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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
☐	☒ 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

For bulk RNA sequencing,total RNA was extracted by the RNeasy Mini Kit (Qiagen, Venlo, Limburg, Netherlands) after cell lysis. To completely remove any possible contaminating DNA, an on-column DNase digestion with the RNase-free DNase set (Qiagen) was performed during total RNA isolation. Quality control of the total RNA was performed using Agilent TapeStation 2200 (Agilent Technologies). RNA integrity (RIN) was routinely found to be optimal (RIN > 7.0). Libraries for transcriptome analysis were prepared using the Smart-seq2 protocol. Briefly, 2 ng total RNA were copied into a first strand cDNA by reverse transcription and template-switching, using oligo (dT) primers and a LNA-containing template-switching oligo (TSO). The resulting cDNA was pre-amplified, purified, and tagmented with Tn5 transposase. cDNA fragments generated after tagmentation were gap-repaired, enriched by PCR and purified to create the final cDNA library. Libraries were sequenced on the Illumina NextSeq 500 in single-read mode (1x75 cycles).
For sc-RNA samples, sorted cells were labelled by using the BD Single-Cell Multiplexing Kit (BD Biosciences), strictly following the manufacturer's protocol (BD Biosciences). Such a procedure allowed us to combine 5 samples (NCP1s, NCP2s, NCP3s, NCP4s and cMoPs) into a single pool. Each sample was washed twice in FACS buffer and resuspended in cold BD Sample Buffer (BD Biosciences). Only samples with high viability (> 85 %, as evaluated by the BD Rhapsody scanner) were used for sequencing. Samples from the same donor were pooled in equal amounts to achieve approximately 15000 cells in 620 µl, and loaded onto a BD Rhapsody cartridge for an incubation of 20 min at room T. Then, Cell Capture Beads (BD Biosciences) were added to the cartridge, incubated at room T for 3 min, and thereafter Cartridges washed. Cell were then lysed, and the released mRNA captured by Cell Capture Beads. mRNAs were then retrieved, to be washed prior to performing reverse transcription and treatment with Exonuclease I. cDNA Libraries were prepared by using the BD Rhapsody Whole Transcriptome Analysis (WTA), Amplification kit and BD Single-Cell Multiplexing kit (BD Biosciences). Size-distribution (Quality) of final libraries was assessed by Agilent 2200 TapeStation with High Sensitivity D5000 ScreenTape and quantified using a Qubit Fluorometer using the Qubit dsDNA HS Kit (ThermoFisher, #Q32854). Sequencing was performed in paired-end mode (2x75 cycles) on a NextSeq 500 System (Illumina). This procedure was utilized for three different BMs.

Data analysis

Sequencing Analysis:
For sc-RNA samples, after demultiplexing of bcl files by using Bcl2fastq2 V2.20 from Illumina and assessment of reads quality, paired-end scRNA-seq reads were then filtered for valid cell barcodes using the barcode whitelist provided by BD bioscience. Then, sequenced reads were

aligned to the hg38 human transcriptome and the expression of transcripts in each cell was quantified via the standard Rhapsody analysis pipeline (BD Biosciences) on Seven Bridges (https://www.sevenbridges.com), following manufacturer's recommendations. After gene expression matrix was created, integration and further analysis were performed using R package Seurat v3.2.2. Pseudo-time trajectories were determined using R package destiny v3.0.1
For bulk RNA sequencing, computational analysis of transcriptome datasets generated by Smart-seq2 has been performed by using the following bioinformatic pipeline. Briefly, after quality filtering, according to the Illumina pipeline, removal of contaminant adapters and base quality trimming were performed using Trim Galore! v0.6.4_dev (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) script. Gene counts were normalized among various samples using DESeq2 v1.26.0, and only genes coding for protein and long non-coding RNA (lncRNA) were retained for downstream analysis. Only genes expressed above 1 FPKM, in at least one sample, were considered as "expressed" genes and thus included for downstream analysis. Differentially expressed genes (DEGs) were identified using DESeq2, by using as selection parameter adjusted P-value lower than 0.01 and likelihood ratio test (LRT). Batch effects were removed using the limma v3.42.2 package's "removeBatchEffect" function before performing principal component analysis (PCA). PCA was performed on DEGs by using Bioconductor/R package pcaExplorer v.2.10.0.
Gene ontology (GO) analysis:
GO analysis was performed on DEGs from bulk RNA-seq and scRNA-seq for the cellular component, biological process and molecular function ontology domains by using the Bioconductor/R package clusterProfiler (version 3.14.3). Over-representation analysis was performed using the Benjamini-Hochberg (BH) procedure, with P value Cutoff = 0.01 and Q-value Cutoff = 0.05. Redundant GO terms were removed by using the "simplify" function in the clusterProfiler package, using the Wang similarity measure and a similarity cutoff of 0.5. The most significant terms were then plotted in R.
R scripts for data processing are available through https://github.com/fbianchetto/Neutrophil-myeloblast

Flow Cytometry:
Flow cytometry quantification was performed using FlowJo v10.7.1
Statistical analysis was performed using GraphPad Prism 7

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Raw and processed datasets have been submitted to the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) and are available under the accession number GSE164687.

Public Datasets Used:
GSE113182: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE113182
GSE153263: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE153263
seurat_COVID19_Neutrophils_cohort2_rhapsody_jonas_FG_2020-08-18.rds: https://beta.fastgenomics.org/datasets/detail-dataset-ee4b1a0f339140ad82f861aea35076f1

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 was chosen based on past experience in the lab and on the standard practices in the field with a minimum of n=3 and was in individual experiments increased to be sufficient to demonstrate statistically significant differences in comparisons between experimental groups by statistical tests described in the text. Further increase in sample size was mainly limited by the availability of human donor material (alloHSCT Bone Marrows). No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications (PMID:32719519; PMID: 32601182; PMID: 31160568)
Data exclusions	No data were excluded
Replication	Experiments were performed at least three times independently. All attempts at replication were successful within a reasonable range of variability due to inter-individuals diversity. The number of replicates for every experiments is indicated in the figure legends
Randomization	No randomisation was performed in experiments with BM cells because not relevant. Samples were allocated into experimental groups based on gating strategy. In each animal experiment, mice were randomly assigned to each group.

Blinding	There was no blinding in our in vitro experiments because for flow citometry experiments, bulk RNAseq and scRNAseq, the analysis was not operator dependent. In bulk RNAseq and scRNAseq experiments unbiased clustering was conducted using the software indicated above

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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

CD14 (clone REA599)-Miltenyi Biotec; Cat# 130-110-524; RRID:AB_2655055; 1:50
CD34 (clone AC136) -Miltenyi Biotec ; Cat# 130-113-182; RRID:AB_2726008; 1:50
CD33 (clone WM53) -Biolegend; Cat# 303416; RRID:AB_2561690; 1:20
CD10 (clone H110a) -Biolegend; Cat# 312218; RRID:AB_2561833; 1:20
CD125 (clone A14) -BD; Cat# 743927; RRID:AB_2741855; 1:20
CD15 (clone W6D3) -Biolegend; Cat# 323040; RRID:AB_2566520; 1:20
CD45 (clone HI30) -Biolegend; Cat# 304036; RRID:AB_2561940; 1:20
CD123 (clone 6H6) -Biolegend; Cat# 306022; RRID:AB_2562068; 1:20
CD15 (clone W8D3) -BD; Cat# 562979; RRID:AB_2744292; 1:20
CD45RA (clone HI100) -Biolegend; Cat# 304136; RRID:AB_2563653; 1:20
CD14 (clone M5E2) -BD; Cat# 563698; RRID:AB_2744287; 1:20
CD115 (clone 9-4D2-1E4) -Biolegend; Cat# 347312; RRID:AB_2566758; 1:20
CD66b (clone G10F5) -Biolegend; Cat# 305104; RRID:AB_314496; 1:20
CD14 (clone REA599) -Miltenyi Biotec; Cat# 130-110-518; RRID:AB_2655049; 1:50
CD141 (clone AD5-14H12) -Miltenyi Biotec; Cat# 130-113-317; RRID:AB_2726094; 1:50
CD1c (clone AD5 8E7) -Miltenyi Biotec; Cat# 130-113-301; RRID:AB_2726080; 1:50
CD303 (clone AC144)-Miltenyi Biotec; Cat# 130-113-192; RRID:AB_2726017; 1:50
CD64 (clone 10.1) -Biolegend; Cat# 305008; RRID:AB_314492; 1:20
CLEC9A (clone 8F9) -Miltenyi Biotec; Cat# 130-097-368; RRID:AB_2657789; 1:50
FCεR1 (clone CRA1) -Miltenyi Biotec; Cat# 130-100-162; RRID:AB_2660603; 1:50
CD10 (clone HI10a) -Biolegend; Cat# 312204; RRID:AB_314915; 1:20
CD114 (clone LMM741) -Biolegend; Cat# 346106; RRID:AB_2083867; 1:20
CD123 (clone AC145) -Miltenyi Biotec; Cat# 130-113-326; RRID:AB_2726103; 1:50
CD1c (clone AD5-8E7) -Miltenyi Biotec; Cat# 130-113-302; RRID:AB_2726081; 1:20
CD45 (clone HI30) -BD; Cat# 562279; RRID:AB_11154577; 1:20
CD45 (clone 5B1) -Miltenyi Biotec; Cat# 130-113-120; RRID:AB_2725948; 1:50
CD16 (clone 3G8) -Biolegend; Cat# 302027; RRID:AB_893262; 1:20
CD45RA (clone HI100) -Biolegend; Cat# 304122; RRID:AB_893357; 1:20
CD11b (clone REA713) -Miltenyi Biotec; Cat# 130-110-555; RRID:AB_2654673; 1:50
CD123 (clone AC145) -Miltenyi Biotec; Cat# 130-113-327; RRID:AB_2726104; 1:50
CD14 (clone REA599) -Miltenyi Biotec; Cat# 130-110-521; RRID:AB_2655061; 1:50
CD141 (clone AD5-14H12) -Miltenyi Biotec; Cat# 130-113-319; RRID:AB_2726096; 1:50
CD19 (clone REA675) -Miltenyi Biotec; Cat# 130-113-647; RRID:AB_2726200; 1:50
CD1c (clone AD5-8E7) -Miltenyi Biotec; Cat# 130-113-303; RRID:AB_2726082; 1:50
CD3 (clone REA613) -Miltenyi Biotec; Cat# 130-113-140; RRID:AB_2725968; 1:50
CD10 (clone HI10a) -Biolegend; Cat# 312210; RRID:AB_314921; 1:20
CD14 (clone REA399) -Miltenyi Biotec; Cat# 130-110-520; RRID:AB_2655053; 1:50
CD19 (clone LT19) -Miltenyi Biotec; Cat# 130-113-165; RRID:AB_2725993; 1:50
CD303 (clone AC144) -Miltenyi Biotec; Cat# 130-113-190; RRID:AB_2726015; 1:50
CD34 (clone AC136) -Miltenyi Biotec; Cat# 130-113-176; RRID:AB_2726003; 1:50
CD66b (clone G10F5) -Biolegend; Cat# 305118; RRID:AB_2566607; 1:20
CD66b (clone G10F5) -Biolegend; Cat# 305114; RRID:AB_2566038; 1:20
CD11b (clone ICRF44) -Biolegend; Cat# 301342; RRID:AB_2563395; 1:20
CD16 (clone 3G8) -Biolegend; Cat# 302018; RRID:AB_314218; 1:20
CD38 (clone HIT2) -Biolegend; Cat# 303534; RRID:AB_2561605; 1:20
CD45RA (clone HI100) -Biolegend; Cat# 304128; RRID:AB_10708880; 1:20
HLA-DR (clone L243) -Biolegend; Cat# 307618; RRID:AB_493586; 1:20
CD3 (clone UCHT1) -Biolegend; Cat#300436; RRID:AB_2562124; 1:20

CD19 (clone HIB19) -Biolegend; Cat#302236; RRID:AB_2563606; 1:20
HLA-A2 (clone BB7.2)- Biolegend; Cat#343316; RRID:AB_2561573; 1:20
mouse CD45.1 (A20)- Thermo Fisher Scientific; Cat#47-0453-82; RRID:AB_1582228; 1:20
CD56 (clone REA196) -Miltenyi Biotec; Cat#130-114-550; RRID:AB_2726696; 1:50
CD123 (clone REA918)-Miltenyi Biotec; Cat#130-115-272; RRID:AB_2726975; 1:50
CD49d (clone MZ18-24A9)-Miltenyi Biotec; Cat#130-125-206; RRID:AB_2857751; 1:50
CD71 (clone CY1G4) -Biolegend; Cat#334110; RRID:AB_2563117; 1:20
CD117 (clone A3C6E2) -Miltenyi Biotec; Cat#130-113-541; RRID:AB_2733335; 1:50
MPO (Rabbit polyclonal) -Agilent Technologies; Cat# A0398; RRID:AB_2335676; 1:5000
ELANE (clone NP57)-Agilent Technologies; Cat# M0752; RRID:AB_630032; 1:200
α-Defensins (clone H-2) - Santa Cruz Biotechnology; Cat# sc-390796; 1:70
LTF (Rabbit polyclonal) -Sigma-Aldrich; Cat# hpa059976; RRID:AB_2684185; 1:500

Validation

All the antibodies are well recognized clones in publications, commercially available and validated by manufacturer (https://www.biolegend.com/en-us/quality/quality-control; https://www.miltenyibiotec.com/US-en/products/macs-antibodies/Antibody-production-development-and-quality-control.html; https://www.thermofisher.com/it/en/home/life-science/antibodies/invitrogen-antibody-validation.html; https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents). See RRIDs for respective websites for each antibody. In addition these antibodies are routinely used in our laboratory with good reproducibility.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Mouse MS-5 cells from German Collection of Microorganisms and Cell Cultures GmbH, DSMZ Cat# ACC-441
HEK293T cells from German Collection of Microorganisms and Cell Cultures GmbH, DSMZ Cat# ACC 635

Authentication

None of the cell lines used have been authenticated.

Mycoplasma contamination

All the cell lines were negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Female NOD.Cg-PrkdcscidIl2rgtm1Sug/JicTac (NOG) mice, 4 weeks old, were used for this study. All the animals were housed in conditions of 22°C and 45-65% humidity with 12 hours light cycle.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve field-collected samples.

Ethics oversight

NOD.Cg-PrkdcscidIl2rgtm1Sug/JicTac (NOG) mice were purchased from Taconic Biosciences.
All mice were maintained under specific pathogen-free conditions in the animal facility of the University of Verona. Animal experiments were performed according to national (protocol number 12722 approved by the Ministerial Decree Number 14/2012-B of January 18, 2012 and protocol number BR15/08 approved by the Ministerial Decree Number 925/2015-PR of August 28, 2015) and Europeans laws and regulations. All animal experiments were approved by Verona University Ethical Committee (https://www.univr.it/it/cirsal) and conducted according to the guidelines of Federation of European Laboratory Animal Science Association (FELASA).

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

Human research participants

Policy information about studies involving human research participants

Population characteristics

Buffy-coats are from anonymous, random gender, random blood group adult donors. BM samples were obtained from healthy donors selected for BM donation purposes according to the Italian Bone Marrow Donor Registry (IBMDR) criteria. BM samples were obtained also from patients undergoing allogeneic hematopoietic stem cell transplantation (alloHSCT) for hematological malignancies. To dissect the different steps of myeloid differentiation, BM samples were collected at day +21 after alloHSCT, when patients have achieved full donor chimerism according to variable number tandem repeats (VNTR) analysis.
BM samples were obtained from healthy donors (n=35, 51% male). Age and gender for every donor are indicated in Supplementary Table 4. Mean age of the donors is 29

Recruitment

Blood from healthy donors was obtained from the Transfusion Center, University hospital, Verona (Italy). Bone marrows from healthy donors and alloHSCT patiens were obtained from the Bone Marrow Transplant Unit, University hospital, Verona (Italy).

Ethics oversight	Human samples were obtained following informed written consent by HDs and alloHSCT patients. The study has been cleared by the Ethic Committee of the Azienda Ospedaliera Universitaria Integrata di Verona (Italy) (CMRI/55742).

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	BM samples were collected under aseptic conditions in heparinized sterile syringe, processed using endotoxin-free polypropylene tubes (Greiner bio-one), and subjected to density gradient centrifugation onto Ficoll-Paque (GE Healthcare). For fluorescence-activated cell sorting of the myeloid progenitors, 50-100*106 BM-LDCs were resuspended at 100*106/ml and labeled for 45 min at 4°. Cells were then washed and resuspended at 30*106/ml in staining buffer, to be ultimately filtered through a 0.35 µM nylon mesh. BM-LDCs, sorted progenitor subpopulations or culture-derived cells were counted, resuspended in 50 µl PBS buffer plus 2 % FBS and 2 mM EDTA (Sigma-Aldrich) , and subsequently processed for flow cytometry. Cells were incubated for 10 min in the presence of 5 % human serum. Cells were then stained for 30 min on ice by fluorochrome-conjugated monoclonal antibodies.
Instrument	Flow cytometry data collection was performed with MACSQuant10 Analyzer (Miltenyi Biotec), LSRFortessa X-20 (BD) or MACSQuant16 Analyzer (Miltenyi Biotec) flow-cytometers. Fuorescence-activated cell sorting was performed by using the FACSAria Fusion (BD) cell sorter.
Software	Flow cytometry data were analyzed using FlowJo v10.7.1.
Cell population abundance	Sorted cell populations displayed a > 95 % purity, as verified by flow cytometry analysis and dead cell exclusion. Usually , immature progenitors or peripheral blood populations were sorted in a range from 2*10^3 to 1*10^6 cells.
Gating strategy	Mature cell populations were identified by sequentially gating on singlets, then on PI- cells, and finally on CD45+ cell. Both lineage-positive cells and immature progenitors were identified by gating on either SSClowCD66b-, or SSChiCD66b+, fraction, as it follows: SSChiCD66b+CD16-CD45hi eosinophils ; ii) SSChiCD66b+ CD16-/+CD45+ neutrophils (from promyelocytes to segmented neutrophils); iii) SSClowCD141+CLEC9A+ cDC1 DCs ; iv) SSClowFceRI+CD1c- basophils ; v) SSClowFceRIdimCD1c+ cDC2 DCs ; vi) SSClowCD45RA+CD33+CD123+CD303+ pDCs; vii) SSClowCD3-CD19-CD56+ NK cells; viii) SSClowCD3-CD19-CD56-CD14+CD16-/CD14dim/-CD16+ total monocytes; ix) SSClowCD3+CD19- T cells; x) SSClowCD3-CD19+ B cells; and xi) SSClowCD45dim cells including CD34+CD38- HSCs, CD34+ and CD34dim/- myeloid/lymphoid progenitors. For immature progenitors within BM-LDCs, analysis was performed on live, SSClowCD66b- cells, CD3/CD19/CD1c/CD141/CD16/CD56(Lin)-negative cells. Within the latter cells, the CD45dim region defines the SSClowLin-CD45dim progenitor pool, from which we could subsequently identify each progenitor population by using an antibody panel comprising key markers conventionally used to detect myeloid and lymphoid progenitors, namely CD34, CD38, CD10, CD123, CD45RA, CD64 and CD115. Fluorescence Minus One (FMO) or isotype control mAbs were used to determine background staining.

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