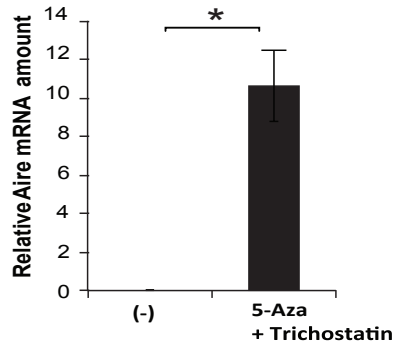


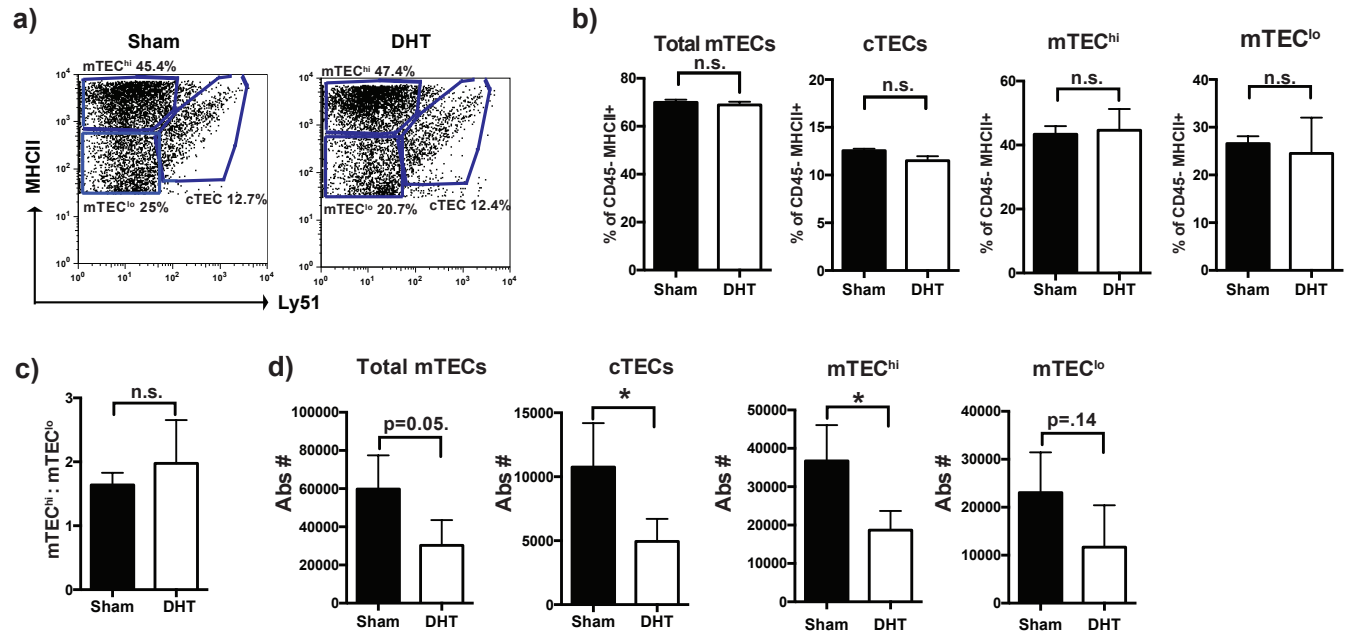
**Supplementary Figure 1: Flutamide blocks DHT mediated increase in Aire expression.**

Relative Aire mRNA expression in primary mouse thymic stromal cells after culture with or without 10nM DHT or vehicle control (-) for 6 h. Cells were pretreated with or without 5 uM flutamide for 2 hours prior to DHT incubation. 10% charcoal-stripped serum was used in culture medium. Relative mRNA expression was measured by quantitative RT-PCR. \*p<0.05.

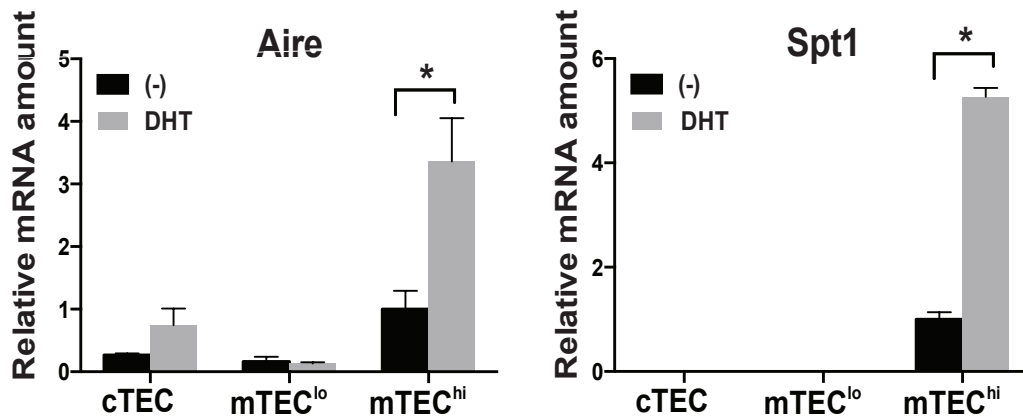


**Supplementary Figure 2: 5 Aza and Trichostatin induce Aire transcription in LnCaP cells.**

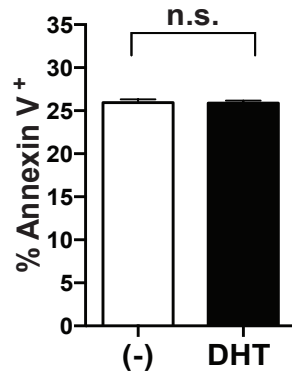
Relative Aire mRNA expression in LNCaP cells treated for 24 hours with 5 mM 5-Aza, a DNA methyltransferase inhibitor, and 100 ng/mL trichostatin, a deacetylase inhibitor. Relative expression was measured by quantitative RT-PCR. \*p<0.05.



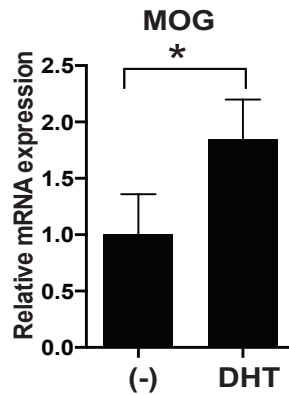
**Supplementary Figure 3: DHT does not alter thymic epithelial subset frequencies, but decreases thymic epithelial numbers globally** (a) Representative flow cytometric plots of MHCII and Ly51 expression in MHCII<sup>+</sup> CD45<sup>-</sup> thymic stromal cells of male mice subjected to sham treatment or DHT pellet insertion. Gating for mTEC<sup>hi</sup>, mTEC<sup>lo</sup>, and cTECs and corresponding frequencies are shown. (b) Average frequencies of total mTEC (mTEC<sup>hi</sup> + mTEC<sup>lo</sup>), cTEC, mTEC<sup>hi</sup> cells, and mTEC<sup>lo</sup> cells. (c) Average ratio of mTEC<sup>hi</sup> : mTEC<sup>lo</sup> cells. (d) Average absolute numbers of subsets in (b). n= 3-4 per group. n.s.= not significant. \*p<0.05.



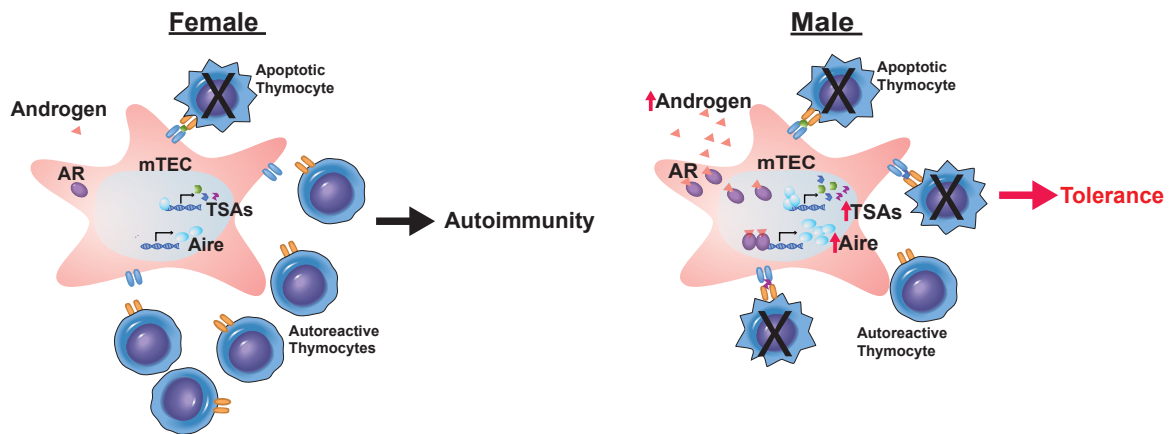
**Supplementary Figure 4: DHT increases Aire and TSA expression in MHCII-high mTECs.** (A and B) Relative Aire (A) and Spt1 (B) expression in sorted MHCII-high mTECs (mTEC<sup>hi</sup>; CD45<sup>-</sup> Ly51<sup>low</sup>, MHCII<sup>high</sup>), MHCII-low mTECs (mTEC<sup>lo</sup>; CD45<sup>-</sup> Ly51<sup>low</sup>, MHCII<sup>low</sup>), or cTEC (CD45<sup>-</sup> Ly51<sup>high</sup>, MHCII<sup>+</sup>) cells by quantitative RT-PCR. Thymi are from mice treated with placebo (black bars) or 10 mg DHT pellet (gray bars). \*p<0.05.



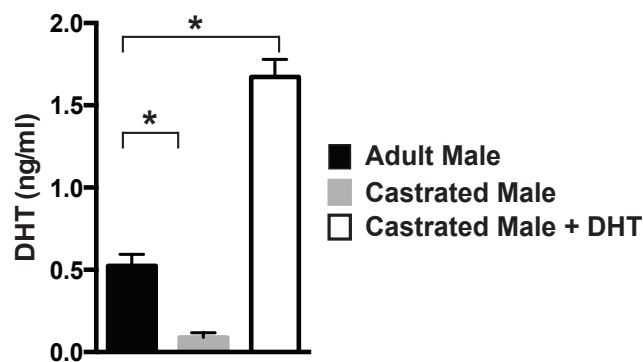
**Supplementary Figure 5: DHT is not directly toxic to thymocytes.** Thymocytes from *Typr1<sup>B-w</sup>* TRP-1 TCR Tg Rag <sup>-/-</sup> mice, which are rescued from negative selection due to TRP-1 deficiency, were incubated with vehicle control (-) or 10nM DHT for six hours. The frequency of Annexin V<sup>+</sup> thymocytes was determined by flow cytometry.



**Supplementary Figure 6: DHT induces MOG expression in thymic stroma.** Thymic stroma isolated from WT male C57BL/6 mice were incubated with either vehicle control (-) or 10nM DHT. Relative MOG mRNA expression was compared by real time RT-PCR.



**Supplementary Figure 7: Model for male protection from autoimmunity.** A proposed model in which higher circulating androgen levels in males induce higher Aire and TSA expression in the thymus. More robust negative selection of self-reactive T cells protects males from autoimmune diseases.



**Supplementary Figure 8: Serum DHT levels.** Circulating levels of DHT in unmanipulated adult mice, castrated male mice, and castrated male mice implanted with DHT pellet. \* $p < 0.05$ .

Aire promoter	Mutations in Androgen Receptor Binding Sites								
	-1032	-994	-970	-943	-391	-339	-260	-236	-52
WT	GCACTGTCC	CTCCTGTCC	GGACACCTT	CTCCTGTCC	GGACAGGGC	TCTCTGTCC	GGACAGCAG	GAAGTGTCC	CCAGTGTCC
Mut1	GCACTGTCC	CTCCTGTCC	GGACACCTT	CTCCTGTCC	<b>CGACAAGGA</b>	<b>TCTCAAAAC</b>	<b>CGCGAACAG</b>	<b>GAAGACCAA</b>	CCAGTGTCC
Mut2	<b>GCAAAAAC</b>	CTCCT <b>AAAC</b>	<b>CGCAACCTT</b>	<b>CTCCAAAAC</b>	GGACAGGGC	TCTCTGTCC	GGACAGCAG	GAAGTGTCC	CCAGTGTCC
Mut3	<b>GCAAAAAC</b>	CTCCT <b>AAAC</b>	<b>CGCAACCTT</b>	<b>CTCCAAAAC</b>	<b>CGACAAGGA</b>	<b>TCTCAAAAC</b>	<b>CGCGAACAG</b>	<b>GAAGACCAA</b>	CCAGTGTCC
Del	-----	-----	-----	-----	-----	-----	-----	-----	-----

**Supplementary Table 1: Sequences of wildtype and mutated AIRE 5' promoter regions in AIRE promoter-luciferase reporter constructs.** Sequences at 9 potential AR binding sites (-1032, -994, -970, -943, -391, -339, -260, -236, and -52 relative to translational start site) in the AIRE 5' promoter region. Wildtype (WT) sequences for AR binding sites are shown at the top. For Mut1-3 constructs, multiple point mutations in the potential AR binding sites are shown in bold. The Del construct contains deletions (-) of all 9 AR binding sites.

	<b>WT+Sham n=19</b>	<b>WT+DHT n=15</b>	<b>GW+Sham n=15</b>	<b>GW+DHT n=16</b>
<b>Mean peak disease severity</b>	<b>3.2</b>	<b>2.4</b>	<b>3.7</b>	<b>3.7</b>
<i>Experiment 1</i>	3.0(0.25) n=8	2.2(0.38) <sup>a</sup> n=5	3.5(0.19) n=5	3.4(0.19) n=5
<i>Experiment 2</i>	3.6(0.2) n=5	2.6(0.5) <sup>a</sup> n=5	3.8(0.2) n=5	4(0) n=5
<i>Experiment 3</i>	3.8(0.4) n=6	3(0.8) <sup>a</sup> n=5	3.8(0.4) n=5	3.3(1.0) n=6
<b>Mean day of onset</b>	<b>8.8</b>	<b>10.8</b>	<b>8.6</b>	<b>9.4</b>
<i>Experiment 1</i>	9.6(0.3)	12.5(1.5) <sup>a</sup>	9.0(0.32)	9.2(0.2)
<i>Experiment 2</i>	8.4(0.2)	8.8(0.4) <sup>a</sup>	8.0(0)	8.6(0.3)
<i>Experiment 3</i>	8.5(0.5)	11(2.2) <sup>a</sup>	8.8(0.4)	10.6(1.3)
<b>Cumulative score*</b>	<b>37.5</b>	<b>20.4</b>	<b>43.9</b>	<b>41.9</b>
<i>Experiment 1</i>	32.6(5.7)	15.8(5.3) <sup>a</sup>	42.0(4.0)	39.0(3.0)
<i>Experiment 2</i>	40.4(2.7)	25(4.0) <sup>a</sup>	45.8(1.4)	44.8(2.4)
<i>Experiment 3</i>	n/a	n/a	n/a	n/a
<b>Death from disease</b>	<b>1/19</b>	<b>0/15</b>	<b>1/15</b>	<b>2/10</b>
<i>Experiment 1</i>	0/8	0/5	0/5	0/5
<i>Experiment 2</i>	1/5	0/5	1/5	2/5
<i>Experiment 3</i>	0/6	0/5	0/5	0/6
<b>Incidence</b>	<b>19/19</b>	<b>14/15</b>	<b>15/15</b>	<b>16/16</b>
<i>Experiment 1</i>	8/8	4/5	5/5	5/5
<i>Experiment 2</i>	5/5	5/5	5/5	5/5
<i>Experiment 3</i>	6/6	5/5	5/5	6/6

**Supplementary Table 2: MOG-induced EAE in male mice treated with DHT.** Clinical EAE scores in DHT or sham treated WT or Aire-deficient Aire<sup>GW/+</sup> male mice. Values in bold are means or sums for three experiments. For each experiment, values are means (SE). <sup>a</sup>Significant difference from genotype-matched sham treated counterpart. \*Sum of scores from Day 0 to 21. Cumulative scores are not available (n/a) for Experiment 3 because mice were sacrificed at Day 14.

	WT female n=15	WT male n=15	GW female n=15	GW male n=16
<b>Mean peak disease severity</b>	<b>3.3</b>	<b>2.3</b>	<b>3.3</b>	<b>3.2</b>
<i>Experiment 1</i>	3.6(0.25) n=5	2.4(0.25) <sup>a</sup> n=5	3.5(0.33) n=5	3.6(0.2) n=5
<i>Experiment 2</i>	2.8(0.5) n=5	1.8(0.25) <sup>a</sup> n=5	2.6(0.25) n=5	2.4(0.25) n=5
<i>Experiment 3</i>	3.5(0.33) n=5	2.6(0.5) <sup>a</sup> n=5	3.8(0.25) n=5	3.7(0.4) n=6
<b>Mean day of onset</b>	<b>14.5</b>	<b>17</b>	<b>13.4</b>	<b>13.5</b>
<i>Experiment 1</i>	12.8(0.6)	13.8(0.8)	11.2(0.8)	12.2(0.4)
<i>Experiment 2</i>	17.4(1.5)	20.8(1.9)	16.2(1.7)	16.2(1.5)
<i>Experiment 3</i>	13.3(0.8)	16.4(1.4) <sup>a</sup>	12.8(0.7)	12.1(1.1)
<b>Cumulative score</b>	<b>19.8</b>	<b>8.9</b>	<b>20.6</b>	<b>19.2</b>
<i>Experiment 1</i>	23.2(3.2)	13.6(2.2) <sup>a</sup>	28.3(4.2)	27.8(3.3)
<i>Experiment 2</i>	16.4(7.5)	4.2(2.2) <sup>a</sup>	12.8(4.0)	10.5(3.9)
<i>Experiment 3</i>	n/a	n/a	n/a	n/a
<b>Death from disease</b>	<b>0/15</b>	<b>0/15</b>	<b>1/15</b>	<b>1/15</b>
<i>Experiment 1</i>	0/5	0/5	0/5	0/5
<i>Experiment 2</i>	0/5	0/5	0/5	0/5
<i>Experiment 3</i>	0/5	0/5	1/5	1/5
<b>Incidence</b>	<b>15/15</b>	<b>15/15</b>	<b>15/15</b>	<b>15/15</b>
<i>Experiment 1</i>	5/5	5/5	5/5	5/5
<i>Experiment 2</i>	5/5	5/5	5/5	5/5
<i>Experiment 3</i>	5/5	5/5	5/5	5/5

**Supplementary Table 3 : MOG-induced EAE in male and female mice.** Clinical EAE scores in male and female WT and Aire<sup>GW/+</sup> mice. Values in bold are cumulative means or sums for three experiments. For each experiment, values are means (SE). <sup>a</sup>Significant difference from genotype-matched female counterpart. \*Sum of scores from Day 0 to 21. Cumulative scores are not available (n/a) for Experiment 3 because mice were sacrificed at Day 14.



<b>Region</b>	<b>Primer sequences</b>	<b>Orientation</b>	<b>Ref (PMID)</b>
Aire promoter (-2916 to -2643)	TGGAGAGAAGGTCAGCAGGT	Forward	
	CCTCGTTGCAGTTGAGACAA	Reverse	
Aire promoter (-1130 to -867)	CTTTTCCGGAGCAATCTCAG	Forward	
	GTGGACGAAGGTACCAGGAA	Reverse	
Aire promoter (-383 to -164)	GCCACATTCGGAAGTGAGAG	Forward	
	GACCACGCAAAGAGCAAAG	Reverse	
Positive control 1: PSA enhancer (-4212 to -4127)	TGGGACAACCTTGCAAACCTG	Forward	16137620
	CCAGAGTAGGTCTGTTTTCAATCCA	Reverse	
Positive control 2: PSA promotor (-273 to -184)	CCTAGATGAAGTCTCCATGAGCTACA	Forward	16137620
	GGGAGGGAGAGCTAGCACTTG	Reverse	
Negative control 1: -1575 to -1275	ACCTTCCTCCCCAGGTTTG	Forward	
	ATTAGGGCTGAAGGGTGGAC	Reverse	
Negative control 2: Aire exon 11	CTCGGGTTCGGGTTTCAGCTA	Forward	
	CCCGCCGACCACGCTCACT	Reverse	

**Supplementary Table 4: Primer sequences for ChIP.**