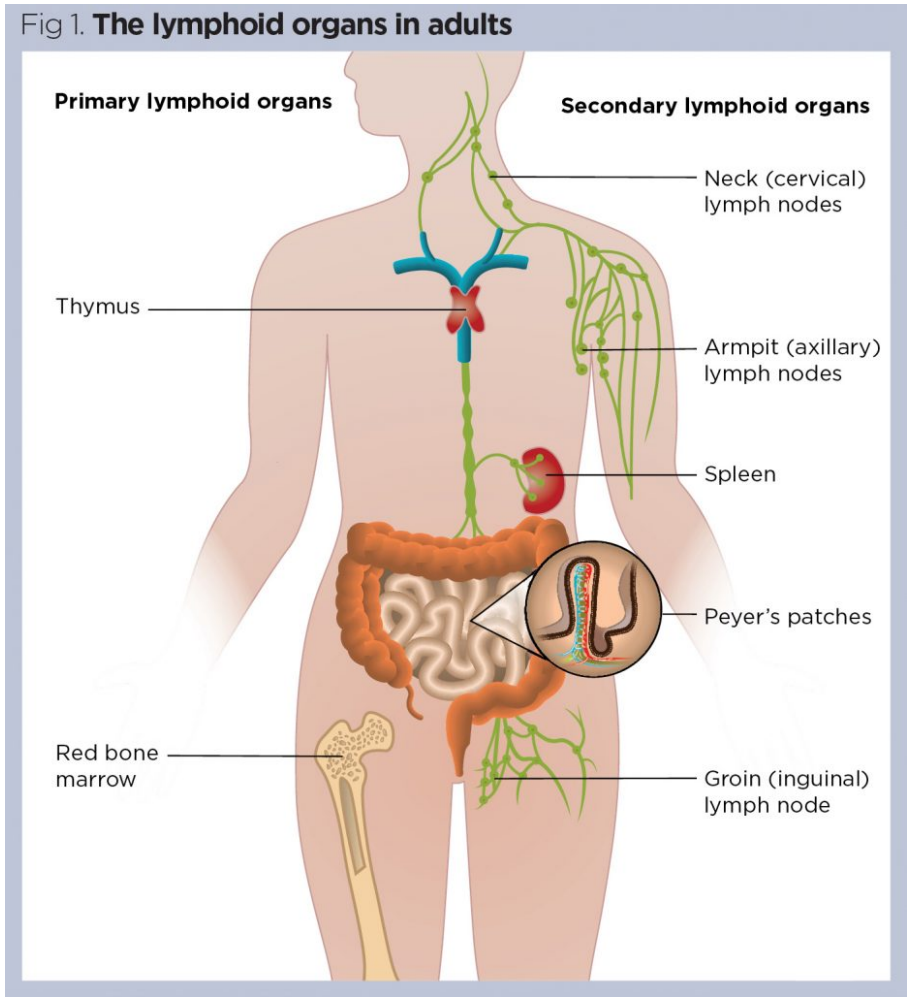
Single-cell transcriptional profiling of human thymic stroma uncovers novel cellular heterogeneity in the thymic medulla

Bautista et al.

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

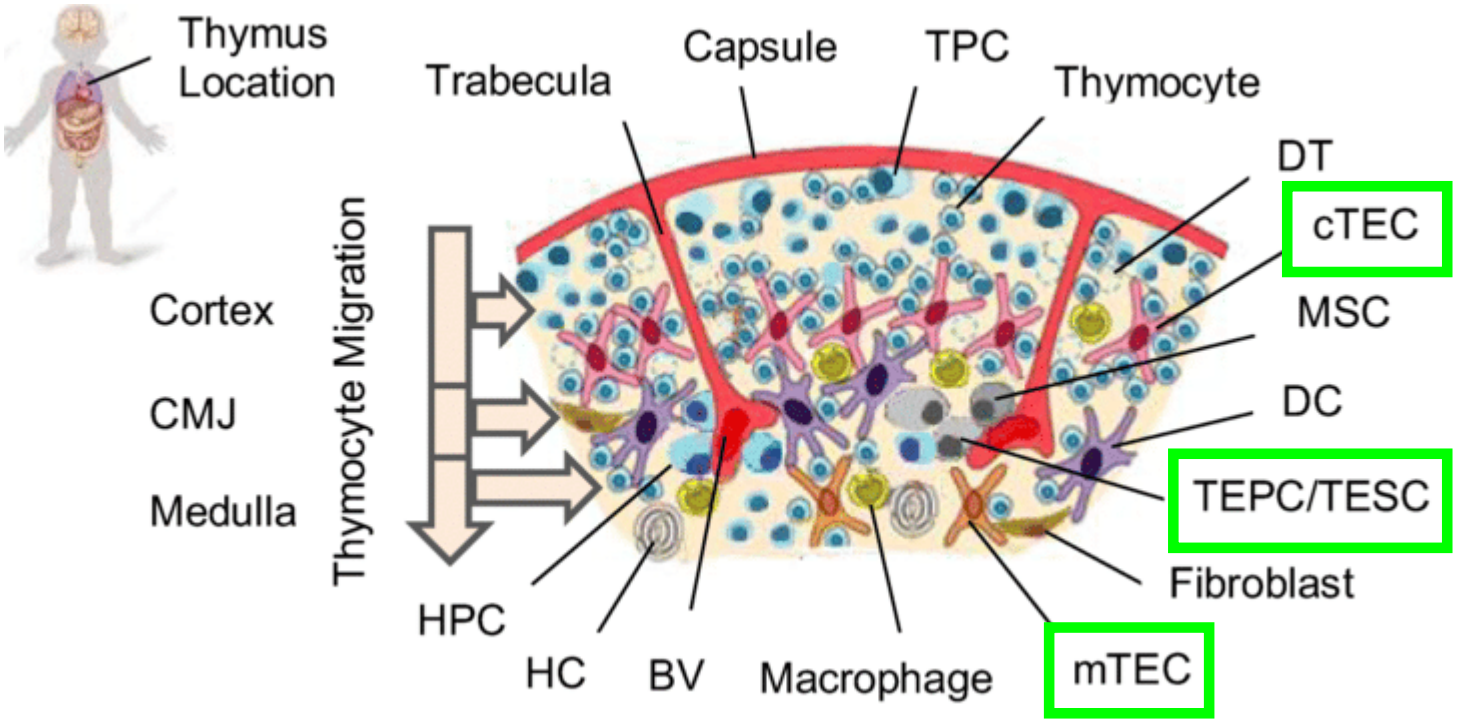
DOI: 10.1038/s41467-021-21346-6

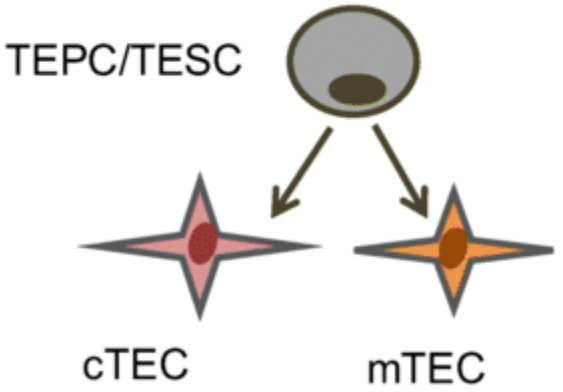
Background- primary lymphoid organs



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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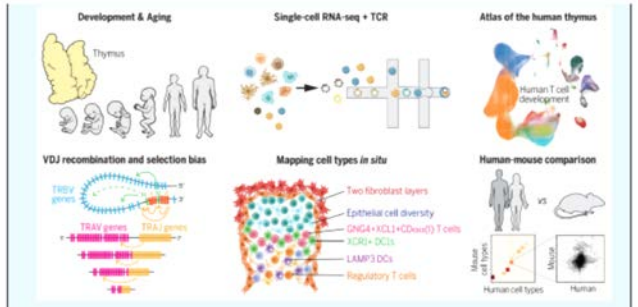


Constructing the human thymus cell atlas.
We analyzed human thymic cells across development and postnatal life using scRNA-seq and spatial methods to delineate the diversity of thymic-derived T cells and the localization of cells constituting the thymus microenvironment. With T cell development trajectory 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

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

Chamutal Bornstein[1,6], Shir Nevo[1,6], Amir Giladi[1,6], Noam Kadouri[1,6], Marie Pouzolles[2], François Gerbe[2], Eyal David[1], Alice Machado[3], Anna Chuprin[1], Beata Toth[4], Orit Goldberg[5], Shalev Itzkovitz[4], Naomi Taylor[2], Phillippe Jay[2], Valérie S. Zimmermann[2], Jakub Abramson[1,7]* & Ido Amit[1,7]*

T cell development and selection are coordinated in the thymus by a specialized niche of diverse stromal populations[1–3]. 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-sequencing[4,5], 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 I–IV) 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

Published in final edited form as:
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

Published in final edited form as:
Cell Rep. 2013 October 17; 5(1): . doi:10.1016/j.celrep.2013.08.038.

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

ARTICLE

OPEN

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

Beth Lucas[1], Andrea J. White[1], Emilie J. Cosway[1], Sonia M. Parnell[1], Kieran D. James[1], Nick D. Jones[1], Izumi Ohigashi[2], Yousuke Takahama[3], William E. Jenkinson[1] & Graham Anderson[1,4]

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^{low} 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 thymic tuft cells which regulate NKT2 via IL-25, while LTβR controls CD104$^+$ CCL21$^+$ mTEC^{low} 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.

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

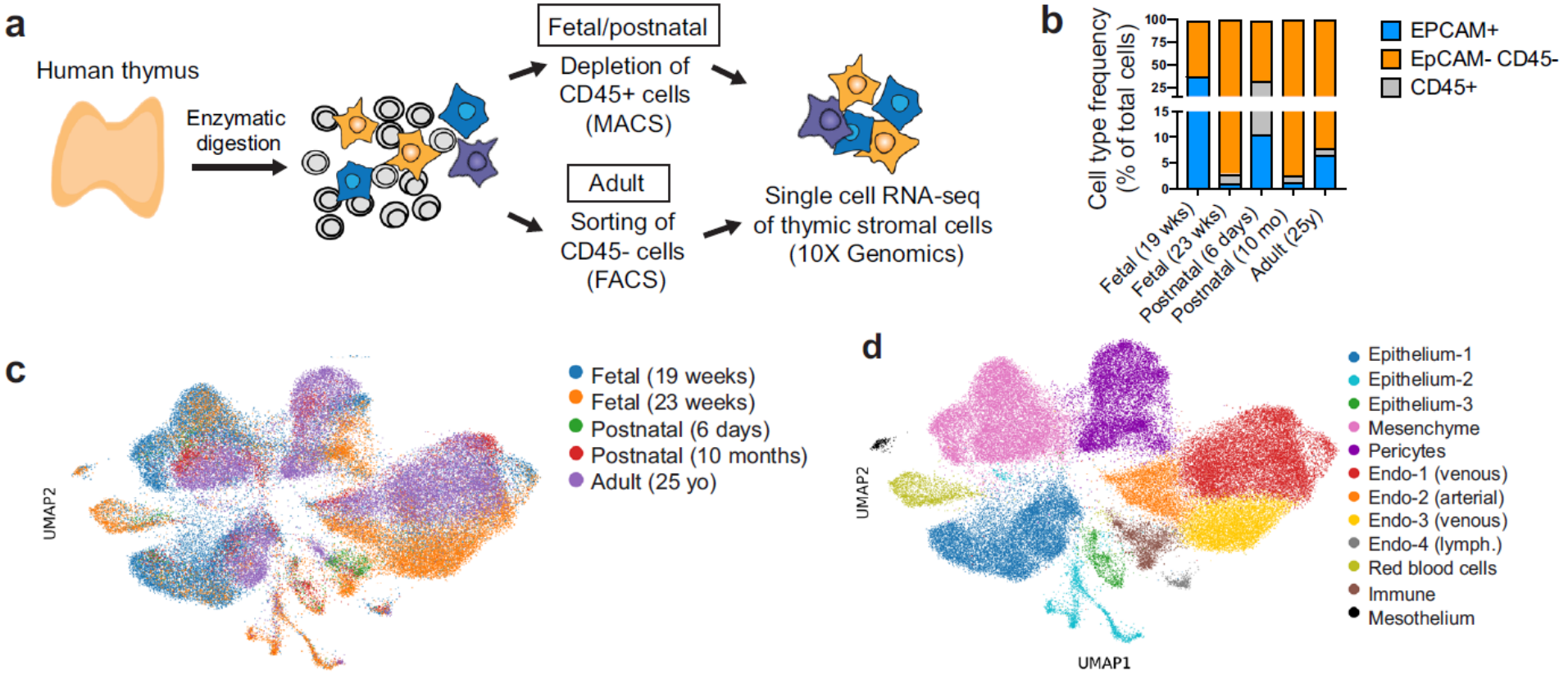
- Mice: Rosa26CAG-stopflox-tdTomato and Ascl1-creERT2, ADIG mice

- 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 ≤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



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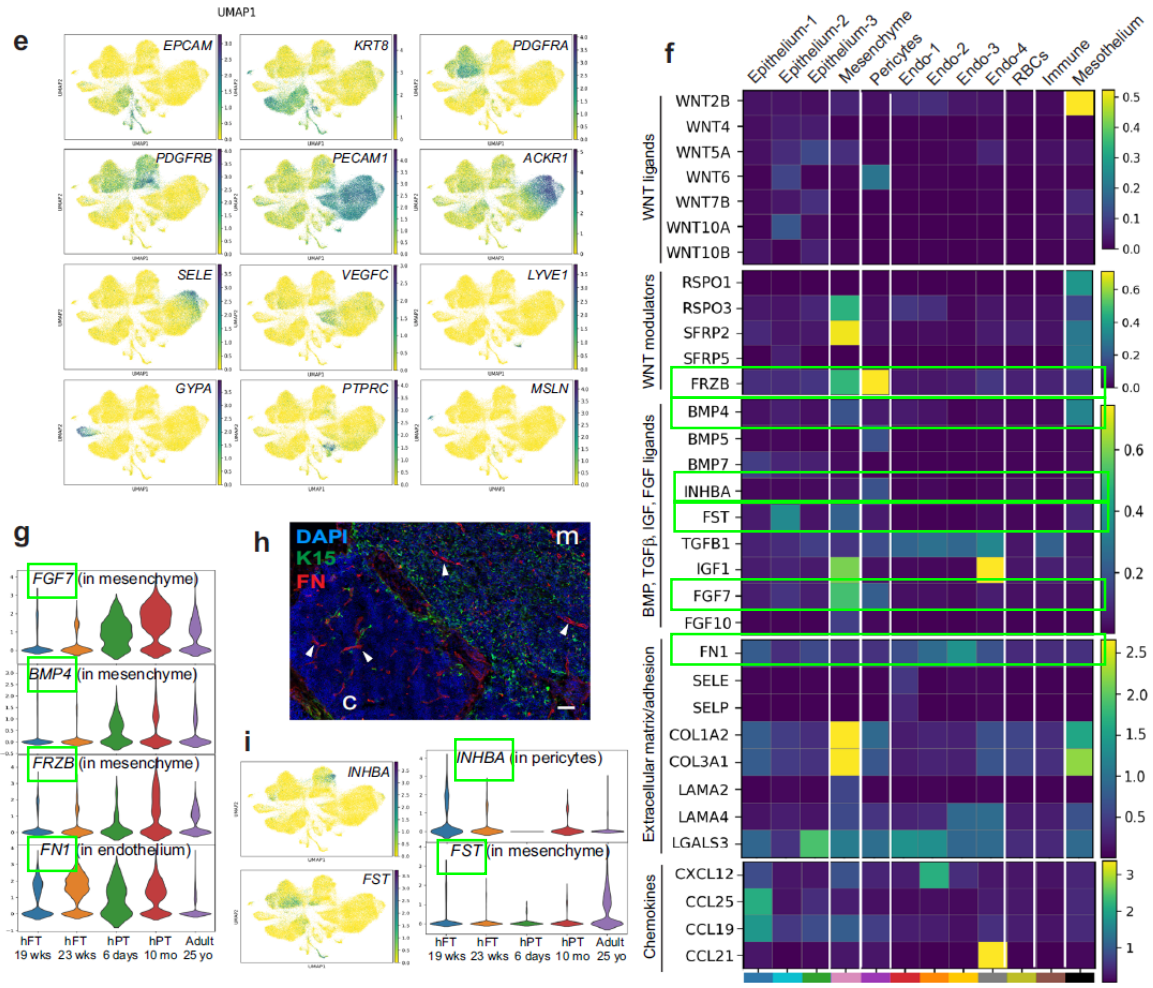
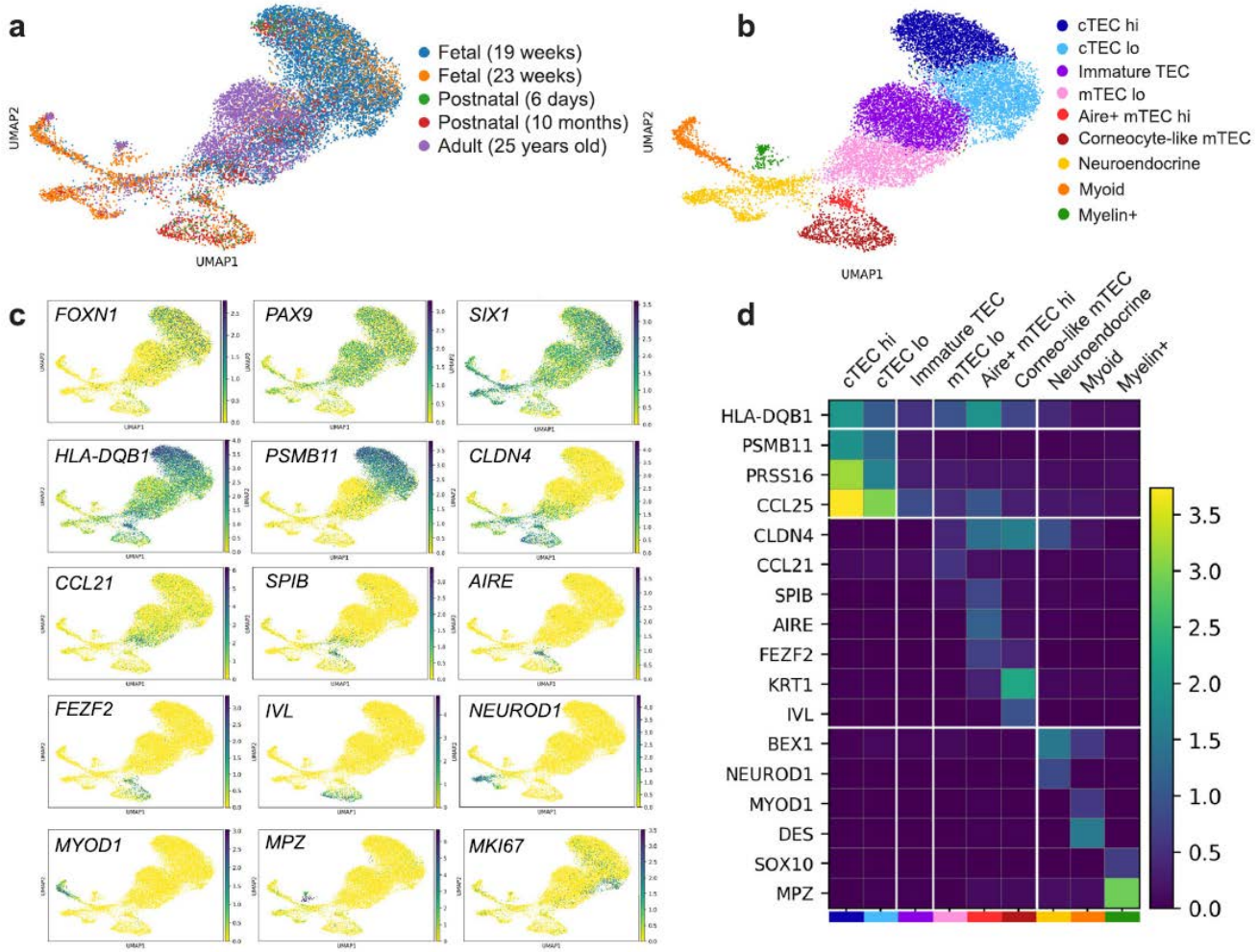


Results
Profiling of human thymic epithelial cells at different stages



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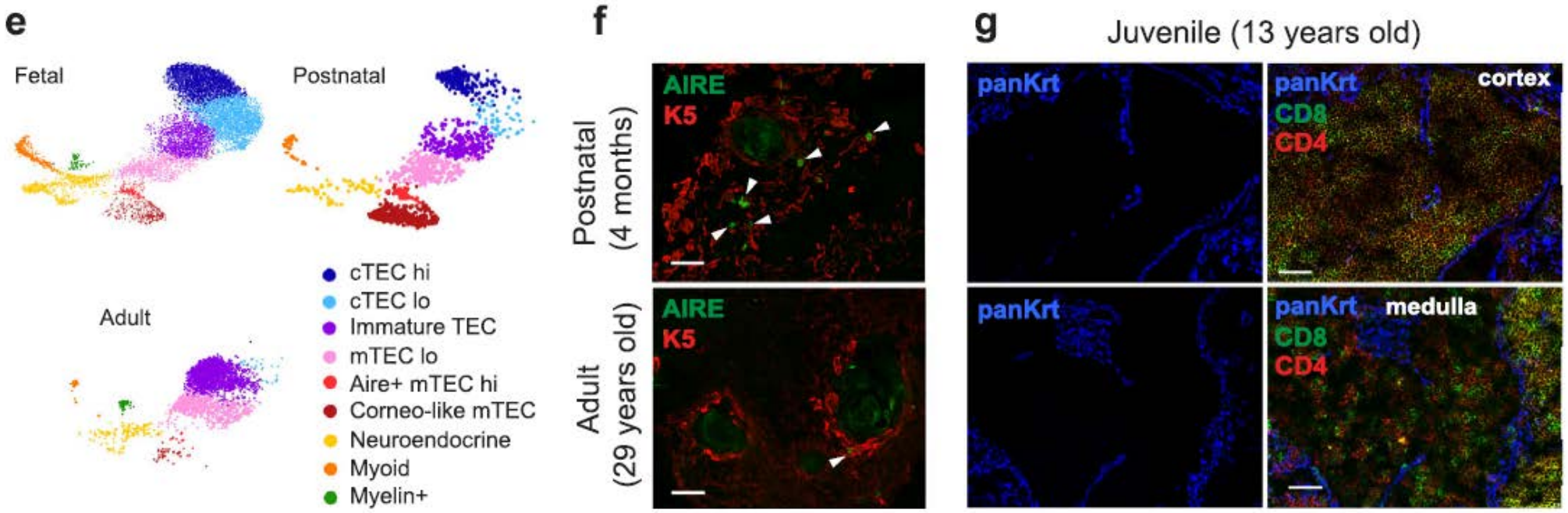
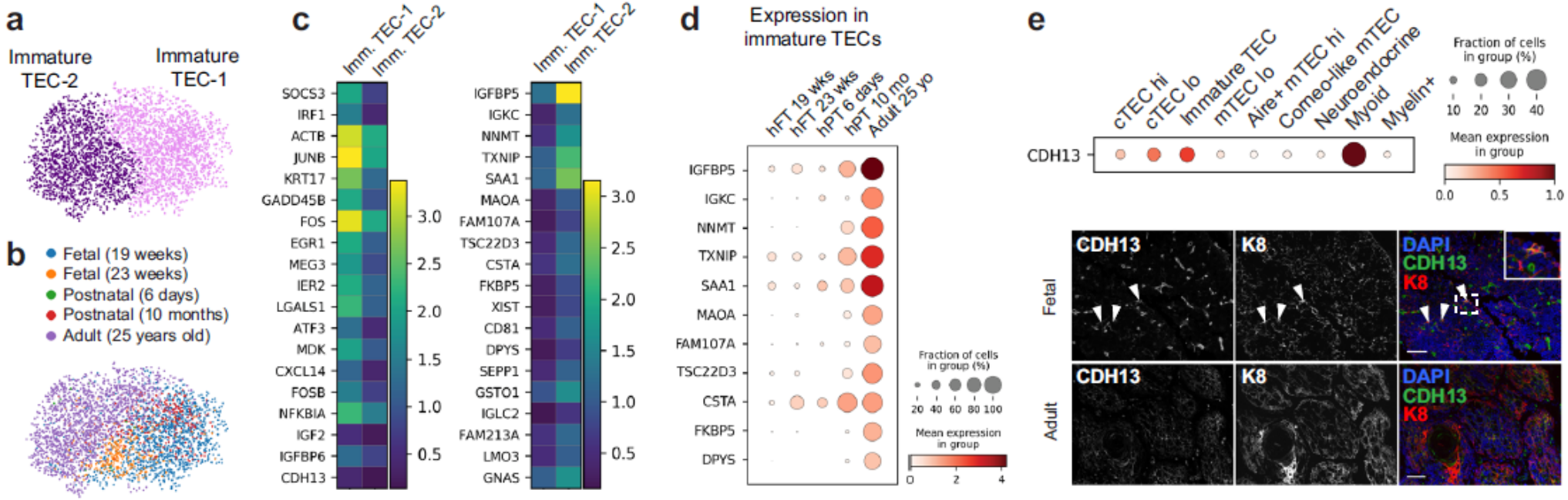
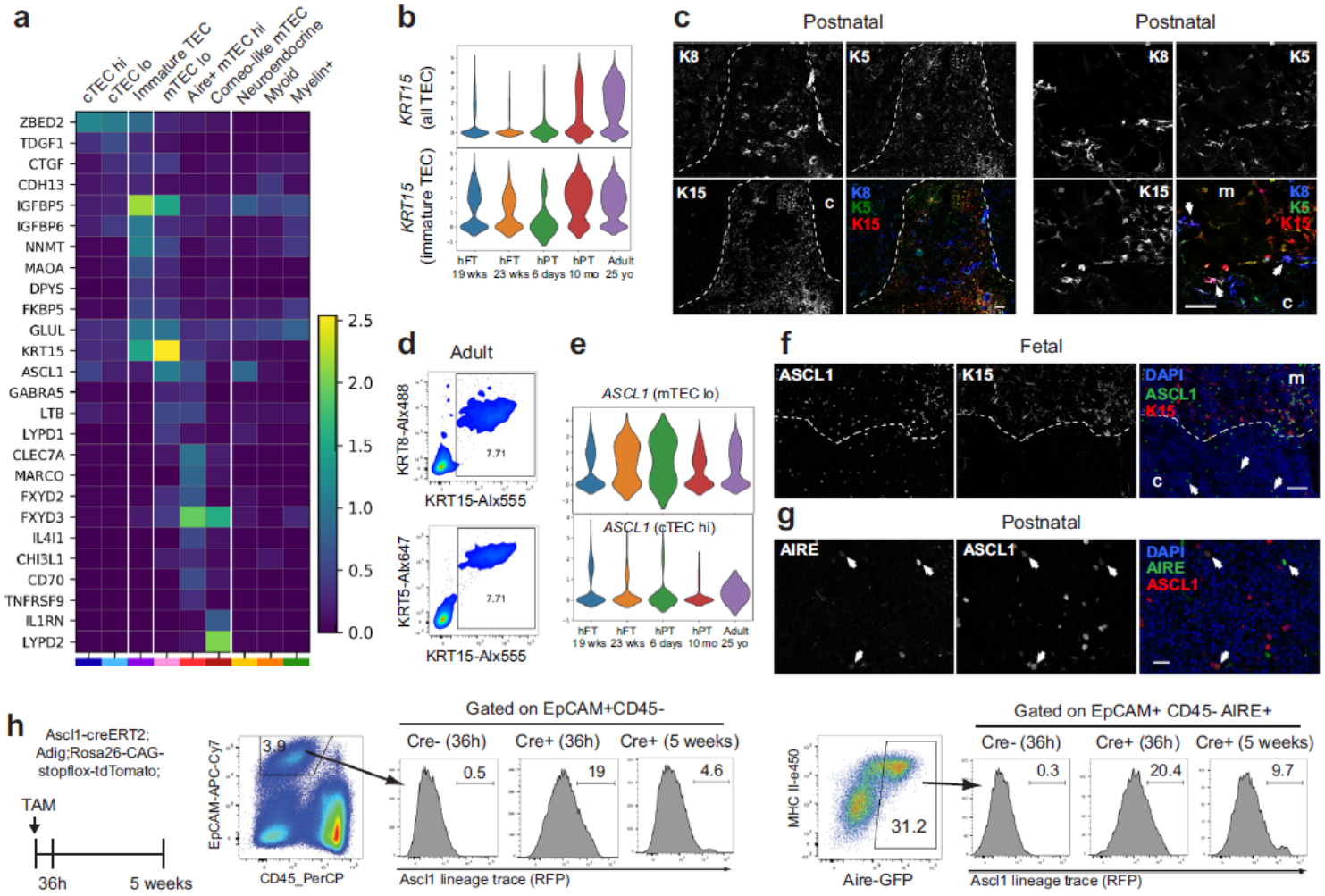


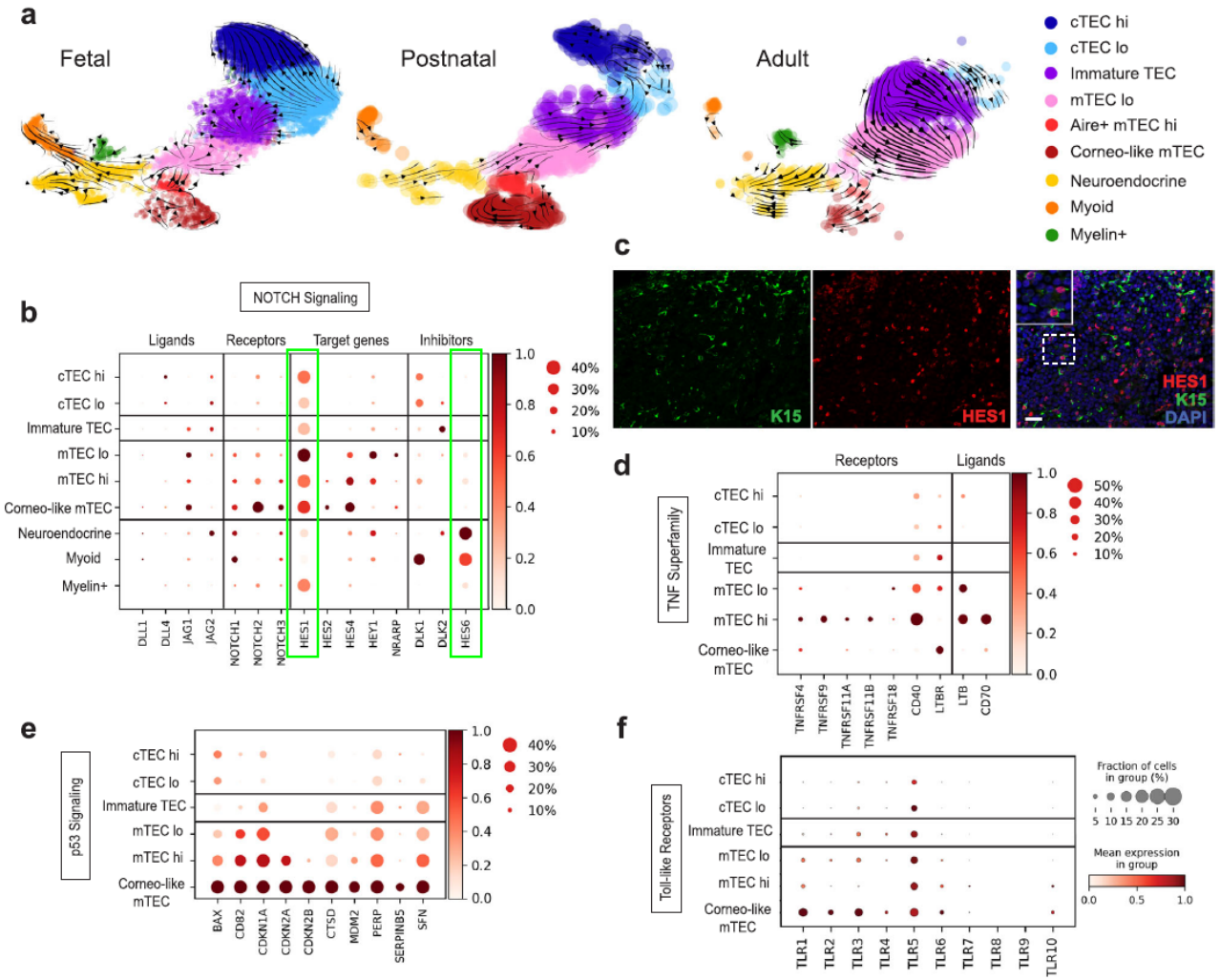
Results
Analysis of immature TECs



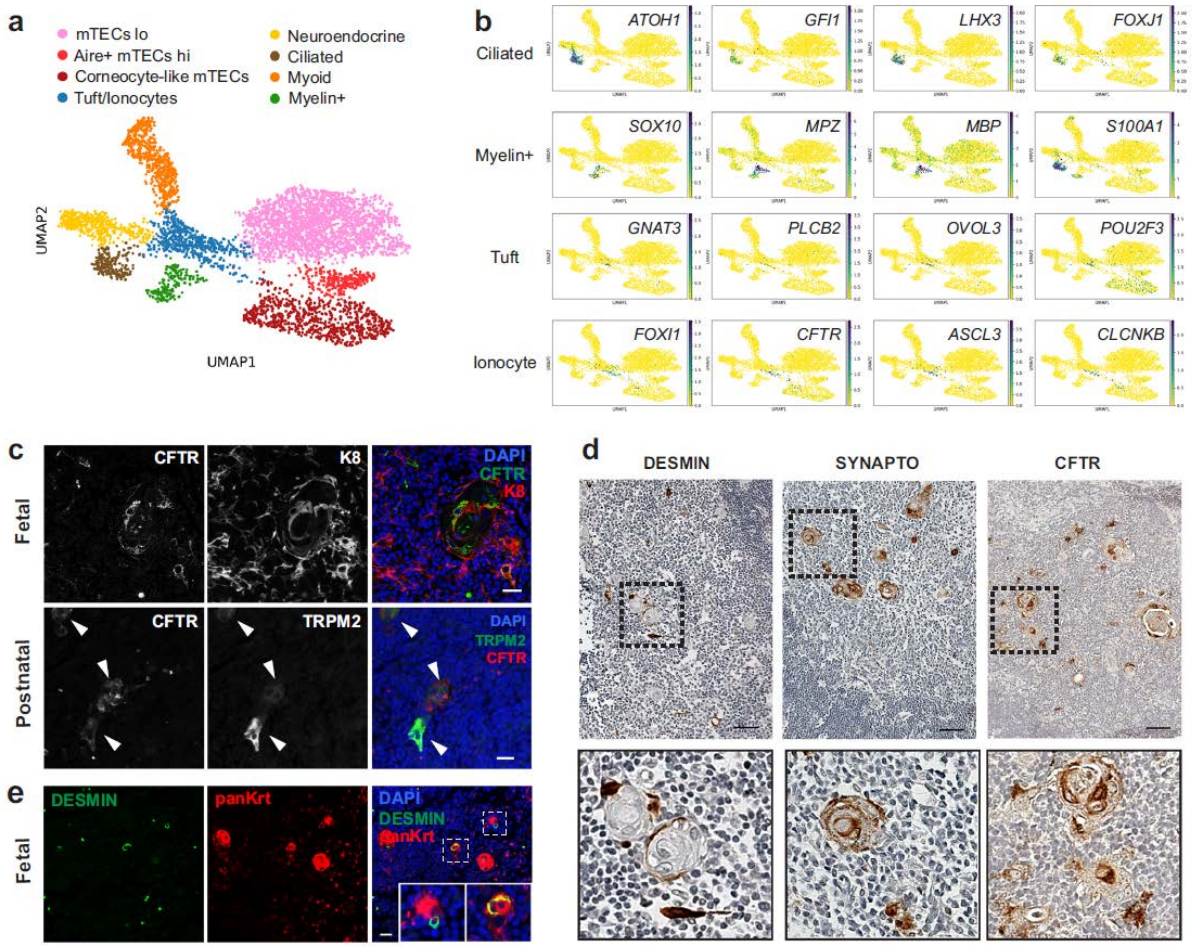
Results
Identification of new TEC markers



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



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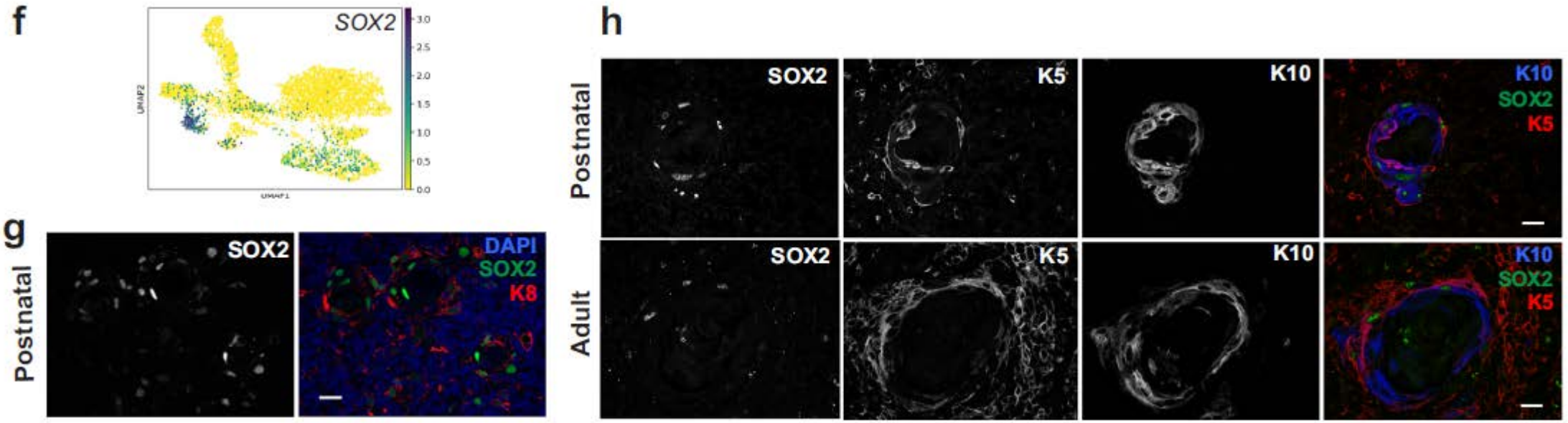
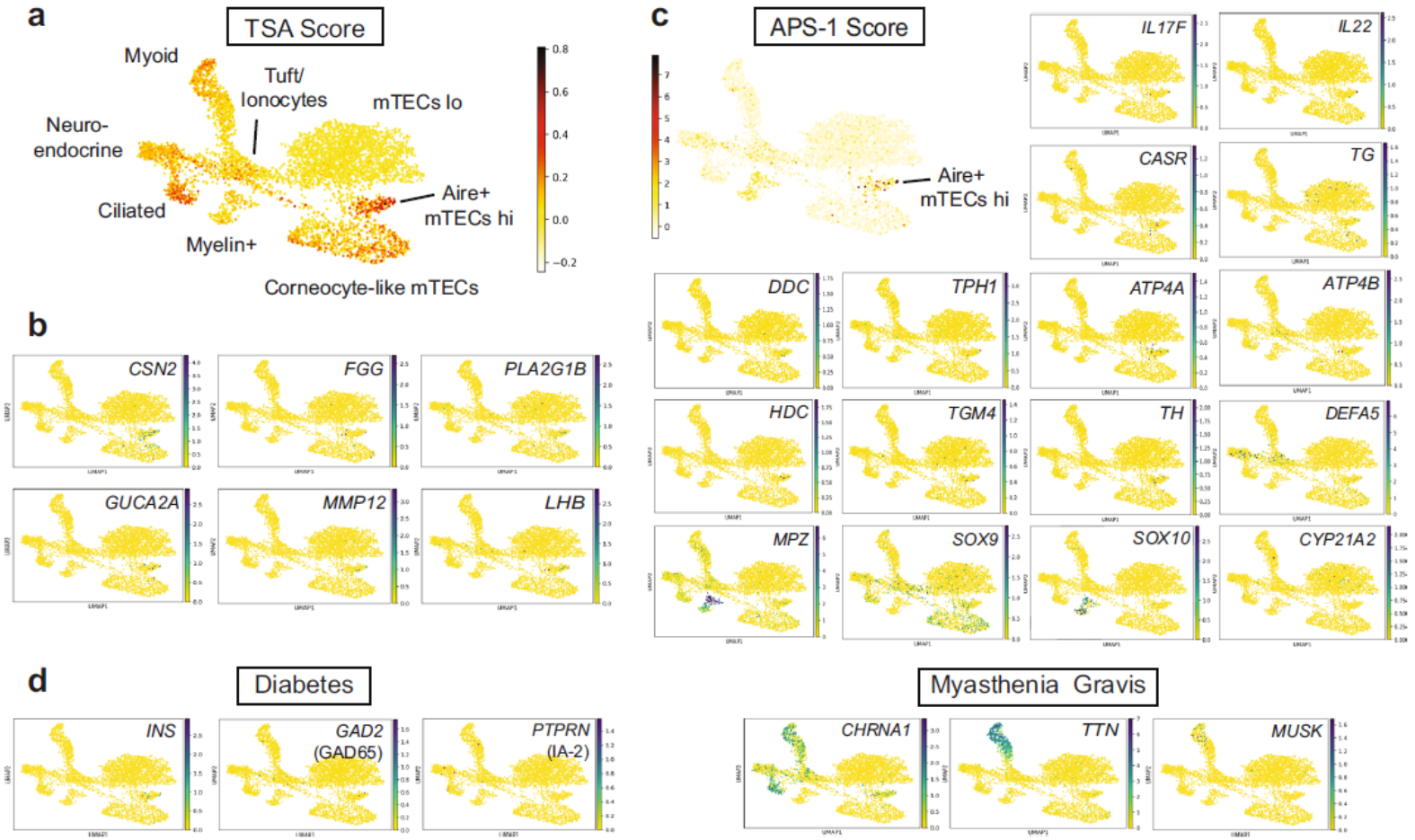


Results
Characterization of tissue-specific antigen expression by human TECs



Discussion

- Ionocytes, Ciliated cells, Schwann cells,
- Crosstalk between TECs and stroma: WNT-pathway (FOXN1), 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