Supplementary Figure 1. Hematopoietic deletion of Tsc1 gave rise to less HSCs, but not CLPs.
(a). Total RNA was isolated from bone marrow cells of wild-type (WT) and Tsc1−/− mice. Tsc1 mRNA was analyzed by quantitative PCR. Data are shown as ratio of Tsc1 to β-actin. (b). Absolute numbers of total BM cells in WT and Tsc1−/− mice. (c) Gating strategy for flow cytometry analysis of HSC and CLPs. (d,e). Frequencies and absolute numbers of HSC and CLPs in WT and Tsc1−/− mice. Values represent mean ± s.d and represent at least three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001.
Supplementary Figure 2. Hematopoietic-specific deletion of Tsc1 disrupted peripheral T-cell hemostasis, rather than development.

(a) Representative flow cytometric plots showing CD3 vs CD19 expression on the splenocytes in WT and Tsc1−/− mice. Bottom, the absolute number of CD3+ T and CD19+ B cells in the spleens of wild-type and Tsc1−/− mice. (b) Analysis of iNKT cells (CD3+CD1d−PBS57+) in the thymus (top) and spleen (bottom) of WT and Tsc1−/− mice. Left panel, representative flow cytometric plots; Right panel, the absolute number of NKT cells in the thymus and spleen of WT and Tsc1−/− mice. (c) Expression of CD62L vs CD44 on splenic CD4+ (top) or CD8+ T cells (bottom) from WT and Tsc1−/− mice. (d) Left panel, Representative flow cytometric plots of thymocytes in WT and Tsc1−/− mice. Right panel, the absolute number of CD4+ single-positive (CD4SP) and CD8+ single-positive (CD8SP) thymocytes. (e) Expression of maturation markers CD62L and CD69 on WT and Tsc1−/− CD4SP (top) or CD8SP (bottom) thymocytes. Each symbol represents an individual mouse; Data are shown as means ± s.d and represent at least three independent experiments. ***p < 0.001, NS, no significant difference.
Supplementary Figure 3. Hematopoietic-specific deletion of Tsc1 blocked early pro-B-cell development.

(a) Top, expression of IgM and CD43 of B220+ B cells from WT and Tsc1−/− mice. Bottom, the absolute number of indicated cells. (b) Top, expression of IgM and B220 of bone marrow cells from WT and Tsc1−/− mice. Bottom, the absolute number of indicated cells. (c) Top, expression of IgM and IgD of splenocytes from WT and Tsc1−/− mice. Bottom, the absolute number of indicated cells. Each symbol represents an individual mouse. All data represent at least three independent experiments and calculated data are shown as means ± s.d. **p < 0.01, ***p < 0.001.
Supplementary Figure 4. The constitutive metabolic activation in the Tsc1−/− mice was NK-cell intrinsic.

(a, b) Tsc1-deleted mice were further bred onto Rag1−/− mice that lack T, and B cells. The cell size, FSC, SSC, and the expression of CD71, CD98, CD69 (a) and the proliferation marker Ki67 (b) of splenic NK cells from WT and Tsc1−/−Rag1−/− mice were analyzed using flow cytometry. Each symbol represents an individual mouse; Data are shown as means ± s.d and are representative of three independent experiments. **p < 0.01, ***p < 0.001.
Supplementary Figure 5. The deletion of Bim failed to correct the increased metabolic activation in the Tsc1−/− mice.

(a, b) Left panel, representative overlaid histograms showing FSC (cell size) and SSC (cell containing) from the indicated mice. Right panel, MFI of FSC and SSC was calculated. Each symbol represents an individual mouse; Data are shown as means ± s.d and are representative of three independent experiments. *p < 0.01; NS, no significant difference.
Supplementary Figure 6. NK-cell fate mapping revealed that Tsc1 was not required for effector functions.

(a) Quantitative RT-PCR analysis of Tsc1 expression in sorted CD3+ NK1.1+EYFP+ cells from indicated mice. (b, i) RosaEYFPfl/+Ncr1-Cre+ and Tsc1fl/flRosaEYFPfl/+Ncr1-Cre+ mice were injected intraperitoneal with polyI:C (10 mg/mg) and sacrificed after 18 hours. Splenic lymphocytes were prepared and co-cultured with an equal number of tumor cell lines, RMA-S and YAC-1, and indicated antibodies; medium alone served as a negative control, and PMA (P) plus ionomycin (I) served as a positive control. Intracellular staining was used for the detection of IFN-γ production by EYFP+ NK cells (b, f). CD107a expression on EYFP+ NK cells was chosen as a marker for NK cell degranulation (d, h). The bar graphs show the averaged percentages of IFN-γ+ cells (c, g) and CD107a+ cells (e, i) of 3–4 experiments. Data represent the mean ± s.d of 3-4 mice per group and are representative of three independent experiments. ***p < 0.001, NS, no significant difference.