

Supplemental Methods

Neural Cell isolation

Gravid adult worms were incubated with 0.5 M NaOH and 1.2% NaClO for 6 minutes at 20°C to release eggs. Eggs were washed with 1X M9 buffer (22.0 mM KH₂PO₄, 42.3 mM Na₂HPO₄, 85.6 mM NaCl, 1 mM MgSO₄) and incubated in 1X M9 overnight at 20°C to allow hatching.

Synchronized L1 worms were plated on 15 cm NGM plates (3000 L1s /plate) and cultured at 20°C for 50 hours to obtain adults. Adult worms were digested with freshly thawed SDS-DTT buffer (200 mM DTT, 0.25% SDS, 20 mM HEPES pH 8.0, 3% sucrose, aliquoted into 1 ml tubes and stored at -20°C) on a nutator at room temperature for 6 minutes. Following centrifugation, worms with damaged cuticle were thoroughly washed with 1X M9 and further digested with freshly made 15 mg/ml and 20 mg/ml Pronase E (Sigma-Aldrich) with egg buffer (118 mM NaCl, 48 mM KCl, 2mM CaCl₂, 2 mM MgCl₂, 25 mM HEPES pH 7.3 adjusted osmolarity to 340mOsm with sucrose). During the 20 min incubation with Pronase E, worms were mechanically disrupted with a 200 µl pipette tip. Digestion was stopped by the addition of 1 x PBS (137.0 mM NaCl, 2.7 mM KCl, 10.1 mM Na₂HPO₄, 3.2 mM KH₂PO₄) + 2 % heat inactivated fetal bovine serum (Gibco). Ice cold 1X PBS was used to pre-wet the 5 µm syringe filter (Millex) and the digested cell suspension was filtered into sterile FACS tubes.

Transgenic worm strains

Transgenic worms expressing the neural Y75B8A.8 reporter with FLAG-tagged wild-type ADR-1 or the ADR-1 binding mutant were generated by microinjecting the *rab3p::gfp::Y75B8A.8* 3' UTR construct and either the *adr-1p::3XFLAG::adr-1 genomic::adr-1* 3' UTR construct or the *adr-1p::3XFLAG::adr-1 genomic* with dsRBM1 KKK-EAA::*adr-1* 3' UTR construct into the gonads of young adult hermaphrodites of BB19 *adr-1(tm668)* to result in strains BB19 *adr-1(tm668)* + blmEx15 [*rab3p::gfp::Y75B8A.8* 3' UTR, 3XFLAG *adr-1 genomic*], BB19 *adr1(tm668)* + blmEx16

[*rab3p::gfp::Y75B8A.8* 3' UTR, 3XFLAG *adr-1* genomic with dsRBM1 KKK-EAA]. The injection mix consisted of the following: 1ng/μl of the *adr-1* transgene of interest and 40ng/μl of the dominant marker (*rab3::gfp::Y75B8A.8* 3' UTR) as well as 1kb DNA ladder (NEB) to make the final concentration of the injection mix to 100ng/μl.

The transgenic animal expressing FLAG ADR-2 within the nervous system was generated by inserting genomic ADR-2 sequence downstream of *rab3p::3X FLAG* within a modified pBluescript plasmid. Transgenic strains were generated by microinjecting this *rab3p::3XFLAG::adr-2 genomic::unc-54* 3' UTR construct along with the *rab3p::gfp::Y75B8A.8* 3' UTR construct into the gonads of young adult hermaphrodites of BB20 *adr-2(ok735)*. During transgenic worm generation, the injection mix consisted of the following: 1ng/μl of the *adr-2* transgene, 20ng/μl of the dominant marker (*rab3::gfp::Y75B8A.8* 3' UTR) as well as 1kb DNA ladder (NEB) to make the final concentration of the injection mix to 100ng/μl. The strain carrying this transgene BB20 (*adr-2(ok735)*) + blm17 [*rab3p::3XFLAG::adr-2 genomic::unc-54* 3' UTR; *rab3p::gfp::Y75B8A.8* 3' UTR] was crossed with BB21 (*adr-1(tm668)*; *adr-2(ok735)*) and genotyped to identify progeny that contained the wild-type *adr-1* locus (named HAH17) or the *adr-1(tm668)* locus (named HAH18).

RNA immunoprecipitation assay

Synchronized L1 and adult worms were washed with IP buffer (50 mM HEPES [pH 7.4], 70 mM K-Acetate, 5 mM Mg-Acetate, 0.05% NP-40 and 10% glycerol) and subjected to UV crosslinking (3 J/cm²) using the Spectrolinker (Spectronics) and frozen at -80°C. These frozen worm pellets were ground with cold motor and pestle on dry ice and the cell lysate was centrifuged at maximum speed to remove cellular debris. Protein concentration was measured with Bradford reagent (Sigma-Aldrich) and 0.75 mg of the L1 lysate and 2 mg of the adult lysate were added to anti-FLAG magnetic beads (Sigma-Aldrich). After incubation for 1 hour on the cold room rotator, protein-bound beads were washed with wash buffer (0.5 M NaCl, 160 mM Tris-HCl [pH

7.5]). A portion of the IP (1/10) was stored in SDS loading buffer and used for immunoblotting. The remaining beads were incubated with 0.5 μ l of 20 mg/ml Proteinase K (Sigma-Aldrich) at 42°C for 20 minutes. RNA was isolated and qPCR was performed as described in main methods section.