#### Supplementary data for Martin-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 agematched (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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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+/- tumourbearing bone marrows compared with B220<sup>+</sup> 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 (CD19phenotype) mice) compared with B220<sup>+</sup> 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<sup>+</sup> bone marrow B cells from WT mice. This shows an inverse correlation.

Supplementary Figure S3: *In vivo* growth of Ba/F3 cells expressing Jak3V<sup>670A</sup> and Jak3<sup>R653H</sup> mutations, respectively,

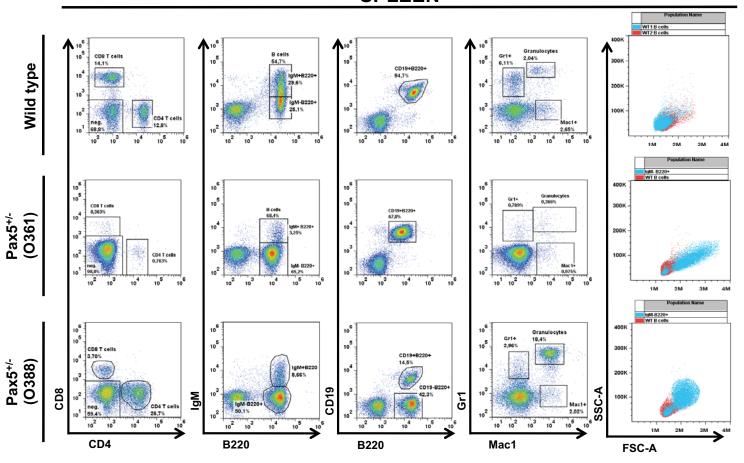
(A) Experimental set up. Nude mice were injected with 1,000,000 Ba/F3 cells harboring Jak3<sup>V670A</sup> (n=5) or Jak3<sup>R653H</sup> (n=4) mutations, respectively. Regular

bleedings were performed to monitor cell growth. (B) Example of splenomegaly observed in 100% (9/9) nu/nu mice injected with Ba/F3 (Jak3<sup>V670A</sup>) cells expressing either Jak3<sup>V670A</sup> or Jak3<sup>R653H</sup>. 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<sup>V670A</sup> or Jak3<sup>R653H</sup> mutations shows the accumulation of Ba/F3 cells (CD25<sup>+</sup>B220<sup>weak</sup>) in PB, BM and spleen. (D) pB-ALL is cured in mouse D009 after RUXOLITINIB treatment. 100,000 leukemic Pax5+/- proB cells harboring Jak3<sup>V670A</sup> 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<sup>low</sup>lgM<sup>-</sup>) 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.

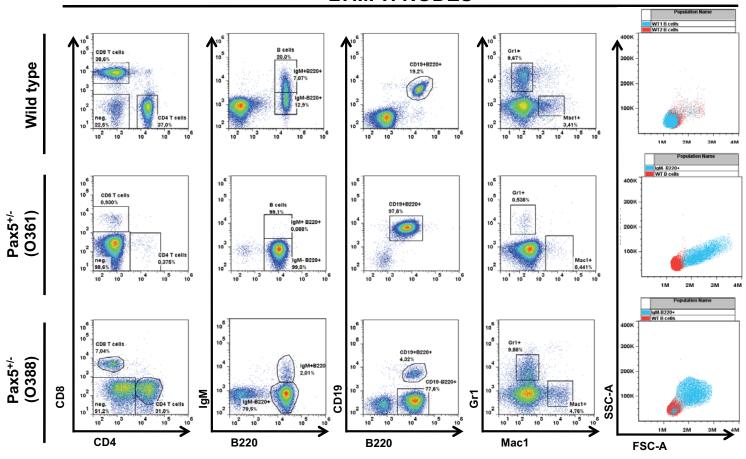
# Supplementary Fig. S1

	CONVENTIONAL FACILITY			SPF FACILITY
Timepoint	moved to conventional facility	1 year	2 year	
VIRUSES				
- MHV	POS	POS	POS	NEG
- MNV	POS	POS	POS	NEG
BACTERIA				
- Helicobacter spp	POS	POS	POS	NEG
PARASITES				
- Trichomonas muris	POS	POS	NEG	NEG

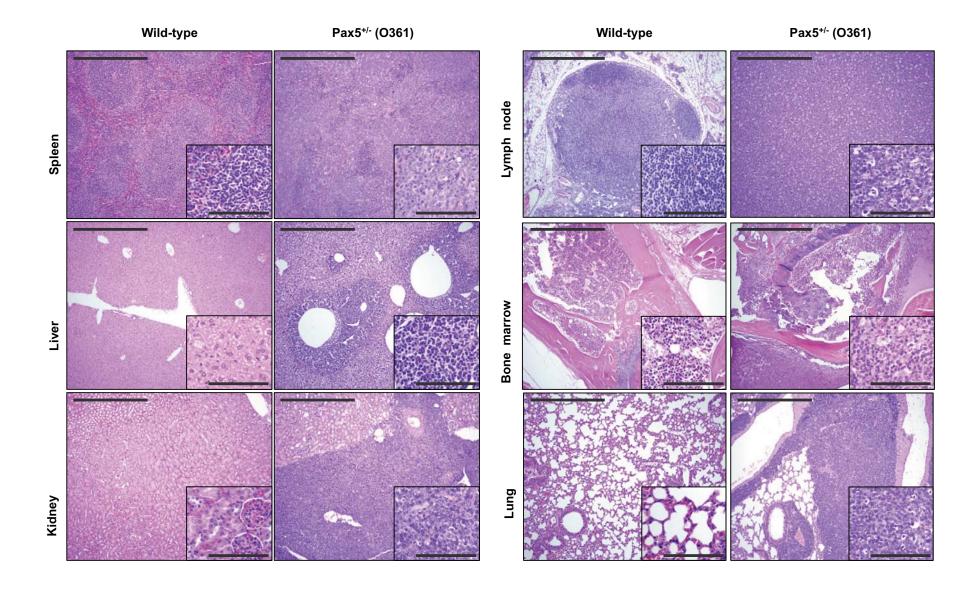
# **SPLEEN**



# LYMPH NODES

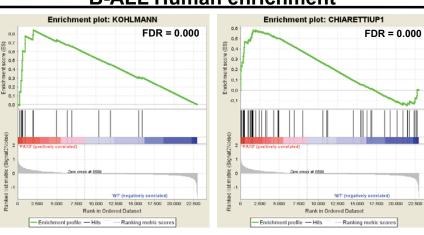


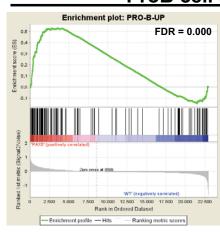
# Supplementary Fig. S2C

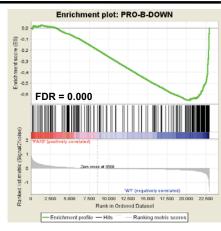


### **B-ALL Human enrichment**

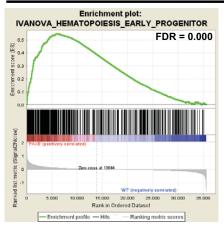
#### **ProB cell enrichment**

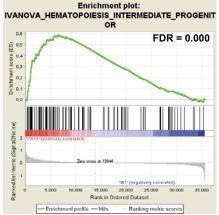


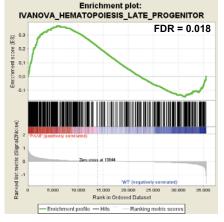


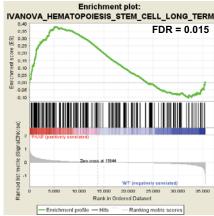


## pB-ALL tumor samples from Pax5+/- mice maintain progenitor signature









#### Supplementary Fig. S2E

Tumor cells phenotype	pB-ALL bearing Pax5+/- mice (n=9)
CD19+	4/9 (44,5%)
CD19-	5/9 (55,6%)

Liu et al., 2014

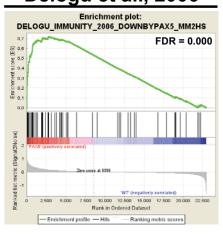
Enrichment plot: LIUPAX5RESTORATIONDOWN

FDR = 0.000

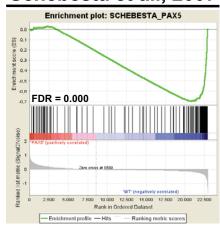
Substituting the plot: LiuPAX5RESTORATIONUP

Substituting

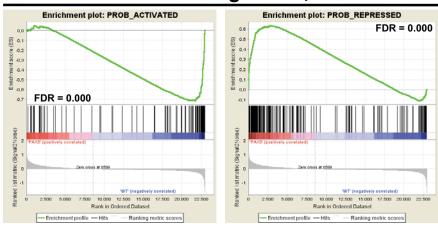
Delogu et al., 2006



Schebesta et al., 2007



Revilla-I-Domingo et al., 2012



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

