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Supplemental information

The transcription factor HSFA7b controls thermomemory at the shoot apical meristem by regulating ethylene biosynthesis and signaling in *Arabidopsis*

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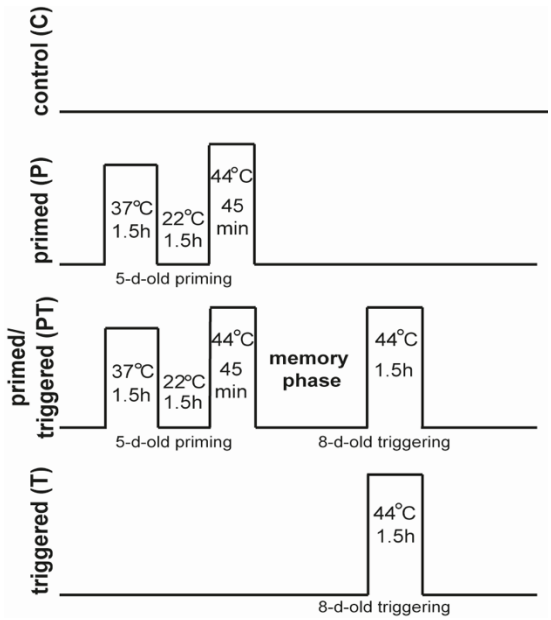
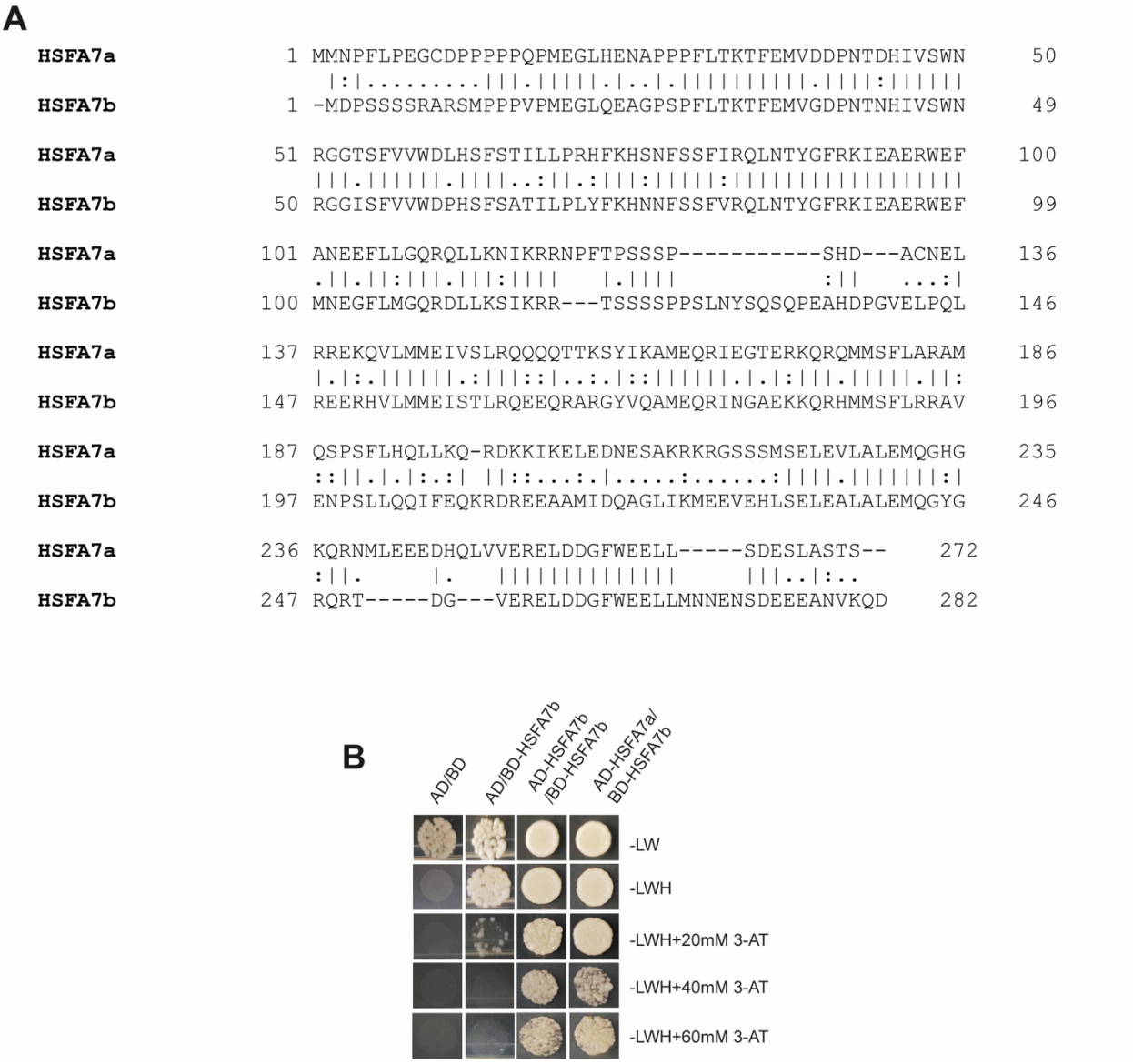
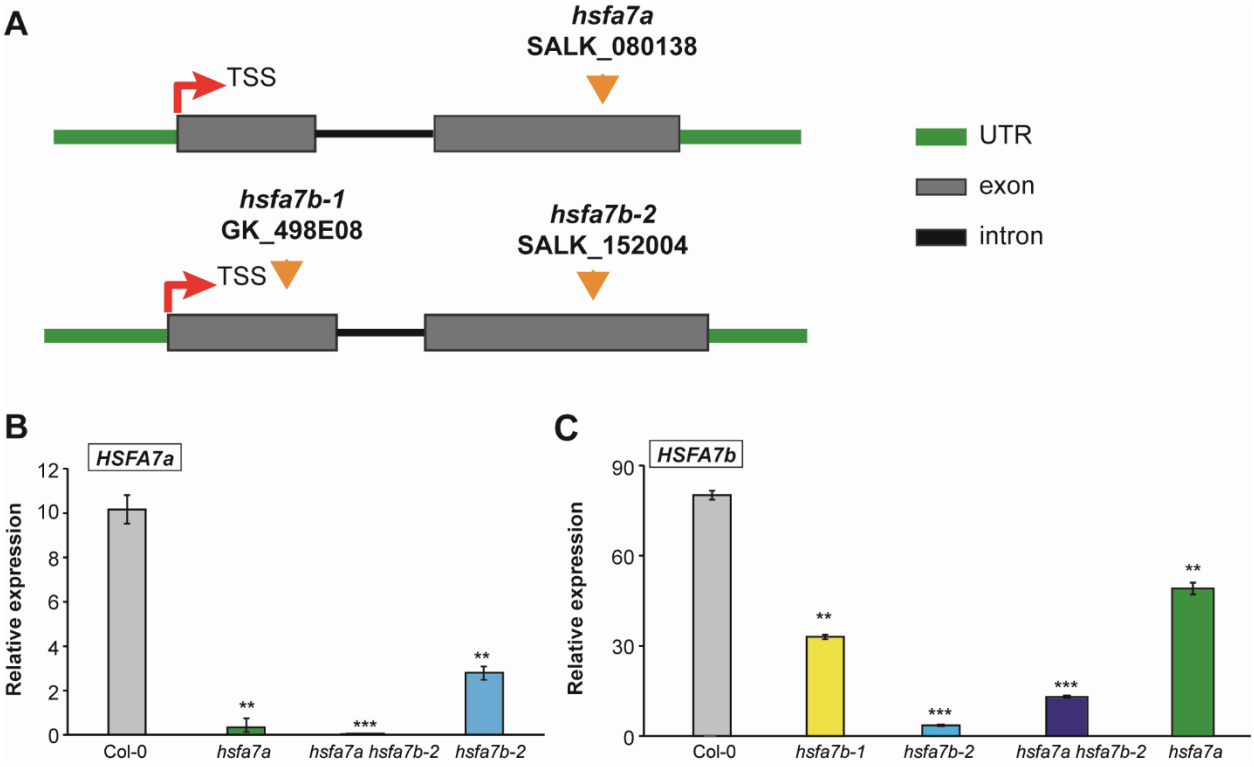


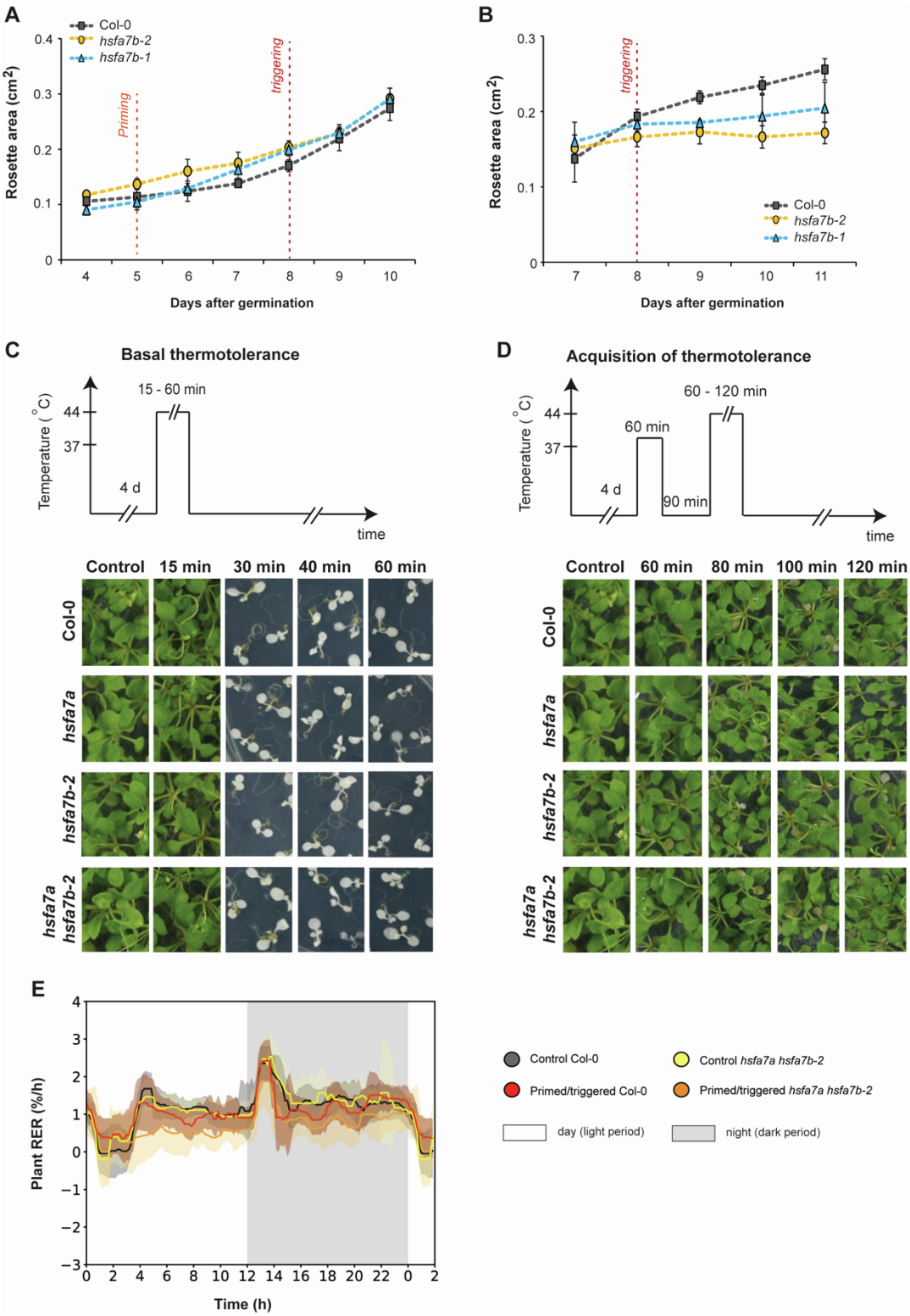
Supplemental Figure 1. Schematic representation of the thermomemory assay. Five-day-old seedlings grown in MS medium with 1% sucrose were used for the experiments. Seedlings that were not subjected to any HS treatment were treated as control (C). Primed (P) seedlings were subjected to a moderate HS treatment called priming (37 °C for 1.5 h followed by 22 °C for 1.5 h, and 44 °C for 45 min). The primed and triggered (PT) seedlings were subjected to priming followed by a 3-day memory/recovery phase, and then subjected to a second triggering HS. The triggered seedlings (T) were directly subjected to triggering on day 8, without prior priming treatment. Time is given in hours (h).



Supplemental Figure 2. HSFA7a and HSFA7b proteins may interact with each other. (A) HSFA7a and HSFA7b protein sequence alignment done using EMBOSS Needle (www.ebi.ac.uk/Tools/psa/emboss_needle). **(B)** Yeast-2-hybrid assay depicts the protein-protein interaction of functional HSFA7b with HSFA7b (homodimer formation) and functional HSFA7b with HSFA7a (heterodimer formation). Abbreviations: –LWH = SD-leucine, tryptophan and histidine. 3-AT = 3-amino-1,2,4-triazole. BD = GAL4 binding domain, AD = GAL4 activation domain. Empty bait (AD) and prey (BD) vectors were used in the negative control. Cell growth on SD medium lacking Trp and Leu (SD-LW) was used as mating control and protein-protein interaction was examined on SD medium lacking Trp, Leu, and His (SD-LWH) with increasing concentrations of 3-AT.

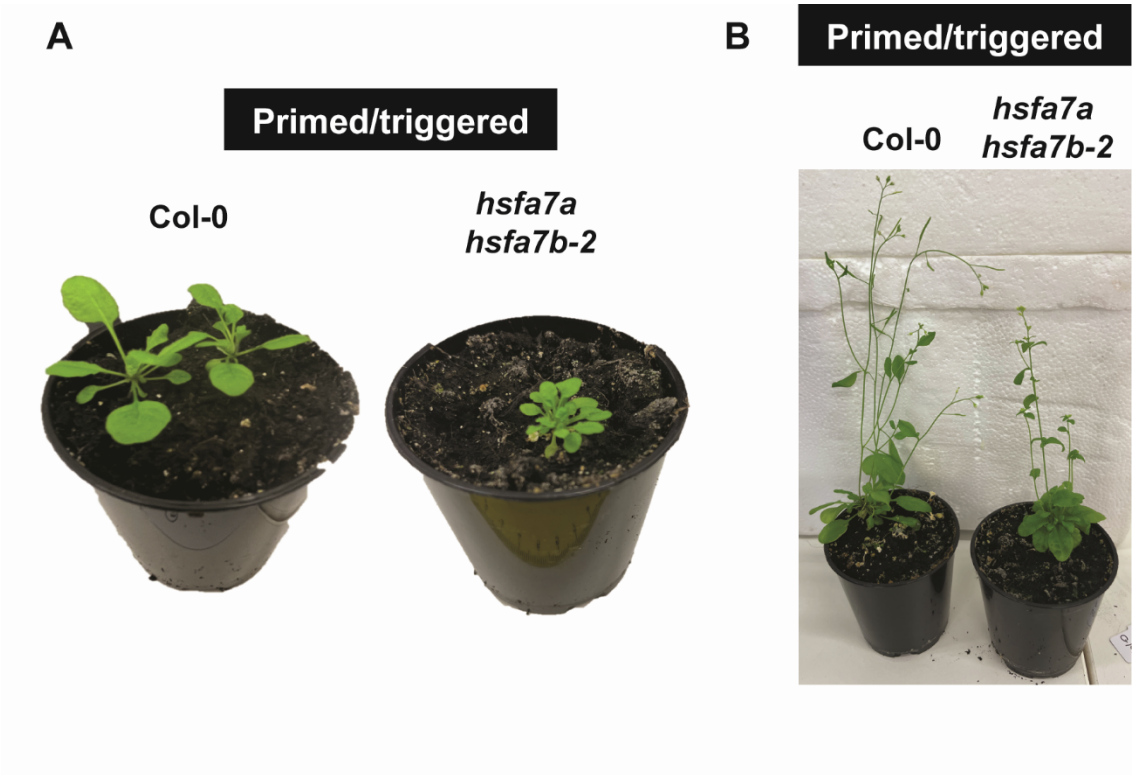


Supplemental Figure 3. Characterization of *hsfa7a* and *hsfa7b* mutants. (A) Schematic representation of the *hsfa7a, hsfa7b-1,* and *hsfa7b-2* mutants depicting the positions of T-DNA insertions. The green boxes represent the 5' and 3' UTRs, the black lines represent introns and the grey boxes represent exons. The red arrow represents the transcription start site (TSS). **(B)** The expression level of *HSFA7a* and *HSFA7b* measured in 5-day-old Col-0, *hsfa7a*, *hsfa7b-1, hsfa7b-2, hsfa7a hsfa7b-2,* and *hsfa7a* mutant plants at 2 h after priming. Error bars indicate s.d. ($n = 3$). Asterisks indicate statistically significant differences (Student *t*-test: $^{**}P \leq 0.01$; $^{***}P \leq 0.001$) compared to Col-0 under the same conditions.

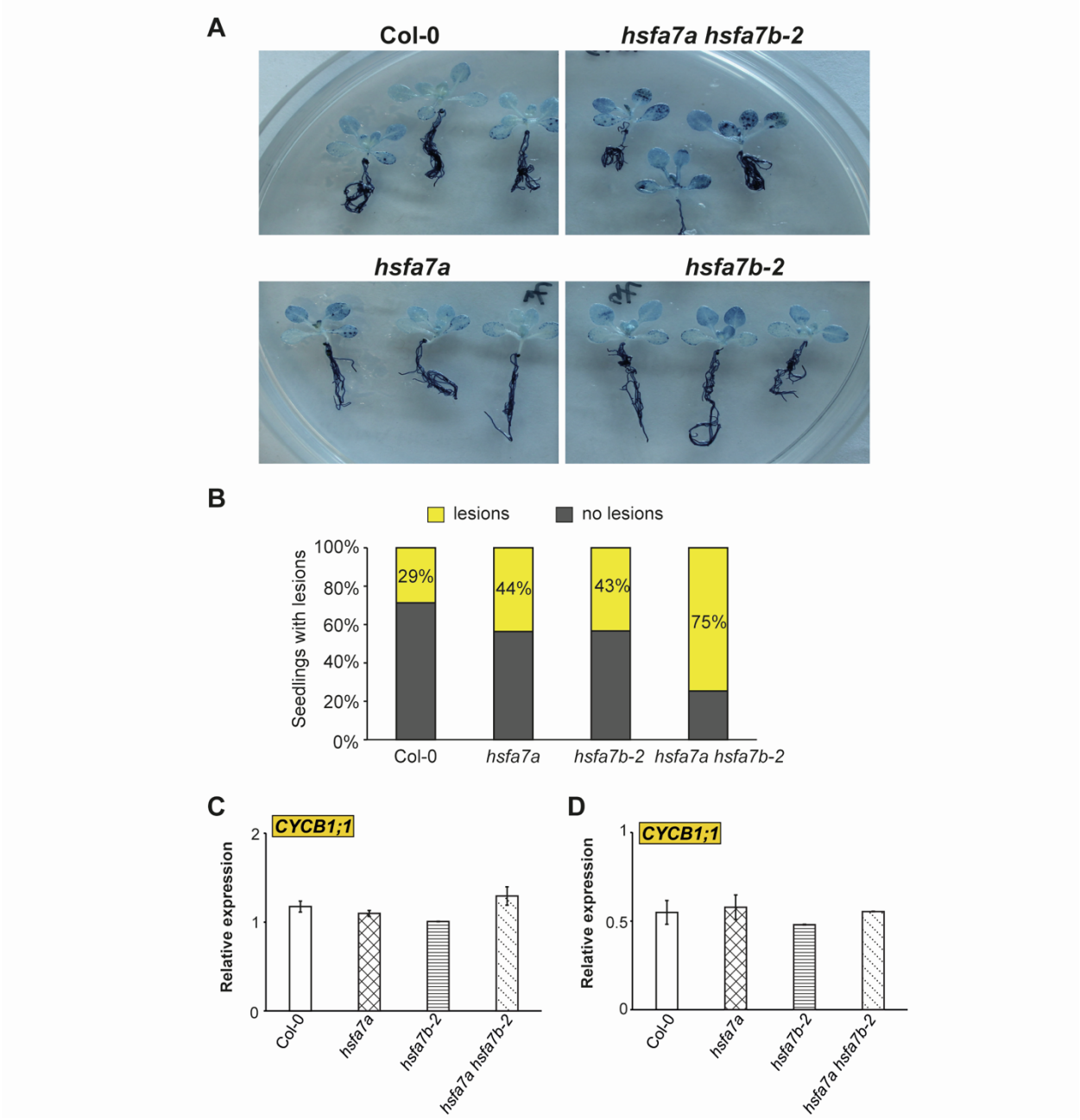


Supplemental Figure 4. Analyses of rosette area, basal thermotolerance, acquisition of thermotolerance, and diurnal relative expansion rate (RER). (A, B) Rosette area of (A) control and (B) primed/triggered (PT) Col-0, *hsfa7b-1*, and *hsfa7b-2* mutant plants analyzed during thermopriming. **(C)** Basal thermotolerance and **(D)** acquisition of thermotolerance of Col-0,

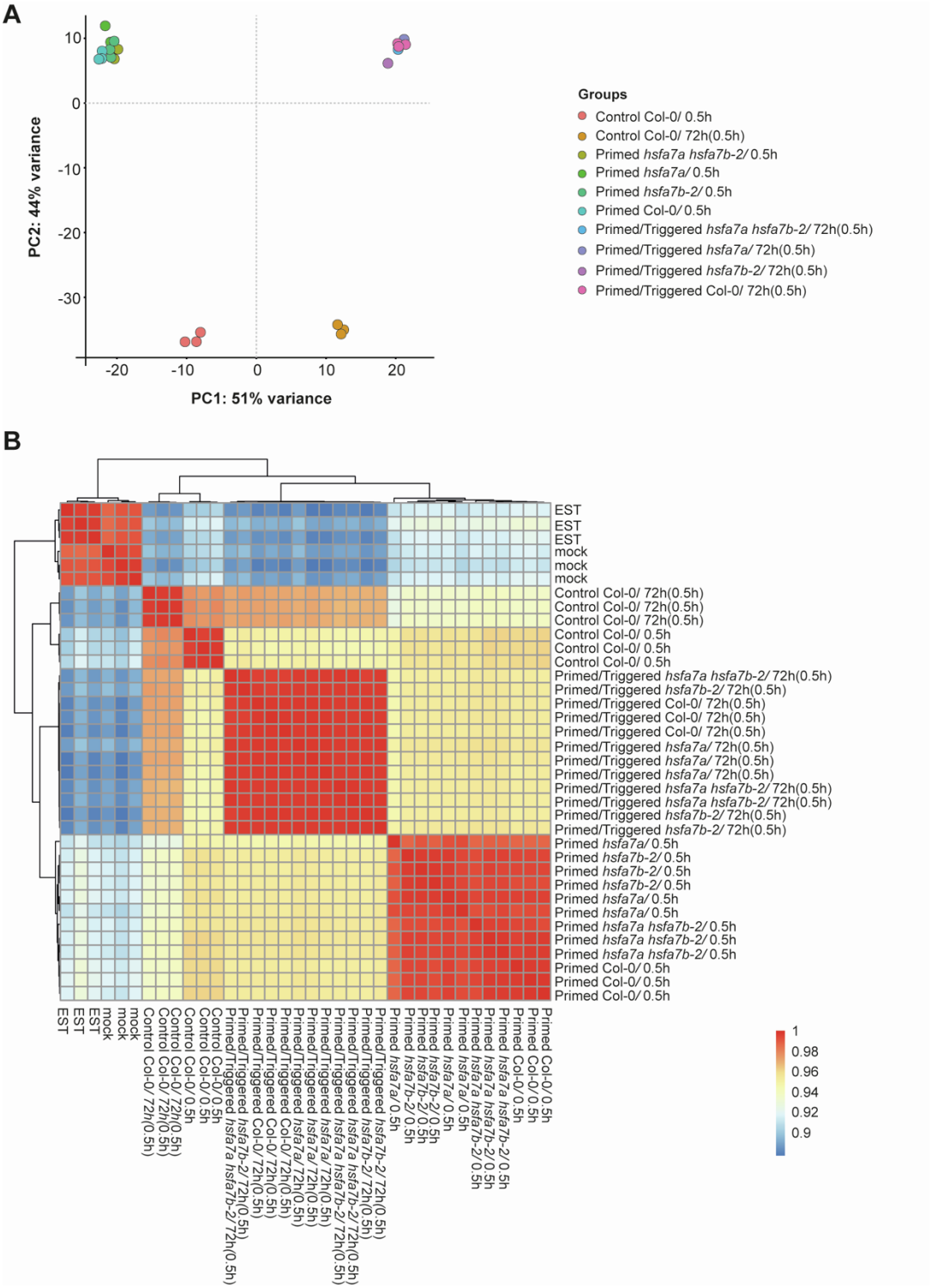
hsfa7a, hsfa7b-2, and *hsfa7a hsfa7b-2* mutant plants. **(E)** Time-resolved RER averaged over seven sequential 24 h periods of control and PT Col-0 wild-type and *hsfa7a hsfa7b-2* mutant plants measured using an 3D imaging system.



Supplemental Figure 5. The phenotype of primed/triggered Col-0 wild-type and *hsfa7a hsfa7b-2* mutant plants grown in long day (16 h light /8 h darkness) photoperiod. Images were taken at (**A**) 21 days after germination (bolting time for Col-0) and (**B**) 55 days after germination.

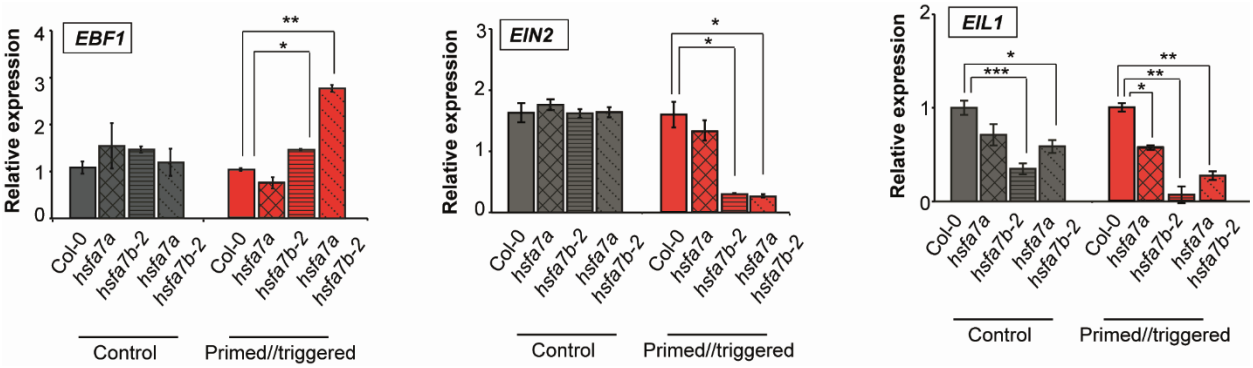


Supplemental Figure 6. Analyses of cell death and cell cycle activity. (A) Trypan Blue staining for cell death in primed/triggered (PT) Col-0, *hsfa7a, hsfa7b-2,* and *hsfa7a hsfa7b-2* mutants at 24 h after triggering treatment. **(B)** Percentage of PT Col-0, *hsfa7a, hsfa7b-2,* and *hsfa7a hsfa7b-2* seedlings with lesions. **(C, D)** Expression level of *CYCLIN B1;1* (*CYCB1;1*) in (C) control and (D) primed Col-0, *hsfa7a, hsfa7b-2,* and *hsfa7a hsfa7b-2* seedlings at 0.5 h after triggering stress. Error bars indicate s.d. (n = 3).

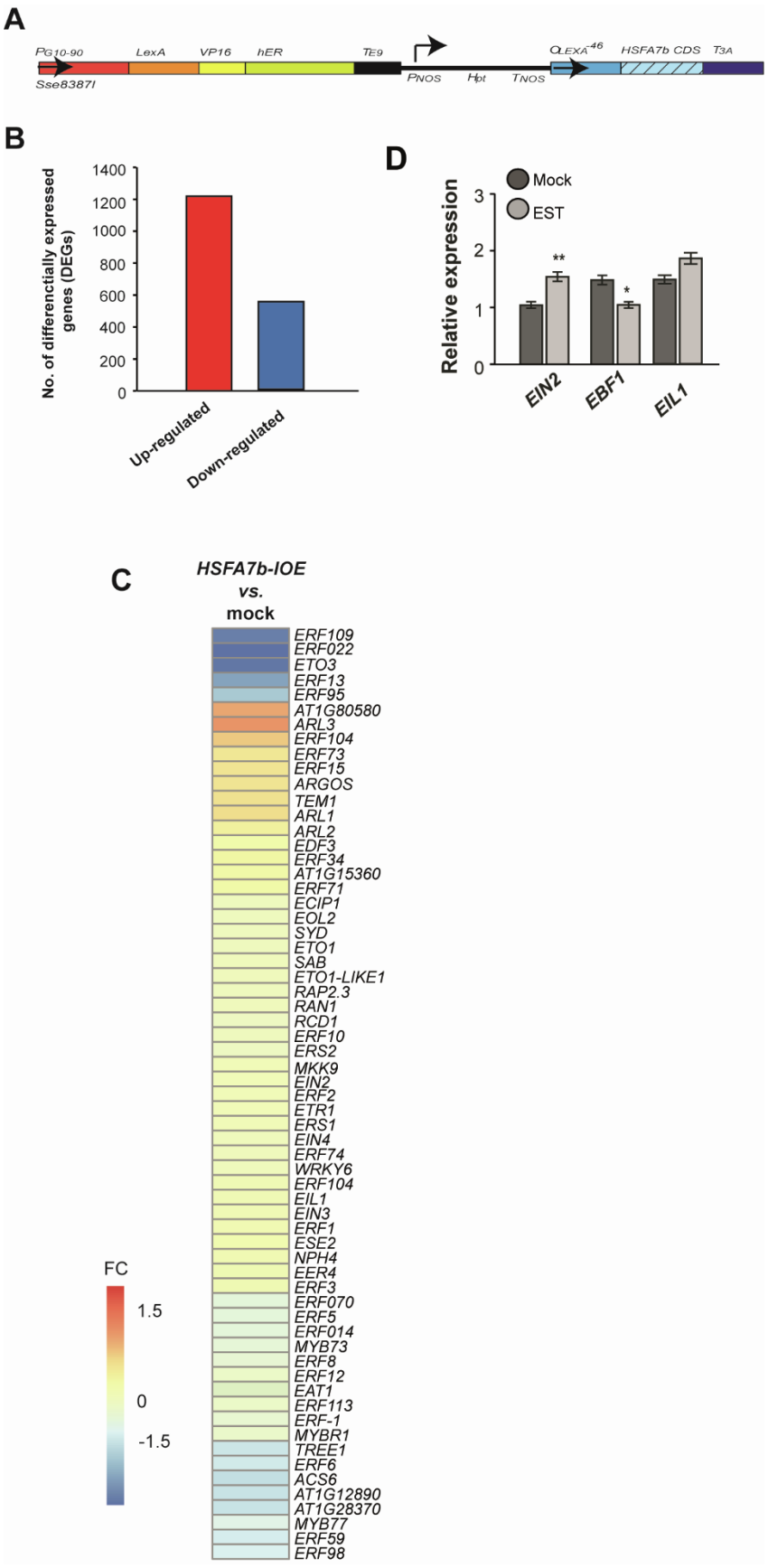


Supplemental Figure 7. Principal component analysis (PCA) and clustering heat map of gene expression analyzed in all samples. (A) PCA describing the relationship between meristem samples of control Col-0, primed and primed/triggered Col-0, *hsfa7a, hsfa7b-2* single, and *hsfa7a hsfa7b-2* double mutants. **(B)** Heat map of the correlation matrix of all samples using pairwise Person correlation. Note that the clustering heat map revealed a weak outlier sample (Primed

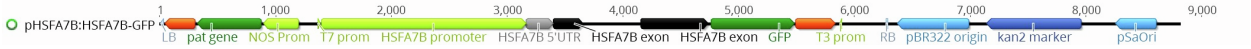
hsfa7a/ 0.5 h) which was removed for further analysis. Heat map and PCA plots were generated with normalized expression values generated by applying variance stabilizing transformation (VST) using DESeq2.



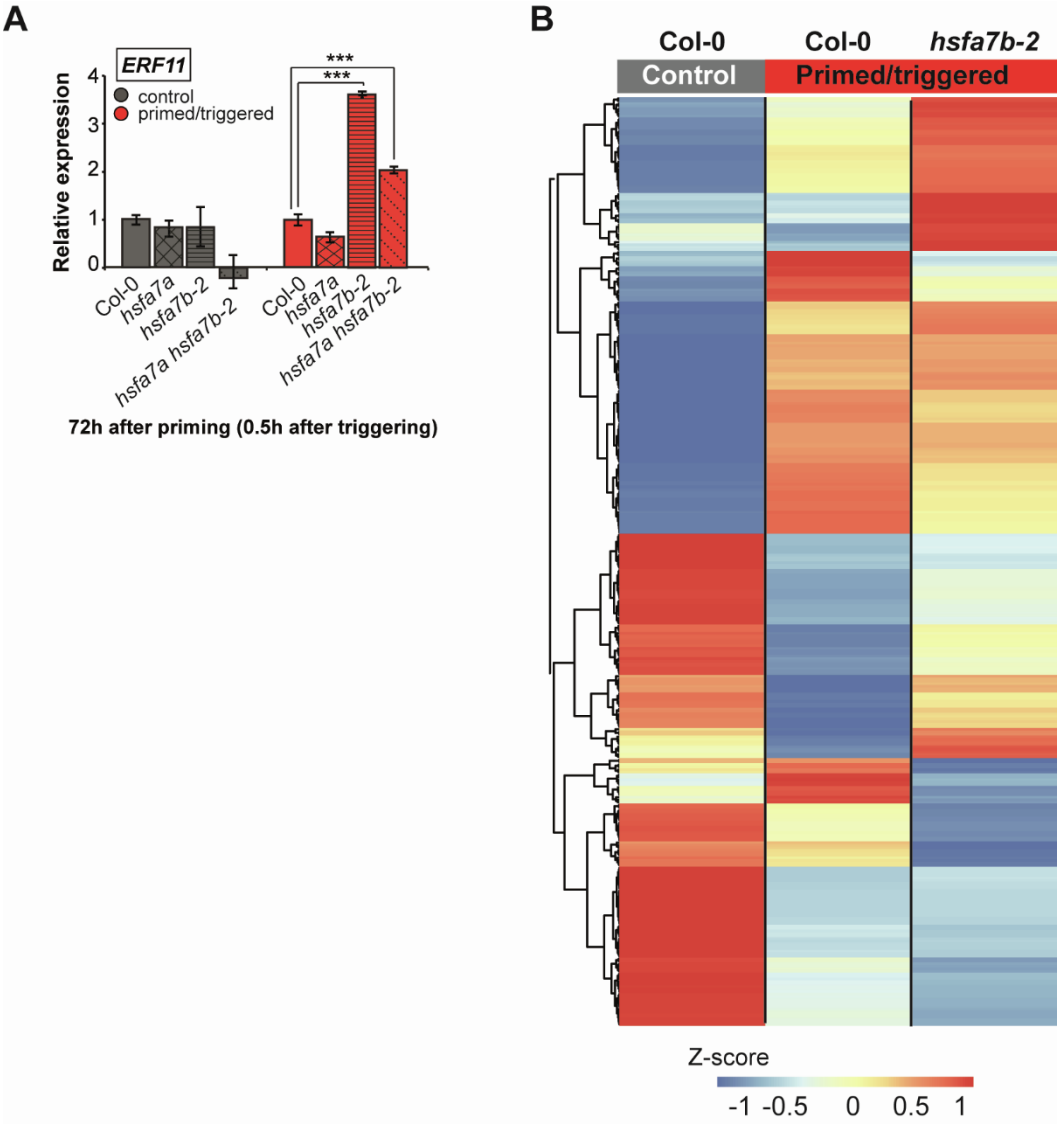
Supplemental Figure 8. Expression of ethylene response gene at the shoot apical meristem (SAM) during thermopriming. (A-C) The expression level of *EIN3-BINDING F BOX PROTEIN 1* (*EBF1*), *ETHYLENE INSENSITIVE 2* (*EIN2*), and *ETHYLENE-INSENSITIVE3-LIKE 1* (*EIL1*) analyzed at the SAM of control and primed/triggered Col-0, *hsfa7a, hsfa7b-2*, and *hsfa7a hsfa7b-2* plants at 0.5 h after triggering (72 h after priming). Error bars represent s.d. (n = 3). Asterisks indicate statistically significant difference (Student´s t-test, *$P \leq 0.05$, **$P \leq 0.01$, and ***$P \leq 0.001$) compared to Col-0 under the same condition.


A
PG10-90
LexA
VP16
hER
TE9
Sse8387I
PNOS
Hpt
TNOS
QLEXA-46
HSFA7b CDS
T3A
B
No. of differenctially expressed genes (DEGs)
1400
1200
1000
800
600
400
200
0
Up-regulated
Down-regulated
D
Relative expression
Mock
EST
3
2
1
0
**
*
EIN2
EBF1
EIL1
C
HSFA7b-IOE
vs.
mock
ERF109
ERF022
ETO3
ERF13
ERF95
AT1G80580
ARL3
ERF104
ERF73
ERF15
ARGOS
TEM1
ARL1
ARL2
EDF3
ERF34
AT1G15360
ERF71
ECIP1
EOL2
SYD
ETO1
SAB
ETO1-LIKE1
RAP2.3
RAN1
RCD1
ERF10
ERS2
MKK9
EIN2
ERF2
ETR1
ERS1
EIN4
ERF74
WRKY6
ERF104
EIL1
EIN3
ERF1
ESE2
NPH4
EER4
ERF3
ERF070
ERF5
ERF014
MYB73
ERF8
ERF12
EAT1
ERF113
ERF-1
MYBR1
TREE1
ERF6
ACS6
AT1G12890
AT1G28370
MYB77
ERF59
ERF98
FC
1.5
0
-1.5

Supplemental Figure 9. *HSFA7b-IOE* transgenic line. (A) *HSFA7b-IOE* construct was generated by inserting the *HSFA7b* coding sequence into the XVE vector (Zuo *et al.,* 2000). Black arrows indicate the direction of the transcription. Abbreviations: $P_{G10\text{-}90,}$ a synthetic promoter controlling *XVE* expression; *LexA,* the transcriptional activation domain of *V16; hER,* human estrogen receptor; *TE9, rbcS E9* poly(A) addition sequence; *Pnos*, nopaline synthase promoter; *Hpt*, hygromycin phosphotransferase II coding sequence; *Tnos*, nopaline synthase poly(A) addition sequence; *OLexA*, eight copies of the *LexA* operator sequence; *HSFA7b CSD*, *HSFA7b* coding sequence; $T_{3A,}$ *rbcs*S*3A* poly(A) addition sequence. **(B)** Number of differentially expressed up- (red) and down-regulated genes in *HSFA7b-IOE*. Note that samples were harvested 16 h after β-estradiol induction. **(C)** Heat map showing the log2 fold change (log2 FC) of the expression of ethylene-related up-regulated (red) or down-regulated (blue) genes in *HSFA7b-IOE* compared to mock-treated plants at 16 h after estradiol induction. **(D)** The expression level of *EIN3-BINDING F BOX PROTEIN 1* (*EBF1*)*, ETHYLENE INSENSITIVE 2* (*EIN2*)*,* and *ETHYLENE-INSENSITIVE3-LIKE 1* (*EIL1*) in β-estradiol (EST)- and mock-treated *HSFA7b-IOE* plants. Error bars represent s.d. (n = 3). Asterisks indicate statistically significant difference (Student´s t-test, $*P \leq 0.05$ and $**P \leq 0.01$) compared to mock treatment (D).



Supplemental Figure 10. *pHSF7b:HSFA7b:GFP* construct. *pHSF7b:HSFA7b:GFP* was generated by cloning the *HSFA7b* promoter (2 kb) and coding sequence (without stop codon) fused to GFP using the pGREENII 0229 vector (Hellens *et al.,* 2000).



Supplemental Figure 11. Expression of ethylene-related genes is affected in the absence of functional HSFA7a/b proteins. (A) Expression of *ETHYLENE RESPONSIVE FACTOR 11* (*ERF11*) at the shoot apical meristem of Col-0 and *hsfa7a/b* mutant plants at 0.5 h after triggering (72 h after priming). Error bars represent s.d. (n = 3). Asterisks indicate a statistically significant difference (Student´s t-test, ***$P \leq 0.001$) compared to Col-0 under the same condition. **(B)** Heat map depicting relative expression (Z-score normalized) of ethylene-related genes that have been reported as direct targets of EIN3 in the Col-0 control and primed/triggered Col-0, and *hsfa7b-2* shoot apices.

Supplemental Table 1. Number of significantly changed genes with $\log_2$ FC > |1.5|.

	0.5 h after priming	**0.5 h after triggering (72 h after priming)**
DEGs: significant		
Col-0 (P) *vs.* Col-0 (C)	12343 (6347↓; 5996↑)	NA
hsfa7a (P) *vs.* Col-0 (P)	1406 (717↓; 689↑)	NA
hsfa7b-2 (P) *vs.* Col-0 (P)	3206 (1688↓;1518↑)	NA
hsfa7a hsfa7b-2 (P) *vs.* Col-0 (P)	810 (529↓; 281↑)	NA
Col-0 (PT) *vs.* Col-0 (C)	-	9845 (5030↓; 4815↑)
hsfa7a (PT) *vs.* Col-0 (PT)	-	274 (161↓;113↑)
hsfa7b-2 (PT) *vs.* Col-0 (PT)	-	153 (67↓; 86↑)
hsfa7a hsfa7b-2 (PT) *vs.* Col-0 (PT)	-	1062 (518↓; 544↑)
DEGs: significant and $\log_2$FC > |1.5|		
Col-0 (P) *vs.* Col-0 (C)	8001 (3758↓; 4243↑)	NA
hsfa7a (P) *vs.* Col-0 (P)	668 (284↓; 384↑)	NA
hsfa7b-2 (P) *vs.* Col-0 (P)	1042 (481↓; 561↑)	NA
hsfa7a hsfa7b-2 (P) *vs.* Col-0 (P)	204 (162↓; 42↑)	NA
Col-0 (PT) *vs.* Col-0 (C)	-	4366 (1576 ↓; 2790↑)
hsfa7a (PT) *vs.* Col-0 (PT)	-	63 (26↓; 37↑)
hsfa7b-2 (PT) *vs.* Col-0 (PT)	-	53 (21↓; 32↑)
hsfa7a hsfa7b-2 (PT) *vs.* Col-0 (PT)	-	252 (26↓; 226↑)

Abbreviations: C, control; P, primed; PT, primed and triggered; T, triggered; NA, not applicable. Downward directed arrows (↓) indicate downregulated genes; upward directed arrows (↑) indicate upregulated genes.

Supplemental Table 2. Oligonucleotides used in this study.

Gene (AGI)	Oligonucleotide	Sequence (5'→3')
Oligonucleotides used for cloning		
ERF1A	ERF1A_F	ATGTCGATGACGGCGGATTC
AT4G17500	ERF1A_R	TTATAAAACCAATAAACGATCGCC
HSFA7a	HSFA7a_F	ATGATGAACCCGTTTCTCCC
AT3G51910	HSFA7a_R	TTAGGAGGTGGAAGCCAAACTC
HSFA7b	HSFA7b_F	ATGGACCCGTCGTCAAGCTCC
AT3G63350	HSFA7b_R	CTAATCTTGCTTCACATTCGC
HSP17.8	HSP17.8_F	ATGTCGCTTATTCCAAGCTTC
AT1G07400	HSP17.8_R	TTAGCCAGAGATATCAATAGAC
Oligonucleotides used for qRT-PCR		
ACS6	ACS6_qRT_F	GCTGCTTCTGCAATCTACGC
AT4G11280	ACS6_qRT_R	ATACGCCAACAGCTTTGCAC
ACS7	ACS7_qRT_F	GAAAGGGAACGCAGGGCTAT
AT4G26200	ACS7_qRT_R	CCTAAACCATCCGACCTCCG
ACS11	ACS11_qRT_F	CCTGAGTTCACCAGCGTTCT
AT4G08040	ACS11_qRT_R	CGGCTGACACCACTTTCTCA
CYCB1;1	CYCB1;1_qRT_F	GCTGCTTCTGCAATCTACGC
AT4G37490	CYCB1;1_qRT_R	ATACGCCAACAGCTTTGCAC
HSFA7a	HSFA7a_qRT_F	ACCACCACCACAACCAATGGAG
AT3G51910	HSFA7a_qRT_R	TCTTGGTCAGAAATGGAGGTGGAG
HSFA7b	HSFA7b_qRT_F	ATGGAGGGATTGCAGGAAGCAG
AT3G63350	HSFA7b_qRT_R	TGGATCACCAACCATCTCGAACG
HSFA2	HSFA2_qRT_F	GCAGCGTTGGATGTGAAAGTGG
AT2G26150	HSFA2_qRT_R	TTGGCTGTCCCAATCCAAAGGC
EBF1	EBF1_qRT_F	CCCTCCAAGCAAGAGATCAC
AT2G25490	EBF1_qRT_R	AACACCCTTCACAATCATCAC
EBF2	EBF2_qRT_F	CCCGATGATTGAAAAACTTGAC
AT5G25350	EBF2_qRT_R	AACCCTCATTCCCAACACC
ERF1A	ERF1A_qRT_F	TTGCGGCGGAGATTAGAGAC
AT4G17500	ERF1A_qRT_R	ATTCAACAAAGCGCGGGAAC
ERF104	ERF104_qRT_F	AGAGAGGCACTACAGGGGAG
AT5G61600	ERF104_qRT_R	GTGTCGTAAGTCCCAAGCCA
EIN2	EIN2_qRT_F	AATGACACCGTGCTTTTGCC
AT5G03280	EIN2_qRT_R	TGACTGCGGTTGTGCATTTG
EIN3	EIN3_qRT_F	TGTCTGGTGGAAGTTGCTCG
AT3G20770	EIN3_qRT_R	ATTCCGAGTTTCCTGCTGGG
EIL1	EIL1_qRT_F	AAGCAACCAAACGCCTCCTA

AT2G27050	EIL1_qRT_R	TTAACCCCGTTGTTCGTCCC
EOL1	EOL1_qRT_F	GCTACTACTGCTTCTTCCCC
AT3G42660	EOL1_qRT_R	CAACAACGCTGAAATCTCTAA C
ETO1	ETO1_qRT_F	TGGCAACACAACTTGACCCT
AT3G51770	ETO1_qRT_R	ATATCGCCCTCGAAAGCTCG
TUB2	TUB_qRT_F	GAGCCTTACAACGCTACTCTGTCTGTC
AT5G62690	TUB_qRT_R	ACACCAGACATAGTAGCAGAAATCAAG
DREB2d	DREB2d _qRT_F	GAGCCTTACAACGCTACTCTGTCTGTC
AT1G75490	DREB2d _qRT_R	ACACCAGACATAGTAGCAGAAATCAAG

Oligonucleotides used for ChIP-qPCR

EIN3	EIN3_F1	TCCATTCAAAGGGACAGGGA
AT3G20770	EIN3_R1	AGACTGATGGAAATAAAGGCGGA
	EIN3_F2	CTAGCTGAGCATGTAGAACAGGT
	EIN3_R2	GTAGTCCACCTGAAACCACCA
	EIN3_NF	CACGCAATGACGCAAATCCT
	EIN3_NR	CACTCGACCTCGTGAACACA
EOL1	EOL1_F1	TTGCTAAGAGCTAGTTCCCCA
AT3G42660	EOL1_R1	CACTTTGGCTCTGGCTTTTGT
	EOL1_F2	GAGAAGCGGTGATGCCAAGA
	EOL1_R2	GAA CACTTTCCCTTTGGACACA
	EOL1_F3	AGGTTAGGGTTTGGTCGAGA
	EOL1_R3	ACCACTTATGTACAGCTGACGG
	EOL1_NF	AACATGGGCTTAGATGGGCTT
	EOL1_NR	AGGGAAGAAACTAGATCATTTGAGG
ETO1	ETO1_F1	CTCAGCTCGCTTCACTTGAG
AT3G51770	ETO1_R1	GTAGACGTGTGCAGCCGAG
	ETO1_F2	TCTCACCCACATGACCATACG
	ETO1_R2	TAAGTCTAATCACTGCTGAGTGG
	ETO1_NF	TTCCCTCCTGGTATGGCTTC
	ETO1_NR	TGGCTATGCTTGCTTTTTCCC

Oligonucleotides used for genotyping

hsfa7a	hsfa7a_LP	GTTCCAGAAGCAAGTTTCGTG
(SALK_080138)	hsfa7a_RP	TTGCTCACTCATGTGGACTTG
	LBb1.3	ATTTTGCCGATTTCGGAAC
hsfa7b-1	hsfa7b_LP	AAACTCCCATCTCTCTGCCTC
(GABI_498E08)	hsfa7b_RP	CCACCAGCAAAAGCAGAGTAC
	LB3	TAGCATCTGAATTTCATAACCAATCTCGATACAC
hsfa7b-2	hsfa7b_LP	TTCTTCGCAAGTTCTGGAAAC
(SALK_152004)	hsfa7b_RP	TCCCATTTTATAAGATTTTCAAGC
	LBb1.3	ATTTTGCCGATTTCGGAAC
erf1a	erf1a_LP	CGTTCCTAACCAAACCCTAGC
(SALK_036267)		

	erf1a_RP	TCCTACTCTTCTCCCTGCTCC
	LBb1.3	ATTTTGCCGATTTCGGAAC
erf11 (SALK_116053)	erf11_LP	CCACACGTCGTCCTTCATATC
	erf11_RP	TGCAAAGCCTAAAATTAAAAACG
	LBb1.3	ATTTTGCCGATTTCGGAAC