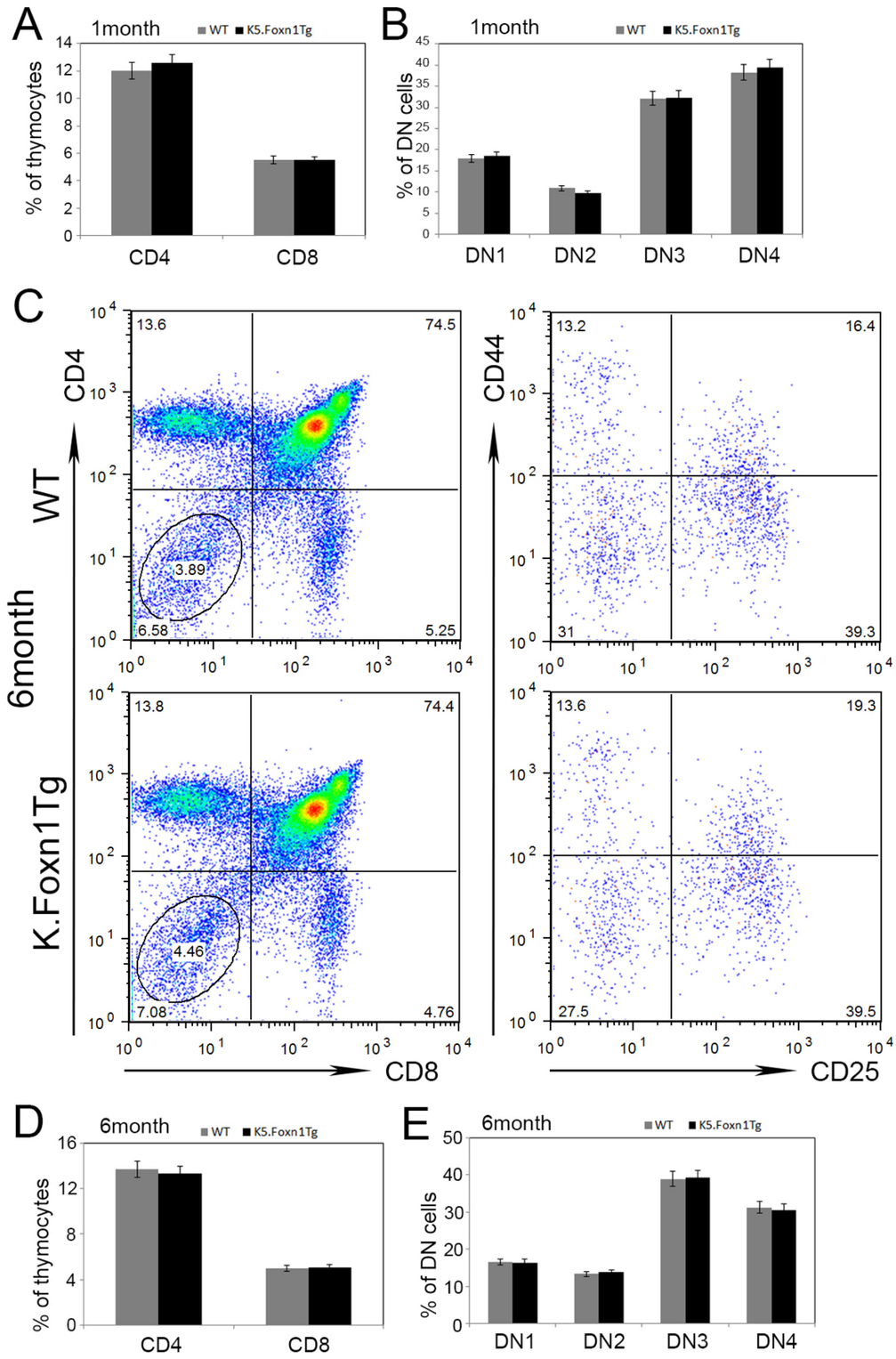

Fig. S1. Thymocytes in Foxn1Tg mice. (A) Percentage of CD4 and CD8 SP cells in one month WT and *K5.Foxn1*Tg thymocytes. **(B)** Percentage of DN1-4 subsets of CD4-CD8- cells defined by CD25 and CD44 in one month WT and *K5.Foxn1* Tg thymocytes. **(C)** Profile of CD4, CD8, CD25, and CD44 expression in *K5.Foxn1* Tg+ and WT thymocytes at six months old. **(D)** Percentage of CD4 and CD8 SP cells in six month WT and *K5.Foxn1* Tg thymocytes. **(E)** Percentage of DN1-4 subsets of CD4-CD8- cells in six month WT and *K5.Foxn1*Tg thymocytes. (n=5)

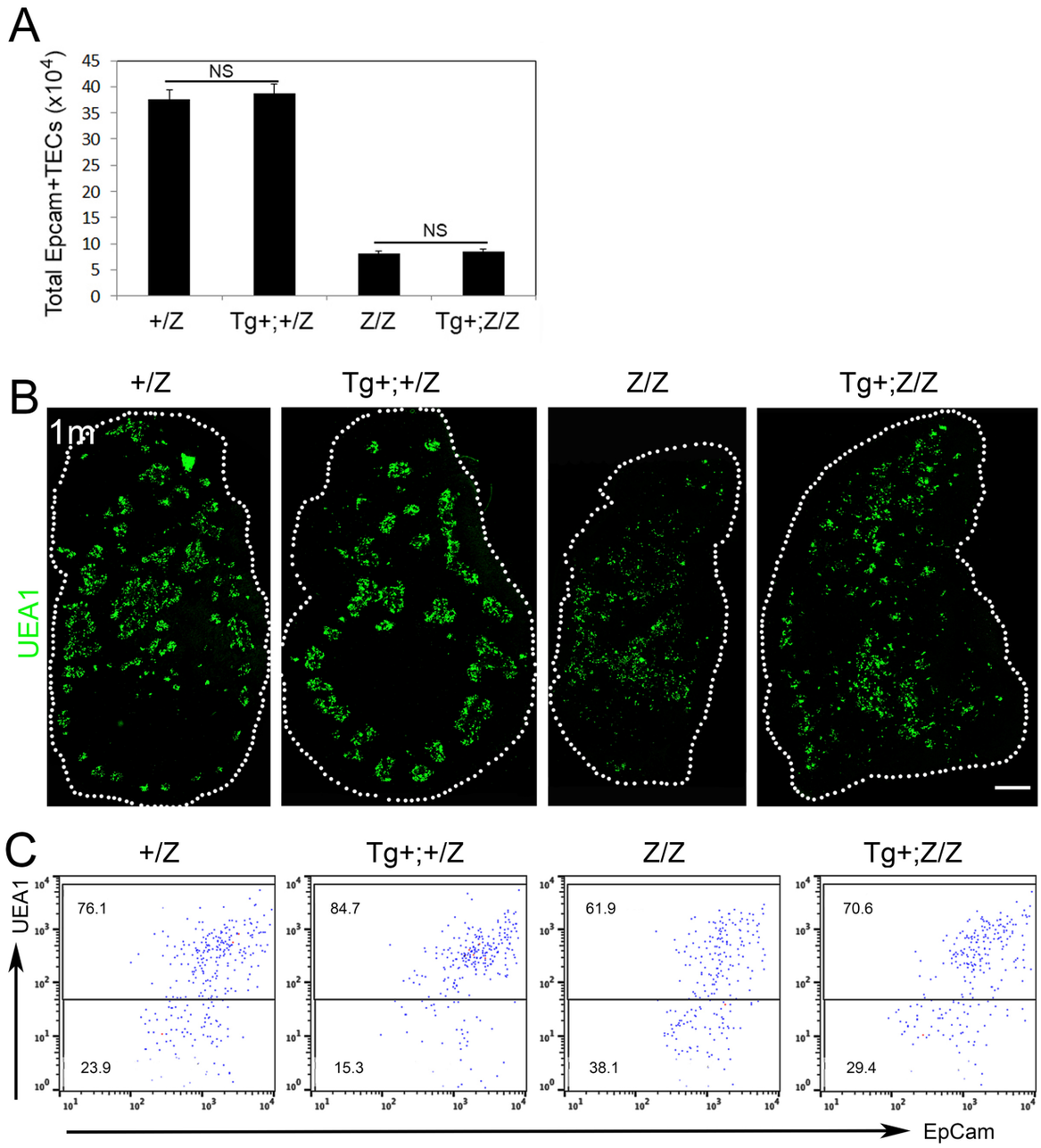

Fig. S2. UEA1 staining in thymi from *Foxn1Z/Z* with and without the *K5.Foxn1* transgene. All thymi are from one month old mice. **(A)** Total thymic epithelial cells numbers in +/Z, Tg+;+/Z, Z/Z and Tg+;Z/Z mice. **(B)** UEA-1 (green) on whole sections. The number and intensity of UEA1+ cells were decreased in the *Foxn1Z/Z* mutant. Scale bar=300µm. **(C)** Profiles of EpCam and UEA1 expression in gated CD45- thymic epithelial cells. (n=5) Statistical anlayses were carried out using one-way Student's t Test. All paired images are shown at same magnification.

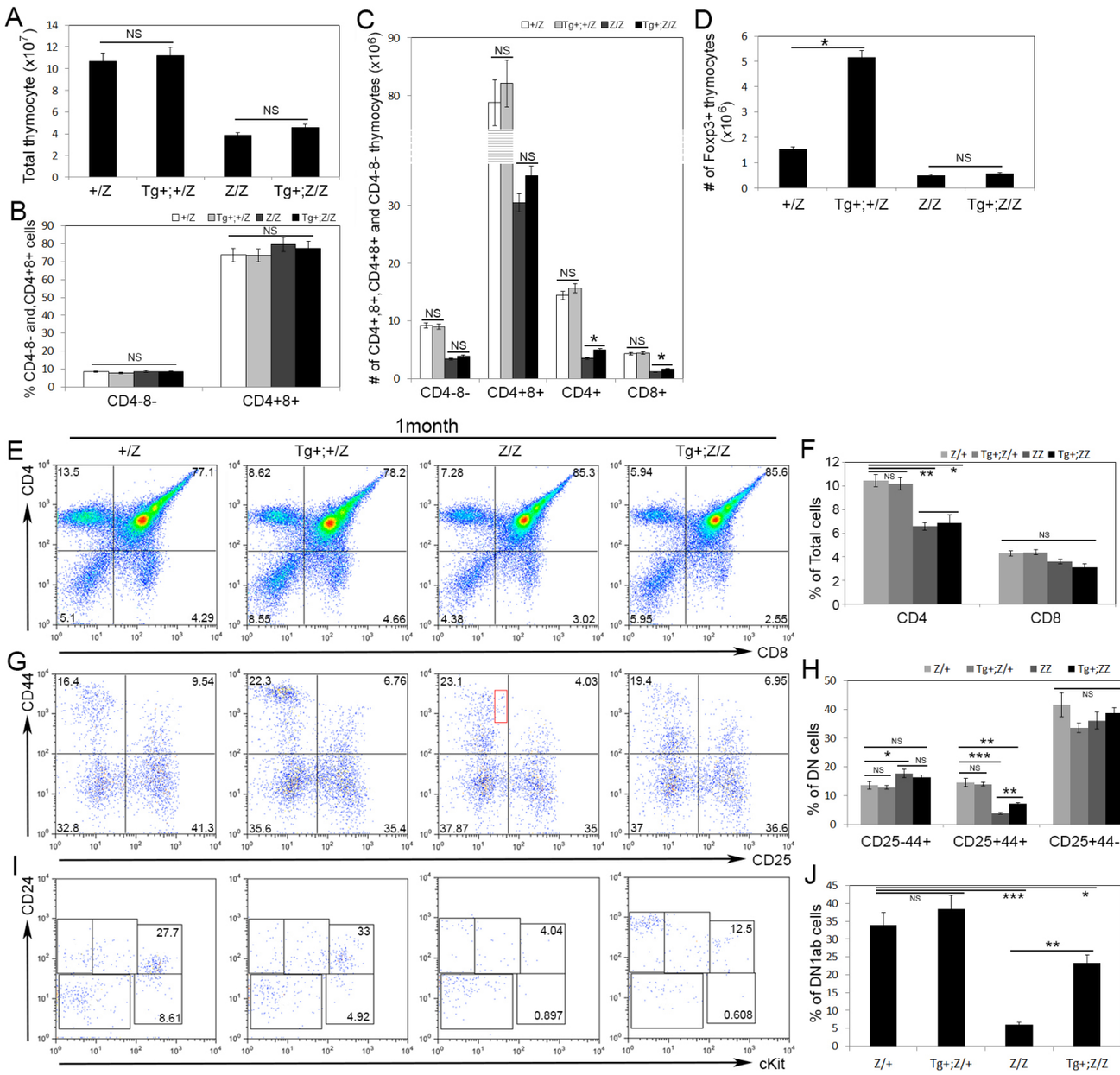

Fig. S3. Thymocyte phenotypes in one and 6 month old *Foxn1Z/Z* with and without the *K5.Foxn1* transgene. Samples were from six month (A-D) (n=8) and one month (E-J) (n=6) mice. **(A)** Total thymocyte numbers in +/Z, Tg+;+/Z, Z/Z and Tg+;Z/Z mice. **(B)** Percentage of CD4-8- and CD4+8+ thymocytes in total thymocytes. **(C)** Numbers of CD4-8-, CD4+8+, CD4+ and CD8+ cells in thymus. *P<0.05. **(D)** Total number of Foxp3+ cells in thymus. *P<0.05. **(E)** Profile of CD4 and CD8 expression in +/Z, Tg+;+/Z, Z/Z and Tg+;Z/Z thymocytes. **(F)** Percentage of CD4 and CD8 SP cells in +/Z, Tg+;+/Z, Z/Z and Tg+;Z/Z thymocytes. *P<0.05, **P<0.01. **(G)** Profile of CD25 and CD44 expression in gated CD4-CD8- thymocytes. **(H)** Percentages of CD4-CD8- subsets defined by CD25 and CD44 expression. *P<0.05, **P<0.01, ***P<0.005. **(I)** Gated lin-CD25-CD44+ thymocytes stained for CD24 and CD117 expression. **(J)** Percentages of CD117+CD24high and CD117+CD24low cells in +/Z, Tg+;+/Z, Z/Z and Tg+;Z/Z mice. *P<0.05, **P<0.01, ***P<0.005. Statistical anlayses were carried out using one-way ANOVA with multiple comparison testing.

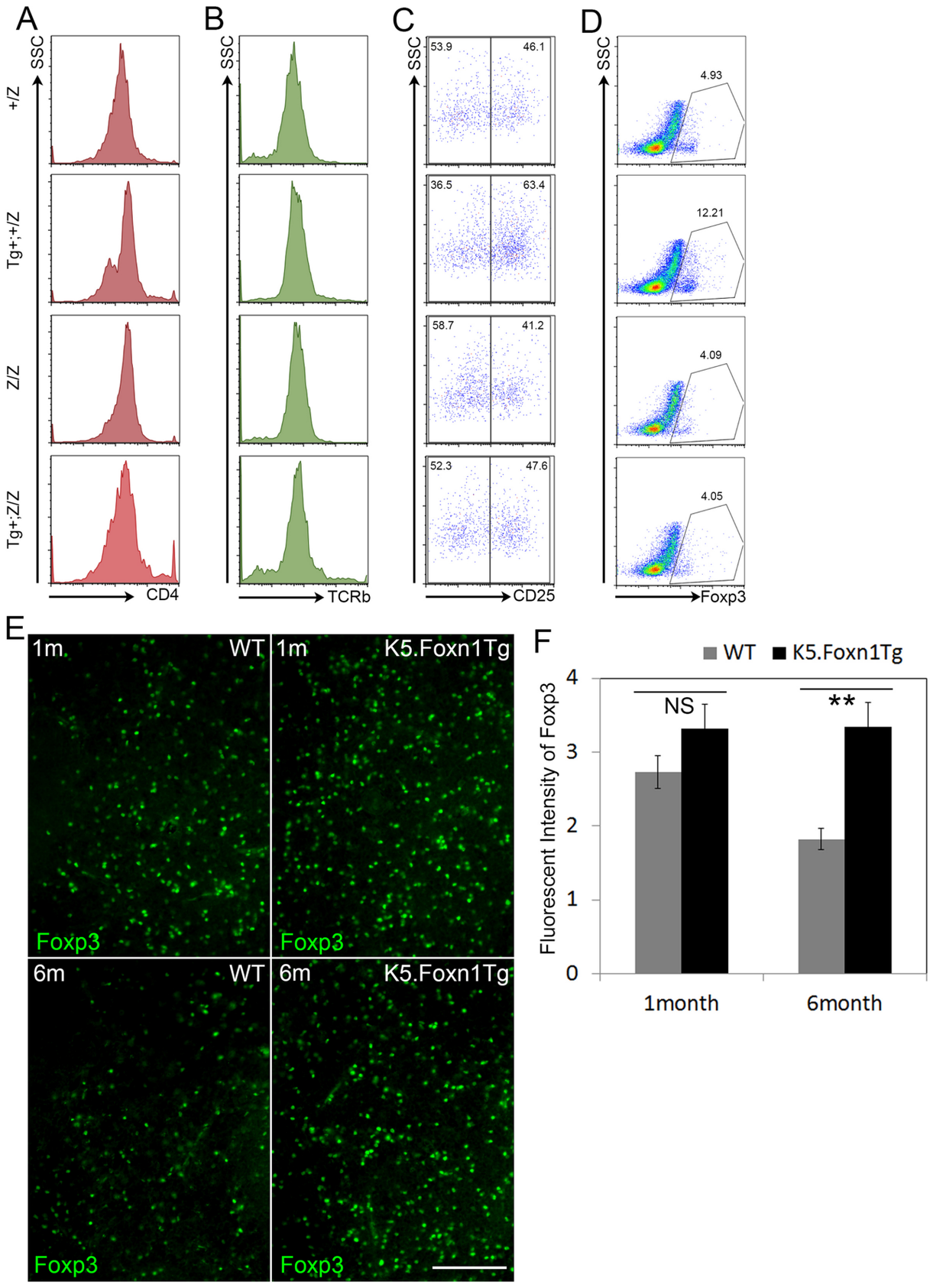

Fig. S4. Characterization of Foxp3+ thymocytes from *Foxn1Z/Z* thymus with and without the *K5.Foxn1* transgene. A-F are from 6 month thymi. **(A-B)** Profiles of gated Foxp3+ cells shows similar MFI of CD4 **(A)** and TCRβ (**B**). **(C)** Profile of CD25 expression in gated Foxp3+ thymocytes. **(D)** Profile of Foxp3 expression in gated CD4+ thymocytes. (n=5) **(E)** Fluorescent immunostaining off FOXP3 on one month and six month old thymus. **(F)** Fluorescent intensity of FOXP3 analyzed by ImageJ. **P<0.01. Scale bar=100µm. (n=7) Statistical anlayses were carried out using one-way Student's t Test. All paired images are shown at same magnification.

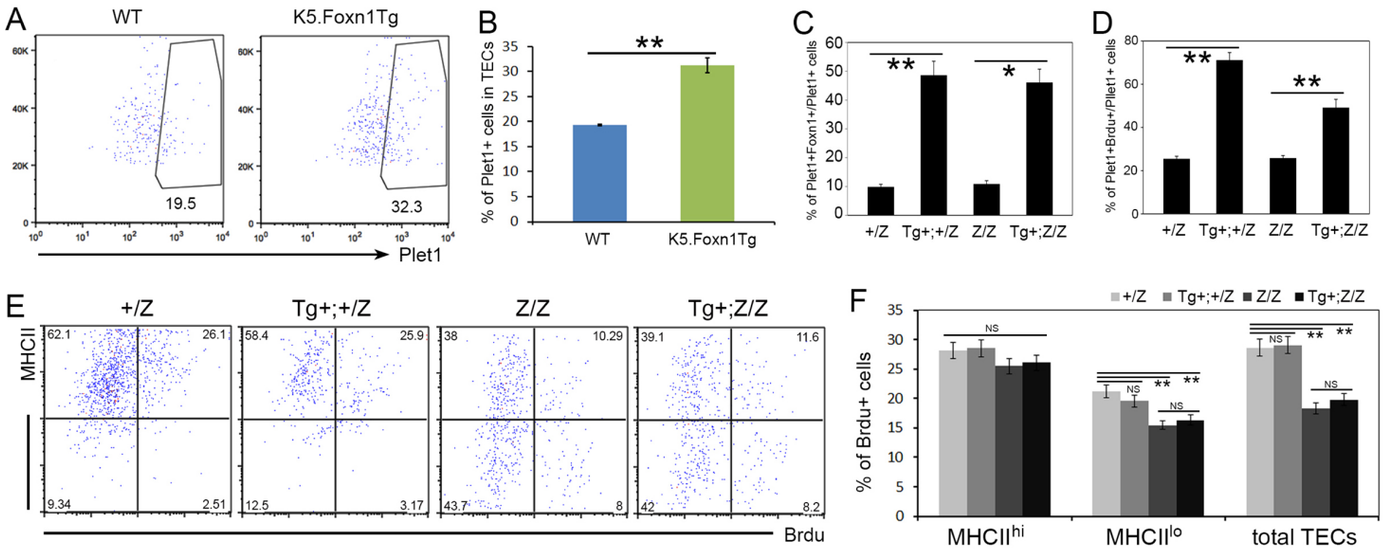

Fig. S5. Characterization of PLET1 and MHCII on TECs from *Foxn1Z/Z* thymus with and without the *K5.Foxn1* transgene. (A) Profile of PLET1 expression and **(B)** percentage of PLET1+ cells in CD45-EpCam+ WT and *K5.Foxn1* thymic epithelial cells. **P<0.01. **(C)** Percentages of PLET1+FOXN1+ cells in PLET1+ thymic epithelial cells and **(D)** of PLET1 +BrdU+ cells in PLET1+ thymic epithelial cells qualtified from IHC shown in Figure 5. *P<0.05, **P<0.01. **(E)** Thymic epithelial cells from one month old BrdU treated mice stained for BrdU and MHCII. **(F)** Percentage of BrdU+ populations in MHC^{hi} and MHC^{lo} thymic epithelial cells from panel E. **P<0.01. (n=7) Statistical anlayses were carried out using one-way ANOVA with multiple comparison testing.

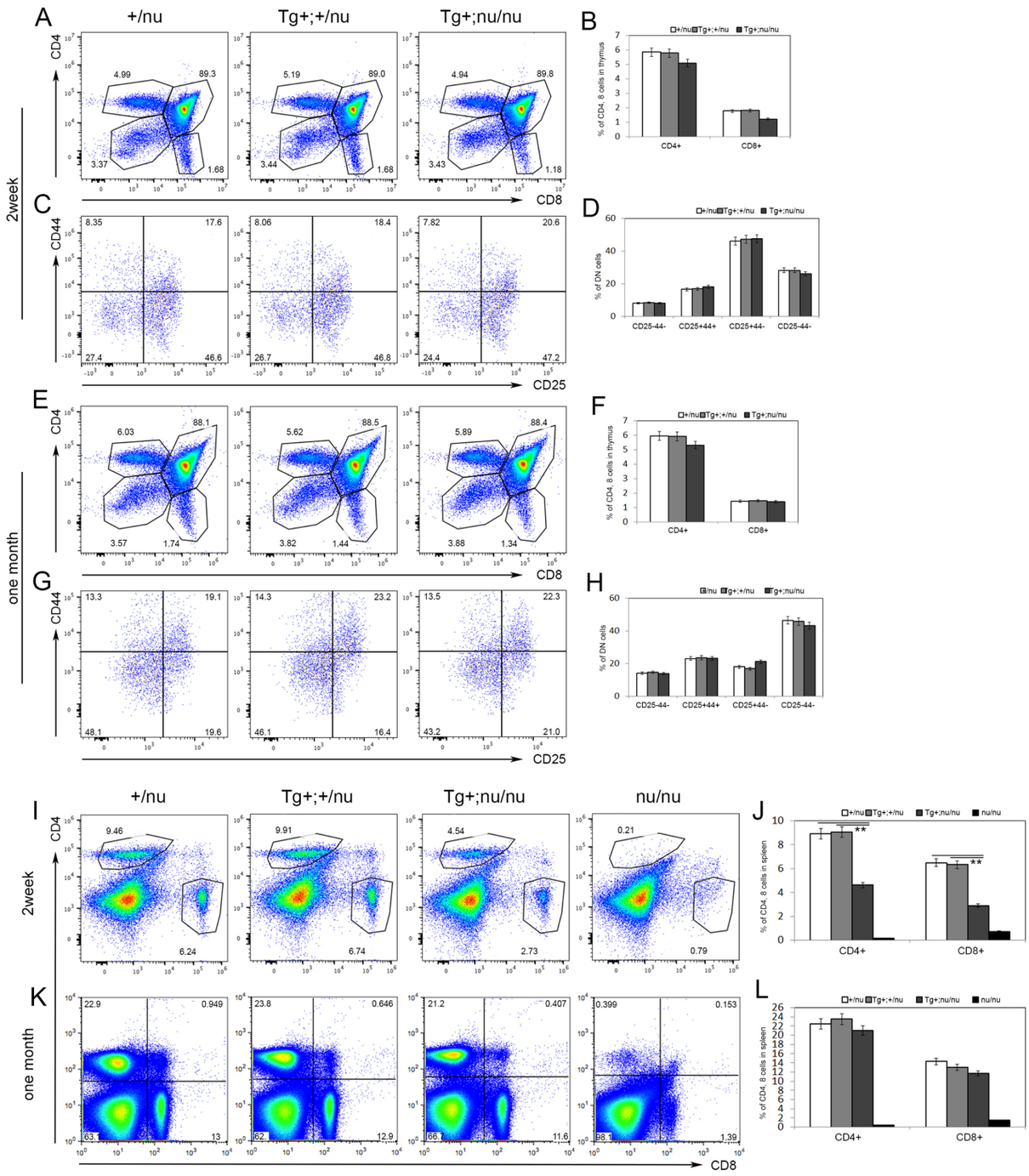

Fig. S6. CD4 and CD8 cells restoration in thymus and spleen in *K5.Foxn1Tg;nu/nu* mice. Samples were from 2 week (A-D, I, J) and one month (E-H, K, L) mice. **(A)** Profile of CD4 and CD8 expression in 2 week-old *+/nu, Tg+;+/nu, Tg+;nu/nu* thymocytes. **(B)** Percentage of CD4+ and CD8+ thymocytes in total thymocytes. **(C)** Profile of CD25 and CD44 expression in gated 2 week-old CD4-CD8- thymocytes. **(D)** Percentages of CD4-CD8- subsets defined by CD25 and CD44 expression from 2 week-old thymus. **(E)** Profile of CD4 and CD8 expression in one month old *+/nu, Tg+;+/nu, Tg+;nu/nu* thymocytes. **(F)** Percentage of CD4+ and CD8+ thymocytes in total thymocytes. **(G)** Profile of CD25 and CD44 expression in gated one month-old CD4-CD8- thymocytes. **(H)** Percentages of CD4-CD8- subsets defined by CD25 and CD44 expression from one month-old thymus. **(I)** Profile of CD4 and CD8 expression in 2 week-old *+/nu, Tg+;+/nu, Tg+;nu/nu* spleen cells. **(J)** Percentage of CD4+ and CD8+ cells in 2 week-old spleen cells. **P<0.01. (K) Profile of CD4 and CD8 expression in one month-old +/*nu*, *Tg*+;+/*nu*, *Tg*+; *nu/nu* spleen cells. (L) Percentage of CD4+ and CD8+ cells in one month-old spleen cells. (n=5) Statistical anlayses were carried out using one-way ANOVA with multiple comparison testing.

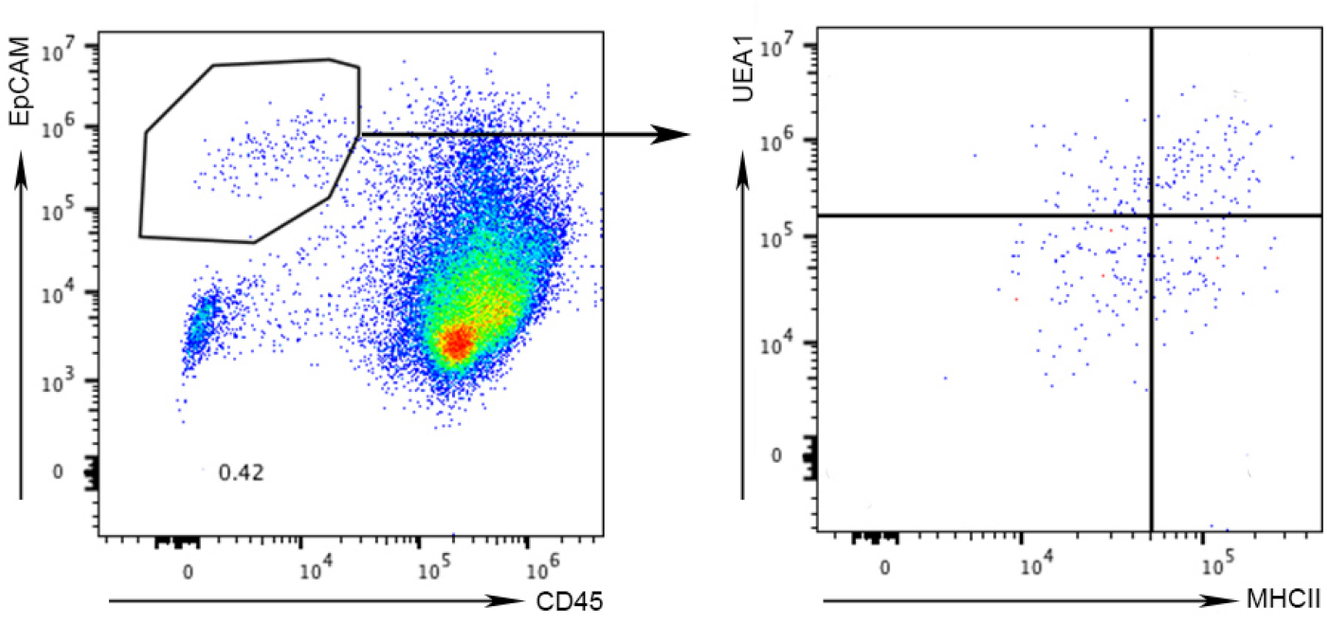

Fig. S7. Cell sorting profiles and gating based on expression of CD45, EpCam, UEA1 and MHCII used for RT-PCR analysis of total *DLL4* expression and *Foxn1* expression.

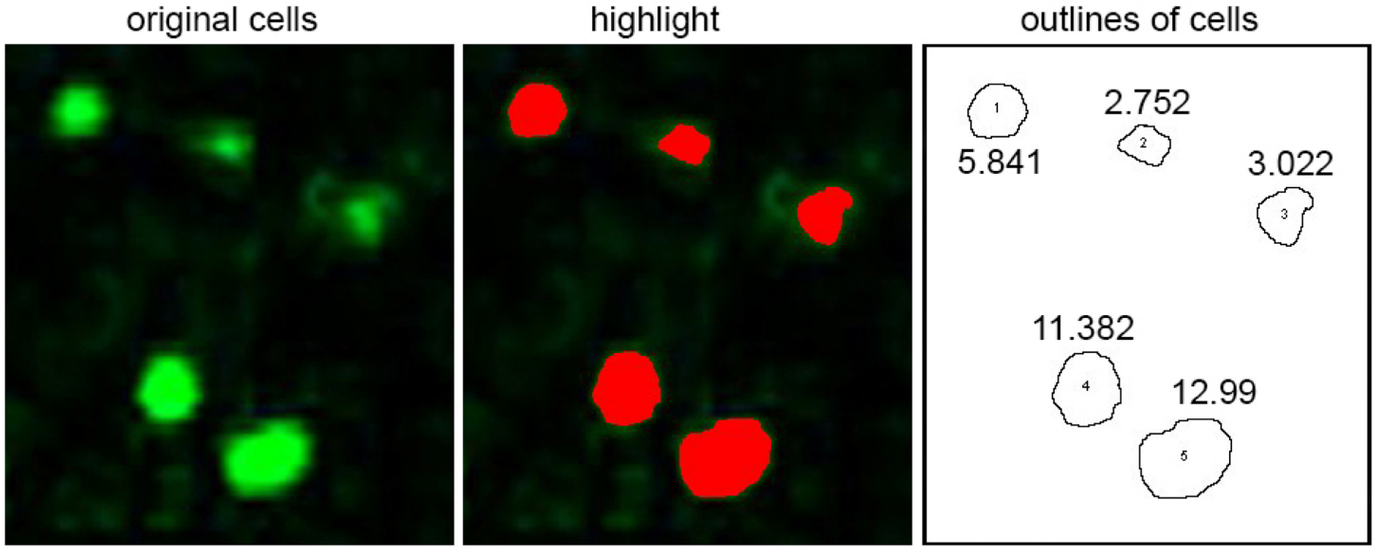

Fig. S8. The measurement of fluorescent intensity on IHC results. Left panel is the original IHC of Foxn1, middle panel shows how ImageJ recognizes each cell with different shapes. The numbers on the right panel are the fluorescent intensity automatically generated by ImageJ.