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Supplemental Data

A 3'UTR Pumilio binding element directs translational activation in olfactory sensory neurons.

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Supplemental Experimental Procedures

GST-FBF-1 purification

Full-length cDNA encoding FBF-1 (yk322e3 from Yuji Kohara) was fused to GST sequences in pGex3T using the BamH1 and EcoR1 sites to generate pJK43. pJK43 was expressed in JM109 cells and 1 liter of cells were grown up overnight at room temperature in TB plus 2% glucose (Kraemer et al., 1999). Cultures were then diluted to an approximate OD_{600} of 0.6, grown for an additional 2 hours at room temperature and induced with 1 mM IPTG for five hours at room temperature. Cells were spun down, frozen in liquid nitrogen and stored at -80°C. Cell pellets were resuspend in 150 ml of Lysis Buffer (Kraemer et al., 1999) containing: 500 mM NaCl, 5 mM DTT, 20 mM Hepes-KOH pH 7.4, 1 mM EDTA, Lysozyme (5 mg/ml) and 0.5 X Complete Protease Inhibitor cocktail (Roche). Cells were cleared from the lysates by passing them through a 0.45 μM filter, then lysates were run over ~2 ml columns of glutathione beads (Amersham Biosciences) and equilibrated with Lysis Buffer. Bound beads were washed with 6 column volumes of Lysis Buffer (Kraemer et al., 1999), followed by 6 column volumes of Binding Buffer: 100 mM NaCl, 5 mM DTT, 20 mM HEPES-KOH, pH 7.4, and 2 mM EDTA. GST-FBF-1 was eluted in 10 ml Binding Buffer containing 50 mM glutathione and 50 mM NaOH pH 7.4. 0.2 ml fractions were collected and those with the most concentrated protein were pooled and dialyzed overnight in Binding Buffer containing 20% glycerol. Concentrated protein was stored at -80°C. The concentration of GST- FBF-1 was determined by running total protein on a comassie gel and comparing the concentration of the full-length band to a BSA standard.

PUM-HD purification A GST-tagged Pum HD vector (a generous gift from Phillip Zamore) was transformed into XL-1 blue cells and grown up in YTA overnight at 37 C°. Cultures were then diluted to an approximate OD_{600} of 0.6, grown for an additional ~2 hours at 37 °C. Cultures were moved to room temperature and induced with 0.5 mM IPTG for two hours. Cells were harvested and GST-PumHD was purified and concentrated as described (Zamore et al., 1997) and stored at -80 °C.

Gel Mobility Shifts
GST-FBF-1 or GST-PUM-HD was incubated with 100 fmoles of 5' P^{32} end-labeled (T4 Polynucleotide kinase from Promega) wild-type or *ky95* mRNA 30 mer oligos (Dharmacon) as described (Bernstein et al., 2005). 4 microliters of 10% Ficoll was added to end the binding reaction and 10 microliters of each reaction was loaded on to a 6% native polyacrylamide gel, resolved for two hours at 4°C and dried on a gel dryer for 1.5 hour. Dry gels were exposed to a phosphorimaging screen (Molecular Dynamics) for ~ 48 hours. Shifted RNA was quantified by Image Quant software on the Storm 860 Imaging System (Molecular Dynamics) and Kd's were estimated by non-linear regression curve fitting by GraphPad Prism™ 4 software.

Construction of the *podr-1* RFP vector
2406 bases of the proposed promoter region 16 bases upstream of the first ATG of ODR-1 was cut out of the clone R01E6.1SC (L' Etoile and Bargmann, 2000) at the HindIII (5') and EarI (3') sites. The EarI site was blunted with T4 polymerase and the fragment was inserted into the HindIII and SmaI sites of the pPD95.75 GFP vector (generous gift from Andy Fire). GFP was replaced with RFP using the KpnI and EcoRI sites.

Expression Analysis
A PCR based fusion technique (Hobert O, 2002) was used to place the coding sequence for GFP downstream of the *fbf-1* promoter. 2 kb of sequence 5' of the translational start site of FBF-1 was amplified by PCR. This product was injected into wild type worms at 50ng/microliter with p*ofm-1*::GFP ((50ng/microliter) which shows expression in the coelomocytes (generous gift from Piali Sengupta)) and p*odr-1*::RFP ((25ng/microliter) to aid in AWC/B identification). It was also injected by itself to reduce risk of misinterpretation due to bleed through from the red to the green channels. All transgenic arrays were generated with *pofm-1*::GFP as the coinjection marker to identify transgenic worms.

Cell-specific rescue
The full-length cDNA encoding FBF-1 was PCR amplified from yk322e3 with KpnI and EcoRI containing primers engineered in to each end. This fragment was inserted into the KpnI and EcoRI sites of p*odr-1*::RFP. This replaced the RFP open reading frame with that of FBF-1. This construct was linearized with Hind III and injected into *fbf-1(ok91)* mutant animals at 2ng/microliter with Sca1pre-digested *fbf-1(ok91)* genomic DNA at 100ng/ microliter (Kelly et al., 1997).

Heat shock rescue
The full-length cDNA encoding FBF-1 was placed under the control of p*hsp-16.2* (Stringham et al., 1992) by PCR amplification using KpnI and SacI containing primers and inserted into the KpnI and SacI sites of the pPD49.78 plasmid (from Andy Fire). *fbf-1(ok91)* animals were injected with 2 ng/microliter of the heat shock construct. Lines carrying the transgene were grown at 25 °C for five days

and placed at 28- 30 C° for 1 hour then placed at room temperature to recover for 1 hour before being subjected to the adaptation protocol. Control, non-heat exposed worms remained at room temperature for the entire two hours.

Kaede Expression vector construction

The *podr-3*::EGL-4 cDNA (L' Etoile et al., 2002) clone was modified such that the EGL-4 open reading frame was flanked with Not I sites yielding the vectors *podr-3*::EGL-4. The *egl-4* 3'UTR was modified by site-directed mutagenesis to generate *ky95* 5'3'Not I (thank you Ting-Wen Cheng). These were the backbones into which reporters were cloned at the Not I site.

The *C. elegans* src myristoylation signal (GSCIGK) (Adler et al., 2006) was added to Kaede (Ando et al., 2002) by PCR of the pKaede-S1 vector. In the same reaction, Not I sites were added to either end of the product. This fragment was used to replace the NotI site flanked EGL-4 open reading frame in the both the wild-type and *ky95* 3'UTR p*odr-3*::EGL-4 5'3'NotI backbones yielding the vectors *podr-3*::E4 wt and *ky95* 5'3'UTRmyr::Kaede.

To place the myristoylated*, egl-4* 3'5' flanked Kaede cDNA (in the vector *podr-3*::E4 5'3'UTRmyr::Kaede) under the AWC-specific, *str-2* promoter (Troemel et al., 1999), we started with *pstr-2*::RFP (generous gift from Miri Van Hoven and Cori Bragmann). We removed the RFP open reading frame by cutting with BamHI and Apa I. The open reading frame for myrKaede was excised from *podr-3*:: E4 5'3'UTRmyr::Kaede with BamHI and Apa I and cloned into the *pstr-2* plasmid. The Not I site just upstream of the myristoylation sequence was removed via Site Directed Mutagenesis (Stratagene). These reporter constructs were injected at 50ng/microliter into wild type worms along with the *pstr-2::RFP* transgene to outline the AWC in red and *pofm-1*::GFP as a coinjection marker.

Kaede Transgene copy number

SYBRgreen Real Time PCR was performed (Applied Biosystems 7300 Real Time PCR system) on three independent genomic DNA preps from either the *egl-4(wt)* 3' UTR or the *egl-4(ky95)* 3'UTR Kaede expressing lines. Each prep was made from the lysate of ~160 adult transgenic worms. The DNA was precipitated with ethanol and resuspended overnight in 100 microliters Tris EDTA (pH 7.4) prior to analysis. Two microliters of DNA was used per reaction. One Kaede-specific primer set was used and the Critical cycle for Kaede was compared to that of the endogenous genes: *HMG-CoA Synthase (F25B4.6) and HMG-CoA Lyase (Y71G12B.10)*. A dilution series of a standard DNA prep was used to allow comparison between the ratios of Kaede transgene copies per genome for each transgenic line analyzed in the Kaede imaging experiments (see Supplemental data Figure 2 for more details).

Kaede Imaging and Quantification

For each animal, the green unconverted Kaede was imaged by excitation with a 488 nM scanning laser and acquiring the image as described in Equipment and

Settings in this section. Using Volocity™ to analyze the Zeiss LSM images, the arbitrary units (A.U.) of green fluorescence within each animal's AWC neuron was determined. The A.U. was divided by the volume of the cell to obtain the A.U./ μm^3. The volume of AWC neuron was determined in Volocity using the Zeiss LSM images by defining the volume occupied by the RFP flourophore (expressed under the p*str2* (Troemel et al., 1999) and photoconverted Kaede. The red fluorophore was excited using a 543 nM scanning laser and the neuron was imaged as described in Equipment and Settings in this section. To reduce the contribution of background green fluorescence, three background readings were taken individually for each image and the background A.U./ μm^3 was defined as the average of these values. The background A.U./ μm^3 was subtracted from the total concentration for each image. The concentration of Kaede within the AWC was determined immediately after photoconversion (denoted as "before recovery") and after 3 hours of recovery (denoted as "after recovery"). Total Change in Kaede expression/ μm^3 during these 3 hours is defined as (A.U./ μm^3 before) – (A.U./ μm^3 after) recovery.

To determine the amount of green Kaede in the cilia and cell body, images were cropped to contain these structures. In some cases, the cell body contained a small part of the axon or dendrite due to an inability to separate these structures. Three background readings were taken as near as possible to the structure and subtracted from the A.U./μm^3 for each structure.

To determine the A.U./μm^3 in the axon and dendrite, the A.U./ μm^3 for the cilia and cell body were added and the total was subtracted from the total A.U./ μm^3 for the whole cell. The remainder was defined as the concentration of A.U. in the axon/dendrite.

Images were discarded if: 1) the animal had moved while image was being taken, 2) the red volume changed from before to after recovery as defined as after/before being less than 0.58, 3) the neuron looked unhealthy or contained abnormal structures, or 4) the recovered animal was not healthy as defined by absence of egg laying and/or movement.

Equipment and settings

All images were obtained on a Zeiss LSM 510 META laser scanning confocal microscope using a Plan-Apochromat 63x/1.4 Oil DIC Objective.
Aquistion information:
Scan Mode: Stack, multi track, 8 bit; Stack size: 512 X 512 X 147, 206.8 μm X 206.8 μm X 53.2 μm;
Scaling: 0.4 X 0.4 x .36
Pixel time: Track 1, 6.40 μs, Track 2, 6.40 μs
Beam Splitters used:
MBS; HFT UV/488/568/433
DBS1: NFT 635 VIS
DBS2: NFT 545
DBS3: Plate
Wavelength emission: T1: 543 nm, 100% T2: 488 nm, 10%
Filters used: Ch3-1: BP 565-615 IR; Ch2-2: BP 500-530 IR

Pinhole size: μm Ch3-1: 108 μm; Ch2-2: 94 μm
Detector Gain: Ch3-1 850; Ch2-2 850
Amplifier gain: Ch3-1 1.00; Ch2-2 1.00
Amplifier offset: Ch3-1 0.00 V; Ch2-2 0.00 V

Statistical Analysis of Kaede recovery
All P values reported for the Kaede A.U./μm^3 data set were obtained from two-tailed unpaired t tests (alpha = 0.05). To determine that the Kaede A.U./μm^3 data set was normal a Kolmogorov-Smirnov (KS) test was performed GraphPad Prism™ 4 software. All sets of data passed the KS test (had P values greater than 0.05) except for the *ky95* cell body data set (P=0.0471). We also performed a two-tailed non-parametric Mann-Whitney test (alpha= 0.05). The medians of the A.U./μm^3 values for the wild type and *ky95* cilia and cell bodies were significantly different (P=0.026 and P=0.0047, respectively) using this test as well.

Total mRNA extraction and analysis of Kaede mRNA abundance
Animals containing the *egl-4(wt)* 3' UTR (*str-2:: myrs-kaede::wt* 3' UTR) in either the wild type or *fbf-1(ok91)* mutant genetic background were grown up on NGM plates containing HB101 for 5 days at 25°C. Animals were synchronized by washing off plates, bleaching, then placing on fresh HB101-containing NGM plates for ~2.5 days at 25°C. Animals were harvested and rinsed 3 times in H_20, frozen in liquid N_2 and stored at -80°C. Total RNA preparation and cDNA synthesis was performed as described in Van Gilst et al., 2005. Reverse Transcriptase PCR was performed on a BioRad iCycler using BioRad 2X Supermix™, 6mM $MgCl_2$, 400 nM random hexamer oligo primer concentration and 200 nM total RNA. In the real time quantitative PCR reaction, primers specific for the Kaede transgene were used (ATT TGA TGG CGT AAA CTT TCC and CAA CAC TCC ATC ACG CAA ATA). Primers specific for the housekeeping gene Y71G12B.10/HMGcoA Lyase 2B4 (Van Gilst et al., 2005) were used to determine the levels of obtain cycle number threshold (Ct) values. Relative Kaede mRNA abundance was normalized to the housekeeping gene Y71G12B.10/HMGcoA Lyase 2B4 as described in the ABI *PRISM 7700* Sequence Detection System USER Bulletin #2.

The amount of Kaede and HMG CoA mRNA was determined by plotting Ct of each sample against a standard curve. All RT-PCR reactions were programmed with the same units of RNA as determined by O.D. 260nM.

Luciferase cloning and expression analysis
The destabilized luciferase gene (luc2CP) from the Promega plasmid pGL4.16 was PCR amplified using the NotI containing primers: GCGGCCGCCACCATGGAAGATGCCAAAAACATT and GCGGCCGCTTAGACGTTGATCCTGGCGCTG. The PCR product was digested with Not I and placed in to the *podr-3*::E4 wt and *ky95* 5'3'UTRmyr::Kaede backbones. The Not I site just upstream of the start of the luciferase sequence

was removed via Site Directed Mutagenesis (Stratagene) by using the primer AGCGACCAGCGGAAAATCAACTGGAAATGGAAGATGCCAAAAACATTAAG and its compatible reverse compliment.

To generate the various permutations of the *egl-4* NR/FBE, we used Site Directed Mutagenesis. To generate the NR/FBE plus 4bp *egl-4* 3' UTR we used the primer CACTCTGTATTAGTTTTTTTTTTCATCACCTCTCCTCATTATGTTCGGTT and its compatible reverse complement. To generate the NR/FBE delete, we used the primer CTCTGTATTAGTTTTTTTTTTCATATATCACCTCTCCTCATTATGTTCG and its compatible reverse complement. To replace the NR/FBE with the *gld-1* FBEa we used the primers CACTCTGTATTAGTTTTTTTTTTCATGTGCCATACATATATCACCTCTCC its compatible reverse complement. To remove the predicted *let-7*/*mir-265/mir-32/mir-84* microRNA binding site GTGTATATATATATATCACCTTATGTTCGGTTAGTGTACCGCCGC. The *unc-54* 3' UTR was PCR'd up from pPD49.78 using the primers, GCAGCAGCAGCGCGGCCGCGGTACCATGGTATTGATATCT and GGCATCCGCTTACAGACAAG. The PCR product was cut with Not1 and Pme1 and placed downstream of the luciferase reporter. Site directed mutagenesis was used to introduce the *gld-1* FBEa into the *unc-54* 3' UTR by using the primer CCCTACATGCTCTTTCTCCCTCATGTGCCATACGTGCTCCCACCCCC and its compatible reverse complement. To introduce *egl-4* NR/FBE into the *unc-54* 3' UTR, we used site directed mutagenesis using the primer CCCTACATGCTCTTTCTCCCTCTGTGTATATATGTGCTCCCACCCCC and its compatible reverse complement. These reporter constructs were injected at 100ng/microliter into wild type and *fbf-1(ok91)* worms *pofm-1*::GFP as a co-injection marker. Luciferase expression was obtained from each line as described in the Methods section. There was a range of expression in wild type animals that contained the wild type 3' UTR and the *gld-1* FBEa that was statistically significant from one another by ANOVA analysis. Basal expression level of all lines including the wild type lines (n=5) and *gld-1* FBEa substituted constructs (n=3) are reported as the average of all lines tested.

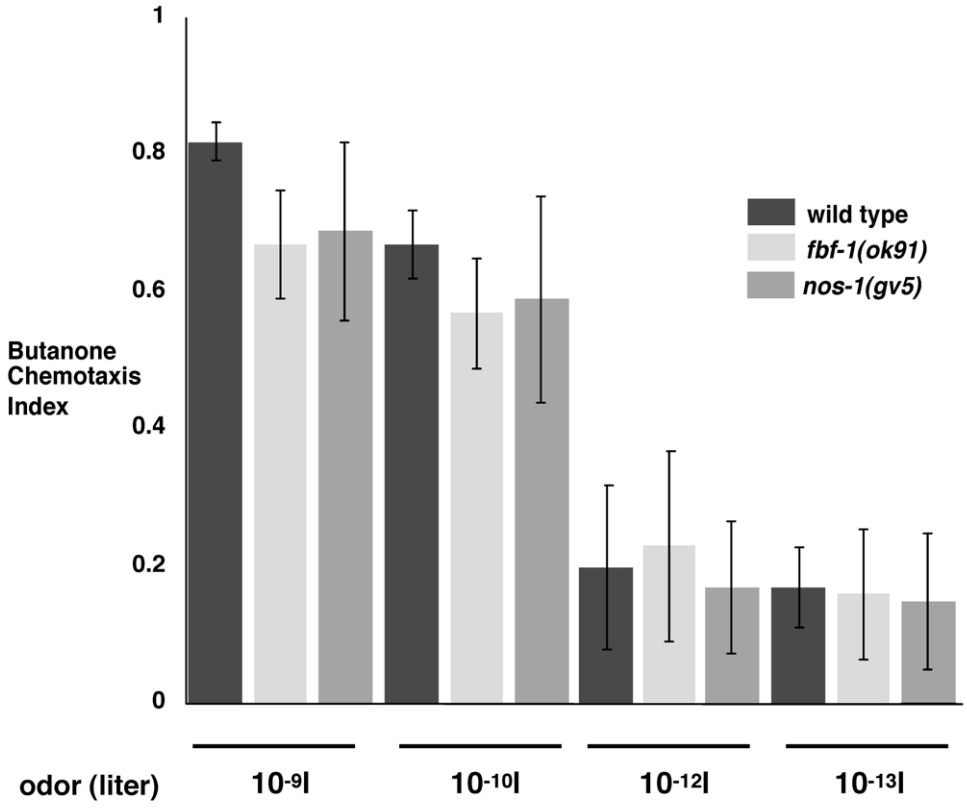
To correlate the chemotaxis and luciferase expression only one line was used for both wild type (this line was the highest expressing wild type line), NR/FBE delete and the *ky95*-mutated 3' UTRS. To determine the correlation between odor exposure and luciferase expression, we used Prism software to determine both a Pearson and Spearmann correlation. All data sets were normal except the *ky95*-mutated 3' UTR data set as determined by a Kolmogorov-Smirnov (KS) test.

Luciferase Transgene copy number

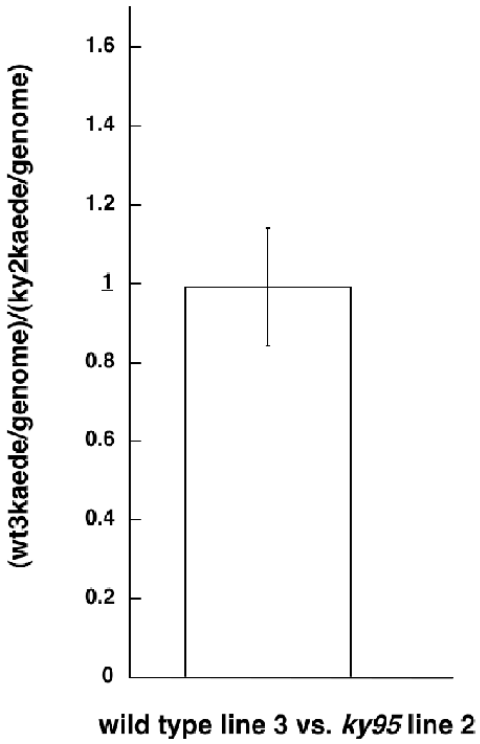
140 ul of SWLB with proteinase K was added to the lysed worm sample that was used for luminometry and frozen at -80. This was flash frozen in liquid N_2, then incubated at 60°C for two hours, heated at ~95°C for 20-30 minutes and spun for

10 minutes. The supernatant was transferred to a new microfuge tube and stored at 4 °C. Using a MJB iCycler, real time PCR was performed using one μl of protienase treated sample in each reaction. Luciferase specific primers (GCCATTCTACCCACTCGAAG and AGCTATTCTCGCTGCACACC) were used in the presence of SYBR green PCR master mix (Applied Biosystems) to amplify the luciferase transgene and the cT was compared to a standard curve to determine total relative transgene concentration.

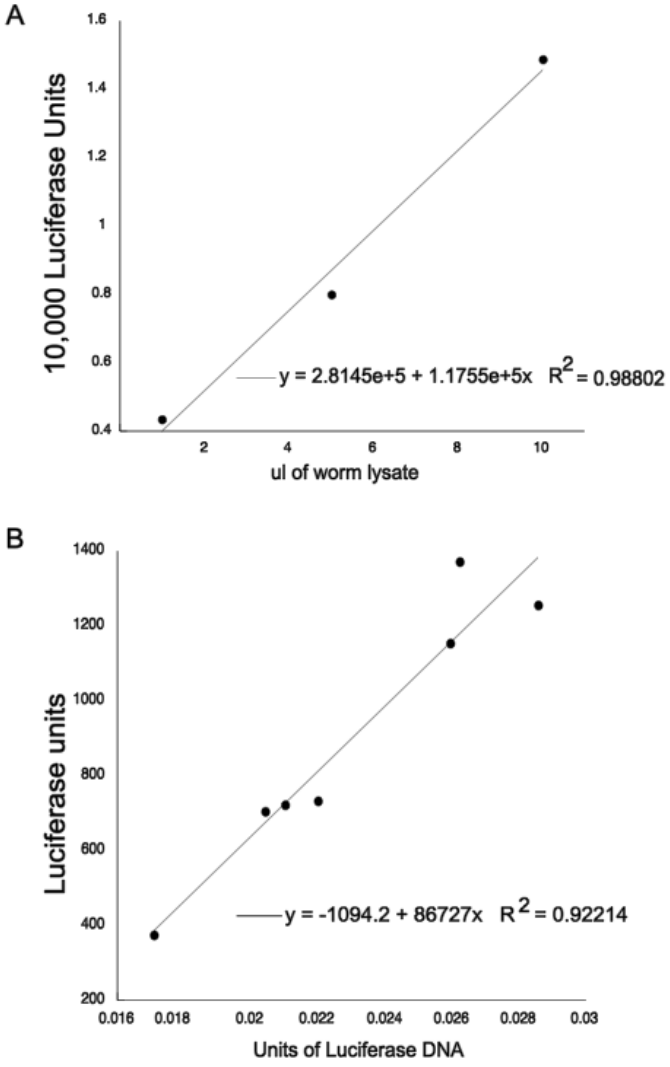
Supplemental Figures and Legends



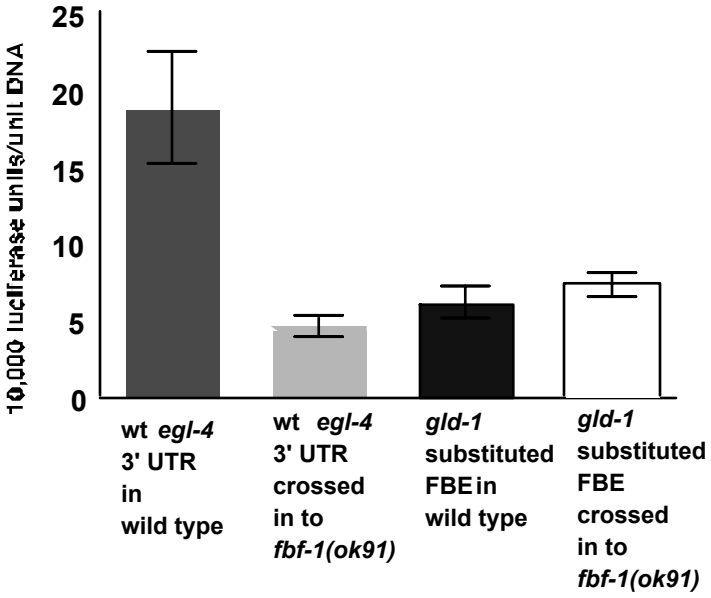
Supplemental Figure 1 *fbf-1(ok91)* and *nos-1(gv5)* mutants are as sensitive to AWC-sensed odors as wild-type worms.
Chemotaxis assays were performed an all three strains at the same time. Butanone was titrated down from the usual amount of 1 nanoliter per plate in ten fold steps. Butanone was diluted in ethanol. Assays were performed on 3 separate days with >100 animals per assay.



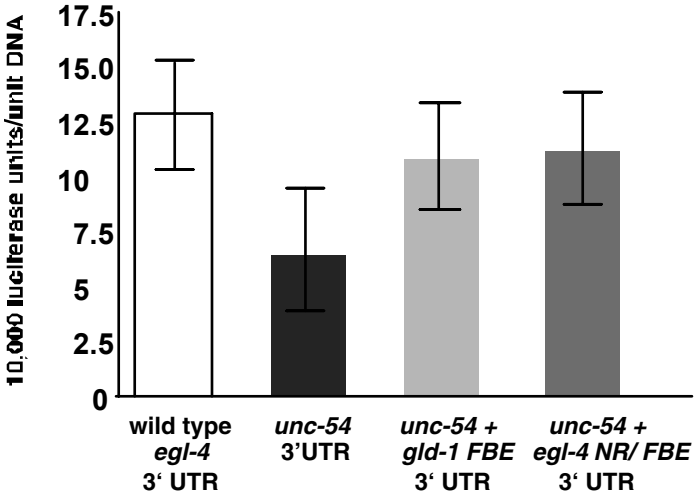
Supplemental Figure 2. Wild-type and *ky95* kaede reporter transgenic lines have the same transgene copy number per worm.
The ratio of Kaede containing transgenes per genome in both wild-type and *ky95* 3'UTR reporter lines was compared to that of a third reporter line using quantitative real time PCR and SYBRGreen (BioRad). The third line was used as a standard and the two experimental strains were compared to it. The Critical cycle threshold (Ct) number for a dilution series of the standard was determined for each set of PCR primers to ensure that the experimental values were in the linear range for the reactions. We determined the fold difference in concentration of each gene in the experimental samples and the standard. The Kaede transgene concentration in the wild-type reporter line (wild type line 3) was at the same concentration relative to the standard as the *ky95* (*ky* line 2) when the concentration of the genomic copies in each sample were normalized to the standard. All worms used in the preparation were transgenic as assessed by their expression of the coinjection marker, *pofm-1*::GFP. The endogenous genes used were, *HMG-CoA Synthase* (F25B4.6) and *HMG-CoA Lyase* (Y71G12B.10).



Supplemental Figure 3. Luciferase expression correlates with concentration of animals and transgene copy number.
A. Destabilized luciferase (half-life 0.5 hours (Promega) was expressed in adult worms, which were harvested as described in the Materials and Methods and subjected to luminometry. The titration of worm supernatant versus luciferase units shows that luciferase units are linear with respect to volume of worms. We found that values >400 luciferase units were linear with respect to volume of worm lysate. **B.** The number of transgenes in a sample (as detected by real-time PCR) correlated well with luciferase units in the same sample.



Supplemental Figure 4. Loss of FBF-1 decreases luciferase expression in animals that contain the wt 3' UTR but not the *gld-1*- substituted 3' UTR. The two highest expressing lines bearing the wild type *egl-4* 3' UTR or the *gld-1*-substituted FBE 3' UTR downstream of luciferase were crossed in to *fbf-1(ok91)* nulls. The loss of FBF-1 decreases expression from the wt *egl-4* 3' UTR by 5.2 fold (compare 1st and 2nd bars) where as loss of FBF-1 in animals bearing the *gld-1*-subsitiutued 3' UTR does not (compare 3rd and 4th bars p= 0.5176).



Supplemental Figure 5. Adding the *egl-4* NR/FBE or the *gld-1* FBE to the *unc-54* 3'UTR is not sufficient to change luciferase expression in animals bearing the *unc-54* 3' UTR. Either the *egl-4* NR/FBE or the *gld-1* FBE was added to the *unc-54* 3' UTR. Either line is not significantly different from the *unc-54* 3' UTR (compare 2nd and 3rd bars, p= 0.1387), (compare 2nd and 4th, p=

0.2111) or from the wild type 3' UTR (compare 1st and 3rd bars, p= 0.5908), (compare 1st and 4th bars, p= 0.8714)

Table S1. The AWC cell fate is not altered in either *fbf-1(ok91)* or *nos-1(gv5)* mutant animals.

Line	% 2 AWC^{OFF}	% 1 AWC^{OFF}/% 1 AWC^{ON}	% 2 AWC^{ON}
fbf-1ok91 line 1	0	100 (5/5)	0
fbf-1 ok91 line 2	0	100 (21/21)	0
fbf-1 ok91 line 3	0	100 (15/15)	0
fbf-1 ok91 line 4	67 (10/15)	33 (5/15)	0
nos-1(qv5) line 1	0	97 (28/29)	3 (1/29)

In the wild-type worm, *pstr-2* drives expression in one AWC whereas in mutants that affect the cell fate, the percent of worms that have *pstr-2* turned ON in neither or both AWCs is increased (Troemel et al., 1999). Thus, the reporter for cell fate is *pstr-2* driving RFP. Transgenic lines carrying the *pstr-2*::RFP array in either the *fbf-1(ok91)* or *nos-1(qv5)* mutant backgrounds were examined microscopically. The percent of the worms examined with each phenotype is presented and the actual numbers are in parenthesis. All worms were grown at 25 °C and examined as L4s or young adults.

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