



HAL
open science

Hematopoiesis in numbers

Jason Cosgrove, Lucie Hustin, Rob de Boer, Leïla Perié

► **To cite this version:**

Jason Cosgrove, Lucie Hustin, Rob de Boer, Leïla Perié. Hematopoiesis in numbers. Trends in Immunology, In press, 10.1016/j.it.2021.10.006 . hal-03425018

HAL Id: hal-03425018

<https://hal.sorbonne-universite.fr/hal-03425018>

Submitted on 10 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Hematopoiesis in numbers

Jason Cosgrove^{1*}, Lucie Hustin^{1*}, Rob de Boer², Leïla Perié¹

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

Author Affiliations

1. Institut Curie, Université PSL, Sorbonne Université, CNRS UMR168, Laboratoire Physico Chimie Curie, Paris, France

2. Theoretical Biology and Bioinformatics, Utrecht University, Utrecht, Netherlands

* both authors contributed equally

Contact information: Leila.Perie@curie.fr

20 **Abstract:** Hematopoiesis is a dynamic process in which stem and progenitor cells give rise to
21 the $\sim 10^{13}$ blood and immune cells distributed throughout the human body. We argue that a
22 quantitative description of hematopoiesis can help to consolidate existing data, identify
23 knowledge gaps, and generate new hypotheses. Here, we review known numbers in murine
24 and, where possible, human hematopoiesis and consolidate murine numbers into a set of
25 reference values. We present estimates of cell numbers, division and differentiation rates, cell
26 size and macromolecular composition for each hematopoietic cell type. We also propose
27 guidelines to improve reporting of measurements and highlight areas where quantitative data
28 are lacking. Overall, we show how quantitative approaches can be used to understand key
29 properties of hematopoiesis.

30 **A quantitative understanding of hematopoiesis**

31
32 Hematopoiesis is the process by which hematopoietic stem and progenitor cells (HSPCs) give
33 rise to blood and immune cells [1]. Here, we argue that numbers are tools to sharpen our
34 understanding of hematopoiesis [2], but it is challenging and time consuming to find robust
35 values in the literature. Numbers come from various studies and it is rarely discussed how they
36 fit together into our broader understanding of hematopoiesis. To address these issues, we
37 review existing numbers in murine and human hematopoiesis, and generate a set of reference
38 values for murine hematopoiesis. Specifically, we discuss current understanding of cell
39 numbers, division and differentiation rates, cell size and macromolecular composition for each
40 hematopoietic cell type. We also highlight limitations of existing measurements, and instances
41 where quantitative data are lacking. Then using simple calculations, we show how this
42 numerical reference can identify key properties of hematopoiesis and provide suggestions for
43 future research.

44 **How many cells are in the hematopoietic system?**

45 Quantifying absolute cell numbers is important to help contextualize changes that occur in
46 hematopoiesis across lifespan and disease. Here, we summarize current measurements of cell
47 numbers across the major hematopoietic organs, lineages and compartments, focusing on the
48 16 week old, 22 gram adult female mouse for which we have the most available data (**File S1**,
49 **Calculation S1a** and [3]). We also summarize known cell numbers in humans. The criteria and
50 sources used to generate reference values are provided in **File S1**, along with metadata for each
51 measurement.

52

53 *How many cells are there in the hematopoietic organs?*

54 Consolidating cell numbers across the hematopoietic system is non-trivial because (i) cells are
55 heterogeneously distributed across tissues, (ii) differences in sample processing can affect cell
56 yield, (iii) most measurements are expressed in relative rather than absolute terms, and (iv)
57 most numbers come from samples rather than entire tissues. Reviewing existing numbers and
58 accounting for these factors (**Table 1**, **Figure 1**, **Calculation S1a**), the bone marrow, blood,
59 spleen, lymph nodes and thymus have 4.5×10^8 , 1.6×10^{10} , 2×10^8 , 7.4×10^7 and 2.1×10^8
60 hematopoietic cells respectively (**Calculation S1a**, **Table 1**). Adding these values, this gives
61 1.7×10^{10} cells in total – likely an underestimate of the true value, as organs such as the liver
62 and gut are not considered in this calculation.

63

64 In humans the standard reference person, historically defined as a male 20–30 years of age,
65 weighing 70 kg and 170 cm in height [4] has $\sim 1.2 \times 10^{12}$ nucleated bone marrow cells and
66 2.8×10^{13} blood cells (**File S1**) [5]. Surprisingly, direct measurements of human spleen and
67 lymph node cellularity are missing. Assuming the average spleen weighs 130-150g [6] and a
68 single cell weighs 1ng [7], there are $\sim 10^{11}$ human splenic cells. A 1974 study extrapolating data

69 from rats to humans reports a total of 1.9×10^{11} lymph node lymphocytes [8], distributed
70 amongst 1200 lymph nodes [9]. This reported number of lymph nodes, obtained from a
71 reference digital image library, [9] is much higher than the 460 lymph nodes cited by [8], for
72 which we could find no primary data. The lack of direct measurements of the spleen and the
73 huge range between reported lymph node numbers illustrate that much work is needed to
74 quantify the human hematopoietic system.

75 *How many Hematopoietic Stem Cells are there?*

76 Flow cytometry analyses suggest that murine **HSCs** ($\text{Lin}^- \text{Kit}^+ \text{Sca-1}^+ \text{CD150}^+ \text{CD48}^-$) represent
77 0.006% of nucleated bone marrow cells and $\sim 16,000$ cells in total [10]. However, this is likely
78 to be an overestimate, as a significant proportion ($\sim 30\text{-}70\%$) of immunophenotypic HSCs do
79 not meet the functional criteria of **multipotency and self-renewal** [11–13]. In addition, only a
80 subset of HSCs actively contribute to hematopoiesis (**differentiation active**), as determined by
81 **fate mapping** [10,14] and **lineage tracing** [15–17] experiments in mice. **Differentiation**
82 **active** murine **HSC** numbers range from 2770 to 22,400 [10,18,19]. Given there are 16,000
83 immunophenotypic **HSCs** in mice and at least 1/3 of Tie2-YFP labelled murine **HSCs** give rise
84 to mature cells, as measured by **fate mapping** [10], we use $\sim 5,200$ as a reference value for the
85 number of **differentiation active** murine **HSCs**.

86

87 In humans, the number of **differentiation active HSCs** in adults ranges from 25,000-1,300,000
88 based on inference from **capture-recapture longitudinal genomic analyses of a 59 year-old**
89 **male** [20] and allele frequency data from a large cohort of blood cancer-free individuals [21].
90 In a gene therapy context, fewer human **HSCs** (1600-4300) actively contribute to **long-term**
91 **hematopoiesis post-transplantation**, as inferred from **lentiviral vector integration site** data
92 in patients with **Wiskott-Aldrich Syndrome** [22].

93 *How many hematopoietic progenitors are there?*

94 Downstream of HSCs are the multipotent progenitors (**MPPs**), a functionally heterogeneous
95 population [23], that harbor multi-lineage potential but lack long-term repopulating capacity
96 post-transplantation [24,25]. Flow cytometry measurements report that the **MPP** compartment
97 is ~9 times bigger than the HSC compartment [10], giving 1.4×10^5 **MPPs** in our reference
98 mouse (**Table 1**).

99

100 Downstream of the **MPP** are the **restricted potential progenitors (RPPs)** which can be
101 subdivided into the common myeloid progenitors (**CMP**), granulocyte-monocyte progenitors
102 (**GMP**) and megakaryocyte-erythroid progenitors (**MEP**), the common lymphoid progenitors
103 (**CLP**) and the megakaryocyte progenitors (**MkP**). Based on flow cytometry measurements
104 [10,26,27] we compute that for each MPP there are 2.9, 3.6, 5.7, 0.2 and 1.8 times more CMPs
105 GMPs, MEPs, MkPs, and CLPs respectively (**Table 1, Calculation S1b**).

106

107 Summing all **HSC, MPP** and **RPP** populations, we estimate that progenitors account for ~0.5%
108 of the murine bone marrow (**Table 1**), while flow cytometry measurements suggest that **lineage**
109 **negative cells** range from 1-5.7% of total bone marrow [12,28–30]. Taking 4.5% as a
110 representative value [29], we estimate that 4% of bone marrow cells (~18 million cells) are not
111 classically defined progenitors, but don't express mature lineage markers. These cells contain
112 both hematopoietic and non-hematopoietic cells, and remain to be better functionally defined.

113

114 In humans, **CD34⁺ progenitors (HSCs, MPPs and RPPs)** represent an average of 2.5% of all
115 mononucleated bone marrow cells [31,32]. Given the total number of nucleated bone marrow
116 cells from [4], this suggests a total of $\sim 3 \times 10^{10}$ CD34⁺ cells in our reference person. Currently,
117 there are conflicting results about the effect of age on the frequency of CD34⁺ cells [33,34],

118 and it is unclear to what extent such frequencies change due to gender, ethnicity, and lifestyle
119 factors.

120 *How many mature hematopoietic cells are there?*

121
122 At the bottom of the hematopoietic hierarchy are the terminally differentiated mature cells. To
123 date, the most extensive measurements in mice account for all mature cells in blood, bone
124 marrow, spleen, thymus, and lymph nodes [35]. For our reference mouse, there are 1.5×10^{10}
125 erythrocytes, 1.5×10^9 platelets, 1.6×10^8 myeloid cells, 1.8×10^8 B lymphocytes, and 2.2×10^8 T
126 lymphocytes (**Table 1**). For megakaryocytes, flow cytometry measurements (0.29% of all
127 nucleated bone marrow cells [36]) suggest a total of 7.7×10^5 cells. Together, this yields a total
128 of 1.7×10^{10} mature hematopoietic cells.

129
130 In humans, a recent metadata analysis [4] calculates that there are 25×10^{12} erythrocytes,
131 1.5×10^{12} platelets, 7×10^{11} T cells, and 3×10^{11} B cells, monocytes 5×10^9 and 6.4×10^{11}
132 neutrophils [4], consolidating measurements from human tissue samples along with
133 measurements from rodents and primates where no human data was available.

134
135 In tallying hematopoietic cell numbers (**Calculation S1, Table 1**), we find that compartment
136 sizes vary by several orders of magnitude within and between lineages in both mice and
137 humans. Notably, progenitors are much rarer than mature cells (**Figure 2, Table 1**), suggesting
138 that significant cell expansion occurs downstream of the RPP compartment, after lineage
139 commitment has occurred. Quantifying hematopoietic cell numbers is a topic that warrants
140 further experimental focus (see ‘outstanding questions’), particularly in humans. When
141 measurements in humans are unavailable, numbers have been extrapolated from rodents.
142 However, cross-species measurements highlight important differences calling for carefulness,

143 for example myeloid cells account for ~9-16% of all blood leukocytes in our reference mouse
144 [3,35], but in humans this number is ~65% [6]. Total cell numbers can help to understand how
145 hematopoiesis changes during disease, complementing other resources such as the human cell
146 atlas, and facilitating cross-species comparisons to understand how the hematopoietic system
147 has evolved.

148 **How many hematopoietic cells do we produce per day?**

149 The rates at which cells divide, differentiate and die are key regulators of cell numbers [10,14],
150 and if perturbed can lead to hematological malignancies [37]. In the following section we
151 discuss current understanding of these parameters, excluding T-cells that have been reviewed
152 elsewhere [38].

153 *Division rate and division number*

154 Division rates can be measured using **division-linked dilution assays**, or reporter molecules
155 that incorporate into newly synthesized DNA [39]. In mice, HSCs are reported to divide every
156 145 days [40]. These values are on the same order of magnitude as estimates derived from **fate-**
157 **mapping** studies [10,14]. However the reported proportion of cells entering S-phase per unit
158 time, using a BrdU EdU sequential labelling technique, suggests a proliferation rate of
159 approximately once every 4 days [41]. Presumably these differences in reported values arise
160 due to differences in labeling techniques or heterogeneity within **HSCs** [14,41,42]. In mice,
161 MPPs reportedly cycle faster than HSCs [14,43] *in vivo* EdU labeling showed that cycling rates
162 for RPPs are higher than those measured in MPPs [43]. These data are consistent with a linear
163 amplification model where proliferation rates increase with early maturation[14]. In humans,
164 HSCs have been estimated to divide once every ~280 days, a value inferred by mathematical
165 modelling of X chromosome inactivation patterns in females [44]. Division rates for human
166 MPPs, and RPPs *in vivo* are lacking however.

167

168 Despite recent progress in our ability to quantify division rates, the number of divisions it takes
169 to differentiate an HSC into a mature cell is unknown. Knowing the size of each cell
170 compartment, it is possible to estimate the minimum number of divisions in this process (Figure
171 **3 Calculation S2a**). Performing this estimation in mice suggests that at least 15-22 divisions
172 are needed to account for the expansion of 5200 HSCs to 10^8 - 10^{10} mature cells (Figure 3
173 **Calculation S2a**), with erythropoiesis requiring ~ 7 more divisions than myelopoiesis.
174 Considering the fraction of B cells that survive after negative and positive selection, we
175 estimate a minimum of 20 divisions are needed to produce all mature B cells (**Figure 3**
176 **Calculation S2b**). We estimate that megakaryocytes derive from HSC in 7 divisions (**Figure**
177 **3 Calculation S2c**), not including **endomitoses** and produce on average 519 platelets each day
178 (**Calculation S2d**). Importantly, these estimates don't consider HSC heterogeneity in clonal
179 expansion, nor the impact of cell death and differentiation, processes that are discussed below.

180 *Lifespans and turnover rates*

181 A key parameter which regulates cell numbers is the rate of cell death, which can be derived
182 from half-life and lifespan measurements (**Table 2**). Half-life measures are mathematically
183 inferred by following the loss of a label over time within a cell population. Cells can be labelled
184 *in vivo* with BrdU, EdU, deuterium isotope or *ex vivo* [45]. Half-lives have not been measured
185 *in vivo* in progenitor populations, as no labelling methods can currently discriminate between
186 death and differentiation. In mature cell types of both humans and mice, results show that most
187 cells are short-lived - on the order of days or weeks (**Tables 2-3**), with some variation within
188 cell types due to maturation or activation state [45] and tissue localization [46].

189

190 While measuring death rates is challenging, estimation of turn-over can help to understand the
191 dynamics of hematopoiesis (Calculation S2e). In steady state conditions, the turnover rate of

192 each lineage can be estimated from population sizes and circulating half-lives (**Tables 2-3**),
193 assuming that cell production and death rates balance each other (Figure 3, **Calculation S2e**).
194 In mice, despite different population sizes, erythroid and granulocyte turnover rates are on the
195 same order of magnitude due to large differences in lifespan (35 days [47] versus 1.2 days [46],
196 **Calculation S2e**). Murine B-lymphocytes however have a 10-fold lower turnover rate
197 (**Calculation S2e**), consistent with the relatively low number of CLPs. Importantly, the steady
198 state assumption we make is not always valid; different studies report that the number of
199 phenotypic HSCs in mice increases with age [48,49].

200

201 In humans, it has been estimated that 3.3×10^{11} cells are produced each day, ~85% of which are
202 hematopoietic [4]. 2.1×10^{11} of these cells are erythroid, 6×10^{10} are neutrophils, 1.5×10^9 are
203 monocytes and 7×10^9 are lymphoid (**Table 3**) [4]. In this study, platelets were not considered
204 a cell type and so were not included in the final analysis. Based on available measurements of
205 total platelet numbers (1.5×10^{12}) as well as lifespan (9.9 days) [50], we estimate a turnover rate
206 of 1.5×10^{11} platelets per day (**Table 3**). In humans, as in mice, the erythromyeloid lineages
207 dominate cell turnover.

208 *Differentiation rates*

209 Apart from division and death rates, the residency times *within*, and transition rates *between*
210 compartments regulate cell dynamics. Inference from a murine **fate mapping** study reports that
211 differentiation rates increase from HSC to MPP and RPP [10], and that an individual MPP is
212 180 times more likely to transition into a CMP than a CLP. While the confidence intervals
213 around these estimates are large, they suggest that lymphoid commitment is rare. Based on
214 these values of MPP to CLP transition [10], a scRNAseq dataset of 1000 murine MPPs contains
215 only 5-6 cells that commit to the lymphoid fate, highlighting the need for enrichment strategies
216 to study early lymphopoiesis [51]. However, it is important to note that estimates derived from

217 **fate mapping** studies vary depending on technical factors such as the labelling system, the
218 gating strategy, or the model used in parameter fitting [14]. In humans, there are no reported *in*
219 *vivo* differentiation rates, although retrospective **lineage-tracing** methods that make use of
220 endogenous barcodes within the genome (e.g. somatic mutations) are emerging [20].

221

222 To summarize, hematopoietic cells are short-lived, and must be constantly regenerated. In both
223 mice and humans, the number of myeloid and erythroid cells produced each day are of the same
224 order of magnitude, even though there are far more erythroid than myeloid cells when we
225 consider total cell numbers. This is due to large differences in their expected lifespan.

226 **How big are different hematopoietic cell types? What does each cell type comprise?**

227 As hematopoietic cells differentiate, they undergo significant remodeling, changing their
228 shapes, sizes and macromolecular composition to fulfill specialized functions, such as oxygen
229 transport, blood clotting, and pathogen killing.

230

231 Cell size can be measured using synthetic beads as an internal control in flow cytometry, or by
232 imaging [52] and results show that cell volumes vary dramatically between cell types (**Table**
233 **S1**). For example, murine HSCs, CMPs, GMPs and MEPs have a volume of $175 \mu\text{m}^3$, $210 \mu\text{m}^3$,
234 $320 \mu\text{m}^3$ and $450 \mu\text{m}^3$, respectively [53]. Differences in cell size impact cellular composition
235 with protein and lipid numbers scaling linearly with cell volume for many mature blood cell
236 types (**Table S1, Figure S2**). As a consequence, quantitative differences in cell size can have
237 major implications for biosynthesis when we consider the large numbers of cells produced each
238 day (see above). Aside from the quantification of protein synthesis rates in progenitors [54],
239 measurements of the ATP and macromolecular requirements for hematopoietic cell production
240 are lacking. However, newly-developed high-sensitivity mass spectrometry-based approaches

241 are emerging to tackle this issue [55]. Using the relative protein content of the different cell
242 types (**Table S1**) and the number of cells produced in humans per day (**Table 3**), we compute
243 the amount of protein and ATP required to fuel cell turnover (**Calculation S2f**). This
244 calculation predicts that myelopoiesis requires ~2.8 times more ATP than erythropoiesis in
245 humans, considering only protein synthesis requirements.

246

247 To summarize, each hematopoietic cell type has a distinct size and shape, helping them to fulfill
248 specialized functions. Consequently, the amount of energy and biomaterial it takes to
249 regenerate each cell type may vary significantly, but many parameters have yet to be quantified.
250 Quantitative hematometabolism is a research topic that warrants further focus, and may
251 facilitate the development of novel dietary interventions to modulate hematopoiesis.

252

253 **Concluding remarks**

254 In this article we have summarized key numbers in hematopoiesis, providing a quantitative
255 reference for the field, and highlighting areas where quantitative information is missing. These
256 numbers can be accessed and updated at the Bionumbers repository
257 (<https://bionumbers.hms.harvard.edu/>).

258

259 In reviewing existing numbers, we learn that in mice, 5.2×10^3 HSCs give rise to 10^{10}
260 hematopoietic cells while in humans 10^4 - 10^6 HSCs give rise to 10^{13} mature hematopoietic cells.
261 Strikingly, in both humans and mice most hematopoietic cells are short-lived – on the order of
262 days or weeks – as such hematopoiesis accounts for 90% of total blood cell turnover [4]. We
263 have also reviewed what is known about hematopoietic cell size and composition, with
264 numbers revealing that each cell type is likely to have very distinct metabolic requirements.

265 For example, we estimate that at least ~36kcal per day, the equivalent of a single strawberry,
266 is needed to maintain RBC homeostasis in humans (Figure S3, **Calculation S2g**). This value
267 is surprising low, given RBCs account for 90% of all cells in the human body, and arises due
268 to the relatively low protein content and low ATP turnover rate of RBCs compared to other
269 cell types.

270

271 We also illustrate how quantitative approaches may improve our ability to: (i) design
272 experiments, as illustrated earlier by the need for novel enrichment strategies to study early
273 lymphopoiesis (ii) consolidate and contextualize data across studies and species, identifying
274 knowledge gaps (see ‘outstanding questions’) and to (iii) make novel predictions about how
275 hematopoiesis is regulated.

276

277 In generating reference values, it is important to understand how each measurement has been
278 made. Conflicting values in the literature may arise due to nuanced technical details about
279 sample processing and analysis, and may be irreflective of true biological variation.
280 Unfortunately, many numbers could not be included in this article due to poor reporting with
281 unclear tissue processing and data transformation. We suggest that best practice is to make raw
282 data available for each figure, along with associated metadata about sample processing, data
283 acquisition and analysis steps.

284

285 To summarize, hematopoiesis is a dynamic process, giving rise to diverse cell types with
286 distinct population sizes, tissue localization, turnover rates, and sizes. We hope that this article
287 illustrates how quantitative approaches can improve our understanding of hematopoiesis, and
288 anticipate that the numbers presented can serve as a reference starting point for the immunology
289 and hematology scientific communities.

290 Acknowledgements:

291 We thank all the Perié lab members, Rob Phillips, Lucie Laplane and Rob Signer for giving
292 their feedback on the manuscript. This work was supported by grants from the *Labex*
293 *Cell(n)Scale* (ANR-11-LABX-0038, ANR-10-IDEX-0001-02 PSL) (to L.P.). This work is part
294 of a project that has received funding from the European Research Council (ERC) under the
295 European Union's Horizon 2020 research and innovation programme 758170-Microbar (to
296 L.P.) J.C. was supported by a Foundation ARC fellowship.

297

298

299

300

301

302

303

304

305

306

307

308

309

310

311

312

313

314

315 **References**

316

- 317 1 Sender, R. *et al.* (2016) Revised Estimates for the Number of Human and Bacteria
318 Cells in the Body. *PLOS Biol.* 14, e1002533
- 319 2 Milo, R. and Phillips, R. (2015) *Cell Biology by the Numbers*, 1 edition. Garland
320 Science.
- 321 3 Grubb, S.C. *et al.* (2009) Mouse Phenome Database. *Nucleic Acids Res.* 37,
322 D720–D730
- 323 4 Sender, R. and Milo, R. (2021) The distribution of cellular turnover in the human
324 body. *Nat. Med.* 27, 45–48
- 325 5 Lahoz-Beneytez, J. *et al.* (2016) Human neutrophil kinetics: modeling of stable
326 isotope labeling data supports short blood neutrophil half-lives. *Blood* 127, 3431–
327 3438
- 328 6 Valentin, J. (2002) Basic anatomical and physiological data for use in radiological
329 protection: reference values: ICRP Publication 89. *Ann. ICRP* 32, 1–277
- 330 7 Makarieva, A.M. *et al.* (2008) Mean mass-specific metabolic rates are strikingly
331 similar across life's major domains: Evidence for life's metabolic optimum. *Proc.*
332 *Natl. Acad. Sci. U. S. A.* 105, 16994–16999
- 333 8 Trepel, F. (1974) Number and distribution of lymphocytes in man. A critical
334 analysis. *Klin. Wochenschr.* 52, 511–515
- 335 9 Qatarneh, S.M. *et al.* (2006) Three-dimensional atlas of lymph node topography
336 based on the visible human data set. *Anat. Rec. B. New Anat.* 289, 98–111
- 337 10 Busch, K. *et al.* (2015) Fundamental properties of unperturbed haematopoiesis
338 from stem cells in vivo. *Nature* 518, 542–546
- 339 11 Wilson, N.K. *et al.* (2015) Combined Single-Cell Functional and Gene Expression
340 Analysis Resolves Heterogeneity within Stem Cell Populations. *Cell Stem Cell* 16,
341 712–724
- 342 12 Challen, G.A. *et al.* (2010) Distinct Hematopoietic Stem Cell Subtypes Are
343 Differentially Regulated by TGF β 1. *Cell Stem Cell* 6, 265–278
- 344 13 Beerman, I. *et al.* (2010) Functionally distinct hematopoietic stem cells modulate
345 hematopoietic lineage potential during aging by a mechanism of clonal expansion.
346 *Proc. Natl. Acad. Sci. U. S. A.* 107, 5465–5470
- 347 14 Sawai, C.M. *et al.* (2016) Hematopoietic Stem Cells Are the Major Source of
348 Multilineage Hematopoiesis in Adult Animals. *Immunity* 45, 597–609
- 349 15 Pei, W. *et al.* (2020) Resolving Fates and Single-Cell Transcriptomes of
350 Hematopoietic Stem Cell Clones by PolyloxExpress Barcoding. *Cell Stem Cell* 27,
351 383–395.e8
- 352 16 Rodriguez-Fraticelli, A.E. *et al.* (2020) Single-cell lineage tracing unveils a role for
353 TCF15 in haematopoiesis. *Nature* 583, 585–589
- 354 17 Bowling, S. *et al.* (2020) An Engineered CRISPR-Cas9 Mouse Line for
355 Simultaneous Readout of Lineage Histories and Gene Expression Profiles in
356 Single Cells. *Cell* 181, 1410–1422.e27
- 357 18 Sun, J. *et al.* (2014) Clonal dynamics of native haematopoiesis. *Nature* 514, 322–
358 327
- 359 19 Abkowitz, J.L. *et al.* (2002) Evidence that the number of hematopoietic stem cells
360 per animal is conserved in mammals. *Blood* 100, 2665–2667
- 361 20 Lee-Six, H. *et al.* (2018) Population dynamics of normal human blood inferred from
362 somatic mutations. *Nature* 561, 473–478

- 363 21 Watson, C.J. *et al.* (2020) The evolutionary dynamics and fitness landscape of
364 clonal hematopoiesis. *Science* 367, 1449–1454
- 365 22 Biasco, L. *et al.* (2016) In Vivo Tracking of Human Hematopoiesis Reveals
366 Patterns of Clonal Dynamics during Early and Steady-State Reconstitution
367 Phases. *Cell Stem Cell* 19, 107–119
- 368 23 Sommerkamp, P. *et al.* (2021) Mouse multipotent progenitor 5 cells are located at
369 the interphase between hematopoietic stem and progenitor cells. *Blood* 137,
370 3218–3224
- 371 24 Morrison, S.J. and Weissman, I.L. (1994) The long-term repopulating subset of
372 hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity* 1,
373 661–673
- 374 25 Morrison, S.J. *et al.* (1997) Identification of a lineage of multipotent hematopoietic
375 progenitors. *Dev. Camb. Engl.* 124, 1929–1939
- 376 26 Akashi, K. *et al.* (2000) A clonogenic common myeloid progenitor that gives rise to
377 all myeloid lineages. *Nature* 404, 193–197
- 378 27 Nakorn, T.N. *et al.* (2003) Characterization of mouse clonogenic megakaryocyte
379 progenitors. *Proc. Natl. Acad. Sci.* 100, 205–210
- 380 28 Harman, B.C. *et al.* (2008) Resolution of Unique Sca-1^{high} c-Kit⁻ Lymphoid-Biased
381 Progenitors in Adult Bone Marrow. *J. Immunol.* 181, 7514–7524
- 382 29 Kumar, R. *et al.* (2008) Lin⁻Sca1+Kit⁻ Bone Marrow Cells Contain Early
383 Lymphoid-Committed Precursors That Are Distinct from Common Lymphoid
384 Progenitors. *J. Immunol.* 181, 7507–7513
- 385 30 Giandomenico, S.D. *et al.* (2019) Megakaryocyte TGFβ1 Partitions Hematopoiesis
386 into Immature Progenitor/Stem Cells and Maturing Precursors. *bioRxiv* DOI:
387 10.1101/689901
- 388 31 Kirby, M.R. and Donahue, R.E. (1993) Rare Event Sorting of CD34+
389 Hematopoietic Cells. *Ann. N. Y. Acad. Sci.* 677, 413–416
- 390 32 Hao, Q.-L. *et al.* (1995) A Functional Comparison of CD34+ CD38⁻ Cells in Cord
391 Blood and Bone Marrow. *Blood* 86, 3745–3753
- 392 33 Farrell, T. *et al.* (2014) Changes in the frequencies of human hematopoietic stem
393 and progenitor cells with age and site. *Exp. Hematol.* 42, 146–154
- 394 34 Povsic, T.J. *et al.* (2010) Aging is not associated with bone marrow-resident
395 progenitor cell depletion. *J. Gerontol. A. Biol. Sci. Med. Sci.* 65, 1042–1050
- 396 35 Boyer, S.W. *et al.* (2019) Clonal and Quantitative In Vivo Assessment of
397 Hematopoietic Stem Cell Differentiation Reveals Strong Erythroid Potential of
398 Multipotent Cells. *Stem Cell Rep.* 12, 801–815
- 399 36 Niswander, L.M. *et al.* (2014) Improved quantitative analysis of primary bone
400 marrow megakaryocytes utilizing imaging flow cytometry. *Cytometry A* 85, 302–
401 312
- 402 37 Basilico, S. and Göttgens, B. (2017) Dysregulation of haematopoietic stem cell
403 regulatory programs in acute myeloid leukaemia. *J. Mol. Med. Berl. Ger.* 95, 719–
404 727
- 405 38 Krueger, A. *et al.* (2017) T Cell Development by the Numbers. *Trends Immunol.*
406 38, 128–139
- 407 39 Romar, G.A. *et al.* (2016) Research Techniques Made Simple: Techniques to
408 Assess Cell Proliferation. *J. Invest. Dermatol.* 136, e1–e7
- 409 40 Wilson, A. *et al.* (2008) Hematopoietic stem cells reversibly switch from dormancy
410 to self-renewal during homeostasis and repair. *Cell* 135, 1118–1129

- 411 41 Akinduro, O. *et al.* (2018) Proliferation dynamics of acute myeloid leukaemia and
412 haematopoietic progenitors competing for bone marrow space. *Nat. Commun.* 9,
413 519
- 414 42 Takahashi, M. *et al.* (2021) Reconciling Flux Experiments for Quantitative
415 Modeling of Normal and Malignant Hematopoietic Stem/Progenitor Dynamics.
416 *Stem Cell Rep.* 16, 741–753
- 417 43 Signer, R.A.J. *et al.* (2016) The rate of protein synthesis in hematopoietic stem
418 cells is limited partly by 4E-BPs. *Genes Dev.* 30, 1698–1703
- 419 44 Catlin, S.N. *et al.* (2011) The replication rate of human hematopoietic stem cells in
420 vivo. *Blood* 117, 4460–4466
- 421 45 Borghans, J.A.M. *et al.* (2018) Current best estimates for the average lifespans of
422 mouse and human leukocytes: reviewing two decades of deuterium-labeling
423 experiments. *Immunol. Rev.* 285, 233–248
- 424 46 Ballesteros, I. *et al.* (2020) Co-option of Neutrophil Fates by Tissue Environments.
425 *Cell* 183, 1282-1297.e18
- 426 47 Verheijen, M. *et al.* (2020) Fate Mapping Quantifies the Dynamics of B Cell
427 Development and Activation throughout Life. *Cell Rep.* 33,
428 48 Bernitz, J.M. *et al.* (2016) Hematopoietic Stem Cells Count and Remember Self-
429 Renewal Divisions. *Cell* 167, 1296-1309.e10
- 430 49 Chambers, S.M. *et al.* (2007) Aging hematopoietic stem cells decline in function
431 and exhibit epigenetic dysregulation. *PLoS Biol.* 5, e201
- 432 50 Harker, L.A. and Finch, C.A. (1969) Thrombokinetis in man. *J. Clin. Invest.* 48,
433 963–974
- 434 51 Amann-Zalcenstein, D. *et al.* (2020) A new lymphoid-primed progenitor marked by
435 Dach1 downregulation identified with single cell multi-omics. *Nat. Immunol.* 21,
436 1574–1584
- 437 52 Model, M.A. (2018) Methods for cell volume measurement. *Cytometry A* 93, 281–
438 296
- 439 53 Hidalgo San Jose, L. *et al.* (2020) Modest Declines in Proteome Quality Impair
440 Hematopoietic Stem Cell Self-Renewal. *Cell Rep.* 30, 69-80.e6
- 441 54 Signer, R.A.J. *et al.* (2014) Haematopoietic stem cells require a highly regulated
442 protein synthesis rate. *Nature* 509, 49–54
- 443 55 Agathocleous, M. *et al.* (2017) Ascorbate regulates haematopoietic stem cell
444 function and leukaemogenesis. *Nature* 549, 476–481
- 445 56 Leidl, K. *et al.* (2008) Mass spectrometric analysis of lipid species of human
446 circulating blood cells. *Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids* 1781,
447 655–664
- 448 57 Radley, J.M. *et al.* (1999) Ultrastructure of primitive hematopoietic stem cells
449 isolated using probes of functional status. *Exp. Hematol.* 27, 365–369
- 450 58 Noris, P. *et al.* (2014) Platelet diameters in inherited thrombocytopenias: analysis
451 of 376 patients with all known disorders. *Blood* 124, e4–e10
- 452 59 H, D. *et al.* (2011) Normal range of mean platelet volume in healthy subjects:
453 Insight from a large epidemiologic study. *Thromb. Res.* 128,
454 60 Bessman, J.D. (1984) The relation of megakaryocyte ploidy to platelet volume. *Am.*
455 *J. Hematol.* 16, 161–170
- 456 61 McLaren, C.E. *et al.* (1987) Statistical and graphical evaluation of erythrocyte
457 volume distributions. *Am. J. Physiol.* 252 Heart Circ. Physiol.
- 458 62 Downey, G.P. *et al.* (1990) Retention of leukocytes in capillaries: role of cell size
459 and deformability. *J. Appl. Physiol. Bethesda Md* 1985 69, 1767–1778

- 460 63 Ting-Beall, H.P. *et al.* (1993) Volume and osmotic properties of human neutrophils.
461 *Blood* 81, 2774–2780
- 462 64 Needham, D. and Hochmuth, R.M. (1990) Rapid flow of passive neutrophils into a
463 4 microns pipet and measurement of cytoplasmic viscosity. *J. Biomech. Eng.* 112,
464 269–276
- 465 65 Kuse, R. *et al.* (1985) Blood lymphocyte volumes and diameters in patients with
466 chronic lymphocytic leukemia and normal controls. *Blut* 50, 243–248
- 467 66 Chervenick, P.A. *et al.* (1968) Quantitative studies of blood and bone marrow
468 neutrophils in normal mice. *Am. J. Physiol.* 215, 353–360
- 469 67 Méndez-Ferrer, S. *et al.* (2010) Mesenchymal and haematopoietic stem cells form
470 a unique bone marrow niche. *Nature* 466, 829–834
- 471 68 Worthley, D.L. *et al.* (2015) Gremlin 1 Identifies a Skeletal Stem Cell with Bone,
472 Cartilage, and Reticular Stromal Potential. *Cell* 160, 269–284
- 473 69 Zhou, B.O. *et al.* (2014) Leptin-Receptor-Expressing Mesenchymal Stromal Cells
474 Represent the Main Source of Bone Formed by Adult Bone Marrow. *Cell Stem Cell*
475 15, 154–168
- 476 70 Gomariz, A. *et al.* (2018) Quantitative spatial analysis of haematopoiesis-
477 regulating stromal cells in the bone marrow microenvironment by 3D microscopy.
478 *Nat. Commun.* 9, 2532
- 479 71 Menees, K.B. *et al.* (2021) Sex- and age-dependent alterations of splenic immune
480 cell profile and NK cell phenotypes and function in C57BL/6J mice. *Immun. Ageing*
481 18, 3
- 482 72 Kaliss, N. and Pressman, D. (2016) Plasma and Blood Volumes of Mouse Organs,
483 As Determined with Radioactive Iodoproteins.*: *Proc. Soc. Exp. Biol. Med.* DOI:
484 10.3181/00379727-75-18083
- 485 73 Furth, J. and Sobel, H. (1946) Hypervolemia Secondary to Grafted Granulosa-Cell
486 Tumor2. *JNCI J. Natl. Cancer Inst.* 7, 103–113
- 487 74 Keighley, G. *et al.* (1962) Response of Normal and Genetically Anaemic Mice to
488 Erythropoietic Stimuli*. *Br. J. Haematol.* 8, 429–441
- 489 75 Riches, A.C. *et al.* (1973) Blood volume determination in the mouse. *J. Physiol.*
490 228, 279–284
- 491 76 Sluiter, W. *et al.* (1984) Determination of blood volume in the mouse with
492 ⁵¹Chromium-labelled erythrocytes. *J. Immunol. Methods* 73, 221–225
- 493 77 Van den Broeck, W. *et al.* (2006) Anatomy and nomenclature of murine lymph
494 nodes: Descriptive study and nomenclatory standardization in BALB/cAnNCrI
495 mice. *J. Immunol. Methods* 312, 12–19
- 496 78 Kawashima, Y. *et al.* (1964) The lymph system in mice. *Subj. Strain Bibliogr.* 1964
- 497 79 Druzd, D. *et al.* (2017) Lymphocyte Circadian Clocks Control Lymph Node
498 Trafficking and Adaptive Immune Responses. *Immunity* 46, 120–132
- 499 80 Hsu, H.-C. *et al.* (2003) Age-related thymic involution in C57BL/6J × DBA/2J
500 recombinant-inbred mice maps to mouse chromosomes 9 and 10. *Genes Immun.*
501 4, 402–410
- 502 81 Allman, D.M. *et al.* (1993) Peripheral B cell maturation. II. Heat-stable antigen(hi)
503 splenic B cells are an immature developmental intermediate in the production of
504 long-lived marrow-derived B cells. *J. Immunol. Baltim. Md* 151, 4431–4444
- 505 82 Kaufman, R.M. *et al.* (1965) Circulating megakaryocytes and platelet release in the
506 lung. *Blood* 26, 720–731
- 507 83 Trowbridge, E.A. *et al.* (1984) The origin of platelet count and volume. *Clin. Phys.*
508 *Physiol. Meas. Off. J. Hosp. Phys. Assoc. Dtsch. Ges. Med. Phys. Eur. Fed. Organ.*
509 *Med. Phys.* 5, 145–170

- 510 84 Buttgereit, F. and Brand, M.D. (1995) A hierarchy of ATP-consuming processes in
511 mammalian cells. *Biochem. J.* 312, 163–167
- 512 85 Argüello, R.J. *et al.* (2020) SCENITH: A Flow Cytometry-Based Method to
513 Functionally Profile Energy Metabolism with Single-Cell Resolution. *Cell Metab.*
514 32, 1063-1075.e7
- 515 86 Brocchieri, L. and Karlin, S. (2005) Protein length in eukaryotic and prokaryotic
516 proteomes. *Nucleic Acids Res.* 33, 3390–3400
- 517 87 Sarpel, G. *et al.* (1982) Erythrocyte phosphate content in Huntington's disease.
518 *Neurosci. Lett.* 31, 91–96
- 519 88 Thore, A. *et al.* (1975) Detection of bacteriuria by luciferase assay of adenosine
520 triphosphate. *J. Clin. Microbiol.* 1, 1–8
- 521 89 Lew, V.L. and Tiffert, T. (2017) On the Mechanism of Human Red Blood Cell
522 Longevity: Roles of Calcium, the Sodium Pump, PIEZO1, and Gardos Channels.
523 *Front. Physiol.* 8, 977
- 524 90 Müller, E. *et al.* (1986) Turnover of phosphomonoester groups and
525 compartmentation of polyphosphoinositides in human erythrocytes. *Biochem. J.*
526 235, 775–783
- 527 91 Feig, S.A. *et al.* (1972) Energy metabolism in human erythrocytes. *J. Clin. Invest.*
528 51, 1547–1554
- 529 92 Child, J.A. *et al.* (1967) A diffraction method for measuring the average volumes
530 and shapes of red blood cells. *Br. J. Haematol.* 13, 364–375
- 531 93 van Meer, G. *et al.* (2008) Membrane lipids: where they are and how they behave.
532 *Nat. Rev. Mol. Cell Biol.* 9, 112–124
- 533 94 Bogue, M.A. *et al.* (2020) Mouse Phenome Database: a data repository and
534 analysis suite for curated primary mouse phenotype data. *Nucleic Acids Res.* 48,
535 D716–D723
- 536 95 Kawashima, Y. *et al.* (1964) THE LYMPH SYSTEM IN MICE.
- 537 96 Druzd, D. *et al.* (2017) Lymphocyte Circadian Clocks Control Lymph Node
538 Trafficking and Adaptive Immune Responses. *Immunity* 0,
- 539 97 Tanaka, S. *et al.* (2020) Tet2 and Tet3 in B cells are required to repress CD86 and
540 prevent autoimmunity. *Nat. Immunol.* 21, 950–961
- 541

542 **Figure Legends**

543 **Figure 1: Generating reference values for total murine hematopoietic cell numbers.** Most
544 studies enumerate cell frequency by measuring a tissue sample. Consequently, these value
545 needs to be transformed to absolute count, scaled up to the entire organism and corrected for
546 sampling and technical artefacts. All the numbers are derived for a reference adult female
547 mouse of 12 weeks weighing 22g. For total cell numbers in blood, we summed the total
548 numbers of leukocytes, platelets and erythrocytes per μl and multiply this by the total blood
549 volume for our reference mouse. In the bone marrow, we scale the measurement of the number
550 of nucleated cells per gram of body weight of a 22g reference mouse and then we add to this
551 number the proportion of anucleated erythrocytes and platelets. For the thymus, the total
552 number of thymocytes at birth is corrected for the loss of thymocytes associated with ageing
553 using the rate of thymocyte loss per day for a 12 weeks old reference mouse. For the lymph
554 node, the number of cells within one lymph node is multiplied by the average total number of
555 lymph nodes in 1 mouse. For the spleen, we take a measurement of the total splenocytes per
556 gram of spleen and then scale it by the mass of the spleen in our reference mouse. A full

557 explanation of these calculations, along with supporting references, is provided in file S1 and
 558 S2.

559
 560

561 **Figure 2: Cell numbers across different hematopoietic compartments in healthy mice (A)**
 562 Calculated total number of cells across the murine hematopoietic hierarchy using values in
 563 Table 1. To help understand the scaling across several orders of magnitude, we place
 564 compartment sizes on a more familiar scale: time. Specifically, we place cells on a time scale
 565 where 1 cell is arbitrarily equal to 1 second. All the numbers are derived for a reference adult
 566 female mouse of 12 weeks weighing 22g (Table 1). HSC = hematopoietic stem cells, MPP =
 567 multi-potent progenitors, RPP = restricted potential progenitor, RBC = red blood cell.
 568

569 **Figure 3. The dynamics of steady-state murine hematopoiesis.** Calculating the minimum
 570 division number between active HSCs and mature cell subsets for red blood cells and myeloid
 571 cells (A). This minimum division number is computed with the logarithmic 2 transformation
 572 of total cell numbers in the body of a certain cell type (N_{mature}) divided by the number of active
 573 hematopoietic stem cells (N_{hsc}). This calculation does not consider the impact of cell death and
 574 differentiation. (B) For B cells, positive and negative selection of mature naïve B cells was
 575 considered and (C) for megakaryocytes, endomitosis has also been accounted for, (D) as well
 576 as platelets fragmentation. (D) Summary of the values for the reference mice, as in file S1. (E)
 577 Estimating the turnover rates of the blood cell lineages, assuming that production and death
 578 rates balance in healthy young adult mice. In this scheme the number of cells produced each
 579 day ($N_{\text{cell produced}}$) is the product of the total cell number (N_{cell}), the production rate and potential
 580 selection (as for B cells) of each cell type per day ($P(D)$). (F) The calculated cell population
 581 sizes in given organs, expected $P(D)$ for each blood cell lineage during hematopoiesis for the
 582 reference mouse, as in Table 2. Details of all these calculations are provided in file S2.
 583

Mouse organ /Cell Population /	Value	Unit	Reference
Reference mouse			
Weight	22	g	[94]
Strain	B6		
Age	84	days	
Sex	female		
Bone Marrow			
Total cell count	4.5E+08	cells	[29,35,66]
Total nucleated cells	2.6E+08	cells	[66]
Lin ⁻ cells	2.0E+07	cells	[10]
Phenotypic HSC ^a (Lin ⁻ Kit ⁺ Sca-1 ⁺ CD150 ⁺ CD48 ⁻)	1.6E+04	cells	[10]
Active HSC	5.2E+03	cells	[10]
MPP ^b (non HSC LSK)	1.4E+05	cells	[10]
Erythro-myeloid progenitors	1.7E+06	cells	
CMP ^c (Lin-Kit ⁺ CD16/32 ⁻ CD34 ⁺)	4.0E+05	cells	[10]
GMP ^d (Lin-Kit ⁺ CD16/32 ⁺ CD34 ⁺)	5.1E+05	cells	[10]

MEP ^e (Lin-Kit ⁺ CD16/32 ⁻ CD34 ⁻)	8.0E+05	cells	[10]
Megakaryocyte progenitors	2.6E+04	cells	[27]
CLP	2.5E+05	cells	[10]
Lin ⁺ cells	4.3E+08	cells	
Erythrocytes	1.8E+08	cells	[35]
Megakaryocytes	7.7E+05	cells	[36]
Platelets	5.6E+06	cells	[35]
Granulomyeloid	1.5E+08	cells	[35]
B cells	8.6E+07	cells	[35]
Peripheral Blood			
Volume	1.5E+03	ul	[76]
Total cell count	1.6E+10	cells	
Erythrocytes	1.5E+10	cells	[35]
Platelets	1.4E+09	cells	[35]
Granulomyeloid	1.7E+06	cells	[35]
B cells	8.3E+06	cells	[35]
T cells	2.6E+03	cells	[35]
Spleen			
Mass	7.9E-02	g	[71]
Total cell count	2.1E+08	cells	
Erythrocytes	8.2E+07	cells	[35]
Platelets	5.1E+07	cells	[35]
Granulomyeloid	2.7E+06	cells	[35]
B cells	4.7E+07	cells	[35]
Follicular Mature B cells	3.3E+07	cells	[35]
T cells	2.0E+07	cells	[35]
Lymph nodes			
Average number lymph nodes in whole body	2.7E+01	lymph nodes	[77,95]
Total cell count	7.6E+07	cells	[96]
Erythrocytes	2.5E+06	cells	[35]
B cells	3.5E+07	cells	[35]
Follicular Mature B cells	2.4E+07	cells	[97]
T cells	3.6E+07	cells	[35]
Others	1.5E+06	cells	[35]
Thymus			
Total cell count	2.1E+08	cells	[80]
Erythrocytes	3.6E+07	cells	[35]
Platelets	2.8E+06	cells	[35]

B cells	8.5E+06	cells	[35]
T cells	1.6E+08	cells	[35]

584

585 **Table 1:** Cellular composition of the main hematopoietic organs in our reference mouse, 16
586 weeks old female C57BL/6J weighing 22g. according to calculations in Box 1. HSC:
587 hematopoietic stem cell ; MPP: multipotent progenitor ; CMP: common myeloid progenitor ;
588 GMP: granulocyte-monocyte progenitor ; MEP: Megakaryocyte- erythroid progenitor. a =
589 Lin⁻Kit⁺Sca-1⁺CD150⁺CD48⁻ ; b = Lin⁻Kit⁺Sca-1⁺CD48⁺, c = Lin-Kit⁺ CD16/32⁻ CD34⁺, d =
590 Lin-Kit⁺ CD16/32⁺ CD34⁺ , e = Lin-Kit⁺ CD16/32⁻ CD34⁻

591

592

593

Cell type	Organ	Cell count	Half-life (days)	Lifespan (days)*	Death Rate (/day)	Developmental Selection Rate	Cells produced (/day)**	Cells produced (/year)**	References
Red blood cells	Blood	1.5E+10	24.3	35.00	0.03	-	4.5E+08	1.6E+11	[59]
Platelets	Blood	1.4E+09	2.5	3.60	0.28	-	3.9E+08	1.4E+11	[59], [60]
Myeloid	BM	1.5E+08	0.84	1.21	0.83	-	1.2E+08	4.5E+10	[46]
B cells (Follicular Mature)	Spleen and LN	5.7E+07	24.3	35.00	0.03	0.03	5.6E+07	2.0E+10	[47,81]

594

595

596 Table 2. Total number and production rate of hematopoietic cells in our reference mouse. Cell counts
597 come from Calculations S1a and Table S1. Half-lives or lifespans were retrieved from the given articles
598 (as indicated in bold) and used to provide an insight of the mature hematopoietic cell population
599 turnover using the following simple relationships: *Lifespan = 1/Death rate = $t_{1/2}/\ln(2)$ ** cells
600 produced = death rate x time period in days x Developmental Selection rate x cell count.

601

602

Cell Type	Estimated Cell Count	Lifespan (days)	Cells produced per day	References
Red blood cells	2.5×10^{13} cells	116	2.1×10^{11}	[4]
Monocytes	5.0×10^9	3.5	1.5×10^9	[4]
Platelets	1.5×10^{12} cells	9.9	1.5×10^{11}	[50]
Neutrophils	6.4×10^{11}	6.6	6×10^{10}	[4]
Mature B cells	3.0×10^{11}	63	5×10^9	[4]
Mature T cells	7.0×10^{11}	323	2×10^9	[4]

603

604 Table 3. Total number and production rate of human hematopoietic cells for our reference person

605

606

607

608

609

Glossary

610

611

Term	Description
Multipotency	The potential to differentiate into diverse blood cell lineages
Self-renewal	Cell division with maintenance of an undifferentiated cell state
Differentiation active	A stem or progenitor cell that is in the process of changing into a more mature hematopoietic cell type
Fate mapping	Labelling cells with a heritable mark to understand developmental processes
Lineage tracing	Fate mapping carried out at single cell resolution, typically by introducing a genetic label
Limited dilution assay	A technique to estimate the frequency of functional hematopoietic stem cells within a heterogeneous starting cell population. In this method a serial dilution of the starting cell population is performed and each subpopulation is functionally assessed by transplantation into a conditioned recipient
Capture-recapture analyses	A technique to estimate population sizes when it is not possible to count each individual cell. The method involves taking a small population of cells and labelling them, then reintroducing labelled cells back into their initial environment and determining the ratio of marked to unmarked cells at a later timepoint.
Post-transplantation hematopoiesis	The formation of new blood cells from stem cells that have been injected into a conditioned (typically irradiated) host.
Lentiviral vector integration site	In gene therapy, a viral vector is used to introduce new genetic material into a host organism. As the integration of the virus is stochastic, the precise location of the new genetic material in the host genome acts as a genetic label to perform fate mapping.
Division-linked dilution assay	A dye that is split equally between daughter cells during cell division. Consequently, the amount of dye in each cell is indicative of how many times it has divided, over a small number of generations.
Restricted Potential Progenitor	A hematopoietic cell that gives rise to multiple cell types, but that is incapable of creating all hematopoietic lineages. These cells therefore have less potential than MPPs and HSCs.
Endomitoses	Chromosome replication in the absence of a cell division, resulting in a polypoidal cell.
Lineage negative	A cell which does not express surface markers associated with the mature blood cell lineages
Restricted-potential progenitor	Hematopoietic progenitors in the murine bone marrow, this population comprises CMPs, CLPs, GMPs, MEPs, MkPs
CD34⁺ progenitor	The human progenitor compartment containing HSCs, MPPs, and restricted-potential progenitors.
HSC	A Hematopoietic Stem Cell, functionally defined as a multipotent cell capable of long-term self-renewal. In this study we consider an immunophenotypic HSC to express the following pattern of markers: Lin ⁻ Kit ⁺ Sca-1 ⁺ CD150 ⁺ CD48 ⁻

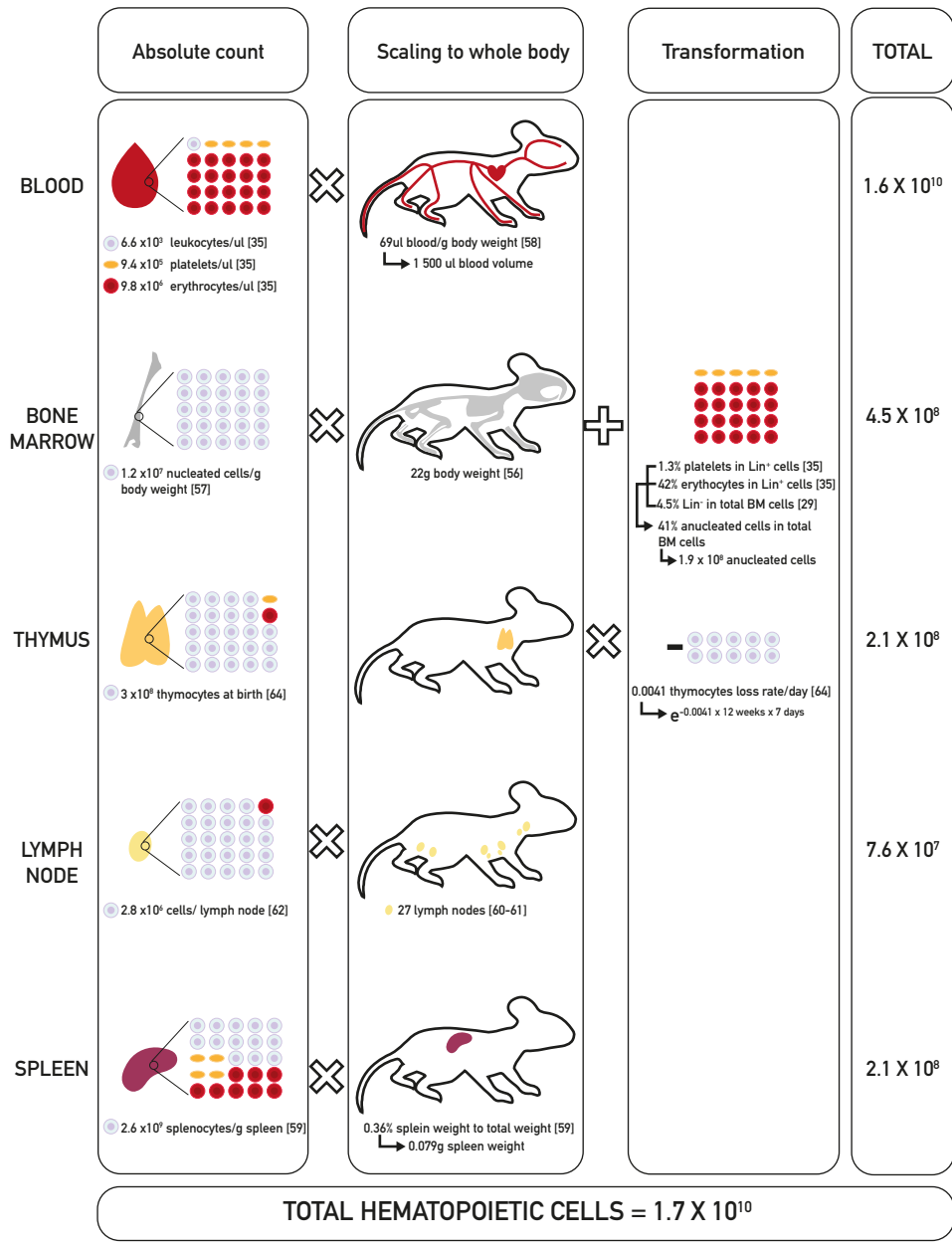
MPP	A Multi-Potent Progenitor, functionally defined as a cell capable of producing all lineages, but lacking long-term self-renewal. In this study we consider that murine MPPs are all Lin ⁻ Kit ⁺ Sca-1 ⁺ cells that are not within the immunophenotypic HSC compartment
CMP	A Common Myeloid Progenitor that gives rise to the erythroid, megakaryocyte and myeloid lineages. In mice expresses the following markers: cKit ⁺ Sca1 ⁻ CD16/32 ⁻ CD34 ⁺
MEP	A Megakaryocyte-Erythroid Progenitor that gives rise to erythroblasts and megakaryocytes. In mice expresses the following markers: cKit ⁺ Sca1 ⁻ CD16/32 ⁻ CD34 ⁻
GMP	A Granulocytic-Monocytic Progenitor that gives rise to the myeloid lineages. In mice expresses the following markers: cKit ⁺ Sca1 ⁻ CD16/32 ⁺ CD34 ⁺
CLP	A Common Lymphoid Progenitor that gives rise to B and T lymphocytes. In mice expresses the following markers: cKit ^{low} Sca1 ^{low} IL7R ⁺ Flk2 ⁻
MkP	A Megakaryocyte progenitor. In mice expresses the following markers: Sca1 ⁻ FcyR ^{low} CD9 ⁺ CD41 ⁺

612

613

614

615



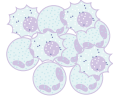
10^4 HSC
4 hours



10^5 MPP
39 hours



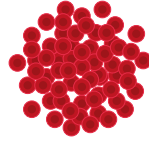
10^6 RPP
23 days



10^8 Granulomyeloids
5 years




10^9 Platelets
48 years

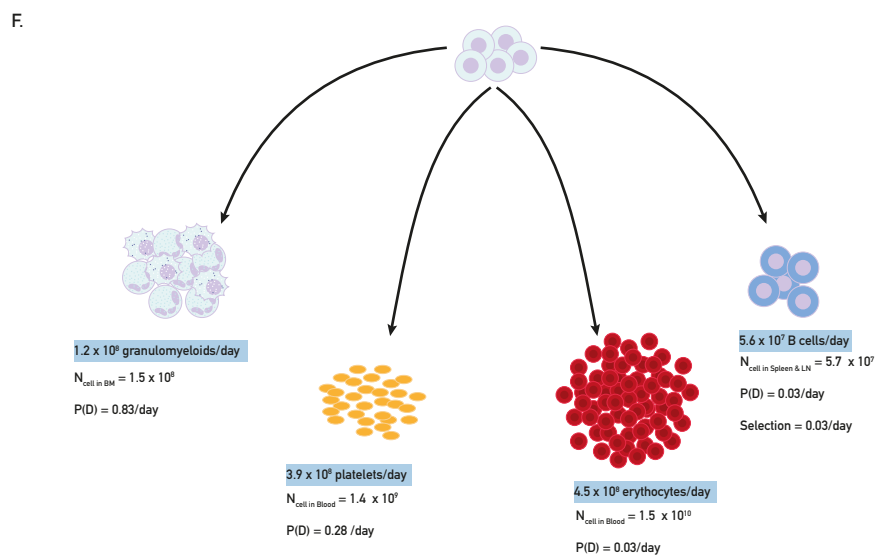
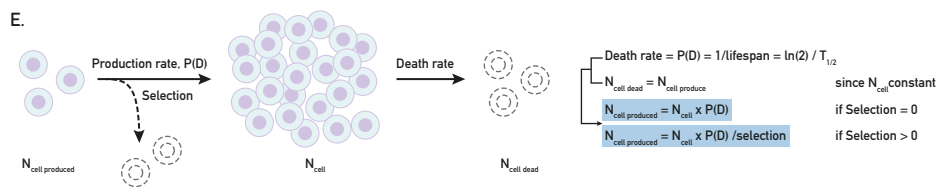
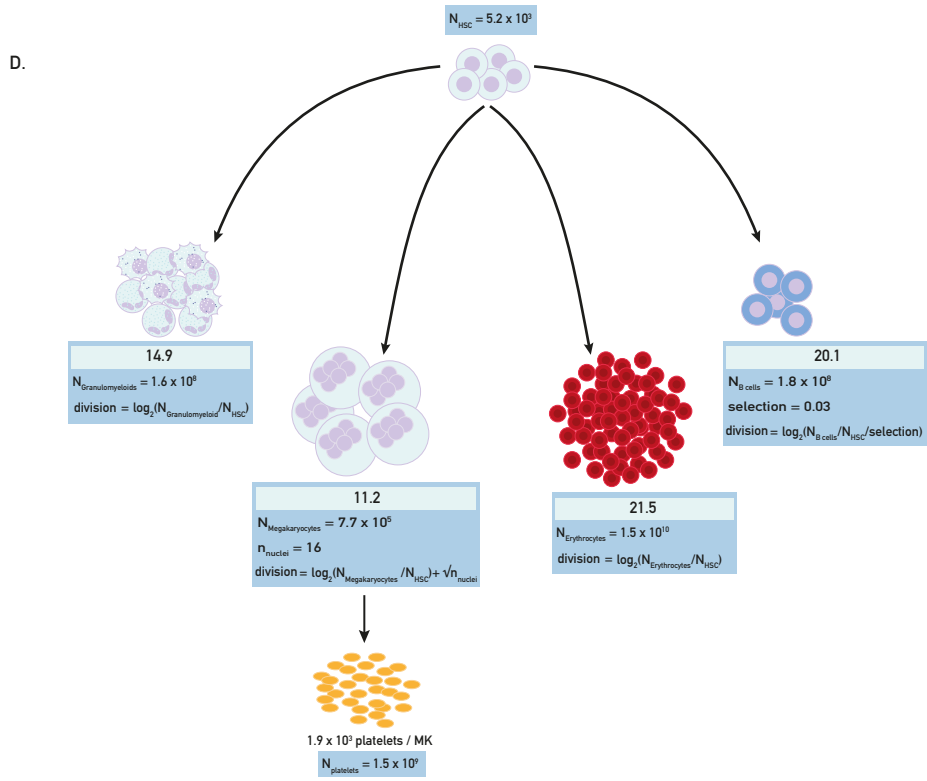
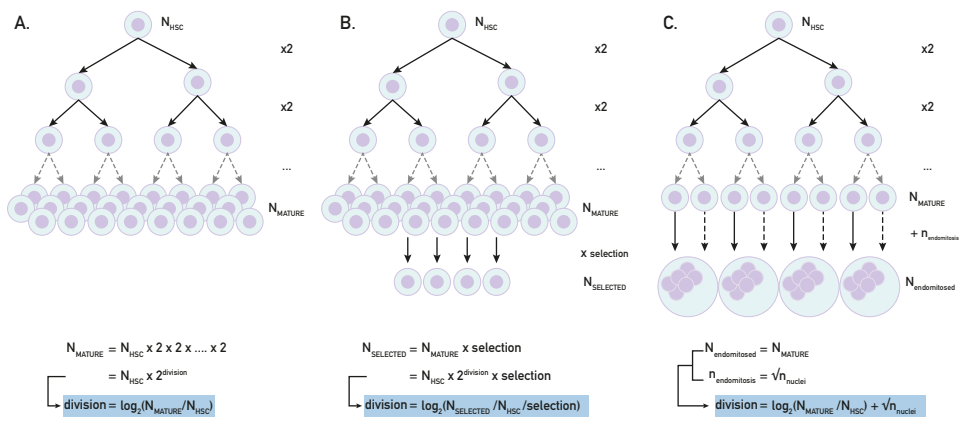


10^{10} Erythrocytes
476 years



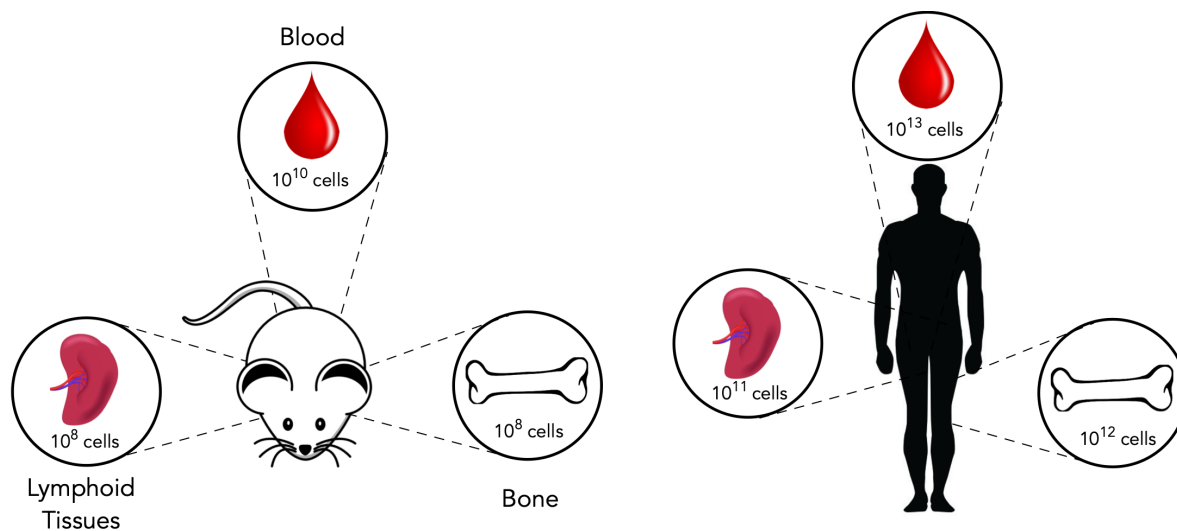
10^8 Lymphoids
11 years

if 1  = 1second



1 **File S2: Supplementary Material**

2



3

4

5 **Figure S1. Calculated cell numbers across different hematopoietic organs at steady state**6 **in mice and humans**(A) Calculated total number of cells in the peripheral blood, bone marrow,

7 and lymphoid tissues (spleen, thymus and lymph nodes) of mice and humans.

8

9

10

11

12

13

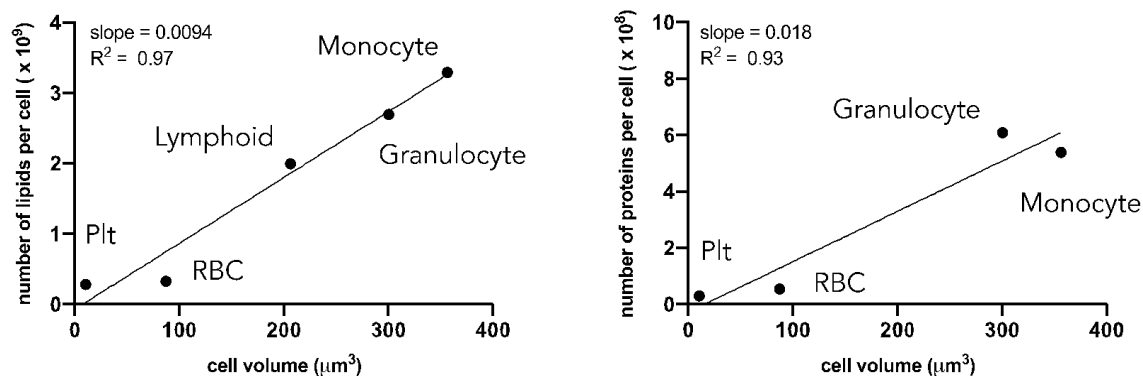
14

15

16

17

18
19
20
21
22



23
24
25
26
27
28
29
30

Figure S2. Linear models relating cell volume to absolute numbers of lipids and proteins in human mature hematopoietic cells taken from peripheral blood. Original data for this figure is derived from [56] and is presented in full in file S1. On the right-hand side, only the erythromyeloid lineages are plotted. The measured protein content of the lymphoid lineage was much higher than the erythromyeloid lineages, and so the Bichinchoninic Acid Assay measurements of lymphoid protein content (**data provided in table 4**) cannot be explained by the linear trend relating cell size to protein content for the erythromyeloid lineages. Plt: platelet ; RBC: red blood cells.

31
32
33
34
35
36
37
38
39
40
41
42
43
44

45
46
47
48
49
50

Cell Type	Diameter (μm)	Volume (μm^3)	Lipids/cell ($\times 10^9$)	Proteins/cell ($\times 10^8$)	Data Source	References
HSC	7*	175	unknown	unknown	Mice	[53], [57]
Platelet	2.6	9.1	0.3	0.3	Human	[56], [58–60]
RBC	7.7	88	0.3	0.6	Human	[56], [61],
Granulocyte	8.4	290	2.7	6.1	Human	[56],[62],[63][64]
Monocyte	8.8	357**	3.3	5.4	Human	[56],[62]
Lymphocyte	7	208	2	9.5	Human	[56], [65],[62]

51
52
53
54
55
56
57

Table S1. Distribution of cell volumes and macromolecular content across different human compartments.

* data taken from mice. **estimated by modelling cell shape as a sphere. Protein and lipid content of human blood cells measured using a Bichinchoninic Acid Assay and Electrospray Ionization Mass-Spectrometry respectively [56]. A full derivation of protein and lipid numbers is provided in calculation S2h-i.

58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76

Sources and criteria for numbers used in our calculations:

In this supplementary section we perform calculations to contextualize key numbers in hematopoiesis and provide directions for future research. The sources and criteria used for the numbers included in this section, along with associated metadata can be found in **file S1**.

Where appropriate we specify whether the calculations refer to human or murine hematopoiesis. Final values are considered representative of either a female mouse aged 12 weeks and weighing 22grams or a 20-30 human male weighing 70kg. Please note that all measurements have been rounded to one digital for the calculations.

The final values obtained from our calculations are also rounded to one decimal place but that rounding of numbers is not performed at intermediate calculation steps. The results of the intermediate steps are only made to help the reader to follow.

Calculation S1. How many hematopoietic cells are there?

Calculation 1a: What are the total number of hematopoietic cells across different tissues of a 22g adult female mouse?

Bone marrow

While current studies focus primarily on quantifying the absolute count of cells recovered from a given bone after flushing or crushing, this is not sufficient to infer the total number of bone marrow nucleated cells. Indeed, in order to quantify the total number of bone marrow nucleated cells, the distribution of bone marrow cells amongst all bones is essential to know, as well as the percentage of bone marrow cells recovered by flushing or crushing. Such numbers were quantified in studies using ^{59}Fe injection and radiation measurement of each bone of the murine skeleton [66], with the assumption that every bone contains a similar percentage of marrow erythropoietic tissue [66]. This assumption is supported by measurements obtained using radioactively labelled iron to estimate the erythroid content of bone marrow from different anatomical sites[66]. It is also important to note that the major hematopoietic organs contain non-hematopoietic stromal cells. In bone marrow, this cell

population represents about 1.5% of the entire nucleated cell compartment [67–69], however there can be significant variation in the proportions of stromal cells recovered depending on how the tissue was processed, [70]. Due to large variability in estimates of stromal cell frequencies, and their low abundance in the major hematopoietic organs (around 1%), which would not influence the order of magnitude of our estimate, they are not considered in our calculations. Accounting for the key factors detailed above, we estimate the total amount of nucleated bone marrow cells in the following manner:

Number of nucleated bone marrow cells/mouse body weight(g) x mouse body weight(g)

$$1.2 \times 10^7 \frac{\text{cells}}{\text{g}} \times 22\text{g} \approx 2.6 \times 10^8 \text{ nucleated cells}$$

However, these measurements only assess nucleated cells. Based on the Boyer et al measurements of the percentage of Ter119+ RBC (42%) and Cd61+ Platelets (1.3%) amongst mature cell lineages [28–30] (Lin+ cells) and the percentage of Lin- cells of whole bone marrow cells (4.5%)[29], we can also derive the total number of hematopoietic cells in the bone marrow in the following manner:

Assuming,

$$\text{Total BM cells} = \text{Nucleated cells} / \% \text{nucleated cells in BM}$$

$$\text{Total BM cells} = \text{Nucleated cells} + \text{Anucleated cells}$$

Then,

$$\% \text{nucleated cells in BM} = 1 - \% \text{anucleated cells in BM} = 1 - (\% \text{RBC in BM} + \% \text{platelets in BM})$$

And,

$$\% \text{RBC in BM} = \% \text{RBC in Lin}^+ \times \% \text{Lin}^+ \text{ in BM} = \% \text{RBC in Lin}^+ (1 - \% \text{Lin}^- \text{ in BM})$$

$$\% \text{platelets in BM} = \% \text{platelets in Lin}^+ \times \% \text{Lin}^+ \text{ in BM} = \% \text{platelets in Lin}^+ (1 - \% \text{Lin}^- \text{ in BM})$$

Therefore,

$$\rightarrow \% \text{nucleated cells in BM} = 1 - [(\% \text{RBC in Lin}^+ + \% \text{platelets in Lin}^+) \times (1 - \% \text{Lin}^- \text{ in BM})]$$

$$\rightarrow \text{Total BM cells} = \text{Nucleated cells} / [1 - [(\% \text{RBC in Lin}^+ + \% \text{platelets in Lin}^+) \times (1 - \% \text{Lin}^- \text{ in BM})]]$$

$$\frac{2.6 \times 10^8 \text{ cells}}{1 - [(42\% + 1.3\%) \times (1 - 4.5\%)]} \approx 4.5 \times 10^8 \text{ total bone marrow cells}$$

Spleen

The spleen is known to be primarily constituted of hematopoietic cells to the extent that changes in spleen weight are regularly used as a biological readout for changes in hematopoietic cell numbers. However, quantifying the number of hematopoietic cells present in the spleen received little attention. We therefore took the approach to relate spleen weight to spleen cellularity using data from [71] in the following manner:

Assuming,

$$\text{Total spleen cells} = \text{number of splenocytes per gram of spleen} \times \text{spleen weight (g)}$$

$$\text{Spleen weight} = \% \text{ spleen weight to total body weight} \times \text{mouse weight (in grams)}$$

Then,

$$\text{Total spleen cells} = \text{number of splenocytes per gram of spleen} \times \text{spleen weight}$$

$$\text{spleen weight to total body weight} \times \text{mouse weight (in grams)}$$

$$0.36\% \times 22\text{g} \times 2.6 \times 10^9 \frac{\text{cells}}{\text{g}} \approx 2.1 \times 10^8 \text{ cells}$$

Peripheral Blood

Estimating the blood cellular content of our reference mouse is non-trivial. While immune cells frequencies in blood are one of the most robust measurements available, quantifying the total blood volume of a mouse is surprisingly challenging and has been disputed for almost a century. The main approach used in published papers is based on the concept that by measuring the dilution of a known component injected in blood, e.g. dye, radioactively labelled proteins or erythrocytes, it is possible to quantify back the total blood volume. However relatively strong experimental biases exist depending on whether the label is diluted in plasma (die or radioactive albumin) or in erythrocytes with values ranging from 29 $\mu\text{l/g}$ to 120 $\mu\text{l/g}$ body weight for various mouse strains [72–76]. In order to stay as closed to our reference mouse (adult female B6 mouse) and to use the most robust data, we decided to use Sluiter *et al.* blood volume measurement (69 $\mu\text{l/g}$ body weight), where a true regression line was inferred from data between blood volume and body weight [76]. Using this value along with cell counts/ μl blood from Boyer *et al.*, we computed the number of hematopoietic cells in the blood as 1.6×10^{10} cells using the following formula:

$$(\text{Granulomyeloids} + \text{platelets} + \text{RBC} + \text{B cells} + \text{T cells})(\text{count}/\mu\text{l}) \times \text{blood volume/body weight} (\mu\text{l} / \text{g}) \times \text{body weight (grams)}$$

$$(1.1 \times 10^3 + 9.4 \times 10^5 + 9.8 \times 10^6 + 5.5 \times 10^3 + 1.7) \frac{\text{cell}}{\mu\text{l}} \times 69 \frac{\mu\text{l}}{\text{g}} \times 22\text{g} \approx 1.6 \times 10^{10} \text{ cells}$$

Lymph Nodes

In the literature we find that each mouse has 22 – 32 lymph nodes [77,78] (i.e. an average of 27 lymph nodes and that each lymph node contains 1.5-4 million cells [79] (i.e., an average of 2.8×10^6 cells). Lymph nodes are difficult to distinguish from the surrounding fat and connective tissue, and so to count all of them lymph nodes were stimulated with adjuvant (activated lymph nodes become enlarged), and colored in vivo by an injection of Indian ink prior to dissection. Lymph node cellularity was measured by flow cytometry but this number is probably an underestimate of the true cellularity, as no quantification beads were used to correct for the volume of liquid recorded by the cytometer:

Number of cells per lymph node (cells) x total numbers of lymph nodes (lymph nodes per mouse)

$$2.8 \times 10^6 \frac{\text{cells}}{\text{lymph node}} \times 27 \text{ lymph nodes} \approx 7.6 \times 10^7 \text{ cells}$$

Thymus

The thymus is known to decrease in size and cellular content over time in a process called thymic involution. In order to infer the amount of thymocytes present in our mouse model, we used the relationship between thymocytes count and female B6J mouse quantified by Hsu *et al* using a negative exponential curve[80]. Assuming our reference mouse is an adult of 12 weeks (84 days) we obtain the following estimate:

thymocyte counts at birth x exp(-thymocyte loss rate per day x mouse age in days)

$$3.0 \times 10^8 \text{ thymocytes} \times e^{-0.0041/\text{day} \times 84 \text{ days}} \approx 2.1 \times 10^8 \text{ cells}$$

Calculation 1b: What are the relative numbers of hematopoietic progenitors in mice?

Before estimating the relative amounts of progenitor populations, we first derive the total number of HSCs in our reference mouse. Flow cytometry analyses from Busch *et al.* suggest that murine HSCs (Lin-Kit+ Sca-1+CD150+CD48-) represent 0.006% of all nucleated bone marrow cells [10] while our calculation in section S1a suggests there are 2.6×10^8 nucleated bone marrow cells. To estimate the total number of HSCs we perform the following calculation:

Number of bone marrow nucleated cells (cells) x HSC frequency (%)

$$(2.6 \times 10^8) \text{ cells} \times 0.006\% \approx 16,000 \text{ HSCs}$$

Furthermore, Busch et al. also estimated that a minimum of 1 in 3 Tie2 induced YFP labelled HSC contribute actively to hematopoiesis. Assuming this characteristic can be extrapolated to all phenotypic HSCs this suggests, as reported by Busch et al, that our reference mice would have a minimum total number of HSCs of:

Number of HSC x minimum active HSC ratio

$$1.6 \times 10^4 \text{ cells} \times \frac{1}{3} \approx 5,200 \text{ active HSCs}$$

To calculate the sizes of the MPP, CMP, GMP, MEP and CLP compartments we scale our reference value for HSCs by flow cytometry measurements of the relative compartment sizes [10] as follows

The MPP compartment is 9 times bigger than the HSC compartment

$$16,000 \times 9 = 1.4 \times 10^5$$

The CMP compartment is 2.9 times bigger than the MPP compartment

$$(1.4 \times 10^5) \times 2.9 = 4 \times 10^5$$

The GMP compartment is 3.6 times bigger than the MPP compartment

$$(1.4 \times 10^5) \times 3.6 = 5.1 \times 10^5$$

The MEP compartment is 5.7 times bigger than the MPP compartment

$$(1.4 \times 10^5) \times 5.7 = 8 \times 10^5$$

The CLP compartment is 1.8 times bigger than the MPP compartment

$$(1.4 \times 10^5) \times 1.8 = 2.5 \times 10^5$$

In this study megakaryocyte progenitors were not measured. To estimate the size of this compartment we use flow cytometry measurements of their frequency amongst all nucleated cells[27] and multiply this value by the total number of nucleated bone marrow cells:

Number of bone marrow nucleated cells (cells) x MkP frequency (%)

$$(2.6 \times 10^8) \times 0.01\% \approx 2.6 \times 10^4 \text{ MkPs}$$

The MkP compartment is 0.2 times bigger than the MPP compartment

$$(1.4 \times 10^5) \times 0.2 = 2.6 \times 10^4$$

Summarising all of this data on a relative scale there are 9 MPPs per HSC, while one MPP will produce on average 2.9 CMPs, 3.6 GMPs, 5.7 MEPs , 0.2 MkP and 1.8 CLP

Calculation S2: The Dynamics and Bioenergetics of Hematopoiesis

Calculation 2a: the *minimum* number of divisions from HSC to myeloid and red blood cells in mice using only population sizes

To calculate the minimum number of divisions required to reach homeostatic cell numbers, we can use the fact that each division produce two cells and derive the number of divisions to produce a given number of mature cells assuming all stem cells contribute equally. This translates into the following equation:

$$N_{\text{mature}} = N_{\text{hsc}} \times 2^{\text{division}}$$

Where *division* is the number of generations it takes to reach steady state mature population sizes (in these calculations rounded to an order of magnitude approximation), not considering cell death and differentiation. N_{mature} and N_{hsc} represent the population sizes of the mature and HSC compartments. Using the number of mature cells and HSCs from the previous section, we can rearrange our formula and calculate minimal division requirements (*division*) for myeloid and red blood cells.

Given that there are 5200 differentiation active HSCs, and that the mature myeloid and RBC compartments have 1.6×10^8 and 1.5×10^{10} (Table 1) cells respectively (as detailed in file S1) we compute the following:

$$\text{HSC} \rightarrow \text{M} \quad \log_2(1.6 \times 10^8 \div 5200) \approx 14.9 \text{ divisions}$$

$$\text{HSC} \rightarrow \text{RBC} \quad \log_2(1.5 \times 10^{10} \div 5200) \approx 21.5 \text{ divisions}$$

For B cells which undergo selection during development, and platelets which fragment from megakaryocytes, the number of divisions the dynamics are more complicated and are discussed in the subsequent sections.

Calculation 2b: the division number from HSC to naïve B cells in mice accounting for selection

A large proportion of B-cells generated do not reach the mature pool due to negative and positive selection. To estimate the effect of this selection, we combine the measurements from the same study of immature B cells produced from the bone marrow each day (1.2×10^7 immature B cells) with the number of cells that enter the

mature naïve B-cell pool (0.6×10^6 mature naïve B cells per day)[81]. It suggests that 1 out of 20 immature B-cells will become a fully mature naïve B cell. Of note, others have measured 0.9×10^6 mature naïve follicular mature (FM) B cells [47] but the measurement of immature B-cells is not available from the same study. Taking into account this selection factor, we calculate the number of divisions from HSC to naïve mature B cell by dividing the number of naïve B cells by the number of HSC. The number of naïve B cells is obtained by dividing total B-cell numbers (1.4×10^8 , detailed in the main text) by our selection factor of 3%:

Given that there are 5000 differentiation active HSCs, and 1.8×10^8

B cells (Table 1) we compute the following:

$$\text{HSC} \rightarrow \text{B} \quad \log_2(1.8 \times 10^8 \div 3\% \div 5200) \approx 20.1 \text{ divisions}$$

Calculation 2c: the minimum division from HSC to megakaryocyte in mice

Platelets are produced from progenitor cells called megakaryocytes. During maturation, megakaryocytes undergo endomitoses and accumulate a large amount of protein and membrane before fragmenting into platelets. Therefore, it is more appropriate to consider megakaryocyte numbers rather than platelet numbers for division numbers. Given that MKs represent 0.29% of nucleated bone marrow cells [36], there are around 8.1×10^5 megakaryocytes in the bone marrow of our reference mouse. For megakaryocytes this gives a minimal division requirement of:

Given that there are 5200 differentiation active HSCs, and 8.1×10^5

megakaryocytes (as detailed in file S1) we compute the following:

$$\text{HSC} \rightarrow \text{Meg} \quad \log_2(7.7 \times 10^5 \div 5200) \approx 7.2 \text{ divisions}$$

Calculation 2d: Number of platelets produced per megakaryocyte in mice

Before giving rise to platelets megakaryocytes first undergo an additional 2-7 endomitoses. Given that most megakaryocytes are 16N [36], we assume that most megakaryocytes undergo 4 endomitoses. Summing the

minimal division number from HSC to Megakaryocytes (7.3 divisions as calculated above) with the 4 endomitoses, we get a total of 11.3 divisions/endomitoses. Given the number of platelets produced each day (**table 2**) and the total number of megakaryocytes (**table 1**), each megakaryocyte produces:

$$\text{Platelets produced per day / number of megakaryocytes}$$

$$4 \times 10^8 \div 7.7 \times 10^5 \approx 519 \text{ platelets produced per megakaryocyte per day}$$

Previously published estimates using the average megakaryocyte volume and the average platelet volume [82,83] or the daily platelet turnover rate in the blood and the total number of megakaryocytes [50] give similar values.

Calculation 2e: How many cells die each day per lineage in mice?

The number of cells that die each day per lineage can be calculated knowing the respective compartment sizes and half-lives. Compartment sizes and half-lives for our reference mouse reference are provided in Table S2, while the sources used in generating these reference values are provided in file S1. Using the following expression, we convert the half-life ($T_{1/2}$) into a death rate ($P(D)$) of each lineage to calculate how many cells die per day per lineage.

$$P(D) = \ln(2) / T_{1/2}$$

Multiplying this death rate $P(D)$ by the total number of cells gives the number of cells that die each day (**Table S2**). As the death rate and production rates are equal at steady state, this value also represents daily bone marrow output:

$$\text{RBC} = 0.03 \times (1.5 \times 10^{10}) \approx 4.5 \times 10^8 \text{ cells per day}$$

$$\text{Plt} = 0.28 \times (1.4 \times 10^9) \approx 3.9 \times 10^8 \text{ cells per day}$$

$$\text{Myeloid} = 0.83 \times (1.5 \times 10^8) \approx 1.2 \times 10^8 \text{ cells per day}$$

For B cells we must also take negative and positive selection into account. We therefore calculate the daily death rate as above:

$$\mathbf{B\ cells} = 0.03 \times (5.7 \times 10^7) \approx 1.7 \times 10^6 \text{ cells per day}$$

and then divide this value by a selection factor which takes the number of immature B cells that enter the mature pool into account

$$3\% \div (1.7 \times 10^6) \approx 5.6 \times 10^7 \text{ B cells produced per day}$$

Calculation 2f: The protein synthesis requirements of erythromyeloid cell production in humans

Given our estimated turnover rates for myeloid cells and RBCs in humans (**table 3**), along with the total protein content of each cell type (**table S1**) we can compute the protein cost of cell production for each lineage.

$$\textit{Protein synthesis requirements} = \textit{Number of cells produced per day} \times \textit{number of proteins per cell}$$

$$\mathbf{RBC} = (2.1 \times 10^{11}) \times (6 \times 10^7) \approx 1.3 \times 10^{19} \text{ proteins per day}$$

$$\mathbf{M} = (6.2 \times 10^{10}) \times (5.8 \times 10^8) \approx 3.6 \times 10^{19} \text{ proteins per day}$$

These differences have important consequences for bioenergetics as approximately half of total ATP budgets is consumed directly by protein synthesis machinery [84,85]. Knowing that a typical protein has 375 amino acids [86], and that translation of a single amino acid requires roughly 6 ATPs [4] we estimate the ATP cost for *de novo* protein synthesis during hematopoiesis with the following expression:

$$\textit{Cost of protein synthesis for each lineage} = \textit{protein synthesis requirements per lineage (proteins required per day)} \times \textit{number of ATPs required to translate a single protein (ATPs per protein)}$$

$$\mathbf{RBC} = (1.3 \times 10^{19}) \times 375 \times 6 \approx 2.9 \times 10^{22} \text{ ATPs per day}$$

$$\mathbf{M} = (3.6 \times 10^{19}) \times 375 \times 6 \approx 8.1 \times 10^{22} \text{ ATPs per day}$$

Calculation 2g: How many calories are required to maintain the human RBC compartment at steady state?

At a fixed moment in time each Human RBC contains 9.7×10^7 ATP molecules, as determined by NMR measurements[87], with results consistent with luciferase assay measurements[88]. The major ATP consuming mechanisms in RBCs are $Na/K/Ca^+$ pumps and cytoskeleton polymerization, with experimental measurements suggesting that these processes lead to an ATP turnover rate of ~ 1 mmol of ATP per liter of cells per hour [89–91]. The volume of an RBC is $\sim 1 \times 10^{-13}$ Liters [92] and thus if we multiply the ATP turnover rate per liter of cells per hour with this volume, we obtain a turnover rate of 1×10^{-16} moles of ATP per cell per hour, which can be transformed from moles into molecules:

ATP turnover rate per liter of cells (moles of ATP per liter of cells per hour) x volume of 1 cell (liters)

$$(1 \times 10^{-3})(1 \times 10^{-13}) = 1 \times 10^{-16} \text{ moles of ATP per cell per hour}$$

Number of moles (ATP per cell per hour) x Avogadro's number =

$$(1 \times 10^{-16})(6 \times 10^{23}) = 6 \times 10^7 \text{ molecules of ATP per cell per hour}$$

Given there is a total of 2.5×10^{13} RBCs in humans we can multiply the ATP turnover rate by the total number of cells to estimate the energetic cost of the RBC compartment:

$$(6 \times 10^7) \times 24 \times (2.5 \times 10^{13}) \approx 3.6 \times 10^{22} \text{ molecules of ATP per day}$$

To convert ATP numbers to a more familiar scale, calories, we perform the following transformation:

(Grams of glucose/molar mass of glucose) x Avogadro's number = the number of glucose molecules in 1 gram of glucose

$$\frac{1}{180.2} \times (6 \times 10^{23}) = 3.3 \times 10^{21} \text{ molecules of glucose in 1 gram}$$

Red blood cells do not have mitochondria and must break down glucose using only glycolysis, a process which yields 2 ATPs per molecule of glucose. Therefore, in red blood cells:

$$1 \text{ gram of glucose} = (3.34 \times 10^{21}) \times 2 = 6.7 \times 10^{21} \text{ ATPs obtained by glycolysis}$$

If the total red blood cell pool needs 3.6×10^{22} ATPs and 1 gram of glucose yields 6.7×10^{21} ATPs then the entire red blood cell compartment requires 5.4 grams of glucose ($3.6 \times 10^{22} \div 6.7 \times 10^{21}$). Each gram of sugar has 4kcal therefore we would need ~22 kcal to sustain RBC energetic requirements using glycolysis alone.

This value does not represent the entire caloric cost however, as it does not account for the cell divisions required to replace the number RBCs that die each day, a parameter that has yet to be measured. We estimate the caloric cost of red blood cell turnover as follows:

The total turnover rate of the red blood cell compartment has been estimated for humans, and converted into a mass scale [4]. This meta-analysis suggests that red blood cell turnover accounts for 20 grams of mass. Assuming a water content of 70% and that proteins account for 60% of dry mass we get the following estimate for the protein turnover needed for erythropoiesis:

$$\begin{aligned} &\text{Total mass turnover rate (grams)} \times \% \text{ of dry mass in a cell} \times \% \text{ of proteins in dry mass of a cell} \\ &20 \times (1 - 0.7) \times 0.6 = 3.6 \text{ grams of protein each day are needed to fuel RBC turnover} \end{aligned}$$

We can then convert this to a caloric scale using the approach described in [4]. In this scheme we assume that it takes 5kJ of energy to make 1 gram of protein[4].

Protein turnover per day x energy cost of protein synthesis

$$3.6 \times 5 = 18 \text{kJ per day}$$

Knowing that there are 0.24 kcal per kJ we can convert to calories:

$$18 \times 0.24 = 4.3 \text{kcal}$$

However, this number only considers protein synthesis, and not the entire ATP cost of making new cells. It has been estimated that protein synthesis accounts for 30% of all ATP use in cells, based on measuring the respiration rate of rat thymocytes following inhibition of different ATP-consuming processes [84]. Assuming that protein synthesis also accounts for 30% of all ATP use in erythroblasts we estimate that:

$$4.3 \times \frac{10}{3} \approx 14 \text{ kcal per day to fuel erythropoiesis}$$

Taken together, our theoretical calculations suggest that just ~22 kcal are needed to maintain existing RBCs, while ~14kcal are needed to create new RBCs. Therefore ~36kcal, the equivalent of a single strawberry is enough to fuel erythroid homeostasis, a striking result considering 90% of the entire cellular content of the human body. However, this remains a theoretical prediction, based on a number of key assumptions (detailed above) and so warrants experimental validation.

Calculation 2h: calculating the numbers of lipids in different subsets

Leidl et al[56] have measured lipid content in different human blood cell types using ESI-MS/MS and measurements were normalized to the protein content of each cell as measured by a BCA assay[56]. In this study total lipids are based on measurements of the following: phospholipids. Phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylethanolamine (PE), PE-based plasmalogens (PE-pl), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), lysophosphatidylcholine (LPC)), ceramide (Cer), cholesteryl esters (CE) and free cholesterol (FC). As these subtypes are highly abundant [93]. In the following section we provide an example calculation showing how total lipid numbers are calculated for monocytes:

Leidl et al report there are 5.5nmol of lipids in 10⁶ monocytes

Moles of lipids per cell x Avogadro's number = number of lipids per monocyte

$$(5.5 \times 10^{-15}) \times (6 \times 10^{23}) = 3.3 \times 10^9 \text{ lipids per monocyte}$$

The same data transformation was applied to all of the other cell lineages.

Calculation 2i: calculating numbers of proteins in different subsets

Using a BCA assay Leidl et al [56] have quantified the amount of proteins in different hematopoietic cell types. However in this publication, the data are reported indirectly, in that they provide the amount of lipids per

mg/protein, and also the amount of lipids per 10^6 cells. Using these numbers we can identify the protein content of each cell type as follows (here we use monocytes as an example):

Leidl et al report that monocytes have 118nmol of lipids per mg/protein and there are 5.5nmol lipid per 10^6 cells.

$$\begin{aligned} & \text{Lipid content per mg protein} \div \text{amount of lipid in } 10^6 \text{ cells} = \\ & 0.046\text{mg protein per } 10^6 \text{ cells or } 4.66 \times 10^{-11} \text{ grams of protein per cell.} \end{aligned}$$

As the average protein is 52kDa (113349) or 86.32×10^{-21} grams we can infer the number of proteins by the following expression:

$$\begin{aligned} & \text{Total protein content} \div \text{size of average protein} \\ & 4.66 \times 10^{-11} \div 86.32 \times 10^{-21} = 5.4 \times 10^8 \text{ proteins per monocyte} \end{aligned}$$

79

80

81

82

83 **References**

84

- 85 1 Sender, R. *et al.* (2016) Revised Estimates for the Number of Human and Bacteria
86 Cells in the Body. *PLOS Biol.* 14, e1002533
- 87 2 Milo, R. and Phillips, R. (2015) *Cell Biology by the Numbers*, 1 edition. Garland
88 Science.
- 89 3 Grubb, S.C. *et al.* (2009) Mouse Phenome Database. *Nucleic Acids Res.* 37,
90 D720–D730
- 91 4 Sender, R. and Milo, R. (2021) The distribution of cellular turnover in the human
92 body. *Nat. Med.* 27, 45–48

- 93 5 Lahoz-Beneytez, J. *et al.* (2016) Human neutrophil kinetics: modeling of stable
94 isotope labeling data supports short blood neutrophil half-lives. *Blood* 127, 3431–
95 3438
- 96 6 Valentin, J. (2002) Basic anatomical and physiological data for use in radiological
97 protection: reference values: ICRP Publication 89. *Ann. ICRP* 32, 1–277
- 98 7 Makarieva, A.M. *et al.* (2008) Mean mass-specific metabolic rates are strikingly
99 similar across life's major domains: Evidence for life's metabolic optimum. *Proc.*
100 *Natl. Acad. Sci. U. S. A.* 105, 16994–16999
- 101 8 Trepel, F. (1974) Number and distribution of lymphocytes in man. A critical
102 analysis. *Klin. Wochenschr.* 52, 511–515
- 103 9 Qatarneh, S.M. *et al.* (2006) Three-dimensional atlas of lymph node topography
104 based on the visible human data set. *Anat. Rec. B. New Anat.* 289, 98–111
- 105 10 Busch, K. *et al.* (2015) Fundamental properties of unperturbed haematopoiesis
106 from stem cells in vivo. *Nature* 518, 542–546
- 107 11 Wilson, N.K. *et al.* (2015) Combined Single-Cell Functional and Gene Expression
108 Analysis Resolves Heterogeneity within Stem Cell Populations. *Cell Stem Cell* 16,
109 712–724
- 110 12 Challen, G.A. *et al.* (2010) Distinct Hematopoietic Stem Cell Subtypes Are
111 Differentially Regulated by TGF β 1. *Cell Stem Cell* 6, 265–278
- 112 13 Beerman, I. *et al.* (2010) Functionally distinct hematopoietic stem cells modulate
113 hematopoietic lineage potential during aging by a mechanism of clonal expansion.
114 *Proc. Natl. Acad. Sci. U. S. A.* 107, 5465–5470
- 115 14 Sawai, C.M. *et al.* (2016) Hematopoietic Stem Cells Are the Major Source of
116 Multilineage Hematopoiesis in Adult Animals. *Immunity* 45, 597–609
- 117 15 Pei, W. *et al.* (2020) Resolving Fates and Single-Cell Transcriptomes of
118 Hematopoietic Stem Cell Clones by PolyloxExpress Barcoding. *Cell Stem Cell* 27,
119 383-395.e8
- 120 16 Rodriguez-Fraticelli, A.E. *et al.* (2020) Single-cell lineage tracing unveils a role for
121 TCF15 in haematopoiesis. *Nature* 583, 585–589
- 122 17 Bowling, S. *et al.* (2020) An Engineered CRISPR-Cas9 Mouse Line for
123 Simultaneous Readout of Lineage Histories and Gene Expression Profiles in
124 Single Cells. *Cell* 181, 1410-1422.e27
- 125 18 Sun, J. *et al.* (2014) Clonal dynamics of native haematopoiesis. *Nature* 514, 322–
126 327
- 127 19 Abkowitz, J.L. *et al.* (2002) Evidence that the number of hematopoietic stem cells
128 per animal is conserved in mammals. *Blood* 100, 2665–2667
- 129 20 Lee-Six, H. *et al.* (2018) Population dynamics of normal human blood inferred from
130 somatic mutations. *Nature* 561, 473–478
- 131 21 Watson, C.J. *et al.* (2020) The evolutionary dynamics and fitness landscape of
132 clonal hematopoiesis. *Science* 367, 1449–1454
- 133 22 Biasco, L. *et al.* (2016) In Vivo Tracking of Human Hematopoiesis Reveals
134 Patterns of Clonal Dynamics during Early and Steady-State Reconstitution
135 Phases. *Cell Stem Cell* 19, 107–119
- 136 23 Sommerkamp, P. *et al.* (2021) Mouse multipotent progenitor 5 cells are located at
137 the interphase between hematopoietic stem and progenitor cells. *Blood* 137,
138 3218–3224
- 139 24 Morrison, S.J. and Weissman, I.L. (1994) The long-term repopulating subset of
140 hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity* 1,
141 661–673

- 142 25 Morrison, S.J. *et al.* (1997) Identification of a lineage of multipotent hematopoietic
143 progenitors. *Dev. Camb. Engl.* 124, 1929–1939
- 144 26 Akashi, K. *et al.* (2000) A clonogenic common myeloid progenitor that gives rise to
145 all myeloid lineages. *Nature* 404, 193–197
- 146 27 Nakorn, T.N. *et al.* (2003) Characterization of mouse clonogenic megakaryocyte
147 progenitors. *Proc. Natl. Acad. Sci.* 100, 205–210
- 148 28 Harman, B.C. *et al.* (2008) Resolution of Unique Sca-1^{high} c-Kit⁻ Lymphoid-Biased
149 Progenitors in Adult Bone Marrow. *J. Immunol.* 181, 7514–7524
- 150 29 Kumar, R. *et al.* (2008) Lin⁻Sca1⁺Kit⁻ Bone Marrow Cells Contain Early
151 Lymphoid-Committed Precursors That Are Distinct from Common Lymphoid
152 Progenitors. *J. Immunol.* 181, 7507–7513
- 153 30 Giandomenico, S.D. *et al.* (2019) Megakaryocyte TGFβ1 Partitions Hematopoiesis
154 into Immature Progenitor/Stem Cells and Maturing Precursors. *bioRxiv* DOI:
155 10.1101/689901
- 156 31 Kirby, M.R. and Donahue, R.E. (1993) Rare Event Sorting of CD34+
157 Hematopoietic Cells. *Ann. N. Y. Acad. Sci.* 677, 413–416
- 158 32 Hao, Q.-L. *et al.* (1995) A Functional Comparison of CD34+ CD38- Cells in Cord
159 Blood and Bone Marrow. *Blood* 86, 3745–3753
- 160 33 Farrell, T. *et al.* (2014) Changes in the frequencies of human hematopoietic stem
161 and progenitor cells with age and site. *Exp. Hematol.* 42, 146–154
- 162 34 Povsic, T.J. *et al.* (2010) Aging is not associated with bone marrow-resident
163 progenitor cell depletion. *J. Gerontol. A. Biol. Sci. Med. Sci.* 65, 1042–1050
- 164 35 Boyer, S.W. *et al.* (2019) Clonal and Quantitative In Vivo Assessment of
165 Hematopoietic Stem Cell Differentiation Reveals Strong Erythroid Potential of
166 Multipotent Cells. *Stem Cell Rep.* 12, 801–815
- 167 36 Niswander, L.M. *et al.* (2014) Improved quantitative analysis of primary bone
168 marrow megakaryocytes utilizing imaging flow cytometry. *Cytometry A* 85, 302–
169 312
- 170 37 Basilico, S. and Göttgens, B. (2017) Dysregulation of haematopoietic stem cell
171 regulatory programs in acute myeloid leukaemia. *J. Mol. Med. Berl. Ger.* 95, 719–
172 727
- 173 38 Krueger, A. *et al.* (2017) T Cell Development by the Numbers. *Trends Immunol.*
174 38, 128–139
- 175 39 Romar, G.A. *et al.* (2016) Research Techniques Made Simple: Techniques to
176 Assess Cell Proliferation. *J. Invest. Dermatol.* 136, e1–e7
- 177 40 Wilson, A. *et al.* (2008) Hematopoietic stem cells reversibly switch from dormancy
178 to self-renewal during homeostasis and repair. *Cell* 135, 1118–1129
- 179 41 Akinduro, O. *et al.* (2018) Proliferation dynamics of acute myeloid leukaemia and
180 haematopoietic progenitors competing for bone marrow space. *Nat. Commun.* 9,
181 519
- 182 42 Takahashi, M. *et al.* (2021) Reconciling Flux Experiments for Quantitative
183 Modeling of Normal and Malignant Hematopoietic Stem/Progenitor Dynamics.
184 *Stem Cell Rep.* 16, 741–753
- 185 43 Signer, R.A.J. *et al.* (2016) The rate of protein synthesis in hematopoietic stem
186 cells is limited partly by 4E-BPs. *Genes Dev.* 30, 1698–1703
- 187 44 Catlin, S.N. *et al.* (2011) The replication rate of human hematopoietic stem cells in
188 vivo. *Blood* 117, 4460–4466
- 189 45 Borghans, J.A.M. *et al.* (2018) Current best estimates for the average lifespans of
190 mouse and human leukocytes: reviewing two decades of deuterium-labeling
191 experiments. *Immunol. Rev.* 285, 233–248

- 192 46 Ballesteros, I. *et al.* (2020) Co-option of Neutrophil Fates by Tissue Environments.
193 *Cell* 183, 1282-1297.e18
- 194 47 Verheijen, M. *et al.* (2020) Fate Mapping Quantifies the Dynamics of B Cell
195 Development and Activation throughout Life. *Cell Rep.* 33,
196 48 Bernitz, J.M. *et al.* (2016) Hematopoietic Stem Cells Count and Remember Self-
197 Renewal Divisions. *Cell* 167, 1296-1309.e10
- 198 49 Chambers, S.M. *et al.* (2007) Aging hematopoietic stem cells decline in function
199 and exhibit epigenetic dysregulation. *PLoS Biol.* 5, e201
- 200 50 Harker, L.A. and Finch, C.A. (1969) Thrombokinetis in man. *J. Clin. Invest.* 48,
201 963–974
- 202 51 Amann-Zalcenstein, D. *et al.* (2020) A new lymphoid-primed progenitor marked by
203 Dach1 downregulation identified with single cell multi-omics. *Nat. Immunol.* 21,
204 1574–1584
- 205 52 Model, M.A. (2018) Methods for cell volume measurement. *Cytometry A* 93, 281–
206 296
- 207 53 Hidalgo San Jose, L. *et al.* (2020) Modest Declines in Proteome Quality Impair
208 Hematopoietic Stem Cell Self-Renewal. *Cell Rep.* 30, 69-80.e6
- 209 54 Signer, R.A.J. *et al.* (2014) Haematopoietic stem cells require a highly regulated
210 protein synthesis rate. *Nature* 509, 49–54
- 211 55 Agathocleous, M. *et al.* (2017) Ascorbate regulates haematopoietic stem cell
212 function and leukaemogenesis. *Nature* 549, 476–481
- 213 56 Leidl, K. *et al.* (2008) Mass spectrometric analysis of lipid species of human
214 circulating blood cells. *Biochim. Biophys. Acta BBA - Mol. Cell Biol. Lipids* 1781,
215 655–664
- 216 57 Radley, J.M. *et al.* (1999) Ultrastructure of primitive hematopoietic stem cells
217 isolated using probes of functional status. *Exp. Hematol.* 27, 365–369
- 218 58 Noris, P. *et al.* (2014) Platelet diameters in inherited thrombocytopenias: analysis
219 of 376 patients with all known disorders. *Blood* 124, e4–e10
- 220 59 H, D. *et al.* (2011) Normal range of mean platelet volume in healthy subjects:
221 Insight from a large epidemiologic study. *Thromb. Res.* 128,
222 60 Bessman, J.D. (1984) The relation of megakaryocyte ploidy to platelet volume. *Am.*
223 *J. Hematol.* 16, 161–170
- 224 61 McLaren, C.E. *et al.* (1987) Statistical and graphical evaluation of erythrocyte
225 volume distributions. *Am. J. Physiol.* 252 Heart Circ. Physiol.
- 226 62 Downey, G.P. *et al.* (1990) Retention of leukocytes in capillaries: role of cell size
227 and deformability. *J. Appl. Physiol. Bethesda Md* 1985 69, 1767–1778
- 228 63 Ting-Beall, H.P. *et al.* (1993) Volume and osmotic properties of human neutrophils.
229 *Blood* 81, 2774–2780
- 230 64 Needham, D. and Hochmuth, R.M. (1990) Rapid flow of passive neutrophils into a
231 4 microns pipet and measurement of cytoplasmic viscosity. *J. Biomech. Eng.* 112,
232 269–276
- 233 65 Kuse, R. *et al.* (1985) Blood lymphocyte volumes and diameters in patients with
234 chronic lymphocytic leukemia and normal controls. *Blut* 50, 243–248
- 235 66 Chervenick, P.A. *et al.* (1968) Quantitative studies of blood and bone marrow
236 neutrophils in normal mice. *Am. J. Physiol.* 215, 353–360
- 237 67 Méndez-Ferrer, S. *et al.* (2010) Mesenchymal and haematopoietic stem cells form
238 a unique bone marrow niche. *Nature* 466, 829–834
- 239 68 Worthley, D.L. *et al.* (2015) Gremlin 1 Identifies a Skeletal Stem Cell with Bone,
240 Cartilage, and Reticular Stromal Potential. *Cell* 160, 269–284

- 241 69 Zhou, B.O. *et al.* (2014) Leptin-Receptor-Expressing Mesenchymal Stromal Cells
242 Represent the Main Source of Bone Formed by Adult Bone Marrow. *Cell Stem Cell*
243 15, 154–168
- 244 70 Gomariz, A. *et al.* (2018) Quantitative spatial analysis of haematopoiesis-
245 regulating stromal cells in the bone marrow microenvironment by 3D microscopy.
246 *Nat. Commun.* 9, 2532
- 247 71 Menees, K.B. *et al.* (2021) Sex- and age-dependent alterations of splenic immune
248 cell profile and NK cell phenotypes and function in C57BL/6J mice. *Immun. Ageing*
249 18, 3
- 250 72 Kaliss, N. and Pressman, D. (2016) Plasma and Blood Volumes of Mouse Organs,
251 As Determined with Radioactive Iodoproteins.*: *Proc. Soc. Exp. Biol. Med.* DOI:
252 10.3181/00379727-75-18083
- 253 73 Furth, J. and Sobel, H. (1946) Hypervolemia Secondary to Grafted Granulosa-Cell
254 Tumor2. *JNCI J. Natl. Cancer Inst.* 7, 103–113
- 255 74 Keighley, G. *et al.* (1962) Response of Normal and Genetically Anaemic Mice to
256 Erythropoietic Stimuli*. *Br. J. Haematol.* 8, 429–441
- 257 75 Riches, A.C. *et al.* (1973) Blood volume determination in the mouse. *J. Physiol.*
258 228, 279–284
- 259 76 Sluiter, W. *et al.* (1984) Determination of blood volume in the mouse with
260 ⁵¹Chromium-labelled erythrocytes. *J. Immunol. Methods* 73, 221–225
- 261 77 Van den Broeck, W. *et al.* (2006) Anatomy and nomenclature of murine lymph
262 nodes: Descriptive study and nomenclatory standardization in BALB/cAnNCrI
263 mice. *J. Immunol. Methods* 312, 12–19
- 264 78 Kawashima, Y. *et al.* (1964) The lymph system in mice. *Subj. Strain Bibliogr.* 1964
- 265 79 Druzd, D. *et al.* (2017) Lymphocyte Circadian Clocks Control Lymph Node
266 Trafficking and Adaptive Immune Responses. *Immunity* 46, 120–132
- 267 80 Hsu, H.-C. *et al.* (2003) Age-related thymic involution in C57BL/6J × DBA/2J
268 recombinant-inbred mice maps to mouse chromosomes 9 and 10. *Genes Immun.*
269 4, 402–410
- 270 81 Allman, D.M. *et al.* (1993) Peripheral B cell maturation. II. Heat-stable antigen(hi)
271 splenic B cells are an immature developmental intermediate in the production of
272 long-lived marrow-derived B cells. *J. Immunol. Baltim. Md* 151, 4431–4444
- 273 82 Kaufman, R.M. *et al.* (1965) Circulating megakaryocytes and platelet release in the
274 lung. *Blood* 26, 720–731
- 275 83 Trowbridge, E.A. *et al.* (1984) The origin of platelet count and volume. *Clin. Phys.*
276 *Physiol. Meas. Off. J. Hosp. Phys. Assoc. Dtsch. Ges. Med. Phys. Eur. Fed. Organ.*
277 *Med. Phys.* 5, 145–170
- 278 84 Buttgereit, F. and Brand, M.D. (1995) A hierarchy of ATP-consuming processes in
279 mammalian cells. *Biochem. J.* 312, 163–167
- 280 85 Argüello, R.J. *et al.* (2020) SCENITH: A Flow Cytometry-Based Method to
281 Functionally Profile Energy Metabolism with Single-Cell Resolution. *Cell Metab.*
282 32, 1063-1075.e7
- 283 86 Brocchieri, L. and Karlin, S. (2005) Protein length in eukaryotic and prokaryotic
284 proteomes. *Nucleic Acids Res.* 33, 3390–3400
- 285 87 Sarpel, G. *et al.* (1982) Erythrocyte phosphate content in Huntington's disease.
286 *Neurosci. Lett.* 31, 91–96
- 287 88 Thore, A. *et al.* (1975) Detection of bacteriuria by luciferase assay of adenosine
288 triphosphate. *J. Clin. Microbiol.* 1, 1–8

- 289 89 Lew, V.L. and Tiffert, T. (2017) On the Mechanism of Human Red Blood Cell
290 Longevity: Roles of Calcium, the Sodium Pump, PIEZO1, and Gardos Channels.
291 *Front. Physiol.* 8, 977
- 292 90 Müller, E. *et al.* (1986) Turnover of phosphomonoester groups and
293 compartmentation of polyphosphoinositides in human erythrocytes. *Biochem. J.*
294 235, 775–783
- 295 91 Feig, S.A. *et al.* (1972) Energy metabolism in human erythrocytes. *J. Clin. Invest.*
296 51, 1547–1554
- 297 92 Child, J.A. *et al.* (1967) A diffraction method for measuring the average volumes
298 and shapes of red blood cells. *Br. J. Haematol.* 13, 364–375
- 299 93 van Meer, G. *et al.* (2008) Membrane lipids: where they are and how they behave.
300 *Nat. Rev. Mol. Cell Biol.* 9, 112–124
- 301 94 Bogue, M.A. *et al.* (2020) Mouse Phenome Database: a data repository and
302 analysis suite for curated primary mouse phenotype data. *Nucleic Acids Res.* 48,
303 D716–D723
- 304 95 Kawashima, Y. *et al.* (1964) THE LYMPH SYSTEM IN MICE.
- 305 96 Druzd, D. *et al.* (2017) Lymphocyte Circadian Clocks Control Lymph Node
306 Trafficking and Adaptive Immune Responses. *Immunity* 0,
- 307 97 Tanaka, S. *et al.* (2020) Tet2 and Tet3 in B cells are required to repress CD86 and
308 prevent autoimmunity. *Nat. Immunol.* 21, 950–961
309

310

311