Supporting information

Methods

Plasmid construction

We constructed four plasmids for the luciferase assays, i.e., pGL3-ECR22-pro, pGL3-In10-pro, pGL3-In10-pro-ΔATG-2, pGL3-In10-pro-ΔATG-all, by the following procedure. The ECR22-promoter fragment (-314 to -610) and the In10-promoter fragment (-1 to -610) were obtained by PCR with the mouse genome used as a template DNA. PCR was performed by using KOD-Plus- (TOYOBO) and a thermal cycler (GeneAmp PCR System 9700). The thermocycling conditions were: 10 sec at 98°C, 30 sec at 58°C, 60 sec at 68°C for 25 cycles. The primers used were: ECR22-Kpn-F, ECR22-Xho-R, and In10-Hind-R containing *Kpn*I, *Xho*I, and *Hind*III restriction enzyme sites, respectively, at the 5' end (underlined in Table S3). The primer sequences are listed in Table S3. The 297 bp PCR product amplified by the ECR22-Kpn-F and ECR22-Xho-R primer pair and the 610 bp PCR product amplified by the ECR22-Kpn-F and In10-Hind-R primer pair were subcloned into the *Kpn*I and *Xho*I restriction enzyme sites and the *Kpn*I and *Hind*III restriction enzyme sites of the pGL3-basic vector (Promega) to obtain pGL3-ECR22-pro and pGL3-In10-pro, respectively. To disrupt the ATG

codon, the adenine nucleotide was deleted by using the one-step site-directed mutagenesis protocol as described in a previous report (Zheng *et al*. 2004). PCR was performed by using KOD-Plus- (TOYOBO) and a thermal cycler (GeneAmp PCR System 9700). pGL3-In10-pro was used as a template DNA. The thermocycling conditions were: 10 sec at 98°C, 6 min at 72°C for 5 cycles, and then 10 sec at 98°C, 6 min at 68°C for 20 cycles. The primer pairs used were: In10-293A-del-F and In10-293A-del-R, In10-186A-del-F and In10-186A-del-R, and In10-77A-del-F and In10-77A-del-R. The primer sequences are listed in Table S3. The adenine nucleotides at the -293 and -186 positions were deleted to obtain pGL3-In10-pro-ΔATG-2, and the adenine nucleotides at the -293, -186, and -77 positions were deleted to obtain pGL3-In10-pro-ΔATG-all.

To visualize promoter activity in the form of production of enhanced green fluorescent protein (EGFP), an EGFP expression vector was constructed by a two-step PCR method, basically as described in a previous study (Ho *et al*. 1989). The plasmid construction strategy is summarized in Fig. S1. PCR was performed by using KOD-Plus- (TOYOBO) and a thermal cycler (GeneAmp PCR System 9700). The primers used were: ECR22-Kpn-F, EGFP-ATG-R, EGFP-ATG-F, and EGFP-TGA-Xba-R. The primer sequences are listed in Table S3. In the first

step of the PCR, the 555 bp PCR product amplified by ECR22-Kpn-F and EGFP-ATG-R was obtained by using the pGL3-In10-pro- ΔATG-2 plasmid as the template DNA, and the 757 bp PCR product amplified by EGFP-ATG-F and EGFP-TGA-Xba-R was obtained by using the pEGFP-C1 plasmid (Clontech) as the template DNA. The thermocycling conditions were: 10 sec at 98°C, 60 sec at 72°C for 5 cycles, and then 10 sec at 98°C, 60 sec at 68°C for 20 cycles. In the second step of the PCR, two PCR products (555 bp and 757 bp) were mixed together and used as the template DNA. PCR was carried out by using the ECR22-Kpn-F and EGFP-TGA-Xba-R primer pair to ligate the two PCR products. The thermocycling conditions were: 10 sec at 98°C, 60 sec at 68°C for 5 cycles, and then 10 sec at 98°C, 30 sec at 60°C for 20 cycles. The 1288 bp PCR product was subcloned into the *Kpn*I and *Xba*I restriction enzyme sites of the pGL3-basic vector to obtain pGL3-In10-EGFP.

Two expression vectors were constructed to express SHANK3c isoforms in HEK293 cells and primary cultured neurons. The plasmid construction strategy is summarized in Fig. S2. PCR was performed by using KOD-Plus- (TOYOBO) and a thermal cycler (GeneAmp PCR System 9700). The primer sequences are listed in Table S3. The thermocycling conditions were: 15 sec at 95°C, 30 sec at 62°C, 60 sec at 68°C for 35 cycles. The 502 bp PCR products amplified by

the In10-77ATG-EcoR-F and Ex14-R primer pair using pGEM-Shank3c-3 as the template DNA were digested with *Eco*RI and *Cla*I restriction enzymes and inserted into pCMV-myc-Shank3, which contains the full-length of *Shank3* cDNA (Uchino *et al.* 2006) and it resulted in the exchange of the amino-terminus of Shank3 sequences. The *Eco*RI/*Not*I *Shank3* fragment from ATG (-77) in intron10 to exon 22 was ligated to the myc-tagged sequence produced by annealing with two synthetic nucleotides, Myc-EcoR-F and Myc-EcoR-R, at the 5'-end, and inserted into mammalian expression vector pCAGGS1, which contains a modified chicken β-actin promoter and a cytomegalovirus-immediate early enhancer (CAG) promoter (Niwa *et al*. 1991), provided by Dr. Miyazaki (Osaka University), to yield pCAGGS-myc-Shank3c-3. Next, the 801 bp PCR products amplified by the Ex12-F and Ex22-TGA-Not-R primer pair were obtained. The pGEM-Shank3c-4 was used as the template DNA, and the thermocycling conditions were: 15 sec at 95°C, 15 sec at 60°C, 60 sec at 68°C for 25 cycles. The PCR product was digested with the *Cla*I and *Not*I restriction enzymes and inserted into pCAGGS-myc-Shank3c-3, which resulted in exchange of the carboxyl-terminus of the *Shnak3* sequences, and yielded pCAGGS-myc-Shank3c-4.

All of the constructs were confirmed by DNA sequencing.

References

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Niwa H., Yamamura K., and Miyazaki J. (1991) Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* **108**, 193-199.

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Figure Legends

Figure S1. Schematic diagram of the construction of EGFP expression vector pGL3-In10-EGFP by a two-step PCR method.

Figure S2. Schematic diagram of the construction of the myc-tagged SHANK3 isoform expression vectors, pCAGGS-myc-Shank3c-3 and pCAGGS-myc-Shank3c-4.

Table S1

Primer sequences used for *Hpa*II-*Mcr*BC PCR and ChIP PCR

Name	Forward primer (5'-3')	Reverse primer (5'-3')	Tm(°C)	Cycles	Size (bp)
CpGP-F / CpGP-R	AGCTCTAGAACCCTCCGTCACC	CGCGTCTTGAAGGCTATGATTGAG	65	35	999
In10-F / CpG2-R	GGCATCGCGTCCGTCACCTACGT	CTAGAGCTTCATGCCAGGAG	63	33	420
CpG3-F / CpG3-R	GGGCGAGGCTGCTTAACTCT	TTGCTTACCCCTTCCCGTCT	63	35	327
CpG4-F / CpG4-R	CCTACCCTGAGCGTCAGAAG	CGCTCATCAATGGAGCGG	63	35	532
CpG5-F / CpG5-R	CTGACCTGACCCAGGATGTT	CGAAGTCTTCCTTGGTGAGC	63	35	664
CpG5-2F / CpG5-R	GCCCTAGACCTCTTGGCTCT	CGAAGTCTTCCTTGGTGAGC	58	45	606
5UTR-F / 5UTR-R	CGGTTAGAAGGGTTGGTCTG	GGTGGAAATCTCCCCAGTCT	63	33	164

Table S2

Primer sequences used for RT-PCR and real-time PCR

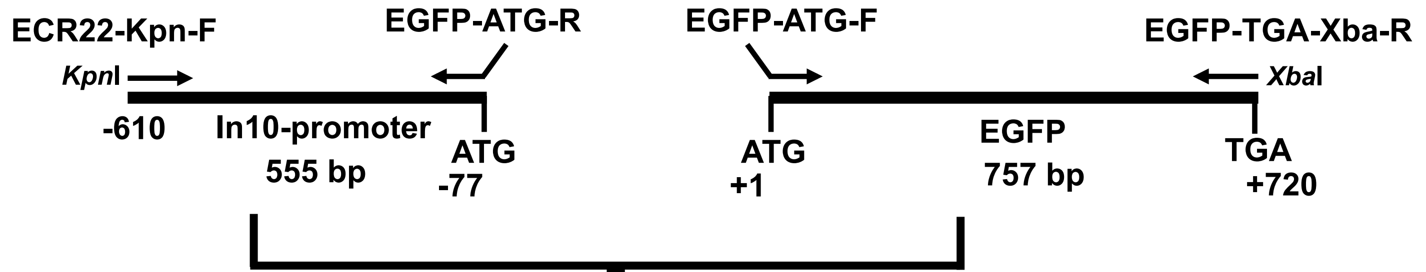
Name	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)
In10-F / Ex14-R	GGCATCGCGTCCGTCACCTACGT	CTTATCATCGATGACATAATCG	441
PSD95-F / PSD95-R	GGCACCGACTACCCCACAG	AACACCATTGACCGACAGGA	261
5UTR-F2 / CpGP-R	GTAGCGCGGTCGGCGGGGCT	CGCGTCTTGAAGGCTATGATTGAG	266
Gapdh-F / Gapdh-R	GTCATCATCTCCGCCCCTTCTGC	GATGCCTGCTTCACCACCTTCTTG	443

Table S3

Primer sequences used for plasmid construction

Name		Sequence (5'-3')
ECR22-Kpn-F	Forward	GGGGTACCACCACAGGAGTCAGGCCCAGAG
ECR22-Xho-R	Reverse	CCGCTCGAGCGCCTCCTCCGCCAGCTC
In10-Hind-R	Reverse	TCCCAAGCTTCTGTAGACGAACACGTAGGTGACGGACGC
In10-293A-del-F	Forward	GAGCGGCCGTGGCTGGAGCGAGCCCGGCG
In10-293A-del-R	Reverse	CGCCGGGCTCGCTCCAGCCACGGCCGCTC
In10-186A-del-F	Forward	CGGCGGCGCGGCCCTGGAGCGGCCCCGGGCC
In10-186A-del-R	Reverse	GGCCCGGGGCCGCTCCAGGGCCGCGCCGCCG
In10-77A-del-F	Forward	GCCGGGCCGTGCGCCTGGAGGCCCCGGGCGCC
In10-77A-del-R	Reverse	GGCGCCCGGGGCCTCCAGGCGCACGGCCCGGC
EGFP-ATG-F	Forward	GCCGTGCGCCATGGTGAGCAAGGGCGA
EGFP-ATG-R	Reverse	CCCTTGCTCACCATGGCGCACGGCCCGGC
EGFP-TGA-Xba-R	Reverse	CGACTCTAGATCACTTGTACAGCTCGTCCATGCCGA
Ex14-R	Reverse	CTTATCATCGATGACATAATCG
Myc-EcoR-F	Forward	AATTCGCCACCATGGCATCAATGCAGAAGCTGATCTCAGAGGAGGACCTGCG
Myc-EcoR-R	Reverse	AATTCGCAGGTCCTCCTCTGAGATCAGCTTCTGCATTGATGCCATGGTGGCG
Ex-12F	Forward	GACAGTATGACACCCGGCATG
Ex22-TGA-Not-R	Reverse	GTAGTTTAGCGGCCGCTCAGCTCACCGAGGCTGCTGAAG

Figure S1



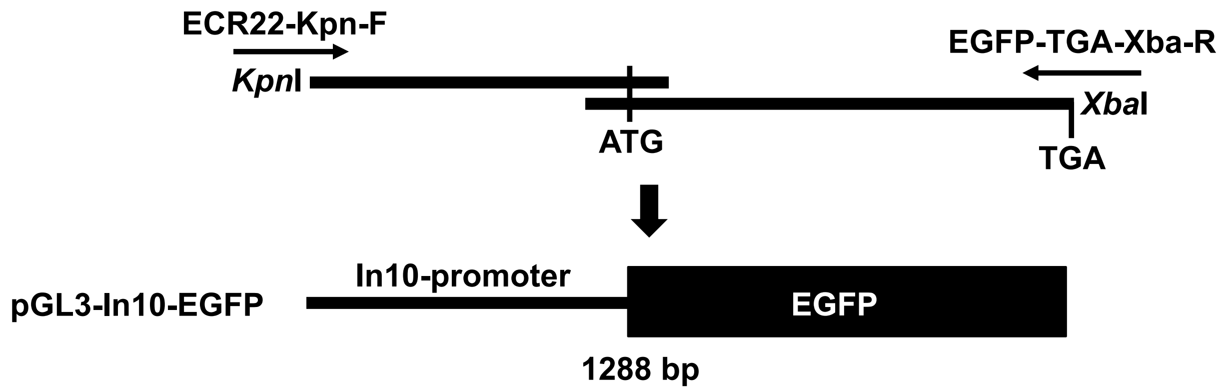


Figure S2