Supplementary Figure Legends

Supplementary Figure 1. Total mTEC number, total aire positive mTEC number, thymic architecture, and thymocyte numbers and percentages are unchanged in G228W heterozygous mice. a) Representative flow cytometry plots of mTECs from wildtype (+/+) and G228W heterozygous (GW/+) mice. Among CD45-, PI- cells, mTECs (encircled) were identified as $G8.8^{+}$, $Ly51^{int}$ staining cells. Numbers next to mTEC gates signify average total numbers of mTECs ± SD. n=3 for each genotype. **b)** Total number of Aire-positive mTECs as determined by flow cytometry and hemocytometer counts. Bars indicate averages ± SD. n=3 for each genotype. **c)** Representative H&E stained frozen thymic sections from +/+ (left column) and GW/+ (right column) mice. All images were taken with a 5x objective. **d)** Flow cytometry plots of lymphocytes in the thymus (left column) and spleen (right column) of +/+ (top row) and GW/+ (bottom row) 6 week old littermates.

Supplementary Figure 2. GW/+ mice in the NOD background develop peripheral neuropathy and insulitis. a) Neuropathic mouse displaying hind limb paralysis with preserved tail tone. **b)** Insulitis scores of 10 week old +/+, +/o, and GW/+ mice in the NOD background.

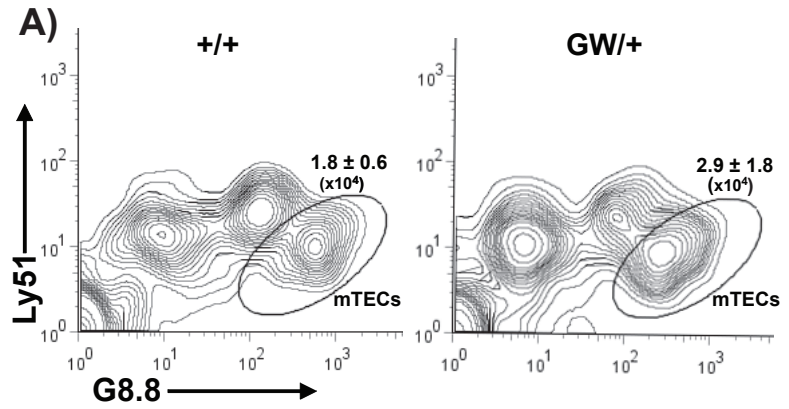
Supplementary Figure 3. GW/+ mice differentially regulate thymic antigens. Heat map of genes differentially regulated in GW/+ mice compared with +/+ littermates. Nonparametric T tests ($p<0.01$) were used to identify genes displayed. Green indicates upregulation, red indicates downregulation.

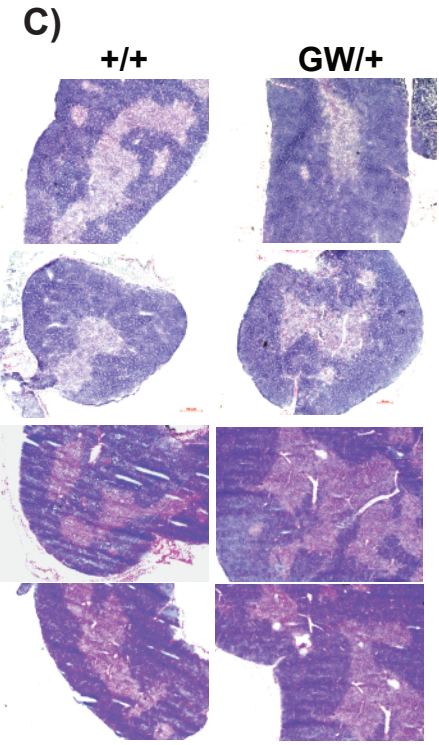
Supplementary Figure 4. Aire-containing aggregates form specifically in G228W mTECs. Ratio of the number of aggregates stained by anti-aire antibody divided by the

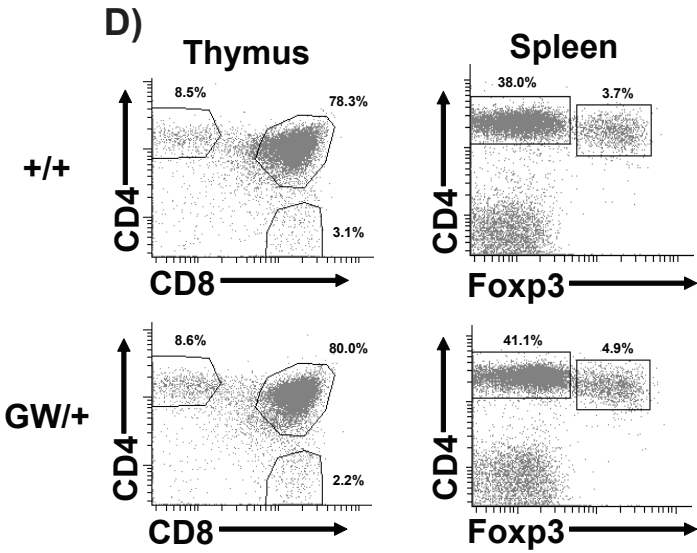
number aire-positive cells for+/+ and GW/+ thymi. Error bars represent standard deviations. (*) indicates P<.0001.

Supplementary Figure 5. Immunohistochemistry of transfected 1C6 cells. Fixed and permeabilized 1C6 cells transfected with either wildtype AIRE (left panel) or G228W AIRE (right panel) were stained with anti-AIRE antibody (green) and DAPI (blue). Staining was visualized by confocal microscopy.

Supplementary Table 1. Sequences of primers and probes used for Real Time RT-PCR.

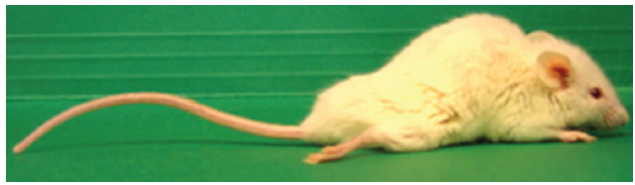






Supplementary Figure 1

A)



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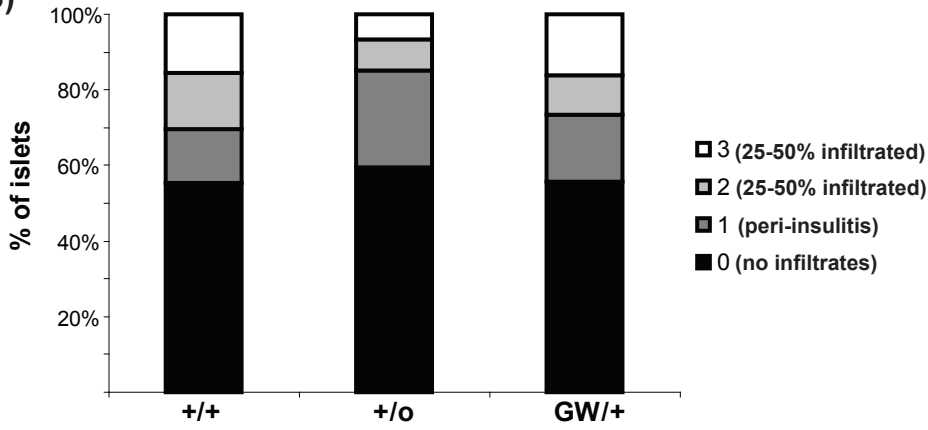
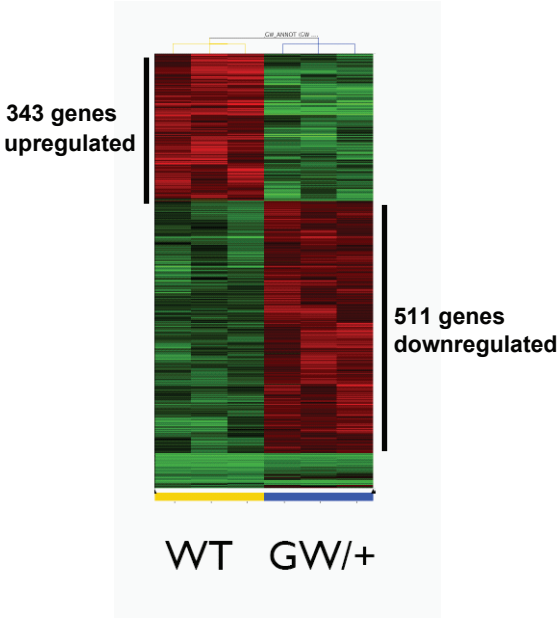
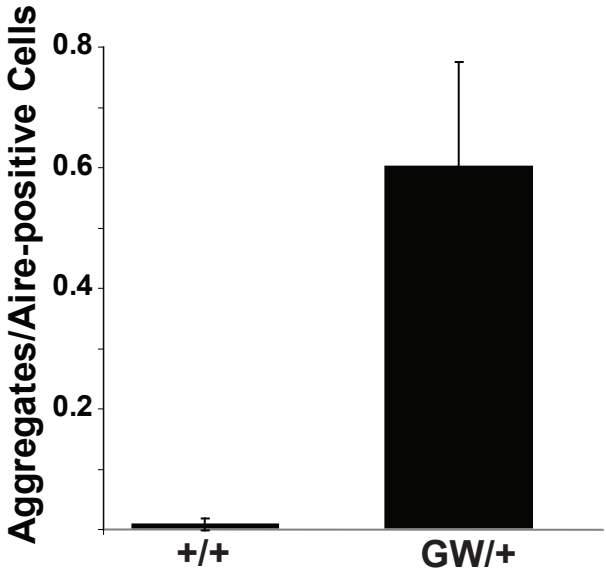


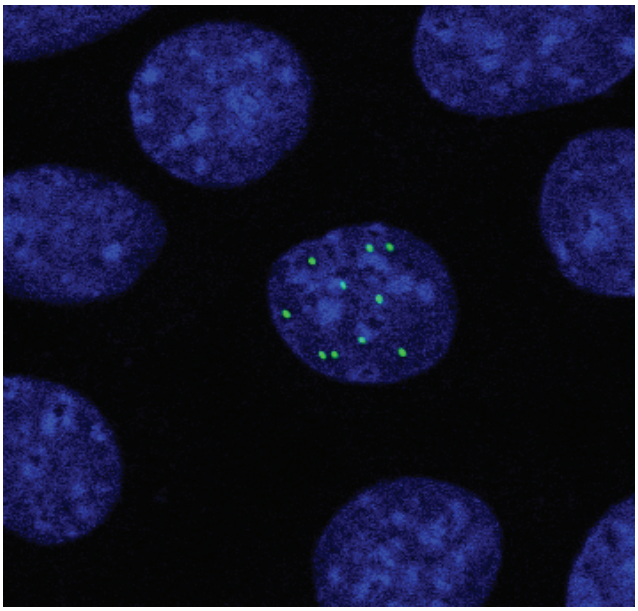
Supplementary Figure 2



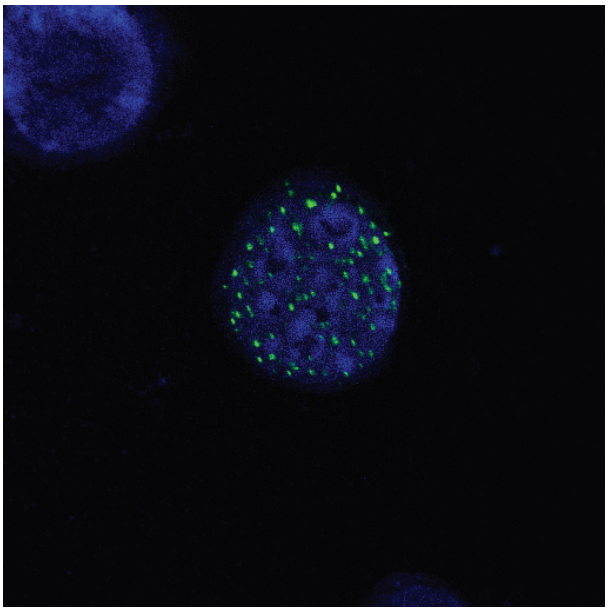
Supplementary Figure 3



Supplementary Figure 4



Wildtype



G228W

Supplementary Figure 5

Real Time RT-PCR Primer and Probe Sequeces

(Sequences are 5'-3')

Hbby-TM-F	GCT AGT CAC TTC GGC AAT GAA TT
Hbby-TM-R	CCC CAG CCA CCA GCT TC
Hbby-TM-Probe	AGC TGA GAT GCA GGC TGC CTG GC
Ptdgs-TM-F	CCT GCC CCA ACC GGA T
Ptdgs-TM-R	GTG ACC AGC CCT CTG ACT GAC
Ptdgs-TM-Probe	AGT GCA TTC AAG AGT AAA CGC AGG TGA GAG
Spt1-TM-F	GCT TGG TGT TTC CAC TAT CCT AGT CT
Spt1-TM-R	AAT CAG CAG TTC CAG AAG TTT CAG T
Spt1-TM-Probe	TTG CCA GGA CCC GGA GAC AAA CA
Spt2-TM-F	CAC CAT GAA GTT CCT GGC ACT
Spt2-TM-R	TCT CCG GGT CCT GGC AA
Spt2-TM-Probe	CTT GTG TTG CTT GGT GTT TCC ACT ATC CTA GTC
DCPP-TM-F	GGAAAATATAACAAGTATCCGGGTATTT
DCPP-TM-R	CACTTGACCGTCCTCGTTGTC
DCPP-TM-Probe	CAGGCTAGATTGATTGTTGGAATTCAGCTCA

Supplementary Table 1