Title: Transcriptional regulation of neonatal neural stem cells is a determinant of social behavior

Short Title: Neonatal origin of social behavior

Authors: Takeshi Hiramoto[1†], Shuken Boku[2†], Gina Kang[1], Seiji Abe[2], Mariel Barbachan e Silva[3], Kenji Tanigaki[4], Masako Nagashima[2], Kenny Ye[5] Takahira Yamauchi[1], Tatyana V. Michurina[6], Pilib Ó Broin[3], Grigori Enikolopov[6,7], Noboru Hiroi[1, 8, 9]*

Affiliations:

[1]Department of Pharmacology, University of Texas Health Science Center at San Antonio; TX 78229, USA.

[2]Department of Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine; Bronx, NY 10461, USA.

[3]School of Mathematical & Statistical Sciences, National University of Ireland Galway; Galway, Ireland.

[4]Research Institute, Shiga Medical Center, 5-4-30 Moriyama, Moriyama-shi, Shiga, Japan.

[5]Department of Epidemiology & Population Health, Albert Einstein College of Medicine; Bronx, NY 10461, USA.

[6]Center for Developmental Genetics, Stony Brook University, Stony Brook, NY 11794, USA.

[7]Department of Anesthesiology, Stony Brook University; Stony Brook, NY 11794, USA.

[8]Department of Cellular and Integrative Physiology, University of Texas Health Science Center at San Antonio; TX 78229, USA.

[9]Department of Cell Systems and Anatomy, University of Texas Health Science Center at San Antonio; TX 78229, USA.

Present addresses:

SB, Department of Neuropsychiatry, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

SA, Department of Hospital Pharmaceutics, School of Pharmacy, Showa University, Tokyo, Japan

TY, Department of Psychiatry, Nara Medical University, Nara, Japan

MN, Department of Palliative Medicine, Teikyo University School of Medicine, Tokyo, Japan

†These two authors contributed equally to this work

*Corresponding author. Email: hiroi@uthscsa.edu

Abstract: Rare gene variants confer a high level of penetrance to neurodevelopmental disorders, but their developmental origin and cellular substrates remain poorly understood. To address this limitation, we explored the role of *TBX1*, a gene encoded in a rare copy number variant, in cell and mouse models. Here, we report that neonatal *Tbx1* deficiency contributes to defective peripubertal social behavior and impairs the proliferation of neonatal neural stem/progenitor cells. Moreover, TBX1 transcriptionally regulates genes linked to post-embryonic neurogenesis and neurodevelopmental disorders associated with other rare gene variants. Our data indicate a precise time window and cell type through which the social dimension is altered by a gene encoded in a rare CNV and provide a potential common mechanistic basis for a group of neurodevelopmental disorders.

One-Sentence Summary: *Tbx1*, a gene affecting neonatal stem cell proliferation, influences peripubertal social behavior.

Main Text:

Identifying the developmental origin of neurodevelopmental disorders and the cell types in which gene variants cause such disorders is essential to develop mechanism-based precision medicine in psychiatry. Rare copy number variants (CNVs) provide a reliable entry point to delve into the pathophysiology of high-penetrance genetic risk factors, linking genes to cellular phenotypes and behavioral consequences. However, CNVs pose conceptual and technical challenges, as each CNV is associated with multiple mental illnesses. A technical challenge is that any CNV might encode many functionally diverse genes. In theory, to determine how CNV-encoded single genes affect neurodevelopmental disorders, isolated dose manipulation of each CNV-encoded single gene (or a set of such genes) and identification of the resulting phenotypes are required. However, such experimental manipulation is not feasible in humans.

As an alternative strategy, CNV-encoded driver genes can be inferred from the protein-truncating variants of each single gene in disease cases. Recent large-scale exome-sequencing studies have identified ultra-rare variants of single genes encoded in CNVs among individuals with schizophrenia and autism spectrum disorder (ASD) (*1, 2*). However, the failure to identify such variants does not necessarily prove their absence. In fact, small-scale exome-sequencing studies have not identified ultra-rare variants that larger scale analyses have. Moreover, ultra-rare variants may also exist in introns and promoter/enhancers of CNV-encoded single genes (*3*). Further, it is possible that such variants simply do not exist in nature but a one-copy deletion of such single genes within a CNV produces certain phenotypes.

Hemizygous deletion at human chromosome 22q11.2 is associated with elevated rates of schizophrenia and diverse neurodevelopmental disorders, including ASD, intellectual disability, and attention-deficit hyperactivity disorder (*4*). Ultra-rare cases of protein-truncating variants of *TBX1* have also been reported in some families, and some of those carriers have been diagnosed with ASD (*5, 6*). However,

these individuals also carry variants of genes outside 22q11.2, precluding the establishment of a causative role for this gene variant (*5*). A recent large-scale exome-sequencing study of schizophrenia cases identified a protein-truncating variant of *TBX1* (*1*). However, power limitations of these human studies render the contribution of *TBX1* to mental illnesses or their dimensions uncertain.

The causal relation between CNVs and phenotypes can also be explored along dimensions crossing clinical boundaries (*7*). Many dimensions of social and cognitive domains common to ASD and schizophrenia are negatively impacted by CNVs (*8, 9*). While animal models do not recapitulate clinically defined mental disorders, a dimensional analysis provides an opportunity to translate human phenotypes to those in experimental animals. Complementing the limitations of human studies, reports on genetic mouse models for single CNV-encoded genes have suggested that some 22q11.2-encoded genes contribute to distinct dimensional aspects of mental illnesses (*10*). Constitutive heterozygosity of *Tbx1* in a homogeneous genetic background impairs social communication and interaction in mice (*11-13*). We capitalized on these observations to further identify the precise developmental time window, critical cell type, and functions associated with *Tbx1* in mouse and cell models.

RESULTS

Critical developmental time points of Tbx1 for hippocampal neurogenesis

Human *TBX1* and its murine homolog *Tbx1* are expressed in the brains of humans (*14*) and mice (*15*), respectively. TBX1 protein levels in the whole mouse brain and hippocampus precipitously declined from the embryonic period to adulthood (**Fig. S1A–C**). Constitutive *Tbx1* heterozygosity alters cortical neurogenesis at embryonic day 13.5 (E13.5) in some, but not all cortical regions (*16, 17*). In the hippocampus of the adult mouse, TBX1 protein is more enriched in the granule cell layer of the hippocampus than in the cortex (*11*). We examined the rate of embryonic neurogenesis in the hippocampus at E18, as embryonic neurogenesis in the hippocampal granule cell layer peaks around E17–E19 in rodents (*18*). BrdU injected at E18 labeled indistinguishable numbers of cells in various hippocampal regions in *Tbx1*$^{+/+}$ and *Tbx1*$^{+/-}$ mice (**Fig. S2**), indicating that *Tbx1* heterozygosity had no detectable impact on embryonic neurogenesis in the hippocampus.

The granule cell layer of the hippocampus undergoes protracted neurogenesis, up to postnatal day 18 (P18), and a small fraction of neurogenesis persists in the subgranular zone toward adulthood (*18*). However, the number of BrdU-positive cells in the hippocampus was statistically indistinguishable between *Tbx1*$^{+/+}$ and *Tbx1*$^{+/-}$ mice at P35 (**Fig. S3**).

Several interpretative issues are associated with BrdU (*19, 20*). A single dose of BrdU labels the DNA synthesis of S-phase cells at the time of injection, but it is not a marker of dividing cells *per se*. While a higher dose or repeated injections of BrdU would label more cells, it could additionally label cell death or repair. Moreover, the number of BrdU-labeled cells could be confounded by altered lengths of the S-phase and total cell cycle. Thus, to reveal the actual neural stem/progenitor cells during the postnatal period, we developed Tg(Nes-EGFP)33Enik/J;*Tbx1*$^{+/-}$ mice (see Supplementary Information) and counted the numbers of GFP-positive neural stem/progenitor cells in the subgranular zone at P35. There were significantly fewer GFP-positive cells in the subgranular zone of the dorsal hippocampus in Tg(Nes-

EGFP)33Enik/J;*Tbx1*$^{+/-}$ mice than in Tg(Nes-EGFP)33Enik/J;*Tbx1*$^{+/+}$ mice (**Fig. 1**). Thus, *Tbx1* heterozygosity reduces the number of stem/progenitor cells in the postnatal hippocampus.

Cellular consequences of Tbx1 deficiency in neonatal neural stem/progenitor cells

We further explored the precise manner through which *Tbx1* heterozygosity impacts the stem/progenitor cell population. To determine the cell-autonomous effect of *Tbx1* deficiency on the cell cycle of stem/progenitor cells, we cultured and isolated stem/progenitor cells derived from the P0 hippocampus of C57BL/6J pups. This developmental time point was chosen because the TBX1 protein level is higher in the hippocampus earlier than later in the post-embryonic period (see **Fig. S1C**), and constitutive *Tbx1* heterozygous mice show defective social communication as early as P7–8 (*11-13*). Cells were made to simultaneously enter the cell cycle simultaneously using the double-thymidine block method (*21*). TBX1 protein levels peaked during the G1/S phase shortly before the levels of cyclin B1—a marker of the G2/M phase of the cell cycle—peaked (**Fig. 2A**).

When we reduced TBX1 expression level using siRNA *in vitro* (**Fig. S1B**) and examined the rate of proliferation of neonatal neural stem/progenitor cells under proliferating conditions, *Tbx1* siRNA significantly reduced the cell proliferation rate (**Fig. 2B**). As this is a selective population of neonatal neural stem/progenitor cells under a maximally proliferative condition *in vitro*, the detection sensitivity of BrdU is expected to be higher than that of *in vivo* samples. *Tbx1* siRNA reduced the number of BrdU-positive cells (**Fig. 2C; Fig. S4**). Moreover, the reduced proliferation rate was not due to apoptosis (**Fig. 2D**). Thus, TBX1 deficiency cell-autonomously reduces the proliferation rate of neonatal hippocampal neural stem/progenitor cells.

Behavioral consequences of Tbx1 deficiency in neonatal neural stem/progenitor cells

Constitutive *Tbx1* heterozygous pups exhibit defective neonatal social communication as early as P7–8 (*11, 13*), and *Tbx1* deficiency blunts the proliferation of neural stem/progenitor cells derived from the P0 hippocampus (see **Fig. 2B**). Therefore, we investigated whether *Tbx1* insufficiency during the neonatal period is critical for the normal development of peripubertal social behavior. To initiate *Tbx1* heterozygosity in neural stem/progenitor cells at specific post-embryonic periods, we developed nestinCreERT;*Tbx1*$^{flox/+}$ mice (c*Tbx1*$^{+/-}$ mice) (see Supplementary Information). *Tbx1* heterozygosity was initiated by tamoxifen at P1–5 and mice were behaviorally tested 1 month later (**Fig. 3A**); it takes at least 1 month for new neurons to be incorporated into the hippocampal circuitry in rodents (*22*). Recombination was found to be selectively induced in newly generated neurons in brain regions with known post-embryonic neurogenesis in response to tamoxifen (**Fig. S6**). As vehicle-treated nestinCreERT;*Tbx1*^{flox} mice and tamoxifen-treated wild-type;*Tbx1*^{flox} mice did not differ in social behavior, they were combined as a single control group (**Fig. S5A–E**). c*Tbx1*$^{+/-}$ mice showed a social approach deficit (**Fig. 3B**), with normal levels of approach behavior toward a novel object (**Fig. 3C**), anxiety-related traits (**Fig. 3D**), and motor behavior (**Fig. 3E**). The predominant form of social approach was sniffing; therefore, we additionally investigated whether the social approach deficit in c*Tbx1*$^{+/-}$ mice was due to their abnormal reaction to social olfactory cues. Control and c*Tbx1*$^{+/-}$ mice were indistinguishable in their responses and habituation to various non-social and social odorants (**Fig. S7AB**). These data show that the reduced levels of social approach in c*Tbx1*$^{+/-}$ mice in the peripubertal period (i.e., 1 month of age) are not due to the generalized deficits in olfactory responses to social cues, novel object approach, anxiety, or motor capacity.

To further examine the specificity of the neonatal period, we initiated tamoxifen treatment at P21–25 in a different group of mice and tested them 1 month later (i.e., at 2 months of age) (**Fig. 3F**); mice of various control genotypes did not differ, and they were combined as a single control group (**Fig. S5F–J**). These c*Tbx1*$^{+/-}$ mice showed no deficits in social approach, an anxiety-related trait, or motor capacity in an open field (**Fig. 3G, I, and J**), although they showed impaired ability to approach a novel non-social

object (**Fig. 3H**). That tamoxifen treatment at P21–25 affected novel object approach indicates that this treatment *per se* was effective, ruling out the possibility that the tamoxifen dose was insufficient to impact adult neurogenesis and behavior.

The ablation of postnatal/adult neural stem/progenitor cells at P28 or later has no effect on the subsequent social approach/interaction *per se* in mice (*23-25*). Our observations are consistent with these findings in that *Tbx1* deletion in stem/progenitor cells at P21–25 had no detectable effect on later social approach/interaction in male mice, but further extend the previous studies by showing that neonatal neurogenesis is more critical for the normal development of social behavior than postnatal neurogenesis and Tbx1 functionally contributes to the development of peripubertal social behavior via its actions during the neonatal period.

Molecular network of TBX1 *in neonatal neural stem/progenitor cells*

Having identified neonatal neural stem/progenitor cells as a cellular substrate through which *Tbx1* deficiency causes social approach deficits later, we next examined the molecular targets of this transcription factor using chromatin immunoprecipitation (ChIP)-seq. We determined TBX1-binding sites under a proliferating condition in neonatal stem/progenitor cells derived from the hippocampus of P0 C57BL/6J mice. We ran two sets of ChIP-seq independently using different protocols. TBX1-binding sites were associated mostly with either two genes (65%) or one gene (30%), and in some instances at sites with no nearby genes (5%) (**Fig. S8A**). This analysis collectively identified 1,257 genes and mRNA variants (**Table S1-1**), comprising 1,039 genes at and near the TBX1-binding sites. The distances between the transcription start site and TBX1 binding sites were most frequently found at 50–500 kb in both directions, followed by 5-50 kb, more than 500 kb, and less than 5 kb in this order (**Fig. S8B**). The majority of TBX1-binding sites were intergenic regions (73.42%), followed by introns (12.12%), upstream regions (10.55%), promoters (2.96%), and exons (0.95%) (**Fig. S9**).

Biological annotations showed the enrichment of G-protein–coupled receptor signaling pathways, epigenetic gene regulation, and chromatin silencing among biological processes (**Fig. S10-1**); apical junction complex and cell–cell junctions among cellular components (**Fig. S10-2**); and transcription factor activity, G-protein–coupled activity, structural molecule activity, and transmembrane receptor activity among the molecular functions (**Fig. S10-3**).

Next, we explored databases of genes relevant to adult hippocampal neurogenesis (MANGO v3.2). It should be cautioned that in the absence of a gene database for neonatal neurogenesis, the MANGO database was used as a proxy. Nonetheless, this search identified 30 TBX1-bound genes that were implicated in adult neurogenesis (**Fig. 4A yellow circle**; **Table S1-2**), 22 of which form molecular networks with TBX1 (**Fig. 4B**). We next asked whether our pool of TBX1-bound genes is involved in ASD (**Table S1-3**) and developmental brain disorders (**Table S1-4)**. This search identified 87 genes (**Fig 4A blue circle**). Tbx1 potentially interacts with 61 such genes (**Fig. S11**), among which 12 genes were also listed in the MANGO database, i.e., *Cacna1d*, *Cnr1*, *Dcc*, *Dcx*, *Disc1*, *Dmd*, *Dyrk1a*, *Foxg1*, *Gria1*, *Pten*, *Tbr1*, and *Tdo2* (**Fig. 4A**), rare variants of which are associated with neurodevelopmental disorders, including ASD.

Molecular networks are based on lenient inclusive criteria; the Search Tool for Retrieval of Interacting Genes/Proteins (STRING) database contains any association among genes or molecules as long as they are mentioned in the same articles (*26*). While such networks are useful for their heuristic value, they may not represent real, functional associations. For example, while a TBX1–PTEN network was identified using the databases of adult neurogenesis (**Fig. 4B**) and neurodevelopmental disorders (**Fig. S11**), this association was not based on any functional validation. Thus, we performed a series of validations. Our ChIP-seq analysis identified the actual binding sites of TBX1 near PTEN (**Table S1-1**). TBX1 was

colocalized with PTEN in neonatal neural stem/progenitor cells derived from the P0 hippocampus of C57BL/6J pups (**Fig. 5A**, left) and in a subpopulation of PTEN-positive cells in the hippocampal granular zone of C57BL/6J mice (**Fig. 5A**, right). The *Pten* promoter was enriched in DNA fragments precipitated by a TBX1 antibody, as determined by ChIP-PCR (**Fig. 5B**). *Tbx1* siRNA significantly reduced *Pten* promoter activity (**Fig. 5C**) and PTEN protein levels (**Fig. 5D**). TBX1 protein was found to be bound 315 bp upstream and 84,805 downstream of the *Pten* transcription start site (**Fig. S12A–B**). The former site is within a reasonable enhancer location distance (*27*). However, the binding locations of transcription factors revealed by ChIP-seq are not reliable in terms of the precise distances from the target genes owing to the size of fragments generated by sonication (*28*). Moreover, the abundance of binding may not necessarily indicate functionally critical loci. Thus, we constructed various promoter sequences of *Pten* with deletions and mutations at different loci (**Fig. 5E**, left) and examined their transcriptional activity. While the *Pten* promoter is constitutively active, its transcriptional activity doubles during proliferation with epidermal growth factor (EGF) (see **Fig. S13**). The presence of the full-length *Pten* promoter was essential for luciferase activity (**Fig. S14**). The transcriptional activation of the *Pten* promoter under this condition was reduced by a deletion or mutation placed at the proximal 35 bp segment (**Fig. 5E**, right), which contains a recognition sequence resembling the T-box consensus sequence (*29*). Our observations that *Tbx1* deficiency results in a blunted increase in the number of neonatal hippocampal neural stem/progenitor cells (see **Fig. 2B**) and reduction in the transcriptional activation and expression levels of PTEN (see **Fig. 5C–D**) are consistent with the eventual depletion of stem/progenitor cells in the adult hippocampal granule cell layer following *Pten* deletion *in vivo* (*30*).

Molecular networks identified by STRING uncovered *Flt1*, *ApoE*, and *Tdo2* as potential indirect interacting molecules that are also implicated in adult neurogenesis (**Fig. 4AB**). While TBX1-binding sites are outside a 2 kb promoter region (**Table S1-1**), *Flt1* was identified in four separate clones of ChIP-seq and thus included. ApoE has not been implicated in neurodevelopmental disorders, but its indirect link with TBX1 via PTEN is a possibility (see **Fig. 4B**). Tryptophan 2,3-dioxygenase (TDO2) is the rate-limiting

enzyme in the catabolism of tryptophan in the kynurenine pathway, the downregulation of which has been implicated in ASD (*31*). We additionally tested *Btg1*, as it was identified in MANGO (**Table S1-2**) but not in databases of neurodevelopmental disorders (see **Table S1-3; S1-4**). We reduced *Tbx1* mRNA expression levels in the culture of neonatal neural stem/progenitor cells using *Tbx1* siRNA. This analysis showed that *Tbx1* siRNA reduced *Tbx1* mRNA by half (**Fig. S15A**) and reduced *Tdo2* mRNA levels but did not affect levels of *Flt1*, *ApoE*, or *Btg1* mRNA (**Fig. S15B–E**). *Tdo2* mRNA is also highly enriched in the hippocampal granule cell layer in the mouse brain (*32*) and is critical for the proliferation of adult neural stem/progenitor cells (*33*).

TBX1 regulates the proliferation of neonatal neural stem/progenitor cells (see **Fig. 2B**), and *Pten* deletion leads to a transitory expansion of hippocampal adult stem/progenitor cells and other stem cells, followed by the exhaustion of the precursor pool, presumably through an imbalanced increase in the extent of G0 to G1 transition (*34, 35*). Therefore, we further explored the potential target genes implicated in the G0/G1 phase among ChIPseq-identified TBX1-bound genes, which revealed *Ecrg4* and *Rnf112* (**Table S1-1; Table S2**). TBX1-binding sites were found near *Ecrg4* and *Rnf112* (**Fig. S17–S18; Table S1-1**). *Tbx1* siRNA significantly reduced the expression of *Ecrg4* and *Rnf112* mRNA (**Fig. S15F–G**). No functional roles of these genes in neonatal neural stem/progenitor cells in the hippocampus are known. However, ECRG4 suppresses the proliferation of stem cells in the mouse dentate gyrus (*36*). Our data identify potential molecular targets whereby TBX1 regulates the cell cycle in hippocampal neonatal neural stem/progenitor cells.

DISCUSSION

We established that neonatal *Tbx1* deficiency impairs peripubertal social behavior and impairs the proliferation of neonatal neural stem/progenitor cells. Therefore, our study provides a potential cellular basis for a social dimension of neurodevelopmental disorders linked to *TBX1* variants and a critical developmental time point at which TBX1 determines the level of a subsequent social dimension. This gene transcriptionally acts on 87 genes that are independently associated with neurodevelopmental disorders; 12 of these genes are also known to be involved in adult neurogenesis. Interestingly, 75 of the 87 genes associated with neurodevelopmental disorders have not been implicated in adult or neonatal neurogenesis. Moreover, 18 TBX1-bound genes that are listed in the adult neurogenesis database have not been known to be associated with neurodevelopmental disorders. This pool of genes provides a means to identify novel genes contributing to the social dimension of neurodevelopmental disorders via neonatal neurogenesis. Thus, our data link *TBX1*, which has its own ultra-rare gene variants and is also encoded in a rare CNV, to a set of other genes, rare variants of which are associated with neurodevelopmental disorders.

How CNV-encoded genes and their biological processes contribute to various psychiatric disorders is still poorly understood. The identification of driver genes within CNVs in humans is limited due to the very rare nature of variants of their encoded genes. Recent large-scale exome sequencing studies have identified more ultra-rare variants (*1, 37*), but these data do not necessarily rule out the possibility that dose alterations--not their own variants—of CNV-encoded genes contribute to phenotypes. Moreover, while embryonic neurogenesis, synaptic functions, and activity-regulated cytoskeleton-associated proteins have emerged as common, robust candidate processes affecting mental illnesses, such analyses are not devoid of technical and interpretative limitations (*38*). Literature-curated databases do not provide genes and biological processes whose functions have not been explored or are not abundantly or commonly represented.

Our approach, starting from a CNV-encoded gene, represents a complementary strategy to circumvent the limitations associated with human studies and to identify driver genes, and their cell functions and dimensional behavioral phenotypes. We capitalized on TBX1, a 22q11.2 CNV-encoded gene, of which several cases of ultra-rare variants exist with variable neurodevelopmental disorders (*1, 5, 6, 39, 40*). The causative roles of *TBX1* in phenotypes have not been established due to their lack of statistical power (*1*) and the presence of other variants among carriers (*5*) in humans. Given that the expression of TBX1 protein is enriched in the sites of post-embryonic neurogenesis (*11*), we focused on the function of this gene in post-embryonic stem cells in mice. This approach provided a theoretical basis for grouping dimensional aspects of neurodevelopmental disorders, based on a shared mechanistic basis, a prerequisite for designing mechanism-based precision medicine to ultimately treat specific dimensions of such disorders in humans (*7*).

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Author Contributions:

Conceptualization: TH, NH

Methodology: TH, SB, GK, SA, KT, MN, TVM, GE

Investigation: TH, NH, BS, GK, SA, KT, MN, MBS, PÓ, KY,

Visualization: KY, TY

Funding acquisition: NH, SB

Project administration: NH

Supervision: NH

Writing – original draft: TH, SB, GK, PÓ, MBS, NH

Writing – review & editing: TH, SB, NH

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Supplementary Materials:

Materials and methods

Table S1–S5

Fig S1–S15

References (41-60)

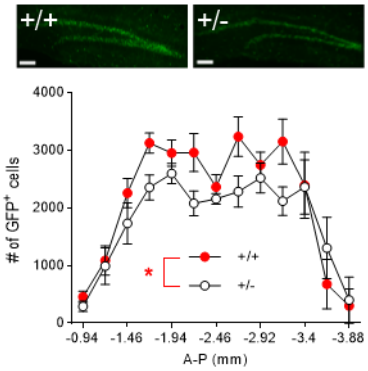

Fig. 1. Effect of constitutive *Tbx1* heterozygosity on adult neural stem/progenitor cells in the mouse hippocampus. (**A**) Representative images of GFP-positive cells in the subgranular zone of the hippocampus of Tg(Nes-EGFP)33Enik/J;*Tbx1*$^{+/+}$ and Tg(Nes-EGFP)33Enik/J;*Tbx1*$^{+/-}$ mice at P35. The scale bar indicates 100 µm. (**B**) More GFP-positive cells were found in the subgranular zone of the hippocampus of Tg(Nes-EGFP)33Enik/J;*Tbx1*$^{+/+}$ mice than in Tg(Nes-EGFP)33Enik/J;*Tbx1*$^{+/-}$ mice (genotype, $F(1,9) = 9.209$, $P = 0.014$; A-P position, $F(12,108) = 16.92$, $P < 0.0001$; genotype × A-P position, $F(12,108) = 1.119$, $P = 0.353$). For clarity, data are presented as means ± SEM but were analyzed using a generalized linear regression model. Tg(Nes-EGFP)33Enik/J;*Tbx1*$^{+/+}$ mice, $N = 6$; Tg(Nes-EGFP)33Enik/J;*Tbx1*$^{+/-}$ mice, $N = 5$. In total, 13 sections were counted per animal.

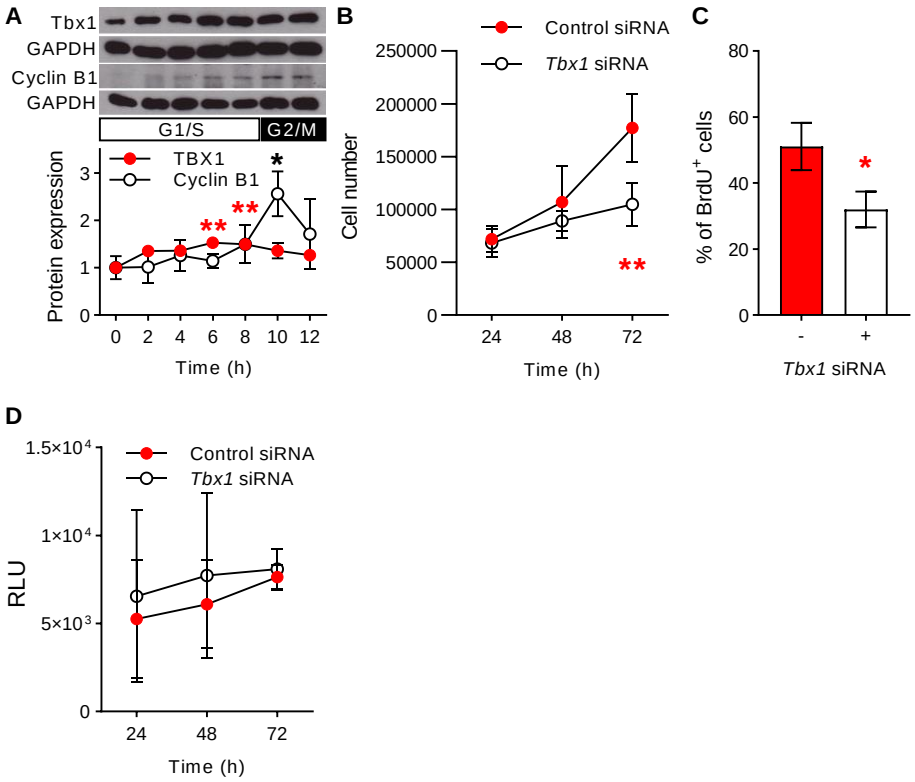

Fig. 2. Effect of *Tbx1* deficiency on neonatal neural stem/progenitor cells. (**A**) TBX1 significantly increased at 6 and 8 h, compared to 0 h (***P* < 0.05). Cyclin B1 expression levels peaked at 10 h, compared to 0 h (**P* < 0.05). Protein expression levels were calculated by dividing the intensity of TBX1 or cyclin B1 signals by that of GAPDH. TBX1; *N* = 6 per time point, except for *N* = 4 at 12 h. Cyclin B1; *N* = 6 per time point, except for *N* = 5 at 6 h and *N* = 4 at 12 h). (**B**) *Tbx1* siRNA blunted neonatal stem/progenitor cell proliferation at 72 h (***P* = 0.001). *N* = 4, 5, and 7 at 24, 48, and 72 h, respectively, for Control siRNA and *Tbx1* siRNA. (**C**) *Tbx1* siRNA significantly reduced BrdU-positive cell number (**P* = 0.04; Control siRNA *N* = 9, *Tbx1* siRNA *N* = 9). (**D**) *Tbx1* siRNA did not alter the apoptosis rate of neonatal stem/progenitor cells, as determined by the RealTime-Glo Annexin V assay (*P* > 0.05 for each time point). RLU, relative light unit. Control siRNA *N* = 9 and *Tbx1* siRNA *N* = 9 for each time point.

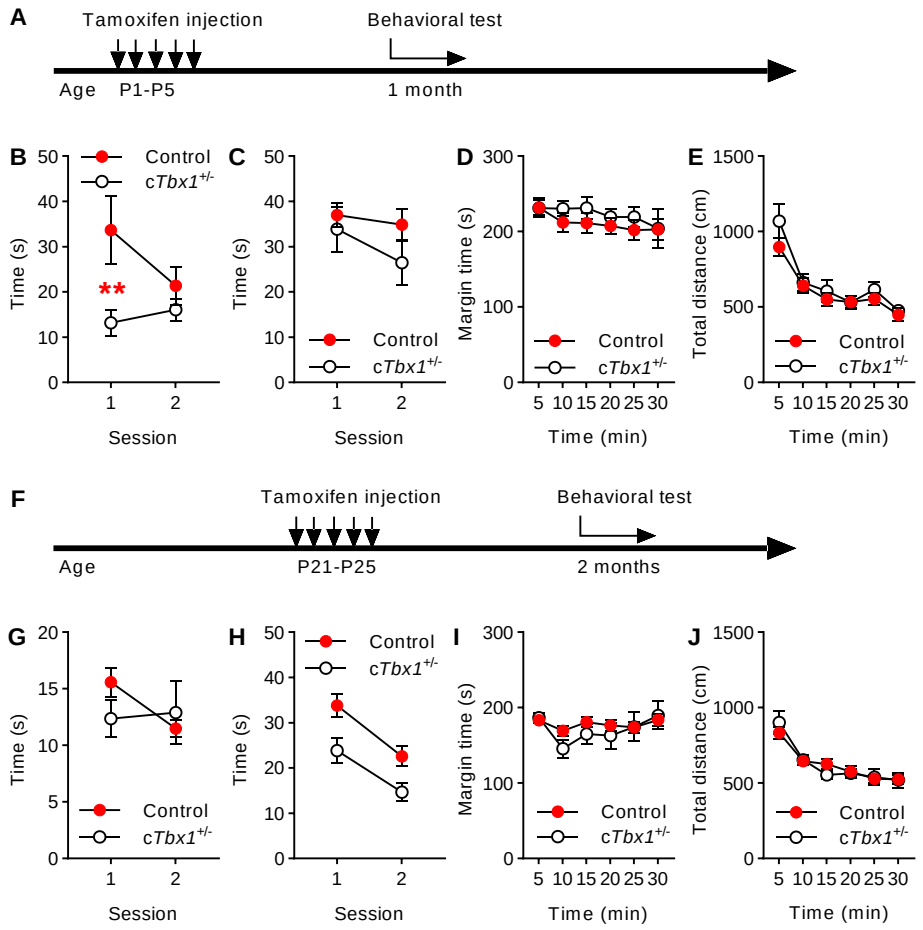

Fig. 3. Behavioral effect of *Tbx1* deletion initiated during neonatal and postnatal periods. Tamoxifen (0 and 83.5 mg/kg body weight, i.p.) was given to lactating mothers of nestinCreERT;*Tbx1*$^{flox/+}$ pups from P1 to P5. Behavioral assays started 1 month later (**A**) for social approach (**B**) (Control, *N* = 19; c*Tbx1*$^{+/-}$, *N* = 12), novel object approach (**C**) (Control, *N* = 23; c*Tbx1*$^{+/-}$, *N* = 12), thigmotaxis (**D**) (Control, *N* = 14; c*Tbx1*$^{+/-}$, *N* = 6), and motor activity (**E**) (Control, *N* = 14; c*Tbx1*$^{+/-}$, *N* = 6). c*Tbx1*$^{+/-}$ mice showed lower levels of social approach at session 1 than control mice did ($P = 0.007$). The groups did not differ in any other tested behavior ($P > 0.05$). Another set of mice were given tamoxifen (0 and 180 mg/kg body weight, i.p.) from P21 to P25 and were tested 1 month later (**F**) for social approach (**G**) (Control, *N* = 35; c*Tbx1*$^{+/-}$, *N* = 11), novel object approach (**H**) (Control, *N* = 33; c*Tbx1*$^{+/-}$, *N* = 12), thigmotaxis (**I**) Control, *N* = 40; c*Tbx1*$^{+/-}$, *N* = 12), and motor activity (**J**) (Control, *N* = 40; c*Tbx1*$^{+/-}$, *N* = 12).

c*Tbx1*$^{+/-}$ mice showed lower levels of novel object approach equally at sessions 1 and 2 compared to control mice (genotype, $F(1,43) = 5.151$, $P = 0.028$; genotype × session, $F(1,43) = 0.635$, $P = 0.43$). The groups did not differ in any other tested behavior ($P > 0.05$).

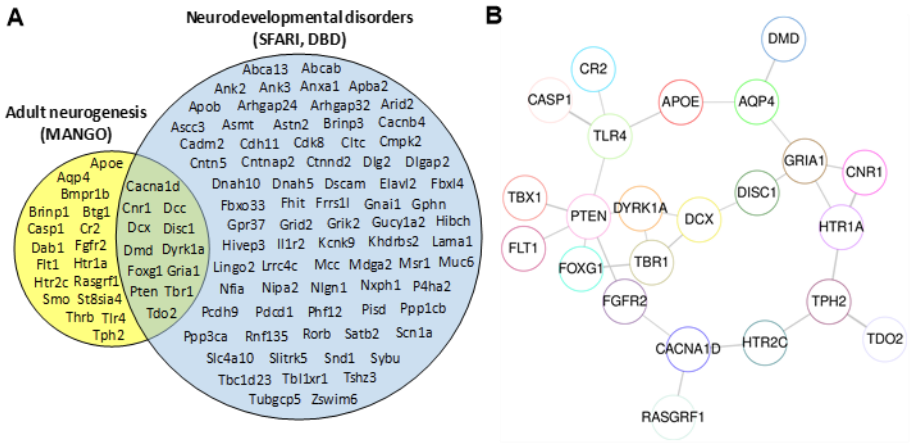

Fig. 4. Genes bound by TBX1 that are implicated in adult neurogenesis and neurodevelopmental disorders. (**A**) The following gene databases were used: (http://mango.adult-neurogenesis.de/documents/annotations?show=20&expression=true; MANGO, left yellow circle). Autism and other neurodevelopmental disorders (SFARI, https://gene.sfari.org; DBD, https://dbd.geisingeradmi.org/) (Right, pale blue circle). (**B)** Potential interactions among the target genes of TBX1 are shown, based on Search Tool for Retrieval of Interacting Genes/Proteins (STRING v11) analysis of MANGO-listed genes (see yellow circle in **A**) (*41*).

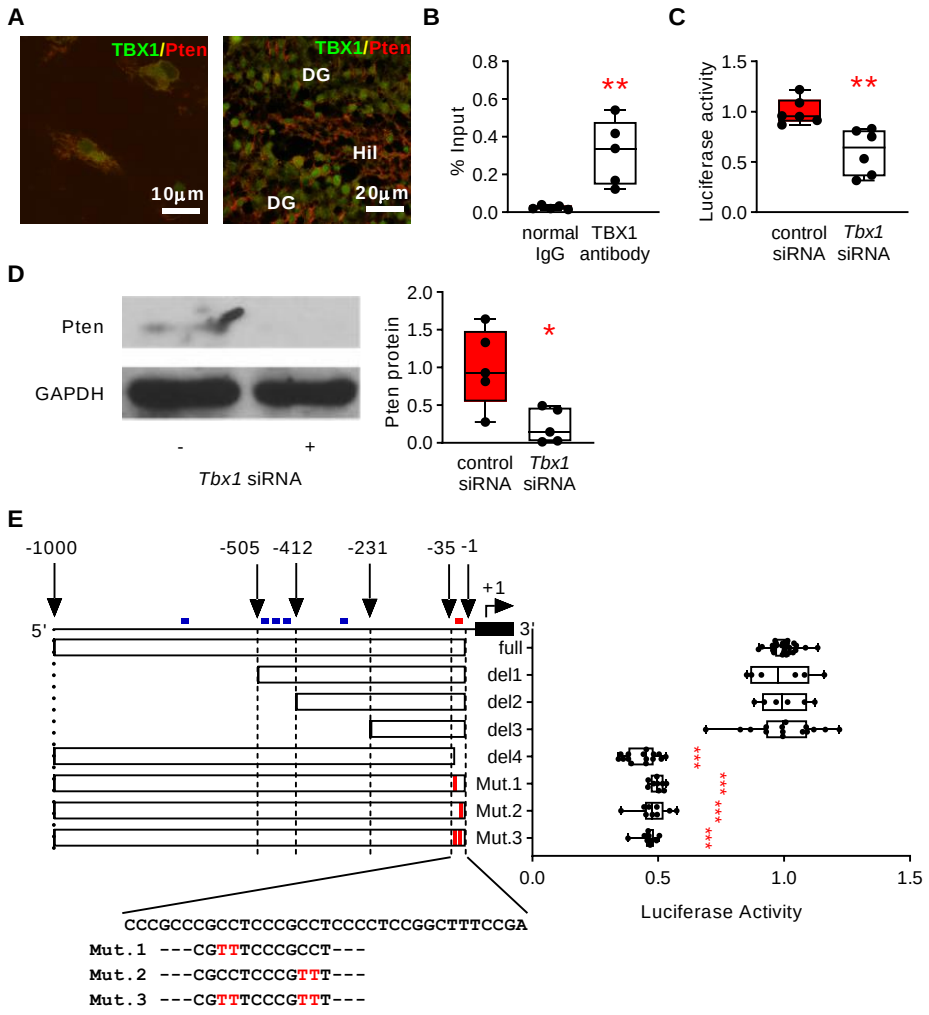

Fig. 5. Transcriptional regulation of *Pten* by TBX1 in neonatal stem/progenitor cells. (**A**) TBX1 expression in PTEN-positive stem/progenitor cells *in vitro* (left) and granule cells in the mouse hippocampus (right). (**B**) ChIP-PCR. TBX1 binds to the *Pten* promoter. Input, pre-immunoprecipitated diluted solution was the control (P = 0.008; N = 5 per group). %input = 2% × 2(C[T] 2%Input Sample − C[T] IP Sample). (**C**) *Tbx1* siRNA significantly reduced transcriptional activity of the 1 kb *Pten* promoter (P = 0.002; N = 6 per group) (see **Fig. S1B** for *Tbx1* siRNA efficacy). (**D**) *Tbx1* siRNA significantly reduced PTEN protein in P0 neural progenitor cells (P = 0.0016; N = 5 per group). (**E**) Deletion and mutation positions within the 1 kb *Pten* promoter (left); their effects on luciferase activity (right). Deletion and mutation of the proximal 35 bp reduced *Pten* promoter activity (***P < 0.001; Mann–Whitney test). T-box-binding consensus

sequence (blue dots): GTGTTATGACACGTGCAAGTGTGAGTGCGA (*42*); T-box–binding-like sequence (red dot): CCGCCTCCCGCCT (*29*). Full, *N* = 27; Del.1, *N* = 6; Del. 2, *N* = 6; Del. 3, *N* = 15; Del. 4, *N* = 15; Mut.1, *N* = 9; Mut. 2, *N* = 9; Mut. 3, *N* = 9.