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Supplemental information

Patterning ganglionic eminences in developing human brain organoids using a morphogen-gradient-inducing device

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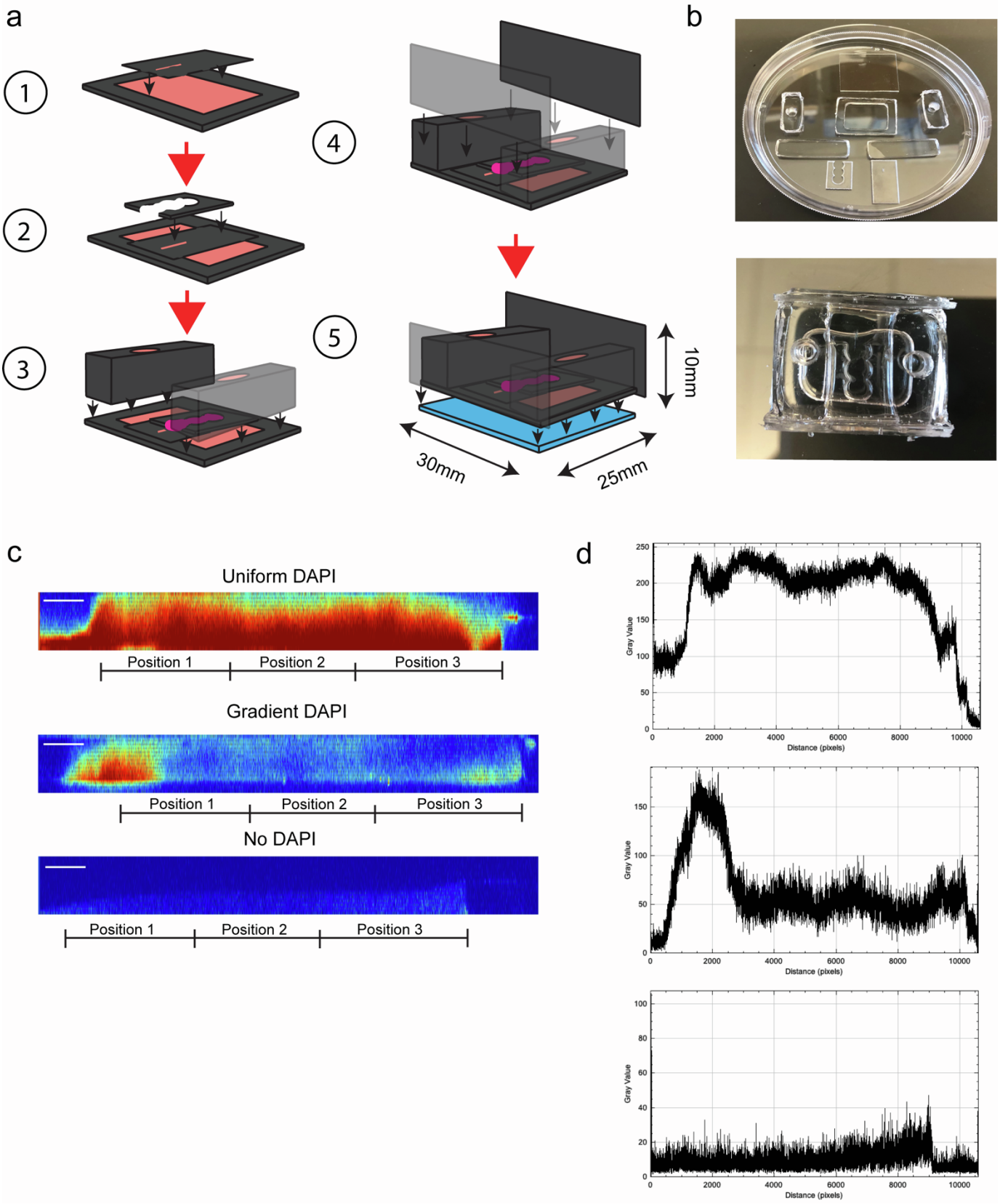

a
1
2
3
4
5
10mm
30mm
25mm
b
c
Uniform DAPI
Position 1
Position 2
Position 3
Gradient DAPI
Position 1
Position 2
Position 3
No DAPI
Position 1
Position 2
Position 3
d
Gray Value
Distance (pixels)
Gray Value
Distance (pixels)
Gray Value
Distance (pixels)

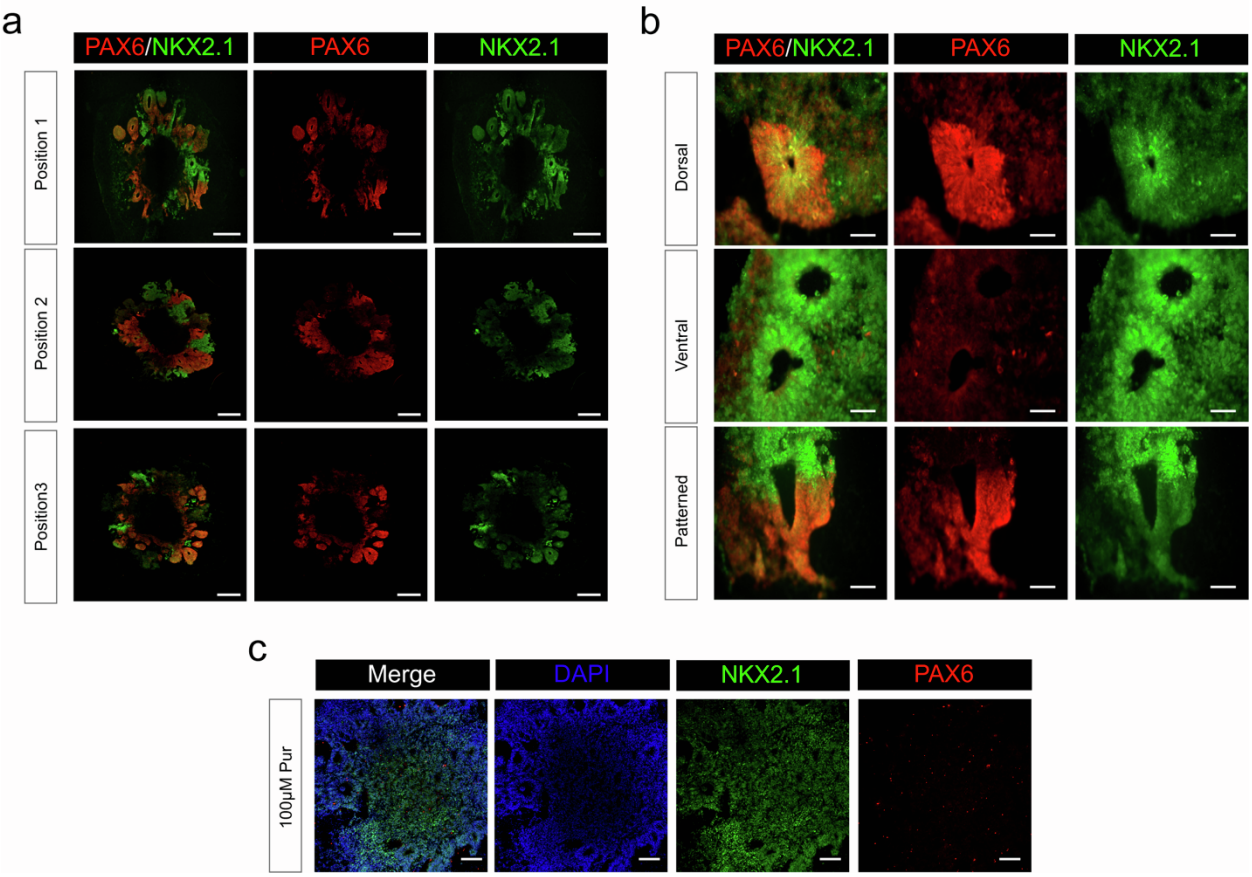
Supplemental FIGURE 1 | PDMS device assembly and gradient quantification (Related to Figure 1).

(A) Numbered, step by step schematic of PDMS device assembly. 1: Culturing area with laser-cut slit placed on chemical chamber base. 2: Matrigel embedding scaffolds placed over culturing area. 3: Inlet/outlet placed on both sides of device. 4: Encapsulating pieces to create a self-containing region for culture medium. 5: Entire device is secured to a glass bottom to fully seal the chemical reservoir.

(B) (Left) Unassembled, Individual PDMS slabs required to make one PDMS device. (Right) Fully assembled PDMS device.

(C) Each condition was carried out characterized after 24 hours in culture. Slice views of compounded and merged Z stack images were used to recreate Matrigel profile. Jet LUT was used to visualize DAPI intensity. (Top) Slice view of DAPI signal retained by Matrigel layer when DAPI containing media is added directly into the culturing area. (Middle) DAPI intensity profile when DAPI containing media is added only into the chemical chamber to elicit passive diffusion. (Bottom) Lack of DAPI signal in Matrigel when no DAPI containing media is added at all. Scale bar is 1000μm.

(D) The profile plot takes the mean intensity of all pixels in a column (y direction) and plots them across distance (x direction).

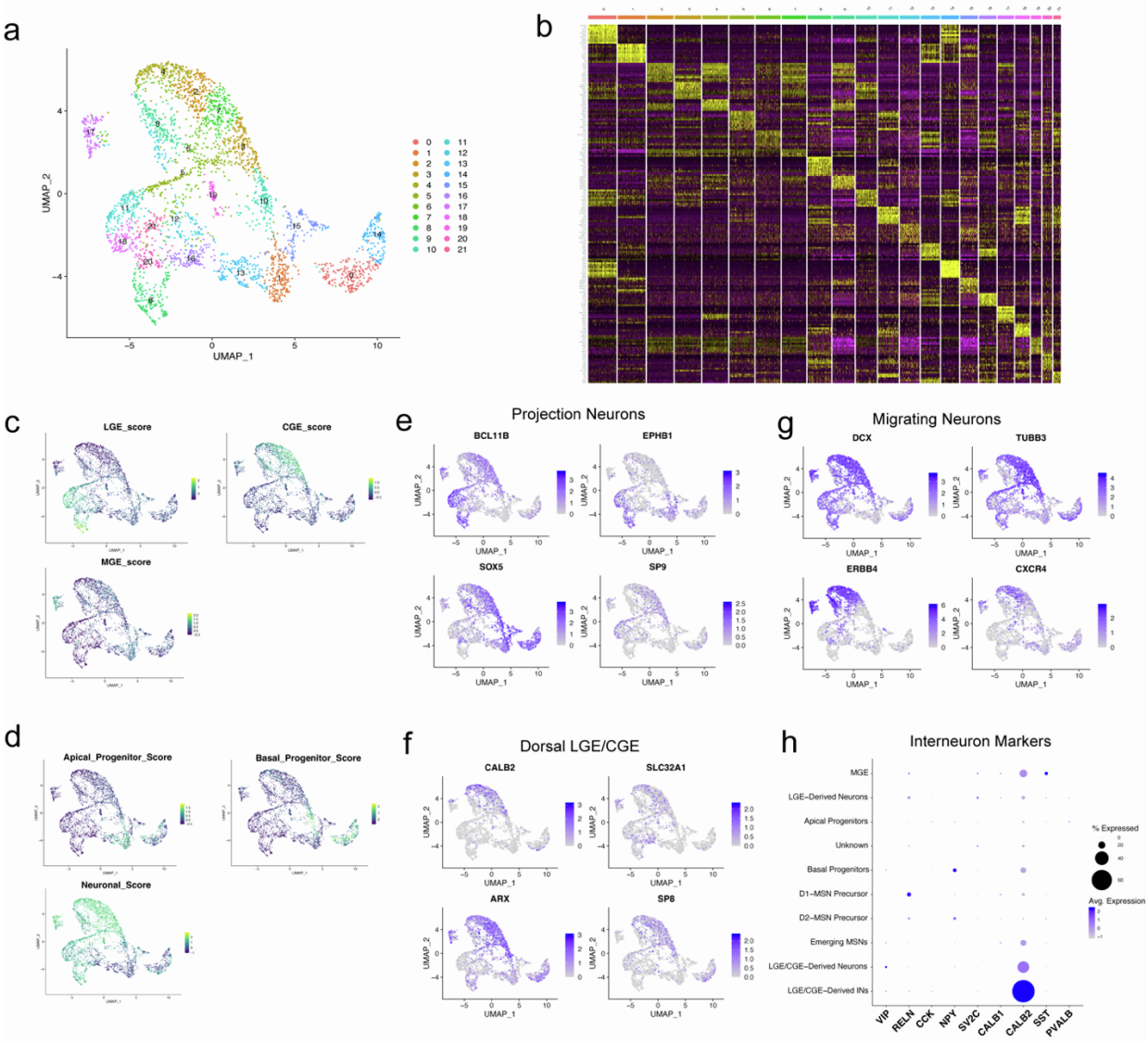


Supplemental FIGURE 2 | Characterizing the effects of purmorphamine (Pur) gradient on forebrain organoids (Related to Figure 2).

(A) Representative confocal images of NKX2.1 and PAX6 staining in device-patterned organoids (1 μM Pur) at different distances from Pur source. 500 μm scale bars.

(B) Representative confocal images displaying various rosette features observed in patterned organoids (1 μM Pur). 50 μm scale bars.

(C) Organoid cultured in PDMS device and exposed to 100μM Pur gradient shows full ventralization. 200μm scale bar.



Supplemental FIGURE 3 | Characterization of mature neuronal subtypes in 4-month device grown MIBOs (Related to Figure 3).

(A) UMAP of 3,366 single cells dissociated from 4-month MIBO before cell annotation. A total of 22 clusters were established using the first 30 principal components and a fine resolution of 2.0.

(B) Heatmap showing top 5 genes expressed for each cluster.

(C) UMAPs showing combined expression for canonical markers for each of the GEs. LGE: ZFHX3, ZNF503, MEIS2, FOXP1, EBF1, ISL1, RBFOX1. CGE: NR2F2, PROX1, SP9, SCGN, PRKCA. MGE: NKX2.1, LHX6, LHX8, SOX6.

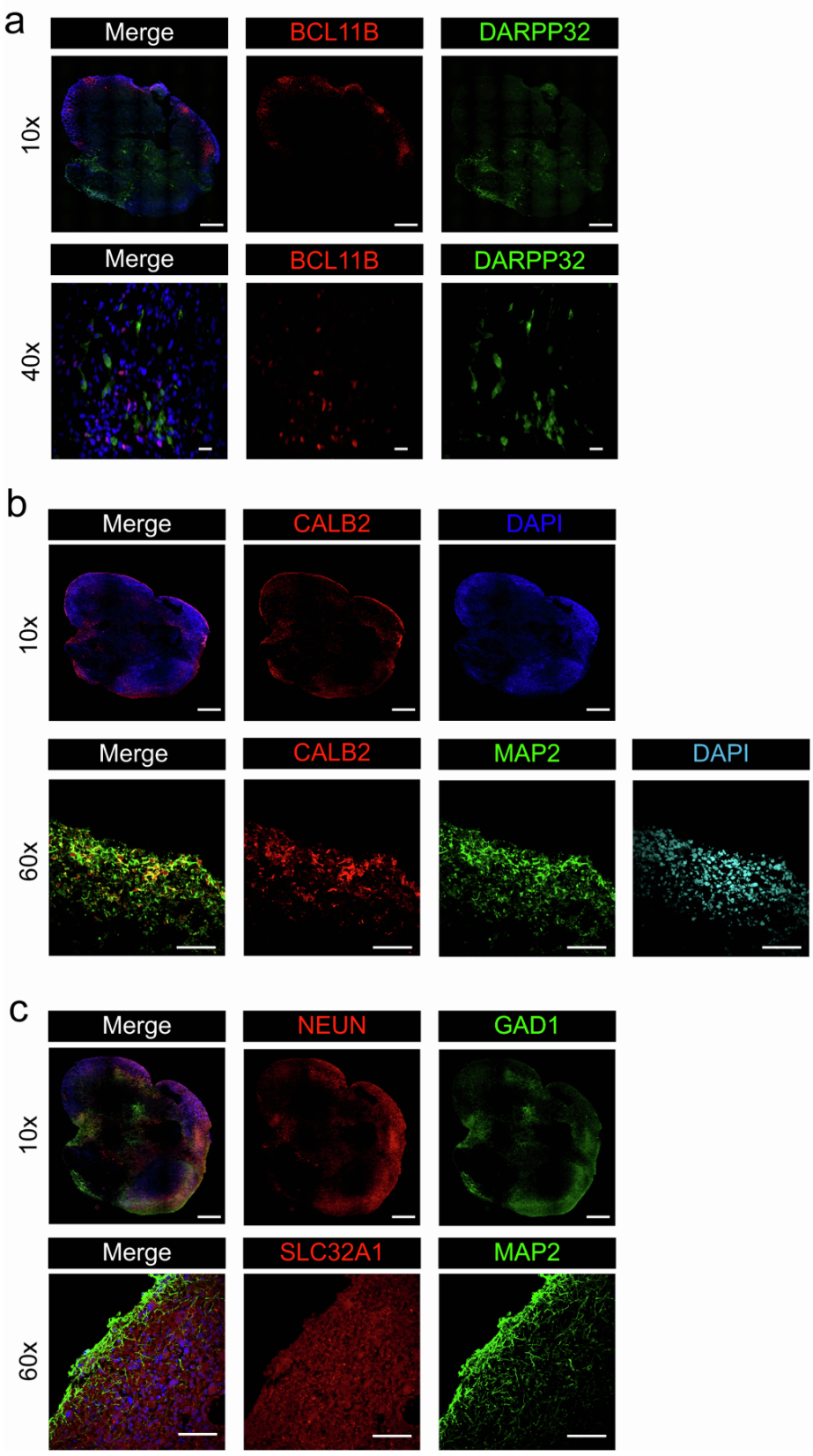
(D) UMAPs showing combined expression for progenitors and mature cell types. Apical progenitors: NES, HES1. Basal progenitors: ASCL1, HES6. Neuronal cells: MAP2, STMN2, DCX.

(E) Feature maps for key marker of projection neurons.

(F) Feature maps identifying dorsal LGE and CGE.

(G) Feature maps showing migrating neurons with top row for general migration and bottom row more specific to IN migration.

(H) Dot plot displaying percentage and average expression of IN associated markers detected within the MIBOs.

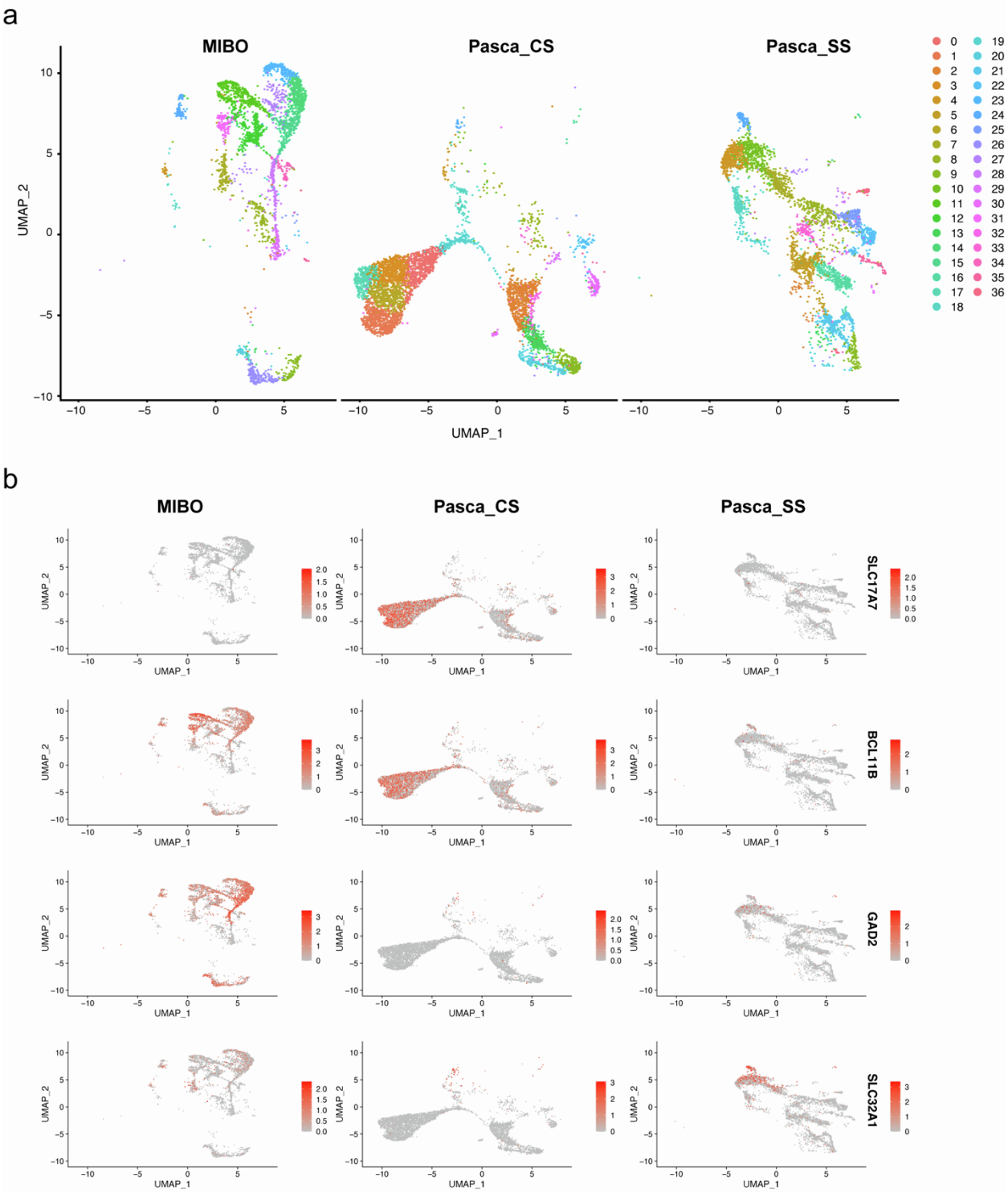

a
Merge
BCL11B
DARPP32
10x
Merge
BCL11B
DARPP32
40x
b
Merge
CALB2
DAPI
10x
Merge
CALB2
MAP2
DAPI
60x
c
Merge
NEUN
GAD1
10x
Merge
SLC32A1
MAP2
60x

Supplemental FIGURE 4 | Characterizing 3.5-month-old MIBOs through immunohistochemistry (Related to Figure 3)

(A) Visualization of emerging MSNs within MIBOs using a combination BCL11B and DARPP32. Scalebar for Merged 10x image is at 500μm and 40x scale bar is 50μm.

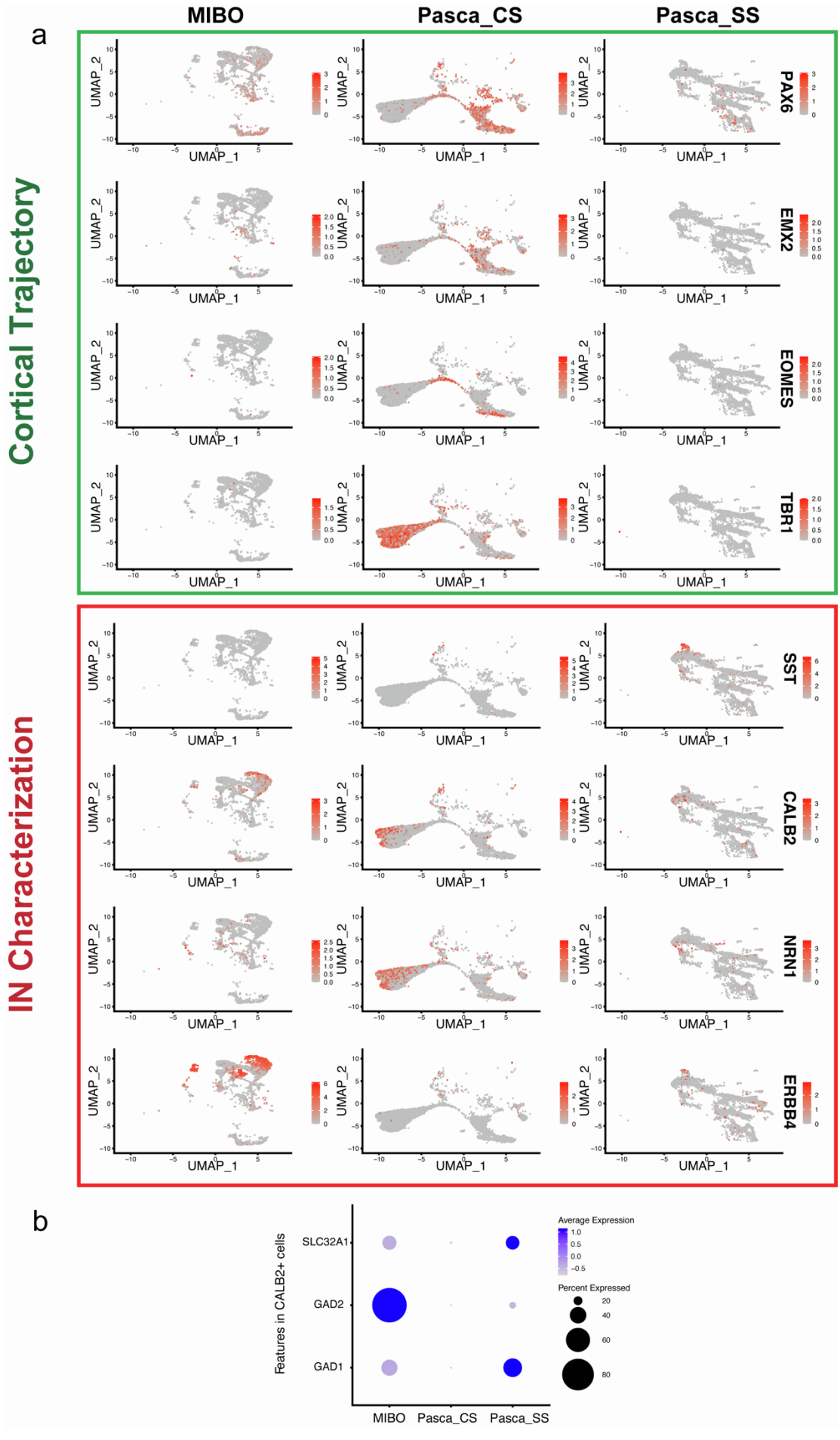
(B) CALB2+ INs can be seen tangentially migrating through the outermost layers of the organoid. Instances of more mature CALB2+ INs can be identified by the co-expression of MAP2. Images captured as 60x contain 50μm scale bars.

(C) Identification of mature GABAergic cells using NEUN (FOXP3), Pan-neuronal marker, GAD1 and SLC32A1 for identifying GABA production, and MAP2, dendritic marker.


a
MIBO
Pasca_CS
Pasca_SS
UMAP_2
UMAP_1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
b
MIBO
Pasca_CS
Pasca_SS
SLC17A7
BCL11B
GAD2
SLC32A1

Supplemental FIGURE 5 | Feature plots used to visualize unique genetic signatures within BCL11B clusters (Related to Figure 5).

(A) Integrated UMAP split to show the individual contribution of each protocol to the total dataset.
(B) Key features used to extrapolate the difference between BCL11B population in MIBO vs Pasca CS.


a
MIBO
Pasca_CS
Pasca_SS
Cortical Trajectory
IN Characterization
PAX6
EMX2
EOMES
TBR1
SST
CALB2
NRN1
ERBB4
UMAP_1
UMAP_2
b
Features in CALB2+ cells
SLC32A1
GAD2
GAD1
MIBO
Pasca_CS
Pasca_SS
Average Expression
Percent Expressed

Supplemental FIGURE 6 | Identifying the developmental trajectory of cortical neurons (Related to Figure 5).

(A) Feature plots used to visualize the unique developmental trajectory from cortical progenitors to mature glutamatergic cortical neurons (Green Box). Feature plots split by sample to characterize IN populations present in each sample.
(B) Dot plot visualizing expression of GABAergic markers within the CALB2+ populations of each sample.