

HSC proliferation: Prospectively sorted HSC (Lineage-CD34⁺CD38⁻CD90⁺CD45RA⁻) were cultured in Stemspan H3000 on hydrogel microwell plates in hydrogels in media containing FL, SCF (FS; 100 ng/ml), Wnt1 (100 ng/ml), Wnt3a (100 ng/ml), Jagged 1 (50 ng/ml), Jagged 2 (50 ng/ml) and DLL4 (50 ng/ml) (collectively “FS/Stem”) in the presence or absence of 40 ng/ml of G-CSF. The plate was then placed in the environmental chamber of an inverted microscope (Zeiss Axiovert 200) equipped with a motorized stage. After cells were randomly distributed and spatially segregated in microwells, the XYZ stage was programmed to repeatedly raster across the microwell array surface, acquiring phase contrast images at 5X magnification of multiple locations in defined time intervals for a period of up to 4 days. The entire surface of the microwell arrays were scanned and the resulting images of the time-lapse experiment were then automatically compiled into a stack (library) using the Volocity software (Improvision). Cells were scored in a blinded fashion and single events were only scored as ‘division’ if there was clear cytokinesis (i.e. one cell became two independently moving cells).

Table S2, related to Fig. 2. Bimodal signaling responses in HSC and progenitors

A likelihood ratio test was applied to response profiles to assess the relative probability of unimodality versus bimodality. Mixture modeling was used to estimate the parameters of the bimodal distribution (see Statistical Methods). p-values represent the likelihood of the null hypothesis being true, i.e. of unimodality, with smaller p-value indicating a more bimodal distribution of response. Significant values are bold.

Sample	HSC						CMP		MEP		
	No Stim/Stat3	G-CSF/Stat3	IL-6/Stat3	No Stim/Stat5	Tpo/Stat5	GM-CSF/Stat5	IL-3/Stat5	No Stim/Stat3	IL-6/Stat3	No Stim/Stat3	No Stim/Stat3
CB030	0.4526	<0.00001	ND	0.32704	<0.00001	<0.00001	ND	ND	ND	ND	ND
CB031	0.931655	0.008355	ND	0.16035	<0.00001	ND	ND	ND	ND	ND	ND
CB033	0.12708	<0.00001	ND	0.220585	ND	ND	ND	ND	ND	ND	ND
CB034	0.31455	0.00298	<0.00001	0.98818	ND	0.00011	ND	0.20358	<0.00001	0.32363	<0.00001
CB035	0.10429	<0.00001	ND	0.00003	ND	<0.00001	ND	ND	ND	ND	ND
CB036	0.71624	<0.00001	0.528375	0.74946	<0.00001	<0.00001	<0.00001	0.110715	0.145945	0.12805	<0.00001
CB037	0.535365	0.04229	<0.00001	0.626005	0.216935	0.00705	0.00574	0.0214	0.66739	0.41627	<0.00001
MPB1082	0.243775	ND	0.00001	0.01027	<0.00001	<0.00001	ND	0.1647	<0.00001	0.28192	<0.00001
MPB1111	0.17814	<0.00001	<0.00001	0.00013	<0.00001	<0.00001	ND	0.065185	<0.00001	0.119835	<0.00001
MPB1140	0.08941	<0.00001	<0.00001	0.00009	<0.00001	<0.00001	<0.00001	0.01306	<0.00001	0.02885	<0.00001
NM1731	0.03683	0.00165	0.351795	0.00512	0.01586	<0.00001	<0.00001	0.00009	<0.00001	0.07394	<0.00001
NM1755	0.00663	0.00005	<0.00001	0.00002	0.00064	<0.00001	<0.00001	0.19656	<0.00001	0.097465	<0.00001
NM1761	0.1616	<0.00001	<0.00001	0.039055	0.00384	<0.00001	<0.00001	0.084735	<0.00001	0.174425	<0.00001

Table S3, related to Fig. 2. Cytokines synergize on hematopoietic stem cells
 Percentage of cells responding, median fluorescent intensity, and 95th percentile of fluorescence for HSC from normal marrow (NM), cord blood (CB) and G-CSF mobilized peripheral blood. p-value from a paired t-test comparing the values of each individual stimulus (FL, SCF, Tpo, IL-3, and IL-6) to the combination (FST36).

		Stat5 (pY694)		
		Percent Cells Responding	Median Fluorescence	95th Percentile Fluorescence
NM1731	No Stim	0	209	389
NM1731	FL	2.2	246	435
NM1731	SCF	3.88	342	493
NM1731	Tpo	73.4	730	1466
NM1731	IL-3	49.6	505	1423
NM1731	IL-6	0.64	265	408
NM1731	FST36	78.8	1114	1969
	p	0.012194969	0.000766088	0.005078043
NM1755	No Stim	0	476	837
NM1755	FL	2.46	505	855
NM1755	SCF	0.76	356	736
NM1755	Tpo	44.5	897	1817
NM1755	IL-3	33.6	686	1940
NM1755	IL-6	0.37	346	613
NM1755	FST36	82	1601	2753
	p	0.001121316	0.00028676	0.002652829
NM1761	No Stim	1.57	247	438
NM1761	FL	0.29	229	428
NM1761	SCF	2.66	277	448
NM1761	Tpo	54.2	597	1010
NM1761	IL-3	54.7	640	1639
NM1761	IL-6	2.3	209	413
NM1761	FST36	87.3	977	2049
	p	0.003767215	0.001681538	0.003171778
CB036	No Stim	0	282	453
CB036	FL	0	218	401
CB036	SCF	2.08	243	546
CB036	Tpo	25.8	409	927
CB036	IL-3	22.6	374	1069
CB036	IL-6	4.69	358	602
CB036	FST36	80.3	939	1730
	p	0.000110359	4.09079E-05	0.000602322
CB037	No Stim	0	311	545
CB037	FL	1.11	212	388
CB037	SCF	0.4	240	449
CB037	Tpo	20.3	533	1063
CB037	IL-3	43.3	703	1460
CB037	IL-6	1.19	275	526
CB037	FST36	81.8	1406	2363
	p	0.00061356	0.00023134	0.000803143
GMPB1082	No Stim	0	154	307
GMPB1082	FL	0	168	300
GMPB1082	SCF	0.58	160	300
GMPB1082	Tpo	41.3	381	1011
GMPB1082	IL-3	4.39	118	391
GMPB1082	IL-6	0.54	171	319
GMPB1082	FST36	67.6	600	1545
	p	0.000956289	0.000493373	0.000712134
GMPB1111	No Stim	0.24	160	341
GMPB1111	FL	0.26	173	313
GMPB1111	SCF	0	186	344
GMPB1111	Tpo	28.7	338	951
GMPB1111	IL-3	19.7	237	950
GMPB1111	IL-6	0.81	162	356
GMPB1111	FST36	61.9	592	1543
	p	0.000491403	0.000162017	0.00153977
GMPB1140	No Stim	0.52	154	315
GMPB1140	FL	0.88	104	181
GMPB1140	SCF	0.66	167	298
GMPB1140	Tpo	48.8	365	981
GMPB1140	IL-3	23.7	218	983
GMPB1140	IL-6	5.67	201	426
GMPB1140	FST36	75.1	673	1474
	p	0.001525387	0.000215722	0.003124156

		Erk (pT202/pY204)		
		Percent Cells Responding	Median Fluorescence	95th Percentile Fluorescence
		1.13	98.7	197
		2.27	99.9	208
		6.29	129	285
		1.67	113	242
		0.54	90.8	168
		0.6	99.2	180
		34.4	206	582
		3.477E-06	5.8787E-05	3.40558E-05
		0.49	124	211
		10.9	158	336
		15.3	150	397
		10.6	129	435
		1.38	128	213
		2.2	117	213
		55.4	279	862
		3.11492E-05	2.36716E-05	0.000147043
		0	105	184
		2.56	106	250
		ND	ND	ND
		5.54	114	292
		0	90.4	165
		0	86.3	152
		41.8	240	599
		3.99536E-05	0.000108277	0.000723323

Table S4, related to Fig. 4. Engraftment characteristics of NOD/SCID/IL-2R γ ^{null} (NSG) mice engrafted with CD114^{neg/lo} or CD114^{pos} subsets from human HSC compartment

Sample	Week 6				Week 14				
	Chimerism	% B Cells	% Myeloid	Engraftment	Chimerism	% B Cells	% Myeloid	Engraftment	
CD114 neg/lo	NM 1	0.19092066	72.6	10.2	Yes	2.94573643	93.2	2.79	Yes
	NM 1	0	•	•	No	0.28694405	87.3	6.46	Yes
	NM 1	0	•	•	No	0	•	•	No
	NM 1	0.10162602	60.8	35.4	Yes	11.4942529	94.2	1.97	Yes
	NM 1	1.93615908	67.2	23.4	Yes	12.6153846	95.2	1.76	Yes
	NM 2	1.13519092	89.3	2.65	Yes	0.99415804	96.1	1.97	Yes
	NM 2	1.01340977	83.7	10.6	Yes	1.45046806	91.5	4.15	Yes
	NM 2	0	•	•	Yes	1.60663656	87	8.43	Yes
	NM 2	8.92307692	85.8	8.13	Yes	0.17567428	89.9	4.93	Yes
	NM 2	0	•	•	No	0	•	•	No
	NM 2	0.36154828	2.38	91.7	Yes	5.19045626	95.9	2.15	Yes
	NM 2	0.26541445	0	91.3	Yes	0.155521	91	7	Yes
	NM 2	0	•	•	No	0.28917211	90.9	3.86	Yes
	NM 2	15.0515464	69.3	20.4	Yes	62.3443983	92.9	4.42	Yes
CD114 hi	NM 1	0.31512605	87	4.11	Yes	0.12317799	95.5	1.72	Yes
	NM 1	0	•	•	No	0	•	•	No
	NM 1	0	•	•	No	0	•	•	No
	NM 1	0	•	•	No	0.7182087	91.2	1.03	Yes
	NM 2	0	•	•	No	0	•	•	No
	NM 2	0.92237538	92.9	0.39	Yes	0	•	•	No
	NM 2	0.12497396	83.7	10.9	Yes	0	•	•	No
	NM 2	0	•	•	No	0	•	•	No
	NM 2	0.53431977	89.8	1.44	Yes	0	•	•	No
	NM 2	0	•	•	No	0	•	•	No

Figure s1, related to Fig. 2. Average response profile by cell type

Heatmap showing the average percent response of each stem and progenitor compartment in all progenitor populations (CB, n=8; GMPB, n=3; BM, n=4) after 15-minute cytokine stimulation. For normal marrow, three nodes (Stat3, Stat5, and Erk) are measured. For MPB and CB, two nodes (Stat3, Stat5) are measured.

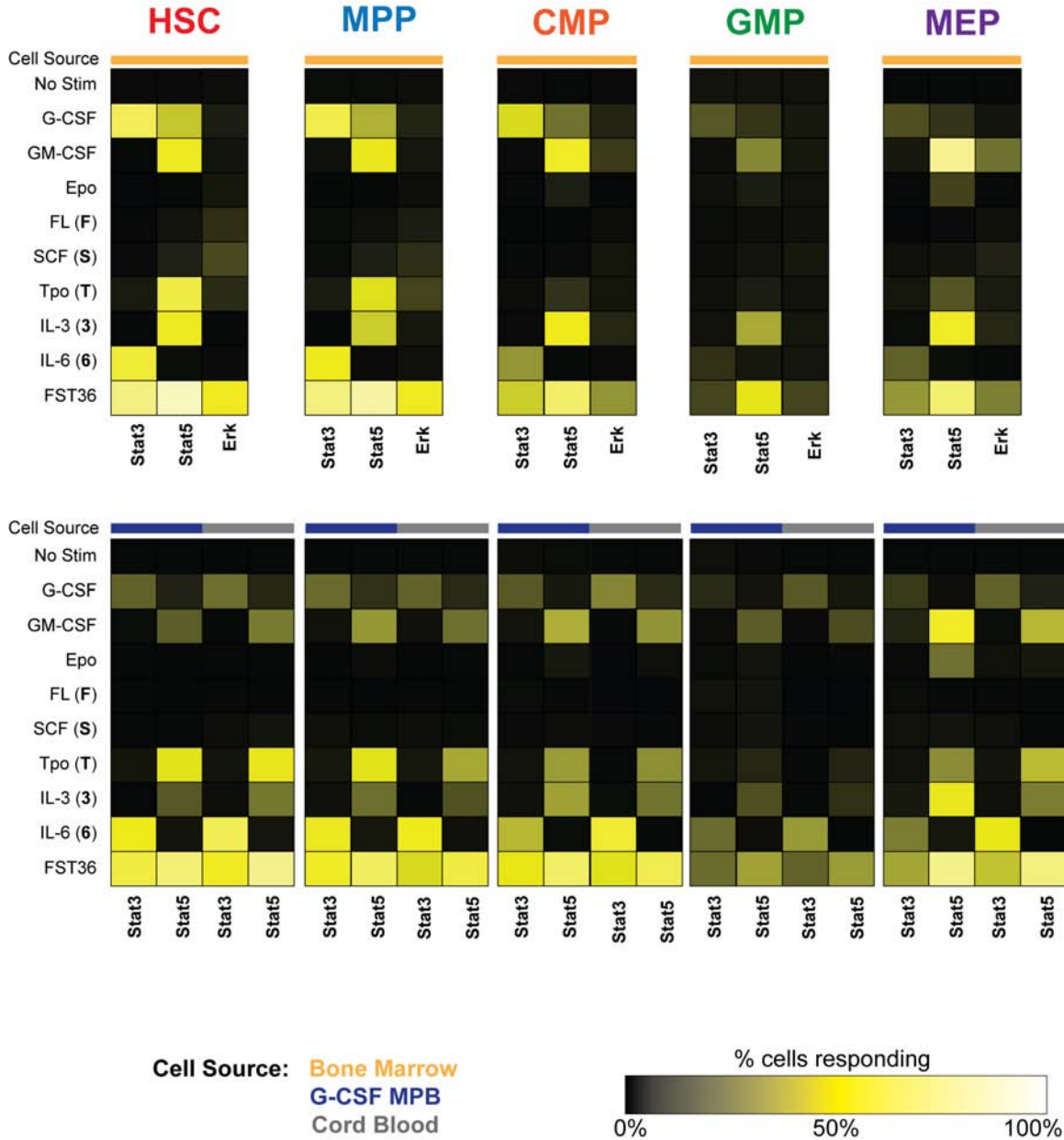


Figure S2, related to Fig. 2. Complete response profile by phospho-node. Phospho-specific response is shown for each stimulus and node tested after 15 minutes of stimulation. Each small bar represents a different biological replicate with HSC, MPP, CMP, GMP, and MEP compartments listed from left to right.

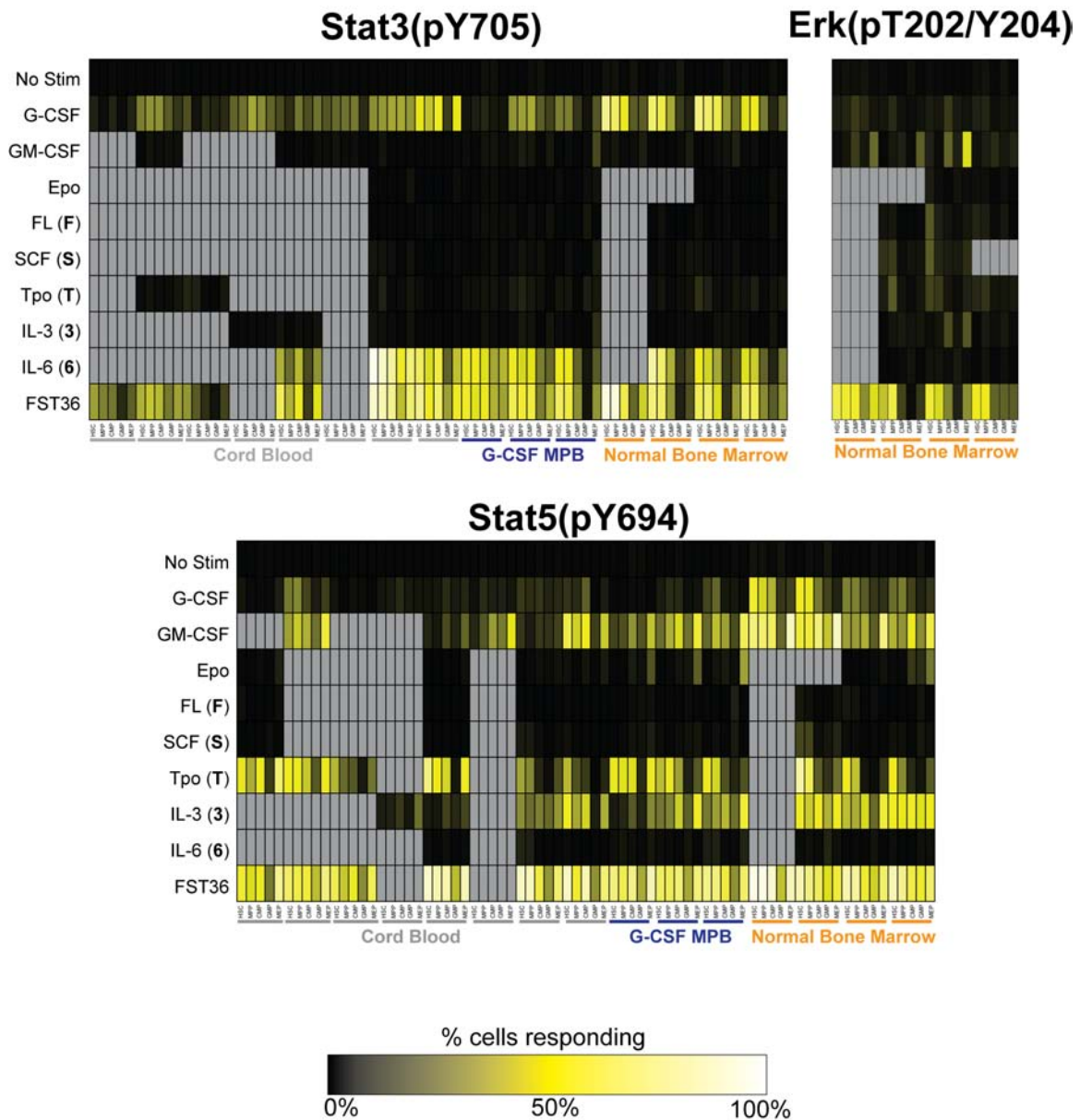


Figure S3, related to Fig. 2. Comparison of JAK/Stat responses in HSC from cord blood, G-CSF mobilized peripheral blood, and normal bone marrow. Percentage of HSC from cord blood, G-CSF mobilized peripheral blood, and normal bone marrow responding to (A) G-CSF via Stat3 phosphorylation, (B) G-CSF via Stat5 phosphorylation, (C) IL-3 via Stat5 phosphorylation, (D) GM-CSF via Stat5 phosphorylation, (E) Tpo via Stat5 phosphorylation, and (F) IL-6 via Stat3 phosphorylation. Statistical differences are noted. p-values determined using pairwise comparison of normal bone marrow to cord blood or G-CSF mobilized peripheral blood by one-tailed t-test.

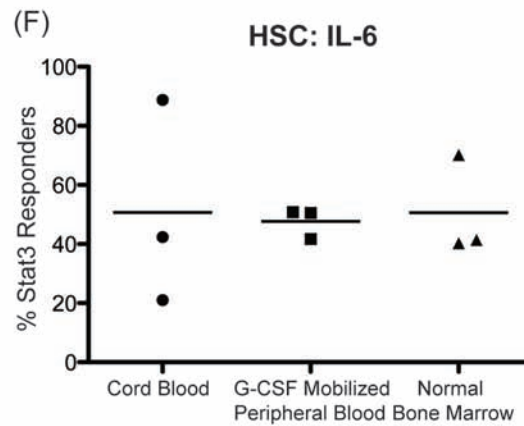
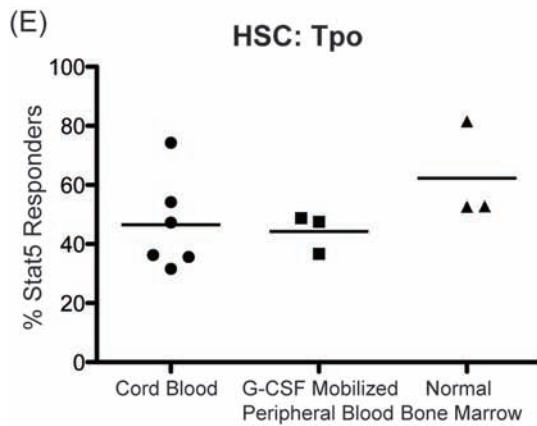
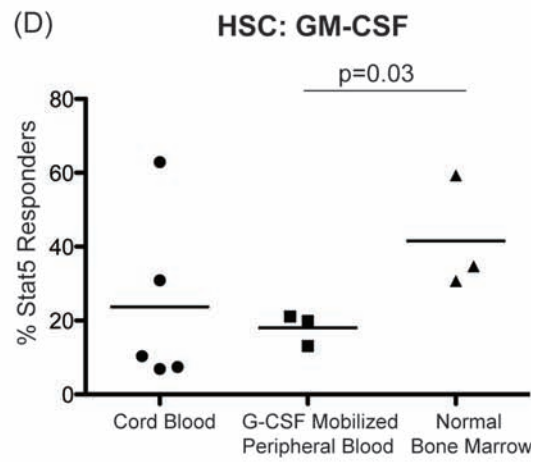
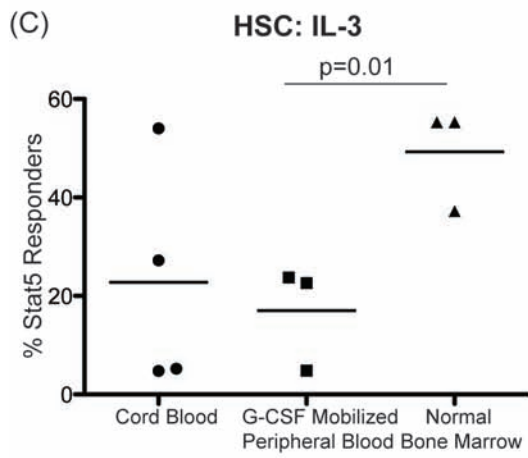
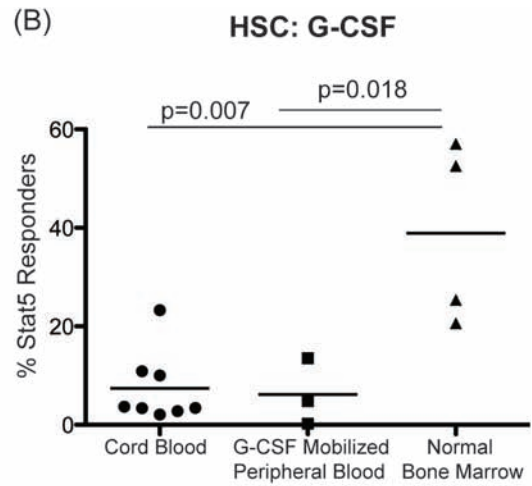
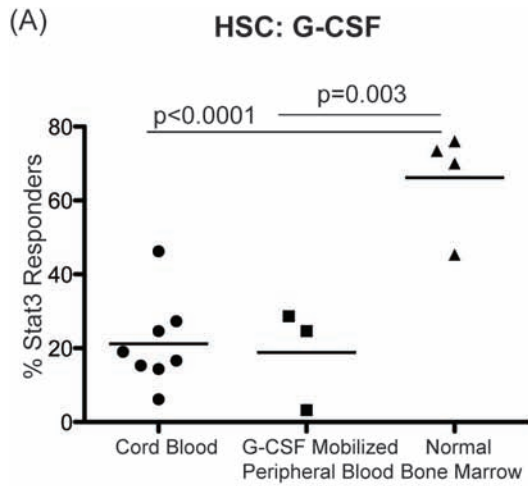
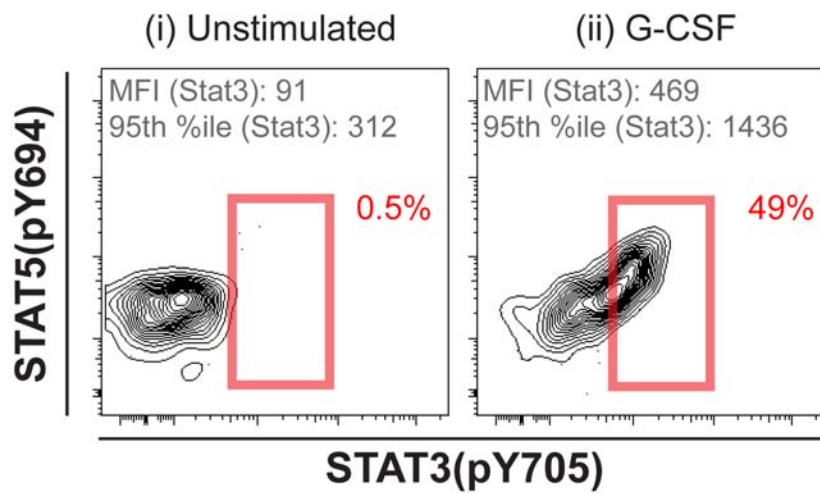


Figure S4, related to Fig. 2. Comparison of G-CSF response in bone marrow HSC and GMP compartments. Flow cytometric plot of phosphorylation of Stat3 (pY705) and Stat5 (pY694) in bone marrow (A) HSC or (B) GMP that were (i) unstimulated or (ii) after 15-minute stimulation by G-CSF. Shaded region represents the percentage of responsive cells in each condition. Stat3 (pY705) median fluorescence and 95th percentile fluorescence are inset for each condition. Plots are representative of four independent biological replicates.

(A) HSC



(B) GMP

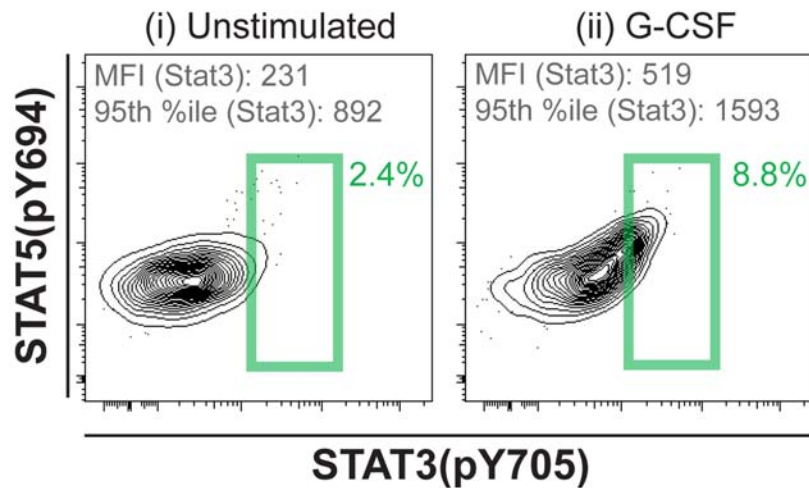


Figure S5, related to Fig. 2. MEP Respond to Epo directly in adult-derived samples. Percentage of MEP from cord blood and adult derived samples (G-CSF mobilized peripheral blood and normal bone marrow) responding to Epo after 15 minute stimulation via Stat5 phosphorylation. p-values determined using pairwise comparison and one-tailed t-test.

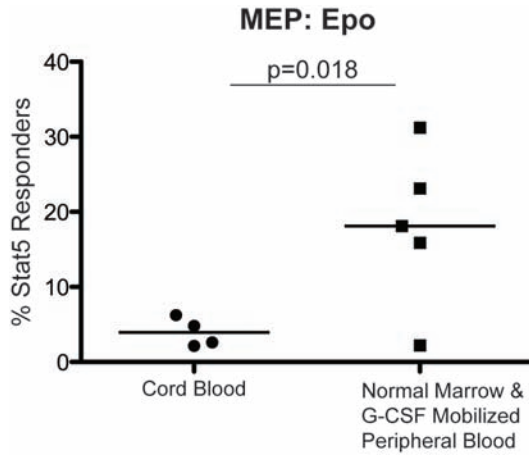


Figure S6, related to Fig. 3. G-CSF drives cord blood HSC to proliferate in the presence of Wnt & Notch ligands. Frequency of proliferation (\geq one cell division) for prospectively sorted, single cord blood HSC plated on hydrogel microwells in serum-free, defined media supplemented with FL, SCF and a cocktail of Wnt and Notch ligands (Wnt1, Wnt3a, Jagged 1, Jagged 2, and DLL4, collectively “FS/Stem”) alone or in combination with 40 ng/ml G-CSF. Differences between proliferation frequency at 80 hours were statistically significant ($p=0.0012$, paired, one-tailed t-test).

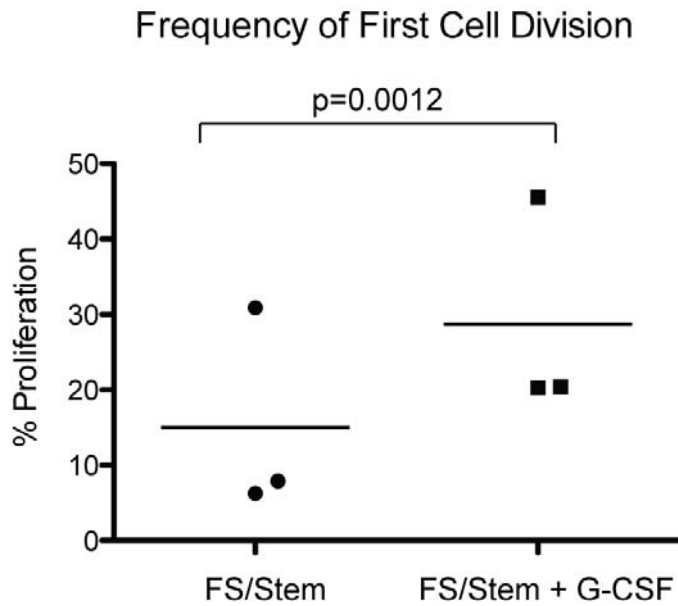
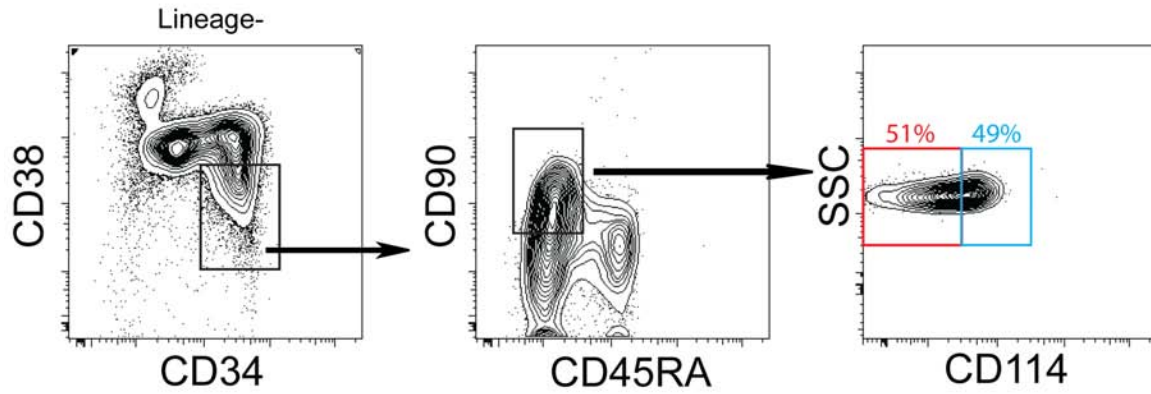
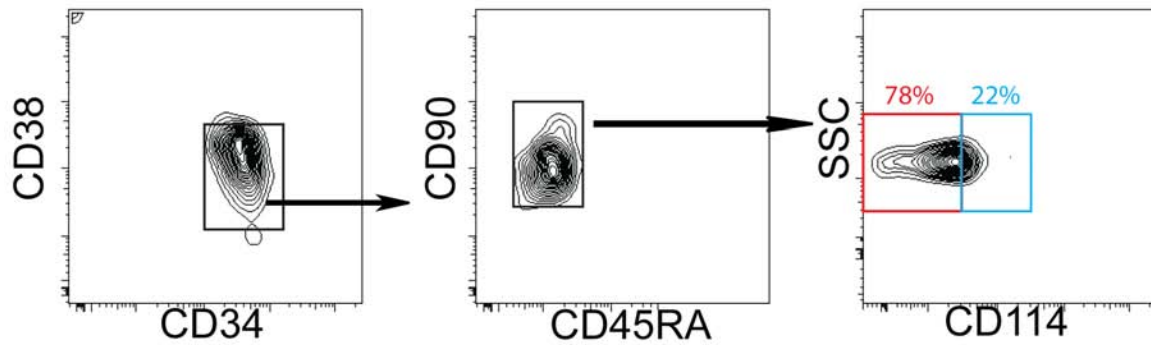


Figure S7, related to Fig. 4. Efficiency of bone marrow HSC purification based on CD114 expression. Flow cytometric analysis of human bone marrow HSC compartment stained for CD114 (a) unsorted, (b) post sort for CD114^{neg/lo}, and (c) post sort for CD114^{pos}. CD114 gates drawn based on staining of isotype control.

(A) Pre-Sort



(B) Post-Sort: CD114 lo



(C) Post-Sort: CD114 hi

