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Supplemental Information

Dominant-Negative Mutations in α-II Spectrin Cause West Syndrome with Severe Cerebral Hypomyelination, Spastic Quadriplegia, and Developmental Delay

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Outline

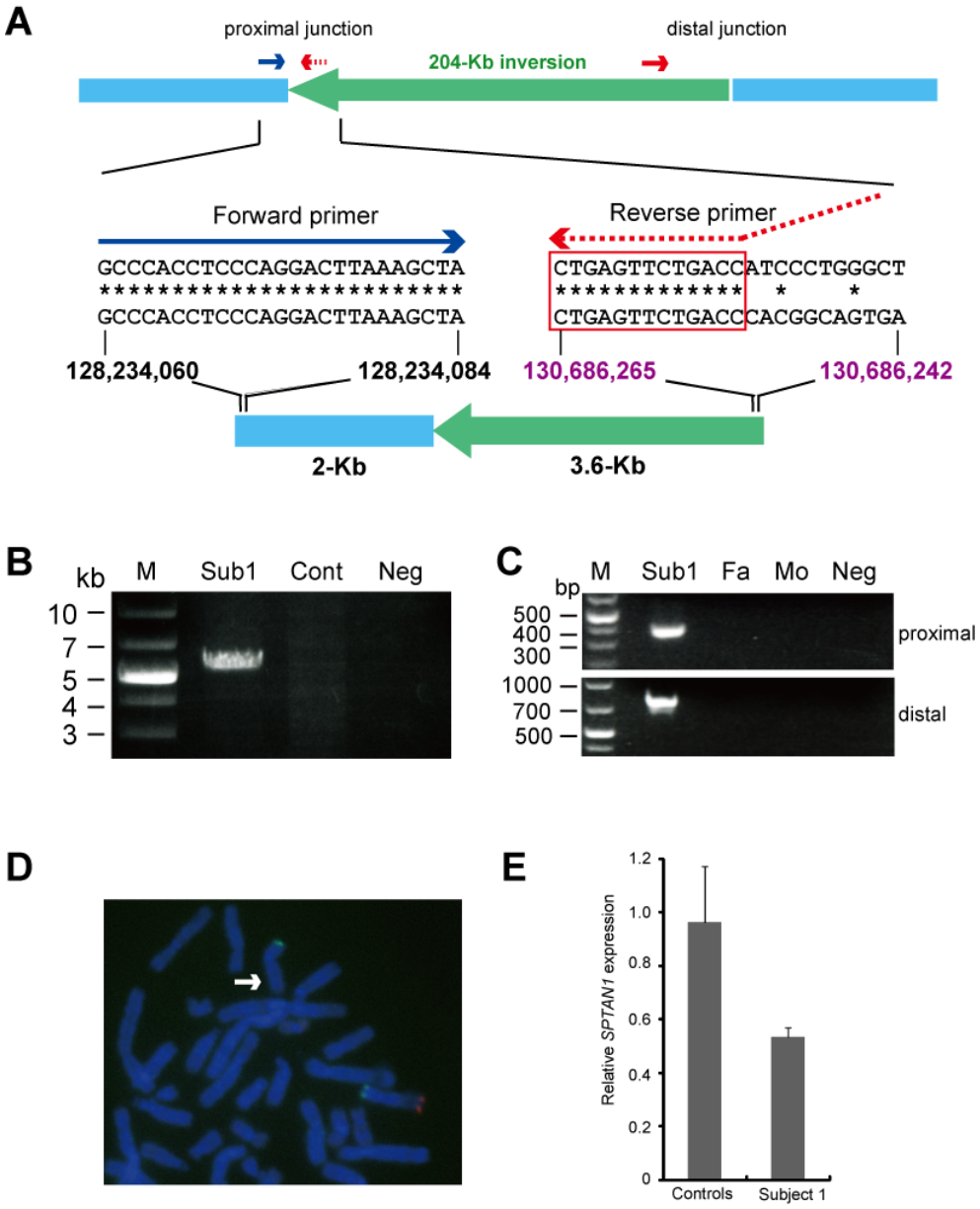

Figure S1. Genomic rearrangements at 9q33.3-q34.11 in subject 1. (A) Deletion junctions were narrowed-down by GeneChip Human Mapping 250K NspI analysis (Affymetrix), which was performed and analysed as previously described.[1] The proximal and distal junctions should map between chr 9:128,205,955 and 128,245,900 (40 kb interval) [UCSC genome browser coordinate (version Mar. 2006)], and between 130,402,182 and 130,493,941 (91.7 kb interval), respectively. Long PCR was performed in a 15 µl volume, containing 100 ng genomic DNA, 1× PCR Buffer for KOD FX, 0.4 mM each dNTP, 0.3 µM each primer, and 0.3 U KOD FX polymerase (Toyobo, Osaka, Japan). Position and sequences of primers used for cloning of the proximal break point was indicated (upper). An unexpected 204-kb inversion and intact genomic regions are shown in green arrow and sky blue, respectively. The forward primer is shown by a blue

arrow. The reverse primer was initially designed to anneal to the position of the red solid arrow, but it accidentally annealed to the position of the red dashed arrow. Close-up view of the proximal breakpoint is also shown (lower). Two aligned nucleotide sequences show primer (upper) and chromosome 9 (lower) sequences with asterisks indicating identical nucleotides between them. Genomic positions of chromosome 9 are according to the UCSC genome browser coordinates (version Mar. 2006). In the reverse primer (red dashed arrow) originally designed without any supposition of the inversion, 13-bp sequence of the 3' side was accidentally matched to the 3.6 kb distal region from the proximal breakpoint in an inverted direction (red box), allowing PCR-amplification. The distal deletion junction fragment (also including another breakpoint of the inversion) was amplified using primers, Forward: 5'-TGAGGTCATCCTGCAAAGTGCGTAT-3' and Reverse: 5'-GAGCCAGGAGTTGGCTGACCTTATC-3' (11.9 kb amplicon). (B) PCR with two primers shown in (A) successfully amplified a 5.6 kb fragment using genomic DNA of subject 1 as a template. Sub1, subject 1; Cont, control DNA, Neg, negative control (no template DNA). (C) Breakpoint-specific PCR analysis of the family of subject 1. Primers specific to proximal (Forward: 5'-AACCCTCACCTGTGACCCAGTCATT-3', Reverse, 5'-TGTTTGAGTTGCTGATTCACAGTTTGCT-3', upper panel) and distal (Forward, 5'-GGCCAGAGAAGTTTCTGAGCACCTG-3', Reverse, 5'-GAGCCAGGAGTTGGCTGACCTTATC-3', lower panel) breakpoints could successfully amplify 381- and 842-bp products, respectively, only from Subject 1 (Sub 1), indicating the genomic rearrangements occurred *de novo*. M, size marker; Fa, father; Mo, mother; Neg, negative control. (D) FISH images of two BAC clones on subject 1's chromosomes are depicted. While RP11-307l3 on the short arm of chromosome 9 (green) showed two signals as a marker of chromosome 9, RP11-589e16 which spans the entire *SPTAN1* gene (red) showed only one signal, indicating heterozygous deletion of *SPTAN1* in subject 1 (white arrow). (F) Relative mRNA levels of *SPTAN1* in lymphoblastoid cells (LCLs) were determined by quantitative real-time RT-PCR using TaqMan Gene expression assays (Hs00162203_m1 for *SPTAN1* and Hs00357333_g1 for β-actin as a control). Levels are shown for four controls and subject 1. Average of duplicated experiments is displayed as a gray bar with an error bar of the standard deviation. LCLs derived from subject 1 showed almost half the level of expression of *SPTAN1* mRNA compared with that of four normal control LCLs.

1. Saitsu, H., Kurosawa, K., Kawara, H., Eguchi, M., Mizuguchi, T., Harada, N., Kaname, T., Kano, H., Miyake, N., Toda, T., et al. (2009). Characterization of the complex 7q21.3 rearrangement in a patient with bilateral split-foot malformation and hearing loss. Am J Med Genet A *149A*, 1224-1230.

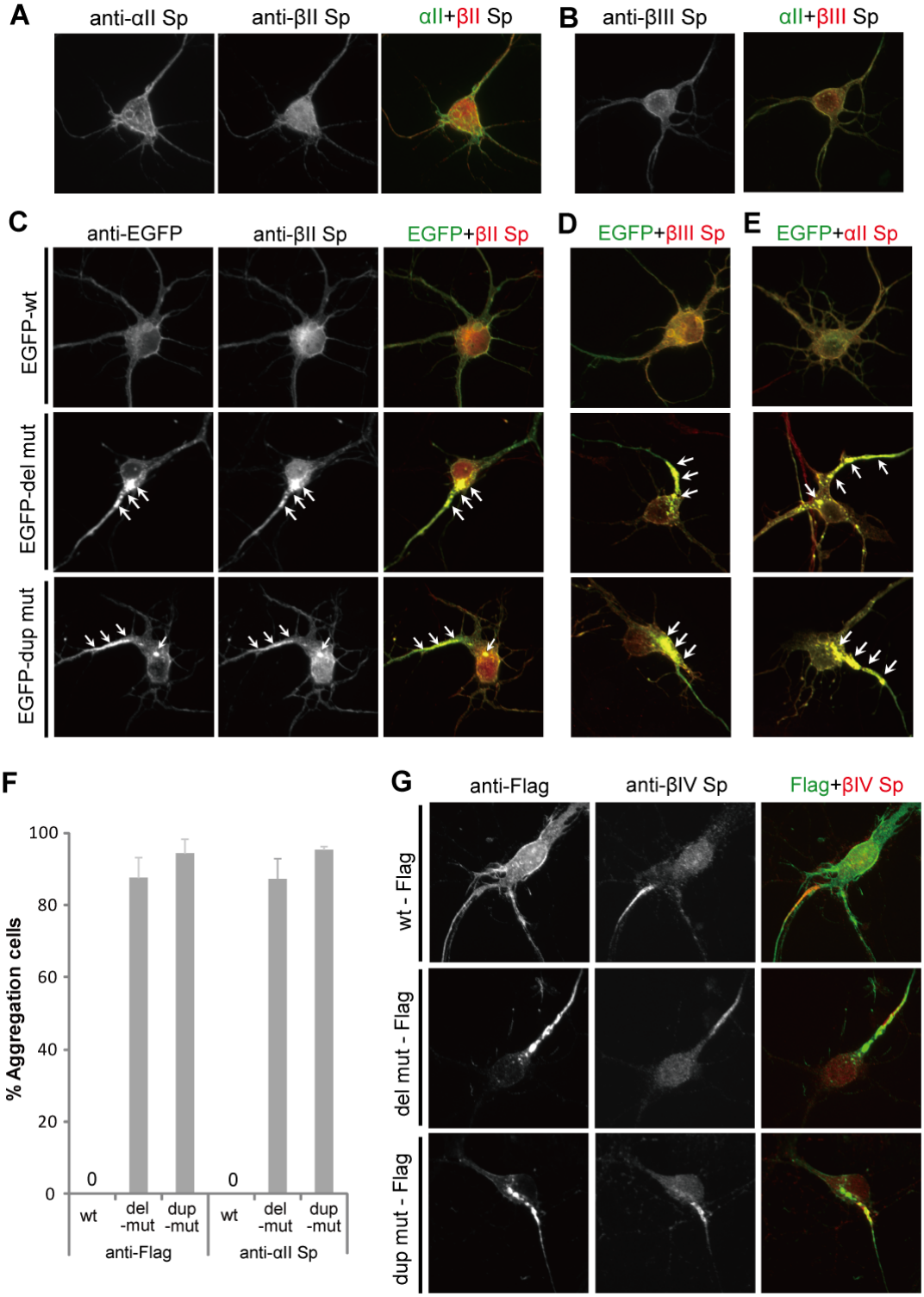

Figure S2. Transient expression experiments of tagged α-II spectrin. Protein expression was evaluated at seven days of maturation in vitro (DIV) (A-F) and nine DIV (G). Double immunostaining of endogenous α-II/β-II spectrins (A), α-II/β-III spectrins

(B) in primary cultured cortical neurons. Expressions of α-II, β-II, and β-III spectrins overlapped at the cell body and neurite. (C and D) Two mutant EGFP-α-II spectrins (EGFP-del mut and EGFP-dup mut) showed aggregation, predominantly in cell bodies and axons (arrows), and these aggregations were co-localized with β-II and β-III spectrins (middle and lower panels), while wild-type EGFP-α-II spectrin (EGFP-wt) did not show any such aggregation (upper panel). Aggregations were also detected with anti-α-II spectrin antibody (E), suggesting that endogenous α-II spectrin was indeed involved in aggregation as an α/β spectrin heterotetramer. (F) Cells showing aggregation were counted using nuclear EGFP as a transfection marker, expressed from the Flag-nucEGFP vector. While aggregations were never seen when wt α-II spectrin was expressed, del mut and dup mut showed aggregation in approximately 87 % and 95 % of transfected cells, respectively (based on three independent experiments, n = 100 neurons in each experiment). The percentage of aggregation detection did not change between utilization of anti-Flag and anti-α-II spectrin antibody. Non-repeated measures using ANOVA followed by a Bonferroni post-test indicated that del mut and dup mut exhibited significant differences in number of cells having aggregation compared with wild-type, respectively ($P<0.01$). (G) Immunostaining against β-IV spectrin did not show its involvement in the mutant aggregation, in contrast to β-II and β-III spectrins. The following primary antibodies were used: mouse anti-α-II spectrin (1:400 dilution; clone D8B7; Abcam, Tokyo, Japan), mouse anti-β-II spectrin (1:600 dilution; clone 42/B-spectrin II; BD Transduction laboratories, San Jose, CA), rabbit anti-β-II spectrin (1:100 dilution; Abcam), rabbit anti-β-III spectrin (1:400 dilution; Abcam), mouse anti-Flag M2 (1:1000 dilution; Sigma, Tokyo, Japan), chick anti-β-IV spectrin (1: 400 dilution),[1] rabbit anti-GFP (1:2000 dilution; MBL, Nagoya, Japan), and chicken anti-GFP (1:2000 dilution; Aves labs, Tigard, OR).

1. Komada, M., and Soriano, P. (2002). βIV-spectrin regulates sodium channel clustering through ankyrin-G at axon initial segments and nodes of Ranvier. J Cell Biol *156*, 337-348.

Table S1. Summary of clinical features in individuals with *SPTAN1* aberrations

Subject Age Sex	*SPTAN1* aberrations	Initial symptoms	Onset of spasms	Initial EEG	Response to therapy	Development	Neurological examination	Brain MRI	OFC (cm)	Others
1 6 y Female	a 2.25-Mb deletion (including *SPTAN1* and *STXBP1*)	Tonic seizure Oral automatism	1.5 m	S-B	Seizure free after TRH injection (5 m)	Visual attention Oral feeding No head control	Profound MR Hypotonic quadriplegia	Slightly decreased WM (at 4 y)	31 (-1.6 SD, at birth) 41.3 (-3.9 SD, at 23 m)	
2 7 y Female	c.6619_6621del (p.E2207del)	No visual attention	3 m	Hyps	Intractable	No visual attention Gastrostomy No head control	Profound MR Spastic quadriplegia	Cortical atrophy Decreased WM Cerebellum & BS atrophy Thin CC (at 6 y)	31.5 (-1.2 SD, at birth) 44.4 (-2.4 SD, at 35 m)	GE reflux
3 Died at 3 y Male	c.6923_6928dup (p.R2308_M2309 dup)	No visual attention	3 m	Hyps	Intractable	No visual attention Tube feeding No head control	Profound MR Spastic quadriplegia	Cortical atrophy Decreased WM Cerebellum & BS atrophy Thin CC (at 3 y)	33 (-0.4 SD, at birth) 42.4 (-4.8 SD, at 36 m)	GE reflux Myo-carditis

y, year(s); m, month(s); EEG, electroencephalogram; S-B, suppression-burst; Hyps, hypsarrhythmia; TRH, thyrotropin-releasing hormone; MR, mental retardation; MRI, magnetic resonance imaging; WM, white matter; BS, brain stem; CC, corpus callosum; OFC, occipitofrontal circumference; SD, standard deviation; GE, gastroesophageal.

Table S2. PCR conditions and primer sequences

	size (bp)	orientation	primer sequence (5'-3')	Annealing (°C)	Extension (°C)
Ex2	338	Forward	CATCTATTTTGGTGCCTATTGGT	56	68
		Reverse	TTTGGGCTCTTGAATTTTGG		
Ex3	231	Forward	ATGCTGATGCTGTGTGGTTG	56	68
		Reverse	TACAATGCTCCACCCAGATG		
Ex4	249	Forward	CCCACAGAGCCAGTTGTGAT	56	68
		Reverse	GGAGTCTCTTCCCTTTTCTTTGA		
Ex5	248	Forward	TGTTTCTGGAAGCCATTGTT	56	68
		Reverse	TGTTGTGAGAAGCATACAGCA		
Ex6	233	Forward	GTTGGAGGAGCCAGAAGTTG	56	68
		Reverse	CTCCAGGTTGAGACCCATTC		
Ex7	244	Forward	AGGGAGTCATCATTGCTGTG	56	68
		Reverse	TTCTCCTGACCCTTGGTAACTT		
Ex8	269	Forward	AAGTTACCAAGGGTCAGGAGAA	56	68
		Reverse	TCTGCTATTCCTGCTTCCAAA		
Ex9	250	Forward	AACCTCCGCTGGAAACATAA	56	68
		Reverse	CCCAACACTGATAAACTGGTG		
Ex10	250	Forward	TGTAAGATGCTGGGCACAAA	56	68
		Reverse	GGTGAATGCGTTCTCTGGAT		
Ex11	239	Forward	ATTGGGGCTGACCTCATCT	60	72
		Reverse	CAAAACACAGGCCCTTCTTC		
Ex12	235	Forward	ACCCTTTGCTTCACAGTCTTAG	56	68
		Reverse	TCTGGCTTACTATAAGGGTCATTTG		
Ex13	222	Forward	CAAGCTTGGGACTAGTGTGCT	56	68
		Reverse	GGGATATCCAGTTGAAGAGACTT		
Ex14	275	Forward	TGGATATCCCTTTGGGAGTG	56	68
		Reverse	TTTGTGAGAGCTGTGTTTAGCTT		
Ex15	317	Forward	GGGAACTTGACGTTCTCAGG	56	68
		Reverse	AAGGGGTATTTAGTTCCCCAAA		
Ex16	292	Forward	ACTGTTCATGGGCAGTGTTG	56	68
		Reverse	GCATTCACTTGCCCAACC		

Ex17	344	Forward	CGATAAAACCACACCCAAAG	56	68
		Reverse	TAGCACAGCCTTTCCCAAGT		
Ex18	245	Forward	CTGGAACCATGGTGGTAGC	56	68
		Reverse	CACCAAGCCATGTCACTGAT		
Ex19	398	Forward	AGTGCCGTGAACACACAGAG	56	68
		Reverse	CGCGCATCACTACTTCTCCT		
Ex20	226	Forward	GGAGGATGGGAGGCTTCAG	56	68
		Reverse	TGATAGGAACACCTGCTGACA		
Ex21	293	Forward	TGGCATTTGAATCTGCTCTG	56	68
		Reverse	CAACCCACAGAAACCCAACT		
Ex22	276	Forward	TCCTTGGATTCTCCCTCTCA	56	68
		Reverse	GGCCCATGTGGAACAGAGTA		
Ex23	248	Forward	CCAACATTAAGCATCCACCA	56	68
		Reverse	CTTCAAGAGAAGCGGCAGAG		
Ex24	294	Forward	GCTAACTGCCTTCCTTGTCC	56	68
		Reverse	TCAAATCTTTAAACAAATTTTCAGC		
Ex25	250	Forward	TCCTTTGACTTTGGCTTGCT	56	68
		Reverse	CACTGCTTGTTGGGCTATGA		
Ex26	230	Forward	TCCTGTGTTTCCAGGTTTGG	56	68
		Reverse	AAGGCATCTGAGGTTCAGGA		
Ex27	167	Forward	GGTGCTTTGTTTCATGGTCA	56	68
		Reverse	GGCACACCAGAAAAGGACAT		
Ex28	220	Forward	CAATGACACTTGCAGCTCAGA	56	68
		Reverse	ACCCGGTTGACAAGAACAAG		
Ex29	248	Forward	ATGCCCTAGCATCTCCTTCA	60	72
		Reverse	GGAAACCAGCAGTCACCTGT		
Ex30	250	Forward	TAATCAAGGCAGTTGTACTTGG	56	68
		Reverse	CTCTTTCTGGGGCAAAAATG		
Ex31	289	Forward	TGCAGAGCATACCCCCTTAC	56	68
		Reverse	GACCCCTACAAAGAAGGCTCT		
Ex32	247	Forward	TGTTTTCCTGGGCAGAAGAT	56	68
		Reverse	ACAAAGGGTGGGAATGAGG		
Ex33	288	Forward	GGCATCTGCTGCCAGTTAC	56	68
		Reverse	CCCTTCAGCAAGGAAACACT		

Ex34	245	Forward	CTTGCTGAAGGGCCTCATT	56	68
		Reverse	GATGTCAATCAGGAAAAATCCA		
Ex35	285	Forward	CCTGCCACCAGCTAGTTCTC	60	72
		Reverse	GCCTTCGTATGAGGGGATG		
Ex36	327	Forward	GTCTCCCCACTTCCTTCTCC	56	68
		Reverse	TGGGTGCAAGGAGATTGTTT		
Ex37	243	Forward	GTTCCCAAATGCTGAGCTTC	56	68
		Reverse	TGAAGAGACACCAGCAAACC		
Ex38	248	Forward	CCTTCCCCCTGAAAAGACAT	56	68
		Reverse	GTGACGGTGGTCATCTTCCT		
Ex39	250	Forward	GAGAGGGACGATTCTTCATAGA	56	68
		Reverse	TGTGCCAAATGACTAGAAATCC		
Ex40	247	Forward	CCACTGGGCAACCTGAATTT	56	68
		Reverse	CCTTAGGAGTCAGGCAGAGC		
Ex41	348	Forward	GCAGATAGCTGTGGGAGACC	60	72
		Reverse	GCCTCACCACTCAACAGTCA		
Ex42	248	Forward	CCACAGTTGACCTGATGTCC	60	72
		Reverse	GGCCCACTCAAACACCTCT		
Ex43	236	Forward	CTGGGAGCTTCGAGACAGAA	60	72
		Reverse	CTGGCCTTCCACTTGTTCC		
Ex44	240	Forward	TCACAATCAAAGCTGGAGGA	56	68
		Reverse	AGGCAGTTGCCAAGAGAGAA		
Ex45	220	Forward	AATAGTGTGCCTTGGCTGCT	56	68
		Reverse	TCCCAGGAAGTGAACTTTGG		
Ex46	295	Forward	CTTTCCAGGGAGGGCATAGT	56	68
		Reverse	ACGGAAGGAGACAAAGAGCA		
Ex47	199	Forward	CCTTTCTAATCCATCTCCACTGA	56	68
		Reverse	GCCACCAGTCATCTCACAGA		
Ex48	299	Forward	TCCAGTCCTGTGGAGTCACC	60	72
		Reverse	GAGCTGTGGGCCAGCTAAG		
Ex49	434	Forward	CACCCCACCTCCTGCACT	60	72
		Reverse	CTGAGCAGGGCCCAGAGA		
Ex50	247	Forward	TGAGCCCATCTGTGAAGGA	60	72
		Reverse	CCCAGGGCTTAAGTACAGCA		

Ex51	115	Forward	GTCCGGACACCACCTTGT	56	68
		Reverse	ACGAGACCCCCACATGAC		
Ex52	227	Forward	CCAGGCATTGCTCTCTCTG	60	72
		Reverse	TTTAAGCCAGGAAGGGAAGG		
Ex53	330	Forward	CTCCTGGGCTGGTGACTG	60	72
		Reverse	CACAGCAGGTGACTGTGTCTT		
Ex54	213	Forward	TGCTGTGTGGAGGGTCTGT	56	68
		Reverse	CCTGCCAGACTTGTCCTTGT		
Ex55	300	Forward	GCCCTCAAAGAATTCAGCAT	60	72
		Reverse	GGCAGAGGCTCACAGCAC		
Ex56	283	Forward	AATTAAGGCCAGGTGCTGTG	56	68
		Reverse	TTGGCTTTAGTTTTCCAAAGGA		
Ex57	260	Forward	GAGGTCGGGTTTGAAGTGG	60	72
		Reverse	CACACAGAGGAGCGGACAT		

Cycling conditions

95°C 1 min
95°C 10 sec
56 / 60°C 20 sec
68 / 72°C 20 sec

***** PCR cycles are determined by monitoring realtime PCR

Table S3. Electrophysiological parameters recorded from cultured cortical neurons expressing either wt or mutant α-II spectrin

	wt -Flag	del mut -Flag	dup mut -Flag	p value
Resting membrane potential (mV)	-61.8 ± 0.7 (7)	-59.7± 1.0 (9)	-58.5 ± 2.1 (7)	0.48
Input resistance (MΩ)	547.7 ± 104.9 (7)	497.2 ± 84 (9)	568.7 ± 79 (7)	0.83
Whole cell capacitance (pF)	33.6 ± 3.2 (7)[a]	28.0 ± 5.8 (9)[a]	29.1 ± 4.0 (7)[a]	0.70
	41.2 ± 5.0 (11)[b]	52.3 ± 4.5 (10)[b]	39.2 ± 7.0 (10)[b]	0.26
Action potential threshold (mV)	-37.9 ± 0.7 (7)	-21.9 ± 1.4 (9)	-23.9 ± 1.8 (7)	<0.0001[c]
Peak sodium current density in activation protocol (pA/pF)	79.0 ± 16.2 (11)	32.7 ± 5.0 (10)	40.2 ± 8.3 (10)	0.016[d]
10-90% Rise time of the sodium current (ms)	1.07 ± 0.08 (11)	1.12 ± 0.12 (10)	1.02 ± 0.11 (10)	0.79

All data are given as mean ± SEM (number of cells). p value was calculated by one-way ANOVA.

[a] whole cell capacitance evaluated in the current clamp recording.

[b] whole cell capacitance evaluated in the voltage clamp recording.,

[c] Results of post-hoc analysis: wt vs. del mut, $p < 0.001$; wt vs. dup mut, $p < 0.001$.

[d] Results of post-hoc analysis: wt vs. del mut, $p < 0.05$; wt vs. dup mut, $p < 0.05$.