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Supplemental Information

Heparan Sulfate Organizes Neuronal Synapses through Neurexin Partnerships

Peng Zhang, Hong Lu, Rui T. Peixoto, Mary K. Pines, Yuan Ge, Shinichiro Oku, Tabrez J. Siddiqui, Yicheng Xie, Wenlan Wu, Stephanie Archer-Hartmann, Keitaro Yoshida, Kenji F. Tanaka, A. Radu Aricescu, Parastoo Azadi, Michael D. Gordon, Bernardo L. Sabatini, Rachel O.L. Wong, and Ann Marie Craig

Table S1. The HS Modification Site on Nrxx is Evolutionary Conserved, Related to Figure 1

(A) Evolutionary conservation in vertebrates.

Nrxn	Species	NCBI/Uniprot	Sequence Alignment
Nrx1	Macaca mulatta	XP_014967815	TTTTLATSTARRGKPPT-KEPISQTTDDILVA SAACP-SD-DEDIDPCEPS
Nrx2	Macaca mulatta	XP_014969352	TTTTMATTTTRGRSPTLRDSTTQNTDILLVA SAACP-SD-DEDLEECEPS
Nrx3	Macaca mulatta	XP_005561989	TTTTMATTTTRKNRSTAS----IQPTSDDLVS SAACS-SD-DEDFVECEP-
Nrx1	Rattus norvegicus	AAA41705	TTTTLATSTARRGKPPT-KEPISQTTDDILVA SAACP-SD-DEDIDPCEPS
Nrx2	Rattus norvegicus	AAA41708	TTTTMATTTTRGRSPTMRDSTTQNTDILLVA SAACP-SD-DEDLEECEPS
Nrx3	Rattus norvegicus	AAA02857	TTTTMATTTTRKNRSTAS----IQPTSDDLVS SAACS-SD-DEDFVECEP-
Nrx1	Bos Taurus	AAA19906	TTTTLATSTARRGNSPT-KEPVSQTTDDILVA SAACP-SD-DEDIDPCEPS
Nrx2	Bos Taurus	XP_010819426	TTTTMATTTTRGRSPTLRDSTTQNTDILLVA SAACP-SD-DEDLEECEPS
Nrx3	Bos Taurus	AAA19908	TTTTMATTTTRKNRSTAS----IQPTSDDLVS SAACS-SD-DEDFVECEPS
Nrx1	Gallus gallus	ACF35428	TTTTLATSTARRGKAPT-KEPIGQTTDDILVA SAACP-SD-DEDIDPCEPS
Nrx3	Gallus gallus	ACF35430	TTTTMATTTTRKNRSPPS----IQT-TDDIVS SAACS-SD-DEDFIDCEPS
Nrx1	Xenopus tropicalis	XP_012819327	TTTTLATST---RKSPT-REPVGQTTDDILVA SAACP-SDDDEDIDPCEPS
Nrx2	Xenopus tropicalis	XP_004913566	TTTTMATTTTRGRSPTMRDSITQNSDILLVA SAACP-SD-DEDLEECEPS
Nrx3	Xenopus tropicalis	XP_012824757	TTTTLATTTTRKNRSSPG----STL-TDDIGS SAECFISDDEDVTDCT-
Nrx1	Danio rerio	ABG25164	TTSNTI---TIT---YSTPADEQQTDELLVA SAACP-SD-DEDIDPCEPS
Nrx2	Danio rerio	ABG25168	TTTTMATTTTRKQRSPTMRDSVTQNTDILLVA SAACP-SD-DEDLEECEPG
Nrx3	Danio rerio	ABG25174	TTTTLSTTTTRKQRSPT----IQT-TDDIVS SAACS-SD-DEDEECDG-

The sequence surrounding the HS-modified serine (red highlight) is conserved in monkey, rat, bovine, chicken, frog, and zebrafish neurexins, as well as in human and mouse neurexins (Figure 1I). Alignment was performed with Clustal; *, conserved residues.

(B) Potential HS modification in *Caenorhabditis elegans*.

VNVNGLMILD	LYENGSNRIH	TIGAPQTTAV	SEQVSSSEEE	DDELAEMMA	HSIDENPNEA	LIESLAPSCL	SLEEQQSCFI
DTDDSTGFFS	PVLPTVANFP	TTRQTEPETT	RRPLSPSKMT	PSLPSPTTTT	LPVFFLSQIT	DGDESEDEFD	<u>SG</u> GDDLFGGD
GVGITAATQP	SVKRTTSISV	VTKVLTTTSE	PSSTHRSTVL	PRPYASVKEA	AEQNPDYLGA	SIWNQVDTLP	EPMVTGPAWR
TNKSLLTTAT	TEASSTKSNR	KTTTTTTTSA	TTTIRIQORP	NYDIDNEVTA	LITSSLAPQK	TRPKSTPHFT	VYVPRPTTPM
GDITTTMQA	ATVTDFFRTP	LIMCSSLAVI	IAIAAVVFFV				

The sequence of *Caenorhabditis elegans* neurexin, Nrxx-1, between the LNS domain (cyan) and the transmembrane region (green) reveals a potential HS modification site (S, red highlight, flanked by underlined acidic residues).

Table S3. Evolutionary Conservation of the Proposed HS Binding Site on NLs, Related to Figure 6

NL	Species	NCBI/Uniprot	Sequence Alignment
NL1	Human	Q8N2Q7	QLYLHIGL K PRVKEHYRANKVNLWLELVPHLHNLNDISQYT
NL2	Human	Q8NFZ4	KQYLHIGL K PRVVDNYRANKVAFWLELVPHLHNLHTELFYT
NL3	Human	Q9NZ94	QLYLHIGL K PRVVDHYRATKVAFWKHLVPHLYNLHDMFHYT
NL4X	Human	Q8N0W4	QLYLHIGL K PRVVDHYRATKVAFWLELVPHLHNLNEIFQYV
NL4Y	Human	Q8NFZ3	QLYLHIGL K PRVVDHYRATKVAFWLELVPHLHNLNEIFQYV
NL1	Macaca mulatta	XP_011753510	QLYLHIGL K PRVKEHYRANKVNLWLELVPHLHNLNDISQYT
NL2	Macaca mulatta	NP_001265378	KQYLHIGL K PRVVDNYRANKVAFWLELVPHLHNLHTELFYT
NL3	Macaca mulatta	XP_014983140	QLYLHIGL K PRVVDHYRATKVAFWKHLVPHLYNLHDMFHYT
NL4X	Macaca mulatta	XP_014982385	QLYLHIGL K PRVVDHYRATKVAFWLELVPHLHNLNEIFQYV
NL4Y	Macaca mulatta	XP_014984076	QLYLHIGL K PRVVDHYRATKVAFWLELVPHLHNLNEIFQYV
NL1	Rattus norvegicus	Q62765	QLYLHIGL K PRVKEHYRANKVNLWLELVPHLHNLNDISQYT
NL2	Rattus norvegicus	Q62888	KQYLHIGL K PRVVDNYRANKVAFWLELVPHLHNLHTELFYT
NL3	Rattus norvegicus	Q62889	QLYLHIGL K PRVVDHYRATKVAFWKHLVPHLYNLHDMFHYT
NL1	Mus musculus	Q99K10	QLYLHIGL K PRVKEHYRANKVNLWLELVPHLHNLNDISQYT
NL2	Mus musculus	Q69ZK9	KQYLHIGL K PRVVDNYRANKVAFWLELVPHLHNLHTELFYT
NL3	Mus musculus	Q8BYM5	QLYLHIGL K PRVVDHYRATKVAFWKHLVPHLYNLHDMFHYT
NL4	Mus musculus	B0F2B4	QLYLHIGL K PRVVDHYRAAKVAFWLELVPHLHGLAADPGAY
NL1	Bos Taurus	NP_001192902	QLYLHIGL K PRVKEHYRANKVNLWLELVPHLHNLNDISQYT
NL2	Bos Taurus	NP_001178171	KQYLHIGL K PRVVDNYRANKVAFWLELVPHLHNLHTELFYT
NL3	Bos Taurus	AAI23786	QLYLHIGL K PRVVDHYRATKVAFWKHLVPHLYNLHDMFHYT
NL1	Gallus gallus	NP_001192902	QLYLHIGL K PRVKEHYRANKVNLWLELVPHLHNLNDISQYT
NL3	Gallus gallus	ADD52425	QLYLHIGL K PRVVDHYRATKVAFWKHLVPHLYNLHDMFHYT
NL4	Gallus gallus	ADD52421	QLYLHIGL K PRVVDHYRATKVAFWLELVPHLHNLNEIFQYV
NL1	Xenopus tropicalis	ADB12662	QLYLHIGL K PRVKEHYRATKVAFWLELVPRRLHVMNDLT---
NL2	Xenopus tropicalis	XP_002944465	KQYLHIGL K PRVVDNYRANKVAFWLELVPHLHNLHTELFYT
NL3	Xenopus tropicalis	ADB12664	QLYLHIGL K PRVVDHYRATKVAFWKHLVPHLYNLHDMFHYT
NL4	Xenopus tropicalis	NP_001165299	QLYLHIGL K PRVVDHYRATKVAFWLELVPHLHNLNEIFQYV
NL1	Danio rerio	NP_001135737	QLYLHIGL K PRVKEHYRANKVNLWLELVPHLHSLNEVSQII
NL2	Danio rerio	XP_005172672	KQYLHIGL K PRVVDNYRANKVAFWLELVPHLHTIHDEIPTY
NL3	Danio rerio	XP_005165239	QLYLHIGL K PRVVDHYRATKVAFWKHLVPHLYNLHDMFHYT
NL4	Danio rerio	XP_021330915	QLYLHIGL K PRVVDHYRATKVAFWLELVPHLHNLNEIFQYV
NL3	Drosophila	NP_001036685	KKYLNFDTKPKLKNHYRAHRLSFWLNLIPLDHPGGDNVPA
NL4	Drosophila	NP_001163661	QKYLEIGMKPRIKNHFRAHQLSIWLRLIPELHRAGMEDVIA
NL1	C. elegans	NP_510283	EAYLEITDRPRVKNYRRAQVGFWNNFIPQLHKNGKETEPV
			: **: : *::: * : : * : : * *:
NL2	Drosophila	NP_523496	QLYVELGN K ANI Q SHYRGHKL S MWLNLI P QLHRHF N INDQS
NL1	Drosophila	NP_731172	QAYLNIGIPPTVGYKYRQIYMNFWNKELPDELNQIAAIQEQ

The three Arg or Lys residues mutated here to disrupt HS binding (red highlight) are conserved in human, monkey, rat, mouse, bovine, chicken, frog, zebrafish, and worm NLs, as well as some isoforms of fly NLs. Fly NL2 may have a weaker HS-binding site composed of a Lys and a Gln whereas fly NL1 seems to lack a consensus HS binding site. Alignment was performed with Clustal.