

Figure S1. Comparison of ATAC-seq and H3s distribution.

(a) Correlation coefficient between replicates of Col-0, asf1a1b, hira-1, fas1-4, and fas2-4 ATAC-seq data. Pearson method was used to calculate correlation coefficient.

(b) Principal component analysis (PCA) from replicates of Col-0, asf1a1b, hira-1, fas1-4, and fas2-4 ATAC-seq data.

Differential (mutant vs Col-0) ATAC-seq signals plotted over Arabidopsis chromosomes in hira-1 (c), fas1-4 (d), fas2-4 (e) and asf1a1b (h). H3.1 (f) and H3.3 (g) ChIP signals in Col-0 over Arabidopsis chromosomes. Reads per million mapping reads (RPKM) is calculated in 100-kb bins and normalized with Col-0 or ChIP input. Boxes correspond to the heterochromatic region.



Figure S2. Distribution of ATAC-seq reads over transposon elements (TE) and genomic distribution of the differentially enriched ATAC-seq peaks.

Metaplot of Col-0 (a), asf1a1b (b), hira-1 (c), fas1-4 (d), and fas2-4 (e) ATAC-seq data over TE (n=32153) including 1 Kb flanking sequence. (f) Genomic distribution of asf1a1b, hira-1, fas1-4, and fas2-4. Highly Accessible Regions (HARs) represent genomic regions where mutants have higher chromatin accessibility than wild-type.

(g) Genomic distribution of asf1a1b, hira-1, fas1-4, and fas2-4. Lowly Accessible Regions (LARs) represent genomic regions where mutants have lower chromatin accessibility than wild-type ones. Enrichment = (number of sample peaks)/(number of randomly selected peaks). (h) Heatmap showing ChIP-seq signals of H3.1/input, H3.3/input over asf1a1b HARs (n=1,996).



Figure S3. Morphology of Col-0, asf1a-1, asf1b-1, hira-1, asf1a1b, hira-1 asf1a-1, hira-1 asf1b-1, and hira-1 asf1a1b.



Figure S4. ASF1 mutation causes a redistribution of H3.3 and H3.1.

(a) Co-immunoprecipitation assay of ASF1A, ASF1B, H3.1, and H3.3. Labels to the right indicate the antibody used for the western blot. (b) Metaplot of H3.1 and H3.3 variation (asf1a1b vs. Col-0) over the gene body and 1 Kb flanking sequence of genes with coding regions lengths over 150bp (n=26816). The Blue and the red line represents H3.1 and H3.3 variation, respectively.

(c) Metaplot of H3.1 and H3.3 variation (asf1a1b vs Col-0) over TE (n=32153) including 1 Kb flanking sequence. The Blue and the red line represents H3.1 and H3.3 variation, respectively.

(d) Metaplot of H3.1 and H3.3 variation (asf1a1b vs Col-0) over 5 chromosomes splitting into 100 Kb bins. The Blue and the red line represents H3.1 and H3.3 variation, respectively.

(e) Variation of ATAC-seq (asf1a1b vs Col-0) over H3.1-unique (n=24841), H3.3-unique (n=28629), and H3.1/H3.3 common (n=15051) peaks.







(a) Morphological phenotype of asf1a1b mutants transformed with ASF1A-Flag and ASF1B-Flag.
 (b-c) Whole-genome distribution of ASF1A and ASF1B ChIP-seq signal over Arabidopsis chromosomes.
 Reads per million mapping reads (RPKM) were calculated in 100-Kb bins and normalized with Col-0 or ChIP input. Boxes correspond to the heterochromatic regions.



Figure S6. Correlation between ASF1A, ASF1B, NRPB1 and H3.1/H3.3 ChIP-seqs.

(a) Genome-wide pairwise scatterplots for ChIP-seq at 1 Kb bins.

(b) Plotting of ASF1A and B ChIP-seq signal over H3.1 unique (n=24841), H3.3 unique (n=28629), and H3.1/H3.3 common (n=15051) peaks.

(c) Correlation of the expression divergence between H3 chaperone mutants. Graph showing correlation of variation in RNA-seq level of genes (n=3412) that are differentially expressed (FC > 2, FDR < 0.05) in at least one mutant in asf1a1b vs hira-1and fas1-4 vs fas2-4. Pearson method was used to calculate correlation coefficient. $R^2 = 0.83$ and P-value < 2.2e-16 for *asf1a1b* vs *hira-1*; $R^2 = 0.93$ and P-value < 2.2e-16 for *fas1-4* vs *fas2-4*.



Figure S7. Effects of asf1a1b on the distribution of epigenetic marks.

(a) Metaplot showing DNA methylation level in Col-0 and asf1a1b over the gene body and 1 Kb flanking sequence.
(b) Metaplot showing differential CG methylation level (asf1a1b minus Col-0) over asf1a1b highly accessible regions (HAR, n=1,996).

(c) Metaplot showing differential of chromatin accessibility (asf1a1b/Col-0) over asf1a1b CG hypomethylated DMRs (n=6197).

(d) Metaplot showing differential CG methylation level (asf1a1b minus Col-0) over asf1a1b lowly accessible regions (LAR, n=914).

(e) Metaplot showing differential of chromatin accessibility (asf1a1b/Col-0) over asf1a1b CG hypermethylated DMRs (n=3867).

(f) Metaplot showing H3K36me2/3 level in Col-0 and asf1a1b over the gene body and 1 Kb flanking sequence.

(g) ChIP-seq (ASF1A, ASF1B, H3.1, H3.3, and H3K36me2/3) and ATAC-seq signal over genes ranked by gene length. Top to bottom display genes from long to short.

(h) Metaplot showing H3.3 ChIP-seq signal in Col-0 over genes according to expression level (Group 1 to Group 10 represent gene expression from low to high).

(i-k) Metaplot of CG methylation difference in hira-1 (i), fas1-4 (j), and fas2-4 (k) vs. Col-0 over genes grouped by gene length (Group 1 to Group 10 represent gene length from short to long).



Figure S8. Subcellular fractionation of ASF1 proteins.

Western blot analysis of nuclear and cytoplasmic fractions using an anti-Myc antibody for ASF1A and ASF1B proteins. Western blot using anti-H3 antibody is a control for nuclear proteins. Numbers on the left indicate the protein ladders' molecular weights (kDa). Three experiments were repeated with similar results.



Figure S9. ASF1B-ZF108 effects on FWA expression and TF footprint.

(a-b) The relative expression level of FWA in five and ASF1B-ZF108 T1 lines and T2 lines. n=3 biologically independent plants examined. Error bars, mean ± standard error. n=3 technical replicates examined. (c) DNA methylation (CG, CHG, and CHH) level over the FWA promoter using bisulfite (BS)-PCR-seq in Col-0 and five-4 controls and the ASF1B-ZF108 T2 line. Light red boxes indicate the ASF1B-ZF108 binding sites. (d-e) McrBC assay of Col-0 and five-4 controls and the ASF1B-ZF108 T1 and T2 lines at the FWA tandem repeat. n=3 biologically independent plants examined. Error bars, mean ± standard error. n=3 biological and technical replicates examined.

(f-h) Screenshot of example genes showing ZF binding assayed by ChIP-seq, chromatin accessibility assayed by ATAC-Seq, and expression level at AT4G08950, AT2G22980, and AT2G26710. Tracks of fwa-4 (orange) and ASF1B-ZF108-3xFlag (blue) are shown overlapped.
 (i) Volcano plot showing differential footprint of 572 plant TF motifs distributed at non-DEG associated, down-regulated off-target sites (n=572). P values calculated by two-sided *t* test.

Supplementary Tables

Supplementary Table 1 Identified ASF1-interacting proteins by immunoprecipitation in combination with mass spectrometry (IP-MS) in ASF1A-3xFlag and ASF1B-9xMyc lines. Values indicate spectral counts values for each protein. Corresponding four wild type control for ASF1A and three wild type control for ASF1B did not show significant enrichment of peptides. N/F represents not identified.

| ID | Protein | ASF1A | | | | ASF1B | | |
|-----------|---------|-------|------|------|------|-------|------|------|
| | | Rep1 | Rep2 | Rep3 | Rep4 | Rep1 | Rep2 | Rep3 |
| AT1G66740 | ASF1A | 84 | 97 | 84 | 92 | N/F | N/F | N/F |
| AT5G38110 | ASF1B | N/F | N/F | N/F | N/F | 143 | 122 | 102 |
| AT4G37210 | NASP | 71 | 60 | 63 | 33 | 1 | 2 | 2 |
| AT3G44530 | HIRA | 62 | 64 | 126 | 153 | 49 | 212 | 199 |
| AT4G32820 | CABIN1 | 33 | 41 | 116 | 127 | 50 | 107 | 68 |
| AT5G20930 | TOUSLED | 18 | 14 | 71 | 47 | 16 | 59 | 51 |
| AT1G21610 | UBN1 | 7 | 10 | 30 | 20 | 19 | 56 | 49 |
| AT1G77310 | UBN2 | N/F | N/F | 23 | 9 | 8 | 12 | 10 |

| asf1a1b fas1-4 (het) | Expected | Observed | asf1a1b fas2-4 (het) | Expected | Observed |
|-------------------------|----------|----------|-------------------------|----------|----------|
| FAS1 | 23.5 | 48 | FAS2 | 18 | 37 |
| fas1-4 | 23.5 | 0 | fas2-4 | 18 | 0 |
| het | 47 | 46 | het | 36 | 35 |

Supplementary Table 2 Segregation ratios of *asf1a-1 asf1b-1 FAS1/fas1-*4 and *asf1a-1 asf1b-1 FAS2/fas2-4* genotyped at F3 population.

Supplementary Table 3 Primers used in this study.

| Name | Sequence | Description |
|-------------------------|----------------------------------------------------|---------------------------------------|
| ENTR-ASF1AgDNA-F | CCGCGGCCGCCCCCTTCACCTGCTATCAAGCACTTGCAAAGTT | gDNA cloning for ASF1A full length |
| ENTR-ASF1AgDNA-R | GGGTCGGCGCGCCCACCCTTTGATTCCTCAGGTTTTGGTTCTACT | with native promoter |
| ENTR-ASF1BgDNA-F | CCGCGGCCGCCCCTTCACCATGCCACTTACTTATGCTTAAAAAA CG | gDNA cloning for ASF1B full length |
| ENTR-ASF1BgDNA-R | GGGTCGGCGCGCCCACCCTTTGTCTCCTGGAGATTTTGTGGCT | with native promoter |
| ASF1A-gDNA-GT-F | GATTGATAAAGTTCAGAGGAACATACTT | gDNA genotyping |
| ASF1A-gDNA-GT-R | AGCCTACATAGATAAGCTTCCATTCCA | primers for ASF1A |
| ASF1B-gDNA-GT-F | TCATCCTGAGAATGAGCAGACTCT | gDNA genotyping |
| ASF1B-gDNA-GT-R | GGCTTACCAGAATGTGATCATG | primers for ASF1B |
| H3.1-flag/myc-F | GTGAACGAAGATGATGAAGATTAA | Cloning primers for |
| H3.1-flag/myc -R | AGCTCTTTCTCCTCTGATTCTCCT | H3.1 (HTR13) |
| H3.3-flag/myc -F | TTACGATTTAGATCCGATATAACA | Cloning primers for |
| H3.3-flag/myc -R | AGCACGTTCTCCTCTGATCCTGCG | H3.3 (HTR5) |
| FWA_McRBc-F | TTGGGTTTAGTGTTTACTTG | McRBc primers for |
| FWA_McRBc-R | GAATGTTGAATGGGATAAGGTA | epiallele region |
| FWA_qRT-PCR-F | GTATGAGTTGCTTCTCCAGCAAAG | qRT PCR primers |
| FWA_qRT-PCR-R | GAGGATGGCTGCAACAAGTGT | epiallele region |
| FWA_bsPCR_region1- F | ТСАТАТАААААААААТТАААТТТСАТТТСАСААТААССАТТ | |
| FWA_bsPCR_region1- R | GTATGGGYTTYGATAAAGAATATATGAGATTYT | BS-PCR primer for FWA region1 |
| FWA_bsPCR_region2- F | СТСАТАТАТАССТТАТСССАТТСААСАТТСАТА | |
| FWA_bsPCR_region2- R | AAGATYTGATATTTGGYTGGAAAAAAYAATAATAAT | BS-PCR primer for FWA region2 |
| FWA_bsPCR_region3- F | CRCTCTTTATCCCATTCAACATTCATAC | |
| FWA_bsPCR_region3- R | TTTGGTTGAAAAAAATAATAAAAAATTTGATTGTYAGTAT | BS-PCR primer for FWA region3 |
| FWA_bsPCR_region5- F | AAGYTGTATTTTTGTGGAAGATAGTATTATTTAAAAATTA | |
| FWA_bsPCR_region5- | CAAACAATTTTAATCATCAAAAAAAAAAACTRAATTCCT | BS-PCR primer for FWA region5 |

| Data | SRA link |
|----------|--------------------------|
| H3K27me3 | SRR10905142 ¹ |
| H3K27ac | SRR1509479 ² |
| H3K9me2 | SRR6410845 ³ |
| H3K4me3 | SRR10905140 ¹ |
| H3Ac | SRR5837284 ⁴ |
| H3K9Ac | SRR4733909 ⁵ |
| H4K16ac | SRR6364625 ⁶ |
| H1 | SRR5364423 ⁷ |
| H2A.Z | SRR8695714 ⁸ |

Supplementary Table 4 Published ChIP-seq data used in this study.

References

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