Supplemental Information

Epidermal Growth Factor Signaling Promotes Sleep through a Combined Series and Parallel Neural Circuit

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Figure S1. RIS-enriched genes for which fluorescence transgene reporters are expressed in RIS. Related to Figure 1.

Validation of RIS enriched genes using fluorescent transgene reporters. Example micrographs for srd-32p::GFP, sbt-1::GFP, nlp-11p::GFP, ser-7::GFP, zig-2::GFP, and their co-localization with flp-11p::mKate2 (the p after the gene name indicates a promoter fusion/ transcriptional fusion, the absence of a p indicates a translational fusion according to the C. elegans nomenclature). Dashed lines display the outlines of the head and pharynx (anterior is left, dorsal is up). RIS is indicated with a white arrow. Scale bar is 50µm.
<table>
<thead>
<tr>
<th>Reporter</th>
<th>flp-11p::mKate2</th>
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<td>ins-27p::GFP</td>
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Figure S2. RIS-enriched genes for which fluorescent transgene reporters do not show RIS expression. Related to Figure 1.

Example micrographs for reporter expression from nlp-8p::GFP, nlp-13p::GFP, nlp-37p::GFP, flp-1p::GFP, ins-24p::GFP, ins-27p::GFP, and their co-localization with expression from flp-11p::mKate2. Dashed lines display the outlines of the head and pharynx (anterior is left, dorsal is up). RIS is indicated with white arrows. Scale bar is 50µm. Lack of reporter gene expression could reflect false positives transcriptome results or false negative reporter expression.
Figure S3. Heat control device. Related to Figures 3, 4, 5, 6, and methods section “Induction of cellular stress by heat shock”.

An agarose microchamber on a glass slide can be placed in the hole of the metal plate (bottom view, right side), which is attached to a Peltier element. Heat is transported by the Peltier element from the metal plate to a metal grid, which is equilibrated with the surrounding air temperature using a small fan (top view). A small petri dish is also glued to the metal plate to allow the filling with agarose, serving as a moisture reservoir and creating contact between the metal plate and the microchambers.
**A**

let-23 (2K1067.1A.1)

**B**

**FRT::let-23::FRT::GFP**

- Arcade cells
- ALA
- RIS

**C**

**let-23 expressing neurons**

- Neurons
- let-23 expression
- No expression

- GABAergic

**D**

**FRT::Cherry::FRT::GFP; unc-47p::FLP**

**E**

**FRT::mCherry::FRT::GFP; unc-47p::FLP**

- RIS
- AVL
- RMEs

**RIS**

**ALA**

**Arcade cells**

**GABAergic**

**let-23 expressing neurons**

**Neurons**

- let-23 expression
- No expression

**tsne 1**

**let-23**

**expression**

**No expression**

**tsne 2**

- 60
- 30
- 0
- 30
- 60

**let-23 expressing neurons**

- GABAergic
- ALA
- RIS
Figure S4. Structure of the conditional let-23 allele and test for recombination specificity of the driver line. Related to Figure 4.

(A) A schematic representation of the generation of the conditional let-23 allele. The section shows let-23 (ZK1067.1A.1) on chromosome II. Insertions that were made to generate let-23(zh131) are color coded; black: genomic sequence, red: FRT sites, orange: mutation of PAM for sgRNA3_let-23tyrK, blue: linker sequence, green: ATG of GFP coding region, yellow: Flag Tag. (B) The conditional let-23::GFP allele shows visible expression in both the ALA and RIS neurons, but not in other neurons. Cells anterior to the neurons likely correspond to the posterior arcade cells [S1]. Dashed lines display the outlines of the head and pharynx (anterior is left, and dorsal is up). Arcade cells, ALA, and RIS are indicated with white arrows. Scale bar represents 50 µm. (C) Single-cell sequencing data reveals that let-23 is expressed mainly in two neurons, ALA and RIS. No consistent let-23 expression could be seen in GABAergic motor neurons. Cells expressing let-23 are colored according to the cluster they belong to. Grey cells do not express let-23. The large central cluster corresponds to mainly cholinergic neurons [S2]. (D-E) mCherry and GFP expression of the recombination reporter FRT::mCherry::FRT::GFP after temperature stimulus with the recombinase (FLPase) expressed in GABAergic neurons, unc-47p::FLP D5. Dashed lines display the outlines of the head and pharynx (anterior is left, and dorsal is up). RMEs, AVL, and RIS are indicated with white arrows. Scale bar represents 50 µm. (D) After the temperature stimulus, mCherry is expressed in all nuclei in which recombination was not complete. GFP is expressed in the nuclei of cells, in which recombination took place. Recombination is seen in the RMEs, AVL, and RIS, but not in ALA. (E) The GFP expression of (D) is shown separately for better clarity and the neurons RMEs, AVL and RIS are indicated by a white arrow. GFP expression was not observed in ALA in any of the worms tested (n = 5).

Supplemental References