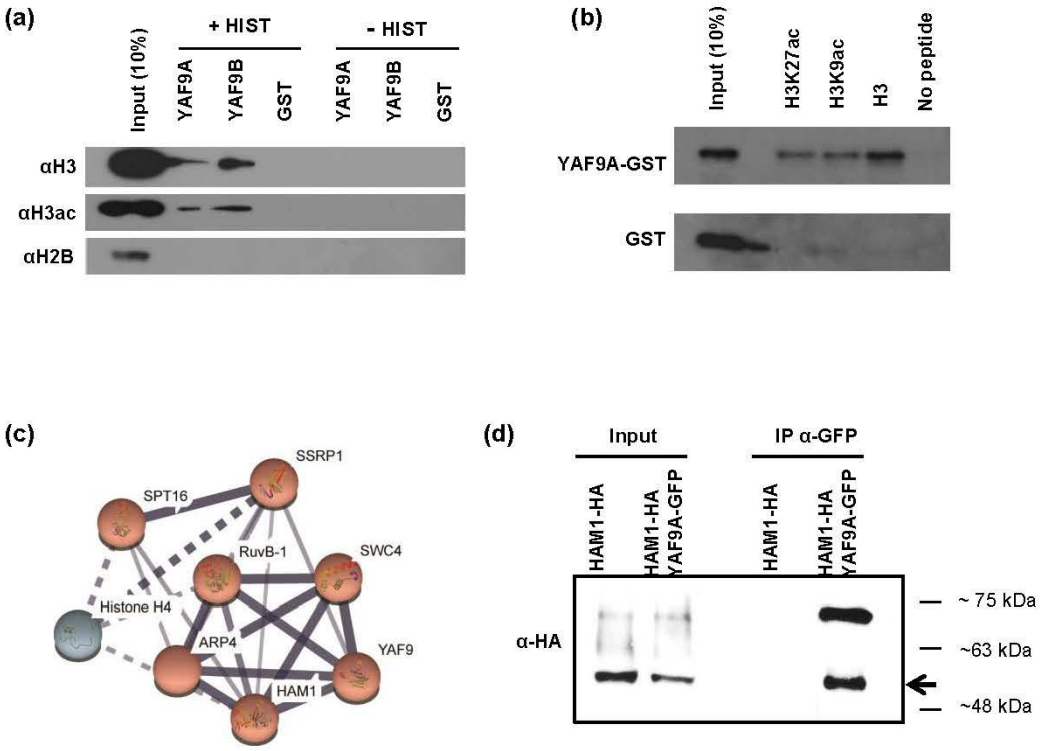
+4260
+6113 +6909
(b)
Relative Enrichment (HTA11-GFP)
Control (Col)
Col HTA11-GFP
yaf9a yaf9b HTA11-GFP
arp6 HTA11-GFP
-335 TSS +232 +456
Distance to FLC TSS
(c)
Relative Enrichment (HTA9)
Col
yaf9a yaf9b
arp6
-1155 -335 TSS +232 +456
Distance to FLC TSS
(d)
Relative Enrichment (H2A.Z Ac)
Col
yaf9a yaf9b
arp6
-1155 -335 TSS +232 +456
Distance to FLC TSS
(e)
Relative Enrichment (H4Ac)
Col
yaf9a yaf9b
arp6
TSS +232 +456
Distance to FLC TSS
(f)
Relative Enrichment (YAF9A)
YAF9A-TAP #3
YAF9A-TAP #10
-1155 -335 TSS +232 +456 +854 +4260 +6113 +6909
Distance to FLC TSS

FIGURE 7


(a)
+ HIST
- HIST
Input (10%)
YAF9A
YAF9B
GST
YAF9A
YAF9B
GST
αH3
αH3ac
αH2B
(b)
Input (10%)
H3K27ac
H3K9ac
H3
No peptide
YAF9A-GST
GST
(c)
SSRP1
SPT16
RuvB-1
SWC4
Histone H4
ARP4
YAF9
HAM1
(d)
Input
IP α-GFP
HAM1-HA
HAM1-HA YAF9A-GFP
HAM1-HA
HAM1-HA YAF9A-GFP
α-HA
~ 75 kDa
~63 kDa
~48 kDa