

Supplementary Figure 1. Zbtb1 is expressed in hematopoietic progenitors and in mature immune cells a) FACS analysis of GFP levels in bone marrow from the indicated mice, Lin (CD11b, GR1, CD3, B220, Ter119, NK1.1). Cells in the represented gates were sorted and tested for Zbtb1 expression by RT-PCR as indicated. **b)** Analysis of GFP levels in the indicated populations, LSK (Lin⁻Sca1⁺cKit⁺); LT-HSC (LSK Flt31⁻CD150⁺); LMPP (LSK Flt31^{hi}); ETP (CD4⁻CD8⁺Lin⁻c-kit^{hi}CD44^{hi}CD25⁻); DN2a(CD4⁻CD8⁺Lin⁻c-kit^{hi}CD44⁺CD25⁺); DN3(CD4⁻CD8⁻ Lin⁻CD44⁻CD25⁺); DN4(CD4⁻CD8⁻ Lin⁻CD44⁺CD25⁻); DP (CD4⁺CD8⁺); CD4SP (CD4⁺CD8⁻TCRb^{hi}); CD8SP (CD4⁻CD8⁺TCRb^{hi}); T-cells (TCRb^{hi}); B-cells (CD19⁺B220⁺); Myeloid (CD11b⁺GR1⁻); Granulocytes (CD11b⁺GR1⁺). Data is representative of three independent experiments. p values corresponding to the significance using T-test are shown n=3.

b Transduced NIH3T3 Transduced NIH3T3 Flag-Zbtb1 Flag-ScanT Empty of Max % of Max Zbtb1 % Flag Thy1.1 βactin Flag -Zbtb1 IRES Thy1.1 Flag-ScanT IRES Thy1.1 ■ IRES Thy1.1 d

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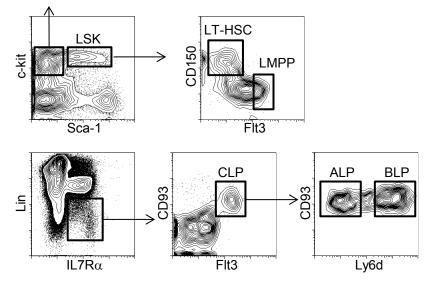
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Transduced NIH3T3 B-cells C57BL/6 ScanT Zbtb1 Flag-Zbtb1 IRES Thy1.1 Flag-ScanT IRES Thy1.1 Flag-ScanT IRES Thy1.1 Flag-ScanT IRES Thy1.1

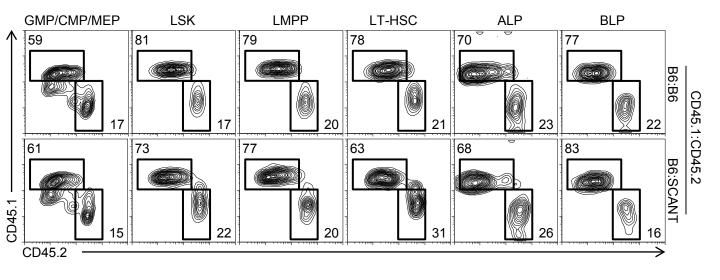
Supplementary Figure 2. The ScanT mutation negatively affects the stability of Zbtb1

a) Staining of Flag and Thy1.1 on NIH3T3 cell lines stably transduced with the indicated constructs. **b)** Western blot analysis to detect Zbtb1 and bactin on transduced NIH3T3 cell lines. **c)** Immunofluorescence of NIH3T3 stable cell lines. **d)** Western-blot analysis on purified B-cells from the indicated strains of mice. Data is representative of two independent experiments.

GMP/CMP/MEP







Supplementary Figure 3. The ScanT mutation of Zbtb1 does not affect the generation of lineage-restricted immune progenitors in hematopoietic bone marrow chimeras

a) Representative gating strategy to identify different immune progenitor populations in bone marrow from mixed bone marrow chimeras generated with a mixture of CD45.1⁺(C57BL/6):CD45.2⁺(C57BL/6 or ScanT) donor cells. Mixed myeloid progenitors (GMP/CMP/MEP); Lin⁻Sca1⁺c-kit⁺ (LSK); Long-term-HSC (LT-HSC); lymphoid-primed multipotent progenitors (LMPP); Common Lymphoid progenitors (CLP); All lymphoid progenitors (ALP); B lymphoid progenitors (BLP). b) Donor chimerism is identified in the indicated populations. Data is representative of four recipient mice per condition.