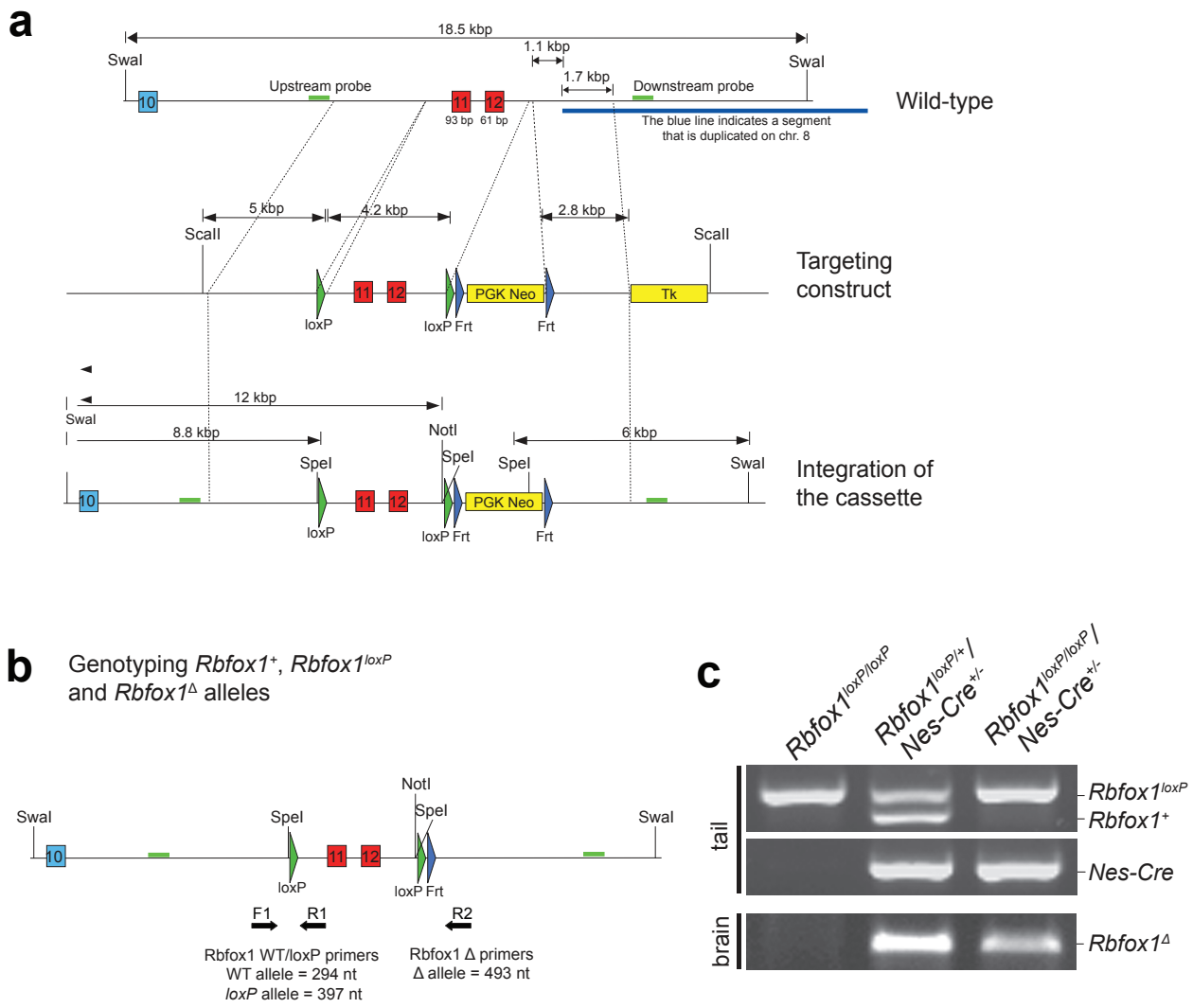
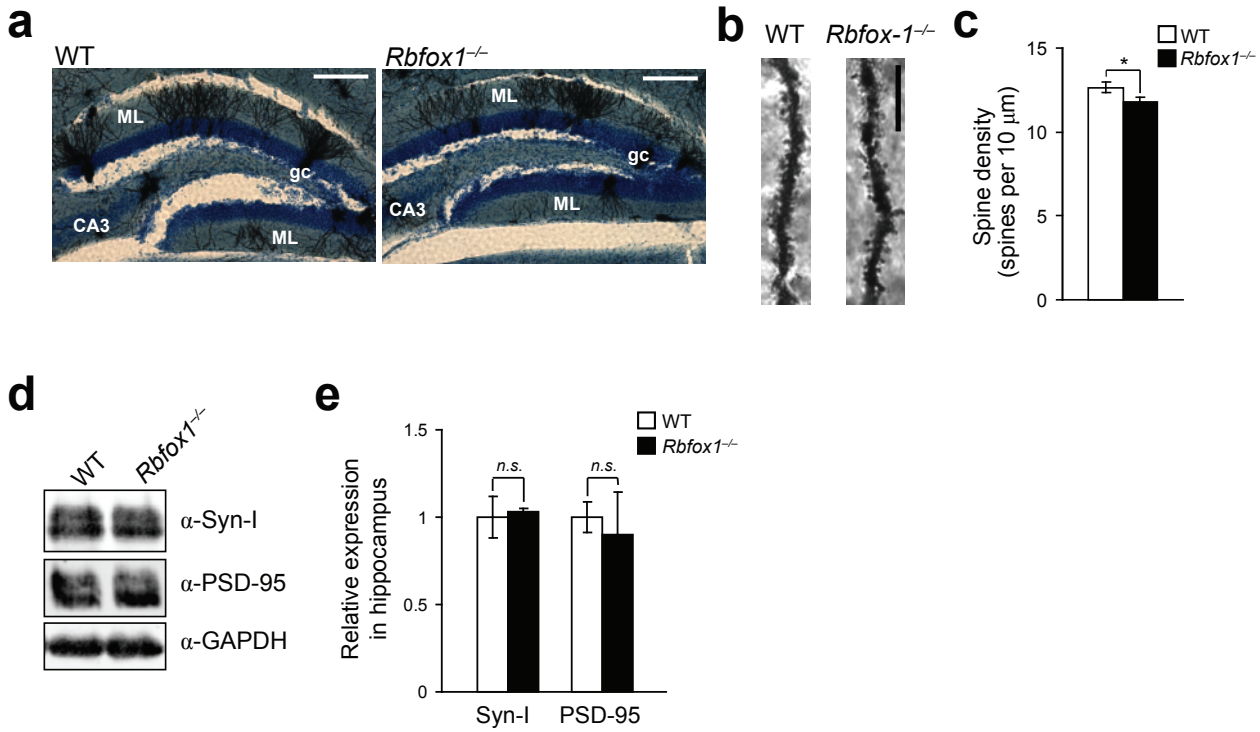
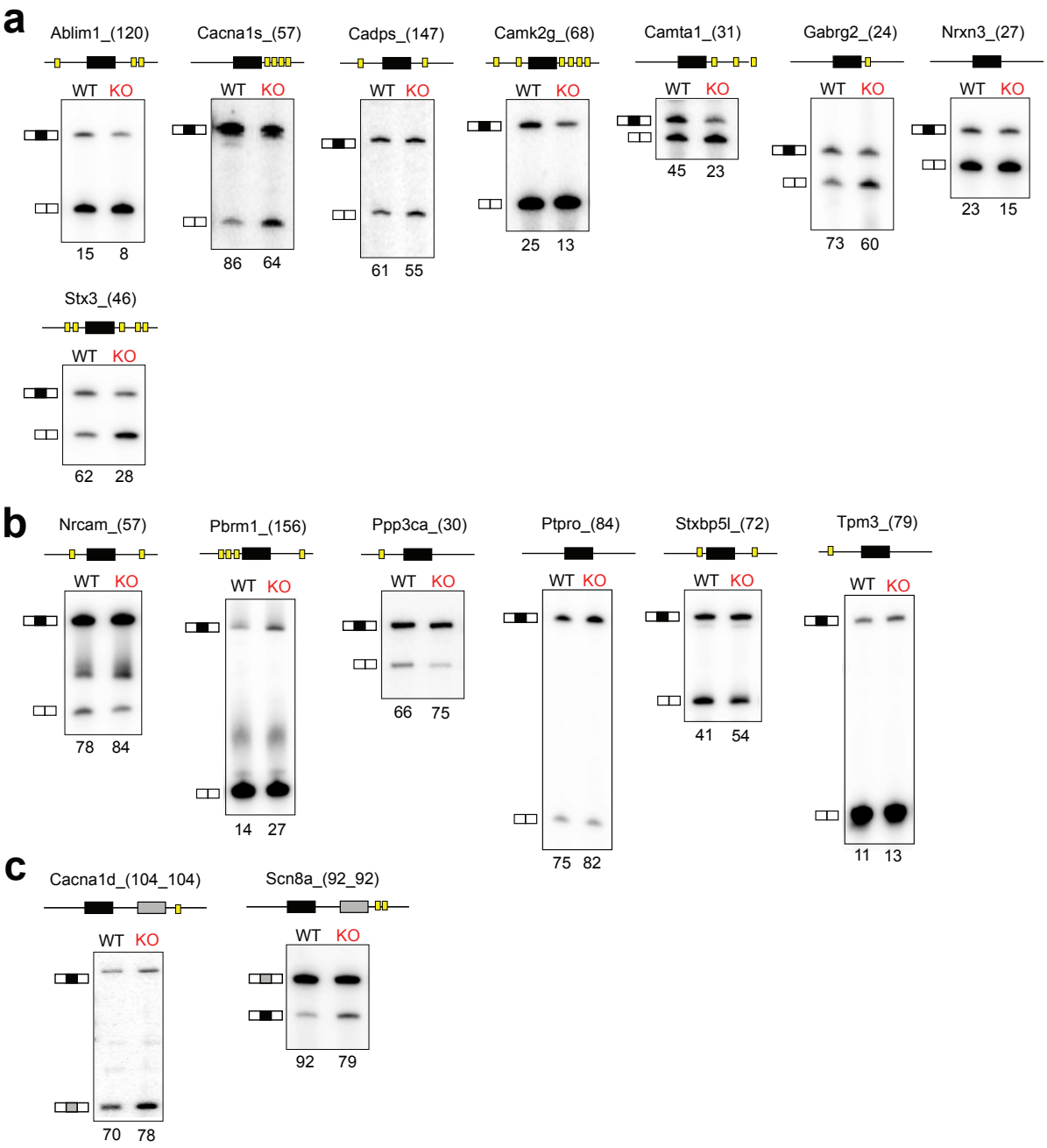


Supplementary Figure 1. Design of the *Rbfox1* targeting constuct and genotyping of the *Rbfox1* alleles. (**a**) Schematics of the relevant portion of the wild-type (WT) *Rbfox1* gene, the targeting construct, and the integrated construct genomic DNA. (**b**) Schematic showing the *Rbfox1*^{loxP} allele after removal of the *neo* selection cassette plus the location of primers for genotyping the WT (*Rbfox1*$^{+}$), *Rbfox1*^{loxP}, and *Rbfox1*$^{\Delta}$ alleles, with PCR product sizes indicated. The *Rbfox1*$^{\Delta}$ allele will only be amplified by PCR after Cre-mediated recombination. (**c**) Agarose gel showing PCR genotyping of DNA extracted from the tail and brain of *Rbfox1*$^{loxP/loxP}$, *Rbfox1*$^{loxP/+}$/*Nestin-Cre*$^{+/-}$, and *Rbfox1*$^{loxP/loxP}$/*Nestin-Cre*$^{+/-}$ mice. Sequences for genotyping primers are listed in Supplementary Table 3.

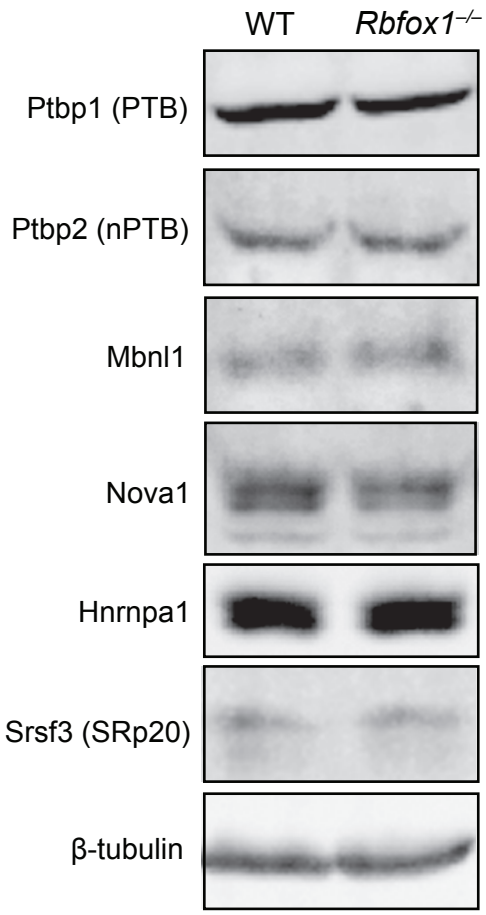


Supplementary Figure 2. *Rbfox1*$^{-/-}$ dentate gyrus granule cells exhibit a very modest decrease in dendritic spine density and no changes in expression of synaptic proteins. (**a**) Representative Golgi-Cox–stained images of adult WT and *Rbfox1*$^{-/-}$ dentate gyrus, counterstained with thionin (blue). Abbreviations: ML, molecular layer of the dentate gyrus; gc, granule cell; CA3, pyramidal layer of the hippocampus. Scale bar, 0.2 mm. (**b**) Representative Golgi-Cox–stained images of dendrites from WT and *Rbfox1*$^{-/-}$ dentate gyrus granule cells. Scale bar, 10 µm. (**c**) Quantification of overall spine density per 10 µm. Results are mean ± s.e.m.; *n* = 28 or 29 dendritic segments from 2 mice for each genotype. **P*=0.031 by one-tailed Student's *t* test. (**d**) Representative immunoblot analysis of Synapsin-I (Syn-I) and PSD-95 in total protein lysates from WT and *Rbfox1*$^{-/-}$ hippocampus. GAPDH was used as a loading control. (**e**) Quantification of relative Syn-I and PSD-95 expression in hippocampus, normalized by GAPDH. Results are mean ± s.e.m.; *n* = 3 animals per genotype. *n.s.*, no significant difference by one-tailed Student's *t* test (*P*=0.404 and *P*=0.363, respectively).



Supplementary Figure 3. Representative RT-PCR gels for all remaining significant splicing changes identified in *Rbfox1*$^{-/-}$ brain. (**a**) and (**b**) Representative RT-PCR gels for alternative cassette exons exhibiting decreased inclusion (a) and increased inclusion (b) in *Rbfox1*$^{-/-}$ brain (KO) compared to WT. (**c**) RT-PCR gels for mutually exclusive exon pairs analyzed by digestion with restriction endonucleases; exon inclusion levels were calculated for the downstream exon. Schematic above each gel shows the relative location of (U)GCAUG sequences (yellow boxes) in flanking introns. For all images, numbers below the gel show mean percentage inclusion levels of the alternative exon.

a



Supplementary Figure 4. The expression of other splicing factors is largely unchanged in the *Rbfox1*$^{-/-}$ brain. (**a**) Immunoblot analysis of six well-characterized splicing factors in total protein lysates isolated from WT and *Rbfox1*$^{-/-}$ brains. β-tubulin was used as a loading control for total protein. Only Nova1 shows a slight decrease (-40%) in *Rbfox1*$^{-/-}$ brain compared to WT.

	WT	*Rbfox1* $^{-/-}$	*Rbfox1* $^{+/-}$	*Rbfox2* $^{-/-}$
Animals with stage 5 seizures[†]	8/10	4/4	4/4	4/4
Latency (minutes) to 1st stage 5 seizure[‡]	48.3±8.9	19.7±5.8*	19.5±2.6*	36.5±7.8[a]
Duration (seconds) of stage 5 seizures	12.4±2.8	73.2±16.8*	61.6±21.0[a]	9.8±3.6[a]
Deaths	0/10	4/4*	4/4*	0/4[a]
Latency to status epilepticus/death[‡]	n/a	31.3±4.8	31.3±2.9	n/a

Supplementary Table 1. Summary of behavioral response to KA-induced seizures.

[†]A stage 5 seizure is defined as a tonic-clonic seizure characterized by continuous rearing and falling[32].

[‡]Latency to 1st stage 5 seizure and to status epilepticus (mean ± s.e.m.) is calculated from the time of KA administration.

*Significantly different from WT (one-tailed Student's *t* test, $P<0.05$).

[a]Not significantly different from WT (one-tailed Student's *t* test, $P>0.05$).

	Alt event ID‡	MJAY Ratio†	RT-PCR ΔPSI (Mean±s.e.m.)	RT-PCR *P* value	Upstream (U)GCAUG	Downstream (U)GCAUG
1	Kcnd3_(57)	+2.47	+28.17±7.53	0.03233*	(-16)	(+83)
2	Camta1_(31)	-1.48	-22.16±1.94	0.00378*	n/a	(+70, +210, +252)
3	Camkk2_(43)	-1.15	+2.42±1.17	0.08697	n/a	(+196, +217, +295)
4	Grin1_(63)	-1.06	-23.29±2.66	0.00641*	n/a	(+8, +262)
5	Stxbp5l_(72)	+1.03	+13.57±4.51	0.04757*	(-13)	(+130)
6	Tmem41b_(118)	-0.98	n/a	n/a	(-185)	n/a
7	Nt5c2_(59)	+0.92	+0.70±0.34	0.08756	n/a	(+254)
8	Scn8a_(92_92)	-0.89	-12.33±0.81	0.00216*	n/a_n/a	n/a_(+114, +192)
9	Pbrm1_(156)	+0.81	+13.22±2.52	0.01729*	(-78, -44, -31)	(+233)
10	Trpm1_(196)	+0.78	n/a	n/a	n/a	n/a
11	Ablim1_(120)	-0.72	-6.93±0.93	0.00871*	(-255)	(+123, +171)
12	Ppp3ca_(30)	+0.71	+8.55±1.97	0.02465*	(-188)	n/a
13	Pld3_(75_84)	+0.67	-1.60±0.72	0.07834	n/a_n/a	n/a_(+123)
14	Ptpro_(84)	+0.65	+6.97±2.22	0.04400*	n/a	n/a
15	Taf1b_(98)	+0.64	+8.22±8.55	0.21890	(-261, -244)	n/a
16	Tpk1_(147)	-0.62	+2.79±1.19	0.07191	(-305)	n/a
17	Tpm3_(79)	+0.61	+1.92±0.31	0.01277*	(-260)	n/a
18	Tmem180_(172)	+0.61	+2.45±2.08	0.18023	n/a	n/a
19	Myom1_(294)	+0.61	-2.31±3.36	0.28156	n/a	(+282)
20	Ccdc38_(183)	+0.60	n/a	n/a	(-115)	n/a
21	Dguok_(113)	+0.60	-0.24±1.27	0.43457	n/a	(+231)
22	Fbf1_(140)	+0.59	-0.15±0.24	0.29756	(-153)	(+57)
23	Nrxn3_(27)	-0.57	-7.61±1.30	0.01400*	n/a	n/a
24	Nrcam_(57)	+0.57	+5.46±0.69	0.00773*	(-136)	(+171)
25	Cacna1d_(60)	-0.56	-20.64±5.03	0.02726*	(-47)	n/a

Supplementary Table 2. Summary of microarray results and RT-PCR validation for splicing changes in *Rbfox1*$^{-/-}$ brain.

‡Alternative events identified by microarray (MJAY) were checked by RT-PCR and mean percentage change in exon inclusion in *Rbfox1*$^{-/-}$ compared to WT were calculated. Location of (U)GCAUG sequences and alternative event nomenclature as in Table 1.

†MJAY ratio is described in Supplementary Methods.

*Significant deviation from WT, as determined by paired, one-tailed Student's *t* test.

	Primer Pair	Purpose / alt event coordinates*	Forward primer†	Reverse primer†
1	Probe-Rbfox1_knpr_frt	Southern blot hybridization probe	AAACCAGATTTCCATACTCATCA	TGTTATTGGGTCTAGGCTGGT
2	5' Probe	Southern blot hybridization probe	ATCAGCACCCTGAGAAATGC	GGCATGGGTAATTTTCCTGT
3	3' Probe	Southern blot hybridization probe	CCTGGTTTGGCTGCATATTT	GGCATGGGTAATTTTCCTGT
4	PL253-Rbfox1	Recombineering primer	tcacagacaatgttggaacttcttatgaaattatttcctat gaggagtgtttgcGTATTGGTCACCACGG CCGAGTTTC	agaccattccccttttggcaaacaggaagctgcaacgt agactggacccaaggaCGGTGGGGTATCGA CAGAGTGCCAG
5	PL452-Rbfox1	Recombineering primer	tcactgacatggttcttatgatgagtatggaaatctggtt tactactacttctaaCTGCAGCCCAATTCCG ATCATATTC	tccaaaggttggtgtatgtatttctaaccaaaatgtacaa ataagcttaaggattTAGAACTAGTGGATCC CCTCGAGGG
6	PL451-Rbfox1	Recombineering primer	acatttataactcttcatataaatgtgaagcaaatttttttt ctggtgatggtggGACGGTATCGATAAGCT TGATATCG	gacatgaagctgtggatgtacatgcagagcatgtacaa gactctaggtccaagtcGCGGCCGCTCTAGA ACTAGTGGATC
7	Rbfox1 WT/loxP alleles	Genotyping primer	ATGCCCATGCAGTGAAAAAT	TGCAGCACATTGAAACCTTC
8	Rbfox1 Δ allele	Genotyping primer	ATGCCCATGCAGTGAAAAAT	AGCCAGTCAGCTGGAGTGAT
9	Nestin-Cre allele	Genotyping primer	CGTGTTGCACTGAACGCTAA	GCAAACGGACAGAAGCATTT
10	Ablim1_(120)	chr19:57,133,575-57,133,694	CTCCATCAACTCCCCTGTGT	TGGGTGGTTTTCGGTAAATG
11	Cacna1d_(104_104)	chr14:30,984,482-30,985,245	CCCAATGGAGGCATCACT	CTTCCAGCTGCTGTTTTTCC
12	Cacna1d_(60)	chr14:30,942,975-30,943,034	CACCAGCGAGACTGAATCTG	TGAGTTTGGATTTCGAGATGG
13	Cacna1s_(57)	chr1:138001147-138001203	TTTGGAGATCCTTGGAATGTG	AGTCTCATGACCCGGAACAG
14	Cadps_(147)	chr14:13,290,197-13,290,343	CGCCCCACTTGTTGTTAGAT	TTTGACGCAGGACTCAATCA
15	Camk2g_(68)	chr14:21,576,888-21,576,955	GGTCTACGGTGGCATCCAT	CCGCCATCTGACTTCTTGTT
16	Camkk2_(43)	chr5:123,186,997-123,187,039	CATGATTCGAAAGCGCTCAT	GTCTTCGCTGCCTTGCTTC
17	Camta1_(31)	chr4:150,445,535-150,445,565	GCCATCCTTATCCAGAGCAA	TCCTTGGCCTTTTTCAATTC
18	Ccdc38_(183)	chr10:93,041,454-93,041,636	TGAGGTCCAGGCTCTTCAGT	AGCATCTCCGATGCAGACTC
19	Dguok_(113)	chr6:83,446,680-83,446,792	GCATTGAAGGCAACATCG	GGCTCCAGCTGCACCTTC
20	Fbf1_(140)	chr11:116,019,334-116,019,473	GGTCTTCCTGCAGAACATGAA	CTTTCCCAGGACCCTTTGC
21	Gabrg2_(24)	chr11:41,727,472-41,727,495	ATTTTGTCAGCAACCGGAAG	ACAGTCCTTGCCATCCAAAC
22	Grin1_(63)	chr2:25,166,863-25,166,925	CCCTACTCCCACCAGTCCAGCGTC	AGCGTCGTCCTCGCTTGCAGAAAGG
23	Kcnd3_(57)	chr3:105,469,880-105,469,936	GGCAAGACCACCTCACTCAT	AGTGGCTGGACAGAGAAGGA
24	Myom1_(294)	chr17:71,431,543-71,431,836	GGTCAGAGCAGTGAATGCAG	ATGTCATAGGGCGCAGATG
25	Nrcam_(57)	chr12:45,646,366-45,646,422	GAGGACACCCGTGAGGACTA	TTTCATTGCCCTCTGGAGTT
26	Nrxn3_(27)	chr12:90,756,350-90,756,376	CTCATCAACGATGCTCTCCA	TCATTGCACTGGTTTCCAGA
27	Nt5c2_(59)	chr19:46,983,980-46,984,038	ACCGAAGTTTAGCCATGGAA	CCTGAGGATAGCCAATGGAA
28	Pbrm1_(156)	chr14:31,927,035-31,927,190	TGGGGACAGAATGGAGAAAC	ATGGGGGCTACTCCTTGATT
29	Pld3_(75_84)	chr7:28,330,205-28,334,117	GGGGCTAGGTTCTGGAGTAGA	AAAGGGGTGGTCCTGAGC
30	Ppp3ca_(30)	chr3:136,594,975-136,595,004	CTGACACTGAAGGGCCTGAC	GAGGTGGCATCCTCTCGTTA
31	Ptpro_(84)	chr6:137,368,845-137,368,928	GGAGCTGGCACGTTTGTTA	TTTCCTCTTCTTTAAGCCATTTTT
32	Scn8a_(92_92)	chr15:100,789,771-100,790,125	GACCCGTGGAACTGGTTAGA	TCCAGATAGCTCTCGTTGAAGTT
33	Snap25_(118_118)	chr2:136,595,478-136,595,910	ATGGCCGAAGACGCAGACATGCGC	TTAACCACTTCCCAGCATCTTTGT
34	Stx3_(46)	chr19:11,864,538-11,864,583	ACAGCCTTCATGGACGAGTT	GTTGTTGGCCCTTTTCTTGA
35	Stxbp5l_(72)	chr16:37,186,737-37,186,808	GCTATTAAGCATGGGGACCA	CCAGGGGAACTGGACTATCA
36	Taf1b_(98)	chr12:25,193,949-25,194,046	CCCCAACACCAAGATCAACT	AGGCCTGTTTGCTCTTCTGA
37	Tmem180_(172)	chr19:46,446,426-46,446,597	GACCACATCTCCCTGTCCAC	AGAAGCAGAGAAGCCAGGTG
38	Tmem41b_(118)	chr7:117,126,170-117,126,287	CCACCGAAATGTTGCACTC	CCATATCTCTCGGAACCTTCA
39	Tpk1_(147)	chr6:43,419,001-43,419,147	CCTGACCAAGACCACACTGA	TGTCTACATGGAGCCTGTGC
40	Tpm3_(79)	chr3:89,894,935-89,895,013	CGTGCTGAGTTTGCTGAAAG	GCTCCTCTTTGGTGCACTTC
41	Trpm1_(196)	chr7:71,344,049-71,344,244	TGCAAAAGGGAATGCATCTT	CCCAAACACCTGCTTCAGTT

Supplementary Table 3. List of primer sequences used for generating Rbfox1 transgenic mice, genotyping *Rbfox1* and *Nestin-Cre* alleles, and for RT-PCR assays.

*Purpose of the primer pair; genomic coordinates (UCSC July 2007 assembly mm9) for the alternative event are listed if the primer pair was used for RT-PCR.

†Primer sequences listed 5’ to 3’.