

Appendix for:

The transcription factor BES1 interacts with HSFA1 to promote heat stress resistance of plants

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Running title: BES1 promotes heat stress resistance

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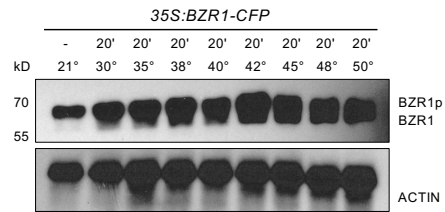
Appendix Figure S1 - Heat stress induces BZR1 de-phosphorylation.

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Appendix Figure S3 - BiFC assay with empty vector controls.

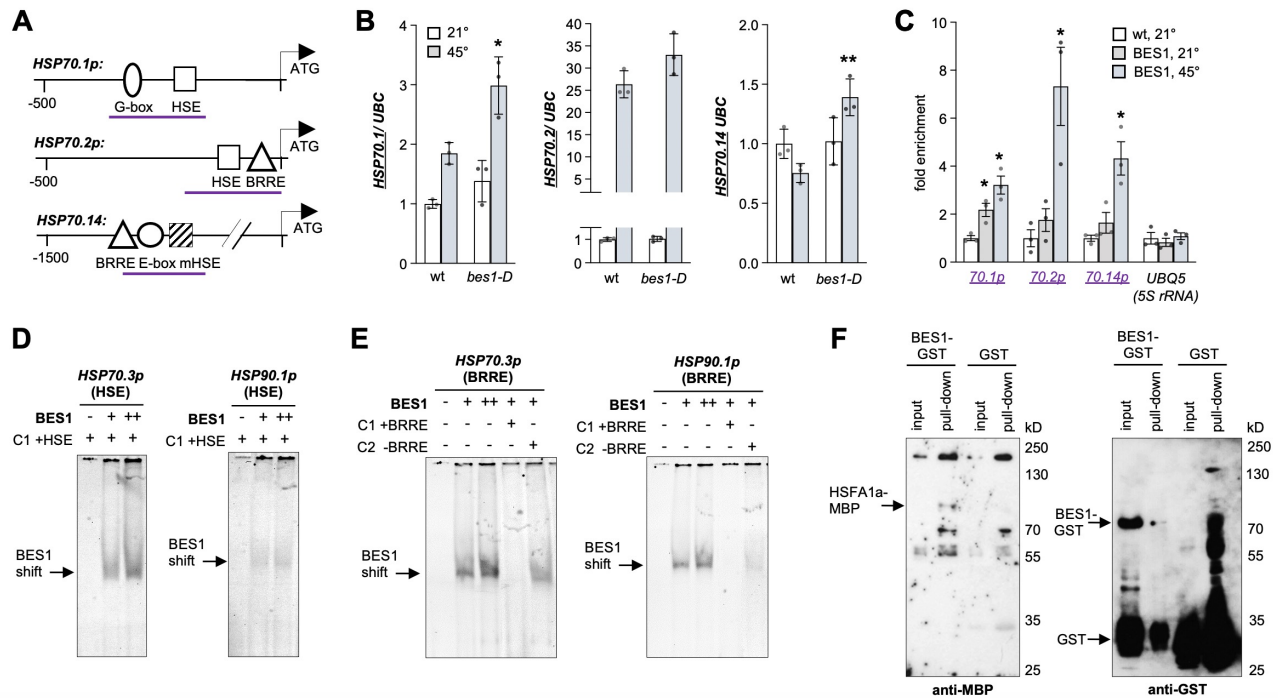
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Appendix Figure S1. Heat stress induces BZR1 de-phosphorylation.

Immunodetection of BZR1-CFP from heat-treated plants. *35S:BZR1-CFP* plants were grown at 21°C and exposed to the indicated temperatures for 20 minutes.



Appendix Figure S2. Heat stress promotes direct binding of BES1 to promoters of *HSP70*s.

A. Illustration of the promoters of *HSP70.1*, *HSP70.2* and *HSP70.14*. Relevant regulatory motifs and the promoter regions that were assessed in ChIP experiments (purple line) are shown.

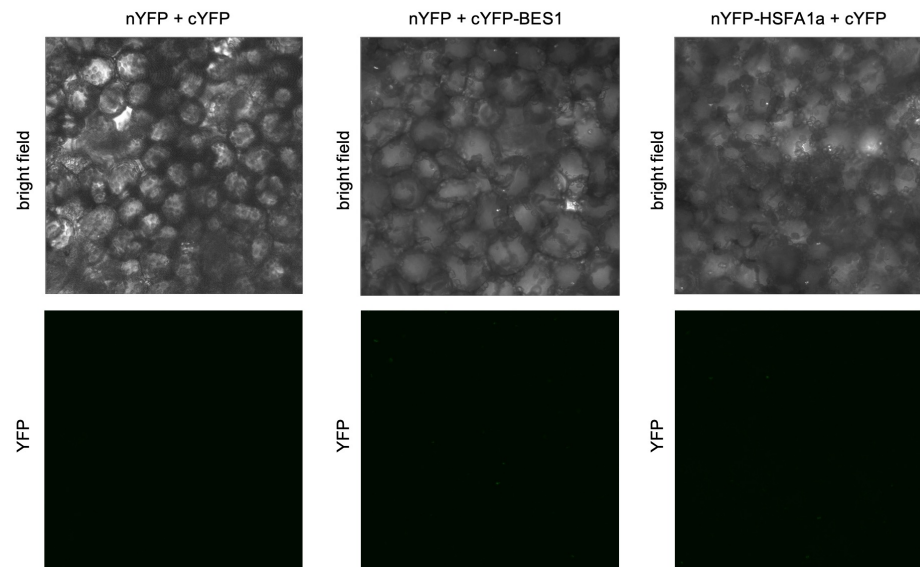
B. Relative expression changes of *HSP70.1*, *HSP70.2* and *HSP70.14* following heat shock in *bes1-D* as compared to untreated wild-type. 14-day-old plants were exposed to 45 °C for 60 minutes, expression was analyzed by qPCR and the results were normalized to *UBC*. Data show the mean $\pm$ SD. n=3 biological replicates measured in 3 technical repeats. The asterisks indicate significant differences compared to the wild-type at 45 °C by Student's *t*-test (* $P \leq 0.05$, ** $P \leq 0.01$).

C. ChIP to determine BES1-CFP enrichment on the promoters of *HSP70.1*, *HSP70.2* and *HSP70.14* following heat stress. 21-day-old 35S:BES1-CFP and wild-type plants were either kept at 21°C or exposed to 45°C for 60 minutes and BES1-CFP was immuno-precipitated with anti-GFP beads. The indicated fragments were quantified in the precipitates by qPCR and the ratios between samples with antibody and samples without antibody were calculated. 5s *rRNA* were used for normalization. *UBQ5* served as an internal control. Data show the mean $\pm$ SE. n=3 biological replicates measured in 3 technical repeats. The asterisks indicate significant differences compared to the wild-type at 21°C by Student's *t*-test (* $P \leq 0.05$).

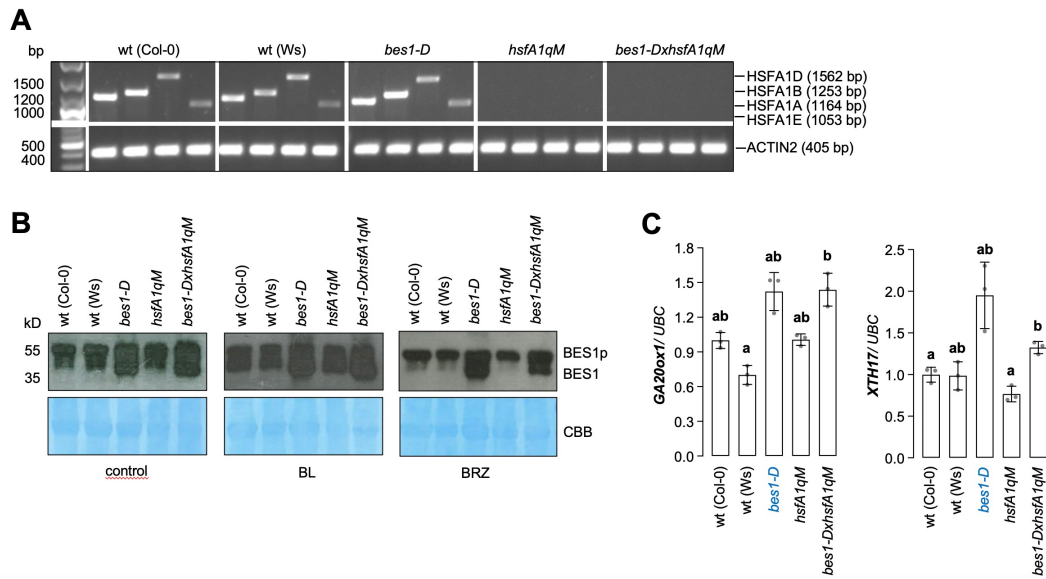
D. EMSAs showing *in vitro* binding of BES1 to HSE-containing fragments of the *HSP70.3* and *HSP90.1* promoters (shown as a grey bars in Fig 4A). Two concentrations of GST-BES1 (or no protein as a control) and 40 bp fragments that contain the HSE were used in DNA binding reactions and analyzed by EMSAs. Arrows indicate BES1-induced shifts of the DNA fragments.

E. Composite images of EMSAs showing *in vitro* binding of BES1 to BRRE-containing fragments of the *HSP70.3* and *HSP90.1* promoters (shown as a grey bar in Fig 4A). Two concentrations of GST-BES1; (or no protein as a control) and 40 bp fragments that contain the BRRE (5'-CGTGT/CG-3') were used in DNA binding reactions. To assess binding to the BRRE, unlabeled competitor fragments with the functional BRRE (C1: +BRRE) or a mutated version (C2: -BRRE) were used in 100x molar excess. Arrows indicate BES1-induced shifts of the DNA fragments.

F. *In vitro* pull-down experiment using BES1-GST as a bait and detecting either HSFA1a-MBP in the immuno-precipitates with anti-MBP antibody (left) or BES1-GST with anti-GST antibody (as a control; right). GST only was used as a bait for control of binding specificity. Input: sample before elution of bait-prey complex. Pull-down: sample after elution of bait-prey complex.



Appendix Figure S3. BiFC assays with nYFP-BES1 and cYFP-HSFA1a, coexpressed with cYFP and nYFP respectively. A combination of nYFP with cYFP was also included as a control. Shown are bright field and YFP filter images of tobacco cells.



Appendix Figure S4. Characterization of the *bes1-DxhsfA1qM* quintuple mutant.

A. Genotyping of the four *HSFA1*s in wild-type, the *bes1-D* and *hsfA1qM* parental lines and the selected homozygous *bes1-DxhsfA1qM* quintuple mutant. Composite images, showing PCR amplicons of the regions in the tested *HSFA1*s that contain T-DNA insertions in the *hsfA1qM*. These T-DNA insertions impair amplicon formation in all relevant *HSFA1*s in the *hsfA1qM* and *bes1-DxhsfA1qM* lines. Actin2 was used as control.

B. Immunodetection of BES1 with an α -BES1 anti-body in 7-day-old seedlings grown on control medium or medium containing 1 μ M 24-epiBL (BL) or 1 μ M BRZ. CBB staining was used for loading control.

C. Expression of BR-regulated genes in *bes1-DxhsfA1qM*, as compared to its parents and respective wild-types analyzed by qPCRs. Data show the mean $\pm$ SD. $n=3$ biological repeats, each measured in 3 technical replicates, normalized to *UBC*. Statistically significant difference at $P \leq 0.05$ of results is indicated with different letters and was determined by Student's *t*-test.

Appendix Table S1 (key resources)

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
GFP Antibody, HRP	Miltenyi Biotec	Cat#130-091-833, RRID:AB_247003
Anti-BES1	Yin et al., 2002	Custom antibody
Actin (plant) antibody produced in mouse	Sigma-Aldrich	Cat#A0480, RRID:AB_476670
Mouse Anti-Glutathione-S-Transferase (GST) Monoclonal, Unconjugated, Clone GST-2	Sigma-Aldrich	Cat#G1160, RRID:AB_259845
Anti-MBP Monoclonal	New England Biolabs	Cat#E8030S, RRID:AB_1559728
Anti-Rabbit IgG (whole molecule)-Alkaline Phosphatase antibody (goat)	Sigma-Aldrich	Cat#A3687, RRID:AB_258103
Anti-Mouse IgG (Fab specific)-Alkaline Phosphatase antibody (goat)	Sigma-Aldrich	Cat# A1293, RRID:AB_257927
Bacterial and Virus Strains		
BL21(DE3) Competent <i>E. coli</i>	New England Biolabs	Cat#C25271
XL1-Blue Supercompetent Cells	Agilent Technologies	Cat# 200236
<i>Agrobacterium tumefaciens</i> GV3101	Poppenberger lab	N/A
Chemicals, Peptides, and Recombinant Proteins		
24-epi-Brassinolide	Apollo Scientific	Cat#BIE0244
Brassinazole, BRZ	Poppenberger lab	N/A
(+)-cis,trans-Abcisic acid	Duchefa	Cat#A0941.0250
Fluridone	Sigma-Aldrich	Cat#45511
Sodium chloride	Sigma-Aldrich	Cat#S7653
Mannitol	Sigma-Aldrich	Cat#M4125
DL-Dithiothreitol	Sigma-Aldrich	Cat#D0632
Bleach (4-5% sodium hypochlorite)	DM	N/A
Triton 100X	Sigma-Aldrich	Cat#T8787
MURASHIGE&SKOOG MEDIUM+VITAMINS/MES	Duchefa Biochemie	Cat#M0255.0001
Sucrose	Sigma-Aldrich	Cat#S7903
PLANT AGAR	Duchefa Biochemie	Cat#P1001
Potassium hydroxide	Sigma-Aldrich	Cat#P1767
Trizma® base	Sigma-Aldrich	Cat#T1503
Sodium chloride	Sigma-Aldrich	Cat#S7653
EGTA	Sigma-Aldrich	Cat#E3889
Magnesium chloride	Sigma-Aldrich	Cat#M8266
Sodium fluoride	Sigma-Aldrich	Cat#S7920
β-Glycerol phosphate	Sigma-Aldrich	Cat#50020
Immobilon-P Membran, PVDF, 0,45 µm	Millipore	Cat#IPVH00010
Sodium pyrophosphate tetrabasic	Sigma-Aldrich	Cat#P8010
cOmplete Protease Inhibitor Cocktail	Roche	Cat#04693124001
Tween® 20	Sigma-Aldrich	Cat#P2287
Glutathione Sepharose 4B, 10 ml	GE Healthcare	Cat#17-0756-01
Amylose Resin	New England Biolabs	Cat#E8021S
Blocked agarose beads	Chromotek	Cat#bab-20

continued

GFP-Trap Agarose	Chromotek	Cat#gta-20
Coomassie Brilliant Blue R-250	Roth	Cat#3862.1
Rotiphorese® Gel 40 (37,5:1)	Roth	Cat#T802.1
ROTI®Quant	Roth	Cat#K015.1
Amersham ECL Select Western Blotting Detection Reagent	GE Healthcare	Cat#RPN2235
PhosphaGLO Reserve AP Substrat	Medac Diagnostic	Cat#55-60-01
Carestream® Kodak® autoradiography GBX fixer/replenisher	Sigma-Aldrich	Cat#P7167-5GA
GBX developer	Sigma-Aldrich	Cat#P7042-1GA
Recombinant protein BES1-GST	Poppenberger lab	-
Recombinant protein BES1-MBP	Poppenberger lab	-
Recombinant protein HSFA1A-MBP	Poppenberger lab	-

Critical Commercial Assays

E.Z.N.A. SP Plant DNA	OMEGA Bio-tek	Cat#D5511-02
E.Z.N.A. Plant RNA	OMEGA Bio-tek	Cat#R6827-02
E.Z.N.A. Plasmid Mini Kit	OMEGA Bio-tek	Cat#D6942-02
E.Z.N.A.® Plasmid Midi Kit	OMEGA Bio-tek	Cat#D6904-03
Dual-Luciferase Reporter Assay System	Promega	Cat#E1910
RevertAid First Strand cDNA Synthesis Kit	Thermoscientific	Cat# K1621

Experimental Models: Organisms/Strains

<i>Arabidopsis thaliana</i> lines		
Col-0	NASC	N1093
Ws	NASC	N6891
<i>bes1-D</i> (Col-0 background)	Ibanese et al., 2009	N/A
<i>bes1-1</i>	NASC	SALK_098634
<i>bes1-2</i>	Lachowiec et al., 2013	WiscDsLox246D02
<i>bri1-1</i>	Li and Chory, 1997	N/A
<i>det2-1</i>	Chory et al, 1991	N/A
<i>bin2-1</i>	Li et al., 2001	N/A
<i>35S:BES1-CFP</i>	Rozhon et al., 2010	N/A
<i>35S:BZR1-CFP</i>	Rozhon et al., 2010	N/A
<i>35S:BES1-CFPxbri1-1</i>	this study	N/A
<i>35S:BES1-CFPxdet2-1</i>	this study	N/A
<i>35S:BES1-CFPxbin2-1</i>	this study	N/A
<i>abitM (abi1-2 hab1-1 pp2ca-1)</i>	Rubio et al., 2009	N/A
<i>hsfA1qM</i>	Liu et al., 2011	N/A
<i>bes1-DxhsfA1qM</i>	this study	N/A
<i>Nicotiana benthamiana</i>	N/A	N/A

Oligonucleotides

Primers for HSFA1a genotyping F: AAGAAGATAAGCCGAGAAAATCT R: ACAAAGTTGCAACCGTACTACTGA	Liu et al., 2011	Customer order
Primers for HSFA1b genotyping F: CCAGCTTCGTCAGACAGTTAAATA R: TAGGAAACTGTCAGGATTGTTGA	Liu et al., 2011	Customer order
Primers for HSFA1d genotyping F: GCATAATAATTTCTCCAGCTTCGT R: AGGTTTTCGCCTAGTTATTGATTG	Liu et al., 2011	Customer order

continued

Primers for HSFA1e genotyping F: TTTTAAGAGGCCAAAAGCAAATAC R: GTTGATTCTTGCTCCACACATTAC	Liu et al., 2011	Customer order
Primers for Actin2 genotyping F: TTGCTGGACGTGACCTTACT R: TGCTCATACGGTCAGCGATA	this study	Customer order
Primers for HSFA1A cloning in pGWR8 vectors F: GAGAGGGCGGCCGCATGTTTGTAATTTCAAATACTTC R: CTCTCTGAGATCTTAGTGTCTGTTTCTGATGTGAG	this study	Customer order
Primers for BES1 cloning in pGWR8 vectors F: GAGAGGGCGGCCGCATGACGTCTGACGGAGCAACGTGC R: TCTCTGAGATCTTCAACTATGAGCTTTACCATTTC	this study	Customer order
Primers for <i>bes1-D</i> sequencing F: GAGAGGGCGGCCGCATGACGTCTGACGGAGCAACGTGC R: TCTCTGAGATCTTCAACTATGAGCTTTACCATTTC	this study	Customer order
Primers for pGWR8 sequencing F: CGAATCTCAAGCAATCAAGC R: CTTATCTGGGAACACTACTCACACA	this study	Customer order
Primers for pGreen R: AGTTGCTCTCCAGCGTTCCA	this study	Customer order
Primers for DWF4 qPCR F: CCGTTGAAGAGCTTAGGGAAGAG R: CATTTCCCAATCGAAGAGTTTC	Poppenberger et al., 2011	Customer order
Primers for ROT3 qPCR F: CTTGTAACCCGGTACAGTTGC R: TCCGCTTCATCTTCACAGTC	Poppenberger et al., 2011	Customer order
Primers for BR6ox2 qPCR F: AGCTTGTTGTGGAACTCTATCGG R: CGATGTTGTTTCTTGCTTGGA	Poppenberger et al., 2011	Customer order
Primers for HSP70.3 qPCR F: AACGGTTGATTGGTGACGC R: TTTGAGCGTGAAGGGCCA	this study	Customer order
Primers for HSP70.4 qPCR F: TGGCTTTTGTGTTACTCTGTTTGC R: TCTTTCATAGGTCAGAGCGAGT	this study	Customer order
Primers for HSP90.1 qPCR F: TGGTTCTGAAAATTCTAATATGTGCG R: TGACACAAACCCAACCCTAGA	this study	Customer order
Primers for HSP70.1 qPCR F: TGTTGGACATTGACCTCTCTCT R: CGTCATCGTAGCTAACTGGT	this study	Customer order
Primers for HSP70.2 qPCR F: TCATTGGTGACCCCTTCTCT R: TCACATTTCTTCGAAGCTTTGTT	this study	Customer order
Primers for HSP70.14 qPCR F: TGAATGACGAGAGAGAGATGGT R: TCCGGGAATAATAGCGAAACC	this study	Customer order
Primers for UBC21 qPCR F: TCAAATGGACCGCTTATC R: CACAGACTGAAGCGTCCAAG	Eremina et al., 2016	Customer order
Primers for GA3ox1 qPCR F: CCCAACATCACCTCAACTACTGC R: GCTAACCACATCAATTTCGATGCC	Unterholzner et al., 2015	Customer order
Primers for GA20ox1 qPCR F: AGATTACTTCTGCGATGCGTTGG R: TCTTGATACACCTTCCCAAATGGC	Unterholzner et al., 2015	Customer order
Primers for IAA5 qPCR F: AAGAGTCAAGTTGTGGGTTGGC R: AATGCAGCTCCATCTACACTCACT	Nakamura et al., 2003	Customer order
Primers for SAUR-AC1 qPCR F: GAGGATTTCATGGCGGTCTATG R: GTTAAGCCGCCCATTTGGATG	Li et al., 2012	Customer order

continued

Primers for XTH17 qPCR F: GTGGTACAAGCTTTGCGTTCTTAC R: AGCTACCCGCATAGACATGCAC	Li et al., 2012	Customer order
Primers for pHSP70.3 ChIP qPCR F: ATGTTGAGCCAATGACCGAC R: TGAATTGCGGCTAGGTTTGT	this study	Customer order
Primers for pHSP70.4 ChIP qPCR F: AAATAAGATCCAACGGCTGAGA R: TCGTCTGTGAGCTTTAAGAGAA	this study	Customer order
Primers for pHSP90.1 ChIP qPCR F: CCGGCGAAATTTCTTTGCCA R: ACCAGTGATTCTGGATTGTTCT	this study	Customer order
Primers for UBQ5 ChIP qPCR F: ACCAAGCCGAAGAAGATCAA R: ATGACTCGCCATGAAAGTCC	this study	Customer order
Primers for 5SRNA ChIP qPCR F: GGATGCGATCATACCAGCACT R: GAGGGATGCAACACGAGGACT	this study	Customer order
Primers for pHSP70.1 ChIP qPCR F: ACAACTTGACGCTGGAAAGA R: CGCCAGGCTGTTTAAATGT	this study	Customer order
Primers for pHSP70.2 ChIP qPCR F: AGCTACCGTCAGATCAACCT R: ATCACACGAAGATAGAAAAAGC	this study	Customer order
Primers for pHSP70.14 ChIP qPCR F: AAAATGGCAGCACACGGT R: GGTATTGCAAATTCACGGTCA	this study	Customer order
Primers for pHSP70.3 _LUC F: ACAAAAGTGGTTAAGCTCACGATGAG R: TGTTAACGCTACTCAGGATTAAGCT	this study	Customer order
Primers for pHSP70.4 _LUC F: GCCACAAGGAGTTGAAGTTTACAGTTG R: TATTAGAGATCAGAATTGTTCCGCGG	this study	Customer order
Primers for pHSP90.1 _LUC F: GGCCTTTCTCTTATCCCTCTCGTCG R: CGCAACGAACCTTGATTCAACGCAA	this study	Customer order
Primers for pHSP70.3 _EMSA_HSE F: TTACATAGCGAGCCATCGAACCTTCTCGAAACTCGAGTACAAGACTC R: GAGTCTTGTA CTGAGT	this study	Customer order
Primers for pHSP70.3 _EMSA_HSEmut F: TTACATAGCGAGCCATCTAACCTTATCTAAACTCGAGTACAAGACTC R: GAGTCTTGTA CTGAGT	this study	Customer order
Primers for pHSP70.3 _EMSA_BRRE F: ATCCAAC TCCAAAAGATCGTGCGTTACATAGCGAGCCAT R: ATGGCTCGCTATGTAAC	this study	Customer order
Primers for pHSP70.3 _EMSA_BRREmut F: ATCCAAC TCCAAAAGATTTTTTTGTTACATAGCGAGCCAT R: ATGGCTCGCTATGTAAC	this study	Customer order
Primers for pHSP90.1 _EMSA_HSE F: GAGTCTCGAAACGAAAAGA A CTTTCTGGAATTCGTTTGCTCACAAAG R: CTTTGTGAGCAAACGAA	this study	Customer order
Primers for pHSP90.1 _EMSA_HSEmut F: GAGTCTCGAAACGAAAATA A CTTTATGTAATTAGTTTGCTCACAAAG R: CTTTGTGAGCAAACGAA	this study	Customer order
Primers for pHSP90.1 _EMSA_BRRE F: TCCATCAGCTACAAAGTCGTGTGCGATTTTGAGTCGTTGT R: ACAACGACTCAAAATCG	this study	Customer order
Primers for pHSP90.1 _EMSA_BRREmut F: TCCATCAGCTACAAAGTTTTTTTCGATTTTGAGTCGTTGT R: ACAACGACTCAAAATCG	this study	Customer order

Recombinant DNA		
pGEM®-T Easy	Promega	Cat#A1360
pGEM-t-Easy-BES1	this study	N/A
pGEM-t-Easy-HSFA1A	this study	N/A
pGEX-4T1	Yaffe et al., 1997	Cat#13279
pGEX-4T1-BES1	Rozhon et al., 2010	N/A
pMAL-c2X	Paul Riggs lab	Cat#75286
pMAL-c2X-BES1	this study	N/A
pMAL-c2X-HSFA1A	this study	N/A
pGWR8 (a derivative of pGreenII)	Rozhon et al., 2010	N/A
pGWR8-BES1	this study	N/A
pGWR8-HSFA1A	this study	N/A
pGreenII-0800-LUC	Hellens et al., 2005	Cat#44468
pGreenII-0800-LUC-pHSP70.3	this study	N/A
pGreenII-0800-LUC-pHSP70.4	this study	N/A
pGreenII-0800-LUC-pHSP90.1	this study	N/A
pGWR8-n-YFP-BES1	this study	N/A
pGWR8-cYFP-HSFA1A	this study	N/A
pGWR8-n-YFP	Poppenberger et al., 2011	N/A
pGWR8-cYFP	Poppenberger et al., 2011	N/A
Software and Algorithms		
ImageJ	Schneider et al., 2012	https://imagej.nih.gov/ij/ RIDD: SCR_003070
Office Excel Power Point 2016	Microsoft	N/A