

Supplementary Data

Topoisomerase II β Activates a Subset of Neuronal Genes That Are Repressed in AT-rich Genomic Environment

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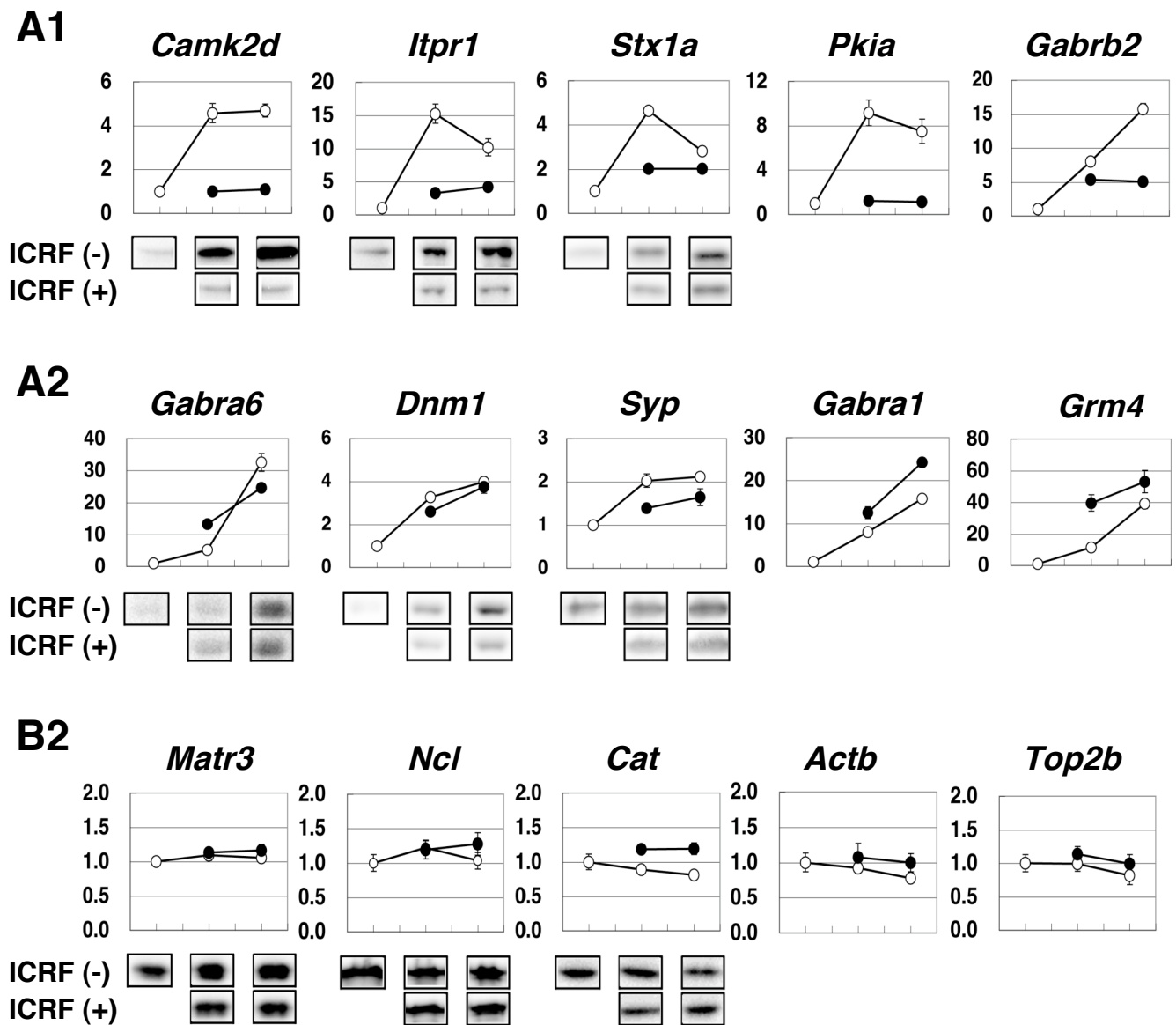


Figure S1. Confirmation of the array-based gene grouping by RT-qPCR and immunoblotting. RNA samples were prepared from the granule cells in culture at day 1, day 3, and day 5 in the presence (filled circles) or absence (open circles) of 10 μ M ICRF-193. Total RNA was used as a template for the cDNA synthesis with M-MLV reverse transcriptase and primer (random hexamer). Representative genes (5 each) from A1, A2, and B2 gene groups that were classified by the expression array experiments were subjected to RT-qPCR analysis to estimate their transcript levels. Constant amounts of cDNA (equivalent to 15 ng of template RNA) were used for qPCR amplification and relative copy numbers of the product with respect to day 1 are plotted (mean \pm s.d., $n = 3$). Sequences of primer pairs used for the amplification are listed in Table S3. Protein levels of some gene products, for which antibody is available, were also estimated by immunoblotting (antibodies and dilutions are given in Table S4). Gene names: *Camk2d*, calcium/calmodulin-dependent protein kinase type II δ ; *Itp1*, inositol 1,4,5-trisphosphate receptor type 1; *Stx1a*, syntaxin 1A; *Pkia*, cAMP-dependent protein kinase inhibitor, α form; *Gabrb2*, gamma-aminobutyric acid receptor (GABA_A), β 2 subunit; *Gabra6*, gamma-aminobutyric acid receptor (GABA_A), α 6 subunit; *Dnm1*, dynamin 1; *Syp*, synaptophysin; *Gabra1*, gamma-aminobutyric acid receptor (GABA_A), α 1 subunit; *Grm4*, metabotropic glutamate receptor 4; *Matr3*, matrin 3; *Ncl*, nucleolin; *Cat*, catalase; *Actb*, beta-actin; *Top2b*, DNA topoisomerase II β .

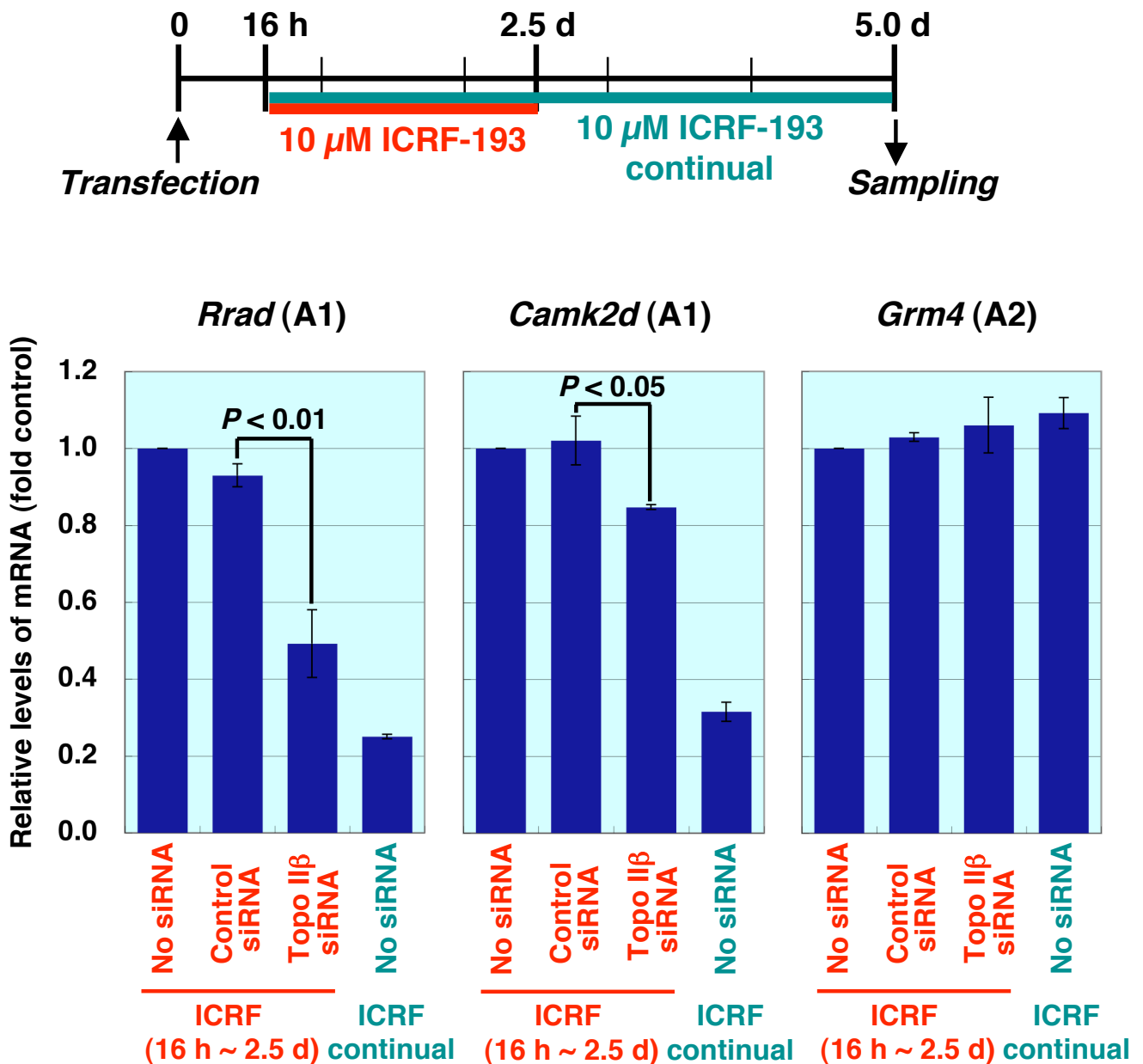


Figure S2. Suppression of transcriptional induction of A1 genes by topo II β siRNA. Effects of topo II β siRNA on mRNA levels of *Rrad* (group A1), *Camk2d* (group A1), and *Grm4* (group A2) genes were examined. Because of the delayed action of siRNA, it was mandatory to use ICRF-193 in the early phase of culture (until 2.5 days) in order to observe the RNAi effect. Since the drug effect is completely reversible, removal of the drug increases the transcript to a native level by day 5 (data not shown). As a positive control, ICRF-193 was added continuously to the culture. At the culture start, siRNAs were transfected to dispersed cerebellar granule cells. ICRF-193 was added after 16 h and removed after 2.5 days. RNA samples were prepared at day 5 and analyzed by RT-qPCR as in Figure S1. Levels of mRNA expression relative to the siRNA-minus control were plotted. Vertical bars designate mean \pm s.d. ($n = 3$). Gene name: ***Rrad***: RAS associated with diabetes (*Rad1*); ***Camk2d***, calcium/calmodulin-dependent protein kinase type II δ ; ***Grm4***, metabotropic glutamate receptor 4. Note that the difference between control and topo II β siRNAs is statistically significant in A1 genes (*Rrad* and *Camk2d*) but not in A2 gene (*Grm4*).

UCSC genome annotation database
Data Freeze : 29-Jul-2007

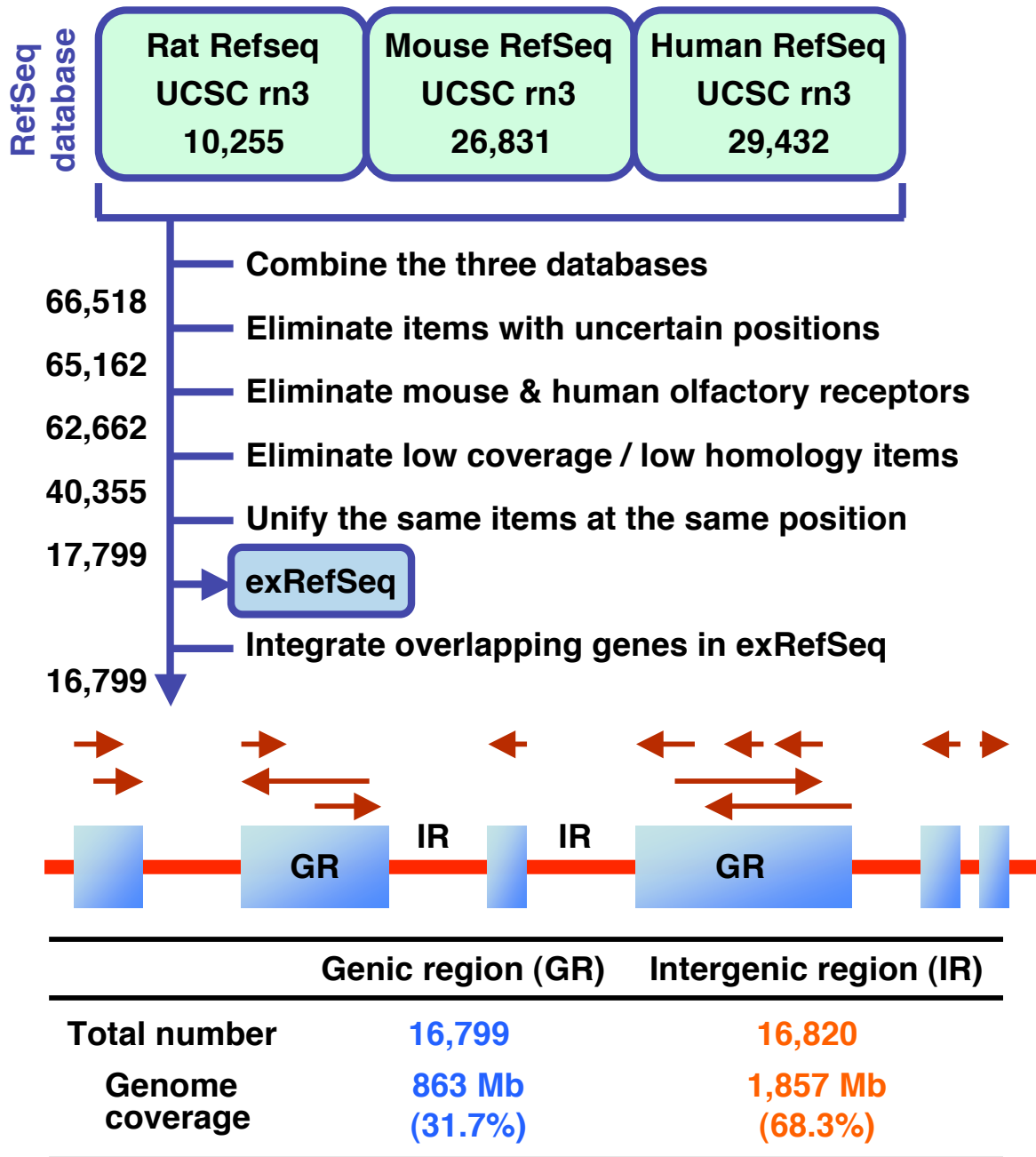


Figure S3. Schematic representation of the procedure for construction of exRefSeq. Using the UCSC genome annotation database (Data freeze: 29-Jul-2007) as the main source, the coordinates for rat, mouse, and human RefSeq were first integrated into one, and then multiple sifting and cleaning steps were applied to construct a rat genome compilation termed exRefSeq. As illustrated here, the genic region was defined as a longest stretch of overlapping transcripts (including reverse directions) in exRefSeq. In the present study, the word “genic” stands for transcribed regions of protein-coding genes. Numbers on the left of the vertical arrow stand for total numbers of remaining items at each step. Detailed description for this procedure is given in Materials and Methods.

	Genic	Intergenic
Number	16,799	16,820
Genome Coverage (%)	863,065,498 bp (31.7)	1,856,856,955 bp (68.3)
Average Length	51,376 bp	110,396 bp
GC content	43.7%	42.2%
Number / Coverage (%)		
Class: LA	2,234 / 428 Mb (13.3 / 49.6)	1,189 / 925 Mb (7.1 / 49.8)
LG	1,786 / 244 Mb (10.6 / 28.3)	1,834 / 600 Mb (10.9 / 32.3)
SA	3,749 / 66 Mb (22.3 / 7.7)	2,929 / 85 Mb (17.4 / 4.6)
SG	9,030 / 124 Mb (53.8 / 14.4)	10,868 / 246 Mb (64.6 / 13.3)

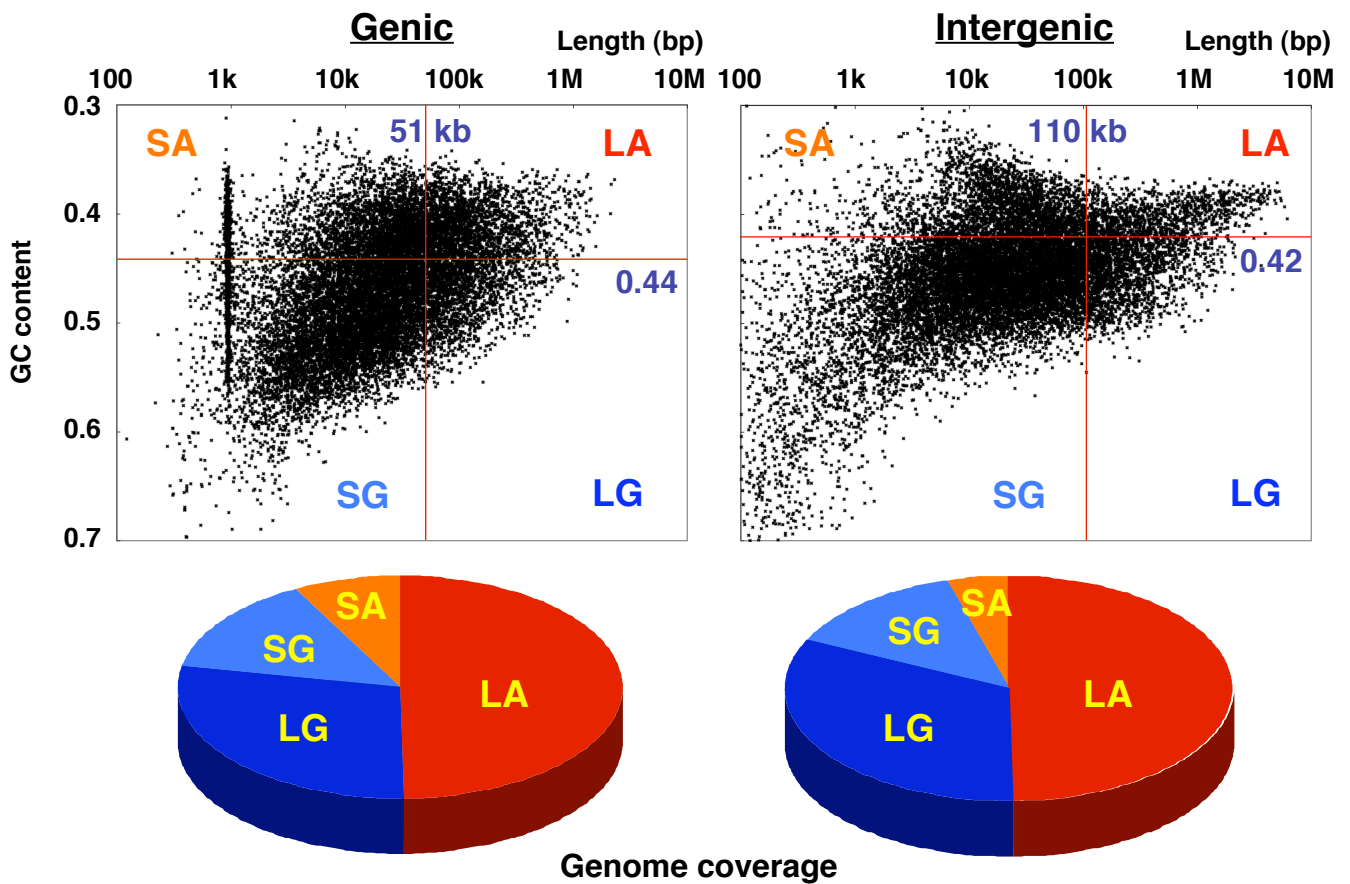


Figure S4. Classification of rat subgenomic regions by length and GC content. Genic and intergenic regions were classified into four classes by their average length (51 kb and 110 kb, respectively) and overall GC content (0.44 and 0.42, respectively). These classes are designated as LA (Long AT-rich), LG (Long GC-rich), SA (Short AT-rich), and SG (Short GC-rich). Accordingly, classified genic regions (GR) and intergenic regions (IR) will be referred to as LAGR, LGGR, SAGR, SGGR, LAIR, LGIR, SAIR and SGIR, respectively.

A

	Number (%)	LAIRp	LAIRd	LAGR	LAIRp & LAGR	LAIRd & LAGR
A1	327 (2.6)	77	250	87	61	26
A2	987 (7.8)	129	858	142	66	76
B2	7,084 (55.7)	521	6,563	929	217	712
D	2,918 (23.0)	309	2,609	292	175	117
Others	1,393 (11.0)	216	1,177	293	84	209
Total	12,709 (100)	1,252	11,457	1,743	603	1,140

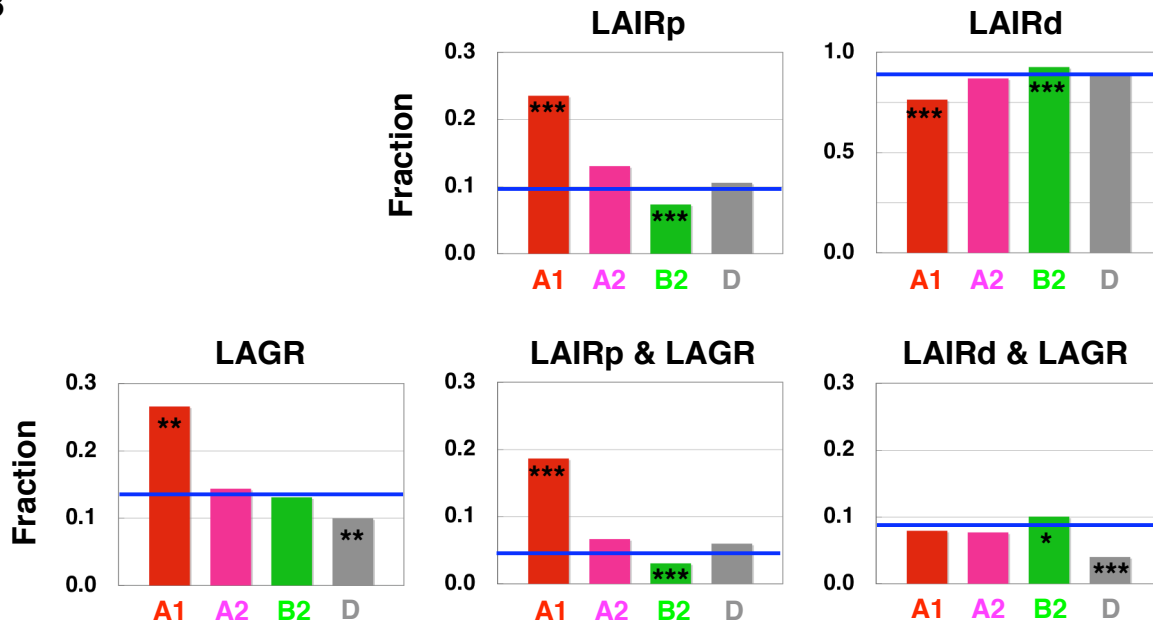
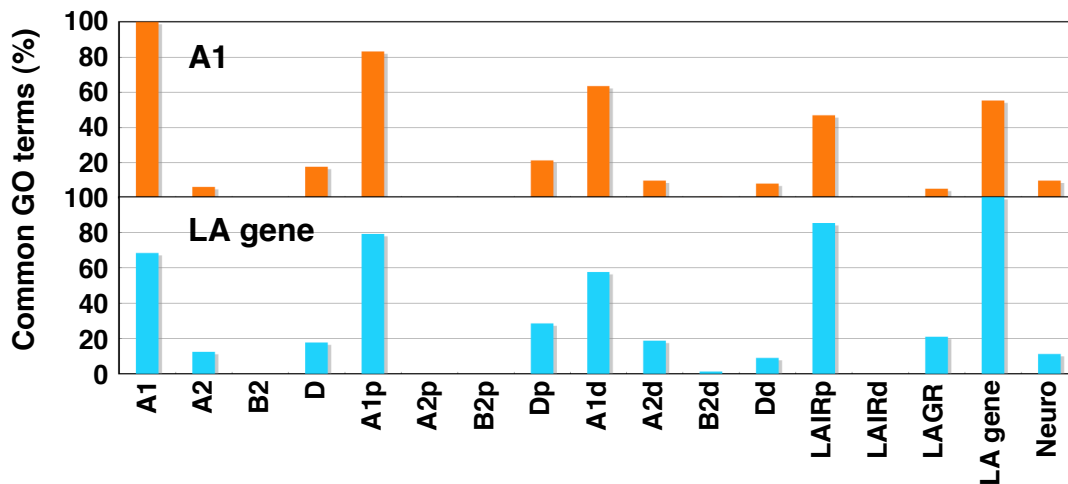
B

Figure S5. Comparison of expression groups in terms of gene's position, length, and GC content. **A**, The expression array data merged to exRefSeq were used to count the number of genes with features indicated in the table. LAIRp, LAIR-proximal; LAIRd, LAIR-distal; LAGR. Total genes in exRefSeq after subtracting the genes without assigned array probes (termed group N) were used for the analysis. "Others" stands for the sum of other minor groups (A3, B1, B3, C1, C2, C3, and Mix). **B**, Fractions of the grouped genes that belong to the featured categories are illustrated in the bar graphs. Levels for total genes are shown by blue lines, which were used as a base for the calculation of statistical significance by chi-square test. $*P < 10^{-5}$, $**P < 10^{-10}$, $***P < 10^{-15}$.

A

	A1	A2	B2	D	A1p	A2p	B2p	Dp	A1d	A2d	B2d	Dd	LAIRp	LAIRd	LAGR	LA gene	Neuro
A1	38	1	0 [-36]	31	20	0	0	6	21	10	4 [-8]	36	16	0 [-22]	2	26	30
A2	1	16	0 [-10]	4	1	1	0	2	1	13	3 [-1]	7	1	0 [-1]	0	2	2
B2	0 [-7]	0 [-14]	115	0 [-76]	0 [-2]	0	0	0 [-8]	3	11 [-1]	104	4 [-11]	0 [-10]	8	8 [-2]	0 [-6]	3 [-8]
D	31	4	0 [-122]	174	17	1	0	27	28	29	15 [-25]	161	24	0 [-30]	2	31	67
A1p	20	1	0 [-22]	17	24	0	0	3	11	9	5 [-6]	22	13	0 [-17]	2	19	20
A2p	0	1	0 [-1]	1	0	1	0	1	0	1	0 [-1]	1	0	0	0	0	0
B2p	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dp	6	2	0 [-27]	27	3	1	0	28	5	8	2 [-10]	25	10	0 [-10]	0	8	17
A1d	21	1	3 [-29]	28	11	0	0	5	33	19	12 [-3]	33	11	0 [-16]	0	19	28
A2d	10 [-4]	13 [-2]	11 [-44]	29 [-12]	9 [-2]	1	0	8 [-4]	19	101	58 [-1]	70	14 [-4]	4 [-17]	7 [-1]	19 [-4]	51 [-4]
B2d	4 [-7]	3 [-22]	104 [-18]	15 [-85]	5 [-2]	0	0	2 [-8]	12	58	541	109 [-7]	8 [-10]	8 [-9]	18 [-2]	7 [-6]	104 [-9]
Dd	36 [-1]	7	4 [-155]	161 [-5]	22	1	0	25 [-1]	33	70	109 [-17]	443	30 [-1]	1 [-37]	9	40 [-1]	164 [-1]
LAIRp	16	1	0 [-31]	24	13	0	0	10	11	14	8 [-9]	30	34	0 [-33]	5	29	27
LAIRd	0 [-6]	0 [-4]	8	0 [-8]	0 [-2]	0	0	0 [-8]	0	4	8	1	0 [-8]	8	0	0 [-6]	0 [-6]
LAGR	2	0	8 [-7]	2 [-12]	2	0	0	0	0	7	18	9 [-1]	5	0 [-5]	38	8	14
LA gene	26	2	0 [-40]	31	19	0	0	8	19	19	7 [-10]	40	29	0 [-36]	8	47	34
Neuro	30	2	3 [-83]	67 [-6]	20	0	0	17	28	51	104 [-15]	164	27	0 [-33]	14	34	302

B**Figure S6. Functional similarity between A1 genes and LA genes as revealed by a GO matrix.**

A, A matrix composed of the numbers of overlapping GO terms was created. GO terms over-represented in the gene groups that were classified by expression or genomic locations were first retrieved by the Gostat program ($P < 10^{-4}$) and their numbers were placed on the diagonal position of the matrix (colored yellow). “Neuro” stands for the neuronal genes selected as described in Materials and Methods. Numbers of common GO terms in every combination of gene groups were then calculated and filled into the matrix at the corresponding non-diagonal positions. Numbers in brackets are under-represented terms. The complete list of GO terms can be found in Table S5. **B**, The entire matrix was converted to relative values by dividing the figures in every rows with the diagonal elements (resulting matrix not shown). The columns for A1 and LA gene (highlighted by orange and light blue, respectively) were plotted in bar graphs representing percentages of overlapping GO terms in various gene groups with respect to A1 gene (upper) or LA gene (lower). The overlap patterns are very similar each other, indicating that these gene groups are functionally similar. No such relationship was observed in other combinations.

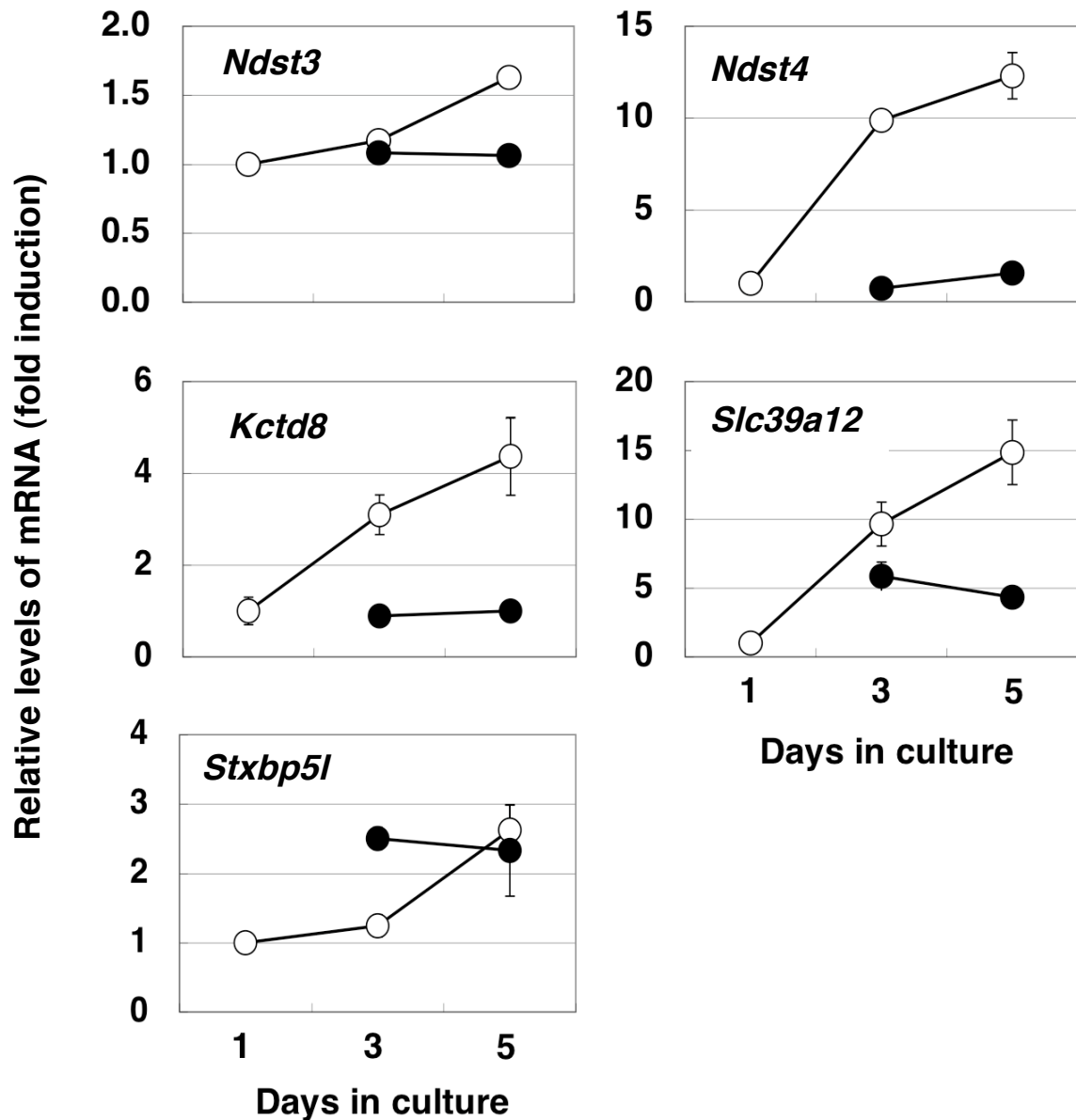


Figure S7. Prediction of A1 genes from positional and functional information. Expression kinetics of five A1 candidate genes selected from group N genes was examined by RT-qPCR using total RNA samples obtained from granule neurons cultivated in the presence (filled circles) or absence (open circles) of ICRF-193. Vertical bars designate mean \pm s.d. ($n = 3$). The following criteria were employed for the selection of candidate genes. 1) LA gene (LAIR-proximal and LAGR), 2) Genes sharing at least 2 GO terms with A1 genes ($P < 10^{-3}$), 3) Genes whose expression is induced to a maximum level in cerebellar granule cells at 7-14 days after birth. To obtain this information, RIKEN Cerebellar Development Transcriptome Database (CDT-DB) was searched. Except for *Stxbp5l*, the genes tested here behave like group A1 as expected. It is worth noting that *Ndst3* and *Ndst4* are located on chromosome 2, flanking a long LAIR enriched with c2 toposites (see Figure S9 for the map). Gene names: *Ndst3*, N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 3; *Ndst4*, N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 4; *Kctd8*, Potassium channel tetramerization domain containing 8; *Slc39a12*, solute carrier family 39 (zinc transporter); *Stxbp5l*, syntaxin binding protein 5-like.

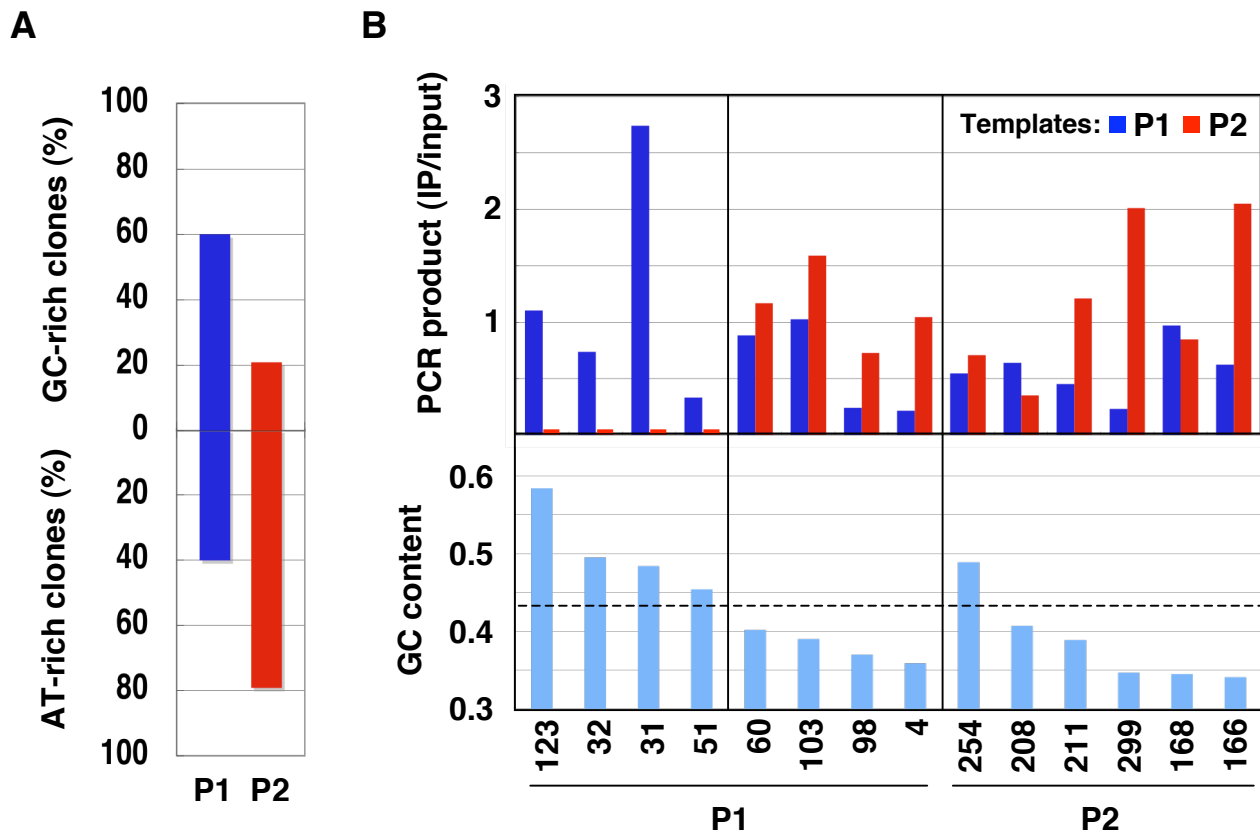
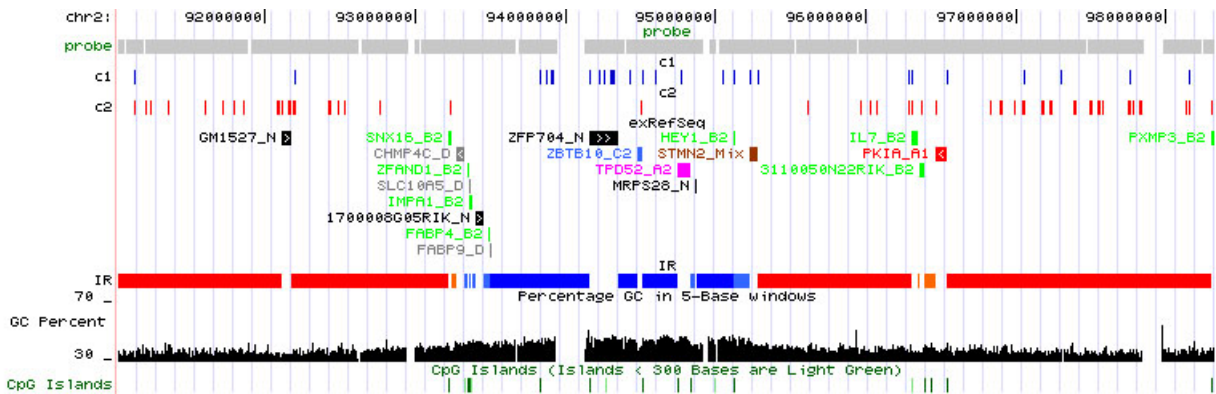
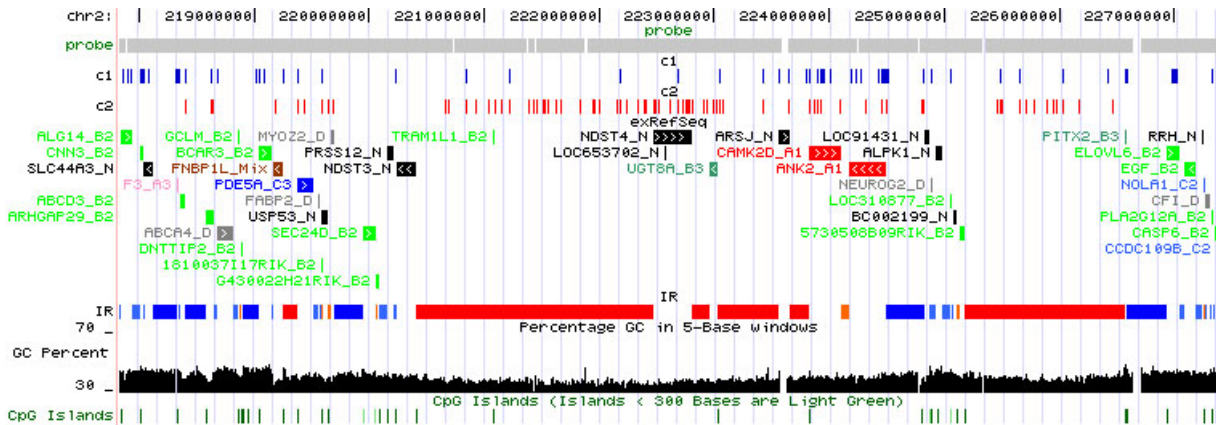


Figure S8. Characterization of eTIP DNA fractions by shotgun cloning and sequencing. The eTIP experiment was performed at the 2nd day of granule cell differentiation. DNA fragments purified from P1 and P2 fractions were cloned into a vector and sequenced as in Materials and Methods. The BLAT search identified 135 and 177 genomic locations for P1 and P2 DNA clones, respectively (complete data is presented in Table S1). **A**, Analysis of nucleotide compositions suggested that P1 DNA is composed of two different populations with distinct GC contents, whereas P2 DNA clones are mostly AT-rich ($P < 10^{-8}$). Local GC contents were calculated for 1,000-bp span (± 500 bp from the center of cloned region) and the numbers of clones with GC contents higher (GC-rich) or lower (AT-rich) than that of entire rat genome (0.427) were counted. The apparent enrichment of AT-rich sequences in P2 clones was estimated by chi-square test. **B**, PCR amplification of eTIP DNA fractions with primers complementary to the cloned fragments. Sequences for eTIP clones with various local GC contents were selected and used to design the primers (summarized in Table S2). Template DNAs from P1 and P2 fractions or IP input (before immunoprecipitation) were amplified with these primer sets. Relative amounts of PCR products for 8 clones from P1 and 6 clones from P2 are shown here. Numbers at the bottom stand for the clone number (listed in Table S1). Local GC contents calculated as above are plotted in the lower panel. Overall GC content of rat genome (0.427) is indicated by broken line. The results confirmed the notion stated above in retrograde: the GC-rich clones from P1 fraction were found exclusively in P1 template, whereas AT-rich clones from P1 or P2 were amplified with both P1 and P2 templates.

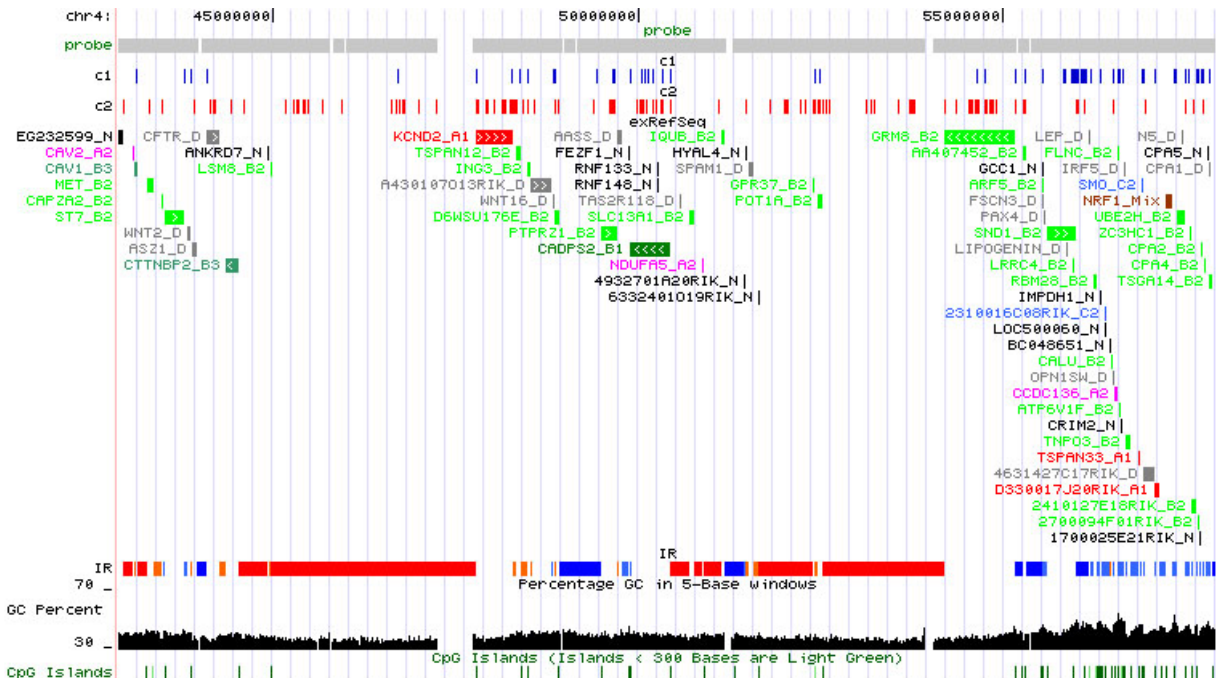
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chr2:217,827,330-227,366,232



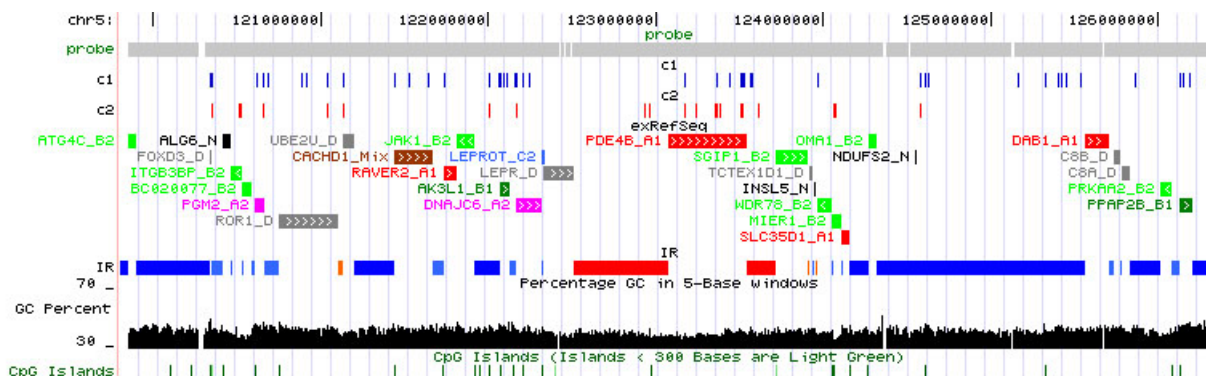
chr4:42,866,895-57,916,607



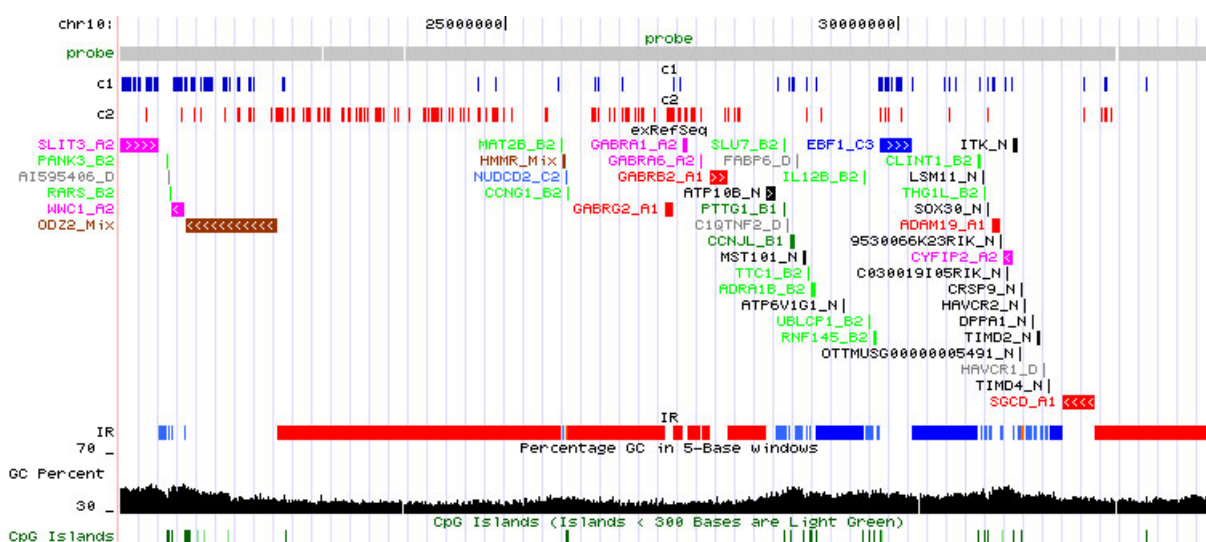
chr4:135,995,247-150,677,540



chr5:119,800,001-126,350,000



chr10:20,098,221-34,042,054



chr19:1-12,000,000

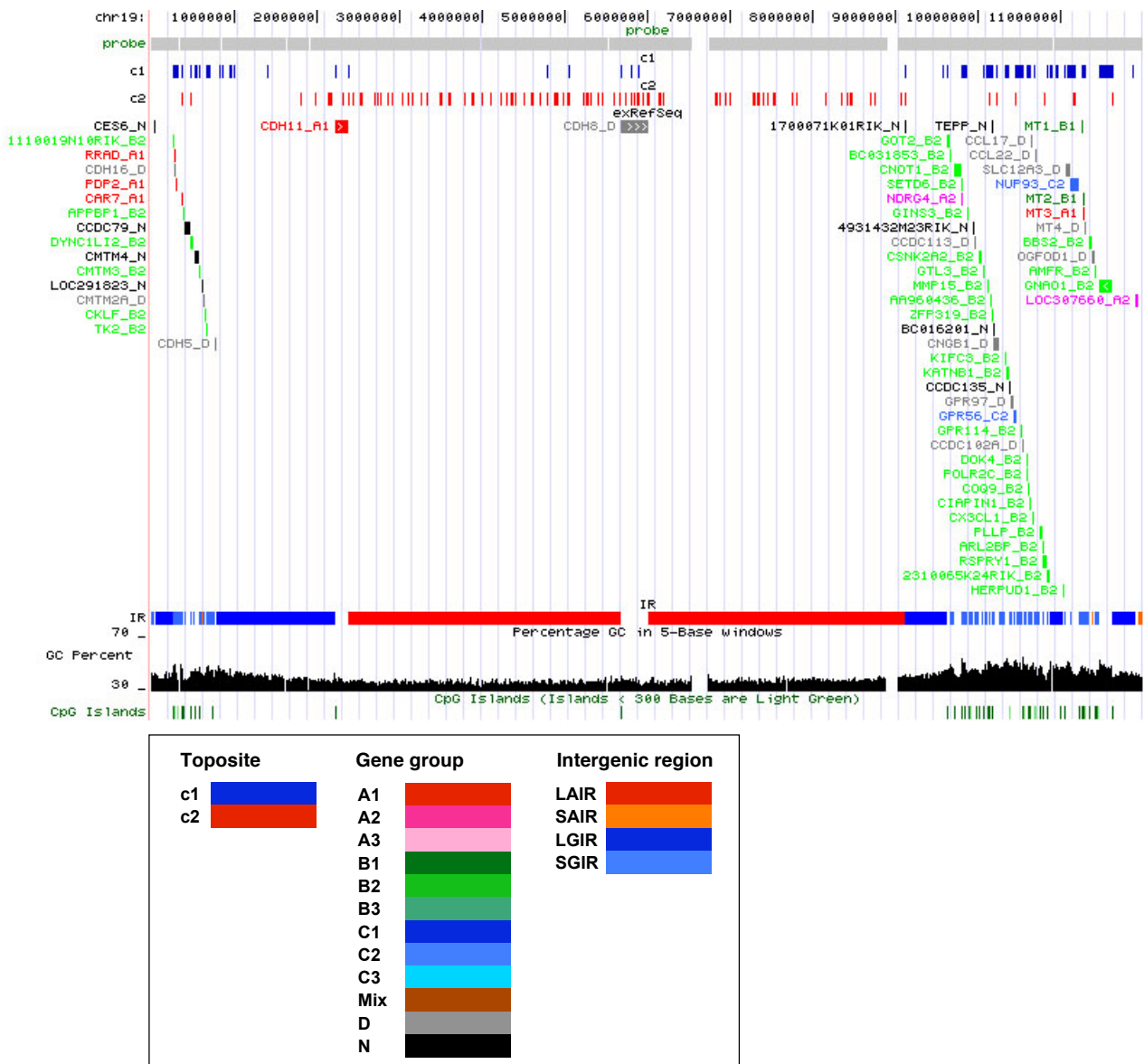


Figure S9. Overview of the topography of toposites and genes in the seven chromosomal regions analyzed by tiling arrays. Positional data for exRefSeq genes and toposites were uploaded and displayed in the custom track of UCSC genome browser. Gray bars on the top row (labeled “probe”) depict the position of array probes. Areas without assigned probes or sequence gaps are blank. Classified toposites (c1, c2) are shown below. The exRefSeq genes, labeled by gene names and the expression group assignments, are discriminated by color codes shown on the bottom of the figure. Toposites and intergenic regions (IR) are also color-coded.

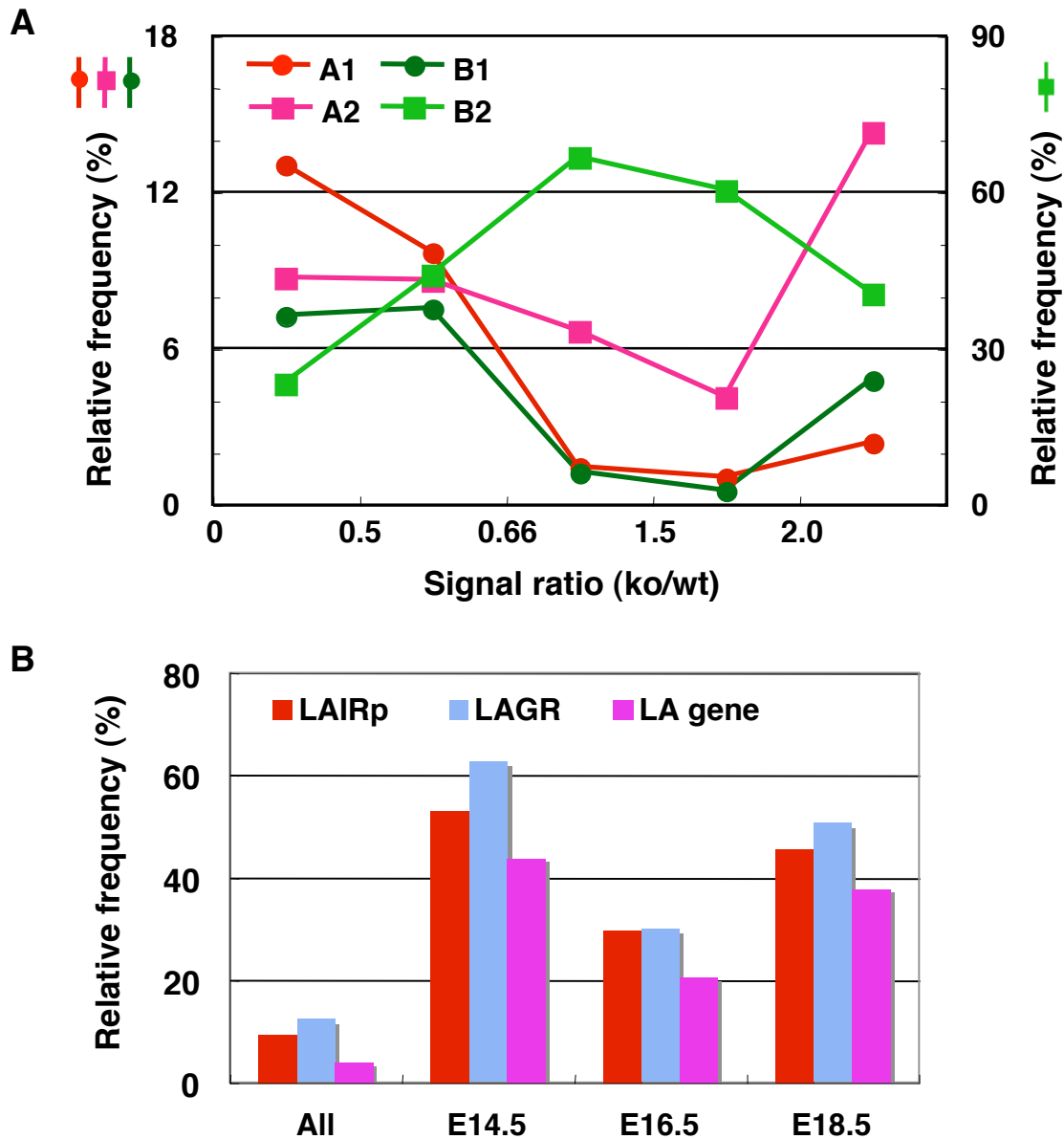


Figure S10. Similarity of expression patterns and genomic locations of relevant gene groups between embryonic brain and cultured granule cells. Our results were compared to the effects of topo II β depletion on the gene expression in mouse embryonic brain [7]. Their array data were obtained from GEO (Accession: GDS2702) or from the supplementary tables. **A**, The 12,433 probes on the array (Affymetrix GeneChip MG-U74A) were converted to RefSeq using g:Convert at the g:Profiler website (<http://biit.cs.ut.ee/gprofiler/>). Corresponding items (10,410) were then extracted from rat exRefSeq, low-signal probes ($P > 0.05$) were removed, and the remaining probes (6,299) were used for the analysis. Data sets for three embryonic days (E14.5, E16.5, E18.5) were averaged in wild type (wt) and the knockout mice (*top2b* ^{$\Delta 2/\Delta 2$}), respectively, and the ratio (average signal intensity)^{ko}/(average signal intensity)^{wt} was calculated. In here, the ratio is sorted into 5 levels and the percentage of gene groups in each level were plotted. The results imply that the genes down-regulated by ICRF-193 in the post-mitotic granule cells (A1 and B1) are also down-regulated in the topo II β -depleted embryonic brain, whereas constitutively expressed genes (B2) are more or less unaffected by the gene knockout. **B**, With respect to three embryonic days (E14.5, E16.5, E18.5), probes differentially expressed in the topo II β -knockout (≥ 1.7 -fold, $P \leq 0.01$) were extracted from the supplementary tables (Table S2-S4 of Ref. 7). Down-regulated probes were then selected, converted to rat exRefSeq, and relative frequencies of LAIR-proximal (LAIRp), LAGR, and LA genes were plotted in the graph. As a reference, those in all arrayed genes (All) are also shown. The results indicate that an extremely large proportion of down-regulated genes belong to LAIR-proximal, LAGR, and LA gene categories.

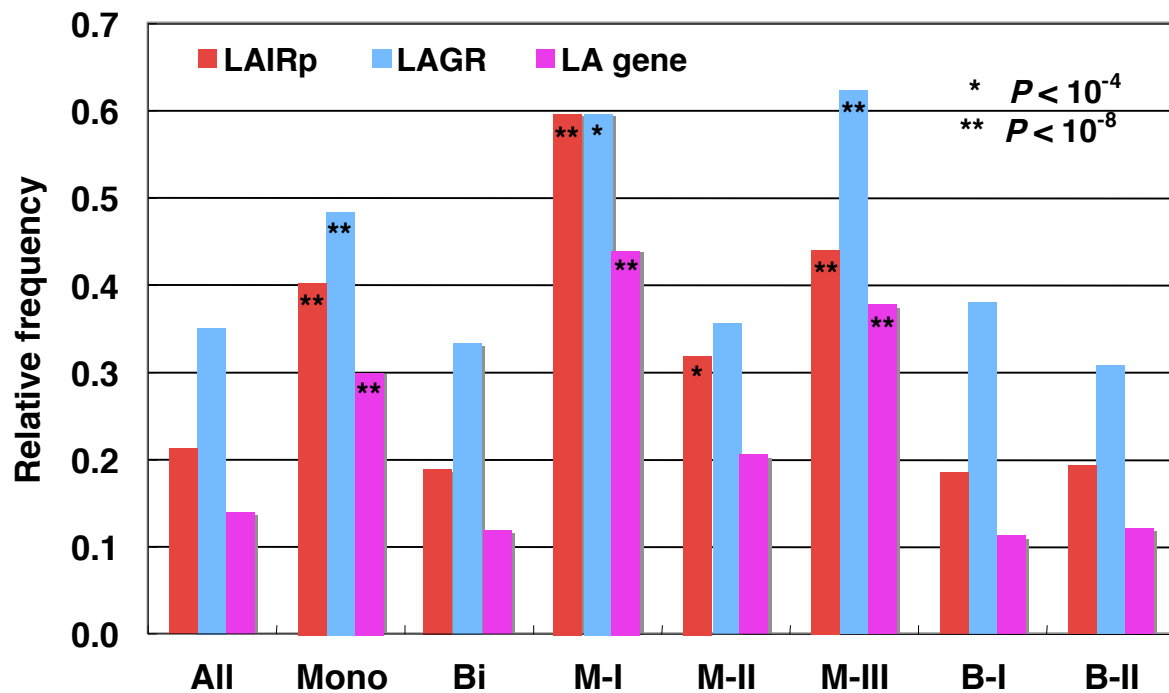


Figure S11. A high incidence of LA genes in monoallelically expressed autosomal genes. Considerably large proportion (5-10%) of human autosomal genes display random monoallelic transcription genome-wide [25]. Functional similarity of monoallelic genes and LA genes made us examine whether these genes also occupy similar positions in the genome. All the genes analyzed in the report (565 monoallelic and 4,407 biallelic) were uploaded to g:Orth at the g:Profiler website to sift out rat homologues. Corresponding items (505 monoallelic and 4,028 biallelic) were then extracted from rat exRefSeq and sorted into LAIR-proximal (LAIRp), LAGR, and LA gene. Monoallelic (Mono) and biallelic (Bi) genes had been classified into three (M-I, M-II, M-III) and two (B-I, B-II) classes, respectively, in the order of decreasing reliability (see the original report for details). The frequency data were plotted in the graph and statistical significance was calculated by the chi-square test against “All”. The results indicate, with high statistical confidence, that a large proportion of monoallelic but not biallelic genes are LA genes.

Table S2. eTIP PCR primers

Clone number	Genomic position		Primer sequence forward	Primer sequence reverse	Amplification target
123	chr19	53221727 53222052	GGGTGCTACTTTGGGGCA	AGCCTCAAGTTTTGATTCCCC	chr19:53221865-53222007
32	chr2	191445729 191446345	GAGAGGTGCCCGAGTCAATG	AGGAAGATGTGCCCGAAGAA	chr2:191445970-191446127
31	chr2	175924263 175925254	CCCCTCACTTGTTAATTTGATGC	TTTCTTTACCTCGCTGACCAGTC	chr2:175924271-175924465
51	chr4	185431956 185432228	CGACTGGCCACACAGTTT	CCGCACTCTATACTTTGCGA	chr4:185431991-185432164
60	chr6	17876320 17876980	AGCTGGTCTCGAGGACTGATG	TTGGTGCAACTGGGTTCTGTT	chr6:17876336-17876501
103	chr15	65790235 65790359	ATTGGATGCAGCAAGAGGTCA	TGAACAAGAGAACCCAGGG	chr2:36841662-36841815
98	chr14	69263930 69264307	GCTTATGCAGGTACATGAGCTGC	CAGAACTACACGTACCAGGCTGTG	chr14:69264141-69264257
4	chr1	106662967 106663512	TTGCAATGCACCTGAAAGCTC	TCTCGGAGAATGTTGATTCTTTG	chr1:106687302-106687444
254	chr9	40586050 40586417	ATCCTCCATTACAGCAACCAGTG	ATTGTTACAGACCTGCTGGAGAGA	chr9:40586051-40586207
208	chr3	129140163 129141010	TGGCCTCTGTGAGAGAGATTATT	CCTCTCTCCAAGTCCACCCCTTA	chr3:129140194-129140319
211	chr4	17771850 17772063	ATCAGCCTGAGTCAGCTTTCT	CAGAGTTTCAAGTCCCAAAGACTG	chr4:17771851-17771951
299	chr15	95586502 95586869	AAAGGTCATGGAATACAGCACAGA	AAGAGAAGAGAGGTAACACGCCA	chr15:95586578-95586718
168	chr1	70762466 70763143	GCACTAAAGCTATCCATTTGGCTC	TGGAATTTCTAGATGAAATGGGTG	chr1:70762881-70763000
166	chr1	63272038 63272142	TGGCTTCTATCTTGATTCTGAGGA	AGCTCGCACAACTCCATAAGT	chr1:63272038-63272142

Column label	Description
Clone_number	serial number of eTIP clones
Genomic_position	chromosome number for the clone location sequence start of the clone sequence end of the clone
Primer_sequence_forward	nucleotide sequence of forward primer
Primer_sequence_reverse	nucleotide sequence of reverse primer
Amplification_target	genomic position of the target

Table S3. RT-qPCR primers

Gene expression group	Target	Forward primer			Reverse primer		
		Length	%GC	Primer sequence	Length	%GC	Primer sequence
A1	<i>Camk2d</i>	21	52.0	GTC AACAGT GCCAGTCTTCA	21	52.0	GCATGGAACATCCGTCCAGTA
A1	<i>ltp1</i>	20	50.0	CAAATGATGATGCTGCTGCC	21	57.0	CGTGTGAGCCTCTAACATGGC
A1	<i>Stx1a</i>	20	55.0	TCCTTTCTGGACCCCAACCT	20	60.0	TGCTCCTAGCACACCAGCAG
A1	<i>Pkia</i>	20	55.0	GACGATGTGGCGAATCTCCT	20	55.0	CAGGCCAGGTGATTTCAACC
A1	<i>Gabrb2</i>	24	54.2	ACTGGAAAGCTCAATGGCATGGGC	24	54.2	TGTCCTCAAGTCCGTCGCAACCTTA
A1	<i>Rrad</i>	20	45.0	AGCGACTGCAGCTGGAACCTC	22	50.0	GGTCCGTCTTCTATACCACCAA
A2	<i>Gabra6</i>	20	50.0	TCCCTGATGCCTTAGTCAA	21	42.9	TCGGGAAATGTCATCAAAGC
A2	<i>Dnm1</i>	19	57.9	TGCCCTCTGTGGTATTGC	19	52.6	TGGAGGTGGCACATTGGAG
A2	<i>Syp</i>	22	50.0	AGACATGGACGTGGTGAATCAG	21	57.0	CCACCCTCTCAGAGTTCTCGA
A2	<i>Gabra1</i>	20	50.0	CACACCCATCAATAGGTTTC	20	55.0	GACAGAGGAGTAAAGGCAGA
A2	<i>Grm4</i>	20	55.0	CAGGACCAACGGACACTGA	20	55.0	GCTGACTGTGGTGCCTCAAA
B2	<i>Matr3</i>	21	52.0	TTGCTGCTGCTACCCAGTCTT	21	57.0	CTGGCCTGGTCTGTATCTCCA
B2	<i>Ncl</i>	20	55.0	CAAAACCCACGGAGAGTCCA	20	55.0	GTGTGGGAACTGCAGCCTTT
B2	<i>Cat</i>	21	47.6	TGCCAAGGAAAAGCTAACCT	21	42.9	TCGGGAAATGTCATCAAAGC
B2	<i>Actb</i>	19	57.9	AACACCCAGCCATGTACG	20	50.0	ATGTCACGCACGATTTCCCT
B2	<i>Top2b</i>	20	45.0	AGTAGAAACGGCTTGCAAAG	20	45.0	CTACATAGCTGCGAAATCCA
N	<i>Ndst3</i>	20	65.0	CGTCAGACCGGAGCTACTCC	19	63.0	CATCCAGGACACAGGCATC
N	<i>Stxbp5l</i>	21	52.0	CTGGCATAACGGAATAGTTGC	20	55.0	AGAGGTCAATGGTCCCCATG
N	<i>Slc39a12</i>	21	48.0	TGAGTACCCCAAAGGCAATGT	21	48.0	CAAAATTTGTCAGGCCATCTC
N	<i>Ndst4</i>	20	55.0	GTCTCGGGAAGAGCAAAGG	20	55.0	TTCGAGAGCTCCAGTTGTG
N	<i>Kctd8</i>	21	52.0	GTGGCAATCTGAACTGCTCCA	21	52.0	ATCAGTTAGGCGGTGACATGG

Table S4. Antibodies used for Western blotting

Target	Source	Host	Type	1st_Ab_conc
<i>Camk2d</i>	calcium/calmodulin-dependent protein kinase type II δ TransGenic Inc.	rabbit	Poly	1/3,000x
<i>ltp1</i>	inositol 1,4,5-trisphosphate receptor type 1 Millipore	rabbit	Poly	0.15 μ g/ml
<i>Stx1a</i>	syntaxin 1A *1	mouse	Mono (clone mAb 6D2)	0.1 μ g/ml
<i>Gabra6</i>	GABA _A receptor, α 6 subunit Millipore	rabbit	Poly	1 μ g/ml
<i>Dnm1</i>	dynamitin 1 BD Transduction Lab.	mouse	Mono (clone 41)	0.125 μ g/ml
<i>Syp</i>	synaptophysin Roche Applied Science	mouse	Mono (clone SY38)	3 μ g/ml
<i>Matr3</i>	matrin 3 *2	rabbit	Poly	1/50,000x
<i>Ncl</i>	nucleolin (C23) *3	rabbit	Poly	0.2 μ g/ml
<i>Cat</i>	catalase Rockland Immunochemicals	rabbit	Poly	1 μ g/ml
<i>Top2b</i>	DNA topoisomerase II β *4	mouse	Mono (clone 3B6)	0.3 μ g/ml

*1. kind gift of Dr. Masami Takahashi (Yoshida, A., Oho, C., Omori, A., Kuwahara, R., Ito, T. & Takahashi, M. HPC-1 is associated with synaptotagmin and omega-conotoxin receptor. J Biol Chem 267, 24925-8 (1992).)

*2. kind gift of Dr. Ryozi Kuwano (Niigata University, Japan)

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