Supplemental Data

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CNTNAP2 and *NRXN1* Are Mutated in Autosomal-Recessive Pitt-Hopkins-like Mental Retardation and Determine the Level of a Common Synaptic Protein in *Drosophila*

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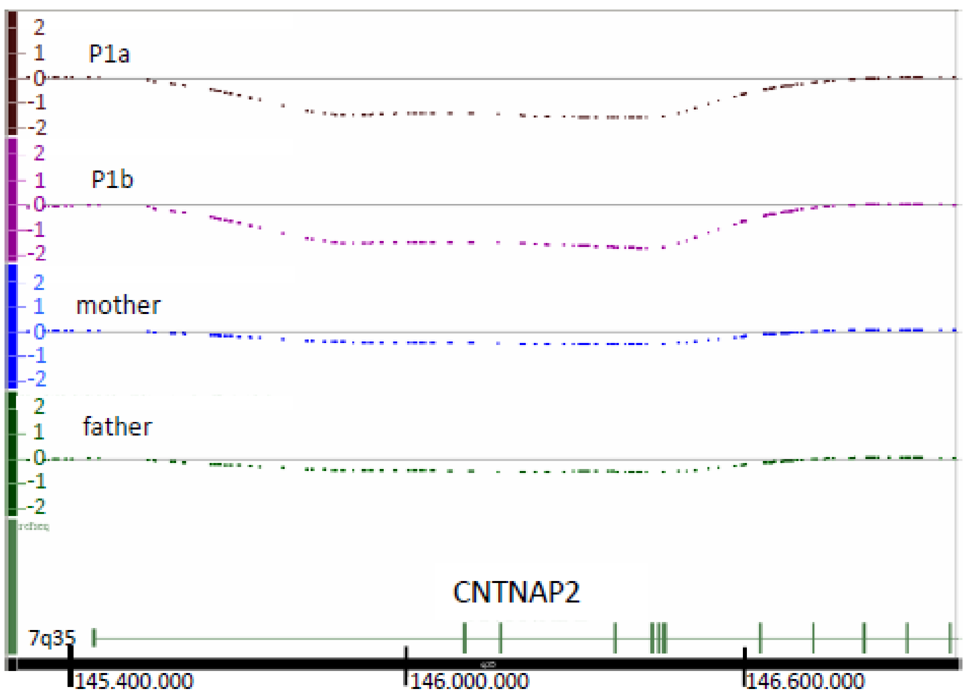
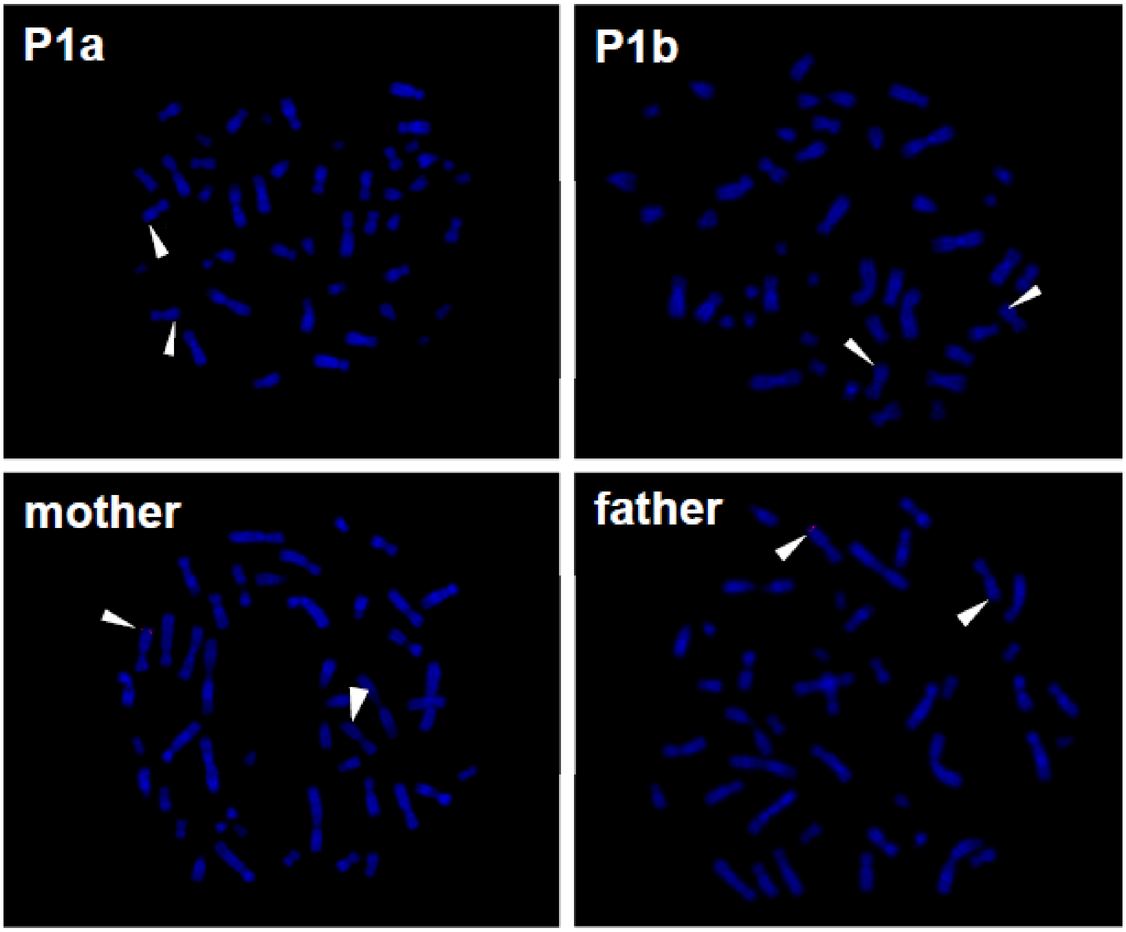
Figure S1. SNP Copy Number Profile in Family 1



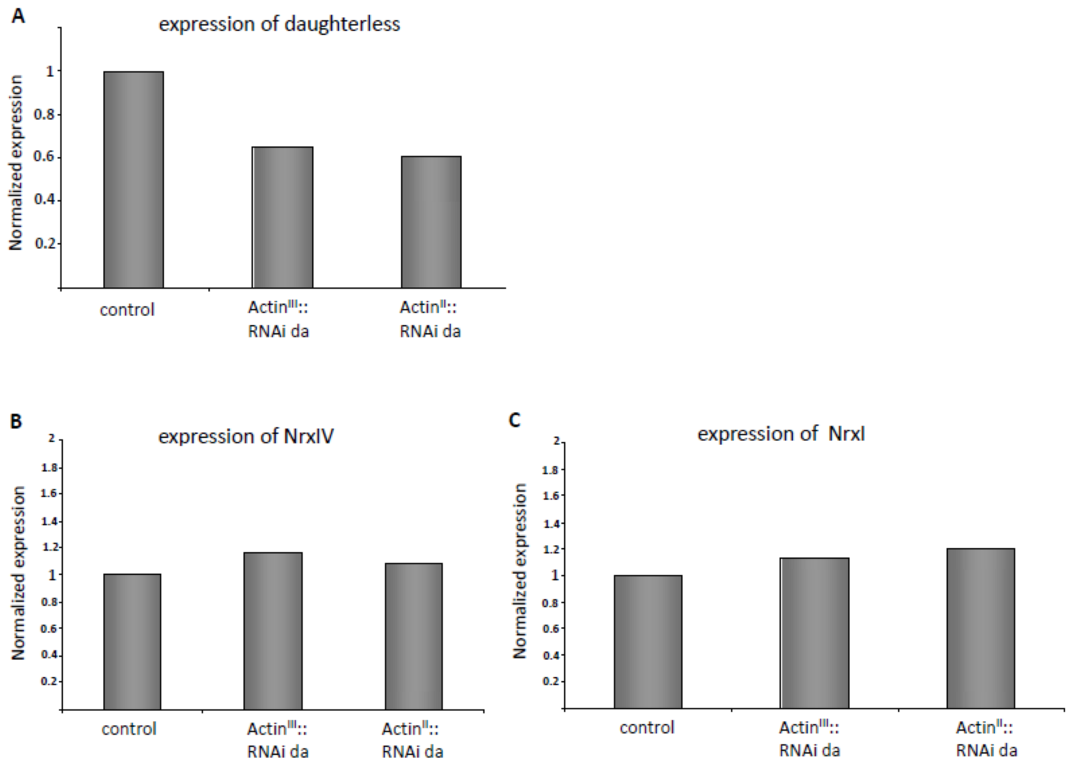
SNP copy number profile (Log2 ratio) of the Affymetrix 250 K array, analyzed with the Genotyping Console 3.0.2 software (Affymetrix) in all members of family 1, showing a homozygous deletion within the *CNTNAP2* gene in both affected children and a heterozygous deletion in both healthy parents.

Figure S2. FISH Analysis in Family 1



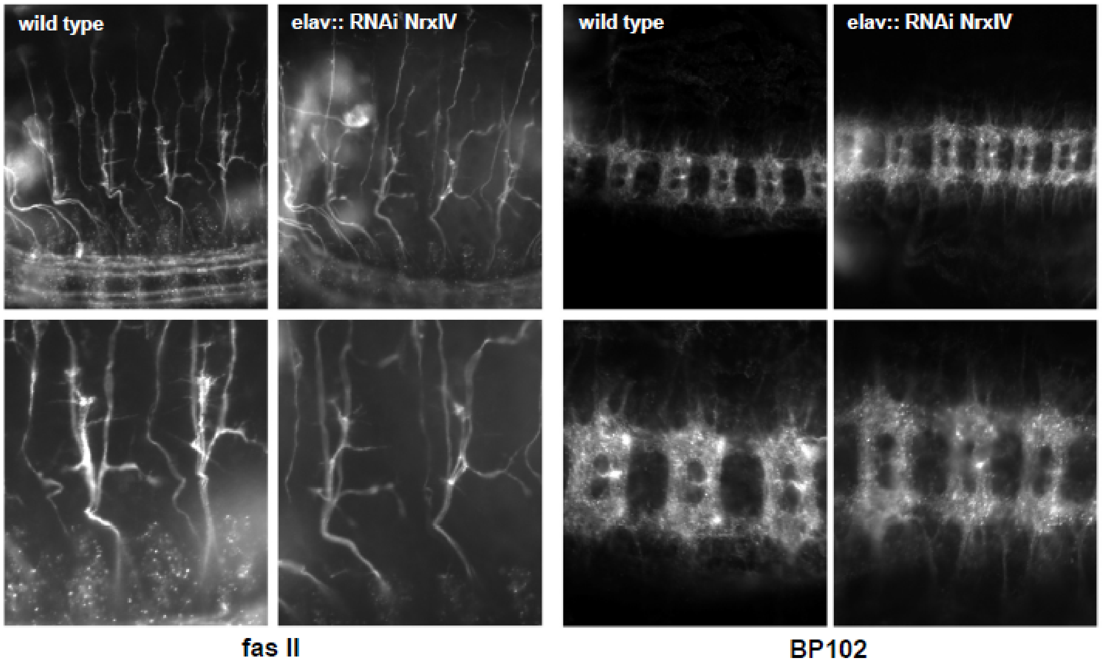
FISH analysis with the directly Cy3-labeled BAC clone RP4-558L10 on metaphase spreads. White arrowheads indicate chromosomes 7. In the patients (P1a, P1b) a homozygous deletion of the BAC clone can be seen, in both parents a heterozygous deletion each.

Figure S3. Expression of Daughterless, Nrx-IV, and Nrx-I in Daughterless Knockdown Larvae



(A) Normalized expression of daughterless after a knockdown with two different ubiquitous drivers on the second (II, Actin-Gal4 17bFO1) and third (III, Actin-Gal4 25FO1) chromosome, respectively. In both cases the knockdown is down to 60% compared to the wt (w1118) (B,C) Normalized expression of Nrx-IV and Nrx-I upon knockdown of daughterless, respectively. No significant changes were detected.

Figure S4. Immunostaining of the Embryonic Nervous System



Examples of immunofluorescence analysis of the nervous system in wild type and Nrx-IV panneuronal knockdown embryos. Anti-fas II stains motor- and central pioneer axons. Antibody BP102 stains axon tracts/the central neuropile region. No apparent morphological or structural alterations were observed in the Nrx-IV knockdown embryos compared to the wild type.

Table S1. Primer Sequences for Amplification and Sequencing of All Coding Exons of *CNTNAP2* and *NRXN1*

exon	primer sequence	amplicon length (bp)
CNTNAP2_e1F	CCTCGCGTATTTGAGGACAG	345
CNTNAP2_e1R	AGTGGCTGCAAGTGTGTGAC	
CNTNAP2_e2F	GAATTGCCTAAATTCCTTTGC	363
CNTNAP2_e2R	TGGTGTCTGCCAACATCTG	
CNTNAP2_e3F	GCACTGCCAAGACCAATTAAG	427
CNTNAP2_e3R	TGATGAATAAATAGTTTCCCAATG	
CNTNAP2_e4F	catggatgaaaagacccaca	481
CNTNAP2_e4R	aaggtagtttattgtcagagaaagca	
CNTNAP2_e5F	tctttgcagacacctgttgg	452
CNTNAP2_e5R	tttttgaatgactaggtgtttcatt	
CNTNAP2_e6F	tcccaggttaactcgaatgg	477
CNTNAP2_e6R	tgaaacgaattaatcaggttttt	
CNTNAP2_e7F	GCCATAGATTTTGGAGGCAG	413
CNTNAP2_e7R	ACATCATTTTGCCCAAACAC	
CNTNAP2_e8F	TCACTGAATCCATGCTCTGC	524
CNTNAP2_e8R	AAAACCTAATCCTGAGCGTGTAAC	
CNTNAP2_e9F	ttgtaagcagcactgtattttcc	497
CNTNAP2_e9R	ggccagaagaatatggtgaca	
CNTNAP2_e10F	GAAACAGTAGTTGGATGTGATGG	408
CNTNAP2_e10R	GAATGGTAATTTCCACCTTACCTG	
CNTNAP2_e11F	CCTTGGTAAGGCAACCTGG	365
CNTNAP2_e11R	GAAATGACAATTGGAATCTTGG	
CNTNAP2_e12F	CTCTTTCCAGGAAGAACTACTCC	369
CNTNAP2_e12R	GCAATATGTTGCTGATTAGATGTTG	
CNTNAP2_e13F	GCTCTCCTTAACACTGTTCTACACC	460
CNTNAP2_e13R	CTTATTTACAGCTTCCTTCCTACTG	
CNTNAP2_e14F	agagtattcctggggaagtgg	440
CNTNAP2_e14R	ttgtcgcactgacctctttct	
CNTNAP2_e15F	CCAAACGATTACTGAAATGTCATC	373
CNTNAP2_e15R	ATCTCTGCTTGGGTTGTGTG	
CNTNAP2_e16F	tgtgaggatttggtccaatg	469
CNTNAP2_e16R	aggcttgtgtgtccacctct	
CNTNAP2_e17F	TCGACCTTTGTAGGACGTGAC	479
CNTNAP2_e17R	GGCCAACACCTTTACTTTTGG	
CNTNAP2_e18F	GCCTTATAGCCTGCAGGGAG	587
CNTNAP2_e18R	GATTAGGAAATGATTTTGGTTGG	
CNTNAP2_e19F	TACTCAGATGCCCTTCCTGG	462
CNTNAP2_e19R	GCCTATGGGGAATAAATAACAAAC	
CNTNAP2_e20F	agcaggaattgaggggatgt	350
CNTNAP2_e20R	ttatgcacttgtaggagaaagtgt	
CNTNAP2_e21F	GAAAACCAGGGTTCAAAGAGTG	314
CNTNAP2_e21R	AAGATATTCGTGACTGGCCC	
CNTNAP2_e22F	gctttggacacaagcattca	462
CNTNAP2_e22R	acgttcctttgccctttctt	
CNTNAP2_e23F	gttgtgattcttgtgggagaca	366
CNTNAP2_e23R	cagcaaaatgaataatgtaaaaacc	
CNTNAP2_e24F	GAGAGGGCTGTGTCTGACG	437
CNTNAP2_e24R	ATATTCCATTGCCTGCCTCC	
NRXN1_ex2aF	CCCTTACCTTTCTGTCTCTCG	499
NRXN1_ex2aR	GTCGATGAAGAGCGTGGTGT	

NRXN1_ex2bF	CGACTTCCTGGAGCTGATTC	696
NRXN1_ex2bR	gagtcccccagaaacaaggt	
NRXN1_ex3F	tgattttgttttccccttg	344
NRXN1_ex3R	gatggcagggttgagaatgt	
NRXN1_ex4F	gtgcagcatatgccagtgtt	446
NRXN1_ex4R	agccacaggaacaaaccaaa	
NRXN1_ex5F	aagccaggctgtctctgcta	386
NRXN1_ex5R	tctggattggtctcgaggtt	
NRXN1_ex6F	ctgtttgactgagacagagttca	596
NRXN1_ex6R	tggcaggaagattcatctcag	
NRXN1_ex7F	ctctgtgggaggcctacttg	418
NRXN1_ex7R	cagatgaaaagaaggaggtcaaa	
NRXN1_ex8F	caggcatatcccaggattaca	341
NRXN1_ex8R	gtgccgtttgactctggaac	
NRXN1_ex9F	tcgttgaaagttacatgagctg	595
NRXN1_ex9R	tcacttttaggaatggcatgg	
NRXN1_ex10F	ttgtctgcctccaaggagtt	573
NRXN1_ex10R	caatggtcagtgcaggtttg	
NRXN1_ex11F	tgaagaagatgaattgattttgtagt	493
NRXN1_ex11R	cccccgaaaacctcaaatta	
NRXN1_ex12F	ttggccatttttaaaaccttc	432
NRXN1_ex12R	gcagtgggaaagtcttcagc	
NRXN1_ex13F	ctctgtgaggttcatttgcttg	380
NRXN1_ex13R	tgtcatttttgaggaaaaacacc	
NRXN1_ex14F	tgtgatattgttatgaaagcctaaa	599
NRXN1_ex14R	tgccttcttatttgtcctcca	
NRXN1_ex15F	ggatggaaccacctgaaaaa	450
NRXN1_ex15R	cagaggcttgtgtgttgtattg	
NRXN1_ex16F	tttagcactttggggaaaaca	433
NRXN1_ex16R	ccaaattgggtatttgaccag	
NRXN1_ex17F	cagcctctcagttcctaattca	429
NRXN1_ex17R	cctttccgtagaaacaactgc	
NRXN1_ex18F	gccatagttttgtgtgtagagttga	416
NRXN1_ex18R	tttcctataacaaagtactggtttctg	
NRXN1_ex19F	gaagtaaaattggaggaaagca	450
NRXN1_ex19R	ggacagcattacattcacatgac	
NRXN1_ex20F	tgctctcattattcaccccata	541
NRXN1_ex20R	ggaagctgtagtgcctaagatca	
NRXN1_ex21F	aaagggaaatagtgaatttggtttc	449
NRXN1_ex21R	aagccctgtgtgctataccc	
NRXN1_ex22F	aggcaaagggatggctacat	550
NRXN1_ex22R	TGTGCTTCATAAAAAGGAAAGTAAA	

Table S2. Probe Sequences for MLPA Analysis of All Coding Exons of *CNTNAP2* and Exon 2 of *NRXN1*

exon	**probe sequence**	**probe length**
CNTNAP2_ex1_F	GGGTTCCCTAAGGGTTGGATGCTGTGGATTGTCAGCAGCT GCCTCT	96
CNTNAP2_ex1_R	GCAGAGCCTGGACGGCTCCCTCCACGTTCTAGATTGGATC TTGCTGGCAC	
CNTNAP2_ex2_F	GGGTTCCCTAAGGGTTGGAGTGATGAGCCACTTGTCTCTG GACTCCCCCA	104
CNTNAP2_ex2_R	TGTGGCTTTCAGCAGCTCCTCCTCCATCTCTTCTAGATTGG ATCTTGCTGGCAC	
CNTNAP2_ex3_F	GGGTTCCCTAAGGGTTGGAGTCTCCATCAGACAGCGACCA TTATCAATGGCT	108
CNTNAP2_ex3_R	TCAGGTTGACTTTGGCAATCGGAAGCAGATCAGTCTAGATT GGATCTTGCTGGCAC	
CNTNAP2_ex4_F	GGGTTCCCTAAGGGTTGGAGAAACATTAACTCTGACGGTGT GGTCCGGCACGAA	112
CNTNAP2_ex4_R	TTACAGCATCCGATTATTGCCCGCTATGTGCGCATTCTAGA TTGGATCTTGCTGGCAC	
CNTNAP2_ex5_F	GGGTTCCCTAAGGGTTGGAGATGGCCATGTTGTATTACCAT ATAGATTCAGAAACAA	116
CNTNAP2_ex5_R	GAAGATGAAAACACTGAAAGATGTCATTGCCTTGAATCTAG ATTGGATCTTGCTGGCAC	
CNTNAP2_ex6_F	GGGTTCCCTAAGGGTTGGAGCATTAACCTCACTCTGGACAG GAGCATGCAGCACTTCC	120
CNTNAP2_ex6_R	GTACCAATGGAGAGTTTGACTACCTGGACTTGGACTATGTC TAGATTGGATCTTGCTGGCAC	
CNTNAP2_ex7_F	GGGTTCCCTAAGGGTTGGAGAGGCATCCCTTTCTCTGGCA AGCCCAGCTC	104
CNTNAP2_ex7_R	CAGCAGTAGAAAGAATTTCAAAGGCTGCATGTCTAGATTGG ATCTTGCTGGCAC	
CNTNAP2_ex8_F	GGGTTCCCTAAGGGTTGGAGGACGGCTTAACCAGGACCTG TTCTCAGTCAGTTTCCAGTT	124
CNTNAP2_ex8_R	TAGGACATGGAACCCCAATGGTCTCCTGGTCTTCAGTCACT TCTAGATTGGATCTTGCTGGCAC	
CNTNAP2_ex9_F	GGGTTCCCTAAGGGTTGGAGAATGATGGACAGTGGCACGA GGTTCGCTTCCT	108
CNTNAP2_ex9_R	AGCCAAGGAAAATTTTGCTATTCTCACCATCGATCTAGATTG GATCTTGCTGGCAC	
CNTNAP2_ex10_F	GGGTTCCCTAAGGGTTGGACATTCCAAGGATGCATGCAGC TCATTCAAGTGGA	112
CNTNAP2_ex10_R	CGATCAACTTGTAAATTTATACGAAGTGGCACAAAGTCTAGA TTGGATCTTGCTGGCAC	
CNTNAP2_ex11_F	GGGTTCCCTAAGGGTTGGAGACAGCTTCAAATGCACTTGTG ATGAGAC	100
CNTNAP2_ex11_R	AGGATACAGTGGGGCCACCTGCCACAACTTCTAGATTGGAT CTTGCTGGCAC	
CNTNAP2_ex12_F	GGGTTCCCTAAGGGTTGGATATCTACGAGCCTTCCTGTGAA GCCTACAAA	104
CNTNAP2_ex12_R	CACCTAGGACAGACATCAAATTATTACTGGATCTAGATTGG ATCTTGCTGGCAC	
CNTNAP2_ex13_F	GGGTTCCCTAAGGGTTGGAGCAGATGCAGACGCCTGTGGT CGGCTACAACCCAGAA	116
CNTNAP2_ex13_R	AAATACTCAGTGACACAGCTCGTTTACAGCGCCTCCATCTA	

	GATTGGATCTTGCTGGCAC	
CNTNAP2_ex14_F	GGGTTCCCTAAGGGTTGGAGCCCTTACACTTGGTGGGTTG GCAAAGCCAACG	108
CNTNAP2_ex14_R	AGAAGCACTACTACTGGGGAGGCTCTGGGCCTGTCTAGAT TGGATCTTGCTGGCAC	
CNTNAP2_ex15_F	GGGTTCCCTAAGGGTTGGATTGGAGATACTGACCGTCAAG GCTCAG	96
CNTNAP2_ex15_R	AAGCCAAATTGAGCGTAGGTCCTCTGCTCTAGATTGGATCT TGCTGGCAC	
CNTNAP2_ex16_F	GGGTTCCCTAAGGGTTGGAGAAACTAGCGCTGACATTTCTT TCTACTTCAAAAC	112
CNTNAP2_ex16_R	ATTAACCCCCTGGGGAGTGTTTCTTGAAAATATGGTCTAGA TTGGATCTTGCTGGCAC	
CNTNAP2_ex17_F	GGGTTCCCTAAGGGTTGGAGTGTCCTTTTCATTTGATGTGG GAAATGGGCCAGTAGAG	120
CNTNAP2_ex17_R	ATTGTAGTGAGGTCACCAACCCCTCTCAACGATGACCAGTC TAGATTGGATCTTGCTGGCAC	
CNTNAP2_ex18_F	GGGTTCCCTAAGGGTTGGACAAAGGTCACATCTGGGTTCAT ATCCGGATGCTCGGGCCAT	124
CNTNAP2_ex18_R	TGCACCAGCTATGGAACAAACTGTGAAAATGGAGGCAAATG TCTAGATTGGATCTTGCTGGCAC	
CNTNAP2_ex19_F	GGGTTCCCTAAGGGTTGGACCTGGCACAGGAGGAGATCCG CTTCAGCTTCAGCACCACCA	124
CNTNAP2_ex19_R	AGGCGCCCTGCATTCTCCTCTACATCAGCTCCTTCACCACA TCTAGATTGGATCTTGCTGGCAC	
CNTNAP2_ex20_F	GGGTTCCCTAAGGGTTGGATGGGTGGCACCCGAGAGCCAT ACAATATT	100
CNTNAP2_ex20_R	GACGTAGACCACAGGAACATGGCCAATGGTCTAGATTGGAT CTTGCTGGCAC	
CNTNAP2_ex21_F	GGGTTCCCTAAGGGTTGGATCCTTCTGTGAGTTACCATCTG CCAAGTT	100
CNTNAP2_ex21_R	CATCCGACACCCTCTTCAATTCTCCCAAGTCTAGATTGGAT CTTGCTGGCAC	
CNTNAP2_ex22_F	GGGTTCCCTAAGGGTTGGACAGGATTCACTGGTTGCCTCTC CAGAGTCCAGTTCAACC	120
CNTNAP2_ex22_R	AGATCGCCCCTCTCAAGGCCGCCTTGAGGCAGACAAACGT CTAGATTGGATCTTGCTGGCAC	
CNTNAP2_ex23_F	GGGTTCCCTAAGGGTTGGAGACAAGGCCAAGCTATAAGAA ATGGAG	96
CNTNAP2_ex23_R	TCAACAGAAACTCGGCTATCATTGGAGTCTAGATTGGATCT TGCTGGCAC	
CNTNAP2_ex24_F	GGGTTCCCTAAGGGTTGGACTGTGGTGATTTTCACCATCCT GTGCACCCTGGTCTT	116
CNTNAP2_ex24_R	CCTGATCCGGTACATGTTCCGCCACAAGGGCACCTACTCTA GATTGGATCTTGCTGGCAC	
NRXN1_ex2_F	GGGTTCCCTAAGGGTTGGACGTGAGGGTCAACTCCTCGCA GG	88
NRXN1_ex2_R	TCCTGCCCGTGGACAGCGGCGAGTCTAGATTGGATCTTGC TGGCAC	

Table S3. Primer Sequences for Quantitative Real-Time PCR

gene	**primer sequence**	**product length (bp)**
Daughterless_F	CTCGCTGCAACAAAAGGAAT	120
Daughterless_R	AAGCAGTTCTGGAACACCTCA	
Neurexin-I_F	AATCTGCGGCTGCAAGTC	105
Neurexin-I_R	GAAGAGACCACCCAGGTGAA	
Neurexin-IV_F	TGCCATACATCAAACAATCCA	111
Neurexin-IV_F	AGGTTCTAGGGGACCACTGC	