Supporting Information

Sanz et al. 10.1073/pnas.0907942107

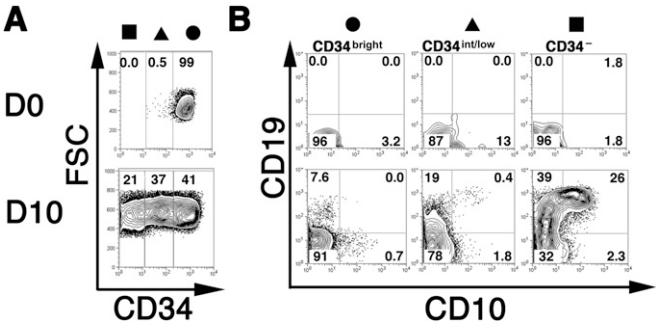

Fig. S1. CD34, CD10, and CD19 expression profiles after 10 days of $CD34^{high}Lin^{-}$ development in S17 cocultures. (*A*) CD34 levels before (D0) or after 10 days (D10) in S17 cocultures. Three levels of CD34 intensity, $CD34^{high}$ (●), $CD34^{int/low}$ (▲), and $CD34^{neg}$ (■), were defined. (*B*) Dot-plots of CD10 and CD19 expression for each subset gated by CD34 levels as in *A* for D0 and D10 time points. Background levels to define the indicated quadrants and statistics were set with isotype-matched reagents. We can ascertain that detection here of two-layered $CD34^{+}$ pathways that generate pro-B cells is not attributable to differences in key materials, using spare frozen samples from the same serum and S17 cell batches that we and others used in long-term cultures "without $CD34^{+}$ cells" [Sanz E, Alvarez-Mon M, Martínez-A C, de la Hera A (2003) Human cord blood CD34+Pax-5+ B-cell progenitors: Single-cell analyses of their gene expression profiles. Blood 101:3424–3430]. Average yields are displayed in Fig. 1. Results are representative of eight experiments from a donor distinct to Fig. 1.

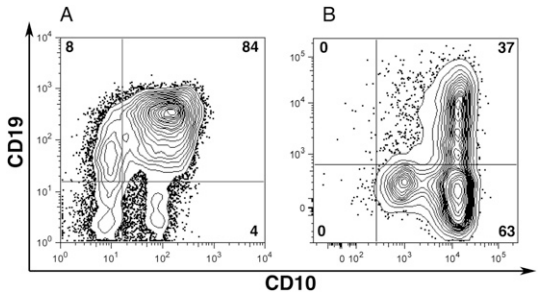

Fig. S2. CD10 and CD19 expression contour-plots after 24 days CD34^{high}Lin^{-}/S17 coculture when initial CD10^{-}CD19^{+} pre/pro-B-cell and late CD10^{+}CD19^{-} CLP/early-B-cell pathways of pro-B-cell development overlap (*A*) or after 21 days of CB CD34^{+}CD10^{+}CD19^{-}/S17 coculture used as a fresh CLP control source free of developing CD34^{+}CD10^{-}CD19^{+} cells (*B*). All our progenitor/S17 cocultures were recombinant cytokine-free. It might be argued that others have analyzed the initial wave of CB CD34^{high} SC development in stroma systems tuned with exogenous cytokines for higher cell yield (~1,500-fold from 2–4 weeks) (1). However, in the latter system, most progeny of the first 2 weeks belong to lineages other than CD19^{+} B-cell progenitors (i.e., <0.02% CD19^{+} cells) and rapid CD34 pool exhaustion occurs that does not allow visualization of the CD34^{+} B-cell progenitors of CD34^{+} pro-B cells (1). Our yield and efficiency of pro-B-cell generation from fresh CB CLPs also compare favorably with quantitative data from valuable multilineage systems using stroma supplemented with cytokines (i.e., 3.6-fold expansion of input cell numbers with 5% CD19^{+} cells in 3-week cultures) (2). Plots are representative of six and three experiments, respectively.

1. Johnson SE, Shah N, Panoskaltsis-Mortari A, LeBien TW (2005) Murine and human IL-7 activate STAT5 and induce proliferation of normal human pro-B cells. *J Immunol* 175:7325–7331.
2. Rossi MI, et al. (2003) B lymphopoiesis is active throughout human life, but there are developmental age-related changes. *Blood* 101:576–584.

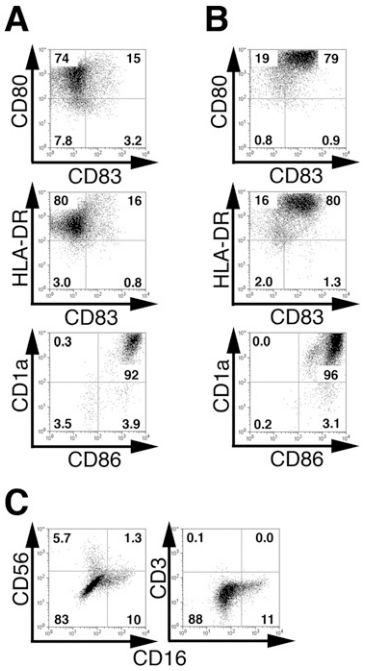

Fig. S3. CD34^{high}CD45RA^{+}CD10^{+}CD19^{-} precursors generated in vitro are CLPs that can develop into DC and NK cell progenies. CD34^{high}CD45RA^{+}CD10^{+}CD19^{-} precursors were sorted from 2-week CB CD34^{high}Lin^{-}/S17 cocultures and replated for 6 days in stroma-free complete medium supplemented with 10% (vol/vol) FBS plus 20 ng/mL IL-4 plus 50 ng/mL GM-CSF (both from R&D Systems) (*A*) or further washed and the immature DCs cultured for an additional 2 days in fresh medium plus 0.5 μg/mL LPS (Sigma) in analyses of DC maturation (*B*). Resulting cells from *A* and *B* were labeled with antibodies to CD80, CD83, CD86, CD1a, and HLA-DR to assess immature DC and mature DC fates. CD34^{high}CD45RA^{+}CD10^{+}CD19^{-} progenitors were CD80^{-}, CD83^{-}, CD86^{-}, CD1a^{-}, and HLA-DR^{low} before exposition to GM-CSF plus IL-4 (Table S1). CD80^{+}CD86^{+}HLA-DR^{bright} large cells CD83^{-}CD1a^{+} were typical immature DCs by phenotype. Mature DCs acquired CD83 and coexpressed much higher levels of CD86 and CD80. (*C*) Sorted CD34^{high}CD45RA^{+}CD10^{+}CD19^{-} progenitors were cultured 6 days in stroma-free complete medium supplemented with 10% FBS plus 25 ng/mL IL-15 (R&D Systems) and labeled with antibodies to CD56, CD16, and CD3. The original sorted population expressed none of the indicated markers. CD34^{+}CD10^{+}CD19^{-} precursors developed in IL-15-generated lymphoid progeny with the phenotype of two NK cell lineages, either CD3^{-}CD56^{+}CD16^{-} or CD3^{-}CD56^{-}CD16^{+}, as reported for human CD34^{+} thymic CLP and lymph node CD34^{int}CD45RA^{int}CD10^{+}CD19^{-} NK cell precursors. Experiments are representative of four different experiment sets with four independent CB samples.

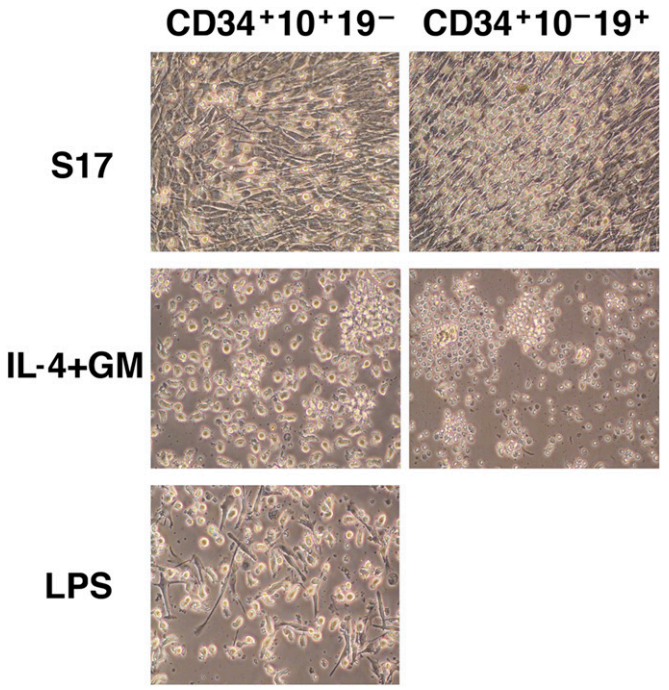

Fig. S4. Phase-contrast microscopy photographs. Sorted CB $CD34^+CD10^+CD19^-$ and $CD34^+CD10^-CD19^+$ cells differentiated under B-lineage (S17 stroma), immature DC (IL-4 + GM stroma-free), and DC maturation (LPS) conditions at the read-out points indicated in Fig. S3. Unlike $CD34^+CD10^+CD19^-$ differentiating cells, $CD34^+CD10^-CD19^+$ cells die in the presence of cytokine but absence of S17 cells. Immature DCs respond to LPS by polarization. Images are representative of four experiments.

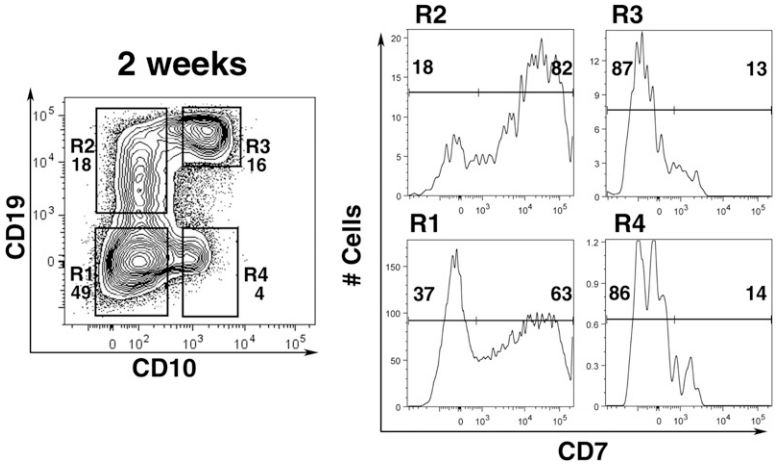

Fig. S5. $CD7^{high}$ expression in the $CD34^+CD10^-CD19^+$ precursors differentiated in vitro. Sorted $CD34^{high}CD10^-CD19^-$ precursors were cultured on S17 stroma for 2 weeks. Their progeny were stained with CD7-, CD10-, and CD19-specific antibodies and submitted to flow cytometry. (*Left*) Quantile plot displays CD10 × CD19 expression gated in total live cells. Numbers associated with R1–R4 regions represent the frequency of positive cells in the region. (*Right*) Histograms of CD7 distribution for cells in the respective R1–R4 subsets. The numbers in the histograms indicate the percentage of $CD7^-$ and $CD7^+$ cells. The tic in the bar shows the negative threshold for statistics, which was set using isotype-matched antibodies and fluorescence-minus-one methods. The input cell number was 10^4 cells per well, and the average yield was $55.38 \pm 5.3 \times 10^4$ cells per well at day 14. Data are representative of results from three independent experiments.

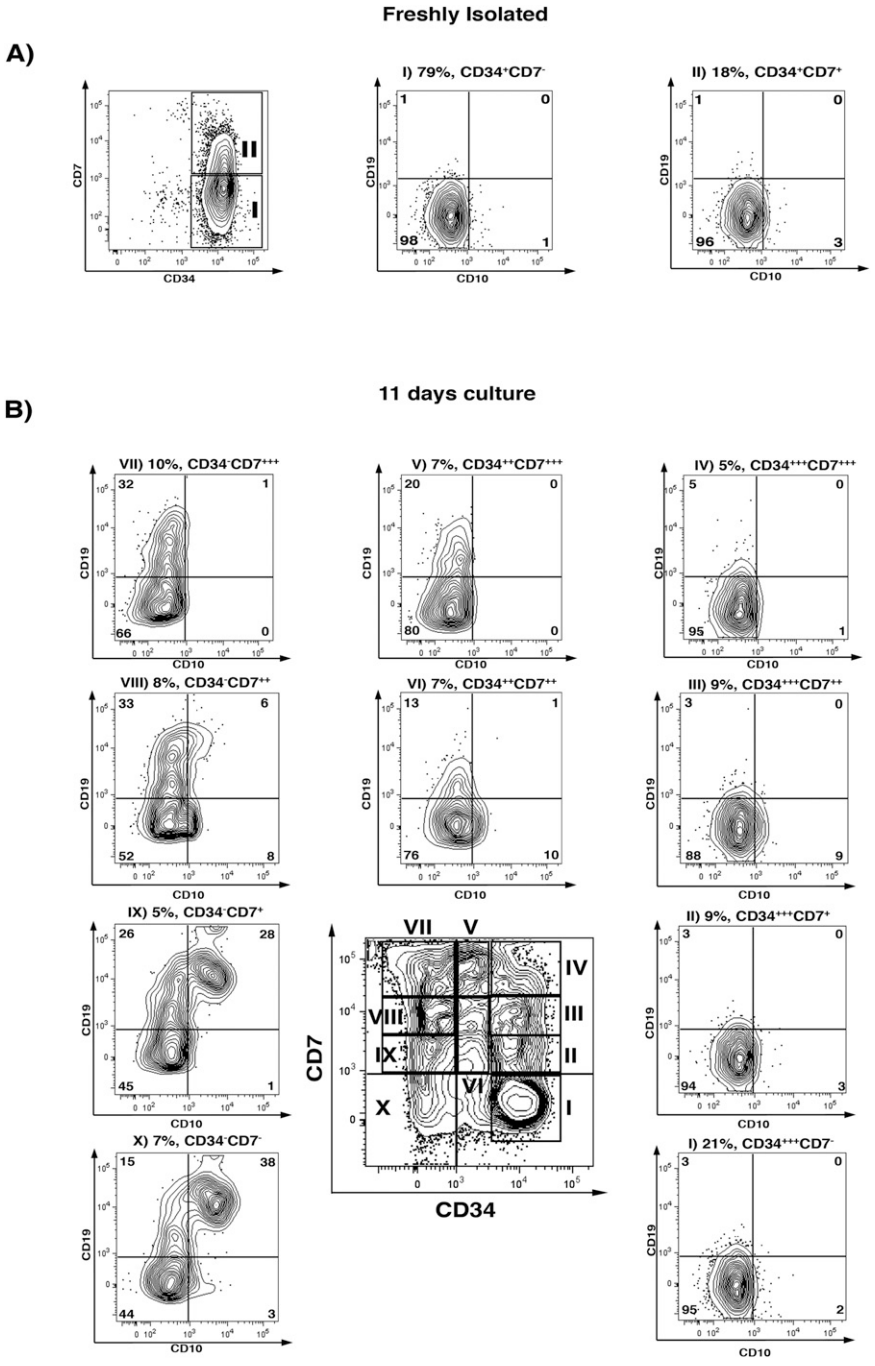

Fig. S6. Quantile plots of CD34, CD7, CD10, and CD19 distribution from either sorted fresh $CD34^{+}CD10^{-}CD19^{-}$ CB cells (*A*) or their progeny after 11 days in S17 coculture (*B*). Two and 10 regions in *A* and *B*, respectively, labeled with roman numerals (I–X) were defined in the CD34 × CD7 "quantile" contour plots [Herzenberg LA, Tung J, Moore WA, Herzenberg LA, Parks DR (2006) Interpreting flow cytometry data: a guide for the perplexed. *Nat Immunol* 7:681–685.], and the indicated gated live-cell subpopulations were analyzed for their correlated CD10 × CD19 distribution. The legend on top of the CD10 × CD19 plots indicates the percentage of the gated cells and their phenotype in the CD34 × CD7 plots. The numbers in the four corners of the CD10 × CD19 plots indicate the percentage of cells in each quadrant. The negative thresholds were set up using specificity controls as explained in *Materials and Methods*. Development events may be ordered as follows: (*i*) $CD34^{++}CD7^{++}CD10^{-}$ cells acquired low CD19 levels; (*ii*) $CD34^{++}CD7^{+++}CD10^{-}$ and $CD34^{-}CD7^{+++}CD10^{-}$ cells showed CD19 stepwise increases to $CD19^{int}$ and $CD19^{high}$ expression levels, respectively, which was coincident with CD34 reduction and maximal CD7 levels; and (*iii*) $CD34^{-}CD19^{high}CD10^{-}$ cells acquired CD10, which was notably coincident with a stage transition to $CD7^{low/-}$ phenotypes ($^{+++}$, ~ high; $^{++}$, ~ int; $^{+}$, ~ low). Data are representative of three similar experiments performed with independent CB donor samples.

Table S1. Expression of DC differentiation and maturation markers by $CD34^{high}CD45RA^{+}CD10^{+}CD19^{-}$ cultured progenitors before exposition to GM-CSF + IL-4

	$CD34^{high}CD45RA^{+}CD10^{+}CD19^{-}$	$CD14^{+}$-derived iDCs	$CD14^{+}$-derived mDCs
CD80	<1	52	99.6
CD83	<1	2–20	90
CD86	<1	96	99
CD1a	<1	98.9	8–16
HLA-DR	80 (low intensity)	90	97

Numbers are given as percentage of positive cells. IDCs, immature DCs; mDCs, mature DCs.