Supporting Information

TSLP or IL-7 provide an IL-7Rα signal that is critical for human B lymphopoiesis

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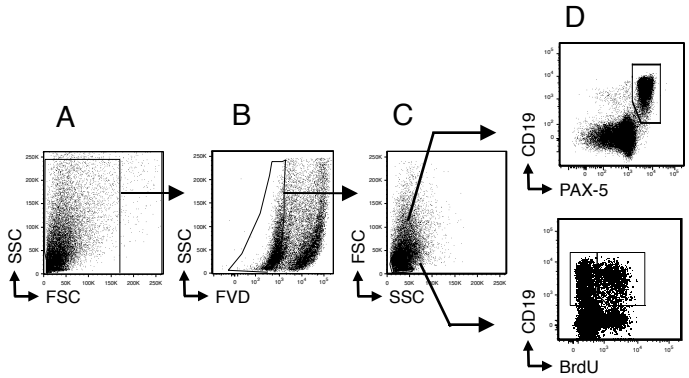

Figure S1. Gating strategy to Identify B cell progenitors generated in human-only selective cytokine co-cultures. Human CB CD34+ cells were cultured with human stromal cells as described in the supporting methods. Cultures were harvested at three weeks and stained for flow cytometry analysis. Cells were gated as shown above: A) Intact cells (non-debris) were gated based on light scatter**.** B) In some but not all experiments, living cells were identified by negative staining using a viability dye (FVD). C) Lymphoid cells were gated based on low forward and side scatter. D) B cell progenitors identified by positive staining for CD19.

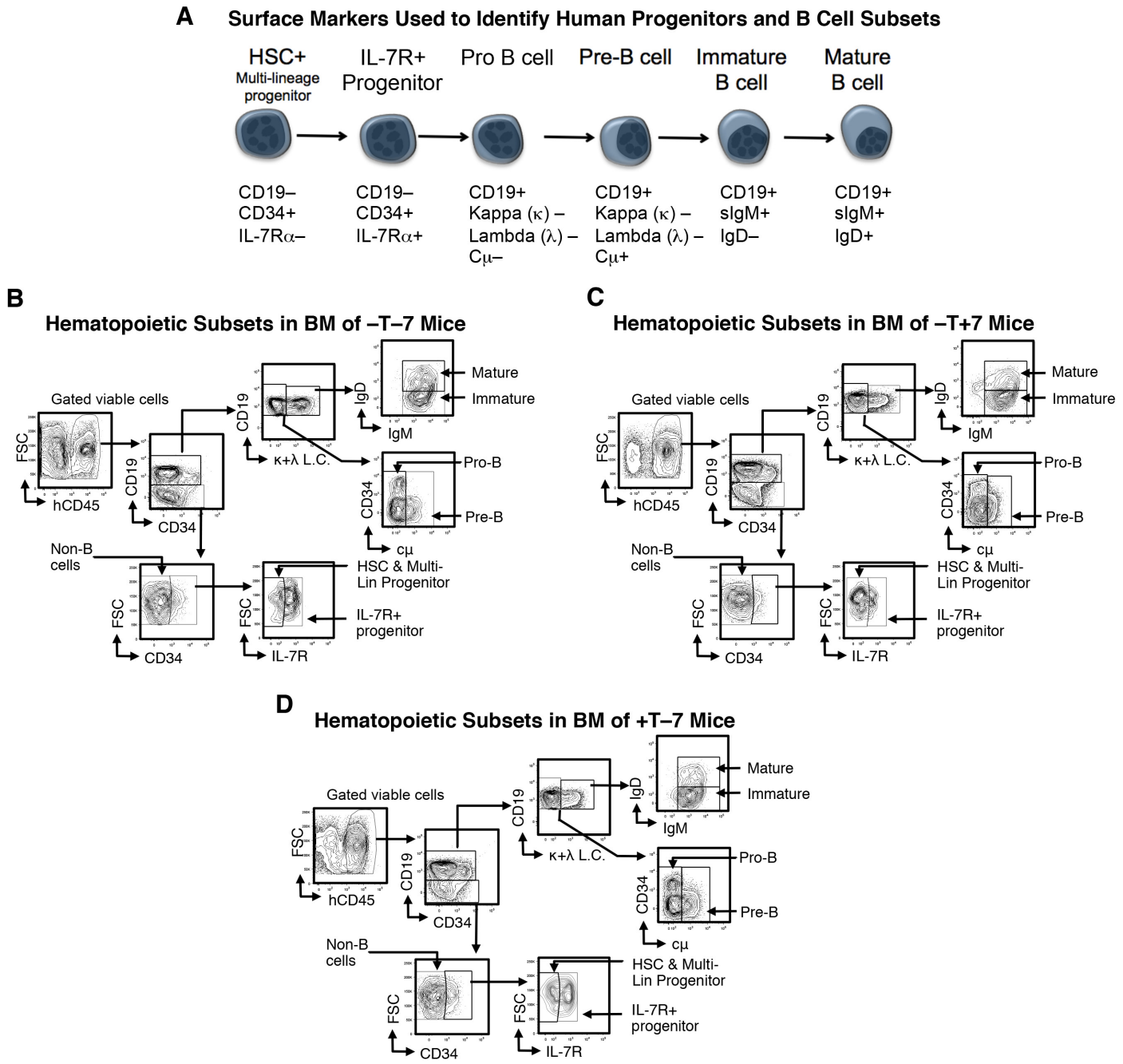

Figure S2. Flow cytometry to Identify hematopoietic subsets in xenografts with selective IL-7 and hTSLP stimulation. Human CB CD34+ cells were injected by tail vein into mice engineered to express hTSLP (+T mice) or that lacked hTSLP (–T mice) as described in methods and previously reported (Francis, *et al* 2015). Beginning 5 weeks after injection of CD34+ cells, mice were treated for two weeks with neutralizing anti-human/mouse IL-7 antibody or isotype-matched control antibody to generate mice with no hTSLP and reduced IL-7 stimulation (–T–7 mice); mice with no hTSLP and normal IL-7 (–T+7 mice); and mice with physiological hTSLP and reduced IL-7 (+T–7 mice). Mice were euthanized seven weeks post-transplant and BM harvested and stained for human specific markers to identify progenitors, developmentally sequential B cell subsets and non-B cells in the BM of indicated xenograft groups. (A) Surface immunophenotypes used to identify indicated hematopoietic subsets in xenograft mice. (B-D) Flow cytometry plots showing gating and representative data from n=4 xenografts in each group (B) –T–7 xenografts (C) –T+7 xenografts and (D) +T–7 xenografts. Gates were set based on isotype controls.