

Ikaros deficiency is associated with aggressive BCR-ABL1 B-cell precursor acute lymphoblastic leukemia independent of the lineage and developmental origin

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Supplementary material

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Supplementary Figure Legends

Figure S1: Comparison of B1 and B2 progenitor cellularity in the BM and Ikaros expression in WT and cKO B1 progenitors. (A) Mean % of BM B1 and B2 progenitors (B220⁺CD43⁺IgM⁻) after birth as indicated. D1: one day after birth. (B) Loss of Ikaros expression in cKO mice was analyzed by intracellular staining with the FoxP3 staining kit (eBioscience) followed by flow cytometry. As staining controls, BM B cells (CD19⁺ IgM⁺) from Ik^{fl/fl}Mb-1-Cre⁺ (cKO), Ik^{fl/fl}Mb-1-Cre⁻ (WT), Ik^{+/fl}Mb-1-Cre⁺ (Ik+/-) and BH1 cells, an Ikaros deficient pre-B cell line (Ik^{-/-}) were used (left panel).¹ Ikaros expression in WT or cKO BM B1 progenitor cells were assessed (right panel). The control in the right panel corresponds to cells stained only with the secondary antibody.

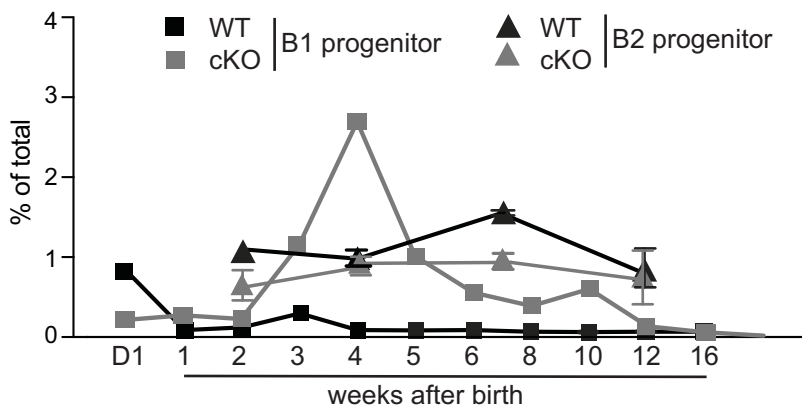
Figure S2: RNA-seq analysis of WT and cKO B1 progenitor cells. RNA-seq analysis of B1 progenitors (Lin⁻IgM⁻CD93⁺CD19⁺B220^{-/lo}) from 3 WT and 3 cKO mice (4 weeks old) as indicated (exp. # 1-3). RNA was extracted (RNeasy, QIAGEN), libraries were prepared (SMART-Seq v4 Ultra Low Input kitRNA, Clontech; NEXTERA XT, Illumina), and sequencing was performed (50bp reads, HiSeq 4000, Illumina). Sequences were aligned onto the *Mus musculus* genome (mm10 assembly) using STAR software version 2.5.3a.² Gene expression quantification was done with htseq-count version 0.6.1p1 using annotations from Ensembl release 90 and comparisons of gene expression between different samples were performed using DESeq2 software version 1.16.1 Bioconductor package.^{3,4} Genes, differentially expressed in each experiment, were selected ($|\text{Log}_2 \text{FC}| > 2$, $p < 0.05$) and visualized by hierarchical clustering generated with Cluster 3.0 and TreeView 1.1.6r4. The positions of specific genes are indicated. Yellow: upregulated, blue: downregulated in cKO.

Figure S3: Expression of TdTomato and BCR-ABL1 in transduced B1 progenitors. (A) BM or FL B1 progenitors from WT and cKO mice were sorted, cultured for 24h, and retrovirally transduced with BCR-ABL1-IRES-TdTomato (BCR-ABL1) or IRES-TdTomato (pMITo).⁵ After

8-10 days, expression of the TdTomato reporter was analyzed by flow cytometry prior to injection. (B) Western blot analysis of BM cKO B1 progenitor cells transduced with pMITo or BCR-ABL1 that were cultured for 5 weeks. Cell extracts were prepared with RIPA buffer, separated by SDS-PAGE and blotted on a PVDF membrane. BCR-ABL1 expression was examined with an anti-Abl antibody (Cell Signaling #2862) using the enhanced chemiluminescence system. β -actin was used as a loading control. (C) Splens from a non-transplanted NSG mouse (NSG) or NSG mice transplanted with BM cKO B1 progenitors transduced with pMITo (cKO pMITo), with BM WT or cKO B1 progenitors transduced with BCR-ABL1 (WT BCR-ABL; sick 56 days after transplantation. cKO BCR-ABL; sick 29 days after transplantation).

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A



B

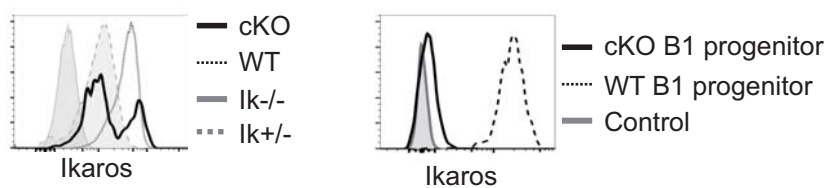


Figure S1

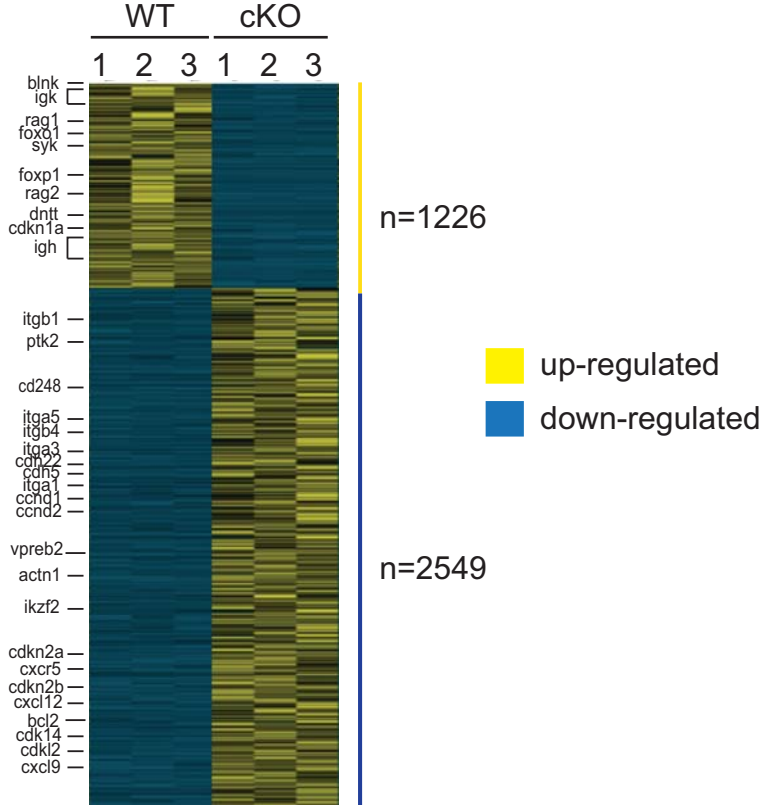
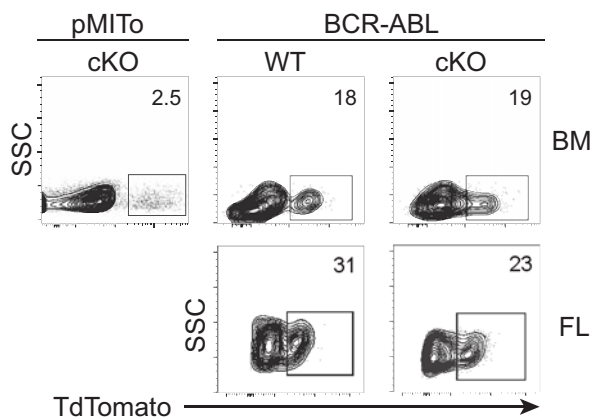
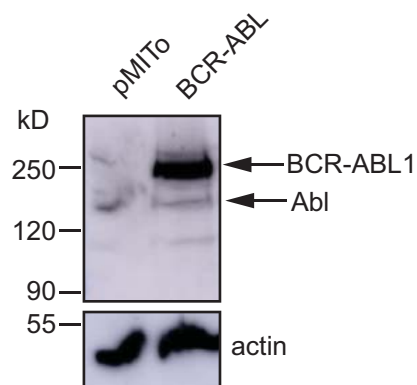


Figure S2

A



B



C

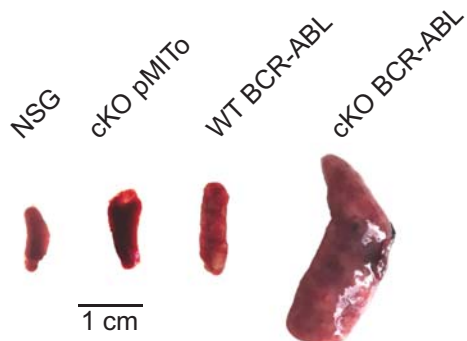


Figure S3