nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used for data collection.
Data analysis	QuantaSoft Software, version 1.7, Regulatory Edition, for use with all QX100™ and QX200™ Droplet Digital™ PCR (ddPCR™) Systems FlowJo Software (https://www.flowjo.com/solutions/flowjo/downloads) LymphoTrack® Software – MiSeq® Version 2.4.3 package is provided with each LymphoTrack assay (Invivoscribe, cat. no. 92270019) TreeMap Software (https://www.TreeMap.com/download/) Past4: Paleontological Statistics Software Version 4 by Øyvind Hammer (https://www.nhm.uio.no/english/research/resources/past/) IMGT (can be found at https://www.imgt.org/)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The TRB and TRG sequencing data were deposited to the Sequence Read Archive (SRA), under accession number: PRJNA926613.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Anonymous samples were used and therefore sex and gender were not reported.
Population characteristics	Population characteristics were not reported since all samples were anonymous.
Recruitment	Donations of cord blood to public cord blood bank are recruited among labors in the obstetric delivery department. Informed consent is signed that cord blood specimens that are not suitable for banking will be used for research. No compensation is granted upon donation.
Ethics oversight	Cord blood (CB)-derived CD34+ HSPC were obtained from Sheba Medical Center CB bank under Institutional Review Board-approved protocols.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	All data represents minimum N=3 and maximum N=14 different donors obtained from CD34 HSPC cord blood samples. Exact p-value calculations are provided, using different test approaches, adjusted to the sample size and configuration.
Data exclusions	There was no data exclusion in this study.
Replication	All experiments contain minimum N=3 independent biological experiments. All replications were successful.
Randomization	Each CD34 donor was used for all control and test groups in a given replicate, thus eliminating the need for randomization.
Blinding	Blinding was not necessary since all analyses were done in an unbiased way by machines and analytical softwares such as immunophenotyping by FlowJo and TCR analysis by NGS

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
[ ]	[x] Antibodies
[x]	[ ] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[x]	[ ] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

PE/Cy7-anti-CD7 (clone: CD7-6B7, BioLegend), BV421-anti-CD5 (clone: UCHT2, BioLegend), PE-anti-CD1a (clone: BL6, Beckman Coulter), APC-anti-NGFR (clone: ME20.4, BioLegend), PE/Cy7-anti-CD4 (clone: RPA-T4, BioLegend), APC-r700-anti-CD8a (clone: RPA-T8, BD Horizon™) and BV421-anti-CD3 (clone: UCHT1, BioLegend)

Validation

PE/Cy7-anti-CD7 (clone: CD7-6B7, BioLegend - https://www.biolegend.com/en-us/products/apc-anti-human-cd7-antibody-6088?GroupID=BLG10166

BV421-anti-CD5 (clone: UCHT2, BioLegend) - https://www.biolegend.com/en-ie/products/brilliant-violet-421-anti-human-cd5-antibody-7308

PE-anti-CD1a (clone: BL6, Beckman Coulter) - https://www.beckman.pt/reagents/coulter-flow-cytometry/antibodies-and-kits/single-color-antibodies/cd1a/A07742

APC-anti-NGFR (clone: ME20.4, BioLegend) - https://www.biolegend.com/nl-nl/products/apc-anti-human-cd271-ngfr-antibody-6877

PE/Cy7-anti-CD4 (clone: RPA-T4, BioLegend) - https://www.biolegend.com/en-us/products/apc-cyanine7-anti-human-cd4-antibody-1933?GroupID=BLG5901

APC-r700-anti-CD8a (clone: RPA-T8, BD Horizon™) - https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-r700-mouse-anti-human-cd8.565165

BV421-anti-CD3 (clone: UCHT1, BioLegend) - https://www.biolegend.com/de-at/products/brilliant-violet-421-anti-human-cd3-antibody-11976

Flow Cytometry

Plots

Confirm that:

[x] The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

[x] The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

[x] All plots are contour plots with outliers or pseudocolor plots.

[x] A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

CD34+ HSPCs were cultured in the StemSpan™ T-Cell Generation Kit (STEMCELL Technologies, Inc.). For the first 14 days, cells were cultured in StemSpan™ SFEM II medium containing Lymphoid Progenitor Expansion Supplement in plates pre-coated with the Lymphoid Differentiation Coating Material. Cells were then harvested and re-seeded for an additional 14 days in StemSpan™ SFEM II medium contanining the T-Cell Progenitor Maturation Supplement on pre-coated plates. Flow cytometry analysis was conducted on days 14 and 28 of IVTD using the LSR Fortessa™ (BD Biosciences). On day 14, cells were stained with PE/Cy7-anti-CD7 (clone: CD7-6B7, BioLegend), BV421-anti-CD5 (clone: UCHT2, BioLegend), PE-anti-CD1a (clone: BL6, Beckman Coulter), and APC-anti-NGFR (clone: ME20.4, BioLegend) antibodies. On day 28, cells were stained with PE/Cy7-anti-CD4 (clone: RPA-T4, BioLegend), APC-r700-anti-CD8a (clone: RPA-T8, BD Horizon™) BV421-anti-CD3 (clone: UCHT1, BioLegend), and APC-anti-NGFR (clone: ME20.4, BioLegend) antibodies

Instrument

LSR Fortessa™ (BD Biosciences)

Software

FlowJo Software (https://www.flowjo.com/solutions/flowjo/downloads)

Cell population abundance

CD34 HSPCs were sorted and found to be 100% in the post-sort population.

Gating strategy

Gating strategies were based on fluorescence minus one (FMO) plus isotype control samples using the following isotypes: PE/Cy7 Mouse IgG2a κ, (BioLegend), BV421 Mouse IgG1 κ (BioLegend), PE Mouse IgG1 κ (BioLegend), PE/Cy7 Mouse IgG1 κ (BioLegend), APC-r700 Mouse IgG1 κ (BD Biosciences), and APC Mouse IgG1 κ (BioLegend).

[x] Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.