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If you have any questions, please consult our Journal Policies and/or contact us: editorial@elifesciences.org.

Sample-size estimation

- You should state whether an appropriate sample size was computed when the study was being designed
- You should state the statistical method of sample size computation and any required assumptions
- If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

No ethical considerations regarding sample size were necessary as the study deals with common laboratory insect models (*Tribolium, Oncopeltus, Gryllus*). The validation of experimental results did not require a prior calculation of sample-size since the described knockdown experiments resulted in highly penetrant phenotypes.

Replicates

- You should report how often each experiment was performed
- You should include a definition of biological versus technical replication
- The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
- If you encountered any outliers, you should describe how these were handled
- Criteria for exclusion/inclusion of data should be clearly stated
- High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:



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The conclusions of the paper rest on RNAi knockdown experiments which were performed according to the highest standards of the field (as in our former studies). The experimental procedure is reported in the "RNAi" section (see Material and Methods) (page 12). As evident from the primer list (Supplemental Material) RNAi knockdown for *Tribolium* genes were repeated using non-overlapping fragments to test for variation in the knockdown strength and potential second site effects. No such effects were observed. Furthermore, the independent knockdown of components of the same pathway produced nearly identical phenotypes. This provides mutual support for the specificity of the knockdown phenotypes including those produced in *Gryllus* and *Oncopeltus*.



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Statistical reporting

- Statistical analysis methods should be described and justified
- Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
- For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
- Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn't apply to your submission:

The numbers of embryos showing particular aspects of the knockdown phenotype are indicated in Figure 2 – figure supplement 4 or in the result section (and 13-15). A Chi-Square test was performed to provide evidence for the knockdown phenotype of T48 (page 14). The developmental delay caused by knockdown of Fog signaling components was analyzed using an unpaired t-test (Figure 10 – figure supplement 1).

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

Group allocation

- Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
- Indicate if masking was used during group allocation, data collection and/or data analysis

Please outline where this information can be found within the submission (e.g., sections o
figure legends), or explain why this information doesn't apply to your submission:
Not applicable.

Additional data files ("source data")

- We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
- Where provided, these should be in the most useful format, and they can be uploaded as "Source data" files linked to a main figure or table
- Include model definition files including the full list of parameters used
- Include code used for data analysis (e.g., R, MatLab)
- Avoid stating that data files are "available upon request"

Please indicate the figures or tables for which source data files have been provided:



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No source data is provided except for exemplary movies (Movie 1-18).