Current Biology, Volume *30*

Supplemental Information

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

Jan Konietzka, Maximilian Fritz, Silvan Spiri, Rebecca McWhirter, Andreas Leha, Sierra Palumbos, Wagner Steuer Costa, Alexandra Oranth, Alexander Gottschalk, David M. Miller III, Alex Hajnal, and Henrik Bringmann

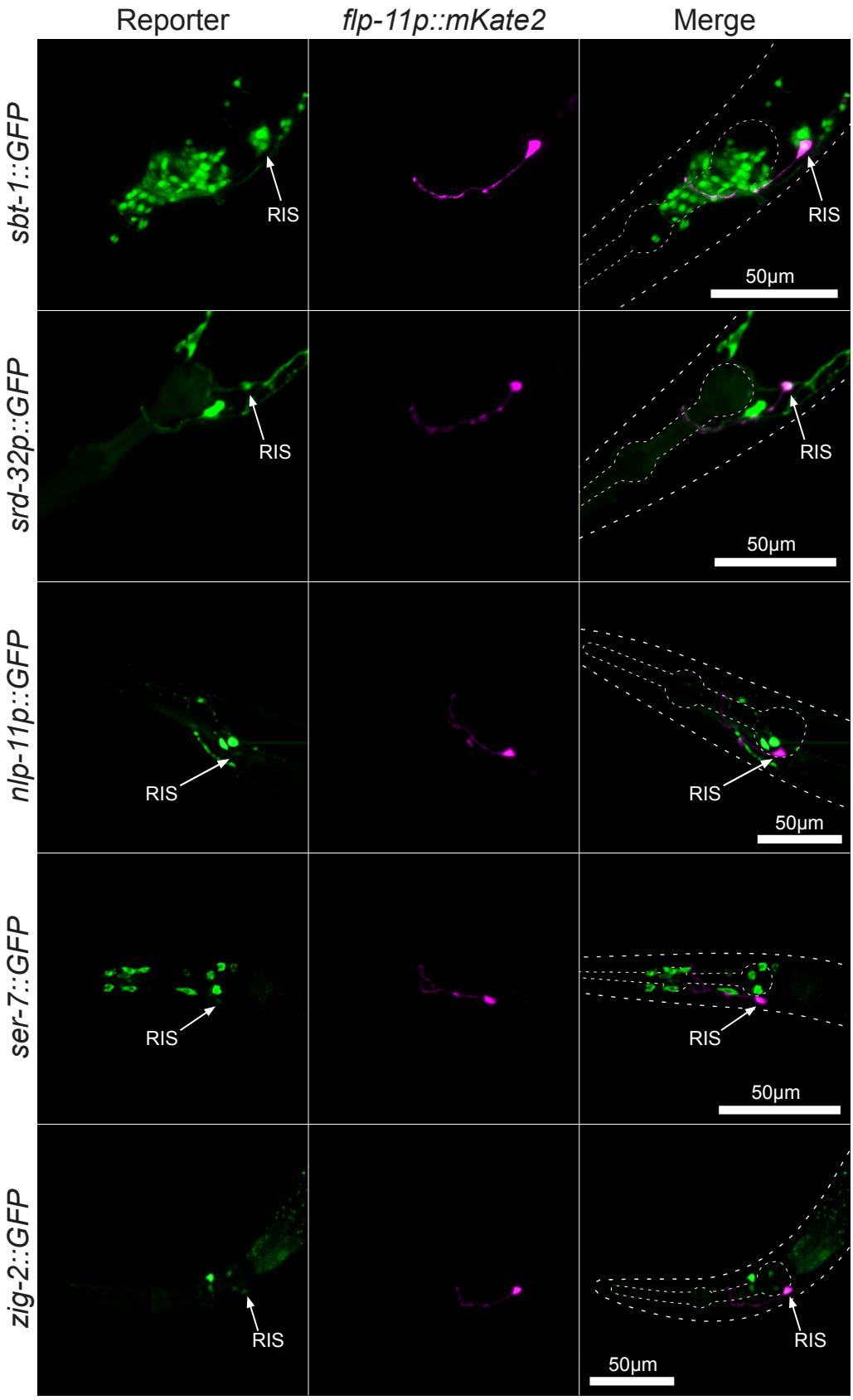
Reporter
flp-11p::mKate2
Merge
sbt-1::GFP
srd-32p::GFP
nlp-11p::GFP
ser-7::GFP
zig-2::GFP
RIS
50μm

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.

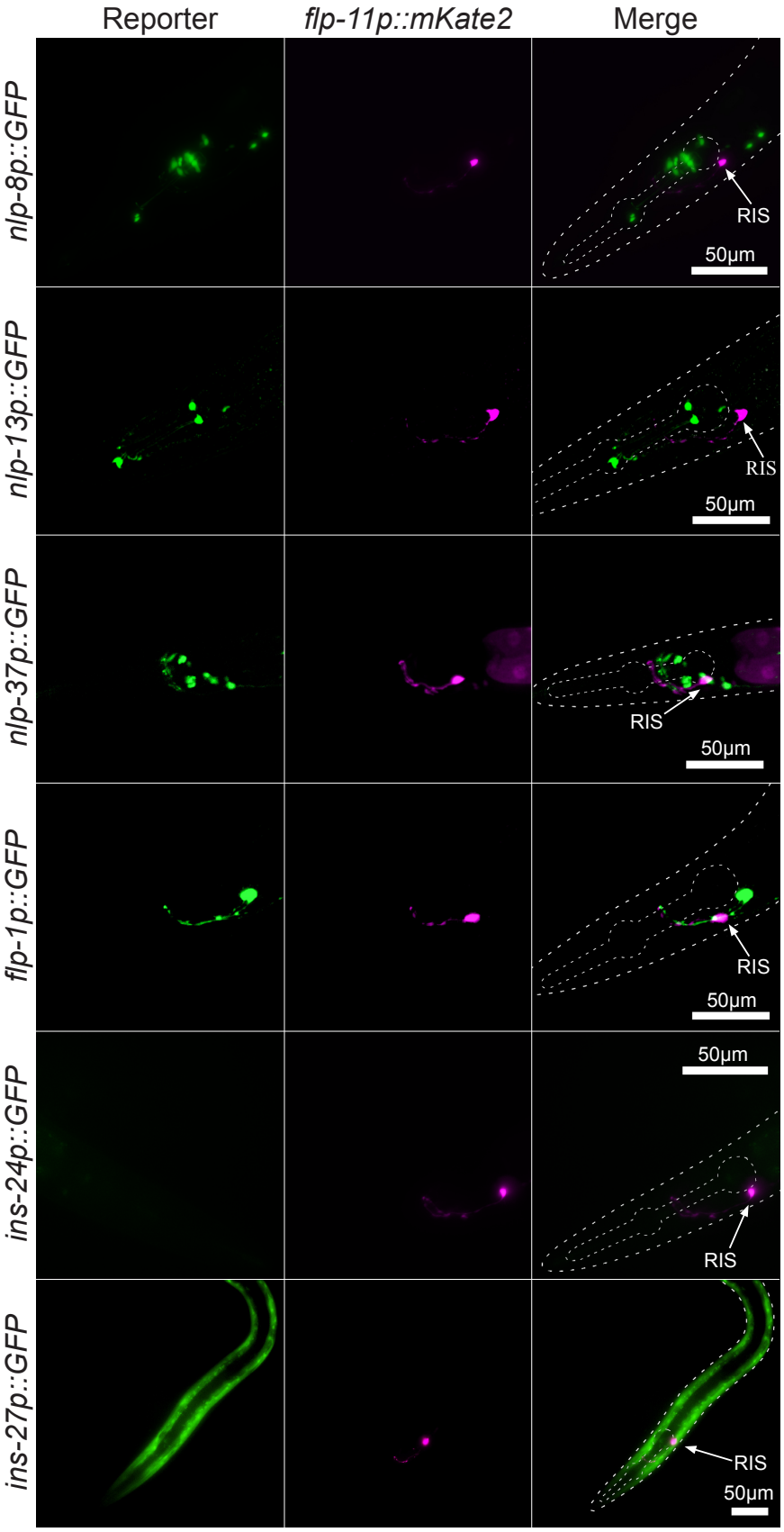
Reporter
flp-11p::mKate2
Merge
nlp-8p::GFP
RIS
50µm
nlp-13p::GFP
RIS
50µm
nlp-37p::GFP
RIS
50µm
flp-1p::GFP
RIS
50µm
ins-24p::GFP
50µm
RIS
ins-27p::GFP
RIS
50µm

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.

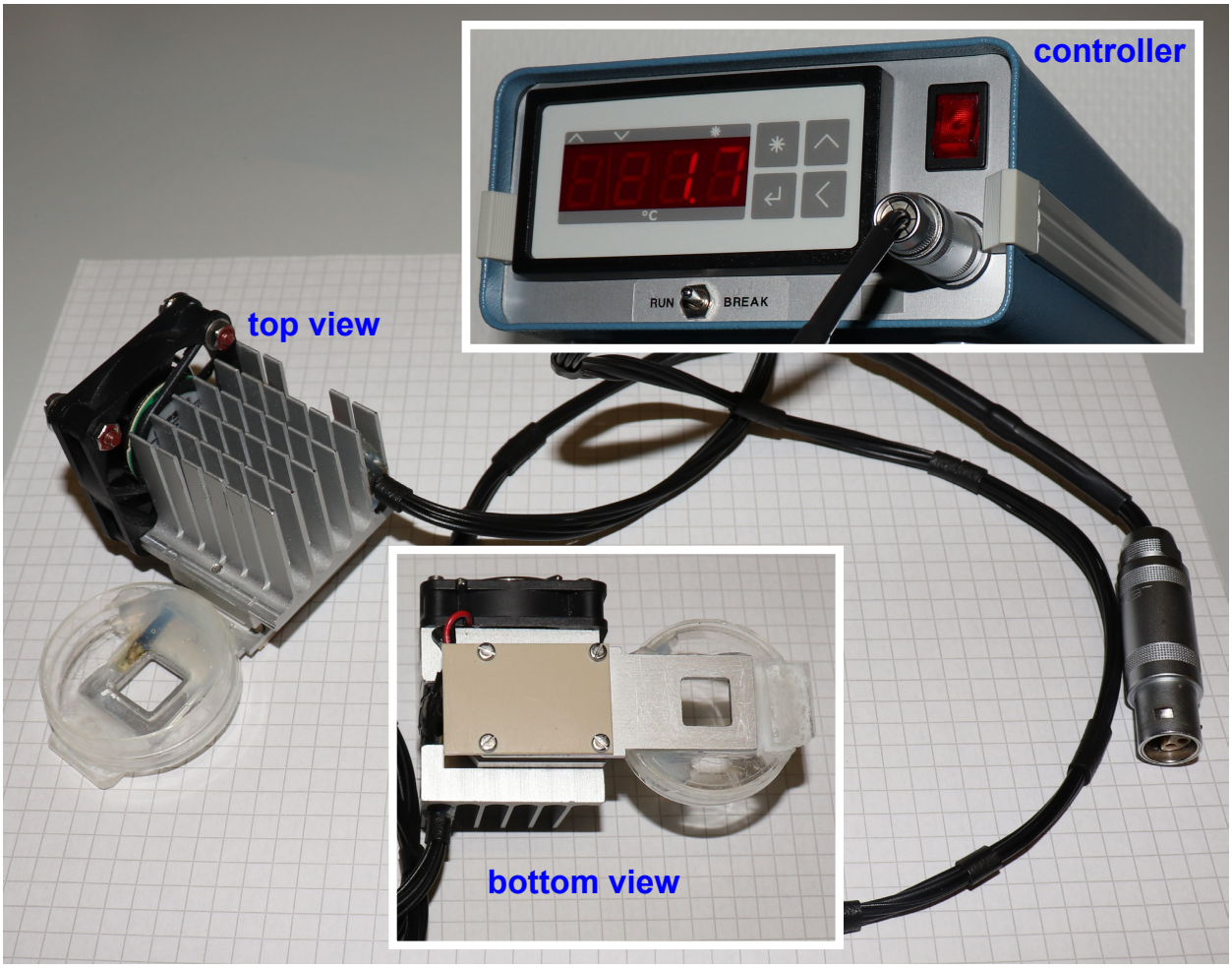

Figure S3. Heat control device. Related to Figures 3, 4, 5, 6, and methods section "Induction of cellular stress by heat shock".

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.

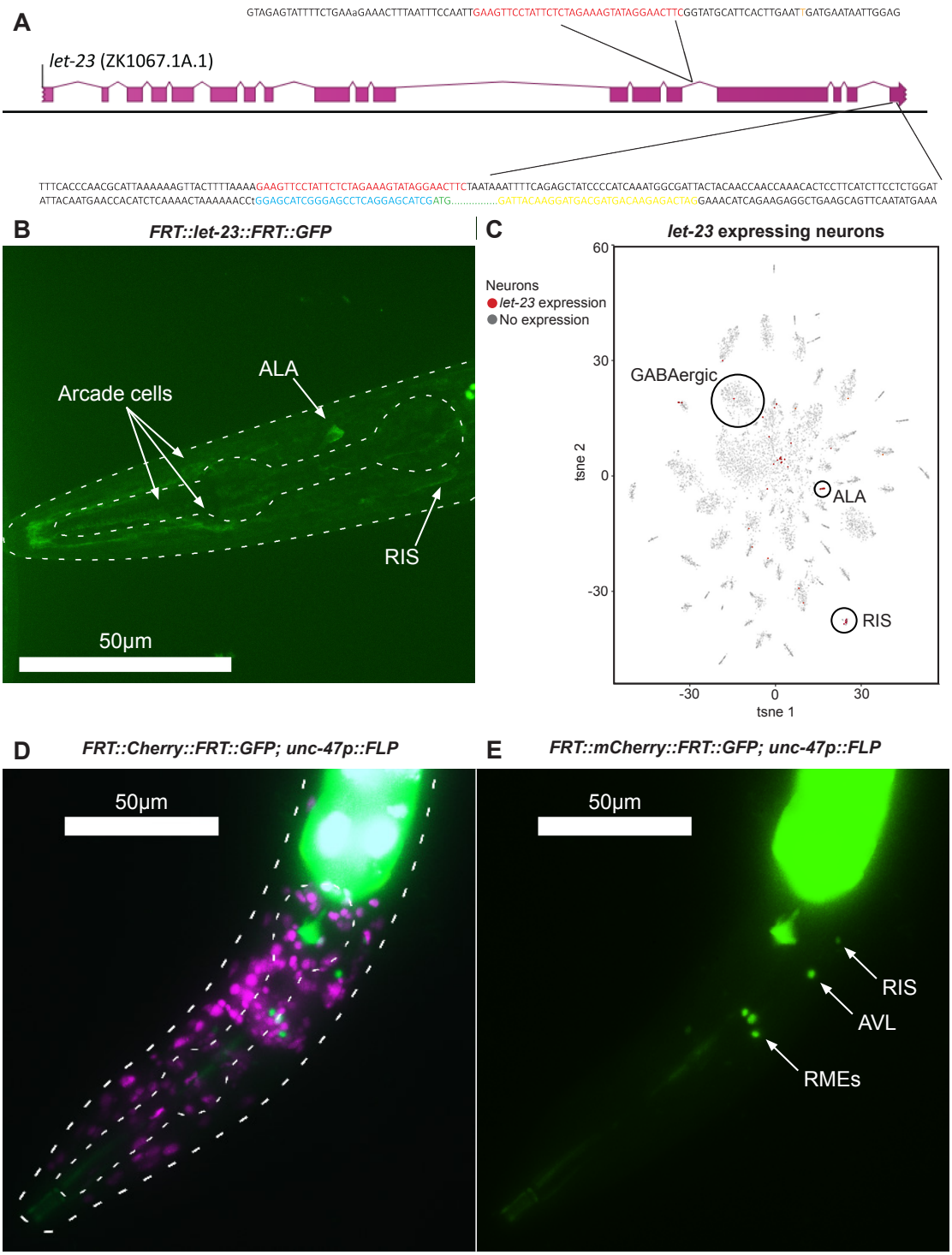
A
GTAGAGTATTTTCTGAAaGAAACTTTAATTTCCAATTGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCGGTATGCATTCACTTGAATTGATGAATAATTGGAG
let-23 (ZK1067.1A.1)
TTTCACCCAACGCATTAAAAAAGTTACTTTTAAAAGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCTAATAAATTTTCAGAGCTATCCCCATCAAATGGCGATTACTACAACCAACCAAACACTCCTTCATCTTCCTCTGGAT
ATTACAATGAACCACATCTCAAAACTAAAAAACCtGGAGCATCGGGAGCCTCAGGAGCATCGATG.....GATTACAAGGATGACGATGACAAGAGACTAGGAAACATCAGAAGAGGCTGAAGCAGTTCAATATGAAA
B
FRT::let-23::FRT::GFP
Arcade cells
ALA
RIS
50µm
C
let-23 expressing neurons
Neurons
let-23 expression
No expression
GABAergic
ALA
RIS
tsne 2
tsne 1
60
30
0
-30
-30
0
30
D
FRT::Cherry::FRT::GFP; unc-47p::FLP
50µm
E
FRT::mCherry::FRT::GFP; unc-47p::FLP
50µm
RIS
AVL
RMEs

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 in 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

S1. Van Buskirk, C., and Sternberg, P.W. (2007). Epidermal growth factor signaling induces behavioral quiescence in Caenorhabditis elegans. Nat Neurosci *10*, 1300-1307.

S2. Cao, J., Packer, J.S., Ramani, V., Cusanovich, D.A., Huynh, C., Daza, R., Qiu, X., Lee, C., Furlan, S.N., Steemers, F.J., et al. (2017). Comprehensive single-cell transcriptional profiling of a multicellular organism. Science *357*, 661-667.