SUPPLEMENTARY MATERIAL

Supplementary Methods
Supplementary clinical information
Supplementary Figures 1, 2, 3, 4, 5
Supplementary Tables 1, 2

Truncating Mutations in NRXN2 and NRXN1 in Autism Spectrum Disorders and Schizophrenia

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Supplementary Methods

DNA constructs. The full-length human NRXN1α cDNA (BC150247, IMAGE:9007472) was obtained from Open Biosystems (Huntsville, AL, Canada). The wild-type (WT) was cloned into pcGlobin2 (Ro et al. 2004). The insACGG mutation was generated by site-directed mutagenesis (Stratagene) and confirmed by sequencing. A FLAG sequence (DYKDDDDK) was inserted just after the signal peptide (between amino acids 33 and 34). Mouse neurexin2α(–S4)-CFP cloned into Clontech ECFP-N1 vector and YFP-LRRTM2 cloned into pLentiLox3.7 vector have been described previously (Siddiqui et al. 2010). Neurexin2α(-S4)delA was generated from neurexin2α(-S4)-CFP by PCR cloning. Rodent HA-neurexin1α(–S4)-CFP expressing the N-terminal 320 aa of rat neurexin fused to the C-terminal 1176 aa of mouse neurexin (Kang et al. 2008) was generated from neurexin1α(-S4)-CFP (Siddiqui et al. 2010) by the overlap PCR method. Rodent HA-neurexin1α(-S4)-CFP H8P, E715K, R813C and InsertACGG were generated from HA-neurexin1α(–S4)-CFP by the overlap PCR method. LRRTM2-alkaline phosphatase (AP) has been described previously (Linhoff et al. 2009). Neuroligin2-Fc was generated by cloning the mature mouse neuroligin2 ectodomain between mouse neurexin1β signal peptide and human Ig Fc domain in a pCDNA4-based vector.

Copy number variant analysis. Whole genome copy number variant analysis on the ASD patients and their parents was performed using the Affymetrix Genome-Wide Human SNP Array 5.0 (Affymetrix, Santa Clara, CA, USA). The Affymetrix 5.0 chip contains all 500,568 single nucleotide polymorphisms (SNPs) from the two-array Mapping 500K Array Set as well as an additional 420,000 non-polymorphic probes that can measure other genetic differences, such as copy number variation. SNPs on the array are present on 200 to 1,100 base pair (bp) Nsp I or StyI digested fragments in the human genome. As per the manufacturer's specifications, two separate tubes, containing 250ng of genomic DNA each, were digested with Sty1, and Nsp1 (New England BioLabs, Ipswich, MA, USA), respectively. After restriction enzyme digestion, adaptors supplied by the manufaturer were ligated onto the products of the previous reactions and PCR amplification was performed according to the Affymetrix Genome-

Wide Human SNP Array 5.0 instructions. The amplified products were pooled and purified with magnetic beads (Agencourt, Beverly, MA). Using reagents supplied by the manufacturer, the purified PCR products where fragmented, end-labeled and hybridized to the Affymetrix 5.0 chip. R-phycoerythrin streptavidin conjugate (Invitrogen, Carlsbad, CA, USA) and antistreptavidin-antibodies (Vector Labs, Burlingame, CA, USA) where used according to the manufacturer's instructions for fluorescence detection. Finally, the chips were scanned on the Affymetrix GeneChip® 3000 Scanner 7G (Affymetrix, Santa Clara, CA). Data analysis was performed using the Chromosome Analysis Suite v1.0 (Affymetrix, Santa Clara, CA). The NSID patients were similarly tested for CNVs using either the Affy 6.0 SNP chip (as described for the Affy 5.0), or by array CGH using a NimbleGen 135K oligo array (done on a clinical basis at the Sainte Justine Hospital, Montreal, Quebec).

Cell culture. Dissociated primary hippocampal neuron cultures were prepared from embryonic day 18 rat embryos essentially as described previously (Goslin et al. 1998; Kaech and Banker 2006). Neurons were plated at a final density of 300,000 cells per dish on poly-L-lysine-coated coverslips in 60 mm culture dishes inverted over a feeder layer of glia. After 2 d, cytosine arabinoside (5 μM) was added to neuron cultures to prevent the overgrowth of glia. For surface expression, neurons were transfected with human Flag-neurexin1α expression plasmids at day *in vitro* (DIV) 0 using electroporation (AMAXA Biosystems) and analysed for surface Flag at 2-5 DIV. For fibroblast-neuron cocultures to assay LRRTM2, neurons were transfected with 4 μg of YFP-LRRTM2 DNA at DIV 0 by electroporation and seeded at a density of 500,000 to 1 million per 60 mm dish. COS7 and human embryonic kidney 293T (HEK293T) cells were cultured in DMEM-H supplemented with 10% fetal bovine serum. HEK293T and COS7 cells were transfected using Fugene 6 (Roche). Cocultures of COS7 cells with neurons were performed as described previously (Graf et al. 2004). Essentially, COS7 cells were harvested by trypsinization 18 to 24 h after transfection, seeded onto neuron coverslips pre-grown for 9–11 DIV, supplemented with 100 μM APV (Research Biochemicals) to limit toxicity, and fixed 20–24 h later.

Surface expression. To determine surface expression, Flag- or HA-neurexin-CFP variants were transfected into COS7 cells, incubated live with anti-Flag or anti-HA antibody for 30 min at room temperature (RT), then fixed in prewarmed 4% formaldehyde/4% sucrose, blocked in 10% BSA in PBS and incubated with Alexa-568-conjugated anti-mouse antibody to detect surface Flag or HA. CFP signal represents total expression level for the missense variants. Truncation mutants were co-transfected with CFP to identify transfected cells. Intracellular expression of Flag-neurexin1α insACGG in COS7 cells was confirmed by incubation with anti-Flag and secondary antibodies after fixing cells and permeabilizing with 0.25% Triton for 5 min. Neurons labeled for surface Flag were permeabilized and co-stained for F-actin with coumarin phalloidin. For the double labeling with surface HA versus permeabilized HA, HA-neurexin1α-CFP or HA-neurexin1αinsACGG-CFP were transfected into COS7, surfaced labelled with rat anti-HA antibody, fixed and permeabilised, then incubated with mouse anti-HA antibody and then species-specific secondary antibodies.

Western blots. COS7 cells grown in 6 cm tissue culture dishes were transfected with 2.5 µg of plasmid DNA. Twenty-four hours after transfection, the culture media was changed to serum-free DMEM, which was collected after twenty-four hours with addition of protease-inhibitor cocktail (Complete, EDTA-free, Roche). Debris was removed by centrifuging at 10,000g for 10 min, and culture media was then concentrated to a final volume of 100 µl (3KDa molecular weight cut-off, Amicon). Cells were washed twice with cold-PBS, lysed in 300 µl of lysis buffer (1% SDS, 10 mM Tris pH7.5, 5 mM EDTA and 1 mM PMSF) and immediately boiled for 3 min to inactivate proteases. Total protein amounts in lysate and concentrated media were measured by the DC Protein Assay (Biorad). 15 µg of lysate and 30 µg of media (~30 µl corresponding to a third of the total media) were electrophoresed in an 8% SDS-PAGE and subsequently blotted onto PVDF membrane (Millipore). The primary antibody used was anti-Flag M2 antibody (Sigma) and the secondary antibody was HRP-conjugated anti-mouse antibody (Southern Biotech). The blots were developed by chemiluminescent HRP substrate Immobilon Western (Millipore) and imaged with a VersaDoc System (Biorad).

Protein binding assays. Expression of the LRRTM2-AP tagged fusion protein or neuroligin2-Fc tagged fusion protein was performed by transient transfection in HEK293T cells essentially as described previously (Siddiqui et al. 2010). Supernatant collected from protein expressing cells was concentrated using Centricon Plus-70 ultrafiltration units (30 kDa cutoff; Millipore). LRRTM2-AP fusion protein also contained Myc and His tags and was purified using Ni-NTA agarose eluting with 200 mM imidazole. Imidazole was removed by overnight dialysis (Spectrapor). Purified LRRTM2-AP fusion protein was quantitated by SDS-PAGE relative to a BSA standard curve using Sypro Ruby gel stain (Invitrogen), UV illumination, and a Bio-Rad gel documentation system. Neuroligin2-Fc fusion protein was purified with protein G (Pierce) beads and eluted in fractions. Purified neuroligin2-Fc protein was quantitated by the RC DC Protein Assay (Bio-Rad).

To determine LRRTM2-AP binding to neurexin variants, transfected cells were incubated with the fusion protein live for 1 h at RT followed by anti-Myc and/or anti-HA antibodies for 30 min. Binding assays were done in buffer: 168 mM NaCl, 2.6 mM KCl, 10 mM HEPES, pH 7.2, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM D-glucose, and 100 µg/ml BSA. Cells were then fixed in prewarmed 4% formaldehyde/4% sucrose, blocked in 10% BSA in PBS, and incubated with Alexa-568-conjugated anti-IgG1 to detect bound Myc and Alexa 488-conjugated anti-IgG2b to detect expressed HA.

To determine neuroligin2-Fc fusion protein binding to neurexin variants, transfected cells were co incubated with fusion protein and anti-HA antibody for 30 min at RT . Cells were then fixed in prewarmed 4% formaldehyde/4% sucrose, blocked in 10% BSA in PBS, and incubated with FITC-conjugated anti-human IgG to detect bound neuroligin2-Fc and/or with Alexa-568-conjugated anti-mouse antibody to detect surface HA.

Immunocytochemistry. COS7 cells and cocultures were fixed for 12–15 min with warm 4% formaldehyde and 4% sucrose in PBS, pH 7.4, followed by permeabilization with PBST (PBS plus 0.25% Triton X-100). Fixed and permeabilized cultures were blocked in 10% BSA in PBS for 30 min at 37°C and primary antibodies applied in 3% BSA in PBS. After overnight incubation at room temperature, the coverslips were washed with PBS and incubated in secondary antibodies in 3% BSA in

PBS for 1 h at 37°C. The coverslips were then washed and mounted in elvanol (Tris-HCl, glycerol, and polyvinyl alcohol, with 2% 1,4-diazabicyclo[2,2,2]octane).

The following primary antibodies were used: affinity purified rabbit anti-neuroligin2 antibody (Graf et al. 2006), rabbit anti-synapsin I (1:2000; Millipore; AB1543P), mouse anti-PSD-95 family (IgG2a; 1:500; clone 6G6-1C9; Thermo Scientific), mouse anti-gephyrin (IgG1; 1:1000; MAb7a; Synaptic Systems). For labeling dendrites, we used anti-MAP2 (chicken polyclonal IgY; 1:2000; Abcam; ab5392). To determine surface localization of N-terminally Flag- or HA-tagged proteins, live staining was performed using mouse anti-Flag (IgG1, 1:100; M2; Sigma) or rat anti-HA antibody 3F10 (Roche) or mouse anti-HA (IgG2b, 1:500; Roche 12CA5). Labeled secondary antibodies used were raised in goat against the appropriate species and monoclonal isotype, highly cross-adsorbed, and conjugated to Alexa-488, Alexa-568, and Alexa-647 dyes (1:500; Invitrogen) or Dylight 594 (1:500; Jackson ImmunoResearch). To visualize dendrites, we used AMCA (7-amino-4methylcoumarin-3-acetic acid)-conjugated anti-chicken IgY (donkey IgG; 1:200; Jackson ImmunoResearch; 703-155-155). Recombinant proteins tagged with human IgG Fc domain were stained with FITC-conjugated donkey anti-human IgG (L+H) (1:150 in live staining; Jackson ImmunoResearch).

Image analysis. Images were acquired on a Zeiss Axioplan2 microscope with a 63x 1.4 numerical aperture oil objective and Photometrics Sensys cooled CCD camera using MetaMorph imaging software (Molecular Devices) and customized filter sets. Images were acquired as grayscale and prepared for presentation using Adobe Photoshop (Adobe Systems). For quantitation, experimental sets were fixed and stained simultaneously and imaged with identical settings. All imaging and analysis were done blind, choosing similar fields among conditions by phase contrast and the transfected protein channel. Analysis was performed using MetaMorph (Molecular Devices), Kaleidagraph (Synergy Software), and InStat3 Graph (GraphPad Software).

To determine surface expression of missense HA-neurexin-CFP proteins, cells were chosen by CFP expression, regions were generated around the perimeter of each cell, then the average gray value of CFP signal, representing total expression and HA signal, representing surface expressed protein

were measured with each region. The average gray value of the off-cell background were subtracted from the average gray values of CFP and HA signals to give the corrected gray values. For the truncation variant which removes the CFP tag, the surface HA signal was reported, not normalized to co-transfected CFP. To determine the binding affinity of soluble proteins to surface-expressed proteins, regions were generated around the exact perimeter of each cell, and the average gray values of bound protein and expressed surface protein were measured within the region. The average gray value of the off-cell background was subtracted, and the corrected average gray values of bound protein and expressed surface protein were measured. For coculture scoring, coverslips were blinded and contacts between expressing neurons and CFP-positive COS7 cells counted. Neurons expressing similar levels of YFP-LRRTM2 in the soma were chosen; note that co-culture with synaptogenic proteins sometimes reduced the levels of YFP-LRRTM2 at endogenous synapses of contacted dendrites. Clustering of the expressed YFP-LRRTM2 or neuroligin2, detected by its antibody in dendrites contacting expressing COS7 cells but absent for pre-synaptic marker synapsin or VGAT was counted as positive, whereas no clustering was counted as negative.

Supplementary clinical information

Patient with the NRXN1 c.4205insACGG mutation. A female diagnosed with disorganized type SCZ. At the age of 17, she developed auditory hallucinations, delusional perceptions, bizarre behaviour and incoherence. During her first hospitalization, organic causes of the psychotic disorders were ruled out and she was diagnosed as having disorganized schizophrenia. She was treated with clozapine (100mg), but the efficacy of the medication was limited. Her schizophrenic positive and negative symptoms progressively worsened over the years and she underwent many hospitalisations. During her illness, she did not manifest any mood disorders or suicidal behaviour and there was no history of alcohol or drug abuse. At the age of 24 years old, she was enrolled in this study. At that time, she was hearing many voices. Her speech was poor, digressive and incoherent. Apathy, mannerisms, blunted affect and the feeling of depersonalisation complete the clinical picture of undifferentiated schizophrenia.

Patient with the NRXN2 c.2853delT mutation. This boy, diagnosed with autistic disorder, has some allergies to benzocaine. He is not on any medications and eats well and is sleeping well. He has soft bowel movements. He had a febrile convulsion when he was 14 months but no further problems. He does not have any skin markings and somewhat small ears but no other minor anomalies. He was delivered by a vaginal vertex delivery and his Apgar's score were 8 and 9. He was noted to have a fast heart rate with possible sepsis and IV antibiotics were given. His developmental milestones were as follows: he sat alone at six months and walked at 13 months. At 10 or 11 months he said Mama and was babbling but words only appeared when he was 2 years 8 months, when he said ball. At the age of 3 years 2 months he did not have any phrase speech.

Supplementary References

Goslin K, Asmussen H, Banker G (1998) Rat hippocampal neurons in low-density culture. In: Banker G, Goslin K (eds) Culturing Nerve Cells, 2nd edn. MIT Press, Cambridge, pp 339-370

Graf ER, Kang Y, Hauner AM, Craig AM (2006) Structure function and splice site analysis of the synaptogenic activity of the neurexin-1 beta LNS domain. J Neurosci 26: 4256-65.

Graf ER, Zhang X, Jin SX, Linhoff MW, Craig AM (2004) Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. Cell 119: 1013-26.

Kaech S, Banker G (2006) Culturing hippocampal neurons. Nat Protoc 1: 2406-15.

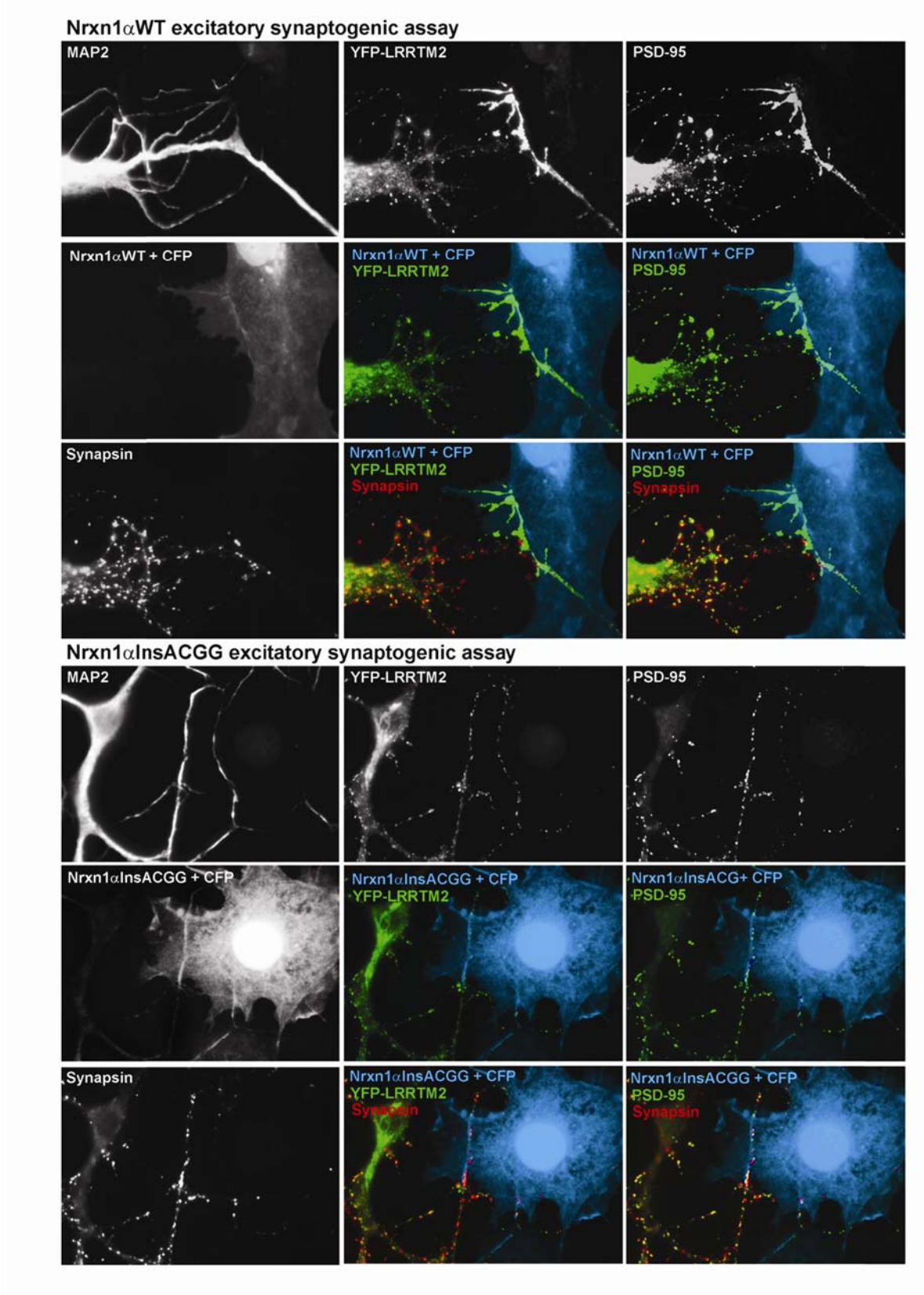
Kang Y, Zhang X, Dobie F, Wu H, Craig AM (2008) Induction of GABAergic postsynaptic differentiation by alpha-neurexins. J Biol Chem 283: 2323-34.

Linhoff MW, Lauren J, Cassidy RM, Dobie FA, Takahashi H, Nygaard HB, Airaksinen MS, Strittmatter SM, Craig AM (2009) An unbiased expression screen for synaptogenic proteins identifies the LRRTM protein family as synaptic organizers. Neuron 61: 734-49.

Ro H, Soun K, Kim EJ, Rhee M (2004) Novel vector systems optimized for injecting in vitro-synthesized mRNA into zebrafish embryos. Mol Cells 17: 373-6.

Siddiqui TJ, Pancaroglu R, Kang Y, Rooyakkers A, Craig AM (2010) LRRTMs and neuroligins bind neurexins with a differential code to cooperate in glutamate synapse development. J Neurosci 30: 7495-506.

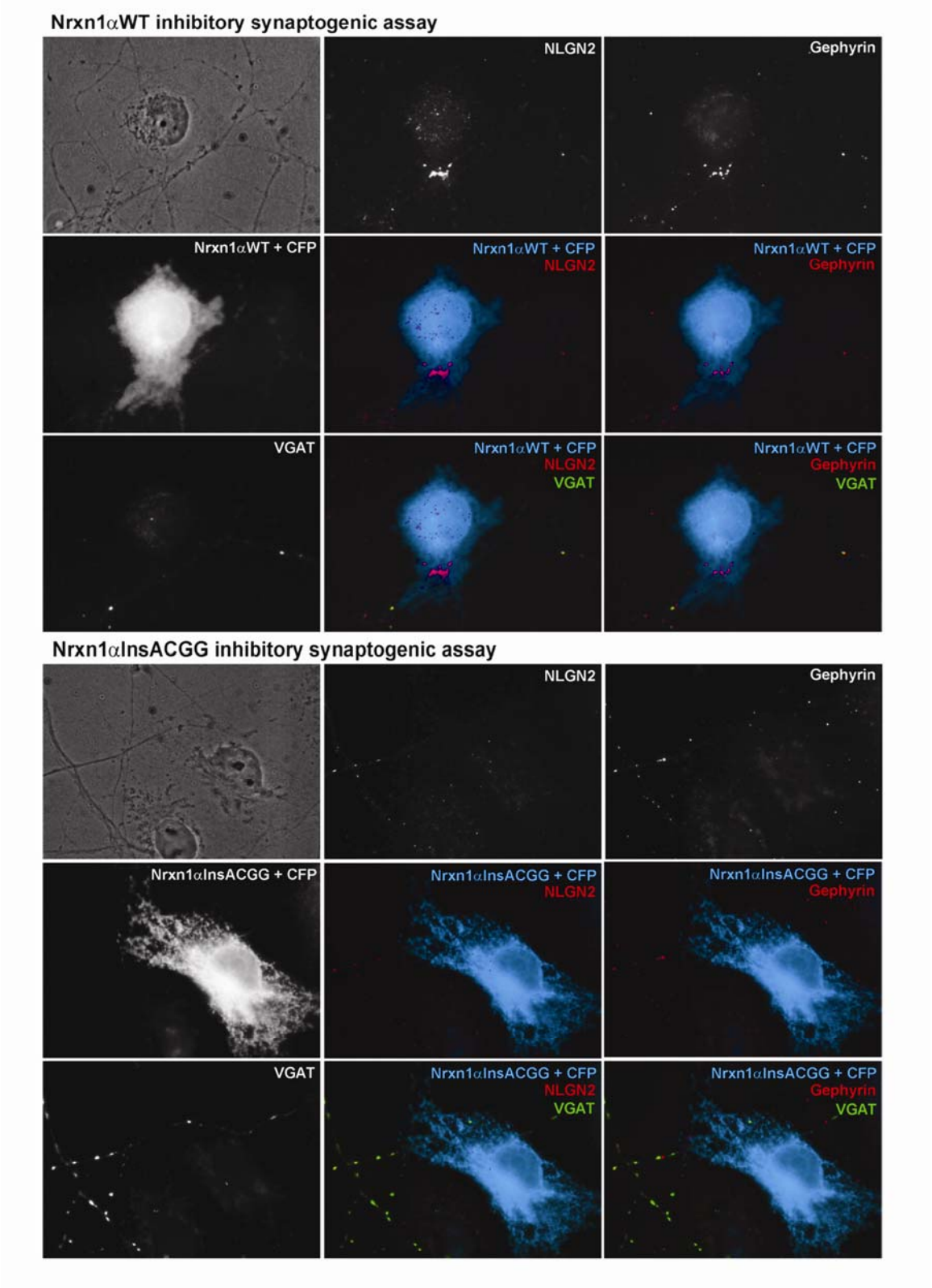
Supplementary Figure 1



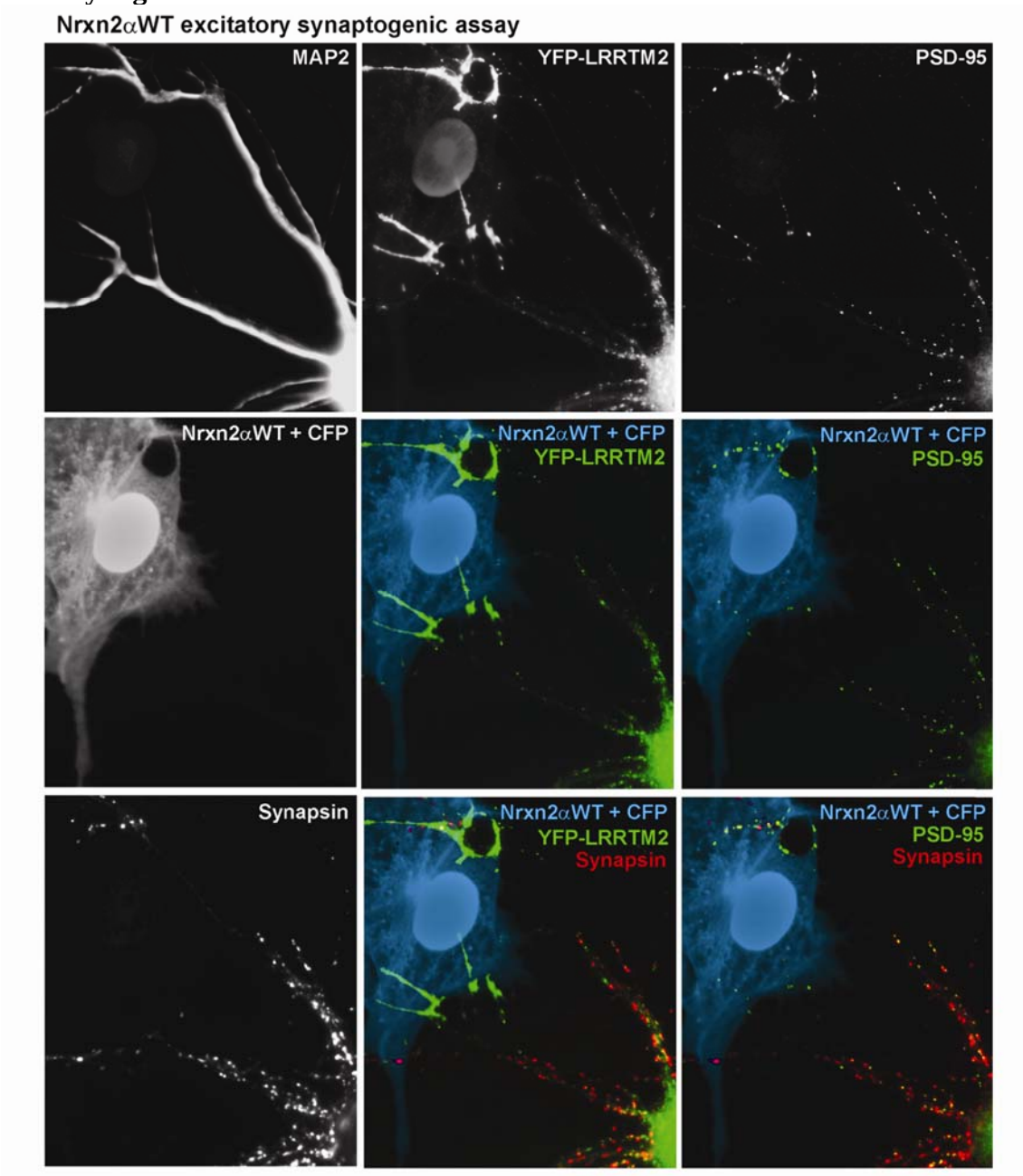
Supplementary Figure 1. NRXN1 c.4205insACGG results in functional deficiency inexcitatory synaptogenic activity in neuron coculture assays. COS cells were transfected with HA-neurexin1α-CFP vectors and CFP to mark the transfected cells and cocultured with rat hippocampal neurons transfected with YFP-LRRTM2. CFP-positive cells cotransfected for HAneurexin1α-CFP wild type (**top**) induced clustering of YFP-LRRTM2 and endogenous PSD-95 at contact sites with transfected neuron dendrites. Induced clusters were distinguished from endogenous synapses by the absence of synapsin. In contrast, in spite of equal contact of the CFP-positive cotransfected cells with MAP2-positive dendrites, the insACGG mutant (**bottom**) failed to induce clustering of either glutamatergic postsynaptic component. Only synapsinpositive clusters of YFP-LRRTM2 and PSD-95 were seen corresponding to endogenous synapses.

Supplementary Figure 2 (below). NRXN1 c.4205insACGG results in functional deficiency in inhibitory synaptogenic activity in neuron coculture assays. CFP-positive cells cotransfected for HAneurexin1α-CFP wild type (**top**) and cocultured with hippocampal neurons induced clustering of endogenous NLGN2 and gephyrin at contact sites with neuron processes. Induced clusters were distinguished from endogenous synapses by the absence of VGAT. In contrast, the insACGG mutant (**bottom**) failed to induce clustering of either GABAergic postsynaptic component. Only VGAT-positive clusters of NLGN2 and gephyrin were seen corresponding to endogenous synapses.

Supplementary Figure 2

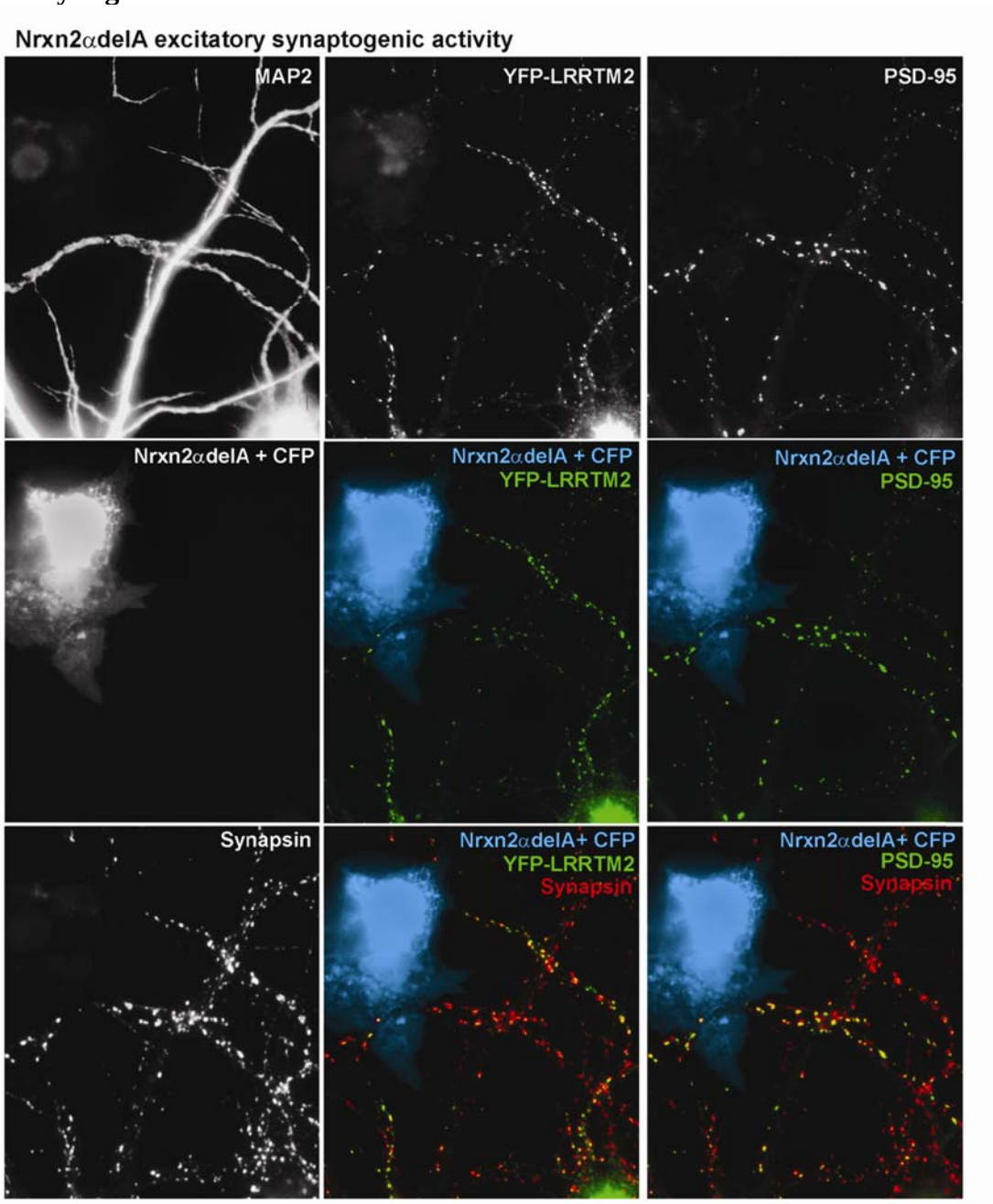


Supplementary Figure 3



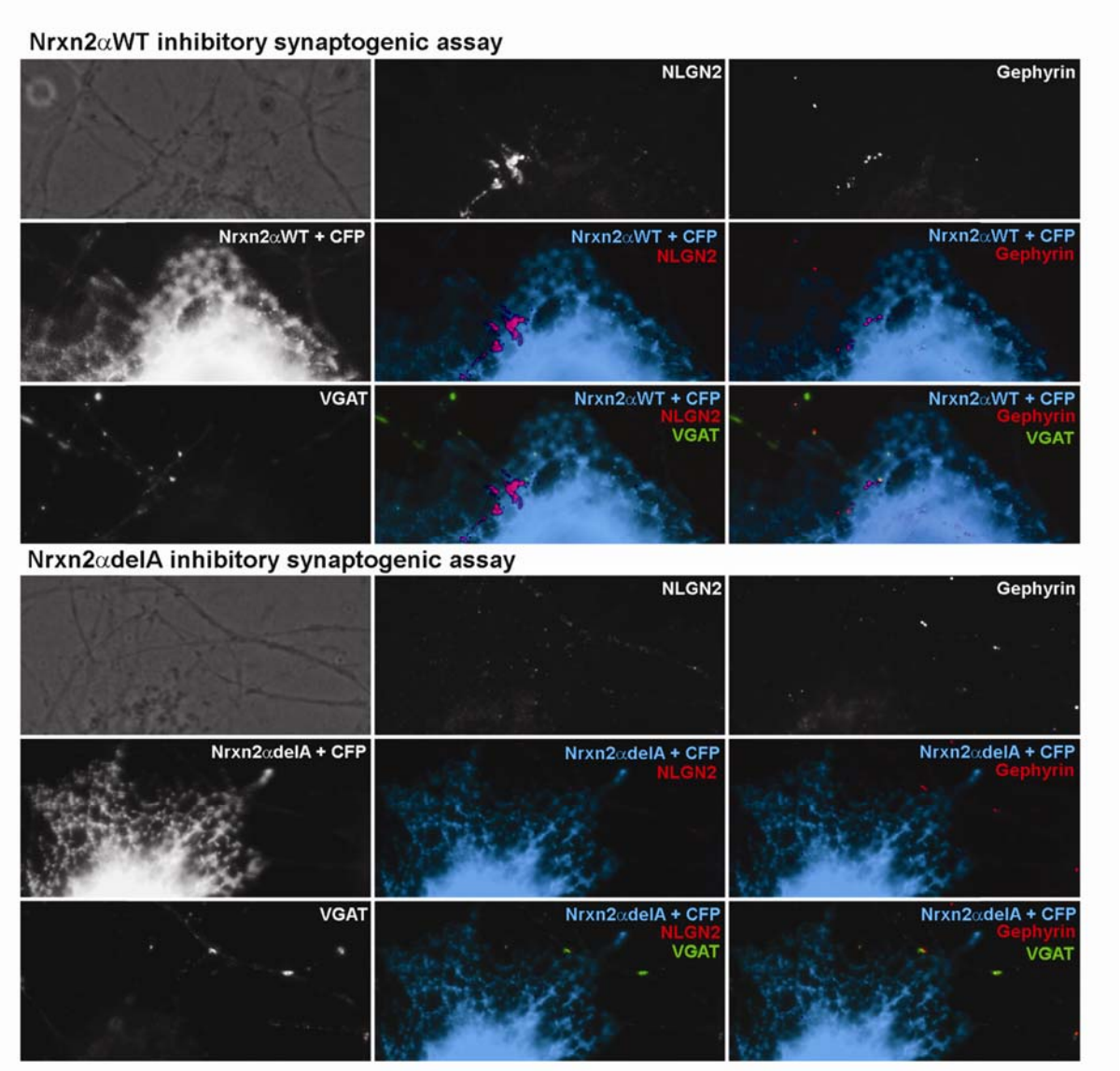
Supplementary Figure 3. NRXN2 exhibits excitatory synaptogenic activity in neuron coculture assays. COS cells were transfected with neurexin2α-CFP wild type and CFP to mark the transfected cells and cocultured with rat hipppocampal neurons transfected with YFP-LRRTM2. CFP-positive cells cotransfected for neurexin2α-CFP induced clustering of YFP--LRRTM2 and endogenous PSD-95 at contact sites with transfected neuron dendritees. Induced clusters were distinguished from endogenous synapses by the absence of synapsin.

Supplementary Figure 4



Supplementary Figure 4. NRXN2 c.2733delT (delA in mouse) results in functional deficiency in excitatory synaptogenic activity in neuron coculture assays. COS cells were transfected with neurexin2α-CFP delA mutant and CFP to mark the transfected cells and cocultured with rat hippocampal neurons transfected with YFP-LRRTM2. In contrast to neurexin2α-CFP wild type (Supp. Figure 3), the delA mutant essentially failed to induce clustering of YFP-LRRTM2 or PSD-95 at contact sites with transfected dendrites. Only synapsin-positive clusters of YFP-LRRTM2 and PSD-95 were seen corresponding to endogenous synapses.

Supplementary Figure 5



Supplementary Figure 5. NRXN2 c.2733delT (delA in mouse) results in functional deficiency in inhibitory synaptogenic activity in neuron coculture assays. CFP-positive cells cotransfected for neurexin2α-CFP wild type (**top**) and cocultured with hippocampal neurons induced clustering of endogenous NLGN2 and gephyrin at contact sites with neuron processes. Induced clusters were distinguished from endogenous synapses by the absence of VGGAT. In contrast, the delA mutant (**bottom**) failed to induce clustering of either GABAergic postsynaptic component. Only VGAT-positive clusters of NLGN2 and gephyrin were seen corresponding to endogenous synapses.

Supplementary Table 1. Primers used to amplify the exons/intronic junctions of *NRXN1*, *NRXN2*, and *NRXN3*

Primer pair Name	Gene	Target RefSeqs	Strand	Exons	Chr	Hg18 Positions F-R)	Forward Primers - F	Reverse Primers -R	Amplicon Size (bp)
G00002_023	NRXN1	NM_138735	+	1	2	50427250-50427503	GCCTTAGGAGCCCAGGAGCG	GCCTGGCCCTGCTTTGGATA	254
G00002_034	NRXN1	BX647616	-	1	2	50054725-50054536	TTTCAGAGGATGGACTAAATTTCC	GCTTCTGGTATCTTAAACCCTGC	190
G00002_037	NRXN1	NM_138735	-	1	2	50427751-50427182	GAGAAGACTGTCCGAAGGAGG	AGAGATAAGTGGCTCGCACC	570
G00002_001	NRXN1	NM_004801	+	2	2	51108292-51108876	CGGCTCATCGTCCAGCTTCA	CTGTGCCTCTCGCTGCTGCT	585
G00002_002	NRXN1	NM_004801	+	2	2	51108021-51108486	TTCCCTCGAAGCGAACTGCC	CGCAGGGACATGACGGTGTT	466
G00002_050	NRXN1	NM_004801	-	2	2	51108643-51108057	CAGCTTCTCCATCTTCTGCG	TAGGAAGACCCCATGCACC	587
G00002_052	NRXN1	EF539882	-	2.1	2	51109058-51108481	GATAAAGGAGGGCACATCCC	CCTGCGCTTGGACTTGAC	578
G00002_049	NRXN1	NM_004801	-	2.3	2	51108483-51108049	AGGGACATGACGGTGTTCAG	GCCAAGATTAGGAAGACCCC	435
G00002_003	NRXN1	NM_004801	+	3	2	51006404-51006783	TTGGGATGGCAGGGTTGAGA	ATGCTGCCAATGGGAGGGAA	380
G00002_051	NRXN1	EF539882	-	3	2	51107233-51106887	CGGCTAATGACATTTATCTGGG	CATTTCAGCCACAAATGCC	347
G00002_004	NRXN1	NM_004801	+	4	2	51002956-51003500	CAGCAACTTCTGATGGTTCTCGG	CCAAAGCATTCCAACCAGGG	545
G00002_036	NRXN1	BC046631	-	5	2	51001229-51000911	GATGGCATAGAGGGAAGAATA	ACACATGCCAACAGAAAACA	319
G00002_048	NRXN1	NM_004801	-	5	2	50711989-50711668	GTCCTGGGTCTCCTTTTGAA	TTATCTTATTTGCCACGAACTGT	322
G00002_006	NRXN1	NM_004801	+	6	2	50703761-50704330	GCCTTTGGGCTCCCAGAACT	TGTTTCTGTTAAGACCTGCTGATTTGC	570
G00002_007	NRXN1	NM_004801	+	7	2	50701552-50702127	CTGGCTGAGCTGCGGTCACT	CCTCTGTGGGAGGCCTACTTGTT	576
G00002_008	NRXN1	NM_004801	+	8	2	50700578-50701110	CGGGTCTTCAGCAAAGGTGC	TTGCAGTAGGGATGATGCTGACA	533
G00002_009	NRXN1	NM_004801	+	9	2	50633382-50633820	TCATCTGTGGGTGCTTGGCA	TTTATTTGCCTGAGAAATGTTGCCTT	439
G00002_010	NRXN1	NM_004801	+	9	2	50633068-50633600	TCTTTCATGGTGTGAAACAGAAGCA	TGAAGATCCATGGAGTGGTGGC	533
G00002_011	NRXN1	NM_004801	+	10	2	50619097-50619565	CAAATCCCTGATGCAGCCCA	TGCATTGACTGAACTGTTTGAGGC	469
G00002_012	NRXN1	NM_004801	+	10	2	50618754-50619308	CAGGTATGGCTGCTCAGCTTGC	GACCTGTGCATTCTCCTCTCTTTGA	555
G00002_013	NRXN1	NM_004801	+	11	2	50611902-50612460	CTAGCTCCAGGCGGAGGGTG	CCACAACTTCGCTGGTGTTTCA	559
G00002_014	NRXN1	NM_004801	+	11	2	50611611-50612119	CCTCACTGACAGACATGGCCTCT	TGCGCTATACCATCCAAGAACTTATCA	509
G00002_015	NRXN1	NM_004801	+	12	2	50609069-50609589	GGAACTGCAGTGGGAAAGTCTTCA	TGCAGCCAGTCTAAGCCAAACA	521
G00002_016	NRXN1	NM_004801	+	13	2	50586843-50587442	TGCAATATTTGATGAGGCACCAAGA	AATTGGTCCAGCCCGCCTCT	600
G00002_017	NRXN1	NM_004801	+	14	2	50578121-50578717	GGTCTTGAAGGTGACAGGATCTGC	GCGTCTCTCTGTGCACTCTCCC	597
G00002_018	NRXN1	NM_004801	+	14	2	50577847-50578350	TTTGTCCTCCACCTGCTCCG	TGGCAGGTGATCATACTAGGCTGG	504
G00002_035	NRXN1	AB011150	-	14	2	50135853-50135382	ATGAATGAATGACAAGTCTGAAAC	GGTAAGATTATTAAACAGCTGGTACG	472
G00002_019	NRXN1	NM_004801	+	15	2	50576499-50577029	GAGACCGTGTGGTGCAAGGC	GGGAGCGCGTGGTGAAAGAT	531
G00002_020	NRXN1	NM_004801	+	15	2	50576386-50576714	GGGAGCAGAGGCTTGTGTGTTG	TGGGAAATGGTGCTAACCTCATCA	329
G00002_021	NRXN1	NM_004801	+	16	2	50552829-50553372	CCAAATTGGGTATTTGACCAGTTATCA	GCCCACATTCCTTCAATTTGACC	544
G00002_022	NRXN1	NM_004801	+	17	2	50545877-50546365	CCAAAGAATCACCATCATCACAGGA	CAGCCTCTCAGTTCCTAATTCAGGTG	489

G00002_024	NRXN1	NM_004801	+	18	2	50317237-50317777	GTTTGCATTGTAAAGAACGCGATTAAC	CAACTTGGTCCACTTTCTTGAGCA	541
G00002_025	NRXN1	NM_004801	+	19	2	50171647-50172243	TCCTTTGTTTAAATGCAAGGATCCAG	GCAGCTGTTGTCCAAAGCACA	597
G00002_026	NRXN1	NM_004801	+	20	2	50134009-50134410	TTGCATGGCAGTGGCTGTTG	GCCAATAATTATGCATTTCATGGACA	402
G00002_027	NRXN1	NM_004801	+	20	2	50133639-50134233	AGCTTGGGCCAGAGGAGGGT	GGGCGTCAGCTCACAATCTTCA	595
G00002_028	NRXN1	NM_004801	+	21	2	50024110-50024663	CCCTGTGTGCTATACCCACTGCT	TGGCTGTCACCATTTCTGCG	554
G00002_029	NRXN1	NM_004801	+	22	2	50002635-50003046	TTCGCACTGCTGGGTTGTTTC	CCATGAGGCAAAGGGATGGC	412
G00002_030	NRXN1	NM_004801	+	22	2	50002285-50002877	TGCGTGCGGTCATCACTGTC	AGGCGGCAGAGAGCCGTATC	593
G00437_025	NRXN2	NM_138734	-	1	11	64166980-64166462	TGGTTTACCTCCGCTTCATC	AAAGCAGAGGGGCCAGG	519
G00437_002	NRXN2	NM_015080	-	3	11	64237450-64236928	ACGCTGCAGCTGGACAC	CAGGAGCCCACACACACTC	523
G00437_003	NRXN2	NM_015080	-	4	11	64221915-64221730	CGTGGTACCTTTGCTAACAGC	AAAGGGAGTCCTTGGGTAGC	186
G00437_004	NRXN2	NM_015080	-	5	11	64217027-64216777	TGGCTGATTTTGATGACTCG	GTGTCAGGACAGGTGGAGG	251
G00437_005	NRXN2	NM_015080	-	6	11	64214598-64214358	CTTCATCCCCTCAAGGCTG	ATGTGCCTGCGTTGACTTG	241
G00437_006	NRXN2	NM_015080	-	7	11	64210068-64209601	GAGGGGAGAGGGAAGCAC	TCCCTCCACCACAAGCTC	468
G00437_007	NRXN2	NM_015080	-	8	11	64201215-64200967	TCCTCGTTCCTTGAGTTTCAC	CAAAAGAGGGAGAATCGAACAG	249
G00437_008	NRXN2	NM_015080	-	9	11	64192727-64192396	GCCTAGCCGTAGCTCTTCC	GACCCAGACAACTAGCCAGG	332
G00437_009	NRXN2	NM_015080	-	10	11	64191808-64191201	CTGCTGGGTGGGAGTGG	GGTGTAGAAGAGACCCGCTG	608
G00437_010	NRXN2	NM_015080	-	11	11	64185301-64184714	CTGCAGGGCACAAGGTG	GCACAGGGATGGAAAGTAGG	588
G00437_011	NRXN2	NM_015080	-	12	11	64184675-64184302	GGCTTTTCGCCCTTGTAATG	GCTTCTCCAGACAGAGGCAG	374
G00437_012	NRXN2	NM_015080	-	13	11	64177864-64177674	AGACACCCCTCCCTGTGAAG	CTCCCTAAATCCCAGCGTC	191
G00437_013	NRXN2	NM_015080	-	14	11	64176290-64176001	GGGAGGCTGTCTGTGTATCC	CCAGGCAGTAGTCACCAAGTAG	290
G00437_014	NRXN2	NM_015080	-	15	11	64175771-64175227	CTCCTGTGGAGGGGAGG	GCCAGGAGAGCTGTATGTGG	545
G00437_015	NRXN2	NM_015080	-	16	11	64174765-64174420	CAGCCATAGCTCCTCCTCAC	CAGGAATGGGGTGATCTCG	346
G00437_016	NRXN2	NM_015080	-	17	11	64173031-64172691	ACCTGACAGCCAGATGCC	CAGAGCAAAGGCTACCTGC	341
G00437_017	NRXN2	NM_015080	-	18	11	64172459-64172176	AAGCTTCCACACCTCCTGG	CTCAGAGGCCCTGTTTCCTC	284
G00437_018	NRXN2	NM_015080	-	19	11	64159576-64159226	GTCACAACCCTGAGGACCTG	AGACTTGAGGTGCTGATCCC	351
G00437_019	NRXN2	NM_015080	-	20	11	64154712-64154378	GAGCTGGGCTAGTGGTGGTC	GCGGCCGCACTCCTATC	335
G00437_020	NRXN2	NM_015080	-	21	11	64150715-64150415	CTTTCCGTGGATGTTGCTG	ATTGGTGAAGGCACGGAG	301
G00437_021	NRXN2	NM_015080	-	22	11	64147224-64146729	CCTCTTTCCTCTCCTTGGC	TGTGGCTATGCAGATATCAACC	496
G00437_022	NRXN2	NM_015080	-	23	11	64144495-64144262	ACCTCAAGTGGCAGCTGTG	CCAGGTGGCAGGGACAG	234
G00437_023	NRXN2	NM_015080	-	24.1	11	64132234-64131615	AACCGATTTGGGTGTGTGAG	GCTCAAAGGACGTGGGG	620
G00437_024	NRXN2	NM_015080	-	24.2	11	64131774-64131141	AACCTCAGGACAGATGGGG	AGCCAGGGAGAGGGTCC	634
G00438_020	NRXN3	AK310235	+	1	14	78150930-78151290	GGCCCTTATGATGCAATTTAG	GGACCTAATGAGATAAAAGTTCAATG	361
G00438_021	NRXN3	AK310235	+	2	14	78181031-78181727	TCACCCCTGCTGCTAGTTTC	CAAGCACAGAGAGAGAATTGAAG	697
G00438_001	NRXN3	NM_004796	+	3	14	78187243-78187514	CCTATGTCCCTCCACCAGG	TGAGGACAGATCCAGTCATTG	272

G00438_002	NRXN3	NM_004796	+	4	14	78245242-78245849	GCCCAGTGAGTGATGGATG	GCATGCAAGGGTGAAAATTAAG	608
G00438_017	NRXN3	NM_001105250	+	4	14	79229134-79229471	AACTCCACCTCTACTAAAGATAAATTG	AACAGTCATTTATGCAGCAGG	338
G00438_003	NRXN3	NM_004796	+	5	14	78250772-78251307	GCTCAGCACTCACCTGTTAGG	CTGCCCAGGGATCCAAC	536
G00438_004	NRXN3	NM_004796	+	6	14	78339638-78339996	CTTAGCCTGAAAGCAAAGGG	CAATCAAACCAATGAAAATCAG	359
G00438_018	NRXN3	AK056530	+	6	14	79396958-79397685	TTGGGTGGCAAGAGGCATACAGA	AGTGGGCAACGGAAGCAAGACTAT	728
G00438_005	NRXN3	NM_004796	+	7	14	78346296-78346565	ATGGCCACATTTCTTACGTG	CCCCACTCCATTGTTAAAAG	270
G00438_006	NRXN3	NM_004796	+	8	14	78493227-78493611	AATTCCTTGAATGCTGCTGG	GACCTTCTTAGCCTTGCCTG	385
G00438_007	NRXN3	NM_004796	+	9	14	78502027-78502617	TGTGGAAACAGGTTACACCC	TGTCAGGAATGGAGGGTAGG	591
G00438_008	NRXN3	NM_004796	+	10	14	78503209-78503569	GGGATTTGAAGCCCACTATG	TGGTTTGCCTCTGGAAATAG	361
G00438_009	NRXN3	NM_004796	+	11	14	78504181-78504512	TGATGAATTATCTCAAGTGTATCAAAC	GGCAGAGGCAGCAAAGAG	332
G00438_010	NRXN3	NM_004796	+	12	14	78524027-78524334	GGTAAATTCAGGTGATTTCCTTG	TTTCAGAAGAGCCATCCTTTTAC	308
G00438_011	NRXN3	NM_004796	+	13	14	79003217-79003612	TCCTCTCTGCAGCTGTTCTG	ATAAGCATGTGCACCTTTGC	396
G00438_012	NRXN3	NM_004796	+	14	14	79199802-79200143	GGAGGATCAAGTTTAAAGGGAG	TGTTCTGGAATTTGGTGACAG	342
G00438_013	NRXN3	NM_004796	+	15	14	79233654-79234119	CCATTCAGACCAGGTAAGGC	TCTTTAGCTACATAAAGCAATGACC	466
G00438_014	NRXN3	NM_004796	+	16	14	79341092-79341476	AATGATGGGAGATGGGACAC	CCATGGGATTTGATGCTTTC	385
G00438_015	NRXN3	NM_004796	+	17	14	79397662-79398133	ATAGTCTTGCTTCCGTTGCC	CTCACCGTCCAAAGATTCATAG	472
G00438_016	NRXN3	NM_001105250	+	1.3	14	78817152-78817704	AAATTTCAGCTCCGGGAAAG	CTACCTGCAAGGCTAGTGGG	553

Supplementary Table 1. Non-synonymous variants identified in *NRXN1, NRXN2 and NRXN3* in patients and control cohorts

									Occurrence								
Gene	Amino Acid Change	Isoform	DNA change	Variant Type	Genomic position[b]	Target Exon	dbSNP	Transmission	ASD (n=142)	SCZ (n= 143)	NSID(n=94)	Controls (n=190)	Feng et al. ASD(n = 264)	Feng et al. Controls (n = 729)	Panther[d]	Sift[d]	PolyPhen[d]
NRXN1	p.T60S	NM_004801.4 NP_004792.1	c.179C>G	Missense	Chr2: 51108737	2	-	Father	0	0	1	0	-	-	NA	0.03	1.72
NRXN1	p.T84R	NM_004801.4 NP_004792.1	c.251C>G	Missense	Chr2: 51108665	2	-	Father	0	0	0	1	-	-	NA	0.17	0.38
NRXN1	p.R88G	NM_004801.4 NP_004792.1	c.262C>G	Missense	Chr2: 51108654	2	-	Mother	0	0	0	1	-	-	NA	0.11	2.08
NRXN1	p.I95V	NM_004801.4 NP_004792.1	c.283A>G	Missense	Chr2: 51108633	2	-	Mother	0	0	1	0	-	-	NA	0.49	0.63
NRXN1	p.P208A	NM_004801.4 NP_004792.1	c.622C>G	Missense	Chr2: 51108294	2	-	Father	1	0	0	0	-	-	NA	1.00	1.93
NRXN1	p.A219_E221del	NM_004801.4 NP_004792.1	c.656delCGGG CGAGG	Deletion	Chr2: 51108252	2	-	Father	0	0	1	0	-	-	NA	NA	NA
NRXN1	p.D392H	NM_004801.4 NP_004792.1	c.1174G>C	Missense	Chr2: 50700810	8	-	Father	0	1	0	1	-	-	-5.98	0.00	2.30
NRXN1	p.P429S	NM_004801.4 NP_004792.1	c.1285C>T	Missense	Chr2: 50700699	8	-	*	2	2	1	0	-	-	-5.17	0.61	2.09
NRXN1	p.I649V	NM_004801.4 NP_004792.1	c.1945A>G	Missense	Chr2: 50619093	10	-	Mother	0	1	0	0	-	-	-5.11	0.50	0.63
NRXN1	p.G667E	NM_004801.4 NP_004792.1	c.2000G>A	Missense	Chr2: 50619038	10	-	Mother	1	0	0	0	-	-	-3.88	0.23	1.55
NRXN1	p.L708I	NM_004801.4 NP_004792.1	c.2122C>A	Missense	Chr2: 50618916	10	-	n.d	2	5	1	3	-	-	-4.32	0.40	1.11

NRXN1	**p.R813C**	**NM_004801.4 NP_004792.1**	**c.2437C>T**	**Missense**	**Chr2: 50587197**	13	**-**	**n.d.**	**0**	**0**	**1**	**0**	**-**	**-**	**-8.09**	**0.04**	**2.51**
NRXN1	p.L869M	NM_004801.4 NP_004792.1	c.2605C>A	Missense	Chr2: 50578249	14	-	*	1	1	0	0	-	-	-5.44	0.34	0.52
NRXN1	p.G17V	NM_138735.2 NP_620072.1	c.50G>T	Missense	Chr2: 50427542	1	rs1341320 5	n.d.	21	17	13	30	>24	>24	NA	0.00	2.02
NRXN1	**p.S14L**	**NM_138735.2 NP_620072.1**	**c.41C>T**	**Missense**	**Chr2: 50427551**	**1**	**-**	**Mother**	**1**	**0**	**0**	**0**	**3**	**0**	**NA**	**0.00**	**1.35**
NRXN1	p.G26del	NM_138735.2 NP_620072.1	c.64delGGC	Deletion	Chr2: 50427516	1	In repeat	n.d	0	0	0	1	-	-	NA	NA	NA
NRXN1	p.G26_A27insGGG G	NM_138735.2 NP_620072.1	c.64insGGC$_{(4)}$	Insertion	Chr2: 50427516	1	In repeat	n.d	0	0	0	1	0	0	NA	NA	NA
NRXN1	p.G26_A27insG	NM_138735.2 NP_620072.1	c.64insGGC	Insertion	Chr2: 50427516	1	In repeat	n.d	0	2	1	0	2	3	NA	NA	NA
NRXN1	p.G26_A27insGG	NM_138735.2 NP_620072.1	c.64insGGC$_{(2)}$	Insertion	Chr2: 50427516	1	In repeat	n.d	1	0	1	0	1	0	NA	NA	NA
NRXN1	p.G22_G26del	NM_138735.2 NP_620072.1	c.64delGGC$_{(5)}$	Deletion	Chr2: 50427528	1	In repeat	n.d	1	1	0	1	6[a]	11	NA	NA	NA
NRXN1	p.I1234M	NM_004801.4 | NP_004792.1	c.3702C>G	Missense	Chr2: 50171981	19	-	*	1	0	1	0	-	-	-5.58	0.11	1.45
NRXN1	**p.G1402fs**	**NM_004801.4 NP_004792.1**	**c.4205insACG G**	**Insertion**	**Chr2: 50002815**	**22**	**-**	***de novo***	**0**	**1**	**0**	**0**	**-**	**-**	**NA**	**NA**	**NA**
NRXN1	**p.H1434R**	**NM_004801.4 NP_004792.1**	**c.4301A>G**	**Missense**	**Chr2: 50002719**	22	**-**	**Mother**	**0**	**1**	**0**	**0**	**-**	**-**	**-6.25**	**0.14**	**2.06**
NRXN2	p.P1696H	NM_015080.3 NP_055895.1	c.5087C>A	MISSENSE	chr11:64131296	23	-	Mother	0	0	1	0	-	-	-5.99	0.09	1.69
NRXN2	p.F1118L	NM_015080.3 NP_055895.1	c.3352T>C	MISSENSE	chr11:64172318	17		n.d.	0	0	0	1			ND	ND	ND

NRXN2	p.H1084Y	NM_015080.3 NP_055895.1	c.3250C>T	MISSENSE	chr11:64172815	16	-	n.d.	1	1	0	0	-	-	-6.52	1.00	1.89
NRXN2	p.D1081N	NM_015080.3 NP_055895.1	c.3241G>A	MISSENSE	chr11:64172824	16	-	Mother	0	1	0	0	-	-	-4.78	0.20	1.70
NRXN2	p.R1031G	NM_015080.3 NP_055895.1	c.3091C>G	MISSENSE	chr11:64174514	15	-	Mother	1	0	0	0	-	-	NA	0.32	2.17
NRXN2	**p.P911fs**	**NM_138732.2 NP_620060.1**	**c.2733delT**	**deletion**	**chr11:64175368**	12	**-**	**Father**	**1**	**0**	**0**	**0**	**-**	**-**	**NA**	**NA**	**NA**
NRXN2	p.V846M	NM_138732.2 NP_620060.1	c.2536G>A	MISSENSE	chr11:64175565	12	-	n.d.	1	1	0	1	-	-	-3.72	0.23	1.07
NRXN2	p.A814V	NM_015080.3 NP_055895.1	c.2441C>T	MISSENSE	chr11:64176178	13	-	Mother	1	0	0	0	-	-	-5.87	0.22	0.55
NRXN2	p.P805S	NM_015080.3 NP_055895.1	c.2413C>T	MISSENSE	chr11:64177747	12	-	Father	1	0	0	0	-	-	-4.70	0.83	0.08
NRXN2	p.V655A	NM_015080.3 NP_055895.1	c.1964T>C	MISSENSE	chr11:64185022	10	-	Mother	1	0	0	0	-	-	-4.77	0.34	1.61
NRXN2	p.G630S	NM_015080.3 NP_055895.1	c.1888G>A	MISSENSE	chr11:64185098	10	-	Mother	0	1	0	0	-	-	-5.74	0.01	1.82
NRXN2	p.V364I	NM_015080.3 NP_055895.1	c.1090G>A	MISSENSE	chr11:64209756	6	-	*	1	0	1	1	-	-	-4.07	0.39	1.07
NRXN2	p.G268V	NM_015080.3 NP_055895.1	c.803G>T	MISSENSE	chr11:64214500	5	-	Mother	0	1	0	0	-	-	NA	0.04	1.36
NRXN2	p.A200T	NM_015080.3 NP_055895.1	c.598G>A	MISSENSE	chr11:64237150	2		Mother	0	1	0	0	-	-	NA	0.58	0.68
NRXN3	p.R308S	NM_004796.4 NP_004787.2	c.924G>C	MISSENSE	chr14:78251234	5	-	Mother	1	0	0	-	-	-	-4.64	0.51	2.02
NRXN3	p.I669L	NM_004796.4 NP_004787.2	c.2005A>C	MISSENSE	chr14:78504424	11	-	Mother	1	0	0	-	-	-	-3.66	0.24	0.65

NRXN3	p.I42L	NM_004796.4 NP_004787.2	c.124A>C	Missense	chr14:78245334	4	-	Father	0	1	0	-	-	-	-4.01	1.00	0.26

Bold; variants tested functionally; n.d.; not determined; NA, not available; [a]found in Afro-American and Controls only, [b]March 2006 Assembly, [c]According to NRXN1-α; [d]Panther score ≤-3 functional significance, >-3 = neutral; SIFT score: <0.05 deleterious, ≥ 0.05 tolerated; Polyphen score: ≥2.00 probably damaging, 1.50-1.99 possibly damaging, <1.50 benign; *transmitted from an unaffected parent.