Supporting Material

Table S1. Oligo-nucleotides used for generation of Hsf mutant constructs in this work. The first column indicates the construct, the second column the name of forward (F) and reverse (R) primers with the restriction sites used for subcloning, and in the third column the oligo-nucletoide sequence in 5' to 3' direction is given with the restriction sites underlined.

Construct	Primer	5'-3' sequence
pRT vector	F270 R179	GACGCACAATCCCACTATCC CACACATTATTCTGGAG
p3HA-HsfA1	R2584(*Sac*I)	GTACCTGGAGCTCGTAATCTGGAAC
pHsfA2-D	F1626(*Avr*II)	GTTGCCTAGGTTTTTCAAGCACAGCAATTTTTCCAGTTTCATTGATCAGC
pA2Δ139-164	F1390(*Sal*I)	CTTGCGTCGACCTTAAGCAGCAACAGCAGAGTA
pA2Δ192-211	F1066(*Sal*I)	TCATTGCCATGGGAGAAAAAATCGAAACACAGGAGAGGAAACAAGTCGACATGATG
pA2Δ167-211	R1067(*Afl*II)	TTCTCCCATGGCAATGATCTGATTCCTCGTACTCTGCTGTTGCTGCTTAAGTTTAAC
pA2Δ167-187	F1058(*Afl*II)	AAACACTTAAGAGGAAACAAGTCGACATG
pA2Δ167-180	F1057(*Afl*II)	ATTGCCCTTAAGGAAAAAATCGAAACAC
pA2Δ167-173	F1056(*Afl*II)	AGAGTCTTAAGAATCAGATCATTGCTATGGG
pA2Δ181-187	R14(*Afl*II)	CTCCTGTGTTTCGATTTTTTCCTTAAGGGCAATG
pA2Δ174-187	R1808(*Afl*II)	GGCAATGATCTGATTCTTAAGACTCTGCTG
pA1:A2DBD and pA2:A1DBD	F618(*Sal*I) R603(*Sal*I)	TTGTGTCGA**C**GTTGGGAAATTTGGGC CCCAACGTCGACACAAGCCCCAAC
pA1:A2CTAD and pA2:A1CTAD	F644(*Sal*I) R645(*Sal*I)	CAGTTTGTCGACCAGCAAAATGAGAGTAATAAGCG TTGCTGGTCGACAAACTGTGCCAAGAATCCAGG
pA2:A1HR-A/B	F1638(*Xho*I)	GTTGGGGCTTGTCTCGAGGTTGGG
pA2:A4bHR-A/B	F1642(*Xho*I) R1645(*Sal*I)	TGTAGCTCCATTGCTCGAGTCTGAGAGAC CTGCTCGCGCTGTCGACGAAATCAGATG

Table S2. Constructs used throughout the manuscript are listed. The first column gives the name of the expression constructs, in the second column the amino acid residues covered by the construct are indicated with their numbers corresponding to the original ORF of the wt form, the third column indicates introduced tags and in the fourth column references are given for all constructs generated previously.

Constructs	Amino acid sequences	Tags	References
HsfA1	23-527	3HA	Treuter et al,1993 (CAA47869)
pHsfA1-D	23-527(R95D)		Bharti et al, 2004
pHsfA1-H	23-162,VD,239-527		Bharti et al, 2004
pHsfA1-A	23-527(W452A, L456A, L457A, F469A, W470A, F473A, F474A)		Bharti et al, 2004
pHsfA1-1m	23-527(W452A, L456A, L457A)		Döring et al, 2000
pHsfA1-2m	23-527(F469A, W470A, F473A, F474A)		Döring et al, 2000
HsfA2	1-351	3HA, GFP	Treuter et al,1993 (CAA47870)
pHsfA2-D	1-351(R85D)	GFP	
pHsfA2-H	1-136,VD,213-351	GFP	Treuter et al, 1993
pHsfA2-A	1-351(W297A, W337E, L341A)	GFP	Döring et al, 2000
pHsfA2-1m	1-351(W297A)		Döring et al, 2000
pHsfA2-2m	1-351(W337E, L341A)		Döring et al, 2000
pA2Δ138-211	identical with pHsfA2-H		
pA2Δ138-164	1-136,VDLK,167-351	GFP	
pA2Δ192-211	1-190,VD,213-351	GFP	
pA2Δ138-187	1-136,VD,188-351(Q192D)		
pA2Δ166-211	1-164,LKRKQVD,213-351		
pA2Δ166-187	1-164,LK,188-351(Q192D)	GFP	
pA2Δ166-180	1-164,LK,181-351	GFP	
pA2Δ166-173	1-164,LK,174-351		
pA2Δ181-187	1-178,LK,188-351(Q192D)		
pA2Δ174-187	1-171,LK,188-351(Q192D)		
pA2Δ174-180	1-171,LK,181-351		
Hybrid Hsfs			
pA1:A2DBD	A1(23-162,VD):A2(139-351)		
pA2:A1DBD	A2(1-136,VD):A1(165-527)		
pA1:A2CTAD	A1(23-236,VD):A2(213-351)		
pA2:A1CTAD	A2(1-210,VD):A1(239-527)		
pA1:A2HR-A/B	A1(23-162,VD):A2(139-210,VD):A1(239-527)		
pA2:A1HR-A/B	A2(1-136,VE):A1(165-236,VD):A2(213-351)		
pA2:A4bHR-A/B	A2(1-136,VE):A4b(127-198,VD):A2(213-351)		

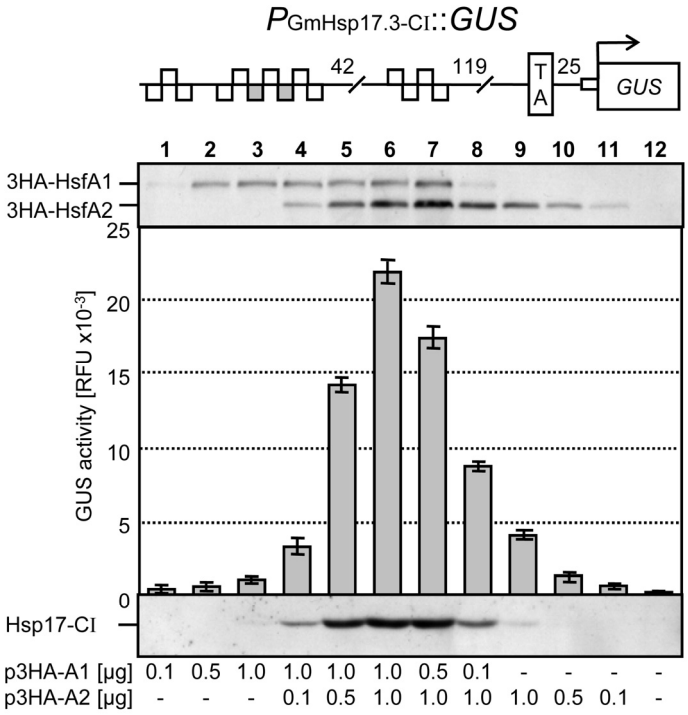

Figure S1. Synergistic activation of Hsp genes by HsfA1 and HsfA2

At the top of the figure, the Hsf dependent GUS reporter construct containing a promoter fragment from the soybean Hsp17.3B-CI gene (*P*$_{Gmhsp17.3B-CI}$*::GUS*) is shown. Blocks within the promoter structure represent heat stress elements (HSE, Nover *et al*, 2001) and the TATA box (TA). Numbers indicate distances in base pairs. The synergistic activation of both plasmid borne and endogenous Hsp17 promoters by tomato HsfA1 and HsfA2 was determined by co-transformation of tobacco protoplasts with the indicated amounts of plasmids. Protein levels of tagged HsfA1 and HsfA2 were monitored using anti-HA antibodies (panel on top of the graph). The graph shows the Hsf-dependent GUS reporter activities. Bars represent mean activity values of three independently transformed samples; error bars indicate the standard deviation of these three samples. Expression levels of induced tobacco Hsp17-CI proteins were detected by Hsp17-CI specific antibodies (panel at the bottom).

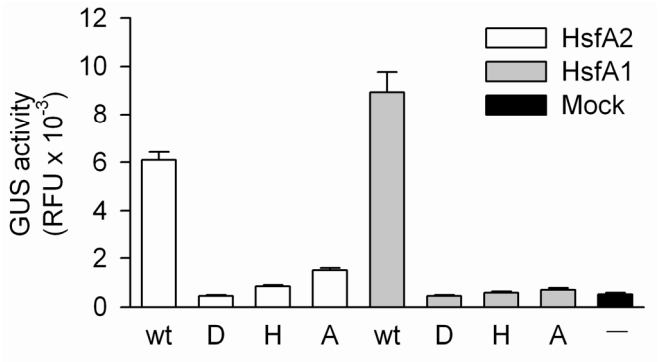

Figure S2. Activity of Hsf mutants

The mutants described in Figure 3 were tested for their activities in the GUS reporter assay. Shown is a representative result for the analysis of the GUS activities in protoplasts transformed with wild type (wt) or mutant forms (D, H, A) of HsfA2 (white bars) or of HsfA1 (grey bars), as well as in mock treated protoplasts transformed with the empty vector (black bar) as control for the activity of endogenous Hsfs.

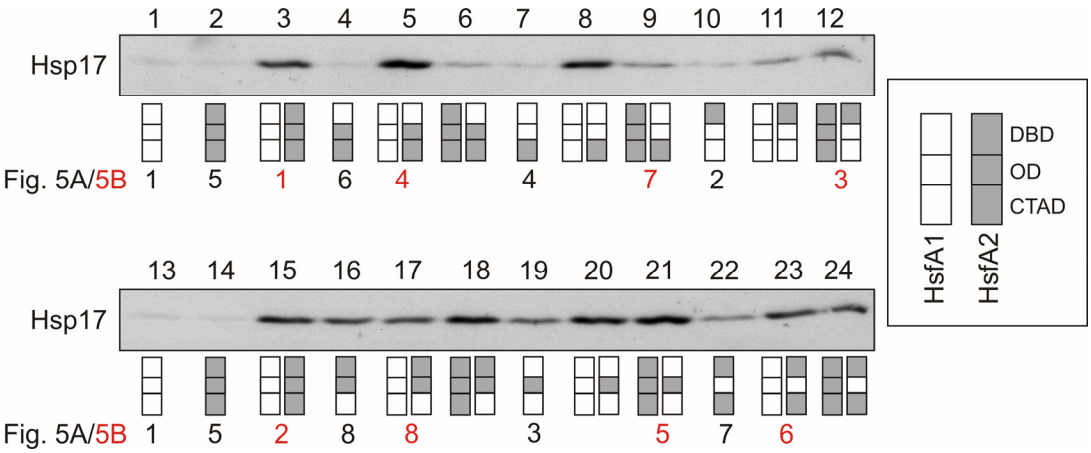

Figure S3. Activation of endogenous Hsp17 expression by domain swapping mutants.

Expression levels of Hsp17-CI were monitored by immunoblotting of protein extracts corresponding to 20.000 protoplasts transformed with domain swapping mutants of HsfA1 or HsfA2, either individually or in combination with wt HsfA1 or HsfA2 as indicated. Lanes 1 to 3 and 13 to 15 are controls for the combination of the wt forms of HsfA1 (white bars) and HsfA2 (gray bars). The wt and mutant forms are illustrated in block diagrams as in Figure 5A and B. Numbers in black refer to the corresponding samples in Fig. 5A where the activity of the mutants in the GUS reporter assay is shown. The numbers given in red indicate the combinations of the mutant forms with wt HsfA1 or HsfA2 that were analyzed in Fig. 5B. In addition, for each domain swapping mutant the effect on endogenous Hsp17 expression in combination with the wt form of the other Hsf was analyzed. The effects observed when exchanging an individual domain to a hetero-oligomeric constellation in the homo-oligomeric complex of HsfA1 (lanes 8, 11, 20) or HsfA2 (lanes 6, 18, and 24) are shown. As expected, the exchange of either the oligomerization (lanes 20 and 24) or the activation domain (lanes 8 and 18) has more pronounced effects for both Hsfs than the exchange of the DBD (lanes 6 and 11).

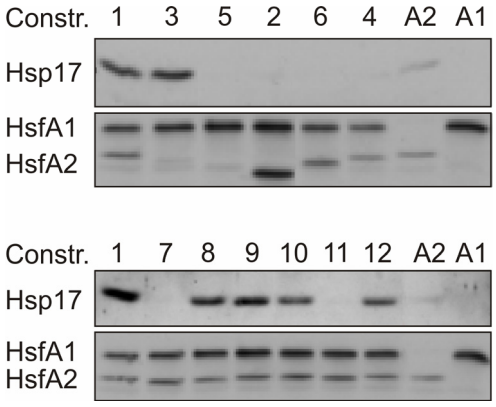

Figure S4. Role of the HsfA2 linker and HR-B region for induction of Hsp17 accumulation

The mutant constructs of HsfA2 indicated at the top by numbers according to Figure 6A were co-transformed with HsfA1 in order to analyze the impact of the HR-A/B domain on the transcriptional activation of endogenous Hsp17-CI genes. HsfA1 (A1) or HsfA2 (A2) was expressed individually in tobacco protoplasts as controls. Immunoblots indicating the accumulation of Hsp17-CI proteins (panels on top) and the amounts of HsfA1 and HsfA2 (panels below) are shown.