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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

All relevant sample sizes are described in the legend to each figure and/or in the materials and methods section. Sample sizes were determined based on previous experience with each of the experimental systems. Statistical tests were not used to pre-determine sample size.

2. Data exclusions

Describe any data exclusions.

Data exclusions are described in detail in the materials and methods section.
In the case of scRNA-seq data, contaminating cell types and putative cell doublets were excluded from analysis based on criteria that are describe in detail in the method section "Exclusion of contaminating cell types and putative cell doublets".
No data was excluded from mouse or cell biology experiments, with the exception of very occasional technical failure in the experimental procedure such as machine failure during a sorting experiment.

3. Replication

Describe whether the experimental findings were reliably reproduced.

At least two independent experiments were performed for the results in the manuscript, except for inDrops experiments, which were performed on a single basal bone marrow sample pooled from the bone marrow of 10 mice, or on a single Epo-stimulated bone -marrow sample from pooled from the bone marrow of 10 mice; or from a single fetal liver sample pooled from the fetal livers of 10 embryos. Each of the inDrops samples included several thousand cells as indicated in each corresponding figure. All reported data were reproduced reliably.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Experiments entailed manipulating very similar primary cell samples, derived from inbred mice of similar age, sex and weight, making randomization not applicable.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was not used. Readouts were quantitative and not subject to subjective judgment of investigators.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

- FACS data was analyzed using the FlowJo 9.9.5 software.
-Western blots were analyzed using the Biorad Image Lab Software 6.0
-Data analysis and graphics: GraphPad Prism 7.0a, Excel version 15.32
- Python (version 2.7), using scripts described in methods section and code available at:
    - https://github.com/indrops/indrops
    - https://github.com/AllonKleinLab/SPRING
    - https://github.com/AllonKleinLab/PBA

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

there are no restrictions on material availability.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

All antibodies used in this study are commercially available.
Where relevant, in addition to validation provided by the supplier, we carried out control experiments with appropriate cell types or biological samples, included in the relevant figures. For example, western blotting for Gata-1 included a positive control of 3T3 cells transduced with the Gata-1 vector. Similarly, western blotting for replication proteins (PCNA, Mcm helicase subunits) included non-dividing (contact inhibited) 3T3 cells as negative controls, and dividing (log-phase expanding) 3T3 cells as positive controls. In the case of flow cytometry antibodies, we relied on supplier validation, and on the expected flow cytometric pattern being obtained for each antibody, since all antibodies in our study are used routinely.
The following antibodies were used in flow cytometry or magnetic bead sorting ora analysis:
anti-CD11b (Clone M1/70 [#557395], BD Biosciences)
anti-Ly-6G and Ly-6C (Clone RB6-8C5 [#553125], BD Biosciences)
anti-CD4 (Clone RM4-5 [#553045], BD Biosciences)
anti-CD8a (Ly-2) (Clone 53-6.7 [#553029], BD Bioscience)
anti-CD19 (Clone 1D3 [#553784], BD Biosciences)
anti-TER119 (Clone TER119 [#553672], BD Biosciences).
CD117- APC Cy7 (Clone 2B8 [#105826], Biolegend)
TER119-BUV395 (Clone TER-119 [#563827], BD Biosciences)
CD71- PE Cy7 (Clone RI7217 [#113812], Biolegend)
CD55-AF647 (Clone RIKO-3 [#131806], Biolegend)
CD105-PE (Clone MJ7/18 [#120408], Biolegend)
CD150-BV650 (Clone TC15-12F12.2 [#115931], Biolgened)
CD41-BV605 (Clone MWReg30 [#133921], Biolegend)
CD49f (=itga6) – BV421 (Clone GoH3 [#313624], Biolegend)
TER119-BV421 (Clone TER-119 , #116233 Biolegend)
CD71- PE Cy7 (Clone RI7217 , #113812] Biolegend)
CD117- APC Cy7 (Clone 2B8 , #105826 Biolegend)
FcεRIα-AF700 (Clone MAR-1,#134323 Biolegend)
CD41-BV605 (Clone MWReg30 , #133921 Biolegend)
Cd11b-PE Cy5 (Clone M1/70, #101209 Biolegend)
Ly 6G/C- FITC (Clone RB6-8C5, #53126 BD Biosciences).

The following antibodies were used in western blotting analysis:
GATA1 (N6, sc-265, Santa Cruz); beta-actin (ab8227, abcam), MCM5 (Bethyl Laboratories, Inc., A300-195A-M), MCM6 (Bethyl Laboratories, Inc., A300-194A), MCM2 (Bethyl Laboratories, Inc., A300-191A), PCNA (PC10) (Santa Cruz, sc-56), IL-17RA/IL-17R (R&D Systems, AF448).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

3T3-L1 cells were used as controls in some western blots. The entire study was otherwise conducted in primary tissue as indicated (bone marrow or fetal liver)

b. Describe the method of cell line authentication used.

None. The precise identity of the 3T3-L1 cells is peripheral to the study; they were used as controls in the following way:
1. their ability to become contact inhibited allowed us to compare cycling and non-cycling cells, which was a useful way to obtain controls either expressing or not expressing proteins characteristic of S phase
2. they were used as vehicles, for transduction of GATA-1, in order to determine the location/mobility of the GATA-1 band on western blotting.

c. Report whether the cell lines were tested for mycoplasma contamination.

No

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Mice
For scRNA-seq studies: the basal state bone marrow sample (bBM), and for the sorted populations P1 to P5, bone marrow was harvested from 8-week old adult BALB/cJ female mice (Jackson Laboratories, Maine, USA). For the Epo-stimulated adult bone-marrow sample (eBM), 8-week old adult Balb/cJ female mice were injected with Epo (Procrit, Amgen corporation) sub-cutaneously once per 24 hours for a total of 48 hours, at 100 Units/25 g. For the fetal liver sample (FL), BALB/cJ female mice were set up for timed pregnancies, and fetal livers were harvested on embryonic day 13.5.

For other experiments: BALB/cJ male or female mice, ages 8 to 12 weeks were used.

For colony formation assays using IL-17RA-deleted mice: To generate the IL-17RA -deleted line, IL-17RA flox/+ mice (El Malki, K. et al. Journal of Investigative Dermatology 133, 441-451, 2013) were bred with CMV-Cre mice (#003465, JAX lab). The generation of il17ra del allele in the F1 generation of il17ra flox/+ × CMV-Cre mating pairs were screened by PCR of tail DNA. To remove the CMV-Cre allele present in the F1 generation, IL-17RA del/+; CMV-Cre+/– mice were outcrossed with C57BL/6 mice. For colony assays with bone marrow of these mice, control mice were C57BL/6 (Jackson laboratories, stock number 000664).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

N/A

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Please see methods section which details these fully.
6. Identify the instrument used for data collection.	LSRII
7. Describe the software used to collect and analyze the flow cytometry data.	Acquisition: BD FACSDiva Analysis: Flowjo NB: in Figure preparation, axis labels do not provide fluorochromes, but these are fully detailed for each antibody in the methods section.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Post-sort fractions are indicated on each flow plot as percentages. Sub-populations P1 to P5 were sorted with purities of >90%.
9. Describe the gating strategy used.	Gating strategies are fully described in the relevant figures (Fig 2a, 6d)

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