Neuron, Volume *70*

Supplemental Information

Neurexin-Neuroligin Transsynaptic Interaction Mediates Learning-Related Synaptic Remodeling and Long-Term Facilitation in *Aplysia*

Yun-Beom Choi, Hsiu-Ling Li, Stefan R. Kassabov, Iksung Jin, Sathyanarayanan V. Puthanveettil, Kevin A. Karl, Yang Lu, Joung-Hun Kim, Craig H. Bailey, and Eric R. Kandel

Supplemental Figures

Figure S1. Sequence Analysis of ApNRX and ApNLG Including Alternative Splicing Features of ApNRX, Related to Figure 1 and Figure 2

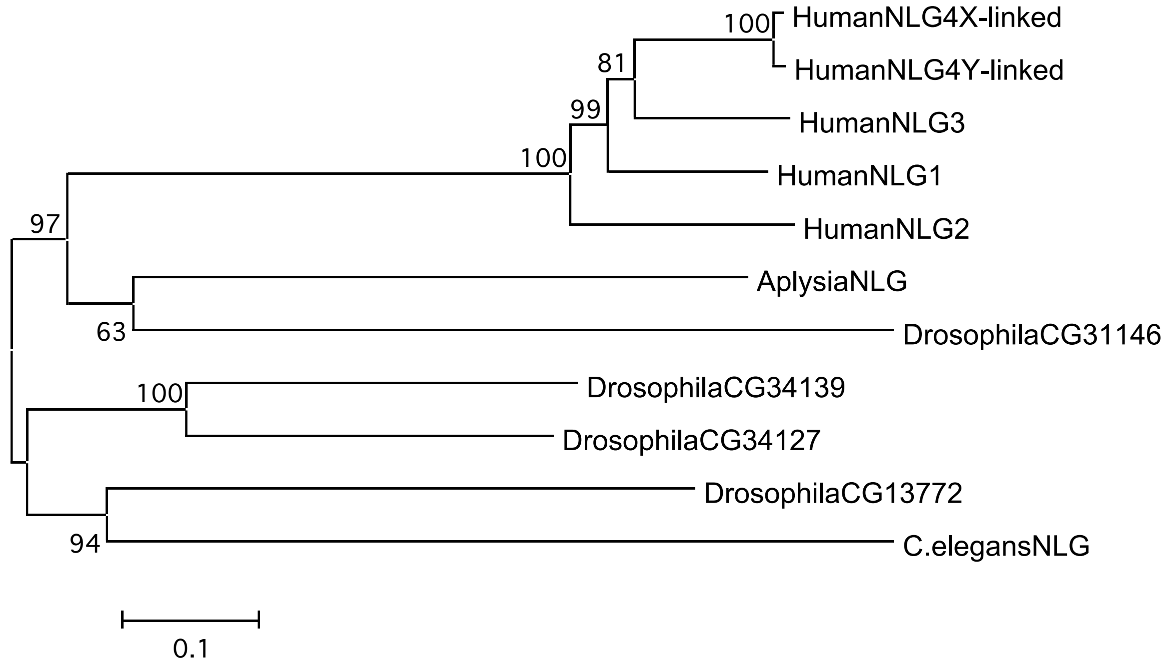
(A and B) Phylogenetic analysis of *Aplysia*, human, *Drosophila*, and *C.elegans* neuroligin (NLG) and neurexin (NRX) protein sequences. The phylogenetic trees were generated using MEGA4 software (Tamura et al, 2007) and represent evolutionary distances of neuroligin or neurexin homologs as the number of amino acid substitution per site.

(C and D) Sequence alignments of neuroligin and neurexin among different species by Clustalw. ApNLG and ApNRX are highlighted in red.

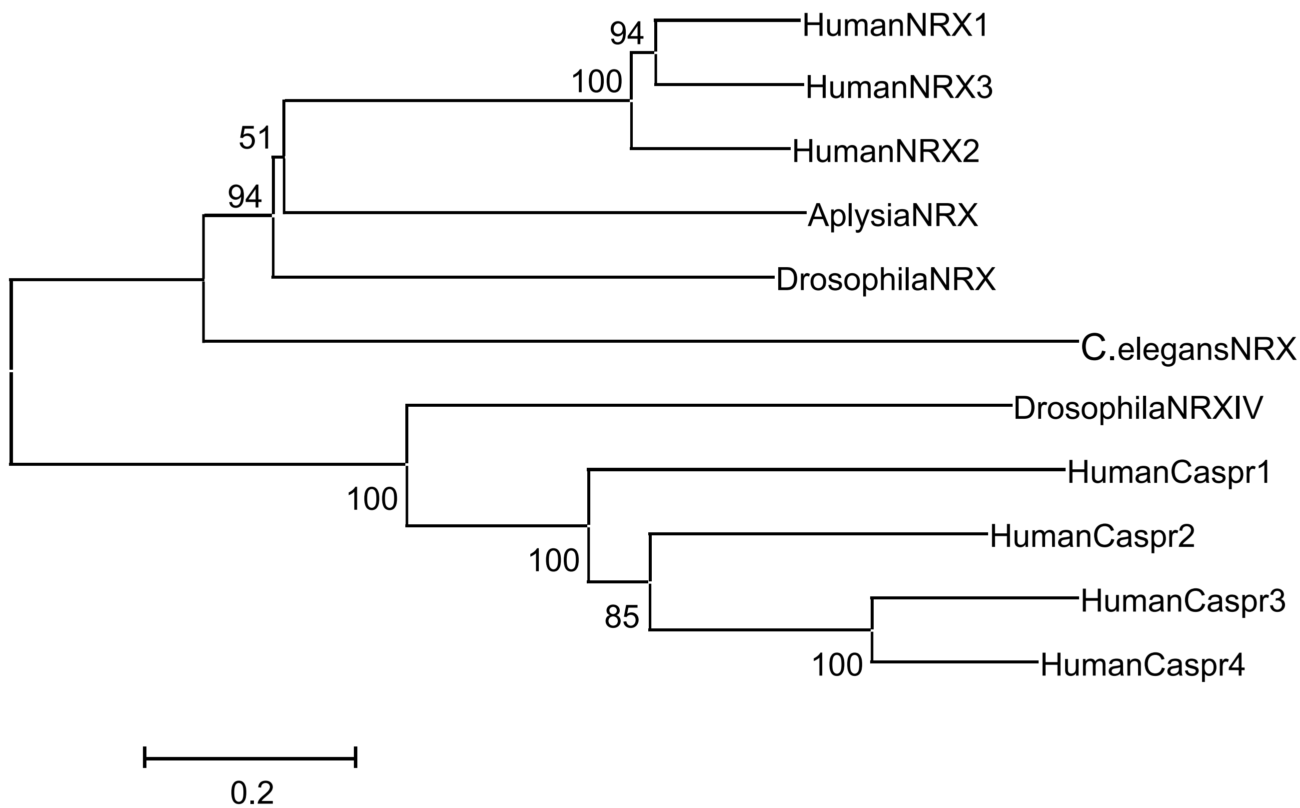
(E) Alternative splicing features of ApNRX. The amino acid sequence of ApNRX and human neurexin-1α (NP_004792.1) were aligned using ClustalW. Positions of the 4 splice sites of ApNRX and 5 splice sites of human neurexin-1α are indicated with numbers above or below their respective sequences. At splice sites where selected splice inserts are included in the sequence, the inserts are boxed with a solid line. Positions of splice sites where inserts are not included in the sequence are indicated with arrowheads. The positions and insert sequences of human neurexin-1α splice sites were extracted from Missler et al, (1998). Neurexin structural features are depicted as follows: LNS(A) domain – light blue; LNS(B) domain – yellow; PDZ domain – green; transmembrane domain – orange. The LNS domains were obtained using the Conserved Domain Database (CDD) tool at NCBI and transmembrane domains determined with the TMHM topology prediction tool.

In ApNRX, as in vertebrate neurexins, one splice site occurs in each of the three LNS(B) domains. The positions of two of the *Aplysia* splice sites (sites 1 and 3) match exactly the positions of the respective sites in human neurexin-1α (sites 2 and 4) in the first and third LNS(B) domains. The other two ApNRX splice sites (sites 2 and 4) occur in regions of much lower conservation between the *Aplysia* and vertebrate sequence – the C-terminal half of the second LNS(B) domain, and the region between the last LNS(B) domain and the transmembrane domain. The position of these two sites is close, but not as precisely matching as compared to the equivalent vertebrate positions (sites 3 and 5). Nevertheless, they are in the same domain regions and could be functionally equivalent. The similarity of the alternative splicing of *Aplysia* and vertebrate neurexins extends to the pattern and type of inserts utilized at the equivalent splice sites (Table S1, Missler et al, 1998). Specifically, the splicing pattern at the sites within the LNS(B) domains is simple consisting of a single short insert for both ApNRX (sites 1-3) and vertebrate neurexins (sites 2-4). In contrast, the splicing at site 4 in ApNRX and the equivalent site 5 in vertebrate neurexins utilize multiple and often long inserts.

A. Phylogenetic Tree of NLG



B. Phylogenetic Tree of NRX



C. Sequence Alignment of NLG

```
DrosophilaCG34127      .......... .......... ..MVMLLSKR QLPPSAATRT RRKVPPTRRR STWALSSLLA
DrosophilaCG34139      .......... .......... .......... .......... .......... ..........
DrosophilaCG13772      MAQTKIIQSR FLHIYGLLCL GSLMGCIKTI GASIAQQTLA DADAPDEAKA KEREAETDAD
HumanNLG4X-linked      .......... .......... .......... .......... .......... ..........
HumanNLG4Y-linked      .......... .......... .......... .......... .......... ..........
HumanNLG1              .......... .......... .......... .......... .......... ..........
HumanNLG3              .......... .......... .......... .......... .......... ..........
HumanNLG2              .......... .......... .......... .......... .......... ..........
AplysiaNLG             .......... .......... .......... .......... .......... ..........
DrosophilaCG31146      .......... .......... ..MKFRLAAF WLFLLTVGGN HKLLSHVSPM GVAAEKQPLH
C.elegansNLG           .......... .......... .......... .......... .......... ..........

DrosophilaCG34127      LVVLDICIAR CLAAGISYSS NSGNLSTSQK SPSSANNGGL VLIGSSSATS SSVPSLSLTS
DrosophilaCG34139      .......... .......... .......... .......... ......MGES QLLLLLRLLL
DrosophilaCG13772      ADAEGDAKAE TETGKGTREH TNGDAEADAR IMATGICIIR LITLKRFLEN SKTEEHNQRR
HumanNLG4X-linked      .......... .......... ......MSRP QGLLWLPLLF TPVCVMLNSN VLLWLTALAI
HumanNLG4Y-linked      .......... .......... ......MLRP QGLLWLPLLF TSVCVMLNSN VLLWITALAI
HumanNLG1              .......... .......... .......MAL PRCTWPNYVW RAVMACLVHR GLGAPLTLCM
HumanNLG3              .......... .......... .......... ........MW LRLGPPSLSL SPKPTVGRSL
HumanNLG2              .......... .......... .......... ........MW LLALCLVGLA GAQRGGGGPG
AplysiaNLG             .......... .......... ...MDTTTRP APSPSSSSSS ASVTLSVRHL TLTLVHLLLL
DrosophilaCG31146      KGNELENAMK LREAPKQSRV IGDITTTIQP DSDPGRSIGH QALRRAKAPP PSLELHFRQN
C.elegansNLG           .......... .......... .......... .......... .........M ERIYLLLLLF

DrosophilaCG34127      SSSAGLSSPG SSSSSSSSST STSGSNSNRS SRPTSNNSNS NSNYSPNHDQ LPAQLSSRII
DrosophilaCG34139      LPTVLISWMH CAGASTAD.. .......... .......... ........IY KGARLGHRIV
DrosophilaCG13772      QQSATSAASA APAGATSPRR RHLHYPGHSA VQPGHPEACA LLVLLLLTSL WPDCCECLHG
HumanNLG4X-linked      KFTLIDS... .......... .......... .......... .......... ...QAQYPVV
HumanNLG4Y-linked      KFTLIDS... .......... .......... .......... .......... ...QAQYPVV
HumanNLG1              LGCLLQAGHV .......... .......... .......... ........LS QKLDDVDPLV
HumanNLG3              CLTLWFLSLA .......... .......... .......... ........LR ASTQAPAPTV
HumanNLG2              GGAPGGPGLG .......... .......... .......... ........LG SLGEERFPVV
AplysiaNLG             LSPLVPPSAS .......... .......... .......... ........VV YFRQMSDRVI
DrosophilaCG31146      LNKLFAGDET THVGHTNATD NTTAAVLATD EGSSEHEPST THHPERRHVV PDKLQYTQEI
C.elegansNLG           LPR....... .......... .......... .......... .......... .IRSYDVRSV

DrosophilaCG34127      NTRNGAISGV IVQLDG.RHL DPVEAYRGIP YASPPVGNLR FMPPVSAA.M WSGVKKADRF
DrosophilaCG34139      QTRYGRLHGL ILPLDSFRFL RSVEVFLGVP YATPPTKQNR FSPTRAPA.P WDGIRISDKY
DrosophilaCG13772      GSNTVKTKYG LLRGIVVRSS PLVEAFLGIP YASPPVGSLR FMPPITPS.T WKTVRSADRF
HumanNLG4X-linked      NTNYGKIRGL RTPLPN.EIL GPVEQYLGVP YASPPTGERR FQPPEPPS.S WTGIRNTTQF
HumanNLG4Y-linked      NTNYGKIQGL RTPLPS.EIL GPVEQYLGVP YASPPTGERR FQPPESPS.S WTGIRNATQF
HumanNLG1              ATNFGKIRGI KKELNN.EIL GPVIQFLGVP YAAPPTGERR FQPPEPPS.P WSDIRNATQF
HumanNLG3              NTHFGKLRGA RVPLPS.EIL GPVDQYLGVP YAAPPIGEKR FLPPEPPP.Y WSGIRNATHF
HumanNLG2              NTAYGRVRGV RRELNN.EIL GPVVQFLGVP YATPPLGARR FQPPEAPA.S WPGVRNATIL
AplysiaNLG             TTRYGKVRGI LVQFEN.KNL KSVEAYLGLR YADLDGGGMR FMPPKNPKDQ WNGIRVAISH
DrosophilaCG31146      QVKQGRLMGI TRRFQVTSGL RQVDQFLGLP YAEAPTGNRR FMPPGAPL.P WQGLKIARHL
C.elegansNLG           TTSWGMVRGE VVSPEG.DDL PPVAQYLGIP YGVAPTGQYR FNMAISAAKW THMPKDARKV

DrosophilaCG34127      SPVCPQRLPD IHNETAALER MPKGRLEYLK RLLPYLQNQS EDCLYLNIYV PIQVGSRDSS
DrosophilaCG34139      SPVCPQRLPN IQNETAALEK MPKGRLEYLK RLLPFLENQS EDCLYLNVFS PVN..AGANE
DrosophilaCG13772      SPVCPQNIPI PPNGPEALLE VPRARLAQLR RLLPLLKNQS EDCLYLNIYV PYETRRQRRN
HumanNLG4X-linked      AAVCPQH... LDERSLLHDM LPIWFTANLD TLMTYVQDQN EDCLYLNIYV PTEDDIHDQ.
HumanNLG4Y-linked      SAVCPQH... LDERFLLHDM LPIWFTTSLD TLMTYVQDQN EDCLYLNIYV PMEDDIHEQ.
HumanNLG1              APVCPQN... IIDGRLPEVM LPVWFTNNLD VVSSYVQDQS EDCLYLNIYV PTEDDIRDS.
HumanNLG3              PPVCPQN... IHT.AVPEVM LPVWFTANLD IVATYIQEPN EDCLYLNVYV PTEDGSGAKK
HumanNLG2              PPACPQN... LHG.ALPAIM LPVWFTDNLE AAATYVQNQS EDCLYLNLYV PTEDGPLTKK
AplysiaNLG             QPVCPQP... TTHERELNQQ LPKGRVDQLR NITPFITEQK EDCLTLNLYV PKQEWNETKP
DrosophilaCG31146      PPVCPQKLP. .DLSPHGSEN MSRARHKHLS RLLPYLRTES EDCLYLNLYV PHEEPQSTPK
C.elegansNLG           SPVCIQTDMP ELSETKAFKH TSAQRFDFNH RLLPHLKKQS EDCLYMNIYV PERLEISRDN

DrosophilaCG34127      GSSSSSSAGS SSSGSGGSSS SSSSSSTSSS SAGSGSPAKY PVLVFVHGES YEWNSGNPYD
DrosophilaCG34139      KK........ .......... .......... .........L PVIVFIHGES FEWSSGNPYD
DrosophilaCG13772      TDDT...... .......... .......... ..TGEPKTKL STVVFIHGES YDWNSGNPYD
```

```
HumanNLG4X-linked      .......... .......... .......... ......NSKK PVMVYIHGGS YMEGTGNMID
HumanNLG4Y-linked      .......... .......... .......... ......NSKK PVMVYIHGGS YMEGTGNMID
HumanNLG1              .......... .......... .......... ......GGPK PVMVYIHGGS YMEGTGNLYD
HumanNLG3              QGEDLADNDG .......... ........DE DEDIRDSGAK PVMVYIHGGS YMEGTGNMID
HumanNLG2              RDEATLN... .......... ........PP DTDIRDPGKK PVMLFLHGGS YMEGTGNMFD
AplysiaNLG             .......... .......... .......... .........M AVMVFVHGES YQTGTGNAYD
DrosophilaCG31146      QY........ .......... .......... .......... AVLVYLHGES FEWNSGNPYD
C.elegansNLG           .......... .......... .......... ........YL PVMVIVHGEE YGWGTGNAFN

DrosophilaCG34127      GSVLASYGQI LVVTINYRLG VLGFLNANTD RYSKL..PAN YGLMDIIAAL HWLKENIAAF
DrosophilaCG34139      GSVLASYGEV VVVTLNYRLG ILGFLNANPN PHAHAR.VAN YGLMDQMAAL HWIQQNIQKF
DrosophilaCG13772      GSELAAHGNV IVVTINFRLG IFGFLKTGGK ESAQG....N FGLMDLVAGL HWLKENLPAF
HumanNLG4X-linked      GSILASYGNV IVITINYRLG ILGFLSTGDQ AAKGN..... YGLLDQIQAL RWIEENVGAF
HumanNLG4Y-linked      GSILASYGNV IVITINYRLG ILGFLSTGDQ AAKGN..... YGLLDQIQAL RWIEENVGAF
HumanNLG1              GSVLASYGNV IVITVNYRLG VLGFLSTGDQ AAKGN..... YGLLDLIQAL RWTSENIGFF
HumanNLG3              GSILASYGNV IVITLNYRVG VLGFLSTGDQ AAKGN..... YGLLDQIQAL RWVSENIAFF
HumanNLG2              GSVLAAYGNV IVATLNYRLG VLGFLSTGDQ AAKGN..... YGLLDQIQAL RWLSENIAHF
AplysiaNLG             GSVLSSFGDV IVVTLNYRLG VLGFLTTEDH AAMGN..... YAMLDITQAL LWLRENIASF
DrosophilaCG31146      GSVLSSYGEV IVVTVNYRLG VLGFLRPSID AHNIAN.... YALLDQIAAL HWIKENIEAF
C.elegansNLG           GTTLAAYGHI IVVTLNYRLG VFGFLGRCES SSCSGN.... SGISDLVSAL TMLNVILPSF

DrosophilaCG34127      GGDPNSITLA GHGTGAACVH FLISSMAVPE GL.......L FNRAILMSGS GLAPWSLVSN
DrosophilaCG34139      GGDPNSVTLA GHGTGAACIN YLMTSPTMVR G........L FHRAILMSGS AYSSWALVED
DrosophilaCG13772      GGDPQSITLL GYGTGAVLAN ILVVSP..VA SD.......L IQRTVLVSGS ALSPWAIQKN
HumanNLG4X-linked      GGDPKRVTIF GSGAGASCVS LLTLSHYSEG .........L FQKAIIQSGT ALSSWAVNYQ
HumanNLG4Y-linked      GGDPKRVTIF GSGAGASCVS LLTLSHYSEG .........L FQKAIIQSGT ALSSWAVNYQ
HumanNLG1              GGDPLRITVF GSGAGGSCVN LLTLSHYSEG NRWSNSTKGL FQRAIAQSGT ALSSWAVSFQ
HumanNLG3              GGDPRRITVF GSGIGASCVS LLTLSHHSEG .........L FQRAIIQSGS ALSSWAVNYQ
HumanNLG2              GGDPERITIF GSGAGASCVN LLILSHHSEG .........L FQKAIAQSGT AISSWSVNYQ
AplysiaNLG             NGDPQRVTLF GHGTGAAIVN LLLLSPFISE DRGK.....Y FQRAILQSGS ASSSWAVSYD
DrosophilaCG31146      GGDNSRVTLM GHSTGAACVN YLMVSPVASG .........L FHRAILMSGS AMSDWAASNQ
C.elegansNLG           GGDSKSVTLA GWGSGASLVS LLMASPLTQP GRR......L FRRAILLDGS ALSPWAISQN

DrosophilaCG34127      PAKYAAIVAH HVNCA..... SDLP....HA HLMKCLREKT LDQLLSVPIR PPEFGFAFGP
DrosophilaCG34139      PVLFAIKLAK EVNCT..... IPDDINRHHE QIVDCLRDVP LEDLYLADIQ APNFLTSFGP
DrosophilaCG13772      PLFVKRRVAE QTGCH..... GDMLY....D DLAPCLRTKS VAELLAVKVD HPRFLVGFAP
HumanNLG4X-linked      PAKYTRILAD KVGCNMLDTT .......... DMVECLRNKN YKELIQQTIT PATYHIAFGP
HumanNLG4Y-linked      PAKYTRILAD KVGCNMLDTT .......... DMVECLKNKN YKELIQQTIT PATYHIAFGP
HumanNLG1              PAKYARMLAT KVGCNVSDTV .......... ELVECLQKKP YKELVDQDIQ PARYHIAFGP
HumanNLG3              PVKYTSLLAD KVGCNVLDTV .......... DMVDCLRQKS AKELVEQDIQ PARYHVAFGP
HumanNLG2              PLKYTRLLAA KVGCDREDSA .......... EAVECLRRKP SRELVDQDVQ PARYHIAFGP
AplysiaNLG             PRWCTEKLAM NVNCSR.... ....HLANSK ALIHCLRERS WAELVSNVPX APKYYSCFAP
DrosophilaCG31146      SLQLTMQIAH ALECPLNEHV EAEDD....D VLLDCLRHRR YQDILHIPTA LTQFSTSLGP
C.elegansNLG           PQQYFMQLAE ELACAPKNRT SSFNDNVDTI VRCMQVHSSE NITKAVLKID VPTFLSGFAP

DrosophilaCG34127      SIDG.....V VIDGGDYVPP APGSPAAQAQ AQASTAAGN. ......GLGG EAGIAAAGGW
DrosophilaCG34139      SVDG.....V VIRPGHSNLD IDDLMARNSR RSSADSGFQS SAGGGGGQGG GAGGGGGGGS
DrosophilaCG13772      FVDG...... .......... TVISPGANPL GSTTLPLG.. .......... ..........
HumanNLG4X-linked      VIDG...... .......... .......... .......... .......... ..........
HumanNLG4Y-linked      VIDG...... .......... .......... .......... .......... ..........
HumanNLG1              VIDG...... .......... .......... .......... .......... ..........
HumanNLG3              VIDG...... .......... .......... .......... .......... ..........
HumanNLG2              VVDG...... .......... .......... .......... .......... ..........
AplysiaNLG             SIDHYT.... .......... .......... .......... .......... ..........
DrosophilaCG31146      IVDG...... .......... .......... .......... .......... ..........
C.elegansNLG           IVDG...... .......... .......... .......... .......... ..........

DrosophilaCG34127      GTPGQLENIV LMRKTAINKL SRYDLMAGVT RAEAFFSFN. SGDVQYGIEA DRRSRILKAY
DrosophilaCG34139      GSSFGGGYFG GSGAGTMNMG GHYDVLFGVV TGESIWRFS. AHDIQNGFEG ERRDKIIRTY
DrosophilaCG13772      ......SAIV STSGIEYANF PKRDLIFCLT SVESYLDLS. AQDLEFGFNE TRRDRILRTF
HumanNLG4X-linked      ....DVIPDD PQILMEQGEF LNYDIMLGVN QGEGLKFVDG IVDNEDGVTP NDFDFSVSNF
HumanNLG4Y-linked      ....DVIPDD PQILMEQGEF LNYDIMLGVN QGEGLKFVDG IVDNEDGVTP NDFDFSVSNF
HumanNLG1              ....DVIPDD PQILMEQGEF LNYDIMLGVN QGEGLKFVEN IVDSDDGISA SDFDFAVSNF
HumanNLG3              ....DVIPDD PEILMEQGEF LNYDIMLGVN QGEGLKFVEG VVDPEDGVSG TDFDYSVSNF
HumanNLG2              ....DVVPDD PEILMQQGEF LNYDMLIGVN QGEGLKFVED SAESEDGVSA SAFDFTVSNF
AplysiaNLG             ...VLPSEVD QMIKERNSKF ASVPVMFGIT KNEAYSYLK. QQEIRKGISD NRKTQIIRTY
```

```
DrosophilaCG31146      ..HVIPNQPY KVMGHYTEHF SRYDLLFGIT ESESYHTLA. ALALEEGLRE NERDNLLRFY
C.elegansNLG           .QLIPNKPQV SFSTQYGSLF REIDLLVGIS SNPSHHMIS. NEDLKVGISK EKRMRIFRSL

DrosophilaCG34127      VRNTYTFHLN ..EIFATIVN EYTDWERPVQ HPINIRDETL EALSDAQVVA PAAQTVDLHS
DrosophilaCG34139      VRNAYNYHLN ..EIFYTIVN EYTDWDRTSQ HPINTRDTAV AALSDAQFVA PIVRAGDILA
DrosophilaCG13772      VRNNFHYHLN ..EIFAVLKN EYTDWEKAIR NPLSSRDATL QFLSDGHTAS PLIKLGYMHS
HumanNLG4X-linked      VDNLYGYPEG KDTLRETIKF MYTDWADK.E NPETRRKTLV ALFTDHQWVA PAVATADLHA
HumanNLG4Y-linked      VDNLYGYPEG KDTLRETIKF MYTDWADK.E NPETRRKTLV ALFTDHQWVA PAVATADLHA
HumanNLG1              VDNLYGYPEG KDVLRETIKF MYTDWADR.H NPETRRKTLL ALFTDHQWVA PAVATADLHS
HumanNLG3              VDNLYGYPEG KDTLRETIKF MYTDWADR.D NPETRRKTLV ALFTDHQWVE PSVVTADLHA
HumanNLG2              VDNLYGYPEG KDVLRETIKF MYTDWADR.D NGEMRRKTLL ALFTDHQWVA PAVATAKLHA
AplysiaNLG             VQNVFRFHRQ ..KIYEILDH HYTDWTHS.S DQISNRDNIL ELLSDGQYVA PLVKVAGLHA
DrosophilaCG31146      MQSRFDIRPD LALAATLKKY QDMYNNPIKA TNLEHRDVVL DILSDARVVG PLLQTGMFHA
C.elegansNLG           VRNLYDFHRE ..EILASIIN EYTDWENPRD HPKSIRNGVL NALSDVLYTA PLIETLRSHS

DrosophilaCG34127      ADHR...... .......... .......... ...NSYLYVF DYQTRFGDYP QRQGCIHGED
DrosophilaCG34139      ANSPPPVSSS STAGSPGANA AASTSAGSTQ PSGRCYFYVF DYQTKDGDYP QRMGTVHGED
DrosophilaCG13772      LRGG...... .......... .......... ...RAYFLHF KHKTIEEEYP QRSGSVRGED
HumanNLG4X-linked      QYGS...... .......... .......... ...PTYFYAF YHHCQSEMKP SWADSAHGDE
HumanNLG4Y-linked      QYGS...... .......... .......... ...PTYFYAF YHHCQSEMKP SWADSAHGDE
HumanNLG1              NFGS...... .......... .......... ...PTYFYAF YHHCQTDQVP AWADAAHGDE
HumanNLG3              RYGS...... .......... .......... ...PTYFYAF YHHCQSLMKP AWSDAAHGDE
HumanNLG2              DYQS...... .......... .......... ...PVYFYTF YHHCQAEGRP EWADAAHGDE
AplysiaNLG             DTAD...... .......... .......... ....TYLYSF AYSTQTEASG .DVQGIHGDE
DrosophilaCG31146      DVNR...... .......... .......... ...RNYMYVF GHNSATGPFA HLPHSIMGEE
C.elegansNLG           ADEVR..... .......... .......... KEANTFMFAF AHETRSWSQE QPNSGIRGSL

DrosophilaCG34127      ....LPYIFG APLVGG.... FNHFTRNYTK TEISLSEVVM FYWSNFVRTG NP.....NEQ
DrosophilaCG34139      ....LPYIFG APLVDG.... FSHFPQNYTK SETALSEAVM IFWTNFARTG NP...NEHHR
DrosophilaCG13772      ....VPFWLG LPMSP..... ..LFPHNYTT QERQIGRLML RYLSNFAKTG NPNQSTAKSV
HumanNLG4X-linked      ....VPYVFG IPMIGP.... TELFSCNFSK NDVMLSAVVM TYWTNFAKTG DPN.....QP
HumanNLG4Y-linked      ....VPYVFG IPMIGP.... TELFSCNFSK NDVMLSAVVM TYWTNFAKTG DPN.....QP
HumanNLG1              ....VPYVLG IPMIGP.... TELFPCNFSK NDVMLSAVVM TYWTNFAKTG DPN.....QP
HumanNLG3              ....VPYVFG VPMVGP.... TDLFPCNFSK NDVMLSAVVM TYWTNFAKTG DPN.....KP
HumanNLG2              ....LPYVFG VPMVGA.... TDLFPCNFSK NDVMLSAVVM TYWTNFAKTG DPN.....QP
AplysiaNLG             ....LPYVFG SPLVDG.... SSPFPSSYTN TEKMLSEAVM TYWTNFAKSG NPN....EPR
DrosophilaCG31146      ....LAFIFG APLAAAG... .PFPSGNYTV QEKLLSEAVM AYWTNFVKTG NPK.......
C.elegansNLG           SGDIVPYIFG YPLAQGD..S EERLYSGFNT DDKGISKVMM HYVSNFVKSG DPSKPNPMSK

DrosophilaCG34127      METEHGSRQE RSRYKT.... .......... .......... .......... ..........
DrosophilaCG34139      QDSSLPVSKE RNRFRS.... .......... .......... .......... ..........
DrosophilaCG13772      LPNPNEVLET ALHQQKKRST SLTHPNLSEA LNLAVLYNQR RSNAMHEKRS YIRRRLRSND
HumanNLG4X-linked      VPQDTKFIHT KPNR...... .......... .......... .......... ..........
HumanNLG4Y-linked      VPQDTKFIHT KPNR...... .......... .......... .......... ..........
HumanNLG1              VPQDTKFIHT KPNR...... .......... .......... .......... ..........
HumanNLG3              VPQDTKFIHT KANR...... .......... .......... .......... ..........
HumanNLG2              VPQDTKFIHT KPNR...... .......... .......... .......... ..........
AplysiaNLG             NQTSVHGDKV NNRFVD.... .......... .......... .......... ..........
DrosophilaCG31146      APWKGIFINS HALEWDR... .......... .......... .......... ..........
C.elegansNLG           NFPMGDVFHS T......... .......... .......... .......... ..........

DrosophilaCG34127      .......... .......... ..IEWTAYE. SVHKKYLNFD TKPKLKNHYR AHRLSFWLNL
DrosophilaCG34139      .......... .......... ..ITWENYD. PLHQKYLEIG MKPRIKNHFR AHQLSIWLRL
DrosophilaCG13772      AAFTQLGISS ERDVGSYDGD ELPFWDAYD. VVNQLYVELG NKANIQSHYR GHKLSMWLNL
HumanNLG4X-linked      .......... .........F EEVAWSKYN. PKDQLYLHIG LKPRVRDHYR ATKVAFWLEL
HumanNLG4Y-linked      .......... .........F EEVAWSKYN. PKDQLYLHIG LKPRVRDHYR ATKVAFWLEL
HumanNLG1              .......... .........F EEVAWTRYS. QKDQLYLHIG LKPRVKEHYR ANKVNLWLEL
HumanNLG3              .......... .........F EEVAWSKYN. PRDQLYLHIG LKPRVRDHYR ATKVAFWKHL
HumanNLG2              .......... .........F EEVVWSKFN. SKEKQYLHIG LKPRVRDNYR ANKVAFWLEL
AplysiaNLG             .......... .......... ..LKWPKYT. RDTQEYLPIG RRPSIRHHYR AQKLALWLDL
DrosophilaCG31146      .......... .......... YDLDWPEFN. RRAQAYLNIG IPPTVGYKYR QIYMNFWNKE
C.elegansNLG           .......... .......... ...AWPQFDQ PNREAYLEIT DRPRVKNYYR NAQVGFWNNF

DrosophilaCG34127      IPDLHKPGGD NVPAAHHQLH DDDDEDNNIP S.....DASV KPLNPPYISR AANAAAMANF
DrosophilaCG34139      IPELHRAGME DVIARHNLFR NHDDMDLYEG .........P VKPDPFGIST AAGASGSSSS
DrosophilaCG13772      IPQLHRHFNI NDQSMRHHQF QDDMNNRDLY EGVVRPQLQT KPAEDDNIII MQRSRTTTPP
```

```
HumanNLG4X-linked      VPHLHNLNEI FQYVSTTTKV PPPDMTSFPY G......... .......TRR SPAKIWPTTK
HumanNLG4Y-linked      VPHLHNLNEI FQYVSTTTKV PPPDMTSFPY G......... .......TRR SPAKIWPTTK
HumanNLG1              VPHLHNLNDI SQYTSTTTKV PSTDITFRPT R......... .......KNS VPVTSAFPTA
HumanNLG3              VPHLYNLHDM FHYTSTTTKV PPPDTTHSSH I......... .......TRR .PNGKTWSTK
HumanNLG2              VPHLHNLHTE LFTTTTRLPP YATRWPPRPP A......... .......GAP GTRRPPPPAT
AplysiaNLG             IPKINRDDGS EHSSHQLLDS DNMSTFDEHE R......... .......... ..........
DrosophilaCG31146      LPDELNQIAA IQEQLQKPGQ EVITGHMSKY GPRDHGAEDP VRTLKLLLQE PSLAGPTQSG
C.elegansNLG           IPQLHKNGKE TEPVGEEHHL LSDHFRKDSY FG........ ..KTRHFSSY ANLPFPPPPM

DrosophilaCG34127      TIFTNQVFSL LNLSSPSSSL QRNGTRYGGG KIYPDDMMD. .......... ..........
DrosophilaCG34139      SSSSSRLLLV DEQLMMKKGR GLNGSAYLNG ILGVTTVEPN NMYTTCIPIG GNYSSSGGVF
DrosophilaCG13772      PPSKNPSTNA TQALNPLGTG TPTTTECGID GAMSVSELTT TQSPKDNRTG IVRVETQKDL
HumanNLG4X-linked      RPAITPANNP KHSKDPHKTG PEDTT..... .......... .......... ..........
HumanNLG4Y-linked      RPAITPANNP KHSKDPHKTG PEDTT..... .......... .......... ..........
HumanNLG1              KQDDPKQQPS PFSVD..... .......... .......... .......... ..........
HumanNLG3              RPAISPAYSN ENAQGSWNGD QDAGP..... .......... .......... ..........
HumanNLG2              LPPEPEPEPG PRAYDRFPGD .......... .......... .......... ..........
AplysiaNLG             .......... .......... .......... .......... .......... ..........
DrosophilaCG31146      ESTETAAENM YNAPPTFGHV HKMQGGSDFE DLVTSTN... .......... ..........
C.elegansNLG           PPSPPPELTT KPKPSESPTT LQTTTE.... .......... .......... ..........

DrosophilaCG34127      .....GDHAG GAASASQDSD GFAAYSTALS VTIAIGCSLL ILNVLIFAGV YYQRDKTRLS
DrosophilaCG34139      APTTLANASS DTLASGFEAA GYAAYSTALS VTIAIGCSLL ILNVLIFAGV YYQRDKTRLE
DrosophilaCG13772      ATASTGIIGN LELLRRLGGK QFQSYTTALI ATVAVGCFLL ILNVLIFAGI YHQREKRARD
HumanNLG4X-linked      .......... ......VLIE TKRDYSTELS VTIAVGASLL FLNILAFAAL YYKKDKRRHE
HumanNLG4Y-linked      .......... ......VLIE TKRDYSTELS VTIAVGASLL FLNILAFAAL YYKKDKRRHE
HumanNLG1              .......... .......... .QRDYSTELS VTIAVGASLL FLNILAFAAL YYKKDKRRHD
HumanNLG3              .......... ......LLVE NPRDYSTELS VTIAVGASIL FLNVLAFAAL YYRKDKRRQE
HumanNLG2              .......... .......... .SRDYSTELS VTVAVGASLL FLNILAFAAL YYKRDRRQEL
AplysiaNLG             .......... .....LIPPL PLSPPPLSLS LSLSLSLSLS LSLSLSFSLW AFLTFVLYKG
DrosophilaCG31146      ......SLES GEDHPQAPPE TVAKSEATMQ LLIALITIII VLNLLIYGTF LLRQRRRRAK
C.elegansNLG           .......... .....SEKAA AGSFTGKALG GVIFIGCGFL IMNVCLLIAV RREWGKKRRN

DrosophilaCG34127      EPRPQTKLKR QENGQMPNNI CGDLETLTIH AKSDPATILS HHHAMQHHQL PPPEFADIPH
DrosophilaCG34139      VKTLQKQYQQ RSLHQQVPYP PEPIKHAHYH MGHSQSSANV IVDVESHQDQ AGQAAMLLQA
DrosophilaCG13772      AKTKEELQEG DNSKNSSILK LNALMGGGGA CGSGPGDIAD AYTLSGSVVD SKGGVGVVFG
HumanNLG4X-linked      THRRPSPQRN TTN....... DIAHIQNEEI MSLQMKQLEH DHECESLQAH DTLR.LTCPP
HumanNLG4Y-linked      THRHPSPQRN TTN....... DITHIQNEEI MSLQMKQLEH DHECESLQAH DTLR.LTCPP
HumanNLG1              VHRRCSPQRT TTN....... DLTHAQEEEI MSLQMKHTDL DHECESIHPH EVVLRTACPP
HumanNLG3              PLRQPSPQRG AWA....... PELGAAPE.E ELAALQLGPT HHECEASPPH DTLR.LTALP
HumanNLG2              RCRRLSPPGG SGSGVPGGGP LLPAAGRELP PEEELVSLQL KRGGGVGADP AEALRPACPP
AplysiaNLG             RKKDSTPLSV QDHLLYFQPT LPHLSEVCLQ ISFFSRSPTS FLQFPLVIPS QASPCHTVGW
DrosophilaCG31146      ALPFPAPKLG GTILSYDGAN DEELKRCSKS RDGDDSFVLE MTRKSNTYEA IKTGQRSLSC
C.elegansNLG           EKKFQLQYQT YNSNHGGGAE QYNSLNSPEP LLSASHKNST SMRPAGISPT CPRHGRAALA

DrosophilaCG34127      RAPPPPKHMK SLQDPGGGGS VSGAVGVSGV VQMPPPHALQ VMGNQCGTLT KKSCMKQTQA
DrosophilaCG34139      AAAAAAAAAN DVKPPPPHIC SNTGMQQQQQ VGGGSGGALN NGGIEGSKVT TDNLGNVTYS
DrosophilaCG13772      EYSCYDEKTK QLKEEKLLVE LPPSSSTGQT SLMIDNWGAC STSTLDLLKT KPGDPIEMVT
HumanNLG4X-linked      DYTLTLRRSP DDIPLMTPNT ITMIPNTLTG .......... .......... ..........
HumanNLG4Y-linked      DYTLTLRRSP DDIPFMTPNT ITMIPNTLMG .......... .......... ..........
HumanNLG1              DYTLAMRRSP DDVPLMTPNT ITMIPNTIPG .......... .......... ..........
HumanNLG3              DYTLTLRRSP DDIPLMTPNT ITMIPNSLVG .......... .......... ..........
HumanNLG2              DYTLALRRAP DDVPLLAPGA LTLLPSGLGP PPPPP..... .......... ..........
AplysiaNLG             LSNSVFNPQP SSFS...... .......... .......... .......... ..........
DrosophilaCG31146      STVDTHTKVC EWMSSQEAPK SGSFNTATPP PQPILSDGRL IICQDIEVAD AALLIPQHMH
C.elegansNLG           LQNSRGNSLT AAQAPTLEEI QV........ .......... .......... ..........

DrosophilaCG34127      QAQQHSPAQQ QQQQQQQQQQ HVVTHQQHLQ NHHNQSMTTV LTTAGGLVVP TPPTVPVIVA
DrosophilaCG34139      TSSKQQQQHQ QQQQQQQREH MQIKGMTGTQ TFGRSGSGSG GGAGGGAGSA SGGASVVVSG
DrosophilaCG13772      YSALPSVMES TSAMSSKRGS FVDTTLQFNS PQQFDYAVQS SDQMSFKAIE DAVKAAAGHD
HumanNLG4X-linked      .......... .......... .......... .......... .......... ..........
HumanNLG4Y-linked      .......... .......... .......... .......... .......... ..........
HumanNLG1              .......... .......... .......... .......... .......... ..........
HumanNLG3              .......... .......... .......... .......... .......... ..........
HumanNLG2              .......... .......... .......... .......... .......... ..........
AplysiaNLG             .......... .......... .......... .......... .......... ..........
```

```
DrosophilaCG31146      EPQHYEMLLQ RQHSALTEPS DEILQQQYPL RNHSHSHSDP VDMILAADEQ VTSFVHADDV
C.elegansNLG           .......... .......... .......... .......... .......... ..........

DrosophilaCG34127      STTQHQQ... .......... .......... .......... .......... ..........
DrosophilaCG34139      SSVSYNPGMM TLPKSGGLHH AATLNYARNT TALNMSGGGT ALVDSRGNVL LTSTAVGPGM
DrosophilaCG13772      SEITRDDDIP EPPPPPRSFL AAQQQQQQQQ QQQQTG.... .......... ..........
HumanNLG4X-linked      .......... .......... .......... .......... .......... ..........
HumanNLG4Y-linked      .......... .......... .......... .......... .......... ..........
HumanNLG1              .......... .......... .......... .......... .......... ..........
HumanNLG3              .......... .......... .......... .......... .......... ..........
HumanNLG2              .......... .......... .......... .......... .......... ..........
AplysiaNLG             .......... .......... .......... .......... .......... ..........
DrosophilaCG31146      DINVTSRDDS DGLEVIPLTS AQQLELLRQR NYPKVLPTEQ DLINSSYKRN SLPPQNFNAP
C.elegansNLG           .......... .......... .......... .......... .......... ..........

DrosophilaCG34127      .......... .......... .......... .......... .......... ..........
DrosophilaCG34139      AGTGAGGGSS GGGGGGDCMT LPRNLGLAAA GRHNPTTELQ QYQQQQQSSK HQSNGAVLTG
DrosophilaCG13772      .......... .......... .......... .......... .......... ..........
HumanNLG4X-linked      .......... .......... .......... .......... .......... ..........
HumanNLG4Y-linked      .......... .......... .......... .......... .......... ..........
HumanNLG1              .......... .......... .......... .......... .......... ..........
HumanNLG3              .......... .......... .......... .......... .......... ..........
HumanNLG2              .......... .......... .......... .......... .......... ..........
AplysiaNLG             .......... .......... .......... .......... .......... ..........
DrosophilaCG31146      LPPPRTISNT LGRRRRDSSN ITTSPLQVAR DCGGEDEDLK EPQITQNTLI VGPIVPKSPA
C.elegansNLG           .......... .......... .......... .......... .......... ..........

DrosophilaCG34127      .......... .......... .......... .......... .......... ..........
DrosophilaCG34139      MQSHHIRGPR PPLRTASSTT TNSSSNNSAM GVAVGVGGGV GNMLLDQTPS GGSSSSGVSS
DrosophilaCG13772      .......... .......... .......... .......... .......... ..........
HumanNLG4X-linked      .......... .......... .......... .......... .......... ..........
HumanNLG4Y-linked      .......... .......... .......... .......... .......... ..........
HumanNLG1              .......... .......... .......... .......... .......... ..........
HumanNLG3              .......... .......... .......... .......... .......... ..........
HumanNLG2              .......... .......... .......... .......... .......... ..........
AplysiaNLG             .......... .......... .......... .......... .......... ..........
DrosophilaCG31146      SSLKRVKRMP ESSAMTALSG SFQSFEAVPP AHETTPPQGG ERTECIYAIR PSPGSCSWAA
C.elegansNLG           .......... .......... .......... .......... .......... ..........

DrosophilaCG34127      .......... .......... .......... ......HQQQ HQQQQHQQQQ QQQQQQHPLM
DrosophilaCG34139      APSSSKGHHT HSHSHAAHLV HSHGDGLVLT SASPVGGQQQ QHPQQHQHPQ QQQQVPQAAM
DrosophilaCG13772      .......... .......... .......... ....ILRQTG AGGTSTSGSA SGSGKKRVHI
HumanNLG4X-linked      .......... .......... .......... ..MQPLHTFN TFSGGQNSTN .....LPHGH
HumanNLG4Y-linked      .......... .......... .......... ..MQPLHTFK TFSGGQNSTN .....LPHGH
HumanNLG1              .......... .......... .......... ..IQPLHTFN TFTGGQNNTL PHPHPHPHSH
HumanNLG3              .......... .......... .......... ..LQTLHPYN TFAAGFNSTG .....LPHSH
HumanNLG2              .......... .......... .......... ..PPSLHPFG PFPPPPPTAT SHNNTLPHPH
AplysiaNLG             .......... .......... .......... .......... .......... ..........
DrosophilaCG31146      PNGDLYAQPM KSSSRNSLIP RPTKPAESQS QATPAGPAGE SLGSTGMATA SRIPQLQRQA
C.elegansNLG           .......... .......... .......... .......... .......... ..........

DrosophilaCG34127      DELRV..... .......... .......... .......... .......... ..........
DrosophilaCG34139      DEMRV..... .......... .......... .......... .......... ..........
DrosophilaCG13772      QEISV..... .......... .......... .......... .......... ..........
HumanNLG4X-linked      STTRV..... .......... .......... .......... .......... ..........
HumanNLG4Y-linked      STTRV..... .......... .......... .......... .......... ..........
HumanNLG1              STTRV..... .......... .......... .......... .......... ..........
HumanNLG3              STTRV..... .......... .......... .......... .......... ..........
HumanNLG2              STTRV..... .......... .......... .......... .......... ..........
AplysiaNLG             .......... .......... .......... .......... .......... ..........
DrosophilaCG31146      SGKDLQARTA TDNTANPLGC QPRTDSTISS GSSASYASTT DSSSSSSTGT VRTELQQFQP
C.elegansNLG           .......... .......... .......... .......... .......... ..........

DrosophilaCG34127      ..........
DrosophilaCG34139      ..........
```

```
DrosophilaCG13772     ..........
HumanNLG4X-linked     ..........
HumanNLG4Y-linked     ..........
HumanNLG1             ..........
HumanNLG3             ..........
HumanNLG2             ..........
AplysiaNLG            ..........
DrosophilaCG31146     APGRSITTNI
C.elegansNLG          ..........
```

D. Sequence Alignment of NRX

```
DrosophilaNRX     MKAPHSATYQ DNYADAAMTA RTRPSMDMDQ QRNRNQAELR LLPAQRTSTS AFESPDLRFN
HumanNRX1         .......... .......... .......... .......... .......... ..........
HumanNRX3         .......... .......... .......... .......... .......... ..........
HumanNRX2         .......... .......... .......... .......... .......... ..........
AplysiaNRX        .......... .......... .......... .......... .......... ..........
C.elegansNRX      .......... .......... .......... .......... .......... ..........

DrosophilaNRX     KARRRRRSEQ VPPVVVEYRR SYRVLIVSAL LVSLAASFVT LSAGFQLDGS QNSFYTFRKW
HumanNRX1         .......... ....MGTALL QRGGCFLLCL SLLLLGCWAE LGSGLEFPGA EGQWTRFPKW
HumanNRX3         .......... .......MSS TLHSVFFTLK VSILLGSLLG LCLGLEFMGL PNQWARYLRW
HumanNRX2         .......... ......MASG SRWRPTPPPL LLLLLALAA RADGLEFGGG PGQWARYARW
AplysiaNRX        .........M AVELFPFSPR HLQCLTLAVA IFAAILCRLP CAHTLNLDGS PGSYVEYPPW
C.elegansNRX      .......... ......MRQK FDDTGQYLTI WLVFTVSLFS VVDSIILTGA PDSYARYPKW

DrosophilaNRX     Y.TGLNGTLE LEFKTEQPNG LVLYTDDGGT YDFFELKLVE G.ALRLRYNL G.GGAQIIT.
HumanNRX1         N.ACCESEMS FQLKTRSARG LVLYFDDEGF CDFLELILTR GGRLQLSFSI FCAEPATLL.
HumanNRX3         D.ASTRSDLS FQFKTNVSTG LLLYLDDGGV CDFLCLSLVD G.RVQLRFSM DCAETAVLS.
HumanNRX2         AGAASSGELS FSLRTNATRA LLLYLDDGGD CDFLELLLVD G.RLRLRFTL SCAEPATLQ.
AplysiaNRX        E.PCLNGSFS FEFRTSEPLS LLLYLNR.GS YSYFEVKLLK G.GIRLRMNL G.ERTMIIR.
C.elegansNRX      A.HSFENSLS MELKTRQSDG MLLYTDDGGT HGNFYSLTIV EGHIQLDFRL GDNSNEFGQR

DrosophilaNRX     ........VG RELHDGHWHK VQVLRNDEQT SLIVDGVSQQ RSTKGKEFQF GKFASNSDVY
HumanNRX1         ........AD TPVNDGAWHS VRIRRQFRNT TLFIDQVEAK WVEVKSKRRD ..MTVFSGLF
HumanNRX3         ........NK Q.VNDSSWHF LMVSRDRLRT VLMLD.GEGQ SGELQPQRPY ..MDVVSDLF
HumanNRX2         ........LD TPVADDRWHM VLLTRDARRT ALAVD.GEAR AAEVRSKRRE ..MQVASDLF
AplysiaNRX        ........AG QNLNDNKWHS VEVVQDGDLT TLIVDGIEHS KTSSGLLHTY NGLANETFLY
C.elegansNRX      RPVNTIRIEE VRIDDDKWHT LTIFQSWENV KLELDYTLVF KILNQRSFVF GNILKNSDVF

DrosophilaNRX     VGGMPNWYS. SKLALLALPS VIFEPRFRGA IRNLVYADQP GGSTRRQEIK QQRDIKCGDV
HumanNRX1         VGGLPPELR. AAALKLTLAS VREREPFKGW IRDVRVNSSQ VLPVDSGEVK LDDEPPNSGG
HumanNRX3         LGGVPTDIR. PSALTLDGVQ AMPG..FKGL ILDLKYGNSE PRLLGSRGVQ MDAEGPCGER
HumanNRX2         VGGIPPDVR. LSALTLSTVK YEPP..FRGL LANLKLGERP PALLGSQGLR GATADPLCAP
AplysiaNRX        LGGLPMEYES KKFNSLALPS VIFEPKFRGS VRNFFYRSCG GEAVRPQPLS SAGLLTTDVD
C.elegansNRX      IGGLPPNMH. .MLPVMSSPL RRYARHLAVN VRNLMYRQYP QGVTSPQLLE SVGTRTNEDD

DrosophilaNRX     PCDHGELPAR ERPLRGVRGG NTTDACERND PCQHGGICIS TDS.GPICEC RNLEYDGQYC
HumanNRX1         GSPC...... .......... .EAGEEGEGG VCLNGGVCSV VDD.QAVCDC SRTGFRGKDC
HumanNRX3         P......... .......... .......... .CENGGICFL LDG.HPTCDC STTGYGGKLC
HumanNRX2         ARN....... .......... .......... PCANGGLCTV LAPGEVGCDC SHTGFGGKFC
AplysiaNRX        LCER...... .......... ........GN PCLNGGRCLT TDN.GVLCDC TATEYRGDRC
C.elegansNRX      HCKS...... .......... .KSMSSREQF VCLNDGECYS SND.GPHCDC QFSDHDGRNC

DrosophilaNRX     EKE....... .......... .......... .......... ...KAPSEAT FRGTQFLSYD
HumanNRX1         SQEDNNVEGL AHLMMG.... .......... ......DQGK SKGKEEYIAT FKGSEYFCYD
HumanNRX3         S......EGL SHLMMS.... .......... ......EQAR ....EENVAT FRGSEYLCYD
HumanNRX2         SEEEHPMEGP AHLTLNSEVG SLLFSEGGAG RGGAGDVHQP TKGKEEFVAT FKGNEFFCYD
AplysiaNRX        DIQ....... .......... .......... .......... ...KIPSDAT FMGTQYFSYN
C.elegansNRX      EIE....... .......... .......... .......... ...KNDGELT FGGDEWVGYD

DrosophilaNRX     LGQTGAEPIV SAQDAISFYF RTRQPNGLLF YTGHG..... ......TDYL NLALRDGGVS
```

```
HumanNRX1          LSQN...PIQ SSSDEITLSF KTLQRNGLML HTGKS..... ......ADYV NLALKNGAVS
HumanNRX3          LSQN...PIQ SSSDEITLSF KTWQRNGLIL HTGKS..... ......ADYV NLALKDGAVS
HumanNRX2          LSHN...PIQ SSTDEITLAF RTLQRNGLML HTGKS..... ......ADYV NLSLKSGAVW
AplysiaNRX         LSSR..GDVM VDQDRVRLDF RTKQANSLLF YTGNS..... ......KDYM TVGLMDGAVF
C.elegansNRX       VSLNISAAVR AKKENLTLTF KTVHGTSMLF YAGDE..... ......KSYL HVMLQDGAII

DrosophilaNRX      LTMGLANG.. .KQEMHIKPS KVRFDDHQWH KVTVHRRIQE ISSITSFCR. .....LVTVV
HumanNRX1          LVINLGSG.. .AFEALVEPV NGKFNDNAWH DVKVTRNLRQ HSGIGHAMV. ......TISV
HumanNRX3          LVINLGSG.. .AFEAIVEPV NGKFNDNAWH DVKVTRNLRQ VT........ .......ISV
HumanNRX2          LVINLGSG.. .AFEALVEPV NGKFNDNAWH DVRVTRNLRQ HAGIGHAMVN KLHYLVTISV
AplysiaNRX         LTINLGSG.. .MYQAEIRPS GTRFDDNRWH QLLIQREARE LPRDAGVCFV T......MEL
C.elegansNRX       ASSKFDGSDA RIIRMFNSFP SQRYDDDSWH TIILERSLQM MT........ .......LIV

DrosophilaNRX      DDVYTDHSHI AGKFTMLSSS .RVYVGGAVN PRALLGARVH TNFVGCLRKV EFSADTLNLN
HumanNRX1          DGILTTTGYT QEDYTMLGSD DFFYVGGSPS TADLPGSPVS NNFMGCLKEV VYKNNDVRLE
HumanNRX3          DGILTTTGYT QEDYTMLGSD DFFYVGGSPS TADLPGSPVS NNFMGCLKEV VYKNNDIRLE
HumanNRX2          DGILTTTGYT QEDYTMLGSD DFFYIGGSPN TADLPGSPVS NNFMGCLKDV VYKNNDFKLE
AplysiaNRX         DGMYRKQGSI TGKFIRLSSN .LLYVAGSPN TETLPGSRVR TNFKGCLRKV RYRADGVLLD
C.elegansNRX       DGRRDEIRQY APELDWISNS ..FGFLGSIP KHNPSKEVNR VSFRGCMKKV RYDIDATRIL

DrosophilaNRX      LIDLAKS..G SKLIQVAGNL EYQ.CPSGDP QDPVTFTTRE SHLVLPPWET GKQ.SSISFK
HumanNRX1          LSRLAKQ..G DPKMKIHGVV AFK.CENVAT LDPITFETPE SFISLPKWNA KKT.GSISFD
HumanNRX3          LSRLARI..A DTKMKIYGEV VFK.CENVAT LDPINFETPE AYISLPKWNT KRM.GSISFD
HumanNRX2          LSRLAKE..G DPKMKLQGDL SFR.CEDVAA LDPVTFESPE AFVALPRWSA KRT.GSISLD
AplysiaNRX         LTDLARR..E HSLMQVTGDV IFDKCQELVE SHPVTFTSPE SHLTIPTWSK PGIRGSLAFQ
C.elegansNRX       LVNLADQSYG GSVVKTEGDI SYSCKNPSQR SDVLSFTETS SYLTLPRWNS LSS.GSLSFH

DrosophilaNRX      FRTKEPNGII VLATG..... ..SKQPRAKN PVLIAIELLN GHIYIHLDLG SGASKVRASR
HumanNRX1          FRTTEPNGLI LFSHGKPRHQ KDAKHPQMIK VDFFAIEMLD GHLYLLLDMG SGTIKIKALL
HumanNRX3          FRTTEPNGLI LFTHGKPQER KDARSQKNTK VDFFAVELLD GNLYLLLDMG SGTIKVKATQ
HumanNRX2          FRTTEPNGLL LFSQGRRAGG GAGSHSSAQR ADYFAMELLD GHLYLLLDMG SGGIKLRASS
AplysiaNRX         FRTVEPGGLM MYSNG..... ......GRDS KDFFALELLD GHLYLVLDMG SGILKIQASK
C.elegansNRX       FRTTSSDGLI LYHGV..... .....MQHNA TDYVAFELID SHLFMIINLG SGVVRLQTTS

DrosophilaNRX      RRVDDGDWHD LILRRNGRDA KVSVDGVWND FRTPGDGTIL ELDGHMYLGG VGPA.YNSVS
HumanNRX1          KKVNDGEWYH VDFQRDGRSG TISVNTLRTP YTAPGESEIL DLDDELYLGG LPEN.KAGLV
HumanNRX3          KKANDGEWYH VDIQRDGRSG TISVNSRRTP FTASGESEIL DLEGDMYLGG LPEN.RAGLI
HumanNRX2          RKVNDGEWCH VDFQRDGRKG SISVNSRSTP FLATGDSEIL DLESELYLGG LPEGGRVDLP
AplysiaNRX         LPVSDGRPHD VYFEFKGYRG DISVDGQKVP FASGRVSDRF DLHGFFYVGG LGSE.INASL
C.elegansNRX       MKVSDGEWHH VQLDRLSRTG SVIVDAIKID FSTPGVSANL IIDDPIFIGN VPNN...SLV

DrosophilaNRX      WPAAIWTATL RQGFVGCLRD LVLSGKAIDI AAFARVQDSA SVKPSCHVQA N..VCNGNPC
HumanNRX1          FPTEVWTALL NYGYVGCIRD LFIDGQSKDI RQMAEVQSTA GVKPSCSKET AK.PCLSNPC
HumanNRX3          LPTELWTAML NYGYVGCIRD LFIDGRSKNI RQLAEMQNAA GVKSSCSRMS AK.QCDSYPC
HumanNRX2          LPPEVWTAAL RAGYVGCVRD LFIDGRSRDL RGLAEAQGAV GVAPFCSRET LK.QCASAPC
AplysiaNRX         LPRELWAVML GLSYVGCLQD LVLDGSKVDL LAAARLQNKS DVAGYCKQTE P..QCVSHPC
C.elegansNRX       YPSSVWSIAL QKGYTGCIKN IRMNGVSTKI GQEFEASNST GIELGCSLSN ELDICEPNPC

DrosophilaNRX      LNGGTCLEGW NRPICDCSAT LYGGPTCGRE LATLAFNGSQ HMTIWLGNGQ GTKTQTEELV
HumanNRX1          KNNGMCRDGW NRYVCDCSGT GYLGRSCERE ATVLSYDGSM FMKIQLP..V VMHTEAEDVS
HumanNRX3          KNNAVCKDGW NRFICDCTGT GYWGRTCERE ASILSYDGSM YMKIIMP..M VMHTEAEDVS
HumanNRX2          RNGGVCREGW NRFICDCIGT GFLGRVCERE ATVLSYDGSM YMKIMLP..N AMHTEAEDVS
AplysiaNRX         NHGGKCVEGW NRFSCDCRAT GFVGAVCQTA AATLQFDGTQ FMKVTMS..R ESVTQAEDVS
C.elegansNRX       QNFGKCSKNL NSFDCDCSNT NFEGKQCELE QTAVEVNGEE SKVHVLA..H TKVSQVEHIK

DrosophilaNRX      IRFKTSRPAG LLLLTSAESN SPDRLEIALV AGRVRASVRL SDR....... ..EKNLLAGQ
HumanNRX1          LRFRSQRAYG ILMATTSR.D SADTLRLELD AGRVKLTVNL DCIRINCNSS KGPETLFAGY
HumanNRX3          FRFMSQRAYG LLVATTSR.D SADTLRLELD GGRVKLMVNL DCIRINCNSS KGPETLYAGQ
HumanNRX2          LRFMSQRAYG LMMATTSR.E SADTLRLELD GGQMKLTVNL DCLRVGCAPS KGPETLFAGH
AplysiaNRX         LRFRSLHPSG LLFLTTGG.. NDNRMQLFLQ QGTLFLSVNV GSG....... ..SKVLSVGH
C.elegansNRX       IRFRTTSSRG VLFDTGAN.G KNDKITVFLN DSQLNLFLQD SSTN...... ....NTFSWG
```

```
DrosophilaNRX      SVLNDNNWHT IRFSRRASNL RLQVDG.... .......... .......... ..APPVRAET
HumanNRX1          NLN.DNEWHT VRVVRRGKSL KLTVD..... .......... .......... ..DQQAMTGQ
HumanNRX3          KLN.DNEWHT VRVVRRGKSL KLTVD..... .......... .......... ..DDVAEG.T
HumanNRX2          KLN.DNEWHT VRVVRRGKSL QLSVD..... .......... .......... ..NVTVEG.Q
AplysiaNRX         RLN.DDRWHT VFIRRRVQTV ELAIDSDRPV IDQLPGSTFS LATNYIFVGH KNPPGTEVAD
C.elegansNRX       KSLSDNHWHE LQVRRLGQKL LLYLDG.... .......... .......... ......FWSH

DrosophilaNRX      ILGRHSTMEI RSVHLGGLF. ..HAEEEIQM TSTMPNFVGQ MQGLVFNGQR YLDIVKSLGP
HumanNRX1          MAGDHTRLEF HNIETG.... ..IITERRYL SSVPSNFIGH LQSLTFNGMA YIDLCKNGDI
HumanNRX3          MVGDHTRLEF HNIETG.... ..IMTEKRYI SVVPSSFIGH LQSLMFNGLL YIDLCKNGDI
HumanNRX2          MAGAHMRLEF HNIETG.... ..IMTERRFI SVVPSNFIGH LSGLVFNGQP YMDQCKDGDI
AplysiaNRX         LPGRARELGL SSVEAGHMVQ DDSGAGTGHS GSDHSGFIGS MQAFIFNNNH FFQMAKSGTG
C.elegansNRX       SIYLQNPISV EIDEVG.... AAFSVHSSAP PPRDEHFKGF LSKLVFNGND YLAKTKMNSA

DrosophilaNRX      ELSALPSATF KLTAR.FVNS PAGQPYHAAT FRSKHSYVGL AMLKAYNSIS IDFRFKTVEP
HumanNRX1          DYCELN.... ......ARFG FRNIIADPVT FKTKSSYVAL ATLQAYTSMH LFFQFKTTSL
HumanNRX3          DYCELK.... ......ARFG LRNIIADPVT FKTKSSYLSL ATLQAYTSMH LFFQFKTTSP
HumanNRX2          TYCELN.... ......ARFG LRAIVADPVT FKSRSSYLAL ATLQAYASMH LFFQFKTTAP
AplysiaNRX         DNIEIS.... .....ARFSS DEYVVRDPVT FKSVDAFAIL PTLQAHEEFS VSLQLKTTES
C.elegansNRX       QLSKSSSRES KGQ...RNVK TRIASISFTN STGYVAFSSD KISGLTGSFR VQFKFQTLMR

DrosophilaNRX      NGLLVFNG.. GRRNDFVAVE LVNGHIHYTF DLGDG...PV TMRDKSRIHM NDNRWHQVSI
HumanNRX1          DGLILYNS.. GDGNDFIVVE LVKGYLHYVF DLGNG...AN LIKGSSNKPL NDNQWHNVMI
HumanNRX3          DGFILFNS.. GDGNDFIAVE LVKGYIHYVF DLGNG...PN VIKGNSDRPL NDNQWHNVVI
HumanNRX2          DGLLLFNS.. GNGNDFIVIE LVKGYIHYVF DLGNG...PS LMKGNSDKPV NDNQWHNVVV
AplysiaNRX         DGLILYNG.. GNENDFFALE LFQGFLYYVY NMGEG...AQ RVKANVQHPI NDNKWHEARI
C.elegansNRX       SALLFFTLPK HDYDQSFRLQ MLNGRLKYTY RTSGQEFHTT SPKLPHRQHL SDMRWHNVLI

DrosophilaNRX      RRPGPKTHTL TVDDSFEIIS LTGNN..MHL ELAGILYIGG VFKDMYSKLP ASISSRSGFE
HumanNRX1          SRDTSNLHTV KIDTKITTQI TAGA...RNL DLKSDLYIGG VAKETYKSLP KLVHAKEGFQ
HumanNRX3          TRDNSNTHSL KVDTKVVTQV INGA...KNL DLKGDLYMAG LAQGMYSNLP KLVASRDGFQ
HumanNRX2          SRDPGNVHTL KIDSRTVTQH SNGA...RNL DLKGELYIGG LSKNMFSNLP KLVASRDGFQ
AplysiaNRX         FRVEKFTQLL RVDDNTPTVD DLTGTKNNRF DLDGFLYIGG VRKTMYPSLP KLVYSRHGFV
C.elegansNRX       YQDEKTHDHV LLVDNSSTTL IIDKIKKVES KMSGKLYFG. ......SNPL GVSRPSNGFR

DrosophilaNRX      GCLASLDLGD ASPSLTSDAV VPSSL..... .......VVS GCEGPT.... ....KCSQNA
HumanNRX1          GCLASVDLNG RLPDLISDAL FCNGQ..... .......IER GCEGPS.... ...TTCQEDS
HumanNRX3          GCLASVDLNG RLPDLINDAL HRSGQ..... .......IER GCEGPS.... ...TTCQEDS
HumanNRX2          GCLASVDLNG RLPDLIADAL HRIGQ..... .......VER GCDGPS.... ...TTCTEES
AplysiaNRX         GCLGSLNLNG YLPNVLREAN PIHES..... .......VGD GCRGPMS... ....KCVNDS
C.elegansNRX       GCISTLRINE NALDLYEDAD SRMKVNRGCS VLTLFELFQF ECKLLNVYNS GPIARCIEDA

DrosophilaNRX      CANRGNCVQQ WNAYACECDM TSYTGPTCYD ESIAYEFGNN KGMVQYTFPE NAQADTEEDN
HumanNRX1          CSNQGVCLQQ WDGFSCDCSM TSFSGPLCND PGTTYIFSKG GGQITYKWPP NDRPSTRADR
HumanNRX3          CANQGVCMQQ WEGFTCDCSM TSYSGNQCND PGATYIFGKS GGLILYTWPA NDRPSTRSDR
HumanNRX2          CANQGVCLQQ WDGFTCDCTM TSYGGPVCND PGTTYIFGKG GALITYTWPP NDRPSTRMDR
AplysiaNRX         CANQGRCVQQ WTSYKCDCDM TSFTGPMCKD ESVSYKFGPG PGLMTFAHLA DHEPSTNFDN
C.elegansNRX       CANHGRCIQL WSSIRCDCSL TAHSGDRCQN PSTTVRFDGS PSAIFYEYAP NERPTTSKDY

DrosophilaNRX      IALGFITTRP DAVLLRVESA TTQ.DYMELE IVEGNIFMVY NIGSVDLPLG EIGTKVNDNA
HumanNRX1          LAIGFSTVQK EAVLVRVDSS SGLGDYLELH IHQGKIGVKF NVGTDDIAIE ESNAIINDGK
HumanNRX3          LAVGFSTTVK DGILVRIDSA PGLGDFLQLH IEQGKIGVVF NIGTVDISIK EERTPVNDGK
HumanNRX2          LAVGFSTHQR SAVLVRVDSA SGLGDYLQLH IDQGTVGVIF NVGTDDITID EPNAIVSDGK
AplysiaNRX         LAFGFKTFLS DAILLRMDS. RAYDDFIQIE LADGYVYLVY NMGSFDHPLG DFYHKVNDGQ
C.elegansNRX       FVFSFRTTQP NGVLISIECA ADQ.DYFTIF LNKGYLNAHY NLGNRDHTTS YHTRILNDGF

DrosophilaNRX      YHVVRFQRKG GNATLQLDDY NVQALTPQS. .......... .......... .........H
HumanNRX1          YHVVRFTRSG GNATLQVDSW PVIERYPAG. .......... .......... .........R
HumanNRX3          YHVVRFTRNG GNATLQVDNW PVNEHYPTG. .......... .......... .........R
HumanNRX2          YHVVRFTRSG GNATLQVDSW PVNERYPAGN FDNERLAIAR QRIPYRLGRV VDEWLLDKGR
AplysiaNRX         YHVVRFTRAG PNATLQIDAD MPQTKHPTG. .......... .......... .........K
C.elegansNRX       PHVIKISRTE ANMTIQVDKL PSLRYRPKR. .......... .......... ........AS
```

```
DrosophilaNRX     HSTVFNTMSN VQVGGKFSRN GR........ ...NRIERPF AGVIAGLSVN KLRILDLAVE
HumanNRX1         QLTIFNSQAT IIIGGKEQGQ .......... ........PF QGQLSGLYYN GLKVLNMAAE
HumanNRX3         QLTIFNTQAQ IAIGGKDKGR .......... ........LF QGQLSGLYYD GLKVLNMAAE
HumanNRX2         QLTIFNSQAA IKIGGRDQGR .......... ........PF QGQVSGLYYN GLKVLALAAE
AplysiaNRX        QAHTFNNQAF VRIGGTKVEN .......... ...GSITQHF EGIISGLVLN GINVFELAKH
C.elegansNRX      DLVLLNMQTR ISIGAAFNTR HLDQKRLLRH RRNTEIFDSY QGEISGVNVN GLMILDLYEN

DrosophilaNRX     RDPHITIRGD VQLVTG.... .......... VLDRNDLQRM QQTPASGYPG ALDDLIFSGA
HumanNRX1         NDANIAIVGN VRLVGEVPS. .......... SMTTES.TAT AMQSEMSTS. .IMETTTTLA
HumanNRX3         NNPNIKINGS VRLVGEVP.. .......... SILGTT.QTT SMPPEMSTT. .VMETTTTMA
HumanNRX2         SDPNVRTEGH LRLVGEGPS. .......... VLLSAETTAT TLLADMATT. .IMETTTTMA
AplysiaNRX        EDPKVRLEGS VSLNQRPQPG PFRPPQLYTD DEDLDEMQST QGHTTPRDGV TDDDIIQAGA
C.elegansNRX      GSNRIHTIGA PQTTAVSEQV SSE.....SE EDDELAEMMM AHSIDENPNE ALIESLAPSC

DrosophilaNRX     GSGCRGDDED ECTPPFESGS GDDLITPVYV PPTKQTTTSQ QGNSLSTGGS SSGGVITNGT
HumanNRX1         TSTARRGKP. PTKEPISQTT DDILVASAEC PSDDE..... .......... ..........
HumanNRX3         TTTTRKNR.. ..STASIQPT SDDLVSSAEC SSDDE..... .......... ..........
HumanNRX2         TTTTRRGRSP TLRDSTTQNT DDLLVASAEC PSDDE..... .......... ..........
AplysiaNRX        GSGCQFDNSE DCSAVGSGIE EIITPVVIVK TTTPPPTTTP KPVPKCQ... ..........
C.elegansNRX      LSLEEQQSCF IDTDDSTGFF SPVLPTVANF PTTRQTEHID N......... ..........

DrosophilaNRX     ERACDDEDCV HGSG...... ....DYGETT EQFTSTSTAR GSESNNEMVT ITTTGRSDVT
HumanNRX1         ....DIDPCE PSSG...... .......... .......... .......... ..........
HumanNRX3         ....DFVECE PSTA...... .......... .......... .......... ..........
HumanNRX2         ....DLEECE PSTGGELILP IITEDSLDPP PVATRSPFVP PPPTFYPFLT GVGATQDTLP
AplysiaNRX        .....GEGCE TSSQLR.... .......... .......... .......... ..........
C.elegansNRX      ....EVTALI TSSLAP.... .......... .......... .......... ..........

DrosophilaNRX     TEQHQGSSSS SSSGSTPSYA TTQSSSSSSS GGSAASTPGQ VSTTSSVATS STQRATSSST
HumanNRX1         .......... .......... .......... .......... .......... ..........
HumanNRX3         .......... .......... .......... .......... .......... ..........
HumanNRX2         PPAARRPPSG GPCQAERDDS DCEEPIEASG FASGEVFDSS LPPTDDEDFY TTFPLVTDRT
AplysiaNRX        .......... ....QRPDPS LPSSTLSPSG SGHDYGGDGG GNGDIDYVGG SGEIEPTDNR
C.elegansNRX      .......... ...QKTRPKS TPHFTVYPVR PTTPMGDTIT TTMQAATVTD FPRTPLIMCS

DrosophilaNRX     STSSSTTSTT TTTTTTQATP PPEIRSTVTE RETTPYDIYI AGGGMGGSGR NDHDRMQLPD
HumanNRX1         .......... .......... .......... .......... .......... ..........
HumanNRX3         .......... .......... .......... .......... .......... ..........
HumanNRX2         TLLSPRKPAP RPNLRTDGAT GAPGVLFAPS APAPNLPAGK MNHRDPLQPL LENPPLGPGA
AplysiaNRX        D......... .......... .......... .......... .......... ..........
C.elegansNRX      .......... .......... .......... .......... .......... ..........

DrosophilaNRX     EHHPLPPLPP PIPPQDPPPY GPYGGNTYNT NMNNYRPKGK GGRINSIEEE RTAMIIGIVA
HumanNRX1         .......... .......... ....GLANPT RAGGREPYPG SAEVIRESSS TTGMVVGIVA
HumanNRX3         .......... .......... ....NPTEPG ....IRRVPG ASEVIRESSS TTGMVVGIVA
HumanNRX2         PTSFEPRRPP PLRPGVTSAP GFPHLPTANP TGPGERGPPG AVEVIRESSS TTGMVVGIVA
AplysiaNRX        .......... .......... ....GYHGHF DKSTTKPGKT TDDDDDDDGV NLVLILGITG
C.elegansNRX      .......... .......... ....SLAVII AIAAVVFFVF KCRQNPPNSE HYTMAMKSQS

DrosophilaNRX     GILIAVVLVI LLVLWLKSNG DRGYKTESEK AAAYGSHNPN AALLGNTSTN GSYHQQRQHH
HumanNRX1         AAALCILILL YAMYKYRNRD EGSYHVDESR NYISNSAQSN GAVVKEKQPS SAKSSN....
HumanNRX3         AAALCILILL YAMYKYRNRD EGSYQVDETR NYISNSAQSN GTLMKEKQ.Q SSKSGH....
HumanNRX2         AAALCILILL YAMYKYRNRD EGSYQVDQSR NYISNSAQSN GAVVKEKAPA APKTPS....
AplysiaNRX        SVLLAVIVLC IVLCKLRGRD EGTYKVDETQ NFSALQSKKS QGNGSLASGG ESCTGK....
C.elegansNRX      GYTAIAPELS PPMNHDRSND SCTQPLLAKP HINGNGYEPL KGAVIANGNG ATATMMRNGN

DrosophilaNRX     MHGGGGGGGA GQQQHHAQQQ MHNGHNGNGN GGGGGGGGMM SSGSGSLGYG SDGRPQMAGL
HumanNRX1         .......... .......... .......... .......... .......... ..........
HumanNRX3         .......... .......... .......... .......... .......... ..........
HumanNRX2         .......... .......... .......... .......... .......... ..........
AplysiaNRX        .......... .......... .......... .......... .......... ..........
C.elegansNRX      GNG....... .......... .......... .......... .......... ..........
```

DrosophilaNRX	VQPKAKKRDS KDVKEWYV
HumanNRX1	......KNKK NKDKEYYV
HumanNRX3	......KKQK NKDREYYV
HumanNRX2	......KAKK NKDKEYYV
AplysiaNRX	......RGKK KDVKEWYV
C.elegansNRX	......VAKK KDFKEWYV

E. ApNRX Alternative Splicing

```
ApNRX        MAVELFPFSPRHLQCLTLAVAIFAAILCRLPCAHTLNLDGSPGSYVEYPPWEPCLNGSFS 60
Human NRX-1  MGTALLQRGGCFLLCLSLLLLGCWAELG-----SGLEFPGAEGQWTRFPKWNACCESEMS 55

ApNRX        FEFRTSEPLSLLLYLNRGSYSYFEVKLLKGGIRLRMNLG---ERTMIIRAGQNLNDNKWH 117
Human NRX-1  FQLKTRSARGLVLYFDDEGFCDFLELILTRGGRLQLSFSIFCAEPATLLADTPVNDGAWH 115

ApNRX        SVEVVQDGDLTTLIVDGIEHSKTSSGLLHTYNGLANETFLYLGGLPMEYESKKFNSLALP 177
Human NRX-1  SVRIRRQFRNTTLFIDQVEAKWVEVKSKRRD--MTVFSGLFVGGLPPELRAAALK-LTLA 172

ApNRX        SVIFEPKFRGSVRNFFYRS-------CGGEAVRPQPLSSAGLLTTDVDLCERGNPCLNGG 230
Human NRX-1  SVREREPFKGWIRDVRVNSSQVLPVDSGEVKLDDEPPNSGGGSPCEAGEEGEGGVCLNGG 232

ApNRX        RCLTTDNGVLCDCTATEYRGDRCDIQKIPSD-------------------ATFMGTQYF 270
Human NRX-1  VCSVVDDQAVCDCSRTGFRGKDCSQEDNNVEGLAHLMMGDQGKSKGKEEYIATFKGSEYF 292
                                                      1

ApNRX        SYNLSSRGDVMVDQDRVRLDFRTKQANSLLFYTGNSKDYMTVGLMDGAVFLTINLGSGMY 330
Human NRX-1  CYDLS-QNPIQSSSDEITLSFKTLQRNGLMLHTGKSADYVNLALKNGAVSLVINLGSGAF 351

                                             1
ApNRX        QAEIRPSGTRFDDNRWHQLLIQREARELPRDAGVCFVTMELDGMYRKQGSITGKFIRLSS 390
Human NRX-1  EALVEPVNGKFNDNAWHDVKVTRNLRQHS-GIGHAMVTISVDGILTTTGYTQEDYTMLGS 410
                                             2

ApNRX        N-LLYVAGSPNTETLPGSRVRTNFKGCLRKVRYRADGVLLDLTDLARREHSLMQVTGDVI 449
Human NRX-1  DDFFYVGGSPSTADLPGSPVSNNFMGCLKEVVYKNNDVRLELSRLAKQGDPKMKIHG-VV 469

ApNRX        FDKCQELVESHPVTFTSPESHLTIPTWSKPGIRGSLAFQFRTVEPGGLMMYSNGGRDSK- 508
Human NRX-1  AFKCENVATLDPITFETPESFISLPKWN-AKKTGSISFDFRTTEPNGLILFSHGKPRHQK 528

ApNRX        ----------DFFALELLDGHLYLVLDMGSGILKIQASKLPVSDGRPHDVYFEFKGYRGD 558
Human NRX-1  DAKHPQMIKVDFFAIEMLDGHLYLLLDMGSGTIKIKALLKKVNDGEWYHVDFQRDGRSGT 588

ApNRX        ISVDGQKVPFASGRVSDRFDLHGFFYVGGLGSEINASLLPRELWAVMLGLSYVGCLQDLV 618
Human NRX-1  ISVNTLRTPYTAPGESEILDLDDELYLGGLPENKAGLVFPTEVWTALLNYGYVGCIRDLF 648

ApNRX        LDGSKVDLLAAARLQNKSDVAG-YCKQTEPQCVSHPCNHGGKCVEGWNRFSCDCRATGFV 677
Human NRX-1  IDGQSKDIRQMAEVQSTAGVKPSCSKETAKPCLSNPCKNNGMCRDGWNRYVCDCSGTGYL 708

ApNRX        GAVCQTAAATLQFDGTQFMKVTMSRESVTQAEDVSLRFRSLHPSGLLFLTTGGN------ 731
Human NRX-1  GRSCEREATVLSYDGSMFMKIQLPVVMHTEAEDVSLRFRSQRAYGILMATTSRDSADTLR 768

ApNRX        ----DNRMQLFLQQGTLFLSVNVGSGSKVLSVGHRLNDDRWHTVFIRRRVQTVELAIDSD 787
Human NRX-1  LELDAGRVKLTVNLDCIRINCNSSKGPETLFAGYNLNDNEWHTVRVVRRGKSLKLTVDDQ 828
                            3
                                            2
ApNRX        RPVIDQLPGSTFSLATNYIFVGHKNPPGTEVADLPGRARELGLSSVEAGHMVQDDSGAGT 847
Human NRX-1  QAMTGQMAG---------------------------DHTRLEFHNIETGIITERR----- 856

ApNRX        GHSGSDHSGFIGSMQAFIFNNNHFFQMAKSGTGDNIEISARFSSDEYVVRDPVTFKSVDA 907
Human NRX-1  -YLSSVPSNFIGHLQSLTFNGMAYIDLCKNGDIDYCELNARFG-FRNIIADPVTFKTKSS 914

ApNRX        FAILPTLQAHEEFSVSLQLKTTESDGLILYNGGNENDFFALELFQGFLYYVYNMGEGAQR 967
Human NRX-1  YVALATLQAYTSMHLFFQFKTTSLDGLILYNSGDGNDFIVVELVKGYLHYVFDLGNGANL 974
```

```
ApNRX         VKANVQHPINDNKWHEARIFRVEKFTQLLRVDDNTPTVDDLTGTKNNRFDLDGFLYIGGV 1027
Human NRX-1   IKGSSNKPLNDNQWHNVMISRDTSNLHTVKIDTKITTQITAG---ARNLDLKSDLYIGGV 1031

ApNRX         RKTMYPSLPKLVYSRHGFVGCLGSLNLNGYLPNVLREANPIHESVGDGCRGPMSKCVNDS 1087
Human NRX-1   AKETYKSLPKLVHAKEGFQGCLASVDLNGRLPDLISDALFCNGQIERGCEGPSTTCQEDS 1091

ApNRX         CANQGRCVQQWTSYKCDCDMTSFTGPMCKDESVSYKFGPGPGLMTFAHLADHEPSTNFDN 1147
Human NRX-1   CSNQGVCLQQWDGFSCDCSMTSFSGPLCNDPGTTYIFSKGGGQITYKWPPNDRPSTRADR 1151

ApNRX         LAFGFKTFLSDAILLRMDSR-AYDDFIQIELADGYVYLVYNMGSFDHPLGDFYHKVNDGQ 1206
Human NRX-1   LAIGFSTVQKEAVLVRVDSSSGLGDYLELHIHQGKIGVKFNVGTDDIAIEESNAIINDGK 1211

                                                  3
ApNRX         YHVVRFTRAGPNATLQIDADMPQTKHPTGKQAHTFNNQAFVRIGGTKVENGSITQHFEGI 1266
Humann NRX-1  YHVVRFTRSGGNATLQVDSWPVIERYPAGRQLTIFNSQATIIIGGKEQG-----QPFQGQ 1266
                                          4                               4
ApNRX         ISGLVLNGINVFELAKHEDPKVRLEGSVSLNQRPQPGPFRPPQLYTDDEDLDEMQSTQGH 1326
Human NRX-1   LSGLYYNGLKVLNMAAENDANIAIVGNVRLVG---------------------------- 1298

ApNRX         TTPRDGVTDDDIIQAGAGSGCQFDNSEDCSAVGSGIEEIITPVVIVKTTTPPPTTTPKPV 1386
Human NRX-1   ---------------------EVPSSMTTESTATAMQSEMSTSIMETTTTLATSTARRGK 1337

ApNRX         PKCQGEGCETSSQLRQRPDPSLPSSTLSPSGSGHDYGGDGGGNGDIDYVGGSGEIEPTDN 1446
Human NRX-1   P-------PTKEPISQTTDDILVASAECPSD-----------DEDIDPCEPSSGGLANPT 1379
                                                                     5
                                                 TM
ApNRX         RDGYHGHFDKSTTKPGKTTDDDDDDDGVNLVLILGITGSVLLAVIVLCIVLCKLRGRDEG 1506
Human NRX-1   RAGGREPYPGSAEVIRESSSTTG--------MVVGIVAAAALCILILLYAMYKYRNRDEG 1431

ApNRX         TYKVDETQNFSALQSKKSQGNGSLASGGESCTGKRGKKKDVKEWYV               1552
Human NRX-1   SYHVDESRNYISNSAQSNGAVVKEKQPSSAKSSNKNKKNKDKEYYV               1477
                                                      PDZ
```

Figure S2. Characterization of ApNRX and ApNLG Antibodies, Related to Figure 1 and 2

(A) HEK293 cells were transfected with VSV-G tagged ApNRX or mock transfected and cell lysates were immunoblotted with anti-VSV-G and anti-ApNRX antibody. Both antibodies detected the same band near 170 kDa (marked with an arrow) corresponding well to the expected size of the VSV-G-ApNRX fusion protein (172 kDa).

(B) *Aplysia* total CNS extract was immunoblotted with the anti-ApNRX antibody, or with the same antibody pre-absorbed with the immunizing peptide as a specific competitor. The anti-ApNRX antibody detected two major high molecular weight bands at 170 and 220 kDa and an additional lower molecular band around 60 kDa (marked with arrows). All of these bands were eliminated by pre-absorption with the specific competitor peptide. The sizes of the high molecular weight bands correspond well to the expected size distribution of the ApNRX isoforms we have detected by RT-PCR (170-220 kDa) and the size of known mammalian and invertebrate α-neurexins. The 60 kDa lower molecular weight band is similar in size to mammalian β-neurexins. Although we have not detected transcripts corresponding to β-neurexins in *Aplysia,* we cannot rule out the possibility that they exist because the *Aplysia* genome is not yet fully sequenced. However, since other invertebrates also lack β-neurexins (Tabuchi and Südhof, 2002), it seems more likely that this band represents a truncated protein generated by a specific proteolytic cleavage which could have similar structure and function as mammalian β-neurexins. The anti-ApNRX antibody also detected a weaker band at 140 kDa (marked with a bar) that was not efficiently competed and therefore most likely represents a cross reactive protein present in the *Aplysia* CNS extract.

(C) HEK293 cells were transfected with GFP tagged ApNLG or mock transfected and cell lysates were immunoblotted with anti-GFP and anti-ApNLG antibody. Both antibodies detected the same band at 110 kDa (marked by an arrow) matching the expected size of the GFP-ApNLG fusion protein (110 kDa). The anti- ApNLG antibody also displayed strong cross-reactivity with several proteins in the 55-65 kDa range in the HEK extract whose identity is unknown.

(D) *Aplysia* total CNS extract was immunoblotted with the anti-ApNLG antibody with or without pre-absorption of the antibody with the recombinant ApNLG protein used for immunization as a specific competitor. The anti-ApNLG antibody detected two major bands at 85 and 120 kDa. The 85 kDa band (marked by an arrow) matches the predicted size of ApNLG (85 kDa) and was efficiently and specifically competed by pre-absorption with ApNLG. The 120 kDa band (marked with a bar) could be due to non-specific cross-reactivity since it was not competed as efficiently by pre-absorption with ApNLG.

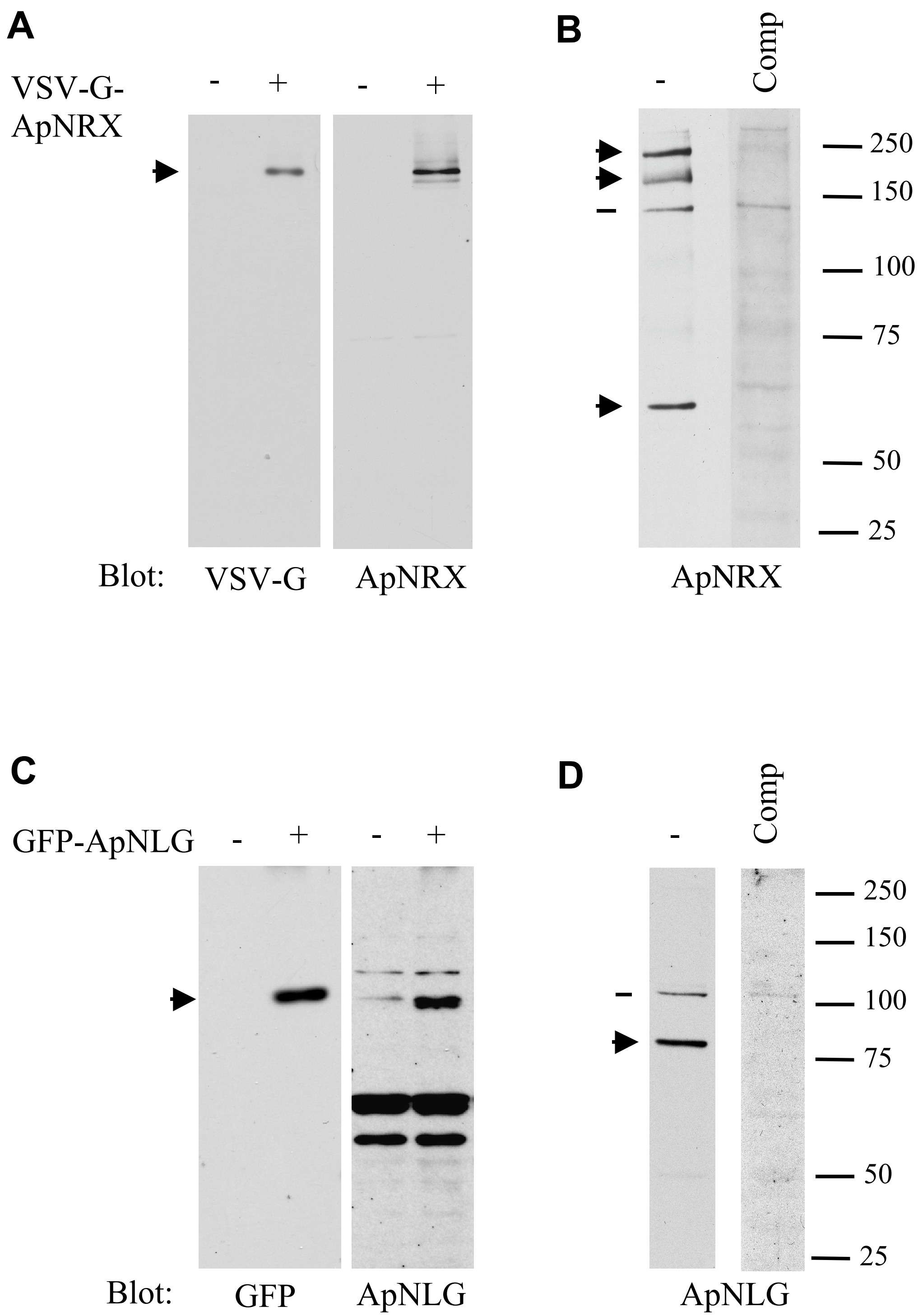

A
VSV-G-
ApNRX
- + - +
Blot: VSV-G ApNRX
B
- Comp
250
150
100
75
50
25
ApNRX
C
GFP-ApNLG - + - +
Blot: GFP ApNLG
D
- Comp
250
150
100
75
50
25
ApNLG

Figure S3. Characterization of ApNRX and ApNLG Antisense Oligonucleotides, Related to Figure 5 and Figure 6

Antisense oligonucleotides were microinjected (50 μg/ml) into *Aplysia* neurons. Neurons were fixed 3 hrs or 12 hrs after injections and immunostained with ApNRX or ApNLG antibodies. (A) Confocal projection images of the initial segment of sensory neurons that were injected with antisense oligonucleotides against ApNRX or uninjected controls. Scale bar 5 μm. (B) Confocal projection images of the initial segment of motor neurons that were injected with antisense oligonucleotides against ApNLG or uninjected controls. Scale bar 10 μm. Summary bar graphs of the average fluorescence intensities of (C) sensory neuron initial segment for ApNRX (at 3 hours after injection: uninjected control 100 ± 8.2%, n = 8, sense 96.1 ± 11.2%, n = 9, antisense 46.3 ± 2.1%, n = 26, $p < 0.001$ vs. control and sense, at 12 hours after injection: uninjected control 100 ± 8.7%, n = 12, sense 92.7 ± 6.5%, n = 13, antisense 38.3 ± 3.5%, n = 14, $p < 0.001$ vs. control and sense) and (D) motor neuron initial segment for ApNLG (at 3 hours after injection: uninjected control 100 ± 10.1%, n = 4, sense 94.7 ± 4.2%, n = 3, antisense 68.7 ± 1.9%, n = 5, $p < 0.05$ vs. control and sense, at 12 hours after injection: uninjected control 100 ± 1.8%, n = 13, sense 99.2 ± 3.2%, n = 15, antisense 67.1 ± 2.3%, n = 22, $p < 0.001$ vs. control and sense).

The error bars represent SEM.

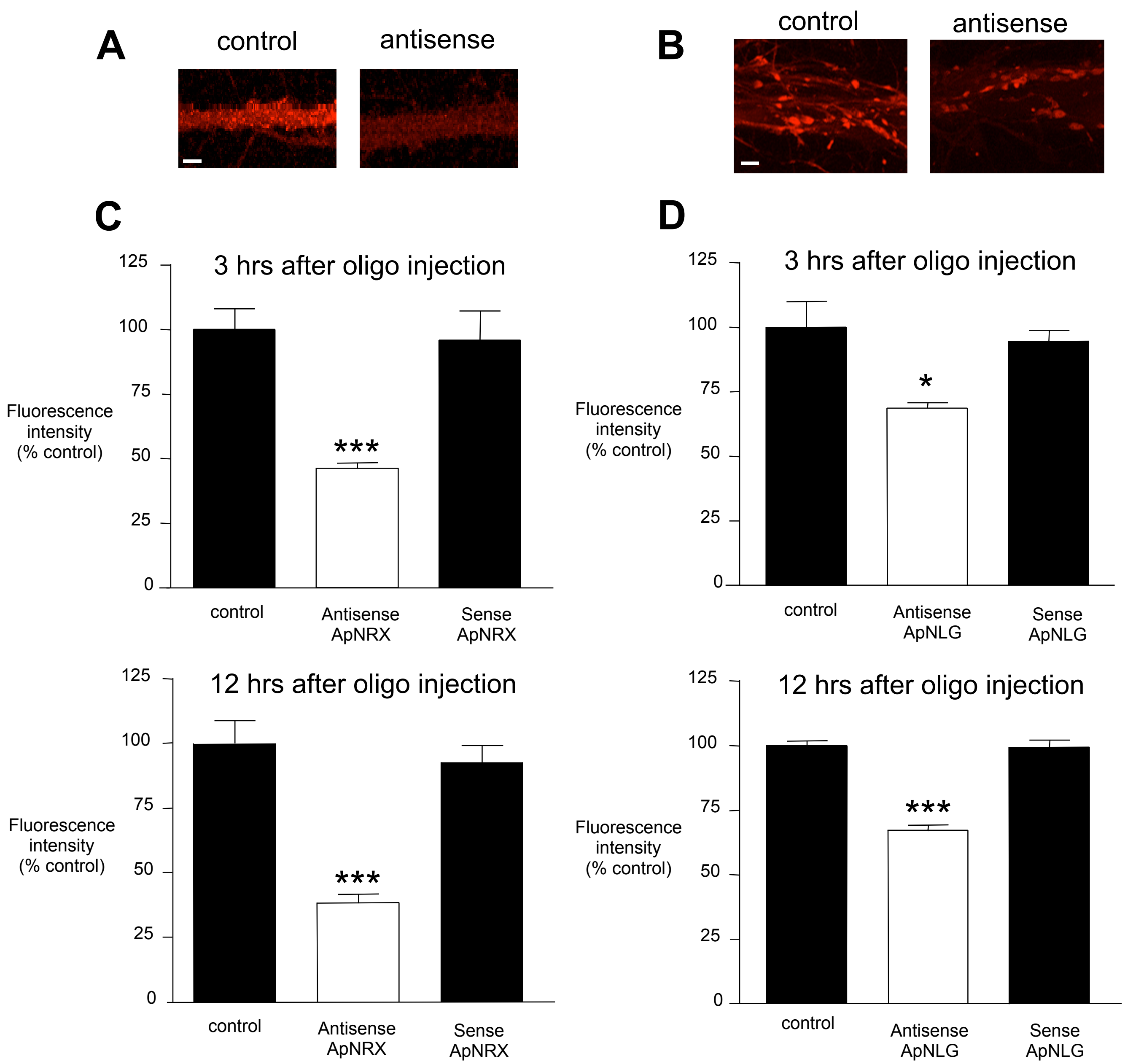

A
control
antisense
B
control
antisense
C
3 hrs after oligo injection
125
100
75
50
25
0
Fluorescence intensity (% control)
***
control
Antisense ApNRX
Sense ApNRX
12 hrs after oligo injection
***
control
Antisense ApNRX
Sense ApNRX
D
3 hrs after oligo injection
*
control
Antisense ApNLG
Sense ApNLG
12 hrs after oligo injection
***
control
Antisense ApNLG
Sense ApNLG

Table S1. ApNRX splice site variants and insert sequences, Related to Figure 2.

ApNRX splice site	Equivalent vertebrate NRX splice site	Variants at each site	Insert sequence(s)
1	2	2	LPRDAGVCF
2	3	2	GHKNPPGTEVADLPGRARELGLSSVEA
3	4	2	SFHSMKRNYLLCDRK
4	5	3	**Insert 1:** RGNPMSPALRRHLLWKSGKIKHHFQMRNSRAKGRRDVRGLRRNGSKHTGVK TPMATKWKPGVKAEEKWRRFTVLEPHRRKYETA; **Insert 2:** RGNPMSPALRRHLLWKSGKIKHHFQMRNSRAKGRRDVRGLRRNGSKHTGVK TPMATKWKPGVKAEEKWRRFTVLEPHRRKYETAVSGAKERRTENVVRETLKEKQEN EVKSMGTYPQYRKDKTSNRVGGYETTKNEDRPWVKNHRSPEFELNHAQQEVHNRTL YIYAPVDKKESSASVRRESNELDASDNDWEDMNSMVKTDFGVIMQPNKPKVRPTPAH QCDYGGPSNLKATAGPAMRGPEKHQKGFGQQTYSSSRRTEESSGIQTHFEHLRGPEPD FGDVIDENAVDNAEYDDIFGVTDRSFAYDSRRVKERQEGLRGRGNTGEDYEHFEETIV KLNELMRSLRVNGDDEDEDEVPEGSGFETVDGEDPSSANDRENLTRWSAHYRQSSEQ TSTIVIPYQTMTSEQPFHLGETSSSSXTLRGAARSYFASETAVSGAKERRTENVVRETLK EKQENEVKSMGTYPQYRKDKTSNRVGGYETTKNEDRPWVKNHRSPEFELNHAQQEV HNRTLYIYAPVDKKESSASVRRESNELDASDNDWEDMNSMVKTDFGVIMQPNKPKVR PTPAHQCDYGGPSNLKATAGPAMRGPEKHQKGFGQQTYSSSRRTEESSGIQTHFEHLRG PEPDFGDVIDENAVDNAEYDDIFGVTDRSFAYDSRRVKERQEGLRGRGNTGEDYEHFE ETIVKLNELMRSLRVNGDDEDEDEVPEGSGFETVDGEDPSSANDRENLTRWSAHYRQS SEQTSTIVIPYQTMTSEQPFHLGETSSSSXTLRGAARSYFAS

ApNRX splice sites 1, 2 and 3 either have or lack a single relatively short insert. ApNRX splice site 4 can have two longer inserts: insert 1 (85 amino acids) and a much longer insert 2 (783 amino acids). Insert 2 is made up of the first insert sequence plus an additional 699 amino acids portion. The functional significance of such long inserts occurring at this site is unclear. However, their presence is confirmed by both long distance RT-PCR amplification with primers flanking the start and stop codon yielding distinct amplification products with the respective ORFs and on the protein level by the ApNRX antibody recognizing bands of the appropriate size – 170 and 220 kDa for the site 4 insert negative and positive isoforms respectively (Figure S2).

Supplemental Experimental Procedures

Cloning and DNA constructs

ApNLG cloning: We used consensus-degenerate hybrid oligonucleotide primers (CODEHOPs – (http://blocks.fhcrc.org/codehop.html), Rose et al., 2003) approach to clone ApNLG. CODEHOP primers that were used to clone an initial 150 bp fragment of ApNLG from *Aplysia* genomic DNA are CACCCACTTCCCTCCCGYNTGYCCNCA and GGGCACGTAGATGTTCAGGTA NARRCARTCYTC. We used this fragment to screen an *Aplysia* CNS cDNA library using 32P labeled probes to isolate a clone contained 5' UTR and approximately 75% of the ApNLG coding sequence. The 3' end of ApNLG coding sequence was cloned by multiple rounds of 3' RACE-PCR using a GeneRacer RACE ready cDNA kit (Invitrogen, CA).

ApNRX cloning: A 1KB EST sequence from the *Aplysia* EST database at the Columbia University Genome Center (http://aplysia.cu-genome.org) with similarity to a region in the extracellular portion of mammalian neurexin was extended in both directions by multiple rounds of 5' and 3' RACE-PCR employing a SMART Race cDNA amplification kit (Clontech/Takara, CA) to obtain the full length ApNRX gene including 5' and 3' UTRs. The full length 4659 base pair ApNRX open reading frame was amplified from *Aplysia* CNS cDNA with the following primers: Forward-ATGGCTGTGGAACTCTTCCCATTCTCGCCTAG; Reverse-TCACACATACCACTCCTTCACATCTTTCTTCTTGCCT using a touch down PCR protocol with the following parameters: 5 cycles: 94° C 30 sec, 72° C 5 min; 5 cycles: 94° C 30 sec, 70° C 30 sec, 72° C 5 min; 30 cycles: 94° C 30 sec, 68° C 30 sec, 72° C 5 min followed by 10 min extension at 72° C. The sequences of ApNLG and ApNRX were deposited into GenBank (accession number: HM448446 for ApNLG and HM461999 for ApNRX). All experiments in this study except for the ones in Figure 3 employed the most abundant ApNRX isoform which lacks inserts at sites 3 and 4 and has inserts at site 1 and 2 (Figure S1E). The ApNRX isoform used in the experiments in Figure 3 lacks inserts at site 1 in addition to sites 3 and 4 and contains the following amino acid substitutions likely resulting from single nucleotide polymorphisms: L207F, T411A and E438V.

ApNLG arginine to cysteine point mutation was generated using QuikChange site-directed mutagenesis kit (Stratagene, CA). GFP-ApNLG and HA-ApNLG were created by inserting GFP or HA peptide sequence into an engineered SmaI site after the 5th amino acid residue from the end of the putative signal peptide. GFP-ApNRX and VSV-G-ApNRX were created by inserting GFP or VSV-G peptide sequence into an engineered EcoRV site after the 3rd amino acid residue from the end of the putative signal peptide. ApNRX-GFP was constructed by amplifying the full length ApNRX excluding the stop codon with primers containing SbfI and XhoI cut site 5' extensions. It was then digested with SbfI and XhoI and ligated in frame with the GFP open reading frame in a pNEX3-GFP expression plasmid linearized with the same two enzymes. ApNRX C-terminal deletion was generated by amplifying the N-terminal 1509 amino acid portion of ApNRX, which deletes C-terminal 44 amino acids, and then ligating it in pNEX3-GFP in the same fashion as described above. All constructs were verified by sequencing. We subcloned all DNA constructs into the *Aplysia* expression vector pNEX3.

Antibody production and Immunoblotting

Rabbit polyclonal antibodies were raised against a synthetic peptide derived from the intracellular cytoplasmic tail of ApNRX [CKLRGRDEGTYKVDETQ] (Covance) and against the extracellular domain of ApNLG recombinantly expressed and purified from *E.Coli* and encompassing the first 615 N-terminal amino acids of the protein. N-terminal cysteine was added to the ApNRX peptide for coupling to KLH and injecting in rabbits. All antibodies were affinity purified onto columns conjugated with the corresponding immunizing peptide or recombinant protein. *Aplysia* proteins were isolated from sensory clusters or pleural ganglia using lysis buffer (50 mM Tris, pH 7.6, 525 mM NaCl, 1 mM EDTA) supplemented with protease inhibitor cocktail (Roche, Basel, Switzerland) and phosphatase inhibitors (Calbiochem, Darmstadt, Germany). Protein concentration was determined using a BCA kit (Pierce, Rockford, IL). 5-10 µg of protein were used for immunoblotting. ApNRX and ApNLG antibodies were used at 1:3000 dilution for immunoblotting.

Immunocytochemistry of *Aplysia* Neurons

We fixed *Aplysia* cultures in 4% paraformaldehyde in PBS at room temperature (RT) for 20 min and then permeabilized in 0.1% Triton X-100 in PBS for 10 min at RT. Free aldehydes were quenched in 50 mM ammonium chloride in PBS for 10 min at RT. After blocking nonspecific antibody binding with incubation in 10% goat serum, we incubated cultures with primary antibody overnight at 4°C followed by incubation with secondary antibody for 1 hr at RT. ApNRX and ApNLG antibodies were used at 1:100 dilution for immunocytochemistry. The secondary antibody was goat anti-rabbit IgG Alexa 568-conjugate (Invitrogen-Molecular Probes, CA) at 1:200 dilution. For immunocytochemistry involving both ApNRX and ApNLG antibodies, we first immunostained cultures with ApNLG antibody, then fixed the cultures again before they were immunostained with ApNRX antibody conjugated to Alexa 488 using Zenon® Rabbit IgG Labeling Kit (Invitrogen-Molecular Probes, CA) at 1:25 dilution.

Construction of HA-ApNLG-Fc and VSV-ApNRX-Fc fusion constructs

For HA-ApNLG-Fc, the HA-tagged extracellular domain of ApNLG (amino acid 1 to 641) was fused with the Fc portion of IgG2A at its C-terminus using the pFuse-mIgG2A-Fc vector (InvivoGen, CA). For VSV-G-ApNRX-Fc, the VSV-G-tagged ApNRX (amino acid 1 to 1457) was fused with the pFuse-mIgG2A-Fc vector. The Fc fusion constructs were transfected into HEK293 cells and the supernatants were harvested at 4 days after transfection. From each T75 flask, 10 ml of supernatants were collected and incubated with 50 % of ammonium sulfate to precipitate the Fc fusion proteins at 4^0 C for at least 4 hours. The precipitates were spun down by centrifugation and resuspended in 300 µl of PBS followed by overnight dialysis against PBS. The PBS equilibrated Fc fusion proteins were used for the binding assays.

Binding Assays

Two days after transfecting HEK293 cells with GFP, GFP-ApNLG or GFP-ApNRX, cells were lysed with ice cold lysis buffer (1 mM phenylmethylsulfonyl fluoride, 100 μg/ml soy bean trypsin inhibitor, 20 μg/ml aprotinin, 100 μg/ml leupeptin, 1% Nonidet P-40 and 0.1% deoxycholate in PBS). Insoluble materials were removed from cell lysates by centrifugation at 12,000 rpm for 10 min. Subsequently, the equal amount of lysates (200 μg) from GFP-ApNLG or GFP-ApNRX transfected cells were incubated with 15 μl of VSV-G-ApNRX-Fc or HA-ApNLG-Fc at 4^0 C overnight. In each condition, GFP transfected cell lysates were used as a control. Protein A agarose (15 μl) were added for an additional 3 hours next day. The precipitates were washed 3 times with lysis buffer followed by once with PBS and then subjected to get electrophoresis. The initial lysates and the subsequent precipitates of GFP-ApNLG and GFP-ApNRX in each binding condition were detected using rabbit anti-GFP antibody (Invitrogen, CA). The inputs of HA-ApNLG-Fc and VSV-G-ApNRX-Fc in the binding assays were detected using mouse monoclonal anti-HA 16B12 (Covance, CA) and anti-VSV-G antibody P5D4 (Sigma, MO), respectively.

Induction and Electrophysiological Assessment of LTF, Intermediate-Term Facilitation and STF in Sensory Neuron-Motor Neuron Co-Cultures

We prepared *Aplysia* sensory-to-motor neuron co-cultures and measured excitatory postsynaptic potentials (EPSPs) as previously described (Montarolo et al., 1986). We evoked the EPSP in L7 motor neuron by stimulating the sensory neuron with a brief depolarizing stimulus using an extracellular electrode. The motor neuron was held at a potential of –30 mV below its resting potential to prevent eliciting action potentials. The synapses with initial EPSPs less than 4 mV were not used for analysis. To induce LTF, we treated cultures with five 5 min pulses of 5-HT (10 µM) at 20 min intervals. Then, the cultures were maintained at 18 °C and the EPSPs were again measured 24 hrs, 48 hrs, and 72 hrs after the initial EPSP measurement. For intermediate term facilitation, EPSPs were measured again 1 hr after the conclusion of 5-HT treatment. To induce STF, we treated cultures with one 5 min pulse of 5-HT (10 µM) after the initial EPSP measurement. EPSP was measured again 5 min after 5-HT treatment.

Microinjection

We dissolved the various DNA constructs (1 µg/µl), oligonucleotides, or Alexa Dextran-594 (Invitrogen-Molecular Probes, CA) in a buffer containing 0.1% fast green, 10 mM Tris-Cl (pH 7.3), and 250 mM KCl. They were injected under visual guidance into the cytoplasm (for oligonucleotides or dyes) or into the nucleus (for DNAs) of *Aplysia* neurons by applying positive air pressure through Picospritzer II (General Valve Co., NJ).

The following oligonucleotides were used:

ApNLG antisense oligonucleotides: GACCAGCTCCGGAATATAACG

ApNLG sense oligonucleotides: CTGGTCGAGGCCTTATATTGC

ApNRX antisense oligonucleotides: CTTGGACTGAAGCGCTGAGAAGTT

ApNRX sense oligonucleotides: AACTTCTCAGCGCTTCAGTCCAAG

Cell Imaging and Quantification of the Structural Changes that Accompany 5-HT-Induced LTF

We acquired images of *Aplysia* neurons using a Zeiss LSM 5 Pascal laser confocal scanning microscopes. Images were taken with a 40x, NA 1.3 objective, and the gains were adjusted to prevent saturation of the detection threshold. A z-series consisting of 30-50 optical sections were collected throughout the entire volume of the sensory neuron varicosities and maximum projections made off-line for analysis with Metamorph (University Imaging Corp, PA). We assessed the long-term structural changes by comparing the images of each sensory neuron before and 24 hr after 5-HT treatment. We identified sensory neuron varicosities as all labeled, elongated spheres (3 μm or more in diameter) in apposition to the initial segment and major neurites of L7 motor neurons. (Bailey and Chen, 1983; Glanzman et al., 1990). The mean pixel intensities of GFP were determined by manually outlining the varicosities fluorescently labeled with Alexa Dextran-594. The maximum mean intensity among all the varicosities in one culture was designated as 100% enrichment index of GFP and the background intensity was designated as 0% enrichment index. The varicosities were binned according to their average fluorescence intensities. We considered varicosities in 0%-10% enrichment index to be “empty varicosities.” Quantitative analysis followed a blind procedure.

Supplemental References

Bailey, C.H., and Chen, M. (1983). Morphological basis of long-term habituation and sensitization in *Aplysia.* Science *220*, 91–93.

Missler, M., Fernandez-Chacon, R., and Südhof, T.C. (1998). The making of neurexins. J. Neurochem. *71*,1339-1347.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. *24*, 1596-1599.