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▶ **To cite this version:**

Marc Dalod, Stefanie Scheu. Dendritic cell functions in vivo: A user's guide to current and next-generation mutant mouse models. European Journal of Immunology, 2022, 10.1002/eji.202149513 . hal-03754422

HAL Id: hal-03754422
https://amu.hal.science/hal-03754422

Submitted on 19 Aug 2022

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HIGHLIGHTS

Basic

REVIEW

Dendritic cell functions in vivo: A user's guide to current and next- generation mutant mouse models

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DCs do not just excel in antigen presentation. They orchestrate information transfer from innate to adaptive immunity by sensing and integrating a variety of danger signals, and translating them to naïve T cells, to mount specifically tailored immune responses. This is accomplished by distinct DC types specialized in different functions and because each DC is functionally plastic, assuming different activation states depending on the input signals received. Mouse models hold the key to untangle this complexity and determine which DC types and activation states contribute to which functions. Here, we aim to provide comprehensive information for selecting the most appropriate mutant mouse strains to address specific research questions on DCs, considering three in vivo experimental approaches: (i) interrogating the roles of DC types through their depletion; (ii) determining the underlying mechanisms by specific genetic manipulations; (iii) deciphering the spatiotemporal dynamics of DC responses. We summarize the advantages, caveats, suggested use, and perspectives for a variety of mutant mouse strains, discussing in more detail the most widely used or accurate models. Finally, we discuss innovative strategies to improve targeting specificity and next-generation mutant mouse models, and briefly address how humanized mouse models can accelerate translation into the clinic.

Keywords: Dendritic cells · Mouse models · Transgenic mice · cre-loxP · Intersectional genetics

Introduction

DCs are innate immune cells that excel in the initial activation or "priming" of naïve T cells and in their functional polarization. This process involves relaying information from pathogens and from the innate immune system to the adaptive immune system. Hence, DCs can be considered as the "chefs d'orchestre" of immunity. DCs are functionally plastic: they can induce either tolerance or immunity and polarize effector immune cells toward different functions. Two complementary mechanisms underlie DC functional plasticity. First, there are distinct DC types specialized in different functions and conserved across warm-blooded vertebrates. Second, each DC type can undergo different activation states, depending on the input signals received, to deliver customized combinations of output signals to activate T cells in the manner best suited to deal with the threat sensed.

The nature of certain DC types is controversial, and the boundaries between distinct DC types versus different activation states of the same DC type are sometimes blurry. Yet, based on our current knowledge, DCs can be classified into six cell types that are evolutionarily conserved between mice and humans: (i) Type 1 conventional DCs (cDC1) excel in $CD8^+$ T-cell priming, in particular, against infected or tumor cells. This is due in part to the high

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efficacy of cDC1 at capturing cell-associated antigens for processing and presentation in association with class I MHC molecules (MHC-I). (ii) Type 2 conventional DCs (cDC2) are especially efficient in the priming of $CD4^+$ T cells and their functional polarization towards type 2 or 17 helper responses (Th2 or Th17), which contribute to defense against parasites or bacterial/fungal infections respectively, or towards follicular helper responses (Tfh) that promote humoral immunity. (iii) Plasmacytoid DCs (pDCs) rapidly produce copious amounts of type I and III IFNs (IFN-I/III) during systemic viral infections. (iv) Monocyte-derived DCs (MoDCs) may contribute to maintain or reactivate effector lymphocytes in nonlymphoid inflamed tissues. (v) Langerhans cells (LCs) are located in the skin epidermis and in certain mucosae, where they can either promote tolerance to the microbiota and innocuous environmental antigens or contribute to induce protective immunity against local infections or cancer. (vi) Transitional DCs (tDCs) have been described only recently and while they share features of both cDCs and pDCs, their function is currently enigmatic [1, 2].

The input signals sensed by DCs include microbial molecular motifs, host danger signals specifically expressed by damaged cells (e.g., infected, tumor or dying cells), cytokines produced by other cells, and metabolites from host or microbial origin. Output signal 1 corresponds to antigen presentation for the triggering of the T-cell receptor. Output signal 2 corresponds to costimulation. Positive costimulation synergizes with signal 1 to boost T-cell proliferation and activation. Negative costimulation prevents overshooting of the T-cell response and programs its contraction. Output signal 3 corresponds to cytokines, which play a key role in instructing T-cell functional polarization. DCs also produce chemokines to recruit other cell types to lymphoid organs or inflamed tissues. DCs can also express metabolic enzymes with immunoregulatory roles. Pathogens or tumors can hijack DC immunoregulatory functions or inhibit DC activating functions to suppress immune responses for their own benefit.

To harness DCs in the clinic safely and effectively to fight infections, cancer, or immunopathology, it is crucial to understand which combination of DC types and activation states contributes to which functions, and how to manipulate it. Hence, we need to advance our basic knowledge of the physiological functions of DC types and their molecular regulation. To achieve this aim, mouse models remain key, considering their versatility to target DCs in vivo genetically or pharmacologically, and the conserved DC biology between mice and humans.

Here, we aim at providing a guideline to help researchers choosing mutant mice depending on their research question and the DC type under study. Since, the focus of our review article is on deciphering DC type functions and their molecular regulation, we will not discuss DC ontogeny that others have recently covered [3–5]. We will discuss the advantages and limitations of a selection of mouse models that are commonly used or may be the most reliable depending on the objective pursued, including the following three main research questions: (i) to interrogate the global role of a given DC type in vivo, through assessing how its depletion affects the process under study, (ii) to identify the specific functions underlying this global effect, by studying the impact of the inactivation of candidate genes, and (iii) to decipher the dynamics of DC responses in vivo, by studying the choreography of the microanatomical redistribution of DC types in relation to their activation and to their establishment of interactions with other cells, using fluorescent reporter mice. We will discuss recent approaches that should improve our ability to interrogate rigorously the role of candidate functions and genes in specific DC types, including intersectional genetics or shield BM chimeric mice. We will also touch on humanized mouse models as preclinical tools for moving one step further toward harnessing DCs for vaccination or immunotherapy.

Depletion models: Determining the specific function of DC types

Conditional depletion of DC types upon diphtheria toxin injection

One widely used approach to achieve DC type-specific ablation in the mouse model organism is the transgenic expression of the primate diphtheria toxin (DT) receptor (DTR; found to be identical with the transmembrane precursor of heparin-binding EGF-like growth factor, HB-EGF) and the administration of the *Corynebacterium diphtheriae* exotoxin DT. Murine cells are resistant to the toxic effects of DT due to several mutations in the DT binding site of the mouse orthologue of HB-EGF [6]. An advantage of the DTR-mediated approach is that cell ablation is conditional and occurs only after DT injection. Hence, compensatory mechanisms found in constitutive depletion settings such as transgenic expression of the DT or gene deficiency in DC type-specific transcription factors are avoided. In addition, depletion can be temporally controlled and, thus, specificity for short-lived versus long-lived cell types can be modified. This allows us to discriminate between the functions of long-lived LCs from those of other, short-lived, DC types in the skin [7]. Accuracy of the ablation critically depends on the choice of DC type-specific markers and their cis-regulatory elements, such as promoter and enhancers/repressors, used for the transgenic DTR expression. Here, the three types of genetic manipulation commonly used for the generation of DTR expressing mouse strains (as well as other mouse strains described throughout this review) differ in the number of control elements that regulate the expression of the DTR-cassette. Earlier approaches, such as the CD11c-DTR transgenic mice, relied on short but well-defined promoter elements of few kilobases (kb) in length [8]. Shortcomings of this approach became clear, when discrepancies between endogenous CD11c expression and cell depletion upon DT administration were found for certain cell types, such as pDCs and NK cells, that were reported not to be depleted despite their intermediate CD11c expression [9, 10]. An improved strategy represents the use of BAC transgenic mice [11], where larger stretches of DNA of 100–300 kb length from the endogenous genomic locus of the DC-specific marker of choice are introduced as transgene.

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Thus, a more complete set of cis-regulatory regions is transferred together with the DTR-expression cassette. Still cis-regulatory elements have been found at distances >300 kb from the gene coding regions [12]. Therefore, knock-in strategies inserting the DTR-cassette into the endogenous locus of the marker gene are considered to achieve the highest faithfulness in DTR expression, although the insertion of a stretch of DNA or an IRES control element could eventually cause some deregulation of the expression of the endogenous marker gene in certain instances. The use of markers, such as surface molecules or transcription factors specific for a certain DC type, not only for depletion of DCs but also for most DC-specific mutant mouse models discussed below, brings with it the inherent problem that these markers change their expression pattern during activation. Thus, the specificity of depletion might change significantly under steady state versus inflammatory conditions and data must be interpreted carefully if several cell types are affected. Further inherent intricacies of the DTR-mediated ablation system have been reviewed recently [13], carefully pointing out DT-independent side-effects of ectopic DTR/HB-EGF expression such as cardiomyocyte apoptosis [14]. Table 1 lists DTR-expressing mouse lines with relevance for addressing DC type-specific questions, pointing out known advantages and caveats. In the following, we will discuss exemplary cases, possible applications, and pitfalls.

Among the earliest DTR-transgenic mouse strains established was the CD11c-DTR (Itgax-DTR) mouse, designed for DC depletion. However, in the meantime it has become clear that besides cDCs and pDCs several other cell types can be affected including metallophilic macrophages, NK cells, and activated T cells [8, 13, 15]. In addition, repeated DT application causes lethality in these mice. Therefore, the generation of BM chimeric mice is required for prolonged DC ablation [16, 17]. A more recent DTR-mouse with high specificity for cDCs, covering both cDC1 and cDC2, but sparing pDCs and monocytes, is the Zbtb46-hDTR mouse strain [18]. However, this mouse also requires the use of BM chimeras, since a single DT injection results in lethality, probably due to *Zbtb46* expression in endothelial cells or other stromal cells [19]. BM chimeras tolerate repeated DT injections for up to 2 weeks without overt side-effects, allowing for extended analyses of the impact of DC ablation on the generation of functional adaptive immune responses against infectious agents and tumor challenges [18]. Different temporal depletion regimes allow for selective depletion of specific DC types depending on their respective cell turnover rates. An example for this is the Lang-DTR/EGFP (CD207-DTR/EGFP) mouse strain, where a single DT injection led to long-lasting depletion of LCs contrasting with the rapid replenishment of cDC1 due to their rapid turnover [7]. Thus, using specific temporal depletion regimes, different DC subpopulations can be targeted.

A very recent report identified dermal-derived LC^{like} cells as a previously described LC fraction found in skin-draining LNs under steady-state conditions [20]. These cells have been found to be affected by DT-depletion in Lang-DTR/EGFP mice in addition to bonafide LCs and dermal cDC1 cells. Using a novel DC-SIGN-DTR mouse strain, the authors could show that these LC^{like} cells exhibit a slow turnover rate over an 8-month observation period and are replaced by BM-derived cells.

One of the more widely used mouse model for the specific depletion of pDCs is the BDCA2-DTR mouse strain that expresses the DTR under the control of the human pDC gene promoter, BDCA-2 [21]. Recently, it has been reported that daily injections of DT over a 5-day period achieved efficient pDC depletion but lead to chronic IFN-I production and severe immunopathology [22]. Further it was shown that a single dose of DT-treatment elicited type IFN production from DTR-expressing pDCs. It remains to be seen whether this phenomenon can occur in other pDC depletion models, which would call for a careful reinterpretation of the data.

Taken together, the DTR-mediated approach allows for diverse settings of time-dependent DC depletion analyses. However, DT-toxicity-mediated side-effects must be carefully controlled in each mouse model.

Models with constitutive depletion of DC types due to DTA expression

Because DCs are extremely potent in priming, sustaining, and recalling T-cell responses [23], protective adaptive immunity against infections or cancer may require only very low numbers of DCs and their transient presence at a specific time point in the immune response. Therefore, the requirement for DCs might be underestimated or even completely missed in experimental settings that rely on their transient and eventually incomplete depletion, as in the case of conditional depletion upon DT injection [24]. Thus, when a long-time depletion of the target DC type is required to answer a research question, it is recommended to use mouse models that are constitutively and specifically devoid of this DC type. One way to achieve constitutive depletion of a DC type is ectopic intracellular expression of the active subunit of DT (DTA) under the direct control of regulatory sequences of a gene specifically expressed in the targeted DC type. To our knowledge, this has only been achieved for LCs, for example, allowing to show that LCs contribute to skin homeostasis by inhibiting contact hypersensitivity and preventing photosensitivity [25, 26]. Another approach is to cross mice expressing the Cre recombinase specifically in the target DC type with mice harboring a knock-in allele for a LoxP-STOP-LoxP-DTA cassette downstream of the ubiquitous Rosa26 promoter [27], leading to DTA expression only in the cells that have undergone Cre-mediated DNA recombination causing their swift death. For example, $Xcr1^{iCre}$; $Rosa26^{LSL\text{-}DTA}$ mice are constitutively and specifically lacking cDC1 [28, 29], harbor altered intestinal immunity [29], and are compromised for antitumor immunity [24, 30]. The use of this strategy requires consideration of potential caveats related to Cre expression and activity, as discussed below. Another potential problem with models aimed at constitutive DC depletion is the possibility of compensatory adaptations of the immune system including the activation of alternative developmental pathways triggered by the availability of the emptied cellular niche. For exam-

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Table 1. DTR-transgenic mouse models for the conditional depletion of DC types upon diphtheria toxin injection

Common name	Official nomenclature[1]	Mutation type[2]	Reporter included[3]	Targeted cells[3]	Other cells affected[4]	Advantages[5]	Caveats[5]	Possible applications/remarks	References[6]
CD64-DTR/EGFP	*Fcgr1*^{tm1Ciphe}	KI	EGFP	Mono, Macs, moDCs, Inf-cDC2	None reported	• Depletion of Macs and Mono-derived cells.		• Depletion of moDCs or inf-cDC2. • Carefully check the eventual confounding impact of Mono and Mac depletion.	NA [117]
CD64^{iDTR}	missing	KI	No	Mono, Macs, moDCs, Inf-cDC2	None reported	• loxP-STOP-loxP-controlled DTR expression allows specific depletion of different Mac and Mono-derived cell types depending on the Cre-driver used.		• Differential and/or tissue-specific depletion of Macs and Mono-derived cell types	NA [118]
CD11c-DTR (Itgax-DTR)	*Tg(Itgax-DTR/EGFP)57Lan*	tg	EGFP	cDC1, cDC2 (pDC)	Mono, Mac subsets, NK cell subsets, effector/memory CTLs, IELs, plasmablasts	• Depletion of both pDC and cDC compartments possible.	• Neutrophilia 6–24h after DT injection. • Lethality of repeated DT injections.	• Use of more specific models is now recommended. • Carefully check off-target effects. • Use BM chimeras for prolonged depletion.	321x [8, 13, 15]
CD11c.LuciDTR	*Tg(Itgax-EGFP,-cre,-HBEGF,-luc)2Gjh*	tg	EGFP, luciferase						2x [15, 119]

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Table 1. (Continued)

Common name	Official nomenclature[1]	Mutation type[2]	Reporter included[3]	Targeted cells[3]	Other cells affected[4]	Advantages[5]	Caveats[5]	Possible applications/remarks	References[6]
CD11c.DOG	*Tg(Itgax-DTR/OVA/EGFP)1Garbi*	tg	EGFP	cDC1, cDC2 (pDC)		• Combined DC antigen presentation/ablation studies.	• Neutrophilia 6–24h after DT injection.	• Use of more specific models is now recommended • Carefully check off-target effects.	26x [15, 120]
Zbtb46-DTR (zDC-DTR)	*Zbtb46$^{tm1(HBEGF)Mnz}$*	KI	mCherry	cDC1, cDC2	Activated Mono, endothelial cells, committed erythroid progenitors	• Depletion of cDCs without affecting pDCs, monocytes and NK cells, in BM chimeras.	• A single DT injection is lethal. • MoDCs might be affected.	• Simultaneous and selective depletion of both cDC1 and cDC2. • Use of BM chimeras is required.	25x [18, 19]
Xcr1-DTR/Venus	*Xcr1$^{tm2(HBEGF/Venus)Ksho}$*	KI	Venus	cDC1	None reported	• Highly specific for cDC1.	• KO of the *Xcr1* gene.	• cDC1 depletion. • Use heterozygous mice for depletion studies.	10x [92]
Karma-DTR	*Gpr141b$^{tm1(HBEGF)Ciphe}$*	KI	tdTomato	cDC1	Skin and peritoneal cavity MCs	• Good specificity for cDC1.	• Expression at a late stage of the differentiation of the cDC1 lineage.	• cDC1 depletion. • Carefully check the eventual confounding impact of MC depletion.	4x [121]
Clec9a-DTR (DNGR1-DTR)	missing	tg	No	cDC1 (pDC)	None reported	Good specificity for cDC1.	• 50% of pDCs depleted.	• cDC1 depletion. • Carefully check the eventual confounding impact of partial pDC depletion.	NA [122]

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Table 1. (Continued)

Common name	Official nomenclature[1]	Mutation type[2]	Reporter included[3]	Targeted cells[3]	Other cells affected[4]	Advantages[5]	Caveats[5]	Possible applications/remarks	References[6]
Clec4A4-DTR	missing	KI	No	cDC2	None reported	• Most specific method reported so far for cDC2 depletion. • Specific depletion of $CD103^+$ $CD11b^+$ DCs in the gut.	• Tissue macrophage subsets might be affected.	• cDC2 depletion.	NA [123]
Mgl2DTR (CD301bGFP-DTR)	*Mgl2*$^{tm1.1(HBEGF/EGFP)Aiwsk}$	KI	GFP	Dermal DC/cDC2 subset	None reported	• Dermal DC/cDC2 depletion.	• Langerhans cells and tissue macrophage subsets might be affected.	• Study of the cDC2 subsets involved in Th2/Th17 induction or wound healing.	11x [124]
CD205-DTR	*Ly75*$^{tm1.1(HBEGF/EGFP)Ksat}$	KI	EGFP	LCs cDC1 dermal DCs	Cortical thymic epithelial cells (cTECs) All mature DCs, including tumor-associated DCs	• cDC1 & migratory/mature DC depletion.		• Use of more specific models is now recommended. • Use of BM chimeras is required to avoid cTEC and LC depletion.	1x [125]
Lang-DTR/EGFP (CD207-DTR/EGFP)	*Cd207*$^{tm3(DTR/GFP)Mal}$	KI	EGFP	LCs dermal DC/cDC1 subsets	LC-like cells	• LC vs. cDC1 (dermal DC) depletion.	• Dermal DCs and LC^{like} cells affected.	• Use of more specific models is now recommended. • Different turn-over rate of LCs and cDC1 enables examining LC function selectively after cDC1 have been reconstituted.	49x [20, 126]

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Table 1. (Continued)

Common name	Official nomenclature[1]	Mutation type[2]	Reporter included[3]	Targeted cells[3]	Other cells affected[4]	Advantages[5]	Caveats[5]	Possible applications/remarks	References[6]
HuLangerin-DTR	*Tg(CD207-HBEGF)#Dhka*	tg	No	LCs	None reported	• High specificity for LCs.		• Depletion of LCs.	2x [127]
DC-SIGN-DTR	missing	KI	No	cDC2 ($CD11b^{high}$) LC	none reported	• LC depletion.		• Selective analysis of LC vs. LC^{like} functions by comparing DC-SIGN-DTR and Lang-DTR mice side by side.	NA [20]
Siglech-DTR	missing	tg	No	pDCs (microglia)	Marginal zone Macs, pDC precursors, other antigen-presenting cells.	• More specific than antibody-mediated pDC depletion.		• Use of more specific models is now recommended. • Carefully check the eventual confounding impact of depletion of macrophage subsets.	NA [122]
Siglech-DTR	*Tg(SiglecH-hDTR-EGFP)NCr*	tg	EGFP						NA [128]
Siglech-DTR/EGFP	*Siglech*$^{tm1.1Ksat}$	KI	EGFP (?)				• KI allele abrogates *Siglech* expression: use heterozygous mice for depletion studies.		5x [129, 130]

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Table 1. (Continued)

Common name	Official nomenclature[1]	Mutation type[2]	Reporter included[3]	Targeted cells[3]	Other cells affected[4]	Advantages[5]	Caveats[5]	Possible applications/remarks	References[6]
CLEC4C-DTR (BDCA2-DTR)	*Tg(CLEC4C-HBEGF)956Cln*	tg	No	pDCs	None reported	• Most specific method reported so far for pDC depletion.	• Multiple DT injections lead to chronic IFN-I production and severe immunopathology.	• pDC depletion. • Improper for long-term pDC depletion. • Carefully check the impact of IFN-I induction triggered upon pDC death.	30x [21, 22, 128]

[1] Official nomenclature can be consulted on the following website: http://www.informatics.jax.org/, where entering a gene name will return the list of all recorded mutant alleles, and clicking on one allele will give details on the different associated nomenclatures as well as the mutation description and the mouse strains bearing it with their availability, and finally a list of the publications where this allele has been used.
[2] KO, knock-out; KI, knock-in; tg, transgenic.
[3] Crossed fluorescent reporter name indicated failure to detect its expression in the corresponding mutant mouse strain. (?) indicates lack of information on reporter expression.
[4] Baso, basophils; CTLs, cytotoxic T lymphocytes, IELs, intraepithelial lymphocytes, LCs, Langerhans cells; Mac, macrophages; MCs, mast cells; Mono, monocytes; moDC, monocytes derived DCs; Neutro, neutrophils; BM, bone marrow.
[5] Specific advantages or caveats beyond specificity issues, the combination of which determines the type of use that is recommended or advised against.
[6] Number of references recorded for the corresponded allele in the Jax database (see footnote 1), and selected references, including the first description of the mouse model and other references illustrating specificity issues, advantages, caveats, or linked to other remarks within the table. NA, not applicable (the strain is not yet recorded in the JAX database).

ple, in immunocompetent mice, all cDC1 are fate-mapped for expression of the Clec9a/DNGR-1 cell-surface marker and derive from a myeloid precursor shared with cDC2 but not with other mononuclear phagocytes, including monocytes or macrophages or with lymphocytes [31]. However, when Clec9a/DNGR-1-expressing cells are depleted by constitutive DTA expression in $Clec9a^{Cre}$;$Rosa26^{LSL\text{-}DTA}$ mice, the cDC pool can be replenished by functional cDC mimetics derived putatively from lymphoid progenitors [32]. Although this observation may appear to be a nonphysiological artifact, it does hold valuable information about the previously overlooked plasticity of precursor cells that could be harnessed in therapeutic and translational approaches.

Models for DC type depletion due to constitutive or conditional knock-out of transcription factors

Transcription factors are decisive regulators in DC lineage commitment, their release from the BM, and survival. Also, the respective key functions of terminally differentiated DC types are controlled by transcription factor activity. Thus, analyses of KO mouse models for transcription factors, either as constitutive or as conditional cell-specific or inducibly targeted alleles, have played key roles in defining not only the development but also the functional roles of DC types. However, when transcription factors that are required in early progenitors are targeted in a KO approach, often not only several DC lineages are affected but also additional hematopoietic and sometimes nonhematopoietic cell types. Deficiencies in transcription factors acting later in cellular ontogeny display a higher lineage specificity. Therefore, to achieve the highest possible specificity, the aim is to choose the mouse model mutated for the most suitable transcription factor to solve the DC-centred research questions. In addition, off-target effects should be carefully evaluated and discussed. In Table 2 part I, we aimed at listing the mouse models mutated for transcription factors that are currently available and could help to elucidate DC biology including their possible applications, advantages, and caveats. Here, we will discuss mutant mouse strains that represent exemplary targeting approaches or where recent novel findings highlighted limitations to their application.

Ikaros represents an early hemopoietic- and lymphoid-restricted transcription factors and mice harboring a homozygous dominant negative mutation in the *Ikzf1* gene lack all DC types additional to cells of the lymphoid lineage [33]. In contrast, mice carrying a hypomorphic mutation in the *Ikzf1* locus ($Ik^{L/L}$) express low levels of Ikaros leading to a lack of peripheral pDCs, but no other DC types, and have been used for evaluating pDC-dependent functions [34]. Still, when using this strain as "pDC-less" mouse model, one has to take into account that other hematopoietic lineages, including T and B cells and neutrophils, are also affected, and that $Ik^{L/L}$ mice are prone to develop thymic lymphomas [35–37]. Another mutant mouse model for the analyses of pDC functions are mice with conditional inactivation of the pDC key transcription factor E2-2 (Tcf4) specifically in $CD11c^{+}$ cells ($Tcf4^{flox/-}$;$ItgaxCre^{+}$ mice), to overcome the peri-

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Table 2. Knock-out mouse models for DC-specific genes for the analysis of physiological functions of DC types and their regulation

Targeted gene	Official nomenclature[1]	Mutation type[2]	Target gene expression pattern[3]	Target gene function in DCs[4] and caveats	Possible applications[5]	References[6]
Part I – Transcription factors						
Spi1 (PU.1)			hematopoietic precursors and macrophages	• In DCs, PU.1 represses pDC development in favour of cDC development • Caveats: Broad impact of PU.1 on lymphoid and myeloid haematopoiesis.	• Analyses of cDC vs. pDC development.	[131–133]
$Spi1^{fl/fl}$;*ItgaxCre*$^{+}$	$Spi^{1tm1.2Nutt}$	floxed allele				
Ikaros			B cells, mast cells, macrophages	• Required for pDC development. • Caveats: cell-intrinsic effects in multiple immune cell lineages such as T and B cells, and neutrophils; thymoma development.	• Analyses of pDC functions. • More specific models available for pDC ablation.	[34–37]
$Ik^{L/L}$	$Ikzf1^{tm1Kast}$	KI of the βgal into *Ikaros* gene, hypomorphic mutation				
Stat1	$Stat1^{tm1Rds}$	KO	wide constitutive expression	• Required for Type I, II, III IFN-induced gene expression.	• Model for severe immunodeficiency.	[134]
Stat3	$Stat3^{tm1Flv}$	floxed allele	Thymocytes, B cells, macrophages, DC	• Floxed in hematopoietic compartment. Positive regulator of Flt3-mediated pDC differentiation by upregulation of E2-2. • Caveats: Negative regulator in macrophage and neutrophil development; embryonic lethality of conventional KO.	• Analyses of STAT network in DC development and function.	[135–138]
Stat5ab	*Del(11Stat5a-Stat5b)1Mam*	KO of Stat5a and b	broad hematopoietic expression	• Positive regulator of $CD103^{+}$ cDC1. Suppresses pDC development. • Caveats: Conventional KO neonatally lethal.	• Analyses of STAT network in DC development and function.	[137, 139]

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Table 2. (Continued)

Targeted gene	Official nomenclature[1]	Mutation type[2]	Target gene expression pattern[3]	Target gene function in DCs[4] and caveats	Possible applications[5]	References[6]
Irf1	*Irf1*^{tm1Mak}	KO	wide constitutive expression, upregulated in IFNAR-/IFNGR-mediated feedback loop	• Involved in chromatin remodelling. Involved in IFN-I and IL-12 expression. • Caveats: Intrinsic function in T-cell differentiation.	• Model for severe immune deficiencies in innate and adaptive immune responses.	[140–143]
Irf3 *Irf3*$^{-/-}$ *Irf3*$^{fl/fl}$	*Irf3*^{tm1Ttg} *Irf3*$^{tm2.1Ttg}$	KO floxed allele	wide constitutive expression, upregulated in IFNAR-mediated feedback loop	• Required for IFN I induction in nonmyeloid cells.	• Model for lack of early IFN-I expression, setting dependent. • Optional use as double KO with *Irf7*.	[144–146]
Irf4 *Irf4*$^{-/-}$ *Irf4*$^{fl/fl}$	*Irf4*^{tm1Mak} *Irf4*^{tm1Rdf}	KO floxed allele	cDC2, pDCs, B cells, T dells	• Required for cDC2 development. • Caveats: Cell intrinsic effects in T- and B-cell lineages.	• Analyses cDC1 vs. cDC2 vs. pDCs development and function. • BM chimeras recommended.	[147–151]
Irf5	*Irf5*^{tm1Ttg}	KO	wide constitutive and inducible expression	• Modulation of IFN-I expression. • Induction of proinflammatory cytokines.	• Analyses of IRF network modulation in IFN expression.	[152, 153]
Irf7	*Irf7*^{tm1Ttg}	KO	constitutively high in pDCs, upregulated IFNAR-mediated feedback loop	• Lack of IFN-α expression - virus dependent. • Caveats: Possible compensation via IRF-5 and IRF-1.	• Modelling lack of IFN-α expression-setting dependent. • Optional use as double KO with *Irf3*.	[70, 145, 154]
Irf7 pDC:IRF7^{+} *Siglech*$^{Irf7/+}$; *Irf3*$^{-/-}$; *Irf7*$^{-/-}$	missing	KI of *Irf7* under control of *Siglech* promoter on *Irf3*$^{-/-}$;*Irf7*$^{-/-}$ double KO background	pDCs [155, 156], macrophage subsets [128], DC progenitors [157]		• Restriction of IFN-I production to pDCs.	[71]

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Table 2. (Continued)

Targeted gene	Official nomenclature[1]	Mutation type[2]	Target gene expression pattern[3]	Target gene function in DCs[4] and caveats	Possible applications[5]	References[6]
Irf8 (ICSBP)			cDC1, pDCs, also B cells	• Required for cDC1 development and pDC function.	• Analyses of cDC1 vs. cDC2 vs. pDCs development and function.	[149, 151, 158]
$Irf8^{-/-}$	$Irf8^{tm1.2Hm}$	KO				
$Irf8^{fl/fl}$	$Irf8^{tm1Hor}$	floxed allele				
$Irf8^{BXH2}$ ($Irf8^{R294C}$)	$Irf8^{myls}$	SPM; hypomorphic allele	cDC1, pDCs, also B cells	• Required for cDC1 development without affecting pDCs.	• Analyses of the role of cDC1.	[159]
Irf8 $+32^{-/-}$	$Irf8^{em1Kmm}$	CRISPR/Cas9 mutations, deletion of IRF8 downstream 32-kb and 41-kb enhancers	cDC1, pDCs, also B cells	• *Irf8* enhancers required for cDC1 development and specification (altered pDC phenotype in Irf8-41- mice).	• Analyses of cDC1 development and function.	[160]
Irf8 $+41^{-/-}$	$Irf8^{em2Kmm}$					
Traf6	$Traf6^{tm1Ywc}$	KO	wide constitutive expression	• Contributes to cDC2 development and function.	• Analyses cDC1 vs. cDC2 vs. pDCs development and function. • BM chimeras recommended.	[161]
Relb			cDC2	• Contributes to cDC2 development. • Caveats: Multiorgan inflammation.	• Analyses cDC1 vs. cDC2 vs. pDCs development and function. • BM chimeras recommended.	[161, 162]
$Relb^{-/-}$	$Relb^{tm1Bru}$	KO				
$Relb^{fl/fl}$	$Relb^{tm1.1Fwei}$	floxed allele				
Rbpj	$Rbpj^{tm1Hon}$	floxed allele crossed to Itgax-Cre	Coexpression of RBP-J and Itgax in pDCs, tDCs, pDC-like cells, monocytes, macrophages, and subsets of B lymphocytes	• Required for presence of splenic $CD8^-$ DCs. • Caveats: check for other cells that might be affected.	• Analyses of the role of splenic cDC2.	[131]

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Table 2. (Continued)

Targeted gene	Official nomenclature[1]	Mutation type[2]	Target gene expression pattern[3]	Target gene function in DCs[4] and caveats	Possible applications[5]	References[6]
Mtg16 (ETO-2, CBFA2T3)	*Cbfa2t3*$^{tm1.1Swh}$	KO	DC precursors, marginal zone B cells, mast cells, cDC1, cDC2, pDC	• Promotes pDC development and pDC IFN-I production. Transcriptional cofactor, no direct DNA binding. • Represses Id2 expression.	• Analyses of cDC vs. pDCs development and function.	[163, 164]
Id2	*Id2*^{tm1Yyk} *Id2*^{tm1Reiz} *Id2*$^{tm1.1(cre/ERT2)Blh}$	KO	Mast cells, NK cells, macrophages, microglia	• Required for cDC1 development. Partially required for LC development. • Caveats: lack of LNs and PPs, NK cells strongly reduced.	• Analyses cDC1 vs. cDC2 vs. pDCs development and function. • BM chimeras recommended.	[164–167]
Tcf4 (E2-2) *Tcf4*$^{-/-}$ *Tcf4*$^{flox/-}$;*ItgaxCre*$^{+}$ *Tcf4*$^{flox/-}$; *R26-CreER*$^{+}$	*Tcf4*^{tm1Zhu} *Tcf4*^{tm1Hmb}	KO floxed allele	Expression of Tcf4 (and Itgax-Cre) in pDCs, tDCs, pDC-like cells, monocytes, macrophages and subsets of B lymphocytes	• Required for pDC development and function BM chimeras. Reduced numbers and IFN production in *Tcf4*$^{+/-}$. • Reduced numbers of pDCs in Itgax-Cre and R26-CreER-driven KOs.	• Analyses of the role of pDCs. • Relevance of optimal numbers of pDCs.	[1, 38, 39, 168, 169]
Tcf4 splice variant	*Tcf4*$^{tm1a(EUCOMM)Wtsi}$	KO of large splice variant	DC specific, additionally in GC B cells (centroblasts) partial lethality at birth	• Involved in pDC development and function • Reduced numbers of pDCs in KO.	• Relevance of optimal numbers of pDCs.	[170]
Bcl11a *Bcl11a*$^{-/-}$ *Bcl11a*$^{fl/fl}$	*Bcl11a*^{tm1Ngc} *Bcl11a*^{tm1Pwt}	KO floxed allele	T, B cells, mono, megakaryocytes	• Required for pDC and B-cell development transcriptional regulator of E2-2, Id2, Mtg16 • Caveats: Perinatal lethality of conventional KO	• Study of pDC functions.	[171, 172]

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Table 2. (Continued)

Targeted gene	Official nomenclature[1]	Mutation type[2]	Target gene expression pattern[3]	Target gene function in DCs[4] and caveats	Possible applications[5]	References[6]
Runx2	*Runx2*^{tm1Mjo}	KO	pDCs	• Required for release of mature Ly49Q^{+} pDCs from BM into periphery. • Caveats: Osteoblast activation; perinatal lethality of conventional KO (failed ossification).	• Study of mature pDC functions in BM chimeras.	[173, 174]
Zfp366	missing	KO	cDCs, LCs, RPMs [175]	• Promotes cDC1 development. Promotes cell-associated Ag capture and cross-presentation by cDC1. • Promotes IL-12 production by cDC1. • Caveats: Check for possible effects in cDC2, LCs and RPMs.	• Analyses the role of cDC1.	[175]
Batf3	*Batf3*^{tm1Kmm}	KO	cDCs, upregulated in T cells after activation [42]	• Required for CD8^{+} cDC1 development Caveats: cell-intrinsic effects on memory CD8^{+} T-cell differentiation and on Tregs. • Compensation by BATF or BATF2 under certain inflammatory conditions.	• Analyses of role of cDC1.	[41–45]
Zbtb46 (zDC)	*Zbtb46*$^{tm2.1Mnz}$	KO	cDCs	• Prevents cDC maturation at steady state.	• Analyses of the role of cDC1.	[18, 176]
Nfil3 (E4BP4)	*Nfil3*^{tm2Look}	KO	NK cells, upregulated in activated macrophages	• Required for development of cDC1 • Caveats: Reduced numbers of mature NK cells; defects in B-cell class switch.	• Analyses of cDC1 development.	[177–179]

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Table 2. (Continued)

Targeted gene	Official nomenclature[1]	Mutation type[2]	Target gene expression pattern[3]	Target gene function in DCs[4] and caveats	Possible applications[5]	References[6]
Klf4 *Klf4*$^{-/-}$ *Klf4*$^{fl/fl}$	*Klf4*$^{tm1.1Khk}$ *Klf4*^{tm1Khk}	KO floxed allele	Monocytes, CD8^{+} T cells, NK cells, and DCs [180]	• Involved in cDC and required for NK cell development • Caveats; Impact also on macrophage and T-cell populations; neonatal lethality of conventional KO.	• Analyses of cDC1 vs. cDC2 vs. pDC development and functions. • Use specific Cre mice.	[180–182]
Zeb2 *Zeb2*$^{-/-}$ *Zeb2*$^{fl/fl}$	*Zeb2*$^{tm1.2Yhi}$ *Zeb2*$^{tm1.1Yhi}$	KO floxed allele	cDC2, pDC	• Required for cDC2 and pDC development represses Id2 • Caveats: Required for NK cell maturation and T-cell differentiation; embryonic lethality of conventional KO between E9.5 and E10.5.	• Analyses of cDC2 and pDC development and function.	[177, 183, 184]
Part II – Non-transcription factor molecules						
Clec9a	*Clec9a*$^{tm1.1Crs}$ *Clec9a*$^{tm1.1Kens}$	KO/reporter KO	• cDC1 [185, 186], pDCs in mice. • Conserved m/h [185–187].	• Promotes recognition of dying cells and cross-presentation of cell-associated antigens through signaling for phagosomal rupture. Dampens neutrophil recruitment to inflamed tissues. • Can paradoxically limit antitumor immunity by limiting recruitment of cDC1. • Caveats: Check for possible effects in pDCs and DC precursors.	• Analyses of the role of cDC1.	[46-49, 51, 52, 188, 189]
Xcr1	*Xcr1*^{tm1Dgen} *Xcr1*^{tm1Ksho}	KO/reporter	• cDC1 [56, 58, 190] • Conserved in vertebrates [191].	• Promotes cDC1 recruitment towards Xcl1-producing cells in inflamed tissues or tumors.	• Analyses of the role of cDC1.	[29, 56, 57, 192]

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Table 2. (Continued)

Targeted gene	Official nomenclature[1]	Mutation type[2]	Target gene expression pattern[3]	Target gene function in DCs[4] and caveats	Possible applications[5]	References[6]
Wdfy4	*Wdfy4*$^{em1(IMPC)J}$	KO	cDC1	• Promotes cell-associated antigen cross-presentation.	• Analyses of the role of Ag cross-presentation by cDC1.	[53]
Rab7b	*Rab7b*^{tm2Ciphe}	KO	• cDC1 [190], MoDCs • Conserved m/h [190].	• Regulates DC migration by linking lysosomes to cytoskeleton.	• Testing the role of DC migration.	[193]
Rab43	*Rab43*$^{tm1.2Kmm}$	KO		• Promotes antigen cross-presentation.	• Testing the requirement for antigen cross-presentation.	[194]
Siglech	*Siglech*$^{tm1.1Cfg}$ *Siglech*$^{tm1.1Ksat}$ *Siglech*$^{tm1.1Cln}$	KO KO/hDTR KO/reporter	• pDCs, [155, 156], macrophage subsets [128], DC progenitors [157] • No ortholog in human	• Limits pDC IFN-I production through DAP12 signaling.	• Modelling the role of pDC deregulation in promoting autoimmune or inflammatory diseases.	[21, 128, 130, 195-197]
Slc15a4	*Slc15a4*^{m1Btlr}	KO/SPM/ENU	pDCs [60], B-cell subsets [65], monocyte/macrophage subsets,	• Promotes TLR7/9-dependent pDC IFN-I production. • Caveats: A phenotype in KO mice cannot be interpreted directly as caused by pDC-specific defects.	• More specific models for pDC-specific loss of IFN-I/III production are needed.	[60, 63, 65, 198]
Pacsin1	*Pacsin1*$^{Gt(OST287788)Lex}$	KO/gene trap	• pDCs [58] and neurons • Conserved m/h [58].	• Promotes TLR7/9-dependent pDC IFN-I production.	• Testing the role of pDC IFN-I/III production.	[66]
Tmem176b	*Tmem176b*^{tm1Ics}	KO	cDC2 [58], RORγt-expressing lymphocytes [199]	• Promotes cDC2 cross-presentation with a tolerogenic outcome.	• Analyses of the role of cDC2.	[199–201]

[1] Official nomenclature, for further information see Table 1 footnote 1.
[2] KO, knock-out; SPM, single-point mutation; ENU, mutant mouse model chemically induced by *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis; gene trap, mutation by insertion of gene trap vector in ES cells.
[3] Target gene expression pattern based on indicated references and/or the public resources bioGPS for various tissues and cell types (http://biogps.org/) and Immgen (https://www.immgen.org) for immune cell types. Conserved m/h, the gene expression pattern is well conserved between mouse and human.
[4] Target gene function in DCs based on current knowledge from literature.
[5] Possible applications, beyond determining the roles of the gene in DC biology.
[6] Selected references, including the first description of the mouse model and further functional studies of the role of the gene in DCs.

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natal lethality of the conventional E2-2-KO mouse strain [38]. However, *Tcf4* and *Itgax* are coexpressed in other cells, additionally to pDCs, not only in the recently described transitional DCs and pDC-like cells whose development is also compromised in the $Tcf4^{flox/-}$;$ItgaxCre^{+}$ mice, but also in subsets of monocytes, macrophages, and B cells [1, 39]. This illustrates the importance of carefully controlling the expression pattern of the driver genes used to target DC types. In addition, the genetic background of different inbred mouse strains as well as gender-specific differences may result in significant alterations in DC and other leukocyte cellularities and have to be considered when working with mutant mouse strains [40].

One of the most widely used mouse models to define the in vivo functions of cDC1 cells are *Batf3*-KO mice [41]. It has recently been shown that besides lacking $CD8\alpha^{+}$ cDCs, BATF3-deficiency has cell-intrinsic effects on memory $CD8^{+}$ T-cell differentiation and on Tregs [42–44]. Another caveat of this mouse model is a possible leakiness of the cDC1 ablation due to compensation by BATF or BATF2 under certain inflammatory conditions [45]. Compensatory mechanisms also might turn out to be relevant for other mouse models where the intricate network of transcription factors significantly changes between steady state and activation.

Models enabling DC type-specific loss- or gain-of-function through gene manipulation

Once constitutive or conditional loss of a specific DC type has been proven to alter a biological process, for example, compromising the ability of the host to fight a viral infection or the development of a tumor, determining the underlying mechanisms will require DC type-specific loss- or gain-of-functions through gene manipulation. This can be achieved through different approaches including studying the consequences of the full KO of candidate genes known to be specifically expressed in the target DC type, restricting the expression of a broadly expressed candidate gene specifically to the target DC type, or, on the contrary, conditionally inactivating candidate genes selectively in the target DC type.

Knock-out mouse strains for genes specifically expressed in DC types

A limited number of genes are expressed rather selectively in specific DC types. Their functional study has shed light on the molecular mechanisms regulating the activation of DC types and their functional specialization, as summarized in Table 2 part II. Here, we will briefly discuss selected examples illustrating the power but also limitations of this approach.

Clec9a is an endocytic receptor of the C-type lectin family, selectively expressed at the surface of cDC1 in both humans and mice, with lower expression also detected on pDCs in mice. The study of *Clec9a*-KO mice revealed that this receptor is a key to promote cross-presentation of antigens from necrotic cells. *Clec9a*-KO mice were impaired in the cross-priming of antiviral $CD8^{+}$ T cells eventually leading to enhanced susceptibility to certain infections [46, 47]. Paradoxically, Clec9a can dampen antitumor immunity by limiting the recruitment of cDC1 [48]. Mechanistically, Clec9a binds the F-actin that gets exposed at the surface of the cells undergoing necrosis [49, 50], an immunogenic cell death that can occur as a consequence of viral infection or cellular transformation. This enables cDC1 to take up antigens from these dying cells and route them intracellularly for cross-presentation upon phagosomal rupture [51]. Hence, *Clec9a*-KO mice could be used to assess the role of antigen cross-presentation by cDC1 in vivo, although possible effects in pDCs or in DC precursors should be carefully checked, as well as other potentially confounding effects such as the dampening of neutrophil recruitment in inflamed tissues [52]. Another tool that could be used to assess the role of antigen cross-presentation in vivo by cDC1 is the *Wdfy4*-KO mouse strain [53], although possible cell-intrinsic effects in $CD8^{+}$ T cells should be carefully checked [54].

Xcr1 is a chemokine receptor that is expressed specifically in cDC1 in a conserved manner across warm-blooded vertebrates [55–58]. Xcr1 ligands, lymphotactins alias Xcl1 and Xcl2, are expressed preferentially by cytotoxic lymphocytes including NK cells and $CD8^{+}$ T cells. *Xcr1*-KO mice mount decreased $CD8^{+}$ T-cell responses to infections with *Listeria monocytogenes* [56] or murine cytomegalovirus (MCMV) [59]. This could result from a direct role of Xcr1/Xcl1 in promoting efficient interactions between cDC1 and $CD8^{+}$ T cells [57]. Alternatively, this could be caused by disrupting the cross-talk between NK cells and cDC1, leading to impaired licensing of cDC1 for functional maturation and migration to the T-cell zone of lymphoid organs, as demonstrated in the MCMV infection model [59].

Slc15a4 is an endolysosome-resident amino acid transporter that was identified in a broad genetic screen as being essential for IFN-I production by pDCs in response to TLR stimulation [60]. Because Slc15a4 expression was thought to be restricted to pDCs, and TLR responses of the other cell types tested did not appear to be affected, *Slc15a4*-deficient mice have been considered as good model to assess requirements for pDC IFN-I production, similar to *TLR7*-deficiency in humans [61]. Since both *Tlr7*-KO- and *Slc15a4*-deficient mice are more susceptible to chronic LCMV infection [62, 63], it was deduced that this must be due to protective effects of pDC IFN-I production [63]. However, neither *TLR7* nor *SLC15A4* are expressed only in pDCs. Indeed, both are also expressed in monocyte or macrophage and B-cell subsets (http://www.immgen.org) and have been demonstrated to promote B-cell functions in a cell-intrinsic manner [64, 65]. Hence, a phenotype in *Tlr7*- or *Slc15a4*-deficient mice cannot directly be attributed to defects in pDC functions; possible cell-intrinsic effects in monocytes/macrophages or B cells should be carefully checked. Another tool that could be used to assess the role of IFN-I production in vivo specifically by pDCs is the *Pacsin1*-KO mouse strain, since this gene is rather selectively expressed in pDCs within hematopoietic cells in a conserved manner between mice and humans [58], and is critical for pDC IFN-I production in response to TLR stimulation [66].

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In conclusion to this section, it is important to emphasize that although several gene-KO mouse strains have been reported as suitable models to assess the requirement of a given function of specific DC types in vivo, such as cross-presentation by cDC1 or IFN-I production by pDCs, results of such experiments must be interpreted with caution. Cell-intrinsic effects of the respective mutation in other cell types should be carefully ruled out. The interpretation of results should be strengthened by assessing whether different models or experimental approaches converge to the same conclusions.

Mutant mouse strains restoring the expression of a knock-out gene specifically in DC

Another approach to investigate the in vivo role of DC types is to determine whether their responses are sufficient to promote health or disease. This can be achieved by restoring in DCs the expression of a gene of interest on a KO-background for this gene.

For example, this method has been applied for MyD88, an adaptor molecule that is essential for signalling downstream of most TLRs as well as of the receptors for the cytokines of the IL-1 family, thus, having broad functions in many cell types. One approach that has been used to decipher the role of MyD88 signaling in distinct cell types was to generate mutant $Myd88^{OFF}$ mice enabling reactivation of the gene in a cell type-specific manner. In $Myd88^{OFF}$ mice, a floxed transcriptional stop was knocked in between exons 1 and 2, leading to loss of expression, but enabling conditional reactivation of Myd88 expression in Cre-expressing cells. Hence, in $Cd11c^{Cre}$;$Myd88^{OFF}$ mice, MyD88 signaling was restored specifically in $CD11c^{+}$ cells including DCs. This approach showed that MyD88 signalling in $CD11c^{+}$ cells was sufficient to protect mice from infections with *Mycobacterium bovis* BC [67], *L. monocytogenes* [68], or MCMV [69]. More specific Cre mouse models need to be used to determine whether these protective effects of MyD88 signalling occur in DCs only and specifically in which DC type.

IRF-3 and IRF-7 are transcription factors that are essential for the induction of IFN-I in potentially any cell type, upon detection of viral replication by cytosolic sensors recognizing DNA or specific RNA conformations in infected cells, or downstream of the detection of viral nucleic acids in the endosomes of mononuclear phagocytes including pDCs. Thus, mice that are double deficient for IRF-3 and IRF-7 generally fail to produce IFN-I and are highly sensitive to many viral infections, although compensation via IRF-5 can occur for viral infection sensing and downstream IFN-I production in cDCs [70]. To determine whether IFN-I production by pDCs is sufficient to protect against viral infections, IRF-7 expression was restored in *Irf3*;*Irf7*-double KO mice under the control of the promoter of the *Siglech* gene that was thought to be specifically expressed in pDCs. This strategy was sufficient to restore the control of dengue and Chikungunya virus infections [71]. However, since *Siglech* is expressed in DC precursors, tDCs, and macrophage subsets, including microglia (http://www.immgen.org), complementary approaches are needed to confirm that the underlying mechanism is IFN-I production by pDCs. In addition, it remains to be determined whether pDC IFN-I production is necessary for the control of viral infections, which is not the case in most of the experimental models examined so far [72–74] with a few exceptions [75, 76].

Conditional knock-out mouse strains for a target gene specifically expressed in DC types

To investigate whether and how DC types are necessary to promote health or cause disease in a variety of pathophysiological models in vivo, one of the most popular methods is conditional inactivation of candidate genes specifically in the target DC type by using the Cre/Lox system. In brief, a conditional mutant allele is generated by flanking key exons with LoxP sequences that are recognized by the Cre recombinase; Cre will excise the flanked genomic sequence, leading to disruption of the gene specifically in the cells expressing this recombinase. This approach has been used broadly for many cell types and candidate genes and has led to numerous key discoveries. An ever-increasing number of Cre driver mutant mouse strains is becoming available to target DC types, as we attempted to summarize in Table 3. However, it is important to emphasize that the Cre/Lox system is not without caveats: several factors can affect the specificity or efficacy of conditional gene inactivation. Many Cre-expressing mutant mouse strains suffer from germline recombination, whereby Cre is active and excises the floxed target sequences during meiosis, leading to a full KO allele. For example, this event is occurring at high frequency in some $Xcr1^{iCre}$ mouse strains, requiring the implementation of specific, cumbersome, and costly breeding strategies to overcome this problem [28]. Germline recombination has also been reported for the $Cd11c^{Cre}$ mouse strain [77, 78]. More generally, even in the absence of overt germline recombination, unfaithful recombination can occur in other cell types than the originally intended one, and these issues differentially affect distinct floxed alleles [79, 80]. Hence, when using a Cre/Lox strategy, several steps are crucial to decrease the risk of overlooking unwanted recombination events and, thus, drawing inaccurate conclusions. First, the use of fluorescent reporters is highly recommended to assess in which cell types Cre activity has occurred and with what penetrance [81]. Second, if the use of these fluorescent reporters shows that germline recombination occurs in a fraction of the mutant mice, especially when the floxed allele and the Cre transgene are present in the same parent/gamete, then all individual mutant mice must be genotyped to discard those that are full KO or partial chimera. Even when no germline recombination is detected, the specificity of the conditional deletion should be checked in the first sets of experiments, by comparing gene excision in the target cell types versus other cell types, where unfaithful gene deletion could cause confounding effects and lead to erroneous interpretation [82]. Depending on the results, the use of alternative Cre drivers or of a tamoxifen-induced Cre activity might be necessary to improve specificity [83]. Regarding the

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Table 3. Selected Cre-expressing mouse strains with their possible applications to study DC types

Common name	Official nomenclature[1]	Mutation Type[2]	Targeted cells[3]	Other cells affected[3]	Advantages[4]	Caveats[4]		Possible applications /remarks	References[6]
						GR[5]	Other issues		
CD11c-Cre-tg (Itgax-Cre)	*Tg(Itgax-cre)1-1Reiz*	tg	cDC1 (>95%) cDC2 (>95%)	pDCs (~90%) Mac (30-95%)[7] Mono (~25%) MCs (~20%) NK cells (~20%) T cells (10-20%) B cells (10-40%)	• Widely used model. • High Cre penetrance in DCs.	Y	• Check for other cells that might be targeted.	• To target all DCs. More specific mouse models are now recommended. • Systematically check for GR in each individual mouse.	468x [77, 78, 131, 202]
	Tg(Itgax-cre,-EGFP)4097Ach	tg	cDCs	Not reported		ND			61x [203]
LysM-Cre	*Lyz2*$^{tm1(cre)Ifo}$	KI	Mac (40-100%) Neutro (60-80%) Mono (~40%)	cDCs (~10%) pDCs (~5%)	• Widely used model.	ND	• KO of the *Lyz2* gene: use heterozygous mice.	• To help identify MoDCs in vivo, discriminating them from cDC2 & inf-cDC2.	1724x [202, 204]
Ms4a3^{Cre}	*Ms4a3*$^{em2(cre)Smoc}$	KI	Monocytic lineage	Neutro	• Targets selectively monocyte-derived cells.	Y	• Recombination efficacy highly dependent on target gene.	• To identify MoDCs through fate mapping.	2x [205]
Cd207-Cre	*Cd207*$^{tm2.1(cre)Bjec}$	KI	LCs	CD207$^+$ cDC1		ND		• To target either LCs or cDC1. • More specific mouse models are now recommended.	15x [206]
Cd207-Cre-tg	*Tg(CD207-cre)1Dhka*	tg	LCs		• Specifically targets LCs, not CD207$^+$ cDC1.	ND		• To target LCs.	21x [207]
Zbtb46-Cre	*Zbtb46*$^{tm3.1(cre)Mnz}$	KI	cDC1 cDC2	Endothelial cells	• Discriminates cDC1 and cDC2 from pDCs and Mo/Mac.	ND	• Requires using BMC mice to overcome recombination in endothelial cells. • Might target MoDCs.	• To simultaneously target cDC1 & cDC2. • Check MoDC targeting.	7x [208]

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Table 3. (Continued)

Common name	Official nomenclature[1]	Mutation Type[2]	Targeted cells[3]	Other cells affected[3]	Advantages[4]	Caveats[4]		Possible applications /remarks	References[6]
						GR[5]	Other issues		
Clec9a-Cre	*Clec9a*$^{tm2.1(icre)Crs}$	KI	cDC1 (>95%) cDC2 (~50%) pDCs (10-20%)	Not reported.	• High Cre penetrance in cDC1.	No		• To target cDC1 or all cDCs and pDCs depending on the Cre penetrance required. • More specific mouse models for cDC1 are now recommended.	9x [31]
Karma-Cre	*Gpr141b*$^{tm2(icre)Ciphe}$	KI	cDC1	MCs	• Relatively high Cre penetrance in cDC1 (less than Xcr1^{iCre}). • No GR (as is the case for Xcr1^{iCre})	No	• Expressed at late stages in the cDC1 lineage. • Target gene deletion might occur too late depending on the biological process studied.	• To target cDC1. • Check the impact of MC targeting.	1x [28, 209]
Xcr1-Cre (Kaisho)	*Xcr1*$^{tm4(cre)Ksho}$	KI, KO	cDC1	Not reported.		Y	• KO for *Xcr1*: use heterozygous mutant mice. • Requires complex breeding schemes to overcome GR issue.	• To target cDC1.	2x [29]
Xcr1^{iCre} (Dalod)	*Xcr1*$^{tm1(icre)Ciphe}$	KI	cDC1	CD4 T cells (<1% in most organs, up to ~14% in skin).	• *Xcr1* expression is retained. Drives Cre expression early in the cDC1 lineage. • High penetrance and specificity of Cre activity in cDC1.	H	• Requires complex breeding schemes to overcome GR issue	• To target cDC1.	2x [24, 28]

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Table 3. (Continued)

Common name	Official nomenclature[1]	Mutation Type[2]	Targeted cells[3]	Other cells affected[3]	Advantages[4]	Caveats[4]		Possible applications /remarks	References[6]
						GR[5]	Other issues		
Xcr1^{iCre} (Malissen)	*Xcr1$^{tm2(icre-mTfp1)Ciphe}$*	KI	cDC1 (Expression of TEAL fluorescent reporter)	CD4 T cells (<1% in most organs, up to ~14% in skin).	• Xcr1 expression is retained. Drives Cre expression early in the cDC1 lineage. High penetrance and specificity of Cre activity in cDC1.	H	• Requires complex breeding schemes to overcome GR issue.	• To target cDC1.	NA [93]
Xcr1^{Cre} (Murphy)	*Xcr1$^{tm1.1(cre)Kmm}$*	KI	cDC1	Not reported.	• *Xcr1* expression is retained.	Y	• May require complex breeding schemes if GR occurs frequently as observed in the other *Xcr1^{Cre}* models.	• To target cDC1.	1x [210]
pDCre-tg	*Gt(ROSA)26Sor^{tm1Hjf} Tg(Siglech-cre,-mCherry)#Spar/Ph*	tg	pDCs (<30%)	cDCs CD11c^{int} BM cells B, T, NK & NK-T cells		Y	• Low efficiency of pDC targeting.	• To target pDCs. Gain-of-function studies. • Check the impact of other cell type targeting.	1x [195]
Siglech^{iCre}	*Siglech$^{tm1.1(iCre)Ciphe}$*	KI	pDCs (>98%)	cDCs CD11c^{int} BM cells B, T, NK & NK-T cells	• Very high efficiency of pDC targeting.	No		• To target pDCs. Loss-of-function studies. • Check the impact of other cell type targeting.	1x [211]
Csf1r-cre-tg	*Tg(Csf1r-cre)1Mnz*	tg	Mononuclear phagocytes			ND		• Specific targeting of cDCs using intersectional genetics.	4x [107]
Csf1r-Cre-tg	*Tg(Csf1r-icre)1Jwp*	tg	Mac	Mono cDCs		ND		• Prospective interest for cDC targeting using intersectional genetics.	39x [212]

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Table 3. (Continued)

Common name	Official nomenclature[1]	Mutation Type[2]	Targeted cells[3]	Other cells affected[3]	Advantages[4]	Caveats[4]		Possible applications /remarks	References[6]
						GR[5]	Other issues		
CD11b-Cre-tg	*Tg(Itgam-cre)AJva*	tg	Mac (40-95%), Mono, cDCs, pDCs, MCs, Baso, Eos (~20% in each cell type)	B cells (~15-30%) T cells (~15-20%) NK cells (~20%)		ND		• Prospective interest for intersectional genetics targeting of cDC2.	15x [202, 213]
CD11b-Cre-tg	*Tg(ITGAM-cre)2781Gkl*	tg	Myeloid cells	Not reported.		ND		• Prospective interest for intersectional genetics targeting of cDC2.	22x [214]
Cx3cr1-Cre-tg	Tg(Cx3cr1-cre)MW126Gsat	tg	Mono (20-60%) Mac (40-80%)	cDC2 (~85%) cDC1 (~25%) pDCs (~10%)		ND		• Prospective interest for intersectional genetics targeting of DC types.	20x [202, 215]
Cx3cr1-Cre	*Cx3cr1$^{tm1.1(cre)Jung}$*	KI	Mono Mac	cDC2, cDC1, pDCs		ND		• Prospective interest for intersectional genetics targeting of DC types.	58x [216]
Cx3cr1-CreER	*Cx3cr1$^{tm2.1(cre/ERT2)Jung}$*	KI			• Tamoxifen-inducible Cre activity, to control gene edition in time.	No	• Tamoxifen side effects.	• Prospective interest for time-controlled targeting of DC types using intersectional genetics.	83x [78, 216]

[1] Official nomenclature, for further information see Table 1 footnote 1.
[2] KO, knock-out; KI, knock-in; tg, transgenic.
[3] Baso, basophils; Eos, eosinophils; LCs, Langerhans cells; Mac, macrophages; MCs, mast cells; Mono, monocytes; Neutro, neutrophils; BM, bone marrow. When available, the percent of targeted cells in each indicated cell type is given, based on published flow cytometry analyses of F1 mice after crossing with a Rosa26-LSL-<fluorescent_reporter> mouse strain.
[4] Specific advantages or caveats, beyond specificity issues, the combination of which determines the type of use that is advisable or on the contrary not recommended.
[5] GR, Germline recombination. H, high frequency of GR; Y, yes, GR or unfaithful recombination have been reported; L, low frequency of GR; No, no GR has been observed so far; ND, Not determined.
[6] Number of references recorded for the corresponded allele in the Jax database (see footnote 1), and selected references, including the first description of the mouse model, and other references illustrating specificity issues, advantages, caveats, or linked to the other remarks included in the table. NA, not applicable (the strain is not yet recorded in the Jax database).
[7] Differential targeting of distinct macrophage types: RPM, ~70%; MZM, ~40%; peritoneal Mac, 30%; alveolar Mac >95%.

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latter option, tamoxifen side-effects, such as hepatotoxicity, need to be considered [84].

Alternative strategies for the inactivation of target functions or genes in a specific DC type

Mixed BM chimeric mice

To overcome some of the caveats of the Cre/Lox system, alternative methods have been developed for the conditional inactivation of candidate genes specifically in the target DC type. Mixed BM chimeric (MBMC) mice can be used to exploit existing conventional KO mice as BM donors in such a manner that in recipient animals, the candidate gene is entirely missing only in the target cell types. For example, mice specifically deficient for IL-15 production by $CD11c^+$ cells were generated by injecting a 1:1 mixture of *Il15*-KO and Cd11c-DTR BM cells into lethally irradiated recipient mice and treating or not the resultant MBMC mice with DT prior to stimulation with a the TLR9 ligand CpG. In control MBMC mice, half of each hematopoietic cell type was WT and the other half *Il15*-KO, whereas in DT-treated MBMC mice, all $CD11c^+$ cells were *Il15*-KO. This strategy enabled the authors to show that, in CpG-injected mice, IL-15 is produced mainly by DCs and that DC-derived IL-15 is essential for CpG-induced prophylactic protection against *L. monocytogenes* infection [85]. A similar strategy has been used to show that DC responsiveness to IFN-I is critical for antitumor [86, 87] and antiviral [88] immunity. One advantage of MBMC mice over the Cre-/LoxP-dependent conditional genetic inactivation strategy is the ability to use existing syngeneic KO mice for potentially any nonlethal genetic mutation, without facing the limitations inherent to the still limited zoo of floxed alleles, and without the need for additional breeding. However, the fact that 50% of all hematopoietic cells are KO in MBMC mice could impact the biological process under study, and further confounding effects might be caused by the total body irradiation of the recipient mice that is necessary for full replacement of their BM with the donor cells.

Shield BM chimeric mice

An interesting alternative to overcome some of the limitations of MBMC mice is the use of shield BM chimeric (SBMC) mice as illustrated in Fig. 1. In brief, recipient mice constitutively and specifically lacking the target DC type (e.g. cDC1, Fig. 1A) undergo hind leg irradiation and BM engraftment from KO donor mice. In these SBMC, cDC1 derive from the KO donor mouse whereas other hematopoietic cells are derived from the recipient mouse and, thus, are WT (Fig. 1B). Like MBMC mouse generation (Fig. 1C), SBMC mouse generation is versatile and cost-efficient, harnessing already available recipient and donor mouse strains without the need to engineer and intercross novel mouse strains. In addition, recipient animals do not suffer from severe immunosuppression requiring antibiotic treatment until immune reconstitution is achieved upon lethal full-body irradiation for MBMC mice. Consequently, SBMC mice can also be used earlier, since DCs are efficiently reconstituted as soon as 4 weeks after transplantation. This strategy enabled us to show that IFN signalling through STAT1 in cDC1 is necessary for immune control in a breast cancer mouse model [24].

Fluorescent reporters for tracking the functions of DC types and their choreography in vivo

To understand the physiological functions of DC types, it is critical to determine how they act and are regulated in time and space in complex in vivo settings. This can be achieved by using mice expressing a fluorescent reporter, enabling the in vivo tracking of a given DC type or of its activity. Two classes of reporter mice can be distinguished here. The first class consists of mice expressing a fluorescent reporter under the direct control of the promoter of a gene expressed in a specific DC type or activation state. It allows real-time tracking of the DC type or of its function in situ. However, for tracking DC types in situ, signal intensity may be limited depending on the strength of the driver promoter or on the intensity of the fluorescent protein used; it may also vary according to the DC activation state. These limitations can be overcome by using the second type, indirect reporter mice. Here, mice expressing the Cre recombinase under the control of a promoter expressed specifically and early in the targeted DC lineage are crossed to mice expressing an inducible fluorescent reporter cassette under the control of a strong, ubiquitous promoter. In this setting, all the targeted cells will express the same, strong level of fluorescence, irrespective of the promoter of the Cre driver and of the cell activation state. A comprehensive list of direct reporter mouse strains available for tracking specific DC types and their functions, such as cytokine or chemokine secretion, can be found in Table 4, where we also point out technical details and possible applications of the individual reporter models. To choose a Cre driver mouse strain for indirect reporter strains, the reader should refer to Table 3. For help in choosing a Rosa26-LSL-fluorescent reporter strain, we recommend reading the paper by Liu et al. [89] and consulting the list of strains listed in the MGI database. Several points must be considered when deciding the suitability of reporter mouse strains for answering a DC-related research question. Possible methods include identification of the DC of interest by flow cytometry analysis and sorting for recultivation in vitro or adaptive transfer in vivo, as well as localization and cellular interactions studied using static imaging or intravital microscopy. The sensitivity of the reporter system depends on the signal to noise ratio of the reporter fluorescence. While for static imaging in confocal microscopy signal amplification strategies using antibody stainings for the fluorescent protein are available, dynamic intravital imaging depends on a bright fluorescence with a reasonable signal-to-noise ratio. For several fluorescent proteins, variants are now available with shorter folding times, such as the Venus variant of YFP [90], that was used in the

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Table 4. Fluorescent-reporter mouse models for the study of spatiotemporal dynamics of DC functions in vivo

Reporter strain	Official nomenclature[1]	Mutation type[2]	Marked cell population[3]	Visualization methods reported	Comments[4]	References[5]
CD11c-Venus (Itgax-Venus, CD11c-EYFP)	*Tg(Itgax-Venus)1Mnz*	tg	$CD11c^{hi}$ $CD86^{+}$ $MHCII^{+}$ DCs low fluorescence in some B & T cells	• FACS • 2-photon & confocal microscopy	• Additional markers are necessary to ensure the identity of the cells studied in microscopy studies.	123x [217]
$Zbtb46^{Gfp}$	*Zbtb46*$^{tm1.1Kmm}$	KI	cDC1, cDC2	• FACS • fluorescence microscopy	• KO of the *Zbtb46* gene: use heterozygous mice for reporters.	22x [19]
$Zfp366^{tdTomato}$	Missing	KI	cDC1, cDC2 (red pulp macrophages)	• FACS	• *Zfp366* expression is retained. • IRES-driven tdTomato KI after STOP of *Zfp366*	[175]
$Zeb2^{EGFP}$ ($Sip1^{SIP1\text{-}EGFP}$)	*Zeb2*$^{tm2.1Yhi}$	KI	MDP, CDP, monocytes, pDCs, few cDC2 and NK cells	• FACS	• Generation of a ZEB2-GFP fusion protein • Precursor populations accessible for sorting and in vitro and in vivo differentiation studies	4x [177, 218]
$ID2^{GFP}$	*Id2*^{tm1Gtbz}	KI	Pre-cDC1, cDC1, cDC2, NK cells	• FACS	• *Id2* expression is retained • Precursor populations accessible for sorting and in vitro and in vivo differentiation studies	18x [177, 219]
$Clec9a^{GFP}$ ($DNGR\text{-}1^{GFP}$)	*Clec9a*$^{tm1.1Crs}$	KI	cDC1, pDCs	• FACS	• KO of the *Clec9a* gene: use heterozygous mice for reporters.	12x [188]
$Snx22^{Gfp}$	*Snx2*$^{2tm1.1Kmm}$	KI	cDC1	• FACS • Multiphoton microscopy	• KO of the *Snx22* gene: use heterozygous mice for reporters. • cDC1 visualized in the kidney.	2x [220]
$Karma^{tdTomato}$ ($Gpr141b^{tdTomato}$)	*Gpr141b*$^{tm1(HBEGF)Ciphe}$	KI	cDC1	• FACS • Confocal microscopy	• *Gpr141b* expression is retained. Mice also express DTR. Fluorescence level limiting for confocal microscopy: use of an anti-RFP antibody recommended to increase signal-to-noise ratio. • In immunohistofluorescence, mast cells can be discriminated from cDC1 upon staining with Avidin.	4x [121]

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Table 4. (Continued)

Reporter strain	Official nomenclature[1]	Mutation type[2]	Marked cell population[3]	Visualization methods reported	Comments[4]	References[5]
Xcr1^{Venus}	*Xcr1*$^{tm2(HBEGF/Venus)Ksho}$	KI	cDC1	• FACS • Confocal microscopy	• KO of the *Xcr1* gene: use heterozygous mice for reporters.	10x [91, 92]
Xcr1^{mTFP1}	*Xcr1*$^{tm2(iCre/mTfp1)Ciphe}$	KI		• FACS	• *Xcr1* expression is retained. • IRES-driven iCre-2A-mTFP1.	[93, 221]
Lang-EGFP (CD207-EGFP)	*Cd207*^{tm2Mal}	KI	LCs dermal DC/cDC1 subsets	• FACS • Confocal microscopy	• *Cd207* expression is retained. • Fluorescence level insufficient for 2-photon microscopy.	26x [126]
Lang-DTR/EGFP (CD207-DTR/EGFP)	*Cd207*$^{tm3(DTR/GFP)Mal}$	KI				49x [126]
SiglecH-eGFP	*Siglech*$^{tm1.1Cln}$	KI	pDCs, cDCs	• FACS • Intravital microscopy (SiglechGFP/GFP)	• KO of the *Siglech* gene: use heterozygous mice for reporters. pDCs EGFP^{hi}, cDCs EGFP^{lo} under steady-state conditions. • *Siglech* expression is rapidly and strongly decreased in activated pDCs, hence, discriminating pDCs from cDCs based on EGFP intensity is expected to be more difficult in infectious or inflammatory contexts.	3x [21, 222]
pDC-Tomato (Siglech^{iCre}; Pacsin1$^{LSL\text{-}tdT}$)	*Siglech*$^{tm1.1(iCre)Ciphe}$; *Pacsin1*$^{tm1(tdT)Ciphe}$	KI	pDCs	• FACS • Confocal microscopy	• *Siglech*-driven CRE activates a tdTomato reporter cassette knocked into the 3'-UTR of the *Pacsin1* gene. • Improved pDC specificity	[108, 211]
DPE^{GFP}	missing	tg	pDCs (T cells)	• FACS, multiphoton microscopy	• GFP is driven under the control of the distal and proximal CD4 enhancers and CD4 promoter. pDCs appear to be the only GFP^{hi} cells among myeloid cells. pDCs are the only GFP-expressing cells in DP^{EGFP};RAG-1$^{-/-}$ mice. • Also available as T-Red mouse strain expressing ds-RedII (not tested for pDCs).	[76, 223-225]

(*Continued*)

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Table 4. (Continued)

Reporter strain	Official nomenclature[1]	Mutation type[2]	Marked cell population[3]	Visualization methods reported	Comments[4]	References[5]
Irf4^{eGFP}	*Irf4*$^{tm1.1Axk}$	KI	T cells, likely also cDC2, pDCs, B cells	• FACS	• *Irf4* expression is retained. Used only in T cell analyses so far. • Precursor populations should be accessible for sorting and in vitro and in vivo differentiation studies.	1x [226]
Irf8^{eGFP}	*Irf8*$^{tm2.1Hm}$	KI	cDC (progenitors), pDC (progenitors), additional hematopoietic cells and precursors	• FACS	• Generation of an IRF8-GFP fusion protein. • Precursor populations accessible for sorting and in vitro and in vivo differentiation studies.	11x [106, 227]
Irf8^{VENUS}	missing	PAC-tg	cDC (progenitors), pDC (progenitors), additional hematopoietic cells and precursors	• FACS • Confocal microscopy	• Endogenous Irf8 expression retained, IRES-driven VENUS inserted into 3'UTR of Irf8 within the PAC-tg *lox*P sites around a *cis*-regulatory element 50 kb upstream of the *Irf8* transcription start site were inserted to allow for conditional removal from the PAC. • Precursor populations accessible for sorting and in vitro and in vivo differentiation studies.	[228]
Mycl^{GFP}	*Mycl*$^{tm1.1Kmm}$	KI	CDPs, precDCs, immature cDCs, pDCs (organ-specific differences)	• FACS • Multiphoton microscopy	• KO of the *Mycl1* gene: use heterozygous mice for reporters. • Specific expression in immature cDCs.	3x [229, 230]
Cxcl9^{RFP};Cxcl10^{BFP} (REX3)	missing	BAC-tg	Cxcl9- and/or Cxcl10-producing cells; stromal cells, cDC1, cDC2, infMo	• Multi-photon intravital microscopy	• Endogenous Cxcl9 and Cxcl10 expression retained. • In situ visualization of DC functions in tissue microenvironment.	[231, 232]

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Table 4. (Continued)

Reporter strain	Official nomenclature[1]	Mutation type[2]	Marked cell population[3]	Visualization methods reported	Comments[4]	References[5]
Ifnb1^{Eyfp}	*Ifnb1*$^{tm1(EYFP)Lky}$	KI	IFN-β-producing cells such as pDCs (monocytes, macrophages)	• FACS • Fluorescence & confocal microscopy	• *Ifnb1* expression is retained. • Coexpression with the majority of IFN-I subtypes, hence, usable as pan-type-I-IFN reporter, at least in pDCs. • Recommended to use as homozygous reporter for highest fluorescence intensity. • Since EYFP has a long half-life and is not secreted, this reporter enables following the fate of the cells after they have secreted all of the IFN-β they produced and stopped expressing the *Ifnb1* gene. • Possibility to sort viable positive cells for functional assays. • For immunohistofluorescence, signal enhancement via anti-GFP staining is recommended.	19x [95, 99, 233]
Ifna6^{Gfp}	*Ifna6*^{tm1Aki}	KI	IFN-α-producing cells including pDCs (macrophages)	• FACS • Fluorescence & confocal microscopy	• *Ifna6* expression is retained. • Coexpression with the majority of IFN-I subtypes, hence, usable as pan-type-I-IFN reporter, at least in pDCs. • For immunohistofluorescence, signal enhancement via anti-GFP staining is recommended. • Possibility to combine with the *Ifnb1*^{Eyfp} reporter strain.	6x [234, 235]
Ifnl2^{Egfp}	missing	KI	IFN-λ2/3-producing cells including pDCs, cDCs (lung epithelial cells, pneumonocytes)	• FACS • Confocal microscopy	• *Ifnl2* translation is retained. • Fluorescence reports stoichiometric IFN-λ2 protein expression, since a T2A-Egfp coding sequence was inserted just before the STOP codon of the *Ifnl2* exon 5 leading to the translation of a self-cleaving polyprotein.	[103]

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Table 4. (Continued)

Reporter strain	Official nomenclature[1]	Mutation type[2]	Marked cell population[3]	Visualization methods reported	Comments[4]	References[5]
Il12b^{GFP} (Get40)	*Il12b$^{tm2.1Lky}$*	KI	IL-12- or IL-23-producing cells including cDC1,cDC2, pDCs (monocytes, macrophages)	• FACS • Fluorescence microscopy	• *Il12b* expression is retained. • Recommended use of homozygous reporter for highest fluorescence intensity. • Possibility to sort viable positive cells for functional assays.	3x [236, 237]
Il12b^{YFP} (Yet40)	*Il12b$^{tm1.1Lky}$*	KI				37x [59, 237]
Il15^{EmGFP} (IL-15-Emerald)	*Tg(Il15/EmGFP)#Lle*	tg	IL-15-producing cells including cDC1	• FACS • Confocal microscopy	• Fluorescence reports Il15 mRNA expression.	3x [238]
Il15^{EmGFP} (IL-15TE)	*Tg(Il15,-EGFP)20Pmr*	tg		• FACS	• Fluorescence reports stoichiometric IL-15 protein expression, since transgene drives equimolar production of IL-15 and EGFP from a self-cleaving polyprotein due to use of a 2A linker.	2x [239]
Il27^{Egfp} (IL-27p28–eGFP)	missing	tg	IL-27p28-producing cells including cDC1 (monocytes, granulocytes)	• FACS • Confocal microscopy		[240]

[1] Official nomenclature, for further information, see Table 1.
[2] BAC, bacterial artificial chromosome; KI, knock-in, PAC, phage artificial chromosome; tg, transgene.
[3] Based on current knowledge from literature.
[4] Information on genetic construction is only given for the mouse strains that are not yet included in the JAX database.
[5] Number of references recorded for the corresponded allele in the Jax database, and selected list of references including the first having described the mouse model as well as some functional studies that used the reporter strain.

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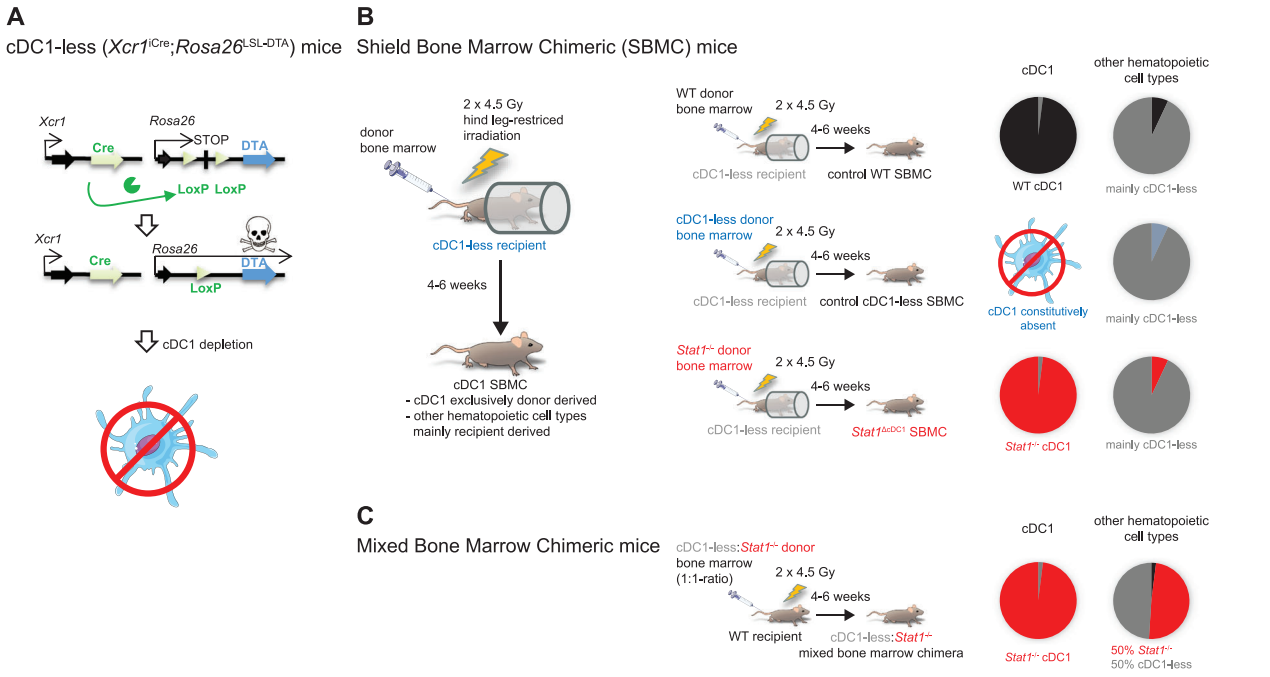


Figure 1. Using shield BM chimeric (SBMC) mice to inactivate genes specifically in cDC1 in vivo. (A) Design of a cDC1-less mouse strain, constitutively and specifically devoid of cDC1. cDC1-less mice were designed as Xcr1^{iCre};Rosa26$^{LSL\text{-}DTA}$. *Xcr1* is expressed specifically and early on in the cDC1 lineage, but it is strongly repressed in mature cDC1. (B) Design of a strategy using shield BM chimeric (SBMC) mice to engraft cDC1-less recipient mice selectively with donor-derived cDC1, with only a minor contribution of donor-derived cells to the other hematopoietic lineages. To generate SBMC mice, cDC1-less recipient mice undergo a partial irradiation restricted to their hind legs, while the other body parts are protected by a lead shield, thus, preserving most of the hematopoietic system. Following this cDC1-less recipient mice are engrafted with the BM of donor mice of a selected genotype. Under the conditions of strong competitive disadvantage against the much more numerous recipient BM cells, the donor BM reconstitutes selectively the hematopoietic lineage specifically lacking in the recipient mice, here the cDC1. Hence, using *Stat1*$^{-/-}$ donor BM allows the generation of *Stat1*$^{\Delta cDC1}$ SBMC mice that have a selective deficiency of *Stat1* in cDC1, whereas the vast majority of the cells are WT for all other cell types. (C) In contrast, in cDC1-less:*Stat1*$^{-/-}$ 1:1 classical mixed BM chimeric mice, 50% of all non-cDC1 hematopoietic cells would be *Stat1*$^{-/-}$, which could impact the biological process to study. WT SBMC and cDC1-less SBMC are used as immunocompetent and immunodeficient controls, respectively. Another advantage of using SBMC mice is the ability to use existing syngeneic gene-targeted mice for potentially any nonlethal genetic mutation, without the need for additional breeding. In contrast, for the Cre-/LoxP-dependent conditional genetic inactivation strategy, floxed alleles are much less frequently available than conventional KO alleles and must be bred with Xcr1^{iCre} mice, notwithstanding the high level of germline recombination observed in several Xcr1^{Cre} mouse strains.

Xcr1^{Venus} reporter mouse strain for the tracking of cDC1 [91, 92]. In an alternative reporter mouse strain for the tracking of the same DC type, the Xcr1^{mTFP1} reporter mouse [93], an optimized CFP variant with high fluorescent brightness and photostability was used, namely mTFP1 (monomeric teal fluorescent protein 1) [94]. On the other hand, the half-life of the fluorescent reporter has to be taken into consideration. While long half-lives can allow for long-term tracking similar to fate mapping [95], short half-lives are required for real-time assessment of the activation of signalling pathways in the target cells and of the resulting functions. Here, the addition of a degradation-inducing PEST sequence has been successfully used to significantly reduce the half-life of a fluorescent protein [96–98].

Reliability of real-time assessment of functional activities heavily relies on faithfulness of the reporter fluorescence regarding cellular specificity as well as the time course of fluorescence.

In essence, three types of reporter approaches exist: (i) Placing a reporter cassette directly behind the start codon of the gene of interest and disrupting the expression of the marker protein itself. This approach can be used as transgene approach or as a knock-in into the endogenous locus. For the latter, this results in a deficiency in the marker gene expression in mice homozygous for the reporter allele, which might impact the biological process examined not only in homozygous reporter/KO mice but also in heterozygous mice in case of a gene dosage effect. (ii) IRES-driven bicistronic reporter constructs placed after the STOP codon of the open reading frame within the marker gene locus, which retains endogenous marker gene expression but uncouples the translation of the marker versus the reporter genes. Therefore, faithfulness of the reporter has to be assessed carefully. However, an advantage of IRES-driven translation is that it might be helpful in amplifying the signal when marker gene expression is rather low. An example for this is the Ifnb1^{Eyfp} reporter mouse [99], where cells that have initiated IFN-β production can be tracked after the cells have terminated expression of the *Ifnb1* gene cells due to the long half-live of YFP that can exceed 24 h [95, 100]. (iii) Stoichiometric amounts of the marker and fluorescent reporter proteins can be achieved by using a self-cleaving viral

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2A peptide sequences, such as the one from thosea asigna virus (T2A) [101], inserted before the STOP codon of the marker gene followed by the fluorescent protein. This is critical when aiming at monitoring real-time protein production under conditions where transcription and translation are uncoupled, for example, for certain chemokines for which mRNA is stored in effector cells to allow very fast production upon stimulation as is the case in NK or T lymphocytes for Ccl5 [102] or Xcl1 [59]. This strategy has been used successfully, for example, in Ifnl2^{Egfp} mice to report IFN-λ2/3 production [103].

The Xcr1^{Cre} and Karma^{Cre} mice were recently reported to be the most faithful indirect reporter strains allowing to track cDC1, as assessed in a side-by-side comparison with the Clec9a^{Cre} strain, both under steady state and inflammatory conditions [28]. The spatiotemporal dynamics of cDC1 activation in vivo during MCMV infection and its control by the Xcr1/Xcl1 signalling axis could then be assessed upon a time course analysis of the microanatomical location of cDC1 as compared to infected cells and NK cells, comparing Karma^{Cre};Rosa26$^{LSL\text{-}RFP}$ mice on a WT or *Xcr1*-KO genetic background [59]. Karma^{Cre};Rosa26$^{LSL\text{-}RFP}$;Il12^{Eyfp} were also used to monitor IL-12 production over time and space by cDC1. This study showed that, early after infection, the Xcr1/Xcl1 signalling axis attracts cDC1 to the sites of viral replication in the spleen marginal zone, where a cross-talk with NK cells further promotes mutual activation of these two cell types, leading to cDC1 licensing by NK cells for increased IL-12 production, CCR7 upregulation, and migration to the T-cell zone, in a manner accelerating the downstream cross-priming of antiviral CD8$^+$ T cells [59].

We would like to point out that there are alternatives to the use of fluorescent proteins to track DC types or function in vivo. Using a firefly luciferase reporter gene placed under the control of the *Ifnb1* promoter, the *Ifnb1*$^{+/\Delta\beta-luc}$ mouse model enables visualizing the kinetics of IFN-β expression in the mouse paralleling the spread of pathogens through the organism under infectious conditions, rather than IFN-β expression at the single-cell level [104]. The same model allows to characterize the impact of IFN-β production by a given cell type when the *Ifnb1*$^{floxβ-luc/floxβ-luc}$ allelic composition is used and crossed to the respective Cre-driver [105]. Here, the *Ifnb1* coding sequence is flanked by loxP sites on both alleles and can be deleted by an intercross with tissue- or cell-specific Cre mice. A further alternative is the use of human surface marker proteins, such as hCD2, that can be detected via specific antibody staining. For example, EBF1-hCD2 reporter mice together with IRF8-eGFP reporter mice were used to define B cell and pDC differentiation potential in progenitor cell subsets [106].

Perspective: Enhancing specificity of the DC type targeting through intersectional genetics

Very few genes are expressed specifically only in one cell type, as mentioned before. This is especially the case for mononuclear phagocytes, as well illustrated by the fact that most of the Cre driver mice that have been generated so far to target DCs turned out to fate-map other cell types beyond the intended target, with only few notable exceptions, such as the Xcr1^{iCre} mice, that are highly specific for cDC1 (Table 3). Hence, novel approaches are being developed to use an intersectional genetic strategy based on the unique coexpression of two genes in the target cell type. This approach should be especially interesting for cDC2 and pDCs that have not been found to express any specific gene not shared with other cell types, but which uniquely coexpress a number of candidate gene pairs.

Application to cDC depletion

To achieve cDC depletion in vivo upon DT injection in a manner overcoming the caveats observed with other models, a novel approach was chosen in Csf1r^{Cre};Zbtb46$^{LSL\text{-}hDTR}$ mice (Fig. 2A), based on the fact that cDCs are the only cells that (i) are fate-mapped for Csf1r expression and (ii) express Zbtb46. This model was reported to achieve a much better specificity than in CD11c-hDTR and Zbtb46-hDTRmice, while preserving a high penetrance in cDCs [107]. Using a similar strategy based on intersectional genetics, we recently succeeded in generating a novel mutant mouse model to specifically identify pDC and track their microanatomical location [108].

Application to Cre-mediated editing of target genes in cDCs

A strategy for conditional gene manipulation in organ-specific blood vessels was recently achieved with a high efficacy and specificity by controlling Cre activity in a manner dependent on the expression of the Dre recombinase in the same cell [109]. Theoretically, conditional gene inactivation could be achieved specifically in cDCs by adapting this strategy based on the same intersectional genetics used for cDC depletion upon DT injection as discussed above (Fig. 2B). The increasing generation of Dre recombinase drivers is expected to rapidly improve accessibility and popularity of this approach [110]. Alternate strategies include the Split-Cre approach, whereby complementation-competent NCre and CCre fragments are expressed from distinct promoters that are coexpressed only in the target cell type, although the resulting penetrance can be limiting [111].

Perspectives and outlook

A range of mouse models from molecular and cellular deficiencies to function and lineage tracking have served as integral tools for many years to generate new findings on the functions of DC types as coordinators of immune responses. Concurrently, caveats were revealed, ranging from off-target effects affecting other cell types than those targeted, to insufficient efficacy to cover the target DC population. Hence, researchers must be aware of the advantages and caveats of the different mutant mouse strains currently available to target DC types, to choose the ones best suited to tackle their research question, and to ensure carefully control

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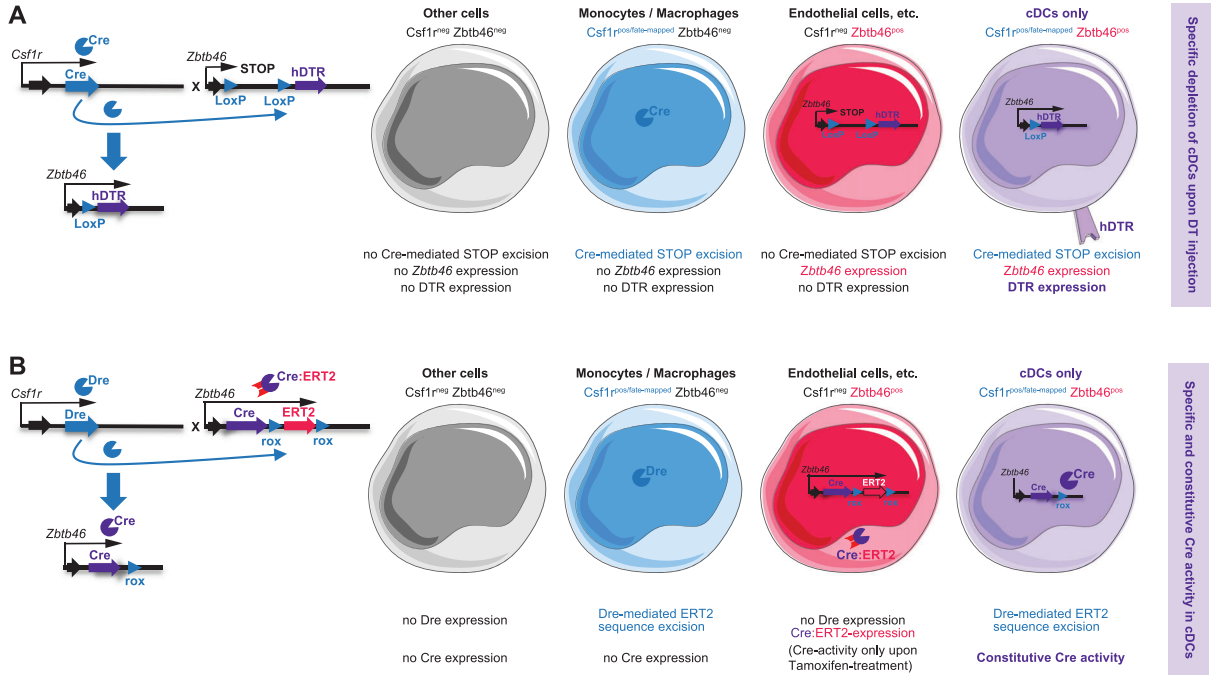


Figure 2. Use of "intersectional genetics" strategies for the specific depletion or genetic manipulation of cDC in vivo. (A) "Intersectional genetics" strategy for the specific depletion of cDC in vivo upon diphtheria toxin injection according to Loschko et al. [107]. Cre is expressed under the control of the *Csf1r* promoter. The human receptor for the diphtheria toxin is knocked-in the 3'-UTR of the *Zbtb46* gene, but downstream of a floxed transcriptional STOP (LSL) cassette. Hence, these Csf1r^{Cre};Zbtb46$^{LSL\text{-}hDTR}$ mice, hDTR expression will be conditional to two events occurring in the same cell: (i) Cre expression at some time point during the ontogeny of the cell to excise the genomic LSL cassette and (ii) expression of the *Zbtb46* gene in the targeted, differentiated cells. Excision of the LSL cassette will occur in all cells which express the *Csfr1r* gene, including monocytes/macrophages, or whose progenitors expressed Csf1r (fate-mapping) including pDC and cDC. However, within the cells expressing or fate-mapped for Csf1r, only those transcribing *Zbtb46* will express hDTR and be depleted upon injection of diphtheria toxin, namely only cDC, in theory. This strategy alleviates the issue of (lethal) depletion of endothelial or other cell types in addition to cDC in straight Zbtb46^{hDTR} mice. (B) Theoretical "intersectional genetics" strategy for the generation of cDC-Cre mice. This is based on the same "intersectional genetics" strategy as shown for (A). Here, the Dre recombinase (instead of Cre) will be expressed under the control of the *Csf1r* promoter, whereas Cre is knocked-into the 3'-UTR of the *Zbtb46* gene. However, Cre nuclear translocation (required for Cre activity) will be conditional to excision of a ERT2 sequence trapping Cre within the cytosol, by the Dre recombinase (Csf1r^{Dre};Zbtb46$^{iCre\text{-}rox\text{-}ERT2\text{-}rox}$ mice). Analogous to (A), Cre activity will be constitutively present only in cDCs.

for potentially confounding effects before drawing definite conclusions. Previous studies on the overall contribution of DCs as CD11c^{+} cells in various anti-infectious, antitumor, or autoinflammatory immune responses can, and should, now be revisited, by using the currently available DC type-specific mouse models. In this regard, it is also important to use different, complementary mouse models or methodological approaches to address through different angles the role of the DC types in the biological process under investigation, to limit the risk of erroneous interpretation and strengthen the conclusions drawn.

In addition, innovative approaches are being developed to accelerate translation of the findings from mouse models into the clinic. Preclinical models of humanized mice are developing rapidly and are very promising. On the one hand, single human genes have been introduced as transgenes into the mouse when the murine orthologue is missing. For example, this is the case for human DC-SIGN, a receptor for pathogen-derived ligands, such as Lewis-type glycans, serving at the same time as an intercellular adhesion molecule [95]. A transgenic mouse strain, where huDC-SIGN expression is driven in DCs by the CD11c-promoter [112] proved a helpful model for testing vaccination strategies implementing glycan modifications of the antigens to specifically direct them to DCs [113]. With a similar aim, the human cDC1-specific chemokine receptor XCR1 was expressed in a mouse strain deficient for its orthologue. Here, coupling antigens to human XCL1 proved efficient to address them specifically to huXCR1-expressing DCs, leading to efficient cross-presentation in vivo [114]. The human immune system (HIS) mouse models allow the development and functions of human DCs to be dissected in a more fully humanized in vivo setting, representing a further step in bridging the gap between preclinical studies of the murine immune system and the clinical implementation of the findings. In essence, in these models, human hematopoietic progenitors are transplanted into immunodeficient mice, genetically humanized for relevant growth factors to provide supportive humanized BM niches, or engrafted with in vitro engineered cellular niches. For example, these humanized mouse models allow the direct assessment of the contribution of DCs to the etiology of hematopoietic malignancies. Specific DC types can also be evaluated as novel therapeutic targets in the treatment of leukemias or of infections with viruses affecting humans. Progress in this field has been reviewed recently by others [115, 116].

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With the advent of next-generation mutant mouse models that can target DC types with increased specificity and versatility, such as those based on SBMC mice or intersectional genetics, new questions can be addressed. At the same time, a revision of several previous findings is warranted to evaluate specific DC types and their functions as potential clinical targets for DC-based immunotherapies and vaccination strategies against cancer or infectious diseases.

Acknowledgments: S.S. is supported by the Deutsche Forschungsgemeinschaft (SCHE692/6-1, SCHE692/8-1, and 270650915/GRK2158) and the Juergen Manchot Foundation (Manchot Graduate School 'Molecules of Infection IV'). Work in the laboratory of M.D. is supported by Institutional fundings from CNRS, Inserm and Aix Marseille Université, and by grants from Agence Nationale de la Recherche (ANR), Agence Nationale de Recherches sur le SIDA et les maladies infectieuses émergentes (ANRS), Fondation pour la Recherche Médicale (FRM) and Fondation ARC pour la Recherche sur le Cancer. Graphics were created using Servier Medical Art according to Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/). Changes were made to the original cartoons.
Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest: The authors declare no commercial or financial conflict of interest.

Data availability statement: Data sharing not applicable – no new data generated.

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Abbreviations: **cDC1**: conventional DCs · **DT**: diphtheria toxin · **DTR**: diphtheria toxin receptor · **HIS**: human immune system · **LCs**: Langerhans cells · **MBMC**: mixed BM chimeric · **MCMV**: murine cytomegalovirus · **MoDCs**: monocyte-derived DCs · **pDCs**: plasmacytoid DCs · **SBMC**: shield BM chimeric · **tDCs**: Transitional DCs · **Tfh**: T follicular helper cells

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Received: 19/11/2021
Accepted: 14/1/2022
Accepted article online: 31/1/2022

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