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Supplemental information

The TELOMERE REPEAT BINDING proteins TRB4 and TRB5 function as transcriptional activators of PRC2-controlled genes to regulate plant development

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Supplementary Information

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Supplementary Table 1:

1A: Protein sequences of species used in **Figure 1B** and **Supplementary Figure 1A**
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Supplementary Table 2:

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2E: Z-score values corresponding to Figure 7D
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Supplementary Table 3:

3A: List of oligos used in this study
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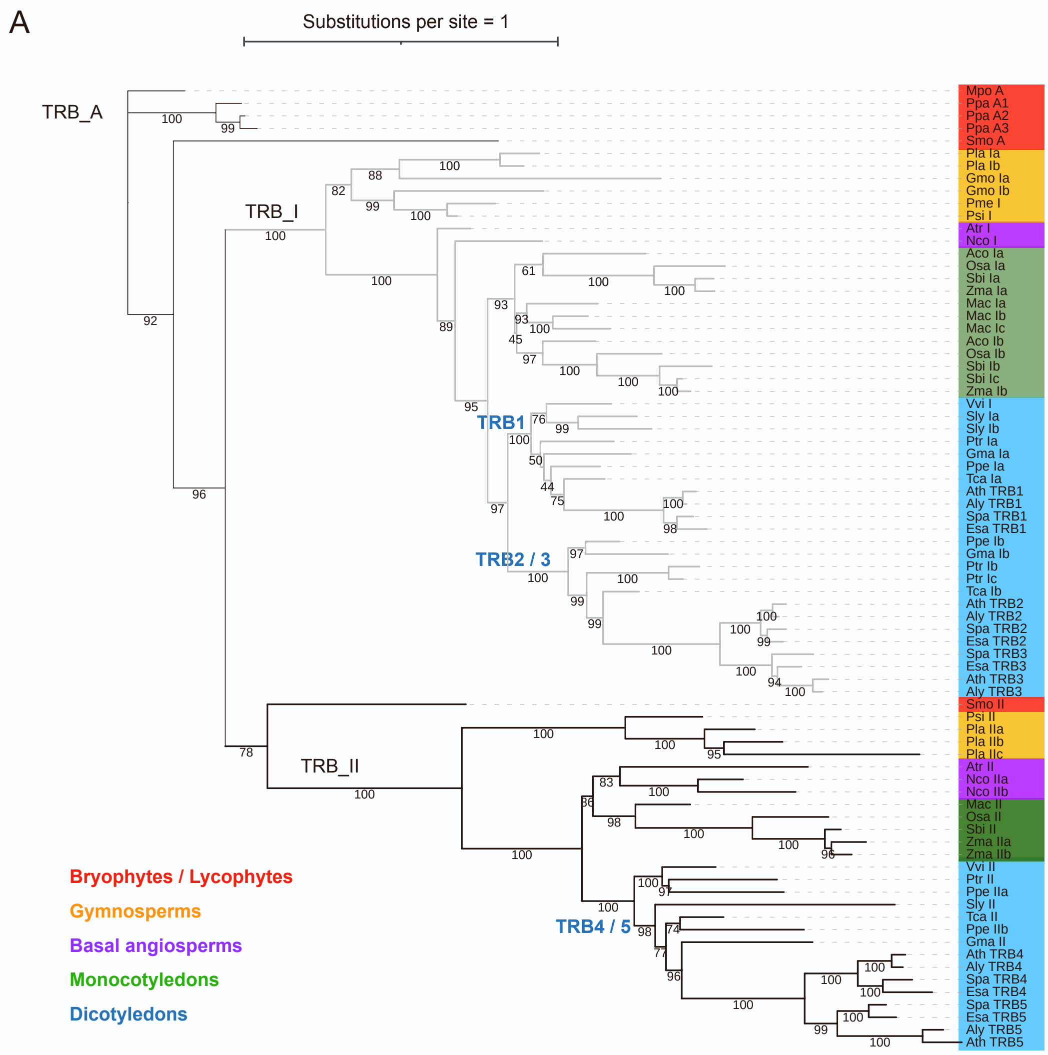
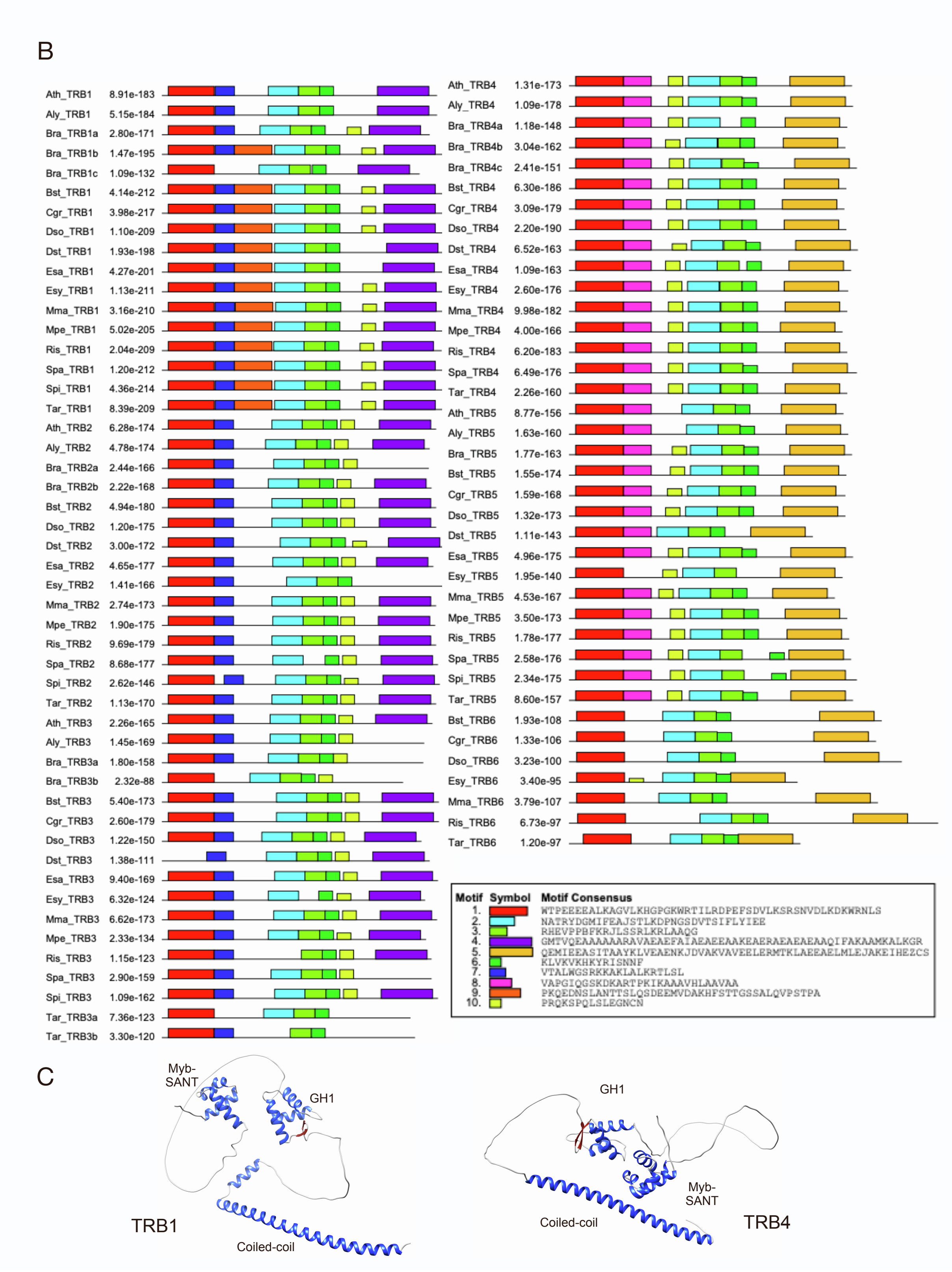
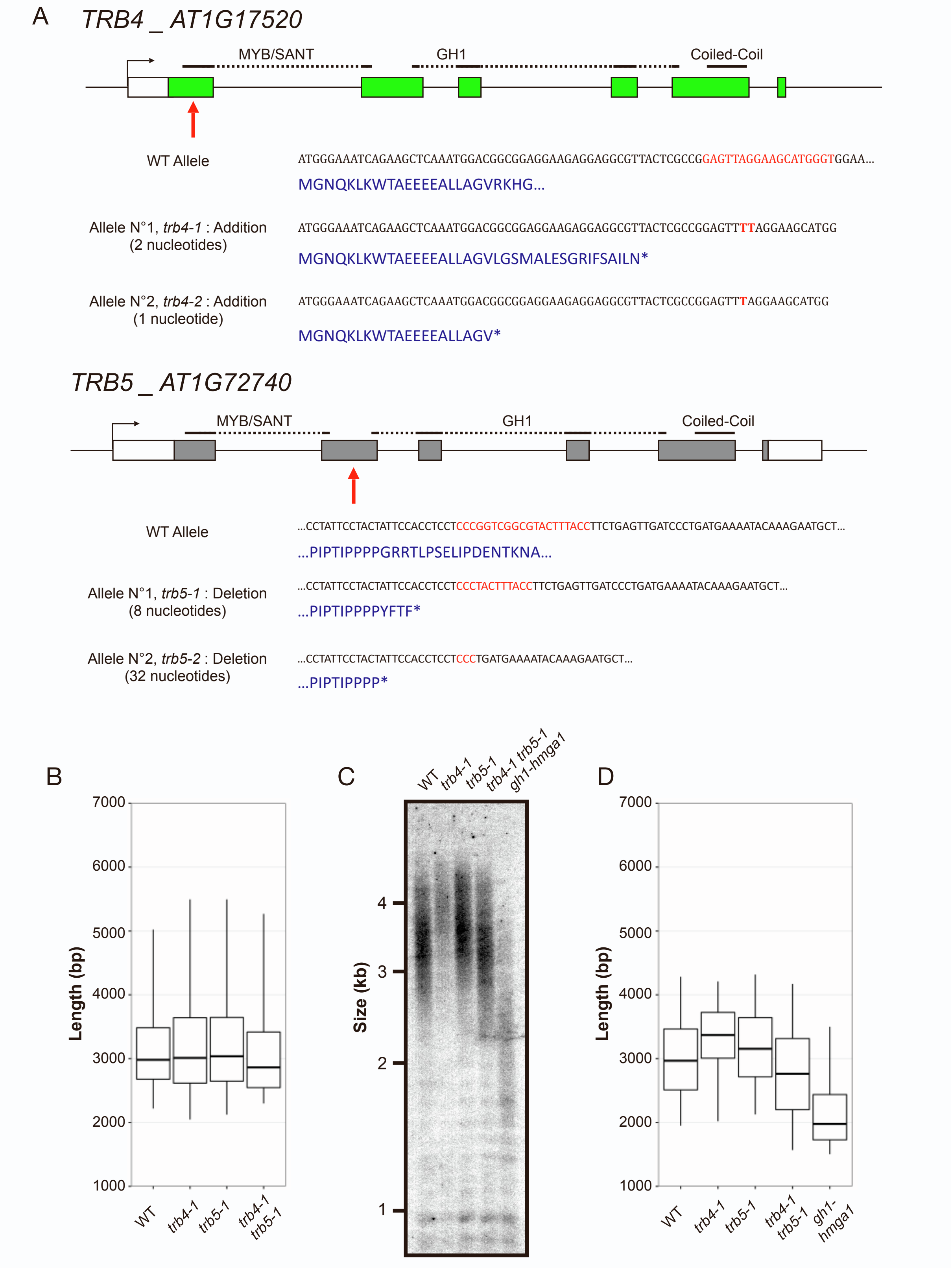
Supplementary Table 4: FPKM of PRC1 and PRC2 genes in WT and *trb4 trb5* mutants

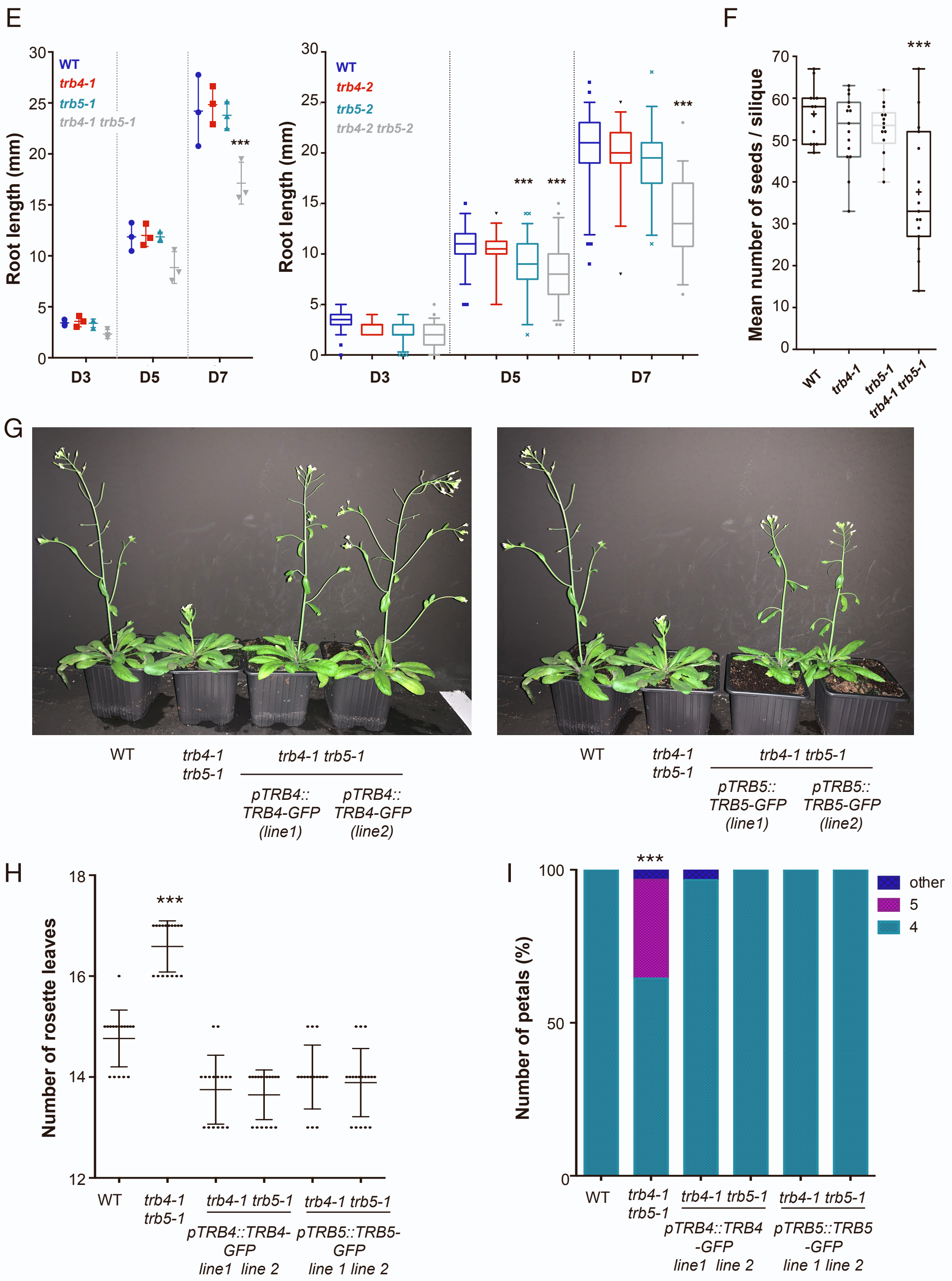

A
Substitutions per site = 1
TRB_A
TRB_I
TRB_II
TRB1
TRB2 / 3
TRB4 / 5
100
99
92
96
88
100
82
99
100
100
100
61
100
100
93
93
100
45
89
97
100
100
100
95
76
99
100
50
44
75
100
97
100
98
97
100
100
99
99
100
100
99
100
100
94
100
78
100
100
100
95
83
100
86
98
100
100
98
100
100
100
97
98
74
77
96
100
100
100
100
100
99
100
Mpo A
Ppa A1
Ppa A2
Ppa A3
Smo A
Pla Ia
Pla Ib
Gmo Ia
Gmo Ib
Pme I
Psi I
Atr I
Nco I
Aco Ia
Osa Ia
Sbi Ia
Zma Ia
Mac Ia
Mac Ib
Mac Ic
Aco Ib
Osa Ib
Sbi Ib
Sbi Ic
Zma Ib
Vvi I
Sly Ia
Sly Ib
Ptr Ia
Gma Ia
Ppe Ia
Tca Ia
Ath TRB1
Aly TRB1
Spa TRB1
Esa TRB1
Ppe Ib
Gma Ib
Ptr Ib
Ptr Ic
Tca Ib
Ath TRB2
Aly TRB2
Spa TRB2
Esa TRB2
Spa TRB3
Esa TRB3
Ath TRB3
Aly TRB3
Smo II
Psi II
Pla IIa
Pla IIb
Pla IIc
Atr II
Nco IIa
Nco IIb
Mac II
Osa II
Sbi II
Zma IIa
Zma IIb
Vvi II
Ptr II
Ppe IIa
Sly II
Tca II
Ppe IIb
Gma II
Ath TRB4
Aly TRB4
Spa TRB4
Esa TRB4
Spa TRB5
Esa TRB5
Aly TRB5
Ath TRB5
Bryophytes / Lycophytes
Gymnosperms
Basal angiosperms
Monocotyledons
Dicotyledons

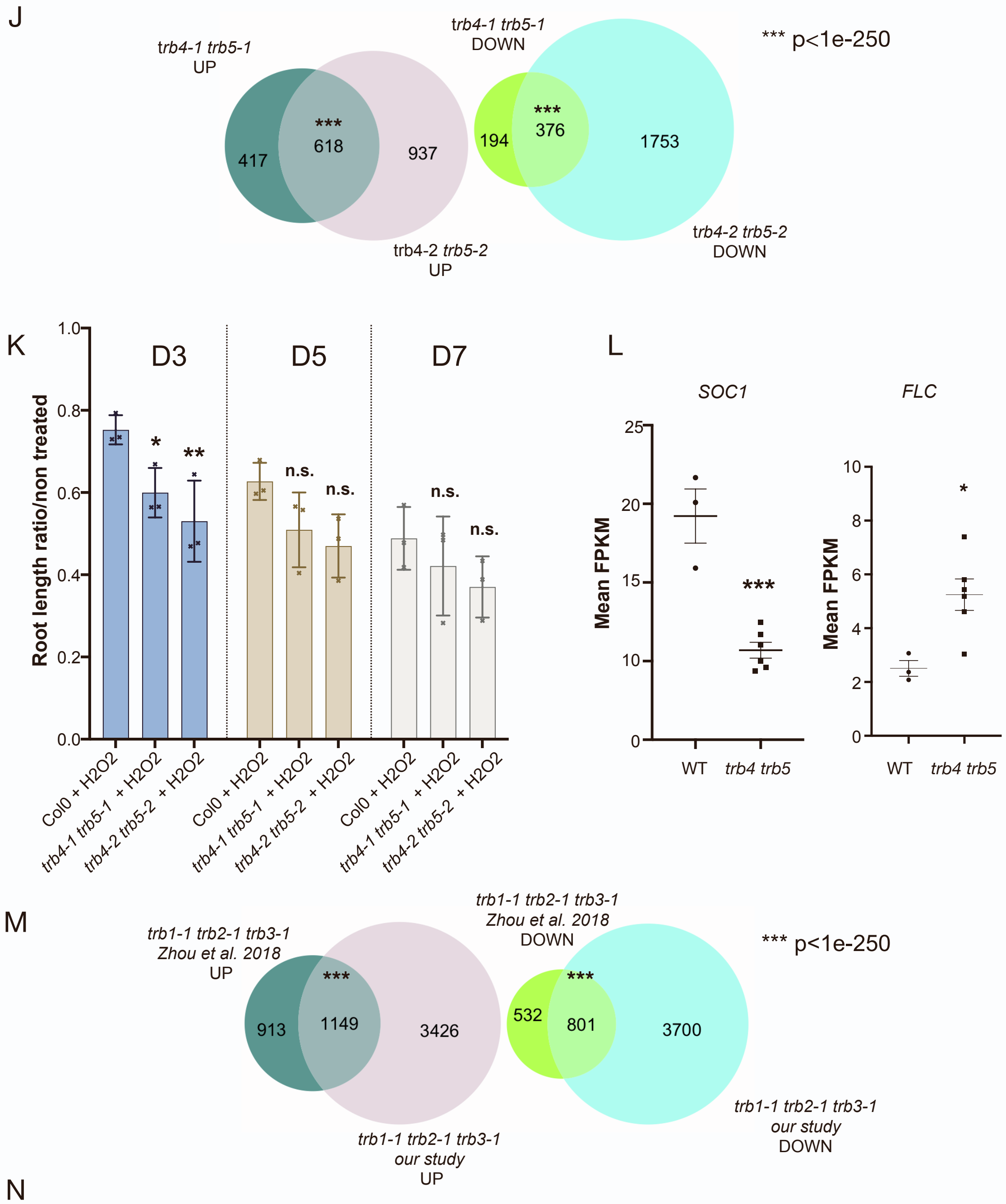


Supplementary Figure 1: *Supplemental information related to Figure 1*

(A) Rooted maximum likelihood phylogenetic tree for TRB orthologs from 24 plant species. Bootstrap values are indicated for each branch. An ancient TRB clade (TRB_A), a clade comprising Arabidopsis TRB1, TRB2 and TRB3 (TRB_I) and a third clade comprising TRB4 and TRB5 (TRB_II) were defined. **(B)** MEME protein motif prediction of the 10 best motifs among the 15 Brassicaceae species. **(C)** Alpha-fold predictions of long alpha helices in the C-terminal domains of Arabidopsis TRB1 and TRB4 proteins.


A
TRB4 _ AT1G17520
MYB/SANT
GH1
Coiled-Coil
WT Allele
ATGGGAAATCAGAAGCTCAAATGGACGGCGGAGGAAGAGGAGGCGTTACTCGCCGGAGTTAGGAAGCATGGGTGGAA...
MGNQKLKWTAEEEEALLAGVRKHG...
Allele N°1, trb4-1 : Addition (2 nucleotides)
ATGGGAAATCAGAAGCTCAAATGGACGGCGGAGGAAGAGGAGGCGTTACTCGCCGGAGTTTTAGGAAGCATGG
MGNQKLKWTAEEEEALLAGVLGSMALESGRIFSAILN*
Allele N°2, trb4-2 : Addition (1 nucleotide)
ATGGGAAATCAGAAGCTCAAATGGACGGCGGAGGAAGAGGAGGCGTTACTCGCCGGAGTTTAGGAAGCATGG
MGNQKLKWTAEEEEALLAGV*
TRB5 _ AT1G72740
MYB/SANT
GH1
Coiled-Coil
WT Allele
...CCTATTCCTACTATTCCACCTCCTCCCGGTCGGCGTACTTTACCTTCTGAGTTGATCCCTGATGAAAATACAAAGAATGCT...
...PIPTIPPPPGRRTLPSELIPDENTKNA...
Allele N°1, trb5-1 : Deletion (8 nucleotides)
...CCTATTCCTACTATTCCACCTCCTCCCTACTTTACCTTCTGAGTTGATCCCTGATGAAAATACAAAGAATGCT...
...PIPTIPPPPYFTF*
Allele N°2, trb5-2 : Deletion (32 nucleotides)
...CCTATTCCTACTATTCCACCTCCTCCCTGATGAAAATACAAAGAATGCT...
...PIPTIPPPP*
B
Length (bp)
7000
6000
5000
4000
3000
2000
1000
WT
trb4-1
trb5-1
trb4-1 trb5-1
C
WT
trb4-1
trb5-1
trb4-1 trb5-1 gh1-hmga1
Size (kb)
4
3
2
1
D
Length (bp)
7000
6000
5000
4000
3000
2000
1000
WT
trb4-1
trb5-1
trb4-1 trb5-1
gh1-hmga1


E
WT
trb4-1
trb5-1
trb4-1 trb5-1
Root length (mm)
0
5
10
15
20
25
30
***
D3
D5
D7
WT
trb4-2
trb5-2
trb4-2 trb5-2
Root length (mm)
***
***
***
F
Mean number of seeds / silique
0
10
20
30
40
50
60
70
***
WT
trb4-1
trb5-1
trb4-1 trb5-1
G
WT
trb4-1
trb5-1
trb4-1 trb5-1
pTRB4::
TRB4-GFP
(line1)
pTRB4::
TRB4-GFP
(line2)
WT
trb4-1
trb5-1
trb4-1 trb5-1
pTRB5::
TRB5-GFP
(line1)
pTRB5::
TRB5-GFP
(line2)
H
Number of rosette leaves
12
14
16
18
***
WT
trb4-1
trb5-1
trb4-1 trb5-1
pTRB4::TRB4-
GFP
line1 line 2
trb4-1 trb5-1
pTRB5::TRB5-
GFP
line 1 line 2
I
Number of petals (%)
0
50
100
***
other
5
4
WT
trb4-1
trb5-1
trb4-1 trb5-1
pTRB4::TRB4
-GFP
line1 line 2
trb4-1 trb5-1
pTRB5::TRB5
-GFP
line 1 line 2



N

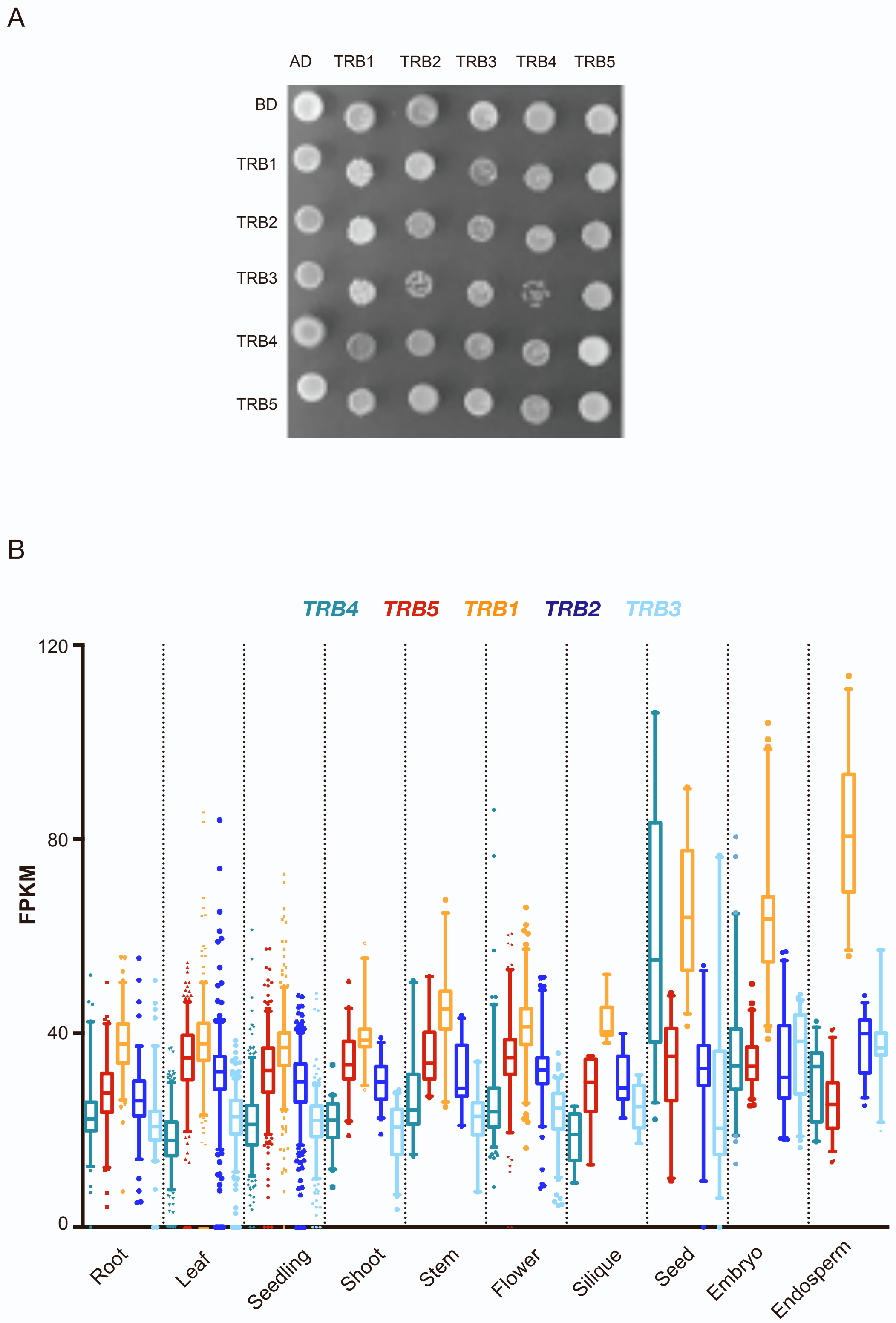
	***trb4-1 trb5-1* UP**	***trb4-1 trb5-1* DOWN**
***trb1-1 trb2-1 trb3-1* UP**	**95**	16
***trb1-1 trb2-1 trb3-1* DOWN**	65	**33**

co-regulated
(total =128)

Oppositely regulated
(total =81)

Supplementary Figure 2: *Supplemental information related to Figure 2*

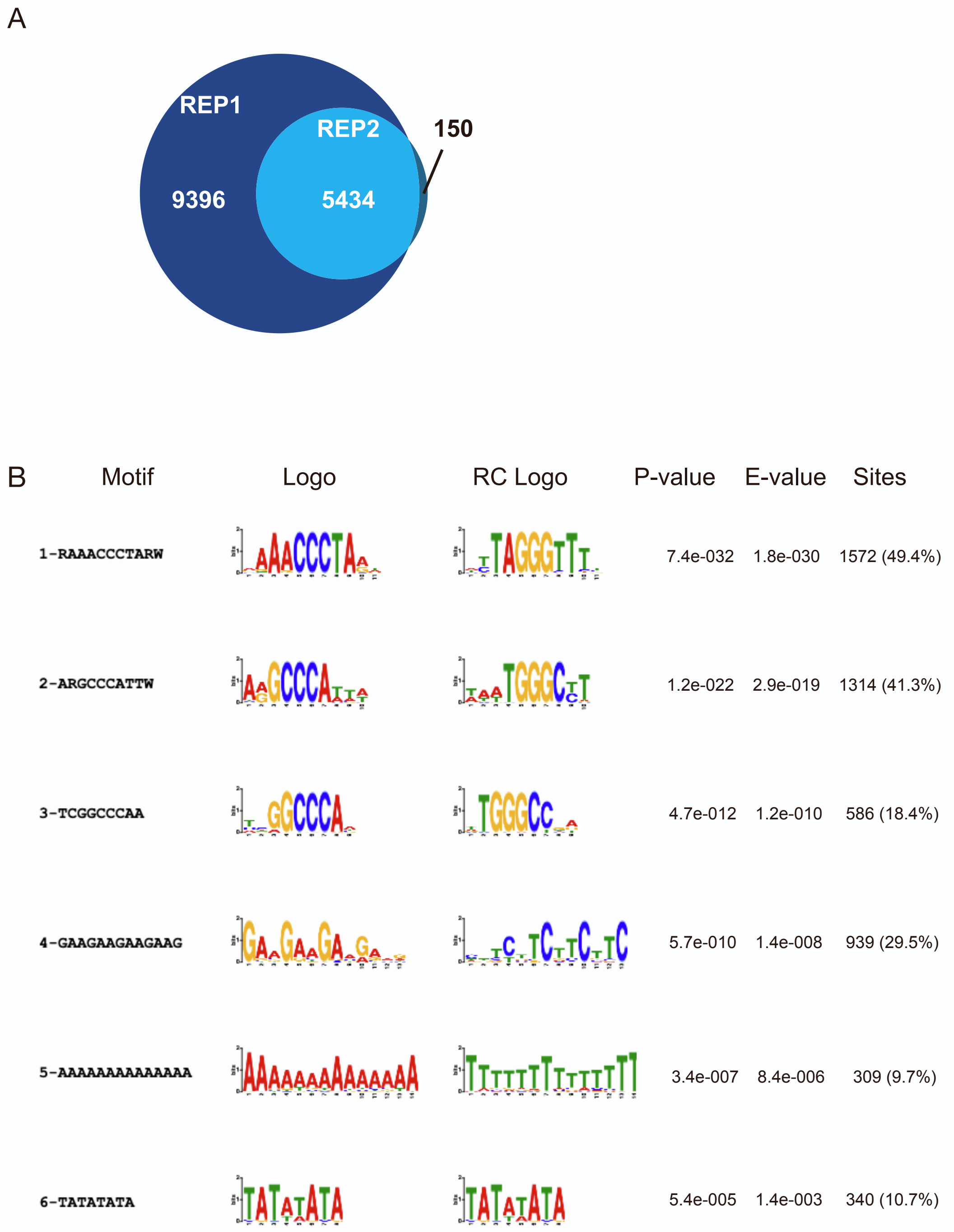
(A) *TRB4* and *TRB5* mutant alleles generated by CRISPR/Cas9. The corresponding nucleotide and the resulting amino acid sequences are shown. In all mutants, premature stop codons are induced. The red arrow indicates the Cas9 target site. **(B)** Telomere Restriction Fragments (TRF) scan analysis from Figure 2C. **(C)** TRF analysis of bulk telomere length in genomic DNA using telomere repeat probes WT, *trb4-1* and *trb5-1*, *trb4-1 trb5-1* and *gh1-hmga1* mutant. **(D)** TRF scan analysis from panel C. **(E)** Quantification of root length from *in vitro* grown WT, *trb4-1* and *trb5-1* single and *trb4-1 trb5-1* double mutant plantlets (left) and for WT, *trb4-2* and *trb5-2* single and *trb4-2 trb5-2* double mutants (right) at day 3 (D3), 5 (D5) and 7 (D7). For each time point, means from three (left) or two (right) replicates comprising 100 plants each are shown. Roots of *trb4-1 trb5-1 or trb4-2 trb5-2* double mutants are significantly shorter (***p < 0.0001, t-test) at D7. **(F)** Mean number of seeds from 15 siliques of five WT plants, *trb4-1* and *trb5-1* single and *trb4-1 trb5-1* double mutants. Double mutants are significantly less fertile (***p = 0.0004, t-test). **(G)** Representative 4-weeks-old WT, *trb4-1 trb5-1* double mutants and four independent transgenic lines expressing either TRB4-GFP or TRB5-GFP under their respective endogenous promoter. The delayed flowering phenotype of *trb4-1 trb5-1* double mutants is complemented. (**H**) Quantification of leaf number at bolting in WT, *trb4-1 trb5-1* double mutants and four independent transgenic lines shown in (**G**). n = 17, N=1, *** < 0.001, t-test. (**I**) Percentage of flowers showing 4, 5 or any other aberrant petal number in the same genotypes as in (**G**). n=100, N = 5, *** < 0.001, t-test. (**J**) Venn diagram showing up- and down regulated genes relative to WT in RNA-seq analysis from 3 replicates of *trb4-1 trb5-1* and *trb4-2 trb5-2* mutants. FC > 0.5, padj < 0.01. **(K)** Quantification of root length from *in vitro* grown WT, *trb4-1 trb5-1 and trb4-2 trb5-2* double mutant plantlets with or without 2,5mM H_2O_2 at day 3 (D3), 5 and 7. For each time point, the mean ratio of the root length of seedlings grown with or without H_2O_2, from three replicates comprising 50 plants each are shown. Root length development is significantly decreased in presence of H_2O_2 in *trb4-1 trb5-1* or *trb4-2 trb5-2* at D3 (*p < 0.05, **p < 0.005, Anova 2-way). (**L**) Expression level (mean FPKM) of *SOC1* and *FLC* in WT and in *trb4 trb5* mutants. ***<0,0005, *<0,05, t-test (**M**) Venn diagram showing up- and down regulated genes relative to WT in RNA-seq analysis from 3 replicates of *trb1-1 trb2-1 trb3-1* in our data set and the dataset from (Zhou et al., 2018), logFC > 0.5, padj < 0.01. **(N)** Comparison of co- or oppositely regulated genes in *trb4 trb5* and *trb1 trb2 trb3* datasets from (**J**) and (**M**).



Supplementary Figure 3: *Supplemental information related to Figure 3*

(A) Growth of zygotes on synthetic medium lacking leucine and tryptophan, selecting for the presence of the bait and prey vectors for interactions scored in Figure 3**. (B)** Expression level of *TRB1, TRB2, TRB3, TRB4* and *TRB5* in different Arabidopsis tissues issued from available RNA-seq datasets (data extracted from Arabidopsis RNA-seq Database -

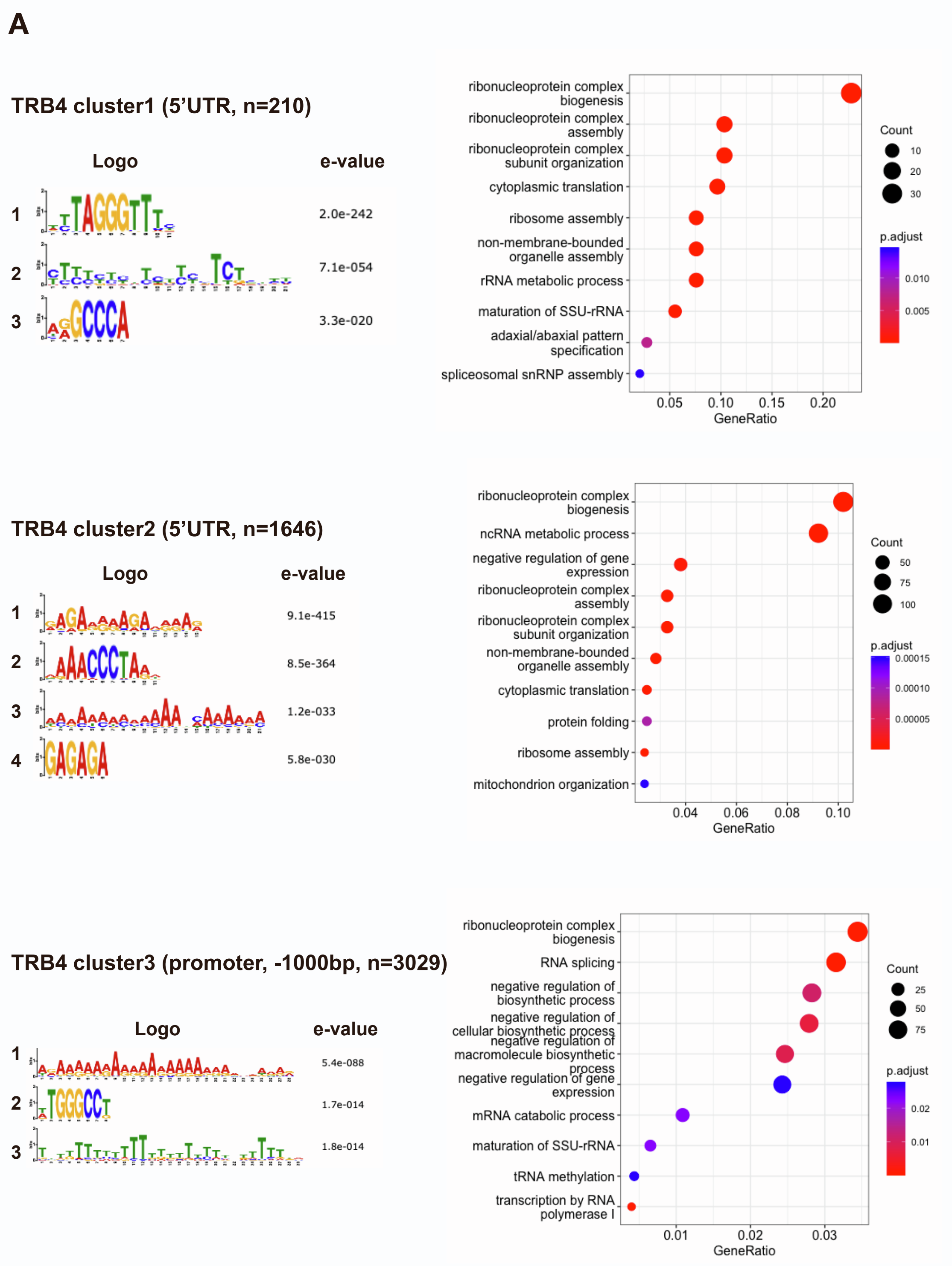
http://ipf.sustech.edu.cn/pub/athrna/). All 5 genes are ubiquitously expressed to similar levels, except in the embryo and endosperm that show higher *TRB1* transcript levels.

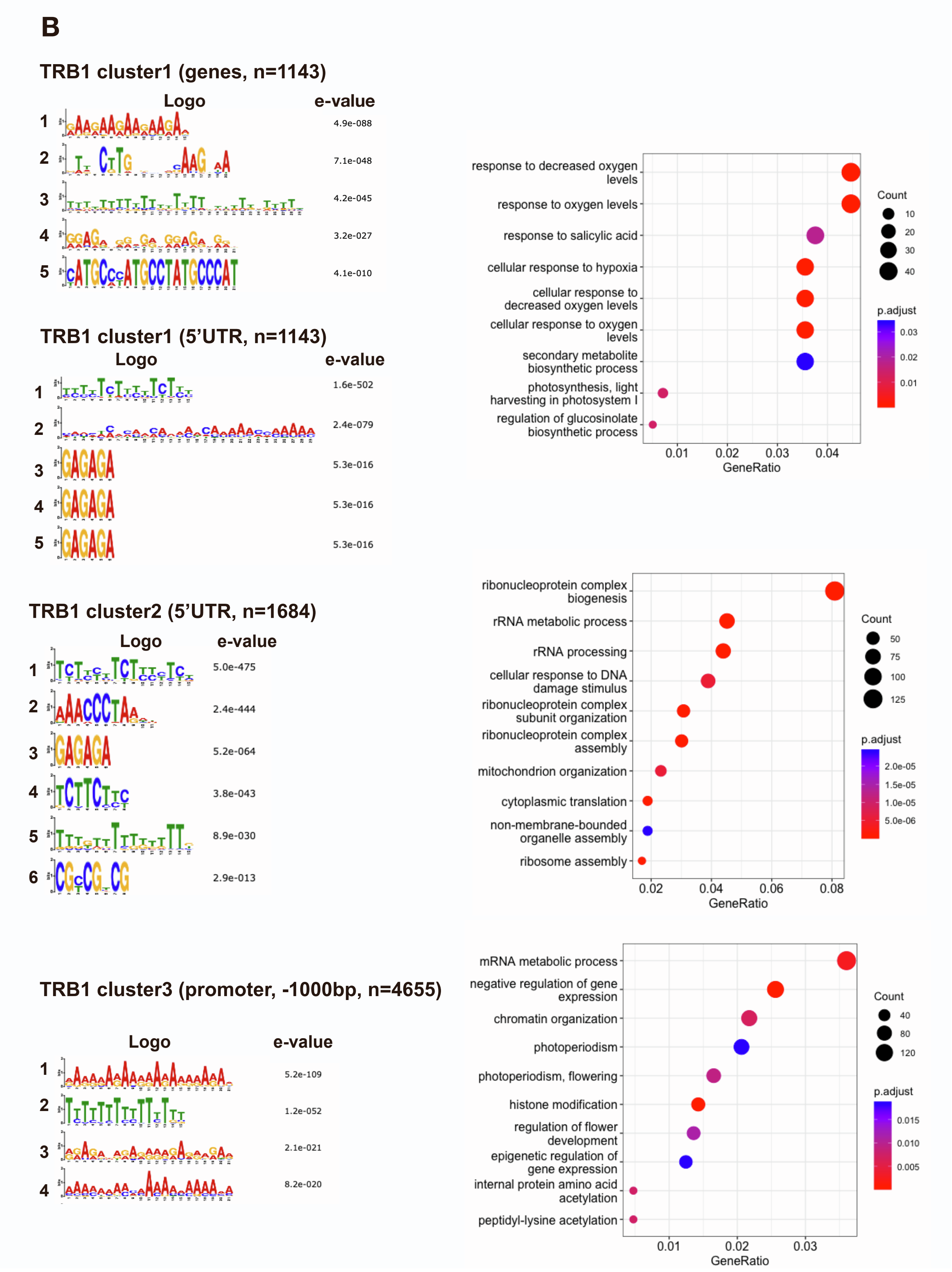


Motif	Logo	RC Logo	P-value	E-value	Sites
1-RAAACCCTARW			7.4e-032	1.8e-030	1572 (49.4%)
2-ARGCCCATTW			1.2e-022	2.9e-019	1314 (41.3%)
3-TCGGCCCAA			4.7e-012	1.2e-010	586 (18.4%)
4-GAAGAAGAAGAAG			5.7e-010	1.4e-008	939 (29.5%)
5-AAAAAAAAAAAAAA			3.4e-007	8.4e-006	309 (9.7%)
6-TATATATA			5.4e-005	1.4e-003	340 (10.7%)

Supplementary Figure 4: *Supplemental information related to Figure 4*

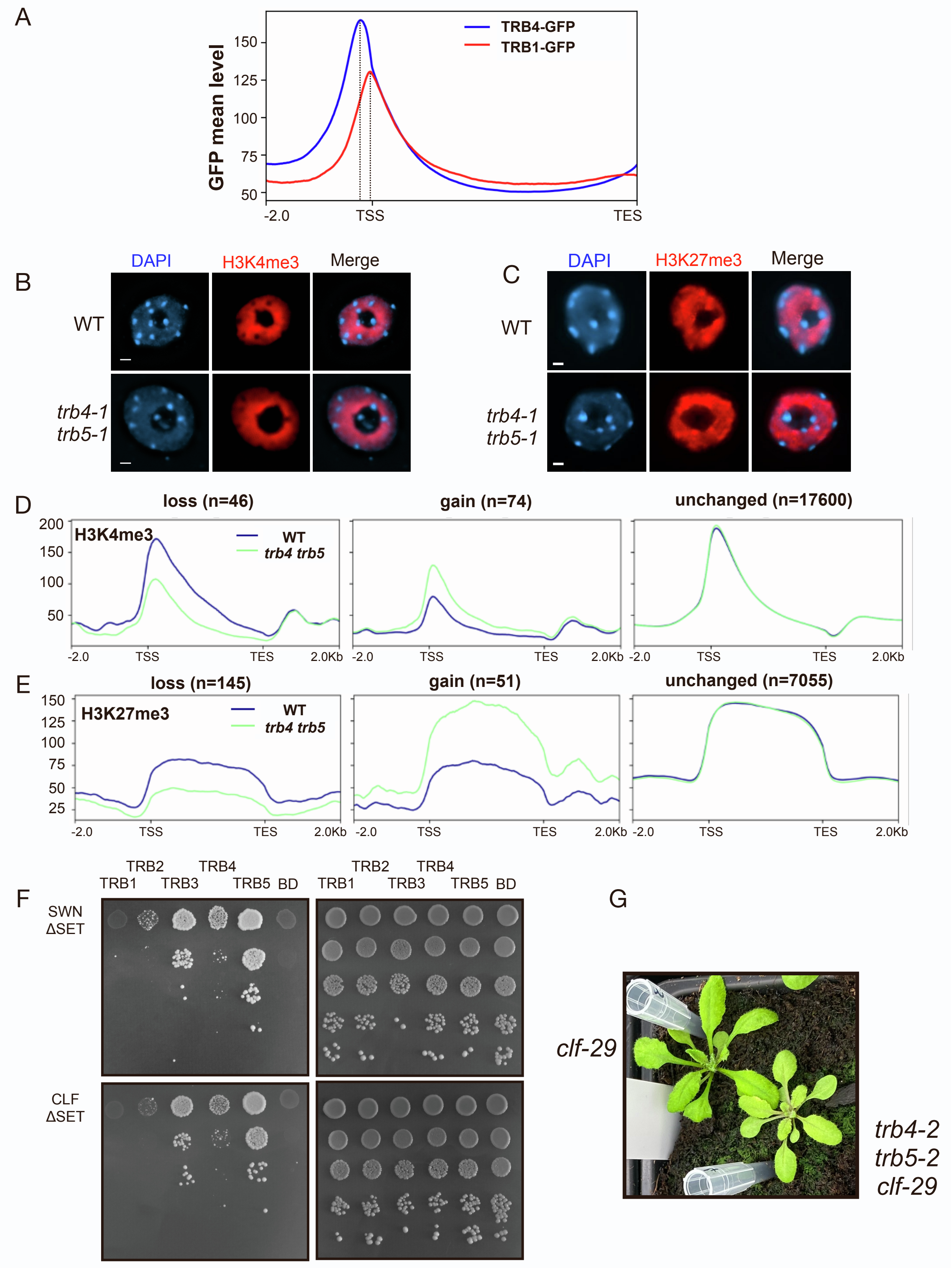
(A) Venn diagram showing overlap between the identified TRB4 targets in the two biological replicates. **(B)** The six most abundant DNA sequence motifs identified by MEME at TRB4 binding sites. Motif 1 corresponds to the telobox, motifs 2 and 3 corresponds to ‘site II motif’ (*TGGGCY*) (Gaspin et al., 2010).


A
TRB4 cluster1 (5'UTR, n=210)
Logo
e-value
1
2.0e-242
2
7.1e-054
3
3.3e-020
ribonucleoprotein complex biogenesis
ribonucleoprotein complex assembly
ribonucleoprotein complex subunit organization
cytoplasmic translation
ribosome assembly
non-membrane-bounded organelle assembly
rRNA metabolic process
maturation of SSU-rRNA
adaxial/abaxial pattern specification
spliceosomal snRNP assembly
0.05
0.10
0.15
0.20
GeneRatio
Count
10
20
30
p.adjust
0.010
0.005
TRB4 cluster2 (5'UTR, n=1646)
Logo
e-value
1
9.1e-415
2
8.5e-364
3
1.2e-033
4
5.8e-030
ribonucleoprotein complex biogenesis
ncRNA metabolic process
negative regulation of gene expression
ribonucleoprotein complex assembly
ribonucleoprotein complex subunit organization
non-membrane-bounded organelle assembly
cytoplasmic translation
protein folding
ribosome assembly
mitochondrion organization
0.04
0.06
0.08
0.10
GeneRatio
Count
50
75
100
p.adjust
0.00015
0.00010
0.00005
TRB4 cluster3 (promoter, -1000bp, n=3029)
Logo
e-value
1
5.4e-088
2
1.7e-014
3
1.8e-014
ribonucleoprotein complex biogenesis
RNA splicing
negative regulation of biosynthetic process
negative regulation of cellular biosynthetic process
negative regulation of macromolecule biosynthetic process
negative regulation of gene expression
mRNA catabolic process
maturation of SSU-rRNA
tRNA methylation
transcription by RNA polymerase I
0.01
0.02
0.03
GeneRatio
Count
25
50
75
p.adjust
0.02
0.01



Supplementary Figure 5: *Supplemental information related to Figure 5*

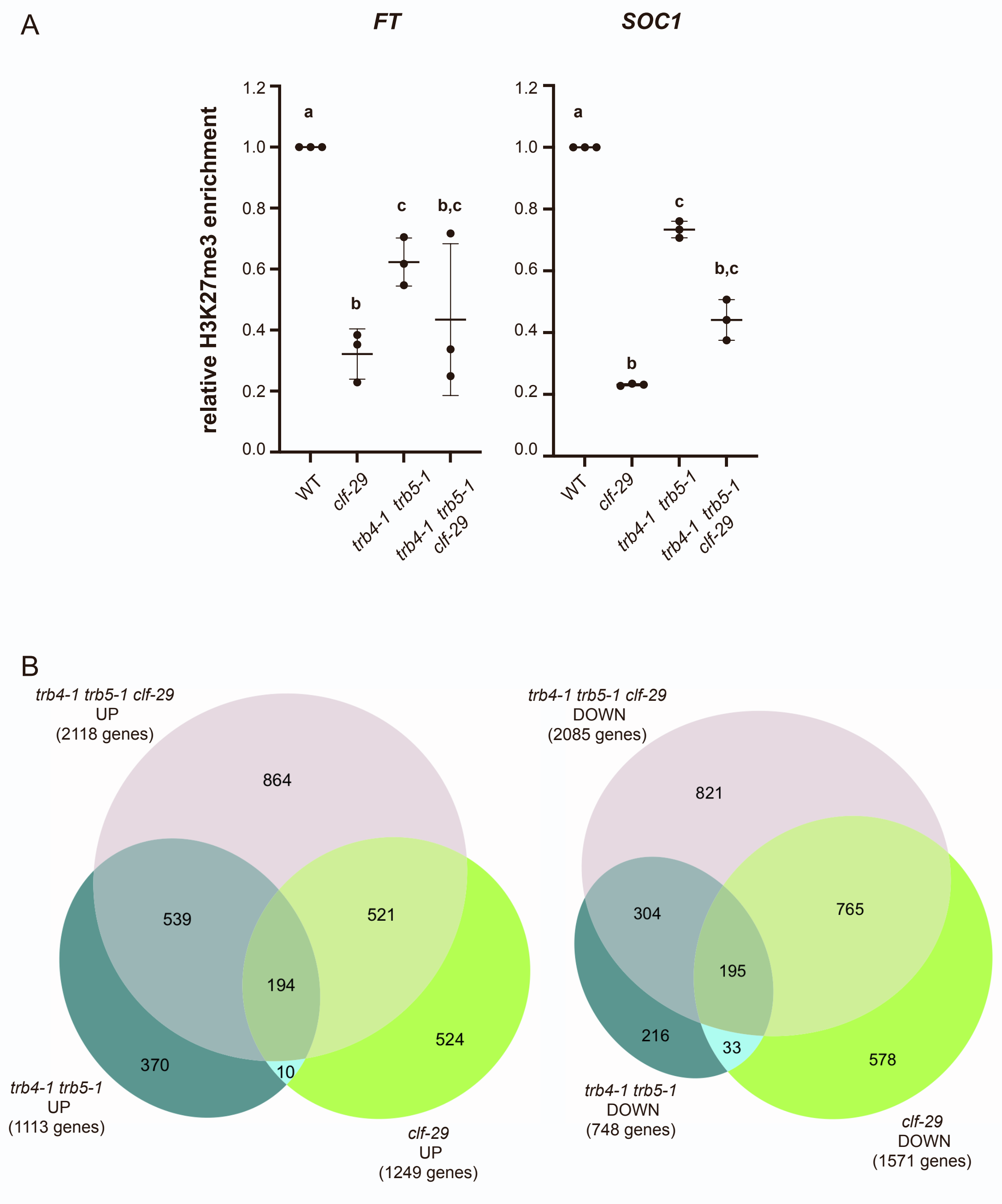
(**A, B**) Left: MEME predictions of up to 6 DNA sequence motifs within the 5'UTR, the promoters (-1000bp from the TTS) of the TRB4 (**A**) or TRB1 (**B**) target genes in the three clusters defined in **Figure 5A-B**. Right: GO-term enrichment of genes corresponding to the three cluster of TRB4 (**A**) and TRB1 (**B**) target genes defined in **Figure 5A-B**.



***Supplementary Figure 6:** Supplemental information related to Figure 6*

(A) Metagene plot showing ChIP-seq signals of TRB4-GFP or TRB1-GFP over genes enriched in H3K4me3. TRB4 peaks upstream of TRB1. (**B**, **C**) Representative mesophyll leaf nuclei from WT and *trb4-1 trb5-1* mutant plants, immunostained for H3K4me3 **(B)** and H3K27me3

(C). Maximum projections are shown. Scale presents 1 µm. **(D, E)** Metagene plot presentations of H3K4me3 **(D)** and H3K27me3 **(E)** enrichment along genes and 2 kb up and downstream of TSS and TTS with loss, gain or unchanged levels of the histone modifications in *trb4-1 trb5-1* mutants. The number of genes presented in each graph is indicated on the top. (**F**) Y2H serial dilutions examining the interaction of Arabidopsis TRB1 to 5 proteins (as bait) with CURLY LEAF (CLF) and SWINGER (SWN) proteins (lacking the SET domain) (as prey). Left panel: yeast strains growing on synthetic medium lacking Leu, Trp, His and Ade reveal interactions. Right panel: growth of zygotes on synthetic medium lacking Leu and Trp, selecting for the presence of the bait and prey vectors for interactions. Note that the first lines of each panel correspond to the Figure 6C. (G) Representative *clf-29* and *trb4-2 trb5-2 clf-29* triple mutant plants at 3 weeks after sowing.



Supplementary Figure 7: *Supplemental information related to Figure 7*

(**A**) Relative enrichment of H3K27me3 normalized to H3 at *FT* and *SOC1* gene promoters in WT, *clf-29*, *trb4-1 trb5-1* and *trb4-1 trb5-1 clf-29* mutants. (**B**) Venn Diagrams showing overlap between up- and down regulated genes in *clf-29*, *trb4-1 trb5-1* and *trb4-1 trb5-1 clf-29* mutants determined by RNA-seq from rosette leaves of 3-week old plants.