

## SUPPLEMENTAL MATERIAL

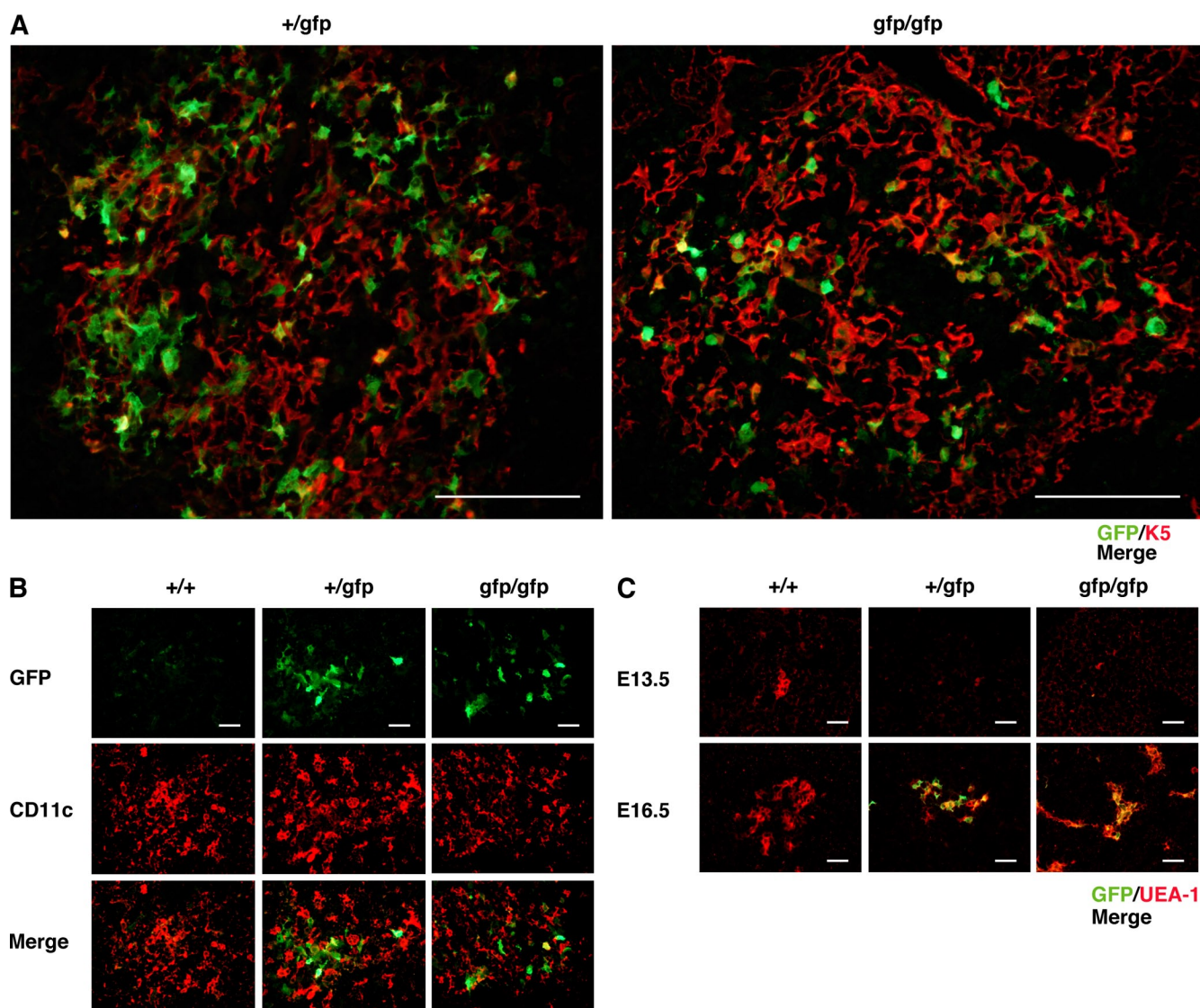
Yano et al., <http://www.jem.org/cgi/content/full/jem.20080046/DC1>

Figure S1. mTECs committed to express Aire in the thymus. (A) mTECs active in *Aire* gene transcription were visualized by immunohistochemistry with anti-GFP Ab (green). The medullary region was identified by staining with anti-K5 Ab (red). Bars, 100  $\mu$ m. (B) Thymic DCs do not express Aire (stained with anti-GFP Ab; green). DCs were identified by staining with anti-CD11c mAb (red). Bars, 20  $\mu$ m. (C) Thymi were harvested at E13.5 and 16.5. The medullary region was identified by staining with UEA-1 (red). mTECs expressing the *Aire* gene were stained with anti-GFP Ab (green). Bars, 20  $\mu$ m. One representative experiment from a total of three repeats is shown.

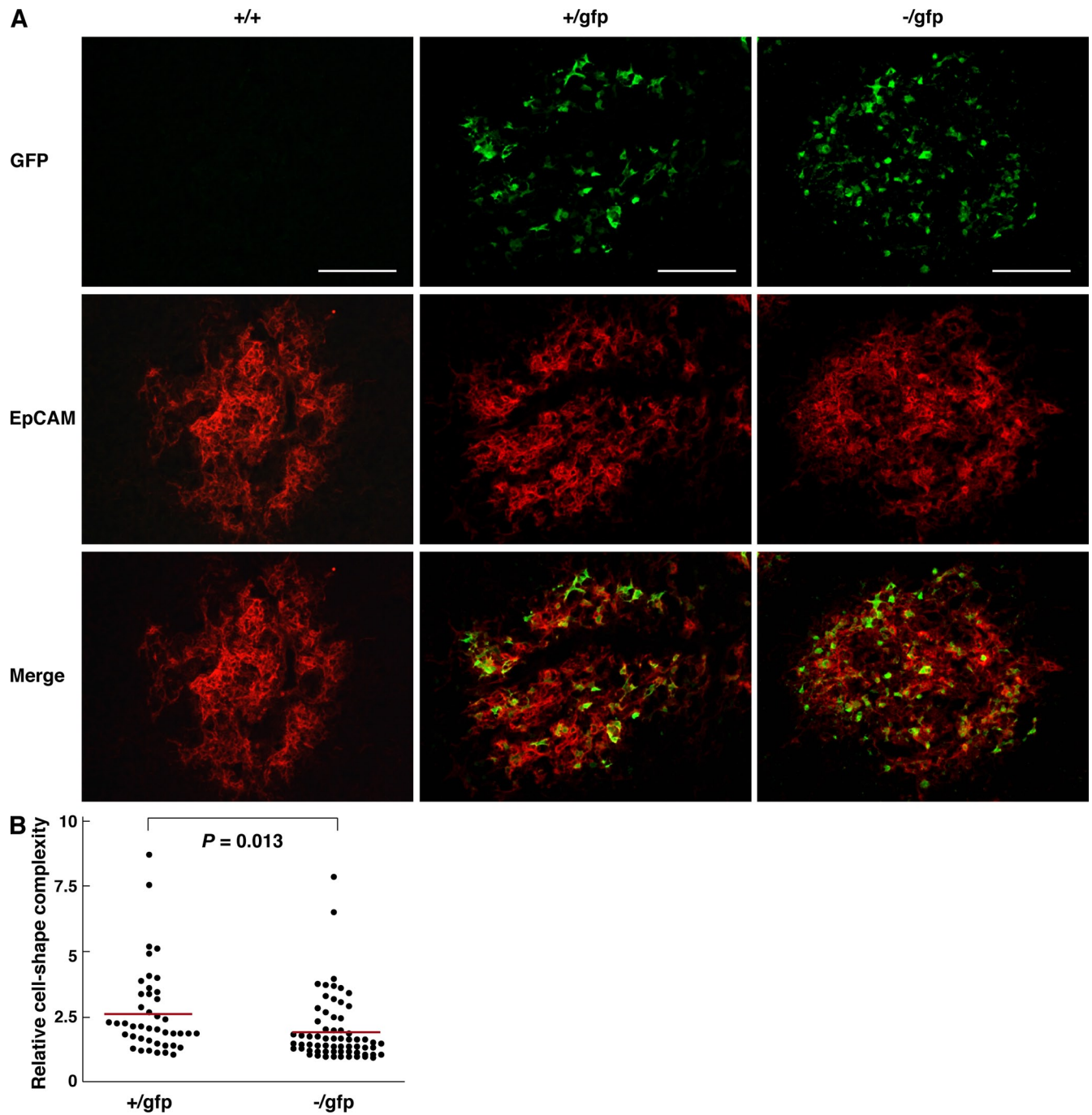


Figure S2. Alteration of the morphology and distribution of mTECs committed to express Aire in *Aire<sup>gfp/gfp</sup>* mouse thymus is not caused by a gene-dosage effect of targeted disruption of the *Aire* gene by the GFP gene. (A) mTECs active in *Aire* gene transcription from *Aire<sup>-gfp</sup>* mice were visualized by immunohistochemistry with anti-GFP Ab (green). The medullary region was identified by staining with anti-EpCAM mAb (red). Bars, 100  $\mu$ m. (B) Morphological changes in the shape of GFP<sup>+</sup> cells from *Aire<sup>+gfp</sup>* and *Aire<sup>-gfp</sup>* thymi, respectively, were statistically analyzed, as described in Fig. 2 D. A total of 43 and 65 GFP<sup>+</sup> cells from *Aire<sup>+gfp</sup>* and *Aire<sup>-gfp</sup>* thymi, respectively, were evaluated. Red lines represent mean values. Two mice for each group were analyzed, and similar results were obtained from a total of three repeats.

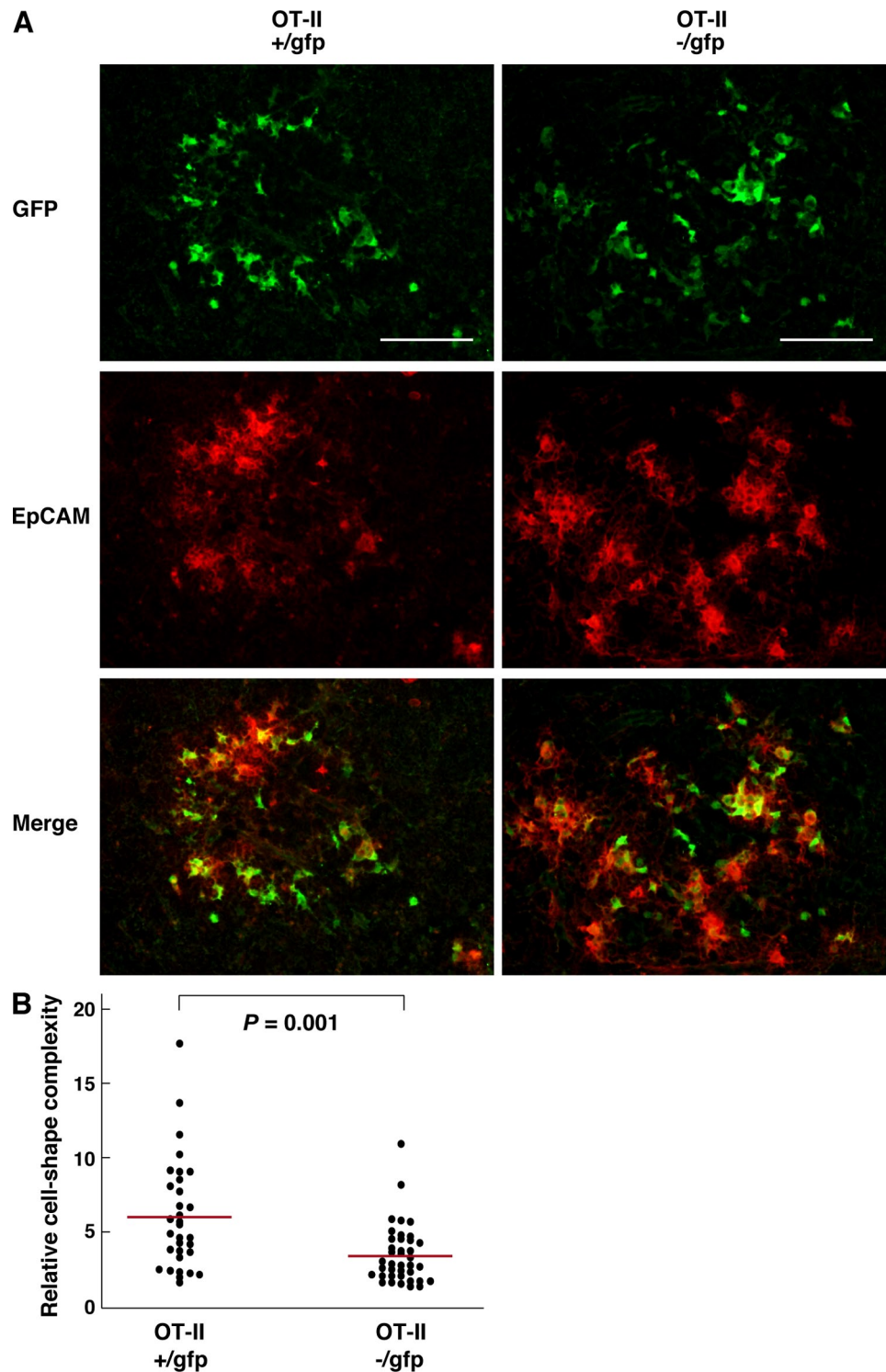


Figure S3. Alteration of the morphology and distribution of mTECs committed to express Aire in the absence of functional Aire protein is not secondary to the autoimmune phenotypes. (A) mTECs showing active *Aire* gene transcription from *Aire*<sup>-/-gfp</sup> mice expressing the nonautoreactive OT-II TCR transgene were visualized by immunohistochemistry with anti-GFP Ab (green). The medullary region was identified by staining with anti-EpCAM mAb (red). Bars, 100  $\mu$ m. (B) Morphological changes in the shape of GFP<sup>+</sup> cells from thymus of *Aire*<sup>-/-gfp</sup> mice expressing OT-II TCR transgene demonstrated in A were statistically analyzed. A total of 32 and 39 GFP<sup>+</sup> cells from thymi of *Aire*<sup>+/gfp</sup> and *Aire*<sup>-/-gfp</sup> mice, respectively, expressing OT-II TCR transgene were evaluated. Red lines represent mean values. Two mice for each group were analyzed, and similar results were obtained from a total of two repeats.



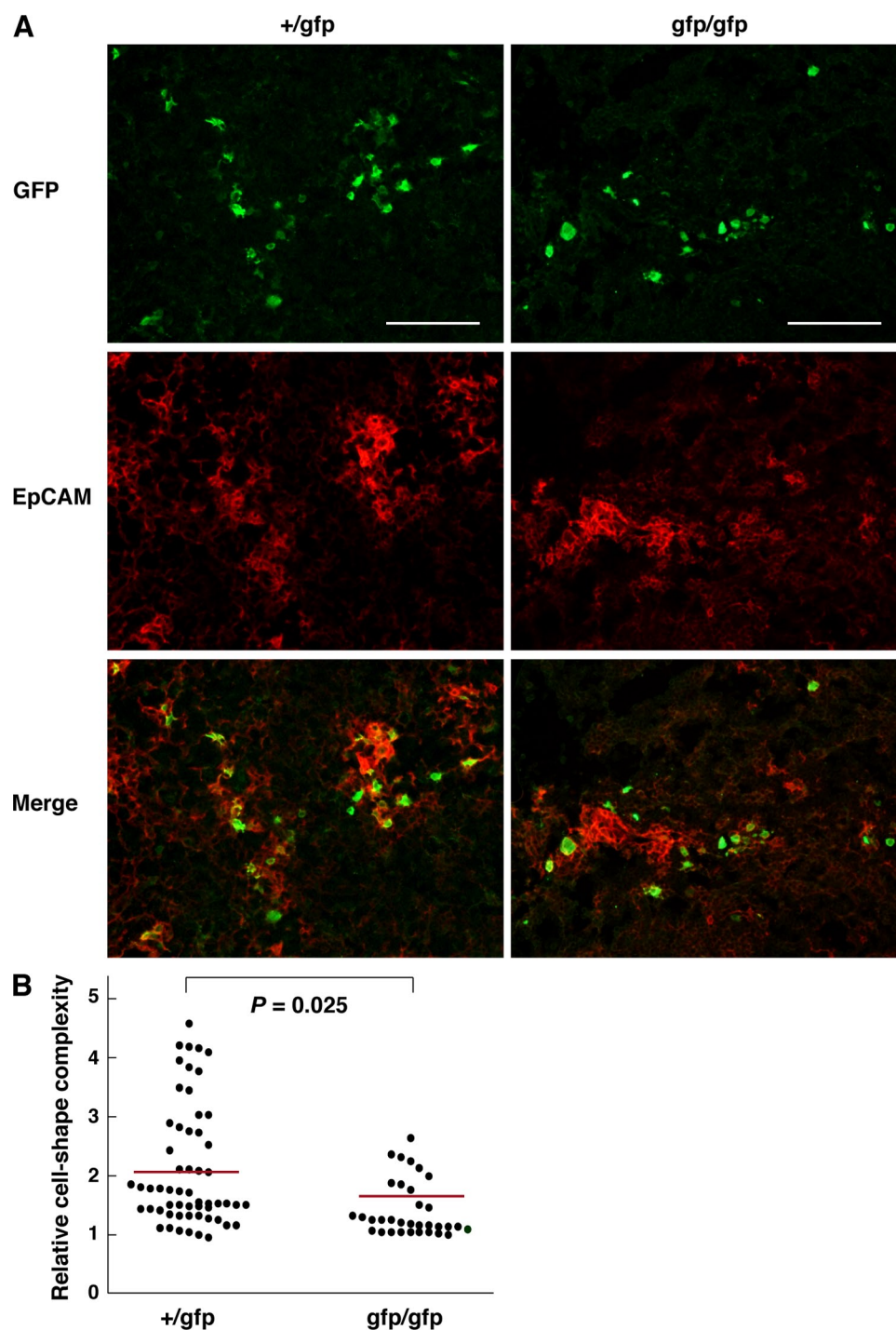


Figure S4. Altered morphology of mTECs committed to express Aire in the absence of functional Aire protein is already evident at the neonatal stage. (A) mTECs showing active *Aire* gene transcription from *Aire<sup>gfp/gfp</sup>* mice at P1 (the neonatal stage) were visualized by immunohistochemistry with anti-GFP Ab (green). The medullary region was identified by staining with anti-EpCAM mAb (red). Bars, 100  $\mu$ m. (B) Morphological changes in the shape of GFP+ cells from neonatal *Aire<sup>gfp/gfp</sup>* mouse thymus demonstrated in A were statistically analyzed. A total of 57 and 33 GFP+ cells from neonatal *Aire<sup>+/gfp</sup>* and *Aire<sup>gfp/gfp</sup>* thymi, respectively, were evaluated. Red lines represent mean values. The results obtained from three mice for each group from a total of three experiments were gathered for the analysis.

Mouse Number	Genotype	Age (wk)/sex	Total number of involucrin-expressing mTECs	Number of sections examined	Involucrin-expressing mTECs per section	Hassall's corpuscle-like structure	Footnote	Depicted in Fig. 3 B
159	+/+	3/F	39	4	9.75	+++	NOD background	No
156	+/-	3/M	22	4	5.5	+++	NOD background	No
161	-/-	3/F	2	4	0.5	-	NOD background	No
416	+gfp	4/M	36	4	9	++	NA	Yes (left)
417A	+/+	4/M	25	4	6.25	+	NA	Yes (left)
418	+/+	4/M	14	4	3.5	++	NA	Yes (left)
419A	gfp/gfp	4/M	0	4	0	-	Small medulla	Yes (left)
421A	gfp/gfp	4/M	0	4	0	-	NA	Yes (left)
402	+/+	8/F	94	4	23.5	++++	NA	Yes (center)
405	+/+	8/F	54	4	13.5	++++	NA	Yes (center)
392	+/+	8/F	37	4	9.25	++++	NA	Yes (center)
404	+gfp	8/F	37	4	9.25	++++	NA	Yes (center)
391	+/+	8/F	27	4	6.75	++++	NA	Yes (center)
417B	+/+	8/M	25	4	6.25	+++	NA	Yes (center)
403	gfp/gfp	8/F	4	4	1	±	NA	Yes (center)
428	-/-	8/M	2	4	0.5	-	NA	Yes (center)
406	gfp/gfp	8/F	2	4	0.5	-	Small thymus	Yes (center)
401	gfp/gfp	8/F	1	4	0.25	-	Small medulla	Yes (center)
419B	+/+	11/F	6	2	3	++	NA	Yes (right)
422	+/-	11/F	5	2	2.5	-	NA	Yes (right)
421B	+/+	11/F	4	2	2	-	NA	Yes (right)
424	-/-	11/M	5	2	2.5	±	NA	Yes (right)
130	-/-	11/F	6	4	1.5	-	NA	Yes (right)
423	-/-	11/F	2	2	1	-	NA	Yes (right)
129	-/-	11/F	0	4	0	-	NA	Yes (right)
425	-/-	11/M	0	2	0	-	NA	Yes (right)
128	+/+	16/F	11	4	2.75	++	NA	No
122	+/+	16/M	10	4	2.5	++	NA	No
113	+/+	17/F	8	4	2	+++	NA	No
127	+/+	16/F	0	4	0	-	Small medulla	No
110	-/-	17/F	5	4	1.25	-	NA	No
124	-/-	15/F	2	4	0.5	-	NA	No
119	-/-	15/M	1	4	0.25	-	NA	No
639	+/+	32/F	4	2	2	-	NA	No
666	+/+	28/M	1	2	0.5	-	NA	No
638	-/-	32/F	0	2	0	-	NA	No
665	-/-	28/M	0	2	0	-	Small thymus	No

NA, not applicable.