EMBO reports

Pyruvate Metabolism Guides Definitive Lineage Specification During Hematopoietic Emergence

Leal Oburoglu, Els Mansell, Isaac Canals, Valgardur Sigurdsson, Carolina Guibentif, Shamit Soneji, and Niels-Bjarne Woods
DOI: 10.15252/embr.202154384

Corresponding author(s): Leal Oburoglu (leal.oburoglu@med.lu.se) , Niels-Bjarne Woods (niels-bjarne.woods@med.lu.se)

Review Timeline:

Submission Date:	24th Nov 21
Editorial Decision:	25th Nov 21
Revision Received:	30th Nov 21
Accepted:	30th Nov 21

Editor: Deniz Senyilmaz Tiebe

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Leal Oburoglu and Niels-Bjarne Woods
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2021-108402

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	No sample size calculation was performed, data was replicated at least 3, up to 6 times and appropriate statistical analyses were applied.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	No sample size calculation was performed, data was replicated with at least 6, up to 16 mice and appropriate statistical analyses were applied.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No data were excluded from the analyses.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Randomization was not required; control and experimental groups derived from the same sample.
For animal studies, include a statement about randomization even if no randomization was used.	Randomization was not required; control and experimental groups derived from the same sample.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Blinding was not relevant to this study.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Blinding was not relevant to this study.
5. For every figure, are statistical tests justified as appropriate?	Significance of differences between conditions were calculated using paired/unpaired t-tests, 1/2-way analysis of variance (ANOVA) tests or Kruskal-Wallis tests with multiple comparisons in GraphPad Prism 6 software, as indicated. p values are indicated in figures with the following abbreviations: ns, not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Gaussian distribution was not assumed unless the n is high enough to perform D'Agostino-Pearson and Shapiro-Wilk normality tests.
Is there an estimate of variation within each group of data?	Standard error of mean is reported for all data in this manuscript.
Is the variance similar between the groups that are being statistically compared?	Statistical analysis were performed by paired t tests (in order to exclude variability due to hematopoietic differentiation efficiency) or ANOVA if more than two groups were analyzed.

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com	Antibodypedia
http://1degreebio.org	1DegreeBio
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repor	ARRIVE Guidelines
http://grants.nih.gov/grants/olaw/olaw.htm	NIH Guidelines in animal use
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm	MRC Guidelines on animal use
http://ClinicalTrials.gov	Clinical Trial registration
http://www.consort-statement.org	CONSORT Flow Diagram
http://www.consort-statement.org/checklists/view/32-consort/66-title	CONSORT Check List
http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tumc	REMARK Reporting Guidelines (marker prognostic studies)
http://datadryad.org	Dryad
http://figshare.com	Figshare
http://www.ncbi.nlm.nih.gov/gap	dbGAP
http://www.ebi.ac.uk/ega	EGA
http://biomodels.net/	Biomodels Database
http://biomodels.net/miriam/	MIRIAM Guidelines
http://jjj.biochem.sun.ac.za	JWS Online
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html	Biosecurity Documents from NIH
http://www.selectagents.gov/	List of Select Agents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Flow cytometry, Anti-human antibodies CD144 (VE-Cadherin), Clone 55-7H1BD, Biosciences, Cat# 561566, RRID:AB_10715835; CD73, Clone AD2, BD Biosciences, Cat# 550257, RRID:AB_393561; CD14, Clone 63D3, BioLegend, Cat# 367104, RRID:AB_2565888; CD11b, Clone ICRF44, BioLegend, Cat# 301310, RRID:AB_314162; CD33, Clone WM-53 (WM53), Thermo Fisher Scientific, Cat# 25-0338-42, RRID:AB_1907380; CD43, Clone 1G10, BD Biosciences, Cat# 655407; CD34, Clone 561, BioLegend, Cat# 343604, RRID:AB_1732005; CD90, Clone 5E10, BD Biosciences, Cat# 562685, RRID:AB_2744468; CD38, Clone HIT2, BioLegend, Cat# 303516, RRID:AB_2072782; GPA, Clone HIR2 (GA-R2), Thermo Fisher Scientific, Cat# 48-9987-42, RRID:AB_2574141; CD45, Clone HI30, BioLegend, Cat# 304024, RRID:AB_493761; CD184 (CXCR4), Clone 12G5, BD Biosciences, Cat# 555976, RRID:AB_398616; 7-AAD, Sigma-Aldrich, Cat# A9400; CD8-BV510, Clone SK1, BioLegend Cat# 344732, RRID:AB_2564624; CD4-APC-Cy7, Clone OKT4, BioLegend Cat# 317418, RRID:AB_571947; CD56, Clone NCAM16.2, BD Biosciences Cat# 564058, RRID:AB_2738569; CD49f, Clone eBioGoH3 (GoH3), Thermo Fisher Scientific Cat# 25-0495-82, RRID:AB_10804881; CD45RA, BD Biosciences Cat# 560362, RRID:AB_1645575; CD19, Clone SJ25C1, BD Biosciences Cat# 562653, RRID:AB_2722592.
	Flow cytometry, Anti-mouse antibodies Ly-6G/Ly-6C (Gr1), Clone RB6-8C5, Biolegend, Cat#108410, RRID:AB_313375; B220, Clone RA3-6B2, Biolegend, Cat#103210 and #103208, RRID: AB_312995 and AB_312995, respectively; CD3e, Clone 145-2C11, Biolegend, Cat#100310 and #100312, RRID: AB_312675 and AB_ 312677, respectively; Ter119, Clone Ter-119, Biolegend, Cat#116210, RRID:AB_313711; Ter119, Clone Ter-119, Thermo Fisher Scientific, Cat#25-5921-82, RRID:AB_ 469661; CD19, Clone 1D3, BD Pharmingen, Cat#557399; CD117 (c-Kit), Thermo Fisher Scientific, Cat#47-1171-82, RRID:AB_ 1272177; Sca1, Clone D7, Biolegend, Cat#108128, RRID:AB_2563064; CD48, Clone HM48-1, Biolegend, Cat#103404, RRID:AB_313019; CD150, Clone TC15-12F12.2, Biolegend, Cat#115927, RRID:AB_11204248; CD71, Clone RI7217, Biolegend, Cat#113806, RRID:AB_313567; CD45.1-PE, Clone A20, BioLegend Cat# 110708, RRID:AB_313497; CD45.1-PE-Cy7, Clone A20, BioLegend Cat# 110730, RRID:AB_1134168. Confocal microscopy antibodies Anti-Histone H4 (acetyl K5 + K8 + K12 + K16) antibody [EPR16606], Abcam, ab177790, RRID:AB_2732882; H3K9ac Monoclonal Antibody, Thermo Fisher Scientific Cat# MA5-23517, RRID:AB_2608308; Donkey anti-mouse AF488, Life Technologies, A21202, RRID:AB_141607; Donkey anti-rabbit AF568, Life Technologies, A10042, RRID:AB_2534017.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	iPSC-CB1RB9 cell line, human (RB9-CBiPS2), developed in our laboratory (Woods, et al. (2011)); Mouse Embryonic Fibroblasts from Merck-Millipore (EmbryoMax PMEF P3, Lot: 2497800); OP9-DL1 murine cell line kindly provided by Dr. Ewa Sitnicka-Quinn (Lund University, Sweden). iPSC-CB1RB9 cell line authentication described in PMID:21544903; OP9-DL1 cell line authentication described in PMID: 26287684. All cell lines tested negative for mycoplasma contamination.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	species: Mus Musculus, strain: C57BL/6xB6.SJL, female, age: 10-14 weeks species: Mus Musculus, strain: C57BL/6xB6.SJL, male, age: 12-18 weeks species: Mus Musculus, strain: B6.SJL, male, age: 12-18 weeks species: Mus Musculus, strain: C57BL/6xB6.SJL x B6.SJL and C57BL/6xB6.SJL x C57BL/6xB6.SJL, female and male foetuses at E14.5 from breeding set up between the females and males specified above. species: Mus Musculus, strain: NOD/Cg-Prkdcscid Il2rgtm1Wjl/SzJ, female, age: 8 weeks (NSG, The Jackson Laboratory)
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	These experiments were done in accordance with ethical approval granted by the Lund University animal ethical committee.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	These experiments were reported in accordance with the ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	No human subjects were used in this study. For the Human Umbilical Cord Blood samples used in this study, informed consents were obtained according to guidelines approved by the regional ethical committee. Skåne University Hospital (Lund and Malmö) and Helsingborg Hospital.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	No human subjects were used in this study. For the Human Umbilical Cord Blood samples used in this study, informed consents were obtained according to guidelines approved by the regional ethical committee. Skåne University Hospital (Lund and Malmö) and Helsingborg Hospital.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	No patient photos were used in this study.
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	No such restrictions for this study.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	This study does not include clinical trials.
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	This study does not include clinical trials.
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	This study does not include tumor marker prognostic studies.

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	A Data Availability section is provided at the end of the Materials & Methods section. The single cell RNA sequencing data presented in this paper is available in the GEO database under the accession number GSE141189.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	The single cell RNA sequencing data presented in this paper is available in the GEO database under the accession number GSE141189.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	No patient data was used in this study.
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	This study does not include computational models-

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	This study does not fall under dual use research restrictions.