Immunofluorescence
Thymi were embedded with OCT, "flash frozen" in liquid nitrogen, and cut at 10 µm. Cryosections were fixed in acetone, rinsed in TBST (0.1% Tween20), blocked in TBS with 0.5% Tween20 and 10% serum, and incubated with the following primary antibodies: Rabbit anti-mouse K5 (Covance), Rabbit anti-mouse K14 (Covance), Rat anti-mouse K8 (Troma-1) or with UEA-1-biotin (Vector) at RT for 2 hours or at 4°C overnight, followed by Donkey anti-rat IgG-FITC or Donkey anti-rabbit IgG-Texas Red secondary antibodies, or Streptavidin-FITC (Jackson Immunoresearch) at RT for 30 minutes. Foxn1 staining was performed with a polyclonal antibody to Foxn1 (WHN G-20, Santa Cruz) in combination with either UEA-1 or anti–MHCII-FITC (BD Pharmigen). Imaging was done on a Zeiss Axioplan 2 microsope with a AxioCam HRm digital camera.

X-gal staining
Frozen thymus sections were rinsed with PBS and fixed with 4% PFA for 5 minutes at 4°C, then washed with PBS and incubated with detergent rinse solution (0.1M PO4, pH7.4, 2mM MgCl2, 0.01% sodium deoxycholate, 0.02% Igepal) for 10 minutes at RT, followed by incubating with staining buffer [0.1M PO4, pH7.4, 2mM MgCl2, 0.01% sodium deoxycholate, 0.02% Igepal, 5mM K3Fe(CN)6, 5mM K4Fe(CN)6] at 37°C for overnight. The sections were then washed with PBS and re-fixed in acetone and counter-stained with eosin.

Quantitative RT-PCR
Total RNA was extracted with trizol (Invitrogen) or micro RNA purification Kit (Qiagen). First-strand cDNA was reverse transcribed with superscript III (Invitrogen) and random primers (Invitrogen), incubated at 42°C for 90 minutes and then at 70°C for 15 minutes. RNaseH and RNaseA (Promega) were used to remove RNA from the transcribed first-strand cDNA. Quantitative PCR was performed on an ABI 7500 real time PCR system with Taqman universal PCR mix (Applied Biosystems) and the following primers purchased from Applied Biosystems: 18S rRNA VIC/TAMRA primer-probe (4310893E), and Foxn1 FAM primer-probe (Forward primer: 5′-CCTCCCTTGCAACATATGTACTGT-3′, Reverse primer: 5′-GGTAGGGCACAGGGTAGCT-3′ , Reporter: 5′-CCTTCCATCAGTACTCCC-3′, Dye: FAM).

Alternatively, PCR was performed with SYBR green PCR master mix and the following primers designed with Primer Express3.0 software (Applied Biosystems): 5′-CGATGCCCTGAGGCTCTTT-3′ and 5′-TGGATGCCACATGATTCCA-3′ for Actin as endogenous control; 5′-TTGCAGTGCACGGCAGATAC-3′ and 5′-ACGCGTGAGCGGTCGTAA-3′ for LacZ; 5′-GCCGCGCGAGATATGG-3′ and 5′-AGCTTGCATGATCTCCGGTATT-3′ for Cre. The PCR condition is as follows: 50°C, 2min; 95°C, 10min; 40 cycles of 95°C for 15sec and 60°C for 1min. Relative quantity of the gene expression was determined using 7500 SDS software (Applied Biosystems).

Primary TEC culture and de-methylation treatment
Enriched TECs were prepared from $Foxn1^{lacZ/Cre}$ thymi as described above and resuspended in culture medium [RPMI 1640 with 2mM L-Glutamine, 10% Fetal Bovine Serum, 100 IU/ml penicillin, 100 mg/ml streptomycin (Gibco)]. The cells were plated onto 100mm cell culture dishes pre-coated with 0.1% gelatin and cultured at 37 °C with 5% CO2. The medium was changed every other day to wash off non-adherent cells. After 5 days' culture, half of the cultures

were treated with 5 μM 5-Aza-dC (Sigma) for 48 hours (medium was changed every 24 hours) and followed by 5 μM 5-Aza-dC and 300nM TSA (sigma) for 24 hours. Cells were then collected for RNA extraction and FACS analysis.