Supplementary data

Table S1: Constructs with primers and cloning procedures

It is essential to clarify the nomenclature of functional and non-functional versions of the Hsf consensus binding sites (Nover, 1987; Nover, 1991; Scharf et al., 2001), which comprise at least two palindromic modules of 5 nucleotides: aGAAnnTTCt. The first half is called the head module (H), whereas the second is called the tail module (T). The exact pattern of the nucleotides and the presence of the invariant G and C residues (underlined) are essential. In addition, for a functional module at least one of the two nucleotides indicated by large case letters (AA, TT) must be present. The flanking adenosine and thymine residues (a, t) are frequently found but are not essential. Clusters of HSE modules, which mediate Hsf binding, are defined by an uninterrupted pattern of several modules (see example in construct no.1). The pattern must be precise, but not all modules of a cluster need to be functional. The nomenclature system is best illustrated by the example of the TATA distal HSE cluster in construct 1.

No	Promoter-leader fragment	Putative HSE configuration (underlined; x, any nucleotide) of promoter with **TATA** box and start codon (ATG) (gene accession number) sequence
Gus reporter constructs		
1	*GmHsp17*-GUS*	Fragment of soybean Hsp17.3B-CI promoter (Schöffl et al., 1984; Treuter et al., 1993) HSE configuration: 20x THtHtHT 42x HTH 33x **TATA** box aagcttggatccgtcgaagaagtccagaatgtttctgaaagtttc agaaaattctagttttgagattttcagaagtacggcatgatgatg cataacaaggactttctcgacctgcagtctagagtcgaccgcaag acccttcctc**TATAT**aaggaagttcatttcatttggagaggacac gctcgagtggccaccATG

MycHsp17.6A-CI reporter constructs For the MycHsp17.6A-CI reporter plasmids, promoter fragments were amplified from Arabidopsis genomic DNA using sequence specific primers and cloned into pRT104-MycHsp17.6A-CI (Kirschner et al., 2000).		
2	pRT104-*Myc Hsp17.6A-CI*	Kirschner et al., 2000
3	CaMV35S promoter	Potential ASF-1/2 binding motifs are bold-faced and HSEs are underlined: 97x HT 6x HTHThT 91x TH 42x HTHtHT 29x HT 16x HT 14x HT 2x **TATA** box aagcttgcatgcctgcaggtcaacatggtggagcacgacactctcgtct actccaagaatatcaaagatacagtctcagaagaccagagggctattga gacttttcaacaaagggtaatatcgggaaacctcctcggattccattgc ccagctatctgtcacttcatcgaaaggacagtagaaaaggaagatggct tctacaaatgccatcattgcgataaaggaaaggctatcgttcaagaatg cctctaccgacagtggtcccaaagatggacccccacccacgaggaacat cgtggaaaaagaagacgttccaacc**acgt**cttcaaagcaagtggat**tga** **tgtgata**tctccac**tgacg**taaggga**tgacg**cacaatcccactatcctt cgcaagacccttcctc**TATAT**aaggaagttcatttcatttggagaggac ctcgagaattcgagctcggtacccggccgcgagaaagagggggATG
4	AtAct2 (actin2) promoter	6x THtH 15x HtH 30x HTTH 48x TH 116x TH 31x HT 11x TH 5x TH 190x **TATA** box, (At3g18780) aagcttatgcatgcaagagtcagcatatgtataattgattcagaatcgt tttgacgagttcggatgtagtagtagccattatttaatgtacatactaa tcgtgaatagtgatatgatgaaacattgtatcttattgtataaatatcc ataaacacatcatgaaagacactttctttcacggtctgaattaattatg atacaattctaatagaaaacgaattaaattacgttgaattgtatgaaat ctaattgaacaagccaaccacgacgacgactaacgttgcctggattgac tcggtttaagttaaccactaaaaaaacggagctgtcatgtaacacgcgg atcgagcaggtcacagtcatgaagccatcaaagcaaaagaactaatcca agggctgagatgattaattagtttaaaaattagttaacacgagggaaaa ggctgtctgacagccaggtcacgttatctttacctgtggtcgaaatgat tcgtgtctgtcgattttaattatttttttgaaaggccgaaaataaagtt gtaagagataaacccgcc**TATATA**aattcatatattttcctctccgctt

		tgaattgtctcgttgtcctcctcactttcatcagccgttttgaatctcc ggcgacttgacagagaagaacaaggaagaagacctcgagtggccacATG
5	AtHsfC1 promoter	30x HT 133x THtH 50x THtHTH 17x HT 13x ThTHHtHT 44x TH 167x TH 115x **TATA** box (At3g24520, Nover et al., 2001) cagctgttttaagttaaaatctgaatacacagcatgttccttctaacgt tttttaacataattgtaaactaaagaaaaattataatataataaattaa attataccctctctattcattgtagaagatttgtttgtttcaccaaatg tgtttttttttataagttgctctcactcttgagagttttggagtattt tgaagaggacgaggttcttttgtgaaaaagtgaaagaaacaaatcatgt caagaattagaaagaaatcatgacaaataattttgtttgtgcagaaatt acaaatgaatcatagatatccttctaattcgggatcggaaaccagttga aaaatcgatggactaagctttctatattgggactagcttacccttagaa ttagtcaagcagcttaaatcatctatgacttaaaattataattaagaaa aaacaatgcctaaatatgcatatatttcaaatgtatcacataacttgtg acataagaaaatataaacaaaacaaaaagggcaaaaaagacctgaaagc ttagaggcacacctgcataggtcccacagttcactcgtgacaccgtaaa aggcaaaacacgaacccgccacgttatcacaaaaagcaagccacgtcaa tatagtctcactgtcaactacacttaacttactattttcacatctcatt ttcctatctt**TATATA**aaccctccaggctcctctttaatttctttacca ccaccaacaacaaacatataaaccataaggaaaacagagctcgagtggc caccATG
6	AtDnaj promoter	46x HTH 58x TH 16x TH 16x THtH 13x TH 118x HT 111x TH 2x HT 5x HT 281x TH 72x HT 10x **TATA** box (At4g36040) gtcgacttttcttctatttgaatgtttagcaaaagaaaaaataaaccga ctttccttgattataacattgacaaccattagtaatctattataataag atacatttgaaaacatttatattacttgatcatttgtattttatcgtaa tctcgtacaaacaccgttagttggtatcatgaaattctgtgagctaaga aaacaatttctcacgaatcaaatttgcagattaccattggctttttata tttccattcctatagtaagaaaataataaagcacataaatattaaaaaa aatgtttgtatggtggaggcaatctttaaatacagtagagacttttctc aatcttctaaaaatctatttctgtcttctcaatatccaacaaacaatac atggtccaaattacgtctccatacaccaattatatttttataaataaa

		agaaaaaaaaatctactcgtaataagaagattctaatttcgaatttcca ccttaaaatactcttctgctaaagaaaattaaaaaaaaaaaaaaatgat aaataactaaaaaccaaaacgtgattagtatttctgtgcaaattaaata ttggattcctttggcattaatatatttttgtaagaatatgttaaaatga caattacagccacagaacaatttggccactatgaataatatcttacgta ctacattcttatctcttctgcaattatttccccaactggataagccttt tttatctgactcagatcacaggatccgacccgactttttacccgacccgt aacttaatcccctatatccgttttagtatgtaattaataaactattcaa aatcttaattaaccaattaatgactaatcgcctcttcgcc**TATAAAT**ta aacccctccattacctttcttcttcaatctttccctctcctcctcgaac aaaaacaacaaacgcagagaaactcaaaactcgaaaacgtttcctcgag tggccaccATG
7	AtHsc70 promoter	177x THtH 8x HtH 10x HT 43x HT 31x HT 95x HTH 50x HT 2x HT 18x **TATA** box (At5g02500) aagctttgaaatagaagaaaaagcctttttccttttgacaacaacatat aaaatcatactcccattaaaaagattttaatgtaaaattctgaatataa gatattttttacaacaacaaccaaaaatatttatttttttcctttttta cagcaacaagaaggaaaaactttttttttgtcaagaaaaggggagatt atgtaaacagataaaacagggaaaataactaaccgaactctcttaatta acatcttcaaataaggaaaattatgatccgcatatttaggaagatcaat gcattaaaacaacttgcacgtggaaagagagactatacgctccacacaa gttgcactaatggtacctctcacaaaccaatcaaaatactgaataatgc caacgtgtacaaattagggttttacctcacaaccatcgaacattctcga aacattttaaacagcctggcgccatagatctaaactctcatcgaccaat tttgaccgtccgatggaaactctagcctcaacccaaaactc**TATATA**a agaaatcttttccttcgttattgcttaccaaatacaaaccctagccgcc ttattcgtcttcttcgttctctagttttttcctcagtctctgttcttag atcccttgtagtttccaaatctcgagtggccaccATG
8	AtHsp70 promoter	129x TH 78x HtHtHthtHtHtH 17x HtH 47x THtHTHT 82x HT 2x HT 108x THTH 54x THT 20x **TATA** box (At1g16030) aagcttaaagcaatcgagttaaaacgagaaattcagtttctttaattct cacagagaacctcagagatgaactatactcaccgagcatttctctgggt ttcgtcggaacaagctgtagatgattaccacgatcgggaactcaataat

		ctgaatatcaacatcaaaacaaaaaggctaaaattaactgaaaaatatc cactagcaaccaggttatgaaagaaagttttagtacccataggagacgc agagtgagagttggatcagaaatgagatcgacagagtatttgttacgga ccacgtgaaatccgaagatcagaaataacccagtaatcacataaacagc aaaagcccccaagttgatatcgtgatactaacggagatttctggattc ttcttctccttcgcctctttcatggcttttcctttctcgtcttcgaaat cacagaacaagtgaagaaagaagacgtaaacaaaatattgaaaatcctc cagaacttacactgggccttttattctatatacgggcctacaagtttat accatatgggctttaataggcccatttaattatcaagcggtcgccggag ataaaatatatcccggtcggtgaatccagaactctcttgtacgtttgcg cgatttctccacctttccacaatcccctgggttgtgccacgaccttttt tctcgaaatgtctcgttcctctcgtcggattcg**TATATA**tagcttcttc catcgtttccgattcttcatcaaacagataaacaaacaaaagaaatcga aaaacctcacttccaatttcattcaattactcgagtggccaccATG
pRT based reporter constructs containing Ds-Red/Gfp as reporters		
9	*AtHsc70 P/L-DsRED*	Subcloning of SalI-XbaI fragment of Ds-red ORF (clonetech) into pRT-*AtHsc70-P/L-MycHsp17.6ACI*
10	*AtHsp70 P/L-GFP*	Subcloning of NcoI-XbaI fragment of Gfp ORF (clonetech) into pRT-*AtHsp70-P/L-MycHsp17.6ACI*
pRT based Hsf expression plasmids		
11	LpHsfA1	Treuter et al., 1993
12	LpHsfA1-M5 (R93>D) (DNA binding mutant)	1.F:5´-gacgcacaatcccacta-3´ 1.R:5´-cagctggtcgacaaagctggaaaagttattatgc-3´ 2.F:5´-agctttgtcgaccagcttaatacttatgg-3´ 2.R:5´-cctgaagagtgactcctgaaacacg-3´ (PCR amplification of 5′ fragment of HsfA1 with mutation of R93 using primers 1.F and 1.R; triple ligation of 5´fragment with 3′ fragment (Pr. 2.F-2.R) into pRT101)
13	LpHsfA1 ΔHRA/B	HsfA1 without oligomerization domain (aa 23-161,VD,239-527) generated by subcloning of SalI-XbaI fragment of pRTHsfA1.71 into pRTHsfA1.8 (Mishra et al., 2002)
14	LpHsfA1-M3	NLS mutant of HsfA1 (Lyck et al., 1997)

15	LpHsfA1ΔC394	HsfA1 with deletion of C-terminal activation domain (Treuter et al., 1993)
16	LpHsfA1-A7	Inactive HsfA1 with hepta alanine substitutions of aromatic and hydrophobic residues in AHA1 and AHA2 motifs (451-IDWQSGLL 12aa DPFWEKFL->451-IDAQSGAA 12aa DPAAEKAA) (Döring et al., 2000)
17	LpHsfA1 (aa23-394) xGal4AD(aa768 -881)	F:5´-tgggtgctagcgccaattttaatcaaagtggg-3´ R:5´-gtatctacgattcatagatctctgc-3´ PCR amplification of Gal4AD using the indicated forward and reverse primers and cloning into pRT-LpHsfA1c394 via NheI-XbaI
18	LpHsfA1 (aa23-394) xVP16(aa412-490)	F:5´-gactggctagcaccgcccccattaccgacg-3´ R:5´-cacacattattctggag-3´ PCR amplification of VP16AD using the indicated forward and reverse primers and cloning into pRT-LpHsfA1c394 via NheI-XbaI
19	Gal4DBD(aa1-147)xLpHsfA1-CTD(aa314-527)	F:5´-cctgaaagtttctcgagtggtgatggttca-3´ R:5´-atatagagcggccgcttgcggactctagatg-3´ PCR amplification of LpHsf C-terminal fragment using the indicated forward and reverse primers and cloning into pBI-Gal4DBD via XhoI-NotI
20	LpHsfA2	Treuter et al., 1993
21	LpHsfB1	Treuter et al., 1993
22	LpHsfB1-M4 (KH(54,55)>EL	DNA binding mutant (Boscheinen et al., 1997)
23	LpHsfB1ΔHRA/B	HsfB1 without oligomerization domain (aa 1-142, VD,214-301) F:5´-tccagcgtcgacgagttggtcaaggaggttgg-3´ R:5´-cacacattattctggag-3´ Cloning of the PCR product into pRTHsfB1.25 (containing a unique SalI site at 144,145) via SalI-XbaI.
24	LpHsfB1ΔNLS	HsfB1 without NLS motif (aa 1-213,VD,268-301)

		F:5´-gagaatgtcgacacttgtggtggacgtgg-3´ R:5´-cacacattattctggag-3´ Cloning of the PCR product into pRTHsfB1.26 (containing a unique SalI site at codons 213,214) via SalI-XbaI.
25	LpHsfB1ΔCC198	Treuter et al., 1993
26	LpHsfB1ΔC294	Treuter et al., 1993
27	LpHsfB1ΔC286	Treuter et al., 1993
28	LpHsfB1ΔC274	Treuter et al., 1993
29	LpHsfB1 (WM286/7>SR)	F:5´-ggactataatggtccctcgaggaaaatgtcttcg-3´ R:5´-cacacattattctggag-3´ PCR mutagenesis of C-terminal fragment of HsfB1 in pRT-LpHsfB1, cloning via XhoI-XbaI
30	LpHsfB1 (Y282>A)	F:5´-atgaaaactgtcgac**gc**taatggtccttgg-3´ R:5´-cacacattattctggag-3´ PCR mutagenesis of C-terminal fragment of HsfB1 in pRT-LpHsfB1, cloning via SalI-XbaI
31	LpHsfB1 (GK274/5>VD)	F:5´-acttgtggtggacgtgtcgacatgatgaaaactgtg-3´ R:5´-cacacattattctggag-3´ PCR mutagenesis of C-terminal fragment of HsfB1 in pRT-LpHsfB1, cloning via SalI-XbaI
32	LpHsfB1 (K275>Q)	F:5´-gagaatgtcgacacttgtggtggacgtgg**cc**a**g**atgat gaaaaac-3´ R:5´-cacacattattctggag-3´ PCR mutagenesis of C-terminal fragment of HsfB1 in pRT-LpHsfB1, cloning via SalI-BamHI
33	LpHsfB1Δ272-279	F:5´-aaaactgt**c**gactataatggtccttgg-3´ R:5´-cacacattattctggag-3´ Cloning via SalI-XbaI into pRTB1.23, containing a unique SalI site at 271,272
34	LpHsfB1 (GRGK272-275>LWTT)	F:5´- gagaatgtcgacacttgtggt**cttt**g**gac**ta**cc**atgat gaaaactgtgg-3´ R:5´-cacacattattctggag-3´ PCR mutagenesis of C-terminal fragment of HsfB1

		in pRT-LpHsfB1, cloning via SalI-XbaI
35	LpHsfB1 (GRGK272-275> GKGR)	F:5´-gagaatgtcgacacttgtggtgga**aaa**ggt**cg**aatgat gaaaactgtgg-3´ R:5´-cacacattattctggag-3´ PCR mutagenesis of C-terminal fragment of HsfB1 in pRT-LpHsfB1, cloning via SalI-XbaI
36	LpHsfB1 (K278>R)	F:5´-acttgtgtcgaccg**c**ggtaaaatgatg**cgt**actgtgg-3´ R:5´-cacacattattctggag-3´ PCR mutagenesis of C-terminal fragment of HsfB1 in pRT-LpHsfB1, cloning via SalI-BamHI
37	LpHsfB1 (aa1-198)xNtHsfB1 (aa204-298)	F:5´-tgatcgctagcatcattagccaaggaacctc-3´ R:5´-atttggtctagatcttcagttacagaccttgttac-3´ PCR amplification of NtHsfB1 CTD using the indicated forward and reverse primers and cloning into pRT-LpHsfB1c198 via NheI-XbaI
38	LpHsfB1 (aa1-198)xGmHsfB1 (aa204-282)	F:5´-agatcgctagcatcatgcggcaaggaag-3´ R:5´-gagcctctagattcagttgcaaaccctgttg-3´ PCR amplification of GmHsfB1 CTD using the indicated forward and reverse primers and cloning into pRT-LpHsfB1c198 via NheI-XbaI
39	LpHsfB1 (aa1-198)xAtHsfB1 (aa200-284)	F:5´-ccggaagctagcgataaaatgatc-3´ R:5´-cacacattattctggag-3´ PCR amplification of AtHsfB1 CTD using the indicated forward and reverse primers and cloning into pRT-LpHsfB1c198 via NheI-XbaI
40	Gal4DBD(aa1-147)xLpHsfB1-CTD(aa200-301)	F:5´-gttgcaccggataagatctaccgtatcatgagcc-3´ R:5´-attagagcggccgctcagttacaaaccttgctg-3´ PCR amplification of C-terminal fragment of LpHsfB1 using the indicated forward and reverse primers and cloning into pBI-Gal4DBD via BglII-NotI
pRT based vectors containing Human and At-CBP and TGA constructs		
41	HsCBP	Subcloning of SacI-NdeI fragment from pcDNA3-CBP (kindly provided by M. Rosenfeld, UCSD) into

		pRT101
42	AtCBP(HAC1)	1.F:5´-gcctaaggcaagactcgagaaaaag-3´ 1.R:5´-aatttcccgggcctaggttacctgcaacgaggtacatggc-3´ (XhoI/BlnI-XbaI/At-cDNA/pRT101)=pRT101-fragCTD 2.F:5´-ggggcaggtgtcgacccctaggactatgtcgggg-3´ 2.R:5´-ctttttctcgagtcttgccttaggc-3´ (XhoI/At-cDNA/pRT101-fragCTD)
43	HAC1-NTD (aa1-988)	F:5´-ggggcaggtgtcgacccctaggactatgtcgggg-3´ R:5´-gtcgccccgggattccctaggttatggaatacaaaagtaatg-3´ PCR amplification of At-cDNA using the indicated forward and reverse primers and cloning of PCR products into pRT101 via BlnI-XbaI
44	HAC1-CTD (aa984-1654)	F: 5´-caccgtaggagcgtcgaccctaggatgtacttttgtattcc-3´ R: 5´-aatttcccgggcctaggttacctgcaacgaggtacatggc-3´ PCR amplification of At-cDNA using the indicated forward and reverse primers and cloning of PCR products into pRT101 via BlnI-XbaI
pJC based bacterial vectors for expression of N and C-terminal His tagged proteins (Clos and Brandau, 1994)		
47	LpHsfA1 - CHis6	Subcloning of EcoRI-ApaI fragment from pRT101-HsfA1LS into pJC20
48	LpHsfB1-CHis6	F: 5´-aagaggcatatgtcgcaaagaacagcg-3´ R: 5´-catagggatccacgcggaaccaagttacaaaccttgctgctttc-3´ PCR amplification of B1 cDNA using the indicated forward and reverse primers and cloning of PCR products into pJC40 via NdeI-XbaI
49	NHis6-At HAC1NTD	F: 5´-ggggcaggtgtcgacccctaggactatgtcgggg-3´ R: 5´-gtcgccccgggattccctaggttatggaatacaaaagtaatg-3´ PCR amplification of At-cDNA using the indicated

		forward and reverse primers and cloning via SalI/XhoI-XbaI
50	NHis6-At HAC1CTD	F: 5´-caccgtaggagcgtcgaccctaggatgtacttttgtattcc -3´ R: 5´-aatttcccgggcctaggttacctgcaacgaggtacatggc-3´ PCR amplification of At-cDNA using the indicated forward and reverse primers and cloning via BlnI-XbaI
pβstop based vectors for in vitro transcription pβstop based vectors containing WT and mutant HsfB1 for in vitro transcription-translation (vector kindly provided by Mr. Michael Jantzen, UC Berkely)		
51	LpHsfB1	Subcloning of XhoI-XbaI fragment of pRT-HsfB1 into pβstop
52	LpHsfB1 Δ272-279	Subcloning of XhoI-XbaI fragment of pRT-HsfB1Δ272-279 into pβstop
pcDNA3 based animal expression vectors All Hsf encoding fragments from the corresponding pRT vectors were cloned via XhoI/XbaI into pcDNA3		
53	LpHsfA1	Heerklotz et al., 2001
54	LpHsfA1-M5	Subcloned from construct no. 12
55	LpHsfA1-A7	Subcloned from construct no. 16
56	LpHsfB1	Subcloned from construct no. 21
57	LpHsfB1-M4	Subcloned from construct no. 22
58	LpHsfB1Δ272 -279	Subcloned from construct no. 33
59	LpHsfB1 (GRGK272 - 275> GKGR)	Subcloned from construct no. 35
60	HsCBP	pcDNA3-HsCBP plasmid was kindly provided by M. Rosenfeld, UC San Diego
61	HsFlagCBP	pcDNA3-HsFlagCBP plasmid was kindly provided by E.Pfitzner, Georg Speyer Haus, Frankfurt
62	p*GmHsp17*-	Luciferase reporter for mammalian cells with

<table>
<tr><td></td><td>LUC</td><td>plant Hsp17.3B promoter fragment; Heerklotz et al., 2001</td></tr>
<tr><td colspan="3">Vecotrs containing inverted repeats
Parts of the ORFs, were cloned into pJawohl vectors using the “Gateway Technology” (Invitrogen). Cloning into pJawohl vector creates inverted repeats of the same domain separated by a neutral intron.This inverted repeat containing vector when transformed into protoplasts generates sequence specific dsRNA.</td></tr>
<tr><td>63</td><td>pIR-AtHAC1</td><td>HAT domain (aa 1173-1611) of At-HAC1</td></tr>
<tr><td>64</td><td>pIR-LpHsfA1</td><td>CTD (aa 351-527) of Lp-HsfA1</td></tr>
<tr><td>65</td><td>pIR-LpHsp 17CII</td><td>Full length ORF of Lp-Hsp17CII</td></tr>
<tr><td colspan="3">Probes used for EMSA</td></tr>
<tr><td>66</td><td>GmHsp17.3B-CI-WT</td><td>THtHtHT50xTATA box
tacgccaagcttggatccgtcgaagaagtccagaatgtttctgaaagtttcagaaaattctagttttgagattttcagaagtacggccggtcgaccgcaaaacccttactcTATATaagg</td></tr>
<tr><td>67</td><td>GmHsp17.3B-CI-mutant</td><td>ththtHT50xTATA box
tacgccaagcttggatccgtcgaattcgtttaaaatgtttaatattgtttcagaaaattctagttttgagattttcagaagtacggccggtcgaccgcaaaacccttactcTATATaagg</td></tr>
</table>