

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](#).

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

► Experimental design

1. Sample size

Describe how sample size was determined.

No statistical method to predetermine sample size was used.

2. Data exclusions

Describe any data exclusions.

No data were excluded from the analysis.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

All experiments were performed at least three times with similar results, except experiments presented in Figure 3 and Figure 7. In Figure 3, the 144 high-quality tracks were derived from one representative video. In Figure 7, data were derived from two independent transplantation experiments of mock control, EPFs, ENFs and decellularized skin.

4. Randomization

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

Mice or embryos were randomly divided into groups.

5. Blinding

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

In EPF/ENF transplantation and de-cellularized skin transplantation experiments, the researcher performed the image analysis is blinded to the initial experimental group allocation. For other experiments, where no relative treatment vs. control conditions exist, investigators were not blinded.

Note: all in vivo studies must report how sample size was determined and 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
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
- ☐ ☒ Test values indicating whether an effect is present
*Provide confidence intervals or give results of significance tests (e.g. *P* values) as exact values whenever appropriate and with effect sizes 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 in all relevant figure captions (with explicit mention of central tendency and variation)

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.

GraphPad v6.0 for statistics,
Summit v4.3 for flow cytometric analysis,
Image J v 1.47 for image analysis,
Imaris v9.1.0 for processing 4D data (time-lapse 3D imaging).

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 third party.

All materials are commercially available, expect for ROSA26(VT2/GK3) reporter mouse, which is available upon request.

9. Antibodies

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

Antibodies used in this study are: Anti-Fibronectin Rabbit polyclonal antibody (Abcam: ab23750, 1:100), Anti-Collagen Type I Rabbit antibody (Rockland: 600-401-103-0.1, 1:00), Anti-CD31 Rabbit antibody (Abcam: ab28364, 1:100), Anti-CD31 Rat APC-conjugated monoclonal antibody (Biolegend: 17-0311-82, Clone: 390, 1:100), Anti-alphaSMA Rabbit polyclonal antibody (Abcam: ab5694, 1:100), Anti-FSP1 (S100A4) Rabbit polyclonal antibody (Abcam: ab41532, 1:100), Anti-Ki67 Rabbit monoclonal antibody (Abcam: ab16667, Clone: SP6, 1:100), Anti-cleaved Caspase 3 (Asp175) Rabbit polyclonal antibody (Cell signaling: 9661, 1:100), Anti-Ter119 Rat AlexaFluor647(R)-conjugated monoclonal antibody (Biolegend: 116218, Clone: TER-119, 1:400), Anti-Lyve-1 Rat eFluor660(R)-conjugated monoclonal antibody (eBioscience: 50-0443-82, Clone: ALY7, 1:400), Anti-CD45 Rat APC-conjugated monoclonal antibody (Biolegend: 103112, Clone: 30-F11, 1:400), Anti-EpCam Rat APC-conjugated monoclonal antibody (Biolegend: 118212, Clone: G8.8, 1:400), Anti-CD202b (Tie-2) Rat APC-conjugated monoclonal antibody (Biolegend: 124010, Clone: TEK4, 1:400), Anti-mouse CD105 PE-Vio770-conjugated antibody (Miltenyi: 130-108-383, Clone: MJ7/18, 1:400), Anti-mouse CD146 PerCP-Vio700-conjugated antibody (Miltenyi: 130-103-865, Clone: ME-9F1, 1:400), Anti-mouse CD140a (PDGFRalpha) PE-Vio770-conjugated antibody (Miltenyi: 130-105-177, Clone: APAS, 1:400), Anti-mouse integrin alpha7 APC-conjugated antibody (Miltenyi: 130-103-356, Clone: 3C12, 1:400), Anti-mouse Sca-1 (Ly-6A/E) PerCP-Cy5.5-conjugated antibody (eBioscience: 45-5981, Clone: D7, 1:400), Anti-mouse CD29 PerCP-eFluor710 (eBioscience: 46-0291, Clone: HMB1-1, 1:400), Anti-GFP Chicken polyclonal antibody (Abcam: ab13970, 1:100), Anti-tdTomato Goat polyclonal antibody (Sicgen: AB8181-200, 1:100), Anti-CD31 Rabbit polyclonal antibody (Novus: NB100-2284, 1:100), Anti-CD26 Rabbit polyclonal antibody (Abcam: ab28340, 1:100), Anti-TNC Rabbit monoclonal antibody (Abcam: ab108930, Clone: EPR4219, 1:100), Anti-Dlk1 Rabbit polyclonal antibody (Abcam: ab21682, 1:100), Anti-Sca-1 Rat monoclonal antibody (BioLegend: BLD-122502, Clone: E13-161.7, 1:100), Anti-FABP4 Rabbit monoclonal antibody (Abcam: ab92501, Clone: EPR3579, 1:100).

All antibodies are well characterised and were applied according to datasheet instructions. Antibodies were additionally validated using respective isotype antibodies in immunofluorescence assays or flow cytometric assays.

10. Eukaryotic cell lines

- State the source of each eukaryotic cell line used.
- Describe the method of cell line authentication used.
- Report whether the cell lines were tested for mycoplasma contamination.
- 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 cell line was used in this study.

No cell line was used in this study.

No cell line was used in this study.

No cell line was used in this study.

► 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 all relevant details on animals and/or animal-derived materials used in the study.

Following mouse strains are used, both males and females, at ages of 8-12 weeks (for adults), or E9.5-E19.5 (for embryos), or P1 (for neonates):
C57BL/6J wild type
Rag2^{-/-}
En1(Cre);R26(mTmG),
En1(Cre);R26(VT2/GK3)
Actin(Cre-ER);R26(VT2/GK3);

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.

The study did not involve human research participants.

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.

Dorsal skin of En1Cre;R26mTmG embryos, neonates or adult mice were harvested and washed with HBSS. Skin was minced into small pieces with surgical scissors and washed again with HBSS. The cells were re-suspended in 2 ml of digestion solution containing 1 mg/ml of Collagenase I, 0.5 mg/ml of Hyaluronidase, and 25 U/ml of DNase I, and incubated in a 37°C water bath for 30 min with agitation. 10 ml of DMEM containing 10% FBS was added to stop the enzymatic reaction and the suspension was filtered through a 100 µm and then 40 µm cell strainer. After centrifugation at 300x g for 5 min, the cell pellet was re-suspended in 1 ml of FACS buffer (0.5% FBS in PBS) and incubated with 1 µg of APC-conjugated anti-mouse CD31 (PECAM-1), CD45, Ter119, Tie2 (CD202b) or EpCam (CD326) antibodies (BioLegend) and eFluor660 conjugated anti-mouse Lyve-1 antibody (Thermo Fisher) on ice for 30 min. A small aliquot of cells was incubated with the respective APC-conjugated isotype controls (BioLegend). After washing with 5 ml FACS buffer, the cell pellet was resuspended in 2 ml FACS buffer containing 1 µl of Sytox blue (Thermo Fisher). The cells were sorted on a BD FACSAria III cell sorter with a 100 µm nozzle. The viable (Sytox blue-), lineage-negative cells (Lin-: CD31-, CD45-, Ter119-, Tie2-, EpCam-, Lyve-1-) were sorted into ENFs (Lin-RFP+GFP-) and EPFs (Lin-RFP-GFP+) based on RFP and GFP fluorescence. The following cell surface antibodies were used in the flow cytometric analysis: anti-mouse CD105-PE-Vio770 (Miltenyi), CD146-PerCP-Vio700 (Miltenyi), CD140a (pdgfra)-PE-Vio770 (Miltenyi), integrin α-7-APC (Miltenyi); and anti-mouse Sca-1-PerCP-Cy5.5 (eBioscience), CD29-PerCP-eFluor710 (eBioscience).

6. Identify the instrument used for data collection.

BD FACSAria III cell sorter

7. Describe the software used to collect and analyze the flow cytometry data.

Summit v4.3

8. Describe the abundance of the relevant cell populations within post-sort fractions.

The purity of sorted EPFs and ENFs were higher than 90%, determined by the re-analysis of sorted cells.

9. Describe the gating strategy used.

1. Cell debris was gated out with primary FSC-A/SSC-A gating.
2. Single cells were gated with FSC-A/FSC-W.
3. Viable cells were defined within Sytox blue negative gate.
4. Lineage negative cells were defined within APC-negative gating (negative for APC conjugated-CD31, CD45, Ter119, Tie2, EpCam, Lyve-1). The APC conjugated isotype stained sample was used as reference.
5. Within the Lineage negative gating, EPFs were gated as GFP+RFP- and ENFs were gated as GFP-RFP+.

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

Editorial Policy Checklist

This form is used to ensure compliance with Nature Research editorial policies related to research ethics and reproducibility in the life sciences. For further information, please see our [Authors & Referees](#) site. All questions on the form must be answered.

► Data availability

Policy information about [availability of data](#)

Data availability statement

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 list of figures that have associated source data
- A description of any restrictions on data availability

☒ A full data availability statement is included in the manuscript.

Required accession codes

Data deposition is mandated for [certain types of data](#). Confirm that all relevant data have been deposited into a public repository and that all accession codes are provided.

☐ Accession codes will be available before publication ☒ No data with mandated deposition ☐ All relevant accession codes are provided

► Data presentation

Image integrity

☒ Confirm that all images comply with our [image integrity policy](#).

Unprocessed data must be provided upon request. Please double-check figure assembly to ensure that all panels are accurate (e.g. all labels are correct, no inadvertent duplications have occurred during preparation, etc.).

Data distribution

Data should be presented in a format that shows data distribution (dot-plots or box-and-whisker plots), with all box-plot elements (e.g. center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; points, outliers) defined. If bar graphs are used, the corresponding dot plots must be overlaid.

☒ Confirm that all data presentation meets these requirements and that individual data points are shown.

► Macromolecular structural data

Policy information about [special considerations](#) for specific types of data

☒ If this study did not involve macromolecular structural data, check here and skip the rest of this section.

Validation report

☐ For all macromolecular structures studied, confirm that you have provided an official validation report from [wwPDB](#).

► Code availability

Policy information about [availability of computer code](#)

Code availability statement

For all studies using custom code, the Methods section must include a statement under the heading "Code availability" describing how readers can access the code, including any access restrictions.

☐ A full code availability statement is included in the manuscript ☒ No custom code used

► Research animals

Policy information about [studies involving animals](#); follow the [ARRIVE guidelines](#) for reporting animal research

☐ If this study did not use animals and/or animal-derived materials for which ethical approval is required, check here and skip the rest of this section.

Ethical compliance

☒ Confirm that you have complied with all relevant ethical regulations and that a statement affirming this is included in the manuscript.

Ethics committee

☒ Confirm that you have stated the name(s) of the board and institution that approved the study protocol in the manuscript.

► Human research participants

Policy information about [studies involving human research participants](#)

☒ If this study did not involve any human research participants, check here and skip the rest of this section.

Ethical compliance

☐ Confirm that you have complied with all relevant ethical regulations and that a statement affirming this is included in the manuscript.

Ethics committee

☐ Confirm that you have stated the name(s) of the board and institution that approved the study protocol in the manuscript.

Informed consent

☐ Confirm that informed consent was obtained from all participants.

Identifiable images

For publication of identifiable images of research participants, confirm that consent to publish was obtained and is noted in the Methods.

Authors must ensure that consent meets the conditions set out in the [Nature Research participant release form](#).

☐ Yes ☐ No identifiable images of human research participants

► Clinical studies

Policy information about [clinical studies](#)

☒ If this study was not a clinical trial, check here and skip the rest of this section.

Clinical trial registration

☐ Confirm that you have provided the trial registration number from [ClinicalTrials.gov](#) or an equivalent agency in the manuscript.

Phase 2 and 3 randomized controlled trials

Confirm that you have provided the [CONSORT checklist](#) with your submission.

☐ Yes ☐ No ☐ Not a phase 2/3 randomized controlled trial

Tumor marker prognostic studies

Did you follow the [REMARK reporting guidelines](#)?

☐ Yes ☐ No ☐ Not a tumor marker prognostic study

► Methods reporting

Nature Research wishes to improve the reproducibility of the work we publish. As part of this effort, all life science manuscripts require a [reporting summary](#); certain types of research require specialized modules.

☒ Confirm that you have provided a complete and accurate [reporting summary](#).

n/a | Confirmed

☒ ☐ For MRI studies, confirm that you have completed the additional [MRI module](#).

☐ ☒ For flow cytometry studies, confirm that you have completed the additional [flow cytometry module](#).

☒ ☐ For ChIP-seq studies, confirm that you have completed the additional [ChIP-seq module](#).

I certify that all the above information is complete and correct.

Typed signature Yuval Rinkevich

Date 29 January 2018