



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 $\Delta\Delta C_t$ 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.