



CENTRE DE RECHERCHE EN CANCÉROLOGIE DE LYON

UMR INSERM-U1052/CNRS-5286/UCBL/CLB

Sub-cellular mechanisms of innate immunity evasion in HBV infection

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PhD, HDR, CRI INSERM*



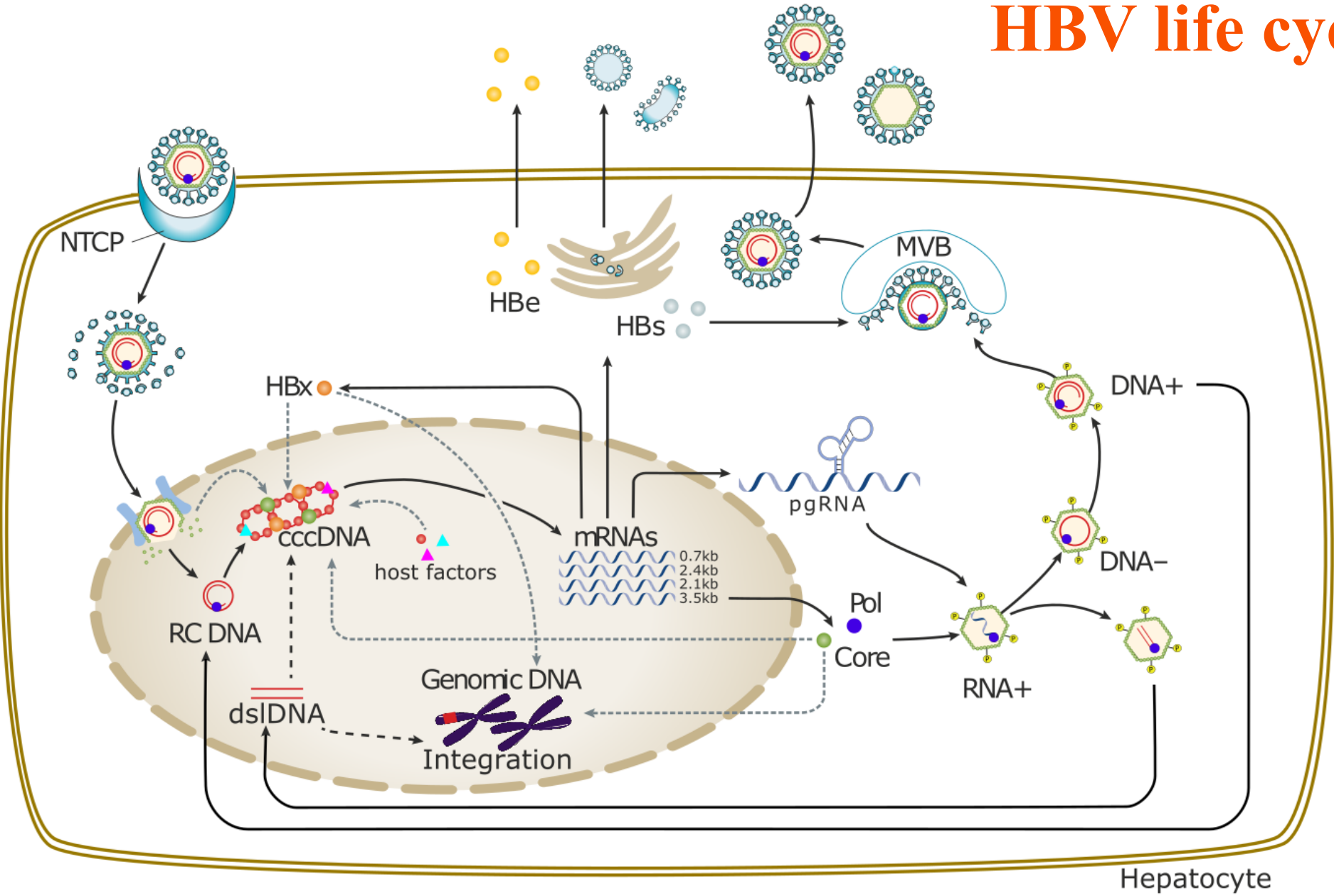
Instituts
thématiques

Inserm

Institut national
de la santé et de la recherche médicale

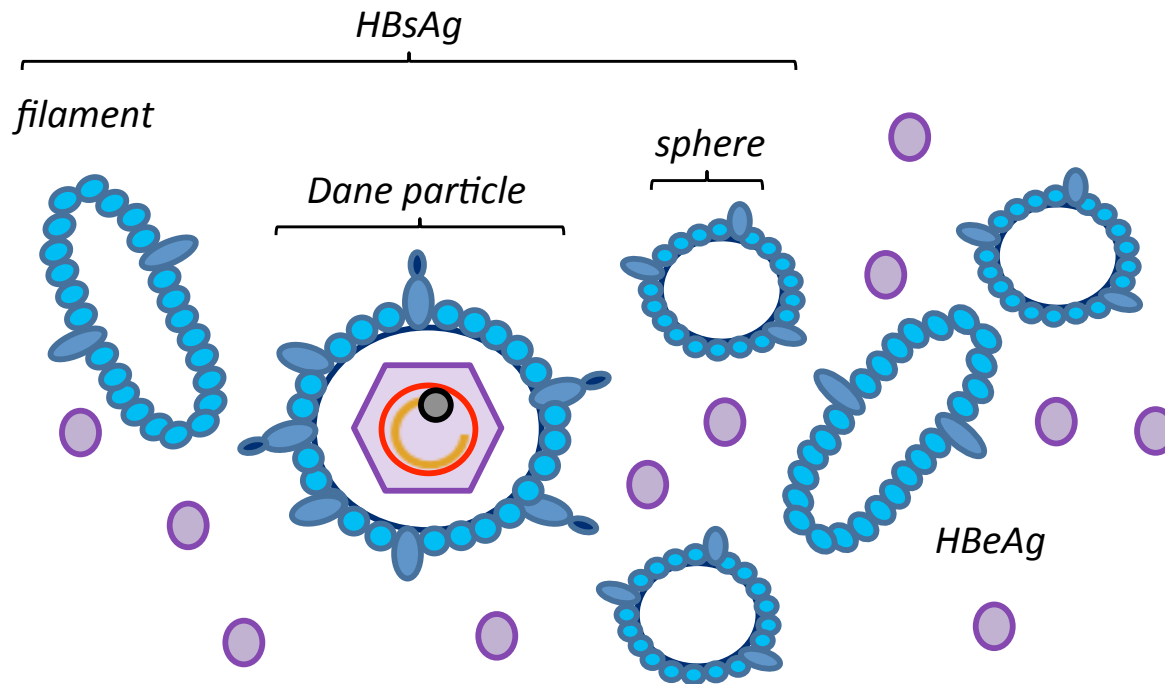


HBV life cycle

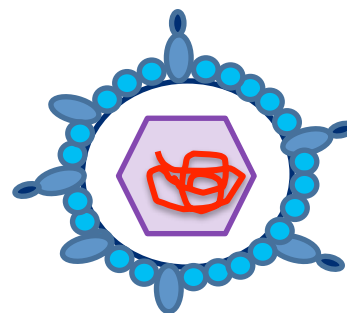


Circulating viral particles (and proteins): a variety of potential PAMPS and/or decoy/escape viral components

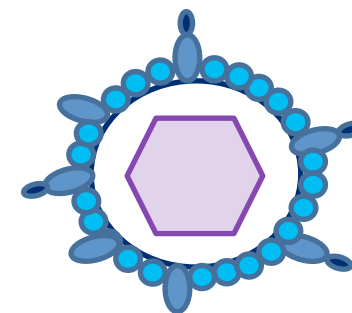
Classic ones:



Newly described:



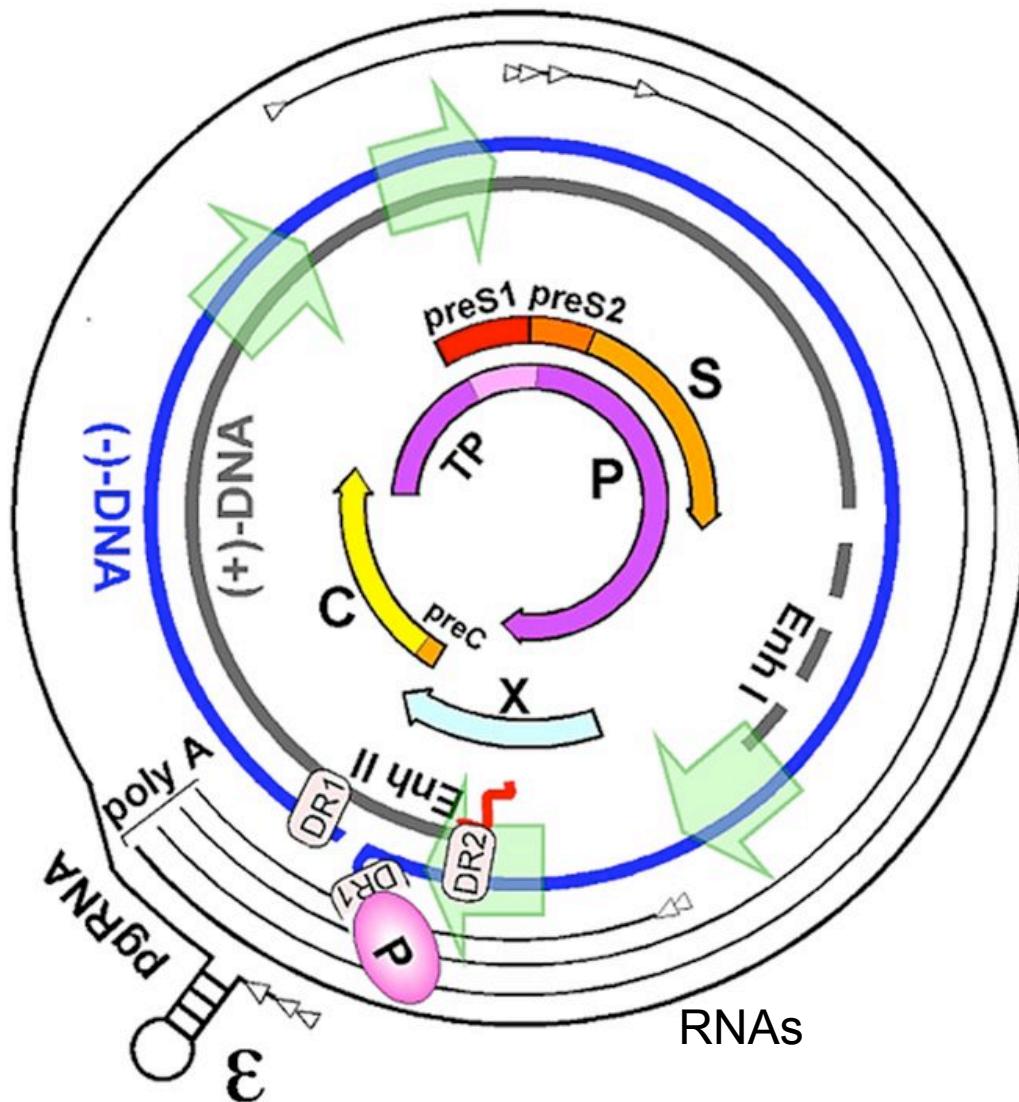
RNA containing particles



Genome free particles

Van Bommel et al, Hepatology 2015;
Hu et al, J Viral Hepatitis 2015

HBV genome structure (in virions) and coding capacity : a damaged molecule !



✓ Relaxed, partially double-stranded circular genome (3.2 kb)

✓ Polymerase covalently linked to genome

✓ 4 ORFs → 4 main transcripts + spliced transcripts

✓ Seven (or 8) proteins encoded

➤ HBeAg (and p22) and HBcAg

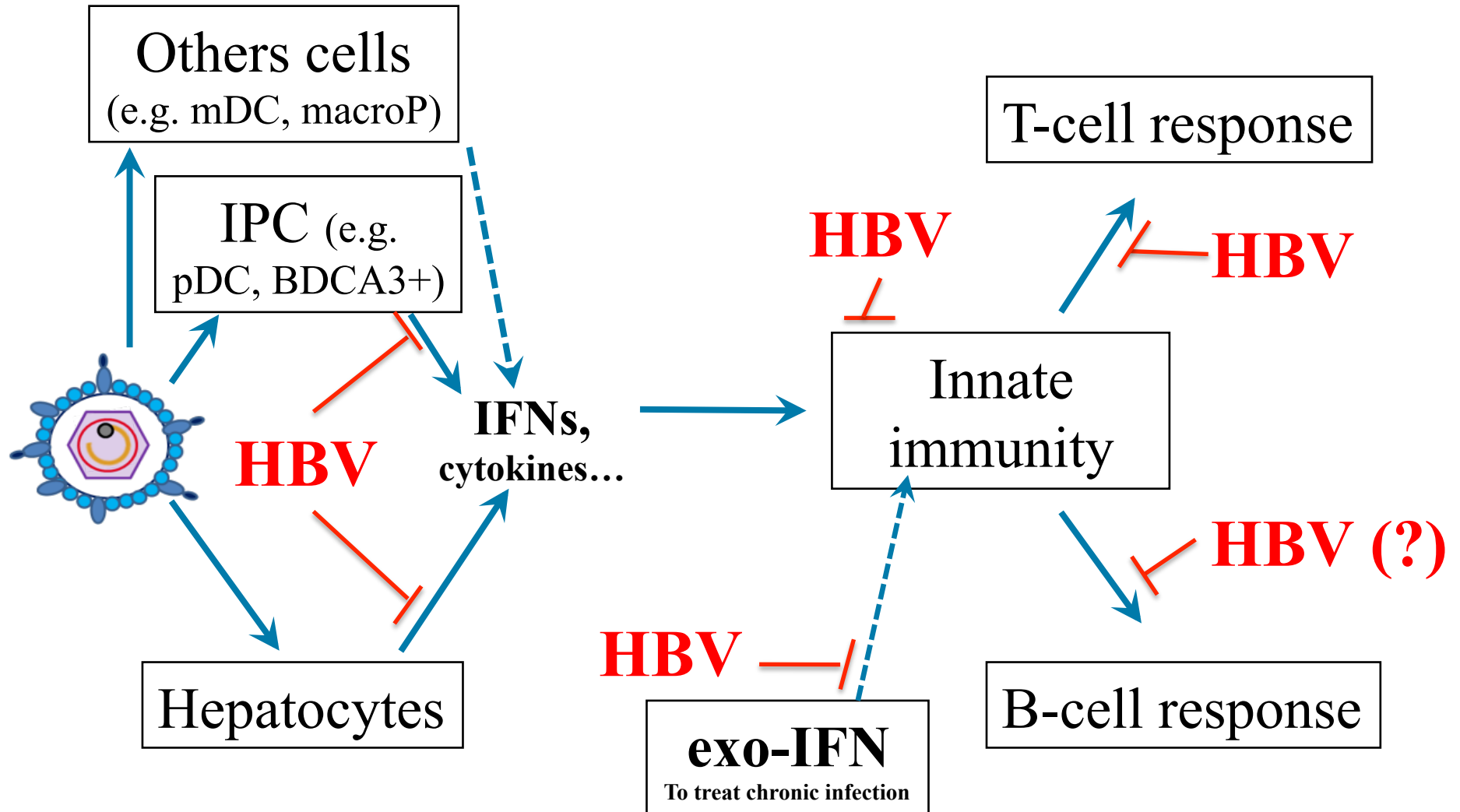
➤ Polymerase

➤ 3 envelope proteins (L/PreS1, M/preS2, and S/HBsAg) with common C-terminus

➤ X protein/HBx

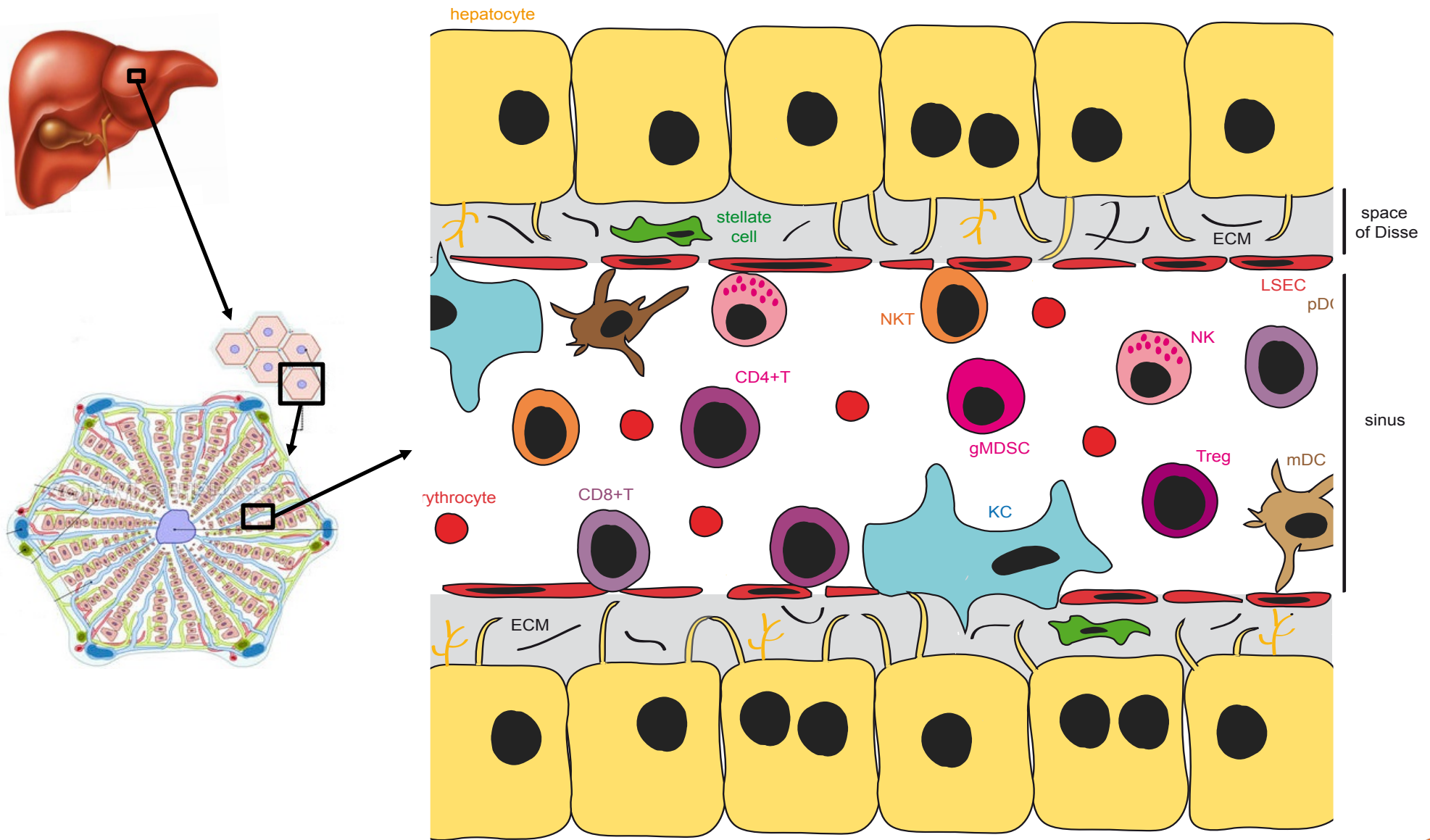
➤ HBSP

HBV impairs immune functions in chronically infected patients

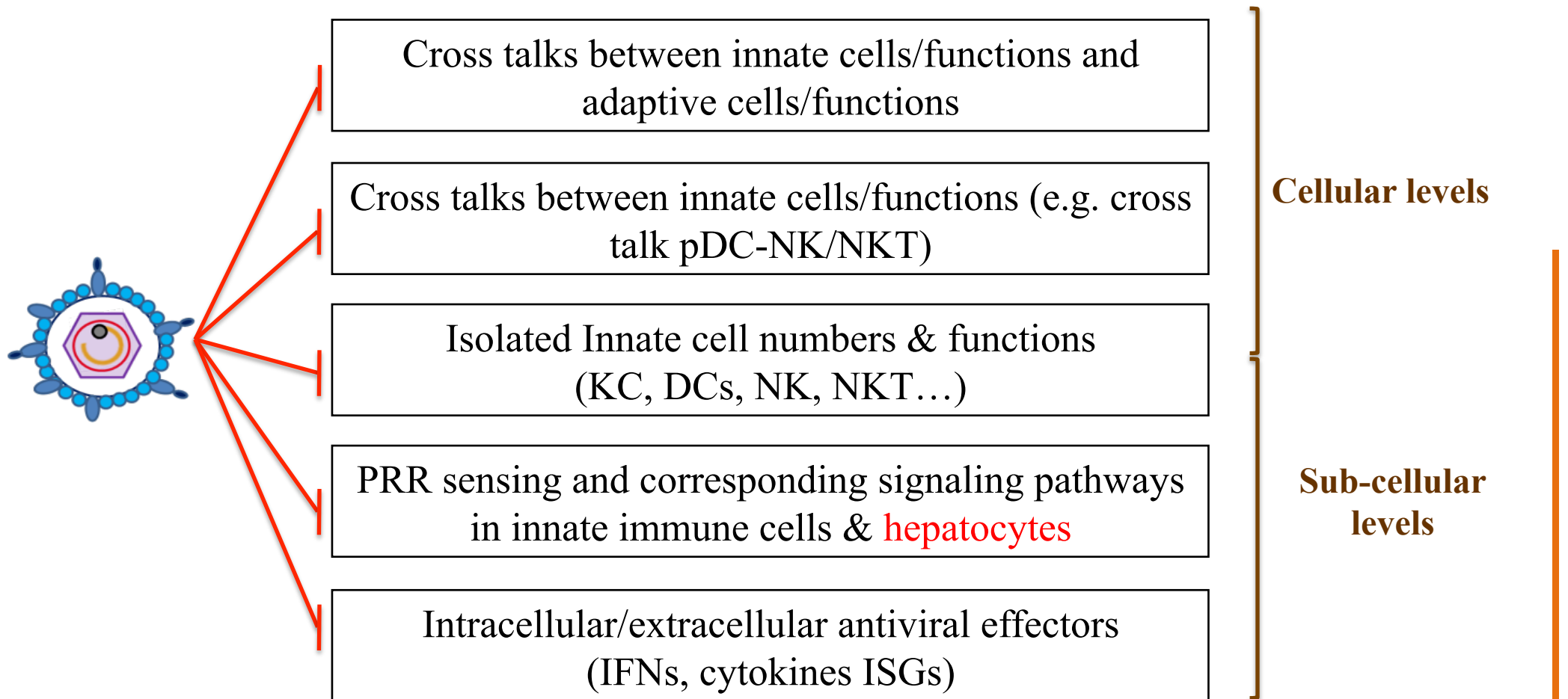


Dusheiko G., *Liver Int.*, 2013; Han et al., *Semin Immunopathol.*, 2013; Bertoletti A, Ferrari C., *Gut*, 2012; Chang et al., *Antiviral Res.*, 2012; Lütgehetmann et al., *Gastroenterology*, 2011; Dunn et al., *Gastroenterology* 2009; Stacey et al., *JVI*, 2009; Christen et al., *JVI*, 2007; and many others...

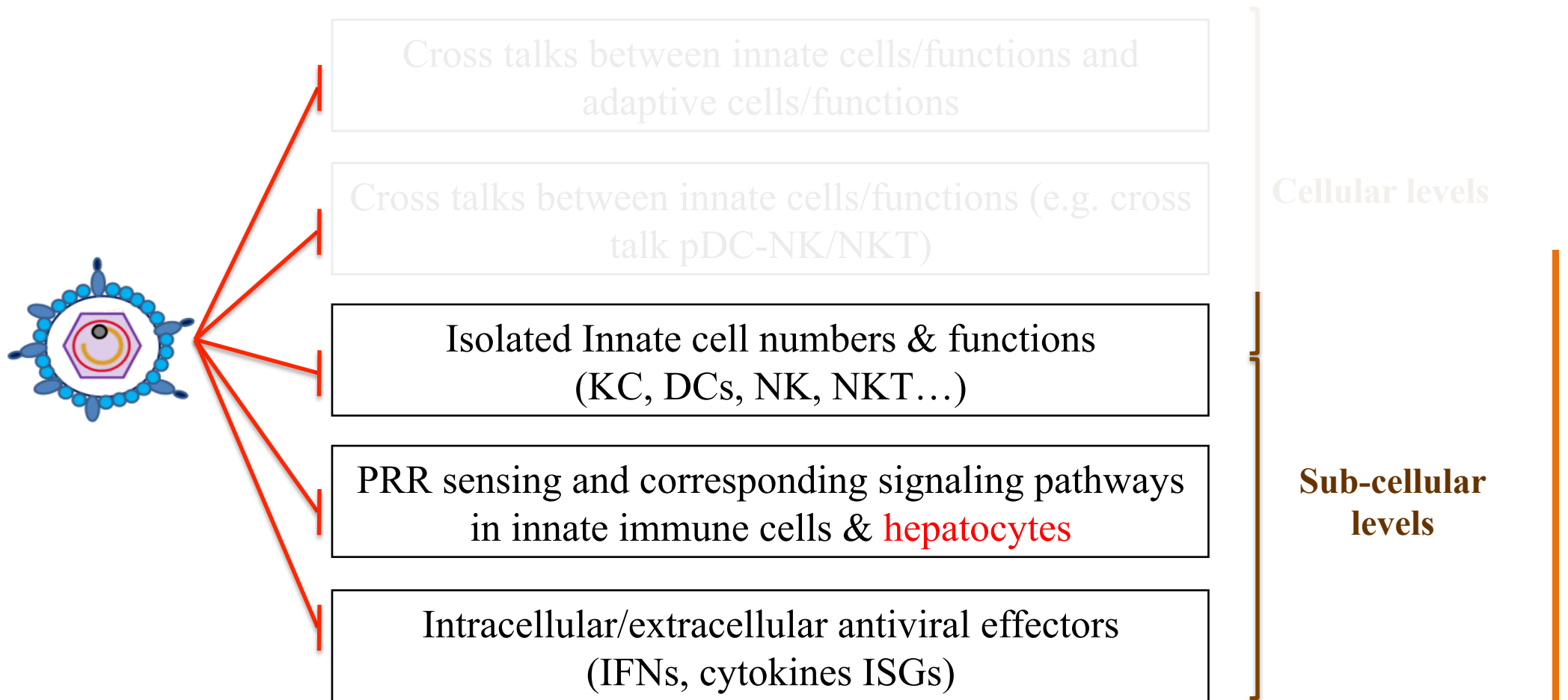
The liver micro-environment



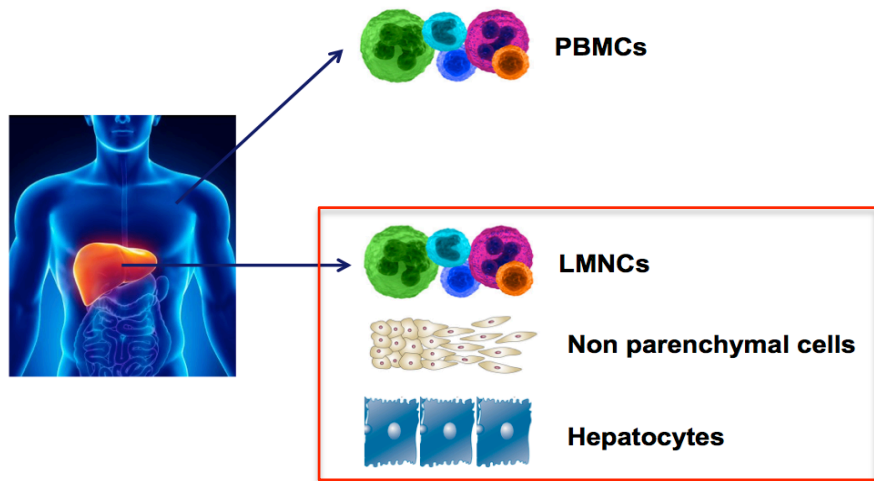
Subversion of immune innate functions: the various possibilities of HBV



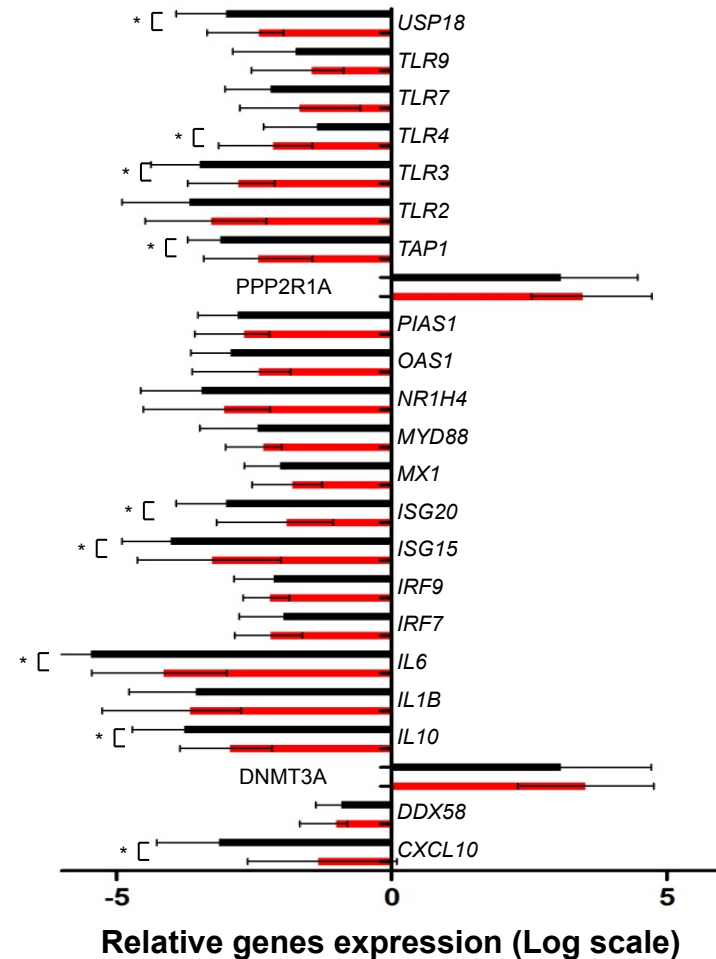
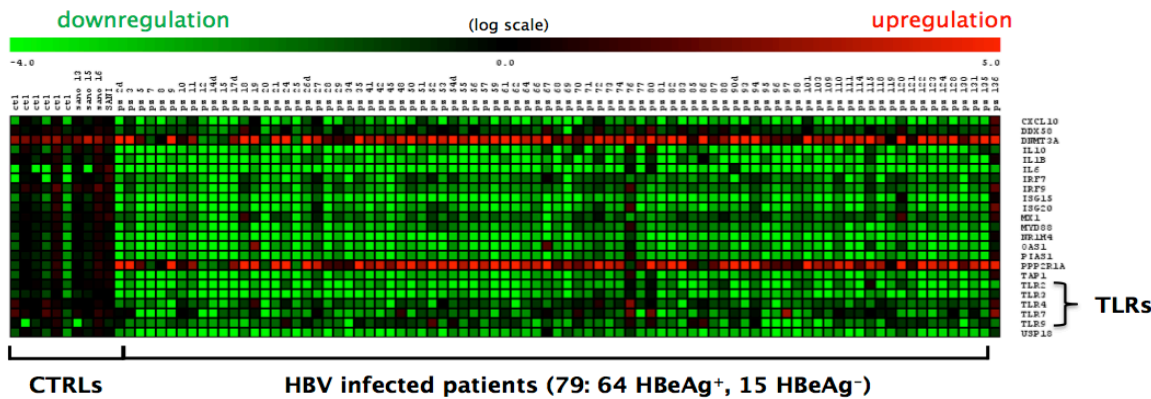
Subversion of immune innate functions: the various possibilities of HBV



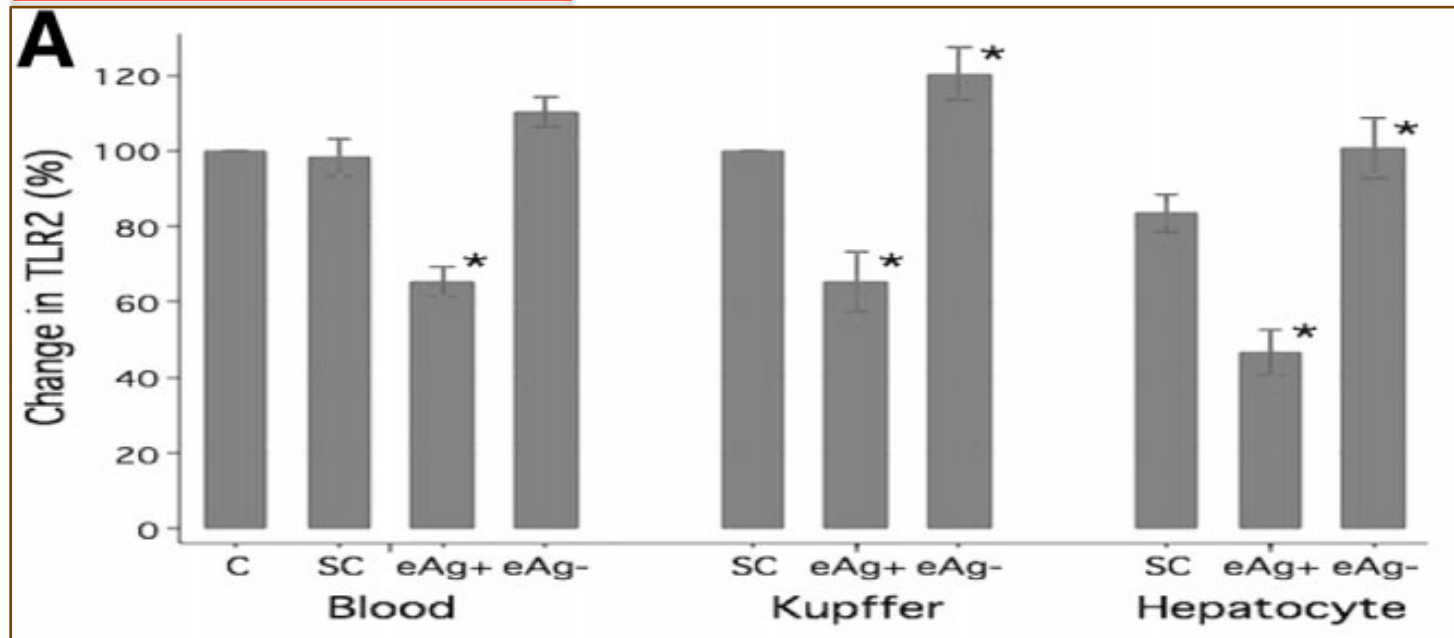
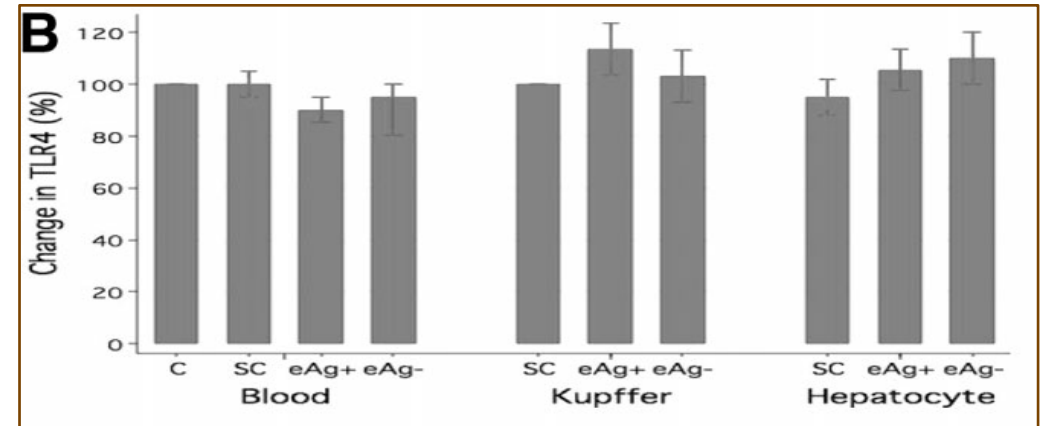
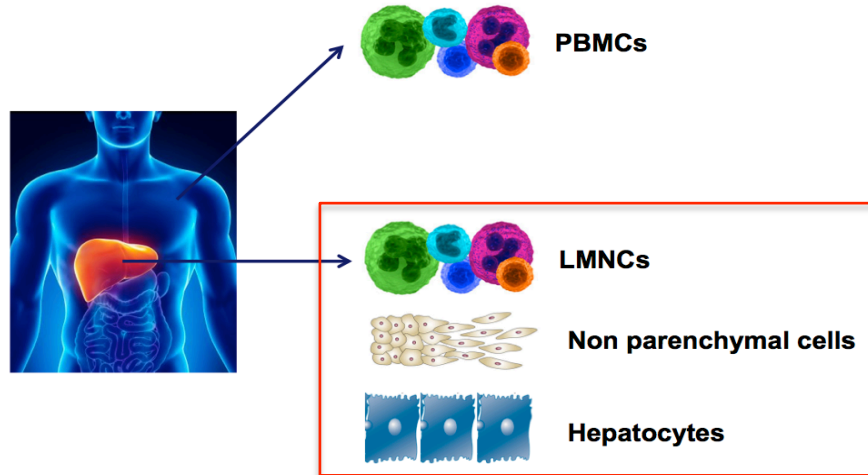
Inhibition of innate immune gene expression in CHB patients



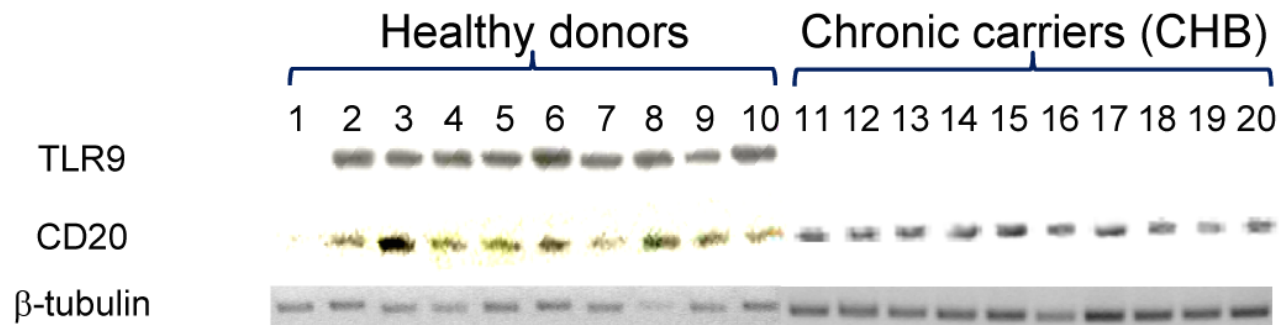
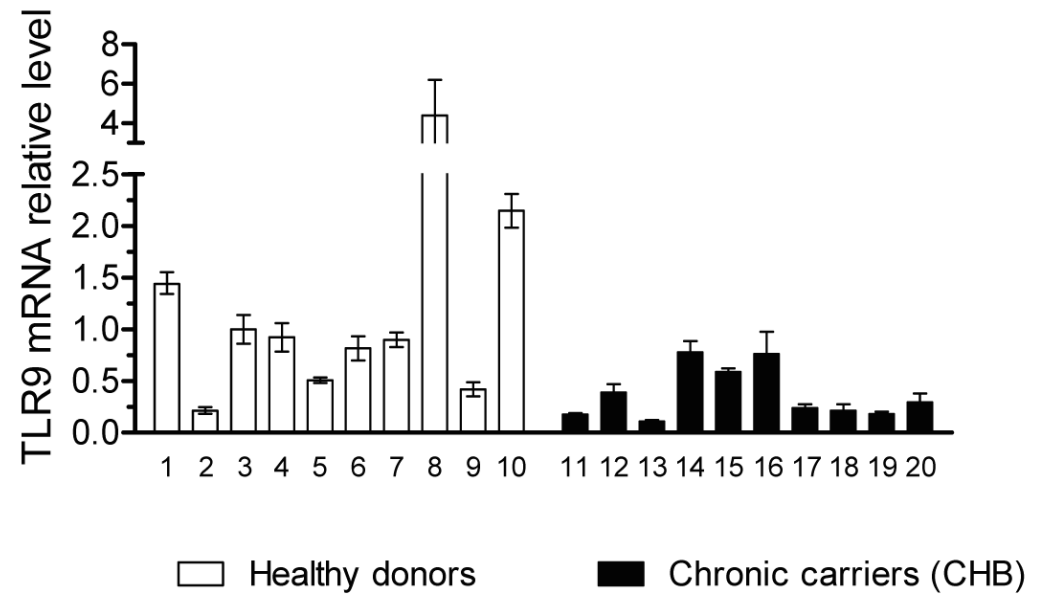
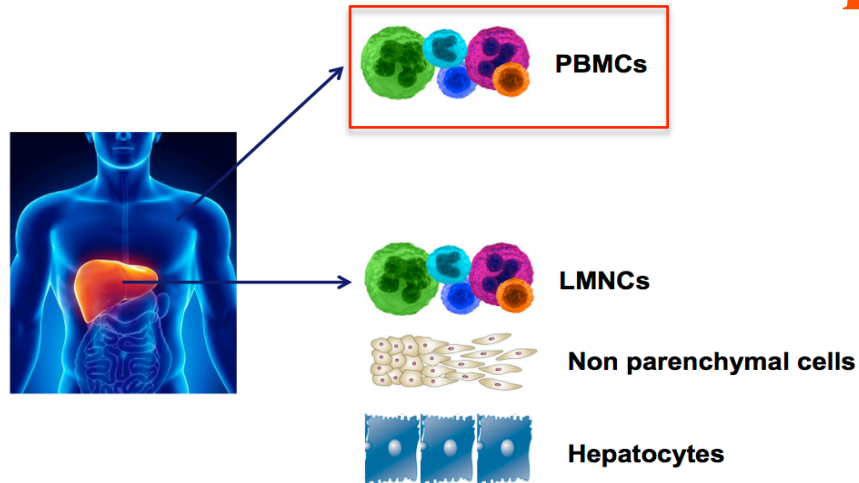
Serum HBV-DNA >20,000 IU/ml and ALT >N (n=52)
Serum HBV-DNA <20,000 IU/ml and/or ALT <N (n=53)



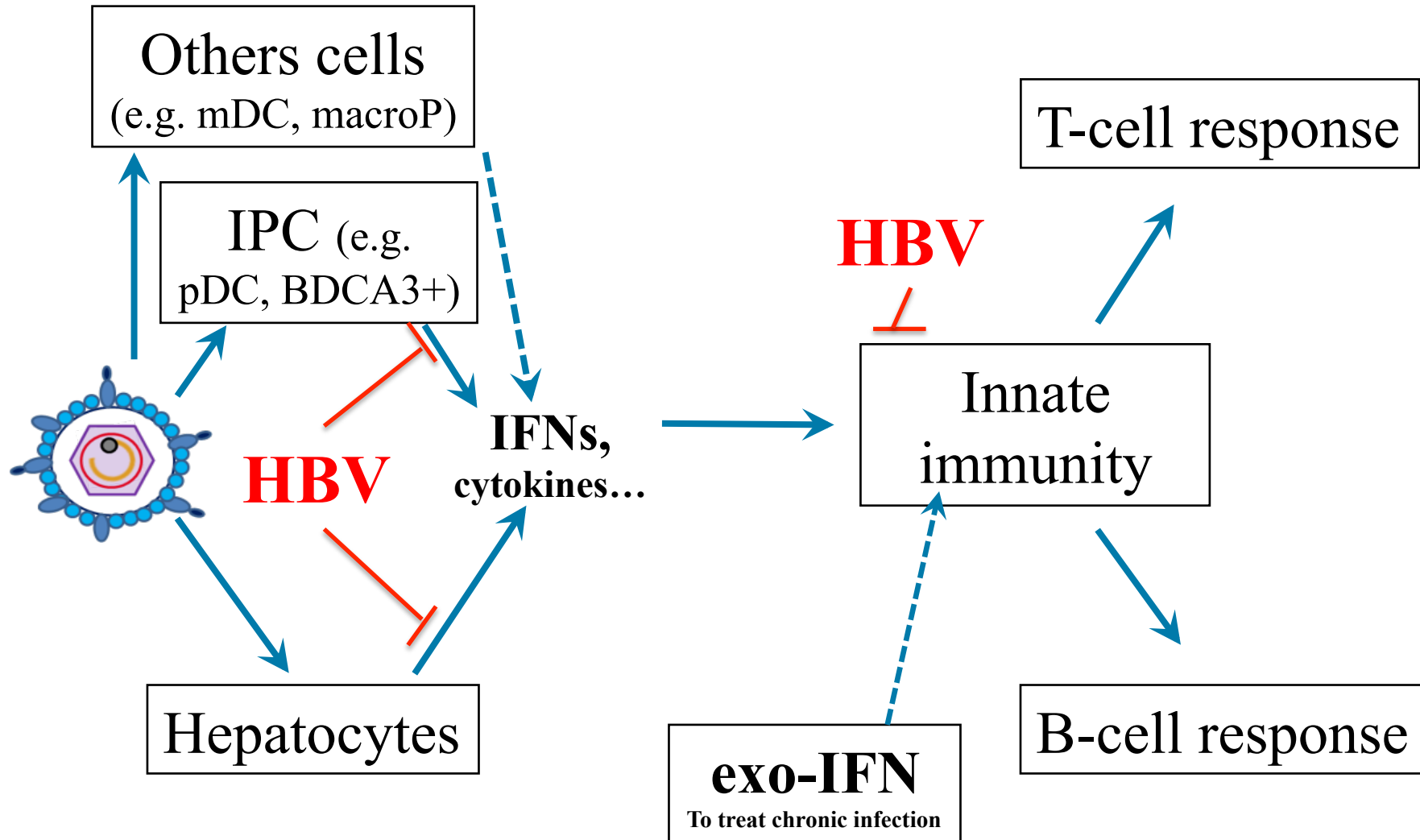
HBV inhibit TLR2 expression/function in liver of chronically infected patients



HBV inhibit TLR9 expression in PBMC of CHB patients



HBV impairs innate immune functions in CHB patients: yet a lot to discover...



Dusheiko G., *Liver Int.*, 2013; Han et al., *Semin Immunopathol.*, 2013; Bertoletti A, Ferrari C., *Gut*, 2012; Chang et al., *Antiviral Res.*, 2012; Lütgehetmann et al., *Gastroenterology*, 2011; Dunn et al., *Gastroenterology* 2009; Stacey et al., *JVI*, 2009; Christen et al., *JVI*, 2007; and many others...

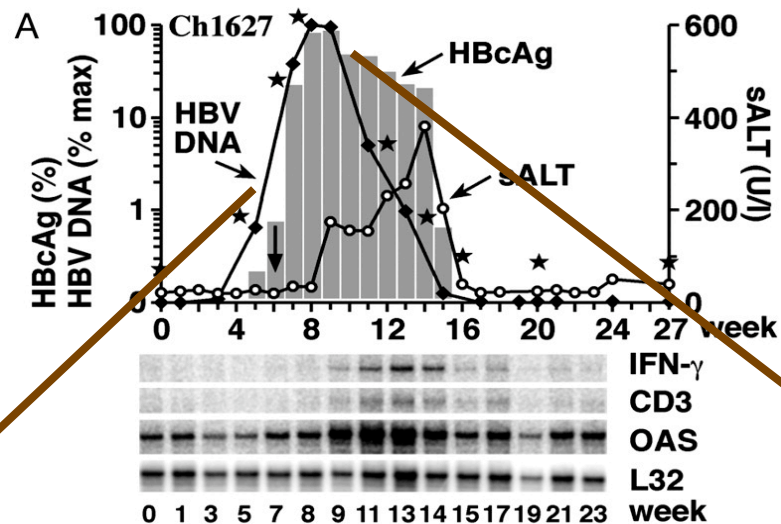
How, when and where to escape innate immunity? HBV has its own way...

- ✓ **Escape by being a « weak inducer »**
- ✓ **Have various strategies to escape**
- ✓ **Use viral protein included in « incoming virions »**
- ✓ **Be capable to impair functions of many cell types**

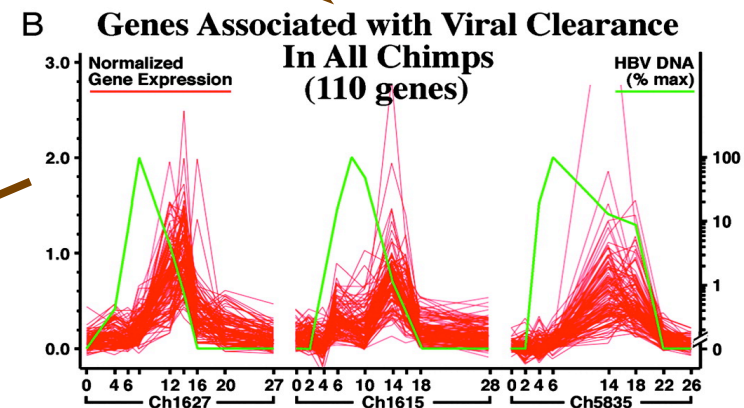
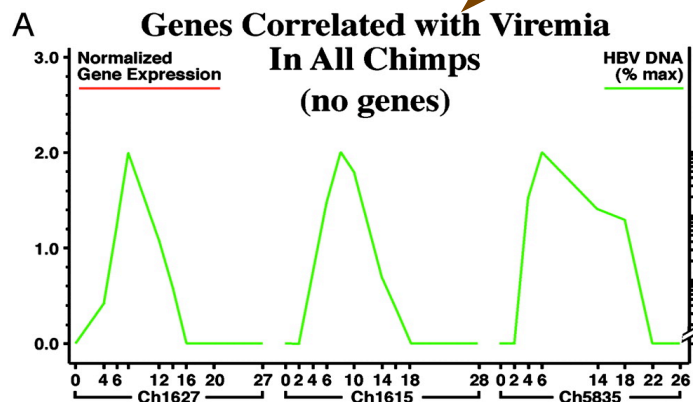
How, when and where to escape innate immunity? HBV has its own way...

- ✓ **Escape by being a « weak inducer »**
 - **No dramatic change in gene expression in acute infection in chimpanzees as compared to HCV or Hav infection (Wieland et al., PNAS 2004; Wieland & Chisari, JVI 2005)**

The concept of a « stealthy » virus



10^{exp8} equi HBV genome/animal



Stealth virus?

OR

Virus capable to impair innate responses

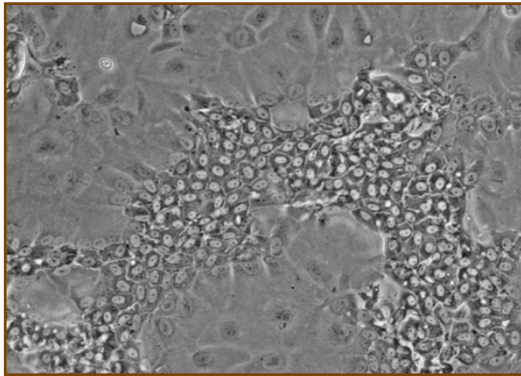
How, when and where to escape innate immunity? HBV has its own way...

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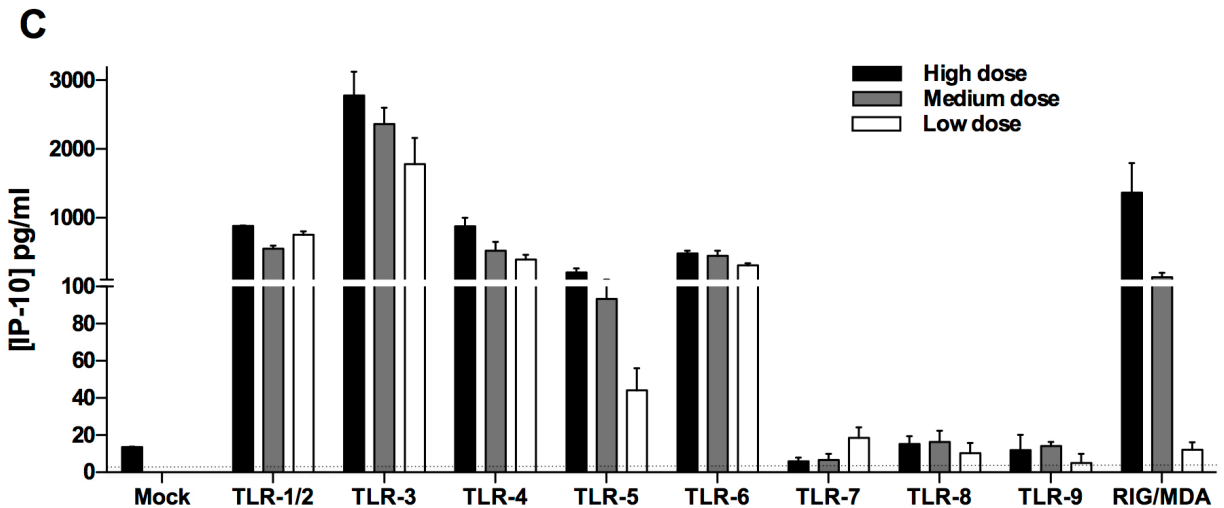
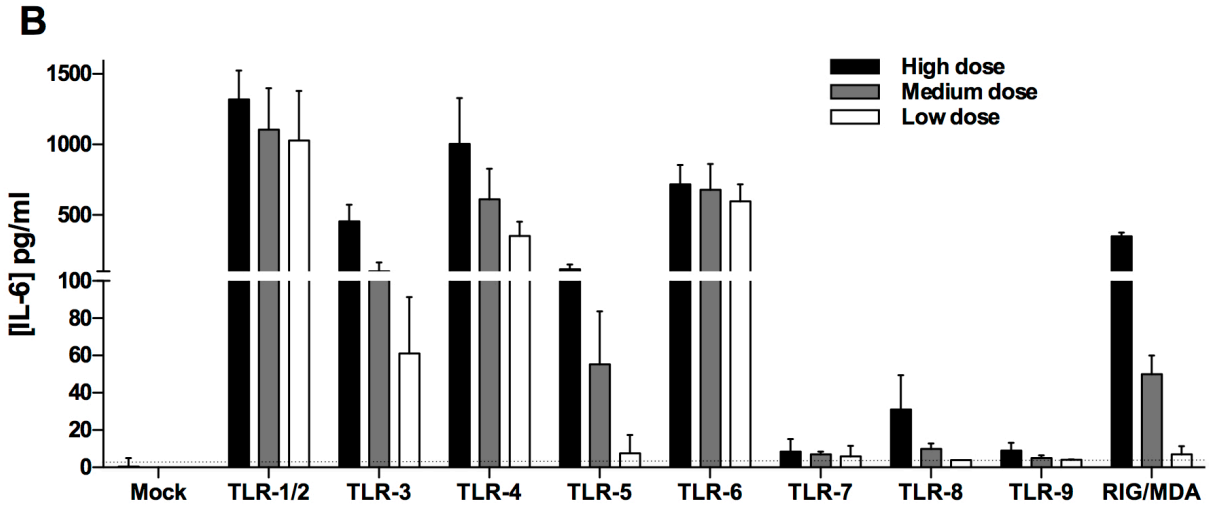
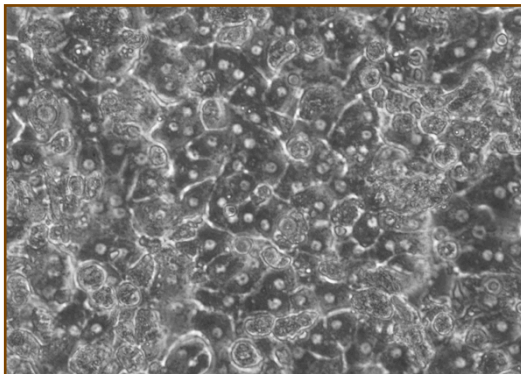
- No dramatic change in gene expression in acute infection in chimpanzees as compared to HCV or Hav infection (Wieland et al., PNAS 2004; Wieland & Chisari, JVI 2005)
- No systemic production of cytokines during acute infection (in contrast to HIV and HCV infections) (Stacey et al., JVI 2009)
- No (or very weak) production of IFNs and proinflammatory cytokines during *in vitro* infection (Hosel et al., Hepatology 2009; Lucifora et al., Hepatology 2010, Luangsay et al., J. Hepatol. 2015b etc...)

Models used: primary or non-transformed, immune competent, hepatic cells

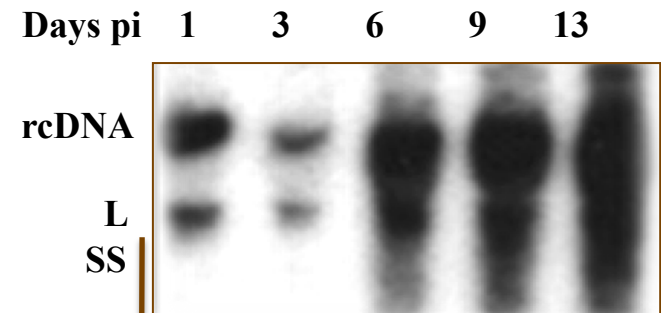
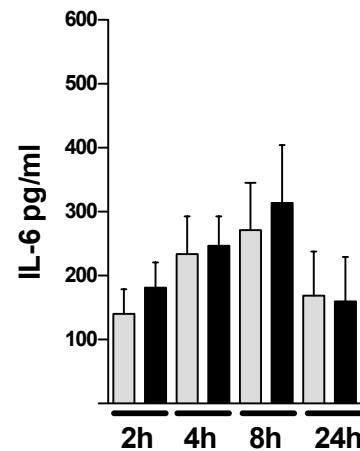
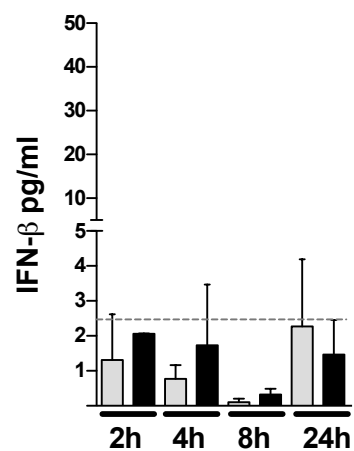
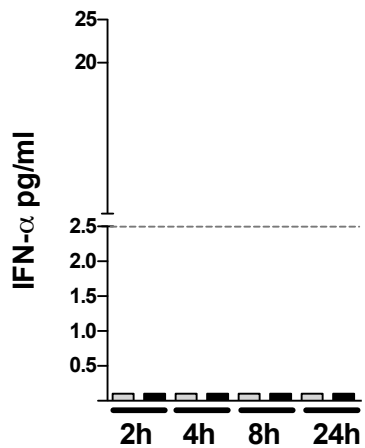
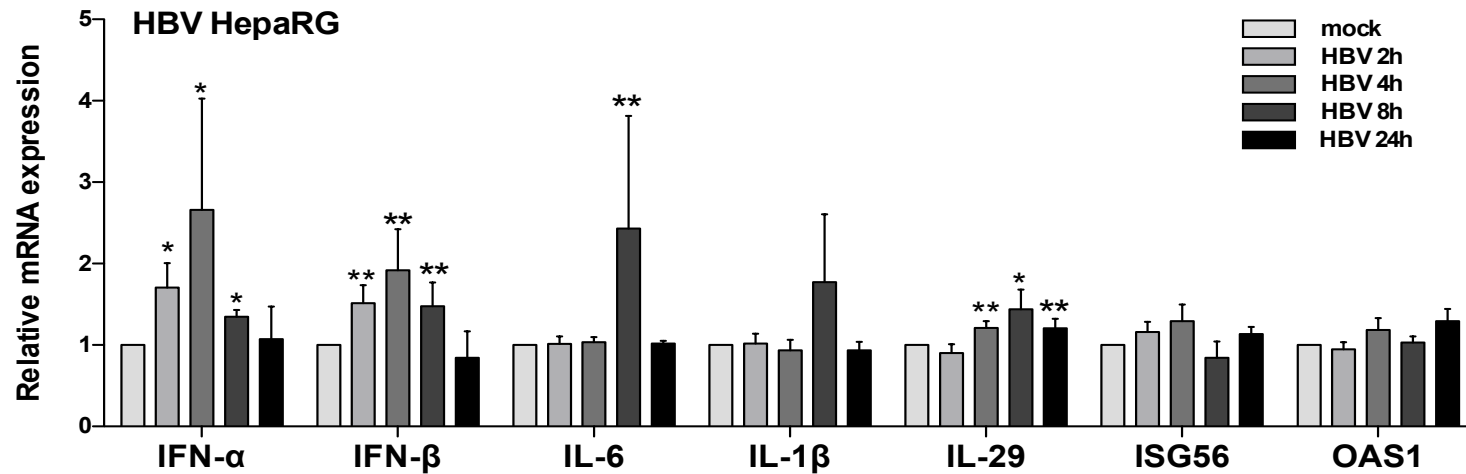
HepaRG differentiated



PHH

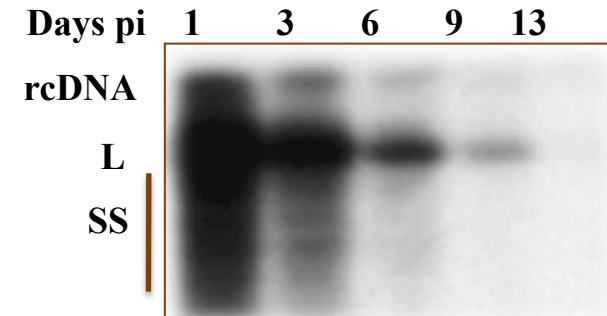
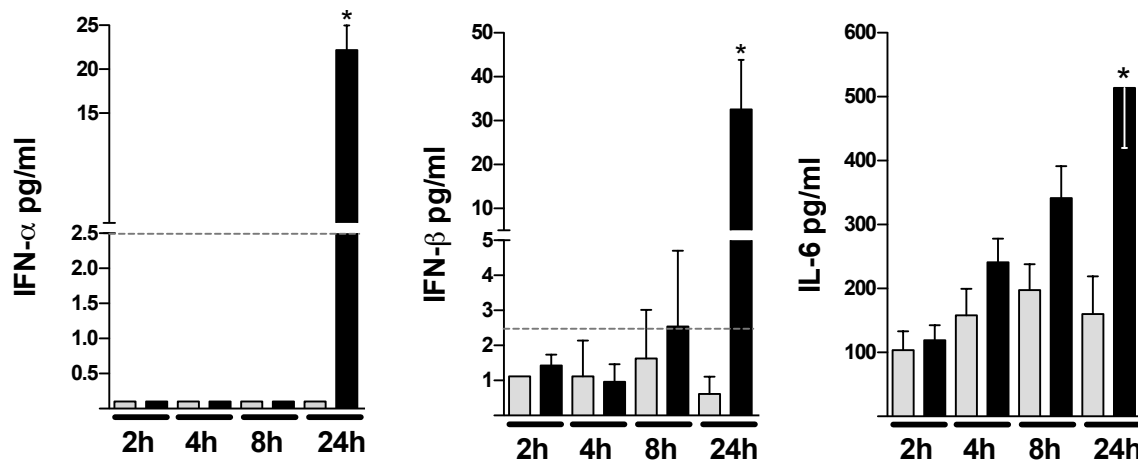
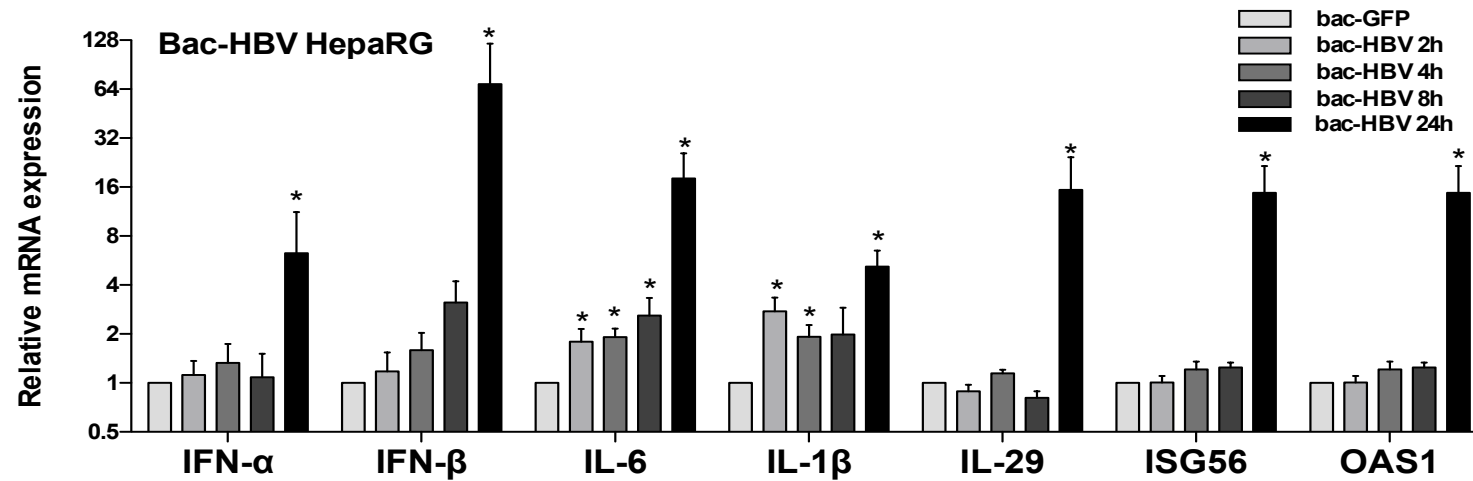


HBV: a weak inducer and likely inhibitor of IFN responses in hepatocytes (our *in vitro* data)



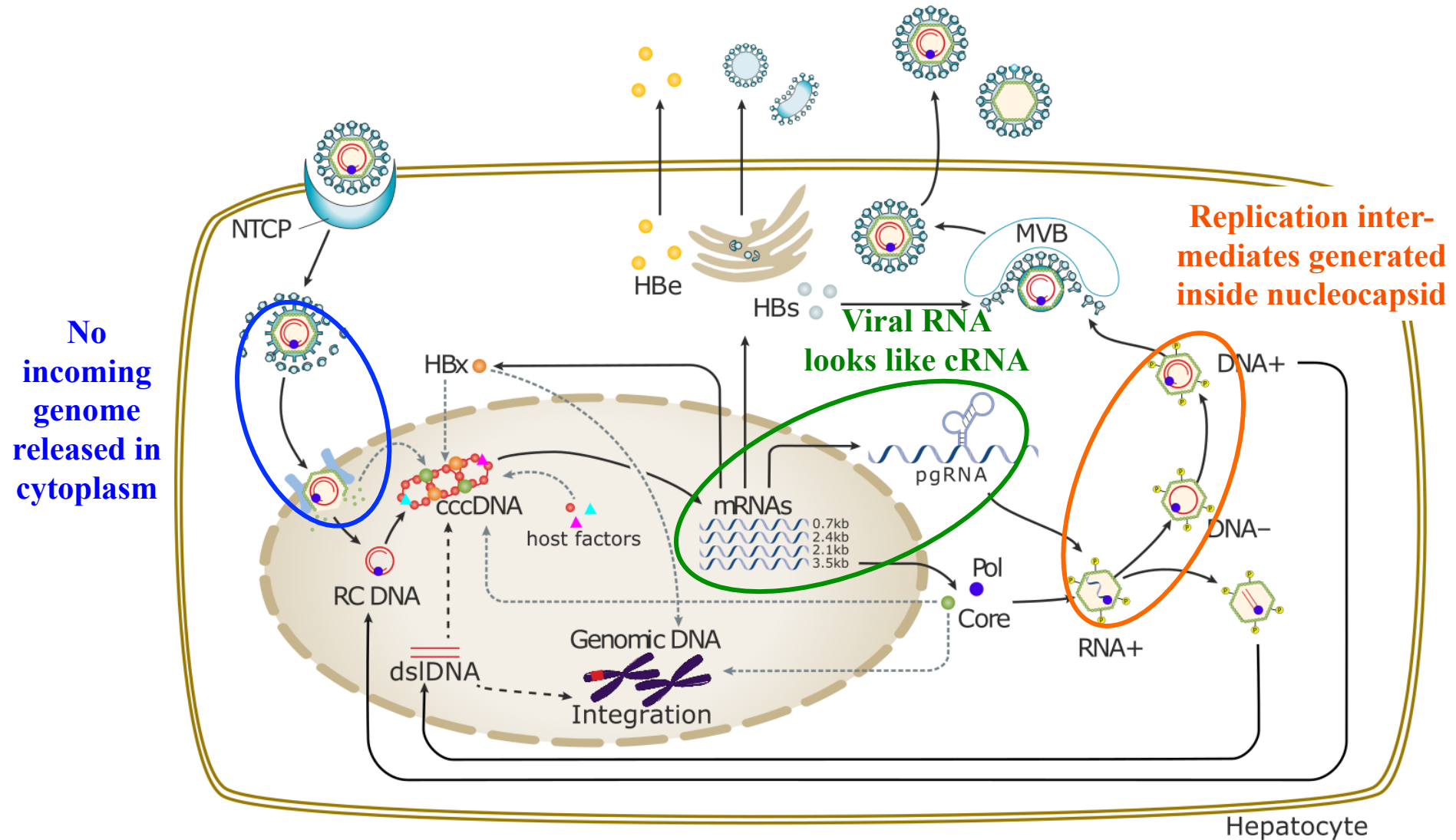
Luangsay et al., *J Hepatol* 2015a and 2015b;
Lucifora, Durantel et al., *Hepatology* 2010

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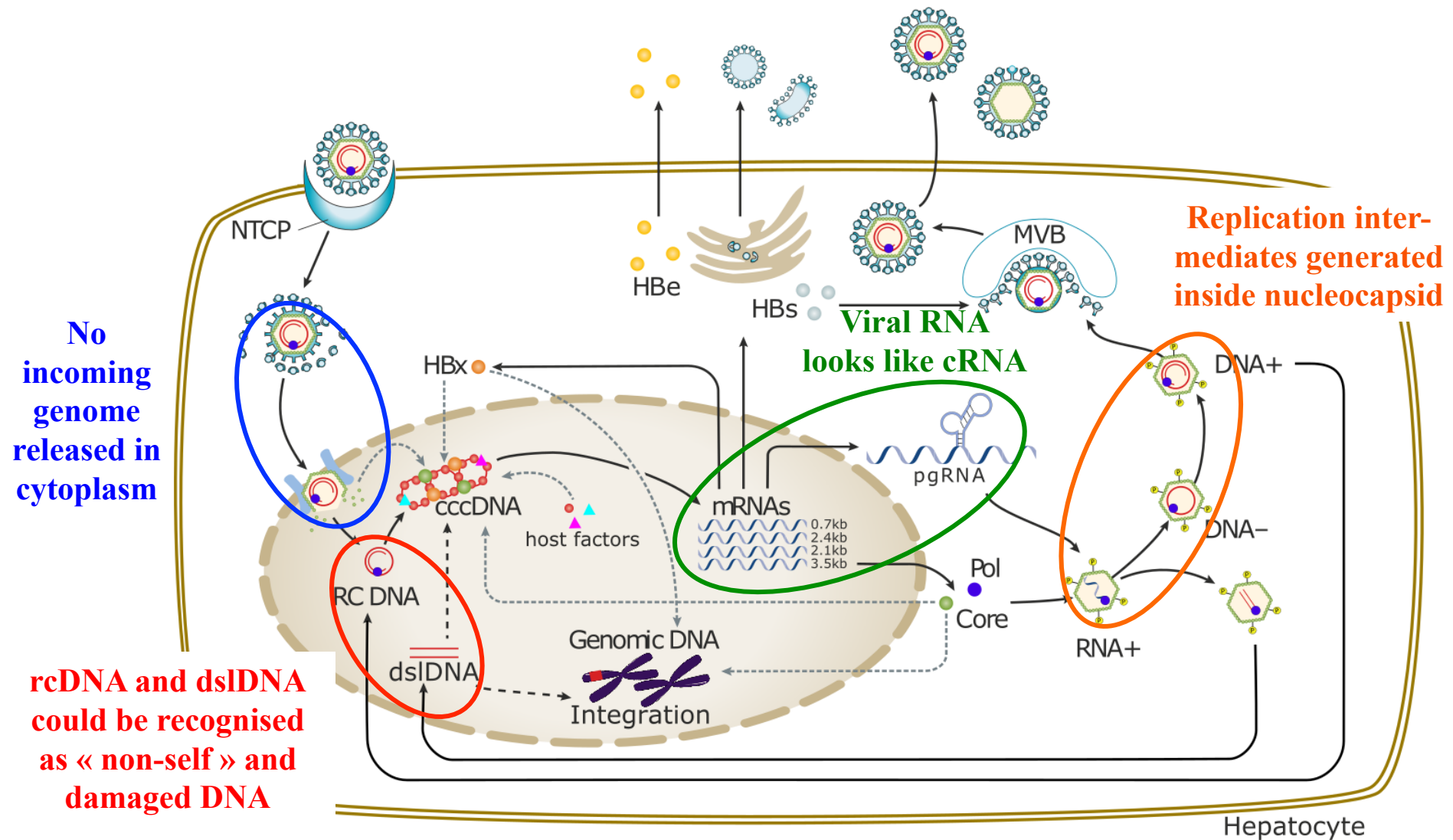


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Hide away your genome and replicative intermediates !



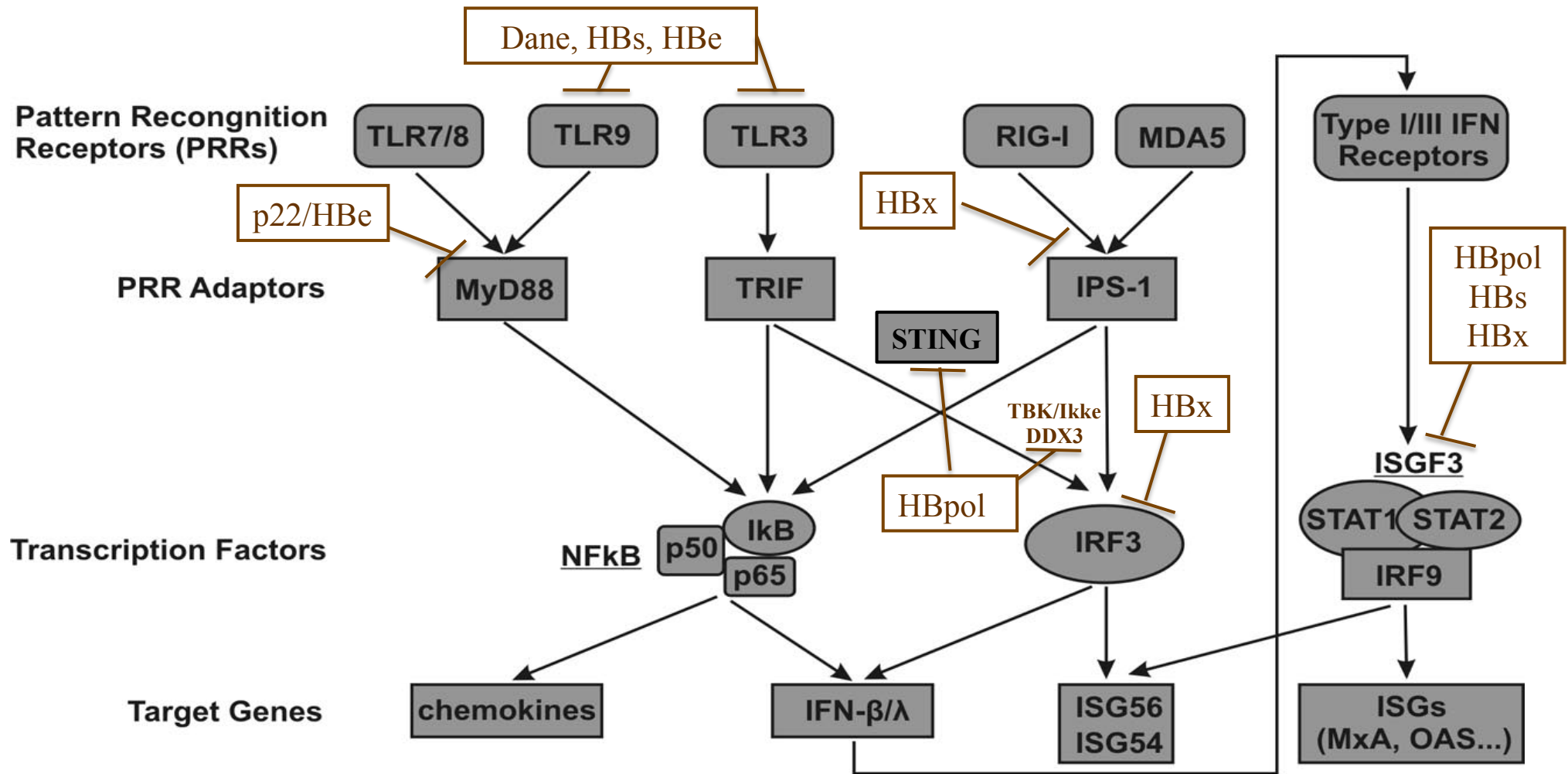
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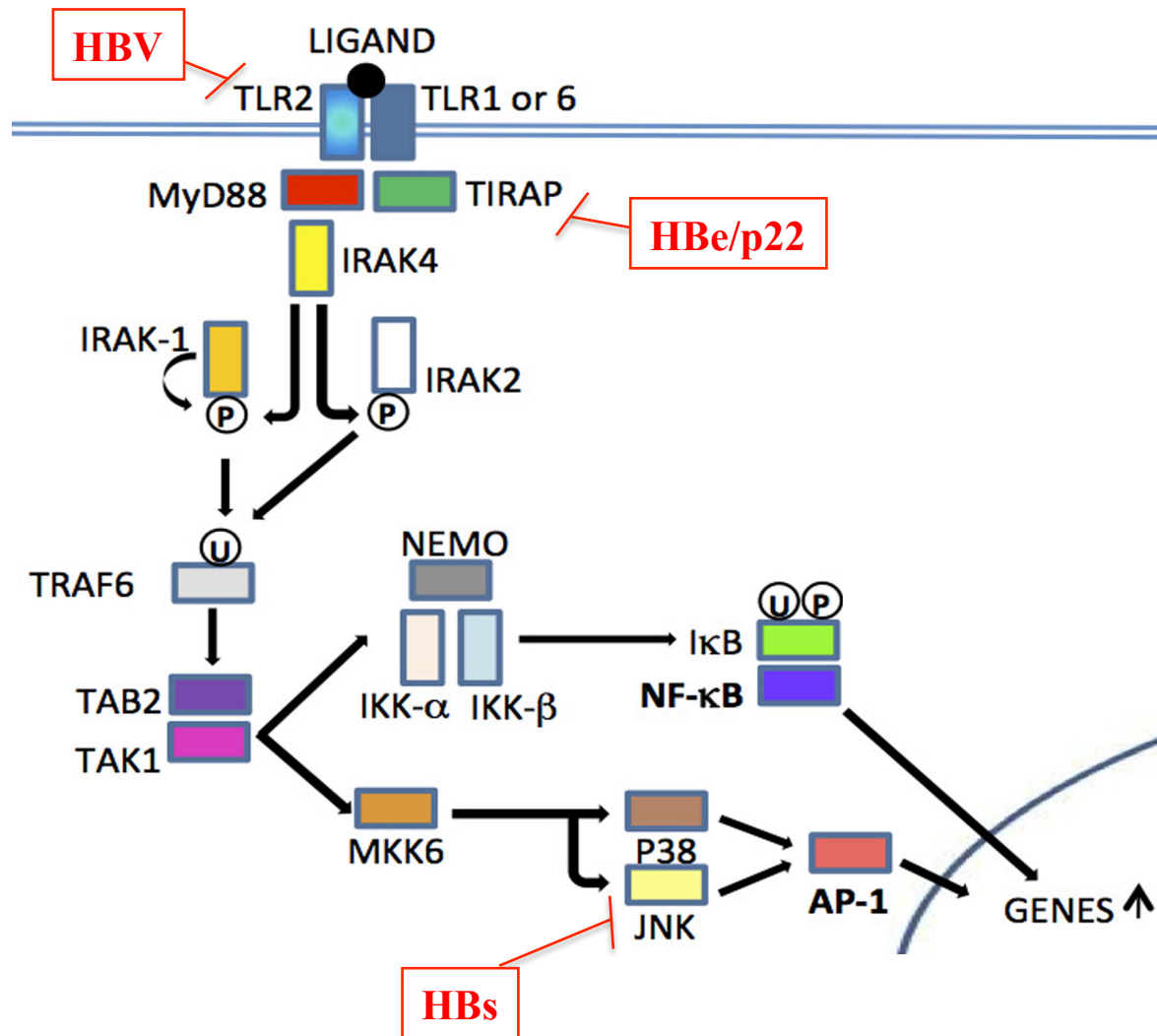
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Evasion by interference with sub-cellular innate immunity: role of HBV viral proteins



Wu et al., *Hepatology* 2009; Wang and Ryu, *PlosPath.* 2010; Wei et al., *J. Immunol.* 2010; Yu et al., *JGV* 2010; Kumar et al., *J Virol.* 2011; Jiang and Tang, *Protein & Cell* 2011; Chang et al., *Antiviral Res.* 2012; Wang et al., *J Immunol.* 2013

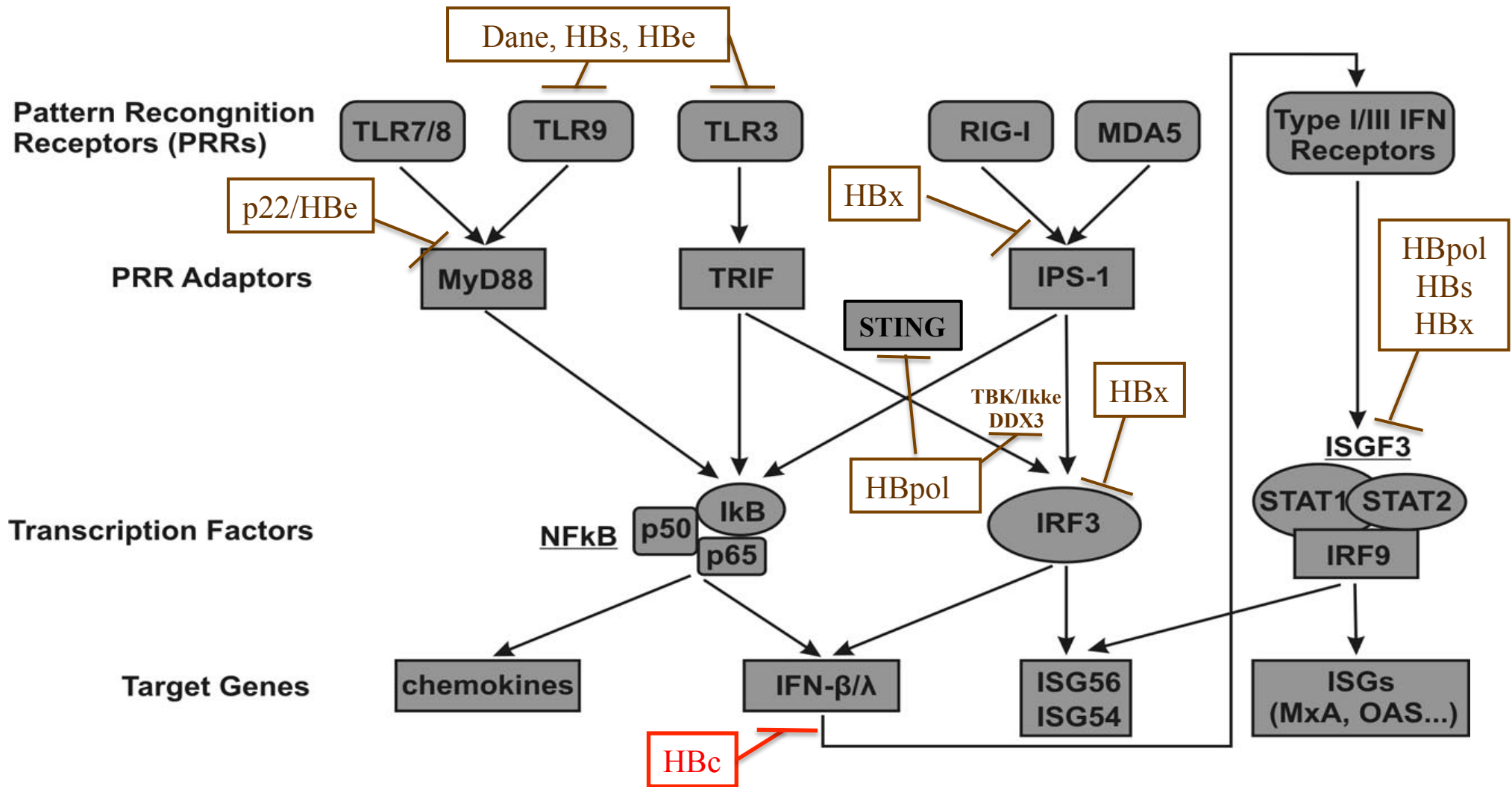
HBV inhibit TLR2 expression/function in chronically infected patients



- ✓ HBs and HBe/p22 would be involved in Inhibition of TLR2 functions
- ✓ HBe/p22 would block TLR2 signaling pathways by binding to Mal/TRAM
- ✓ HBs would act through JNK inhibition to prevent IL-12 production

Visvanathan et al., *Hepatology* 2007; Lang et al., *J. Hepatol.* 2011; Wang et al., *J. immunol.* 2013

Evasion by interference with sub-cellular innate immunity: role of HBV viral proteins

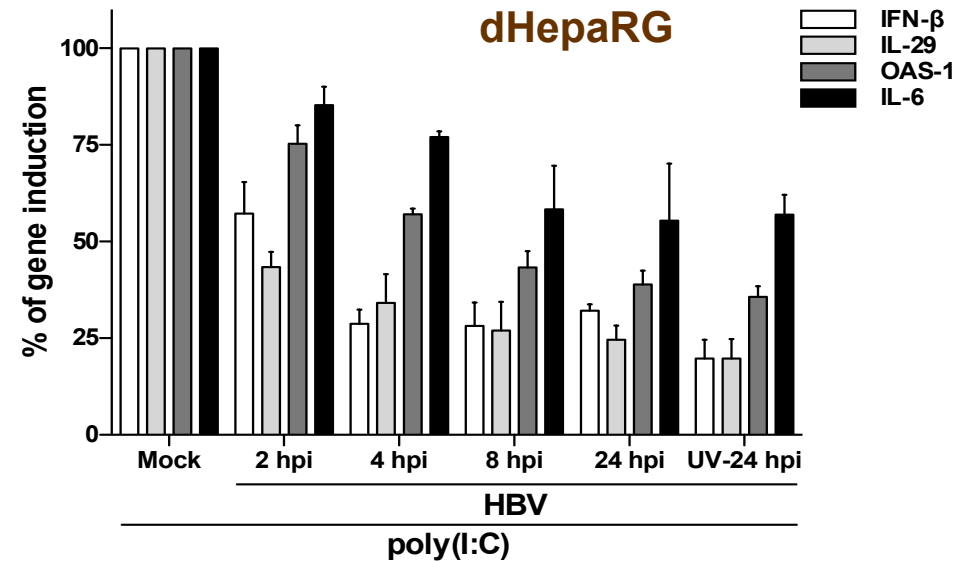
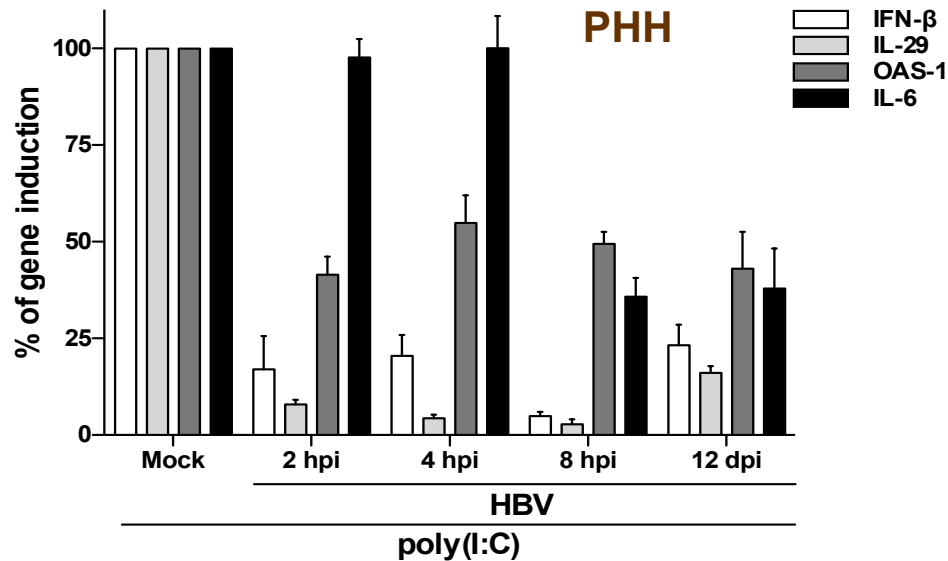
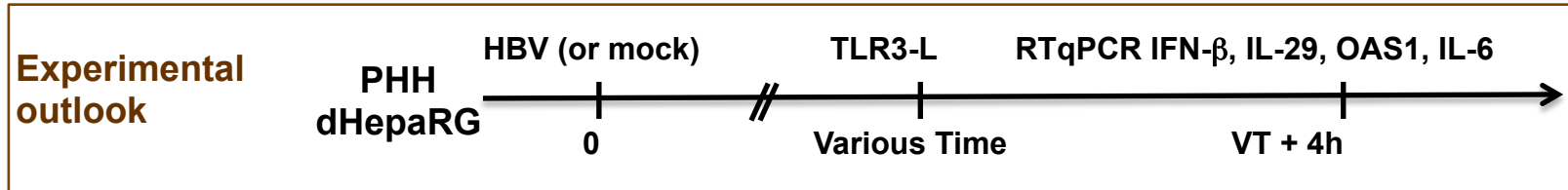


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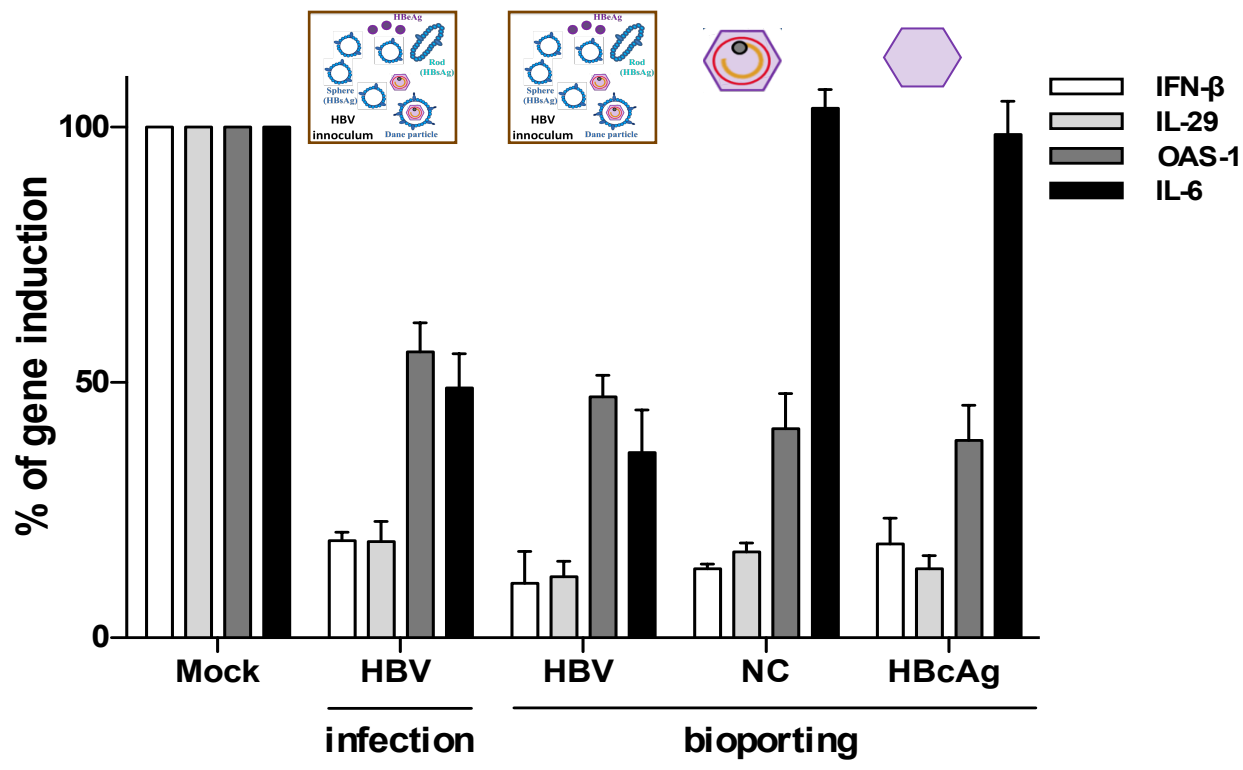
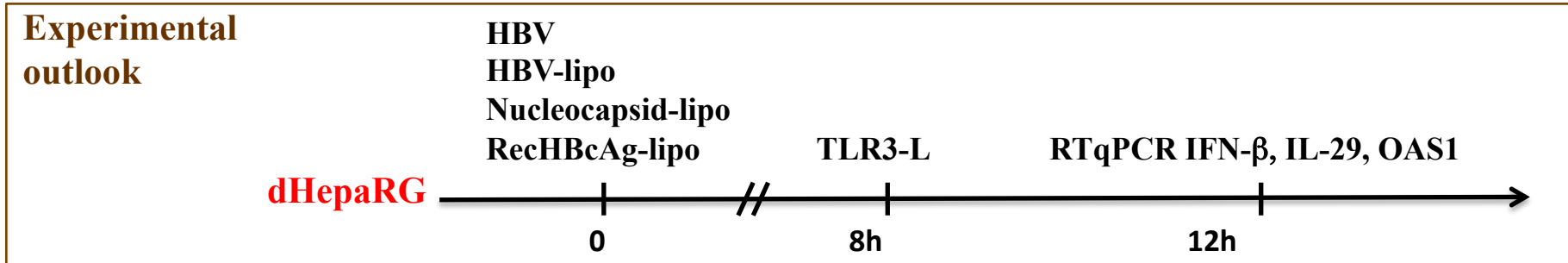
Inhibition of dsRNA-mediated IFN response occurs very early after infection of PHH by HBV (and is long lasting)



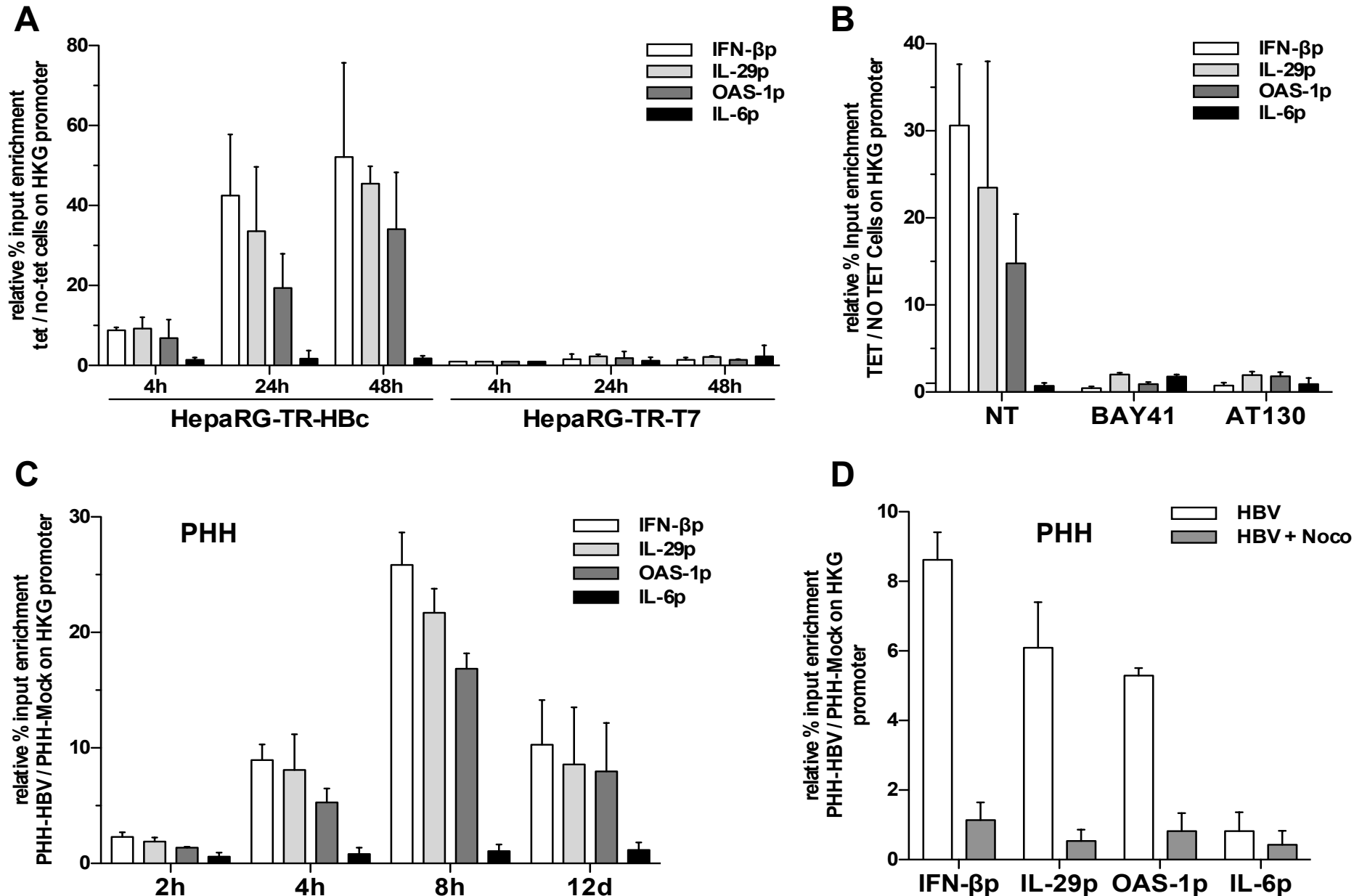
- ✓ Very early inhibition
- ✓ No need for protein neo-synthesis

- ✓ Long lasting inhibition

« Incoming » HBc is responsible for the early inhibition of dsRNA mediated IFN response in hepatocytes

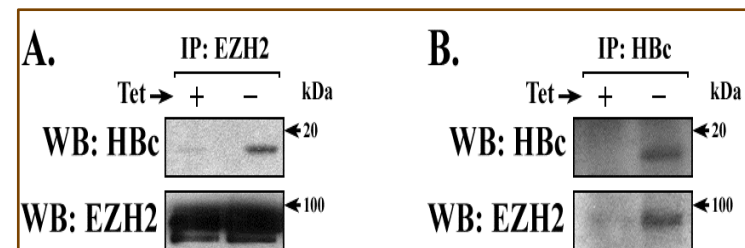
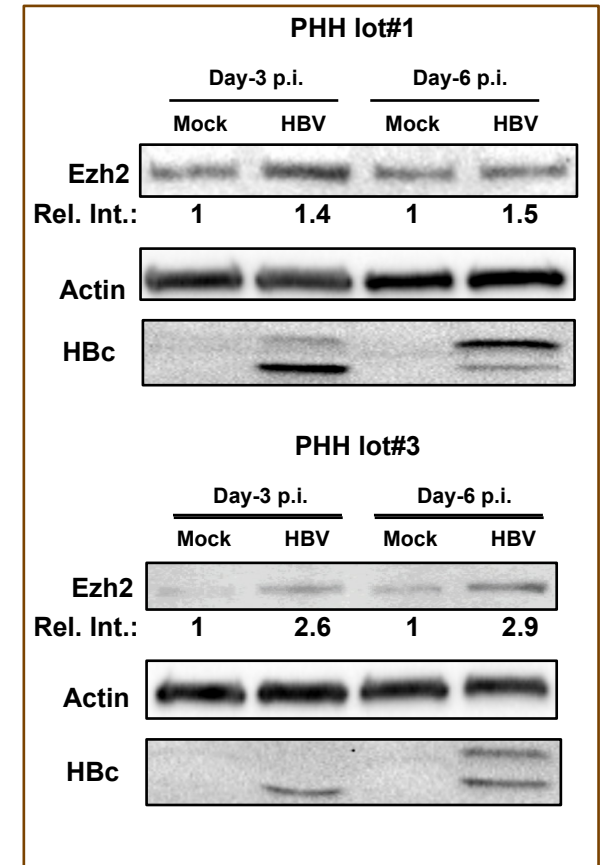
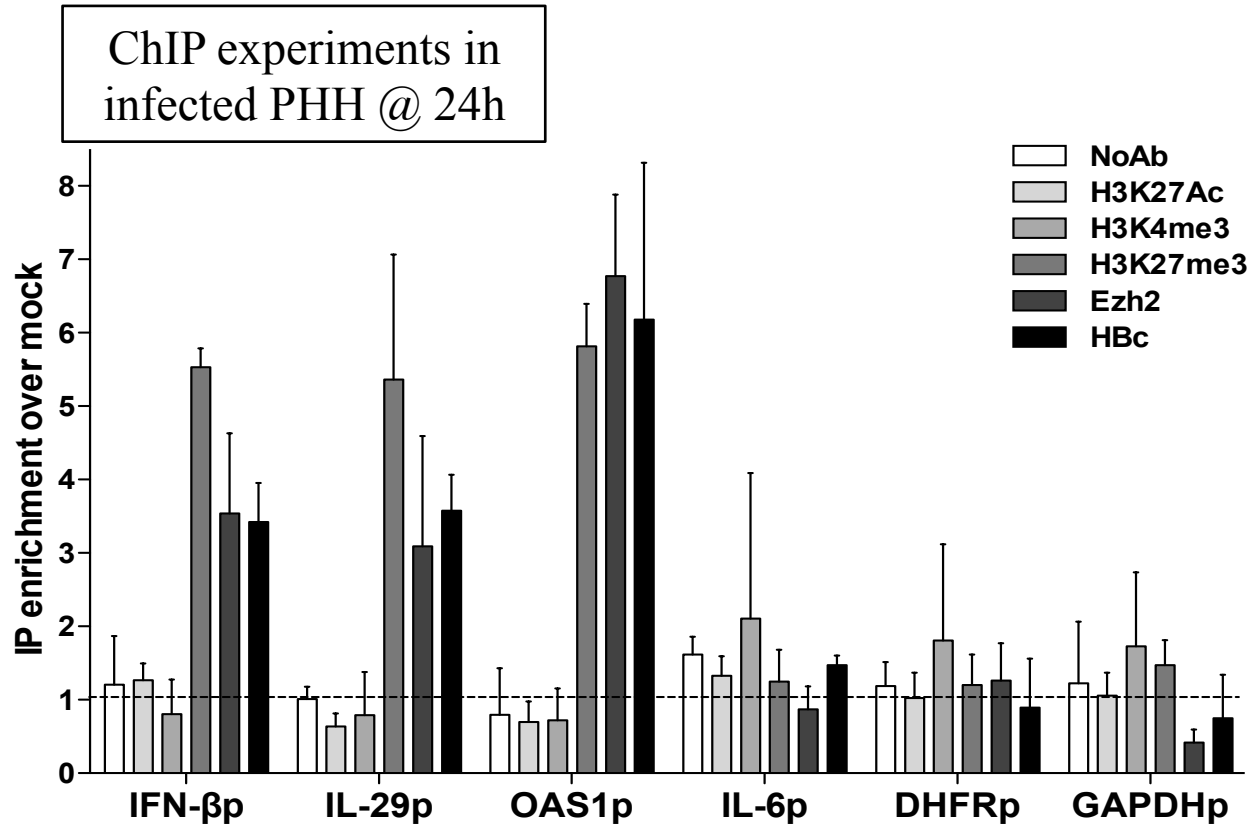


HBc binds to promoters of innate immunity genes



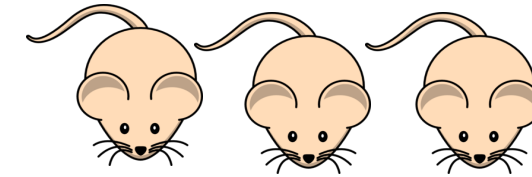
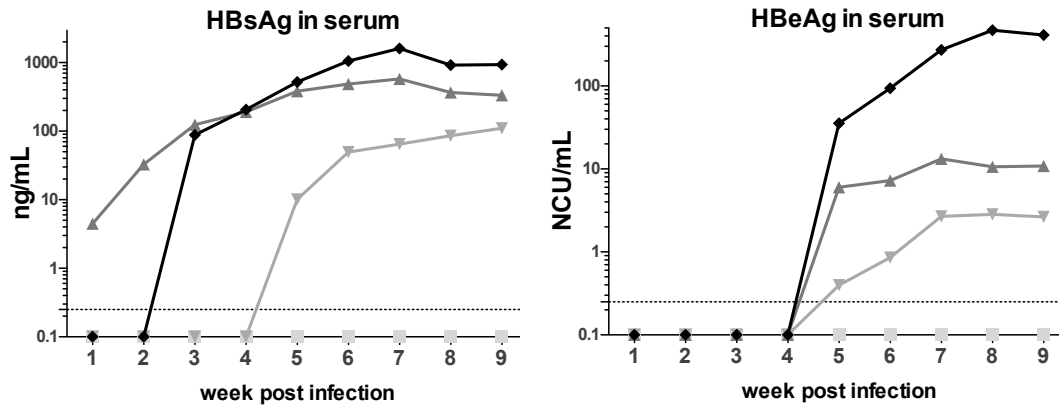
Gruffaz, Testoni et al., unpublished data

HBc recruits histone-methyl-transferases on target promoters leading to repressive epigenetic marks in HBV-infected PHH

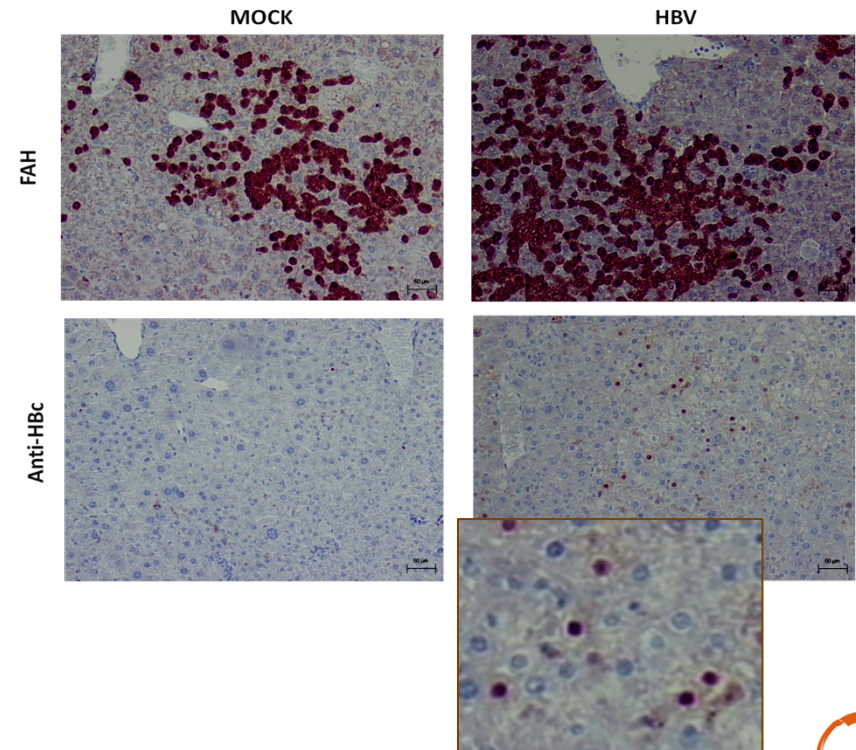
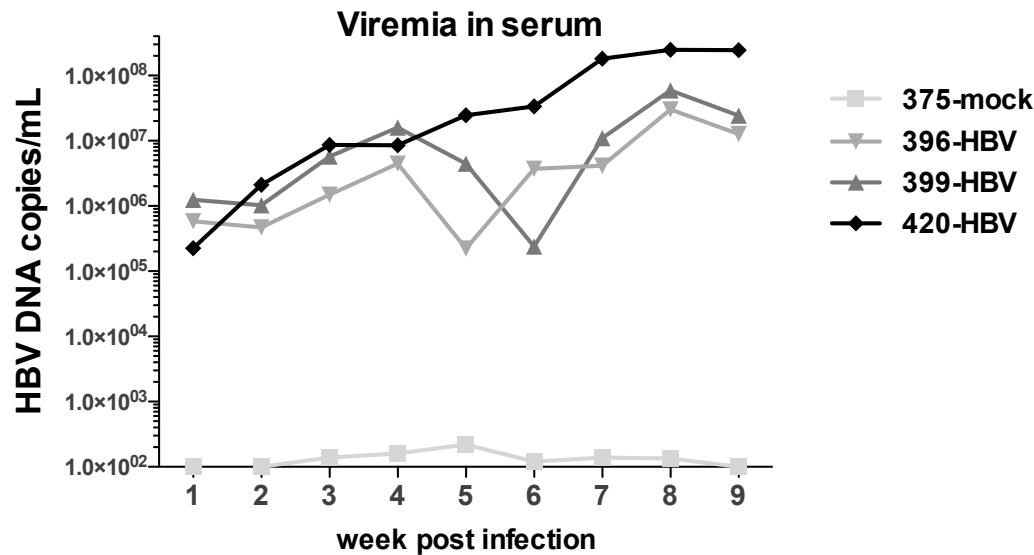


Gruffaz, Testoni et al., unpublished data

HBc remains bound to target promoters long after the onset of infection in (infected hepatocytes) and in HBV-infected liver-humanized mice

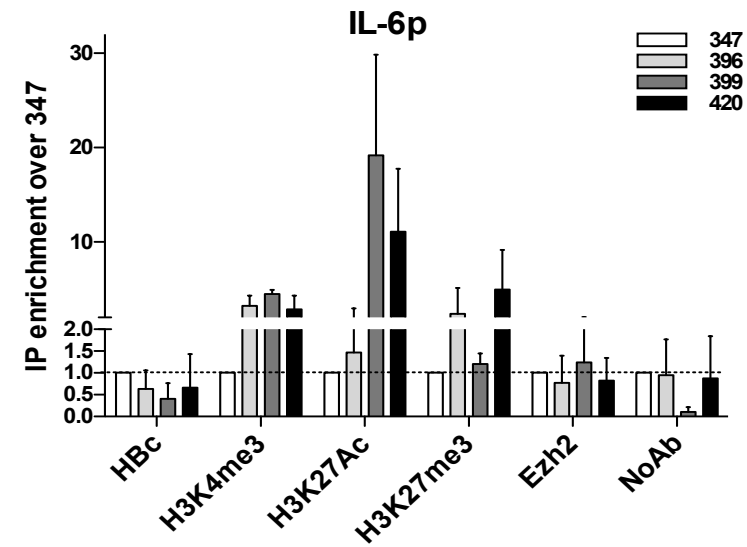
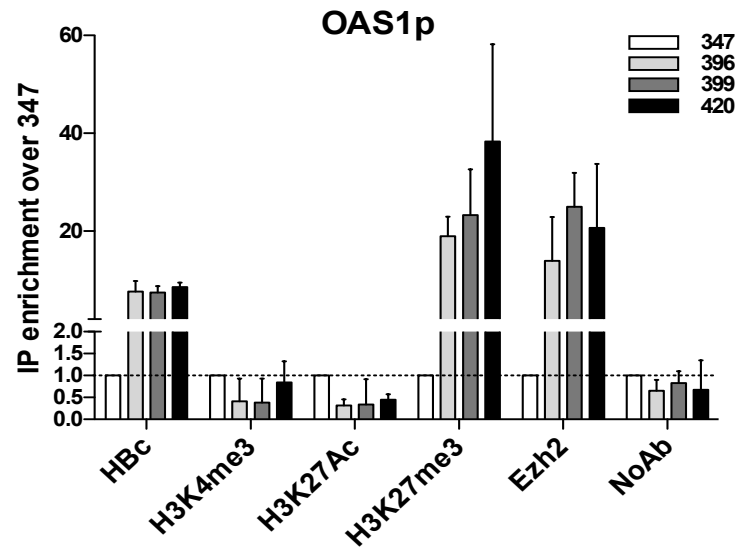
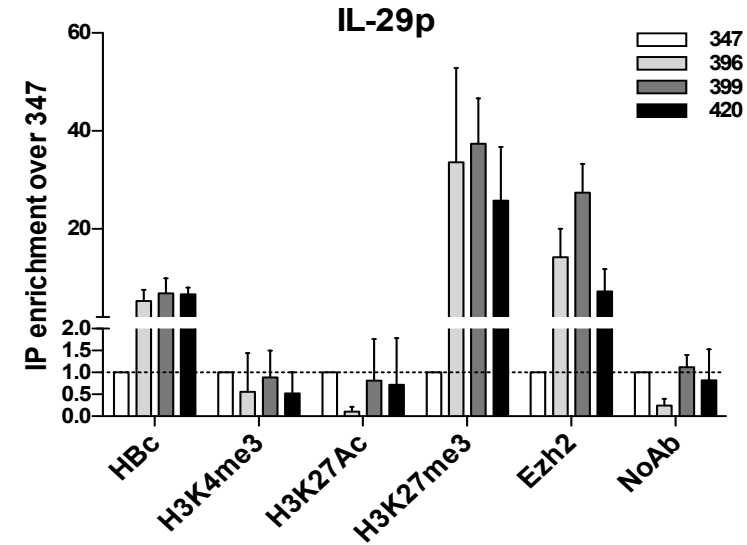
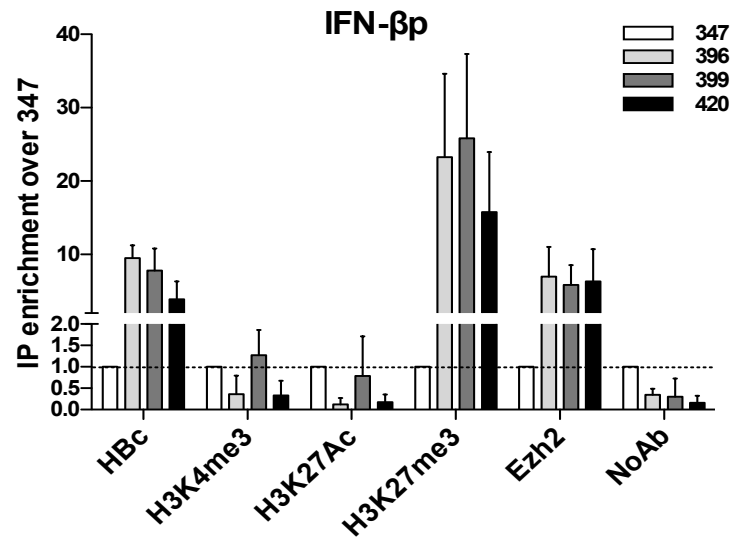


Exp. in liver humanized FRG mice



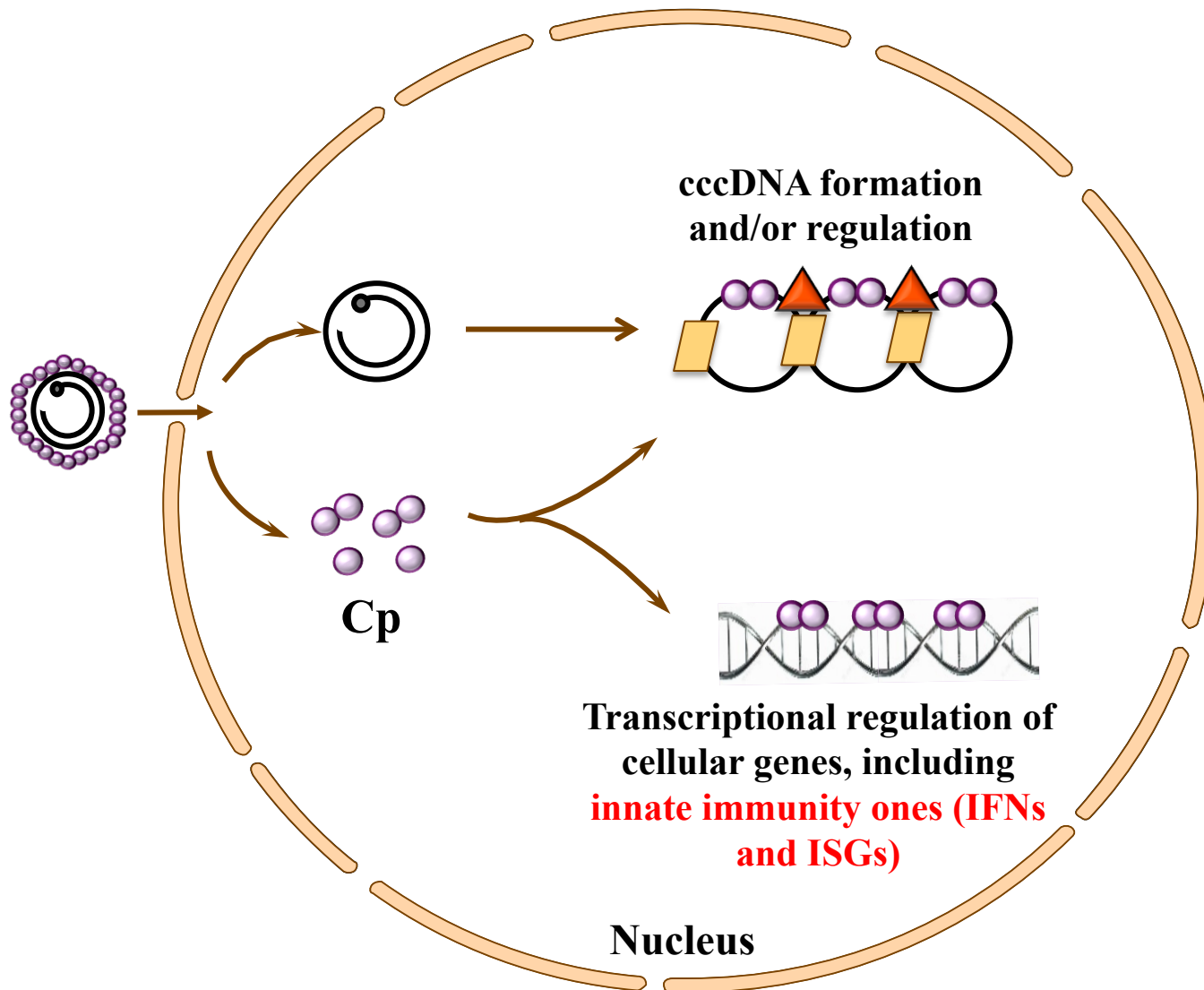
Data obtained thanks to a collaboration with the laboratory of Dr *François-Loïc Cosset*

HBc binds to innate immunity gene promoters in infected FRG mice



Gruffaz, Testoni et al., unpublished data

Nuclearly located HBC is responsible for inhibition of innate gene (IFN response) expression



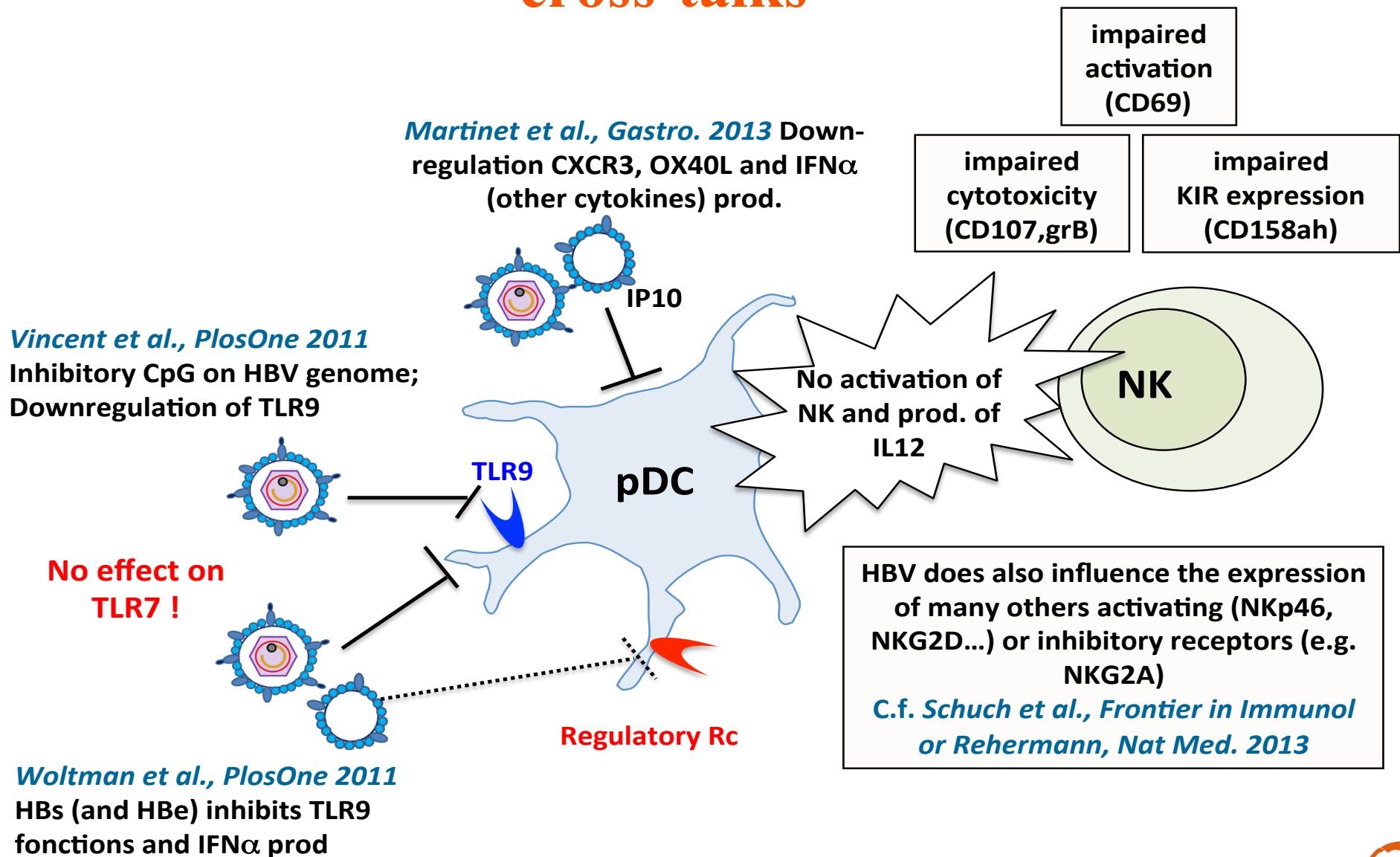
- ✓ Incoming (and recycled) HBC binds to innate immunity gene promoters
- ✓ HBC recruits EZH2 to these promoters
- ✓ EZH2 establishes epigenetic repressive marks (H3K27me3)
- ✓ Innate immunity genes are less inducible by IFN-inducing PRR ligands

How, when and where to escape innate immunity?

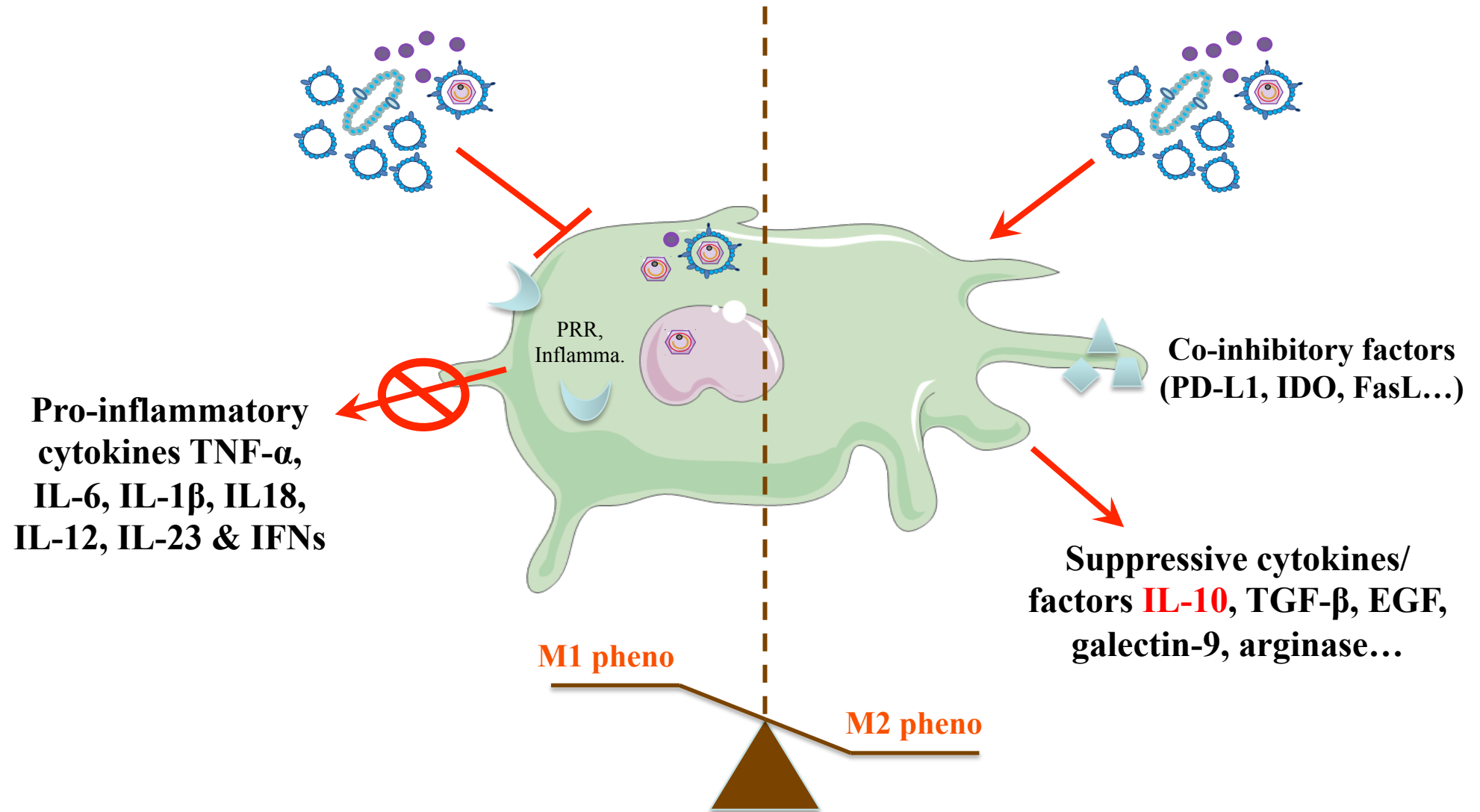
HBV has its own way...

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Alteration of pDC and NK/NKT functions & cross-talks

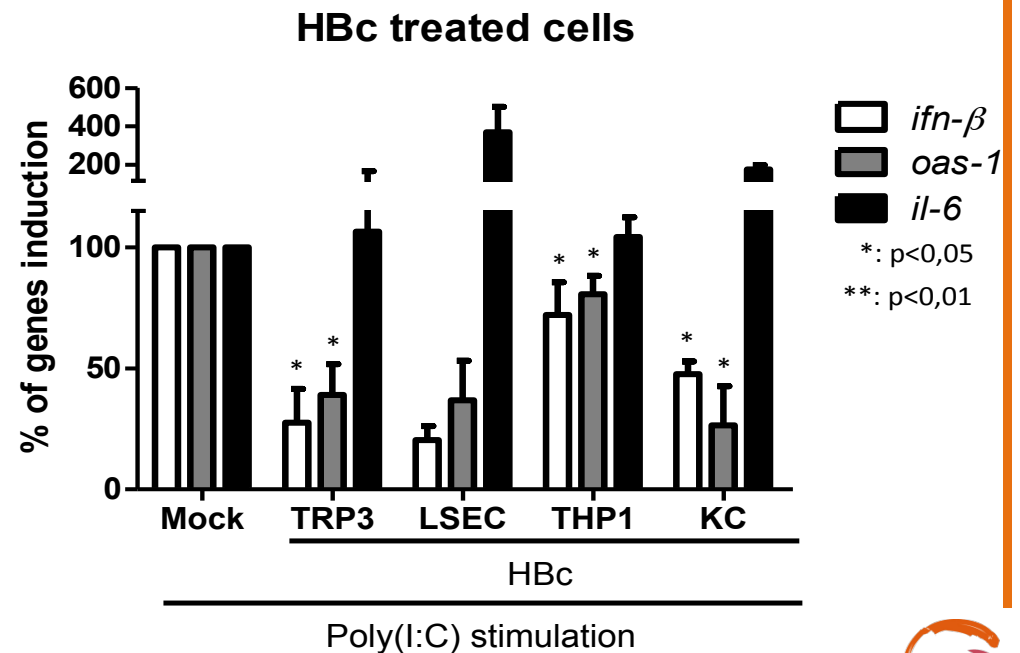
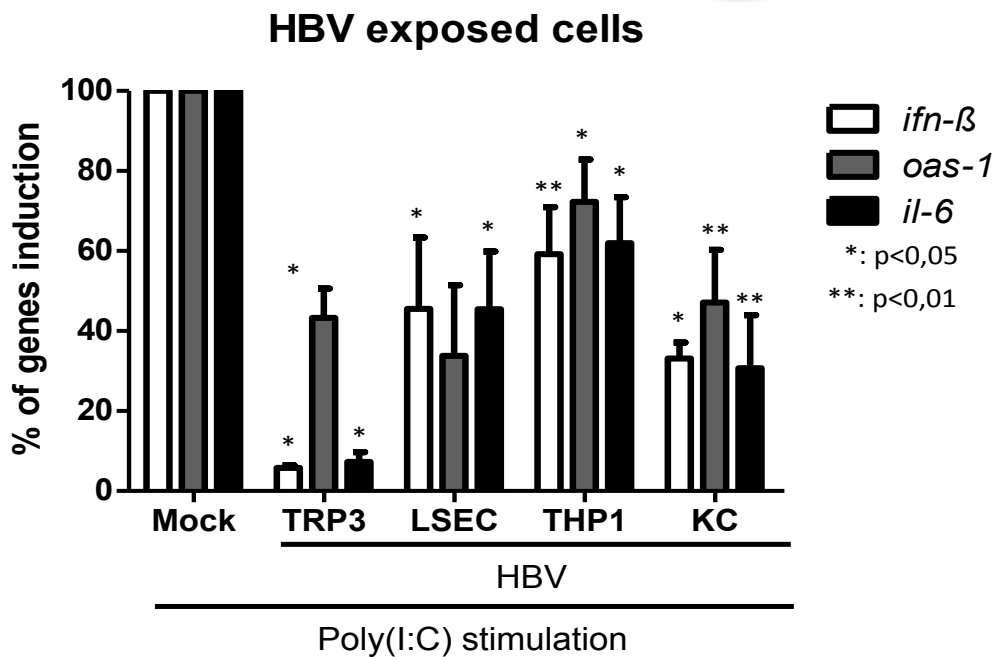
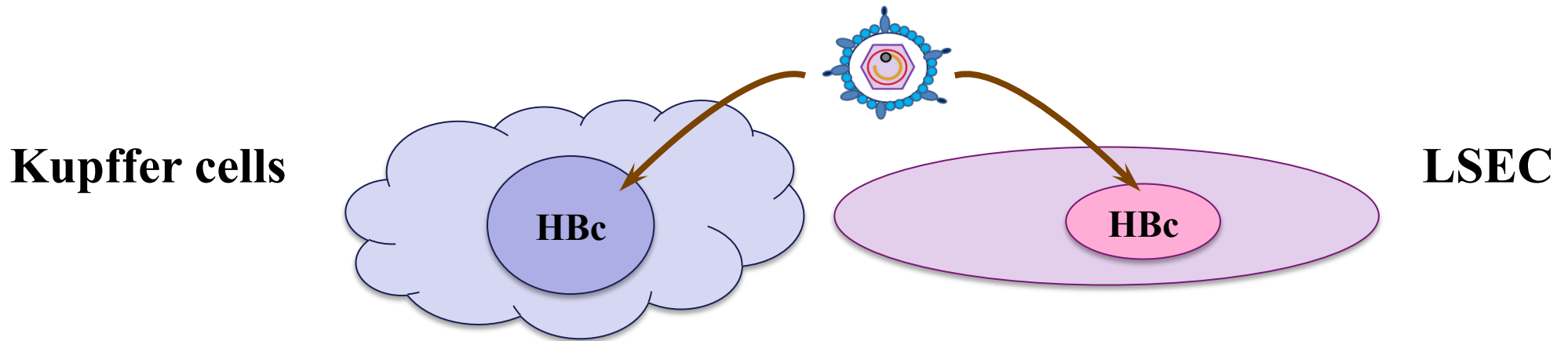


Modulation of liver macrophages (KC and other myeloid) by HBV

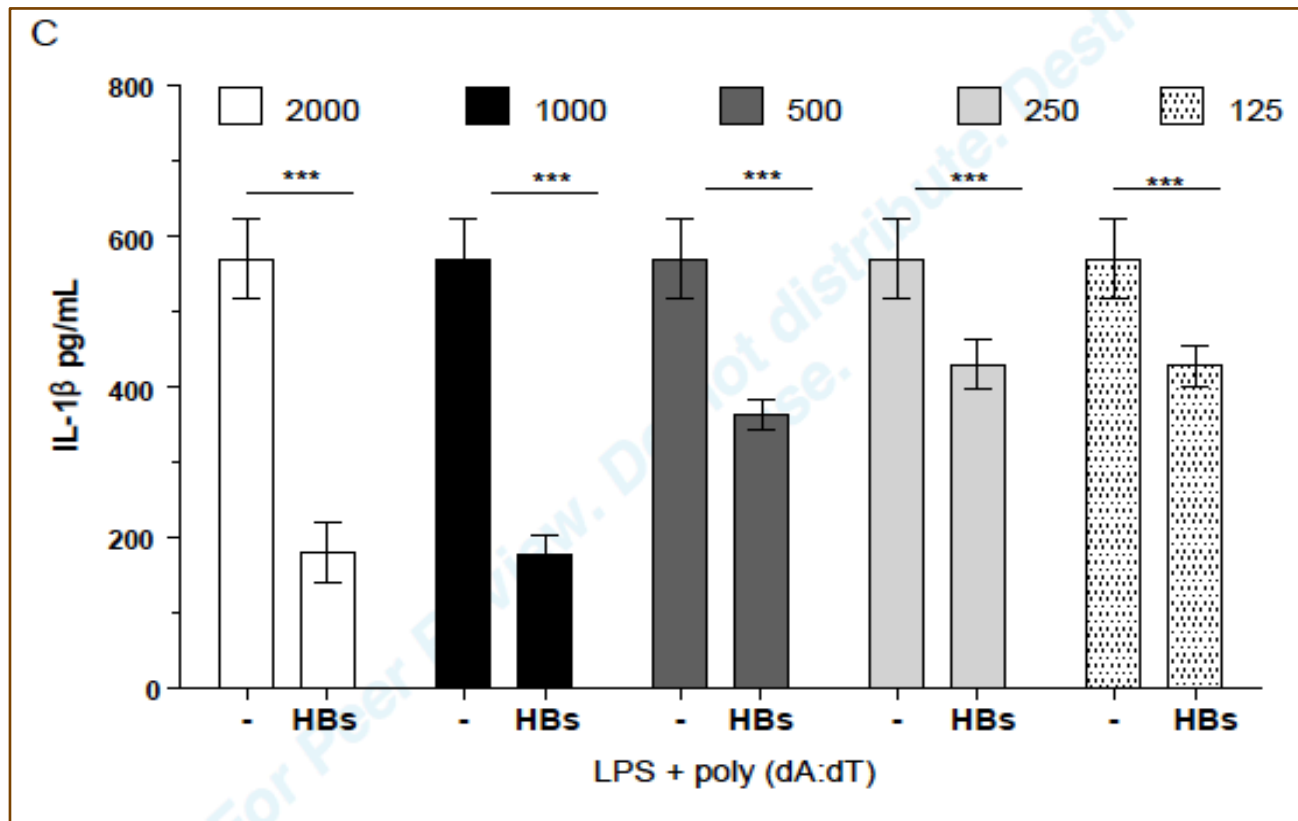


Xu et al., Hepatology 2014; Boljes et al., J Hepatol. 2014; Bility et al., PlosPath. 2014; Wang et al., J. Immunol. 2013; Wu et al. Hepatology 2009; Our unpublished work

HBc can be delivered to liver immune cells (KC and LSEC) to inhibit IFN response



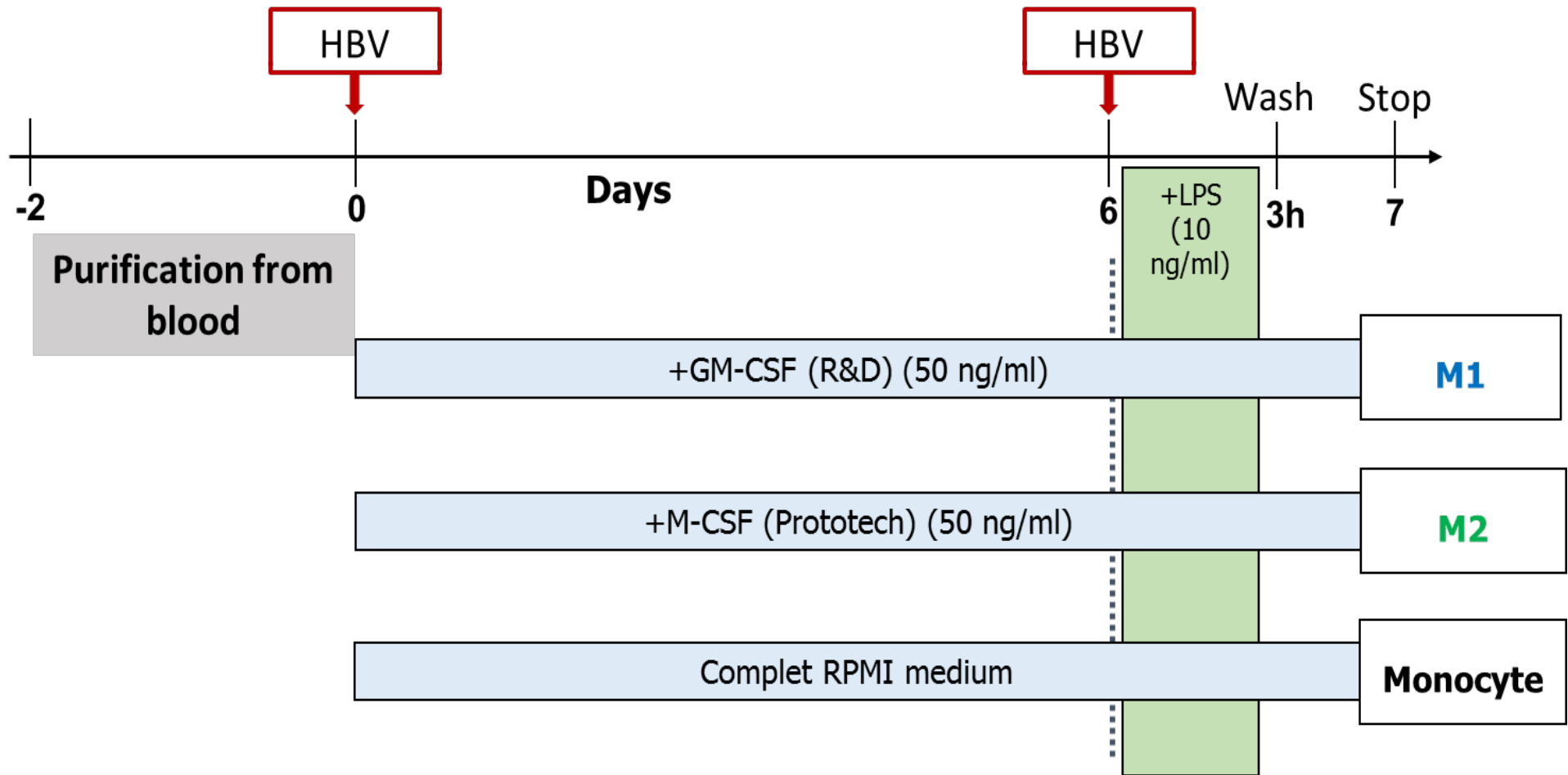
HBV inhibit also secretion of IL-1 β in KC and in monocytes-derived macrophages...



- ✓ HBV inhibit Aim2-mediated IL-1 β secretion
- ✓ Dane particles and HBs are responsible for this inhibition
- ✓ Short exposure to HBV is necessary to get this inhibition
- ✓ HBsAg is involved in the inhibitory phenotype
- ✓ Role of HBc delivery to cell: HBc induce reduction of AIM2 expression (data not shown)

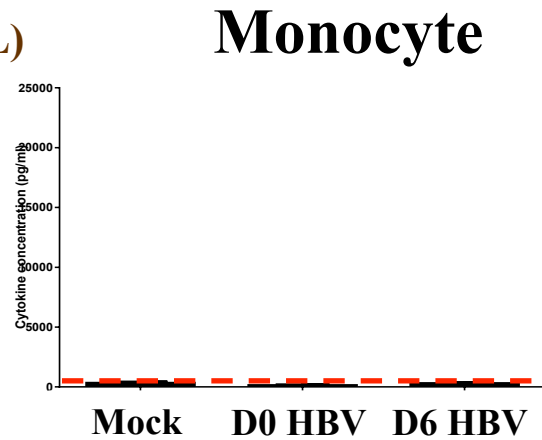
Effect of HBV on M1/ M2 differentiation?

Effect of HBV on M1/M2 activation?

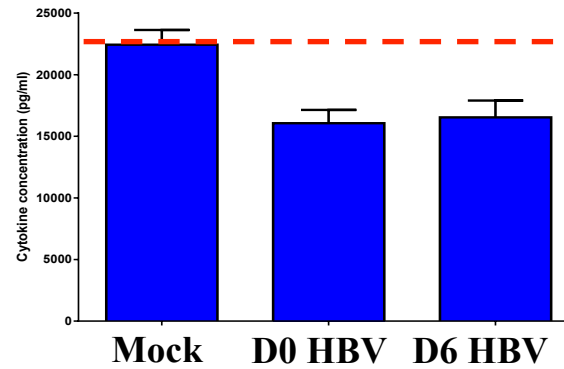


... and promote IL-10 secretion

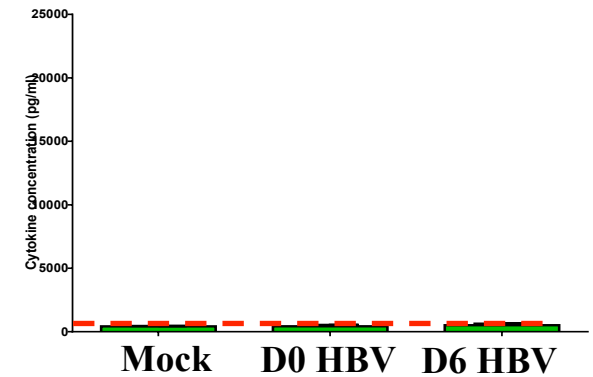
IL-1 β
(pg/mL)



M1

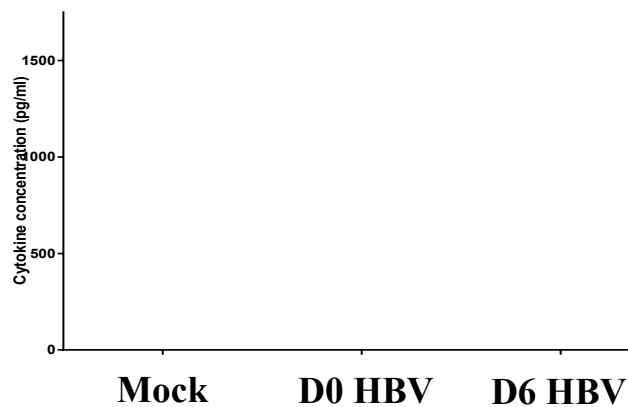


M2

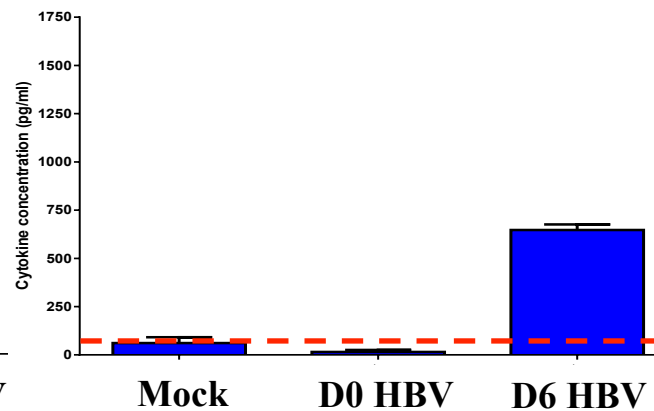


IL-10
(pg/mL)

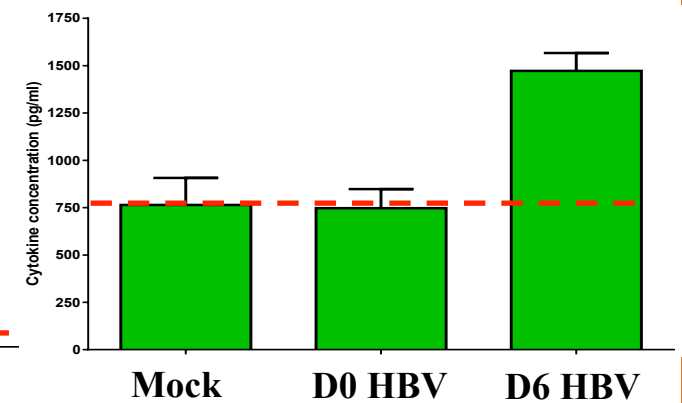
Monocyte



M1



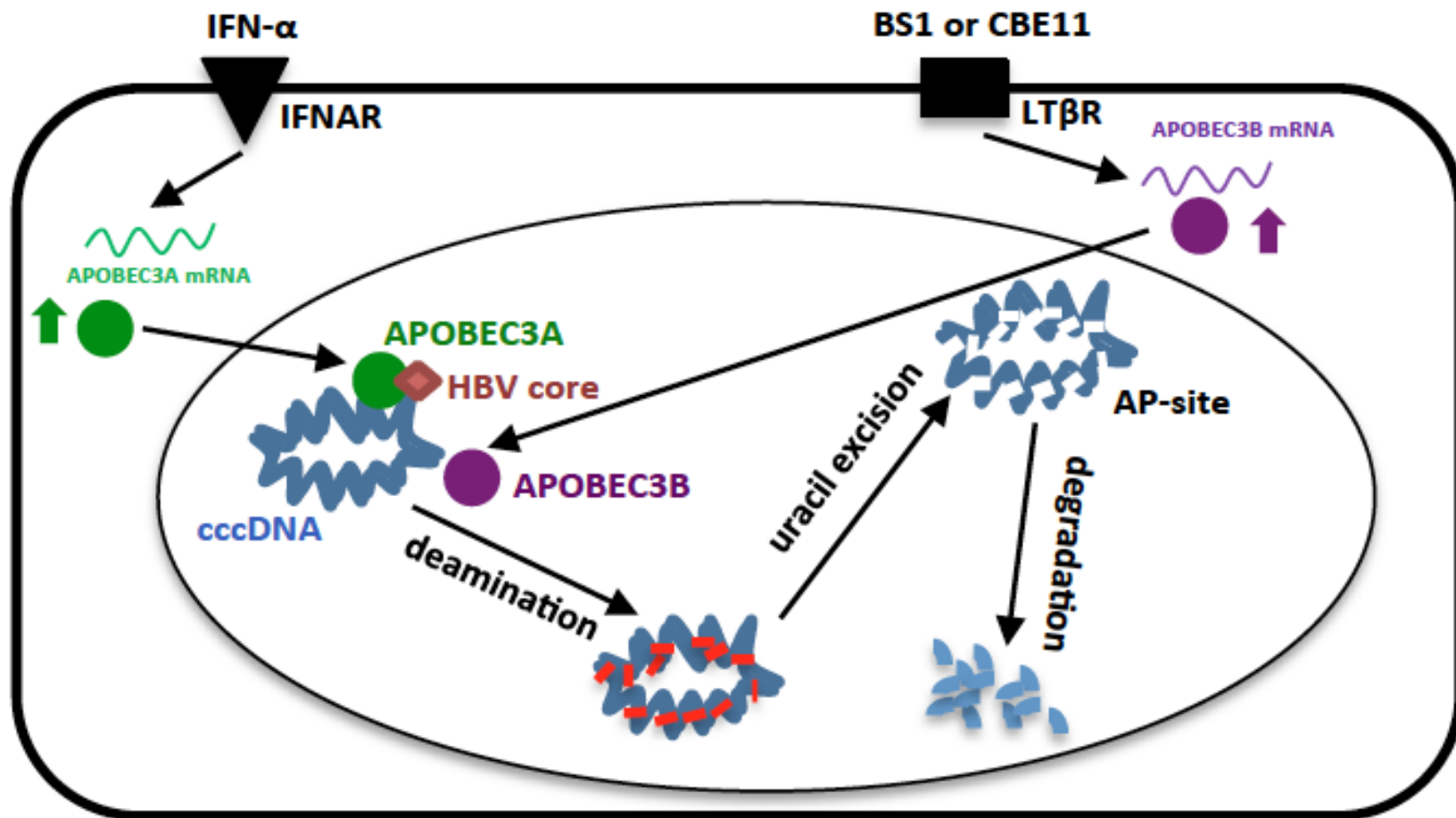
M2



Therapeutic implications

- ✓ **Better knowledge of escape mechanisms could help selecting the right pathway to target with PRR agonists**
- ✓ **Studies in pDC led to the development of TLR7-L as potential immune-therapeutics**
- ✓ **TLR7-L (GS9620) is in current phase-II trial**
 - **Potent inhibition of WHB (woodchuck model) replication in mono-therapy**
 - **Effect on cccDNA → Long lasting effect off drug and HBs seroconversion**
 - **Direct effect of innate immunity cytokines/IFNs on cccDNA clearance in a non cytopathic manner?**

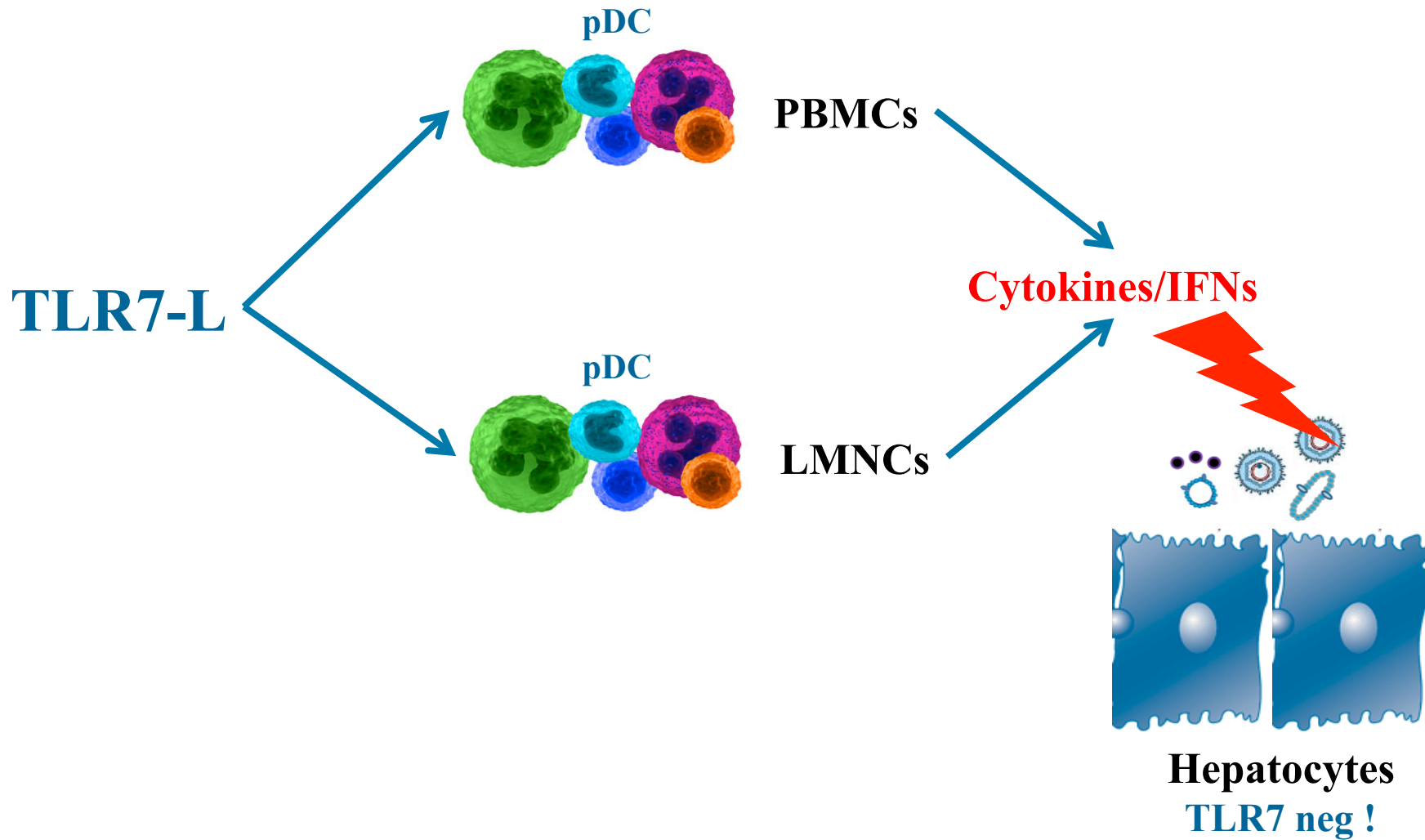
IFN- α and Lymphotoxin- β agonists can induce cccDNA degradation via an APOBEC-3A/B mechanism: POC that cccDNA can be targeted



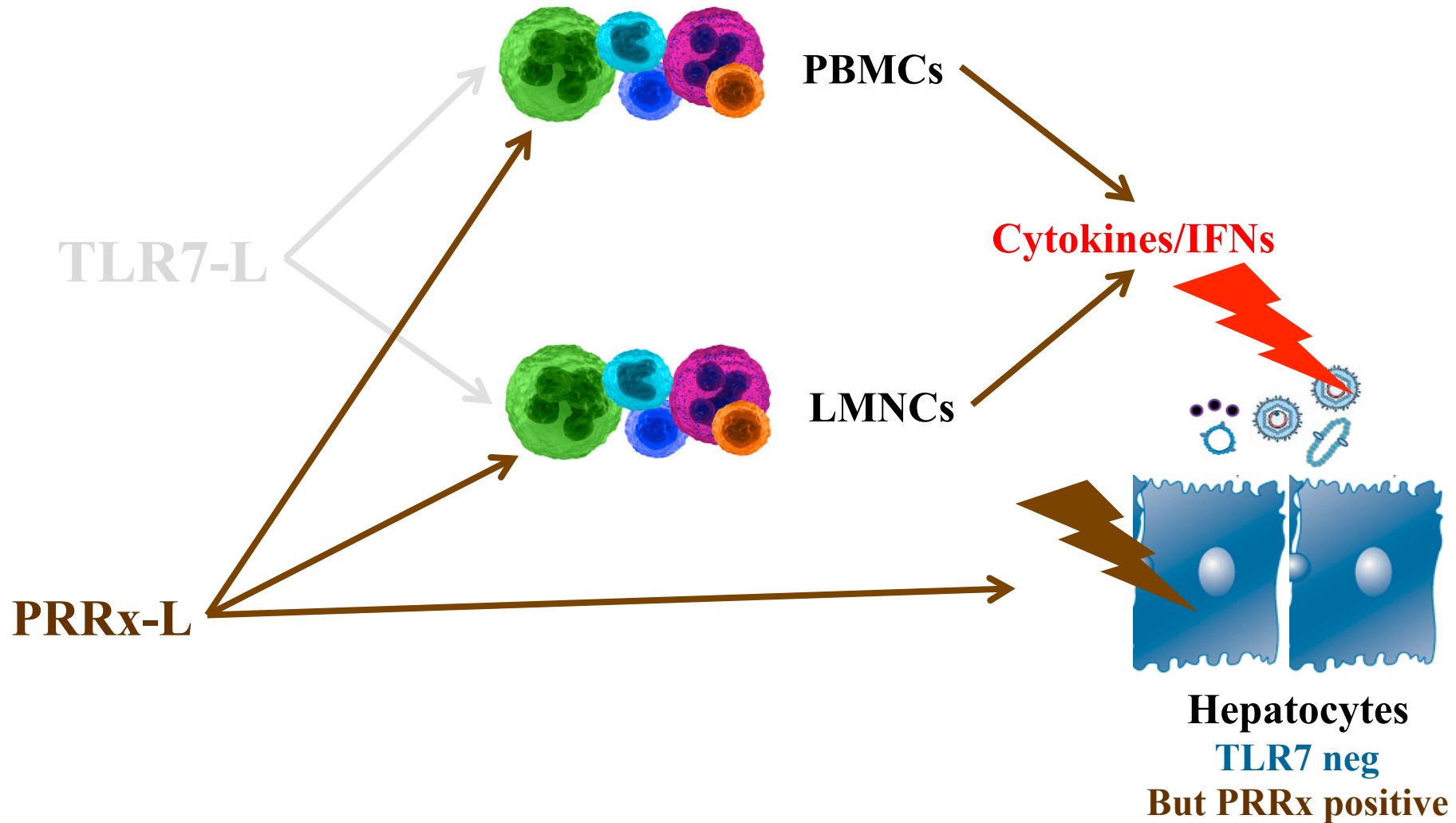
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- ✓ **Other ligands to be considered?**

TLR7-L acts on immune cells, not on hepatocytes themselves



PRRx-L that could act on both types of cells: a better choice?



Acknowledgements



**CRCL (Dir A. Puisieux),
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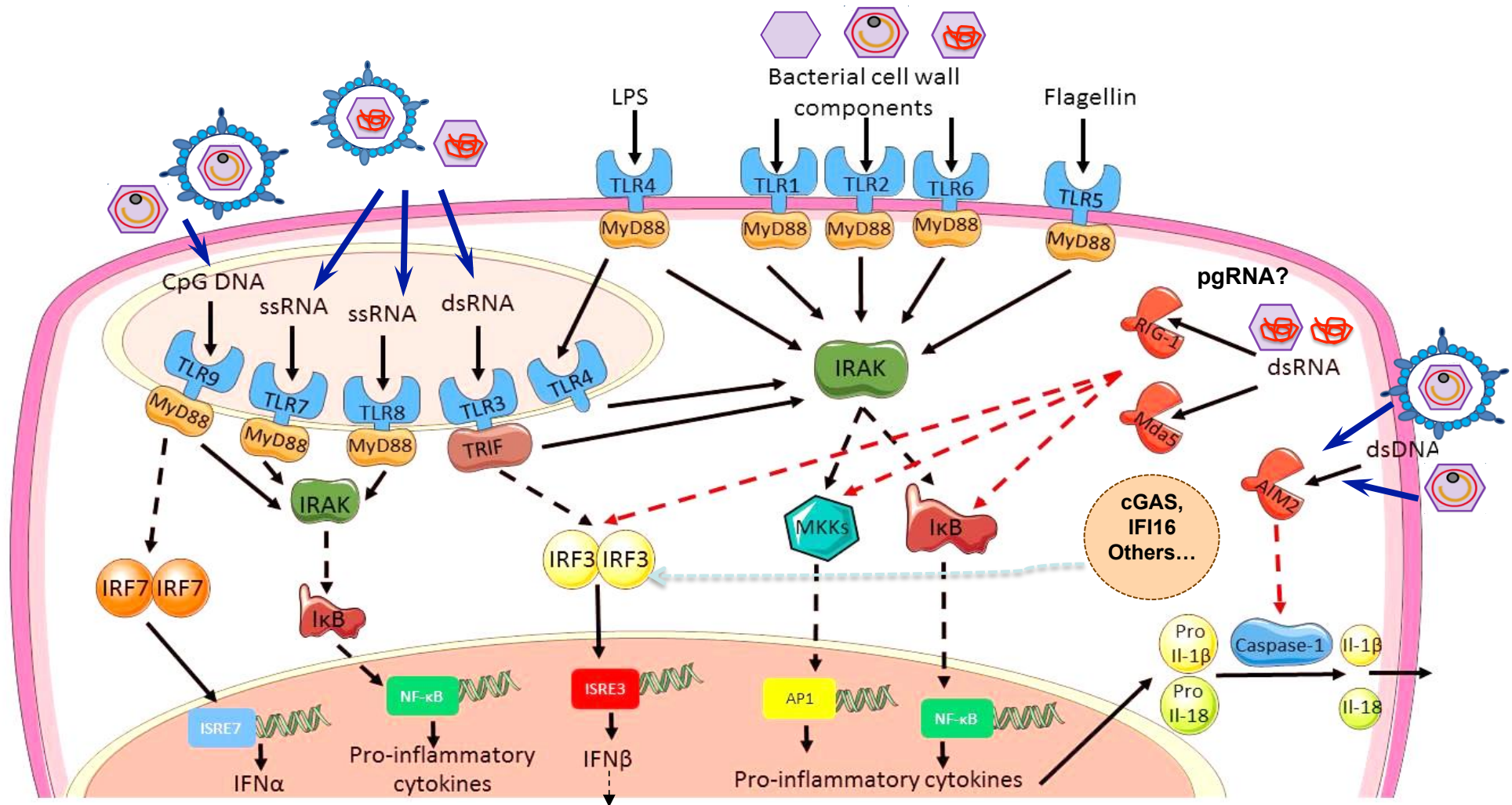
Collaborations

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Academic funding bodies

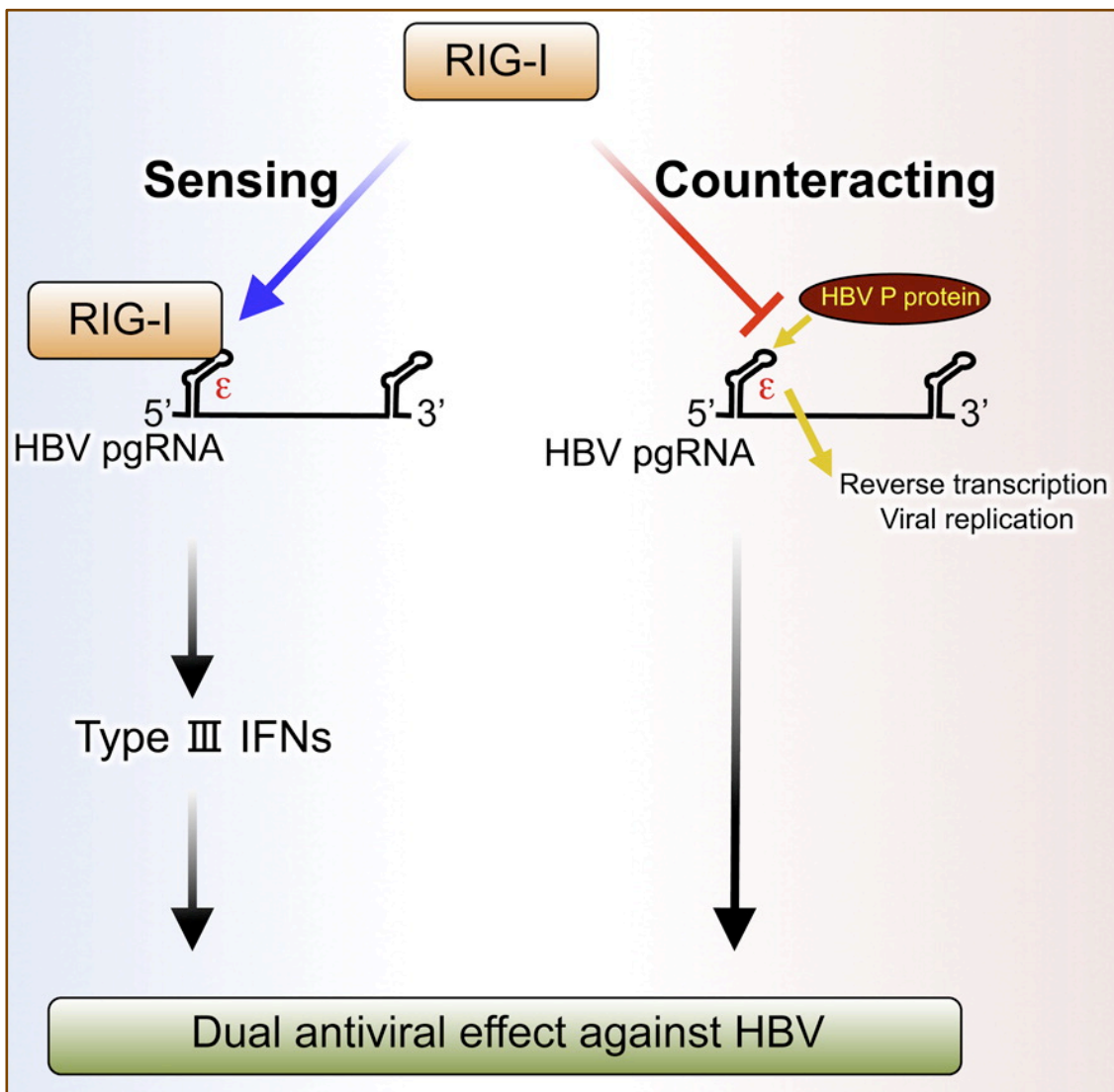


PRRs and PAMPs involved in HBV sensing/ detection: few data available...

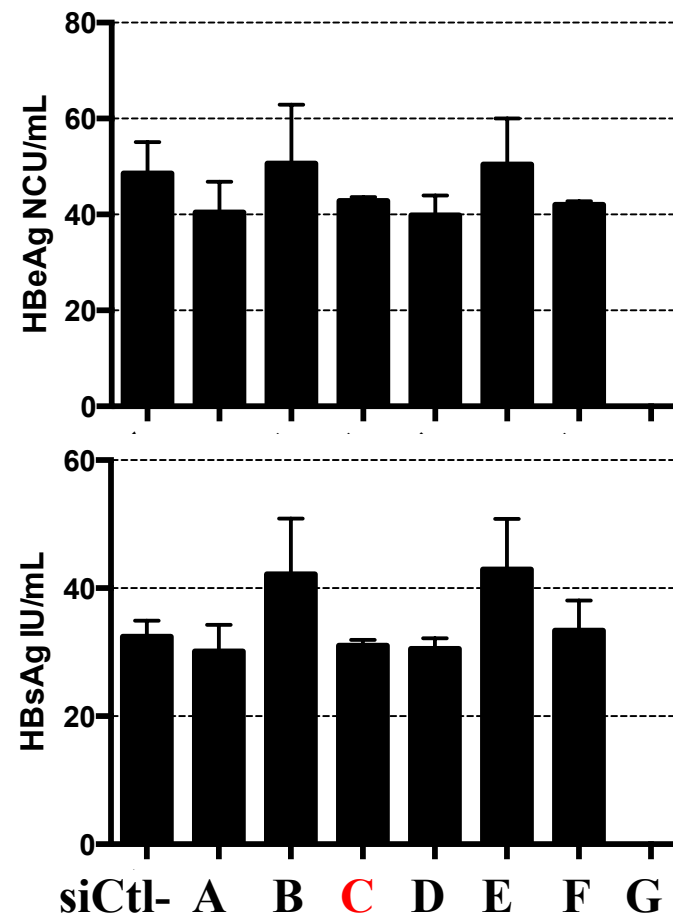


Sato et al., *Immunity* 2015; Liu and Liao, *J. Immunol*, 2013; Chang et al., *Antiviral Res*, 2012; Ait-Goughoulte et al., *Viruses* 2010; Cooper et al., *J. Immunol* 2005

Dual role of RIG-I?



C = siRNA RIG-I
G = positive siRNA



✓ KO of RIG-I in primary hepatocytes does not seem to lead to enhanced replication