

# Sca-1<sup>negative</sup> haematopoietic progenitor cells markedly differ in proliferation rate and are vital for blood cell production

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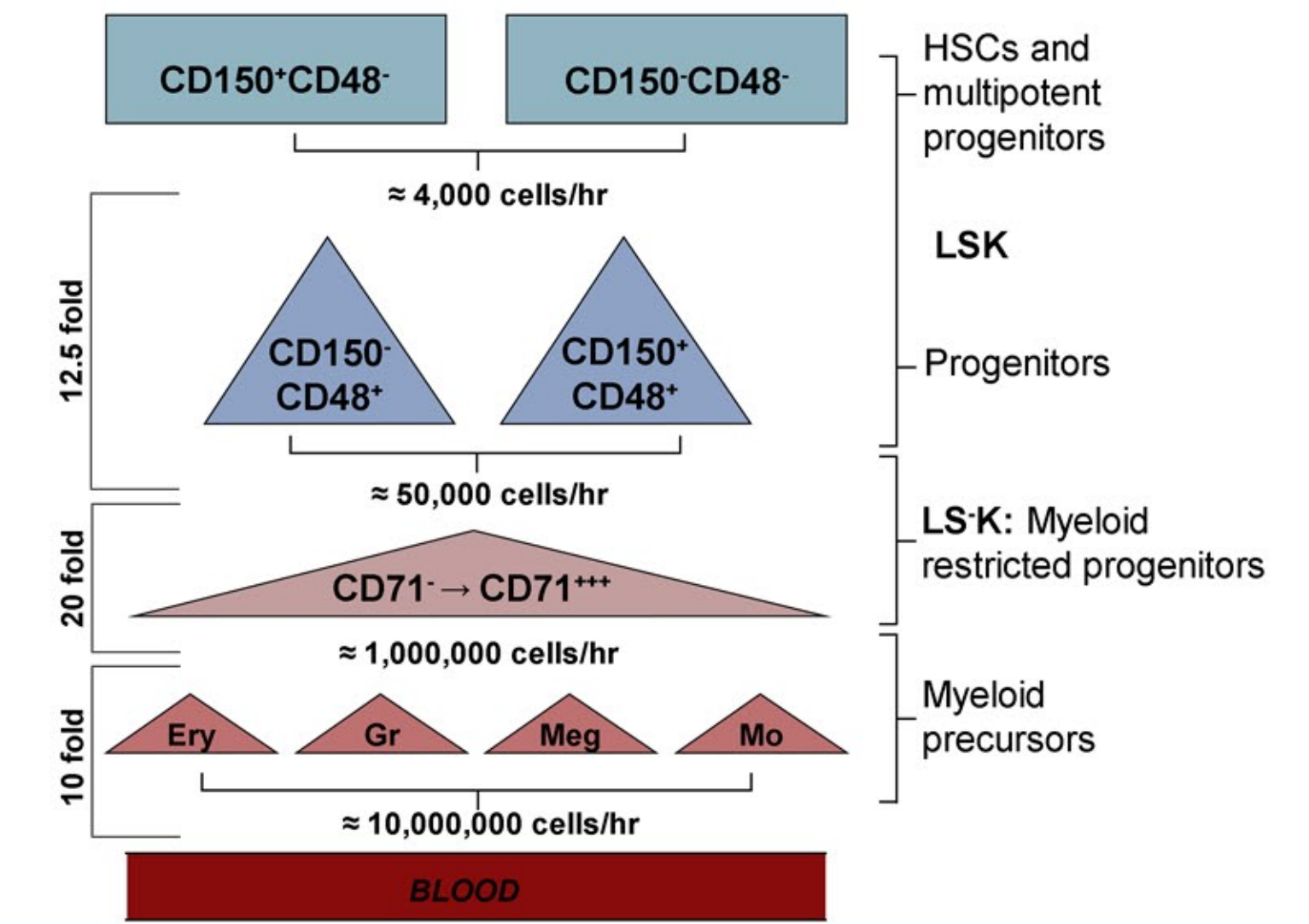
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## Introduction

Haematopoiesis steadily generates a large number of blood cells by intensive cell proliferation. Haematopoietic stem cells (HSCs) are very rare cells and divide infrequently, hence the production of mature blood cells stems from the proliferation of progenitors. In the mouse, various types of progenitors were characterized, and the aim of our study was to find out to which extent these distinct populations contribute to mature haematopoiesis.

## Conclusions

Sca-1<sup>negative</sup> haematopoietic immature cells demonstrate an enormous range of proliferation intensities. The CD71 expression level is very tightly associated with the proliferation rate of haematopoietic immature cells and also with their developmental commitment. The production rate in various types of haematopoietic progenitors and precursors was estimated and is shown in the adjacent figure.



## Materials and methods

Mice: C57BL/6

Flow cytometry: BD FACS Aria IIu, Canto II

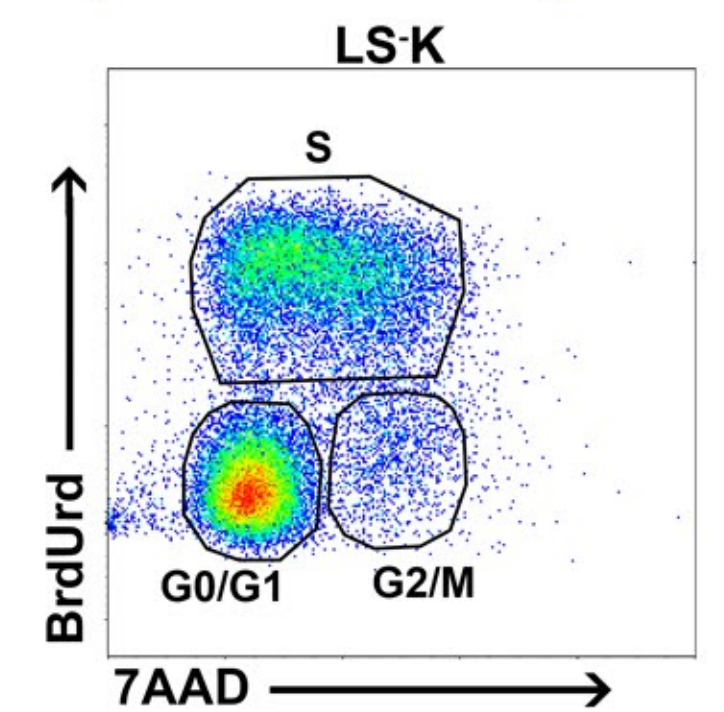
Antibodies: against lineage markers (Lineage cocktail containing anti-B220, -CD3, -Gr-1, -Mac-1, -Ter119), Sca-1, c-Kit, CD48, CD150, CD71 (transferrin receptor), IL7 receptor (IL7R), CD16/32 (FcγRIII/II), CD34

Cell cycle analysis: in vitro 5-bromo-2-deoxyuridine (BrdUrd) labeling \*

Estimation of the cell production rate (assuming S-phase lasting 5 hours):

$$(\text{number of various progenitors in whole organism} \times \% \text{ of S phase cells} / 5 / 100) \times 2 = \text{cell production per hour}$$

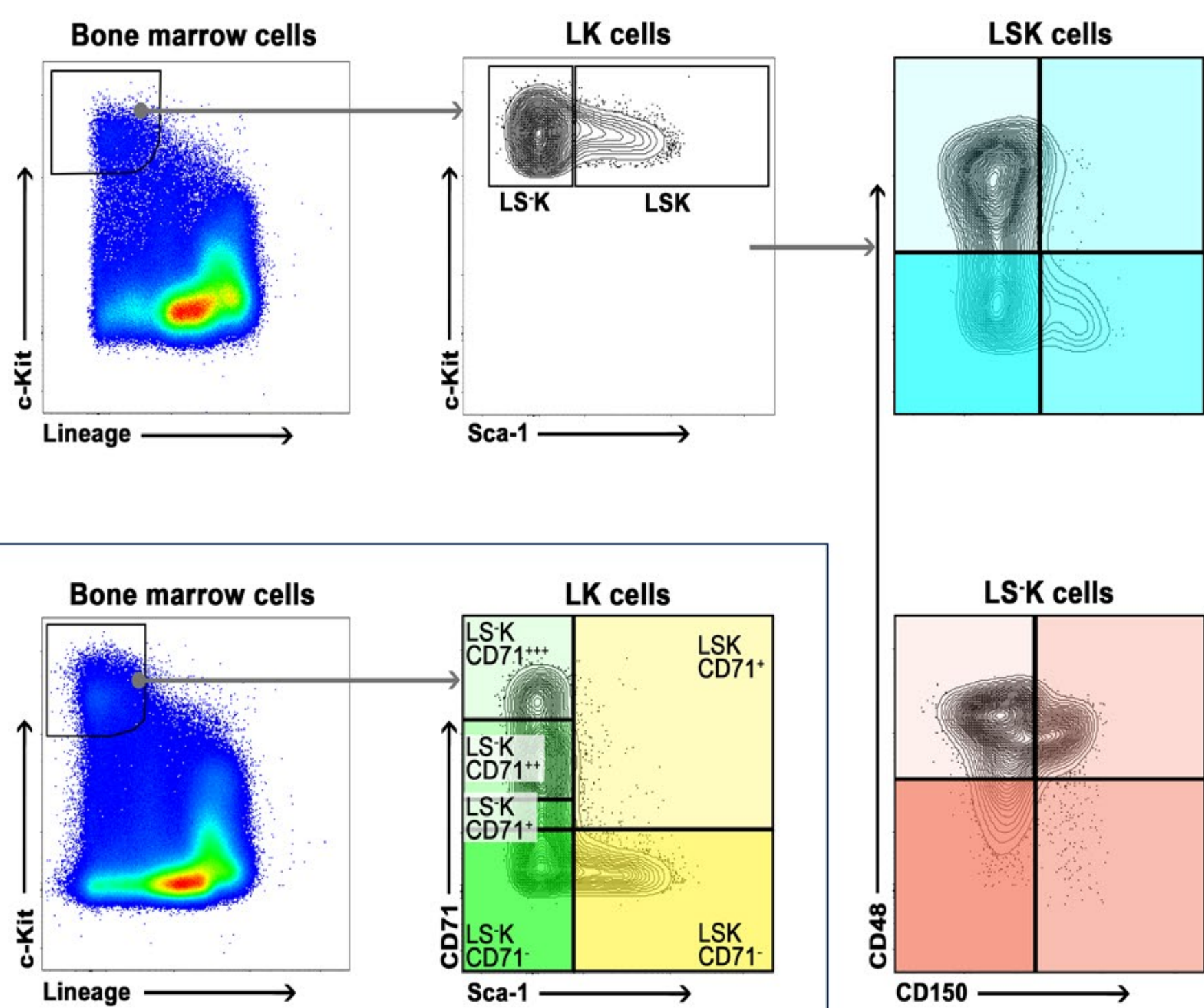
\* Example of the cell cycle analysis



## Results

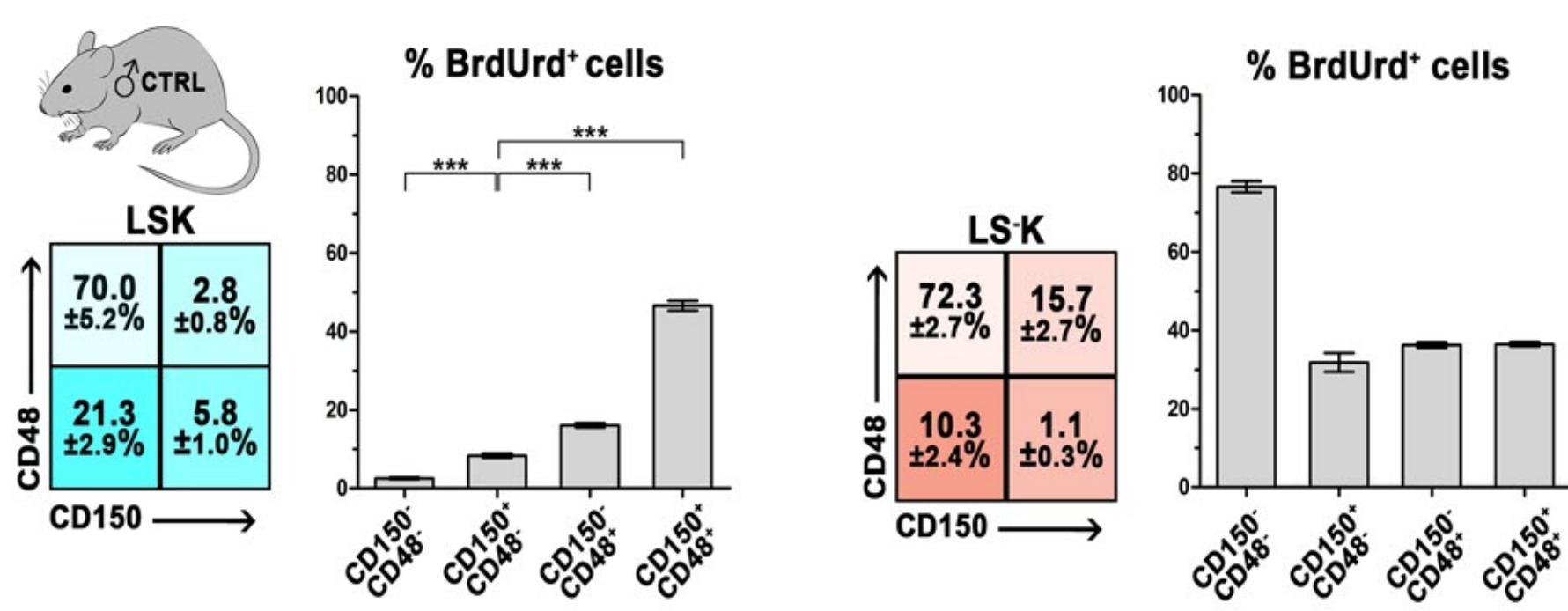
### 1. Proliferation rate significantly differs among Lin<sup>c-Kit</sup> (LK) cells with specific immunophenotypes

#### 1A) Gating strategy of LK cells

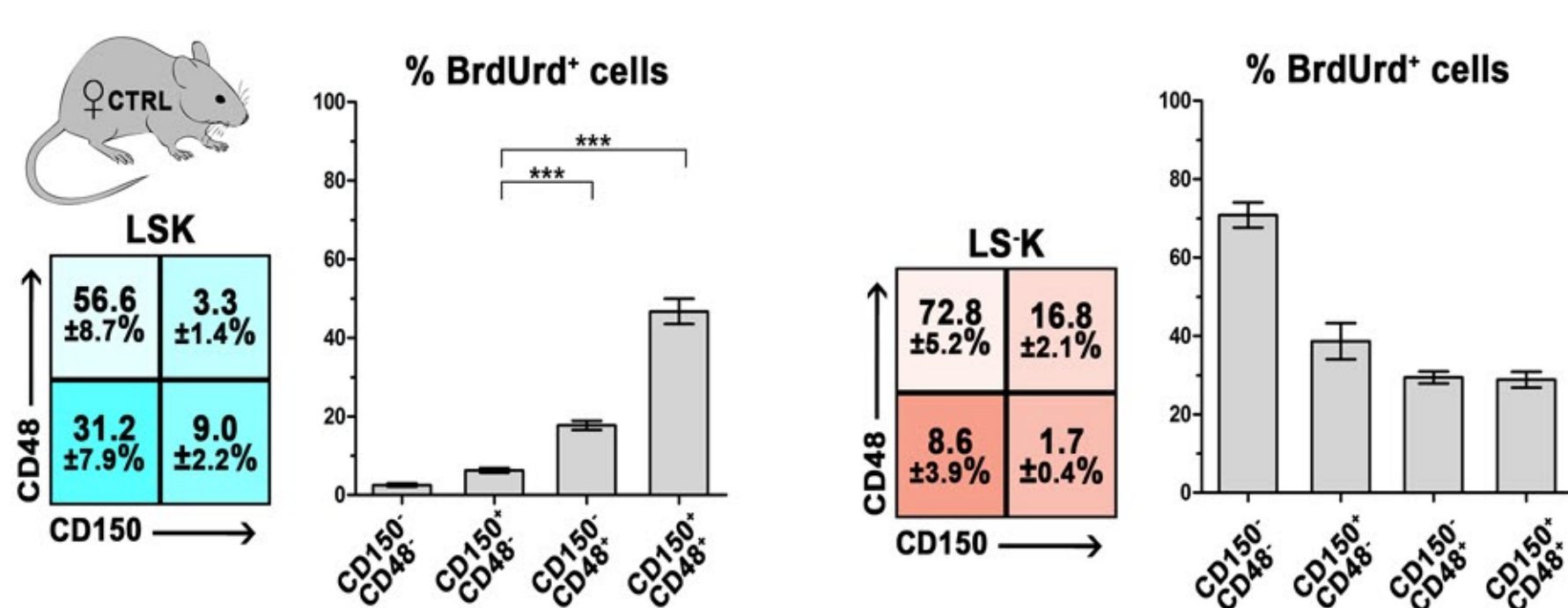


#### 1B) Frequency of CD150/CD48 subtypes of Lin<sup>c-Kit</sup> (LSK) and Lin<sup>c-Kit</sup> (LS-K) cells, and their proliferation rate

Males

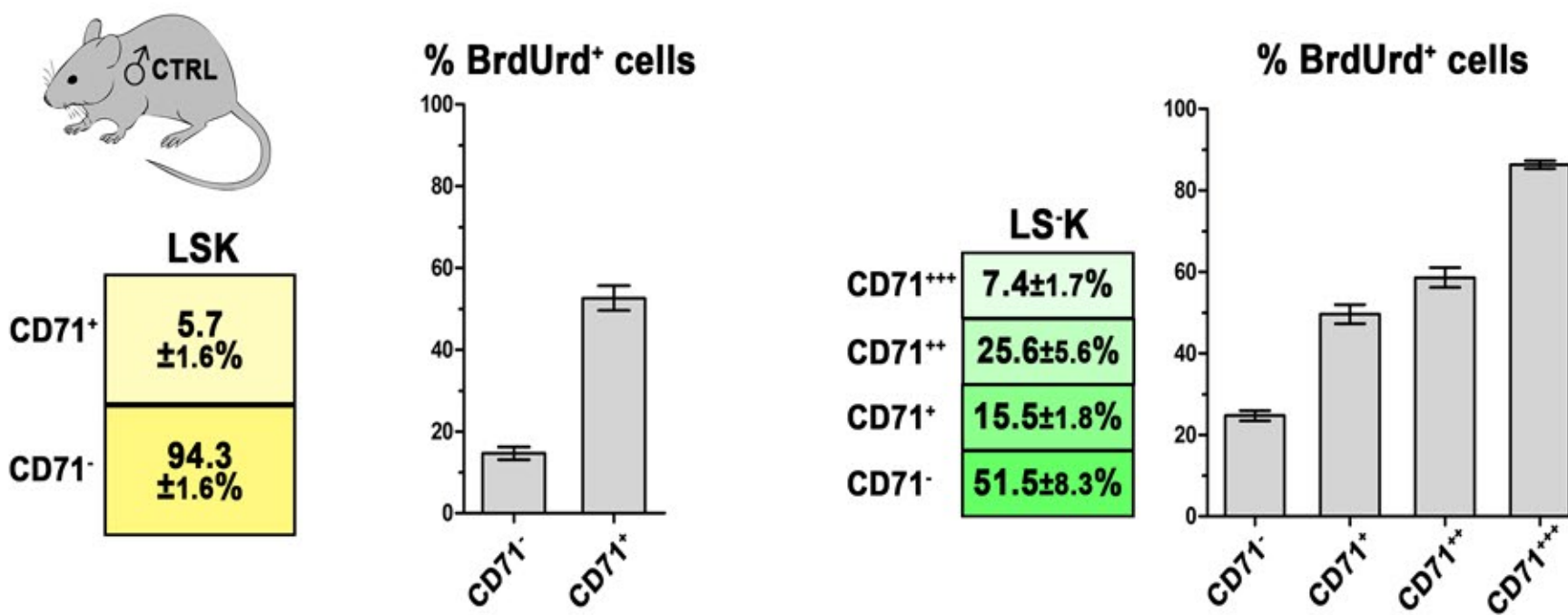


Females



### 1C) Frequency of CD71 subtypes of Lin<sup>c-Kit</sup> (LSK) and Lin<sup>c-Kit</sup> (LS-K) cells, and their proliferation rate

Males

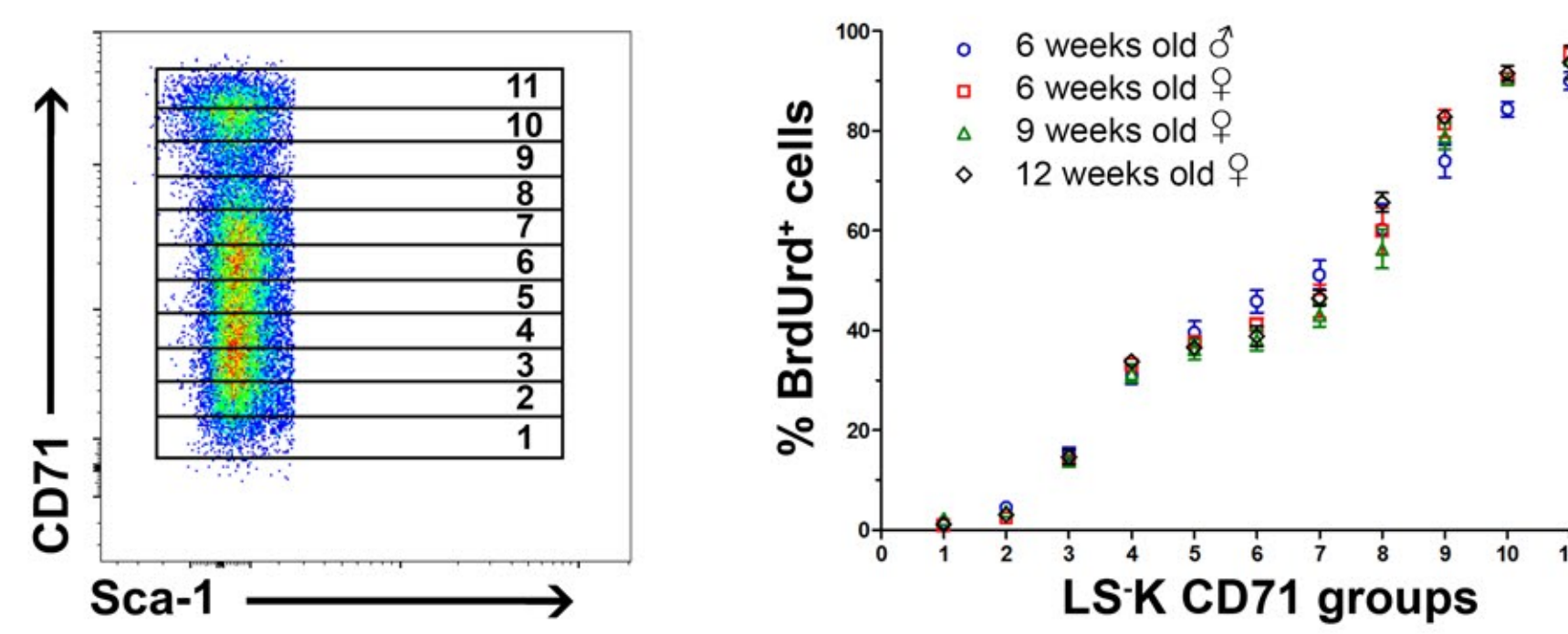


### 1D) Total cell number and number of LK, LSK and LS-K cells in bone marrow (BM)

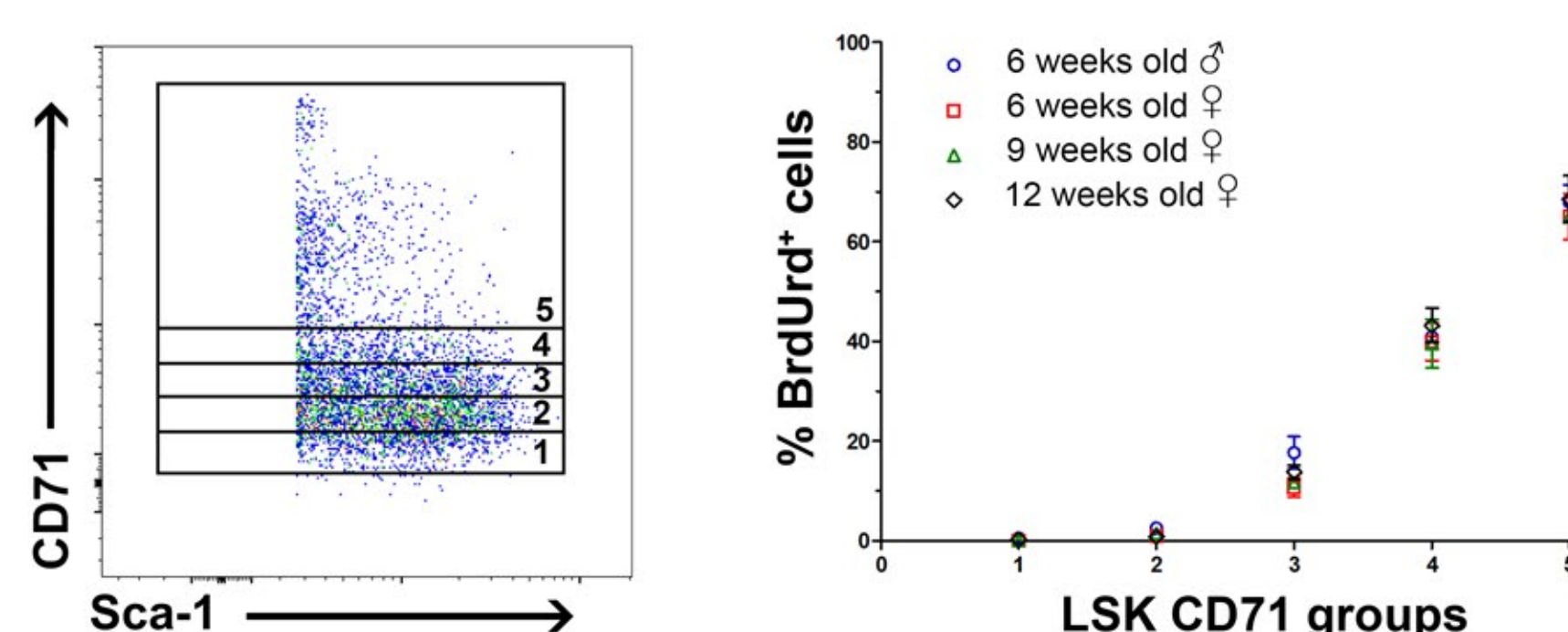
	BM cells/femur (x10 <sup>6</sup> )	LK cells/femur (x10 <sup>2</sup> )	LSK cells/femur (x10 <sup>3</sup> )	LS-K cells/femur (x10 <sup>3</sup> )
Males	26.51 ± 4.22	559.4 ± 153.8	63.3 ± 17.7	478.8 ± 153.3
Females	24.47 ± 1.56	504.9 ± 183.1	46.9 ± 9.5	404.0 ± 160.0

### 2. The CD71 expression level correlates tightly with the proliferation rate of immature haematopoietic cells

LS-K cells

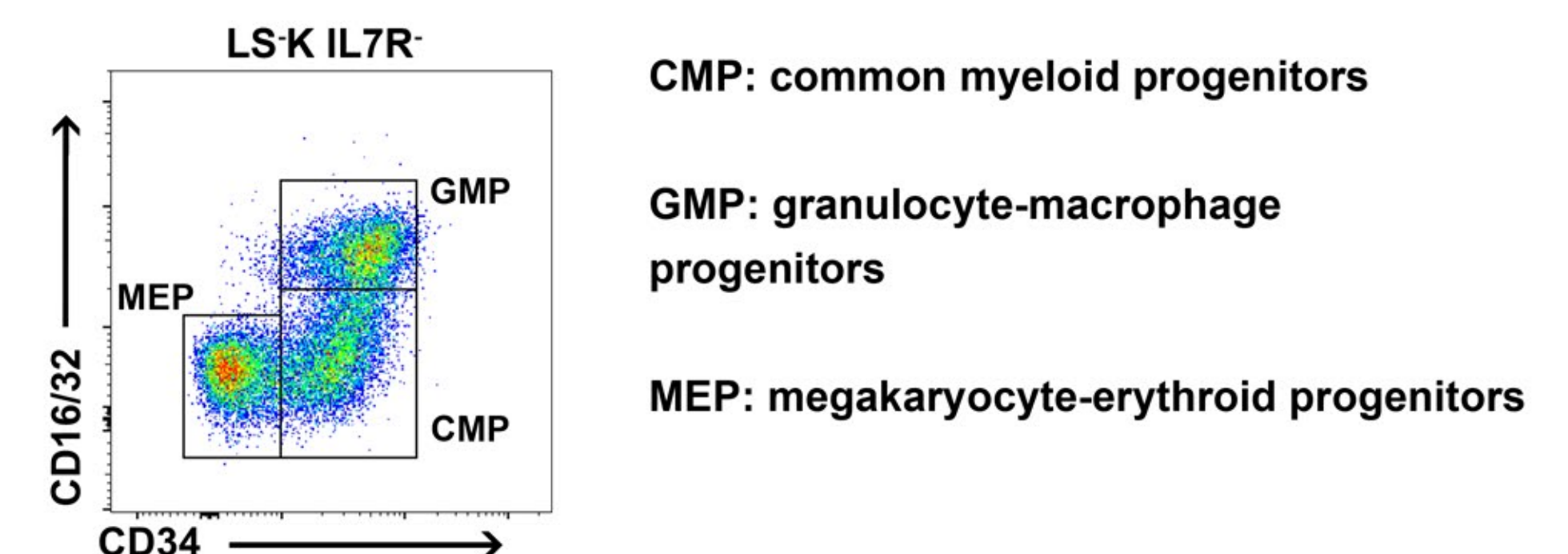


LSK cells

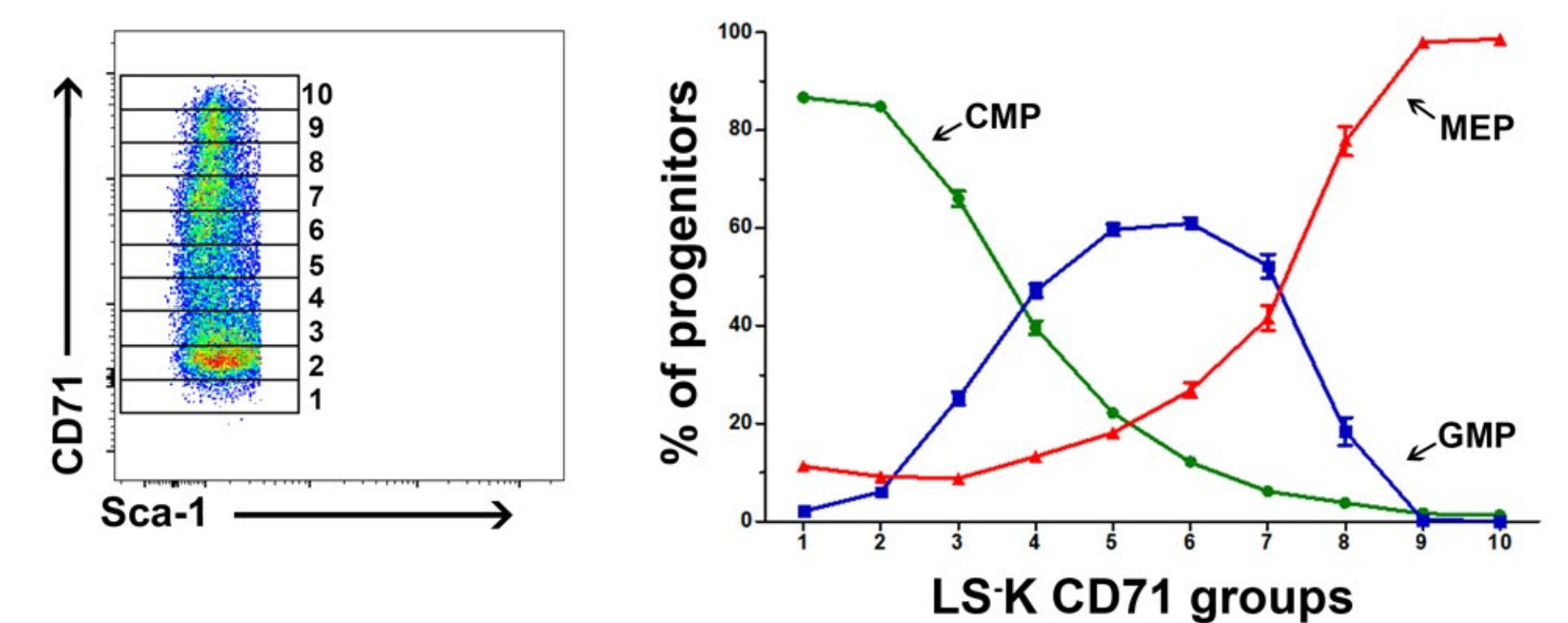


### 3. The CD71 expression level increases not only in the megakaryocyte-erythroid progenitors (MEP), but also in granulocyte-macrophage progenitors (GMP)

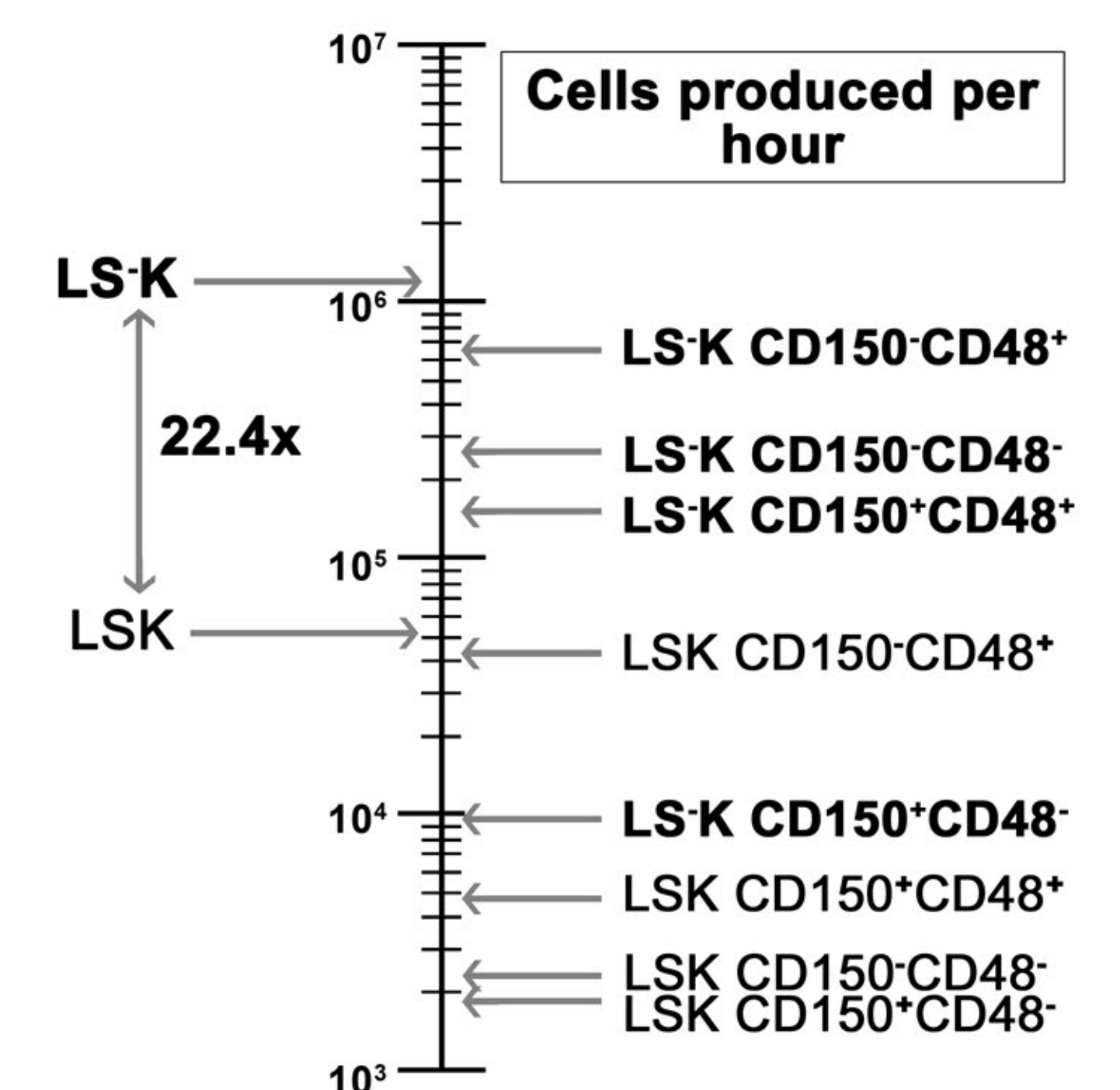
Example of the myeloid progenitor gating strategy



Myeloid progenitor percentage in LS-K groups with various CD71 intensities



### 4. The number of cells generated by various types of immature haematopoietic cells (in the whole bone marrow)



## Acknowledgement

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