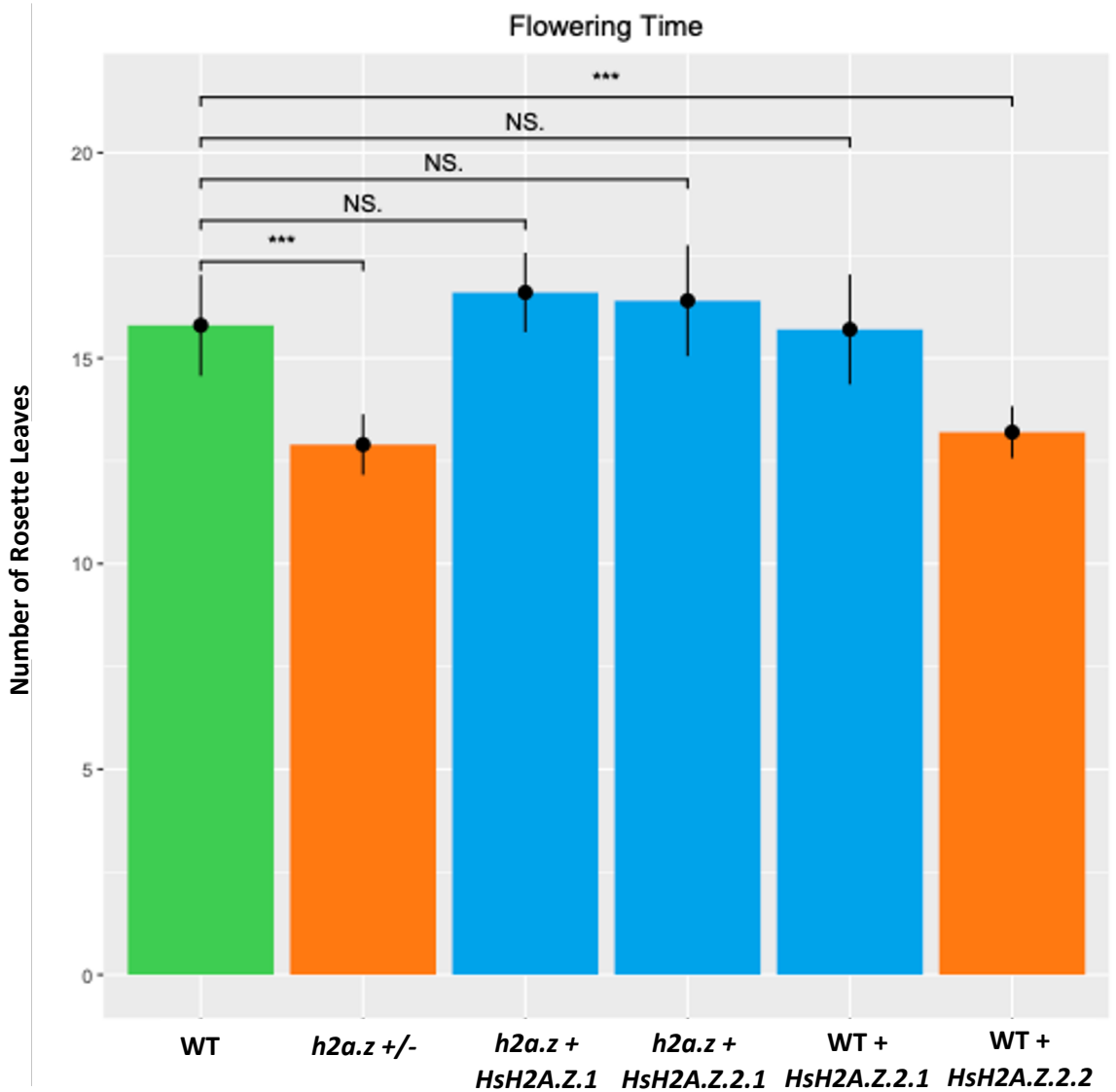
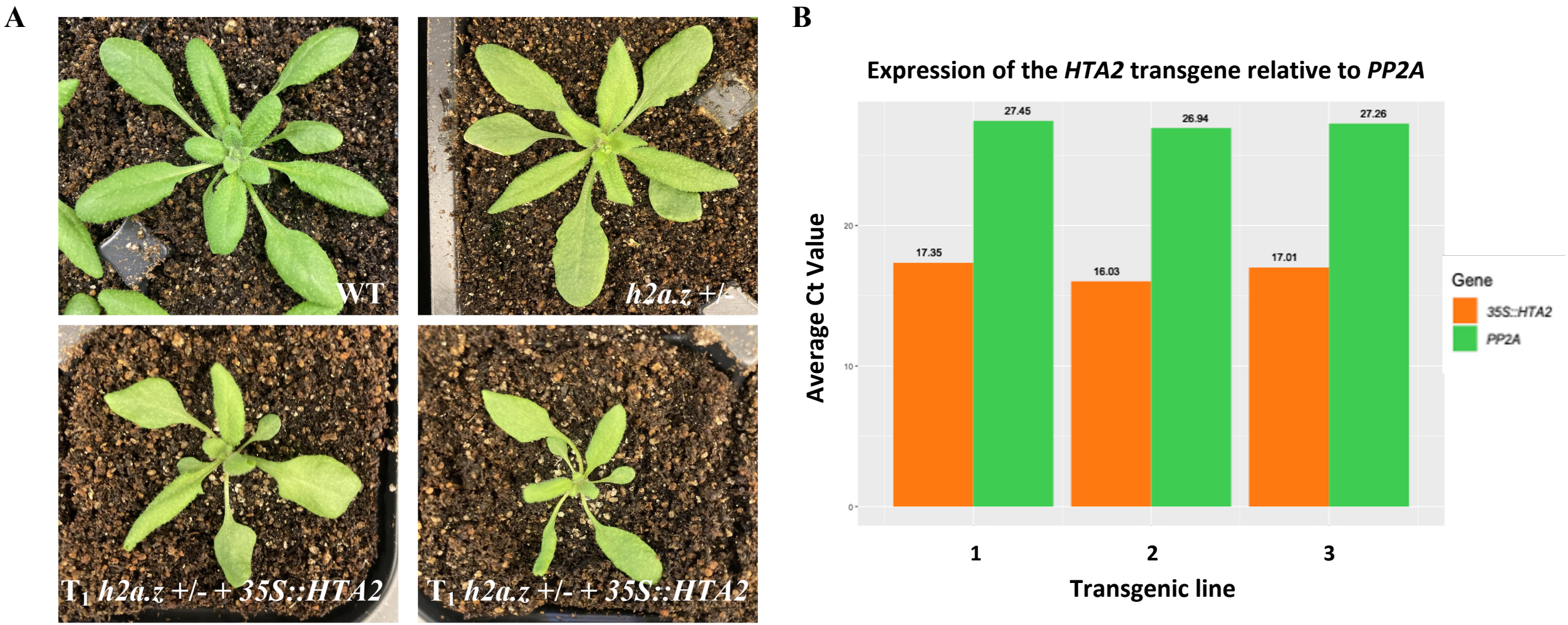
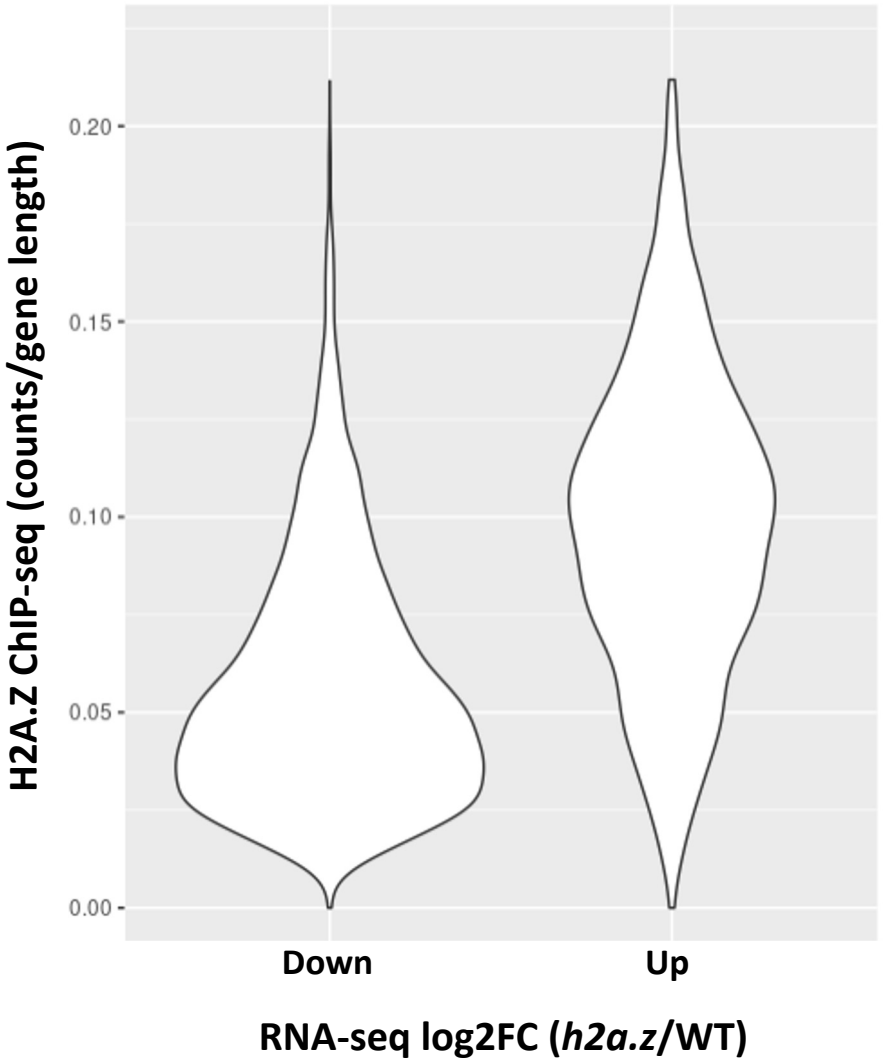
S1 Figure. Complete loss-of-function *h2a.z* mutant plants have severe developmental defects. WT (left), *h2a.z* +/- (middle), and *h2a.z* (right) plants, grown under long-day conditions, were individually photographed at four time points over eight weeks of growth. *h2a.z* mutant plants are dwarfed and have severely delayed development compared to *h2a.z* +/- and WT plants.



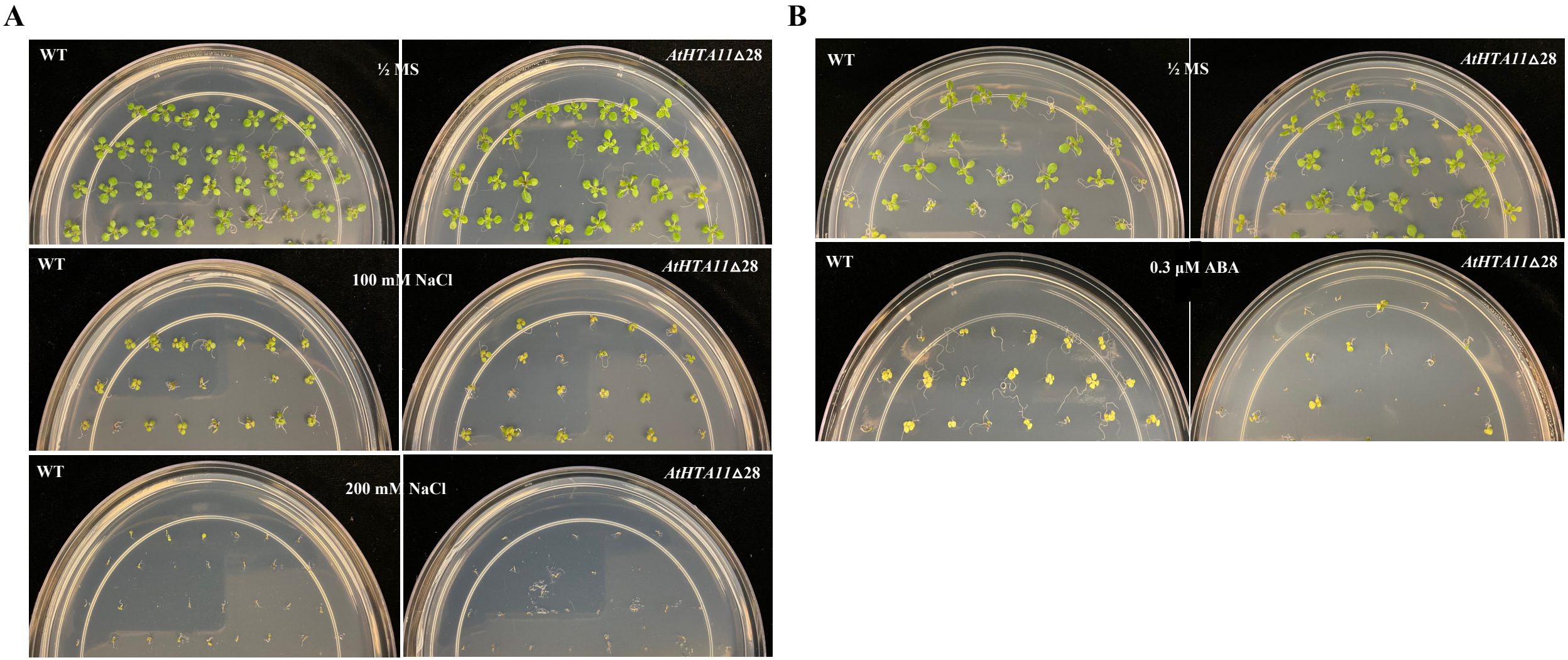
S2 Figure. The average number of rosette leaves of WT plants, *h2a.z +/-*, *h2a.z* + *HsH2A.Z.1*, *h2a.z* + *HsH2A.Z.2.1*, WT + *HsH2A.Z.2.1*, and WT + *HsH2A.Z.2.2* transgenic plants at flowering. Flowering time (assayed as the average number of rosette leaves from 10 different plants per genotype at 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.



S3 Figure. Overexpression of Arabidopsis canonical H2A histone HTA2 does not rescue *h2a.z +/-* phenotypic defects. (A) Two week old plants were grown under long-day conditions and individually photographed. **(B)** The cycle threshold (Ct) values of RT-qPCR assays 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 RT-qPCR. Each biological replicate/transgenic plant had two technical RT-qPCR replicates.



S4 Figure. **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.



S5 Figure. *AtHTA11Δ28* plants are more sensitive to high salt and ABA stresses than WT plants. Eleven-day-old seedlings grown on ½ MS agar plates or plates supplemented with either 100 mM or 200 mM NaCl **(A)** or supplemented with 0.3 µM Abscisic acid (ABA) **(B)**, were photographed and characterized for their ability to germinate and to produce green tissue. Each experiment was performed in duplicate and representative plates are shown here.

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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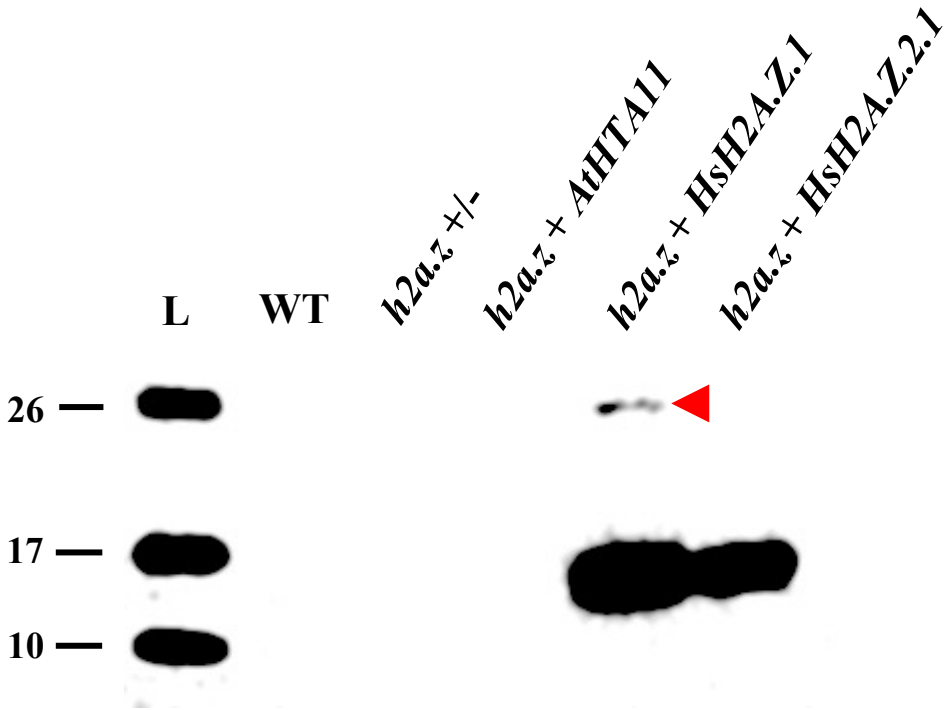
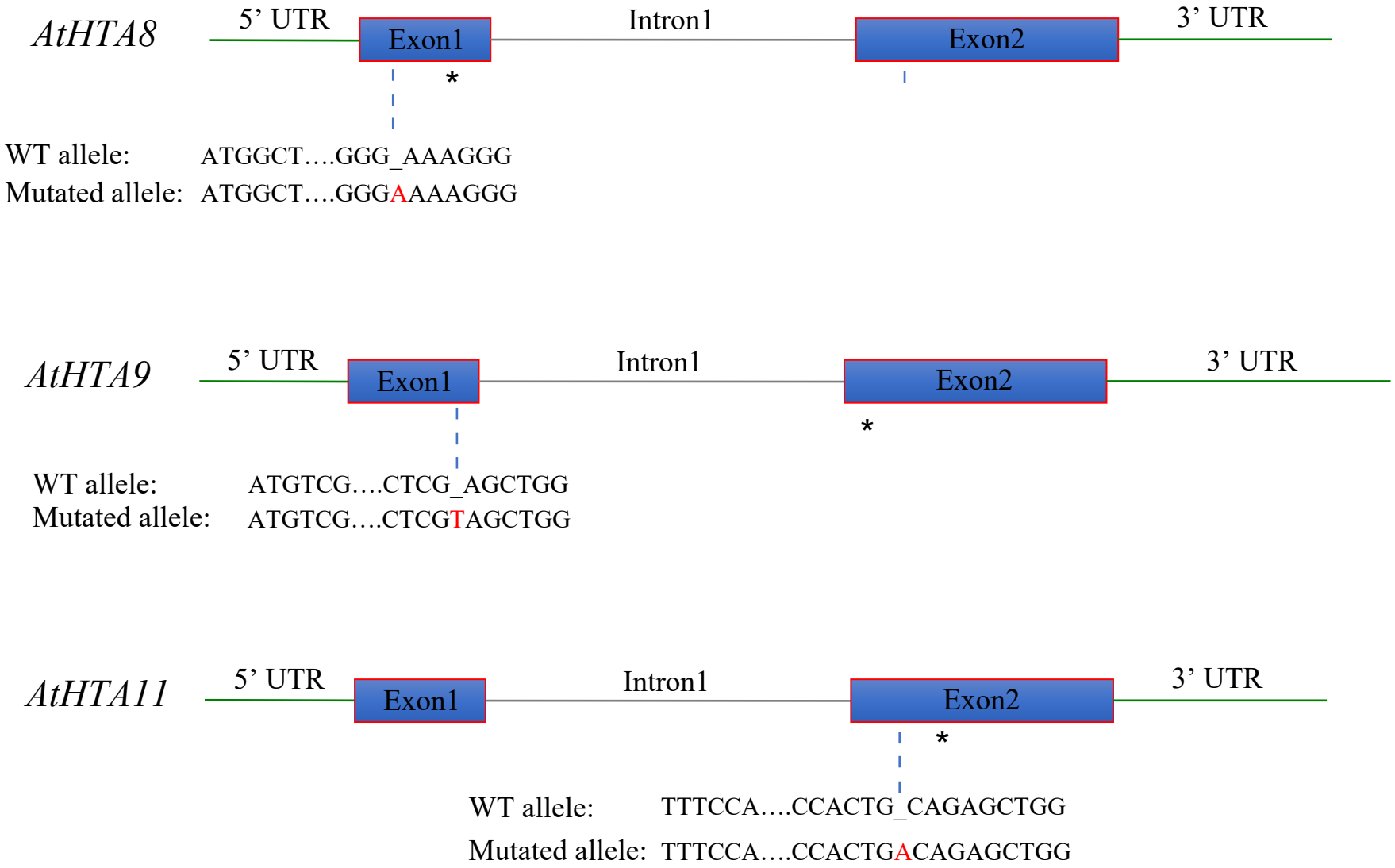
S6 Figure. Conserved amino acids that contribute to H2A.Z unique function are found in both human and Arabidopsis H2A.Zs but not in Arabidopsis histone H2A. (A) Clustal Omega alignment between Arabidopsis HTA11 (H2A.Z) and HTA2 (core H2A) histones. Amino acids that are important for H2A.Z identity are highlighted in bold within the AtHTA11 sequence and amino acids exclusively found in AtHTA11 are highlighted in bold red and are not present in HTA2. **(B)** Clustal Omega alignment between human and Arabidopsis H2A.Zs. Amino acids that contribute to unique H2A.Z functions 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.



S7 Figure. Human HsH2A.Z.1 appears to be monoubiquitinated when expressed in plants. The same western blot as shown in Figure 2C (middle panel, probed with an antibody against human H2A.Z) but overexposed, with the red arrowhead denoting the likely monoubiquitinated form of human HsH2A.Z.1 detected in the *h2a.z +HsH2A.Z.1* transgenic plants.



S8 Figure. 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 (marked with an asterisk).