

Supplementary table 1. Conservation of *C. elegans* genes with human autism-associated genes

<i>C. elegans</i> gene	Human gene	SFARI rank	EAGLE score	~identity (%)
<i>nrx-1</i>	<i>NRXN1</i>	1	143.75	27
	<i>NRXN2</i>	1	7	24
	<i>NRXN3</i>	1	11.1	24
<i>nlg-1</i>	<i>NLGN3</i>	1	6.5	29
	<i>NLGN4X</i>	1	12	29
	<i>NLGN2</i>	1	3	27
	<i>NLGN4Y</i>	2	/	28
<i>glr-1</i>	<i>NLGN1</i>	2	/	28
	<i>GRIA2</i>	1	12	37
	<i>GRIA3</i>	S	/	37
<i>glr-2</i>	<i>GRIA1</i>	2	/	37
	<i>GRIA2</i>	1	12	37
	<i>GRIA3</i>	S	/	35
<i>avr-15</i>	<i>GRIA1</i>	2	/	37
	<i>GLRA2</i>	2	/	39
	<i>GABRA3</i>	S	/	32
	<i>GABRB2</i>	1	0.3	31
	<i>GABRB3</i>	1	/	32
	<i>GABRA4</i>	2	/	30

Note: Conservation identified from homology searches on flybase, ortholist2, and previous work (refs). SFARI rank and EAGLE score from SFARI gene. % identity from DIOPT comparison of amino acid sequences.

Supplementary Table 2. *C. elegans* strains by Figure

Figure	Figure reference	Strain identifier	Genotype	Source	injection concentration*
1	<i>npr-1</i>	MPH39	<i>him-8(e1489) IV; npr-1(ad609) X; otIs525[lim-6int4::gfp]</i>	Hart Lab	
	<i>npr-1</i>	DA609	<i>npr-1(ad609) X</i>	CGC	
	<i>nrx-1(null); npr-1</i>	MPH40	<i>unc-119(ed3) III; him-8(e1489) IV; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; otIs525[lim-6int4::gfp]</i>	Hart Lab	
	<i>nrx-1(null); npr-1</i>	MPH49	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X</i>	this study	
	<i>nrx-1(α mut); npr-1</i>	MPH50	<i>him-8(e1489) IV; nrx-1(gk246237) V; npr-1(ad609) X; otIs525[lim-6int4::gfp]</i>	this study	
	<i>nrx-1(α del); npr-1</i>	MPH51	<i>nrx-1(nu485) V; npr-1(ad609) X</i>	this study	
	solitary controls	OH15098	<i>him-8(e1489) IV; otIs525[lim-6int4::gfp]</i>	Hart & Hobert 2018	

	<i>nrx-1(null)</i>	TV13570	<i>unc-119(ed3) III, nrx-1(wy778[unc-119(+)] V</i>	CGC	
	<i>nrx-1(α mut)</i>	OH15116	<i>him-8(e1489) IV; nrx-1(gk246237) V; otIs525[lim-6int4::gfp]</i>	Hart & Hobert 2018	
	<i>nrx-1(α del)</i>	TV22997	<i>nrx-1(nu485) V</i>	Tong et al. 2017	
2	<i>npr-1; nrx-1(null); ric-19p::nrx-1(γ)</i>	MPH52	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; hpmEx3[ric-19p::sfGFP::nrx-1(γ); ttx-3::mCherry]</i>	this study	
	<i>npr-1; nrx-1(null); ric-19p::nrx-1(α)</i>	IV870	<i>unc-119(ed3) III, him-8(e1489) IV; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; otIs525[lim-6int4::gfp]; ueEx601[ric-19p::sfGFP::nrx-1(α); unc-122p::dsRed]</i>	this study	
	<i>npr-1; nrx-1(null); flp-21p::nrx-1(α)</i>	IV874	<i>unc-119(ed3) III, him-8(e1489) IV; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; otIs525[lim-6int4::gfp]; ueEx605[flp-21p::sfGFP::nrx-1(α); unc-122::dsRed]</i>	this study	
	<i>npr-1; nrx-1(null); nhr-79p::nrx-1(α)</i>	IV878	<i>unc-119(ed3) III, him-8(e1489) IV; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; otIs525[lim-6int4::gfp]; ueEx609[nhr-79p::sfGFP::nrx-1(α); unc-122::dsRed]</i>	this study	
	<i>npr-1; nrx-1(null); ric-19p::nrx-1(α)</i>	MPH53	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; ueEx611[ric-19p::sfGFP::nrx-1(α); unc-122p::dsRed]</i>	this study	
	<i>npr-1; nrx-1(null); nhr-79p::nrx-1(α)</i>	MPH54	<i>unc-119(ed3) III, nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; ueEx609[nhr-79p::sfGFP::nrx-1(α); unc-122::dsRed]</i>	this study	
	<i>npr-1; nrx-1(null); srv-3p::nrx-1(α)</i>	MPH55	<i>unc-119(ed3) III, nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; hpmEx9[srv-3p::sfGFP::nrx-1(α); lin-44::gfp]</i>	this study	
	<i>npr-1; nrx-1(null); srv-3p::nrx-1(α); sra-6p::nrx-1(α)</i>	MPH56	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; hpmEx9[srv-3p::sfGFP::nrx-1(α); lin-44::gfp]</i>	this study	45 ng/ μ l
	<i>npr-1; nrx-1(null); sra-</i>	MPH57	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; him-8(e1489) IV;</i>	this study	45 ng/ μ l

	<i>6p::nrx-1(α)</i>		<i>otIs525[lim-6int4::gfp]; hpmEx10[sra-6p::sfGFP::nrx-1(α); unc-122::dsRed]</i>		
3	<i>npr-1; nlg-1</i>	MPH43	<i>him-8(e1489) IV; npr-1(ad609) X; nlg-1(ok259) X; otIs525[lim-6int4::gfp]</i>	Hart Lab	
	solitary controls	N2	Bristol lab control strain	CGC	
	<i>nlg-1</i>	VC228	<i>nlg-1(ok259) X</i>	CGC	
	<i>npr-1; nlg-1; ric-19p::nlg-1</i>	IV930	<i>npr-1(ad609) X; nlg-1(ok259) X; ueEx645[ric-19p::sfGFP::nlg-1; lin-44::gfp]</i>	this study	
	<i>npr-1; nlg-1; nhr-79p::nlg-1</i>	MPH58	<i>him-8(e1489)IV; npr-1(ad609) X; nlg-1(ok259) X; otIs525[lim-6int4::gfp]; hpmEx11[nhr-79p::sfGFP::nlg-1; lin-44::gfp]</i>	this study	
	<i>npr-1; nlg-1; nlp-56p::nlg-1</i>	MPH59	<i>him-8(e1489) IV; npr-1(ad609) X; nlg-1(ok259) X; otIs525[lim-6int4::gfp]; hpmEx12[nlp-56p::sfGFP::nlg-1; ttx-3::mCherry]</i>	this study	40 ng/μl
	<i>npr-1; nrx-1; nlg-1</i>	MPH44	<i>unc-119(ed3) III; him-8(e1489) IV; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) nlg-1(ok259) X; otIs525[lim-6int4::gfp]</i>	this study	
4	<i>npr-1; eat-4</i>	MPH60	<i>eat-4(ky5) III; npr-1(ad609) X; otIs525[lim-6int4::gfp]</i>	this study	
	<i>npr-1; eat-4; nhr-79p::eat-4</i>	MPH61	<i>eat-4(ky5) III; npr-1(ad609) X; otIs525[lim-6int4::gfp]; hpmEx13[nhr-79p::eat-4::SL2::gfp; ttx-3::mCherry]</i>	this study	
	<i>npr-1; eat-4; srv-3p::eat-4</i>	MPH62	<i>eat-4(ky5) III; npr-1(ad609) X; otIs525[lim-6int4::gfp]; hpmEx14[srv-3p::eat-4::SL2::gfp; ttx-3::mCherry]</i>	this study	
	<i>npr-1; eat-4; sra-6p::eat-4</i>	MPH63	<i>eat-4(ky5) III; npr-1(ad609) X; otIs525[lim-6int4::gfp]; hpmEx15[sra-6p::eat-4::SL2::gfp; ttx-3::mCherry]</i>	this study	
	<i>npr-1; nrx-1; eat-4</i>	MPH64	<i>eat-4(ky5) III; unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; otIs525[lim-6int4::gfp]</i>	this study	
	<i>npr-1; nrx-1; eat-4; nhr-79p::eat-4</i>	MPH65	<i>eat-4(ky5) III; unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; otIs525[lim-6int4::gfp]; hpmEx13[nhr-79p::eat-4::SL2::gfp; ttx-3::mCherry]</i>	this study	
	<i>npr-1; nrx-1; eat-4; nhr-79p::nrx-1(α)</i>	MPH66	<i>eat-4(ky5) III; unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; ueEx609[nhr-79p::sfGFP::nrx-1(α); unc-122::dsRed]</i>	this study	
	<i>eat-4</i>	MT6308	<i>eat-4(ky5) III</i>	CGC	

	<i>npr-1; nlg-1; eat-4</i>	MPH67	<i>eat-4(ky5) III; npr-1(ad609) nlg-1(ok259) X; otIs525[lim-6int4::gfp]</i>	this study	
	<i>npr-1; glr-1</i>	MPH68	<i>glr-1(n2461) III; npr-1(ad609) X</i>	this study	
	<i>npr-1; glr-2</i>	MPH69	<i>glr-2(ok2342) III; npr-1(ad609) X</i>	this study	
	<i>npr-1; avr-15</i>	MPH70	<i>avr-15(ad1051) V; npr-1(ad609) X</i>	this study	
	<i>npr-1; nrx-1; glr-1</i>	MPH71	<i>glr-1(n2461) unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; otIs525[lim-6int4::gfp]</i>	this study	
	<i>npr-1; nrx-1; glr-2</i>	MPH72	<i>glr-2(ok2342) unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; otIs525[lim-6int4::gfp]</i>	this study	
	<i>npr-1; nrx-1; avr-15</i>	MPH73	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] avr-15(ad1051) V; npr-1(ad609) X; otIs525[lim-6int4::gfp]</i>	this study	
5	solitary controls	CX16921	<i>kyls673[sra-6:eat-4::pHluorin; unc-122:dsRed]</i>	Bargmann lab	
	<i>nrx-1(null)</i>	MPH74	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; kyls673[sra-6:eat-4::pHluorin; unc-122:dsRed]</i>	this study	
	<i>npr-1</i>	MPH75	<i>npr-1(ad609) X; kyls673[sra-6:eat-4::pHluorin; unc-122:dsRed]</i>	this study	
	<i>npr-1; nrx-1(null)</i>	MPH76	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; kyls673[sra-6:eat-4::pHluorin; unc-122:dsRed]</i>	this study	
6	solitary controls	MPH77	<i>hpmEx16[svr-3p::gfp::cla-1; lin-44::gfp]</i>	this study	
	<i>nrx-1(null)</i>	MPH78	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; hpmEx16[svr-3p::gfp::cla-1; lin-44::gfp]</i>	this study	
	<i>npr-1</i>	MPH79	<i>npr-1(ad609) X; hpmEx16[svr-3p::gfp::cla-1; lin-44::gfp]</i>	this study	
	<i>npr-1; nrx-1(null)</i>	MPH80	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; hpmEx16[svr-3p::gfp::cla-1; lin-44::gfp]</i>	this study	
	solitary controls	MPH81	<i>hpmEx17[sra-6:gfp::cla-1; lin-44::gfp]</i>	this study	
	<i>nrx-1(null)</i>	MPH82	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; hpmEx17[sra-6:gfp::cla-1; lin-44::gfp]</i>	this study	
	<i>npr-1</i>	MPH83	<i>npr-1(ad609) X; hpmEx17[sra-6:gfp::cla-1; lin-44::gfp]</i>	this study	
	<i>npr-1; nrx-1(null)</i>	MPH84	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-</i>	this study	

			<i>1(ad609) X; hpmEx17[sra-6:gfp::cla-1; lin-44::gfp]</i>		
	<i>npr-1; nrx-1(α mut)</i>	MPH85	<i>nrx-1(gk246237) V; npr-1(ad609) X; hpmEx17[sra-6:gfp::cla-1; lin-44::gfp]</i>	this study	
7	<i>flp-21p::pkc-1(gf)</i>	CX10252	<i>kyEx2385[flp-21p::pkc-1(gf)::sl2::gfp; ofm-1::dsred]</i>	Bargmann lab	
	<i>nrx-1(null); flp-21p::pkc-1(gf)</i>	MPH98	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; kyEx2385[flp-21p::pkc-1(gf)::sl2::gfp; ofm-1::dsred]</i>	this study	
Supp 1	<i>qglR1</i>	QG1	<i>qglR1 (X, CB4856>N2, npr-1) X</i>	this study	
	<i>qglR1; nrx-1(null)</i>	MPH86	<i>unc-119(ed3) III, him-8(e1489) IV; nrx-1(wy778[unc-119(+)] V; qglR1 (X, CB4856>N2, npr-1) X; otIs525[lim-6int4::gfp]</i>	this study	
	<i>npr-1; nrx-1(null); osm-6p::nrx-1(α)</i>	MPH87	<i>unc-119(ed3) III, him-8(e1489) IV; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; otIs525[lim-6int4::gfp]; ueEx603[osm-6p::sfGFP::nrx-1(α); unc-122::dsred]</i>	this study	
	<i>npr-1; nrx-1(null); sre-1p::nrx-1(α)</i>	MPH88	<i>unc-119(ed3) III, him-8(e1489) IV; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; otIs525[lim-6int4::gfp]; hpmEx18[sre-1p::sfGFP::nrx-1(α); unc-122::dsRed]</i>	this study	
	<i>npr-1; nrx-1(null); gcy-36p::nrx-1(α)</i>	MPH89	<i>unc-119(ed3) III, him-8(e1489) IV; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; hpmEx19[gcy-36p::sfGFP::nrx-1(α); unc-122::dsRed]</i>	this study	
	<i>npr-1; nrx-1(null); flp-8::nrx-1(α)</i>	MPH90	<i>unc-119(ed3) III, him-8(e1489) IV; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; hpmEx20[flp-8p::sfGFP::nrx-1(α); unc-122::dsRed]</i>	this study	
	<i>npr-1; nrx-1(null); nlp-56p::nrx-1(α)</i>	MPH91	<i>unc-119(ed3) III, him-8(e1489) IV; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; otIs525[lim-6int4::gfp]; hpmEx21[nlp-56p::sfGFP::nrx-1(α); ttx-3::mCherry]</i>	this study	40 ng/μl
	<i>npr-1; nrx-1(null); osm-6p::nrx-1(α)</i>	MPH20	<i>unc-119(ed3) III; nrx-1(wy778[unc-119(+)] V; ueEx603[osm-6p::sfGFP::nrx-1(α); unc-122::dsred]</i>	this study	
	<i>npr-1; nrx-1(null); srv-</i>	MPH97	<i>unc-119(ed3) III, him-8(e1489) IV; nrx-1(wy778[unc-119(+)] V; npr-1(ad609) X; otIs525[lim-</i>	this study	

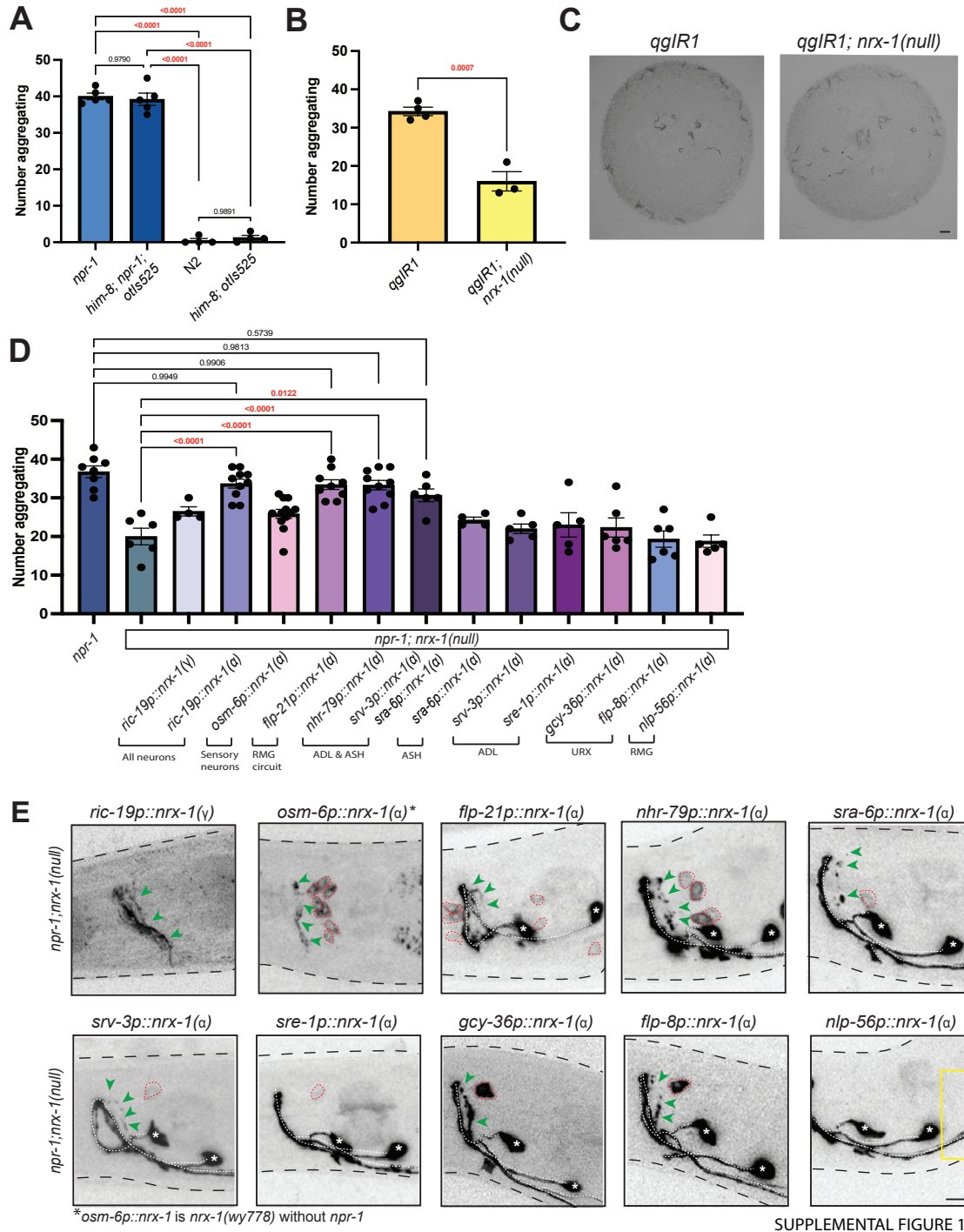
	<i>3p::nrx-1(α)</i>		<i>6int4::gfp; hpmEx24[srv-3p::sfGFP::nrx-1(α); lin-44::gfp]</i>		
Supp 2	<i>npr-1; nlg-1; sra-6p::nlg-1</i>	MPH93	<i>him-8(e1489) IV; npr-1(ad609) nlg-1(ok259) X; otIs525[lim-6int4::gfp]; hpmEx22[sra-6p::sfGFP::nlg-1; lin-44::gfp]</i>	this study	40 ng/μl
	<i>npr-1; nlg-1; srv-3p::nlg-1</i>	MPH94	<i>him-8(e1489) IV; npr-1(ad609) nlg-1(ok259) X; otIs525[lim-6int4::gfp]; hpmEx23[srv-3p::sfGFP::nlg-1; lin-44::gfp]</i>	this study	40 ng/μl
	<i>npr-1; nlg-1; ins-1p::nlg-1</i>	MPH92	<i>npr-1(ad609) nlg-1(ok259) X; ueEx651[ins-1p::sfGFP::nlg-1; lin-44::gfp]</i>	this study	
	<i>nlg-1; sra-6p::nlg-1</i>	IV937	<i>nlg-1(ok259) X; ueEx651[ins-1p::sfGFP::nlg-1; lin-44::gfp]</i>	this study	
Supp 3	<i>npr-1; nmr-2</i>	MPH95	<i>nmr-2(ok3324) V; npr-1(ad609) X</i>	this study	
	<i>npr-1; mgl-1</i>	MPH96	<i>mgl-1(tm1811) X; npr-1(ad609) X</i>	this study	

* 20 ng/μl if not noted

Supplementary Table 3. Plasmids and promoters

Identifier	Construct	promoter forward primer	promoter reverse primer	promoter size	from
pMPH34	<i>ric-19p::sfGF P::nrx-1(α)</i>	CATTAAAGAGTGTGCTC CACGAGCC	GTTCAAAGTGAAGAGCT CTCTCGAC	147	Hart Lab
pMPH35	<i>ric-19p::sfGF P::nrx-1(γ)</i>	CATTAAAGAGTGTGCTC CACGAGCC	GTTCAAAGTGAAGAGCT CTCTCGAC	147	Hart Lab
pMPH38	<i>osm-6p::sfGFP ::nrx-1(α)</i>	TCCATACGGCATCTGTT GCATTC	TGAAGGTAATAGCTTGA AAGAGA	2082	Hart Lab
pMPH41	<i>flp-21p::sfGF P::nrx-1(α)</i>	TGAGGTCACGCAACTTG ATGATCATTTTAT	GAAAATGACTTTTTTGGGA TTTTGGAGCAATG	4099	this study
pMPH42	<i>nhr-79p::sfGF P::nrx-1(α)</i>	CACGATCATTTTAAGCC AAGTTGTGGCCGT	TTTTATGCTAAAAATCGA TAAATCAAGGAA	3000	this study
pMPH43	<i>srv-3p::sfGFP ::nrx-1(α)</i>	TCACATTTGCCACCAA TTGCCGTTGCCA	TTTTGGAGGAGAAAGTT GAGCAAATAGTAG	770	this study
pMPH44	<i>sra-6p::sfGFP ::nrx-1(α)</i>	CTGAGGTGCATTTGCGA GGGGCACTTCAGA	GGCAAATCTGAAATAAT AAATATTAATT	2408	this study
pMPH45	<i>ric-19p::sfGF P::nlg-1</i>	CATTAAAGAGTGTGCTC CACGAGCC	GTTCAAAGTGAAGAGCT CTCTCGAC	147	this study
pMPH46	<i>nhr-79p::sfGF P::nlg-1</i>	CACGATCATTTTAAGCC AAGTTGTGGCCGT	TTTTATGCTAAAAATCGA TAAATCAAGGAA	3000	this study
pMPH47	<i>nlp-56p::sfGF P::nlg-1</i>	TTCCAAATCCGAACTTC CAGCTCAAATGAC	CTGGAAGAGTTGAATCA TATGGTTTAGAAG	721	this study

pMPH48	<i>nhr-79p::eat-4::SL2::gf p</i>	CACGATCATTTTAAGCC AAGTTGTGGCCGT	TTTTATGCTAAAAATCGA TAAATCAAGGAA	3000	this study
pMPH49	<i>srv-3p::eat-4::SL2::gf p</i>	TCACATTTGCCACCAAA TTGCCGGTTGCCA	TTTTGGAGGAGAAAGTT GAGCAAATAGTAG	770	this study
pMPH50	<i>sra-6p::eat-4::SL2::gf p</i>	CTGAGGTGCATTTGCGA GGGGCACTTCAGA	GGCAAAATCTGAAATAAT AAATATTAAT	2408	this study
pMPH51	<i>srv-3p::gfp::cla-1</i>	TCACATTTGCCACCAAA TTGCCGGTTGCCA	TTTTGGAGGAGAAAGTT GAGCAAATAGTAG	770	this study
pMPH52	<i>sra-6:gfp::cla-1</i>	CTGAGGTGCATTTGCGA GGGGCACTTCAGA	GGCAAAATCTGAAATAAT AAATATTAAT	2408	this study
pMPH53	<i>sre-1p::sfGFP ::nrx-1(α)</i>	GGGCGGGGCTATCTGC AAACAATGCAATGC	GAGGACATTTAAAAACC GGCGAGTATTGTA	1100	this study
pMPH54	<i>gcy-36p::sfGF P::nrx-1(α)</i>	ATGATGTTGGTAGATGG GGTTTGGATTCAT	TGTTGGGTAGCCCTTGT TTGAATTTACCAC	1087	this study
pMPH55	<i>flp-8p::sfGFP ::nrx-1(α)</i>	AGTGCTCAAATGGAGTC TGCATGAAAATGA	TTTCTACTTGAAAAGTGT GGACTGAGCACT	3165	this study
pMPH56	<i>nlp-56p::sfGF P::nrx-1(α)</i>	TTCAAATCCGAACTTC CAGCTCAAATGAC	CTGGAAGAGTTGAATCA TATGGTTTAGAAG	721	this study

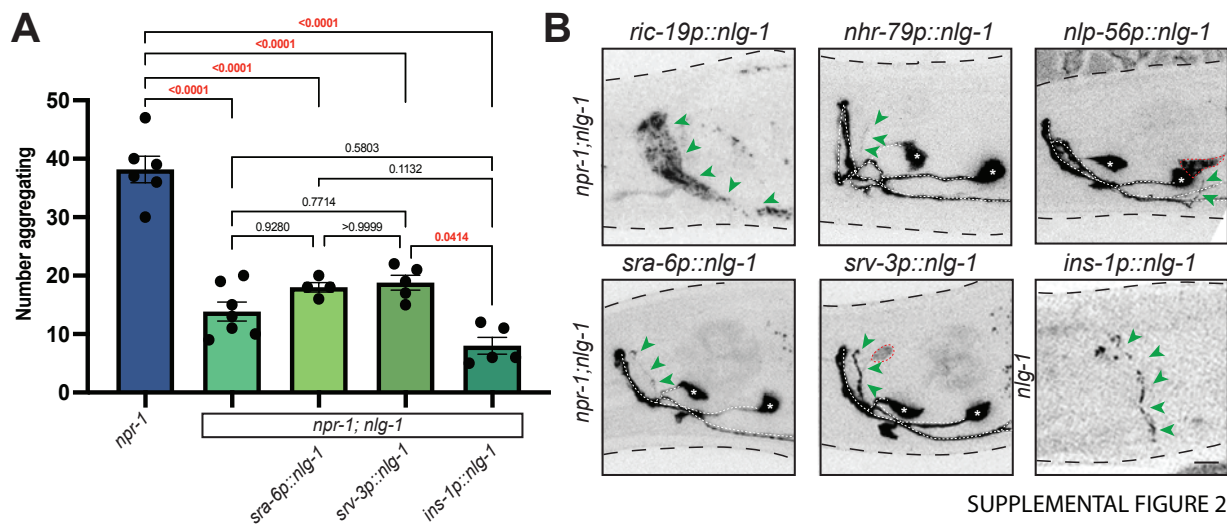


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2 **Supplemental Figure 1. Confirming role for, expression, and localization, of NRX-1 in**
 3 **aggregation behavior**

4 A) Graph showing aggregation behavior levels in *npr-1(ad609)* animals compared to *npr-*
 5 *1(ad609); otIs525;him-8* animals and N2 compared to *otIs525;him-8*. Aggregation behavior was

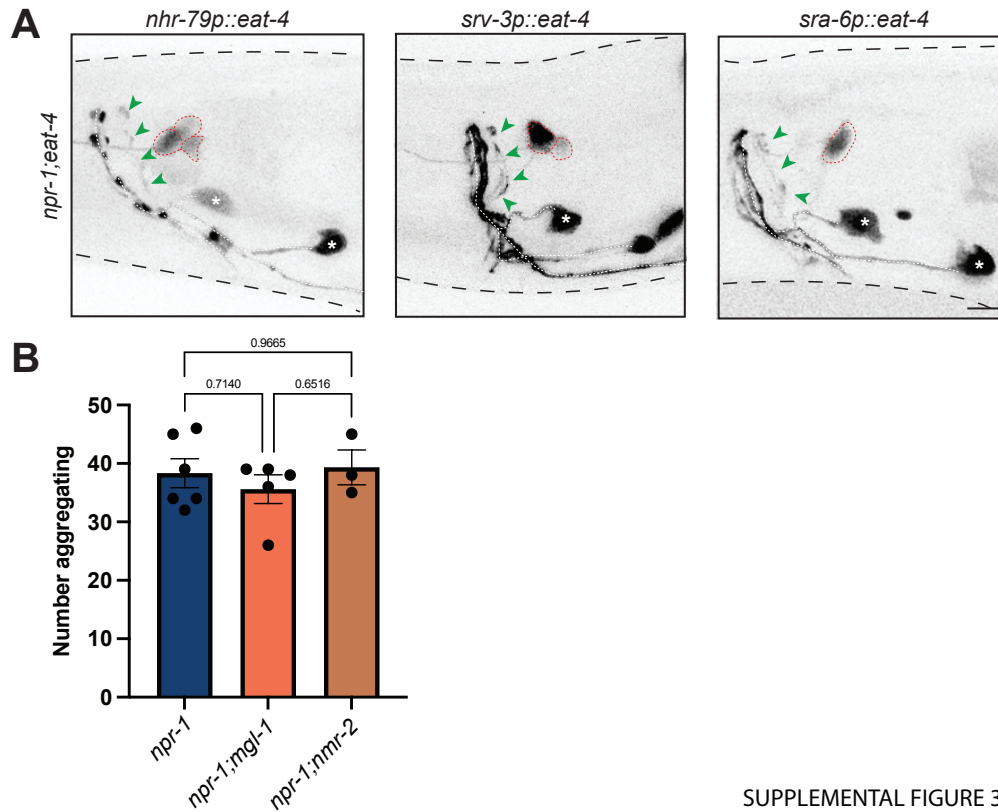
1 not changed by the presence of *otIs525* or *him-8*. Graph showing number of aggregating animals
 2 **(B)** and representative images **(C)** of QG1 (*qgIR1*) strain compared to *qgIR1;nrx-*
 3 *1(wy778);otIs525;him-8* mutants (Scale bar = 1mm). **D)** Graph showing number of aggregating
 4 animals in *npr-1(ad609)*, *npr-1(ad609);nrx-1(wy778)*, and *npr-1(ad609);nrx-1(wy778)* animals
 5 with NRX-1(γ) driven under the *ric-19* promoter and NRX-1(α) driven under promoters indicated.
 6 **E)** Expression of NRX-1 tagged with sfGFP driven under various promoters. Green arrows
 7 indicate NRX-1 axonal expression. Red dashed lines show cell bodies. White dashed line indicates
 8 *lim-6^{int4}::gfp* which drives expression in RIS and AVL axons. White asterisks indicate RIS and
 9 AVL cell bodies. Yellow box in *nlp-56p::nrx-1(α)* indicates area where RMG should be located.
 10 Expression of *nrx-1* under this promoter is not seen. *osm-6p::nrx-1(α)* imaging performed in *nrx-*
 11 *1(wy778)*(Scale bar = 10 μ m).



12
 13 **Supplemental Figure 2. Expression and localization of NLG-1 in aggregation behavior**

14 **A)** Graph showing number of aggregating animals in *npr-1(ad609)*, *npr-1(ad609);nlg-1(ok259)*,
 15 and *npr-1(ad609);nlg-1(ok259)* with NLG-1 driven under *sra-6*, *srv-3*, and *ins-1* promoters. **B)**
 16 Expression of NLG-1 tagged with sfGFP driven under various promoters. Green arrows
 17 indicate NRX-1 axonal expression. Red dashed lines show cell bodies. White dashed line indicates

- 1 *lim-6^{int4}::gfp* which drives expression in RIS and AVL axons. White asterisks indicate RIS and
- 2 AVL cell bodies (Scale bar = 10 μ m).



SUPPLEMENTAL FIGURE 3

- 3
- 4 **Supplemental Figure 3. Expression of EAT-4 and analysis of glutamate receptors in**
- 5 **aggregation behavior**

6 A) Expression of EAT-4 tagged with sfGFP driven under *nhr-79p*, *srv-3p*, and *sra-6p* promoters.

7 Green arrows indicate NRX-1 axonal expression. Red dashed lines show cell bodies. White dashed

8 line indicates *lim-6^{int4}::gfp* which drives expression in RIS and AVL axons. White asterisks indicate

9 RIS and AVL cell bodies (Scale bar = 10 μ m). B) Graph showing number of aggregating animals

10 in *npr-1(ad609)*, *npr-1(ad609);mgl-1(tm1811)* and *npr-1(ad609);nmr-2(ok3324)*.

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