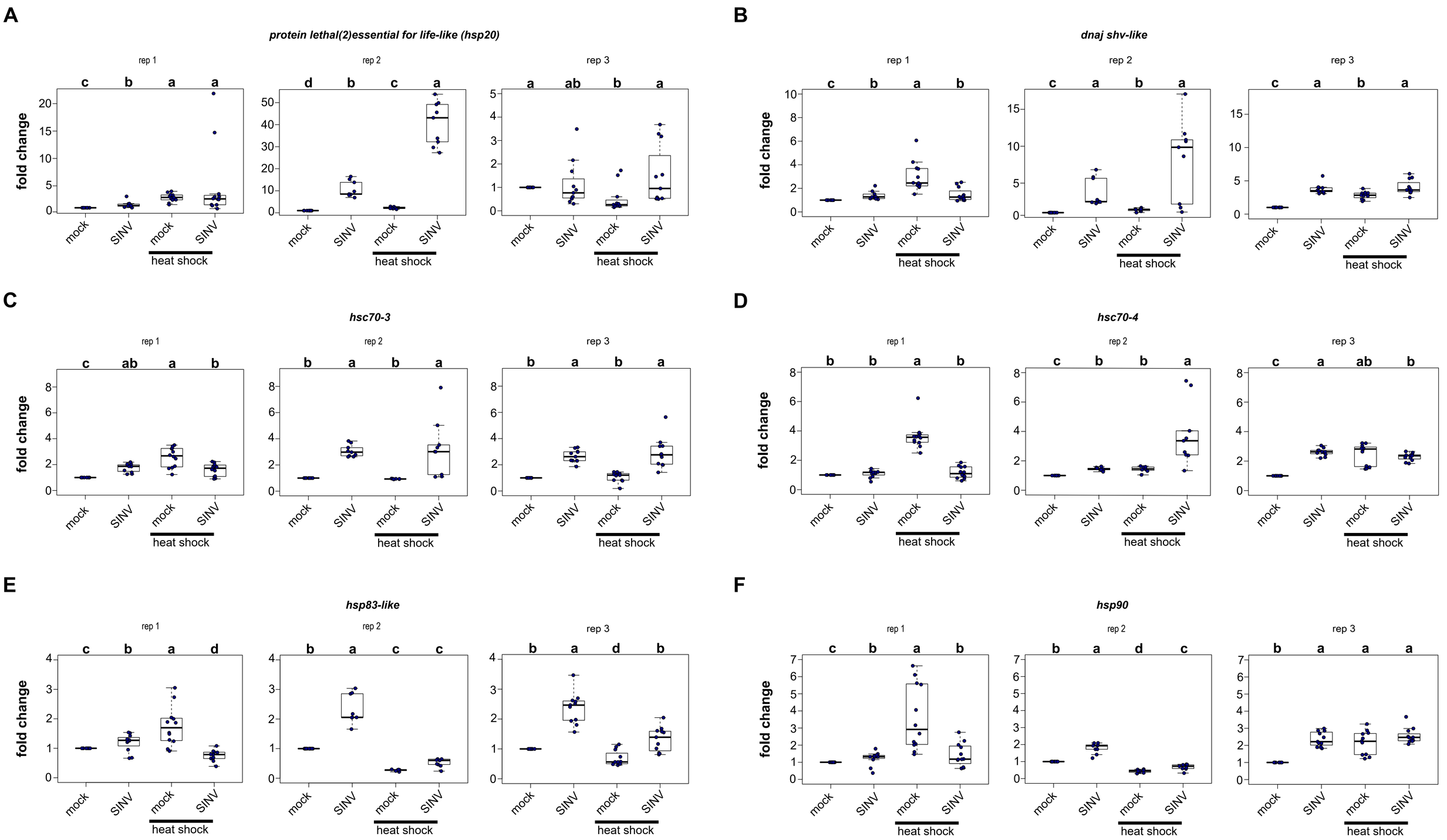


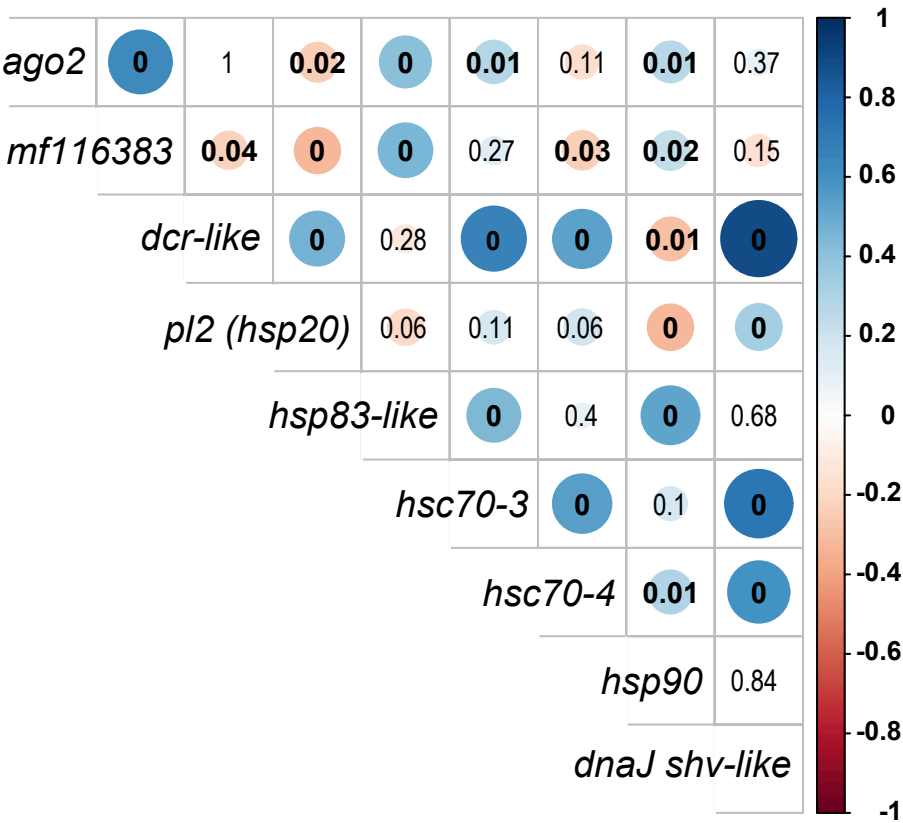
Supplementary Figure S1. Induction of heat shock proteins in 3 replicates

The relative expression of honey bee heat shock protein encoding genes was assessed by qPCR using the ΔΔCt method with normalization to rpl8 and relative to mock-infected bees maintained at a constant temperature. Unless otherwise stated, the following comparisons are relative to mock-infected honey bees.

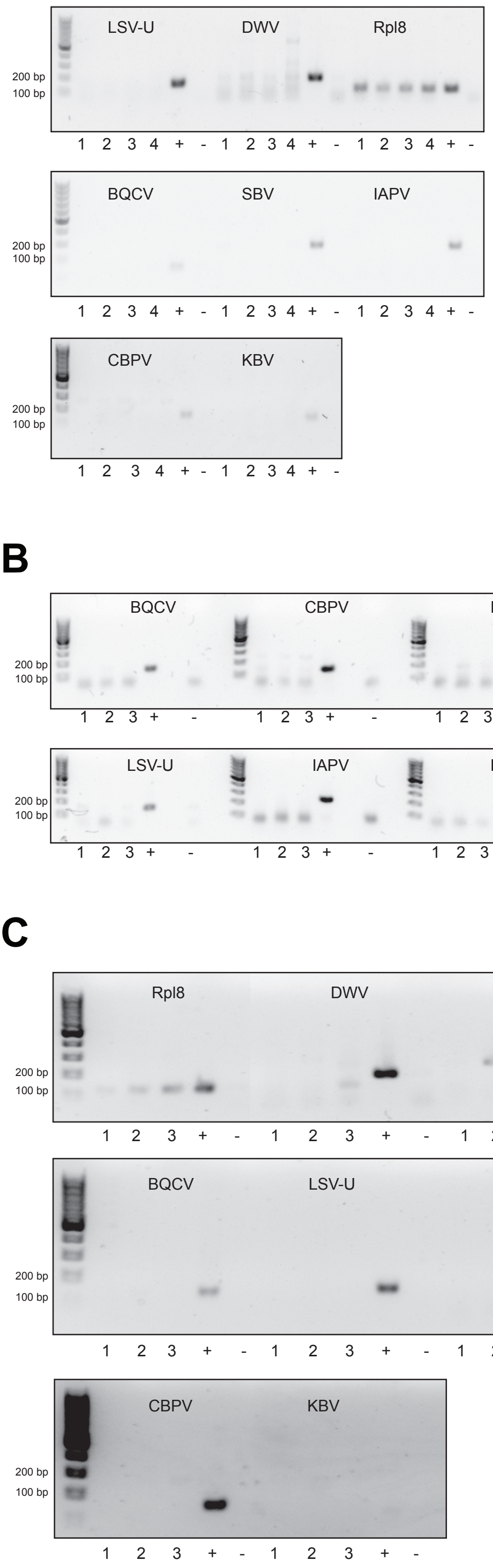
(A) Protein lethal(2)essential for life-like (hsp20) was significantly increased in expression by both viral infection and heat shock in replicates 1 and 2, but not replicate 3. (B) Dnaj shv-like was significantly increased in expression by both viral infection and heat shock in all three replicates, including when the stressors were combined. (C) While heat shock 70-kDa protein cognate 3 (hsc70-3) expression was induced by viral infection in all three replicates, its expression was only increased by heat shock in replicate 1. However, hsc70-3 expression was increased in all three replicates where bees received both viral infection and heat shock. (D) Heat shock 70-kDa protein cognate 4, hsc70-4, expression was increased in heat shocked bees in all three replicates and viral infection induced this gene in replicates 2 and 3, but not in replicate 1. Likewise, combined heat shock and viral infection induced hsc70-4 expression in replicates 2 and 3, but not in replicate 1.

(E) Heat shock protein 83-like (hsp83-like) expression was induced by viral infection in all three replicates, while heat shock induced expression in replicate 1 but reduced its expression in both replicates 2 and 3. The combination of virus-infected and heat shock resulted in reduced hsp83-like expression relative to viral infection alone in all three replicates, indicating heat shock may suppress expression.

(F) Heat shock protein 90 (hsp90) expression was increased in virus-infected bees in all three replicates and mock-infected, heat shocked bees in replicates 1 and 3, whereas expression was reduced in replicate 2. Expression of hsp90 was induced by virus-infected, heat shocked bees in replicates 1 and 3, but its expression was reduced in replicate 2. Data were analyzed by a pairwise Wilcoxon Rank Sums with a Benjamini-Hochberg correction for multiple comparisons within replicates. Shared letters above two treatments denote there is no difference while different letters denote a statistical difference.



Supplementary Figure S2. Honey bee gene co-expression p-value matrix
The co-expression of genes in virus-infected heat shocked honey bees was analyzed by calculating the correlation coefficient for each pairwise comparison, each of which were assessed by calculating a corresponding p-value, which are shown in each cell. Correlation coefficients (r-values) are determined to be significant when their corresponding p-value is below 0.05. Shaded blue circles represent positive correlations while shaded red circles denote negative correlations. The larger the circle, the stronger the correlation. The matrix illustrates several significant positive correlations between immune genes (dcr-like, ago2, and mf116383) and heat shock proteins (pl2, hsp83-like, hsc70-3, hsc70-4, hsp90, dnaj shv-like), including dcr-like and dnaj shv-like, hsc70-3 and dnaj shv-like, and dcr-like and hsc70-3.



Supplementary Figure S3. Honey bee pathogen test results

Pathogen-specific PCR was used to test for presence of potential confounding, pre-existing infections in honey bees obtained from the same colony at the same time as the honey bees utilized in the experiments described herein. RNA was isolated from individual, untreated bees that were housed in conditions identical to the bees in the non-heat shocked treatment groups for the duration of the study. The quality of the cDNA was assessed using primers for the honey bee *rpl8* gene and the presence of the following viruses was assessed by PCR using primers listed in Supplementary Table S1 (i.e., LSV-U (Lake Sinai virus - universal), DWV (deformed wing virus), BQCV (black queen cell virus), SBV (sacbrood virus), IAPV (Israeli acute paralysis virus), CBPV (chronic bee paralysis virus), ABPV (acute bee paralysis virus) and KBV (Kashmir bee virus). +, positive control; -, negative control). Pathogen testing was performed on pooled cDNA samples representing four individual bees. PCR products were products were analyzed by agarose gel electrophoresis. (A) Pathogen test results of pooled cDNA samples from biological replicates 1 and 2 (Figures 1-4). Specifically, pools 1 and 2 are from rep1 bees, pool 3 consists of samples from reps 1 and 2, and pool 4 consists of rep2 cDNA samples. The PCR results for these samples indicate that the virus levels in biological replicates 1 and 2 were below the limit of detection. (B) Pathogen test results of three unique sets of pooled cDNA samples, representing 12 bees, from biological replicate 3 (Figures 1-4). The PCR results pooled cDNA samples 1, 2, and 3 indicate that the virus levels in biological replicate 3 were below the limit of detection. (C) Pathogen test results of three unique sets of pooled cDNA samples, representing 12 bees, from biological replicate 2 of Figure 5. The PCR results from pooled cDNA samples 1, 2, and 3 indicate that the virus levels were below the limit of detection for the majority of the samples, faint bands corresponding to SBV were detected in pools 2 and 3.