Lactotransferrin-Cre reporter mice trace neutrophils, monocytes/macrophages and distinct subtypes of dendritic cells

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Supplementary Data

Supplementary Methods:

Bone marrow transplantation
TdRFP⁺ cells from the BM of three Ltf-reporter⁺ mice were double-sorted via FACS AriaIII and intravenously injected into lethally irradiated (10 Gy) B6129F1 recipient mice (4 x 10⁵ cells/mouse). In parallel, tdRFP⁻ cells of the same pool were injected into control mice. After four months, surviving mice were sacrificed and hematopoietic organs (BM, PB, spleen) analysed by FACS for tdRFP⁺ cells.

Cell and tissue preparation
Femurs and tibiae were isolated from mice and cut at both ends. Bone marrow cells were flushed out with 1x PBS using a 22-gauge needle. A plunger from a 20 ml syringe was used to crush spleens through a 70µm cell strainer into 50ml Falcon tubes. Peripheral blood erythrocytes were lysed in 1xEry-Lysis buffer. Nucleated cells were collected by centrifugation and resuspended in 1xPBS. For tissue mRNA analysis, organs were excised, cut into 2mm x 2mm pieces and immediately frozen in liquid nitrogen.

Cell staining and light microscopy
TdRFP⁺ subpopulations of granulocytes were FACS-purified using cell-surface marker combinations according to Table S1. Cells were centrifuged on a glass slide in a cytopsin centrifuge, fixed and stained by Diff-Quik® staining (Medion Diagnostics) in accordance with the manufacturer’s instructions. Pictures were taken with a Motic Type 102M light microscope (magnifications 40x and 100x) and Motic Images Plus 2.0 software. Grouped images in figure 5A and S4 were obtained from individual micrographs taken from the same
specimen as described previously (52). No adjustments of brightness, contrast, or color balance have been performed. Images were processed with Adobe Photoshop CS5 and grouped with Adobe Illustrator CS5.

**qPCR analysis**

Total RNA was extracted using the TriFast reagent (peqGOLD) according to the manufacturer’s protocol. Tissues were minced using the ULTRA-TURRAX homogenizer. Samples were treated with DNaseI (RQ1) to remove traces of DNA. Total RNA (900ng-1µg) was reverse-transcribed using the iScript First Strand cDNA Synthesis Kit (Biorad) and the Q-PCRs was performed in triplicates on a Mastercycler realplex (Eppendorf) using following conditions: 4mM MgCl2, 200µM dNTPs (each), 300µM primers, 100nM labeled probe and 1U HotFire DNA polymerase. Following primers were used for Q-PCR analysis: Ube2d2 (forward: AGGTCTCTGGAGATGATATGT, reverse: TTGGGAAATGAATTGTCAAGAAA, FAM: CCAATGCAGCCCTATCAGGGTG), iCre (forward: TACCTGTTCGGGGGTCA, reverse: CACCATAAGTCGGCGGTGG, FAM: TCCCAACTGTCCACCCGGGC), Ltf (forward: TGCTTGCTAACCAGACAGA, reverse: ACCAATACACAGGGGACAGA, FAM: TGCCCCTGGTGCTGACCCCA).

**Colony-forming unit (CFU) assays**

Bone marrow cells of 5 individual mice were pooled and sorted either for tdRFP+ and tdRFP- cells. Single cell suspensions were plated at 4x10^5 cells in growth factor-free methylcellulose (MethoCult 3231, Stem Cell Technologies) supplemented either with GM-CSF (3ng/ml) or a combination of SCF (50ng/ml), mIL-3 (2ng/ml), mIL-6 (5ng/ml), GM-CSF (3ng/ml), IgF-I (40ng/ml), Tpo (20ng/ml) and Epo (1U/ml). Colonies were counted by light microscopy (Leica Fluovert microscope, 4 x magnification).
### Supplementary Table:

**Table S1**

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<th>population</th>
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<th>additional markers</th>
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**Table S1** | Phenotypic characteristics of the populations evaluated in this study.
Supplementary Figures:

Figure S1

A

$i$Cre tissue expression in Ltf-Cre mice

B

gated: lin$^-$/IL7Rc$^+$ / c-kit$^+$ / Sca-1$^+$

C

% of total cells

D

wildtype

Ltf-Cre

CD19

CD3e

Gr1$^+$

CD11b

CD11b

CD11b
Figure S1 | Effects caused by transgenic Cre-expression in cells of hematopoietic and non-hematopoietic organs.

A, qPCR-analysis showing iCre-expression in distinct tissues of Ltf-Cre<sup>+</sup> mice. Ltf mRNA expression is shown relative to the house-keeping gene Ube2d2. Control cDNA was isolated from a peripheral blood sample after lysis of erythrocytes. B, FACS-plots indicating the relative percentages of CMPs, GMPs and MEPs in the BM of wildtype and Ltf-Cre<sup>+</sup> littermates. One representative FACS-plot out of three is depicted. No significant differences were observed (n=12). C, Distribution of CMPs, GMPs and MEPs as indicated by the percentage of total cells in the BMs of wildtype and Ltf-Cre<sup>+</sup> littermates (n=12). No significant differences were observed. D, Normal distribution of peripheral blood lymphoid cells (CD19<sup>+</sup>/CD3ε<sup>+</sup> - B/T cells) and myeloid cells (Gr-1<sup>+</sup>/CD11b<sup>+</sup> - granulocytes/monocytes/macrophages) between wildtype and Ltf-Cre<sup>+</sup> mice. One representative FACS-plot is indicated.
Figure S2 | Phenotypical analysis of myeloerythroid populations in Ltf-reporter\(^+\) mice.

**A**, Proportion of tdRFP\(^+\) GMPs as defined by gating on lin\(^-\)/c-kit\(^+\)/Sca-1\(^-\)/(KLS\(^-\))/CD41\(^-\)/CD150\(^-\)/FcyRII/III\(^+\) cells. **B**, gating strategy for cDCs: distinct subpopulations express Ltf-reporter. Representative Facs-plots are shown. **C**, gating strategy for pDCs: only a tiny population of CD11c\(^{int}\)/CD11b\(^+\)/B220\(^+\)/SiglecH\(^+\) pDCs express Ltf-reporter. Representative Facs-plots are shown. **D**, gating strategy for iKDCs: no Ltf-reporter expression was detectable. **E**, Mean percentages of tdRFP\(^+\) cells among cDCs (n=12) and pDCs (n=4) in the spleens of Ltf-reporter mice. Error bars indicate means ± SEM. **F**, Contribution of tdRFP\(^+\) cells to early dendritic lineage stages in the BM of Ltf-reporter mice. Boxes and whiskers indicate One-way Anova ± Tukey post-test within the populations (n=12).
Figure S3
corresponding to Figure 4
Figure S3 | *Ltf-reporter* BM cells have multilineage potential *in vitro.*

Representative examples of CFU-GEMM, CFU-GM, CFU-G, CFU-M, CFU-E, BFU-E, CFU-pro-B and CFU-Meg arising from *Ltf-reporter* BM cells are depicted.
Figure S4
corresponding to Figure 5

A

B
Figure S4 | Distinct stages of monocytic and eosinophilic development in populations II and IV.

A, Morphology of population II compartment was analyzed by Grunwald Giemsa stainings of cytospins from sorted cells. Representative cells from individual images of the same specimen were grouped with regard to their morphology as described previously (52). B, Morphology of population IV compartment was determined by Grunwald Giemsa stainings. Note that population IV was Ltf-reporter. Representative cell populations are depicted.
References: