

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

Boris Kovacic,<sup>1,2\*</sup> Andrea Hoelbl-Kovacic,<sup>3</sup> Katrin M. Fischhuber,<sup>1</sup> Nicole R. Leitner,<sup>1</sup> Dagmar Gotthardt,<sup>3</sup> Emilio Casanova,<sup>4,5</sup> Veronika Sexl,<sup>3</sup> and Mathias Müller<sup>1</sup>

<sup>1</sup>Institute of Animal Breeding and Genetics, University of Veterinary Medicine Vienna; <sup>2</sup>Research Institute of Molecular Pathology (I.M.P.), Vienna; <sup>3</sup>Institute of Pharmacology and Toxicology, University of Veterinary Medicine, Vienna; <sup>4</sup>Ludwig Boltzmann Institute for Cancer Research (LBI-CR), Vienna; and <sup>5</sup>Institute of Pharmacology, Center of Physiology and Pharmacology, Comprehensive Cancer Center, Medical University of Vienna, Austria

*\*present address: Institute of Medical Genetics, Medical University of Vienna, Austria*

---

©2014 Ferrata Storti Foundation. This is an open-access paper. doi:10.3324/haematol.2013.097154  
Manuscript received on August 23, 2013. Manuscript accepted on February 14, 2014.  
Correspondence: boris.kovacic@meduniwien.ac.at or mathias.mueller@vetmeduni.ac.at

## **Supplementary Data**

### **Supplementary Methods:**

#### **Bone marrow transplantation**

TdRFP<sup>+</sup> cells from the BM of three *Ltf*-reporter<sup>+</sup> mice were double-sorted via FACSAriaIII and intravenously injected into lethally irradiated (10 Gy) *B6129F1* recipient mice (4 x 10<sup>5</sup> cells/mouse). In parallel, tdRFP<sup>-</sup> 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<sup>+</sup> 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<sup>+</sup> subpopulations of granulocytes were FACS-purified using cell-surface marker combinations according to Table S1. Cells were centrifuged on a glass slide in a cytospin centrifuge, fixed and stained by Diff-Quik<sup>®</sup> 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 MgCl<sub>2</sub>, 200µM dNTPs (each), 300µM primers, 100nM labeled probe and 1U HotFire DNA polymerase. Following primers were used for Q-PCR analysis: Ube2d2 (forward: AGGTCCTGTTGGAGATGATATGT, reverse: TTGGGAAATGAATTGTCAAGAAA, FAM: CCAAATGACAGCCCCTATCAGGGTGG), iCre (forward: TACCTGTTCTGCCGGGTCA, reverse: CACCATAGATCAGGCGGTGG, FAM: TCCCAACTGTCCACCCGGGC), Ltf (forward: TGCTTGCTAACCAGACCAGA, reverse: ACCAATACACAGGGCACAGA, FAM: TGCCCCTGGTGCTGACCCCA).

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

Bone marrow cells of 5 individual mice were pooled and sorted either for tdRFP<sup>+</sup> and tdRFP<sup>-</sup> cells. Single cell suspensions were plated at 4x10<sup>5</sup> 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:

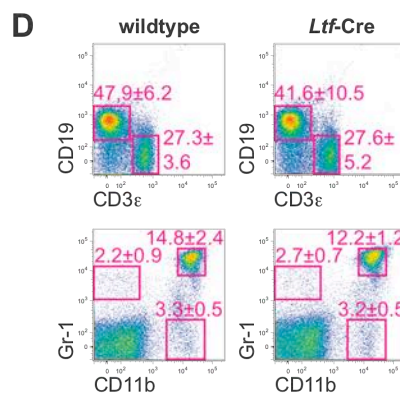
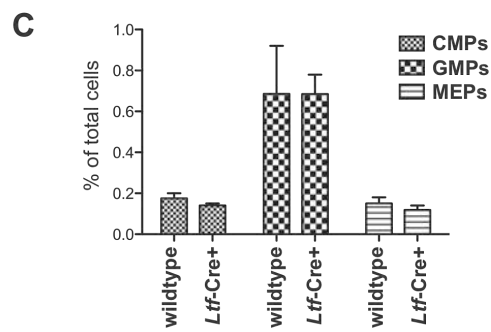
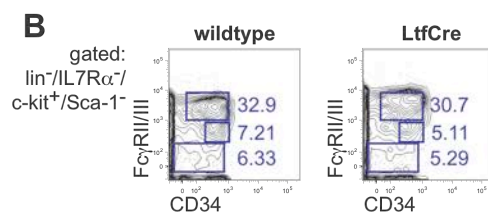
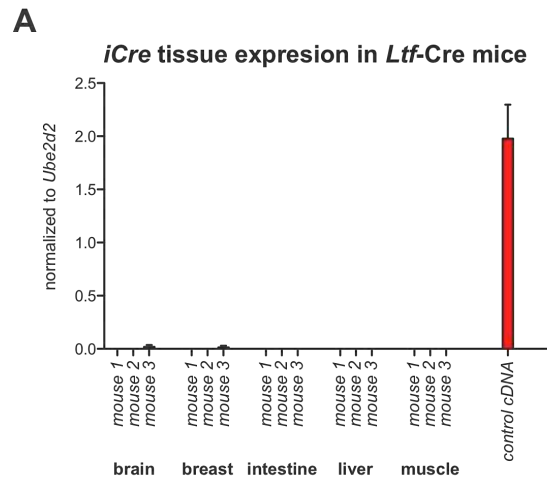
Table S1

<i>population</i>	<i>lineage-cocktail (depletion)</i>	<i>additional markers</i>
LT-HSCs	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b	c-kit <sup>+</sup> , Sca-1 <sup>+</sup> , Thy1.2 <sup>low</sup> , Flt3 <sup>+</sup>
ST-HSCs	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b	c-kit <sup>+</sup> , Sca-1 <sup>+</sup> , Thy1.2 <sup>low</sup> , Flt3 <sup>+</sup>
MPPs	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b	c-kit <sup>+</sup> , Sca-1 <sup>+</sup> , Thy1.2 <sup>+</sup> , Flt3 <sup>+</sup>
CMPs	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b, IL-7R $\alpha$	c-kit <sup>+</sup> , Sca-1 <sup>+</sup> , CD34 <sup>+</sup> , Fc $\gamma$ R1/III <sup>int</sup>
GMPs	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b, IL-7R $\alpha$	c-kit <sup>+</sup> , Sca-1 <sup>+</sup> , CD34 <sup>+</sup> , Fc $\gamma$ R1/III <sup>high</sup>
MEPs	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b, IL-7R $\alpha$	c-kit <sup>+</sup> , Sca-1 <sup>+</sup> , CD34 <sup>low</sup> , Fc $\gamma$ R1/III <sup>-</sup>
CLPs	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b	c-kit <sup>int</sup> , Sca-1 <sup>low</sup> , IL-7R $\alpha$ <sup>+</sup>
MPs	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b, CD11c, CD19, NK1.1, Sca-1	c-kit <sup>high</sup> , Flt3 <sup>+</sup> , CD115 <sup>low</sup>
MDPs	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b, CD11c, CD19, NK1.1, Sca-1	c-kit <sup>high</sup> , Flt3 <sup>+</sup> , CD115 <sup>high</sup>
CDPs	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b, CD11c, CD19, NK1.1, Sca-1	c-kit <sup>low</sup> , Flt3 <sup>+</sup> , CD115 <sup>high</sup>
MpK	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b, IL-7R $\alpha$	c-kit <sup>+</sup> , Sca-1 <sup>+</sup> , CD150 <sup>+</sup> , CD41 <sup>+</sup>
pre-Meg/E	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b, IL-7R $\alpha$	c-kit <sup>+</sup> , Sca-1 <sup>+</sup> , CD150 <sup>+</sup> , CD41 <sup>+</sup> , CD105 <sup>+</sup> , Fc $\gamma$ R1/III <sup>-</sup>
pre-CFU-E	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b, IL-7R $\alpha$	c-kit <sup>+</sup> , Sca-1 <sup>+</sup> , CD150 <sup>+</sup> , CD41 <sup>+</sup> , CD105 <sup>+</sup> , Fc $\gamma$ R1/III <sup>-</sup>
GMPs <sup>CD150-</sup>	CD3 <sub>E</sub> , B220, Ter119, Gr-1, CD11b, IL-7R $\alpha$	c-kit <sup>+</sup> , Sca-1 <sup>+</sup> , CD150 <sup>+</sup> , CD41 <sup>+</sup> , Fc $\gamma$ R1/III <sup>-</sup>
pre-cDCs	CD3 <sub>E</sub> , B220, Ter119, CD19, NK1.1	CD11c <sup>+</sup> , MHC-II <sup>+</sup> , Flt3 <sup>+</sup> , SIRP $\alpha$ <sup>int</sup>
CD8 $\alpha$ <sup>+</sup> cDCs	CD3 <sub>E</sub> , Ter119, CD19, NK1.1	CD11c <sup>high/int</sup> , CD11b <sup>+</sup> , MHC-II <sup>+</sup> , CD8 $\alpha$ <sup>+</sup>
CD8 $\alpha$ <sup>-</sup> cDCs	CD3 <sub>E</sub> , Ter119, CD19, NK1.1	CD11c <sup>high/int</sup> , CD11b <sup>+</sup> , MHC-II <sup>+</sup> , CD8 $\alpha$ <sup>-</sup>
pDCs	CD3 <sub>E</sub> , Ter119, CD19, NK1.1	CD11c <sup>int</sup> , CD11b <sup>+</sup> , B220 <sup>+</sup> , SiglecH <sup>+</sup>

Table S1 | Phenotypic characteristics of the populations evaluated in this study.

**Supplementary Figures:**

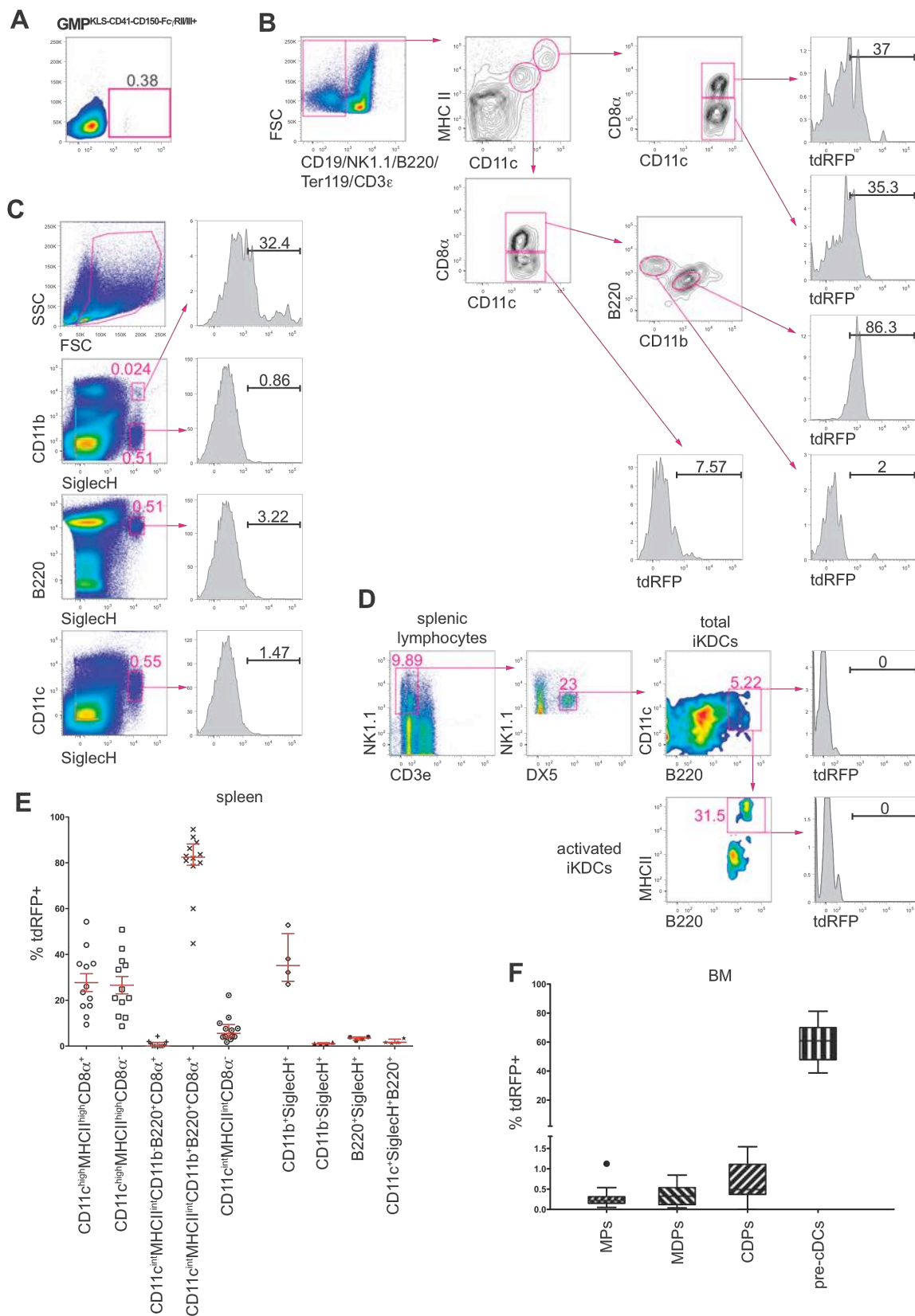
**Figure S1**



**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**

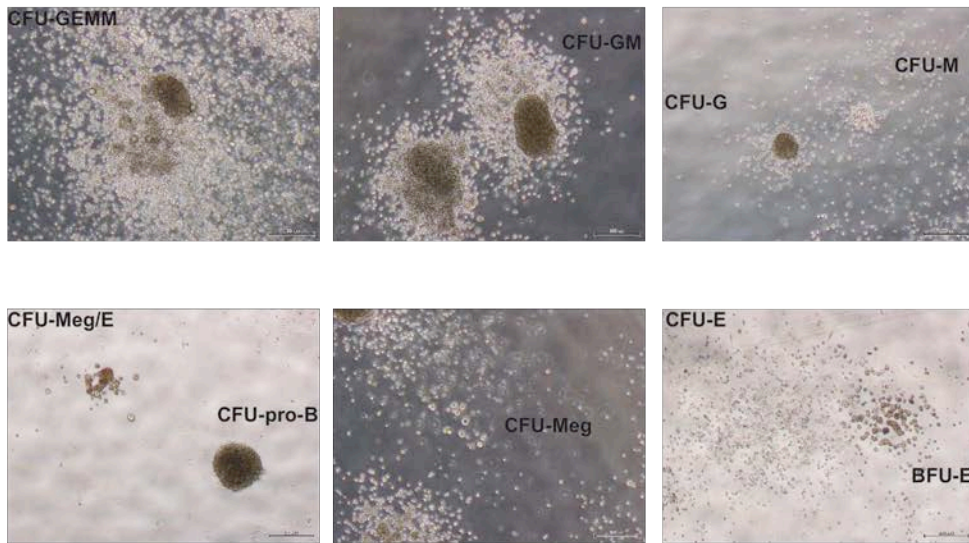


**Figure S2 | Phenotypical analysis of myeloerythroid populations in *Ltf*-reporter<sup>+</sup> mice.**

**A**, Proportion of tdRFP<sup>+</sup> GMPs as defined by gating on lin<sup>-</sup>/c-kit<sup>+</sup>/Sca-1<sup>-</sup>(KLS<sup>-</sup>)/CD41<sup>-</sup>/CD150<sup>-</sup>/FcγRII/III<sup>+</sup> 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<sup>int</sup>/CD11b<sup>+</sup>/B220<sup>+</sup>/SiglecH<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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).



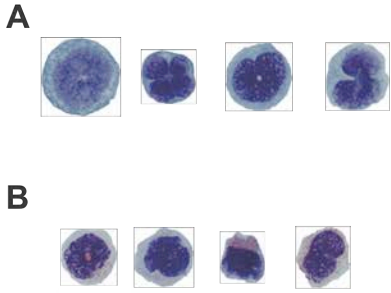
**Figure S3**  
corresponding to **Figure 4**



**Figure S3 | *Ltf*-reporter<sup>-</sup> 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<sup>-</sup> BM cells are depicted.

**Figure S4**  
corresponding to Figure 5



**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 cytopins 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<sup>-</sup>. Representative cell populations are depicted.

## **References:**

52. Pronk CJ, Rossi DJ, Mansson R, Attema JL, Norddahl GL, Chan CK, et al. Elucidation of the phenotypic, functional, and molecular topography of a myeloerythroid progenitor cell hierarchy. *Cell Stem Cell*. 2007 Oct 11;1(4):428-42.