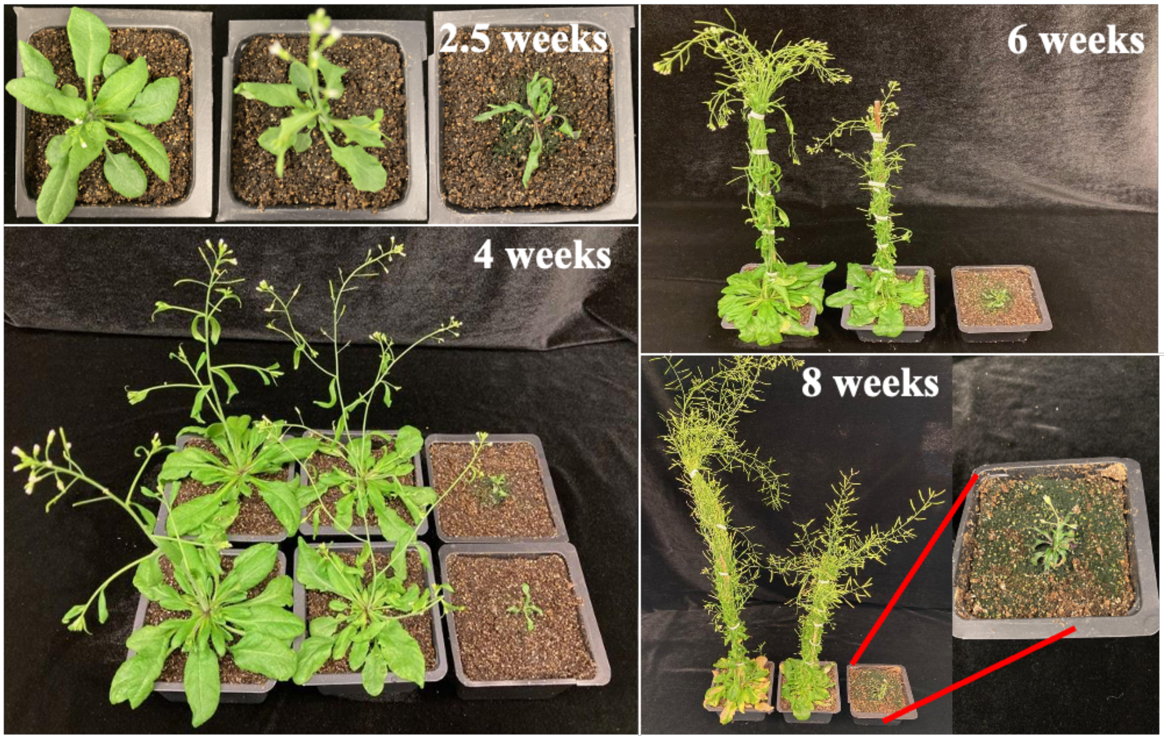
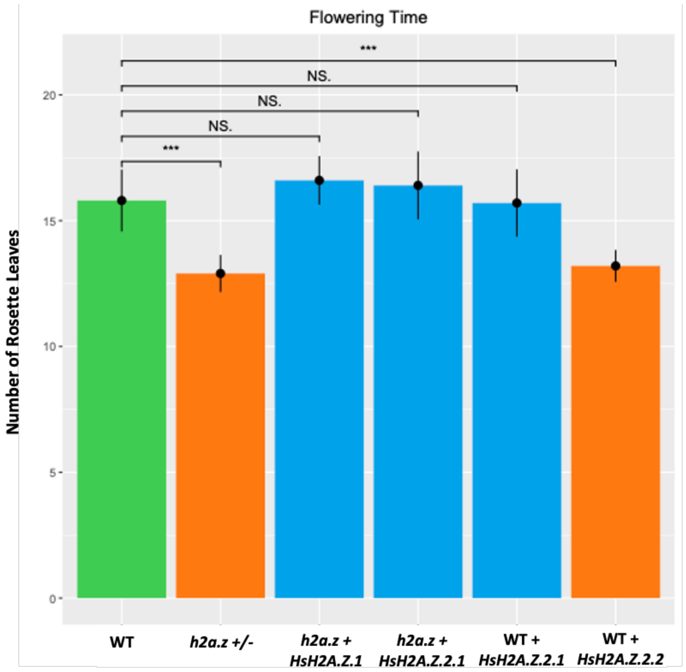
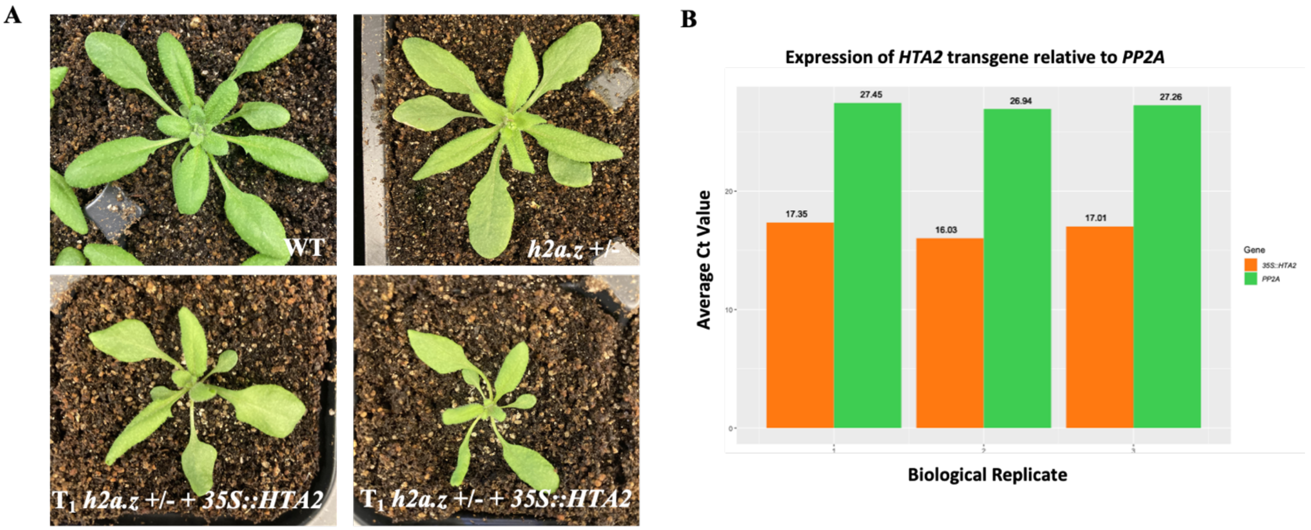
SUPPLEMENTAL FIGURES



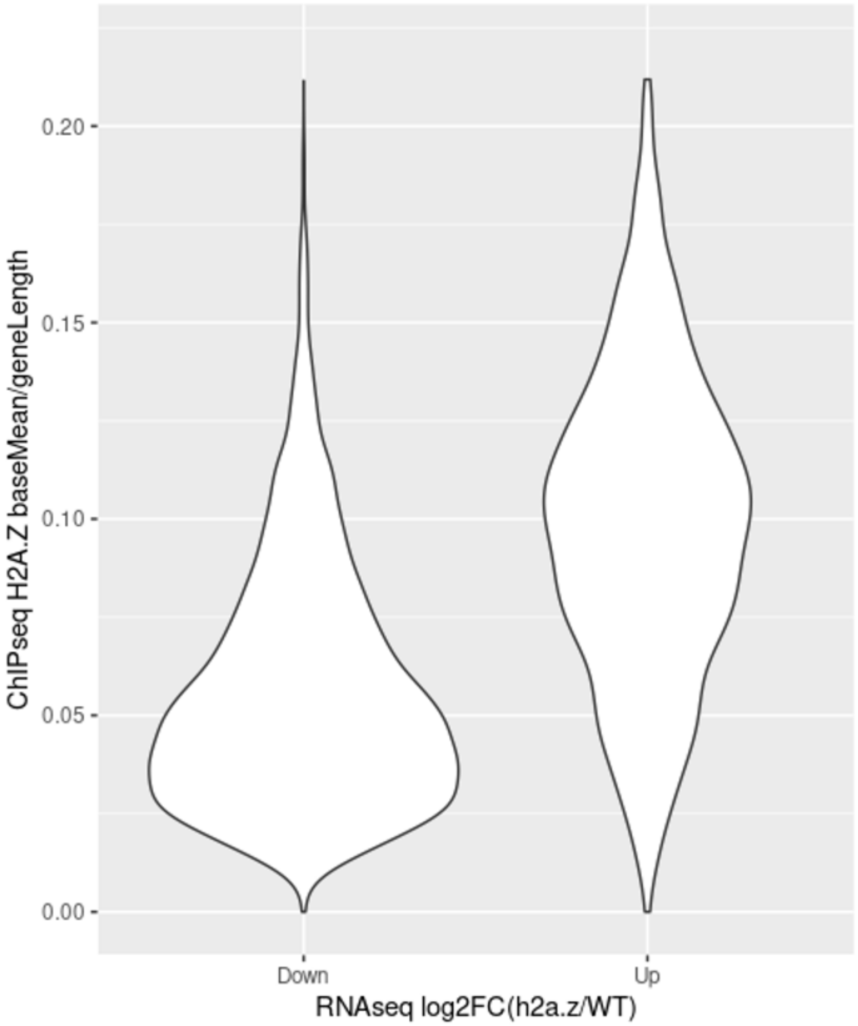
Supplemental Figure 1. Complete loss-of-function *h2a.z* mutant plants have severe developmental defects. WT (left), *h2a.z* +/- (middle), and *h2a.z* (right) plants, grown under long-day conditions, were individually photographed at four time points over eight weeks of growth. *h2a.z* mutant plants are dwarfed and have severely delayed development compared to *h2a.z* +/- and WT plants.



Supplemental Figure 2. The average number of rosette leaves of WT plants, *h2a.z +/-*, *h2a.z + HsH2A.Z.1, h2a.z* + *HsH2A.Z.2.1*, WT + *HsH2A.Z.2.1*, and WT + *HsH2A.Z.2.2* transgenic plants at flowering. Flowering time (assayed as an average number of rosette leaves from 10 different plants per genotype at the time of bolting) is significantly different between WT and h2a.z +/- (with p value=1.305e-05), as well as between WT and WT + *HsH2A.Z.2.2* plants (with p value=4.204e-05). T-test was used for statistical analysis.



Supplemental Figure 3. Overexpression of Arabidopsis canonical H2A histone HTA2 does not rescue *h2a.z +/-* phenotypic defects. (A) Two weeks old plants were grown under long-day conditions and individually photographed. **(B)** The Ct values **(**expression) of the *HTA2* transgene (orange bars) in three individual T_1 plants (three biological replicates) relative to the Ct values of the endogenous control gene *PP2A* (green bars), as measured by qRT-PCR. Each biological replicate/transgenic plant had two technical qRT-PCR replicates.



Supplemental Figure 4. **WT H2A.Z levels are higher at upregulated genes vs downregulated genes.** Violin plots showing average H2A.Z enrichment in WT across the bodies of genes either downregulated (n = 3714) or upregulated (n =4685) in *h2a.z* plants. Counts are averaged over 3 DESeq2 normalized ChIP-seq replicates and corrected for gene length. Down and Up genes are defined as |L2FC| > 0.6 and padj < 0.05 with outliers excluded from each group.

A

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HTA11      MAGKGGKGLVAAKTMAANKDKDKDKKKPISRSARAGIQFPVGRIHRQLKTRVSAHGRVGA      60
HTA2       MAGRGKQ------------LGSGAAKKSTSRSSKAGLQFPVGRIARFLKAGK-YAERVGA      47
           ***:* :              .   **  ***::**:******* * **:      ****

HTA11      TAAVYTASILEYLTAEVLELAGNASKDLKVKRITPRHLQLAIRGDEELDTLIK-GTIAGG      119
HTA2       GAPVYLAAVLEYLAAEVLELAGNAARDNKKTRIVPRHIQLAVRNDEELSKLLGDVTIANG      107
            * ** *::****:**********::* * .**.***:***:*.****..*:   ***.*

HTA11      GVIPHIHKSLINKTTKE-------     136
HTA2       GVMPNIHNLLLPKKAGSSKPTEED     131
           **:*:**: *: *.: .
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B

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H2A.Z.2.2     ---------MAGG--KAGKDSGKAKAKAVSRSQRAGLQFPVGRIHRHLKTRTTSHGRVGA     49
H2A.Z.1       ---------MAGG--KAGKDSGKAKTKAVSRSQRAGLQFPVGRIHRHLKSRTTSHGRVGA     49
H2A.Z.2.1     ---------MAGG--KAGKDSGKAKAKAVSRSQRAGLQFPVGRIHRHLKTRTTSHGRVGA     49
HTA9          MSGKGAKGLIMGKPSG--SDKDKDKKKPITRSSRAGLQFPVGRVHRLLKTRSTAHGRVGA     58
HTA8          MAGKGGKGLLAAKTTAAAANKDSVKKKSISRSSRAGIQFPVGRIHRQLKQRVSAHGRVGA     60
HTA11         MAGKGGKGLVAAKTMAANKDKDKDKKKPISRSARAGIQFPVGRIHRQLKTRVSAHGRVGA     60
                       : .       :... * * ::** ***:******:** ** * ::******

H2A.Z.2.2     TAAVYSAAILEYLTAEVLELAGNASKDLKVKRITPRHLQLAIRGDEELDSLKA-TIAGGE     108
H2A.Z.1       TAAVYSAAILEYLTAEVLELAGNASKDLKVKRITPRHLQLAIRGDEELDSLIKATIAGGG     109
H2A.Z.2.1     TAAVYSAAILEYLTAEVLELAGNASKDLKVKRITPRHLQLAIRGDEELDSLIKATIAGGG     109
HTA9          TAAVYTAAILEYLTAEVLELAGNASKDLKVKRISPRHLQLAIRGDEELDTLIKGTIAGGG     118
HTA8          TAAVYTASILEYLTAEVLELAGNASKDLKVKRITPRHLQLAIRGDEELDTLIKGTIAGGG     120
HTA11         TAAVYTASILEYLTAEVLELAGNASKDLKVKRITPRHLQLAIRGDEELDTLIKGTIAGGG     120
              *****:*:*************************:***************:*   *****

H2A.Z.2.2     KRRCS--------------     113
H2A.Z.1       VIPHIHKSLIGKKGQQKTV     128
H2A.Z.2.1     VIPHIHKSLIGKKGQQKTA     128
HTA9          VIPHIHKSLINKSAKE---     134
HTA8          VIPHIHKSLVNKVTKD---     136
HTA11         VIPHIHKSLINKTTKE---     136
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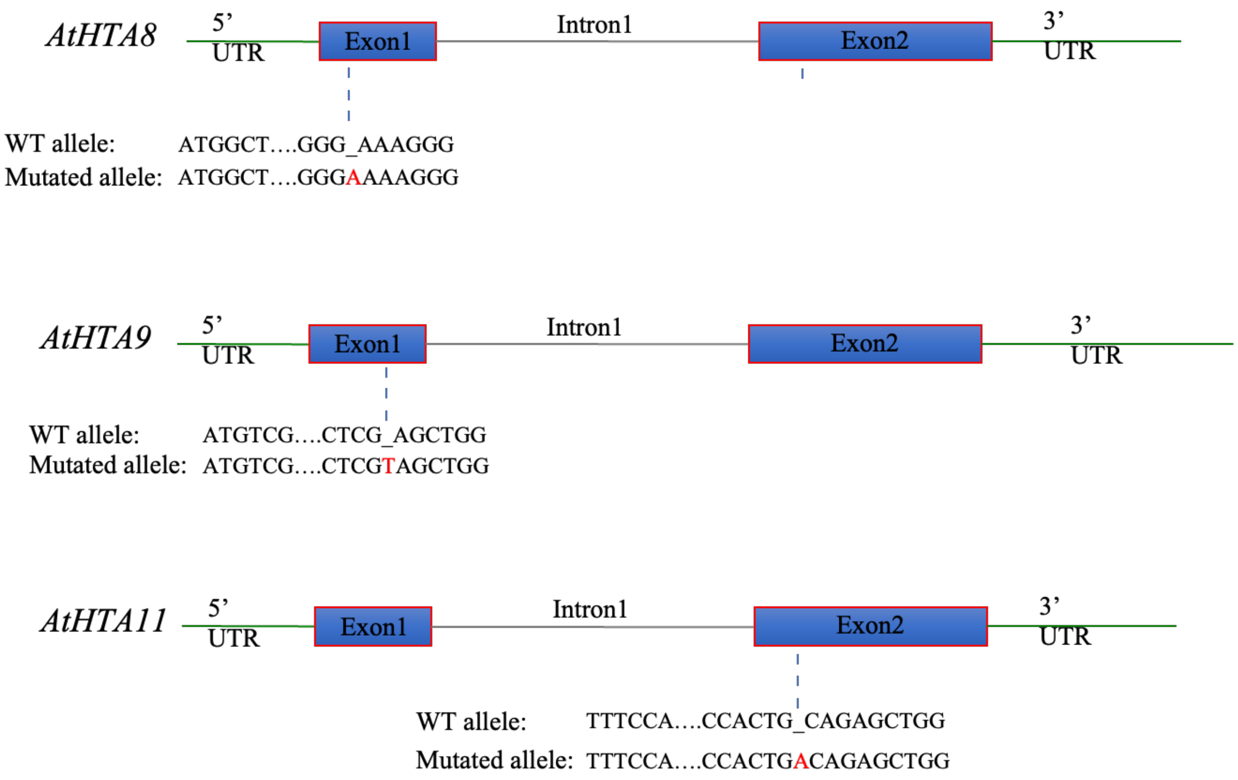
Supplemental Figure 5. Conserved amino acids that contribute to H2A.Z unique function are found in both human and Arabidopsis H2A.Zs but not in Arabidopsis H2A histone. (A) Clustal Omega alignment between Arabidopsis HTA11 (H2A.Z) and HTA2 (core H2A) histones. Amino acids that are important for H2A.Z identity are highlighted in bold within the AtHTA11 sequence and are not present in HTA2. **(B)** Clustal Omega alignment between human and Arabidopsis H2A.Zs. Amino acids that contribute to the H2A.Z unique function are highlighted in bold and are found in all Arabidopsis H2A.Zs and human H2A.Z.1 and H2A.Z.2.1, while in human H2A.Z.2.2. several key conserved residues at the C-terminal end are missing.



Supplemental Figure 6. Graphical representation of Arabidopsis *h2a.z* CRISPR mutant alleles. For each H2A.Z gene the location and the type of CRISPR mutation is shown. All three genes have an addition of a single base pair causing a frame shift that leads to a premature stop codon.