Supplementary Information

PDF-1 neuropeptide signaling modulates a neural circuit for mate-searching behavior in *C. elegans*.

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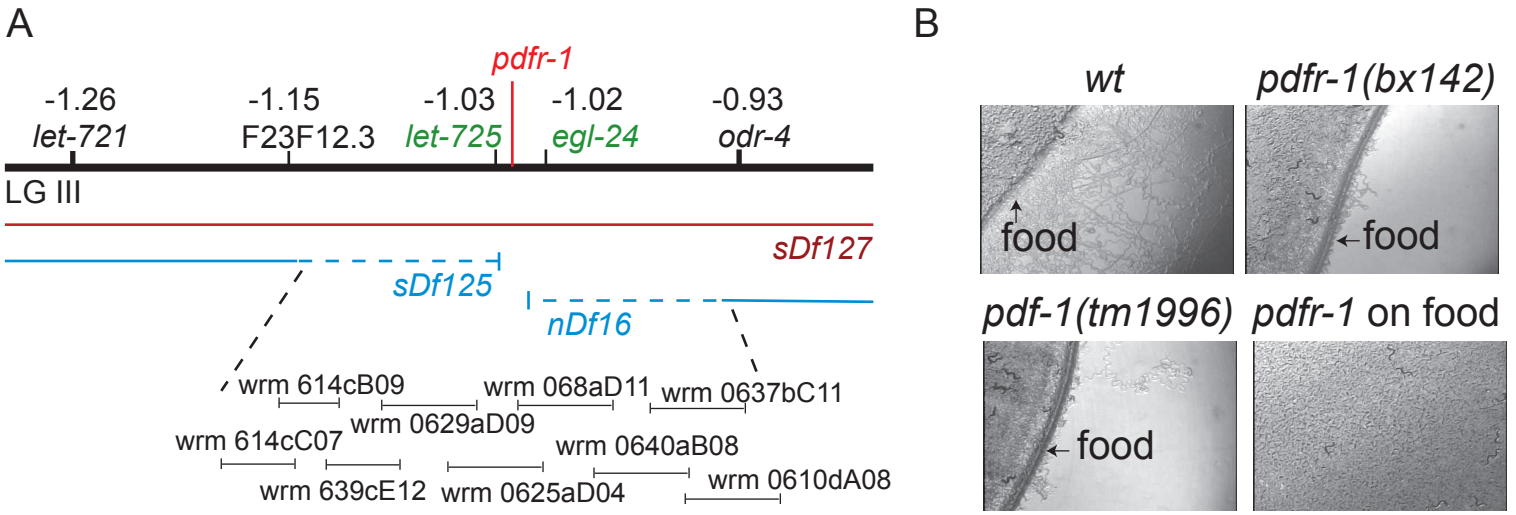
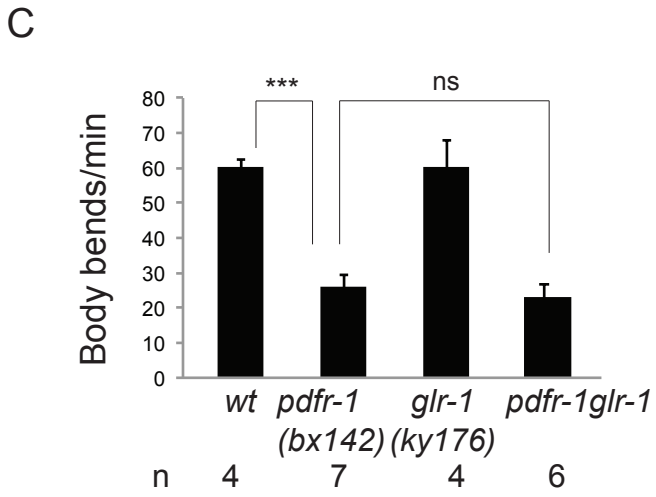
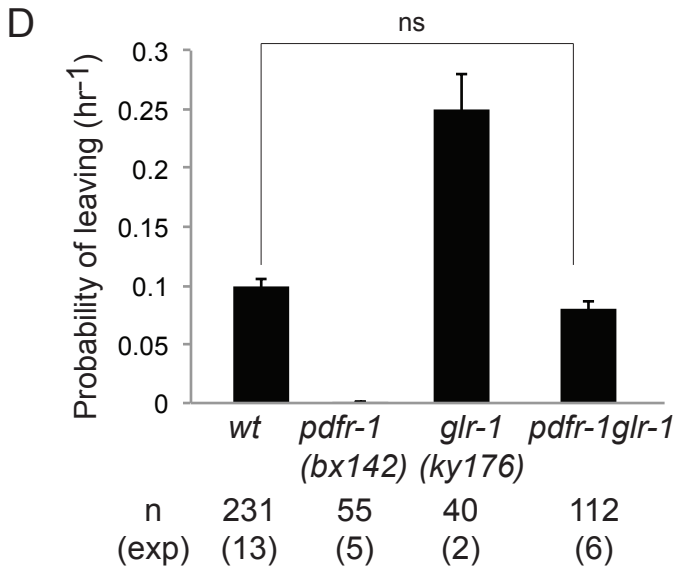
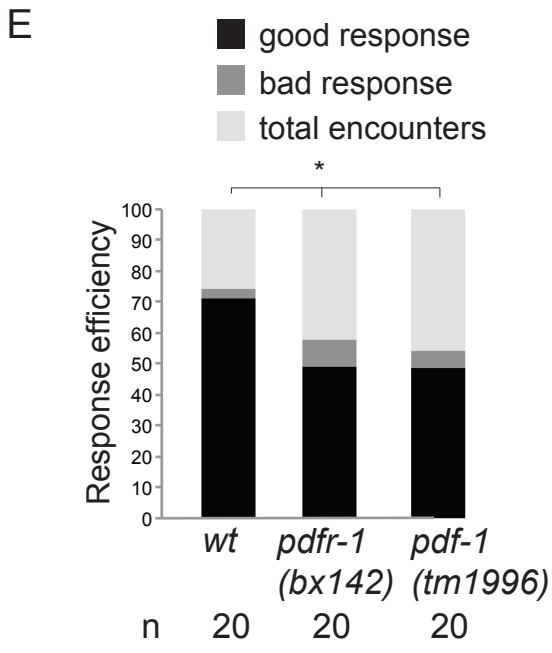
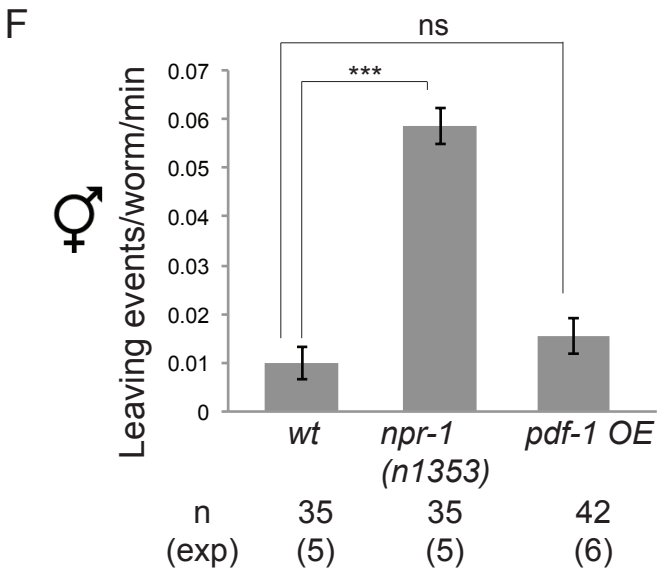
Supplementary Figure 1











SFigure. 1. The PDF-1/PDFR-1 signaling pathway promotes male mate-searching behavior

A. Genetic mapping of *bx142*. A region of chromosome III (LGIII) between *dpy-17* and *unc-32* (not in the diagram) is shown in black. Genetic positions of landmark genes are indicated above. Genes for whom the physical position in the chromosome is known are indicated in black. Genes for whom only the genetic position is known are indicated in green. Regions deleted by deficiencies (Df) are labeled in red (deletes *bx142*) or blue (does not delete *bx142*). Dashed lines indicate the regions deleted by the deficiencies that have not been confirmed by physical position. A pool of DNA fosmids encoding genes in the region rescues (3 of 5 independent lines) when transformed into *bx142*.

B. Photographs show the tracks produced by 25 males during 15 hours on agar plates with food. Wild-type (wt) males explore inside and outside the food lawn. *pdfr-1(bx142)* and *pdf-1(tm1996)* mutants only explore within the limits of the food lawn.

C and **D** show speed on food (measured by propagation of sinusoidal wave, body bends/minute) (C) and mate-searching behavior (measured by probability of leaving food (P_L)) (D) of wild type (wt), *pdfr-1(bx142)*, *glr-1(ky176)* and *pdfr-1(bx142)glr-1(ky176)* mutant males. The *glr-1(ky176)* null mutation restores mate-searching behavior in *pdfr-1(bx142)* mutants without suppressing the slow speed phenotype.

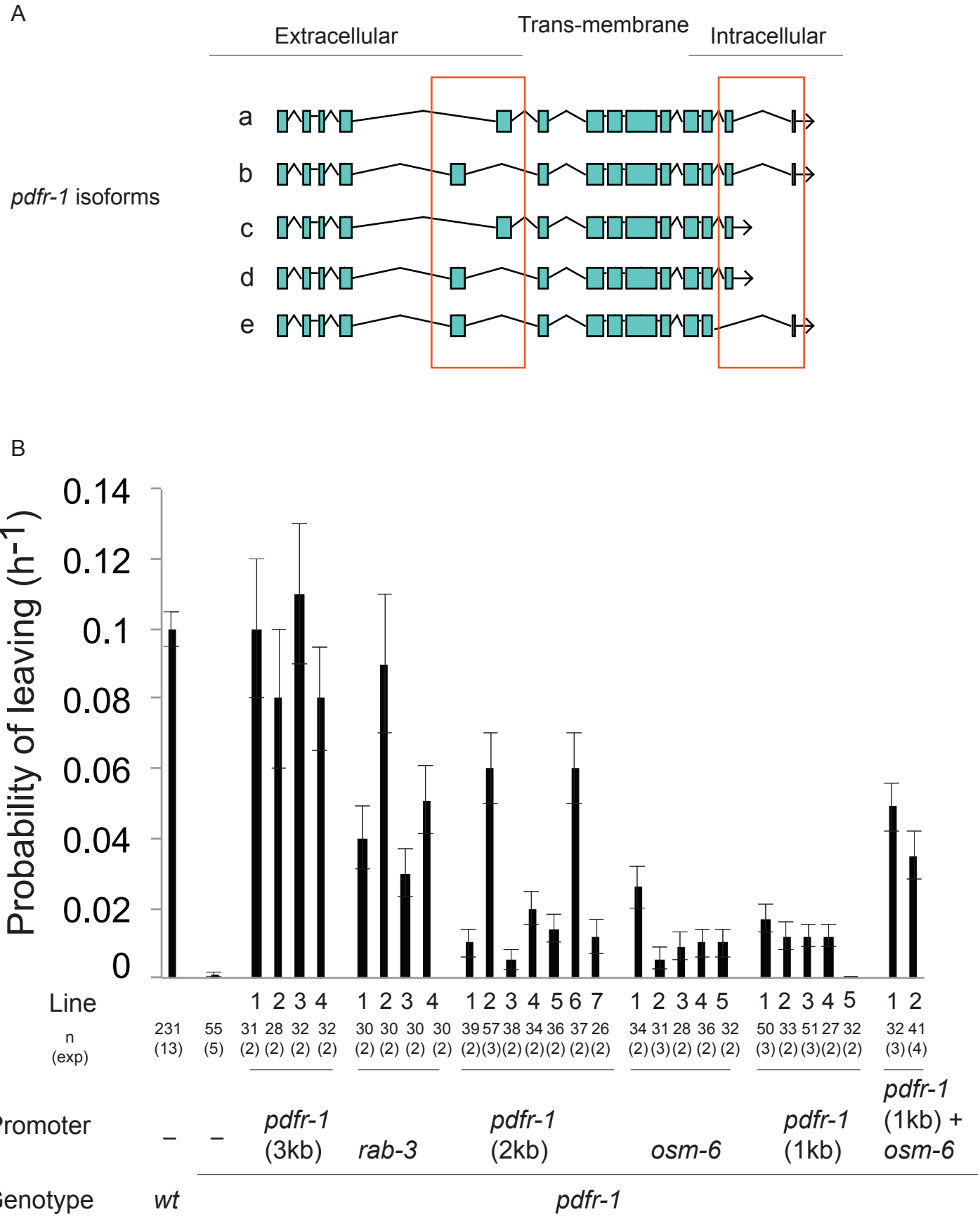
E. Response efficiency to mate contact of wt, *pdfr-1(bx142)* and *pdf-1(tm1996)* mutant males. Graphs show the average response efficiency per male for each encounter with a mate. *pdfr-1* and *pdf-1* males required more encounters with a mate before initiating the mating sequence. Good response indicates that the male placed its tail ventral down on the mate body and backed to make a turn. Bad response indicates that the male lost contact during backing to the opposite end of the mate body.

Mann-Whitney test was used for statistical analysis in B, E and F. Maximum likelihood statistical analysis was used to compare P_L values in C. n indicates the total number of animals tested; (exp) indicates the number of independent

experiments. ***p<0.001, *p<0.05, ns: no statistically significant difference (p≥0.05).

F. The rate of food-leaving events per minute per worm is shown for wt, *npr-1(n1353)* mutant and *pdf-1*-overexpressing (*pdf-1* OE) hermaphrodites. Unlike wild-type hermaphrodites, *npr-1* mutant hermaphrodites produce a high rate of exploratory events outside the food lawn. Hermaphrodites overexpressing *pdf-1* did not produce a significant increase in the rate of exploratory events compared to wild-type hermaphrodites. Error bars indicate SEM; n indicates total number of animals tested; (exp) indicates the number of independent population-based experiments. Kruskal Wallis test was used for statistical analysis. ***p<0.001, ns: no statistically significant difference (p≥0.05).

Supplementary Figure 2



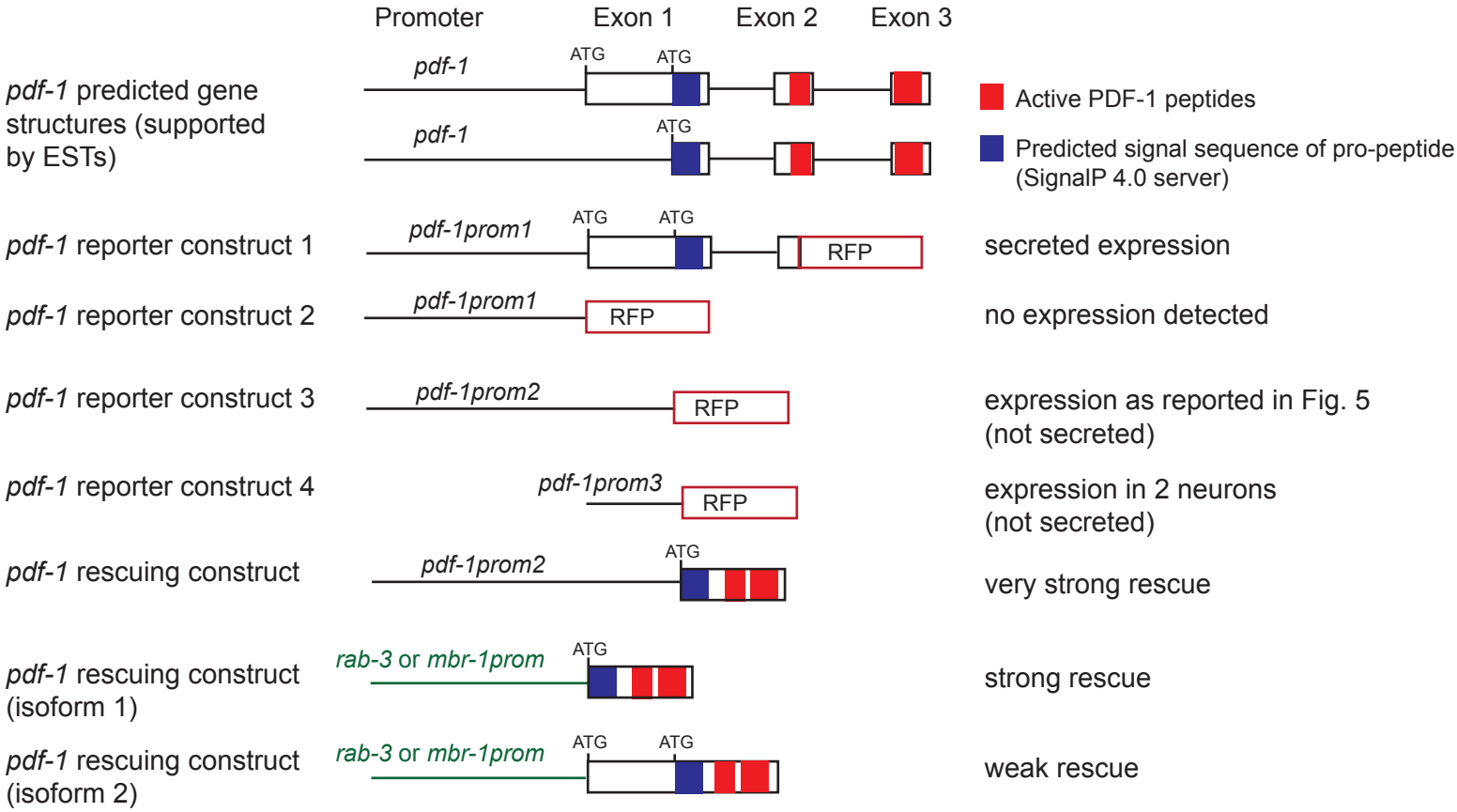
SFigure 2. Isoforms of *pdfr-1* and rescue experiments.

A. *pdfr-1* isoforms. Boxes represent exons and lines represent introns. The domains of the G protein-coupled receptor encoded by the exons are labeled. Three different isoforms of *pdfr-1* (a,b and c) have been previously reported [Janssen, T. *et al. J. Biol. Chem.* **283**, 15241–15249 (2008)]. From a mixed age and mixed gender mRNA pool we isolated those three isoforms plus two new isoforms, d and e. Isoforms differ in exons (within orange boxes) that encode for the extracellular and intracellular domains of the protein.

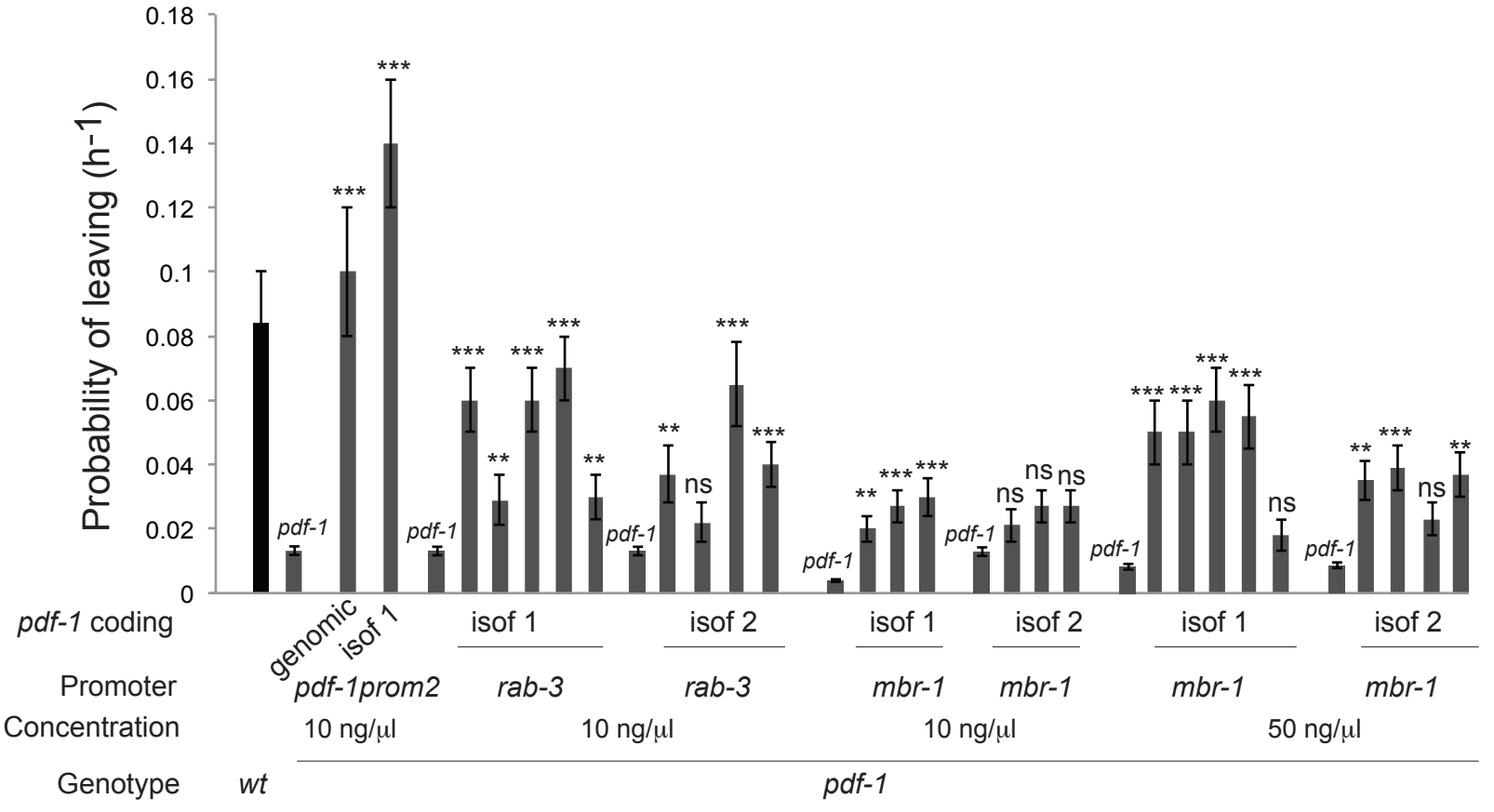
B. Rescue of mate-searching behavior in *pdfr-1(bx142)* mutants with *pdfr-1* bashed and heterologous promoters driving expression of *pdfr-1* isoforms b and d. Graphs show P_L values of several independent lines for some of the constructs listed in Figure 4 E. Error bars indicate SEM; n indicates total number of animals tested; (exp) indicates the number of independent population-based experiments.

Supplementary Figure 3

A



B



SFigure 3. Annotation of the *pdf-1* locus and rescue experiments.

A. Diagrams depict the structure of the *pdf-1* locus, expression and rescue constructs. Exons are shown as boxes. The region encoding active PDF-1 peptides is marked in red [Janssen, T. *et al. Journal of Neurochemistry* **111**, 228–241 (2009)]. The predicted signal sequence that targets the pro-peptide to the secretory cellular machinery is marked in blue and was identified by the SignalP 4.0 online software program. The 279 nt region between the two predicted translational start sites contains essential regulatory elements for the expression of a reporter gene (RFP). A *pdf-1* isoform that does not contain this 279 nt region fully rescues the mate-searching defects of *pdf-1* mutants when expressed under other neuronal promoters.

B. Rescue of mate-searching behavior in *pdf-1(tm1996)* mutants with endogenous and heterologous promoters driving either of the two predicted *pdf-1* isoforms. Note that *pdf-1prom2* contains the 279 nt region between the two predicted start sites. Graphs show P_L values of several independent lines, and includes some of the constructs listed in Figure 5C. Error bars indicate SEM; each line was tested at least in two independent population-based experiments with at least 15 animals in each experiment. Maximum likelihood statistical analysis was used to compare P_L values against *pdf-1* mutants assayed in parallel, ***p<0.001, **p<0.01, ns: no statistically significant difference (p≥0.05).