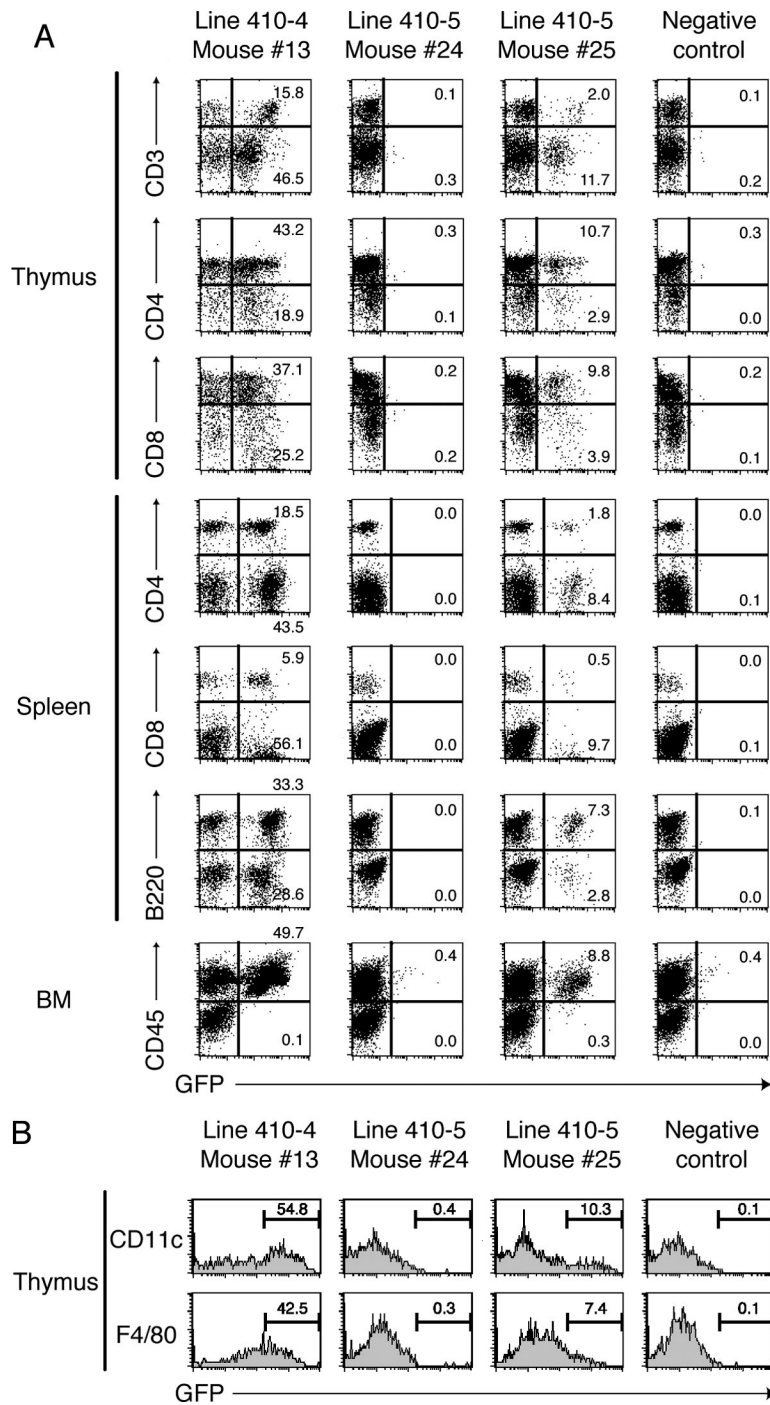
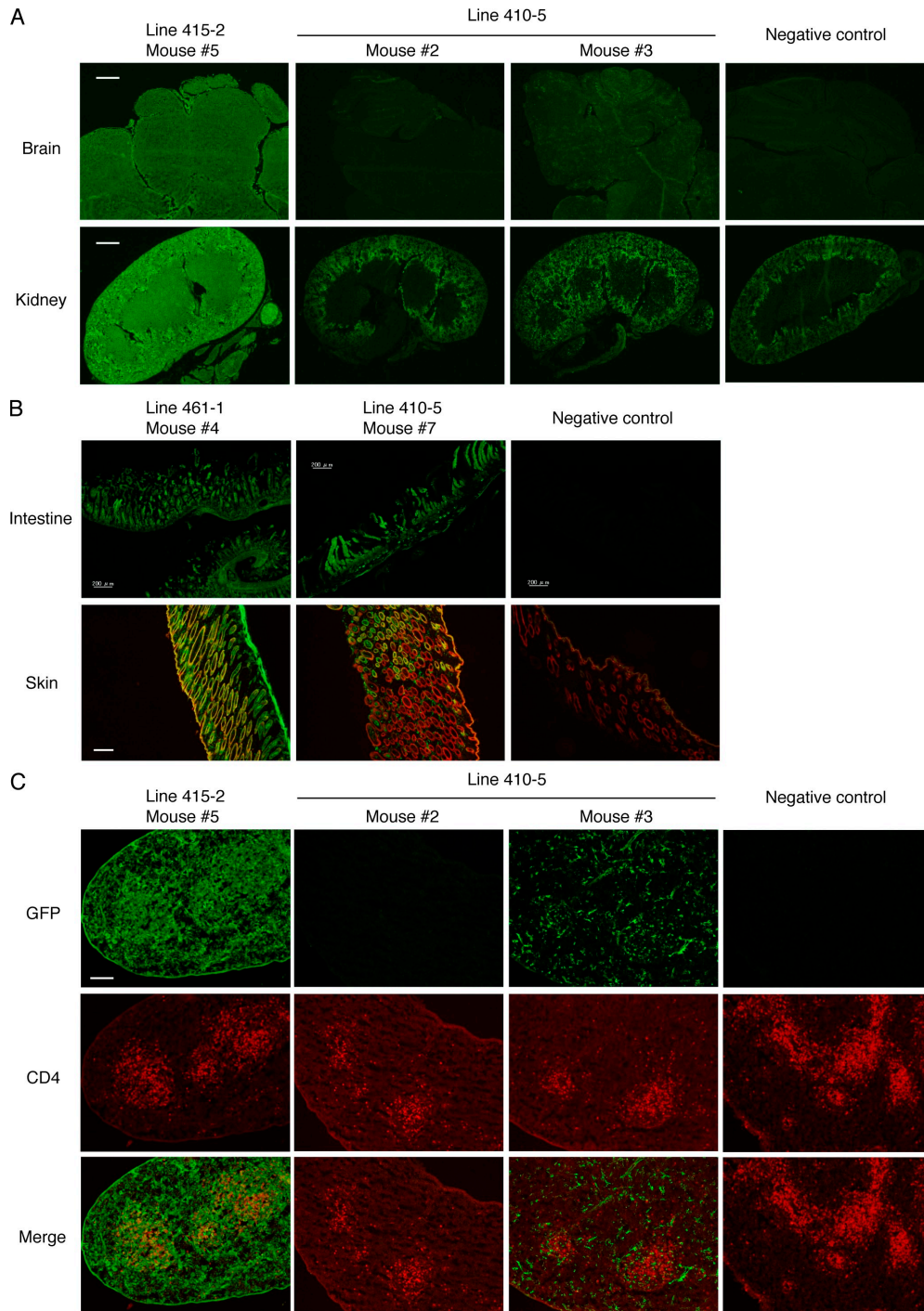


SUPPLEMENTAL MATERIAL

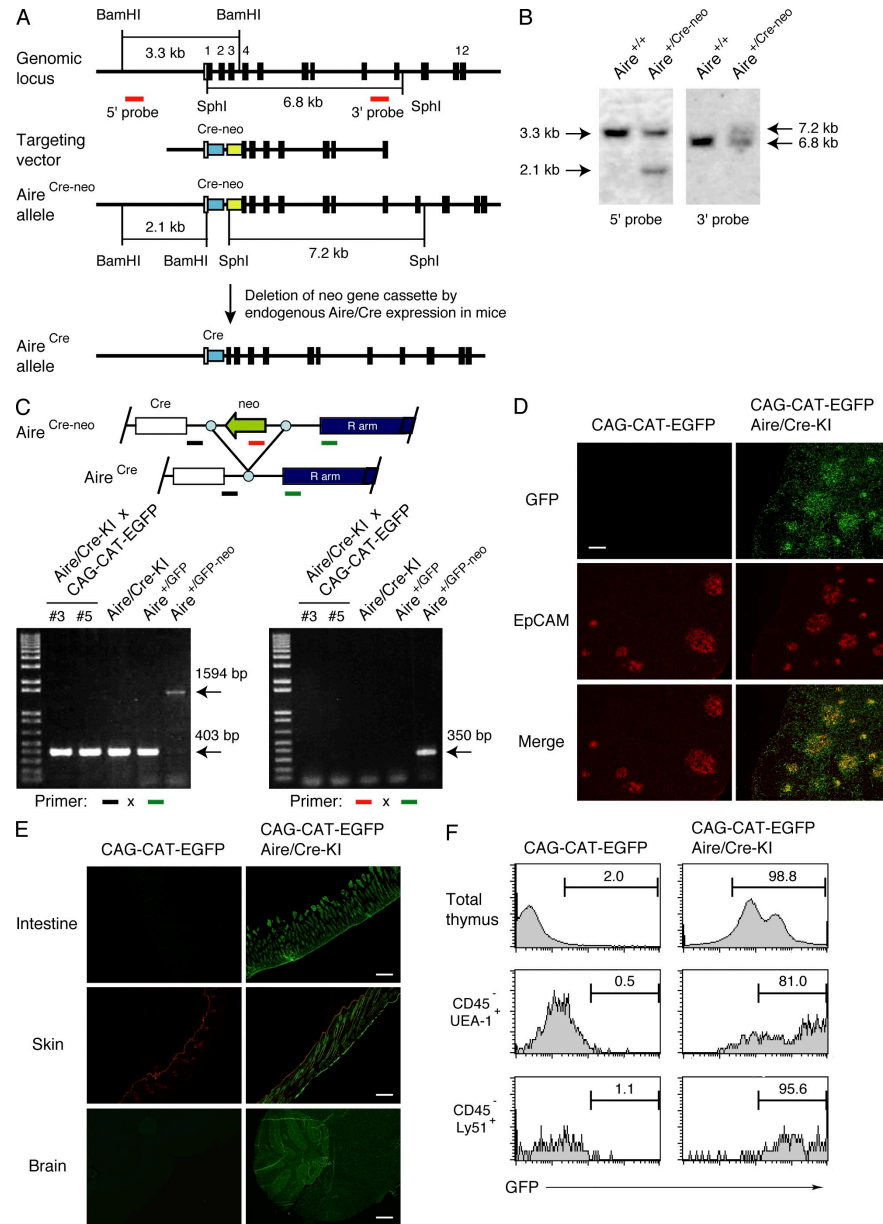
Nishikawa et al., <http://www.jem.org/cgi/content/full/jem.20092144/DC1>



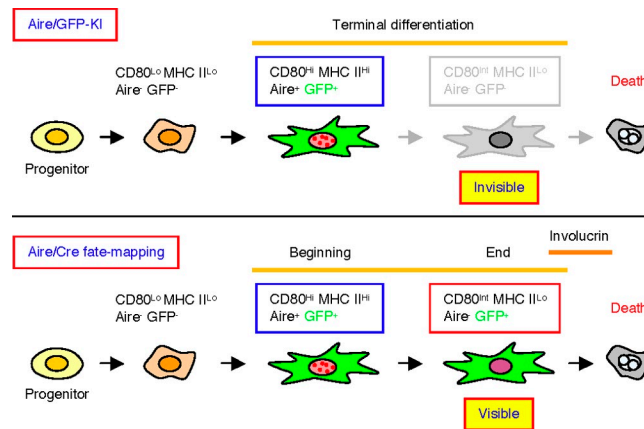
**Figure S1. Flow cytometric analysis of GFP-expressing hemopoietic cells in the thymus, spleen, and BM.** (A) GFP expression from hemopoietic cells was determined for ubiquitous type (mouse #13, line 410-4), mTEC type (mouse #24, line 410-5), mosaic type (mouse #25, line 410-5), and negative control (CAG-CAT-EGFP). Ubiquitous and mosaic types contained GFP<sup>+</sup> hemopoietic cells in all the organs examined, whereas mTEC type possessed no such cells. (B) GFP expression from DCs (top) and macrophages (bottom) in the thymus was determined after making a gate for CD11c<sup>+</sup> and F4/80<sup>+</sup> cells, respectively. Cells from a GFP reporter strain (CAG-CAT-EGFP) served as negative control. Percentages of the cells in the indicated areas are included. One representative result from a total of three repeats is shown.



**Figure S2. Extra-thymic expression of Aire/Cre BAC-Tg.** (A) GFP expression from the brain (top) and kidney (bottom) was examined by immunohistochemistry with anti-GFP Ab (green). Mouse #5 from line 415-2 represents a ubiquitous type for Aire/Cre BAC-Tg expression, whereas mice #2 and #3, both from line 410-5, represent mTEC type and mosaic type, respectively. Sections from a GFP reporter strain (CAG-CAT-EGFP) served as negative control from A to C. Note that cortex of the kidney showed nonspecific signals. Bars, 200  $\mu$ m. (B) Mosaicism of Aire/Cre BAC-Tg expression from intestine (top) and skin (bottom) in mouse #7 from line 410-5 (middle). Mouse #4 from line 461-1 represents a ubiquitous type (left). Staining with anti-GFP Ab (green) and with anti-K5 Ab (red) is merged for the skin. Bars, 200  $\mu$ m. (C) GFP expression in the spleen (green). White pulps were identified by staining with anti-CD4 mAb (red). Ubiquitous type (mouse #5, line 415-2) showed GFP expression from the entire spleen, whereas mTEC type (mouse #2, line 410-5) and mosaic type (mouse #3, line 410-5) showed no and scattered GFP expression, respectively. Bar, 100  $\mu$ m. One representative result from a total of three repeats is shown.



**Figure S3. Ubiquitous GFP expression in *Aire/Cre* knockin mice crossed with a GFP reporter strain.** (A) Targeted insertion of the *Cre recombinase* gene into the *Aire* gene locus by homologous recombination. Note that the neo gene cassette in the targeted allele was deleted in mice, probably as a result of autonomous expression of the *Aire/Cre* gene during early embryogenesis and/or spermatogenesis. (B) Southern blot analysis of genomic DNA from targeted ES cells. DNA was digested with BamHI and SphI and hybridized, respectively, with the 5' probe and 3' probe shown in A. (C) Spontaneous deletion of the neo gene cassette in the *Aire/Cre-KI* line. Primers were designed to detect the presence or absence of the neo gene cassette. Mice used for the analysis shown in panels D-F (mice #3 and #5), together with their parental male (marked as *Aire/Cre-KI*, third lanes in each), had no neo gene cassette. DNA extracted from the *Aire/GFP-KI* line before and after crossing with a general deleter *Cre recombinase*-expressing Tg line (Yano et al. 2008. *J. Exp. Med.* doi:10.1084/jem.20080046) indicated the presence and absence of the neo gene cassette, respectively (fifth and fourth lanes, left). (D) GFP expression in the thymus was examined by immunohistochemistry with anti-GFP Ab (green). The medullary region was identified by staining with anti-EpCAM mAb (red). GFP expression was observed from the entire region of the thymus of offspring from *Aire/Cre* knockin mice (*Aire/Cre-KI*) crossed with a GFP reporter strain. Sections from a GFP reporter strain (CAG-CAT-EGFP) served as negative control. Bar, 200  $\mu$ m. (E) GFP expression from intestine (top), skin (middle), and brain (bottom) in *Aire/Cre-KI* crossed with a GFP reporter strain. Staining with anti-K5 Ab is in red and merged with GFP staining for the skin. Bars, 200  $\mu$ m. (F) Flow cytometric analysis of GFP-expressing cells in the thymus. Cells were isolated enzymatically (top) and CD45<sup>+</sup> cells were stained with markers for mTECs (UEA-1; medium) and cTECs (anti-Ly51; bottom). *Aire/Cre-KI* crossed with a GFP reporter strain contained GFP<sup>+</sup> cells in all the fractions. Percentages of the cells in the indicated regions are included. One representative result from a total of two repeats is shown.



**Figure S4. Aire is expressed before end-stage terminal differentiation in mTECs.** Schematic representation of the differentiation program of Aire-expressing cell lineages suggested by the present study. Aire<sup>+</sup>CD80<sup>high</sup>MHC class II<sup>high</sup> mTECs develop from Aire<sup>-</sup>CD80<sup>low</sup>MHC class II<sup>low</sup> immature mTECs (Gäbler et al. 2007. *Eur. J. Immunol.* doi:10.1002/eji.200737131; Gray et al. 2007. *J. Exp. Med.* doi:10.1084/jem.20070795; Rossi et al. 2007. *J. Exp. Med.* doi:10.1084/jem.20062497). Differential cell marking with GFP arising from Aire/GFP-KI (Yano et al. 2008. *J. Exp. Med.* doi:10.1084/jem.20080046) and the fate-mapping system reveals the existence of Aire<sup>-</sup>CD80<sup>int</sup>MHC class II<sup>low</sup> end-stage cells before cell death. The results also suggest that Aire expression is not constitutive but that it is expressed transiently at the beginning of terminal differentiation. Note that involucrin expression (Yano et al. 2008. *J. Exp. Med.* doi:10.1084/jem.20080046) might follow post-Aire stages because GFP and involucrin were scarcely coexpressed in the mTECs from mTEC-type fate-mapping mice if involucrin and Aire are expressed by the same lineages of mTECs.

**Table S1.** List of mice presented in the fate-mapping study

Line	Mouse	Thymus	Other organs	Reference
410-3	#5	Mosaic by IHC	Mosaic (Int, Sk)	Not depicted
410-4	#7	Ubiquitous by IHC	Ubiquitous (Br, Ki, Int, Sk)	Not depicted
410-4	#13	Ubiquitous by FACS	Ubiquitous (Sp)	Fig. S1
410-4	#17	Ubiquitous by FACS	Ubiquitous (PBL)	Fig. 3
410-5	#2	mTEC by IHC	N.D. (Br, Ki, Int, Sp)	Fig. 1 and Fig. S2 (A and C)
410-5	#3	Mosaic by IHC	Mosaic (Br, Kid, Int, Sk, Sp)	Fig. S2 (A and C)
410-5	#4	Mosaic by IHC	Mosaic (Int, Sk)	Not depicted
410-5	#5	Mosaic by IHC	Mosaic (Int, Sk)	Fig. 1 A
410-5	#7	Mosaic by IHC	Mosaic (Int, Sk)	Fig. S2 B
410-5	#24	mTEC by IHC/FACS	N.D. (Sk, Sp, BM)	Figs. 4 and S1
410-5	#25	Mosaic by FACS	Mosaic (Sk, Sp, BM)	Fig. S1
410-5	#28	Mosaic by IHC/FACS	Mosaic (PBL)	Not depicted
410-5	#29	mTEC by IHC/FACS	N.D. (PBL)	Figs. 3, 4, and 5
410-5	#33	Mosaic by IHC/FACS	Mosaic (PBL)	Not depicted
410-5	#38	mTEC by FACS	N.D. (PBL)	Not depicted
410-5	#39	mTEC by FACS	N.D. (PBL)	Not depicted
410-5	#43	Mosaic by FACS	Mosaic (PBL)	Not depicted
410-5	#44	mTEC by FACS	N.D. (PBL)	Fig. 4
410-5	#46	mTEC by FACS	N.D. (PBL)	Fig. 4
410-5	#52	mTEC by FACS	N.D. (PBL)	Fig. 4
410-5	#72	mTEC by FACS	N.D. (PBL)	Not depicted
410-5	#81	mTEC by FACS	N.D. (PBL)	Fig. 4
410-5	#84	N.A.	Mosaic (PBL)	Not depicted
410-5	#96	mTEC by FACS	N.D. (PBL)	Fig. 4
410-5	#157	mTEC by FACS	N.A.	Fig. 4
410-5	#162	Mosaic by FACS	N.A.	Not depicted
410-5	#163	mTEC by FACS	N.A.	Not depicted
410-5	#164	Mosaic by FACS	N.A.	Not depicted
413-3	#3	Ubiquitous by IHC	Ubiquitous (Int, Sk)	Not depicted
413-3	#6	Ubiquitous by IHC	Ubiquitous (Int, Sk)	Not depicted
415-2	#5	Ubiquitous by IHC	Ubiquitous (Br, Kid, Sp)	Fig. S2 (A and C)
415-2	#7	Ubiquitous by IHC	Ubiquitous (Br, Ki, Int, Sk)	Not depicted
461-1	#2	Ubiquitous by IHC	Ubiquitous (Int, Sk, Sp)	Fig. 1 A
461-1	#4	Ubiquitous by IHC	Ubiquitous (Int, Sk)	Fig. S2 B
461-1	#7	Ubiquitous by IHC	N.A.	Not depicted

IHC, immunohistochemistry; Br, brain; Kid, kidney; Int, intestine; Sk, skin; Sp, spleen; PBL, peripheral blood lymphocyte; N.D., not detected; N.A., not assessed.