



# PPRV infection hinders ovine monocyte-derived dendritic cells maturation: functionality and transcriptomics analysis

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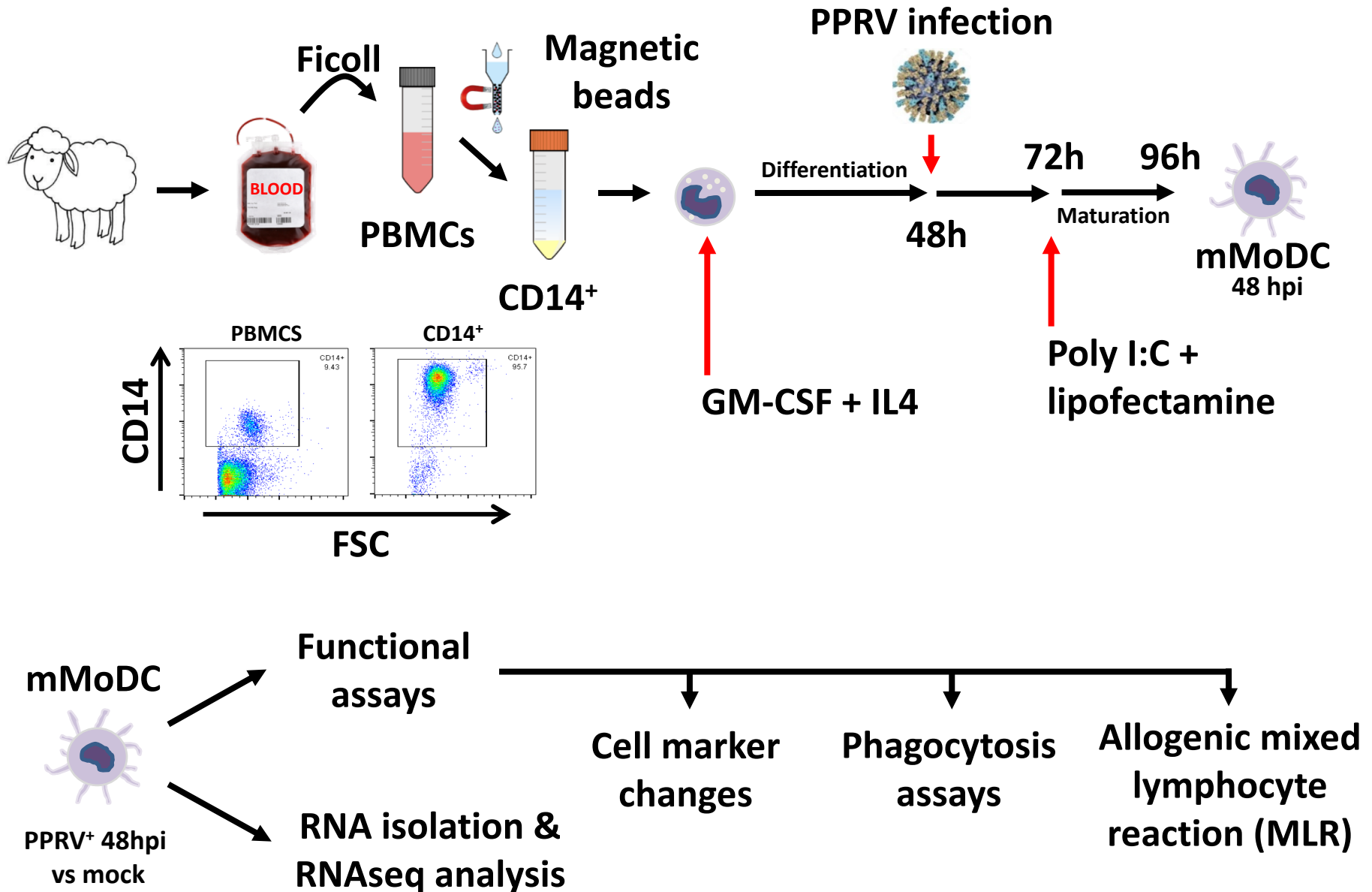
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# ABSTRACT

**Peste des Petits Ruminants (PPR)** is an economically important disease, especially in developing countries, affecting sheep and goats. **Peste des Petits Ruminants virus (PPRV)** is the causative agent of PPR, closely related to other Morbillivirus like measles, rinderpest virus and canine distemper virus, within the *Paramyxoviridae* family. **PPRV targets** immune cells, including **dendritic cells (DC)**. Our goal was to **assess** whether **PPRV infection affects the maturation process from immature DC to mature DC**, an essential step for the development of adequate adaptive immunity to the infection. To do so, a number of **functional assays and RNAseq analysis** were performed. Immature monocyte-derived DC (iMoDC) were obtained by a 48-hours differentiation process of positive-selected **CD14<sup>+</sup> cells** (monocytes) from peripheral blood mononuclear cells (PBMCs), employing **GM-CSF and IL-4** cytokines. iMoDC were then PPRV- or mock-infected and after 24 hours cultures were transfected overnight with **Poly I:C** for **maturation**. **PPRV-infected** mature monocyte-derived DC (**mMoDC**) showed an **increased expression** of **CD14, CD11b, CD11c and CD209 cell markers**, while **CD80, CD86, MHC-I and MHC-II** expression levels were **reduced**, compared to mock-infected mMoDCs. PPRV-infected mMoDC showed as well a **decrease in antigen presentation**, as detected by **allogeneic MLR assays**. **RNAseq** analysis was performed using RNA extracted from PPRV and mock-infected mMoDCs cultures from four different sheep. **PPRV-infected mMoDCs** showed **453 up-regulated** and **179 down-regulated genes** compared to mock-infected counterparts. **KEGG analysis** revealed **31 different up-regulated pathways**, including some involving **autophagy** or mitophagy mechanisms and signalling pathways related to **viral infection responses** like **TNF, mTOR or IL-17 pathways**, among others. Taken together, these data indicate that **PPRV can target DC maturation to hinder adaptive immunity** and thus contribute to the immunosuppressive effects of PPRV infection on its natural hosts.

# MATERIAL AND METHODS

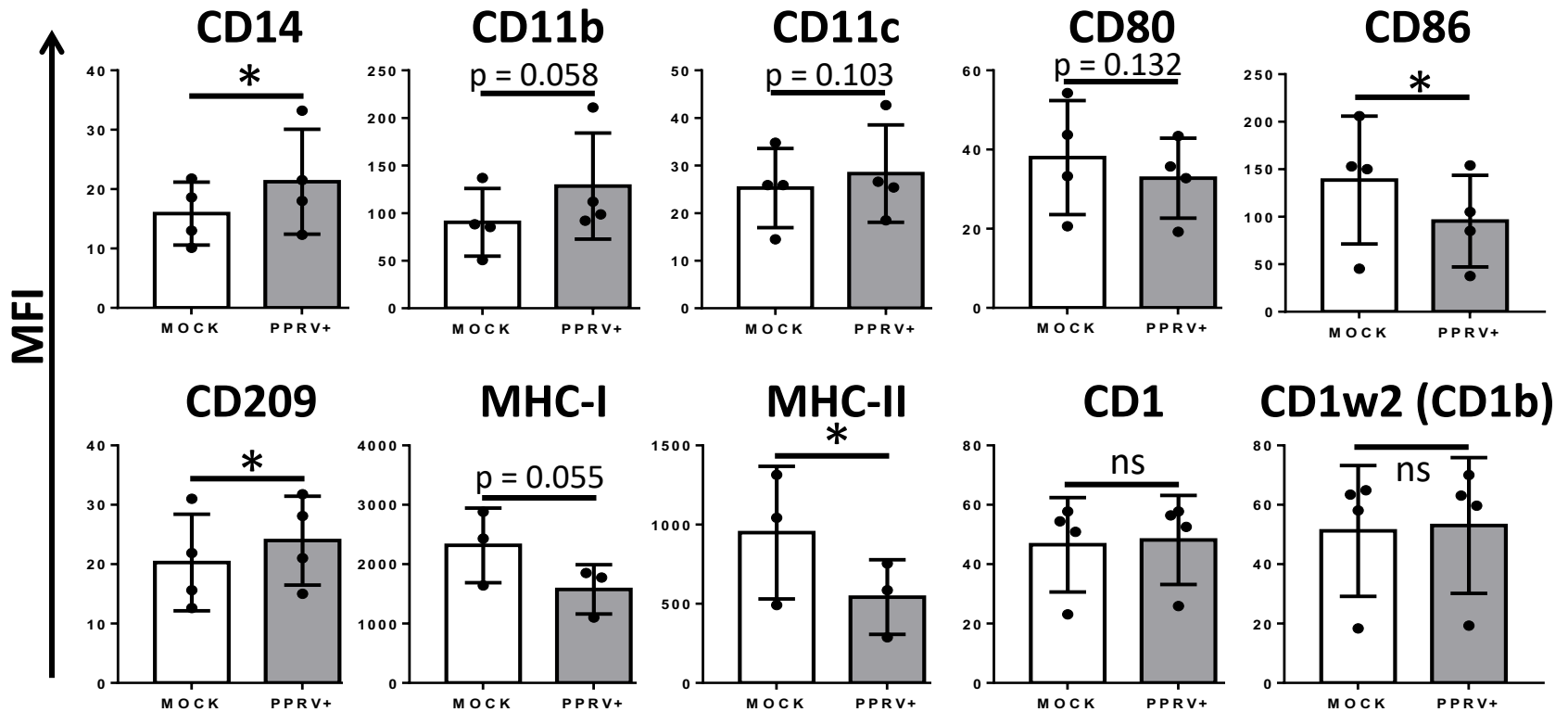


# PPRV EFFECTS ON mMoDC MATURATION

## FUNCTIONAL ASSAYS

### mMoDC cell marker changes at 48hpi

Mean fluorescence intensity of cell surface markers (mean  $\pm$  SD) measured by flow cytometry in 3-4 donor sheep. Paired t test, \* $p < 0.05$



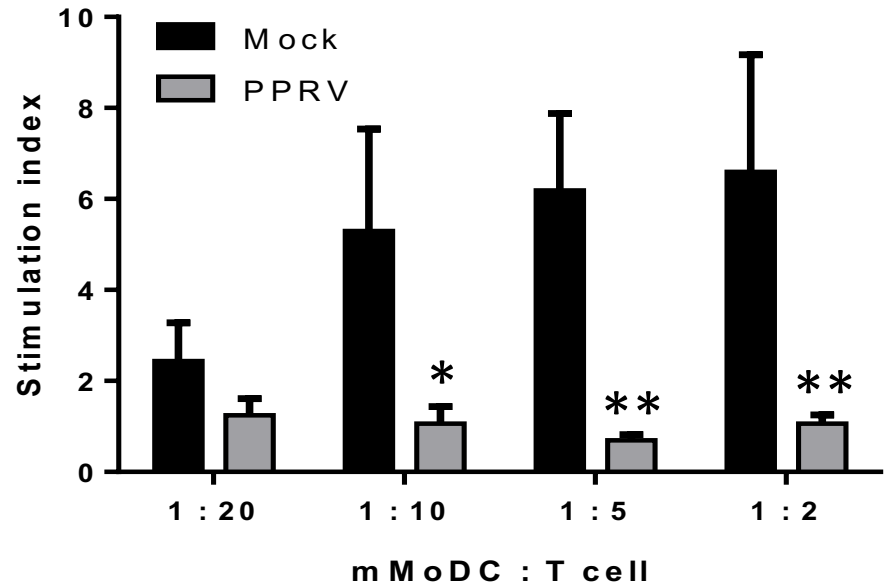
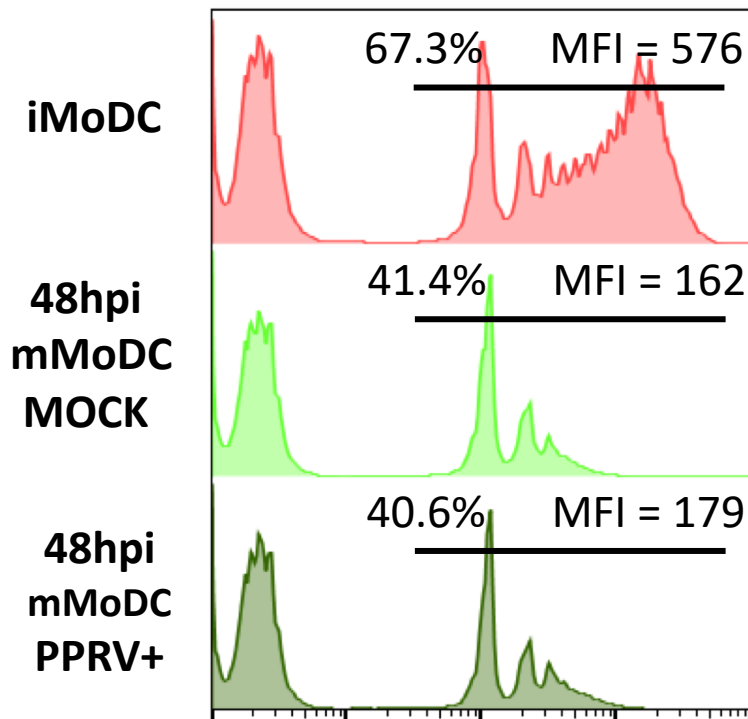
EXPRESSION LEVELS:  $\uparrow$  CD14, CD11b, CD11c & CD209  $\downarrow$  CD80, CD86, MHC-I & MHC-II

# PPRV EFFECTS ON mMoDC MATURATION

## FUNCTIONAL ASSAYS

mMoDC microsphere  
phagocytosis assays (48hpi)

Allogenic mixed lymphocyte reaction  
(MLR) on PPRV infected mMoDC



T cell proliferation was assessed by  $^3\text{H}$ -thymidine incorporation in 5 day co-cultures and presented as stimulation index.

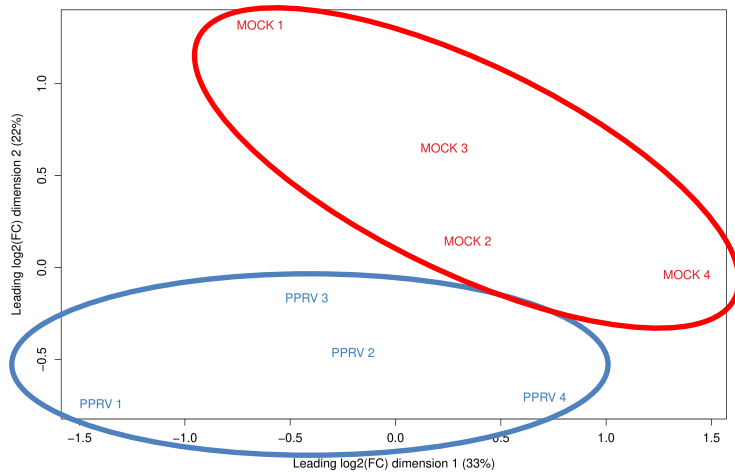
- No differences in microsphere phagocytosis and between PPRV- and mock-infected mMoDC

- PPRV infection impairs the ability of mMoDC to activate T cells

# PPRV EFFECTS ON mMoDC MATURATION

## RNAseq ANALYSIS Differentially expressed genes (DEG)

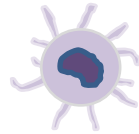
### Principal Coordinate Analysis (PCoA)



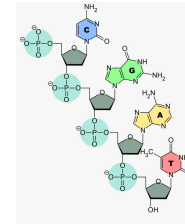
RNAseq analysis comparing PPRV<sup>+</sup> mMoDC vs mock-infected controls from 4 sheep:

- **453 up-regulated genes**
- **179 down-regulated genes**

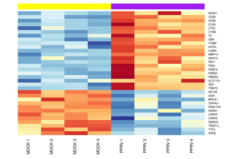
mMoDC



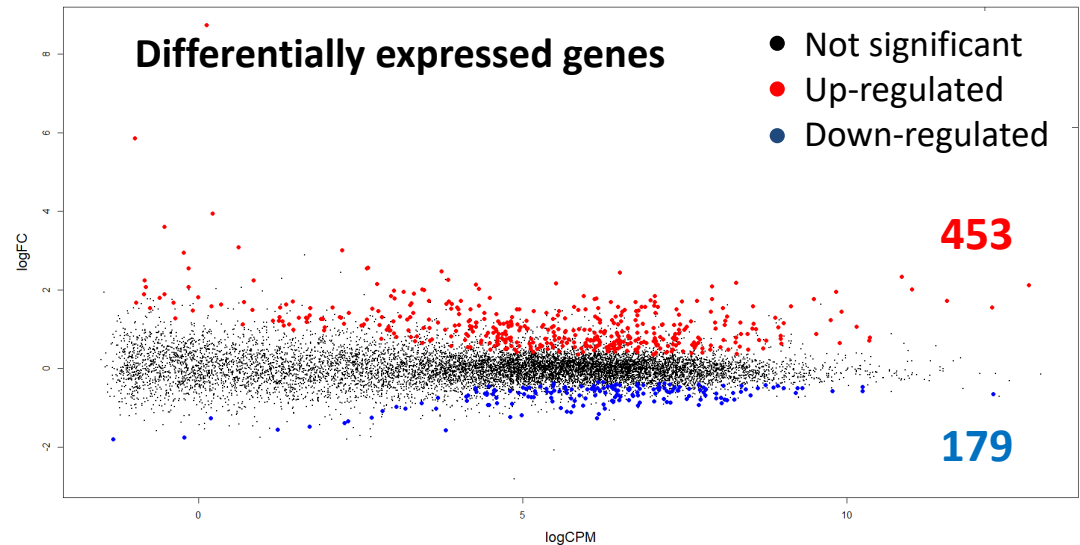
PPRV<sup>+</sup> 48hpi  
vs mock



RNA  
isolation



RNAseq  
analysis



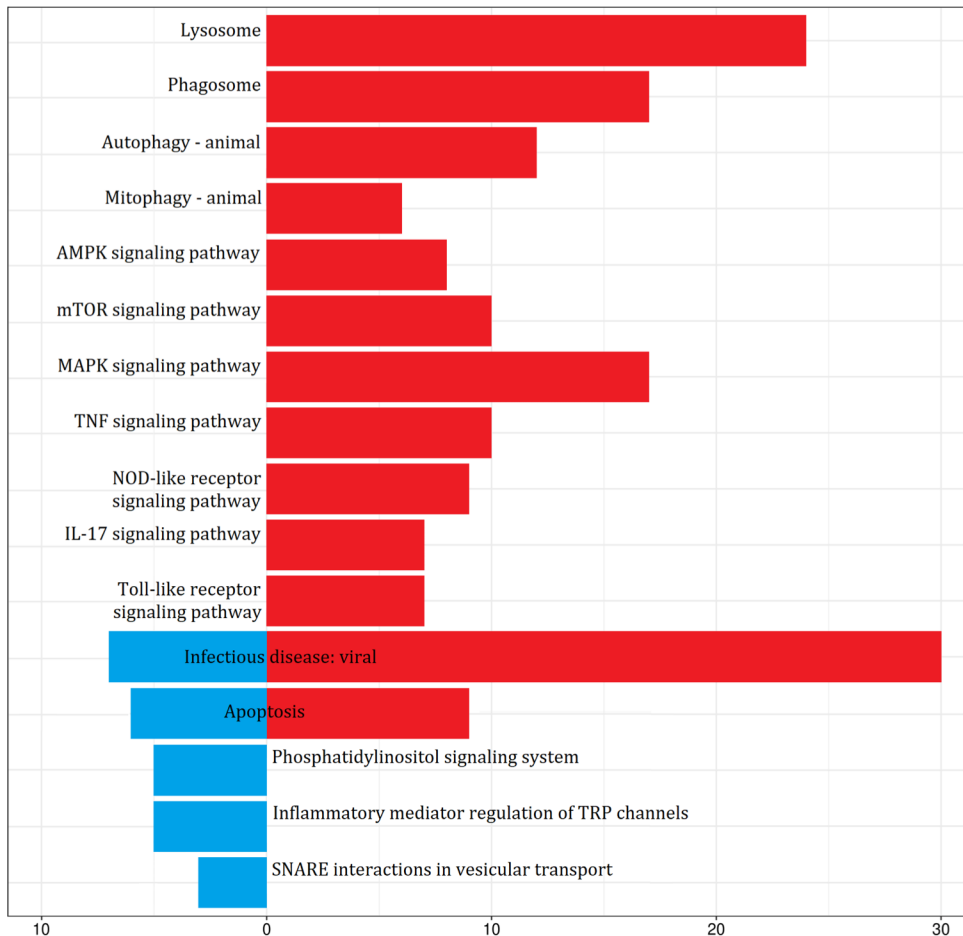
# PPRV EFFECTS ON mMoDC MATURATION

## RNAseq ANALYSIS

### Selection of relevant KEGG pathway analysis

Number of genes

← Down-regulated    Up-regulated →



mMoDC populations infected with PPRV showed:

- 31 up-regulated pathways
- 10 down-regulated pathways

Among them, pathways with important roles in the infection process:

- Viral infection response (TLR, IL-17, TNF, NOD)
- Apoptosis / MAPK
- Virus replication and spread (Autophagy, mitophagy)

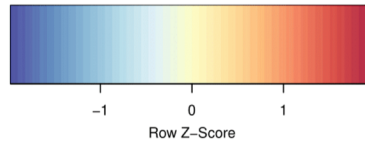
# PPRV EFFECTS ON mMoDC MATURATION

## RNAseq ANALYSIS

Two relevant heatmaps of PPRV-infected mMoDC organized according to gene ontology

### INTEFERON-RELATED GENES

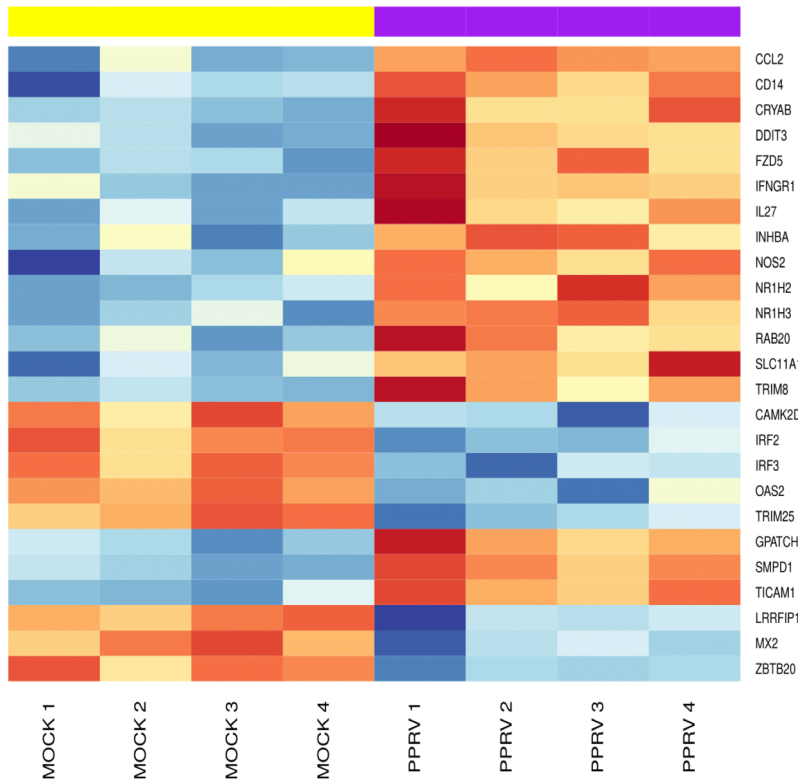
Color Key



### ANTIGEN PROCESSING AND PRESENTATION GENES

Mock CNTs

PPRV<sup>+</sup>

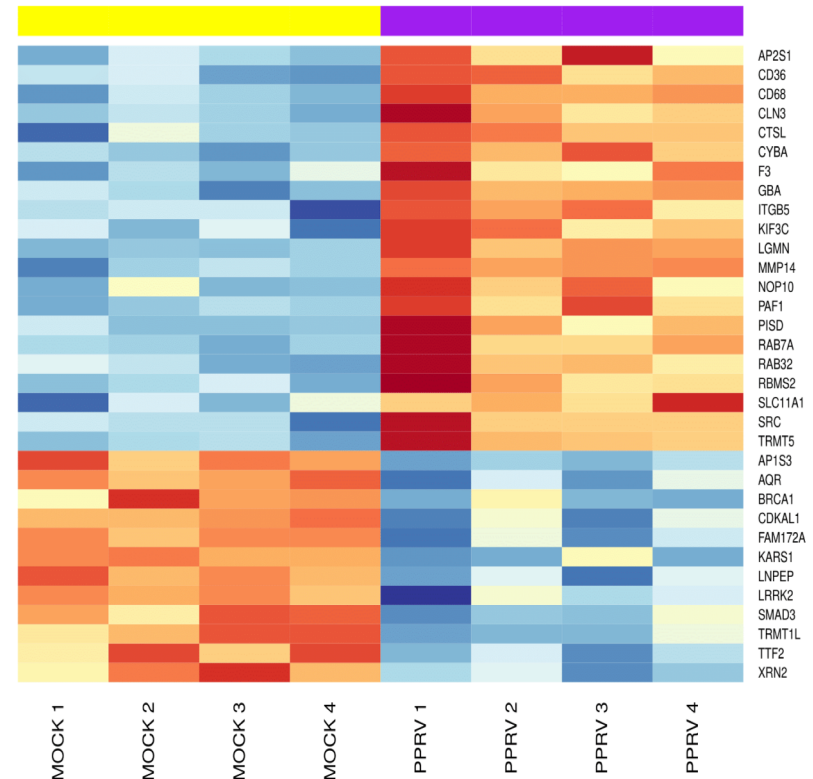


IFN- $\gamma$

IFN- $\alpha$   
IFN- $\beta$

Mock CNTs

PPRV<sup>+</sup>





## CONCLUSIONS

**mMoDC functionality is affected by PPRV infection:**

- ↓ DC cell markers**
- ↓ T-cell activation / proliferation**

**PPRV infection induces changes in mMoDC gene expression:**

- Up- (453) and down- (179) regulation of genes**
- Induction of pathways associated with viral infection response, apoptosis, viral replication, among others**


# ACKNOWLEDGEMENTS



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