

Supplementary Material

to

**Conservation of anatomically restricted glycosaminoglycan structures in divergent
nematode species**

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Running title: conserved glycosaminoglycan structures

3 Figures

1 Table

2 Supplementary Figures

2 Supplementary Tables

Supplementary Materials

Mutants: hse-5(tm472) III (OH1487), hst-2(ok595) X (OH1876), hst-6(ok273) X (OH1421), hst-3.1(tm734) II (EB649), hst-3.2(dz171) X.

Integrated transgenic strains:

EB1119: *dzIs12 [Punc-122::HS3A8::GFP(S65T); pRF4]*
EB669: *dzIs15 [Punc-122::EW4E9::GFP(S65T); pRF4]*
EB703: *dzIs16 [Punc-122::MPB49::GFP(S65T); pRF4] (EB703)*
EB1968: *dzEx1031 [Punc-122::EW3G6::2xsfGFP; pRF4]*
EB1981: *dzEx1042 [Punc-122::EW4A4::2xsfGFP; pRF4]*
EB1782: *dzEx876 [Punc-122::EW4A4::2xGFP; Ptx-3::mCherry; pRF4]*
EB1350: *dzEx597 [Punc-122::EW3G6::2xsfGFP; Pglr-6::mCherry; dpy-20(+)]*
EB1974: *dzEx1037 [Punc-122::EW3G6::2xsfGFP; Pgcy-13::mCherry; pRF4]*
EB2226: *dzEx1249 [Punc-122::EW4A4::2xsfGFP; Pinx-1::mCherry; pRF4]*
EB2228: *dzEx1251 [Punc-122::EW4A4::2xsfGFP; Pgcy-13::mCherry; pRF4].*

Extrachromosomal transgenes for surveying 33 different HS-specific scFv antibodies (if not mentioned elsewhere):

EB889-90: *dzEx468-9 [Punc-122::EW4E10::GFP(S65T);pRF4]*
EB1408-10: *dzEx625-7 [Punc-122::HS3B7::GFP(S65T);pRF4]*
EB1406-7: *dzEx623-4 [Punc-122::EW4A4::GFP(S65T);pRF4]*
EB1475: *dzEx671 [Punc-122::EW4B7::GFP(S65T);pRF4]*
EB1404-5: *dzEx621-2 [Punc-122::HS3G8::GFP(S65T);pRF4]*
EB888: *dzEx467 [Punc-122::EW4B10::GFP(S65T);pRF4]*
EB604-5: *dzEx363-4 [Punc-122::RB4EA12::GFP(S65T);pRF4]*
EB550-1: *dzEx326-7 [Punc-122::EV3B2::GFP(S65T);pRF4]*

EB539-42: *dzEx315-8 [Punc-122::EW3D3::GFP(S65T);pRF4]*

EB552-3: *dzEx328-9 [Punc-122::EW3E4::GFP(S65T);pRF4]*

EB554-6: *dzEx330-2 [Punc-122::EW3F5::GFP(S65T);pRF4]*

EB571: *dzEx343 [Punc-122::EW3G6::GFP(S65T);pRF4]*

EB916-8: *dzEx474-6 [Punc-122::EW3G6::2xsfGFP;pRF4]*

EB1459: *dzIs34 [Punc-122::EW3G6::2xsfGFP;pRF4]*

EB1969: *dzEx1032 [Punc-122::EW3G6::2xsfGFP;pRF4]*

EB547-9: *dzEx323-5 [Punc-122::EW4A11::GFP(S65T);pRF4]*

EB543-6: *dzEx319-22 [Punc-122::EW4E1::GFP(S65T);pRF4]*

EB532-3: *dzEx308-9 [Punc-122::EW4E9::GFP(S65T);pRF4]*

EB757: *dzIs15 [Punc-122::EW4E9::GFP(S65T);pRF4]*

EB1114: *dzEx519 [Punc-122::EW4E9::GFP(S65T);pRF4]*

EB574-6: *dzEx344-6 [Punc-122::EW4G2::GFP(S65T);pRF4]*

EB1204-8: *dzIs27-31 [Punc-122::EW4G2::GFP(S65T);pRF4]*

EB1325, EB1327: *dzIs32-3 [Punc-122::EW4G2::GFP(S65T);pRF4]*

EB568-70: *dzEx340-2 [Punc-122::HS3A8::GFP(S65T);pRF4]*

EB666-7: *dzIs12-3 [Punc-122::HS3A8::GFP(S65T);pRF4]*

EB313-9: *dzEx195-201 [Punc-122::HS4C3::GFP(S65T);pRF4]*

EB502-3: *dzIs10-1 [Punc-122::HS4C3::GFP(S65T);pRF4]*

EB536-7: *dzEx312-4 [Punc-122::HS4E4::GFP(S65T);pRF4]*

EB1104: *dzEx511 [Punc-122::HS4E4::GFP(S65T);pRF4]*

EB1105: *dzEx512 [Punc-122::LKIV69::GFP(S65T);pRF4]*

EB1202-3: *dzIs25-6 [Punc-122::LKIV69::GFP(S65T);pRF4]*

EB1333-4: *dzEx580-1 [Punc-122::EV3C3::GFP(S65T);pRF4]*

EB1399-403: *dzEx616-620 [Punc-122::EW4G1::GFP(S65T);pRF4]*

EB1110-1: *dzEx516-7 [Punc-122::HS4D10::GFP(S65T);pRF4]*

EB1472-3: *dzEx668-9 [Punc-122::EW4B5::GFP(S65T);pRF4]*
EB1474,1601-4: *dzEx670,760-3 [Punc-122::EW4C10::GFP(S65T);pRF4]*
EB749-50,891-2: *dzEx403-4,470-1 [Punc-122::EW4D5::GFP(S65T);pRF4]*
EB1476-7: *dzEx672-3 [Punc-122::EW4G10::GFP(S65T);pRF4]*
EB636-8: *dzEx368-370 [Punc-122::AO4B08::GFP(S65T);pRF4]*
EB668: *dzIs14 [Punc-122::AO4B08::GFP(S65T);pRF4]*
EB534-5,664-5: *dzEx310-1,376-7 [Punc-122::EV3D1::GFP(S65T);pRF4]*
EB1398: *dzEx615 [Punc-122::MW3G3::GFP(S65T);pRF4]*
EB1723: *dzEx834 [Punc-122::NS4F5::GFP(S65T);pRF4]*
EB1595: *dzEx759 [Punc-122::HS4D4::GFP(S65T);pRF4]*
EB1347-9,1724-5: *dzEx594-6,835-6 [Punc-122::HS4A5::GFP(S65T);pRF4]*

Transgenes for cell identification of EW3G6:

EB1106-7: *dzEx513-4 [Punc-122::ew3g6::2xgfp 25ng/ μ l; Ptx-3::mCherry 75ng/ μ l; pRF4 75ng/ μ l]*. Similar DNA concentrations were used in all following strains, except for strains with complex arrays (identifiable as containing gDNA N2).

EB1121-2: *dzEx518+dzEx520 [Punc-122::ew3g6::2xgfp; Ptx-3::mCherry; pRF4]*
EB1772-3: *dzEx866-7 [Punc-122::EW3G6::2xGFP; Pflp-8::mCherry; pRF4]*
EB1344: *dzEx591 [Punc-122::ew3g6::2xsfGFP; Ppept-3::mCherry; dpy-20(+)]*
EB1353: *dzEx599 [Punc-122::ew3g6::2xsfGFP; Ppept-3::mCherry; dpy-20(+)]*
EB1338: *dzEx585 [Punc-122::ew3g6::2xsfGFP; Pceh-16::mCherry; dpy-20(+)]*
EB1339: *dzEx586 [Punc-122::ew3g6::2xsfGFP; Pceh-16::mCherry; dpy-20(+)]*
EB1330: *otIs181; dzEx475 [Punc-122::ew3g6::2xsfGFP]*
EB1329: *otIs266; dzEx475 [Punc-122::ew3g6::2xsfGFP]*
EB1350-1: *dzEx597-8 [Punc-122::ew3g6::2xsfGFP; Pglr-6::mCherry; dpy-20(+)]*
EB1774-5: *dzEx868-9 [Punc-122::EW3G6::2xGFP; Ptdc-1::mCherry; pRF4]*

EB1768-9: dzEx862-3 [*Punc-122::EW3G6::2xGFP; Pzig-3::mCherry; pRF4*]

EB1770-1: dzEx864-5 [*Punc-122::EW3G6::2xGFP; Psra-11::mCherry; pRF4*]

EB1972-3: dzEx1035-6 [*complex array Punc-122::ew3g6::2xsfGFP; Ptbh-1::mCherry PCR fusion; pRF4; N2 gDNA*]

EB1976: dzEx1039 [*complex array Punc-122::ew3g6::2xsfGFP; Pgcy-28::mCherry PCR fusion; pRF4; N2 gDNA*]

EB1974-5: dzEx1037-8 [*complex array Punc-122::ew3g6::2xsfGFP; Pgcy-13::mCherry PCR fusion; pRF4; N2 gDNA*]

EB1970-1: dzEx1033-4 [*complex array Punc-122::ew3g6::2xsfGFP; Pinx-1::mCherry PCR fusion; pRF4; N2 gDNA*]

EB2299-2300: dzEx1285-6 [*complex array: Punc-122::ew3g6::gfp Pgcy-18::mCherry; pRF4; N2 gDNA*]

Transgenes for cell identification of EW4A4:

EB1592-4: dzIs34; dzEx756-8 [*Punc-122::ew4a4::DsRed2; Pttx-3::mCherry; pBS*]

EB1782-3: dzEx876-7 [*Punc-122::EW4A4::2xGFP; Pttx-3::mCherry; pRF4*]

EB1776-7: dzEx870-1 [*Punc-122::EW4A4::2xGFP; Pser-2.2::mCherry; pRF4*]

EB1778: dzEx872 [*Punc-122::EW4A4::2xGFP; Pflp-8::mCherry; pRF4*]

EB1779: dzEx873 [*Punc-122::EW4A4::2xGFP; Pfax-1::mCherry; pRF4*]

EB1784-5: dzEx878-9 [*Punc-122::EW4A4::2xGFP; Ptdc-1::mCherry; pRF4*]

EB1780-1: dzEx874-5 [*Punc-122::EW4A4::2xGFP; Pgcy-32::mCherry; pRF4*]

EB2228-9: dzEx1251-2 [*complex array: Punc-122::EW4A4::2xsfGFP; Pgcy-13::mCherry; pRF4; N2 gDNA*]

EB2226-7: dzEx1249-50 [*complex array: Punc-122::EW4A4::2xsfGFP; Pinx-1::mCherry; pRF4; N2 gDNA*]

Transgenes in the *C. elegans* CB4856 strain:

EB1613: *dzEx772* [*Punc-122::EW4A4::2xsfGFP*; *pRF4*]

EB1618: *dzEx777* [*Punc-122::EW3G6::2xsfGFP*; *pRF4*]

Transgenes in *C. briggsae* (AF16):

dzEx1024 [*Punc-122::HS3A8::GFP(S65T)*]

dzEx1025 [*Punc-122::EW4A4::2xsfGFP*; *pRF4*]

dzEx1026 [*Punc-122::EW3G6::2xsfGFP*; *pRF4*].

Supplementary Figures

Figure S1. Triangulation with cell specific markers was used to identify the location of the specific label in EW4A4::2xsfGFP and EW3G6::2xsfGFP transgenic animals.

- a. – c'. Epifluorescent optical sections of a medial/sagittal (a-c) and a lateral/parasagittal plane (a'-c') of animals transgenically expressing the EW4A4::2xsfGFP scFv antibody fusion and mCherry as a cytoplasmic marker in the bilateral pair of AIYL/R interneurons under control of the *Pttx-3* promoter (Altun-Gultekin, Z., Andachi, Y., et al. 2001). Images of the green (a,a') and red (b,b') channels with a merged image (c,c') are shown. Green and red arrowheads point to labeling of the respective processes. Anterior is to the left, ventral down in all images and a scale bar indicates 10 μm in all images (a-f').
- b. – f'. Epifluorescent optical sections of a medial/sagittal (d-f) and a lateral/parasagittal plane (d'-f') of animals transgenically expressing the EW3G6::2xsfGFP scFv antibody fusion and mCherry as a cytoplasmic marker in the bilateral pair of RIAL/R interneurons under control of the *Pglr-6* promoter (Brockie, P.J., Madsen, D.M., et al. 2001). Images of the green (d,d') and red (e,e') channels with a merged image (f,f') are shown. Green and red arrowheads point to labeling of the respective processes.
- g. Schematic of the dorsal portion of the *C. elegans* nerve cords from transverse sections with the names of processes indicated. Orange lines exemplify the triangulation strategy using AIY and RIA neurons (indicated in magenta) pointing to the area in the vicinity of AIB and RIM neurons (indicated in green). Modified after Fig.20c in (White, J.G., Southgate, E., et al. 1986).

Figure S2. EW3G6::2xsfGFP and EW4A4::2xsfGFP bind distinct epitopes in proximity to the synaptic connection between AIB and RIM interneurons in the *C. elegans* nerve ring.

- a. – b. Schematics of RIM interneurons (left panel) and AIB motor/interneurons (right panel) with synaptic output and input between both, respectively, indicated by blue arrows. Modified after www.wormatlas.org.
- c. – e'. Epifluorescent optical sections of a medial/sagittal (c,d,e) and a lateral/parasagittal plane (c',d',e') of animals transgenically expressing the EW3G6::2xsfGFP and mCherry as a cytoplasmic marker in RIML/R under control of the *Pgcy-13* promoter (Ortiz, C.O., Etchberger, J.F., et al. 2006). Similar results were obtained with the *Ptdc-1* promoter, which is also expressed in RIM (Alkema, M.J., Hunter-Ensor, M., et al. 2005). Images of the green (c,c') and red (d,d') channels with a merged image (e,e') are shown. Green and red arrowheads point to labeling of the respective processes. Anterior is to the left, ventral down and a scale bar indicates 10 μ m in all panels (c-h'). The reciprocal experiment between EW4A4 and AIBL/R is shown in Fig.2.
- f. – h'. Epifluorescent optical sections of a medial/sagittal (f,g,h) and a lateral/parasagittal plane (f',g',h') of animals transgenically expressing the EW4A4::2xsfGFP and mCherry as a cytoplasmic marker in the bilateral pair AIBL/R under control of the *Pinx-1* promoter (Altun, Z.F., Chen, B., et al. 2009). Images of the green (f,f') and red (g,g') channels with a merged image (h,h') are shown. Green and red arrowheads point to labeling of the respective processes and indicate the more anterior descending (a), and posterior ascending (p) neurites of AIB, respectively. The reciprocal experiment between EW3G6 and RIML/R is shown in Fig.2.

- i. Schematic representations of the synaptic connectivity of AIBL and RIMR, modified from (White, J.G., Southgate, E., et al. 1986). Synaptic output to and input from, are indicated by blue arrows for AIBL and RIMR, respectively.

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Table S1. HS scFv properties and binding characteristics *in vitro* and *in vivo*

				HS modifications required for binding ^a											anatomical binding in <i>C. elegans</i> ^b								
Name of scFv	V _H ^c	CDR3	isolat. against ^d	<i>in vitro</i>						In vivo					nervous system				alimentary system		refs. ^e		
				NAC	NS	C5	2S	6S	3S	NAC	NS	C5	2S	6S	3S	nr	vnc	dnc	comments	pm, pi valve		gut	
EV3B2 (EW4D2)	DP-38	GKMKLNLR	hiHS/iHep	+	+	+	+																(Dennissen, M.A., Jenniskens, G.J., et al. 2002, van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
EW3D3 (EW3D10)	DP-38	GRTVGRN	iHep	+				+															(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
EW4E1	DP-38	GRRHKLIR	iHep																				(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
EW4B5	DP-45	GRLHLPRK	iHep																				(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
EW4D5	DP-45	PVSHRKWRVTV	iHep																				(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
HS4C3	DP-38	GRRLKD	bkHS	+		(+)	+	+				(+)	+	+									(+) (Attreed, M., Desbois, M., et al. 2012, Smits, N.C., Lensen, J.F., et al. 2006, Wijnhoven, T.J., van de Westerlo, E.M., et al. 2008)
HS3A8 (EW3H12)	DP-38	GMRPRL	bkHS	+	+	+	+					+	+										(+) (Attreed, M., Desbois, M., et al. 2012, Dennissen, M.A., Jenniskens, G.J., et al. 2002, van de Westerlo, E.M., Smetsers, T.F., et al. 2002, Wijnhoven, T.J., van de Westerlo, E.M., et al. 2008)
EW4G2	DP-38	GKVLPN	iHep	+				+															(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
EW3F5	DP-38	SGRQARQGRFPK	iHep																				(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
HS4E4	DP-38	HAPLRNTRTNT	bkHS	+	+	+	+	in.															(Dennissen, M.A., Jenniskens, G.J., et al. 2002, Wijnhoven, T.J., van de Westerlo, E.M., et al. 2008)
EW4C10	DP-45	ARMTGHVRNVM I	iHep																				(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
EW4G1	DP-42	GARLKR	iHep																				(+) (van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
AO4B08 ^f	DP-47	SLRMNGWRAHQ	mskHS	+	+	+	+					+	+										(+) (Attreed, M., Desbois, M., et al. 2012, Dennissen, M.A., Jenniskens, G.J., et al. 2002, Wijnhoven, T.J., van de Westerlo, E.M., et al. 2008)
RB4EA12	DP-32	RRYALDY	hsmHS	+	+	+	in.	+															(Dennissen, M.A., Jenniskens, G.J., et al. 2002, Wijnhoven, T.J., van de Westerlo, E.M., et al. 2008)
LKIV69	DP-38	GSRSSR	bkHS	+	+	+																	(Wijnhoven, T.J., van de Westerlo, E.M., et al. 2008)
HS4D4	DP-58	GMRPRL	bkHS	+	+	+	+	(+)															(Dennissen, M.A., Jenniskens, G.J., et al. 2002)
EW4A4	DP-08	GTKLKMTK	iHep	-								+	+	+ ^g									(van de Westerlo, E.M., Smetsers, T.F., et al. 2002), this study.
EW3G6	DP-38	GGTRIRK	iHep	(+)								+	in.	+/- ^h									(van de Westerlo, E.M., Smetsers, T.F., et al. 2002), this study.
EW4E9	DP-38	LRGTKMFRH	iHep																				(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
EV3C3	DP-42	GYRPRF	hiHS																				(Dennissen, M.A., Jenniskens, G.J., et al. 2002)
EV3D1	DP-47	SISMNGVGVR I Q	hiHS	+	+		(+)	(+)															(Dennissen, M.A., Jenniskens, G.J., et al. 2002)
EW4E10	DP-03	SRKTPKPFMRK	iHep																				(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
HS3B7	DP-03	SRKTRKPFMRK	bkHS	+	+	+	in.	+															(Dennissen, M.A., Jenniskens, G.J., et al. 2002)
EW4B7	DP-08	SSSRHRLHR	iHep																				(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
HS3G8	DP-08	YYHYKVN	bkHS ⁱ																				(van Kuppevelt, T.H., Dennissen, M.A., et al. 1998)
EW4B10	DP-25	QRWKPAVTPKLV	iHep																				(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)

EW3E4	DP-38	DRRNTQKTRYRT	iHep				weak/absent	(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
EW4A11	DP-38	ERNTIIR	iHep				weak/absent	(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
HS4D10	DP-42	SLRMNGCGAHQ	bkHS				weak/absent	(van Kuppevelt, T.H., Dennissen, M.A., et al. 1998)
EW4G10	DP-45	GTKKLGK	iHep				weak/absent	(van de Westerlo, E.M., Smetsers, T.F., et al. 2002)
MW3G3	DP-47	QKKRPRF	AS	+	+	+	weak/absent	(ten Dam, G.B., van de Westerlo, E.M., et al. 2004)
NS4F5	DP-53	SGRKGRMR	hlHS	+	+	+	weak/absent	(Smits, N.C., Kurup, S., et al. 2010)
HS4A5	DP-65	WVTEP	bkHS		+	+	weak/absent	(Dennissen, M.A., Jenniskens, G.J., et al. 2002)
MPB49	DB-38	WRNDRQ	na				neg. contr.	(van Kuppevelt, T.H., Dennissen, M.A., et al. 1998)

^a HS modifications required for binding of the scFvs based on competitive binding assays (*in vitro*) or as determined genetically (*in vivo*). +, required; (+) weakly required; -, not required, in., inhibitory. ^b Abbreviations used: nr, nerve ring; vnc, ventral nerve cord; dnc, dorsal nerve cord; pi, pharyngeal intestinal; pm, pharyngeal muscle; +, expressed; (+), weakly expressed. ^c V_H: variable heavy chain type, with the sequence of the complementary determining region 3 indicated in the adjacent column CDR3. ^d Target material to isolate scFvs in panning experiments is indicated: iHep, immobilized heparin; bkHS, bovine kidney HS; hsmHS, human skeletal muscle HS; hlHS, human lung HS; mskHS, mouse skeletal muscle HS; AS, acharan sulfate. ^e References are indicated that initially describe isolation, biochemical characterization, or in the case of HS4C3, HS3A8, and AO4B08 prior genetic characterization in *C. elegans*. ^f Note that AO4B08 shows some additional staining in the M-lines of the muscle and the dense bodies. ^g Both type I and II HS 3-O-sulfotransferases are required for binding *in vivo*. ^h HS 3-O-sulfation introduced by type I HS 3-O-sulfotransferases acts to inhibit whereas HS 3-O-sulfation introduced by type II HS 3-O-sulfotransferases is required for binding in *C. elegans* (this study). ⁱ HS3G8 was counterselected against chondroitin and dermatan sulfate.

Table SII. Cell specific reporters used for triangulation experiments.

Gene ^a	neurons labeled ^b	tested with		5' primer used for PCR fusion	3' fusion used for PCR fusion	size	ref ^d
		EW3G6 ^c	EW4A4 ^c				
<i>ttx-3*</i>	AIY	yes (4)	yes (5)	na	na		(Altun-Gultekin, Z., Andachi, Y., et al. 2001)
<i>ser-2.2*</i>	AIY AIZ RID DVA BDU SIAD SIAV RME PVT	No	yes (2)	na	na		(Tsalik, E.L., Niacaris, T., et al. 2003)
<i>flp-8</i>	ADA CP9 URX RMG AUA PVM	yes (2)	yes (1)	TCTTCGTGATGGAGTTCGC	ATCCTCCTCGCCCTTGCTCACCATT TTCTACTTGAAAAGTGTGG	3.0kb	(Kim, K. and Li, C. 2004)
<i>opt-3 (pept-3)</i>	AVE	yes (2)	no	CGATAAAAAATAACAGAATTAGT AAGAAGGTGGG	ATCCTCCTCGCCCTTGCTCACCATG GTTAATTGAGATTCTTTTGC	2.8kb	(Fei, Y.J., Romero, M.F., et al. 2000)
<i>ceh-16</i>	GLRD	yes (2)	no	AAGGCGCATAAAATGTATTTCG	ATCCTCCTCGCCCTTGCTCACCATA ATACCGAATTTTCAGAATCAT	3.1kb	na
<i>dat-1 (otIs181)**</i>	PDE CEPV CEPD ADE	yes (1)	no	na	na		(Bertrand, V. and Hobert, O. 2009)
<i>che-1 (otIs232)**</i>	ASE ASH AUA	yes (1)	no	na	na		(Didiano, D., Cochella, L., et al. 2010)
<i>fax-1*</i>	AVK AVE SABD AVB CEPD SIBD RIC SMBV SABV AVA AIY URX PVP DVA	no	yes (1)	na	na		(Much, J.W., Slade, D.J., et al. 2000)
<i>glr-6</i>	RIA	yes (2)	no	GGCCCGAGTCACAAATACCGC	ATCCTCCTCGCCCTTGCTCACCATT TCAAATGCATTTGTTTCAGC	4.0kb	(Brockie, P.J., Madsen, D.M., et al. 2001)
<i>tdc-1</i>	RIC RIM	yes (2)	yes (2)	AAGTAGTTGGTGTACATAGTTT CGG	ATCCTCCTCGCCCTTGCTCACCATT TGGGCGGTCTGAAAAATGCACCG	3.2kb	(Alkema, M.J., Hunter-Ensor, M., et al. 2005)
<i>zig-3</i>	PVT AIM ASI	yes (2)	no	GCTCGCCAAGAAACATTCCTTT ACCC	ATCCTCCTCGCCCTTGCTCACCATT TTTCCAAATGCAAAATATAG	4.5kb	(Aurelio, O., Hall, D.H., et al. 2002)
<i>sra-11</i>	AIA AIY AVB RIG RIF	yes (2)	no	ACTAAACTAATTAACAAGTCAC GG	ATCCTCCTCGCCCTTGCTCACCATG GTTAGCTGAAATATAAGGG	4.2kb	(Altun-Gultekin, Z., Andachi, Y., et al. 2001)
<i>gcy-32</i>	URX AQR PQR	no	yes (2)	GTTTTCCAAAGGCGAAACGTGG GG	ATCCTCCTCGCCCTTGCTCACCATT CTATAATAACAATCGTGATCTTCGC	4.1kb	(Yu, S., Avery, L., et al. 1997)
<i>tbh-1</i>	RIC	yes (2)	no	GCCGCGTTGGCCGAATTTT	ATCCTCCTCGCCCTTGCTCACCATA GCAACGGCACTTCTCAT	5.0kb	(Alkema, M.J., Hunter-Ensor, M., et al. 2005)
<i>gcy-28</i>	AIA	yes (1)	no	TACAATTGTAGTGAGCTTCG	ATCCTCCTCGCCCTTGCTCACCATT TCGCACTCATCTCACCATTCC	2.8kb	(Shinkai, Y., Yamamoto, Y., et al. 2011)
<i>gcy-13</i>	RIM	yes (2)	yes (2)	CTCAAAATCCATTGTGTAAGTT C	ATCCTCCTCGCCCTTGCTCACCATC CATCCTACAGAGGAGGC	2.3kb	(Ortiz, C.O., Etchberger, J.F., et al. 2006)
<i>inx-1</i>	AIB AIY	yes (2)	yes (2)	CAGATTCATAACTGTCTATG	ATCCTCCTCGCCCTTGCTCACCATC TTGTCCACAAAATCGTCGTC	0.9kb	(Altun, Z.F., Chen, B., et al. 2009)
<i>gcy-18</i>	AFD	yes (2)	no	GGTCGAGAGCAGCAAATC	ATCCTCCTCGCCCTTGCTCACCATT TTCTGATGCTCCGAC	0.8kb	(Inada, H., Ito, H., et al. 2006)

^a Promoters of genes used to drive cytoplasmic mCherry for triangulation experiments with EW3G6-2xsfGFP and EW4A4-2xsfGFP in the nerve ring using existing constructs (*) or integrated reporter lines (**). All other experiments were conducted using promoter fusions obtained by a PCR fusion technique (Hobert, O. 2002) with the primers indicating the extent of the 5'UTR. ^b Labeled neurons as reported on www.wormbase.org. ^c Number of transgenic lines with comparable results indicated in parentheses. ^d References are indicated that either describe the constructs or were used as a basis to design primers. na: not applicable.

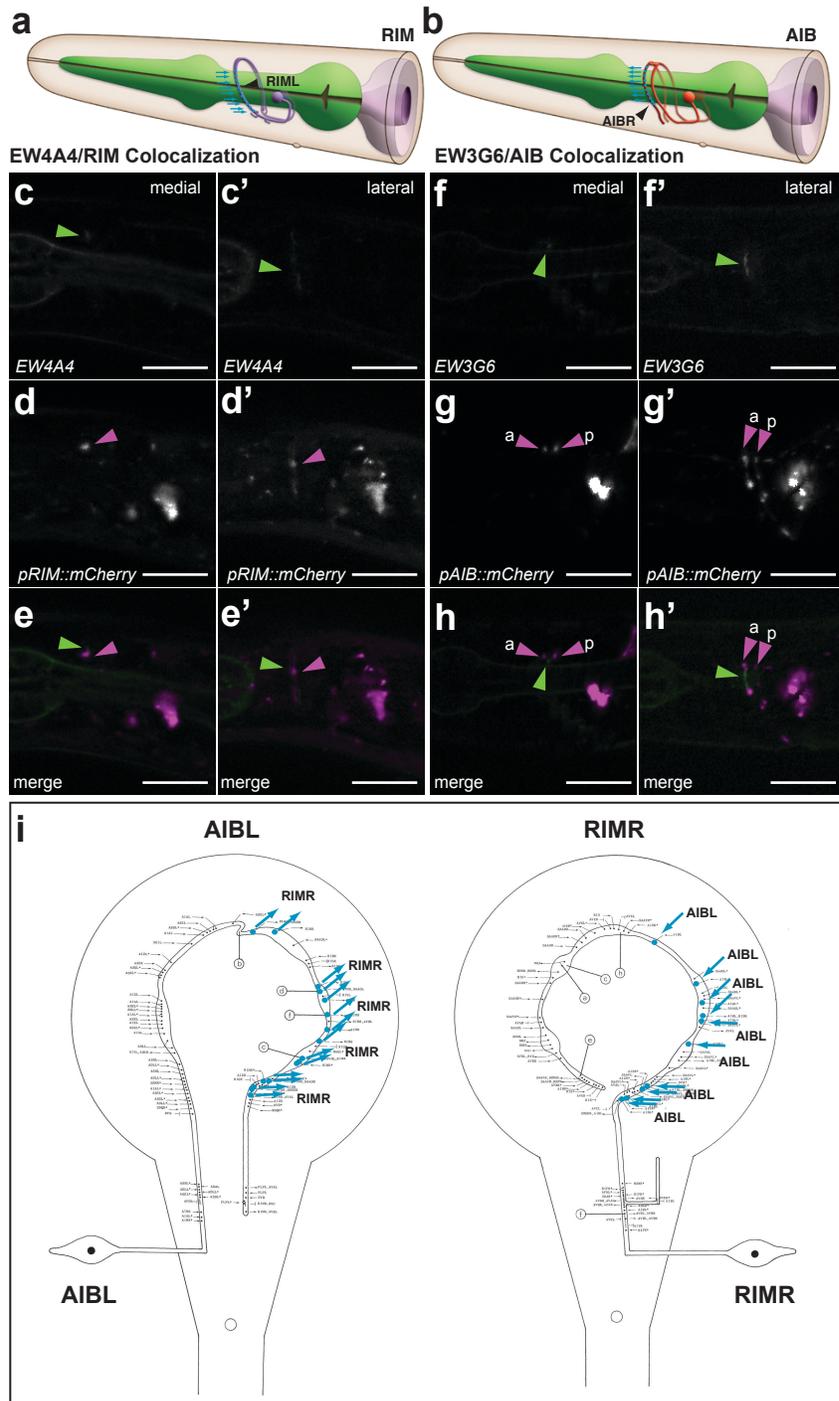


figure S2