

ZBTB1 is a determinant of lymphoid development

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In this study, we describe a chemically induced mouse mutation that caused a complete and cell-intrinsic T cell deficiency. Development of other lymphoid lineages was also partially impaired and was severely compromised under competitive conditions. Positional cloning, retroviral transduction, and a somatic reversion event revealed that the causative mutation lay within *Zbtb1* (zinc finger and BTB domain containing 1), a gene conserved throughout vertebrate evolution. Our data establish ZBTB1 as a critical determinant of T cell development and lymphopoiesis in general, most likely by acting as a transcriptional regulator.

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Abbreviations used: BTB-ZF, Broad complex, Tramtrack, and Bric à brac–zinc finger; CLP, common lymphoid progenitor; EdU, 5-ethynyl-2'-deoxyuridine; ENU, *N*-ethyl-*N*-nitrosourea; ETP, early thymic precursor; HSC, hematopoietic stem cell.

Lymphocytes arise from multipotent progenitors in the bone marrow (Kondo et al., 1997), deviating from the myeloid lineage and completing their differentiation under the influence of a suite of transcriptional regulators (Busslinger, 2004; Rothenberg and Taghon, 2005), including Ikaros (Georgopoulos et al., 1994), Pax5 (Nutt et al., 1999), and Notch1 (Radtke et al., 1999). The transcriptional regulators that guide this process are of special interest because many are also involved in lymphoid tumorigenesis (Ye et al., 1993; Weng et al., 2004; Mullighan et al., 2007, 2008), whereas others can be manipulated for directed reprogramming of cell fate (Cobaleda et al., 2007).

Among all known transcriptional regulators of lymphopoiesis, the BTB-ZF (Broad complex, Tramtrack, and Bric à brac–zinc finger) family of proteins is well represented. These proteins influence lymphoid development from the common lymphoid progenitor (CLP) stage (Kosan et al., 2010), to the T versus B fate decision (Maeda et al., 2007), to CD4⁺ versus CD8⁺ T cell selection (He et al., 2005), and even as late as the differentiation of Tfh (Johnston et al., 2009; Nurieva et al., 2009; Yu et al., 2009) and NKT (Kovalovsky et al., 2008; Savage et al., 2008) effector lineages.

In this study, we have used chemical mutagenesis in mice to reveal the physiological function of

the BTB-ZF protein ZBTB1 (zinc finger and BTB domain containing 1). ZBTB1 was essential for T cell development and also for the development of B and NK cells under competitive conditions.

RESULTS AND DISCUSSION Severe T cell lymphopenia

While surveying the descendants of chemically mutagenized mice for lymphocyte deficiencies (Siggs et al., 2011), we identified a single male mouse devoid of T cells. This phenotype, nicknamed *scanT* because of the low (or scant) frequency of T cells, was transmitted as a recessive trait (Fig. 1, A and B). *scanT* mutant mice were born at the expected Mendelian ratio, were typically fertile, and were outwardly normal in behavior.

scanT thymi were severely hypoplastic (Fig. 1 C), containing around 2% of the number of wild-type cells, and lacked corticomedullary definition (Fig. 1 D). Thymocyte development was impaired from the early thymic precursor (ETP) stage and beyond (Fig. 1, E and F). Of the lymphocytes that were present in the thymus, almost all were B cells, representing a 20-fold increase over wild-type numbers (Fig. 1 F).

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Mutation of a previously uncharacterized zinc finger protein

We mapped the *scanT* genetic lesion first by genome-wide linkage to chromosome 12 (Fig. 2 A) and then by fine mapping to a 5-Mbp interval between markers D12Mit33 and D12Mit4 (Fig. 2 B). Because none of the 35 annotated protein-encoding genes in the interval (Table S1) had previously been implicated in T cell development, we sequenced the coding exons and flanking splice junctions of three (*Zbtb1*, *Zbtb25*, and *Ppp2r5e*) based on their predominant expression in

lymphocytes (<http://biogps.gnf.org/>). 93.4% of all target nucleotides (6,081/6,511) were covered on both strands of wild-type and *scanT* samples with a Phred quality score of >30, and a single missense transition was identified in *Zbtb1* (C74R; Fig. 2 C). PolyPhen-2 (Adzhubei et al., 2010) assigned a score of 0.954 to this mutation, predicting a deleterious effect with 93% confidence. 43.2% of the total coding critical region (59,447/137,702 nt) was also covered at least three times by SOLiD 3 sequencing, with no additional mutations found.

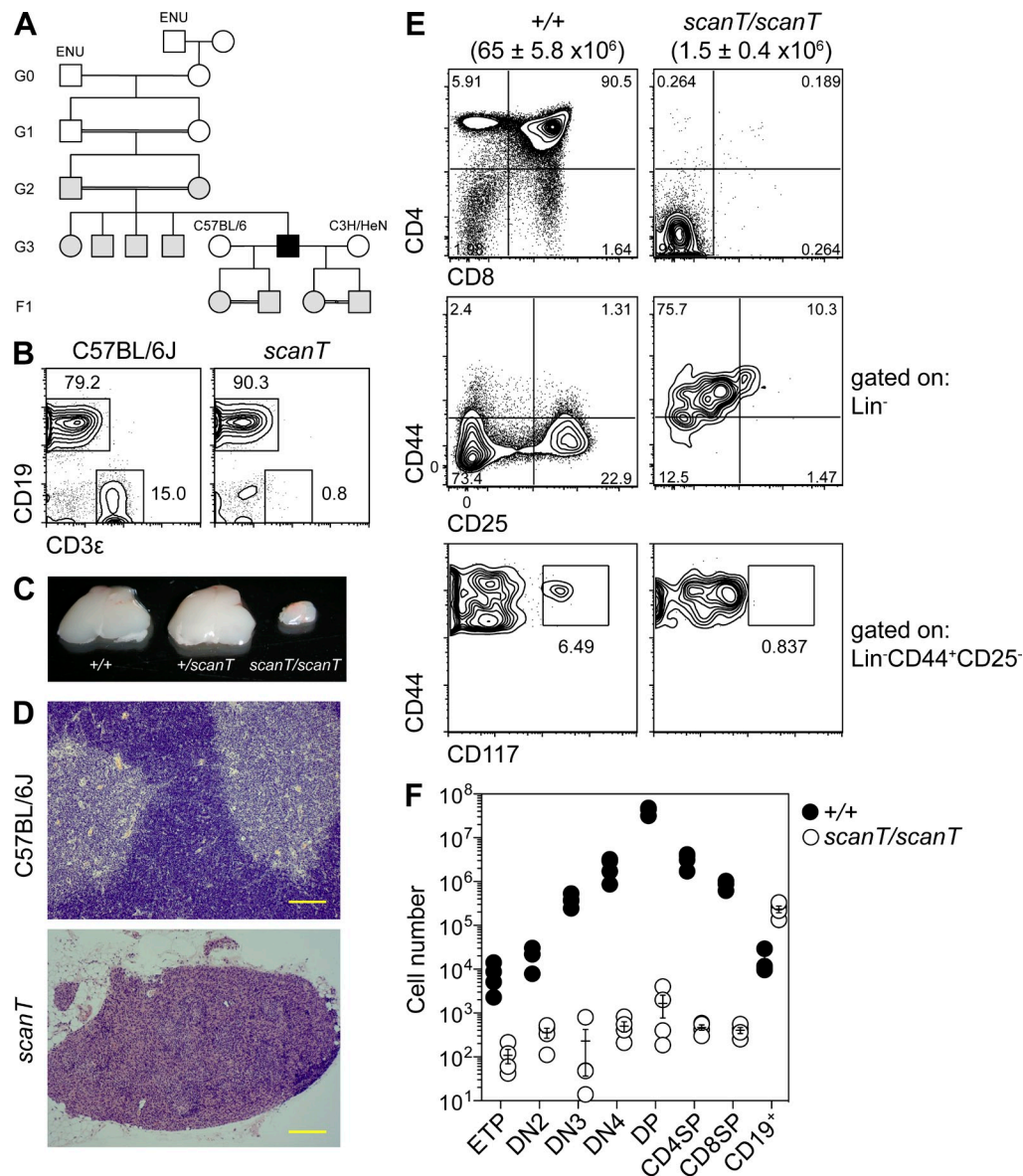


Figure 1. T cell aplasia in *scanT* mice. (A) Initial generations of the *scanT* pedigree. Black symbols, T-deficient phenotype; gray symbols, wild-type phenotype; open symbols, not tested. (B) Percentages of B (CD19⁺) and T (CD3ε⁺) cells in the peripheral blood of 8-wk-old mice as measured by flow cytometry. (C–F) Relative size (C), histological appearance (H&E; D), and cellular composition (E and F) of the thymus of wild-type and mutant siblings at 8 wk of age. Lineage markers in E and F were CD11b, CD3ε, B220, Ter119, Ly6G, NK1.1, and CD8α. Subsets in F were gated as follows: ETP (Lin⁻CD44⁺CD25⁻CD117⁺), DN2 (Lin⁻CD44⁺CD25⁺), DN3 (Lin⁻CD44⁺CD25⁺), DN4 (Lin⁻CD44⁺CD25⁻), DP (CD4⁺CD8α⁺), CD4SP (CD4⁺CD8α⁻), and CD8SP (CD4⁻CD8α⁺). Data are representative of one (A and D), two to three (E and F), or more than three (B and C) independent experiments. Error bars represent standard error, and symbols in F represent individual mice. Numbers in parentheses in E represent mean thymic cellularity and standard error. Bars, 100 μm.

Zbtb1 encodes a 713-aa member of the POK (POZ [Poxviruses and zinc finger] and Krüppel) or BTB-ZF family of transcriptional regulators and had no previously described function. Mouse ZBTB1 is 94% identical to its counterpart in man. Two characteristic domains are shared by BTB-ZF family members: an N-terminal BTB/POZ domain and a series of C-terminal C₂H₂ Krüppel-type zinc finger motifs. ZBTB1 itself contains an N-terminal BTB domain, eight zinc finger motifs, and two nuclear localization sequences (Matic et al., 2010). The residue mutated in the *scanT* pedigree (C74) is predicted to lie within the A3 helix of the BTB domain (Fig. 2 D and Fig. S1; Stogios et al., 2005) and is

highly conserved across the vertebrate lineage (Fig. 2 E). Two unique transcripts are predicted to arise from the *Zbtb1*/*ZBTB1* locus, both of which harbor the *scanT* mutation, yet only one of which (Ensembl release 65 accession no. ENST00000394712) is predominantly expressed in T cells and other leukocytes in mouse and man (Fig. S2). The other (Ensembl release 65 accession no. ENST00000358738) is known to be transcribed in HeLa cells by the nonconventional single-polypeptide nuclear RNA polymerase IV (Kravchenko et al., 2005).

To confirm that the mutation in *Zbtb1* was responsible for the *scanT* phenotype, wild-type *Zbtb1* was retrovirally

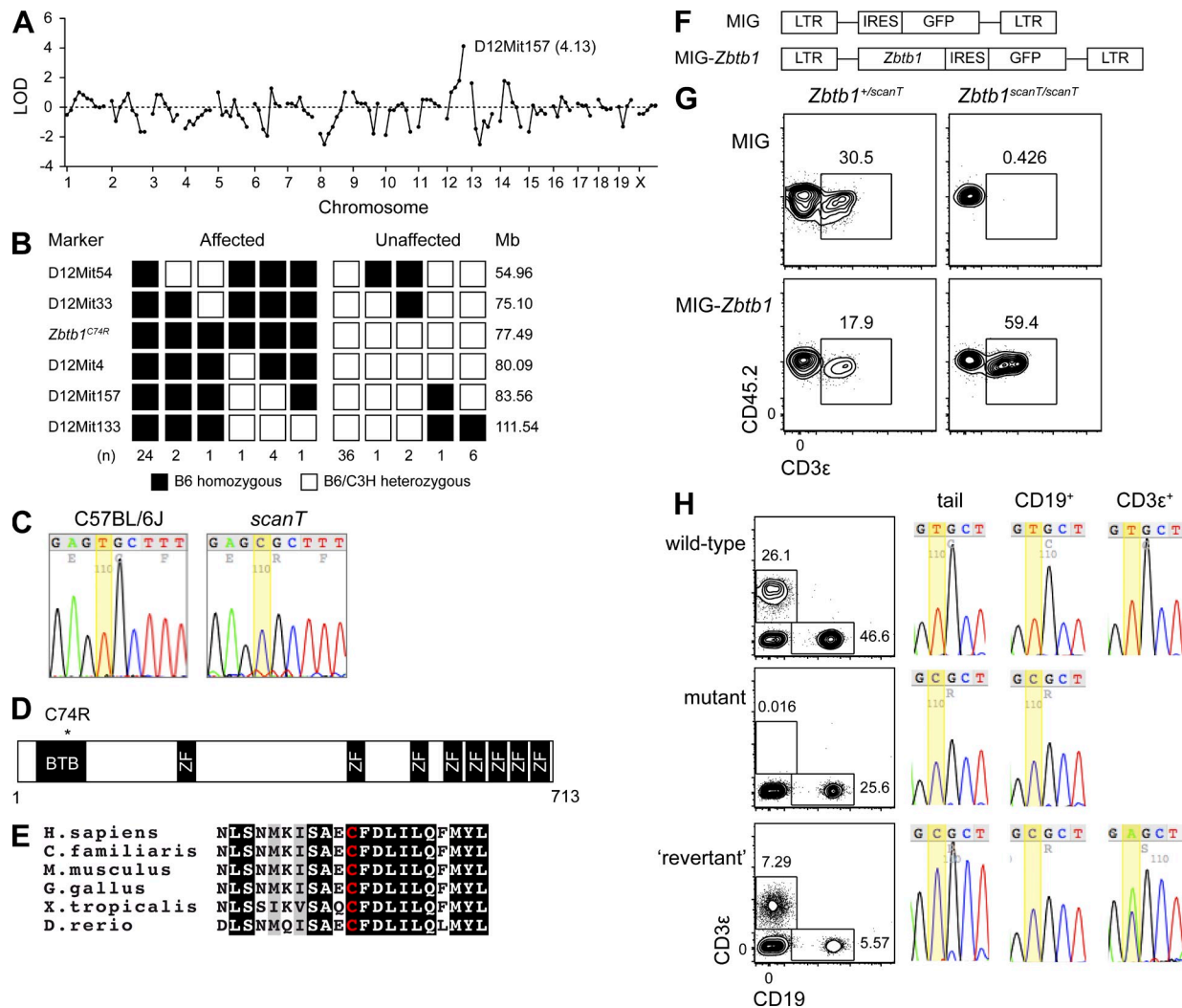


Figure 2. Identification of a missense mutation in ZBTB1. (A and B) Chromosomal mapping (A) and fine mapping (B) of the *scanT* phenotype. LOD, logarithm of odds score. (C) DNA sequence chromatograms of the mutated nucleotide in *Zbtb1* (thymine to cytosine), resulting in the substitution of arginine for cysteine at codon 74. (D) Predicted ZBTB1 protein domain structure. (E) Conservation of ZBTB1 sequence across multiple vertebrates, with the amino acid corresponding to ZBTB1^{C74} highlighted in red. ZBTB1 counterparts were not found in nonvertebrate proteomes. (F) Schematic of the MIG-Zbtb1 and control retroviral constructs. MIG, MSCV-IRES-GFP; LTR, long terminal repeat. (G) Bone marrow cells from *scanT* mice or heterozygous controls (CD45.2⁺) were transduced with a retroviral construct expressing *Zbtb1* and transferred into irradiated CD45.1⁺ recipients (~2 × 10⁶ cells/mouse). Transduced CD3ε⁺ cells were identified in blood 4 mo after transfer by coexpression of GFP. (H) Phenotypic reversion associated with somatic mutation of *Zbtb1*. DNA was prepared from tail clippings and flow-sorted CD19⁺ and CD3ε⁺ lymph node cells and sequenced at the locus mutated in the *scanT* pedigree. Contour plots were obtained from blood. Data in G are representative of three independent experiments. (C and H) Yellow highlighting indicates the position of the nucleotide mutated in the *scanT* pedigree.

transduced into *scanT* hematopoietic progenitors (Fig. 2 F). GFP⁺ T cells were recovered from recipients of *Zbtb1*-transduced *scanT* progenitors (Fig. 2 G), presumably as a consequence of restored thymic development. These data indicated that the mutation in *Zbtb1* could account for T cell deficiency in *scanT* mice.

In a minority of cases (3/16 in a 3-mo-old cohort of *Zbtb1*^{scanT} homozygotes), T cells were detected at diminished frequencies in the blood of germline mutant mice.

We hypothesized that these cells arose from a somatic mutation that suppressed the *scanT* phenotype, considering the strong selective advantage that such a mutation would confer and given that a similar phenomenon is known to occur in human T cell-deficient SCID (Hirschhorn et al., 1996). To test for somatic suppressor mutations, DNA was isolated from sorted T and B cells. Sequencing of the mutated *Zbtb1* locus revealed a heterozygous mutation within the T cell compartment of one phenotypic revertant (but not the other

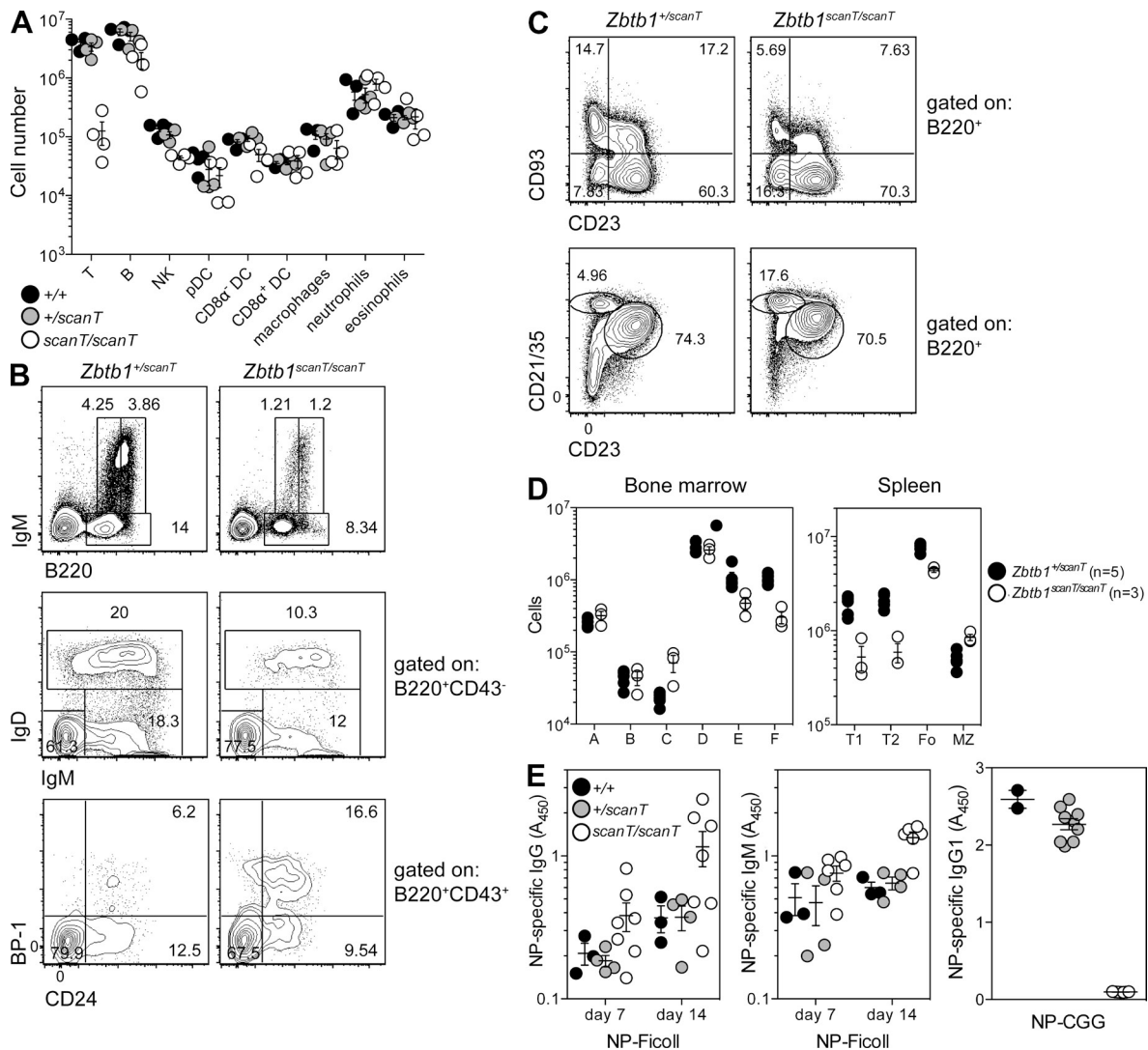


Figure 3. *scanT* mice have generalized deficiencies of lymphoid but not myeloid cells. (A) Total numbers of major lymphoid and myeloid cell subsets in the spleen of 8-wk-old sex-matched littermates, gated as follows: T (CD3⁺), B (CD19⁺), NK (NK1.1⁺CD3⁺), plasmacytoid DCs (pDC; Lin⁺PDCA1⁺SiglecH⁺), CD8 α ⁺ DCs (Lin⁺CD11c⁺CD11b⁺CD8 α ⁺), CD8 α ⁺ DCs (Lin⁺CD11c⁺CD11b⁺CD8 α ⁺), macrophages (Lin⁺CD11b⁺F4/80⁺SSC^{lo}), neutrophils (Lin⁺CD11b⁺F4/80⁺SSC^{hi}), and eosinophils (Lin⁺CD11b⁺F4/80⁺SSC^{hi}). Lineage markers included CD19, TCR- β , NK1.1, and the viability dye 7-AAD. (B–D) Frequencies (B and C) and numbers (D) of major B cell subsets in bone marrow and spleen. Subsets in bone marrow were gated as follows: A (CD11b⁺CD3⁺Ter119⁺Ly6G⁺NK1.1⁺IgM⁺B220⁺CD19⁺CD24⁺BP-1⁺), B (B220⁺IgM⁺CD43⁺CD24⁺BP-1⁺), C (B220⁺IgM⁺CD43⁺CD24⁺BP-1⁺), D (B220⁺CD43⁺IgM⁺IgD⁺), E (B220⁺CD43⁺IgM⁺IgD⁺), and F (B220⁺CD43⁺IgM⁺IgD⁺). Subsets in spleen were gated as follows: T1 (B220⁺CD93⁺CD23⁺), T2 (B220⁺CD93⁺CD23⁺), Fo (follicular; B220⁺CD93⁺CD23⁺CD21/35^{int}), and MZ (marginal zone; B220⁺CD93⁺CD23⁺CD21/35^{hi}). (E) NP (4-hydroxy-3-nitrophenylacetyl)-specific antibodies in the serum of mice immunized with NP-Ficoll or NP-CGG at 7 or 14 d (NP-Ficoll) or 14 d (NP-CGG) after immunization, presented as absorbance at 450 nm (A₄₅₀). (A–E) Data are representative of one (E) or two (A–D) independent experiments with four or more mice per group. Error bars represent standard error, and symbols represent individual mice.

two; Fig. 2 H), indicating that most, if not all, T cells in this mouse were clonally derived. This mutation either occurred after the CLP stage or did not confer a selective advantage in the B cell lineage because it was not observed in DNA from CD19⁺ cells (Fig. 2 H). The mutation in question occurred at the same nucleotide as the *scanT* mutation (the first base of codon 74), yet rather than a reversion to the wild-type codon (TGC, encoding cysteine) changed the codon to serine (AGC). Given the physical similarities of serine and cysteine, we propose that *Zbtb1*^{C74S} is permissive to T cell development and T cell clonal expansion, whereas *Zbtb1*^{C74R} is not.

Defects in lymphoid but not myeloid development

The effects of *Zbtb1* mutation were not restricted to the T cell lineage. Numbers of all lymphocyte lineages (T, B, and NK) were reduced in the spleens of mutant mice, whereas numbers of myeloid cells were not (Fig. 3 A). An examination of B cell development in mutant bone marrow revealed an accumulation of B cell progenitors at the Hardy Fraction C stage, followed by a reduction of progenitors at each subsequent stage (Fig. 3, B and D). In the mutant spleen, numbers of transitional and follicular B cells were significantly reduced by an average of 72% and 44%, respectively (Fig. 3, C and D). The relatively minor reduction in follicular as compared with transitional B cell numbers may indicate mild compensatory expansion in the follicular

compartment. Mutant B cells nevertheless responded to T-independent (but not T dependent) immunization (Fig. 3 E), implying a developmental rather than a functional B cell defect.

A cell-intrinsic defect

To distinguish between potential hematopoietic and non-hematopoietic origins of the *Zbtb1* mutant phenotype, we created radiation chimeras. *Rag1* mutants reconstituted with *Zbtb1* mutant bone marrow developed B cells but not T cells (Fig. 4 A). This defect was intrinsic to *Zbtb1* mutant T cell progenitors, rather than other radiosensitive hematopoietic cells, because T cells in mixed bone marrow chimeras were exclusively derived from wild-type donors (Fig. 4 B). In fact, all blood lymphocytes (but not CD11b⁺ myeloid cells) were wild-type donor derived, implying a general competitive failure of *Zbtb1* mutant lymphoid progenitors (Fig. 4 B). Finer phenotyping of the spleen, bone marrow, and thymus of mixed bone marrow chimeric mice confirmed this to be the case. B, T, and NK cells in the spleen were exclusively wild-type derived (CD45.1⁺), whereas little or no competitive disadvantage was seen in the myeloid compartments (Fig. 4 C). Cells of wild-type origin dominated the B cell compartment from the Fraction A stage onward and similarly outcompeted *Zbtb1* mutant NK progenitors in bone marrow and T cell progenitors in the thymus (Fig. 4 C).

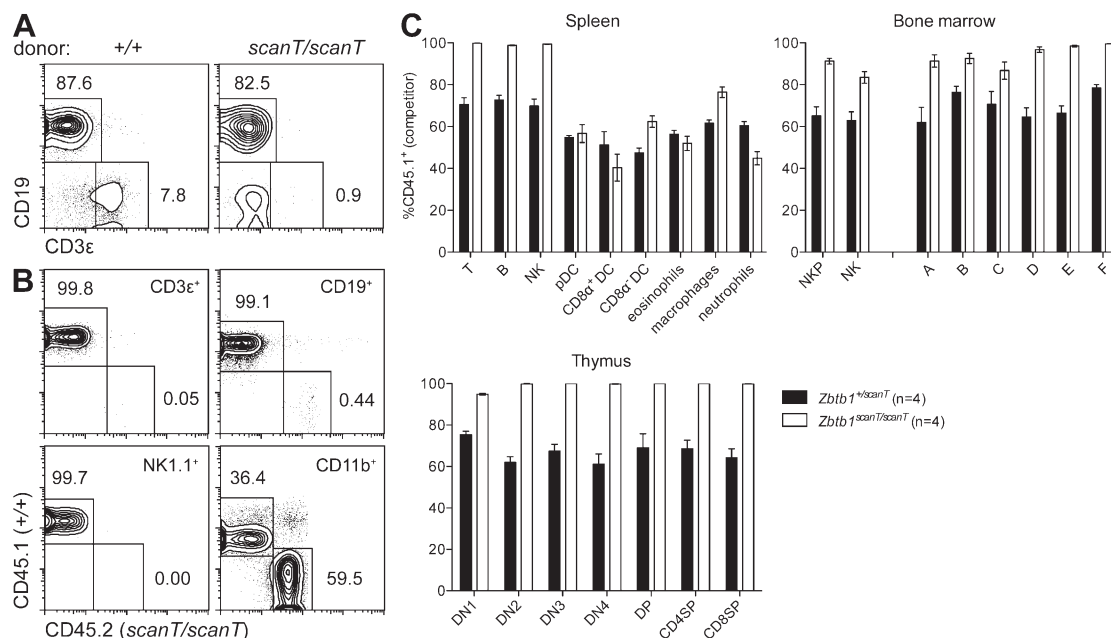
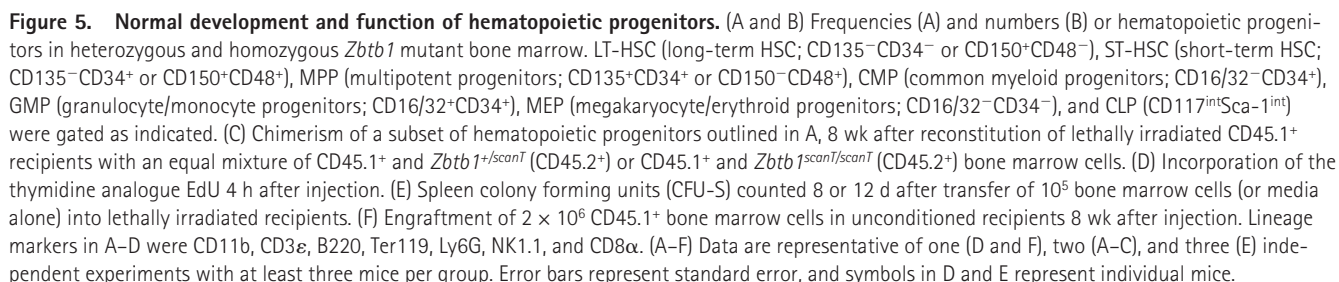


Figure 4. A cell-intrinsic T cell deficiency and competitive failure of lymphoid reconstitution. (A and B) Lethally irradiated *Rag1* mutant mice (CD45.2⁺) were reconstituted with unmixed *scanT* or wild-type bone marrow (CD45.2⁺; A) or a mixture of *scanT* or wild-type marrow (CD45.2⁺) with wild-type bone marrow (CD45.1⁺; B). Chimerism was measured 8 wk after transplant. Panels in B have been gated on the indicated cell subset. (C) Lethally irradiated wild-type recipients (CD45.1⁺) were transplanted with an equal mixture of wild-type (CD45.1⁺) and heterozygous or homozygous mutant (CD45.2⁺) bone marrow. Wild-type donor chimerism (CD45.1⁺) was measured 8 wk later in the spleen, bone marrow, and thymus. Subsets were gated as in Fig. 3, with the addition of NK progenitors (NKP; 7-AAD⁻CD19⁻TCR-β⁻CD122⁺NK1.1⁻). (A–C) Data are representative of one (A) or three (B and C) independent experiments with at least three mice per group. Error bars represent standard error.

rates in wild-type and mutant mice (Fig. 5 D). Wild-type and *Zbtb1* mutant bone marrow also generated equivalent numbers of hematopoietic colonies in the spleens of lethally irradiated recipients, both at days 8 (indicative of megakaryocyte/erythroid progenitor function) and 12 (reflective of multipotent progenitor function; Fig. 5 E; Na Nakorn et al., 2002). Myeloid engraftment did not occur in unconditioned *Zbtb1* mutant recipients of wild-type bone marrow, which is consistent with the interpretation of intact HSC function (Fig. 5 F).



In certain aspects, *Zbtb1* mutants reflect the phenotype of mice lacking *Notch1*. Both *Zbtb1* and *Notch1* mutants are profoundly T cell deficient and show an accumulation of B cells in the thymic rudiment (Radtke et al., 1999). Yet although *Zbtb1* mutants have a competitive disadvantage in the B and NK lineages, *Notch1* mutants do not (Radtke et al., 1999). Germline mutation of *Zbtb1* appears permissive to embryonic development, whereas mutation of *Notch1* is not (Swiatek et al., 1994), indicating a more specialized role for ZBTB1 in determining lymphoid fate. This is consistent with the conservation of *Zbtb1* only among vertebrate genomes and might implicate the human ZBTB1 locus in genetically obscure cases of T cell-deficient SCID (Fischer, 2007).

Although there are no previous studies of a physiological role for ZBTB1, one describes some of its biochemical characteristics (Matic et al., 2010). ZBTB1, like many of its BTB-ZF counterparts, acts as a potent transcriptional repressor, as determined by the repressive activity of a Gal4-ZBTB1 fusion protein. This repressive activity, as well as the colocalization of ZBTB1 with the SMRT transcriptional repressor, is also regulated by SUMOylation (Matic et al., 2010).

Our data raise several questions about the function of ZBTB1 in T cell and lymphoid specification. In particular, why is ZBTB1 critical for T cell development but only essential for B and NK development under competition? Can the reduction in ETP numbers be explained by their failure to migrate to the thymus, by a failure to proliferate within it, or a combination of both? Programmed death of T cell progenitors is unlikely to account for this because T cells also fail to develop in the absence of the proapoptotic protein BIM (which can rescue erythropoiesis in mice deficient for the BTB-ZF relative LRF; Fig. S3; Maeda et al., 2009).

In summary, our work defines ZBTB1 as a regulator of lymphoid development, joining the ranks of several other lymphoid-promoting BTB-ZF proteins. As is the case with other BTB-ZF family members, ZBTB1 presumably acts as a transcriptional suppressor, and the determination of precisely which genes it regulates will be of central importance to our understanding of lymphopoiesis.

MATERIALS AND METHODS

Mice and positional cloning. *Zbtb1*^{scanT} was generated on a pure C57BL/6J (The Jackson Laboratory) background by *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis as previously described (Georgel et al., 2008). The index *scanT* mutant (C57BL/6J) was outcrossed to C3H/HeN females (Taconic), and F1 progeny were intercrossed. Blood was collected from the retroorbital plexus of 26 F2 progeny, and mice were grouped into mutant and wild-type cohorts (7 and 19 mice, respectively) based on relative percentages of CD19⁺ and CD3e⁺ cells as measured by flow cytometry. Individual mice were typed at 128 microsatellite markers spaced across the genome (complete list available in the Supplemental material), with expected and observed genotype frequencies used to calculate LOD (logarithm of odds) scores at each marker. For fine mapping, an extra 71 F3 backcross progeny were genotyped at additional loci within the critical region. Candidate gene amplicons from wild-type and *scanT* genomic DNA were sequenced using a capillary sequencer (3730xl; Applied Biosystems). Genomic DNA from a single *scanT* homozygote was used to create a fragment library for single slide whole-genome sequencing on the SOLiD 3 platform (Applied Biosystems). *Rag1*^{maladaptive} mice

(C57BL/6J, MGI:3851764) were generated in-house by ENU mutagenesis, and B6.SJL-*Ptprca*^d *Pep3*^b/BoyJ (CD45.1, backcrossed >22 generations to C57BL/6), *Bcl2l1*^{tm1.1As} (backcrossed 13 generations to C57BL/6; Bouillet et al., 1999), and C57BL/6J males used for mutagenesis were obtained from the Jackson Laboratory. All other C57BL/6J mice were obtained from The Scripps Research Institute breeding colony. All animal procedures were performed in accordance with guidelines of the Institutional Animal Care and Use Committee of The Scripps Research Institute, and all experiments were conducted with homozygous mutants and sex-matched littermate controls.

Flow cytometry. Blood from the retroorbital plexus of isoflurane-anesthetized mice was collected in cluster tubes (Costar) containing 20 μ l of 6% (wt/vol) EDTA in water. 50 μ l of blood was subjected to two rounds of red blood cell lysis with ammonium chloride before staining. Lymphocyte suspensions from bone marrow (femurs and tibias from one hind leg), spleen, and thymus were counted (Z2 Coulter Counter; Beckman Coulter) and were stained with a combination of the following mouse-specific antibodies: FITC-conjugated IgM (goat polyclonal; 1020-02; SouthernBiotech); FITC-conjugated CD24 (30-F1), CD48 (HM48-1), F4/80 (BM8), CD127 (A7R34); PE-conjugated CD122 (5H4), CD135 (A2F10), CD150 (9D1), IgD (11-26), PDCA1 (927), TCR- β (H57-597; PerCP-Cy5.5); CD117 (2B8; eFluor710), APC-conjugated CD8 α (53-6.7), CD11b (M1/70), CD93 (AA4.1), IgM (II/41), Ly6G (RB6-8C5), NK1.1 (PK136), TER119 (TER-119; eBioscience); FITC-conjugated CD4 (GK1.5), CD23 (B3B4), CD25 (7D4), CD34 (RAM34), PE-conjugated BP-1 (BP-1), CD11b (M1/70), CD19 (1D3), CD21/35 (7G6), CD44 (IM7), PerCP-Cy5.5-conjugated B220 (RA3-6B2), CD8 α (53-6.7), CD19 (1D3), NK1.1 (PK136), APC-conjugated CD11c (HL3) and CD43 (S7), CD44 (IM7; Horizon V500; BD); APC-conjugated SiglecH (551), CD3e (145-2C11), APC-Cy7-conjugated Sca-1 (D7) and CD45.2 (104), PE-Cy7-conjugated CD16/32 (93) and CD19 (6D5), CD45.1 (A20; Pacific blue), and CD16/32 (93; purified; BioLegend). Samples were acquired on a FACSCalibur or LSRFortessa (BD), and data were analyzed with FlowJo software (Tree Star). 7-AAD was purchased from eBioscience, and EdU labeling and staining were performed according to the manufacturer's instructions (Invitrogen) 4 h after a single intraperitoneal injection of 100 mg/kg EdU. For sorting experiments, lymph node or suspensions were labeled with antibodies, and target populations were sorted by a FACSARIA II (BD) into serum for genomic DNA extraction.

Immunizations. Immunizations were performed as described previously (Siggs et al., 2011).

Hematopoietic chimeras. Recipient mice were γ -irradiated with a split dose of 11 Gy (2 \times 5.5 Gy, ¹³⁷Cs source). The next day, mice were injected with 2 \times 10⁶ bone marrow cells via the retroorbital plexus and were maintained on trimethoprim/sulfamethoxazole antibiotic water until sacrificed for analysis 8 wk later. For colony forming unit assays, mice received 10⁵ bone marrow cells and were sacrificed 8 or 12 d later. Spleens were fixed overnight in Bouin's solution (Sigma-Aldrich), and colonies were counted the next day. For unconditioned chimeras, unconditioned recipients received a single retroorbital injection of 2 \times 10⁶ bone marrow cells.

Retroviral transduction. The open reading frame of *Zbtb1* was cloned into the BglII-EcoRI site of pMIG (MSCV2.2-IRES-GFP; Addgene #9044, deposited by W. Hahn [Dana-Farber Cancer Institute, Boston, MA]) to generate the pMIG-*Zbtb1* vector. Vectors were transfected into the Plat-E packaging cell line using Eugene 6 (Roche), and retrovirus-containing supernatant was collected 24 and 48 h later. Bone marrow donor mice were primed 5 d before harvest with 150 mg/kg 5-fluorouracil (Sigma-Aldrich), and bone marrow cells were prestimulated overnight with 10 ng/ml of mouse recombinant IL-3, 10 ng/ml IL-6, 50 ng/ml SCF, 50 ng/ml TPO, and 5 ng/ml FLT3L (all from PeproTech). The next day, 3 ml/well of retroviral supernatant was centrifuged (2,000 rpm for 2 h at 32°C) onto RetroNectin-coated 6-well plates (20 μ g/ml; Takara Bio Inc.), and cells were

cultured on coated plates in the presence of 4 µg/ml Polybrene (Sigma-Aldrich) for 48 h. Recipient CD45.1⁺ mice were γ-irradiated with a split dose of 11 Gy (¹³⁷Cs source) and the next day injected with ~2 × 10⁶ transduced bone marrow cells via the retroorbital plexus.

Histology. Thymi were fixed in 10% neutral buffered formalin and embedded in paraffin wax, and 5-µm sections were stained with hematoxylin and eosin (H&E).

PolyPhen-2. The deleterious effect of the *scanT* mutation was predicted using the HumVar-trained PolyPhen-2 server (version 2.1.0; Adzhubei et al., 2010).

Online supplemental material. Fig. S1 compares the sequence of ZBTB1 with other BTB-ZF proteins. Fig. S2 shows the tissue-specific expression of *Zbtb1* transcripts. Fig. S3 shows flow cytometric analysis of lymphocyte populations in *Zbtb1*; *Bcl2l1* double mutant mice. Table S1 is a list of protein-encoding genes in the *scanT* critical region. The whole genome mapping panel for C57BL/6J versus C3H/HeN is also included in the supplemental material. Online supplemental material is available at <http://www.jem.org/cgi/content/full/jem.20112084/DC1>.

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Author contributions: O.M. Siggs designed research, performed all experiments, analyzed data, and wrote the manuscript. X. Li propagated the *scanT* strain by intracytoplasmic sperm injection, Y. Xia provided bioinformatics support, and B. Beutler managed the mutagenesis operation and corrected the manuscript.

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