Title

Plasmacytoid dendritic cells are dispensable or detrimental in murine systemic or respiratory viral infections

Author list

Clemence Ngo[1], Khalissa Rahmani[1]*, Michael Valente[1]*, Nils Collinet[1], Gilles Bessou[1], Manon Fabregue[2], Sarah Sharkaoui[2], Sophie Mazzoli[2], Camille Pierini-Malosse[1,2], Amandine Sansoni[2], Frederic Fiore[2], Caroline Laprie[3], Mauro Gaya[1], Claude Gregoire[1], Achille Broggi[1], Pierre Milpied[1], Bertrand Escalière[1], Thien Phong Vu Manh [1], Mathieu Fallet[1], Lionel Chasson[1], Hien Tran[4], Marc Le Bert[5], Bernard Malissen[1], Ana Zarubica[2,$], Marc Dalod[1,$], Elena Tomasello[1,$].

[1]Aix-Marseille University, CNRS, INSERM, CIML, Centre d'Immunologie de Marseille-Luminy, Turing Center for Living Systems, Marseille, France

[2]Centre d'Immunophénomique, Aix Marseille Université, Inserm, CNRS, PHENOMIN, Marseille, France

[3]Laboratoire Vet-Histo, 122 Av Joseph Vidal, 13008 Marseille

[4]Department of Microbiology, Immunology and Glycobiology, Institute of Laboratory Medicine, Lund University, 223 62 Lund, Sweden

[5]Immuno-Neuro Modulation, Université d'Orléans Campus CNRS, 3B rue de la Ferollerie, 45071 ORLEANS, France

* Equal contribution

[$] These authors jointly supervised this work

M.V. current address: Veracyte, Luminy biotech entreprises, case 923, 163 avenue de Luminy, F-13288 Marseille Cedex 09, France.

N.C. current address: Plateforme d'histopathologie expérimentale ICEP, Centre Recherche Cancerologie Marseille-Institut Paoli Calmettes, 27 Bd Leï Roure, CS 30059, 13273 Marseille Cedex 09

Corresponding authors: AZ, MD and ET

Abstract (150 words)

Plasmacytoid dendritic cells (pDCs) are major producers of type I/III interferons. As these cytokines are crucial for antiviral defense, it is assumed to be also the case for pDCs. However, robust evidence supporting this dogma is scarce. Genetic mutations or pharmacological manipulations causing pDC loss or disrupting their interferon production affect other immune cells, which could confound interpretation. To overcome this bottleneck, we engineered pDC-less mice, specifically and constitutively devoid of pDCs as expressing diphtheria toxin under coordinated control of the *Siglech* and *Pacsin1* genes co-expressed uniquely in pDCs. pDC-less mice mounted protective intrinsic and innate immune responses against systemic infection with mouse Cytomegalovirus, and were more resistant to intranasal infection with influenza virus and SARS-CoV2. Thus, contrary to dogma, pDCs and their interferon production proved dispensable or deleterious during systemic or respiratory viral infections, respectively. pDC-less mice will enable rigorously revisiting the roles of pDCs in health and disease.

Introduction

Vertebrate antiviral immunity heavily depends on type I and type III interferons (IFN-I/IIIs)[1]. IFN-I/IIIs promote all the three arms of antiviral immune defenses: intrinsic, innate and adaptive immunity. IFN-I/III binding to their respective receptors activate a major common pathway involving the activation by phosphorylation of three transcription factors, STAT1, STAT2 and IRF9, and their hetero-trimerization into the complex ISGF3 (interferon stimulated gene factor 3). ISGF3 binds to IFN-stimulated response elements (ISRE) in genomic promoter or enhancer regions, driving the expression of hundreds of genes called IFN-stimulated genes (ISGs). Many ISGs act as viral restriction factors able to block specific phases of the life cycle of virus families in infected cells, thus being the effector molecules of antiviral intrinsic immunity. The receptors for IFN-I and IFN-III have a complementary, largely reciprocal expression, mostly on epithelial cells in all barrier tissues for the latter and in all other nucleated cells of the body for the former. Hence, IFN-I/IIIs protect the host from viral infection in a large part by reinforcing antiviral intrinsic immunity in putatively all nucleated cells of the body[1]. IFN-Is also contribute to the activation or functional polarization of immune cells, hence promoting protective innate and adaptive anti-viral immune responses [1].

IFN-I/III responses are generally beneficial during viral infections. However, when these responses are deregulated, they can turn deleterious[1]. This is the case when they are inappropriately activated in certain inflammatory or autoimmune diseases, in the absence of detectable viral infection. It is also the case during certain viral infections, if their induction is not properly controlled in time and space, as the proinflammatory functions of IFN-I/IIIs can promote virally-induced immunopathology, contribute to immunosuppression, or increase susceptibility to secondary bacterial infections by preventing the induction of proper adaptive immune responses or delaying the healing of infection-induced tissue damage in epithelial barriers[1, 2, 3]. One strategy to promote the beneficial functions of IFN-I/IIIs and limit their deleterious effects would be to determine whether and how these distinct functions are linked to the modalities of their production. This includes identifying which cell types produce IFN-I/IIIs, when, where, with what functional consequences, and under the instruction of which sensing pathway. Indeed, if specific cell types or sensing pathways are driving deleterious IFN-I/III responses in certain viral infections or autoimmune/inflammatory diseases, their specific targeting would allow dampening detrimental inflammation or misfired immune responses while preserving protective antiviral immunity. This would improve disease treatment by avoiding the general immunosuppression and the associated infectious risks induced by broad anti-inflammatory drugs such as corticosteroids or JAK/STAT inhibitors.

Amongst cellular sources of IFN-I/III, plasmacytoid dendritic cells (pDCs) are special because they rapidly produce very high amounts of all subtypes of IFN-I/IIIs upon engulfment and endosomal sensing of nucleic acids derived from viral particles or material from infected cells, while being themselves highly resistant to viral infection[1, 4]. Specifically, pDCs express the endosomal TLR7 and TLR9 receptors that are able to recognize viral single stranded RNA and unmethylated CpG DNA sequences, respectively. TLR7/9 engagement induces IFN-I/III production via a MYD88-IRAK4-IRF7 signaling pathway[1]. In contrast, infected cells sense cytosolic or nuclear genome intermediates from the viruses replicating endogenously in their cytosol or nucleus, through specific helicases or the cGAS enzyme converging on the activation of a STING-IRF3 signaling pathway promoting the production of IFN-β and only few subtypes and low amounts of IFN-αs, with susceptibility to inhibitory viral immune-evasion mechanisms [1, 5]. Hence, it is currently thought that pDCs generally play a critical and beneficial role in antiviral immunity as a major source of IFN-I/IIIs early after infection, indispensable for the reinforcement of intrinsic antiviral immunity and for proper orchestration of innate and adaptive immune responses[5, 6, 7, 8]. Yet, as we reviewed recently [4] and will summarize in the next paragraph, direct and robust experimental evidence supporting this dogma is scarce, in a large part because methodological bottlenecks are preventing rigorous and definitive evaluation of the specific role of pDCs in antiviral immunity both in humans and in mice.

In humans, primary immune deficiencies abrogating pDC ability to produce IFN-I/IIIs, including loss-of-function mutations in TLR7, MYD88, IRAK4 or IRF7, have been associated primarily with a heightened susceptibility to *Mycobacterium tuberculosis* or a few other pyogenic bacteria but not to viral infections, except with the respiratory viruses Influenza and SARS-Cov2 [9, 10, 11, 12, 13, 14, 15, 16, 17]. This contrasts with the much broader susceptibility to viral infections of patients suffering from inborn errors in genes necessary for the response to IFN-Is or IFN-IIIs, such *as STAT1, STAT2, IRF9, IFNAR1* or *IFNAR2*[18, 19]. Hence, these data show that pDCs but not IFN-I/III responses are largely redundant for antiviral immunity in modern humans in the current hygiene and health care context. In mice, contrasting results were obtained regarding the consequences on antiviral immunity of the genetic inactivation of *Tlr7/9* or *Myd88,* depending on the combination of the virus and mouse strains studied, on differences in the inoculum dose or route, as well as on the type of readout measured. Indeed, the analysis of intrinsic antiviral immunity and side-by-side comparisons with mice deficient for IFN-I/III responses were seldom performed [1, 20]. In any case, whether in humans or mice, genetic inactivation of TLR7/9, MyD88, IRAK4 or IRF7 affects other cells and biological processes beyond pDC IFN-I/III production. For example, TLR7 and IRF7 are also expressed and functional for IFN-I induction in monocytes and macrophages, and Myd88 is required for the responses to IL-1 cytokine family members in many cell types[4].

Several approaches have been developed to deplete pDCs in vivo in mice to assess the functional impact of their loss. However, most of these approaches are not specific enough and have off-target effects. This confounds rigorous interpretation of the contribution to phenotypes observed as dependent on pDCs as opposed to other cell types also directly impacted by the mutation or treatment, as summarized in [21]. For example, in *Ikaros*$^{L/L}$ mice [22] many cell types beyond pDCs are directly affected; this is the case for DC precursors and subpopulations of DCs and macrophages in *Siglech*-hDTR mice [23, 24], for subpopulations of DCs, macrophages and possibly B cells in *Itgax*^{Cre};*Tcf4*$^{-/fl}$ mice [25, 26, 27, 28, 29], for plasma cells, subpopulations of activated B cells, DCs, monocytes and macrophages in mice treated with anti-BST2/PDCA1 depleting antibodies. Studies performed using these tools supported the notion that pDCs were a major source of IFN-Is in infected mice, but varied in the assessment of the effective contribution of pDCs to anti-viral immunity, from beneficial to dispensable or even detrimental, depending on the infection route and the combination of virus and mouse strains[4]. Currently, pDC depletion can be achieved with high specificity and efficacy in one mutant mouse model, the BDCA2-hDTR mice, upon diphtheria toxin (DT) administration[30]. However, triggering of hDTR on the pDCs from these mice induces their IFN-I production before their death[31]. This might confound the interpretation of the requirement of pDCs in antiviral immunity or IFN-I responses when using this model. Moreover, repeated DT injections in BDCA2-hDTR mice have been reported to induce a severe morbidity proposed to be dependent on chronic pDC IFN-I production [31]. Therefore, there is still an unmet scientific need to determine rigorously whether and how pDCs modulate host antiviral defense *in vivo* by selectively depleting pDCs limiting off target and confounding effects.

Here, we reported the generation and characterization of pDC-less mice, a new mutant mouse model allowing specific and constitutive depletion of pDCs. pDC-less mice were engineered through an intersectional genetic strategy targeting diphtheria toxin A (DTA) expression in cells co-expressing the genes *Siglech* and *Pacsin1*, which we had previously validated with a fluorescent reporter mouse model [32]. pDC-less mice were confirmed to be specifically devoid of pDCs in all tissues examined both at steady state and during viral infections. Yet, pDC-less mice remained able to establish protective anti-viral immunity during systemic infection with Mouse Cytomegalovirus (MCMV), a natural rodent pathogen. Moreover, pDC-less mice showed an enhanced resistance to respiratory infections with influenza viruses or SARS-CoV2 and IFN-I production by pDCs was deleterious in the Influenza infection model. Thus, our study revealed a detrimental role of pDCs and their IFN-I production during respiratory viral infections. In summary, we report here the generation of a novel mutant mouse model specifically and constitutively devoid of pDCs and its use demonstrating that these cells are either dispensable or detrimental in murine systemic or respiratory viral infections. This

challenges the current dogma proposing that pDCs generally play a crucial protective role in antiviral immunity. This new mouse model will be a critical tool for future studies aiming at rigorously determining the physiological functions of pDCs and their molecular regulation, in other models of infections and in inflammatory or autoimmune diseases.

Results

The pDC-less mice are constitutively and selectively devoid of pDCs

We recently generated Siglech^{iCre} x Pacsin1^{LSLtdTomato} double Knock-In mice, called pDC-Tom, in which tdTomato fluorescent protein was exclusively expressed in "bona fide" pDCs[32]. Specific pDC targeting was achieved using intersectional genetic approach based on the co-expression of *SiglecH* and *Pacsin1* genes uniquely in mouse pDCs. We adapted our two gene-based strategy by generating *Pacsin1*$^{LoxP\text{-}STOP\text{-}LoxP\text{-}DTA}$ (Pacsin1$^{LSL\text{-}DTA}$) knock-in mice, in which a floxed cassette containing the gene encoding the A subunit of the diphtheria toxin (DTA) was located at the 3' of *Pacsin1* gene (**Fig. 1a**). Pacsin1$^{LSL\text{-}DTA}$ were then bred with Siglech^{iCre}, thus allowing the removal of STOP sequence and lethal expression of DTA exclusively in SiglecH^{+}Pacsin1^{+} cells, corresponding to pDCs. Indeed, pDCs were ablated in both lymphoid and non-lymphoid organs of double heterozygous Siglech^{iCre}; Pacsin1$^{LSL\text{-}DTA}$ mice, hereafter called pDC-less mice (**Fig. 1b** and **Extended Data Fig. 1a**). pDC-less mice developed and grew normally **(Extended Data Fig. 1b)**. Moreover, the cell numbers isolated from lymphoid and non-lymphoid organs of control versus pDC-less mice were comparable (**Fig. 1c**). The development of several other lymphoid and myeloid lineages was not affected, as exemplified in the spleen (**Fig. 1d-e**), thus confirming the selective ablation of pDCs. As pDCs and tDCs share a common hematopoietic precursor [29], we extended our analysis by using an optimized staining panel allowing to discriminate "bona fide" pDCs from other cDCs and tDC^{hi} and tDC^{lo} cell subsets expressing high or low levels of CD11c, respectively [27, 29] (**Extended Data Fig. 1c).** We confirmed the lack of "bona fide" CD11c^{lo} Bst2^{hi} Ly6D^{+} CX3CR1^{neg} pDCs[32] in our pDC-less mice (**Fig. 1f**). cDC2s were unaffected, while cDC1s were significantly increased in pDC-less mice, consistent with the previously reported enhanced expansion of cDC1s when the development of pDC-committed precursors is inhibited[33]. Finally, tDC^{lo}/pDC-like were slightly, but significantly reduced, consistent with low, but detectable Tomato expression in late pDC-committed precursors isolated from pDC-Tom mice[32], likely including recently described pro-pDC/tDC precursors [29, 32]. Altogether, our results demonstrate that pDCs are selectively and constitutively absent in pDC-less mice.

During MCMV infection pDCs are the main source of IFN-I, but they are dispensable for cell-intrinsic and innate anti-viral immunity

During systemic infection by mouse cytomegalovirus (MCMV), a natural rodent pathogen, IFN-Is are mainly produced by pDCs[30, 34, 35, 36]. Indeed, both IFN-α and IFN-β were undetectable in the sera of MCMV-infected pDC-less mice at the peak of IFN-I production detectable in infected control mice (1.5 days after infection). However, the production of other pro-inflammatory cytokines, such as IL-12, TNF-α, IFN-γ, CXCL10 and CCL2 was unaffected, thus supporting the existence of other sources of these cytokines redundant with pDCs (**Fig. 2a** and **Extended Data Fig. 2a**). We then evaluated the impact of the loss of pDCs and, especially of their IFNs, on anti-viral immunity. pDC-less mice survived MCMV infection, even at high viral loads (**Fig. 2b** and **Extended Data Figure 2b**) and efficiently controlled viral dissemination in all host organs analyzed (**Fig. 2c)**. Next, we analyzed the kinetics of ISG expression during MCMV infection. In infected control mice *Mx2* gene was induced at higher levels in the spleen than in the lungs and in the liver and its expression peaked in the spleen and the lungs at 1.5 days after infection, while it was delayed of 12 hours in the liver (**Extended Data Figure 2c**). When compared to uninfected mice, *Irf7*, *Mx2*, *Isg15* and *Ifit2* expression was upregulated with similar kinetics in the organs of MCMV-infected control and pDC-less mice, but poorly in the organs isolated from infected Ifnar1^{KO} mice (**Figure 2d** and **Extended data Figure 2d**). Splenic pDCs were also found to be the main source of IFN-III during MCMV infection (**Extended Data Figure 2d**), consistent with enhanced expression of *Il28a* and *Il28b* genes in IFN-I-producing pDCs[36]. IFNs produced by pDCs during MCMV infection promote the activation of NK cells and cDCs, two innate immune lineages crucial for the induction of efficient antiviral responses during MCMV infection[20, 30, 37, 38]. However, granzyme B, IFN-γ and CD69 were similarly upregulated in splenic NK cells isolated from MCMV-infected control and pDC-less mice, whatever time point after infection analyzed (**Figure 2e**). Moreover, splenic cDC1 and cDC2 isolated from MCMV-infected control and pDC-less mice expressed comparable amounts of IFN-induced CD86 activation marker (**Extended Data Fig. 2e**).

Altogether, our results thus demonstrate that during systemic MCMV infection pDCs are dispensable for mounting protective intrinsic and innate anti-viral immunity, despite being the main IFN-I source.

pDCs and the response to IFN-I are both detrimental in respiratory infection by influenza A virus

pDCs were considered critical for enhanced susceptibility of *IRF7* mutant patients to the respiratory influenza A virus (IAV) infection, as defective in IFN-I production in vitro upon TLR7

triggering[12, 14]. In IAV-infected pDC-depleted mice the scenario appeared more complex, supporting evidence of dispensable, protective or even detrimental functions of pDCs[39, 40, 41, 42, 43, 44]. However, in all mouse studies other cells, besides pDCs, were depleted, thus confounding the interpretation of the results obtained. Moreover, both mouse genetic background and viral strain differentially affected mice resistance to IAV infection. We thus investigated the specific contribution of pDCs by infecting control and pDC-less mice with a IAV strain (H3N2 A/Scotland/20/74) inducing severe pneumonia in mice on C57BL/6 background[45]. When infected with LD50 of this strain, half of control mice died, while all pDC-less mice recovered from infection and survived (**Fig. 3a** and **Extended Data Fig. 3a**). We extended our analyses to BDCA2-hDTR mice[30]. Upon DT administration, most of IAV-infected BDCA2-hDTR mice survived, while 80% of control C57BL/6 mice died of IAV-induced immunopathology (**Fig. 3b** and **Extended Data Fig. 3b**), thus confirming in two distinct mutant mice that pDCs were deleterious in this infection model. IAV replication in the lungs did not differ between control and pDC-less mice, as observed analyzing the expression of IAV *M1* gene at different time points after infection (**Fig. 3c**). Moreover, *Irf7*, *Oas2* and *Isg15* ISG genes were similarly induced in the lungs of infected control and pDC-less mice (**Fig. 3d**), as analyzed on bulk RNASeq data performed on RNA extracted from the lungs of control vs pDC-less mice at days 0, 3 and 6 after infection. Altogether, these data exclude a key role of pDCs in intrinsic anti-viral immunity induced during pulmonary IAV infection.

In most murine strains, including C57BL/6, Mx1, the main virulence restriction factor against IAV, is defective, due to a genetic non-functional mutation. Yet, when C57BL/6 mice express at least one functional *Mx1* allele, they become resistant to IAV, even at high viral loads, via a mechanism proposed to depend on TLR7-Myd88[41]. Lung viral titers were increased in IAV-infected Mx1+ mice when depleted of BST2+ cells, proposing a protective role of pDCs[41]. We thus investigated the impact of selective pDC depletion in Mx1+ mice. To this aim, we bred our Pacsin1$^{LSL\text{-}DTA}$ mice with B6.A2G-Mx1+/+, obtaining Pacsin1$^{LSL\text{-}DTA}$ Mx1+/+ mice, then we crossed them with *Siglech*^{iCre} mice, obtaining Mx1+/- pDC-less mice. Both Mx1+ Pacsin1$^{LSL\text{-}DTA}$, used as control, and Mx1+/- pDC-less mice survived to the infection with high viral loads of IAV and displayed poor morbidity (**Fig. 3e** and **Extended Data Fig. 3c**), excluding an essential role of pDCs in IAV-infected Mx1+ hosts. Life-threatening lung immunopathology induced by pathogenic IAV strains was abrogated in *Ifnar1*^{KO} [42, 43], supporting a deleterious role of the response to IFN-I. When infected with LD50 of H3N2 A/Scotland/20/74 strain, 90 % of Ifnar1^{KO} mice survived the infection, while all Stat1^{KO} mice succumbed by day 8 (**Fig. 3f** and **Extended Data Fig. 3d**), confirming the detrimental role of IFN-I and revealing a protective role of IFN-II and/or IFN-III. The administration of synthetic TLR7 antagonists was shown to inhibit IAV-dependent lung immunopathology, thus suggesting that deleterious IFN-Is were

mainly produced via a TLR7-dependent pathway[44]. Accordingly, TLR7^{KO} survived IAV infection (**Fig. 3g** and **Extended Data Fig. 3e**).

In conclusion, the infection with the pathogenic H3N2 A/Scotland/20/74 IAV strain induces in mice a life-threatening pneumonia promoted by pDCs and the response to IFN-Is, likely via TLR7-dependent mechanisms.

During IAV infection lung pDCs increase and are a major source of IFN-α

As both pDCs and IFN-Is were harmful during IAV infection, we wondered whether pDCs could be a detrimental source of these cytokines. We determined whether and when lung pDCs produced IFN-I during IAV infection by using *Ifnb*^{EYFP} reporter mice[46]. pDCs, as well as tDCs and cDCs, were rare within CD45+ cells isolated from the lungs of uninfected mice, while their percentages increased starting from day 4 after infection (**Fig. 4a** and **Extended Fig. 4a-b**). We analyzed YFP expression, correlating with IFN-I production[36, 46], in these different DC subsets. Within DCs isolated from the lungs of IAV-infected *Ifnb*^{EYFP} mice only pDCs contained YFP+ cells (**Extended Fig.4a**) that reached the plateau between day 4 and 6 after infection (**Fig. 4b**), confirming pDC contribution to IFN-Is produced in the lungs of IAV-infected mice. We then evaluated the impact of pDC loss on whole IFNs detectable in the lungs at the transcriptional and protein level. First, we analyzed the expression of genes encoding for IFN-I/IIIs in bulk RNASeq samples derived from the lungs isolated from control vs pDC-less mice at days 0, 3 and 6 after IAV infection (**Extended Data Fig.4c**). The expression of all genes encoding IFN-Is and IFN-IIIs was detectable exclusively in infected mice. *Ifnb1*, *Ifna4*, *Ifnl2* and *Ifnl3* were expressed at comparable levels in both mouse strains, while we observed a tendency to a lower expression of *Ifna1, Ifna2, Ifna6* and *Ifna5* in the lungs of infected pDC-less mice (**Extended Data Fig.4c**). We then analyzed IFN-α and IFN-β protein contents in bronchioalveolar lavages (BAL) isolated from control vs pDC-less mice at different days after IAV infection (**Fig. 4c**). Consistent with our transcriptional data, both IFN-I subtypes were detectable in the BAL starting day 3 after infection. However, only IFN-α were significantly reduced in the absence of pDCs, while IFN-β levels were comparable between infected control and pDC-less mice (**Fig. 4c**). Taken together, these results confirm that pDCs are a main source of IFN-α in infected lungs, although other cells produce other IFN-I subtypes, as well as IFN-III. Breeding of *Ifnb*^{EYFP} reporter with our pDC-Tom reporter mice generated SCRIPT mice that discriminate IFN+ (Tom+ YFP+) from IFN- (Tom+ YFP-) pDCs in tissues[32]. We quantified the distribution of whole pDCs vs IFN+ ones in the lungs of IAV-infected SCRIPT mice. Heterogeneous CD45 staining allowed to distinguish CD45^{low} (lowly inflamed) vs CD45^{high} (highly inflamed) areas, the latter ones resembling to inflammatory foci (**Fig. 4d-e, j**

and **Extended Data Fig. 4d-e**). Highly inflamed areas contained also the highest numbers of infected cells expressing viral hemagglutinin (HA+) (**Fig. 4d-e, k** and **Extended Data Fig. 4f**). Both CD45+ and HA+ cell numbers increased during the infection. pDC frequency significantly increased in highly inflamed areas starting from day 4 after infection (**Fig. 4f-g, l** and **Extended Data Fig. 4g**), consistent with our flow cytometry data (**Fig. 4a** and **Extended Data Fig. 4a-b**). IFN+ pDCs (YFP+ Tom+) were detected only in highly inflamed areas and peaked at day 4 after infection (**Fig. 4h-i, m** and **Extended Data Fig. 4h-j**). Thus, during IAV infection lung pDCs produce IFN-Is and are preferentially located in highly inflamed and infected areas.

IFN production by pDCs is mainly responsible of their deleterious role during IAV infection

pDCs are a major, but not the unique source of IFN-Is in the lungs during IAV infection (**Fig. 4** and **Extended Fig. 4**). Studying the specific role of IFN-I produced by pDCs in IAV-induced pneumonia would require the inactivation of genes involved in the TLR7/Myd88/IRF7 signaling cascade uniquely in pDCs. However, none of currently existing conditional Cre mice, including our Siglech^{iCre} mice[32, 47], allows the selective targeting of pDCs. To overcome this roadblock, we developed a novel chimeric mouse model, the pDC Shield Bone Marrow Chimeras (pDC SBMC) mice (**Fig. 5a**). To achieve this purpose, we lethally irradiated the hind legs of pDC-less mice, while the rest of their body was protected by a lead shield. This created a local perturbation that favored the engraftment of donor BM cells, especially for cell lineages absent in recipient mice, in our case pDCs. Indeed, four weeks after reconstitution, pDC SBMC mice were replenished with pDCs derived from donor BM, while the other immune lineages were still mostly composed by recipient cells, as validated by using CD45.2 donor BM cells in CD45.1+ pDC-less mice used as SBMC recipient (**Fig. 5b**). pDC SBMC thus allow to study the impact of the loss of a candidate gene selectively in pDCs. As cytokine production by pDCs is Myd88-dependent[47], we evaluated the resistance to IAV infection of pDC SBMC reconstituted with Myd88^{KO} pDC. We generated CTR, pDC-less and Myd88^{KO} pDC SBMC by reconstituting shield-irradiated CD45.1+ pDC-less mice with CD45.2+ C57BL/6, pDC-less or Myd88^{KO} BM cells, respectively. When infected with IAV, half of CTR pDC SBMC succumbed, while both pDC-less and Myd88^{KO} pDC SBMC mostly survived (**Fig. 5c** and **Extended Fig. 5a**). TLR7^{KO} pDC SBMC were also resistant to IAV infection, when compared to infected CTR pDC SBMC (**Fig. 5d** and **Extended Fig. 5b**). pDC-intrinsic lack of TLR7/Myd88 completely abrogates TLR7-dependent cytokine and chemokine production, while pDC-intrinsic deficiency in IRF7 abolishes exclusively IFN production [47]. We thus generated IRF7^{KO}, as well as CTR, pDC-less pDC SBMC and infected them with IAV. All IRF7^{KO} and pDC-less pDC SBMC

survived, while 60% of CTR pDC SBMC died (**Fig. 5e** and **Extended Fig.5c**). Hence, selective inactivation of TLR7/Myd88/IRF7 signaling in pDCs was sufficient per se to restore the resistance to IAV infection and confirmed the detrimental contribution of IFNs produced by lung pDCs.

During IAV infection lung inflammation and cytokine contents are reduced in the absence of pDCs

During IAV infection the recruitment of immune cells to the infected lungs and their activation are essential to establish protective anti-viral immunity. However, unbridled and sustained inflammatory responses can lead to severe pneumonia and acute respiratory distress syndrome (ARDS). Therefore, we performed anatomopathological analysis of lung sections isolated from IAV-infected control and pDC-less mice to evaluate the impact of pDC loss on lung inflammation and tissue integrity. We harvested our samples 8 days after IAV infection, when survival and morbidity start to differ between these two mouse strains (**Fig. 3a**). Most of the lungs isolated from infected control mice showed a marked broncho-interstitial pneumonia (**Fig. 6a**), eventually associated with bronchiolar filling with degenerated epithelial cell and high alveolar inflammation (**Fig. 6b**). In contrast, lungs isolated from infected pDC-less mice displayed milder pneumonia and inflammation (**Fig. 6c-d**). Consistently, semi-quantitative score of lung sections, taking in account inflammation and necrosis in interstitial, alveolar, bronchial and vascular areas (**Suppl Table 1**), was significantly higher in control than in pDC-less mice (**Fig. 6e**). In IAV-infected mice the depletion of BST2^{+} cells correlated with reduced recruitment of inflammatory leukocytes in infected lungs[42]. Absolute numbers of whole lung cells and, especially, of CD45+ cells increased during infection and peaked at 6 days p.i., following a similar trend in infected control and pDC-less mice (**Fig. 6f** and **Extended Fig. 6a-c**). The percentages of most myeloid and lymphoid lineages isolated from infected control and pDC-less mice were comparable, except for pDCs, as expected (**Fig. 6g** and **Extended Data Fig. 6a-d**). However, both neutrophils and eosinophils were significantly increased at days 6-8 after IAV infection in the absence of pDCs (**Fig. 6g**). Neutrophils were proposed to exert detrimental vs protective roles upon IAV infection[44, 48]. We thus determined whether neutrophils were required for enhanced resistance of pDC-less mice to IAV infection by injecting IAV-infected control and pDC-less mice with isotype control (IC) or neutrophil-depleting antibodies (1A8) at days 5 and 6 after infection. Neutrophil depletion severely affected the survival of both control and pDC-less infected mice (**Figure 6h**), showing that in our infection model neutrophils were protective, but via pDC-independent mechanisms. Indeed, selective pDC

depletion did not inhibit the recruitment of immune cells into infected lungs, but it is rather associated with neutrophilia and eosinophilia.

IFN-Is produced by pDCs activate both innate and adaptive immune cells[1], by inducing the expression of activation molecules, such as CD86 and CD69. The expression of both these markers was induced during infection on lung myeloid and lymphoid cells, but it was only transiently affected in the absence of pDCs (**Extended Data Fig. 7a-b**). Depletion of BST2+ cells was also shown to reduce the amounts of proinflammatory cytokines detected in bronchioalveolar lavages (BAL) of IAV-infected mice and, consequently, lung immunopathology[42]. The analysis of our bulk RNASeq data showed that mRNAs encoding most cytokine and chemokines analyzed were similarly upregulated in infected control and pDC-less mice at days 3 and 6 after infection (**Extended Data Fig. 7c**). BAL isolated from both strains of mice at days 2, 3 and 6 after infection did not differ in their content for proinflammatory, such as CXCL10, CCL2 and IFN-γ, and anti-inflammatory cytokines, such as IL-10 (**Fig. 6i** and **Extended Data Fig. 7d**). However, at day 8 after infection the amounts of most cytokines detectable in the BAL were significantly lower in the absence of pDCs (**Fig. 6i** and **Extended Data Fig. 7d**). Hence, during late phases of IAV infection enhanced resistance of pDC-less mice correlates with reduced bronchioalveolar amounts of cytokines known to promote ARSD.

The alterations of lung integrity and permeability induced upon IAV infection are less severe in the absence of pDCs

Unbridled cytokine-dependent inflammation induced by IAV infection can promote severe lung lesions, fluid leakage and defaults in respiratory capacity leading to ARDS. Reduced cytokine levels in the BAL of IAV-infected pDC-less mice could indicate reduced tissue damage and leakage in the bronchial tree in the absence of pDCs, consistent with milder histopathological score in the lungs of infected pDC-less mice (**Fig. 6e**). Bronchi were clearly identified in the lung sections of uninfected control and pDC-less mice by staining with antibodies recognizing Epithelial Cell Adhesion Molecule (EpCAM), a marker of bronchiolar cells (**Fig. 7a**). However, while in uninfected lungs EpCAM staining was continuous and homogeneous in both strains (**Fig. 7a**, red arrows), it was often disrupted in the lungs of infected mice (**Fig. 7a**, white arrows). On the whole lung sections the percentages of EpCAM+ areas were significantly higher in infected pDC-less mice (**Fig. 7b**), while the percentages of bronchi with disrupted (open) EpCAM staining were higher in infected control mice (**Fig. 7c**), thus suggesting an increased breaching of the bronchial tree and, potentially, of fluid leakage in the presence of pDCs. Pulmonary permeability can be analyzed by measuring the leakage into the plasma of

fluorescent proteins instilled in the airways (**Fig. 7d**)[49]. We thus intratracheally instilled 10KDa FITC-Dextran control and pDC-less mice uninfected or 8 days after IAV infection. While the fluorescence was absent in the plasma of uninfected mice, as well as of PBS-instilled IAV-infected mice, it was clearly detectable in the plasma of IAV-infected mice and significantly lower in pDC-less than in control mice (**Fig. 7e**). pDCs thus contribute to promote lung lesions and fluid leakage during IAV infection.

During mouse SARS-CoV2 infection pDCs are not required for mice survival and can exert detrimental functions

Patients harboring genetic defects in *MYD88*, *IRAK4, IRF7* or *TLR7* genes were highly susceptible to the infection by several respiratory viruses, including SARS-CoV2[13, 14, 15, 16, 17]. As blood pDCs of these patients were defective in vitro in TLR7-dependent IFN production, patient susceptibility was attributed to impaired pDC-dependent viral control [14, 17]. However, other studies proposed rather a detrimental role of pDCs during SARS-CoV2 infection, by promoting via their IFN-Is macrophage-dependent cytokine storm[50, 51, 52, 53]. We thus decided to address the specific role of pDCs by using our pDC-less mice. C57BL/6 mice are resistant to SARS-CoV2 infection, as the virus cannot use mouse angiotensin-converting enzyme 2 (ACE2) as receptor for cell entry. However, ectopic expression of human ACE2 under the control of the cytokeratin 18 (K18) promoter render transgenic K18:hACE C57BL/6 mice highly susceptible to SARS-CoV infections[54]. Hence, we first generated K18:hACE x Pacsin1$^{LSL\text{-}DTA}$ mice, then we bred them with Siglech^{iCre} mice. Offspring triple heterozygous mice, hereafter called K18:hACE pDC-less, as well as control K18:hACE mice, were infected with SARS-CoV2. Infected male K18:hACE survived less than females, consistent with higher morbidity and mortality observed in male SARS-CoV2-infected patients[55] (**Fig. 8a** and **Extended Data Fig. 8a**). We did not detect any significant difference in survival when comparing infected females K18:hACE mice and K18:hACE pDC-less mice. In contrast, infected males K18:hACE pDC-less mice were significantly more resistant than gender-matched infected K18:hACE mice (**Fig. 8a** and **Extended Data Fig. 8a**). Thus, pDCs are not required for K18:hACE mice survival to SARS-Cov2 infection and exert detrimental functions in infected males.

The response to IFN-I is essential for human survival to viral infections, including to SARS-CoV2[15]. However, IFN-I-responsiveness is deleterious in IAV infected-mice (**Fig. 3** and **Fig. 5**). When infected with SARS-CoV2, K18:hACE *Ifnar1*^{KO} mice were even more susceptible than K18:hACE (**Fig. 8b** and **Extended Data Fig. 8b**), thus supporting a main protective role of IFN-I in this infection model. In contrast, plain *Ifnar1*^{KO} mice were resistant, as lacking hACE receptor. Patients with TLR7 mutations, especially males, were found highly susceptible to

SARS-CoV2 infection[16]. We thus addressed TLR7 role in K18:hACE mice, by breeding them with *Tlr7*^{KO} mice. TLR7 deficiency significantly worsened the susceptibility of K18:hACE mice to SARS-CoV2 infection and this independently of mouse gender, showing a protective role of TLR7 (**Fig. 8c** and **Extended Data Fig. 8c**). Altogether, these results demonstrate that the survival of K18-hACE mice to SARS-CoV2 infection requires the response to IFN-I sources that are TLR7-dependent, but pDC-independent.

The timing and the magnitude of IFN production during SARS-CoV2 infection varies between patients developing moderate versus severe COVID[50]. In mice infected with SARS-CoV1 and MERS-CoV2, IFNs were beneficial in very early phases of the infection, but detrimental later on[56, 57]. ISG expression in SARS-CoV2-infected lungs was induced as soon as 1-2 days after infection, consistent with SARS-CoV2 detection in infected tissues (**Fig. 8d-e** and **Extended Fig. 8d**). ISG induction was not affected in infected K18:pDC-less mice (**Fig. 8e**), according with their dispensable role for viral control. In contrast, ISG upregulation was affected at variable extent in infected K18:hACE *Ifnar1*^{KO} (**Fig. 8e**), likely due to partial compensation by other IFNs, such as IFN-II and IFN-III. We then analyzed the kinetic of IFN production in pDCs by infecting with SARS-CoV2 K18:hACE SCRIPT mice. pDCs isolated from the lungs of infected K18:hACE SCRIPT mice started to express YFP protein at day 1 after infection, but they reached their maximal peak later on, between day 2 and 4 (**Fig. 8f**). Hence, IFN production by pDCs was delayed with respect to ISG induction and sustained during late phases of the infection, potentially fueling IFN-dependent detrimental functions, as proposed in humans[53].

In conclusion, pDCs were dispensable in female and detrimental in male infected mice, while both IFN-I and TLR7 were protective during SARS-CoV2 infection of K18:hACE mice.

Discussion

In this study, we reported the generation of a new mouse model, the pDC-less mice, allowing specific and constitutive ablation of pDCs. pDC-less mice developed normally and were healthy, thus excluding a critical role of pDCs for host fitness in homeostatic conditions, at least in an environment free of specific pathogens. We did not detect any major disruption of other immune cell types in our mice, consistent with our intersectional genetic approach specifically targeting late pDC-committed precursors without affecting other lineages[32]. Only tDC^{lo} were slightly but significantly reduced in pDC-less mice, consistent with the identification of a shared pDC/tDC-committed bone marrow precursor [29]. Hence, in pDC-less mice, the specificity of pDC depletion is highly improved, as compared to previous methods used to target pDCs but also affecting DC precursors, tDCs and subsets of monocytes/macrophages or B cells [21], including the administration of anti-PDCA1/BST2 antibodies or the use of other mutant mice encompassing CD11c-Cre; Tcf4-/fl (CKO) animals[25, 29] or *Siglech*-hDTR animals[23, 24]. In addition, pDC-less mice allow evaluating the long-term impact of pDC loss without the confounding side-effects caused by repeated DT injections, as observed in BDCA2-hDTR mice[30, 31]. Moreover, the use of pDC-less mice as recipient for the generation of shield BM chimeras allowed to inactivate candidate genes selectively in pDCs, for the first time to our knowledge, thus representing an excellent surrogate method to overcome the current lack of pDC-specific Cre mice.

pDCs are highly conserved in vertebrates, both in their molecular make-up [58] and in their functional specialization, namely professional production of IFNs, with rapid and massive release of all subtypes of these cytokines upon exposure to virus-type stimuli[1, 47]. This implies that pDC functions benefit vertebrates in a manner enhancing their reproductive fitness, which is currently thought to be via rapid IFN-dependent reinforcement of antiviral intrinsic immunity throughout the body to promote early viral control and host resistance upon primary acute infections[6, 7]. Yet, robust experimental data supporting this hypothesis are scarce, both in humans and mice. Indeed, unlike patients who do not respond to IFN-I/III[18, 19], patients with homozygous loss-of-function mutations in TLR7, MYD88, IRAK-4 or IRF7 do not show increased susceptibility to most viral infections, with the exception of Influenza or SARS-Cov2[11, 12, 13, 14, 15, 16, 17]. However, the role of loss of pDC IFN production in this increased susceptibility is unclear, since other cell types and biological processes are also directly affected by the same mutations. In mice, although pDCs are a major IFN sources during many viral infections, their depletion or functional inactivation did not compromise host survival, except upon systemic infection by HSV-2 or ocular infection with HSV-1 [59, 60]. Here, we showed that during systemic infection by MCMV, a natural rodent pathogen, pDCs were the main source of both IFN-Is and IFN-IIIs, consistent with previous studies[30, 34]. However, the selective

ablation of pDCs compromised neither the IFN-dependent induction of ISG, nor viral control or host survival. These results are consistent with our previous work showing a greater impact of Ifnar1 inactivation over Myd88 deficiency on ISG expression and host survival in MCMV-infected mice[20]. Moreover, we also showed that ISG were induced early in the lungs of mice infected by Influenza or SARS-CoV2, irrespective of the presence of pDCs, and that pDCs were not required for resistance to these infections. This was also true in mice expressing a functional allele of *Mx1*, the key restriction factor against IAV. Hence, the increase in IAV titers observed previously in Mx1+ mice depleted with anti-Bst2 antibodies [41] was unlikely to have been caused by pDC depletion but rather resulted from the perturbation of other cell types also targeted by this treatment. Thus, contrary to the current dogma in the field, our results demonstrate that pDCs are redundant with other cellular sources of IFN for boosting antiviral intrinsic immunity and promoting overall host resistance to several systemic or respiratory viral infections in mice, which is also likely the case in humans.

Although immune cell activation was impaired in the absence of pDCs in several models of viral infections, including those used in our study[20, 30, 37, 59, 61], these defects were transient and had very limited impact on host resistance to infection, suggesting the existence of other redundant mechanisms able to preserve the induction of protective innate and adaptive antiviral immune responses in the absence of pDC functions. Our findings thus emphasize the need to reevaluate whether and how pDC functions could benefit the host during viral infections. The use of pDC-less mice and of shield BM chimeras derived thereof will certainly be key to address these questions in future studies.

In the present study, during IAV or SARS-CoV2 infections of mice, we not only showed that pDCs were dispensable but that they could actually be detrimental for host survival. Specifically, in K18:hACE infected mice, pDCs were dispensable in females, but deleterious in males. This appears contradictory with the prominent hypothesis in the field that pDCs are protective against human respiratory infections [11]. However, the picture is more complex since the role of pDCs in respiratory Influenza and SARS-CoV infections is debated. In mouse models of Influenza infection, different studies have drawn contrasting conclusions, proposing that pDCs are beneficial [41] dispensable [39, 40] or deleterious [42, 43, 44]. However, these studies used experimental strategies to deplete pDCs or impair their functions that were not specific enough and also targeted other cell types or biological processes, potentially confounding the interpretation of the results in terms of the contribution of pDCs to the phenotypes observed. For example, previous studies showed a deleterious role during murine IAV infection of Bst2+ cells [42, 43, 44], encompassing not only pDCs but also inflammatory monocytes, activated DCs and B cell subsets. In patients with severe COVID, blood pDCs were numerically reduced and defective in IFN production upon ex vivo stimulation [16, 62, 63, 64]. Moreover, an enhanced

frequency of severe COVID was detected in patients with loss-of-function mutations disrupting the TLR7-to-MYD88-to-IRF7 signaling pathway that is essential for pDC IFN production. These observations led to the proposal that pDCs are protective against COVID in most patients and that disruption of their responses contribute to disease severity. However, alternate interpretations should be considered. The decrease in pDC numbers and the loss of their ability to produce IFN in the blood of severe COVID patients could reflect the recruitment and activation of these cells into the infected tissues where they could contribute to fuel inflammation by producing IFN locally, as has been reported to occur during infections of macaques or humans by immunodeficiency viruses[65, 66]. The protective role of the TLR7-to-MYD88-to-IRF7 signaling pathway observed in human respiratory viral infections could occur in other cells than pDCs, in particular in monocytes or macrophages that also express, and respond to, these molecules [16, 67, 68]. Indeed, other studies proposed that pDCs are a deleterious source of IFNs promoting exacerbated activation of monocytes/macrophages responsible of life-threatening immunopathology [50, 51, 52, 53]. In SARS-CoV2-infected K18:hACE infected mice, our results do show contrasting roles of pDCs (deleterious) versus of overall IFN production and responses (protective), which supports the hypothesis that, in humans, the beneficial role TLR7 and IFN responses against COVID occurs in other cells than pDCs, whereas pDCs may be redundant or even deleterious. Future studies are required to test this hypothesis, especially to compare the kinetics and spectrum of the TLR7-dependent production of IFN subtypes between pDCs and other cells in vivo during SARS-CoV2 infection. These experiments could be achieved in mice but also perhaps in macaques, as the animal model naturally susceptible to this infection that is the closest to humans. This has important implications as it has been proposed to boost pDC IFN production to treat severe human respiratory infections[8], which could lead to an outcome exactly opposite to expectations if pDCs are deleterious in these diseases as we propose. On the contrary, understanding whether and how pDC IFN production could worsen disease may uniquely allow to disentangle the mechanisms underlying the beneficial versus deleterious effects of IFN responses in these diseases. This could ultimately allow designing intervention strategies to inhibit the deleterious effects of IFNs while still preserving their beneficial functions.

Most IFN-α subtypes, except IFN-α4, but not IFN-β, were reduced in the lungs of IAV-infected pDC-less mice. The production of most IFN-α subtypes requires IRF7 that is constitutively expressed in pDCs but not in most other cell types, whereas IFN-α4/β can be quickly released by IAV-infected cells in an IRF3-dependent IRF7-independent manner[69, 70]. Although all IFN-Is interact with the same IFNAR receptor complex, IFN-I subtypes differ in their affinity and duration of binding to IFNAR, leading to differences in the intensity, kinetics and array of ISG induction. In addition, differently from most infected cells, pDCs are highly motile, especially

after activation[61], thus being able to release their IFNs to specific cells in a precise time and location, as we previously showed during MCMV infection[32, 36]. Hence, a division of labor may exist between different types of IFN-producing cells. Virally-infected cells could be mainly involved in rapidly reinforcing intrinsic immunity locally, in all neighboring cells, to control viral propagation. As a complementary response, pDCs could travel within infected tissues and across organs to deliver IFNs at slightly later time points to conventional DCs or other immune cell types to exert unique immunostimulatory functions. This pDC response could be generally beneficial but would turn detrimental during infections causing IFN-dependent immunopathology or autoimmunity.

In conclusion, contrary to the dogma currently prevailing in the field, we show that, in mice, pDCs, appear to be a largely dispensable source of IFN-I for host resistance to primary acute viral infections, similarly to what had been proposed several years ago in humans[9, 10, 71]. These findings illustrate how redundancy contributes to the robustness of immune responses [20, 72], including for the signaling pathways and cellular sources leading to the production of IFNs during viral infections which is vital for the host[73]. However, our results also raise the question of the identification of the exact benefit for the host of the evolutionary conservation of pDCs, especially considering that current state of knowledge supports their detrimental roles in both infection-induced and sterile inflammation, whereas rigorous experimental proof are scarce of any beneficial role of pDCs in the natural history of diseases. Several hypotheses can be formulated. First, pDCs might be required for the protection of specific immune-privileged organs unable to produce protective IFN otherwise, as recently shown for the eye in a mouse model of cornel infection with HSV-1[60]. Second, rather than being required for resistance against a primary acute infection with a first virus, pDCs may be required to induce a body-wide state of antiviral resistance including in barrier tissues remote from the initial site of infection[74], in order to prevent superinfections by other viruses that could otherwise constitute a second lethal hit for the host. Third, pDC IFN production during a first primary acute viral infection could improve the induction of adaptive immune memory to enhance host protection to secondary infections, explaining in part the enhanced immune resistance of mice exposed to a normalized microbial environment [75]. Further studies will be necessary to investigate these hypotheses, which will require the generation of a novel mouse model of conditional rather than constitutive pDC depletion, not suffering from the confounding side effects reported for existing models [31, 34], and complex experimental set-up of sequential exposure to different pathogens [76].

Methods

Mice

All animal experiments were performed in accordance with national and international laws for laboratory animal welfare and experimentation (EEC Council Directive 2010/63/EU, September 2010). Protocols were approved by the Marseille Ethical Committee for Animal Experimentation (registered by the Comité National de Réflexion Ethique sur l'Expérimentation Animale under no. 14; APAFIS no. 21626-2019072606014177 v.4 and APAFIS #35098-2022020215455814 v4, APAFIS#26484-2020062213431976 v6). C57BL/6J (B6) mice were purchased from Janvier Labs. Siglech^{iCre} (*B6-Siglech$^{tm1(iCre)Ciphe}$*), Ifnar1-KO (B6.129S2-Ifnar^{1tm1agt}) and SCRIPT (Siglech^{iCre};Pacsin1$^{LSL-tdT}$;*Ifnb*^{EYFP}) mice were previously described[32, 47]. *Pacsin1*$^{LoxP-STOP-LoxP-DTA}$ (*B6-Pacsin1$^{tm1(DTA)Ciphe}$*, Pacsin$^{LSL-DTA}$) and Stat1-KO (B6-Stat1$^{tm1d(EUCOMM)Ciphe}$) were generated by CIPHE. Homozygous Pacsin1$^{LSL-DTA}$ and Siglech^{iCre} were bred together and their double heterozygous Siglech^{iCre}; Pacsin1$^{LSL-DTA}$ progeny was named pDC-less mice. Siglech^{iCre} and Pacsin$^{LSL-DTA}$ were also bred with CD45.1 congenic mice, then interbred to generate 5.1 pDC-less mice used as recipient for Shield Bone Marrow Chimera (SMBC) generation. TLR7^{KO} (*B6-Tlr7^{tm1Flv})* were kindly provided by L. Alexopoulou, CIML. B6.A2G-Mx1 congenic mice were generated and provided by M. Le Bert, TAAM, Orleans, France. IR7^{KO} (*B6-Irf7^{tm1Ttg})* were kindly provided by C. Svanborg, Lund, Sweden. *Ifnb*^{EYFP} (B6.129-Ifnb1^{tm1Lky}), BDCA2-hDTR (C57BL/6-Tg (CLEC4C-HBEGF)956Cln/J) and heterozygous K18-hACE C57BL/6J mice (strain: 2B6. Cg-Tg (K18-ACE2)2Prlmn/J) were obtained from Jackson Laboratories, USA. Pacsin$^{LSL-DTA}$ and B6.A2G-Mx1 mice were intercrossed to generate double homozygous mice, then bred with Siglech^{iCre} to obtain as progeny Mx1+ pDC-less mice. Control mice used in all experiments were Pacsin$^{LSL-DTA}$, Siglech^{iCre} or B6 mice. All mouse strains were on B6 genetic background and bred at the Centre d'ImmunoPhénomique (CIPHE) or the Centre d'Immunologie de Marseille-Luminy (CIML), under specific pathogen free-conditions and in accordance with animal care and use regulations. Mice were housed under a 12 h dark:12 h light cycle, with a temperature range of 20–22 °C and a humidity range of 40–70%. All animals used were sex and age matched (8-12 weeks of age for all experiments, except for SBMC).

Shield Bone Marrow Chimera (SMBC) generation

5 weeks-old CD45.1+ pDC-less were anesthetized, then their hind legs were 9 Gy irradiated, while the rest of the body was protected with a lead shield. At the end of the irradiation, mice were intravenously injected with 15 x 10^6 cells of BM isolated from indicated CD45.2+ donor

mice. 4 weeks after BM engraft mice were bled to test the reconstitution with donor BM cells, then used for experimentation.

Viruses, viral infections and mice treatment

MCMV Smith stocks were prepared from salivary gland extracts of 3-week-old, MCMV-infected BALB/c mice. Mice were infected intraperitoneally with 10^5 Plaque-Forming Units (PFU) and sacrificed at the indicated time points. For survival experiments with MCMV, mice were infected with 1.4 x 10^5 PFU. H3N2 A/Scotland/20/74 IAV strain was produced and titrated in vitro by using Madin-Darby Canine Kidney (MDCK) cell line. Mice were anesthetized with ketamine/xylazine, then intranasally infected with 40µl of DMEM medium containing 160 PFU of IAV strain and euthanized at indicated time points. For survival studies mice were infected with 145 PFU of the same IAV strain. β CoV/France/IDF0372/2020 SARS-Cov2 strain was supplied by the National Reference Centre for Respiratory Viruses hosted by the Institut Pasteur (Paris, France). This strain was isolated from a human sample provided by the Bichat Hospital, Paris, France and corresponded to the original Wuhan/D614 SARS-Cov2 strain detected at the beginning of COVID19 pandemic. Infectious stocks were grown by inoculating Vero E6 cells and collecting supernatants upon observation of the cytopathic effect. Debris was removed by centrifugation and passage through a 0.22 mm filter. Supernatants were stored at -80 °C. Mice were anesthetized with 150µL of ketamine/xylazine, then intranasally infected with 30µl of medium containing 200 PFU of β CoV/France/IDF0372/2020 SARS-CoV2. For Bulk RNASeq studies mice were infected with a high dose (1.1 x10^5 PFU) of SARS-CoV2 strain. IAV-infected and SARS-CoV2 infected mice were monitored daily for morbidity (body weight) and mortality (survival). During the monitoring period, mice were scored for clinical symptoms (weight loss, eye closure, appearance of the fur, posture, and respiration). Mice obtaining a clinical score defined as reaching the experimental end-point were humanely euthanized, according to experimental protocols approved by ethical committee. For pDC depletion in BDCA2-hDTR mice, mice were intraperitoneally injected with 15ng/g of Dyphtheria Toxin, Merck, at day 2 after IAV infection. For neutrophil depletion mice were intraperitoneally injected at day 5 and 6 after IAV infection with 500 µg/mouse of anti-Ly6G (1A8) or isotype control antibodies, both purchased from Biolegend.

Cell preparation

Mice were euthanized and perfused with PBS 1×. Spleen and lymph nodes were digested for 25 min at 37 °C with Collagenase IV (Worthington biochemical) and DNase I (Roche Diagnostics). Organs were then mechanically crushed and filtered over 70-µm cell strainers

(Corning). Bone marrow cells were flushed from mouse femurs. Livers and lungs were harvested, minced and mechanically digested with Collagenase IV and DNase I by using GentleMACS (Miltenyi, program 37_m_LIDK_1 for the liver, 37_m_LDK_1 for the lungs). At the end of the digestion, lung cell suspensions were filtered over 70-µm cell strainers and cells were pelleted by centrifugation. Liver cells were submitted to a 80:40 Percoll gradient. Small intestines were harvested, opened longitudinally, cut into 1-cm pieces and washed extensively with PBS 1×, then incubated 3× at 37 °C on shaking (200 r.p.m.) with PBS 1× containing 2% fetal calf serum (FCS) and 5 mM EDTA. At the end of each incubation, supernatants were collected and centrifuged. Pelleted cells from the three incubations were pooled together and submitted to a 67:44 Percoll gradient. Bronchoalveolar lavages (BAL) were performed upon intratracheal injection of 1 ml of cold PBS, repeated once. BAL were then centrifuged, fluids harvested and frozen at -80°C until their use. RBC lysis was performed with RBC lysis buffer (Thermofisher). For the lungs, the left lobe was harvested for RNA extraction, while the other lobes were used for flow cytometry.

Flow cytometry analysis

Cells were first incubated with 2.4G2 mAb for 10 minutes at 4°C, then we performed extracellular staining in staining buffer, PBS 1× supplemented with 2 mM EDTA (Sigma-Aldrich) and 2% FCS for 30 min at 4 °C. Dead cell staining (LIVE/DEAD Fixable Aqua Dead Cell Stain, Life Technologies) was performed in PBS 1× according to the manufacturer's recommendations. All antibodies used and their related dilution have been listed in the Reporting Summary of this manuscript. Before acquisition samples were fixed with Cytofix/Cytoperm BD 1X (eBioscience) or with a 2% formaldehyde solution in PBS 1× when using cells expressing EYFP protein. Samples were acquired with a FACS LSR UV (BD Biosciences) using BD Diva v.9.0. All data were analyzed with FlowJo v.10.8.1 software.

RNA extraction and RT-qPCR

Harvested organs were incubated in RNAlater (Thermofisher) at 4°C overnight. Total RNAs from lung cells were extracted using RNeasy Plus Mini Kit (Qiagen) following the manufacturer protocol. Retrotranscription into complementary DNA (cDNA) was performed using the Quantitect Reverse Transcription Kit (Qiagen). The expression levels of the following murine genes were determined by quantitative PCR (qPCR) using the SYBR® Premix Ex TaqTM kit and analysed using the Prism 7500 Fast PCR System. Relative gene expression was calculated using the ΔΔCt method with *Actinb* as housekeeping gene for normalization. The primers used were as it follows: *Actinb* forward 5'-GGCTGTATTCCCCTCCATCG-3'; reverse 5'-CCAGTTGGTAACAATGCCATGT-3'; *Il28b* forward 5'— GGAGGCCCAGAGCAAGGA-3' ;

reverse 5'- TTGAAACAGGTTGGAGGTGACA-3' ; *Irf7* forward 5'— CCACGCTATACCATCTACCTGG-3' ; reverse 5'- GCTGCTATCCAGGGAAGACAC-3' ; *Isg15* forward 5'— GGTGTCCGTGACTAACTCCAT-3' ; reverse 5'- TGGAAAGGGTAAGACCGTCCT-3' ; *Mx2* forward 5'— AGAGGGAGAATGTCGCCTATT-3' ; reverse 5'- CGTCCACGGTACTGCTTTTCA-3' and *Oas2* forward 5'— TTGAAGAGGAATACATGCGGAAG-3' ; reverse 5'GGGTCTGCATTACTGGCACTT-3'. Viral titration of MCMV was performed as previously described[20]. For viral titration of IAV, we performed RT-qPCR by testing the expression of *M1* IAV gene, using the following primers: forward 5'— AAGACCAATCCTGTCACCTCTGA-3' ; reverse 5'- CAAAGCGTCTACGCTGCAGTCC-3'. For viral titration of SARS-CoV2, tissues were homogenized with ceramic beads in a tissue homogenizer (Precellys, Bertin Instruments) in 0.5 mL RLT buffer. RNA was extracted using the RNeasy Mini Kit (QIAGEN) and reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Amplification was carried out using OneGreen Fast qPCR Premix (OZYME) according to the manufacturer's recommendations. Copy numbers of the RNA-dependent RNA polymerase (*RdRp*) SARS-CoV2 gene were determined using the following primers: forward 5'- CATGTGTGGCGGTTCACTAT-3', reverse 5'- GTTGTGGCATCTCCTGATGA-3'. This region was included in a cDNA standard to determine the copy number determination down to 100 copies/reaction. SARS-CoV-2 copy numbers were compared and quantified using a standard curve and normalized to total RNA levels. An external control (mock-infected wild type animal) and a positive control (SARS-CoV2 cDNA containing the targeted region of the *RdRp* gene at a concentration of 10^4 copies/µl [1.94×10^4 copies/µl detected in the assay]) were used in the RT-qPCR analysis to validate the assay.

Bulk RNA-Seq

RNA was isolated from the lungs of uninfected and IAV-infected control and pDC-less mice (160 PFU, days 0, 3 and 6 after infection) or of uninfected and SARS-CoV2 infected K18:hACE2 mice (1.1 10^5 PFU, days 0, 1, 2, 4 and 6 after infection), as described in RNA extraction method. For Bulk RNASeq 500 ng of lung RNA/sample were used to prepare libraries for sequencing by using KAPA RNA HyperPrep Kits (Roche). Libraries generated with IAV-infected samples were sequenced by Macrogen, while libraries generated from SARS-CoV2 infected samples were sequenced at the Genomic facility of the CIML. All libraries were sequenced using Illumina platforms. Alignments of fastq files obtained after sequencing were generated with STAR 2.7.9a software using the GRCm38 reference genome. The number of reads mapped to each gene was determined with featureCounts v2.0.1 using

Mus_musculus.GRCm38.100.gtf annotation file. Gene models (gene symbols of the type "Gmxxxx") were then removed from the feature count file and TPM normalization was applied using the Scuttle package of R 1.0.34.

FITC-Dextran

To assess lung permeability, isoflurane-anesthetized mice were administered intratracheally with 10 µg/mouse (50µl of a solution at 200µg/ml in PBS 1x) of FITC-dextran (Sigma) 8 days after IAV infection. 1 hour later, blood was collected from anesthetized mice from the retro-orbital sinus and the plasma was separated by centrifugation. Plasma were diluted 1:2 in PBS 1X. Dextran leakage in the bloodstream was measured as FITC fluorescence in the plasma. Fluorescence was read at 520 nm with excitation at 490 nm by using a Mithras LB 940 plate reader (Berthold Technologies).

Cytokine quantification

Cytokines and chemokines present in the sera of uninfected vs MCMV-infected mice and in the BAL of uninfected vs IAV-infected mice were analyzed using the LEGENDplex Mouse Anti-Virus Response Panel (BioLegend). Samples and standards were plated in technical duplicates and the assay was executed according to the manufacturer's protocol. Data were collected using the CANTO II (BD Biosciences) and analyzed using the cloud-specific LEGENDplex Data Analysis Software Qognit (BioLegend).

Anatomopathological analysis

Lungs were harvested from control and pDC-less mice 8 days after IAV infection and kept with 10% buffered formalin (VWR Chemical) for 24 hours, then dehydrated and embedded in paraffin (Fischer Histoplast). 3,5µm-thick sections were cut using the microtome Leica RM2245. Hematoxylin-eosin staining was made automatically with Leica Autostainer XL. Finally, the slides were mounted with Entellan mounting medium (Merck) and kept at room temperature. Tissue sections were blindly assessed by a certified veterinary pathologist. Microscopic examination was done using a Nikon Eclipse E400 microscope. Pictures were taken with a Nikon DS-Fi2 camera and NIS Elements imaging software (Nikon, Japan). Histopathological semi-quantitative lung inflammation scoring system was performed according to Supplementary Table 1.

Immunohistofluorescence, confocal microscopy and image analysis

Mouse lungs were perfused with a mixture of 1:2 of AntigenFix (Diapath, containing 4% paraformaldehyde) and 1:2 of Optimal Cutting Temperature (OCT, Sakura Fineteck), collected, fixed in AntigenFix for 4h at 4°C and then washed several times in phosphate buffer (PB; 0.025 M NaH2PO4 and 0.1 M Na2HPO4). Lungs were incubated in PB solution containing 30% Sucrose overnight at 4°C and then embedded in OCT freezing medium, snap frozen and stored at −80 °C. 30 µm-thick cryosections were performed using a microtome (Leica 3050s Cryostat) at temperatures between −24 °C and −20 °C.

For immunostaining, lung sections were blocked with PB, 0.1% Triton X-100 and 2% BSA for 1h at room temperature and then stained overnight at 4°C with primary antibodies diluted in PB, 0.1% Triton X-100 and 2% BSA. After several washings with PB, lung sections were then stained in PB, 0.1% Triton X-100 and 2% BSA with secondary antibodies for 2h at 4°C. To detect YFP signal, after the incubation with secondary antibodies, sections were washed and incubated with PB, 0.1% Triton X-100, 2% BSA and 5% rabbit serum for 45 min at room temperature. Sections were then stained with Alexa 488-conjugated anti-GFP antibodies for 1h at 4°C. Finally, lung sections were washed with PB and mounted with a coverslip and Prolong Antifade Gold mounting medium (Life Technologies). Stained lung sections were acquired using spectral confocal microscopes (Zeiss LSM880 or Zeiss LSM980) with x10/0.45 or x20/0.8 objectives, respectively. Images were acquired using the ZEN blue software (Carl Zeiss Microscopy), in 16-bit format, in spectral mode to suppress autofluorescence for each fluorochrome used. Images were then analyzed using ImageJ software [77]. For CD45 quantification areas expressing low versus high CD45 signal were qualitatively identified in each whole lung section, then a region of interest (ROI) was created for each area and CD45 mean fluorescence intensity (MFI) was measured. In highly inflamed areas CD45 MFI was at least twice of that detected in the lowly inflamed areas. We then used QuPath v0.4.3 [78] for cell quantifications. CD45+ cells, IAV-infected HA+ cells and Tom+ pDCs were quantified using the QuPath cell detection module. Then, using the QuPath classifier module, we quantified among the Tom+ pDCs, the IFN+ (Tom+ YFP+) pDCs expressing at least a threshold value of 2216 in YFP channel. For lung integrity analysis, EpCAM+ areas were defined as areas expressing at least a 5000 threshold for EpCAM channel, then their percentage was calculated on the whole lung surface analyzed. EpCAM+ bronchi were classified as closed when EpCAM staining was continuous and as opened when EpCAM was disrupted. Both types of EpCAM+ bronchi were manually quantified and corresponding percentages were calculated.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 10 software. All results are expressed as mean ± s.e.m. All quantifications were performed with awareness of experimental groups, meaning not in a blinded fashion. Animals were matched in age and gender between experimental groups and each cage was randomly assigned to a treatment group. No animals or data were excluded under any circumstances. Statistical parameters including the exact value of n (number of biological replicates and total number of experiments) and the types of statistical tests are reported in corresponding legends. Comparisons between two groups were assessed using unpaired and non-parametric Mann–Whitney test. Multiple comparisons were assessed using a Kruskal-Wallis test followed by Dunn's post-hoc test. Survival curves were analyzed using Log-rank (Mantel-Cox) test. Comparisons between groups were planned before statistical testing and target effect sizes were not predetermined. Each dot indicates an individual. p values have been specified in each panel.

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Author contributions

C.N. designed, performed and analyzed most of the experiments, and contributed to write the manuscript. K.R. performed RT-qPCR experiments on MCMV- and IAV-infected samples. M.V. prepared the libraries used for Bulk RNA-Seq samples and contributed to perform the experiments on SARS-CoV2-infected samples. N.C. genotyped and stabilized mutant pDC-less mice. G.B. performed viral titration on MCMV-infected samples. M.Fab., S.S., S.M., C.P.M. and A.S. bred K18:hACE and related double/triple mutant mice and performed the experiments with SARS-CoV2-infected mice and samples. F.F. directed, designed and analysed the generation of *Pacsin1*$^{LSL-DTA}$ mice. C.L. performed anatomopathological analysis of lung sections from IAV-infected mice. M.G., C.G. and A.B. provided advice and shared reagents and experimental protocols. P.M. provided advice and technical support for Bulk RNA-Seq experiments. B.E. and T.P.V.M. performed the mapping and the first analysis of Bulk RNA-Seq data. M. Fal. contributed to the setting of the analysis of confocal microscopy images. L.C.

performed paraffin-embedding, prepared and stained lung sections used for anatomopathological studies. H.T. genotyped and provided *Irf7*^{KO} mice. M.L.B. provided B6.A2G-Mx1 mice. B.M. contributed to direct and fund the study. A.Z., M.D. and E.T. contributed to fund the study and directed it, designed experiments, performed some experiments, analysed data and wrote the manuscript.

Competing interest

The authors declare no competing interests.

Legends to Figures

Fig. 1: The pDC-less mice allow selective and constitutive depletion of pDCs in different organs. a, Scheme illustrating the strategy used to generate pDC-less mice. LoxP is the sequence recognized by Cre recombinase. 'STOP' corresponds to a transcriptional stop sequence. DTA= Diphtheria Toxin Subunit A. **b,** Dot plots showing the absence of lin- CD11b- CD11c^{int} Bst2^{hi} pDCs in cells isolated from indicated organs of control (CTR) vs pDC-less mice. pDC percentages (mean +/- s.e.m.) were pooled from two independent experiments (n=5 for each strain). **c,** Absolute numbers of total cells isolated from the indicated organs of CTR vs pDC-less mice (n= 9 for the spleen and lymph nodes (LN), n=5 for the bone marrow (BM) and liver). **d-f**, Percentages within CD45+ splenic cells of indicated lymphoid (**d**) and myeloid (**e,f**) lineages isolated from control vs pDC-less mice. RPM: red pulp macrophage cMo: conventional monocyte. The data shown (mean + s.e.m.) are pooled from two independent experiments (n = 9). An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak method for correction was used for the statistical analysis.

Fig. 2: pDCs exert a dispensable role during MCMV infection. Control and pDC-less mice were infected (i.p) with 1x10^5 PFU of MCMV Smith and analyzed at indicated time points after infection. **a,** Cytometric bead arrays was performed to quantify cytokine concentrations in the sera isolated from uninfected (0) and 36h infected (1.5) control and pDC-less mice. Each dot represents an individual animal. The data shown (mean +/- s.e.m.) are pooled from two independent experiments (n=3 for 0, n=7 for 1.5). An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak correction method was used for the statistical analysis. **b,** Control and pDC-less mice were infected with 1.4 x10^5 PFU of MCMV Smith and daily monitored for their survival, as shown in Kaplan-Meier curve (n= 10 for control, =8 for pDC-

less). Log-rank (Mantel-Cox) test was used for statistical analysis. **c,** Viral titers were measured by RT-qPCR in the spleen, liver and lungs isolated from control and pDC-less mice 3 days after MCMV infection. Data shown are pooled from two independent experiments (9 CTR and 7 pDC-less for the spleen, 7 CTR and 6 pDC-less for the liver, n=8 for both strains for the lungs). Means are indicated by a horizontal bar. An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak correction method was used for the statistical analysis. **d,** Expression levels of indicated genes was analyzed by RT-qPCR in indicated organs isolated from uninfected or MCMV-infected CTR, pDC-less and *Ifnar1*^{KO} mice at indicated days after infection. Data were normalized to expression level of *Actin* gene. The data (mean + s.e.m.) shown are from two independent experiments (n=4 for each condition at each time points). A nonparametric One-Way ANOVA (Kruskal-Wallis test with Dunn's correction) was used for the statistical analysis. **e,** The percentages of GranzymeB (GzmB)$^{+}$, IFNγ^{+} and CD69^{+} cells within splenic NK cells (mean +/- s.e.m.) were determined in control and pDC-less mice at indicated days after MCMV infection (n=4 for 0, =8 for 1.5, =7 for 2 and =10 for 3 days after infection for both strains of mice). An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak correction method was used for the statistical analysis.

Fig. 3: Both pDCs and the response to IFN-I are detrimental during IAV infection. Mice were infected with 145 PFU (i.n). in **a,b** and in **f, g**, while in **e**, they were infected i.n. with 1x10^{6} PFU of IAV strain H3N2A/Scotland/20/74. IAV-infected mice were monitored daily to assess their morbidity and survival. BDCA2-DTR mice were inoculated with 15 ng/g of diphtheria toxin (DT) two days after IAV infection. Kaplan-Meier survival plot shows the percentages of survival of indicated mouse strains. **a**, n= 10 for both strains, **b**, n=9 for both strains, **e**, n=8 for Mx1 CTR and 10 for Mx1 pDC-less mice, **f**, n= 10 CTR, 8 *Ifnar1*^{KO} and 9 *Stat1*^{KO} mice, **g**, n= 10 CTR, 9 *Tlr7*^{KO} mice. Log-rank (Mantel-Cox) test was used for statistical analysis. **c**, Expression levels of IAV M1 gene was analyzed by RT-qPCR in lungs isolated from uninfected or IAV-infected CTR and pDC-less mice at indicated days after infection. Data were normalized to expression level of *Actin* gene. The data (mean + s.e.m.) shown are from two independent experiments (n=4 for each condition at each time points). n= 6 at 0, 3 at day 1, 5 at day 2, 14 at day 6 and 3 at day 8 after infection. **d**, Normalized expression (log2) of indicated genes based on Bulk RNASeq samples obtained by whole lung samples isolated from CTR and pDC-less mice at day 0 (n=3/strain), 3 and 6 (n=4/strain) after IAV infection.

Fig. 4: During IAV infection, pDCs increase in infected lungs and produce IFN-I. a-b, *Ifnb1*^{EYFP} mice were infected (i.n) with 160 PFU of IAV strain H3N2A/Scotland/20/74 and analyzed at different time points after infection. **a**, The percentages of cDCs, pDCs and tDCs within CD45+ cells (mean +/- s.e.m.) isolated from the lungs of infected *Ifnb1*^{EYFP} mice were determined at the indicated time points. One-Way ANOVA (Kruskal-Wallis test followed by

Dunn's post-hoc test) was used for the statistical analysis. The percentages of YFP+ pDCs (**b**) at the indicated days after infection have been represented. N=2 for day 0, =3 for day 2, =6 for days 4 and 6, =4 for day 8 after infection. A two-tailed non-parametric Mann-Whitney test was used for the statistical analysis. **c,** Cytometric bead arrays was performed to quantify IFNα and IFNβ concentrations in the BAL isolated from CTR and pDC-less mice uninfected (0) or at indicated days after infection. Each dot represents an individual animal. The data shown (mean +/- s.e.m.) are pooled from two independent experiments (n=3 CTR and 4 pDC-less at day 0, 3 CTR and 2 pDC-less at day 2, 7 at day 3, 6 at day 6 and 7 at day 8 for both strains). An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak method for correction was used for the statistical analysis. **d-i**, Representative images of histological sections isolated from the lungs of SCRIPT mice at day 4 after IAV infection. 30µm-thick lung sections were stained with anti-CD45 (white), anti-Hemagglutinin (HA) of IAV (orange), anti-Tomato (Tom, purple), anti-YFP (green). On each image acquired, the MFI of CD45 in highly inflamed areas was at least twice of that of lowly inflamed areas. The numbers of CD45+ cells (**j**), HA (IAV)+ cells (**k**), pDCs (**l**) and YFP+ pDCs (**m**) per mm² were quantified in highly and lowly inflamed areas in IAV-infected lungs. The data shown (mean +/- s.e.m.) are from n=5 at day 2, n=5 at day 3, n=10 at day 4 and n=4 at day 6, with two sections of the lung analyzed per mouse. An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak correction method was used for the statistical analysis.

Fig. 5: IFN-I produced by pDCs during IAV infection are deleterious. a, Scheme illustrating the generation of pDC Shield BM chimeric (SBMC) mice. Only hind limbs of pDC-less mice are irradiated, while the rest of the body is protected by a lead shield (gray oval). Upon local irradiation-induced distress, donor BM cells preferentially reconstitute immune cell types absent in the recipient, that is pDCs. **B**, Recipient CD45.1+ (gray) pDC-less mice are used as recipient of pDC SBMC reconstituted with donor CD45.2+ (black) BM cells. The percentage of donor (black) vs recipient (gray) cells was analyzed in indicated lineages 4 weeks after reconstitution (n= 4). **c-e,** pDC SBMC mice reconstituted with CTR, pDC-less or *Myd88*^{KO} BM (**c**), with CTR or *TLR7*^{KO} BM (**d**) or with CTR, pDC-less or *Irf7*^{KO} were infected (i.n). with 145 PFU of IAV strain H3N2A/Scotland/20/74 IAV-infected mice and daily monitored to assess their morbidity and survival. Kaplan-Meier survival plots show the percentages of survival of indicated genotypes. **c-d,** Pool of two independent experiments (n=8 for each strain); **e,** Pool of three independent experiments (n=12 for each strain). Log-rank (Mantel-Cox) test was used for statistical analysis.

Fig. 6: During IAV infection, lung inflammation and cytokine production are reduced in the absence of pDCs. a-d, Representative images of H&E-stained sections of lungs of control (**a-b**) and pDC-less (**c-d**) mice isolated at day 8 after infection. (**a-b**)= marked broncho-interstitial pneumonia with terminal bronchiole filled with degenerated neutrophils; (**c-d**)= mild broncho-interstitial pneumonia. Magnification is indicated on each image. **e,** Lung sections of all mice were semi-quantitatively assessed using a cumulative score as normal (0), mild (1), moderate (2), marked (3) and severe (4) for inflammation in interstitium, intra-alveolar, peribronchial, intra-bronchial and perivascular areas, and as normal (0), focal (1), multifocal (2), extensive (3) for hemorragic necrosis, emphysema and pleura. The data (mean +/- s.e.m.) shown are pooled from two independent experiments, n=8 CTR, 9 pDC-less mice. A two-tailed nonparametric Mann-Whitney test was used for the statistical analysis. **f,** Absolute numbers of all lung cells and of CD45+ cells isolated from the lungs of control and pDC-less mice at the indicated days after infection. Data are represented as mean +/- s.e.m. of the following numbers of individuals pooled from two independent experiments for each time point (n= 5 at day 0, n= 7 at day 3, n= 8 at day 6 and at day 8 for each mouse strain). **g,** Percentages of myeloid and lymphoid immune cells isolated from the lungs of control and pDC-less mice isolated at the indicated day after infection. For each mouse strain, n= 5 at day 0, n= 7 at day 3, n= 8 at day 6 and n= 4 at day 8, except n= 8 at day 8 for neutrophils, eosinophils, alveolar macrophages (AM) and monocytes (cMo). For **f** and **g**, an unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak correction method was used for the statistical analysis. **h,** Control and pDC-less mice were infected with 145 PFU of IAV strain and treated with anti-Ly6G (1A8) antibody (triangle) or isotype control (IC,circle) at days 5 and 6 after infection. Survival and morbidity were daily monitored. Data are pooled from two independent experiments. Kaplan-Meier survival plot shows percentage survival of indicated conditions. For each mouse strain, n= 8 for mice treated with isotype control (IC) and n=9 for mice treated with 1A8 antibodies. **i,** Cytometric bead arrays was performed to quantify the concentrations of indicated cytokines in the BAL isolated from control and pDC-less mice at indicated days after IAV infection. Each dot represents an individual. Data shown (mean + s.e.m.) are pooled from two experiments. n= 3 CTR and 4 pDC-less mice at day 0, n= 3 CTR and 2 pDC-less mice at day 2, n= 7 at day 3, n= 6 at day 6 and n= 7 at day 8 for both mouse strain. An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak method for correction was used for the statistical analysis.

Fig. 7: During IAV infection lung integrity and permeability are less affected in the absence of pDCs. a, Representative images of histological sections of lungs isolated from control and pDC-less mice uninfected (left) or at day 8 after IAV infection (right). 30µm-thick

sections were stained with anti-CD45 (white) and anti-EpCAM (red) antibodies. EpCAM+ bronchi were classified as closed (red arrow) when EpCAM staining was uninterrupted and as opened (white arrow) when EpCAM was discontinuous. **b-c**, The percentages of the EpCAM+ area over the entire surface of each lobe (**b**) and the percentages of closed vs opened bronchi (**c**) were quantified in lung sections isolated from uninfected vs IAV-infected control vs pDC-less mice. The data (mean +/- s.e.m.) shown in **b-c** are from n=1 at day 0 and n=4 at day 8 after infection for both mouse strains. We analyzed 2 lobe sections at day 0 and 9 lobe sections at day 8 for control mice, 3 lobe sections at day 0 and 8 lobe sections at day 8 for pDC-less. An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak correction method was used for the statistical analysis. **d-e,** 8 days after IAV infection control and pDC-less mice were i.t. treated with fluorescein isothiocyanate (FITC-Dextran) (10µg per mouse). Barrier permeability was measured as relative fluorescent units (RFU) of FITC-dextran leaked in the plasma 1 hour after i.t. FITC-dextran administration. Each dot represents an individual. Data shown (mean + s.e.m.) are pooled from two experiments (n = 2 for each strain and condition for uninfected mice, as well as for PBS-treated infected mice, n= 9 for FITC-treated infected CTR and 8 for pDC-less mice). An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak method for correction was used for the statistical analysis.

Fig. 8: pDCs are not required for the resistance to SARS-CoV2 infection and can exert detrimental functions. **a-c,** K18:hACE transgenic mice bred or not with indicated mutant mice were infected with 200 PFU (i.n). of SARS-CoV2 strain. Infected mice were daily monitored to assess their morbidity and survival. Kaplan-Meier survival plot shows the percentages of survival of indicated mouse strains. **a**, n= 28 for female K18:hACE and K18:hACE pDC-less; n= 19 for male K18:hACE, = 26 for male K18:hACE pDC-less, **b**, n=10 for K18:hACE and plain *Ifnar1*^{KO}, =15 for K18:hACE *Ifnar1*^{KO}, **c**, n= 12 for female K18:hACE, n= 9 for female K18:hACE *Tlr7*^{KO}; n= 11 for male K18:hACE, n = 7 for male K18:hACE *Tlr7*^{KO}. **d**, Normalized expression (log2) of indicated genes based on Bulk RNASeq samples obtained by whole lung samples isolated from transgenic K18:hACE mice infected with 1.1 10^5 PFU of SARS-CoV2 at indicated days after infection. Data (mean +/- s.e.m.) shown are from n= 3 at days 0 and 1, = 5 at day 2 and 4, =2 at day 6 after infection. **e**, Expression levels of indicated genes was analyzed by RT-qPCR in lungs isolated from uninfected or SARS-CoV2-infected K18:hACE mice alone or bred with pDC-less or with *Ifnar1*^{KO} mice at indicated days after infection. Data were normalized to expression level of *Actin* gene. The data (mean + s.e.m.) shown are from two independent experiments (n=2 for all strains at day 0, n=10 for K18:hACE and K18:hACE pDC-less, =5 for K18:hACE *Ifnar1*^{KO} and *Ifnar1*^{KO} at day 2, n= 7 for K18:hACE and K18:hACE pDC-less, =5 for K18:hACE *Ifnar1*^{KO} and =3 for *Ifnar1*^{KO} at day 5). A nonparametric one-way ANOVA (Kruskal-

Wallis test followed by Dunn's post-hoc test) was used for the statistical analysis. **f**, The percentages of YFP+ pDCs at the indicated days after infection have been represented. Data are pooled of three independent experiments. n=10 at day 0, =12 at day 1, =7 at day 2, =12 at day 4 after infection. A two-tailed non-parametric Mann-Whitney test was used for the statistical analysis.

Extended Fig. 1: Gating strategy for the identification of myeloid and lymphoid lineages. a, c, Gating strategy used to identify myeloid and lymphoid immune cell types studied. **b**, The weight of control vs pDC-less was measured along time. Weight mean was represented at each time point analyzed for each strain of mice (n=5).

Extended Fig. 2: Complementary analysis of MCMV-infected control and pDC-less mice a, Cytometric bead arrays was performed to quantify CCL2 concentrations in sera isolated from uninfected (0) and 36h infected (1.5) control and pDC-less mice. Each dot represents an individual animal. The data shown (mean +/- s.e.m.) are pooled from two independent experiments (n=3 for 0, n=7 for 1.5). **b**, Weight curves of mice represented in **Fig. 2a** (10 CTR, 8 pDC-less). **c**, Relative expression of *Mx2* gene (mean +/- s.e.m.) was analyzed in the spleens of C57BL/6 infected with MCMV at indicated time points (n=3 for each time point). **d,** Expression levels of indicated genes was analyzed by RT-qPCR in indicated organs isolated from uninfected or MCMV-infected CTR, pDC-less and *Ifnar1*^{KO} mice at indicated days after infection. Data were normalized to expression level of *Actin* gene. The data (mean + s.e.m.) shown are from two independent experiments (n=4 for each condition at each time point). Statistical analysis was performed as described in **Fig. 2d**. **e**, CD86 MFI values on indicated cDC subsets (mean +/- s.e.m.) were determined in control and pDC-less mice at indicated days after MCMV infection (n=4 for 0, =8 for 1.5, =7 for 2 and =10 for 3 days after infection for both strains). Statistical analysis was performed as described in **Fig. 2e**.

Extended Fig. 3: Weight curves of IAV-infected mice deficient in pDCs, in the response to IFN-I or to all IFNs, or in TLR7 a-e, The weight of indicated IAV-infected mice was regularly measured up to 2-3 weeks after infection. Data are represented as percentages of the weight at day 0 of the infection. Mice reaching the stop points were euthanized. **a**, n= 10 for both strains, **b**, =n=9 for both strains, **c**, n=8 Mx1 CTR ,10 Mx1 pDC-less mice, **d**, n= 10 CTR, 8 *Ifnar1*^{KO} and 9 *Stat1*^{KO} mice, **e**, n= 10 CTR, 9 *Tlr7*^{KO} mice.

Extended Fig. 4: During IAV infection pDCs are increased in the lungs and are a main source of IFNα. a-b, Gating strategy for (**a**) and percentages of (**b**) pDCs, cDCs and tDCs isolated from the lungs of IAV-infected *Ifnb1*^{EYFP} mice. n=2 for day 0, =3 for day 2, =6 for days 4 and 6, =4 for day 8 after infection. **c**, Normalized expression (log2) of indicated genes based on Bulk RNASeq samples obtained by whole lung samples isolated from CTR and pDC-less mice at day 0 (n=3/strain), 3 and 6 (n=4/strain) after IAV infection. **d**, Highly vs lowly inflamed areas were defined taking in account the CD45 MFI. For each tissue section CD45 MFI was at least twice in highly inflamed areas, as compared to lowly ones. **e-h**, The numbers of CD45+ cells (**e**), IAV+ cells (**f**), pDCs (**g**) and YFP+ pDCs (**h**) per mm² were quantified in highly and lowly inflamed areas in IAV-infected lungs. The data shown (mean +/- s.e.m.) in **d-h** are the pool of the analysis of two tissue sections/lung lobe/mouse for the following numbers of infected SCRIPT mice: n=5 at day 2, n=5 at day 3, n=10 at day 4 and n=4 at day 6 after infection. An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak method for correction was used for the statistical analysis. **i-j**, Staining exclusively with anti-YFP (green) **(i)** or with anti-tdT (magenta) **(j)** on the image represented in **Fig. 4i**.

Extended Fig. 5: Weight curves of IAV-infected pDC SBMC mice deficient in pDCs or in molecules involved in TLR7/Myd88/IRF7 signaling pathway. a-c, The weight of indicated IAV-infected mice was regularly measured up to 3-4 weeks after infection. Data are represented as percentages of the weight at day 0 of the infection. Mice reaching the stop points were euthanized. **c-d,** Pool of two independent experiments (n=8 for each strain); **e,** Pool of three independent experiments (n=12 for each strain).

Extended Fig. 6: Gating strategy used to identify immune cell types in cells isolated from the lungs of uninfected vs IAV-infected control and pDC-less mice. a-b, Cells were isolated after enzymatic digestion of lungs of uninfected or IAV-infected animals and analyzed by flow cytometry. Gating strategy used to define indicated immune cell types has been depicted. Plots shown are from one representative mouse out of 40 analyzed. **c,d**, Percentages of CD45+ and lymphoid immune cells isolated from the lungs of control and pDC-less mice isolated at the indicated day after infection. For each mouse strain, n= 5 at day 0, n= 7 at day 3, n= 8 at day 6 and n= 4 at day 8. An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak correction method was used for the statistical analysis.

Extended Fig. 7: Activation state of immune cells and cytokine production in the lungs of control vs pDC-less mice during IAV infection. a-b, Mean Fluorescence intensity (MFI) of CD86 (**a**) and of CD69 (**b**) molecules expressed by indicated immune cell types isolated from the lungs of control or pDC-less mice at indicated days after IAV infection. **c**, Normalized expression (log2) of indicated cytokine genes based on Bulk RNASeq samples obtained by whole lung samples isolated from CTR and pDC-less mice at day 0 (n=3/strain), 3 and 6 (n=4/strain) after IAV infection. **d,** Cytometric bead arrays was performed to quantify cytokine concentrations in the BAL isolated from CTR and pDC-less mice uninfected (0) or at indicated days after infection. Each dot represents an individual animal. The data shown (mean +/- s.e.m.) are pooled from two independent experiments (n=3 CTR and 4 pDC-less at day 0, n=3 CTR and 2 pDC-less at day 2, n=7 at day 3, =6 at day 6, =7 for day 8 for both strains). An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak method for correction was used for the statistical analysis.

Extended Fig. 8: Impact of the loss of pDCs, *Ifnar1* or *Tlr7* on the weight curves of SARS-CoV2-infected K18:hACE. a-c, The weight of indicated SARS-CoV2-infected mice was regularly measured up to 12 days after infection. Data are represented as percentages of the weight at day 0 of the infection. Mice reaching a clinical score defined as reaching the experimental end-point were humanely euthanized. Mice cohort numbers were indicated in the legend of **Fig. 8**. **d,** SARS-CoV-2 viral burdens were measured by RT-qPCR as genome equivalent of RdP protein/ng of total RNA of lungs isolated from K18:hACE mice at 0, 2 and 5 days post infection. Each plotted dot represents the viral burden for an individual animal and bars represent median +/- s.e.m.

Supplementary Table 1 : Semi-quantitative scoring of lung inflammation in IAV-infected mice.

Compartment And/or assessed criteria	**Semi-quantitative scoring**				
Interstitium	Normal	Less than 25 % of interstitial parenchyma affected	25 to 50 %	51 to 75 %	> 75 %
	0	1	2	3	4
Alveoli Intra-alveolar inflammatory cells (neutrophils, macrophages)	Absence	< 10 per HPF	10 to 20 per HPF	21 to 50 per HPF	> 50 per HPF
	0	1	2	3	4
Peribronchial inflammation	None	Less than 25 % of bronchi affected	25 to 50 % of bronchi affected	51 to 75 %, of bronchi affected	> 75 % of bronchi affected
	0	1	2	3	4
Intra-bronchial inflammation	None	Less than 25 % of bronchi filled with rare necrotic or inflammatory cells	25 to 50 % bronchi filled with necrotic or inflammatory cells	51 to 75 % bronchi filled with necrotic or inflammatory cells,	> 75 % bronchi filled with inflammatory or cellular debris
	0	1	2	3	4
Perivascular inflammation	None	Perivascular cuffs on less than 25 % of vessels	Perivascular cuffs on 25 to 50 % of vessels	Perivascular cuffs on 51 to 75 % of vessels	Perivascular cuffs on > 75 % of vessels
	0	1	2	3	4
Vasculitis inflammatory cells within the wall	No		Yes		
	0		1		
Hemorragic necrosis	Absence	Focal	Multifocal	Extensive	
	0	1	2	3	
Emphysema	Absence	<25 % of parenchyma	26 to 50 % of parenchyma	51 to 75 % of parenchyma	
	0	1	2	3	
Pleura	Normal	Focal pleural involvement	Multifocal pleural involvement	Diffuse pleural involvement	
	0	1	2	3	

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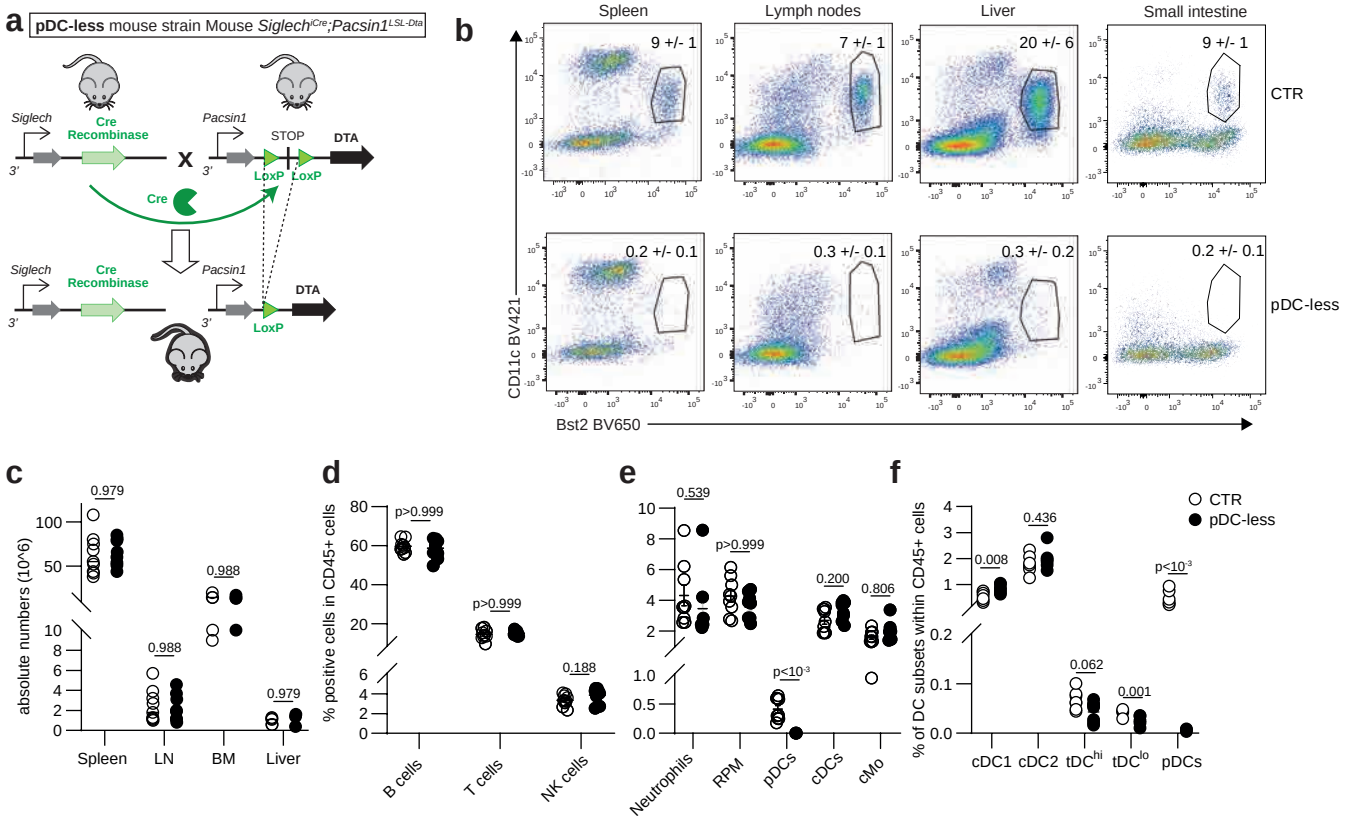

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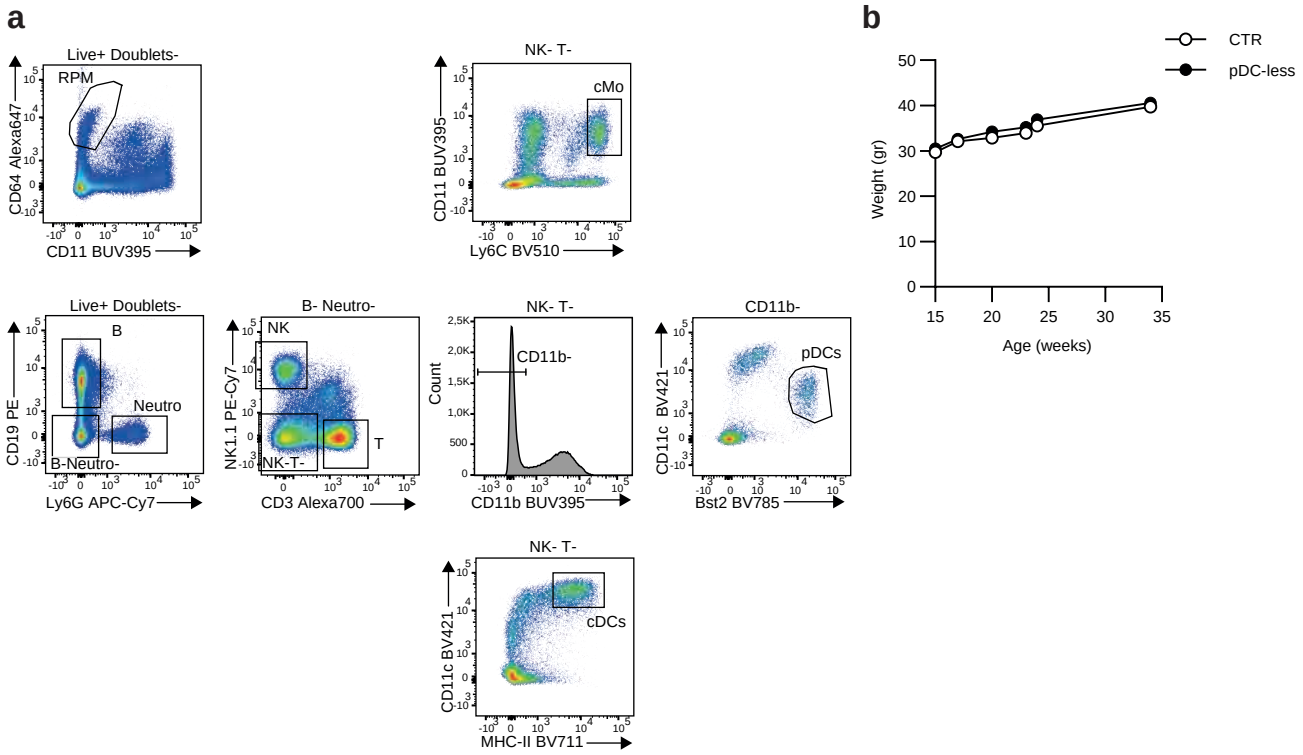

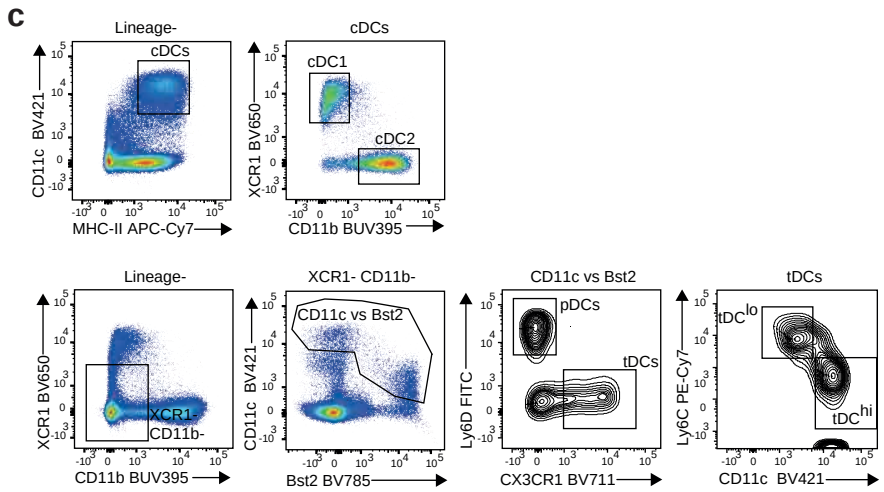

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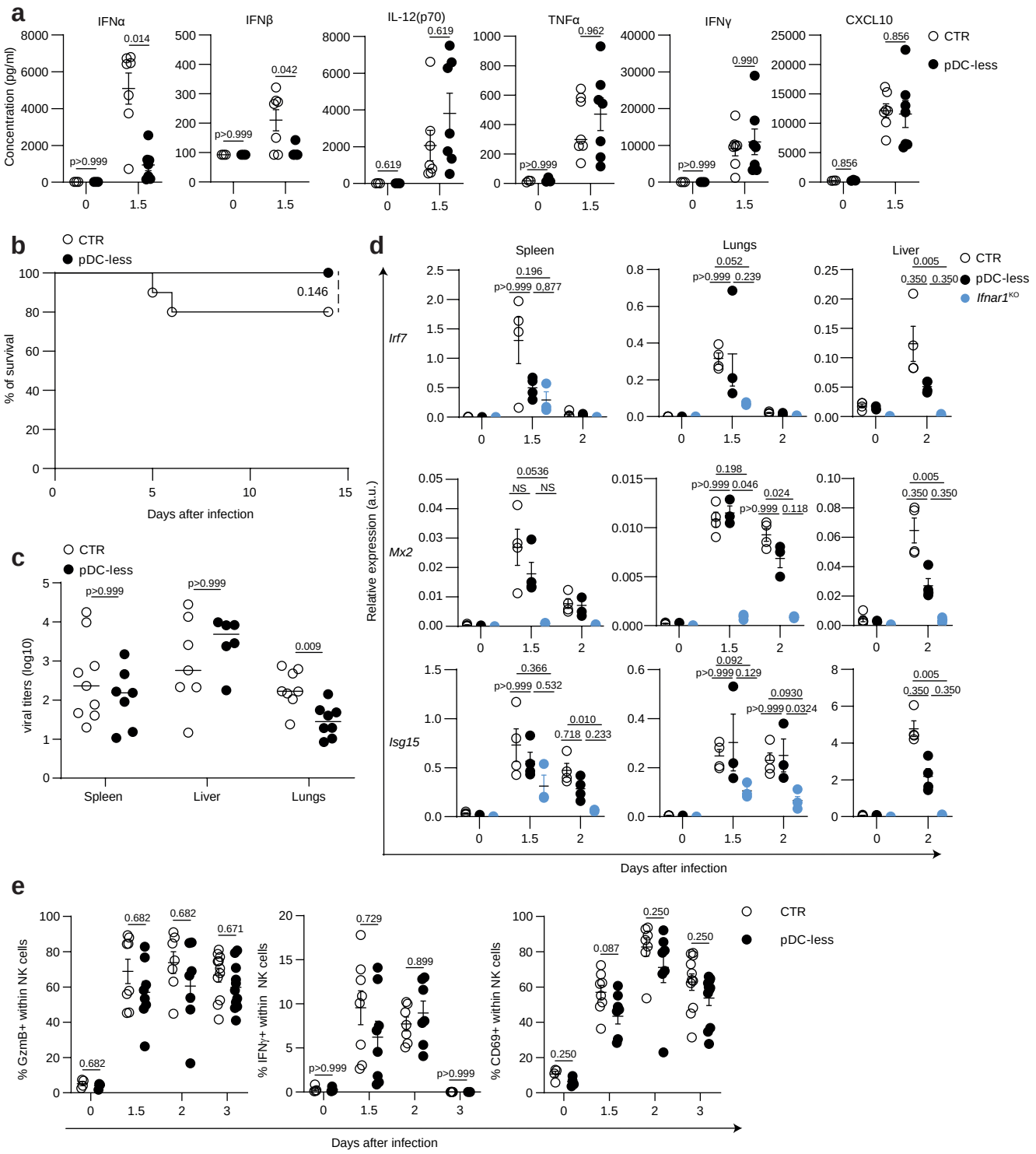

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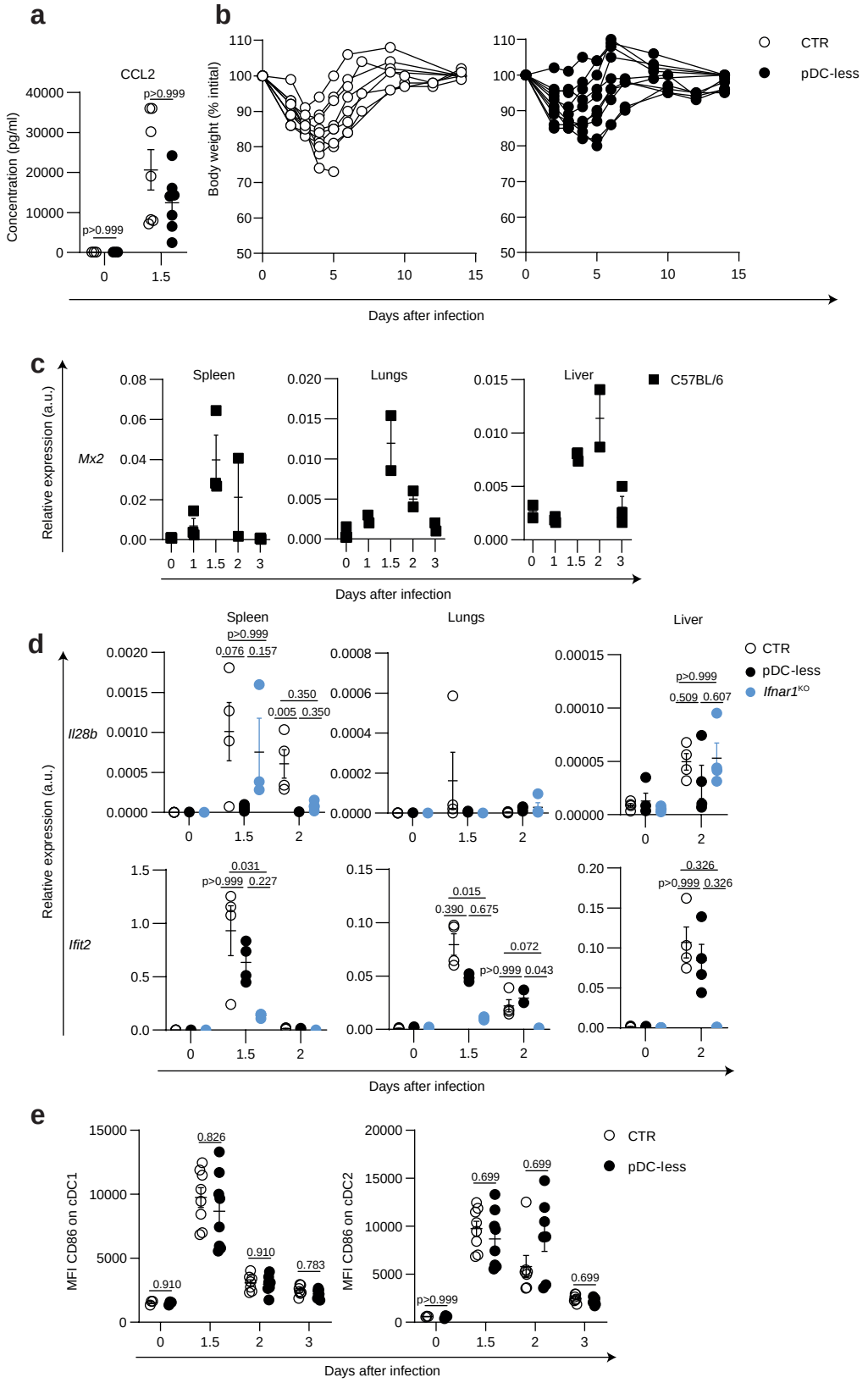

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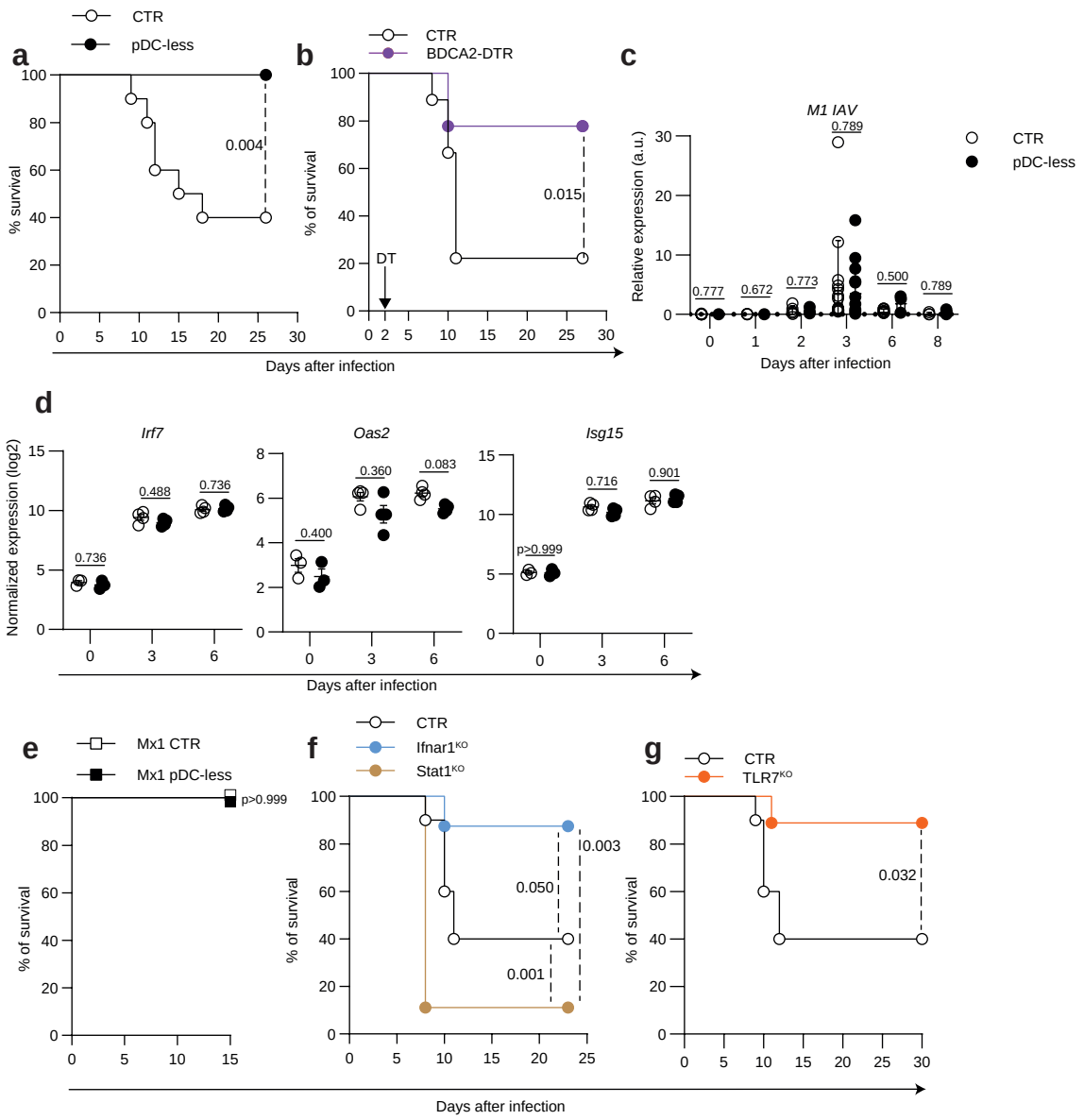

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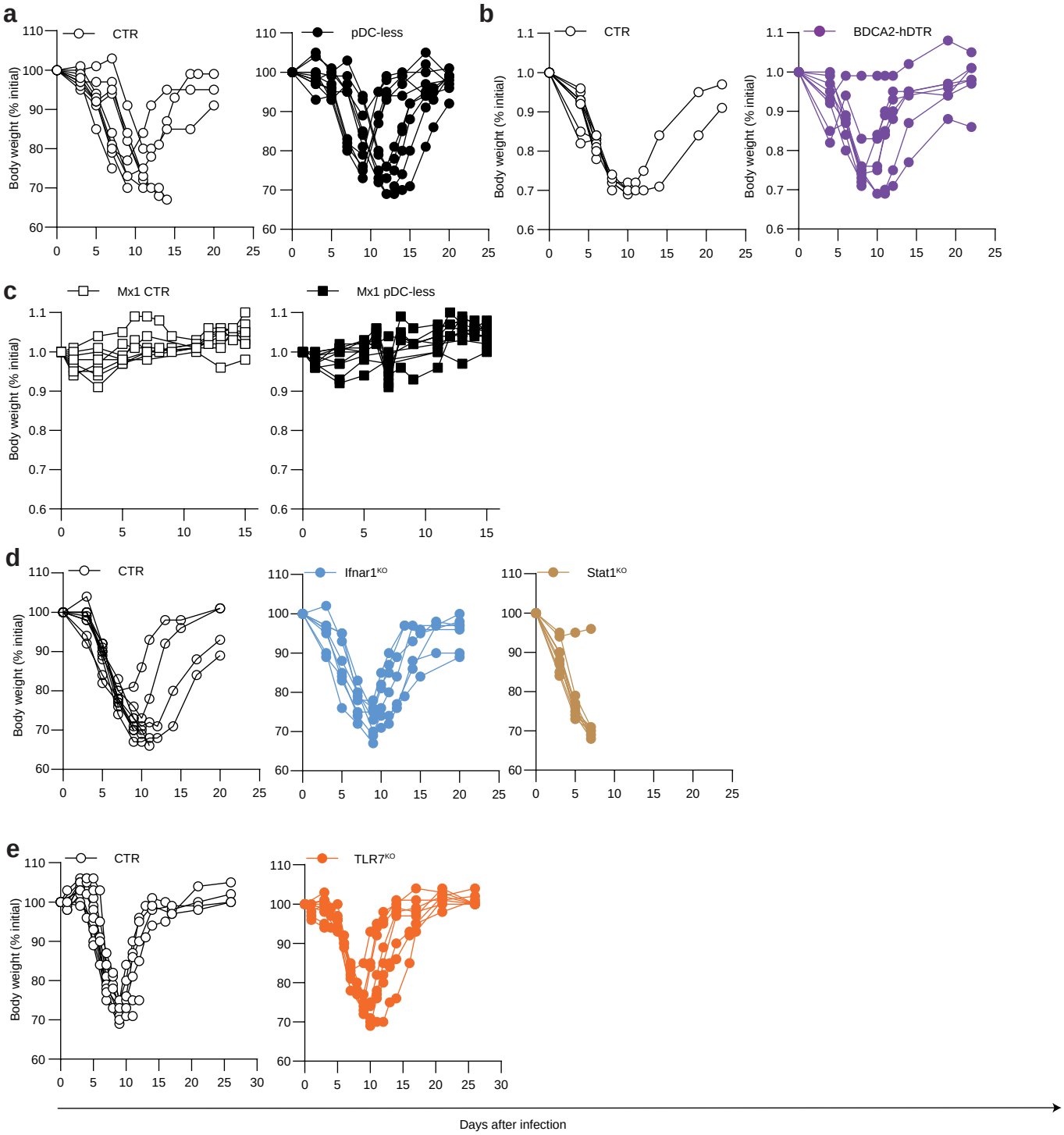

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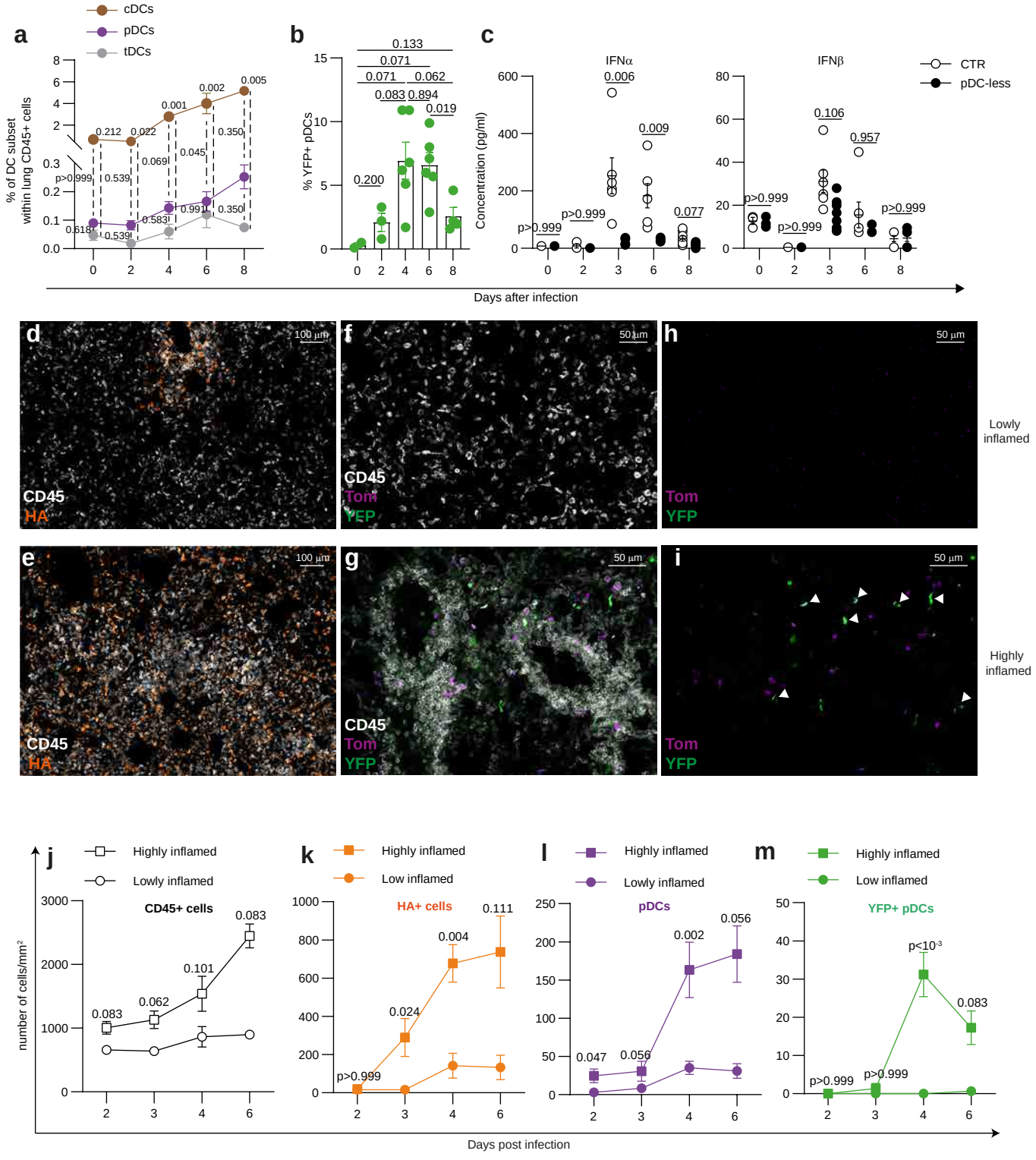

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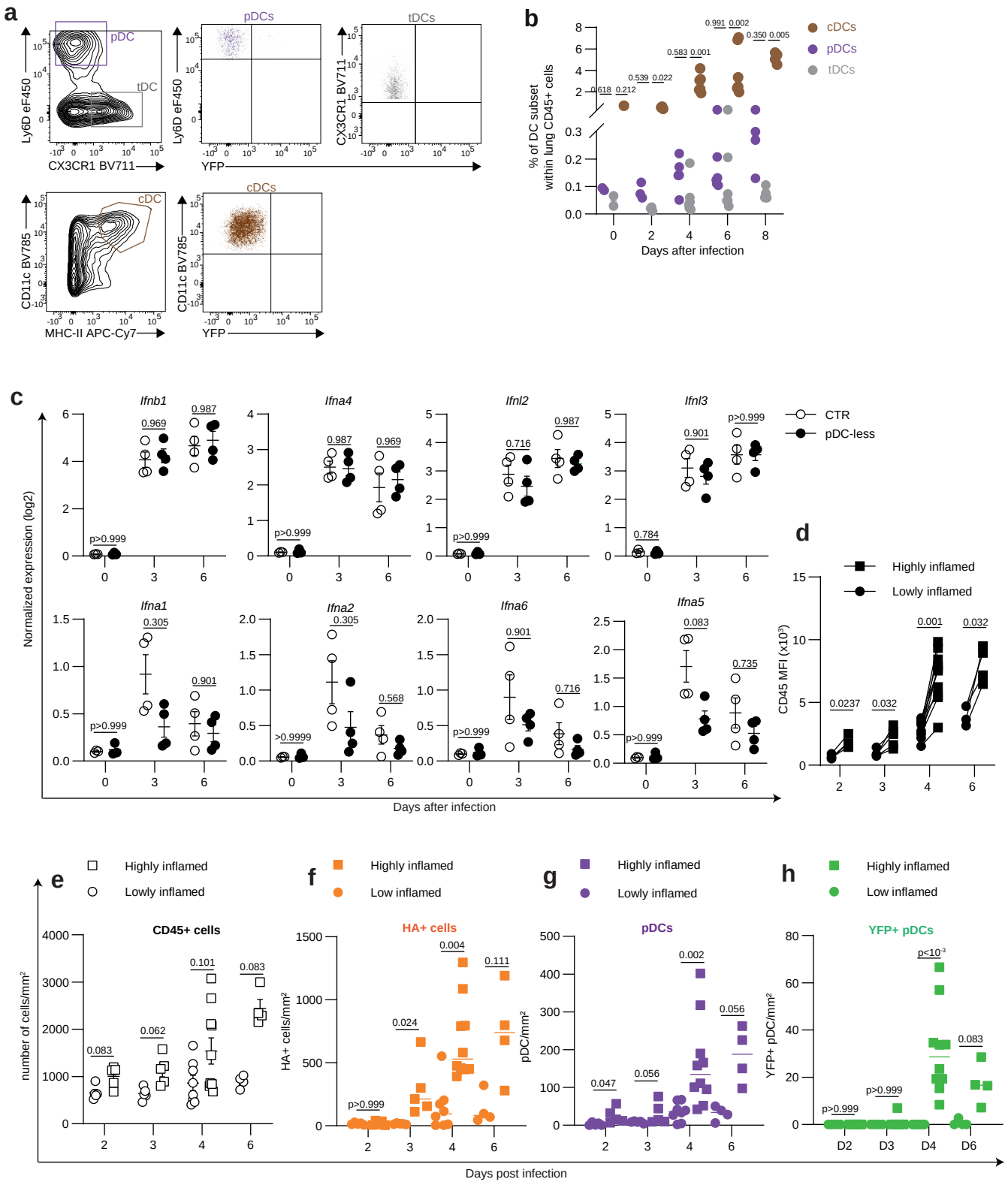

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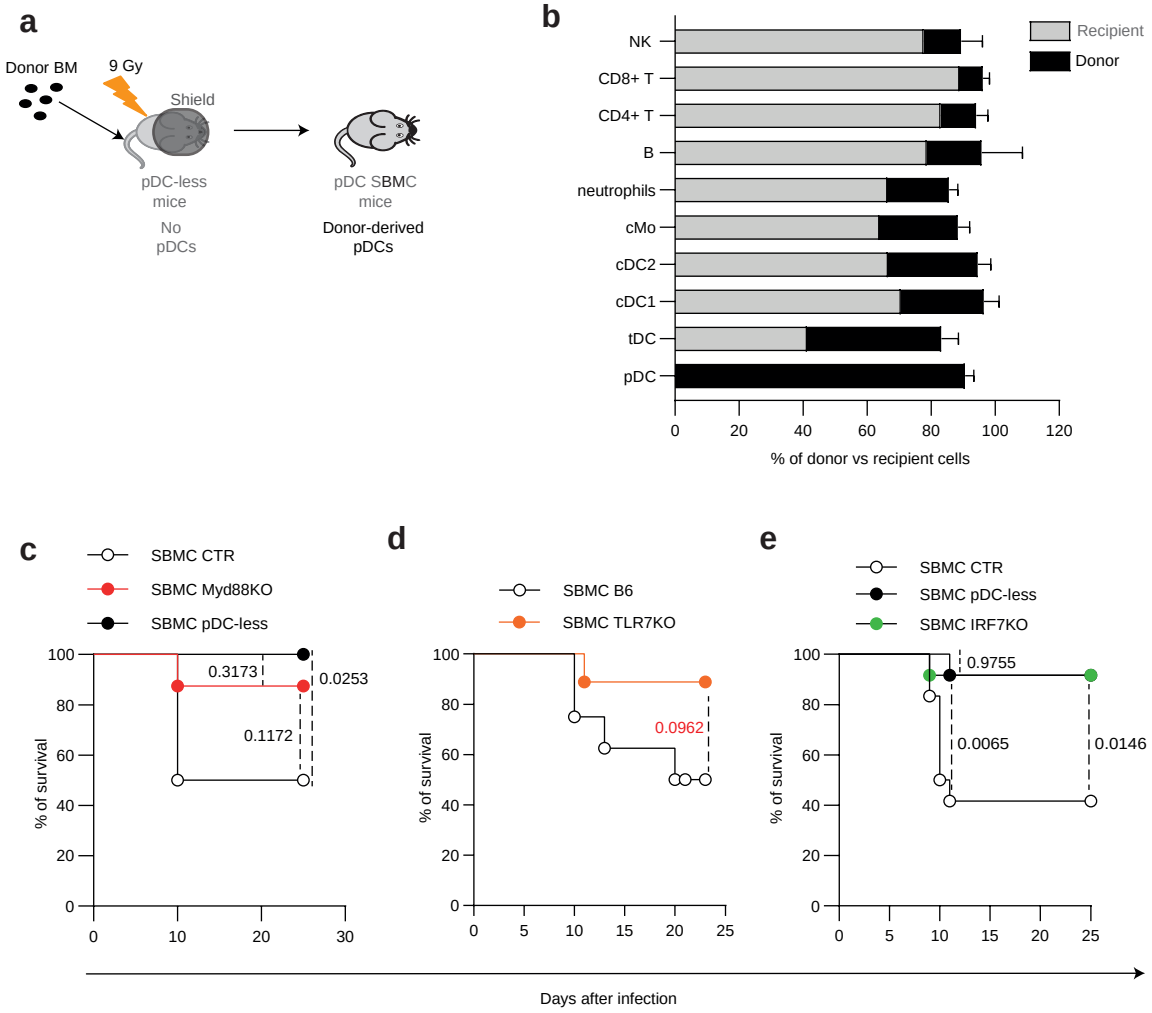

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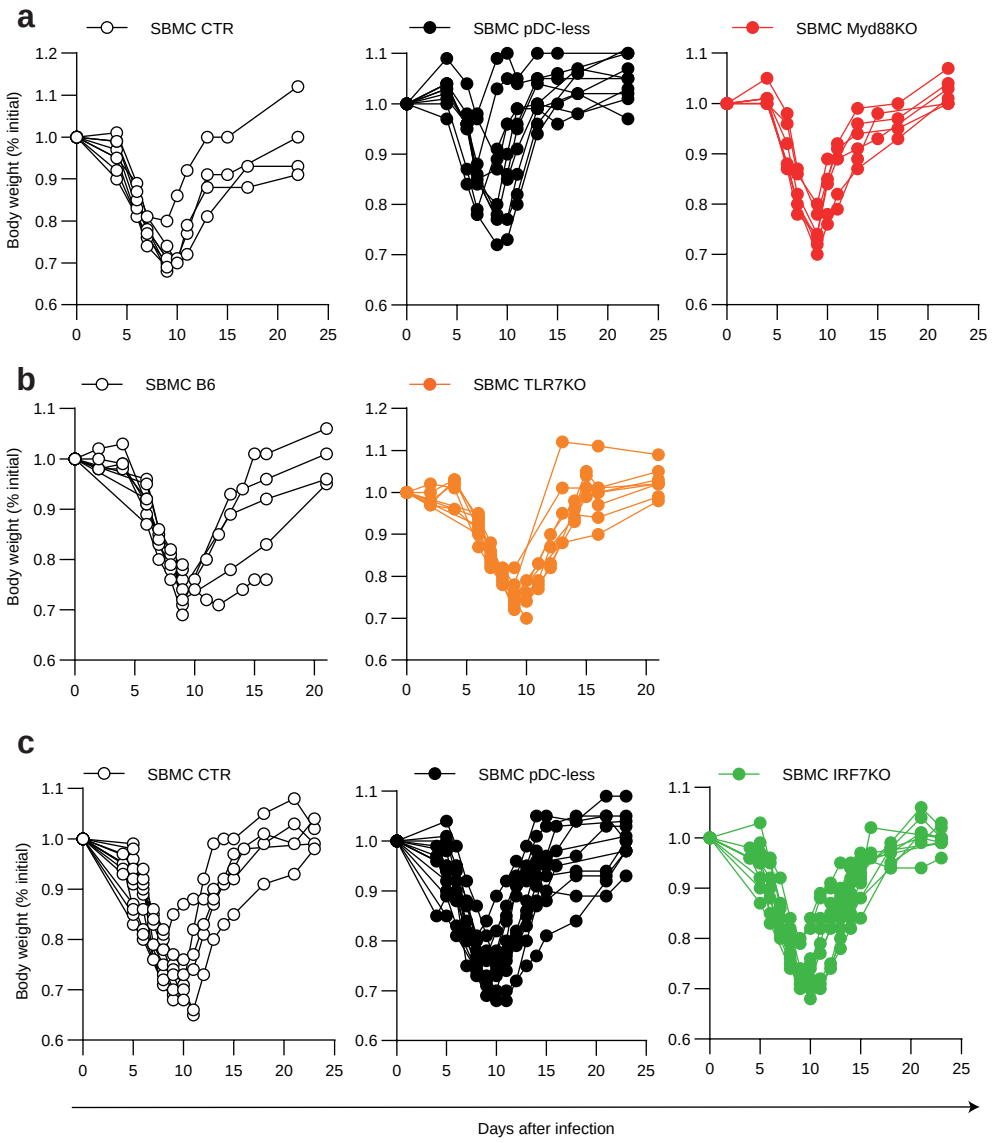

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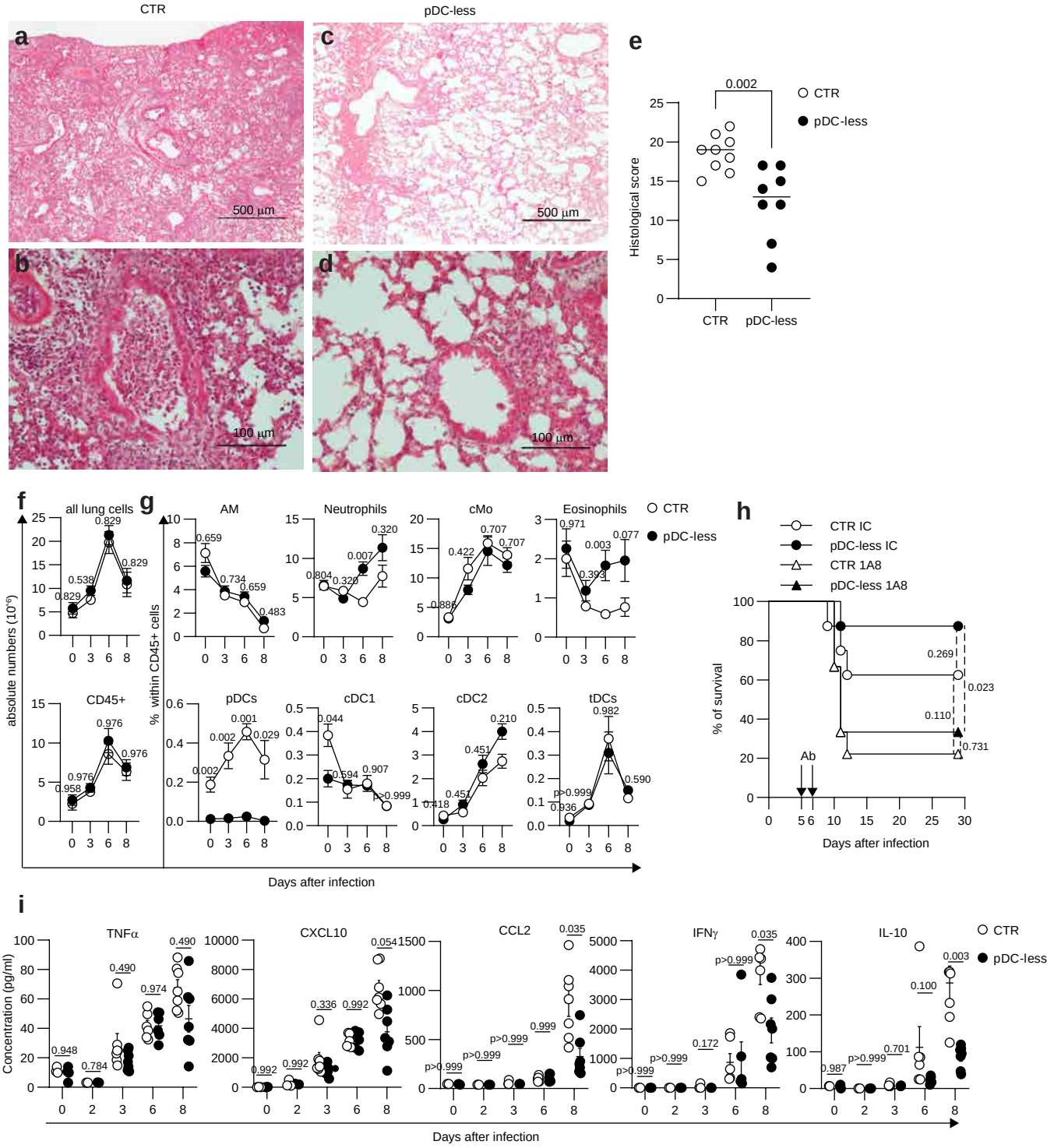

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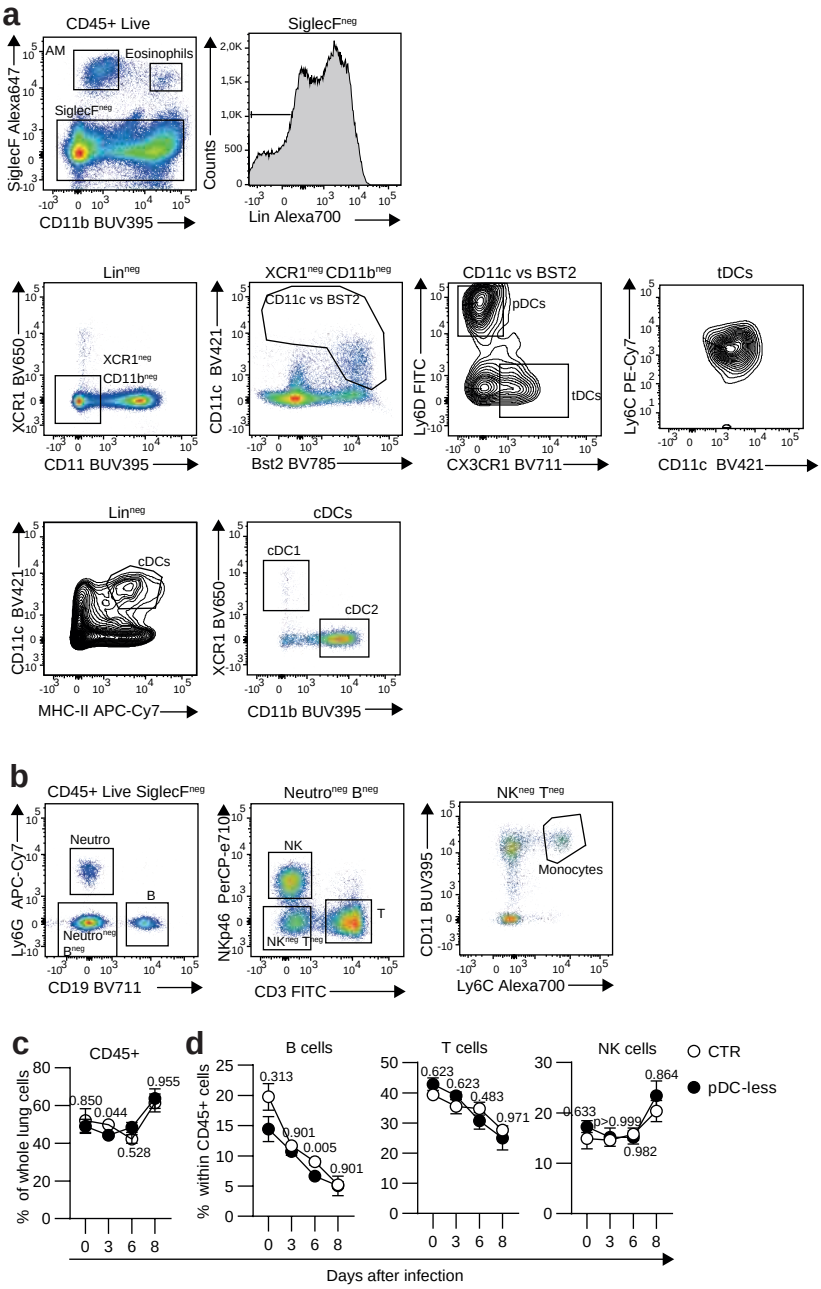

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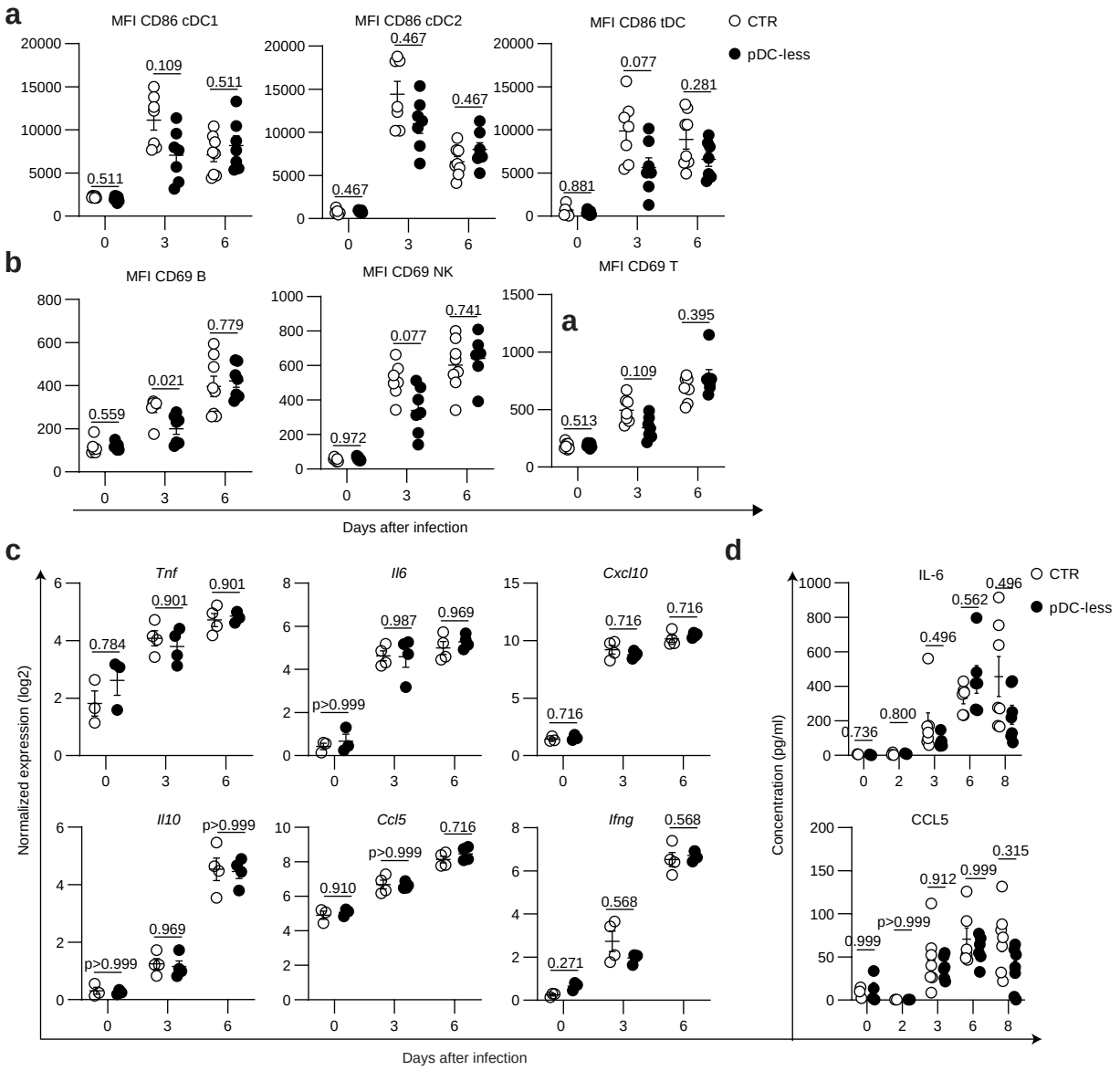

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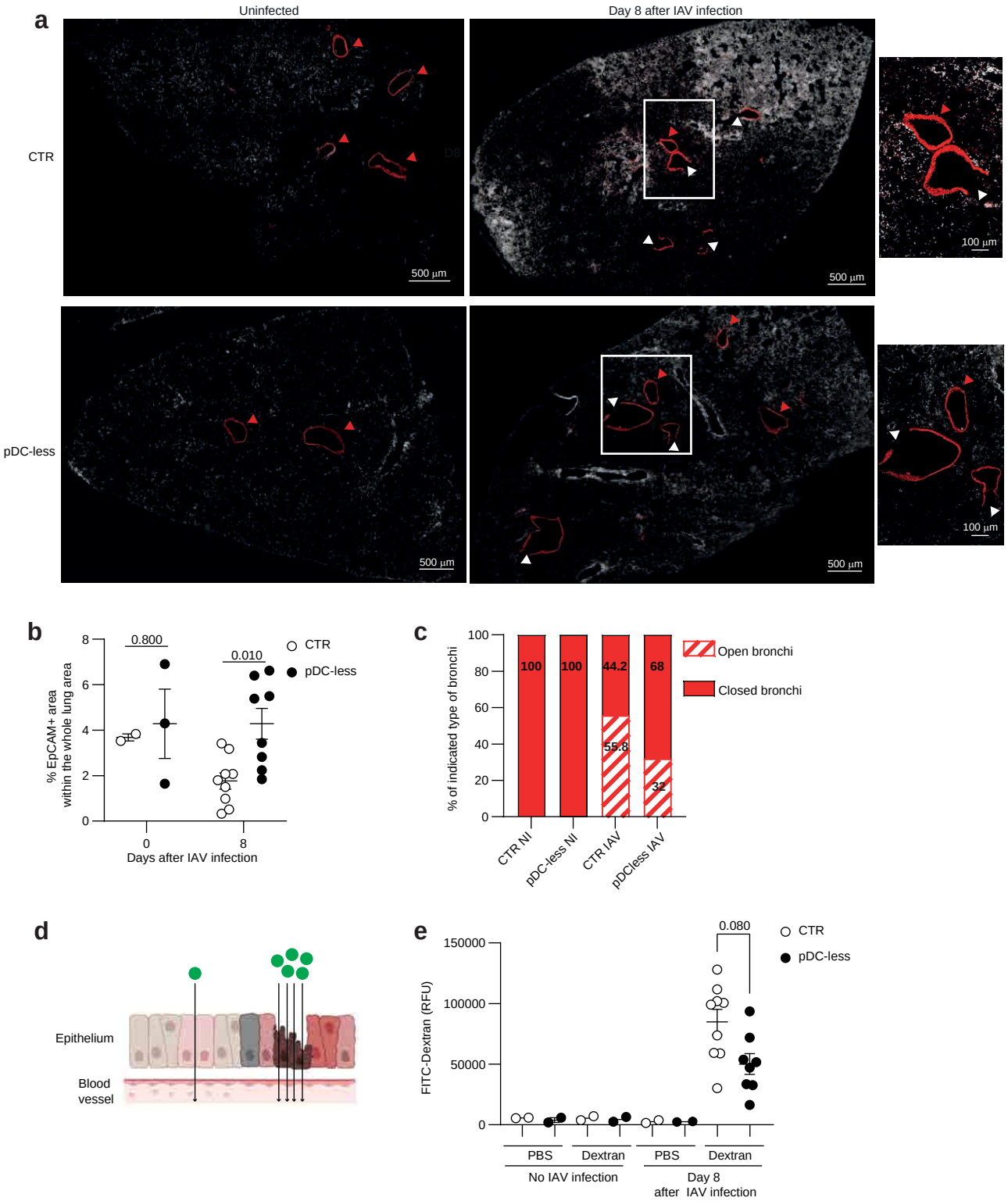

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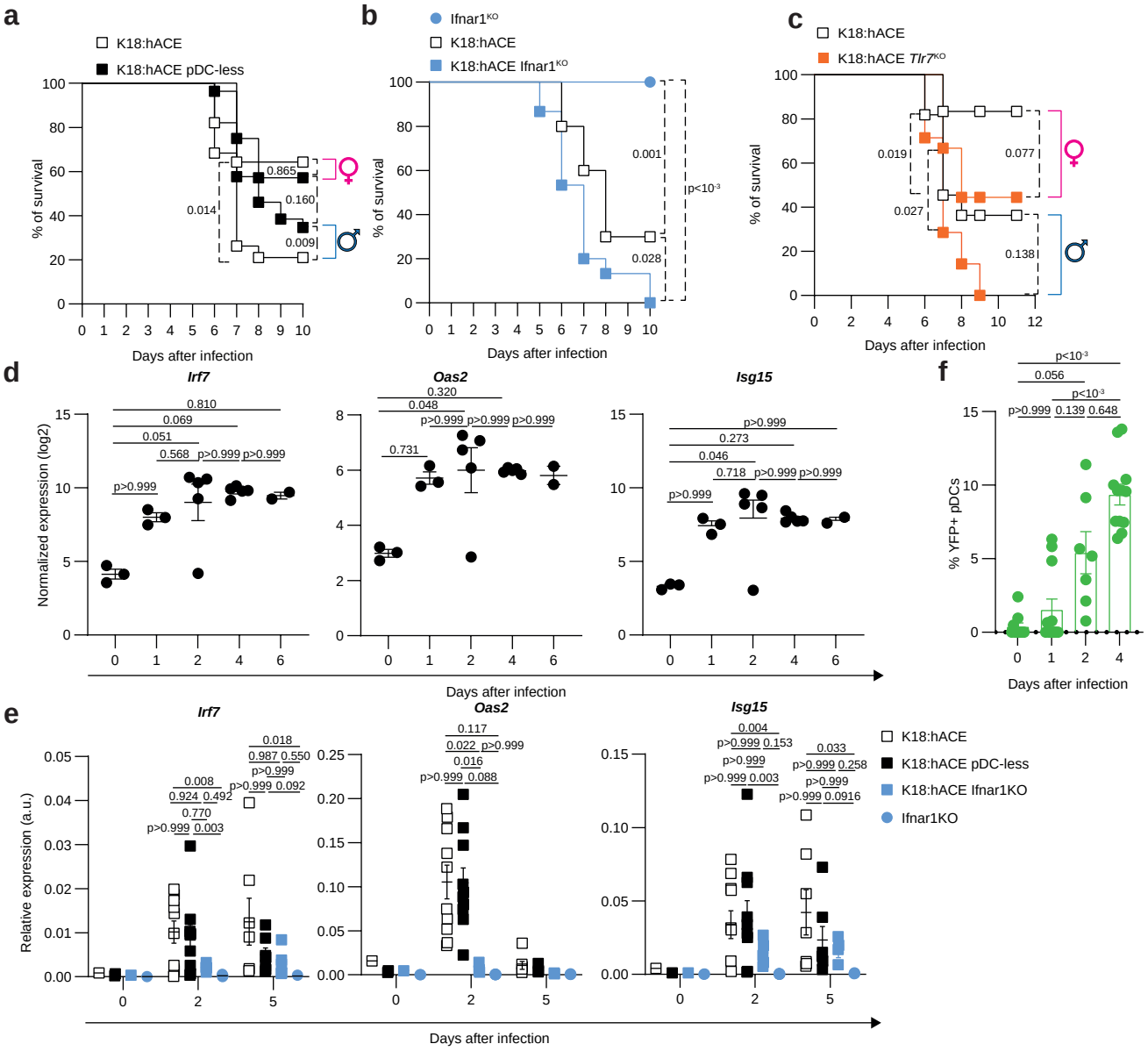

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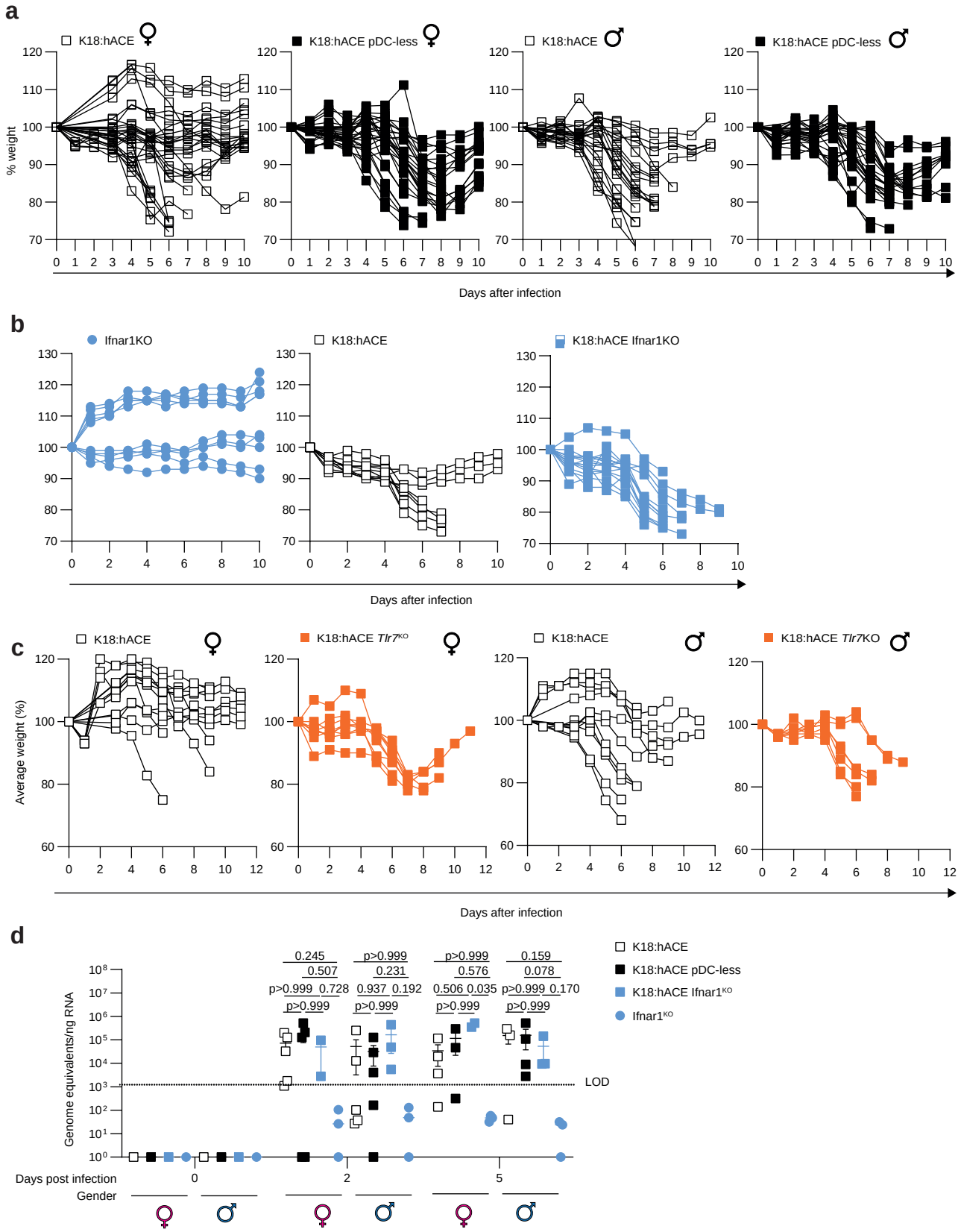

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