SUPPLEMENTAL MATERIAL

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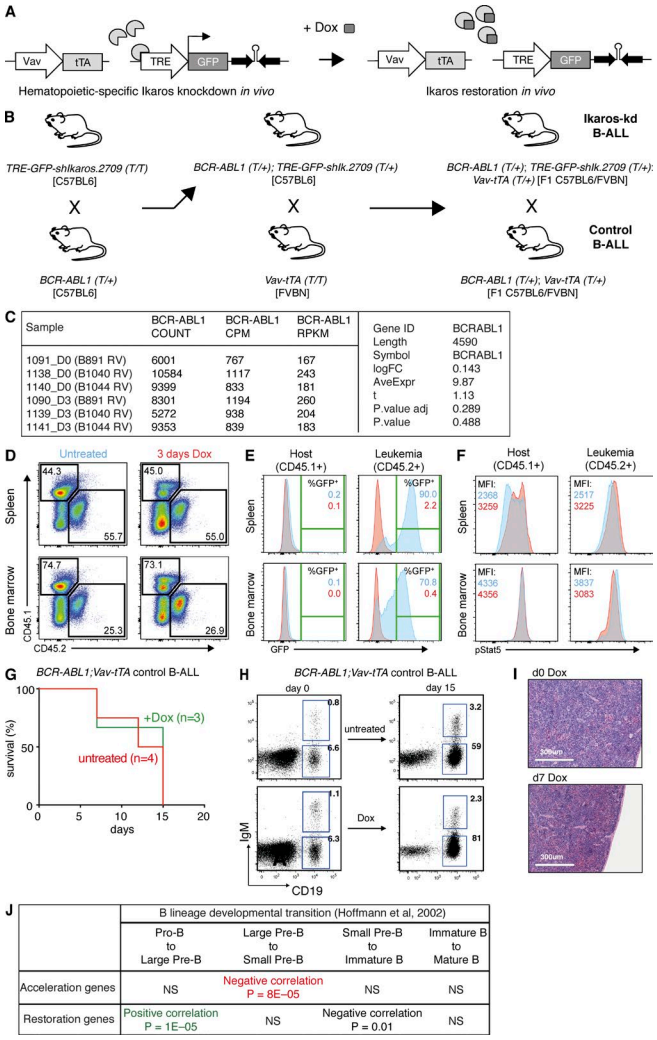

Figure S1. **Generation and analysis of primary transgenic BCR/ABL1$^+$ Ikaros-kd and control B-ALL.** (A) Schematic of genetic elements allowing tet-regulated shRNA-mediated suppression of Ikaros expression in the hematopoietic system in vivo. (B) Crossing strategy to generate Ikaros-kd leukemia. Homozygous *TRE-GFP-shIkaros* transgenic mice (C57BL/6 strain background) were mated with heterozygous *BCR/ABL1^{P190}* transgenic mice (C57BL/6). *BCR/ABL1^{P190};TRE-GFP-shIkaros* bitransgenic progeny (C57BL/6) were mated with homozygous *Vav-tTA* transgenic mice (FVBN), generating two groups of progeny on an identical C57BL/6/FVBN F1 genetic background: *BCR/ABL1^{P190};Vav-tTA;TRE-GFP-shIkaros* triple transgenic mice that develop accelerated Ikaros-kd B-ALL; and *BCR/ABL1^{P190};Vav-tTA* littermates that develop control B-ALL. *T* denotes transgenic and + denotes wild type. All primary leukemias were generated in CD45.2$^+$ mice and transplanted into CD45.1$^+$Rag1$^{-/-}$ recipient mice to enable in vivo tracking of B-ALL cells by flow cytometry. The immunodeficiency of these recipients allowed engraftment of C57BL/6/FVBN F1 strain background cells. (C) RNA-seq reads mapping to the coding region of human BCR-ABL1 and statistical analysis showing no differential expression of BCR-ABL1 upon Ikaros restoration in the retroviral B-ALL model. (D–F) Flow cytometry analysis of spleen (top) and bone marrow (bottom) harvested from 2 CD45.1$^+$Rag1$^{-/-}$ recipient mice transplanted with equal numbers of the same *BCR/ABL1^{P190};Vav-tTA;TRE-GFP-shIkaros* triple transgenic leukemia B031 (CD45.1$^+$), and either left untreated or Dox-treated for 3 d upon leukemia development. CD45.1 and CD45.2 flow cytometry (D) allowed identification of host and leukemia cells, respectively. GFP expression in leukemia cells was shut off by Dox treatement as indicated (E). Flow cytometry of phosphorylated STAT5 (F) identified positive and negative peaks in host splenocytes, whereas the host bone marrow harbored a single pSTAT5$^+$ population. Leukemia cells in the spleen and bone marrow were uniformly pSTAT5$^+$, and this was unaffected by Dox-induced Ikaros restoration. (G) Kaplan-Meier survival analysis of recipient mice transplanted with *BCR/ABL1^{P190};Vav-tTA* control B-ALL cells and either left untreated or Dox-treated upon disease onset (three independent primary control B-ALLs, one recipient per condition for each primary B-ALL). Median survival 13.5 d for untreated versus 15 d for Dox treated, P = ns, log-rank test. (H) Flow cytometry of Cd19 and IgM expression in peripheral blood of control B-ALL transplant recipient mice with indicated treatments initiated upon disease establishment. (I) Spleen histology for control B-ALL transplant recipient mice with Dox treatments indicated. (J) Roast gene set testing results comparing Acceleration and Restoration genes to previously reported expression profiles for transitions between consecutive stages of normal B lymphocyte development in the murine bone marrow: from pre–BI to large pre–BII (pro–B to large cycling pre–B); large pre–BII to small pre–BII (large cycling preB to small resting pre–B); small resting preB to immature B; and immature to mature B (Hoffmann et al., 2002). Notable correlations are highlighted. As Acceleration genes compare Ikaros-kd to control leukemias, a negative correlation with the large preB to small preB transition suggests that Ikaros-kd leukemias are less mature than control leukemias.

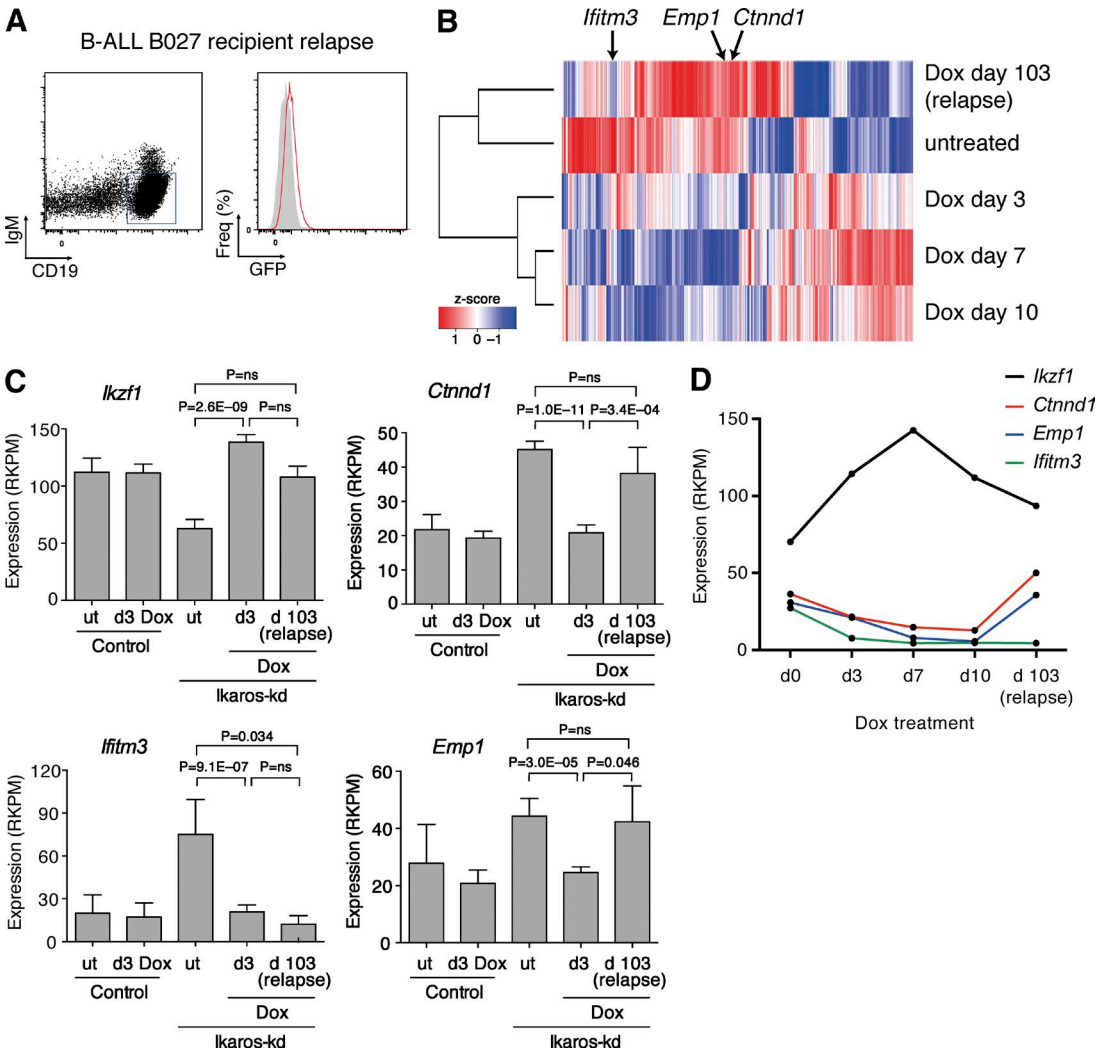

Figure S2. **Analysis of relapsed Ikaros-kd B-ALL harvested from Dox-treated mice.** (A) Flow cytometry profile of CD19/IgM (left) and GFP (right) expression of splenocytes isolated from a recipient mouse initially transplanted with Ikaros-kd B-ALL B027, Dox-treated at disease onset, and then harvested at relapse after 103 d of Dox treatment. The GFP histogram shows expression in CD45.1$^+$ host cells (gray) and CD45.2$^+$CD19$^+$IgM$^-$ donor-derived splenocytes (red). (B) Heat map showing expression of Ikaros-repressed and Ikaros-activated genes in B-ALL cells harvested from several recipient mice originally transplanted with Ikaros-kd B-ALL B027 and Dox treated as indicated. Z-scores are $\log_2$-RPKM standardized to have mean 0 and standard deviation 1 for each gene. Samples are ordered by hierarchical clustering with Pearson correlation distances. The plot shows that relapsed B-ALL is transcriptionally more similar to untreated B-ALL than acute Dox-treated B-ALL. *Ctnnd1*, *Emp1*, and *Ifitm3* are indicated. (C) TMM-normalized RNA-seq expression (RPKM) of *Ikzf1*, *Ctnnd1*, *Ifitm3*, and *Emp1* in control and Ikaros-kd B-ALLs incorporating relapsed samples harvested from three recipient mice, each bearing independent primary Ikaros knockdown B-ALLs and after extended Dox treatment. Moderated t-statistic P-values are indicated. (D) Temporal mRNA expression (RPKM) of *Ikzf1*, *Ctnnd1*, *Ifitm3*, and *Emp1* in B-ALL cells harvested from a series of recipient mice originally transplanted with Ikaros-kd B-ALL B027 with Dox treatments indicated.

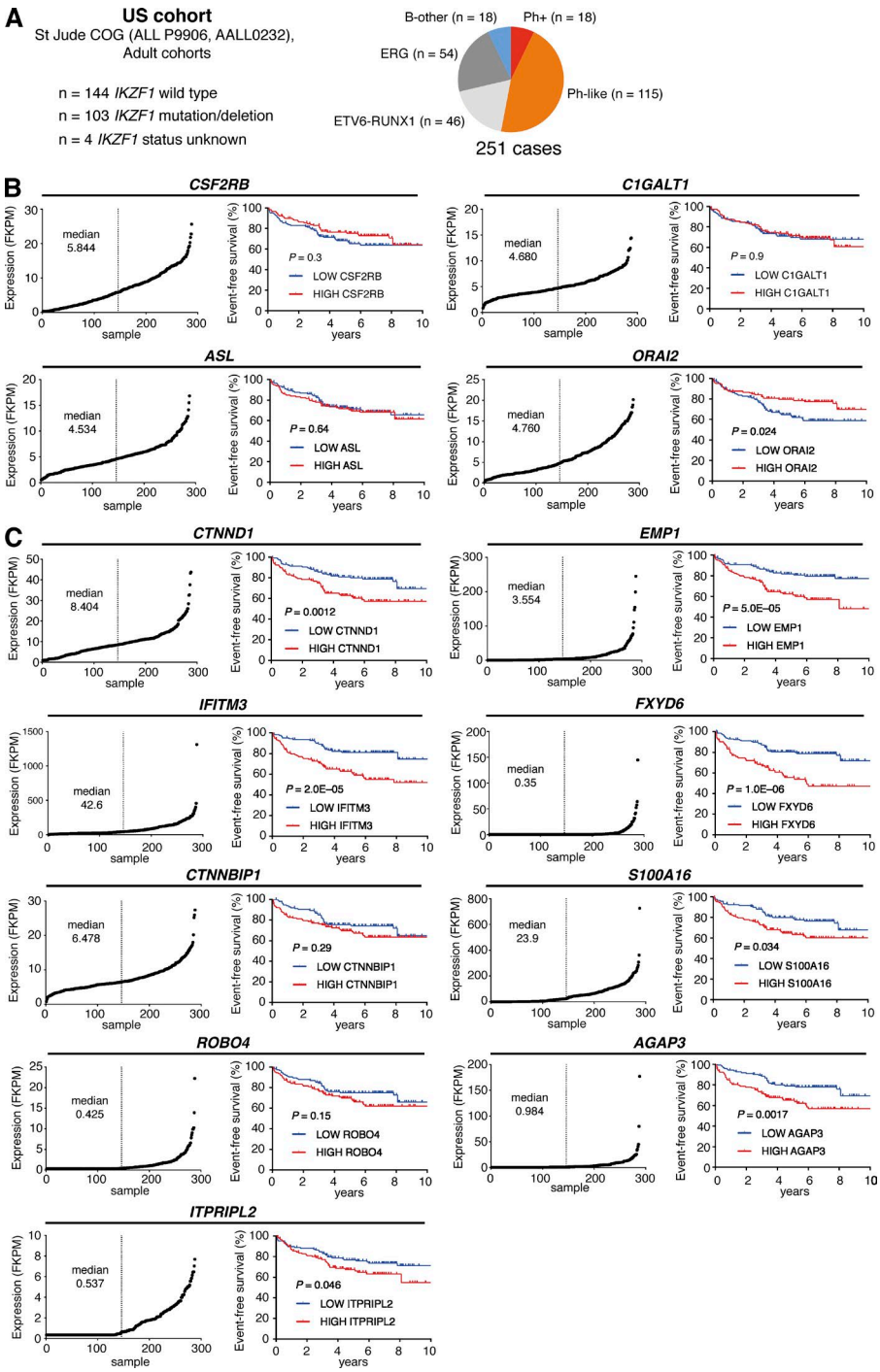

Figure S3. **Event-free survival association with expression of conserved IKAROS-repressed and IKAROS-activated genes in the US patient cohort.** (A) B-ALL subtype composition of the US patient cohort. (B, left) Conserved IKAROS-activated RNA-seq gene expression across all 289 samples in the cohort, with the median indicated. (right) 10-yr EFS in patients with LOW (< median) or HIGH (> median) gene expression. Log-rank P-values are indicated. (C) As in B, but for conserved IKAROS-repressed genes.

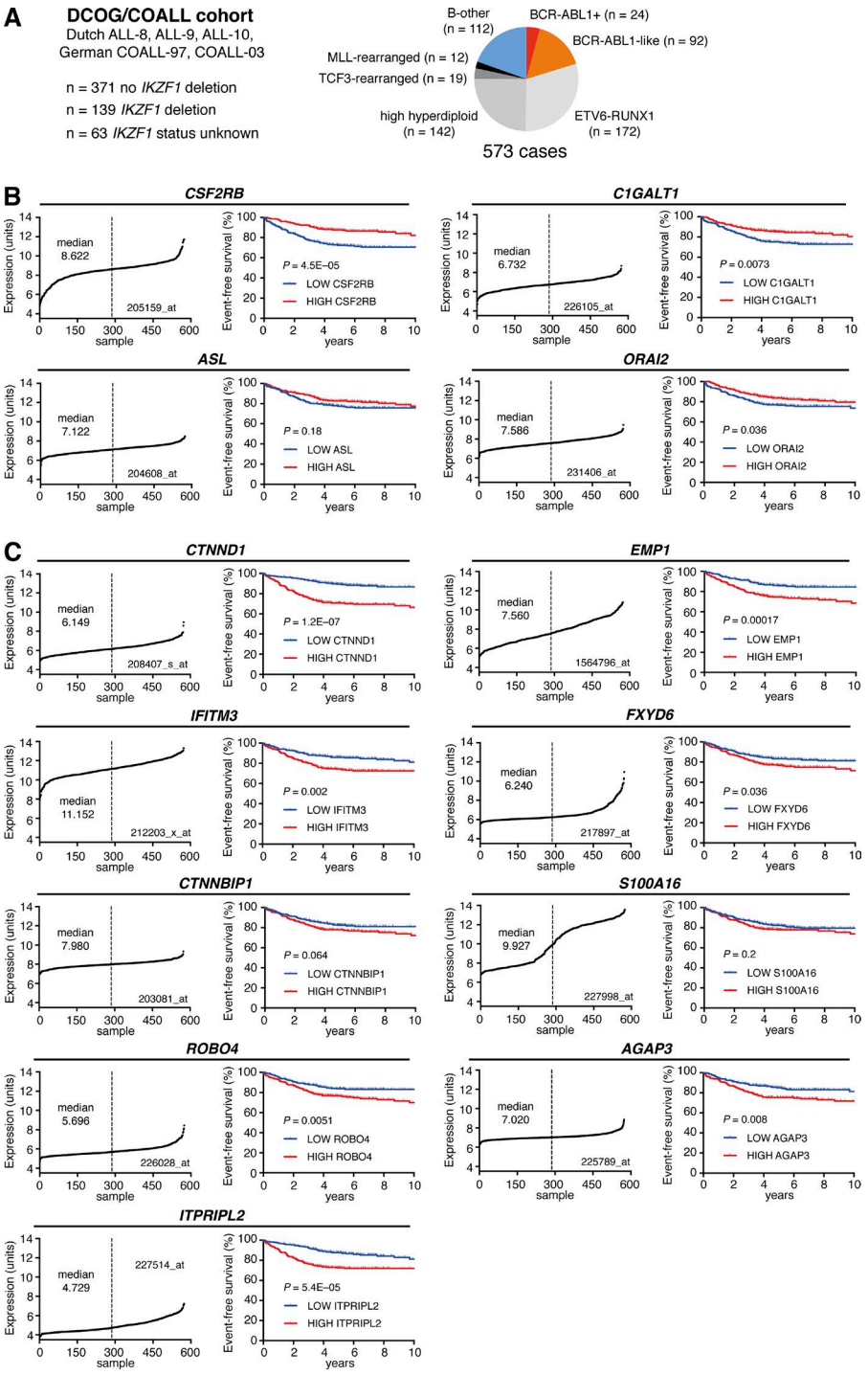

Figure S4. **Event-free survival association with expression of conserved IKAROS-repressed and IKAROS-activated genes in the DCOG/COALL patient cohort.** (A) B-ALL subtype composition of the DCOG/COALL patient cohort. (B, left) Conserved IKAROS-activated gene expression (microarray probeset indicated) across all 573 samples in the cohort, with the median indicated. (right) 10-yr EFS in patients with LOW (< median) or HIGH (> median) gene expression. Log-rank P-values are indicated. (C) As in B, but for conserved IKAROS-repressed genes.

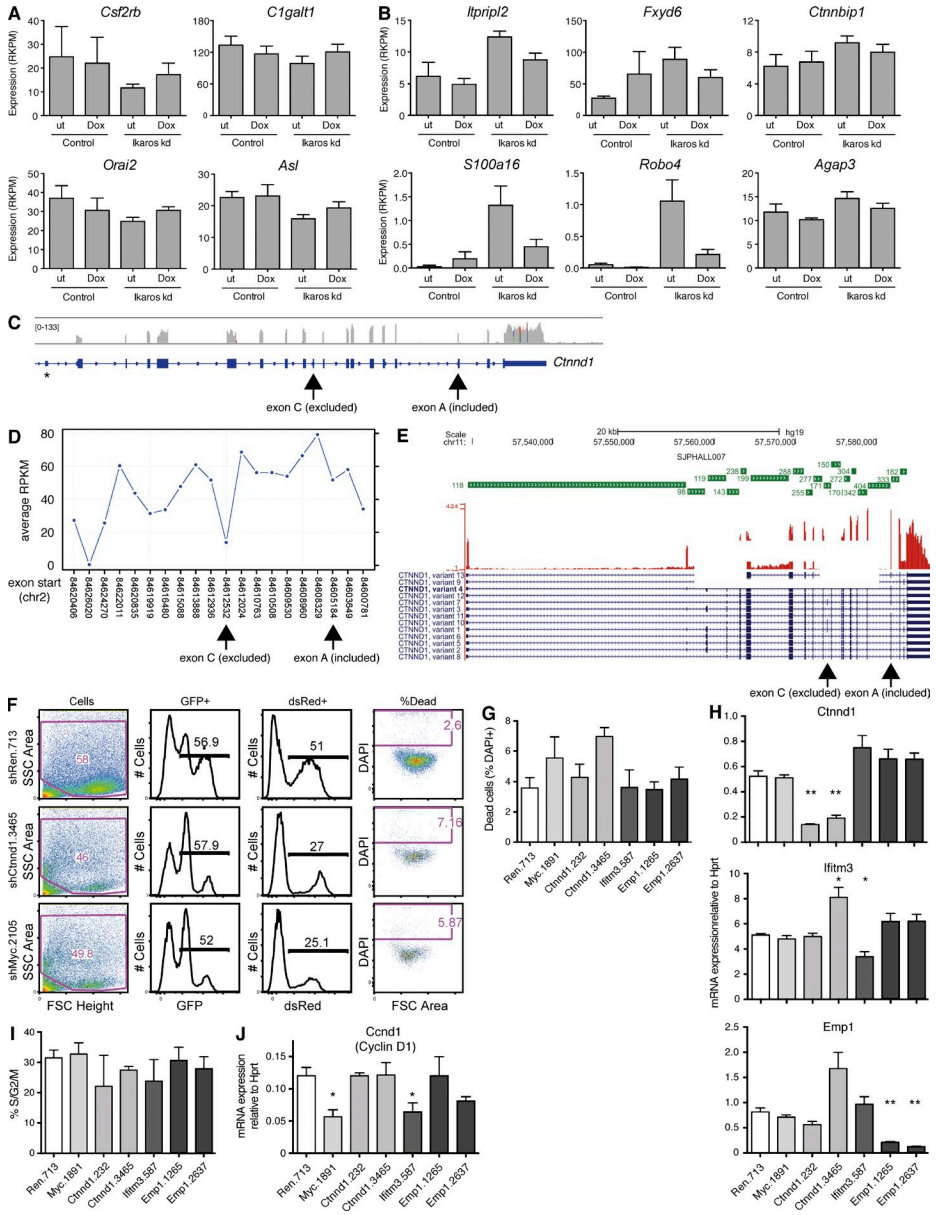

Figure S5. **Expression of conserved IKAROS-regulated genes in murine B-ALL, RNA-seq analysis of Ctnnd1/CTNND1 mRNA isoform expression in mouse and human BCR-ABL1$^+$ B-ALL, and characterization of cultured BCR-ABL1$^+$ B-ALL cells upon knockdown of IKAROS-repressed genes.** (A and B) RNA-seq expression (RPKM) of the four conserved Ikaros-activated genes (A) and six of the nine conserved Ikaros-repressed genes (B) listed in Fig. 3 (D and E). Data for the remaining Ikaros-repressed genes *Ctnnd1*, *Emp1*, and *Ifitm3* are shown in Fig. 5 (A–C). (C) RNA-seq track showing Ctnnd1 expression in B-ALL cells isolated from a representative mouse transplanted with Ikaros-kd leukemia B035. Exons A and C are indicated on the gene model. Coding exons are shown (exon 1 is upstream, untranslated, and not depicted). The asterisk indicates an excluded untranslated exon. (D) Exon level expression analysis (RPKM) showing average counts from the Ikaros-kd leukemias B031 and B035, with exon start positions (chr2) indicated. The asterisk indicates the excluded untranslated exon also marked in C. (E) RNA-seq track showing CTNND1 expression in three representative human *IKZF1* altered B-ALL cases. Exons A and C are indicated on the gene model. The main isoform expressed in all cases is NM_001085460.1 (transcript variant 4) encoding CTNND1 isoform 1A. The numbers in the green isoform schematic refer to the number of reads spanning the indicated intron. (F) Flow cytometry analysis of a culture-adapted Ikaros-kd BCR-ABL1$^+$ B-ALL cell line infected with TRMPV-shRNAs as shown in Fig. 6 F, after 3 d of Dox treatment to induce dsRed-linked shRNA expression. The gating strategy for assessing the proportion of dead (DAPI$^+$) cells within the GFP$^+$dsRed$^+$ gate is shown. (G) Percentage of dead (DAPI$^+$) cells in the GFP$^+$dsRed$^+$ gate after 3 d of Dox treatment. Mean ± SEM, $n = 5$ independent experiments. *, $P < 0.05$; **, $P < 0.01$ relative to Ren.713, unpaired Student's *t* test. (H) RT-qPCR expression of Ctnnd1 (left), Ifitm3 (middle), and Emp1 (right) in sorted GFP$^+$dsRed$^+$ B-ALL cells after 10 d of Dox treatment. Mean ± SEM. $n = 3$. *, $P < 0.05$; **, $P < 0.01$ relative to Ren.713, unpaired Student's *t* test. (I) Proportion of proliferating GFP$^+$dsRed$^+$ B-ALL cells (in S/G2/M phases of the cell cycle as determined by DAPI staining) after 10 d of Dox treatment. Mean ± SEM. $n = 3$. (J) RT-qPCR expression of Ccnd1 (Cyclin D1) in sorted GFP$^+$dsRed$^+$ B-ALL cells after 10 d of Dox treatment. Mean ± SEM, $n = 3$. *, $P < 0.05$, relative to Ren.713, unpaired Student's *t* test.

Tables S1–S3 list murine B-ALL Acceleration, Restoration, and Ikaros-regulated genes. Tables S4 and S5 include gene ontology and KEGG analysis. Tables S6 and S7 list human B-ALL IKAROS-regulated genes. Table S8 lists IKAROS-regulated genes common to both human B-ALL cohorts. Tables S1–S8 are available as Excel Files.