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SUPPLEMENTAL FIGURE LEGENDS

Fig. S1 (Associated with Fig. 4): Known $\gamma\delta$ T cell ligands were not Aire-dependent. **A.** Microarray-based quantification of transcripts encoding H2-T10 and -T22 in MEC^{hi} from *Aire*^{+/+} and *Aire*^{-/-} littermates. Data are from (Yang et al., 2015). **B.** Flow cytometric analysis of IL-17A and T22-tetramer staining in $\gamma\delta$ thymocyte compartments. Left, representative cytofluorometric dot plots. n=4. Center and right, summary data on fractional representation and numbers. **C, D.** Same as A, B, except PCR-based quantification of *Skint-1* expression and flow cytometry analysis of V γ 5⁺ T cells. n=3. Dot plot scales for this figure are all the same.

Fig. S2 (Associated with Fig. 5): Characterization of V γ 6⁺V δ 1⁺ TCRtg mice. **A.** Flow-cytometric analysis of neonatal thymocytes from V γ 6⁺V δ 1⁺ TCRtg mice that were either *Aire*^{+/+} or *Aire*^{-/-}, and wild-type non-transgenic controls. Representative plots highlighting $\gamma\delta$ T cells (top), the V γ 6⁺ subset (middle) or IL-17A producers (bottom). **B.** Summary plot for IL-17A-producing cells (as per panel A, bottom row). Left, percentage; right, numbers. **C.** Flow-cytometric quantification of lymphocytes isolated from the eye or lacrimal gland of 5-week-old *Aire*^{-/-} mice that either did or did not carry the V γ 6⁺V δ 1⁺ TCR transgenes. Dot plot scales for this figure are all the same.

Fig. S3 (Associated with Fig. 6): $\alpha\beta$ T cells were not required for T $\gamma\delta$ 17 cell infiltration of parenchymal tissues. Summary plots for numbers of V γ 1,2,4,5⁺CD27⁻ (i.e. IL-17⁺V γ 6⁺-enriched) $\gamma\delta$ T cells from 7-week-old littermates that were either *Tcra*^{+/+} or *Tcra*^{-/-}.

Figure S1
Associated with Figure 4

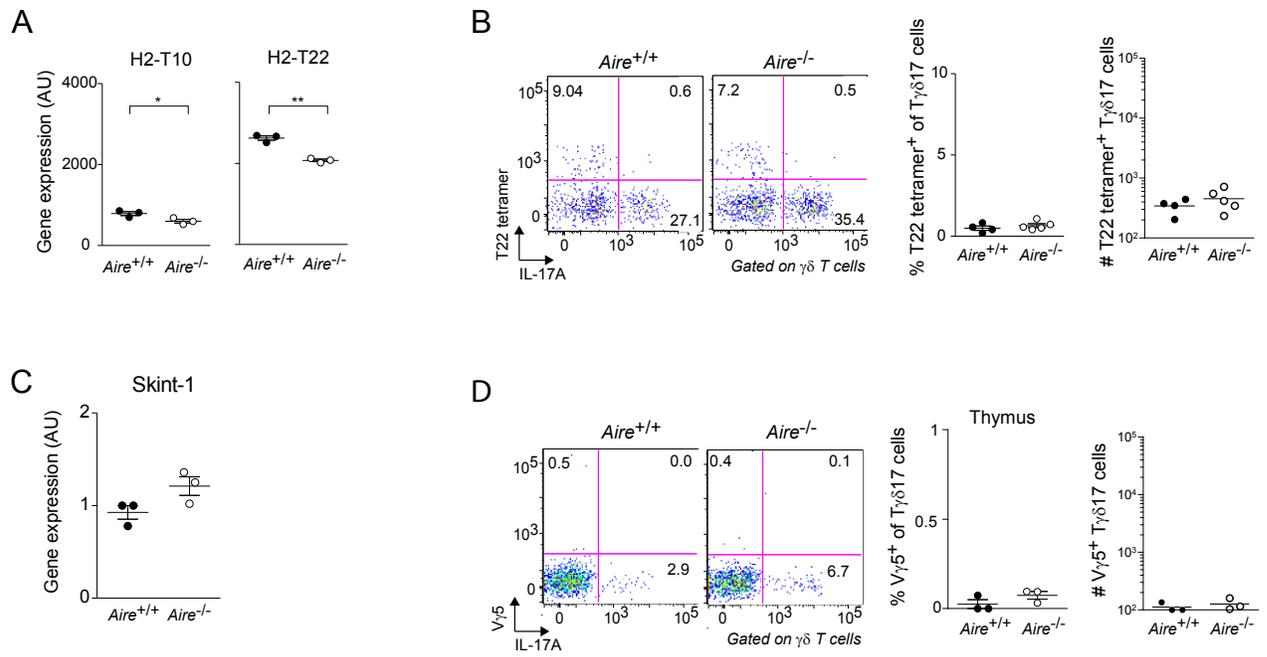


Figure S2
Associated with Figure 5

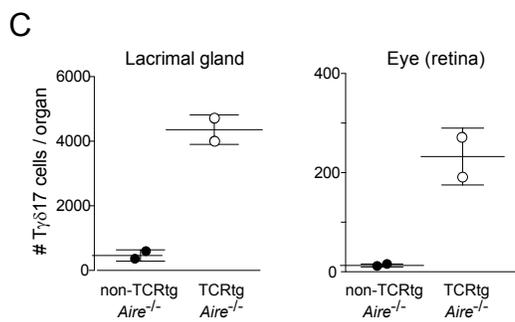
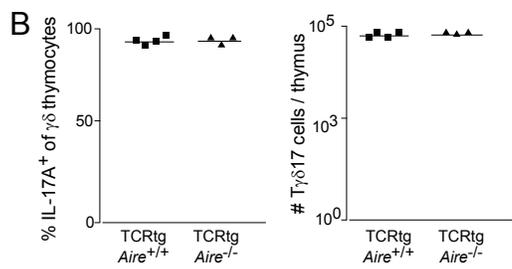
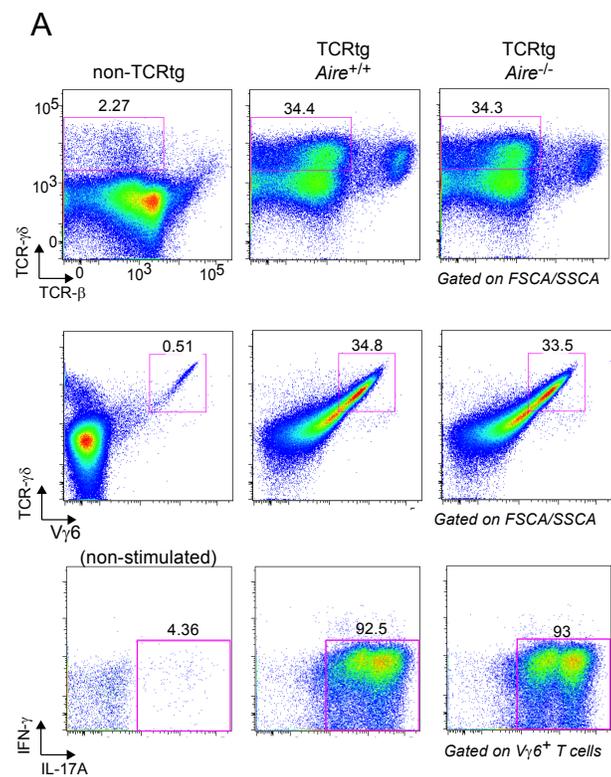


Figure S3
Associated with Figure 6

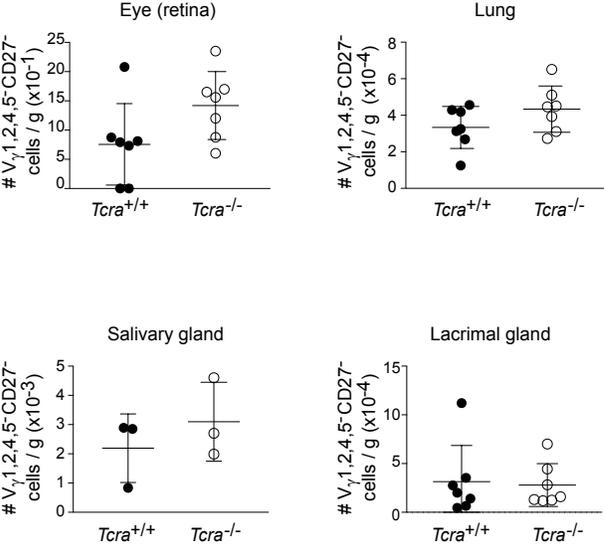


Table S1: V γ and V δ CDR3 sequences from *Aire*^{+/+} and *Aire*^{-/-} thymocytes Associated with Figure 3.

TCR γ	V	J	CDR3 length	CDR3 sequence	# sequences	
					<i>Aire</i> ^{+/+}	<i>Aire</i> ^{-/-}
V6	J1		11	ACWDSSGFHKV	70	57
Total					70	57

TCR δ	V	J	D	CDR3 length	CDR3 sequence	# sequences	
						<i>Aire</i> ^{+/+}	<i>Aire</i> ^{-/-}
V4	J2	D2		15	GSDIGGSSWDTRQMF	52	49
V4	J2	D2		18	GSDGSGRDGSSWDTRQMF	1	
V4	J2	D2		18	GSDVSGRGGSSWDTRQMF	1	
V4	J2	D2		16	GSDIGGSSSSWDTRQMF	1	
V4	J2	D2		15	GSDIGGSSWDTRPDV	1	
V4	J1	D2		14	GSDISEAYATDKLV	1	
V4	J2	D2		16	GSDIGGIRSWDTRQMF	1	
V4	J2	D2		15	GSEVGGSSWDTRQMF		1
V4	J2	D2		15	GSEIGGSSWDTRQMF		1
V4	J2	D2		15	GSDMGGSSWDPRQMF		1
V4	J2	D2		15	GSDIGGSSWDTRQKF		1
V4	J2	D2		15	GSDIGGSSWDTRQTF		1
V4	J2	D2		15	GSDFGGSSWDTGTVV		1
V4	J1	D2		13	GSDIDWRATDKLV		1
V4	J1	D2		13	GSDIDRRDTDKLV		1
V4	J1	D2		12	VSEYRRDTDKLV		1
V4	J1	D2		12	GSDMEGYTDKLV		1
V4	J1	D2		11	GSDRRDTDKLV		1
V4	J2	D2		18	GSDIDRRDTSSWDTRQMF		2
V4	J2	D2		15	GSDIGGSSWVTRQMF		2
V4	J2	D2		15	GSDIGGSSCDTRQMF		2
V4	J1	D2		14	GSDIGGIRATDKLV		5
Total						58	72

Table S2 : V γ and V δ CDR3 sequences from *Aire*^{+/+} and *Aire*^{-/-} peripheral cells. Associated with Figure 3.

TCR δ	V	J	D	CDR3 length	CDR3 sequence	# sequences	
						<i>Aire</i> ^{+/+}	<i>Aire</i> ^{-/-}
Lung (5 weeks)							
	V4	J2	D2	15	GSDIGGSSWDTRQMF	31	21
	V4	J1	D2	14	GSDMGGIRATDKLV	1	
	V4	J2	D2	15	GSEIGGSSWATRQMF		1
	V4	J1	D2	12	GSDMGYATDKLV		1
	V4	J2	D2	18	GSDIDRRDTSSWDTRQMF		1
	V4	J2	D2	15	GSDIGGSSCDTRQMF		1
	V4	J1	D2	12	GSDTEGYTDKLV		1
					Total	32	26
Eye (7 weeks)							
	V4	J2	D2	15	GSDIGGSSWDTRQMF	15	50
	V4	J2	D2	15	GSDRRDSSWDTRQMF	1	
	V4	J2	D2	15	GSDIGGSSWDTRQML		4
	V4	J2	D2	16	GSDIIGGSSWDTRQMF		1
	V4	J1	D2	14	GSDIGGIRATDKLV		1
	V4	J1	D2	14	GSDIGEIRAPDKLV		1
					Total	16	57
Eye (13-15 weeks)							
	V4	J2	D2	15	GSDIGGSSWDTRQMF	10	39
	V4	J2	D2	12	GSDRRDTTDKLV	1	
	V4	J2	D2	16	GSDIGGSSWDTRTDV		1
	V4	J1	D2	14	GSDISEGYATDKLV		1
	V4	J1	D2	12	GSGGIRATDKLV		1
	V4	J2	D2	15	GSDRRDSSWDTRQMF		1
	V4	J1	D2	12	GSDIGDATDKLV		1
	V4	J2	D2	15	GSDIGGSSWHTRQMF		1
	V4	J2	D2	15	GSDIRESSWDTRQMF		1
	V4	J2	D2	15	GSDIGGSSWDTRQTL		1
					Total	11	47

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Antibodies and flow cytometry – mice

Abs used for cytofluorometric analyses were: anti-TCR β (H57-597), -TCR $\gamma\delta$ (GL3), -IL-17A (TC11-18H10.1), -IFN- γ (XMG1.2), -CD24 (M1/69), -CD27 (LG.3A10), -V γ 1 (Vg1.1, 2.11), V γ 1+2 (V γ 1.1+1.2, 4B2.9), -V γ 4 (V γ 2, UC3-10A6), -V γ 5 (Vg3, 536), -CD45, -Ly51, -MHC-II (all from BioLegend); and anti-IL-7R (CD127, B12-1) (BD Pharmingen). The 17D1 hybridoma, kindly provided by Drs. Bob Tigelaar and Julia Lewis, specifically recognizes V γ 6⁺ TCRs after staining with the anti-C δ mAb, GL3, as described in (Roark et al., 2004). As a secondary Ab for 17D1, we used goat-anti-rat IgM (Jackson Immunoresearch Laboratories). T22-tetramers were generated by the NIH tetramer core facility. For intracellular staining of IL-17A and IFN- γ , cells were first stimulated in culture medium containing 10 ng/ml pokeweed mitogen antigen and 1 μ g/ml ionomycin for 3-4h at 37°C, 5% CO₂, in the presence of Brefeldin-A: BD GolgiPlug (BD Biosciences). After surface staining, cells were pre-fixed by using 4% paraformaldehyde/PBS, and permeabilized by using BD Cytotfix/Cytoperm (BD Biosciences) according to the manufacturer's protocol. Flow cytometric analysis was performed on an LSR II, sorting on a FACSAria (BD Bioscience) or a MoFlo (Beckman Coulter). Data were analyzed using FlowJo software (Tree Star).

Antibodies and flow cytometry – humans

Whole blood was harvested into EDTA tubes; red blood cells were lysed using BD FACS Lysing Solution (BD Biosciences); and staining for flow cytometry was performed using the following mAbs: FITC-conjugated anti-TCR $\alpha:\beta$ (clone WT31) (BD Biosciences), PE-conjugated anti-TCR $\gamma\delta$ (clone 11F2) (BD Biosciences), PE-Cy7-conjugated anti-CD3 (clone UCHT1) (eBioscience) and APC-eFluor780-conjugated anti-CD45 (clone HI30) (eBioscience). Staining was performed on ice for 30 min, and the cells were then washed with PBS. Data were acquired on a FACSanto (BD Biosciences).

To quantify IL-17A-producing cells, we isolated peripheral blood mononuclear cells (PBMCs) from whole blood of APECED patients and healthy donors using Lymphocyte Separation Medium (Lonza) per the manufacturer's instructions. PBMCs were cultured in RPMI 1640 with 10% FBS, 2mM L-glutamine, 100 U/ml of penicillin, and 100 μ g/mL of streptomycin (Gibco) at 37°C in a humidified 5% CO₂ incubator. Intracellular IL-17A staining was performed on PBMCs stimulated for 6 hours with PMA (20 ng/ml; Sigma) and ionomycin (2 μ M; Life Technologies) in the presence of Brefeldin A (10 μ l/ml; Sigma). Surface staining was performed using the following mAbs which were added for the final 30 minutes of culture before the fix and permeabilization step: PE-Cy7-conjugated anti-CD3 (clone UCHT1) (eBioscience), PerCP-eFluor710-conjugated anti-TCR $\gamma\delta$ (clone B1.1) (eBioscience), APC-eFluor780-conjugated anti-CD4 (clone PA-T4) (eBioscience), FITC-conjugated anti-V δ 2 (clone B6) (BioLegend) and APC-conjugated anti-V γ 9 (clone B3) (BioLegend). Cells were washed with PBS and then fixed and permeabilized with Foxp3 Staining

Set (eBioscience) according to the manufacturer's instructions. Cells were then incubated with PE-conjugated anti-IL-17A (clone eBio64CAP17) (eBioscience) for 1 hour at 4°C, and washed with PBS. Data were acquired on a FACSCanto (BD Biosciences).

SUPPLEMENTAL REFERENCES

Roark, C.L., Aydintug, M.K., Lewis, J., Yin, X., Lahn, M., Hahn, Y.S., Born, W.K., Tigelaar, R.E., and O'Brien, R.L. (2004). Subset-specific, uniform activation among V gamma 6/V delta 1+ gamma delta T cells elicited by inflammation. *J. Leukoc. Biol.* 75, 68-75.