Neuron

Supplemental Information

Differential NOVA2-mediated splicing in excitatory and inhibitory neurons regulates cortical development and cerebellar function

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Saito et al., Figure S1

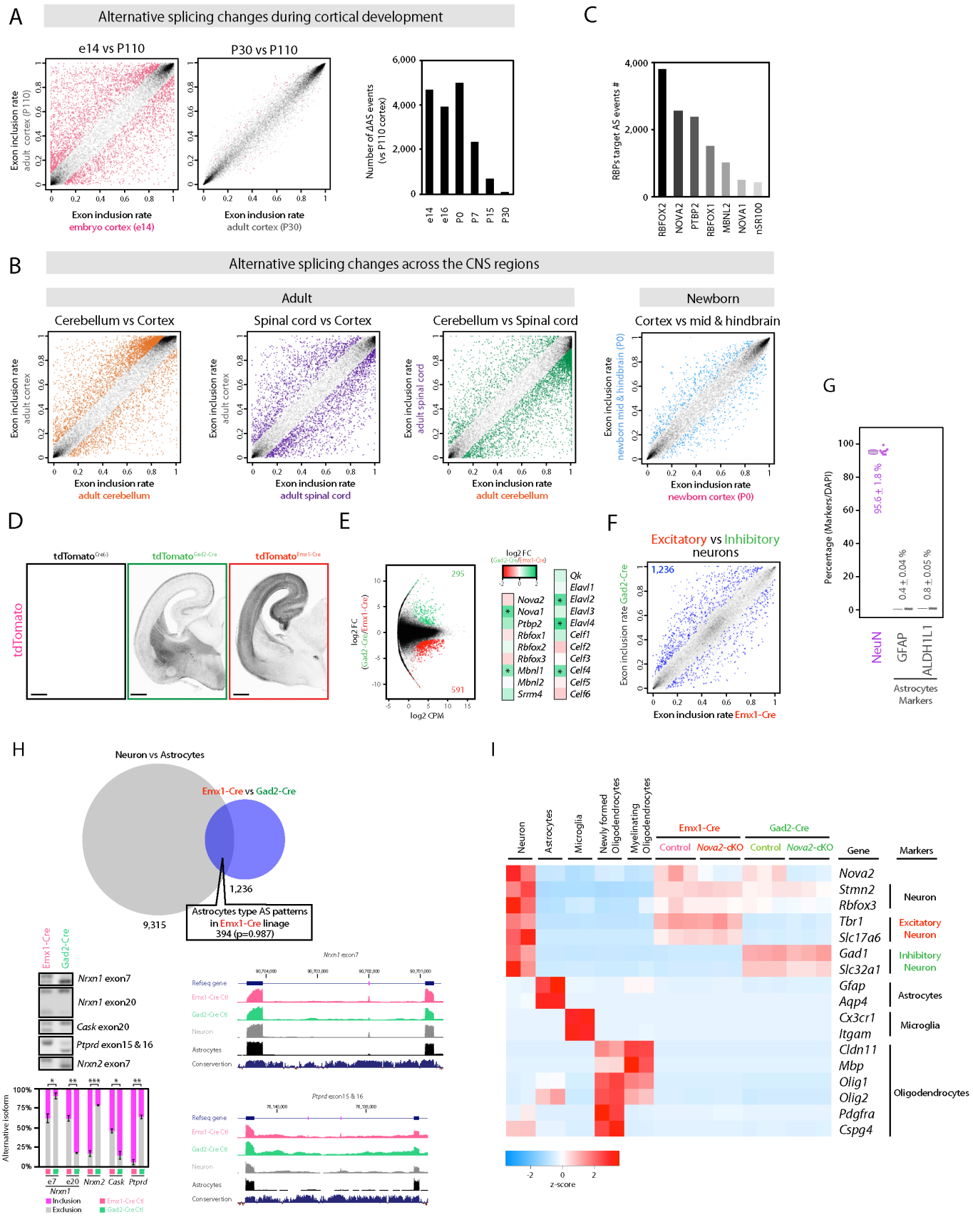

Figure S1. Related to Figure 1. Diversity of AS across the CNS regions and neuronal types.
(A) AS changes during mouse cortical development. The examples of developmentally regulated cassette-type AS events in the mouse cortex between e14 or P30 and P110 mouse cortex. *Pink* dot indicates the significantly changed AS events (FDR<0.05, |⊿I|>=0.1) (*Left* two scatter plot). Summary of total cassette-type AS number that significantly changed when compared with P110 mouse cortex (FDR<0.05, |⊿I|>=0.1) (*right*). (B) AS diversity across the CNS regions. Scatter plots show the exon inclusion rate of cassette-type AS events between the CNS regions. Each *Orange*, *purple*, *green*, or *light blue* dot indicates the significantly changed AS event between adult cortex and cerebellum, adult cortex and spinal cord, adult cerebellum and spinal cord, and newborn cortex and the mixture of midbrain, hindbrain, and cerebellum, respectively (FDR<0.05, |⊿I|>=0.1). (C) Total AS events number significantly changing in each RBP-KO mouse brain. AS changes were identified from publically available RNA-seq datasets (FDR<0.05, |⊿I|>=0.1). (D) Cre dependent tdTomato expression. tdTomato immunofluorescence staining images in e18.5 brain sections of $Control^{tdTomato;\ Cre(-)}$,$Control^{tdTomato;\ Gad2\text{-}Cre}$, and $Control^{tdTomato;\ Emx1\text{-}Cre}$ mouse. Scale bars: 500 μm. (E) RBPs mRNA expression difference between neuronal linages. Asterisks show the significant changes (FDR<0.05, |log2FC|>=1). (F) AS comparison between e18.5 Gad2-Cre and Emx1-Cre linage. *Blue* dot indicates a significantly changed AS event determined by RNA-seq (FDR<0.05, |⊿I|>=0.1). (G) Count of neuron and non-neuronal cell number in e18.5 cortex. NeuN (neuron marker), GFAP (astrocyte marker), and ALDH1L1 (astrocyte marker) (n>=4, counted cell number >= 77,802 for each). (H) No significant overlap of AS difference between Emx1-Cre vs Gad2-Cre linage and neuron vs astrocytes (*left*). Examples of AS difference between Emx1-Cre and Gad2-Cre linage (right, n=3).

Saito et al., Figure S2

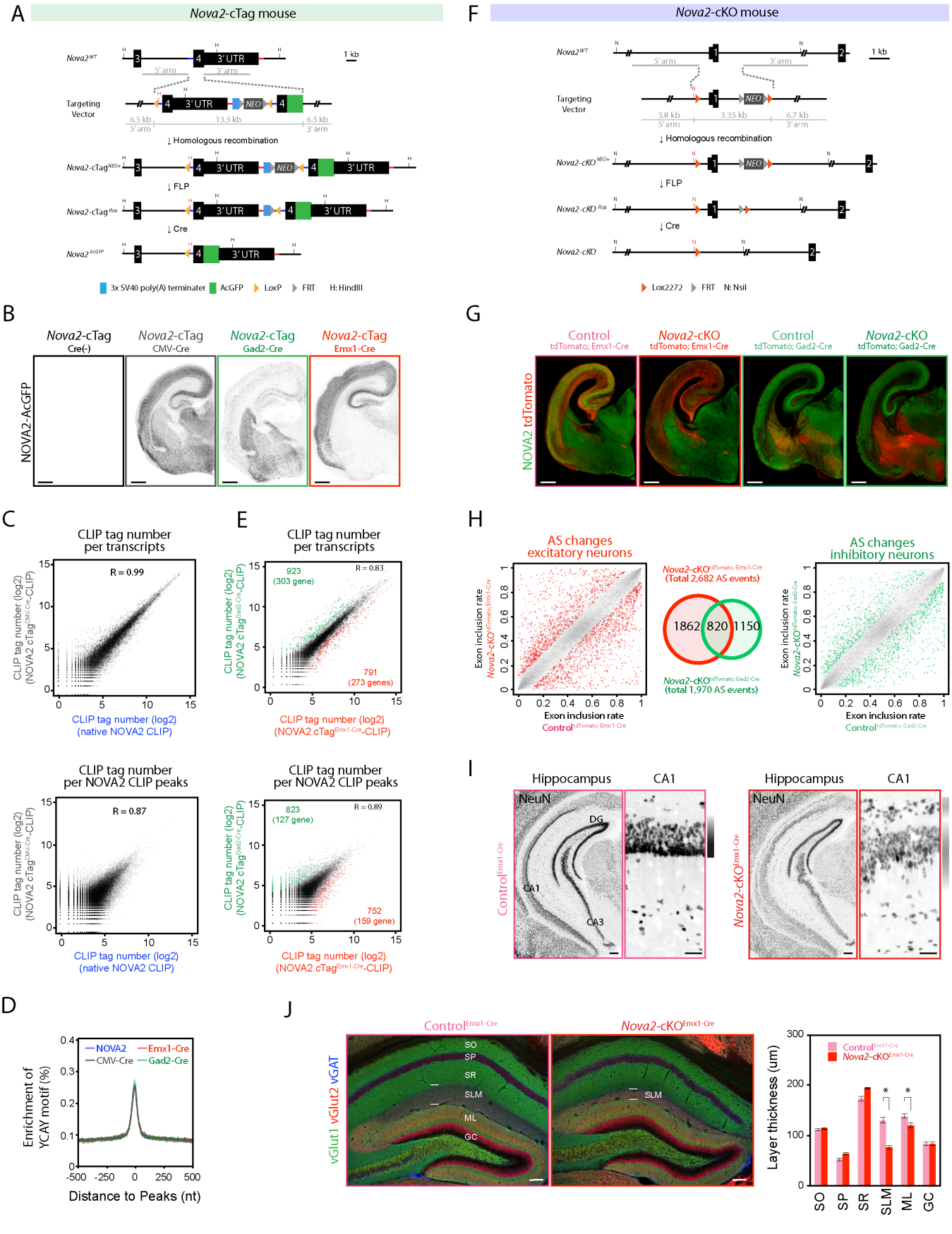

Figure S2. Related to Figure 2. Mice models development providing neuronal linage selective and transcriptome-wide NOVA2 analysis. (A) Targeting strategy for generating the knock-in *Nova2*-cTag mouse. FLP: Flippase. FRT: Flippase recognition target. *NEO*: Neomycin resistance gene. (B) Cre-dependent NOVA2-AcGFP protein expression. NOVA2-AcGFP immunofluorescent staining images with anti-GFP antibody in e18.5 *Nova2*-cTag$^{Cre(-)}$, *Nova2*-cTag$^{CMV-Cre}$, *Nova2*-cTag$^{Gad2-Cre}$, and *Nova2*-cTag$^{Emx1-Cre}$ mice brain. Scale bars: 500 μm. (C) The comparison of NOVA2 and NOVA2-AcGFP binding property to RNA. Scatter plots show the correlation of read counts between conventional NOVA2-CLIP to NOVA2 cTag$^{CMV-Cre}$-CLIP. Correlation of total CLIP read counts on transcripts (*Left*) and on NOVA2 CLIP peaks (*right*). Each dot represents a transcript (*left*) or CLIP peak (*right*). R: correlation coefficient. (D) Enrichment of YCAY motif near conventional NOVA2 CLIP and NOVA cTag-CLIP peaks. (E) The comparison of neuronal linage specific NOVA2 cTag-CLIP between cortical excitatory (Emx1-Cre) and inhibitory neurons (Gad2-Cre). Scatter plots show the correlation of read counts between NOVA2 cTag$^{Gad2-Cre}$-CLIP to NOVA2 cTag$^{Emx1-Cre}$-CLIP; data are compiled from three biologic replicates of mouse brain E18.5 cortex. Correlation of total CLIP read counts on transcripts (*Left*). Correlation of total CLIP read counts on NOVA2 CLIP peaks (*right*). Each *black* dot represents a comparable transcript (*left*) or CLIP peak (*right*) between two neuronal linages. Each *green* or *red* dot represents a significantly enriched transcript (*left*) or CLIP peak (*right*) in NOVA2 cTag$^{Gad2-Cre}$-CLIP or NOVA2 cTag$^{Emx1-Cre}$-CLIP, respectively (FDR<0.05, |log2 FC|>=1). R: correlation coefficient. (F) Targeting strategy for generating the *Nova2*-cKO mouse. FLP: Flippase. FRT: Flippase recognition target. *NEO*: Neomycin resistance gene. (G) NOVA2 depletion from selective neuronal populations. NOVA2 (*green*) and tdTomato (*red*) immunofluorescent staining images in the e18.5 Control$^{tdTomato;\ Emx1-Cre}$, *Nova2*-cKO$^{tdTomato;\ Emx1-Cre}$, Control$^{tdTomato;\ Gad2-Cre}$, and *Nova2*-cKO$^{tdTomato;\ Gad2-Cre}$ mice brain. Scale bars: 500 μm. (H) NOVA2 mediated AS events in restricted neuronal population. *Left and right* scatter plots show the exon inclusion rate of the e18.5 cortical Control$^{tdTomato;\ Emx1-Cre}$, *Nova2*-cKO$^{tdTomato;\ Emx1-Cre}$, Control$^{tdTomato;\ Gad2-Cre}$, and *Nova2*-cKO$^{tdTomato;\ Gad2-Cre}$. Each *grey* dot represents an AS event. Each *Red* and *green* dot indicates the significantly changed AS event in Emx1-Cre or Gad2-Cre lineage, respectively (FDR<0.05, $|\Delta I|$>=0.1). *Middle* Venn diagram representing NOVA2 target AS events number overlapping or differentially regulated between two neuronal-linages. (I) The disorganization of hippocampal laminar structure in *Nova2*-cKO$^{Emx1-Cre}$ mice. NeuN immunostaining in the hippocampus of 3 weeks old Control$^{Emx1-Cre}$ (*left, pink*) or *Nova2*-cKO$^{Emx1-Cre}$ mice (*right, red*). Scale bars: hippocampus; 200 μm, CA1; 50 μm. (J) CA1 molecular layer disorganization in 3 weeks old Emx1-Cre; *Nova2*-cKO mice. Hippocampal layer visualized by immunostaining with anti-vGlut1, vGlut2, and vGAT antibodies in 3 weeks old Control$^{Emx1-Cre}$ or *Nova2*-cKO$^{Emx1-Cre}$ mice (*left images*). Quantification and comparison of hippocampal layer thickness between Control$^{Emx1-Cre}$ or *Nova2*-cKO$^{Emx1-Cre}$ mice (*right*). *: p<0.05. SO; stratum oriens, SP; apical dendrites stratum pyramidal, SR; stratum radiatum, SLM; stratum lacunosum moleculare, ML; molecular layer, GC; granule cell. Scale bars: 100 μm.

Saito et al., Figure S3

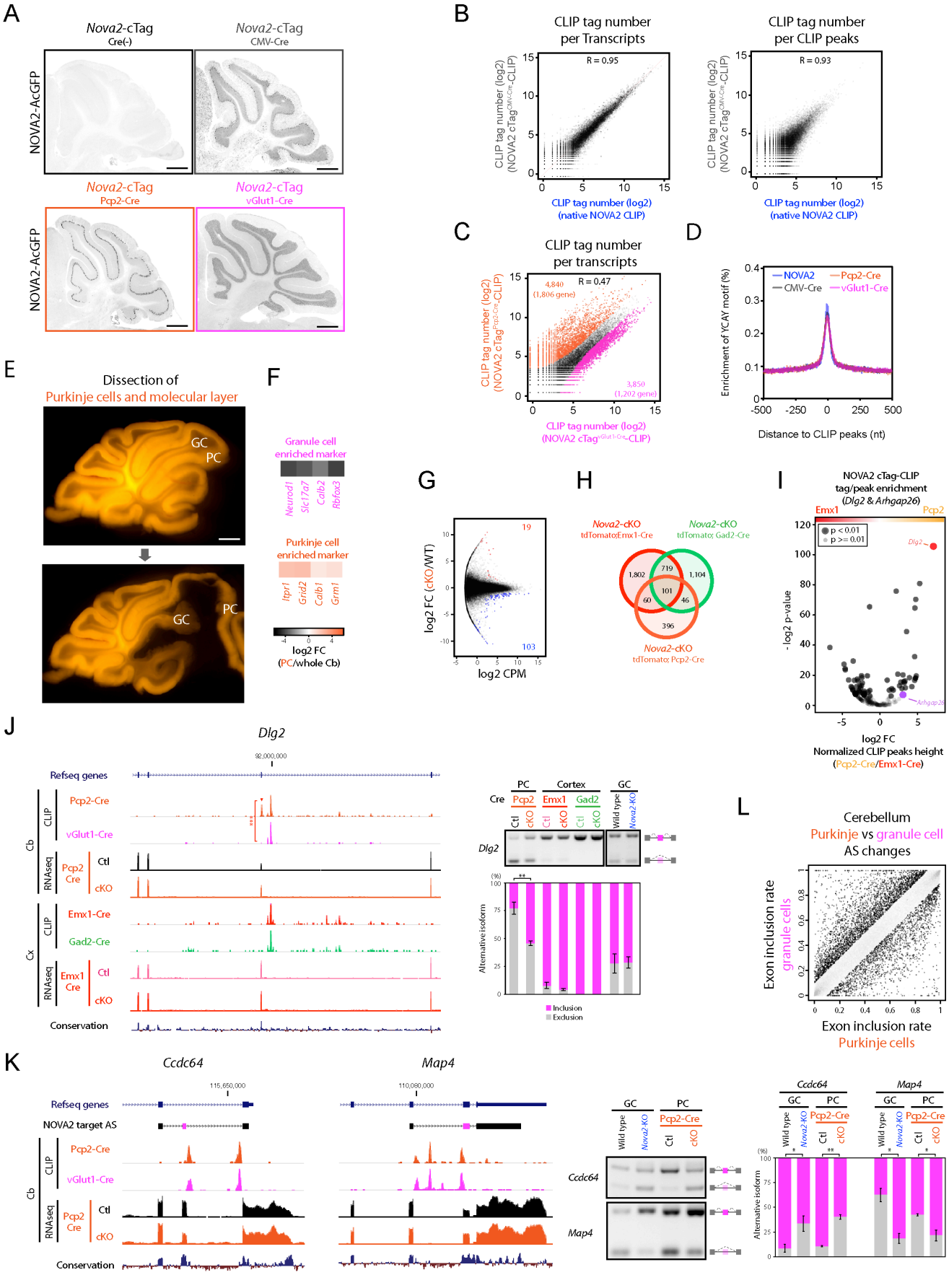

Figure S3. Related to Figure 3. Neuronal type specific analysis in adult cerebellum. (A) Cre-dependent NOVA2-AcGFP expression in cerebellum. Mouse brain slices prepared from 4 weeks old *Nova2*-cTag mice mated with CMV-Cre, Pcp2-Cre (Purkinje cell), vGlut1-Cre (granule cell) driver mice were subjected to immunostaining with anti-GFP antibody. Scale bars: 500 μm. (B) The comparison of NOVA2 and NOVA2-AcGFP binding property to RNA in adult cerebellum. Scatter plots show the correlation of read counts between conventional NOVA2-CLIP to NOVA2 cTag-CLIP (CMV-Cre). Correlation of total CLIP read counts on transcripts (*Left*) and on NOVA2 CLIP peaks (*right*). Each dot represents a transcript (*left*) or CLIP peak (*right*). R: correlation coefficient. (C) The comparison of neuronal type specific NOVA2 cTag-CLIP between cerebellar granule (vGluT1-Cre) and Purkinje cells (Pcp2-Cre). Scatter plot shows the correlation of read counts on CLIP peaks between NOVA2 $cTag^{vGlut1\text{-}Cre}$-CLIP to NOVA2 $cTag^{Pcp2\text{-}Cre}$-CLIP. Each *black* dot represents a comparable CLIP peak between two neuronal type. Each *magenta* or *orange* dot represents a significantly enriched CLIP peak in either NOVA2 $cTag^{vGlut1\text{-}Cre}$-CLIP or $cTag^{Pcp2\text{-}Cre}$-CLIP, respectively (FDR<0.05, |log2 FC|>=1). R: correlation coefficient. (D) Enrichment of YCAY motifs around NOVA2 cTag-CLIP peaks. (E) Manual dissection images of Purkinje cell layer from cerebellar slices of $Control^{tdTomato;\ Pcp2\text{-}Cre}$ reporter mice. Scale bars: 500 μm. (F) Neuronal-type marker enrichment in and depletion from RNA-seq libraries prepared from dissected out Purkinje cell layer. Purkinje cell enriched markers (*orange*). Granule cell enriched markers (*magenta*). (G) Transcript abundance changes upon NOVA2 depletion from Purkinje cells determined by RNA-seq. Each *black* dot represents a transcript. Each *blue* or *red* dot represents a significantly decreased or increased transcript in the Purkinje cell layer of *Nova2*-$cKO^{Pcp2\text{-}Cre}$ mouse, respectively (n=3, FDR<0.01, |log2FC|>=1, TPM average >= 2). (H) Venn diagram representing NOVA2 target AS events number overlapping or differentially regulated among three neuronal-linages. (I and J) (I) NOVA2 cTag-CLIP peak enrichment normalized to RNA abundance and functional NOVA2 protein abundance in *Arhgap26* and *Dlg2*. Each dot indicates an individual NOVA2 CLIP peak (Fisher's exact test). (J) Discovery of PCs selective NOVA2 mediated AS event. UCSC genome browser view of around *Dlg2* AS events (*left*). RT-PCR confirmation of AS change detected by RNA-seq (*right panels*) (n=3). **; p<0.01. (K) Common NOVA2 mediated AS events in PCs and GCs. UCSC genome browser views of *Ccdc64* and *Map4* (*left panels*) and RT-PCR confirmation and quantification of AS changes (*right panels*) (n=3). *; p<0.05, **; p<0.01 (L) AS changes between Purkinje cells and granule cells. Scatter plot shows the exon inclusion rate of Purkinje cells and granule cells determined by TRAP (Mellén et al., 2012). Each *grey* dot represents a comparative AS event. Each *black* dot indicates a significantly changed AS event (total 6,163 events) (n=4, FDR<0.05, $|\Delta I|$>=0.1).

Saito et al., Figure S4

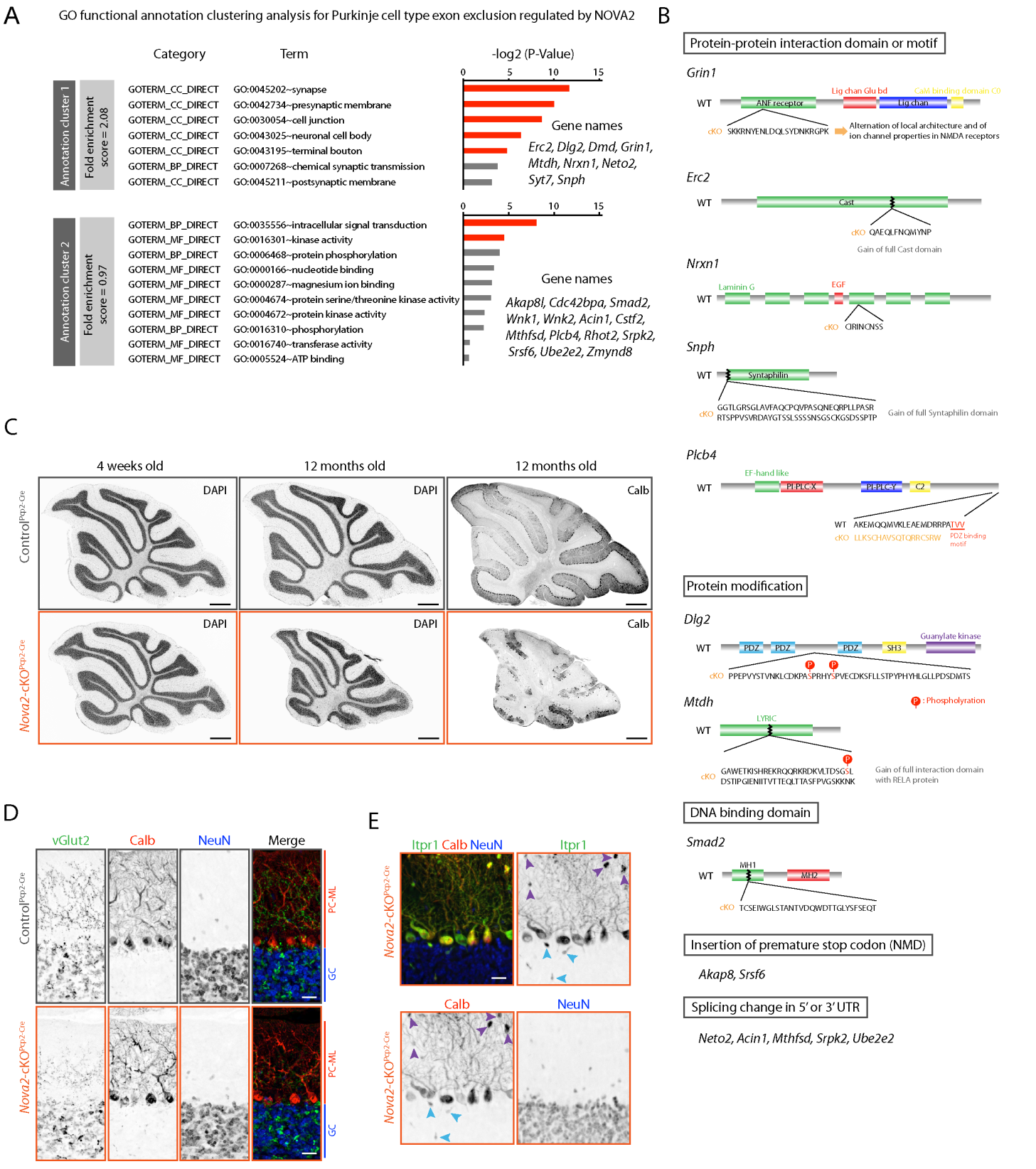

Figure S4. Related to Figure 4. Purkinje cell specific *Nova2* deficiency leads progressive motor discoordination and cerebellar atrophy. (A) GO functional annotation clustering analysis for the genes used in Figure 3H. *Red bars* represent the GO term having $p<0.05$. (B) Effects of alternative splicing on protein domains, modification, and RNA stoichiometry and regulation. Differential amino acid sequences are listed. (C) Comparative granule cell layer thickness. DAPI staining images in 4 weeks and 12 months old Control$^{tdTomato;\ Pcp2\text{-}Cre}$ and *Nova2*-cKO$^{tdTomato;\ Pcp2\text{-}Cre}$ and Calbindin D28 immunostaining images at 12 months old. Scale bars: 500 μm. (D) The climbing fiber innervation defect and reduced Purkinje molecular layer thickness upon Purkinje cell specific *Nova2* deficiency. vGlut2 (*green*, climbing fiber terminal marker), calbindin D28 (*red*, Purkinje cells marker), and NeuN (*blue*, granule cell marker) immunofluorescent staining images in the 4 weeks old Control$^{Pcp2\text{-}Cre}$ and *Nova2*-cKO$^{Pcp2\text{-}Cre}$. Scale bars: 25 μm. (E) The swelling Purkinje cell's neurites upon *Nova2* deficiency. ITPR1 (*green*), Caibindin D28 (*red*), and NeuN (*blue*) immunofluorescent staining images from 16 weeks old *Nova2*-cKO$^{Pcp2\text{-}Cre}$ mouse. *Purple* and *blue* arrowheads represent swelling neurites in molecular layer or granule cell layer, respectively. Scale bars: 25 μm.

Saito et al., Figure S5

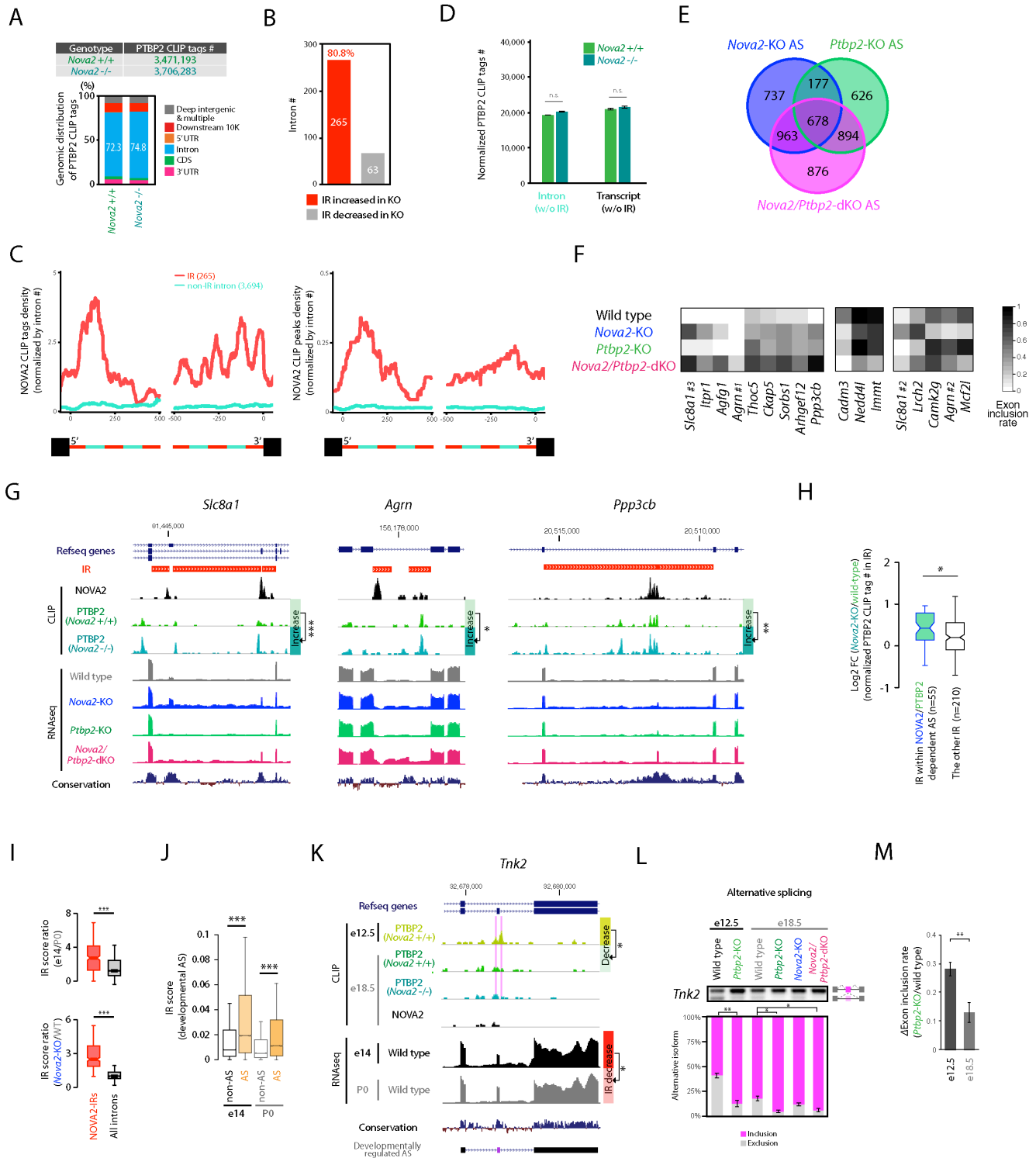

Figure S5. Related to Figure 5. NOVA2 regulates IR to serve as a *cis*-acting scaffold element for AS factor PTBP2. (A) PTBP2 CLIP results in wild type and *Nova2*-KO mouse. Total PTBP2 unique CLIP tag number from the e18.5 cortex of wild type and *Nova2*-KO mouse (*upper panel*) (three independent

CLIP experiments used three biological replicates). Genomic distribution of PTBP2 unique CLIP tags (*lower graph*). (B) The number of NOVA2-dependent increased and decreased IR in *Nova2*-KO. (C) NOVA2 CLIP tags or peaks distribution on IR or non-retained intron. Normalized NOVA2 CLIP tag (*upper*) or peak (*lower*) density on IR or non-retained intron. (D) PTBP2 CLIP tag number counted on all introns excepting IR, and full-length transcript excepting IR, which were normalized by total PTBP2 CLIP tag number of each replicate (n=3). Transcripts retaining IR or the other introns from these were subjected to analysis. (E) Venn diagram representing NOVA2, PTBP2, and NOVA2/PTBP2 target AS events number overlapping or differentially regulated among e18.5 cortex of *Nova2-KO*, *Ptbp2-KO*, and *Nova2*/*Ptbp2*-dKO. (F) Examples of AS changes in *Nova2*-KO, *Ptbp2*-KO, and *Nova2/Ptbp2*-dKO. Heatmaps represent the exon inclusion rate in either NOVA2 or PTBP2, or both absence condition *in vivo*. *Left* 9 columns show the additively or synergistically skipped by NOVA2 and PTBP2 in normal condition. *Middle* 3 columns show the additively or synergistically included by NOVA2 and PTBP2 in wild type. *Right* 5 columns show the attenuated-type AS events which are significantly changed in single-KO mouse but comparative in *Nova2*/*Ptbp2*-dKO with wild type. (G) UCSC genome browser views of transcripts containing the NOVA2-regulated IR with PTBP2 CLIP peaks changes. (H) Higher fold change of PTBP2 CLIP tag number in NOVA2-dependent IR with NOVA2/PTBP2-dependent AS events. (I) Higher decrease degree of NOVA2-IR as cortical developmental proceeds. ***; $p < 0.001$. (J) Higher intron retention within developmentally regulated alternative splicing events. (K) UCSC genome browser view of developmentally regulated *Tnk2* alternative splicing which independent on NOVA2. (L and M) PTBP2 dependent *Tnk2* AS difference at e12.5 and e18.5.

Saito et al., Figure S6

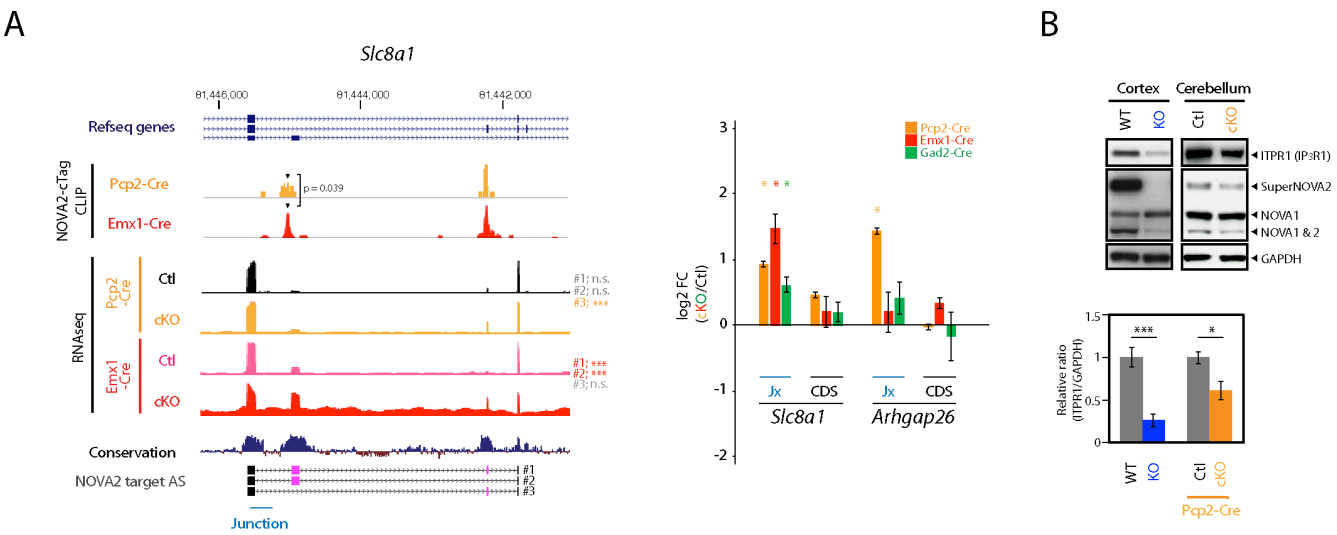

Figure S6. **Related to Figure 6. Multiple NOVA2-mediated target RNA metabolism coupling to IR.** (A) Neuronal subtype selective and NOVA2-dependent AS changes coupling with IR and CLIP peak enrichment. UCSC genome browser views (mm10) of examples (*Slc8a1*) (*left*). Quantification of transcripts abundance or it retaining exon-intron junction by qPCR (n=3, *; p<0.05). *Orange*; Purkinje cells, *red*; cortical excitatory neurons, *green*; cortical inhibitory neurons. *(B)* Decreased ITPR1 protein levels both in the cortex and cerebellum. Immunoblot analysis with anti-ITPR1 antibody in e18.5 cortex of wild type and *Nova*2-KO and in 16 weeks old whole cerebellum of Control$^{Pcp2\text{-}Cre}$ and *Nova2*-cKO$^{Pcp2\text{-}Cre}$ (n=3, *; p<0.05, ***; p<0.001).

Table S2. Primers list. Primer names, sequences, and expected PCR product size were listed. Related to Figure 2,3, and 5.

Genotyping

Primer_Name	Sequence	wild-type (bp)	mutant (bp)
Emx1-Cre_genotyping1	AAG GTG TGG TTC CAG AAT CG	378	102
Emx1-Cre_genotyping2	CTC TCC ACC AGA AGG CTG AG		
Emx1-Cre_genotyping3	GCG GTC TGG CAG TAA AAA CTA TC		
Emx1-Cre_genotyping4	GTG AAA CAG CAT TGC TGT CAC TT		
Gad2-Cre_genotyping1	CTT CTT CCG CAT GGT CAT CT	250	352
Gad2-Cre_genotyping2	AAA GCA ATA GCA TCA CAA ATT TCA		
Gad2-Cre_genotyping3	CAC CCC ACT GGT TTT GAT TT		
CMV-Cre_genotyping1	GCG GTC TGG CAG TAA AAA CTA TC	-	100
CMV-Cre_genotyping2	GTG AAA CAG CAT TGC TGT CAC TT		
Pcp2-Cre_genotyping1	GCG GTC TGG CAG TAA AAA CTA TC	-	100
Pcp2-Cre_genotyping2	GTG AAA CAG CAT TGC TGT CAC TT		
vGlut1-Cre_genotyping1	ATGAGCGAGGAGAAGTGTGG	218	344
vGlut1-Cre_genotyping2	GTGGAAGTCCTGGAAACTGC		
vGlut1-Cre_genotyping3	CCCTAGGAATGCTCGTCAAG		
Rosa-tdTomato_geno1	AAG GGA GCT GCA GTG GAG TA	297	196
Rosa-tdTomato_geno2	CCG AAA ATC TGT GGG AAG TC		
Rosa-tdTomato_geno3	GGC ATT AAA GCA GCG TAT CC		
Rosa-tdTomato_geno4	CTG TTC CTG TAC GGC ATG G		
Nova2-cTag_genotyping1	CAGAGCTCACACAGGCAGAAGTTGG	126	164
Nova2-cTag_genotyping2	CTTATCTCTGGGACCTGAAATGTCTCTACG		
Nova2-cKO_genotyping1	GAGCCTCGCGGTCCTTATTATACCC	130	168
Nova2-cKO_genotyping2	GCAGTCAAGGCCACGCCCT		

RT-PCR (Alternative splicing)

Primer_Name	Sequence
Nrxn1_AS1_e7_F	gaagcactagtggagcctgtgaat
Nrxn1_AS1_e7_R	gtgtaatcttcttgcgtgtagcccg
Nrxn1_AS2_e20_F	caaggagtggtggcaatgcca
Nrxn1_AS2_e20_R	cctgctctttcccgccaattat
Nrxn2_AS_e7_F	tggaacccgtcaatggcaagttc
Nrxn2_AS_e7_R	agaagaagtcatcagagcccagca
Ptprd_AS_exon15-16_F	gcggtaatccggatccagaaatcac
Ptprd_AS_exon15-16_R	ctctgttcgatctgcagcgctc
Dab1-AS_F	CCGTGATCCTGAAACAGAAGAGAACATT
Dab1-AS_R	AGCAGCAGTGCCGAAAGACATAGA
Cask-AS_F	GGCGATGTATCAACAGAGAAACTGGG
Cask-AS_R	ATGTGTGGATGCTTCAGCATATGACAG
Arhgap26-AS_F	CTAGCAGCAGCCTGCAGCCCAATCTGAAT
Arhgap26-AS_R	AAGACGGTGCCCGCTGTGAAGGAGAG
Dlg2_AS_exon10_F	catctactgtctggtaacaatggcaca
Dlg2_AS_exon10_R	agttgcagtactgtgctggg

Itpr1-AS1_F	TCCTCTTGGCAATCTTAGACTGTGTCC
Itpr1-AS1_R	GAAGCCTCCTCCCCGCAGC
Itpr1-AS2_F	ACAGCCCTGAACTGGCGGTTATC
Itpr1-AS2_R	GAACATCCACGAGCACAGACAGCT
Mcf2l-AS_F	CACAAAAGGGAACACAAGAAATGTCA
Mcf2l-AS_R	CAGCTTCTTAGGGCCCACTCCAC
Gphn_AS1_exon8_F	Cagtggtgtagcttcaacagaagatagtt
Gphn_AS1_exon8_R	aggagtagtgctaagggaggctg
Gphn_AS2_exon9-11_F	cagcctcccttagcactactcct
Gphn_AS2_exon9-11_R	ggctcttagaatgttctccttgctgc
Tnk2_AS_F	tcctgacctgctgagtgtgg
Tnk2_AS_R	ctcaggccaagcctcttgaag
MAP4_AS_F	gcccaagccaaagtgggatc
MAP4_AS_R	catttgcccggaacgtcagc
Ccdc64_AS_F	aaactgaacctctctcagcagc
Ccdc64_AS_R	ctgaagaaggagaagagccgttt

qPCR (IR and CDS)

Primer_Name	Sequence	
Itpr1-CDS-qPCR_F	GCGTTTCCACTGGAGAGAATGCTC	
Itpr1-CDS-qPCR_R	GTCTGTGCAAACCTCTCCTTAATTTCATC	
Mcf2l-CDS-qPCR_F	ggctgtgaacgactccatgca	
Mcf2l-CDS-qPCR_R	gcttgaacctggctagctcct	
Gphn-CDS-qPCR_F	ctggaggaacggggtttgca	
Gphn-CDS-qPCR_R	tccacacactggtctagagagcat	
Arhgap26-CDS-qPCR_F	ggccaatgttgctaacaaccacaa	
Arhgap26 -CDS-qPCR_R	ttccgggacaggtgtagctgg	
Slc8a1-CDS-qPCR_F	tccattgctgccatctaccatgcg	
Slc8a1-CDS-qPCR_R	gctcacctcctatttctggcctc	
Actb-CDS_F	GCTGTGCTGTCCCTGTATGCCTCT	
Actb-CDS_R	CTCAGCTGTGGTGGTGAAGC	
Itpr1-junction1_F	TGGAGATGTCAAGACCAGGATGTTCTG	
Itpr1-junction1_R	GAAGCCTCCTCCCCGCAGC	(Same with Itpr1-AS1_R)
Itpr1-junction2_F	ACAGCCCTGAACTGGCGGTTATC	(Same with Itpr1-AS2_F)
Itpr1-junction2_R	CACAGTGTGAGGAGGCCTATGCC	
Mcf2l-junction_F	CACAAAAGGGAACACAAGAAATGTCA	(Same with Mcf2l-AS_F)
Mcf2l-junction_R	ccttctatgtgaccagtgtgctga	
Arhgap26-junction_F	GAGAATTGTGGCTTCTTAGAGCGTGT	
Arhgap26-junction_R	AAGACGGTGCCCGCTGTGAAGGAGAG	(Same with Arhgap26-AS_R)
Slc8a1-junction_F	ACCTTCTTCATTGAGATTGGAGAGCC	
Slc8a1-junction_R	ccactgatgccacagcgaaa	
Gphn-junction1_F	Cagtggtgtagcttcaacagaagatagtt	(Same with Gphn_AS1_exon8_F)
Gphn-junction1_R	Ctgtaccaggagagaggcattgat	
Gphn-junction2_F	Cctgggctgatctcagattcacat	
Gphn-junction2_R	Ggctcttagaatgttctccttgctgc	(Same with Gphn_AS2_exon9-11_R)

Table S3. NOVA2 cTag-CLIP and RNA-seq lists used in this study. Mouse genotype, age, tissue, obtained unique CLIP tag number, Accession number, and references were listed. Related to Figure 1-3, and 5-6.

CLIP: GSE103316				
CLIP_name	Mouse_Genotype	age	Tissue (# of tissue)	CLIP.uniq.tag#
NOVA2 cTag$^{CMV\text{-}Cre}$-CLIP_1	CMV-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	e18.5	Cortex (2)	1,112,127
NOVA2 cTag$^{CMV\text{-}Cre}$-CLIP_2	CMV-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	e18.5	Cortex (2)	605,981
NOVA2 cTag$^{CMV\text{-}Cre}$-CLIP_3	CMV-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	e18.5	Cortex (2)	1,138,717
NOVA2 cTag$^{Gad2\text{-}Cre}$-CLIP_1	Gad2-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	e18.5	Cortex (3)	484,733
NOVA2 cTag$^{Gad2\text{-}Cre}$-CLIP_2	Gad2-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	e18.5	Cortex (3)	665,816
NOVA2 cTag$^{Gad2\text{-}Cre}$-CLIP_3	Gad2-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	e18.5	Cortex (3)	533,227
NOVA2 cTag$^{Emx1\text{-}Cre}$-CLIP_1	Emx1-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	e18.5	Cortex (3)	447,882
NOVA2 cTag$^{Emx1\text{-}Cre}$-CLIP_2	Emx1-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	e18.5	Cortex (3)	826,576
NOVA2 cTag$^{Emx1\text{-}Cre}$-CLIP_3	Emx1-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	e18.5	Cortex (3)	402,321
NOVA2 CLIP_1	Wild type	P28	Cerebellum (2)	618,550
NOVA2 CLIP_2	Wild type	P28	Cerebellum (2)	436,857
NOVA2 CLIP_3	Wild type	P28	Cerebellum (2)	371,590
NOVA2 cTag$^{CMV\text{-}Cre}$-CLIP_1	CMV-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	P28	Cerebellum (3)	148,516
NOVA2 cTag$^{CMV\text{-}Cre}$-CLIP_2	CMV-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	P28	Cerebellum (3)	979,110
NOVA2 cTag$^{CMV\text{-}Cre}$-CLIP_3	CMV-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	P28	Cerebellum (3)	758,256
NOVA2 cTag$^{vGlut1\text{-}Cre}$-CLIP_1	vGlut1-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	P28	Cerebellum (3)	141,263
NOVA2 cTag$^{vGlut1\text{-}Cre}$-CLIP_2	vGlut1-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	P28	Cerebellum (3)	1,071,274
NOVA2 cTag$^{vGlut1\text{-}Cre}$-CLIP_3	vGlut1-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	P28	Cerebellum (3)	711,719
NOVA2 cTag$^{Pcp2\text{-}Cre}$-CLIP_1	Pcp2-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	P28	Cerebellum (7)	268,648
NOVA2 cTag$^{Pcp2\text{-}Cre}$-CLIP_2	Pcp2-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	P28	Cerebellum (7)	475,913
NOVA2 cTag$^{Pcp2\text{-}Cre}$-CLIP_3	Pcp2-Cre $^{+/-}$; Nova2-cTag $^{Flox/wt}$	P28	Cerebellum (7)	497,991
PTBP2 CLIP_wt_1	Wild type	e18.5	Cortex (1)	1,241,813
PTBP2 CLIP_wt_2	Wild type	e18.5	Cortex (1)	942,468
PTBP2 CLIP_wt_3	Wild type	e18.5	Cortex (1)	1,289,458
PTBP2 CLIP_Nova2-KO_1	Nova2-KO	e18.5	Cortex (1)	990,027
PTBP2 CLIP_Nova2-KO_2	Nova2-KO	e18.5	Cortex (1)	1,462,221
PTBP2 CLIP_Nova2-KO_3	Nova2-KO	e18.5	Cortex (1)	1,256,337
PTBP2 CLIP_wt_1	Wild type	e12.5	Cortex (4)	826,934
PTBP2 CLIP_wt_2	Wild type	e12.5	Cortex (4.5)	1,073,842
PTBP2 CLIP_wt_3	Wild type	e12.5	Cortex (4.5)	884,118
RBPs_Name	Reference	age	Tissue	Accession #
NOVA2	Saito et al., 2016, eLIFE	e18.5	Cortex	GSE69711
RBFOX1/2/3	Weyn-Vanhentenryck et al., 2014	P15	Whole Brain	SRP035321
MBNL1	Wang et al., 2012	16 W	Whole Brain	GSE39911
nSR100/SRRM4	Raj et al., 2014	N2a cells		GSE57278

RNA-seq				
Mouse	Age	Tissues	Accession #	References
Wild type	e14	Cortex	SRP055008	Yan et al., 2015
Wild type	e16			
Wild type	P0			
Wild type	P7			
Wild type	P15			
Wild type	P30			
Wild type	P110			
Wild type	P60	Cerebellum	GSE60731	Frank et al., 2015
Wild type	P90	Spinal Cord	GSE103316	This paper
Nova1-control	P0	Midbrain & hindbrain & cerebellum	GSE69711	Saito et al., 2016
Nova1-KO	P0	Midbrain & hindbrain & cerebellum		
Nova2-control_Ribozero	e18.5	Cortex	GSE69711	Saito et al., 2016
Nova2-KO_Ribozero	e18.5	Cortex		
Nova2-control_Poly(A)+	e18.5	Cortex	GSE103316	This paper
Nova2-KO_Poly(A)+	e18.5	Cortex	GSE103316	This paper
Ptbp2-KO	e18.5	Cortex	GSE94054	Hwang et al., 2017
Nova2/Ptbp2-dKO	e18.5	Cortex	GSE103316	This paper
Rbfox1-control	1 M	Whole brain	SRP030031	Lovci et al., 2013
Rbfox1-KO				
Rbfox2-control				
Rbfox2-KO				
nSR100/SRR4-control	e18.5	Cortex	GSE65818	Quesnel-Vallieres et al., 2015
nSR100/SRR4-KO				
Mbnl2-control	2-3 M	Hippocampus	GSE38497	Charizanis K, et al., 2012
Mbnl2-KO				
TRAP.granule_cells	7-11 W	Cerebellum	GSE42880	Mellén et al., 2012
TRAP.Purkinje_cells				
Control; tdTomato;Emx1-Cre	e18.5	Cortex_tdTomato+(FACS)	GSE103316	This paper
Nova2-cKO; tdTomato;Emx1-Cre	e18.5	Cortex_tdTomato+(FACS)		
Control; tdTomato;Gad2-Cre	e18.5	Cortex_tdTomato+(FACS)		
Nova2-cKO; tdTomato;Gad2-Cre	e18.5	Cortex_tdTomato+(FACS)		
Control; tdTomato;Pcp2-Cre	P28	Cerebellum_Purkinje cell layer		
Nova2-cKO; tdTomato;Pcp2-Cre	P28	Cerebellum_Purkinje cell layer		