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Supplemental Information

MyT1 Counteracts the Neural Progenitor Program to Promote Vertebrate Neurogenesis

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MyT1 expression in Ascl1 LoF and GoF microarrays				
		Linear FC	P value	Reference
Ascl1 LoF	NS cells	-5.00	4.56×10^{-2}	Table S7 on Castro et al., 2011
	Telencephalon	-2.99	1.46×10^{-4}	Table S2 on Castro et al., 2011
Ascl1 GoF	NS cells	7.08	5.76×10^{-5}	Table S1 on Raposo et al., 2015
	Telencephalon	1.47	2.28×10^{-2}	Gohlke et al., 2008 Table S3 on Castro et al., 2011

Figure S1. MyT1 expression data from Ascl1 GoF and LoF experiments in mouse embryonic telencephalon and in cultured NS cells (related to Figure 1)
Ascl1 LoF in telencephalon, DNA arrays on RNA extracted from E13.5 ventral telencephalon of wild-type and Ascl1 knock-out mice (Castro et al. 2011). Ascl1 GoF in telencephalon, DNA arrays on RNA extracted from In utero electroporated E10.5 mouse telencephalon with control or Ascl1-expressing vectors (Castro et al., 2011; Gohlke et al., 2008). Ascl1 LoF in NS cells, DNA arrays on RNA extracted from NS5 cells electroporated with control or bHLH domain of Ascl1 fused to EnR domain expressing vectors (Castro et al., 2011). Ascl1 GoF in NS cells, DNA arrays on NS5 Ascl1-ERT2 cells before and after Tam induction (Raposo et al., 2015). FC, Fold Change.

Figure S2

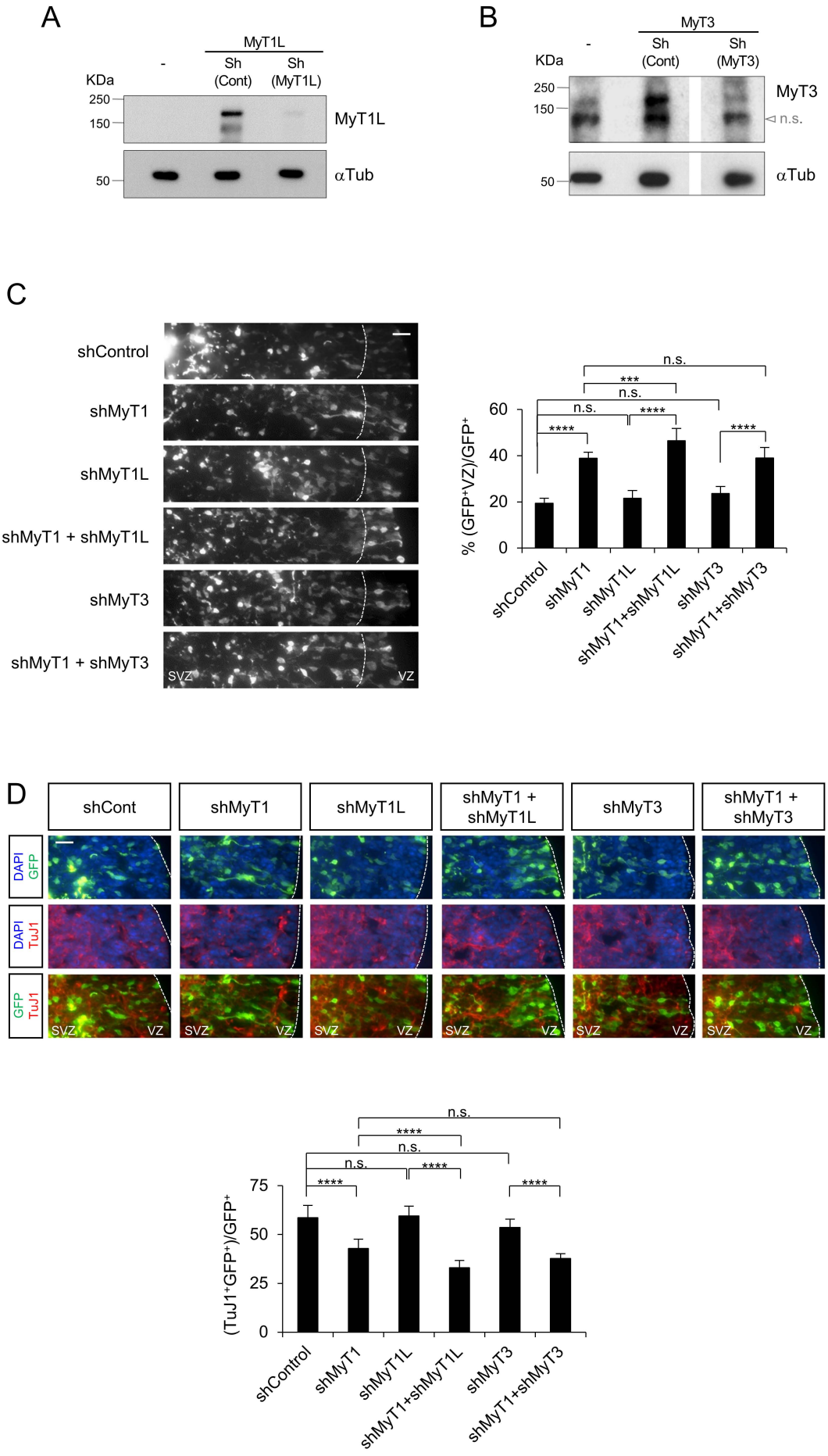
A
MyT1L
-
Sh (Cont)
Sh (MyT1L)
KDa
250
150
50
MyT1L
αTub
B
MyT3
-
Sh (Cont)
Sh (MyT3)
KDa
250
150
50
MyT3
n.s.
αTub
C
shControl
shMyT1
shMyT1L
shMyT1 + shMyT1L
shMyT3
shMyT1 + shMyT3
SVZ
VZ
% (GFP+VZ)/GFP+
n.s.
***
n.s.
n.s.
****
****
****
60
40
20
0
shControl
shMyT1
shMyT1L
shMyT1+shMyT1L
shMyT3
shMyT1+shMyT3
D
shCont
shMyT1
shMyT1L
shMyT1 + shMyT1L
shMyT3
shMyT1 + shMyT3
DAPI GFP
DAPI TuJ1
GFP TuJ1
SVZ
VZ
(TuJ1+GFP+)/GFP+
n.s.
****
n.s.
n.s.
****
****
****
75
50
25
0
shControl
shMyT1
shMyT1L
shMyT1+shMyT1L
shMyT3
shMyT1+shMyT3

Figure S2. Combined knock-down of MyT1 family members by in utero electroporation in ventral telencephalon (related to Figure 2)
Analysis by Western blot of MyT1L **(A)** or MyT3 **(B)** protein levels in P19 cells co-transfected with scramble shRNA (shControl), MyT1L ShRNA (shMyT1L) **(A)** or MyT3 ShRNA (shMyT3) **(B)**. α-tubulin was used as loading control. n.s.: non-specific band. **(C-D)** In utero electroporation of control (shControl), MyT1 (shMyT1), MyT1L (shMyT1L) and/or MyT3 (shMyT3) ShRNA vectors in E12.5 mouse ventral telencephalon. Immunofluorescence analysis on coronal section of the telencephalon for GFP (green or grey) and TuJ1 (red) 2 days post electroporation (E14.5). Cell nuclei are labeled with DAPI (blue). Scale bars, 20μm and 50μm (C and D). VZ: ventricular zone, SVZ: subventricular zone. Histograms represent the quantification of cell migration based on the fraction of GFP^+ cells that are retained in the VZ (GFP^+VZ/GFP^+) **(C)** and of neuronal differentiation based on the fraction of GFP^+ cells that express TuJ1 (($TuJ1^+GFP^+$)/GFP^+) **(D).** Mean ± SD. n.s. for P>0.05,* for P<0.05, ** for P<0.01, *** for P<0.001, **** for P<0.0001 according to one-way ANOVA with Bonferroni correction for multiple testing.

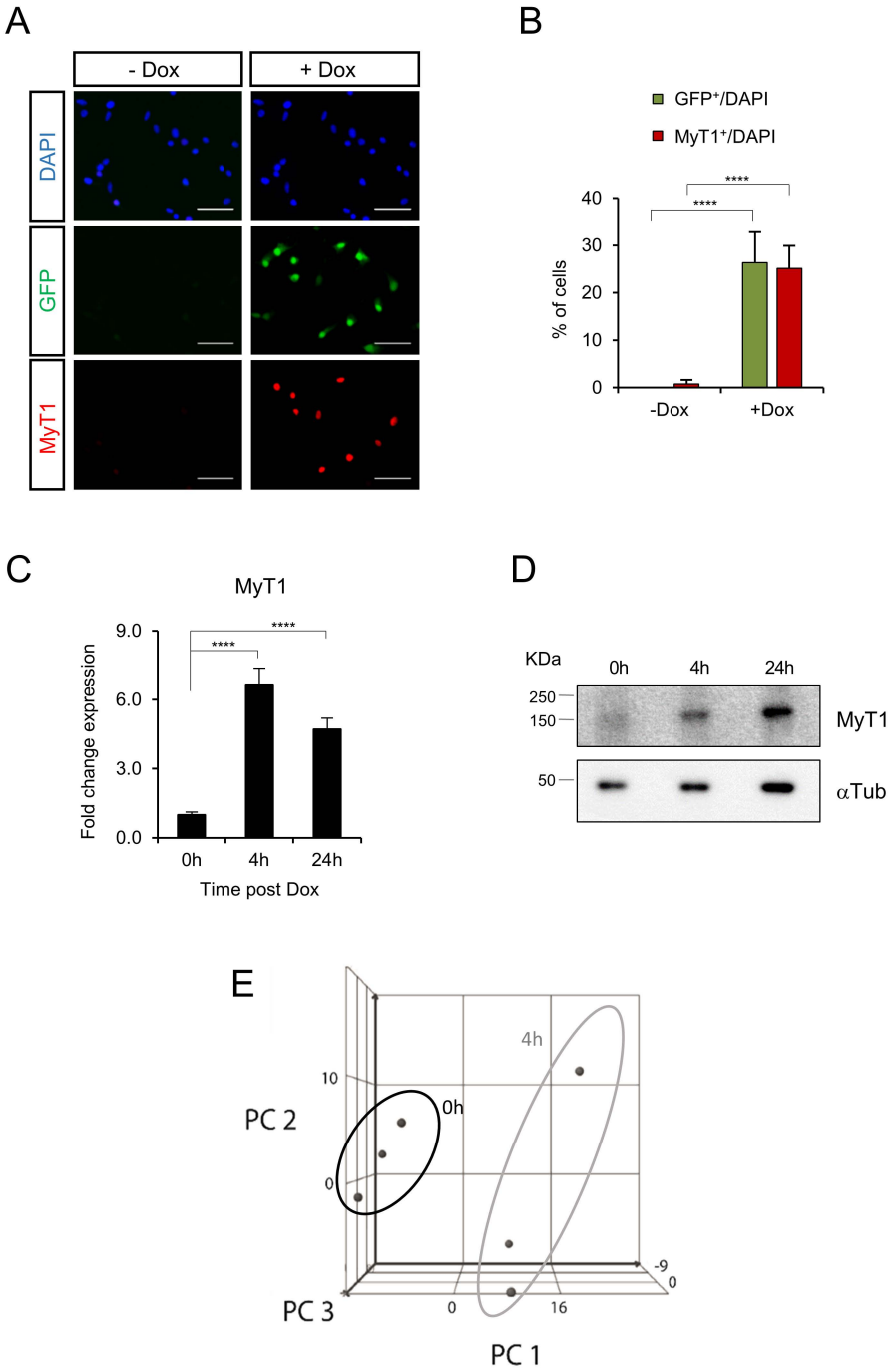

Figure S3. MyT1 GoF in NS5 cells using a Dox-inducible system (related to Figure 3) **(A)** Immunocytochemistry analysis for MyT1 (red) and GFP (green) before (-Dox) and 24h post Dox (+Dox) addition to NS5 MyT1-HA inducible cell cultures. Nuclei were stained with DAPI (blue). Scale bar, 40µm. **(B)** Histogram represents the percentage of GFP$^+$ cells (GFP$^+$/DAPI) and of MyT1 overexpressing cells (MyT1$^+$/DAPI). Mean ± SD for at least 1000 cells on each condition. **** for P<0.0001 according to Student's t-test. **(C)** Analysis of MyT1 RNA expression by qPCR. Mean ± SD of triplicate assays. **** for P<0.0001 according to one-way ANOVA test with Bonferroni correction for multiple testing. **(D)** Analysis of MyT1 protein levels by Western blot post-Dox induction. α-tubulin was used as loading control. **(E)** Principal component analysis (PCA) of technical replicates of MyT1 GoF gene expression DNA arrays 0h and 4h post Dox.

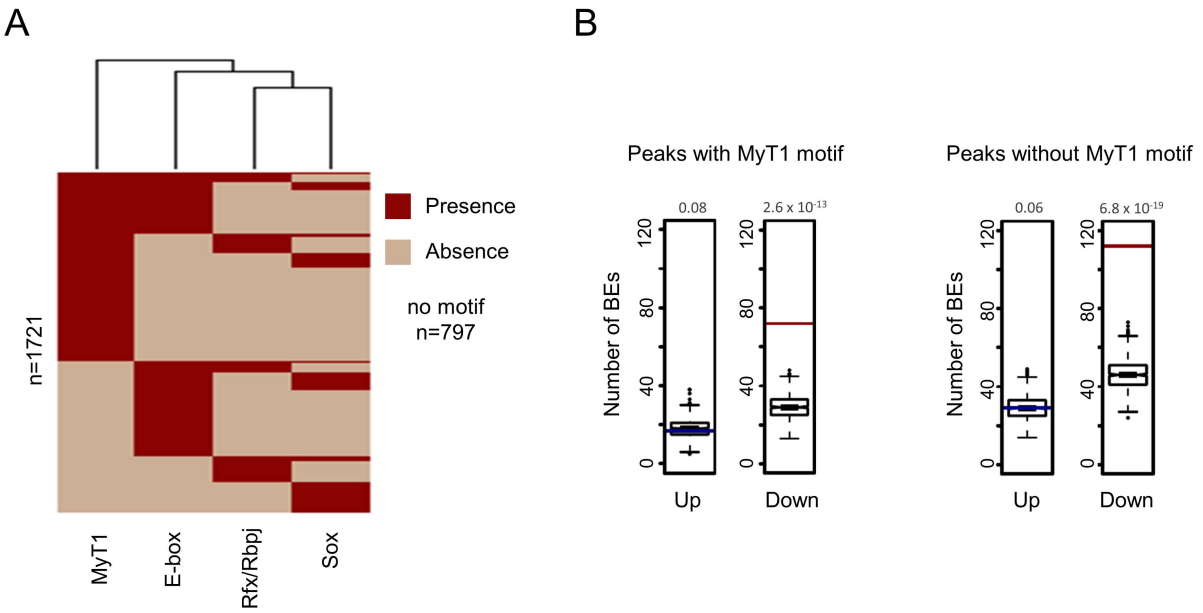

Figure S4. DNA binding motif analysis of MyT1 binding (related to Figure 3)
(A) Hierarchical clustering of MyT1 peaks based on the presence (red) or absence (tan) of MyT1, E-box, RFX and Sox motifs. **(B)** Number of MyT1 BEs containing (left) or not containing (right) MyT1 motif associated with up- (blue bar) or downregulated (red bar) genes in MyT1 GoF DNA arrays. Test data represented as box with median of test and 1st and 3rd quartiles; whiskers, ±1.5xIQR. P-values are indicated above each box.

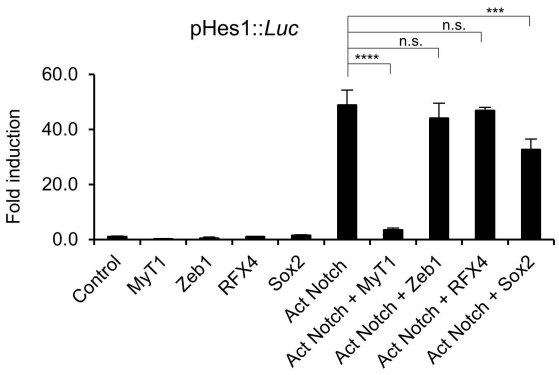

Figure S5. Control experiments demonstrating specificity of MyT1 inhibition of *Hes1* promoter (related to Figure 5)

Transcriptional assay in P19 cells co-transfected with control, MyT1, Zeb1, RFX4, Sox2 and/or Act Notch expression vectors and pHes1*::Luc*. Mean ± SD of quadruplicate assays. n.s. for P>0.05, *** for P<0.001, **** for P<0.0001 according to one-way ANOVA test with Bonferroni correction for multiple testing.

Figure S6

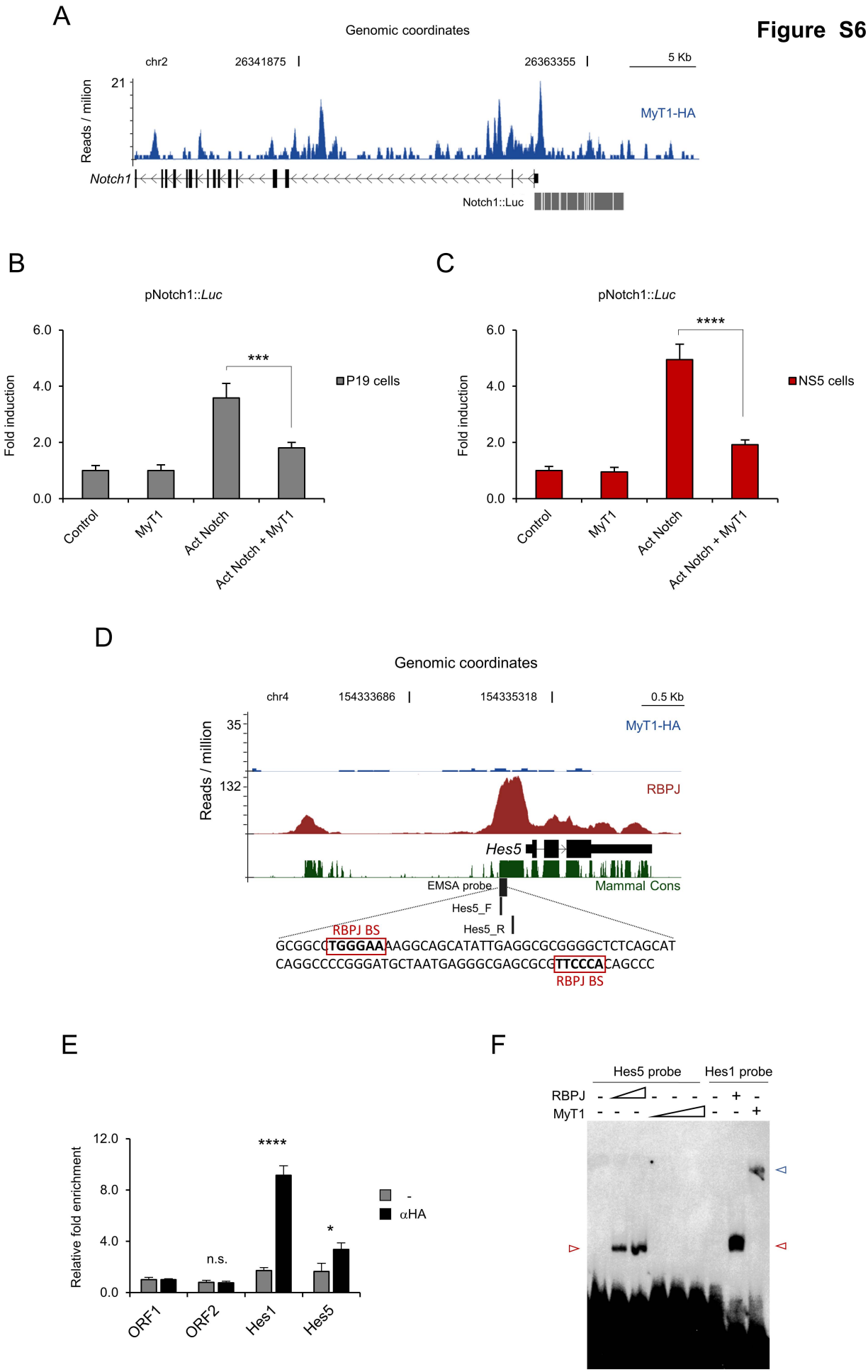
A
Genomic coordinates
chr2
26341875
26363355
5 Kb
Reads / milion
21
MyT1-HA
Notch1
Notch1::Luc
B
pNotch1::Luc
Fold induction
6.0
4.0
2.0
0.0
***
P19 cells
Control
MyT1
Act Notch
Act Notch + MyT1
C
pNotch1::Luc
****
NS5 cells
D
Genomic coordinates
chr4
154333686
154335318
0.5 Kb
35
132
MyT1-HA
RBPJ
Hes5
EMSA probe
Mammal Cons
Hes5_F
Hes5_R
RBPJ BS
GCGGCCTGGGAAAAGGCAGCATATTGAGGCGCGGGGCTCTCAGCAT
CAGGCCCCGGGATGCTAATGAGGGCGAGCGCGTTCCCACAGCCC
RBPJ BS
E
Relative fold enrichment
12.0
8.0
4.0
0.0
****
*
n.s.
-
αHA
ORF1
ORF2
Hes1
Hes5
F
Hes5 probe
Hes1 probe
RBPJ
MyT1

Figure S6. Analysis of MyT1 binding and regulation of *Notch1* and *Hes5* regulatory regions (related to Figure 7)

(A) MyT1 ChIP-Seq enrichment profiles at vicinity of *Notch1* gene. Genomic region contained in pNotch1*::Luc* (Yashiro-Ohtani et al., 2009) is indicated below figure; white vertical bars represent the position of MyT1 motifs. **(B-C)** Transcriptional assays in P19 cells **(B)** and NS5 cells **(C)** co-transfected with control, MyT1 and/or Act Notch expression vectors and a reporter construct expressing luciferase under the control of *Notch1* proximal promoter region pNotch1*::Luc.* Mean ± SD of quadruplicate assays. **(D)** MyT1 (blue) and RBPJ (red) ChIP-Seq enrichment profiles at vicinity of Hes5 gene. Genomic location of the ChIP-qPCR primers (Hes5_F, Hes5_R) and EMSA probe are indicated. Sequence of *Hes5* EMSA probe and position of RBPJ BSs are indicated below. Green track, Mammal conservation by PhastCons. **(E)** ChIP-qPCR using an anti-HA antibody in NS5 cells upon MyT1 GoF. ORF1 and ORF2, negative control regions. Mean ± SD of triplicate assays. **(F)** EMSA testing MyT1 binding to *Hes5* probe. *Hes1* promoter probe and RBPJ-expressing reticulocytes were used as positive controls. Blue arrowhead indicates MyT1 binding to *Hes1* probe. Red arrowheads indicate RBPJ binding to *Hes1* and *Hes5* probes. Different exposure times for different parts of the same gel are shown. n.s. for $P>0.05$,* for $P<0.05$, *** for $P<0.001$, **** for $P<0.0001$ according to Student's t-test **(C,E)**.

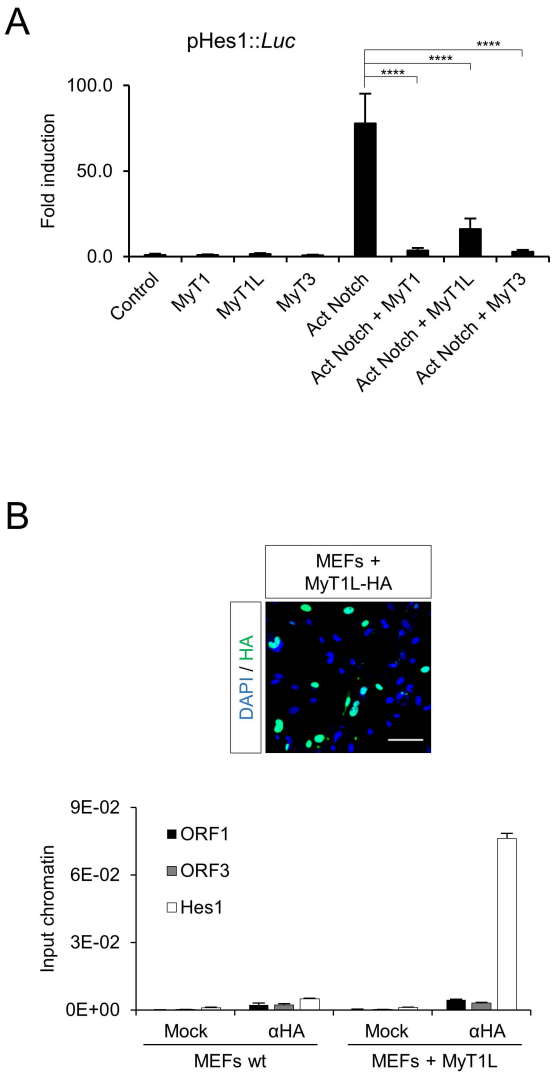

Figure S7. Functional conservation of Hes1 regulation by MyT1 family members (related to Figure 5)

(A) Transcriptional assay in P19 cells co-transfected with control, MyT1, MyT1L, MyT3 and/or Act Notch expression vectors and pHes1*::Luc*. Mean ± SD of quadruplicate assays. **** for P<0.0001 according to one-way ANOVA test with Bonferroni correction for multiple testing.

(B) Top panel: immunocytochemical analysis with anti-HA in MEFs 48h upon infection with a MyT1L-HA-expressing lentivirus. Bottom panel: analysis of MyT1L-HA binding in the *Hes1* proximal promoter region by ChIP-qPCR in chromatin extracted from MEFs wild-type (not infected) and MEFs MyT1L-HA (48h upon infection). ORF1 and ORF3, negative control regions. Mean ± SD of triplicate assays.

List of Supplemental Tables

Table S1. Genes deregulated 4h after MyT1 GoF in NS5 cells (related to Figure 3).

Table S2. Genomic coordinates of MyT1-HA binding events determined by ChIP-Seq (related to Figure 3).

Table S3. Genes bound and repressed by MyT1 GoF (related to Figure 3).

Table S4. Identity of MyT1 target genes associated with enriched Gene Ontology terms (related to Figure 3)

Table S5. Expression profiling dataset upon Notch inhibition (related to Figure 5).

Table S6. Genomic coordinates of RBPJ binding events determined by ChIP-Seq (related to Figure 7).

Supplemental experimental procedures

1. Plasmids

pCAG-MyT1-IRES-GFP
The full-length cDNA of mouse MyT1 was excised from pMycMyT1-7ZF-IRES/Red vector with EcoRI and subcloned (blunt ended) into the EcoRV site of pCAG-LinkerA-IRES-NLS-GFP.

MyT1-HA TetON-FUW
An HA-tag encoding oligonucleotide with restriction sites for XhoI and NotI at each end and EcoRI and AgeI sites downstream the HA-tag was inserted into the XhoI and NotI sites of pPyCAG-MCS-MyT1-V5. The ECoRI fragment of MyT1-V5 was subcloned into the EcoRI site of TetON-FUW.

FLAG-Act Notch TetON-FUW
Activated Notch1 tagged N-terminally with FLAG-tag was excised from pCAG-IRES-GFP-FLAG-Act Notch with BamHI, and cloned upon Klenow fill in reaction into the EcoR1 site of TetON-FUW.

MyT1L-HA TetON-FUW
An HA-tag oligonucleotide was inserted into the pCAG-MyT1L by PCR amplification using primers containing the HA-tag, STOP codon, one EcoRI site and one BglII site. The amplified fragment and the pCAG-MyT1L were digested with SacI and BglII. MyT1L-HA was excised from pCAG-MyT1L-HA vector using EcoRI and cloned into TetON-FUW.

Site-directed mutagenesis of pHes1*::Luc*
The mutations on the MyT1 BSs on the Hes1 promoter luciferase reporter (pHes1*::Luc*) were generated by site-directed mutagenesis using the plasmid pHes1*::Luc* and the primers listed on the Table S7. PCR reactions were performed with 50nM of each

primer, 100ng of plasmid, 100µM dNTPs, 7.5U of Cloned Pfu polymerase and Pfu buffer with $MgSO_4$ (Stratagene). Reaction was run under the following cycling conditions: 1 cycle 95ºC/5min; 18 cycles (95ºC/50sec; 60ºC/50sec; 72ºC/10min); 1 cycle 72ºC/25min, followed by treatment with DpnI for 3-4h at 37ºC.

Table S7 Oligonucleotides used for site-directed mutagenesis of pHes1*::Luc* (related to Figure 5)

Primer	Sequence
MyT1_BS1_mut_FW	CTCTTCCTCCCATTGGCTGAACCCTACTGTGGGAAAGAAAGTTTG
MyT1_BS1_mut_RV	CAAACTTTCTTTCCCACAGTAGGGTTCAGCCAATGGGAGGAAGAG
MyT1_BS2_mut_FW	GAAAGTTACTGTGGGAAAGAACCATTGGGAAGTTTCACACGAGCC
MyT1_BS2_mut_RV	GGCTCGTGTGAAACTTCCCAATGGTTCTTTCCCACAGTAACTTTC
MyT1_BS3_mut_FW	GAAAGAAAGTTTGGGAACCTTCACACGAGCCGTTCG
MyT1_BS3_mut_RV	CGAACGGCTCGTGTGAAGGTTCCCAAACTTTCTTTC
MyT1_BS1+2+3_mut_FW	GAAAGAACCATTGGGAACCTTCACACGAGCCGTTC
MyT1_BS1+2+3_mut_RV	GAACGGCTCGTGTGAAGGTTCCCAATGGTTCTTTC

2. Electromobility shift assay

Table S8 Oligonucleotides used as EMSA probes (related to Figure 5)

EMSA probe primers	Sequence
Hes1_WT_F	TGGCTGAAAGTTACTGTGGGAAAGAAAGTTTGGGAAGTTTCACACGAGCC
Hes1_WT_R	GGCTCGTGTGAAACTTCCCAAACTTTCTTTCCCACAGTAACTTTCAGCCA
Hes1_MyT1_BS1_mut_F	TGGCTGAACCCTACTGTGGGAAAGAAAGTTTGGGAAGTTTCACACGAGCC
Hes1_MyT1_BS1_mut_R	GGCTCGTGTGAAACTTCCCAAACTTTCTTTCCCACAGTAGGGTTCAGCCA
Hes1_MyT1_BS2_mut_F	TGGCTGAAAGTTACTGTGGGAAAGAACCATTGGGAAGTTTCACACGAGCC
Hes1_MyT1_BS2_mut_R	GGCTCGTGTGAAACTTCCCAATGGTTCTTTCCCACAGTAACTTTCAGCCA
Hes1_MyT1_BS3_mut_F	TGGCTGAAAGTTACTGTGGGAAAGAAAGTTTGGGAACCTTCACACGAGCC
Hes1_MyT1_BS3_mut_R	GGCTCGTGTGAAGGTTCCCAAACTTTCTTTCCCACAGTAACTTTCAGCCA
Hes1_MyT1_BS1+2+3_mut_F	TGGCTGAACCCTACTGTGGGAAAGAACCATTGGGAACCTTCACACGAGCC
Hes1_MyT1_BS1+2+3_mut_R	GGCTCGTGTGAAGGTTCCCAATGGTTCTTTCCCACAGTAGGGTTCAGCCA
Hes5_F	GCGGCCTGGGAAAAGGCAGCATATTGAGGCGCGGGGCTCTCAGCATCAGGCCCCGGGATGCTAATGAGGGCGAGCGCGTTCCCACAGCCC
Hes5_R	GGGCTGTGGGAACGCGCTCGCCCTCATTAGCATCCCGGGGCCTGATGCTGAGAGCCCCGCGCCTCAATATGCTGCCTTTTCCCAGGCCGC

3. Expression q-PCR

Table S9 Expression real-time PCR primers (related to Figures 1, 3 and 7)

Gene	Forward Primer	Reverse primer
ActinB	CTAAGGCCAACCGTGAAAAG	ACCAGAGGCATAGGGACA
Dll1	GGGCTTCTCTGGCTTCAAC	TAAGAGTTGCCGAGGTCCAC
GAPDH	GGGTTCCTATAAATACGGACTGC	CCATTTTGTCTACGGGACGA
Hes1	TGAAGGATTCCAAAATAAAATTCTCTGGG	CGCCTCTTCTCCTGATAGGCTTTGATGAC
Hes5	AAGTACCGTGGCGGTGGAGAT	CGCTGGAAGTGGTAAAGCAGC
Id3	TCATAGACTACATCCTCGACCTTC	CACAAGTTCCGGAGTGAGC
Lfng	CCACTCCCACCTAGAGAACCT	ACTGCGTTCCGCTTGTTC
Lmcd1	GATCCATCCAAAGAAGTGGAA	TGTCAGCGTAGACCACAGG
MyT1	GGCCATGCATGAAAATGTACT	GCAATGGGACATCCAGATAAA
Notch1	CTGGACCCCATGGACATC	GGATGACTGCACACATTGC
Olig1	CAGGCCCAGTTCTCCAAG	GGGAAGATTGGCTGAGGTC
Sox2	AAGCGCCTTCATGGTATGGTC	TATAATCCGGGTGCTCCTTC
Tubb3	GCGCATCAGCGTATACTACAA	CATGGTTCCAGGTTCCAAGT

4. ChIP q-PCR

Table S10 ChIP-qPCR primers (related to Figures 1, 3 and 7)

Primers	Forward Primer	Reverse primer
Dll1 ORF (ORF1)	GTCTCAGGACCTTCACAGTAG	GAGCAACCTTCTCCGTAGTAG
Fbxw7 ORF (ORF2)	CTCGTCACATTGGAGAGTGG	CAGGAGCTTGGTTTCCTCAG
Hes1 ORF (ORF3)	CACTTTCTGCCTTCTGTGGA	AGAGGATGGAGGAGTCATGG
Hes1	GGGAAAGAAAGTTTGGGAAGT	GTTATCAGCACCAGCTCCAG
Hes5	GGGAAAAGGCAGCATATTGAGGCG	CACGCTAAATTGCCTGTGAATTGGCG
Id3	GAAAGGTTGCCTGGGACA	GTCTGCGCTGTTTTTGTTTC
Lfng	CTCCCCCACCACTAAGGAG	GGAGAGACACACAGGAAGCA
Lmcd1	ACAGGAAGGGCTGTTACCAT	CTGTTTGCTCTGTGTCTCTGG
MyT1	CTGGCAACACAATTCCAAAG	AGGGGTCATGCTGCTTCTAT
Notch1_1	ATTTGGCCAGAATTTGCATT	GCGCCACATTTAAACTCCTG
Notch1_2	CAGACCTGCTTAATTGGCTTC	GGAGACAGAGAAGGCTCCAG
Olig1	GTGAACAGTCCCCCTTCTGT	GCTGCCAAACCTTCAGTCTA
Sox2	CCGGAAACCCATTTATTCC	TGCAAACACTCTCTTCTCTGC

5. Cell culture

P19 and HEK293T cells were transfected by using linear polyethylenimine (PEI) (Sigma-Aldrich) in the proportion of DNA:PEI (w/w) of 1:2.5 for P19 cells and 1:3 for HEK293T cells. Total amount of DNA/cm^2, 500 ng. Medium was replaced with fresh medium 4-6h after transfection.

Mouse embryonic fibroblasts (MEFs) were isolated from E12.5 embryos. The head, vertebral column (containing the spinal cord), dorsal root ganglia and all internal organs were removed and discarded to ensure the removal of all cells with neurogenic potential from the cultures. The remaining tissue was manually dissociated and incubated 0.25% trypsin (Gibco) for 10-15 min to create a single cell suspension. The cells from each embryo were plated onto a T150 flask with MEF medium (Dulbecco's Modified Eagle Medium (DMEM) (BioWest) / High glucose containing 10% fetal bovine serum (FBS) (BioWest), 2mML-Glutamine (Gibco) and 100U/mL Penicillin/Streptomycin (Gibco)).

6. Antibodies

Table S11 Antibodies used in this study

Antigen (Species)	Working dilution IF: immunofluorescence WB: Western blot	Catalog number	Company / Reference
GFP (chicken)	1:1000 (IF)	06-896	Millipore
HA-tag (rabbit)	1:1000 (IF)	ab9110	Abcam
MyT1 (rabbit)	1:1000 (IF) ; 1:5000(WB)		Wang et al., 2007
MyT1L (guinea pig)	1:1000 (WB)		Tennant et al., 2012
MyT3 (rabbit)	1:2000 (WB)		Guogiang Gu et al.
Sox2 (rabbit)	1:500 (IF)	AB5603	Millipore
TubulinB III (mouse)	1:500 (IF)	MAB1637	Millipore
Alexa Fluor 488 Goat Anti-Chicken IgG	1:1000 (IF)		Life Technologies
Alexa Fluor 488 Goat Anti-mouse IgG	1:1000 (IF)		Life Technologies
Alexa Fluor 568 Goat Anti-rabbit IgG	1:1000 (IF)		Life Technologies
Alexa Fluor 568 Goat Anti-mouse IgG	1:1000 (IF)		Life Technologies
α-tubulin (mouse)	1:10 000 (WB)	T6074	Sigma
Goat Anti-Rabitt IgG (H+L) Poly-HRP	1:4000 (WB)		Jackson ImmunoResearch
Donkey Anti-Mouse IgG (H+L) Poly-HRP	1:4000 (WB)		Jackson ImmunoResearch

7. Image analysis and fluorescence quantification

All images were treated using ImageJ. The number of DAPI, Sox2-, MyT1-HA-positive cells was quantified using Threshold, Watershed and Analyze particles tools from ImageJ. The number of TuJ1-positive cells was quantified using the Cell Counter plugin from ImageJ. The number of cells counted per condition is mentioned in figures legends. Data is presented as mean ± SD.

8. Bioinformatics

Location analysis and expression data integration

MyT1 ChIP-Seq peak overlap with expression data from MyT1 GoF microarrays was calculated and plotted as heat maps with R/Bioconductor packages “genomeIntervals”, “gplots”, and in-house developed scripts.

Motif finding and Gene Ontology analysis

De novo search for differentially enriched motifs was performed using CisFinder (Sharov and Ko, 2009). Searches were run against a control dataset of identical length located 2Kb upstream (FDR<0.05%; Match threshold for clustering, 0.55). Motif fold enrichment (peaks/control) and percentage of peaks with motif were determined using the abundance tables obtained from CisFinder. Frequency distributions were plotted using the frequency tables obtained with CisFinder upon search within 2000bp regions centered on peak summits. Gene Ontology Biological Process analysis with functional annotation clustering was carried out using DAVID v6.7 (Dennis et al., 2003), using the whole microarray (MoGene 1.0 ST v1) as control (enrichment score (EASE)<0.05; similarity threshold for clustering, 0.8).

Density plots

ChIP-Seq normalized tag signals were calculated using a 10bp sliding window over the ± 2kb region around each peak summit to generate the occupancy profiles (in-house developed algorithm). These were plotted as heat maps of signal density using R/Bioconductor packages (http://www.Rproject.org/ and http://CRAN.R-project.org/package=gplots).

Gene expression analysis

NS5 Ascl1-ERT2 cells were plated in 6-well plates (600 000 cells/ well). Differentiation was induced 24h after plating by reducing EGF concentration to 5ng/mL and by adding 4-hydroxy-tamoxifen (TAM) (Sigma-Aldrich) (50nm). Samples were collected in triplicates 0, 4, 12, 24 and 48h post TAM.

Gene expression analysis using datasets from embryonic mouse telencephalon

Expression RNA-Seq data sets from single cells isolated from E11.5 mouse cortex are from (Hagey and Muhr, 2014). Analysis of the correlation of MyT1 and Hes1 expression was performed as previously described (Hagey and Muhr, 2014). Expression RNA-Seq data sets from cortical layers of the developing mouse brain at E14.5 are accessible at NCBI GEO database (Fietz et al., 2012), accession GSE38805. Hierarchical clustering of in vivo expression of genes bound and downregulated by MyT1 in culture was performed using distances based on the Pearson's correlation coefficient and plotted as a row-scaled heat map with R/Bioconductor “hclus” and “gplots” packages.

Hierarchical clustering of motif-containing ChIP-Seq peaks

Hierarchical clustering of ChIP-Seq peaks based on the presence or absence of the represented motifs. MyT1 motif and E-box were searched as consensus motifs. Rfx and Sox motifs were searched as positional weight matrices. Abundance tables obtained with the Cisfinder Search tool (Sharov and Ko, 2009) were converted to binary (1-presence, 0- absence). Only the peaks that have at least one of the motifs

searched are represented. Hierarchical clustering was plotted as heat maps with R/Bioconductor “hclust”, “heatmap” packages. P(MyT1 ChIP-Seq)>10^{-10}.

9. Publicly available data sets used in this study

Table S12 Data sets previously generated used in this study

Dataset	Reference
Ascl1-ERT2 ChIP-Seq in NS5 Ascl1-ERT2 cells, t=18h	Wapinski et al., 2013
H3K27ac ChIP-Seq in NS5 Ascl1-ERT2 cells, t=0h	Raposo et al., 2015
H3K4me1 in NS5 Ascl1-ERT2 cells, t=0h	Raposo et al., 2015
H3K4me3 ChIP-Seq in neural progenitor cells	Mikkelsen et al., 2007
H3K27me3 ChIP-Seq in neural progenitor cells	Mikkelsen et al., 2007
DNAse-Seq in proliferating and differentiating neural progenitor cells	Raposo et al., 2015

10. Supplemental references

Castro, D.S., Martynoga, B., Parras, C., Ramesh, V., Pacary, E., Johnston, C., Drechsel, D., Lebel-Potter, M., Garcia, L.G., Hunt, C., Dolle, D., Bithell, A., Ettwiller, L., Buckley, N., Guillemot, F., 2011. A novel function of the proneural factor Ascl1 in progenitor proliferation identified by genome-wide characterization of its targets. Genes Dev. 25, 930–45.

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Gohlke, J.M., Armant, O., Parham, F.M., Smith, M. V, Zimmer, C., Castro, D.S., Nguyen, L., Parker, J.S., Gradwohl, G., Portier, C.J., Guillemot, F., 2008. Characterization of the proneural gene regulatory network during mouse telencephalon development. BMC Biol. 6, 15.

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