Supplementary data for Martín-Lorenzo et al.

Contents:

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 $Jak3V^{670A}$ and $Jak3^{R653H}$ mutations, respectively,

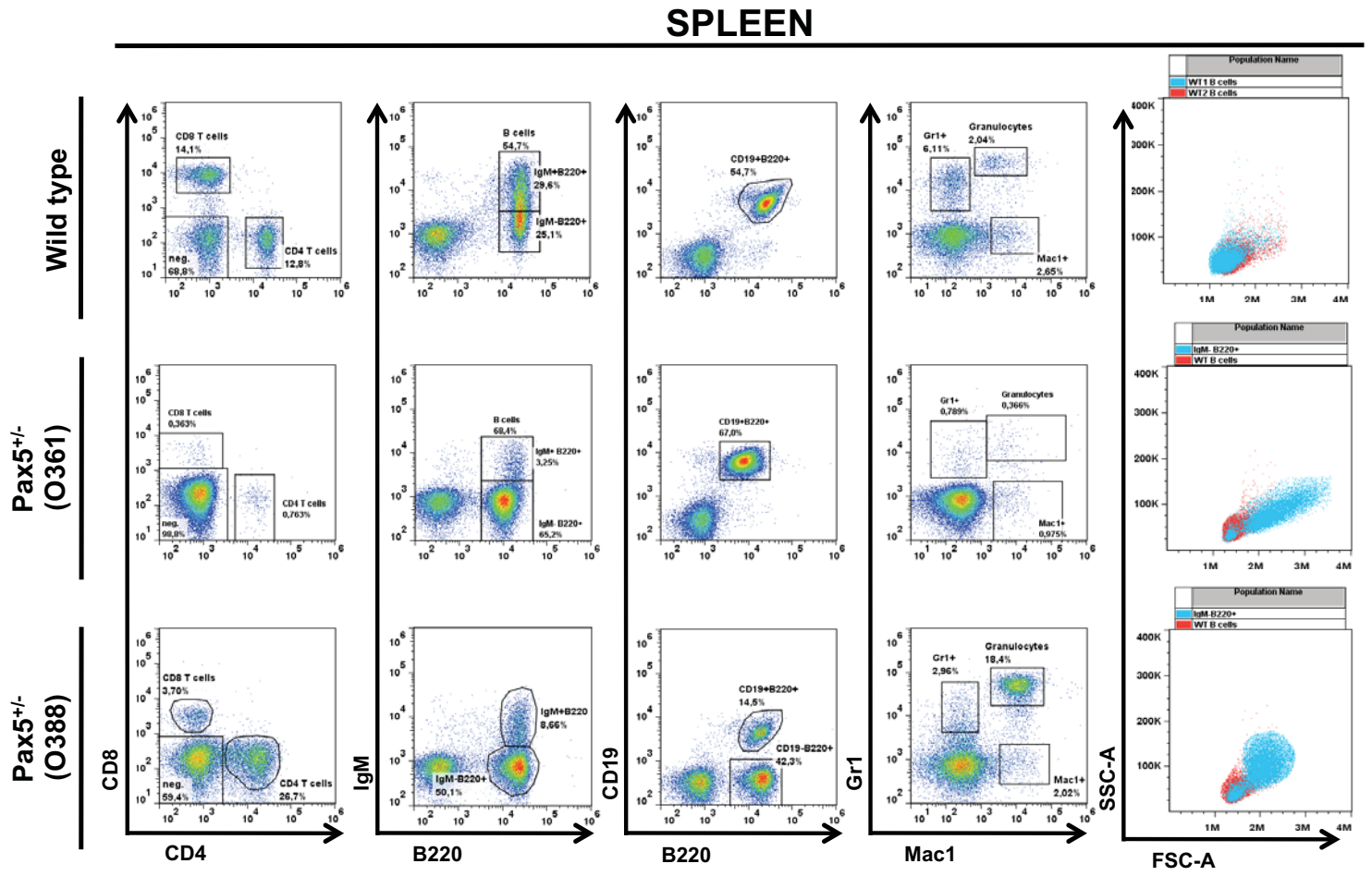
(A) Experimental set up. Nude mice were injected with 1,000,000 Ba/F3 cells harboring $Jak3^{V670A}$ (n=5) or $Jak3^{R653H}$ (n=4) mutations, respectively. Regular

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

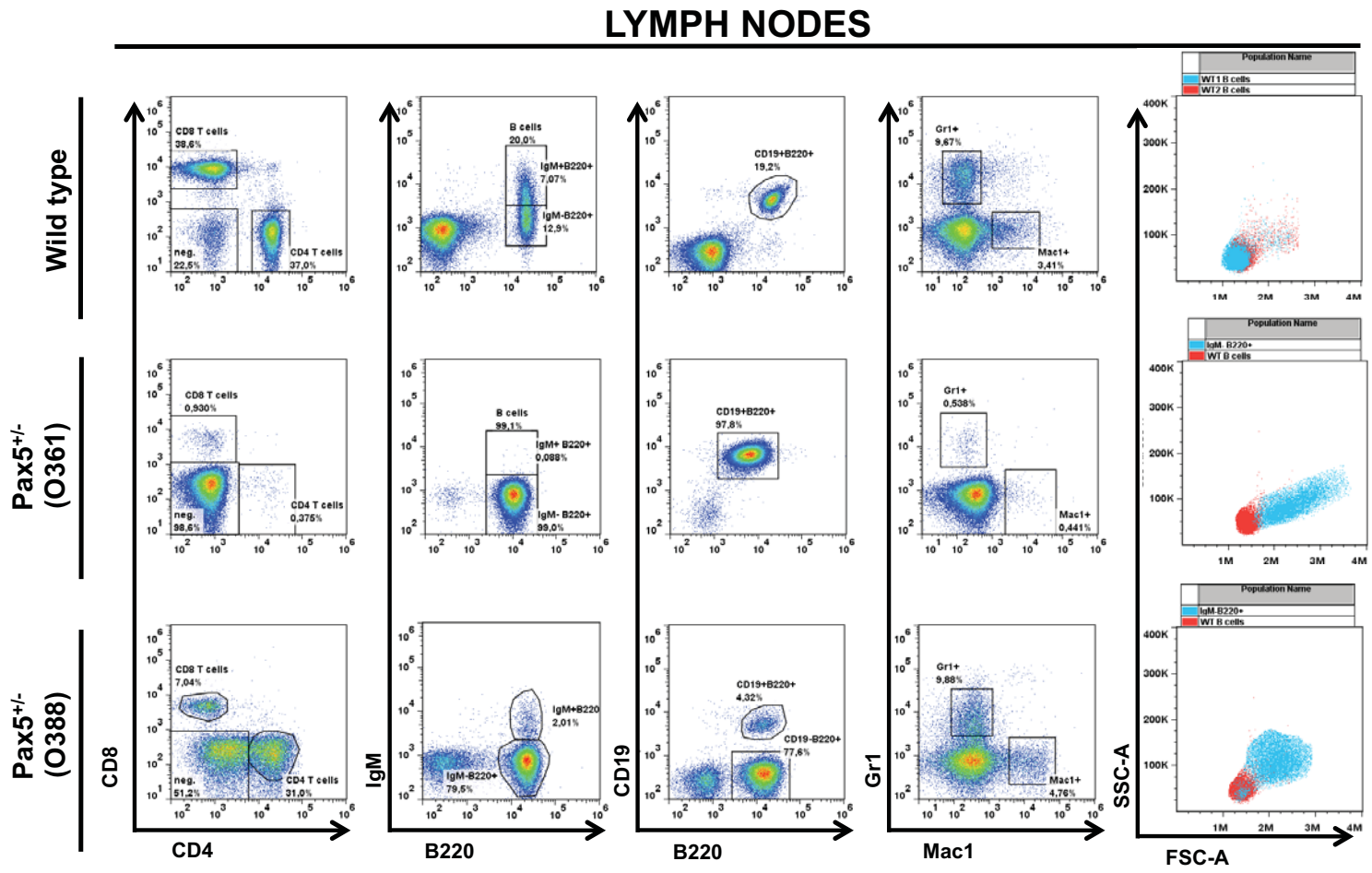
Supplementary Fig. S1

	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*

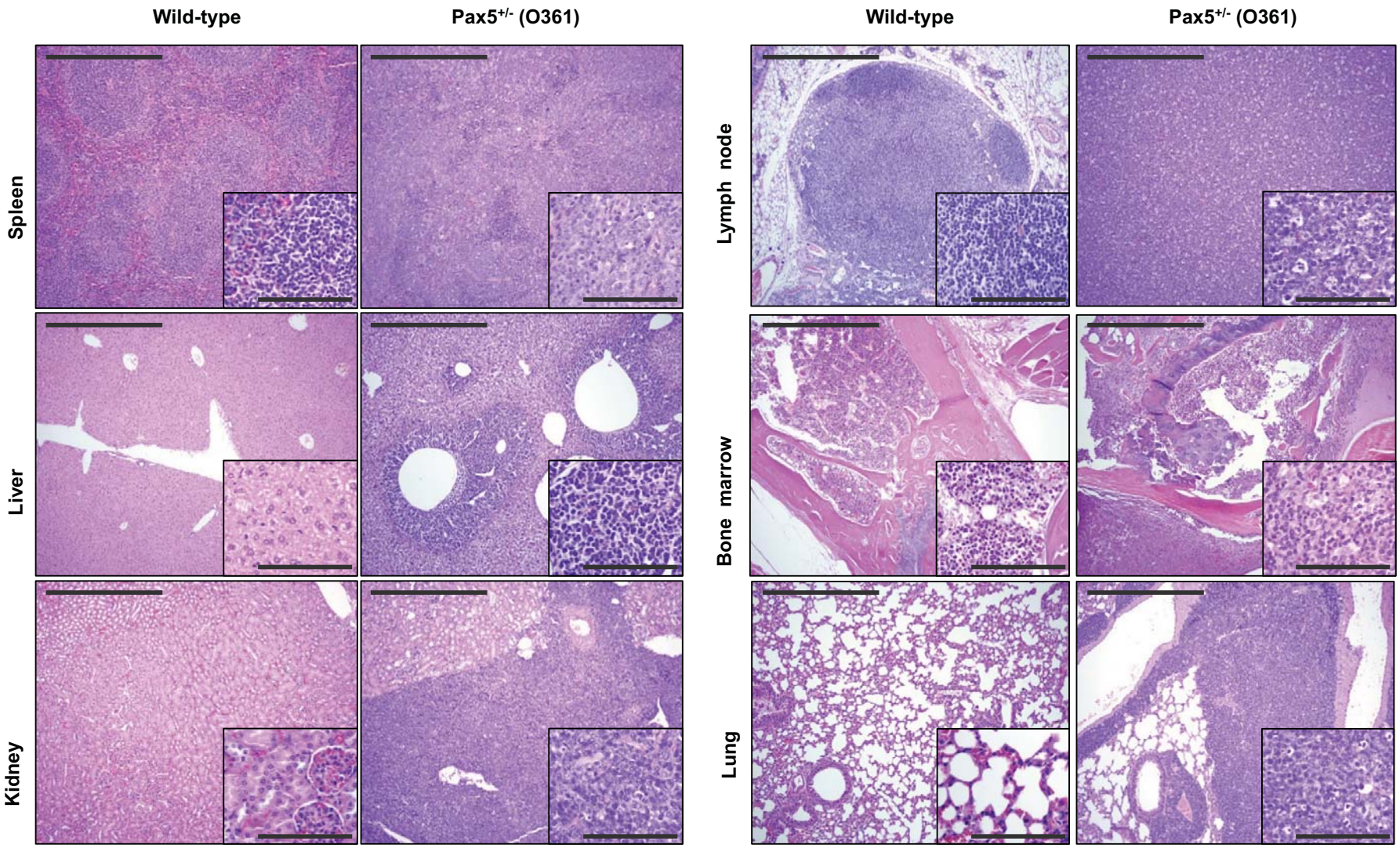
Supplementary Fig. S2A



Supplementary Fig. S2B

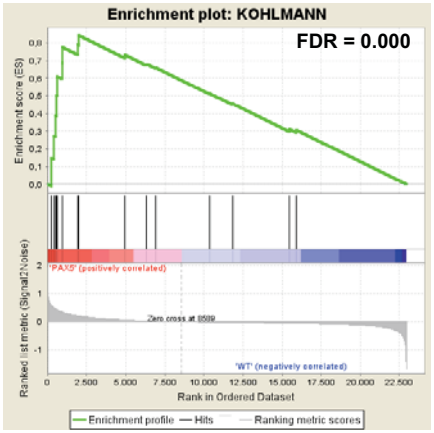


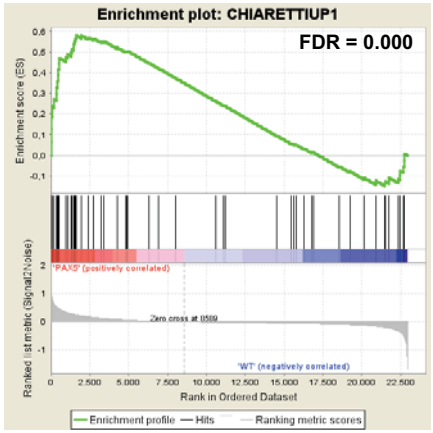
Supplementary Fig. S2C



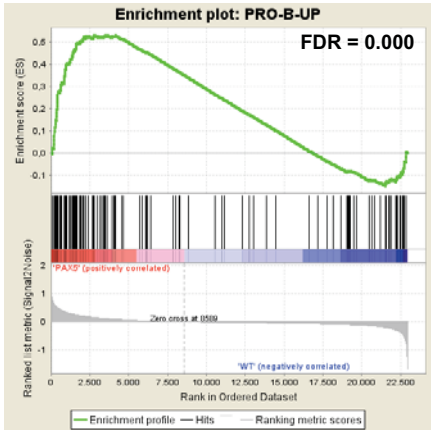
Supplementary Fig. S2D

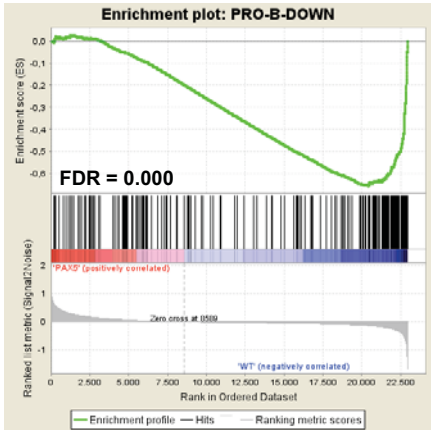
B-ALL Human enrichment



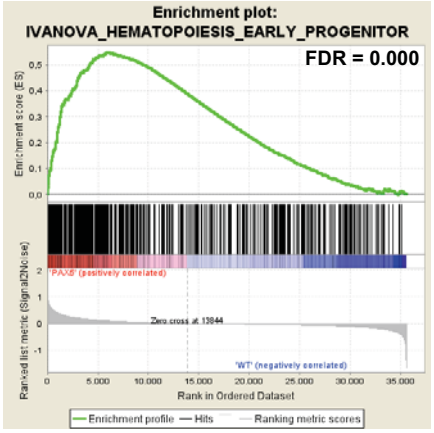


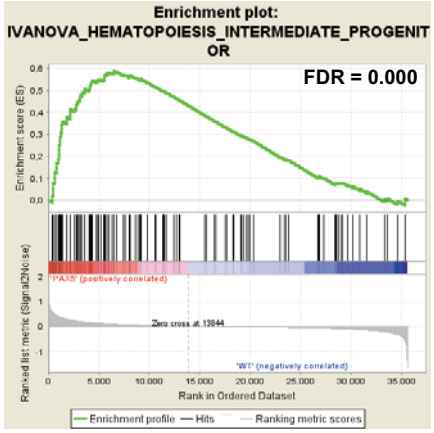
ProB cell enrichment

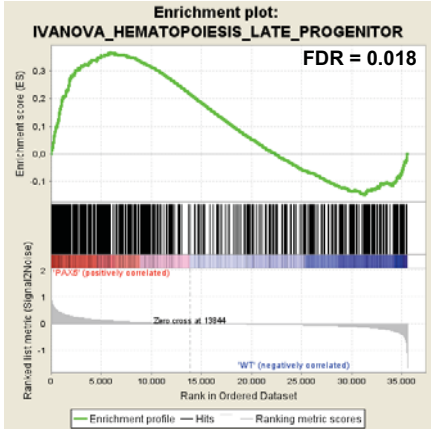


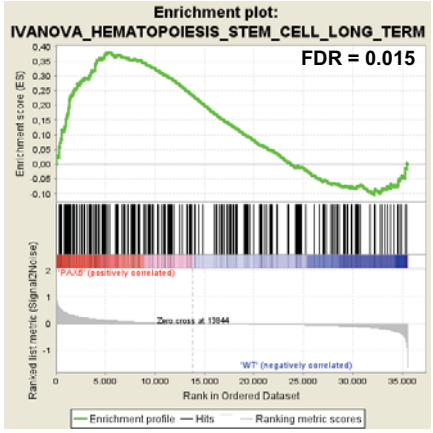


pB-ALL tumor samples from *Pax5*+/- mice maintain progenitor signature





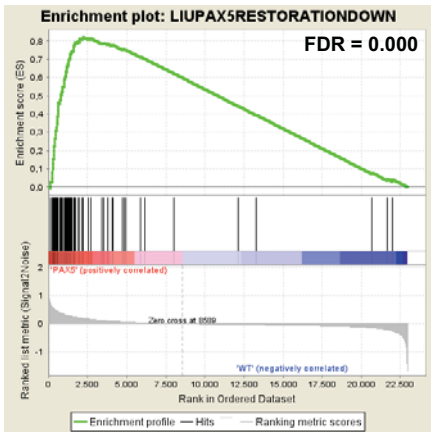


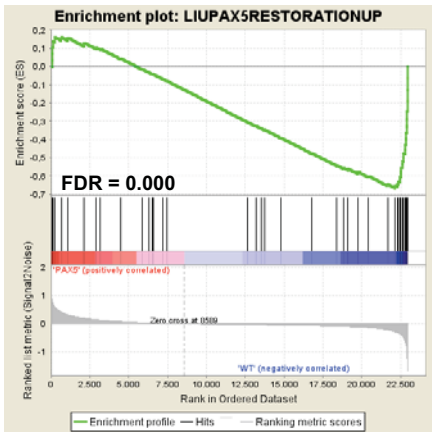


Supplementary Fig. S2E

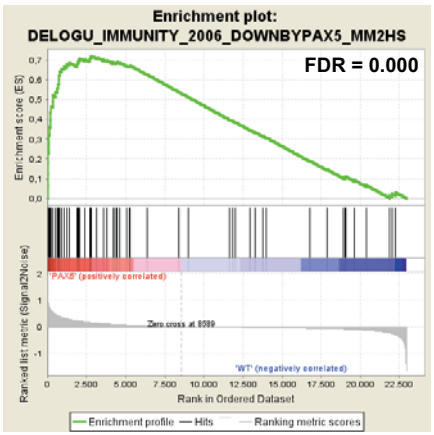
Tumor cells phenotype	pB-ALL bearing Pax5+/- mice (n=9)
CD19+	4/9 (44,5%)
CD19-	5/9 (55,6%)

Liu et al., 2014

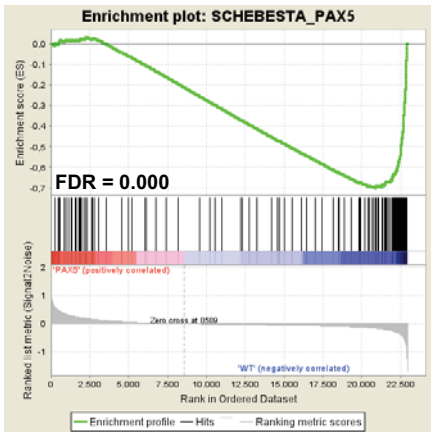




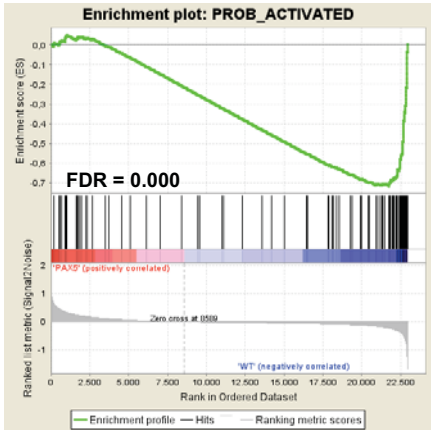
Delogu et al., 2006

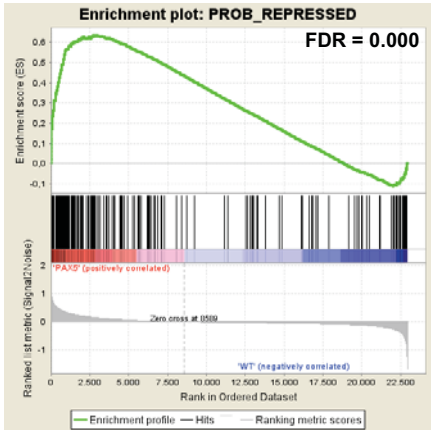


Schebesta et al., 2007



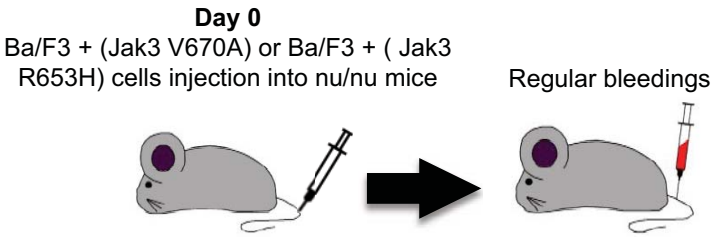
Revilla-I-Domingo et al., 2012



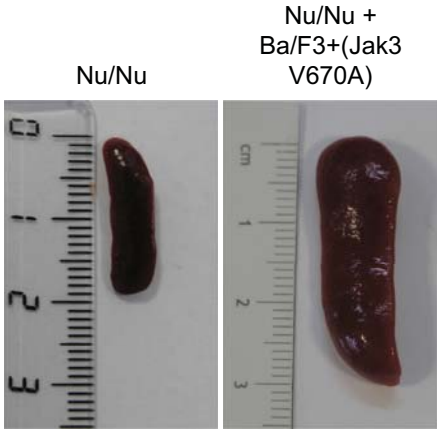


Supplementary Fig. S3

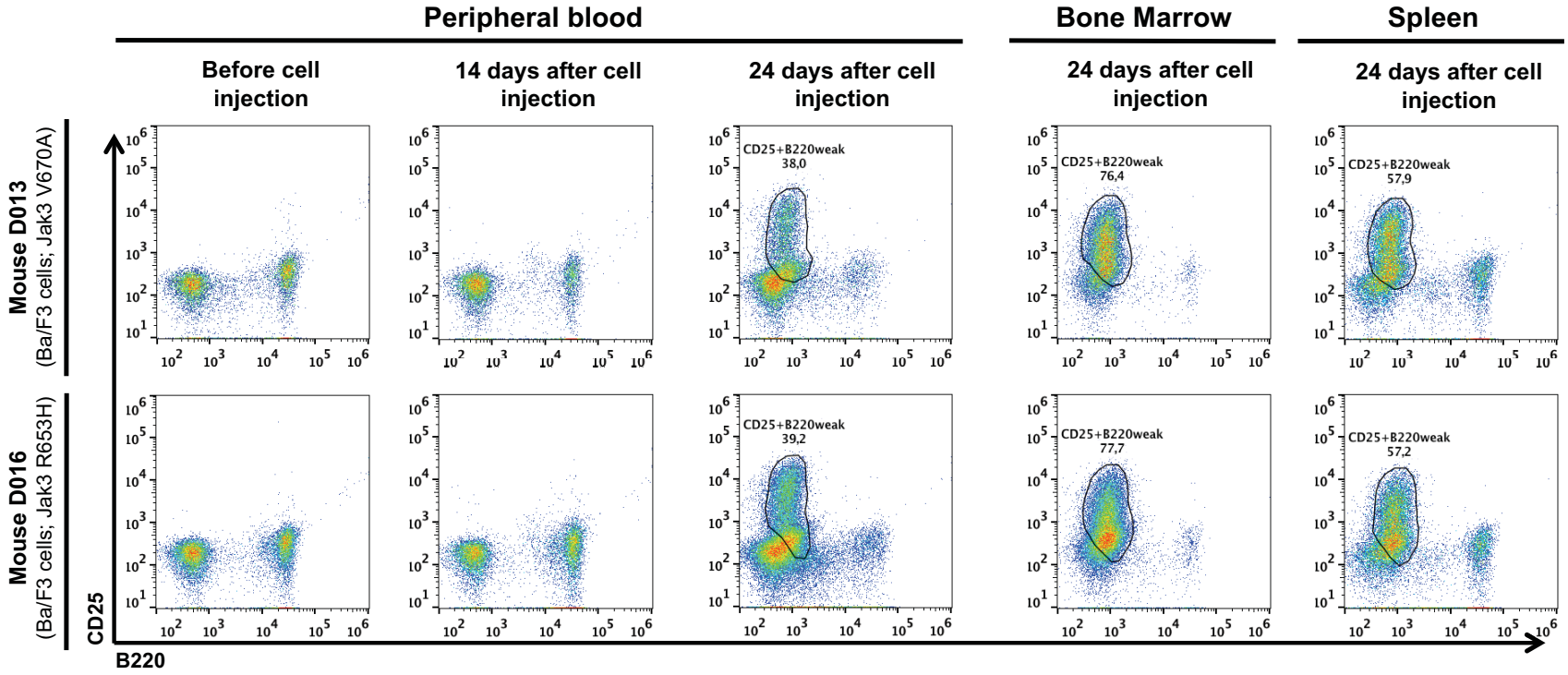
A



B



C



Supplementary Fig. S3D