**Extended Material and Methods**

**Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)**

Termite queens were treated using siRNA and RNA was extracted as described in the methods section of the main text. The concentration and purity of the extracted RNA was checked using a nanodrop (Nanodrop ND-1000) and all the samples were then standardized to a concentration of 25 ng/µl. We had a total of 17 samples (5 target, 6 sham and 6 untreated). In addition to the test sample, we also extracted RNA from a pool of three separate queens which we used to calibrate the qRT-PCR run.

For HDAC3 amplification, we used Primer3web version 4.1.0 (<https://primer3.ut.ee/> last accessed on 10.08.2024) for primer design. Using the complete HDAC3 coding sequence (Csec\_G08074), we designed the forward and reverse primer sequences 5’-GAGGCGTCGGGATTCTGTT-3’ and 5’-CCACTCTCTGCACCCACTTC-3’ targeting a region of 224 bp within the coding sequence. To normalize the expression of HDAC3, *γ*-Tubulin forward and reverse primers 5’-CCCTTGATTCCCACACCTCG-3’ and 5’-AGCATTACGGTCGTGAGCC-3’ previously designed in our lab were also used.

The qRT-PCR amplification was done using SYBR Green one-step qRT-PCR kit according to the manufacturer’s instructions. Briefly, we set up a 20 µl qRT-PCR reaction containing 10 µl (2×) SYBR green reaction mix, 0.2 µl (units) of reverse transcriptase, 1 µl (0.5µM) each of the forward and reverse primers 6.2 µl of nuclease free water and 1.6 µl (40ng) of RNA. The reaction was run in a LightCycler® 96 (ROCHE) with the following steps: Reverse transcription at 50° C for 30 min, initial inactivation at 95° C for 15 min, 3 amplification steps of 95° C for 15 s, 62° C for 30 s and 72° C for 70 s during which the signal was also captured. Finally, a melting step of 95°C for 10s, 65° C for 60 s and 97°C for 1 s was performed.

The resulting qPCR products were first analyzed using the LightCycler® 96 software version 1.1.0.1320 (ROCHE) and the normalized expression data were exported to R where statistical analyses were performed. We used the Wilcoxon rank sum test to analyze the data, testing for expression differences between the HDAC3 target treatment versus sham treatment, HDAC3 target treatment versus control and sham treatment versus control. We also tested for the efficiency of the *HDAC3* silencing by analyzing the percentage reduction in *HDAC3* expression in the target and sham samples using the control samples as a baseline. The R scripts data and qPCR data used are available on GitHub (<https://github.com/AllanOkwaro/Csec_HDAC3_Analysis>).

**Supplementary Results**

The qRT-PCR analysis showed a 48 % reduction in *HDAC3* expression between the target and the sham treatment and a 72 % reduction between the target and the control; both of which were significant differences (Wilcoxon rank-sum test: p < 0.05). For the RNAseq samples, we performed a Wilcoxon rank-sum test on the read counts data obtained from the experimental groups. Our values are typical for RNAi studies via feeding, which often have an efficiency 50 % - 90 % depending on the species (Huvenne & Smagghe, 2010; Zhou et al., 2008).

**Supplementary Figures**



**Figure 1: Boxplots for the analysis of HDAC3 after silencing**. **A**) quantitative polymerase chain reaction (qPCR) analysis showing significant downregulation of *HDAC3* expression in the target groupcompared to the sham and untreated group (Wilcoxon signed-rank test: p-value < 0.05). **B**) Shows the *DESeq2* rlognormalized read counts of *HDAC3* gene from the RNA sequencing reads. The small letters above each boxplot indicates the level of significance in a pairwise comparison based on Wilcoxon signed-rank test. Similar letter means that there is no significant difference between the comparisons (p-value > 0.05). Also check beta actin



**Figure 2**: **Effect of the *HDAC3* silencing experiment on expression of TI-J-LiFe genes**. The heatmap shows the expression of all genes of the IIS, JH, Fecundity and CHC synthesis pathways for the different treatment groups (shown at the bottom). The values inside the boxes represent the FDR (false discovery rate)-adjusted *p-*values while its color indicates the direction of gene expression based on Log2FoldChange (blue = downregulated in the first compared to the second treatment; red = upregulated in the first compared to the second treatment. \* indicates significantly differentially expressed genes

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Huvenne, H., & Smagghe, G. (2010). Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: A review. In Journal of Insect Physiology (Vol. 56, Issue 3, pp. 227–235).

Zhou, X., Wheeler, M. M., Oi, F. M., & Scharf, M. E. (2008). RNA interference in the termite *Reticulitermes flavipes* through ingestion of double-stranded RNA. Insect Biochemistry and Molecular Biology, 38(8), 805–815