SUPPLEMENTAL MATERIAL

Cohen et al., http://www.jem.org/cgi/content/full/jem.20092465/DC1

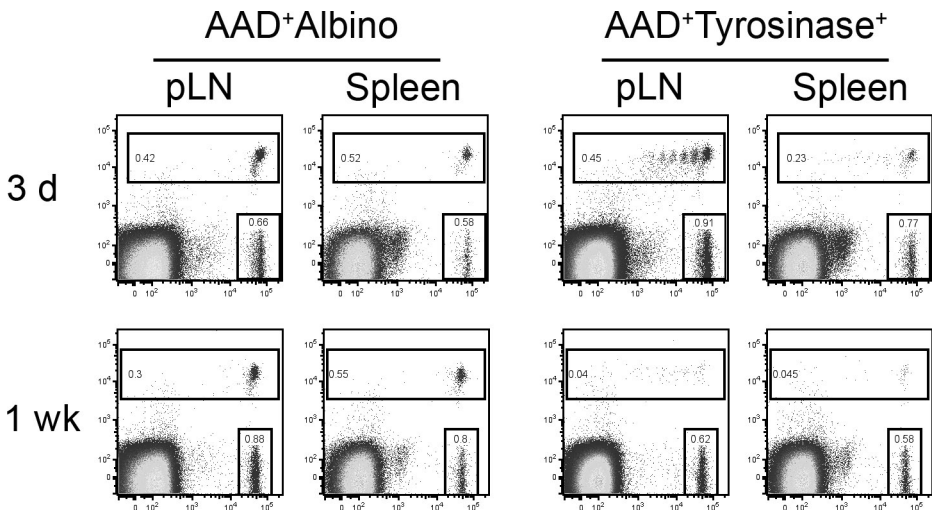

Figure S1. FH cells undergo activation and deletion in AAD+Tyrosinase+ but not AAD+ albino recipients. 10^6 CD8-enriched Thy1.2+ FH and control Thy1.1+ cells were CFSE-labeled and adoptively transferred into AAD+ albino or AAD+Tyrosinase+ recipients. At 3 and 7 d after transfer, pooled LNs and spleens were harvested and stained for CD8 and Thy1.2 and assessed for CFSE dilution. Data are representative of four independent experiments with two mice for each condition.

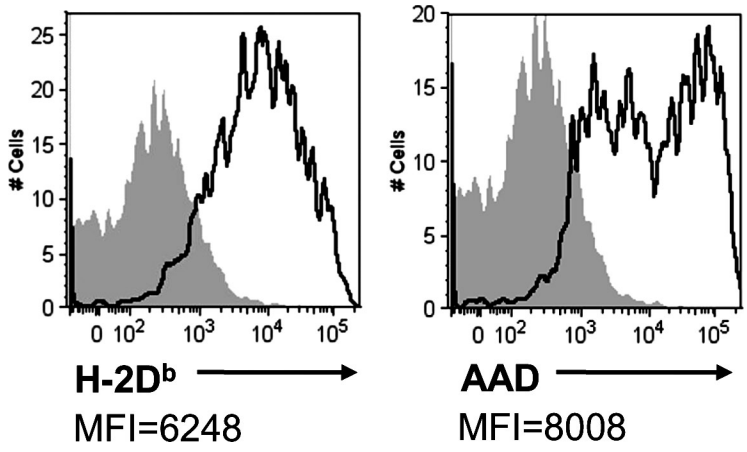

Figure S2. Expression of AAD and H-2D^b molecules on LN stromal subpopulations. Gp38+CD31+ lymphatic endothelial cells were surface stained for expression of H-2D^b and AAD. Pooled peripheral and mesenteric LN of AAD+ mice were digested and LN stromal cells negatively selected using CD45 beads (Miltenyi biotec). CD45^{neg} cells were stained with mAbs against gp38 (8.1.1; Developmental Studies Hybridoma Bank), CD31 (eBioscience), H-2D^b (eBioscience), and anti–HLA-A2 (α1 and α2 domains of AAD; clone CR11-351). Secondary reagents used were anti–goat FITC (Jackson ImmunoResearch Laboratories) and anti–mouse PE (BD).

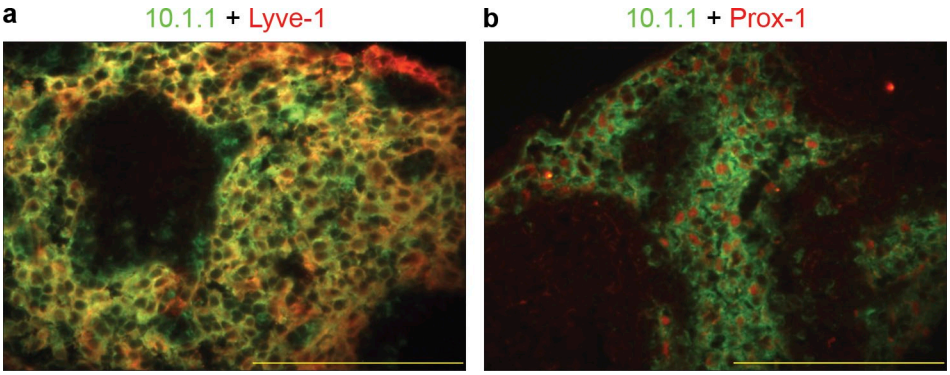

Figure S3. 10.1.1⁺ cells in LN are LEC based on costaining with additional LEC-specific markers. Immunofluorescence microscopy of representative LN medullary sections stained with antibodies against 10.1.1 and the LEC markers Lyve-1 (a) and Prox-1 (b; 400×). Tissue sections were blocked with PBS containing 3% H_2O_2 and 0.1% NaN_3 and stained with 10.1.1, followed by FITC anti-Syrian hamster IgG (Jackson ImmunoResearch Laboratories). Fluorescein and biotin tyramide signal amplification (PerkinElmer) was performed as per the manufacturer's instructions. Tissue sections were then permeabilized and stained with unlabeled anti-Lyve-1 (Millipore) or anti-Prox-1 (Abcam), followed by biotinylated anti-rabbit IgG (Vector Laboratories) and Texas red streptavidin (SouthernBiotech). Staining is representative of multiple magnifications and fields from one experiment consisting of two separate LNs from one mouse. Bars, 400 µm.

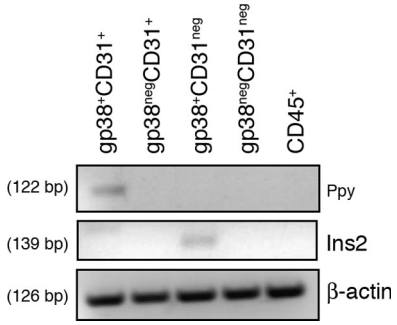

Figure S4. Expression of pancreatic polypeptide and preproinsulin 2 PTA in LN stromal cell subsets. Pancreatic polypeptide (Ppy), preproinsulin 2 (Ins2), and β-actin mRNAs were amplified by 40-cycle RT-PCR from the indicated subpopulations of LN stromal cells isolated from C57BL/6 mice. Primers used were the following: pancreatic polypeptide forward, 5′-GACTATGCGACACCTGAGCA-3′, and reverse, 5′-CCAGGAAGTCCACCTGTGTT-3′; preproinsulin 2 forward, 5′-ACCTGGTGGAGGCTCTCTA-3′, and reverse, 5′-AAGGTCTGAAGGTCACCTGCT-3′; and β-actin forward, 5′-ACGTAGCCATCCAGGCTGTG-3′, and reverse, 5′-TGGCGTGAGGGAGAGCAT-3′. Data are representative of two independent experiments.

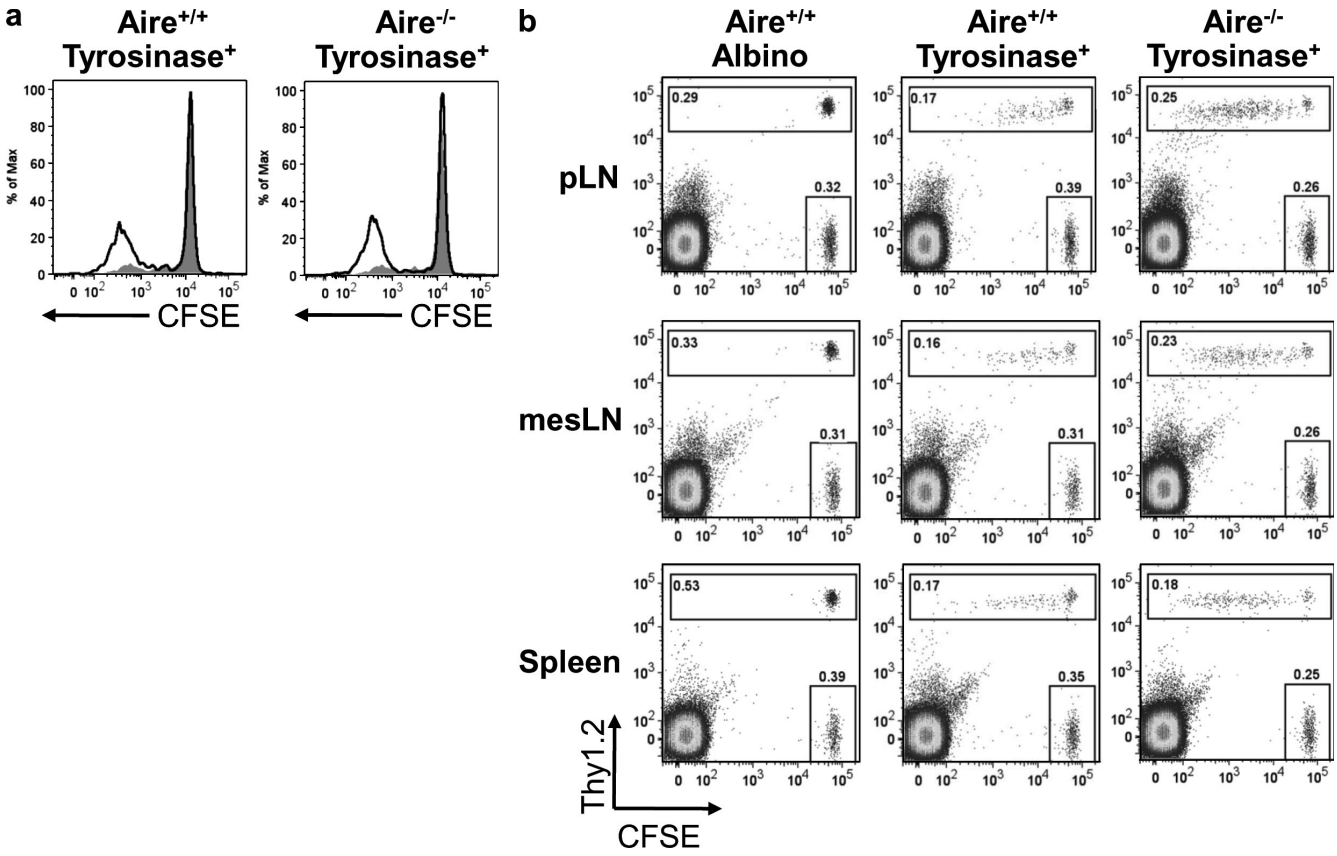

Figure S5. Aire is not required for the presentation of the Tyr_{369} to FH T cells. (a) Proliferation of CFSE-labeled naive Thy1.2$^+$ FH T cells co-cultured with Thy1.1$^+$ lymphatic endothelial cells from albino (gray), tyrosinase$^+$, and Aire$^{-/-}$ animals (solid lines) was assessed at 86 h. Data are representative of one independent experiment. (b) 10^6 CD8-enriched Thy1.2$^+$ FH and control Thy1.1$^+$ cells were CFSE labeled and adoptively transferred into AAD$^+$albino, AAD$^+$Tyrosinase$^+$, or Aire$^{-/-}$AAD$^+$Tyrosinase$^+$ recipients. At 4 d after transfer, pooled peripheral LNs (pLNs), mesenteric LNs (mesLNs), and spleens were harvested and stained for CD8 and Thy1.2 and assessed for CFSE dilution. Data are representative of one independent experiment with two mice for each condition.