

Letter to the Editor

Dicer is indispensable for the development of murine mast cells

To the Editor:

Mast cells (MCs) are major effector cells of type I allergy.¹ In addition, there is growing evidence for the involvement of MCs in infection, autoimmunity, and tumor development. Murine MCs are categorized into connective tissue–type mast cells (CTMCs) and mucosal mast cells (MMC). CTMCs reside predominantly in the dermis, peritoneal cavity, and submucosa of the gastrointestinal tract and mainly express mouse mast cell protease (mMCP) -4, -5, and -6, whereas MMCs are exclusively located in the mucosal epithelia of the airways and the gastrointestinal tract and primarily express mMCP-1 and mMCP-2. MicroRNAs (miRNAs) are small noncoding RNAs with critical functions in regulating the mammalian immune system by binding protein-coding mRNA targets, resulting in translational repression and mRNA decay.² Specific miRNAs, including miR-221/miR-222, miR-146, and miR-21, have been reported to regulate *in vitro* MC proliferation, cell cycling, cytokine production, and formation of the actin cytoskeleton.³ During miRNA biogenesis, the RNase III endoribonuclease Dicer is responsible for processing of mature miRNA duplexes. Ubiquitous deletion of *Dicer* in mice leads to early embryonic lethality, demonstrating a vital role for miRNAs in development.²

To explore the role of Dicer in MCs *in vivo*, we generated mice with conditional deletion of *Dicer* in CTMCs. In particular, we analyzed MC counts and MC-specific responses. Furthermore, the development of MCs from Dicer-deficient bone marrow was assessed *in vitro*.

To generate MC-specific *Dicer* knockout mice, we crossed the *Mcpt5Cre* strain,⁴ which expresses Cre recombinase under control of the *Mcpt5* promoter, to *Dicer*^{fl/fl} mice.⁵ Enumeration of MCs in different tissues was performed by means of microscopic counting and flow cytometry. MMC counts were additionally quantified by measurement of MMC-specific mMCP-1 levels in sera and corresponding *Mcpt1* and *Mcpt2* transcript levels in the small intestines of IL-3–treated mice. Passive systemic anaphylaxis after intraperitoneal injection with dinitrophenol (DNP)–specific IgE and subsequent challenge with dinitrophenylated human serum albumin (DNP-HSA) was analyzed by measurement of rectal body temperature. Blood cell counts were determined with the hematology system Hemavet 950FS (Drew Scientific, Waterbury, Conn), and numbers of myeloid and lymphoid cells in the spleen and peritoneal cavity were assessed by means of flow cytometry. Development of bone marrow–derived mast cells (BMMCs) was investigated in *Mx1Cre/Dicer*^{fl/fl} mice. In these mice Cre expression under control of the *Mx1* promoter was induced by administration of polyinosine-polycytidylic acid (polyI:C), leading to *Dicer* deletion in all interferon-responsive hematopoietic cells.⁶ The methods used are further detailed in the [Methods](#) section in this article's Online Repository at www.jacionline.org.

Enumeration of MCs in different tissues revealed comparable MC counts in *Mcpt5Cre/Dicer*^{fl/wt} mice and *Dicer*^{fl/wt} control animals (Fig 1, A–E). Interestingly, homozygous deletion of

Dicer significantly reduced the number of CTMCs in the peritoneal cavity (85.8%) and resulted in nearly complete ablation (93.4% to 100%) of CTMCs in the dermis of back skin, ears, mesentery, muscularis of the glandular stomach, tongue, and heart compared with levels seen in *Dicer*^{fl/fl} control animals (Fig 1, A–G, and see Fig E1 in this article's Online Repository at www.jacionline.org). In contrast to these alterations in CTMCs, MMC numbers were comparable between *Mcpt5Cre/Dicer*^{fl/fl} mice and *Dicer*^{fl/fl} control animals, as indicated by unchanged serum levels of mMCP-1 (Fig 1, H) and comparable expression of *Mcpt1* and *Mcpt2* mRNA in the small intestine in both groups (Fig 1, I and J).

Because MCs are major effector cells of anaphylaxis, we next sought to investigate the response of *Mcpt5Cre/Dicer*^{fl/fl} mice in IgE-mediated passive systemic anaphylaxis (Fig 1, K). We observed that *Mcpt5Cre/Dicer*^{fl/fl} mice were completely protected from the anaphylaxis-associated decrease in body temperature, whereas *Dicer*^{fl/fl} control mice showed the expected temperature decrease. These findings demonstrate that ablation of CTMCs is highly efficient in *Mcpt5Cre/Dicer*^{fl/fl} mice and results in loss of systemic MC functions.

We observed no alterations in MC counts upon deletion of *Dicer* in mature MCs *in vivo* using polyI:C-treated *Mx1Cre/Dicer*^{fl/fl} mice (see Fig E2 in this article's Online Repository at www.jacionline.org), suggesting that Dicer might be dispensable for survival of MCs. To further investigate the role of Dicer for MC development, we also assessed MC differentiation from bone marrow of polyI:C-treated *Mx1Cre/Dicer*^{fl/fl} mice (Fig 1, L–N). In *Mx1Cre/Dicer*^{fl/fl} cultures we observed very low cell numbers and massive cell death (Fig 1, L and M). Furthermore, analysis of Kit⁺ and FcεRIα⁺ cells after 4 weeks in culture revealed that only 3.09% ± 0.05% MCs developed in *Mx1Cre/Dicer*^{fl/fl} cultures (Fig 1, N). In contrast, *Dicer*^{fl/fl} cultures showed expression of Kit and FcεRIα in 91.29% ± 1.18% of cells. Similarly, basophils did not develop, whereas low numbers of dendritic cells arose from the bone marrow of *Mx1Cre/Dicer*^{fl/fl} mice (see Fig E3 in this article's Online Repository at www.jacionline.org). These findings indicate that Dicer is indispensable for development of MCs, as well as basophils, but not for survival of hematopoietic stem cells. Different miRNAs, such as miR-342-3p, miR-125b-5p, and miR-132, have recently been reported to be crucially involved in the development of MCs.⁷ Hence the absence of these miRNAs upon deletion of *Dicer* might explain the deficiency of CTMCs. In line with our findings, Dicer ablation *in vivo* has been shown to also affect maturation of granulocyte/macrophage progenitors toward neutrophils, macrophages, and dendritic cells, as well as T-cell differentiation, B-cell development, and germinal center formation.^{2,8}

Expression of *Mcpt5Cre* has previously been demonstrated to be highly specific for CTMCs,⁴ with the exception of T_H2 cell–mediated expansion of MMCs.⁹ To further assess the specificity of *Mcpt5Cre*-mediated deletion of *Dicer*, we next analyzed the number of different myeloid and lymphoid cells in peripheral blood, the spleen, and the peritoneal cavity (Fig 2). We found that all these cell populations showed comparable numbers in *Mcpt5Cre/Dicer*^{fl/fl} mice and *Dicer*^{fl/fl} control animals, confirming that *Mcpt5Cre* is specifically expressed in MCs.

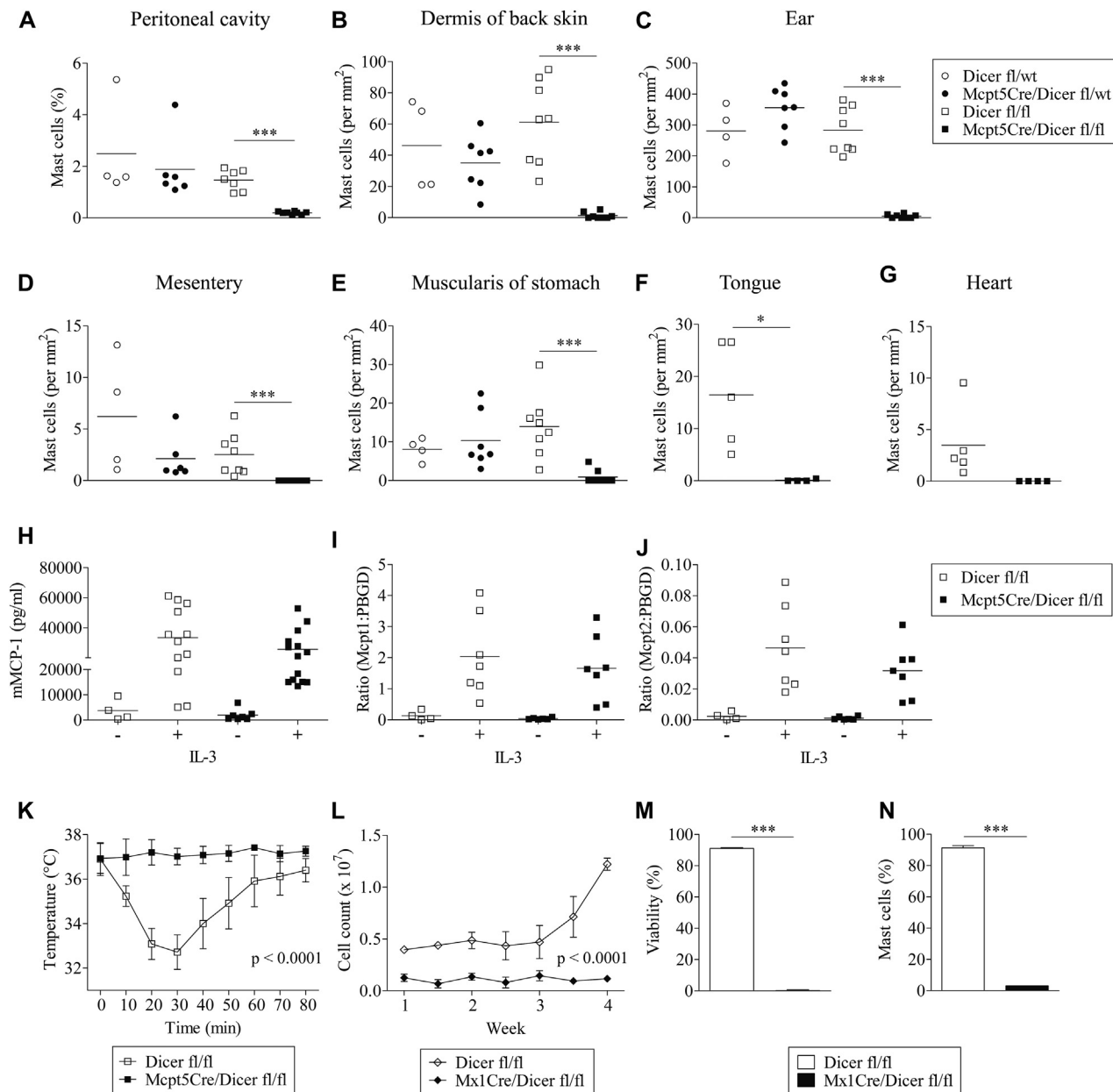


FIG 1. *Mcpt5Cre*-mediated deletion of *Dicer* leads to the absence of CTMC and MC-specific responses. **A-G**, MC counts (n = 4-8). **H**, Serum levels of mMCP-1 (n = 4-7) upon injection of IL-3. **I** and **J**, Expression ratio of *Mcpt1* and *Mcpt2* mRNA relative to porphobilinogen deaminase (*PBGD*) mRNA in the small intestine (n = 4-7) upon injection of IL-3. **K**, IgE-mediated passive systemic anaphylaxis (n = 6-7, mean ± SD). **L**, BMMCs were cultured from *Dicer*^{fl/fl} and *Mx1Cre/Dicer*^{fl/fl} mice, and cell counts were assessed twice a week (n = 3, mean ± SD). **M** and **N**, Viability and numbers of MCs (Kit⁺FcεR1α⁺) in BMMC cultures were determined after 4 weeks of cultivation (100% refers to all cells in the culture; n = 3, mean ± SD). *P < .05, **P < .01, and ***P < .001.

For previous investigations of MC functions, the *Kit* mutant mouse strains WBB6F1-*Kit*^{W^W-v} and C57BL/6-*Kit*^{W^W-sh/W^W-sh} have been widely used. However, these strains also exhibit other major alterations in the hematopoietic system and in fertility. To overcome these limitations, we and others recently generated mouse models of more specific deficiency of MCs, which depend on the Cre/loxP recombination system.^{1,4} The reported *Cpa3Cre*-dependent mouse models exhibited deficiency of MCs and a reduction in basophil counts. In contrast, crossing *Mcpt5Cre* to

R-DTA mice, which express diphtheria toxin A in Cre-expressing cells, induced depletion of CTMCs. The present study reporting on *Mcpt5Cre*-mediated deletion of *Dicer* thus introduces a new mouse model of highly specific and efficient CTMC deficiency.

In conclusion, our study demonstrates that posttranscriptional regulation by Dicer-dependent mechanisms is essential for development of the MC lineage. Furthermore, the *Mcpt5Cre/Dicer*^{fl/fl} cross represents a novel mouse model with specific CTMC deficiency.

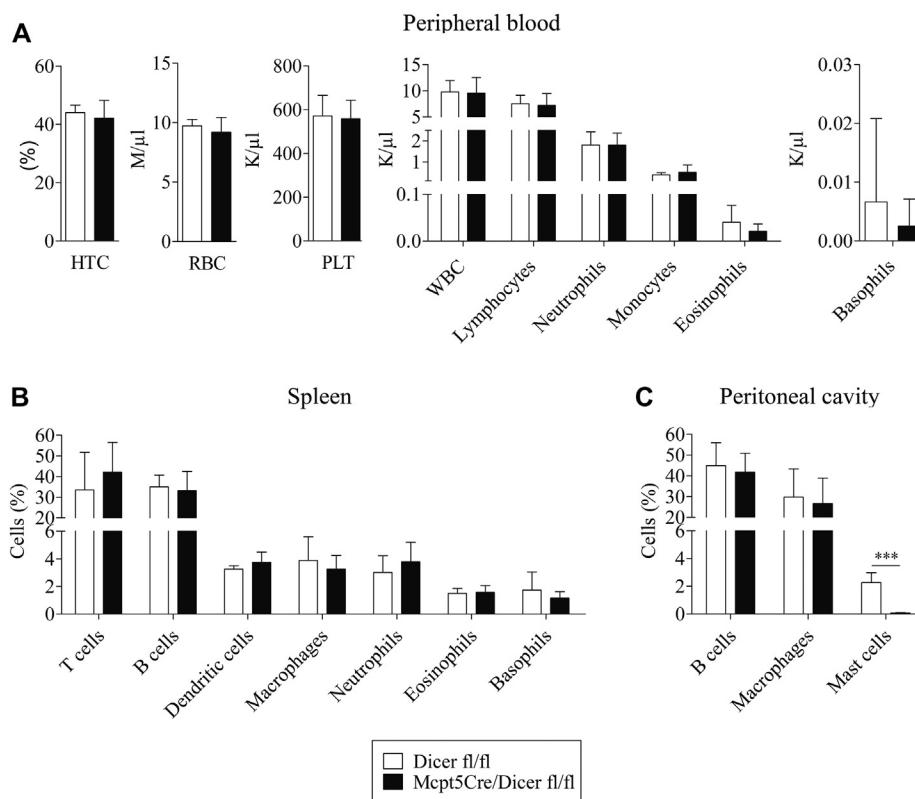


FIG 2. Numbers of other hematopoietic cell populations are unaffected in *Mcpt5Cre/Dicer^{fl/fl}* mice. **A**, Hematocrit (HTC) and numbers of red blood cells (RBC), platelets (PLT), and the indicated white blood cell (WBC) subclasses in venous blood (n = 8-9). **B** and **C**, Numbers of myeloid and lymphoid cells in the spleen (Fig 2, B) and peritoneal cavity (Fig 2, C) were determined in *Dicer^{fl/fl}* and *Mcpt5Cre/Dicer^{fl/fl}* mice (n = 6) (mean ± SD). ***P < .001.

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Novartis, and Shire. The rest of the authors declare that they have no relevant conflicts of interest.

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METHODS

Mice

All mice were kept in the animal facilities of the Institute for Medical Microbiology, Immunology and Hygiene and the Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany. *Dicer*^{fl/fl} mice^{E1} were crossed to heterozygous *Mcpt5Cre*^{E2} or *Mx1Cre*^{E3} mice. Littermates negative for the respective Cre served as control mice. All mice were on a C57BL/6 background and used for experiments at the age of 8 to 12 weeks. The experiments were carried out in accordance with institutional and state guidelines (approvals AZ 84-02.04.2011.A302 and 84-02.04.2013.A064). *Mcpt5Cre/Dicer*^{fl/fl}, *Mcpt5Cre/Dicer*^{fl/wt}, and *Mx1Cre/Dicer*^{fl/fl} mice were viable and fertile. Weekly monitoring of body weight, fur and skin condition over 10 weeks revealed no apparent defects in *Mcpt5Cre/Dicer*^{fl/fl} or *Mcpt5Cre/Dicer*^{fl/wt} mice compared with *Dicer*^{fl/fl} control animals. *Mx1Cre/Dicer*^{fl/fl} mice and *Dicer*^{fl/fl} littermate control mice were injected intraperitoneally twice within 2 days with polyI:C (GE Healthcare, Fairfield, Conn) to induce Cre expression in interferon-responsive hematopoietic cells.

Enumeration of MCs in different tissues

For enumeration of MCs in different tissues, paraffin-embedded sections were stained with Giemsa, and MCs were counted in 15 medium-power fields ($\times 200$ magnification, dermis of back skin, tongue, and stomach) and 15 high-power fields ($\times 400$ magnification, ear) of Giemsa-stained, paraffin-embedded sections by using a Leica DM4000B microscope (Leica Microsystems, Wetzlar, Germany) with Diskus software (Hilgers, Königswinter, Germany). MC counts in the peritoneal cavity (Kit⁺ and FcεRIα⁺) were determined by means of flow cytometry.

Analysis of MC proteases expressed by MMCs

Mice were injected intraperitoneally for 5 consecutive days with 0.5 mL of IL-3-containing supernatant of X63/0 cells, as previously described,^{E4} to induce expansion of intestinal MCs. Three days later, mice were killed to harvest serum, as well as the small intestine, for measurement of MC proteases, which are specific for MMCs. mMCP-1 serum levels were determined by using the mMCP-1 ELISA (eBioscience, Frankfurt, Germany), according to the manufacturer's instructions. To quantify expression of *Mcpt1* and *Mcpt2* mRNA as a marker for MMCs in the small intestine, quantitative real-time PCR was used, as previously described.^{E4} Porphobilinogen deaminase (*PBGD*) was used as a housekeeping gene.

IgE-mediated passive systemic anaphylaxis

Mice were injected intraperitoneally with 150 μL of DNP-specific IgE (83 μg/mL; clone SPE7, Sigma-Aldrich, Steinheim, Germany) and challenged 24 hours later with 200 μL of DNP-HSA (1 mg/mL). The extent of anaphylaxis was measured based on rectal body temperature every 10 minutes for 80 minutes.

Cell culture

Five days after the first polyI:C injection, femoral and tibial bone marrow was isolated from *Mx1Cre/Dicer*^{fl/fl} mice and *Dicer*^{fl/fl} littermate control animals. To gain BMMCs, cells were cultured in Dulbecco modified Eagle medium (high glucose; Gibco, Invitrogen, Carlsbad, Calif) supplemented with 20% FCS, 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin (Biochrom AG, Berlin, Germany), and 30%

WEHI-3B-conditioned medium (as a source of IL-3) for at least 4 weeks. After 3 weeks in culture, 0.5% CHO-conditioned medium (as a source of stem cell factor) was added. Dendritic cells were cultured in RPMI 1640 medium (Biochrom AG) supplemented with 10% heat-inactivated FCS, 2 mmol/L L-glutamine, 50 μmol/L 2-mercaptoethanol (Gibco, Invitrogen), 100 U/mL penicillin, 100 μg/mL streptomycin (Gibco, Invitrogen), and 20 ng/mL rmGM-CSF (rhesus macaque GM-CSF; PeproTech, Hamburg, Germany). Basophils were cultured with 30 ng/mL IL-3 (PeproTech) instead of rmGM-CSF.

Analysis of different cell populations in peripheral blood, the spleen, and the peritoneal cavity

Whole blood samples were taken from the superficial temporal vein. Counting of blood cells was performed with the hematology system Hemavet 950FS (Drew Scientific), according to the manufacturer's protocol. Numbers of T cells (CD3e⁺), B cells (CD19⁺), dendritic cells (CD11c⁺), macrophages (F4/80⁺), neutrophils (Gr-1⁺), eosinophils (Siglec-F⁺), and basophils (CD49b⁺ and FcεRIα⁺) in the spleen and numbers of B cells (CD19⁺), macrophages (F4/80⁺), and MCs (Kit⁺ and FcεRIα⁺) in the peritoneal cavity were determined by using flow cytometry.

Flow cytometry

The following antibodies were used for flow cytometry: anti-mouse CD16/32 (clone 93; eBioscience, San Diego, Calif), phycoerythrin (PE)-conjugated anti-mouse FcεRIα (clone MAR-1; eBioscience), allophycocyanin (APC)-conjugated anti-mouse CD117 (Kit; clone 2B8, eBioscience), PE-conjugated anti-mouse CD3e (clone 145-2C11, eBioscience), fluorescein isothiocyanate-conjugated anti-mouse CD19 (clone 1D3, eBioscience), PE-conjugated anti-mouse CD11c (clone N418, eBioscience), fluorescein isothiocyanate-conjugated anti-mouse F4/80 (clone BM8, eBioscience), PE-conjugated anti-mouse Gr-1 (clone RB6-8C5; Miltenyi Biotec, Bergisch-Gladbach, Germany), PE-conjugated anti-mouse Siglec-F (clone E50-2440; BD Biosciences, Heidelberg, Germany), and APC-conjugated anti-mouse CD49b (integrin alpha 2; clone DX5, eBioscience). Propidium iodide (Sigma-Aldrich) and APC-conjugated Annexin V (eBioscience) were used to determine viability and apoptosis, respectively.

Statistical analysis

Statistical analysis was performed with the Student *t* test for unpaired values. Two-way ANOVA was used to compare time courses. A *P* value of less than .05 was considered statistically significant.

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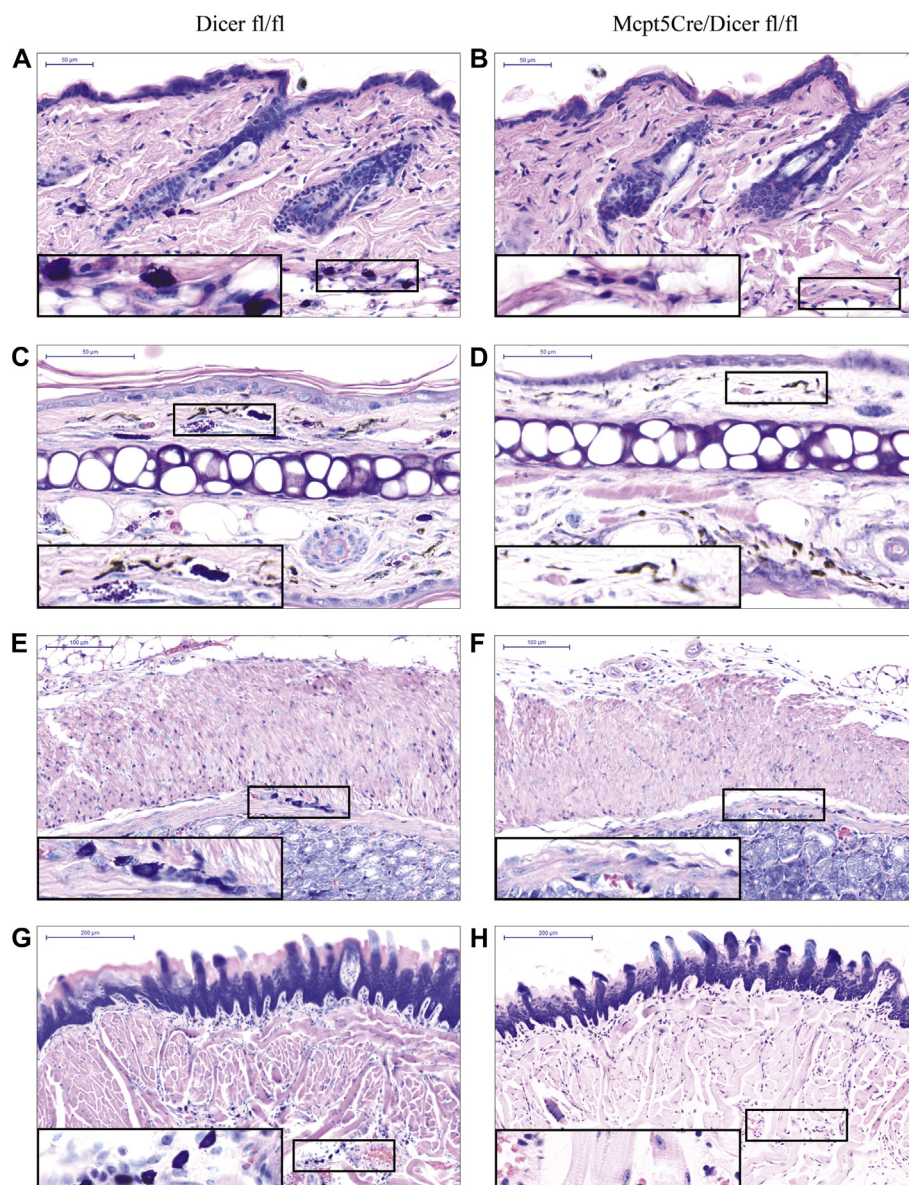


FIG E1. Histologic analysis of different tissues reveals ablation of CTMCs in *Mcpt5Cre/Dicer^{fl/fl}* mice. Representative Giemsa staining of dermis of back skin ($\times 430$ magnification; *insert* = $\times 1100$ magnification; **A** and **B**), ears ($\times 800$ magnification; *insert* = $\times 1300$ magnification; **C** and **D**), stomachs ($\times 300$ magnification; *insert* = $\times 800$ magnification; **E** and **F**), and tongues ($\times 200$ magnification; *insert* = $800\times$; **G** and **H**) of *Dicer^{fl/fl}* and *Mcpt5Cre/Dicer^{fl/fl}* mice are depicted.

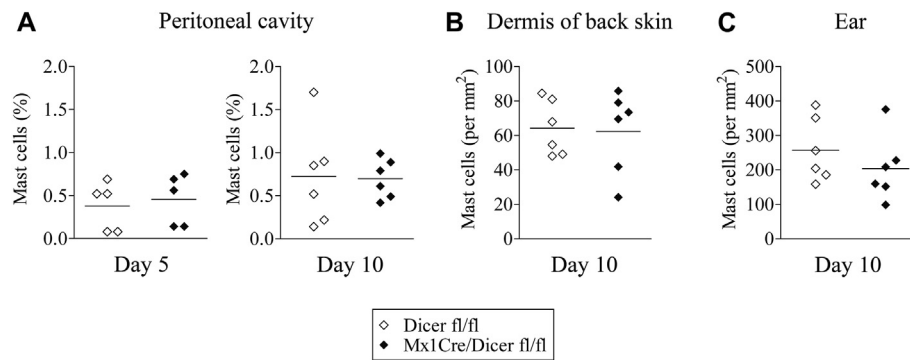


FIG E2. Deletion of *Dicer* in mature MCs has no effect on MC counts. *Mx1Cre/Dicer*^{fl/fl} (n = 6) and *Dicer*^{fl/fl} mice (n = 6) were injected intraperitoneally with polyI:C. Numbers of MCs in the peritoneal cavity (A), dermis of back skin (B), and ears (C) were analyzed by using flow cytometry and histology.

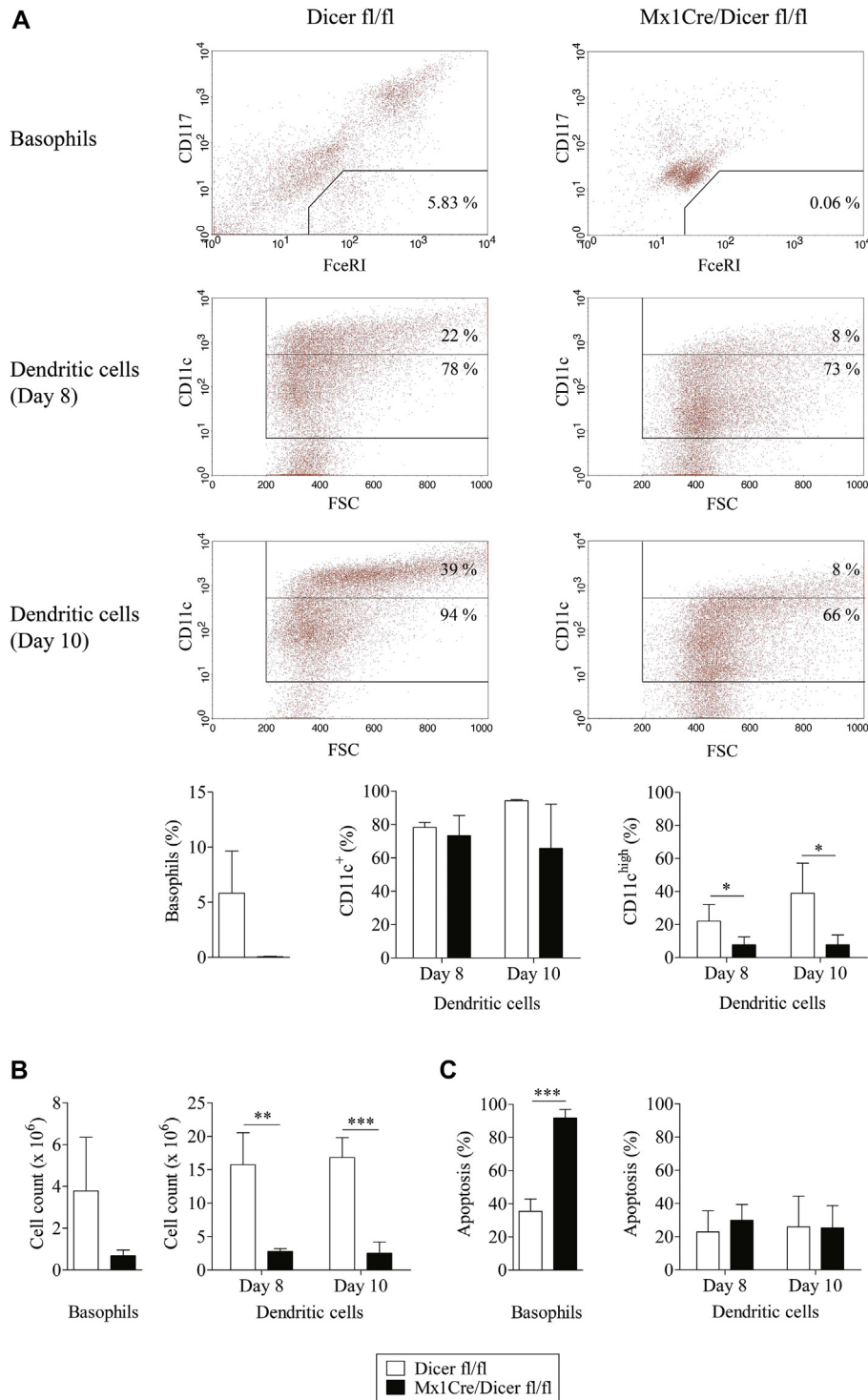


FIG E3. Dendritic cells develop from the bone marrow of *Mx1Cre/Dicer^{fl/fl}* mice. Basophils and dendritic cells were cultured from bone marrow of *Mx1Cre/Dicer^{fl/fl}* and *Dicer^{fl/fl}* mice on induction of Cre expression. **A**, Representative fluorescence-activated cell sorting plots show gating of basophils (CD117 [Kit]⁺ FcεRI⁺) and CD11c⁺ and CD11c^{high} dendritic cells. Percentages of basophils (day 10) and dendritic cells (day 8 and 10) are depicted in bar charts. **B** and **C**, Cell counts (Fig E3, B) and apoptosis (Fig E3, C) were analyzed in cultures of basophils and dendritic cells by means of microscopic counting and flow cytometry (n = 3-4, mean ± SD). **P* < .05, ***P* < .01, and ****P* < .001.