Supplementary Information for Sorrells et al.
Immature excitatory neurons develop during adolescence in the human amygdala

Supplementary Figures 1–8

Supplementary Table 1: Case List

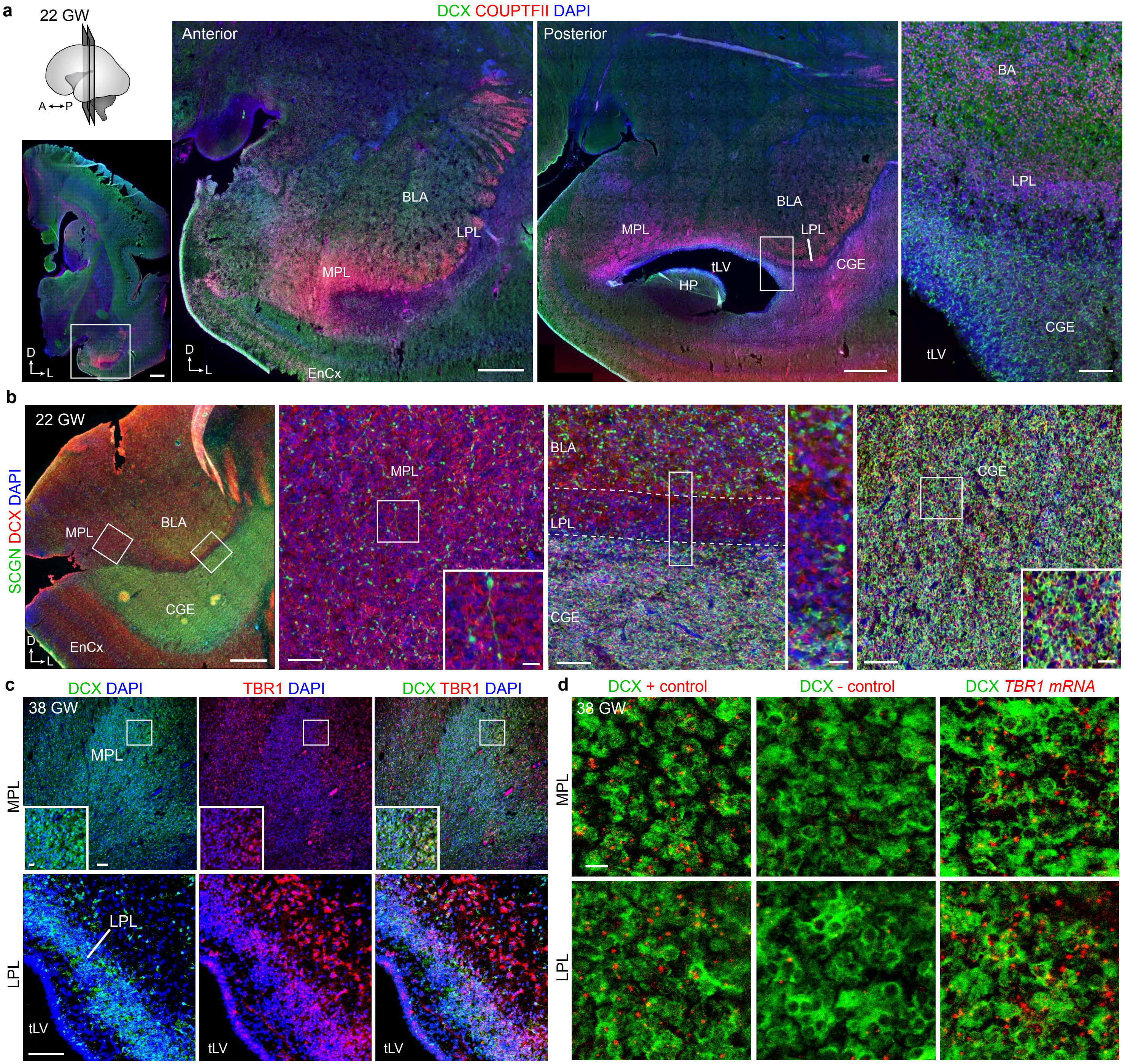
Supplementary Table 2: Antibodies

Supplementary Table 3: Genes differentially expressed in immature PL neurons in ASD

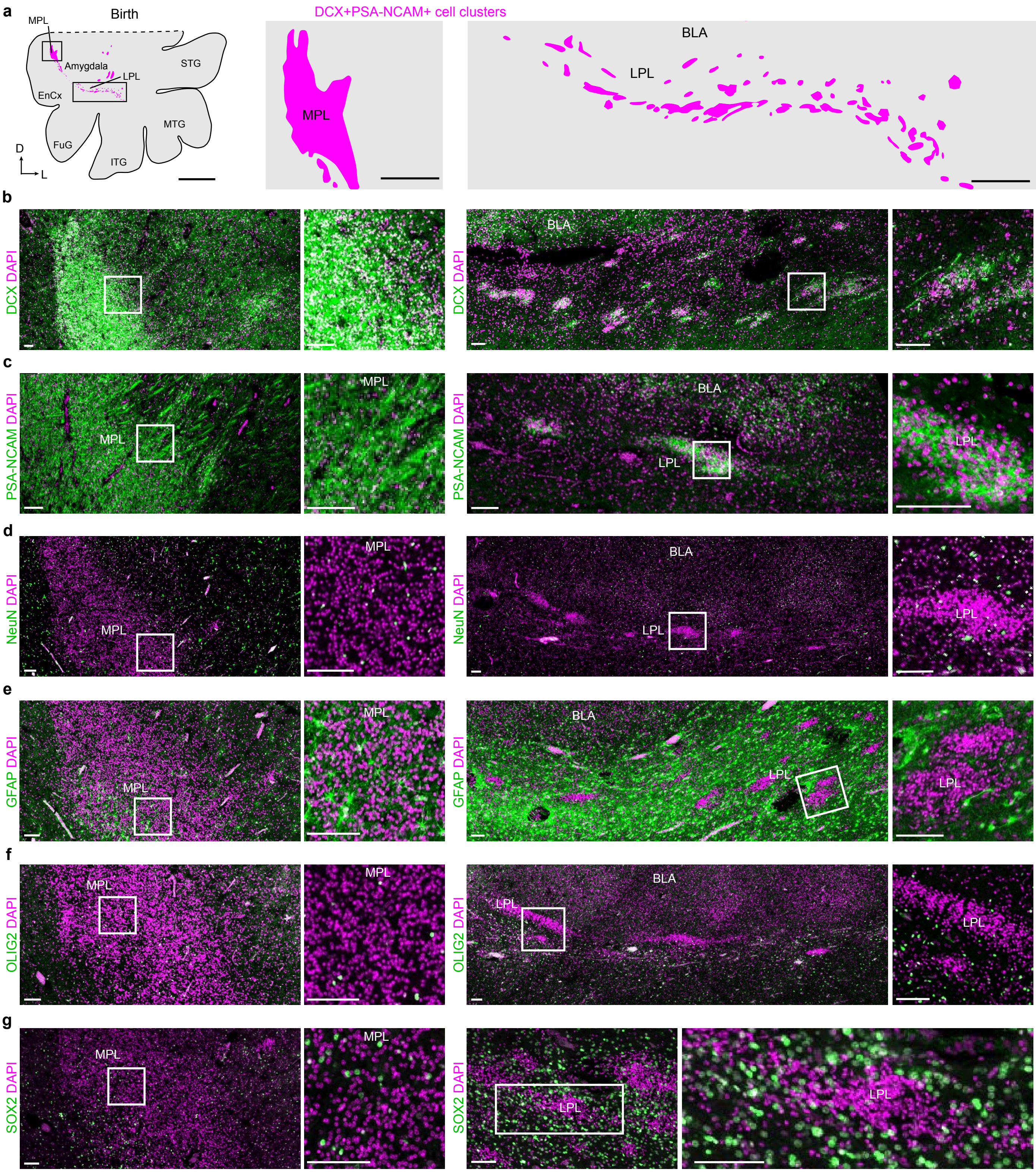
Supplementary Data 1: Cell metadata for the amygdala snRNA-seq dataset

Supplementary Data 2: Mixed linear models

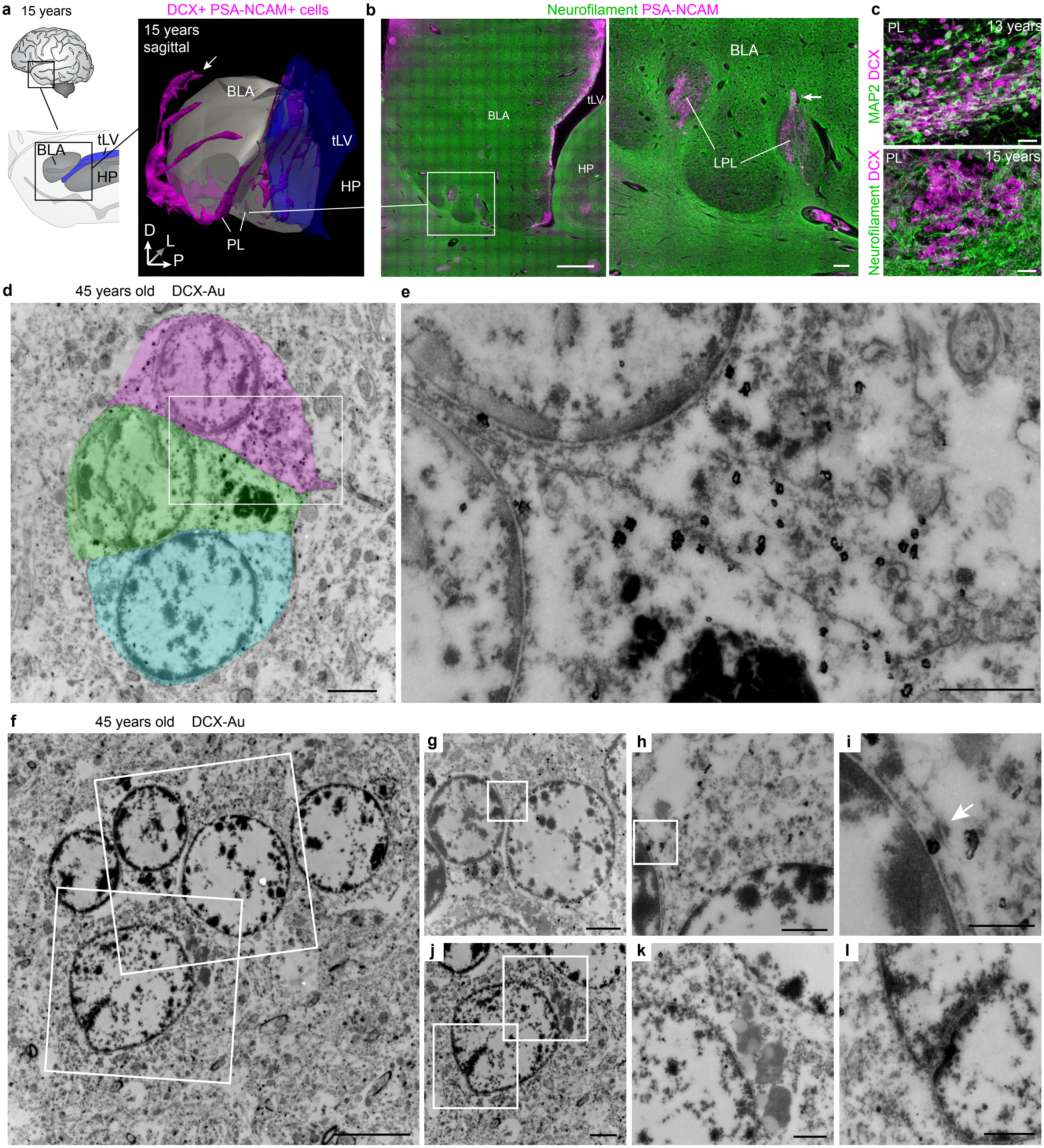
Supplementary Data 3: List of markers of immature PL neurons identified by unbiased analysis of snRNA-seq data



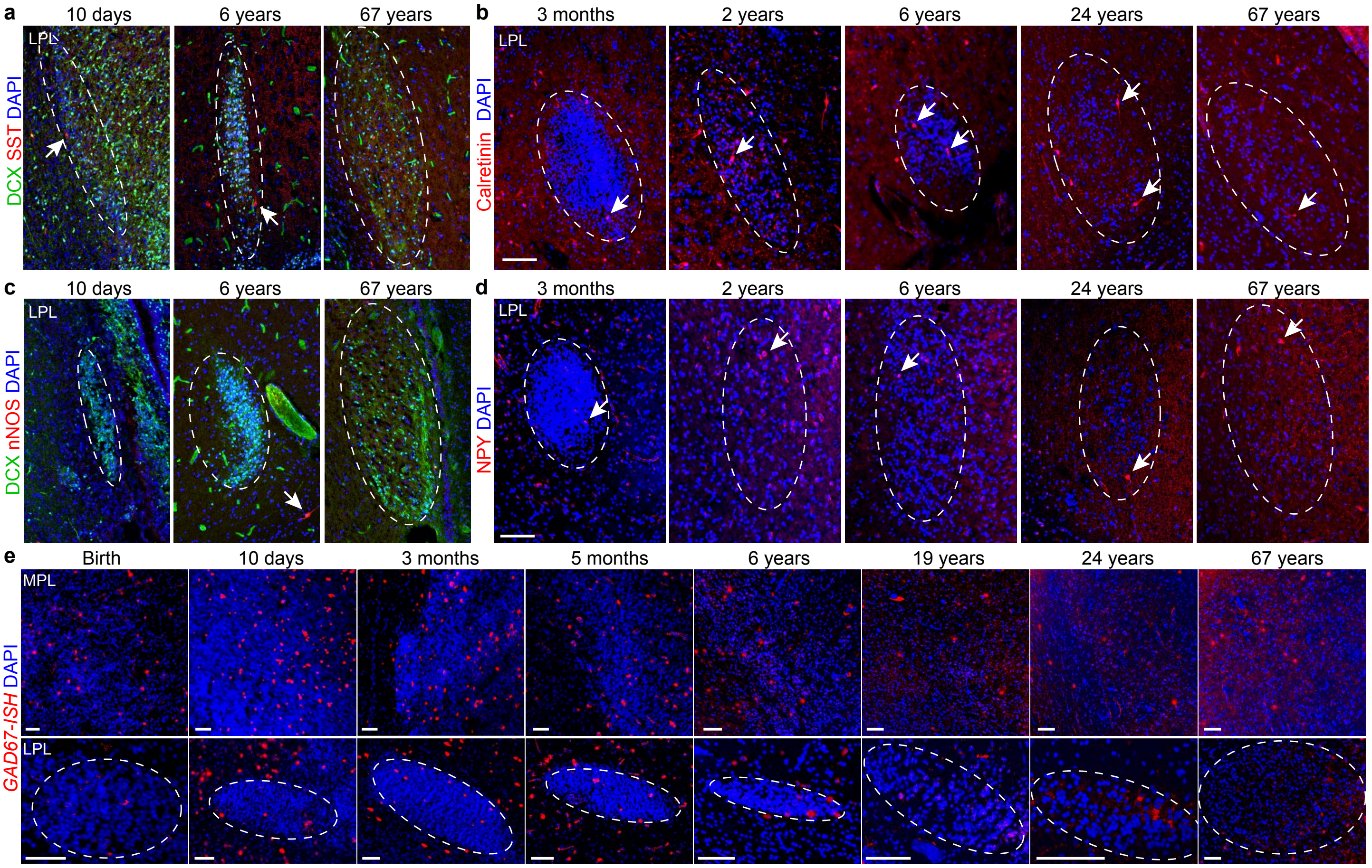
Supplementary Figure 1: DCX⁺COUP-TFII⁺ cells expressing *TBR1* in the PL at 22 GW. a, Coronal sections at 22 GW of the anterior (left) and posterior (right) human amygdala separated by 1 mm. DCX⁺COUP-TFII⁺ cells are present in the medial and lateral PL regions (MPL and LPL respectively) and in the CGE. **b,** (Left) SCGN⁺DCX⁺ cells in the temporal lobe at 22 GW. The MPL (Middle left) and LPL (Middle right) contain few of these cells compared to the BLA or CGE (Right). **c,** DCX⁺TBR1⁺ cells in the MPL and LPL at 38 GW. **d,** Single-molecule detection of *TBR1* mRNA (or the positive control PPIB) in the DCX⁺ cells within the MPL and LPL at 38 GW. Scale bars: 2 mm (**a** lower left), 1 mm (**a** middle left, **a** middle right, **b** left), 100 µm (**b** middle left, **b** middle right, **b** right, **c**), 20 µm (**b** insets, **c** insets), 10 µm (**d**).



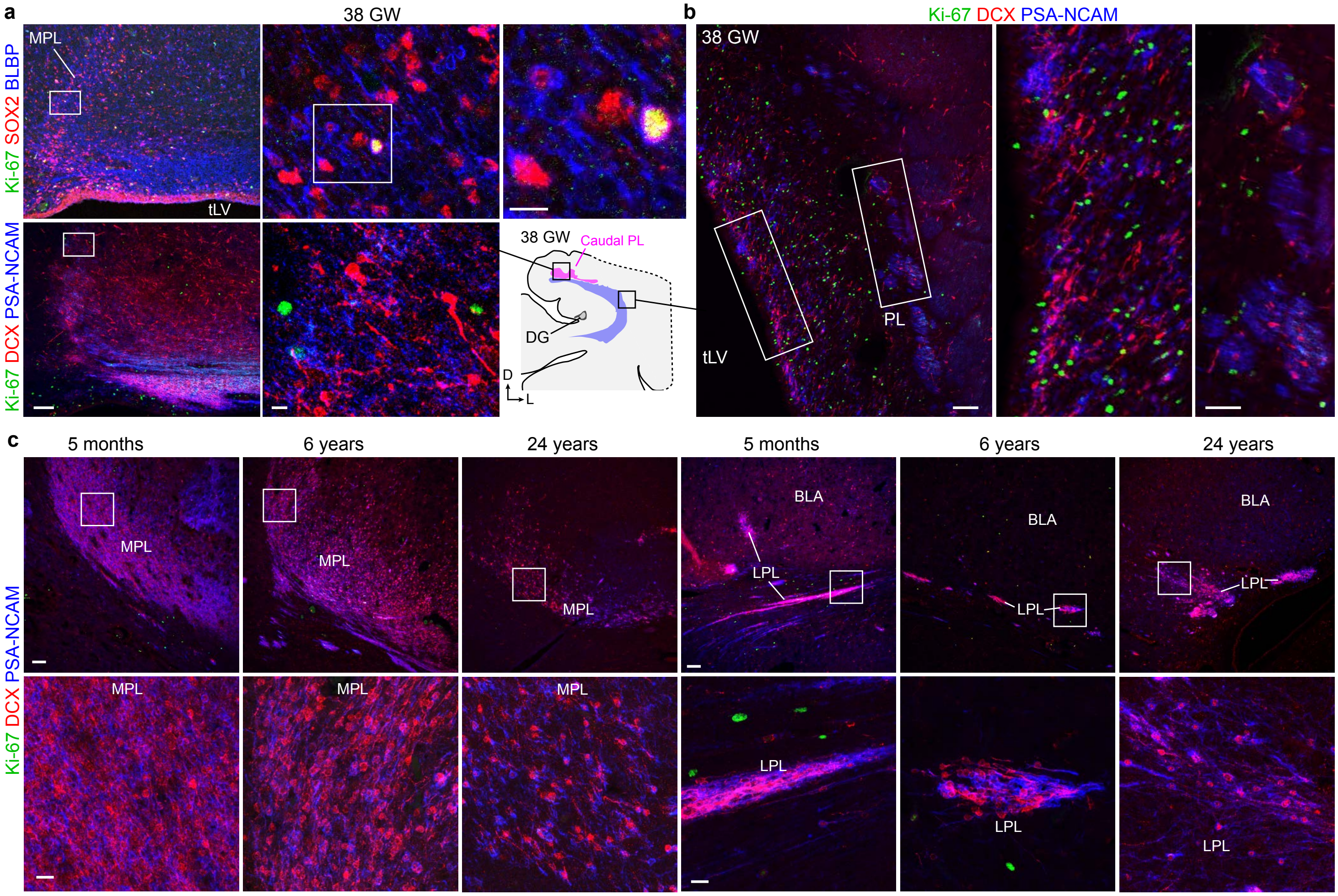
Supplementary Figure 2: DCX⁺PSA-NCAM⁺ young neurons in the PL at birth. a, Diagram of human temporal lobe at birth indicating clusters of DCX⁺PSA-NCAM⁺ cells in the MPL and LPL (higher magnification of **Fig. 3e** section i). (Right) MPL and LPL clusters correspond to regions stained in (**b–g**). **b,** DCX⁺ cells in the MPL and LPL clusters at birth. **c,** PSA-NCAM⁺ cells and fibers in the MPL and LPL at birth. **d,** NeuN⁺ cells in the MPL and LPL at birth. **e,** GFAP⁺ cells within the MPL and surrounding the LPL at birth. **f,** OLIG2⁺ cells in the MPL and LPL, and in the white matter surrounding the LPL at birth. **g,** SOX2⁺ cells in the MPL and LPL and surrounding the LPL at birth. Scale bars: 5 mm (**a** left), 500 µm (**a** middle, **a** right), 100 µm (**c**).



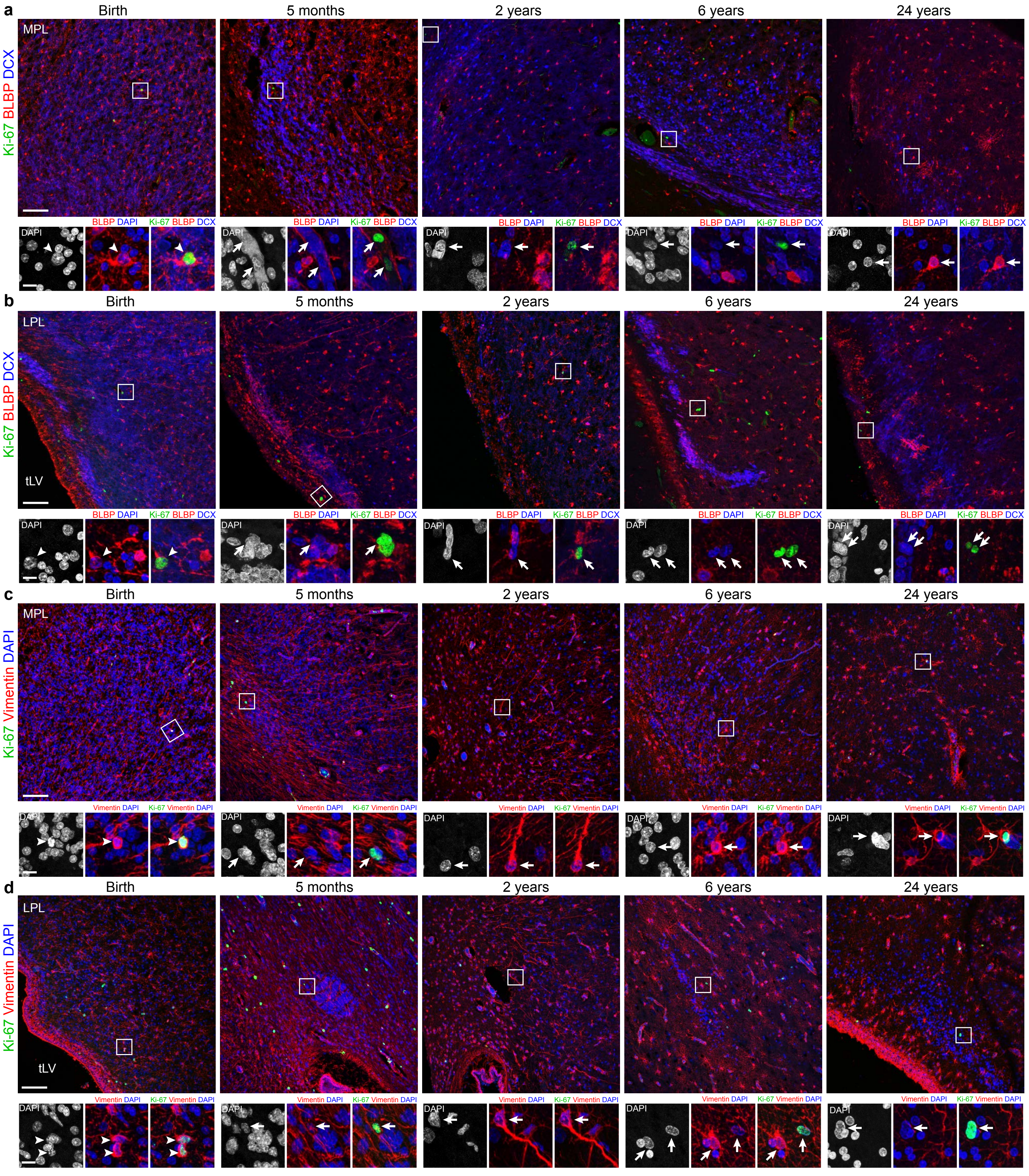
Supplementary Figure 3: Connectivity of DCX⁺ cells in the PL. a, (Left) Diagram of human brain at 15 years showing sagittal perspective of the temporal lobe. (Right) Sagittal view of a 3-D reconstruction of the right temporal lobe showing the location of DCX⁺PSA-NCAM⁺ cell clusters in the PL surrounding and protruding into the BLA at 15 years of age. The location of the intercalated cell nuclei is indicated (arrow). **b,** Sagittal section through the BLA at the level of the two LPL clusters indicated in (a). Staining for neurofilament and PSA-NCAM revealed fibers extruding from the angular bundle and wrapping around the LPL clusters. PSA-NCAM⁺ and neurofilament⁺ fibers are present on the dorsal side of the LPL cluster on the right (arrow). **c,** (Top) A subset of the DCX+ cells in the LPL at 13 years are MAP2⁺. (Bottom) neurofilament+ fibers surround and extend into the LPL at 15 years. (**d–l**)**,** The ultrastructural analysis of DCX+ cells at 45 years of age detected small clusters of cells, similar to those observed by fluorescence microscopy, with features of immature neurons. At higher magnification most of these cells have small nuclei and their cytosol contains few organelles, although they display a high ribosome density (**g–i**). We occasionally found adherens junctions between these cells, a hallmark of immature neurons that are in contact (arrow). More rarely, we found cells in these clusters with a phenotype more similar to mature neurons: a higher variety of organelles, accumulation of lipofuscin and even nuclear invaginations (**j–l**). Scale bars: 2 mm (**b** left), 200 μm (**b** right), 20 μm (**c**), 5 μm (**f**), 2 μm (**d**, **g**, **j**), 1 μm (**e**, **h**, **k**, **l**), 500 nm (**i**).



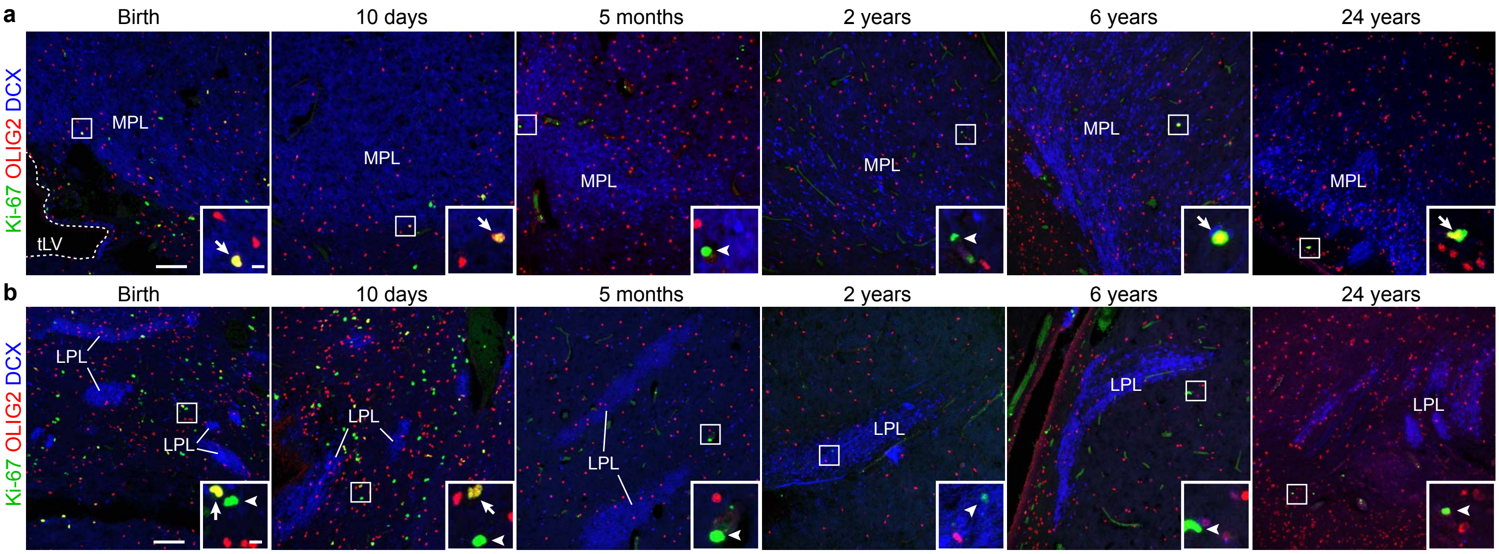
Supplementary Figure 4: Interneurons in the PL throughout life. a, SST⁺ cells (arrows) near the DCX⁺ cells in the LPL at 10 days and 6 years. **b,** Calretinin⁺ cells (arrows) in the LPL from 3 months to 67 years. **c,** Infrequent nNOS⁺ cells (arrow) near the DCX⁺ cells in the LPL at 10 days, 6 years, and 67 years. **d,** NPY⁺ cells (arrows) in the LPL from 3 months to 67 years. **e,** In situ hybridization for human *GAD67* in the MPL and LPL from birth to 67 years of age. Scale bars: 100 µm (**a–e**).



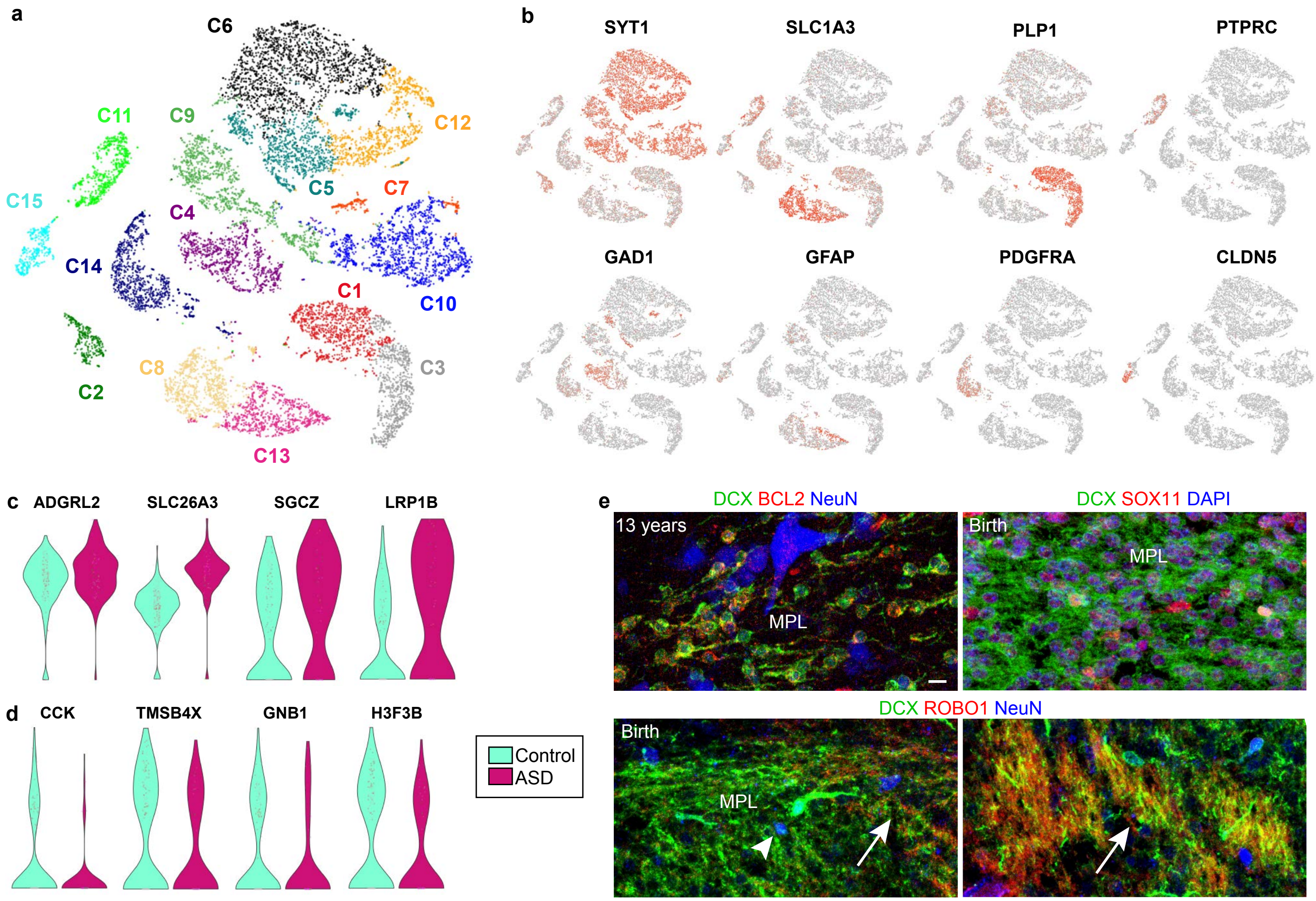
Supplementary Figure 5: DCX⁺PSA-NCAM⁺ cells are Ki-67⁻ in the postnatal PL. a, (Top) Ki-67⁺SOX2⁺BLBP⁺ cell in the MPL at 38 GW at the location in the caudal MPL indicated in the diagram in the lower right (the former site of high Ki-67+ cell density during gestation, see **Fig. 1a,b**. Coronal section level corresponds to (vi) from **Fig. 2a**.) (Bottom) Ki-67+ cells near DCX+PSA-NCAM+ cells in an adjacent section. **b,** Ki-67+ cells near DCX+PSA-NCAM+ cells in the lateral wall of the temporal lobe lateral ventricle (tLV) (Middle) and the caudal LPL (Right). **c,** Ki-67⁺ and DCX⁺PSA-NCAM⁺ cells in the MPL (Left) and LPL (Right) from 5 months to 24 years of age in the sections used to make the maps in (**Fig. 6a**). Scale bars: 100 μm (**a** left, **b** left, **c** top), 50 μm (**b** insets), 20 μm (**c** bottom), 10 μm (**a** middle, **a** top right).



Supplementary Figure 6: Ki-67⁺BLBP⁺ and Ki-67⁺vimentin⁺ cells in the PL decline between birth and 5 months. a, Ki-67⁺BLBP⁺ cells in the MPL at birth (arrowheads), and single-positive Ki-67⁺ BLBP⁻ or Ki-67⁻ BLBP⁺ cells (arrows) in ages 5 months and older. **b,** Ki-67⁺BLBP⁺ cells in the LPL at birth (arrowheads), and single-positive Ki-67⁺BLBP⁻ or Ki-67⁻BLBP⁺ cells (arrows) in ages 5 months and older. **c,** Ki-67⁺vimentin⁺ cells in the MPL at birth (arrowheads), and single-positive Ki-67⁺vimentin⁻ or Ki-67⁻vimentin⁺ cells (arrows) in ages 5 months and older. **d,** Ki-67⁺vimentin⁺ cells in the LPL at birth (arrowheads), and single-positive Ki-67⁺vimentin⁻ or Ki-67⁻vimentin⁺ cells (arrows) in ages 5 months and older. Scale bars: 100 μm (**a–d** top), 10 μm (**a–d** bottom).



Supplementary Figure 7: Ki-67⁺ OLIG2⁺ cells decline in the postnatal PL. a,b, Ki-67$^+$OLIG2$^+$ cells (arrows) and Ki-67$^+$OLIG2$^-$ cells (arrowheads) in the MPL (**a**) and LPL (**b**) from birth to 24 years of age. Scale bars: 100 µm (**a,b**), 10 µm (insets).



Supplementary Figure 8: Clustering of snRNA-seq data from human amygdala and ASD-associated gene expression changes. a, 15 cell clusters identified though unbiased clustering of single nuclei gene expression profiles. **b,** Markers of neuronal and glial cell types in the human amygdala snRNA-seq dataset. **c,d,** ASD-associated differentially expressed genes in immature PL neurons (cluster C2). **e,** Immunofluorescent validation of different markers identified in C2: $DCX^{+}BCL2^{+}$ cells in the MPL at 13 years of age, $DCX^{+}SOX11^{+}$ cells at birth, and DCX^{+} processes that are $ROBO1^{+}$ at birth (arrows) near DCX^{+} cell bodies that are $ROBO1^{-}$. Scale bar: 10 μm (**e**).

Supplementary Table 1- Case list

Case no.	Age	Gender	PMI	Experimental Use	Neuropathology diagnosis	Clinical history
1	20 GW	M	42h	1,2	control	spontaneous abortion
2	21 GW	F	14h	1,2	control	osteogenesis imperfecta
3	22 GW	M	24h	1–3,9	control	urethral stenosis
4	22 GW	M	48h	1–3,9	control	spontaneous abortion
5	26 GW	F	24h	1,2	control	spontaneous abortion
6	34 GW	F	42h	1,2	control	lung malformation
7	37 GW	M	48h	6,8	control	VATER malformation
8	38 GW	M	50h	1–7,9	control	bronchopulmonary dysplasia
9	39 GW	F	48h	3,4	control	intrauterine demise
10	39 GW	M	24h	3,4	control	congenital diaphragmatic hernia
11	term	M	14h	1–7	control	chondrodysplasia
12	term	M	11h	1–3,5,7	control	cardiac dysfunction
13	term	M	5h	1–7	control	respiratory failure
14	term	M	24h	1–7	control	hydronephrosis
15	1 day	F	36h	1–3,5,7	control	renal hypoplasia; hypoplastic lung
16	2 day	M	36h	1–7,11	control	hypoplastic L heart
17	10 days	F	19h	10	control	respiratory failure
18	1 month	M	10h	2	control	congenital renal agenesis
19	1.5 months	F	21h	1–7	control	congenital diaphragmatic hernia
20	3 months	M	16h	1–7	control	diaphragmatic hernia
21	3 months	M	20h	10	control	diaphragmatic hernia
22	5 months	M	44h	1–7,10	control	congenital diaphragmatic hernia
23	6 months	M	14h	7	control	VATER malformation
24	1 year	F	48h	6,7	control	right pulmonary vein stenosis
25	2 years	M	19h	1–7,10	control	leukemia
26	3 years	M	25h	7	control	esophageal atresia, tracheoesophageal fistula
27	6 years	F	36h	1–8,10	control	congenital left diaphragmatic hernia
28	6 years	M	16h	7,12	control	drowning
29	7 years	M	3h	7,12	ASD	complications of cancer
30	7 years	M	21h	11	control	septic shock from acute appendicitis
31	8 years	M	12h	7,12	ASD	drowning
32	8 years	M	12h	7,12	control	drowning
33	11 years	M	27h	7,12	ASD	acute hemorrhagic tracheobronchitis
34	12 years	M	13h	7,12	control	drowning
35	13 years	M	12h	3–9	control	focal segmental glomerulosclerosis
36	15 years	M	9h	7,12	control	cardiac arrhythmia due to conduction system
37	15 years	F	13h	7,12	ASD	drowning associated with seizure disorder
38	15 years	M	22h	7,8	control	acute lymphoblastic leukemia
39	19 years	M	48h	10	control	systemic fungal infection
40	24 years	F	16h	1–8,10	control	acute kidney injury, multiorgan failure
41	36 years	M	56h	7	control	interstitial lung disease
42	45 years	M	2h	11	control	pleural cancer
43	48 years	M	5h	11	control	autoimmune nephritis w/ transplant
44	49 years	F	23h	7,8	control	undifferentiated round cell sarcoma
45	55 years	M	36h	7	control	heart failure
46	57 years	F	9h	10	control	papillary thyroid carcinoma
47	67 years	F	19h	10	control	chronic obstructive lung disease
48	77 years	M	50h	7,8	control	VHL with hemangiomas
49	78 years	F	23h	7	control	hypertension, history of aortic dissection

Key to experimental use:

1 Ki-67;SP8;COUPTFII;PROX1
2 DCX;COUPTFII;NeuN
3 Ki-67;SOX2;DCX
4 Ki-67;BLBP;Vimentin
5 Ki-67;DCX;PSA-NCAM
6 Ki-67;Olig2;DCX
7 DCX;PSA-NCAM;NeuN
8 DCX;CB
9 DCX;TBR1
10 DCX; interneurons
11 DCX; immunogold
12 snSeq

Supplementary Table 2 - Antibodies

Antigen	Species	Dilution	Manufacturer	Cat. No.	Antigen Retrieval	Lot No.	Manufacturer Specificity Description
ALDH1L1	Mouse	1:500	NeuroMab	N103/39	None	N103/31	Human reactivity
BCL2	Mouse	1:100	Santa Cruz Biotech	sc-7382 (C-2)	10 min	K1218	Raised against human Bcl-2
BLBP	Rabbit	1:200	EMD Millipore	ABN14	10 min	2299161	Human reactivity predicted based on sequence
BLBP	Mouse	1:200	Abcam	ab131137	10 min	AT1D1	Human reactivity
Calbindin	Rabbit	1:1000	Swant	CB-38a	10 min	9.03	Human reactivity
Calretinin	Mouse	1:1000	Swant	6B3	10 min	7699/4	Human reactivity
CoupTFII	Mouse	1:250	R&D Systems	PP-H7147-00	10 min	A-2	Raised against human COUPTFII
Doublecortin	Rabbit	1:200	Cell Signaling	4604S	None	42798	Human reactivity
Doublecortin	Guinea pig	1:200	EMD Millipore	AB2253	None	2787730	Human reactivity predicted based on sequence
GFAP	Chicken	1:750	Abcam	ab4674	None	GR267558-1	Human reactivity
Iba1	Rabbit	1:100	Wako	019-1974	None	LKJ2979	Reactivity to human Iba1
Ki-67	Mouse	1:200	BD Pharmigen	556003	10 min	6110925	QC resting: human
Ki-67	Rabbit	1:500	Novocastra	NCL-Ki67p	10 min	6029714	Specificity: human Ki-67
Ki-67	Rabbit	1:1000	Vector Labs	VP-K451	None	6013873	Specificity: human Ki-67
MAP2	Chicken	1:500	Abcam	ab5392	None	GR286806-6	Human reactivity
Nestin	Mouse	1:250	Covance	MMS-570p	None	14683401	Human reactivity
NeuN	Chicken	1:500	EMD Millipore	ABN91	None	2620673	Human reactivity
NeuN	Rabbit	1:1000	Novus Biologicals	R-3770-100	None	201605-SH	Raised against human FOX3
Neurofilament	Mouse	1:1000	Abcam	ab24574	None	GR191433-7	Human reactivity
NKX2.1	Rabbit	1:500	Santa Cruz Biotech	sc-13040 (H-190)	10 min	B2216	Raised against human NKX2.1
nNOS	Rabbit	1:500	EMD Millipore	AB5380	None	2519293	Raised against human nNOS
NPY	Rabbit	1:500	Abcam	ab30914	None	GR212905-1	Human reactivity
OLIG2	Rabbit	1:750	EMD Millipore	AB9610	None	2519344	Human reactivity
PROX1	Rabbit	1:500	EMD Millipore	AB5475	8 min	LV1354325	Human reactivity
PROX1	Goat	1:500	R&D Systems	AF2727	10 min	VIY0216011	Human reactivity
PSA-NCAM	Mouse	1:1000	EMD Millipore	MAB5324	None	2201402	Human reactivity
ROBO1	Mouse	1:100	Santa Cruz Biotech	sc-293444	10 min	D2817	Raised against human ROBO1
SCGN	Rabbit	1:1000	Sigma-Aldrich	HPA006641	None	A106808	Human reactivity
SOX2	Goat	1:200	Santa Cruz Biotech	sc-17320 (Y-17)	10 min	H2914	Reactivity to human SOX2
SOX2	Rabbit	1:200	Cell Signaling	2748S	10 min	2	Reactivity to human SOX2
SOX11	Rabbit	1:500	EMD Millipore	AB5776	10 min	3054693	Human reactivity
SP8	Goat	1:200	Santa Cruz Biotech	sc-104661 (C-18)	8 min	G0516	Human reactivity
SST	Goat	1:250	Santa Cruz Biotech	sc-7819 (D-20)	None	G0716	Human reactivity
TBR1	Chicken	1:200	EMD Millipore	AB2261	None	2893188	Human reactivity
TUJ1	Mouse	1:200	Covance	MMS-435P	10 min	TU1	Human reactivity
VGLUT2	Guinea pig	1:200	EMD Millipore	AB5907	None	2894024	Rat (protein seq. 98% identical to human)
Vimentin	Mouse	1:1000	Sigma-Aldrich	V5255	None	045K4826	Tested in human appendix/ tonsil

Supplementary Table 3 - Genes differentially expressed in immature PL neurons in ASD

gene.IDs	gene.names	gene.biotypes	FCs	FDRs	FC_raw
ENSG00000091138	SLC26A3	protein_coding	5.850149658	0.0001871921422	1.847488373
ENSG00000168702	LRP1B	protein_coding	1.922913347	0.001339982591	1.232924153
ENSG00000138670	RASGEF1B	protein_coding	1.801840449	0.001572375975	1.299544569
ENSG00000184305	CCSER1	protein_coding	4.217426847	0.006826692191	0.4825503699
ENSG00000205542	TMSB4X	protein_coding	-1.233572225	0.01281857797	-0.4848494181
ENSG00000150756	FAM173B	protein_coding	0.2820599983	0.01721292848	0.1579495104
ENSG00000187094	CCK	protein_coding	-2.478100813	0.01873110543	-0.521808086
ENSG00000117114	ADGRL2	protein_coding	0.3843599182	0.02164326496	0.3808905808
ENSG00000275443	RP11-759A24.3	lincRNA	-0.9530566461	0.02723474619	-0.165065828
ENSG00000078369	GNB1	protein_coding	-0.5159267986	0.02827392498	-0.3942154221
ENSG00000111011	RSRC2	protein_coding	-2.144482041	0.02848930642	-0.3959280793
ENSG00000105649	RAB3A	protein_coding	-2.54553859	0.02903743238	-0.265926041
ENSG00000128989	ARPP19	protein_coding	-1.422262201	0.03169490743	-0.5170711773
ENSG00000100554	ATP6V1D	protein_coding	-2.254287096	0.03182949225	-0.2666499763
ENSG00000179889	PDXDC1	protein_coding	-1.108149114	0.03182949225	-0.4178134235
ENSG00000185053	SGCZ	protein_coding	1.308263765	0.03562456606	1.148732649
ENSG00000164919	COX6C	protein_coding	-2.477741609	0.03570725607	-0.1755408476
ENSG00000136267	DGKB	protein_coding	0.9101420031	0.03619039185	0.8408377592
ENSG00000154174	TOMM70A	protein_coding	0.804467377	0.03619039185	0.3046166414
ENSG00000179104	TMTC2	protein_coding	0.7391567743	0.03619039185	0.700715308
ENSG00000236824	BCYRN1	lincRNA	0.2701744597	0.03619039185	0.6806952491
ENSG00000132475	H3F3B	protein_coding	-1.036045754	0.03712499988	-0.5241241606
ENSG00000175395	ZNF25	protein_coding	-2.129270255	0.03782169224	-0.1595052421
ENSG00000232573	RPL3P4	processed_pseudogene	-0.8582949501	0.03782169224	-0.3671730924
ENSG00000070785	EIF2B3	protein_coding	-3.267738217	0.03821687899	-0.309978585
ENSG00000126457	PRMT1	protein_coding	-2.335274072	0.03821687899	-0.1931841097
ENSG00000145416	MARCH1	protein_coding	1.075637619	0.03892016793	0.7278414853
ENSG00000147684	NDUFB9	protein_coding	-1.242078339	0.03960912211	-0.1711299453
ENSG00000151376	ME3	protein_coding	-2.634126749	0.04435063816	-0.5051282709
ENSG00000152784	PRDM8	protein_coding	-2.441363985	0.04585269919	-0.188538258
ENSG00000170396	ZNF804A	protein_coding	0.7594812117	0.04585269919	0.6881329164
ENSG00000259589	RP11-317G6.1	antisense	-2.502210026	0.04648736138	-0.1641248921
ENSG00000114784	EIF1B	protein_coding	-1.474803654	0.04881442074	-0.5132531336