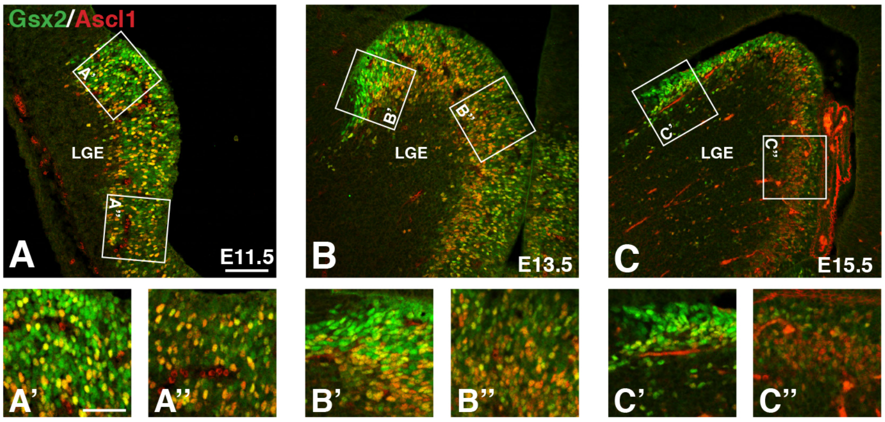
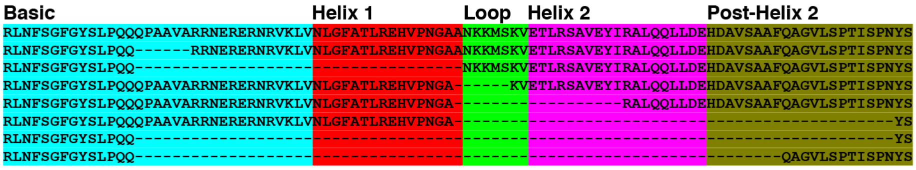


Figure S1. Co-expression of Gsx2 and Ascl1 in LGE progenitors over early neurogenic timepoints. Double immunohistochemistry for Gsx2 and Ascl1 in tissue sections from E11.5 (A), E13.5 (B) and E15.5 (C) mouse telencephalon. Boxed areas represent the VZ regions quantified for the dLGE (A’, B’, C’) and the vLGE (A”, B”, C”). Boxes have been rotated so that the apical surface is at the top of all images in A’, A”, B’, B”, C’ and C”. Results from the quantification are presented in Fig. 1H, I. Scale bars in A-C=100μm; A’-C”=50μm.

Ascl1 bHLH



```
RLNFSGFGYSLPQQQPAAVARRNERERNRVKLVNLGFATLREHVPNGAANKKMSKVETLRSAVEYIRALQQLLDEHDAVSAAFQAGVLSPTISPNYS
RLNFSGFGYSLPQQ------RRNERERNRVKLVNLGFATLREHVPNGAANKKMSKVETLRSAVEYIRALQQLLDEHDAVSAAFQAGVLSPTISPNYS
RLNFSGFGYSLPQQ-----------------------------------NKKMSKVETLRSAVEYIRALQQLLDEHDAVSAAFQAGVLSPTISPNYS
RLNFSGFGYSLPQQQPAAVARRNERERNRVKLVNLGFATLREHVPNGA------KVETLRSAVEYIRALQQLLDEHDAVSAAFQAGVLSPTISPNYS
RLNFSGFGYSLPQQQPAAVARRNERERNRVKLVNLGFATLREHVPNGA-------------------RALQQLLDEHDAVSAAFQAGVLSPTISPNYS
RLNFSGFGYSLPQQQPAAVARRNERERNRVKLVNLGFATLREHVPNGA-------------------------------------------------YS
RLNFSGFGYSLPQQ-------------------------------------------------------------------------------YS
RLNFSGFGYSLPQQ------------------------------------------------------------------QAGVLSPTISPNYS
```

Figure S2. Deletion constructs in the Ascl1 bHLH. Amino acid sequence of the bHLH domain in Ascl1 and subsequent deletion mutations tested in the yeast 2-hybrid interaction assays with Gsx2 shown in Fig. 5F.

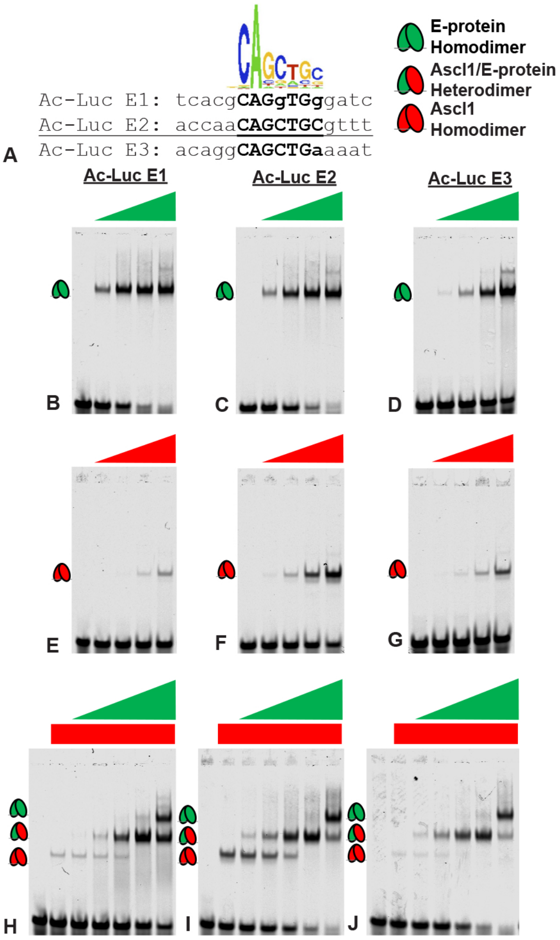


Figure S3. Ascl1 homodimers and heterodimers bind the 3 E-boxes from the Ac-luciferase reporter with varying affinities. A) Sequence logo for Ascl1 binding determined via ChIP on CHIP assay (Castro et al., 2011), and the E-box sequences from the Ac-Luciferase reporter used as probes in EMSAs. Matches to perfect Ascl1 binding site are capitalized. B-D) Binding of increasing levels of E-protein as E47-E47 homodimers to E-boxes from Ac-Luc B) E1, C) E2, and D) E3. E-G) Binding of increasing levels of Ascl1 as Ascl1-Ascl1 homodimers to E-boxes from Ac-Luc E) E1, F) E2, and G) E3. H-J) Binding of Ascl1 added at constant levels with increasing levels of E-protein as Ascl1-Ascl1 homodimers, Ascl1-E47 heterodimers, and E47-E47 homodimers to E-boxes from Ac-Luc H) E1, I) E2, and J) E3.

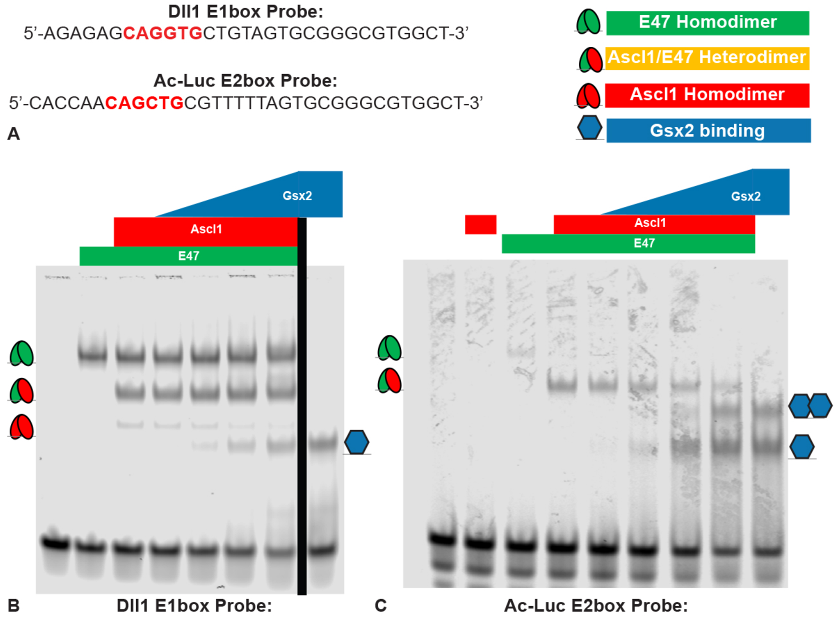


Figure S4. Wild type Gsx2 protein interferes with Ascl1 homodimer and heterodimer binding to E-box DNA sequences. A) Sequences of the E-box DNA probes used in EMSAs shown in B-C, and cartoons indicating the different complexes observed in EMSAs. B) Ascl1 (5 pmoles) and E-protein (1.25 pmoles) were added in each lane marked by red and green bars respectively with increasing levels of Gsx2 protein from 0 to 30 pmoles indicated by the blue triangle. Each lane contained 0.64 pmoles of the Dll1 E1box DNA probe. Note, the black line separates the Gsx2 only lane at right that was run on the same gel but moved adjacent to the other lanes for comparative purposes. C) Ascl1 (0.156 pmoles) and E-protein (0.078 pmoles) were added in each lane marked by red and green bars respectively with increasing levels of Gsx2 protein from 0 to 40 pmoles. Each lane contained 0.04 pmoles of the E2box DNA probe from Ac-Luc.

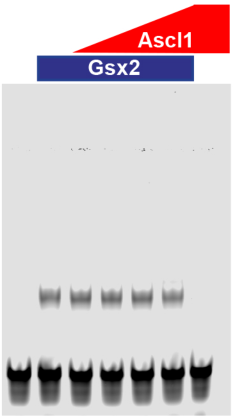


Figure S5. Ascl1 has no effect on Gsx2 DNA binding Protein binding microarray data for mouse Gsx2 was used to generate a predicted, high affinity Gsx2 binding site (see Berger et al., 2008). EMSA shows binding of a constant amount of Gsx2 to a probe containing this high affinity site with increasing levels of Ascl1 added in each lane.

Table S1. Genes enriched in distinct cell clusters in the developing mouse ventral telencephalon at E12.5.

Click here to download Table S1

Table S2. Genes enriched in Gsx2+ and/or Ascl1+ LGE progenitors.

Gene	p_val	avg_logFC	pct.1	pct.2	p_val_adj	Cluster
Gsx2	0	0.86161	1.000	0.107	0	Gsx2+Ascl1+
Ascl1	2.19E-191	0.65296	1.000	0.566	4.08E-187	Gsx2+Ascl1+
Gadd45g	2.70E-71	0.56279	0.599	0.343	5.02E-67	Gsx2+Ascl1+
Dlx1	2.39E-63	0.45537	0.732	0.502	4.44E-59	Gsx2+Ascl1+
Dlx2	2.78E-74	0.43268	0.832	0.604	5.18E-70	Gsx2+Ascl1+
Hes6	3.62E-72	0.41834	0.818	0.588	6.73E-68	Gsx2+Ascl1+
Sox4	1.28E-69	0.36637	0.995	0.964	2.38E-65	Gsx2+Ascl1+
Dll1	7.80E-65	0.29207	0.474	0.233	1.45E-60	Gsx2+Ascl1+
Helt	2.57E-47	0.29037	0.242	0.092	4.78E-43	Gsx2+Ascl1+
Dll3	3.62E-48	0.27996	0.424	0.224	6.72E-44	Gsx2+Ascl1+
Dleu7	2.73E-35	0.25662	0.406	0.237	5.07E-31	Gsx2+Ascl1+
Dlx6os1	5.12E-38	0.45336	0.366	0.217	9.51E-34	Ascl1+
Ascl1	3.01E-189	0.44689	1.000	0.473	5.60E-185	Ascl1+
Abracl	2.46E-39	0.43215	0.873	0.810	4.57E-35	Ascl1+
Sp9	1.30E-38	0.41413	0.419	0.256	2.42E-34	Ascl1+
Tubb3	2.20E-42	0.38288	0.835	0.748	4.09E-38	Ascl1+
Sox11	1.01E-60	0.36496	0.967	0.935	1.88E-56	Ascl1+
Cited2	8.90E-50	0.34948	0.715	0.553	1.65E-45	Ascl1+
Dlx5	1.75E-35	0.34670	0.363	0.211	3.25E-31	Ascl1+
Insm1	5.37E-57	0.33358	0.577	0.361	9.97E-53	Ascl1+
Gad2	1.19E-32	0.31832	0.395	0.252	2.20E-28	Ascl1+
Igfbpl1	4.42E-27	0.31543	0.346	0.224	8.22E-23	Ascl1+
Mpped2	3.16E-16	0.31180	0.610	0.542	5.87E-12	Ascl1+
Cd24a	1.24E-33	0.29979	0.712	0.603	2.30E-29	Ascl1+
Pak3	2.56E-30	0.29945	0.676	0.562	4.77E-26	Ascl1+
Nrxn3	1.15E-30	0.29811	0.393	0.257	2.13E-26	Ascl1+
Dlx1	9.60E-36	0.28847	0.659	0.503	1.78E-31	Ascl1+
Arx	2.89E-23	0.28840	0.541	0.432	5.37E-19	Ascl1+
Gadd45g	4.32E-29	0.28682	0.508	0.351	8.03E-25	Ascl1+
Dpysl3	8.96E-21	0.28124	0.569	0.477	1.67E-16	Ascl1+
Ccnd2	7.31E-32	0.27983	0.942	0.907	1.36E-27	Ascl1+
Elavl3	7.52E-38	0.27416	0.757	0.643	1.40E-33	Ascl1+
Dlx2	2.91E-32	0.27287	0.736	0.622	5.42E-28	Ascl1+
Mfng	2.87E-47	0.26772	0.580	0.389	5.34E-43	Ascl1+
Hes6	2.76E-34	0.26415	0.731	0.599	5.14E-30	Ascl1+
Sox4	5.11E-45	0.25396	0.989	0.962	9.50E-41	Ascl1+
Top2b	7.62E-38	0.25070	0.869	0.797	1.42E-33	Ascl1+
Fabp7	4.43E-72	1.05892	0.959	0.831	8.23E-68	Gsx2+
Ptn	1.21E-68	0.76159	0.949	0.744	2.24E-64	Gsx2+
Mt3	2.35E-42	0.61585	0.888	0.661	4.36E-38	Gsx2+
Mest	1.18E-37	0.58710	0.788	0.570	2.19E-33	Gsx2+
Slc1a3	1.65E-54	0.52919	0.781	0.420	3.07E-50	Gsx2+
Gsx2	2.29E-147	0.51121	1.000	0.304	4.25E-143	Gsx2+
Ttyh1	5.18E-51	0.50879	0.808	0.491	9.63E-47	Gsx2+
Rgcc	3.57E-44	0.50487	0.835	0.535	6.64E-40	Gsx2+
Epha5	1.56E-31	0.47750	0.803	0.603	2.90E-27	Gsx2+
Dbi	2.31E-44	0.45934	0.993	0.976	4.30E-40	Gsx2+
Ptprz1	1.02E-40	0.45419	0.976	0.846	1.89E-36	Gsx2+
Hmga2	8.59E-45	0.45133	0.781	0.476	1.60E-40	Gsx2+
Pmm1	1.17E-40	0.44712	0.827	0.558	2.17E-36	Gsx2+

Rbp1	1.36E-37	0.44106	0.978	0.916	2.53E-33	Gsx2+
Ppp1r1a	9.26E-49	0.43181	0.603	0.276	1.72E-44	Gsx2+
Fgfbp3	1.24E-42	0.42843	0.720	0.395	2.30E-38	Gsx2+
Fam181b	7.84E-38	0.42655	0.732	0.459	1.46E-33	Gsx2+
Ntrk2	2.51E-27	0.40735	0.538	0.295	4.66E-23	Gsx2+
Ddah1	1.43E-40	0.40346	0.951	0.817	2.66E-36	Gsx2+
Sparc	3.16E-37	0.40186	0.757	0.452	5.87E-33	Gsx2+
Id4	1.77E-38	0.39535	0.835	0.537	3.30E-34	Gsx2+
Vim	1.63E-46	0.39429	0.993	0.985	3.03E-42	Gsx2+
Rorb	3.19E-39	0.38459	0.684	0.388	5.94E-35	Gsx2+
Ptx3	2.12E-35	0.38445	0.616	0.323	3.94E-31	Gsx2+
Pea15a	1.75E-36	0.38231	0.895	0.689	3.26E-32	Gsx2+
Arhgap5	1.46E-32	0.37832	0.864	0.708	2.72E-28	Gsx2+
Phgdh	1.09E-34	0.37781	0.951	0.825	2.02E-30	Gsx2+
Asrgl1	4.33E-31	0.37290	0.710	0.471	8.04E-27	Gsx2+
Lix1	6.80E-20	0.35816	0.757	0.609	1.26E-15	Gsx2+
Sfrp1	2.95E-34	0.34454	0.959	0.806	5.48E-30	Gsx2+
Lxn	1.96E-23	0.33412	0.577	0.342	3.64E-19	Gsx2+
Sall3	4.31E-29	0.33288	0.706	0.443	8.02E-25	Gsx2+
Tubb2a	3.65E-18	0.33287	0.766	0.625	6.78E-14	Gsx2+
Psat1	1.92E-25	0.31479	0.791	0.576	3.57E-21	Gsx2+
Ldha	6.84E-15	0.30961	0.922	0.836	1.27E-10	Gsx2+
Pkdcc	1.77E-23	0.30786	0.594	0.348	3.30E-19	Gsx2+
Cpe	5.06E-22	0.30004	0.822	0.631	9.41E-18	Gsx2+
Cst3	5.42E-25	0.29959	0.946	0.876	1.01E-20	Gsx2+
Slc12a2	1.06E-16	0.29949	0.662	0.463	1.97E-12	Gsx2+
Jun	9.92E-23	0.29926	0.910	0.738	1.84E-18	Gsx2+
Hspa5	6.22E-25	0.29834	0.949	0.852	1.16E-20	Gsx2+
Calr	1.15E-22	0.29660	0.951	0.867	2.13E-18	Gsx2+
Hs3st1	2.41E-58	0.29633	0.282	0.061	4.49E-54	Gsx2+
Aldoa	1.09E-18	0.29528	0.910	0.831	2.03E-14	Gsx2+
Stk39	3.70E-25	0.28882	0.618	0.386	6.88E-21	Gsx2+
Oat	9.21E-23	0.28616	0.835	0.662	1.71E-18	Gsx2+
Kbtbd11	2.70E-26	0.28598	0.504	0.263	5.02E-22	Gsx2+
Khdrbs3	9.30E-10	0.28582	0.827	0.758	1.73E-05	Gsx2+
Ldhb	2.70E-22	0.28575	0.976	0.916	5.03E-18	Gsx2+
Hes1	1.02E-21	0.28019	0.664	0.420	1.89E-17	Gsx2+
Gpm6b	4.18E-22	0.27427	0.888	0.769	7.77E-18	Gsx2+
Ednrb	6.96E-24	0.27208	0.735	0.488	1.29E-19	Gsx2+
Ntm	1.28E-20	0.26880	0.460	0.259	2.38E-16	Gsx2+
Plpp3	1.65E-22	0.26862	0.837	0.619	3.06E-18	Gsx2+
Tpi1	3.15E-18	0.26843	0.910	0.802	5.86E-14	Gsx2+
Pcdh10	1.35E-20	0.26816	0.655	0.428	2.52E-16	Gsx2+
Creb5	4.09E-22	0.26106	0.596	0.357	7.61E-18	Gsx2+
Cspg5	1.46E-20	0.26000	0.637	0.401	2.71E-16	Gsx2+
Hey1	5.39E-22	0.25648	0.406	0.211	1.00E-17	Gsx2+
Sox9	2.79E-19	0.25236	0.808	0.617	5.19E-15	Gsx2+
Fabp7	5.24E-66	0.63531	0.904	0.822	9.73E-62	double negative
Id4	7.58E-114	0.61691	0.750	0.501	1.41E-109	double negative
Mest	5.17E-64	0.56229	0.725	0.544	9.62E-60	double negative
Ptn	2.83E-62	0.50068	0.866	0.727	5.26E-58	double negative
Mt3	5.71E-48	0.44937	0.775	0.648	1.06E-43	double negative
Fgfbp3	1.24E-55	0.44364	0.582	0.370	2.30E-51	double negative

Sparc	7.97E-55	0.42793	0.639	0.425	1.48E-50	double negative
Ptx3	1.90E-57	0.39797	0.516	0.293	3.53E-53	double negative
Pmm1	1.91E-62	0.38890	0.721	0.535	3.56E-58	double negative
Slc1a3	7.63E-47	0.38272	0.596	0.402	1.42E-42	double negative
Ttyh1	6.54E-51	0.37876	0.676	0.465	1.22E-46	double negative
Hmga2	1.24E-51	0.37028	0.646	0.454	2.31E-47	double negative
Calr	9.75E-59	0.36151	0.905	0.864	1.81E-54	double negative
Hspa5	2.42E-46	0.34914	0.884	0.852	4.50E-42	double negative
Rgcc	3.44E-40	0.34766	0.696	0.515	6.40E-36	double negative
Cntnap2	8.85E-54	0.34079	0.343	0.156	1.65E-49	double negative
Pea15a	1.10E-50	0.33050	0.806	0.673	2.04E-46	double negative
Hes1	8.15E-38	0.32452	0.580	0.395	1.51E-33	double negative
Vim	4.60E-59	0.32189	0.982	0.987	8.55E-55	double negative
Fam181b	3.29E-27	0.31553	0.565	0.453	6.12E-23	double negative
Ptprz1	1.69E-36	0.31268	0.877	0.849	3.14E-32	double negative
Gm42418	1.31E-20	0.30261	0.994	1.000	2.44E-16	double negative
Pkdcc	6.18E-39	0.29685	0.511	0.323	1.15E-34	double negative
Pdia6	3.29E-36	0.29599	0.874	0.866	6.12E-32	double negative
Jun	1.62E-25	0.28298	0.804	0.735	3.01E-21	double negative
Sfrp1	2.04E-34	0.27544	0.867	0.802	3.80E-30	double negative
Ppp1r1a	1.70E-30	0.26908	0.421	0.264	3.15E-26	double negative
Ddah1	2.16E-34	0.26881	0.858	0.818	4.02E-30	double negative
Tubb2a	1.44E-20	0.26877	0.695	0.617	2.68E-16	double negative
Psat1	9.21E-30	0.26121	0.686	0.564	1.71E-25	double negative
Scrn1	5.66E-28	0.25714	0.470	0.330	1.05E-23	double negative
Pdia3	1.72E-28	0.25566	0.792	0.743	3.19E-24	double negative

Table S3. Plasmids generated for the experiments

Insert	Amino Acids	Source	Destination Vector	Integration site	Method
Gsx2	1-305 (Full length)	pBS SK- mouse clone	pGBKT7	EcoR1-BamH1	PCR
Gsx1	1-261 (Full length)	pBS SK- mouse clone	pGBKT7	EcoR1-BamH1	PCR
Ascl1	1-231 (Full length)	Embryonic mouse telencephalon RNA	pGADT7	EcoR1-BamH1	RT-PCR
Olig2	1-323 (Full length)	pCAGGS	pGADT7	EcoR1-BamH1	PCR
Ascl1 NT	1-131	pGADT7 FL clone	pGADT7	EcoR1-BamH1	PCR
Ascl1 bHLH	97-193	pGADT7 FL clone	pGADT7	EcoR1-BamH1	PCR
Ascl1 CT	193-231	pGADT7 FL clone	pGADT7	EcoR1-BamH1	PCR
Ascl1 Δ111-116	Δ 111-116	pGADT7 FL clone	pGADT7	EcoR1-BamH1	PCR
Ascl1 Δ 111-145	Δ 111-145	pGADT7 FL clone	pGADT7	EcoR1-BamH1	PCR

Ascl1 Δ 145-150	Δ 145-150	pGADT7 FL clone	pGADT7	EcoR1-BamH1	PCR
Ascl1 Δ 145-162	Δ 145-162	pGADT7 FL clone	pGADT7	EcoR1-BamH1	PCR
Ascl1 Δ 145-191	Δ 145-191	pGADT7 FL clone	pGADT7	EcoR1-BamH1	PCR
Ascl1 Δ 111-191	Δ 111-191	pGADT7 FL clone	pGADT7	EcoR1-BamH1	PCR
Ascl1 Δ 111-179	Δ 111-179	pGADT7 FL clone	pGADT7	EcoR1-BamH1	PCR
Gsx2 Y3H MCS1	1-305 (Full length)	pCDNA6 FL clone	pBridge	EcoR1-BamH1	PCR
TCF3 Y3H MCS2	1-653 (isoform-1)	Mouse brain RNA	pBridge	Not1-BglII	RT-PCR
Gsx2 Y3H MCS2	1-305 (Full length)	pCDNA6 FL clone	pBridge	Not1-BglII	PCR
Tcf3 Y3H MCS1	1-653 (isoform-1)	Mouse brain RNA	pBridge	EcoR1-BamH1	RT-PCR

Tcf3 Y3H MCS1, Gsx2-MCS2	1-653 (isoform-1)	Mouse brain RNA	pBridge with Gsx2 Y3H MCS2	EcoR1-BamH1	PCR
Tcf3 Y3H MCS1	1-653 (isoform-1)	Mouse brain RNA	pBridge with Gsx2 Y3H MCS2	EcoR1-BamH1	PCR
Ascl1	1-231 (Full length)	pGADT7 clone	pAC5.1	EcoRI-XhoI	Subcloning
Gsx2	1-305 (Full length)	pCDNA6 FL clone	pAC5.1	XhoI-XbaI	Subcloning
Gsx2	N253A 1-305 (Full length)	pGBKT7 clone	pAC5.1	EcoRV-XhoI	Subcloning
Gsx2	167-305	pGBKT7 clone	pET14b (modified)	BamH1-NotI	PCR
Gsx2	N253A 167-305	pGBKT7 N253A clone	pET14b (modified)	BamH1-NotI	PCR
Ascl1	115-231	pGADT7 clone	pET14b (modified)	NdeI-KpnI	PCR

Ascl1	1-231 (Full length)	pGADT7 FL clone	pCDNA6	EcoRI-XhoI	PCR
Gsx2	1-305 (Full length)	pGBKT7 clone	pCDNA6	EcoRV-XhoI	subcloning
Gsx2	N253A 1-305 (Full length)	pGBKT7 clone	pCDNA6	EcoRV-XhoI	subcloning
6xE2box Luciferase		Synthesized oligonucleotides from IDT	Modified pGL3basic	KpnI-BamHI	Cloning of annealed oligonucleotides

Table S4. PCR primers used in cloning

Construct (amino acids)	destination vector	Forward primer	Reverse primer
Ascl1(1-231)	pAc5.1	CAGTGTGGTGGAATTCATGGAGAGCTCTGGCAAGAT	GCCCTCTAGACTCGAGTCAGAACCAGTTGGTAAAGTCCA
Gsx2N253A (1-305)	pGBKT7	CTGAAACCAGATTTTTCACCTGC	AAAATCTGGTTTCAGGCCCGTCGCGTGAAGCACAAGA
Gsx2 (167-305)	pET14b	GTACGGATCCGCAGCACCACGCACCTGTC	GTACGCGGCCGCTTACAAGGGGGAAATCTCCTTGTC
Gsx2N253A (167-305)	pET14b	GTACGGATCCGCAGCACCACGCACCTGTC	GTACGCGGCCGCTTACAAGGGGGAAATCTCCTTGTC
Ascl1(115-231)	pET14b	GTACATATGGTGGCGCGCCGCAACGAGCGC	GTAGGTACCTCAGAACCAGTTGGTAAAGTCCAG
E47(430-648)	pET14b	GTACATATGTCACTGGGCGGGCGGCATGCC	GTAGGTACCTCACAGGTGCCCGGCTGGGTTG
Gsx2(1-305)	pGBKT7	CATGGAGGCCGAATTCCAGGACAAGCCATCCATCGAC	GCAGGTCGACGGATCCCAGAAGGAGCCTCTGCCTTT
Gsx1(1-261)	pGBKT7	CATGGAGGCCGAATTCATGCCGCGCTCCTT	GCAGGTCGACGGATCCCTACGGAGTGACTG
Ascl1(1-231)	pGADT7	GGAGGCCAGTGAATTCATGGAGAGCTCTGGCAAGAT	CGAGCTCGATGGATCCTCAGAACCAGTTGGTAAAGTCCA
Olig2(1-323)	pGADT7	GGAGGCCAGTGAATTCATGGACTCGGACGCCAGCCT	CGAGCTCGATGGATCCTCACTTGGCGTCGGAGGTG
Ascl1 NT (1-131)	pGADT7	GGAGGCCAGTGAATTCATGGAGAGCTCTGGCAAGAT	CGAGCTCGATGGATCCCAGGTTGACCAACTTGAC
Ascl1 bHLH (97-193)	pGADT7	GGAGGCCAGTGAATTCGCTCAACTTCAGCGGCTT	CGAGCTCGATGGATCCGGAGTAGTTGGGGGAGAT
Ascl1 CT (193-231)	pGADT7	GGAGGCCAGTGAATTCTCCAACGACTTGAACTCT	CGAGCTCGATGGATCCTCAGAACCAGTTGGTAAAGTCCA
Ascl1 Δ111-116	pGADT7	GGCTACAGCCTGCCAGTGGCGCGCCGCAACGAG	TGGCAGGCTGTAGCCGAAGCCGCTGAAGTTGAG
Ascl1 Δ111-145	pGADT7	GGCTACAGCCTGCCAGGCGCGGCCAACAAGAAG	TGGCAGGCTGTAGCCGAAGCCGCTGAAGTTGAG
Ascl1 Δ145-150	pGADT7	GAGCATGTCCCCAACATGAGCAAGGTGGAGACG	GTTGGGGACATGCTCCCGGAGGGTGGCAAAACC
Ascl1 Δ145-162	pGADT7	GAGCATGTCCCCAACGAGTACATCCGCGCGCTG	GTTGGGGACATGCTCCCGGAGGGTGGCAAAACC
Ascl1 Δ145-191	pGADT7	GAGCATGTCCCCAACTCCCCCAACTACTCCAAC	GTTGGGGACATGCTCCCGGAGGGTGGCAAAACC
Ascl1 Δ111-191	pGADT7	GCTACAGCCTGCCAGCTGCCTTTCAGGCGGGC	GTTGGGGACATGCTCCCGGAGGGTGGCAAAACC
Ascl1 Δ111-179	pGADT7	GCTACAGCCTGCCAGCTGCCTTTCAGGCGGGC	TGGCAGGCTGTAGCCGAAGCCGCTGAAGTTGAG
Gsx2 (1-305)	pBridge MCS1	TGTATCGCCGGAATTCAGGACAAGCCATCCATCGAC	GCAGGTCGACGGATCCAGAAGGAGCCTCTGCCTTT
TCF3 (1-653)	pBridge MCS2	AGAAAGGTGGCGGCCGCGAACCAGCCGCAGAGGAT	CGGGCTAATGCGGCCCGGAGGCATACCTTTCACAT
Gsx2 (1-305)	pBridge MCS2	AGAAAGGTGGCGGCCCAGGACAAGCCATCCATCGAC	CGGGCTAATGCGGCCCAGAAGGAGCCTCTGCCTTT
Tcf3 (1-653)	pBridge MCS1	TGTATCGCCGGAATTGAACCAGCCGCAGAGGAT	GCAGGTCGACGGATCCGGAGGCATACCTTTCACAT
6xE2box Luciferase	pGL3basic modified	AACAGCTGCGTTTACCAACAGCTGCGTTTACCAACAGCTGCGGTACCGTAG	GATCCTACGGTACCGCAGCTGTTGGTAAACGCAGCTGTTGGTAAACGCAGCTGTTGTAC

Table S5. Contents of EMSAs in Figure 6

Lane	Ascl1 (pmoles)	Tcf3 (pmoles)	Gsx2 N253A (pmoles)	probe (pmoles)
1	0	0	0	0.04
2	2.5	0	0	0.04
3	2.5	0	20	0.04
4	2.5	0	40	0.04
5	2.5	0	80	0.04
6	0	0	0	0.04
7	2.5	0	0	0.04
8	0	0.078125	0	0.04
9	2.5	0.078125	0	0.04
10	2.5	0.078125	10	0.04
11	2.5	0.078125	20	0.04
12	2.5	0.078125	40	0.04
13	2.5	0.078125	80	0.04
14	0	0	0	0.04
15	0	0.3125	0	0.04
16	0.15625	0.3125	0	0.04
17	0.15625	0.3125	10	0.04
18	0.15625	0.3125	20	0.04
19	0.15625	0.3125	40	0.04
20	0.15625	0.3125	80	0.04
21	0	0	80	0.04
22	0	0	0	0.04

23	0.026	0	0	0.04
24	0	0.3125	0	0.04
25	0.026	0.3125	0	0.04
26	0.026	0.3125	20	0.04
27	0.026	0.3125	40	0.04
28	0.026	0.3125	80	0.04
29	0	0	0	0.04
30	0	0.3125	0	0.04
31	0	0.3125	20	0.04
32	0	0.3125	40	0.04
33	0	0.3125	80	0.04