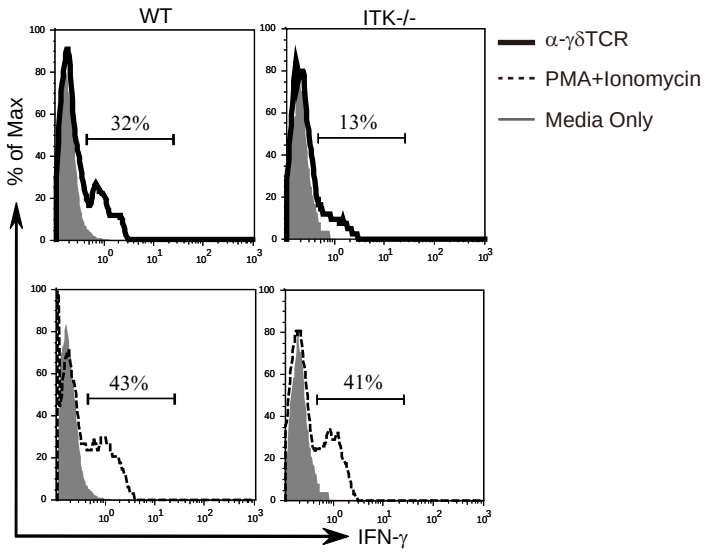
Supplementary Data

Supplementary Figure 1. Impaired production of IFN-γ by $ITK^{-/-}$ sIELs in response to anti- anti-γδ TCR stimulation. sIELs of $ITK^{-/-}$ and wild type mice were purified by cell sorting and cultured for 24 hours in the presence of anti-γδ TCR (1 μg/ml, GL4) or PMA+Ionomycin. 3 μg/ml Brefeldin A was added for 12 hours prior to the staining. IFN-γ was determined by the intracellular staining and analyzed by flow cytometry. The percentages of IFN-γ^+ cells are as shown. Data presented is one representative from two independent experiments.

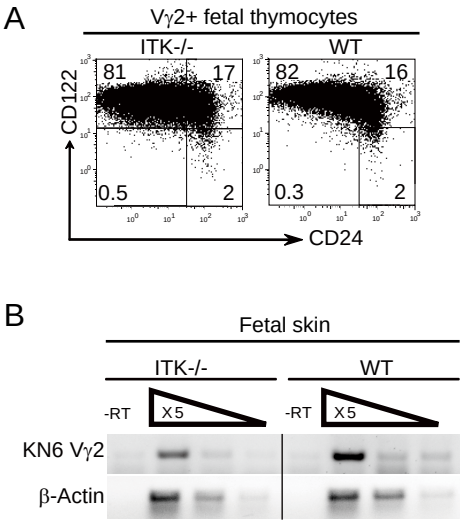
Supplementary Figure 2. Normal maturation but impaired skin-seeding of the $ITK^{-/-}$ fetal thymic KN6 transgenic γδ T cells. **A.** Flow cytometric analysis of E16-17 fetal thymic KN6 transgenic $V\gamma2^+$ γδ cells for CD122 and CD24 expression. The histographs were gated on $V\gamma2^+$ cells. Data presented is one representative from three independent experiments. **B**. Total RNA of fetal skin was reverse transcribed into cDNA, which were 5 fold serially diluted and subject to PCR to determine the expression of KN6 TCRγ2 gene. β-actin was performed as controls. Data presented is one representative from three mice of each genotype.

Supplementary Figure 3. In situ TUNEL staining of ear epidermal sheets of ITK-sufficient and deficient KN6 mice to detect apoptotic transgenic sIELs. Ear epidermal sheets were stained by TMR red TUNEL staining mixture and FITC-conjugated anti-Vγ2 antibody, and analyzed by a confocal fluorescent microscope (Olympus FluoView

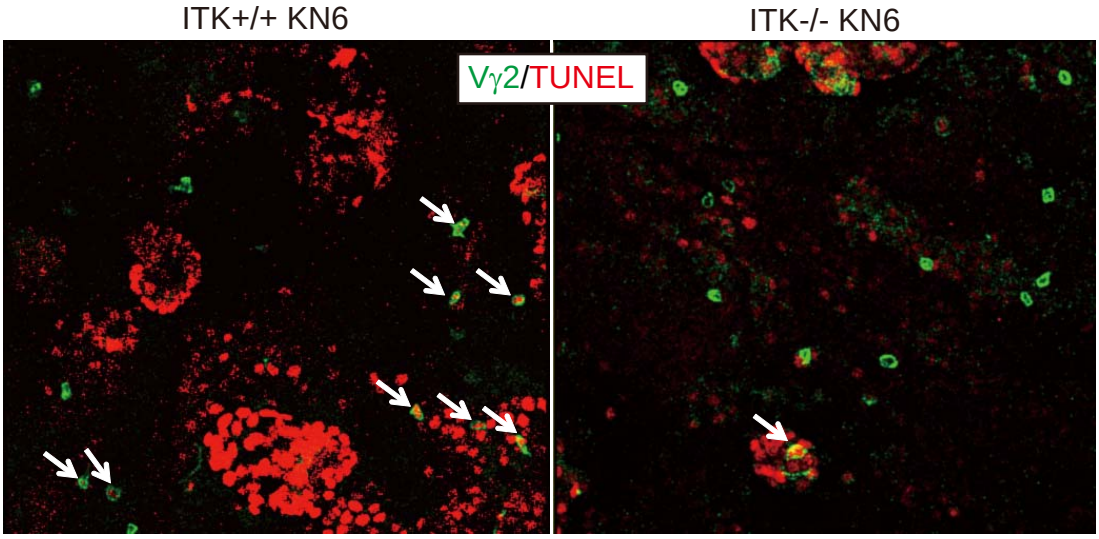
TM FV300). Apoptotic sIELs were double positive for green and red, indicated by arrows. Note that there were many other apoptotic skin cells besides the apoptotic sIELs due to high rates of epidermal cell turnover. Data presented is one representative from two independent experiments.



Supplementary Fig. 1



Xia, et al, Supplementary Fig. 2



Xia, et al, Supplementary Fig. 3