Appendix

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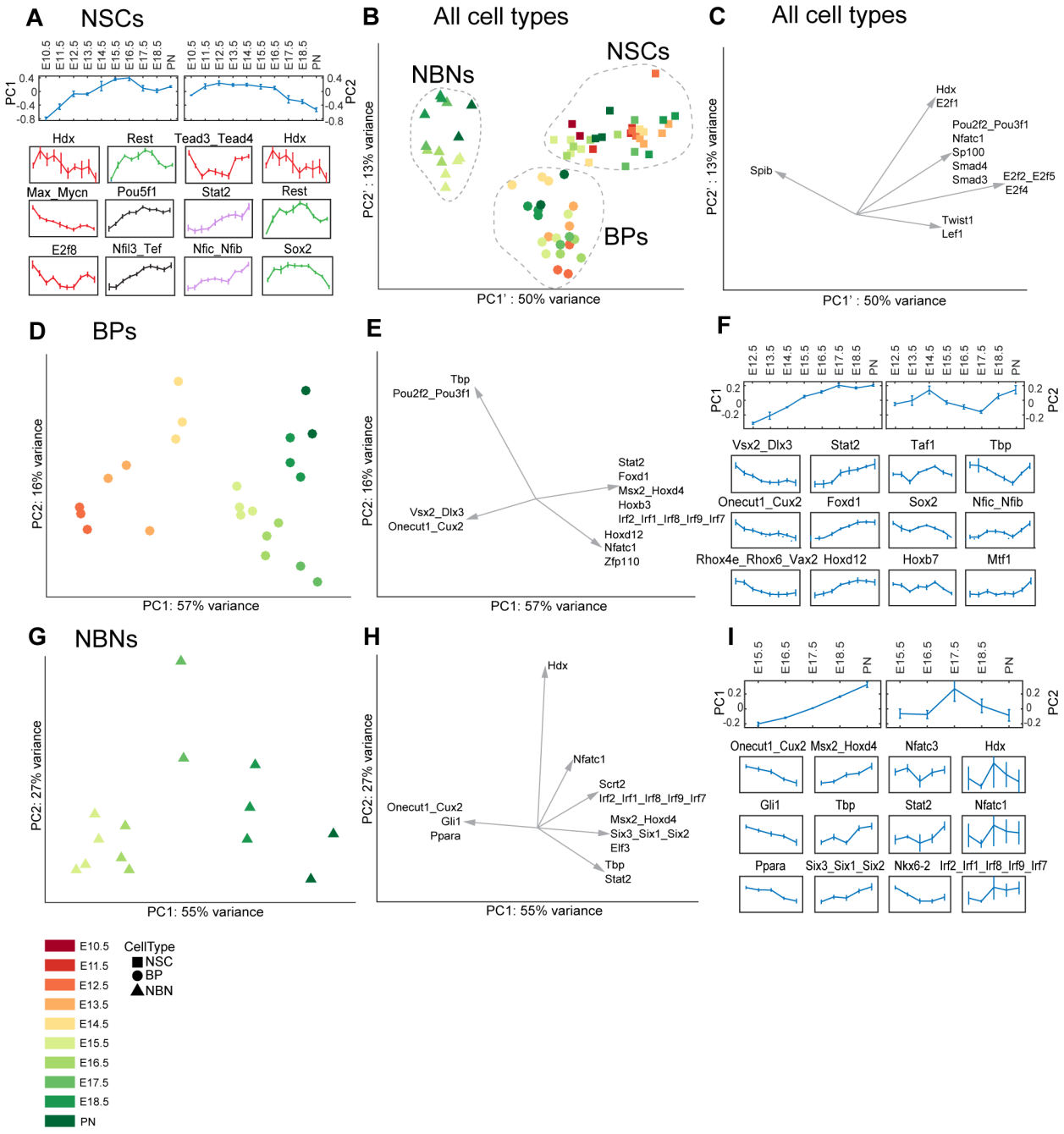
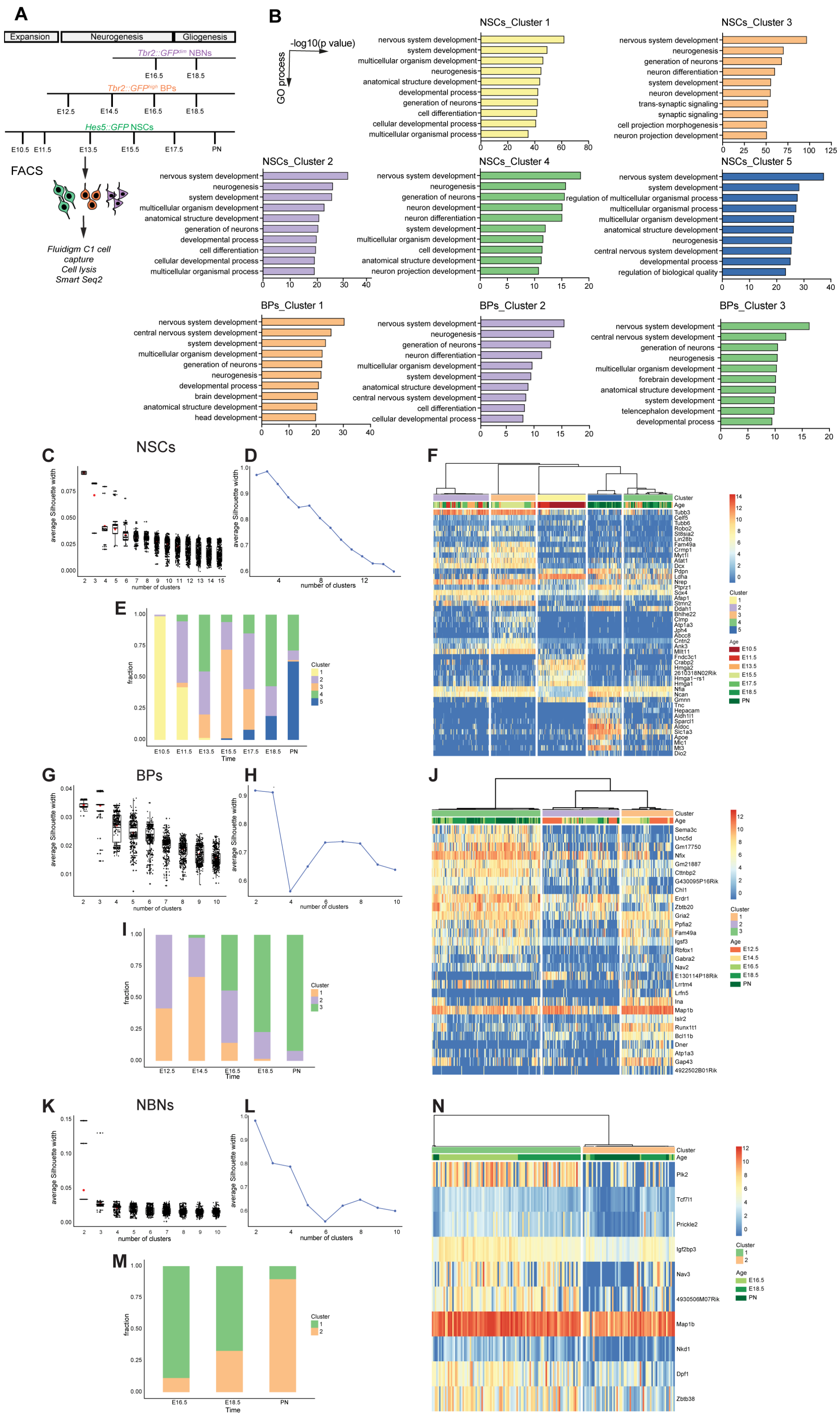


Appendix Figure S1: Dynamics of transcriptional network changes with ISMARA in different populations over time.

(A) Examples of dynamic motifs based on the PCA of NSCs (Figure 3B). Plots show the replicate average of samples across the sampling time for the first two principal components separately, and for the three motifs contributing the most to the first and second principal component, positively and negatively, separately.

(B) PCA plot for all cell types (NSCs, BPs and NBNs) after removing the first two components of NSC variance (from Figure 3B).
(C) Plot showing the projections of each cell type sample on the replicate average of motif activity, representing 63% of the total variance.
(D) PCA analysis on motif activity for BPs for all time points, on the first two components, representing 73% of the total variance.
(E) Top 12 motifs contributing the most to the first two principal components, projected on the first two principal components.
(F) Examples of dynamic motifs based on the PCA of BPs. Plots show the replicate average of samples across the sampling time for the first two principal components separately, and for the three motifs contributing the most to the first and second principal component, positively and negatively, separately.
(G) PCA on motif activity for NBNs for all time points, on the first two components, representing 82% of the total variance.
(H) Plot showing the projections of each cell type sample on the replicate average of motif activity, representing 82% of the total variance.
(I) Examples of dynamic motifs based on the PCA of NBNs. Plots show the replicate average of samples across the sampling time for the first two principal components separately, and for the three motifs contributing the most to the first and second principal component, positively and negatively, separately.
Data Information: In A, F and I bottom, the y-axis is the embryonic day and x-axis is log2(TPM) expression values. The lines define the SD. 3-4 biological replicates were collected for each time point.


A
Expansion
Neurogenesis
Gliogenesis
Tbr2::GFP^dim NBNs
E16.5
E18.5
Tbr2::GFP^high BPs
E12.5
E14.5
E16.5
E18.5
Hes5::GFP NSCs
E10.5
E11.5
E13.5
E15.5
E17.5
PN
FACS
Fluidigm C1 cell capture
Cell lysis
Smart Seq2
B
-log10(p value)
GO process
NSCs_Cluster 1
nervous system development
system development
multicellular organism development
neurogenesis
anatomical structure development
developmental process
generation of neurons
cell differentiation
cellular developmental process
multicellular organismal process
NSCs_Cluster 3
nervous system development
neurogenesis
generation of neurons
neuron differentiation
system development
neuron development
trans-synaptic signaling
synaptic signaling
cell projection morphogenesis
neuron projection development
NSCs_Cluster 2
nervous system development
neurogenesis
system development
multicellular organism development
anatomical structure development
generation of neurons
developmental process
cell differentiation
cellular developmental process
multicellular organismal process
NSCs_Cluster 4
nervous system development
neurogenesis
generation of neurons
neuron development
neuron differentiation
system development
multicellular organism development
cell development
anatomical structure development
neuron projection development
NSCs_Cluster 5
nervous system development
system development
regulation of multicellular organismal process
multicellular organismal process
multicellular organism development
anatomical structure development
neurogenesis
central nervous system development
developmental process
regulation of biological quality
BPs_Cluster 1
nervous system development
central nervous system development
system development
multicellular organism development
generation of neurons
neurogenesis
developmental process
brain development
anatomical structure development
head development
BPs_Cluster 2
nervous system development
neurogenesis
generation of neurons
neuron differentiation
multicellular organism development
system development
anatomical structure development
central nervous system development
cell differentiation
cellular developmental process
BPs_Cluster 3
nervous system development
central nervous system development
generation of neurons
neurogenesis
multicellular organism development
forebrain development
anatomical structure development
system development
telencephalon development
developmental process
C
NSCs
average Silhouette width
number of clusters
D
average Silhouette width
number of clusters
E
fraction
Time
Cluster
F
Cluster
Age
E10.5
E11.5
E13.5
E15.5
E17.5
E18.5
PN
G
BPs
average Silhouette width
number of clusters
H
average Silhouette width
number of clusters
I
fraction
Time
Cluster
J
Cluster
Age
Sema3c
Unc5d
Gm17750
Nfix
Gm21887
Cttnbp2
G430095P16Rik
Chl1
Erdr1
Zbtb20
Gria2
Ppfia2
Fam49a
Igsf3
Rbfox1
Gabra2
Nav2
E130114P18Rik
Lrrtm4
Lrfn5
Ina
Map1b
Islr2
Runx1t1
Bcl11b
Dner
Atp1a3
Gap43
4922502B01Rik
E12.5
E14.5
E16.5
E18.5
PN
K
NBNs
average Silhouette width
number of clusters
L
average Silhouette width
number of clusters
M
fraction
Time
Cluster
E16.5
E18.5
PN
N
Cluster
Age
Plk2
Tcf7l1
Prickle2
Igf2bp3
Nav3
4930506M07Rik
Map1b
Nkd1
Dpf1
Zbtb38

Appendix Figure S2: NSC, BP and NBN heterogeneity at single-cell level.

(A) Schematic representation of the experimental approach used for single cell collection used to isolate *Hes5::GFP* and *Tbr2::GFP* cells for single cell sequencing using Fluidigm C1 platform.

(B) Gene Ontology (GO) analysis of the biological process in different clusters of NSC` and BP single cells. Metacore Software was used, -log10(p value) is indicated. The analyses were performed only on the clusters which are composed by 50 or more genes.

(C) Silhouette analysis where points represent the average Silhouette width of k-means clusters of NSC single cells for each k for a random initial number. For each k, 500 k-means clustering applied with different initial values. Central band is the median, red dot defines the mean, the whiskers define the upper and lower limit, and the box defines the interquartile ranges.

(D) Silhouette coefficient of hierarchal clustering of the assignment matrix of NSC single cells for different k.

(E) Bar plot shows the fractions of NSC cells at each cluster at different time points.

(F) Signature genes identified for each NSC single cell cluster.

(G) Silhouette analysis where points represent the average Silhouette width of k-means clusters of NSC single cells for each k for a random initial number. For each k, 500 k-means clustering applied with different initial values. Central band is the median, red dot defines the mean, the whiskers define the upper and lower limit, and the box defines the interquartile ranges.

(H) Silhouette coefficient of hierarchal clustering of the assignment matrix of BP single cells for different k.

(I) Bar plot shows the fractions of BP cells at each cluster at different time points.

(J) Signature genes identified for each BP single cell cluster.

(K) Silhouette analysis where points represent the average Silhouette width of k-means clusters of NSC single cells for each k for a random initial number. For each k, 500 k-means clustering applied with different initial values. Central band is the median, red dot defines the mean, the whiskers define the upper and lower limit, and the box defines the interquartile ranges.

(L) Silhouette coefficient of hierarchal clustering of the assignment matrix of BP single cells for different k.

(M) Bar plot shows the fractions of NBN cells at each cluster at different time points.

(N) Signature genes identified for each NBN single cells cluster.

Appendix Figure S3: UMAP clustering of NSCs over time.

(A) NSC clusters based on UMAP visualization. UMAP clustering visualization of NSCs based on time points to be used as a reference for the feature plots in the figure.

(B) Feature plots of NSCs, for Hbb sub-units to validate to PCA analyses. Y-axis is the log normalized expression.

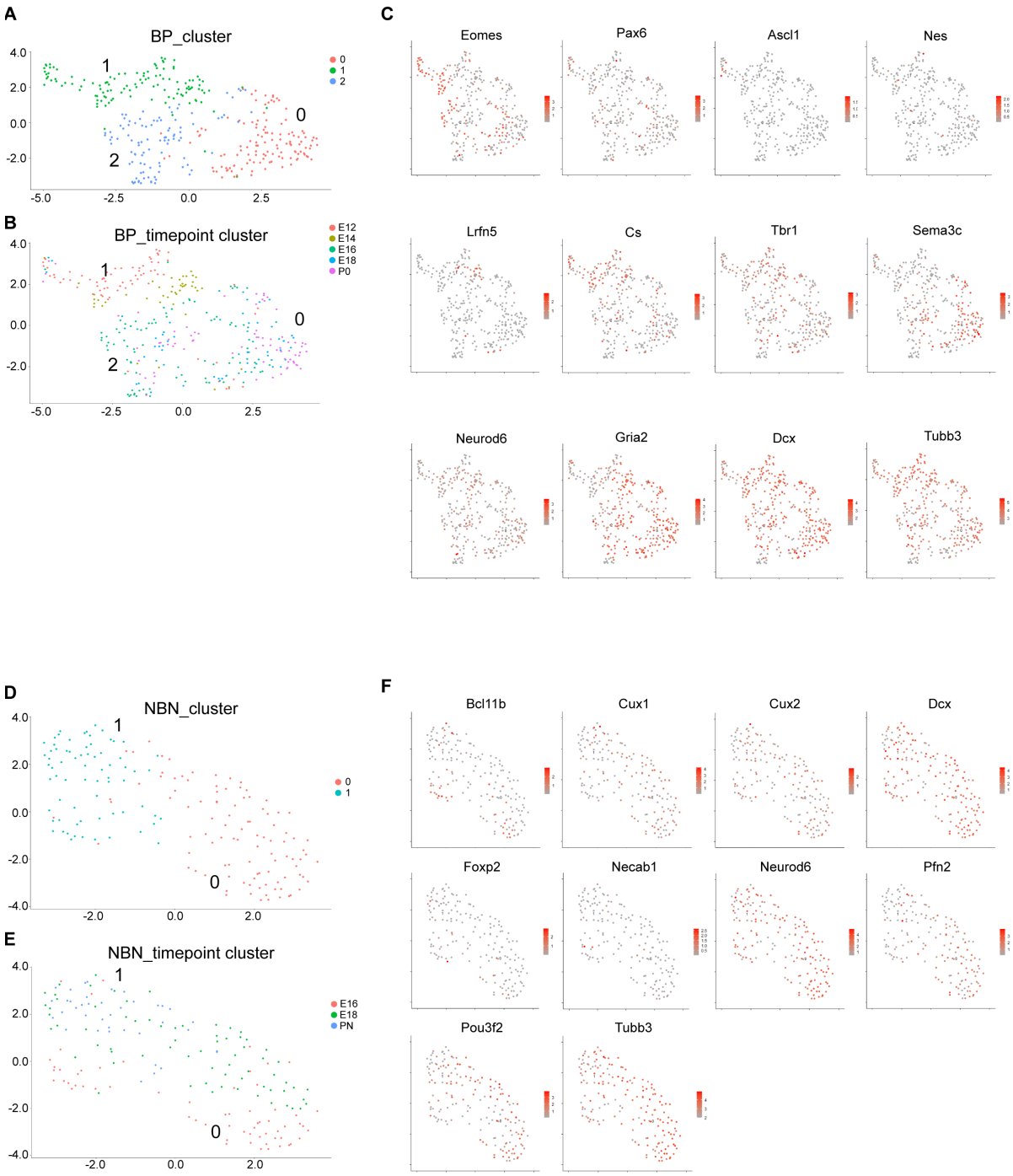
(C) Examples of feature plots of NSCs, for known progenitor markers, to show similarities with PCA.

(D) Feature plots of NSCs for excitatory neuron markers, highlighting the onset of neurogenic program in NSCs as observed by sc-PCA and bulk-RNA sequencing.

(E) Feature plots of NSCs for inhibitory neuron markers validating the presence of a small cluster of Dlx+ cells in the neurogenic NSCs.

(F) Feature plots of NSCs for astrocyte glial lineage shows clear segregation on UMAP clustering similar to PCA.

Feature plots of NSCs for oligodendrocyte lineage shows a cluster on UMAP similar to PCA.



Appendix Figure S4: UMAP clustering of BPs and NBNs over time.

(A) BP KNN clusters visualized with UMAP.

(B) Collection timepoint identity of BP clusters

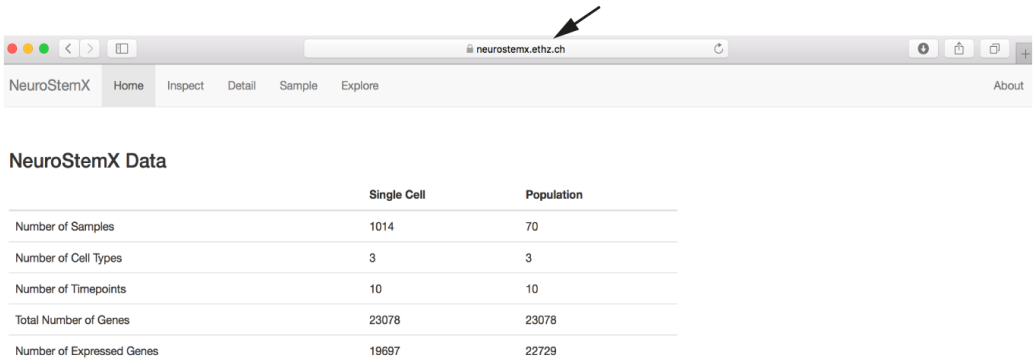
(C) Feature plots as example genes to show comparable expression of markers across clusters as previously observed using K-means and tSNE. Y-axis is the log normalized expression.

(D) NBN clusters visualized using UMAP.

(E) UMAP visualization of NBN timepoint collection identities two clusters.

(F) Feature plots as examples to show congruent expression of markers as previously observed using tSNE.

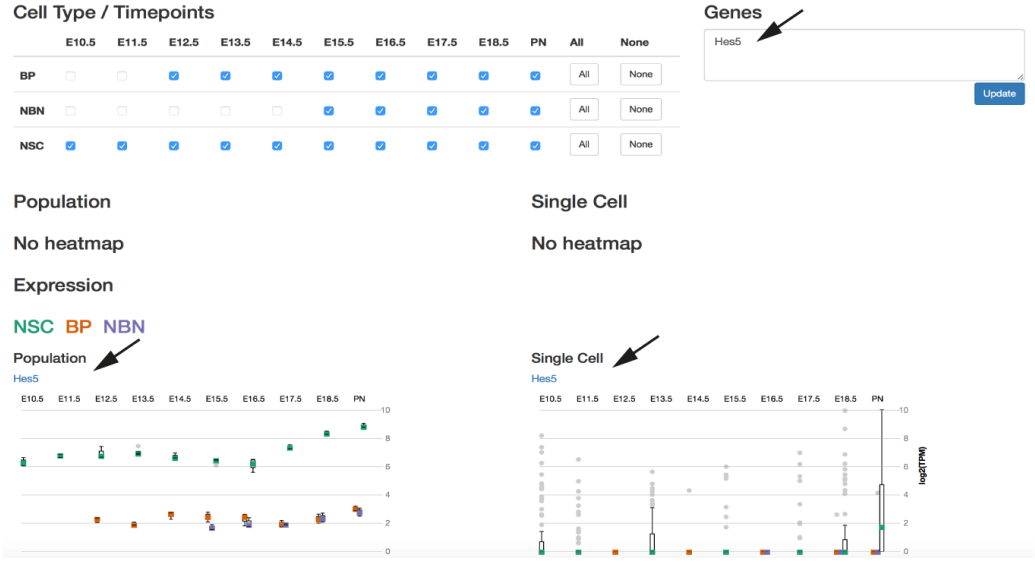
A



	Single Cell	Population
Number of Samples	1014	70
Number of Cell Types	3	3
Number of Timepoints	10	10
Total Number of Genes	23078	23078
Number of Expressed Genes	19697	22729

B

C



Appendix Figure S5: The NeuroStemX website.

(A) The online browser (http://neurostemx.ethz.ch/) directs to detailed population and single cell RNA sequencing analyses for NSCs, BPs and NBNs.

(B) Going through the Inspect tab, one can select the time points or genes one is interested in and click on Update. The website in real-time processes the request and displays the desired heatmaps.

(C) Example showing a query for a single gene, here, *Hes5*, yields two types of plots- population and single cell for all cell types.

(The data are in log2(TPM), color code as mentioned in the key).