Received: 26 August 2019 | Revised: 3 January 2020 | Accepted: 7 January 2020

DOI: 10.1111/ejn.14674

RESEARCH REPORT

EJN European Journal of Neuroscience FENS WILEY

The basic helix-loop-helix transcription factor TCF4 impacts brain architecture as well as neuronal morphology and differentiation

Melanie Schoof[1,2] | **Malte Hellwig**[1,2] | **Luke Harrison**[3] | **Dörthe Holdhof**[1,2] | **Marlen C. Lauffer**[3] | **Judith Niesen**[1,2] | **Sanamjeet Virdi**[4] | **Daniela Indenbirken**[4] | **Ulrich Schüller**[1,2,5]

[1]Department of Pediatric Hematology and Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[2]Research Institute, Children's Cancer Center Hamburg, Hamburg, Germany

[3]Center for Neuropathology, Ludwig-Maximilians-University, Munich, Germany

[4]Heinrich-Pette-Institute, Leibniz Institute for Experimental Virology, Hamburg, Germany

[5]Institute of Neuropathology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Correspondence
Ulrich Schüller, Research Institute Children's Cancer Center Hamburg, Martinistrasse 52, N63 (HPI), D-20251 Hamburg, Germany.
Email: u.schueller@uke.de

Present address
Luke Harrison, Institute of Diabetes and Obesity, Helmholtz Center, Neuherberg, Germany

Marlen C. Lauffer, Department of Psychiatry, Erasmus University Medical Center, Rotterdam, The Netherlands

Funding information
Wilhelm-Sander Stiftung; Research Institute Children's Cancer Center; Hans Brökel Stiftung für Wissenschaft und Kultur; Burkhard Meyer Stiftung

Abstract

Germline mutations in the basic helix-loop-helix transcription factor 4 (*TCF4*) cause the Pitt–Hopkins syndrome (PTHS), a developmental disorder with severe intellectual disability. Here, we report findings from a new mouse model with a central nervous system-specific truncation of *Tcf4* leading to severe phenotypic abnormalities. Furthermore, it allows the study of a complete TCF4 knockout in adult mice, circumventing early postnatal lethality of previously published mouse models. Our data suggest that a TCF4 truncation results in an impaired hippocampal architecture affecting both the dentate gyrus as well as the cornu ammonis. In the cerebral cortex, loss of TCF4 generates a severe differentiation delay of neural precursors. Furthermore, neuronal morphology was critically affected with shortened apical dendrites and significantly increased branching of dendrites. Our data provide novel information about the role of *Tcf4* in brain development and may help to understand the mechanisms leading to intellectual deficits observed in patients suffering from PTHS.

KEYWORDS

brain, E2-2, mouse, neurodevelopment, Pitt–Hopkins syndrome

Abbreviations: bHLH, basic helix-loop-helix; CA, cornu ammonis; CNS, central nervous system; DNE, dentate neuroepithelium; E-box, Ephrussi box; hGFAP, human glial fibrillary acidic protein; ID, intellectual disability; PTHS, Pitt–Hopkins syndrome; SVZ, subventricular zone; Tcf4, transcription factor 4; TF, transcription factor; VZ, ventricular zone.

Schoof and Hellwig contributed equally to this work.

Edited by Yoland Smith.

The peer review history for this article is available at https://publons.com/publon/10.1111/EJN.14674

1 | INTRODUCTION

Heterozygous germline mutations of the transcription factor 4 (*TCF4*, also known as *SEF2*, *ITF2*, *E2-2*, *ME2* and others) lead to the Pitt–Hopkins syndrome (PTHS), a congenital autism spectrum disorder associated with intellectual disability (ID). ID is a symptom of almost all developmental disorders and, although a lot of research was done, the molecular mechanisms of ID in most congenital syndromes remain widely unknown.

PTHS patients present with a number of different symptoms including ID, a global developmental delay, certain characteristic facial features, short stature and microcephaly (Sweatt, 2013). PTHS was first described in 1978 (Pitt & Hopkins, 1978) and in 2007, germline mutations in the gene encoding TCF4 were identified as the cause of the syndrome (Amiel et al., 2007; Brockschmidt et al., 2007; Zweier et al., 2007). Besides, a tumour-suppressive role of *TCF4* in medulloblastoma was described, highlighting the importance of the transcription factor (TF) in developmental processes (Hellwig et al., 2019).

TCF4 is a member of the family of basic helix-loop-helix (bHLH) TFs. These proteins have a DNA-binding domain and form homo- or heterodimers to regulate gene expression. The dimers of TFs can exert different functions depending on their dimerization partners (Jones, 2004). Within the family of bHLH TFs, TCF4 belongs to the subgroup of E-proteins, which share the recognition of the pseudo-palindromic Ephrussi box (E-box) DNA element (Massari & Murre, 2000). TCF4 can dimerize with numerous other TFs, and interactions of TCF4 with ATHO1 (MATH1), HASH1 and NEUROD1 have been described in the brain (Flora, Garcia, Thaller, & Zoghbi, 2007; Navarrete et al., 2013). Mutations in PTHS patients affect different domains of the protein; however, the bHLH domain is described as a mutational hotspot (Whalen et al., 2012). Consequences of the mutations range from decreased binding ability to DNA or its dimerization partner to a loss-of-function or even a dominant-negative effect of the mutated protein (Sepp, Pruunsild, & Timmusk, 2012). In this regard, the authors like to emphasize not to confuse the *TCF4* (Gene ID:6925) discussed in this manuscript with the *transcription factor 7-like 2* gene (Gene ID:6934, official gene symbol *TCF7L2*). The latter is often referred to as *T-cell factor 4* which is commonly but mistakenly abbreviated as *TCF4*.

To understand TCF4 functions and PTHS pathobiology, different mouse models have been developed. Conventional homozygous deletion is lethal shortly after birth (Flora et al., 2007; Zhuang, Cheng, & Weintraub, 1996), and so previous work attempting to understand the function of TCF4 in brain development used heterozygous knockout mouse models, mimicking the situation in PTHS patients. It has been shown that these animals recapitulate different aspects of the human syndrome, such as an overall developmental delay, problems in social interactions and vocal articulation (Kennedy et al., 2016; Rannals, Page, et al., 2016b; Thaxton et al., 2018). Also, *Jung et al.* described the importance of TCF4 in the context of hippocampal and cortical neuron development and plasticity (Jung et al., 2018). That TCF4 might play a critical part in keeping up neuronal connectivity and excitatory-inhibitory balance was well described before by Sepp et al. (2017). In 2019, Li et al. used conventional knockout mice to study the consequences of a TCF4 deletion in the brain (Li et al., 2019). Newborn homozygous as well as newborn and adult heterozygous knockout mice were employed to describe a role of TCF4 in neural differentiation and migration, but their mouse model did not allow the study of a complete deletion of TCF4 in adult postnatal animals (Li et al., 2019). They also show abnormal dendrite and synapse formation in their mouse model, which was also reported by D'Rozario et al. (2016). It is therefore consequent that TCF4 is not only discussed in the context of PTHS, but has also been associated with different neurodevelopmental disorders such as ID, the autism spectrum disorder and schizophrenia (Brzozka, Radyushkin, Wichert, Ehrenreich, & Rossner, 2010; Forrest et al., 2018; Hasi et al., 2011; Moen et al., 2017; O'Donnell et al., 2010).

In this study, we used a central nervous system (CNS)-specific TCF4 knockout mouse model (*hGFAP-cre::Tcf4*$^{Fl/Fl}$), which allowed us to investigate an organ-specific homozygous knockout of TCF4 at different ages including adult animals in order to specifically identify structures affected by the knockout. Our data suggest a function of TCF4 in the formation of hippocampal and cortical architecture, in the differentiation of neuronal precursors, and in neuronal morphology in both, developing and adult mice. These findings complement previous studies on TCF4 and help to further understand its role in the pathophysiology of PTHS and in brain development in general.

2 | MATERIAL AND METHODS

2.1 | Mice

hGFAP-cre mice (Brenner, Kisseberth, Su, Besnard, & Messing, 1994; Zhuo et al., 2001) were purchased from The Jackson Laboratory. *Tcf4*$^{Fl/Fl}$ mice (Bergqvist et al., 2000) have been obtained from Dan Holmberg. Genotyping was performed with genomic DNA from ear biopsies with primer pairs described before (Bergqvist et al., 2000; Zhuo et al., 2001). Mice were bred on a C57BL/6 background and kept on a 12 hr dark/light cycle; water and food were available ad libitum. Animals of both sexes were used for the experiments. All experimental procedures were approved by the City of Hamburg (Reference: 113/16).

2.2 | Immunohistochemistry and quantification

Animals were decapitated under anaesthesia, and the brains were dissected. After fixating the brain tissues in 4% paraformaldehyde/PBS over night at room temperature, the tissue was dehydrated, embedded in paraffin and sectioned at 4 μm. Immunohistochemistry was subsequently performed using standard protocols with the following primary antibodies: PROX1 (ab199359, abcam, 1:500), SOX2 (ab97959, abcam, 1:200), CRE (PRB-106P, Biolegend, 1:3,000), KI67 (ab15580, abcam, 1:100), NEUN (MAB377, Merck Millipore, 1:25), CTIP2 (ab18465, abcam, 1:100), RELN (MAB5364, Merck Millipore, 1:500) and CUX1 (11733-1-AP, Proteintech, 1:200). Detection was performed using the SuperVision 2 HRP Kit (DCS Diagnostics) according to the standard protocol provided by the manufacturer. Cell nuclei were counterstained using haematoxylin. For quantifications, up to five representative pictures of the indicated region of three mice per genotype were taken and quantified. Image J 1.48a (Fiji)(NIH) was used by a blinded observer for quantification of thickness, distance and cell counting.

2.3 | Golgi–Cox staining and quantification

Golgi–Cox staining of adult mouse brains was performed using the FD Rapid GolgiStain Kit (FD NeuroTechnologies, Inc.) according to manufacturer's instructions. Briefly, freshly dissected brains were impregnated with a premade solution of mercuric chloride, potassium dichromate and potassium chromate for 1 week, cut in 200 μm slices with a vibrating blade microtome (Leica VT 1000S), and the impregnation was visualized with a solution provided by the manufacturer. For quantification of dendrite length and the number of branches, 10 representative pictures per mouse brain were taken and three mice per genotype were used.

2.4 | RNA-sequencing

After isolation of total RNA, the RNA integrity was analysed with the RNA 6,000 Nano Chip on an Agilent 2100 Bioanalyzer (Agilent Technologies). From total RNA, mRNA was extracted using the NEBNext Poly(A) mRNA Magnetic Isolation module (New England Biolabs) and RNA-Seq libraries were generated using the NEXTFLEX Rapid Directional qRNA-Seq Kit (Bioo Scientific) as per the manufacturer's recommendations. Concentrations of all samples were measured with a Qubit 2.0 Fluorometer (Thermo Fisher Scientific), and fragment lengths distribution of the final libraries was analysed with the DNA High Sensitivity Chip on an Agilent 2100 Bioanalyzer (Agilent Technologies). All samples were normalized to 2nM and pooled equimolar. The library pool was sequenced on the NextSeq500 (Illumina) with 1 × 75 bp, with 16.5 to 18.2 mio reads per sample.

2.5 | RNA-sequencing analysis

Gene abundance was quantified using salmon (v0.12.0) (Patro, Duggal, Love, Irizarry, & Kingsford, 2017) and mouse gene annotations from ENSEMBL (version 97) for GRCm38 genome assembly, and imported using R package tximport (Soneson, Love, & Robinson, 2015). Counts normalization and differential gene expression analysis were performed using DESeq2 package which automatically filters for low-expression genes and differentiates them for noise (Love, Huber, & Anders, 2014). Null variance of Wald test statistic output by DESeq2 was re-estimated using R package fdrtool (Strimmer, 2008) to calculate *p*-values (and adjusted using Benjamini-Hochburg method) for the final list of differentially expressed gene (DEG). An FDR (BH-adjusted *p*-values) <.1 was used a criteria for the final DE gene list. Gene ontology enrichment analysis was performed on the obtained gene list using topGO (Alexa & Rahnenfuhrer, 2019). Ten non-DE genes having similar mean expression level per each DE gene were selected for enrichment analysis as background using R package genefilter (Bourgon & Huber). GO plots for enrichment of biological process were plotted using R package clusterProfiler (Yu, Wang, Han, & He, 2012).

2.6 | Statistics

All data presented are mean ± *SEM* (standard error of the mean), and each data point represents an individual animal. Statistical analyses were done using the Prism 7 software (Graph Pad). p values ≤ .05 were considered significant ($*p \leq .05$, $**p \leq .01$, $***p \leq .001$, $****p \leq .0001$). The unpaired t test (two-tailed) was applied to compare the means of two groups. In order to compare percentages, values were arcsin transformed for statistical analysis.

3 | RESULTS

3.1 | CNS-specific deletion of TCF4 results in microcephaly

In this study, we employed a CNS-specific TCF4 knockout mouse model to study the protein function of TCF4. The CNS specificity was achieved with an inducible Cre/LoxP system,

in which the Cre recombinase is driven by the human glial fibrillary acidic protein (*hGFAP*) promoter and therefore starts being expressed on embryonic day E 13 (Zhuo et al., 2001). HGFAP expression is mainly found in astrocytes of adult brains, but previous studies by Schüller et al. and Spassky et al. demonstrated that also radial glia cells, which generate most cerebellar cell types, including (cerebellar) granule neuron precursors, interneurons, forebrain stem cells and astrocytes, express the Cre recombinase under the *hGFAP* promoter (Schüller et al., 2008; Spassky et al., 2008). Thereby, recombination leads to an effect in most, but not all cells of the adult nervous system. The Cre-driven recombination results in a truncated TCF4 protein, which lacks the protein domains transcribed from the exons 18, 19 and parts of exon 20 (Figure 1a). The translated protein misses the bHLH domain, which results in an abolished DNA-binding ability of the TF. Previous work on TCF4 showed that the bHLH domain is a mutational hotspot in PTHS patients (Whalen et al., 2012), emphasizing the previously established essential function of this protein domain (Murre, McCaw, & Baltimore, 1989; Voronova & Baltimore, 1990). The successful recombination was ensured by PCR: as expected, bands for the recombined TCF4 allele were visible in *hGFAP-cre::Tcf4*$^{Fl/wt}$ and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ but not in control mice carrying only the *hGFAP-cre* transgene (Figure 1b).

We generated control mice carrying only the *hGFAP-cre* transgene, mice carrying a recombined *Tcf4* gene on one allele (*hGFAP-cre::Tcf4*$^{Fl/wt}$) and mice carrying the truncating mutation homozygously (*hGFAP-cre::Tcf4*$^{Fl/Fl}$), henceforth referred to as mutant mice. With respect to the overall brain morphology, mice carrying the heterozygous mutation were indistinguishable from their control littermates (Figure 1c). However, in mice with homozygous *Tcf4* mutations, the brain appears smaller, mainly due to a considerably diminished telencephalon (Figure 1c). To quantify this observation, we weighed the freshly dissected brains of *hGFAP-cre*, *hGFAP-cre::Tcf4*$^{Fl/wt}$ and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice at three different time points during postnatal development (Figure 1d). In line with the visual impressions, *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice had a significantly reduced brain weight at all three developmental stages investigated (postnatal day (P) 7, 14 and adulthood). As the mutant mice were phenotypically distinct from the used controls but still viable beyond sexual maturity, and because a homozygous TCF4 loss in adult mice has not been investigated yet, we decided to focus our analyses on *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice.

3.2 | Differentiation and migration of neuronal precursors are dependent on Tcf4 function

In order to study the functions of TCF4 during embryonic development, we analysed prenatal mouse brains. We compared two embryonic stages: early after the start of the *hGFAP*-driven recombination activity at E14.5 and at E16.5. The comparison of the overall brain architecture (Figure 2a1,b1,c1,d1) did not reveal any major differences between control and mutant littermates. We then focused our investigations on two brain regions that are particularly important

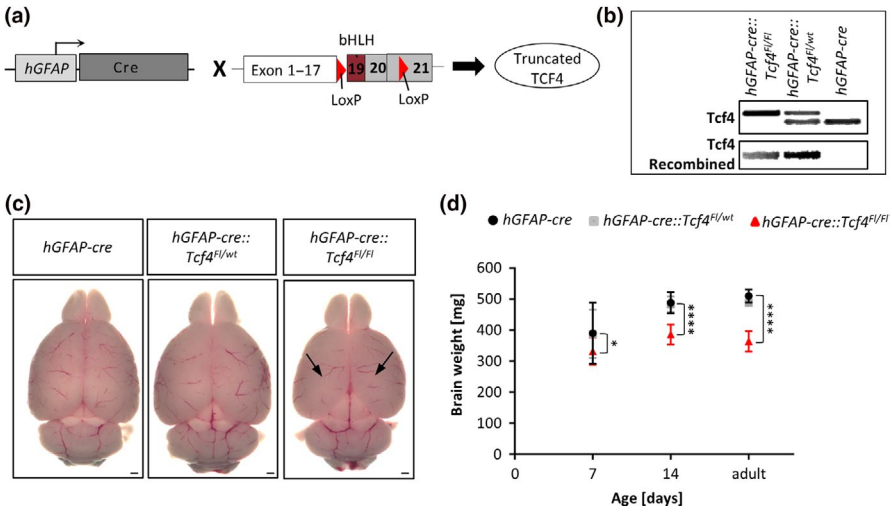


FIGURE 1 Description of *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice. (a) Schematic drawing of the mouse model used in this study. Cre-mediated recombination of the DNA sequence between the loxP sites (red triangles) results in a truncated TCF4 protein without the essential bHLH domain (dark red). (b) Agarose gel images showing the results of a PCR targeting the floxed TCF4 gene and the recombined allele. Mice carrying only wild-type TCF4 allele show no band in the recombination PCR. (c) Whole-mount photographs of brains from control mice and mice with a heterozygous or homozygous loss of Tcf4. Arrows point towards the smaller cerebral hemispheres. Scale bar = 250 μm. (d) Fresh brain weights of *hGFAP-cre*, *hGFAP-cre::Tcf4*$^{Fl/wt}$ and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice at different ages (n = 3 for P7, n = 8–15 for P14 and n = 3 for adult animals). *$p < .05$, ****$p < .0001$

in cognition: the cerebral cortex and the hippocampus. In comparison to the intact overall brain morphology of control mice, we detected a differentiation and migration deficit in neural precursor cells of mice without functional TCF4.

At E14.5, no differences were detectable, neither in morphology (Figure 2a2–a3/b2–b3) nor in the expression of PROX1, a marker for hippocampal granule cells (Figure 2a4/b4), or SOX2 and CRE (as a surrogate for hGFAP), markers for neural stem cells (Figure 2a5/b5,a6/b6).

We also examined the germinal centre of the developing cortex, the ventricular zone (VZ), visualized by the expression of SOX2. At E14.5, we did not see a difference in the thickness of the VZ (Figure 2a7/b7). We neither observed a difference in the amount or location of proliferating ($KI67^+$) cells (Figure 2a8/b8) nor regarding *hGFAP*-cre expressing stem cells (Figure 2a9/b9).

However, 2 days later in embryonic development, the control and the mutant mice became histologically distinct. In the control animals, the developing dentate gyrus with its PROX1-positive granular cells already had established its characteristic U-shape with the two blades of the dentate gyrus, whereas the PROX1-positive cells in the mutant mice did not form this anatomic structure (Figure 2c4/d4). The developing hippocampus was visibly smaller in *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice, whereas the adjacent germinal centre, the dentate neuroepithelium (DNE), was significantly thicker (Figure 2c2,c5/d2,d5,f). In agreement with the thickened DNE, significantly fewer SOX2-positive neuronal precursor cells were detectable within the developing dentate gyrus (2ry and 3ry matrix) in the mutant animals compared with healthy control mice (Figure 2c5/d5,g). These data suggest that the migration of neural precursor cells in the developing hippocampus from their germinal centre, the DNE, to their destination, the dentate gyrus, is disturbed in mice without a functional TCF4. Additionally, we evaluated the amount of further differentiated cells in the developing hippocampus, indicated by positivity of the neuronal marker NEUN. We observed that the number of NEUN-positive cells was significantly decreased in the mutant compared with the wild-type situation, in which cells of the developing dentate gyrus were differentiating and becoming NEUN-positive by E16.5 (Figure 2c6/d6,h).

We also studied the VZ as the germinal centre of the developing cortex for morphology and neural marker expression. The overall cortical architecture seemed to be intact independent of functional TCF4 (Figure 2c3/d3), but the thickness of the VZ was affected by a TCF4 deletion. The VZ was identified by the expression of the neural stem cell marker SOX2, and the thickness was measured (Figure 2c7/d7,e). In control mice, the thickness of the VZ decreased from E14.5 to E16.5. This decrease in VZ thickness was not detected in *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice, which results in a significantly thicker VZ at E16.5 in comparison to controls (Figure 2e).

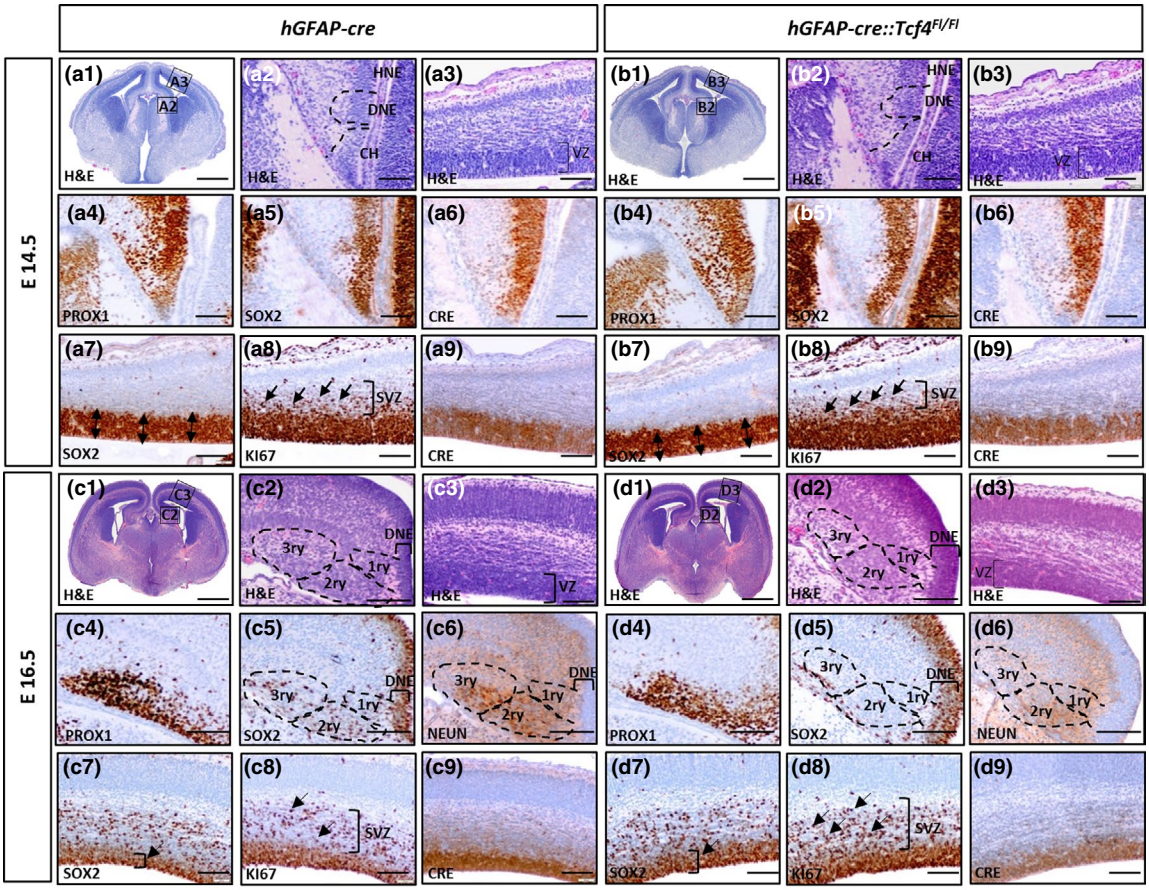
When taking a closer look at the proliferation in the subventricular zone (SVZ) at E16.5, indicated by KI67-positivity, we speculated about a higher rate of proliferation in mutant mice in comparison with control animals (Figure 2c8/d8). However, quantification of $KI67^+$ did not result in statistical significant differences (data not shown). Still, our finding support the notion that precursor cells in the mutant remain longer in their germinal centre and show a delayed differentiation compared with the control.

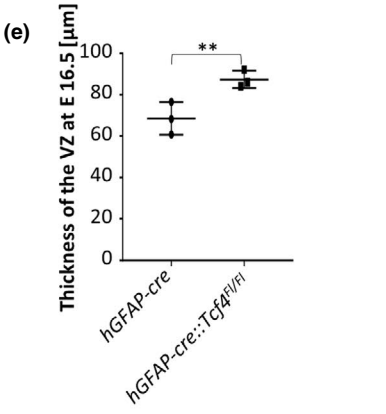
3.3 | TCF4 function is important for the differentiation of progenitor cells in the hippocampus and the hippocampal architecture

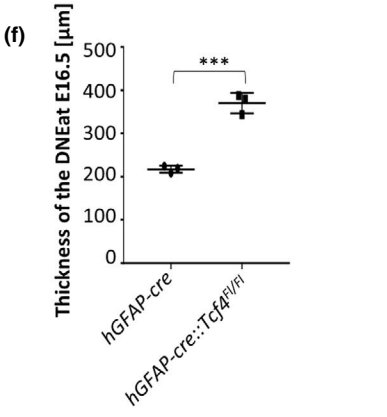
As one of the two neurogenic regions in the adult brain, the hippocampus is especially important for memory and cognition. Therefore, we extended our investigation of the embryonic development of *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice to an investigation of the postnatal hippocampus at three different postnatal developmental stages: newborn mice (P0), young mice (P7) and adult mice (P37). At all three analysed points in time, *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice were morphologically clearly distinguishable from the control animals. The entire hippocampus was visibly smaller, and the cellular architecture was disturbed. This is visible in H&E stains (Figure 3a1,a4/b1,b4;c1,c4/d1,d4;e1,e4/f1,f4) and in stains of the granule cell marker PROX1 (Figure 3a2/b2,c2/d2,e2/f2).

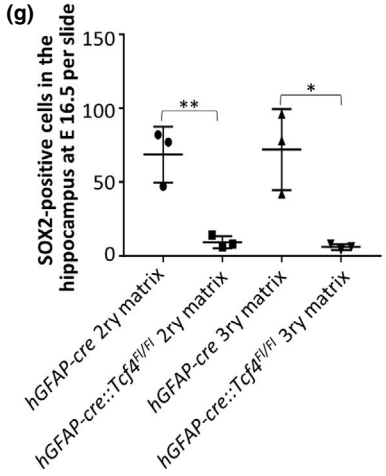
In line with the differentiation deficit that we observed at the embryonic stages, we also detected a delay in differentiation in the postnatal hippocampus. In the *hGFAP-cre* animals, the cornu ammonis as well as the dentate gyrus of a newborn animal contained NEUN-positive cells (Figure 3a3), and at P7, all granule cells of the hippocampus differentiated into NEUN-positive neurons (Figure 3c3). In contrast, in the *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice, no NEUN-positive cells were detected at P0 (Figure 3b3). At P7, some cells have differentiated into NEUN-positive cells, which is comparable to the amount of NEUN-positive cells at P0 in *hGFAP-cre* mice (Figure 3a3). At P37, all cells of the hippocampus in *hGFAP-cre* and in *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice were NEUN-positive (Figure 3e3/f3), thereby showing a differentiation delay in TCF4-deficient cells but no differentiation failure.

The granular cells of the cornu ammonis were highly dispersed in the mutant, whereas a tight band of cells formed the stratum pyramidalis in control brains (Figure 3a4–a5,c4–c5/b4–b5,d4–d5). This effect was more pronounced in the young animals, whereas in the adult, the cells were no longer widely dispersed throughout the entire cornu ammonis, but rather spots of dispersed cells throughout the stratum pyramidalis could be detected (Figure 3f7).


hGFAP-cre
hGFAP-cre::Tcf4Fl/Fl
E 14.5
E 16.5
H&E
PROX1
SOX2
CRE
KI67
NEUN
HNE
DNE
CH
VZ
SVZ
A2
A3
B2
B3
C2
C3
D2
D3
1ry
2ry
3ry


(e)
Thickness of the VZ at E 16.5 [µm]
hGFAP-cre
hGFAP-cre::Tcf4Fl/Fl


(f)
Thickness of the DNEat E16.5 [µm]
hGFAP-cre
hGFAP-cre::Tcf4Fl/Fl


(g)
SOX2-positive cells in the hippocampus at E 16.5 per slide
hGFAP-cre 2ry matrix
hGFAP-cre::Tcf4Fl/Fl 2ry matrix
hGFAP-cre 3ry matrix
hGFAP-cre::Tcf4Fl/Fl 3ry matrix

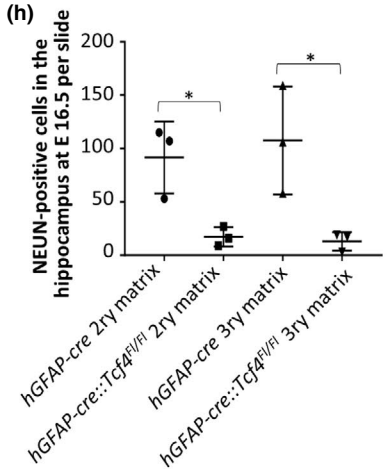

(h)
NEUN-positive cells in the hippocampus at E 16.5 per slide
hGFAP-cre 2ry matrix
hGFAP-cre::Tcf4Fl/Fl 2ry matrix
hGFAP-cre 3ry matrix
hGFAP-cre::Tcf4Fl/Fl 3ry matrix

FIGURE 2 TCF4 is important for embryonic development and differentiation. (a–b) Representative haematoxylin/eosin (H&E) stains and immunohistochemistry using antibodies against PROX1, SOX2, CRE and KI67 showing different brain regions of E14.5 *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice: the whole brain (a1/b1), the dorsal telencephalon (a2/b2, a4–a6/b4–b6) and the developing cortex (a3/b3, a7–a9/b7–b9). Arrows point towards the equal thickness of the VZ (a7/b7) and the similar number of proliferating cells above the VZ (a8/b8). CRE antibodies in a6, a9, b6 and b9 mark recombinase expression and serve as a surrogate marker for GFAP. (c–d) Representative H&E stains and immunohistochemistry using antibodies against PROX1, SOX2, CRE, NEUN and KI67 displaying different regions of the developing brain from E16.5 *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice: the whole brain (c1/d1), the dorsal telencephalon (c2/d2, c4–c6/d4–d6) and the developing cortex (c3/d3, c7–c9/d7–d9). PROX1$^+$ granule neuron precursor cells, SOX2$^+$ cells and NEUN$^+$ cells of the hippocampus are depicted in c4/d4, c5/d5 and c6/d6, respectively. The ventricular zone, identified by SOX2-positivity, is significantly thinner in the mutant mice (c7/d7). Proliferating (KI67$^+$) cells can be found within the subventricular zone (SVZ) of both control and mutant mice and do not differ significantly (c8/d8). CRE staining in c9 and d9 indicates recombinase activity in the VZ. (e–f) Quantification of the thickness of the VZ (e) and the DNE (f) at E16.5. Both zones were identified by SOX2-positive cells ($n = 3$, two-tailed unpaired t test). (g) Total number of SOX2-positive cells in the area of 2ry and 3ry matrix of the developing hippocampus at E16.5 per slide ($n = 3$, two-tailed unpaired t test). (h) Total number of NEUN-positive cells in the 2ry and 3ry matrix of the developing hippocampus at E16.5 per slide ($n = 3$, two-tailed unpaired t test). HNE = hippocampal neuroepithelium, DNE = dentate neuroepithelium, CH = cortical hem, VZ = ventricular zone, SVZ = subventricular zone, scale bar in a1/b1 = 500 μm, in a2–a9/b2–b9 = 100 μm, in c1/d1 = 500 μm, and in c2–c9/d2–d9 = 100 μm. *$p < .05$; **$p < .01$; ***$p < .001$

We took a closer look at the dispersion of cells of the CA1 region of the hippocampus and employed the granular cell marker CTIP2 and RELN as a marker for Cajal–Retzius cells and RELN-expressing interneurons of the hippocampus (Figure 3a8–a9, c8–c9,e8–e9/b8–b9,d8–d9,f8–f9). Specific analysis revealed that in control animals, RELN-expressing interneurons were evenly distributed throughout the stratum oriens of the CA1 region and CTIP2-positive granular cells formed the underlying stratum pyramidalis. In *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice, this layer structure was destroyed with up to 60% of misplaced RELN-expressing interneurons being located between CTIP2-positive neurons (Figure 3g). Our results intensify speculations about the putative role of TCF4 in the differentiation process of neuronal precursor cells.

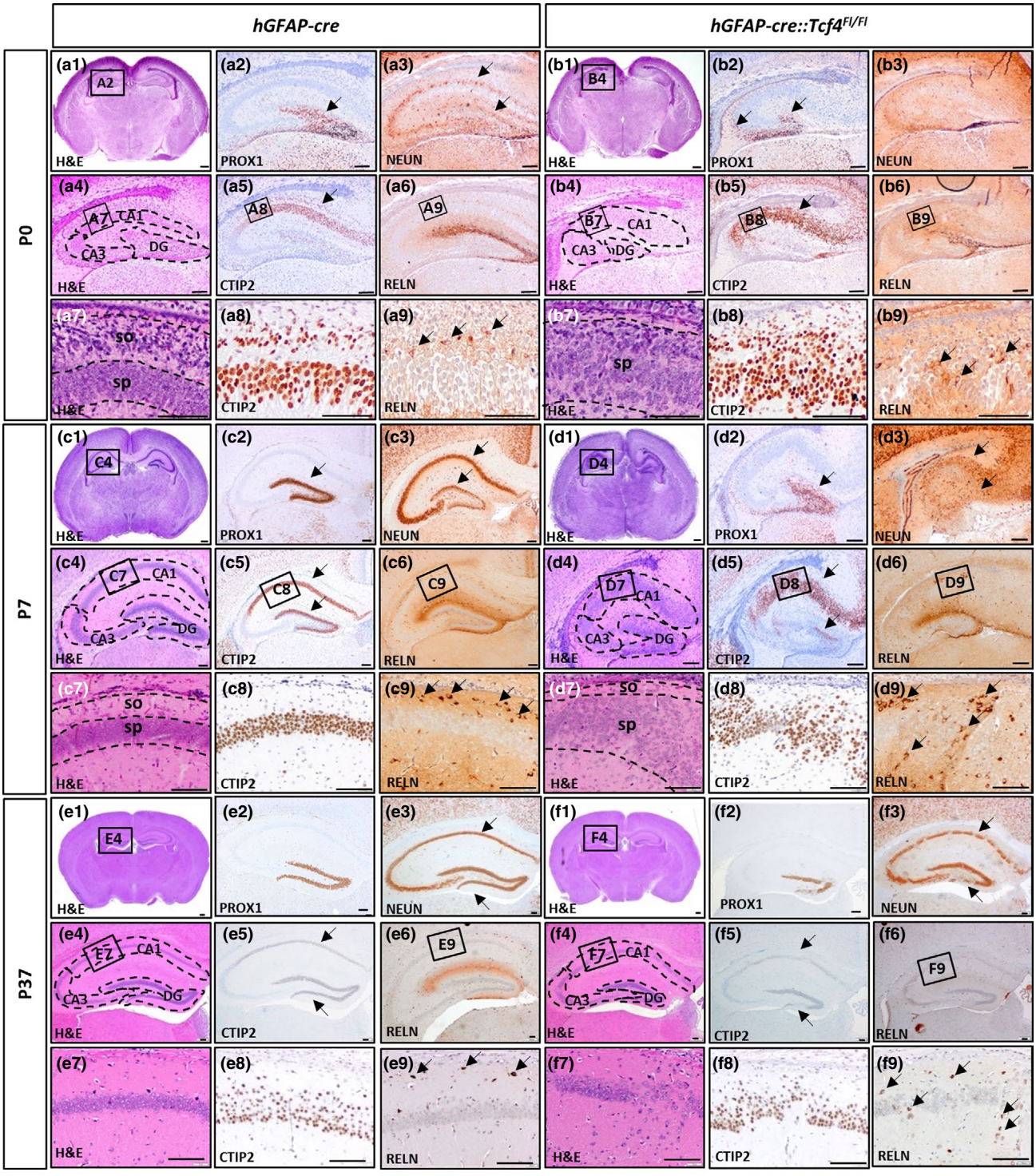
3.4 | Large fibre tracts are diminished in TCF4-deficient animals

As not only neurons but also other cell types of the developing brain are affected by the TCF4 deletion, we also analysed the corpus callosum as a large fibre tract in the mammalian brain (Figure 4). We investigated the myelination by employing the Klüver–Barrera (or Luxol fast blue) staining and the oligodendrocytes with the pan-oligodendrocyte marker Olig2. The CC develops postnatally, so that no difference of the overall morphology or myelination in the brain of newborn mice of either genotype can be observed (Figure 4a/b). However, at P7, the CC is clearly visible in the control animal (Figure 4c1) although not completely myelinated (Figure 4c2), whereas in the mutant, no CC is observable in the same sectioning plane (Figure 4d). In the adult animals, the CC developed also in the mutant animal (Figure 4f1), but is significantly thinner (Figure 4g). The myelination is weaker in mutant animals (Figure 4e2/f2), and the percentage of Olig2-positive oligodendrocytes is reduced in the TCF4-deficient animals (Figure 4e3/f3,h).

3.5 | The cortex layer structure and neuron morphology are affected by a TCF4 deletion, which is supported by the connection of TCF4 signalling and neuron differentiation and development in gene expression analysis

Additionally, we investigated the cerebral cortex in order to reveal effects of a TCF4 deletion. The overall architecture was preserved in mutant animals (Figure 5a1,c1,e1/b1,d1,f1). Therefore, we examined the cortical layer structure by staining for the neuronal markers CTIP2 and CUX1. CTIP2 was expressed by neurons of the deeper layers V and VI (Arlotta et al., 2005), whereas CUX1 was expressed in the upper layers II, III and IV (Nieto et al., 2004). For comparing the thickness of deeper and upper layers between mice, the absolute thickness of the respective layers was measured and respective values of the control mice were defined as 100% thickness. Subsequently, the relative thickness of the deeper and upper layers of mutant mice was calculated. The deep layers V and VI of mutant mice did not seem to be affected by the deletion (Figure 5a2,c2,e2/b2,d2,f2) and did not alter in thickness from the controls (data not shown), whereas the upper layers II/III were significantly thinner across all observed developmental stages in mutant mice (Figure 5a3,c3,e3/b3,d3,f3,g).

In order to gain more information about the underlying mechanisms of the ID in PTHS patients, we analysed the neuronal morphology of murine cortical neurons, which were stained by Golgi–Cox impregnation. We investigated the morphology of the pyramidal neurons of the cortical layer II and III (Figure 5h1). Abnormalities in neuronal morphology have been associated with different neurodevelopmental and neurodegenerative diseases (reviewed for example in Dierssen & Ramakers, 2006). We observed a trend of a shorter apical dendrite in *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice (Figure 5h2) and a significant increase in the number of branches (Figure 5h3). The formation of the deep


hGFAP-cre
hGFAP-cre::Tcf4Fl/Fl
P0
P7
P37
H&E
PROX1
NEUN
CTIP2
RELN
CA1
CA3
DG
so
sp

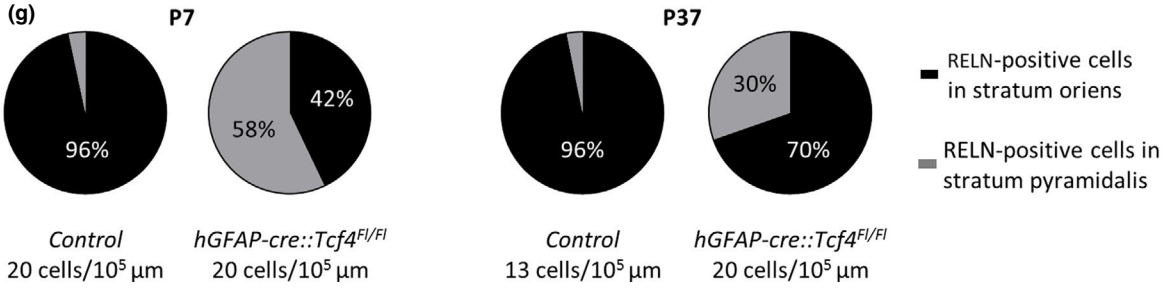

(g)
P7
P37
96%
58%
42%
96%
30%
70%
RELN-positive cells in stratum oriens
RELN-positive cells in stratum pyramidalis
Control
20 cells/10⁵ µm
hGFAP-cre::Tcf4Fl/Fl
20 cells/10⁵ µm
Control
13 cells/10⁵ µm
hGFAP-cre::Tcf4Fl/Fl
20 cells/10⁵ µm

FIGURE 3 Truncation of TCF4 leads to a developmental delay and a disturbance of the architecture in the postnatal hippocampus. (a–b) Representative haematoxylin/eosin (H&E) stains and immunohistochemistry using antibodies against PROX1, NEUN, CTIP2 and RELN of newborn *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mouse brains. Shown are sections through the whole brain (a1/b1), the hippocampus (a2–a6/b2–b6) and the CA1 region (a7–a9/b7–b9). Arrows point towards the PROX1-positive granule cells of the developing DG (a2/b2), NEUN-positive differentiated cells in the hippocampus (a3/b3), the CTIP2-positive cells of the cornu ammonis (a5/b5) and RELN-positive interneurons in the CA1 region (a9/b9). (c–d) Representative H&E stains and immunohistochemistry using antibodies against PROX1, NEUN, CTIP2 and RELN of postnatal day 7 (P7) *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mouse brains: the whole brain (c1/d1), the hippocampus (c2–c6/d2–d6) and the CA1 region (c7–c9/d7–d9). Arrows point towards PROX1-positive cells in the DG (c2/d2), NEUN-positive differentiated cells in the hippocampus (c3/d3), CTIP2-positive cells of CA1 and DG (c5/d5) and RELN-positive interneurons of the CA1 region (c9/d9). (e–f) Representative H&E stains and immunohistochemistry using antibodies against PROX1, NEUN, CTIP2 and RELN of adult (P37) *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mouse brains: the whole brain (e1/f1), the hippocampus (e2–e6/f2–f6) and the CA1 region (e7–e9/f7–f9). Arrows point towards NEUN-positive differentiated cells in the hippocampus (e3/f3), CTIP2-positive granule cells of CA1 and DG (e5/e5) and RELN-positive interneurons in the CA1 region (e9/f9). (g) Quantification of RELN-positive cells in the hippocampus at P7 and P37. CA = cornu ammonis, DG = dentate gyrus, so = stratum oriens, sp = stratum pyramidalis. Scale bar in a1/b1/c1/d1/e1/f1 = 300 μm, in all other images = 100 μm

cortical layers starts at embryonic day E10 (Angevine & Sidman, 1961), and therefore, 3 days before the used *hGFAP* promoter becomes active (Zhuo et al., 2001). Thus, we suspected the knockdown of TCF4 to not affect the deep cortical layers, as the greater part of neurons in these layers have been generated days before the knockdown. Still, analysis on mutant layer V pyramidal neurons showed the same tendency to shorter apical dendrites and a significantly increased number of branches (data not shown).

Finally, we were interested in the consequences of a CNS-specific TCF4 deletion regarding global gene expression and therefore performed RNA-sequencing (RNA-seq.) on the forebrains of newborn *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice. We employed three mice per genotype and included both sexes in our analysis. We identified 542 DEGs with an FDR (false discovery rate) smaller than 0.1 (Table S1) and thereby discriminated control and mutant samples (Figure 5h1). When we subjected the identified DEG to a GO term enrichment analysis, we found an enrichment of genes associated with neuron development, differentiation and function (Figure 5h2). This illustrated the importance of TCF4 in the correct development and maintenance of the nervous system, which is also demonstrated in the developing animal and in the analysed brain structures of cortex, corpus callosum and hippocampus.

4 | DISCUSSION

We employed a conditional knockout mouse model that allowed us to investigate the function of TCF4 not only in mouse brain development but also in adult mice. We describe here a role for TCF4 in the differentiation of neural progenitor cells, the establishment of proper cortical and hippocampal architecture and neuron morphology.

Whereas it might seem counterintuitive to use a homozygous knockout mouse model for TCF4 loss when PTHS is caused by a heterozygous mutation, we purposely decided to use the homozygous mice for different reasons. Firstly, heterozygous *hGFAP-cre::Tcf4*$^{Fl/wt}$ mice were indistinguishable from control animals in their brain morphology. This was unexpected to a certain extent, as for example mouse models with heterozygous *Tcf4* loss described by *Thaxton et al.* showed reduced brain- and bodyweights in comparison with wild-type controls (Thaxton et al., 2018). Likely, this can be explained by the used promoters in their study that become active several days before the used *hGFAP* promoter in this study and thus affect a larger number of cell types during the developmental process (Mignone, Kukekov, Chiang, Steindler, & Enikolopov, 2004; Shmerling et al., 2005). In addition, the weight measurements in *Thaxton et al.* were performed on mice aged P70-90. This leaves room for speculations, as stronger effects on brain weights might also be observed in *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice at later stages. Secondly, our used CNS-specific deletion of TCF4 is exceptional as it circumvents the early postnatal lethality of conventional TCF4 knockout mouse models (Flora et al., 2007; Zhuang et al., 1996) and allows us to study the effects of a homozygous TCF4 loss in adult mice. Although genotypically different from the situation found in PTHS patients, we observe a distinct CNS phenotype with anatomical rearrangements not found in other Tcf4 mouse models, without neglecting certain aspects of the human PTHS, such as the microcephaly, which affects 72% of all PTHS patients (Sweatt, 2013). After all, none of the published mouse models for PTHS features all characteristics of the syndrome. Therefore, we suggest our *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice can be a convincing tool to investigate a homozygous *Tcf4* loss in the CNS. It gives us the opportunity to study the affected structures in detail and to shed further light on the function of *Tcf4* during CNS development, helping us to understand the pathophysiology of PTHS eventually.

In order to study the effects of TCF4 on brain development and neuron differentiation further, we studied two embryonic stages of mouse development, E14.5 and E16.5. Based on the histological analysis of the embryonic brains of *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice, we conclude that TCF4 is important for the correct differentiation of neuronal precursor cells. The cells destined to build up the hippocampus and

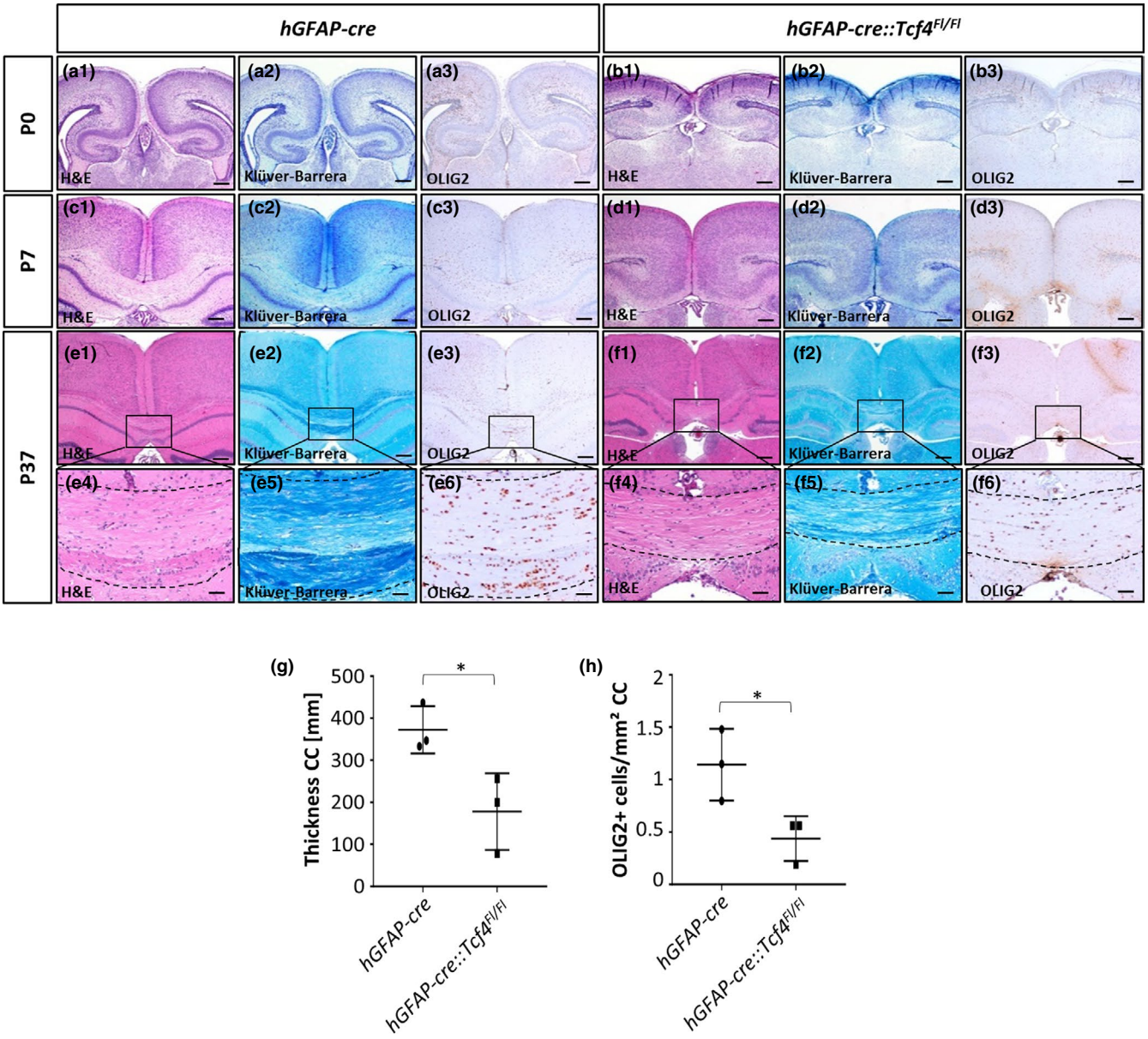


FIGURE 4 The corpus callosum is severely disturbed by a loss of TCF4. (a–b) Representative haematoxylin/eosin (H&E) stains, Klüver–Barrera stains, and immunohistochemistry using antibodies against OLIG2 of frontal newborn *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mouse brain slices. At this early age, no clear CC is visible neither in control, nor in mutant mice. (c–d) Representative haematoxylin/eosin (H&E) stains, Klüver–Barrera stains and immunohistochemistry using antibodies against OLIG2 of frontal 7 days old *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mouse brain slices. As opposed to the control, no CC is visible in the same plane in TCF4-deficient mice at this age. (e–f) Representative haematoxylin/eosin (H&E) stains, Klüver–Barrera stains and immunohistochemistry using antibodies against OLIG2 of frontal adult *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mouse brain slices. Shown are overviews of the CC (e1–3/f1–3) and magnifications of the central CC (e4–6/f4–6). A dotted line marks the borders of the CC (e4–6/f4–6), which is distinctly thinner in the mutant. (g) The thickness of the CC, measured in H&E stains of adult animals, is significantly reduced in TCF4-deficient animals. (h) The percentage of OLIG2-positive cells per mm^2 is decreased in mutant animals. CC = corpus callosum. Scale bar = 200 μm (a–d, e1–3, f1–3); 50 μm (e3–6/f3–6). *$p < .05$

cortex appear to remain longer in the germinal centres (DNE and VZ) and do not migrate out and differentiate properly.

Being mainly expressed during brain development (Jung et al., 2018; Rannals, Hamersky, et al., 2016a), numerous research groups have shed light upon different functions of TCF4 in this critical process. For example, in the SVZ, a zone of postnatal neurogenesis, TCF4 expression is described to be enriched and essential for the differentiation of progenitor cells (Fischer et al., 2014). Furthermore, previous studies have suggested an association of TCF4 functions with neuronal development as well as differentiation and migration of cortical progenitors (Chen et al., 2016; Flora et al., 2007; Forrest, Waite, Martin-Rendon, & Blake, 2013; Hill et al., 2017; Li et al., 2019; Page et al., 2018). One potential

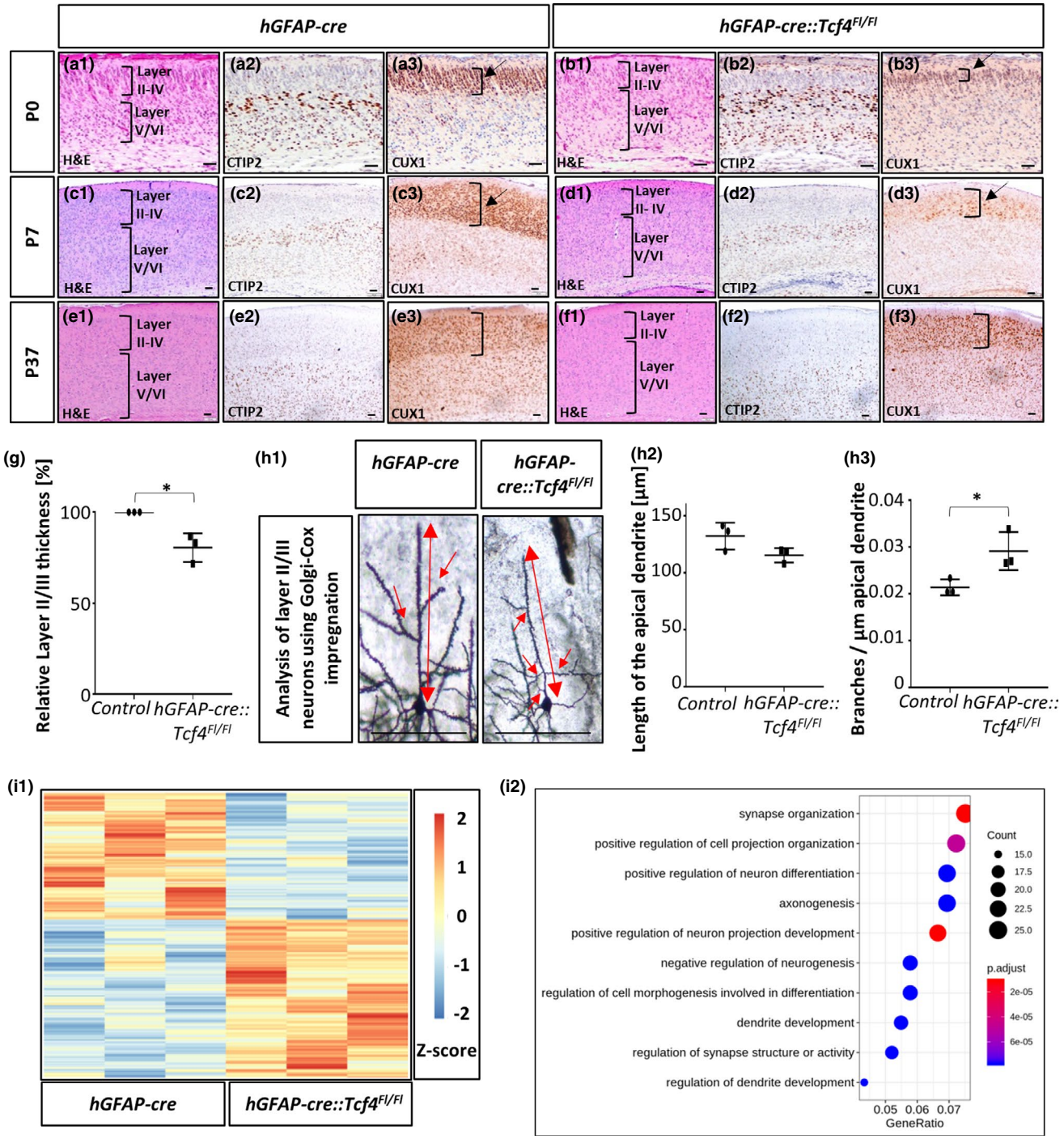


FIGURE 5 Truncation of TCF4 results in cortical layer abnormalities and defects in neuronal morphology and affects genes responsible for differentiation and maturation. (a–f) Representative H&E stains and immunohistochemistry using antibodies against CTIP2 and CUX1 of newborn (a1–a3/b1–b3), seven-day-old (c1–c3/d1–d3) and adult (e1–e3/f1–f3) *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mouse brains. Arrows point towards the thickness of the CUX1-positive neurons of the cortical layers II and III (a2/b2, c2/d2, e2/f2). (g) Quantification of layer II/III thickness: absolute layer thickness of the CUX1 positive cortical layer II/II was measured across all three age stages. Layer thickness of control mice was defined as 100%, and the relative layer thickness of mutant mice was calculated. Compared to the wild-type control, *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice showed a significant reduction in layer II/III thickness ($n = 5$). (h) Representative images of Golgi–Cox impregnated layer II/III neurons of adult *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mouse brains. Arrows point towards the dendrite length and branches of the apical dendrite (h1). Measurement of the length of apical dendrites (h2) and number of branches of the apical dendrite (h3) of *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice. $n = 3$, 40 neurons per mouse. (i) Analysis of differential gene expression in the forebrain of newborn *hGFAP-cre* and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice via RNA-sequencing. In total, 542 DEGs with a false discovery rate of <0.1 were identified. The differential expression of genes discriminates control and mutant mice (i1). GO term enrichment correlates Tcf4 with neuron differentiation and development (i2). Scale bar = 100 µm. *$p < .05$

mechanism how TCF4 regulates differentiation is the association with other TFs (Flora et al., 2007). Other investigations on TCF4 conclusively showed that TCF4 is crucial for the correct neuronal migration in the developing cortex and that a knockout of TCF4 causes a differentiation delay (Li et al., 2019; Page et al., 2018). By using conventional heterozygous and homozygous knockout mouse models, the effects of a homozygous deletion were so far only studied embryonically and in early postnatal animals. Hence, our observations in adult mice carrying a CNS-specific homozygous deletion in the *Tcf4* gene further complement the understanding of TCF4 function. In summary, our results are in line with previous evidence and support the hypothesis that TCF4 is important for neuronal differentiation and that a differentiation deficit is a part of the pathobiology of PTHS.

When we analysed the postnatal hippocampus of *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice, we noticed that it resembles aspects of the well-characterized Reeler mouse. The Reeler mouse was first described in 1951 (Falconer, 1951) as a spontaneous mutant with severe neurological deficits and RELN was identified as the causing protein in 1995 (D'Arcangelo et al., 1995; Ogawa et al., 1995). These mice have a disrupted laminar organization of the cortex, a disturbed hippocampal architecture and a hypoplastic cerebellum (Fatemi, 2001). Mice with mutations in other members of the Reelin signalling pathway such as DAB1 show similar phenotypes (for example: Kojima, Nakajima, & Mikoshiba, 2000; Sheldon et al., 1997). Mutations in the human *RELN* gene are an underlying cause of lissencephaly (Chang et al., 2007) and, additionally, *RELN* mutations are associated with schizophrenia, mood disorders, autism, polymicrogyria, epilepsy and Alzheimer's disease (reviewed for example in Ishii, Kubo, & Nakajima, 2016). The study of RELN-expressing cells in our mouse model revealed no obvious reduction in their number, but a misplacement of RELN-expressing interneurons of the CA1 region (Figure 3a9,c9,e9/b9,d9,f9,g). As Reelin signalling is important for correct migration of neuronal precursor cells in the cortex and the hippocampus (Katsuyama & Terashima, 2009), it is feasible to speculate if the described misplacement of RELN-expressing cells is the underlying cause of the granular cell dispersion in our mouse model. The TFs converting the Reln signal into gene expression are not fully known. The serum response factor (SRF) was identified as a downstream target of Reelin signalling, and both the hippocampus of SRF-depleted mice and our TCF4 mutant mice show similar characteristics: a generally smaller hippocampus and dispersed granular cells with RELN-expressing interneurons within the granular cell layer of the stratum pyramidalis (Stritt & Knöll, 2010). This similarity raises the question if TCF4, like SRF, might be a downstream target of RELN. That TCF4 function might be crucial for several genes preferentially expressed by neurons of the hippocampal CA1 region was proposed before (Xia et al., 2018). A possible connection between the expression of TCF4 and its interaction with Reelin signalling can be established via NOTCH1, whose expression has been shown to be affected by altered TCF4 expression in different in vitro experiments (Forrest et al., 2013; Xia et al., 2018). In turn, NOTCH1 itself interacts with Reelin signalling (Hashimoto-Torii et al., 2008). Also, analysis of publicly available RNA-seq data from *Li et al.* illustrates that both heterozygous and homozygous *Tcf4* knockout mice show a certain modification in reelin signalling (Figure S1). Certainly, this proposition requires further investigations, but if a link between RELN and TCF4 signalling was established, it might help to further understand the molecular mechanisms underlying the pathology of the PTHS.

In addition to the hippocampus, we analysed the neocortex of TCF4-deficient animals. We noted a thinning of the upper layers (CUX1^{+}), whereas the deeper layers (CTIP2^{+}) remain unimpaired by the deletion (Figure 4). These observations fit to the results described by Li et al. Their described conventional homozygous TCF4 knockout mouse models show hardly any CUX1-expressing cells in the upper layer of the neocortex except for regions of the frontal/motor cortex (Li et al., 2019). The differences in the effects on the different layers in our model might also at least be partly explained by the promoter activity of the *hGFAP* promoter. It is known that the promoter, and thereby the recombination mediated by CRE, becomes active around E13 (Zhuo et al., 2001). The generation of cortical neurons in the mouse starts around E10.5 (Hanashima & Toma, 2015), and consequently, early generated neurons, which build up the deeper layers V and VI, might not be as strongly affected by the Cre-driven deletion of TCF4.

We hypothesized before that TCF4 might be linked to RELN signalling and thereby affects the hippocampal architecture. We have described similarities between the phenotype of Reeler and *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice and a misplacement of RELN-expressing cells, which might be causing the granular cell dispersion in the hippocampus. In the cortex, the situation seems to be different. Whereas the cortical structure in the Reeler mice is completely disrupted (Fatemi, 2001), the cortex of *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice is intact, except for a thinning of the upper layers. Possible explanations for those observed differences between cortex and hippocampus might be an independence of RELN signalling from TCF4 in the cortex or the different developmental time points these structures start to develop. The cortical layer structure is established from E10.5 onwards by the migration of Cajal–Retzius cells to the area, which becomes the cortical layer I later in development (Hanashima & Toma, 2015). These cells secrete RELN and thereby organize the inside-out migration of cortical neurons. The hippocampus development, in comparison, only starts at E12.5 with the migration of Cajal–Retzius cells to the presumptive DNE (reviewed in Urbán & Guillemot, 2014). It seems obvious to argue that the hippocampus

development might be more severely affected as the expression of the Cre recombinase, and thus the loss of functional TCF4 starts early enough to interfere with early hippocampal, but not cortical development. Still, it is important to consider that the layering of the cortex might be at least partially independent of the presence of Cajal–Retzius cells in the layer I of the cortex (Yoshida, Assimacopoulos, Jones, & Grove, 2006). Certainly, these discrepancies need to be investigated in future studies. Apart from this, taking into account the complexity of the hippocampal architecture, it is reasonable to suspect numerous TFs to be needed to orchestrate the assembly of the hippocampus and that a loss of only one of those TFs, like TCF4, could hamper proper Reelin signalling, leading to the observed misconstruction of the hippocampus.

The CC is a major fibre tract in the mammalian brain, connecting both hemispheres. Abnormalities of the CC are associated with intellectual disability, autism and schizophrenia (reviewed for example in Paul et al., 2007). Furthermore, hypoplasia of the CC is a common clinical feature of PTHS patients (Marangi et al., 2012), and this is also reflected by our mouse model, which shows a delayed development of the CC and a reduced size (Figure 4). Additional to the reduced overall size of the CC, the percentage of Olig2-positive oligodendrocytes is reduced in animals without functional TCF4. Obviously, a reduced amount of oligodendrocytes is associated with a reduced myelination and differentiation of the CC, and this is in line with the differentiation delay observed in the other brain structures and the overall developmental delay in PTHS patients. This is in line with results from *Kennedy et al.*, as their heterozygous Tcf4 knockout mouse model shows a significant downregulation of numerous genes associated with myelination (Kennedy et al., 2016).

We were able to generate in vivo data of postmitotic neurons in adult homozygous knockout mice. This complements findings regarding the impact of TCF4 on neuron function and morphology previously generated by others. It has been shown before that TCF4 is important for the excitability and the long-term potentiation of neurons (Jung et al., 2018; Rannals, Hamersky, et al., 2016a). Additionally, it was demonstrated that TCF4 is also able to regulate dendritic spine density and spine morphology (Crux, Herms, & Dorostkar, 2018). It is believed that a disturbed neuronal morphology is a common feature in ID and might cause cognitive impairments (reviewed for example in Dierssen & Ramakers, 2006). The effect of TCF4 on dendritic branching has been described in 2016 by D`Rozario et al., who proposed that TCF4 restricts neurite branching at least partly through the repression of Neurexin. They showed that upon TCF4 knockout in postmitotic neurons, the number of dendritic branches is significantly increased, whereas the length of the longest dendrite (corresponding to the apical dendrite) is not altered (D'Rozario et al., 2016). Our in vivo data from *hGFAP-cre::Tcf4*$^{Fl/Fl}$ mice are in line with their observations despite using a different model. Li et al. analysed neuronal morphology in a heterozygous conventional knockout mouse model and observed an increased apical dendrite length and a reduced dendritic branching (Li et al., 2019). Although their results seem to contradict the observations made in our mouse model, together our data support the notion that TCF4 is able to shape neuron morphology and thereby influences neuron function. Therefore, we hypothesize that a disturbance of neuronal morphology is an important factor in the development of ID in PTHS patients.

The effect of TCF4 signalling on neuronal morphology and differentiation is also reflected in our global gene expression analysis of the forebrains of newborn mice. In total, 542 DEGs with an FDR < 0.1 were identified by RNA-sequencing which also shows the global effects of a TCF4 deletion. The GO term enrichment of the DEGs shows that especially genes involved in neuron differentiation and dendrite development are affected by the TCF4 deletion, which fits to the observed phenotypical abnormalities. In the past years, there have been several different studies trying to unravel the complex network of genes regulated by TCF4. Matching our data, differences in the expression of genes associated with axon/neuronal development (Xia et al., 2018), axon guidance (Doostparast Torshizi et al., 2019), neuronal differentiation (Quevedo et al., 2019) and growth (Doostparast Torshizi et al., 2019) have been described following TCF4 knockdown in vitro. Other studies have reported that TCF4 knockdown in vitro rather leads to a differential expression of genes mainly involved in cell cycle regulation and apoptosis (Hill et al., 2017; Moen et al., 2017), especially in the context of the putative tumour-suppressive role of TCF4 in cancer development (Hellwig et al., 2019; Herbst, Helferich, Behrens, Goke, & Kolligs, 2009). This might seem incompatible with the other studies. However, many of the genes found to be altered are not only associated with cell cycling, but also with schizophrenia (Hill et al., 2017; Moen et al., 2017). Adding to this, there have been in vivo studies describing severe differences in gene expression following heterozygous and also homozygous knockout of *Tcf4* in mice. In line with our results, conventional heterozygous *Tcf4* knockdown resulted mainly in dysregulation of biochemical pathways linked to neuronal plasticity, axon guidance and neurogenesis (Kennedy et al., 2016). Beyond that, analysis of newborn homozygous *Tcf4* knockout mice revealed a critical downregulation of genes involved in synapse and neuron formation as well as neurite outgrowth further supporting an involvement of TCF4 in these processes (Li et al., 2019). Additionally, they demonstrate that a deletion of TCF4 results in a shift towards a more immature gene signature (Li et al., 2019). The results from our own global gene expression analysis and from the data generated by others are in line with the here described histologically observed differentiation delay in hippocampus, corpus callosum and cortex and the abnormalities in neuron morphology.

Together, the data from the different developmental stages and the global gene expression data strongly suggest that TCF4 is important for the correct development and differentiation of the nervous system including neurons and other cell types like oligodendrocytes. The understanding of the global function of TCF4 and the analysis of different aspects like the disturbances in hippocampal architecture and the abnormalities of dendrite morphology and CC hypoplasia might help to understand the mechanisms underlying the ID of PTHS patients. We believe that our results add to a full understanding of the role of TCF4 in brain development in general. The unveiling of such molecular mechanisms leading to the clinical presentation of the PTHS will help us to understand the disorder properly, improve the life of patients, pave the way for future studies and lead to the development of therapeutic agents eventually.

ACKNOWLEDGEMENTS

The authors are indebted to Margarethe Gregersen, Gundula Pilnitz-Stolze, Michael Schmidt, Anne Reichstein, Jacqueline Kolanski and Kristin Hartmann for excellent technical support. We thank Dr. Dan Holmberg (Lund University, Sweden) for providing *Tcf4*$^{Fl/Fl}$ mice. This work was supported by the Fördergemeinschaft Kinderkrebs-Zentrum Hamburg, the Roggenbuck Stiftung and the Wilhelm-Sander-Stiftung. MH was supported by the Burkhard Meyer Stiftung and MS by the Hans Brökel Stiftung für Wissenschaft und Kultur.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conceptualization and design of the study: M. S., M. H., U. S.; Methodology, Formal Analysis, and Investigation: M. S., M. H., L. H., D. H., M. C. L., J. N., S.V., and D.I.; Writing and visualization: M. S., M. H. and U. S.; Supervision: U. S.

DATA AVAILABILITY STATEMENT

All data generated and analysed in this study are available from the corresponding author upon reasonable request.

ORCID

Malte Hellwig https://orcid.org/0000-0002-9239-956X

REFERENCES

Alexa, A., & Rahnenfuhrer, J. (2019). *topGO: Enrichment Analysis for Gene Ontology*. R package version 2.36.0.

Amiel, J., Rio, M., de Pontual, L., Redon, R., Malan, V., Boddaert, N., … Colleaux, L. (2007). Mutations in TCF4, encoding a class I basic helix-loop-helix transcription factor, are responsible for Pitt-Hopkins syndrome, a severe epileptic encephalopathy associated with autonomic dysfunction. *American Journal of Human Genetics*, *80*, 988–993. https://doi.org/10.1086/515582

Angevine, J. B. Jr., & Sidman, R. L. (1961). Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature*, *192*, 766–768. https://doi.org/10.1038/192766b0

Arlotta, P., Molyneaux, B. J., Chen, J., Inoue, J., Kominami, R., & Macklis, J. D. (2005). Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. *Neuron*, *45*, 207–221. https://doi.org/10.1016/j.neuron.2004.12.036

Bergqvist, I., Eriksson, M., Saarikettu, J., Eriksson, B., Corneliussen, B., Grundstrom, T., & Holmberg, D. (2000). The basic helix-loop-helix transcription factor E2–2 is involved in T lymphocyte development. *European Journal of Immunology*, *30*, 2857–2863. https://doi.org/10.1002/1521-4141(200010)30:10<2857:AID-IMMU2857>3.0.CO;2-G

Bourgon, R. & Huber, W. https://www.rdocumentation.org/packages/genefilter/versions/1.54.2.

Brenner, M., Kisseberth, W. C., Su, Y., Besnard, F., & Messing, A. (1994). GFAP promoter directs astrocyte-specific expression in transgenic mice. *Journal of Neuroscience*, *14*, 1030–1037. https://doi.org/10.1523/JNEUROSCI.14-03-01030.1994

Brockschmidt, A., Todt, U., Ryu, S., Hoischen, A., Landwehr, C., Birnbaum, S., … Weber, R. G. (2007). Severe mental retardation with breathing abnormalities (Pitt-Hopkins syndrome) is caused by haploinsufficiency of the neuronal bHLH transcription factor TCF4. *Human Molecular Genetics*, *16*, 1488–1494. https://doi.org/10.1093/hmg/ddm099

Brzozka, M. M., Radyushkin, K., Wichert, S. P., Ehrenreich, H., & Rossner, M. J. (2010). Cognitive and sensorimotor gating impairments in transgenic mice overexpressing the schizophrenia susceptibility gene Tcf4 in the brain. *Biological Psychiatry*, *68*, 33–40. https://doi.org/10.1016/j.biopsych.2010.03.015

Chang, B. S., Duzcan, F., Kim, S., Cinbis, M., Aggarwal, A., Apse, K. A., … Walsh, C. A. (2007). The role of RELN in lissencephaly and neuropsychiatric disease. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics.*, *144b*, 58–63. https://doi.org/10.1002/ajmg.b.30392

Chen, T., Wu, Q., Zhang, Y., Lu, T., Yue, W., & Zhang, D. (2016). Tcf4 controls neuronal migration of the cerebral cortex through regulation of Bmp7. *Front Mol Neurosci*, *9*, 94. https://doi.org/10.3389/fnmol.2016.00094

Crux, S., Herms, J., & Dorostkar, M. M. (2018). Tcf4 regulates dendritic spine density and morphology in the adult brain. *PLoS ONE*, *13*, e0199359. https://doi.org/10.1371/journal.pone.0199359

D'Arcangelo, G., Miao, G. G., Chen, S. C., Soares, H. D., Morgan, J. I., & Curran, T. (1995). A protein related to extracellular matrix proteins deleted in the mouse mutant reeler. *Nature*, *374*, 719–723. https://doi.org/10.1038/374719a0

Dierssen, M., & Ramakers, G. J. A. (2006). Dendritic pathology in mental retardation: From molecular genetics to neurobiology. *Genes, Brain and Behavior*, *5*, 48–60. https://doi.org/10.1111/j.1601-183X.2006.00224.x

Doostparast Torshizi, A., Armoskus, C., Zhang, H., Forrest, M. P., Zhang, S., Souaiaia, T., … Wang, K. (2019). Deconvolution of transcriptional networks identifies TCF4 as a master regulator in schizophrenia. *Science Advances*, *5*, eaau4139.

D'Rozario, M., Zhang, T., Waddell, E. A., Zhang, Y., Sahin, C., Sharoni, M., … Marenda, D. R. (2016). Type I bHLH proteins daughterless and Tcf4 restrict neurite branching and synapse formation by repressing neurexin in postmitotic neurons. *Cell Reports*, *15*, 386–397. https://doi.org/10.1016/j.celrep.2016.03.034

Falconer, D. S. (1951). Two new mutants, 'trembler' and 'reeler', with neurological actions in the house mouse (*Mus musculus* L.). *Journal of Genetics*, *50*, 192–201. https://doi.org/10.1007/BF02996215

Fatemi, S. H. (2001). Reelin mutations in mouse and man: From reeler mouse to schizophrenia, mood disorders, autism and lissencephaly. *Molecular Psychiatry*, *6*, 129–133. https://doi.org/10.1038/sj.mp.4000129

Fischer, B., Azim, K., Hurtado-Chong, A., Ramelli, S., Fernandez, M., & Raineteau, O. (2014). E-proteins orchestrate the progression of neural stem cell differentiation in the postnatal forebrain. *Neural Development*, *9*, 23. https://doi.org/10.1186/1749-8104-9-23

Flora, A., Garcia, J. J., Thaller, C., & Zoghbi, H. Y. (2007). The E-protein Tcf4 interacts with Math1 to regulate differentiation of a specific subset of neuronal progenitors. *Proceedings of the National Academy of Sciences of the United States of America*, *104*, 15382–15387. https://doi.org/10.1073/pnas.0707456104

Forrest, M. P., Hill, M. J., Kavanagh, D. H., Tansey, K. E., Waite, A. J., & Blake, D. J. (2018). The psychiatric risk gene transcription factor 4 (TCF4) regulates neurodevelopmental pathways associated with schizophrenia, autism, and intellectual disability. *Schizophrenia Bulletin*, *44*, 1100–1110. https://doi.org/10.1093/schbul/sbx164

Forrest, M. P., Waite, A. J., Martin-Rendon, E., & Blake, D. J. (2013). Knockdown of human TCF4 affects multiple signaling pathways involved in cell survival, epithelial to mesenchymal transition and neuronal differentiation. *PLoS ONE*, *8*, e73169. https://doi.org/10.1371/journal.pone.0073169

Hanashima, C., & Toma, K. (2015). Switching modes in corticogenesis: Mechanisms of neuronal subtype transitions and integration in the cerebral cortex. *Frontiers in Neuroscience*, *9*. https://doi.org/10.3389/fnins.2015.00274

Hashimoto-Torii, K., Torii, M., Sarkisian, M. R., Bartley, C. M., Shen, J., Radtke, F., … Rakic, P. (2008). Interaction between Reelin and Notch signaling regulates neuronal migration in the cerebral cortex. *Neuron*, *60*, 273–284. https://doi.org/10.1016/j.neuron.2008.09.026

Hasi, M., Soileau, B., Sebold, C., Hill, A., Hale, D. E., O'Donnell, L., & Cody, J. D. (2011). The role of the TCF4 gene in the phenotype of individuals with 18q segmental deletions. *Human Genetics*, *130*, 777–787. https://doi.org/10.1007/s00439-011-1020-y

Hellwig, M., Lauffer, M. C., Bockmayr, M., Spohn, M., Merk, D. J., Harrison, L., … Schüller, U. (2019). TCF4 (E2–2) harbors tumor suppressive functions in SHH medulloblastoma. *Acta Neuropathologica*, *137*, 657–673. https://doi.org/10.1007/s00401-019-01982-5

Herbst, A., Helferich, S., Behrens, A., Goke, B., & Kolligs, F. T. (2009). The transcription factor ITF-2A induces cell cycle arrest via p21(Cip1). *Biochemical and Biophysical Research Communications*, *387*, 736–740. https://doi.org/10.1016/j.bbrc.2009.07.102

Hill, M. J., Killick, R., Navarrete, K., Maruszak, A., McLaughlin, G. M., Williams, B. P., & Bray, N. J. (2017). Knockdown of the schizophrenia susceptibility gene TCF4 alters gene expression and proliferation of progenitor cells from the developing human neocortex. *Journal of Psychiatry and Neuroscience*, *42*, 181–188. https://doi.org/10.1503/jpn.160073

Ishii, K., Kubo, K.-I., & Nakajima, K. (2016). Reelin and neuropsychiatric disorders. *Frontiers in Cellular Neuroscience*, *10*, 229–229. https://doi.org/10.3389/fncel.2016.00229

Jones, S. (2004). An overview of the basic helix-loop-helix proteins. *Genome Biology*, *5*, 226.

Jung, M., Haberle, B. M., Tschaikowsky, T., Wittmann, M. T., Balta, E. A., Stadler, V. C., … Lie, D. C. (2018). Analysis of the expression pattern of the schizophrenia-risk and intellectual disability gene TCF4 in the developing and adult brain suggests a role in development and plasticity of cortical and hippocampal neurons. *Molecular Autism*, *9*, 20. https://doi.org/10.1186/s13229-018-0200-1

Katsuyama, Y., & Terashima, T. (2009). Developmental anatomy of reeler mutant mouse. *Development, Growth & Differentiation*, *51*, 271–286. https://doi.org/10.1111/j.1440-169X.2009.01102.x

Kennedy, A. J., Rahn, E. J., Paulukaitis, B. S., Savell, K. E., Kordasiewicz, H. B., Wang, J., … Sweatt, J. D. (2016). Tcf4 regulates synaptic plasticity, DNA methylation, and memory function. *Cell Reports*, *16*, 2666–2685. https://doi.org/10.1016/j.celrep.2016.08.004

Kojima, T., Nakajima, K., & Mikoshiba, K. (2000). The disabled 1 gene is disrupted by a replacement with L1 fragment in yotari mice. *Molecular Brain Research*, *75*, 121–127. https://doi.org/10.1016/S0169-328X(99)00313-7

Li, H., Zhu, Y., Morozov, Y. M., Chen, X., Page, S. C., Rannals, M. D., … Rakic, P. (2019). Disruption of TCF4 regulatory networks leads to abnormal cortical development and mental disabilities. *Molecular Psychiatry*, *24*, 1235–1246. https://doi.org/10.1038/s41380-019-0353-0

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*, 550. https://doi.org/10.1186/s13059-014-0550-8

Marangi, G., Ricciardi, S., Orteschi, D., Tenconi, R., Monica, M. D., Scarano, G., … Zollino, M. (2012). Proposal of a clinical score for the molecular test for Pitt-Hopkins syndrome. *American Journal of Medical Genetics. Part A*, *158A*, 1604–1611. https://doi.org/10.1002/ajmg.a.35419

Massari, M. E., & Murre, C. (2000). Helix-loop-helix proteins: Regulators of transcription in Eucaryotic organisms. *Molecular and Cellular Biology*, *20*, 429–440. https://doi.org/10.1128/MCB.20.2.429-440.2000

Mignone, J. L., Kukekov, V., Chiang, A. S., Steindler, D., & Enikolopov, G. (2004). Neural stem and progenitor cells in nestin-GFP transgenic mice. *The Journal of Comparative Neurology*, *469*, 311–324. https://doi.org/10.1002/cne.10964

Moen, M. J., Adams, H. H. H., Brandsma, J. H., Dekkers, D. H. W., Akinci, U., Karkampouna, S., … Poot, R. A. (2017). An interaction network of mental disorder proteins in neural stem cells. *Translational Psychiatry*, *7*, e1082. https://doi.org/10.1038/tp.2017.52

Murre, C., McCaw, P. S., & Baltimore, D. (1989). A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. *Cell*, *56*, 777–783. https://doi.org/10.1016/0092-8674(89)90682-X

Navarrete, K., Pedroso, I., De Jong, S., Stefansson, H., Steinberg, S., Stefansson, K., … Collier, D. A. (2013). TCF4 (e2–2; ITF2): a schizophrenia-associated gene with pleiotropic effects on human disease. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*, *162b*, 1–16.

Nieto, M., Monuki, E. S., Tang, H., Imitola, J., Haubst, N., Khoury, S. J., … Walsh, C. A. (2004). Expression of Cux-1 and Cux-2 in the subventricular zone and upper layers II-IV of the cerebral cortex. *The Journal of Comparative Neurology*, *479*, 168–180. https://doi.org/10.1002/cne.20322

O'Donnell, L., Soileau, B., Heard, P., Carter, E., Sebold, C., Gelfond, J., … Cody, J. D. (2010). Genetic determinants of autism in individuals with deletions of 18q. *Human Genetics*, *128*, 155–164. https://doi.org/10.1007/s00439-010-0839-y

Ogawa, M., Miyata, T., Nakajimat, K., Yagyu, K., Seike, M., Ikenaka, K., … Mikoshibat, K. (1995). The reeler gene-associated antigen on cajal-retzius neurons is a crucial molecule for laminar organization of cortical neurons. *Neuron*, *14*, 899–912. https://doi.org/10.1016/0896-6273(95)90329-1

Page, S. C., Hamersky, G. R., Gallo, R. A., Rannals, M. D., Calcaterra, N. E., Campbell, M. N., … Maher, B. J. (2018). The schizophrenia- and autism-associated gene, transcription factor 4 regulates the columnar distribution of layer 2/3 prefrontal pyramidal neurons in an activity-dependent manner. *Molecular Psychiatry*, *23*, 304–315. https://doi.org/10.1038/mp.2017.37

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods*, *14*, 417–419. https://doi.org/10.1038/nmeth.4197

Paul, L. K., Brown, W. S., Adolphs, R., Tyszka, J. M., Richards, L. J., Mukherjee, P., & Sherr, E. H. (2007). Agenesis of the corpus callosum: Genetic, developmental and functional aspects of connectivity. *Nature Reviews Neuroscience*, *8*, 287–299. https://doi.org/10.1038/nrn2107

Pitt, D., & Hopkins, I. (1978). A syndrome of mental retardation, wide mouth and intermittent overbreathing. *Journal of Paediatrics and Child Health*, *14*, 182–184. https://doi.org/10.1111/jpc.1978.14.3.182

Quevedo, M., Meert, L., Dekker, M. R., Dekkers, D. H. W., Brandsma, J. H., van den Berg, D. L. C., … Poot, R. A. (2019). Publisher correction: Mediator complex interaction partners organize the transcriptional network that defines neural stem cells. *Nature Communications*, *10*, 3318. https://doi.org/10.1038/s41467-019-11254-1

Rannals, M. D., Hamersky, G. R., Page, S. C., Campbell, M. N., Briley, A., Gallo, R. A., … Maher, B. J. (2016a). Psychiatric risk gene transcription factor 4 regulates intrinsic excitability of prefrontal neurons via repression of SCN10a and KCNQ1. *Neuron*, *90*, 43–55. https://doi.org/10.1016/j.neuron.2016.02.021

Rannals, M. D., Page, S. C., Campbell, M. N., Gallo, R. A., Mayfield, B., & Maher, B. J. (2016b). Neurodevelopmental models of transcription factor 4 deficiency converge on a common ion channel as a potential therapeutic target for Pitt Hopkins syndrome. *Rare Diseases*, *4*, e1220468. https://doi.org/10.1080/21675511.2016.1220468

Schüller, U., Heine, V. M., Mao, J., Kho, A. T., Dillon, A. K., Han, Y. G., … Ligon, K. L. (2008). Acquisition of granule neuron precursor identity is a critical determinant of progenitor cell competence to form Shh-induced medulloblastoma. *Cancer Cell*, *14*, 123–134. https://doi.org/10.1016/j.ccr.2008.07.005

Sepp, M., Pruunsild, P., & Timmusk, T. (2012). Pitt-Hopkins syndrome-associated mutations in TCF4 lead to variable impairment of the transcription factor function ranging from hypomorphic to dominant-negative effects. *Human Molecular Genetics*, *21*, 2873–2888. https://doi.org/10.1093/hmg/dds112

Sepp, M., Vihma, H., Nurm, K., Urb, M., Page, S. C., Roots, K., … Timmusk, T. (2017). The intellectual disability and schizophrenia associated transcription factor TCF4 is regulated by neuronal activity and protein kinase A. *Journal of Neuroscience*, *37*, 10516–10527. https://doi.org/10.1523/JNEUROSCI.1151-17.2017

Sheldon, M., Rice, D. S., D'Arcangelo, G., Yoneshima, H., Nakajima, K., Mikoshiba, K., … Curran, T. (1997). Scrambler and yotari disrupt the disabled gene and produce a reeler -like phenotype in mice. *Nature*, *389*, 730–733. https://doi.org/10.1038/39601

Shmerling, D., Danzer, C. P., Mao, X., Boisclair, J., Haffner, M., Lemaistre, M., … Muller, M. (2005). Strong and ubiquitous expression of transgenes targeted into the beta-actin locus by Cre/lox cassette replacement. *Genesis*, *42*, 229–235.

Soneson, C., Love, M. I., & Robinson, M. D. (2015). Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Research*, *4*, 1521. https://doi.org/10.12688/f1000research.7563.1

Spassky, N., Han, Y. G., Aguilar, A., Strehl, L., Besse, L., Laclef, C., … Alvarez-Buylla, A. (2008). Primary cilia are required for cerebellar development and Shh-dependent expansion of progenitor pool. *Developmental Biology*, *317*, 246–259. https://doi.org/10.1016/j.ydbio.2008.02.026

Strimmer, K. (2008). fdrtool: A versatile R package for estimating local and tail area-based false discovery rates. *Bioinformatics*, *24*, 1461–1462. https://doi.org/10.1093/bioinformatics/btn209

Stritt, C., & Knöll, B. (2010). Serum response factor regulates hippocampal lamination and dendrite development and is connected with reelin signaling. *Molecular and Cellular Biology*, *30*, 1828–1837. https://doi.org/10.1128/MCB.01434-09

Sweatt, J. D. (2013). Pitt-Hopkins syndrome: Intellectual disability due to loss of TCF4-regulated gene transcription. *Experimental & Molecular Medicine*, *45*, e21. https://doi.org/10.1038/emm.2013.32

Thaxton, C., Kloth, A. D., Clark, E. P., Moy, S. S., Chitwood, R. A., & Philpot, B. D. (2018). Common pathophysiology in multiple mouse models of Pitt-Hopkins syndrome. *The Journal of Neuroscience*, *38*, 918–936. https://doi.org/10.1523/JNEUROSCI.1305-17.2017

Urbán, N., & Guillemot, F. (2014). Neurogenesis in the embryonic and adult brain: Same regulators, different roles. *Frontiers in Cellular Neuroscience*, *8*, 396–396. https://doi.org/10.3389/fncel.2014.00396

Voronova, A., & Baltimore, D. (1990). Mutations that disrupt DNA binding and dimer formation in the E47 helix-loop-helix protein map to distinct domains. *Proceedings of the National Academy of Sciences of the United States of America*, *87*, 4722–4726. https://doi.org/10.1073/pnas.87.12.4722

Whalen, S., Heron, D., Gaillon, T., Moldovan, O., Rossi, M., Devillard, F., … Giurgea, I. (2012). Novel comprehensive diagnostic strategy in Pitt-Hopkins syndrome: Clinical score and further delineation of the TCF4 mutational spectrum. *Human Mutation*, *33*, 64–72. https://doi.org/10.1002/humu.21639

Xia, H., Jahr, F. M., Kim, N. K., Xie, L., Shabalin, A. A., Bryois, J., … McClay, J. L. (2018). Building a schizophrenia genetic network: Transcription factor 4 regulates genes involved in neuronal development and schizophrenia risk. *Human Molecular Genetics*, *27*, 3246–3256. https://doi.org/10.1093/hmg/ddy222

Yoshida, M., Assimacopoulos, S., Jones, K. R., & Grove, E. A. (2006). Massive loss of Cajal-Retzius cells does not disrupt neocortical layer order. *Development*, *133*, 537–545. https://doi.org/10.1242/dev.02209

Yu, G., Wang, L.-G., Han, Y., & He, Q.-Y. (2012). clusterProfiler: An R package for comparing biological themes among gene clusters. *Omics : A Journal of Integrative Biology*, *16*, 284–287. https://doi.org/10.1089/omi.2011.0118

Zhuang, Y., Cheng, P., & Weintraub, H. (1996). B-lymphocyte development is regulated by the combined dosage of three basic helix-loop-helix genes, E2A, E2–2, and HEB. *Molecular and Cellular Biology*, *16*, 2898–2905. https://doi.org/10.1128/MCB.16.6.2898

Zhuo, L., Theis, M., Alvarez-Maya, I., Brenner, M., Willecke, K., & Messing, A. (2001). hGFAP-cre transgenic mice for manipulation of glial and neuronal function in vivo. *Genesis*, *31*, 85–94. https://doi.org/10.1002/gene.10008

Zweier, C., Peippo, M. M., Hoyer, J., Sousa, S., Bottani, A., Clayton-Smith, J., … Rauch, A. (2007). Haploinsufficiency of TCF4 causes syndromal mental retardation with intermittent hyperventilation (Pitt-Hopkins syndrome). *American Journal of Human Genetics*, *80*, 994–1001. https://doi.org/10.1086/515583

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Schoof M, Hellwig M, Harrison L, et al. The basic helix-loop-helix transcription factor TCF4 impacts brain architecture as well as neuronal morphology and differentiation. *Eur J Neurosci*. 2020;00:1–17. https://doi.org/10.1111/ejn.14674