

## Figure S1. Germlines of L4 larval hermaphrodites do not respond to ascr#10. Related to Figure 1.

Number of GPCs in L4 larval hermaphrodites on and off ascr#10. Each dot represents one animal. Black bars denote the mean. See Data S1 for primary data and details of statistical analyses.



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## Figure S2. Exploratory behavior in mutants with compromised reproduction. Related to Figure 2.

(A) Exploratory behavior of *fem-3(q96)*. This gain-of-function allele masculinizes the germline, resulting in exclusive sperm production. (B) Exploratory behavior of DR730 *dpy-13(e184); ama-1(m118m238)* hermaphrodites that have dramatically reduced brood size (<3 offspring in the course of this experiment vs. >13 in N2 animals). During the 12 hours of the experiment, only ~1/2 of these animals produced any offspring (in comparison, all N2 hermaphrodites of comparable age produce offspring). Separating DR730 hermaphrodites into those that did vs. those that did not have offspring, suggests that despite marginal mobility characterizing this strain, exposure to ascr#10 further reduced exploration in animals that have started to reproduce. (C) The number of eggs laid by hermaphrodites in which HSN has been silenced. These results are from the same animals in Figure 2E. In **A**, **B**, each square represents one animal. In **C**, each diamond represents the number of eggs laid by a single animal. Black bars denote the mean. \*\*\* p<0.001. See Data S1 for primary data and details of statistical analyses.







⊳ control

⊳ascr#10

## Figure S3. Functional egg-laying apparatus regulates response to ascr#10. Related to Figure 3.

(A) Exploratory behavior in *egl-19(n582)* and *egl-19(ad695)* (egg-laying defective and egg-laying constitutive alleles, respectively). Neither strain responds to ascr#10. Number of GPCs in (B) *unc-103(e1597)*, (C) *egl-2(n693)*, and (D) *lin-12(wy750)*. These mutants were counted earlier because of increased egg retention and internal hatching. (E) Egg-laying in hermaphrodites in which vm2 vulval muscles were silenced soon after L4/adult molt, but prior to onset of egg-laying. These results are from the same animals in Figure 3D. (F) Number of GPCs in hermaphrodites with silenced vm2 vulval muscles on and off ascr#10. (G) Exposure to histamine does not alter expression of *tph-1::YFP* response to ascr#10 in NSM or (H) HSN neurons in OH12495. Arrows point to the cell body. Anterior is to the left and ventral is down. Quantification of fluorescence is shown below micrographs. Each square (in A) and each dot (in B-D and F) represents one animal. In E, each diamond represents the number of eggs laid by one animal. In G, H, each triangle represents one neuron. Black bars denote the mean. \* p<0.05, \*\* p<0.01, \*\* p<0.001. See Data S1 for primary data and details of statistical analyses.



## Figure S4. Consequences of chemogenetic silencing of vm2 vulva muscles. Related to Figure 4.

(A) Number of eggs laid by hermaphrodites with functional vm2 vulva muscles. (B) Number of eggs laid by the same hermaphrodites as in A, but with silenced vm2 vulva muscles. The experiment in A was carried out on animals that were between 48 and 60h after release from L1 arrest; in B, the same animals between 60 and 72h. Each diamond represents the number of eggs laid by one animal. Black bars denote the mean. See Data S1 for primary data and details of statistical analyses.