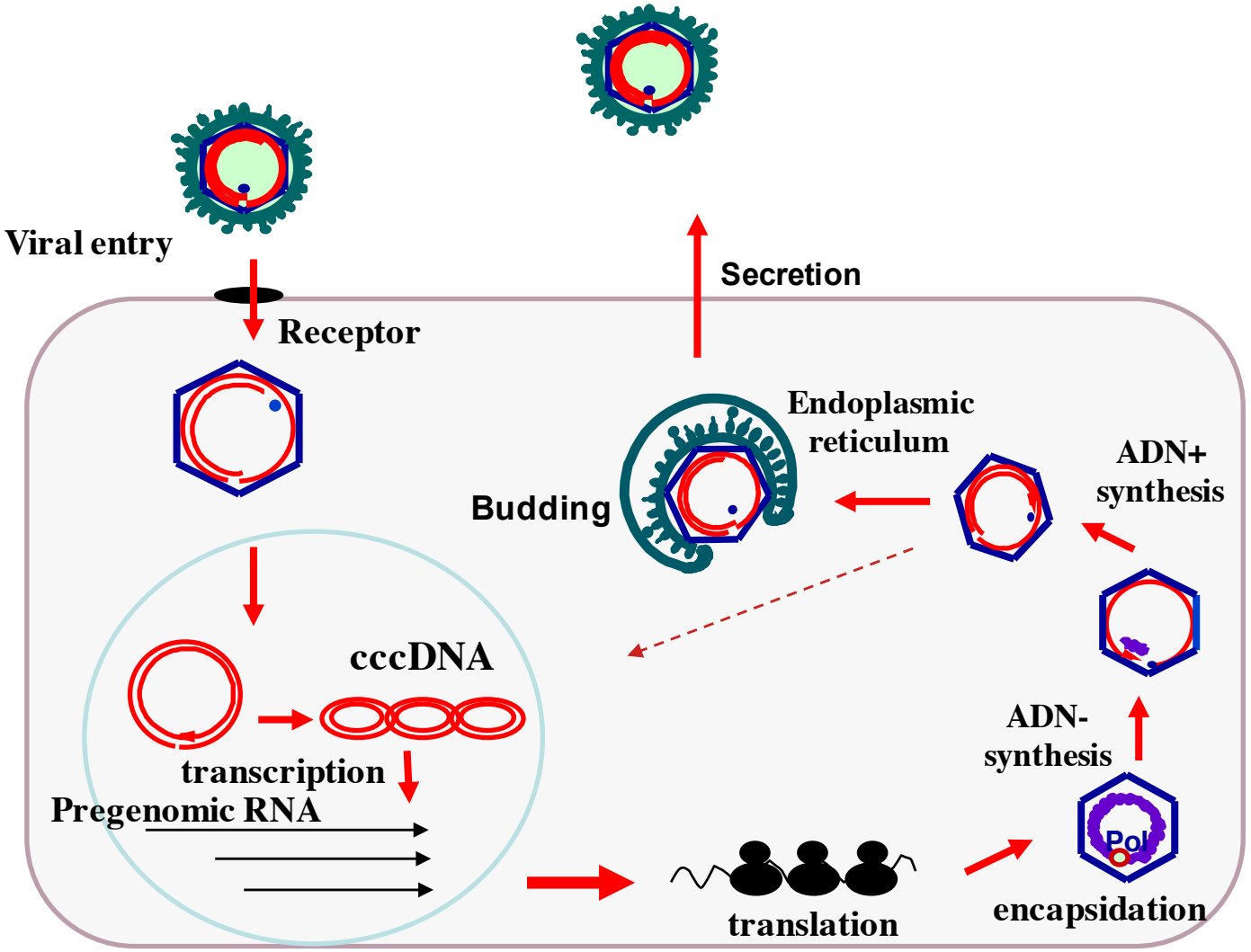
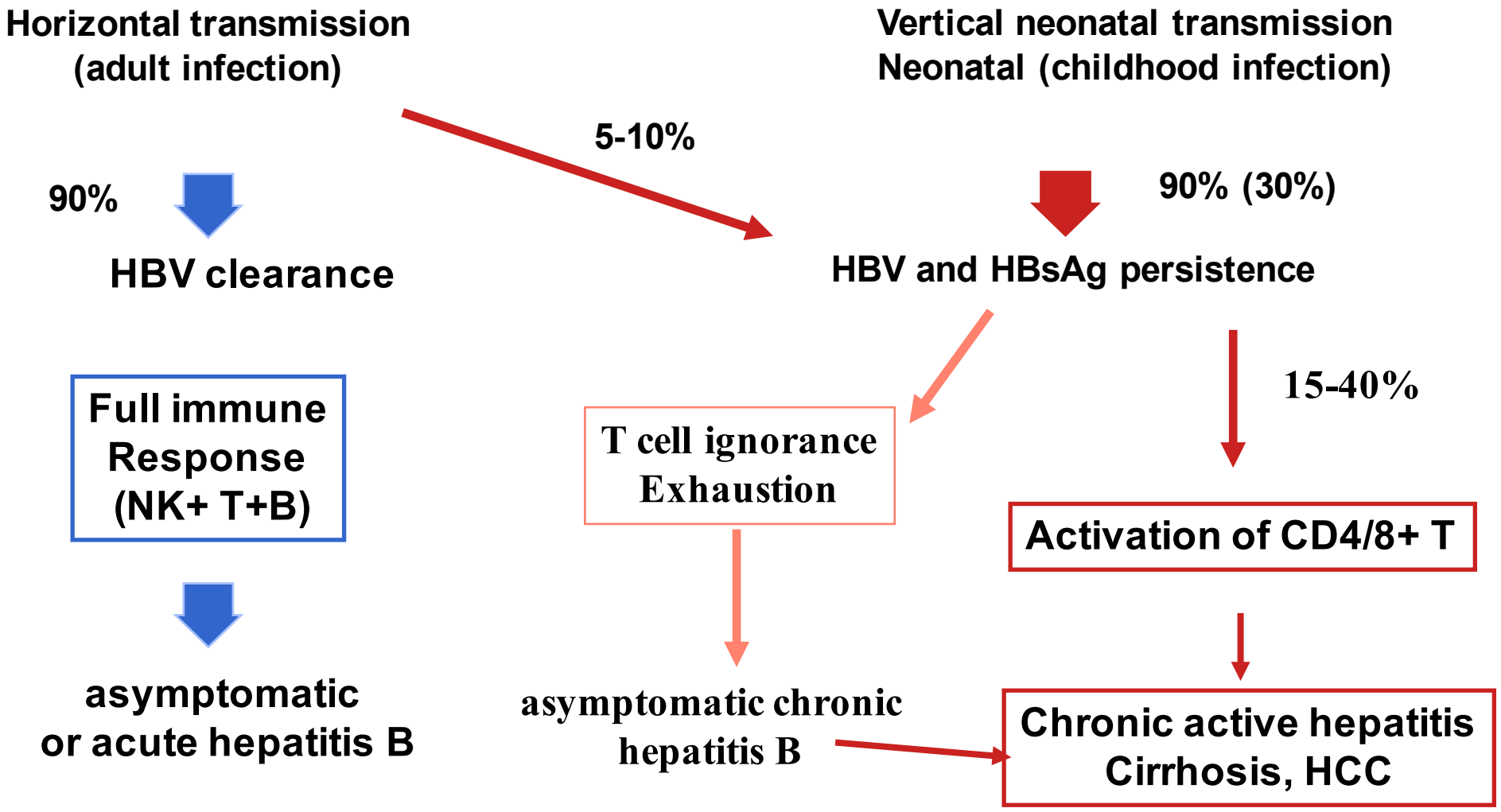
The regulatory HBx protein contributes to evasion from intrinsic antiviral response

Hepacivirus et Immunité Innée
Institut Pasteur
Paris

HBV replication cycle

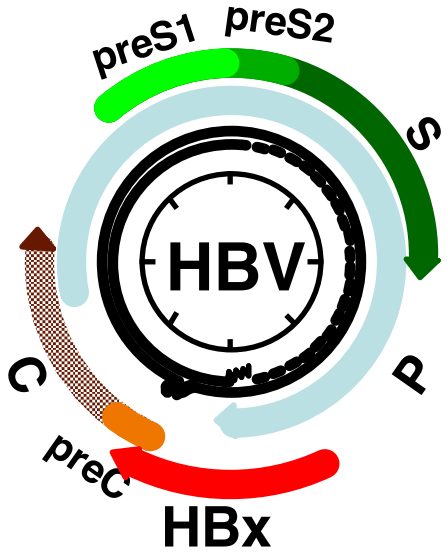


Natural history of HBV infection



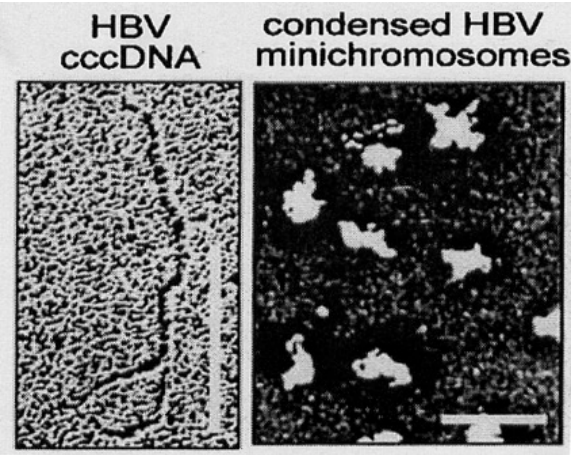
Virus outcome: balance between cellular antiviral responses and mechanisms develop by the virus to escape from these antiviral responses

HBx regulatory protein

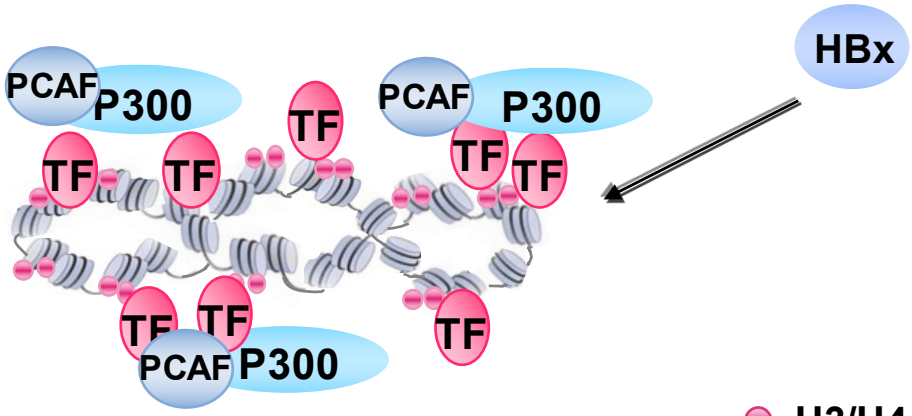


- Viral HBx is a regulatory protein (17kD)
- HBx is essential for viral replication *in vivo* *(Zoulim et al.,1994)*
- HBx has pleiotropic activities (signal transduction, cell cycle, apoptosis, transcription)

HBx is involved in cccDNA transcriptional regulation

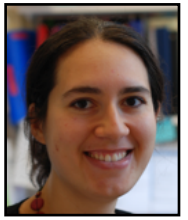


Bock et al, JMB 2001



ADNccc

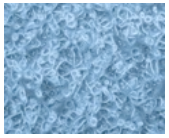
- HBV cccDNA is organized into chromatin-like structure as a viral minichromosome: up to 20 nucleosomes containing histones et non histone proteins.
- HBx is required for the initiation and maintenance of HBV transcription in the setting of infection (Lucifora et al., 2011)
- HBx expression correlates with HBV transcription and histone H3 hyperacetylation (Lucifora et al., 2011; Belloni et al., 2009)

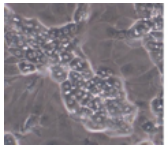


Molecular mechanisms involved in HBV cccDNA silencing in infected hepatocytes

Lise Rivière

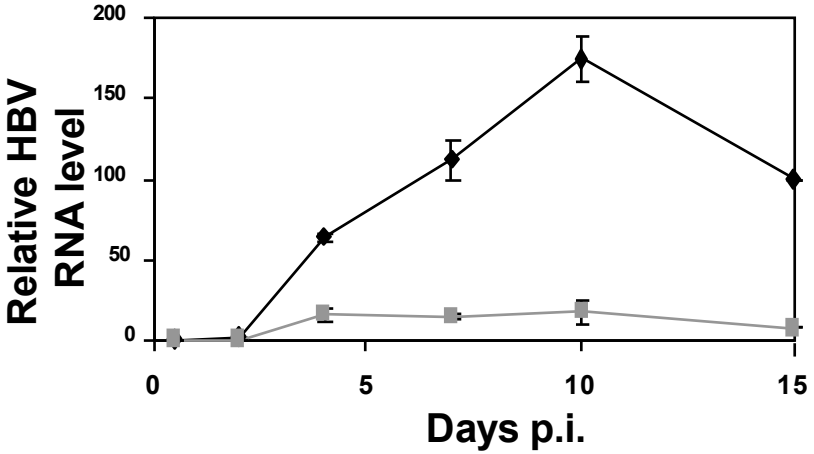
HBx increases the level of HBV RNAs

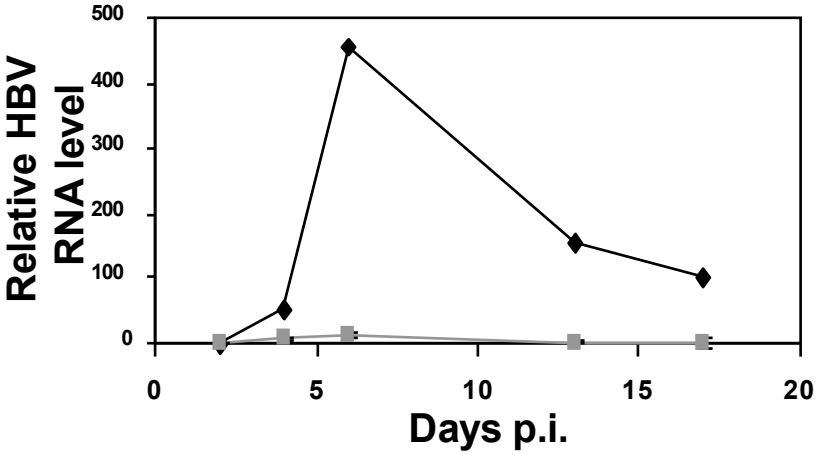
dHepaRG 

PHH 

HBV WT
HBV X-

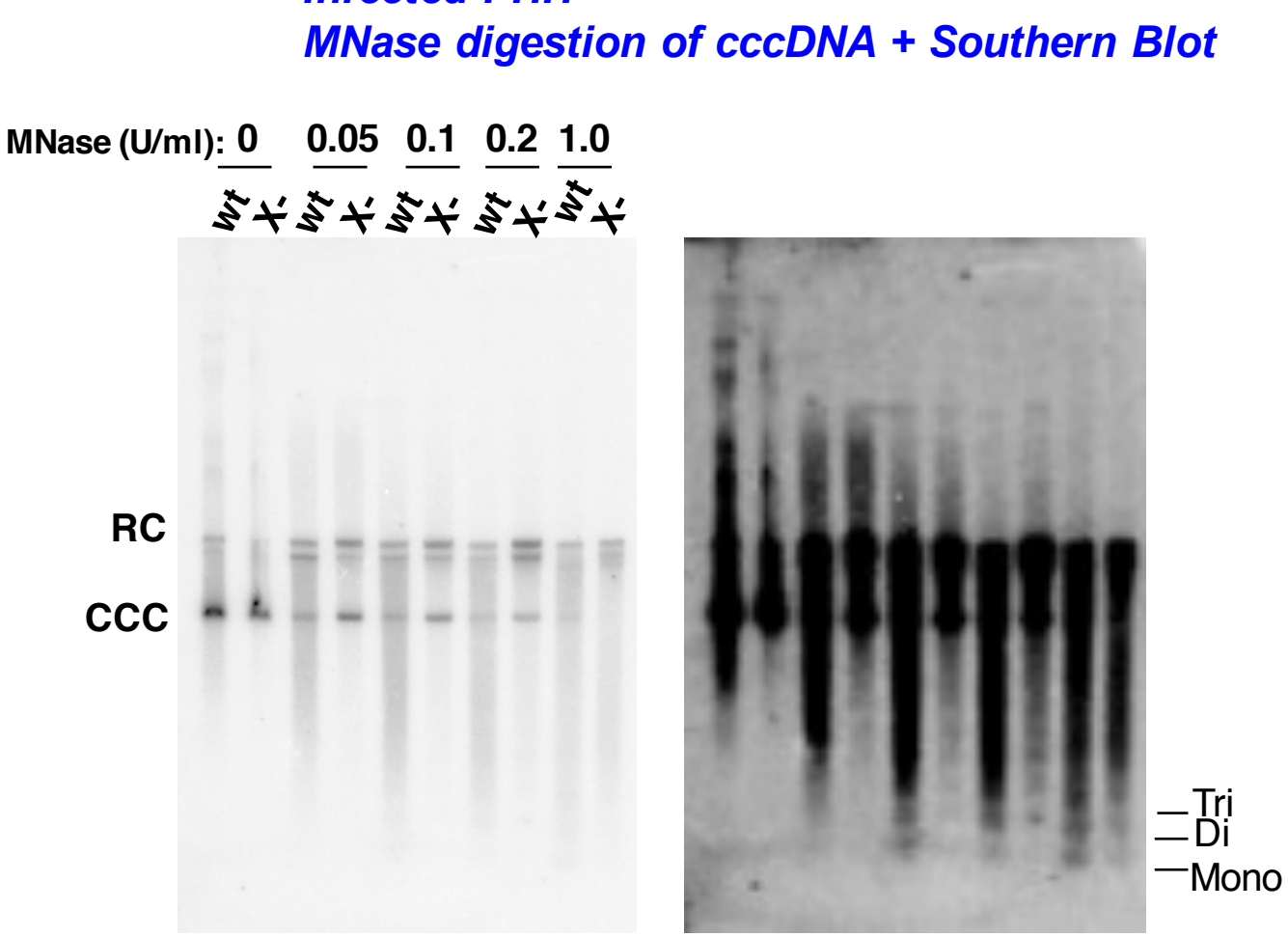
HBV RT-QPCR



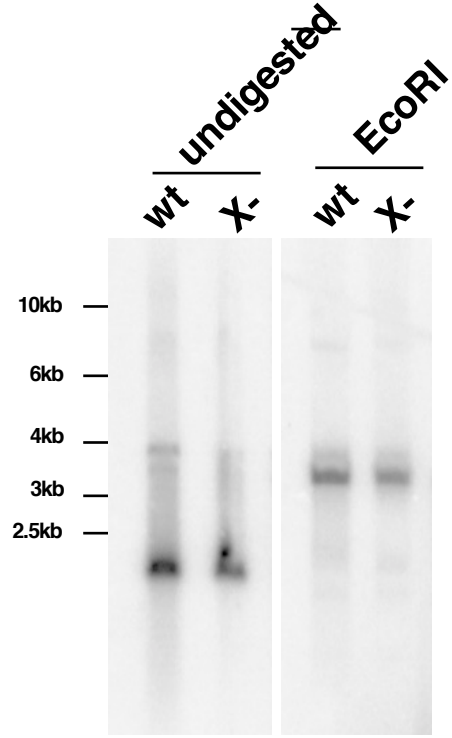


HBV wt and X- cccDNAs show different sensitivities to MNase digestion

Infected PHH
MNase digestion of cccDNA + Southern Blot



Long exposure



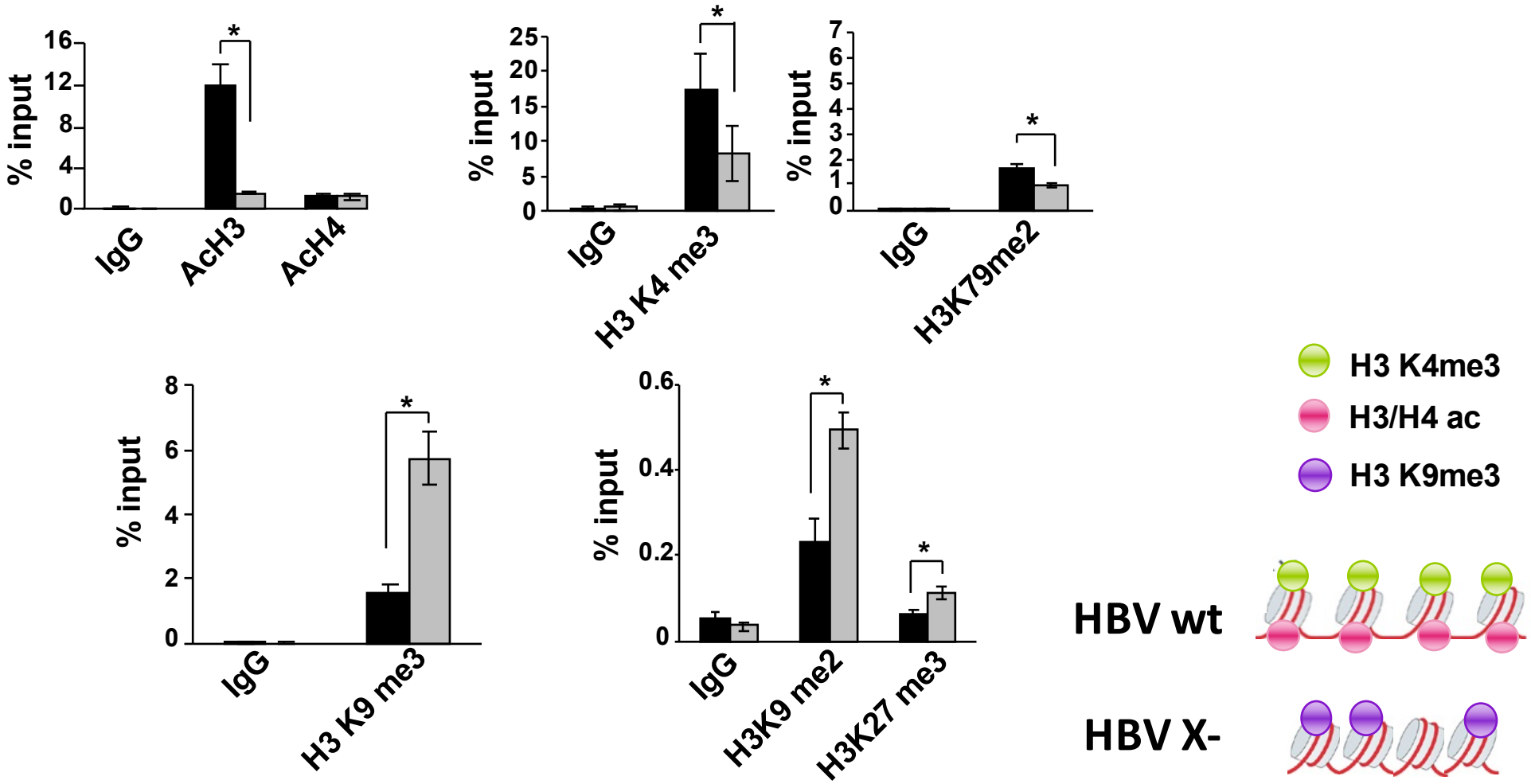
Micrococcal Nuclease (MNase)

Silenced HBV X- cccDNA is associated with repressive histone marks

ChIP Q-PCR

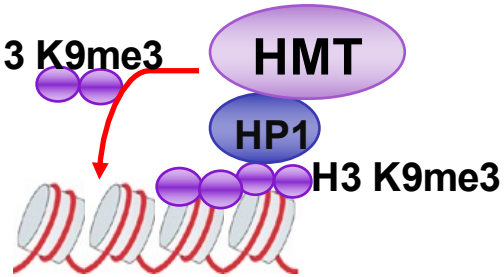
dHepaRG

HBV cccDNA



Histone methyltransferase SETDB1 mediates the deposition of H3K9me3 on the cccDNA

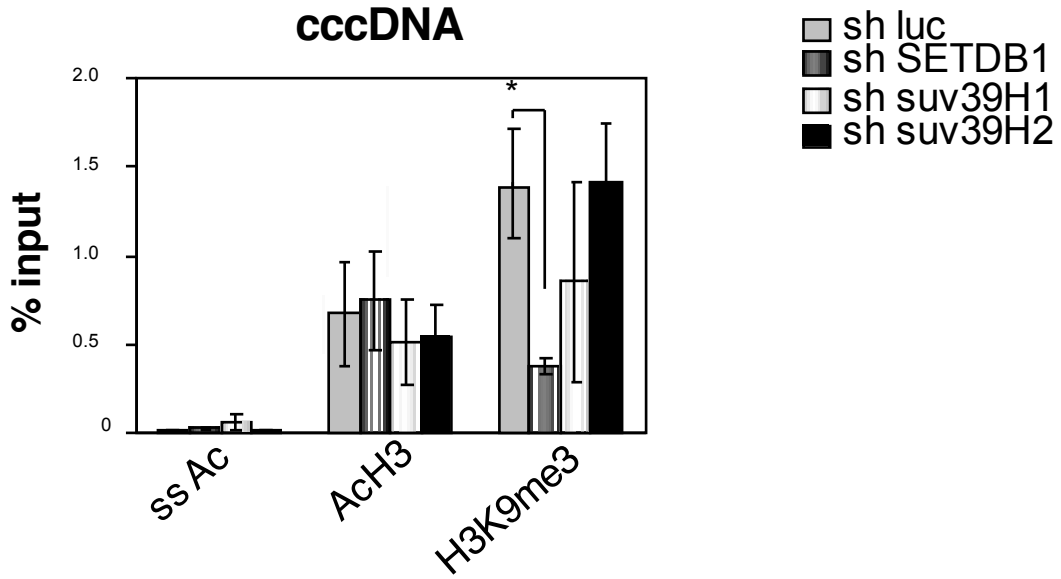
HBV X-



histone methyltransferase ?

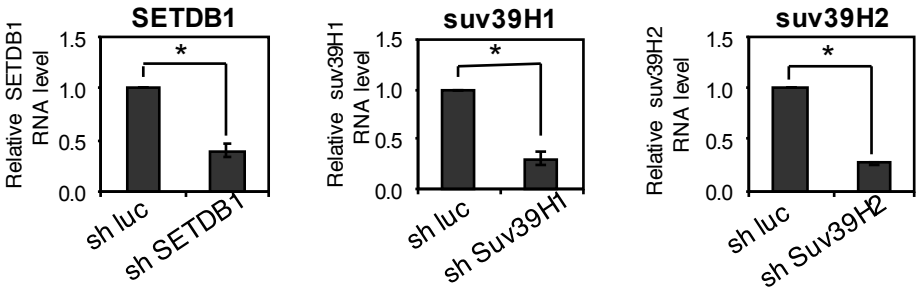
HepaRG

ChIP Q-PCR



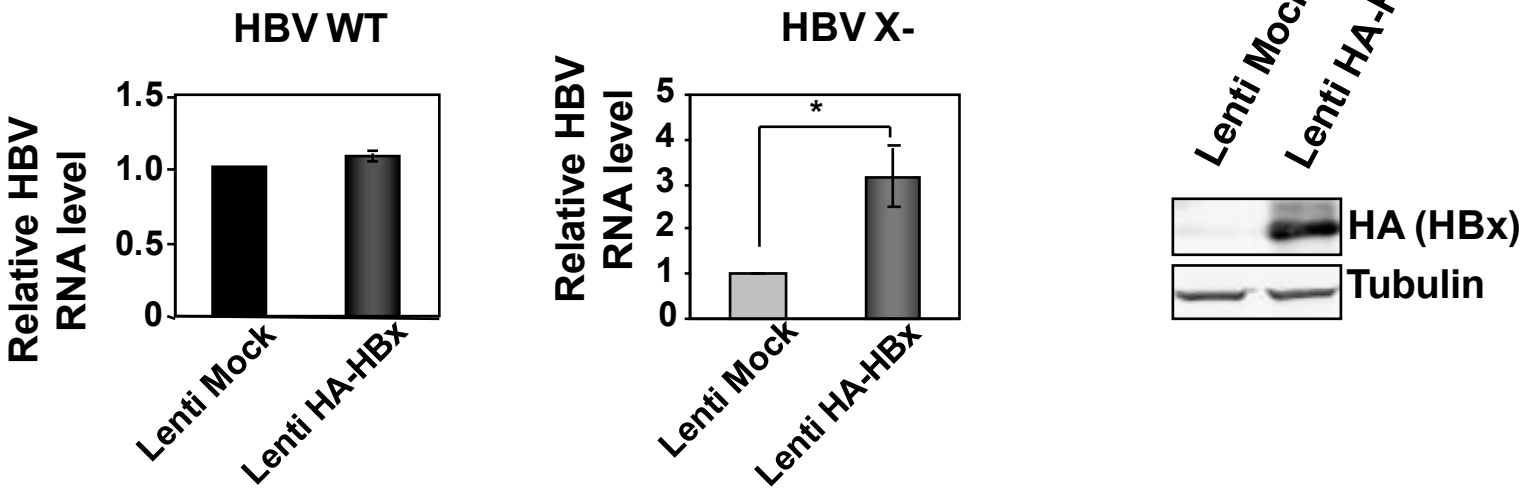
RT-QPCR

Control of silencing

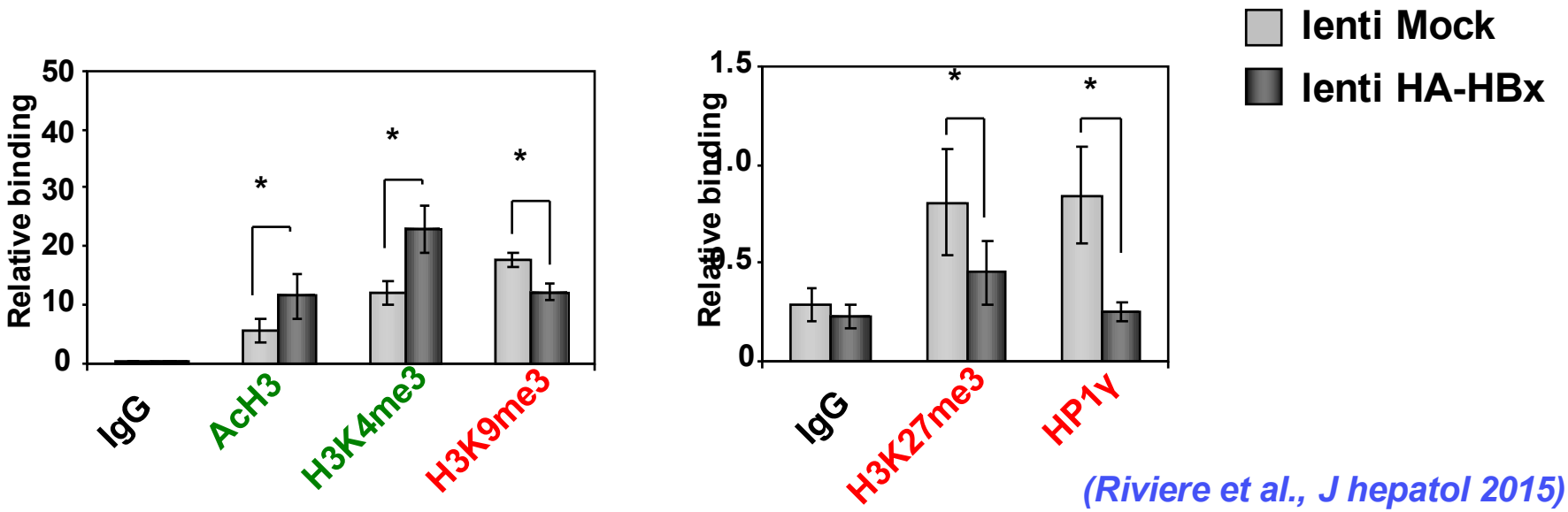


HBx restores cccDNA transcription and alleviates chromatin repression

HBV transcription (RT-qPCR)



ChIP qPCR
HBV X- + lentivector



(Riviere et al., J hepatol 2015)

Conclusion

✓ HBx is necessary for viral transcription and replication, in the context of cellular infection by HBV

✓HBx expression correlates with the deposition of activating histone modifications on HBV cccDNA (histone acetylation, H3K4 me3)

✓ In the absence of HBx, repressive epigenetic marks are deposited on HBV cccDNA (H3K9 me3, histone hypoacetylation, recruitment of HP1γ)

✓HBV silencing is in part mediated by SETDB1

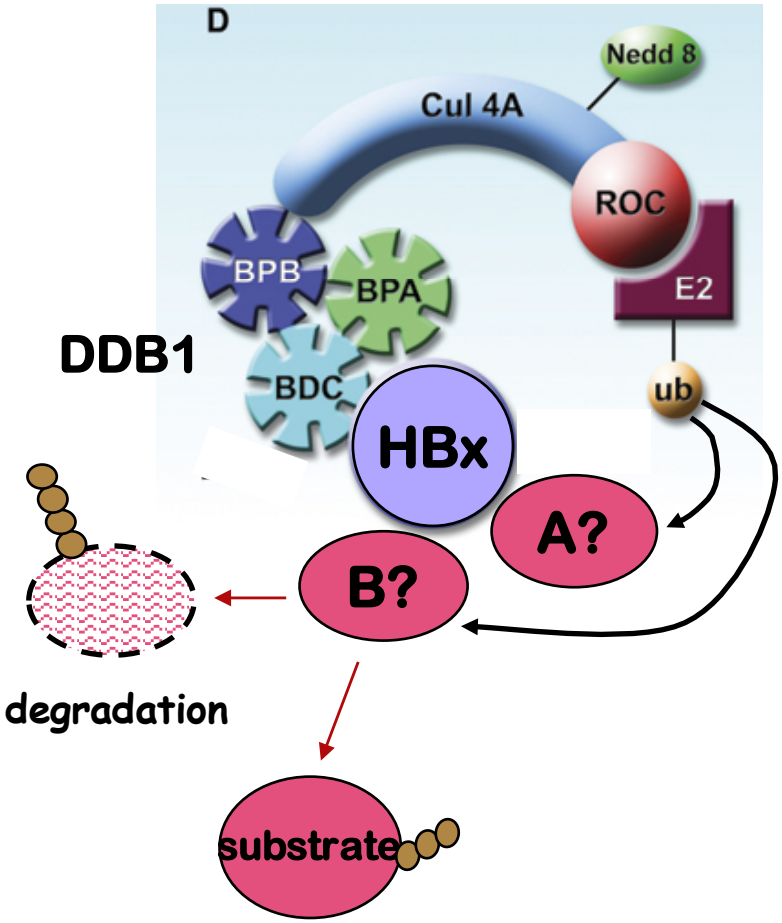
✓ **HBV repression can be reverted by the reexpression of HBx**

⇒ **HBx may counteract an antiviral response that prevents viral transcription via the establishment of repressed chromatin**

Working model

Common viral manipulation: subversion of E3 ubiquitin ligase activity

DDB1 : a core subunit of a Cul4a-based E3 ubiquitin ligase complex.



Ubiquitination of cellular protein(s):

Degradation of cellular substrates or modification of their functions

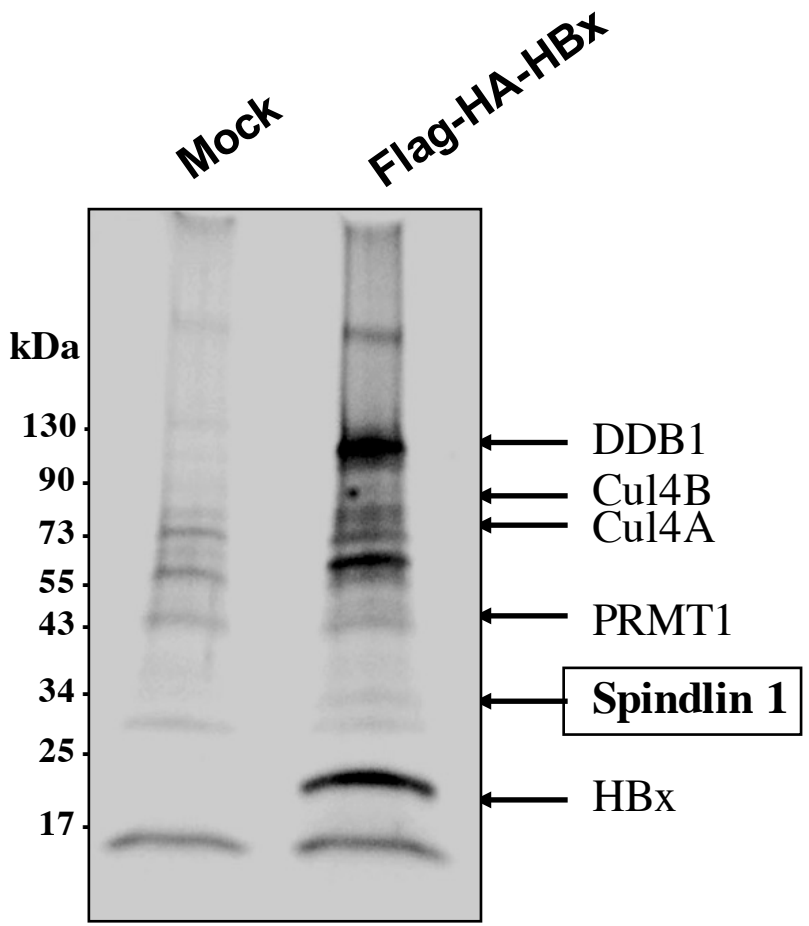
- ↗ of virus replication
- Induction of apoptosis
- Transcriptional activity

Cellular factors involved in HBV silencing and counteracted by HBX

Aurélie Ducroux

Identification of Spindlin 1 as a new HBx interacting-protein

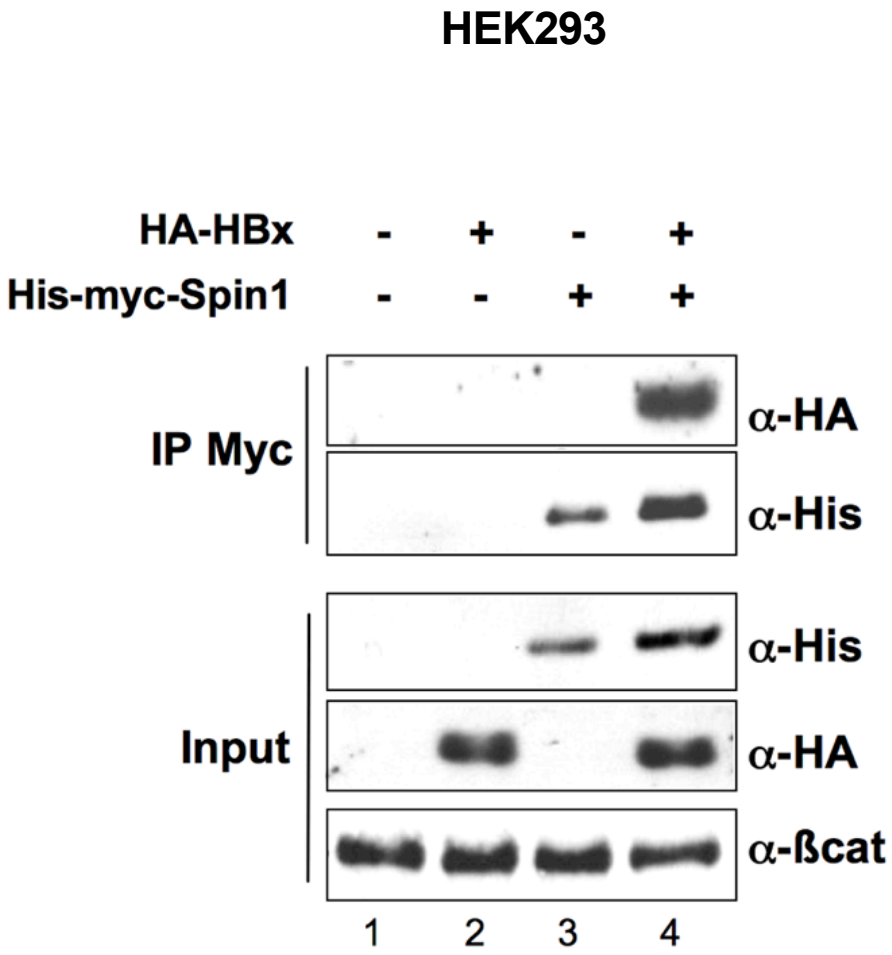
HepG2
Tap-tag

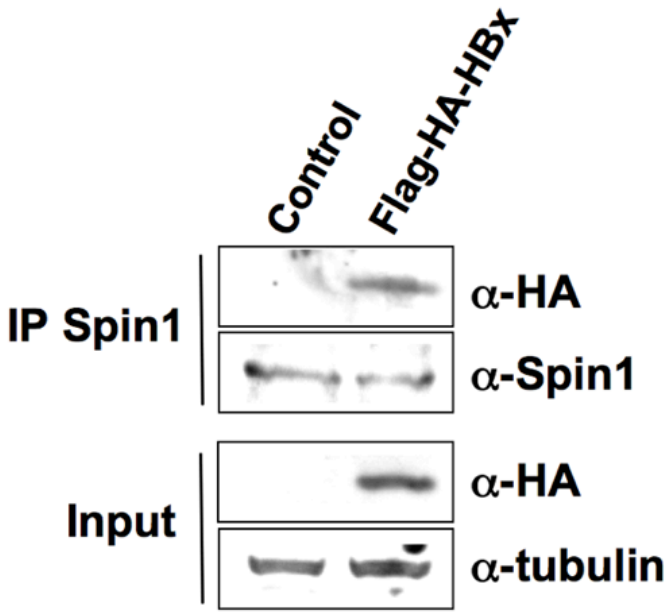


Spindlin1:

- Associated with meiotic spindles
- SPIN/SSTY family
- Contains three Tudor-like domains
- Histone reader for dual H3 K4me3-R8me2a methylation pattern
- Increases rRNA trancription
- Activator of Wnt/ β-catenin pathway

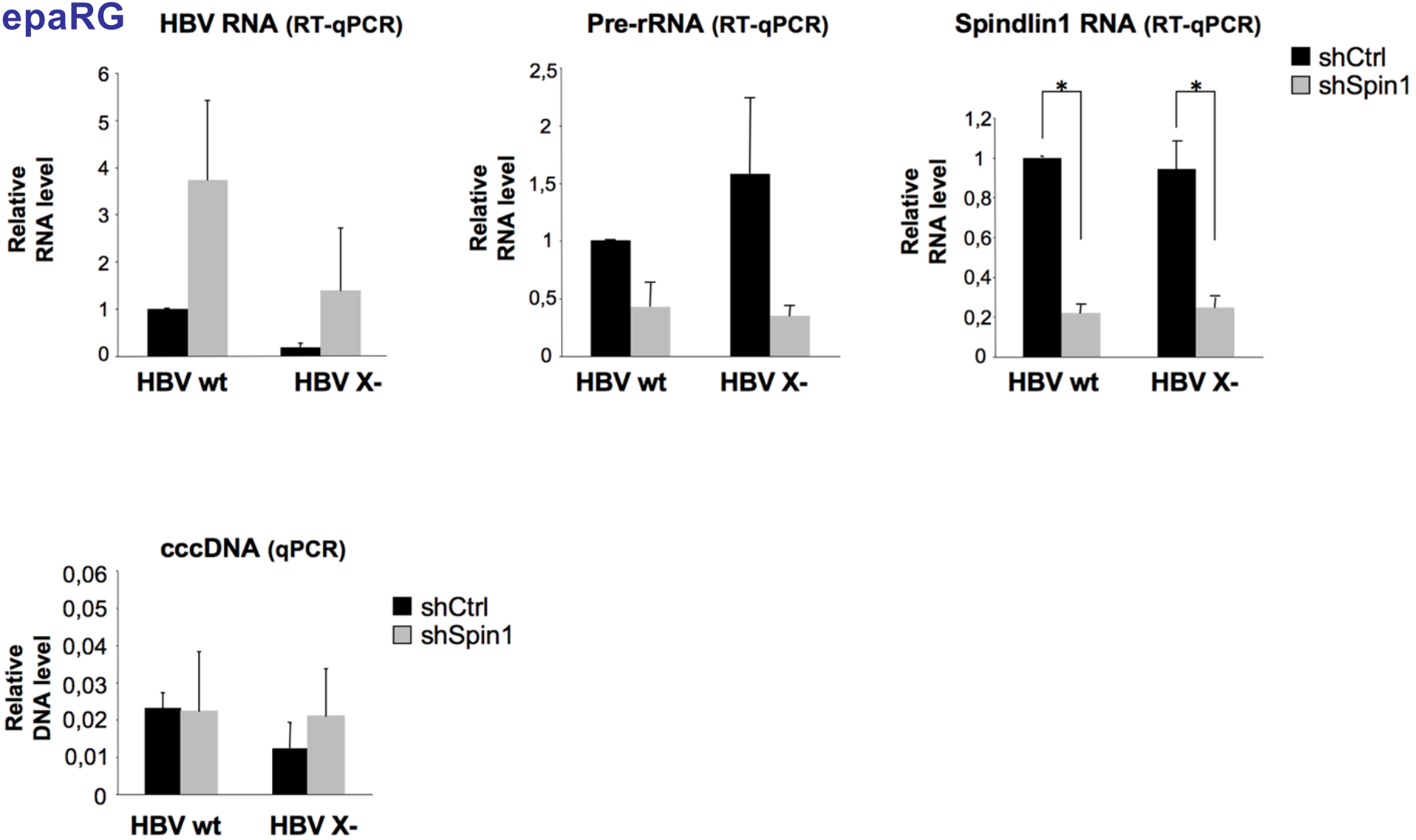
HBx interacts with endogenous Spindlin1





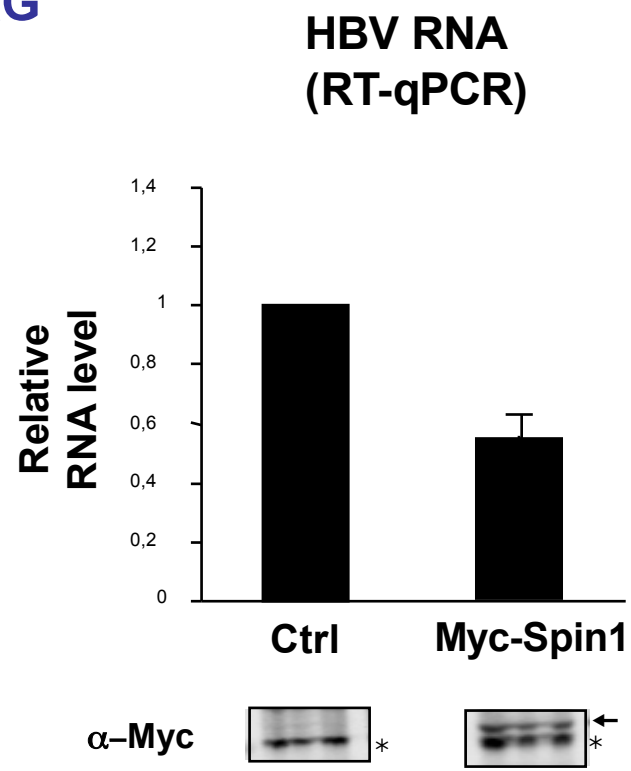
Spindlin1 represses HBV transcription in the setting of infection

dHepaRG



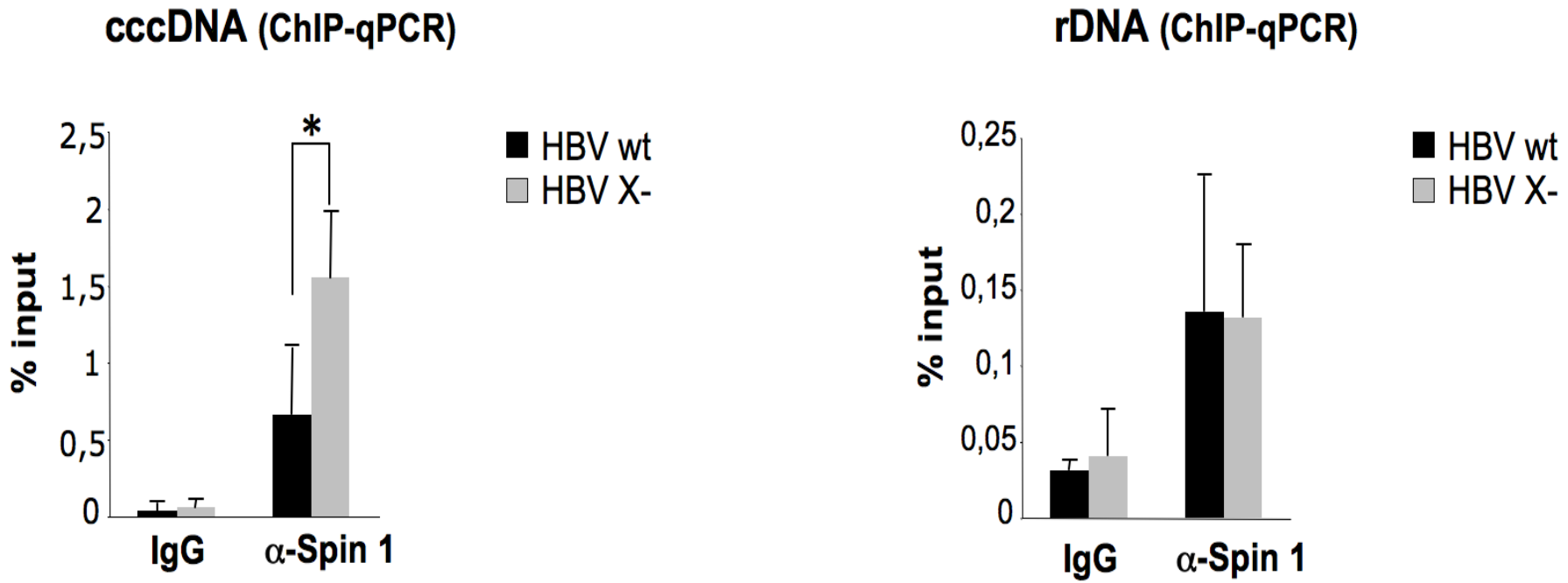
Spindlin1 expression represses HBV transcription in the setting of infection

dHepaRG



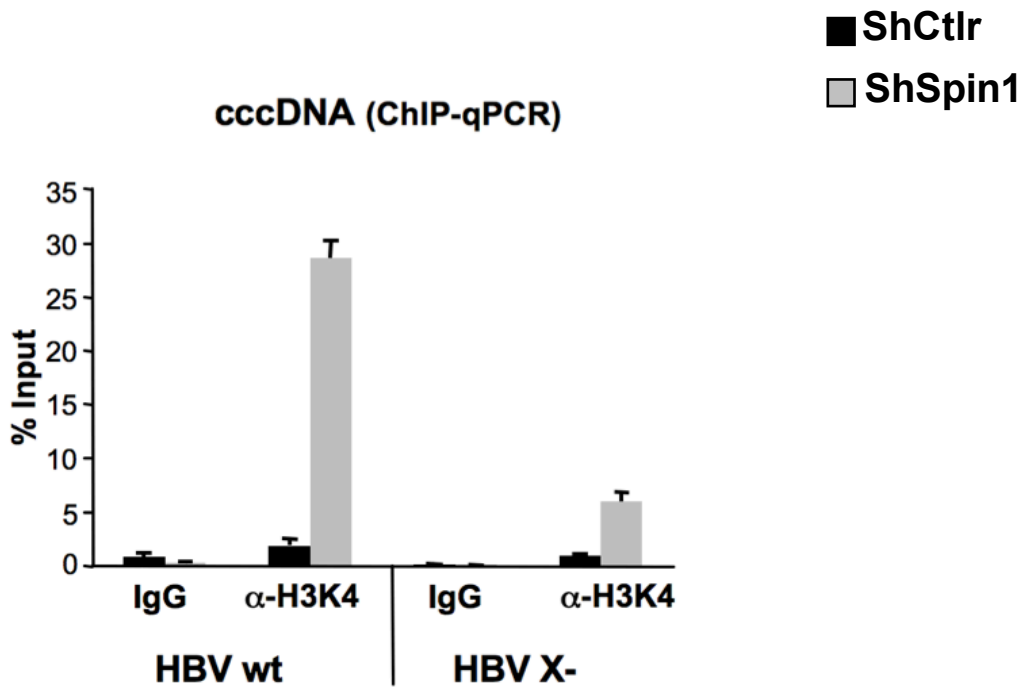
HBx decreases recruitment of Spindlin1 to cccDNA

dHepaRG cells



Depletion of Spindlin1 increases H3K4me3 on the cccDNA

dHepaRG cells

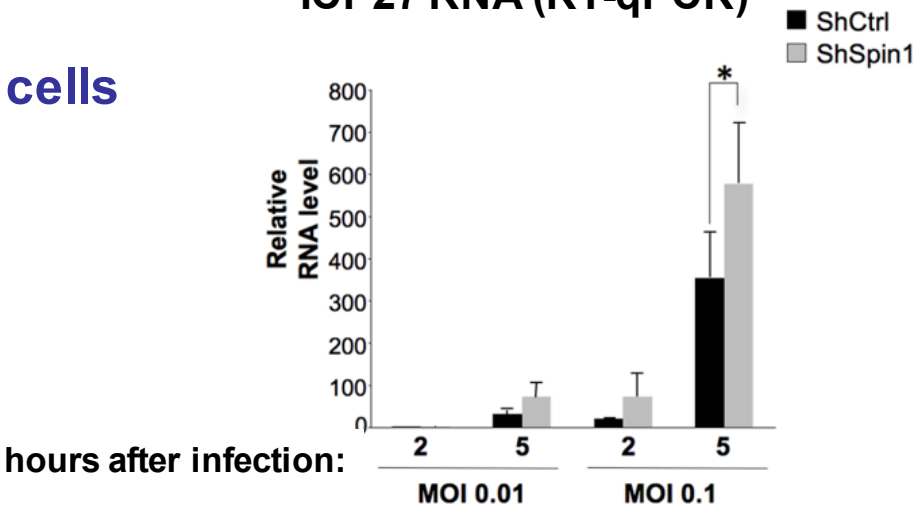


Spindlin1 represses the transcription of HSV-1 during infection

A

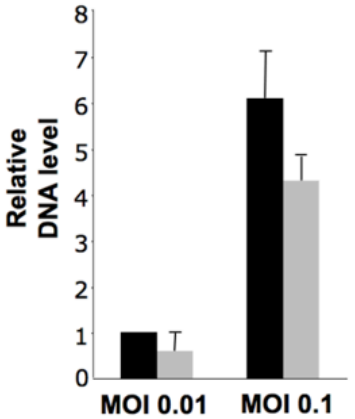
HepaRG cells

ICP27 RNA (RT-qPCR)

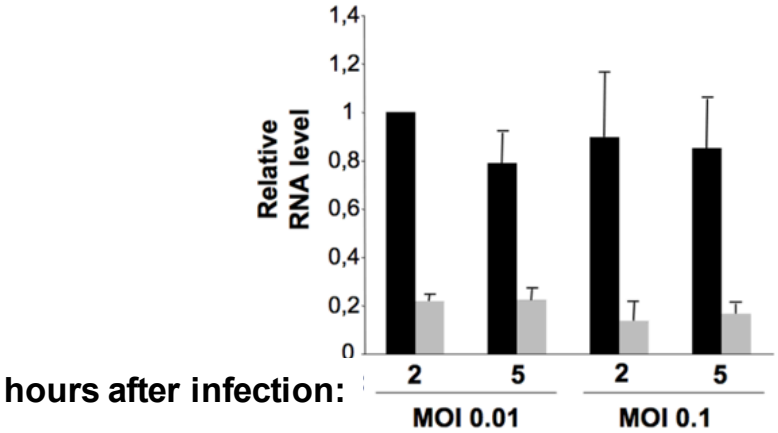


B

HSV1 DNA(qPCR)



Spindlin1 RNA (RT-qPCR)



ShCtlr

ShSpin1

(Ducroux et al.,Plos Pathogen 2014)

Conclusions

✓**Spindlin1 is recruited on the cccDNA and represses its transcription in the context of HBV infection**

✓ **Spindlin1 represses more severely HBV X- virus than wild type virus, suggesting that HBx counteracts Spindlin1 activity on HBV.**

✓**Spindlin1 represses the transcription of Herpes Simplex Virus type 1 in the setting of infection**

➔ **Spindlin1 is a new component of the intrinsic antiviral defense**

✓ **How Spindlin 1 is recruited on the cccDNA?**

✓**How HBx counteracts spindlin1 activity?**

✓ **Mechanisms involved in cccDNA repression?**

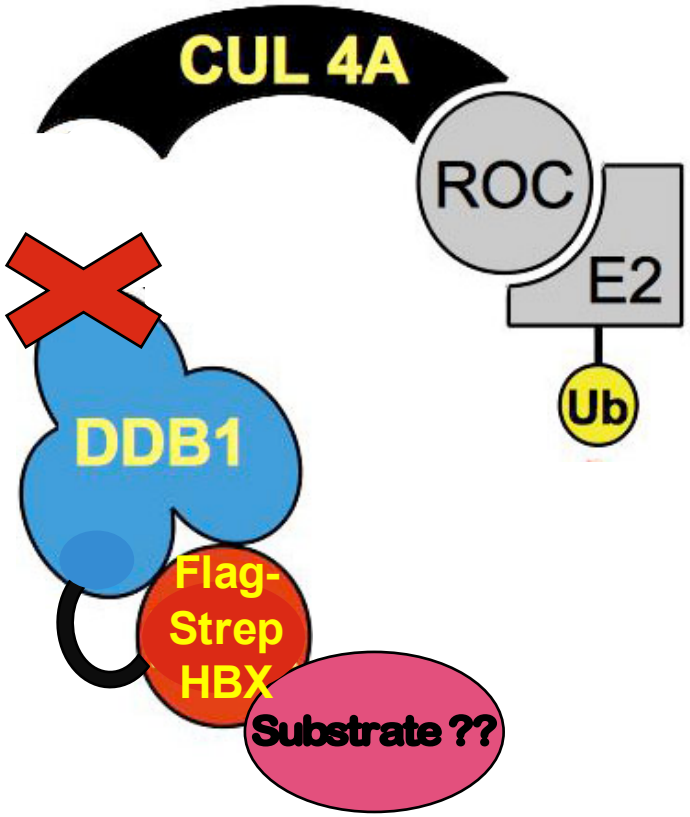
Hepatitis B virus X protein identifies the Smc5/6 complex as a host restriction factor

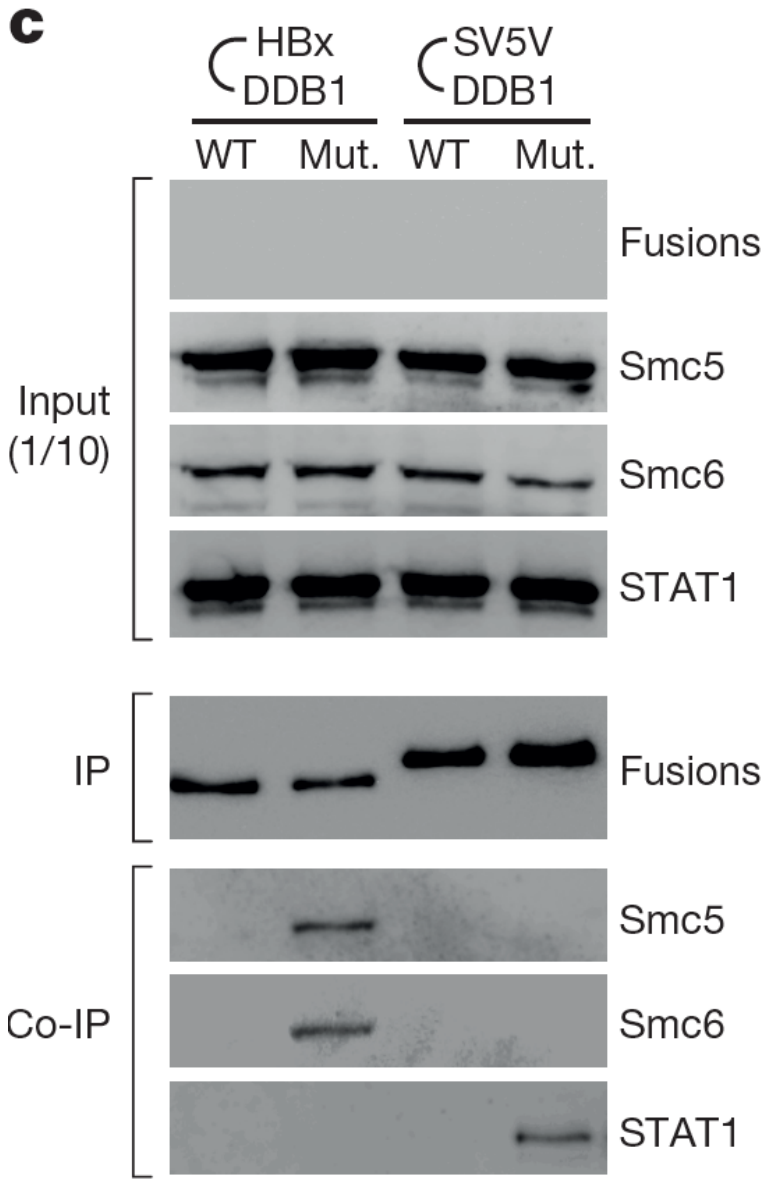
Adrien Decorsière1*, Henrik Mueller1†*, Pieter C. van Breugel1†*, Fabien Abdul1*, Laetitia Gerossier2, Rudolf K. Beran3, Christine M. Livingston3, Congrong Niu3, **Simon P. Fletcher3**, **Olivier Hantz2 & Michel Strubin**

NATURE, VOL 531, 2016

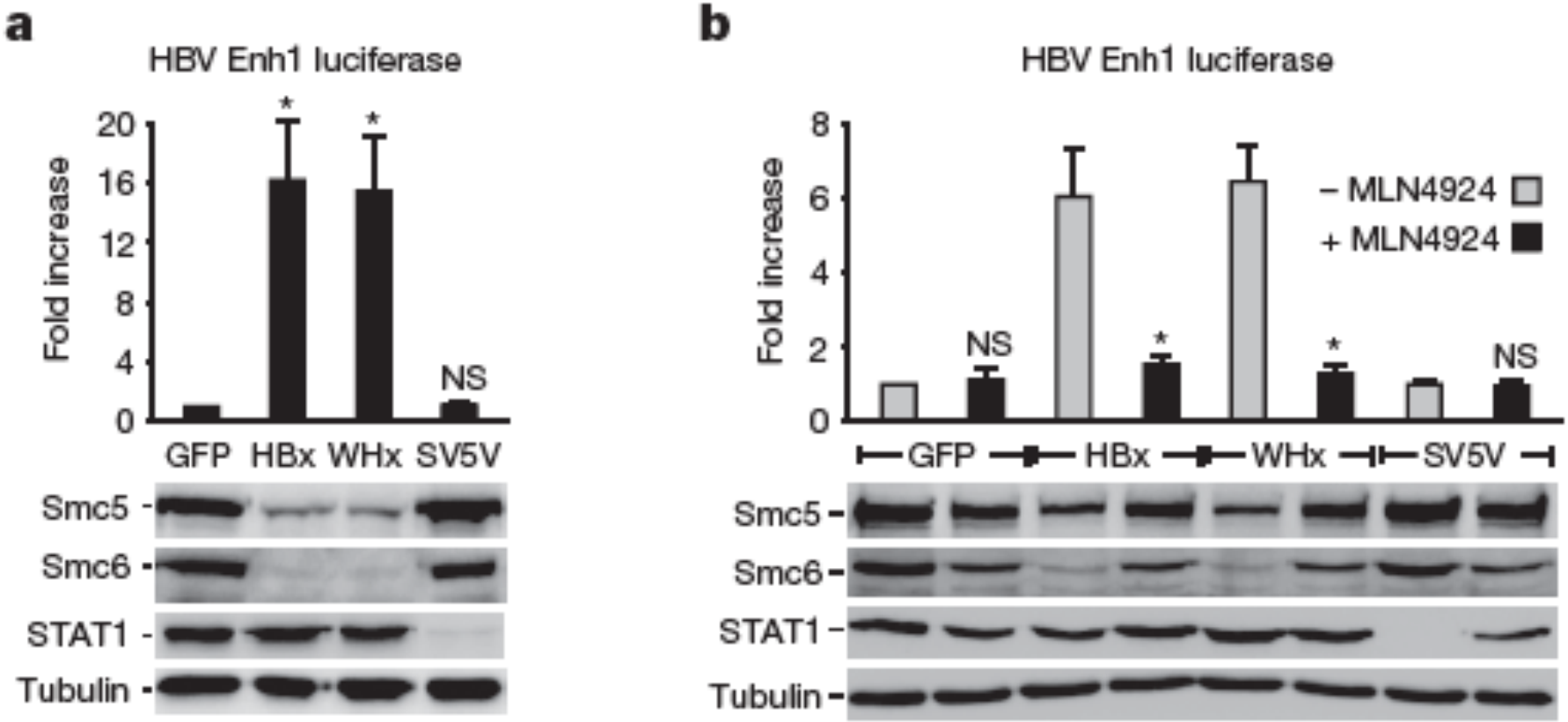
Indentification of Smc5/6 complex as an HBx interacting partner

Strategy: Tandem affinity purification assay (TAP) hepG2 cells

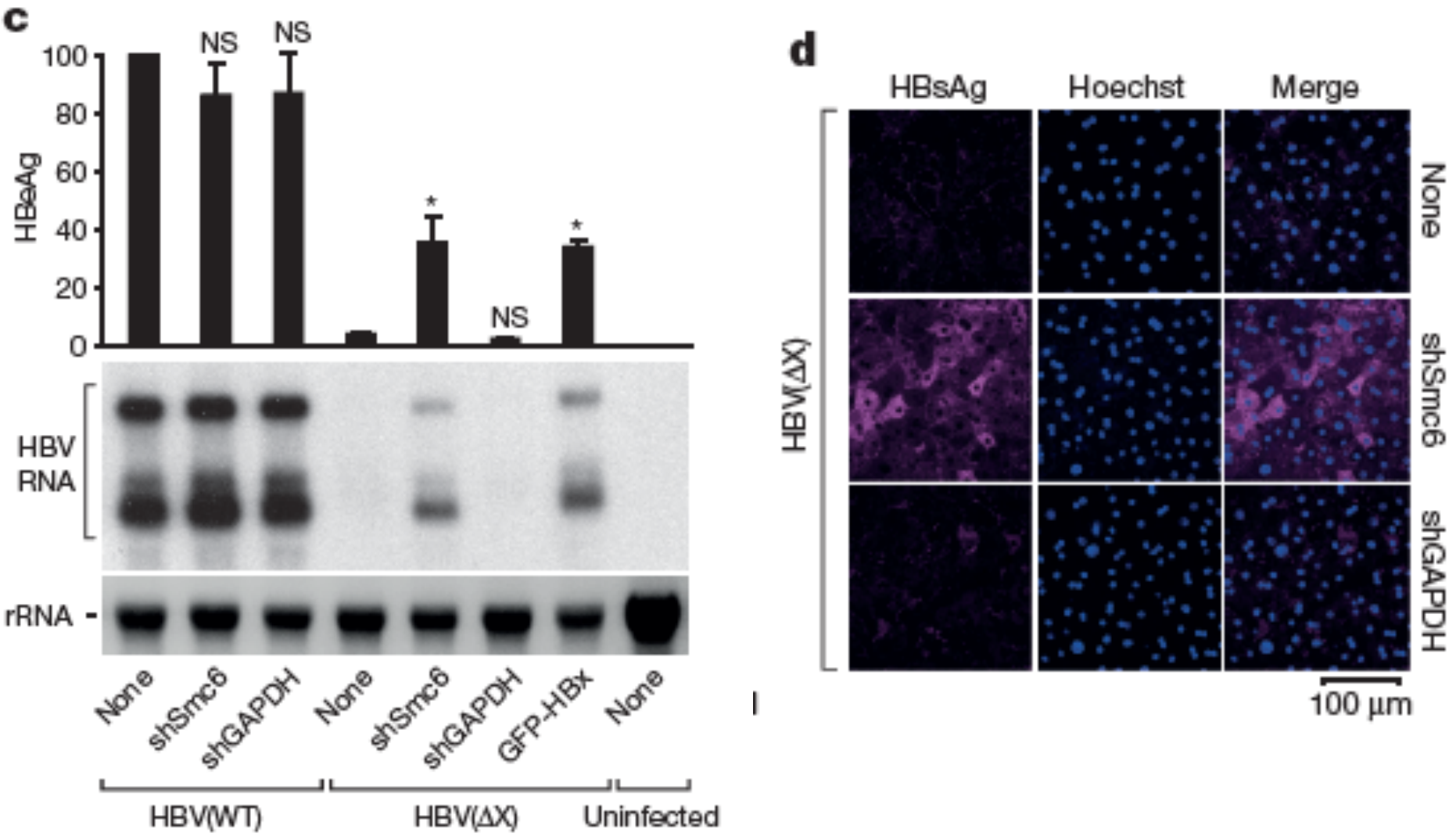




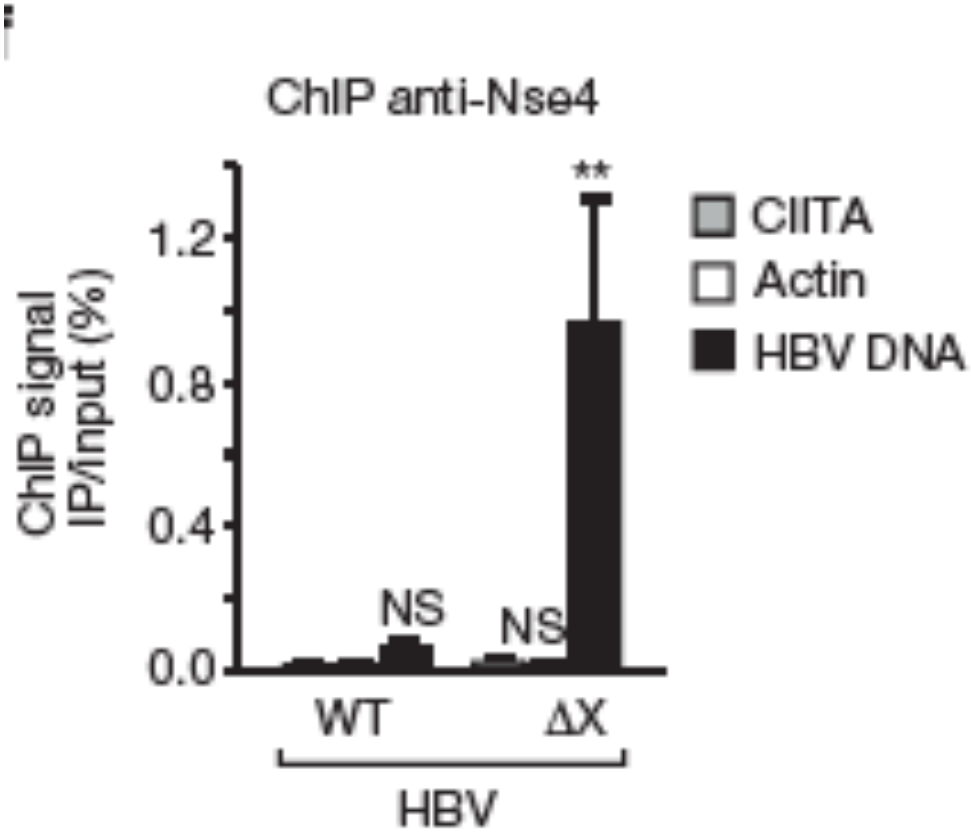
Smc5/6 complexe is a bona fide substrate of the Cul4/DDB1/HBx complexe



Smc5/6 is a restriction factor for HBV replication and its activity is counteracted by HBx



Nse4 a component of Smc5/6 complex is recruited on cccDNA



Conclusions

✓ **Smc5/6 complex is a new HBV restriction factor**

✓ **HBx counteracts the repressive activity of Smc5/6 complex via the induction of its degradation by the CulA/DDB1 ubiquitin ligase**

✓ **How Smc5/6 is recruited on the cccDNA or episomal DNA?**

✓ **Mechanisms involved in cccDNA repression?**

Acknowledgements

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