**Supplementary information**

Fundamental parameters of the developing thymic epithelium in the mouse

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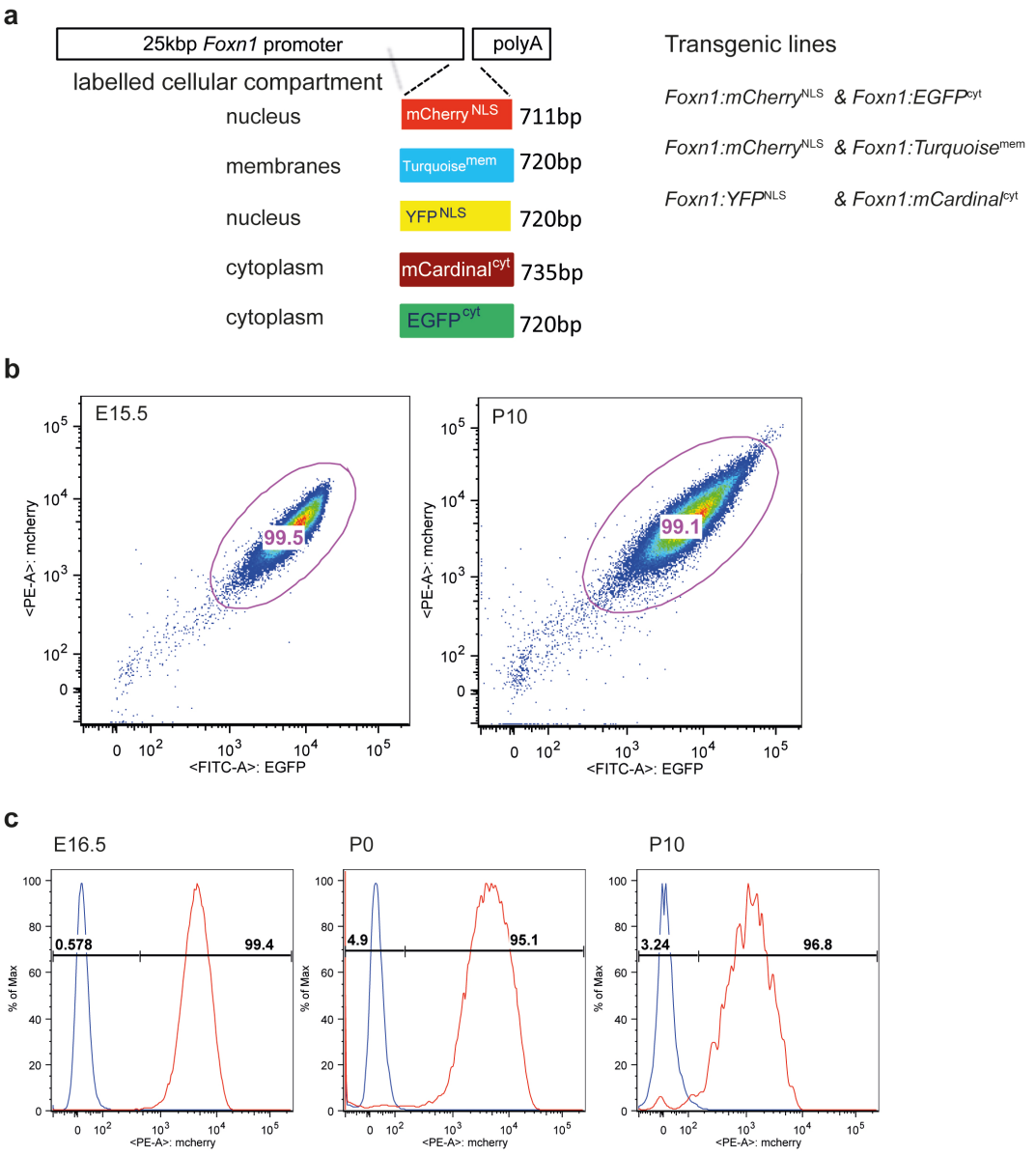
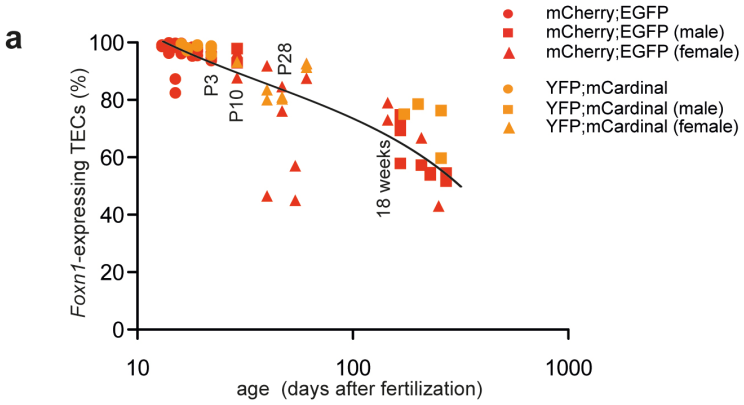
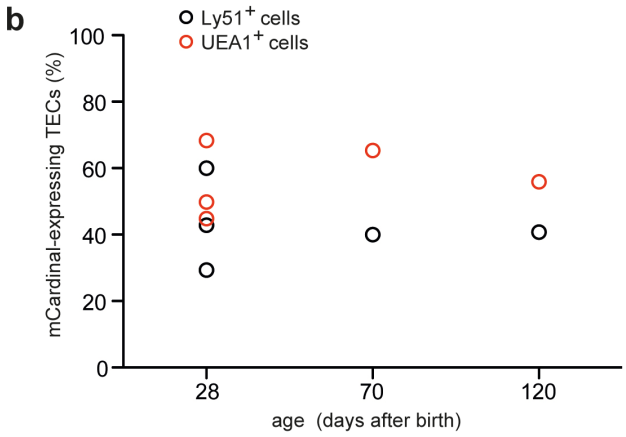
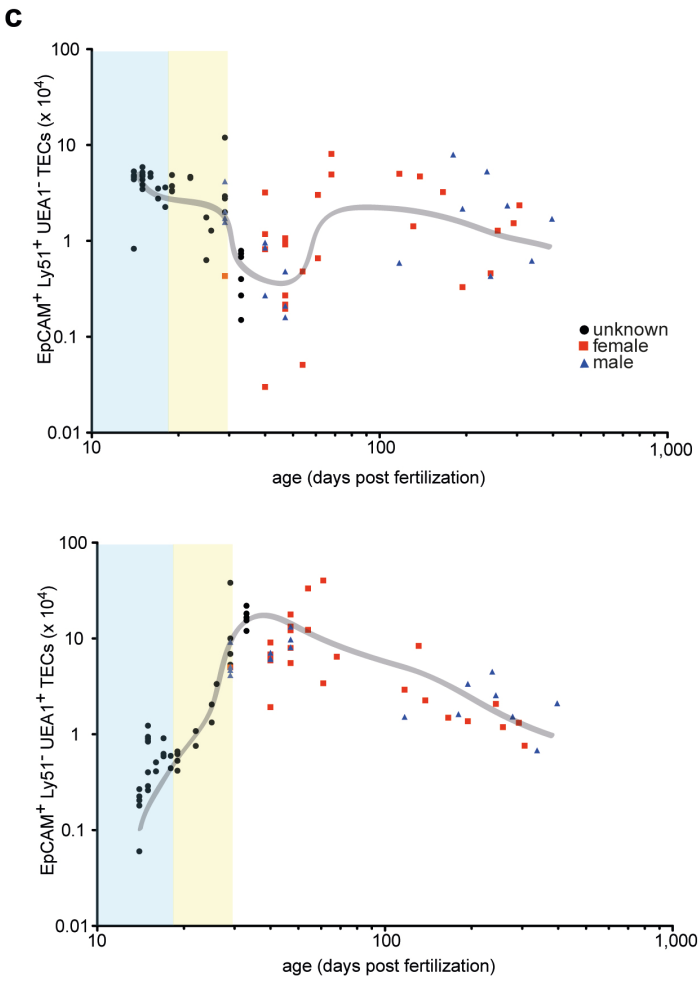



**Supplementary Figure 1**. Characterization of novel transgenic reporter lines. (**a**) Schematic outline of transgenic constructs, consisting of a ~25kb Foxn1 promoter fragment, cDNA fragments encoding different fluorescent proteins, and poly(A) addition signal (see Methods). The three co-injected (and subsequently co-integrated) construct combinations are indicated. Although not exploited here, the transgenic line expressing the mCardinal protein should enable non-invasive monitoring of the TEC compartment[26]. (**b**) Co-expression of different fluorescent proteins in TECs of the *Foxn1:mCherry*$^{NLS}$*;Foxn1:EGFP*$^{cyt}$ transgenic line at two time points; cells liberated from enzymatically dissociated thymi were stained and the depicted plots are gated on EpCAM$^{+}$;CD45$^{-}$ cells. (**c**) All EpCAM$^{+}$;CD45$^{-}$ cells of *Foxn1:mCherry*$^{NLS}$*;Foxn1:EGFP*$^{cyt}$ transgenic thymi express the fluorescent proteins

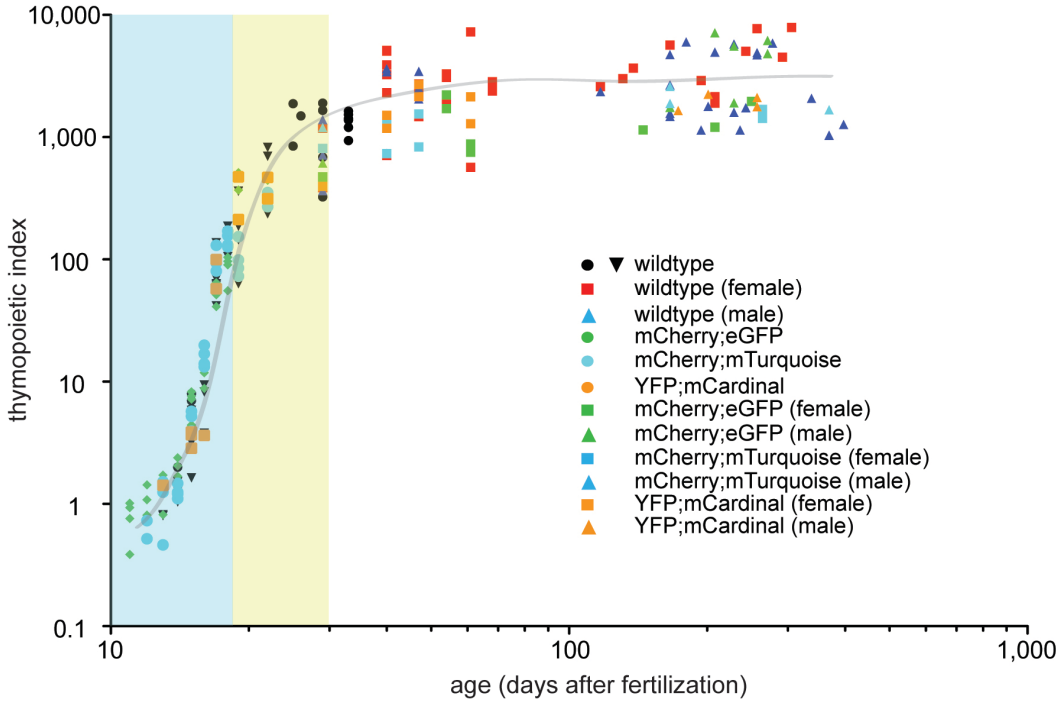
up to at least P10. Blue histograms are from non-transgenic, red histograms are from transgenic mice.


a
mCherry;EGFP
mCherry;EGFP (male)
mCherry;EGFP (female)
YFP;mCardinal
YFP;mCardinal (male)
YFP;mCardinal (female)
Foxn1-expressing TECs (%)
P3
P10
P28
18 weeks
age (days after fertilization)

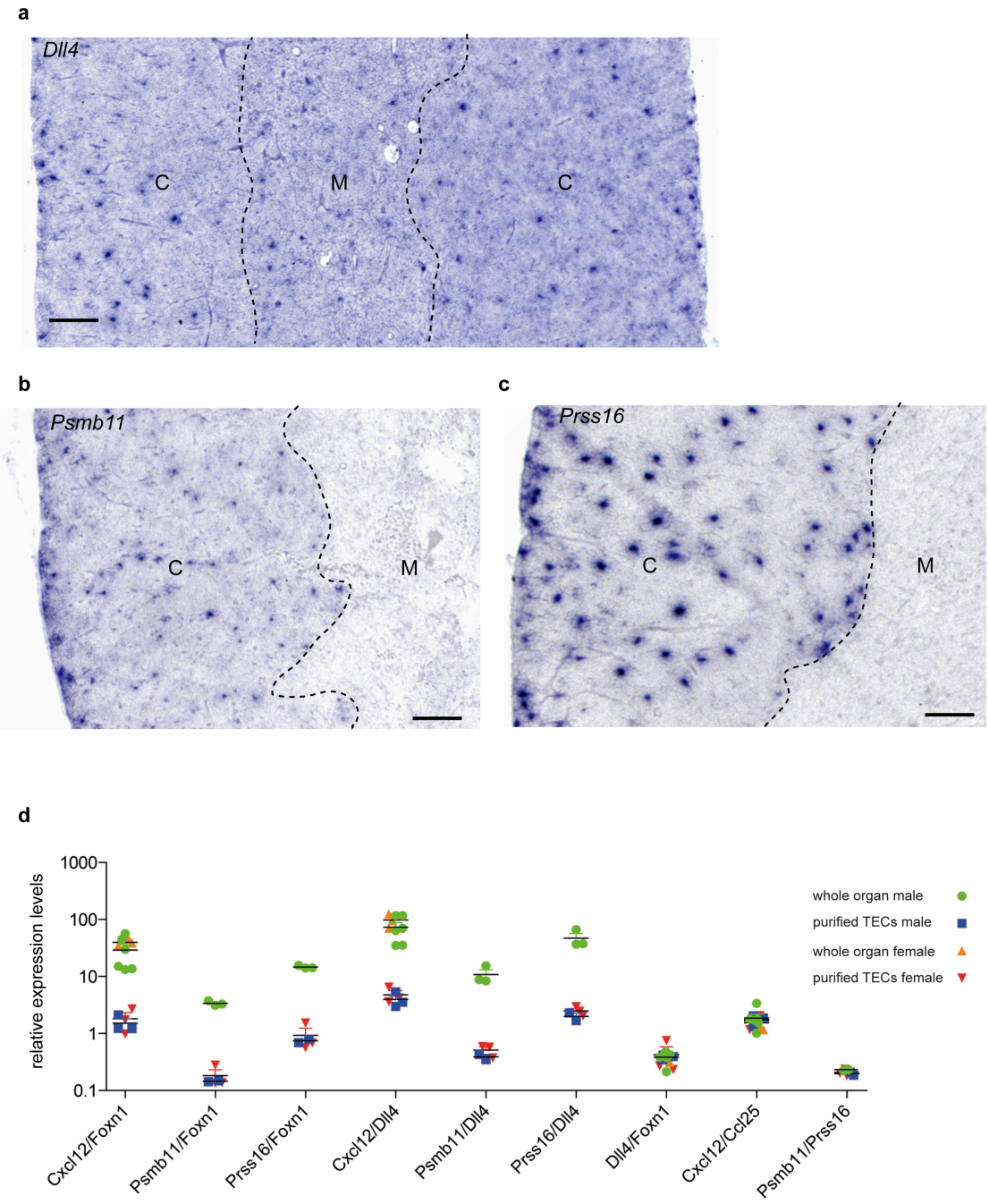

b
Ly51+ cells
UEA1+ cells
mCardinal-expressing TECs (%)
age (days after birth)


c
EpCAM+ Ly51+ UEA1- TECs (x 10⁴)
unknown
female
male
age (days post fertilization)
EpCAM+ Ly51- UEA1+ TECs (x 10⁴)
age (days post fertilization)

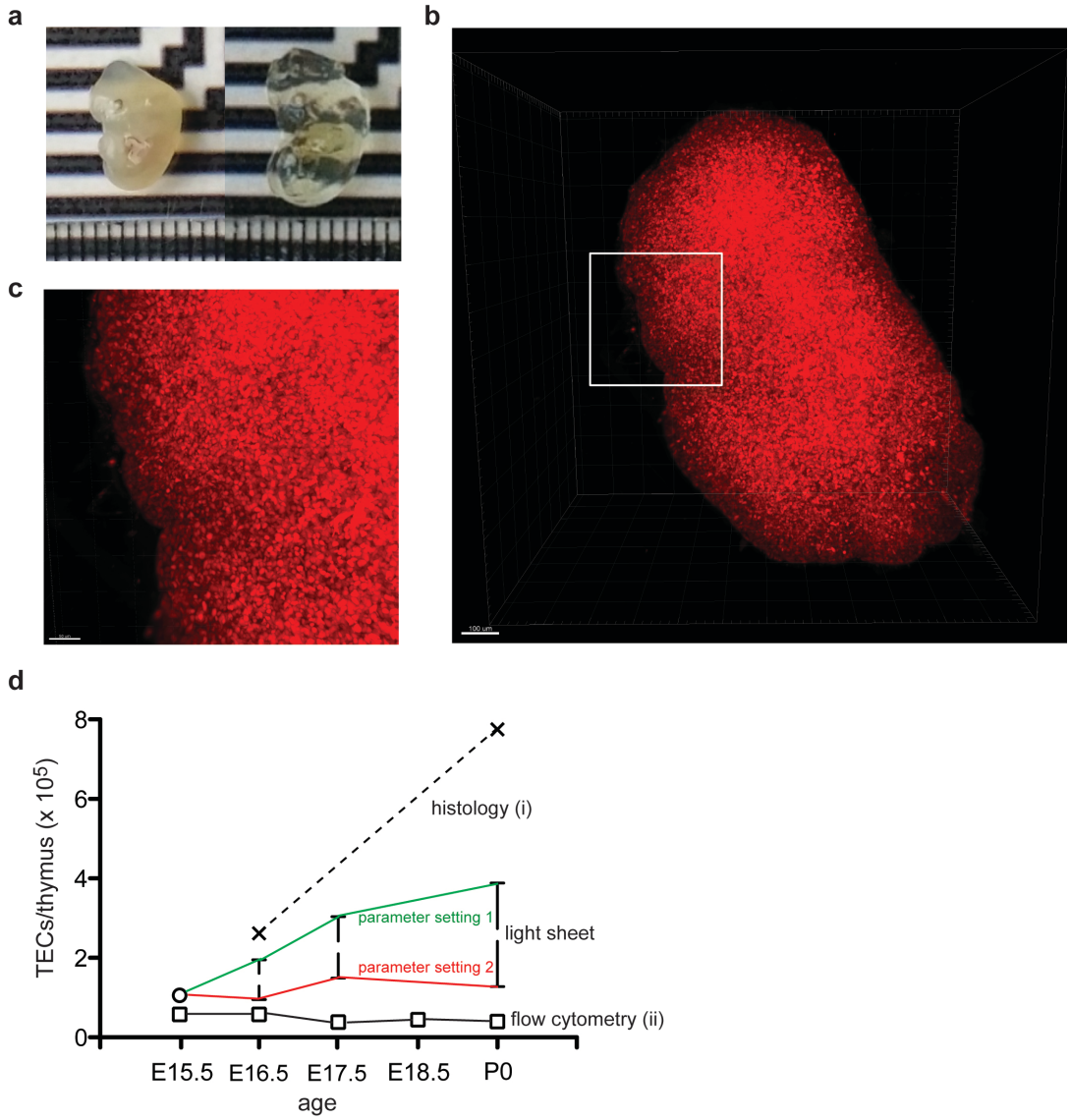
**Supplementary Figure 2**. Characteristics of thymic epithelial cells. (**a**) Fraction of TECs expressing *Foxn1* as a function of age, as determined by transgene fluorescence in the indicated genotypes. Note that TECs of males and females do not appreciably differ with respect to the fraction of *Foxn1*-negative cells. (**b**) Both $Ly51^+$ and $UEA1^+$ cells lose *Foxn1* expression. TECs were isolated from the thymi of *Foxn1:YFP*$^{NLS}$*;Foxn1:mCardinal*; *Foxn1:Cre*;*RosaR26SLSYFP* quadruple transgenic mice to record both past and present *Foxn1* expression (see text for details). (**c**) Absolute numbers of $Ly51^+UEA1^-$ cells (**c**) and $Ly51^-UEA1^+$ cells (**d**) among $EpCAM^+$ TECs as determined by flow cytometry (see Fig. 2).



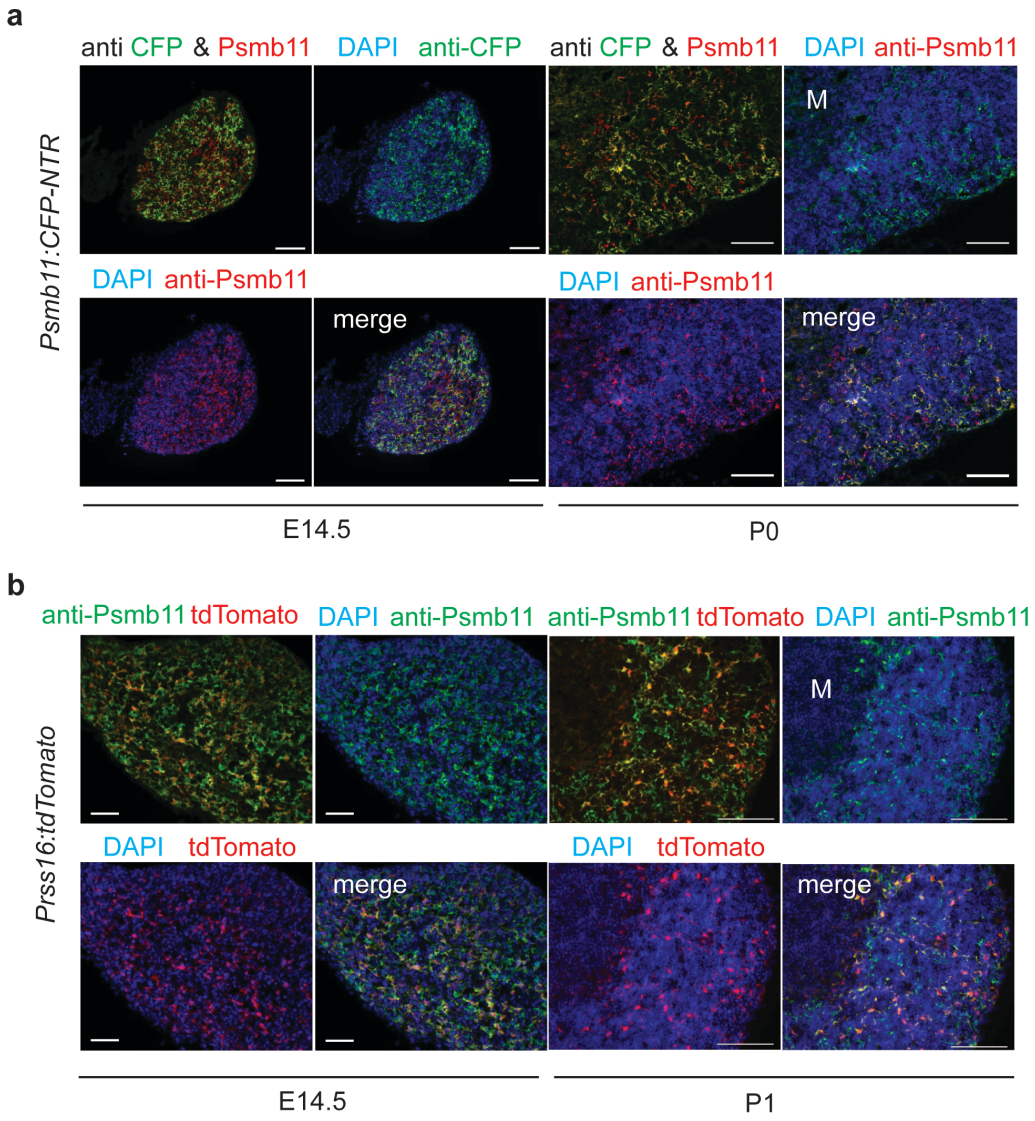

**Supplementary Figure 3**. Age-dependent changes of the thymopoietic index, as determined by flow cytometry following enzymatic tissue dissociation. The thymopoietic index is expressed as the ratio of $CD45^+$ and $EpCAM^+$ cells. Each symbol represents a single animal with genotypes and sex (where known) indicated in the figure key; the two wild-type symbols represent mice of two cohorts analysed in different years. The blue shading denotes the embryonic phase, the yellow shading marks the perinatal period until P10.



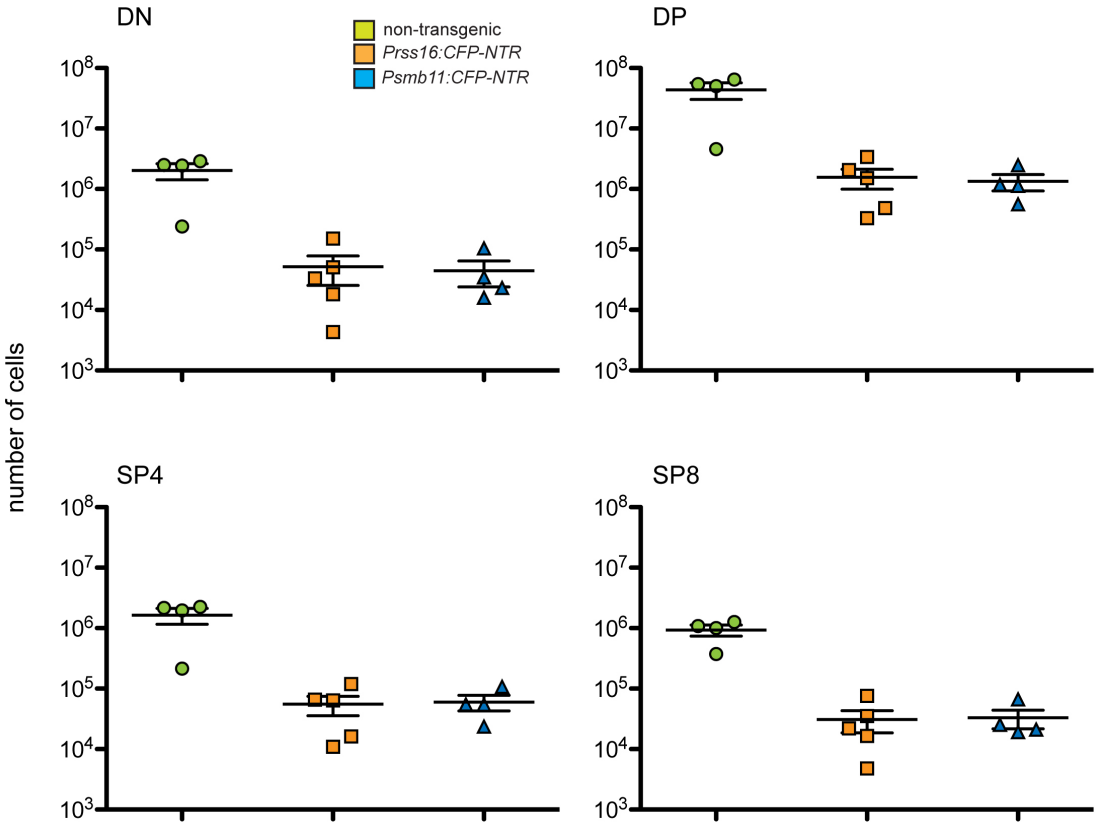

**Supplementary Figure 4**. Expression signature of TEC-specific genes. RNA *in situ* hybridization to localize TECs expressing *Dll4* (**a**), *Psmb11* (**b**), and *Prss16* (**c**) in sections of a 4-week-old mouse. The positions of cortex (C) and medulla (M) are indicated. Scale bars, 0.1mm. (**d**) Expression levels of epithelial-specific genes with a regio-specific signature in the thymus using RNA extracted from purified TECs or whole thymi. Each symbol represents a single mouse. The fold changes between whole organ and purified TECs are indicated.




**Supplementary Figure 5**. Determination of the absolute number of TECs. (**a**) Demonstration of the effectiveness of tissue clearing of E12.5 embryos. The left embryos was fixed with further treatment, whereas the right embryo was additionally subjected to clearing after fixation. (**b**) Reconstructed image indicating the distribution of TEC nuclei of *Foxn1:mCherry*$^{NLS}$*;Foxn1:Turquoise*$^{mem}$ transgenic mice in the intact organ as determined by light-sheet microscopy. Scale bar, 0.1 mm. (**c**) Higher magnification of a selected region of (b). Scale bar, 0.05 mm. (**d**) Summary of three different methods to determine the absolute number of TECs. Data are taken (i) for the histological procedure from Figure 3c,d; (ii) for flow cytometric measurements are taken from Figure 2b. The values presented for the four time points of the light-sheet determinations are from two different parameters for nucleus size; 6 mm (green line), 10 mm (red line). For details, see text.




**Supplementary Figure 6**. Characterization of cTEC-specific transgenic lines. (**a**) The activity of the *Psmb11* promoter is restricted to thymic epithelial cells. The anti-CFP staining identifies expression of the transgene, the anti-Psmb11 antibody marks epithelial progenitor and cTECs. Note that most TECs in the cortex are double-positive, indicating that the transgenic construct largely reflects the endogenous *Psmb11* expression pattern. (**b**) The activity of the *Prss16* promoter is restricted to a subset of cortical thymic epithelial cells. The red fluorescence identifies expression of the transgene, the anti-Psmb11 antibody marks epithelial progenitor and cTECs. Note that a large fraction of transgene-expressing cells is also positive for Psmb11 (yellow); some cTECs are positive for either the transgene (red) or Psmb11 (green). Scale bars, 0.1mm.




**Supplementary Figure 7.** Thymocyte subsets of regenerating thymi after subtotal ablation of cTEC-like cells. For experimental strategy, see Fig. 5. Total number of TECs (left panel) and $CD45^+$ haematopoietic cells as determined by flow cytometry following enzymatic dissociation at P7; each symbol represents a single mouse. Although the absolute numbers of cells in all subsets are significantly lower in regenerating thymi as compared to control (at least $P<0.04$), the relative ratios of subsets are not significantly different from each other in all three conditions with the exception of a significant elevation ($P=0.03$) of the percentage of CD4 single-positive cells in *Psmb11:CFP-NTR* transgenics relative to the *Prss16:CFP-NTR* transgenics.