

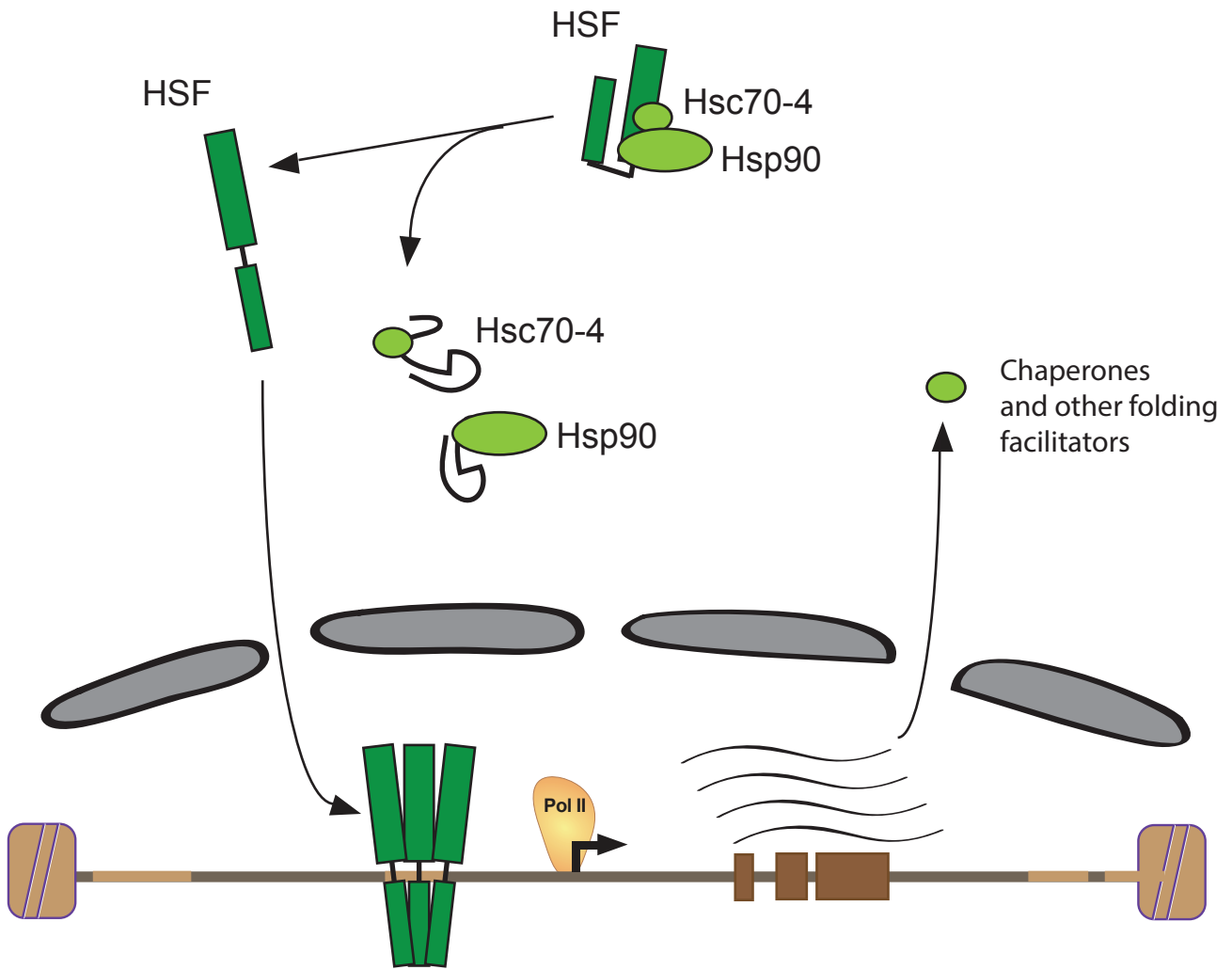
1 **The heat shock response and humoral immune response are mutually antagonistic**
2 **in honey bees**

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4 W. Snow^{1*}

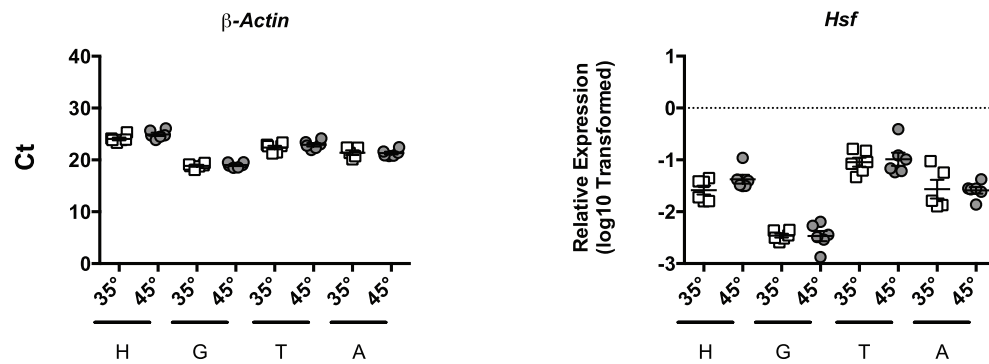
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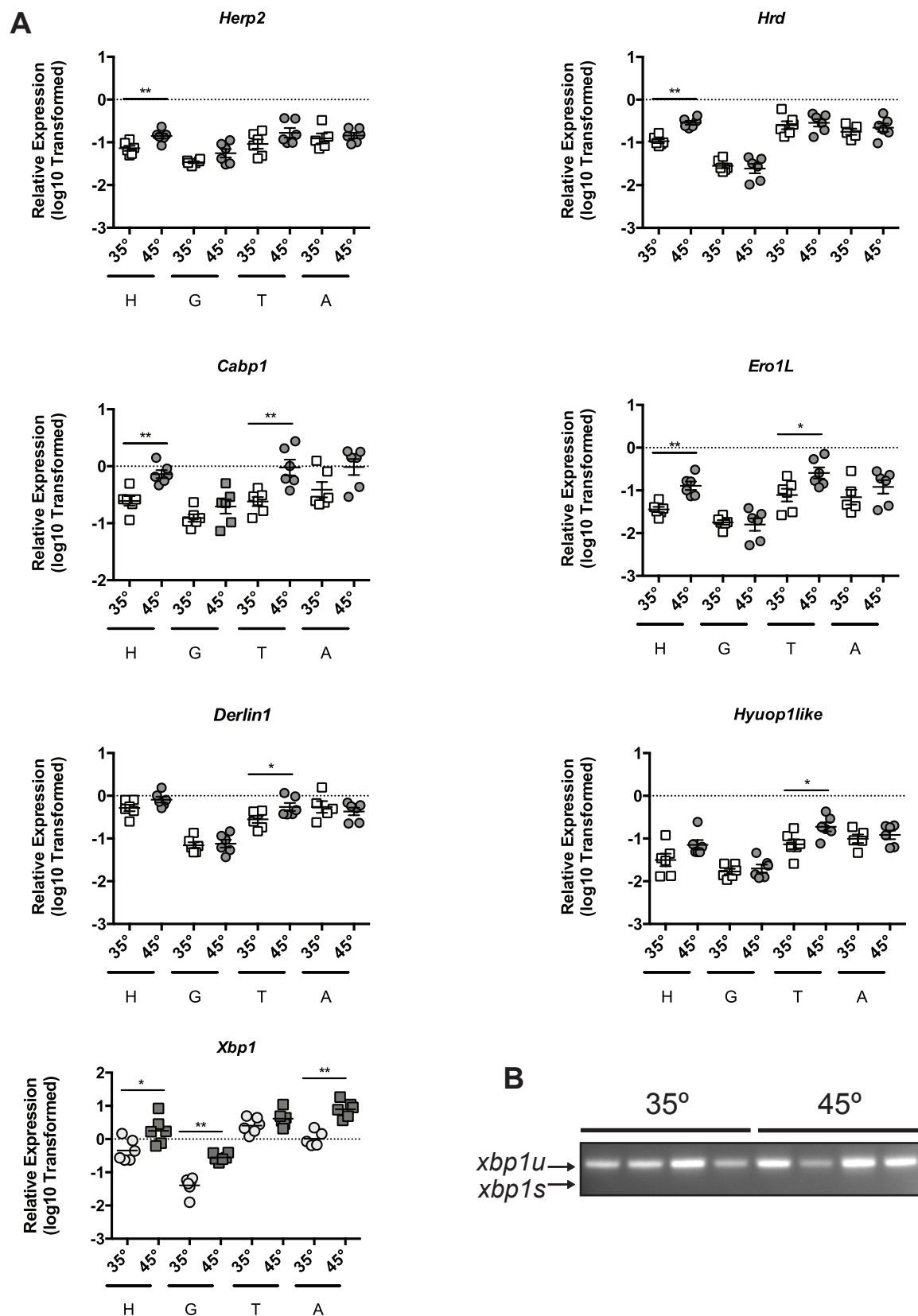
7 **Supplemental Information**



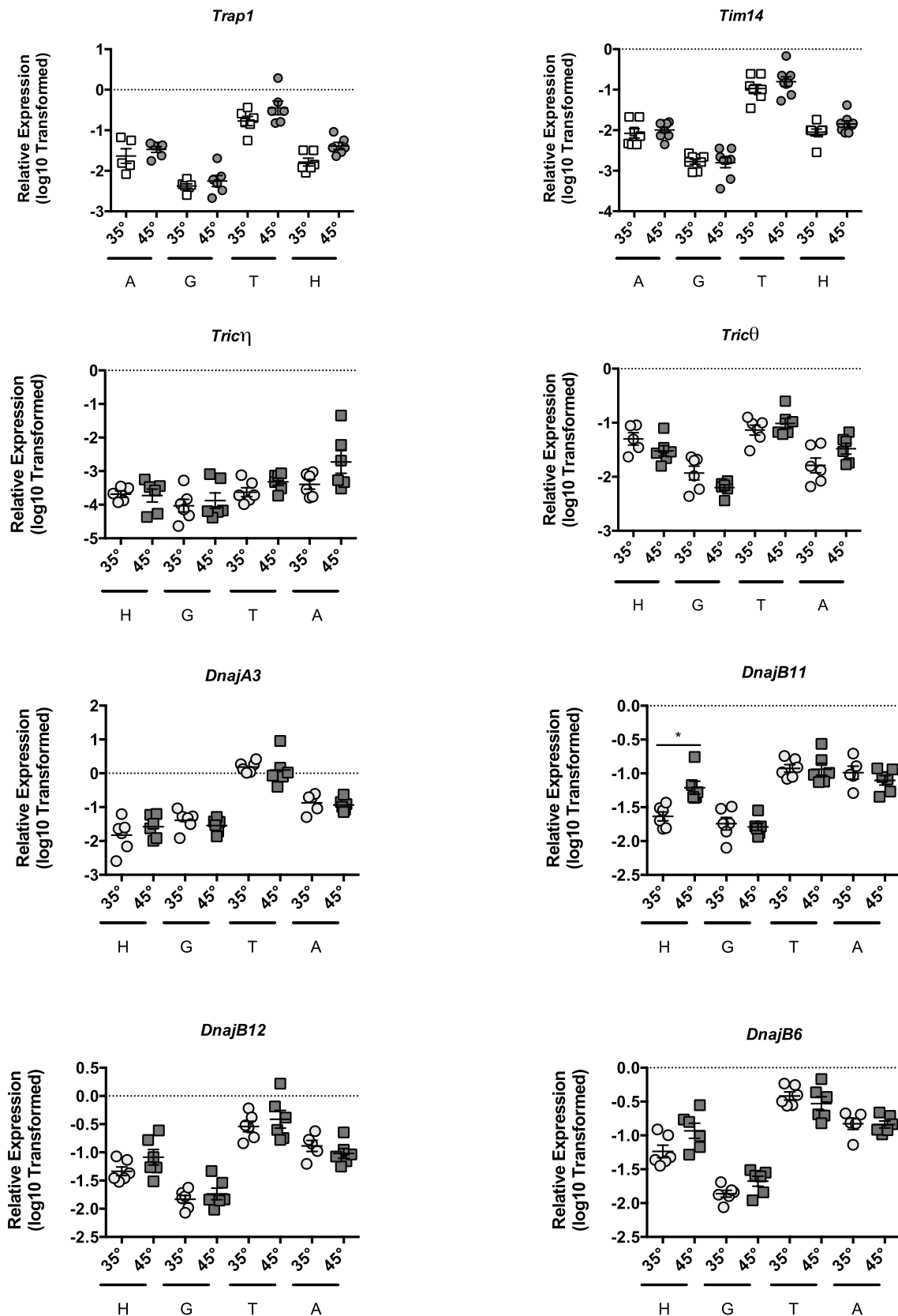
Supplemental Figure 1. Diagram of components of the HSR pathway conserved in honey bees.



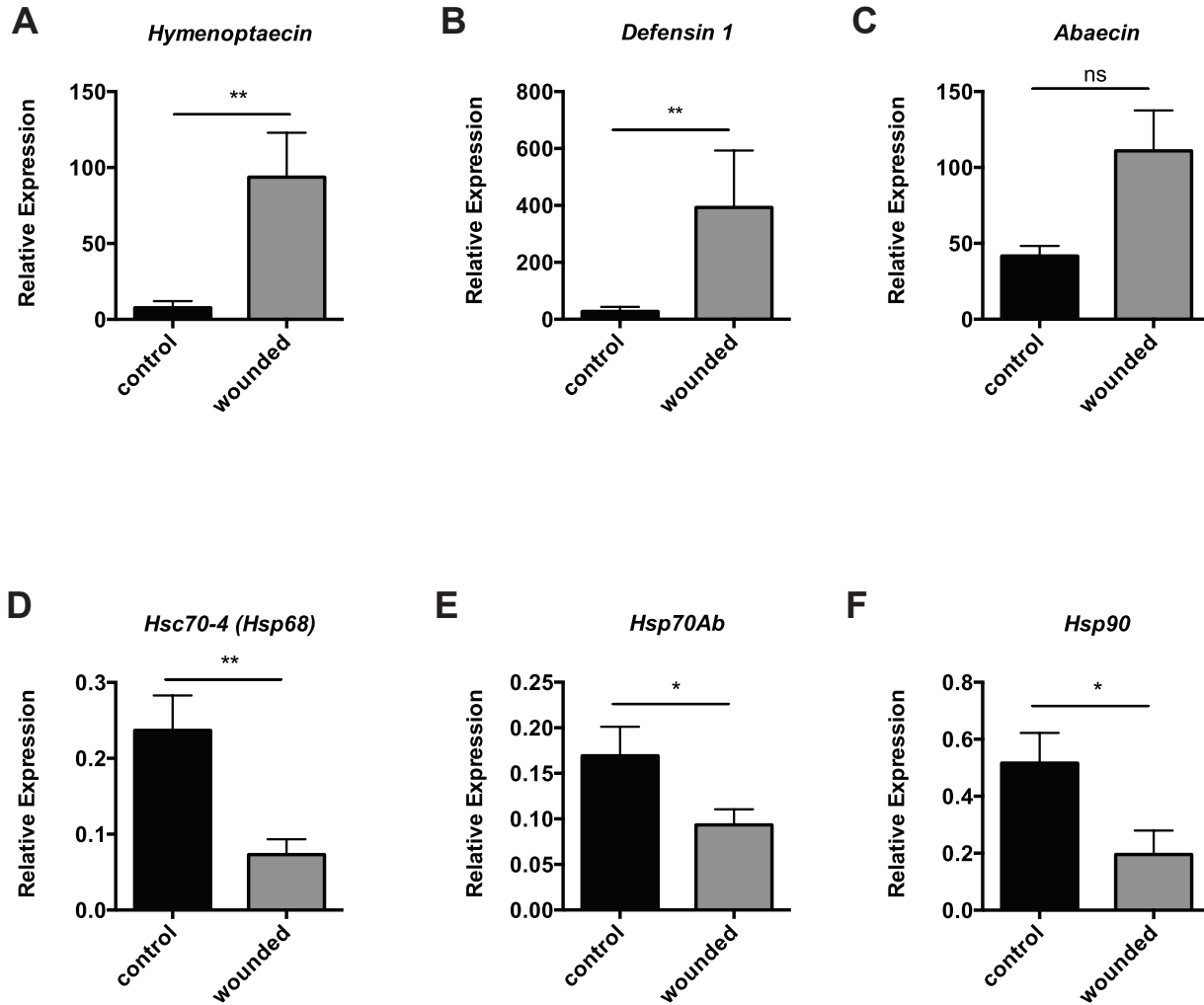
Supplemental Figure 2. Mean threshold cycle (Ct) for β -actin and transcript levels of HSR transcriptional regulator *Hsf* relative to β -actin in head tissue (H, predominantly brain and sensory organ tissue), midgut (G), thorax tissue (T, predominantly flight muscle), and abdominal wall (A, predominantly fat body) from bees maintained at 35° C or heat-shocked at 45°. Symbols represent expression values of the genes of interest calculated using the $\Delta\Delta$ CT method for individual bees. Mean \pm SEM is also shown. Statistical significance was assessed using unpaired t-tests with Welch's and is noted as *p < 0.05, and **p < 0.01.



Supplemental Figure 3. (A) Transcript levels of UPR_{ER} target genes *Herp2*, *Hrd*, *Cabp1*, and *Ero1L*, *Derlin1*, *Hyuop1* and transcriptional regulator *Xbp1* relative to β -actin in head tissue (H, predominantly brain and sensory organ tissue), midgut (G), thorax tissue (T, predominantly flight muscle), and abdominal wall (A, predominantly fat body) from bees maintained for four hours in cages at either 35 ° or 45 ° C . Symbols represent expression values of the genes of interest calculated using the $\Delta\Delta$ CT method for individual bees. Mean \pm SEM is also shown. Statistical significance was assessed using unpaired t-tests with Welch's and is noted as * $p < 0.05$, and ** $p < 0.01$. (B) *Xbp1* mRNA splicing by IRE1 in midgut tissue from individual bees maintained for 4 hours at 35° or 45° C.



Supplemental Figure 4. Transcript levels of UPR_{MT} target genes *Trap1*, *Tim14*, the TRiC genes *Tric1* and *Tric0*, and the DNAJ-containing proteins *DnajA3*, *DnajB11*, *DnajB12*, *DnajB6* relative to β -actin in head tissue (H, predominantly brain and sensory organ tissue), midgut (G), thorax tissue (T, predominantly flight muscle), and abdominal wall (A, predominantly fat body) from bees maintained for four hours in cages at either 35 ° or 45 ° C . Symbols represent expression values of the genes of interest calculated using the $\Delta\Delta$ CT method for individual bees. Mean \pm SEM is also shown. Statistical significance was assessed using unpaired t-tests with Welch's and is noted as * $p < 0.05$, and ** $p < 0.01$.



Supplemental Figure 5. Wounding represses select HSR target genes at the site of injury. Transcript levels of *Hymenoptaecin* (A), *Defensin 1* (B), and *Abaecin* (C), *Hsc70-4* (D), *Hsp70Ab* (E), and *Hsp90* (F) relative to β -actin in abdominal wall (predominantly fat body) from bees receiving a sterile wound or control bees after four hours. Bar and error bars represent the Mean \pm SEM for expression values of the genes of interest calculated using the $\Delta\Delta$ CT method. Statistical significance was assessed using unpaired t-tests with Welch's and is noted as *p < 0.05, and **p < 0.01.

Supplemental Table 1. Primer Sequences Developed for Use in this Study

Name	Gene ID	Role	5' primer	3' primer	Reference
<i>Heat shock factor (Hsf)</i>	411854	signal transduction / transcription factor	AAGAAACGCGGATGTAAGGGT	GCCATTGCTGCGAGTTTTGA	this study
<i>Heat shock protein 90 (Hsp90)</i>	408928	chaperone	CATGGCTAATGCCGGAGAGG	CTGCACCAGCTTGAAGAGC	this study
<i>Heat shock protein 83-like (Hsp83)</i>	411700	chaperone	TACTCAACGCGCTCTTCAT	TGAGCAATCTCTGCCTGGAA	this study
<i>Heat shock protein 70Ab-like (Hsp70Ab-like)</i>	410620	chaperone	CTTGCGTGGGTGTTTTCCAG	TATCATCGAAGCGACGACCG	this study
<i>Heat shock protein 70Cb ortholog (Hsp110)</i>	408706	chaperone	CATGGCTGCTATGTCCTGTA	TTTTTGGCTGCCACACCAAG	this study
<i>Heat shock protein cognate 4 (Hsc70-4, Hsp68)</i>	409418	chaperone	CTTGCGTGGGTGTTTTCCAG	TATCATCGAAGCGACGACCG	Johnston, 2016
<i>Heat shock protein 10 (Hsp10)</i>	552531	chaperone	ACGCCATGCATGATCTTAAAGC	TGCCAGTATATCTGACTCACGG	this study
<i>DnaJ protein homolog 1-like (DnajB4)</i>	411071	chaperone	CACAGCTTACGCGACACTTT	TCTCTTCTGCTCCAGCACTTC	this study
<i>DnaJ homolog subfamily A member 1 (DnajA1)</i>	724368	chaperone	AGGCTCGTATTCTGCACTCA	AGGTACGTGCAGCTTCATCC	this study
<i>Heat shock 70 kDa protein cognate 3</i>	409587	chaperone	TCGAAGTCGTGCTGAGAGTA	CCATCAGCAGTAAATGCCACA	Johnston, 2016
<i>Glycoprotein 93 (Gp93)</i>	412150	chaperone	TAGCCGCGAAGGATCTCGAA	CGATTGACTTCGGTTTGAAAAGT	Johnston, 2016
<i>p58ipk</i>	413441	chaperone	TCAGGAACCAACTCAGGCAA	GATGCTGTTCTTGGCACACC	Johnston, 2016
<i>T-complex chaperonin Epsilon</i>	409825	chaperonin subunit	TGCCATTTGAACCTCCAA	GGAACAATACGTCCACCCGT	this study
<i>T-complex chaperonin Delta</i>	551275	chaperonin subunit	GATGGCGAAATGCTCATGG	CGTTCTGCAGCTTCCAAAAGA	this study
<i>T-complex chaperonin Eta</i>	550694	chaperonin subunit	ACTTCCGTAAAGTGTGAACGAT	TTGTGCAGCATCTGTGCCCT	this study
<i>T-complex chaperonin Theta</i>	551436	chaperonin subunit	CGATGTCGCTGTAATCGCAA	GCCATTTGGGCCATAAGCAG	this study
<i>Heat shock protein cognate 5 (Hsc70-5)</i>	408605	chaperone	CAAGATGCTGACTGCTGCAAG	TGGAGTCGTTCTGTATCCTTC	this study
<i>Heat shock protein 60 (Hsp60)</i>	409384	chaperone	TACTAGCGAGACAACGTGCG	TTGTAATTGGCGCAAGGCTG	this study
<i>Hypoxia up-regulated protein 1-like (Hyo1-like)</i>	551763	chaperone	ACGTACGTGTACCTATGCCT	CCCCTACTTGAGCATCTTCTCC	this study
<i>Tumor Necrosis Factor Receptor-Associated Protein-1 (Trap)</i>	550968	chaperone	CGAAAGCATGGCTACCGTTC	TTCAAGTGCATCACTCGCATT	this study
<i>Protein tumorous imaginal discs, mitochondria (DnajA3)</i>	411359	chaperone	CGGCCTGGTACTTCATCTCA	CGCTTGAGTTTTACCTTGGA	this study
<i>DnaJ homolog subfamily B member 11</i>	552223	chaperone	AACGCTGCTAGAATGGCAGC	ATTGCATGTTGGCTTGCTGT	this study
<i>DnaJ homolog subfamily B member 12</i>	413120	chaperone	ATGAGGCGGAGCGATGTATG	TGGATGCAGTTTGACGTTTCC	this study
<i>DnaJ homolog subfamily B member 6-like/6-B</i>	408966	chaperone	AGCACGTCTCTTTGGTCC	CGAGATATCGTGGCACTGT	this study
<i>Mitochondrial import inner membrane translocase (Tim14)</i>	726653	chaperone	TGAAGATAACGCGTGCCAGT	ACTAGCTTCACGTCGTGTCA	this study
<i>Abaecin</i>	406144	antimicrobial peptide	CGTATGTACCATTACCAAATGTACCA	GACCAGGAAACGTTGGAAAC	this study
<i>Defensin 1</i>	406143	antimicrobial peptide	TGCGCTGCTAACTGTCTCAG	AATGGCACTTAACCGAAACG	Evans, 2006
<i>Hymenoptaecin</i>	406142	antimicrobial peptide	CTCTTCTGTGCCGTTGCATA	GCGTCTCTGTGCTATTCCATT	Evans, 2006
<i>Universal Bacteria 16S</i>	NA	bacteria	ACTCCTACGGGAGGCAGCAGT	ATTACCGCGCTGCTGGC	Hartman, 2009
<i>Gfp</i>	NA	db11 bacteria gfp	GTCAGTGGAGAGGGTGAAGG	TACATAACCTTCGGGCATGG	this study

	Signal Transduction / Transcription Factor
	Predicted Core HSR Target Genes
	Other Potential HSR Target Genes
	Other Genes in This Study

Supplemental Table 2. All genes encoding HSP70 proteins in the honey bee genome.

Name	<i>A. mellifera</i> Gene ID	Gene of <i>H. sapiens</i> closest homolog	Signal Sequence (SignalP 4.1)	M, S (TargetP 1.1)	Prosites (KDEL)	localization	Heat Shock
<i>Heat shock protein 70Ab-like (Hsp70Ab-like)</i>	410620	<i>Hspa1a, Hspa8, Hspa1</i>	N	N	N	C	increased
<i>Heat shock protein 70Cb ortholog (Hsp110)</i>	408706	<i>Hsph1</i>	N	N	N	C	increased
<i>Heat shock protein cognate 4 (Hsc70-4, Hsp68)</i>	409418	<i>Hspa1a, Hspa8, Hspa1</i>	N	N	N	C	increased
<i>Heat shock 70 kDa protein cognate 3 (Hsc70-3)</i>	409587	<i>Grp78</i>	Y	S	Y	ER	increased
<i>Heat shock protein cognate 5 (Hsc70-5)</i>	408605	<i>mHsp70</i>	N	M	N	M	increased
<i>Hypoxia up-regulated protein 1-like (Hyuop1-like)</i>	551763	<i>Grp170</i>	Y	N	N	ER	no change

C = cytoplasm

ER = endoplasmic Reticulum

M = mitochondria

S = secretory

Y = found

N = none found

NT = not tested

Supplemental Table 3. All genes encoding HSP90 proteins in the honey bee genome.

Name	<i>A. mellifera</i> Gene ID	Gene of <i>H. sapiens</i> closest homolog	Signal Sequence (SignalP 4.1)	M, S (TargetP 1.1)	Prosites (KDEL)	localization	Heat Shock
<i>Heat shock protein 90 (Hsp90)</i>	408928	<i>Hsp90</i>	N	N	N	C	increased
<i>Heat shock protein 83-like (Hsp83like)</i>	411700	<i>Hsp90</i>	N	N	N	C	no change
<i>Glycoprotein 93 (Gp93)</i>	412150	<i>Grp94</i>	Y	S	Y	ER	increased
<i>Tumor Necrosis Factor Receptor-Associated Protein-1 (Trap1)</i>	550968	<i>Trap1</i>	N	M	N	M	no change

C = cytoplasm

ER = endoplasmic Reticulum

M = mitochondria

S = secretory

Y = found

N = none found

NT = not tested

Supplemental Table 4. All genes encoding DNAJ-containing proteins in the honey bee genome.

Name	<i>A. mellifera</i> Gene ID	class	client binding	Gene of <i>H. sapiens</i> closest homolog	Signal Sequence (SignalP 4.1)	M, S (TargetP 1.1)	Prosit (KDEL)	localization	Heat Shock
<i>DnaJ</i> homolog subfamily A member 1	724368	A	promiscuous	<i>DnajA1</i>	N	N	N	C	increased
protein tumorous imaginal Discs, mitochondria	411359	A	promiscuous	<i>DnajA3</i>	N	M	N	M/ER	no change
<i>DnaJ</i> protein homolog 1-like	411071	B	promiscuous	<i>DnajB4</i>	N	N	N	C	no change
<i>DnaJ</i> homolog subfamily B member 11	552223	B	promiscuous	<i>DnajB11</i>	N	S	N	ER	no change
<i>DnaJ</i> homolog subfamily B member 12	413120	B	wide	<i>DnajB12</i>	N	N	N	C/ER	no change
<i>DnaJ</i> homolog subfamily B member 6-like/6-B	408966	B	wide	<i>DnajB2 (Hsj1)</i>	N	N	N	C/ER	no change
<i>DnaJ</i> homolog Dnj-5	409594	C	specific	<i>DnajC14</i>	N	N	N	C/ER/M	NT
<i>DnaJ</i> homolog subfamily C member 16	412118	C	specific	<i>DnajC16</i>	N	N	N	U	NT
<i>DnaJ</i> homolog subfamily C member 17	552846	C	specific	<i>DnajC17</i>	N	N	N	U	NT
<i>DnaJ</i> homolog subfamily C member 21	100578360	C	specific	<i>DnajC21</i>	N	N	N	U	NT
<i>DnaJ</i> homolog subfamily C member 3	413441	C	specific	<i>DnajC3 (p58iik)</i>	Y	S	N	ER	increased
<i>DnaJ</i> homolog subfamily C member 5	724965	C	specific	<i>DnajC5</i>	N	N	N	exo	NT
<i>DnaJ</i> homolog subfamily C member 7	410037	C	specific	<i>DnajC7</i>	N	N	N	C	NT
Cyclin-G-associateD kinase	413462	C	specific	<i>DnajC26</i>	N	N	N	C/N	NT
<i>DnaJ</i> homolog subfamily C member 22	410730	C	specific	<i>DnajC22</i>	N	N	N	C	NT
<i>DnaJ</i> homolog subfamily C member 24	724329	C	specific	<i>DnajC24</i>	N	N	N	C	NT
<i>DnaJ</i> homolog subfamily B member 13	727642	C	unclear	<i>DnajB13</i>	N	N	N	C	NT
<i>DnaJ</i> homolog subfamily C member 11	409991	C	unclear	<i>DnajC11</i>	N	N	N	M	NT
<i>DnaJ</i> homolog subfamily C member 13	411060	C	unclear	<i>DnajC13 (Rem8)</i>	N	N	N	C/M	NT
<i>DnaJ</i> homolog subfamily C member 8	410294	C	unclear	<i>DnajC8 (Sfp3b)</i>	N	N	N	C	NT
<i>DnaJ</i> homolog subfamily C member 9-like	409892	C	unclear	<i>DnajC9</i>	N	N	N	N	NT
<i>DnaJ</i> homolog subfamily C member 1-like	552151	C	no	<i>DnajC1 (Erdj1)</i>	Y	S	N	ER	NT
J Domain-containing protein	408863	C	no	<i>DnajC12 (Jpd1)</i>	N	N	N	U	NT
Mitochondrial import inner membrane translocase	726653	C	no	<i>DnajC19 (Tim14)</i>	N	S	N	M	no change
<i>DnaJ</i> homolog subfamily C member 2	413209	C	no	<i>DnajC2 (Zuotin)</i>	N	N	N	C	NT
SEC63 homolog	412505	C	no	<i>DnajC23 (Sec63)</i>	N	N	N	U	NT
<i>DnaJ</i> homolog subfamily C member 18	102654159	?	?	ND	N	N	N	U	NT

C = cytoplasm
ER = endoplasmic Reticulum
M = mitochondria
S = secretory
Y = found
N = none found
NT = not tested

Supplemental Table 5. All genes encoding alpha-crystallin domain containing proteins in the honey bee genome.

Name	<i>A. mellifera</i> Gene ID	Gene of <i>H. sapiens</i> closest homolog	Signal Sequence (SignalP 4.1)	M, S (TargetP 1.1)	Prosite (KDEL)	localization	Heat Shock
<i>Heat shock protein beta-1</i>	408875	<i>alpha-crystallin B chain</i>	N	N	N	C	NT
<i>Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase</i>	413078	<i>Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase</i>	N	N	N	C	NT
<i>SGT1 homolog</i>	551201	<i>SGT1</i>	N	N	N	C	NT
<i>Lethal(2)essential for life-like (l(2)efl)</i>	724488	<i>HSPB1, clust</i>	N	N	N	C	NT
<i>Lethal(2)essential for life-like (l(2)efl)</i>	724405	<i>HSPB5 (CRYAB) clust</i>	N	N	N	C	NT
<i>Lethal(2)essential for life-like (l(2)efl)</i>	724367	<i>HSPB5 (CRYAB) clust</i>	Y	S	N	ER	NT
<i>Lethal(2)essential for life-like (l(2)efl)</i>	724274	<i>HSPB5 (CRYAB) clust</i>	N	N	N	C	NT
<i>Lethal(2)essential for life-like (l(2)efl)</i>	410857	<i>HSPB5</i>	N	N	N	C	NT
<i>Lethal(2)essential for life-like (l(2)efl)</i>	724449	<i>HSPB1 clust</i>	N	N	N	C	NT
<i>Lethal(2)essential for life-like (l(2)efl)</i>	412197	<i>HSPB5 (CRYAB)</i>	N	N	N	C	NT
<i>uncharacterized LOC410087</i>	410087	<i>HSPB5 (CRYAB)</i>	N	M	N	C	NT
<i>PIH1 domain containing 1</i>	726775	<i>PIH1 domain containing 1</i>	N	N	N	C	NT
<i>PIH1D3-like</i>	102655121	<i>PIH1D3</i>	N	N	N	C	NT

C = cytoplasm

ER = endoplasmic Reticulum

M = mitochondria

S = secretory

Y = found

N = none found

NT = not tested

Supplemental Table 6. All genes encoding chaperonin proteins in the honey bee genome.

Name	<i>A. mellifera</i> Gene ID	<i>H. sapiens</i> closest homolog	Signal Sequence (SignalP 4.1)	M, S (TargetP 1.1)	Prosites (KDEL)	localization	Heat Shock
<i>T-complex protein 1 subunit alpha</i>	409134	<i>TCP1 alpha</i>	N	M	N	C/M	NT
<i>T-complex protein 1 subunit beta</i>	409809	<i>TCP1 beta</i>	N	N	N	C	NT
<i>T-complex chaperonin delta</i>	551275	<i>TCP1 delta</i>	N	N	N	C	up
<i>T-complex chaperonin epsilon (Cct5)</i>	409825	<i>TCP1 epsilon</i>	N	N	N	C	up
<i>T-complex protein 1 subunit gamma</i>	409296	<i>TCP1 gamma</i>	N	N	N	C	NT
<i>T-complex protein 1 subunit eta</i>	550694	<i>TCP1 eta</i>	N	N	N	C	no change
<i>T-complex protein 1 subunit theta</i>	551436	<i>TCP1 theta</i>	N	N	N	C	no change
<i>T-complex protein 1 subunit zeta</i>	551295	<i>TCP1 zeta</i>	N	N	N	C	NT
<i>Heat shock protein 60 (Hsp60)</i>	409384	<i>Hsp60</i>	N	M	N	M	up
<i>Heat shock protein 10 (Hsp10)</i>	552531	<i>Hsp10</i>	N	M	N	M	up

C = cytoplasm

ER = endoplasmic Reticulum

M = mitochondria

S = secretory

Y = found

N = none found

NT = not tested