Supplementary data for Martín-Lorenzo et al.

Contents:

Supplementary Table 1: Conventional Facility Health Monitoring Report. The table shows the pathogens tested to monitor the health status of the animals housed in the conventional facility during the time the animals have been studied.

Supplementary Table 2: Differentially expressed genes in total bone marrow from pB-ALL bearing Pax5+/- mice compared to wild-type B220+ bone marrow B cells.

Supplementary Table 3: Pax5 target genes differentially express in p-BALL bearing Pax5+/- mice according to Delogu, A. et al. 2006; Schebesta, A. et al. 2007 and Revilla-i-Domingo, R. et al. 2012.

Supplementary Table 4: Total numbers and percentage of hematopoietic subsets in young Pax5+/- and wild type mice.

Supplementary Table 5: Somatic SNVs detected by whole exome analysis in the three index mice.

Supplementary Figure S1. Health report of three murine Pax5+/- pB-ALL. Health report of Pax5+/- mice held at the SPF – and conventional animal facility. Indicated are pathogens to which the mice were exposed when transferred to the conventional animal facility. Murine Norovirus (MNV), Murine hepatitis Virus (MHV).
Supplementary Figure S2: Flow cytometric, histological and gene set enrichment characterization in *Pax5*+/- mice.

(A) Representative plots show accumulation of blast B cells in *Pax5*+/- mice (n=9; age: 6-16 months) compared to control littermate wild-type mice age-matched (n=4, age: 8-16 months). (B) Representative plots show accumulation of blast B cells in *Pax5*+/- mice (n=9; age: 6-16 months) compared to control littermate wild-type mice age-matched (n=4, age: 8-16 months). (C) Haematoxylin and eosin staining of WT mice and tumour-bearing *Pax5*+/- mice showing infiltrating blast cells in spleen, liver, kidney, lymph node, bone marrow and lung. Loss of normal architecture resulting with cells morphologically resembling lymphoblasts can be shown (n=9). Scale bar represents 500 µm (=100X) for large panels and 100 µm (=400X) for inset. (D) Gene set enrichment analysis of leukemic mice. GSEA identified significant enrichment in human B-ALL gene sets (extracted from (18, 19)) in *Pax5*+/- tumor-bearing bone marrows (derived from O361, W634 and S748 -CD19+ phenotype-; S665, O388 and W362 -CD19- phenotype-) compared to B220⁺ bone marrow B cells from WT mice (GSEA FDR = 0.000 and FDR = 0.000). GSEA also shows significant enrichment of the normal proB cell signature (20, 21) (GSEA up genes FDR = 0.000, down genes FDR=0,000). (E) Reduced transcriptional activity of *Pax5*+/- in pB-ALL tumors. CD19 expression is lost in tumor cells of 55.6% *Pax5*+/- mice. GSEA identified significant enrichment in “downregulated genes upon Pax5 restoration” (extracted from (17)) in *Pax5*+/- tumour-bearing bone marrows (derived from O361, W634 and S748 (CD19+ phenotype); S665, O388 and W362 (CD19- phenotype) mice) compared with B220⁺ bone marrow.
B cells from WT mice. Also, it was identified significant negative enrichment in “upregulated genes upon Pax5 restoration” (extracted from (17)) in Pax5+/- tumour-bearing bone marrows compared with B220⁺ bone marrow B cells from WT mice. This shows an inverse correlation. GSEA identified significant enrichment in “downregulated genes by Pax5 gene set (extracted from (45)) in Pax5+/- tumour-bearing bone marrows (derived from O361, W634 and S748 (CD19+ phenotype); S665, O388 and W362 (CD19- phenotype) mice) compared with B220⁺ bone marrow B cells from WT mice. Also, GSEA identified significant negative enrichment in upregulated genes by Pax5 in pro-B cells (Schebesta_Pax5 gene set; extracted from (46)) in Pax5+/- tumour-bearing bone marrows compared with B220⁺ bone marrow B cells from WT mice. This shows an inverse correlation. GSEA identified significant negative enrichment in pro-B cell genes activated by Pax5 (pro-B_activated gene set; extracted from (47)) in Pax5+/- tumour-bearing bone marrows (derived from O361, W634 and S748 (CD19+ phenotype); S665, O388 and W362 (CD19- phenotype) mice) compared with B220⁺ bone marrow B cells from WT mice. Also, it was identified significant enrichment in pro-B cell genes repressed by Pax5 (pro-B_repressed gene set, extracted from (8)) in Pax5+/- tumour-bearing bone marrows compared with B220⁺ bone marrow B cells from WT mice. This shows an inverse correlation.

**Supplementary Figure S3: In vivo growth of Ba/F3 cells expressing Jak3V⁶⁷⁰A and Jak3R⁶⁵³H mutations, respectively,**

(A) Experimental set up. Nude mice were injected with 1,000,000 Ba/F3 cells harboring Jak3V⁶⁷⁰A (n=5) or Jak3R⁶⁵³H (n=4) mutations, respectively. Regular
bleedings were performed to monitor cell growth. (B) Example of splenomegaly observed in 100% (9/9) nu/nu mice injected with Ba/F3 (Jak3\textsuperscript{V670A}) cells expressing either Jak3\textsuperscript{V670A} or Jak3\textsuperscript{R653H}. A spleen from a control nu/nu mouse is shown for reference. (C) Representative flow cyometric analysis of mice injected with Ba/F3 cells harboring Jak3\textsuperscript{V670A} or Jak3\textsuperscript{R653H} mutations shows the accumulation of Ba/F3 cells (CD25\textsuperscript{*}B220\textsuperscript{weak}) in PB, BM and spleen. (D) PB-ALL is cured in mouse D009 after RUXOLITINIB treatment. 100,000 leukemic Pax5\textsuperscript{+/-} proB cells harboring Jak3\textsuperscript{V670A} mutation were injected into sublethaly irradiated WT syngeneic mice. Regular bleedings were performed in order to monitor the development of the PB-ALL. When PB-ALL cells (B220\textsuperscript{low}IgM\textsuperscript{-}) were detected in PB, the mouse treatment with RUXOLITINIB started. Mice were treated with RUXOLITINIB for only 5 days. FACS analysis of the peripheral blood was used to verify disease remission after therapy. Disseminated leukemia, documented by clinical criteria, and FACS invariably, ensued except in 1 out 5 mice (mouse D009) that was alive and healthy 33 days after discontinuation of treatment when the mouse was sacrificed and FACS analysis confirmed that blast cells were not present in the PB and BM.
### Supplementary Fig. S1

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**Key:**
- **POS**: Positive
- **NEG**: Negative
Supplementary Fig. S2B

LYMPH NODES

Wild type

Pax5\textsuperscript{+/-} (O361)

Pax5\textsuperscript{+/-} (O388)

CD4

B220

CD19

Gr1

Mac1

FSC-A

SSC-A
Supplementary Fig. S2C
pB-ALL tumor samples from *Pax5*+/- mice maintain progenitor signature
Supplementary Fig. S2E

<table>
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<th>Tumor cells phenotype</th>
<th>pB-ALL bearing Pax5+-/ mice (n=9)</th>
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<tr>
<td>CD19+</td>
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<tr>
<td>CD19-</td>
<td>5/9 (55.6%)</td>
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</table>

- **Liu et al., 2014**
  - Enrichment plot: LIUPAXSRESTORATIONDOWN
  - FDR = 0.000

- **Delogu et al., 2006**
  - Enrichment plot: DELGU_IMMUNITY_2005_DOWNBPAX5_MM2HS
  - FDR = 0.000

- **Schebesta et al., 2007**
  - Enrichment plot: SCHEBESTA_PAX5
  - FDR = 0.000

- **Revilla-I-Domingo et al., 2012**
  - Enrichment plot: PROB_ACTIVATED
  - FDR = 0.000

  - Enrichment plot: PROB_REPRESSED
  - FDR = 0.000
Supplementary Fig. S3

A

Day 0
Ba/F3 + (Jak3 V670A) or Ba/F3 + (Jak3 R653H) cells injection into nu/nu mice
Regular bleedings

B

Nu/Nu + Ba/F3+(Jak3 V670A)

C

Peripheral blood

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<th>14 days after cell injection</th>
<th>24 days after cell injection</th>
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Mouse D013
(Ba/F3 cells; Jak3 V670A)

Mouse D016
(Ba/F3 cells; Jak3 R653H)

Bone Marrow

Spleen

Day 0
Mouse D013

Regular bleedings

Supplementary Fig. S3
Supplementary Fig. S3D

Mouse D009

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