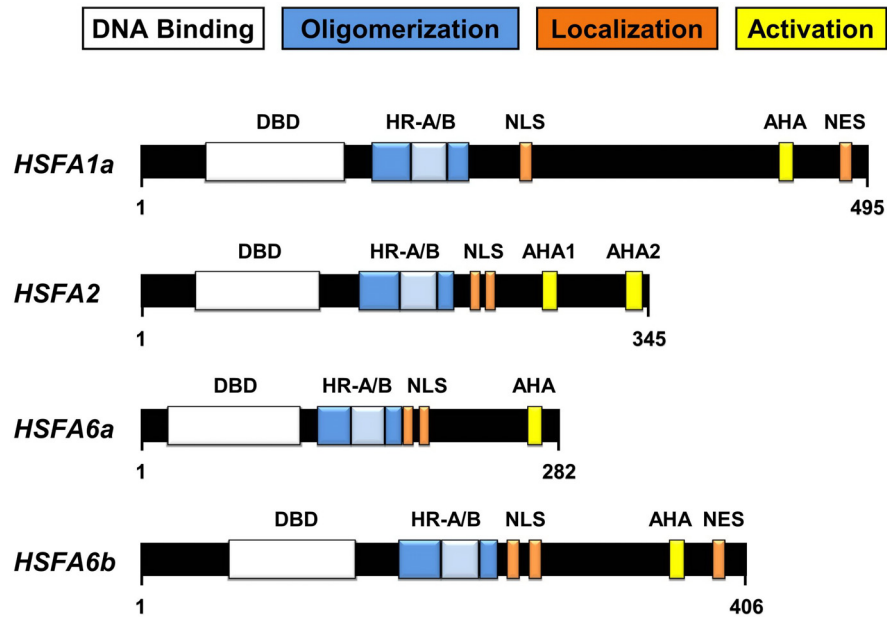
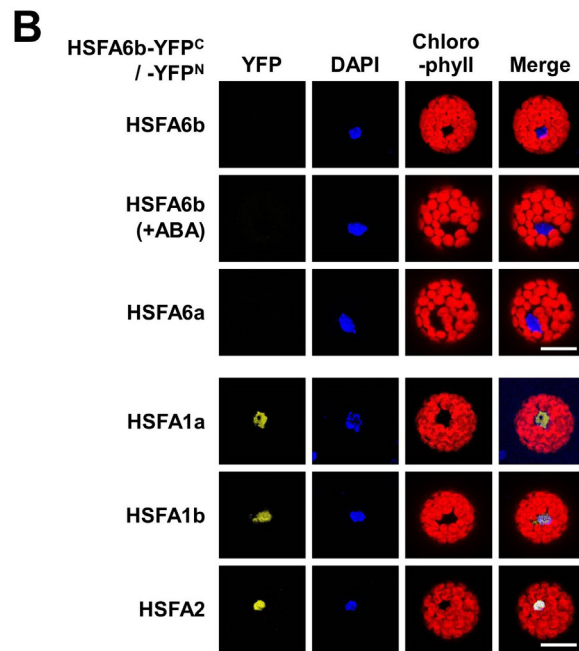
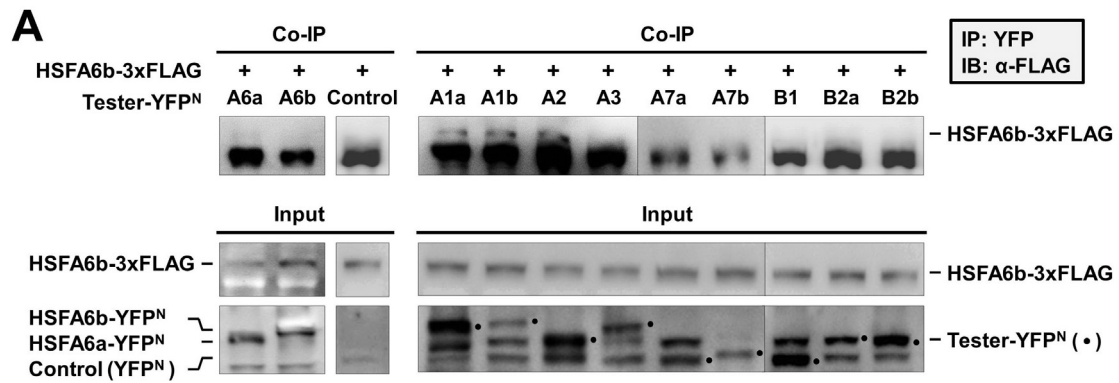


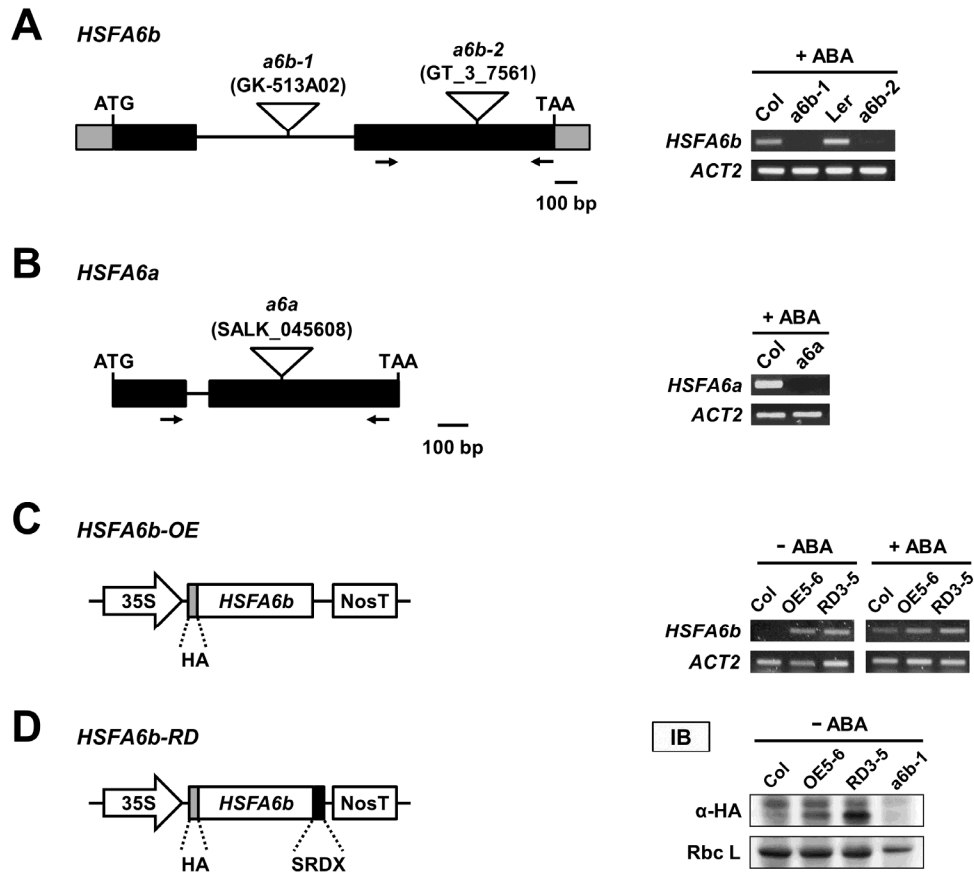
**Supplemental Figure S1. The expression profiles of Arabidopsis 21 heat shock factor (HSF) genes under abiotic stresses and during development. (A and B)** Normalized and averaged signals were analyzed as “heat maps” with GeneSpring 7.3 (Silicon Genetics). The color code of signal intensities corresponds to the abundance of transcripts, from low (blue) to high (red) expression. Root samples: 1-h heat stress (38°C in incubator); 6-h salt (150 mM NaCl), drought (15 min dry air stream until 10% loss of fresh weight) or osmotic (300 mM mannitol) stress; 12-h cold (4°C). Shoot samples: 1-h heat stress, 12-h salt, drought, cold or osmotic stress. Developmental samples: roots and leaves of 15-d-old plants; second stem internode, 21 days; flowers at stage 12; mature seeds at stage 10. The detailed microarray data was provided by the AtGenExpress consortium (<http://www.arabidopsis.org/info/expression/ATGenExpress.jsp>). The expression profiles of *HSFA6a*, *HSFA6b*, and *AREB/ABF* transcripts by time-series abiotic stresses and ABA treatment, as indicated. Note that the *AREB1* probe was not available in the ATH1 GeneChip. **(C)** Nine-d-old seedlings were incubated in H<sub>2</sub>O containing 200 mM NaCl for 6 h or 10 μM ABA for 3 h. The transcription levels of *HSFA6a* and *HSFA6b* were analyzed by qRT-PCR. The fold expression was normalized relative to the *HSFA6a* level in H<sub>2</sub>O treatment. Data are means ± SD of 3 biological replicates. *PP2A* was an internal control.



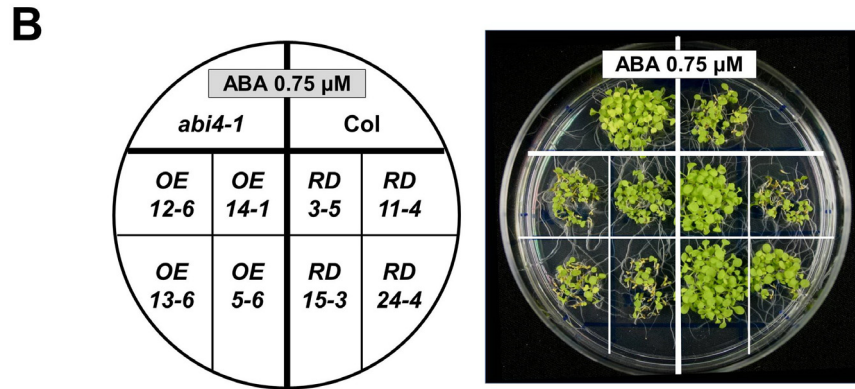
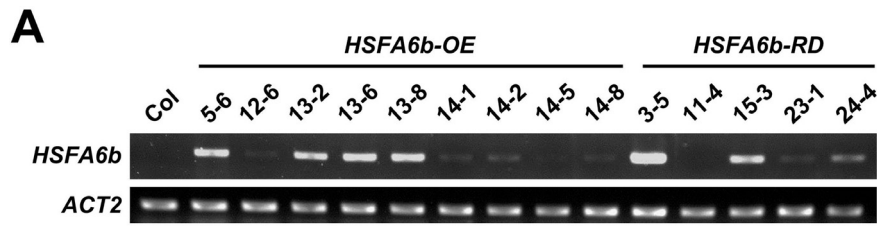
**Supplemental Figure S2. Modular structures of Arabidopsis HSFs HSFA6a and HSFA6b.** The well-studied class-A heat shock factors HSFA1a and HSFA2 were used as references for the conserved HSF signatures comparison. The block diagrams represent their conserved functional domains. N-terminal DNA-binding domain (DBD, white) is a helix-turn-helix motif, which binds to the HSEs of HS-responsive genes. HR-A/B (blue), a heptad-repeat region of hydrophobic amino acid residues, is required for homo- or hetero-oligomerization of HSFs. Nuclear-cytoplasmic shuttling sequence (brown), is a nuclear localization signal (NLS) and nuclear export signal (NES). AHA motifs (yellow), are a short domain enriched in aromatic and large hydrophobic amino acid residues embedded in an acidic surrounding, transcriptional activity of class-A HSFs. Adapted from Nover et al. (2001). HSFA6b harbors NLS and NES, but HSFA6a only contains NLS.



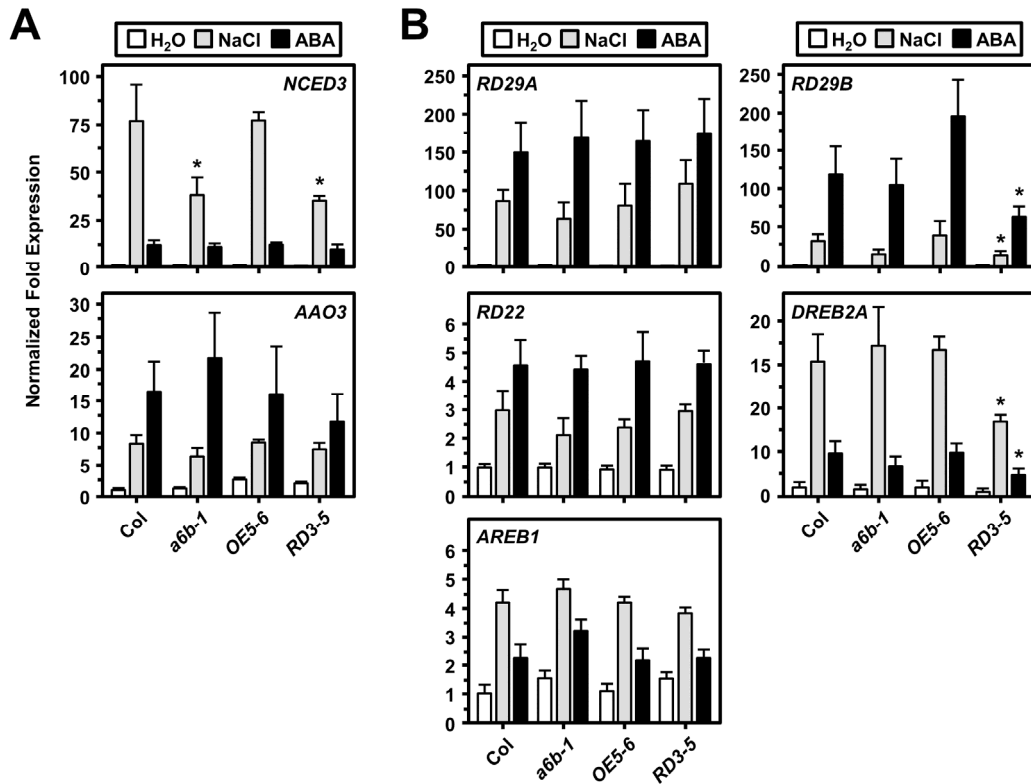
**Supplemental Figure S3. Interaction of HSFA6b and different HSFs. (A)** Co-immunoprecipitation (Co-IP) assay of HSFA6b-3XFLAG and tester (HSFs)-YFP<sup>N</sup> in Arabidopsis leaf mesophyll protoplasts, as indicated. Immunoprecipitation (IP) with anti-GFP antibody and the interacted HSFA6b-3XFLAG were analyzed by immunoblotting (IB) with anti-FLAG antibody. Input protein (50%) was used as control of immunoblotting in the transfected cell lysates. **(B)** Confocal micrographs of protoplasts showing the interaction of HSFA6b-YFP<sup>C</sup> and HSFs-YFP<sup>N</sup> by using BiFC analysis without or with 10-μM ABA for 2 h treatment (+ABA), as indicated in **Figure 2**.



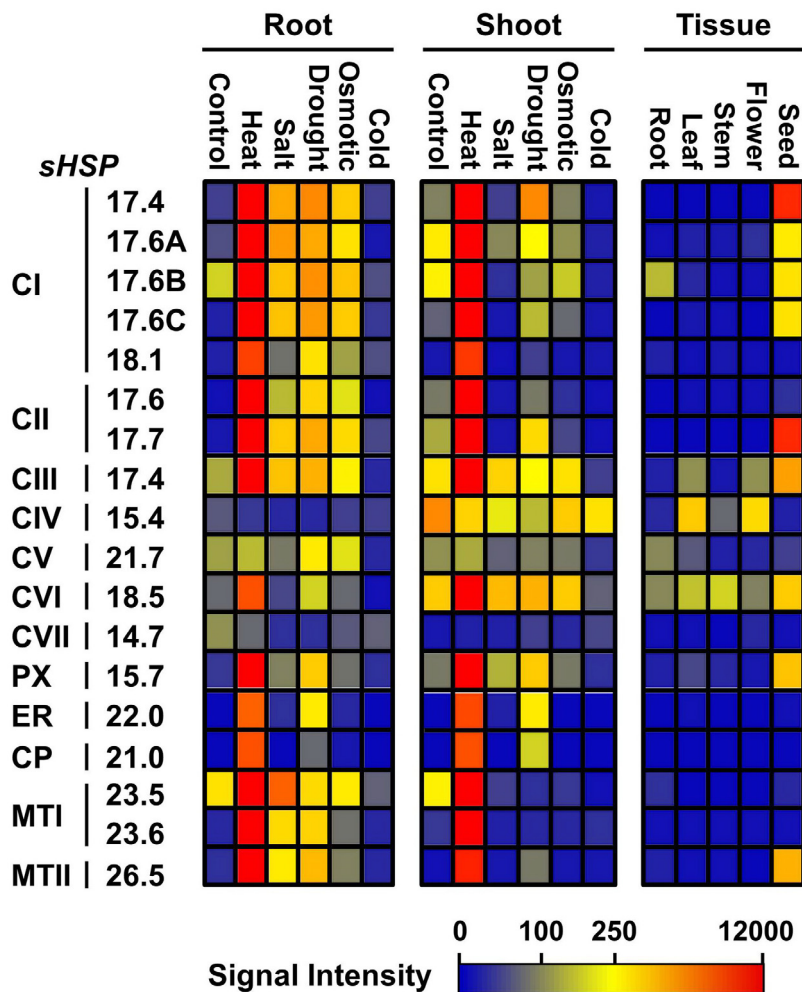
**Supplemental Figure S4. Characterization of *HSFA6b* and *HSFA6b* T-DNA insertion, overexpression, and dominant-negative mutant lines. (A and B)** Schematic maps of *HSFA6b* and *HSFA6a* gene structure and T-DNA insertion sites. Exon, UTR, and T-DNA are indicated by black, gray, and triangle, respectively. The specific primers for genotyping and RT-PCR are shown with black arrows. ATG and TAA are initiation and stop codons. **(C)** *HSFA6b*-overexpression line *HSFA6b-OE*. *HSFA6b* was fused with the hemagglutinin tag (HA; YPYDVPDYA) to the 5' end and driven by the CaMV 35S promoter. **(D)** *HSFA6b*-dominant-negative line *HSFA6b-RD*. The EAR-motif repression domain SRDX (GLDLLELRGFA) was fused to the 3' end of the HA-*HSFA6b*. The expression level of *HSFA6a* and *HSFA6b* in wild-type, Col and Ler, plants and *HSFA6b* mutants *OE5-6* and *RD3-5* were analyzed by RT-PCR and immunoblotting (IB; with anti-HA antibody and the Rubisco large subunit, Rbc L, shown for equal loading). Plants without (-ABA) or with 3-h 20- $\mu$ M ABA treatment (+ABA) are indicated. *ACT2* was a loading control for RT-PCR.



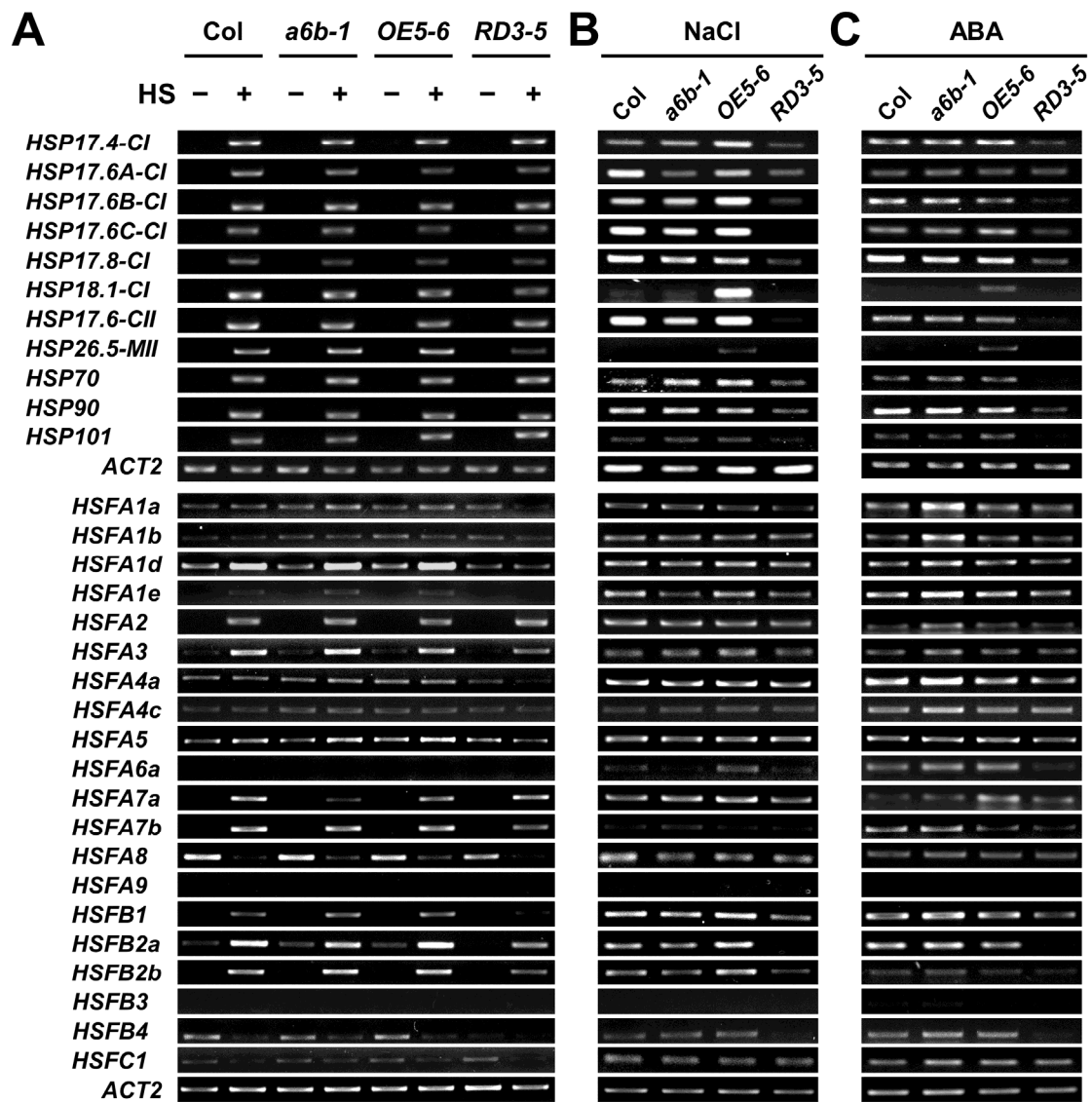
**Supplemental Figure S5. The expression levels of *HSFA6b* and ABA sensitivity in *HSFA6b* mutant lines. (A)** The *HSFA6b* transcription levels were analyzed by RT-PCR, under normal growth condition without ABA treatment, in *HSFA6b-OE* and *HSFA6b-RD* mutant lines. *ACT2* was a loading control. **(B)** Seeds with different expression levels of *HSFA6b*, as indicated in **panel A**, were planted in medium containing 0.75  $\mu$ M ABA, and then photographed at day 12. *abi4-1*, an ABA insensitive mutant (Col ecotype), was used as reference.



**Supplemental Figure S6. The expression levels of ABA biosynthesis and responsive genes in response to salt and ABA treatments in *HSFA6b* mutants. (A and B)** Nine-d-old seedlings were incubated in H<sub>2</sub>O containing 200 mM NaCl for 6 h or 20 μM ABA for 3 h. The transcription levels of ABA biosynthesis genes *NCED3* and *AAO3*, ABA-responsive genes *RD29A*, *RD29B*, and *RD22*, as well as *DREB2A* and *AREB1*, were analyzed by qRT-PCR. The fold expression was normalized relative to that of Col H<sub>2</sub>O treatment. Data are means ± SD of 3 biological replicates. \*, Significant at  $P < 0.05$  compared with Col. *PP2A* was an internal control.

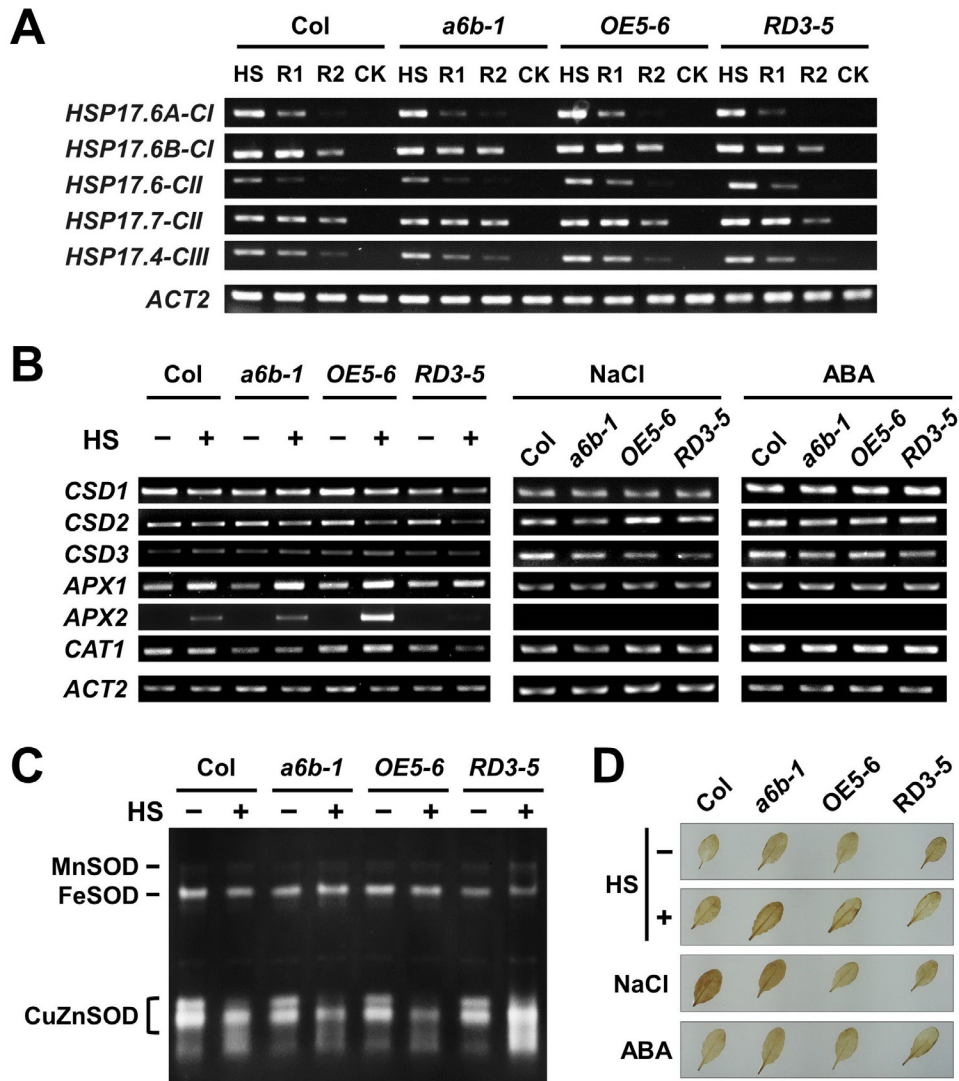


**Supplemental Figure S7. The expression profiles of Arabidopsis 18 small heat shock protein (sHSP) genes under various abiotic stresses and during development.** Normalized and averaged signals are presented as “heat maps”, as indicated in **Supplemental Figure S1**. Because of the absence of a specific probe for *HSP17.8-CI*, only 18 sHSP genes are presented. The *HSP18.5-CIV*, *15.4-CV*, and *21.7-CVI* genes reported by Siddique et al. (2008) were revised as *HSP15.4-CIV*, *21.7-CV*, and *18.5-CVI*, respectively (Waters et al., 2008). CI to CVII (class I to VII subfamily), cytoplasmic/nuclear localization; CP, plastids; ER, endoplasmic reticulum; MT, mitochondria; PX, peroxisomes.

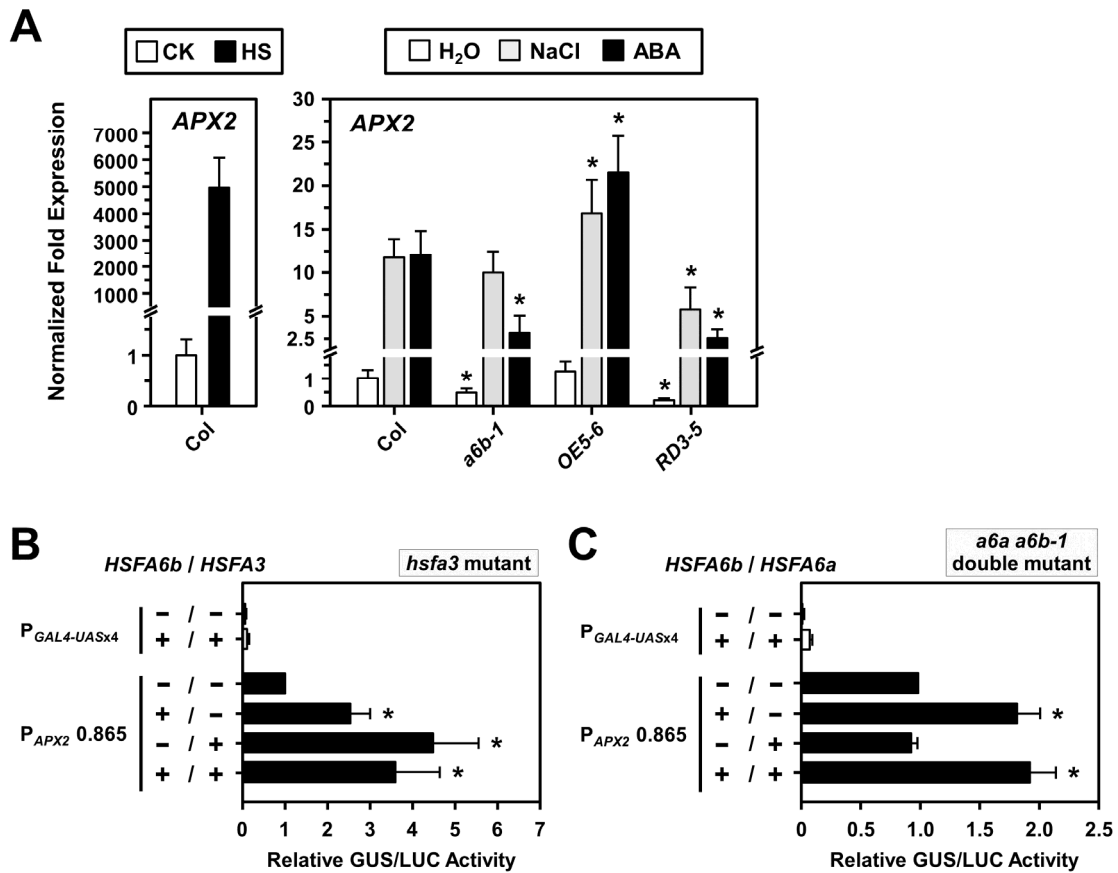


**Supplemental Figure S8. The expression of HS-related genes in response to HS, salt, and ABA treatments in *HSFA6b* mutants. (A to C)** Nine-d-old seedlings were treated without or with HS at 37°C for 1-h, incubated in H<sub>2</sub>O containing 150-mM NaCl for 6 h, and incubated in H<sub>2</sub>O containing 20-μM ABA for 3 h, respectively. The transcription levels of 11 *HSPs* and 20 *HSFs* were analyzed by RT-PCR. *ACT2* was a loading control.

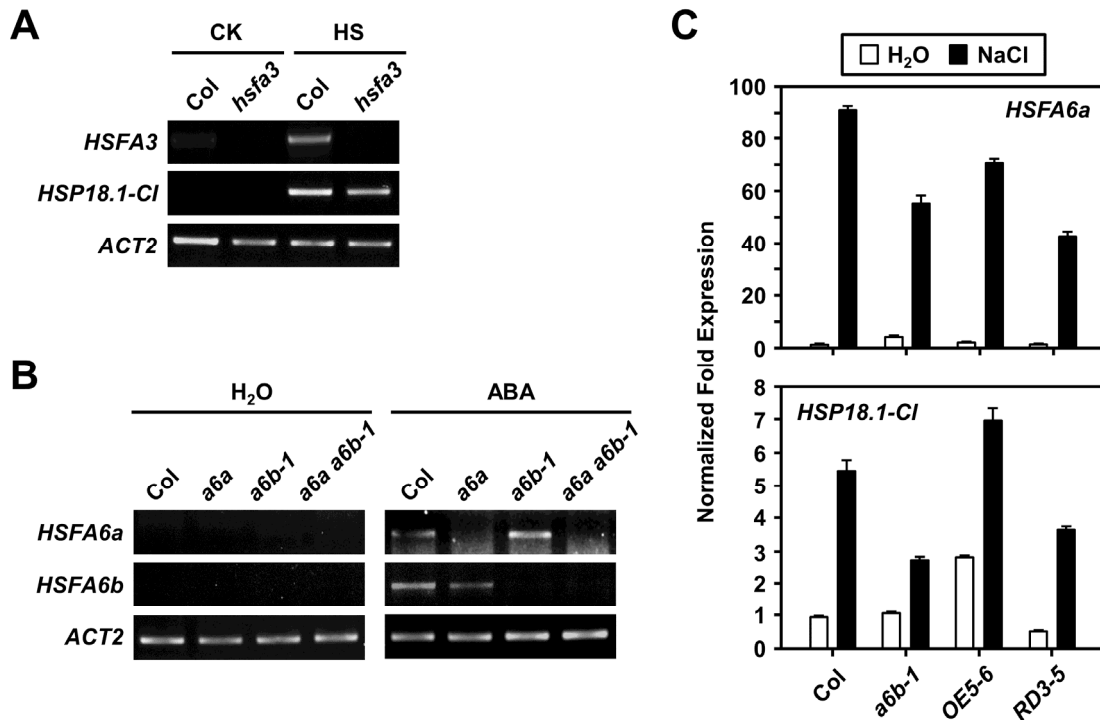




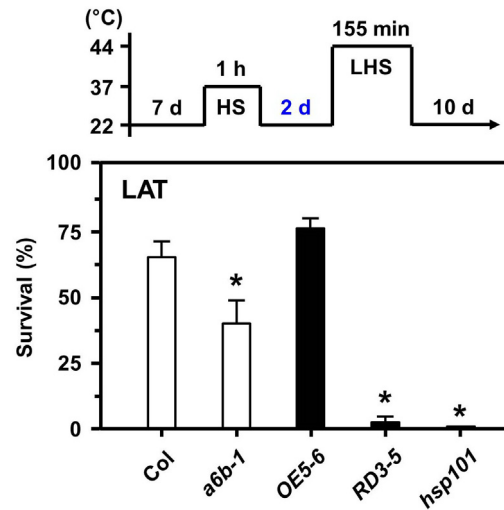
Supplemental Figure S9. The expression levels of HS and oxidative related genes, superoxide dismutase (SOD) activity, and H<sub>2</sub>O<sub>2</sub> detection in response to HS, NaCl, and ABA treatments in *HSFA6b* mutants. Nine-d-old seedlings were treated without or with 1-h 37°C HS and then underwent recovery for 1 or 2 h (R1 and R2). The NaCl and ABA treatments, as indicated in Supplemental Figure S8. (A and B) The transcription levels of 5 HS-related and 6 oxidative-related genes were analyzed by RT-PCR, respectively. (C) In-gel CuZnSOD, FeSOD, and MnSOD activity assay. (D) H<sub>2</sub>O<sub>2</sub> detection by DAB staining.



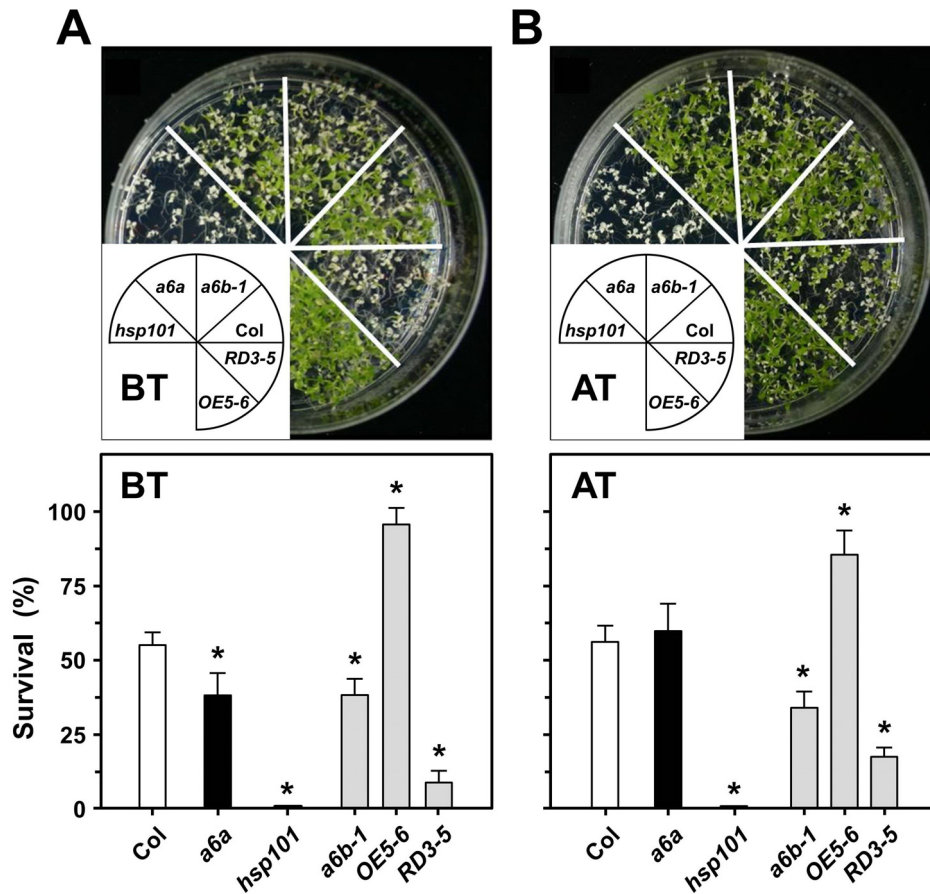
**Supplemental Figure S10. The expression levels of *APX2* in response to HS, NaCl, and ABA treatment in *HSFA6b* mutants, as well as *HSFA6b* mediated *HSFA3* activation of the *APX2* promoter. (A)** Nine-d-old seedlings were treated without (control) or with 1-h 37°C HS. The NaCl and ABA treatments are as indicated in **Supplemental Figure S8**. The transcription levels of *APX2* were analyzed by qRT-PCR. The fold expression was normalized to that of wild-type (Col) plants with mock (control or H<sub>2</sub>O) treatment. Data are means  $\pm$  SD of 3 independent replicates. \*, Significant at  $P < 0.05$  compared with the Col. *PP2A* was an internal control. **(B and C)** *hsfa3* and *hsfa6a hsfa6b* double (*a6a a6b-1*) mutant protoplasts were used for the transcriptional activation assay.  $P_{GAL4-UASx4}$ , was a control, and 0.856-kb length of the *APX2* promoter was fused with a *GUS* reporter gene, then transfected with or without effectors *HSFA6b*, *HSFA3*, and *HSFA6a*, as indicated. The fold expression was normalized to that with the transfection of  $P_{APX2}$  without effectors, as described in **Figure 4**. Data are means  $\pm$  SD of 3 independent replicates. \*  $P < 0.05$ .



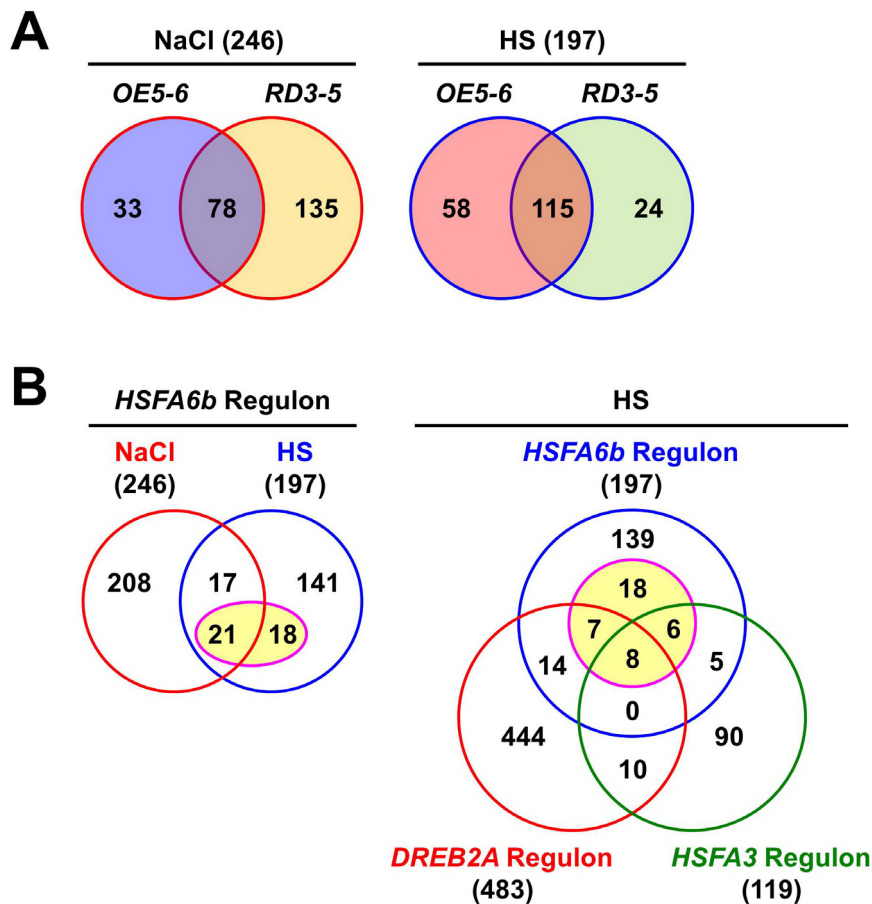
**Supplemental Figure S11. Characterization of *hsf3* and *hsf6a hsf6b* double mutant lines, as well as the *HSFA6a* and *HSP18.1-CI* transcription levels in response to NaCl treatment in *HSFA6b* mutants.** The HS or ABA treatment was as indicated in **Supplemental Figure S8**. **(A and B)** The expression levels of *HSFA3*, *HSFA6a*, and *HSFA6b* in *hsf3* (SALK\_01117) and *a6a a6b-1* double mutant lines were analyzed by RT-PCR. *HSP18.1-CI*, used as a reference, is a HS-responsive gene. *ACT2* was a loading control. **(C)** The *HSFA6a* and *HSP18.1-CI* transcription levels in response to 200-mM NaCl for 6 h in *HSFA6b* mutants were analyzed by qRT-PCR. The fold expression was normalized to that of the *HSFA6a* level in Col H<sub>2</sub>O treatment. Data are means  $\pm$  SD of 3 biological replicates. *PP2A* was an internal control.



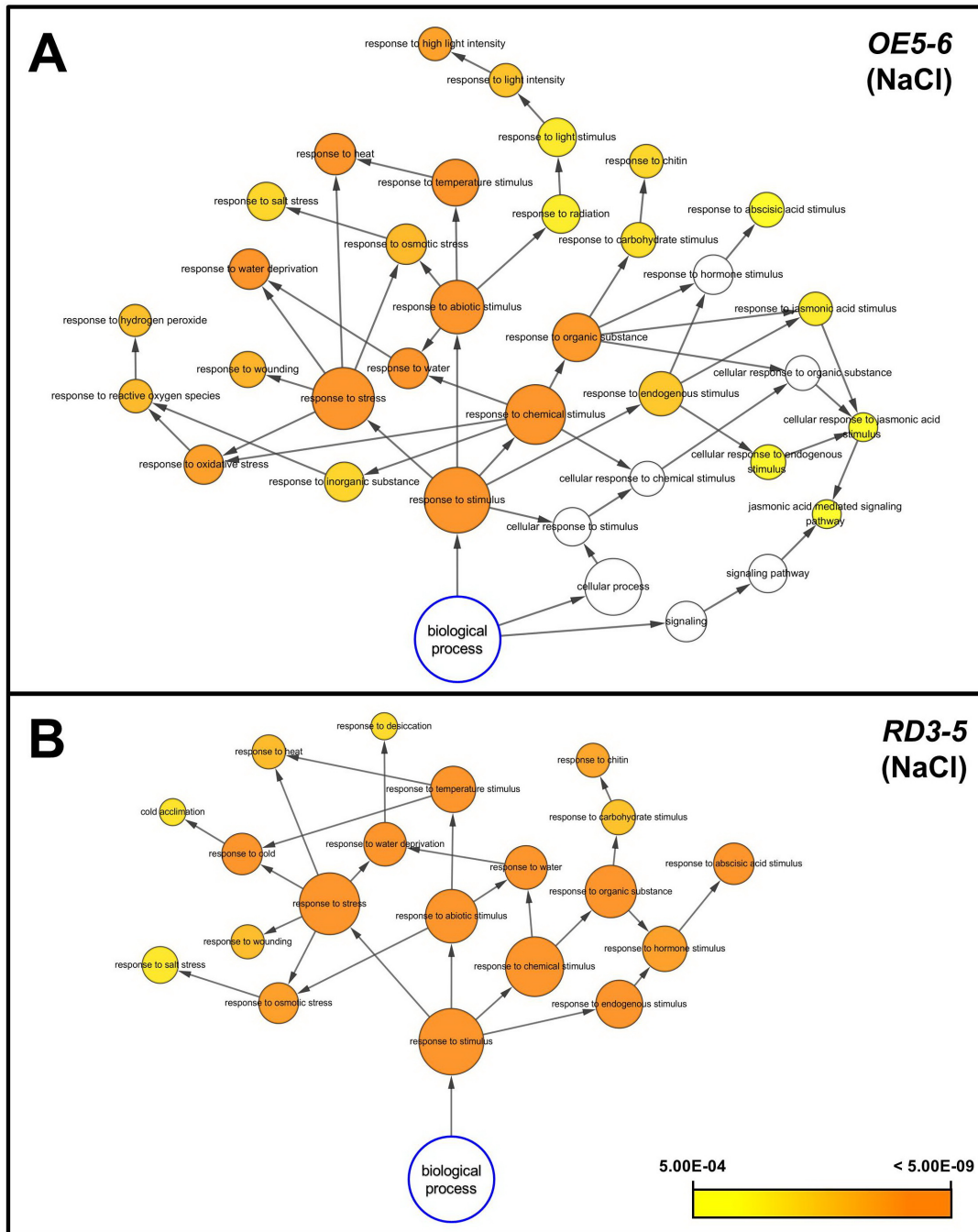
**Supplemental Figure S12. Thermotolerance test in *HSFA6b* mutants.** Seven-d-old seedlings were analyzed for long-term acquired thermotolerance (LAT). The pictogram shows the HS regime. Survival (%) was measured at day 10 after HS treatment, as described in **Figure 8**. Data are means  $\pm$  SD of 3 independent replicates. \*, Significant at  $P < 0.05$  compared with the Col.



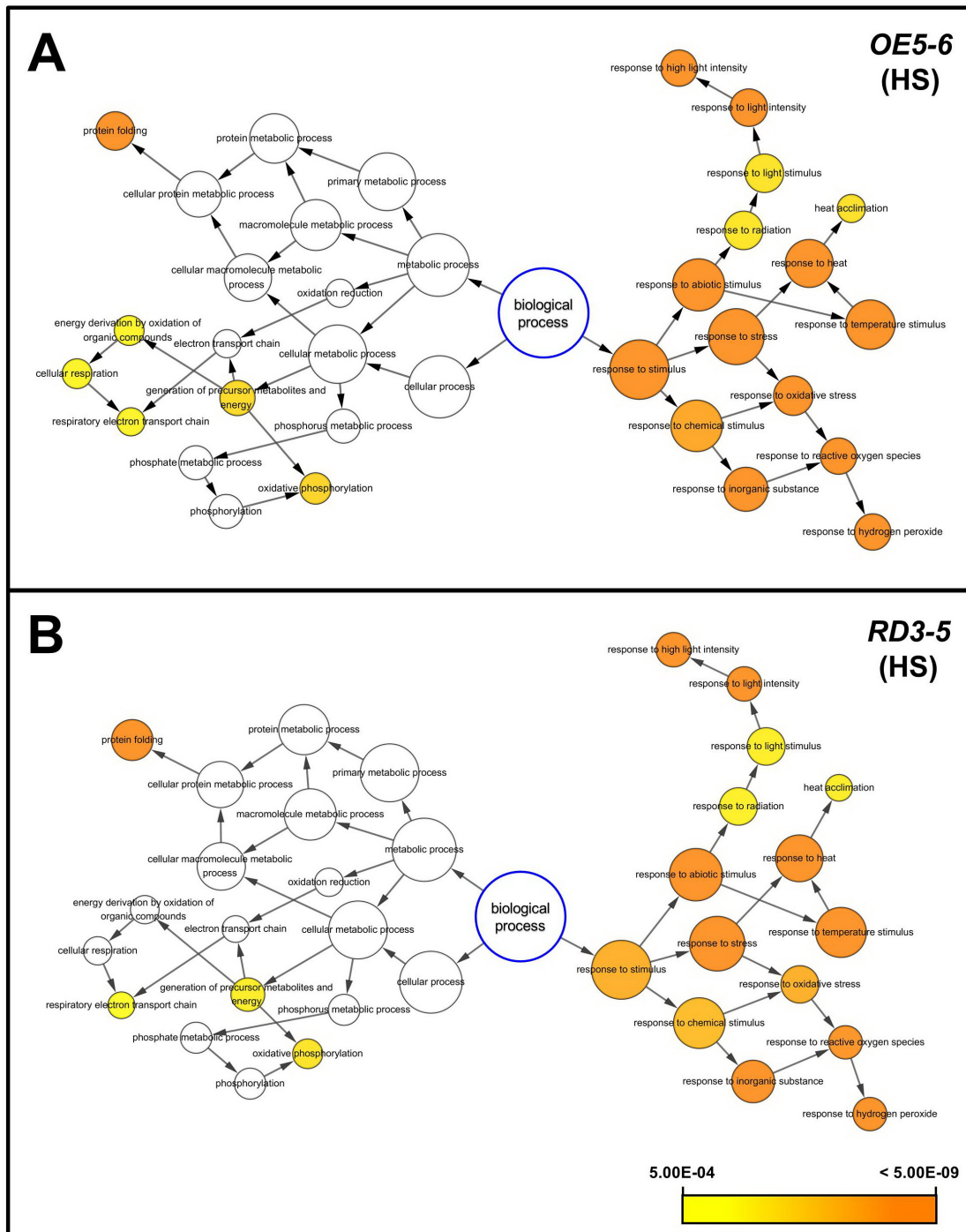
**Supplemental Figure S13. Thermotolerance test in *HSFA6a* and *HSFA6b* mutants. (A and B)** Nine-d-old seedlings were analyzed for BT and AT, respectively, as described in **Figure 8**. Seedlings were photographed (**top**) and survival (%) was measured (**bottom**). Data are means  $\pm$  SD of 3 independent replicates. \*, Significant  $P < 0.05$  compared with the Col.



**Supplemental Figure S14. Venn diagram of gene transcripts in response to salt or HS treatment in *HSFA6b* mutants.** Venn diagram based on TAIR locus identifier. **(A)** The expression changed > 6-fold at  $p < 0.10$ , in *HSFA6b* mutants *OE5-6* and *RD3-5* with 150 mM NaCl for 6 h or 37°C HS for 1 h treatment, as compared with Col plants at 22°C-control treatment. **(B)** The top 39 DEGs with expression changed > 30-fold at  $p < 0.05$  of the *HSFA6b* HS response regulon (highlighted in yellow) overlapping with NaCl-response regulon (**left**), and the HS-response regulon of *DREB2A* and *HSFA3* (**right**) (Sakuma et al., 2006; Yoshida et al., 2008). At4g36990 and At4g36988 shared the same probeset number, 246214\_at, in ATH1 GeneChip array.

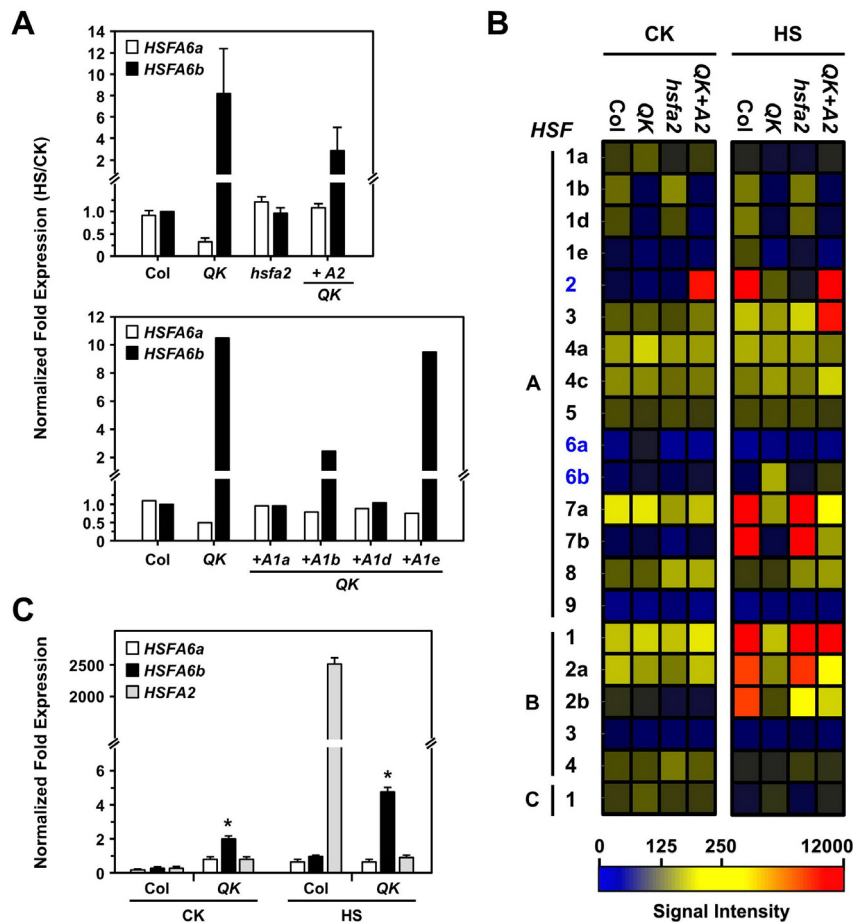


**Supplemental Figure S15. GO enrichment analysis of gene transcripts in response to salt treatment in *HSFA6b* mutants. (A and B) Network graphs of enriched GO terms in response to 150 mM NaCl for 6 h in *OE5-6* and *RD3-5* mutants, respectively, as indicated in Supplemental Figure S14A. The yellow to orange color of the circles correspond to the level of significance of the overrepresented GO category at  $p \leq 5.00E-04$  according to a multiple  $t$  test with false discovery rate-corrected  $p$  value. The size of the circle is proportional to the number of genes in the category.**



**Supplemental Figure S16. GO enrichment analysis of gene transcripts in response to HS treatment in *HSFA6b* mutants. (A and B) Network graphs of enriched GO terms in response to 37°C HS for 1 h in *OE5-6* and *RD3-5* mutants, respectively, as indicated in Supplemental Figures S14b and S15.**





**Supplemental Figure S17.** The expression levels of *HSFA6a* and *HSFA6b* in response to HS treatment in *hsfa1a/b/d/e* quadruple-KO (*QK*) and *hsfa2* mutant lines. **(A)** Wild-type Col, *QK*, and *hsfa2*, as well as the *QK* plants complemented (+) with *HSFA2* and different member of the *HSFA1s*, are as indicated. The 1-h 37°C treatment served as HS and 22°C treatment was a control (CK). The fold expression (HS/CK) was normalized to that of the *HSFA6b* level in Col. Normalized and averaged signals were collected from the Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo>) from the National Center for Biotechnology Information (NCBI) accession number GSE26266 and GSE44655 (Liu et al., 2011; 2013). **(B)** The heat maps show the expression level of *HSFA6a* and *HSFA6b*, in addition to that of the HSFs. **(C)** The expression levels of *HSFA6a*, *HSFA6b*, and *HSFA2* were analyzed by qRT-PCR, which was normalized to that of the *HSFA6b* level in Col HS treatment. Data are means  $\pm$  SD of 3 independent replicates. \*, Significant at  $P < 0.05$  (the *HSFA6b* level in *QK* compared with the Col).

**Supplemental Table S1. Primers used for genotyping, cloning, RT-PCR and real-time quantitative PCR, as well as accession numbers can be found in the TAIR database.**

<b>Primer</b>	<b>Sequence (5' to 3')</b>
<b>Genotyping</b>	
HSFA6a_Fw	GAACCAGAGACTTTTGCCCTAAT
HSFA6a_Rv	CTCCCCTTGTCTTGATGCTC
HSFA6b-1_KO_Fw	TTGTCCGCCAGCTCAACACA
HSFA6b-1_KO_Rv	ACTCCACCGATCCGGATTCACC
HSFA6b-2_KO_Fw	GGCAAAGCATCTCCTCAAG
HSFA6b-2_KO_Rv	CTTCCCAAAAACCTTCACCA
<b>HSPs</b>	
HSP17.4-CI_Fw	AAATACGCCAACGCACATAA
HSP17.4-CI_Rv	AACTATCAATGGATCAAAACACTC
HSP17.6A-CI_Fw	AACTTCAATTCCTCGTCATCG
HSP17.6A-CI_Rv	ATCCAAATACACACATTTCTCCAC
HSP17.6B-CI_Fw	CTTGCCTGGATTGAAGAAGG
HSP17.6B-CI_Rv	AGGATCAAATGTATCCGATACATC
HSP17.6C-CI_Fw	GATTGGAGGGAGACACCTGA
HSP17.6C-CI_Rv	ACAAAGCACACTCTTATTAAGATT
HSP17.8-CI_Fw	AAACATCGGCGATAACGAAC
HSP17.8-CI_Rv	AATATAAAGGCCTTGCTTAATTAT
HSP18.2-CI_Fw	ACGTCTTTGATCCGTTCTCG

HSP18.2-CI_Rv	ATTCATAACACAACAAGCCAAG
HSP17.6-CII_Fw	GACCCGCAACAACCCTTCAC
HSP17.6-CII_Rv	ACCATATCCCTCACGCATTCC
HSP26.5-MII_Fw	TCACTCCCACCCTAAATGAG
HSP26.5-MII_Rv	TTCTCCGTCCTAGGAATCAC
HSP70b_Fw (70-5)	AGACAATCAACCAGGCGTTCT
HSP70b_Rv (70-5)	TCGCCTTCTCAATCTTCTGCT
HSP90.1_Fw	GCGAGGTCTGGAACAAAAGAG
HSP90.1_Rv	ATCGGTTTCTGCTTGTTGATG
HSP101_Fw	ACCGAGAAGAAGTCTCTGGC
HSP101_Rv	TTTCCCACGAAGCTTCTCAAC
<b>HSFs</b>	
HSFA1a_Fw	CGAGGCCAATAAGAAGCGGAGACT
HSFA1a_Rv	CTGAATCGGGACTGAACCACCATC
HSFA1b_Fw	ACCGCCAGATTGTTAGATA
HSFA1b_Rv	CCGGGCAGCTTAGGGACTC
HSFA1d_Fw	CGGCGATGATGGTTTCCTTCTA
HSFA1d_Rv	TTGTCCCATCCATTTTGTTCCTG
HSFA1e_Fw	GTGGGTGCGTGCGTTGAAGT
HSFA1e_Rv	GAAGCCTGCAGGAATGGATGAAA
HSFA2_Fw	TGAGGCAACAGCAACACAGC
HSFA2_Rv	CAAAGGCGAACCAACCAAATCT
HSFA3_Fw	TGGAAAAGGCGAGAAAGAAGTT

HSFA3_Rv	TTGGCGTATTATCATCATCAC
HSFA4a_Fw	GCCAGGGCTTGCTTTGAAC
HSFA4a_Rv	TCCGGCTTTATCTTTATCGTCTTT
HSFA4c_Fw	ACGGAATCAGAAAGACGGAGCAT
HSFA4c_Rv	GAGCAGGCGCAGTAACGGTAAC
HSFA5_Fw	CACGCCTCCTTCTTCCAACCTACT
HSFA5_Rv	CGCGTCTCGAGCTACCACTACTAT
HSFA7a_Fw	AGATGGGAATTTGCAAACGAA
HSFA7a_Rv	TCCCAGAAACCATCATCCAACCT
HSFA7b_Fw	TCCCCTCCTTCGCTTAACTACTCT
HSFA7b_Rv	ACCCGTCGTAAGCTCCCTCTC
HSFA8_Fw	CAGGAGGTGACGGATACTAAGA
HSFA8_Rv	GAAGGGCTCAACAACAAAAG
HSFA9_Fw	GAAGCGAGTACCACCACCACGACA
HSFA9_Rv	TATCCTCCCATTACATCCATTGA
HSFB1_Fw	ACGGCGGGGAAATGTGTTGTTGT
HSFB1_Rv	CTTTTCATCCCGGTCCCTCTTTTT
HSFB2a_Fw	GTACCGGATCGATGGGAGTTTTTC
HSFB2a_Rv	ACCGCCGTCGTCTTCACCT
HSFB2b_Fw	CGCCGGGAATAGTGGTGGAAGTAG
HSFB2b_Rv	CCGCAGGCGAAGAATTAGACGATA
HSFB3_Fw	GTTCCGCCAGCTCAATACTTACG
HSFB3_Rv	AGCCCTTCATCTTCTTCATCATC

HSFB4_Fw	GAAATCCACCGCCGTAAAACATC
HSFB4_Rv	AGTGGCATTGACAGTGGCAGTATT
HSFC1_Fw	CGGACCGGTGGGAGTTTG
HSFC1_Rv	ATGACGGCGACGGTGTGATGAT
<b>Oxidative stress responsive genes</b>	
APX1_Fw	CAATGAGGTTTCCGGCTGAGCAAGC
APX1_Rv	CTGCAGTTAAGCATCAGCAAACCCAAGC
APX2_Fw	AGCCTTCAACGATTTCCAGAAG
APX2_Rv	TCGAATCCTGAACGCTCCTT
CAT1_Fw	CAGTCTCACCTAAAAATCGC
CAT1_Rv	ACTTGGGATTCGGTTTCAAT
CSD1_Fw	TCTGAGCTCATGGCGAAAGGAGTTGCAGTTTTG
CSD1_Rv	TTAGCCCTGGAGACCAATGATGC
CSD2_Fw	TCTCCCGGGATGGCTGCCACCAACACAATCC
CSD2_Rv	TCTGGATCCGAGCGGCGTCAAGCCAATCA
CSD3_Fw	TCTGAGCTCATGGAAGCTCCTAGAGGAAATCTGAGAG
CSD3_Rv	TCTCCCGGGTAGTTTAGCATCCGCAGATGATTGAAGT
<b>Cloning</b>	
HSFA6b_cDNA_Fw	TCTGTGCGACTATGGATCCTTCATTTAGGTT
HSFA6b_cDNA_Rv	TCTCTGCAGTTAATTAGTGTGTGAACTAG
HA_Fw	TCTCCCGGGATGTACCCATACGATGTTCCA
HSFA6b_ORF_Rv	TCTCCCGGGATTAGTGTGTGAACTAGAA
HSFA6b_ORF_Fw	ATGGATCCTTCATTTAGGTTTCATTA

HSFA6b_ORF_Rv	ATTAGTGTGTGAACTAGAACCCAAA
AREB1_Fw	AAGTCGACATGGATGGTAGTATGAATTTG
AREB1_Rv	AAGGATCCTCACCAAGGTCCCG
ProHSFA6b_1.0_Fw	CTCGAGGTTTTAAAAAATTGGATAACAACAC
ProHSFA6b_1.0_Rv	CCATGGGGTTTTAAATTTAAACAAAGATTTTTTTAT
ProHSFA6b_1.7_Fw	CTCGAGTTCAATTTTCTCGTTATTGTTTTTAGG
ProHSFA6b_1.7_Rv	CCATGGGGTTTTAAATTTAAACAAAGATTTTTTTAT
ProHSP18.1_1.0_Fw	GGATCCATTTGCAGTGTTATGAGTGAATTTT
ProHSP18.1_1.0_Rv	CTC GAGTGTTTCGTTGCTTTTCGGGAGACTTTT
ProDREB2A_1.0_Fw	CTCGAGTAAAATGGACGACACCCATCCAAT
ProDREB2A_1.0_Rv	CCATGGAAGGAAGACAAAGTATCTACGGAGT
ProAPX2_0.856_Fw	AAGGATCCTAATCTCGTCTGCTTTGG
ProAPX2_0.856_Rv	AACCATGGTTTTTTCAAATTCGCTTCC
<b>Real-time Quantitative PCR</b>	
qHSFA2_Fw	CAGCGTTGGATGTGAAAGTG
qHSFA2_Rv	CAGCGTTGGATGTGAAAGTG
qHSFA6a_Fw	CAGCGTTGGATGTGAAAGTG
qHSFA6a_Rv	CAGCGTTGGATGTGAAAGTG
qHSFA6b_Fw	GTGATGAAAGTGGTTATGGGAATG
qHSFA6b_Rv	TCCGACATCTCGAATTCAGACAT
qRD22_Fw	ATTGTGCGACGTCTTTGGAGT
qRD22_Rv	TGCGTTCTTCTTAGCCACCTC
qRD29A_Fw	TGGATCTGAAGAACGAATCTGATATC

qRD29A_Rv	GGTCTTCCCTTCGCCAGAA
qRD29B_Fw	AGTCGCCACGGTCCGTTGAAG
qRD29B_Rv	CCGCCACTGCCTCCCAACTC
qNCED3_Fw	AGCCGCCATTATCGTCTTCTC
qNCED3_Rv	GGAGTGTGAAGCGCAGATGAA
qAAO3_Fw	TCCATCATGGACTGCTCCTTC
qAAO3_Rv	CGAGACACTAGCGCCAAGAAA
qDREB2A_Fw	GACCTAAATGGCGACGATGT
qDREB2A_Rv	TCGAGCTGAAACGGAGGTAT
qAREB1_Fw	GAGAGAAGGCCAAAGGAGAATGA
qAREB1_Rv	CTTCAAGCTCCACGGTGTAAG
qAPX2_Fw	CGGGTTTGCTGACAAGGAGTA
qAPX2_Rv	GAGGGAACAAGAATCAAGGAGGTA
qHSP18.1_Fw	AAGGCAACAATGGAGAATGG
qHSP18.1_Rv	GCACACAAGCTTTTTATTTGACA
<b>ChIP</b>	
qPCR_Fw	ATTTTAAAAAGGTTTAAACT
qPCR_Rv	TCCGTTTCGACGAGTGTCTC
18S rDNA_Fw	CCTGCGGCTTAATTTGACTC
18S rDNA_Rv	GACAAATCGCTCCACCAACT
<b>Controls</b>	
UBQ10_Fw	GATCTTTGCCGAAAACAATTGGAGGATGGT
UBQ10_Rv	CGACTTGTCATTAGAAAGAAAGAGATAACAGG

ACT2_Fw	ATGAAGCACAATCCAAGAGAGGTATTCTTA
ACT2_Rv	GAGCTTCTCCTTGATGTCTTACAATTC
qPP2A_Fw	CCTGCGGTAATAACTGCATCT
qPP2A_Rv	CTTCACTTAGCTCCACCAAGCA

**Sequence data in this article can be found in the TAIR database under the following accession numbers:** AAO3 (At2g27150), APX1 (At1g07890), APX2 (At3g09640), AREB1/ABF2 (At1g45249), CAT1 (At1g20630), CSD1 (At1g08830), CSD2 (At2g28190), CSD3 (At5g18100), DREB2A (At5g05410), NCED3 (At3g14440), RD22 (At5g25610), RD29A (At5g52310), RD29B (At5g52300).

**HSP genes:** HSP17.4-CI (At3g46230), HSP17.6A-CI (At1g59860), HSP17.6B-CI (At2g29500), HSP17.6C-CI (At1g53540), HSP17.8-CI (At1g07400), HSP18.2-CI (At5g59720), HSP17.6-CII (At5g12020), HSP26.5-MII (At1g52560), HSP70b (At1g16030), HSP90.1 (At5g52640), HSP101 (At1g74310).

**HSF genes:** HSFA1a (At4g17750), HSFA1b (At5g16820), HSFA1d (At1g32330), HSFA1e (At3g02990), HSFA2 (At2g26150), HSFA3 (At5g03270), HSFA4a (At4g18880), HSFA4c (At5g45710), HSFA5 (At4g13980), HSFA7a (At3g51910), HSFA7b (At3g63350), HSFA8 (At1g67970), HSFA9 (At5g54070), HSFB1 (At4g36990), HSFB2a (At5g62020), HSFB2b (At4g11660), HSFB3 (At2g41690), HSFB4 (At1g46264), HSFC1 (At3g24520).













**Supplemental Table S3.** Enriched GO terms in differential expression genes (DEGs) expression changed more than 6 fold in transgenic plants harboring HSF6b-OE and HSF6b-RD under Salr or HS treatment. Enriched GO terms were identified using BINGO and GO\_full annotation (Maere et al., 2005). Significantly overrepresented terms with a  $p$ -value less than 5.00E-04 following a Benjamini and Hochberg false discovery rate correction are shown. BP, biological process. CC, cellular component. MF, molecular function.

**(A) Transgenic plants harboring HSF6b-OE in SALT condition**

GO ID	GO Type	GO description	corrected p-value
6950	BP	response to stress	1.17E-19
42221	BP	response to chemical stimulus	1.88E-17
50896	BP	response to stimulus	1.28E-16
9266	BP	response to temperature stimulus	2.57E-15
9408	BP	response to heat	2.16E-14
9628	BP	response to abiotic stimulus	1.21E-13
9414	BP	response to water deprivation	5.69E-11
9415	BP	response to water	8.48E-11
10033	BP	response to organic substance	7.78E-09
6979	BP	response to oxidative stress	1.96E-08
9644	BP	response to high light intensity	2.04E-08
9611	BP	response to wounding	1.63E-07
6970	BP	response to osmotic stress	2.44E-07
302	BP	response to reactive oxygen species	2.89E-07
42542	BP	response to hydrogen peroxide	5.58E-07
9642	BP	response to light intensity	7.09E-07
9719	BP	response to endogenous substance	1.24E-06
10035	BP	response to inorganic substance	5.71E-06
10200	BP	response to chitin	6.39E-06
9651	BP	response to salt stress	6.43E-06
9743	BP	response to carbohydrate stimulus	1.63E-05
9416	BP	response to light stimulus	5.76E-05
9753	BP	response to jasmonic acid stimulus	5.76E-05
9314	BP	response to radiation	7.40E-05
71495	BP	cellular response to endogenous stimulus	2.01E-04
9737	BP	response to abscisic acid stimulus	3.17E-04
9867	BP	jasmonic acid mediated signaling pathway	3.76E-04
71395	BP	cellular response to jasmonic acid stimulus	3.76E-04
16563	MF	transcription activator activity	3.87E-04

**(B) Transgenic plants harboring HSF6b-RD in SALT condition**

GO ID	GO Type	GO description	corrected p-value
9414	BP	response to water deprivation	3.69E-18
6950	BP	response to stress	3.69E-18
9415	BP	response to water	3.69E-18
9266	BP	response to temperature stimulus	1.31E-16
42221	BP	response to chemical stimulus	2.01E-16
50896	BP	response to stimulus	7.49E-14
10033	BP	response to organic substance	1.34E-13
9628	BP	response to abiotic stimulus	1.78E-13
9409	BP	response to cold	3.99E-13
9737	BP	response to abscisic acid stimulus	3.61E-12
9719	BP	response to endogenous substance	2.03E-10
6970	BP	response to osmotic stress	1.60E-08
9725	BP	response to hormone stimulus	1.98E-08
10200	BP	response to chitin	3.46E-08
9408	BP	response to heat	3.84E-07
9611	BP	response to wounding	4.22E-07
9743	BP	response to carbohydrate stimulus	7.74E-07
9269	BP	response to desiccation	1.07E-05
9631	BP	cold acclimation	2.37E-05
9651	BP	response to salt stress	4.12E-05

**(C) Transgenic plants harboring HSF6b-OE in HS condition**

GO ID	GO Type	GO description	corrected p-value
9408	BP	response to heat	1.99E-28
9266	BP	response to temperature stimulus	8.84E-24
6457	BP	protein folding	1.18E-13
9628	BP	response to abiotic stimulus	4.64E-13
9644	BP	response to high light intensity	5.85E-12
42542	BP	response to hydrogen peroxide	1.67E-10
6950	BP	response to stress	2.74E-10
10035	BP	response to inorganic substance	5.93E-10
9642	BP	response to light intensity	1.05E-09
302	BP	response to reactive oxygen species	7.05E-09
50896	BP	response to stimulus	1.04E-07
6979	BP	response to oxidative stress	1.05E-07
42221	BP	response to chemical stimulus	4.17E-07
6119	BP	oxidative phosphorylation	3.06E-05
10286	BP	heat acclimation	7.82E-05
9416	BP	response to light stimulus	8.80E-05
6091	BP	generation of precursor metabolites and energy	8.80E-05
9314	BP	response to radiation	1.09E-04
22904	BP	respiratory electron transport chain	3.55E-04
5618	CC	cell wall	8.93E-05
30312	CC	external encapsulating structure	9.30E-05
30554	MF	adenyl nucleotide binding	1.24E-04
1883	MF	purine nucleotide binding	1.24E-04
1882	MF	nucleotide binding	1.25E-04
5524	MF	ATP binding	1.52E-04
32559	MF	adenyl ribonucleotide binding	1.52E-04

**(D) Transgenic plants harboring HSF6b-RD in HS condition**

GO ID	GO Type	GO description	corrected p-value
9408	BP	response to heat	5.29E-28
9266	BP	response to temperature stimulus	1.28E-20
42542	BP	response to hydrogen peroxide	3.32E-13
9644	BP	response to high light intensity	4.23E-13
9628	BP	response to abiotic stimulus	1.76E-12
9642	BP	response to light intensity	2.20E-12
6950	BP	response to stress	4.67E-12
302	BP	response to reactive oxygen species	1.55E-11
10035	BP	response to inorganic substance	4.10E-11
6457	BP	protein folding	1.83E-09
6979	BP	response to oxidative stress	3.56E-09
50896	BP	response to stimulus	3.59E-09
42221	BP	response to chemical stimulus	3.83E-08
6119	BP	oxidative phosphorylation	5.60E-06
6091	BP	generation of precursor metabolites and energy	9.30E-06
10286	BP	heat acclimation	2.24E-05
9416	BP	response to light stimulus	2.63E-05
9314	BP	response to radiation	3.56E-05
22904	BP	respiratory electron transport chain	1.50E-04
15980	BP	energy derivation by oxidation of organic compounds	1.71E-04
45333	BP	cellular respiration	1.71E-04

**Supplementary TableS4.** The top 39 DEGs with levels changed > 30-fold at  $p < 0.05$  in HSF6b mutants OE5-6 and RD3-5 after 150 mM NaCl for 6 h or 37°C HS for 1 h treatment as compared with the Col control (CK) treatment.

Treatment line Locus Identifier	CK WT		CK ATHSFA6b-OE		CK ATHSFA6b-RD		SALT WT		SALT ATHSFA6b-OE		SALT ATHSFA6b-RD		HS WT		HS ATHSFA6b-OE		HS ATHSFA6b-RD		Symbol	Array Element	Gene Function
	Normalized	p-value	Normalized	p-value	Normalized	p-value	Normalized	p-value	Normalized	p-value	Normalized	p-value	Normalized	p-value	Normalized	p-value	Normalized	p-value			
AT2G19310	0.99	0.95	1.98	0.13	0.83	0.30	9.58	0.08	9.98	0.05	4.16	0.06	56.67	0.00	44.68	0.01	41.74	0.02	HSP18.5	267336_at	HSP20-like chaperones superfamily protein
AT5G25450	1.00	0.97	1.83	0.03	1.09	0.41	12.33	0.07	12.46	0.02	5.45	0.05	98.55	0.00	74.69	0.01	78.50	0.03		246944_at	Cytochrome bd ubiquinol oxidase, 14kDa subunit
AT2G29500	0.92	0.87	2.25	0.23	0.58	0.47	15.64	0.00	19.44	0.00	3.34	0.20	134.48	0.00	120.65	0.00	104.98	0.03	HSP17.6B	266294_at	HSP20-like chaperones superfamily protein
AT2G47180	0.99	0.95	1.32	0.30	0.54	0.18	5.21	0.18	6.87	0.00	1.82	0.17	34.01	0.00	29.85	0.00	26.19	0.04	GOLS1	263320_at	Galactinol synthase 1
AT4G36990	0.97	0.92	1.60	0.20	0.52	0.14	5.36	0.06	8.94	0.06	3.44	0.00	77.52	0.01	74.90	0.01	57.55	0.01	HSFB1	246214_at	Heat shock transcription factor B1
AT5G58770	1.00	0.97	1.66	0.18	0.90	0.55	6.14	0.21	7.19	0.14	4.45	0.15	24.64	0.08	31.03	0.04	16.55	0.01		247780_at	Undecaprenyl pyrophosphate synthetase family protein
AT2G32120	0.99	0.97	0.99	0.95	0.87	0.17	12.06	0.11	10.86	0.03	2.93	0.14	126.82	0.01	104.49	0.01	96.54	0.03	HSP70T-2	265675_at	Heat-shock protein 70T-2
AT5G10695	0.95	0.90	1.10	0.76	0.84	0.80	9.07	0.04	7.60	0.02	3.31	0.06	113.47	0.01	83.39	0.01	82.34	0.03		246018_at	Unknown protein
AT3G25230	0.98	0.94	0.88	0.06	0.92	0.74	4.48	0.12	4.76	0.12	2.72	0.02	34.39	0.02	33.16	0.01	31.95	0.03	ROF1	257822_at	Rotamase FKBP 1
AT3G51910	0.99	0.96	0.91	0.24	1.06	0.70	4.60	0.01	4.32	0.02	2.65	0.04	34.01	0.01	25.64	0.02	30.31	0.03	HSFA7A	252081_at	Heat shock transcription factor A7A
AT1G16030	1.00	0.99	1.16	0.22	0.98	0.74	26.67	0.03	34.48	0.03	6.63	0.18	340.51	0.00	301.70	0.01	286.78	0.02	HSP70b	261838_at	Heat shock protein 70B
AT1G26800	1.00	1.00	0.95	0.17	1.23	0.36	10.17	0.03	10.86	0.02	3.92	0.04	48.05	0.00	40.84	0.01	35.43	0.02		261265_at	RING/U-box superfamily protein
AT1G30070	1.00	0.98	0.96	0.51	1.06	0.61	15.65	0.10	12.33	0.08	5.80	0.00	142.27	0.01	113.05	0.00	130.83	0.01		260025_at	SGS domain-containing protein
AT5G35320	0.99	0.95	1.03	0.85	1.08	0.32	13.94	0.05	12.89	0.04	6.27	0.03	92.65	0.02	80.82	0.01	85.69	0.03		246612_at	Unknown protein
AT3G16050	0.99	0.95	0.86	0.58	1.26	0.65	14.86	0.06	15.43	0.00	6.92	0.05	162.86	0.00	149.22	0.00	147.97	0.03	PDX1.2	258336_at	Pyridoxine biosynthesis 1.2
AT3G12050	0.98	0.94	1.01	0.84	1.02	0.80	5.43	0.07	5.97	0.02	3.25	0.01	35.77	0.00	30.38	0.01	28.28	0.02		256663_at	aha1 domain-containing protein
AT5G09590	0.99	0.95	0.97	0.01	0.81	0.22	7.69	0.00	9.04	0.01	4.75	0.04	80.59	0.01	71.41	0.00	68.11	0.02	MTHSC70-2	250502_at	Mitochondrial HSO70 2
AT1G07350	0.99	0.95	0.95	0.81	1.02	0.43	12.86	0.08	12.75	0.03	8.63	0.00	48.95	0.02	43.22	0.00	37.32	0.02	SR45a	261081_at	RNA-binding (RRM/RBD/RNP motifs) family protein
AT3G09440	1.00	0.98	1.06	0.59	0.94	0.44	15.75	0.03	12.69	0.09	9.01	0.08	56.78	0.02	49.70	0.02	47.36	0.02		258979_at	Heat shock protein 70 (Hsp 70) family protein
AT4G12400	1.00	0.98	1.19	0.41	0.99	0.98	32.71	0.06	30.27	0.05	13.00	0.05	171.53	0.00	152.08	0.00	145.27	0.01	HOP3	254839_at	Stress-inducible protein, putative
AT3G07090	1.00	0.97	0.99	0.83	0.74	0.40	9.43	0.08	9.97	0.07	5.61	0.04	45.97	0.00	37.63	0.01	41.28	0.03		258830_at	PPPDE putative thiol peptidase family protein
AT4G23570	0.95	0.90	0.94	0.69	1.23	0.46	7.65	0.13	7.42	0.11	6.26	0.14	43.25	0.05	30.51	0.00	37.74	0.02	SGT1A	254211_at	Phosphatase-related
AT1G54050	1.00	1.00	0.98	0.78	1.32	0.31	71.51	0.00	71.52	0.00	36.53	0.03	195.21	0.00	156.21	0.00	151.22	0.02	HSP17.4	263150_at	HSP20-like chaperones superfamily protein
AT3G09350	0.99	0.95	1.17	0.57	1.42	0.25	108.66	0.02	93.26	0.02	76.75	0.00	187.54	0.01	155.87	0.00	165.78	0.02	Fes1A	259037_at	Fes1A
AT3G24500	0.97	0.92	1.39	0.65	0.62	0.16	86.33	0.04	85.07	0.01	35.92	0.01	240.40	0.01	190.96	0.00	184.66	0.03	MBF1C	258133_at	Multiprotein bridging factor 1C
AT5G52640	1.00	0.99	1.13	0.49	0.69	0.03	49.50	0.01	51.41	0.00	24.06	0.05	176.39	0.00	149.88	0.01	134.73	0.03	HSP90.1	248332_at	Heat shock protein 90.1
AT3G12580	0.97	0.93	1.49	0.00	0.41	0.18	34.48	0.04	42.84	0.01	19.39	0.03	83.76	0.01	75.19	0.01	68.70	0.03	HSP70	256245_at	Heat shock protein 70
AT5G48570	1.00	0.99	1.33	0.06	0.53	0.21	45.26	0.03	46.35	0.01	11.95	0.00	143.37	0.00	128.58	0.01	117.63	0.02	ROF2	248657_at	FKBP-type peptidyl-prolyl cis-trans isomerase family protein
AT5G05410	0.99	0.96	1.30	0.01	0.64	0.07	85.07	0.02	95.38	0.00	56.15	0.02	66.95	0.02	35.83	0.00	37.61	0.05	DREB2A	250781_at	DRE-binding protein 2A
AT1G67360	0.99	0.95	1.56	0.27	0.54	0.03	4.29	0.28	11.14	0.12	0.42	0.09	75.03	0.01	68.90	0.00	49.41	0.02		264968_at	Rubber elongation factor protein (REF)
AT5G12110	1.00	0.99	1.85	0.00	0.51	0.06	4.12	0.05	6.52	0.06	0.60	0.47	38.62	0.02	35.94	0.00	26.57	0.02		250304_at	Glutathione S-transferase
AT1G17870	1.00	0.99	1.91	0.16	0.50	0.08	4.96	0.19	7.21	0.05	1.11	0.61	32.09	0.06	34.92	0.01	34.07	0.02	EGY3	255891_at	Ethylene-dependent gravitropism-deficient and yellow-green-like 3
AT1G03070	0.96	0.91	1.12	0.58	1.09	0.73	3.70	0.25	6.19	0.10	0.85	0.77	168.09	0.03	180.81	0.02	119.03	0.01		263164_at	Bax inhibitor-1 family protein
AT4G26270	1.00	0.99	0.94	0.77	0.74	0.32	1.45	0.14	1.66	0.18	0.91	0.64	34.58	0.02	30.64	0.03	21.60	0.05	PFK3	253987_at	Phosphofructokinase 3
AT5G47610	0.98	0.93	1.29	0.36	0.41	0.20	1.44	0.61	2.19	0.37	0.29	0.10	28.57	0.06	30.47	0.01	22.71	0.03		248759_at	RING/U-box superfamily protein
AT5G59720	1.00	0.97	7.05	0.02	0.72	0.22	4.64	0.00	6.94	0.08	1.20	0.42	259.01	0.00	225.01	0.00	195.59	0.02	HSP18.1	247691_at	Heat shock protein 18.2
AT4G25340	0.97	0.92	1.14	0.47	0.87	0.79	0.76	0.58	0.86	0.70	0.50	0.24	32.27	0.01	30.23	0.00	20.23	0.00	FKBP53	254076_at	FK506 BINDING PROTEIN 53
AT5G43170	0.99	0.97	1.41	0.16	0.88	0.50	2.34	0.04	3.06	0.03	2.20	0.04	47.00	0.00	50.63	0.01	9.32	0.04	ZF3	249139_at	Zinc-finger protein 3