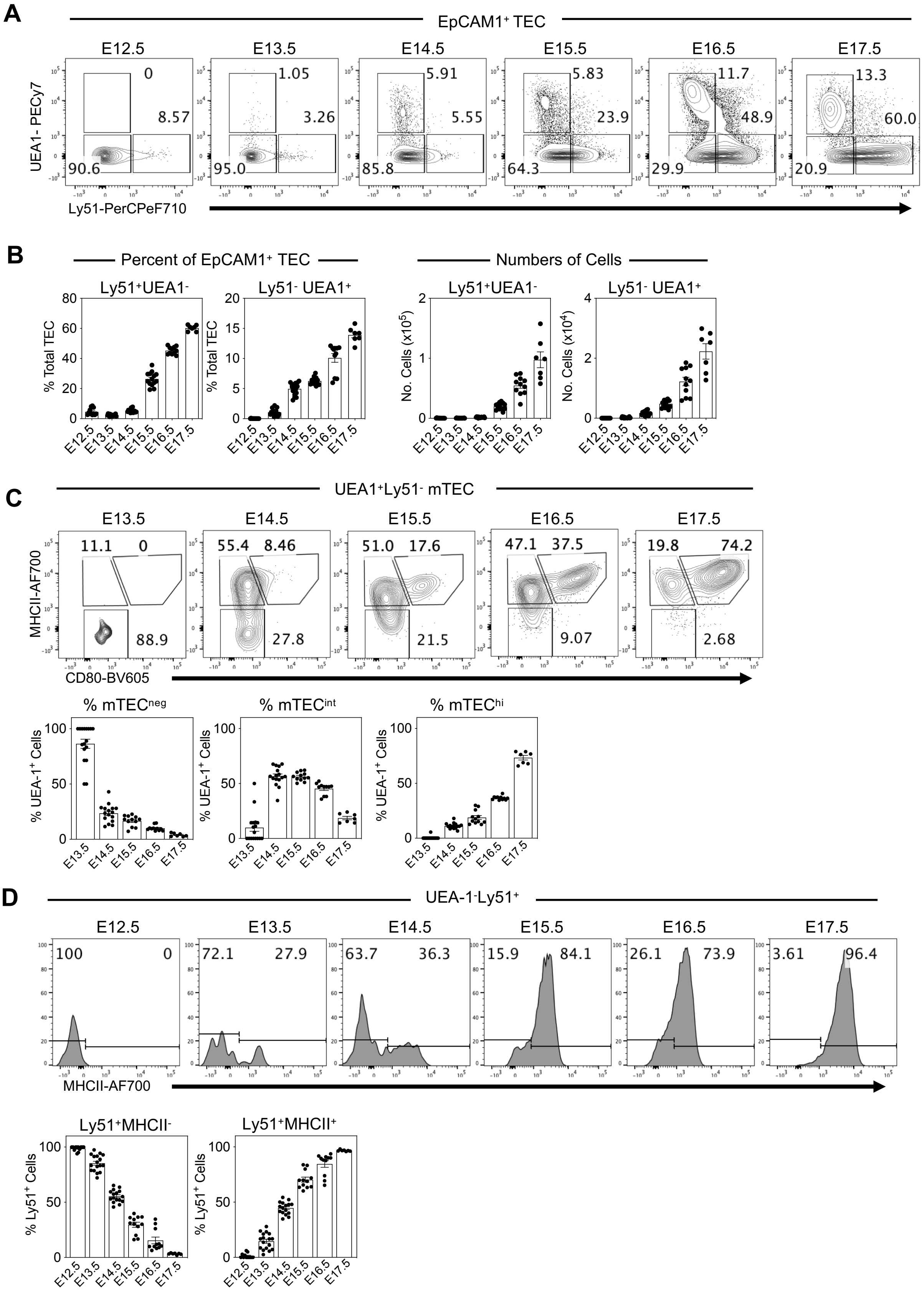
Sup. Fig. 1

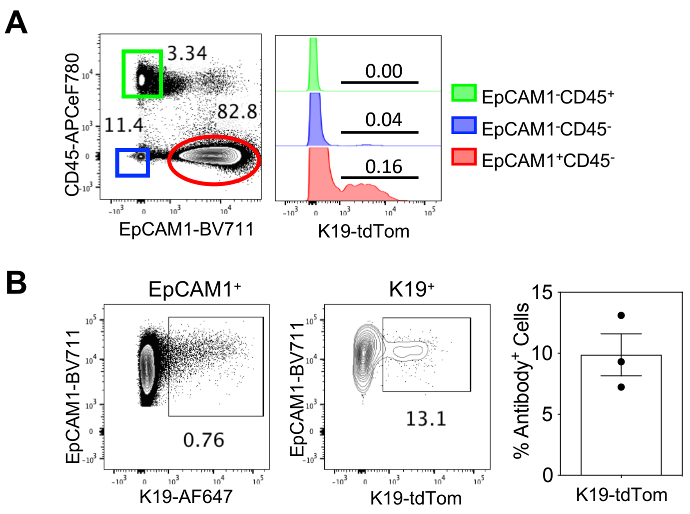


Supplementary Figure 1. Defining Stages in mTEC and cTEC Ontogeny.

A Representative FACS plots showing expression of UEA1 and Ly51 by EpCAM1$^+$ cells during ontogeny.

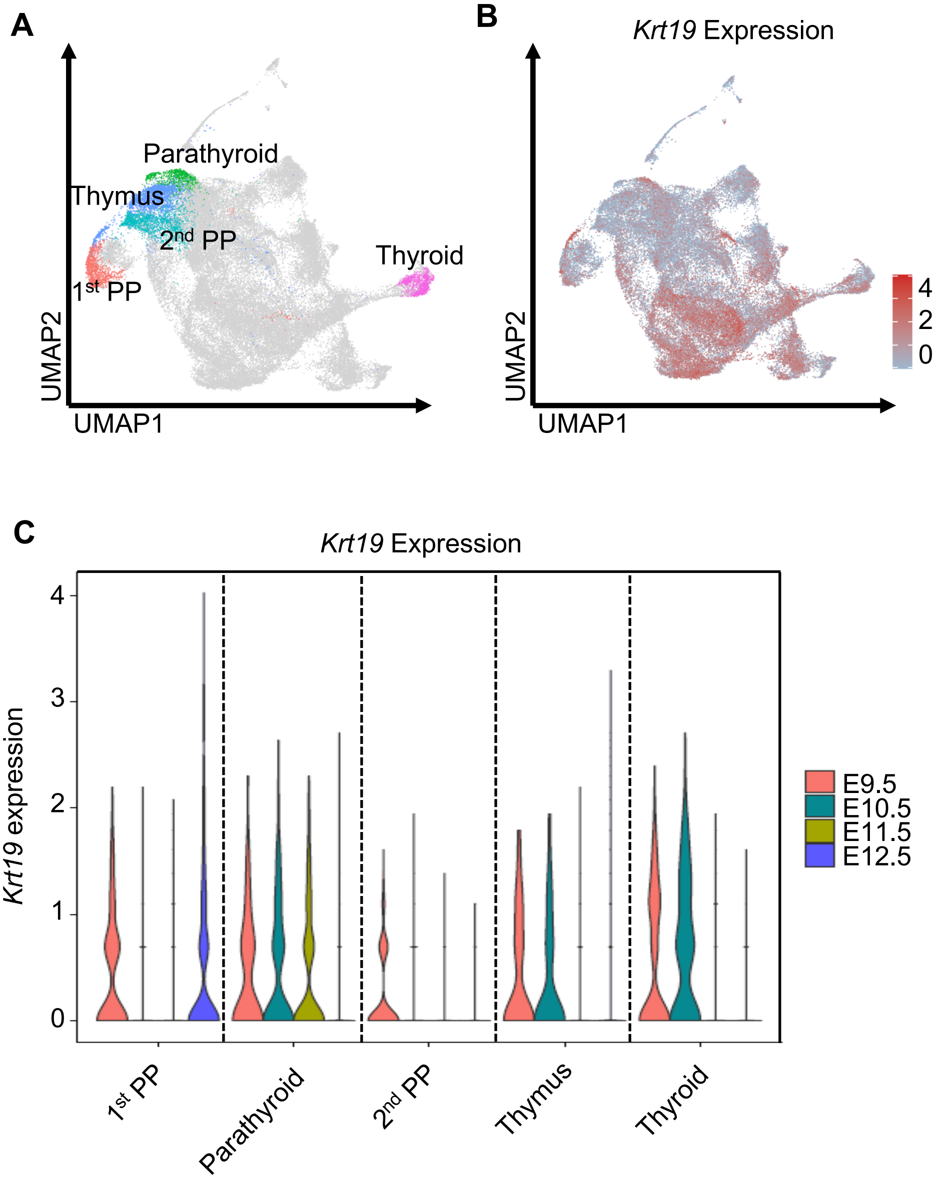
B Quantification of Ly51$^+$UEA1$^-$, and Ly51$^-$UEA1$^+$ subsets within EpCAM1$^+$ cells. **C** Representative FACS plots showing expression of MHCII and CD80 by UEA1$^+$Ly51$^-$ mTEC, to identify mTEC^{neg} (MHCII$^-$CD80$^-$), mTEC^{int} (MHCII^{int}CD80^{int}) and mTEC^{hi} (MHCII^{hi}CD80^{hi}), with corresponding quantitation E12.5 n=12, E13.5 n=17, E14.5 n=16, E15.5 n=12, E16.5 n=11, E17.5 n=7. **D** Representative FACS plots showing expression of MHCII by UEA1$^-$Ly51$^+$ TEC, with corresponding quantitation E12.5 n=12, E13.5 n=17, E14.5 n=16, E15.5 n=12, E16.5 n=11, E17.5 n=7. All data is from 3 independent experiments per gestational age. The data are shown as mean ± SEM.

Sup. Fig. 2



Supplementary Figure 2. Specificity and efficiency of *Krt19*$^{\mathrm{ERTCre}}$
Fate mapping was induced in *Krt19*$^{\mathrm{ERTCre}}$tdTom embryos at E15.5 by a single tamoxifen gavage, and embryonic thymi were analysed the following day, n=3. **A** K19-tdTom expression was determined in CD45^{+} haematopoietic cells (green), CD45^{-}EpCAM^{-}cells (blue) and EpCAM1^{+} TEC (red) by flow cytometry. **B** Representative FACS plots and quantitation showing expression of K19tdTom within K19-antibody^{+} EpCAM1^{+} cells. The data are shown as mean ± SEM.

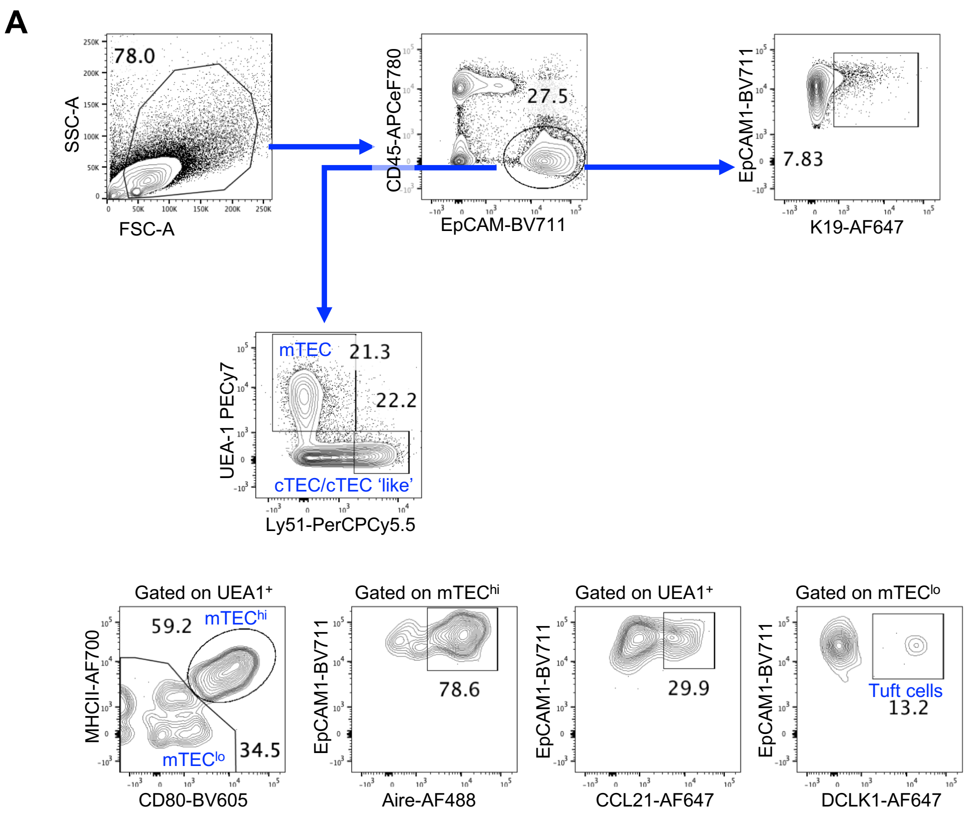
Sup. Fig. 3



Supplementary Figure 3. *Krt19* expression prior to E12.5

A UMAP plot of single cell transcriptomic data of pharyngeal endoderm from E9.5-E12.5 showing clusters which have been annotated using published marker genes (ref - Magaletta et al. 2022). **B** UMAP plot showing *Krt19* expression across the pharyngeal endoderm. **C** Violin plots showing *Krt19* expression within different cell types within the pharyngeal endoderm between E9.5 and E12.5.

Sup. Fig. 4



Supplementary Figure 4. Gating strategies used for flow cytometry

A Gating for live cells based on FSC/SSC, and identification of EpCAM1$^+$ thymic epithelial cells (TEC), K19$^+$ TEC, UEA1$^+$ mTEC, Ly51$^+$ cTEC/cTEC 'like', mTEC^{lo}, mTEC^{hi}, Aire$^+$ mTEC^{hi}, CCL21$^+$ mTEC, and thymic tuft cells. Gating strategy used in Fig1, 3-9.