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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. — we used minimal sample size for statistical comparisons

2. Data exclusions

   Describe any data exclusions. — No data were excluded

3. Replication

   Describe whether the experimental findings were reliably reproduced. — All attempt at replication were successful

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups. — Randomization is not relevant to this study because no comparisons between experimental groups were made.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis. — For teratoma assay the investigator was blinded to the group allocation (with no prior knowledge about the cell lines and treatments) during the experiments.

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.	Softwares used in this study for data analysis were described in the method section.

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.	no unique materials were used

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Primary antibodies: Anti-KLF17 antibody produced in rabbit (1:100) (HPA024629, SIGMA), GKLF/EKLF/LKLF Antibody (F-8) (1:100) (sc-166238, Santa Cruz), Mouse Anti-Stella Antibody (1:100) (MAB4388, EMD Millipore), Nanog (D73G4) XP® Rabbit mAb #4903 (1:300) (4903S, Cell Signaling), Oct-3/4 Antibody (C-10) (1:300) (sc-5279, Santa Cruz), Annexin V (1:100) (APC, BD Bioscience 550474), ?H2AX (1:100) (Alexa Fluor 647, BD Bioscience 560447), THY-1 (1:100) (PE anti-human CD90 (Biolegend 328110)) and CD75 (1:100) (Purified anti-human CD75 (LN-1, 326901 Biolegend). β-ACTIN (1:10000) (Cell Signaling Technology, clone 13E5, cat. #5125); ERK1/2 (1:1000) (Cell Signaling Technology, 137F5, 4695); p-ERK1/2 (1:1000) (Cell Signaling Technology, D13.14.4E, 4370). Secondary antibodies: Goat anti-Rabbit IgG (H+L) (Alexa Fluor 488, A11008, Thermo Fisher), Goat anti-Mouse IgG (H+L) (Alexa Fluor 546, A11003, Thermo Fisher), Goat anti-Mouse IgM Heavy Chain Secondary Antibody, Alexa Fluor® 647 conjugate (A-21238, Thermo Fisher). Antibodies were validated by the company; refer to the company website for detailed validation analysis.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	Human primed WIBR3 cells were obtained from Dr Rudolf Jaenisch (Whitehead Institute, Boston). UCLA1, UCLA3, UCLA4, UCLA5, UCLA9, UCLA17 hESC lines from Dr. Kathrin Plath lab (UCLA, Los Angeles).
b. Describe the method of cell line authentication used.	UCLA1, UCLA3, UCLA4, UCLA5, UCLA9, UCLA17 hESC lines were authenticated by the Human Embryonic and Induced Pluripotent Stem Cell Core at UCLA. The WIBR3 cell line by the Whitehead Institute.
c. Report whether the cell lines were tested for mycoplasma contamination.	Cell lines were tested weekly for mycoplasma contamination using the LONZA Mycoplasma Detection Kit. All cell lines tested negative for Mycoplasma contamination.
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 commonly misidentified cell lines were used.

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.	9 immunodeficient NOD/SCID mice from Jackson laboratory were used for Teratoma assay.

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.	This study did not involve human research participants

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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.	In all flow experiments cells were detached using Accutase, centrifuged in DMEM medium containing 10% FBS, filtered through a 0.45uM strained and resuspended in FACS buffer (PBS + 5% FBS). Cell permeabilization was performed using the Fix and Perm Cell fixation and cell permeabilization Kit (ThermoFisher Scientific, GAS003) following the manufacturer instructions.
6. Identify the instrument used for data collection.	LSRII FACS BD Bioscience
7. Describe the software used to collect and analyze the flow cytometry data.	Collection: Diva v6.1.2 (BD Biosciences). Analysis: FlowJo software v10 (TreeStar)
8. Describe the abundance of the relevant cell populations within post-sort fractions.	N/A
9. Describe the gating strategy used.	Cell debris was excluded using a FSC vs SSC gate; aggregates were excluded via a FSC-H vs FSC-A approach; dead cells were defined as DAPI high/positve and gated out. Data were represented as Empty-channel/GFP;Empty-channel/APC or CD75-APC/THY-1-PE

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