Supporting Information

Milward et al. 10.1073/pnas.1106134109

SI Methods

Strains. The strains used were wild-type N2 (Bristol) from the Caenorhabditis Genetics Center:

PR694, *tax-2* (p694);
CX2948, *osm-9* (ky10);
AX1031, *ocr-2* (ak47);
CX2948, *tax-4* (p678);
AX2157, *tax-2*(p694); *lin-15*(n765ts); dbEx[*pflp-17::tax-2 cDNA::Sl2gfp,lin-15(+)*] (a kind gift from A. Bretscher, University of California, San Francisco, CA);
AX2159, *tax-2*(p694); *lin-15*(n765ts); dbEx[*pgcy-32::tax-2 cDNA::Sl2gfp,lin-15(+)*] (a kind gift from A. Bretscher);
AX2161, *tax-2*(p694); *lin-15*(n765ts); dbEx[*pflp-6::tax-2 c+ DNA::Sl2gfp,lin-15(+)*] (a kind gift from A. Bretscher);
AX2178, *tax-2*(p694); *lin-15*(n765ts); dbEx[*pgcy-8::tax-2 cDNA::Sl2gfp,lin-15(+)*] (a kind gift from A. Bretscher);
PR680, *che-1*(p680);
XA2262, dbEx[*pgcy-32::egl-1, pgcy-35::gfp*];
AX2125, *ttx-1*(p767);
AX2172, *ttx-1*(p767); dbEx[*pgcy-33::egl-1,punc-122::dsRed*];
AX2051, dbEx[*pgcy-33::egl-1, punc-122::dsRed*].
CX6241, *ocr-2*(ak47); kyEx687[*psra-6::ocr-2, punc-122::gfp*] (a kind gift from R. Gatsi, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland);
CX6382, *ocr-2*(ak47); kyEx693[*psrh-220::ocr-2, punc-122::gfp*] (a kind gift from R. Gatsi);
CX6237, *ocr-2*(ak47); kyEx686[*psrh-142::ocr-2, punc-122::gfp*] (a kind gift from R. Gatsi);
CX6235, *ocr-2*(ak47); kyEx685[*podr-10::ocr-2, punc-122::gfp*] (a kind gift from R. Gatsi);
CB1372, *daf-7*(e1372);
DA2202, *daf-7*(e1372); adEx2202[*pgpa-4::daf-7,rol-6p::gfp*] (a kind gift from Y.-J. You, Virginia Commonwealth University Medical Center, Richmond, VA, and L. Avery, University of Texas Southwestern Medical Center, Dallas, TX);
CB1370, *daf-2*(e1370);
CF1038, *daf-16*(mu86);
GR1309, *daf-16*(mgDf47);*daf-2*(e1370);
TJ1052, *age-1*(hx546);
daf-16 (mu86);Ex[*pmyo-3::gfpdaf-16, punc122::gfp*]; *daf-16* (mu86);Ex[*pges-1::gfp daf-16, punc-122::gfp*]; *daf-16*(mu86);Ex [*punc-119::gfp daf-16,punc-122::gfp*];
age-1(hx546); Ex[*punc-119::age-1,pges-1::gfp*] (a kind gift from I. Mori, Nagoya University, Nagoya, Japan);
age-1(hx546);Ex[*posm-6::age-1,pges-1::gfp*] (a kind gift from I. Mori);
FK234, *egl-4*(ks62) (a kind gift from Y.-J. You and L. Avery);
DA2143, *egl-4*(ks62); adEx2143[*ptax-4::egl-4,prol-6::gfp*] (a kind gift from Y.-J. You and L. Avery);
DA2149, *egl-4*(ks62);adEx2149[*podr-3::egl-4,prol-6::gfp*] (a kind gift from Y.-J. You and L. Avery);
DA2186, *egl-4*(ks62);adEx2186[*pgcy-32::egl-4,prol-6::gfp*] (a kind gift from Y.-J. You and L. Avery);
AX204, *npr-1*(ad609);
AX1295, *gcy-35*(ok769);
AX1230, *gcy-35*(ok769); *npr-1*(ad609);dbEx[*pgcy-32::gcy-35, punc122::gfp*]; AX1744, *npr-4*(tm1782) ox6;
AX1775, *npr-5*(ok1583) ox6;
AX1412, *flp-21*(pk1601);*flp-18*(db99);
npr-1(ad609); *lin-15*(n765ts);dbEx[*pnpr-1::npr-1-gfp*];
CX9586, *npr-1*(ad609);kyEx[*pgcy-32::npr-1*];
CX9645, *npr-1*(ad609); kyEx[*pncs-1::npr-1*];
AX1823, *npr-1*(ad609);*lin-15*(n765ts);dbEx[*pgcy-7::npr-1-gfp*];
AX1820, *npr-1*(ad609);*lin-15*(n765ts);dbEx[*pgcy-5::npr-1-gfp*;
AX2311, *npr-1*(ad609) *lite-1* (*ce314*);dbEx[*pgcy-32::channelrhodopsin*];
AX2415, *che-1*(p680);*ttx-1*(p767);Ex[*pgcy-33::egl-1 punc122::dsRed*].

Transgenic Strain Construction. For *daf-16* rescue, *pmyo-3::gfp daf-16*, *pges-1::gfp daf-16*, *or punc-119::gfp daf-16* constructs (1) were injected at 30 ng/μL into CF1038 *daf-16* (mu86) with *punc-122::gfp* (CCGFP) as a coinjection marker. Three independent rescue lines for each construct were used. The RMG rescue of *npr-1* was made by coinjecting *pncs-1::nCre* and *pflp-21::LoxStopLox::npr-1* (2) together with pJMZ *lin-15* marker into *npr-1*(ad609); *lin-15* (n765ts). Two independent lines were used. For ASH rescue of *npr-1*, *psra-6::npr-1* (npr-1 genomic DNA a kind gift from Marina Ezcurra, Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK) was injected at 20 ng/μL with pJMZ *lin-15* coinjection marker into *npr-1*(ad609), *lin-15(n765ts)*. Two independent lines were assayed.

The *gcy-5* and *gcy-7* promoters (a kind gift from Yoshinori Tanizawa, Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK) were placed upstream of *npr-1* cDNA with polycistronic GFP, to follow expression, and injected *punc-122::gfp* (CCGFP) as a coinjection marker into *npr-1 (ad609)*. Three independent lines were analyzed.

Channelrhodopsin Expression and Activation. Channelrhodopsin that was C-terminally tagged with mCitrine was expressed in the *npr-1 (ad609) lite-1 (ce314)* background from the *gcy-32* promoter. Worms were grown on plates supplemented with 50 μL of 5 mM all-transretinal (Sigma), dissolved in ethanol. Control animals were grown on plates supplemented with the same amount of ethanol. For assays, 10–13 adult hermaphrodites were transferred to low peptone nematode growth medium plates seeded with 30 μL of *Escherichia coli* OP50 2 d before assay, and left foraging for 1 h. Ten minutes before the recording, the plate was transferred into a Perspex chamber and kept at 11% O_2 (balance N_2). Animals were filmed using dim white light 3 min before and 3 min after the stimulation. For stimulation, the animals were exposed to blue light (13 mW/cm^2) for 3 min.

Analysis of Behavioral Characteristics. Recorded worms were tracked using DIAS software (Solltech). Worm object paths were generated. For each worm object, the centroid x and y coordinates, maximum length, mean width, perimeter, and roundness were extracted and these parameters were used in a custom-written program in MATLAB (Mathworks) to calculate speed, reversal rate per animal per minute, proportion of worms leaving after entering border area, border arrival rate per animal per minute and the average time worms spend at the border. Immobile and merged objects were excluded in the analysis. Further details are available on request.

1. Libina N, Berman JR, Kenyon C (2003) Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. *Cell* 115:489–502.
2. Macosko EZ, et al. (2009) A hub-and-spoke circuit drives pheromone attraction and social behaviour in *C. elegans*. *Nature* 458:1171–1175.

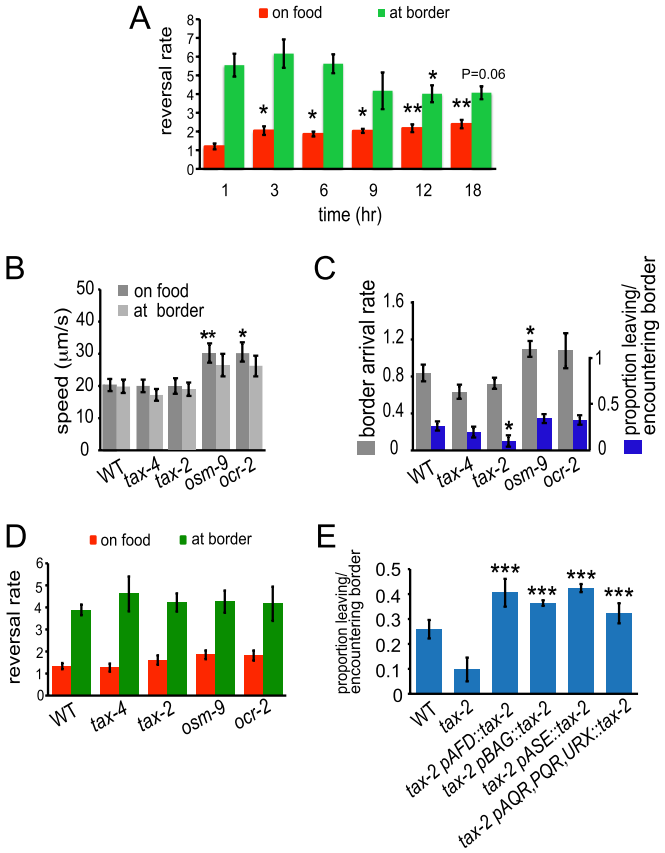

Fig. S1. Behavioral parameters. (*A*) The reversal rate of wild-type animals on food increases over time and there is a trend of reduced reversals at the border. (*B*) The *osm-9* mutants show increased speed and border arrival rate compared with wild-type, whereas *tax-2* mutants show reduced likelihood of leaving food per encounter with the border (*C*). (*D*) No significant changes in reversal rates are seen in *tax-2*, *tax-4*, *osm-9*, and *ocr-2* mutants compared with wild-type animals, but the proportion leaving from the border of those encountering the border is reduced in *tax-2* mutants ($P < 0.05$) but rescued with expression of *tax-2* is AFD, BAG, ASE or AQR, PQR, and URX (*E*). ***$P < 0.005$; **$P < 0.01$; *$P < 0.05$. $n = 6$ or more per genotype.

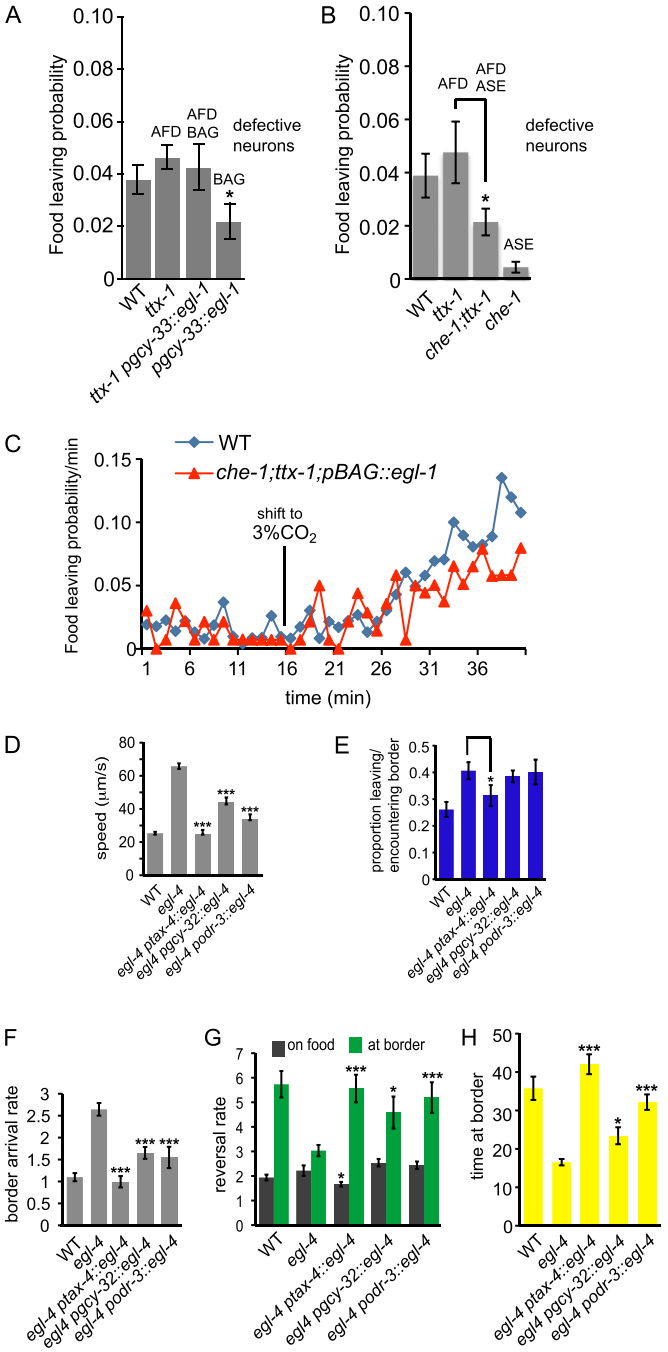

Fig. S2. (*A*) The *ttx-1* mutation suppresses food-leaving defects in BAG-ablated animals and in *che-1* mutants (*B*). (*C*) Time line of food-leaving when wild-type and ASE-, AFD-, BAG-defective animals are subjected to high CO_2. High CO_2 increases food-leaving in wild-type animals but less so in *che-1; ttx-1; pBAG::egl-1* mutants. The animals were left to feed for 6 h. The animals were then placed in a in a Perspex chamber and recorded at 21% oxygen followed by a shift to 3% CO_2 (in 21% oxygen) after 15 min and recorded for another 25 min. (*D*) Transgenic rescue of the *egl-4* speed phenotype. (*E*) The proportion of *egl-4* animals leaving food per encounter with the border is reduced when *egl-4* is selectively expressed in the *tax-4* expressing neurons. (*F*) The *egl-4* border arrival rate is higher, which is rescued in *egl-4* add-back lines. (*G*) Reversal rates at the border are reduced in *egl-4* mutants ($P < 0.005$) and rescued in *egl-4* add-back lines. (*H*) The average time *egl-4* loss-of-function mutants spend at the border is strongly shortened ($P < 0.005$). This defect is rescued by expression *egl-4* under the *tax-4* promoter and partially rescued when *egl-4* is expressed from the *odr-3* and *gcy-32* promoter. Asterisks indicate *P* value for pair-wise comparison between *egl-4* and rescue lines. (*D–H*) $P < 0.005$ comparing WT and *egl-4* mutant animals. ***$P < 0.005$; *$P < 0.05$. $n = 6$ or more per genotype.

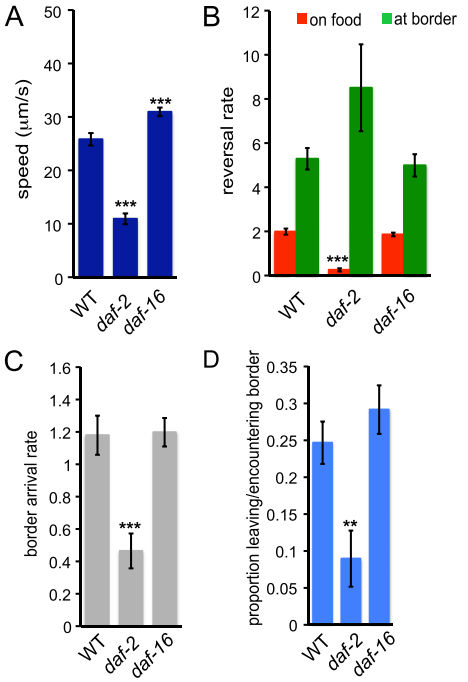

Fig. S3. Behavioral parameters of wild type and *daf-2* and *daf-16* mutant animals. (*A*) Speed is significantly reduced in *daf-2* mutants and increased in *daf-16* mutants. (*B*) Reversal rates on food are reduced in *daf-2*. (*C*) The *daf-2* mutant animals show reduced border arrival rate and reduced proportion of animals leaving after entering the border region (*D*). ***$P < 0.005$; **$P < 0.01$. $n = 6$ or more per genotype.

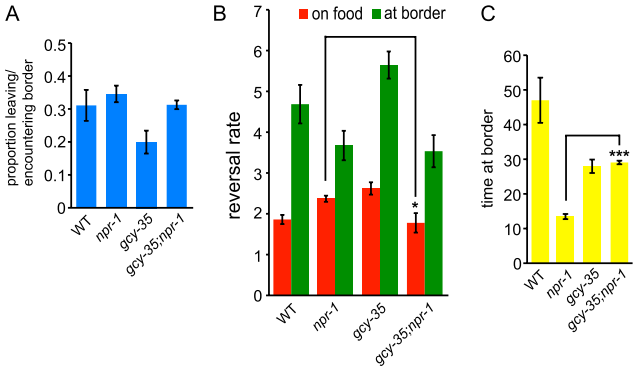

Fig. S4. Behavioral parameters of wild-type animals, *gcy-35*, *npr-1*, and *gcy-35:npr-1* mutant animals. (*A*) The proportion of animals leaving after encountering the border is not significantly different. (*B*) The reversal rates on food are increased in *gcy-35* and *npr-1* mutants (both $P < 0.005$ compared with WT). (*C*) The average time at the border is reduced in *npr-1* ($P < 0.005$) and *gcy-35* ($P < 0.05$). *gcy-35; npr-1* mutants show increased decision time compared with *npr-1*. ***$P < 0.005$; *$P < 0.05$. $n = 6$ or more per genotype.