Supplementary Information

Supplementary Figure 1. ThPOK expression by peripheral γδ cells and Lin- DN thymocytes. (A) ThPOK mRNA levels in indicated sorted subsets from thymus, spleen and peripheral blood, as determined by real-time RT PCR. Error bars indicate SD of duplicate samples. (B) GFP expression by Lin- DN thymocytes from DRE-GFP transgenic mouse. Top 4 panels show GFP expression by gated Lin- DN subsets, as indicated. Bottom panel depicts GFP versus ckit expression for gated CD25- Lin- DN cells, which includes both DN1 and DN4 subsets. Note that GFP+ cells are all negative for ckit.

Supplementary Figure 2. ThPOK is induced in early thymic lymphoma cell line by TCR signaling. Scid.adh cells transduced with the KN6 γδTCR were cultured for 24 hrs in the presence or absence of anti-γδTCR or –CD3ε antibodies, then analyzed by FACS for expression of CD69 and CD25 (left-hand panels), and ThPOK mRNA levels assessed by real-time RT PCR (right panel). Error bars indicate SD of duplicate samples. Results are representative of 2 experiments.

Supplementary Figure 3. Id3 deficiency impairs ThPOK induction in γδ thymocytes. (A) RT PCR analysis of ThPOK expression in sorted mature (CD24- CD44+) and immature (CD24+ CD44-) γδTCR+ thymocytes from Id3-/- and wt littermate. Error bars indicate SD of duplicate samples. Results are representative of 2 experiments. (B) FACS
analysis of thymocytes from Id3-/− and wt mice stained with anti-CD4, -CD8, -γδTCR, -TCRβ, -CD24, -CD44 and –Vγ1.1. Subsets were gated as indicated.

**Supplementary Figure 4.** GFP<sup>hi</sup> γδ thymocytes are particularly sensitive to ThPOK deficiency. FACS analysis of GFP expression by gated CD24+ and CD24- γδTCR+ DN thymocytes from DRE-GFP mice on HD/HD, HD/+ and ThPOK<sup>const</sup> backgrounds. Shaded histograms represent equivalent gated populations from non-transgenic control mice.

**Supplementary Figure 5.** ThPOK deficiency causes partial block of development of Vγ1.1+ and Vγ2+ thymocytes at CD24<sup>+</sup> > CD24<sup>−</sup> transition. Absolute numbers of indicated γδTCR+ thymocyte subsets from 6 week-old HD/HD (n=8) and HD/+ littermates (n=7). Error bars indicate SD.

**Supplementary Figure 6.** ThPOK deficiency impairs Vγ2+ thymocyte maturation in neonates. (A) FACS analysis of thymocytes from neonatal HD-/− and HD+/- littermates, showing expression of CD4, CD8, CD24, γδTCR, and Vγ2 (left-hand panels). (C) Ratio of mature (CD24-) versus immature (CD24+) Vγ2+ thymocytes from HD/HD and HD/+ neonatal mice.

**Supplementary Figure 7.** Impaired development of KN6 thymocytes in HD/HD mice. FACS analysis of total thymocytes from KN6+ H-2<sup>bd</sup> mice on HD/HD or HD/+ backgrounds, showing expression of CD4, CD8, γδTCR, and CD24 (left-hand panels), or
of DN thymocytes, spleen and lymph node cells from the same mice, showing expression of TCRβ and γδTCR.

**Supplementary Figure 8.** Constitutive ThPOK expression promotes development of CD4+ γδ cells and disappearance of CD8+ γδ cells. (A) ThPOK^const transgene expression in DN thymocyte subsets. Real-time RT PCR analysis of ThPOK expression in indicated sorted TCR^- DN subsets from ThPOK^const and wt mice. Error bars indicate SD of duplicate samples. (B) FACS analysis of γδTCR+ DN splenocytes from 1 month old ThPOK^const and wt littermates stained with anti-γδTCR, and anti-Vγ1.1. (C) FACS analysis of gated γδTCR+ thymus, lymph node or spleen cells, as indicated, stained with anti-CD4, and -CD8.

**Supplementary Figure 9.** (A) BrdU incorporation by γδ thymocyte subsets from HD/HD, ThPOK^const and wt mice. FACS analysis of BrdU incorporation by indicated gated γδ thymocyte subsets from mice treated with BrdU for indicated time periods. (B) Intracellular expression of IFNγ and IL17 by NK1.1+ and CCR6+ thymus or spleen γδ subsets, as indicated, following stimulation with PMA/ionomycin. Thymocyte data was obtained from mature (CD24-) cells, as immature (CD24+) cells do not produce cytokines in response to activation. HD/HD mice did not yield sufficient mature γδ thymocytes to carry out this analysis. Data for CCR6+ and NK1.1+ subsets are shown superimposed in red and blue, respectively (percentages in red and blue quadrants correspond to proportions of CCR6+ and NK1.1+ subpopulations, respectively, not γδ cells as a whole). Experiments were carried out at least twice with similar results.
Supplementary Figure 10. ThPOK expression by peripheral γδ subsets. (A) FACS analysis of total peritoneal cavity lymphocytes or gated γδTCR+ cells stained with anti-Thy1, -γδTCR, -CD122 and –CD25. (B) Relative proportions of CD122+ and CD25+ peritoneal cavity γδ cells in HD/HD versus HD+/- mice. (C) FACS analysis of GFP expression by gated CD25+ and CD122+ γδTCR+ peritoneal cavity subsets from DRE-GFP mice on HD/HD or HD/+ backgrounds. Green and red histograms represent equivalent gated populations from transgenic and non-transgenic mice, respectively.
Supplementary Fig. 1

(a) Relative mRNA levels (arbitrary units)

(b) GFP expression in different cell populations:
- DN1
- DN2
- DN3
- DN4

CD25^+ Lin^- DN (DN1 + DN4)

CD117 (c-kit)

GFP

wt
DRE-GFP
Supplementary Fig. 4

Supplementary Fig. 5
Supplementary Fig. 8

(a) ThPOK mRNA levels (arbitrary units)

(b) Vγ1.1

(c) DN, Spl

ThPOKconst

γδ TCR+

CD24- γδ TCR+

CD8

CD8

CD4

LN

Spl
Supplementary Fig. 9

**a**

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**b**

Flow cytometry plots showing the expression of Thy1, Spl, IL17, and IFNγ in wt, HD/HD, and ThPOK<sup>const</sup> conditions.

Legend:
- Thy: Thy1
- Spl: Spl
- IL17: IL17
- IFNγ: IFNγ
- Thy1+ DN thymocytes
- CD24-CD44+ (mature)
Supplementary Fig. 10

(a) Graphs showing Thy1 expression in +/HD and HD/HD mice. The Thymus lineage marker Thy1 is depicted on the x-axis.

(b) Graphs showing the percentage of Per Cava+ cells in +/HD and HD/HD mice. The x-axis represents percentage of cells, and the y-axis represents different markers.

(c) Graphs showing CD122 and CD25 expression in +/HD mice. The x-axis represents different markers, and the y-axis represents percentage of cells.

Legend:
- O = +/HD
- ● = HD/HD

littermate pairs: 1/3, 2/4