Supplemental Table 1. Histological evaluations of mice

Genotype	Sex	Pancreas		Liver	Lung
		Periinsular lymphocytic infiltration	Atrophy of acinar tissues	Lymphoid cell infiltration	Lymphoid cell infiltration
+/+	F	++	-	-	-
+/+	F	+	-	-	-
+/+	F	++	-	-	-
+/+	M	+	-	-	-
+/+	M	+	-	+	-
+/+	M	+	-	-	-
+/+	M	+	-	-	-
+/-	F	+	-	-	-
+/-	F	++	-	+	-
+/-	F	++	-	-	+
+/-	M	+	-	-	-
+/-	M	+	-	-	-
+/-	M	++	-	-	+
+/-	M	++	-	N.A.	-
+/-	M	-	-	-	-
+/-	M	++	-	-	-
-/-	F	++	++	+	++
-/-	F	++	++	+	++
-/-	F	++	++	+	++
-/-	F	N.A.	N.A.	+	++
-/-	F	N.A.	N.A.	+	+
-/-	F	+	-	+	+
-/-	F	N.A.	N.A.	+	++
-/-	F	++	+	+	++
-/-	F	++	-	+	++
-/-	F	-	-	+	++
-/-	F	+	++	+	+
-/-	F	++	++	+	++
-/-	M	++	++	+	++
-/-	M	++	++	+	++
-/-	M	++	++	+	++
-/-	M	+	-	+	++
-/-	M	++	+	+	++
-/-	M	++	++	+	-
-/-	M	++	++	-	++
-/-	M	++	++	+	++
-/-	M	+	++	-	+
-/-	M	++	+	+	++
-/-	M	++	++	+	++
-/-	M	++	++	+	++

Formalin-fixed tissue sections were subjected to H&E staining, and two pathologists independently evaluated the histology without being informed of the condition of each individual mouse. Histological evaluations were made 6 to 21 wks after birth. Mice backcrossed onto NOD mice for 6 to 9 generations were used.
N.A., not assessed.
++, severe; +, moderate; -, not remarkable.

Supplemental Table 2. Expression of tissue-specific genes from Aire-deficient NOD mouse thymus

Genotype	Foxn1/Hprt	Ins/Hprt	SP1/Hprt	FABP/Hprt	CRP/Hprt	GAD67/Hprt	PDIp/Hrpt
+/+	1.86	2.83	1.04	0.78	1.41	52.2	0.74
-/-	1.65	9.77×10^{-2}	4.96×10^{-3}	3.49×10^{-2}	1.60	185	0.62
Relative abundance (Aire-KO/control)	0.89	1/29.0	1/210	1/22.5	1.13	3.54	0.85

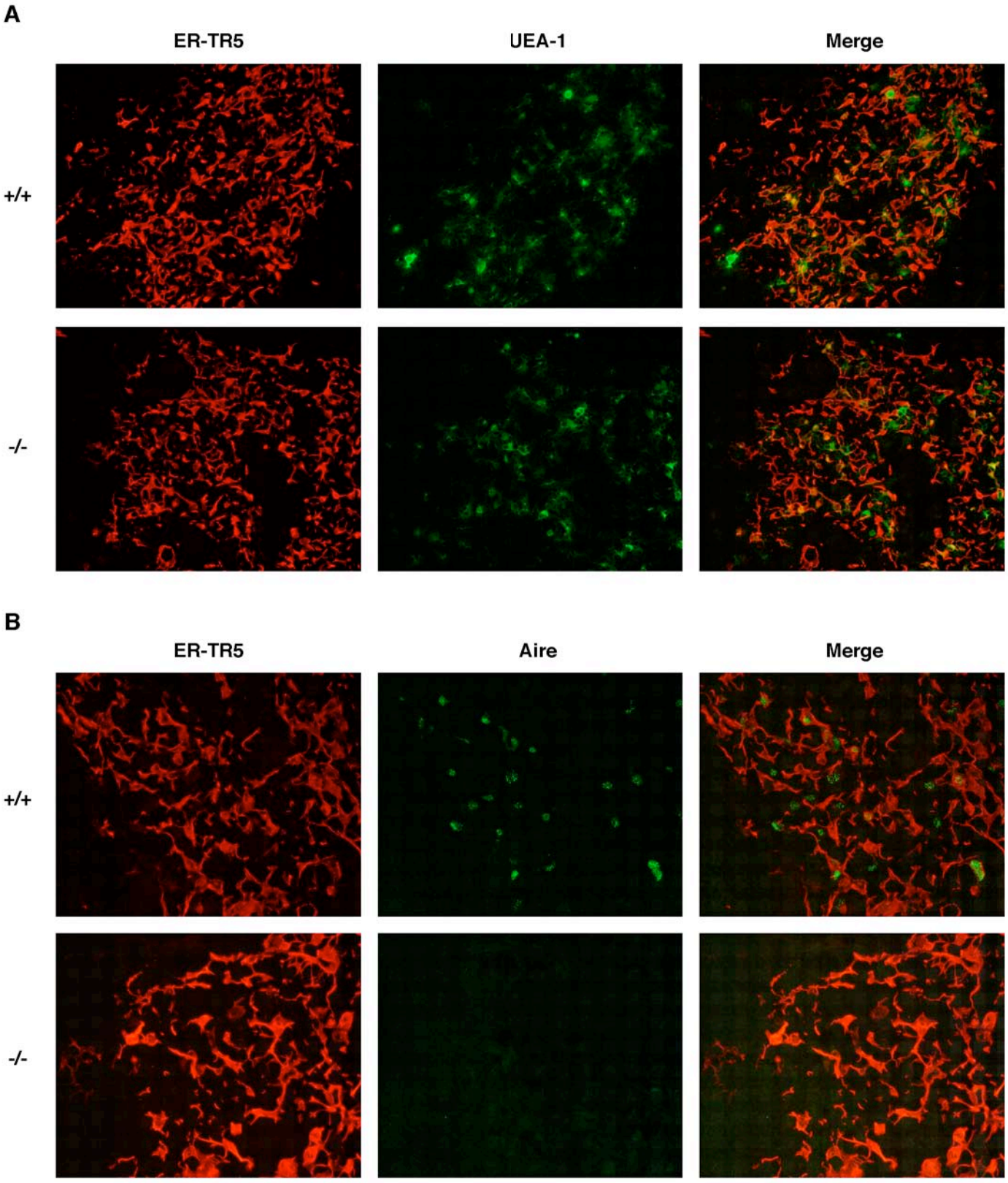
Real-time PCR for *Foxn1* and peripheral tissue-specific genes was performed using total thymus RNAs from control and Aire-deficient NOD mice. The relative abundance of each gene was calculated as described in Table 1. Mice backcrossed onto NOD mice for 6 generations were used.

Supplemental Table 3. Serum auto-Abs against various organs detected with immuno-fluorescence

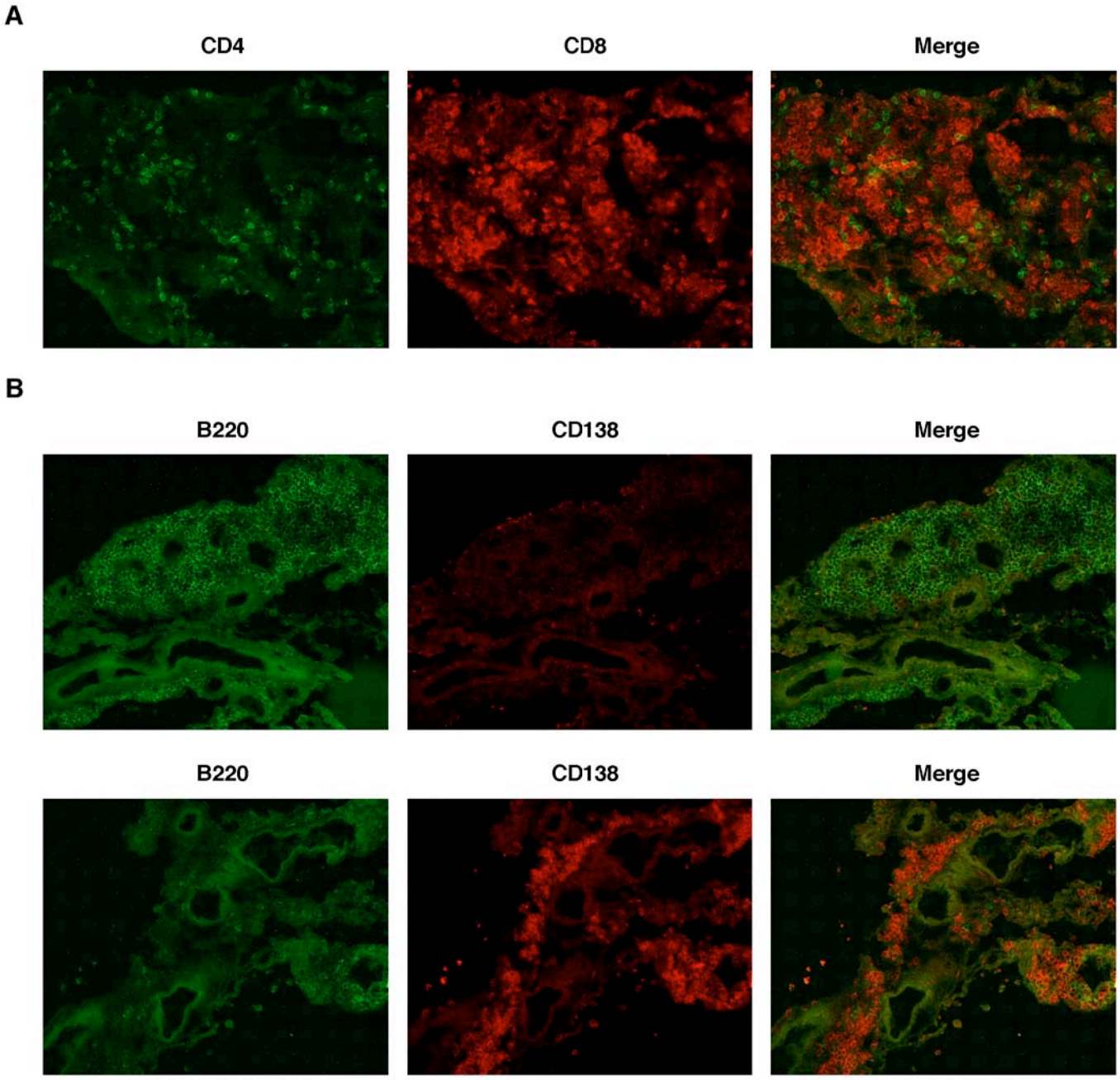
Genotype	Pancreas	Stomach	Kidney	Liver
+/+	-	-	-	-
+/-	-	-	-	-
+/-	-	-	-	-
+/-	-	-	-	-
+/-	-	-	-	-
+/-	-	-	-	-
-/-	+	++	+	++
-/-	++	++	+	++
-/-	++	+	+	++
-/-	++	++	+	++
-/-	++	++	+	+
-/-	+	+	+	+
-/-	++	++	++	++

Mouse serum was incubated with various organs obtained from Rag2-deficient mice, and reactivity was determined by immuno-fluorescence. Mice backcrossed onto NOD mice for 6 generations were used.
++, strongly positive; +, moderately positive; -, negative.

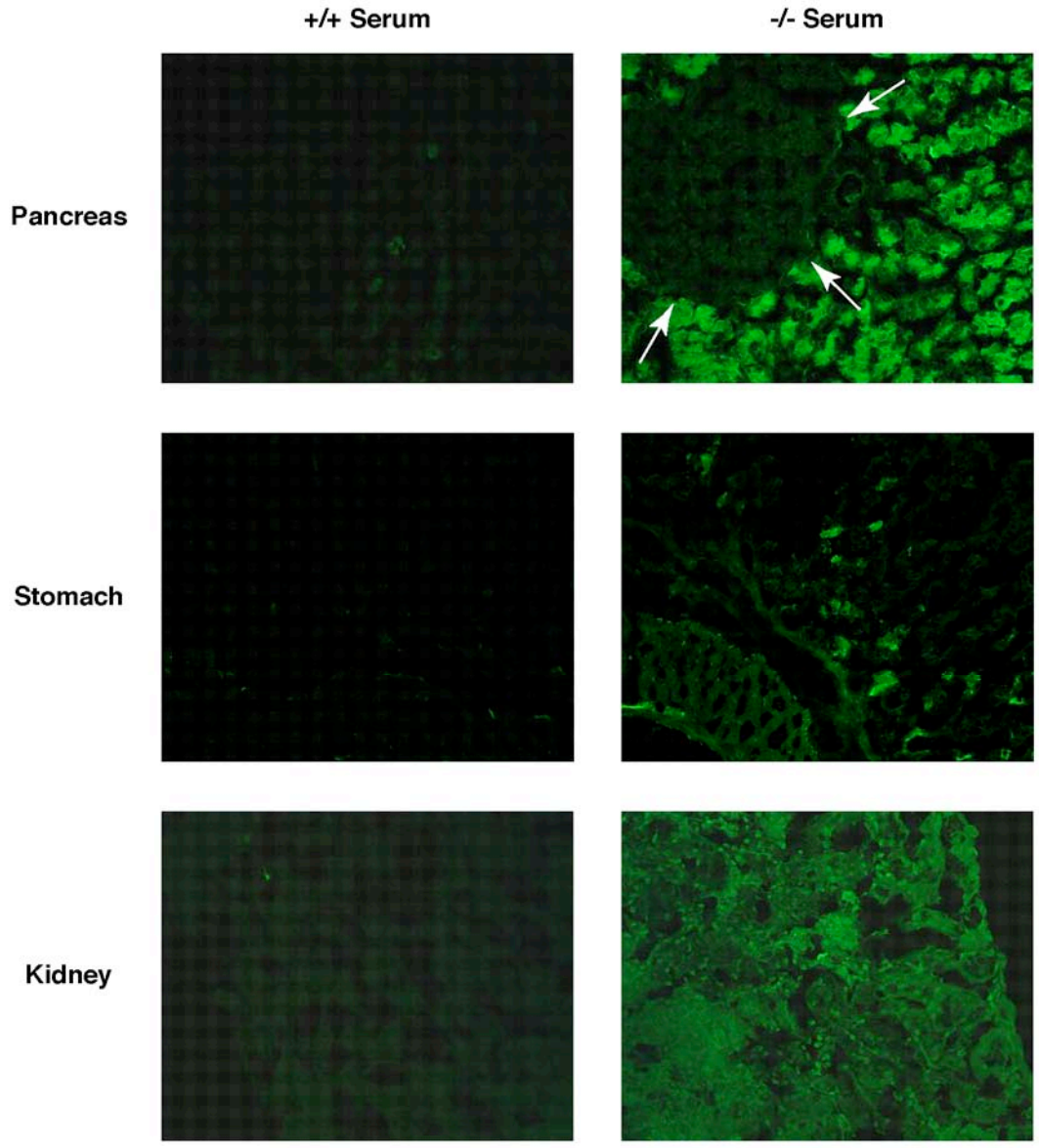
Supplemental Figure 1. Matsumoto M, et al



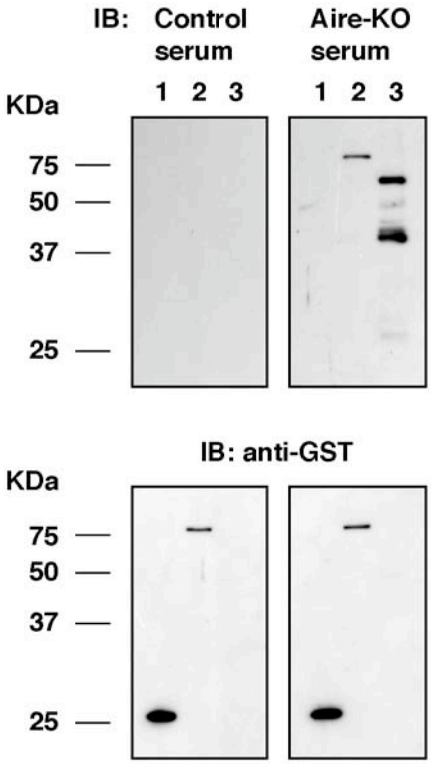
Supplemental Figure 2. Matsumoto M, et al



Supplemental Figure 3. Matsumoto M, et al



Supplemental Figure 4. Matsumoto M, et al



Supplemental Data

Figure S1. Retained thymic structure in both Aire-sufficient and Aire-deficient NOD mice. (A) Thymic medulla, identified as ER-TR5$^+$ areas (stained in red), contained normal numbers and distribution of UEA-1$^+$ cells (stained in green) in both Aire-sufficient and Aire-deficient NOD mice. Original magnification, x 200. (B) Aire$^+$ cells were normally distributed within the medullary TECs in NOD mice. The subcellular distribution of Aire nuclear-dots within the cell was also unaltered. Aire-deficient NOD thymus served as a negative control for staining with anti-Aire Ab. Original magnification, x 400.

Figure S2. Lymphoid cell infiltration in the pancreas from Aire-deficient NOD mice. (A) Both CD4$^+$ and CD8$^+$ cells infiltrated in the pancreas from Aire-deficient NOD mice. (B) B220$^+$ cells, which do not express CD138, were recognized in the pancreas from Aire-deficient NOD mice (upper panels). Clusters of CD138$^+$ cells, which are B220^{low}, were also identified among these lymphoid cell infiltrations (lower panels). Original magnification, x 200.

Figure S3. Auto-Ab production in Aire-deficient NOD mice. Serum from Aire-deficient NOD mice, but not from control littermates, contained IgG class auto-Abs against pancreas, stomach and kidney as detected with immuno-fluorescence. Arrows in the upper right panel indicate a β-cell islet. Original magnification, x 200.

Figure S4. Production of anti-PDIp auto-Ab in Aire-deficient NOD mice. Aire-deficient NOD mouse serum (top right), but not control mouse serum (top left), showed reactivity against bacterially expressed GST-PDIp fusion protein (lane 2), but not against GST alone (lane 1), by Western blot analysis. Protein extracted from pancreas was used as a positive control (lane 3). The same blot was probed with anti-GST Ab (bottom).