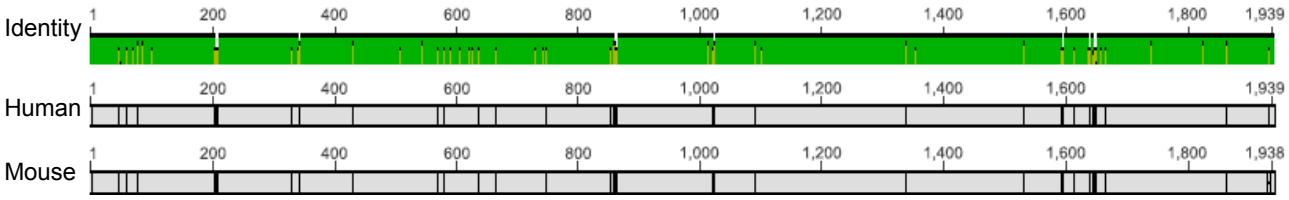
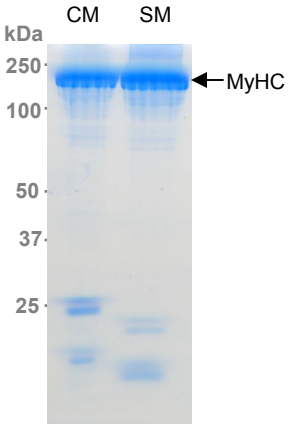
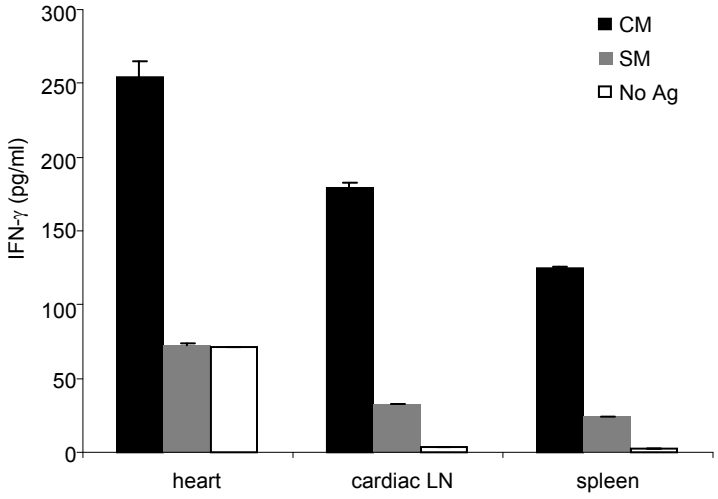


Supplemental Figure 1. Amino acid sequence alignment of α-MyHC. The alignment was created using the ClustalW algorithm. The alignment statistics are 96.9% for both pairwise identity and percent identical sites. The upper panel is the quality line with identical positions in green and mismatches in broken lines.

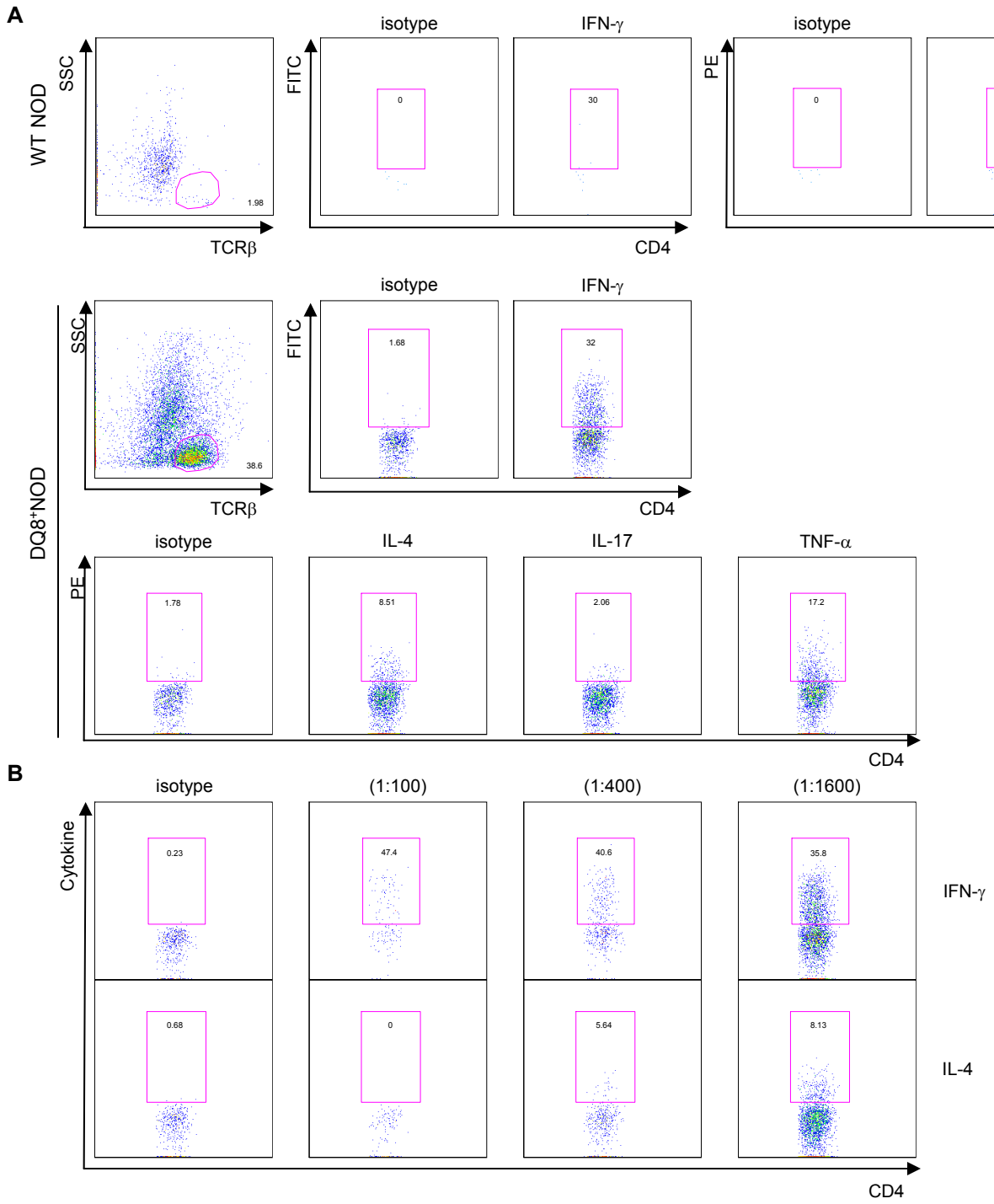


Supplemental Figure 2. Purified mouse cardiac myosin and soleus myosin.

Myosin was prepared side-by-side from the heart and soleus muscle of NOD mice. 15 μg of cardiac myosin (left lane, CM) or soleus myosin (right lane, SM) was loaded on a 10% SDS-polyacrylamide gel and stained with Coomassie G-250.

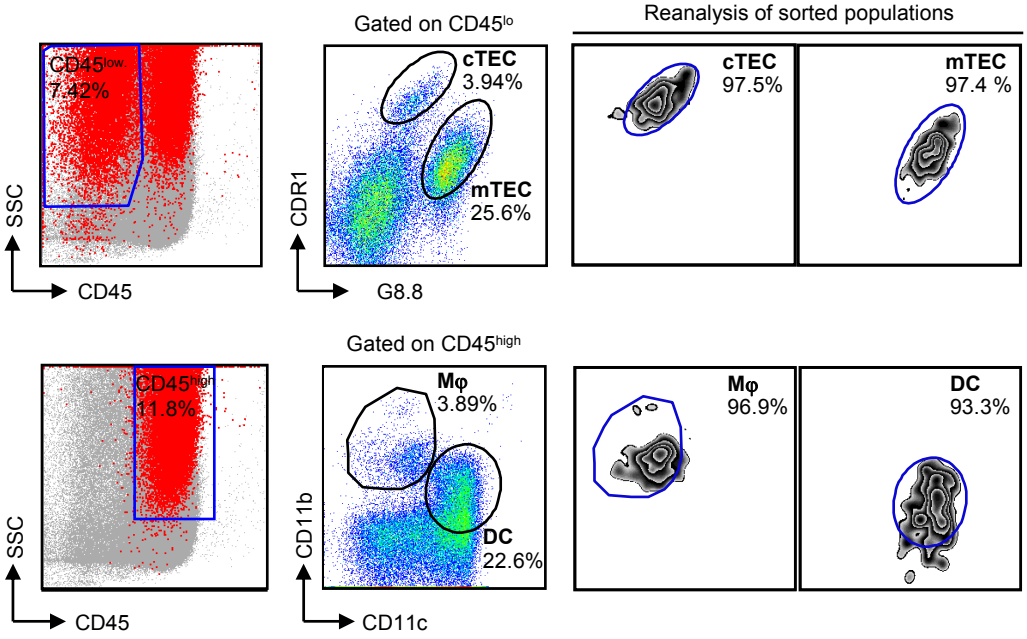


Supplemental Figure 3. Antigen-specificity of bulk cardiac-infiltrating and peripheral lymphoid T cells in DQ8^{+}NOD mice. Mononuclear cells were isolated from the heart, cardiac-draining lymph nodes (LNs), and spleen of an 11-wk-old female DQ8^{+}NOD mouse. Cells were stimulated *in vitro* with mouse cardiac myosin (CM) or soleus myosin (SM) for 96 h. Responses were tested in duplicate by IFN-γ ELISA: data is representative of 7 mice.

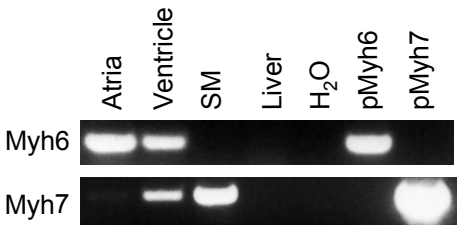


Supplemental Figure 4. Intracellular cytokine profiles of cardiac-infiltrating CD4⁺ T cells in myocarditic mice.

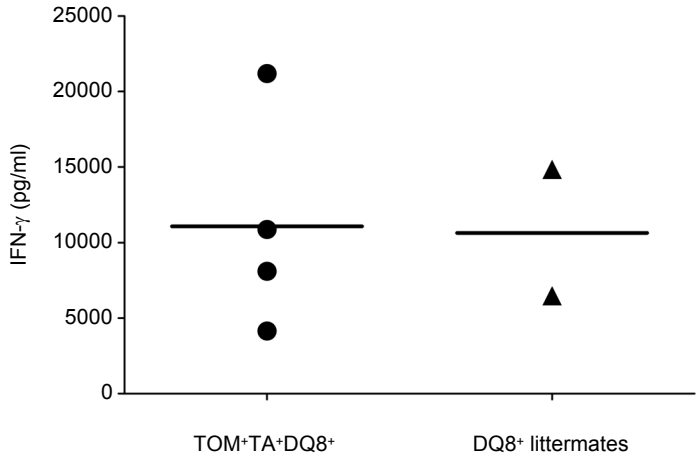
(A) Mononuclear cells (MNCs) isolated from the hearts of a 19-wk-old female NOD and a 12-wk-old female DQ8⁺NOD mouse (1:1,600 cardiac myosin autoantibody titer) were pulsed with PMA/ionomycin, followed by intracellular cytokine staining with the indicated anti-cytokine antibodies. **(B)** Dynamics of intracellular cytokine profile of heart-infiltrating CD4⁺ T cells. Intralesional mononuclear cells were isolated from the hearts of individual DQ8⁺NOD mice with various disease severities (as determined by anti-cardiac myosin antibody titers, shown in brackets), followed by intracellular cytokine staining.



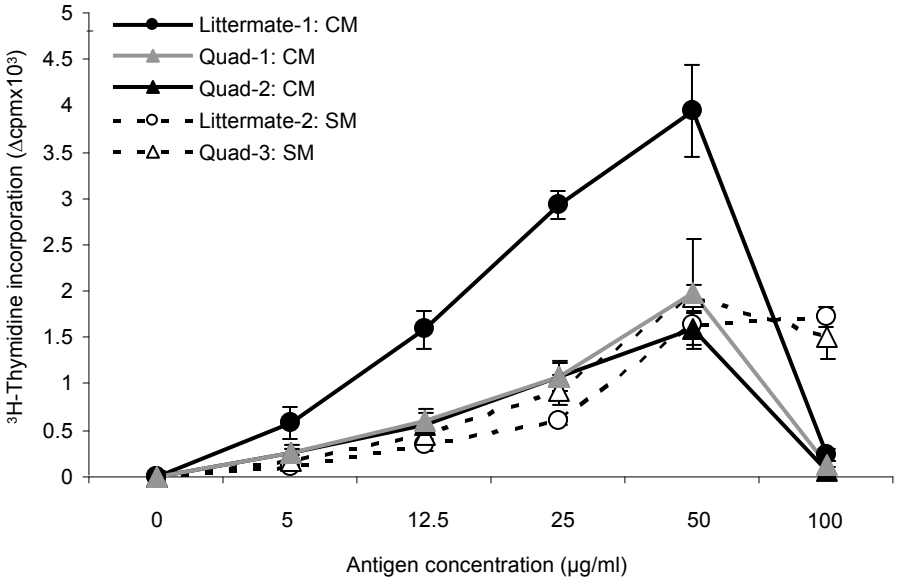
Supplemental Figure 5. Cell sorting regions selected for isolation of thymic stromal cell subsets. Appropriate side-scatter gating together with CD45 gating was used to exclude small thymocytes and select for myeloid or epithelial cells, respectively. Typically the purity of sorted thymic stromal populations was greater than 93% upon reanalysis.



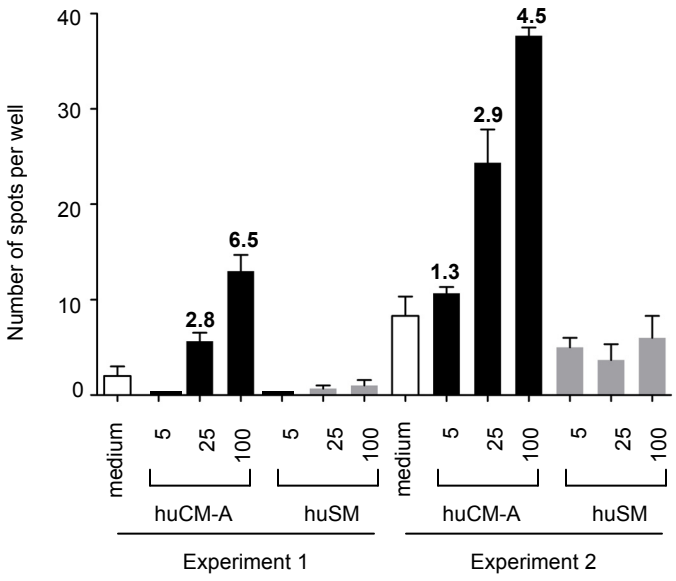
Supplemental Figure 6**. Demonstration of the specificity of Myh6 and Myh7 PCR amplification in Figure 5A.** Positive and negative control mouse tissues and cDNAs encoding Myh6 (pMyh6) and Myh7 (pMyh7) were used as templates in PCR reactions.



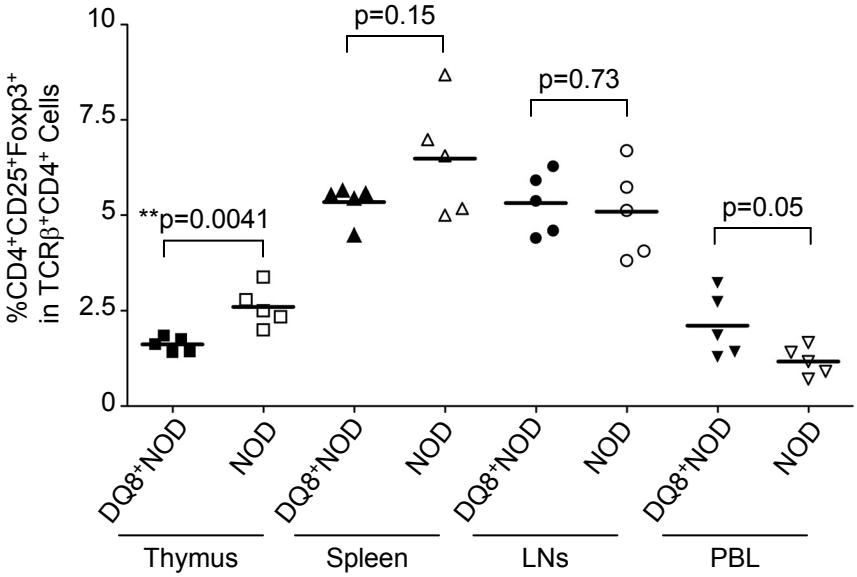
Supplemental Figure 7. Responsiveness of splenic T cells from TOM$^+$TA$^+$DQ8$^+$ and control DQ8$^+$ littermates to anti-CD3/anti-CD28 stimulation. Approximately 1 x 10^6 spleen cells were stimulated with 0.1 μg/ml of anti-mouse CD3 and 0.2 μg/ml of anti-mouse CD28 for 96 h and responses were tested by IFN-γ ELISA.



Supplemental Figure 8. T cell tolerance to cardiac myosin as assessed by *in vitro* recall proliferative responses. Thymic α-MyHC-expressing TOM$^+$TA$^+$DQ8$^+$NOD ("Quad") and non-thymic α-MyHC-expressing (TOM$^+$TA$^-$ or TOM$^-$TA$^+$)DQ8$^+$NOD ("Littermate") mice were immunized with purified cardiac myosin (CM) or soleus myosin (SM). Lymphocytes isolated from draining LNs 10 d after immunization were restimulated *in vitro* with the same antigen used in the immunization. Data represent mean ± s.d. of responses measured in triplicates.



Supplemental Figure 9. Independent IFN-γ ELISPOT analyses of fresh PBMCs from healthy control subject C1, obtained 11 months apart. Each bar represents the number of spots. Numbers at the top of each bar are stimulation indexes (= number of spots/number of spots in medium control).



Supplemental Figure 10. Treg cell populations in DQ8$^+$NOD mice. DQ8$^+$NOD mice have peripheral Treg cell populations comparable to NOD mice, with slight decrease in the percentage of thymic Treg cells compared to NOD mice. Anti-Foxp3 antibody (FJK-16s) was purchased from eBioscience and intracellular staining was performed according to the manufacture's protocol.

Supplemental Table 1. Microarray data of genes expressed in medullary thymic epithelial cells

Gene Symbol	Probe Name	Expression Value	Gene Symbol	Probe Name	Expression Value
Actb	101578_f_at	540	Myh6	101071_at	3.7
Aire	97159_at	183	Myh7	98616_f_at	28
Ctsl	101963_at	240	Tnnt2	100593_at	44
Ctss	98543_at	584	Tnni3	100921_at	24
Ins2	161849_r_at	68	Chrna1	100021_at	4.5

Probe names and expression values from the gene chips of genes listed in figure 5(A). These numbers are expression values from the Affymetrix murine U74Av2 gene chips hybridized with RNA from wild type C57BL/6 mTEC. This data is available from the NCBI GEO data repository, accession number GSE85.

Supplementary Table 2. Microarray data of genes expressed in extrathymic Aire-expressing cells

Gene ID	Gene name	Gene symbol	Probe name	WT signal	KO signal
17888	**myosin, heavy polypeptide 6, cardiac muscle, alpha**	**Myh6**	**1417729_at**	**7.1**	**6.8**
140781	**myosin, heavy polypeptide 7, cardiac muscle, beta**	**Myh7**	**1448553_at**	**70.9**	**308.5**
11461	actin, beta	Actb	1419734_at	21616	20537.9
16783	lysosomal-associated membrane protein 1	Lamp1	1415880_a_at	13175.2	12032.4
14960	histocompatibility 2, class II antigen A, alpha	H2-Aa	1435290_x_at	10419.5	10183.3
11634	autoimmune regulator (autoimmune polyendocrinopathy candidiasis ectodermal dystrophy)	Aire	1419241_a_at	4058.7	1373
14813	glutamate receptor, ionotropic, NMDA2C (epsilon 3)	Grin2c	1449245_at	997.6	470.9
12638	cystic fibrosis transmembrane conductance regulator homolog	Cftr	1420579_s_at	533	232

Microarray data of the expression of mouse Myh6, Myh7, and positive control genes in extrathymic Aire-expressing cells (eTACs) on $Aire^{+/+}$ (WT) and $Aire^{-/-}$ (KO) background. Myh6 transcripts were absent in eTACs, while Myh7 transcripts were expressed at low level in these cells. Color code: blue represents highly expressed genes and yellow represents tissue-specific genes expressed in eTACs. Background = 0-20.