Single-cell RNA-Seq Profiling Identified Molecular Signatures And Transcriptional Networks Regulating Lung Maturation

Yan Xu

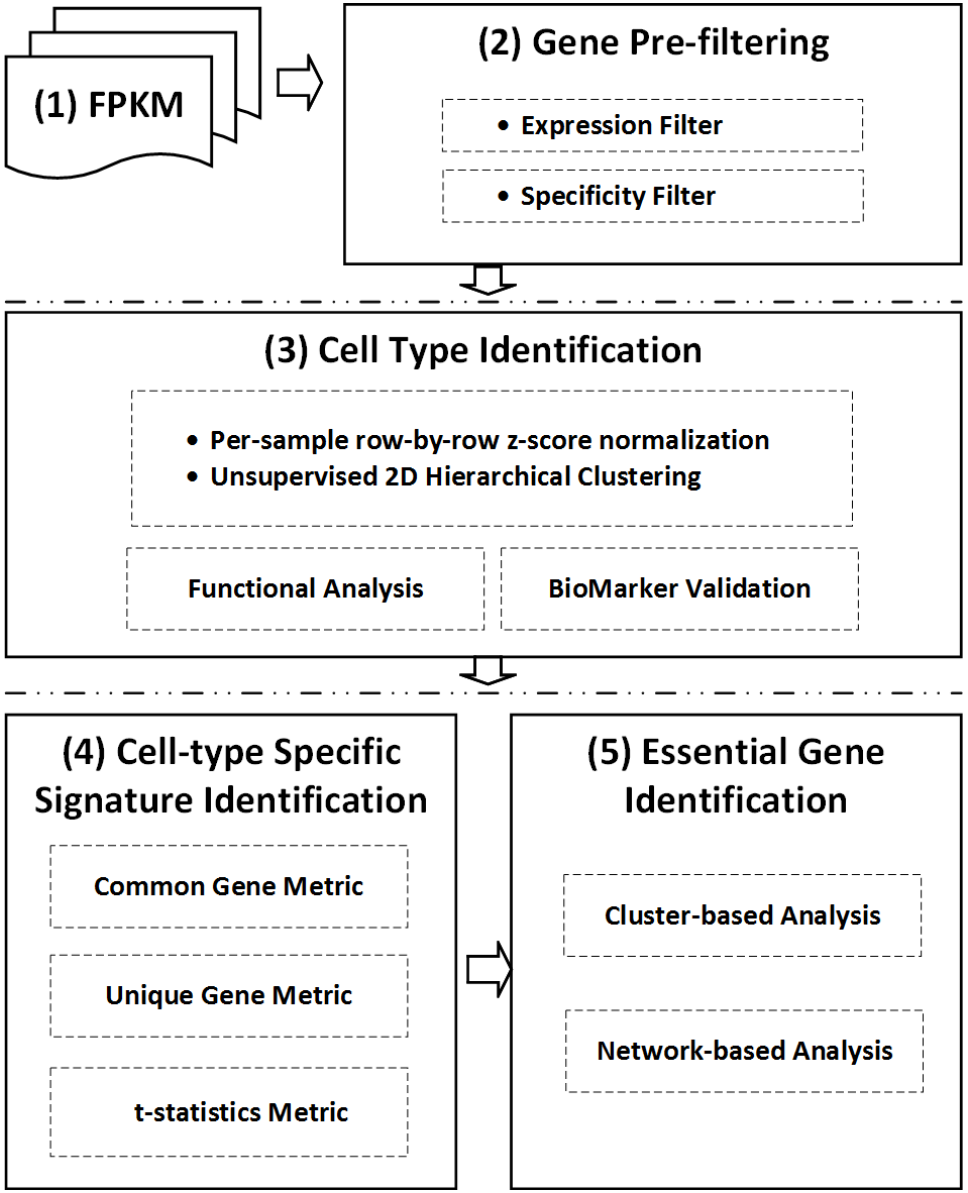
Sept, 8, 2014

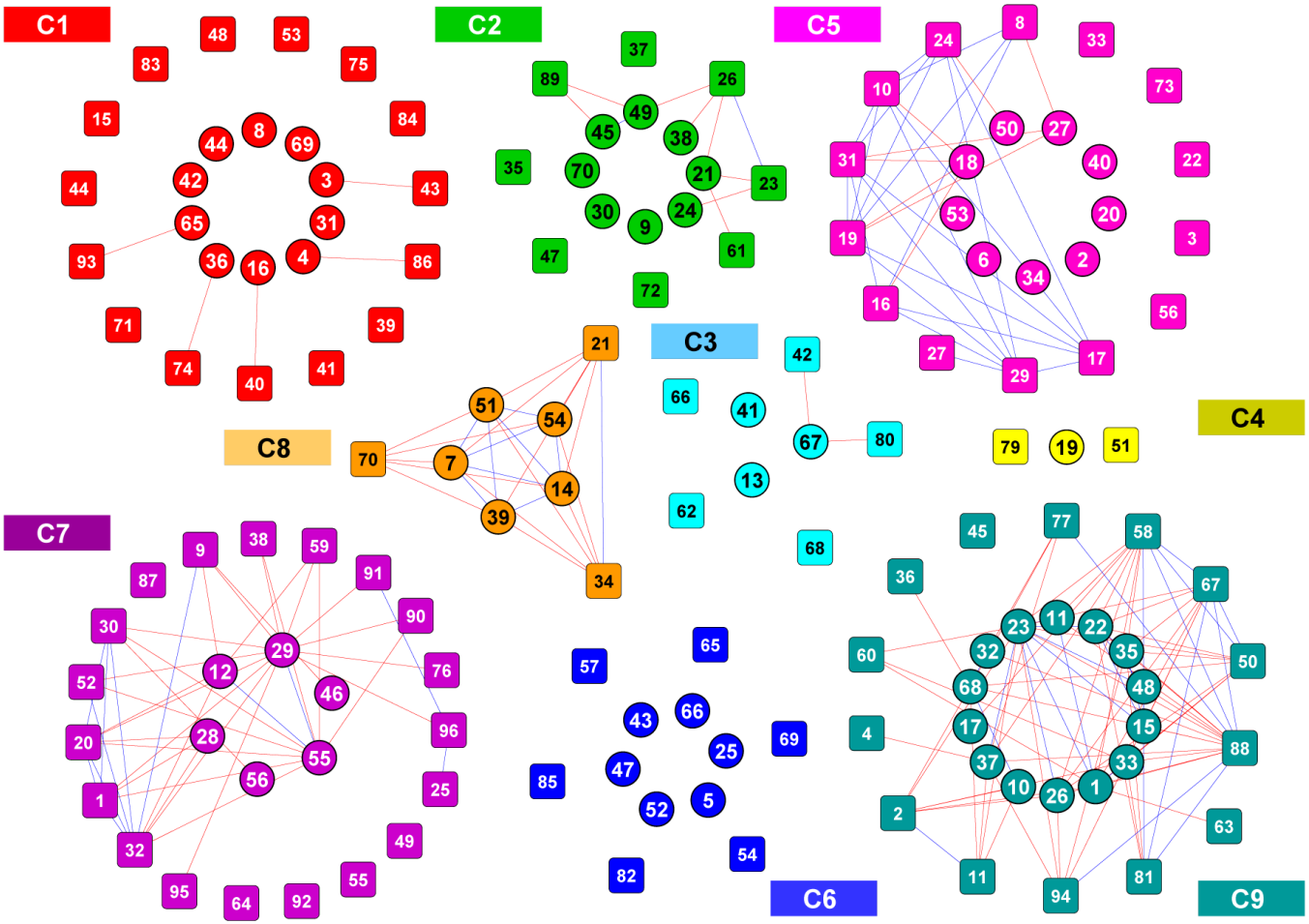
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Samples and Pipeline

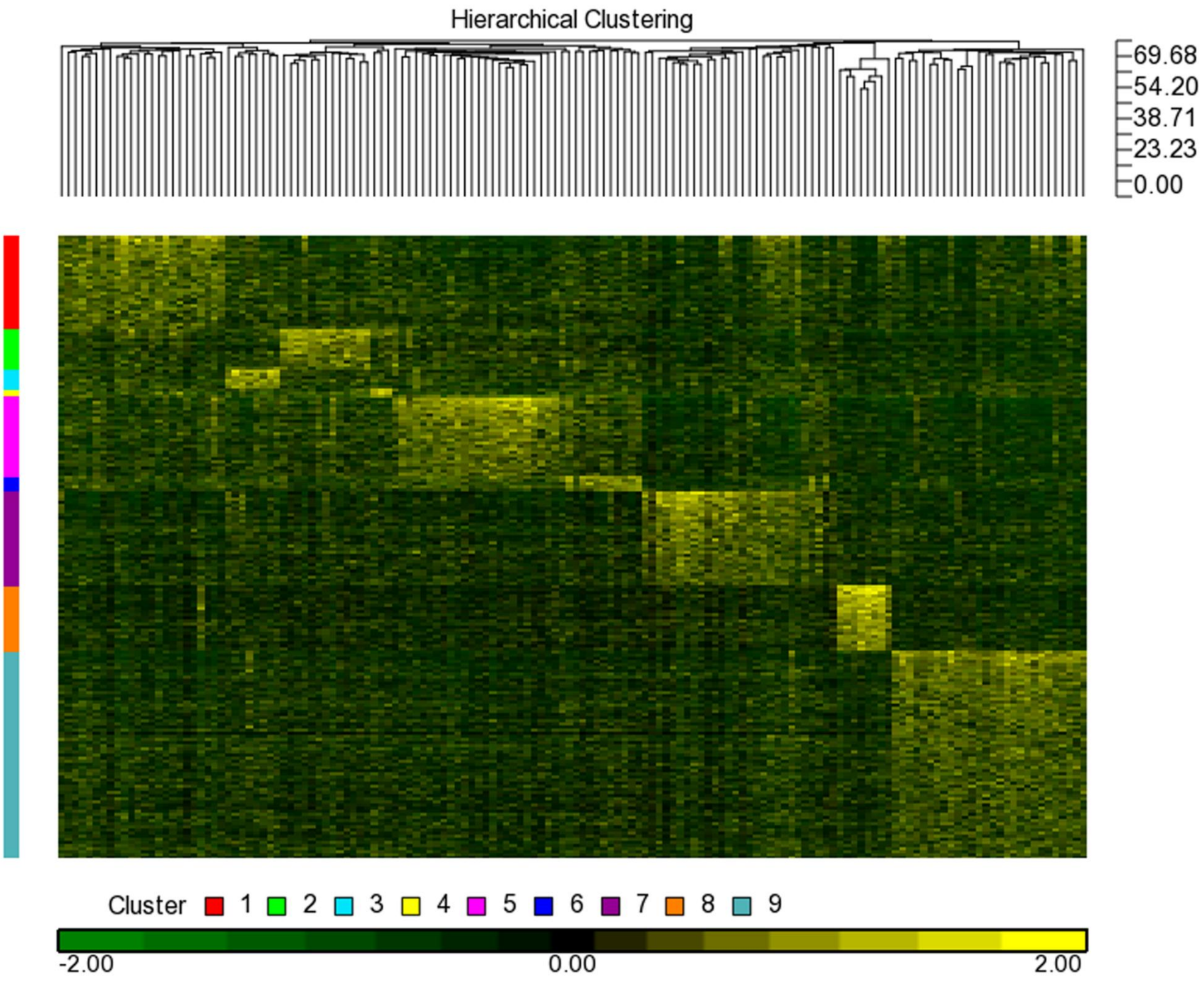
- Mouse Lung E16.5
- Fluidigm™ C1™ System
- Data for Pipeline Analysis
  - 148 lung single cells
    - S1: 86 samples
    - S2: 62 samples
  - 36188 Expression Profiles
- Expression Filter
  - $\delta_i^S(\theta) \geq N$
- cell specificity index τ_i^S
  - $x_{ij}^S = \frac{E_{ij}^S - \min(E_i^S)}{max(E_i^S) - \min(E_i^S)}$
  - $y_{ij}^S = \frac{y_{ij}^S}{\max\{y_{ij}^S | j=1,\ldots,N^S\}}$
  - $\tau_i^S = \frac{\sum_{j=1}^{N^S}(1-y_{ij}^S)}{N^S-1}$

[Yanai et al, 2005]

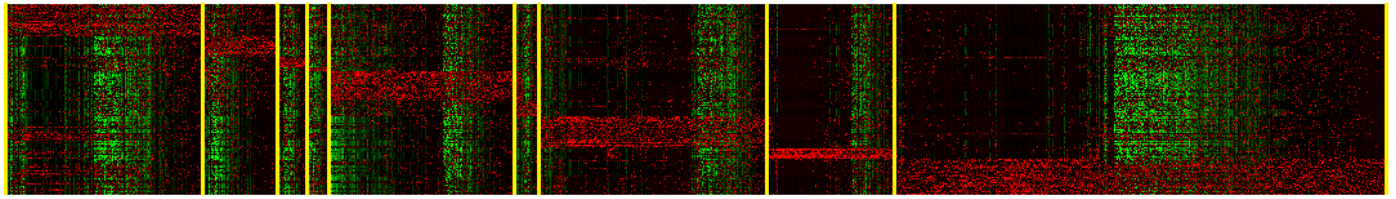




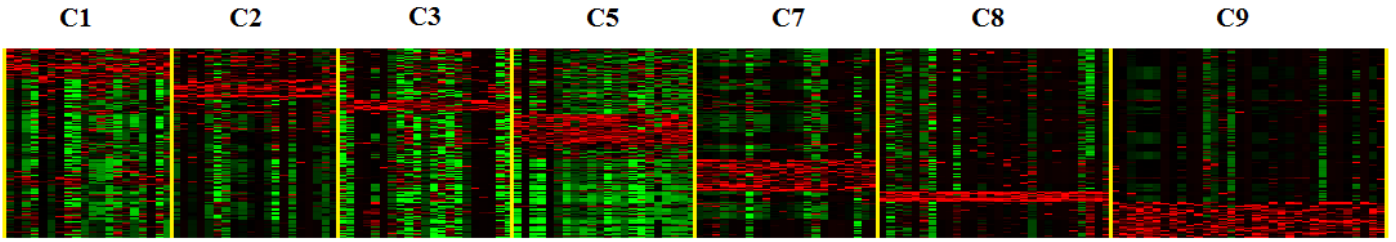
C1	Proliferative fibroblast
C2	Myofibroblast/smooth muscle-like
C3	Pericyte
C4	Undefined fibroblast
C5	Matrix fibroblast
C6	Intermediate fibroblast
C7	Endothelial cells
C8	Myeloid/immune cells
C9	Epithelial cells


Hierarchical Clustering
69.68
54.20
38.71
23.23
0.00
Cluster 1 2 3 4 5 6 7 8 9
-2.00
0.00
2.00

(a)

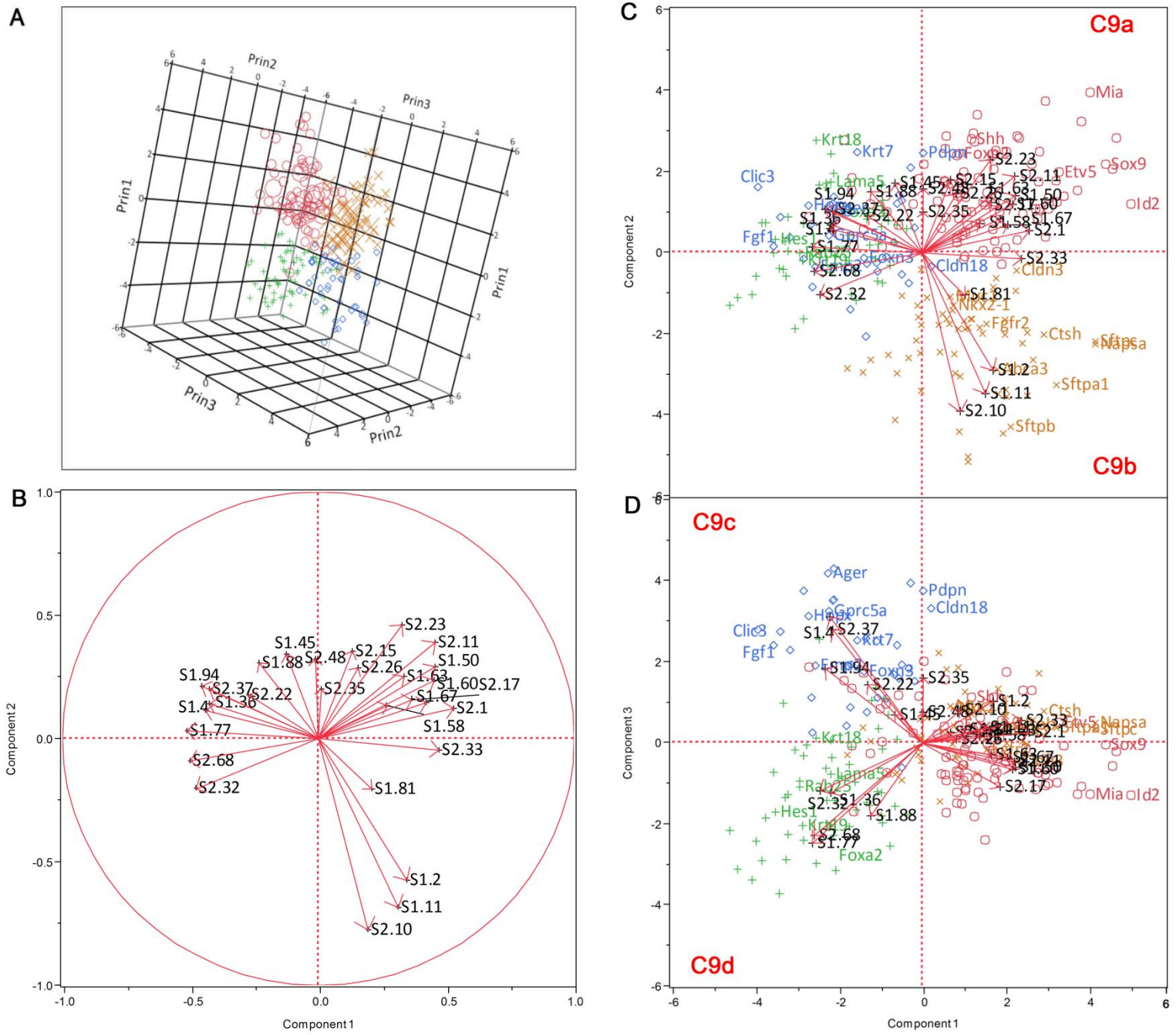


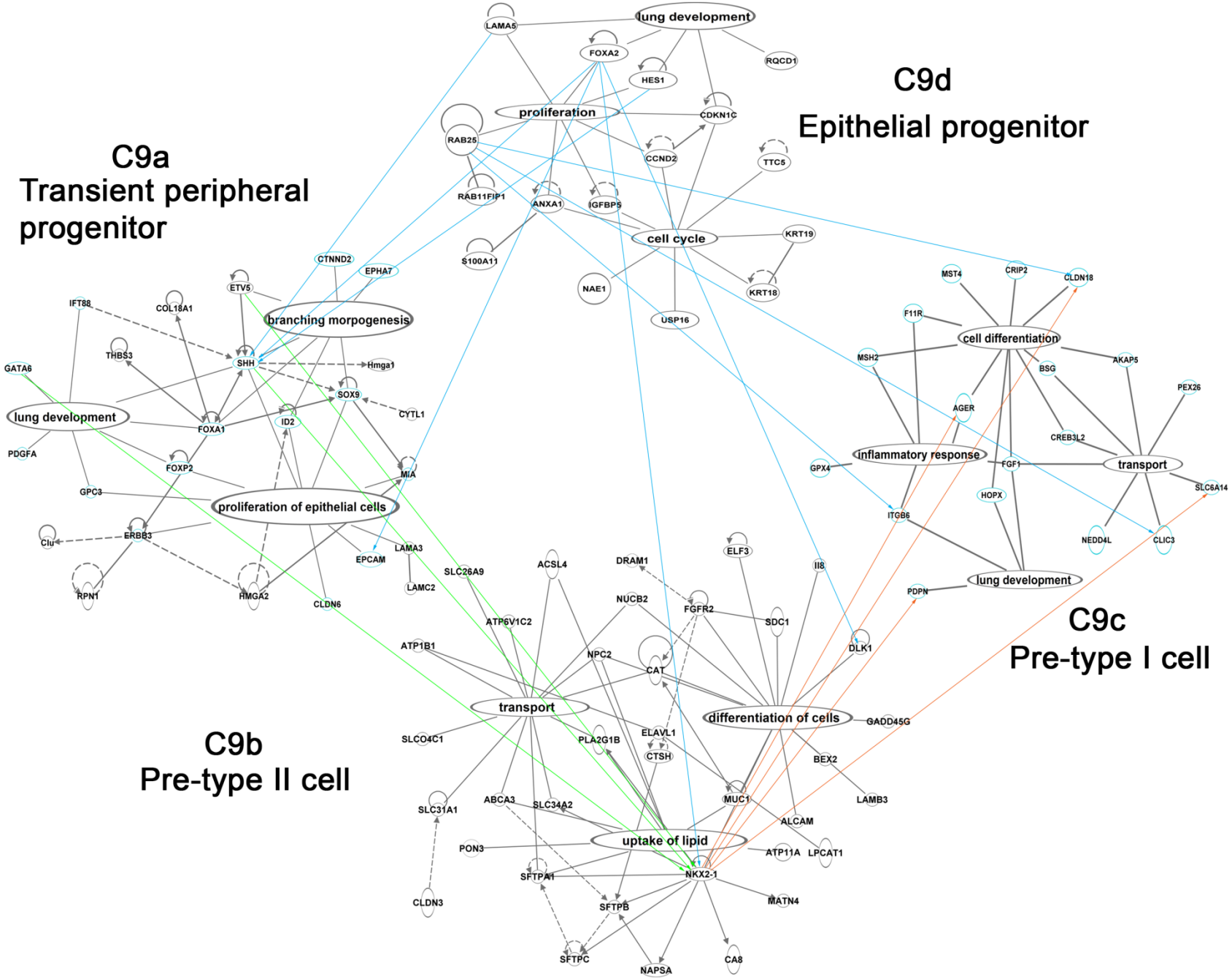
(b)



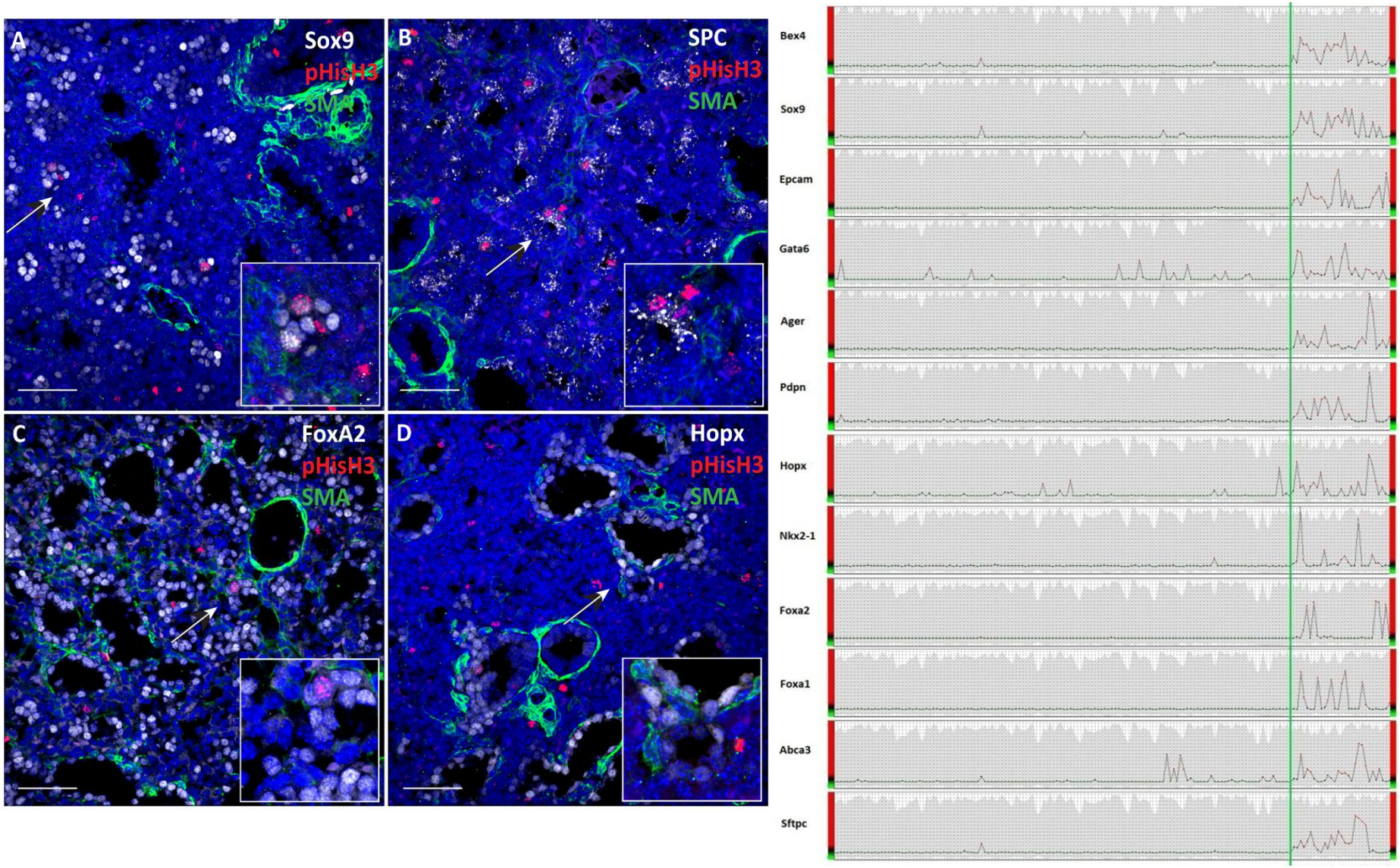
Bmp5	Acta2	Acta1	B4galt1	Calcrl	B2m	Abca3
Bub1	Actg2	Cald1	Bgn	Cav1	Cst3	Ager
Ccna2	Cd248	Cdh2	Cdh11	Cd34	Cyba	Bex1
Cks2	Ednrb	Cox4i2	Col1a1	Cd93	Fcer1g	Bex4
Dek	Fstl1	Dlk1	Col6a2	Cdh5	Fcgr1	Bsg
Dkk3	Ghr	Ebf1	Fgf10	Clec14a	Ftl1	Cd9
Fgf7	Igf1	F2r	Fhl1	Clecla	Fyb	Cdh1
Foxm1	Itga9	Gnas	Figf	Egfl7	Gm2a	Cgn
H2afz	Mfap2	Gng11	Fn1	Emcn	Gmfg	Cldn18
Hhip	Mgp	Grcc10	Gyg	Fli1	Laptm5	Cldn3
Hmgb2	Myh11	Gucy1a3	Limch1	Flt1	Lcp1	Cldn6
Hoxb5	Myl9	Lgals1	Macf1	Flt4	Ly86	Elf5
Pdgfra	Nrep	Marcks	Mfap4	Gimap6	Man2b1	Epcam
Sdc2	Pdgfra	Nme1	Nebl	Kdr	Mef2c	Fgfr2
Smc2	Rgs2	Notch3	Nr2f2	Lyve1	Ncf2	Foxa1
Sox11	Robo1	Pdgfrb	Pmp22	Mest	Ptpn6	Foxa2
Tbx3	Scara5	Postn	Tcf21	Pecam1	Rac2	Foxp1
Top2a	Tagln	Rgs5	Tns1	Ramp2	Rassf4	Gata6
Tuba1b	Tbx5	Tax1bp3	Vcam1	Tek	Sat1	Hopx
Tubb6	Tgfbi	Tuba1a	Zyx	Tie1	Tyrobp	Id2

Itgb6
Klf5
Krt18
Krt8
Lpcat1
Mia
Muc1
Napsa
Nkx2-1
Pdgfa
Pdpn
Plp2
Rbm47
Sftpa1
Sftpb
Sftpc
Shh
Slc34a2
Sox9
Tmsb4x

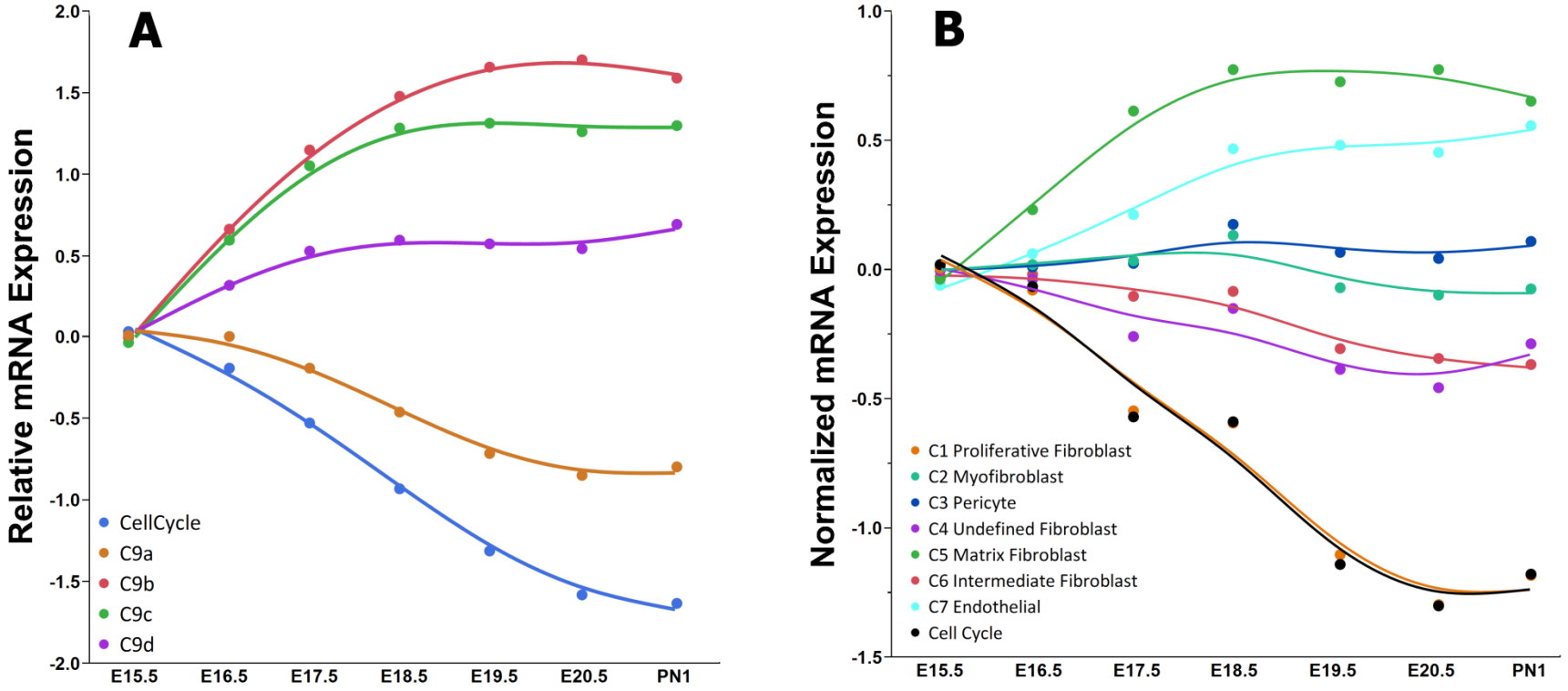

A
Prin2
Prin3
Prin1
Prin3
Prin2
B
Component 2
Component 1
S2.23
S1.45
S2.11
S1.88
S2.48
S2.15
S2.26
S1.50
S1.94
S1.63
S1.60
S2.17
S2.37
S2.22
S2.35
S1.67
S1.4
S1.36
S2.1
S1.77
S1.58
S2.68
S2.33
S2.32
S1.81
S1.2
S1.11
S2.10
C
C9a
C9b
Component 2
Component 1
Mia
Krt18
Krt7
Pdpn
Foxa2
Shh
S2.23
Sox9
Etv5
Clic3
Lama5
S1.45
S2.15
S2.11
S1.94
S1.88
S2.48
S2.26
S1.50
Id2
S2.37
S2.22
S2.35
S1.67
Fgf1
Hes1
Gprc5a
S1.77
S2.1
Foxn3
S2.33
S2.68
Cldn18
Cldn3
S2.32
S1.81
Nkx2-1
Fgfr2
Ctsh
Napsa
S1.2
Sftpa1
S1.11
S2.10
Sftpb
D
C9c
C9d
Component 3
Component 1
Ager
Pdpn
Cldn18
Gprc5a
Clic3
S2.37
Krt7
Fgf1
S1.94
Foxn3
S2.22
S2.35
S1.2
Ctsh
S2.10
S2.33
Etv5
Napsa
Sftpc
Krt18
Sox9
Lama5
S2.17
Mia
Id2
Rab25
S2.32
S1.36
S1.88
Hes1
S2.68
S1.77
Foxa2

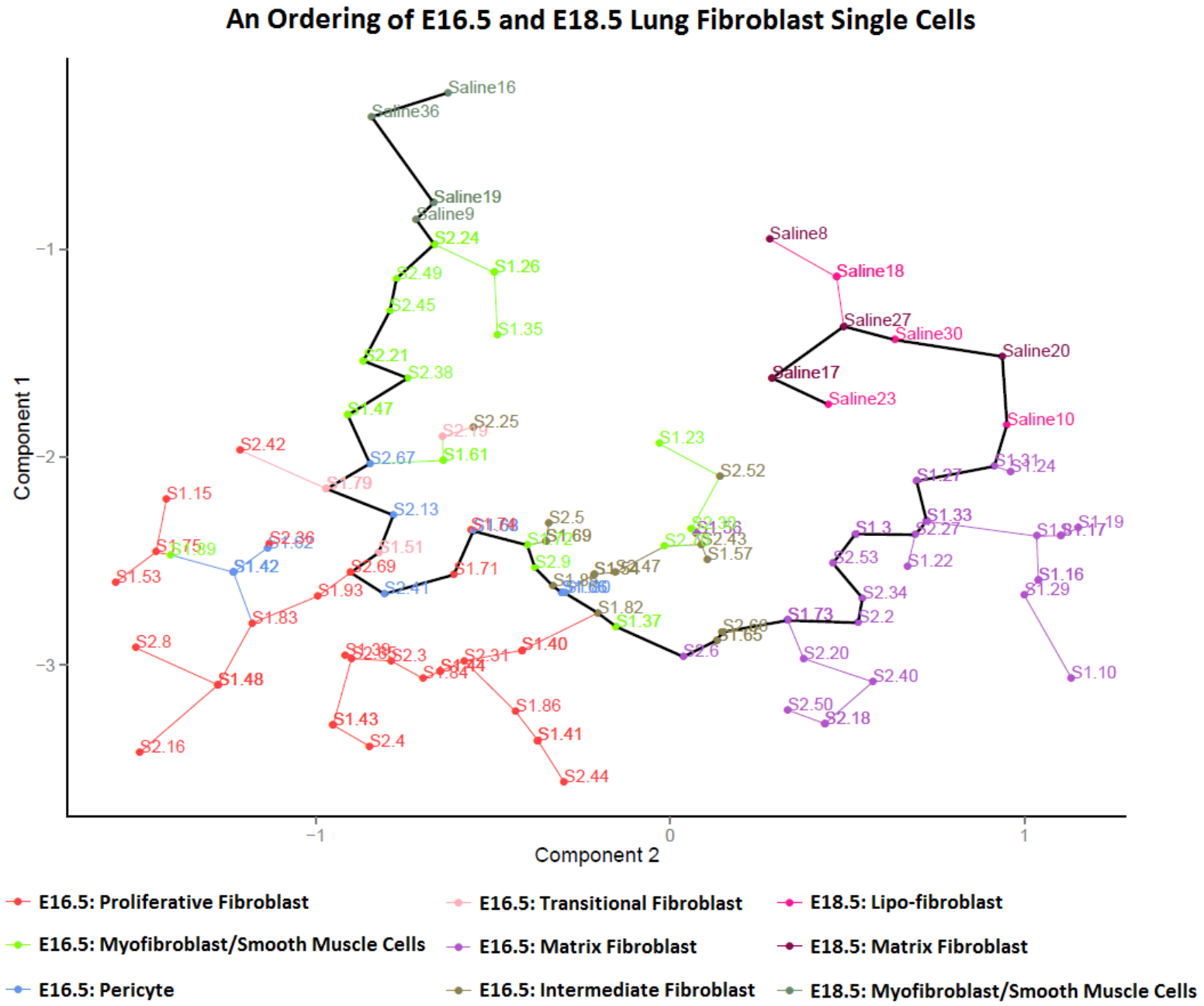

C9a
Transient peripheral progenitor
C9d
Epithelial progenitor
C9b
Pre-type II cell
C9c
Pre-type I cell
lung development
LAMA5
FOXA2
RQCD1
HES1
proliferation
CDKN1C
RAB25
CCND2
TTC5
RAB11FIP1
ANXA1
IGFBP5
cell cycle
KRT19
S100A11
NAE1
KRT18
USP16
CTNND2
EPHA7
ETV5
IFT88
COL18A1
branching morpogenesis
THBS3
SHH
Hmga1
GATA6
SOX9
CYTL1
lung development
FOXA1
ID2
PDGFA
FOXP2
MIA
GPC3
proliferation of epithelial cells
Clu
ERBB3
EPCAM
LAMA3
LAMC2
RPN1
HMGA2
CLDN6
MST4
CRIP2
CLDN18
F11R
cell differentiation
MSH2
BSG
AKAP5
PEX26
AGER
CREB3L2
inflammatory response
GPX4
FGF1
transport
SLC6A14
HOPX
ITGB6
NEDD4L
CLIC3
lung development
PDPN
ELF3
II8
DRAM1
ACSL4
SLC26A9
NUCB2
FGFR2
SDC1
ATP6V1C2
DLK1
ATP1B1
NPC2
CAT
transport
differentiation of cells
GADD45G
SLCO4C1
PLA2G1B
ELAVL1
CTSH
BEX2
LAMB3
MUC1
SLC31A1
ABCA3
SLC34A2
ALCAM
uptake of lipid
PON3
ATP11A
LPCAT1
SFTPA1
NKX2-1
CLDN3
SFTPB
MATN4
SFTPC
NAPSA
CA8

Anatomic Location Of Distinct Epithelial Cell Subtypes And Representative Epithelial Signature Genes

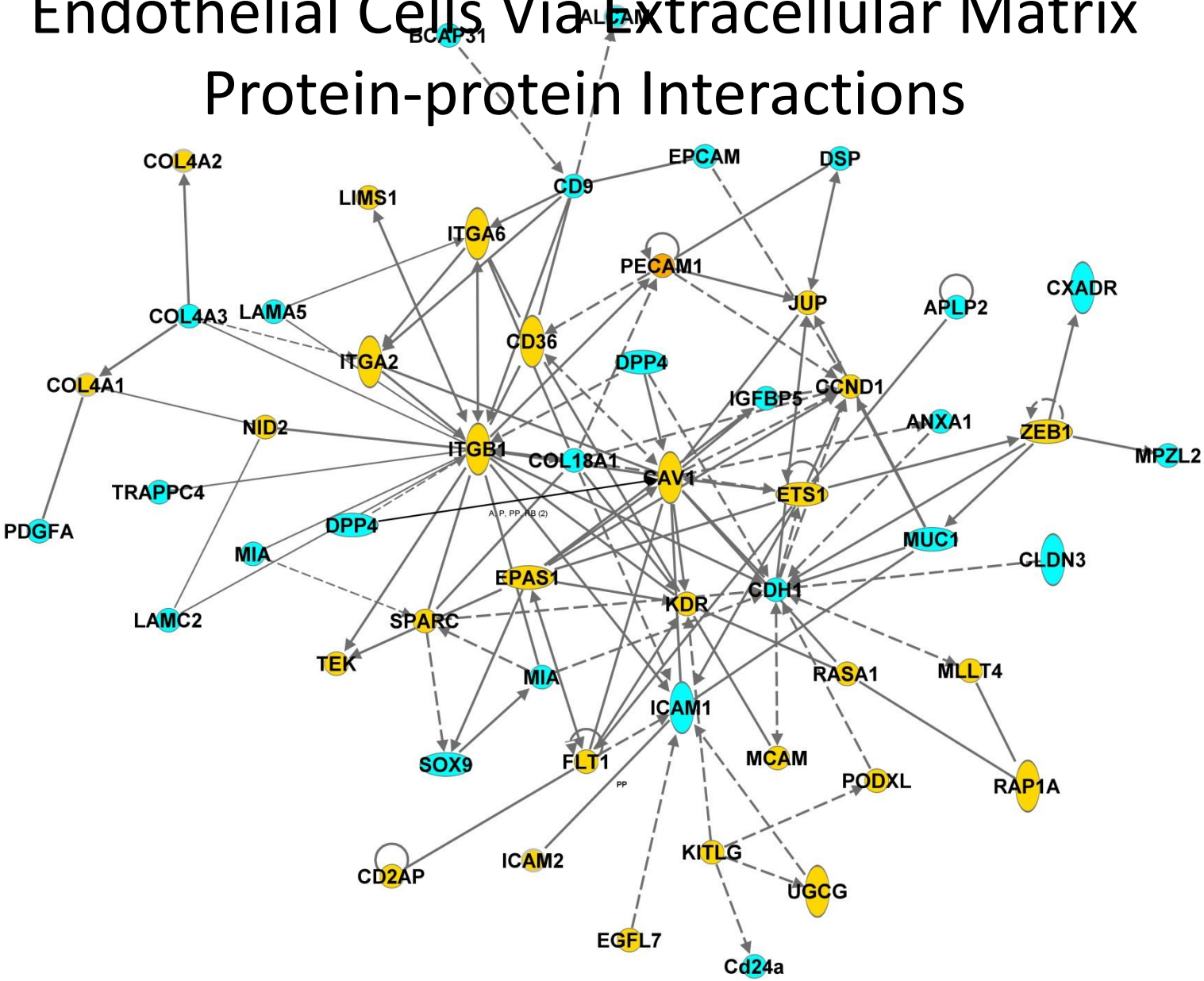


Ontogenic Changes In RNAs Defining Distinct Lung Cell Clusters During Lung Maturation

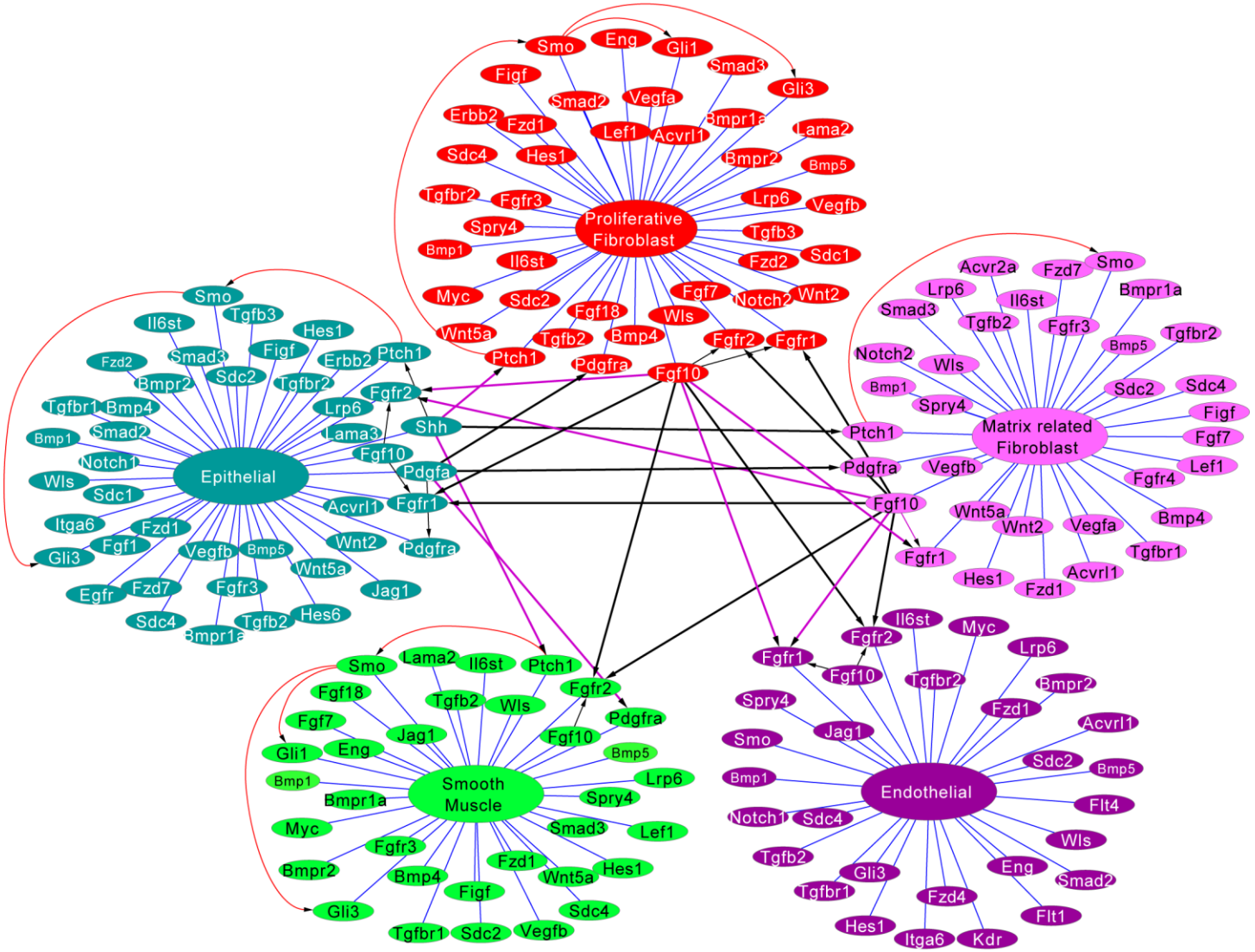



An Ordering of E16.5 and E18.5 Lung Fibroblast Single Cells
Component 1
Component 2
−1
−2
−3
−1
0
1
Saline16
Saline36
Saline19
Saline9
S2.24
S1.26
S2.49
S2.45
S1.35
S2.21
S2.38
S1.47
S2.25
S2.42
S1.61
S2.67
S1.79
S1.15
S2.13
S1.75
S1.42
S1.53
S1.51
S2.69
S1.93
S1.71
S1.83
S2.8
S1.48
S2.16
S2.3
S1.40
S1.43
S2.4
S1.86
S1.41
S2.44
S1.82
S1.37
S2.6
S1.23
S2.52
S2.5
S2.9
S1.57
S1.73
S2.20
S2.40
S2.50
S2.18
Saline8
Saline18
Saline27
Saline30
Saline20
Saline17
Saline23
Saline10
S1.31
S1.24
S1.27
S1.33
S1.3
S2.27
S1.19
S2.53
S1.22
S1.16
S1.29
S2.34
S2.2
S1.10
E16.5: Proliferative Fibroblast
E16.5: Transitional Fibroblast
E18.5: Lipo-fibroblast
E16.5: Myofibroblast/Smooth Muscle Cells
E16.5: Matrix Fibroblast
E18.5: Matrix Fibroblast
E16.5: Pericyte
E16.5: Intermediate Fibroblast
E18.5: Myofibroblast/Smooth Muscle Cells

Cross Talk Between Lung Epithelial Cells And Endothelial Cells Via Extracellular Matrix Protein-protein Interactions

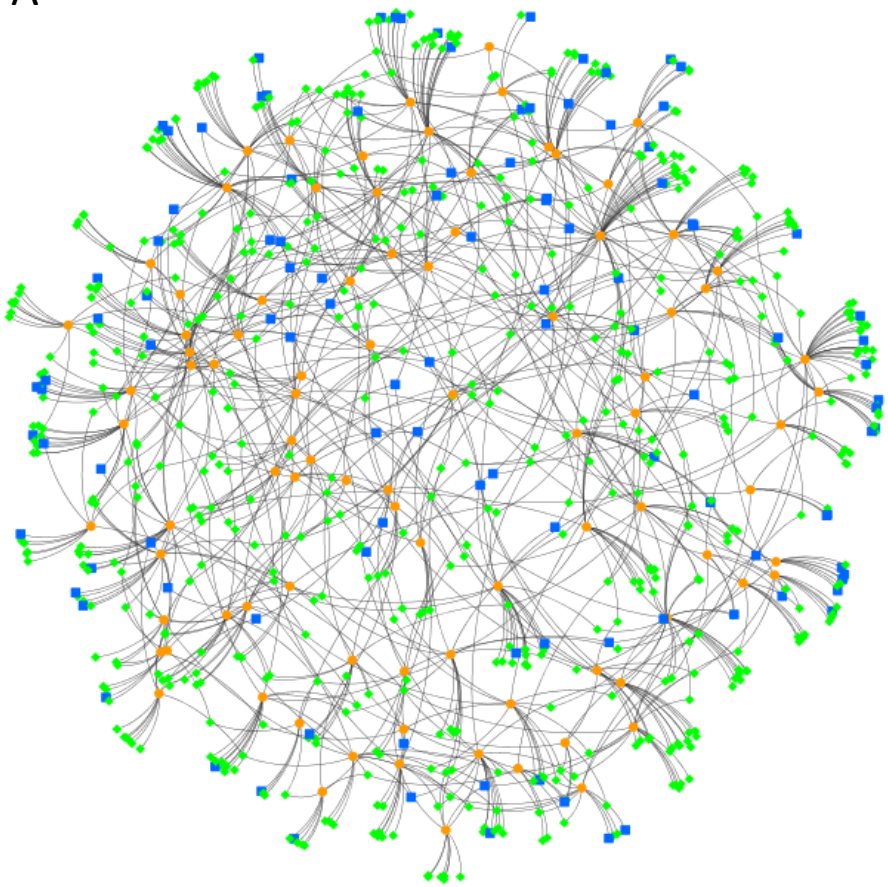


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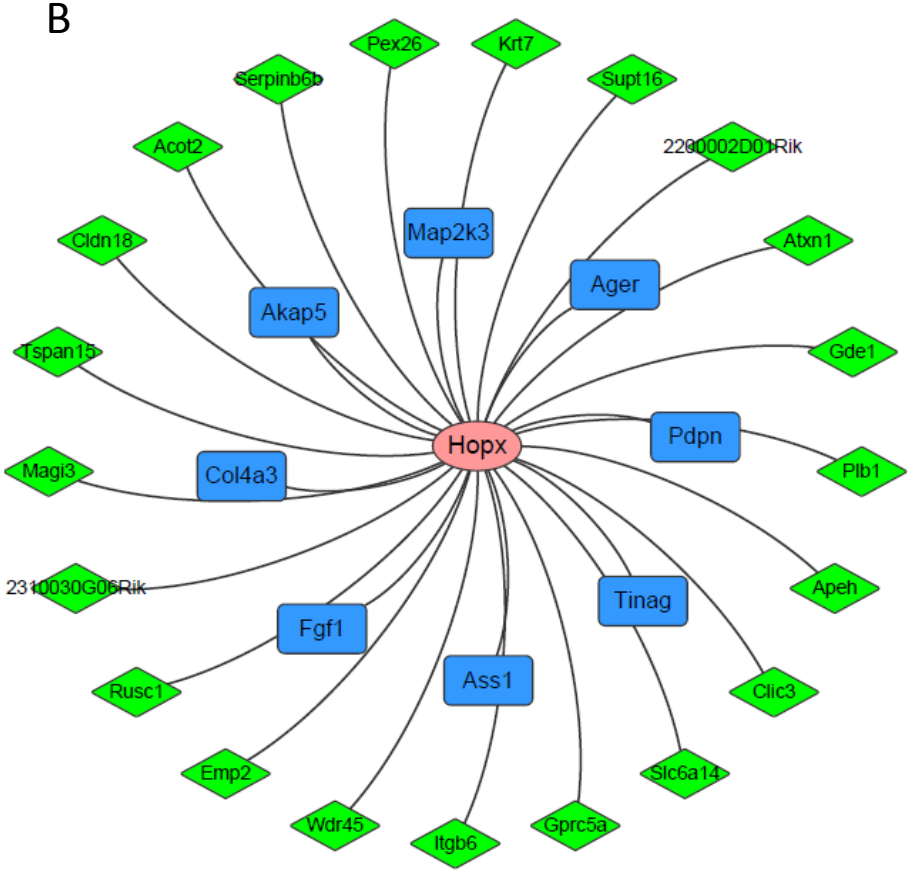

Proliferative Fibroblast
Smo
Eng
Gli1
Smad3
Gli3
Figf
Smad2
Vegfa
Bmpr1a
Lama2
Erbb2
Fzd1
Lef1
Acvrl1
Sdc4
Hes1
Bmpr2
Bmp5
Tgfbr2
Fgfr3
Lrp6
Vegfb
Spry4
Tgfb3
Bmp1
Il6st
Fzd2
Sdc1
Myc
Sdc2
Fgf7
Notch2
Wnt2
Fgf18
Wls
Wnt5a
Tgfb2
Bmp4
Fgfr2
Fgfr1
Ptch1
Pdgfra
Fgf10
Epithelial
Smo
Tgfb3
Il6st
Hes1
Figf
Smad3
Erbb2
Ptch1
Fzd2
Bmpr2
Sdc2
Tgfbr2
Fgfr2
Tgfbr1
Bmp4
Lrp6
Bmp1
Smad2
Lama3
Shh
Notch1
Fgf10
Wls
Pdgfra
Sdc1
Acvrl1
Fgfr1
Itga6
Fzd1
Fgf1
Vegfb
Bmp5
Wnt2
Pdgfra
Gli3
Wnt5a
Egfr
Fzd7
Fgfr3
Jag1
Sdc4
Tgfb2
Hes6
Bmpr1a
Matrix related Fibroblast
Acvr2a
Fzd7
Smo
Lrp6
Il6st
Bmpr1a
Smad3
Tgfb2
Fgfr3
Tgfbr2
Bmp5
Notch2
Wls
Bmp1
Sdc2
Sdc4
Spry4
Figf
Ptch1
Fgf7
Pdgfra
Vegfb
Lef1
Fgfr4
Fgf10
Wnt5a
Bmp4
Wnt2
Vegfa
Tgfbr1
Fgfr1
Hes1
Acvrl1
Fzd1
Smooth Muscle
Smo
Lama2
Il6st
Ptch1
Fgfr2
Fgf18
Tgfb2
Wls
Pdgfra
Fgf7
Jag1
Fgf10
Gli1
Eng
Bmp5
Bmp1
Lrp6
Bmpr1a
Spry4
Myc
Smad3
Lef1
Fgfr3
Fzd1
Bmpr2
Hes1
Bmp4
Wnt5a
Figf
Gli3
Sdc4
Tgfbr1
Sdc2
Vegfb
Endothelial
Il6st
Myc
Fgfr2
Lrp6
Fgfr1
Fgf10
Tgfbr2
Bmpr2
Spry4
Fzd1
Jag1
Acvrl1
Smo
Sdc2
Bmp5
Bmp1
Flt4
Notch1
Sdc4
Wls
Tgfb2
Gli3
Eng
Smad2
Tgfbr1
Fzd4
Flt1
Hes1
Itga6
Kdr

Epithelial TF-TG Network

A



B



Summary and Conclusions

- We have developed an analytic pipeline for the analysis of single-cell RNA-Seq data
- We identified major cell types in the fetal mouse lung, four sub-types of epithelial, four subtypes of fibroblast, endothelial, smooth muscle, pericyte and myeloid cells were classified by their expression & function similarity within cell groups.
- We identified cell-specific gene signatures, key regulators, surface markers, bioprocesses and functional profiles associated with each cell type
- The study provided the framework to delineate cell signaling and communications across cell types via paracrine and autocrine signaling as well as protein-protein interaction.
- Algorithms were developed to map cell type specific transcriptional regulatory networks and identify driving forces for individual cell types.
- The data provide a knowledge base facilitating the understanding of lung maturation at high resolution.

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NIH/NHLBI



LungMAP
Molecular Atlas of Lung Development Program

UNIVERSITY OF Cincinnati

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