

# **Novel mouse models based on intersectional genetics to identify and characterize plasmacytoid dendritic cells**

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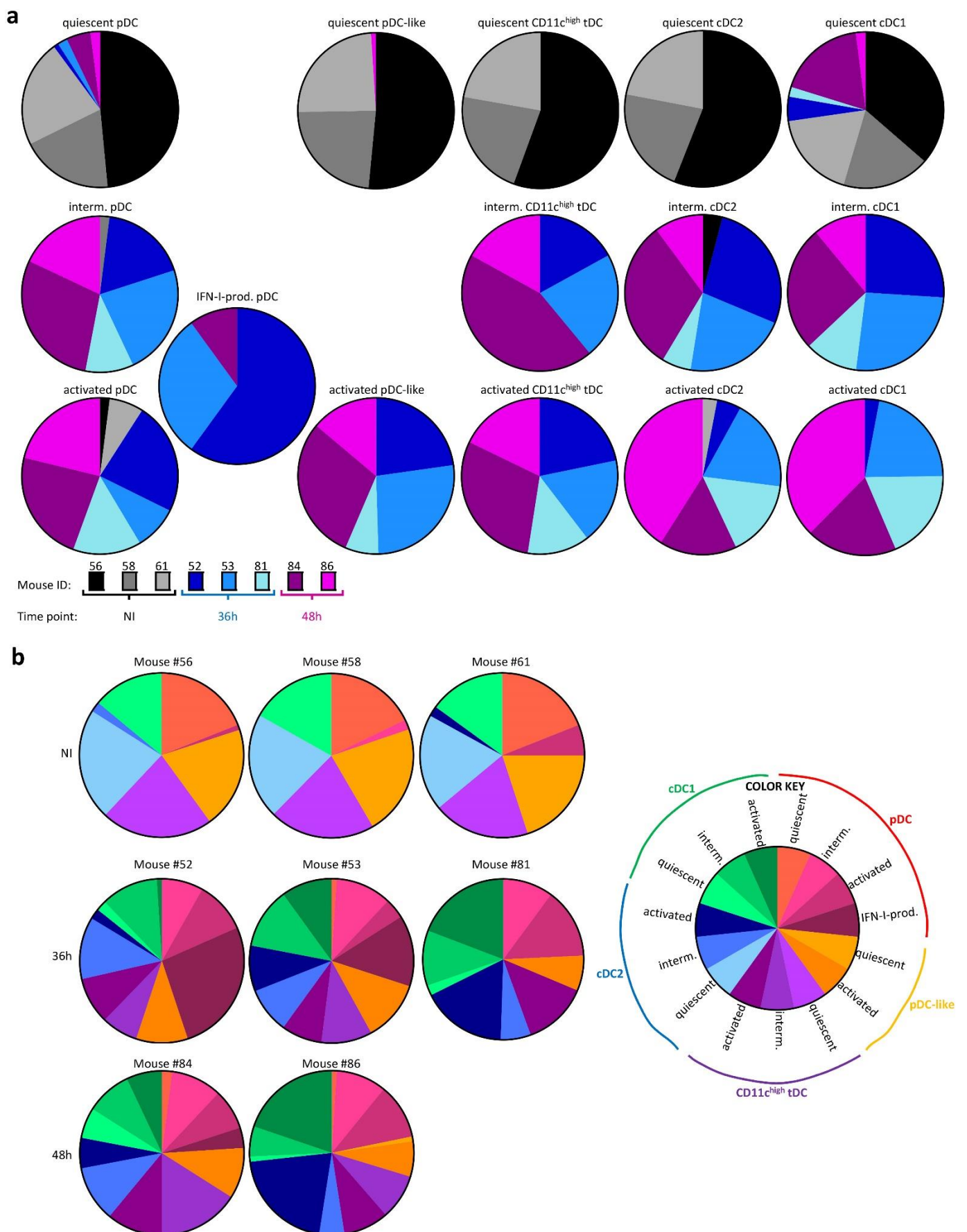
In the format provided by the  
authors and unedited

**Table of content**

Supplementary Fig. 1

Supplementary Fig. 2

Description of Supplementary Table.



**Supplementary Fig. 1. Analysis of scRNA-seq data for individual mice.** **a**, Pie charts showing the contribution of each mouse (color code) to each of the combinations of cell type and activation state (individual pie charts) as identified in Fig. 8a. **b**, Pie charts showing the fraction of each combination of cell type and activation state (color code, as in Fig. 8a) for all the annotated cells of each mouse (individual pie charts).

Supplementary Figure 2. Strategy and parameters used for the computational analyses of the FB5P-seq single cell RNA sequencing dataset.

|                             | Function / aim                                                                                                              | analysis step                                                                                             | cells of uninfected mice only,<br>starting #of cells: 384                                                                                                                                                                                                                                | whole dataset: cells of uninfected & infected mice,<br>starting #of cells: 1343                                                                                                                                                                            |
|-----------------------------|-----------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Seurat analysis<br>(Step#1) | Seurat version                                                                                                              |                                                                                                           | 3.2.0                                                                                                                                                                                                                                                                                    | 3.2.0                                                                                                                                                                                                                                                      |
|                             | genes expressed in at least                                                                                                 |                                                                                                           | 3 cells                                                                                                                                                                                                                                                                                  | 7 cells                                                                                                                                                                                                                                                    |
|                             | cells expressing at least #of genes                                                                                         |                                                                                                           | 600 genes                                                                                                                                                                                                                                                                                | 600 genes                                                                                                                                                                                                                                                  |
|                             | cells expressing at maximum #of genes                                                                                       |                                                                                                           | 5000 genes                                                                                                                                                                                                                                                                               | 5000 genes                                                                                                                                                                                                                                                 |
|                             | % of mitochondrial genes                                                                                                    |                                                                                                           | <median + 3 MAD (median absolute deviation)                                                                                                                                                                                                                                              | <median + 3 MAD                                                                                                                                                                                                                                            |
|                             | {remaining cells/ genes}                                                                                                    |                                                                                                           | 345 cells / 12381 genes                                                                                                                                                                                                                                                                  | 1132 cells / 13326 genes                                                                                                                                                                                                                                   |
| Seurat analysis<br>(Step#2) | NormalizedData                                                                                                              | pre-processing, normalization,<br>dimensionality reduction, clustering,<br>identification of marker genes | normalization.method = "LogNormalize", scale.factor = 1e6<br>selection.method = "vst", mean.function = ExpMean,<br>dispersion.function = LogVMR, num.bin = 20, binning.method =<br>"equal_width", nfeatures = 1000                                                                       | normalization.method = "LogNormalize", scale.factor = 1e6<br>selection.method = "vst", mean.function = ExpMean,<br>dispersion.function = LogVMR, num.bin = 20, binning.method =<br>"equal_width", nfeatures = 1000                                         |
|                             | FindVariableFeatures                                                                                                        |                                                                                                           | 30 pcs                                                                                                                                                                                                                                                                                   | 30 pcs                                                                                                                                                                                                                                                     |
|                             | RunPCA                                                                                                                      |                                                                                                           | 5 dims, k.param=5, compute.SNN=TRUE, force.recalc=TRUE<br>resolution=0.2, random.seed=0, algorithm=1                                                                                                                                                                                     | 11 dims, k.param=7, compute.SNN=TRUE, force.recalc=TRUE<br>resolution=0.6, random.seed=0, algorithm=1                                                                                                                                                      |
|                             | FindNeighbors                                                                                                               |                                                                                                           | umap.method="uwot", 5 dims, seed.use=10, n.components=2,<br>n.neighbors = 4, spread=1                                                                                                                                                                                                    | umap.method="uwot", 9 dims, seed.use=10, n.components=2,<br>n.neighbors = 4L, spread=1                                                                                                                                                                     |
|                             | RunUMAP                                                                                                                     |                                                                                                           | test.use = "bimod"                                                                                                                                                                                                                                                                       | test.use = "bimod"                                                                                                                                                                                                                                         |
|                             | FindMarkers                                                                                                                 | removal of contaminants                                                                                   | NA                                                                                                                                                                                                                                                                                       | clusters 6, 9, 10, 12                                                                                                                                                                                                                                      |
| Seurat analysis<br>(Step#3) | clusters of contaminating cells<br>{remaining cells/ genes}                                                                 |                                                                                                           | 345 cells / 12381 genes                                                                                                                                                                                                                                                                  | 951 cells / 13326 genes                                                                                                                                                                                                                                    |
|                             | FindVariableFeatures                                                                                                        | re-analysis of dataset after contaminant<br>removal                                                       | NA                                                                                                                                                                                                                                                                                       | selection.method = "vst", mean.function = ExpMean,<br>dispersion.function = LogVMR, num.bin = 20, binning.method =<br>"equal_width", nfeatures = 2000                                                                                                      |
|                             | RunPCA                                                                                                                      |                                                                                                           | NA                                                                                                                                                                                                                                                                                       | 30 pcs                                                                                                                                                                                                                                                     |
|                             | FindNeighbors                                                                                                               |                                                                                                           | NA                                                                                                                                                                                                                                                                                       | 9 dims, k.param=9, compute.SNN=TRUE, force.recalc=TRUE<br>resolution=0.7, random.seed=0, algorithm=1                                                                                                                                                       |
|                             | FindClusters                                                                                                                |                                                                                                           | NA                                                                                                                                                                                                                                                                                       | umap.method="uwot", 9 dims, seed.use=10, n.components=2,<br>n.neighbors = 4L, spread=1                                                                                                                                                                     |
|                             | RunUMAP                                                                                                                     |                                                                                                           | NA                                                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                            |
| Rphenograph<br>analysis     | removal of cells<br>lacking index sorting information<br>{remaining cells with transcript and<br>protein level information} | integration of index sorting information<br>and clustering                                                | 2 cells (plate12_BC12 and plate13_BC23)                                                                                                                                                                                                                                                  | 3 cells (plate4_BC22, plate12_BC12 and plate13_BC23)                                                                                                                                                                                                       |
|                             | Rphenograph                                                                                                                 |                                                                                                           | 343 cells<br>k = 20                                                                                                                                                                                                                                                                      | 948 cells<br>k = 50                                                                                                                                                                                                                                        |
| CMAP analysis               | signatures used                                                                                                             | cell type identification based on gene<br>expression                                                      | tDC, cDC2, cDC1, pDC, pDC vs_pDClike<br>(external signatures generated from public data from PMIDs:<br>26903243, 29925996)                                                                                                                                                               | unambiguous relative DC_type-specific signatures,<br>from the analysis of the cells from uninfected mice                                                                                                                                                   |
|                             | {#cells identified as bona fide pDC, pDC-<br>like, tDC, cDC1, cDC2}                                                         |                                                                                                           | 205 cells (Extended Data Fig. 6f);<br>103 pDC, 23 cDC2, 34 cDC1, 26 pDC-like, 19 tDC;<br>identified based on combined analysis of Seurat and Rphenograph<br>clusters (Extended Data Fig. 6d), refined by selecting cells on cMap<br>scores <sup>footnote_1</sup> (Extended Data Fig. 6e) | 851 cells (Fig. 4e);<br>310 pDC, 103 cDC2, 122 cDC1, 167 pDC-like, 149 tDC;<br>identified based on combined analysis of Seurat and Rphenograph<br>clusters (Extended Data Fig. 7e), and globally confirmed by the CMAP<br>analysis (Extended Data Fig. 7f) |
| Seurat analysis<br>(Step#3) | FindMarkers                                                                                                                 | adj. P-value<0.05 in both cases                                                                           | test.use = "wilcox"<br>(generation of unambiguous relative DC_type-specific signatures)                                                                                                                                                                                                  | test.use = "wilcox"<br>(generation of DEGs between activation states of a given cell type)                                                                                                                                                                 |

<sup>footnote\_1</sup>Precise rules used to assign a DC\_type to each cell.

\*for pDC: (i) belonging to SeuratRphenograph clusters Inc or Inc or Inf, and (ii) CMAP scores obeying the following rules, AND(sgCMAP\_pDC<0; sgCMAP\_pDC vs\_pDClike>0; sgCMAP\_tDC<0; sgCMAP\_cDC2<0; sgCMAP\_cDC1<0).

\*for pDC-like: (i) belonging to SeuratRphenograph clusters Ilng, and (ii) CMAP scores obeying the following rules, AND(sgCMAP\_pDC vs\_pDClike<0; sgCMAP\_cDC2<0; sgCMAP\_cDC1<0).

\*for tDC: (i) belonging to SeuratRphenograph clusters Ilina or Vna, and (ii) CMAP scores obeying the following rules, AND(sgCMAP\_tDC<0; sgCMAP\_cDC2<0; sgCMAP\_cDC1<0; sgCMAP\_pDC<0).

\*for cDC2 (i) belonging to SeuratRphenograph clusters Ilina or Vna, and (ii) CMAP scores obeying the following rules, AND(sgCMAP\_cDC2<0; sgCMAP\_cDC1<0; sgCMAP\_pDC<0; sgCMAP\_tDC<0; sgCMAP\_pDC vs\_pDClike<0).

\*for cDC1 (i) belonging to SeuratRphenograph clusters Vna, and (ii) CMAP scores obeying the following rules, AND(sgCMAP\_cDC1>0; sgCMAP\_cDC2<0; sgCMAP\_pDC<0; sgCMAP\_tDC<0; sgCMAP\_pDC vs\_pDClike<0).

Supplementary Fig 2. Strategy and parameters used for the computational analyses of the FB5P-seq single cell RNA sequencing dataset.

**Description of Supplementary Table.** The first spreadsheet gives the absolute numbers of DC types in the spleen early during MCMV infection. These data are derived from the spectral flow cytometry experiments. For each spleen, the percentages of each DC type within the live cell spectral flow cytometry gate was retrieved from the analysis illustrated in Extended Data Fig. 5 and multiplied by the absolute number of viable cells. Statistical analyses were performed with unpaired t test. The second spreadsheet gives the antibody panels used for the immunohistofluorescence (IHF) analyses, for each of the corresponding figure. The subsequent spreadsheets give the metadata for the single cell RNA sequencing experiment. Specifically, the third sheet lists the names of each of the following sheets and describes their content. The fourth sheet indicates, for each cell, the experimental condition (“timepoint”), the identity of the mouse it was isolated from (“mouse\_ID”), its initial sorting phenotype, the results of the quality controls, its belonging to Seurat or Rphenograph clusters for the different analyses performed, its final assignment to a DC type and activation state, and its fluorescence intensity for the cell surface markers used for sorting. Gene markers for Seurat clusters, and the lists of genes differentially expressed between Seurat clusters or sub-clusters, according to the different bioinformatics analyses performed, are given in the following spreadsheets, as are the signatures used for the CMAP analyses, as listed and described in the third sheet.