# SUPPLEMENTAL INFORMATION
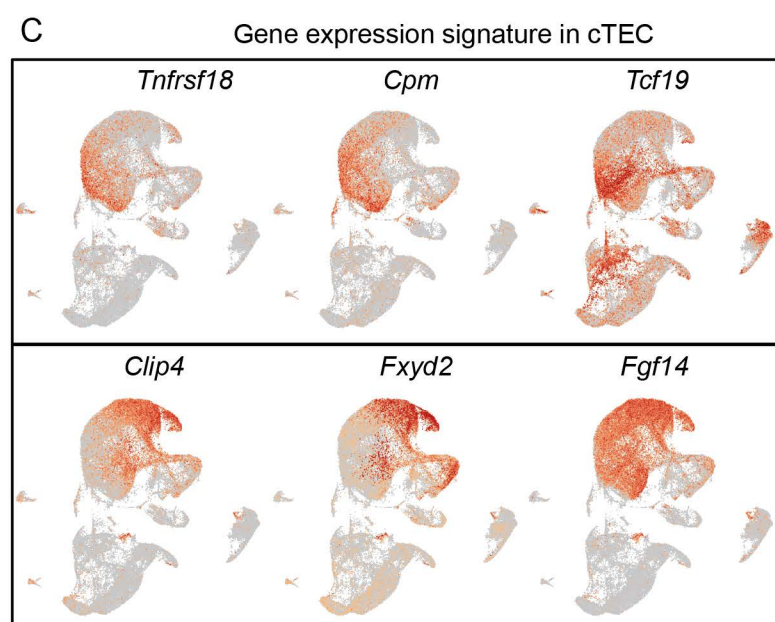# SUPPLEMENTAL FIGURES
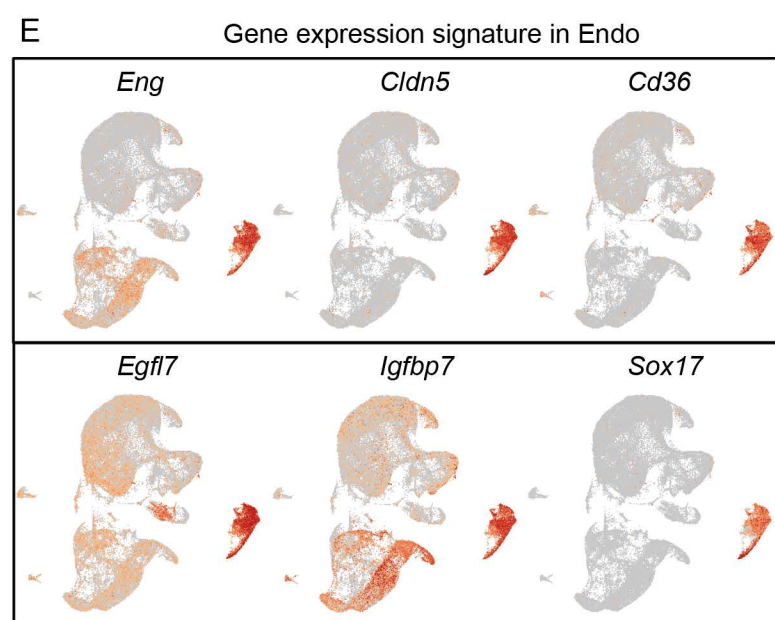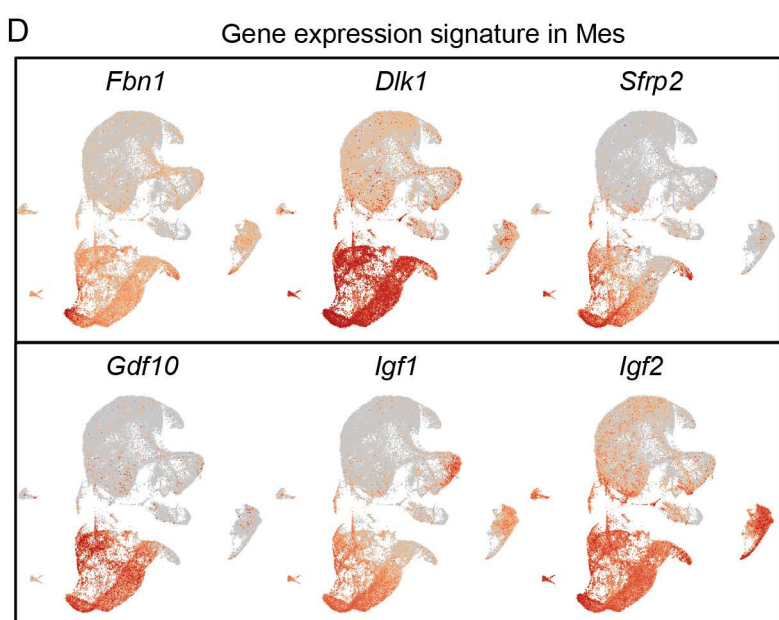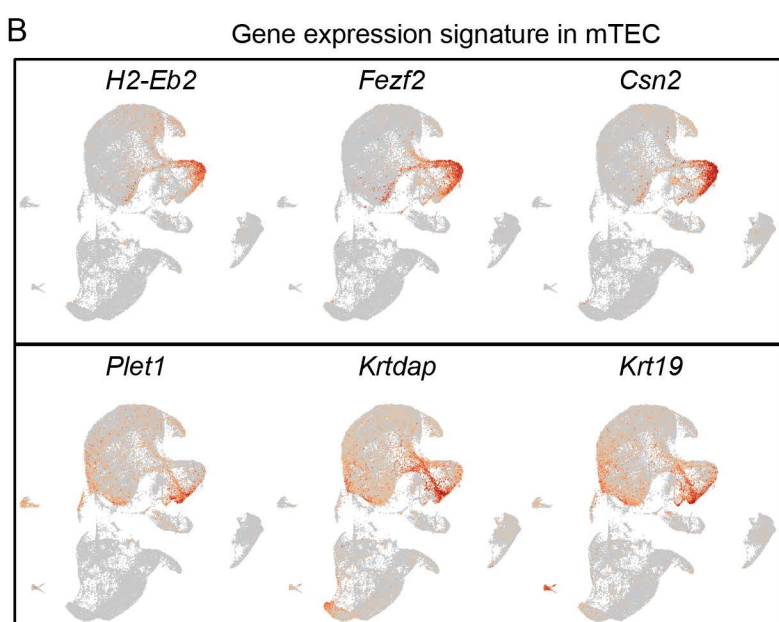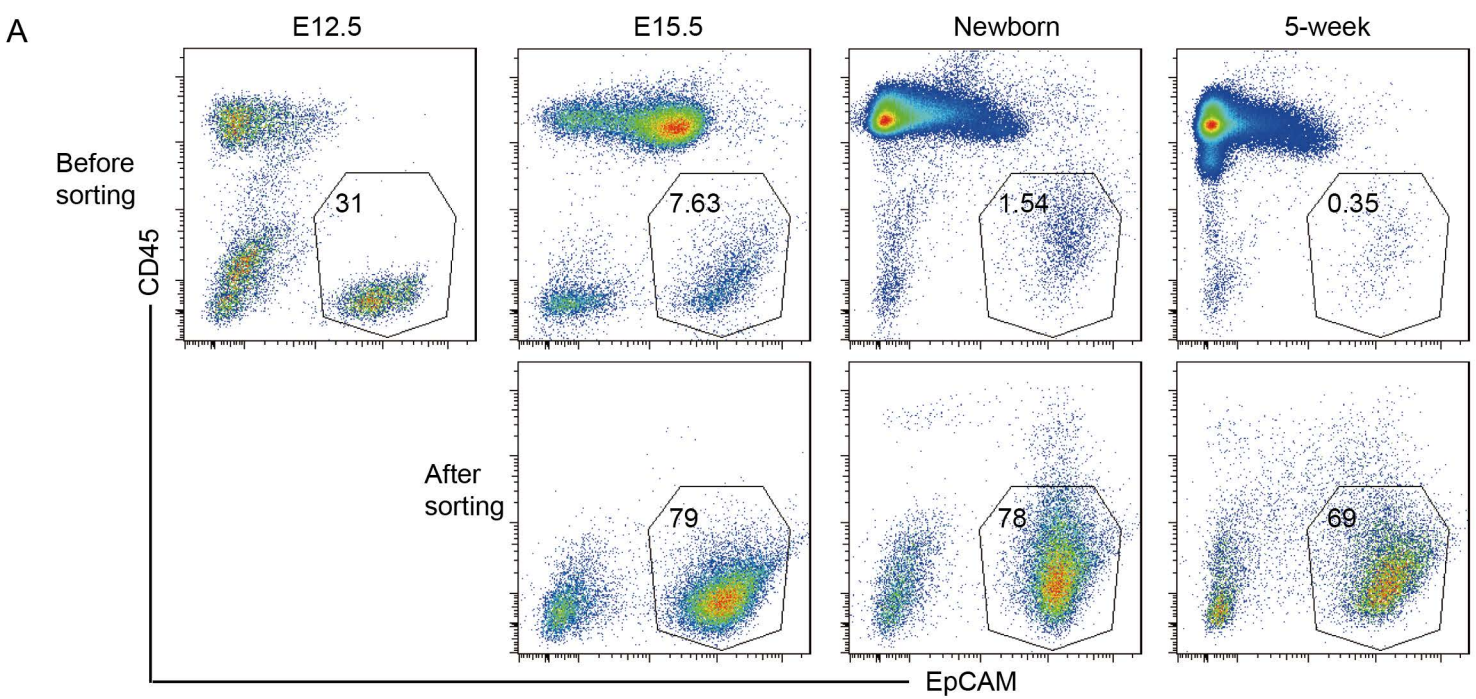
**Figure. S1 Feature gene expression of the major cell types.**

**(A)** Representative FACS analysis of Cd45$^-$ EpCAM$^+$ TECs before and after magnetic depletion of hematopoietic cells. (n=3).

**(B-E)** UMAP visualization of the expression of curated feature genes specific for **(B)** mTEC, **(C)** cTEC, **(D)** endothelial cell (Endo), **(E)** mesenchymal fibroblasts (Mes).
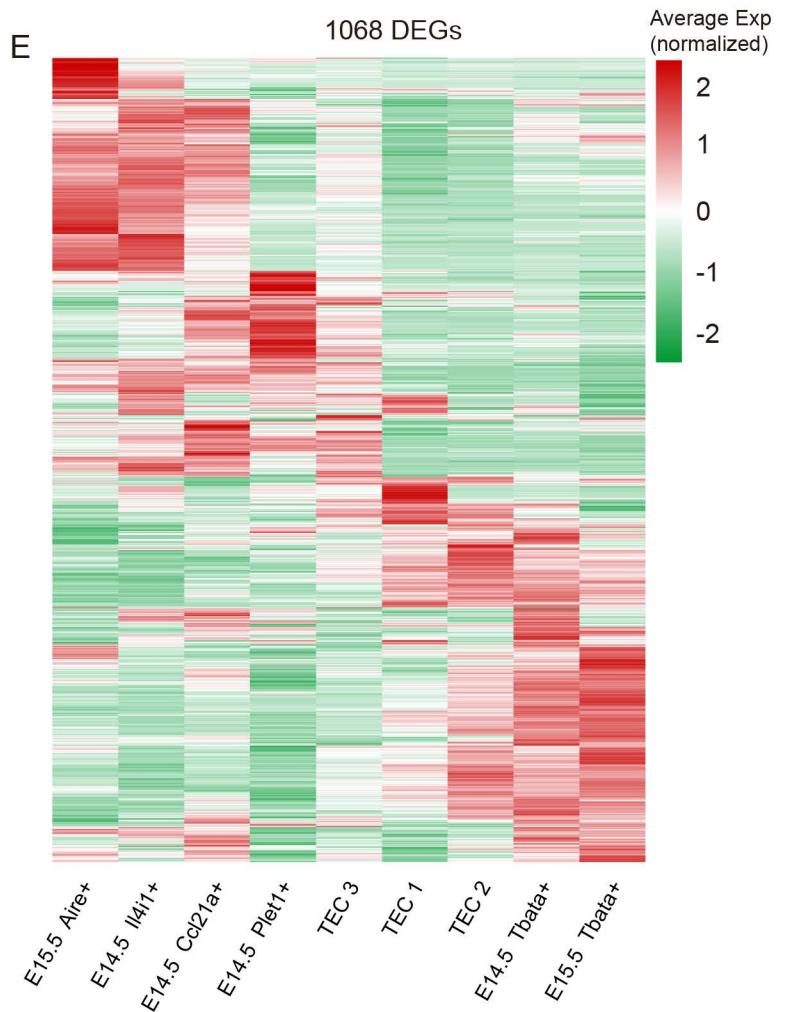
**A**

Average Exp (normalized)

*Pax9*
*Hmga2*
*Ccl25*
*Eya2*
*Psmb11*
*Prss16*
*Wnt4*
*Il17*

*Krt5*
*Krt17*
*Zfp750*
*Upk2*
*Aldh3b2*
*Pvrl4*
*Clu*

*Igfbp2*
*Sox2*
*Klf5*
*Krt7*
*Igfbp5*
*Cldn6*
*Anxa8*
*Foxe1*

Sox2+ cells | TEC 3 | TEC 1 | TEC 2

**B**

Log2 Exp  0.0 – 8.0  E11.5  E12.5  E13.5

*Krt8*
*Ctsl*
*Psma7*

**C**

Log2 Exp  0.0 – 8.0  E11.5  E12.5  E13.5

*Pax1*
*Psmb11*
*Ccl25*
*Pltp*

**D**

*Ctsl*
*Psma7*
*Krt8*
*Krt18*
*Epcam*
*Six1*
*Pdlim1*
*Fxyd3*
*Sfn*
*Cdc34*
*Pax9*
*Mtf2*
*Kdelr2*
*Bnip3l*
*Sox4*
*Cbx1*
*Shisa2*
*Il7*
*Kcnn3*
*Gpx8*
*Scd2*
*Pax1*
*Pltp*
*Ccdc34*
*Wnt4*
*Psmb11*
*Prss16*
*Cxcl12*
*Gm1673*
*Mast4*
*Spock2*
*Ccl25*
*Eya2*
*Comt*
*Ndufa11*
*Foxn1*

Average Exp (normalized)

5-week | Newborn | TEC 3 | TEC 1 | TEC 2 | Newborn | 5-week
Mature mTEC | Mature cTEC

**E**

1068 DEGs

Average Exp (normalized)

E15.5 Aire+ | E14.5 Il4i1+ | E14.5 Ccl21a+ | E14.5 Plet1+ | TEC 3 | TEC 1 | TEC 2 | E14.5 Tbata+ | E15.5 Tbata+
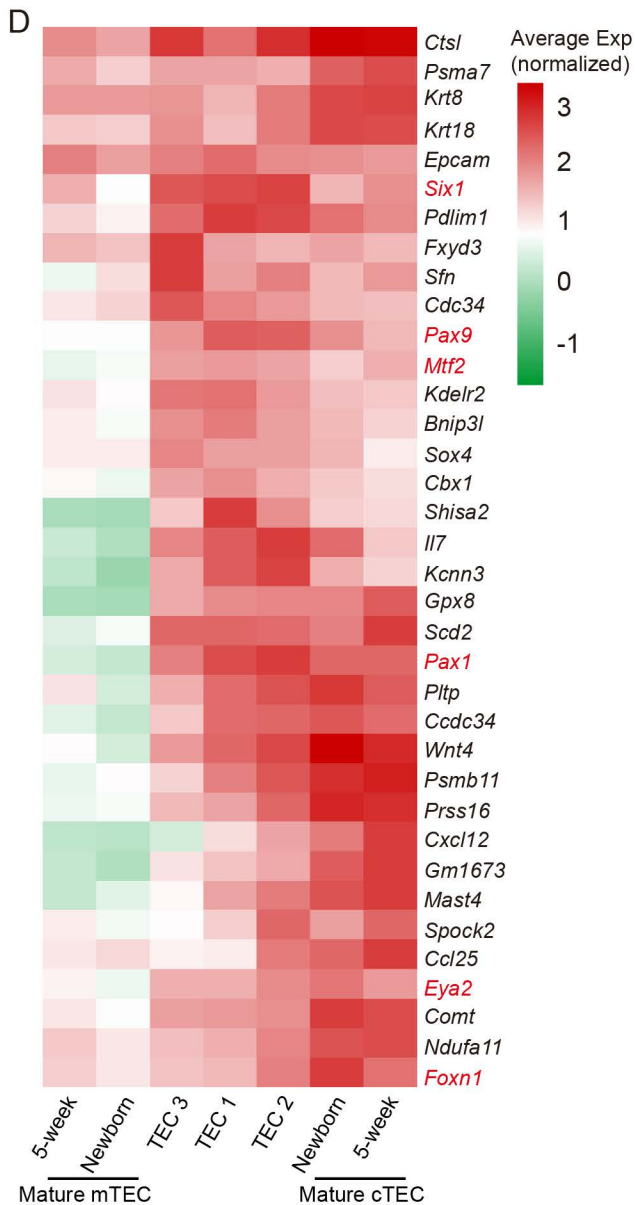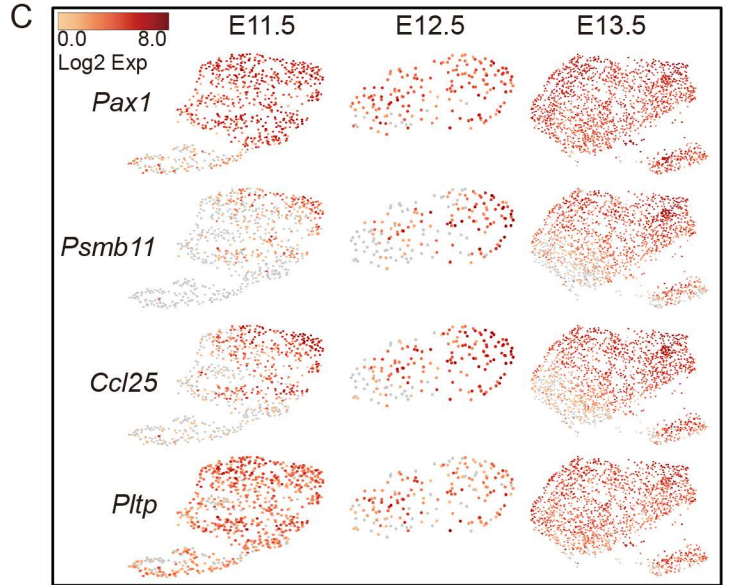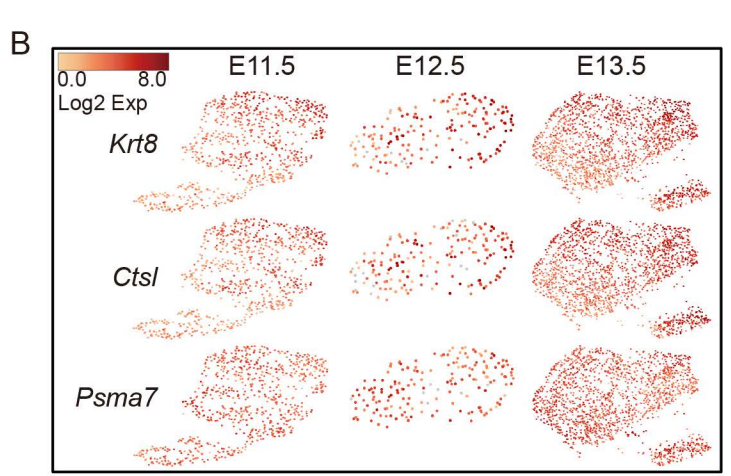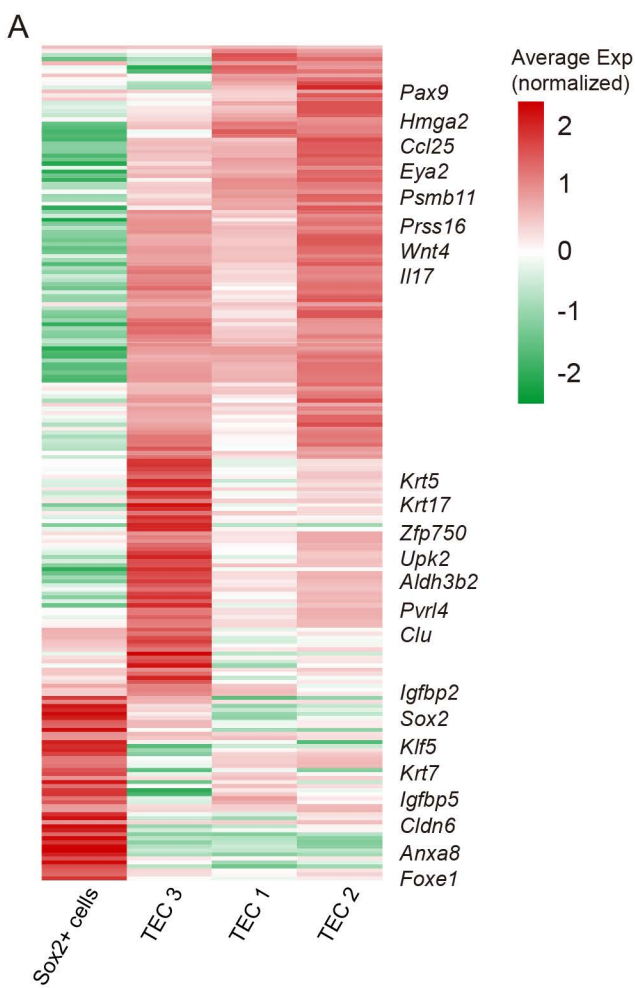
**Figure. S2 Gene expression profiling of the early TECs at E11.5-E13.5.**

**(A)** Heat map showing differentially expressed genes in TEC 1, TEC 2, TEC 3 and Sox2$^{+}$ cells of early thymus (E11.5-E13.5). Gene expression levels were maximum-normalized and smoothed. Genes were grouped by their expression patterns.

**(B-C)** UMAP visualization of the expression of cTEC feature genes in early TECs.

**(D)** Heat map showing expression of mature cTEC-specific genes in TEC 1, TEC 2, TEC 3 from E11.5-E13.5, mature mTEC and mature cTEC from newborn and 5-week thymus. Genes were grouped by their expression patterns. Genes in red are transcription factors.

**(E)** Heat map showing 1,068 differentially expressed genes in clusters of TEC 1, TEC 2, TEC 3 from E11.5-E13.5 thymus, and mTEC, cTEC from E14.5, E15.5 thymus. Expression levels were maximum-normalized and smoothed. Genes were grouped by their expression patterns. (DEGs, differentially expressed genes).

**A**

|  | E13.5 | E14.5 | E15.5 | Newborn | 5-week |
|---|---|---|---|---|---|
|  | 176 | 433 | 1,152 | 1,527 | 2,169 |

mTEC

Pax1

Ccl21a

Emp2

Pdpn

Aire

UMAP2

UMAP1 →

0 ——— Max  Log2 Exp

**C**

C1 C2 C3 C4 C5 C9 C8 C7 C6

Pax1
Ccl21a
Krt5
Emp2

Ube2c
Cenpf
Top2a
Cdk1
Ccnd2
Ascl1
Fgf21
Hnf1a
Il4i1
Cdx1
Fezf2
Aire
H2-Eb2
Cd52
Csn2
Gp2
Hamp
Fabp1
Ccr5
Ccl9
Ccl20
Ccl6
Wfdc18
Dclk1
Pou2f3
Il25
Avil
Lrmp
Gnb3
Krt19
Cxcl17
Bpifa1
Wfdc2
Gsto1
Plet1

Spink5
Dmkn
Clic3
Ly6d
Lypd3
Sbsn
Krt16

Average Exp (normalized)

-2  -1  0  1  2

**B**

| Krt5 | Emp2 | Krt8 | Merged | Krt5/Emp2/Krt8 |
|---|---|---|---|---|

Newborn

50µm   50µm   50µm   C  M  M  50µm   M

E18.5

50µm   50µm   50µm   M  M  C  50µm   M  M

**D**

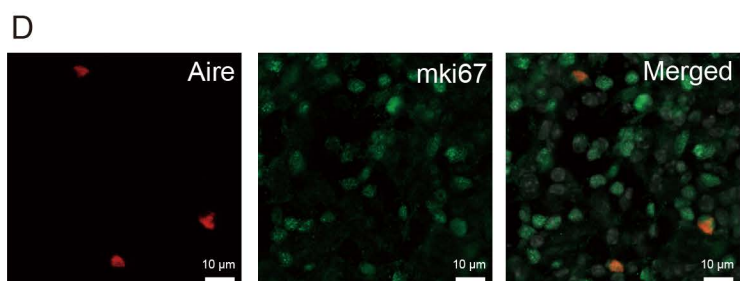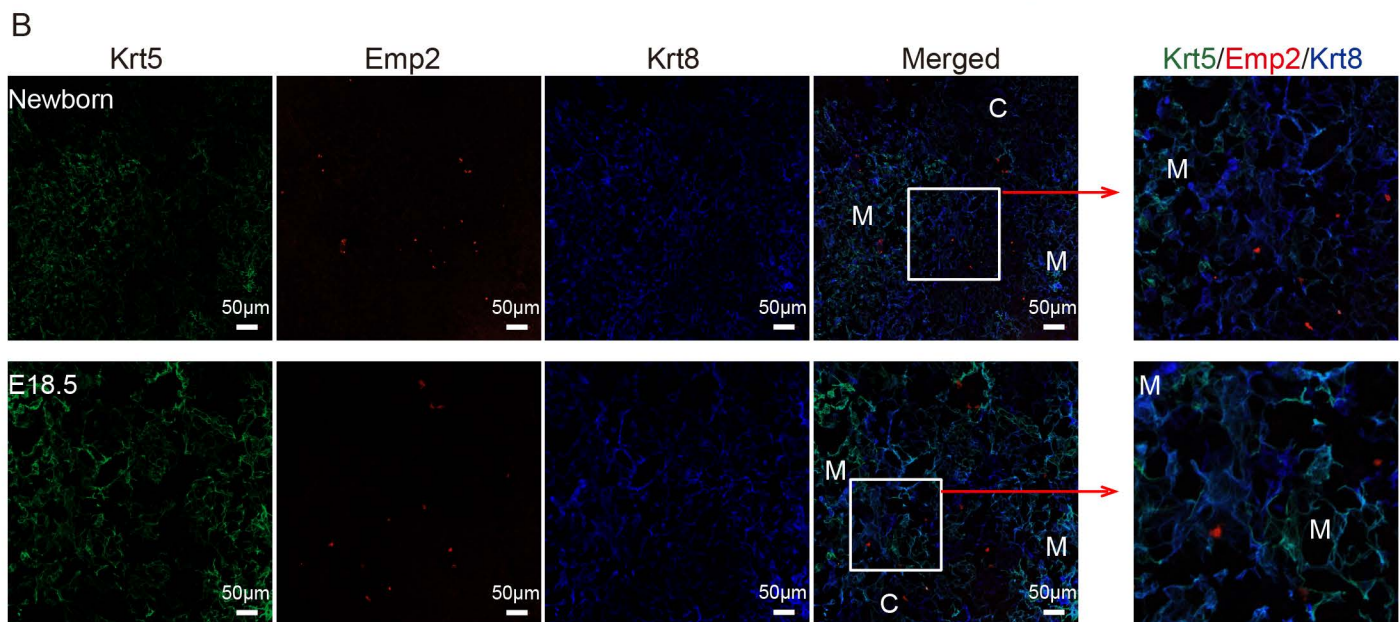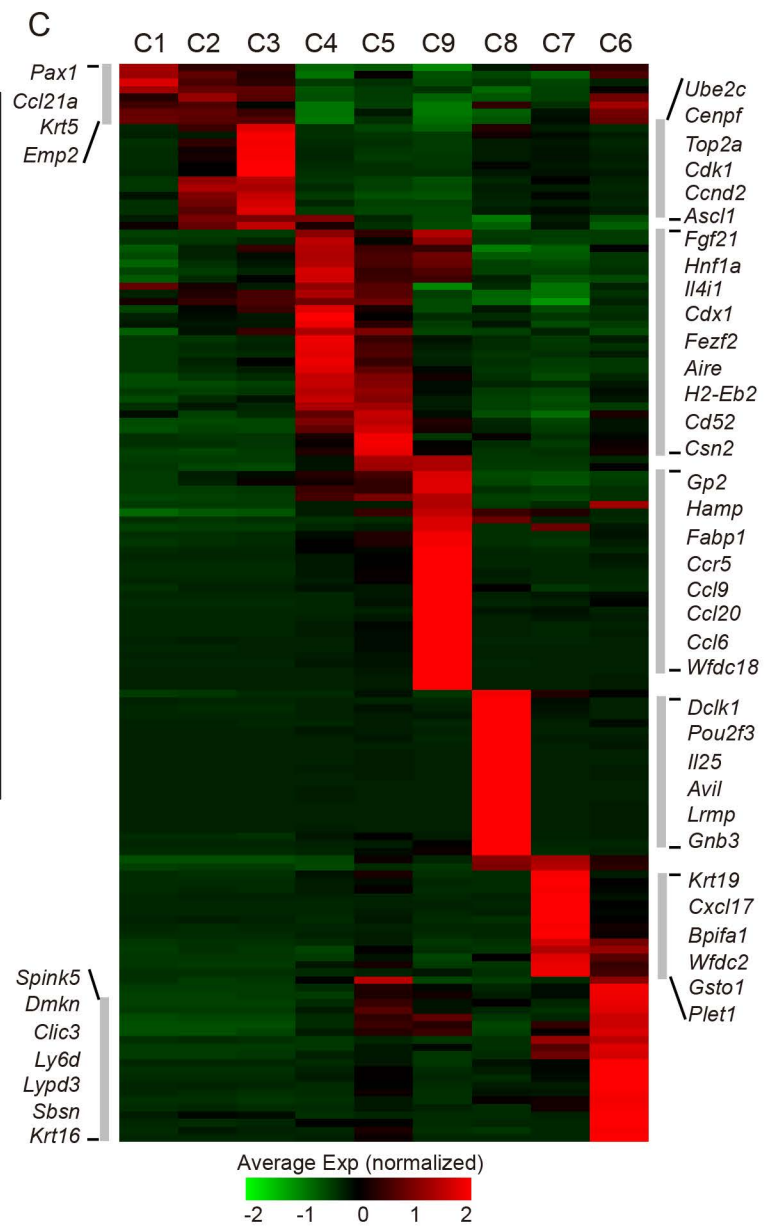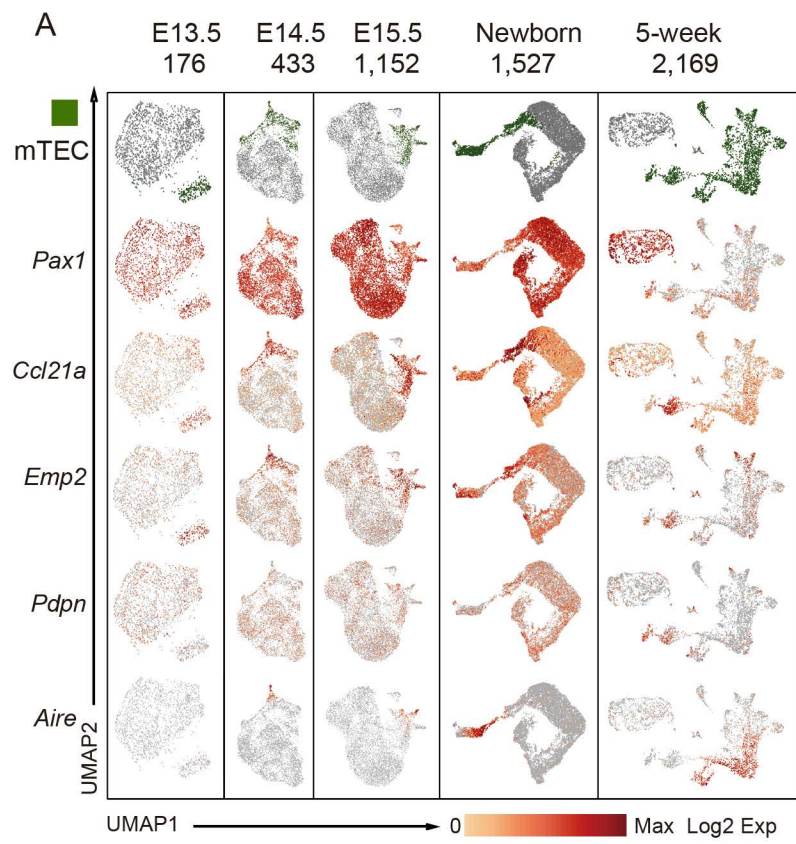| Aire | mki67 | Merged |
|---|---|---|

10 µm   10 µm   10 µm

**Figure. S3 The molecular characteristics of mTEC diversity during development.**

**(A)** UMAP visualization of the expression of *Pax1*, *Ccl21a*, *Emp2*, *Pdpn*, and *Aire* in mTECs at each developmental stage. Numbers on the diagram indicated the number of mTECs in our data. Green points indicated subpopulations of mTECs.

**(B)** Immunofluorescence staining of newborn and E18.5 thymus slides with antibodies to Krt5 (green), Emp2 (red), and Krt8 (blue). All staining experiments were replicated at least 2 times, and 2 biological replicates for each experiment.

**(C)** Heat map showing differentially expressed genes in 9 clusters of mTECs. Genes were grouped by their expression patterns.

**(D)** Immunofluorescence staining of 5-week thymus slides with antibodies to mKi67 (green), Aire (red). All staining experiments were replicated at least 2 times, and 2 biological replicates for each experiment.
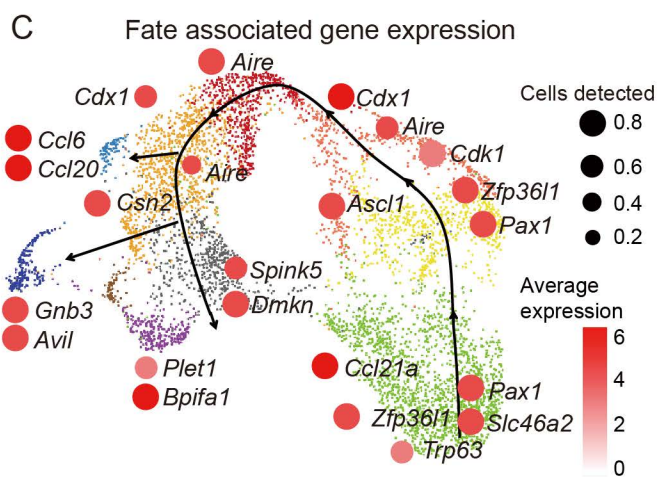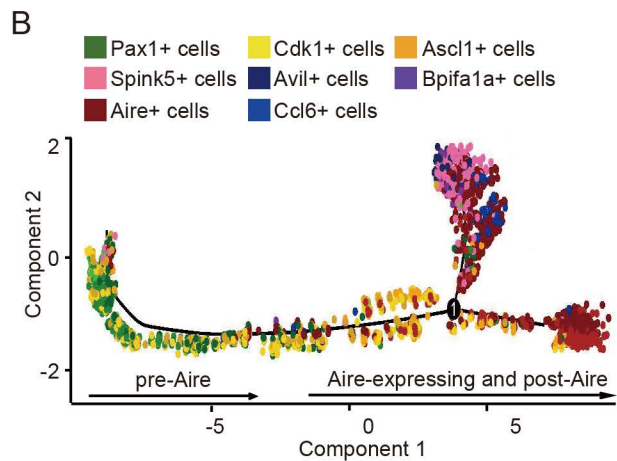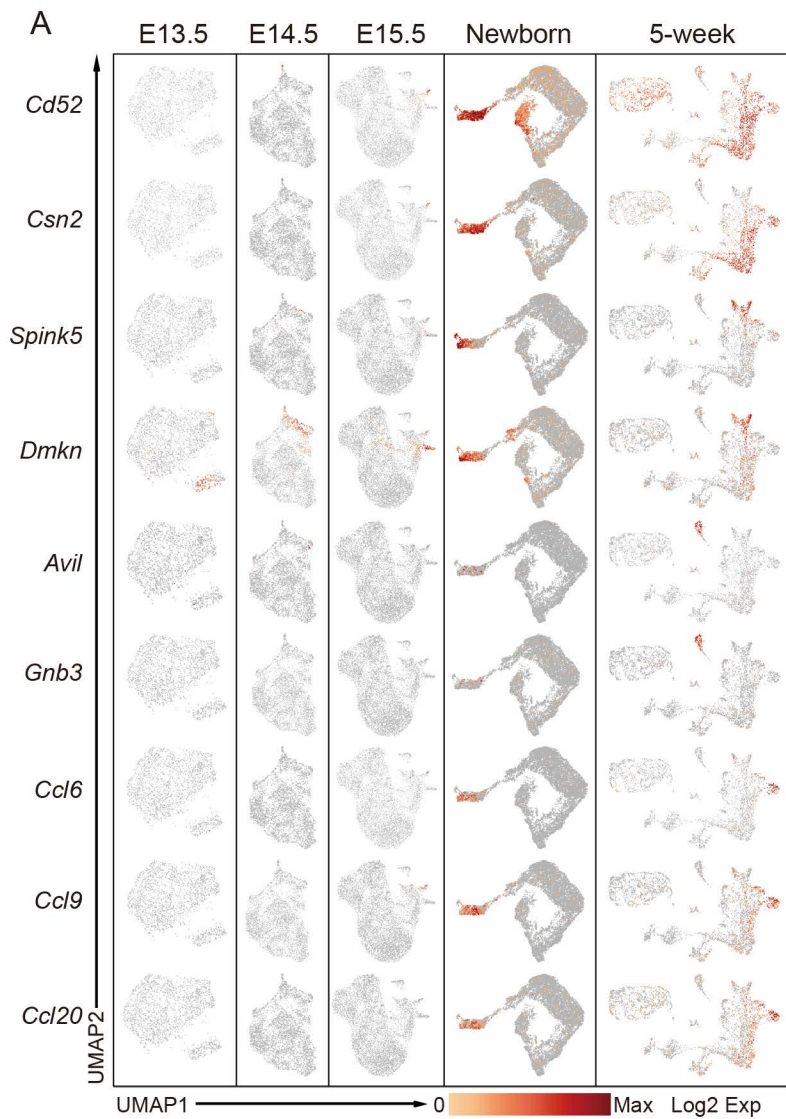
**A**

| | E13.5 | E14.5 | E15.5 | Newborn | 5-week |
|---|---|---|---|---|---|
| *Cd52* | | | | | |
| *Csn2* | | | | | |
| *Spink5* | | | | | |
| *Dmkn* | | | | | |
| *Avil* | | | | | |
| *Gnb3* | | | | | |
| *Ccl6* | | | | | |
| *Ccl9* | | | | | |
| *Ccl20* | | | | | |

UMAP2 ↑

UMAP1 ⟶  0 ▬▬▬ Max  Log2 Exp

**B**

Pax1+ cells
Spink5+ cells
Aire+ cells
Cdk1+ cells
Avil+ cells
Ccl6+ cells
Ascl1+ cells
Bpifa1a+ cells

pre-Aire ⟶          Aire-expressing and post-Aire ⟶

Component 2
Component 1

**C**  Fate associated gene expression

*Cdx1* *Aire* *Cdx1* *Aire*
*Ccl6* *Cdk1*
*Ccl20* *Aire* *Ascl1* *Zfp36l1*
*Csn2* *Pax1*
*Spink5*
*Dmkn*
*Gnb3* *Ccl21a*
*Avil* *Plet1* *Pax1*
*Bpifa1* *Zfp36l1* *Slc46a2*
*Trp63*

Cells detected
0.8
0.6
0.4
0.2

Average expression
6
4
2
0

**Figure. S4 Pseudotime trajectory analysis for mTECs.**

**(A)** UMAP visualization of the expression of *Cd52*, *Csn2*, *Spink5*, *Dmkn*, *Avil*, *Gnb3*, *Ccl6*, *Ccl9*, and *Ccl20* in mTECs at each developmental stage.

**(B)** The ordering of mTECs from E13.5 to adult thymus along pseudotime in a two-dimensional state-space defined by Monocle2. Cell orders were inferred from the expression of most dispersed genes across mTECs. Each point corresponded to a single cell, and each color represented a cell cluster.

**(C)** Dot plot showing expression of fate associated genes in mTEC clusters. Color represented maximumnormalized mean expression of genes in each cell cluster, and size indicated the proportion of cells expressing marker genes.
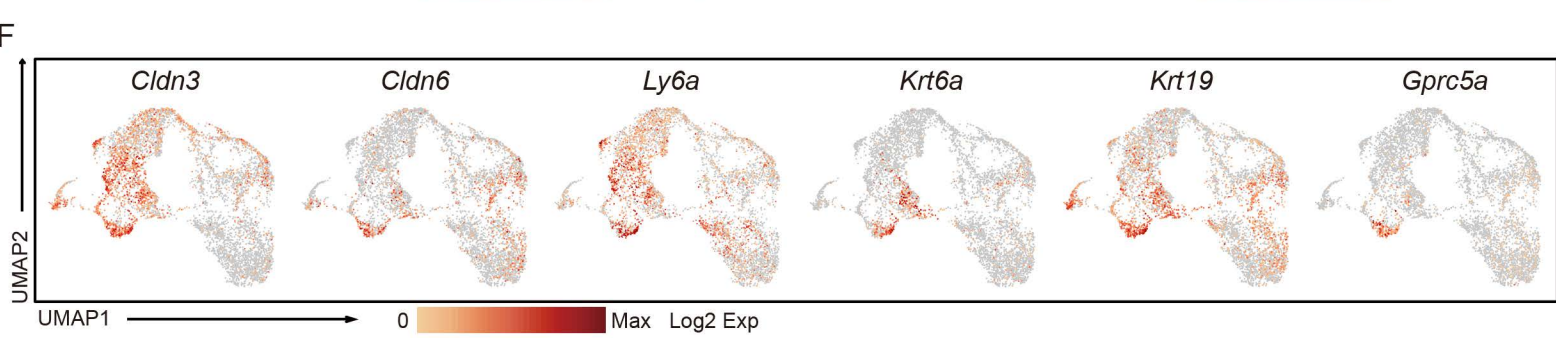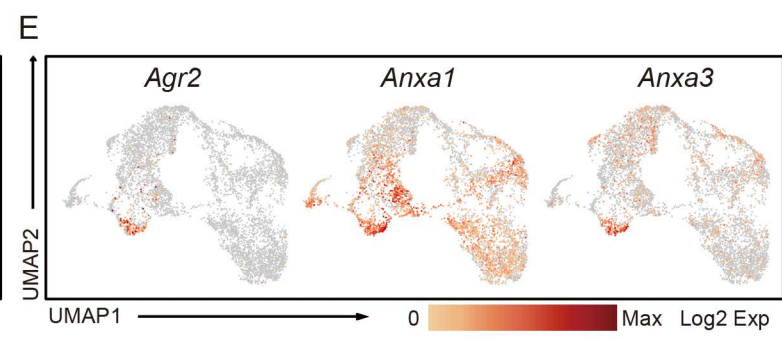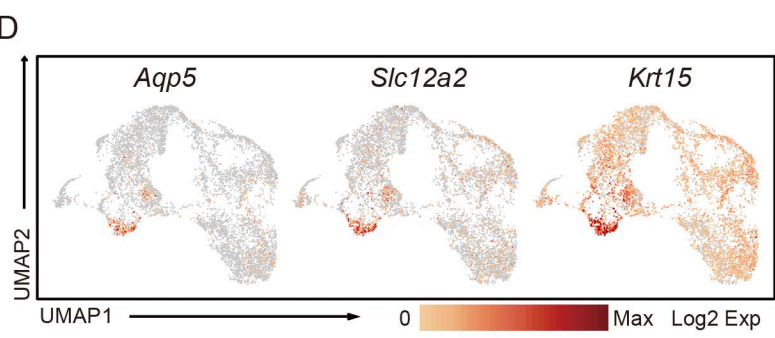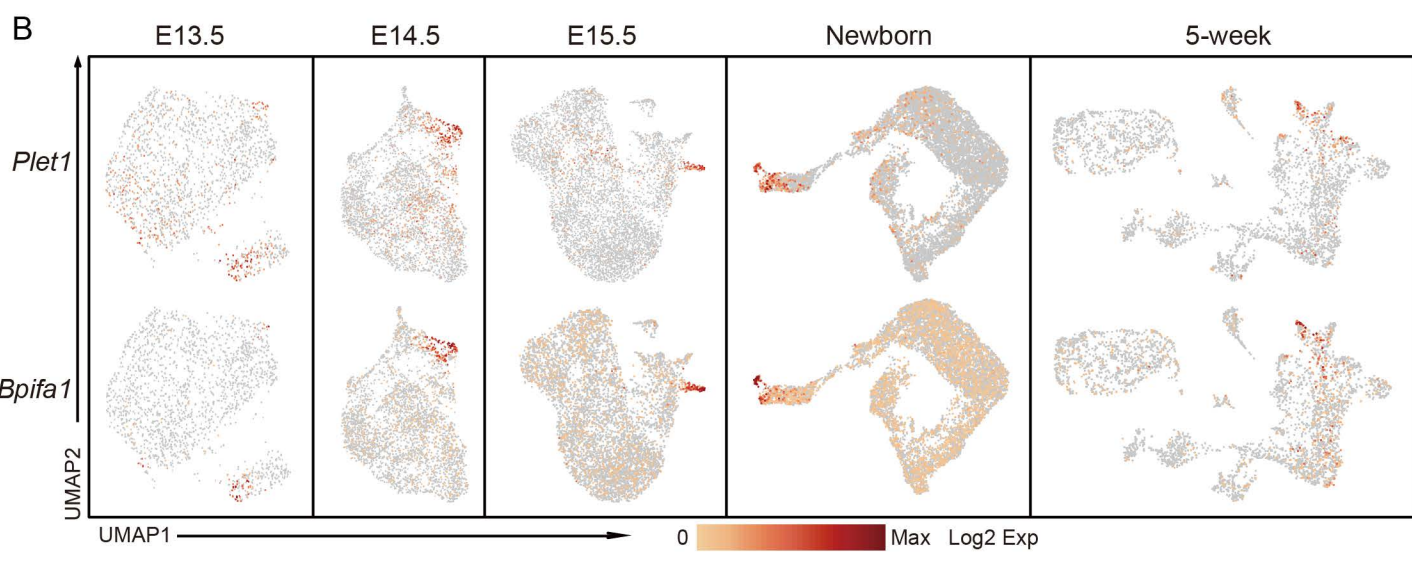
A

E11.5　E13.5　E14.5　E15.5　Newborn　5-week

Normalized expression (counts)

Plet1+ cells sellected (>2 counts in cell)

C

Spink5　Bpifa1　Plet1

Expression (counts)

E11.5

E13.5

E14.5

E15.5

Newborn

5-week

B

E13.5　E14.5　E15.5　Newborn　5-week

Plet1

Bpifa1

UMAP2

UMAP1

0　Max Log2 Exp

D

Aqp5　Slc12a2　Krt15

UMAP2

UMAP1

0　Max Log2 Exp

E

Agr2　Anxa1　Anxa3

UMAP2

UMAP1

0　Max Log2 Exp

F

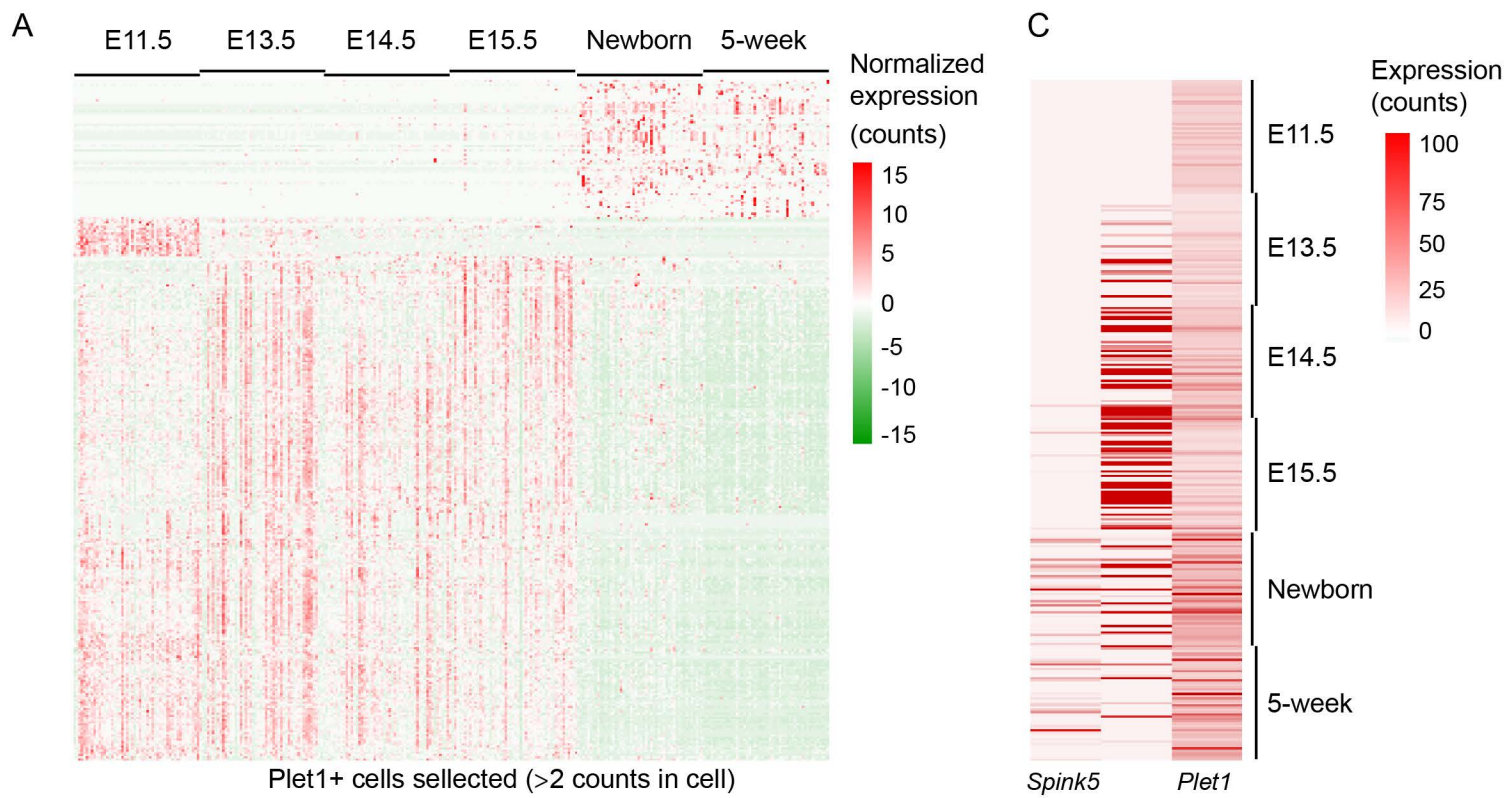Cldn3　Cldn6　Ly6a　Krt6a　Krt19　Gprc5a

UMAP2

UMAP1

0　Max Log2 Exp

**Figure. S5 Molecular characteristics of Plet1$^+$ cells during development.**

**(A)** Clustering analysis of differentially expressed genes in Plet1$^+$ cells from 6 developmental stages.

**(B)** UMAP visualization of the expression of *Bpifa1* and *Plet1* in indidcated developmental stages.

**(C)** Expression of selected genes in Plet1$^+$ cells.

**(D-F)** UMAP visualization of the expression of indicated genes that specific to Bpifa$^+$ Plet1$^+$ cells.
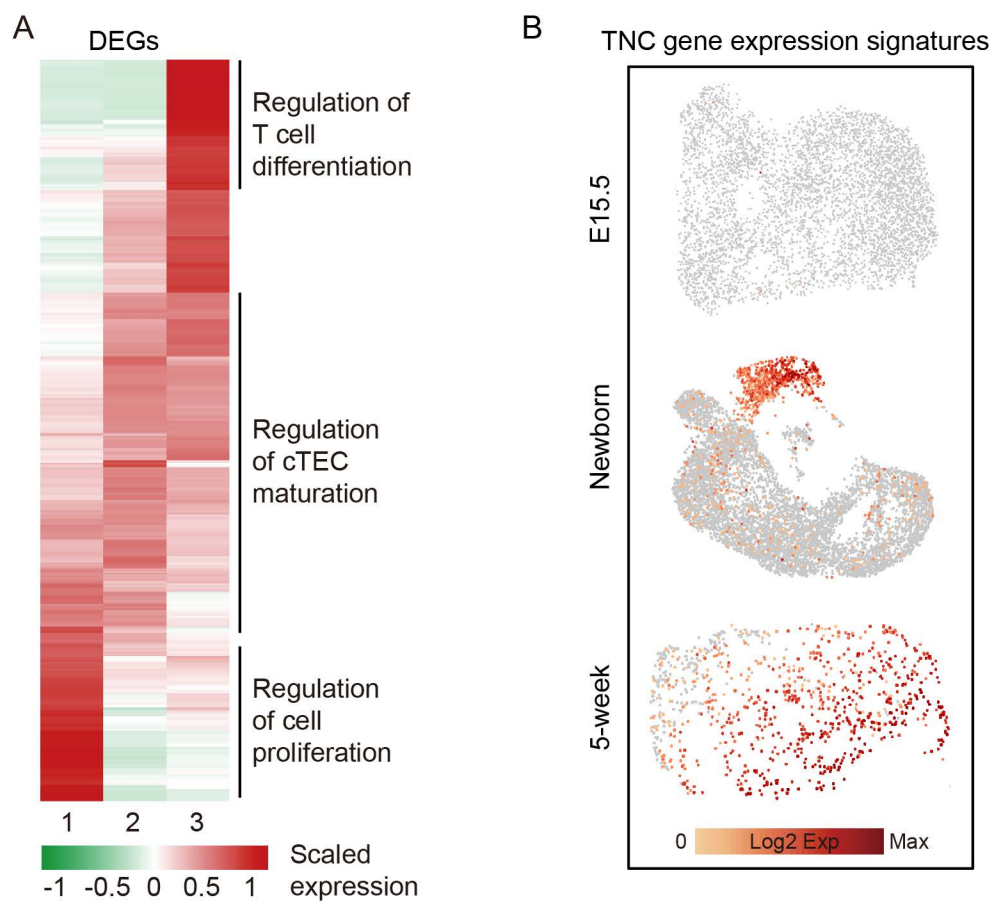
**Figure. S6 Gene expression profiling of 3 subclusters of cTECs.**

**(A)** Heat map showing differentially expressed genes in subtypes of cTECs. Genes were grouped by their functional categories and expression patterns. (DEGs, differentially expressed genes).

**(B)** UMAP visualization of the expression of the TNC feature genes in cTECs.
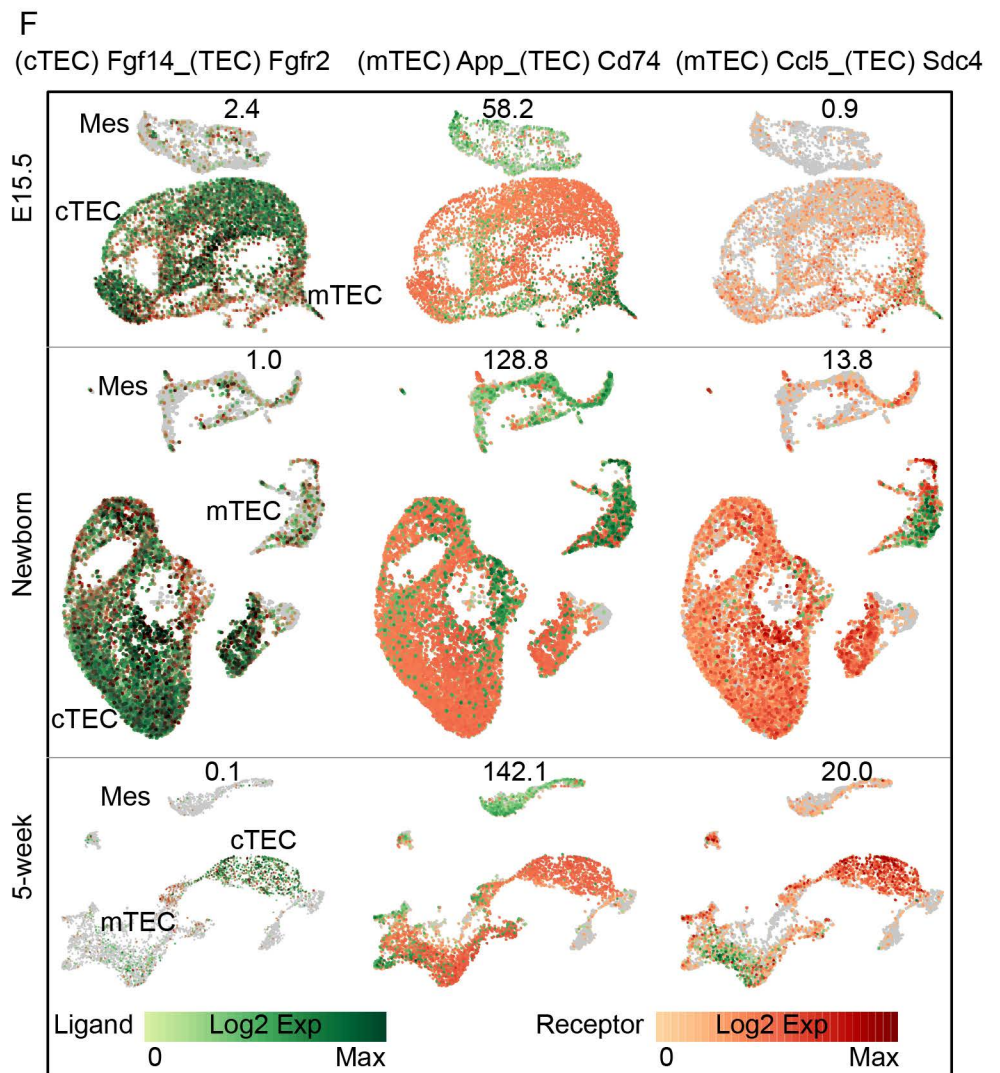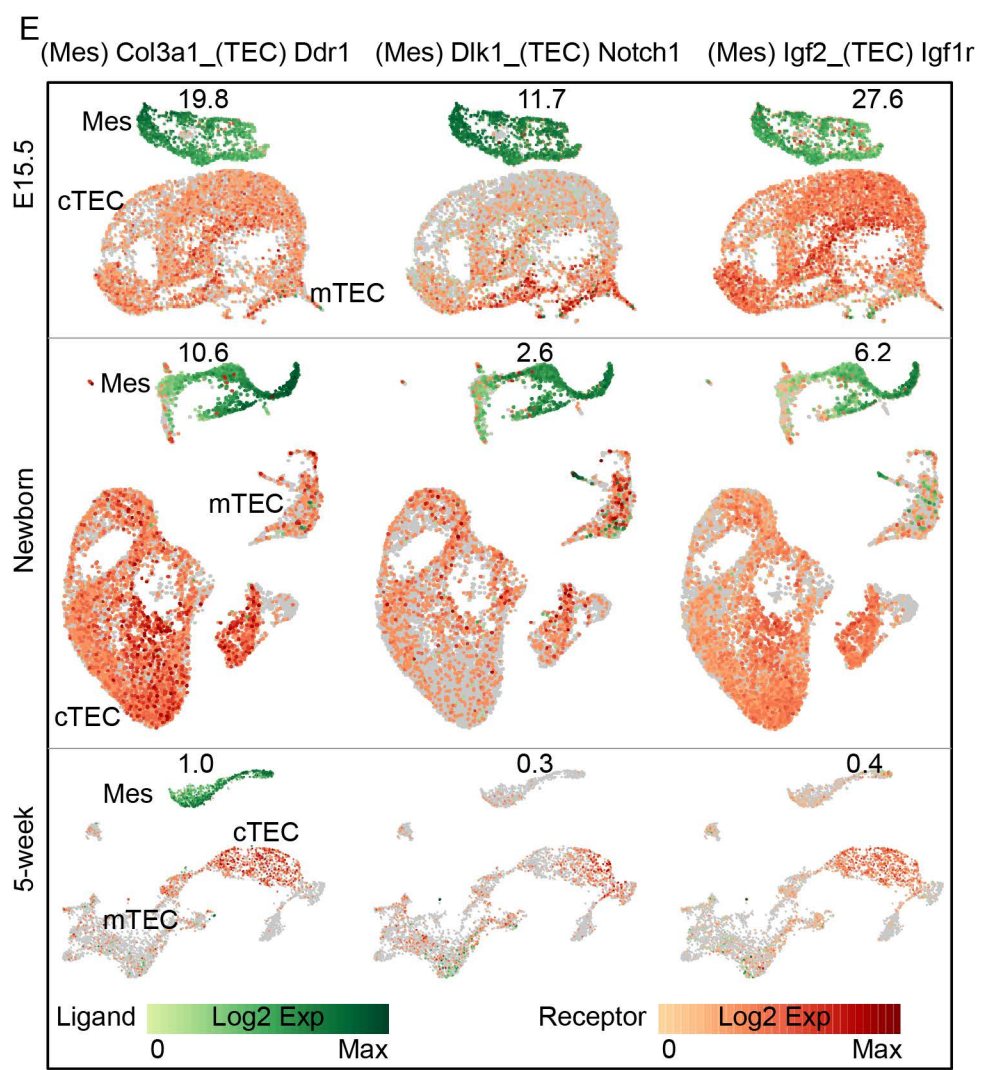
**A** Mesenchymal fibroblast cells

1
2
3

E11.5
E13.5
E14.5
E15.5
Newborn
5-week

UMAP2

UMAP1

**B**

*Top2a*

*Ccnb2*

UMAP2

UMAP1

**C**

*Gdf10*

*Igf1*

UMAP2

UMAP1

**D**

*Ndufa4l2*

*Cox4i2*

UMAP2

UMAP1

**E**

(Mes) Col3a1_(TEC) Ddr1    (Mes) Dlk1_(TEC) Notch1    (Mes) Igf2_(TEC) Igf1r

E15.5

Mes    19.8        11.7        27.6

cTEC

mTEC

Newborn

Mes    10.6        2.6        6.2

mTEC

cTEC

5-week

Mes    1.0        0.3        0.4

cTEC

mTEC

Ligand  Log2 Exp        Receptor  Log2 Exp
0        Max            0          Max

**F**

(cTEC) Fgf14_(TEC) Fgfr2    (mTEC) App_(TEC) Cd74    (mTEC) Ccl5_(TEC) Sdc4

E15.5

Mes    2.4        58.2        0.9

cTEC

mTEC

Newborn

Mes    1.0        128.8        13.8

mTEC

cTEC

5-week

Mes    0.1        142.1        20.0

cTEC

mTEC

Ligand  Log2 Exp        Receptor  Log2 Exp
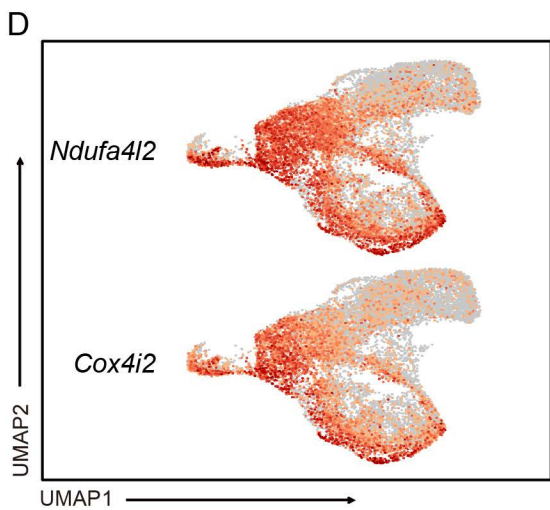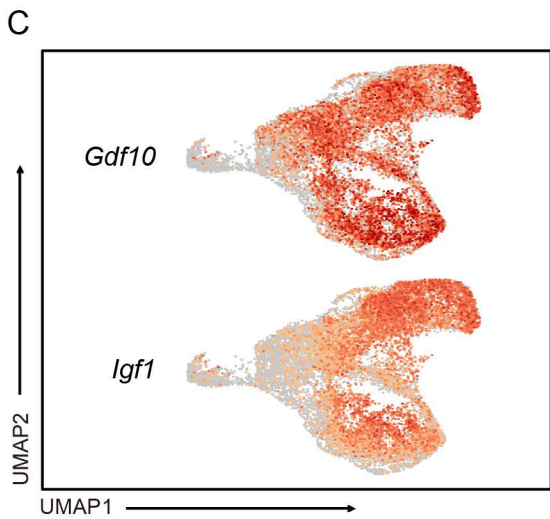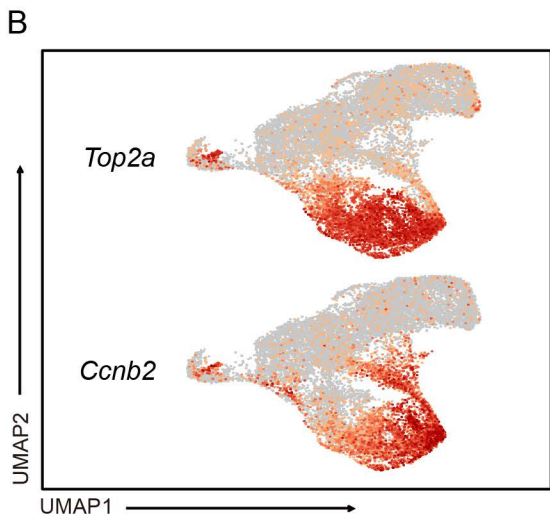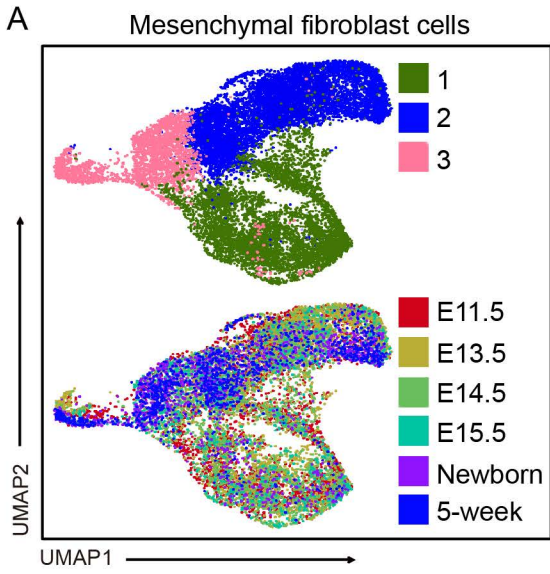0        Max            0          Max

**Figure. S7 Ligand-receptor interactions between mesenchymal fibroblast and TECs.**

**(A)** UMAP visualization of mesenchymal fibroblast cells, with each cell colorcoded for each subcluster (upper panel), and the sample origin (lower panel).

**(B-D)** UMAP visualization of the expression of **(B)** *Top2a*, *Ccnb2*, **(C)** *Gdf10*, *Igf1*, **(D)** *Ndufa4l2*, *Cox4i2*.

**(E)** UMAP visualization of the expression of ligand-receptor pairs (Col3a1_Ddr1, Dlk1_Notch1, Igf2_Igf1r), with each cell colorcoded for mesenchyme expressed ligands (green) and TEC expressed receptors (red).

**(F)** UMAP visualization of the expression of ligand-receptor pairs (Fgf14_Fgfr2, App_Cd74, Ccl5_Sdc4), with each cell colorcoded for TEC expressed ligands (green) and receptors (red). Numbers on the diagram were ligand-receptor interaction scores as indicated.