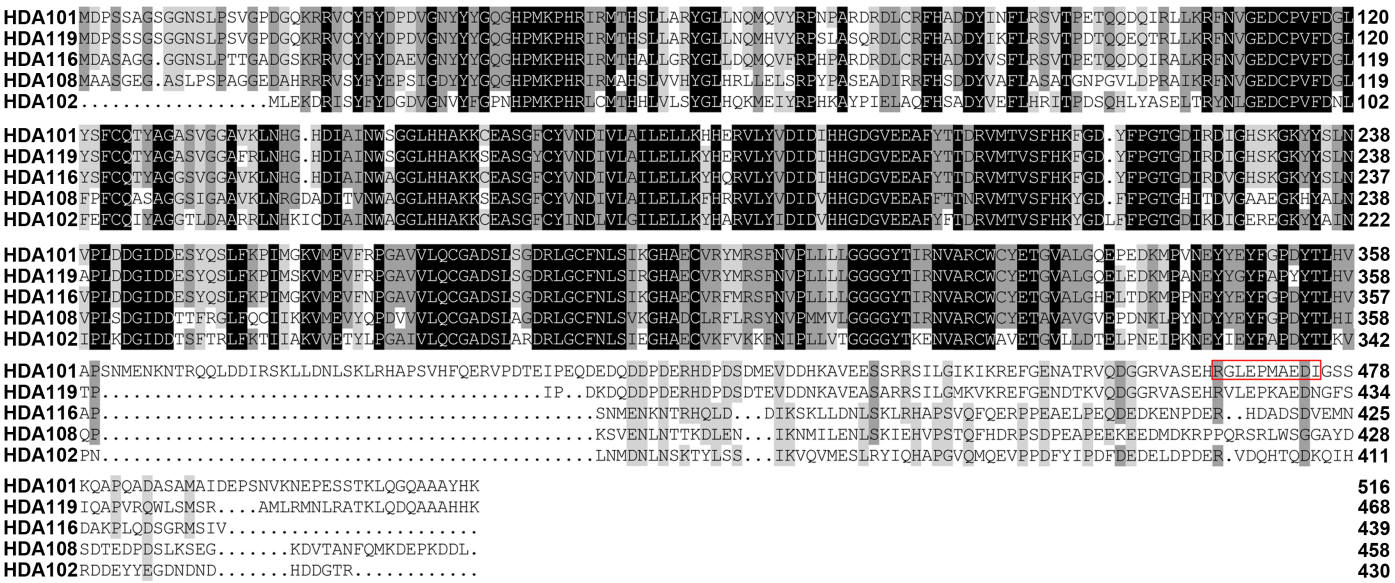
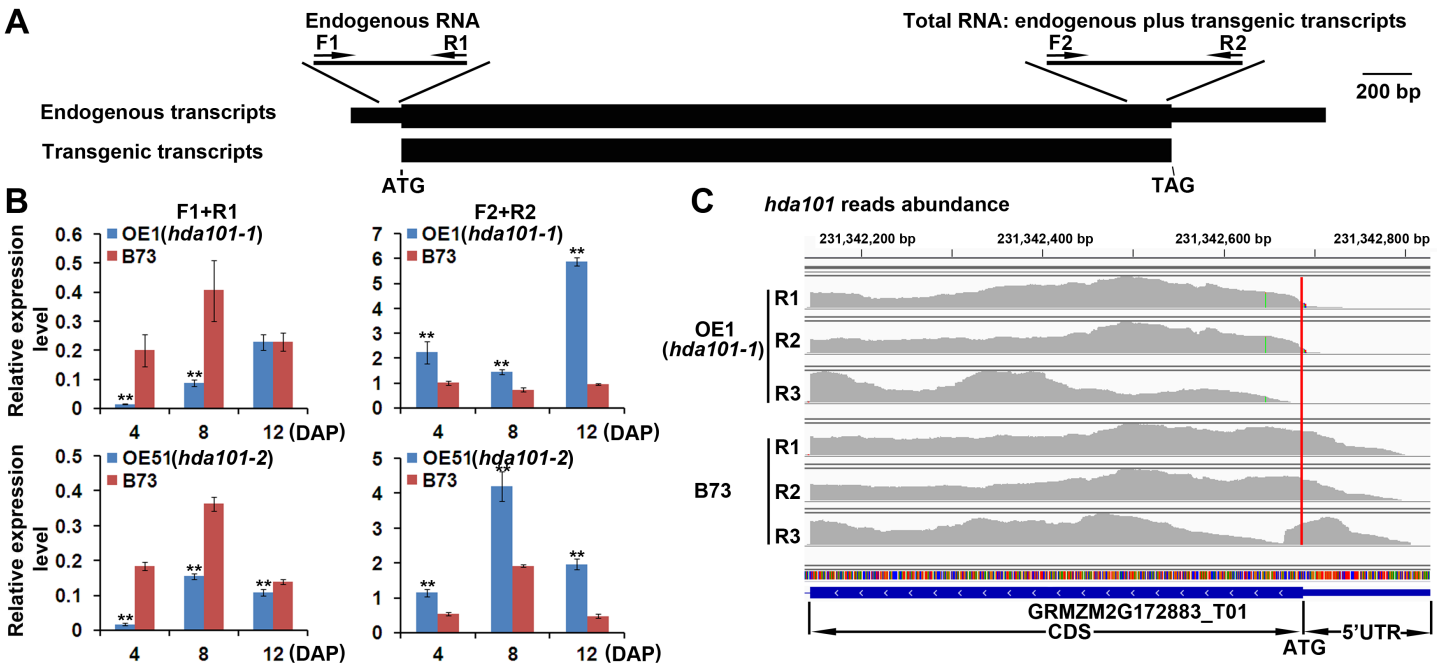
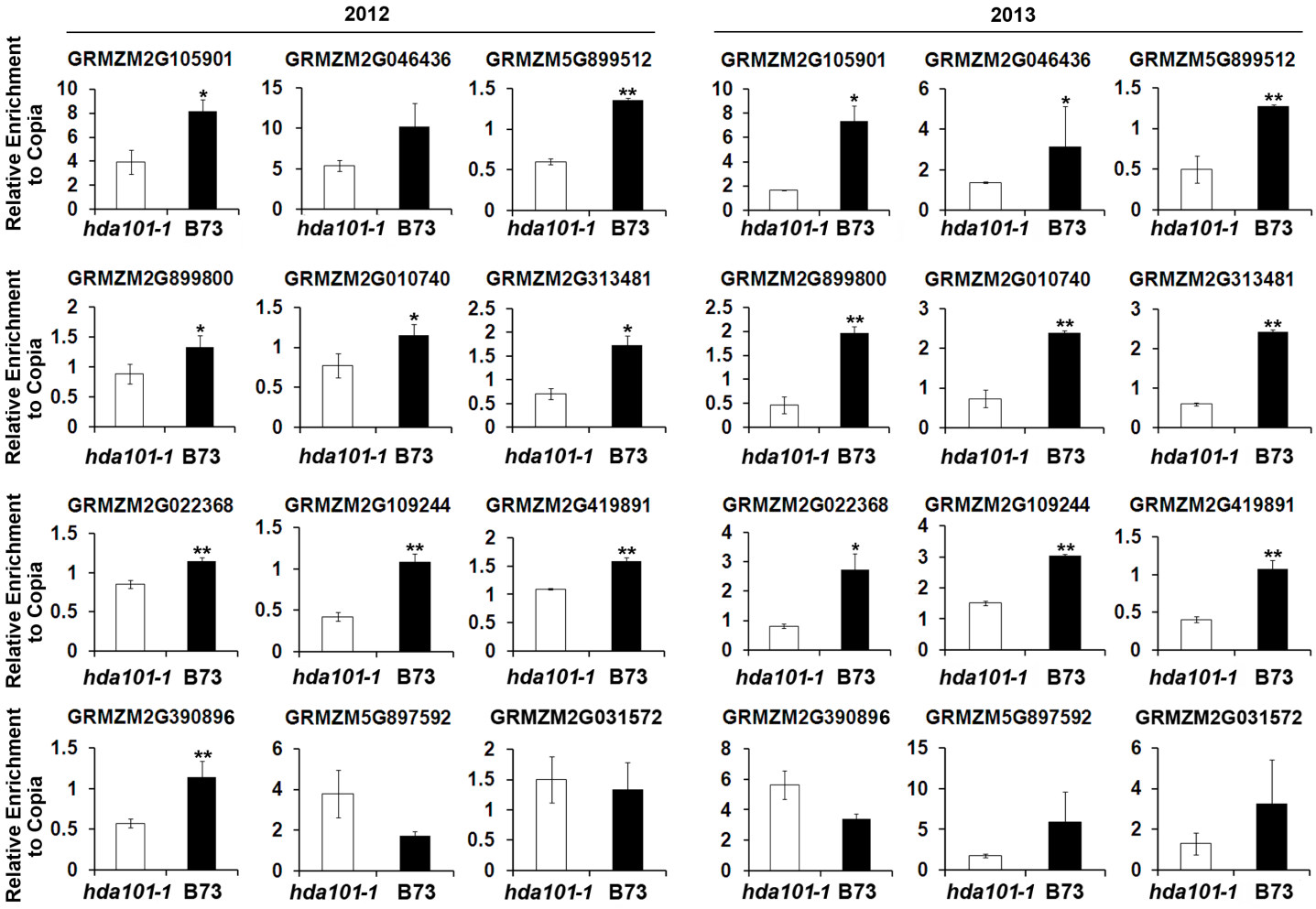


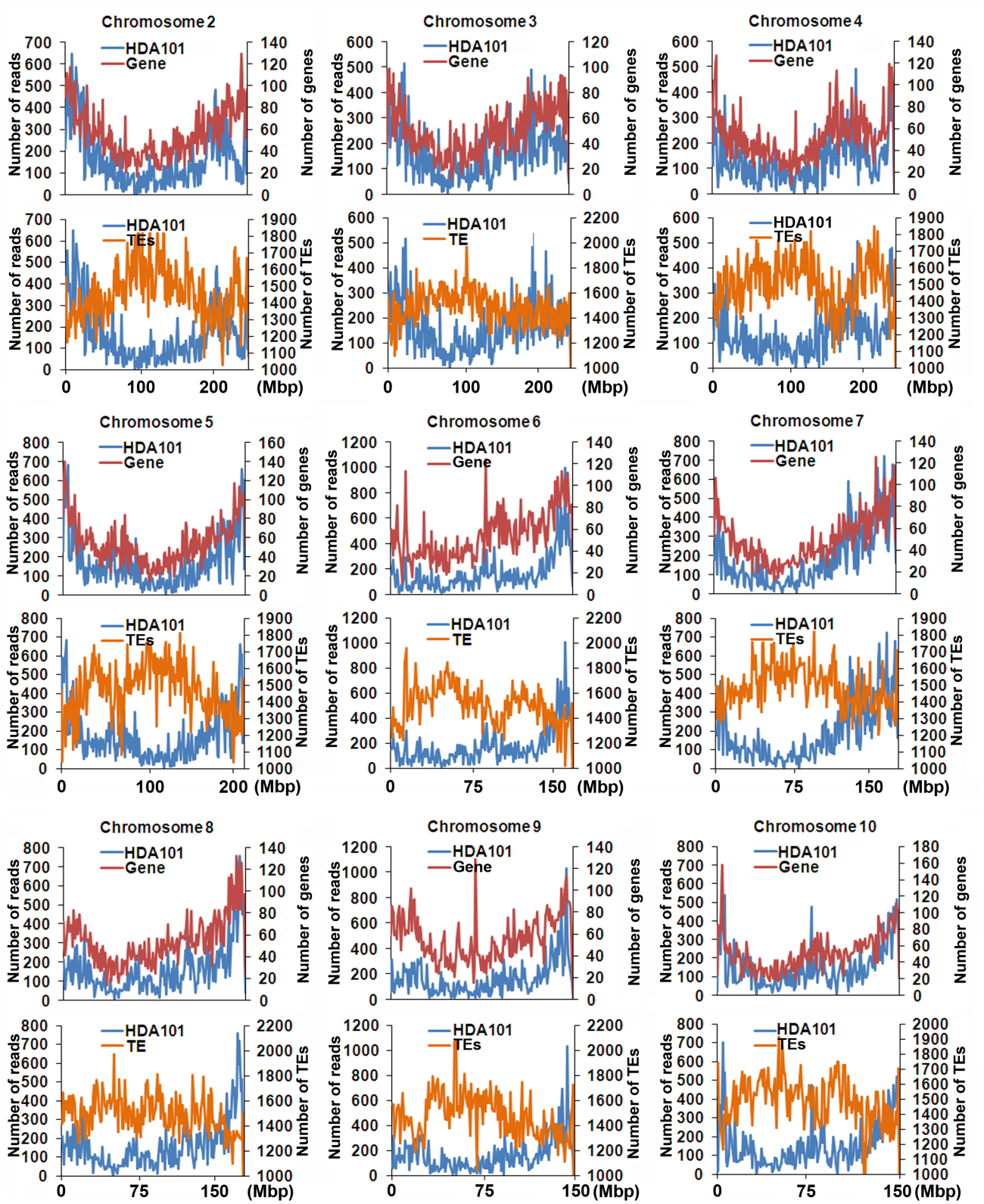
Supplemental Figure 1. Alignment of amino acid sequence of different maize Rpd3-type HDACs. The red box indicates the regions used for the production of the HDA101-specific monoclonal antibody.



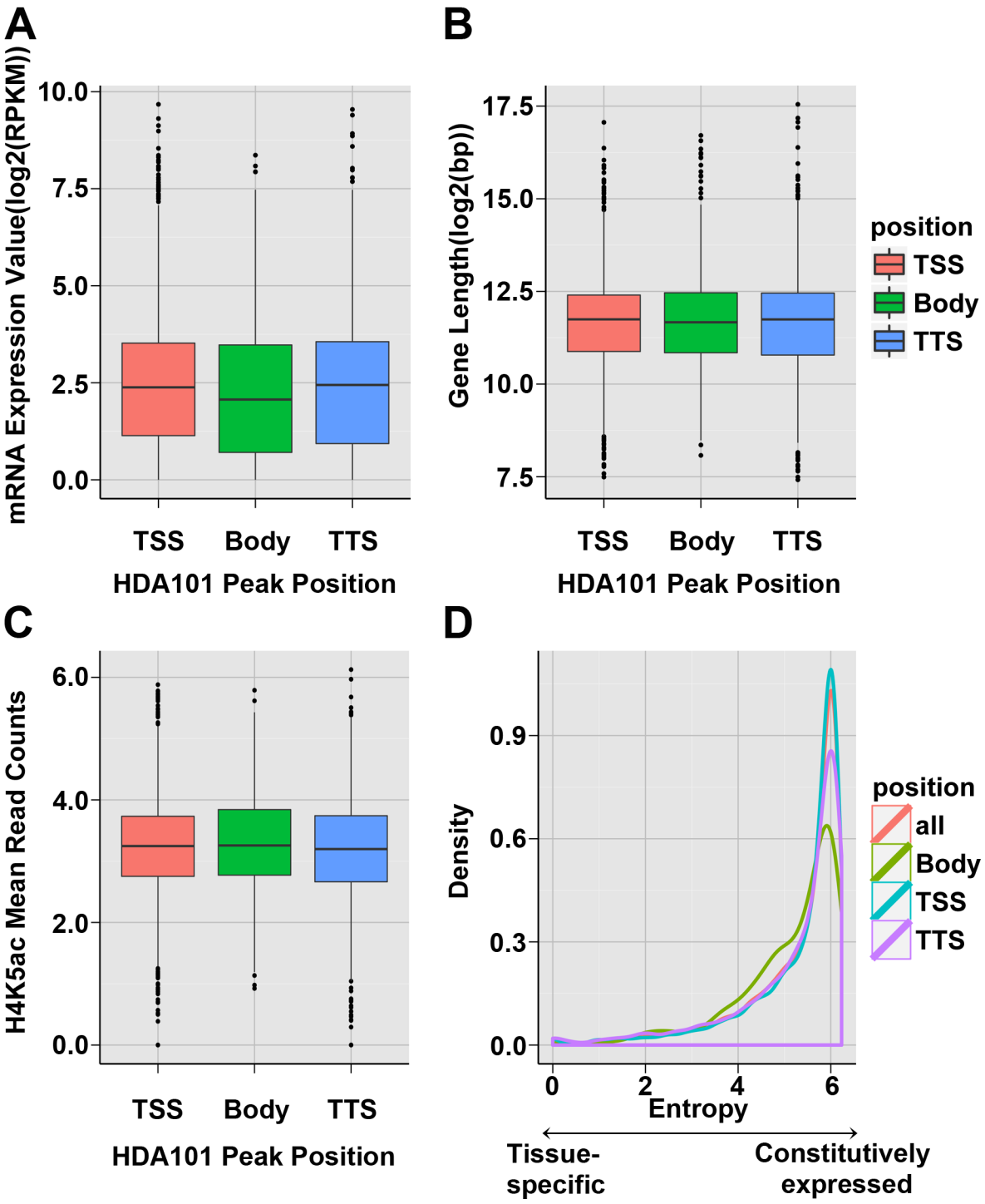
Supplemental Figure 2. Analysis of the *hda101* transcripts level in transgenic lines. The level of the endogenous and of the total (i.e. endogenous plus transgenic) *hda101* transcript in seeds harvested at 4, 8, and 12 DAP of OE1 (*hda101-1*) and OE51 (*hda101-2*) transgenic lines, as well as of B73 wild-type plants. **(A)** Gene structure of endogenous transcript, including 5′ and 3′ untranslated region and of the transgenic transcript produced by the plasmid used by Rossi et al., (2007) for the production of the *hda101* transgenic lines. Position of primers F1+R1 and F2+R2, used to detect the endogenous and total *hda101* transcript, respectively are shown. **(B)** RT-qPCR analysis of *hda101* transcripts in seeds harvested at 4, 8, and 12 DAP of transgenic OE1 (*hda101-1*) and OE51 (*hda101-2*) lines and of B73 wild-type plants. **(C)** The RNA-seq reads mapped to *hda101* region in B73 and *hda101-1* mutant. The red line represents the translation start codon ATG.



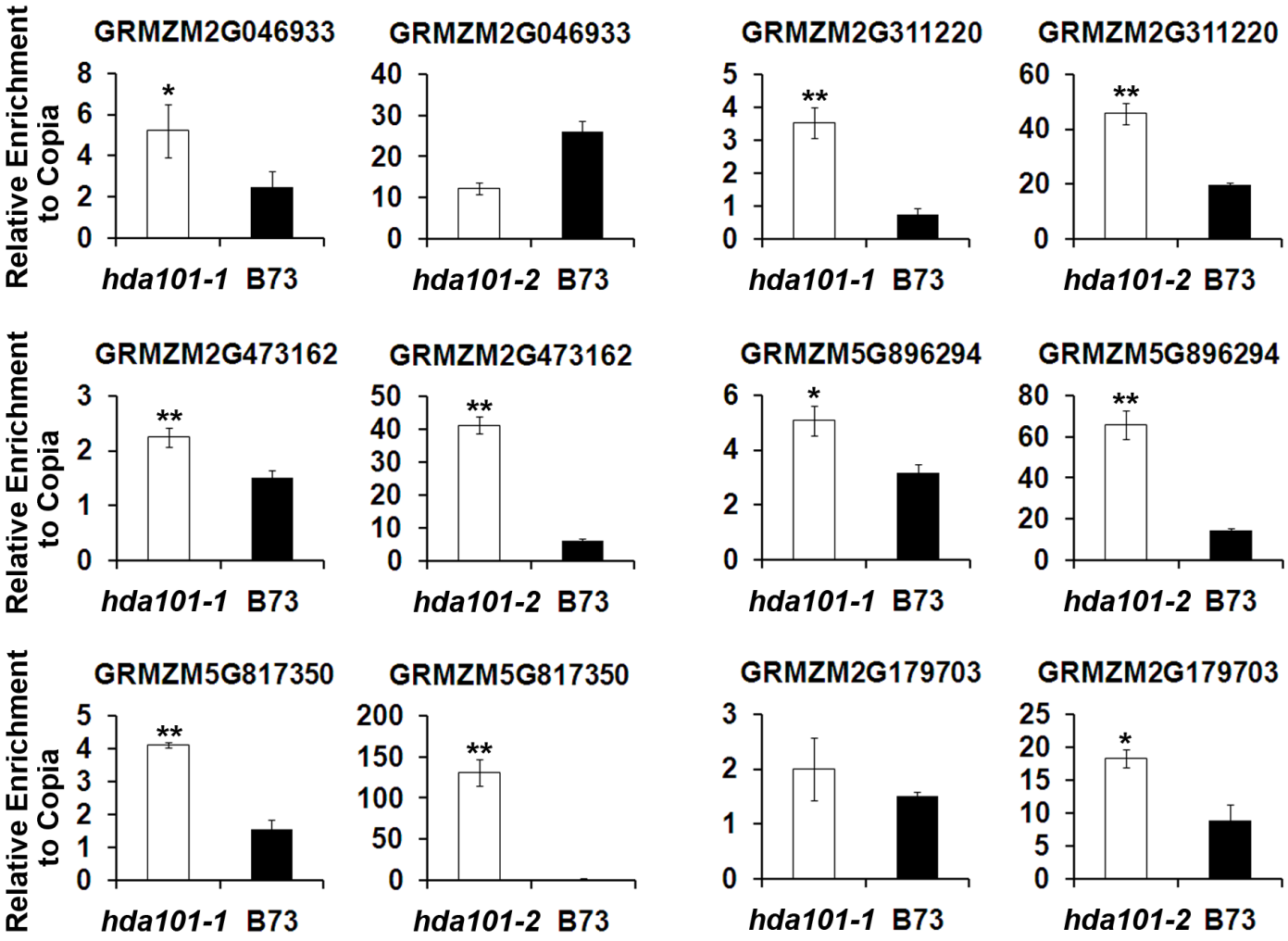
Supplemental Figure 3. ChIP–quantitative PCR assay for twelve loci bound by HDA101. The HDA101 binding enrichment (*y*-axis) was normalized to input and the non-target transposon *Copia* (AF398212) and represented as means ± SEM (n=3). Twelve loci were randomly selected based on ChIP-seq data of HDA101 binding. The seeds at 4 DAP of B73 and *hda101-1* mutant were collected in both 2012 and 2013. In each year, three biological replications were performed and the results showed similar trends, thus one replication was shown.



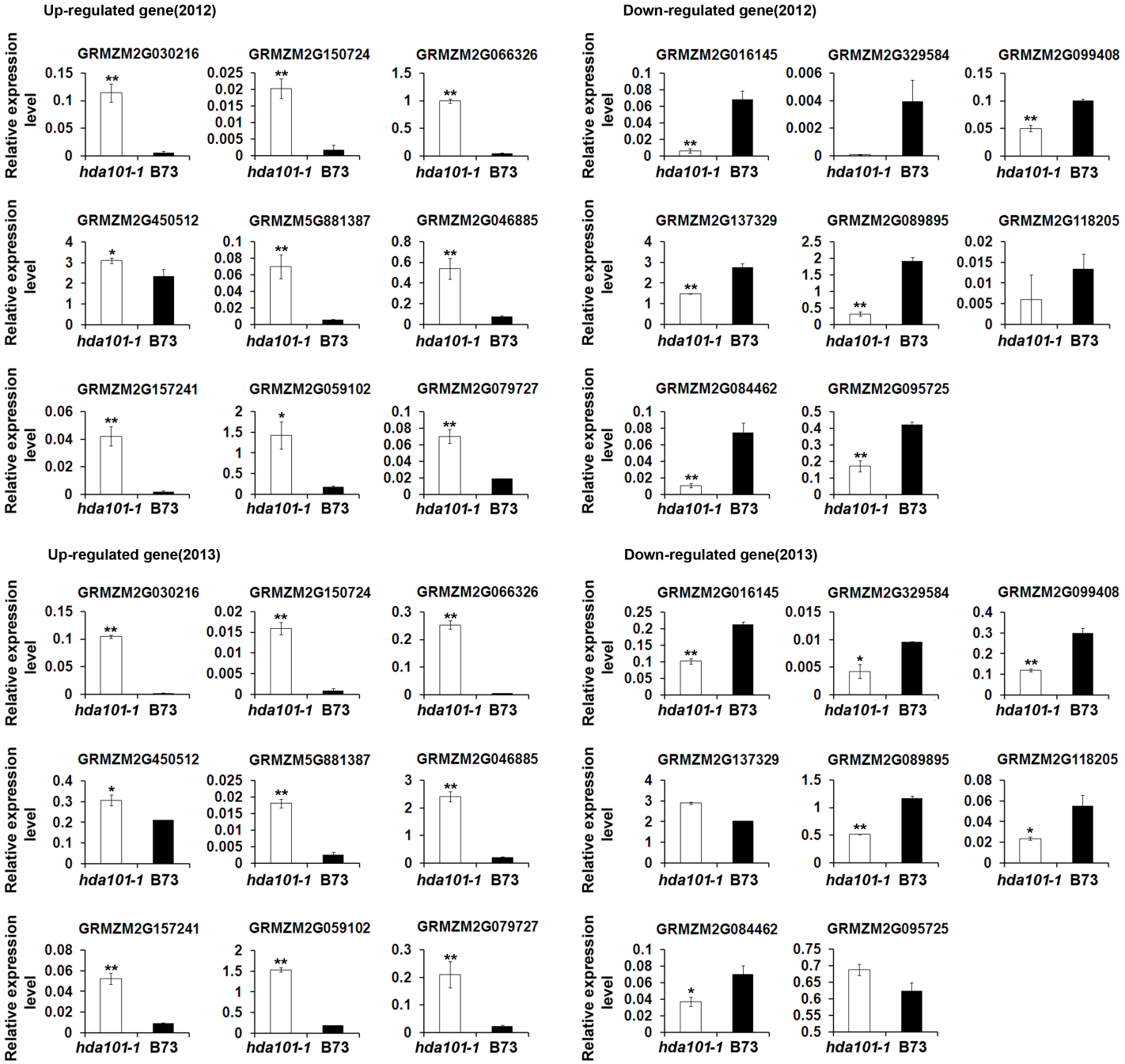
Supplemental Figure 4. Distribution of HDA101 protein binding sites with respect to genes and to transposable elements (TEs) along maize chromosomes 2-10. For each chromosome, *x*-axis represents different position in chromome; *y*-axis of left-side scale represents number of HDA101 binding reads per 1 Mb; *y*-axis of right-side scale represents number of genes per 1Mb (upper part) and number of repeat region per 1 Mb (bottom part).



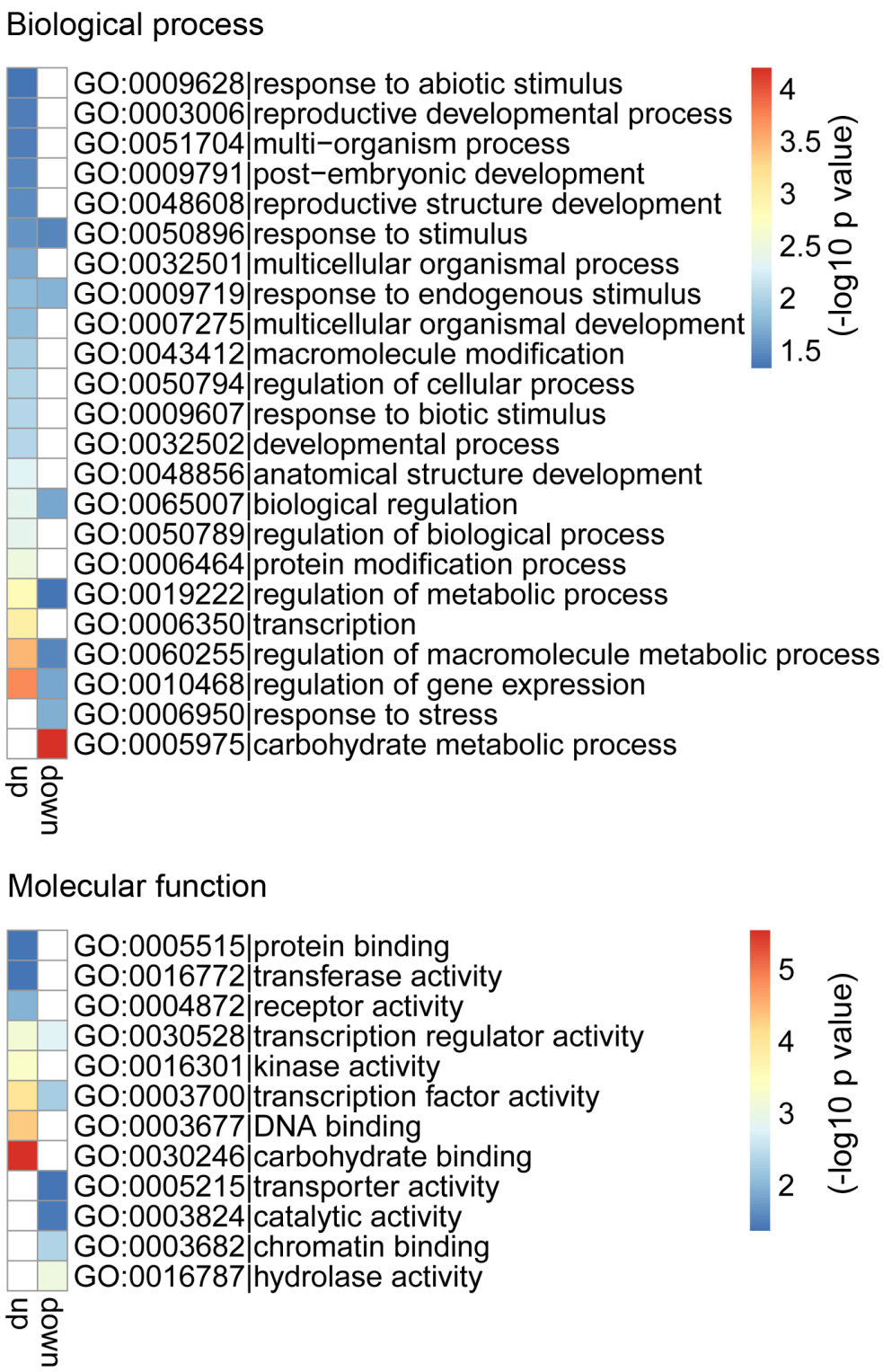
Supplemental Figure 5. Relationship between HDA101 binding location in TSS, gene body and TTS region with gene expression, gene length, and tissue specificity of the target genes. (A) Relationship between HDA101 binding location with target gene expression. **(B)** Relationship between HDA101 binding location with target gene length. **(C)** Relationship between HDA101 binding location with H4K5ac level of target gene. **(D)** Relationship between HDA101 binding location with tissue specificity of target genes.



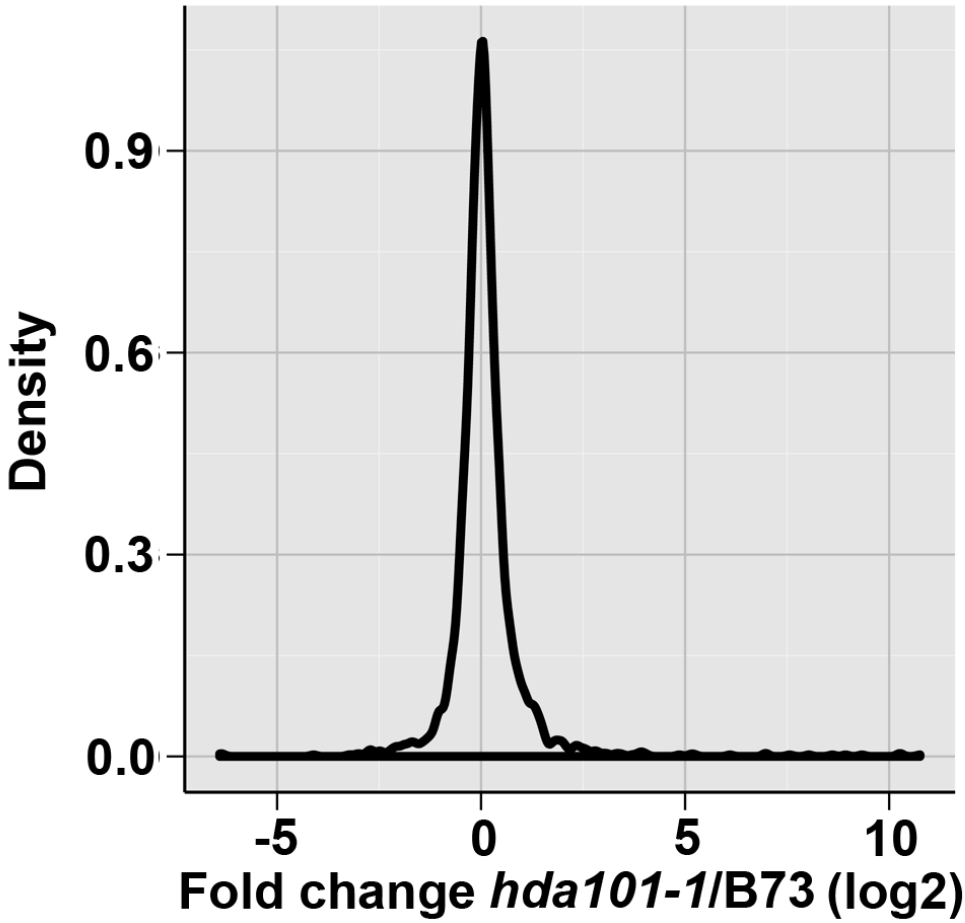
Supplemental Figure 6. H4K5ac level in a selected number of HDA101 target genes. ChIP assays were performed using chromatin extracted from 4 DAP seeds of *hda101-1* and *hda101-2* mutants and wild-type B73 plants with three biological replications and the results showed similar trends, thus one replication was shown. H4K5ac enrichment (*y*-axis) was normalized to input and the non-target transposon *Copia* (AF398212) and represented as means ± SEM (n=3).



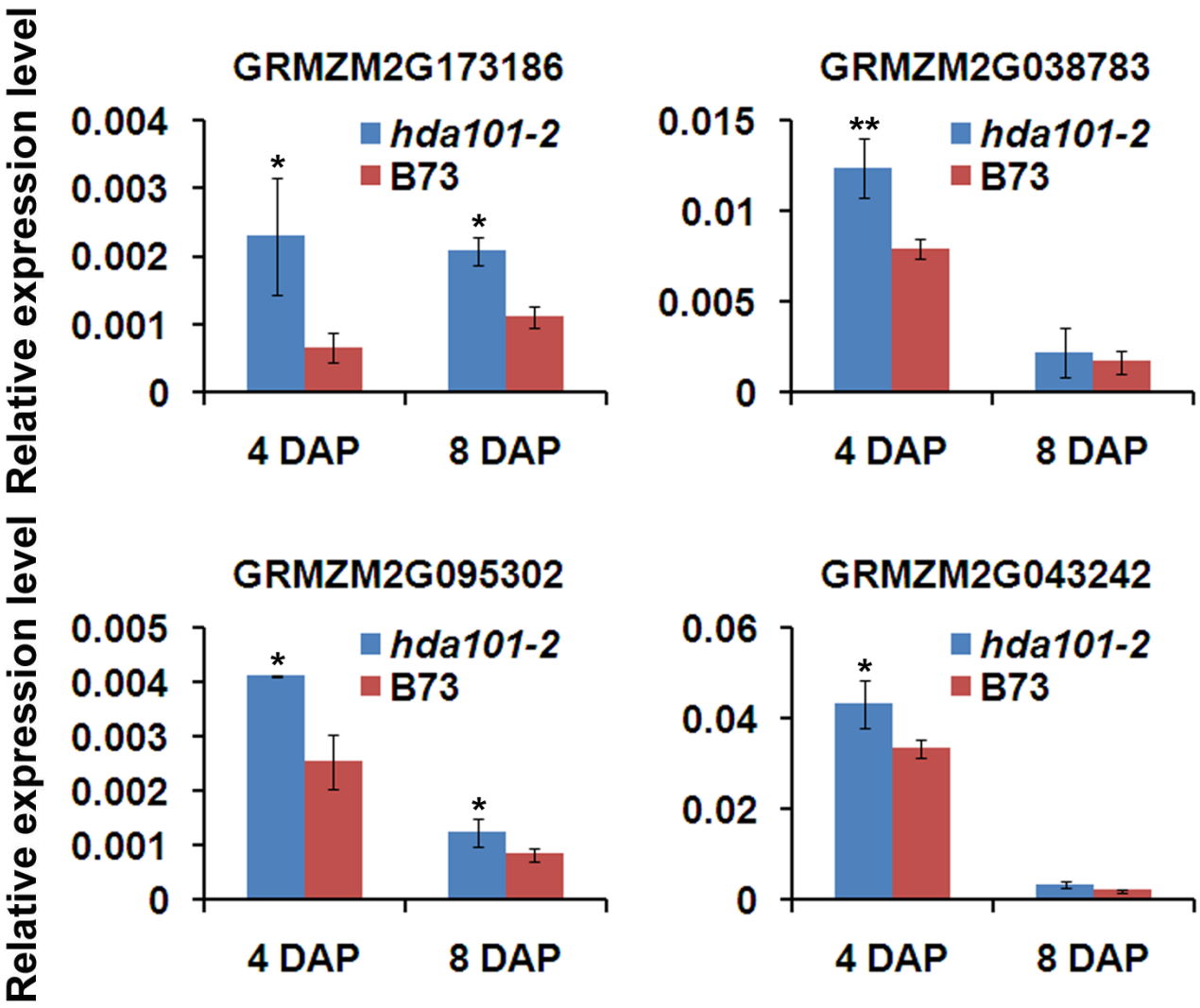
Supplemental Figure 7. RT-qPCR analysis of 17 sequences differently expressed in 4 DAP seeds of *hda101-1* mutant and B73 plants. The seeds at 4 DAP of B73 and *hda101-1* mutant were collected in both 2012 and 2013. In each year, three biological replications were performed and the results showed similar trends, thus one replication was shown. The relative expression of gene (*y*-axis) was normalized to *Actin* (*GRMZM2G126010*) and represented as means ± SEM (n=3). Up-regulated and down-regulated gene mean the genes are up-reguated and down-regulated in *hda101-1* mutant compared to wild-type B73.



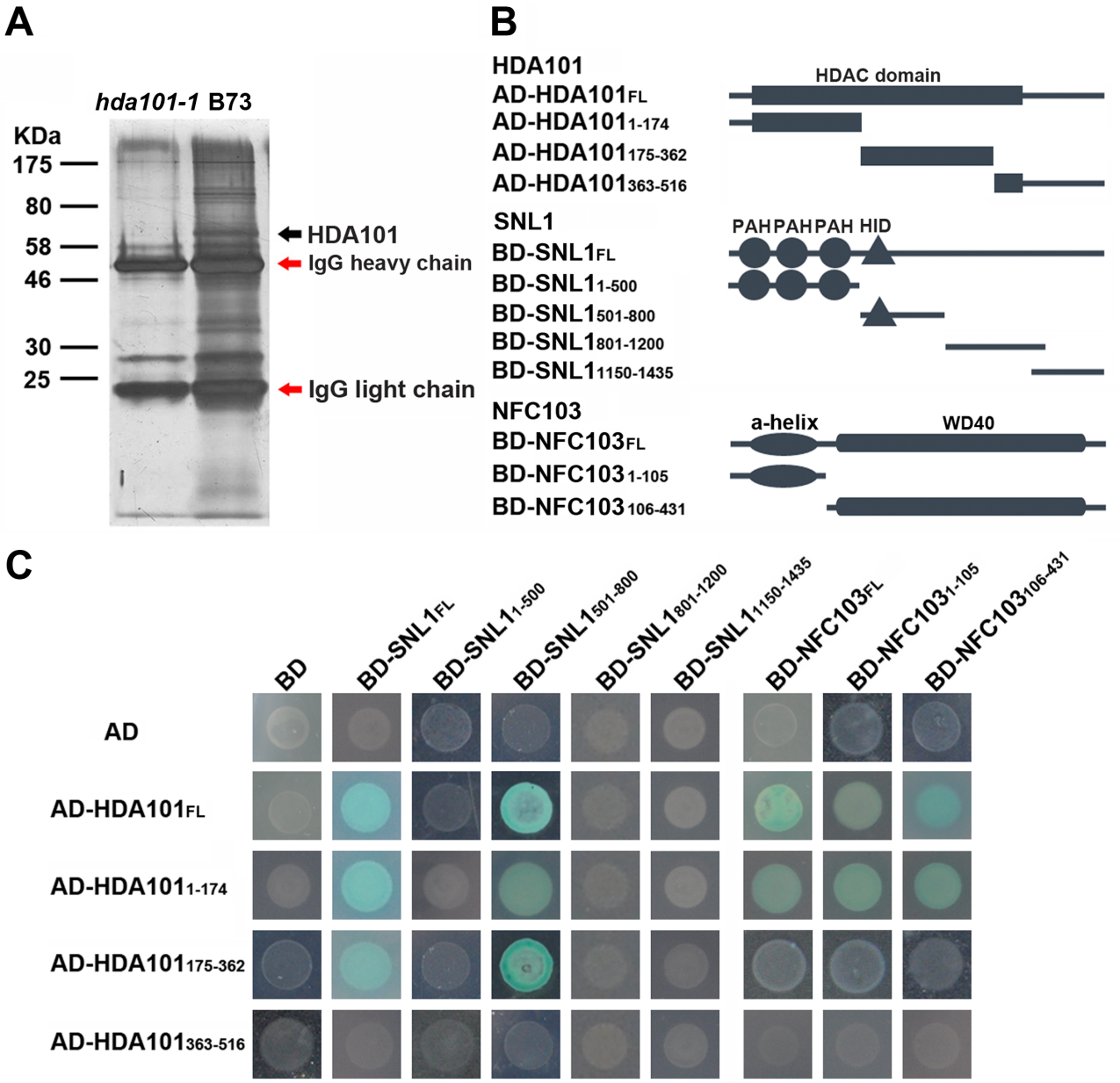
Supplemental Figure 8. GO functional analysis of genes differentially expressed in 4 DAP seeds of *hda101-1* mutant and B73 plants. The color in each cell indicates -log10 (P values) of the GO (gene ontology) enrichment and blank cell indicates no significance.



Supplemental Figure 9. Expression alteration of HDA101 target genes in the *hda101-1* mutant compared to B73. These target genes show an increased H4K5ac level with loss of HDA101 in *hda101-1* mutant. *X*-axis represents the fold-change in expression of these genes in *hda101-1* mutant compared to wild-type B73 and the *y*-axis represents the fraction of genes with corresponding expression change.



Supplemental Figure 10. The RNA level of four HDA101 target genes in seeds at 4 and 8 DAP of the *hda101-2* mutant and wild-type B73. These genes are negatively regulated during seed development and upregulated in transcription with H4K5ac increase in *hda101* mutants. The seeds at 4 and 8 DAP of B73 and *hda101-2* mutant were collected and three biological replications were performed; the results showed similar trends, thus one replication was shown. The relative expression of gene (*y*-axis) was normalized to *GRMZM2G027378* and represented as means ± SEM (n=3).



Supplemental Figure 11. Identification of HDA101-interacting proteins. (A) Immunoprecipitation of HDA101-interacting proteins. The Co-IP was performed with extracts from 4 DAP kernels of B73 and extracts from the *hda101-1* mutant were used as a negative control. The immunoprecipitated proteins were separated by SDS gels and the polypeptides were visualized by silver staining. The black arrow indicates the HDA101 protein and the red arrows indicate the IgG heavy and light chains, which were discarded for LC-MS/MS mass spectrometry. **(B)** Diagrams of the constructs used to analyze the interactions between HDA101 and NFC103 or SNL1 proteins. Schemes of different deletions are shown. Boxes represent the HDAC protein domains in HDA101; the black circle and black rectangle represent the PAH and HID domains in SNL1; elliptical boxes and rounded rectangles represent the a-helix and WD40 domains of NFC103. **(C)** HDA101 interacts with NFC103 and SNL1 in yeast two-hybrid assays. The *hda101* cDNA was fused to the GAL4 activation domain (AD); the *snl1* and *nfc103* cDNA and their deletions were fused to the GAL4 binding domain (BD).