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Puerto Vallarta/Mexico



# Influence of Host Cell Defence during Influenza Vaccine Production in MDCK Cells

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# Influenza Virus



MAX-PLANCK-GESELLSCHAFT

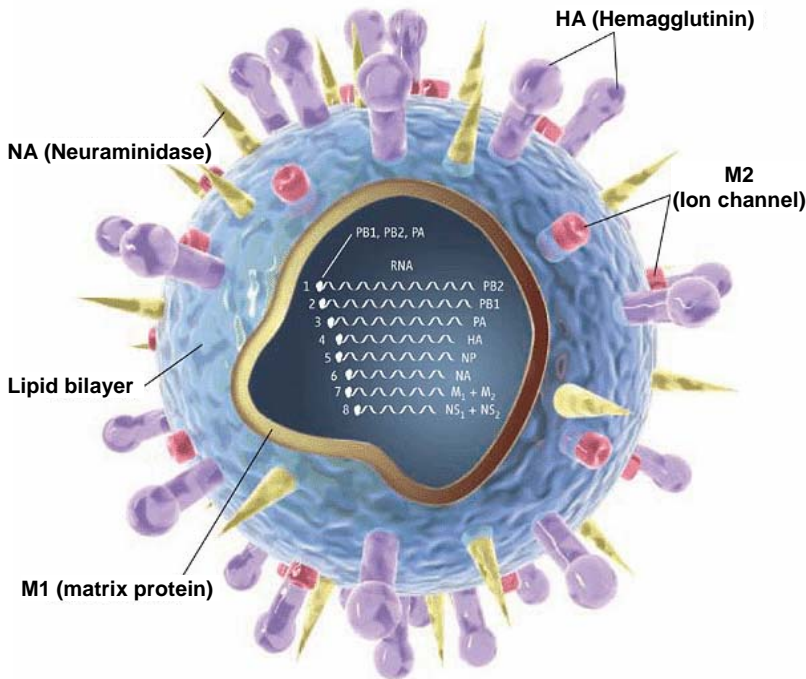


Illustration: Chris Bickel/Science  
(21 April 2006) © 2006 by AAAS

*Orthomyxoviridea*: segmented single-stranded RNA genome

divided into influenza A, B and C viruses

annually 3-5 million cases of severe illness worldwide and 250,000-500,000 deaths (WHO)

best protection by vaccination annually with trivalent seasonal influenza vaccine

## Egg-based vaccine production

Table 3 Scale-up of influenza vaccine production

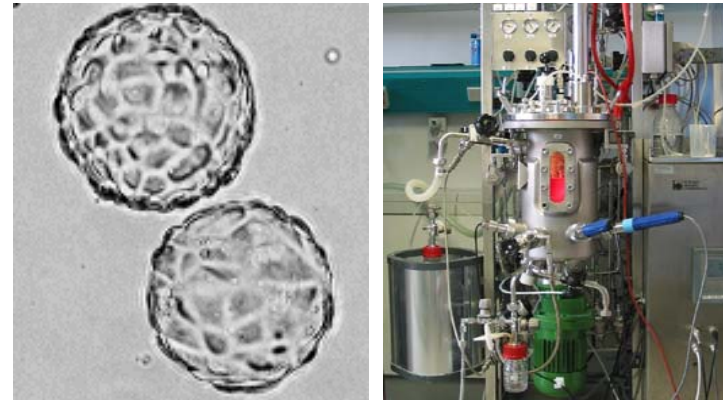
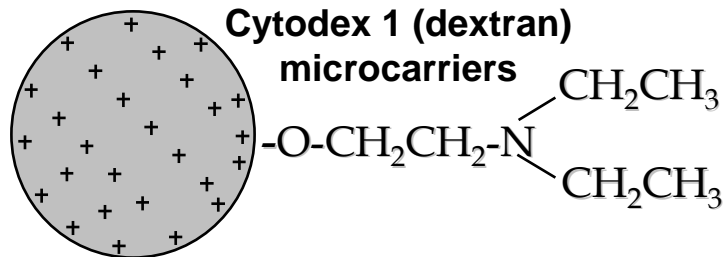
Production time (including lead time)	Worldwide capacity (monovalent doses of 15 µg)	Worldwide coverage (%)
1 year	~1,000,000,000	~17
2 years	~2,500,000,000	~40
4 years and 9 months	~6,500,000,000	100

Ulmer et al., 2006



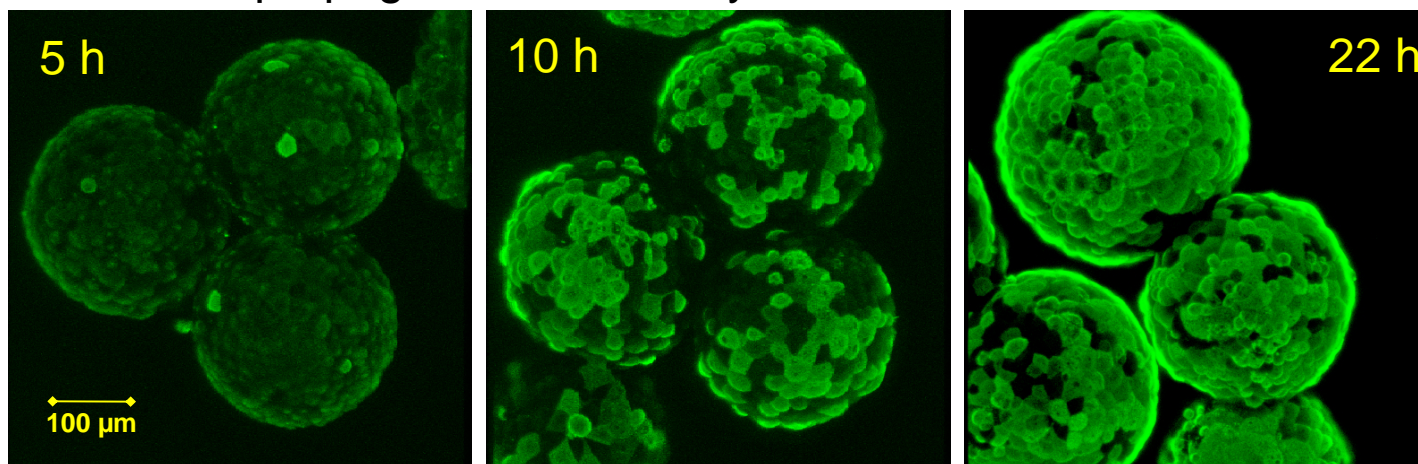
## Cell culture-based vaccine production

MDCK (Madin-Darby canine kidney) cells



- 4 days of cell growth to approx.  $2.0 \times 10^6$  cells/mL
- medium exchange and virus infection with MOI 0.025
- virus propagation for 2-3 days

Genzel et al., 2004



# Influenza A/PuertoRico/8/34

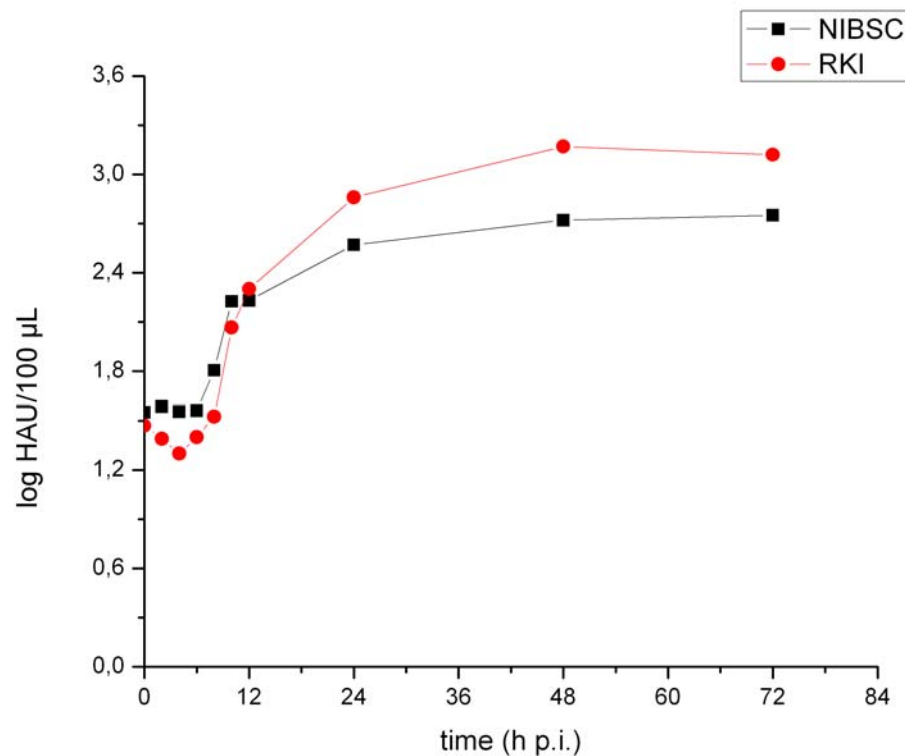


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## Influenza virus strain PR8 variants:

A/PR/8/34 RKI (Robert Koch Institute)

A/PR/8/34 NIBSC (National Institute for Biological Standards and Control)



## PR8 variants differ in:

- yield
- replication kinetics
- apoptosis induction

Schulze-Horsel et al., 2009

- amount of differentially expressed host proteins

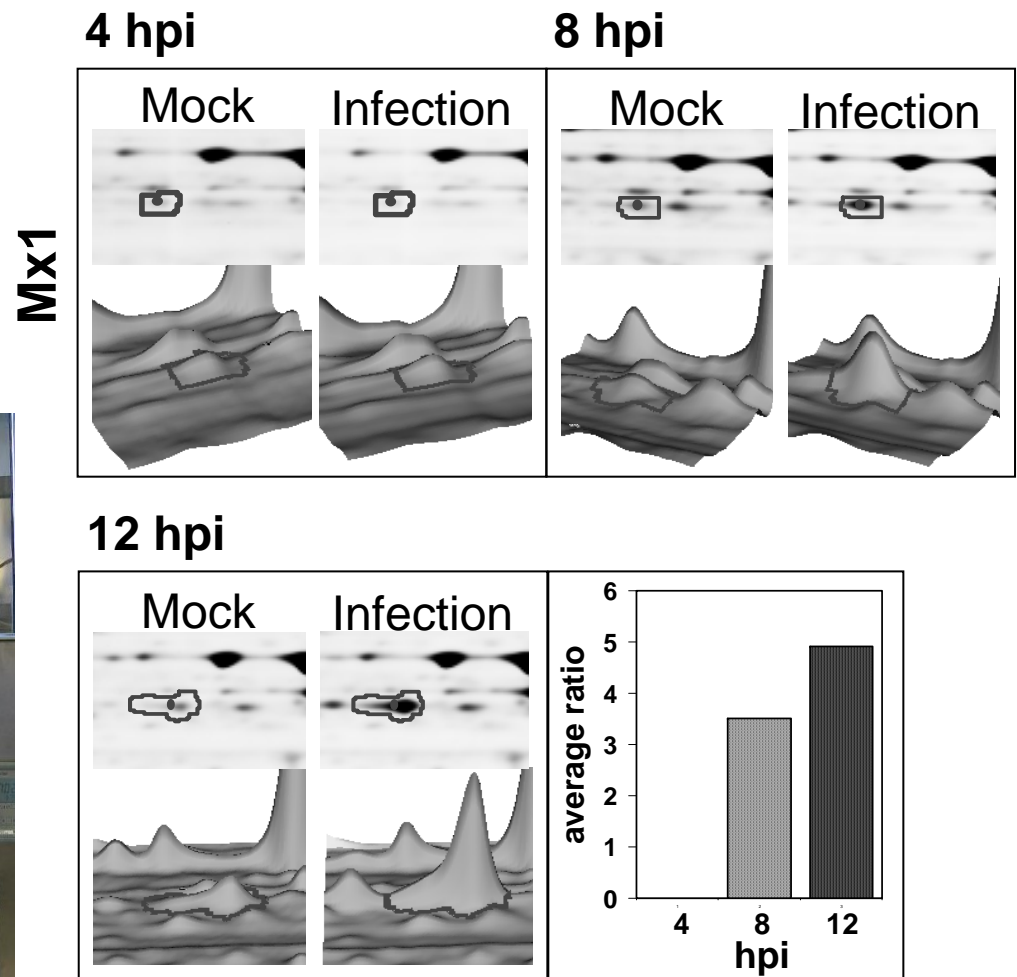
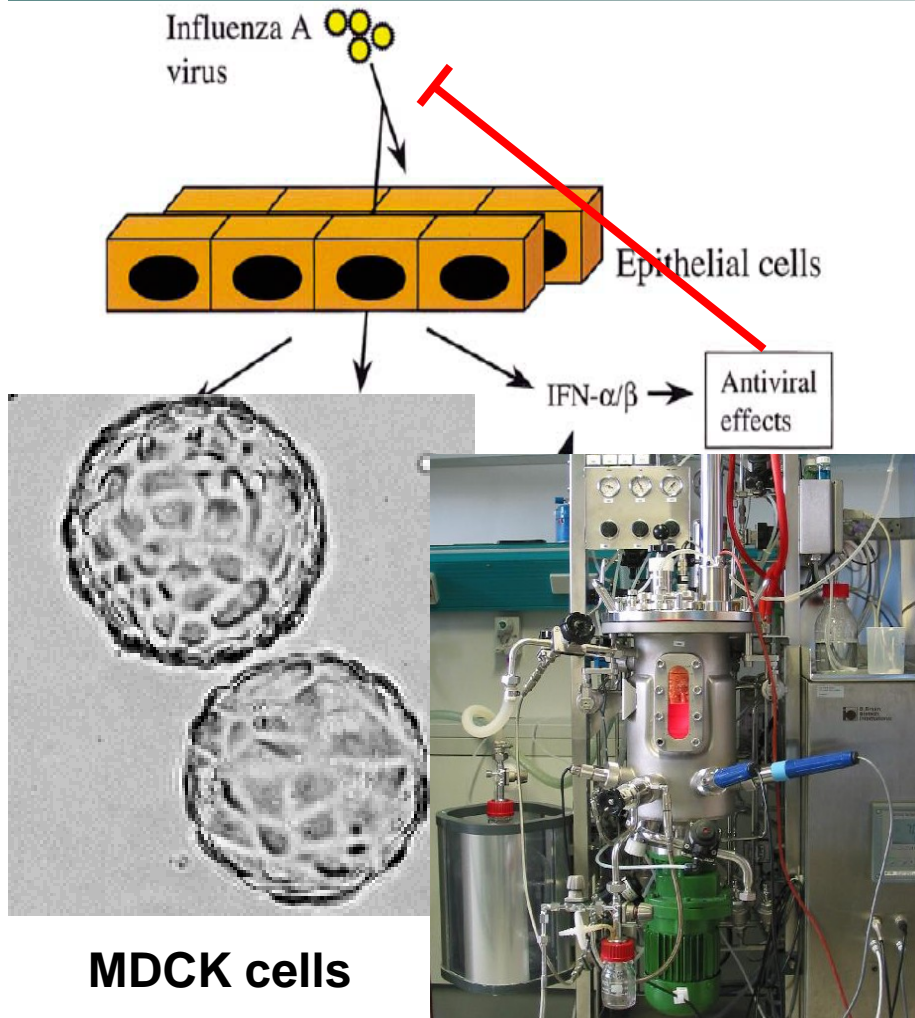
Vester et al., 2010



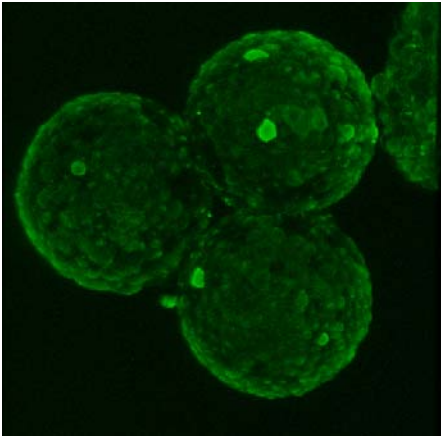
# Limitations by Innate Immune Response?



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Vester et al., 2009

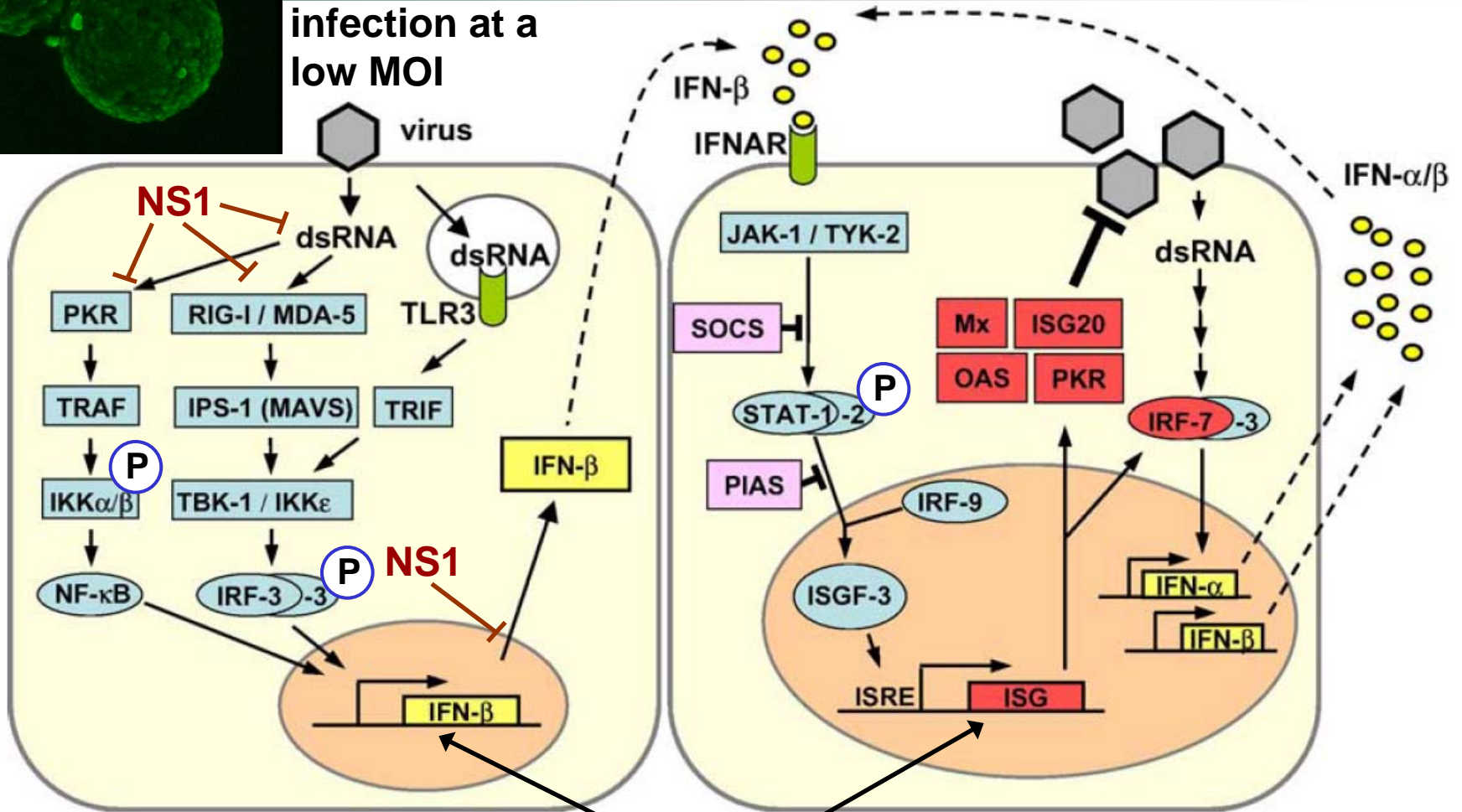


# Signalling Pathways



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infection at a low MOI



Haller et al., 2006

Western blots (P)

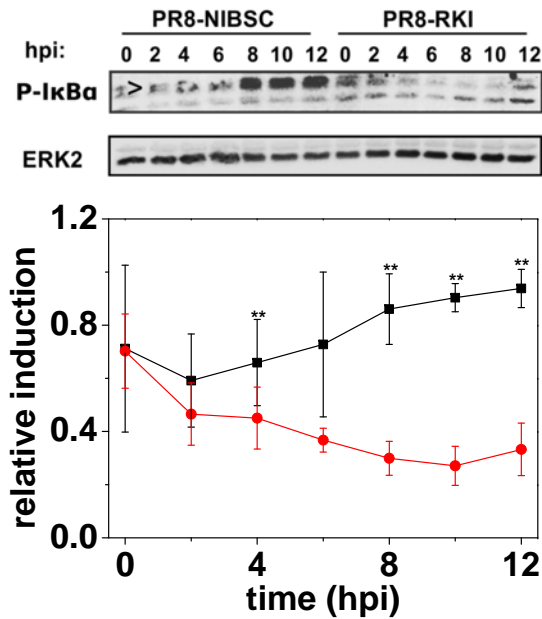
qRT-PCR for IFN-β and cMx1

# Interferon Induction

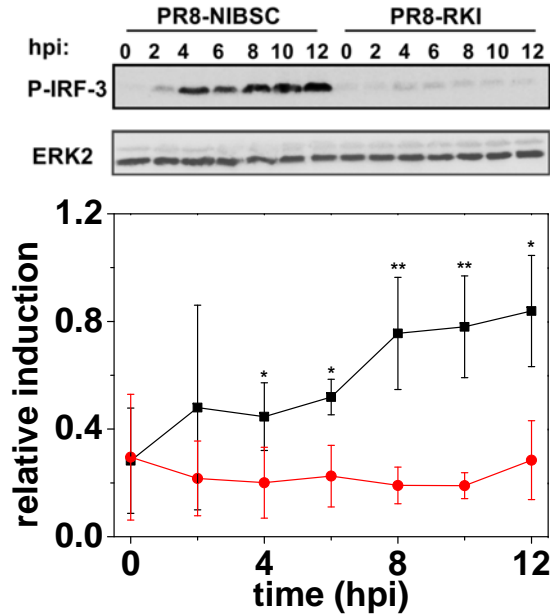


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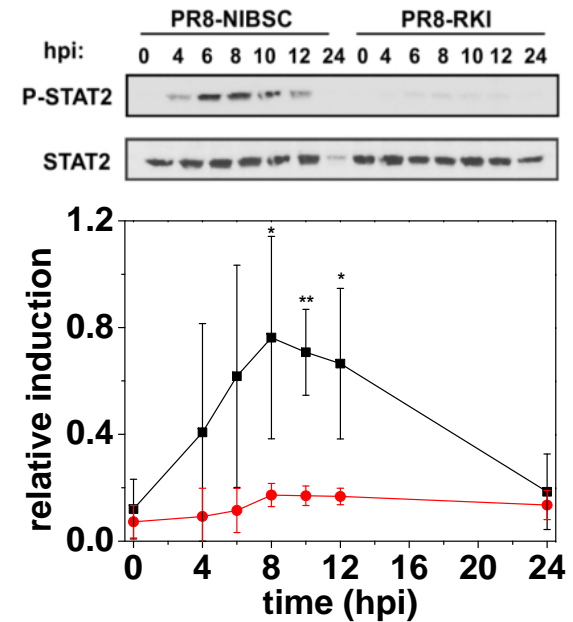
## I $\kappa$ B $\alpha$



## IRF-3



## IFN-mediated signalling / activation of the antiviral state



Björn Heynisch

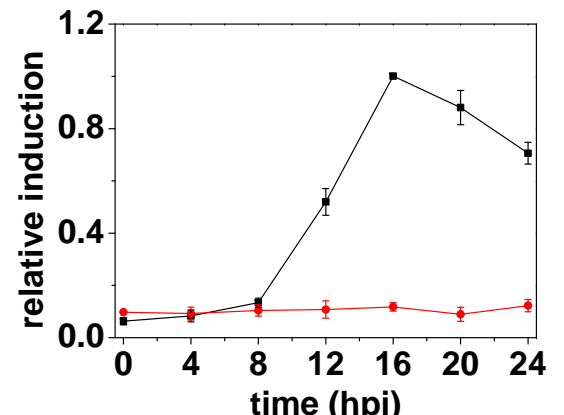
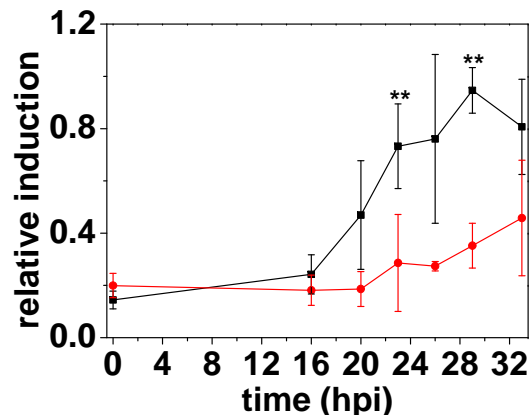
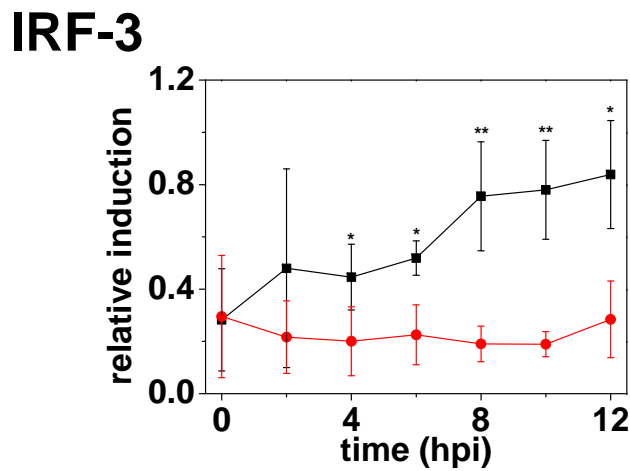
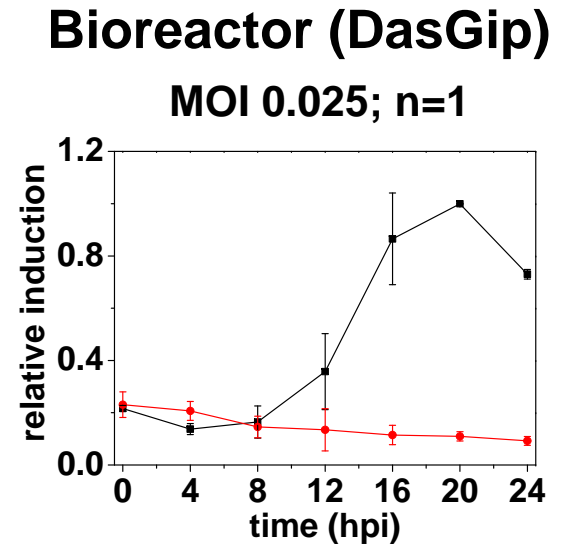
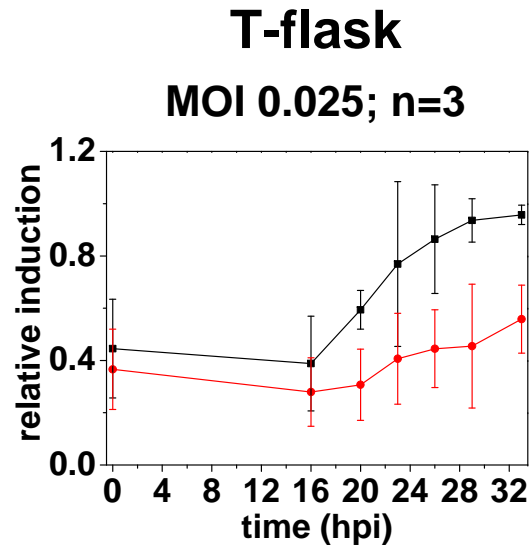
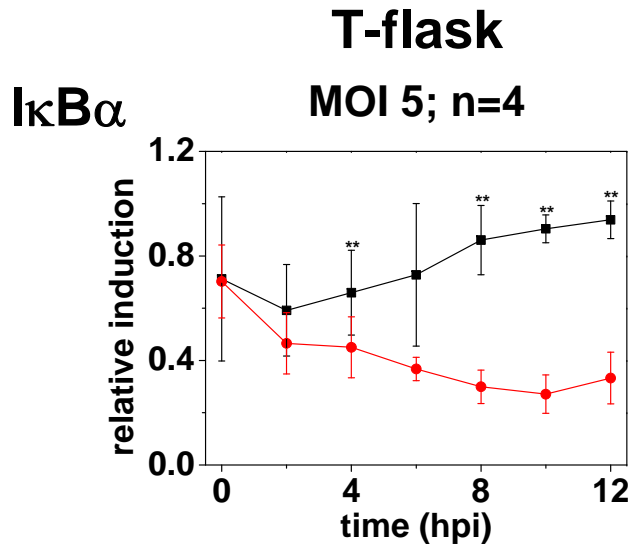
T-flask experiments with an MOI 5; n=4

Heynisch et al., 2009 submitted

# Interferon Induction



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■ PR8-NIBSC  
● PR8-RKI



# Interferon Induction

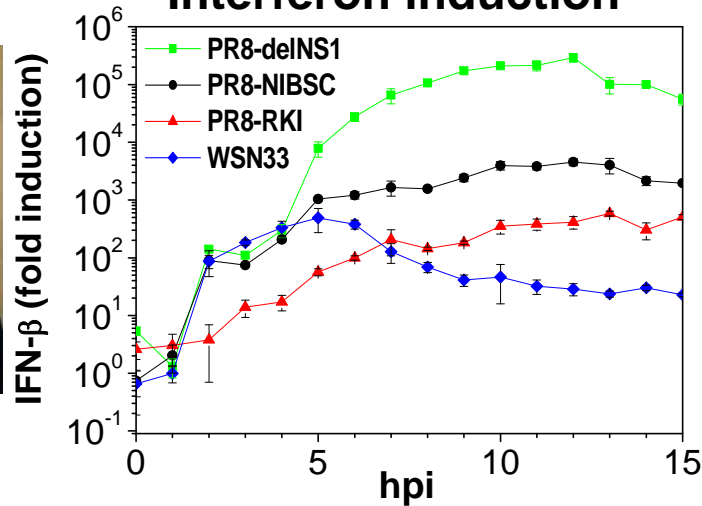


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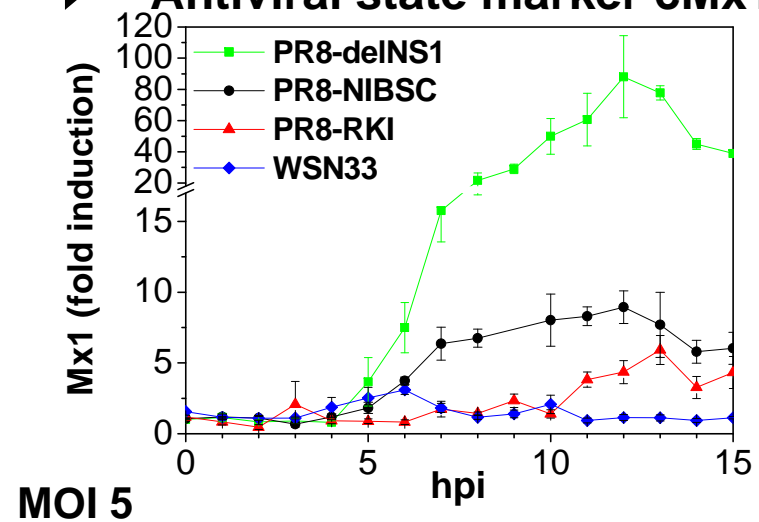


Claudius Seitz

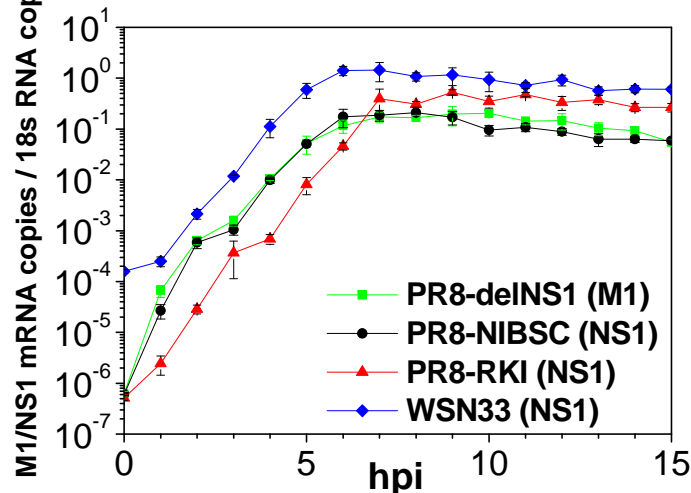
## Interferon induction



## Antiviral state marker cMx1



## Intracellular viral mRNA



### loss of function:

- Does the inhibition of host defence increase the virus titre?

### gain of function:

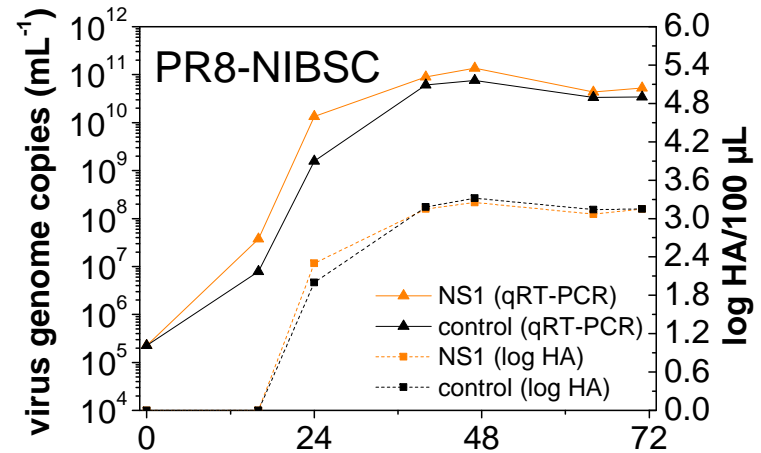
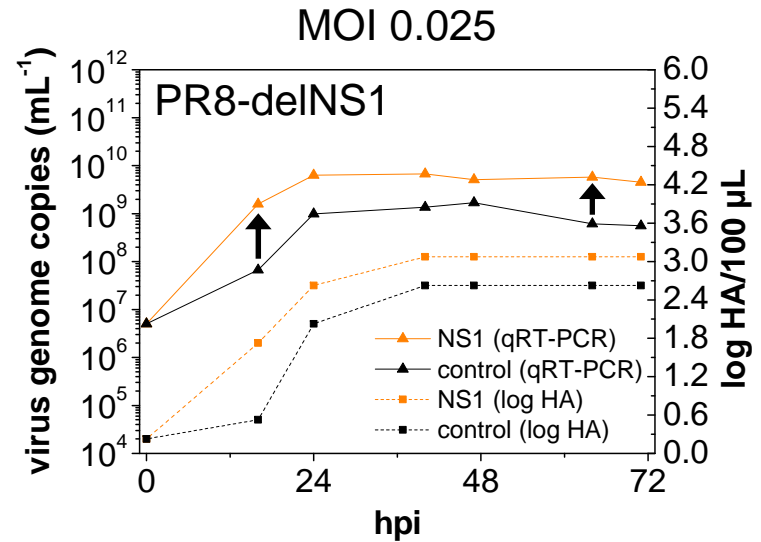
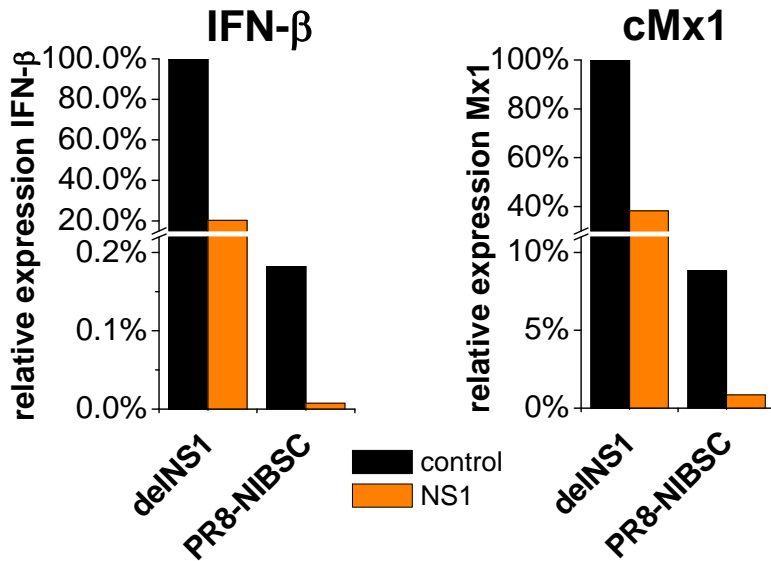
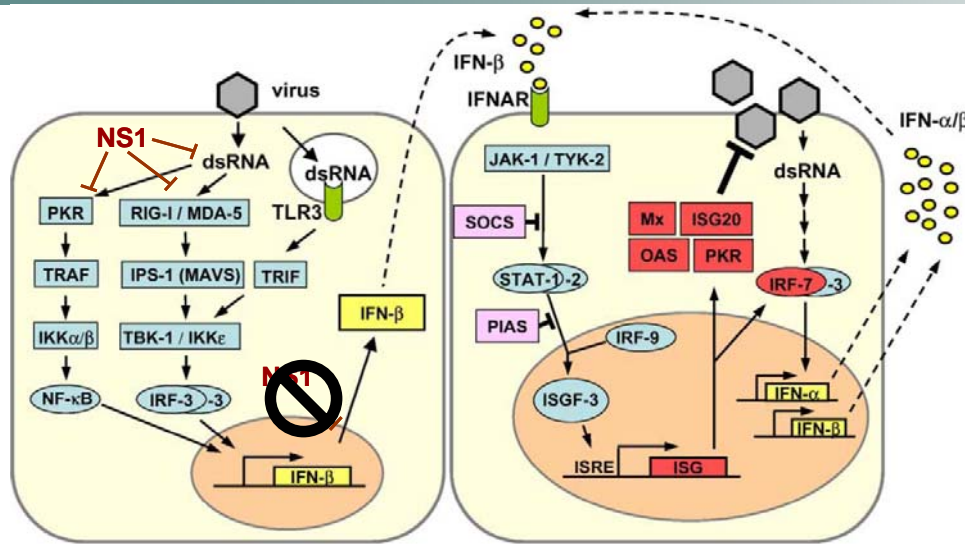
- Does the activation of the antiviral state reduce the virus yield?

Seitz & Frensing et al., 2010

# Loss of function: viral antagonist NS1



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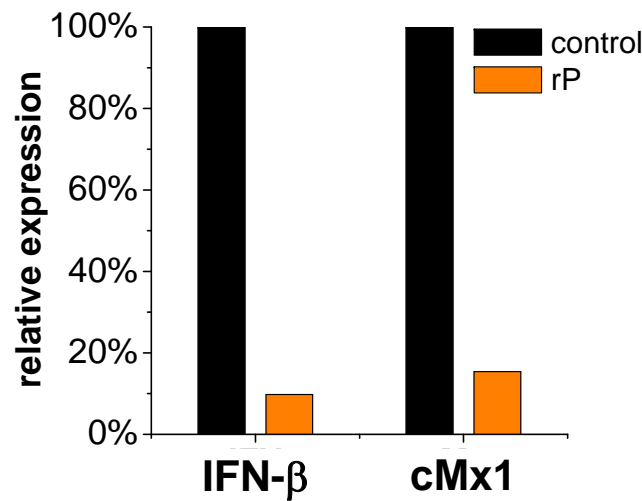
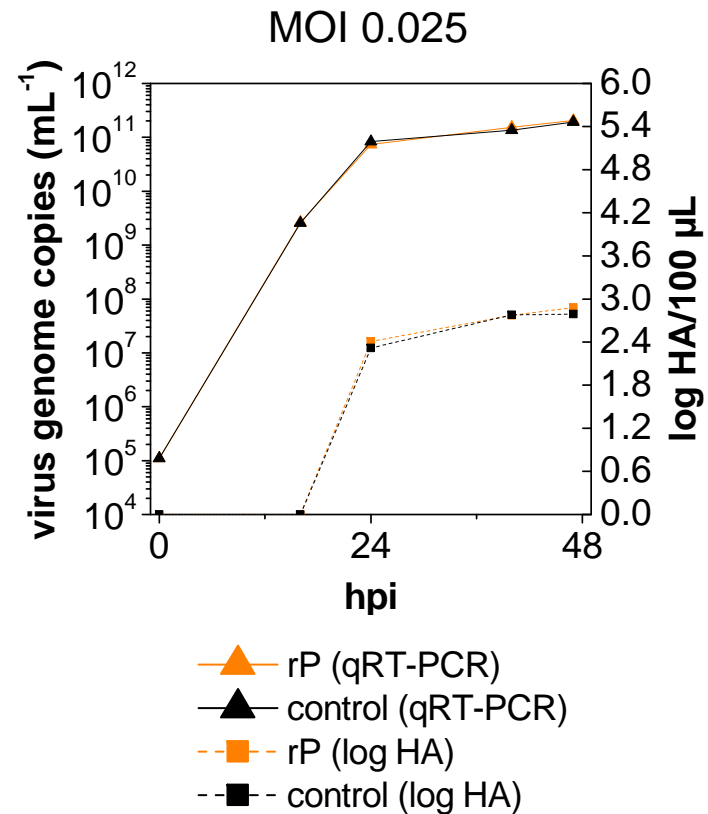
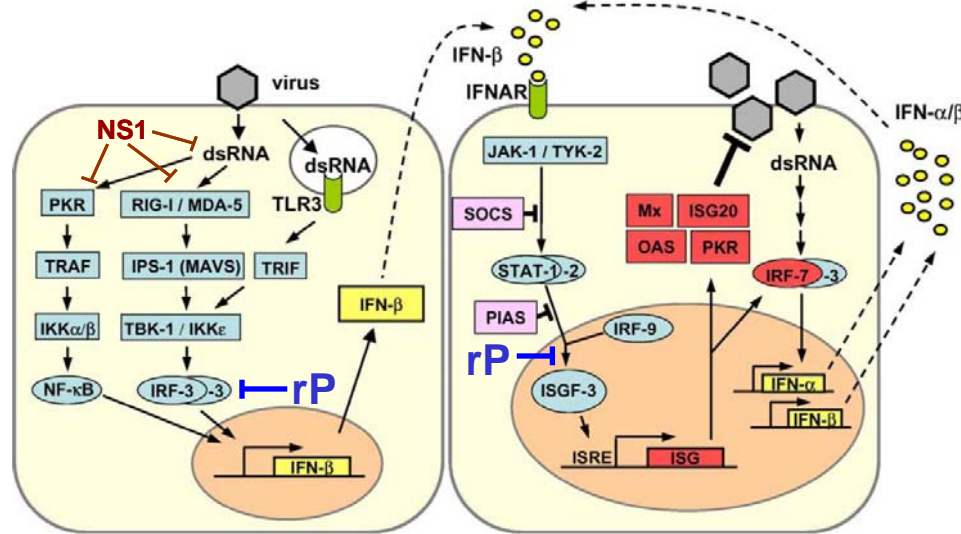


but: NS1 promotes viral mRNAs translation. Which NS1 function enhanced the virus titre?

# Loss of function: viral antagonist rP



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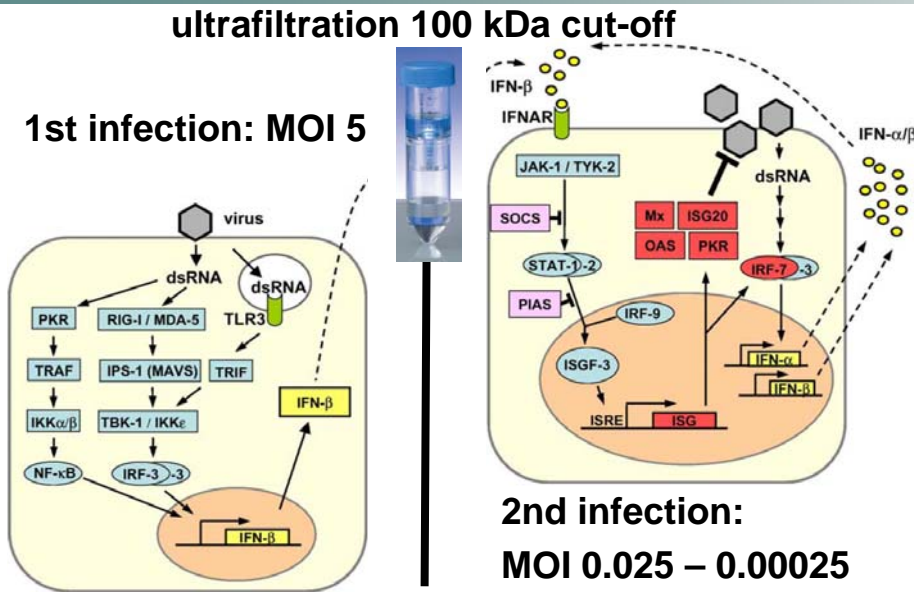


**Inhibition of IFN signalling does not result in higher virus yields**

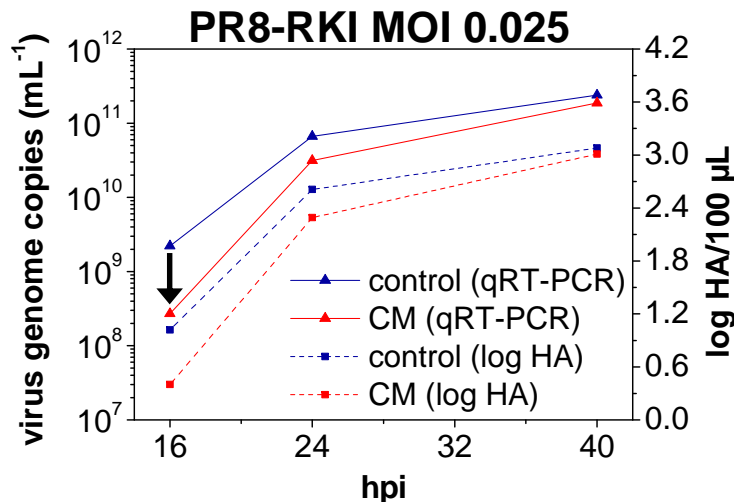
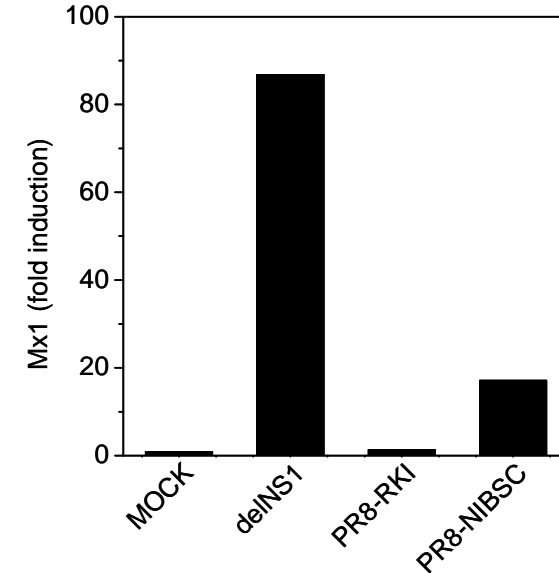
# Gain of function: cytokine stimulation



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**cMx1 induction**



**Virus replication is slowed down, but almost the same titre is reached**

- active virus titre (TCID50)
- MOIs (0.025 / 0.0025 / 0.00025)
- PR8-NIBSC, A/WSN/33

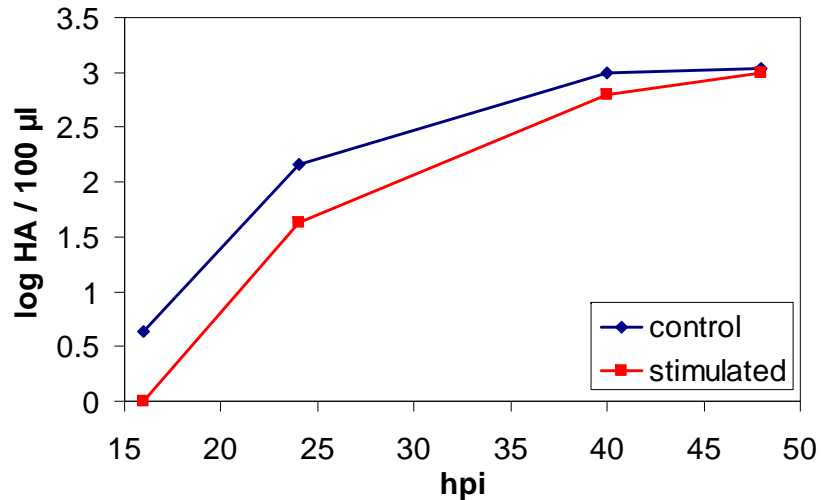


# Gain of function: cytokine stimulation

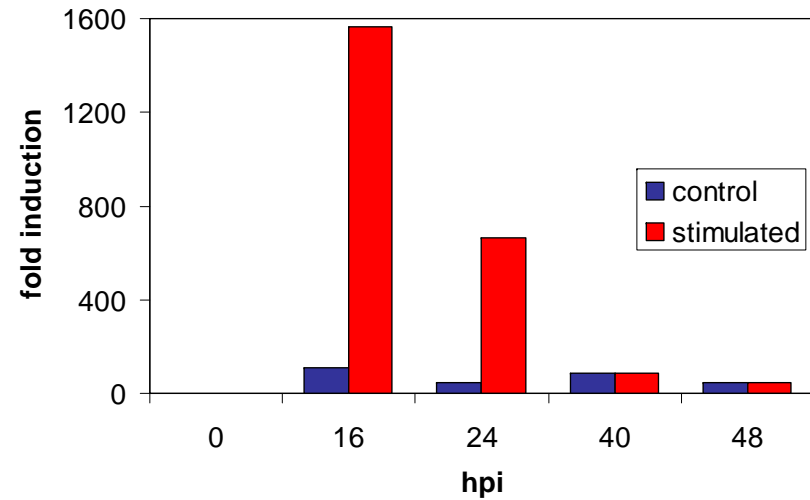


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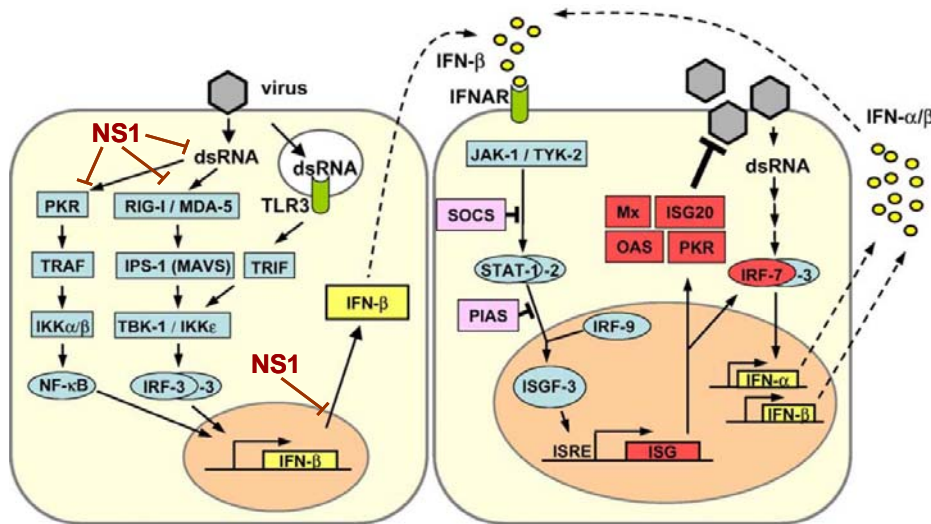
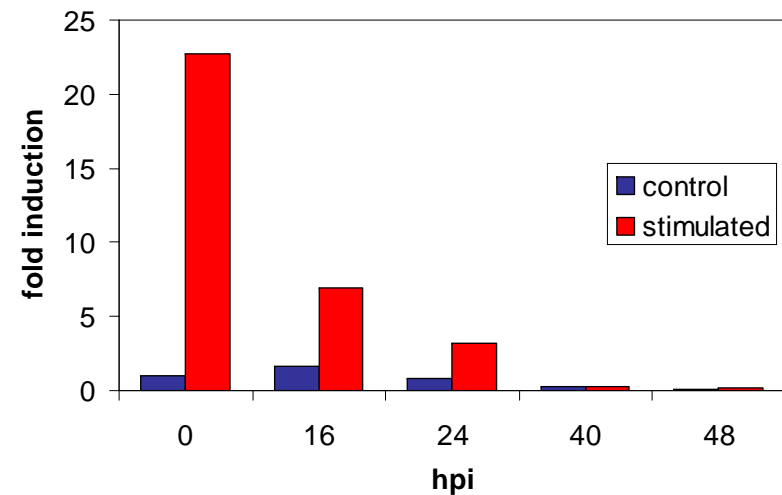
B/Malaysia/2506/2004 (MOI 0.025)



IFN induction



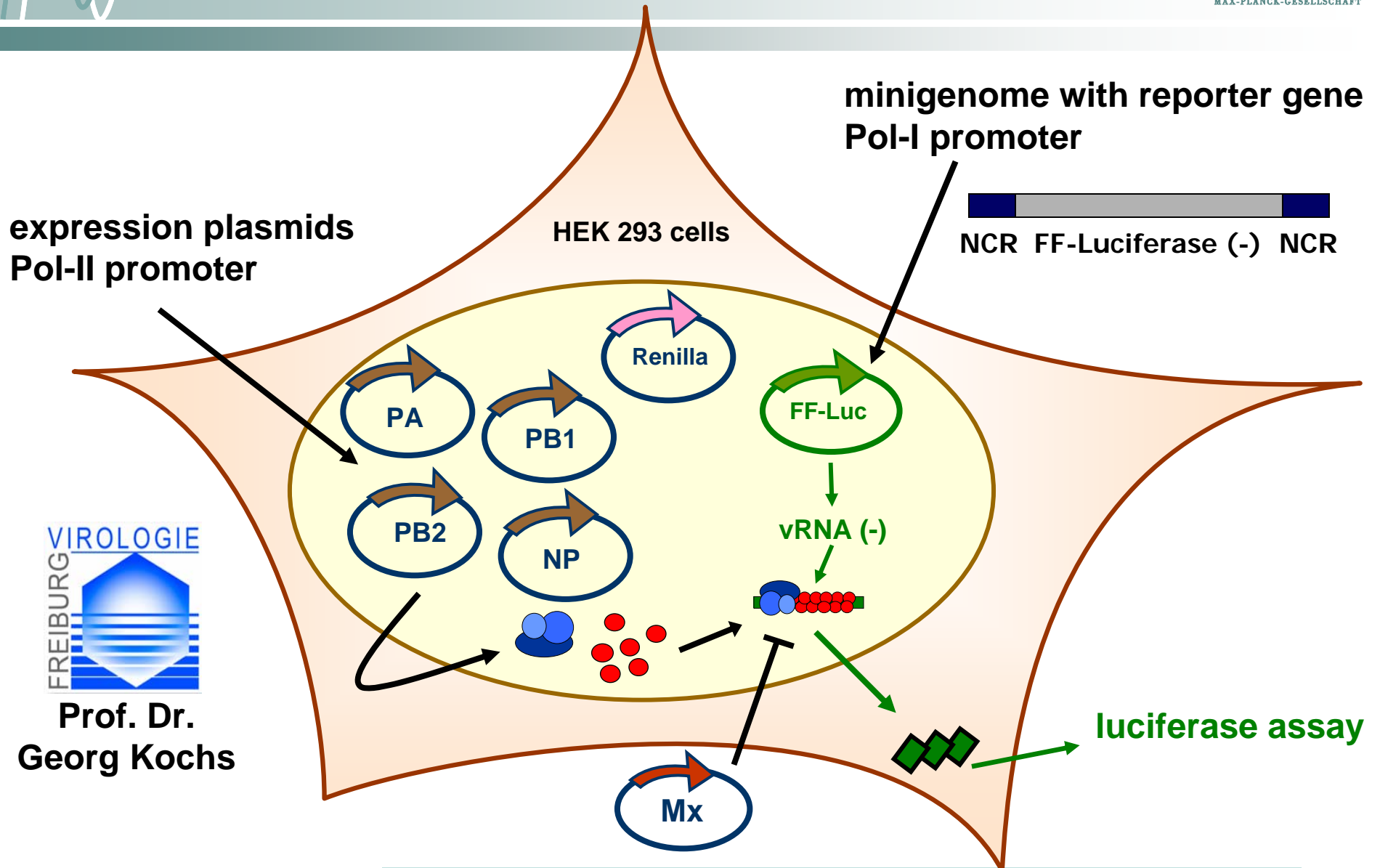
cMx1 induction



# Minireplicon Assay



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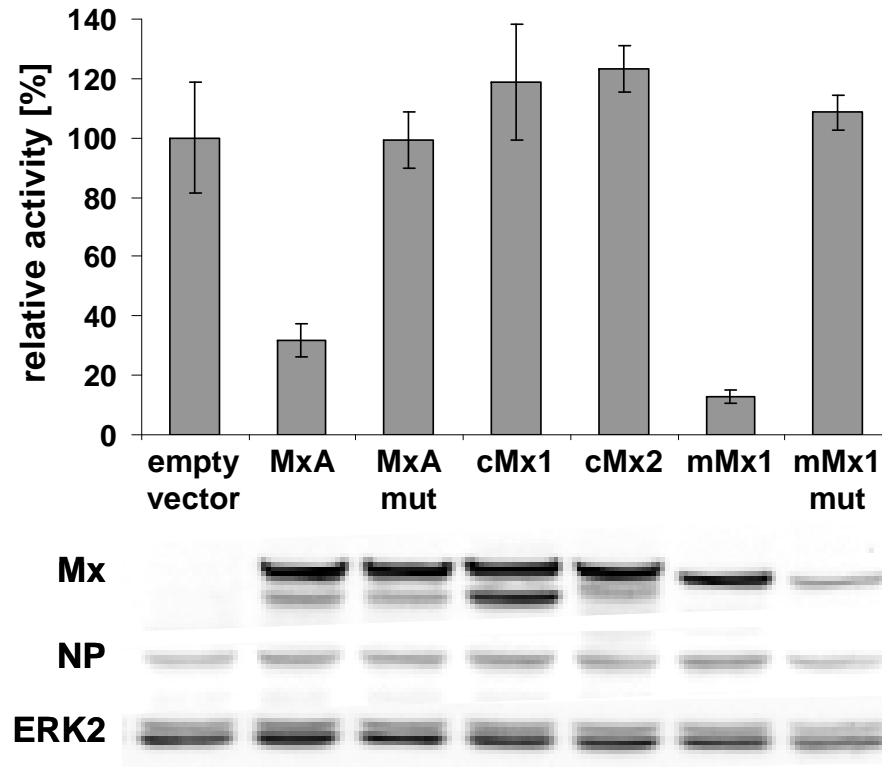


Prof. Dr.  
Georg Kochs

# Minireplicon Assay



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**No inhibitory effects of canine Mx proteins**

**Seitz, C. & Frensing, T., Höper, D., Kochs, G., Reichl, U. (2010).**

High yields of Influenza A virus in MDCK cells are promoted by an insufficient IFN-induced antiviral state. *Journal of General Virology*, accepted

# Summary



MAX-PLANCK-GESELLSCHAFT

- **considerable IFN signalling in influenza virus infected MDCK cells**
- **no impact on final virus titres by inhibition or stimulation of IFN**
- **lack of inhibitory potential of canine Mx proteins against influenza virus**



**IFN signalling has only a minor effect on influenza virus replication in MDCK cells, which makes these cells an ideal system for high yield vaccine production.**



# Acknowledgment



MAX-PLANCK-GESELLSCHAFT

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Bianca Kaps

## former students:

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Anja Fincke

Stefan Heldt

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# Acknowledgment



MAX-PLANCK-GESELLSCHAFT

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