Supplemental Materials:

The BET inhibitor CPI203 promotes ex-vivo expansion of cord blood long-term repopulating HSCs and megakaryocytes

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Supplemental Methods

Cell isolation

Human umbilical cord blood (UCB) was collected from the John Radcliffe Hospital, Oxford, UK or provided via the NHS Cord Blood Bank, London, and used with informed, written pre-consent and ethical approval (REC Ref. no. 15/SC/0027) from the South Central Oxford C and Berkshire Ethical Committees and approval of the NHSBT R&D committee. Mononuclear cells (MNCs; density <1.077g/ml) were isolated by density gradient centrifugation no more than 24 hours after UCB collection. Human CD133+ hematopoietic stem and progenitor cells (HSPC) were enriched by MACS using the CD133 direct microbead kits (Miltenyi Biotec GmbH) and cryopreserved until use[s1,s2]. Purity of the cell

isolation was assessed by flow cytometry and only donor samples with >90% UCB CD133+ cell purity were used for expansion experiments.

Expansion culture

Cells were thawed and cultured overnight (20-24hs) in 24-well flat bottom plates at 50,000 cells/ml in serum-free Stem Span ACF media (Stem Cell Technologies) supplemented cytokines (SCF, FLT-3L (both at 100ng/ml) and TPO (20ng/ml) all from R&D Systems) at 37^{o}C, 5% CO_2, 95% humidity. On day 0, cells were harvested, counted using Countbright absolute counting beads (Molecular Probes) by flow cytometry and plated onto Corning 6 or 24 well plates at an optimised cell density of 5000 cells/ml in Stem Span ACF supplemented with 3 cytokines as described[s3]. Cells were either treated with the small-molecules[s4,s5,s6] at various concentrations in DMSO or an equivalent amount of vehicle (0.1% DMSO, Sigma) then harvested for downstream assays on days 2-5. For *in vivo* studies, in all cases 5000 cells were seeded per well in 24-well plates. On day 5, one-tenth of the cells were harvested from one well and these were considered to be the progeny of 500 cells. Similarly, one-fifth of one well was considered to be the progeny of 1000 cells. Cells from two wells were considered to be the progeny of 10000 cells and 10 wells as the progeny of 50000 cells.

For the extended expansion culture without CPI203, expanded cells from either vehicle or CPI203 treated conditions were harvested at day 5 and resuspended in megakaryocyte expansion media (100ng/ml TPO and SCF 50ng/ml) for another five days for MK and ploidy analysis. The media used were Stemspan SFEM (StemCell Technologies #09650) + 1% Pen/Strep supplemented with recombinant human cytokine cocktail mixes (Peprotech) as previously described[s7].

Flow cytometry and immunofluorescent staining

Multicolour flow cytometry characterisation of cells was performed according to Notta *et al.* [s3,s8]. The antibodies used are listed in Supplemental Table 1. Briefly, cells were resuspended in human FcR blocking reagent diluted in MACS buffer (both from Miltenyi Biotec) and incubated for 10 min on ice. The cells were then incubated with a mixture of fluorescently labeled antibodies diluted in MACS buffer for 20 min on ice. Cells were washed once, resuspended in MACS buffer and acquired by flow cytometer. DAPI (Invitrogen) was added at 100ng/ml directly before acquisition to distinguish live and dead cells. HSPC subsets were determined by gating on single viable, Lin- (CD2, CD3, CD4, CD7, CD8a, CD10, CD11b, CD14, CD19, CD20, CD56 and CD235ab) cells, and defined as HSC: Lin-CD38low\-CD34+CD45RA-CD90+CD49f+; MPP: Lin-CD38low\-CD34+CD45RA-CD90-; LMPP: Lin-CD38low\-CD34+CD45RA+CD90-; CMP: Lin-CD38+CD34+CD45RA-CD123+; GMP: Lin-CD38+CD34+CD45RA+CD123+; and MEP: Lin-CD38+CD34+CD45RA-CD123-. For megakaryocyte ploidy analysis, cells were stained with CD41 and CD42 for 20 minutes at 4°C, then centrifuged, suspended and incubate with Hoechst for 30 minutes at 37°C.

Flow cytometer was performed on FACSLSRII or X50 (BD Biosciences) using FACSDIVA™ software v.8.0.3. Gates were set using strict fluorescence-minus-one controls run for each sample and experiment. Data were analyzed on FlowJo software v.10.5.3 (Tree Star). In some cases, sorting of these subpopulations was carried out on a FACS Aria FusionII (BD Biosciences). All cell counting was performed on aliquots of cell suspension using Countbright absolute counting beads (Molecular Probes) by flow cytometry.

Apoptosis assay

After 5 days of culture, cells were resuspended in wells by pipetting up and down. 200ul of cell suspension was taken from each well and stained with Annexin V FITC (BD Biosciences) according to the manufacturer's instructions. Propidium iodide was added at 100ng/ml directly before acquisition on a LSRII flow cytometer (BD Biosciences). Cells were characterised as apoptotic (AnnexinV+/PI-) and dead (AnnexinV+/PI+) cells.

LTC-IC and CFU assays

Long-term culture-initiating cells (LTC-ICs) assay were analysed following the protocol described previously[s3]. The murine stromal cell lines (M2-10B4 and SL/SL mixed at 1:1) were irradiated with 8000cGy and plated into 96-well collagen-coated microtiter plates (5,000 cells/well of each cell line) and cultured in long-term culture medium (MyeloCult H5100; Stem Cell Technologies) supplemented with hydrocortisone 21–hemi-succinate (10^{-6}M). The Lin-CD34+CD38-CD45RA-CD90+CD49f+ cells were plated at 10, 26, 50, 100, 150 and 200 cells per well by flow sorting (BD FACSAria Fusion II, 70uM nozzle, single cell mode, n=4-10). Co-cultures were maintained at 37°C in high humidity and with 50% medium exchange every week. After 6 weeks, all cells were plated in methylcellulose cultures supplemented with complete methylcellulose-based medium (MyeloCult H4435; Stem Cell Technologies). Long-term culture colony-forming cells (LTC-CFCs; readout of LTC-IC assay) were scored after an additional 14-16 days of culture. Only wells observed with colonies from all lineages (GM and BFU-E and/or GEMM) were counted as positive. The number of negative wells were analyzed by LDA software for estimating the frequency of LTC-IC (http://bioinf.wehi.edu.au/software/elda/)[s9].

High-throughput RNA sequencing and bioinformatics analysis

RNA was isolated from 100,000 CPI203/cytokines (150nM) or vehicle/cytokines (0.1% DMSO) expanded CB CD133+ cells using RNeasy Plus Mini Kit (QIAGEN) according to the manufacturer's protocol. mRNA capture and cDNA synthesis were performed using the KAPA mRNA Hyper Kit for illumine platform (KAPA) according to the manufacturer's protocol. After a quality check using the Qubit High-sensitivity kit (Life Technologies), 16 cycles of PCR were performed for amplifying each library. Each experiment starting from cell expansion was performed in duplicate using cells from three different biological donors. Amplified cDNA libraries were sequenced on an Illumina HiSeq 4000 machine using paired end reads (2 x 150 bp) at Novogene, China. Forty (40) million reads per sample were gained and raw files of the

same sample from different lanes were merged into a single file using Samtools (1.3). Processed files were then aligned using STAR (2.4.2a) against the human genome (UCSC hg38) and the quality was also checked by fastQC (0.10.1). The nonadjusted read counts for each gene were assessed statistically for global differential expression between the specified populations using the edgeR package (3.22.2) on R (3.5.1). Genes that are significant at a 1% false discovery rate (calculated using a BenajminiHochberg adjusted p-value) were considered differentially expressed between populations described[s10]. Gene ontology enrichment was assessed using the topGO package (2.32.0) and pathway enrichment was analysed on the ReactomePA package (1.24.0). Sequencing data have been submitted to the NCBI Gene Expression Omnibus (GSE GSE140813). UM171 and SR1 expanded cells' RNA sequencing results were obtain as raw fastq files from SRA (UM171: 35nM for 72h treatment, GSM1379040, SR1:500nM, 72h, GSM1379039 and DMSO: GSM1379038[s11,s12]). Reads were aligned using STAR (2.4.2a) against the human genome (UCSC hg38) and the read counts (combined with our data of CPI203 and vehicle). RNA sequencing results of primary human bone marrow subpopulations were obtained Gene Expression Omnibus (GEO) via accession GSE74912 and reads were aligned using STAR (2.4.2a) against the human genome (UCSC hg38) and the read counts (combined with our data of CPI203 and vehicle). When combining data sets, batch correction was performed using RUVSeq R package[s13] with the RUVg method and GAPDH, SDHA, B2M as housekeeping genes. Each gene was assessed statistically for global differential expression between the specified populations using the edgeR package (3.22.2) on R (3.5.1). Gene expression data of primary human bone marrow subpopulations (GSE74912) was used to create HSC/MPP UP and DOWN data sets. HSC+MPP samples were compared pairwise against each progenitor cell populations (GMP, CMP, LMPP, CLP, MEP). Genes that were significantly upregulated in all comparisons where used to create the HSC/MPP UP gene set while genes that were significantly down regulated in all comparisons were used to create the HSC/MPP DOWN gene set. Using these gene sets GSEA was performed using the fGSEA R package[s14].

In vivo analyses

Cord blood CD133+ cells with purity higher than 90% were used for *in vivo* analysis. Eight to twelve-week old NOD,B6.SCID Il2rg$^{-/-}$Kit$^{W41/W41}$ (NBSGW) mice[s15] were transplanted with the specified number of cells or the progeny of expansion. Cells were injected into the tail vein. After 20-22 weeks, mice were euthanised by cervical dislocation and peripheral blood (PB), spleen and bone marrow (both femurs, BM) were collected. Spleens were mashed and filtered before analysis. Femurs were mashed with the back end of a syringe plunger in a 24 well plate, washed five times with 2ml PBS and BM collected through a 70mm filter before analysis. PB was collected in tubes containing heparin. Samples were either lysed with 3ml 1x RBC lysis buffer for 5 minutes or were unprocessed for erythrocyte analysis prior to antibody staining. Cell aliquots were stained as above in the Flow Cytometry protocol and the human grafts were analyzed by flow cytometry (BD FACS Canto II). The antibodies used are listed in Supplementary Table 1.

For secondary transplants, bone marrow cells from primary recipient mice in unexpanded, vehicle/cytokine-expanded or CPI203/cytokine-expanded groups (from mice in cell doses of 500, 1000 and 10000 cells) were pooled respectively and then 5 million cells were injected into each secondary recipient mouse. The engraftment of human cells were analysed 22 weeks post-transplantation. All mouse experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986 and using protocols approved by the Committee on Animal Care and Ethical Review at the University of Oxford, under PPL number P8869535A.

Statistical analysis

Statistical analysis was performed on GraphPad Prism 8.0. Data are shown as mean ± SEM. Two-tailed Student's t-tests were performed for statistical analysis between two groups. One-way ANOVA with multi-comparison (Fisher's LSD test) was used to compare differences in means among three groups. N.S $p>0.05$; * $p\leq0.05$; **, $p\leq0.01$; ***, $p\leq0.005$.

Supplemental Table 1. Antibodies Used for Flow Cytometry

Name	Clone	Isotype	Conj.	Company	Dilution
hCD34	581	mIgG1	AF700/APC	BD	1:60
hCD34	Ef780	41211	47-0349 42	Invitrogen	1:20
hCD38	HIT2	mIgG1	PETxR/BB515	BD	1:40
hCD45RA	HI100	mIgG2bk	APC-H7	BD	1:50
hCD90	5E10	mIgG1	PE	Biolegend	1:50
hCD49f	GoH3	rIgG2a	PE-Cy7	Biolegend	1:120
hCD133	293C3	mIgG2b	APC	Miltenyi	1:30
hCD123	6H6	mIgG1k	PerCP-Cy5.5	Biolegend	1:50
hCD2	RPA-2.10	mIgG1	PE-Cy5	Biolegend	1:150
hCD3	HIT3a	mIgG2a	PE-Cy5	Biolegend	1:200
hCD4	RPA-T4	mIgG1	PE-Cy5	Biolegend	1:150
hCD7	CD7-6B7	mIgG1	PE-Cy5	Biolegend	1:150
hCD8a	RPA-T8	mIgG1	PE-Cy5	Biolegend	1:200
hCD10	HI10a	mIgG1	PE-Cy5	Biolegend	1:80
hCD11b	ICRF44	mIgG1	PE-Cy5/APC	Biolegend/Invitrogen	1:80
hCD14	61D3	mIgG2a	PE-Cy5/APC	eBio	1:80
hCD19	HIB19	mIgG1	PE-Cy5	Biolegend	1:200
hCD20	2H7	mIgG2a	PE-Cy5	Biolegend	1:150
hCD56	B159	mIgG1	PE-Cy5	BD	1:20
hCD235ab	HIR2	mIgG2b	PE-Cy5/eFluor450	Biolegend	1:1200
hCD41a	HIP8	mIgG1	APC	eBioscience	1:20
hCD41	HIP8	mIgG1k	PE-Cy7	Biolegend	1:20
hCD42b	HIP1	mIgG1k	PE	Biolegend	1:20
hCD71	M-A712	mIgG2a	AF700	BD	1:20
DAPI				Biolegend	100ng/ml
7AAD				eBio	1:200
Hoechst				Thermo Fisher	1:100
hCD45	HI30	mIgG1	APC	Invitrogen	1:10
mCD45	30-F11	RIgG2b	eFluor450	eBio	1:10
hCD3	HIT3a	mIgG2a	PE-Cy5	Biolegend	1:200
hCD19	HIB19	mIgG1	PE-Cy5	Biolegend	1:200
hCD33	HIB19	mIgG1	PE-Cy5	Biolegend	1:200
mT119	TER-119	Rat / IgG2b	FITC	eBio	1:50
Anti-Human Lineage Cocktail 1				BD	1:50

Supplemental Table 2. Determination of SRC (SCID-repopulating cells) frequency in vehicle or CPI203 treated group.

Culture Condition	Number of injected cells		Number of mice with >1% human CD45+ cell chimerism/total number of mice	1/SRC frequency in total number of cells transplanted	80% confidence interval (upper, lower)
Unexpanded	500		1/3	1790	773,4144
	1,000		1/3		
	10,000		3/3		
	50,000		3/3		
	Equivalent starting cell number*	**Actual number of cells injected***			
Vehicle/cytokines-expanded	500	$8.0x10^3±2.5x10^2$	1/3	1790	773,4144
	1,000	$1.6x10^4±1.1x10^3$	1/3		
	10,000	$1.6x10^5±2.3x10^3$	3/3		
	50,000	$8.0x10^5±1.7x10^3$	3/3		
CPI203/cytokines-expanded	500	$3.0x10^3±5.1x10^2$	4/4	1	1,462
	1,000	$5.9x10^3±2.2x10^2$	4/4		
	10,000	$6.1x10^4±0.5x10^3$	3/3		
	50,000	$3.0x10^5±2.6x10^3$	3/3		

* For *in vivo* studies, in all cases 5000 cells were seeded per well in 24-well plates. On day 5, one-tenth of the cells were harvested from one well and these were considered to be the progeny of 500 cells. Similarly, one-fifth of one well was considered to be the progeny of 1000 cells. Cells from two wells were considered to be the progeny of 10000 cells and 10 wells as the progeny of 50000 cells.

Supplemental Table 3. Frequencies of human cells in the NBSGW mouse bone marrow after primary transplantation.

Group	Initiating cell number	% in bone marrow (no RBC) Mean±SEM					% in bone marrow (before RBC-lysis) Mean±SEM
		hCD45+	hCD33+	hCD19+	hCD3+	hCD34+Lin-*	hCD235a+
Unexpanded	50000	84.8±5.5	21.2±4.4	62.3±6.0	1±0.8	11.9±1.3	84.4±1.0
Unexpanded	10000	69.6±5.0	13.7±3.6	52.1±4.7	1.2±0.6	8.4±0.9	80.4±3.6
Unexpanded	1000	8.1±8.2	0±0	0±0	0±0	0.1±0.1	4.8±4.8
Unexpanded	500	0.7±0.6	0±0	0±0	0±0	0±0	3.3±3.1
Vehicle/cytokines-expanded	50000	87.9±8.3	12.4±2.2	67.7±7.6	0.7±0.4	11.8±1.9	87.8±3.7
Vehicle/cytokines-expanded	10000	54.9±23.5	3.2±1.2	46.1±20.4	0.2±0.1	3.7±2.1	63.4±19.2
Vehicle/cytokines-expanded	1000	1.2±0.4	0±0	0.9±0.2	0±0	0±0	3.7±3.5
Vehicle/cytokines-expanded	500	1.5±1.4	0±0	1.5±1.4	0±0	0±0	7.5±6
CPI203/cytokines-expanded	50000	89.8±6.2	12.2±3.9	69.0±6.2	1.2±1.1	14.1±0.9	85.7±3.5
CPI203/cytokines-expanded	10000	60.2±21.7	6.2±2.8	47.4±16.6	0.4±0.1	4.6±2.3	68.6±17.4
CPI203/cytokines-expanded	1000	35.8±10.0	2.1±1.1	31.8±8.8	0.2±0.1	0.2±0.1	25.9±9.6
CPI203/cytokines-expanded	500	23.1±5.9	0.7±0.2	20.1±5.9	1.4±1.2	0.2±0.1	40.1±15.2

*Lineage here includes CD3, CD14, CD16, CD19, CD20 and CD56

Supplemental Table 4. Frequencies of human cells in the NSBGW mouse spleen and peripheral blood after primary transplantation.

Group	Initiating cell number	% in Spleen (no RBC) Mean±SEM	% in peripheral blood (no RBC) Mean±SEM
		hCD45+	hCD45+
Unexpanded	50000	91.4±3.5	34.6±12.8
Unexpanded	10000	75.6±8.6	20.1±4.6
Unexpanded	1000	7.6±7.4	0.4±0.4
Unexpanded	500	2.1±1.4	0.0±0.0
Vehicle/cytokines-expanded	50000	87.3±10.6	47±21.1
Vehicle/cytokines-expanded	10000	51.4±22.8	47±21.1
Vehicle/cytokines-expanded	1000	3.9±3.2	0.4±0.1
Vehicle/cytokines-expanded	500	4.5±3.3	0.2±0.2
CPI203/cytokines-expanded	50000	91.3±5.4	41.3±21.3
CPI203/cytokines-expanded	10000	50.9±15	7.0±4.5
CPI203/cytokines-expanded	1000	38.6±13.1	3.5±2.7
CPI203/cytokines-expanded	500	37.6±13.2	5.8±3.2

Supplemental Table 5. Frequencies of human cells in NBSGW mice after secondary transplantation.

Group	% in bone marrow Mean±SEM						% in Spleen (no RBC) Mean±SEM	% in peripheral blood (no RBC) Mean±SEM
	hCD45+	hCD33+	hCD19+	hCD3+	hCD34+ Lin-*	hCD235a+	hCD45+	hCD45+
Unexpanded	0.4±0.1	0.3±0.1	0.0±0.0	0.19±0.09	0.0±0.0	0.9±0.3	0.2±0.1	0.0±0.0
Vehicle/cytokines-expanded	0.3±0.2	0.3±0.2	0.0±0.0	0.01±0.00	0.0±0.0	0.3±0.0	0.5±0.5	0.1±0.0
CPI203/cytokines-expanded	7.2±2.9	2.0±1.1	2.4±2.1	0.02±0.01	0.4±0.2	3.1±1.0	3.5±1.7	0.8±0.2

*Lineage here includes CD3, CD14, CD16, CD19, CD20 and CD56

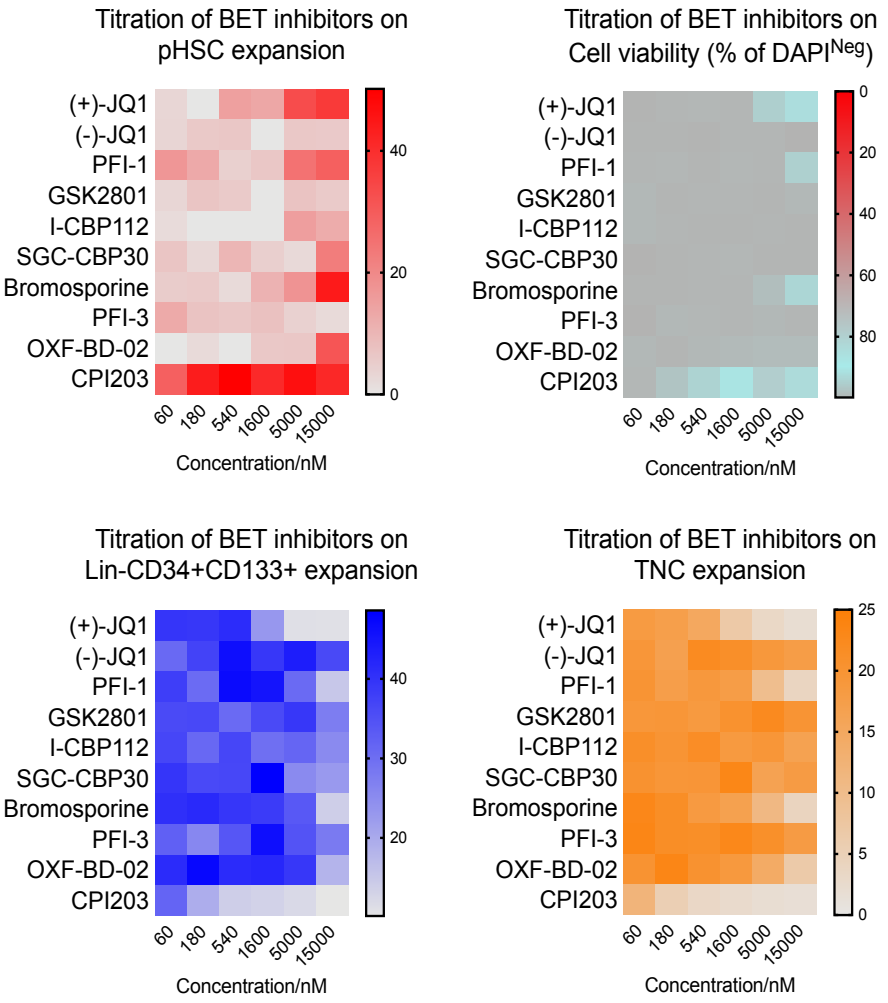
Supplemental Table 6. Top 20 GO biological processes enriched in the differentially expressed genes when comparing vehicle and CPI203 expanded cells

	GO.ID	Term	Annotated	Significant	Expected	Rank in cl	classic	KS	weight
1	GO:0006952	defense response	1518	213	114.44	2	3.6e-20	< 1e-30	3.5e-15
2	GO:0035588	G-protein coupled purinergic receptor si..	23	13	1.73	28	1.3e-09	0.5570	4.9e-09
3	GO:0031295	T cell costimulation	79	22	5.96	56	5.1e-08	0.0023	1.5e-07
4	GO:0015671	oxygen transport	15	9	1.13	75	2.5e-07	0.9804	2.5e-07
5	GO:0032729	positive regulation of interferon-gamma ..	63	17	4.75	102	2.7e-06	3.5e-09	2.7e-06
6	GO:0045055	regulated exocytosis	739	96	55.71	60	8.7e-08	4.2e-17	3.6e-06
7	GO:0006955	immune response	1995	248	150.4	4	1.4e-16	< 1e-30	2.1e-05
8	GO:0048469	cell maturation	155	29	11.69	109	4.3e-06	1.5e-09	2.9e-05
9	GO:0032496	response to lipopolysaccharide	304	46	22.92	114	4.4e-06	2.5e-28	3.4e-05
10	GO:0070374	positive regulation of ERK1 and ERK2 ca	181	30	13.65	165	3.5e-05	1.1e-27	3.5e-05
11	GO:0042493	response to drug	408	54	30.76	166	3.6e-05	1.4e-27	3.6e-05
12	GO:0010771	negative regulation of cell morphogenesi.	76	17	5.73	169	4.0e-05	0.0016	4.0e-05
13	GO:0061614	pri-miRNA transcription from RNA polyme	30	10	2.26	174	4.2e-05	6.0e-05	4.2e-05
14	GO:0007155	cell adhesion	1343	182	101.25	5	1.1e-15	< 1e-30	5.2e-05
15	GO:0045058	T cell selection	46	14	3.47	110	4.3e-06	1.4e-05	5.7e-05
16	GO:0003018	vascular process in circulatory system	154	26	11.61	192	8.3e-05	1.1e-16	5.9e-05
17	GO:0042102	positive regulation of T cell proliferat...	95	19	7.16	189	7.4e-05	1.4e-12	7.4e-05
18	GO:1902105	regulation of leukocyte differentiation	239	38	18.02	130	9.2e-06	3.1e-28	8.6e-05
19	GO:0007626	locomotory behavior	192	30	14.47	202	0.00011	8.6e-08	9.7e-05
20	GO:0010863	positive regulation of phospholipase C a..	35	10	2.64	222	0.00018	0.0096	0.00018

A

Name	Target
(+)-JQ1	BRD2, BRD3, BRD4, BRDT
(-)-JQ1	BRD2, BRD3, BRD4, BRDT (- ent inactive)
PFI-1	BRD2, BRD3, BRD4, BRDT (BET)
GSK2801	BAZ2A, BAZ2B
I-CBP112	CREBBP, EP300
SGC-CBP30	CREBBP, EP300
Bromosporine	pan-Bromodomain
PFI-3	SMARCA,PB1
OXF-BD-02	BRD2, BRD3, BRD4, BRDT (BET)
CPI203	BRD4

B



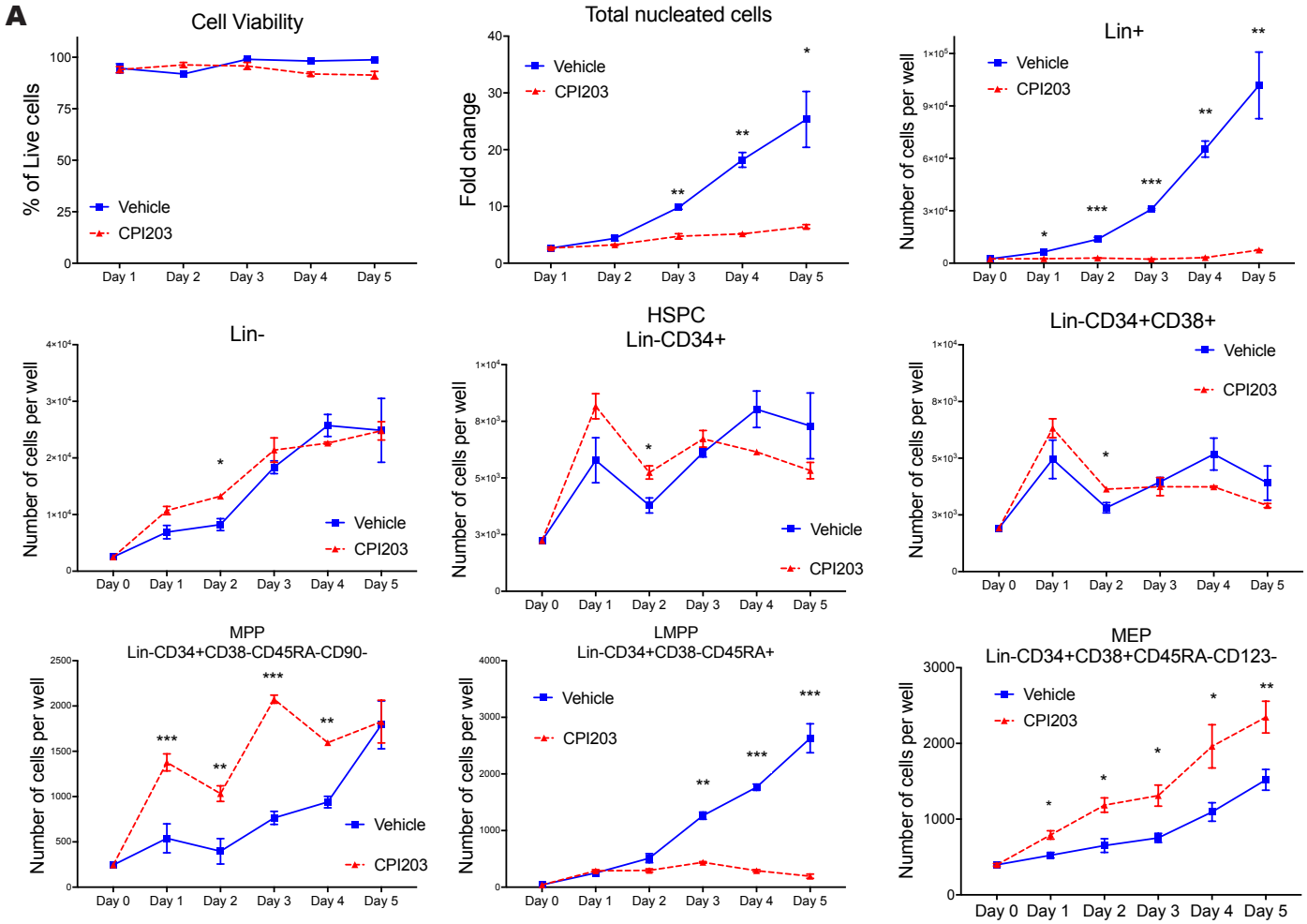
Supplemental Figure 1. Selected BCP inhibitors and the effect on UCB CD133+ cells during 5-day expansion

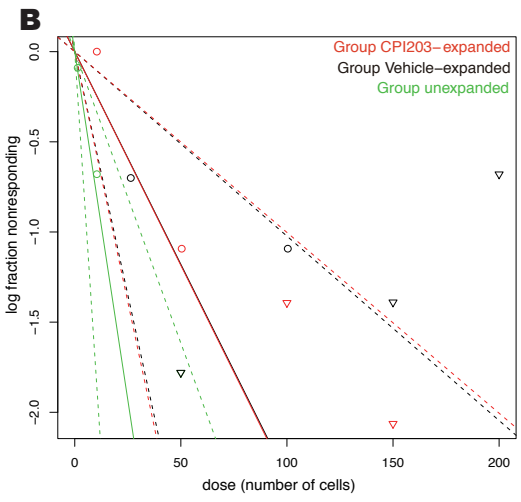
A) Name, structure and targets of selected BCP inhibitors. All BCP inhibitors were tested on CD133+ cells origining from six different biological donors respectively. B) Fold change of pHSC: Lin-CD38-CD34+CD45RA-CD90+CD49f+, Lin-CD34+CD133+ cells, total nucleated cells and the proportion of cells negative with DAPI (before spin down) were shown in various concentrations of BCP inhibitors from 60nM to 15000nM.

Supplemental Figure 2. Assessment of UCB CD133+ HSC and progenitor cells expansion during 5 days culture with cytokines and CPI203 or vehicle

A) Fold change of total nucleated cells, proportion of cells negative with DAPI, absolute number of HSPC: Lin-CD34+, Lin+, Lin- cells, Lin-CD34+CD38+/- cells, MPP: Lin-CD38-CD34+CD45RA-CD90-, LMPP: Lin-CD38-CD34+CD45RA+CD90- and MEP: Lin-CD34+CD38+CD123-CD45RA- were analyzed from 24 hours after treatment until day 5 (n=2-5, *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.005$. Each well seeded with 5000 cells). **B**) Limiting dilution analysis (LDA) for quantitating LTC-ICs present in the unexpanded and CPI203 or vehicle expanded Lin-CD34+CD38-CD45RA-CD90+CD49f+ cell subsets (n=4-10). **C**) Flow plots showing Annexin V+PI- (Apoptotic) and Annexin V+PI+ (Dead) cells in cytokine containing medium supplemented with CPI203 or the vehicle control (n=3) did not differ on day 5 of expansion.

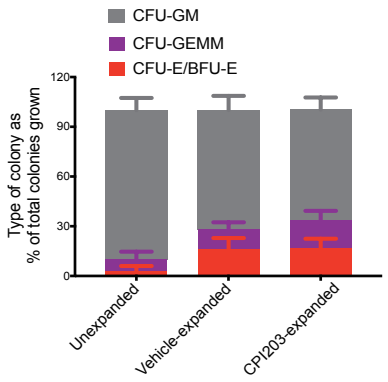
Supplemental Figure.2

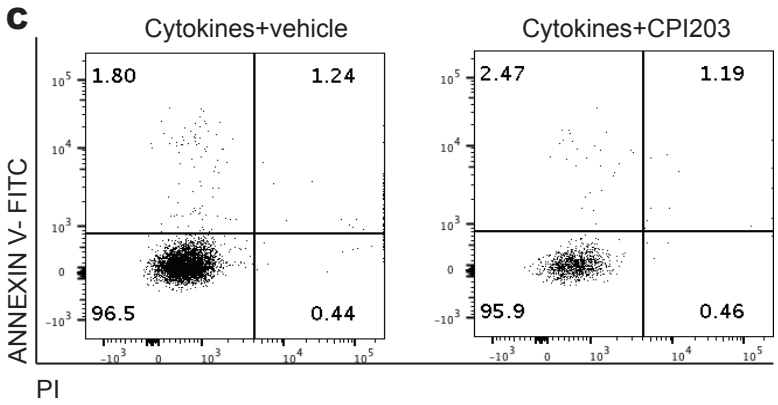




Confidence intervals for 1/(stem cell frequency)

Group	Lower	Estimate	Upper
CPI203-expanded	97.8	42.4	18.5
Vehicle-expanded	99.7	42.2	18.0
unexpanded	30.9	12.9	5.6

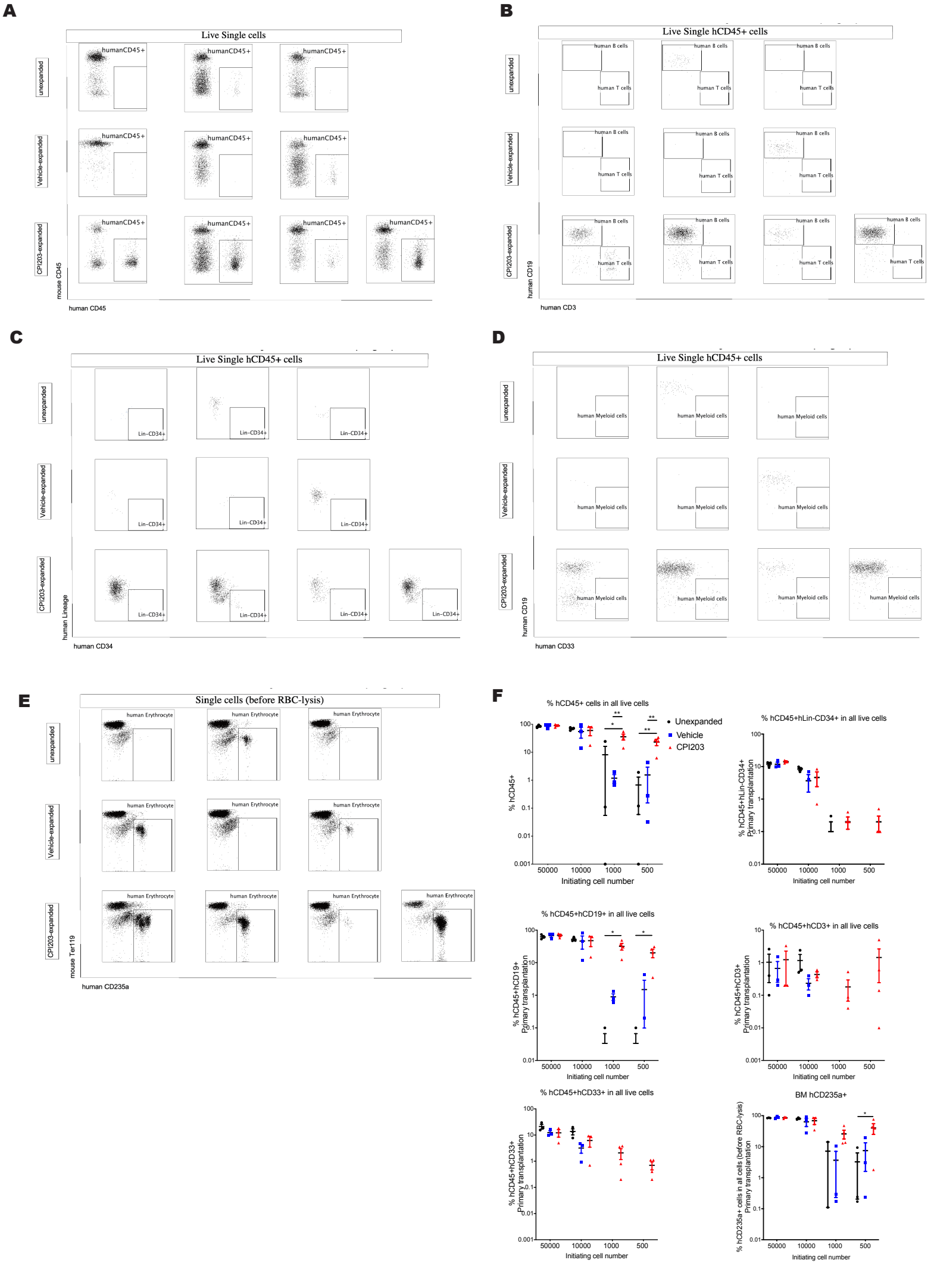


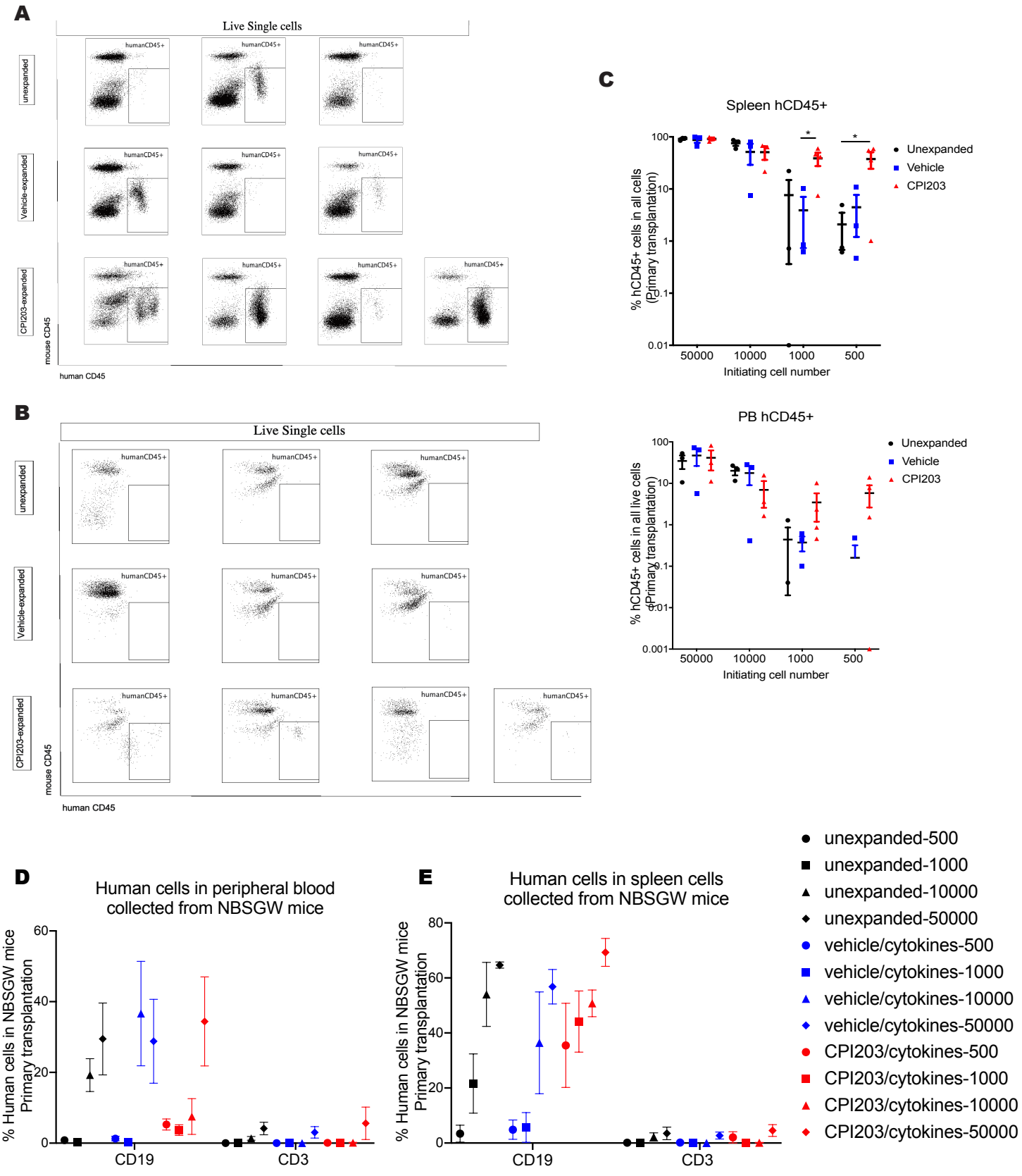


Supplemental Figure 3. *In vivo* analysis of the engraftment capacity in bone marrow of expanded cells in primary transplanted mice

Bone marrow cells harvested from each mouse injected with 500 cells or progeny of that number show significant more human cells in CPI203/cytokines-expansion conditions: **A**) human CD45+; **B**) CD3+ T cells and CD19+ B cells; **C**) Lin-CD34+ HSPC; **D**) CD33+ cells; **E**) CD235a+ cells. **F**) Dot plots showing the quantitative data in all bone marrow cells collected from each mouse injected with varying cell doses (n=3-4; *, $p \leq 0.05$; **, $p \leq 0.01$; One-way ANOVA with multiple comparison (Fisher's LSD)).

Supplemental Figure.3





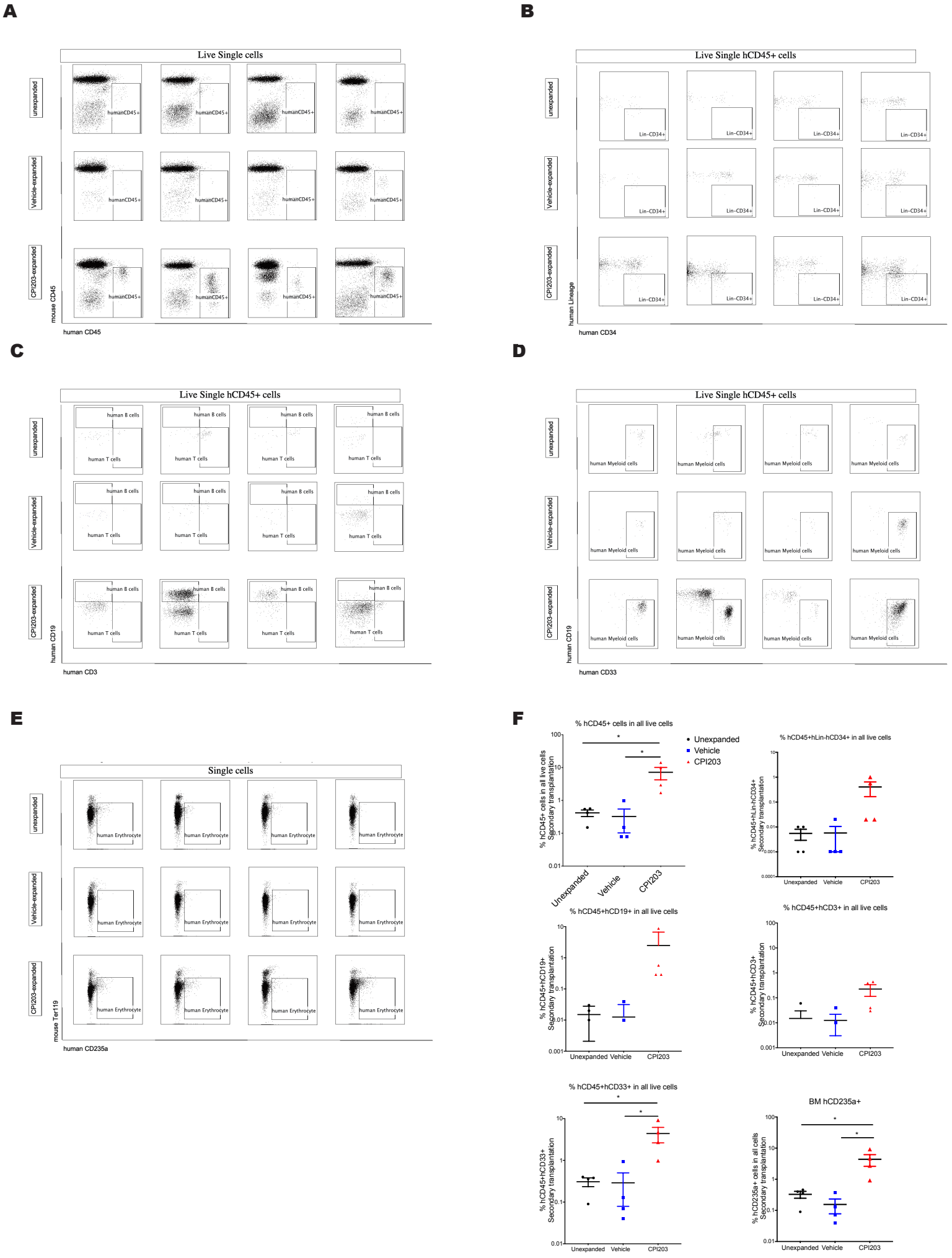
Supplemental Figure 4. In vivo analysis of the engraftment capacity of expanded cells in primary transplanted mice

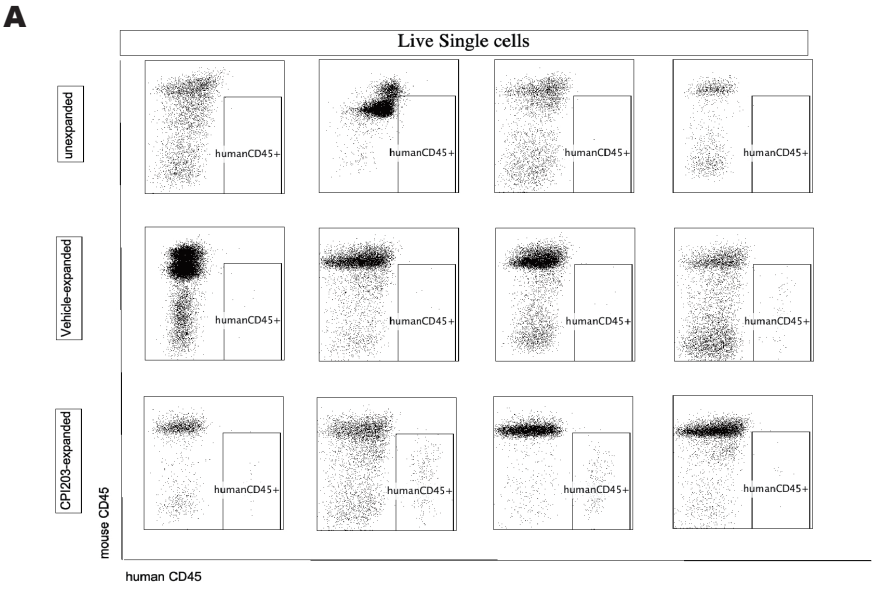
A) Spleen cells harvested from each mouse injected with 500 cells or progeny of that number show significant more human cells in CPI203/cytokines expansion conditions. B) Peripheral blood cells harvested from 500-cell initiated mice show significantly more human cells in CPI203-expansion conditions. C) Dot plots show the percentage of hCD45+ human cells in all spleen or peripheral blood cells analyzed (n=3-4; *, $p<0.05$; One-way ANOVA with multiple comparison (Fisher's LSD)). D)The average reconstitution level of human CD19 and CD3 in peripheral blood samples collected from each group of mice (error bar shows SEM, n=3-4). E) The average reconstitution level of human CD19 and CD3 in spleen samples collected from each group of mice (error bar shows SEM, n=3-4).

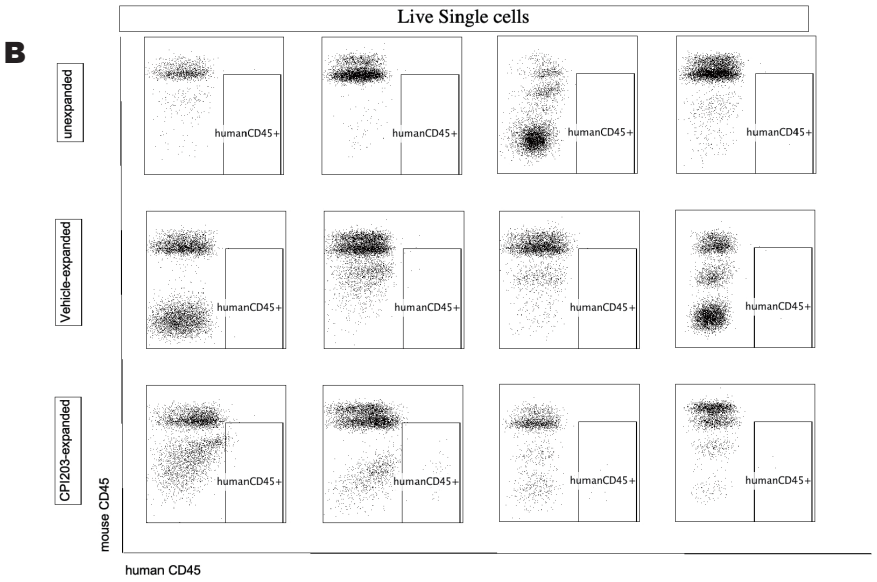
Supplemental Figure 5. *In vivo* analysis of the engraftment capacity of expanded cells in secondary transplanted mice

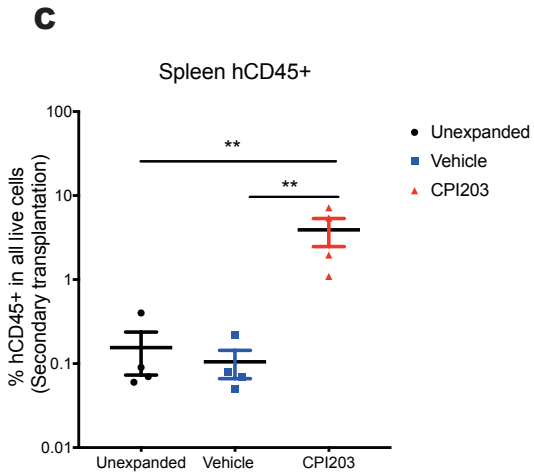
A) Cells from each mouse injected with same amount of bone marrow cells harvested from primary transplanted mice show significantly more human leukocytes in CPI203/cytokines-expanded conditions. **A**) human CD45+ cells; **B**) Lin-CD34+ cells ; **C**) CD3+ cells and CD19+ cells; **D**) CD33+ cells; **E**) CD235a+ cells. **F**) Dot plots showing the quantitative data from all bone marrow cells collected from each mouse (n=3-4; *, $p \leq 0.05$; One-way ANOVA with multiple comparison (Fisher's LSD)).

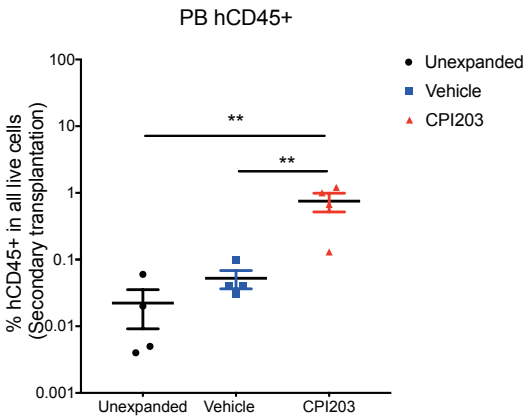
Supplemental Figure.5











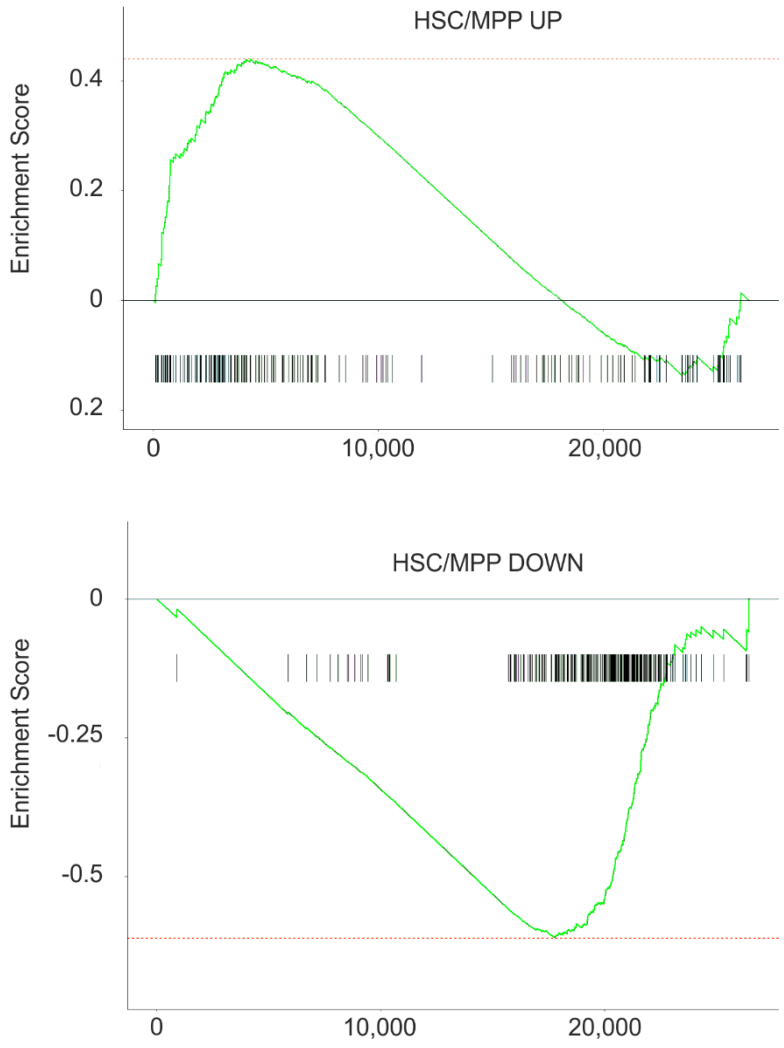
Supplemental Figure 6. In vivo analysis of the engraftment capacity of expanded cells in secondary transplanted mice

A) Spleen cells from each mouse injected with same amount of bone marrow cells harvested from primary transplanted mice show significantly more human cells in CPI203/cytokine-expanded condition. B) Peripheral blood cells from each mouse injected with same amount of bone marrow cells harvested from primary transplanted mice show significantly more human cells in CPI203/cytokines-expanded condition. C) Dot plots show the percentage of hCD45+ human cells in all spleen or peripheral blood cells analysed (n=3-4; **, $p<0.01$; One-way ANOVA with multiple comparison (Fisher's LSD)).

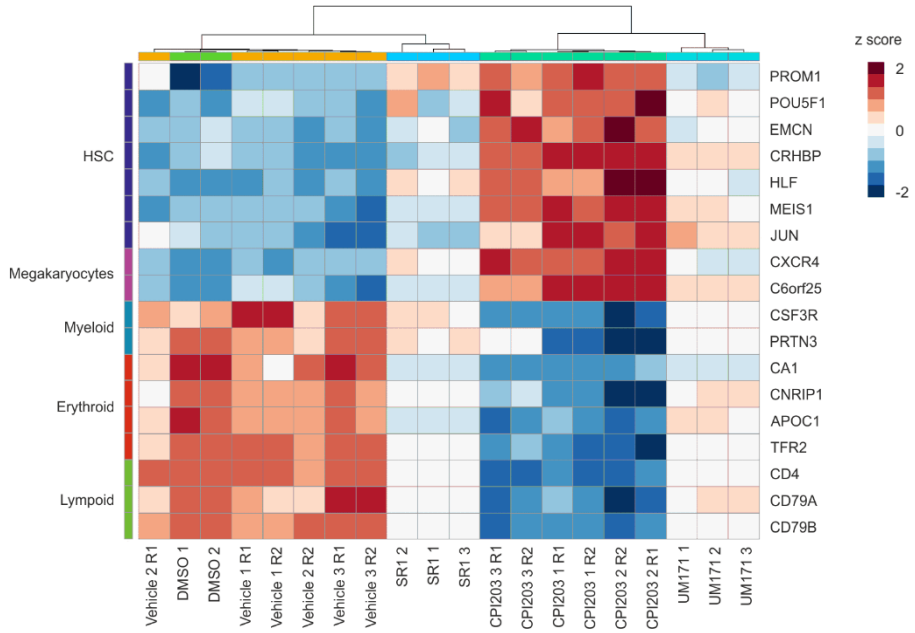
Supplemental Figure7: CPI203 treated cord blood cells maintain an HSC/MPP gene signature.

A) GSEA shows that genes up regulated by CPI203 treatment are significantly ($p < 0.005$) enriched for genes in an HSC/MPP gene set which contain genes up regulated in HSC/MPP cells compared to downstream progenitors (191 genes). Conversely, CPI203 treatment led to down regulation of genes in a gene set which contained genes down regulated in HSC/MPP cells compared to downstream progenitors (226 genes). **B**) Heatmap shows the expression level of selected genes in cord blood cells treated with CPI203 with corresponding vehicle control samples as well as UM171 (35nM, 72h, GM1379040) and SR1 (500nM, 72h, GM13790389) treated cells with corresponding DMSO treated controls (GM13790388).

A.


HSC/MPP UP
Enrichment Score
0.4
0.2
0
0.2
0
10,000
20,000
HSC/MPP DOWN
Enrichment Score
0
-0.25
-0.5
0
10,000
20,000

B.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0
-2
HSC
Megakaryocytes
Myeloid
Erythroid
Lympoid
PROM1
POU5F1
EMCN
CRHBP
HLF
MEIS1
JUN
CXCR4
C6orf25
CSF3R
PRTN3
CA1
CNRIP1
APOC1
TFR2
CD4
CD79A
CD79B
Vehicle 2 R1
DMSO 1
DMSO 2
Vehicle 1 R1
Vehicle 1 R2
Vehicle 2 R2
Vehicle 3 R1
Vehicle 3 R2
SR1 2
SR1 1
SR1 3
CPI203 3 R1
CPI203 3 R2
CPI203 1 R1
CPI203 1 R2
CPI203 2 R2
CPI203 2 R1
UM171 1
UM171 2
UM171 3

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