Expanded View Figures

Figure EV1. Experimental paradigm and validation of the transcriptional analyses.

A *Hes5::GFP* and *Tbr2::GFP* transgenic mice used for cell isolation.

B Expression of *Hes5::GFP* and *Tbr2::GFP* embryonic cortices at E17.5. Scale bar = 100 μm. Arrowheads pointing to *Tbr2::GFP* cells in VZ. Immunostainings for Pax6 and Tbr2 with *Hes5::GFP* and *Tbr2::GFP* coronal sections from E17.5. Arrows point to GFP+Pax6+ cells and arrowheads point to GFP+Tbr2+ cells. Scale bare = 100 μm.

C Examples of FACS plots for GFP positive cell sorting at E14.5 *Hes5::GFP* and E15.5 *Tbr2::GFP*.

D–F Expression validation of *Hes5::GFP* and *Tbr2::GFP* positive cells after FAC sorting *in vitro*. Scale bar = 20 μm.

G Expression plots of some known markers of NSCs. Each dot defines the mean and lines define the SD. Three to four biological replicates were collected for each time point.

H Heatmap showing differentially expressed genes in three cell populations illustrating NSCs, BPs and NBNs vary in expression, based on z-scored log2(TPM) expression values.

I Bar plot representing the proportion of variance covered by each PC in PCA of all cell types.

J, L, N Volcano plots for DEG analysis for NSCs versus BPs, NSCs versus NBNs and BPs versus NBNs, respectively. Significantly DEGs are colored as gray and top 100 DEGs are colored by red.

K, M, O Top 10 DEGs for NSCs versus BPs, NSCs versus NBNs, and BPs versus NBNs, respectively. Central band is the median, the whiskers define the upper and lower limit, and the box defines the interquartile ranges. (J–O) are related to analysis of Fig 1E. The range of *P*-values is very different: NSC (0.01–0.4%), BP (1.6–4.9%), NBN (0.06–0.2%). There are no good marker genes for BPs as their gene expression tends to be similar to either NSC or NBN.

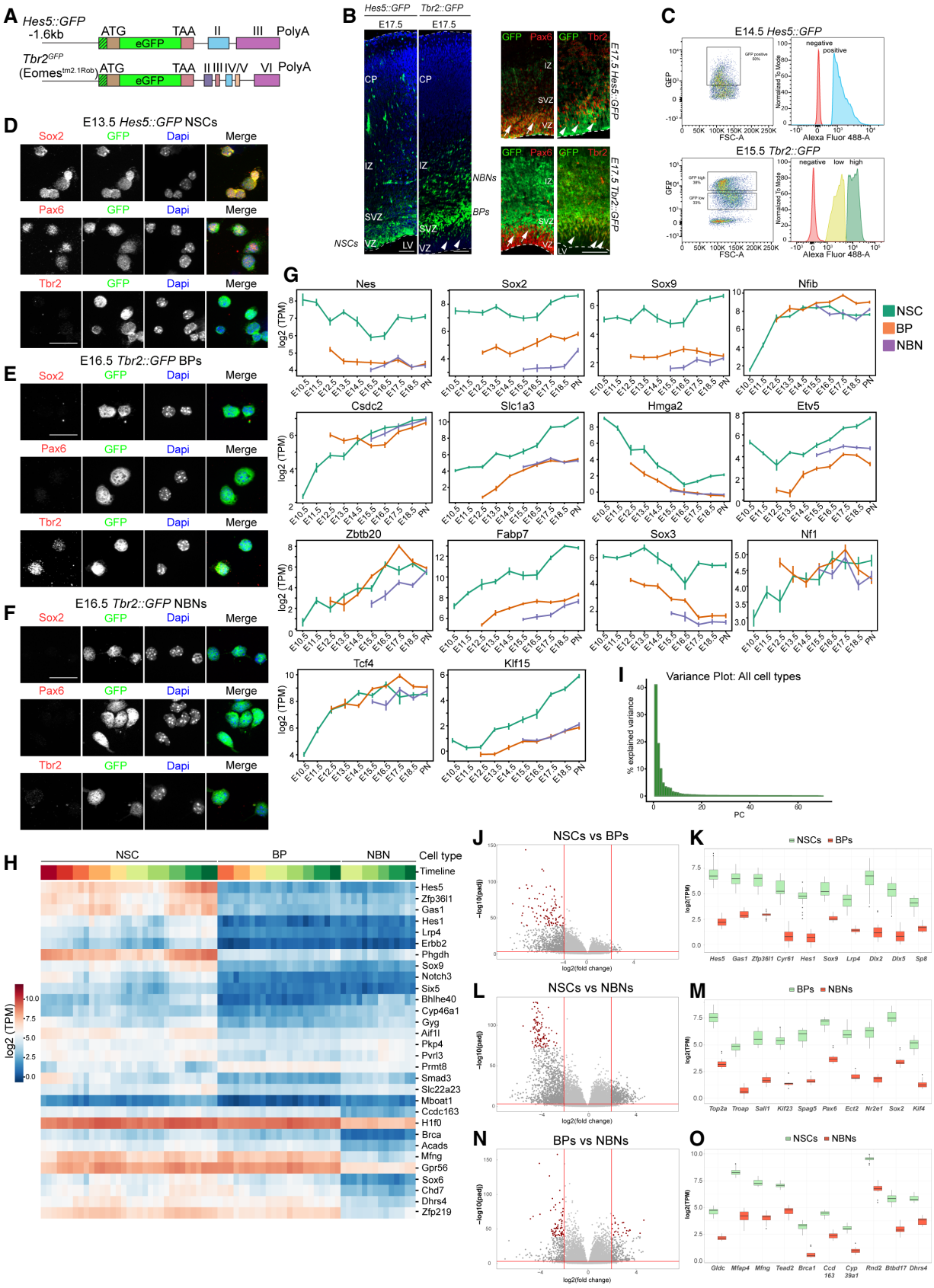
A
Hes5::GFP
-1.6kb
ATG
TAA
eGFP
II
III
PolyA
Tbr2GFP
(Eomestm2.1Rob)
ATG
TAA
eGFP
II III IV/V
VI
PolyA
B
Hes5::GFP
Tbr2::GFP
E17.5
E17.5
CP
IZ
SVZ
VZ
LV
NBNs
BPs
NSCs
GFP Pax6
GFP Tbr2
E17.5 Hes5::GFP
E17.5 Tbr2::GFP
C
E14.5 Hes5::GFP
negative
positive
GFP
FSC-A
Alexa Fluor 488-A
E15.5 Tbr2::GFP
negative
low
high
D
E13.5 Hes5::GFP NSCs
Sox2
GFP
Dapi
Merge
Pax6
Tbr2
E
E16.5 Tbr2::GFP BPs
F
E16.5 Tbr2::GFP NBNs
G
Nes
Sox2
Sox9
Nfib
Csdc2
Slc1a3
Hmga2
Etv5
Zbtb20
Fabp7
Sox3
Nf1
Tcf4
Klf15
log2 (TPM)
E10.5
E11.5
E12.5
E13.5
E14.5
E15.5
E16.5
E17.5
E18.5
PN
NSC
BP
NBN
I
Variance Plot: All cell types
% explained variance
PC
H
NSC
BP
NBN
Cell type
Timeline
Hes5
Zfp36l1
Gas1
Hes1
Lrp4
Erbb2
Phgdh
Sox9
Notch3
Six5
Bhlhe40
Cyp46a1
Gyg
Aif1l
Pkp4
Pvrl3
Prmt8
Smad3
Slc22a23
Mboat1
Ccdc163
H1f0
Brca
Acads
Mfng
Gpr56
Sox6
Chd7
Dhrs4
Zfp219
log2 (TPM)
J
NSCs vs BPs
−log10(padj)
log2(fold change)
K
NSCs
BPs
log2(TPM)
Hes5
Gas1
Zfp36l1
Cyr61
Hes1
Sox9
Lrp4
Dlx2
Dlx5
Sp8
L
NSCs vs NBNs
M
BPs
NBNs
Top2a
Troap
Sall1
Kif23
Spag5
Pax6
Ect2
Nr2e1
Sox2
Klf4
N
BPs vs NBNs
O
Gldc
Mfap4
Mfng
Tead2
Brca1
Ccd 163
Cyp 39a1
Rnd2
Btbd17
Dhrs4

Figure EV1.

Figure EV2. Experimental validation of transcriptional profile changes in NSCs over time.

A Bar plot representing the variance coverage by PC corresponding to PCA plot in Fig 2A.

B Heatmap illustrating the expression changes in signature genes in time points corresponding to expansion, neurogenesis, and gliogenesis.

C qPCR validation of signature genes in three zones. Each time point has samples varying from $N = 3$ to $N = 7$ biological replicates. (Statistical test used- Unpaired Student's *t*-test, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).

D–G k-Means clustering of z-scored log2 (TPM) gene expression profiles over developmental time course in NSCs with genes showing upregulation, e.g., Cspg4, downregulation, e.g., Shh, transient downregulation, e.g., Jag1, transient upregulation, e.g., Neurog2.

H Bar plot representing the variance coverage by PC corresponding to PCA plot in Fig 2E.

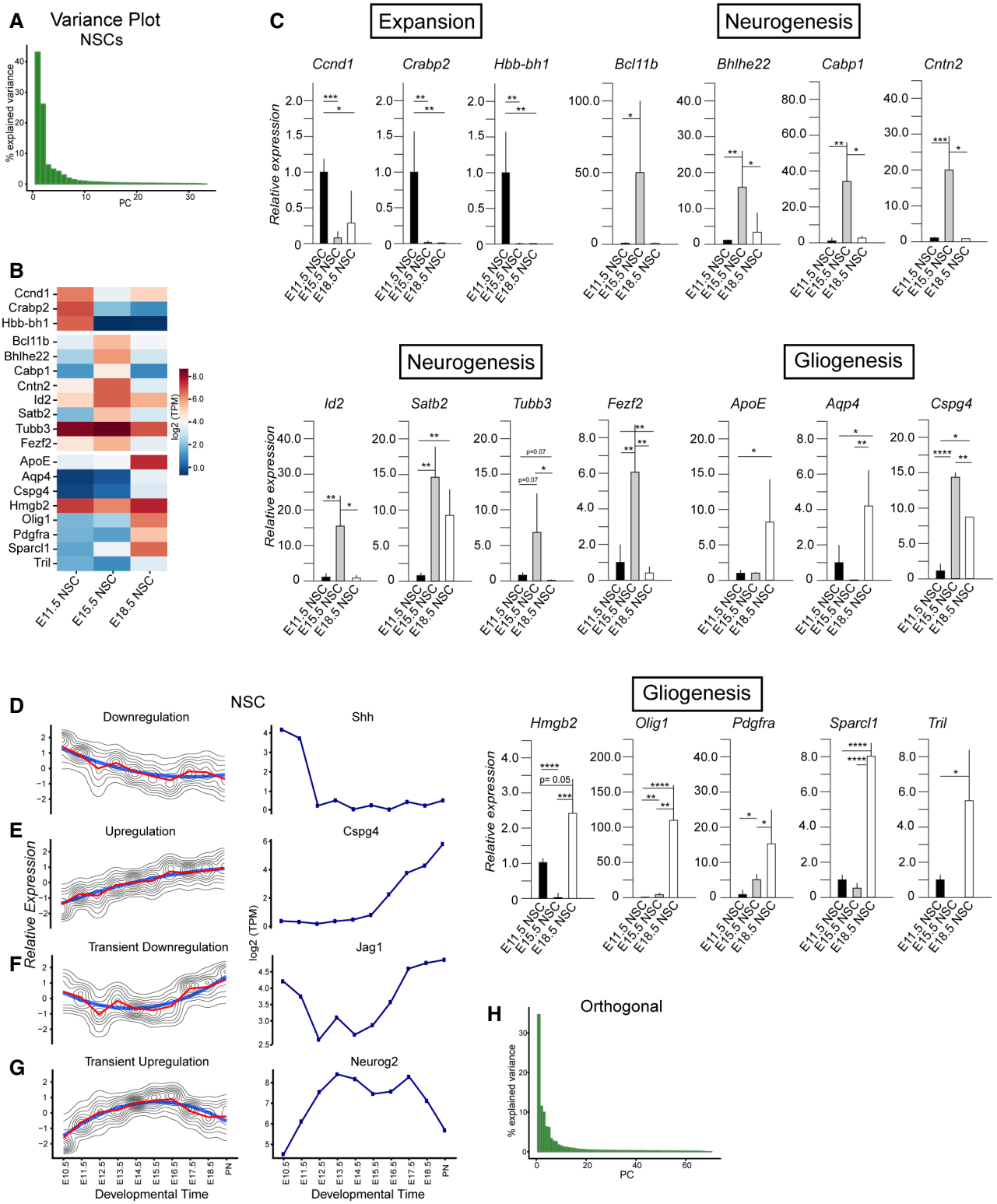
A
Variance Plot
NSCs
% explained variance
PC
B
Ccnd1
Crabp2
Hbb-bh1
Bcl11b
Bhlhe22
Cabp1
Cntn2
Id2
Satb2
Tubb3
Fezf2
ApoE
Aqp4
Cspg4
Hmgb2
Olig1
Pdgfra
Sparcl1
Tril
log2 (TPM)
E11.5 NSC
E15.5 NSC
E18.5 NSC
C
Expansion
Neurogenesis
Gliogenesis
Relative expression
p= 0.05
p=0.07
D
NSC
Downregulation
Shh
E
Upregulation
Cspg4
Relative Expression
log2 (TPM)
F
Transient Downregulation
Jag1
G
Transient Upregulation
Neurog2
Developmental Time
H
Orthogonal

Figure EV2.

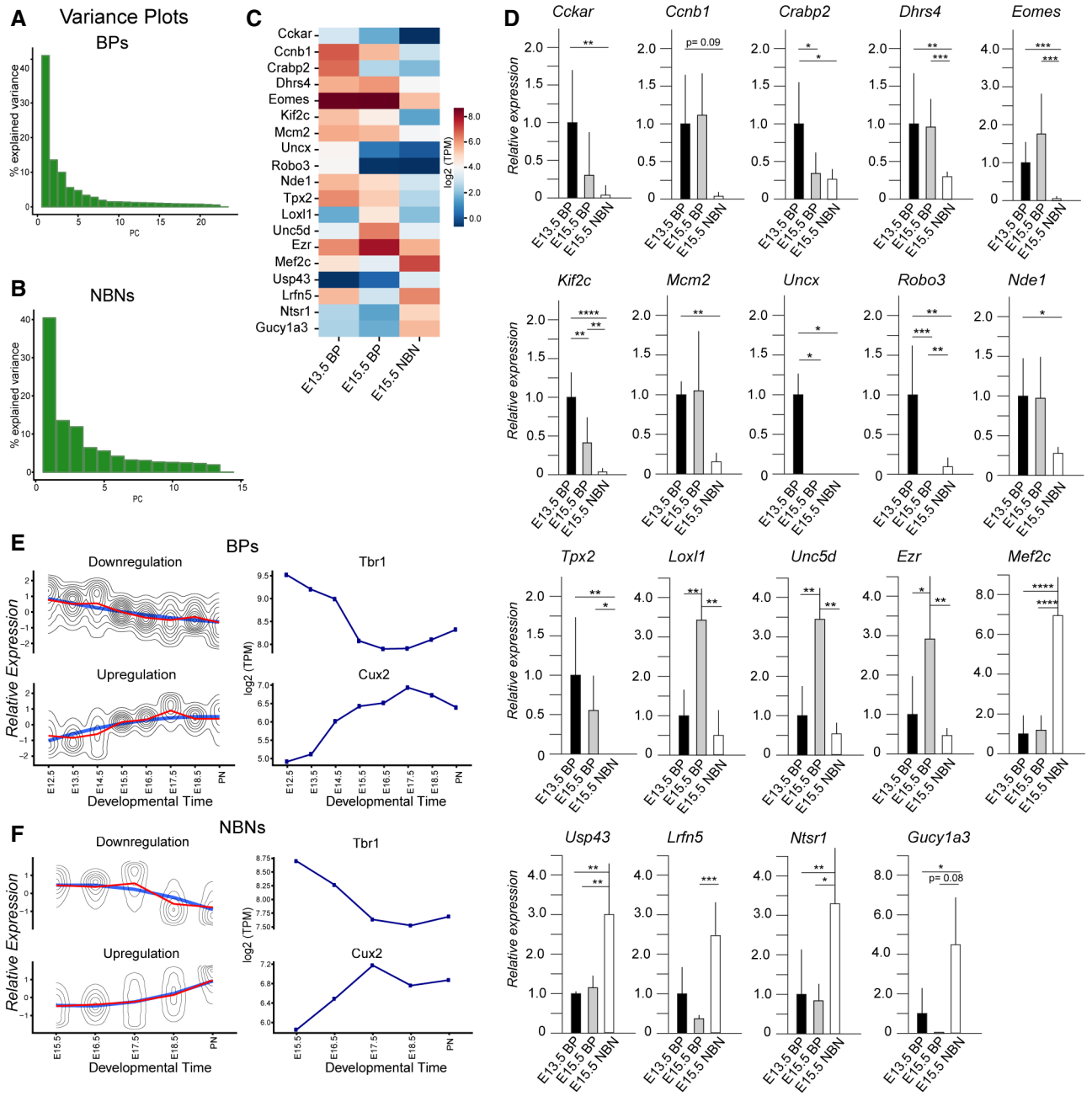

Figure EV3. Experimental validation of transcriptional profile changes in BPs and NBNs over time.

A, B Bar plots representing the variance coverage by PCs corresponding to PCA plot in Fig 2I and M.

C Heatmap illustrating the expression changes in signature genes in time points corresponding to early BPs, mid-BPs, and NBNs.

D qPCR validation of signature genes for three sample types. Each time point has samples varying from $N = 3$ to $N = 7$ biological replicates (statistical test used—Unpaired Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$).

E k-Means clustering of z-scored log2(TPM) gene expression profiles over developmental time course in BPs with genes showing downregulation, e.g., Tbr1 and upregulation, e.g., Cux2.

F k-Means clustering of z-scored log2(TPM) gene expression profiles over developmental time course in NBNs with genes showing downregulation, e.g., Tbr1 and upregulation e.g., Cux2.

Figure EV4. C1 data integration and comparison with Linnarsson dorsal cortex data (La Manno *et al*, 2021).

A UMAP visualization of identified clusters of NSCs, BPs and NBNs when analyzed together, segregating all cells in eight clusters—four NSC, two BP, and two NBN clusters.
B UMAP visualization of post CCA integrated merged dataset containing C1 and dissected forebrain and dorsal forebrain cells; Linnarsson dataset from (La Manno *et al*, 2021), into 10 clusters.
C UMAP clustering visualization of Linnarsson dataset with C1 data post CCA. Cells are labeled with established "Class" from original manuscript. Our NSCs and NBNs fall in Linnarsson radial glia and neuronal clusters.
D C1 data maps onto Linnarsson clusters.
E UMAP clustering visualization after CCA. Cells are labeled by our previously identified clusters. Our C1 clusters integrate mostly with Linnarsson radial glia and neuronal clusters and maintain their separate clustering.
F Example feature plots showing consistent expression of markers in NSCs, BPs and NBNs between two datasets. *Y*-axis is the log normalized expression.
G UMAP clustering visualization post CCA integration of C1 NSCs and radial glial classed cells from Linnarsson dataset, segregating in five clusters.
H Positional mapping of C1 onto Linnarsson clusters.
I After CCA integration, we find C1 NSCs integrate well with Linnarsson radial glial cells. Cells labeled with our previously identified five NSC clusters.
J Example feature plots showing consistent expression of markers in NSCs and radial glial cells, between two datasets.
K Example feature plots showing consistent expression of Hbb subunits in NSCs and radial glial cells, between two datasets.

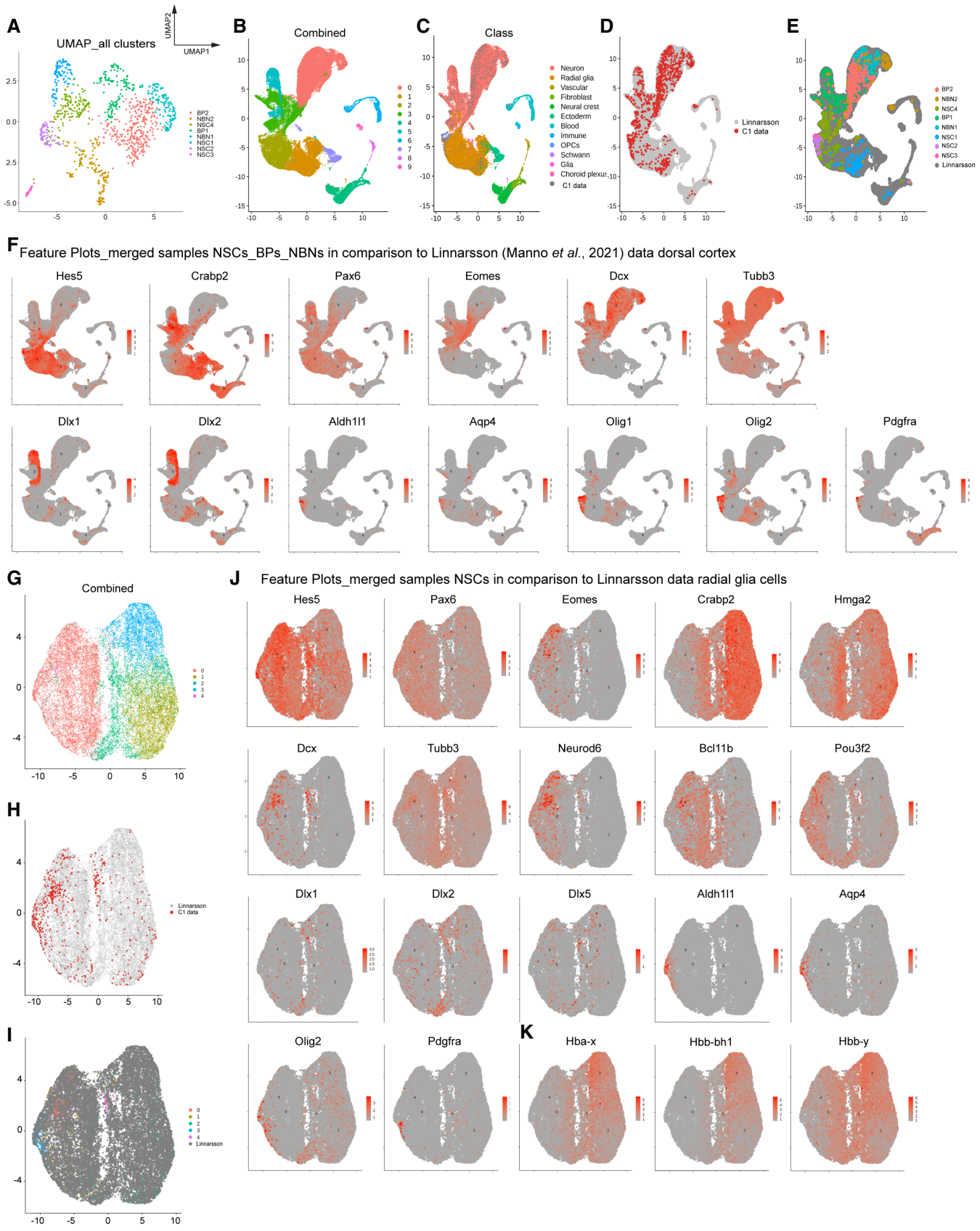

Figure EV4.

Figure EV5. Heterogeneity of cortical layering marker expression in NSCs, BPs and NBNs.

A Heatmap of cortical layer markers in NSC single cells, based on z-scored log2(TPM) expression values.
B Heatmap of cortical layer markers in BP single cells, based on z-scored log2(TPM) expression values.
C Heatmap of cortical layer markers in NBN single cells, based on z-scored log2(TPM) expression values.
D Temporal distribution of NSC single cells along the deep or upper layer markers.
E Temporal distribution of BP single cells along the deep or upper layer markers.
F Temporal distribution of NBN single cells along the deep or upper layer markers.

Data Information: In (D–F), *X* axis: deep layer markers- Bcl11b, Tbr1, Lhx2, Lix1, Sox5, and *Y* axis- Cux2, Satb2, Bhlhe22, Mef2c, Mdga1.

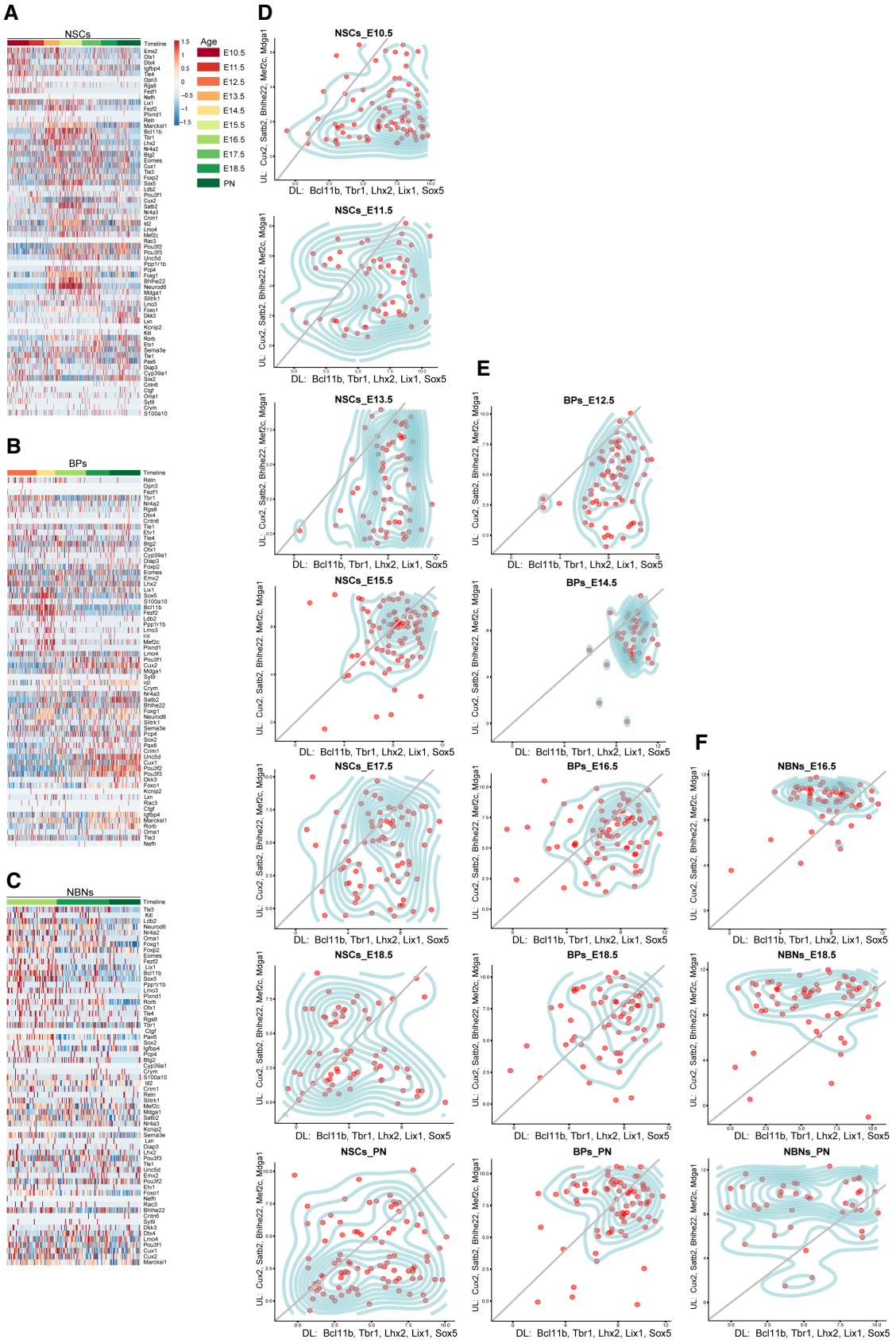

Figure EV5.