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Supplemental Information

Molecular Profiling Reveals Involvement of ESCO2 in Intermediate Progenitor Cell Maintenance in the Developing Mouse Cortex

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SUPPLEMENTAL DATA

Figure S1

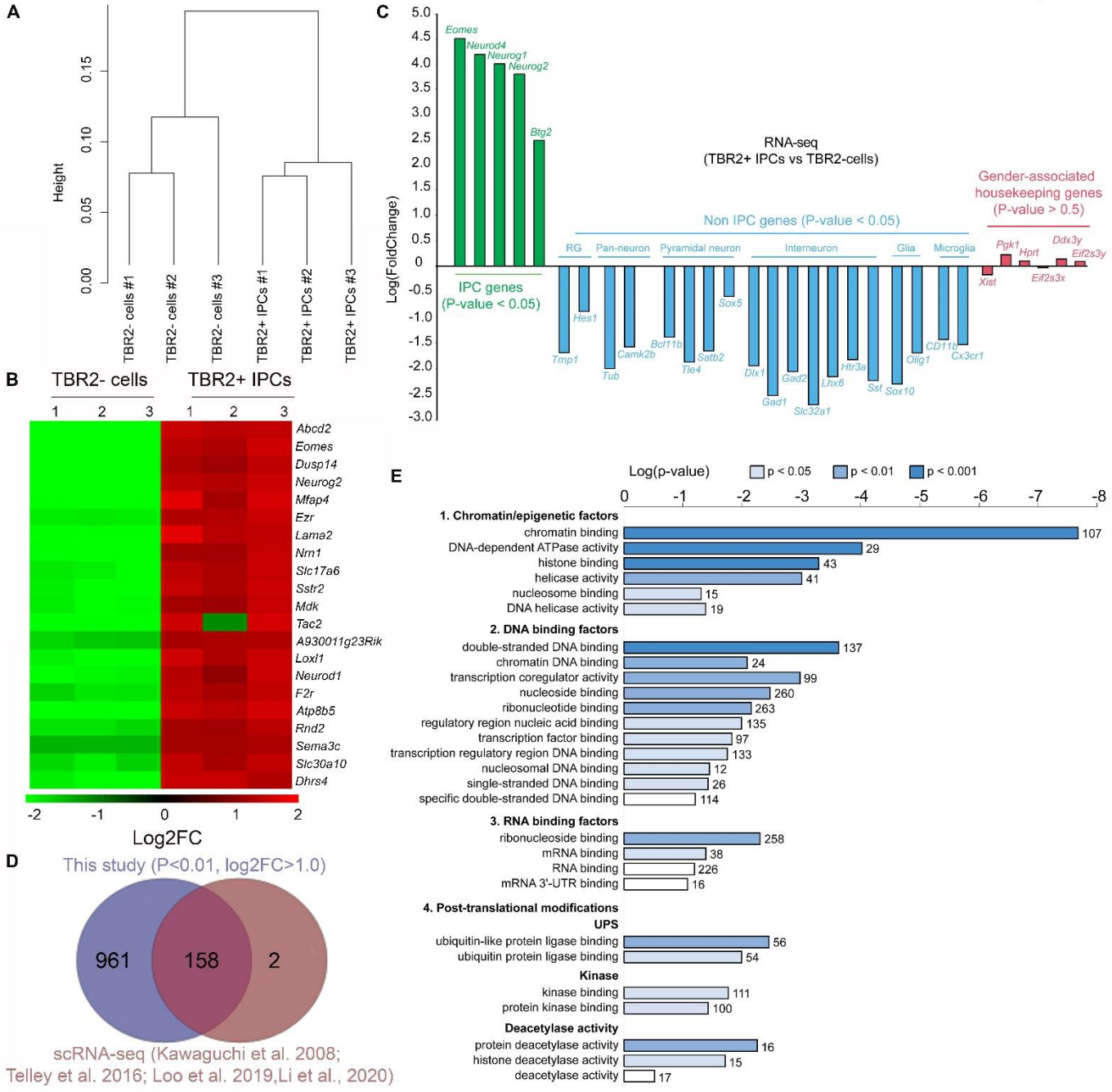


Figure S1 (related to figure 1). RNA-seq analysis of mouse TBR2+ IPCs and TBR2- cells. (A) Cluster dendrogram analysis of RNA-seq for TBR2+ and TBR2- sorted cell samples. (B) Heatmap showing the top 20 genes with high expression (enriched/upregulated) in TBR2+ IPCs and low expression in TBR2- cells. (C) Bar graph showing expression of selected IPC (in green), non-IPC (in blue) and gender-associated housekeeping genes (in red) in IPCs (TBR2+) and non IPCs (TBR2-) in the E16.5 mouse cortex. (D) An indication of the increased number of IPC-enriched genes identified in our study compared with previous studies performed at the IP single cell level. (E) Bar graph showing the molecular pathway analysis in TBR2+ IPCs and the number of genes belonging to each category of pathway.

Figure S2

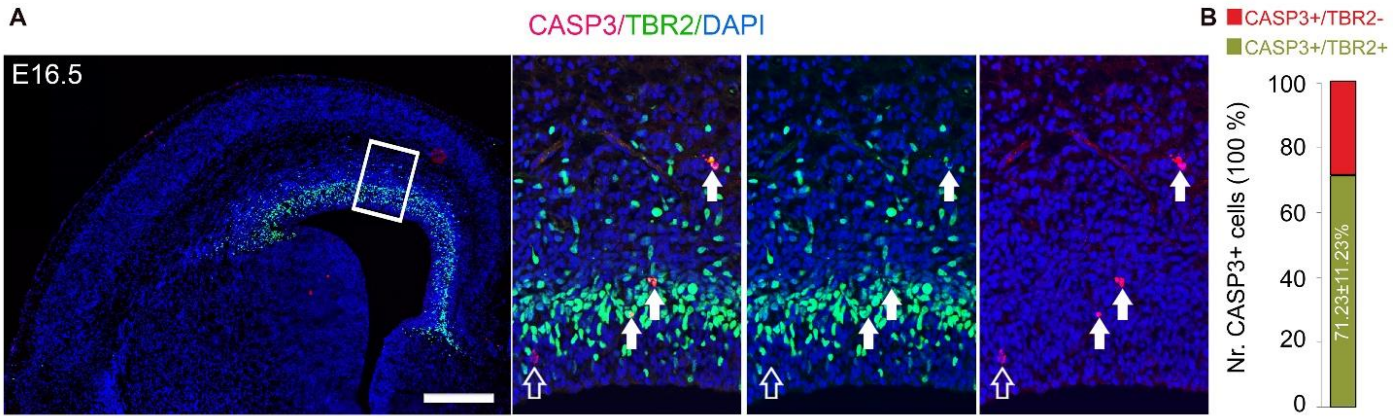

A
CASP3/TBR2/DAPI
E16.5
B
CASP3+/TBR2-
CASP3+/TBR2+
Nr. CASP3+ cells (100 %)
100
80
60
40
20
0
71.23±11.23%

Figure S2 (related to figure 3). TBR2+ IPCs in mouse display prominent apoptotic activity. (A) Immunohistochemical micrographs showing an overview of E16.5 mouse cortex and highly magnified germinal zone stained with TBR2 and CASP3 antibodies. The zoomed area is indicated with white inserted box. Filled arrows point to TBR2+ IPCs undergoing apoptosis whereas empty arrow indicates apoptotic activity in a TBR2- cell. Counterstaining was done with DAPI. (B) Bar graph showing the proportion of TBR2+ and TBR2- cells undergoing apoptosis. Experimental replicates (n) = 6 (B). Scale bar = 100 µm.

Figure S3

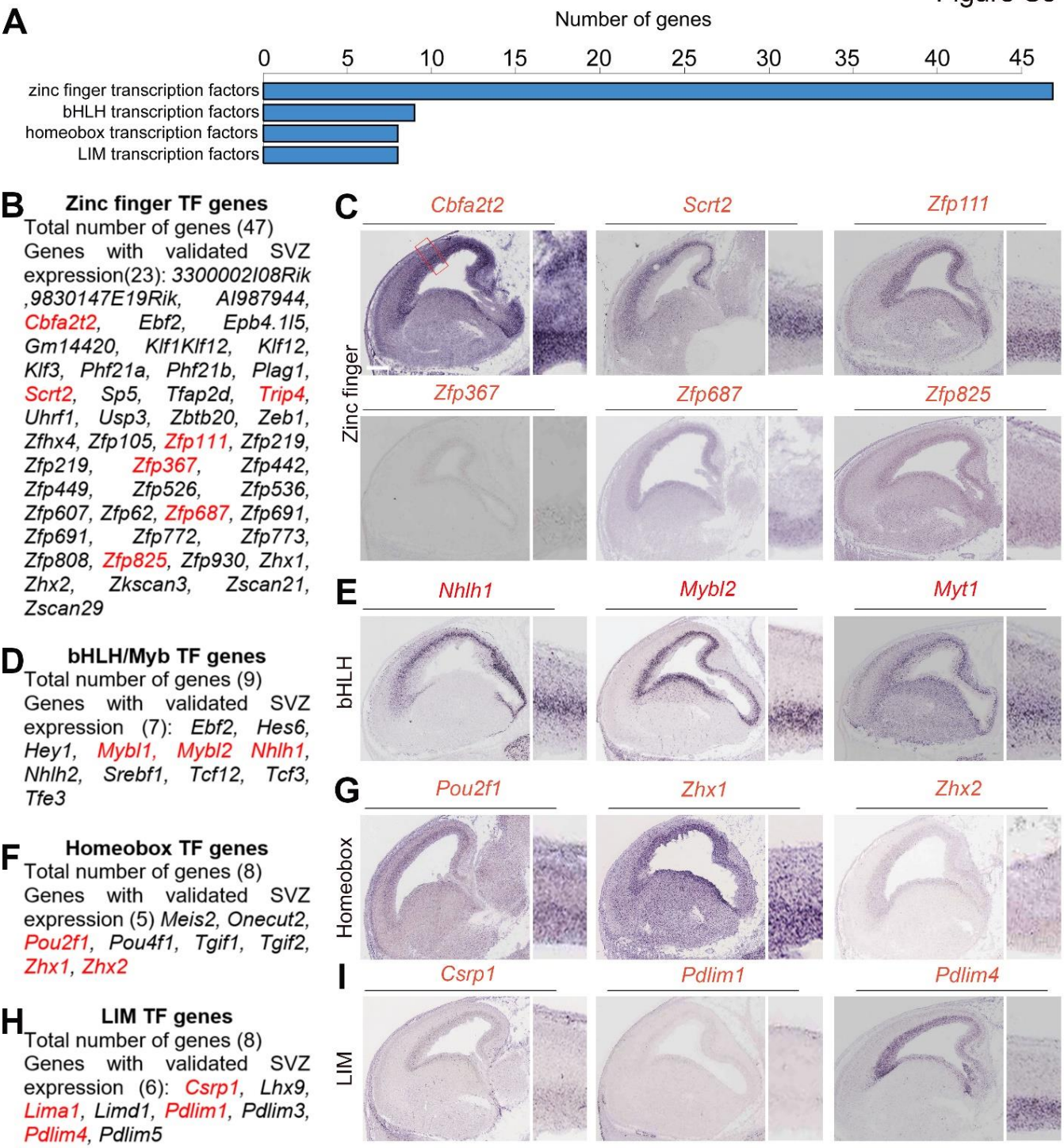
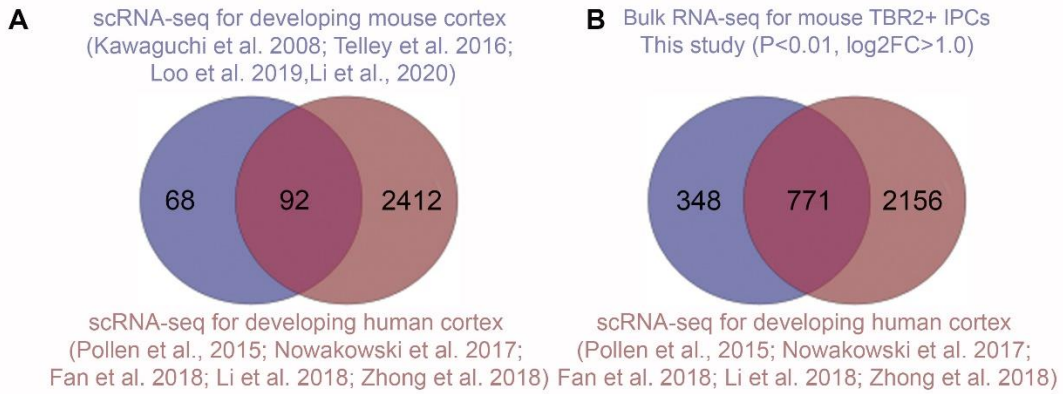
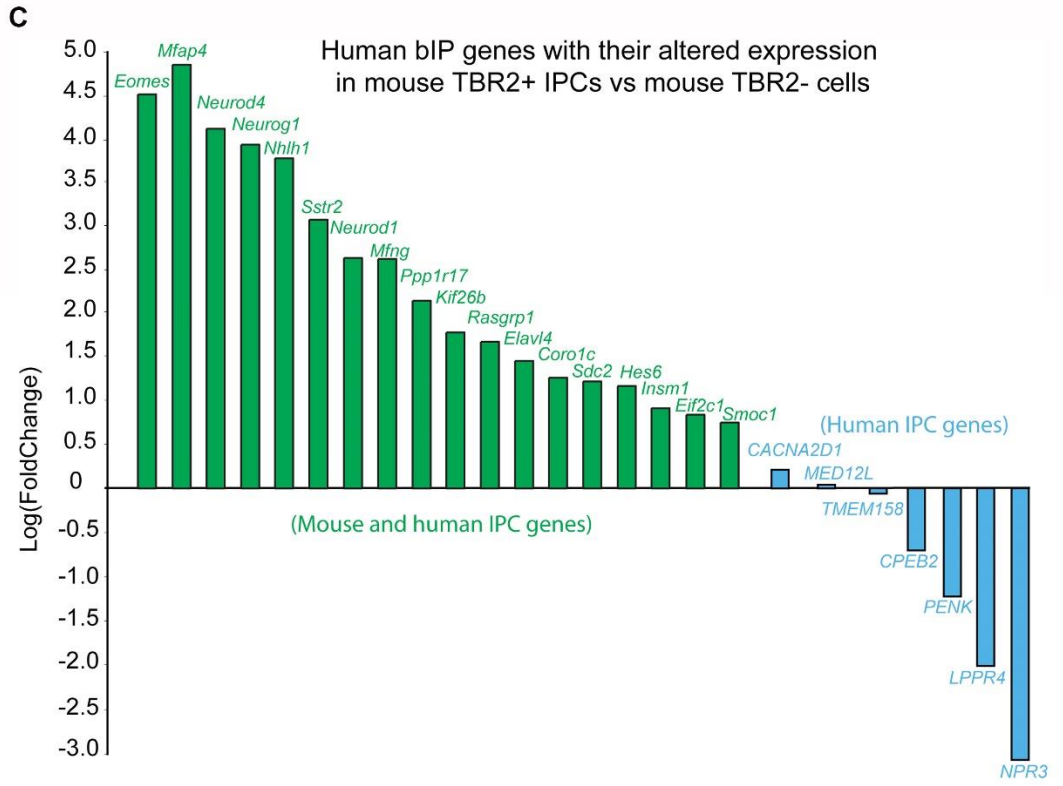

A
Number of genes
0 5 10 15 20 25 30 35 40 45
zinc finger transcription factors
bHLH transcription factors
homeobox transcription factors
LIM transcription factors
B
Zinc finger TF genes
Total number of genes (47)
Genes with validated SVZ expression(23): 3300002I08Rik ,9830147E19Rik, AI987944, Cbfa2t2, Ebf2, Epb4.1l5, Gm14420, Klf1Klf12, Klf12, Klf3, Phf21a, Phf21b, Plag1, Scrt2, Sp5, Tfap2d, Trip4, Uhrf1, Usp3, Zbtb20, Zeb1, Zfhx4, Zfp105, Zfp111, Zfp219, Zfp219, Zfp367, Zfp442, Zfp449, Zfp526, Zfp536, Zfp607, Zfp62, Zfp687, Zfp691, Zfp691, Zfp772, Zfp773, Zfp808, Zfp825, Zfp930, Zhx1, Zhx2, Zkscan3, Zscan21, Zscan29
D
bHLH/Myb TF genes
Total number of genes (9)
Genes with validated SVZ expression (7): Ebf2, Hes6, Hey1, Mybl1, Mybl2 Nhlh1, Nhlh2, Srebf1, Tcf12, Tcf3, Tfe3
F
Homeobox TF genes
Total number of genes (8)
Genes with validated SVZ expression (5) Meis2, Onecut2, Pou2f1, Pou4f1, Tgif1, Tgif2, Zhx1, Zhx2
H
LIM TF genes
Total number of genes (8)
Genes with validated SVZ expression (6): Csrp1, Lhx9, Lima1, Limd1, Pdlim1, Pdlim3, Pdlim4, Pdlim5
C
Cbfa2t2
Scrt2
Zfp111
Zinc finger
Zfp367
Zfp687
Zfp825
E
Nhlh1
Mybl2
Myt1
bHLH
G
Pou2f1
Zhx1
Zhx2
Homeobox
I
Csrp1
Pdlim1
Pdlim4
LIM

Figure S3 (related to figure 4). Enrichment of transcription factor genes in TBR2+ IPCs. (A) Bar graph showing the number of genes in IPCs under the indicated classes of transcription factor. (B, D, F, H) Categories of transcription factors with corresponding list of newly identified IPC-enriched genes. (C, E, G, I) Respective micrographs showing *in situ* hybridization of examples of the identified transcription factor genes (highlighted red in the adjoining gene list) with expression endowment in the developing mouse cortical subventricular zone. Magnified cortical region is shown with red box in (C). Scale bar = 100 µm.

Figure S4


A
scRNA-seq for developing mouse cortex
(Kawaguchi et al. 2008; Telley et al. 2016;
Loo et al. 2019,Li et al., 2020)
68
92
2412
scRNA-seq for developing human cortex
(Pollen et al., 2015; Nowakowski et al. 2017;
Fan et al. 2018; Li et al. 2018; Zhong et al. 2018)
B
Bulk RNA-seq for mouse TBR2+ IPCs
This study (P<0.01, log2FC>1.0)
348
771
2156
scRNA-seq for developing human cortex
(Pollen et al., 2015; Nowakowski et al. 2017;
Fan et al. 2018; Li et al. 2018; Zhong et al. 2018)


C
Human bIP genes with their altered expression
in mouse TBR2+ IPCs vs mouse TBR2- cells
Log(FoldChange)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
Mfap4
Eomes
Neurod4
Neurog1
Nhlh1
Sstr2
Neurod1
Mfng
Ppp1r17
Kif26b
Rasgrp1
Elavl4
Coro1c
Sdc2
Hes6
Insm1
Eif2c1
Smoc1
CACNA2D1
MED12L
TMEM158
CPEB2
PENK
LPPR4
NPR3
(Mouse and human IPC genes)
(Human IPC genes)

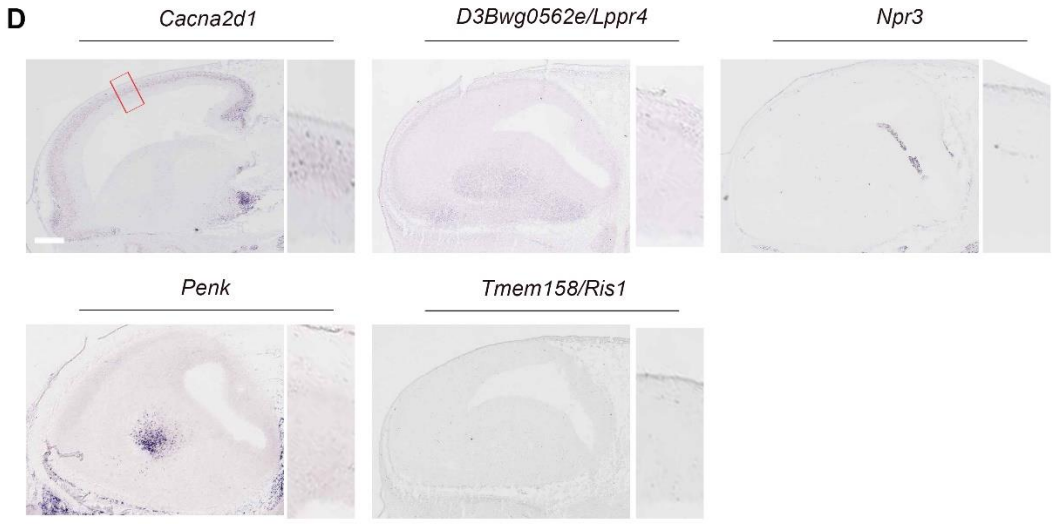

D
Cacna2d1
D3Bwg0562e/Lppr4
Npr3
Penk
Tmem158/Ris1

Figure S4 (related to figure 5). Many human bIP genes are upregulated in mouse TBR2+ IPCs while others are downregulated or absent. (A, B) Overlap between the number of human IPC genes from scRNA-seq analysis of the human developing cortex (Fan et al., 2018; Li et al., 2018; Nowakowski et al., 2017; Pollen et al., 2015; Zhong et al., 2018) and number of mouse IPC genes, which was recently identified by scRNA-seq analysis (Kawaguchi et al., 2008; Li et al., 2020; Loo et al., 2019; Telley et al., 2016) (A), and by bulk RNA-seq (B, this study). (C) Bar graph showing both upregulated (enriched) and downregulated human bIP genes in TBR2+ IPCs compared with TBR2- cells in mouse cortex. (D) *In situ* hybridization micrographs showing the E14.5 mouse cortex riboprobed for the indicated human bIP genes. Magnified cortical region is shown with red box. Scale bar = 100 µm.

Figure S5

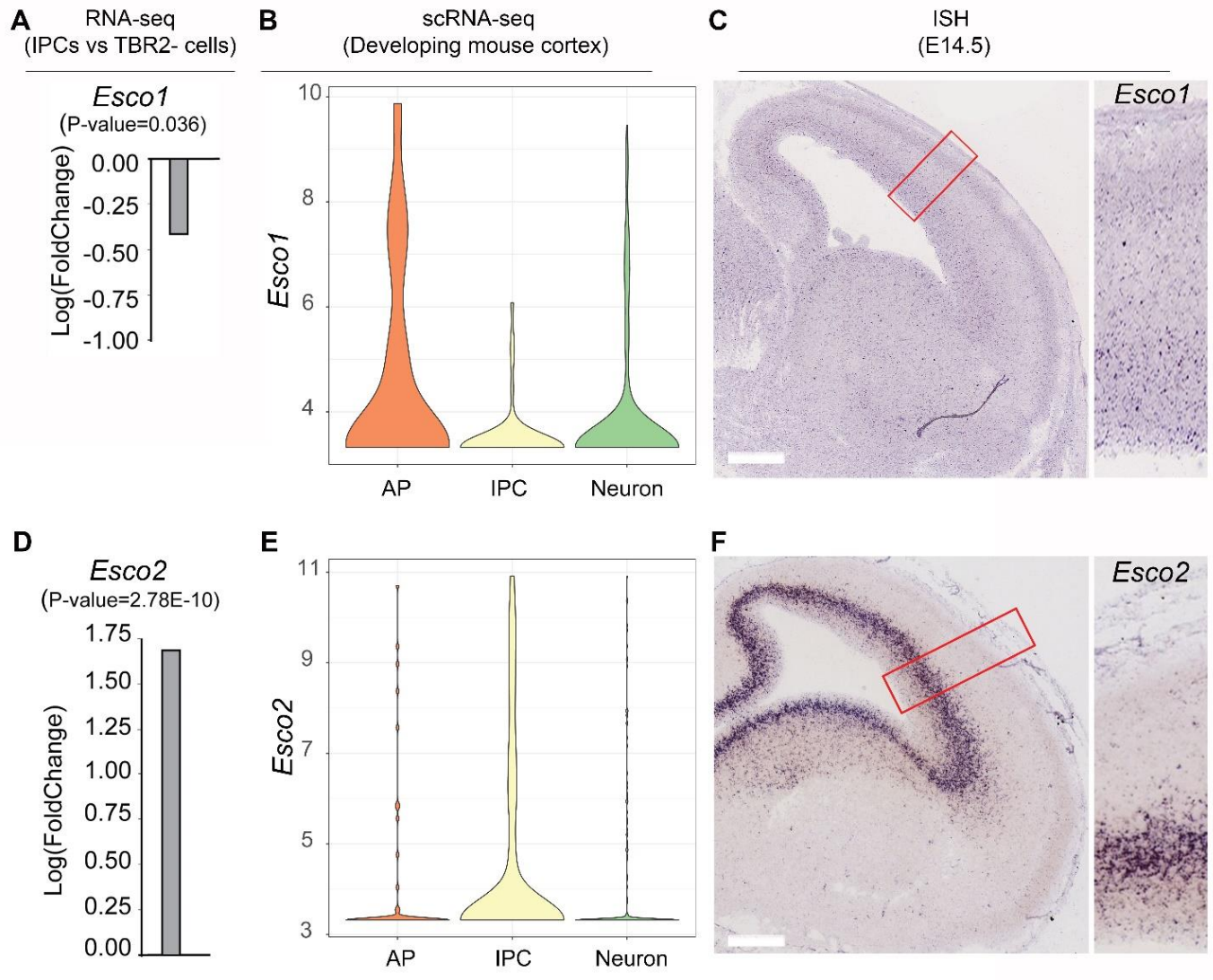

A
RNA-seq
(IPCs vs TBR2- cells)
Esco1
(P-value=0.036)
Log(FoldChange)
0.00
-0.25
-0.50
-0.75
-1.00
B
scRNA-seq
(Developing mouse cortex)
Esco1
10
8
6
4
AP
IPC
Neuron
C
ISH
(E14.5)
Esco1
D
Esco2
(P-value=2.78E-10)
Log(FoldChange)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
E
Esco2
11
9
7
5
3
AP
IPC
Neuron
F
Esco2

Figure S5 (related to figure 6). Expression of ESCO1 and ESCO2 in the developing mouse cortex. (A–F) Expression of ESCO1 (A–C) and ESCO2 (D–F) were evaluated by RNA-seq with TBR2+ and TBR2- samples (A, D), single-cell (sc)RNA-seq (B, E), and ISH (C, F). (A, D) Bar graph indicating significant differential expression of ESCO1 (A) and ESCO2 (D) in TBR2+ IPCs compared with TBR2- cells in RNA-seq analysis. (B, E) Expression of *Esco1* (B) *Esco2* (E) based on a published single-cell scRNA-seq dataset of the developing mouse cortex (Telley et al., 2016). The graph-plots were generated using the Seurat package of R (Macosko et al., 2015) (http://genebrowser.unige.ch/science2016/). (C, F) Micrograph of *in situ* hybridization (ISH) staining showing prominence of *Esco1* (C) and *Esco2* (F) expression in VZ and SVZ of E14.5 mouse cortex, respectively. Magnified cortical region is shown with red box. Note that in contrast to a highly-enriched expression of *Esco1* in RGCs in VZ, expression of *Esco2* is mostly restricted in IPCs in SVZ. Abbreviations: RGC (Radial glial progenitor cell), IPC (Intermediate progenitor cell). Scale bar = 100 μm.

Figure S6

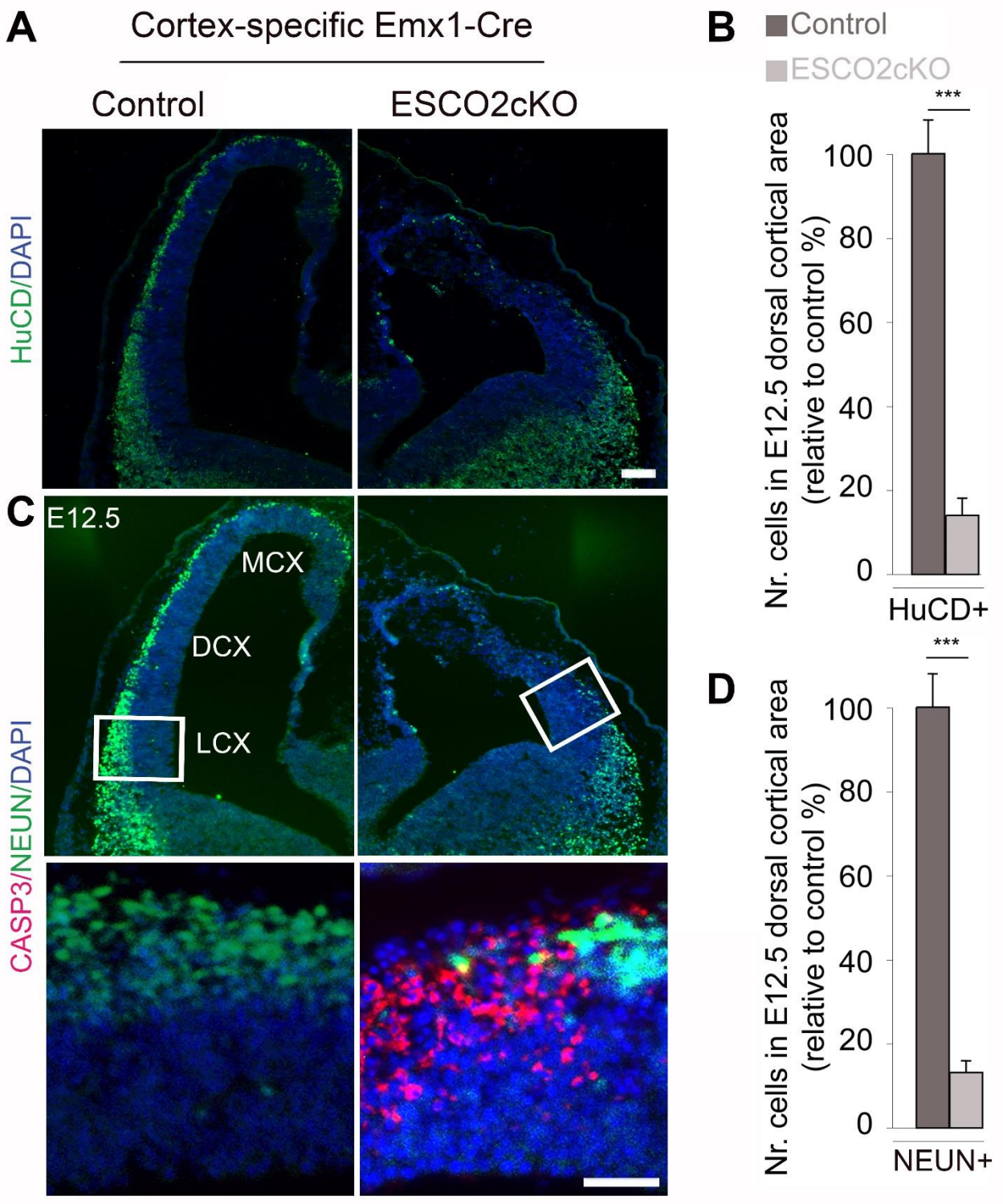

A
Cortex-specific Emx1-Cre
Control
ESCO2cKO
HuCD/DAPI
C
E12.5
MCX
DCX
LCX
CASP3/NEUN/DAPI
B
Control
ESCO2cKO
***
Nr. cells in E12.5 dorsal cortical area (relative to control %)
100
80
60
40
20
0
HuCD+
D
***
Nr. cells in E12.5 dorsal cortical area (relative to control %)
100
80
60
40
20
0
NEUN+

Figure S6 (related to figure 7). Deletion of ESCO2 in developing cortex causes depletion of post-mitotic neurons via apoptosis. (A, C) Immunohistochemical micrographs showing staining of the E12.5 mouse cortex with the antibodies HuCD and NeuN (pan-neuronal markers), and CASP3 to mark post-mitotic neurons undergoing apoptotic cell death. (B, D) Bar charts showing quantification of the number of HuCD+ and NeuN+ cells in the lateral aspect of the E12.5 cortex (marked with white box). The medial (MCX), dorsal (DCX), and lateral (LCX) cortical areas are indicated. *** *p*-value < 0.001, Experimental replicates (n) = 4 (B, D). Scale bar = 200 µm (A), 50 µm (C, lower panel).

- **Table S1 (related to figure 1).** Differential gene expression between TBR2+ IPCs and TBR2- cells (as a Supplemental Spreadsheet).
- **Table S2 (related to figure 1).** Selected gene ontology categories significantly enriched for TBR2+ IPC genes compared with TBR2- cell genes (as a supplemental spreadsheet).
- **Table S3 (related to figure 1).** List of validated IPC genes with their restricted expression in SVZ (as a Supplemental Spreadsheet).
- **Table S4 (related to figure 2).** List of cell cycle and chromosome segregation -related gene enriched in TBR2+ IPCs (as a Supplemental Spreadsheet).
- **Table S5 (related to figure 3).** List of apoptosis-related gene enriched in TBR2+ IPCs (as a Supplemental Spreadsheet).
- **Table S6 (related to figure 4).** List of TF-, chromatin remodeling-, epigenetic-, and lncRNA related genes enriched in TBR2+ IPCs (as a Supplemental Spreadsheet).
- **Table S7 (related to figure 5).** List of microcephaly-linked gene enriched in TBR2+ (as a Supplemental Spreadsheet).
- **Table S8 (related to figures 1, 6, 7, S2, S6)**. Statistical analyses (as a Supplemental Spreadsheet).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES.

Plasmids

Plasmids used in this study: CAG-GFP-IRES-CRE (Zhao et al. 2006) from Addgene.

Antibodies

The following polyclonal (pAb) and monoclonal (mAb) primary antibodies used in this study were obtained from the indicated commercial sources: CASP3 rabbit pAb (1:100; Cell Signaling), ESCO2 (Whelan et al., 2012), GFP chick pAb (1:400; Abcam), HuCD mouse mAb (1:20; Invitrogen), NEUN mouse mAb (1:200, Chemicon), PAX6 mouse mAb (1:100; Developmental Studies Hybridoma Bank), PAX6 rabbit pAb (1:200; Covance), TBR2 rabbit pAb (1:200; Abcam), TBR2 rat 923 mAb (1:200; eBioscience).

Secondary antibodies used were Alexa 488-, Alexa 568-, Alexa 594- and Alexa 647-conjugated IgG (various species, 1:400; Molecular Probes).

Functional enrichment analysis of IPC genes

The lists of IPC genes (p-Value<0.01, log2FoldChange>1.0) were uploaded to the DAVID functional annotation tool (https://david.ncifcrf.gov/). Then, representative enriched biological functional terms were manually selected. To perform the Gene Set Enrichment Analysis (GSEA) analysis, the list of upregulated genes was uploaded to GSEA. Using FDR q-value<0.05 as a cut-off generated the enriched biological and cellular component terms. The Fisher's exact test was applied to identify the terms showing a statistically significant difference for the upregulated genes.

Identification of IPC-enriched lnRNAs

The lists of IPC genes (p-Value<0.01, log2FoldChange>1.0) were uploaded to the MGI gene nomenclature analysis tool (http://www.informatics.jax.org/batch). List of lnRNAs protein-encoding genes was extracted from each other.

Protein-Protein interaction network

The combined list of IPC-enrriched genes encoding for TFs (chromatin re modeling and epigenetic factors, Fig. 4J) was uploaded to the STRING database (http://string-db.org/). The protein-protein interactions from STRING were visualized by Cytoscape (https://cytoscape.org/; version: 3.3.0).

SUPPLEMENTAL DISCUSSION

Prominence of cell cycle-related factors in IPCs

Further unanswered questions that need to be addressed to increase our understanding of ICP cell biology include (1) the reasons behind shorter S-phase and longer G1-phase, and total length of cell cycle in IPCs than in RGCs, and (2) what factors drive the proliferation of some IPCs in cell cycle? In developing cortex, the length of G1 is increased in neurogenic progenitor cells compared with proliferative progenitors (Caviness et al., 2003; Dehay and Kennedy, 2007; Lukaszewicz et al., 2005; Salomoni and Calegari, 2010). As such, basal progenitors, including IPCs, are known to display a more extended G1 phase than RGCs (Calegari et al., 2005; Salomoni and Calegari, 2010). The increased G1 phase may support the more differentiative capacity of IPCs compared with RGCs, thus likely promoting IPC fate (Dehay and Kennedy, 2007; Lange et al., 2009). Indeed, functional manipulation of G1 length was shown to have effects that either support (i) IPC genesis leading to neurogenic division and premature neurogenesis (Calegari et al., 2005) or (ii) increased proliferative divisions, resulting in progenitor pool expansion, which manifests in cortical layer phenotypes later in development (Lange et al., 2009; Pilaz et al., 2009). Of note, we found a high expression of genes encoding for CDKs (*Cdk2*, *Cdk4*) with their regulator proteins (CCND1, CCND2, CCNE1, CCNE2), which drive the G1 phase of cycling IPCs. Hence, it is worth to examine the proliferation capacity of IPCs, in which the expression of these G1-phase factors is specifically manipulated.

Identification of new IPC-specific transcription factors

An intriguing molecular difference between the TBR2+ IPCs and the TBR2- cells in the developing mouse cortex is the differential expression of genes, which encode for different TF families (Table S5).

The zinc finger TFs form the largest protein family, having a wide range of molecular functions, and are involved in the development and differentiation of several cell lineages (Cassandri et al., 2017). Expression of many genes encoding for this TF family is enriched in IPCs. The role of the zinc finger proteins identified in this study in neurogenesis are largely unknown, except for that reported for the function of Uhrf1 in adult neurogenesis (Blanchart et al., 2018; Murao et al., 2019).

The second largest family of TFs is the bHLH TFs, which play key roles in various developmental processes in organisms from yeast to humans (Jones, 2004). Class I bHLH proteins are ubiquitously expressed, whereas class II bHLH proteins are tissue-specific. The nervous system-specific bHLH factors can further be classified into proneural and neural differentiation genes (Dennis et al., 2019). Two closely-related nescient helix loop helix 1 (*Nhlh1/bHLHa35*) and 2 (*Nhlh2/bHLHa34*) genes belong to the neural differentiation bHLH/Nscl subfamily genes (Dennis et al., 2019). In chicken, misexpression of *Nhlh1* leads to an abnormal brain structure with an underdeveloped cerebellum and a larger tectum caused by changes in cell proliferation (Li et al., 1999). *Nhlh1*-deficient mice exhibit a predisposition to arrhythmia leading to an early death due to autonomic nervous system dysfunction (Cogliati et al., 2002). The phenotype was more severe when *Nhlh1*-knockout mice were also heterozygous for *Nhlh2*. The specific and high expression of *Nhlh1* and *Nhlh2* in IPCs (Table. S5) suggests that the two factors act together to control the IPC differentiation. Expression of other bHLH TFs, including *Hes6/bHLHb41*, *Tcf3/bHLHb21*, *Tcf12/bHLHb20*, *Hey1/bHLHb31*, and *Ebf2*, *Srebf1/bHLHd1* were found to be enriched in IPCs. Among them, *Hes6*, *Tcf3*, *Tcf12*, *Hey1* and *Ebf2* were reported to be involved in neural development (Chuang et al., 2011; Gribble et al., 2009; Methot et al., 2013; Nam et al., 2016; Sakamoto et al., 2003; Uittenbogaard and Chiaramello, 2002; Yang et al., 2015). For *Srebf1*, no studies have yet been published on its role in neural development, albeit associations with Schizophrenia and Parkinson's disease have been reported, making a future investigation into its role in brain development even more interesting (Le Hellard et al., 2010; Lou et al., 2019; Yang et al., 2016).

The homeobox TFs were also found to be highly expressed in IPCs. Homeobox genes are important for the embryonic development of diverse animals, and are often comparatively analyzed to investigate evolution of animal development (Holland et al., 2007). Our investigation of genes enriched in IPC revealed three transcription factors that belong to the homeobox gene family. For two of the homeobox genes enriched in IPC, Meis homeobox 2 (*Meis2*) and POU domain, class 4, transcription factor 1 (*Pou4f1*, also called *Brn3a*), a role for neural development has already been described. MEIS2 has been described as a regulator of dorsal midbrain development interacting with the paired-box transcription factors PAX3 and PAX7 (Agoston et al., 2012). In humans, *MEIS2* mutations can cause intellectual disability (Douglas et al., 2018; Giliberti et al., 2019; Louw et al., 2015). In the developing nervous system, *Pou4f1* was shown to be essential for the generation of dorsal root ganglia sensory neurons and the regulation of sensory afferent projections (Zou et al., 2012). The other homeobox gene is the one cut domain family member 2 (*Onecut2*), which is well known as a master regulator in cancer (Lu et al., 2018; Rotinen et al., 2018). Function of ONECUT2 in brain development has not yet been described, however, *Onecut2* overexpression was shown to induce a neuron-like morphology and neuronal gene expression in fibroblasts making its role in neural development plausible (van der Raadt et al., 2019).

Another group of TF genes found to be enriched in IPC lineage is the myeloblastosis oncogene-like (Myb-like) transcription factor. The transcription factor MYBL1 (also called A-MYB) is known as a master regulator of meiosis (Bolcun-Filas et al., 2011), and in mice, it plays a vital role in spermatogenesis and mammary gland development (Toscani et al., 1997). Although *Mybl1* expression in neuronal progenitor cells has already been described (Trauth et al., 1994), its specific role in brain development is far from clear. Similarly, another Myb-like transcription factor, named MYBL2 or B-MYB was found to be enriched in IPC. MYBL2 is involved in cell proliferation and survival, however, these roles have been investigated mainly in cancer research and a possible function in neurogenesis has not been determined so far (Chen and Chen, 2018; Musa et al., 2017).

Altogether, our investigation of genes specific to IPCs revealed the expression of many transcription factors that were previously not known and, thus, are putative genetic

determinants of this cohort of neuronal progenitor cell type. Understanding the function of these IPC-enriched TFs would not only shed light on the mechanisms of cortical development, but also provide suggestions for ways to generate this cell type by direct reprograming from other cell linages.

Mutation of IPC-enriched genes is implicated in human neurodevelopmental disorders and neuropsychiatric diseases

The single-cell transcriptomic analysis of human developing cortex has identified a set of IPC genes (Fan et al., 2018; Li et al., 2018; Nowakowski et al., 2017; Pollen et al., 2015; Zhong et al., 2018). As part of further investigations, we compared in silico expression of such human IPC genes with our identified mouse IPC transcriptome to identify the developmental and evolutionary origin of the transcriptional signature of IPC cells. Our data suggest the existence of both conserved and non-conserved transcriptional signatures of IPCs in mammalian evolution. Consistent with this line of evidence, previous studies have shown that expression of TBR2 was found specifically in IPCs in lissencephalic rodent brain (Englund et al., 2005). In gyrencephalic ferret or primates, TBR2 labeled IPCs, and almost half of SOX2+, PAX6+ bRGC population (Betizeau et al., 2013; Fietz et al., 2010; Florio and Huttner, 2014; Hansen et al., 2010; Turrero Garcia et al., 2016). Furthermore, in rodent cortex, IPCs are predominately-neurogenic progenitors. However, in gyrencephalic species, IPCs are capable of self-amplification through symmetric proliferative divisions before their terminal division to generate neurons (Florio and Huttner, 2014; Lui et al., 2011). Beyond simply marking IPCs as transient progenitor cell type, future studies may have to relate and delve into the heterogeneity in the molecular milieu of IPCs in different species to afford elucidation of their contributions to cortical morphogenesis.

Advances in genetics and genomics studies in recent times have made it possible to identify many genetic coding and non-coding variants that cause neurodevelopmental disorders (D'Gama and Walsh, 2018; Hu et al., 2014; Juric-Sekhar and Hevner, 2019), with increased risk of neuropsychiatric disturbances (Sestan and State, 2018; Sullivan and Geschwind, 2019). Although we now have better insights into the genetic architecture

of neuropsychiatric perturbations, we still lack a comprehensive description of the underlying molecular and cellular mechanisms, mainly because of the heterogeneity of risk loci, and the involvement of multiple cell types and brain regions. Therefore, knowledge of the regulatory networks and the spatiotemporal distribution of these networks in the brain, is essential for elucidating which cell types are relevant in the etiology and possible treatment of these neurodevelopment- and neuropsychiatry-related disorders. Moreover, the clarification of the mechanistic underpinnings of any given neurological disorder also requires detailed understanding of the developmental events that are disrupted in the course of the disease, non-genic causatives (environmental or epigenetic) of the anomalies, and dissection of the eventual phenotype.

Gene co-expression analyses have also revealed that the developing human (Kang et al., 2011; Miller et al., 2014; Pletikos et al., 2014) or mouse (Loo et al., 2019) brain transcriptome can be organized into distinct co-expression networks with often prominent spatiotemporal patterns, and enriched for distinct biological functions. By probing the transcriptome of mouse IPCs and performing further analysis in the form of phenotype association categorization, we found strong connection between the identified IPC genes and known human neurodevelopmental disorders (Fig. 5). This can be explained by the essential role of IPCs in cortical development. A great proportion of cortical neurons can be traced to IPCs. IPC-derived neurons predominately form the upper cortical layers and their axons constitute the large interhemispheric commissural system (i.e., the corpus callosum). Cortical expansion and evolutionary changes have been attributed to the tremendous neurogenic output of TBR2-expressing IPCs and their diversity, especially in human. It is mainly for these reasons that disruption in the production of IPCs can lead to a wide range of cortical malformations and diverse neurological perturbations in the mammalian cortex. Our data thus suggest that disease-linked mutations of IPC genes might form robust groupings based on their GO profiles. These diseases clearly link to neurodevelopmental defects, e.g. cortical size-associated disorders (microcephaly, macrocephaly, and abnormal cortical gyration), corpus callosum defects (dysplastic, agenesis, aplasia, hypoplasia of corpus callosum, and abnormality of the cerebral white matter), and neurological deficits (intellectual disability, psychomotor developmental delay, schizophrenia, autism, and epilepsy).

Despite the recent great interest in elucidating the principles underlying the IPC-mediated evolutionary expansion of the neocortex and the consequence of related dysregulation, relatively less attention is accorded to dissecting disease-linked mutations of IPC genes to elucidate the pathophysiology of the attendant neurological disorder. By employing mouse model for the novel IPC gene *Esco2*, we were able to identify that IPCs may centrally rely on ESCO2 for survival and maintenance of their pool in the developing cortex. The absence of ESCO2, which is rather needed for the correct segregation of chromatids and therefore the genetic material into the progenies of dividing IPCs, may have triggered the massive apoptosis of the resultant ESCO2-deficient IPCs and the resultant overt cortical dysgenesis. Interestingly, *Esco2* mutations in human have been linked to neurological phenotypes, including microcephaly and cognitive deficits. The said pivotal role played by ESCO2 in IPC genesis and cortical morphogenesis recapitulated similar critical function of TBR2 in brain morphogenesis. Mutations that abolish *Tbr2* expression can cause severe neurodevelopmental abnormalities, including microcephaly, severe motor and cognitive delay, hypotonia, callosal agenesis, polymicrogyria, and cerebellar hypoplasia in rodent (Arnold et al., 2008; Sessa et al., 2008) and human (Baala et al. 2007). For future studies, linkage mapping and/or exome sequencing in human is expected to identify more IPC-related mutations and dysregulated genes associated with aberrant cortical architecture and growth.

To minimize gender bias in sampling for RNA-seq, we altogether used tissue from 15 embryos for 3 replicates. In addition, to examine the relative gender contribution in our samples, we compared the expression of chromosome X (*Xist*, *Pgk1*, *Hprt*, *Eif2s3x*) and chromosome Y (*Ddx3y*, *Eif2s3y*) - located genes, which are known housekeeping genes in the developing forebrain (Dewing et al. 2003; Cheung et al. 2017). The comparison revealed their comparable expression level in samples from TBR2+ IPCs and TBR2- cells (Fig. S1C). The data suggests that TBR2+ and TBR2- cell populations were derived from a similar number of female and male embryos. Nevertheless, the expression pattern of chromosome X/Y-located IPC genes should be validated by either IHC or ISH afore further investigation.

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