Supporting Information

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SI Materials and Methods

Mice. β5t-Venus knock-in mice (1), CAG-loxP-stop-loxP-EGFP-transgenic mice (2), and Rosa26 knock-in mice that were engineered to contain the CAG-loxP-stop-loxP-ZsGreen sequence (3) were described previously. Flp-deleter (129S4/SvJaeSor-Gt (ROSA)26Sor$^{tm1(FLP1)Dym/J}$) mice were obtained from The Jackson Laboratory. For developmental staging, the day of the vaginal plug was designated as E0.5. Mice were maintained under specific pathogen-free conditions and experiments were carried out under the approval of the Institutional Animal Care and Use Committee of the University of Tokushima and according to Swiss cantonal and federal regulations and permissions.

Generation of β5t-Cre Knock-In Mice. β5t-Cre targeting construct was prepared by subcloning cDNA encoding the codon-improved Cre recombinase (iCre, a gift from Dr. R. Sprengel, Heidelberg, Germany) into the targeting vector with β5t homology arms (1) containing a PGK-neo cassette in a transcriptionally opposite direction that is flanked by flippase recognition target sites. The linearized vector was electroporated into 129xC57BL/6 ES cells and correctly targeted clones were identified by PCR and verified by Southern blotting. Chimeric mice were generated using standard techniques and further bred to a C57BL/6 background. The genotypes of β5t-Cre mice were determined by PCR analysis of tail DNA using the following three primers: β5t 5′ untranslated region forward primer, 5′-ATCCCTCACCAGCCAATTCCAAAGCC-3′; β5t coding sequence reverse primer, 5′- TGGTGCACAGGAATGACCTTCCGT-3′; and iCre reverse primer, 5′- GAGATGTCCTTCACTCTGATTC-3′. The amplified products were electrophoresed on 1.0% agarose gel and visualized with ethidium bromide.

Immunohistology. Tissues were fixed in 4% (g/vol) paraformaldehyde, embedded in optimum cutting temperature (OCT) compound (Sakura Finetek), and frozen. Thymuses and embryos were sliced into 5-μm-thick and 10-μm-thick sections, respectively. The sections were stained using antibodies specific for GFP (Invitrogen), keratin (K) 5 (Covance), K14 (Covance), CD249 (Ly51, eBioscience), CD326 (EpCAM, BioLegend), β5t (1), and Alexa Fluor 647-conjugated anti-Aire antibody (eBioscience). Alexa-Fluor conjugated anti-IgG antibodies (Invitrogen) were used as secondary reagents. Images were analyzed with a TSC SP2 confocal laser-scanning microscope and Leica Confocal software (version 2.6, Leica).

Cell Preparation. Minced fragments of the thymus and other organs were digested with 0.125% collagenase D (Roche) in the presence of 0.01% DNase I (Roche), as described previously (4). For the analysis and isolation of TECs, CD45$^+$ cells were depleted using magnetic bead–conjugated anti-CD45 antibody (Miltenyi Biotech).

Flow Cytometry. Multicolor flow cytometry and cell sorting were performed with FACSAria II (BD Bioscience). For the analysis of TECs, cells were stained for the expression of CD326 (EpCAM, BioLegend), CD205 (eBioscience), CD249 (Ly51, eBioscience), and CD45 (eBioscience) and for reactivity with UEA1 (Vector Laboratories). For intracellular Aire staining, cells were surface-stained for CD45, CD326, and UEA1 reactivity, fixed in 2% paraformaldehyde, permeabilized with 0.1% saponin, and stained with Alexa Fluor 647-conjugated anti-Aire antibody (eBioscience). For intracellular Cre staining, cells were surface-stained for CD45, CD326, CD80, and CD249, fixed in 2% paraformaldehyde, permeabilized with 0.1% saponin, and stained with biotinylated anti-Cre antibody (Covance) followed by allophycocyanin-conjugated streptavidin. For the analysis of thymocytes, cells were stained with allophycocyanin-conjugated anti-CD4 antibody (BioLegend) and biotinylated anti-CD8 antibody (BioLegend) followed by phycoerythrin-conjugated streptavidin.

Quantitative mRNA Analysis. Total cellular RNA was reverse-transcribed with oligo-dT primer and SuperScript III reverse transcriptase (Invitrogen). Quantitative real-time PCR was performed using SYBR Premix Ex Taq (TaKaRa) and a 7900HT Sequence Detection System (Applied Biosystems). The primers used were as follows: Cre, 5′-GACTACCTCCTGTACCTGCA-3′ and 5′-GAGATGTCCTTCACTCTGATTC-3′; EGFP, 5′-AGCAAGGGCGAGGAGCTGTT-3′ and 5′-GTAGGTCAGGGTGGTCACGA-3′; β5t, 5′-CTCTGTGGCTGGGACCACTC-3′ and 5′-TCCGCTCTCCCGAACGTGG-3′; and GAPDH, 5′-TTGTCAGCAATGCATCCTGCAC-3′ and 5′-GAAGGCCATGCCAGTGAGCTTC-3′. The amplified products were confirmed to be single bands by gel electrophoresis and normalized to the amount of GAPDH amplification products.

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2. Kawamoto S, et al. (2000) A novel reporter mouse strain that expresses enhanced green fluorescent protein upon Cre-mediated recombination. *FEBS Lett* 470(3):263–268.
3. Madisen L, et al. (2010) A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci* 13(1):133–140.
4. Gray DH, et al. (2006) Developmental kinetics, turnover, and stimulatory capacity of thymic epithelial cells. *Blood* 108(12):3777–3785.

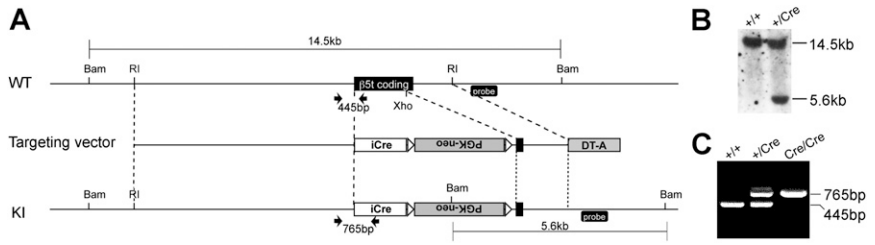

Fig. S1. Generation of β5t-Cre knock-in mice. (*A*) Schematic illustration of β5t genomic locus, targeting vector, and targeted locus. Bam, BamHI; DT-A, diphtheria toxin A; iCre, codon-improved Cre; KI, knock-in; PGK-neo, phosphoglycerate kinase I promoter-driven neomycin resistance gene; RI, EcoRI. Open triangles represent the flippase recognition target (frt) sites. In the KI allele, iCre-encoding cDNA replaces the β5t-encoding sequence. (*B*) Southern blot analysis of BamHI-digested genomic DNA from WT (+/+) and β5t-Cre knock-in heterozygous (+/Cre) mice. The probe is shown in *A*. (*C*) PCR analysis of genomic DNA isolated from WT (+/+), β5t-Cre knock-in heterozygous (+/Cre), and homozygous (Cre/Cre) mice. PCR primers are shown in *A*.