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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.

The sample size for the in vivo studies to achieve statistical significance was not calculated before the studies as the survival of the hypo-cells in the different models was unknown prior. It was reasoned that 5-10 mice per group in individual experiments would indicate valid efficacy. Sample sizes in vitro were determined by three or more samples for comparisons between one or multiple groups, followed by the statistical test. Again, the sample size to achieve statistical significance was not calculated before the studies for the reason described above.

2. Data exclusions

Describe any data exclusions.

No pre-established data exclusion method was used.

3. Replication

Describe whether the experimental findings were reliably reproduced.

The experimental findings can be reliably reproduced. Some key data generated by one co-author were repeated by other co-authors.

4. Randomization

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

All samples were number coded until the readout was finalized. The numbers were assigned prior to the experiment and determined the group/ treatment/ condition. Animals were number coded and assigned to a group prior to the surgical procedure.

5. Blinding

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

Group allocation for cell transplantations were performed by blinded investigators. For in vivo imaging and teratoma measurement, the investigators doing the readouts (Core Facility) were not blinded, but not familiar with the experimental setup of this study. For immunofluorescent images, the animal group that each cell type belonged to was unknown at the time to the individual doing the imaging.

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.	FlowJo7 was used to analyze flow cytometric data. Prism7 or Excel 2010 was used for graphing and statistical analysis. LivingImage3.1 was used for quantification of bioluminescence imaging. Elispots were enumerated by Immunospot software.

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 restriction. Standard MTA needed.

9. Antibodies

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

The following antibodies were used for immunostaining: brachyury (polyclonal, catalog# ab20680, Abcam), cytokeratin 8 (clone: EP1628Y, AF488, catalog#ab192467), GFAP (clone: GA5, catalog#3670, Cell Signaling), CD31 (polyclonal, catalog # ab28364, abcam), Ve-Cadherin (polyclonal, catalog# sc-6458, Santa Cruz Biotechnology), smooth muscle actin (polyclonal, catalog #ab21027, Abcam), sm22 (polyclonal, catalog #ab14106, Abcam), α-sarcomeric actinin (clone: EA-53, catalog# ab9465, Abcam), troponin I (polyclonal, catalog #ab47003, Abcam), luciferase (polyclonal, catalog #ab21176, abcam), CD3 (clone: SP7, catalog #ab16669, abcam). Following corresponding Secondary antibodies were used: donkey-anti-mouse IgG (polyclonal, AF555, catalog #A31570, Invitrogen), donkey-anti-mouse IgG (polyclonal, AF488, catalog #A21202, Invitrogen), donkey-anti-rabbit IgG (polyclonal, AF555, catalog #A31572, Invitrogen), donkey-anti-rabbit IgG (polyclonal, AF488, catalog #A21206, Invitrogen), goat-anti-rabbit IgG (polyclonal, AF555, catalog #A21430, Invitrogen), goat-anti-rabbit IgG (polyclonal, AF488, catalog #A11070, Invitrogen), goat-anti-mouse IgG (polyclonal, AF488, catalog #A11017, Invitrogen).

For Flow cytometry analysis: MHC class I (clone AF6-88.5.5.3, PerCP-eFlour710,catalog#46-5958-82, eBioscience), MHC class II (clone: M5/114.15.2, PerCP-eFlour710, catalog#46-5321-82, eBioscience) Cd47 (clone: miap301, Alexa Fluor 647, catalog #563584, BD Biosciences), TRA-160 (clone XXX,) HLA-A,B,C (clone G46_2.6, APC, catalog #562006, BD Biosciences), HLA-DR,DP,DQ (clone Tu3a, AF647, catalog #563591, BD Biosciences), CD47 (clone B6H12, PerCP-Cy5, catalog #561261, BD Biosciences), VE-Cadherin (clone F-8, catalog #SC-9989, Santa Cruz Biotechnology), smooth muscle actin (polyclonal, catalog #ab5694, Abcam) and troponin I (clone CT3, PE, catalog #sc-20025, Santa Cruz).

For NK-stimulation assays: NKG2D (catalog #1299-NK-050, R&D Systems), Nkp80 (catalog #1900-NK-050, R&D Systems), Nkp46 (catalog #1850-NK-025, R&D Systems), Nkp44 (catalog #2249-NK-050, R&D Systems), or Nkp30 (catalog #1849-NK-025, R&D Systems).

Characterization of human pluripotent stem cells were conducted using the Applied stem cell Human ES/iPS Cell Characterization Kit (ASK-3006). Mouse pluripotent stem cells were characterized by Applied stem cell Mouse ES/iPS Cell Characterization Kit (ASK-3005). For cell selection TRA-1-60 Alexa Fluor™ 488 Conjugate Kit for Live Cell Imaging (A25618, Invitrogen) was used.

10. Eukaryotic cell lines

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

The Human Episomal iPSC Line was purchased from Thermo Fisher Scientific (Waltham, MA). Mouse iPSCs were reprogramed from C57BL/6 mice. Mouse YAC-1 and human K-562 were purchased from ATCC (Manassas, VA) and used as controls in appropriate NK cell assays. Irradiated CF1 Mouse Embryonic Fibroblasts (MEFs) were used as feeder cells for mouse iPSCs and purchased from Thermo Fisher Scientific.

b. Describe the method of cell line authentication used.

None of the cell lines used have been authenticated.

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

All cell lines were tested and negative for mycoplasma contamination using the Universal Mycoplasma test kit from ATCC.

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.

Male C57BL/6J, BALB/cAnNCrl, SCID-beige (CBySmn.CB17-Prkdcscid/J) and NSG (NOD.Cg-Prkdc-scid IL2rg-tm1Wjl/SzJ mice 6-12 weeks of age and humanized NSG-SGM3 mice 18-30 weeks were purchased from Jackson Laboratories (Bar Harbor, Maine, USA). All mice were maintained in pathogen-free, ventilated cages with irradiated food and autoclaved water at the University of California San Francisco (UCSF) or at the University Hamburg (HH). Experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at UCSF or the "Amt fuer Gesundheit und Verbraucherschutz" at HH. Mice were monitored daily and euthanized by CO2 asphyxiation and cervical dislocation prior to any signs of distress. BLT mice were generated at the University of North Carolina (UNC) and were approved by the local IACUC at UNC.

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 subjects.