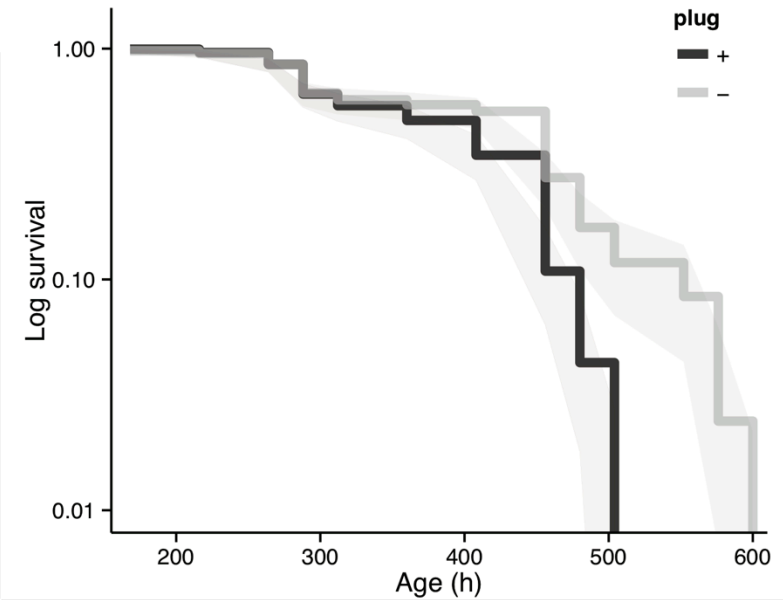

Figure S1, related to Figure 1. Plugged excretory pores reduce male life span. Longevity of QG71 males (372 in total) was assayed in groups of four according to presence of a plugged excretory pore, after 3 days in male-only populations. Shading shows 95% confidence intervals from a Cox proportional hazard model ($P = 0.0005$).

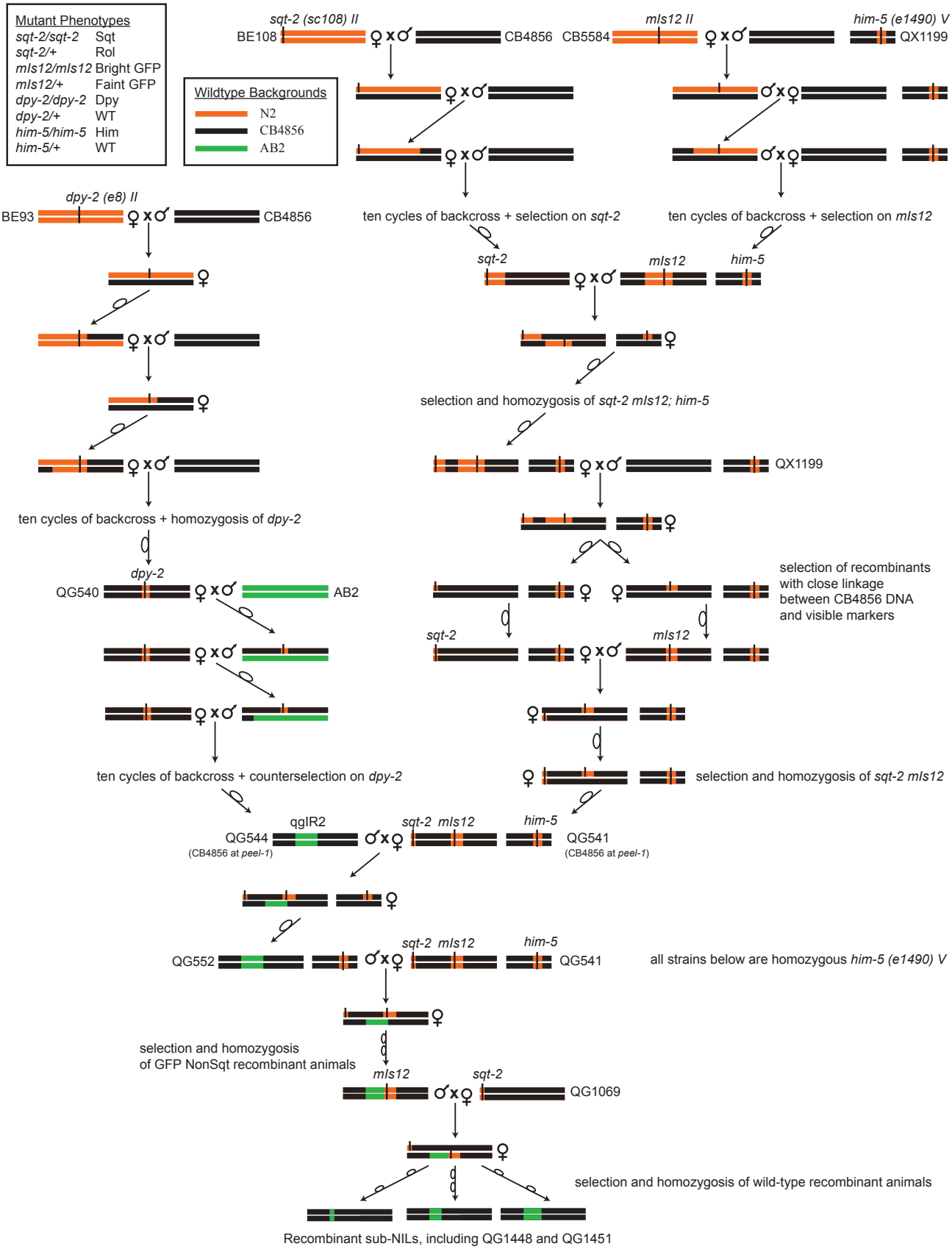

Figure S2, Related to Figure 2. Crosses used to generate Near Isogenic Lines.

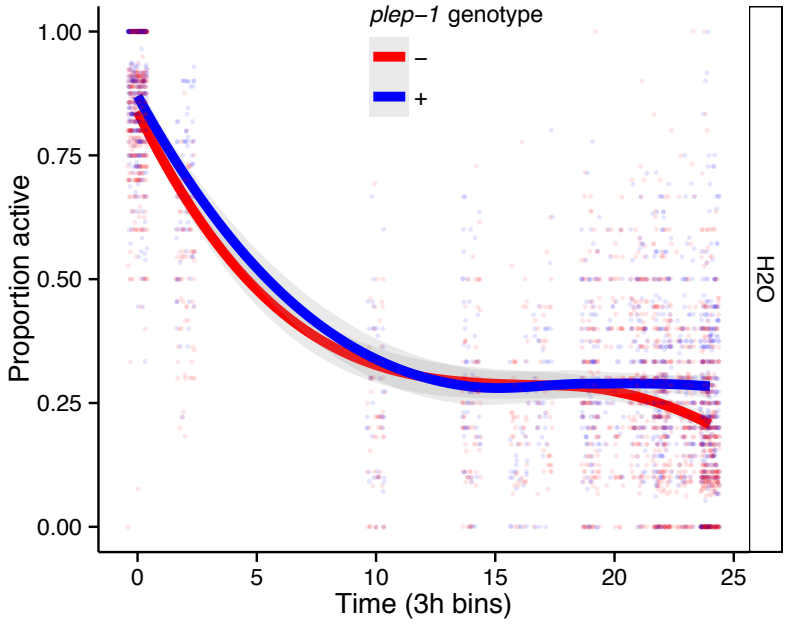

Figure S3, related to Figure 3. Hermaphrodite activity is impaired during starvation in liquid culture. Data are proportions of active young adult hermaphrodites of mean 10 per well (minimum 7, maximum 13) at 24 hours in H_2O, by *plep-1* genotype. Loess smoothed means with 99.9% confidence intervals are plotted over data points (10 assays, n = 5,849 worms). Strains assayed are N2 and QG2456 (*plep-1*$^+$), and QG2457, QG2458, QG2462 (*plep-1*$^-$). *plep-1*$^+$ strains are not significantly different at 24 hours (Generalized linear mixed effects model, $P = 0.4015$), while all mutants are individually significant different from N2 at $P < 0.01$).

Supplemental Experimental Procedures

***C. elegans* Strains**

Strain	Genotype	Reference
DF89	*him-5(e1490)* V > CB4856	[S1, S2]
CB4108	*fog-2(q71)* V	[S3]
CB4088	*him-5(e1490)* V	[S1]
DP38	*unc-119(ed3)* III	[S4]
VS8	*dhs-28(hj8)* X	[S5]
CB234	*unc-18(e234)* X	[S6, S7]
BE108	*sqt-2(sc108)* II	[S8]
BE93	*dpy-2(e8)* II	[S6, S9]
CB5584	*mIs12*[$p_{pharynx}$*::GFP*] II	www.cgc.cbs.umn.edu/strain.php?id=7556
VC40058	*Y52E8A.4(gk140536)* II	[S10]
IE53821	*Y52E8A.4(ttTi53821)* II	[S11]
IE36640	*Y52E8A.6(ttTi36640)* II	[S11]

Mos mutant strains (*ttTi* alleles) were generated by the Ewbank and Segalat labs as part of the NEMAGENETAG project funded by the European Community. They were distributed by M. Carre-Pierrat at the UMS 3421, supported by the CNRS, and by Patricia Kuwabara at the University of Bristol. The other strains were provided by the *Caenorhabditis* Genetics Center, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

Wild Isolates (See Tab 1 of the Supplemental Data File for additional details). Association: N2, CB4856, AB2, CB4858$_{CGC}$, EG4348, JU1088, JU1171, JU345, MY1, MY16, MY18, PB306, RC301. V278D Genotyping: AB1, AB4, CB3198, CB4507, CB4852, CB4853, CB4855$_{CGC}$, CX11258, CX11305, CX11317, DL238, ED3011, ED3021, ED3042, ED3046, ED3075, ED3077, EG4347, EG4948, JU1172, JU1522, JU258, JU310, JU319, JU371, JU400, NIC195, NIC196, NIC197, NIC198, NIC200, PX174$_{CGC}$, PX179, QG2075, QG536, QG537, QG538, QG556, QG557, QG558, QX1211.

New Strains

QX1199	*him-5(e1490)* V > CB4856
QG5	*him-5(e1490)* V > AB2; carries N2 *peel-1* region.
QG9-QG104	RILs from QG5 x QX1199 cross
QG693	*mIs12* II > QX1199

QG1434-1437; QG1440-1456: AB2 > QX1199 II NILs. See Figure S1 and Tab 9 of the Supplemental Data File .

QG2285	*dhs-28(hj8)* X > QG71
QG2286-7	*dhs-28*(N2) X > QG71
QG2288	*plg-1*(N2) III > QG71
QG2452	*Y52E8A.4(ttTi53821)* II; *him-5 (e1490)* V
QG2456	*Y52E8A.4*(N2) II > N2; outcrossing control
QG2457	*Y52E8A.4(gk140536)* II > N2; outcrossed mutation
QG2458	*Y52E8A.4(ttTi53821)* II > N2; outcrossed mutation
QG2462	*Y52E8A.4*(AB2) II > N2
QG2465-6	*qgEx6-7[*p_{plep-1}*::GFP]* > N2

RIL construction and genotyping
We generated recombinant inbred lines from a cross of QX1199 and QG5. QX1199 is *him-5(e1490)* in the CB4856 background. We started with DF89, a gift from David Fitch, which carries the *e1490* allele introgressed through many backcrosses into the CB4856 background. Because DF89 carries the N2 alleles of *zeel-1* and *peel-1*, we derived QX1199 by crossing CB4856 males to DF89 hermaphrodites, selfing F_1s, and recovering a Him F_2 "escaper" that is homozygous for the CB4856 haplotype of *zeel-1* and *peel-1*, by PCR assay. QG5 carries *e1490* introgressed into the AB2 background from CB4088 via seven cycles of backcrossing.

A region on the right side of Chr V has few markers because it is not segregating among the RILs; the founding strains share the N2 *him-5* haplotype across that region.

Strain construction for trans-plugging assays
To introgress the N2 loss-of-function *plg-1* allele into RIL QG71, we first introgressed *unc-119(ed3)*III into QG71 by 10 cycles of backcrossing and selection for Uncs to yield QG1604. The source of the *ed3* allele was QG529, which carried *ed3* from DP38 introgressed into CB4856 by 10 cycles of backcrossing and selection. We then introgressed *plg-1*(N2) III into QG1604 by 10 cycles of backcrossing and counterselection against Uncs. The resulting strain QG2288 does not produce copulatory plugs [S12].

We next generated a GFP-marked CB4856 strain by introgressing transgene *mIs12* II, which drives strong pharyngeal GFP, into QX1199, the CB4856 *him-5* strain used as a parent to the RILs. The resulting *mIs12* II; *him-5* V > CB4856 strain, QG693, was derived from a cross of QX1199 and QG541 (see Figure S2) and selection of GFP Him nonSqt animals.

F_2 assays
Each worm was genotyped by PCR at an indel polymorphism at 4.057 Mb on chromosome II, close to the QTL peak at the SNP marker at 4.317 Mb. Each worm was also genotyped at indels at 3.715, 5.304, and 6.443 Mb, yielding 27 recombinant animals out of 376 scored. These recombinants localize the QTL between 4.057 and 5.304. Data are in Tab 5 of the Supplemental Data File.

Indel Genotyping Primers

3.175	TGAAAAACTCTTGGGTTGGG	AATGCTTGGGGTCTTCACAG
4.057	GATATTGCGCTCGAAGGAAC	TTTGAAATTGGTGGAGGAGG
5.304	TTTGTCACTCAACGACTCGG	TGAGAGCTGGAAAATTGGAAG
6.443	TATGCCCAACACTCAGTGGA	GACACATCAACATCCGTTGC

***dhs-28* introgressions**
To introgress *dhs-28 (hj8)* X from VS8 into the QG71 background, we first introgressed *unc-18(e234)* X into QG71 by phenotypic selection through 11 generations of backcrosses, yielding strain QG1601. We next crossed *dhs-28(hj8)* hermaphrodites to

QG71 males, generating F_1 males carrying *hj8*-bearing X chromosomes. These we crossed to QG1601 hermaphrodites, and selected against *unc-18* through 10 generations of backcrosses, yielding QG2285 (*dhs-28* X > QG71), which we confirmed by PCR and phenotype is *dhs-28(hj8)* X *plep-1*(AB2) II *plg-1(e2001)* III *him-5(e1490)* V.

In parallel, we introgressed the N2 allele of *dhs-28* into the QG71 background in two fully independent lines by the same protocol, yielding QG2286 and QG2287. These strains control for the effect of wild-type N2 DNA linked to the *dhs-28* locus in QG2285, and are *dhs-28*(N2) X *plep-1*(AB2) II *plg-1(e2001)* III *him-5(e1490)* V. We assayed four plates of 40 L4 males of strain QG2285 in parallel with three plates each of QG2286 and QG2287.

Identifying associated variants
Two strains, CB4858 and PB306, exhibited high levels of Plep comparable to AB2, and eight strains exhibited little or no excretory pore plugging. The CB4858 strain included in our survey originated at the CGC, where it was apparently subject to a mix-up with AB2 (or the nearly identical strains AB3 and AB4) [S13]; we excluded it from further consideration, with no effect on our association findings. We treated the phenotype as binary to identify alleles shared by PB306 and AB2, but not carried by CB4856 or any of the other 8 non-Plep strains.

RNAi experiments
We cloned a genomic fragment of *Y52E8A.4* into the pL4440 feeding-RNAi vector from the Fire Lab Vector Kit. We amplified the fragment with the following primers:
5'-GCGCACTAGTTGATTTTTGGCGGAGTTTTC
5'-GCGCCTCGAGCATTGGGGAATGTCAAGGAG
We cut the PCR product and pL4440 DNA with *Spe*I and *Xho*I, ligated the cut fragments, and transformed DH5α cells with the reaction product. We validated the resulting plasmid by digestion and then transferred it to HT115(DE3) cells.

Wild Isolate Genotypes
To genotype V278D, we PCR amplified a 695bp fragment with primers GGGGAATGTCAAGGAGATCA and TGATTTTTGGCGGAGTTTTC and digested the product with *Eco*RV, which cuts the D allele but not the V allele.

Hermaphrodite Activity Assays
To manufacture these strains, we first generated QG2290, *sqt-2(sc108) mIs12* II; *him-5(e1490)* V, by crossing CB4088 (*e1490*) and QG1072 (*sc108 mIs12*, each independently outcrossed to N2 for 12 generations and then linked). We then made QG2458 by crossing a *ttTi53821* homozygous strain to QG2290 and selecting against *sqt-2 mIs12* for 8 backcrosses. The resulting *plep-1(ttTi53281)* II; *him-5(e1490)* V strain, QG2452, was then crossed to QG1072 to remove *him-5* from the background, yielding QG2458. QG2457 was constructed similarly, by first crossing VC40058, a Million Mutation Project strain, to QG2290 and selecting against *sqt-2 mIs12* eight times. The resulting *plep-1(gk140536)* II; *him-5(e1490)* V strain, Q2451, was then crossed to QG1072 to remove *him-5*. QG2456, our N2 control strain, derives from a cross of N2 to QG2290 and

selection of a nonSqt nonGFP *him-5* strain, QG2450, which we then crossed to QG1072 to remove *him-5*. QG2456 does not carry any known mutations in the N2 background, but derives from the same strain intermediates as QG2457 and QG2458. Finally, QG2462 is derived from 8 backcrosses of QG2288 to QG2290 with selection against *sqt-2 mIs12* to yield *plep-1(V278D)* (and flanking regions from the AB2 strain) in the N2 *him-5* background.

Gene expression analysis

The two differentially expressed genes mentioned as implicated in neuropeptide biosynthesis or activity are a predicted peptide amidating monooxygenase *pgal-1*, which is coexpressed with FMRFamide-like peptide encoding genes (Bonferroni corrected $P = 1.18\times10^{-29}$) and other behavioral signaling genes; and *C45G9.6*, a predicted transmembrane protein with homology to human NOTCH1, which also shows significant expression correlation with genes annotated to neuropeptide signaling ($P = 1.30\times10^{-9}$) and regulation of behavior. Both show higher expression in the *plep-1* mutant. A third significantly downregulated gene, *basl-1*, is a predicted catalytically inactive paralog of *bas-1*, a serotonin and dopamine biosynthetic aromatic amino acid decarboxylase. *bas-1* itself may be weakly downregulated at the level of whole animals (adjusted $P = 0.16$, ranked 75 by *t* statistic).

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