SUPPLEMENTAL MATERIAL

Breton et al., http://www.jem.org/cgi/content/full/jem.20141441/DC1

a Limiting Dilution Assay of CB CD34$^+$ HSPCs

Cell #/ well	# of wells plated	# of Positive wells	% of Negative wells
1	59	9	84.7
2	59	20	66.1
4	26	14	46.2
8	12	12	0.0
16	12	12	0.0
32	6	6	0.0
100	6	6	0.0

b CB CD34$^+$ HSPC Clonal Output

G	M	DC	M+DC	G+M	G/M/DC + L
4	3	22	8	4	2

c DC positive clones output phenotype

CD1c$^+$	CD141$^+$	cDCs	pDC +cDC	pDC
9	0	3	8	2

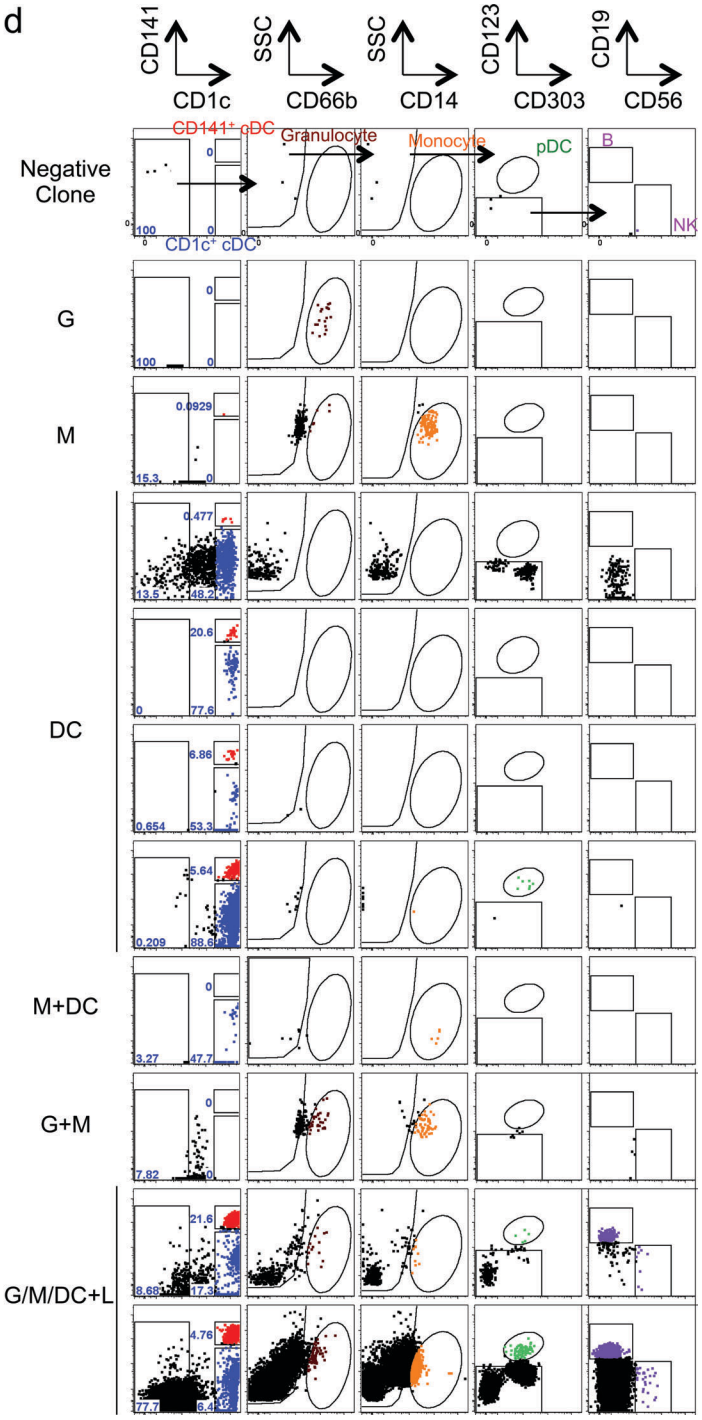

Figure S1. Clonal output of cord blood CD34$^+$ HSPCs. (a) CB CD34$^+$ HSPCs were cultured at 1, 2, 4, 8, or 16 cells per well for 14 d on MS5+FSG. Each well was analyzed for its positivity and lineage potential based on surface marker analysis. Positive wells were determined based on the presence of CD45$^+$ cells (Materials and methods) by flow cytometry. Red font indicates wells with single clones (cell no. per well < 4.57 cells) analyzed for lineage output. (b) Table summarizes the number of clones giving rise to the specified lineages. (c) Clones generating only DCs were broken down into their respective subsets and combinations (CD1c$^+$ cDCs, CD141$^+$ cDCs, cDCs, pDCs+cDCs, or pDCs alone). (d) Representative flow cytometry plots of gated live human CD45$^+$ cells show clones failing to generate any of the lineages (negative clones), as well as positivity for granulocytes alone (G), monocytes alone (M), DCs alone, M+DC, G+M, or Myeloid (G/M/ or DC) + Lymphoid (B or NK, L).

a Limiting Dilution Assay of CB hpre-cDCs

Cell #/ well	# of wells plated	# of Positive wells	% of Negative wells
1	126	10	92.1
5	138	71	48.6
10	34	19	44.1

b CB hpre-cDC Clonal Output

G	M	DC	M+DC	G+M	G/M/DC + L
0	7	71	3	0	0

c DC positive clones output phenotype

CD1c$^+$	CD141$^+$	cDCs	pDC +cDC	pDC
40	26	5	0	0

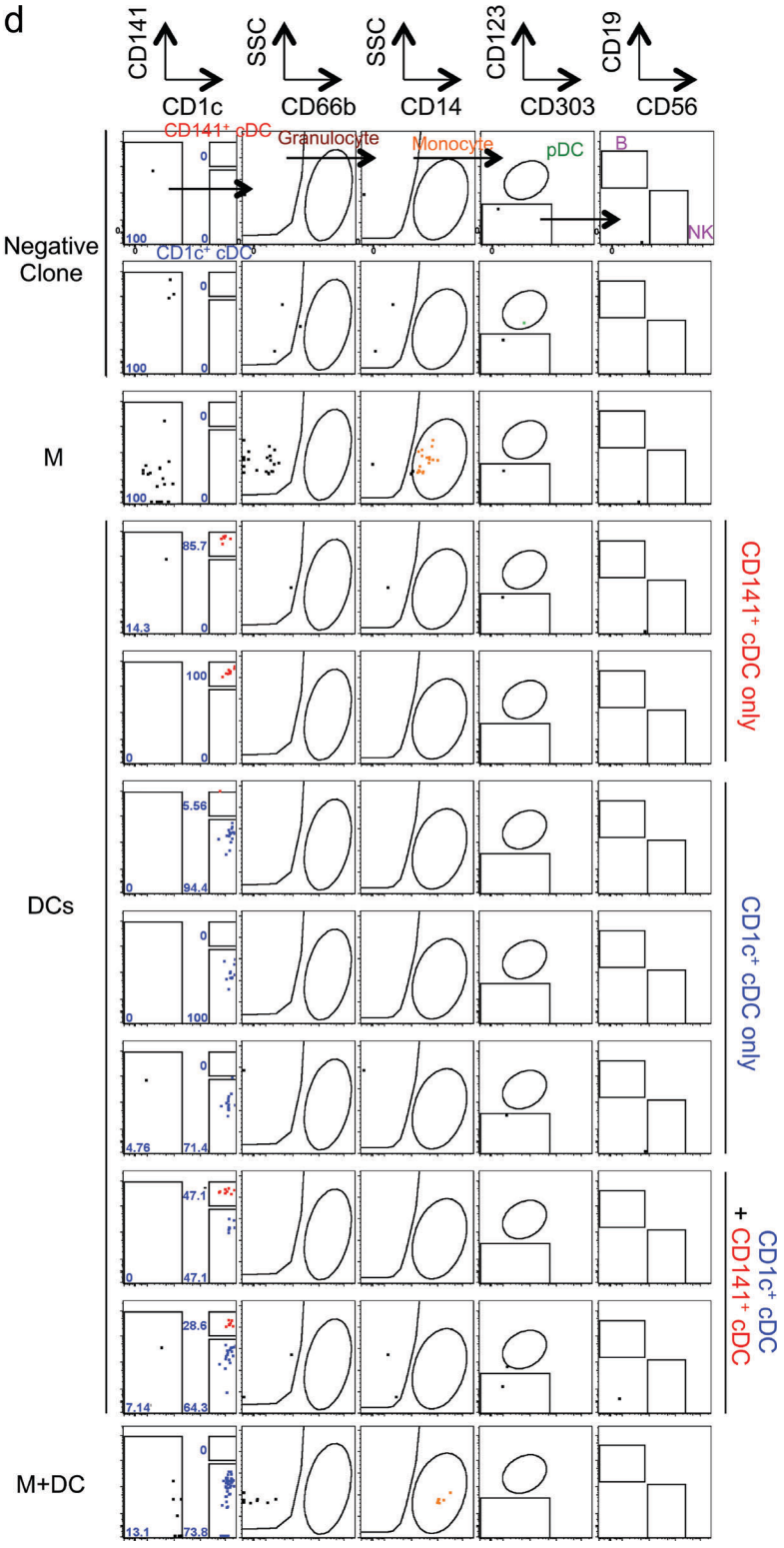

Figure S2. Clonal output of cord blood (CB) hpre-CDCs. (a) CB hpre-CDCs were cultured at 1, 5, or 10 cells per well for 5 d on MS5+FSG. Each well was analyzed for its positivity and lineage potential based on surface marker analysis. Positive wells were determined based on the presence of CD45$^+$ cells by flow cytometry. Red font indicates wells with single clones (cell no. per well < 7.84 cells) analyzed for lineage output. (b) Table summarizes the number of clones giving rise to the specified lineages. (c) Clones generating only DCs were broken down into their respective subsets and combinations (CD1c$^+$ cDCs, CD141$^+$ cDCs, cDCs, pDCs+cDCs, or pDCs alone). (d) Representative flow cytometry plots of gated live human CD45$^+$ cells show clones failing to generate any of the lineages (negative clones), as well as positivity for monocytes alone (M), DCs alone, or M+DC.

a Limiting Dilution Assay of PB hpre-cDC

Cell #/ well	# of wells plated	# of Positive wells	% of Negative wells
1	143	24	83.2
5	157	81	48.4

b PB hpre-cDC Clonal Output

G	M	DC	M+DC	G+M	G/M/DC + L
0	5	94	6	0	0

c DC positive clones output phenotype

$CD1c^+$	$CD141^+$	cDCs	pDC +cDC	pDC
68	11	15	0	0

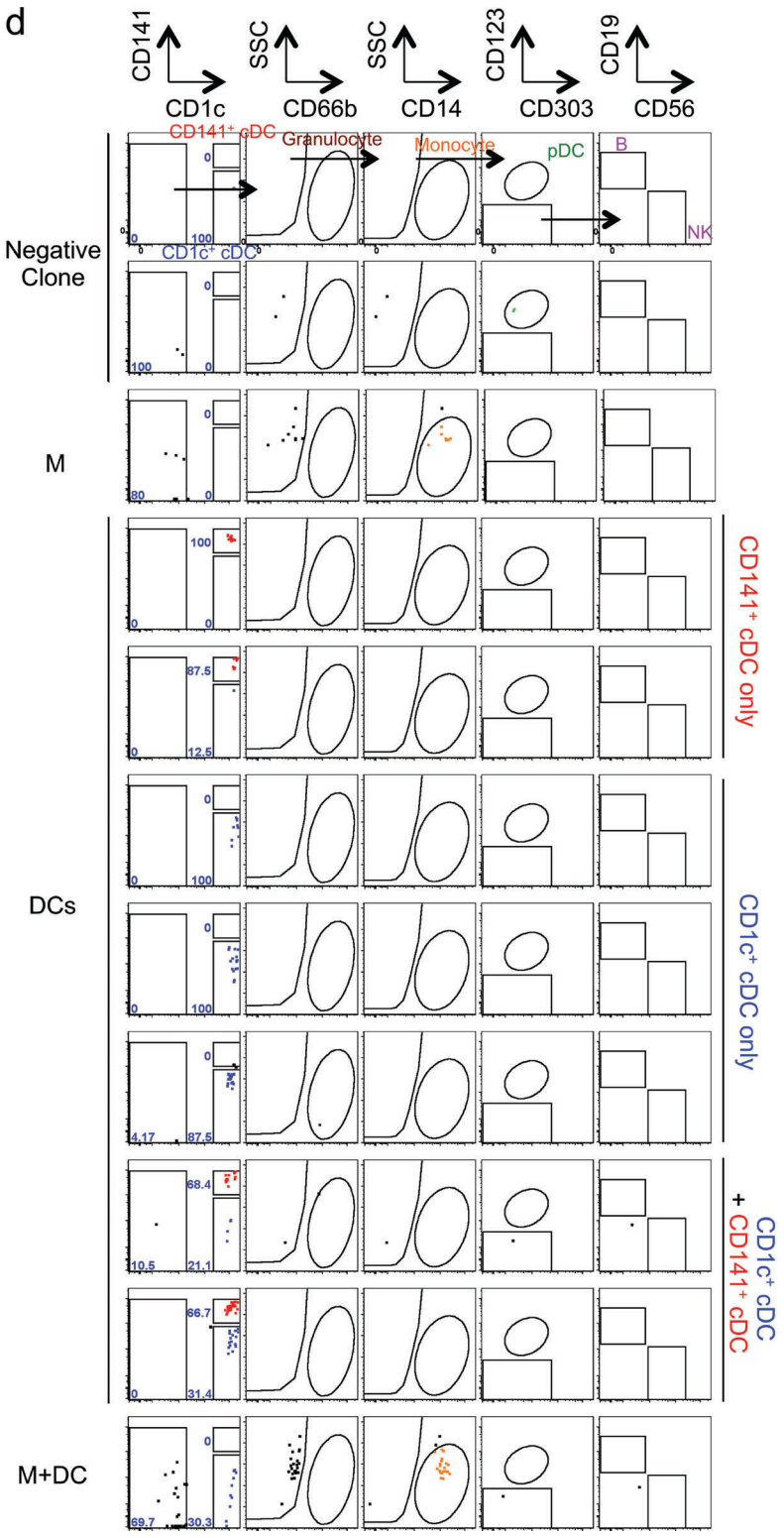

Figure S3. Clonal output of peripheral blood (PB) hpre-CDCs. (a) PB hpre-CDCs were cultured at 1 or 5 cells per well for 5 d on MS5+FSG. Each well was analyzed for its positivity and lineage potential based on surface marker analysis. Positive wells were determined based on the presence of $CD45^+$ cells by flow cytometry. Red font indicates wells with single clones (cell number per well < 6.55 cells) analyzed for lineage output. (b) Table summarizes the number of clones giving rise to the specified lineages. (c) Clones generating only DCs were broken down into its respective subsets and combinations ($CD1c^+$ cDCs, $CD141^+$ cDCs, cDCs, pDCs+cDCs, or pDCs alone). (d) Representative flow cytometry plots of gated live human $CD45^+$ cells show clones failing to generate any of the lineages (negative clones), as well as positivity for monocytes alone (M), DCs alone, or M+DC.

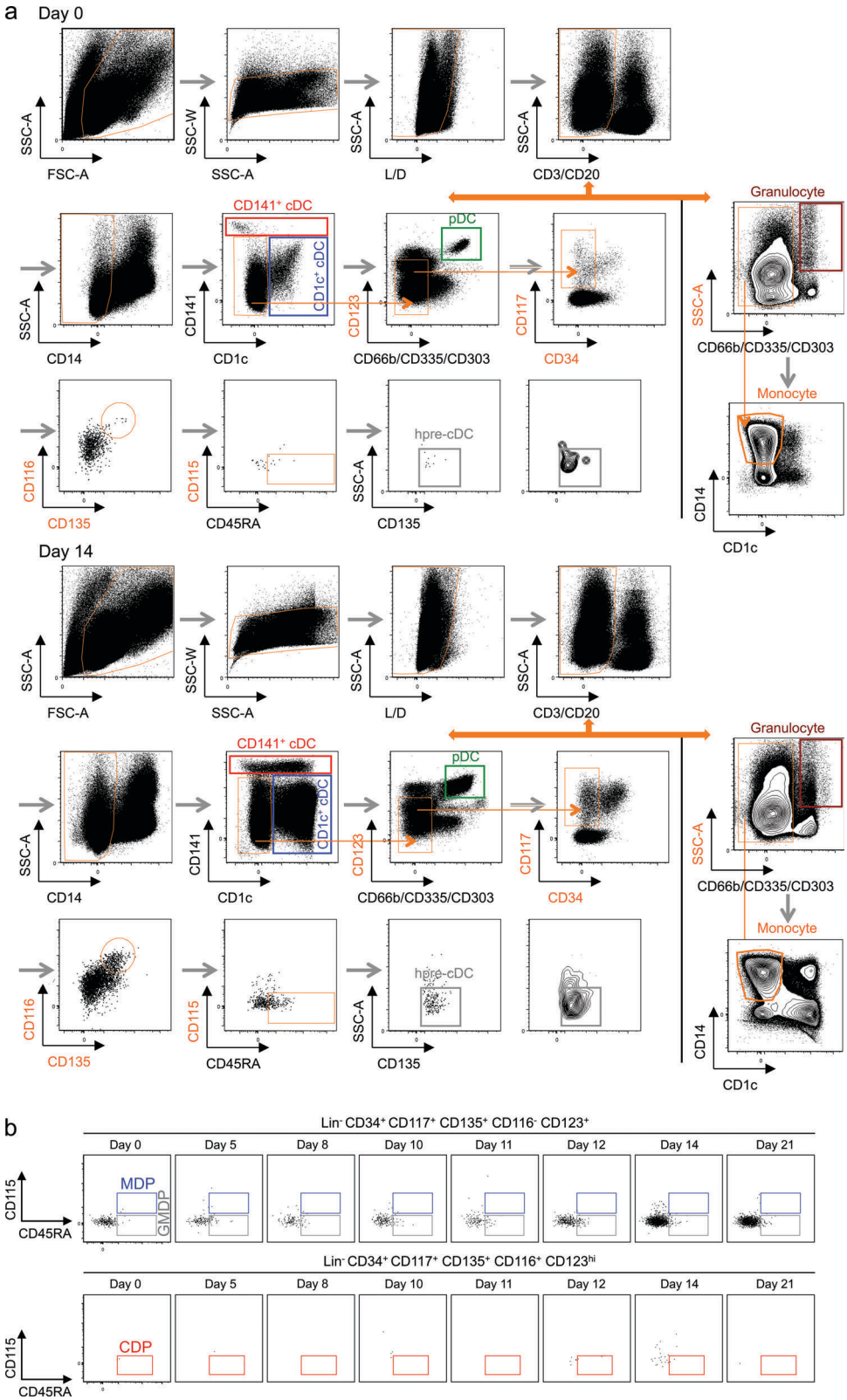

Figure S4. 14-color gating strategy for identification of human hpre-CDCs in blood. (a) PBMCs were isolated from heparinized blood using Ficoll-Hypaque. Live CD3$^-$CD19$^-$CD335$^-$CD66b$^-$CD14$^-$CD1c$^-$CD141$^-$CD303$^-$ cells, i.e., T, B, NK, neutrophils, monocytes, and DC cell-depleted, were stained for CD34, CD117, CD123, CD135, CD116, CD115, and CD45RA markers. Blood CD34$^-$CD117$^+$CD123$^+$CD135$^+$CD116$^+$CD115$^-$CD45RA$^+$ hpre-CDCs are shown before (Day 0) and after (Day 14) Flt3L administration. (b) hCDPs, hMDPs, and hGMDPs are undetectable in the blood before and after Flt3L administration in healthy volunteers. Flow cytometry plots of gated Lin$^-$CD34$^+$CD117$^+$CD135$^+$CD116$^+$CD123^{hi} cells show lack of hCDPs in blood of Flt3L-injected volunteers. Flow cytometry plots of gated Lin$^-$CD34$^+$CD117$^+$CD135$^+$CD116$^-$CD123$^+$ cells show lack of hMDPs and hGMDPs in blood of Flt3L injected volunteers.