

Supporting Information

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SI Methods

Mice. mCD4-ThPOK (line 198) mice, referred to as ThPOK^{const} mice throughout the study, have been described previously (1). To assess lymphoma development, animals were monitored by biweekly physical examinations and PBL analyses. Lymphoid and nonlymphoid organs were subjected to FACS analysis using antibodies against Thy1, TCR β , $\gamma\delta$ TCR, CD4, CD8, CD25, and CD44. KN6⁺ RAG2^{-/-} d/d and KN6⁺ RAG2^{-/-} B2m^{-/-} mice were described previously (2). hCD2-ThPOK transgenics have been reported previously (1). For transgenic expression of ThPOK cDNA under mProxLck regulatory elements, the full-length cDNA insert was cloned into a vector containing mouse proximal promoter/enhancer elements (3).

Immunohistochemical Staining. Hematoxylin/eosin staining was carried out according to standard procedures. Anti-CD3 ϵ antibody (eBioscience) was used according to manufacturer's instructions.

Antibody Injection of RAG^{-/-} Mice. Groups of juvenile ThPOK^{const} RAG^{-/-} mice (2 wk of age) and control RAG^{-/-} mice were injected either with anti-CD3 ϵ antibody (clone 2C11, 9 μ g of antibody per gram of mouse weight) or with PBS alone as a control. A mouse of each group at 3 wk was killed to verify occurrence of thymocyte expansion and also at 15 wk to check for persistence of post-DN3 cells generated by antibody stimulation. Remaining mice were monitored for 7 mo by biweekly physical examination for signs of lymphoma development.

Adoptive Transfer of Bone Marrow and Lymphoma Cells from ThPOK^{const} Donors. Bone marrow cells were isolated from tibias and femora of ThPOK^{const} mice (or control nontransgenic mice) and depleted of CD4⁺ and CD8⁺ cells by magnetic cell separation system (Miltenyi Biotec). One million cells in 0.2 mL of DMEM + Hepes media were injected i.v. into RAG2^{-/-} recipients that had been irradiated (900 rads, delivered in two doses of 450 rads) 24 h previously. Peripheral blood samples were analyzed 6 wk after bone marrow transfer to verify hematopoietic reconstitution. Mice were monitored weekly by physical examination and monthly by PBL analysis for signs of lymphoma development. Adoptive transfers of 10⁵ ThPOK^{const} tumor cells into RAG2^{-/-} hosts were carried out according to the same protocol.

PCR Assays. Primers for PCR are as follows: Sequences of Notch1 gene were obtained from genomic DNA following PCR with specific primers: exon 26: F, GCGAGTCGCCAAGCACATTT and R, CACCTCCAGGGTTAAGCTGGT; exon 27: F, GGC-TAGGGAGTCAGAGCTGGT and R, TTGAACCCCTGTCC-TCTGCAA; exon 28: F, AGCCACAGCATCACAC and R, ATGGGCTTCTGGAGCTAAG; exon 34 PEST: F, CACAG-CAGCCTCTCCACAA and R, CCCAGCATCTGAAGC-ACT; and exon 34 TAD: F, CATGCTGTCGCCTGTGGACT and R, CTGCCGAGTCACACTGAGG.

In some cases Notch1 sequences were amplified from cDNA, using the same primers, except for exons 26–28 (F, ACTGTGAC-AGCCAGTGCAAC and R, CACAAAGAACAGGAGCACGA).

D β 2-J β 2.7 rearrangements were PCR amplified using specific primers for:

D β 2 5'-GTAGGCACCTGTGGGGAAGAAACT-3' and J β 2.7 5'-TGAGAGCTGTCTCCTACTATCGATT-3'.

Indicated commercial Taqman Gene expression assays were used to measure expression of the following murine genes: Mm00478361_m1 for Ptcra, Mm01345646_m1 for Notch3, Mm01342805_m1 for Hes1, Mm00492297_m1 for Dtx1, and Mm00490666_m1 for Runx3. For RT-PCR measurement of murine ThPOK expression, we designed the following primers and probe: F, AGAAGCC-CTTGCCCTGTGA and R, TGTGGATCTTCAGCTTGTCATTC; P: 6fam-TCTGCGGCGTCCGCTTAC-Bhq1.

Microarray Analysis. Gene expression profiling of mouse tumors. We profiled three Thpok transgenic-derived T-ALLs and an additional 11 T-cell lymphoblastic tumors from well-characterized T-ALL models including E2A knockout, TAL1 transgenic, TAL1 and LMO1 double transgenic, and Ikaros plastic mutant mice. Gene-expression profiling was performed using Affymetrix Mouse Genome 430A 2.0 Array following standard procedures (www.affymetrix.com/support/technical/manuals.affx). Raw data are available in GEO www.ncbi.nlm.nih.gov/geo/ (accession no. GSE33550). Array normalization was performed by DNA-Chip Analyzer (dChip) (4). Following interarray normalization the microarray data were preprocessed and analyzed for differential expression using the Genepattern platform (www.broadinstitute.org/cancer/software/genepattern/) for microarray analysis (5). Significant differentially expressed genes were calculated by using the Comparative Marker Selection Genepattern tool (6) and *t* test statistical test and non-parametric *P* value calculation (1,000 random permutations).

CGH Array Analysis. Genomic imbalances were identified using array-CGH (comparative genomic analysis), which was performed according to Agilent's oligonucleotide array-based CGH protocol for Genomic DNA Analysis, version 4.0. Genomic DNA (0.5–3 μ g) from tumors that were digested with AluI and RsaI restriction endonucleases. The digested DNA was labeled using Agilent's Genomic DNA Labeling Kit PLUS. Tumor and normal (tail) reference DNA samples were labeled with either cyanine 5- or cyanine 3-dUTP, according to the manufacturer's suggestions. The labeled DNA products were purified using Microcon YM-30 filtration devices (Millipore). DNA yield and level of dye incorporation were measured using a ND-1000 spectrophotometer. Appropriate cyanine 5- and cyanine 3-labeled DNA sample pairs were combined and mixed with mouse Cot-1 DNA, Agilent 10 \times blocking agent, and Agilent 2 \times hybridization buffer. Then each labeled target solution was hybridized to an Agilent 244K Mouse Genome CGH Microarray (G4415A) using SureHyb chambers. Following hybridization, the microarrays were washed and dried as outlined in Agilent's protocol. Microarray slides were then scanned using an Agilent microarray scanner. Data for individual features were extracted from the scanned image using Agilent's Feature Extraction Software, and output files were imported into Agilent's CGH Analytics for DNA copy number analysis.

1. He X, et al. (2005) The zinc finger transcription factor Th-POK regulates CD4 versus CD8 T-cell lineage commitment. *Nature* 433(7028):826–833.
2. Haks MC, et al. (2005) Attenuation of gamma delta TCR signaling efficiently diverts thymocytes to the alpha beta lineage. *Immunity* 22(5):595–606.
3. Chaffin KE, et al. (1990) Dissection of thymocyte signaling pathways by in vivo expression of pertussis toxin ADP-ribosyltransferase. *EMBO J* 9(12):3821–3829.

4. Li C, Wong WH (2001) Model-based analysis of oligonucleotide arrays: Expression index computation and outlier detection. *Proc Natl Acad Sci USA* 98(1):31–36.
5. Reich M, et al. (2006) GenePattern 2.0. *Nat Genet* 38(5):500–501.
6. Gould J, Getz G, Monti S, Reich M, Mesirov JP (2006) Comparative gene marker selection suite. *Bioinformatics* 22(15):1924–1925.

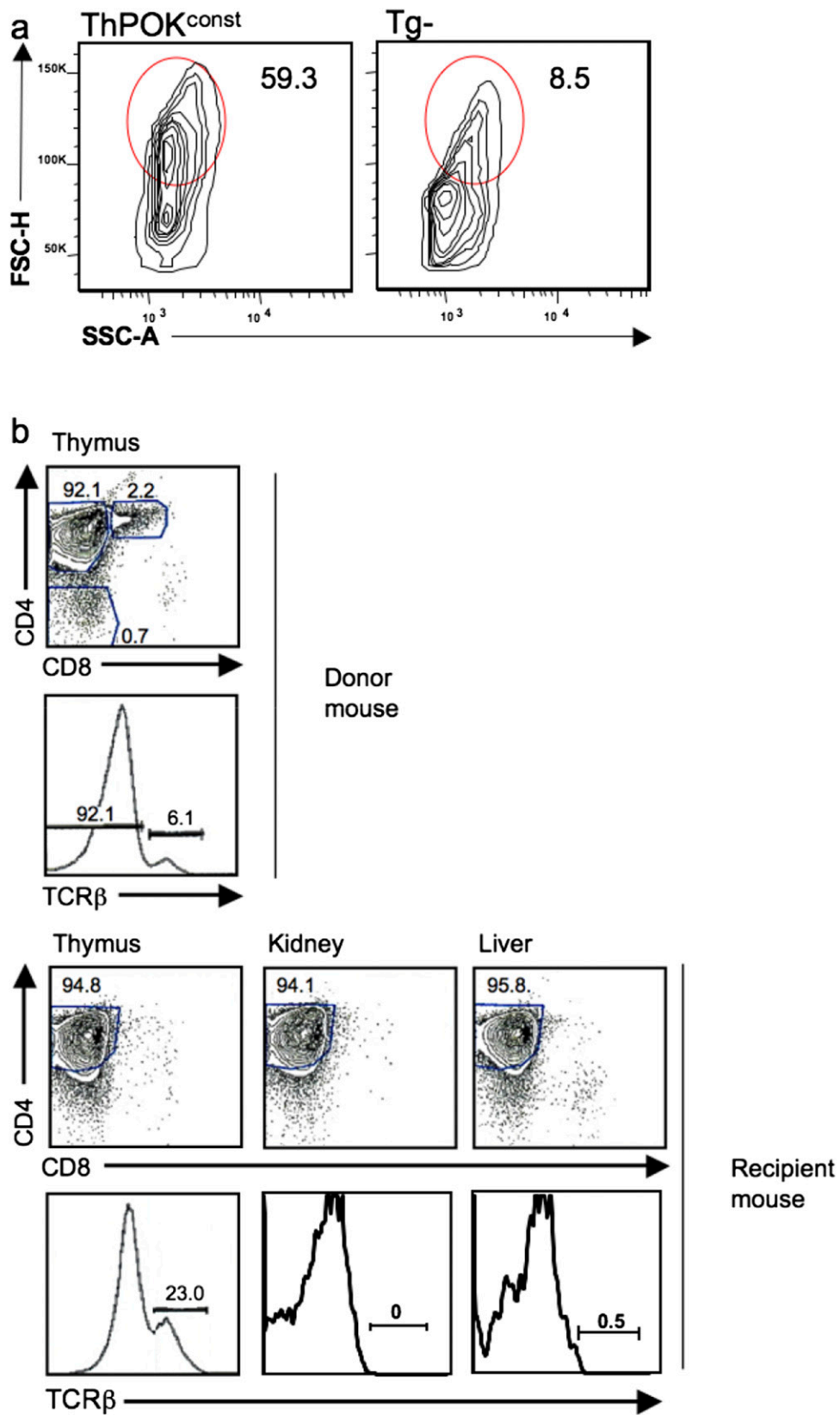


Fig. S1. ThPOK^{const} lymphomas exhibit increased cell size and can be adoptively transferred to immunodeficient hosts. (A) Forward/side scatterplots of Thy1⁺ PBLs from ThPOK^{const} and nontransgenic littermates. (B) The 10⁵ Thy1⁺ cells from the thymus of a sick 4-mo-old ThPOK^{const} mouse were transferred i.v. into sublethally irradiated Rag2^{-/-} hosts. Organs were harvested 6 wk after adoptive transfer and examined by flow cytometry for presence of lymphoma cells, based on expression of CD4, CD8, and TCRβ. Note high proportion of CD4⁺ TCRβ⁻ cells in all host organs after adoptive transfer (Bottom row), closely resembling the phenotype of original donor lymphoma cells (Top row) (no such cells were detected in Rag2^{-/-} mice that did not receive tumor cell transplants). This experiment was repeated three times with similar results.

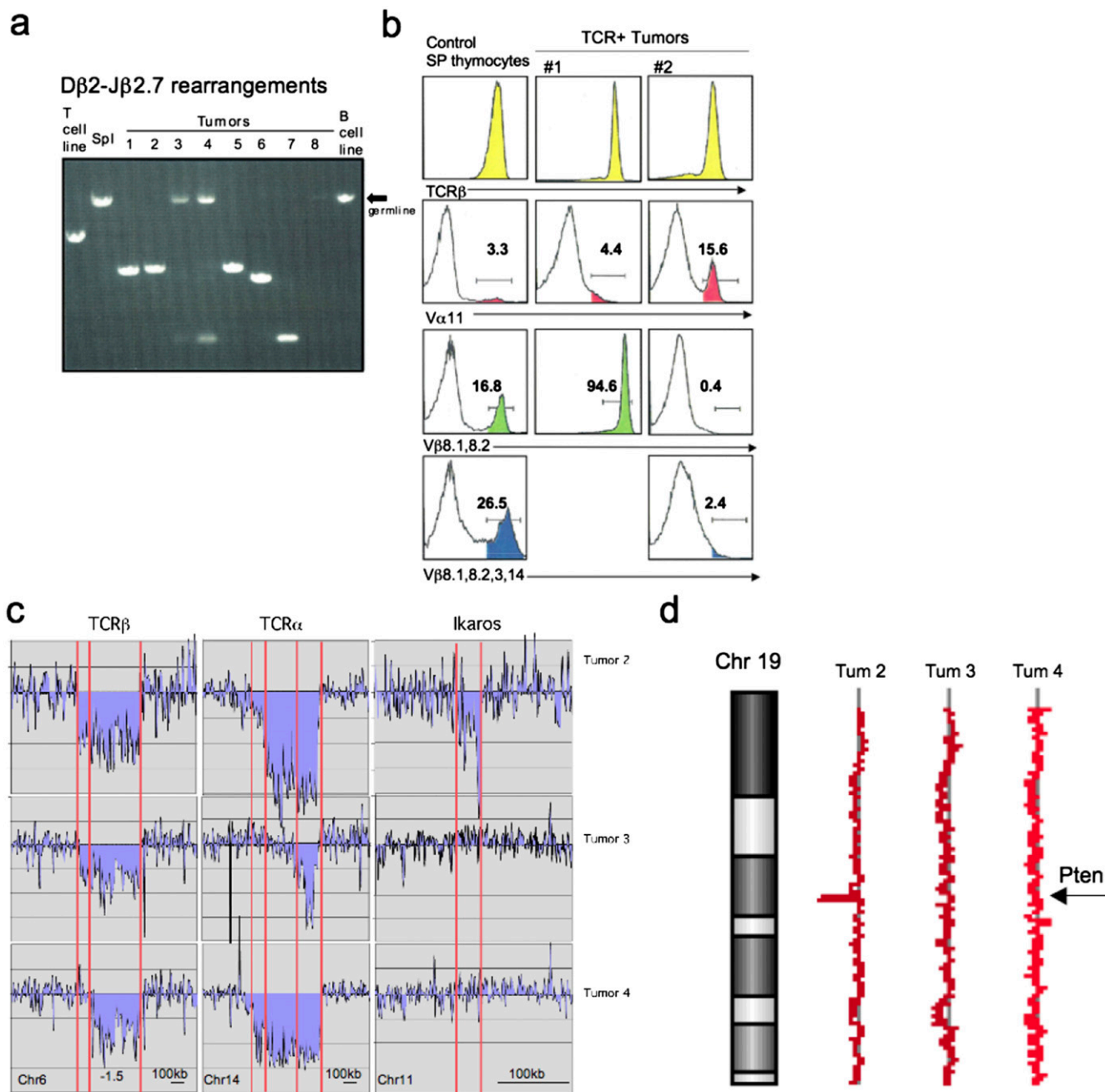


Fig. S2. Clonality of ThPOK^{const} T-cell lymphomas. (A) PCR analysis of D β 2-J β 2.7 rearrangement from eight independent ThPOK^{const} T-cell lymphomas. Note that each tumor sample exhibits a single rearrangement product (tumors 3 and 4 also retain some germ-line configuration TCR β). (B) FACS analysis of two independent ThPOK^{const} T-cell lymphomas. Note that samples 1 and 2 express V β 8 on 95% or <1% of all cells, compared with 17% by normal WT T cells (combined SP CD4 and CD8 populations from C57BL/6 mouse). (C) Comparative genomic hybridization (CGH) array analysis of representative ThPOK^{const} T-cell lymphoma samples showing deletions of TCR β , TCR α , and Ikaros loci (plotted as Log₂ ratio). (D) CGH array analysis of whole chromosome 19 in representative ThPOK^{const} T-cell lymphoma samples showing deletions of Pten locus in sample 2.

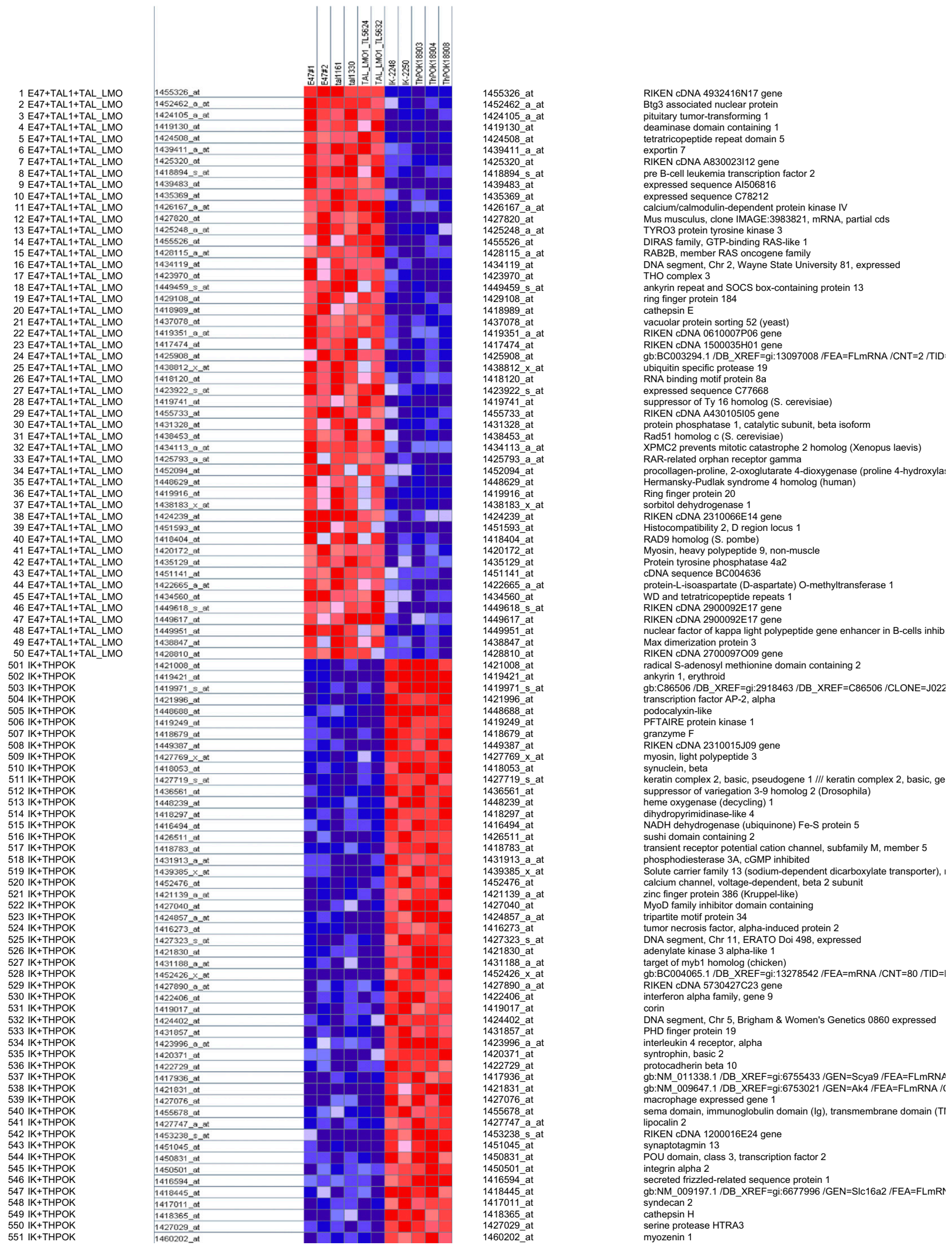


Fig. S6. ThPOK^{const} lymphomas share a robust gene expression profile with Ikaros plastic tumors. Oligonucleotide microarray gene expression comparison of ThPOK^{const} lymphomas with other mouse T-cell lymphomas. Unsupervised hierarchical clustering indicates closest similarity to Ikaros DN lymphomas. This analysis is the same as in Fig. 2C, but limited to the 100 most robust gene expression differences.

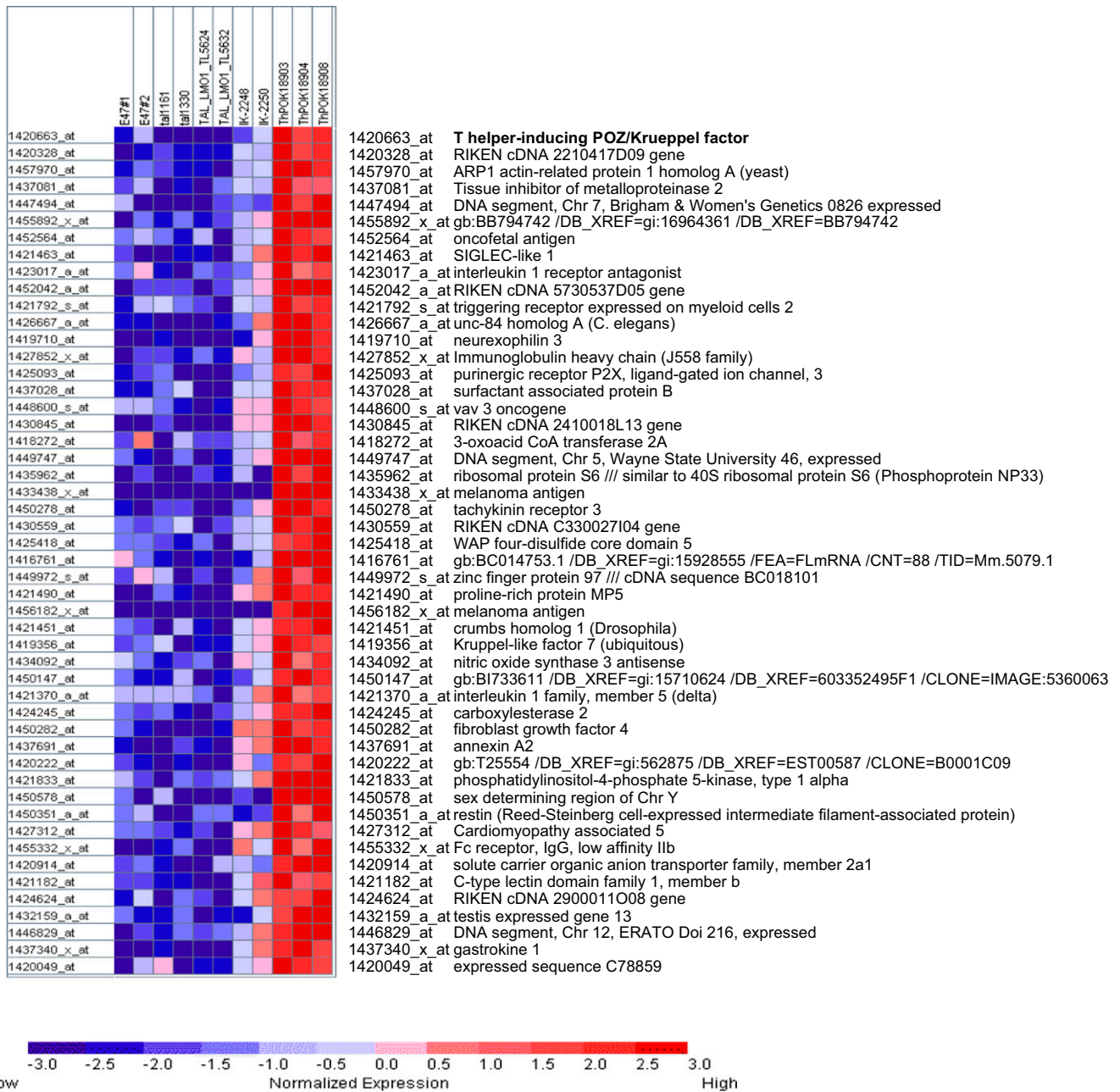


Fig. S7. Genes most highly induced in ThPOK^{const} lymphomas. Oligonucleotide microarray gene expression comparison of ThPOK^{const} lymphomas with other mouse T-cell lymphomas. Comparative expression levels of 50 genes that are most highly overexpressed in ThPOK^{const} lymphomas relative to a panel of other mouse T-cell lymphomas.