Transcriptomic Mapping of Neural Diversity, Differentiation and Functional Trajectory in iPSC-Derived 3D Brain Organoid Models

Kiavash Kiaee [a,b,], Yasamin A. Jodat [a,b], Nicole J. Bassous [a], Navneet Matharu [c,d,e], Su Ryon Shin [a,*]*

**Table S1.** Gene subsets governing specific neural functions.

Axon Guidance	Axon Development	Neuronal Action Potential	Neuron Neuron Synaptic Transmission	NOTCH Signaling	WNT Signaling	Neuron Differentiation	BMP Signaling
ABL1	ABL1	ANK3	DLGAP2	ADAM17	APC	ABI2	ACVR2A
ABLIM1	ACTB	CACNA1	DRD2	APH1A	APC2	ABITRAM	ACVR2B
ABLIM2	ACTBL2	G	KIF1B	CIR1	AXIN1	ABL1	ACVRL1
ABLIM3	ADARB1	CACNA1	PTEN	CREBBP	AXIN2	ABL2	AMH
ARHGEF1	ADCY1	H	TMOD2	CTBP1	BTRC	ACAP3	AMHR2
2	ADGRB1	CACNA1I	VDAC1	CTBP2	CACYBP	ACP4	BMP10
CDC42	AFG3L2	CHRNA1		DLL1	CAMK2A	ACSL3	BMP2
CDK5	ALCAM	FGF12		DLL3	CAMK2B	ACSL4	BMPR1A
CFL1	AMIGO1	FMR1		DLL4	CAMK2D	ACTB	BMPR1B
CFL2	ANAPC2	GBA		DTX1	CAMK2G	ACTBL2	BMPR2
CHP1	ANK3	GPER1		DTX2	CCND1	ACTL6B	CER1
CHP2	ANOS1	GPR35		DTX3	CCND2	ACTR2	CHRDL1
CXCL12	APBB1	GPR88		DTX3L	CCND3	ADAM10	FSTL1
CXCR4	APOA1	GRIK2		DTX4	CER1	ADARB1	GDF2
DCC	APOD	KCNA1		DVL1	CHD8	ADCY1	GREM2
DPYSL2	APOE	KCNA2		DVL2	CHP1	ADCY6	NOG
DPYSL5	APP	KCND2		DVL3	CHP2	ADCYAP1	SKI
EFNA1	ARHGAP35	KCNMB2		EP300	CREBBP	ADGRB1	SMAD1
EFNA2	ARHGAP4	KCNMB3		HDAC1	CSNK1A1	ADGRB3	SMAD4
EFNA3	ARHGDIA	KCNMB4		HDAC2	CSNK1A1	ADGRF1	SMAD5
EFNA4	ARTN	MTNR1B		HES1	L	ADGRV1	SMAD6
EFNA5	ARX	MYH14		HES5	CSNK1E	ADM	SMAD7
EFNB1	ATL1	P2RX1		JAG1	CSNK2A1	ADNP2	SMAD9
EFNB2	ATOH1	P2RX3		JAG2	CSNK2A2	ADORA2A	SMURF1
EFNB3	ATP8A2	P2RX4		KAT2A	CSNK2B	ADRA2B	SMURF2
EPHA1	AUTS2	SCN10A		KAT2B	CTBP1	ADRA2C	UBE2D1
EPHA2	B3GNT2	SCN11A		LFNG	CTBP2	AFG3L2	UBE2D3
EPHA3	B4GALT5	SCN1A		MAML1	CTNNB1	AGBL4	ZFYVE16
EPHA4	B4GALT6	SCN2A		MAML2	CTNNBIP	AGER	
EPHA5	BAIAP2	SCN3A		MAML3	1	AGRN	

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EPHA6	BARHL2	SCN4A	MFNG	CUL1	AGTPBP1
EPHA7	BCL11B	SCN5A	NCOR2	CXXC4	AHI1
EPHA8	BCL2	SCN7A	NCSTN	DAAM1	AKT1
EPHB1	BDNF	SCN8A	NOTCH1	DAAM2	ALCAM
EPHB2	BMP7	SCN9A	NOTCH2	DKK1	ALDH1A2
EPHB3	BMPR1B		NOTCH3	DKK2	ALK
EPHB4	BMPR2		NOTCH4	DKK4	ALKAL1
EPHB6	BOC		NUMB	DVL1	ALKAL2
FES	BRSK1		NUMBL	DVL2	ALKBH1
FYN	BRSK2		PSEN1	DVL3	ALS2
GNAI1	BSG		PSEN2	EP300	AMIGO1
GNAI2	C12orf57		PSENEN	FBXW11	ANAPC2
GNAI3	C9orf72		PTCRA	FOSL1	ANK3
GSK3B	CAMSAP2		RBPJ	FRAT1	ANKRD1
HRAS	CASP3			FRAT2	ANKRD27
ITGB1	CCK			FZD1	ANKS1A
KRAS	CCKAR			FZD10	ANOS1
L1CAM	CDH11			FZD2	AP2A1
LIMK1	CDH2			FZD3	APBB1
LIMK2				FZD4	
				FZD5	

Table S2. Comparison between the sample properties of the datasets used in this study.

scRNA-seq data	Reference	Identifier	No. of cells	No. of Batches	Differentiation induction period (days)	Neural maturation start day	Notable steps/signaling pathways
Human Brain Organoids	Trujillo et al., 2019	GSE130238	3,480	1	10	17	Inhibition of BMP, TGFβ
	Velasco et al. 2019	GSE129519	99,760	15	18	35	Inhibition of WNT, TGFβ

						Matrigel coating
	Giandomenico et al., 2019	GSE124174	12,824	1	11	20	Matrigel coating
	Fiddes et al., 2018	GSE106245	13,747	2	18	24	Inhibition of SHH, WNT, BMP, TGFβ
	Madhavan et al., 2018	GSE110006	3,743	1	6	25	Inhibition of BMP, TGFβ
	Quadrato et al., 2017	GSE86153	43,706	26	4	8	Matrigel coating
	Birey et al., 2017	GSE93811, GSE96045	4,953	1	6	25	Inhibition of BMP, TGFβ
	Xiang et al.,2017	GSE97882	23,707	2	10	18	Inhibition of WNT, BMP, TGFβ

| **Fetal Brain**	**Reference**	**Identifier**	**No. of cells**	**No. of Batches**	**Cell count**	
	Zhong et al. 2020	GSE104276	2,394	101	Female	Male
			Early stage (week 8-13)		278	136
			Mid-early stage (week 16-19)		889	0
			Middle stage (week 23-26)		928	143
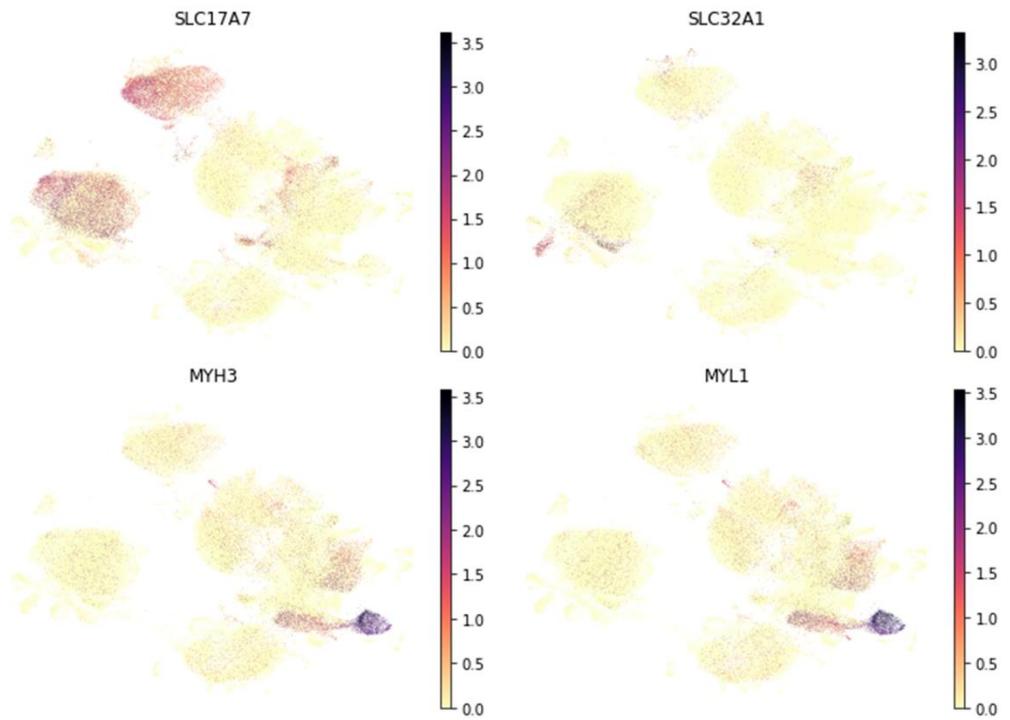
Figure S1. Visualization of various genes (top: Glutamatergic, bottom: GABAergic) on the integrated UMAP from organoid and fetal brain samples.
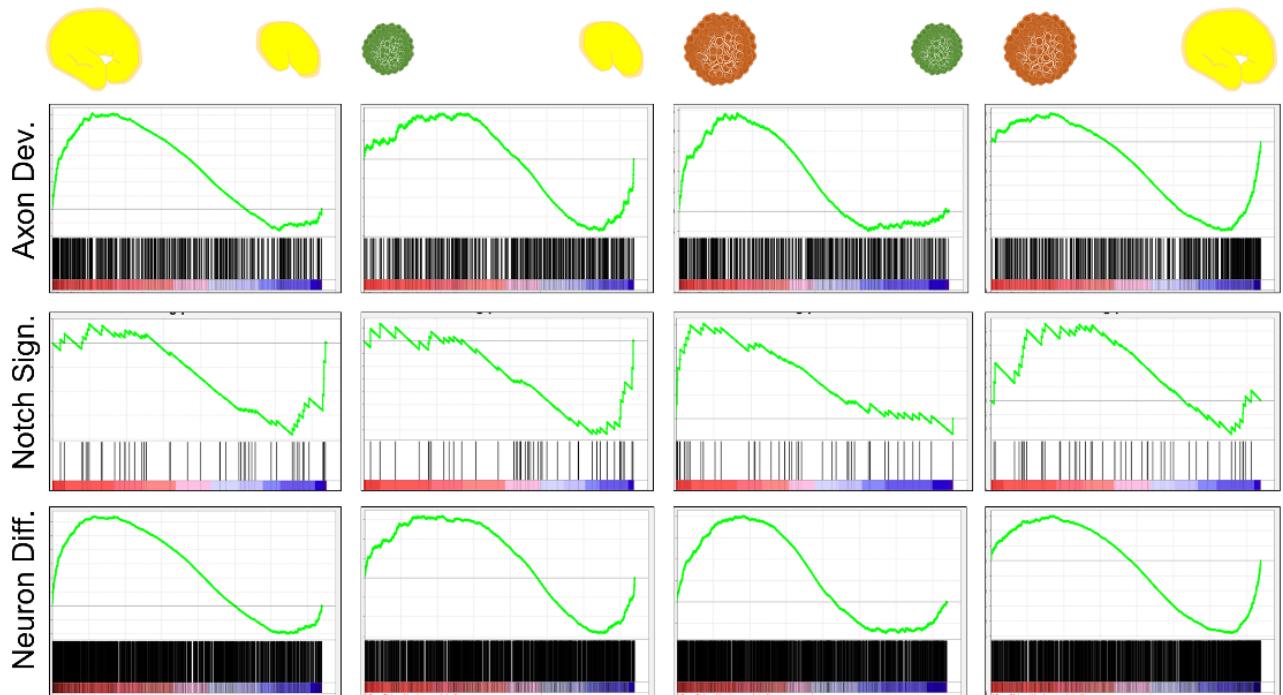
Figure S2. GSEA analysis of developmental genesets (axon development, notch signaling and neuron differentiation) between organoid and fetal groups. From left to right: fetal 6-month vs 3-month, organoid 3-month vs fetal 3-month, organoid 6-month vs organoid 3-month, organoid 6-month vs fetal 6-month.
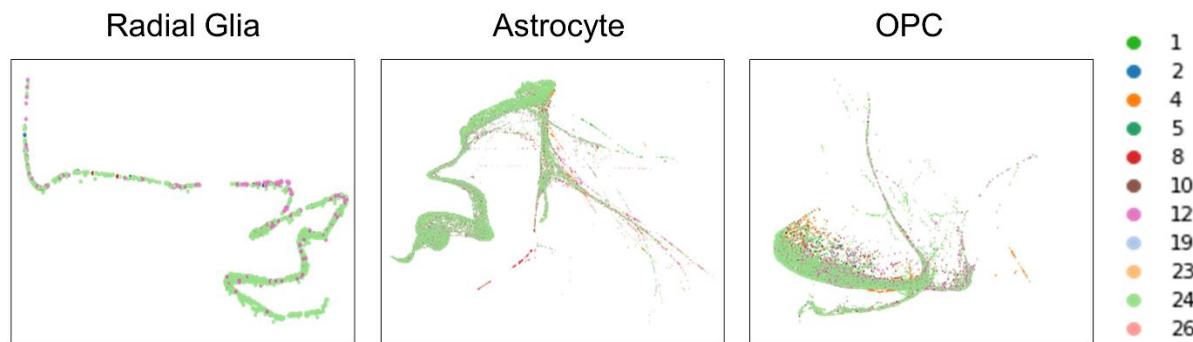
Figure S3. Cell type specific developmental trajectory in radial glia, astrocytes and OPCs across timepoints of 1-26 weeks.
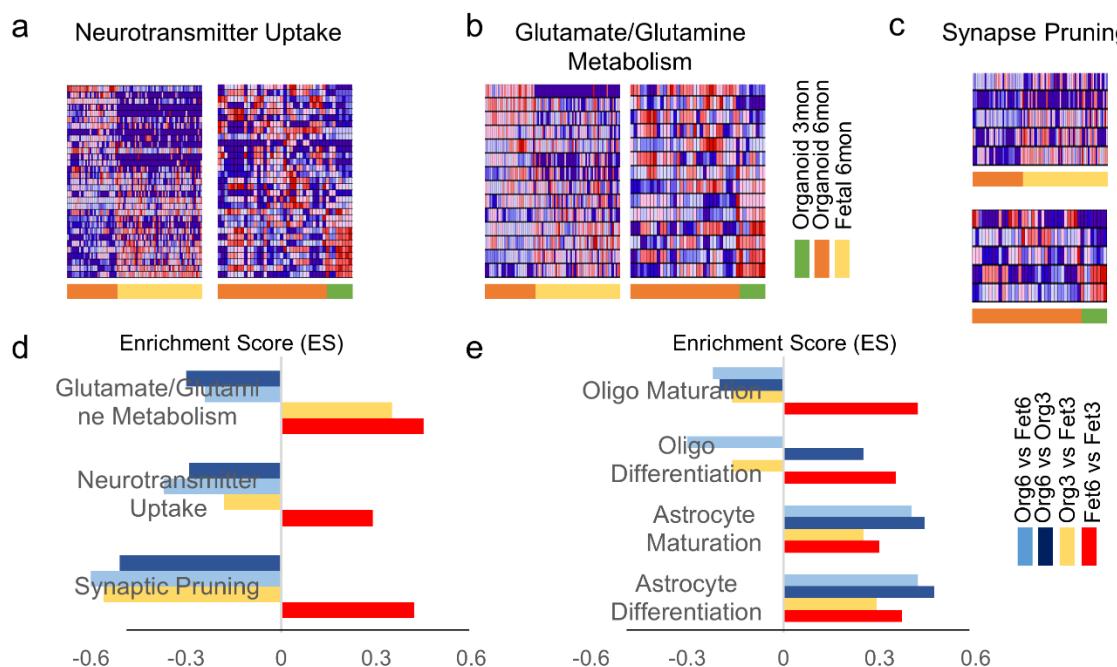
Figure S4. Gene set enrichment analysis of functional pathways (a-d), differentiation and maturation (e) relevant to astrocytes in organoid cultures and fetal brain at 3-month and 6-month timepoints. The differentiation gene set in (e) refers to the transformation process of relatively unspecialized cells to astrocytes [oligodendrocytes]. The maturation

gene set refers to the progression of astrocytes [oligodendrocytes] from initial commitment to the astrocyte

[oligodendrocyte] fate to a fully functional differentiated cell.