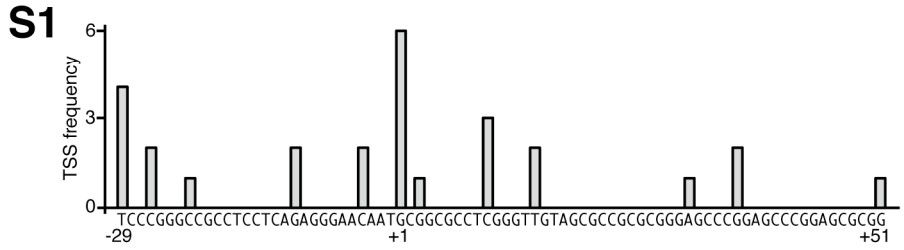

Fig. S1. Dispersed transcriptional start sites of GnT-IX gene. 5'-RACE was performed using 5'-Full RACE Core Set (TAKARA) and total RNA from 20-week-old mouse brain. For synthesis of 1st strand cDNA, we used 5'-phosphorylated primer (5'-CCCATCTGGGTTGAC). After circularization of the cDNA, nested PCR was performed, and then the product was cloned and sequenced. Primers for the 1st PCR, TTCGTGGTTCCCGAGGCGGT and CTCCCCGATTCACATTTTGG, for the 2nd PCR, GCTTCTCGCCCAGACAAGTT and TCTGCCTGCTAGGCTCGCTG. GnT-IX mRNA transcription is initiated at several genomic sites which is often seen for a vertebrate TATA-less dispersed core promoter. Totally, 30 clones were sequenced, and 27 out of 30 mRNAs were initiated in this region (from -29 to +51). One of major TSS was designated as +1.

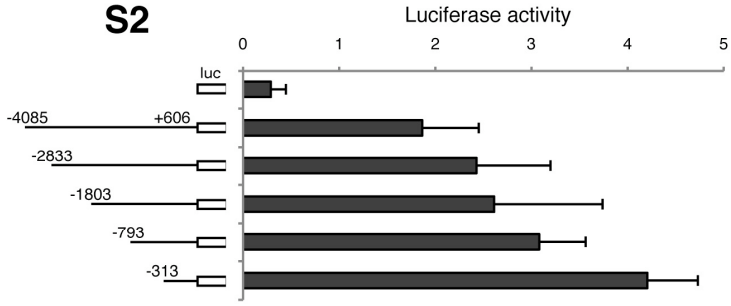

Fig. S2. Promoter activity of GnT-IX gene in Neuro2A cells. Activity of mouse GnT-IX P1 was analyzed by luciferase reporter assay in Neuro2A cells. Relative luciferase activities against internal control Renilla luciferase are shown as the mean ± S. E. (n=3). Promoter regions analyzed are shown to the left of the graph. There are no apparent enhancer elements in this region.

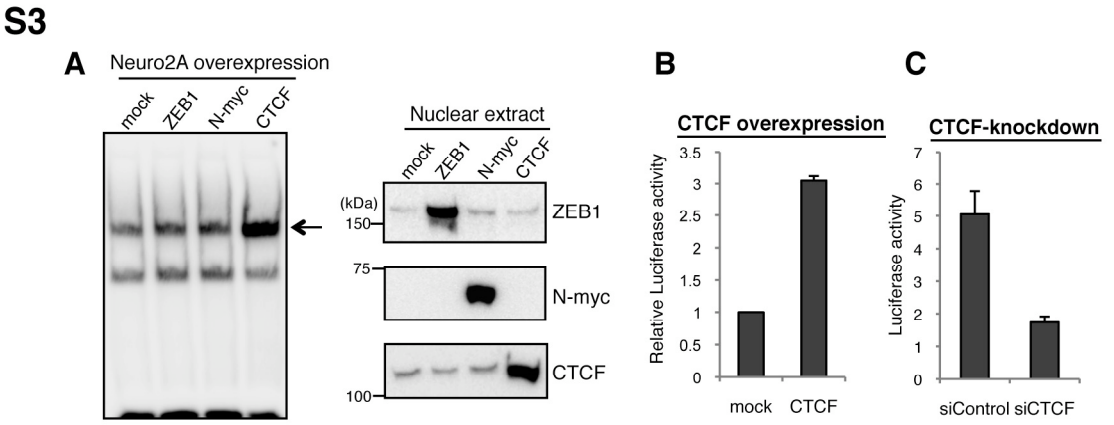

Fig. S3. Binding of CTCF activates GnT-IX promoter. (A) EMSA was performed using a biotinylated oligoDNA probe (from -82 to -44 in P1). Nuclear extracts of Neuro2A cells transfected with pcDNA6 (mock), ZEB1, N-myc or CTCF expression plasmid were used. An arrow indicates increased CTCF signal after overexpression. Overexpression of each factor was confirmed by western blotting using the same nuclear extracts with anti-ZEB1, anti-N-myc and anti-CTCF antibodies (right). (B, C) Neuro2A cells were transfected with part of the mouse P1 construct (-313 to +606) inserted into a luciferase vector. Luciferase activity was then measured after overexpression (B) or knockdown (C) of CTCF. For overexpression, CTCF expression vector or pcDNA6 (mock) was co-transfected with reporter plasmids. Relative luciferase activity against mock sample is shown as the mean ± S. E. (n=3) in the left graph. For knockdown, siRNA specific for mouse CTCF or negative control siRNA was transfected 24 h before transfection of the reporter plasmids. Data are presented as the mean ± S. E. (n=3) (right).

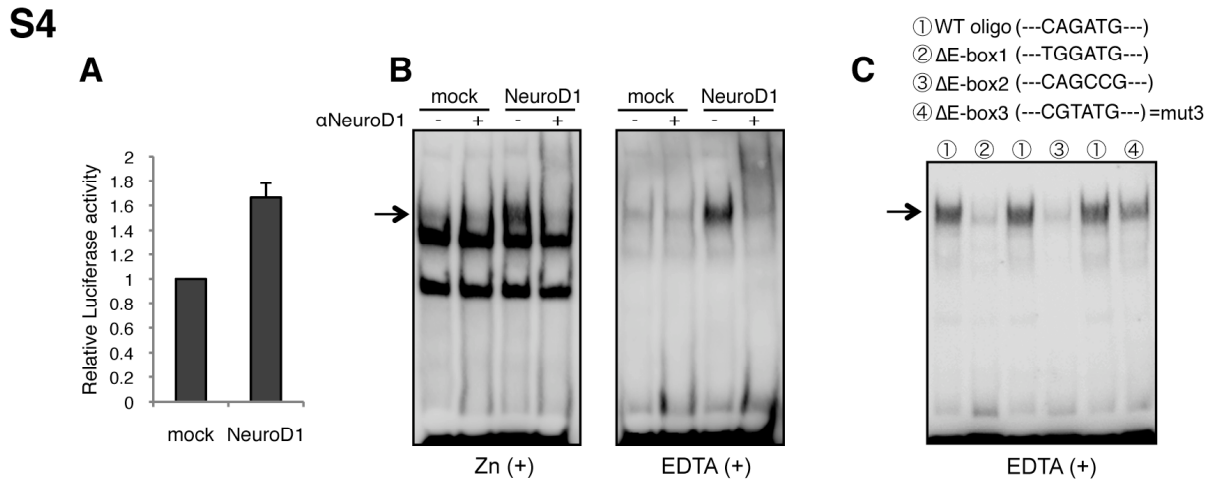

Fig. S4. Activation of GnT-IX promoter by NeuroD1 overexpression. (A) Neuro2A cells were transfected with part of the mouse P1 construct (-313 to +606) inserted into a luciferase vector and a NeuroD1 expression vector or pcDNA6 (mock). Luciferase activity was then measured. Relative luciferase activity against mock sample is shown as the mean ± S. E. (n=3). (B) Nuclear extracts of Neuro2A cells that had been transfected with pcDNA6 (mock) or NeuroD1 expression plasmid were prepared. EMSA was performed using biotinylated oligonucleotide probe (from -82 to -44 in P1) (left). Binding of NeuroD1 to DNA was validated by addition of anti-NeuroD1 antibody. Right panel shows the result of the same experiment but without divalent cation and with EDTA. An arrow indicates increased signal of NeuroD1 after overexpression. (C) Nuclear extracts of Neuro2A cells that had been transfected with NeuroD1 expression plasmid were prepared. EMSA was performed in the presence of EDTA using three kinds of biotinylated oligonucleotide probe (from -82 to -44 in P1). In ΔE-box1, 2 and 3, the E-box sequence was mutated as shown at the top of the panel. ΔE-box3 had the same mutation in mut3 in Fig. 3B. An arrow indicates the reduced signal of NeuroD1.

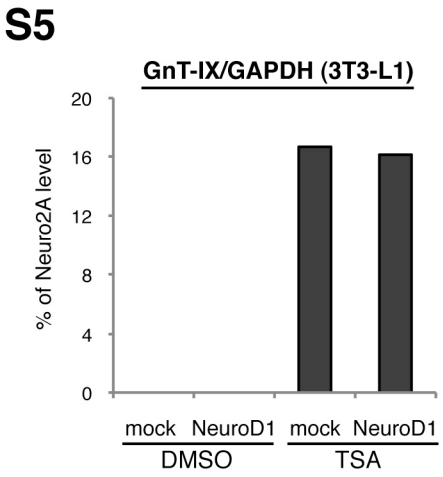

Fig. S5. Overexpression of NeuroD1 did not induce GnT-IX expression in 3T3-L1 cells. 3T3-L1 cells were transfected with pcDNA6 (mock) or NeuroD1 expression plasmid. After 24 h, DMSO or TSA (final 0.1 μM) was added to the culture medium followed by further 24 h incubation, and then GnT-IX mRNA was quantified by real-time PCR relative to GAPDH mRNA. Expression level is shown as ratio to that in Neuro2A cells.

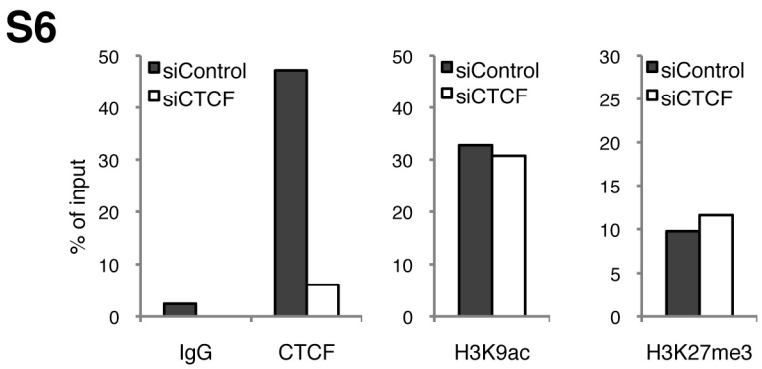

Fig. S6. Chromatin state around the GnT-IX core promoter after CTCF-depletion. Neuro2A cells were transfected with control or CTCF-specific siRNA and ChIP experiments were performed using anti-CTCF antibody (left), anti-H3K9ac antibody (middle) or anti-H3K27me3 antibody (right). Precipitated GnT-IX core promoter, including the CTCF binding site, was quantified by real-time PCR. Relative percentage against 1% input sample was shown.

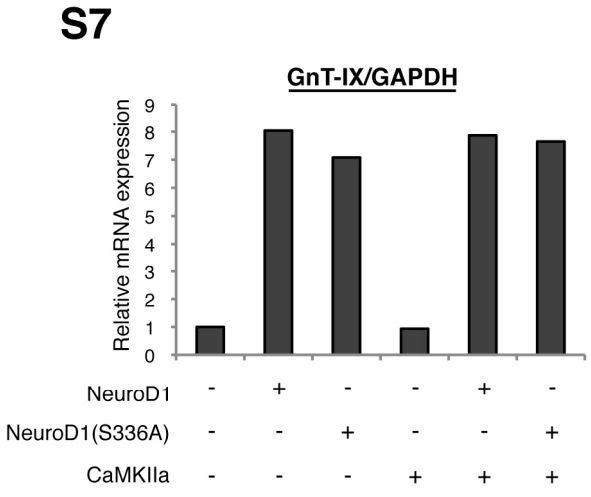

Fig. S7. Ability of NeuroD1 to activate GnT-IX is unaltered by CaMKII. NeuroD1, mutant NeuroD1 (serine336 was mutated to alanine), CaMKIIα, or combinations thereof were overexpressed in Neuro2A cells and then GnT-IX mRNA was quantified by real-time PCR relative to that of GAPDH. Relative expression levels to that of the mock transfected cells are shown.

S8 Primers for DNA construction

Promoter1 for cloning	TCAGTGGCTTTACTGGTAGGTTTGACCCAC, CATTGTTCAAACTTGTCTGGGCGAGAAGCC
pGV/-4085-+606	AAAACGCGTCTGGTCCAAGACCCTGAATA (underline: MluI site) , TTTCTCGAGAACTTGTCTGGGCGAGAAGC (primer1) (underline: XhoI site)
pGV/-2833-+606	AAAACGCGTAAGAGCGCTGTCTTACCAGG (underline: MluI site) , primer1
pGV/-1803-+606	AAAACGCGTCCTTGTAGGTCCTGAGGTCA (underline: MluI site) , primer1
pGV/-793-+606	AAAACGCGTCTGGCTAAGGAAATGTCTGA (underline: MluI site) , primer1
pGV/-313-+606	AAAACGCGTCCATAGGTACGGAAGCTCTT (underline: MluI site) , primer1
pGV/-106-+606	AAAACGCGTCGCACAGCCCGGGGAGGGAG (underline: MluI site) , primer1
pGV/-79-+606	AAAACGCGTAGACTGCGGCCACCAGATGG (underline: MluI site) , primer1
pGV/-53-+606	AAAACGCGTGGCGCCGAGGAAACGCGAGG (underline: MluI site) , primer1
pGV/+1-+606	AAAACGCGTGCGGCGCCTCGGGTTGTAGC (underline: MluI site) , primer1
Promoter2 for cloning	GGTGCCCCTGAGATGTTACCTGTGT , GACAAACAGCCTGCAGGGAGAGAGG
pGV/promoter2	AGTGGTACCAAGCAGCTCTTGGGAATCTC (underline: KpnI site) , ACTGCTAGCATAGCAAGTTCCAGCCCAGC (underline: NheI site)
pGV/-313-+606 mut1	GAGCGCAGCGAGACTGCGGCCGTCAGATGGCGCTCAGGCGCCGA and its complementary sequence
pGV/-313-+606 mut2	CGCAGCGAGACTGCGGCCACCGTATGGCGCTCAGGCGCCGAGGA and its complementary sequence
pGV/-313-+606 mut3	TGCGGCCACCAGATGGCGCTCGTGCGCCGAGGAAACGCGAGGCC and its complementary sequence
Mouse ZEB1	GACGAATTCGAGAGGATCATGGCGGATGG (underline: EcoRI site) , AGAACTCCTAAGCTTCATTTGTC
Human N-myc	CCGGAATTCAGGCGAGCCGATGCCGAGCTGCT (underline: EcoRI site) , AGCGTCTAGCAAGTCCGAGCGTGTT
Human CTCF	GGCAGGGGAAATGGAAGGTGATGCA , CCGCCATCACCGGTCCATCATGCTG
Mouse NeuroD1	CAACAGGAAGTGGAAACATGACCAA , AAACTGACGTGCCTCTAATCGTGAA
Mouse NeuroD2	CTTGACTCCTCTCTGAGGCACCATG , GGCGCGAGGTCTCAGTTATGGAAAA
Mouse Math2	AAGAACCATGTTAACACTACCGTTTG , TTAGAGTGGGAGGTGAATGACCACT
Mouse Neurogenin1	GTCCTGCACACTGCAAGATGCCTGC , CCTTACAAAGGCCTAGTGGTATGG
Mouse Olig1	CGCCCCAGATGTACTATGCGATTTC , AGCCAGCCCTCACTTGGAGAACTGG
Mouse CaMKIIa	CCCAGTGCCAGGATGGCTACCATCA, GGCCTGGTCCTTCAATGCGGCAGGA
NeuroD1(S336A)	GATCCCCATAGACAACATTATGGCTTTCGATAGCCATTCGCATCA and its complementary sequence

Primers for RT-PCR

Mouse GnT-IX	GAAGATGCCTGGTCACCCTG , GGCCAGCATATCCATGCGCT (primer3)
Mouse GnT-Vb	ACCCTGCTCCTCTCTCCCTG , primer3
Mouse GAPDH	TCCACCACCCTGTTGCTGTA , ACCACAGTCCATGCCATCAC
Human GnT-IX	AGAAGATGCCTGGTCACCCT , AGCGATGTCGGAGACGTTCT (primer4)
Human GnT-Vb	ATGGCCCTTCCTGCCCTCCT , primer4
Human beta-actin	TGGCACCCAGCACAATGAA , CTAAGTCATAGTCCGCCTAGAAGCA
Mouse ST6Gal-I	GCATAACGGAGACCAAGCGT , TGTGGAGCTTGGCACAGCTG
Mouse Beta4GalT-I	CCCACCACCACTGGACTGTT , GTCGAGTTGCTGGCGCTGAA
Mouse GlcAT-P	GCGACACTGGCCTCAACTAC , ACACCCTTCTTGTGCTGCGC
Mouse ST8Sia-IV	GGCTCCACCATCTTCCAACA , ACAGGTCTTAAACCTGCGG

Primers and probes for ChIP analysis

GnT-IX primer	TAGGAAGTTGCATGGTCCAG, CCGGGCTCCCGCGCGGCGCT
GnT-IX probe	5'-FAM-TCAGAGGGAACAATGCGGCG-MGB-3'
ST6Gal-I primer	GAGGCAGCATTTGGGCTTCC, GAGGACGCTTGGTCTCCGTT
ST6Gal-I probe	5'-FAM-AGCAGGCACCTTTAACCTTG-MGB-3'
β4GalT-I primer	CAGGTTCCGCGAACAGCCTT, CCACAGCGATCGAAGGAGGG
β4GalT-I probe	5'-FAM-CTGATCTCTCCAAAGTCCCG-MGB-3'
GlcAT-P primer	AGAGCAGGGAGGTGATACCA, CGCGGCTGTGACAGCGCCT
GlcAT-P probe	5'FAM-AGGTTGGTACGGACCGCGAG-MGB-3'
ST8Sia-IV primer	CCGAGCTCACAACGACTCTC, CCCTGGGGCTCAGGTTTCTT
ST8Sia-IV probe	5'-FAM-TTATCGTTTCCCTGCTACGG-MGB-3'

Fig. S8. Primers used for plasmid construction, RT-PCR and ChIP analyses.