

Figure S1

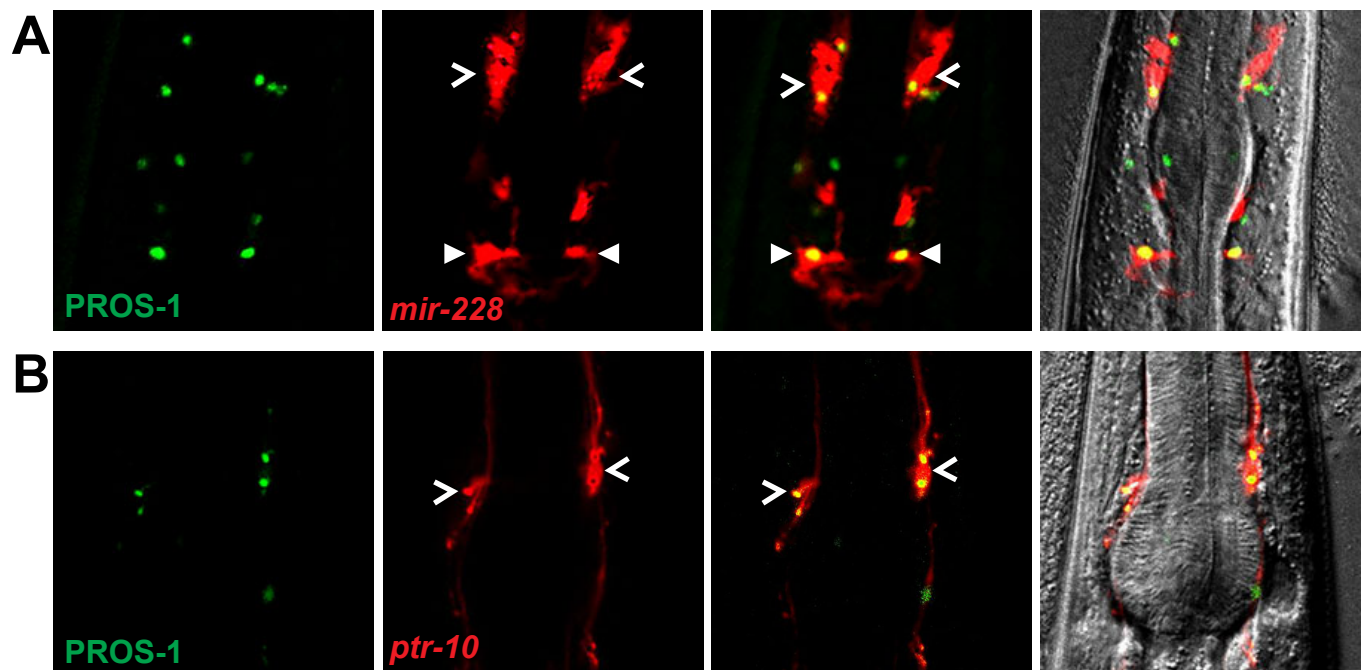


Figure S1. *pros-1* is expressed in CEPsh glia and anterior glia, related to Figure 5. (A) *pros-1*::GFP is expressed in *mir-228*::mCherry-positive CEPsh and anterior (inner labial and outer labial) glia. (B) *pros-1*::GFP is expressed in *ptr-10*::RFP-positive anterior glia. Filled arrowheads indicate CEPsh glia, open arrowheads indicate anterior glia.

Figure S2

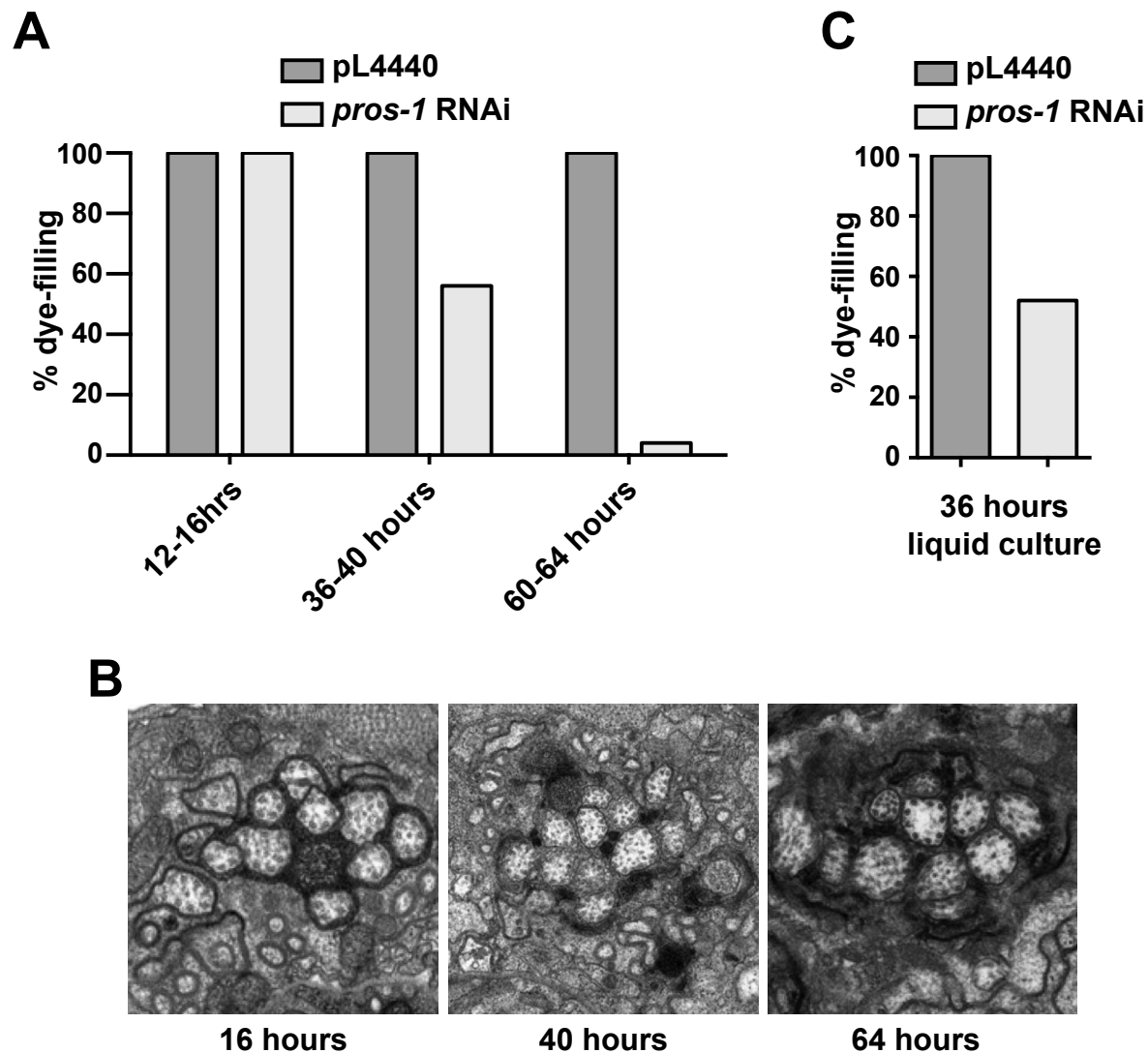


Figure S2. *pros-1* RNAi defects are apparent after 36-42 hours of RNAi treatment, related to Figure 6 . (A-B) Animals grown post-embryonically on standard RNAi plates. (A) Amphid neuron dye-filling. n=100. (B) Electron micrographs of *pros-1* RNAi-treated animals. Note the apparently normal matrix-filled amphid channel compartment at 16 hours of treatment, which is absent at 40 hours treatment. (C) Animals grown in modified liquid culture for RNAi treatment of large numbers of animals. Amphid dye-filling scored at 36 hours, n>80.

Figure S3

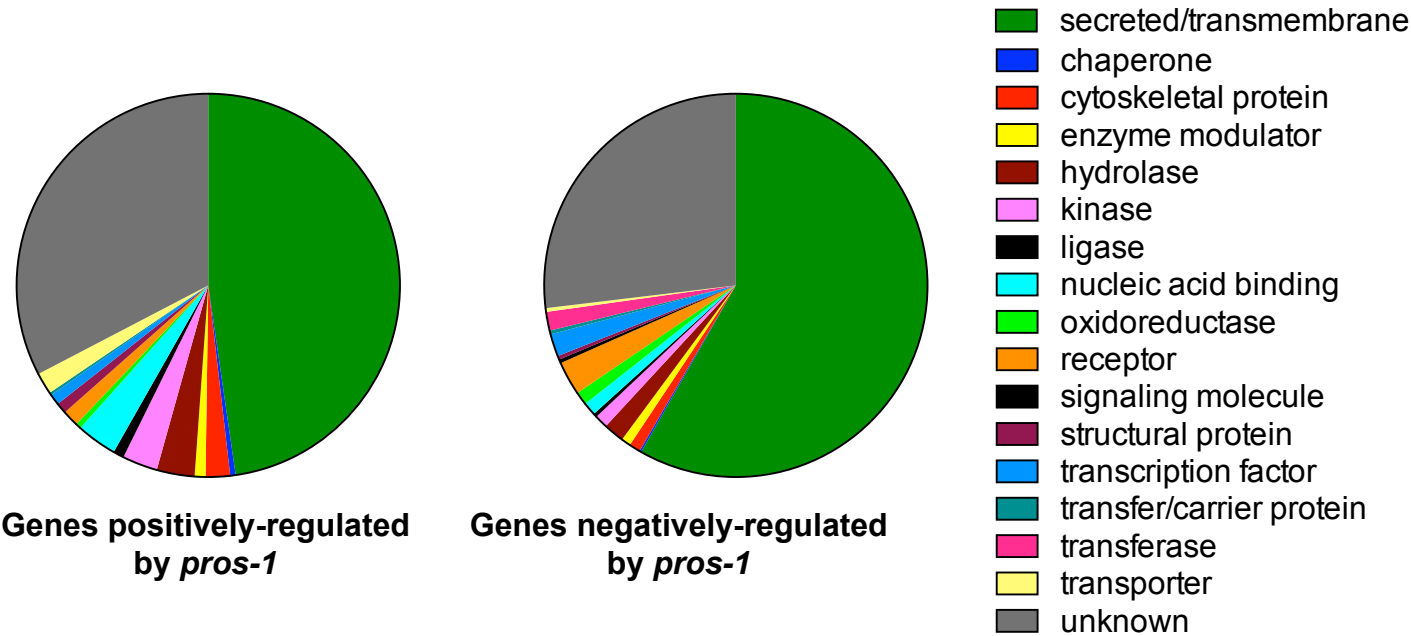


Figure S3. Protein classes of all PROS-1 AMsh glia targets (>2 fold change), related to Figure 6.

SUPPLEMENTAL TABLE LEGENDS

Table S1. List of AMsh-enriched genes, related to Figure 1. Fold-enrichment and adjusted p-values shown. Most genes were identified using DESeq analysis. Additional genes identified using voom analysis are listed below. See Experimental Procedures for details of statistical analysis. TM/SP indicates presence of signal peptide or transmembrane domain.

Table S2. List of *pros-1*-dependent genes that show decreased expression in AMsh glia following *pros-1* RNAi, related to Figure 6. Fold-change and adjusted p-values shown. Most genes were identified using DESeq analysis. Additional genes identified using voom analysis are listed below. See Experimental Procedures for details of statistical analysis. TM/SP indicates presence of signal peptide or transmembrane domain.

Table S3. List of *pros-1*-dependent genes that show increased expression in AMsh glia following *pros-1* RNAi, related to Figure 6. Fold-change and adjusted p-values shown. Most genes were identified using DESeq analysis. Additional genes identified using voom analysis are listed below. See Experimental Procedures for details of statistical analysis. TM/SP indicates presence of signal peptide or transmembrane domain.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Strains and Plasmids

Alleles used in this study are: *rde-1(ne219)* (Tabara et al., 1999), *rrf-3(pk1426)* (Sijen et al., 2001), *pros-1(tm258)* (National BioResource Project, Japan), *fig-1(ns306)* – a 16-bp insertion in exon 7 that results in a frameshift and formation of a predicted truncation protein of 1112 amino acids.

Integrated transgenic strains used in this study are:

nsIs143 X (*F16F9.3::dsRed*)
oysIs45 V (*odr-1::YFP*)
ntlIs1 V (*gcy-5::GFP*)
nsIs109 (*F16F9.3::DT-A(G53E)*) (AMsh-ablated strain (Bacaj et al., 2008))
nsIs198 (*mir-228::GFP*) (Miska et al., 2007)
nsIs390 (*rab-3::mCherry*)
vsIs48 (*unc-17::GFP*)
nsIs108 (*ptr-10::RFP*)
cbIs1 I (*vap-1::GFP*)
nsIs228 I (*srtx-1::GFP*)
wgIs500 (CEH-26::TY1::EGFP::3xFLAG) (recombineered fosmid with GFP inserted at C-terminus of PROS-1, Regulatory Element Project (Niu et al., 2011)).
nsIs360 (DAF-6::TY1::EGFP::3xFLAG) (recombineered fosmid with GFP inserted at C-terminus of DAF-6, Regulatory Element Project (Niu et al., 2011)).
nsIs338 (*T02B11.3::LIT-1C-GFP*)

Extrachromosomal arrays used in this study are:

nsEx5120 (pGO17 = *lit-1::NLS-RFP*)
nsEx4236 (pAS406 = *srtx-1B::mCherry*)
nsEx5118 (pSW70 = *K02E11.4::GFP* + *elt-2::mCherry*)
nsEx5119 (pSW71 = *R11D1.3::GFP* + *elt-2::mCherry*)
nsEx864 (*F11C7.2::GFP*)
nsEx1758 (*fig-1::GFP*)
nsEx3747 (pSW7 = *F16F9.3::rde-1* + *T02B11.3::GFP*)
nsEx3961 (fosmid WRM0617bG01 + *F16F9.3::GFP* + *elt-2::mCherry*)
nsEx5121 (pSW25 = *mir-228::mCherry*)

Plasmids generated in this study are:

pSW7 – *F16F9.3::RDE-1*
pSW25 – *mir-228::mCherry*
pSW70 - *K02E11.4::GFP* (800 bp promoter fragment upstream of start codon)
pSW71 – *R11D1.3::GFP* (900 bp promoter fragment upstream of start codon)

Behavioral Assays

All behavior assays were performed on first-day adults. Chemotaxis assays were carried out on circular assay plates as previously described (Bargmann et al., 1993; Ward, 1973). Concentrations of odors used were: benzaldehyde 0.5% (in ethanol), methylpyrazine 0.25% (in ethanol). For sodium chloride chemotaxis, a salt gradient was created by soaking a 5 mm agar plug in 200 mM NaCl for 1 hour, then adding the plug to the chemotaxis assay plate for 16-20 hours. Avoidance assays were carried out on square assay plates, as described previously (Chao et al., 2004). 1-octanol was used undiluted. Chemotaxis and avoidance assays were run for 60 minutes. Chemotaxis index (CI) presented is mean of independent repeats, with statistical significance calculated using t-test (GraphPad). Thermotaxis assays were carried out on 18-26°C linear temperature gradients (Ryu and Samuel, 2002). Assays were carried out on square plates with 6x6 grid marks to define 6 temperature bins. 5 ml of glycerol was added to the bottom of the

plate before placing on the temperature gradient to improve thermal conductivity. Assays were run for 45 minutes.

Fluorescence and Electron Microscopy

Fluorescence imaging was performed using an inverted TCS SP8 laser scanning confocal microscope (Leica) with PlanApo 40x/1.10 NA objective (Rockefeller University Bio-Imaging Resource Center). Images shown are projections of one to several adjacent sections, processed using Image J software. Alternatively, imaging was performed using an Axioplan II fluorescence microscope (Zeiss) equipped with an AxioCam camera. Images were processed for figure presentation using Photoshop (Adobe). Transmission electron microscopy was carried out on animals prepared and sectioned using standard methods (Perens and Shaham, 2005). Samples were imaged using an FEI Tecnai G2 Spirit BioTwin transmission electron microscope equipped with a Gatan 4Kx4K digital camera.

Cell Isolation and FACS

Synchronized larvae were grown from L1 arrest, using a modified liquid culture protocol for RNAi. Overnight starter cultures of *E. coli* HT115 (with pL4440 empty vector or pL4440-*pros-1* RNAi, Ahringer RNAi library) were used to inoculate (1:1000) 500 ml LB (+ ampicillin 50 µg/ml). This culture was grown to OD₆₀₀ = 0.4-0.6 (approx. 6 hours, 37 °C, 220 rpm), followed by induction of dsRNA expression by addition of IPTG (1 mM) and growth for 12 hours at 30°C, 200 rpm. Bacteria were pelleted at 5000g, 4 °C, 20 mins, and resuspended thoroughly by vortexing in 50 ml S-Medium (Stiernagle, 2006). Approximately 500,000 L1 animals (strain *rrf-3(pk1426); nsIs143*, enhanced RNAi efficiency, deRed transgene expression in AMsh) were added to the culture and incubated at 20°C with moderate shaking (150 rpm). These conditions allow animals to grow robustly to the L4 stage without starving or entering dauer. Efficiency of RNAi was assessed by performing dye-filling assays on *pros-1* RNAi-treated animals (Figure S2). Typically 2 cultures (1,000,000 animals) were prepared in this way for each replicate sort.

After growth for 36-42 hours, cells were isolated from late-stage larvae (L3 and L4) as described previously (Zhang and Kuhn, 2013), with modifications (Menachem Katz, personal communications). Specifically, animals were transferred to 15 ml tubes and washed extensively in M9 to remove excess bacteria. Each wash consisted of a brief (10 second, 1300 rpm) centrifugation, such that most animals were pelleted, but bacteria stayed in suspension. This was repeated 12 times to remove as much bacteria as possible. Following washing, animals pellets were treated as described (Zhang and Kuhn, 2013). Pronase E treatment was carried out by incubating *C. elegans* pellets in 2X pellet volume pronase E (15 mg/ml) at RT with rotation, followed by 10-20 minute gentle homogenization on ice (2 ml dounce homogenizer, pestle clearance 0.013-0.064 mm, Kimble Chase). After washes with egg buffer (Zhang and Kuhn, 2013) to remove pronase E, cells were filtered through a 5 µm filter to remove undigested animal fragments.

AMsh glia expressing a dsRed transgene (*nsIs143*) were sorted using a BD FACS Aria sorter equipped with 561 nm laser (Rockefeller University Flow Cytometry Resource Center), with egg buffer as the sheath buffer to preserve cell viability. Dead cell exclusion was carried out using DAPI. Gates for size and granularity were adjusted to exclude cell aggregates and debris. Gates for fluorescence were established using wild-type (N2) non-fluorescent animals. 80,000 – 200,000 dsRed-positive events were sorted per replicate, which represented 0.1-0.4% of total events (after scatter exclusion), which is roughly the expected labeled-cell frequency in the animal. dsRed-negative events were also sorted for comparison. Cells were sorted directly into Trizol LS (Ambion).

RNA-Seq Quality Assessment and Read Alignment

Fastq files were generated with CASAVA v1.8.2 (illumina), and examined using the FASTQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) application for sequence quality. Reads were aligned to the *C. elegans* WS244 reference genome (ftp://ftp.wormbase.org/pub/wormbase/releases/WS244/species/c_elegans/PRJNA13758/c_elegans.PRJNA13758.WS244.genomic.fa.gz) using the STAR v2.3 aligner with parameters (--outFilterMultimapNmax 10 --outFilterMultimapScoreRange 1) (Dobin et al., 2013). All samples had > 20 million uniquely mapped reads. The alignment results were evaluated through RNA-SeQC v1.17 to make sure all samples had a consistent alignment rate and no obvious 5' or 3' bias (DeLuca et al., 2012). Aligned reads were summarized through featureCounts (Liao et al., 2014) with gene models from Ensemble (Caenorhabditis_elegans.WBcel235.77.gtf) at gene level unstrand: specifically, the uniquely mapped reads

(NH 'tag' in bam file) that overlapped with an exon (feature) by at least 1 bp on either strand were counted and then the counts of all exons annotated to an Ensemble gene (meta features) were summed into a single number. rRNA genes, mitochondrial genes and genes with length <40bp were excluded from downstream analysis.

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