

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

|                                     |                                                                                                                                                                                                                                                                                                |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| n/a                                 | Confirmed                                                                                                                                                                                                                                                                                      |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                                |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                                      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated                                                                                                                                                          |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

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

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-----------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data collection | Flow cytometry data were collected on FACS Aria IIIu, Celesta, or LSR Fortessa (Becton Dickinson). Confocal and super-resolution images were acquired on N-SIM super-resolution confocal microscope with a 100× objective (Nikon). TEM and immuno-EM images were collected on JEM-1400Flash electron microscope (JEOL). Cytokine data were collected on Bio-Plex 200 analyzer (Bio-Rad). Seahorse assay data were collected using Seahorse XFe96 Extracellular Flux Analyzer (Agilent).                                |
| Data analysis   | Statistical analyses were performed in GraphPad Prism (v10.4.0) and R (v4.0.2). RNA-seq: FastQC (v0.12.0), bcl2fastq (v2.20), HISAT2 (v2.2.1), StringTie (v2.2.3), edgeR (v3.30.3); GSEA (v4.3.0). ATAC-seq: Bowtie2 (v2.5.3), MACS2 (v2.2.7.1), bedtools (v2.31.0), edgeR. Flow cytometry: FlowJo (v10.10.0). Confocal images were visualized and analyzed with NIS-Elements Viewer (v4.11.0) and ImageJ (v1.53c). Morpheus was used for heatmaps. Seahorse data were processed with Seahorse Analytics (v1.0.0-520). |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

## Data

Policy information about [availability of data](#)

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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All source data supporting the findings are provided with this paper. Bulk RNA-seq data and ATAC-seq data from have been deposited at Gene Expression Omnibus and are publicly available under the accession number GSE285111.

## Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

|                                                                    |                                  |
|--------------------------------------------------------------------|----------------------------------|
| Reporting on sex and gender                                        | <input type="text" value="n/a"/> |
| Reporting on race, ethnicity, or other socially relevant groupings | <input type="text" value="n/a"/> |
| Population characteristics                                         | <input type="text" value="n/a"/> |
| Recruitment                                                        | <input type="text" value="n/a"/> |
| Ethics oversight                                                   | <input type="text" value="n/a"/> |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

|                 |                                                                                                                                                                                                                                                                                                         |
|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample size     | No statistical methods were used to predetermine sample size. Sample sizes were chosen based on prior experience with similar hematopoietic stem cell experiments and are sufficient to detect biologically meaningful differences. Exact n values for each experiment are indicated in figure legends. |
| Data exclusions | A few mice that developed idiopathic tumors were excluded from analyses to ensure evaluation of normal hematopoiesis. For transplantation experiments, outliers and non-engrafted animals were excluded based on the exclusion criteria described in the Methods section.                               |
| Replication     | All experiments were performed with multiple replicates. The number of replicates (n) and independent experiments performed were detailed in the figure legends.                                                                                                                                        |
| Randomization   | Samples were randomly allocated to experimental groups.                                                                                                                                                                                                                                                 |
| Blinding        | Blinding was not feasible because animals were grouped by genotype. However, objective outcome measures and standardized protocols were used to minimize potential bias.                                                                                                                                |

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials &amp; experimental systems

| n/a                                 | Involved in the study                                           |
|-------------------------------------|-----------------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines       |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                                 |

## Methods

| n/a                                 | Involved in the study                              |
|-------------------------------------|----------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging    |

## Antibodies

## Antibodies used

Armenian hamster anti-mouse CD3ε-PECy5 (145-2C11), Thermo Fisher Scientific, 15-0031-83; AB\_468691  
 Armenian hamster anti-mouse CD3ε-PECy7 (145-2C11), Thermo Fisher Scientific, 25-0031-82; AB\_469572  
 Armenian hamster anti-mouse CD48-AF700 (HM48-1), BioLegend, 103425; AB\_10612754  
 Armenian hamster anti-mouse CD48-APCeF780 (HM48-1), Thermo Fisher Scientific, 47-0481-82; AB\_2573962  
 Donkey anti-goat IgG (H+L)-AF488, Abcam, ab150129; AB\_2687506  
 Goat anti-mouse IgG (H+L)-AF647, Thermo Fisher Scientific, A-21235; AB\_2535804  
 Goat anti-mouse IgG-AF488, Thermo Fisher Scientific, A-11001; AB\_2534069  
 Goat anti-mouse neogenin-1 (NEO-1), Bio-Techne, AF1079; AB\_2151002  
 Goat anti-rabbit IgG (H+L)-AF594, Thermo Fisher Scientific, A-11012; AB\_2534079  
 Goat anti-rabbit IgG-5nm gold, BBI Solutions, EM GAR5/1; AB\_1769142  
 Mouse anti-mouse CD45.1-APCeF780 (A20), Thermo Fisher Scientific, 47-0453-82; AB\_1582228  
 Mouse anti-mouse CD45.2-BV786 (104), Thermo Fisher Scientific, 417-0454-82; AB\_2929112  
 Mouse anti-mouse CD45.2-FITC (104), Thermo Fisher Scientific, 11-0454-85; AB\_465062  
 Mouse anti-mouse COX-IV (3C7D2), Proteintech, 60251-1-Ig; AB\_2881372  
 Mouse anti-mouse MLKL (E7V4W), Cell Signaling Technology, 26539; AB\_3608292  
 Mouse anti-mouse γH2AX (JBW301), Millipore, 05-636  
 Rabbit anti-FLAG (DYKDDDDK), Thermo Fisher Scientific, 740001; AB\_2610628  
 Rabbit anti-mouse GPR183-FITC, Thermo Fisher Scientific, AGR-063; AB\_2925069  
 Rabbit anti-mouse p-MLKL (S345) (D6E3G), Cell Signaling Technology, 37333; AB\_2799112  
 Rat anti-mouse B220-AF700 (RA3-6B2), Thermo Fisher Scientific, 56-0452-82; AB\_891458  
 Rat anti-mouse B220-BV605 (RA3-6B2), Thermo Fisher Scientific, 406-0452-82; AB\_2937168  
 Rat anti-mouse B220-PECy5 (RA3-6B2), Thermo Fisher Scientific, 15-0452-83; AB\_468756  
 Rat anti-mouse c-Kit-APC (2B8), Thermo Fisher Scientific, 17-1171-83; AB\_469431  
 Rat anti-mouse CD150-BV650 (TC15-12F12.2), BioLegend, 115932; AB\_2715765  
 Rat anti-mouse CD150-PE (TC15-12F12.2), BioLegend, 115904; AB\_313683  
 Rat anti-mouse CD34-FITC (RAM34), Thermo Fisher Scientific, 11-0341-85; AB\_465022  
 Rat anti-mouse CD4-PECy5 (GK1.5), Thermo Fisher Scientific, 15-0041-83; AB\_468696  
 Rat anti-mouse CD41-BV421 (MWReg30), BioLegend, 133911; AB\_10960744  
 Rat anti-mouse CD41-FITC (MWReg30), BD Biosciences, 553848; AB\_395085  
 Rat anti-mouse CD5-PECy5 (53-7.3), BioLegend, 100610; AB\_312739  
 Rat anti-mouse CD8α-PECy5 (53-6.7), Thermo Fisher Scientific, 15-0081-83; AB\_468707  
 Rat anti-mouse EPCR-PE (eBio1560), Thermo Fisher Scientific, 12-2012-82; AB\_914317  
 Rat anti-mouse FcγR (93), BioLegend, 101302; AB\_312801  
 Rat anti-mouse FcγR-BV510 (93), BioLegend, 101333; AB\_2563692  
 Rat anti-mouse Flk2-Bio (A2F10), Thermo Fisher Scientific, 13-1351-85; AB\_466600  
 Rat anti-mouse Flk2-BV421 (A2F10), BioLegend, 135315; AB\_2571919  
 Rat anti-mouse Gr-1-eF450 (RB6-8C5), Thermo Fisher Scientific, 48-5931-82; AB\_1548788  
 Rat anti-mouse Gr-1-PECy5 (RB6-8C5), Thermo Fisher Scientific, 15-5931-83; AB\_468814  
 Rat anti-mouse IL-7Rα-APCCy7 (A7R34), BioLegend, 135039; AB\_2566160  
 Rat anti-mouse IL-7Rα-PE (A7R34), Thermo Fisher Scientific, 12-1271-83; AB\_465845  
 Rat anti-mouse Ki-67-FITC (SolA15), Thermo Fisher Scientific, 11-5698-82; AB\_11151330  
 Rat anti-mouse Mac-1-APC (M1/70), Thermo Fisher Scientific, 17-0112-83; AB\_469344  
 Rat anti-mouse Mac-1-Bio (M1/70), Tonbo Biosciences, 30-0112; AB\_2621639  
 Rat anti-mouse Mac-1-PECy5 (M1/70), Thermo Fisher Scientific, 15-0112-83; AB\_468715  
 Rat anti-mouse P-selectin-PE (RMP-1), BioLegend, 161204; AB\_2876576  
 Rat anti-mouse Sca-1-Bio (D7), BioLegend, 108104; AB\_313340  
 Rat anti-mouse Sca-1-PECy7 (D7), Thermo Fisher Scientific, 25-5981-82; AB\_469669  
 Rat anti-mouse Ter119-PECy5 (TER-119), Thermo Fisher Scientific, 15-5921-83; AB\_468811  
 See Method section for antibody amount/dilution information.

## Validation

All primary antibodies were commercially available and validated by the manufacturers for the indicated applications. Specificity of immunofluorescent signals was confirmed using knockout cells as shown in Fig. 6c, 6d, Supplementary Fig. 3m, and 8b.

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

|                                                                      |                                                                                                                                                                                                             |
|----------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell line source(s)                                                  | Plat-E packaging cells were kindly provided by Yosuke Tanaka (the Institute of Medical Science, University of Tokyo) and 293GPG packaging cells were kindly provided by Goro Sashida (Kumamoto University). |
| Authentication                                                       | Cell lines were not independently authenticated, as they were obtained from reliable sources and used solely for standard retroviral packaging.                                                             |
| Mycoplasma contamination                                             | All cell lines tested were negative for mycoplasma contamination                                                                                                                                            |
| Commonly misidentified lines<br>(See <a href="#">ICLAC</a> register) | n/a                                                                                                                                                                                                         |

## Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

|                         |                                                                                                                                                                                                                                                                                                                                                                          |
|-------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Laboratory animals      | All animals used in this study are described in the Method section and listed in Supplementary Table 1.                                                                                                                                                                                                                                                                  |
| Wild animals            | No wild animals were used in this study.                                                                                                                                                                                                                                                                                                                                 |
| Reporting on sex        | Both male and female animals were used indiscriminately in this study                                                                                                                                                                                                                                                                                                    |
| Field-collected samples | No field-collected samples were used in this study.                                                                                                                                                                                                                                                                                                                      |
| Ethics oversight        | All animal experiments comply with all relevant ethical regulations and were performed in accordance with protocols approved by the Animal Care and Use Committee at the Institute of Medical Science, University of Tokyo (protocol number: PA19-07) and the St. Jude Children's Research Hospital Institutional Animal Care and Use Committee (protocol number: 3252). |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Plants

|                       |     |
|-----------------------|-----|
| Seed stocks           | n/a |
| Novel plant genotypes | n/a |
| Authentication        | n/a |

## Flow Cytometry

### Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ 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).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

### Methodology

|                    |                                                                                                                                                                                                                                                                                                                                                     |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample preparation | Single-cell suspensions were prepared from mouse bone marrow, spleen, and peripheral blood in PBS containing 2% heat-inactivated FBS. Red blood cells were lysed with ACK buffer, and cells were stained with fluorochrome-conjugated antibodies before flow cytometric analysis. Detailed staining protocols are described in the Methods section. |
| Instrument         | Flow cytometric data were acquired using BD FACSCelesta and BD FACSria III (BD Biosciences) at the Institute of Medical Science, the University of Tokyo, and BD LSRFortessa (BD Biosciences) at St. Jude Children's Research Hospital.                                                                                                             |

## Software

Flow cytometry data were analyzed using FlowJo software (v10.10.0; BD Biosciences).

## Cell population abundance

Cell population frequencies were determined by flow cytometry, and absolute cell numbers were calculated based on viable cell counts obtained with a Vi-CELL analyzer (Beckman Coulter).

## Gating strategy

Debris were excluded based on FSC-A, and dead cells (PI+) were excluded except for Annexin V dying/dead cell analysis. Lin<sup>−</sup> cells were defined based on the backgating of Sca-1+/c-Kit+ cells. Gating strategies to enrich individual immature hematopoietic populations are provided in Supplementary Fig. 1a. Boundaries between positive and negative were defined based on appropriate negative control staining. FRET/CFP-high cells were identified based on increase of the FRET/CFP ratio and decrease of CFP signal, usually corresponding to the top 10% of the FRET/CFP ratio in control samples.

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