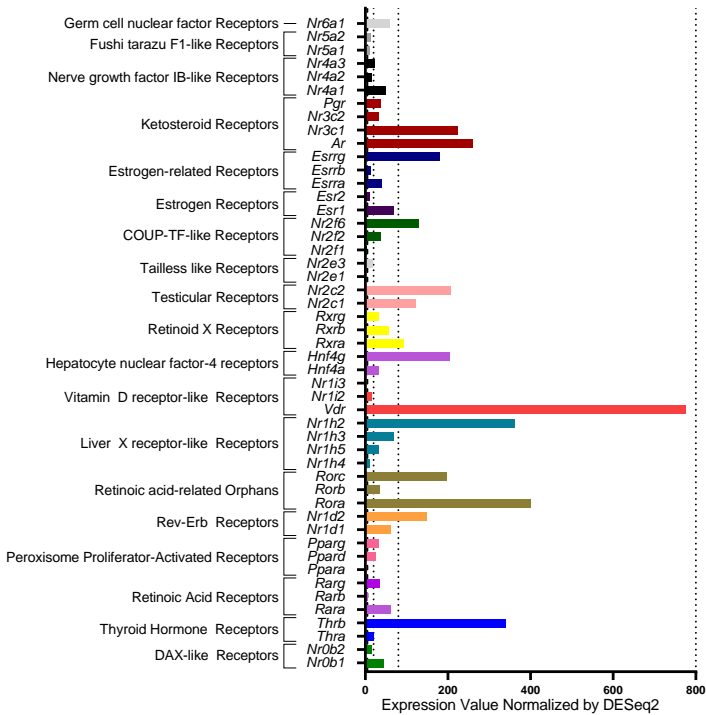
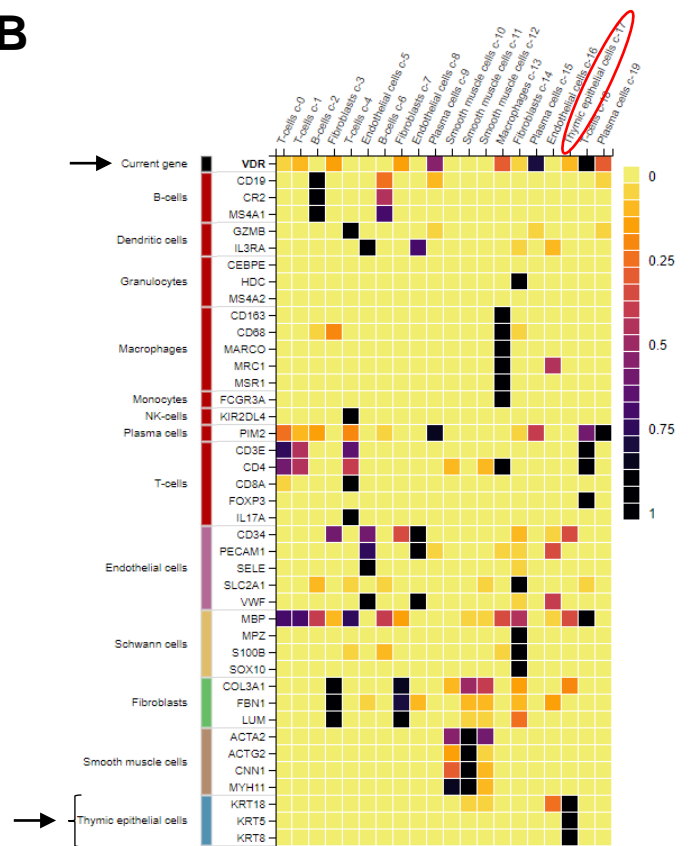
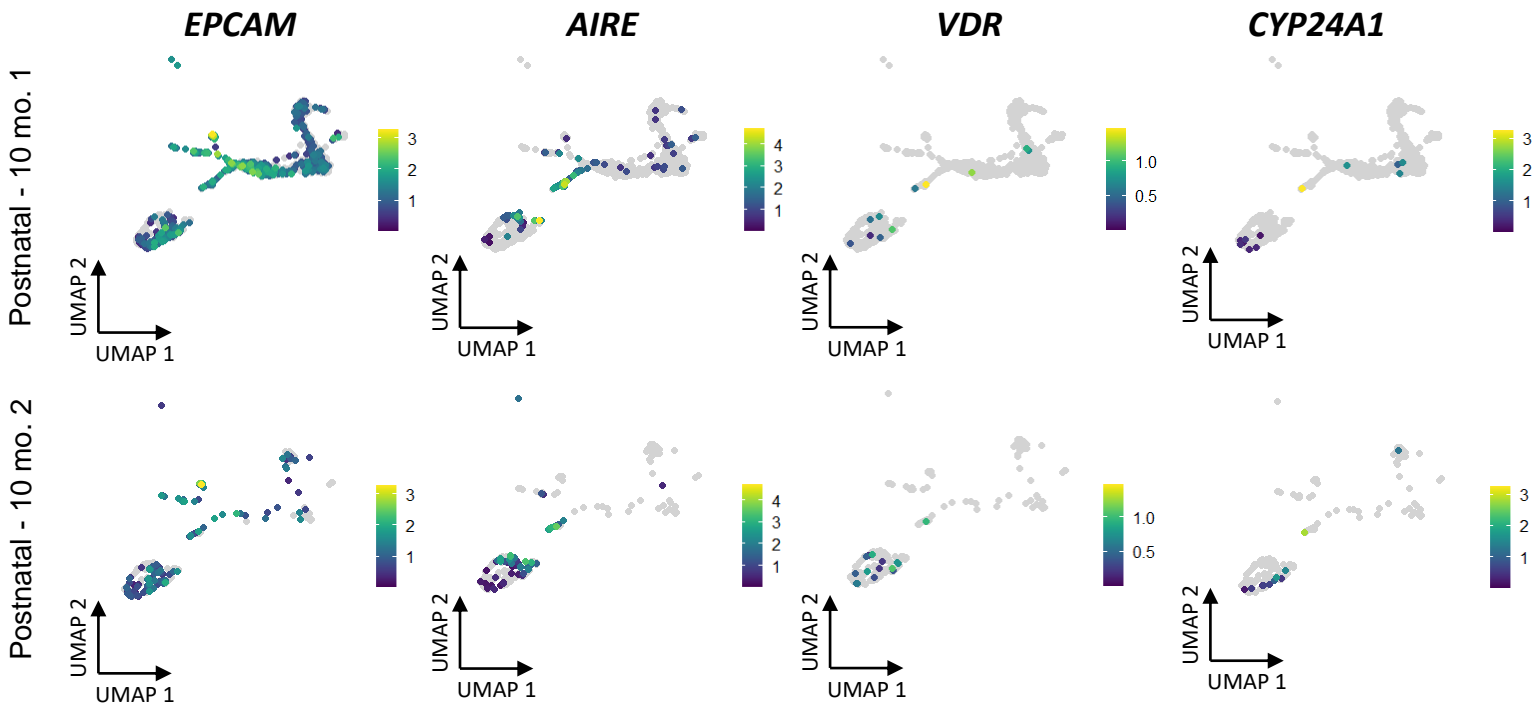
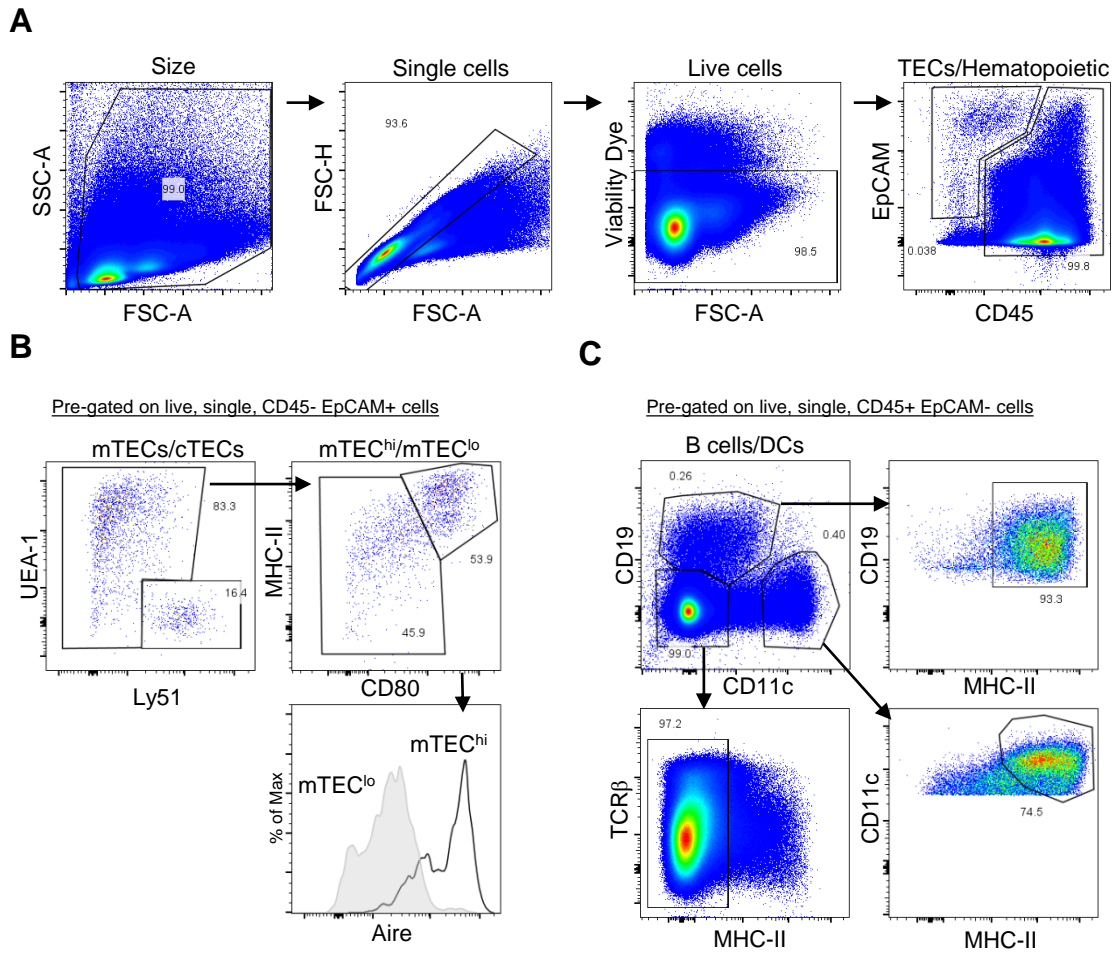


Supplemental Fig 1 : Active 1,25D signaling in the thymus. **A.** RNA sequencing data showing *Vdr* mRNA expression in different mouse thymic and splenic cell types [13]. **B.** RNA sequencing data showing *Cyp27b1* mRNA expression in different mouse thymic and splenic cell types [13]. **C.** Detection of *Vdr* and *Cyp27b1* mRNA in mouse thymi (top) or sorted TECs cultured for 6h (bottom), gene expression normalized to 18S and shown as Δ Ct values. **D.** Immunohistochemical staining of mouse thymus showing cortical (c) and medullary (m) *Vdr* staining in thymocytes (CD3+) and mTECs (CK5+). **E.** IF microscopy showing *Vdr* staining in Aire+ mTECs, cTECs, thymocytes, and dendritic cells. **F.** RNA sequencing data showing *Cyp24a1* mRNA expression in different mouse thymic and splenic cell types [13]. **G.** UCSC browser image showing mouse intestine VDR ChIP-seq tracks (GEO accession: GSE69179) at the *Rnase1* locus. The areas surrounding the VDR binding sites up- and downstream (regions highlighted in blue) of the genes are shown. The binding site chosen for ChIP analysis is indicated with the asterisk. RNA sequencing data shown in Figs A,B and F was derived from data assembled by the ImmGen consortium [13]. Data are individual mice ($N \geq 3$, ≥ 1 experiment).

A**Nuclear receptor mRNA expression in sorted mTEC^{hi} cells****B****C****Supplemental Figure 2****Nuclear receptor mRNA expression in sorted mTEC^{hi} cells.**

A. RNA sequencing data showing mRNA expression of various nuclear receptors in sorted mouse MHC-II^{hi} mTECs (This data was derived from data assembled by the ImmGen consortium) [13]. **B.** RNA sequencing data of human thymic cells indicating VDR mRNA expression (arrow) in human TECs. TEC markers KRT5, 8 and 18 are used as positive controls for TECs (double arrow) (Human Protein Atlas proteinatlas.org, image credit: Human Protein Atlas, <https://www.proteinatlas.org/ENSG00000111424-VDR/single+cell+type/thymus>, Tabula Sapiens, C., R.C. Jones, et al. 2022. The Tabula Sapiens: A multiple-organ, single-cell transcriptomic atlas of humans. Science. 376(6594): p. eabl4896). **C.** scRNAseq analysis of TEC samples from duplicate 10-month-old human post-natal thymi (GSM4466784/5) [14].



Supplemental Figure 3

Flow cytometry gating strategy for TEC subtypes and various hematopoietic cells

A-C. Representative flow cytometry gating strategy including pre-gating on live, single, and stromal or hematopoietic cells (A); stromal cells (B) including mTEC^{hi} cells (UEA-1⁺, Ly51⁻, MHC-II^{hi}, CD80⁺), mTEC^{lo} cells (UEA-1⁺, Ly51⁻, MHC-II^{lo}, CD80⁻), cTECs (Ly51⁺, UEA-1⁻); hematopoietic cells (C) including B cells (CD19⁺, MHC-II⁺, CD11c⁻), DCs (CD11c⁺, MHC-II⁺, CD19⁻), and thymocytes (TCRβ⁺, MHC-II⁺, CD19⁻, CD11c⁻).

	Target	Conjugate	Host	Clone	Company	Application	Reference
Primary antibodies	CD11c	Unconjugated	Armenian Hamster	N418	ThermoFisher	IF	14-0114-81
	Cyp27b1	Unconjugated	Rabbit	Polyclonal	ThermoFisher	IF/FC	PA5-79128
	Cytokeratin 5	Unconjugated	Rabbit	Poly19055	Biologend	IF	905503
	TROMA-1 (Cytokeratin 8)	Unconjugated	Rat	N/A	DSHB	IF	DSHB
	Mouse/Human Vdr	Unconjugated	mouse	D-6	Santa Cruz	WB/ChIP	sc-13133
	Mouse/Human Aire	Unconjugated	Rat	5H12	ThermoFisher	ChIP/CoIP	14-5934-82
	CD16/32 (Fc block)	Unconjugated	Rat	S17011E	Biologend	FC	156603
	EpCAM	PeCy7		G8.8	ThermoFisher	FC	25-5791-80
	CD45	eFluor 450		30-F11	ThermoFisher	FC	48-0451-82
	Aire	eFluor660		5H12	ThermoFisher	IF/FC	50-5934-80
	MHC Class II	APC-eFluor780		M5/114.15.2	ThermoFisher	FC	47-5321-80
	CD19	AF700		1D3	ThermoFisher	FC	56-0193-82
	CD3	eFluor660		17A2	ThermoFisher	IF/FC	50-0032-80
	Ly51	eFluor 710		6C3	ThermoFisher	FC	46-5891-80
	NK1.1	PE		PK136	Biologend	FC	108707
	CD80	BV650		16-10A1	Biologend	FC	104732
	CD11c	BV785		N418	Biologend	FC	117335
TCR β	BV711		H57-597	Biologend	FC	109243	
UEA-1	Dylight 594		N/A	Vector laboratories	FC	DL-1067	
Secondary antibodies	horse anti mouse IgG	HRP		N/A	Cell Signaling	WB	#7076
	goat anti armenian hamster IgG	AF594		N/A	Biologend	IF	405512
	goat anti rabbit IgG	AF488		N/A	ThermoFisher	IF/FC	A11034
	goat anti rat IgG	AF546		N/A	ThermoFisher	IF	A11081
Other reagents	DAPI	N/A		N/A	ThermoFisher	IF	D1306

Supplemental Table 1 – List of antibodies and reagents used.