



70185, Sweden.
Email: josefin.sundh@oru.se

ORCID

Josefin Sundh  <https://orcid.org/0000-0003-1926-8464>

REFERENCES

1. Asthma Gf. 2017 GINA Report, Global Strategy for Asthma Management and Prevention.
2. Nathan RA, Sorkness CA, Kosinski M, et al. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004;113(1):59-65.
3. Kämpfe M, Lisspers K, Ställberg B, Sundh J, Montgomery S, Janson C. Determinants of uncontrolled asthma in a Swedish asthma population: cross-sectional observational study. *Eur Clin Respir J* 2014;1(1):24109.
4. Stanford RH, Gilsenan AW, Ziemiecki R, Zhou X, Lincourt WR, Ortega H. Predictors of uncontrolled asthma in adult and pediatric patients: analysis of the asthma control characteristics and prevalence survey studies (ACCESS). *J Asthma* 2010;47(3):257-262.
5. Panek M, Mokros Ł, Pietras T, Kuna P. The epidemiology of asthma and its comorbidities in Poland-health problems of patients with severe asthma as evidenced in the province of Lodz. *Respir Med* 2016;112:31-38.
6. Katsura H, Yamada K, Kida K. Both generic and disease specific health-related quality of life are deteriorated in patients with underweight COPD. *Respir Med* 2005;99(5):624-630.
7. Sundh J, Ställberg B, Lisspers K, Montgomery SM, Janson C. Comorbidity, body mass index and quality of life in COPD using the clinical COPD questionnaire. *COPD* 2011;8(3):173-181.
8. Bousquet J, Khaltaev N, Cruz AA, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the world health organization, GA(2)LEN and AllerGen). *Allergy* 2008;63(Suppl 86):8-160.
9. Gaugris S, Sazonov-Kocevar V, Thomas M. Burden of concomitant allergic rhinitis in adults with asthma. *J Asthma* 2006;43(1):1-7.
10. Clatworthy J, Price D, Ryan D, Haughney J, Horne R. The value of self-report assessment of adherence, rhinitis and smoking in relation to asthma control. *Prim Care Respir J* 2009;18(4):300-305.
11. Lisspers K, Janson C, Larsson K, et al. Comorbidity, disease burden and mortality across age groups in a Swedish primary care asthma population: An epidemiological register study (PACEHR). *Respir Med* 2018;136:15-20.

DOI: 10.1111/all.13981

CD203c distinguishes the erythroid and mast cell-basophil differentiation trajectories among human FcεRI⁺ bone marrow progenitors

To the Editor,

IgE molecules that bind their specific antigen crosslink FcεRI receptors present on mast cells and basophils. Downstream signaling results in cell activation and subsequent release of diverse compounds that exhibit potential to trigger allergic symptoms. Although mature FcεRI⁺ cells have been extensively studied, less is known about the FcεRI⁺ progenitors and their differentiation capacity.¹ Here, we analyzed the FcεRI⁺ progenitor population from human bone marrow with multicolor flow cytometry and fate assays. The results revealed distinct subpopulations of FcεRI⁺ progenitors, all showing capacity to form mast cells and basophil-like cells but not granulocytes or monocytes. The CD203c⁻ subsets displayed erythroid potential, whereas the CD203c⁺ subset did not, altogether providing early evidence for a common mast cell-basophil-erythroid differentiation trajectory in human, distinct from the granulocyte-monocyte axis.

The CD34⁺ CD117⁺ FcεRI⁺ phenotype identifies the human mast cell progenitor population in blood.² Other characteristics include expression of the IL-3 receptor and the absence of CD45RA, positioning the cells among common myeloid progenitors (CMPs) when analyzing the progenitors with flow cytometry.^{2,3} In contrast to blood, we recently demonstrated that CMPs^{FcεRI+} in bone marrow do not exclusively form CD117^{hi} mast cells.⁴ This observation

warranted further characterization of the bone marrow CMPs^{FcεRI+} phenotype and cell-forming potential.

Morphologic assessment following cell sorting and May-Grünwald Giemsa stain revealed that the CMP^{FcεRI+} population was heterogeneous (Figure 1A,C; see Methods S1 for materials and methods). Some cells exhibited a blast-like phenotype with little cytoplasm, whereas others displayed numerous metachromatic granules (Figure 1C). The cell heterogeneity prompted us to design a multicolor flow cytometry panel that further characterizes the progenitors. Analyzing the CD203c and integrin β7 expression patterns revealed subpopulations of CMP^{FcεRI+} cells (Figure S1). Three CMP^{FcεRI+} subpopulations—CD203c⁺, integrin β7⁺ CD203c⁻, and integrin β7⁻ CD203c⁻ cells—exhibited distinct protein expression profiles and were studied further (Figure 1B,D). These three populations, along with CMPs^{FcεRI-} and granulocyte/monocyte progenitors (GMPs), were sorted and cultured to investigate their cell-forming potential (Figure 2A). The five bone marrow progenitor populations were first cultured with IL-3 and IL-6. These conditions support mast cell progenitors from peripheral blood to form CD117^{hi} FcεRI⁺ mast cells.³ We analyzed the cultured bone marrow cells with a flow cytometry panel that distinguished three subsets of FcεRI⁺ cells separated based on the CD117 expression, CD235a⁺ erythroid cells, and

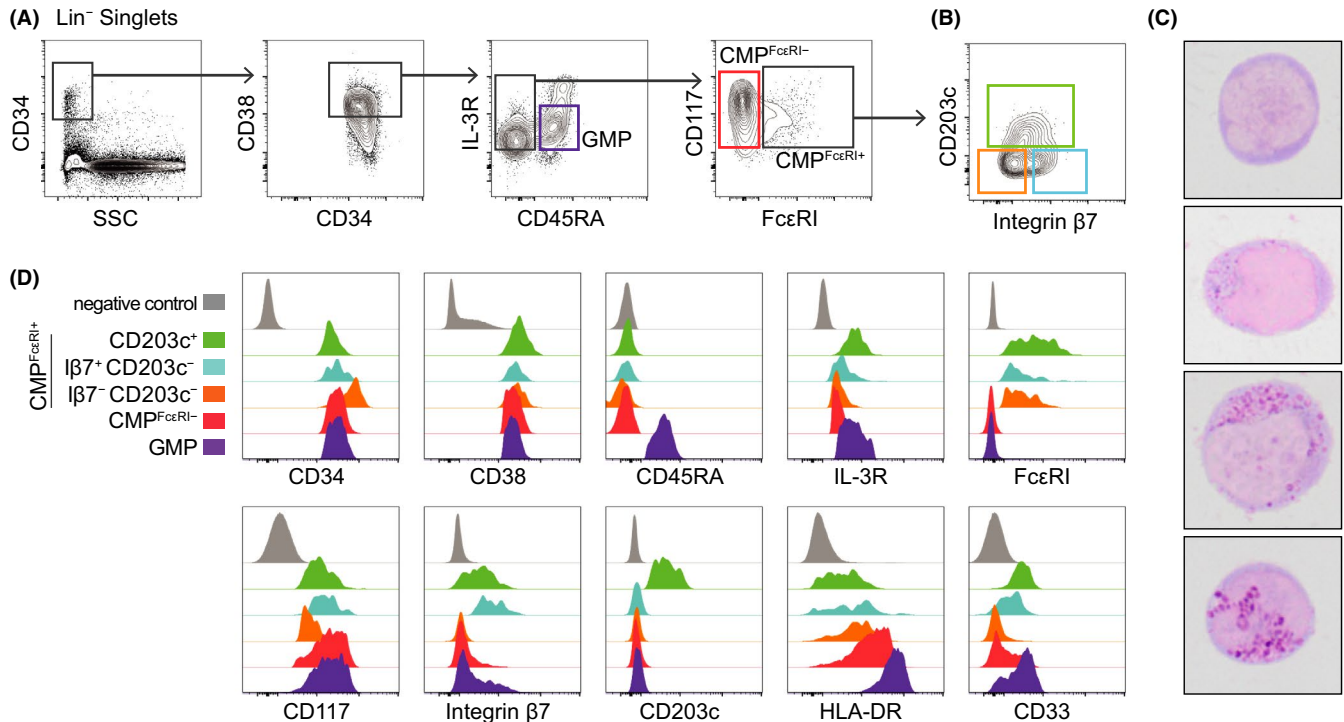


FIGURE 1 Bone marrow CMPs^{FcεRI+} comprises three distinct progenitor populations. A, Flow cytometry analysis of human bone marrow cells. B, CD203c and integrin β7 distinguishes three distinct CMP^{FcεRI+} subpopulations. C, May-Grünwald Giemsa staining of CMPs^{FcεRI+}. D, Surface expression analysis of CD203c⁺ (green), Iβ7⁺ CD203c⁻ (blue), and Iβ7⁻ CD203c⁻ (orange) CMP^{FcεRI+} compared with CMPs^{FcεRI-} (red), and GMPs (purple). Negative controls (gray) represent internal control populations from the sample that are negative for the marker of interest. One representative bone marrow sample is shown

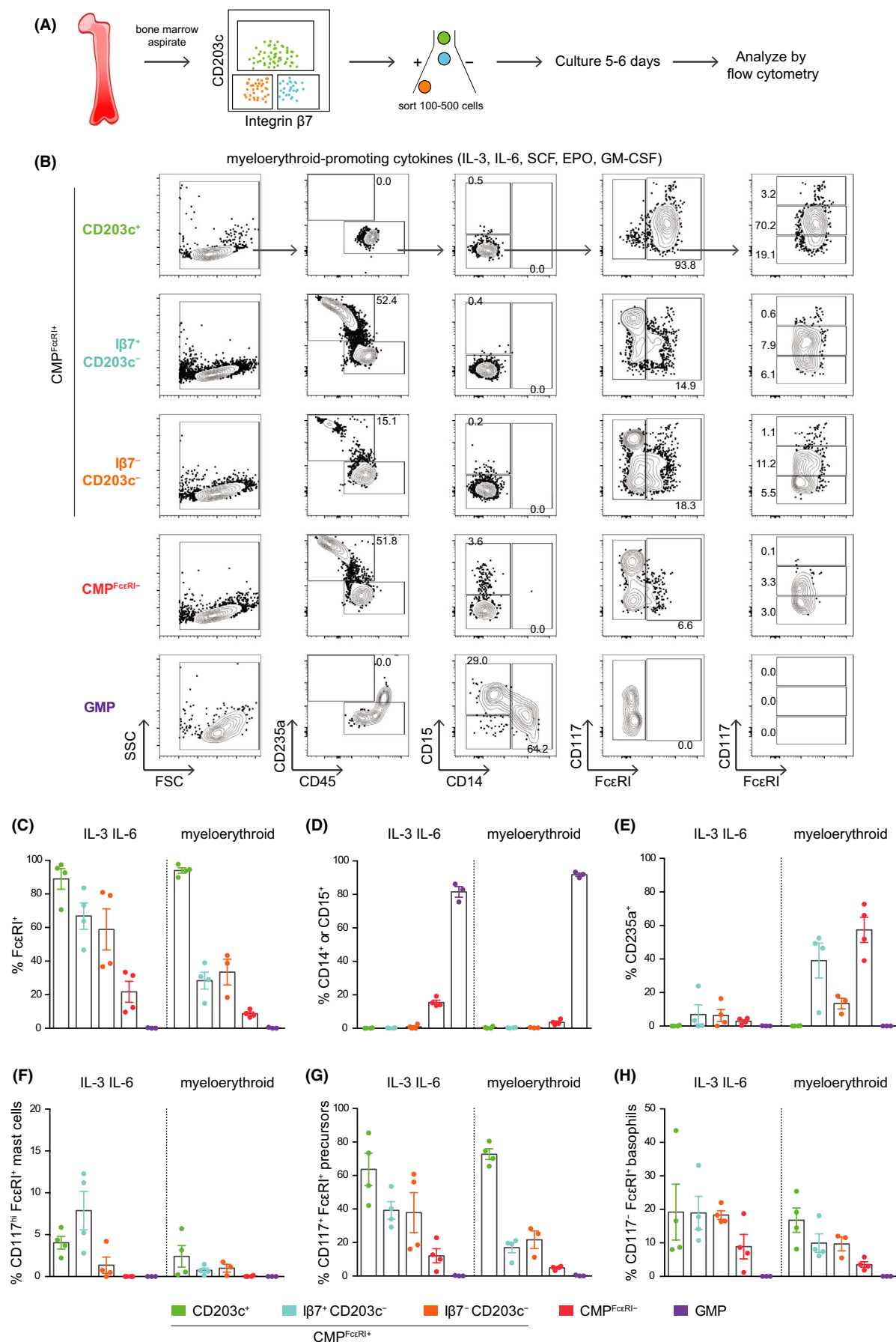
CD14⁺ or CD15⁺ granulocyte-monocyte output (Figure 2B visualizes the gating strategy). The CMP^{FcεRI+} largely maintained their FcεRI⁺ phenotype during culture (Figure 2C). None of the three CMP^{FcεRI+} subpopulations produced pure populations of CD117^{hi} mast cells or CD117⁻ basophil-like cells, but instead constituted a mix of cells with variable CD117 expression (Figure 2F-H).

The three CMP^{FcεRI+} populations did not display granulocyte-monocyte potential (Figure 2D). By contrast, culture of CMPs^{FcεRI-} and GMPs resulted in substantial granulocyte-monocyte output. The CMP^{FcεRI-} population included progenitors with potential to upregulate FcεRI expression, suggesting that this population contains precursors of CMPs^{FcεRI+}. GMPs did not produce FcεRI⁺ cells, indicating that this population lacks mast cell and basophil-forming capacity (Figure 2C). Bühring et al previously reported that the CD34⁺ CD203c⁺ progenitors exhibit mast cell-forming and high basophil-forming potential, agreeing with our results that the CD203c⁺ subset of the CMPs^{FcεRI+} form these cell types.⁵ However, the CD34⁺ CD203c⁺ progenitors cultured in the study by Bühring et al exhibit residual granulocyte-monocyte-forming

potential and were proposed to contain multipotent progenitors.⁵ We sorted and cultured CD203c⁺ cells from the CMP^{FcεRI+} fraction, constituting cells that likely are more differentiated than CD34⁺ CD203c⁺ progenitors in general, which could explain the observation that CD203c⁺ CMP^{FcεRI+} cells lack granulocyte-monocyte potential.

No or few erythroid cells developed from any of the starting populations in the IL-3 and IL-6 conditions (Figure 2E), which is in agreement with lack of sufficient stimulus for erythroid development. Through a combined single-cell RNA sequencing and cell culture-based approach, Tusi et al⁶ recently identified progenitors with combined erythroid and mast cell-basophil output in mouse bone marrow. Hence, we investigate whether the CMP^{FcεRI+} populations exhibited erythroid potential. We cultured the CMPs^{FcεRI+} with the myeloerythroid-promoting cytokines IL-3, IL-6, SCF, EPO, and GM-CSF. Cell culture assays in these conditions revealed that all CMP^{FcεRI+} subpopulations still maintained FcεRI⁺ cells (Figure 2B-C). Notably, we observed clear erythroid output when culturing the two CD203c⁻ subpopulations (Figure 2E). A population of FcεRI⁻ CD117^{hi} cells was also present in cultures of the

FIGURE 2 Cell fate assays reveal the cell-forming potential of the CMP^{FcεRI+} populations. A, Schematic diagram indicating the cell fate assay methodology. B, Gating strategy of the cultured cells. Cells cultured for 5-6 d with the myeloerythroid-promoting cytokines IL-3, IL-6, SCF, EPO, and GM-CSF are shown. Percentages of (C) FcεRI⁺, (D) CD14⁺ or CD15⁺, and (E) CD235a⁺ (Glycophorin A⁺) cells after 5-6 d of culture. Percentages of (F) CD117^{hi} FcεRI⁺ mast cells, (G) CD117⁺ FcεRI⁺ precursors, and (H) CD117⁻ FcεRI⁺ basophils after 5-6 d of culture. Panels C-H display cells cultured with IL-3 and IL-6 (left) or the myeloerythroid-promoting cytokines IL-3, IL-6, SCF, EPO, GM-CSF (right). The cell fate assay was performed from 3-4 donors per population and condition as indicated



CD203c[−] subpopulations, cells with dim CD45 expression that likely constituted erythroid precursors (Figure S2A–B).

We cannot exclude that unipotent erythroid progenitors contaminated the CMP^{FcεRI+} sort gate. However, performing single-cell culture experiments to resolve this question is complicated by the poor proliferation capacity of human mast cell progenitors,² making it difficult to identify and characterize mixed colonies. Though, it is worth to point out that erythrocytes were not present in cultures derived from the CD203c⁺ subpopulation across cell fate assays of 4 donors, suggesting that CD203c upregulation is associated with loss of erythroid potential.

Progressive loss of proliferation capacity with mast cell differentiation may explain the relatively low frequency of mast cells derived from CD203c⁺ compared with integrin β7⁺ CD203c[−] CMP^{FcεRI+} cells in IL-3 and IL-6 conditions.

Resolving the differentiation trajectories from hematopoietic stem cells to FcεRI⁺ mast cells and basophils could significantly improve our understanding of, for example, IgE-mediated allergic diseases as well as the mast cell-driven disease systemic mastocytosis. The frequency of putative bone marrow mast cell progenitors was recently demonstrated to be elevated in systemic mastocytosis patients compared with healthy subjects.⁷ Thus, the establishment of flow cytometry gating strategies for the identification of progenitors with basophil and mast cell-forming capacity can help us to understand why mature cells accumulate in disease. Taken together, the results presented here provide early evidence that the mast cell and basophil differentiation trajectories are closely linked to the erythropoiesis in human. Further studies on the topic are warranted, and fate assays of individual cells coupled with single-cell transcriptomics represent a promising way forward.

ACKNOWLEDGMENTS

This study was supported by Vetenskapsrådet, Cancerfonden, Radiumhemets Forskningsfonder, Tore Nilsons Stiftelse för Medicinsk Forskning, Magnus Bergvalls Stiftelse, and Karolinska Institutet. Furthermore, the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and the Karolinska Institutet, and ALF-funding from Uppsala University Hospital supported the study. CW was supported by a China Scholarship Council grant.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

Jennine Grootens¹ 

Johanna S. Ungerstedt^{2,3}

Chenyang Wu¹

Kerstin Hamberg Levedahl⁴

Gunnar Nilsson^{1,5} 

Joakim S. Dahlin¹ 

¹Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden

²Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden

³Hematology Center, Karolinska University Hospital, Stockholm, Sweden

⁴Division of Hematology, Uppsala University Hospital, Uppsala, Sweden

⁵Department of Medical Sciences, Uppsala University, Uppsala, Sweden

Correspondence

Joakim Dahlin and Gunnar Nilsson, NKS BioClinicum, J7:30, Visionsgatan 4, Solna 171 64, Sweden.

Emails: joakim.dahlin@ki.se; gunnar.p.nilsson@ki.se

ORCID

Jennine Grootens  <https://orcid.org/0000-0003-4469-8589>

Gunnar Nilsson  <https://orcid.org/0000-0001-6795-5512>

Joakim S. Dahlin  <https://orcid.org/0000-0003-3007-9875>

REFERENCES

- Grootens J, Ungerstedt JS, Nilsson G, Dahlin JS. Deciphering the differentiation trajectory from hematopoietic stem cells to mast cells. *Blood Adv*. 2018;2:2273–2281.
- Dahlin JS, Malinovsky A, Ohrvik H, et al. Lin[−] CD34hi CD117int/hi FcεRI⁺ cells in human blood constitute a rare population of mast cell progenitors. *Blood*. 2016;127:383–391.
- Dahlin JS, Ekoff M, Grootens J, et al. KIT signaling is dispensable for human mast cell progenitor development. *Blood*. 2017;130:1785–1794.
- Grootens J, Ungerstedt JS, Ekoff M, et al. Single-cell analysis reveals the KIT D816V mutation in haematopoietic stem and progenitor cells in systemic mastocytosis. *EBioMedicine*. 2019;43:150–158.
- Buhring HJ, Simmons PJ, Pudney M, et al. The monoclonal antibody 97A6 defines a novel surface antigen expressed on human basophils and their multipotent and unipotent progenitors. *Blood*. 1999;94:2343–2356.
- Tusi BK, Wolock SL, Weinreb C, et al. Population snapshots predict early haematopoietic and erythroid hierarchies. *Nature*. 2018;555:54–60.
- Mayado A, Teodosio C, Dasilva-Freire N, et al. Characterization of CD34(+) hematopoietic cells in systemic mastocytosis: Potential role in disease dissemination. *Allergy*. 2018;73:1294–1304.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

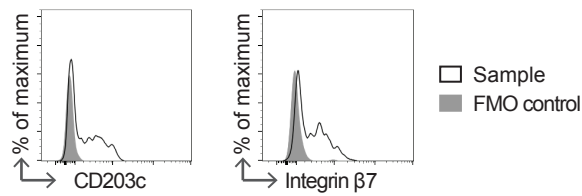
Supplementary figure legends

Supplementary Figure 1. CD203c and integrin $\beta 7$ expression analysis of CMP^{Fc ϵ RI⁺} cells.

Flow cytometry analysis of CMP^{Fc ϵ RI⁺} cells. Fluorescent minus one (FMO) controls are shown.

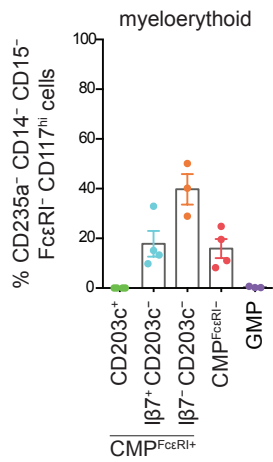
Supplementary Figure 2. Flow cytometry analysis demonstrates the absence of CD235a⁻ CD14⁻ CD15⁻ Fc ϵ RI⁻ CD117^{hi} cells after culturing CD203c⁺ CMPs^{Fc ϵ RI⁺}. (A) Percentages of CD235a⁻ CD14⁻ CD15⁻ Fc ϵ RI⁻ CD117^{hi} cells after 5-6 days in culture with myeloerythroid-promoting cytokines. (B) Flow cytometry profile of the CD235a⁻ CD14⁻ CD15⁻ Fc ϵ RI⁻ CD117^{hi} population compared with all live cells cultured in the same condition. CD235a⁻ CD14⁻ CD15⁻ Fc ϵ RI⁻ CD117^{hi} cells were absent when CD203c⁺ CMPs^{Fc ϵ RI⁺} and GMPs were cultured.

Supplementary figure 1

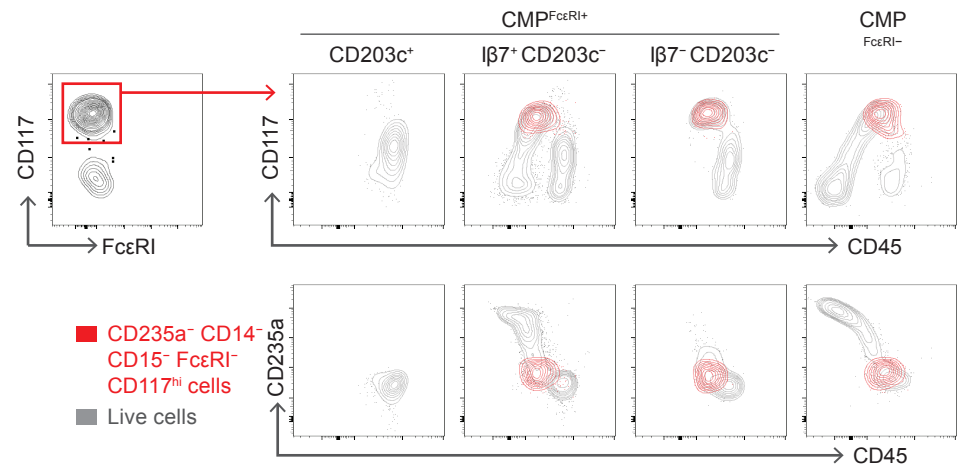


Supplementary figure 2

A



B



Supplementary materials and methods

Sample preparation

Bone marrow aspirates were collected from healthy volunteers or patients under evaluation for systemic mastocytosis. The Stockholm and Uppsala Regional Ethics committee approved the study. We obtained informed consent from each healthy volunteer and patient, and followed the World Medical Association Declaration of Helsinki. Samples were processed at the day of collection by red blood cell lysis using PharmLyse buffer (BD Biosciences, Franklin Lakes, NJ) and washed with PBS containing 2% fetal calf serum (Thermo Fisher Scientific, Waltham, MA).

Fluorescent activated cell sorting

Cells were stained in Brilliant Stain Buffer (BD Biosciences) with a combination of fluorophore-conjugated antibodies: CD3 (SK7), CD14 (M5E2), and CD19 (HIB19) as lineage markers, and CD33 (WM53), CD34 (581), CD38 (HB7), CD45RA (HI100), CD117 (104D2), CD123/IL-3R (6H6), CD203c (E-NPP3), FcεRI (CRA-1), HLA-DR (L243), and Integrin β7 (FIB504) (all from BD Biosciences and Biolegend, San Diego, CA). Cells were sorted following a two-step sorting protocol using the FACS Aria Fusion (BD Biosciences). In the first round, cells were sorted into sterile-filtered PBS containing 2% fetal calf serum using a yield precision mode, and the second sorting step using the purity precision mode directly into medium and transferred to culture plates. Post-sort analysis of 20-100 cells sorted into sterile-filtered PBS with 2% fetal calf serum confirmed the purity. FlowJo software version 10 (TreeStar, Ashland, OR) was used for data analysis.

Cell culture

Cells were cultured in Gibco StemPro-34 serum free medium (Thermo Fisher Scientific) with 2 mM L-glutamine (Sigma-Aldrich, St. Louis, MO), 100 U/mL penicillin (Sigma-Aldrich), and 0.1 mg/mL streptomycin (Sigma-Aldrich). The medium was supplemented with recombinant cytokines as indicated in the figures and included 10 ng/mL IL-3 (PeproTech, Rocky Hill, NJ), 10 ng/mL IL-6 (PeproTech), 5 ng/mL SCF (kindly provided by Swedish Orphan Biovitrum, Stockholm, Sweden), 10 ng/mL GM-SCF (PeproTech), 1 IU/mL EPO (Janssen-Cilag AB, Solna, Sweden). The cells were

analyzed with the LSRFortessa flow cytometer (BD Biosciences) following staining with a panel of antibodies including CD14 (M5E2), CD15 (W6D3), CD45 (HI30), CD117 (104D2), CD235a/Glycophorin A (HIR-2), FcεRI (CRA-1) (all from BD Biosciences and Biolegend). 7-AAD (Thermo Fisher Scientific) was used to select for live cells. May-Grünwald Giemsa staining (Sigma-Aldrich) visualized the cells of the cytospin preparations.