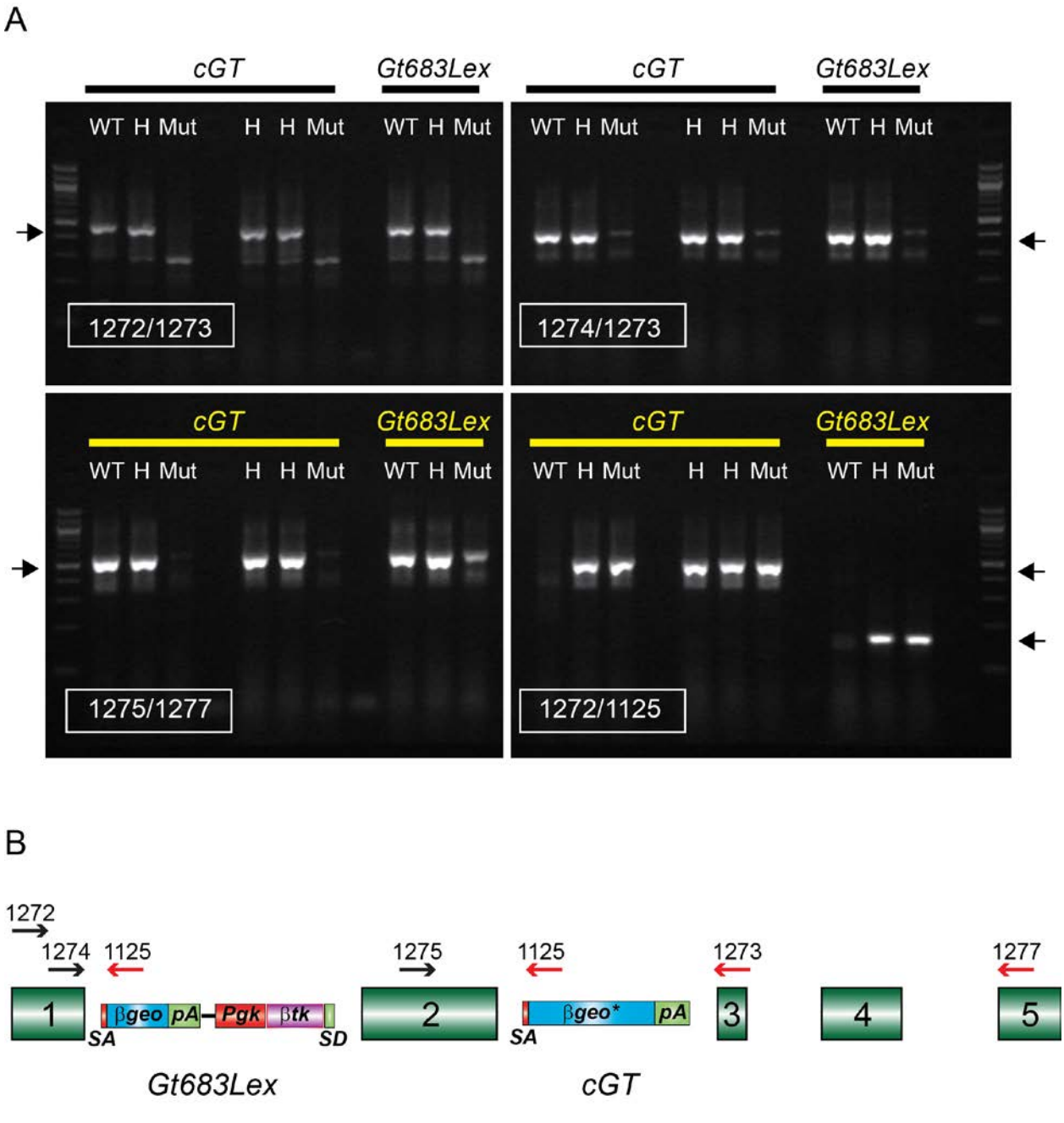
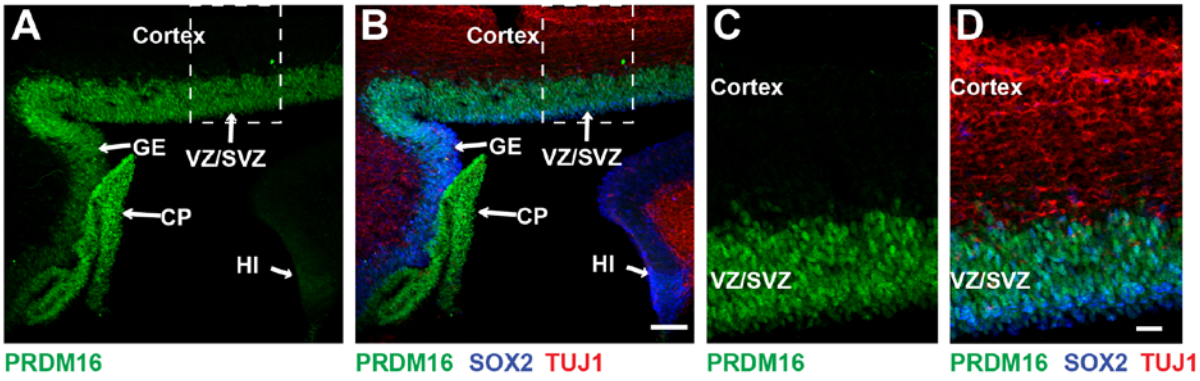
SUPPLEMENTAL DATA

Supplemental Figure 1



Supplemental Figure 1. The *Prdm16*^{cGT} and *Lex* alleles are gene trap null alleles with different insertion sites. (**A**) Gel images of One-step reverse transcriptase (RT)-PCR amplification from *Prdm16*^{cGT} and *Prdm16*^{Gt683Lex} total RNA derived from wt, heterozygous and mutant embryos using various exonic primers that span multiple introns OR primers within the *βgeo* cassette. Each exonic primer pair amplifies the expected amplicon in wt and heterozygous samples but not mutants as is expected with complete trapping of downstream splicing by the gene trap cassettes. The primers oBB 1272 (exon 1) and oBB 1125 (*βgeo*) detect the mutant *Prdm16-βgeo* fusion transcript produced in both alleles. For the *Prdm16*^{cGT} allele, this fusion product is amplified in heterozygous and mutant embryos but not wt, and the product is larger due to the gene trap insertion site being located in intron 2 versus intron 1 for the *Prdm16*^{Gt683Lex} allele. (**B**) Schematic depicting the gene trap insertion sites for the *Prdm16*^{cGT} and *Prdm16*^{Gt683Lex} alleles and the forward (black arrows) and reverse (red arrows) oligonucleotide primer locations used in the RT-PCR analyses above.

Supplemental Figure 2



Supplemental figure 2. PRDM16 is co-localized with SOX2 in the embryonic forebrain. (**A, B**) Coronal sections through E13.5 mouse brain showing cortex, ganglionic eminence, hippocampus and choroid plexus. Co-immunolabeling of PRDM16 (green), neural progenitor marker SOX2 (blue) and neuronal marker TUJ1 (red) shows co-localization of PRDM16 and SOX2 expression in multiple progenitor zones, including ventricular zone (VZ), sub-ventricular zone (SVZ), Ganglionic eminence (GE) and Hippocampus (HI). Additionally, PRDM16 is expressed in the choroid plexus (CP) where SOX2 is absent. Dashed rectangles in **A** and **B** are shown at higher magnification in **C** and **D**. Scale bar: 50 µM.

Supplemental Table 1. Plasmid clones used in this study

pBB#	Clone Name	Description	Vector	Host	Antibiotic
119	Prdm16in2-5'ENTR-L4-R1	oBB1040/1042 (attB4-B1) from MHPN 168g07 cloned into pDONR-P4-P1R with BP Clonase	pDONR P4-P1R	Stbl3	Kanamycin
123	Prdm16in2-3'ENTR-R2-L3	oBB1043/1044 (att2BR-B3) from MHPN 168g07 cloned into pDONR-2PR-P3 with BP Clonase	pDONR 2PR-P3	Stbl3	Kanamycin
130	pDONR-P4-P1R	Multi-fragment Gateway Donor plasmid	pDONR-P4-P1R	DB3.1	Kanamycin/ Chloramphenicol
131	pDONR-P2R-P3	Multi-fragment Gateway Donor plasmid	pDONR-P2R-P3	DB3.1	Kanamycin/ Chloramphenicol
132	pDONR221	Multi-fragment Gateway Donor plasmid	pDONR 221	DB3.1	Kanamycin/ Chloramphenicol
133	pDEST-R4-R3	Multi-fragment Gateway Entry plasmid	pDEST-R4-R3	DB3.1	Kanamycin/ Chloramphenicol
126	pPNT-DEST-R4-R3	DEST R4-R3 cassette cloned into the NotI/XbaI sites of pPNT, replaces Neo	pPNT	DB3.1	Ampicillin/ Chloramphenicol
134	prsFlpROSAbGeo* 1	conditional gene trap plasmid for ORF +1 w/ loxP/lox5171 and frt/f3 and bgeo*	pBabeSrf	TOP10	Ampicillin/G418
135	prsFlpROSAbGeo* 0	conditional gene trap plasmid for ORF +0 w/ loxP/lox5171 and frt/f3 and bgeo*	pBabeSrf	TOP10	Ampicillin/G418
136	prsFlpROSAbGeo* 2	conditional gene trap plasmid for ORF +2 w/ loxP/lox5171 and frt/f3 and bgeo*	pBabeSrf	TOP10	Ampicillin/G418
137	prsFlpROSAbGeo* TT 0	conditional gene trap plasmid for ORF +0 with unique ApaI and SnaBI cloning sites for targeted trapping	pBabeSrf	TOP10	Ampicillin/G418
138	prsFlpROSAbGeo* TT1	conditional gene trap plasmid for ORF +1 with unique ApaI and SnaBI cloning sites for targeted trapping	pBabeSrf	TOP10	Ampicillin/G418
139	prsFlpROSAbGeo* TT2	conditional gene trap plasmid for ORF +2 with unique ApaI and SnaBI cloning sites for targeted trapping	pBabeSrf	TOP10	Ampicillin/G418
140	prsFlpROSAbGeo* TT 0-ENTR	ApaI/NotI conditional gene trap cassette cloned into the XmnI/NotI sites of pENTR11	pENTR11	TOP10	Kanamycin
141	prsFlpROSAbGeo* TT 1-ENTR	ApaI/NotI conditional gene trap cassette cloned into the XmnI/NotI sites of pENTR11	pENTR11	TOP10	Kanamycin
142	prsFlpROSAbGeo* TT 2-ENTR	ApaI/NotI conditional gene trap cassette cloned into the XmnI/NotI sites of pENTR11	pENTR11	TOP10	Kanamycin
152	Prdm16in2-cGT	Prdm16 intron 2 conditional gene trap targeting construct	pPNT	Stbl3	Ampicillin/G418

Supplemental Table 2. Oligonucleotide primers used in this study

oBB #	Alias	Sequence	Nt	Tm
965	Ad-SA-XhoI_R	TGA AGC CGC TCG AGACTGGAAAGACCGCGAAGA		
1040	MHPN168g07-P16in2cGT-5'attB4_F1	GGGG ACA ACT TTG TAT AGA AAA GTT GCCTTAGAATCTTGTCAGGAAGTCTG	51	59.84
1042	MHPN168g07-P16in2cGT-5'attB1_R1	GGGG AC TGC TTT TTT GTA CAA ACT TGAGGACAAAGGATTCTCCAACAA	48	59.98
1043	MHPN168g07-P16in2cGT-3'attB2_F	GGGG ACA GCT TTC TTG TAC AAA GTG GAGGCCATCAATCACAGAGAGA	47	59.82
1044	MHPN168g07-P16in2cGT-3'attB3_R	GGGG AC AAC TTT GTA TAA TAA AGT TGTCTTTGGTCCAGTAGGACTCTCA	49	60.29
1054	PspOMI-LS-attR4_F	ATAAGAATGGGCCCGGCGCGCCTTAATTAAGGCCGGCCGTTTAAACGCGGCCGCATTTAAATCAGGAA ACAGCTATGAC	81	
1055	NheI-attR3_R	CCGTAGCTAGCGTAAAACGACGGCCAG	27	
1067	1040-1042L-R	AGGAAGAATACGGCCCTTGT	20	59.96
1069	1040-1-2R-F	CCTAGTCAAGGGTGTCAAGAGG	22	60.16
1073	1043-4R-F	ATCAGTGTGCTCCACCCTTC	20	60.12
1084	pENTR11_F	TCGTTGCAACAAATTGATAAGC	22	60.14
1085	pENTR11_R	GCAGCTGGATGGCAAATAAT	20	60.07
1086	1040-1-2intF2	TCTGAGCTTCTGAATCACAATGTA	24	59.04
1087	1043-4LR2	TCACGCTCTGGATAGCTCAA	20	59.7
1112	mPrdm16in2cGT-3'TargetPCR_F	GGGAGGATTGGGAAGACAAT	20	60.13
1113	mPrdm16in2cGT-3'TargetPCR_R	ACACTCCAGAGAGGGCAGAG	20	59.58
1114	mPrdm16in2cGT-5'TargetPCR_F	CTAGTCCACTGGCACAGCCTAC	22	61.24
1115	mPrdm16in2cGT-5'TargetPCR_R	ACTGGAAAGACCGCGAAGAG	20	61.84
1116	P16cGT-5RACE_F1	GAGACCGAAGACGGCATCCT	20	63.94
1117	GTprimer353	CAGGGTTTTCCCAGTCACGAC	21	64.04
1125	bGeo_RT-R1	CGCCATGTCACAGATCATCAAG	22	63.89
1272	P16x1RT-F1	CTGGGCTCAAGGAGGAGGAG	20	63.66
1273	P16x3RT-R1	TGCAGCTCTCCTGGGATGAC	20	63.89
1274	P16x1RT-F2	GCGAGGGCGAGGAAGCTA	18	63.99
1275	P16x2RT-F1	GAGACCGAAGACGGCATCCT	20	63.94
1277	P16x5RT-R1	GGCCATGACACCCAAGGAG	19	63.86
1278	P16_5hom-cons-aa1_F	CTGTTTTCCGTGGACCTGTT	20	60.01
1279	P16_5hom-cons-aa1_R	GGGAAAATGCCTCCTCTTTT	20	59.53

1280	P16_5hom-cons-aa2_F	CAGCCTATGCAGCACATAAAAG	22	59.93
1281	P16_5hom-cons-aa2_R	GCATGCTCTGTGGAACTCCT	20	60.42
1282	P16_5hom-cons-a1_F	GTGGGGACCTGTTCAGTGAC	20	60.4
1283	P16_5hom-cons-a1_R	AATGCAAACTGTGCAAGTCG	20	59.91
1284	P16_5hom-cons-b1_F	GCCAAAATGAGTTCTGGGAAT	21	60.31
1285	P16_5hom-cons-b1_R	GTCTAGGGGCTGATCAGGAA	20	59.24
1286	P16_5hom-cons-c1_F	CTGGGGCTAGAACAGGGAAT	20	60.46
1287	P16_5hom-cons-c1_R	GCACTCCAAGGGGAGAGAAT	20	60.6
1288	P16_5hom-cons-c2_F	CGGAGTGTCAGAGCAGTGAA	20	60.18
1289	P16_5hom-cons-c2_R	GAGTGAGGTCCTCCAGGTCA	20	60.24
1290	P16_5hom-cons-d1_F	GGGTTCCTCCAGTCATGGTA	20	59.78
1291	P16_5hom-cons-d1_R	TGTGGGCATGTAATGGATGT	20	59.65
1292	P16_3hom-cons-a1_F	AAACGAATGTCACTTTCAAGAGC	23	59.82
1293	P16_3hom-cons-a1_R	AGGCTGGGATAGCCCTAAAC	20	59.58
1324	hb-Actin-Cre_F (RA47)	GCA GAA CCT GAA GAT GTT CGC	21	66
1325	hb-Actin-Cre_R (RA48)	ACA CCA GAG ACG GAA ATC CAT C	22	66
	attB1-forward-5'	GGGG ACA AGT TTG TAC AAA AAA GCA GGC TNN--template specific seq.		
	attB2-forward-5'	GGGG ACA GCT TTC TTG TAC AAA GTG GNN--template specific seq.		
	attB4-forward-5'	GGGG ACA ACT TTG TAT AGA AAA GTT GNN--template specific seq.		
	attB1-reverse-5'	GGGG AC TGC TTT TTT GTA CAA ACT TGN-template specific seq.		
	attB2-reverse-5'	GGGG AC CAC TTT GTA CAA GAA AGC TGG GTN-template specific seq.		
	attB3-reverse-5'	GGGG AC AAC TTT GTA TAA TAA AGT TGN-template specific seq.		
	hsv-tkF	GTACCGAGCTCGAATTCCTA		
	pUC_R	GTGGAGCTCCAGCTTTTGTT		
	pUC_F	CTAGTCCACTGGCACAGCCTAC		
	hsv-tk_R	ACACTCCAGAGAGGGCAGAG		