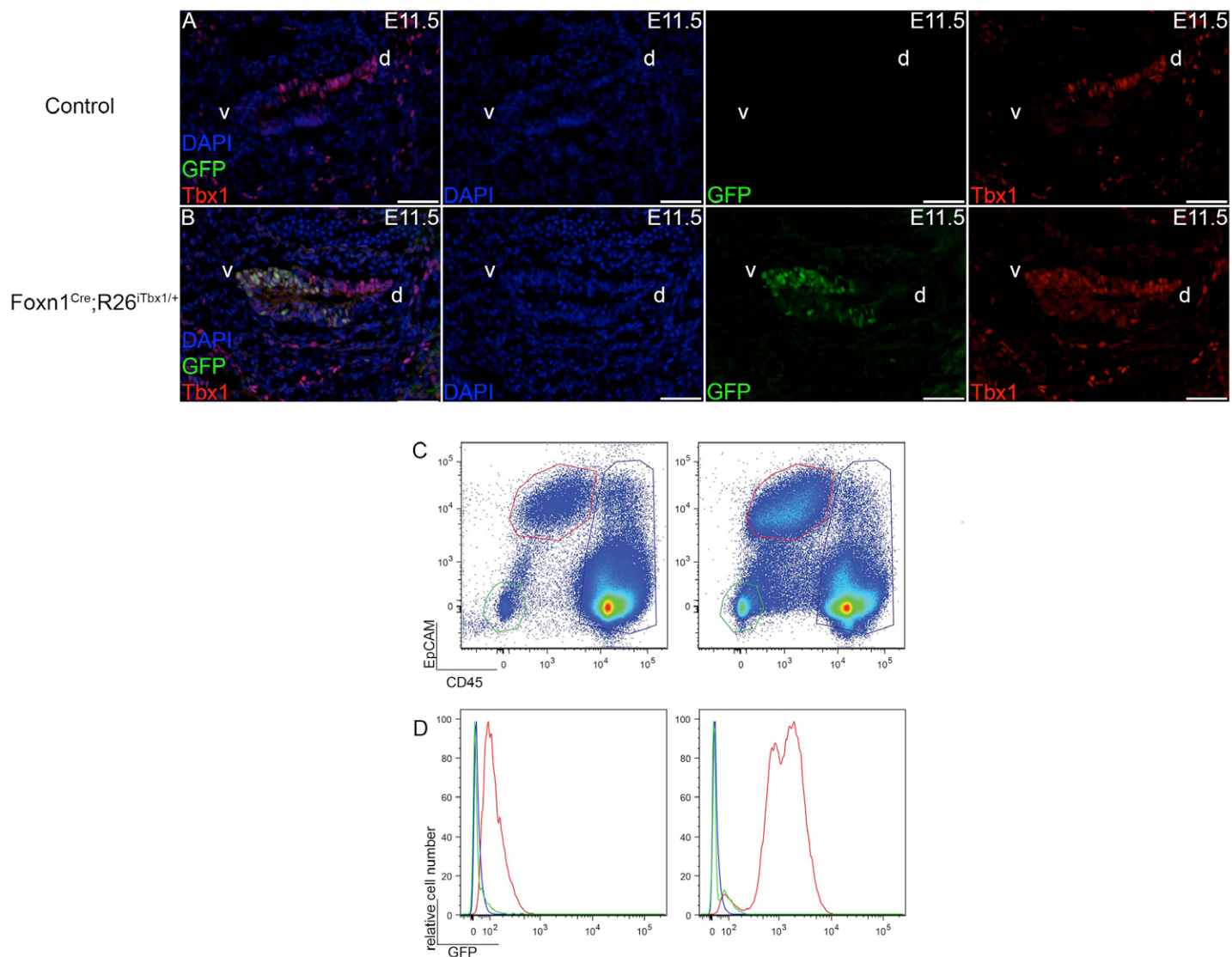


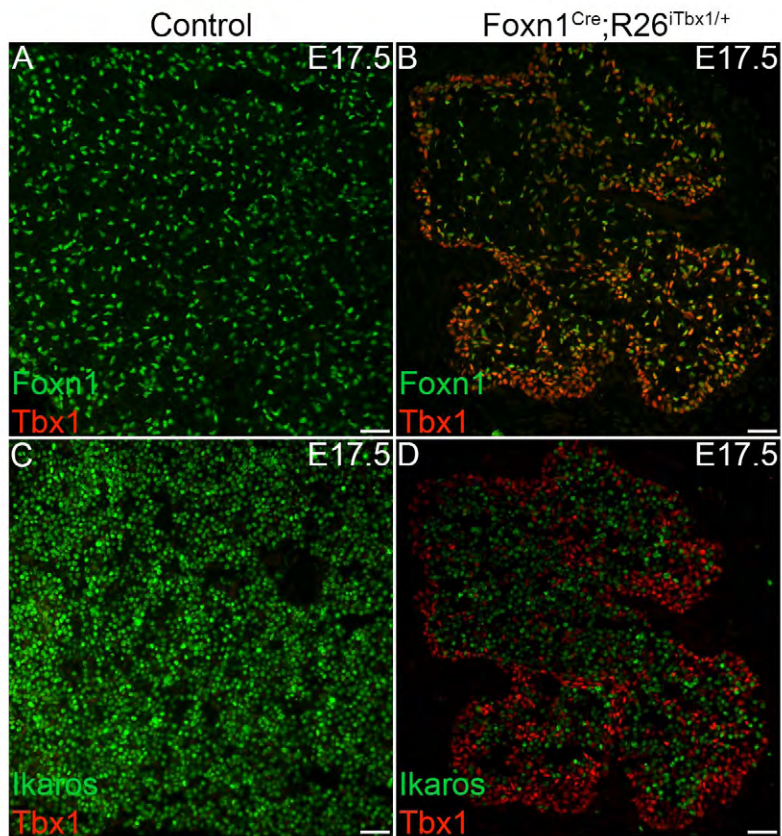
**Fig. S1. Generation of *Rosa26Tbx1* conditional knock-in mice.**

(A) Schematic illustration of the *Rosa26* genomic wild-type (WT) locus, targeting vector and targeted knock-in (KI) locus. Black triangles represent the loxP sites. The black bar represents the 5' probe used for the Southern Blot screen. Arrows represent the primers used for the PCR screen for the targeting event, which amplify a 1.2 kb PCR product from the KI allele, but not the wild-type allele. (B) Southern blot analysis of genomic DNA from Neo resistant ES cell lines after electroporation. The genomic DNA samples were digested with Hind III and hybridized with the 5' probe shown in S1A. The 5' probe hybridizes with a 4.3 kb band from the *Rosa26* WT allele and a 3.6 kb band from the *R26<sup>iTbx1</sup>* allele. (C) PCR genotyping analysis of genomic DNA from *R26<sup>iTbx1</sup>* strain in which a 298bp band represents the WT allele and a 456bp band represents the mutant allele.



**Fig. S2. Ectopic *Tbx1* is expressed in the thymus-fated domain of the 3<sup>rd</sup> pp and in TECs, as reflected by GFP expression.**

(A,B) Representative IHC stains of sagittal sections from E11.5 3<sup>rd</sup> pp show GFP and TBX1 expression in the *R26<sup>iTbx1</sup>* mutant (B) but not the control (A) and verifying ectopic expression of *Tbx1* in the ventral, but not dorsal, domain of the pouch. (C) FACS analysis of E11.5 control and *Foxn1Cre;R26<sup>iTbx1/+</sup>* thymi showing electronic gates set around EpCAM<sup>+</sup> CD45<sup>-</sup> TECs (red), CD45<sup>+</sup> thymocytes (blue) and EpCAM<sup>-</sup>CD45<sup>-</sup> non-TEC stromal cells (green). (D) Histograms showing GFP expression in the corresponding TEC, thymocyte and non-TEC stromal cell subsets. The GFP signal in *Foxn1Cre;R26<sup>iTbx1/+</sup>* TECs confirms continued expression of the *R26<sup>iTbx1</sup>* allele in mutant TECs. The data also confirm that the GFP signal is restricted to EpCAM<sup>+</sup> CD45<sup>-</sup> TEC subset in the mutant thymus and is not detected in control TECs,



**Fig. S3. Thymocytes co-localize with TECs expressing low levels of FOXN1 in E17.5 *Foxn1Cre;R26<sup>iTbx1/+</sup>* thymic lobes.** Representative IHC stains of serial transverse sections showing co-stains for FOXN1 and TBX1 (A,C) and for IKAROS and TBX1 (B,D) in control (A,C) and *Foxn1Cre;R26<sup>iTbx1/+</sup>* (B,D) E17.5 thymic lobes.