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                                     S26
TRAB1 -----MGGGLGKDFGSMNMDELLRSIWT
Os bZIP23 MDFPGGSGRQQQLPMTPLPLARQGSVYSLTFDEFQSTLGGVGKDFGSMNMDELLRSIWT
Os bZIP46 -----MELPADGSALARQGSIIYSLTFDEFQSALGSAEKDFGSMNMDELLRNIWT
                                     * . ***** . ***

                                     S98      S106
TRAB1 AEESQAMASASAAAA-----AAEGLQRQGS LTLPR TISVKT VDEVWRDLERE
Os bZIP23 AEESHAVGAATTTTATTASVAAAEHAAV GAPPVQRQGS LTLPR TISQKT VDEVWRDMMCF
Os bZIP46 AEESQAIAPAAAAASAAAV---VGDAQOQQOPIQRQGS LTLPR TISQKT VDEVWRDIMGL
****:*:. *::: : : ***** *****:

                                     T143
TRAB1 ASPGAAAADGGGGGGEQQQPRRQETL GEMTLEEF LVRAGVVRENTAAAAAMVAAAAPPV
Os bZIP23 GGGGASTA--PAAAEP PPAHRQQT LGEITLEEF LVRAGVVREDMSVPPVPPAPTPTAAA
Os bZIP46 GGSDDDP--AAAAAAPAQRQETL GEMTLEEF LVRAGVVREDMGQTIVLPPQ-----
..          ...          : ** *****:*****: . .

TRAB1 APRSIPAVNNSSIFFGNYGGVNDAAAAAAGAMGFSPVGIGDPTMGNRLMSGVAGIGGGAI
Os bZIP23 VPPPPPPQQQTPMLFGQSNV-----FPPMVPPLSLGNGLVSGAVGHGGGA
Os bZIP46 -----AQALFPGSNV-----V---APAMQLANGMLPGVVG VAPGAA
          : : *          : . * : : * . * . * .

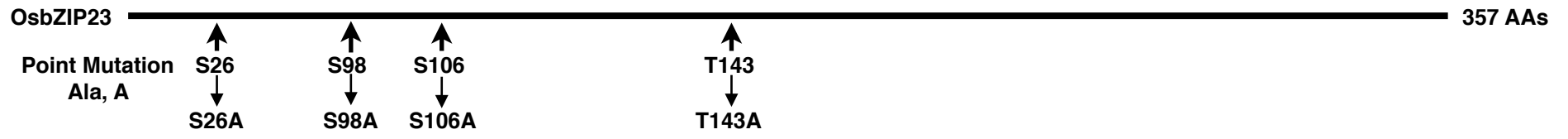
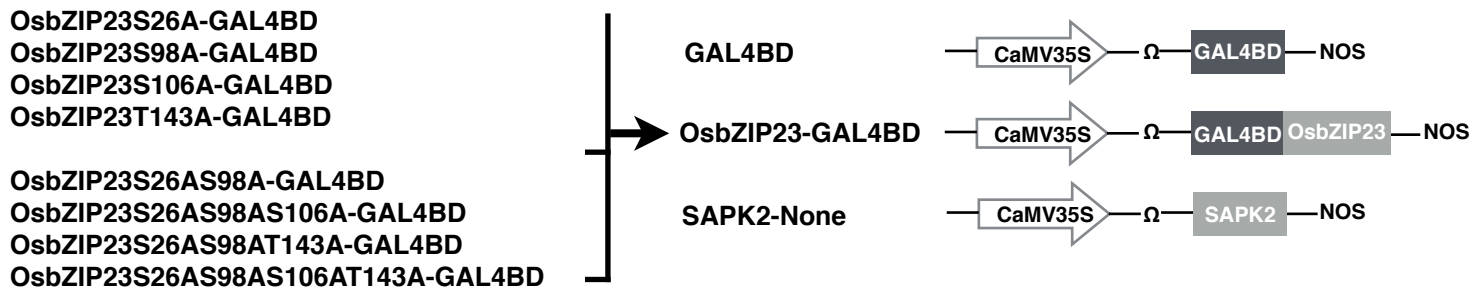
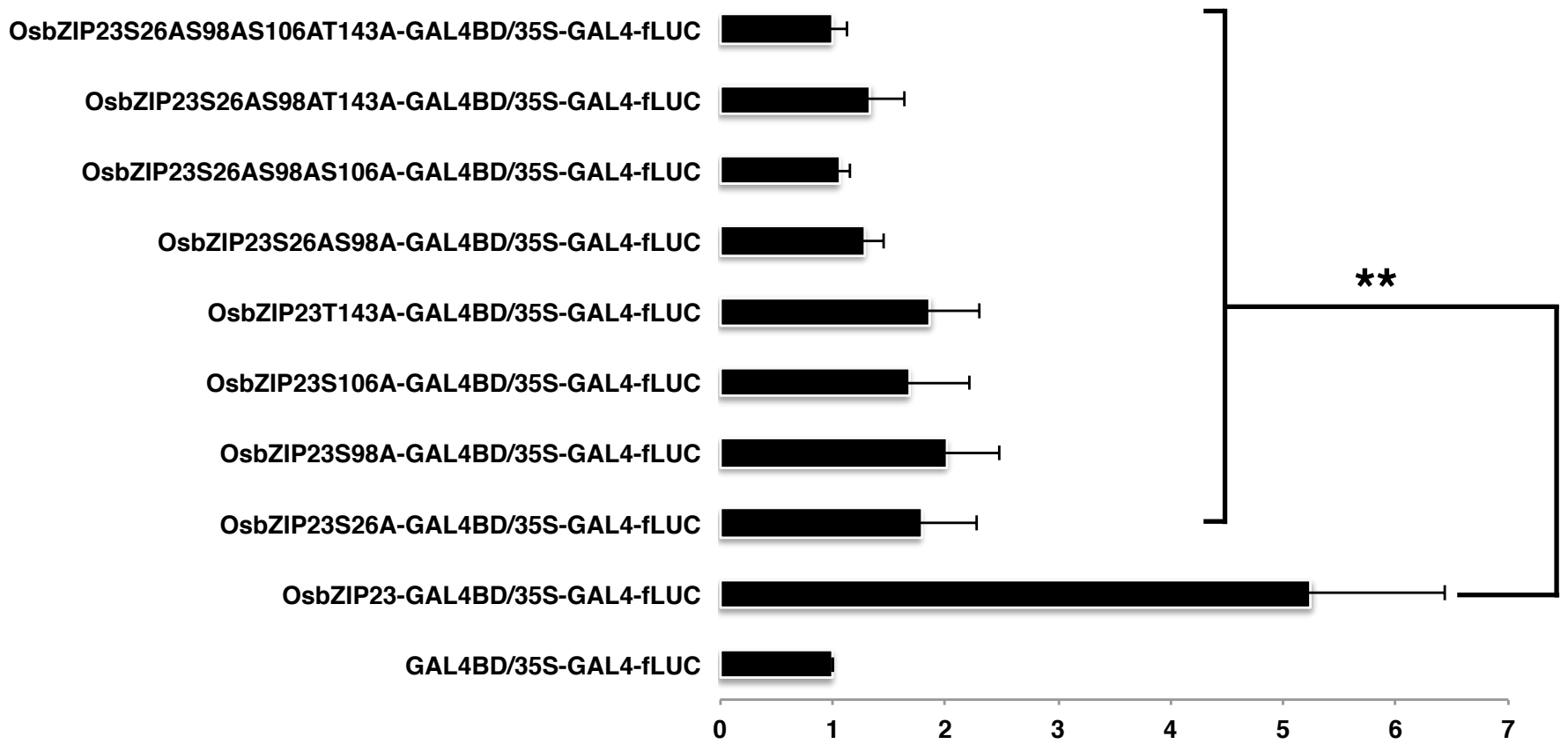
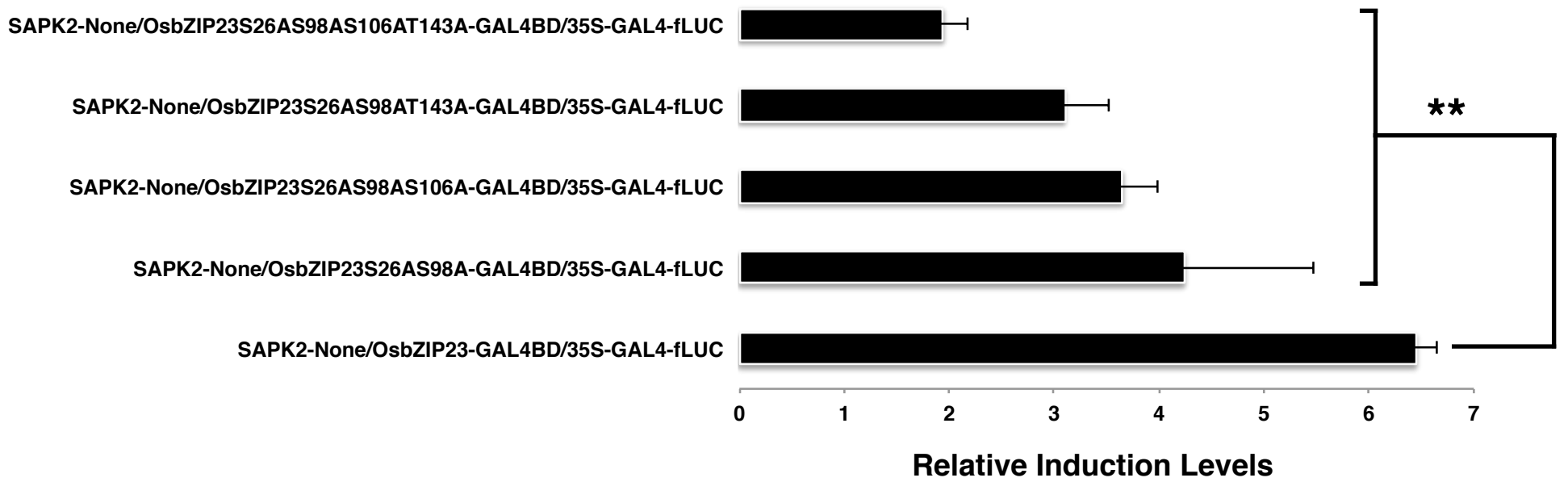
TRAB1 TVAPVDTSVGQMSAGK--G-DGDLSS-PMAPVPYPFEGVIRGRRSGGNVEKVVERRQRR
Os bZIP23 --ASLVSPVRPVSSNGFGKMEGGDLSSLSPSPVPYVFKGGLRGRK-APGIEKVVERRQRR
Os bZIP46 AAMTVAAPATPVVLNGLGKVEGGDLSSLSP--VPYPFD TALVRK-GPTVEKVVERRQRR
          : : . : *          *****          *** * . : * * : . :*****

TRAB1 MIKNRESAARSRARKQAYTMELEAEVQKLKEQNMELOKKQEEIMEMQKNFFPEMQKNQVL
Os bZIP23 MIKNRESAARSRQRKQAYMMELEAEVAKLKELNDELQKKQDEMLEQQKNE-----VL
Os bZIP46 MIKNRESAARSRARKQAYIMELEAEVAKLKEQKAELQKKQVEMIQKONDE-----VM
***** ***** ***** ***** : ***** *::: *:::          *:

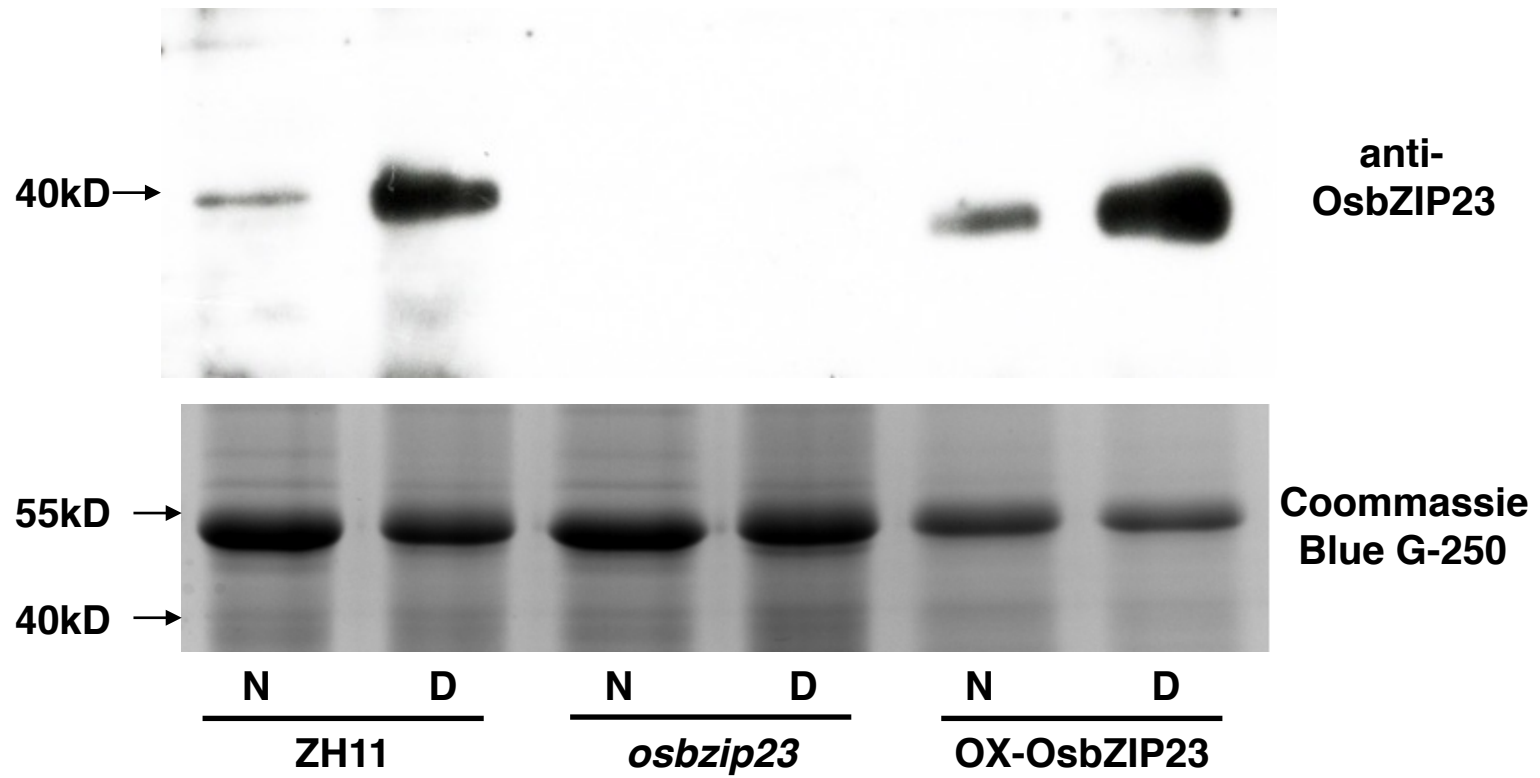
TRAB1 EAVNNPYG--QKKRCLRRTL TGPW
Os bZIP23 ERMSRQVGPTAKRICLRRTL TGPW
Os bZIP46 ERITQQLGPKAKRFCLRRTL TGPW
* :.. * * : *****

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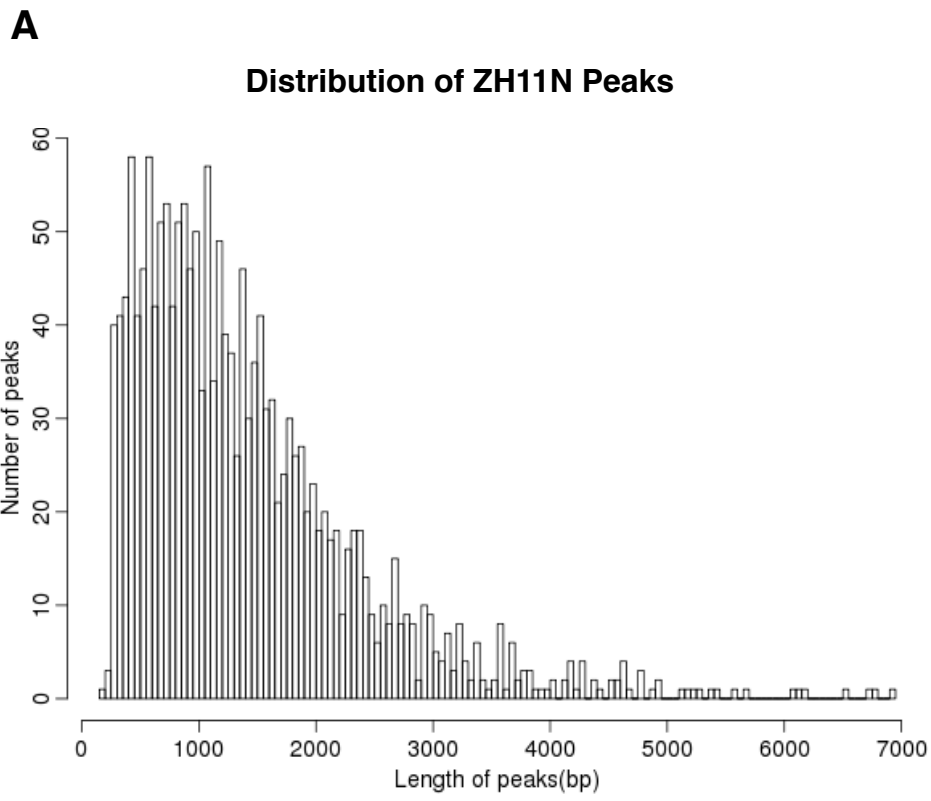
Supplemental Figure 1. Protein sequence alignment of OsbZIP23, OsbZIP46, and TRAB1. The red rectangles represent the Ser/Thr in the conserved RQXS/T motif, and the blue rectangle indicates the conserved Ser102 of TRAB1. The numbers represent the Ser/Thr location sites in OsbZIP23.

A**B****Effector****Reporter****Internal Control****C****D**

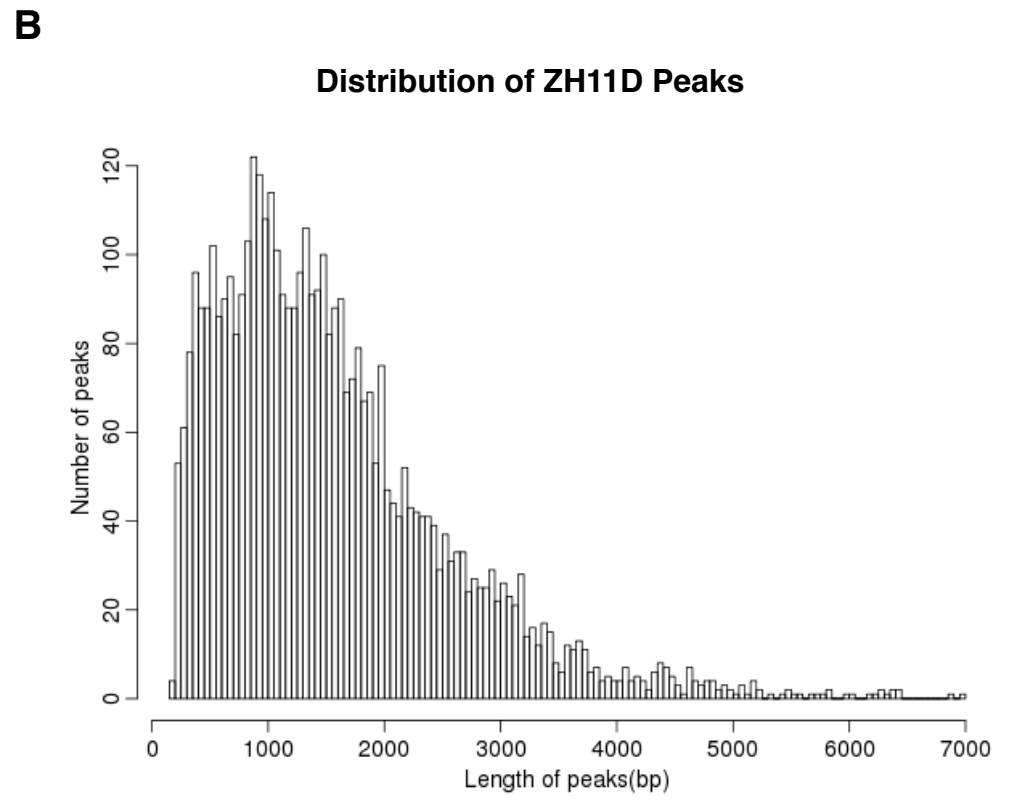
Supplemental Figure 2. Effects of Ser/Thr substitution on SAPK2 mediated transcriptional activity activation of OsbZIP23. A, Schematic diagram of amino acid substitution of OsbZIP23. B, Scheme of the constructs used in the rice protoplast co-transfection assay. C, Ser/Thr substitution significantly decrease the transcriptional activation activity of OsbZIP23 under ABA treatment. The relative fold enrichment represents the luciferase activity changes between normal and ABA treatment conditions. D, Ser/Thr substitution significantly decreases the transcriptional activation caused by SAPK2 under normal condition. The values in each column are the mean of three independent replicates and error bars indicate SD. The asterisks represent a significantly difference determined by the Student's *t* test. Two asterisks represents $t < 0.01$.



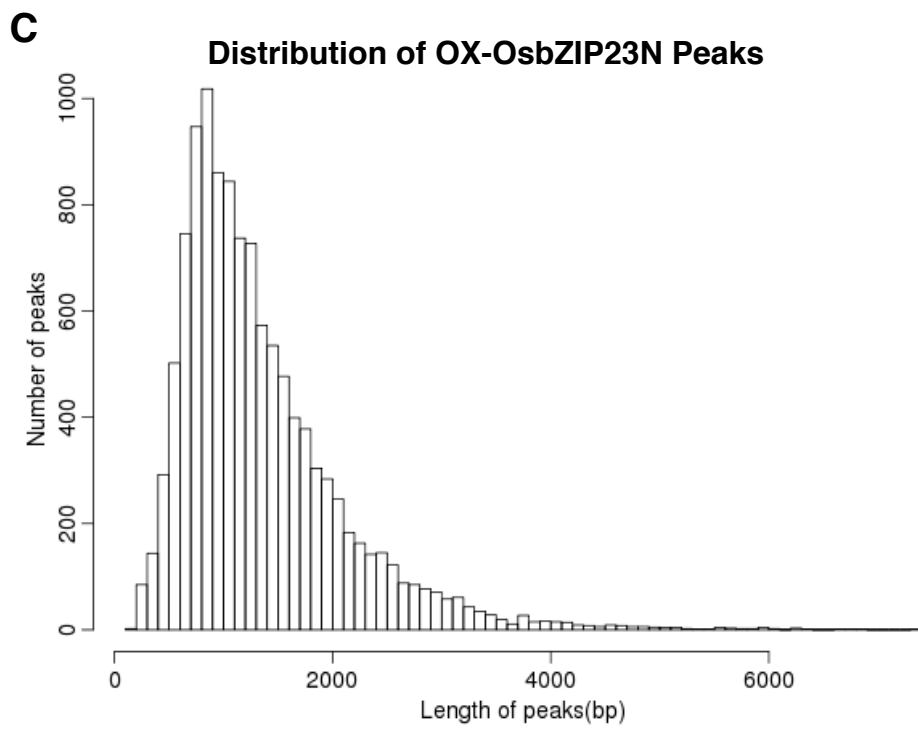
Supplemental Figure 3. Immunoblotting detects the specificity of the OsbZIP23 antibody. Wild type (ZH11), *osbzip23* and over expressed OsbZIP23 (OX-OsbZIP23) seedlings under both normal and drought stress conditions were used for nuclear protein extraction. OsbZIP23 antibody was made by Abmart (Shanghai, China) Company. After transformation, the PVDF membrane was blocked by non-fat milk and then incubated with OsbZIP23 antibody (1:5000). Finally, HRP conjugated goat anti-Rabbit 2nd antibody were used. The signal was detected by X-ray film (FUJIFILM).



Sample	Peak Numbers	Average Length
ZH11N	1738	1413

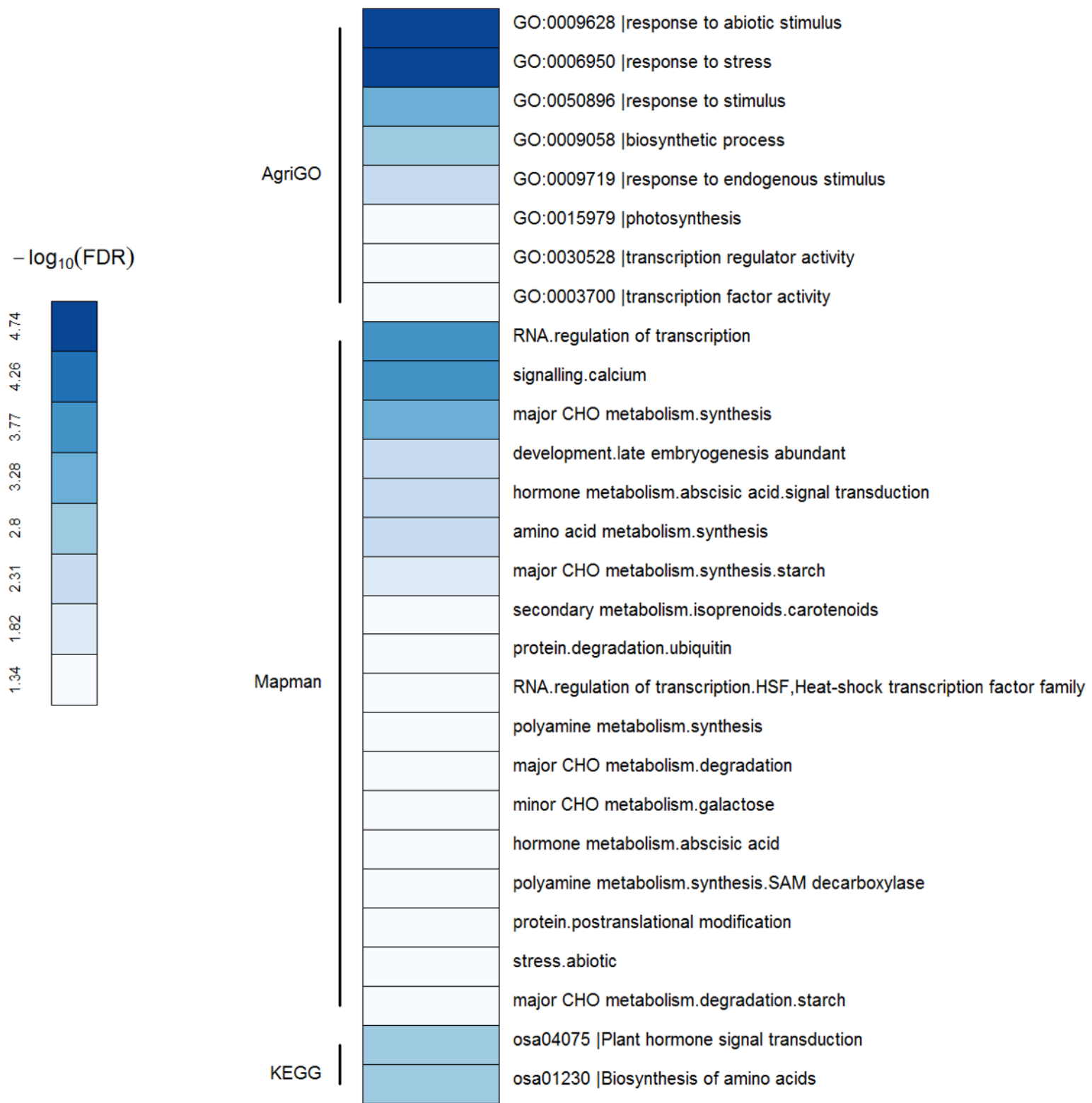


Sample	Peak Numbers	Average Length
ZH11D	4260	1548

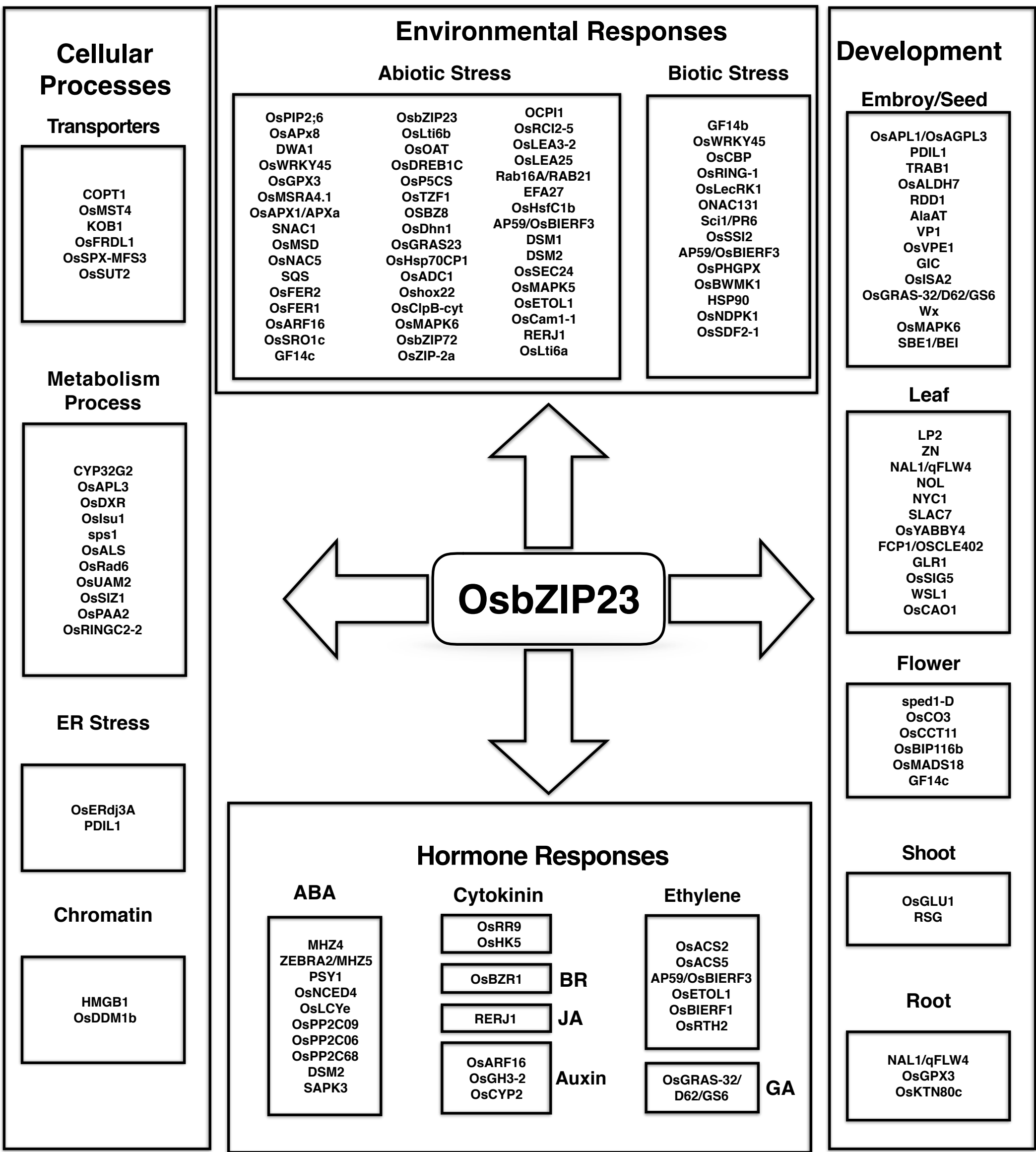


Sample	Peak Numbers	Average Length
OX-OsbZIP23	11611	1338

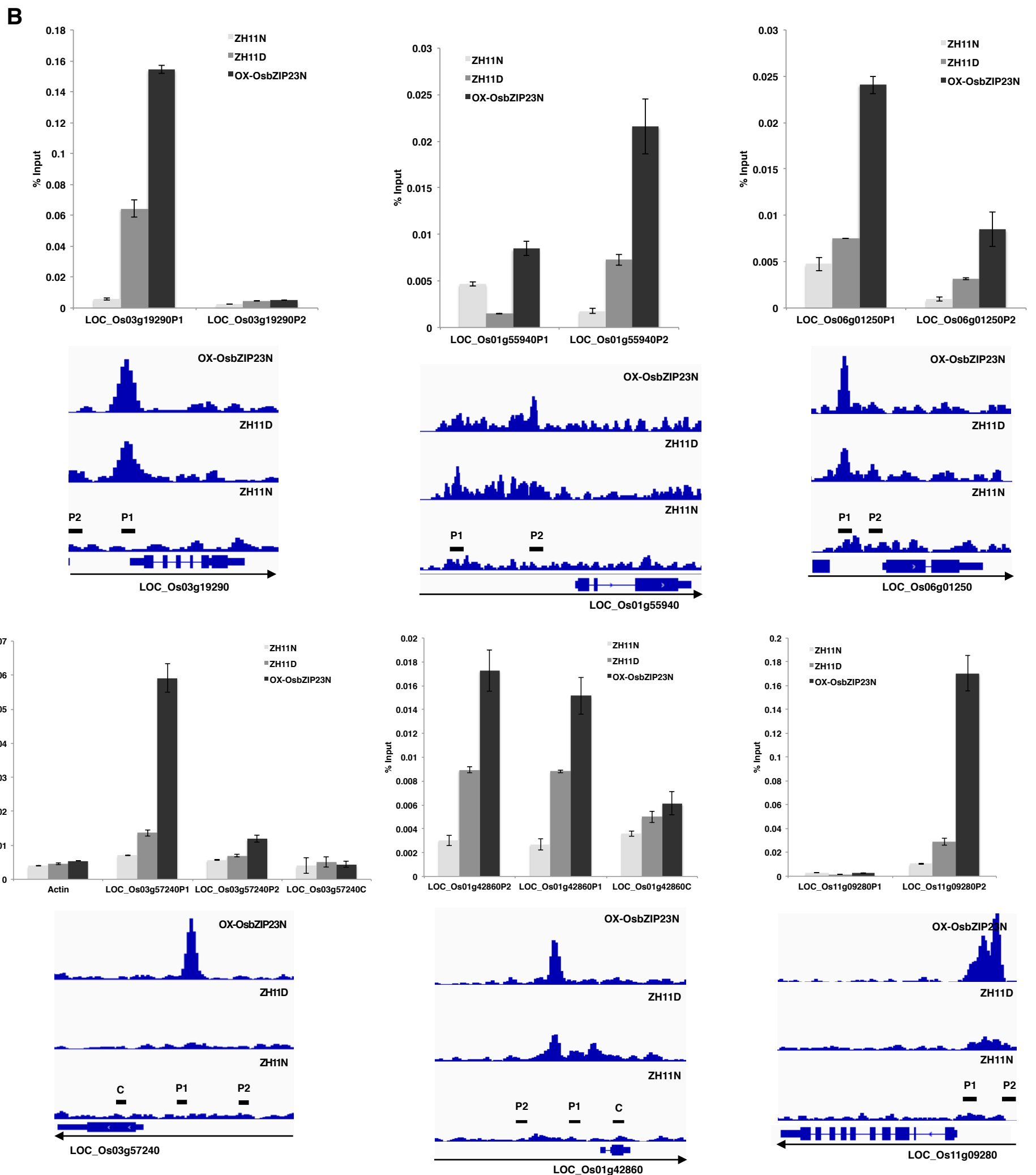
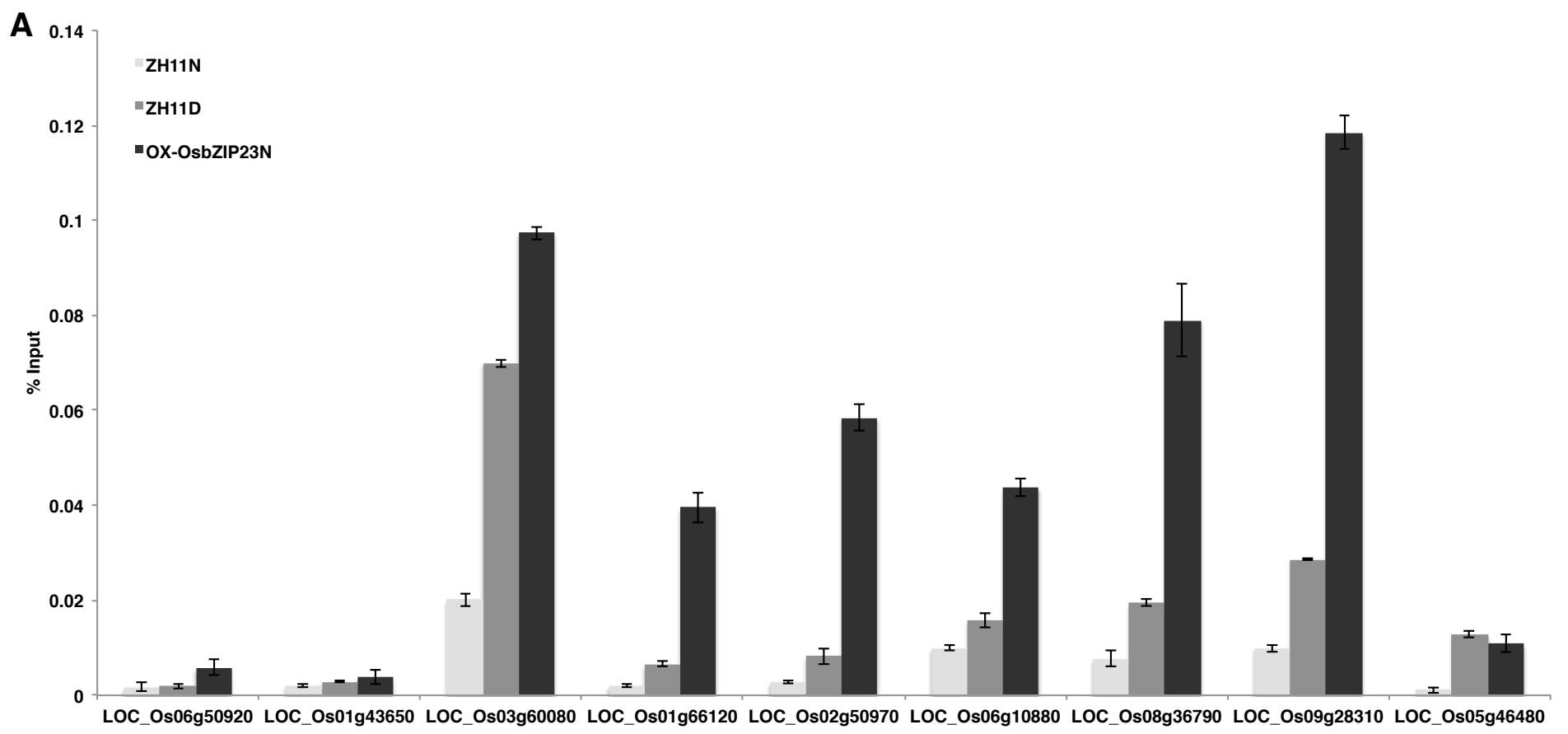
Supplemental Figure 4. Statistical analysis of the length of the OsbZIP23 bound regions.



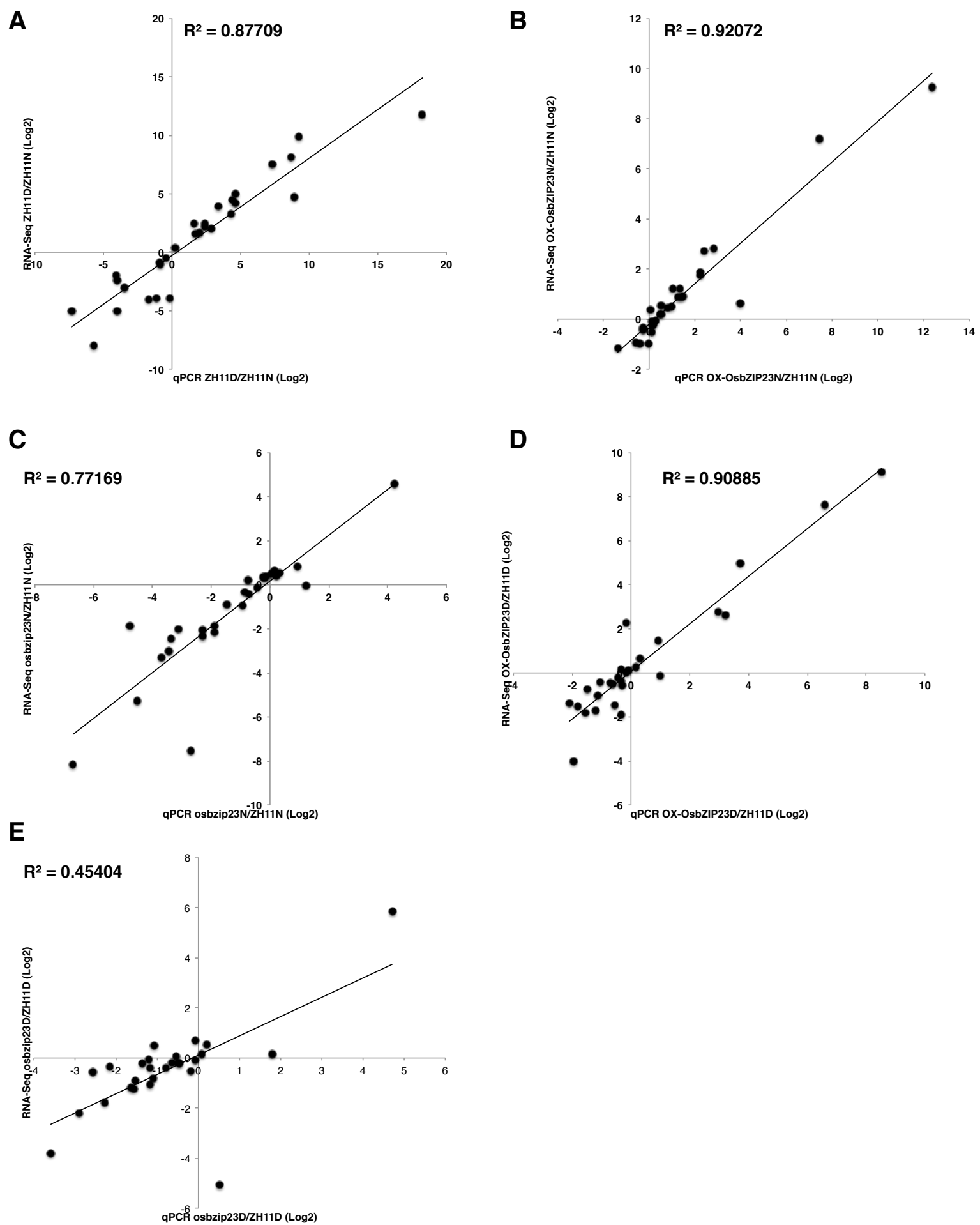
Supplemental Figure 5. Functional categories of OsbZIP23 bound genes in drought stress.



Supplemental Figure 6. OsbZIP23BGDs with known functions in various cellular processes and responses (Supplemental File 1). References for each gene can be found in RicENcode, <http://ricencode.ncpgr.cn>.



Supplemental Figure 7. ChIP-qPCR verification of the ChIP-Seq results. A, Enrichment of 7 OsbZIP23 bound and 2 unbound genes (LOC_Os06g50920 and LOC_Os01g43650) were examined by ChIP-qPCR. B, ChIP-qPCR verification of the OsbZIP23 bound genes. The OsbZIP23 binding profiles in the promoter of these six genes were arranged below the histogram. The primers used in the qPCR assay are represented with black lines in the promoter region. The values in each column are the means of three independent replicates and error bars represent the SD. The primers used in this experiment are listed in Supplemental Table S4.

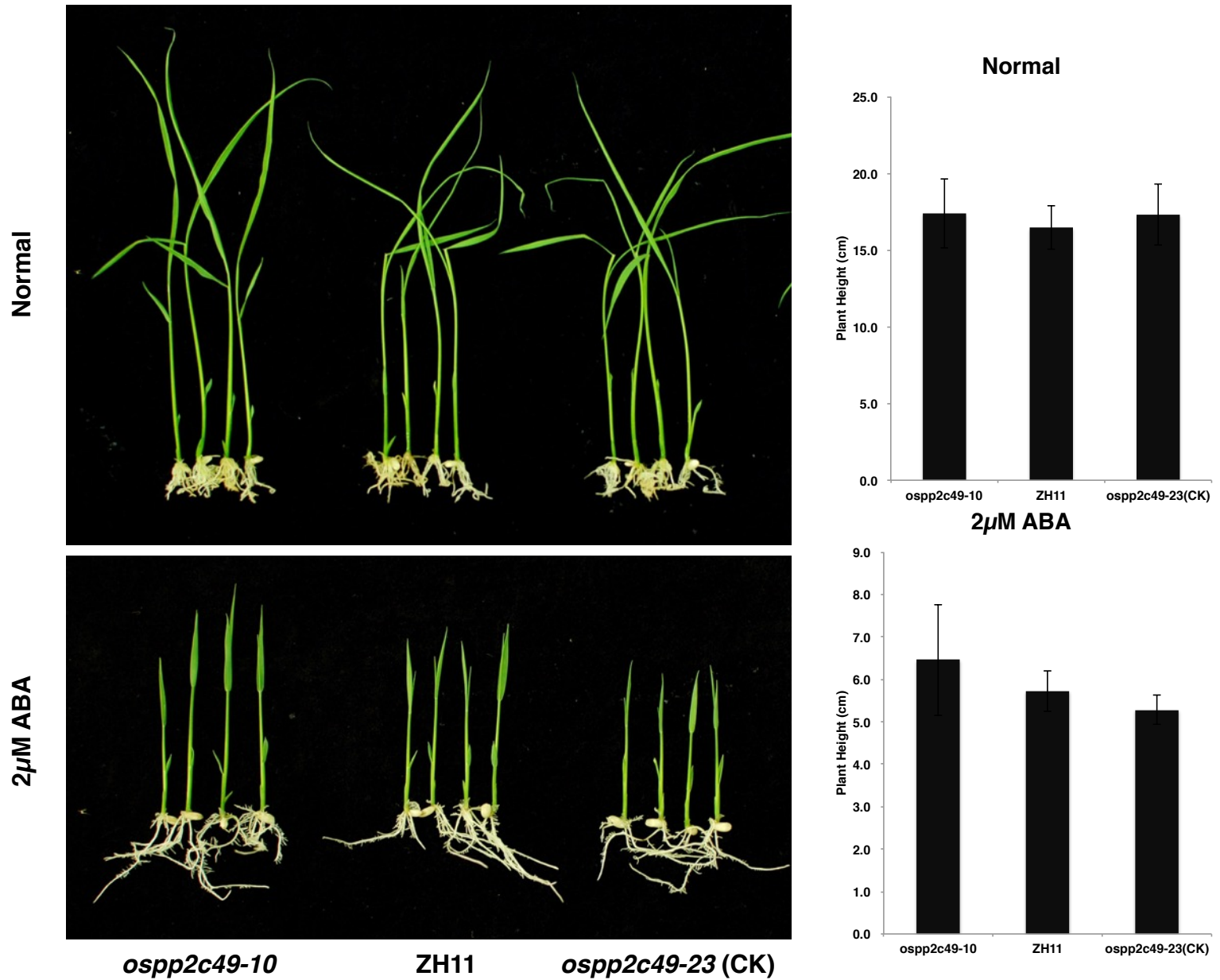


Supplemental Figure 8. Correlation analysis between the RT-qPCR and the RNA-Seq data. A to E, 29 genes were used in the validation of the RNA-Seq results by RT-qPCR. The primers used in this experiment are listed in Supplemental Table S4.

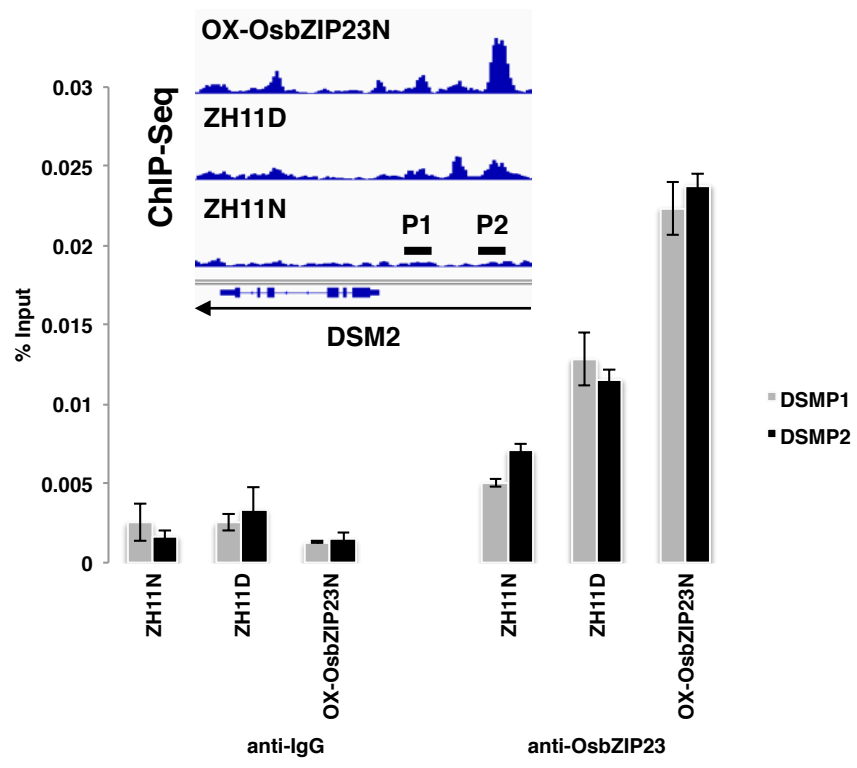
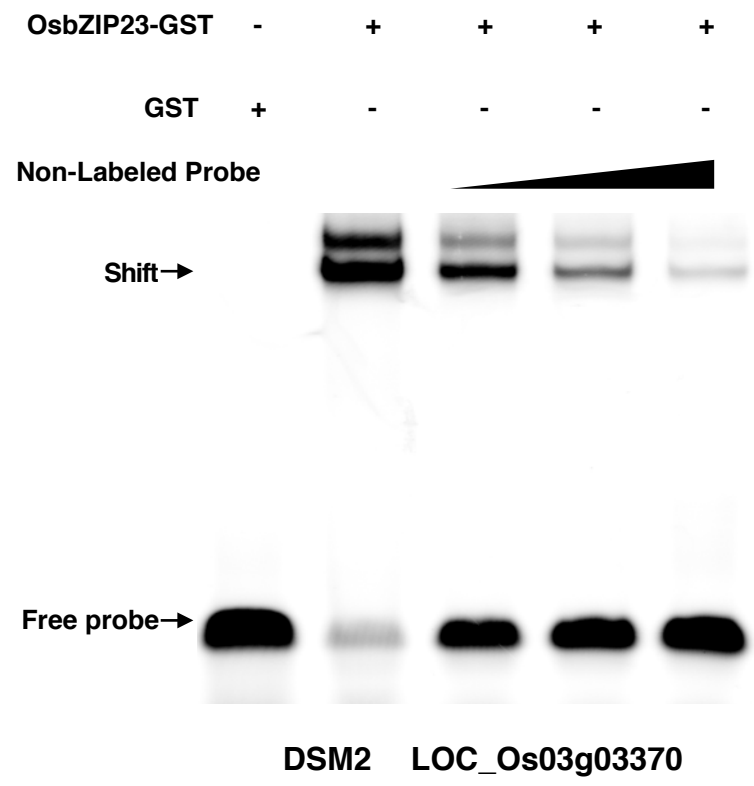
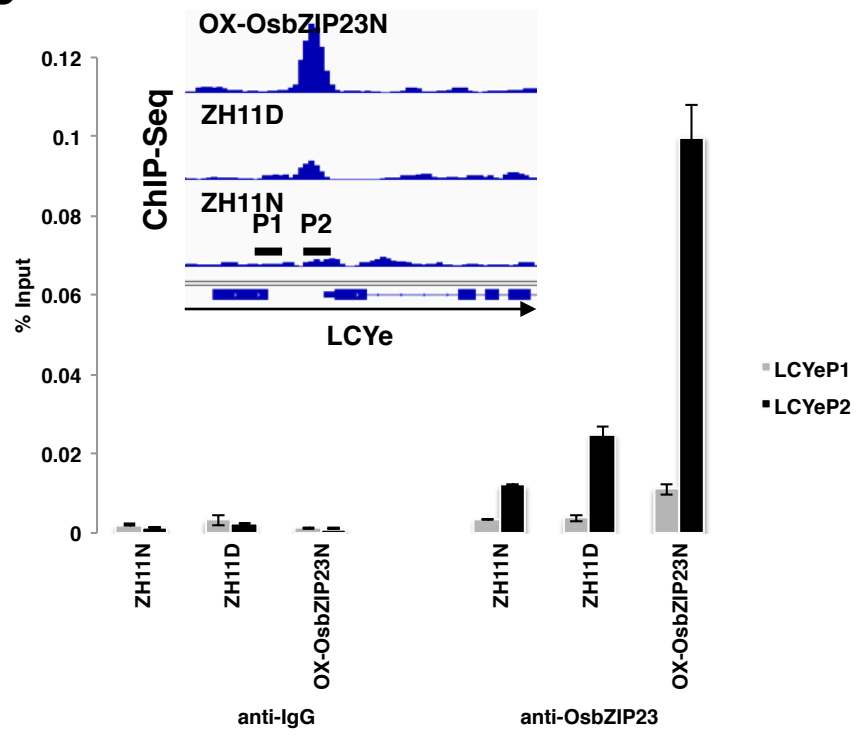
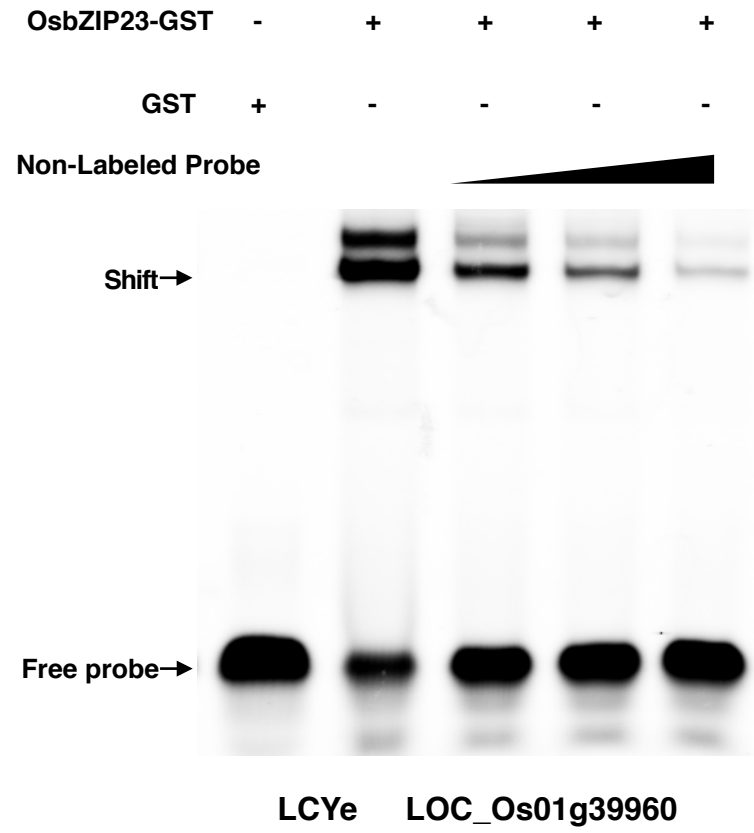
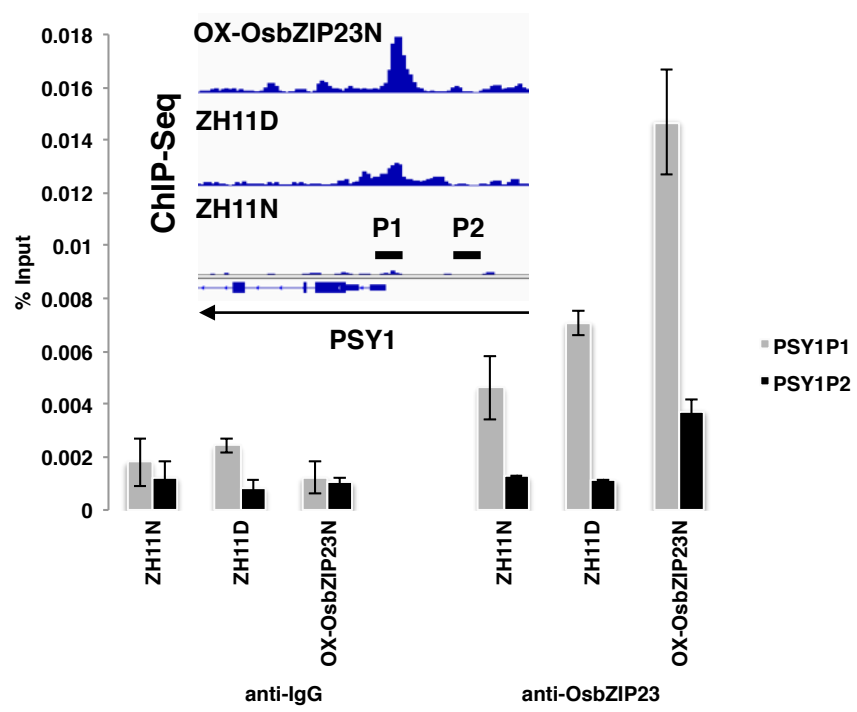
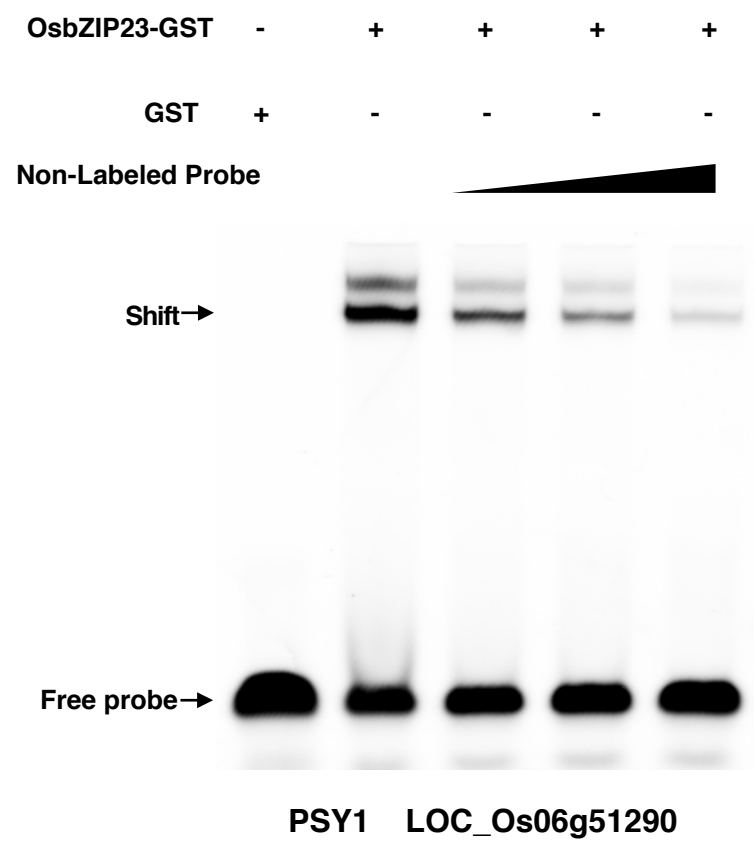
A

OsPP2C49 GGGCTCGACCGCCGTTGTCGCGG-TCGTCGGTCC
 980 |
ospp2c49-10 GGGCTCGACCGCCGTTGTCGCGG TCGTCGGTCC

B



Supplemental Figure 9. The *ospp2c49* showed similar ABA sensitivity with the wild-type. A, Mutation sites of *ospp2c49*. The underlined sequences indicate the sgRNA and the dash represents the insertion site. B, Performance of *ospp2c49-10*, ZH11 and control line (*ospp2c49-23*) in 1/2 strength MS medium containing 2µM ABA. Plant height was measured after growth for 14d. Bar represent the SD (n>6).

A**B****C****D****E****F**

Supplemental Figure 10. OsbZIP23 directly binds to the promoters of the ABA biosynthesis genes. A, Validation of OsbZIP23 direct binding sites in the *DSM2* promoter by ChIP-qPCR analysis. B, EMSA showing that OsbZIP23 could directly bind to the promoter of *DSM2*. C, Validation of OsbZIP23 direct binding sites in the *LCYe* promoter by ChIP-qPCR analysis. D, EMSA showing that OsbZIP23 could directly bind to the promoter of *LCYe*. E, Validation of OsbZIP23 direct binding sites in the *PSY1* promoter by ChIP-qPCR analysis. F, EMSA showing that OsbZIP23 could directly bind to the promoter of *PSY1*. In the ChIP-qPCR assay, the Rabbit IgG was used as a control and the primers used in the qPCR assay were represented by black lines in the promoter region of each gene. In the EMSA assay, 5-, 10- and 30- fold excess non-labeled probe was used for competition.

Supplemental Table S1. Summary statistics for ChIP-Seq library alignment.

Sample	Total Reads	Mapped Reads	Unique Mapped Reads
ZH11N	22269610	9773237 (43.89%)	7797925 (35.02%)
ZH11D	20707873	13053956 (63.04%)	9927059 (47.94%)
OX-OsbZIP23N	20756525	8457039 (40.74%)	6963151 (33.55%)

Supplemental Table S2. OsbZIP23 directly binds to the promoter of clade A PP2Cs in rice.

Gene Symbol	MSU ID 7.0	ZH11N Peak	ChIP-Seq Data		RNA-Seq Data	
			ZH11D Peak	OX-OsbZIP23N Peak	ZH11N/OX-OsbZIP23N	ZH11N/ZH11D
OsPP2C06	LOC_Os01g40094	N	Y	Y	N/A	3.107146928
OsPP2C08	LOC_Os01g46760	N	Y	Y	N/A	N/A
OsPP2C09	LOC_Os01g62760	Y	Y	Y	N/A	2.096307912
OsPP2C30	LOC_Os03g16170	Y	Y	Y	N/A	5.2083152
OsPP2C37	LOC_Os04g08560	N	N	N	N/A	N/A
OsPP2C49	LOC_Os05g38290	Y	Y	Y	2.819251871	5.409323663
OsPP2C50	LOC_Os05g46040	N	N	Y	N/A	4.241217471
OsPP2C51	LOC_Os05g49730	N	Y	Y	2.725393679	9.823846266
OsPP2C53	LOC_Os05g51510	N	Y	Y	N/A	N/A
OsPP2C68	LOC_Os09g15670	Y	Y	Y	N/A	6.627762714

Supplemental Table S3. OsPYLs and SAPKs in OsbZIP23BGDs.

Gene Symbol	MSU ID 7.0	ZH11N Peak	ChIP-Seq Data		OX-OsbZIP23N Peak	RNA-Seq Data
			ZH11D Peak	ZH11N/ZH11D		
OsPYLs						
OsPYL1	LOC_Os10g42280	N	N	N	N	N
OsPYL2	LOC_Os06g36670	N	N	N	N	N
OsPYL3	LOC_Os02g13330	N	N	N	N	N
OsPYL4	LOC_Os01g61210	N	N	Y	Y	N
OsPYL5	LOC_Os05g39580	N	N	N	N	N
OsPYL6	LOC_Os03g18600	N	N	N	N	-4.019988316
OsPYL7	LOC_Os06g33480	N	N	N	N	N
OsPYL8	LOC_Os06g33640	N	N	N	N	N
OsPYL9	LOC_Os06g33690	N	N	N	N	N
OsPYL10	LOC_Os02g15640	N	N	N	N	N
OsPYL11	LOC_Os05g12260	N	N	N	N	N
OsPYL12	LOC_Os02g15620	N	N	N	N	N
SAPKS						
SAPK1	LOC_Os03g27280	N	N	Y	Y	3.755917517
SAPK2	LOC_Os07g42940	N	N	N	N	N
SAPK3	LOC_Os10g41490	Y	Y	Y	Y	N
SAPK4	LOC_Os01g64970	N	N	Y	Y	3.8734785
SAPK5	LOC_Os04g59450	N	N	Y	Y	N
SAPK6	LOC_Os02g34600	N	N	Y	Y	2.06026931
SAPK7	LOC_Os04g35240	N	N	N	N	-2.513927433
SAPK8	LOC_Os03g55600	N	N	N	N	N
SAPK9	LOC_Os12g39630	N	N	N	N	2.506073579
SAPK10	LOC_Os03g41460	N	N	Y	Y	2.021317306