June 28, 2022

Neuro2022 Supplemental Info

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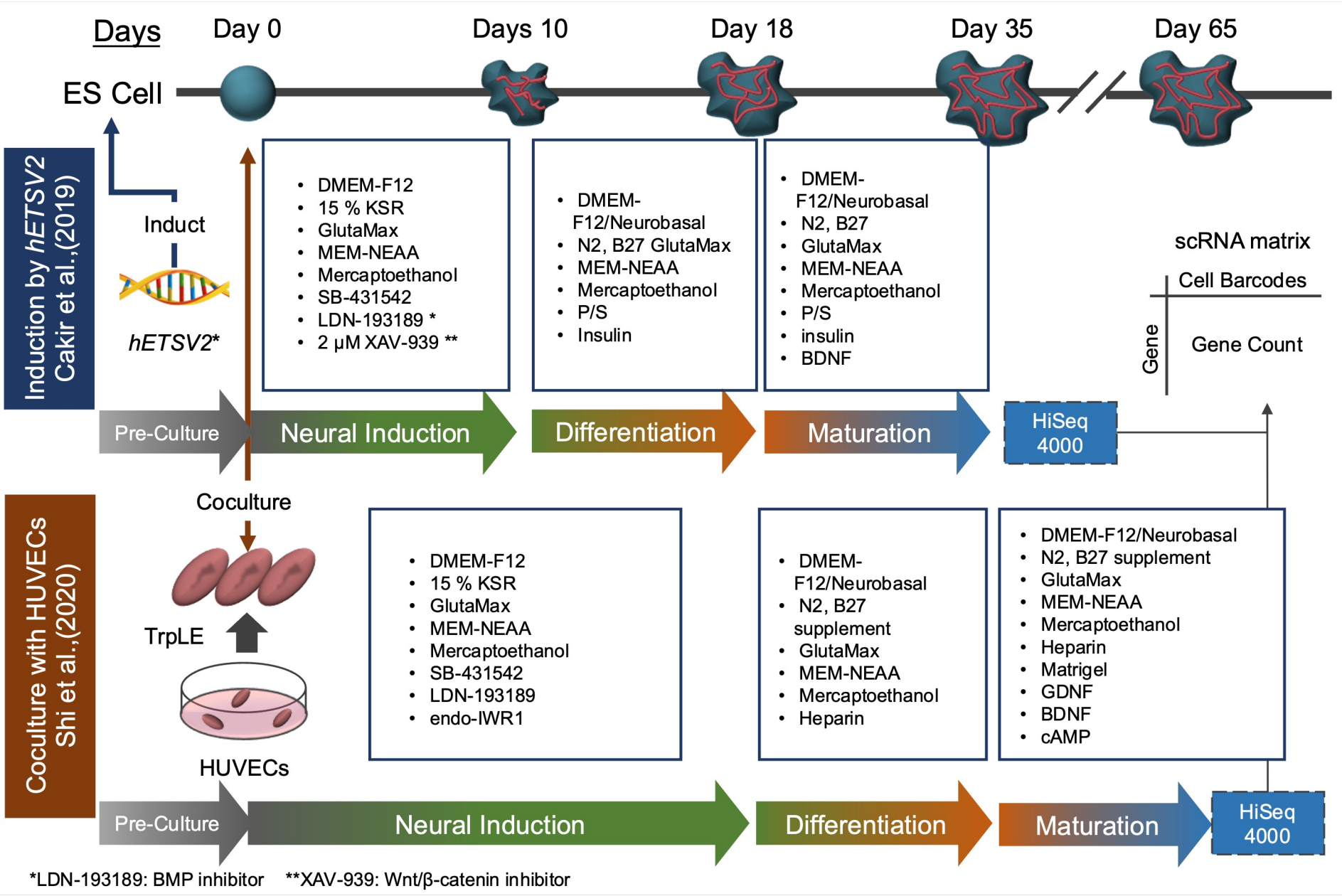
Applied Biology, Developmental & Genomic Science

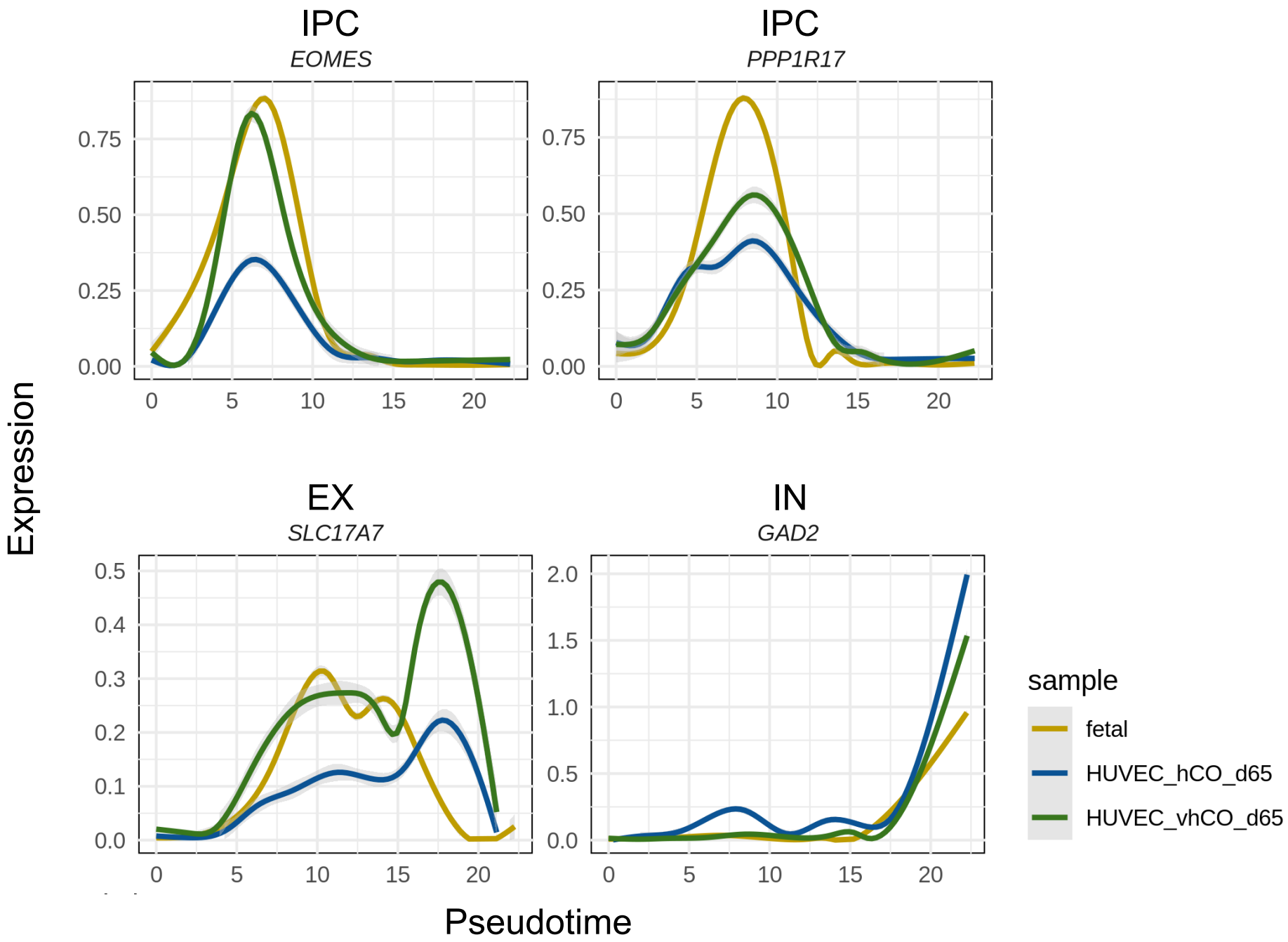
Protocols

Protocol	Cakir et al., (2019)	Shi et al., (2020)
Treatment	induction by hETSV2	coculturing with HUVEC
Accession Number	PRJNA553561	PRJNA542649
Total Maturation Day	18 days	35 days
Equipment	HiSeq4000	HiSeq4000
Target Cell Type	ES cell	ES cell
Samples	hCO / vhCO	hCO / vhCO
Organism	Homo sapiens	Homo sapiens

Protocols


Days
Day 0
Days 10
Day 18
Day 35
Day 65
ES Cell
Induction by hETSV2 Cakir et al.,(2019)
Induct
hETSV2*
• DMEM-F12
• 15 % KSR
• GlutaMax
• MEM-NEAA
• Mercaptoethanol
• SB-431542
• LDN-193189 *
• 2 μM XAV-939 **
• DMEM-F12/Neurobasal
• N2, B27 GlutaMax
• MEM-NEAA
• Mercaptoethanol
• P/S
• Insulin
• DMEM-F12/Neurobasal
• N2, B27
• GlutaMax
• MEM-NEAA
• Mercaptoethanol
• P/S
• insulin
• BDNF
scRNA matrix
Cell Barcodes
Gene
Gene Count
Pre-Culture
Neural Induction
Differentiation
Maturation
HiSeq 4000
Coculture with HUVECs Shi et al.,(2020)
Coculture
TrpLE
HUVECs
• DMEM-F12
• 15 % KSR
• GlutaMax
• MEM-NEAA
• Mercaptoethanol
• SB-431542
• LDN-193189
• endo-IWR1
• DMEM-F12/Neurobasal
• N2, B27 supplement
• GlutaMax
• MEM-NEAA
• Mercaptoethanol
• Heparin
• DMEM-F12/Neurobasal
• N2, B27 supplement
• GlutaMax
• MEM-NEAA
• Mercaptoethanol
• Heparin
• Matrigel
• GDNF
• BDNF
• cAMP
Pre-Culture
Neural Induction
Differentiation
Maturation
HiSeq 4000
*LDN-193189: BMP inhibitor
**XAV-939: Wnt/β-catenin inhibitor

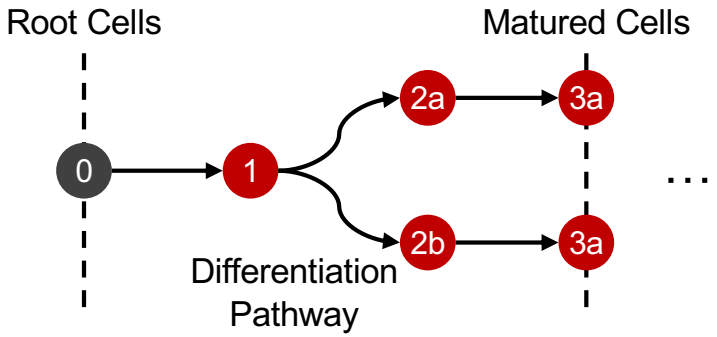
Neuro-marker expression alteration with vascularization



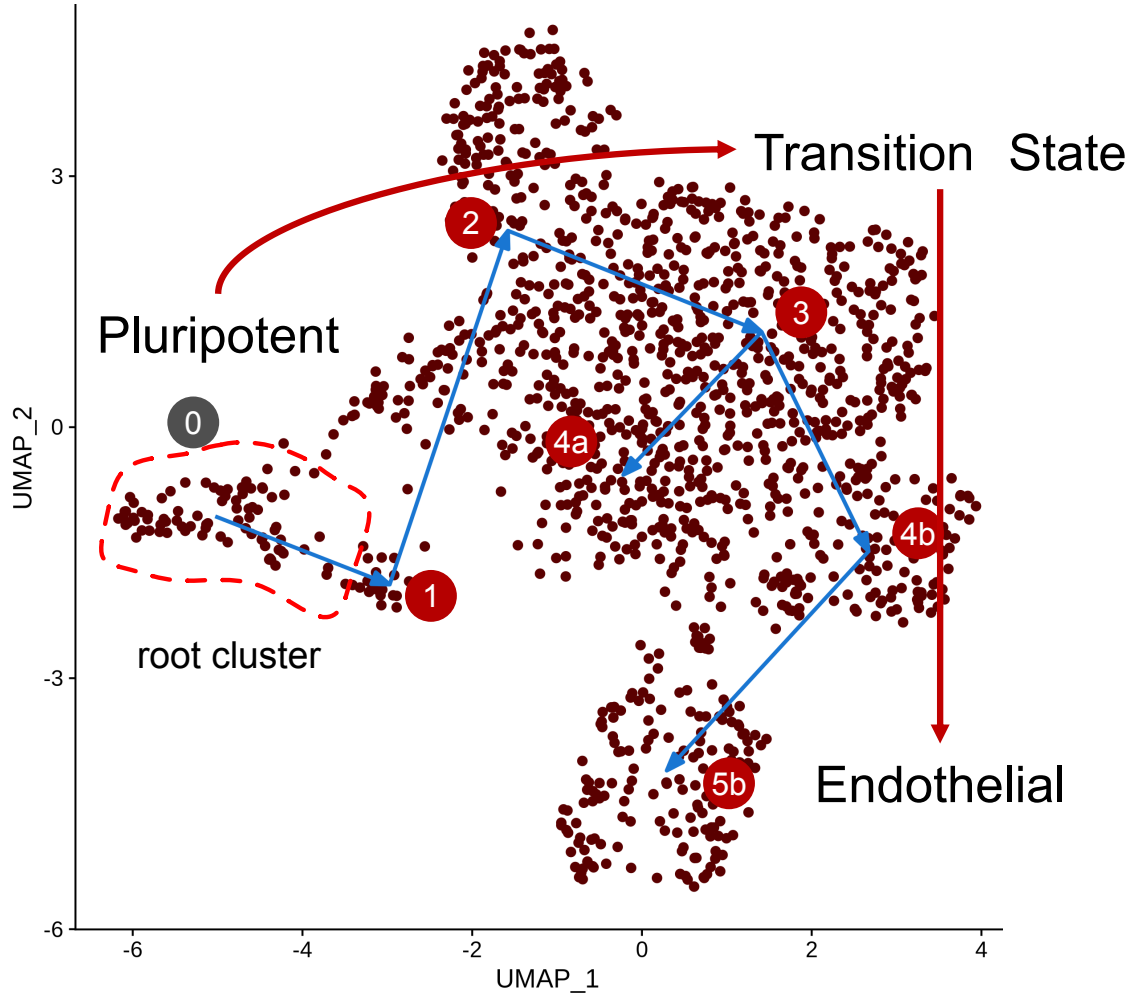
Identification of Differentiation Trajectories

Trajectory Analysis

Predict cell differentiation order with MST* algorithm

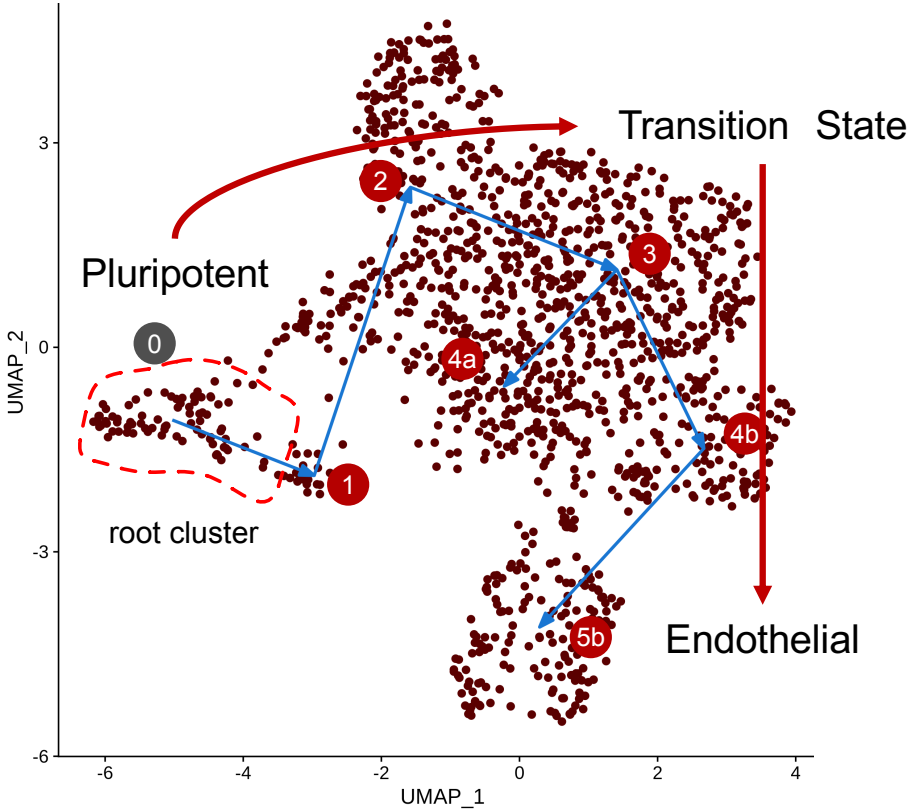


* MST: Minimum Spanning Tree



Identified differentiation order with Trajectory Analysis (Slingshot)

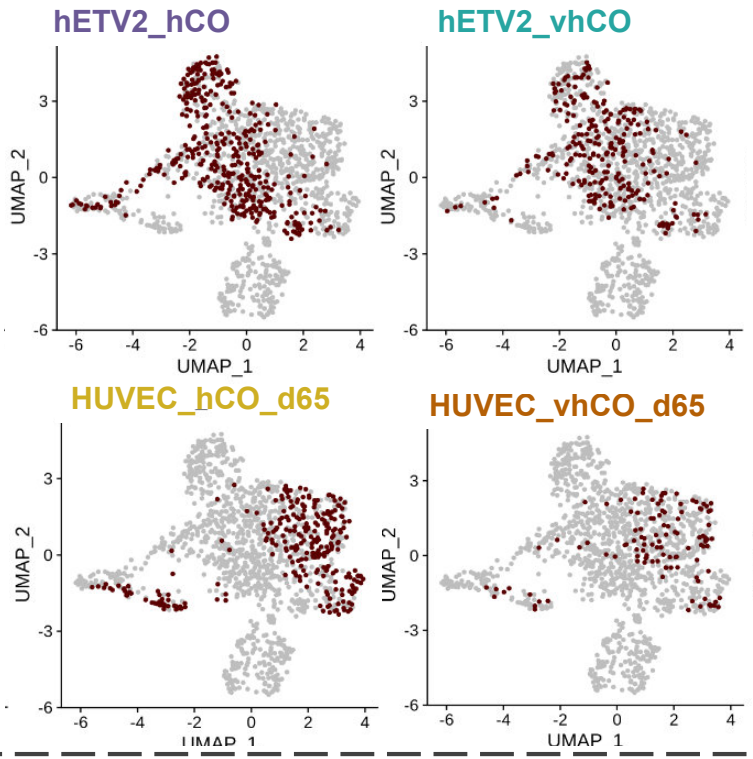
Organoid ECs are potentially progenitor cells



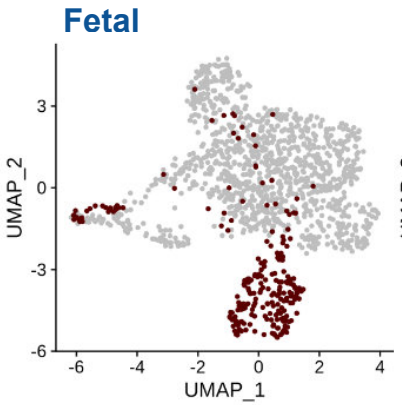
High Pluripotency

Day 30

Day 65



Real Brain



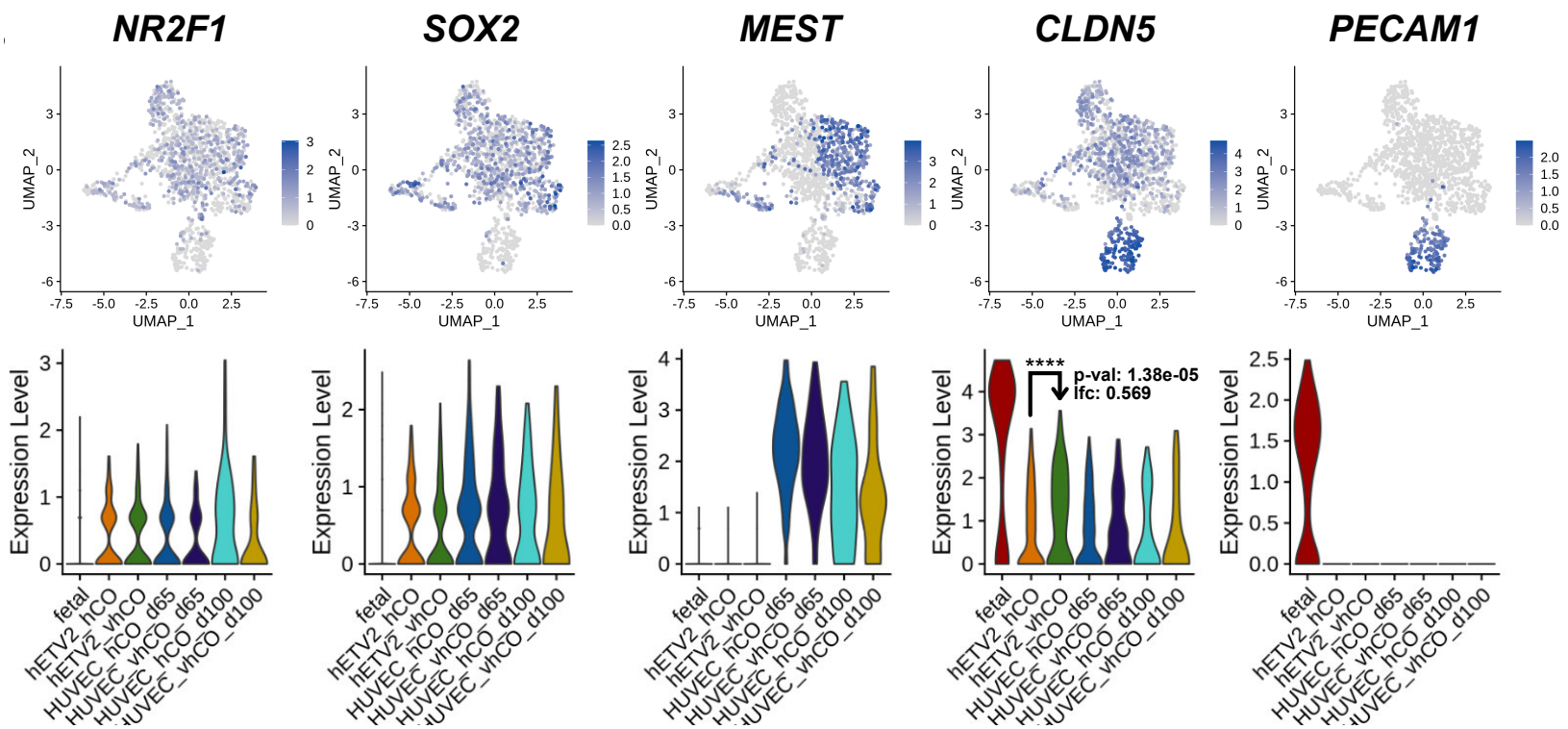
Endothelial Property

ECs in organoids:
Cells transitioning from pluripotent to endothelial cells

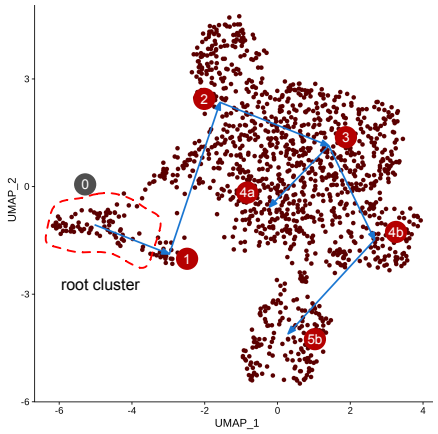
Organoid ECs are potentially progenitor cells

Pluripotency → Endothelial

Pluripotent Marker | Mesoderm marker | Endothelial marker



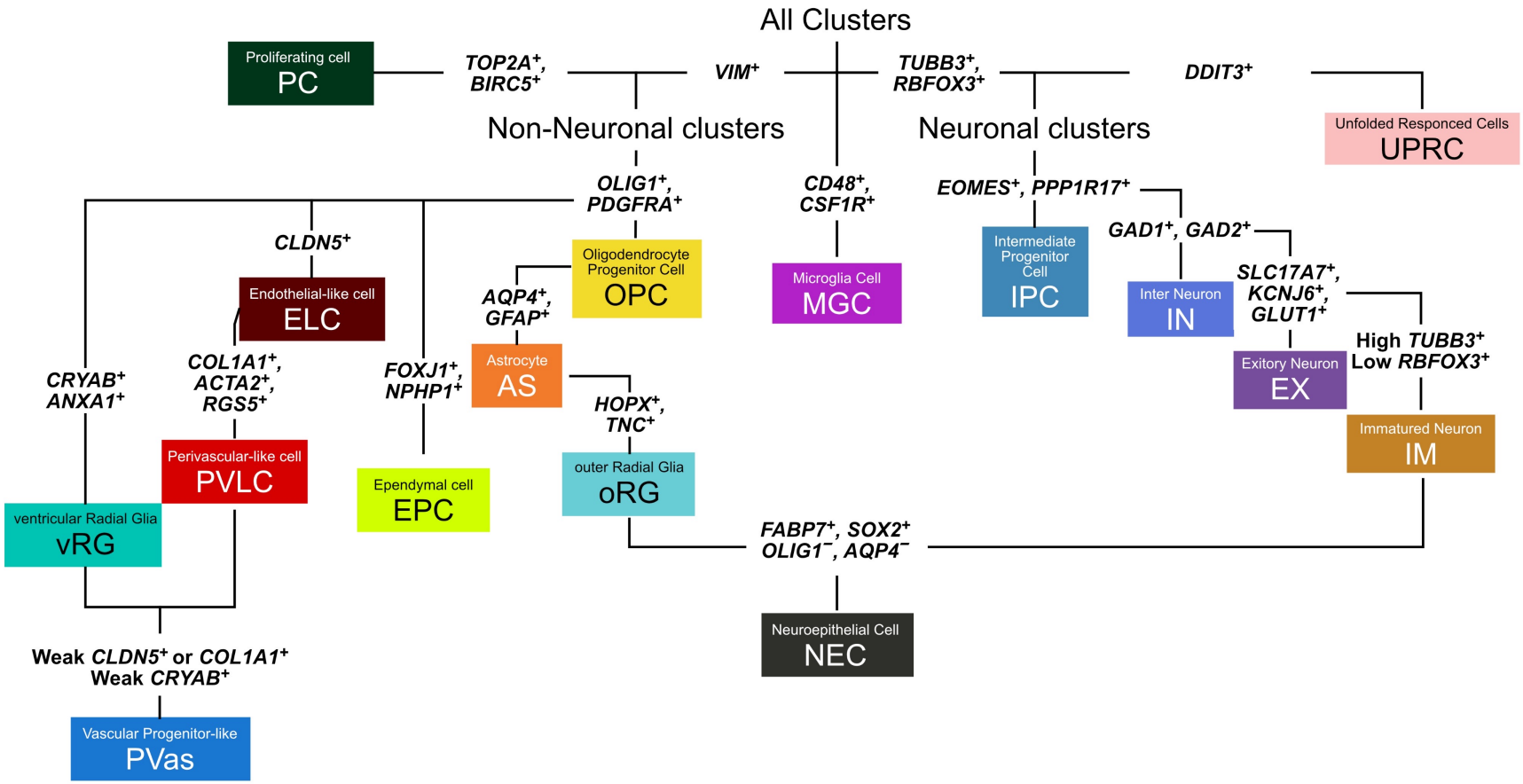
Samples



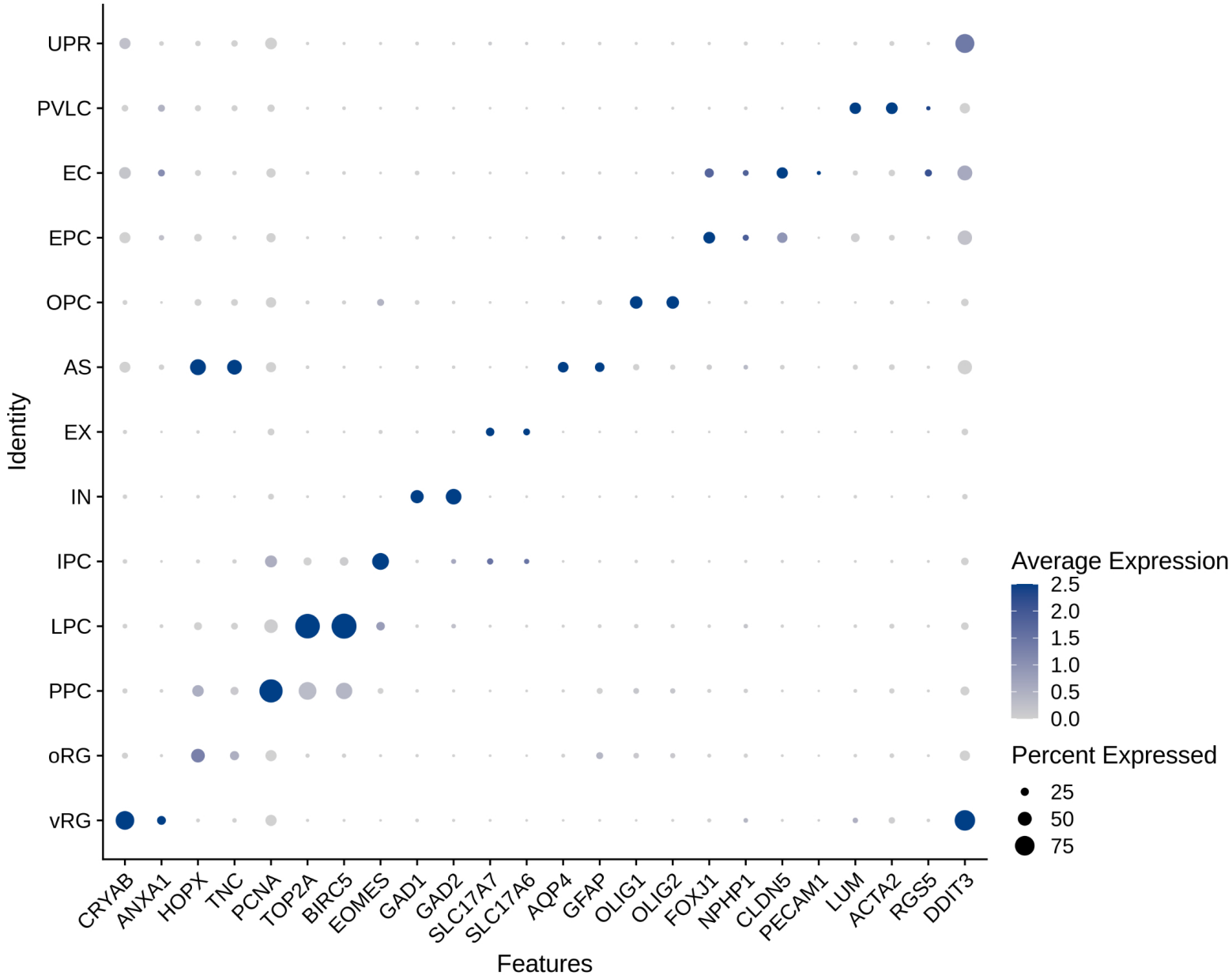
Conclusion

ECs in organoids are potentially progenitor cells of Endothelial cells

Cell Decision Tree



Evidence


UPR
PVLC
EC
EPC
OPC
AS
EX
IN
IPC
LPC
PPC
oRG
vRG
Identity
CRYAB
ANXA1
HOPX
TNC
PCNA
TOP2A
BIRC5
EOMES
GAD1
GAD2
SLC17A7
SLC17A6
AQP4
GFAP
OLIG1
OLIG2
FOXJ1
NPHP1
CLDN5
PECAM1
LUM
ACTA2
RGS5
DDIT3
Features
Average Expression
2.5
2.0
1.5
1.0
0.5
0.0
Percent Expressed
25
50
75

Quality Info