**Supplementary table S1:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S.N. | Primers | **Direction** | **Sequence** | **Amplicon Length** |
| 1 | Krupple homolog (*Kr-h1*)-long | Forward | TTGCATCAGGTTGCCCACTA | 668 |
| Reverse | CACCGTTTTCTTGGAGGGAGA |
| 2 | *Kr-h1*–T7 nested | Forward | TAATACGACTCACTATAGGGAGAAAAACTCATTCAGACTCATCGGT | 440 |
| Reverse | TAATACGACTCACTATAGGGAGATACTCCCTCTGTCTTTCTTCTTCG |
| 3 | pGEMT–T7 nested | Forward | TAATACGACTCACTATAGGGAGAAAGATACCAGGCGTTTCCCC | 433 |
| Reverse | TAATACGACTCACTATAGGGAGAGCCGGATCAAGAGCTACCAA |
| 4 | *Kr-h1* RT-PCR | Forward | TGAAGGTACATACCCGCACG | 109 |
| Reverse | TAGTGGGCAACCTGATGCAA |
| 5 | Elongation factor (Ef-1α)-RT-PCR | Forward | CGTTTACCGCTTCAGGACGT | 91 |
| Reverse | GCATGCCTGGTTTCAGAATA |

Chart, waterfall chart

Description automatically generated

Chart, box and whisker chart

Description automatically generated

**Supplementary Figure S2. The influence of the days of dsRNA injection on ovarian activity.** (**A**) A summary of experiments in which we tested different amounts of dsRNA (1-3µg, as shown on the X axis). We loaded plasmid (ds-pG) or *Bombus terrestris* *Kr-h1* dsRNA onto 0.1pmol PFCnp. Ovarian activity was assessed on day 7 of the experiment. We compared each treatment group with the control group (C) using Unpaired t test with Welch's correction for multiple comparisons. *\* - α<0.05, \*\* - α<0.001, \*\*\* - α<0.0001*. (**B**) A summary of experiments in which we injected 1 µg control or *Kr-h1* dsRNA loaded to 0.1µg/µl PFCnp. Sample sizes is shown below each box plot. Treatments marked with different small letters are statistically different in a Kruskal-Wallis H Test, followed by Dunn’s post-hoc analysis comparing each combination of injection days. For additional details, see Supplementary Figure S1.

Chart, box and whisker chart

Description automatically generated

Chart, bar chart

Description automatically generated

**Supplementary Figure S1: The influence of PFCnp concentration on ovarian activity and on dsRNA binding efficiency**. (**A**) Ovarian activity and survival. The x-axis shows increasing concentration of PFCnp (not loaded with RNA) injected to bees. The number of bees surviving, out of a total of 8 injected bees, is shown in parentheses. Treatments with different letters are statistically different in a Kruskal-Wallis test followed by pairwise Dunn posthoc test. The box plots show ovarian activity at the age of 7 days. Each box plot shows the median (—), mean (+), and the box frame spans over the first to the third quartile. The whiskers depict the 5th/95th percentile; outliers are depicted with black dots. (**B**) dsRNA loading efficiency. The darkness of the bar corresponds to the amount of PFCnp onto which the dsRNA was loaded. The bars and whiskers depict mean ± SE, N=5. Further details as in Figure 1. Bars marked with different small letters are significantly different in Two-way ANOVA and Bonferroni posthoc tests using log 10 transformed data.

Diagram

Description automatically generated

Diagram

Description automatically generated

**Supplementary figure S3: The influence of naked *Kr-h1* dsRNA injection on JH-regulated physiology and behavior.** (A) general outline of the experiment. (B) Fat body *Kr-h1* mRNA levels. (C) Wax weight at the end of experiment. (D) Ovarian activity at 7 days of age. The vertical bars in panels B-D depict mean ± SE. (E) The amounts of threatening displays performed before and after the 2nd dsRNA injections. The details of the box plots in panel E are as described in Supplementary Figure S1. Treatments marked with different small letters are statistically different in either one-way ANOVA followed by Tukey post-hoc analysis for parametric data (B & C), or Kruskal Wallis H tests followed by Dunn’s post-hoc analysis for non-parametric data (D & E). Numbers below the bars depict the sample size for each group; the sample size in C is the number of cages from which wax amount was measured.

**Supplementary table S2: Summary of the three-way ANOVA analysis of the figure 2 B & C.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Variations** | **F** | **DF** | ***P value*** |
| Ovarian maturation | Treatment | 6.689 | 3 | *<0.001* |
| Trial | 0.856 | 1 | *0.356* |
| DMF vs. JH | 10.266 | 1 | *0.002* |
| Treatment x Trial | 0.814 | 3 | *0.488* |
| Treatment x DMF vs JH | 0.099 | 3 | *0.960* |
| Trial x DMF vs JH | 2.257 | 1 | *0.135* |
| Treatment x Trial x DMF vs JH | 0.343 | 3 | *p = 0.794* |
| Wax secretion | Treatment | 16.646 | 3 | *<0.001* |
| Trial | 2.692 | 1 | *0.111* |
| DMF vs. JH | 2.040 | 1 | *0.164* |
| Treatment x Trial | 1.289 | 3 | *0.296* |
| Treatment x DMF vs JH | 0.282 | 3 | *0.838* |
| Trial x DMF vs JH | 0.003 | 1 | *0.953* |
| Treatment x Trial x DMF vs JH | 0.737 | 3 | *p = 0.538* |

**Diagram

Description automatically generated**

**Supplementary Figure S4: The influence dsRNA mediated *Kr-h1* knock-down on dominance and agonistic behavior – separate analyses for each observation day.** Top row: Threatening displays. Middle row: Dominance index. Lower row: Dominance rank. The right, central, and left columns summarize the observations on Day 3, 4, and 5, respectively. Other details as in Fig. 3.