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# Life Sciences Reporting Summary

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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.

Sample size was calculated by a professional biostatistician by power analysis (Rosner B. Fundamentals of biostatistics.7th Edition Boston, MA. Brooks/Cole, 2011) and was approved by the Institutional Animal Care and Use Committee (IACUC) of San Raffaele Hospital Scientific Institute (Milan, IT) and by the Italian Governmental Health Institute (Rome, IT). A statement on results of sample-size calculation by power analysis has been included in the Methods section.

2. Data exclusions

Describe any data exclusions.

No data were excluded from analysis in each experiment.

3. Replication

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

All attempts at replication were successful in each experiment.

4. Randomization

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

Mice were allocated to experimental groups randomly.

5. Blinding

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

Technicians performing infusions into mice, sampling, preparation and flow cytometry analysis were blinded to experimental groups.

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<br>*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<br>*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. | Prism Software 5, GraphPad.<br>CellRanger v1.3 (10X Genomics). https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger<br>R environment (v3.3.2). https://www.r-project.org<br>Seurat v2.1. http://satijalab.org/seurat/ |

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. | No unique material was used. |

9. Antibodies

| | |
|---|---|
| Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species). | Mouse monoclonal Abs specific for human CD3 (BV510-conjugated, clone OKT3, Biolegend, lot nr. B226707, Cat 317332, dilution 1:100; APC-Cy7-conjugated, clone SK7, Biolegend, lot nr. B225054, Cat 344818, dilution 1:100), CD4 (PerCP-conjugated, clone SK3, BD Biosciences, lot nr. 23-5127-01, Cat 344624, dilution 1:100); CD8 (APC-Cy7-conjugated, clone SK1, Biolegend, lot nr. B209571, Cat 344714, dilution 1:100); CD14 (PerCP-conjugated, clone M P9, BD Biosciences, lot nr. 23-5143-01, Cat 345786, dilution 1:50); CD15 (BV510-conjugated, clone W6D3, Biolegend, lot nr. B201379, Cat 323028, dilution 1:100); CD19 (PE-conjugated, clone HIB19, Biolegend, lot nr. B188908, Cat 302208, dilution 1:100); CD33 (PE-conjugated, clone WM53, Biolegend, lot nr. B195145, Cat 303404, dilution 1:100); CD44v6 (PE-conjugated, clone 2F10, R&D, lot nr. YAV0616061, Cat FAB3660P, dilution 1:50; APC-conjugated, clone 2F10, R&D, lot nr. YAW0515041, Cat FAB3660A, dilution 1:50); CD45 (APC-Cy7-conjugated, clone HI30, Biolegend, lot nr. B214034, Cat 304014, dilution 1:200; PE-Cy7-conjugated, clone HI30, Biolegend, lot nr. B210429, Cat 304016, dilution 1:200); CD45RA (FITC-conjugated, clone HI100, Biolegend, lot nr. B202186, Cat 304106, dilution 1:100), CD62L (APC-conjugated, clone DREG-56, Biolegend, lot nr. B230061, Cat 304810, dilution 1:100); CD95 (PE-conjugated, clone DX2, Biolegend, lot nr. B2013943, Cat 305608, dilution 1:100); NGFR (PE-conjugated, clone C40-1457, BD Biosciences, lot nr. 7068641, Cat 557196, dilution 1:50); IL-6 (PE-conjugated, clone MQ2-13A5, Miltenyi Biotec, lot nr.5171106502, Cat 130-096-086, dilution 1:50); IL-1 (APC-conjugated, clone 364-3B3-14, Miltenyi Biotec, lot nr. 5171106567, Cat 130-109-227, dilution 1:50), and a rat mAb specific for mouse CD45 (Ly5.1; PerCP-conjugated, clone 30-F11, Biolegend, lot nr. B214531, Cat 103130, dilution 1:200) were purchased from commercial vendors. Samples were run through a FACS Canto II flow cytometer (BD Biosciences) and data were analyzed with the FlowJo software (LLC). Prior to use, all antibodies were validated and titrated on human peripheral blood and cord blood mononuclear cells or on mouse peripheral blood. |

10. Eukaryotic cell lines

| | |
|---|---|
| a. State the source of each eukaryotic cell line used. | Cell lines were purchased from ATCC. Patient-derived ALL-CM leukemic cells were kindly provided by Fred Falkenburg (Leiden University Medical Center, The Netherlands). 293T packaging cells were kindly provided by Luigi Naldini (Telethon Institute, San Raffaele, Milano IT). |
| b. Describe the method of cell line authentication used. | No cell line was authenticated. Prior to use, each cell line was however validated for surface antigen expression by flow cytometry and for xeno-engraftment in NSG mice (take, latency, biodistribution in relevant organs). |
| c. Report whether the cell lines were tested for mycoplasma contamination. | All cell lines were routinely tested for mycoplasma contamination by PCR and proved negative. |
| 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 line was 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 all relevant details on animals and/or animal-derived materials used in the study.

Eight-to-ten weeks old female or males NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl) or SGM3 mice (NSG TgCMV-IL3,CSF2,KITLG1Eav/MloySzJ) were purchased from Jackson Laboratories.

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.

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☐ Initial submission ☐ Revised version ☒ Final submission

# 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. | Human peripheral blood or cord blood mononuclear cells were isolated by density gradient centrifugation, washed extensively with PBS 2% BSA and stained at 4°C for 20min. Before acquisition, mouse peripheral blood, bone-marrow and spleen single-cell suspensions were treated with ACK solution to lyse red blood cells. |
| 6. Identify the instrument used for data collection. | BD FACSCanto II, BD Biosciences |
| 7. Describe the software used to collect and analyze the flow cytometry data. | FlowJo Software, LLC |
| 8. Describe the abundance of the relevant cell populations within post-sort fractions. | In each experiment, at least 20,000 human CD3+ or CD45+ events were acquired. |
| 9. Describe the gating strategy used. | Mouse peripheral blood, bone-marrow or spleen single-cell suspensions were serially gated as follows: FSC-A/FSC-H, then FSC-A/SSC-A, then human CD45/Ly5.1, then human CD3/CD19 (or any other relevant combination). Beckman-Coulter Flow-Count fluorospheres were gated as follows: FSC-A/FSC-H, then Pacific Blue/PE relevant fluorescence channel. |

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