

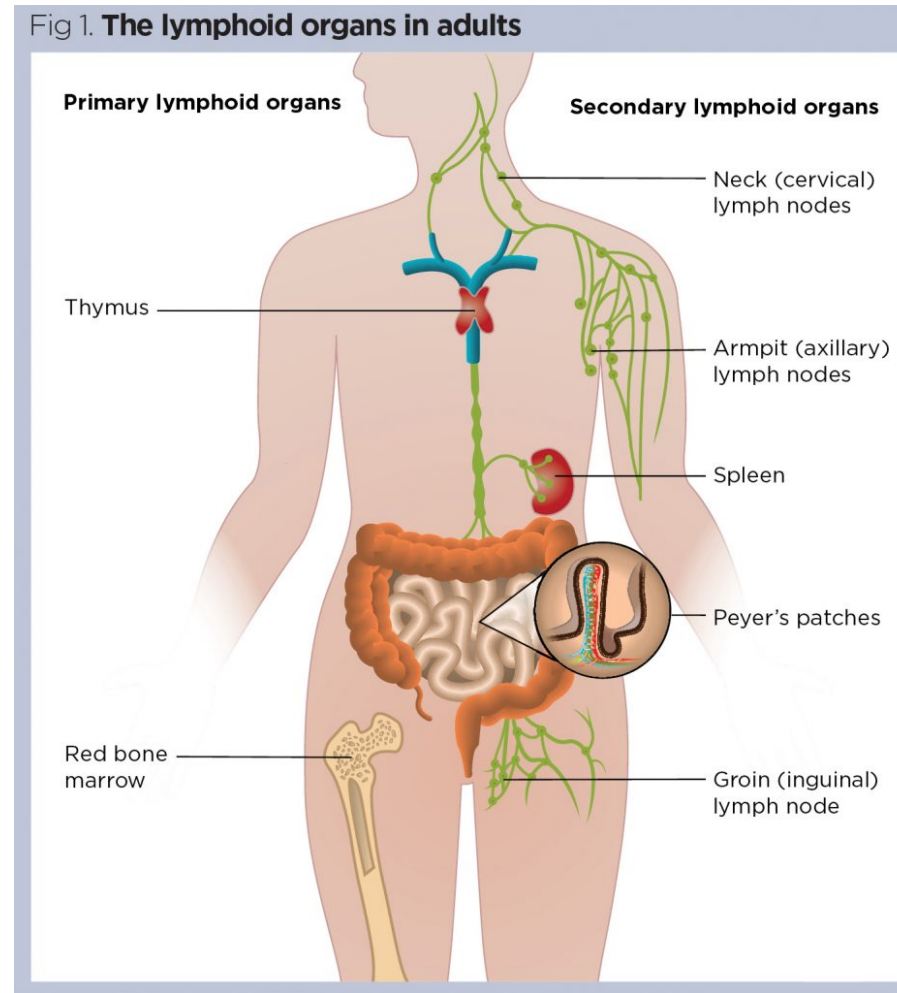
# Single-cell transcriptional profiling of human thymic stroma uncovers novel cellular heterogeneity in the thymic medulla

Bautista et al.

NATURE COMMUNICATIONS | Feb 17;12(1):1096

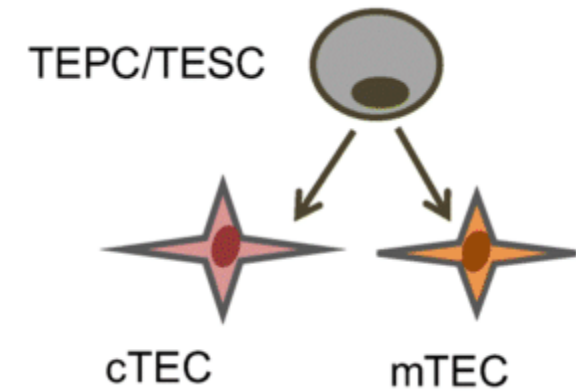
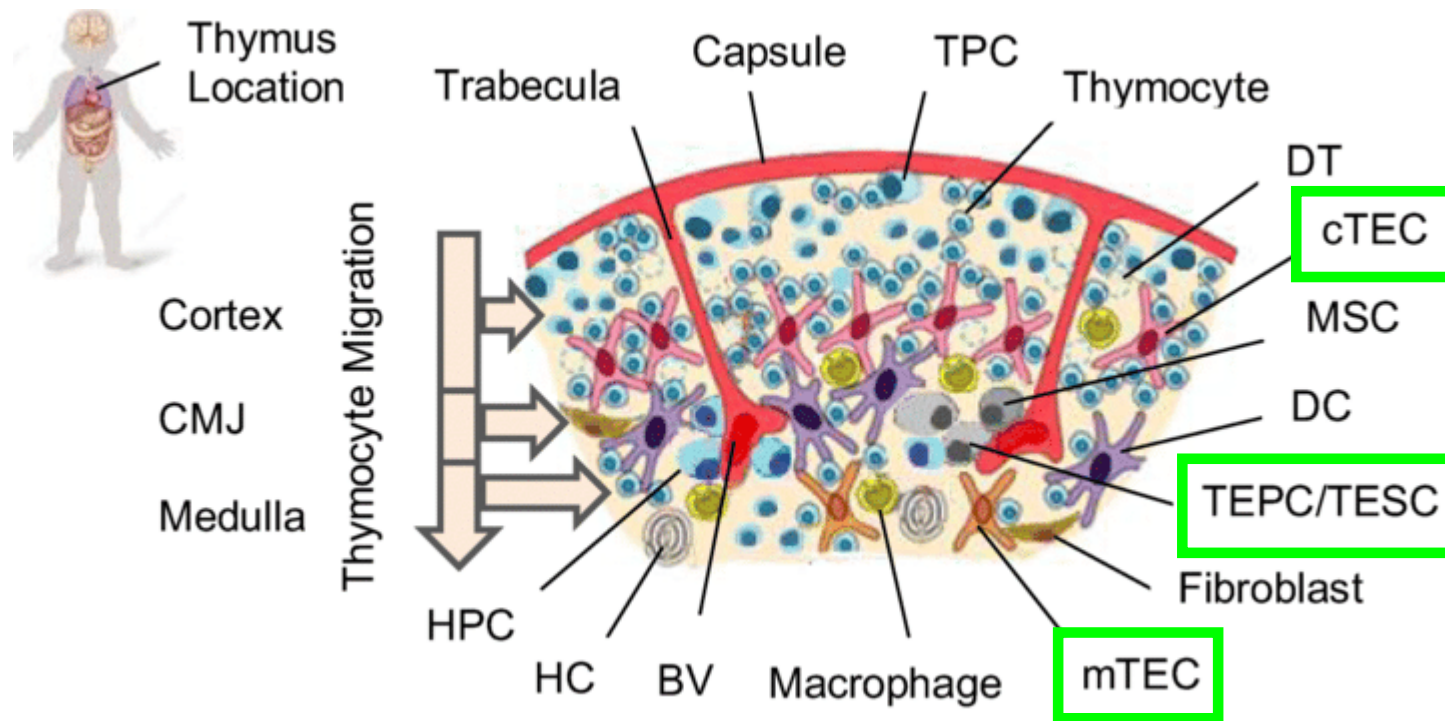
DOI: [10.1038/s41467-021-21346-6](https://doi.org/10.1038/s41467-021-21346-6)

# Background- primary lymphoid organs



<https://www.nursingtimes.net/clinical-archive/immunology/the-lymphatic-system-2-structure-and-function-of-the-lymphoid-organs-26-10-2020/>

# Background

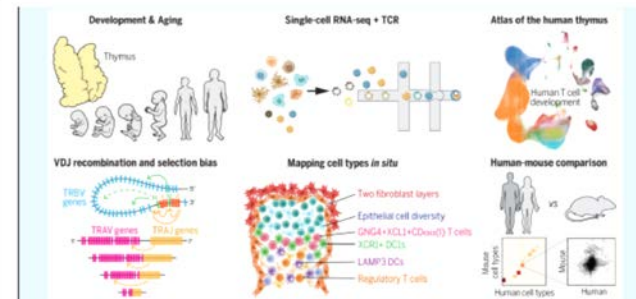


Shichkin, V.P., Antica, M. Thymus Regeneration and Future Challenges. *Stem Cell Rev and Rep* 16, 239–250 (2020). <https://doi.org/10.1007/s12015-020-09955-y>

# Background- Heterogeneity of the thymic medulla

## A cell atlas of human thymic development defines T cell repertoire formation

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### Constructing the human thymus cell atlas.

We analyzed human thymic cells across development and postnatal life using scRNA-seq and spatial methods to delineate the diversity of thymic-derived T cells and the localization of cells constituting the thymus microenvironment. With T cell development trajectory reconstituted at singlecell resolution combined with TCR sequence, we investigated the bias in the VDJ recombination and selection of human TCR repertoires. Finally, we provide a systematic comparison between human and mouse thymic cell atlases.

## LETTER

<https://doi.org/10.1038/s41586-018-0345-1>

## Single-cell mapping of the thymic stroma identifies IL-25-producing tuft epithelial cells

Chamutal Bornstein<sup>1,4</sup>, Shir Nevo<sup>1,4</sup>, Amir Giladi<sup>1,4</sup>, Noam Kadoury<sup>4</sup>, Marie Pourouf<sup>1,5</sup>, François Gerbe<sup>1</sup>, Eyal David<sup>1</sup>, Alice Machado<sup>1</sup>, Anna Chapurin<sup>1</sup>, Beata Toty<sup>1</sup>, Ori Goldberg<sup>1</sup>, Shalev Itzkovitz<sup>1</sup>, Naomi Taylor<sup>1</sup>, Philippe Jay<sup>1</sup>, Valérie S. Zimmermann<sup>1</sup>, Jakub Abramson<sup>1,2,3</sup> & Idit Amir<sup>1,2,3\*</sup>

T cell development and selection are coordinated in the thymus by a specialized niche of diverse stromal populations<sup>1–3</sup>. Although much progress has been made over the years in identifying the functions of the different cell types of the thymic stromal compartment, there is no comprehensive characterization of their diversity and heterogeneity. Here we combined massively parallel single-cell RNA-seq<sup>4,5</sup>, spatial mapping, chromatin profiling and gene targeting to characterize *de novo* the entire stromal compartment of the mouse thymus. We identified dozens of cell states, with thymic epithelial cells (TECs) showing the highest degree of heterogeneity. Our analysis highlights four major medullary TEC (mTEC, LVI) populations, with distinct molecular functions, epigenetic landscapes and lineage regulators. Specifically, mTEC IV constitutes a new and highly divergent TEC lineage with molecular characteristics of the gut chemosensory epithelial tuft cells. Mice deficient for this lineage show impaired T cell development and selection, and elevated levels of autoantibodies, demonstrating the critical role of this lineage in thymic selection and the development of a diverse T cell repertoire.

## NIH Public Access

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## Lineage tracing and cell ablation identifies a post-Aire expressing thymic epithelial cell population

### ARTICLE

<https://doi.org/10.1016/j.celrep.2013.08.038>

OPEN

## Diversity in medullary thymic epithelial cells controls the activity and availability of iNKT cells

Beth Lucas<sup>1</sup>, Andrea J. White<sup>1</sup>, Emilie J. Cosway<sup>1</sup>, Sonia M. Parnell<sup>1</sup>, Kieran D. James<sup>1</sup>, Nick D. Jones<sup>1</sup>, Izumi Ohigashi<sup>2</sup>, Yousuke Takahama<sup>2</sup>, William E. Jenkinson<sup>1</sup> & Graham Anderson<sup>1,3,4\*</sup>

The thymus supports multiple αβ T cell lineages that are functionally distinct, but mechanisms that control this multifaceted development are poorly understood. Here we examine medullary thymic epithelial cell (mTEC) heterogeneity and its influence on CD1d-restricted iNKT cells. We find three distinct mTEC<sup>hi</sup> subsets distinguished by surface, intracellular and secreted molecules, and identify LTβR as a cell-autonomous controller of their development. Importantly, this mTEC heterogeneity enables the thymus to differentially control iNKT sublineages possessing distinct effector properties. mTEC expression of LTβR is essential for the development of thymic tuft cells which regulate NKT2 via IL-25, while LTβR controls CD104<sup>hi</sup> CCL21<sup>hi</sup> mTEC<sup>hi</sup> that are capable of IL-15 transpresentation for regulating NKT1 and NKT17. Finally, mTECs regulate both iNKT-mediated activation of thymic dendritic cells, and iNKT availability in extrathymic sites. In conclusion, mTEC specialization controls intrathymic iNKT cell development and function, and determines iNKT pool size in peripheral tissues.



## HHS Public Access

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Nature. Author manuscript; available in PMC 2019 January 18.

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Nature 2018 July; 559(7715): 627–631. doi:10.1038/s41586-018-0345-2.

## Thymic tuft cells promote an IL4-enriched medulla and shape thymocyte development

Sten L. Wells<sup>5</sup>, Aparna R. Ghosh<sup>1,2,1</sup>, Todd C. Metzger<sup>1,2,1</sup>, Wighton<sup>3</sup>, Audrey V. Parent<sup>1,2</sup>, Steinmetz<sup>5</sup>, Richard M.

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for the purposes of academic research, please refer to the original source.

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reviewed and designed experiments with informed bulk RNA-seq, provided Flow25 performed single cell RNA-seq and analyzed

role in enforcing central tolerance (TSAs) and deletion of regulator (Aire), a transcriptional D80 and MHC II expression and an *Aire-DTR* transgenic line, we which quickly undergo RANK-  
Ck, and using an inducible *Aire-Cre* subset of mTECs that showed n, and preferentially migrates a distinct stage of mTEC a key role in maintaining tolerance.

in preventing T cell reactivity to self and  
2) Medullary thymic epithelial cells pe for guiding central tolerance, which of tissue-specific self-antigens (TSAs)  
). TSA expression depends in part on identified as the defective gene in the autoimmune Polyglandular Syndrome Type  
While the molecular mechanisms by et fully elucidated, many individual central tolerance (DeVoss et al., 2006, ed that transgenic Aire-dependent ly through negative selection (Liston et and that endogenous Aire-dependent of autoreactive epitope-specific T cell (Ohigashi et al., 2012). More recently, have also been found to serve as

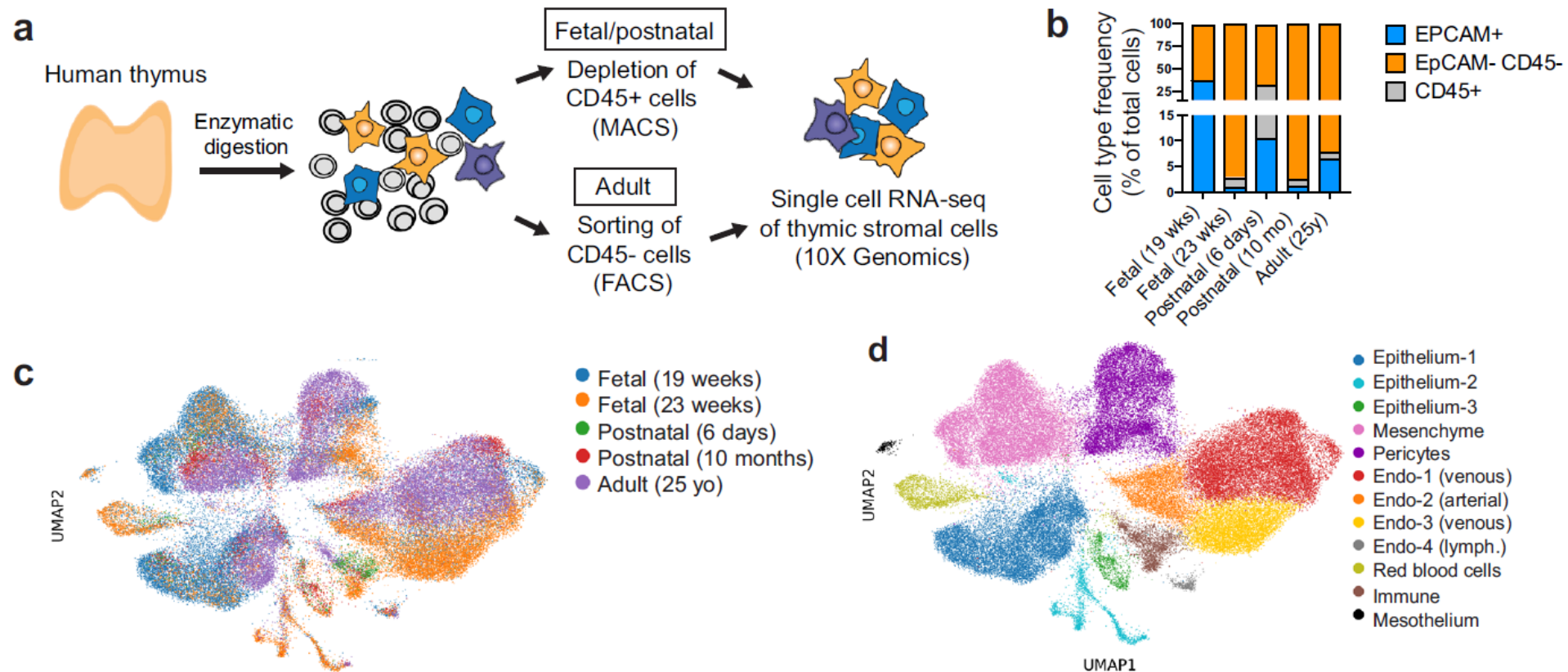
# Methods

- Human thymic tissue:
  - 19-23 gestational-week (legal, elective termination of pregnancy)
  - pediatric tissue (corrective cardiothoracic surgery)
  - Adult thymic tissue: organ acquisition for clinical transplantation
- Mice: Rosa26CAG-stopflox-tdTomato and Ascl1-creERT2, ADIG mice
- Tissue preparation: DNase, Liberase, gentle MACs (spleen program), ca. 1,5 h digestion,
- Stromal cell enrichment: FACS or magentic column (CD45, EPCAM/CD45)
- Single cell: 10x 3' v2 or 3, Novaseq 600, Cellranger v 2.0.0, 2.1.1, 3.0.2
  - Data analysis: Scanpy (v1.4.4, 1.6.0), quality: 200-500 features, <10% MT + features  $\leq 3$  cells
  - Total cells: 68008
  - Clustering: Leiden algorithm, res:main 0.7, sub 0.5
  - Park et al.: res: main 0.51, sub 0.25
  - Trajectory: RNA Velocity
- IF-stainings
- Immunohistochemistry
- Flow cytometry



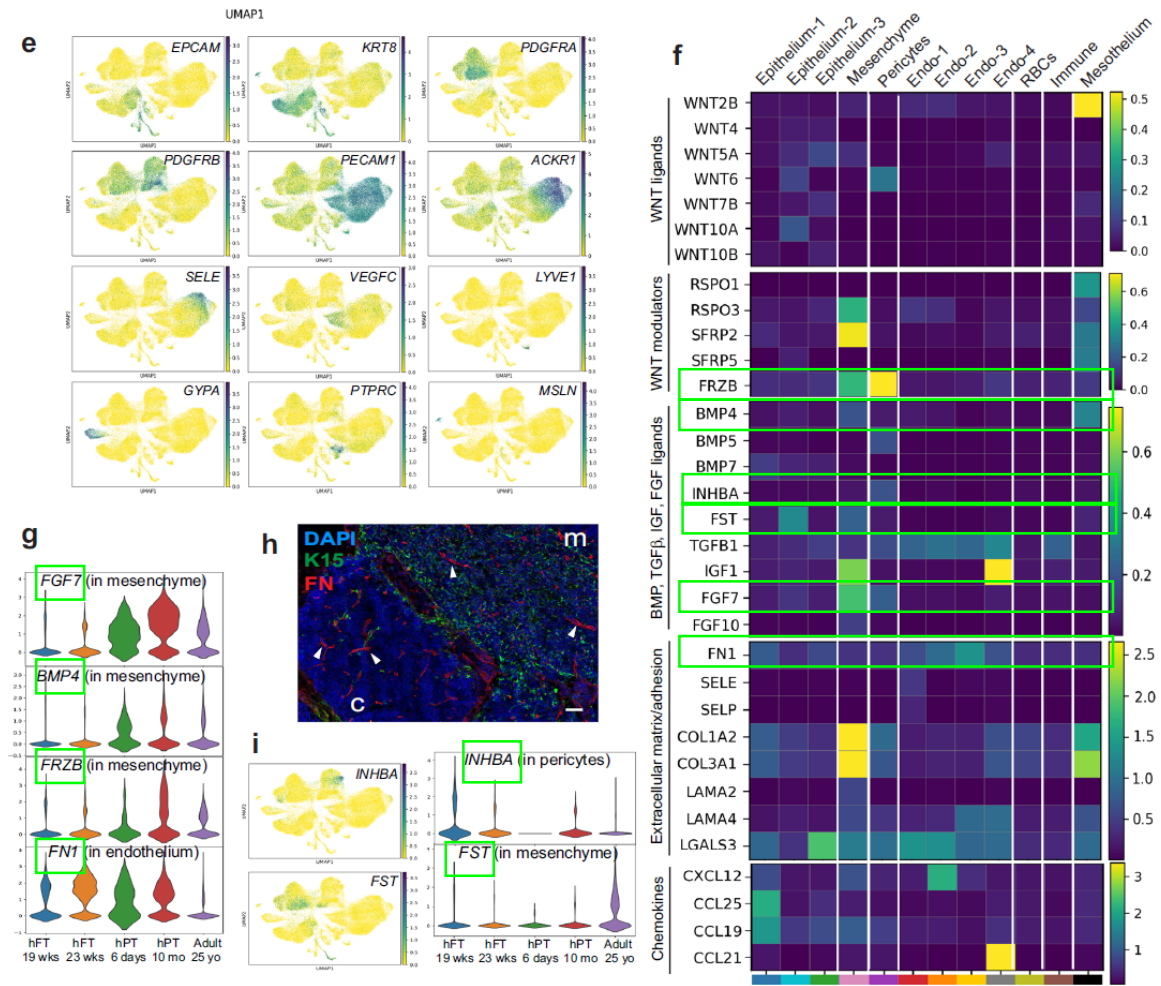
# Results

## Single-cell profiling of stromal cells from human thymus



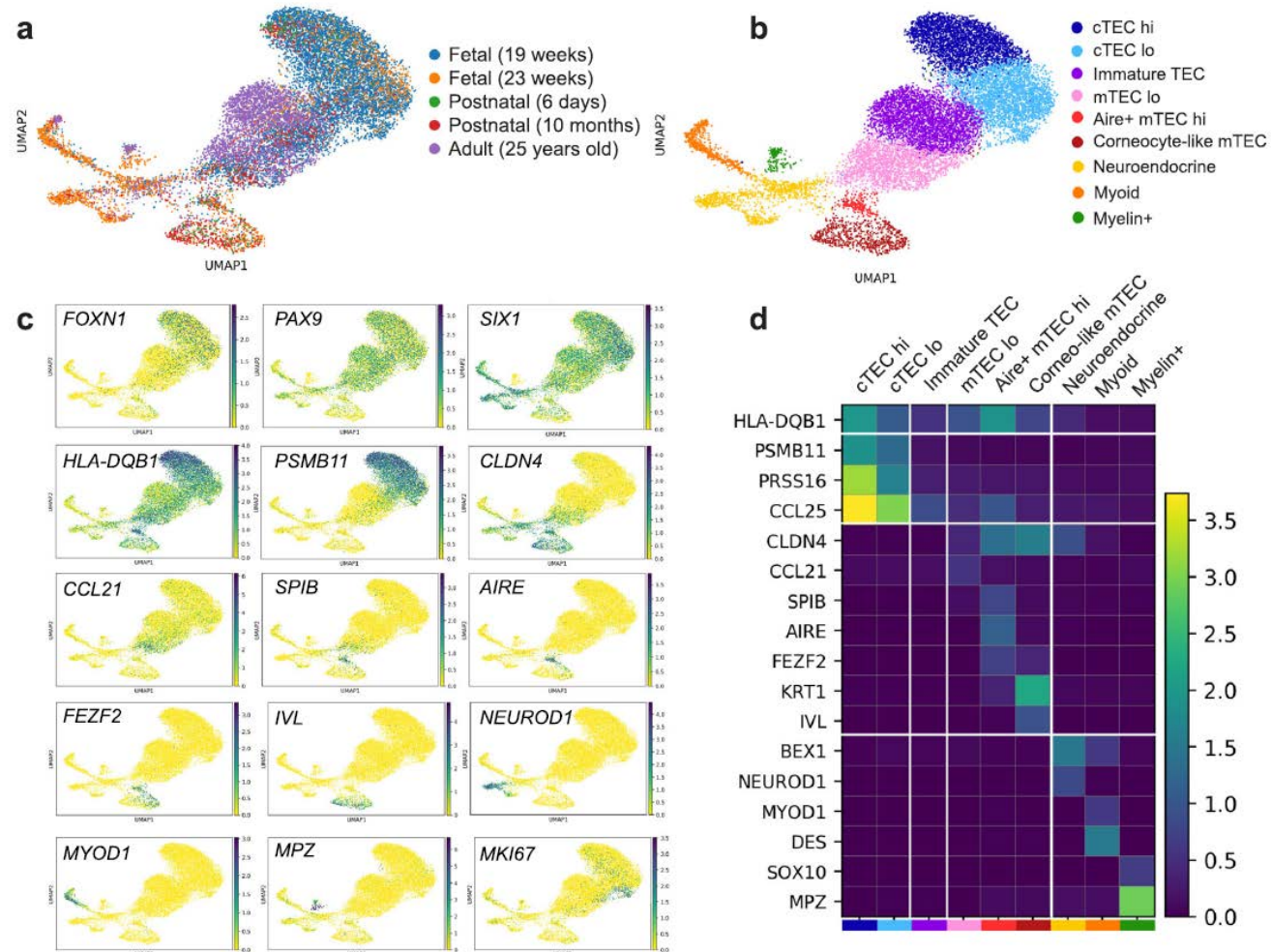
# Results

## Single-cell profiling of stromal cells from human thymus



# Results

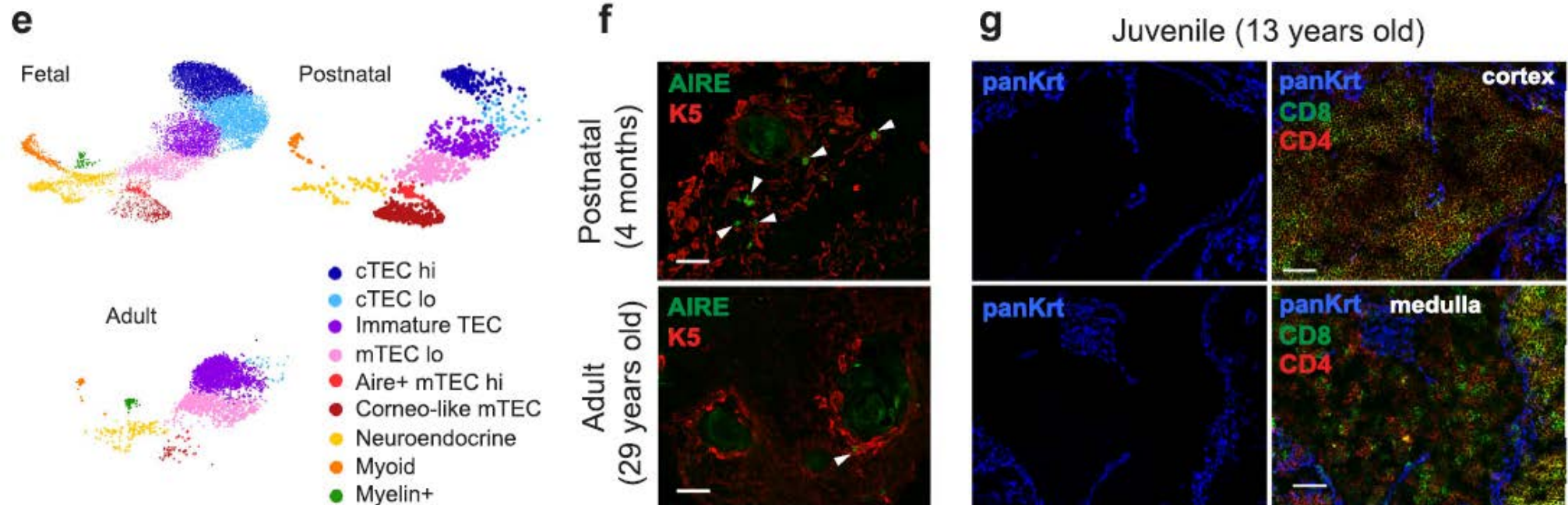
## Profiling of human thymic epithelial cells at different stages





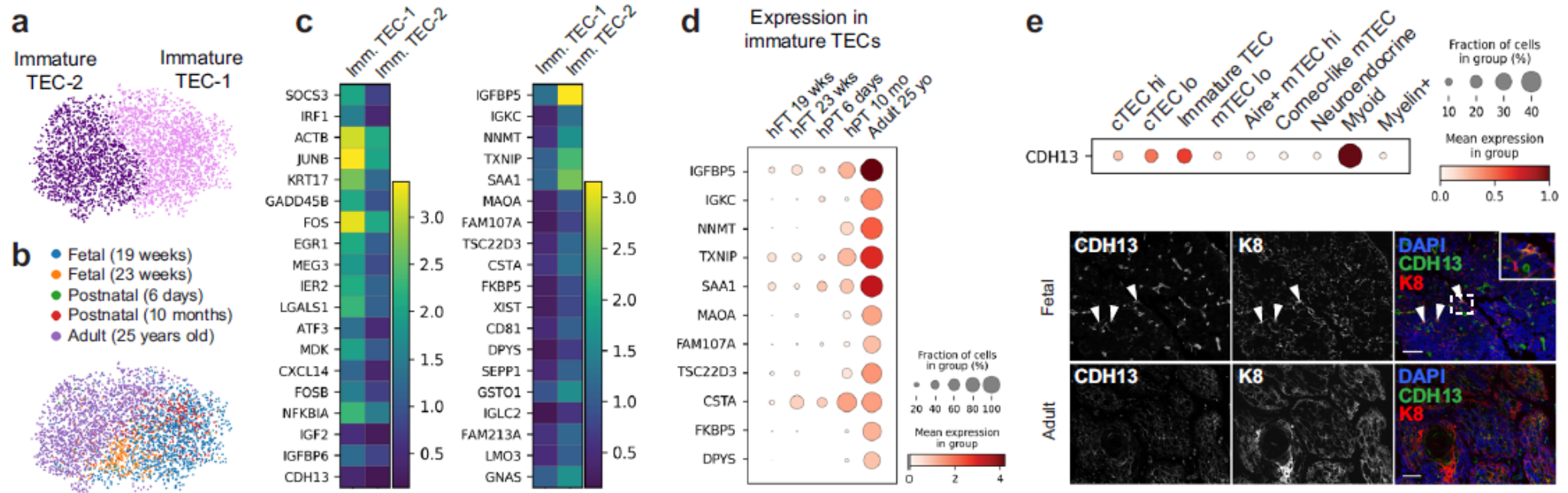
# Results

## Profiling of human thymic epithelial cells at different stages



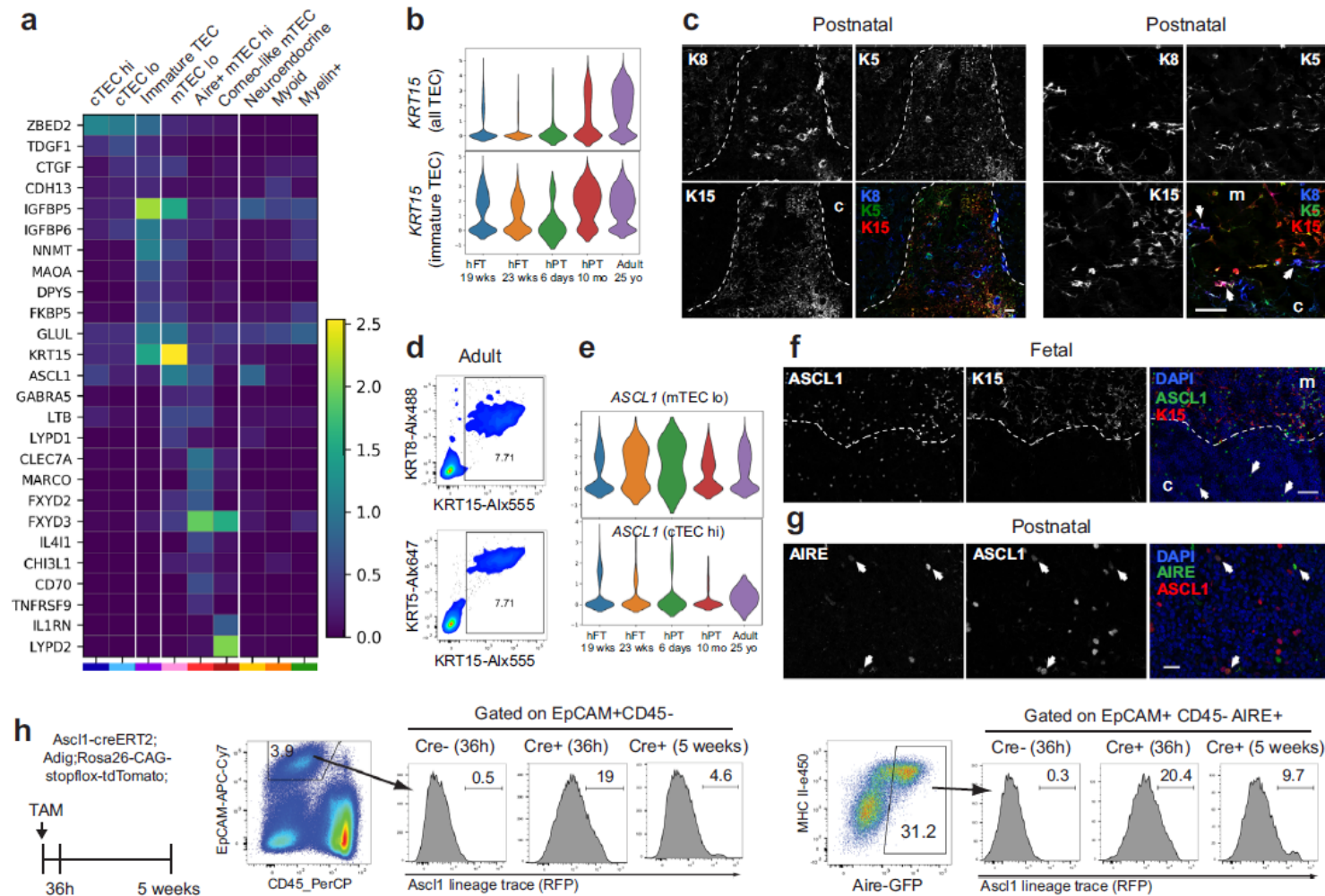
# Results

## Analysis of immature TECs



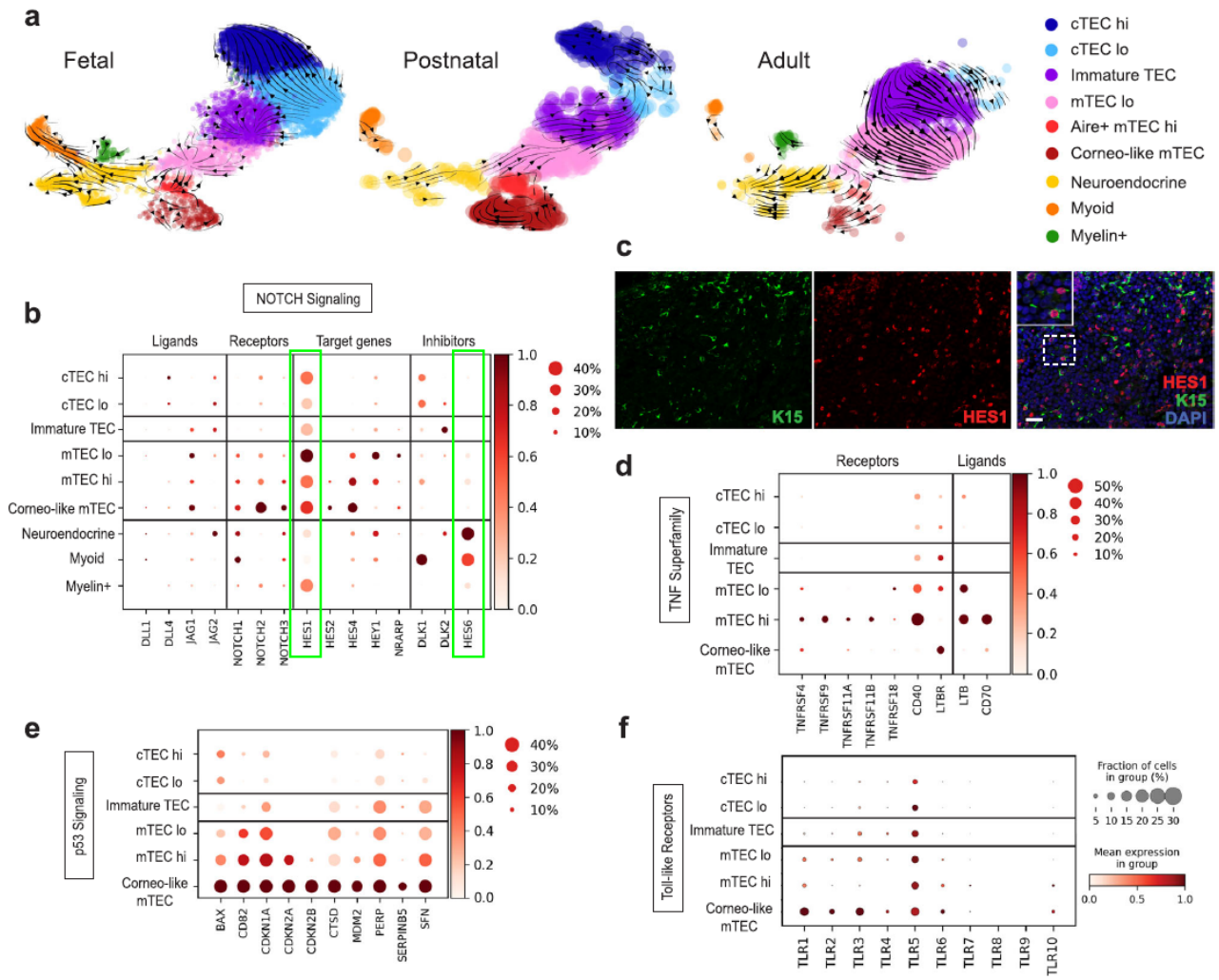
# Results

## Identification of new TEC markers



# Results

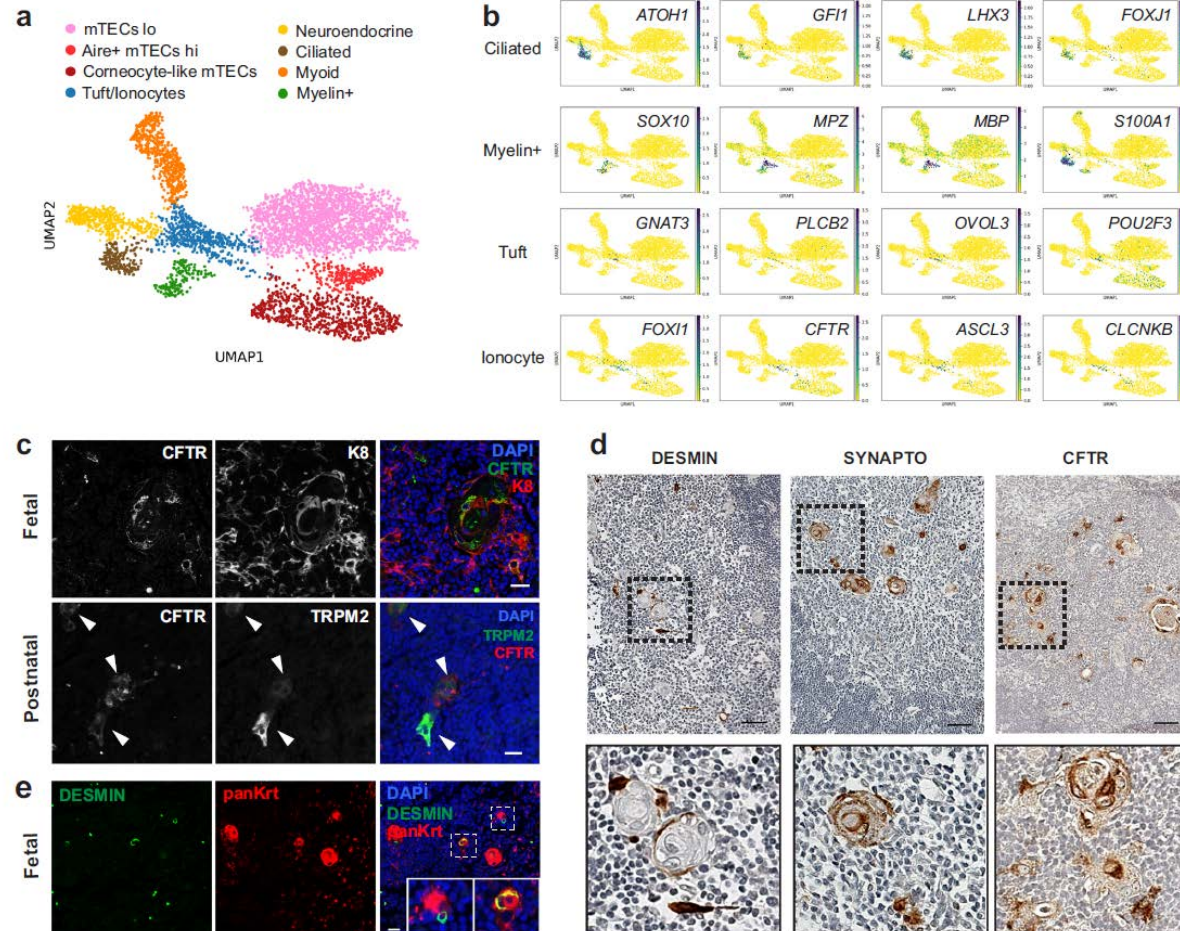
## Lineage decisions within the thymic epithelial compartment





# Results

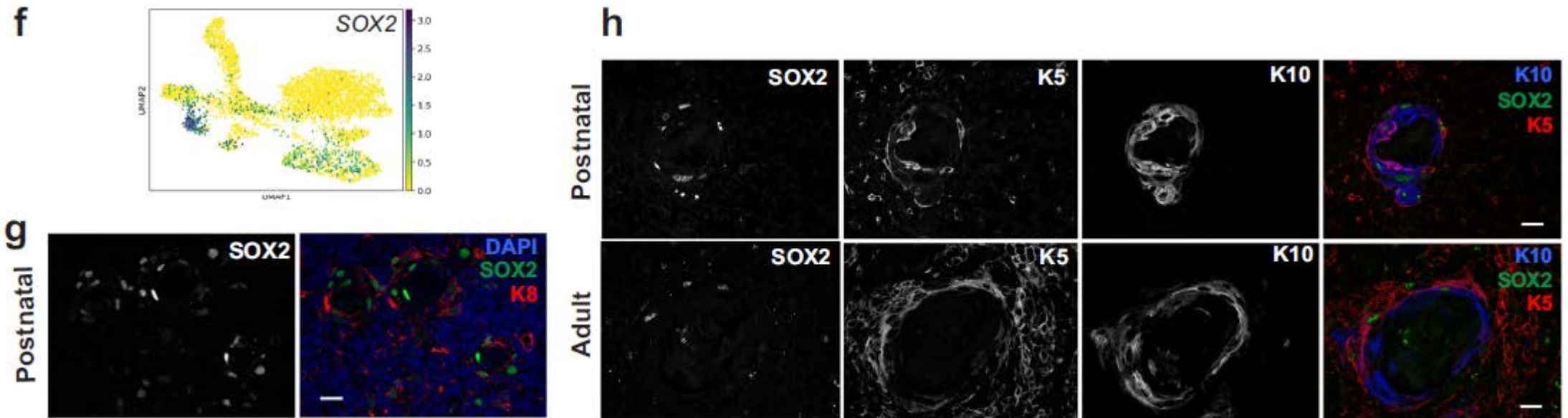
## Tuft cells, ionocytes, ciliated cells, and myelin-expressing cells are present in the human thymic medulla





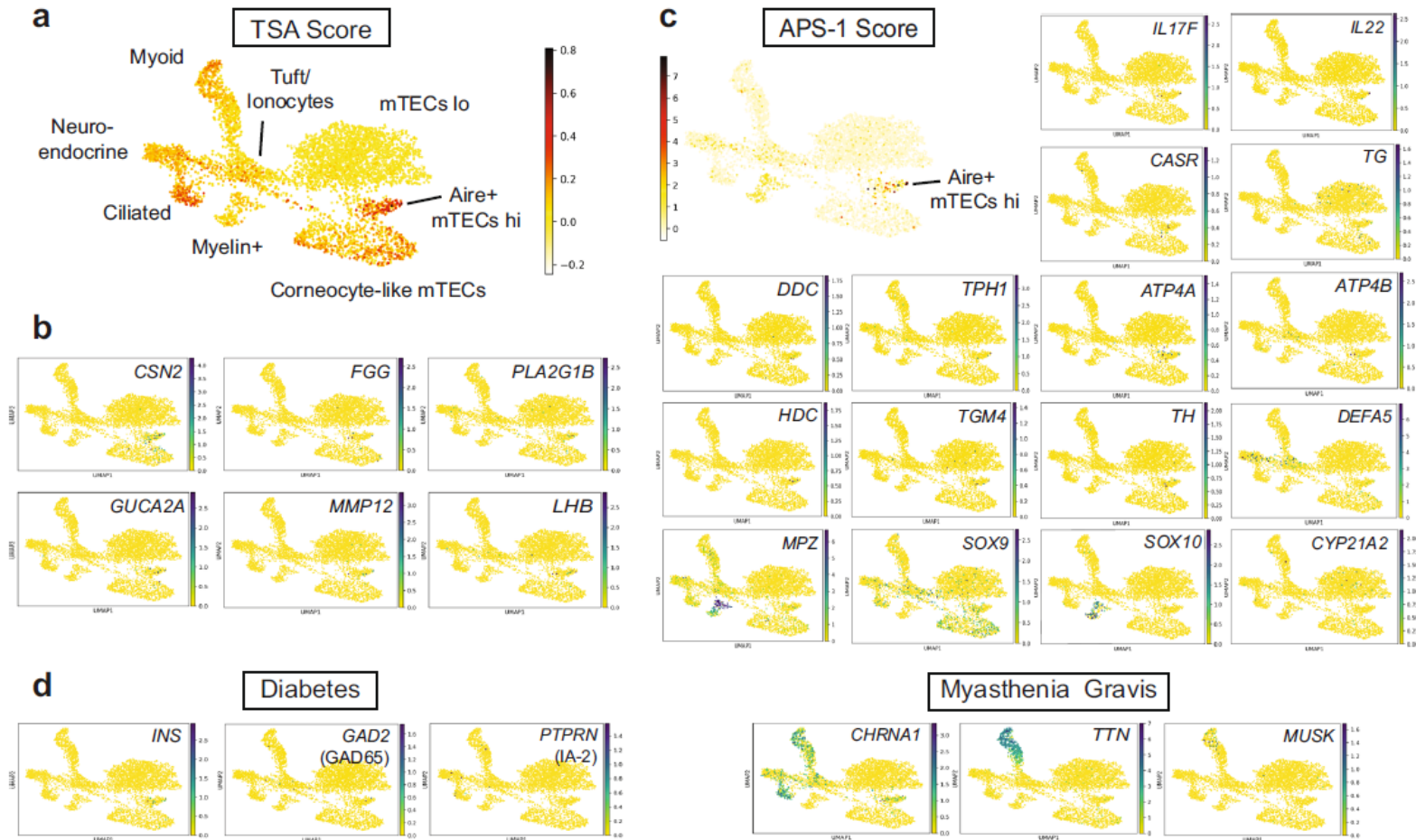
# Results

Tuft cells, ionocytes, ciliated cells, and myelin-expressing cells are present in the human thymic medulla



# Results

## Characterization of tissue-specific antigen expression by human TECs





# Discussion

- Ionocytes, Ciliated cells, Schwann cells,
- Crosstalk between TECs and stroma: WNT-pathway (FOXP1), activin A...
- similar progenitors of ionocytes, neuroendocrine and tuft cells
- Notch signaling -> TEC specification (HES6 inhibition of HES1)
- APS-1 antigens are AIRE-dependent
- Myoid cells as the main source of muscle antigens in the human thymic medulla

# Conclusion

- Reference transcriptomic maps for TEC
- Evidence of greater heterogeneity among medullary TECs
- Platform to study the expression of disease-relevant antigens
- Insight on the relevance of the heterogeneity to induce immune tolerance and human autoimmune disease