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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

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
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ 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 all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ 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)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), 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 was collected using FACSDiva Software (BD) and using FACS Aria II (BD Biosciences) or FACS Symphony (BD Biosciences) instruments. Plasma immunoassay data was collected by instruments at the Stanford Human Immune Monitoring Center (HIMC).
Data analysis	All graphs were generated and analyzed statistically with GraphPad Prism version 9.4.1 for macOS (GraphPad Software) or with SPSS Statistics version 28.0.0.1 (IBM). The flow cytometry results were analyzed using FlowJo v10.8 Software (BD Life Sciences). Flow-cytometry computational analysis was conducted with the Spectre package (PMID: 33840138) using R version 4.2.2. Publicly available gene-expression data was analyzed using Microsoft Excel applied to pre-processed data or data processed by GREIN (PMID: 31110304), GEO2R (PMID: 11752295), or Phantasus (doi: 10.18129/B9.bioc.phantasus). For RNA-sequencing of purified mouse HSCs, libraries were prepared using Takara SMART-Seq v4 Ultra low Input RNA kit and sequencing was performed with NovaSeq with approximately 20 million paired reads per sample by MedGenome Inc. Differential gene expression was performed using DESeq2 (PMID: 25516281) with fold change shrinkage. Heatmaps were generated using Phantasus v1.21.5 (doi:10.1101/2022.12.10.519861) with FPKM values as input and Limma (PMID: 25605792) to define differentially expressed genes. GSEA was conducted on genes ranked by DESeq2 test statistic using WEB-based GEne SeT AnaLysis Toolkit (WebGestalt 2019, PMID: 31114916) with default parameters using a custom list of curated gene-signatures.

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

Data for all graphical representations in figures is provide in Source Data files. HSC RNA-sequencing data has been deposited at the Gene Expression Omnibus at GSE252062 and at the Sequence Read Archive (SRA) with BioProject ID: PRJNA1054066. The following publicly available datasets were used, with web links provided in the manuscript: GSE4372924, GSE3955334, GSE4889335, GSE10954636, GSE2768637, GSE4492338, GSE12805039, GSE4781940, GSE13050427, GSE11276932, E-MEXP-393531, GSE327192, GSE104406108, GSE69408150, GSE115348151, GSE107594152, GSE11141093, GSE5568994, GSE7424695, GSE132040156, GSE87633157, GSE10042826.

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	Sex or gender was not considered in study design. Bone marrow mononuclear cells were from young-adult male donors that were commercially obtained from AllCells, Inc. All donors were male, but this was determined by sample availability and not predetermined.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity, or other socially relevant groupings was not considered in study design. Bone marrow mononuclear cells were from young-adult male donors that were commercially obtained from AllCells, Inc.
Population characteristics	Bone marrow mononuclear cells were from male young-adult donors. All donors were male, but this was determined by sample availability and not predetermined.
Recruitment	Participants were not recruited for this study. Bone marrow mononuclear cells from young-adult donors (ages 26-33) were commercially obtained from AllCells, Inc. All donors were male, but this was determined by sample availability and not predetermined.
Ethics oversight	All human samples were commercially obtained from AllCells, Inc.

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

Life sciences study design

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

Sample size	The sample size for in vivo experiments, including antibody-depletion, viral infection, and transplantation was decided based on prior publications with similar experiments by our laboratories or others, including PMID: 27510901; PMID: 31204177; PMID: 18033883; PMID: 31754028; PMID: 20304793; PMID: 9930867; PMID: 9658099; PMID: 10873767.
Data exclusions	For plasma immunoassay analysis, MFI average values were compared after removal of statistical outliers using the extreme studentized deviate (ESD) Grubbs statistical test (α=0.0001). For animal experiments, sick or diseased mice were excluded from the analysis.
Replication	All attempts at replication were successful. Replicated experiments were identically or similarly designed. Some experiments were replicated at independent institutions (Stanford or Rocky Mountain Labs) with independent mouse strains. Data depicts combined results of at least 2 independent experiments (Fig. 1b–c, Fig. 1i–k, Fig. 3b, Fig. 4b–e, Fig. 5h–5i, Ex. Fig. 2g–j, Ex. Fig. 4j–q, Ex. Fig. 7a–c, Ex. Fig. 9b, Ex. Fig. 9i–k, Ex. Fig 10s), or is representative of four or more experiments (Fig. 1d–h, Ex. Fig. 2b–c), three experiments (Fig. 2c, Fig. 2f, Fig. 5f–g, Ex. Fig. 10g–j, Ex. Fig. 10o), two experiments (Fig. 3c–e, Fig. 3g, Ex. Fig. 7f–j, Ex. Fig. 8a–c, Ex. Fig. 10k–m, Ex. Fig. 10p), or one experiment (2a–b, Fig. 2d–e, Fig. 2g–k, 3f, 3h–j, 4a, Ex. Fig. 2d–f, Ex. Fig. 2k, Ex. Fig. 3a–n, Ex. Fig. 4a–i, Ex. Fig. 4r–x, Ex. Fig. 5c–s, Ex. Fig. 6a–n, Ex. Fig 7d–e, Ex. Fig. 8d–l, Ex. Fig. 9c–h, Ex. Fig. 10q–r). Biological replicates (n = individual mice) are reported in the figure legends.
Randomization	For all experiments, allocation of mice into experimental groups was randomized after matching for age and sex.

Blinding

Investigators were not blinded during data collection or analysis as all experiments involved internal control groups. Experimental and control animals were treated equally.

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 & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

HSPC FACS analysis: anti-FLT3 APC 1:50 (ThermoFisher; 17-1351-82) or anti-FLT3 PerCP-eFluor710 1:50 (eBioscience; 46-1351-82), goat anti-mouse NEO1 15ug/mL (R&D; AF1079), anti-CD150 PE-Cy7 1:50 (BioLegend; 115914; clone TC15-12F12.2), anti-IL7Ra PE-Cy5 1:25 (ThermoFisher; 15-1271-82 or BioLegend; 135016) or anti-IL7Ra APC 1:25 (BioLegend; 135012), anti-CD16/32 BV510 1:50 (BioLegend; 101308), anti-cKit APC-eFluor780 1:50 (ThermoFischer; 47-1171-82), anti-mouse Lineage Cocktail (includes anti-CD3, anti-Ly-6G/C, anti-CD11b, anti-CD45R, anti-Ter-119) AF700 1:10 (BioLegend; 133313), anti-CD48 BV711 1:50 (BD; 740687), anti-CD41 BV650 1:50 (BD; 740504), anti-CD34 biotin 1:50 (ThermoFisher; 13-0341-85), anti-SCA1 BUV395 1:50 (BD; 744328), followed by Streptavidin BUV737 1:50 (BD; 612775) and donkey anti-goat IgG H&L AF488 1:100 (abcam; ab150129). In some instances, anti-CD150 clone mShad150 PE 1:50 (eBioscience; 12-1502-80) or PE-Cy7 (eBioscience; 25-1502-82), anti-CD150 clone 9D1 PE 1:50 (eBioscience; 12-1501-80), anti-CD150 clone Q38-480 PE 1:50 (BD; 562651), anti-CD62p PE 1:50 (BioLegend; 148308), or anti-Ly6D PE 1:50 (eBioscience; 12-5974-80), were included. For testing of candidate my-HSC markers, the following antibodies were used: anti-CD51 PE 1:50 (12-0512-81; ThermoFisher), anti-CD61 PE 1:50 (561910; BD), anti-CD31 PE 1:50 (561073; BD), anti-CD38 PE 1:50 (12-0381-81; ThermoFisher), anti-CD47 clone MIAP301 PE 1:50 (127507; BioLegend), anti-CD47 clone MIAP410 PE 1:50 (LS-C810701-25; LSBio), anti-CD62p PE 1:50 (148305; BioLegend), anti-ALCAM PE 1:50 (12-1661-82; ThermoFisher), anti-CD9 PE 1:50 (124805; BioLegend), anti-ESAM PE 1:50 (136203, BioLegend), anti-TIE2 PE 1:50 (124007; BioLegend), anti-CD201 PE 1:50 (141503; BioLegend), or anti-cKIT clone ACK2 PE 1:50 (135105; BioLegend). Isotype control rat IgG 1mg/mL (ab37361; abcam) was used as a blocking antibody control.

T cell analysis: anti-Helios AF647 1:200 (BD; 563951), anti-CD3 APC-Cy7 1:200 (BioLegend; 100222), anti-Ki67 R718 1:150 (BD; 566963), anti-CD43 AF488 1:400 (BioLegend; 121210), anti-CD8 BUV395 1:400 (BD; 563786), anti-Foxp3 eF450 1:150 (Invitrogen; 48-5773-82), anti-CD4 BV510 1:400 (BioLegend; 100559), anti-CD44 BV605 1:400 (BD; 563058), anti-CD62L BV711 1:1,000 (BioLegend; 104445), anti-EOMES PE 1:200 (Invitrogen; 12-4875-82), anti-PD1 PE-CF594 1:200 (BD; 562523), anti-Tbet PE-Cy7 1:200 (Invitrogen; 25-5825-82), anti-CD25 PerCP-Cy5.5 1:200 (BioLegend; 102030). FV-specific CD8+ T cells were identified using H-2Db/Abu-Abu-L-Abu-LTVFL APC- or PE-DbgagL-MHC Dextramer (Immudex, Copenhagen, Denmark) at 1:25 during surface staining.
B cell analysis: anti-CD43 APC 1:400 (BioLegend; 121214), anti-CD21/CD35 APC-Cy7 1:400 (BioLegend; 123418), anti-CD5 AF700 1:200 (BioLegend; 100636), anti-IgM FITC 1:400 (Invitrogen; 11-5790-81), anti-CD19 BUV395 1:400 (BD; 563557), anti-IgD eFluor450 1:400 (eBioscience; 48-5993-82), anti-CD11b BV510 1:400 (BioLegend; 101245), anti-MHCII BV605 1:400 (BD; 563413), anti-CD40 BV711 1:400 (BD; 740700), anti-PDL1 PE 1:200 (Invitrogen; 12-5982-82), anti-CD93 PE-Cy7 1:400 (BioLegend; 136506), anti-CD23 PerCP-Cy5.5 1:200 (BioLegend; 101618), and anti-CD45R/B220 PE-CF594 1:800 (BD; 562290).

Peripheral blood chimerism analysis: anti-CD8a Spark UV387 1:50 (BioLegend; 100797), anti-CD4 BUV737 1:200 (BD, 612843), anti-Gr1 Pacific Blue 1:100 (BioLegend, 108429), anti-CD3 BV711 1:100 (BioLegend, 100241), anti-CD44 BV785 1:300 (BioLegend, 103059), anti-CD45.2 AF488 1:100 (BioLegend, 109815), anti-Foxp3 PE 1:100 (eBioscience, 12-5773-82), anti-CD62L PE/Dazzle™ 594 1:200 (BioLegend, 104447), anti-CD19 PE-Cy7 1:100 (BioLegend, 115519), anti-CD45.1 APC 1:50 (BioLegend 110713), anti-CD11b APC-Cy7 1:100 (BioLegend, 101225). Intracellular staining was performed with Foxp3 Transcription Factor Staining Buffer Set (eBioscience, 00-5523-00) per manufacturer's protocol. Red blood cells were removed with ACK lysis buffer (Gibco, a1049201). Non-specific binding was prevented with TruStain FcX PLUS (BioLegend cat# 156604). To determine viability of fixed cells, cells were incubated in buffer containing LIVE/DEAD™ Fixable Aqua Dead Cell (ThermoFisher; L34957).

For erythroid cell analysis, spleen cells were first incubated for 30 min with mAb 34, a mouse IgG2b specific for the FV glycoGag protein expressed on infected cells (PMID: 6787798), then stained with anti-mouse IgG2b FITC (BD; 553395) and anti-Ter119 PE-Cy7 (Invitrogen; 25-5921-82).

Human HSC analysis: anti-lineage panel PE-Cy5: [anti-CD3 1:200 (BD; 555341), anti-CD4 1:200 (BD; 555348), anti-CD8 1:200 (BD; 555368), anti-CD11b 1:200 (BD; 555389), anti-CD14 1:200 (ThermoFisher; MHCD1406), anti-CD19 1:200 (BD; 555414), anti-CD20 1:200 (BD; 555624), anti-CD56 1:200 (BD; 555517), anti-CD235a 1:200 (BD; 559944)], anti-CD34 APC-Cy7 1:25 (343514; Biolegend), anti-CD45RA BV-785 1:25 (304139; Biolegend), anti-CD38 APC 1:10 (555462; BD), anti-CD90 FITC 1:50 (328107; Biolegend), and one of anti-human PE: anti-CD62P clone AK4 1:20 (304905; Biolegend), anti-CD62P clone Psel.KO2.3 1:20 (12-0626-82; eBioscience), anti-

CD62P clone AC1.2 1:5 (550561; BD), anti-CD150 1:20 (306307; Biolegend), anti-TIE2 1:20 (CD202b, 334205; Biolegend), anti-ESAM 1:10 (408519; Novus), anti-CD166 1:20 (ALCAM, 343903; Biolegend), anti-CD9 1:20 (312105; Biolegend), anti-CD105 1:20 (Endoglin, 800503; Biolegend), or anti-CD304 1:20 (Neuropilin-1, 354503; Biolegend).

In vivo experiments: Isotype control antibodies: mouse IgG1 (clone MOPC-21, Bio X Cell), rat IgG2b (clone LTF-2, Bio X Cell), or rat IgG2a (clone RTK2758, BioLegend). Experimental antibodies: rat IgG2a anti-CD150 (clone TC15-12F12.2, BioLegend), rat IgG2b anti-CD150 (clone mShad150, eBioscience), anti-CD62p (clone RMP-1, BioLegend), goat anti-NEO1 (polyclonal cat# AF1079, R&D), mouse IgG2a anti-goat (SB115d; SouthernBiotech), mouse IgG2b anti-goat (SB115h; SouthernBiotech), mouse IgG1 anti-CD47 (clone MIAP410, Bio X Cell), rat anti-cKIT (clone ACK2, Bio X Cell).

Validation

All antibodies used in this study have been previously validated by commercial manufactures, previous publications, and/or this study.

All antibodies used for mouse HSC, HSPC, T & B cells analysis were validated by commercial manufacturer or have been cited in the literature:
anti-FLT3 APC (ThermoFisher; 17-1351-82), reported to recognize mouse FLT3 (manufacturer's website)
anti-FLT3 PerCP-eFluor710 (eBioscience; 46-1351-82), reported to recognize mouse FLT3 (manufacturer's website)
goat anti-mouse NEO1 (R&D;AF1079), reported to recognize mouse Neogenin (manufacturer's website). See additional validation details below.
anti-CD150 PE-Cy7 (BioLegend; 115914; clone TC15-12F12.2), reported to recognize mouse CD150 (manufacturer's website)
anti-IL7Ra PE-Cy5 (ThermoFisher; 15-1271-82), reported to recognize mouse IL-7 receptor (manufacturer's website)
anti-IL7Ra PE-Cy5 (BioLegend; 135016), reported to recognize mouse IL-7Ra (manufacturer's website)
anti-IL7Ra APC (BioLegend; 135012), reported to recognize mouse IL-7Ra (manufacturer's website)
anti-CD16/32 BV510 (BioLegend; 101308), reported to recognize mouse CD16/32 (manufacturer's website)
anti-cKit APC-eFluor780 (ThermoFisher; 47-1171-82), reported to recognize mouse c-Kit (manufacturer's website)
anti-mouse Lineage Cocktail (includes anti-CD3, anti-Ly-6G/C, anti-CD11b, anti-CD45R, anti-Ter-119) AF700 (BioLegend; 133313), reported to recognize mouse CD3, mouse Ly-6G/Ly-6C, mouse CD11b, mouse CD45R/B220, mouse TER-119 (manufacturer's website)
anti-CD48 BV711 (BD; 740687), reported to recognize mouse CD48 (manufacturer's website)
anti-CD41 BV650 (BD; 740504), reported to recognize mouse CD41 (manufacturer's website)
anti-CD34 biotin (ThermoFisher; 13-0341-85), reported to recognize mouse CD34 (manufacturer's website)
anti-SCA1 BUV395 (BD; 744328), reported to recognize mouse SCA1 (manufacturer's website)
Streptavidin BUV737 (BD; 612775)
donkey anti-Goat IgG H&L AF488 (abcam; ab150129), reported to recognize goat IgG (manufacturer's website)
anti-CD150 clone mShad150 PE (eBioscience; 12-1502-80), reported to recognize mouse CD150 (manufacturer's website)
anti-CD150 clone mShad150 PE-Cy7 (eBioscience; 25-1502-82), reported to recognize mouse CD150 (manufacturer's website)
anti-CD150 clone 9D1 PE (eBioscience; 12-1501-80), reported to recognize mouse CD150 (manufacturer's website)
anti-CD150 clone Q38-480 PE (BD; 562651), reported to recognize mouse CD150 (manufacturer's website)
anti-CD62p PE (BioLegend; 148308), reported to recognize mouse CD62p (manufacturer's website)
anti-Ly6D PE (eBioscience; 12-5974-80), reported to recognize mouse Ly-6D (manufacturer's website)
anti-CD51 PE (12-0512-81; ThermoFisher), reported to recognize mouse CD51 (manufacturer's website)
anti-CD61 PE (561910; BD), reported to recognize mouse CD61 (manufacturer's website)
anti-CD31 PE (561073; BD), reported to recognize mouse CD31 (manufacturer's website)
anti-CD38 (12-0381-81; ThermoFisher), reported to recognize mouse CD38 (manufacturer's website)
anti-CD47 clone MIAP301 PE (127507; BioLegend), reported to recognize mouse CD47 (manufacturer's website)
anti-CD47 clone MIAP410 PE (LS-C810701-25; LSBio), reported to recognize mouse CD47 (manufacturer's website)
anti-CD62p PE (148305;BioLegend), reported to recognize mouse CD62p (manufacturer's website)
anti-ALCAM PE (12-1661-82; ThermoFisher), reported to recognize mouse ALCAM (manufacturer's website)
anti-CD9 (124805; BioLegend), reported to recognize mouse CD9 (manufacturer's website)
anti-ESAM (136203, BioLegend), reported to recognize mouse ESAM (manufacturer's website)
anti-TIE2 (124007; BioLegend), reported to recognize mouse TIE-2 (manufacturer's website)
anti-CD201 (141503; BioLegend), reported to recognize mouse CD201 (manufacturer's website)
anti-cKIT clone ACK2 (135105; BioLegend), reported to recognize mouse c-KIT (manufacturer's website)
anti-Helios AF647 (BD; 563951), reported to recognize mouse Helios (manufacturer's website)
anti-CD3 APC-Cy7 (BioLegend; 100222), reported to recognize mouse CD3 (manufacturer's website)
anti-Ki67 R718 (BD; 566963), reported to recognize mouse Ki-67 (manufacturer's website)
anti-CD43 AF488 (BioLegend; 121210), reported to recognize mouse CD43 (manufacturer's website)
anti-CD8 BUV395 (BD; 563786), reported to recognize mouse CD8a (manufacturer's website)
anti-Foxp3 eF450 (Invitrogen; 48-5773-82), reported to recognize mouse FOXP3 (manufacturer's website)
anti-CD4 BV510 (BioLegend; 100559), reported to recognize mouse CD4 (manufacturer's website)
anti-CD44 BV605 (BD; 563058), reported to recognize mouse CD44 (manufacturer's website)
anti-CD62L BV711 (BioLegend; 104445), reported to recognize mouse CD62L (manufacturer's website)
anti-EOMES PE (Invitrogen; 12-4875-82), reported to recognize mouse EOMES (manufacturer's website)
anti-PD1 PE-CF594 (BD; 562523), reported to recognize mouse PD-1 (manufacturer's website)
anti-Tbet PE-Cy7(Invitrogen; 25-5825-82), reported to recognize mouse T-bet (manufacturer's website)
anti-CD25 PerCP-Cy5.5 (BioLegend; 102030), reported to recognize mouse CD25 (manufacturer's website)
anti-CD43 APC (BioLegend; 121214), reported to recognize mouse CD43 (manufacturer's website)
anti-CD21/CD35 APC-Cy7 (BioLegend; 123418), reported to recognize mouse CD21/CD35 (manufacturer's website)
anti-CD5 AF700 (BioLegend; 100636), reported to recognize mouse CD5 (manufacturer's website)
anti-IgM FITC (Invitrogen; 11-5790-81), reported to recognize mouse IgM (manufacturer's website)
anti-CD19 BUV395 (BD; 563557), reported to recognize mouse CD19 (manufacturer's website)
anti-IgD eFluor450 (eBioscience; 48-5993-82), reported to recognize mouse IgD (manufacturer's website)
anti-CD11b BV510 (BioLegend; 101245), reported to recognize mouse CD11b (manufacturer's website)
anti-MHCII BV605 (BD; 563413), reported to recognize mouse MHC II (manufacturer's website)
anti-CD40 BV711 (BD; 740700), reported to recognize mouse CD40 (manufacturer's website)
anti-PDL1 PE (Invitrogen; 12-5982-82), reported to recognize mouse PD-L1 (manufacturer's website)
anti-CD93 PE-Cy7 (BioLegend; 136506), reported to recognize mouse CD93 (manufacturer's website)

anti-CD23 PerCP-Cy5.5(BioLegend; 101618), reported to recognize mouse CD23 (manufacturer's website)
anti-CD45R/B220 PE-CF594 (BD; 562290), reported to recognize mouse B220/CD45R (manufacturer's website)
anti-CD8a Spark UV 387 (BioLegend; 100797), reported to recognize mouse CD8a (manufacturer's website)
anti-CD4 BUV 737 (BD, 612843), reported to recognize mouse CD4 (manufacturer's website)
anti-Gr1 Pacific Blue (BioLegend, 108429), reported to recognize mouse Gr-1 (manufacturer's website)
anti-CD3 BV 711 (BioLegend, 100241), reported to recognize mouse CD3 (manufacturer's website)
anti-CD44 BV785 (BioLegend, 103059), reported to recognize mouse CD44 (manufacturer's website)
anti-CD45.2 AF488 (BioLegend, 109815), reported to recognize mouse CD45.2 (manufacturer's website)
anti-Foxp3 PE (eBioscience, 12-5773-82), reported to recognize mouse Foxp3 (manufacturer's website)
anti-CD62L PE/Dazzle™ 594 (BioLegend, 104447), reported to recognize mouse CD62L (manufacturer's website)
CD19 PE-Cy7 (BioLegend, 115519), reported to recognize mouse CD19 (manufacturer's website)
anti-CD45.1 APC (BioLegend 110713), reported to recognize mouse CD45.1 (manufacturer's website)
anti-CD11b APC-Cy7 (BioLegend, 101225), reported to recognize mouse CD11b (manufacturer's website)

All antibodies for erythroid cell analysis or infectious analysis were validated by commercial manufacturer or have been cited in the literature: mAb 34 (PMID: 6787798), F-MuLV envelope-specific Mab 72015 (PMID: 1744218).
goat anti-mouse (H+L) HRP (EMD Millipore; AP308P), reported to recognize mouse IgG (manufacturer's website)
anti-mouse IgG2b FITC (BD; 553395), reported to recognize mouse IgG2b (manufacturer's website)
anti-Ter119 PE-Cy7 (Invitrogen; 25-5921-82), reported to recognize mouse Ter119 (manufacturer's website)

All antibodies used for human HSC analysis were validated by commercial manufacturer or have been cited in the literature:
anti-lineage panel PE-Cy5:
anti-CD3 (BD; 555341), reported to recognize human CD3 (manufacturer's website)
anti-CD4 (BD; 555348), reported to recognize human CD4 (manufacturer's website)
anti-CD8 (BD; 555368), reported to recognize human CD8 (manufacturer's website)
anti-CD11b (BD; 555389), reported to recognize human CD11b (manufacturer's website)
anti-CD14 (ThermoFisher; MHCD1406), reported to recognize human CD14 (manufacturer's website)
anti-CD19 (BD; 555414), reported to recognize human CD19 (manufacturer's website)
anti-CD20 (BD; 555624), reported to recognize human CD20 (manufacturer's website)
anti-CD56 (BD; 555517), reported to recognize human CD56 (manufacturer's website)
anti-CD235a (BD; 559944), reported to recognize human CD235a (manufacturer's website)

anti-CD34 APC-Cy7 (343514; Biolegend), reported to recognize human CD34 (manufacturer's website)
anti-CD45RA BV-785 (304139; Biolegend), reported to recognize human CD45RA (manufacturer's website)
anti-CD38 APC (555462; BD), reported to recognize human CD38 (manufacturer's website)
anti-CD90 FITC (328107;Biolegend), reported to recognize human CD90 (manufacturer's website)
anti-CD62P PE clone AK4 (304905; Biolegend), reported to recognize human CD62p (manufacturer's website)
anti-CD62P PE clone Psel.KO2.3 (12-0626-82; eBioscience), reported to recognize human CD62p (manufacturer's website)
anti-CD62P PE clone AC1.2 (550561; BD), reported to recognize human CD62p (manufacturer's website)
anti-CD150 PE (306307; Biolegend), reported to recognize human CD150 (manufacturer's website)
anti-TIE2 PE (334205; Biolegend), reported to recognize human Tie2 (manufacturer's website)
anti-ESAM PE (408519; Novus), reported to recognize human ESAM (manufacturer's website)
anti-CD166 (ALCAM) PE, (343903; Biolegend), reported to recognize human CD166 (ALCAM) (manufacturer's website)
anti-CD9 PE (312105; Biolegend), reported to recognize human CD9 (manufacturer's website)
anti-CD105 Endoglin PE (800503; Biolegend), reported to recognize human CD105 (manufacturer's website)
anti-CD304 (Neuropilin-1) PE (354503; Biolegend), reported to recognize human CD304 (manufacturer's website)

All antibodies used for in vivo antibody conditioning experiments were validated by commercial manufacturer or have been cited in the literature:

Isotype control antibodies:
mouse IgG1 (clone MOPC-21, Bio X Cell), reported to be a non-reactive isotype-matched control for mouse IgG1 antibodies (manufacturer's website)
rat IgG2b (clone LTF-2, Bio X Cell), reported to recognize keyhole limpet hemocyanin, which is not expressed by mammals, so useful for isotype-matched control for rat IgG2b antibodies (manufacturer's website)
rat IgG2a (clone RTK2758, BioLegend), reported to recognize KLH and tested as chosen as an isotype control on resting, activated, live, and fixed mouse tissues (manufacturer's website)

Experimental antibodies:
rat IgG2a anti-CD150 (clone TC15-12F12.2, BioLegend), reported to recognize mouse CD150 (manufacturer's website)
rat IgG2b anti-CD150 (clone mShad150, eBioscience), reported to recognize mouse CD150 (manufacturer's website)
anti-CD62p (clone RMP-1, BioLegend), reported to recognize mouse CD62p (manufacturer's website)
mouse IgG2a anti-goat (SB115d; SouthernBiotech), reported to recognize goat IgG (manufacturer's website)
mouse IgG2b anti-goat (SB115h; SouthernBiotech), reported to recognize goat IgG (manufacturer's website)
mouse IgG1 anti-CD47 (clone MIAP410, BioX Cell), reported to recognize mouse CD47 (manufacturer's website)
rat anti-cKIT (clone ACK2, Bio X Cell), reported to recognize mouse c-KIT (manufacturer's website)

goat anti-NEO1 (polyclonal cat# AF1079, R&D): Reported to recognize mouse Neogenin (manufacturer's website). Specificity for mouse Neogenin validated by loss of staining in NEO1-KO mice, comparing Nestin-Cre:Neogenin-fl/fl mice to control mice (Neuron 2020. PMID: 32562661); validated by FACS by labeling mouse HSCs by flow-cytometry that express higher NEO1 mRNA compared to unlabeled cells (PMID: 31754028); in vivo/in vitro functional neutralization (PMID: 22412855; PMID: 30479344; PMID: 35186922; PMID: 25029243; PMID: 31042165); validated by detecting mouse Neogenin by Western and IHC (R&D Systems); IHC (PMID: 33740419; PMID: 23457482); in vitro FACS antibody titration (this study, Extended Data Fig. 5c-h), FACS secondary antibody co-labeling on HSCs (this study, Extended Data Fig. 5n-s), and in vivo functional dose-response relationship (this study, Extended Data Fig. 5i-m).

Extensive additional characterization of antibodies was performed in this study:
To identify anti-CD150 antibodies that are not blocked by anti-CD150 antibody clone 1 (TC15-12F12.2, TC15), bone-marrow HSPC stained cells were incubated with saturating concentrations (200ug/mL) of unlabeled anti-CD150 antibody clone TC15 and then stained with PE-conjugated anti-CD150 clones 2 (Q38), 3 (9D1), or 4 (mShad150); PE-Cy7 conjugated anti-CD150 clone TC15 was used as a control. To confirm if any anti-CD150 clones identify the same population of cells as anti-CD150 antibody clone 1 (TC15) by flow-cytometry, bone-marrow HSPC stained cells were incubated with PECy-7 anti-CD150 antibody clone 1 (TC15) and with either PE-conjugated anti-CD150 clone 2 (Q38), 3 (9D1), or 4 (mShad150). To confirm that anti-CD150 antibody clone 4 (mShad150) does not block anti-CD150 clone 2 (Q38), bone-marrow cells were incubated with saturating concentrations (200ug/mL) of unlabeled anti-CD150 clone mShad150 and then stained with PE-conjugated anti-CD150 clone Q38; PE-Cy7 conjugated anti-CD150 clone mShad150 was used as a control. To confirm that anti-CD150 clone mShad150 and clone Q38 identify the same populations by flow-cytometry, bone-marrow cells were incubated with PECy-7 anti-CD150 clone mShad150 and with PE anti-CD150 clone 2 (Q38).

To confirm that mouse IgG2a (SB115d; SouthernBiotech) and IgG2b (SB115h; SouthernBiotech) anti-goat antibodies do not block donkey anti-goat IgG AF488 (abcam; ab150129), bone-marrow HSPC stained cells were incubated with saturating concentrations (100ug/mL) of unlabeled mouse IgG2a (6158-01; SouthernBiotech) or IgG2b (6157-01; SouthernBiotech) anti-goat antibodies and then stained with donkey anti-goat AF488. To confirm that mouse IgG2a and IgG2b anti-goat antibodies identify the same populations as donkey anti-goat IgG AF488 by flow-cytometry, bone-marrow HSPC stained cells were incubated with mouse IgG2a AF555 (6158-32; SouthernBiotech) or IgG2b PE (6157-09; SouthernBiotech) anti-goat antibodies, and with donkey anti-goat AF488.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Mus dunni cells, originally derived from an adult female M. dunni animal (PMID: 6092693), were used for in vitro experiments. The Mus dunni cells used in this study were obtained from ATCC (American Type Culture Collection).
Authentication	The Mus dunni cells were obtained from American Type Culture Collection (ATCC) and were not independently authenticated beyond the identity provided by ATCC.
Mycoplasma contamination	The Mus dunni cells used in this study tested negative for mycoplasma contamination upon receipt from ATCC.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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 mice were C57BL/6 or (C57BL/10×A.BY)F1 (H-2b/b, Fv1b, Rfv3r/s) and between 8-weeks to 120-weeks old. For transplant experiments, C57BL/6J (Jackson Labs, Strain #000664) CD45.2 mice were donors and B6.SJL-Ptprca Pepcb/BoyJ (Jackson Labs, Strain #002014) CD45.1 mice were recipients. Mice were bred and maintained at Stanford University's Research Animal Facility or at the Rocky Mountain Laboratories. All animal experiments were performed according to guidelines established by the Administrative Panel on Laboratory Animal Care of Stanford University or on an Animal Study Proposal approved by the Animal Care and Use Committee of the Rocky Mountain Laboratories (RML 2018-058, RML 2021-046) and carried out by certified staff in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited facility according to the institution's guidelines for animal use, the basic principles in the NIH Guide for the Care and Use of Laboratory Animals, the Animal Welfare Act, and the United States Department of Agriculture and the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals. Mice were housed in individually ventilated cages, same-sex, on a 12-hour light cycle, with temperature and humidity control, enrichment material, and ab libitum rodent chow and water.
Wild animals	No wild animals were used in this study.
Reporting on sex	All experiments were conducted with sex-matched animals, without bias to either sex. Sex-based analysis was not performed.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal experiments were performed according to guidelines established by the Administrative Panel on Laboratory Animal Care of Stanford University or on an Animal Study Proposal approved by the Animal Care and Use Committee of the Rocky Mountain Laboratories.

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

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	Mouse bone marrow cells: mice were euthanized and bone marrow harvested following one of two methods. The unilateral or bilateral femurs, tibias, and pelvises were dissected, cleaned, and collected in a mortar bowl containing PBS supplemented with 2% FBS (FACS-buffer) and 1mg/mL DNAse-I (LS002007; Worthington). Bones were crushed, and the resulting cell suspension was passed through a 40um filter. Alternatively, the femurs and tibias were dissected, cleaned, and cut at the joints and the bone marrow was flushed using an inserted 25-gauge needle and phosphate-buffered balanced salt solution (PBBS) with cells passed through a 100um filter. Cells were collected by centrifugation and washed with FACS-buffer multiple times. Red blood cells were depleted by ACK-lysis or by cKIT enrichment. For ACK-lysis, cells were resuspended in 1mL ACK Lysing Buffer (A1049201; ThermoFisher) and incubated for 10 minutes at room-temperature for 10 min. For cKIT enrichment, cells were Fc-blocked by incubation with 1mg/mL rat IgG (ab37361; abcam) for 30 minutes on ice, followed by the addition of anti-cKIT APC-eFluor780 (47-1171-82; ThermoFisher) for 30 minutes. Cells were collected by centrifugation and resuspended in FACS-buffer containing 10uL anti-APC MicroBeads (130-090-855; Miltenyi Biotec) and incubated for 20 minutes on ice. Cells were then washed and isolated with LS Columns (130-042-401; Miltenyi Biotec) using a MACS Separator (Miltenyi Biotec) according to manufacturer instructions, and then stained for flow cytometry as indicated in the methods section. For erythroid cell analysis, spleen cells were first incubated for 30 min with mAb 34, a mouse IgG2b specific for the FV glycoGag protein expressed on infected cells, then stained with anti-mouse IgG2b FITC (BD; 553395) and anti-Ter119 PE-Cy7 (Invitrogen; 25-5921-82). For HSC transplant experiments, 100 total HSCs (KLS FLT3–CD34–CD150+) were FACS-sorted from CD45.2 aged control mice, or CD45.2 aged mice that received antibody-conditioning 9 days earlier and injected retro-orbitally into 2-month-old recipient CD45.1 mice together with 1x106 whole-bone marrow CD45.1 support cells in 100uL PBS. Recipient mice received split-dose irradiation 24 hours prior to transplantation with 2 doses of 4.5Gy 4 hours apart. For chimerism analysis, peripheral blood was collected from the facial vein. HSC-derived donor cells were identified based on CD45.2 expression and host recipient cells were identified based on CD45.1 expression. Human bone marrow cells: bone marrow mononuclear cells from young-adult donors (ages 26-33) were commercially obtained (AllCells, Inc.). CD34-positive cells were enriched with CD34 MicroBead Kit (130-046-702; Miltenyi Biotec) according to manufacturer instructions, and then stained for flow cytometry as indicated in the methods section. Mouse blood cell isolation: mouse peripheral blood was collected in EDTA tubes after removal of cells through centrifugation at 500 RCF for 10 min and red blood cells were depleted with ACK-lysis, followed by a PBS wash, and then stained for flow cytometry as indicated in the methods section.
Instrument	Flow-cytometry was performed on a FACS Aria II (BD Biosciences) or FACS Symphony (BD Biosciences) at Stanford University or at the Rocky Mountain Labs.
Software	Flow cytometry data was collected using FACSDiva Software (BD) and and analyzed with FlowJo v10.8 Software (BD Life Sciences). Flow-cytometry computational analysis was conducted with the Spectre package (PMID: 33840138) using R version 4.2.2.
Cell population abundance	Cell population abundances are provided in the figures depicting gating strategies. For RNA-seq of purified HSCs, cells were directly sorted into lysis buffer. To evaluate sort purity, independent pilot experiments using the same gating strategies were conducted with post-sort purity of approximately 90%.
Gating strategy	FACS gating strategies for analysis are supplied in Extended Data Fig. 2a (mouse HSPCs), Extended Data Fig. 8a–c (T cells), Extended Data Fig. 8d–e (B cells), Extended Data Fig. 8g–i (mature/progenitor B cells), Extended Data Fig. 8j–l (mature myeloid cells), Extended Data Fig. 9b (Ter119+ and Ag34-infected cells), and Extended Data Fig. 10g–h (human HSCs and HPCs). FACS gating strategies for cell sorting are supplied in Extended Data Fig. 3m–n (total HSC: Aged vs. Aged +Conditioning). Population definitions are stated in the manuscript main text or methods. For analysis of human HSCs, for some antibodies the gating was determined by florescence-minus-one (FMO). For analysis of candidate mouse or human my-HSC markers, florescence-minus-one (FMO) controls were used to define gates. For erythroid/infected cell analysis, cells from uninfected controls were used for gating strategy. Previously published gating strategies were used and referenced to define and analyze mouse HSCs and HPCs (PMID: 33236985; PMID: 31754028; PMID: 26863982; PMID: 22123971), T cells (PMID: 31457092), B cells (PMID: 10429672;PMID: 21562046; PMID: 23410004; PMID: 33639971), and human HSCs (PMID: 22123971; PMID: 19180077). For peripheral blood chimerism analysis, donor cells were identified based on CD45.2 expression and host recipient cells were identified based on CD45.1 expression; Myeloid cells were defined as CD11b+CD19-CD3- cells and Lymphoid cells were defined as CD19+ or CD3+ (PMID: 31754028)

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