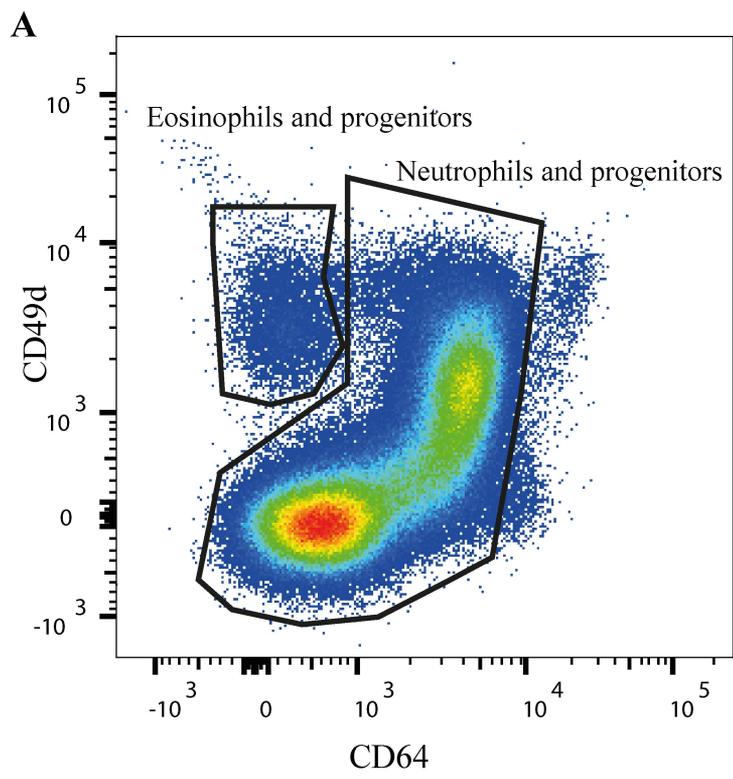


**Characterization of the phenotype of human eosinophils and their progenitors in the bone marrow of healthy individuals**

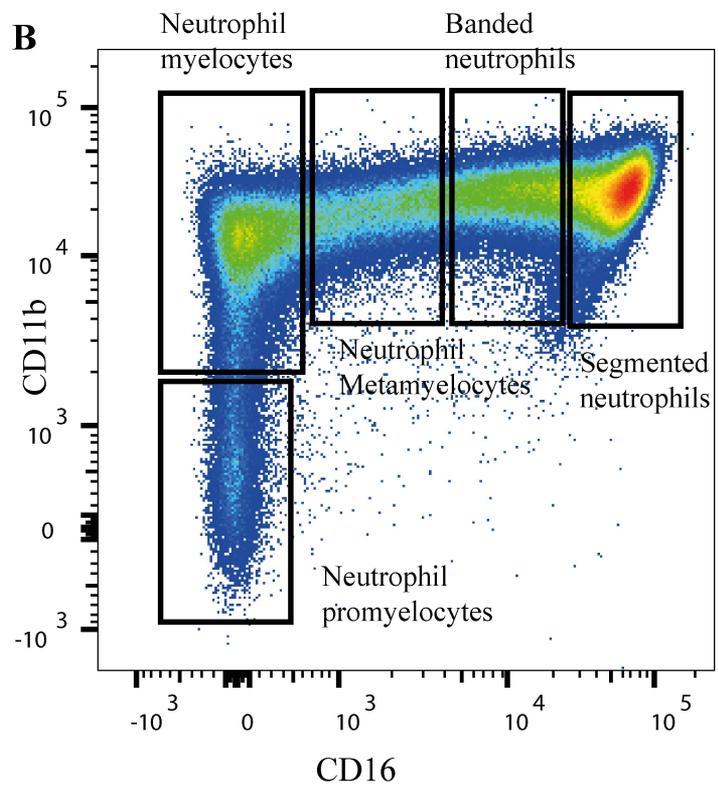
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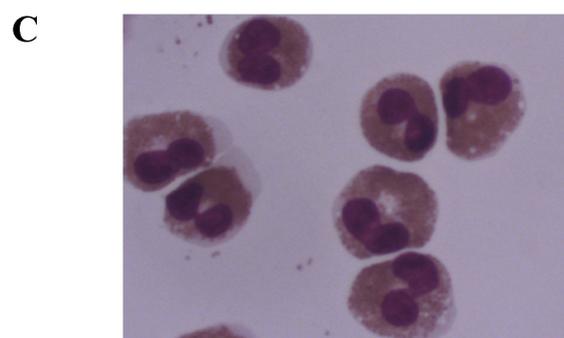
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doi:10.3324/haematol.2019.219048*



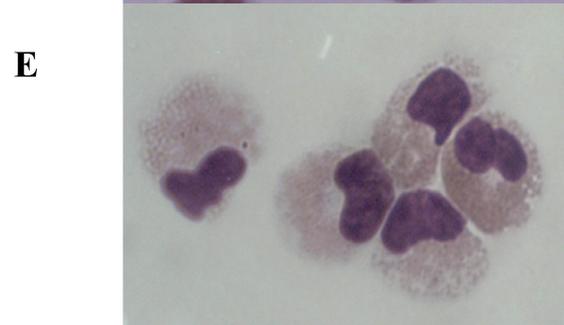
Eosinophil lineage



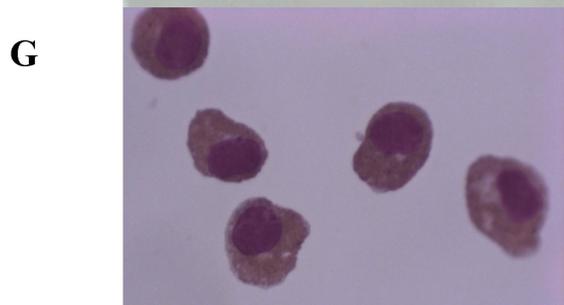
Neutrophil lineage



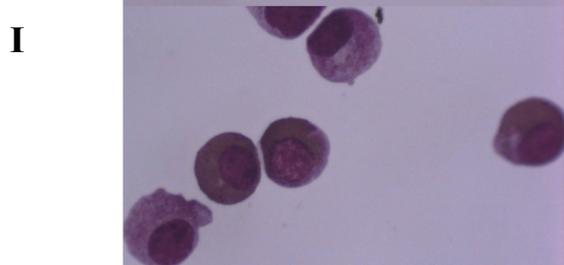
Mature



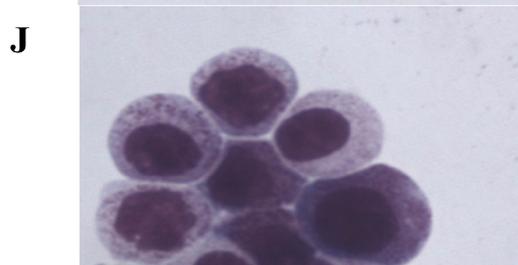
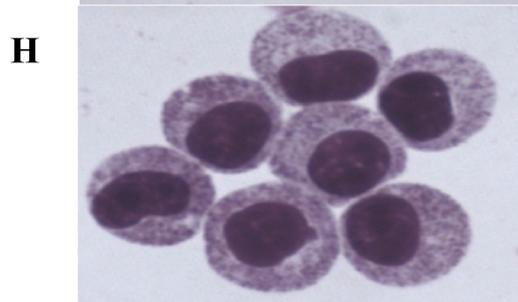
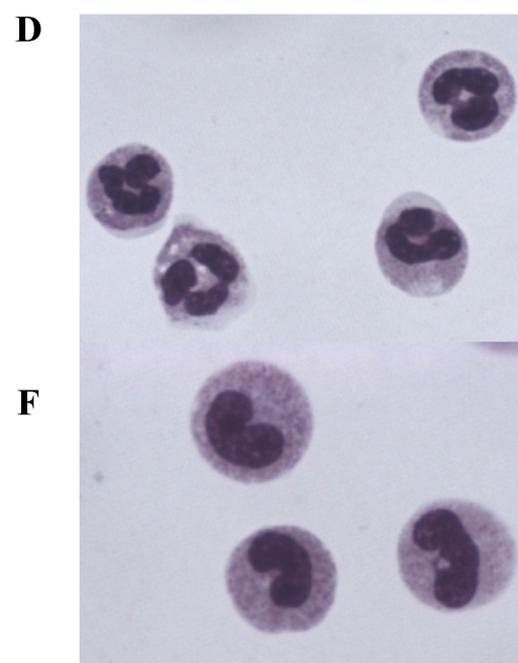
Metamyelocytes



Myelocytes



Promyelocytes

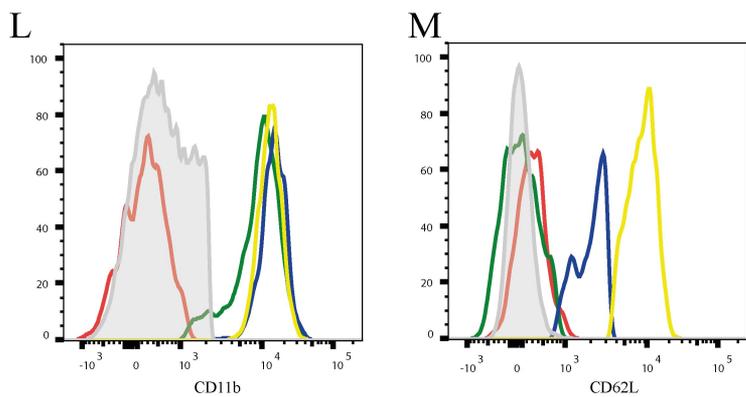
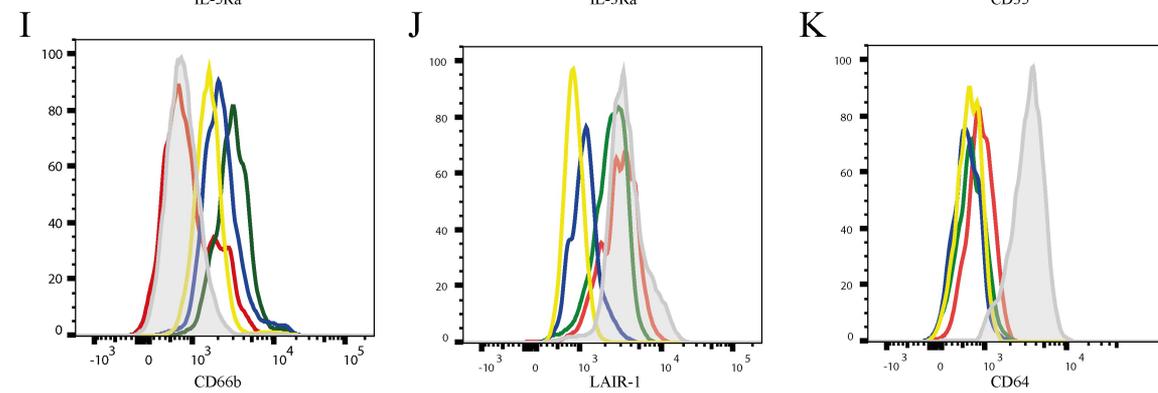
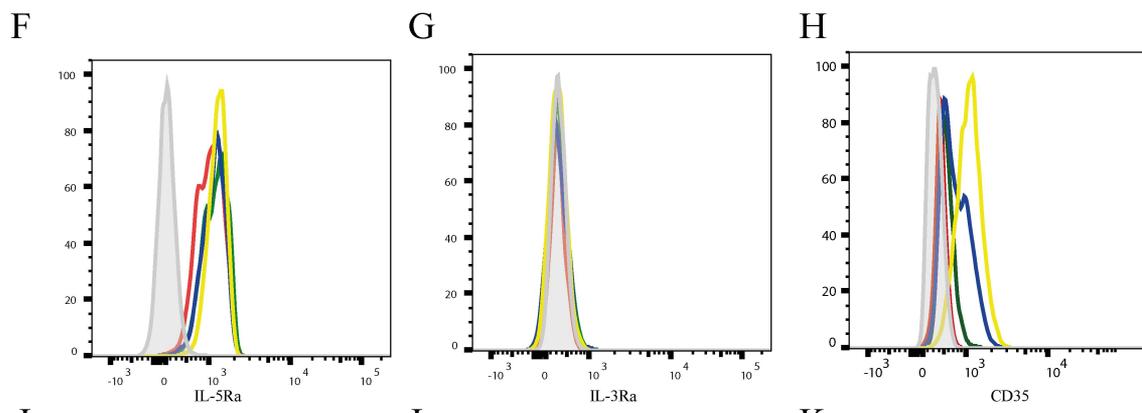
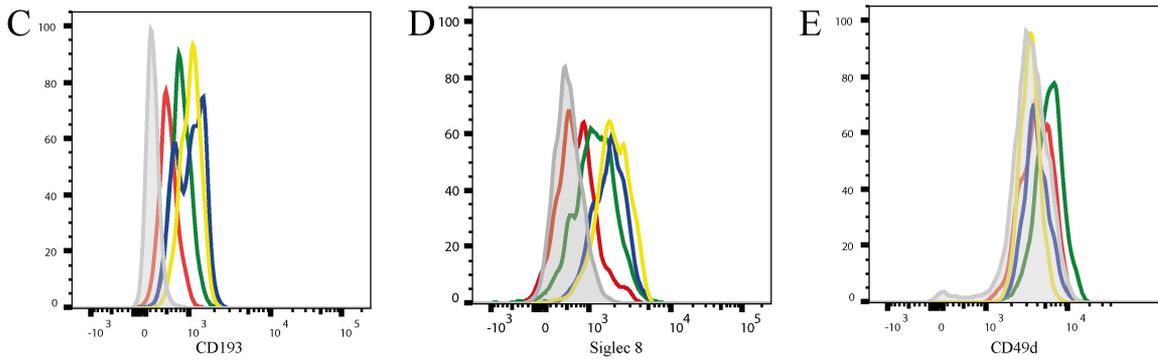
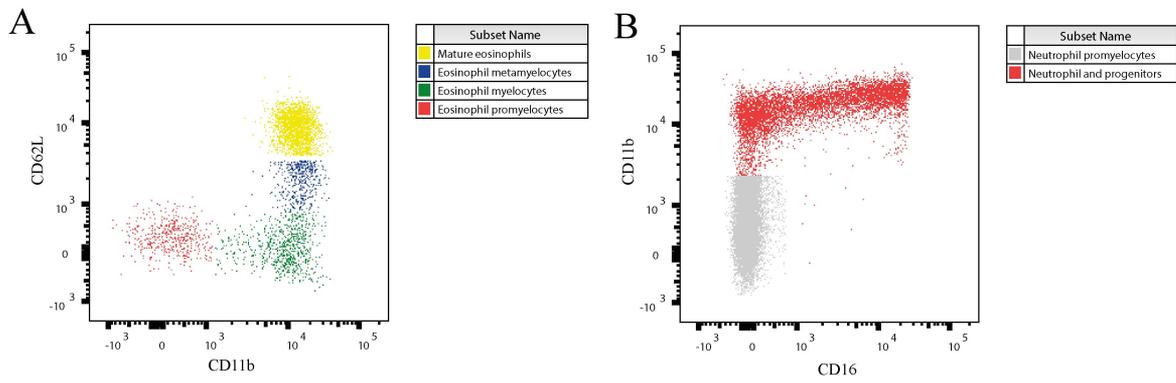


**Supplementary Figure 1. Alternative gating of eosinophil and neutrophils and their progenitors in the bone marrow.**

**A.** An example of gating eosinophils and neutrophils and their progenitors is shown. This plot was obtained after gating cells subsequently for singlets, excluding debris, high SSC and excluding CD64<sup>++</sup> like is shown in Figure 1. However CD64 and CD49d is now used instead of CD193 to distinguish both neutrophil and eosinophil lineages.

**B.** After gating all neutrophils and their progenitors as is shown in Supplementary Figure 1A, neutrophil precursors are gated like described before.<sup>6</sup> Neutrophilic promyelocytes are CD16<sup>-</sup> and CD11b<sup>-</sup>, neutrophilic myelocytes are CD16<sup>-</sup> and CD11b<sup>+</sup>, neutrophil metamyelocytes are CD16<sup>dim</sup> and CD11b<sup>+</sup>, neutrophil bandeds are CD16<sup>+</sup> and CD11b<sup>+</sup> and mature neutrophils are CD16<sup>++</sup> and CD11b<sup>+</sup>.

**C-J.** Representative cytopins of sorted neutrophilic and eosinophilic precursors and mature cells are shown after staining with May-Grünwald-Giemsa (Objective used: 100x, Axioskop 40, Light microscopy, Carl Zeiss, Goettingen, Germany). The distinction between eosinophilic promyelocytes (C) and myelocytes (E) and neutrophilic promyelocytes (D) and myelocytes (F) can be clearly seen as eosinophilic precursors contain the for eosinophils specific (dark) orange granules that are lacking in the neutrophilic precursors.



**Supplementary Figure 2. Representative histograms for all measured expression markers on the different eosinophil maturation stages and neutrophilic promyelocytes.**

**A-B.** Eosinophils and their progenitors are gated like shown in Figure 1 and neutrophilic promyelocytes are gated like shown in Supplementry Figure 1.

Yellow histograms depict mature eosinophils, blue depict eosinophilic metamyelocytes, green depict eosinophilic myelocytes, red depict eosinophilic promyelocytes and grey

depictneutrophilic promyelocytes. **C.** CCR3 (CD193), **D.** Siglec-8, **E.** CD49d, **F.** IL-5R $\alpha$  (CD125), **G.** IL-3R $\alpha$  (CD123), **H.** CR1 (CD35), **I.** CEACAM-8 (CD66b), **J.** LAIR1 (CD305),

**K.** Fc $\gamma$ RI (CD64), **L.** MAC-1 (CD11b), **M.** L-Selectin (CD62L).