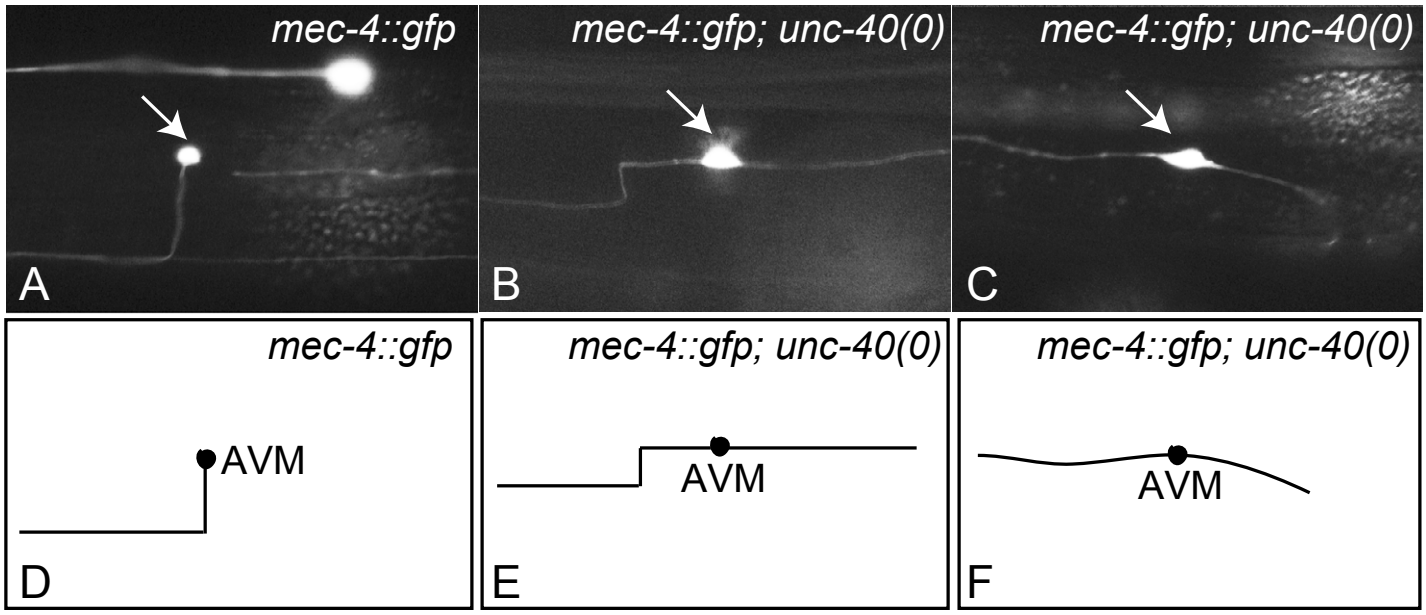

Fig. S1. The AVM migration defects of *unc-40* mutants. Photomicrographs (**A-C**) and corresponding schematics (**D-F**) of animals showing the AVM cell body (arrow) and axon projections as marked by *mec-4::gfp*. Anterior is to the left and dorsal is up. In wild-type animals (A,D), the AVM axon first migrates ventrally and then turns anteriorly to migrate along the ventral nerve cord. (B,C,E,F) Examples of AVM axon migration defects in *unc-40* mutants.

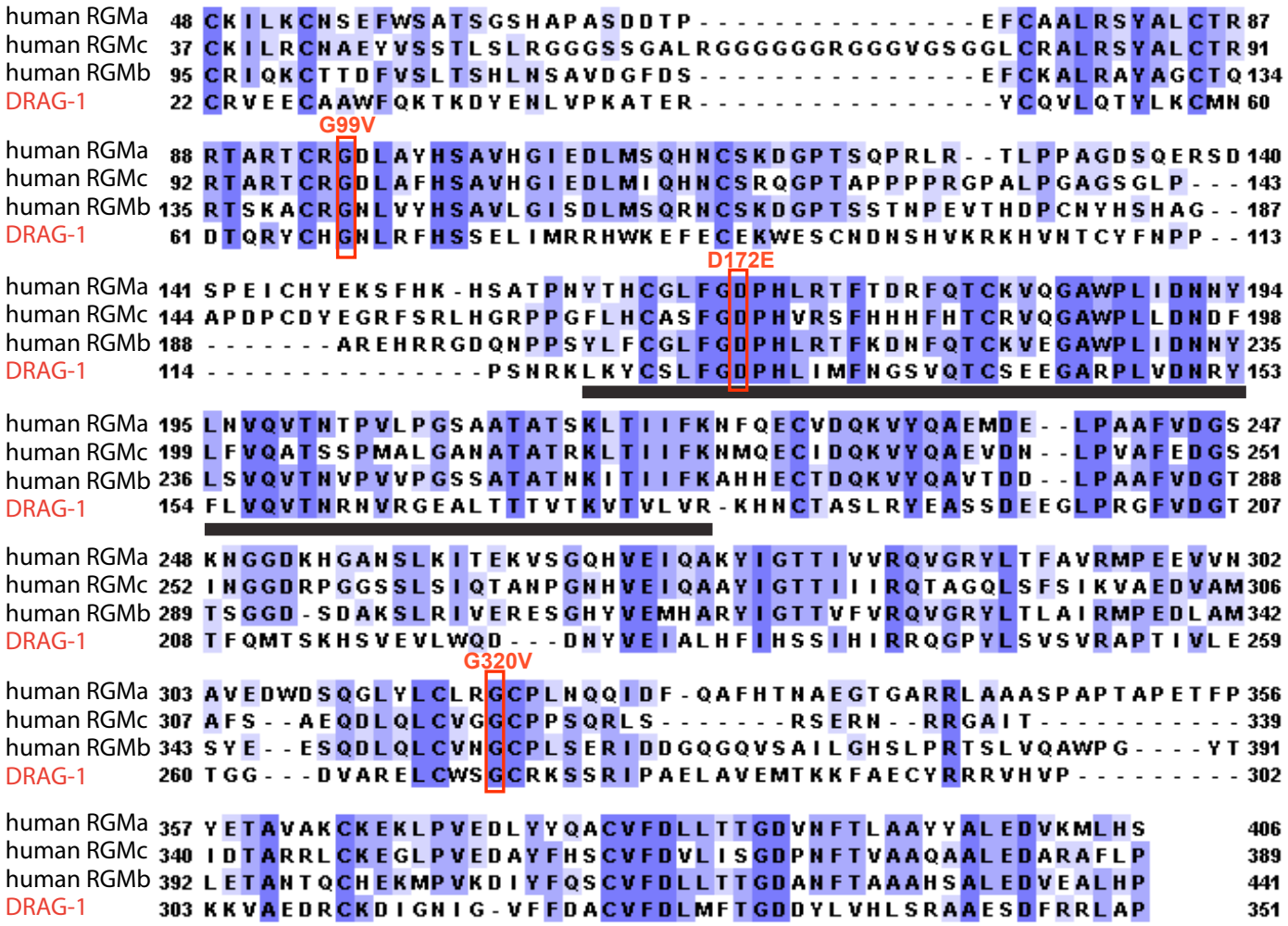

Fig. S2. DRAG-1 shares conserved features with human RGM proteins. Sequence comparison between mature human RGMa, RGMb and RGMc (HFE2) and *C. elegans* DRAG-1 proteins, highlighting conserved residues (blue). The N- and C-terminal signal sequences are not shown. The black line underlines the partial vWF type D domain. The three JH (juvenile hemochromatosis) disease-associated hypomorphic mutations discussed in this paper are boxed in red (Lanzara et al., 2004).

Table S1. Plasmid constructs

Constructs for tissue-specific expression of *unc-40*
pCXT261, unc-40p::unc-40 cDNA::gfp::unc-54 3'UTR
pCXT262, elt-3p::unc-40 cDNA::gfp::unc-54 3'UTR
pCXT264, rol-6p::unc-40 cDNA::gfp::unc-54 3'UTR
pCXT266, hlh-8p::unc-40cDNA::gfp::unc-54 3'UTR
pCXT265, unc-119p::unc-40cDNA::gfp::unc-54 3'UTR
pCXT289, myo-3p::unc-40 cDNA::gfp::unc-54 3'UTR
Mammalian cell culture expression constructs (all cloned into the pSecTag vector)
pCXT239 (DRAG-1-FLAG), *pCMV::signal peptide::DRAG-1 mature region (aa22-360)::FLAG*
pJKL962 (Myc-DRAG-1), *pCMV::signal peptide::cMyc-His::DRAG-1 mature region (aa22-360)*
pCXT271 (DRAG-1^{G272V}-FLAG), *pCMV::signal peptide::DRAG-1 mature region (aa22-360)^{G272V}::FLAG*
pCXT245 (Myc-FN5,6), *pCMV::signal peptide::cMyc-His-UNC-40 FNIII 5-6 (aa852-1081)*
pCXT249(Myc-FN5,6 C-C'loop), *pCMV::signal peptide::cMyc-His-UNC-40 FNIII 5-6 (aa852-1081) with aa987-993 mutated from DRASLAD to LDKNIPI*
pCXT299 (Myc-FN5,6 C'strand), *pCMV::signal peptide::cMyc-His-UNC-40 FNIII 5-6 (aa852-1081) with aa997-1002 mutated from TINYVA to IMETIS*
pCXT300 (Myc-FN5,6 E-Floop), *pCMV::signal peptide::cMyc-His-UNC-40 FNIII 5-6 (aa852-1081) with aa1011-1014 mutated from SNLL to MDLN*
pJKL964 (Myc-SMA-6), *pCMV::signal peptide::cMyc-His-SMA-6 full length (aa27–stop)*
pCXT230 (Myc-DAF-4), *pCMV::signal sequence::cMyc-His-DAF-4 EXD- TM-partial ICD (aa33–468)*
pCXT241 (FLAG-DBL-1), *pCMV::signal sequence::DBL-1 prodomain (aa32-238)::FLAG-DBL-1 mature region (aa239–stop)*
Constructs for making DRAG-1 and UNC-40 vertebrate and *C. elegans* hybrids
pCXT161, *drag-1p (–3977 to –1 and 4 to 1123)::drag-1 signal sequence:: mRgmb mature region::drag-1 C-signal sequence::unc-54 3'UTR*, has aa23-375 of DRAG-1 replaced by aa59-414 of mouse Rgmb in pCXT15 (Tian et al., 2010)
pCXT185, *drag-1p (–3977 to –1 and 4 to 1123)::drag-1 signal sequence:: hHJV mature region::drag-1 C-signal sequence::unc-54 3'UTR*, has aa23-375 of DRAG-1 replaced by aa38-398 of human RGMc/HJV in pCXT15
pCXT278, *unc-40p (–610 to –1)::unc-40 signal sequence::mDCC mature region::unc-54 3'UTR*, has aa36-stop of UNC-40 replaced by aa34-stop of mDCC
pCXT279, *unc-40p (–610 to –1)::unc-40 signal sequence::mNeo1 mature region::unc-54 3'UTR*, has aa36-stop of UNC-40 replaced by aa58-stop of mouse Neo1
Constructs for *in vivo* structure-function analysis of UNC-40 All constructs are derived from pZF22, a functional *unc-40::gfp* fusion described by Chan et al. (Chan et al., 1996)
pCXT253(ΔFN5), *unc-40::gfp without aa852-939*
pCXT254(ΔFN6), *unc-40::gfp without aa946-1041*
pCXT255(ΔFN5,6), *unc-40::gfp without aa852-1041*
pCXT260 (UNC-40 EXD), *unc-40 (aa1-1079)::gfp*
pCXT269(UNC-40 C-C' loop), *unc-40::gfp with aa987-993 mutated from DRASLAD to LDKNIPI*
pCXT285(UNC-40 C' strand), *unc-40::gfp with aa997-1002 mutated from TINYVA to IMETIS*
pCXT286(UNC-40 E-F loop), *unc-40::gfp with aa1011-1014 mutated from SNLL to MDLN*
Constructs for expressing wild-type and mutant *drag-1* using the MosSCI technique
pCXT208: *wildtype DRAG-1 in pCFJ151*
pCXT224: *DRAG-1G68V (GGT to GTT) in pCFJ151*
pCXT225: *DRAG-1D117E (GAT to GAA) in pCFJ151*
pCXT223: *DRAG-1G272V (GGA to GTA) in pCFJ151*