Supplemental Figure Legends

Supplemental Figure S1. Two independent transient expression experiments of *AtHSBP-GFP* in mesophyll protoplasts. Wild-type Arabidopsis protoplasts were transfected with the *AtHSBP-GFP* construct and treated without HS (CK) or with HS at 37°C for 1 h (H1R0) and then recovered from HS for 1 h (H1R1) or 2 h (H1R2) as described in Fig. 5A. A. Two independent experiments with similar results are shown in Fig. 5A. B, Five continuous confocal scans with 1 μm optical sectioning thickness and the confocal plane of sequential-3 as the representative shown in Fig. 5A, H1R1. Scale bars indicate 20 μm.

Supplemental Figure S2. Transient expression of a control construct, *GUS-GFP*, in mesophyll protoplasts. Wild-type mesophyll protoplasts were transfected with the *GUS-GFP* construct and were treated without HS (CK) or with HS at 37°C for 1 h (H1R0) then recovered from HS for 1 h (H1R1). Similar results were obtained from 3 independent replicates, and representative images are shown. The scale bar indicates 20 μm.

Supplemental Figure S3. Transient expression of the *AtHSBP-GFP* in *Athsbp-1* mutant mesophyll protoplasts. The *Athsbp-1* protoplasts were transfected with the construct of *AtHSBP-GFP*, and treated without HS (CK) or with HS at 37°C for 1 h (H1R0) and then recovered from HS for 1 h (H1R1) or 2 h (H1R2) as described in Fig. 5. The scale bar indicates 20 μm.

Supplemental Figure S4. *AtHSBP* and *HSP* gene expression were analyzed by real-time quantitative PCR and normalized by the internal control, *EF1α*. *EF1α* (At5g60390) transcript was the quantitative control compared with *AtACT2* for *AtHSBP* (A) and *HSP* (B) gene expression analysis, as shown in the Figs. 2A and 8A, respectively.

Supplemental Table S1. Comparison (percent identity, percent similarity) of the amino acid sequences of the α-helix region for AtHSBP and other organisms.

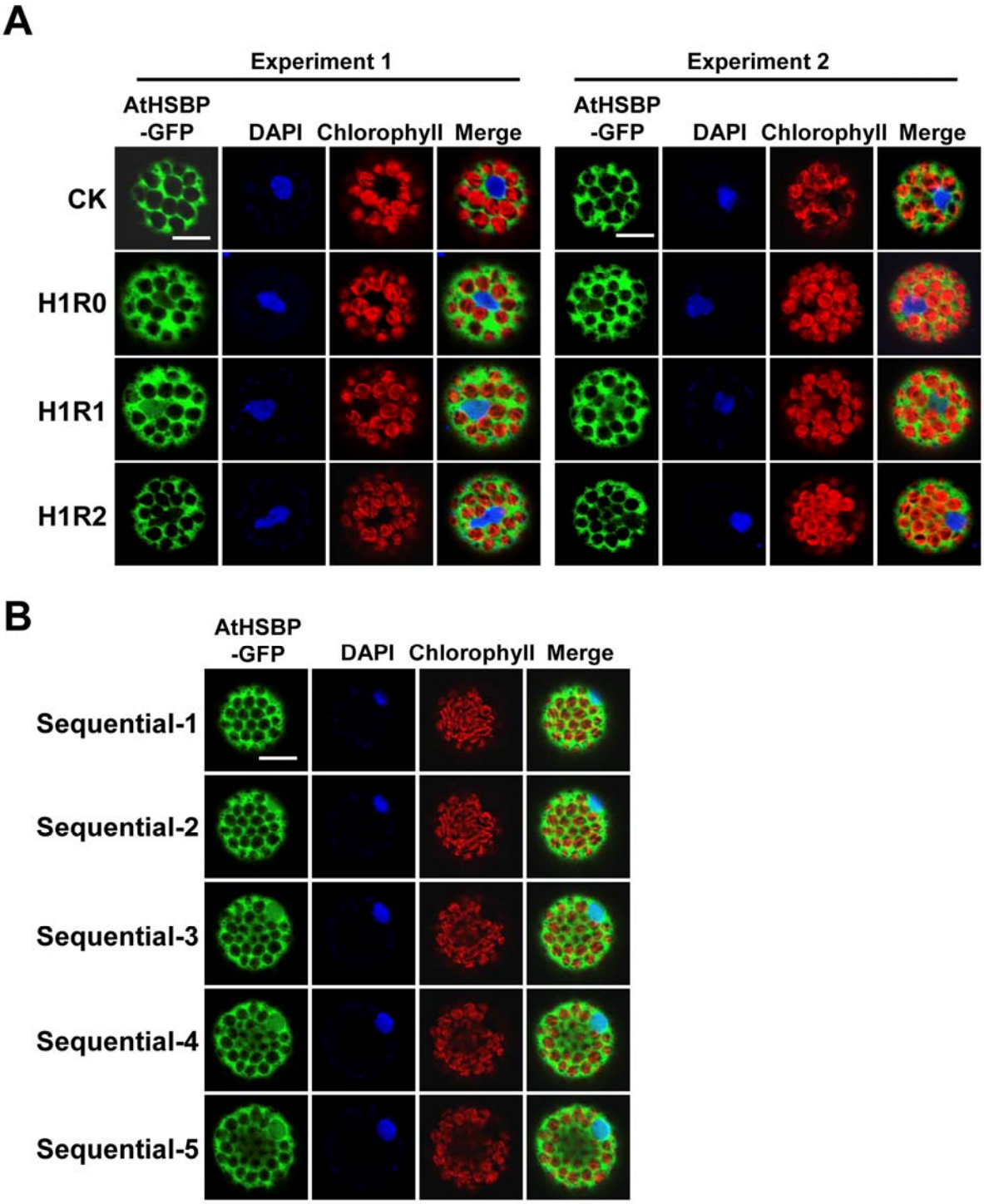
Gene	Local Name	Accession Number	Genus Species	% Identity	% Similarity
AtHSBP	Arabidopsis	NP_849392.4	*Arabidopsis thaliana*		
LeHSBP1	Tomato	AW624356	*Lycopersicon esculentum*	84	93
ZmHSBP1 (EMP2)	Maize	AAM15929	*Zea mays*	76	91
ZmHSBP2	Maize	AAR18070	*Zea mays*	78	91
OsHSBP1	Rice	AU075659	*Oryza sativa*	71	91
OsHSBP2	Rice	BE040146	*Oryza sativa*	80	93
HsHSBP1	Human	NP_001528	*Homo sapiens*	62	84
MmHSBP1	Mouse	NP_077181	*Mus musculus*	58	80
XtHSBP1	Xenopus	NP_001011422	*Xenopus tropicalis*	64	82
DrHSBP1	Zebrafish	AAH59566	*Danio rerio*	62	82
CeHSBP1	C. elegans	NP_502406	*Caenorhabditis elegans*	58	78

The comparison of the α-helix region among different organisms was analyzed by Vector NTI 10 (Invitrogen).

Supplemental Table S2. Primers for genotyping, cloning, mutation, real-time quantitative PCR and EMSA. Restriction enzyme sites were underlined as indicated.

Primer	Sequence (5' to 3')	Tm (^{o}C)
Genotyping		
HSBP-5'UTR-Fw	GTCTCTCATCGCTTTTCGCTG	62.5
HSBP-CDS-Rv	AGAGGAACTAGCCGGTGTTTT	59.3
Cloning		
HSBP-CDS-NcoI-Fw	TCTCCATGGATGGTCATGATTCTGAGGATA	70.8
HSBP-CDS-NcoI-Rv	TCTCCATGGCAGAGGAACTAGCCGGTGTTT	75.2
HSBP-Pro-XhoI-Fw	TCTCTCGAGTGGAAGCCGAGACTGTAGAAG	71.3
HSBP-Pro-NcoI-Rv	TCTCCATGGCAGAGGAACTAGCCGGTGTTT	75.2
AtHSBP$_{38-86}$-Fw	TCTGAATTCATCACAAAGATTGATGACATGG	57.4
AtHSBP$_{39-86}$-Rv	TCTGAATTCAGAGGAACTAGCCGGTGTTTT	59.3
Mutation of S35A		
HSBP-CDS-NcoI-Fw	TCTCCATGGATGGTCATGATTCTGAGGATA	70.8
HSBP-CDS-NcoI-Rv	TCTCCATGGCAGAGGAACTAGCCGGTGTTT	75.2
S35A-Fw	CAGACAATGGCCGACTCCATCATCACAAAG	74.7
S35A-Rv	GATGGAGTCGGCCATTGTCTGGAACCTGGT	77.2
Real-Time Quantitative PCR		
AtHSP101 (At1g74310)	CACCTCCTTGCAGGGCTAACT	62.9
	CGAACACCACAATCTCGTCAA	62.1
AtHSP70 (At2g32120)	GGATGAGATATACAAAGGCGTGAA	61.5
	AGGTGTGGCTTGTATGGTTAACAG	61.5
AtsHSP18.2 (At5g59720)	GGATTCTTCACGCCATCTTCTG	63.2
	ATGTGCCTCCGGCGTTT	62.3
AtsHSP17.4 (At3g46230)	CGTGTTCGACCCATTTTCACT	62.2
	CCTCCAGTCCACTTTAGCGTTT	61.9
AtACT2 (At3g18780)	TTGCACCAAGCAGCATGAA	62.6
	GCTGAGGGAAGCAAGAATGG	62.2
AtPP2A (At1g13320)	CCTGCGGTAATAACTGCATCT	59.3
	CTTCACTTAGCTCCACCAAGCA	61.8
EF1α (At5g60390)	ATTGCCACACCTCTCACATTG	61.0
	ATACCAGCGTCACCATTCTTC	59.0
HSE Probe for EMSA		
HSE-Fw	GGCGGCTTCAAGAAGCTTCTCTTCAAGAAGCTTCT	-
HSE-Rv	GGCGGAGAAGCTTCTTGAAGAGAAGCTTCTTGAAG	-

Supplemental Figure S1



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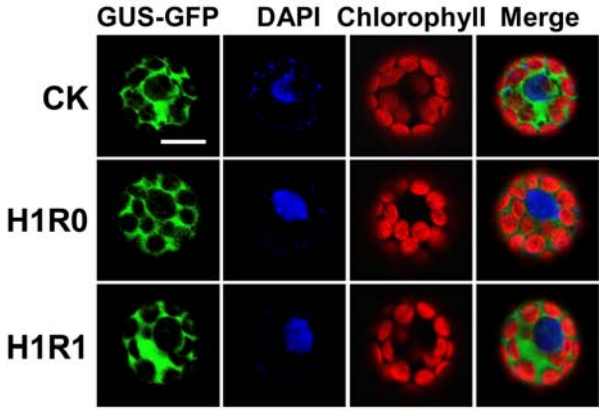

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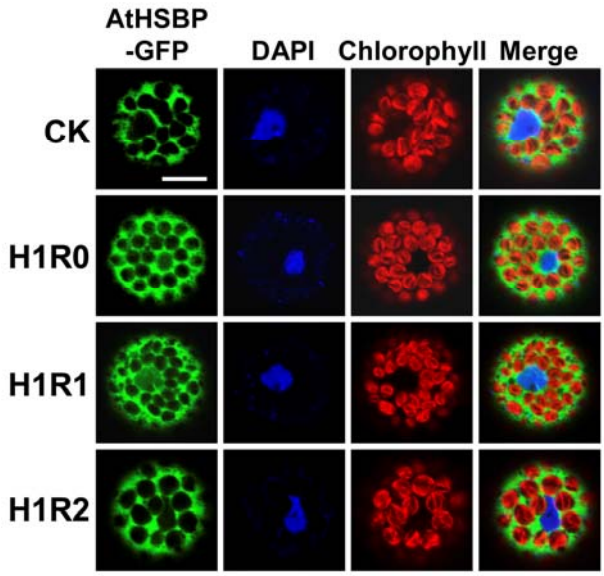
Supplemental Figure S3



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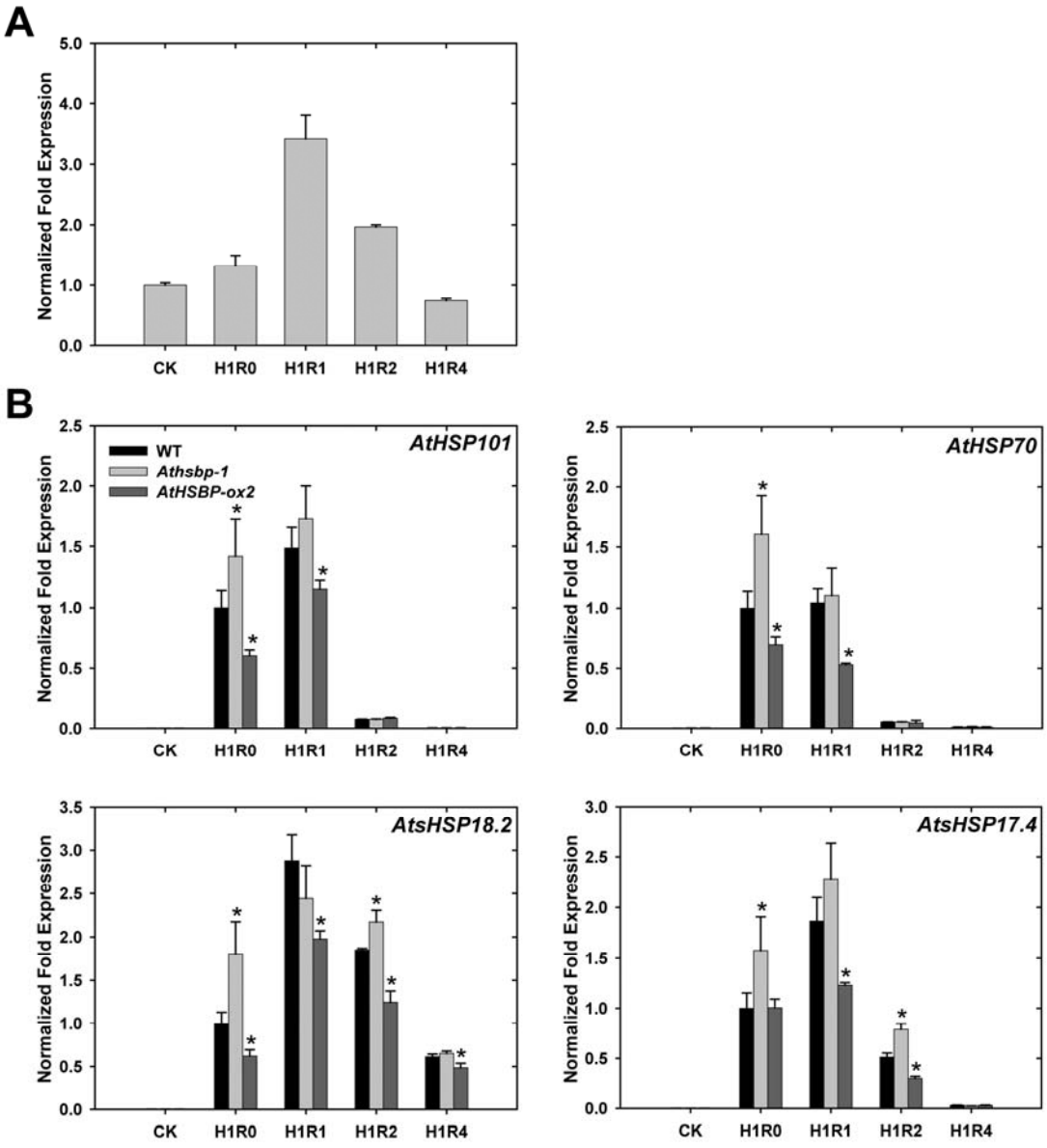
Supplemental Figure S4



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