Supplementary Data

Supplementary Data 1

Supplementary Experimental Procedures

Human cord blood cell isolation

Human umbilical cord blood (CB) was collected from the John Radcliffe Hospital, Oxford, United Kingdom, or the NHS Cord Blood Bank, London, United Kingdom, and used with informed consent and ethical approval from the South Central Oxford C and Berkshire Ethical Committees and approval of the NHSBT R&D committee. Mononuclear cells (MNCs) were isolated by density centrifugation on Ficoll-Histopaque (density <1.077 g/mL) and $CD133^+$ cells were enriched using direct microbead kits (Miltenyi Biotec, Bergisch-Gladbach, Germany) [1–3]. Cell number and viability were assessed using CountBright beads (ThermoFisher) and DAPI (Invitrogen Ltd.), respectively, as described [1,4]. CD133-enriched cells were incubated with FcR block and the relevant antibodies, viz, mouse (m) anti-human PE- or APC-CD133/2 (293C3) and APC- or PerCP-CD34 (AC136 or 8G12, respectively; all from Miltenyi Biotec) or relevant isotype controls (PE- or APC-mIgG2b-PE, APC- or PerCP-mIgG2a; Miltenyi Biotec, or APC- or PeCy5-mIgG2a or APC-mIgG1-APC; BD Biosciences) [1–3]. DAPI (1:50,000 dilution) was added as a viability stain before cells were analyzed on a BD LSR II flow cytometer using the BD FACSDiva 6 software program (both from BD Biosciences) or using FlowJo software (TreeStar, Inc.) as previously described [1–3]. Cell purity precryopreservation was on average 93% ± 3% (mean ± SD; $n = 20$ donors). The cells were cryopreserved in 10% DMSO in fetal calf serum (FCS) and stored at ≤−150°C until use.

Thawing of cryopreserved cells for the CombiCult® screen

Cells were thawed into media consisting of StemSpan SFEM, 10 ng/mL human recombinant thrombopoietin (TPO), 10 ng/mL heparin, 10 ng/mL human recombinant fibroblast growth factor 1 (FGF-1), 20 ng/mL human recombinant stem cell factor (SCF), 100 ng/mL human recombinant insulin growth factor-binding protein 2 (IGFBP2), and 500 ng/mL human recombinant angiopoietin-like 5 (ANGPTL5) (all from R&D Systems) and plated into 96-well round bottom suspension plates (Bibby Sterilin) at 20,000 cells/well in 150 μL of media above and incubated for 1–3 days at 37°C in a humidified incubator with 5% CO_2.

Selection of cytokine combinations for analysis and algorithm development for CombiCult screen design

The experimental matrix comprised three split-pool cycles, with medium changes performed on days 1, 4, and 7. Ten conditions were tested in each cycle, resulting in an experimental complexity of $10 \times 10 \times 10$, which equals 1,000 different combinations. StemSpan SFEM (Stem Cell Technologies), supplemented with 10 ng/mL TPO (R&D Systems), 20 ng/mL SCF (R&D Systems), and 10 ng/mL heparin (Sigma Aldrich Ltd.), was used throughout the entire experiment as basal media.

Medium components for each condition were selected using the following algorithm:

(1) For a chosen number of components, generate all possible combinations of that size from the full set of components, that is, choose exactly k components from a total of n. The number of combinations is given by the binomial coefficient:

$$^{n}C_{k} = \frac{n!}{k!(n-k)!}$$

(2) Zero or more filters are specified and applied to each of the combinations. When the test performed by at least one of these filters satisfied, the combination is removed from the results. Each filter consists of a three-way check per component: PRESENT, MISSING, or EITHER. A test is satisfied when a generated combination matches all such checks.

(3) The remaining combinations are sorted alphabetically by component and tabulated for subsequent analysis.

Thus, certain pairings of components known a priori to be undesirable can be excluded from the screening matrix. In a very general manner, other combinatorial tests can be performed.

In this instance, each condition comprised basal media containing two cytokines and supplemented with four additional cytokines or growth factors known to have stimulatory effects on CB HSC expansion. Initially, all combinations of four components were generated from the set:

{ANGPTL5, DLL1, FGF-1, Flt3L, IGFBP2, OSM; all from R&D Systems}

Subsequently, these ${}^{7}C_{4} = 35$ combinations were filtered by removing any containing one or more of the following pairings, leaving an experimental matrix width of 10 conditions:

FGF-1 + Flt3L
FGF-1 + DLL1
Flt3L + Wnt5a
DLL1 + Wnt5a

An additional software analysis tool was implemented to analyze component commonality across two or more split-pool cycles. First, all possible pathways were generated through the split-pool matrix (for a matrix of width, w, and number of cycles, c, this amounts to a total of w^c possible pathways). Subsequently, a set-wise intersection of the components across all steps in each path was made such that a tally of common component groupings could be made over the entire screen. This operation permitted rapid analysis of the relative frequencies of different conditions given the chosen filtering.

In the first instance, the data were tabulated so that for each hit bead, the presence or absence of each component across all experimental stages is represented horizontally with a binary value. For a chosen number of clusters, the K-medoids algorithm selects cluster centers and then assigns beads to those clusters such that the total Euclidean distance between centers and cluster members is minimized over the entire dataset. Different initial (random) configurations can lead to different final clusterings, so multiple runs are typically performed to ensure optimality.

Supplementary References

1. Gullo F, M Van Der Garde, G Russo, M Pennisi, S Motta, F Pappalardo and S Watt (2015). Computational modeling of the expansion of human cord blood CD133+ hematopoietic stem/progenitor cells with different cytokine combinations. Bioinformatics 31:2514–2522.
2. Pepperell EE and SM Watt (2013). A novel application for a 3-dimensional timelapse assay that distinguishes chemotactic from chemokinetic responses of hematopoietic CD133(+) stem/progenitor cells(). Stem Cell Res 11:707–720.
3. Chang C-H, SJ Hale, CV Cox, A Blair, B Kronsteiner, R Grabowska, Y Zhang, D Cook, CP Khoo, et al. (2016). Junctional adhesion molecule-A is highly expressed on human hematopoietic repopulating cells and associates with the key hematopoietic chemokine receptor cxcr4. Stem Cells 34:1664–1678.
4. van der Garde M, Y van Hensbergen, A Brand, MC Slot, A de Graaf-Dijkstra, A Mulder, SM Watt and JJ Zwaginga (2015). Thrombopoietin treatment of one graft in a double cord blood transplant provides early platelet recovery while contributing to long-term engraftment in NSG mice. Stem Cells Dev 24:67–76.

Supplementary Table S1. Protocols for Validation

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Protocol	*747*	*379*	*245*	*776*	*187*	*277*	*74+*	*3K*	*4K1 (241)*	*444*	*777*	*P1*	*P2*	*Positive control*	*Negative control*
Day 1	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	Flt3L	Flt3L	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	Basal
	IGFBP2	DLL	DLL	IGFBP2	DLL1	DLL1	IGFBP2	DLL1	DLL1	IGFBP2	IGFBP2	IGFBP2	OSM	IGFBP2	
	Flt3L	IGFBP2	Flt3L	Flt3L	Flt3L	Flt3L	Flt3L	OSM	OSM	FGF-1	Flt3L	OSM		FGF-1	
	OSM	OSM	OSM	OSM	IGFB2P	OSM	OSM		ANGL5	OSM	OSM				
							FGF-1								
Day 4	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	ANGLPT5	ANGPTL5	FGF-1	FGF-1	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	Basal
	IGFBP2	IGFBP2	IGFBP2	IGFBP2	OSM	IGFBP2	IGFBP2	ANGL5	ANGL5	IGFBP2	IGFBP2	IGFBP2	OSM	IGFBP2	
	FGF-1	Flt3L	FGF-1	Flt3L	IGFBP2	Flt3L	Flt3L	IGFBP2	IGFBP2	FGF-1	Flt3L	OSM		FGF-1	
	OSM	OSM	OSM	OSM	Wnt-5a	OSM	OSM		OSM	OSM	OSM				
							FGF-1								
Day 7	ANGPTL5	OSM	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	Flt3L	Flt3L	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	ANGPTL5	Basal
	IGFBP2	IGFBP2	IGFBP2	OSM	IGFBP2	IGFBP2	IGFBP2	IGFBP2	IGFBP2	IGFBP2	IGFBP2	IGFBP2	OSM	IGFBP2	
	Flt3L	DLL1	Wnt-5a	Wnt-5a	Flt3L	Flt3L	Flt3L	ANGPTL5	ANGPTL5	FGF-1	Flt3L	OSM		FGF-1	
	OSM	Flt3L	FGF-1	FGF-1	OSM	OSM	OSM		DLL1	OSM	OSM				
							FGF-1								

Basal media: StemSpan SFEM, 20 ng/mL SCF, 10 ng/mL TPO, and 1-ng/mL heparin.

Supplementary Table S2. Media Components Used for Each of 3 Stages of Expansion for Protocol Validation

Protocol	*747*	*7+4*	*379*	*3+7+9*
Day 1	ANGPTL5 IGFBP2 Flt3L OSM	ANGPTL5 IGFBP2 Flt3L OSM FGF-1	ANGPTL5 IGFBP2 OSM DLL1	ANGPTL5 IGFBP2 Flt3L OSM DLL1
Day 4	ANGPTL5 IGFBP2 FGF-1 OSM	ANGPTL5 IGFBP2 Flt3L OSM FGF-1	ANGPTL5 IGFBP2 Flt3L OSM	ANGPTL5 IGFBP2 Flt3L OSM DLL1
Day 7	ANGPTL5 IGFBP2 Flt3L OSM	ANGPTL5 IGFBP2 Flt3L OSM FGF-1	IGFBP2 Flt3L OSM DLL1	ANGPTL5 IGFBP2 Flt3L OSM DLL1

Supplementary Table S3. Schema for Cytokine Addition on Days 0, 2, and 5 to Scaffold-Based Cultures

Protocol and day of medium change	*Cytokine (ng/mL)*								
	SCF	*TPO*	*FLT3L*	*FGF-1*	*OSM*	*ANGPTL5*	*IGFBP2*	*DLL1*	*Wnt5A*
379									
Day 0	20	10	0	0	50	500	100	50	0
Day 2	20	10	100	0	50	500	100	0	0
Day 5	20	10	100	10	50	0	100	50	0
747									
Day 0	20	10	100	0	50	500	100	0	0
Day 2	20	10	0	10	50	500	100	0	0
Day 5	20	10	100	0	50	500	100	0	0
245									
Day 0	20	10	100	0	50	500	0	50	0
Day 2	20	10	0	10	50	500	100	0	0
Day 5	20	10	0	10	0	500	100	0	500
241									
Day 0	20	10	100	0	50	500	0	50	0
Day 2	20	10	0	10	50	500	100	0	0
Day 5	20	10	100	0	0	500	100	50	0
Basal									
Day 0	20	10	0	0	0	0	0	0	0
Day 2	20	10	0	0	0	0	0	0	0
Day 5	20	10	0	0	0	0	0	0	0

Combinations of cytokines added at days 0, 2, and 5 to basal media comprising SCF and TPO in StemSpan ACF serum-free media. Readout at day 8.

Supplementary Data 2

MIFlowCyt Configuration File for Scaffold-Expanded Cells

Experimental overview

1. Purpose

The purpose of the experiment was the analysis of the fold expansion of CB $CD133^{+}CD34^{+}$ and hematopoietic stem and progenitor cell (HSPC) subsets cultured with different cytokine combinations by performing quantitative flow cytometry experiments of 3D nanofiber scaffold cultured cells. This method was capable of determining the importance of sequential exposure of cells to different cytokine signaling inputs. To test the benefits of this method in our 3D scaffold culture, we included our previous observation that the addition of SCF and TPO served as an important starting point in our formulation

of an optimal cocktail for CB HSPC expansion [1]. In the experiments presented here, we chose four formulations from the CombiCult screen for validation and compared this with the basal control, which contained SCF and TPO only. Importantly, we aimed to discover if combinations of factors added at different times throughout the culture period were more powerful at promoting the proliferation of the CB $CD133^+CD34^+$ cells and of the HSC, MMP, CLP, CMP, GMP, and MEP HSPC subsets in ex vivo culture based on phenotypic markers ahead of future in vivo studies in surrogate models.

2. Keywords

Expansion, hematopoietic stem and progenitor cells, cytokines, 3D scaffold, HSPC subsets.

3. Experimental variables

• Fold expansion of total nucleated cells (TNCs)

The fold expansion of TNCs was calculated by dividing the number of viable cells after culture (day 8) by the number of cells before culture (day 0). The average was calculated for three to five CB donors for each cytokine combination.

• Fold expansion of $Lin^-CD133^+CD34^+$ cells

The fold expansion of $Lin^-CD133^+CD34^+$ was calculated by multiplying the fold expansion of TNCs with the percentage of viable $Lin^-CD133^+CD34^+$ cells, which was determined by flow cytometry.

• Fold expansion of HSPC subsets

Cell subsets were defined as single viable cells based on FSC and SSC and DAPI negativity (Fig. 4A). Subsets were then defined as HSC ($Lin^-CD34^+CD38^{dim}CD45RA^-CD90^{++}$), MPP ($Lin^-CD34^+CD38^{dim}CD45RA^-CD90^-$), CLP ($Lin^-CD34^+CD38^{dim}CD45RA^+CD90^-$), CMP ($Lin^-CD34^+CD38^+CD45RA^-CD123^+$), GMP ($Lin^-CD34^+CD38^+CD45RA^+CD123^+$), and MEP ($Lin^-CD34^+CD38^+CD45RA^-CD123^-$). Fold expansion was calculated as described above. The antibodies used are defined below in Supplementary Table S4.

Supplementary Table S4. Antibodies Used for Flow Cytometry

Name	*Clone*	*Isotype*	*Fluorochrome*	*Company*	*Catalogue number*	*Typical dilution used and/or final conc. (μg/mL)*
CD34	581	mIgG1	AF700	BD	561440	1:60
CD38	HIT2	mIgG1	PE TxR	Caltag	MHCD3817	1:40
CD45RA	HI100	mIgG2bk	APC-H7	BD	560674	1:50
CD90	5E10	IgG1	PE	Biolegend	328109	1:50 or 1:25 0.5 μg/mL
CD49f	GoH3	rIgG2a	PE-Cy7	Biolegend	313622	1:120 0.8 μg/mL
CD133	293C3	IgG2b	APC	Miltenyi	130-090-854	1:30
CD123	6H6	mIgG1	PerCp-Cy5.5	Biolegend	306016	1:50 or 1:100
CD2	RPA-2.10	mIgG1	PE-Cy5	Biolegend	300210	1:150 0.16 μg/mL
CD3	HIT3a	mIgG2a	PE-Cy5	Biolegend	300310	1:200 0.1 μg/mL
CD4	RPA-T4	mIgG1	PE-Cy5	Biolegend	300510	1:150 0.6 μg/mL
CD7	CD7-6B7	mIgG1	PE-Cy5	Biolegend	343110	1:150 0.08 μg/mL
CD8a	RPA-T8	mIgG1	PE-Cy5	Biolegend	301010	1:200 0.125 μg/mL
CD10	HI10a	mIgG1	PE-Cy5	Biolegend	312206	1:80 0.625 μg/mL
CD11b	ICRF44	mIgG1	PE-Cy5	Biolegend	301308	1:80 0.625 μg/mL
CD14	6103	mIgG2a	PE-Cy5	eBiocience	15-0149-42	1:80
CD19	HIB19	mIgG1	PE-Cy5	Biolegend	302209	1:200 0.125 μg/mL
CD20	2H7	mIgG2a	PE-Cy5	Biolegend	302307	1:150 0.13 μg/mL
CD56	B159	mIgG1	PE-Cy5	BD	555517	1:20
CD235ab	HIR2	mIgG2b	PE-Cy5	Biolegend	306606	1:1200 0.16 μg/mL
CD133	293C3	mIgG2b	PE	Miltenyi	130-090-853	1:22 for assessing purity of cells after CD133 selection
DAPI			UV 440/40			0.1 μg/mL

4. Organization

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Date:

Experiments were performed between March 2013 and December 2015.

Conclusions:

The fold expansion depended on the number of cytokines and their combination in the experiments presented here.

5. Quality control measures

The performance of the flow cytometer was analyzed daily by running Cytometer Setup and Tracking beads (Life Technologies) according to the manufacturer's recommendations. Appropriate isotype controls for the initial $CD133^+$ cell purity assessment and FMOs were used to assess the level of background staining of the antibodies and to adjust compensation and set gates.

Sample/specimen material description

1. Biological samples

Biological sample description:

70–150 mL of umbilical cord blood (CB) was collected in the John Radcliffe or specified London Hospitals (UCH, Barnett, Watford General, St George's or Northwick Park hospitals) with written consent from the mother and ethical permission and stored at room temperature (20°C–22°C) for up to 24 h until processing.

Biological Sample Source Description:

Homo Sapiens

Biological Sample Source Organism Description:

Taxonomy: Homo Sapiens

Age: Blood was drawn from the placenta and umbilical cord from newborn babies over 38 weeks of gestation

Gender: Unknown

Phenotype: Not applicable

Genotype: Not applicable

Treatment: Mononuclear cells (MNCs) were isolated from cord blood (CB) by density gradient separation. $CD133^+$ cells were isolated from the MNCs using MACS magnetic bead separation. Cells were frozen in 10% DMSO with 90% FCS at −150°C until use.

2. Sample characteristics

On days 0 and 8, cells were analyzed for HSC and HPC subset markers (see list attached and Supplementary Table S3 above).

3. Sample treatment description

- Before expansion: $CD133^+$ cells were isolated using MACS-CD133 beads (Miltenyi Biotec) and assayed for CD133 purity using CD133-PE or CD133-APC antibodies described above (Supplementary Table S3) and DAPI for viability and then cryopreserved. At day −1, cells were thawed by diluting the cell suspension 5× by dropwise addition of warm media (37°C). Cells were rested at RT for 20 min, then washed by centrifugation, and resuspended in StemSpan ACF serum-free expansion medium.
- Cells were precultured for 18–24 h in basal medium comprising StemSpan ACF serum-free medium containing SCF and TPO in 96-well plates at 20,000 cells/well.
- After preculture: Cells were harvested, counted using CountBright beads by flow cytometry, and plated at 2,500 cells/well on 3D scaffold-coated 24-well plates. Cells were cultured in StemSpan ACF serum-free medium supplemented with SCF and TPO and different combinations of cytokines (Supplementary Table S3).
- After expansion: Cells were harvested from the plate and counted using CountBright beads to obtain TNC counts after 8 days of expansion and phenotyping was performed by flow cytometry.
- TNC Quantification: CountBright Absolute counting beads (Molecular Probes) were added to 200 μL of cell suspension (either undiluted or diluted depending on the expected concentration). When quantifying the cells directly after culture (without centrifugation), 200 μL of the harvested cell suspension was added to a 5-mL Falcon tube together with 20 μL of CountBright beads. Just before flow cytometry, 2 μL of DAPI (10 μg/mL) was added to the tube. When quantifying the cells in one sample together with the surface markers, 10 μL of CountBright beads was added with DAPI as above.

- Multicolor flow cytometry staining: Cells were stained in 5-mL Falcon tubes or 96-well V bottom plates depending on the number of samples to be processed. Fc-receptors were blocked with Miltenyi human FcR blocking reagent (1:10 diluted in MACS buffer, 25–100 µL) and cells were incubated for 10 min at 4°C. Antibodies were either added directly to the cell suspension (25 µL cells +25 µL Ab 2×) or alternatively the cells were centrifuged and resuspended in antibody solution (50–100 µL). Final antibody dilutions or concentrations are as indicated in Table S4. Cells were incubated for 30 min and then washed with 1 mL MACS buffer if using 5-mL Falcon tubes or 100 µL MACS buffer if using 96-well plates. After centrifugation at 1,500 rpm for 4.5 min, the supernatant was removed and the cells re-suspended in 200 µL MACS buffer. Just before flow cytometry, 2 µL of DAPI (10 µg/mL) was added to the cells.

4. Multiparameter flow cytometry for HSPC subsets:

Fluorescence Reagent Description

Subset markers are described above and in Supplementary Table S4 and their staining is illustrated in Fig. 4, and lasers and detectors are described below.

Compensation:

Compensation was performed using BD CompBeads (cat no 552843; BD Biosciences), using single stains for antibodies and one unstained sample as well as FMO controls.

Instrument details

1. Instrument manufacturer and model

BD Biosciences, BD LSR II Flow Cytometer.

2. Instrument configurations and settings (Supplementary Tables S5–S7)
3. Flow cells and fluidics

The instrument has not been altered.

4. Light sources

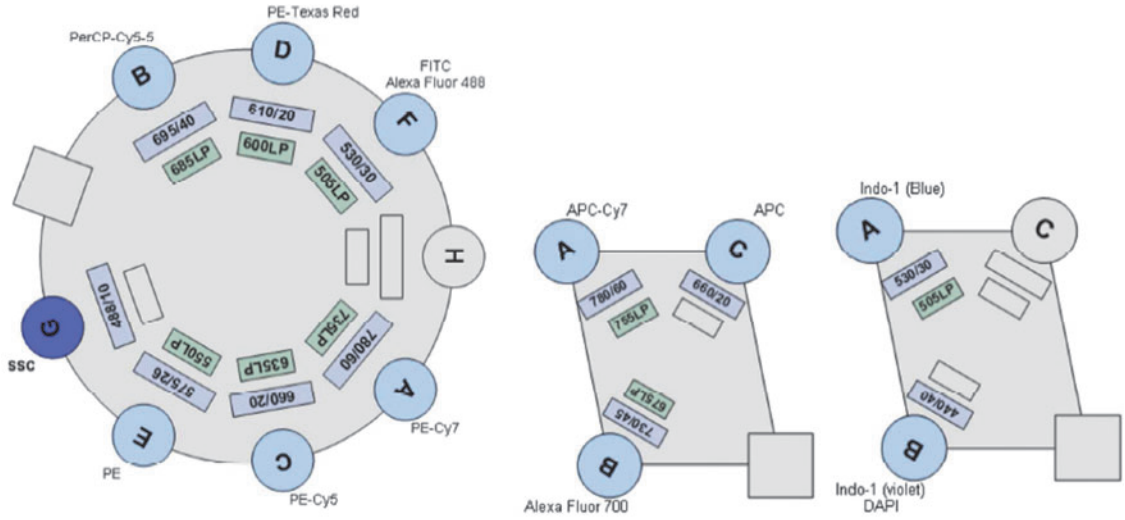
SUPPLEMENTARY TABLE S5. LIGHT SOURCES

Laser		*Type*	*Wavelength*	*Power (mW)*
Blue	Coherent Sapphire	Solid state	488	20
Red	JDS Uniphase	HeNe gas	633	17
UV	Lightwave Xcyte	Solid state	355	20

5. Excitation

The instrument has not been altered.

Optical Paths and Filters



blue laser red laser UV laser

Supplementary Table S6. Flow Cytometer Details

Detector array	*PMT*	*LP mirror*	*BP filter*	*Intended dye*
Octagon, blue laser 488nm	A	735	780/60	PE-Cy7
	B	685	695/40	PerCP-Cy5.5
	C	635	660/20	Pe-Cy5
	D	600	610/20	PE-Texas Red
	E	550	575/26	PE
	F	505	530/30	FITC
	G	—	488/10	SSC
Trigon, red laser 633nm	A	755	780/60	APC-Cy7
	B	675	730/45	Alexa Fluor 700
	C	—	660/20	APC
Trigon, UV laser 355nm	A	505	530/30	Indo-1 (blue)
	B	—	440/40	DAPI

Supplementary Table S7. Detector Voltages

Cytometer parameters	*Fluorophore*	*Voltage*
FSC-A		340
FSC-H		340
FSC-W		340
SSC-A		300
SSC-H		300
SSC-W		300
PE-A	PE	586
PE-Texas Red-A	PE-Texas Red	625
PE-Cy5-A	PE-Cy5	700
PerCP-Cy5-5-A	PerCP-Cy5-5	680
PE-Cy7-A	PE-Cy7	827
Indo-1 (violet)-A	Indo-1 (violet)	350
APC-A	APC	646
Alexa Fluor 700-A	Alexa Fluor 700	597
APC-Cy7-A	APC-Cy7	551

Data analysis details

1. List-mode data files

FSC files can be obtained by contacting Prof. S.M.W. after this work has been published.

2. Compensation

The compensation matrix as shown in Supplementary Table S8 was exported from FlowJoV10.0.6.

Supplementary Table S8. Compensation for Multiparameter Analyses

Compensation LSRII	*PE*	*PE-Texas Red*	*PE-Cy5*	*PerCP-Cy5-5*	*PE-Cy7*	*APC*	*Alexa Fluor 700*	*APC-Cy7*
PE	100	30.06825	28.62304	12.52708	1.888783	0	0.459494	0.08047
PE-Texas Red	13.8167	100	113.7357	57.85912	12.35224	0	0.666783	0.670753
PE-Cy5	1.2061	0.438597	100	47.9836	12.7516	0	0.1321	12.0677
PerCP-Cy5-5	0.2061	0.082454	30.7141	100	33.4804	0.1348	0	2.7106
PE-Cy7	0.8399	0.222049	0.251	0.1844	100	0.1316	0.178	1.5782
APC	0	1	0	0	0	100	11.138	3.3749
Alexa Fluor 700	0	0	0	0	3.4776	1.4718	100	22.867
APC-Cy7	0	0	3.0198	0.3169	4.2881	5.6092	5.4635	100

Data transformation details

1. Data transformation description

FlowJo version 10.0.6 has been used for visualization of the data.

FSC and SCC were displayed with linear scales, all fluorescence parameters were displayed with logarithmic parameters.

Number of decades to display linear converted data: 3
Number of decades to display log converted data: 5
2. Relevant transformation details
FlowJo version 10.0.6 running on Windows 7 was used for transforming the data.

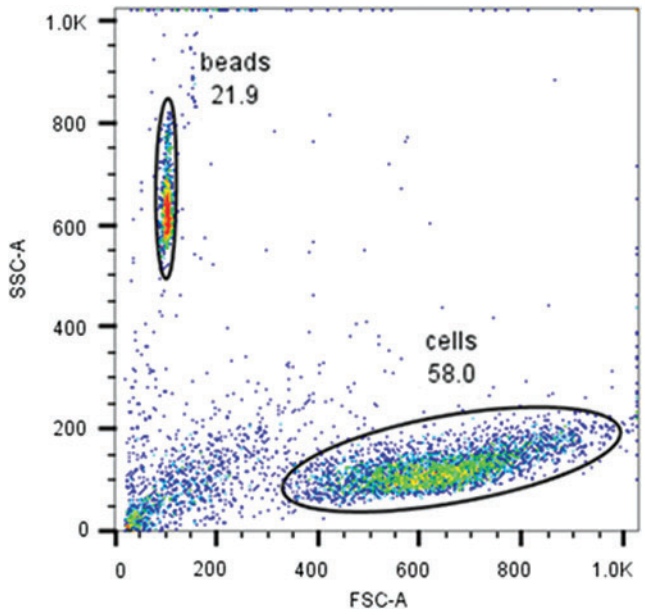
Gating details

1. Gate description
The gating strategy involved the following gates:

- FSC/SSC gate to define cells
- FSC/SSC gate to define CountBright beads
- DAPI/SSC gate to define viable cells
- Gating for cell surface markers as in Fig. 4.

2. Gate statistics
Data can be obtained from FCS files.
3. Gate boundaries

- Those for cell number quantitation through CountBright beads and viability staining are shown in Supplementary Fig. S1.
- Those for multiparameter analyses are shown in Fig. 4A.



SUPPLEMENTARY FIG. S1. FSC/SSC gate to define cells and CounBright beads for cell quantitation.