

Supplemental material

Deletion 9p drives B-ALL through the heterozygous inactivation of Pax5 and Cd72 in pre-leukemic cells

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Supplemental data

Supplemental Tables

Supplemental Table 1: Differentially expressed genes in preleukemic *CD72^{+/-}; Pax5^{+/-}* versus preleukemic *Pax5^{+/-}* proB cells. 312 genes-probesets.

Supplemental Table 2: Differentially expressed genes in preleukemic *CD72^{+/-}; Pax5^{+/-}* versus WT proB cells. 255 genes-probesets.

Supplemental Table 3: Differentially expressed genes in preleukemic *Pax5^{+/-}* versus WT proB cells. 246 genes-probesets.

Supplemental Table 4. Genetic Background of the mice using the miniMUGA array

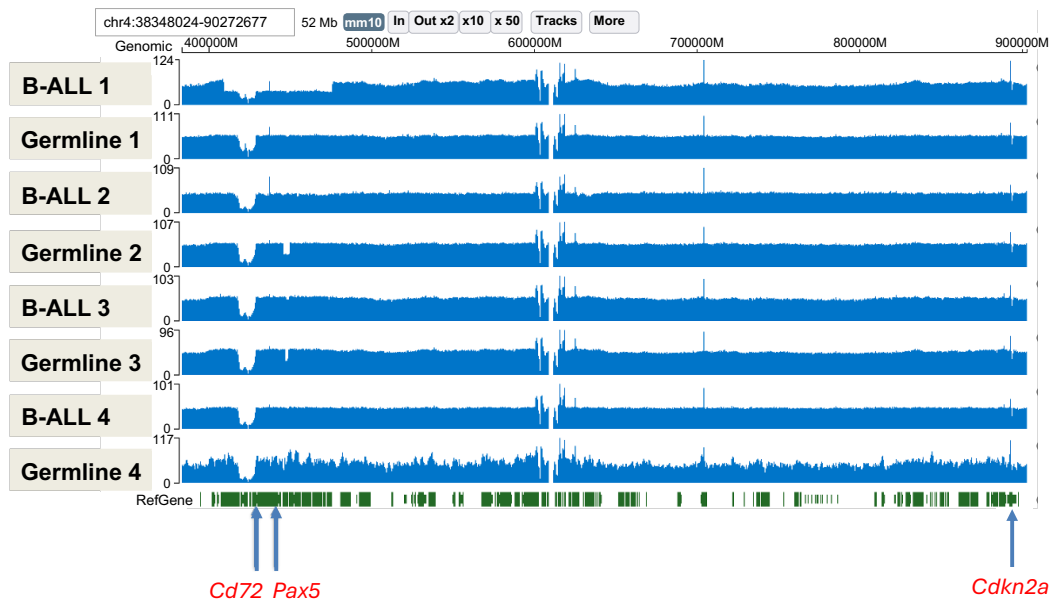
Supplemental Table 5. Cytokine serum levels of IL-6, IL-2, IL-4, IL-10, IL-17A, TNF α and IFN γ in *Cd72^{+/-};Pax5^{+/-}* and *Cd72^{+/-}* mice over time (3, 5, 7, 9, 11 and 13 months of age).

Supplemental zip file.

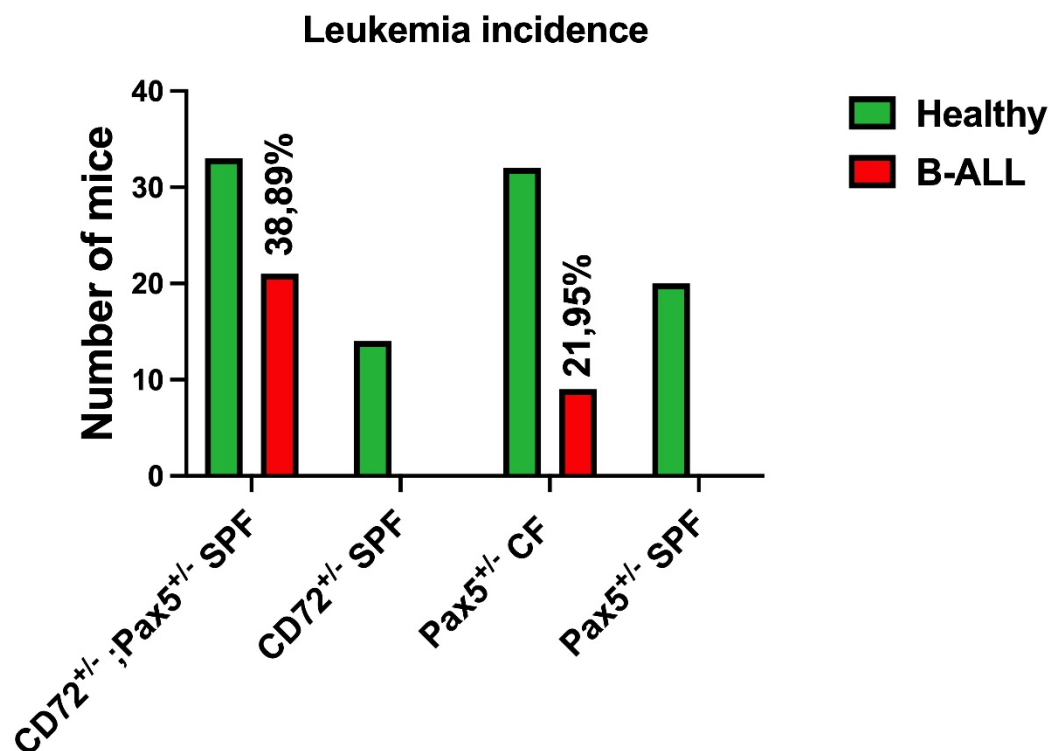
Supplemental Strain_Genotyping.zip. Details of the strain genotyping results diagnosed by SNPs, using the MiniMUGA Background Analysis v2.3.1, for the mice listed in Supplemental Table 4.

Supplemental Figures

Supplemental Figure 1

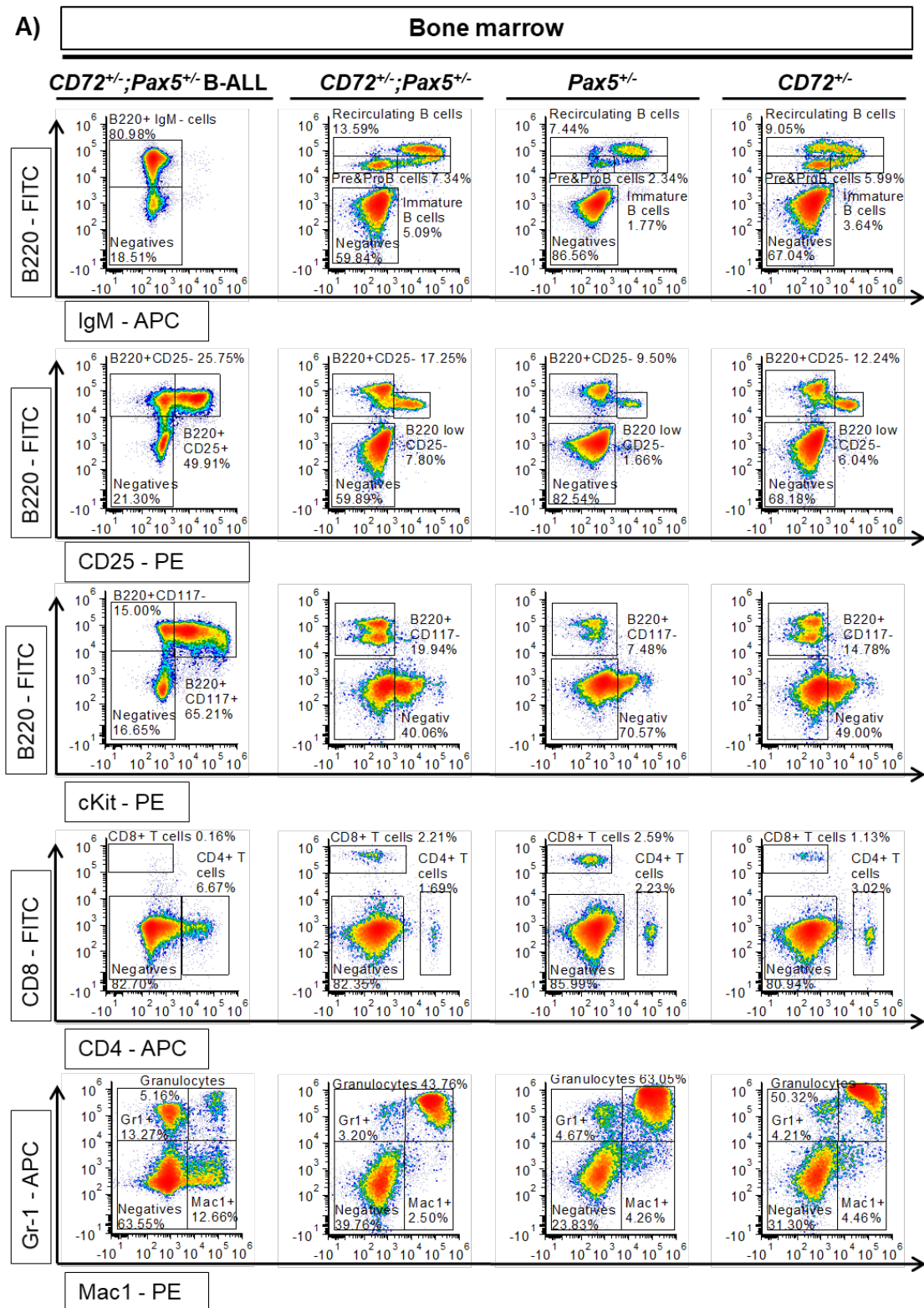


Supplemental Figure 1: Representative chromosomal 4 alterations affecting the *Pax5* gene in murine B-ALL showing DNA deletion encompassing the *Pax5* and *Cd72* loci. Genome-wide copy number data are shown for B-cell acute lymphoblastic leukemia (B-ALL) samples (top track) compared to matched germline DNA (bottom track), visualized using the UCSC Genome Browser (mm10 assembly). The genomic coordinates span chr4: 38348024-90272677 (52 Mb). The blue read-depth signal represents normalized copy number, with relative gains and losses detectable by comparison between tracks. Focal deletion is observed in the B-ALL sample around the *Pax5* locus. Additional deletions affecting *Cd72* and other genes are also annotated. These somatic copy number losses suggest a pathogenic role in leukemogenesis.



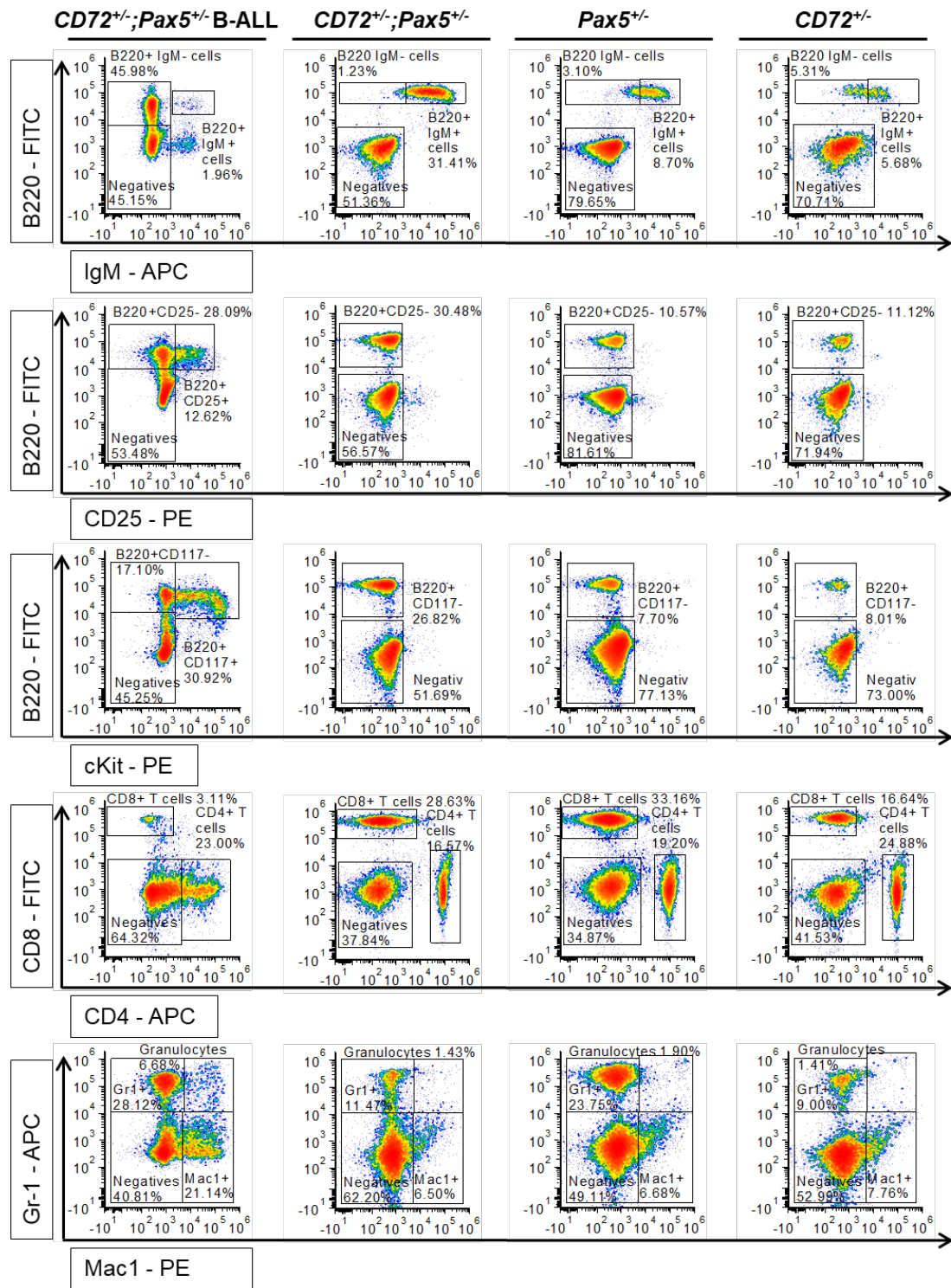
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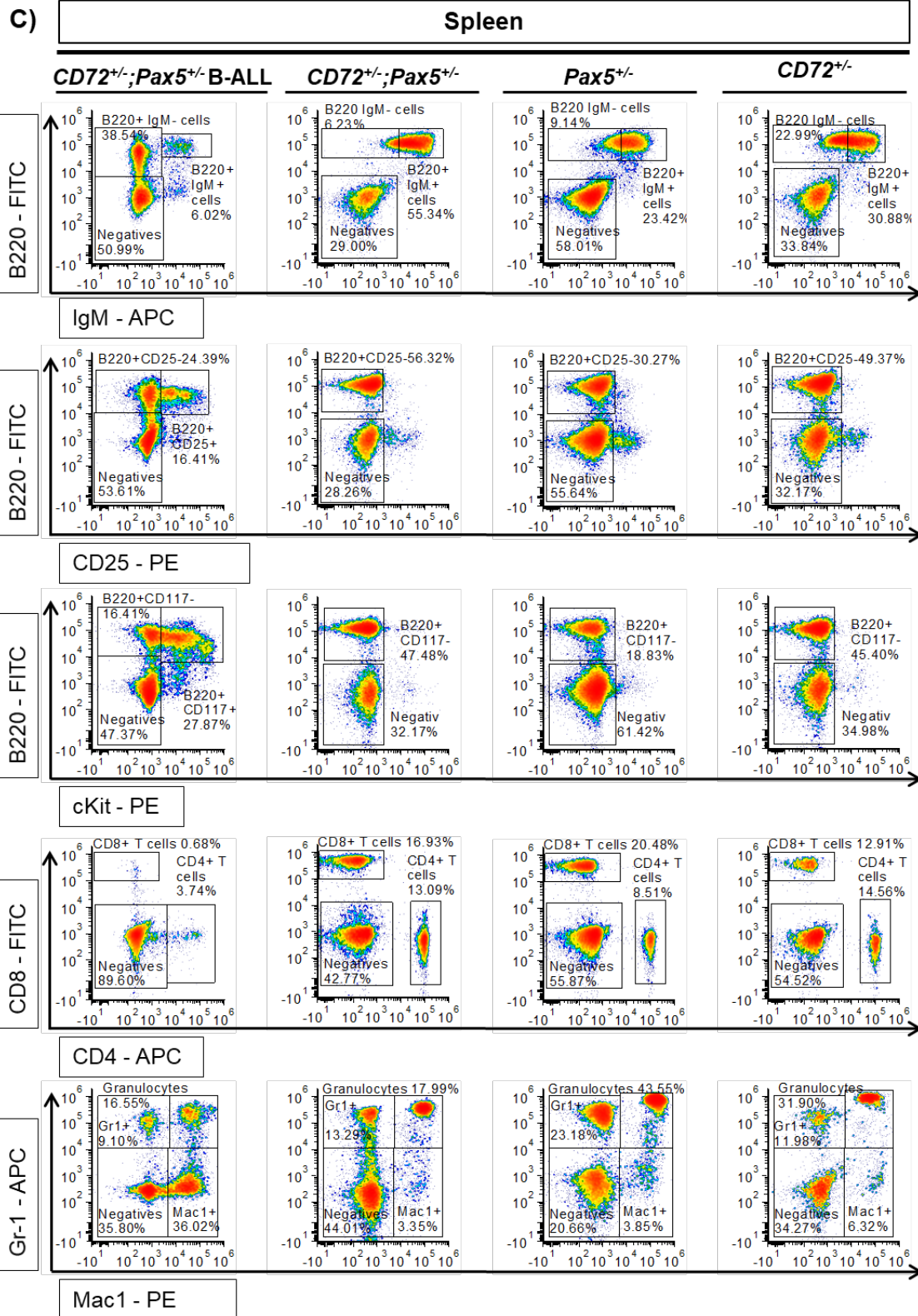
Supplemental Figure 2: $CD72^{+/-}; Pax5^{+/-}$ mice housed in SPF conditions developed leukemia. $CD72^{+/-}; Pax5^{+/-}$ mice (n= 54) that were not exposed to infections, develop B-ALL with an incidence of 38,89%, which is slightly higher than that of $Pax5^{+/-}$ mice (n= 41) when exposed to infection in a conventional facility (CF) (21,95%). None of the $CD72^{+/-}$ (n= 14) or $Pax5^{+/-}$ (n= 15) mice housed in SPF conditions developed the disease. (SPF: specific pathogen free; CF: conventional facility).



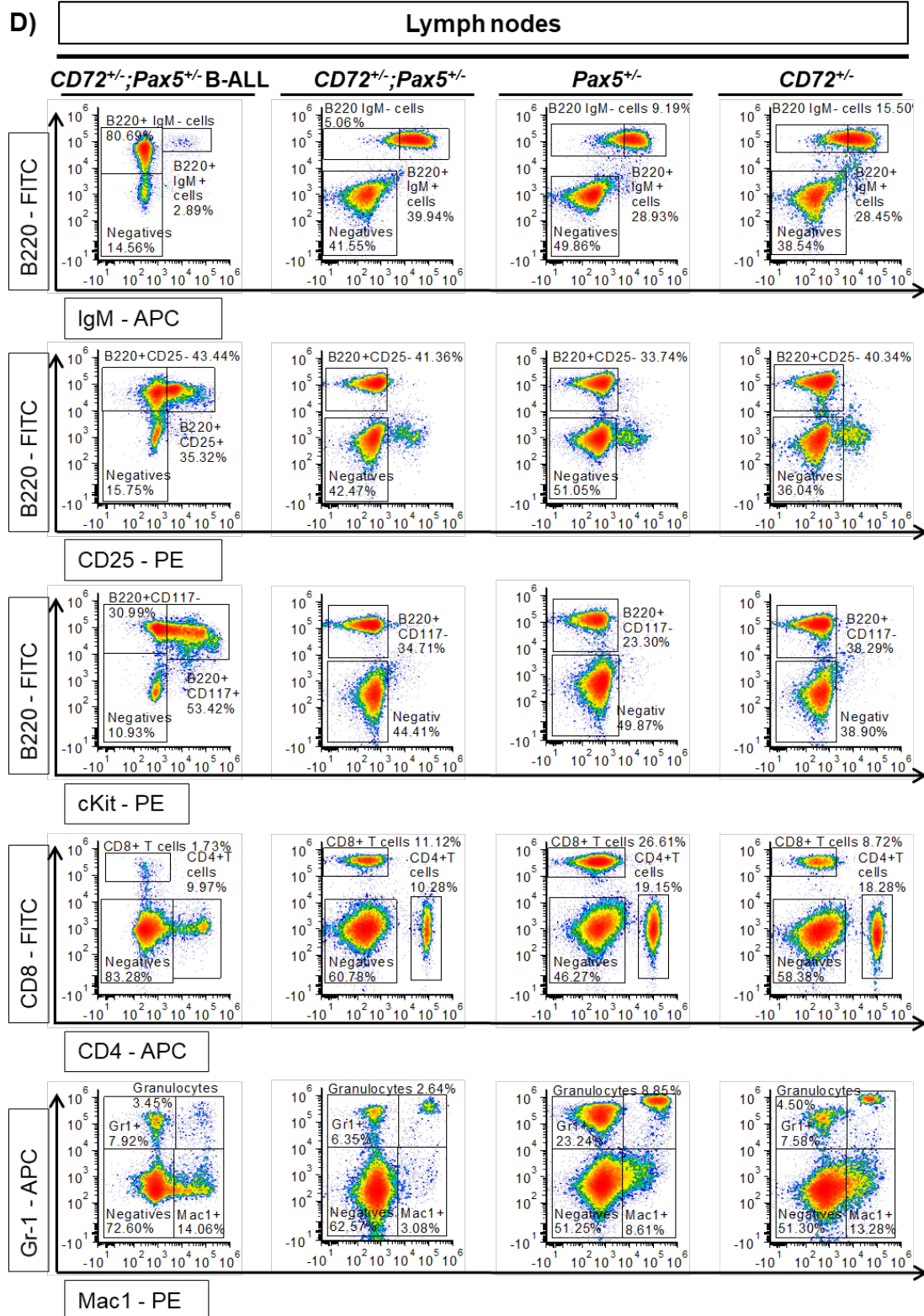
B)

Peripheral blood

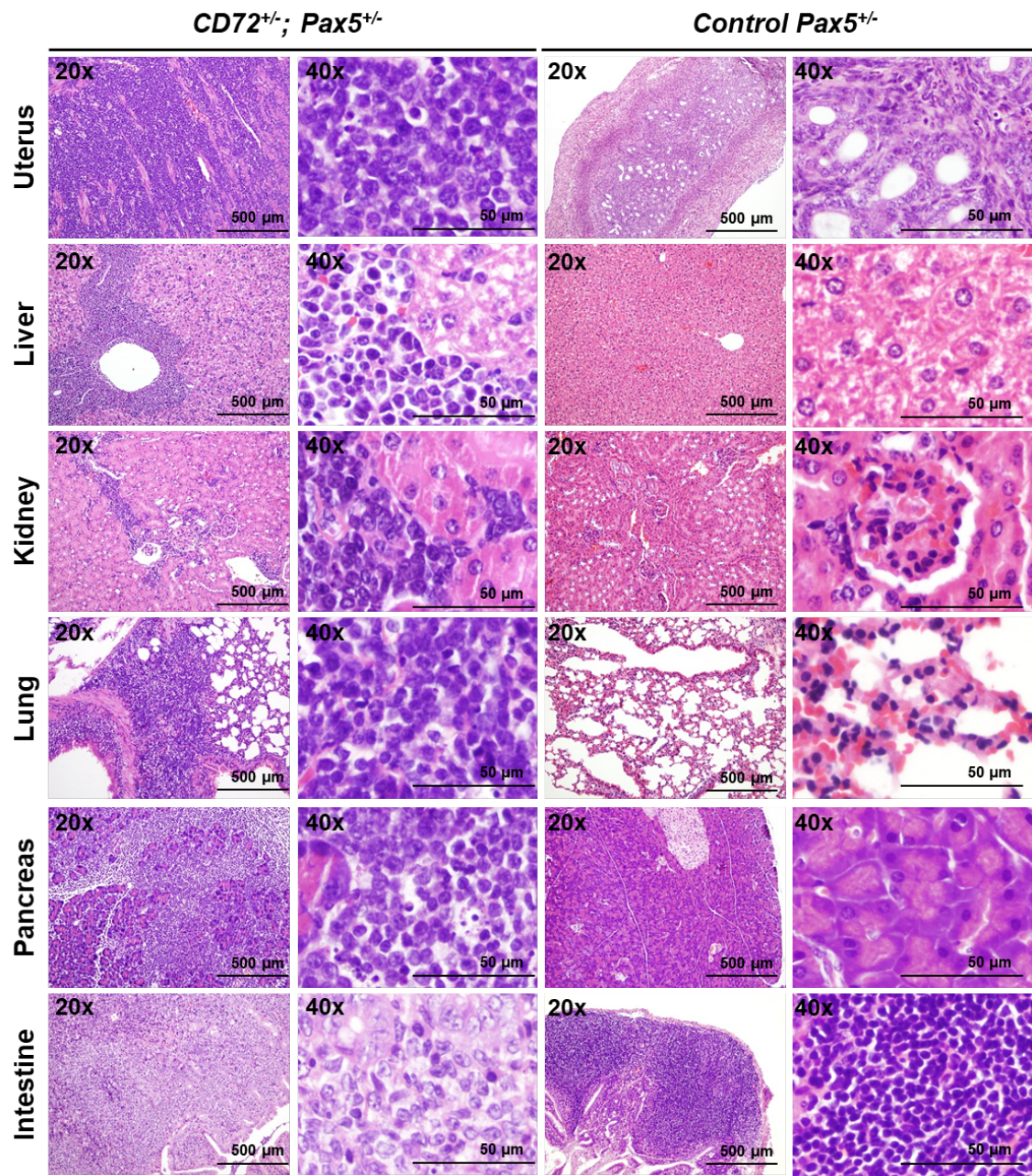




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Supplemental Figure 3: B-ALL in *CD72*^{+/-}; *Pax5*^{+/-} mice. Flow cytometric analysis of hematopoietic subsets in diseased *CD72*^{+/-}; *Pax5*^{+/-} mice. Representative plots of cell subsets from the bone marrow (A), peripheral blood (B), spleen (C), and lymph nodes (D) show accumulation of blast B cells in a leukemic *CD72*^{+/-}; *Pax5*^{+/-} mouse (B834) compared to age-matched healthy littermates *CD72*^{+/-}; *Pax5*^{+/-} (B855), *Pax5*^{+/-} (S907) and *CD72*^{+/-} (B924) mice.



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140 **Supplemental Figure 4: B-ALL in a *CD72^{+/-}; Pax5^{+/-}* mouse.** Haematoxylin
141 and eosin staining of tumour-bearing *CD72^{+/-}; Pax5^{+/-}* mice (A072) housed in a
142 specific pathogen-free facility (SPF), reveals infiltrating blast cells in the uterus,
143 liver, kidney, lung, pancreas and intestine. Loss of normal tissue architecture is
144 observed with cells morphologically resembling lymphoblasts (n=1). Tissues
145 from a control *Pax5^{+/-}* littermate mouse are included for reference. Magnification
146 and corresponding scale bars are indicated in each case.

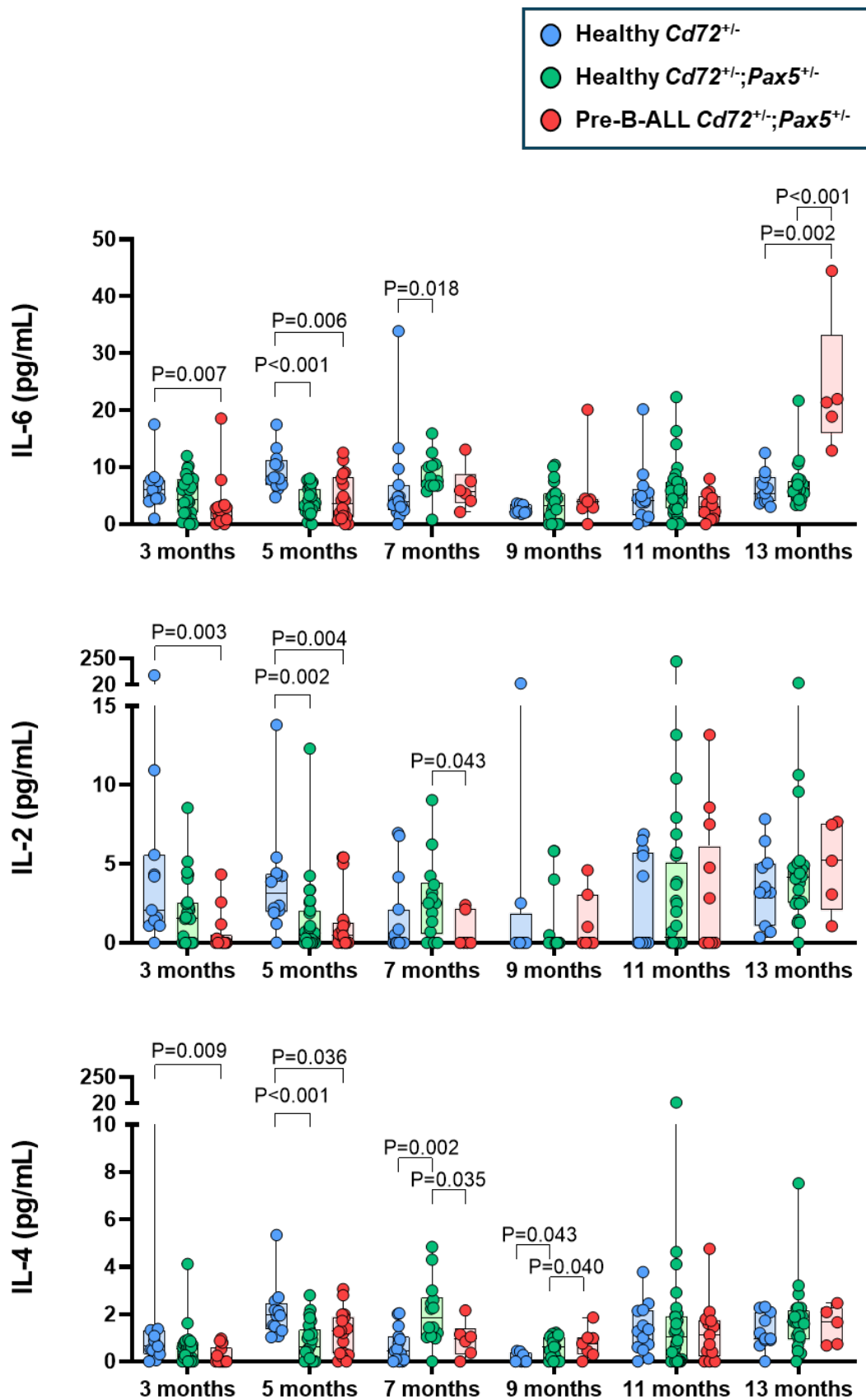
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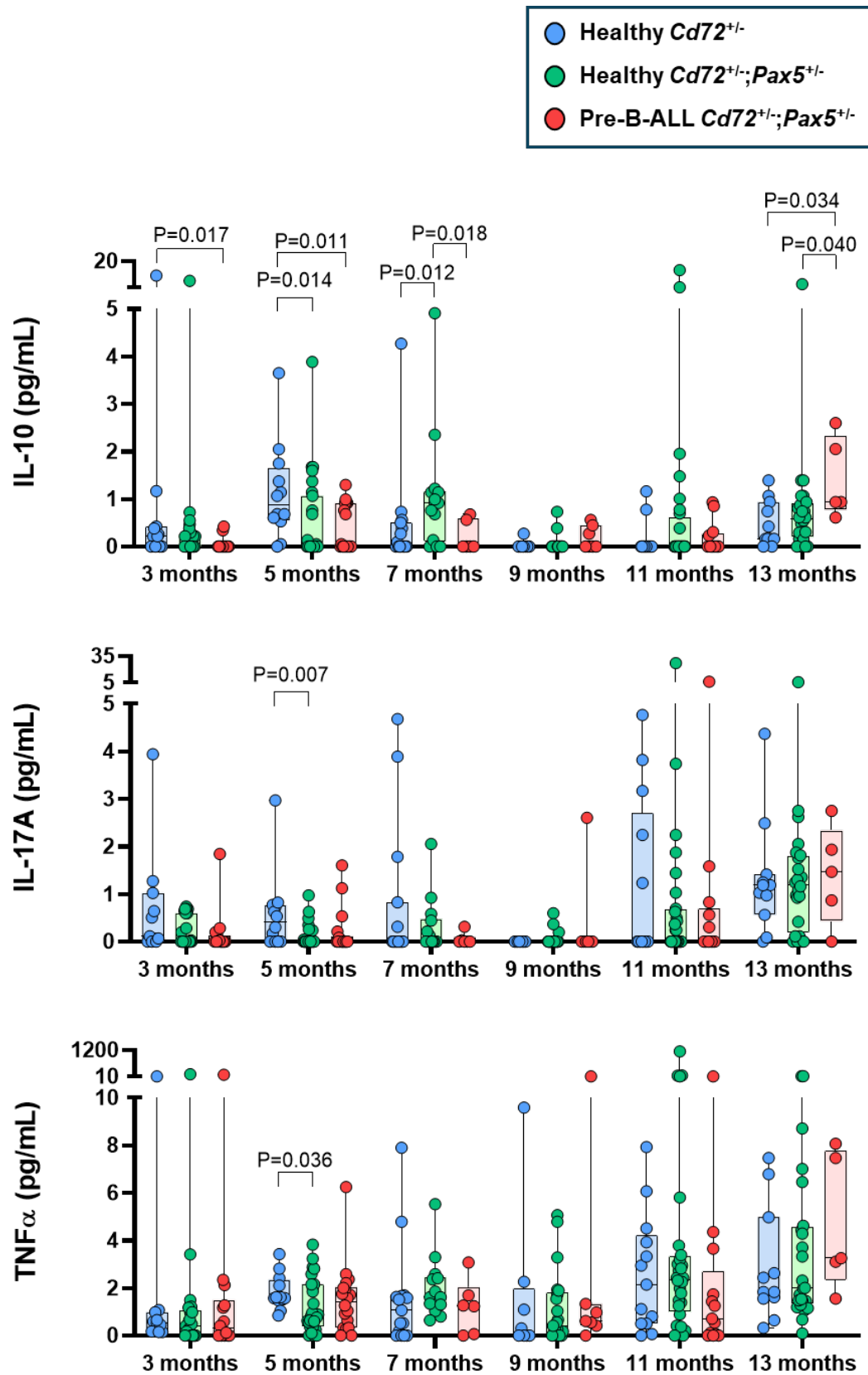
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Supplemental Figure 5



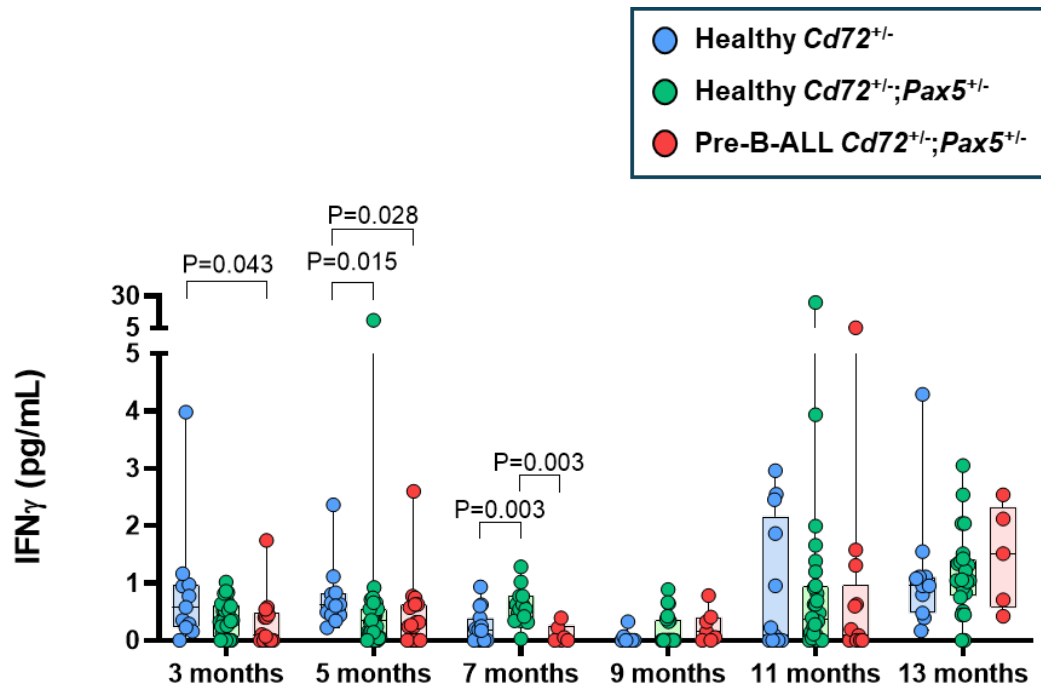
Supplemental Figure 5: Focal somatic amplification of the *Flt3* locus in a murine B-ALL tumor from a *Cd72^{+/-};Pax5^{+/-}* mouse. Copy number profiles of a B-ALL tumor (B834) and matched germline DNA (tail) are shown across chr5:145,000,000–149,000,000 (mm10 assembly), visualized using the UCSC Genome Browser. Read-depth signals (blue) reveal a focal amplification at the *Flt3* locus (highlighted in red), absent in the germline sample. *Flt3* encodes a receptor tyrosine kinase critical for hematopoietic stem and progenitor cell survival and proliferation.





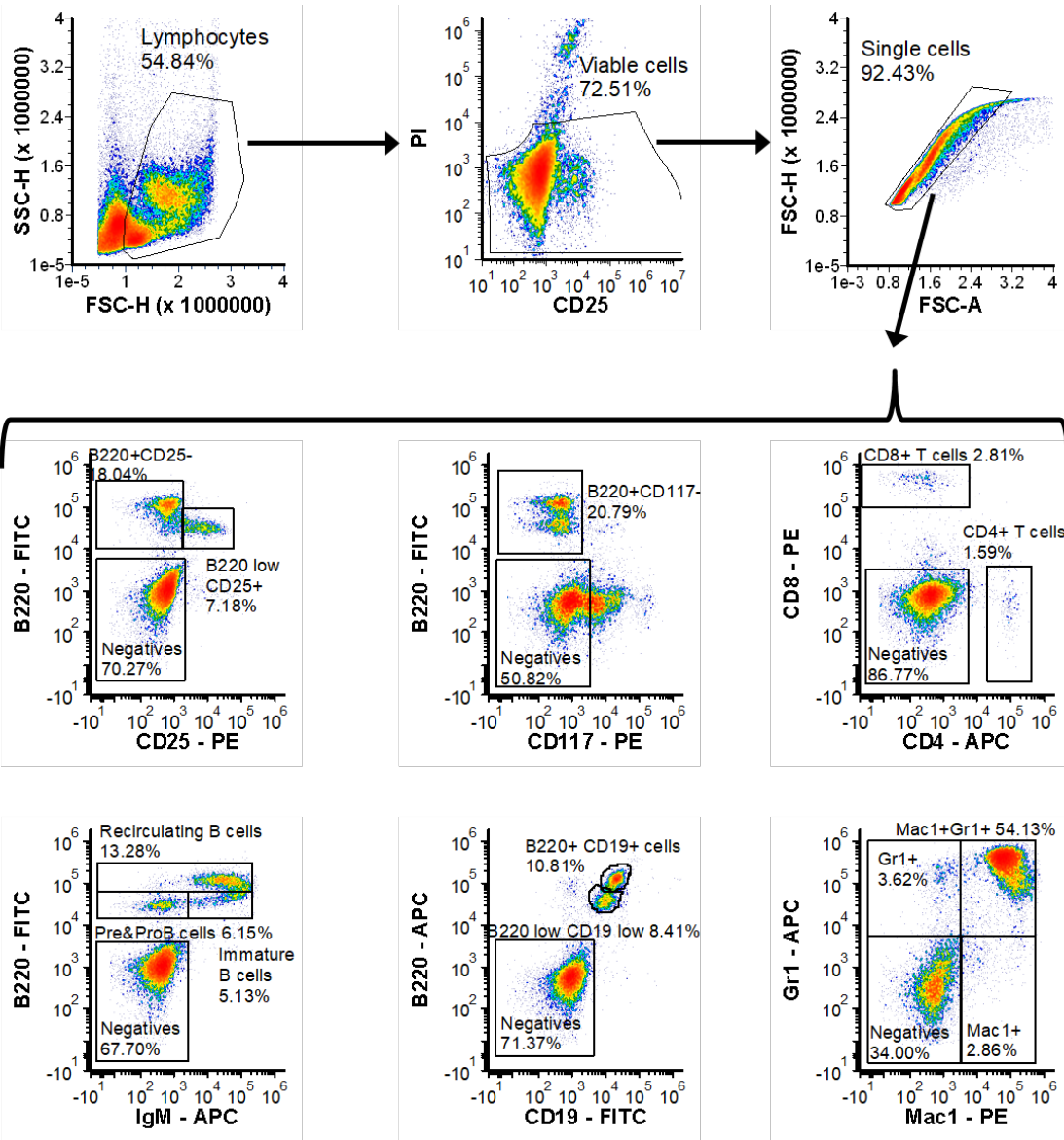
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Supplemental Figure 6: Cytokine serum levels in $Cd72^{+/-}; Pax5^{+/-}$ mice over time. Serum concentrations of IL-6, IL-2, IL-4, IL-10, IL-17A, TNF α and IFN γ were quantified in $Cd72^{+/-}; Pax5^{+/-}$ mice that developed B-ALL (red) and compared with healthy $Cd72^{+/-}; Pax5^{+/-}$ (green) and healthy $Cd72^{+/-}$ (blue) littermates at 3, 5, 7, 9, 11 and 13 months of age using a Cytometric Bead Array immunoassay (CBA, BD Biosciences). All animals were housed under specific-pathogen-free (SPF) conditions, as detailed in the Methods section. Each dot represents an individual mouse and box-and-whisker plots indicate median, interquartile range and range. P values above brackets indicate statistically significant differences between the indicated groups at each time point (U de Mann-Whitney test for each time point).

Supplemental Figure 7



Supplemental Figure 7: Gating strategy used in FACS analysis. This figure exemplifies the gating strategy used in all cytometric analyses. For each analysis, a total of at least 100.000 viable cells (PI-; propidium iodide negative cells) were assessed. Singlets were selected prior gating strategy that is specific for each population. Bone marrow cells stained with the indicated antibodies are shown as an example. The same gating strategy was used for all FACS analysis presented in Figure 1C and Supplemental Figure 3.