SUPPLEMENTARY METHODS

Oligonucleotide templates

Ninety-six oligonucleotides (Lumina) were individually synthesized according to the format
GCTGGCGCAGAAATATACAGGTCGGACCTCAGCTG$(NNNN)_4$
CCTCAGCACCTCC in which NNNN represents one of the four nucleotide words AATC, ATCA, TCTA, CAAA, TTAC, TACT, ACAT, or CTTT. We amplified template mixtures of known diversities between 1 and 96 (containing 1 fmol of each component sequence) with primers flanking the variable region of the templates (P1 and P2, both at 0.3 μM final concentration) in 100 μL PCR reactions with 1.5 mM $MgCl_2$, using 40 cycles and an annealing temperature of 55ºC, yielding 52 bp products. We used the method of Thompson et al.[1] to isolate homoduplexes: first-round PCR products were diluted 1:10 in fresh reaction mixes and further amplified for three cycles. Finally, we removed single-stranded DNA by incubating with 40 units of exonuclease I (NEB) at 37ºC for 60 min.

Mouse strains

C57BL/6 (Jackson Labs) and terminal deoxynucleotidyl transferase knockout mice[2] B6.129S2-*Dntt*^{tm1Gfn} (Taconic) were housed in modified specific-pathogen-free conditions. The protocol was approved by the UCSF Institutional Animal Care and Use Committee.

Isolation and sort purification of splenic T and non-T cell subpopulations

Spleens and thymi were dissociated through 70 μm mesh screens; mononuclear cells (primarily lymphocytes) were further purified with a Histopaque-1083 gradient (Sigma). For the diversity titration experiment, we sorted splenic T and non-T cells on a FACSVantage (Becton-Dickinson) using a FITC-CD3 antibody (Becton-Dickinson) and propidium iodide to exclude dead cells. For all other experiments, splenic T cells were enriched using a negative selection kit for CD3+ cells (Cell Sep; Stem Cell). Memory and naïve splenic T cells and various thymocyte subpopulations were sorted using the antibodies PerCP-CD4, APC-CD8a, FITC-CD44, and PE-CD62L (Becton-Dickinson) along with propidium iodide exclusion. All sorts exceeded 99% purity. Cells were counted with a Coulter counter, suspended in RNeasy buffer RLT (Qiagen), and homogenized with QiaShredder columns before freezing at –80°C.

Real-time PCR

cDNA samples were amplified using universal primers to the C region of the TCRβ chain (CQ1 and CQ2). Results were normalized to parallel reactions using primers to β-actin (Maxim Biotech; proprietary sequences). Samples were amplified using SYBR Green mastermix (Applied Biosystems) on an ABI 7700 real-time PCR machine.

Nondenaturing gel electrophoresis

After incubation at the annealing temperature, we loaded samples and references onto an 8% polyacrylamide TBE minigel (Invitrogen). We visualized duplexes using the SYBR Green dye already present in the annealing mix, by scanning with a Typhoon imager (Amersham). The entire image was adjusted in Photoshop (Adobe) to increase contrast.

Immunoscope

V-J PCR products were generated as with the AmpliCot protocol, except that the J primer was 5' labeled with 6-FAM. 1 uL of the PCR reaction, mixed with 9 uL formamide and ROX-350 markers (Applied Biosystems), was run on an ABI3100 capillary electrophoresis machine (Applied Biosystems) and analyzed using GeneMapper software[3].

Oligonucleotide sequences

Name	Sequence
P1	TACAGGTCGGACCTCAGCTG
P2	GGCGGAGGTGCTGAGG
CQ1	GCAGTTCCATGGGCTTTCAG
CQ2	ACAGTCTGCTCGGCCCCAG
V4	GGCCACATACTTCTGTGCCAGC
C	GGGTGGAGTCACATTTCTCAGATCCT
J1-1	CTGGTTCCTTTACCAAAGAAGACT

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1. Thompson, J.R., Marcelino, L.A. & Polz, M.F. Heteroduplexes in mixed-template amplifications: formation, consequence and elimination by 'reconditioning PCR'. *Nucleic Acids Research* **30**, 2083-2088 (2002).
2. Gilfillan, S., Dierich, A., Lemeur, M., Benoist, C. & Mathis, D. Mice lacking TdT: mature animals with an immature lymphocyte repertoire. *Science* **261**, 1175-1178 (1993).
3. Pannetier, C., Even, J. & Kourilsky, P. T-cell repertoire diversity and clonal expansions in normal and clinical samples. *Immunol Today* **16**, 176-181 (1995).