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Supplementary information

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

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Supplementary methods

RNA extraction and RT-qPCR on brain samples.

Brains were harvested from euthanized mice after intracardiac perfusion with PBS 1x. Right hemispheres were then put in TRIzol (Invitrogen) and kept at -80°C until RNA extraction. Total RNAs cells were extracted following the protocol provided by the TRIzol Reagent user guide. The quantity of total RNA extracted was quantified using a Thermo Scientific NanoDrop. Purified RNA was reverse transcribed into cDNA by using an iScript cDNA Synthesis kit (Life Technologies).

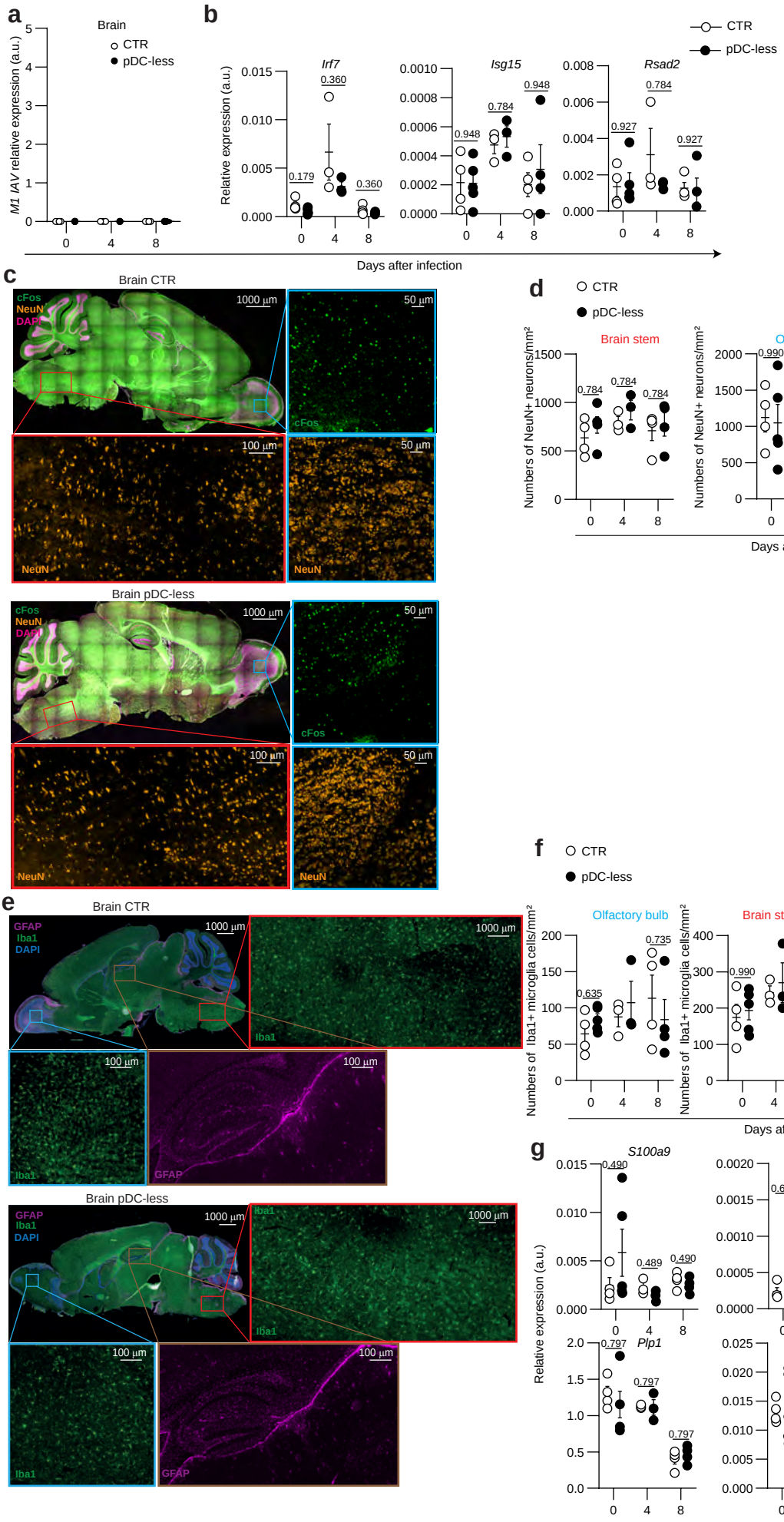
The expression levels of the following murine genes: *Actin, Irf7, Isg15, Rsad2, S100a9, Ngp, Plp1, Dcx* as well as that of the influenza virus matrix protein gene (*M1*) were determined by quantitative PCR (qPCR) using the SYBR® Premix Ex TaqTM kit and analysed using the Prism 7500 Fast PCR System. Relative gene expression was calculated using the ΔΔCt method with *Actinb* as housekeeping gene for normalization. The primers used were as follows: *Actinb* forward 5'-GGCTGTATTCCCCTCCATCG-3'; reverse 5'-CCAGTTGGTAACAATGCCATGT-3'; *Irf7* forward 5'— CCACGCTATACCATCTACCTGG-3' ; reverse 5'-GCTGCTATCCAGGGAAGACAC-3' ; *Isg15* forward 5'— GGTGTCCGTGACTAACTCCAT-3' ; reverse 5'-TGGAAAGGGTAAGACCGTCCT-3' ; *Rsad2* forward 5'—TGCTGGCTGAGAATAGCATTAGG-3' ; reverse 5'-GCTGAGTGCTGTTCCCATCT-3' ; *M1 protein IAV* forward 5'-AAGACCAATCCTGTCACCTCTGA-3'; reverse 5'-CAAAGCGTCTACGCTGCAGTCC-3' ; *S100a9* forward 5'-CAAATGGTGGAAGCACAGTT-3' ; reverse 5'-AGCATCATACACTCCTCAAAGC-3' ; *Ngp* forward 5'-AGACCTTTGTATTGGTGGTGGC-3' ; reverse 5'-GGTTGTATGCCTCTATGGCTCTA-3' ; *Plp1* forward 5'-TGGAGTCAGAGTGCCAAAGACA-3' ; reverse 5'-GACATACTGGAAAGCATGAATCACA-3' ; *Dcx* forward 5'-AACCAGAACCTTGCAGGCATTA-3' ; reverse 5'-CATAGCTTTCCCCTTCTTCCAGT-3'.

Brain immunohistofluorescence, microscopy and image analysis

Mice were euthanized with overdose of ketamine-xylazine anesthetic and perfused transcardially with phosphate buffered saline (PBS) for antibody staining. Skull caps and meninges were removed, and following a brief wash in PBS, the brains were sectioned sagittally to keep left side for microscopic analysis and right side for RNA extraction. The leftbrain parts were incubated for 2 days in formalin 2,5% diluted in PBS 1x (Sigma), then overnight in 30% sucrose solution diluted in PBS 1x (Sigma). Brains were then embedded in OCT freezing medium (CellPath), snap frozen and stored at −80 °C. 25 µm-thicked

cryosections were performed using a microtome (Leica 3050s Cryostat) at temperatures between −23 °C and −20 °C.

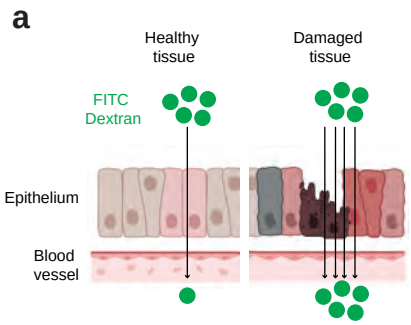
For immunostaining, brain sections were blocked with PBS, 0.5% Triton X-100 and 2% Fetal Bovin Serum (FBS) for 1h at room temperature, then stained overnight at 4°C with primary antibodies diluted in PBS, 0.5% Triton X-100 and 2% FBS, with the exception of the anti-cFos antibody, which was incubated alone for 5h at 37°C before adding the other primary antibodies. The day after, brain sections were washed for 30 s and stained with secondary antibodies 2h at room temperature. Finally, brain sections were placed in mounting medium (FluorSave Reagent with DAPI) on a glass slide (Superfrost Plus, ThermoScientific) and cover-slipped. Brain sections stained for anti-mouse NP, anti-mouse NeuN and anti-mouse cFos were acquired by confocal microscope (Zeiss LSM880 Indimo) with 10x. Brain sections stained for anti-mouse Iba1 and anti-mouse GFAP were acquired by slide scanner (Sysmex, Pannoramic Scan SC 150). Pictures were analyzed using QuPath.



Ngo et al. Supplementary Fig.1

Legend Suppl. Figure 1: Histological and transcriptional analysis of brains isolated from uninfected versus IAV-infected control and pDC-less mice

a-b, Expression levels of IAV M1 gene (**a**) or of indicated ISGs (**b**) was analyzed by RT-qPCR in brains 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. **c,** Representative images of whole brains or of brain stem (red outline) or olfactory bulb (blue outline) of indicated mice at day 4 after infection. **d**, Positive cells for indicated markers were quantified in indicated brain areas. First, the grid scale option was activated to determine the annotations of the following regions: brainstem (6x4 squares) and olfactory bulb (2x2 squares) on the brain section. Then, for each region, the different cell types were quantified by cell detection with a threshold of 10 for NeuN and for cFos. NeuN stains mature neurons, c-Fos activated neurons. **e,** Representative images of whole brains or of brain stem (red outline), olfactory bulb (blue outline) and dentate gyrus (brown outline) of indicated mice at day 4 after infection**. f)** Positive cells for indicated markers were quantified in indicated brain areas. First, the grid scale option was activated to determine the annotations of the following regions: brainstem (6x4 squares), olfactory bulb (2x2 squares) and dentate gyrus (5x3 squares) on the brain section. Then, for each region, the different cell types were quantified by cell detection with a threshold of 12000 for GFAP and 10000 for Iba1. GFAP stains astrocytes, Iba1 stains microglia. **g,** Expression of indicated genes was analyzed by RT-qPCR in brains isolated from uninfected or IAV-infected CTR and pDC-less mice at indicated days after infection. In all experiments n=5 at day 0, 3 at day 4 and 4 at day 8 after infection. *S100a9* and *Ngp* expression correlates with global brain inflammation state, *Plp1* and *Dcx* are key brain genes. An unpaired and nonparametric multiple t-test (Mann-Whitney) with Holm-Sidak method for correction was used for all statistical analysis.



Ngo et al. Supplementary Fig.2

Legend Suppl. Figure 2: Explicative scheme of FITC-dextran protocol

In healthy lungs intratracheal administration of FITC-dextran leads to poor leakage of FITC-dextran in the blood circulation. However, when lungs are damaged, as during IAV infection, the leakage of FITC-dextran in the blood circulation is significantly increased and can be detected by measuring FITC fluorescence in the plasma.

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	