



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.

human RGMa	48	CKILKCNSEFW	SATSGSHAPASDDTP	-----	EFCAALRSYAL	CTR	87	
human RGMc	37	CKILRCNAEYV	SSLTLSLRGGSSGALRGGGGGGRGGGVGSGGL	-----	CRALRSYAL	CTR	91	
human RGMb	95	CRIQKCTTDFV	SLTSHLNSAVDGFDS	-----	EFCKALRAYAG	CTQ	134	
DRAG-1	22	CRVEECAAWFQ	KTKDYENLVPKATER	-----	YCQVLQTYLK	CMN	60	
			G99V					
human RGMa	88	RTARTCRGDLA	YHSAVHGI EDLMSQHNCSKDGPTSQPRLR	- -	TLPPAGDSQERSD		140	
human RGMc	92	RTARTCRGDLA	FHSAVHGI EDLMIQHNCSTRQGPTAPPPRGPALP		GAGSGLP	- - -	143	
human RGMb	135	RTSKACRGNLV	YHSAVLGISDLMSQRNCSKDGPTSSSTNPEVTHDP		CNYHSHAG	- -	187	
DRAG-1	61	DTQRYCHGNLR	FHSSSELIMRRHWKEFECEKWCSCNDNSHVKRKHVNTCYFNPP	- -			113	
			D172E					
human RGMa	141	SPEICHYEKSF	HK-HSATPNYTHCGLFGD	PHLRTFTDRFQ	TCKVQGAWPL	IDNNY	194	
human RGMc	144	APDPCDYEGRF	SRLHGRPPGFLHCASF	GDPHVRSFHHFH	FTCRVQGWAWPL	LDNDF	198	
human RGMb	188	- - - - -	AREHRRGDQNP	SYLFCGLFGD	PHLRTFKDNF	QCKVEGAWPL	IDNNY	235
DRAG-1	114	- - - - -	PSNRKLLKYCS	LFGD	PHLIMFNGSV	QTCSEEGARPL	VDRY	153
human RGMa	195	LVQVTNTPTVL	PGSAATATSKLTIIFKNFQEC	VQKVVYQAEMDE	- -	LPAAFVDGS	247	
human RGMc	199	LFVQATSSPMAL	GANATATRKLTIIFKNMQEC	IDQKVVYQAEVDN	- -	LPVAFEDGS	251	
human RGMb	236	LSVQVTNVPVVP	GSSATATNKITIIIFKAHHECT	DQKVVYQAVTDD	- -	LPAAFVDGT	288	
DRAG-1	154	FLVQVTNRNVR	GEALTTVTKVTVLVR	-KHNCTASLRYEASSDEEGL	PRGFVDGT		207	
human RGMa	248	KHGGDKHGANSL	KITEKVSQGHVEIQAKYIGTTI	VVRQVGRYLTFAVRMPEE	VVN		302	
human RGMc	252	INGGDRPGGSSLS	IQTANPGNHVEIQAAAYIGTTI	IIRQTAGQLSFSIKVAED	VAM		306	
human RGMb	289	TSGGD-SDAKSLR	IVERESGHYVEMHARYIGTTV	FVRQVGRYLTLAIRMPED	LAM		342	
DRAG-1	208	TFQMTSKHSVEV	LWQD--DNYVEIALHFIHSSI	HIRRQGPYLSVSVRAPTI	VLE		259	
			G320V					
human RGMa	303	AVEDWDSQGLYL	CLRGCPLNQQIDF-QAFHTNAEGT	GARRLAAASPAPTAPETFP			356	
human RGMc	307	AFS--AEQDLQL	CVGGCPPSQRLS-----RSERN--	RRGAI	T-----		339	
human RGMb	343	SYE--ESQDLQL	CVNGCPLSERIDDGQGVSA	ILGHSLPRTSLVQAWPG	- - - -	YT	391	
DRAG-1	260	TGG--DVARELC	WSGCRKSSRIPAELAVEMTKK	FAECYRRRVHVP	- - - - -		302	
human RGMa	357	YETA	VAKCKEKL	PVEDLYYQACVFDLLTTGD	VHFTLAAYY	ALEDV	KMLHS	406
human RGMc	340	IDTARRL	CKEGLP	VEDAYFHSCVFDVLISGDP	NFTVAAQA	ALEDAR	FLP	389
human RGMb	392	LETANTQ	CHEKMP	VKDIYFQSCVFDLLTTGD	ANFTAAAH	SALED	VEALHP	441
DRAG-1	303	KKVAEDR	CKDIGNIG-VFFD	ACVFDLMFTGDDYL	VHLSRAAES	DFRRL	LAP	351

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