Supplementary Materials and methods

Primary cell cultures

Primary human thymic epithelial cell (TEC) cultures were established following the protocol previously described in several published manuscripts (40, 41, 86). Briefly, after removal of the thymic capsule, fresh human thymic tissue was mechanically minced with scissors in Hanks buffer to obtain 0.5 mm explants. Explants were washed twice with hanks buffer to remove as much as possible thymocytes. Explants were set down onto 75 cm2 flasks for 20 min to allow proper adhesion on the flask surface, and grown in RPMI 1640 medium (Invitrogen) supplemented with L-glutamine, Ultroser-g and 20% of horse sera. Minor changes to the published model are provided, the replacement of horse serum by charcoal-treated SVF and the RPMI medium used was a phenol free RPMI medium, in order to avoid phenol hormone-like effects. The percentage of medullary epithelial cells was assessed throughout the culture. Preliminary experiments indicated that the optimal time for high mTEC percentage was between days 6 and 8. As an example of a representative experiment the percentage of mTEC was estimated by immunofluorescence using an anti-Claudin 4 and anti-Keratin 5/14 antibodies, both known as specific markers of mTEC (19, 87, 88) and was over 85% (**Supplemental Figure S6**). The other cells were either contaminating fibroblasts or residual thymocytes.

Thus, all experiments were performed at day 7, in order to achieve experiments only with high enriched mTECs. Under a percentage of 80% cells being Keratin 14 positive, the cell culture was discarded. After 7 days of culture, mTECs were trypsinized and transferred in 24-well plates at 10^5 cells/well. The cells were let to attach overnight and were then treated with several doses of sex hormones (estrogen, testosterone, DHT or progesterone) for 24 hours.

Isolation of murine thymic epithelial cells

Murine thymuses were prepared from 6 to 9 weeks old C57BL/6 mice (Janvier Laboratory, Saint Berthevin, France), as previously described by Gray et al. (24). Connective and fat tissue were removed from each individual thymus and the capsule was incised with fine scissors. Thymuses were then cut in RPMI-1640 (Life Technologies, Carlsbad, USA), to slowly remove as many thymocytes as possible. The supernatant was collected and replaced as it became visibly cloudy and kept on ice. Thymuses were then incubated at 37 °C for 5 minutes in 5 ml of RPMI-1640 containing 0.15% (w/v) liberase DH and 0.1% (w/v) DNAse I (Roche, Meylan, France). A regular and gentle agitation was performed. Fragments were allowed to settle. The supernatant was collected, kept on ice, and the digestion repeated with fresh enzyme mixture using the remaining settled thymic fragments. After 3 digestions, the remaining aggregates were incubated for 10 min with 5 ml of RPMI-1640, containing 0.125% (w/v) Collagenase/Dispase (Roche, Meylan France) and 300 µl of 0.1%(w/v) DNAse I. Cells from all supernatant fractions were then centrifuged at 472 ×g for 5 min, pooled, and resuspended in cold EDTA (5mM EDA/PBS with 5% FCS). Cells were filtered through 70 µm mesh and then stained. mTECs were sorted on a FACSAria II (Becton Dickinson, Le Pont de Claix, France) according to the phenotype $CD45^-$ $MHC^+Ly51^-UEA^+$ as described by Gray et al (24).

The staining was performed with fluorescein coupled ulex europaeus agglutinin I (UEA I) (Vector Laboratories, Burlingame, USA), RPE coupled-anti-mouse Ly-51 (clone 6C3/BP-1; Biolegend, San Diego, USA), RPE-Cy7 coupled anti-mouse I-A/I-E (clone M5/114.15.2, Biolegend, San Diego, USA), APC coupled anti-mouse CD45 (clone 30-F11, Becton Dickinson, Le Pont de Claix, France), and Live/Dead fixable IR dead markers (Becton Dickinson, Le Pont de Claix, France).

Fetal Thymus Organ Culture (FTOC)

Fetal mouse thymuses were extracted from wild type C57BL6 fetal mice at gestational day 12 (GD12). Thymic lobes were placed on membrane filters (Millipore) in petri dishes with 3 ml complete medium (89) containing complete RPMI (Invitrogen, Carlsbad, CA) containing 12% FCS (HyClone, Logan, UT), 100 U/ml penicillin and streptomycin (Invitrogen, Carlsbad, CA), and 50 µ Logan, UT), 100 U/ml penicillin and streptomycin. After 7 days of culture, thymuses were treated with several doses of estrogen for 24 to 72 hours.

NSG mouse experiments

To generate xenografts, 4 fragments of human thymuses from immunologically normal patients (less than 1 year old) were grafted s.c. into both flanks of 6 week-old female severe combined immunodeficient (NOD Scid gamma C-/- (NSG)) mice provided by Janvier Laboratory (Saint Berthevin, France). The treatment began the day of the graft. To assess estrogen effects, we injected daily in intra-peritoneal, 200 µg of E_2 (Sigma-Aldrich, Lyon, France). At day 4, mRNA expression level in two grafted human thymic explants was analyzed. Control mice were injected with vehicle daily.

Flow cytometry

For cytometry analysis of the Aire protein in mTECs, we used the protocol described by Monte et al. (90) for optimal staining in the nucleus. Nuclei were isolated from cultured mTECs in cold nuclei extraction buffer (320 mM sucrose, 5 mM $MgCl_2$, 10 mM HEPES, 1% Triton X-100 at pH 7.4). Cells were gently vortexed for 10 seconds and allowed to incubate at 4°C for 10 minutes. Nuclei were then pelleted by centrifugation at 2000 g and washed twice with nuclei wash buffer (320 mM sucrose, 5 mM MgCl2, 10 mM HEPES at pH 7.4). Nuclei quality and integrity were confirmed by microscopic examination with trypan blue staining. Nuclei were

incubated overnight with uncoupled goat anti-AIRE (clone D17, sc-17986, Santa Cruz Biotechnologies, Heidelberg, Germany) and washed with nuclei wash buffer. Aire staining was detected by PeCy7 coupled rabbit anti goat IgG (Dako, Trappes, France). The labeling was performed in nuclei wash buffer supplemented with 1% BSA and 0.1% sodium azide, at 4°C. Nuclei were analyzed on FACSVerse using the Facs suite software (Becton Dickinson, Le Pont de Claix, France).

Immunofluorescence microscopy

Cortical and medullary thymic surface analyses

Cryostat serial sections (7 µm) of frozen mouse and human thymic tissues were affixed to glass superfrost slides, dried overnight, and stained by hematoxylin. The whole slide pictures were done using Axio Vision software (Carl Zeiss, Le Pecq, France). Quantification of medulla and cortex surfaces was obtained with Image J software.

Quantification of Aire expressing cells

To quantify Aire-positive cells in mouse and human thymuses, frozen thymic sections were incubated with a goat anti Aire antibody (clone D-17, sc-17986, Santa Cruz Biotechnologies, Heidelberg, Germany) and a rabbit anti-K14 antibody (Eurogentec, Seraing, Belgium) for 2 hours at room temperature. The labeled cells were revealed with Alexa 488 coupled chicken anti goat IgG and Alexa 594 coupled donkey anti rabbit IgG.

For each thymus, $AIRE^+$ cells were counted in the medullary area on the whole section (about 20-25 mm^2) as shown in **supplemental Figure S2**. Since the number of K14+ cells was generally higher than 3000 in the thymic entire section, these cells were not counted in the whole section but in four different representative medullary areas and then normalized to the whole section. The surface of these areas, as well as the whole medullary area, were estimated by image

j analysis. The number of K14+ cells in the whole medullary area of the section was calculated as follows: number of K14+ cells/surface of the zones counted multiplied by the total medullary surface. The number of AIRE+ cells was then normalized to the number of K14+ cells in the whole section. Alternatively, the number of AIRE+ cells was calculated per mm^2 of thymic section or per mm^2 of thymic medulla. All countings of AIRE+ cells were done in blind by the same experimenter directly on the microscope in order to be able to change the focus, when required. The individual number of AIRE+ cells from males and females is detailed for humans and mice in **supplemental Tables S2 and S4,** respectively. Of note, due to ageing process, in adult human thymuses, the thymic medulla was dispersed and its surface could not be correctly evaluated. In this case, the normalization was done to the thymic section total surface only.

Quantification of cell infiltrates in the thyroid

To quantify cell infiltration in mouse thyroids, we analyzed CD8 T cell infiltrates in mouse thyroids (54). Frozen thyroid sections were incubated with a rat anti-mouse CD8 antibody (Clone: 53-6.7, Becton Dickinson, Le Pont de Claix, France) for 2 hours at room temperature. The labeled cells were revealed with a donkey anti-rat IgG coupled to an Alexa 594. Frozen thyroid sections were also incubated with a rat anti mouse F4/80 (glycoprotein express in mouse macrophage) antibody (Clone: BM8, eBiosciences, San Diego, USA) for 2 hours at room temperature. The labeled cells were revealed with Alexa 488 coupled donkey anti rat IgG. In the thyroid, F4/80 positive cells were counted and normalized per thyroid surface unit. Images were acquired with a Zeiss Axio Observer Z1 Inverted Microscope using 20X magnification. IHC analyses in the mouse thyroids were done in blind.

Demethylation and deacetylation experiments

Primary human TECs were seeded at 2.10^5 cells per well in a 12 well-plate, incubated for 24h in RPMI 1640 medium with 5% charcoal-treated serum, and then supplemented with fresh medium containing 5-aza-2'-deoxycytidine (10 µM) (Sigma-Aldrich, Lyon, France) or trichostatin A (50 nM) (Sigma-Aldrich, Lyon, France) for 24h.

DNA isolation and bisulfite genomic DNA sequencing

The methylation status of CpG sites of AIRE human promoters was performed on bisulfite treated genomic DNA. Genomic DNA was extracted from cultured primary human TECs using the DNeasy Qiagen kit following the manufacturer's instruction. The DNA was bisulfite-treated and purified using the Epitect Bisulfite kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. The AIRE promoter region was amplified as previously reported by Murumagi et al. (44). The amplified DNA fragment was purified using QIAquick Gel Extraction kit (Qiagen, Courtaboeuf, France) and sequenced.

Silencing Aire gene transcript

To reduce AIRE levels, we took advantage of the BLOCK-iTTM Pol II miR Invitrogen system in order to generate plasmid vectors expressing artificial miRNA which were engineered to have 100% homology to mouse AIRE, resulting in efficient AIRE mRNA cleavage. Using the BLOCK-iT RNAi Designer, three BLOCK-iT™ Pol II miR RNAi sequences which target mouse AIRE (NM_009646.1) were designed. One negative miRNAi control (CTL-miRNA) was also synthesized. Sequences are listed in **supplemental table S5.** Each sequence was cloned into the pcDNA™ 6.2-GW/EmGFP-miR vector, in order to allow co-cistronically expression of miRNA and EGFP, regulated by the CMV promoter (BLOCK-iT Pol II miR RNAi Expression Vector Kits user manual; Invitrogen).

To test the downregulation efficiency of each artificial miRNA and to select the most *e*fficient miRNA expression clone, proliferating cells were transfected with the Lipofectamine 2000 transfection reagent (Life technologies, Carlsbad, USA) following manufacturer's instructions. Recombinant sc AAV9 carrying the AIRE-850-miRNA, AIRE 288-miRNA or the CTL-miRNA were produced using the tri-transfection method, as previously described by Duque et al. (50).

Briefly, each scAAV9 was produced by helper virus-free three-plasmid transfection in HEK293 cells, using the adenovirus helper plasmid, the AAV packaging plasmid encoding the rep2 and cap9 genes (p5E18-VD2/9) and the AAV2 plasmid expressing the miRNA under control of the CMV promoter. The viruses were purified by ultracentrifugation on an iodixanol density gradient. Viral preparation was desalted and concentrated using Amicon Ultra cell 100 K filters units (Millipore, Billerica, USA). Aliquots were stored at -80°C until use. Vector titers were determined by real-time PCR and expressed as viral genomes per milliliter (vg/ml).

RNA extraction

Thymuses were homogenized with the FastPrep FP120 instrument (Qbiogen, Illkirch, France). Total RNA was prepared from the thymus and TECs using the trizol RNA Isolation kit (Invitrogen, Cergy-Pontoise, France). The quality and concentration of RNA were analyzed with a NanoDrop ND-1000 spectrophotometer (LabTech, Palaiseau, France). RNA samples presenting a minimal ratio of 1.9 and 2 for respectively 260/280 and 260/230 were also controlled on a gel. When the samples were degraded even partially, they were excluded.

Real-Time PCR

Total mRNA was reverse-transcribed using the SuperScript II RT (Invitrogen Cergy-Pontoise, France) according to the manufacturer's instructions. PCR reactions were performed using the LightCycler apparatus as previously described (42). The mRNA expression levels were

measured on thymuses (human and mouse) and thymic epithelial cells. The primers used for the real-time PCR are listed in **supplemental table S6**. Each RNA sample was run at least in duplicate. For Aire analysis in the human thymus, the experiments were repeated three times. Mouse and human samples were normalized as specified on the figure legends.

Experimental Autoimmune Thyroiditis

EAT were developed as previously described (47). Female and male SJL mice, 8-9 weeks old, were challenged (s.c.) at the base of the tail with 100 nmol of p2340 in complete Freund's adjuvant emulsion (Sigma-Aldrich, Lyon, France) and boosted 21 days later with 50 nmol of peptide in incomplete Freund's adjuvant emulsion (Sigma-Aldrich, Lyon, France). The p2340 hTg peptide (QVAALTWVQTHIRGFGGDPR) was synthesized by Genecust Europe (Dudelange, Luxembourg). P2340 N and C-terminals were respectively blocked with an acetyl and an amide group.Once a week after the initial challenge, the serum was collected for ELISA. The thyroids were removed for EAT assessment at day 30 after the first challenge.

Elisa

96 well plates (Nunc, Langenselbold, Germany) were coated overnight at 4°C with 5 mg/ml of peptide p2340 in 100 ml of carbonate/bicarbonate buffer 0.1 M, pH 9.6. After washing, the wells were blocked 2 hours with 0.1% BSA in PBS. Mouse sera (1/100) were incubated for 90 min at room temperature and, subsequently, 1/10,000 diluted biotinylated anti-mouse IgGs (Dako, Trappes, France) and streptavidin-horseradish peroxidase (Dako, Trappes, France) were added. Tetramethylbenzedine (Sigma-Aldrich, Lyon, France) was used for color development. The O.D. was measured at 405 nm using a Dynatech MR5000 microplate reader (Dynatech, Chantilly, VA).

Supplementary Materials:

Figure S1. Compared analysis of thymic gene expression in men and women by microarray. Expression ratios of putative AIRE-dependent TSA genes spotted on the arrays. Each dot corresponds to the median of ratios of replicates (five for women or four for men) for a given gene. The lists of genes are given in supplementary table 1. P values were obtained using the Wilcoxon paired test.

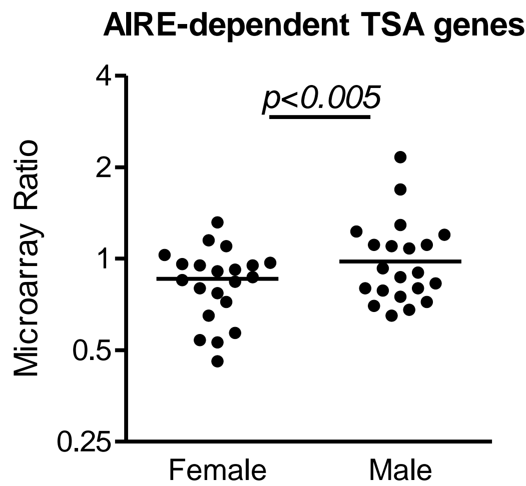
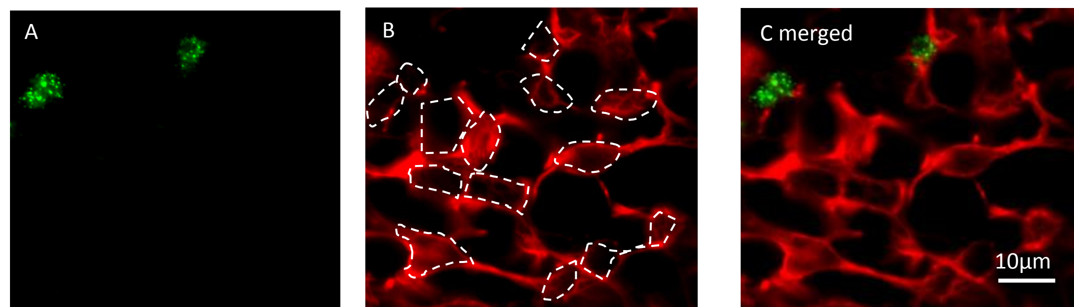


Figure S2. Immunofluorescence analysis of AIRE and K14 cells in human thymic section.

(A) Total number of $AIRE^+$ cells and $K14^+$ cells were counted by immunofluorescence in pubescent human thymic whole section as described in Material and Methods.

Briefly, frozen human thymus sections were co-labeled with goat anti-AIRE (a) and rabbit anti-keratin-14 (b) antibodies. The labeled cells were clearly identified using Alexa 488 coupled chicken anti-goat IgG and alexa 594 coupled donkey anti-rabbit IgG. Images were acquired with a Zeiss Axio Observer Z1 Inverted microscope. The number of $AIRE^+$ cells among medullary $K14^+$ epithelial was evaluated.



(B) According to the counting method described above, the percentage of $AIRE^+$ cells in $K14^+$ cells in thymic medulla was significantly decreased in pubescent female (n=8) compared to pubescent male (n=8) while it was similar in male and female children. These data corroborate what has been observed in whole thymus section (**Figure 1E**) or in thymic medulla area (**Figure 1F**). P values were obtained using the Mann-Whitney test. ** *p<0.01*

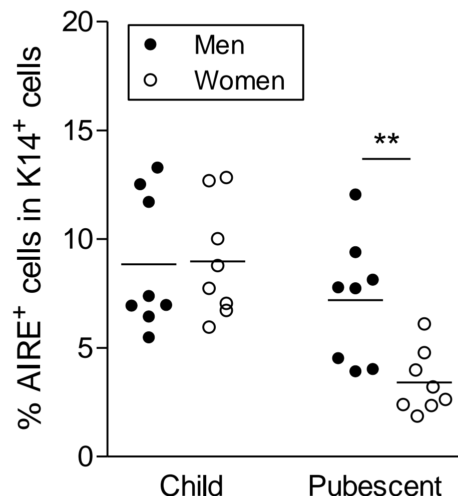


Figure S3. Representative medullary areas of thymic sections from male and female SJL mice.

Frozen mouse thymus sections were co-labeled with goat anti-Aire and rabbit anti-Keratin-14 antibodies. The labeled cells were revealed with Alexa 488 coupled chicken anti-goat IgG (green) and alexa 594 coupled donkey anti-rabbit IgG (red). Images were acquired with a Zeiss Axio Observer Z1 Inverted Microscope.

Here are shown representative medullary areas of thymic sections from 3 male and 3 female mice. The number of $Aire^+$ cells counted in $K14^+$ cells in each medullary area is given in the figure.

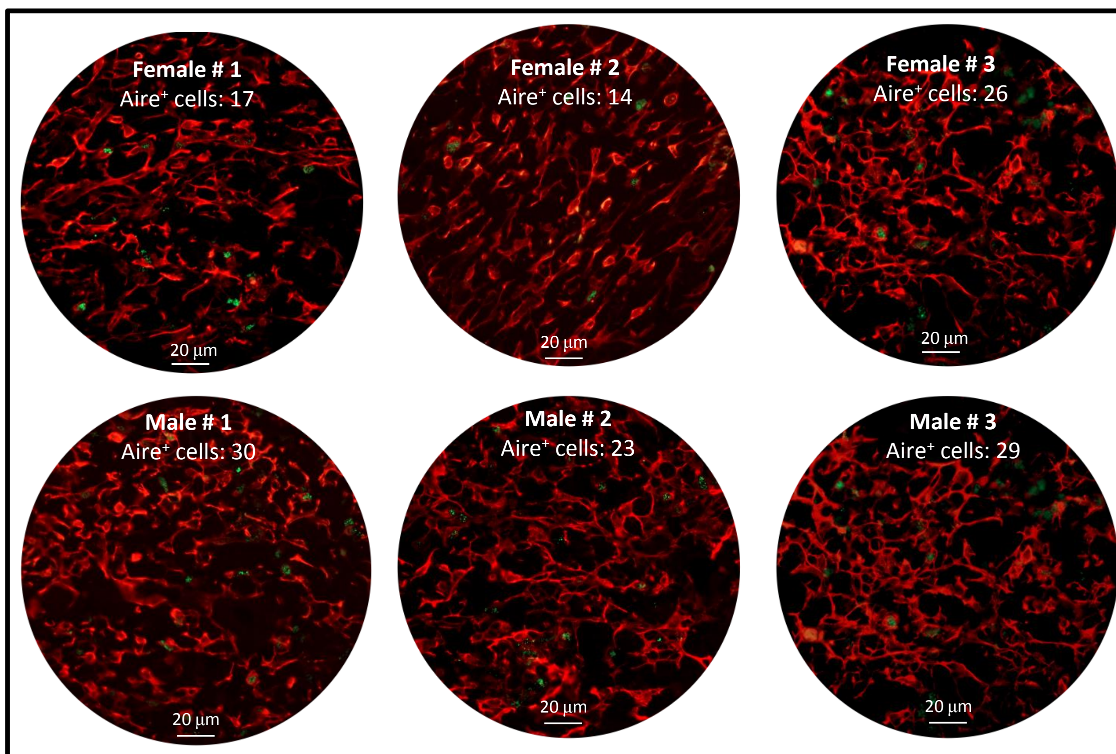


Figure S4. Histological and immunofluorescence analyses of C3H mouse thymuses. Number of Aire^{+} cells per thymic area (A) or per thymic medulla area (B) of C3H control male and female mice. P values were obtained using the Mann-Whitney test.

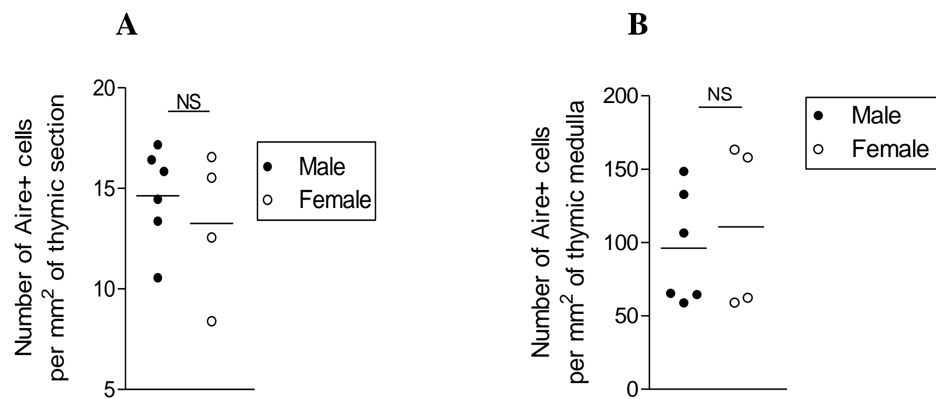


Figure S5. Representative plot of FACS-sorted murine mTECs. Murine thymic stromal cells were isolated enzymatically and mTECs were sorted on a FACSAria II (Becton Dickinson, Le Pont de Claix, France) according to the phenotype $CD45^{-}MHC^{+}Ly51^{-}UEA^{+}$ as described by Gray (2008).

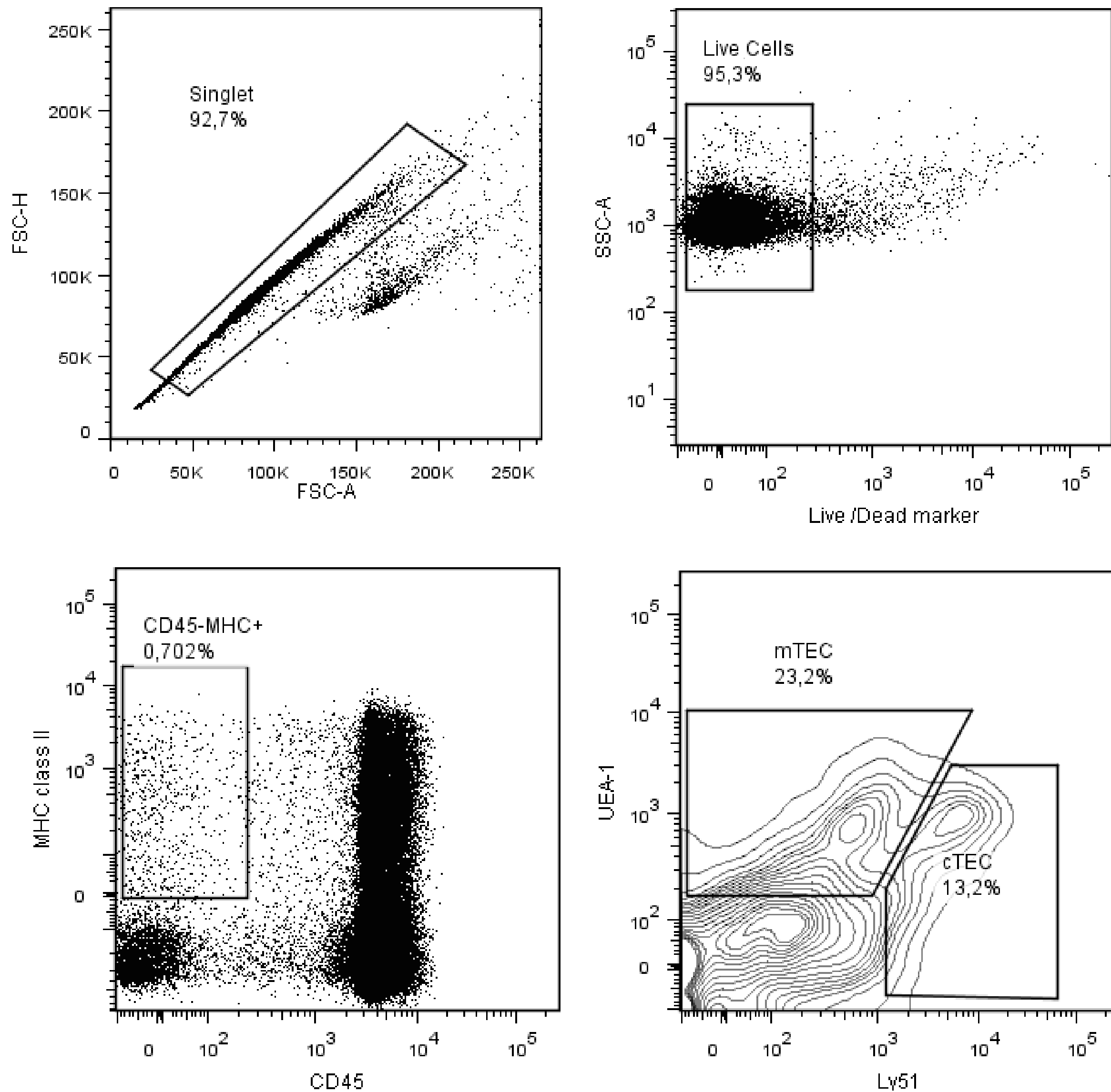


Figure S6. Histological and immunofluorescence analyses of SJL mouse thymuses after castration. Percentage of medullary area (A) and number of $K14^+$ cells (B) per thymus section of SJL control and castrated male and female mice. Thymic Aire mRNA expression level (C), number of $Aire^+$ cells per thymic area (D) or per thymic medulla area (E) of SJL control and castrated female mice. P values were obtained using the one-way ANOVA test (A-B) and the Mann-Whitney test (C-E). Although the medullary area was significantly decreased in thymus of castrated males, the total number of $K14^+$ cells was not affected suggesting that castration did not change the medullary epithelial cell content.

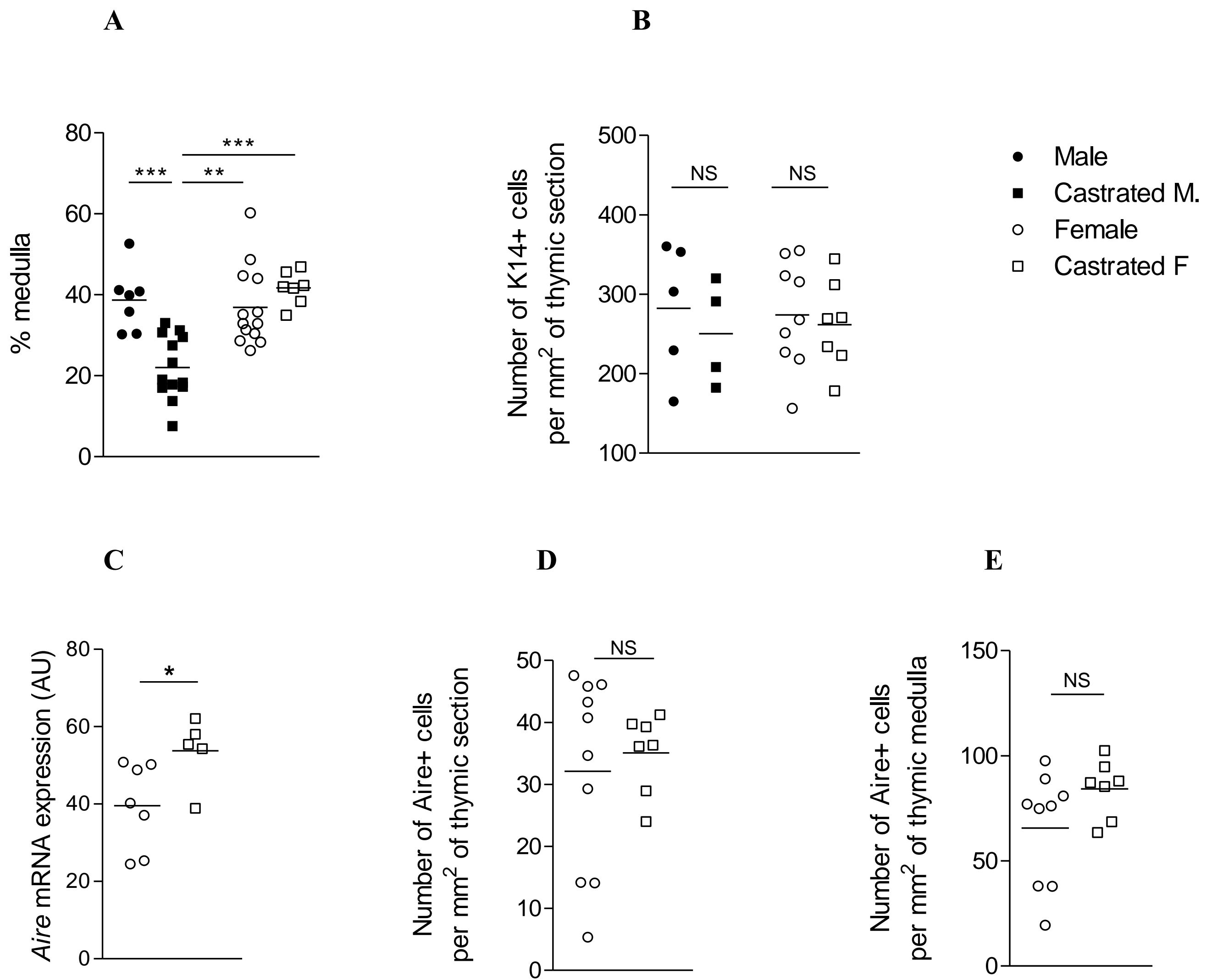
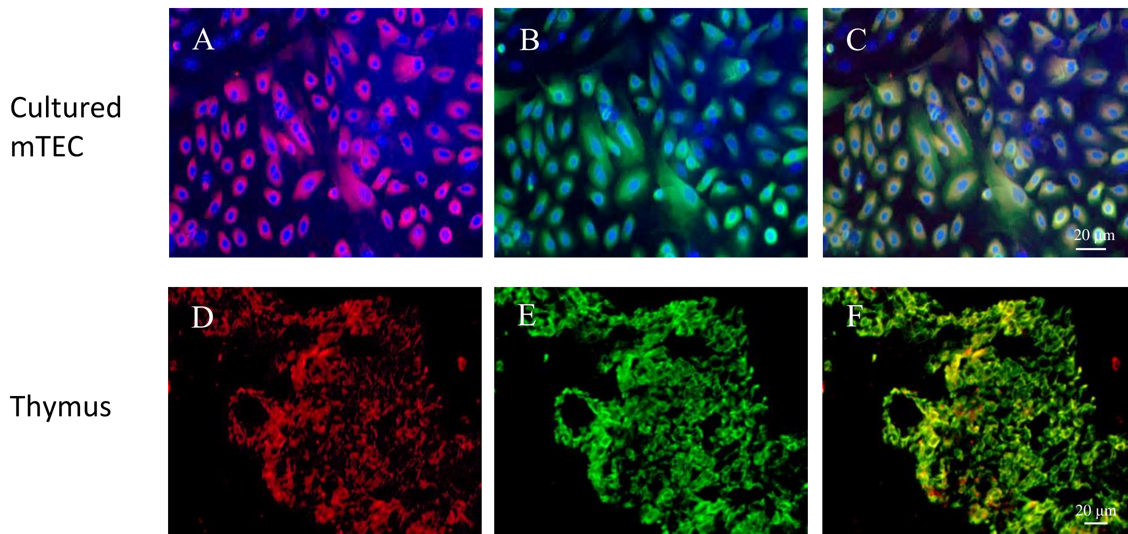


Figure S7. Immunohistochemical analysis of cultured primary human mTECs at day 7. Primary human mTECs and thymic frozen sections were acetone fixed and co-labeled with mouse anti-Claudin 4 (A and D, respectively) and rabbit anti-keratin-14 antibodies (B and E, respectively). Labeled cells were revealed with Alexa 488 coupled chicken anti-mouse IgG and Alexa 594 coupled donkey anti-rabbit IgG. The nucleus of mTECs were stained with the DAPI (blue) (A-C). Merged images are shown in C and F for mTEC and thymic sections respectively. Images were acquired with a Zeiss Axio Observer Z1 Inverted Microscope. ImageJ software was used to display the digital pictures and to count manually the labeled cells. As expected and corroborating published data regarding the human primary cultured mTEC model (88, 91), most cultured cells (A-C) expressed the same markers as the TEC located in the thymic medullary area (D-F), validating hence our primary cell culture mTEC as a model of mTEC culture. The following table summarizes the immunohistochemical analysis of the culture mTECs (**Figures S7A-C**) including the total number of cells, the number of K14+ cells and that of Claudin 4+ cells in the microscope field.



		Number of cells in the field	% of cells
Fig S7	**Total cells**	102	100

Fig S7A	Claudin 4^{+} cells	93	91,18
Fig S7B	Keratin 14^{+} cells	95	93,14

Figure S8. Histological and Immunofluorescence analyses of ERα $^{-/-}$ thymuses. Percentage of medullary area (A) and number of Keratin 14^{+} cells (B) in medullary area per thymus section in Erα-/- male and female. In deficient mice for estrogen receptor α, the percentage of medullary area and the number of K14^{+} cells in mouse thymuses were similar in males and females. P values were obtained using the Mann-Whitney test.

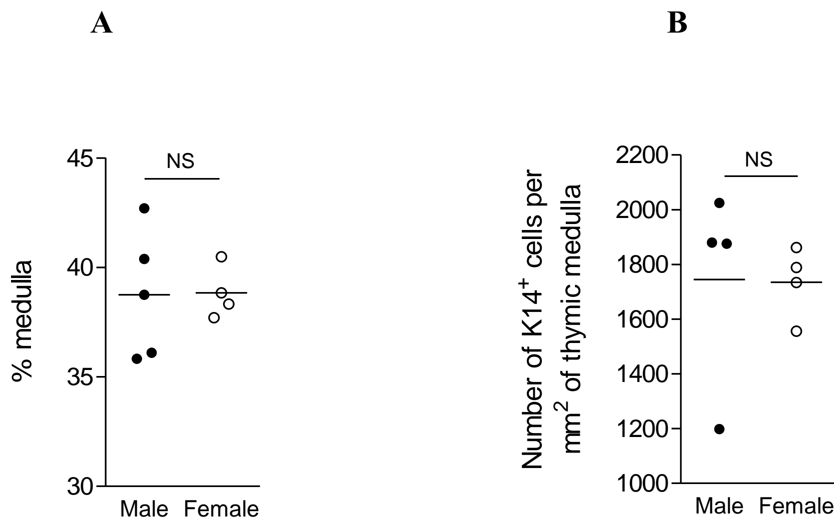


Figure S9. Cell transfection with Silencing Aire RNA. Aire mRNA expression was analyzed by real-time PCR in cells transfected with 3 different Aire artificial miRNA. The Aire silencing RNA 288 and 850 induced a significant decrease in Aire expression and thus were used for in vivo experiments.

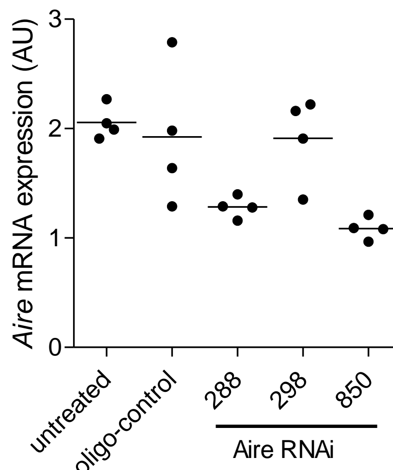


Figure S10. Immunohistochemical analysis of SJL mouse thymus treated with AAV vectors containing Aire iRNA 850. Male SJL mice, 7 weeks old, received a 10µl intrathymic injection of a vector solution (8 to 10.10^{12} vg/ml). Frozen thymus sections were co-labeled with an anti-GFP antibody for AAV-miRNA (A) and a rabbit anti-keratin-5/14 antibody for medullary thymic epithelial cells (B). Images were acquired with a Zeiss Axio Observer Z1 inverted microscope. As expected, the AAV-miRNA infected the stroma thymic cells among them the medullary thymic epithelial cells.

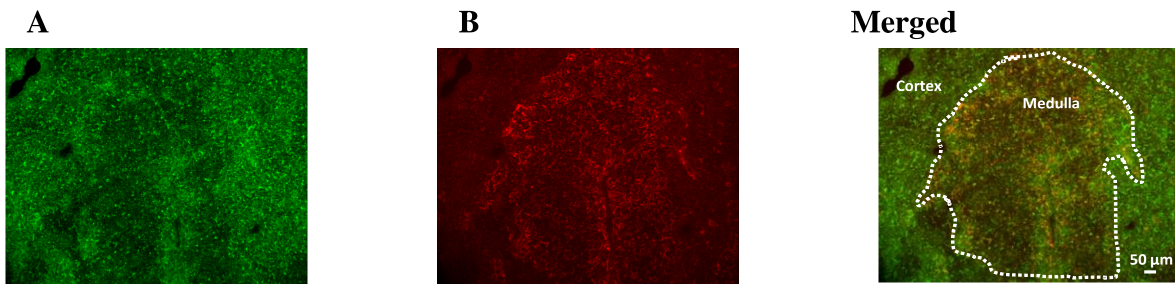


Figure S11. **Quantification of cell infiltration in mouse thyroids.** The number of infiltrating F4/80 positive cells was counted and normalized per surface of thyroid (n=4-6 mice per group). The number of F4/80 infiltrating cells was lower in males compared to females, but increased in male mice treated with Aire RNAi compared to control RNAi. These results suggest that decreased thymic Aire expression may favor thyroid cell infiltrations in SJL mice immunized with p2340 thyroglobulin peptide.

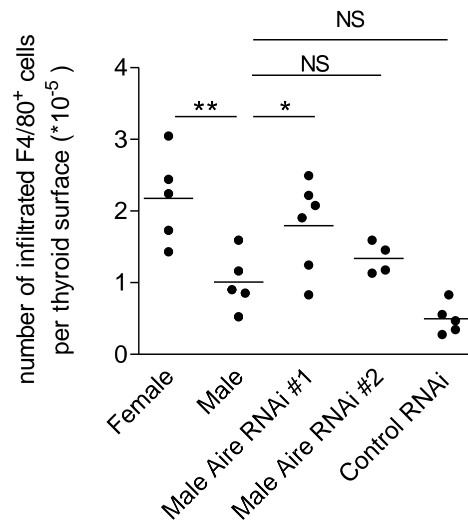


Figure S12. **Representative schedule for male SJL castration, thymectomy, estradiol administration and EAT challenge.** Male SJL mice, 3 weeks old, were first bilaterally castrated and allowed to recover for 1 week. Then after, castrated male mice were thymectomized. Mice were allowed to recover for 2 weeks before being challenged for EAT with 100 nmol of thyroglobulin peptide P2340. Following the castration surgery male SJL mice were treated, by sub-cutaneous injections, every 2 days with 1 µg of Estradiol (79) (85).

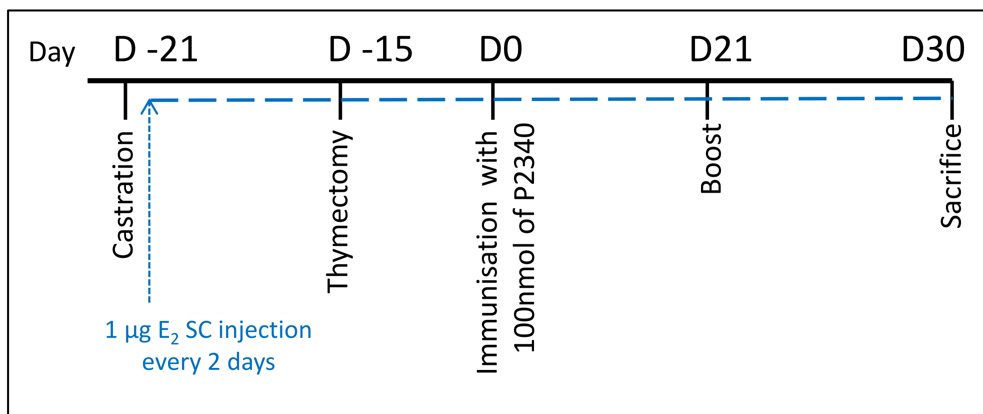


Table S1. List of putative AIRE-dependent TSAs spotted on the array.

	Gene name	Genbank	Fold Change (M/W)
W>M	aldo-keto reductase family 1, member B10	U37100	0,8
	fatty acid binding protein 5 (psoriasis-associated)	M94856	0,8
W=M	aldo-keto reductase family 1, member B1	BF213317	0,9
	human mRNA for protein HC (alpha-1-microglobulin).	X04225	0,9
	chemokine (C-C motif) ligand 11, (eotaxin)	AA127067	1
	ATPase, H+/K+ exchanging, beta polypeptide	BI760752	1
	keratin 6	L42598	1
	hemoglobin, gamma G	AI133196	1,1
	insulin-like growth factor IGFII	X53038	1,1
	cytokeratin type II.	Y17282	1,1
W<M	cytochrome P450, family 1, subfamily A, polypeptide 2 (CYP1a2)	M55053	1,2
	trefoil factor 3 (intestinal)	AA633399	1,2
	insulin (INS)	L15440	1,3
	lactotransferrin	BI021407	1,3
	casein alpha s1	X78416	1,4
	collagen, type III, alpha 1	X14420	1,7
	thyroid peroxidase	M17755	1,7
	keratin 2A	NM_000423	2
	type II cytokeratin	BAA85657	2,1
	keratin 6E	BG681462	2,1
	regulator of G-protein signaling 13	AF030107	2,1

Identification of tissue-specific genes

Gene expression data from the public database http://symatlas.gnf.org were taken at the starting point for identifying tissue-restricted genes among the total number of genes over-expressed in male adults compared to female adults in combination with the SOURCE (http://genome-www5.stanford.edu/cgi-bin/source/sourceSearch) and the literature. Genes were assigned to tissues of their predominant expression when applicable. Genes with expression restricted to less than five tissues were designated as tissue restricted. Among these genes, expression in a single tissue was rare (e.g. casein alpha s1), whereas expression in two to four tissues represented the most cases (e.g. low density lipoprotein receptor, annexin A1, ryanodine receptor 1). Raw microarray data are available on the Array Express database at (www.ebi.ac.uk/arrayexpress) (accession no. E-MEXP-518). For each array, raw data were corrected by a Lowess transformation using the TIGR Microarray Data Analysis System (www.tigr.org.).

Table S2. Number of Aire$^+$ cells in human thymuses.

Total number of AIRE$^+$ cells was counted by immunofluorescence and peroxidase staining in the pubescent human thymic whole section. The counting method is described in detail in "Supplemental Materials and Methods" section. Pubescent females displayed significantly less AIRE$^+$ cells per area of mm^2 of thymic medulla independently of the analysis method used.

	Peroxidase	Fluorescence
	AIRE$^+$/per mm^2 of thymic medulla	AIRE$^+$/per mm^2 of thymic medulla
Pubescent female	40,9	53,6
	33,9	33,8
	37,5	37,9
	45,2	41,5
	42,9	55,5
	37,2	33,7
	49,1	31,0
	33,2	33,3
Pubescent male	65,5	78,1
	83,3	79,9
	58,1	63,5
	102,5	59,4
	64,3	93,0
	102,2	93,6
	35,7	42,7
	76,4	53,2

Table S3. Intervariability of AIRE expression in human thymuses regarding gender and age. Thymic AIRE mRNA expression in human regarding gender and age. AIRE expression was analyzed by real-time PCR and normalized to 28S. Total number of AIRE$^+$ cells in thymic medulla area was counted by immunofluorescence in the pubescent human thymic whole section. The counting method is described in detail in "Supplemental Materials and Methods" section. The inter-individual variability is much higher at the mRNA expression than at the protein level.

		All individuals (*5 months to 46 years old*)	Child (*5 months to 11 years old*)		Pubescent (*13 to 17 years old*)		Adult (*over 18 years old*)	
			Women	Men	Women	Men	Women	Men
RNA analysis (AU/28S)	N	48	8	7	9	11	5	8
	Mean	73,2	100,8	118,2	34,9	86,7	33,4	55,4
	Median	67,6	112,3	121,6	31	81,3	29,7	44,7
	Max	186,9	126,9	173,8	81,7	186,9	43,8	115
	Min	14,7	67,4	20,6	16,4	14,7	24,1	30,3
	Max/Min	**12,7**	**1,9**	**8,4**	**5**	**12,7**	**1,8**	**3,8**
Protein analysis (Aire+ cells per area of thymic section)	N	36	6	6	8	8	4	4
	Mean	12,3	11,5	17,3	6,7	12,7	7,4	9,9
	Median	11,5	14,9	16,1	6,3	12,1	7,3	9,7
	Max	27,8	27,8	27,3	11,4	19,6	8,7	10,8
	Min	4,3	12,0	11,5	4,3	7,4	6,2	9,3
	Max/Min	**6,5**	**2,3**	**2,4**	**2,6**	**2,6**	**1,4**	**1,2**

Table S4. Number of Aire$^+$ and K14$^+$ cells in SJL mouse thymuses

The total number of Aire$^+$ cells and K14$^+$ cells were counted by immunofluorescence in mouse thymic whole section (n= 5 per gender). For each thymus section, the number of Aire$^+$ cells and the thymic medulla surface as well as the thymus section surface were measured. The number of K14$^+$ cells was also counted. The counting method is described in detail in "Material and Methods" section. The number of Aire$^+$ was normalized to the number of K14$^+$ cells, the surface of the medullary area as well as to the whole thymus section. Female displayed significantly less Aire$^+$ cells in thymic medulla area than male mice, irrespective to the normalization factor.

	Aire$^+$ cells counted in the whole section	K14$^+$ in medulla	% of Aire$^+$ /K14$^+$	Aire$^+$/mm^2 of thymus section	Aire$^+$/mm^2 of thymic medulla
Female	357	3108	11,5	40,8	76,9
	131	1447	9,1	14,2	38,1
	137	2120	6,5	14,1	38,0
	228	1763	12,9	34,7	80,9
	431	3142	13,7	43,3	74,8
Male	838	3687	22,7	80,3	172,8
	246	1580	15,6	25,7	78,3
	514	3036	16,9	61,0	120,4
	271	1665	16,3	49,4	118,9
	148	1066	13,9	31,8	89,7

Table S5. List of RNAi sequences used in the study

Control	5'-GCTGAAATGTACTGCGCGTGGAGACGTTTTGGCCACTGACTGACGTCTCCACGCAGTACATTT-3'
288	5'-TGCTGTTAAAGAGAATCCTCCAGAAAGTTTTGGCCACTGACTGACTTTCTGGAATTCTCTTTAA-3'
298	5'-TGCTGATTGTAGTCCTTAAAGAGAATGTTTTGGCCACTGACTGACATTCTCTTAGGACTACAAT-3
850	5'- TGCTGATCTCTACCAGGTATAGTGACGTTTTGGCCACTGACTGACGTCACTATCTGGTGAGAT- 3'

Table S6. List of primers used in the study

GENE NAME	**SPECIES**	**FORWARD PRIMER**	**REVERSE PRIMER**
28S	HUMAN	GGTAGGGACAGTGGGAATCT	CGGGTAAACGGCGGGAGTAA
ACHR-α		AAGCTACTGTGAGATCATCGTCAC	TGACGAAGTGGTAGGTGATGTCCA
AIRE		ATGACACTGCCAGTCACGAG	AGGAGGTGTCCTTCTCAGCA
GAD 67		CACAAGGTGGCTCCAAAAAT	TTACAGATCCTGGCCCAGTC
KERATIN 5		GGTTGATGCACTGATGGATG	TCCTCATACTGGGCCTTGAC
KERATIN 8		TGAGGTCAAGGCACAGTACG	TGATGTTCCGGTTCATCTCA
KERATIN 14		TTCTGAACGAGATGCGTGAC	GCAGCTCAATCTCCAGGTTC
THYROGLOBULIN		CCTGCTGGCTCCACCTTG	CCTTGTTCTGAGCCTCCC
AChR-α	mouse	GTGCTGGGCTCTTTCATCTC	TTCTGTGCGCGTTCTCATAC
Aire		CCCGGCCGGGGACCAATCTC	AGTCGTCCCCTACCTTGGCAAGC
Casein a		TGACTGGACCCTCCATTCTC	CCTTGATTCTCTCCGCTCAG
Gad 67		CACAAACTCAGCGGCATAGA	CTGGAAGAGGTAGCCTGCAC
Keratin 5		TCAAGAAGCAGTGTGCCAAC	TCCAGCAGCTTCCTGTAGGT
Thyroglobulin		CTGTGGTGTTCTCAGCCTCA	TTGGCCTGAGTAGCAGAGGT