

Identification and functional analysis of heat-shock promoters from *Spodoptera frugiperda*

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Table S1 Accession of *SfHsp* sequences on NCBI.

Sequence	Accession number
<i>SfHsp11.2</i>	MN735761
<i>SfHsp15.82</i>	MN735762
<i>SfHsp19.07</i>	MN735763
<i>SfHsp19.35</i>	MN735764
<i>SfHsp19.66</i>	MN735765
<i>SfHsp19.74</i>	MN735766
<i>SfHsp20.15</i>	MN735767
<i>SfHsp20.71</i>	MN735768
<i>SfHsp21.37</i>	MN735769
<i>SfHsp21.38</i>	MN735770
<i>SfHsp21.96</i>	MN735771
<i>SfHsp24.35</i>	MN735772
<i>SfHsp26.61</i>	MN735773
<i>SfHsp29.00</i>	MN735774
<i>SfHsp60</i>	MN735775
<i>SfHsp70A</i>	MN735776
<i>SfHsp70B</i>	MN735777
<i>SfHsp70C</i>	MN735778
<i>SfHsp70D</i>	MN735779
<i>SfHsp75</i>	MN735780
<i>SfHsp83</i>	MN735781
<i>SfHsp97</i>	MN735782
<i>SfHsp19.07</i> -P790	MN735783
<i>SfHsp19.74</i> -P1385	MN735784
<i>SfHsp20.15</i> -P872	MN735785
<i>SfHsp20.71</i> -P1638	MN735786
<i>SfHsp21.37</i> -P1369	MN735787
<i>SfHsp29.00</i> -P499	MN735788
<i>SfHsp70A</i> -P1218	MN735789
<i>SfHsp70D</i> -P1403	MN735790

Table S2 Primers used in this study.

Primers	Sequence (5'-3')	Purpose
SfHsp11.2-QF	AGGGCTGAAGCAGTAACCAA	qPCR
SfHsp11.2-QR	TCGCTCTCTCGGAATAGGTG	qPCR
SfHsp15.82-QF	GTGCACGACTATGCTCCTGA	qPCR
SfHsp15.82-QR	TGCGAGGAACAGAGATGATG	qPCR
SfHsp19.07-QF	CCC GTTCTTCAGAGATCCAG	qPCR
SfHsp19.07-QR	ACCGTGATCTCGTTTCCAAC	qPCR
SfHsp19.35-QF	GGACCAGAACTTCGGACTGA	qPCR
SfHsp19.35-QR	TGGCCTTCAACCACAATGTA	qPCR
SfHsp19.66-QF	TTGCCTGAAGGGTGTATTCC	qPCR
SfHsp19.66-QR	ACTTCGGTTTTTCGTGGTTTG	qPCR
SfHsp19.74-QF	GGGTATATTTCCCGCCAGTT	qPCR
SfHsp19.74-QR	CTGGTCCTTGACTTCCTTGC	qPCR
SfHsp20.15-QF	CCGCAAGACCAGTTAACCAT	qPCR
SfHsp20.15-QR	GATGAATCCGTGCTGATCCT	qPCR
SfHsp20.71-QF	AGCCAAGCATGAGGAGAAGA	qPCR
SfHsp20.71-QR	CTGGTCCTTGATCTCCTTGC	qPCR
SfHsp21.37-QF	CCTTTTGTTCTCGGCTACGA	qPCR
SfHsp21.37-QR	ATCCTTGTCGGACTTGATGC	qPCR
SfHsp21.38-QF	CGACACTGAATTCTCCAGCA	qPCR
SfHsp21.38-QR	CACTGTGCTGTGACGATGTG	qPCR
SfHsp21.96-QF	CGAGGAGAAACAGGATGAGC	qPCR
SfHsp21.96-QR	CTGTCTTGGTGATGGGGACT	qPCR
SfHsp24.35-QF	GCTGACATTGGACCCAGAAT	qPCR
SfHsp24.35-QR	CTCGAGGAGTTGGCTGGTAG	qPCR
SfHsp26.61-QF	GGTGTTCGACAAGGGAGTGT	qPCR
SfHsp26.61-QR	TGGCTTTGTCTTGGTCTCCT	qPCR
SfHsp29.00-QF	GAGGAAGTCCTGGAGGAACC	qPCR
SfHsp29.00-QR	GGTCACTTCTTCCTGCTTGC	qPCR
SfHsp75-QF	AGACAGACAGGCCTCTGGAA	qPCR
SfHsp75-QR	TGCCCTGCTTTTTGATCTCT	qPCR
SfHsp83-QF	GAAGCGCAAGAACAACATCA	qPCR
SfHsp83-QR	TTCTGTTGGAGCATCTCACG	qPCR
SfHsp97-QF	TGAGAACAGAAGGCGAACCT	qPCR
SfHsp97-QR	GCCTCTGCCACTTTCTGAAC	qPCR
SfHsp70A/B-QF	GTGGCTATGGAGGAAACCAG	qPCR
SfHsp70A/B-QR	CGCACATTTTCTCTGTTGA	qPCR
SfHsp70C-QF	GGCCAACTGTGGAAGAAGTC	qPCR
SfHsp70C-QR	TGTGGGTGTGTTAGCGTTTG	qPCR
SfHsp70D-QF	CAAGCAAGCGAAGAGAAGAGA	qPCR

SfHsp70D-QR	CACACGAGTAGGTCGTTCCA	qPCR
SfHSP70A-P1218F	ATCGGGGTACCCACCTACACAC	Promoter
SfHSP70A-P1218R	AGTTCGAATATTGC	amplification
SfHSP70D-P1403F	CCATGCCATGGTTTGTCTT	Promoter
SfHSP70D-P1403R	CAGATTCTCTTCAC	amplification
SfHSP19.07-P790F	ATCGGGGTACCAACTCAATAAC	Promoter
SfHSP19.07-P790R	CGGTTGGGTAAGC	amplification
SfHSP19.74-P1385F	CCATGCCATGGTTTTCTTGATTA	Promoter
SfHSP19.74-P1385R	TATTTCTCTTTATTAC	amplification
SfHSP20.15-P872F	ATCGGGGTACCAGTTAATAGTCA	Promoter
SfHSP20.15-P872R	CCTACACTCGGAAG	amplification
SfHSP20.71-P1638F	CCATGCCATGGTTTGGTCGTTTTG	Promoter
SfHSP20.71-P1638R	TTTATTTCGTTG	amplification
SfHSP21.37-P1369F	ATCGGGGTACCTTCTGCATAGTCT	Promoter
SfHSP21.37-P1369R	ATGGATATCGG	amplification
SfHSP29.00-P499F	CCATGCCATGGTTTTCTTATTTTCG	Promoter
SfHSP29.00-P499R	TTTTCAACTGAC	amplification
ie1-F	ATCGGGGTACCTACTGGATGTATCT	Promoter
ie1-R	AGAGTGCTCC	amplification
ie2-F	CCATGCCATGGCTTCACTGGATAAT	Promoter
ie2-R	ACTGATAATC	amplification
hr5/ie1-F	ATCGGGGTACCAACACTGACATGAA	Promoter
hr5/ie1-R	GACACATGG	amplification
	ATCCCAAGCTTTTTGCTTGATTCTAA	Promoter
	CACAACAAC	amplification
	ATCGGGGTACCACATACATTTCAAAG	Promoter
	TAAGTCACG	amplification
	CCATGCCATGGTTTGTGAGTGTTGA	Promoter
	GTATTCTTC	amplification
	ATCGGGTACCGAGTAGAGTAACCTCT	Promoter
	ACTCTCC	amplification
	CCATGCCATGGCGTTCGTTGCG	Promoter
	TTGTTGTTGCTTTC	amplification
	ATCGGGGTACCCGATGTCTTTG	Promoter
	TGATGCGCGCGAC	amplification
	CCATGCCATGGCTTGGTTGTTCA	Promoter
	CGATCTTGTCG	amplification
	ATCGGGGTACCCATGATGATAAA	Promoter
	CAATGTATGG	amplification
	CCATGCCATGGAACAGATGCTGT	Promoter
	TCAACTGTGT	amplification
	ATCGGGGTACCCATTGCTTGTCAT	Promoter
	TTATTAATTTGG	amplification
	CCATGCCATGGCTTGGTTGTTTAC	Promoter
	GATCTTGTCG	amplification

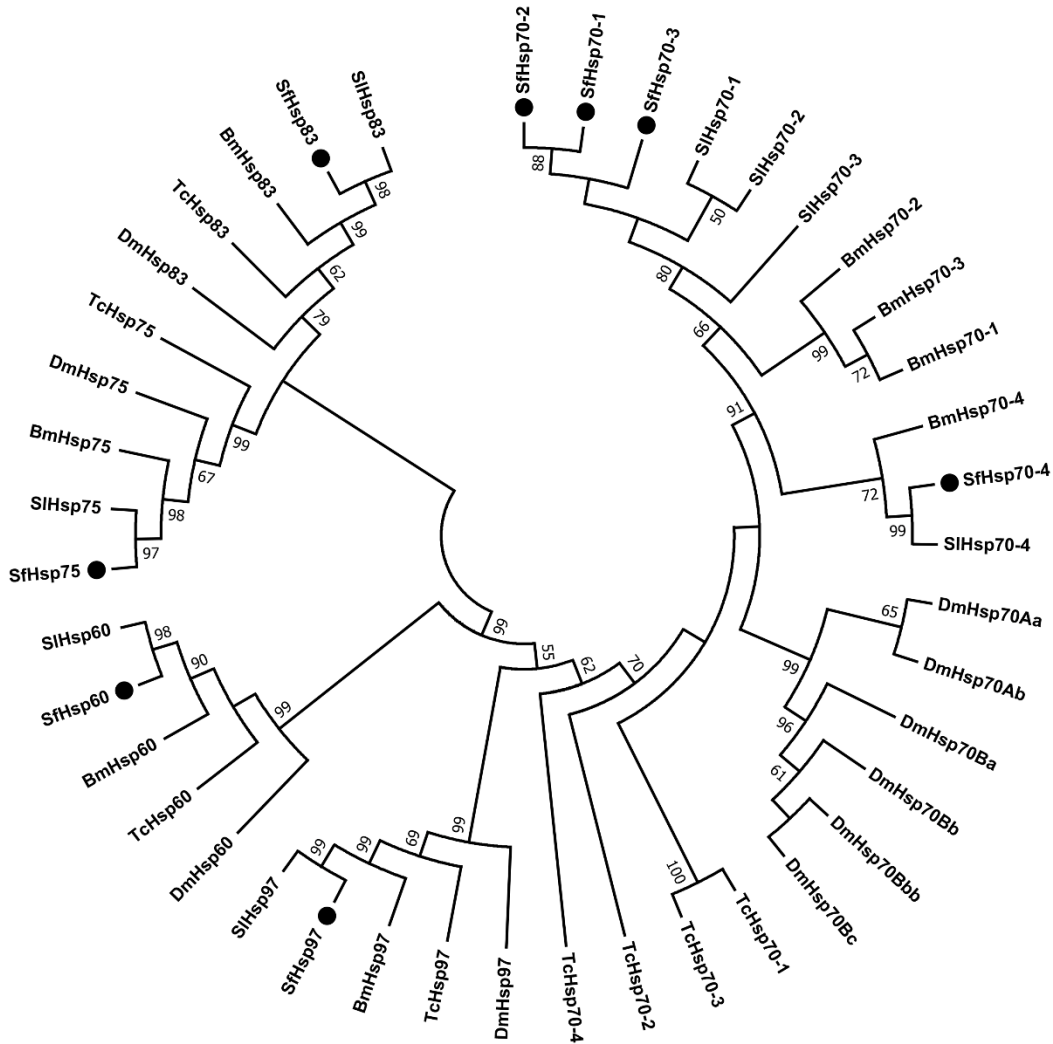


Figure S2. Phylogenetic analysis of insect Hsp60, Hsp70, Hsp75, Hsp83, and Hsp90. Amino acid sequences of Hsp60, Hsp70, Hsp75, Hsp83, and Hsp90 from *B. mori* (Bm), *Danaus plexippus* (Dp), *P. xylostella* (Px), *S. frugiperda* (Sf), and *S. litura* (Sl) were obtained from the non-redundant database on NCBI. The phylogenetic tree was established using MEGA7 (<https://www.megasoftware.net>) by the neighbor-joining method with a bootstrap test of 1,000 replicates. Only percentage bootstrap values over 50 are indicated on the nodes of the tree. The *S. frugiperda* Hsp are labeled with black dots.

CTGGATATGGCAATCGAGACGTCTAGCCAGTAAAAGATAAACCTAATGGGTATTTTTTTGGTATAAGCTAGTGAACGAGCAGGCAATTTTT
 TTTGTAAATGTGTAAACTAAACACTGACATGAAGACACATGGTAAACATATTTATTTTTCCAGAAATAACTAACAGCTGATAGTGATTCAGCT
 ACAAATCGTATTGTTTACAATGTAGGTTAGTAGATAAAGGTGATAATTGGTATTACCCGTCAGTTAAATCATCAAGAAATATTATATCGCTA
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 CCTACCAAACTAACGCAATAACAAAACGCAAGTCATCATATACATCATTGTCATAAAAAATAAATTATGTAACCTCAAAACGAATTCAAA
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 GTGTAACTTGAGAACGAGTAAGTAAATCATTATTGCGAATTTTATTTTATTGAAATATGCTAATATATTTTTTTGATCTTATATTTATTGGTA
 TACTTGGCCGATGTCACTATTTATCAGAACTATTTCTCAGTAGAATTCATATATTTAACTTATAATTTAAGAAGTAAACAACGAATTTAAAT
 CGTAGGATAACGAAGACTAAGAACATCGCCGCTTAAATACCAAAAACAAAAAAAACCTATTTCTAAGTTACCTACTTACTTCAATAATATA
 AATGTCAACTCTAATAACTTCATCCATTTACTTATCTTTTCTAAATTTGGAATATTTATTATCTAAAATTATAAATAAATATGAAACAAACCAC
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 TACTATACACAAACACAAACAACAGGTACAACAGCTGACTTGTGTTTGGTTTCGAGAATTTTCTACTAGATACTAGATGGCGGTGTTGCCAAT
 ATAAAAGGCGGCCGAGCGCGGCACGGGCATCAGTTTGA AAAACAAGCGAACAAAGTGATACAAACTTCAAGCGCTCTCAAAGCGACTCG
 AGGATATTTCTCAGCGATTTTTGTGAAGGAAGAAAGTTGTTGTTGTTGTTAGAAATCAAGCAAA

Figure S3. Promoter sequence of SfHsp20.71 gene with potential HSEs marked. The 1783 bp nucleic acid sequence with three types of HSE elements present upstream to the ATG of SfHsp20.71 gene are shown. Two types of HSEs, tail-tail and step/gap, indicated by red and blue bold lines respectively are shown on the top of the sequence. Another type of HSE, head-head, represented by pink bold lines, is displayed below the sequence.

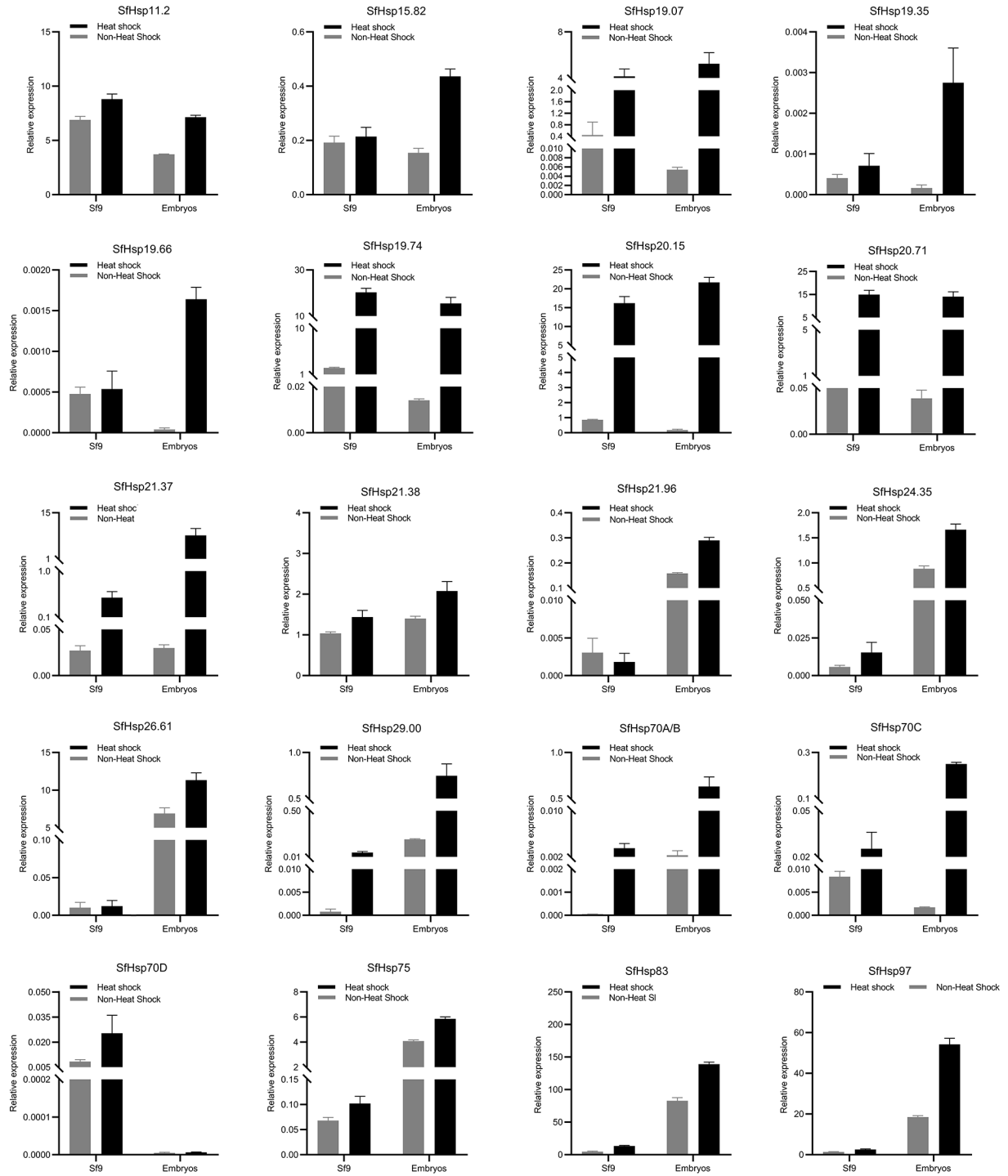


Figure S4. Relative mRNA levels of *SfHsp* genes in Sf9 cells and embryos. The Sf9 cells or embryos were exposed to 37°C for 1 hr, then let them recover at 27°C for 1 hr. Cells and fresh embryos kept at

27 °C were used as non-heat-shock control. Total RNA was isolated, converted to cDNA and used in RT-qPCR to quantify mRNA levels. 28 srRNA gene as the reference gene. Mean \pm SD (n=3) are shown.

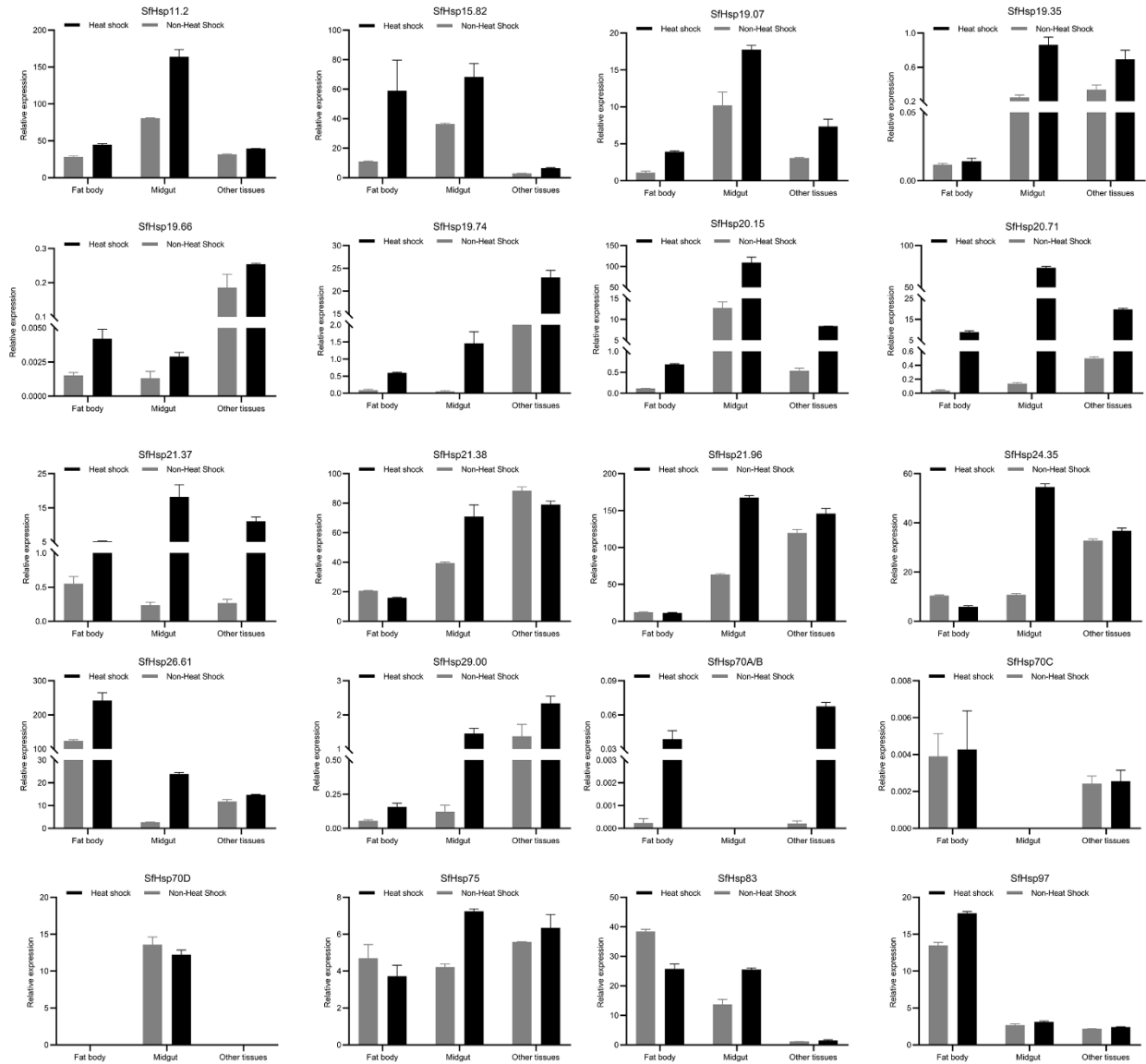


Figure S5. Relative mRNA expression levels of *SfHsp* genes in different larval tissues. The 6th instar larvae were exposed to 37 °C for 1 hr, then recovered at 27°C for 1 hr. Fat body, midgut, and remaining tissues were dissected. Tissues dissected from larvae raised at 27°C were used as non-heat-shock control. Mean \pm SD (n=3) are shown.

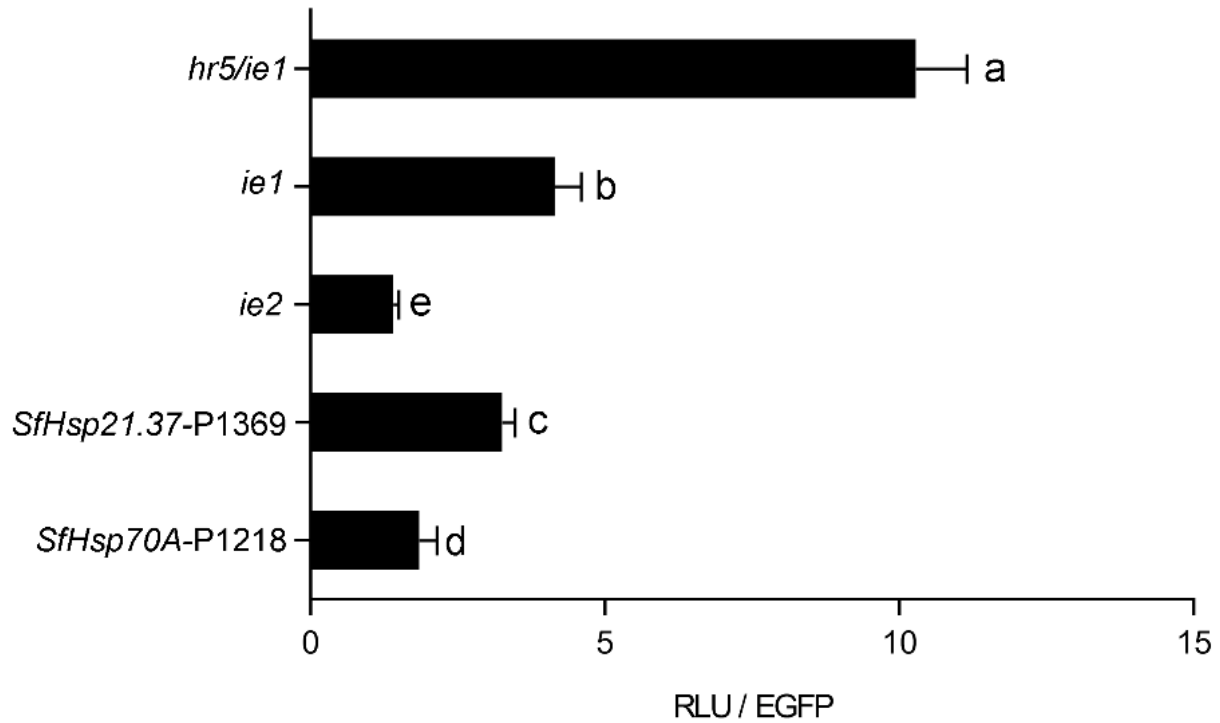


Figure S6. Comparison of highly heat-inducible *S. frugiperda* heat-shock promoters with commercially used promoters in Sf9 cells. Top two heat-shock promoter constructs that showed heat-inducible activity in Sf9 cells, and *ie1*, *ie2*, and *hr5/ie1* constructs were co-transfected into Sf9 cells with a construct expressing EGFP. The transfected cells were processed as described in Figure 4. The fluorescence intensity of EGFP in each sample was determined using SpectraMax i3x with excitation maximum of 484 nm and emission maximum of 509 nm. The luciferase activity was normalized by dividing with the EGFP activity in the same sample. Mean \pm SD (n=4) are shown. Different letters beside each column indicate significant differences ($p < 0.05$) among groups determined using One-way ANOVA followed by the Tukey HSD test.