

# Role of H2A.Z histone variants on HBV replication

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MSD Avenir, ANRS

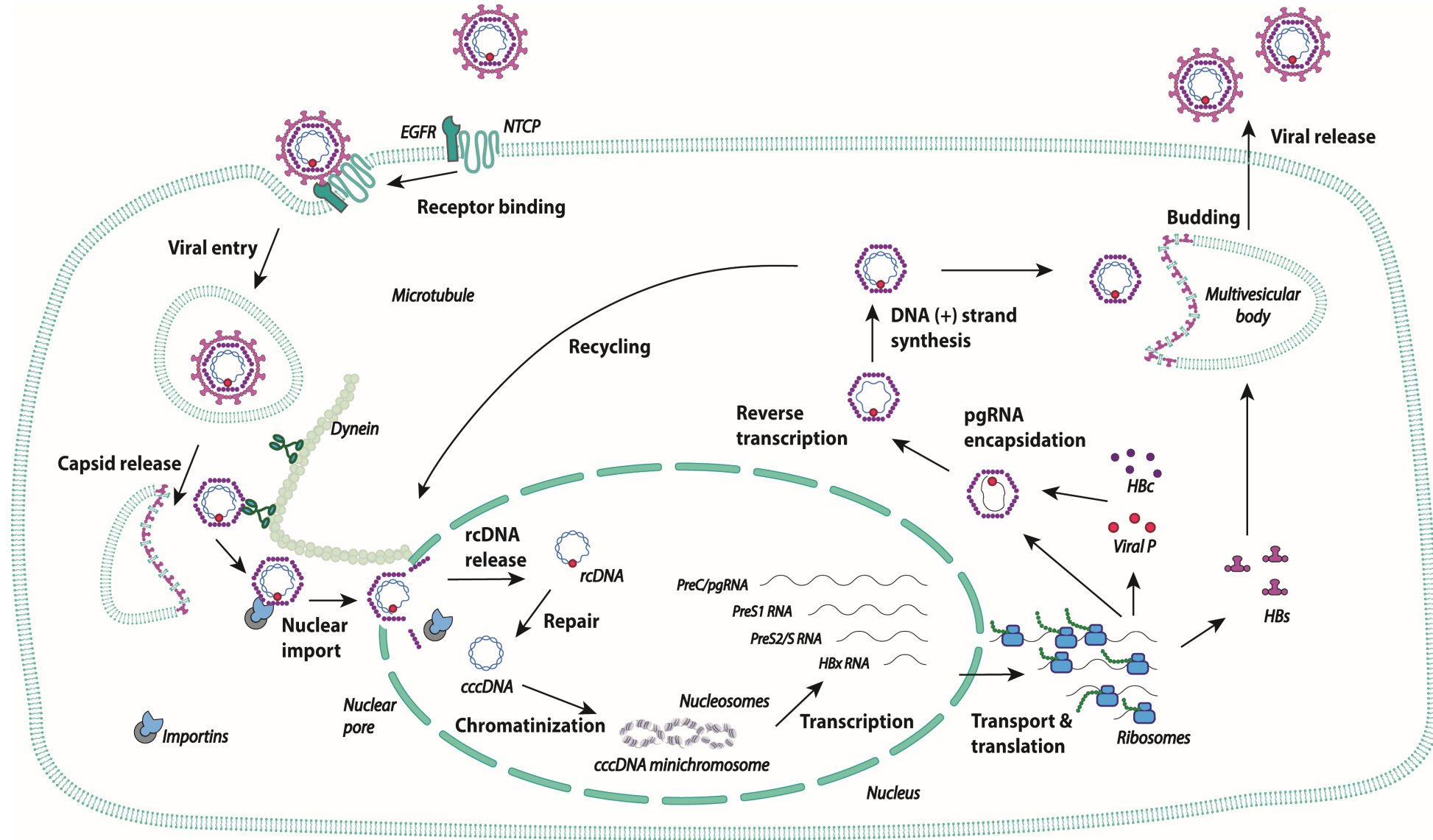
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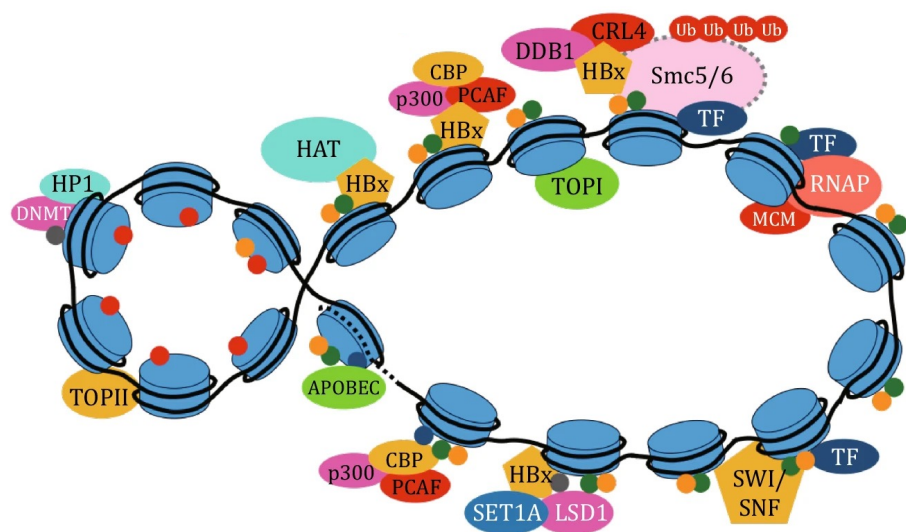


# The hepatitis B viral cycle

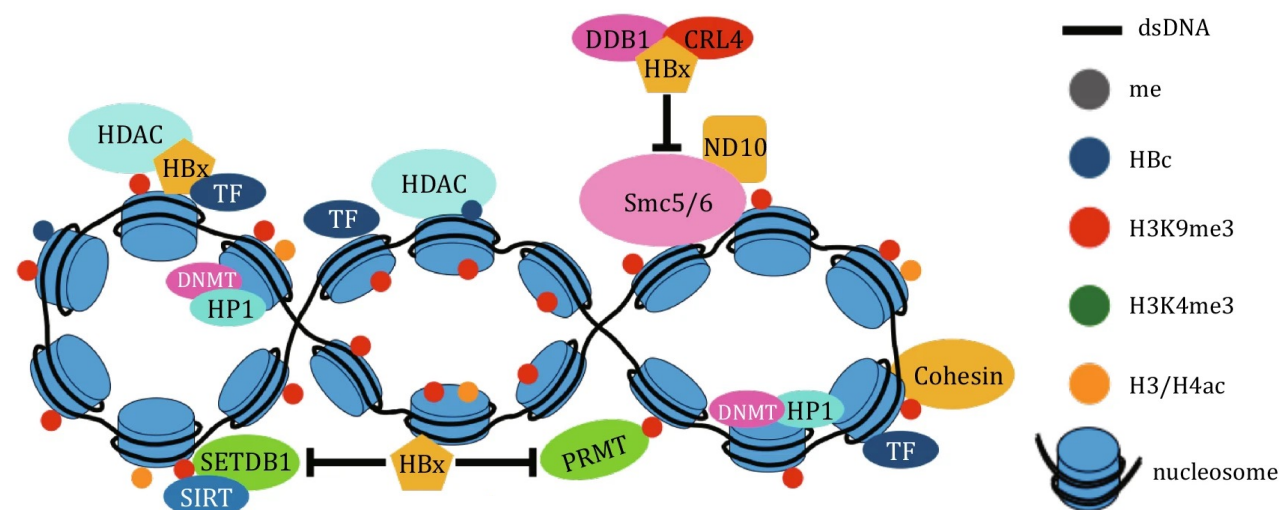


# HBV chromatinization is essential to the HBV life cycle

## Active chromatin



## Repressive chromatin



The makeup of the minichromosome protein landscape is one of the factors determining the viral transcriptional activity

# H2A.Z is present on HBV DNA in infected cells

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cccDNA was purified from primary human hepatocytes and differentiated HepaRG on an iodixanol gradient by ultracentrifugation and the associated proteins were identified by mass spectrometry

## Mass spectrometry results

Name	Score (WT infected cells)	Score (HBx-infected cells)	Previous work
Hepatocyte nuclear factor 4-alpha (HNF4- $\alpha$ )	24,20	N/A	Raney AK, Johnson JL, Palmer CN, McLachlan A. <i>J Virol</i> , 1997
Non-structural maintenance of chromosomes element 1 (NSE1, part of the SMC6 complex)	N/A	35	Decorsière A, et al. <i>Nature</i> , 2016

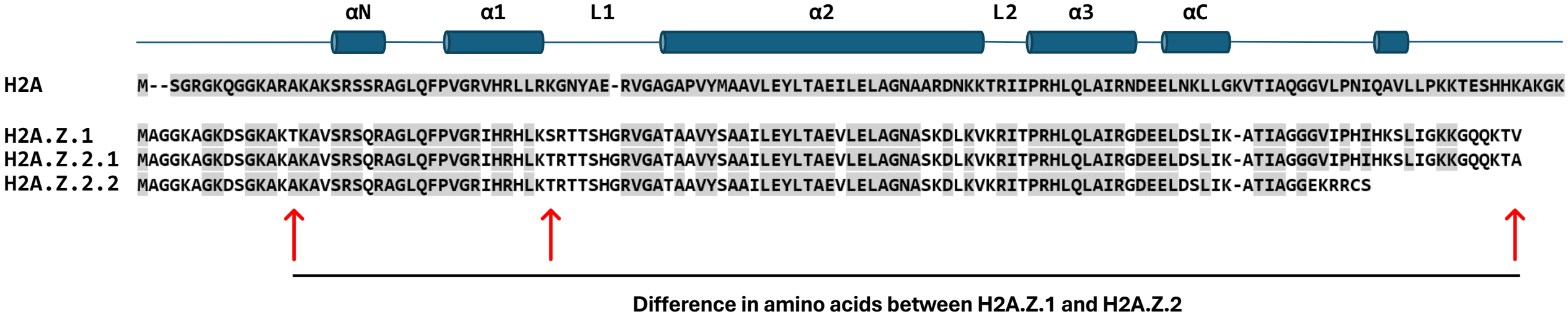
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H2A.Z histone variant 1 (H2A.Z.1)	335,35	N/A	
Snf2 Related CREBBP Activator Protein (SRCAP)	40,54	N/A	
E1A Binding Protein P400 (ep400)	40,86	N/A	

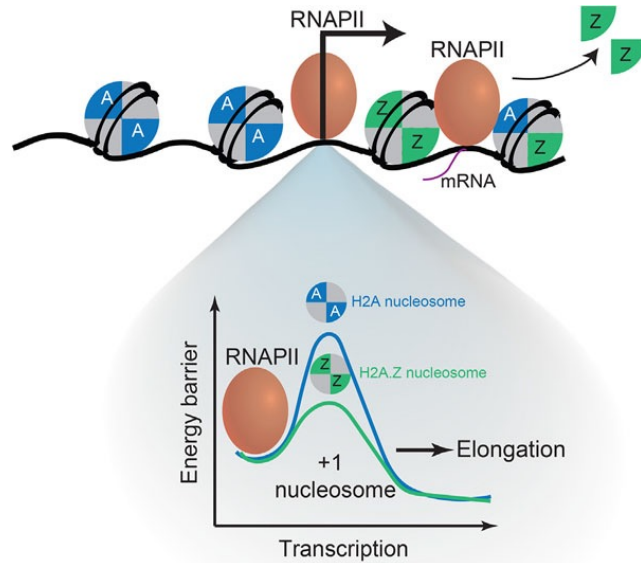
# H2A.Z variants and isoforms in primates





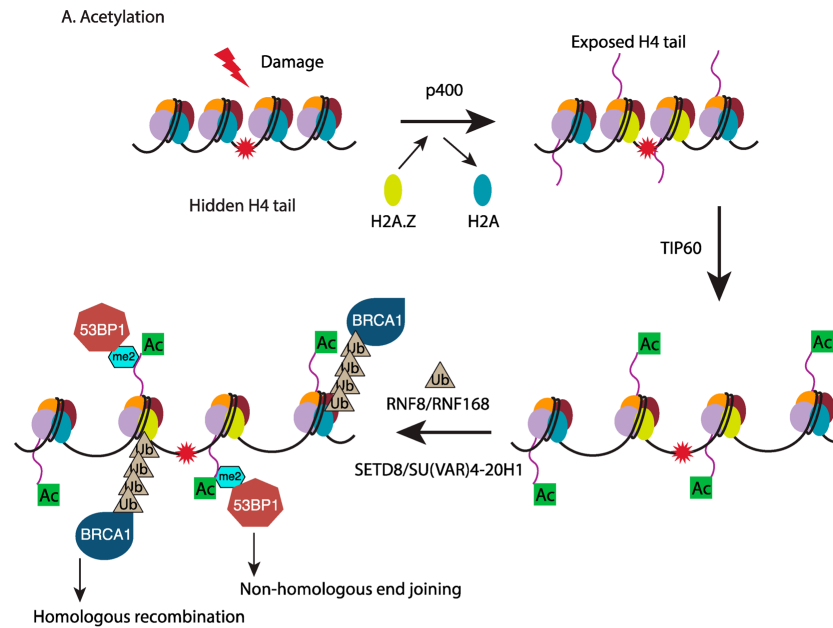
# H2A.Z has roles in DNA transcription, repair and replication

## Transcription



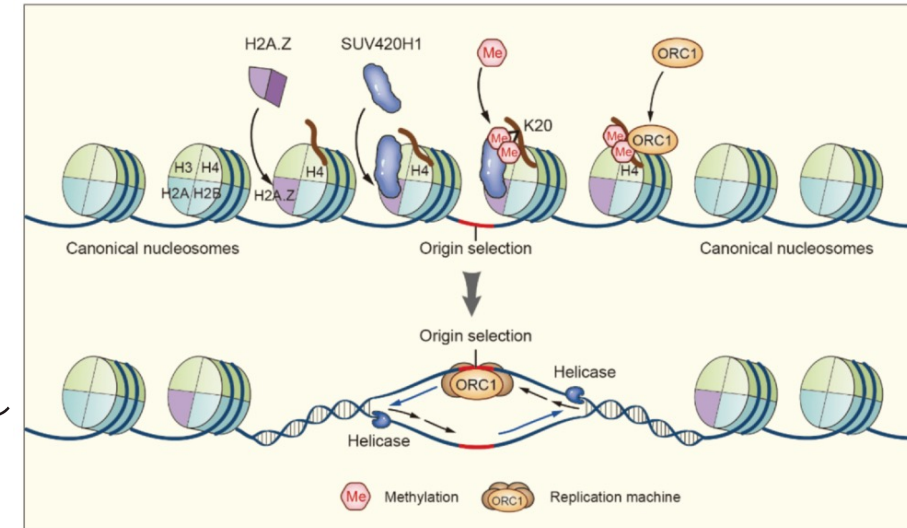
Subramanian V, et al. *F1000Prime Reports*. 2015

## Repair



Nair N, et al. *International Journal of Molecular Sciences*. 2017

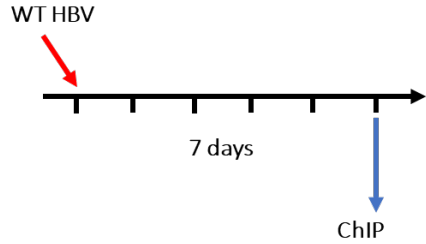
## Replication



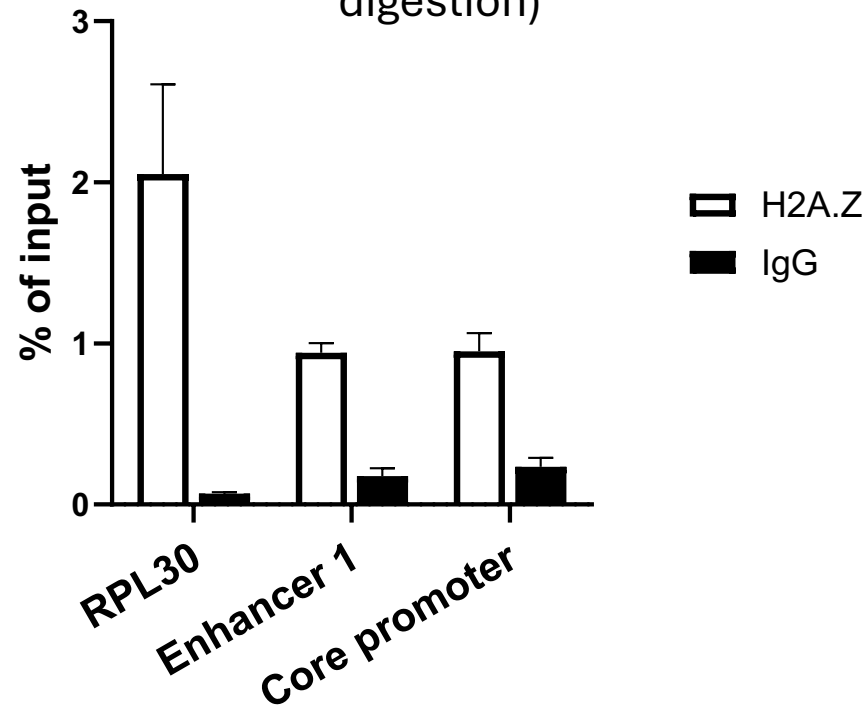
Qiu L, Zeng X, Han J. *Science China Life Sciences*. 2020

# H2A.Z is recruited to the viral chromatin in our infection model

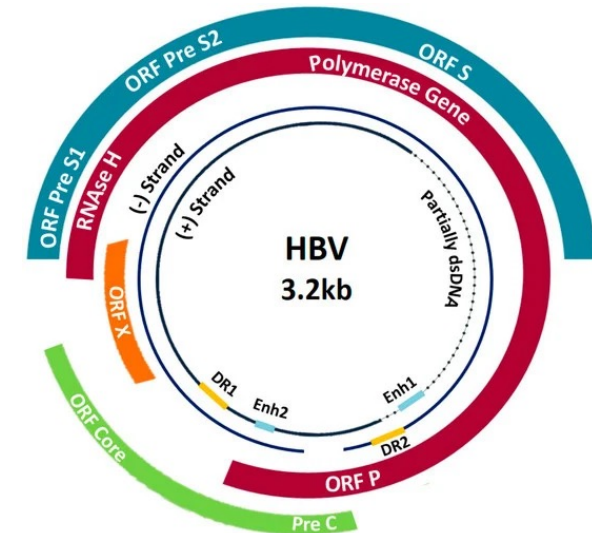
## Experimental design



Chromatin immunoprecipitation in WT HBV infected HepG2 NTCP sec (MNase digestion)



Schematic representation of the HBV genome

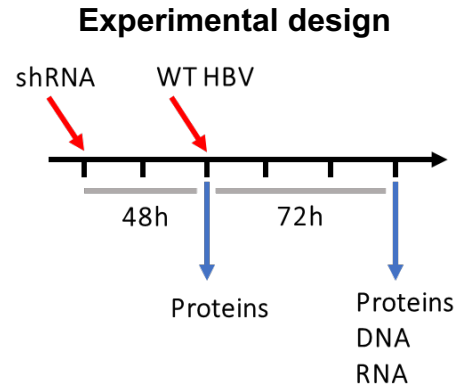


Al-Sadeq DW, et al. *Pathogens*. 2019

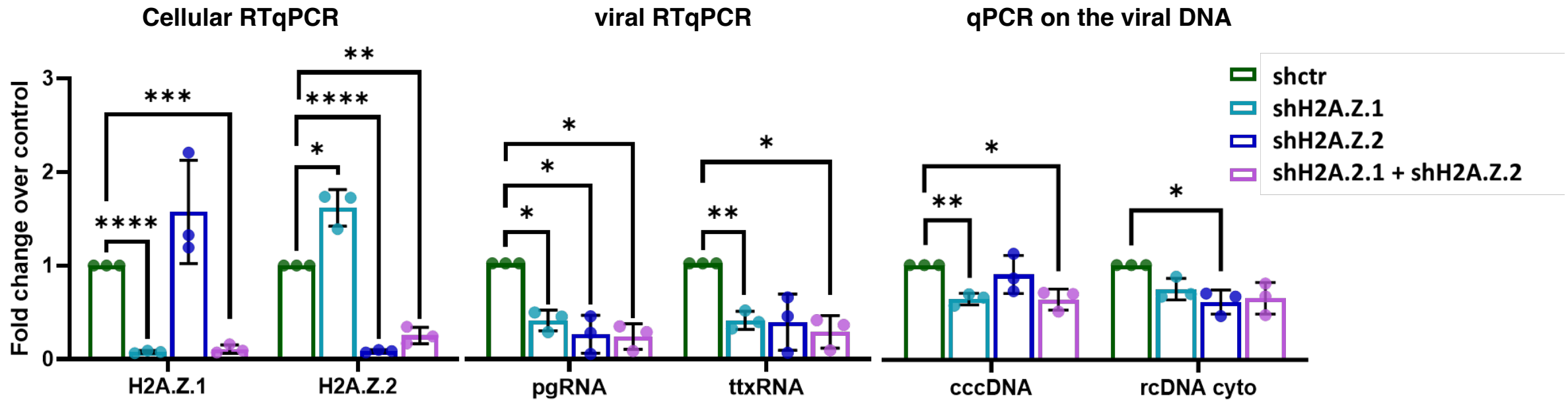
➔ H2A.Z is present on the viral chromatin in our cell model



# Role of H2A.Z.1 and/or H2A.Z.2 on the viral replication



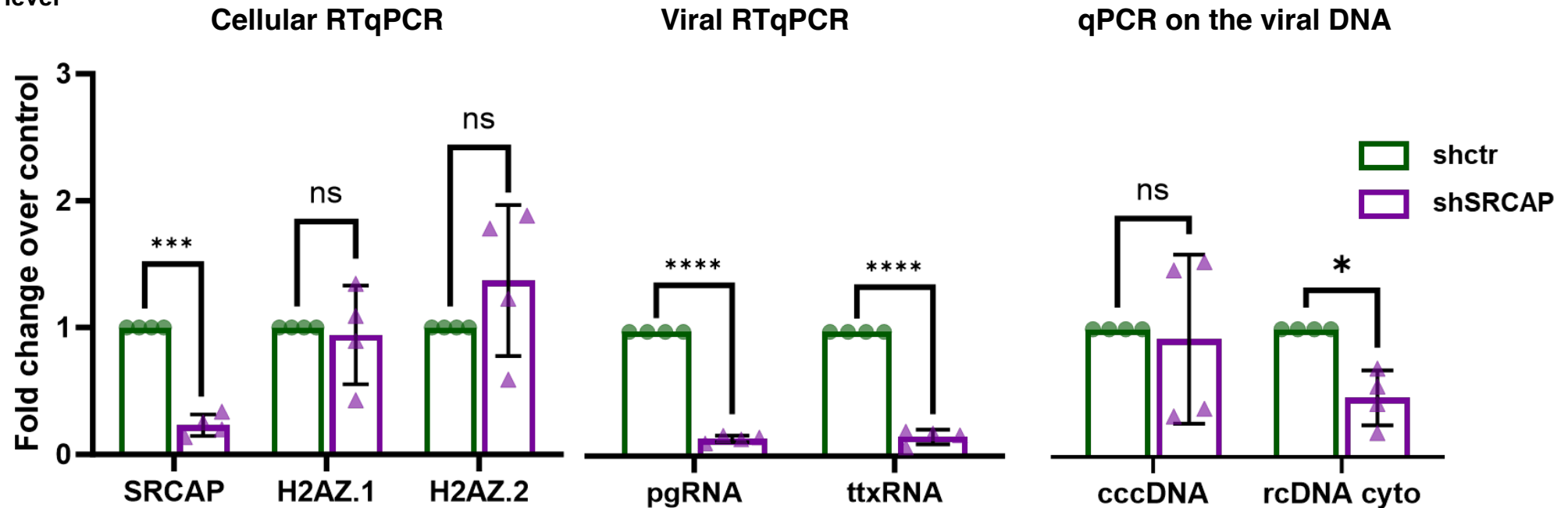
**Knockdown efficiency at protein level**



A decrease in H2A.Z.1 and/or H2A.Z.2 leads to a decrease in viral RNA while only the H2A.Z.1 knock-down seem to affect the cccDNA levels

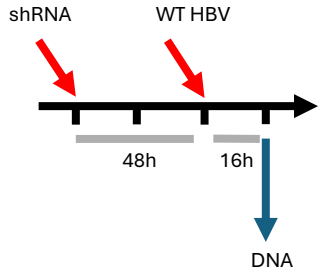
# Role of SRCAP on the viral replication

Knockdown efficiency at protein level

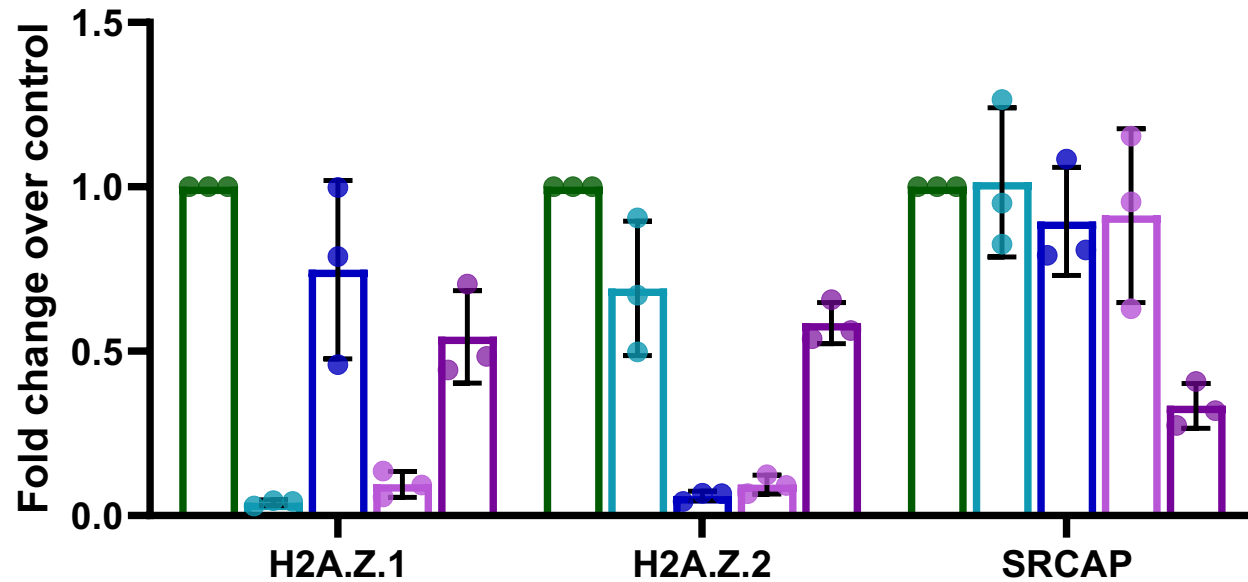


A decrease in SRCAP leads to a sharp decrease in viral RNAs and in the amount of cytoplasmic rcDNA

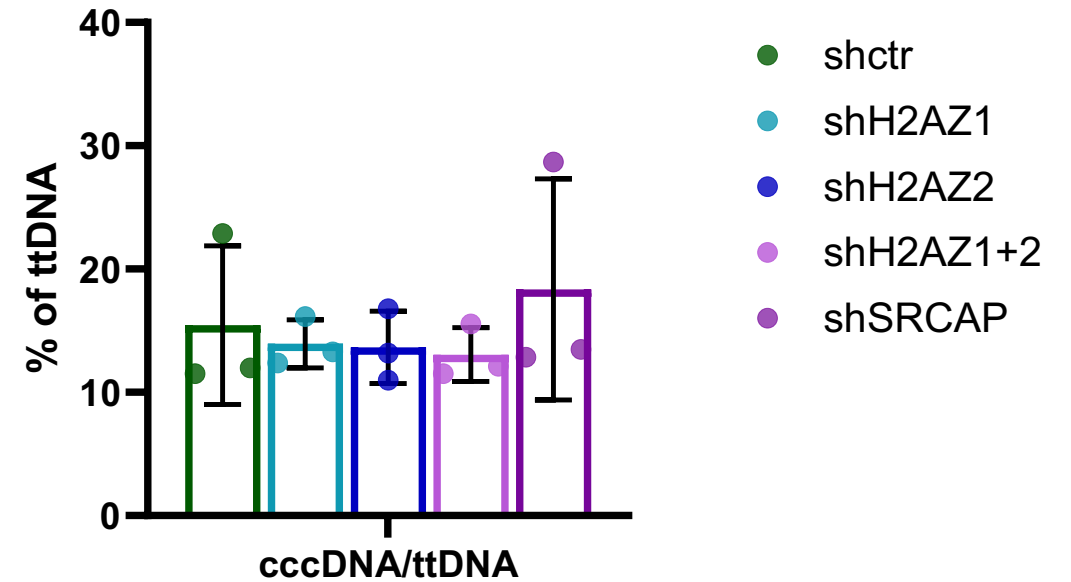
# Impact of H2A.Z and SRCAP on early amounts of viral DNA



Cellular RTqPCR



Viral ddPCR

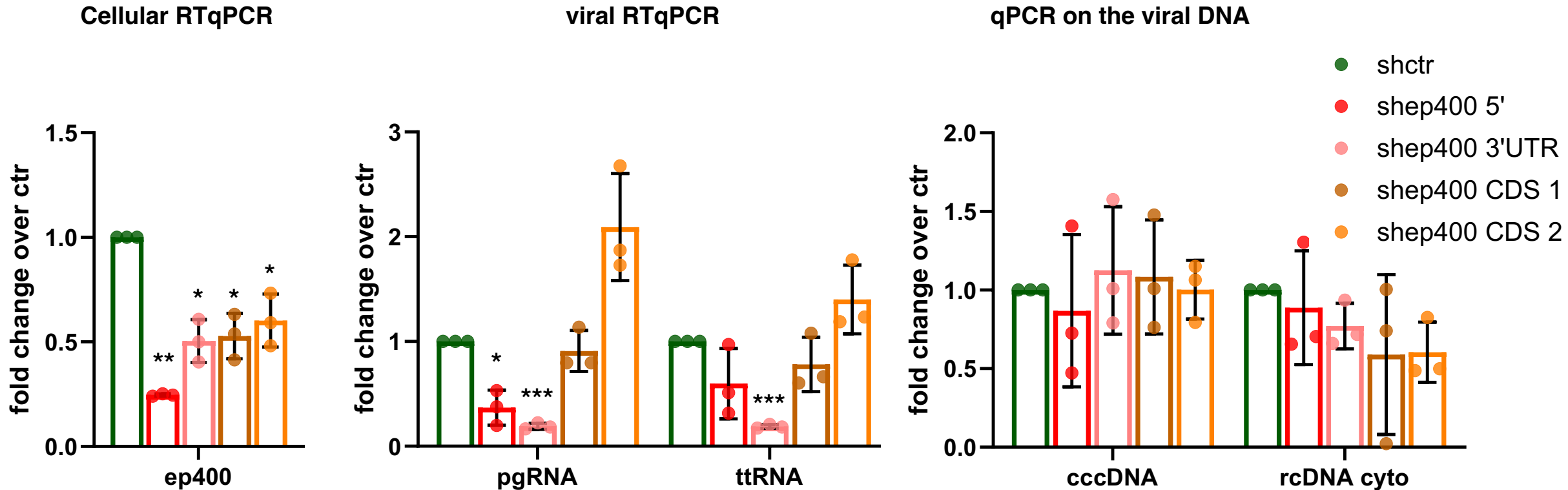


- shctr
- shH2AZ1
- shH2AZ2
- shH2AZ1+2
- shSRCAP



Decreasing the amounts of H2A.Z.1, H2A.Z.2 and SRCAP does not seem to have an impact on early amounts of cccDNA in the nuclei

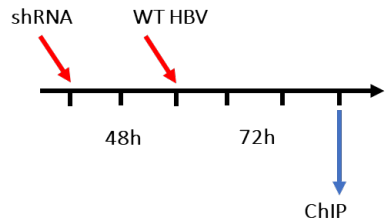
# Role of ep400 activity on the viral replication



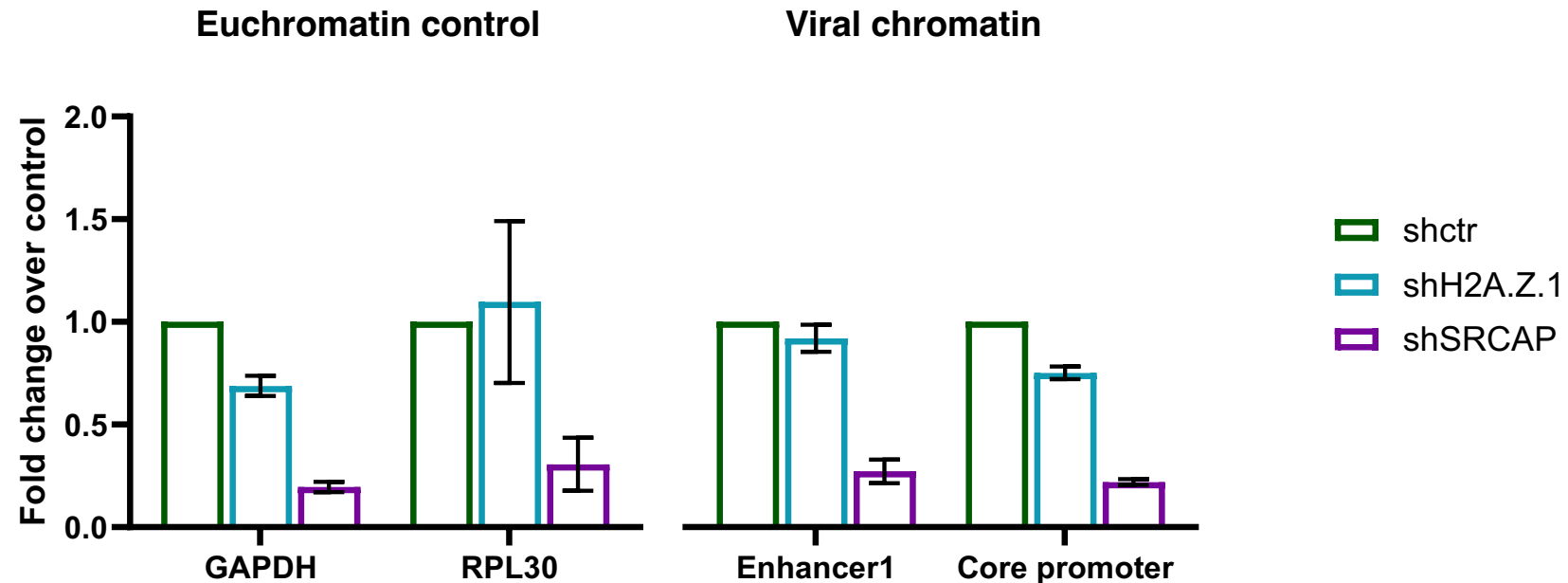
Knocking-down ep400 does not reliably lead to a decrease in viral RNA or DNA levels

# Role of H2A.Z on HBV chromatin status using silencing approaches

## Experimental design



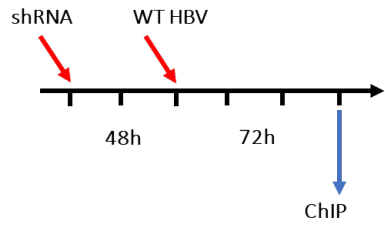
## Chromatin immunoprecipitation of H2A.Z associated DNA in WT HBV infected cells



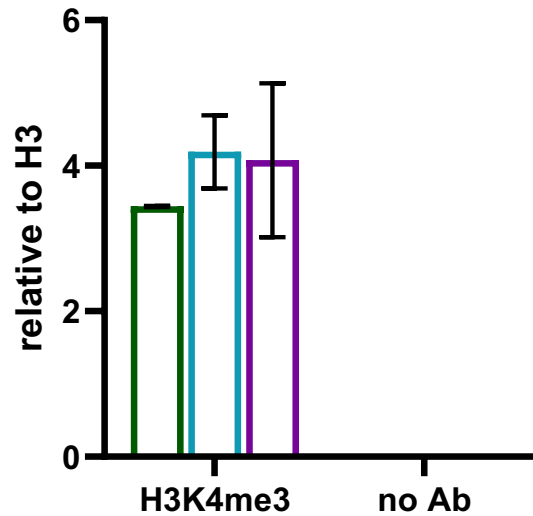
**Knocking-down H2A.Z.1 or SRCAP leads to a decrease in H2A.Z occupancy of the cellular and viral chromatin**

# Role of H2A.Z on HBV chromatin status using silencing approaches

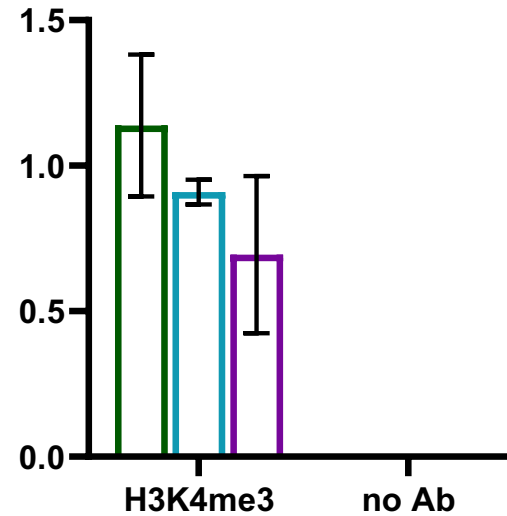
## Experimental design



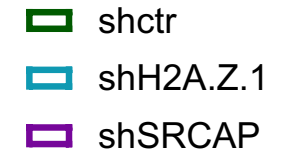
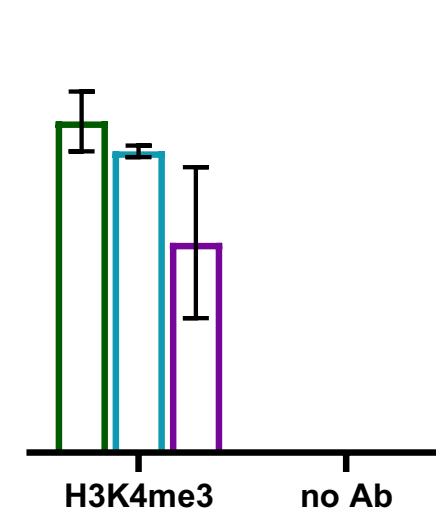
Euchromatin control (RPL30)



HBV enhancer 1



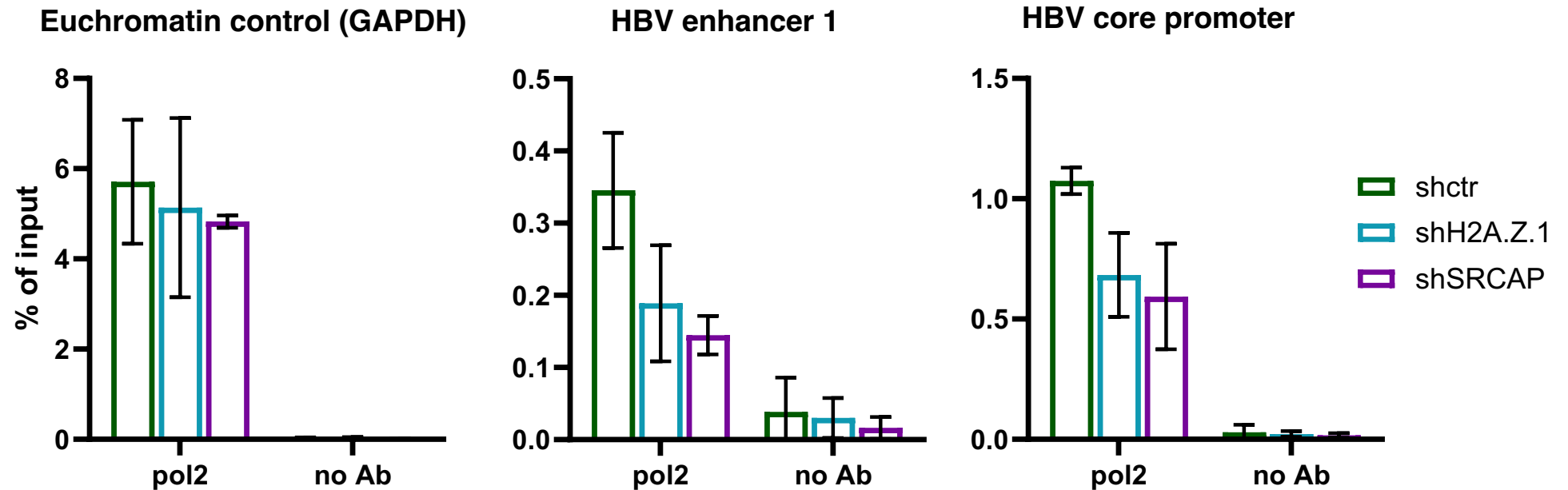
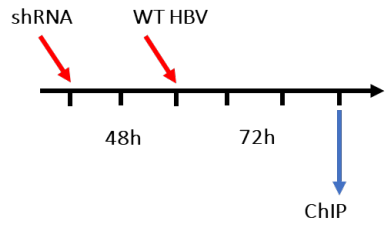
HBV core promoter



**Knocking-down H2A.Z.1 or SRCAP decreases the presence of the H3K4me3 histone mark on the viral chromatin**

# Impact of H2A.Z on RNAPolII recruitment on the viral chromatin

## Experimental design

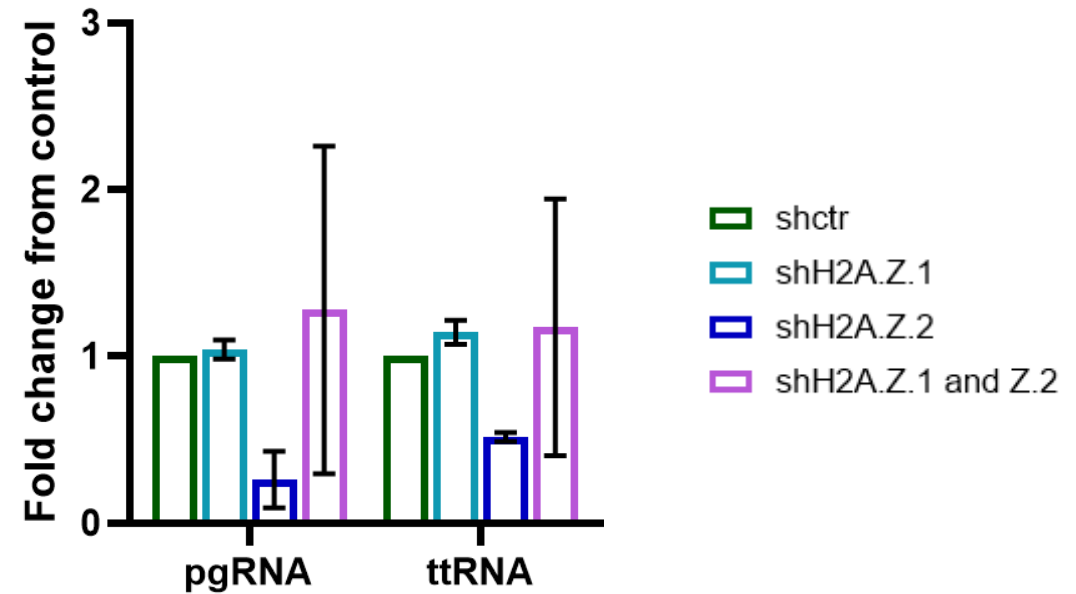
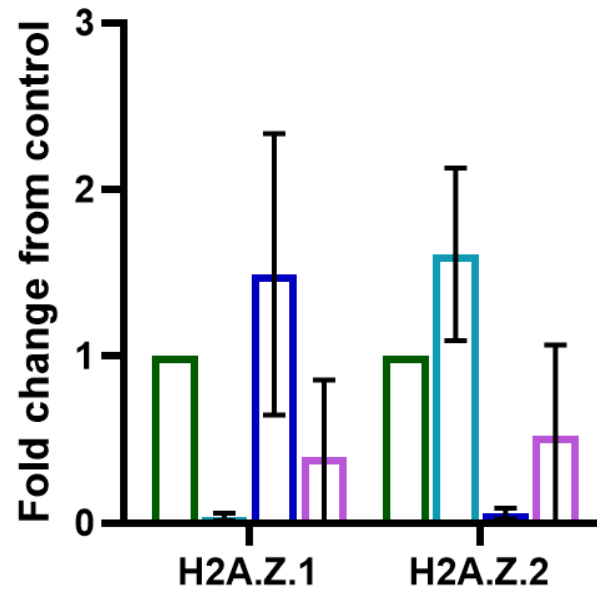
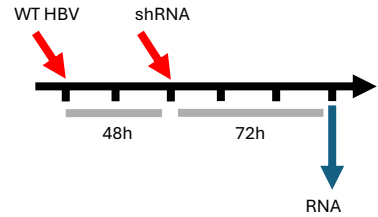


**Knocking-down H2A.Z.1 or SRCAP decreases the presence of the RNAPolII on the viral chromatin**



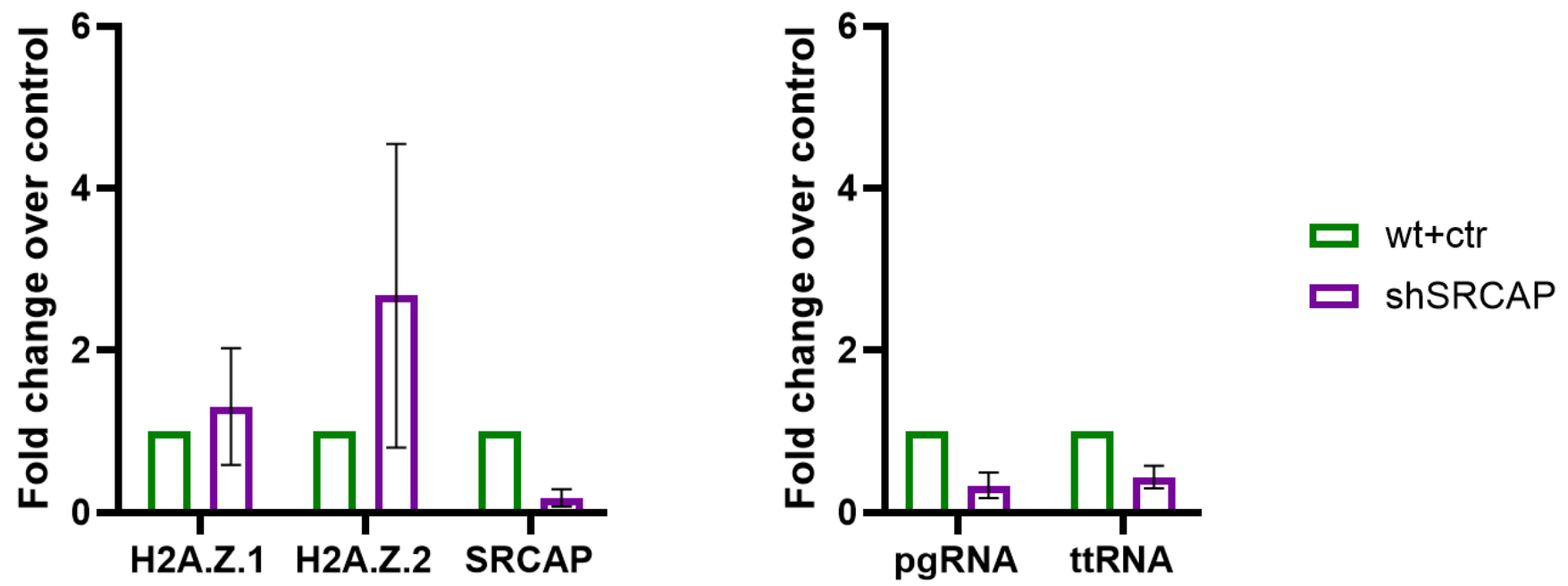
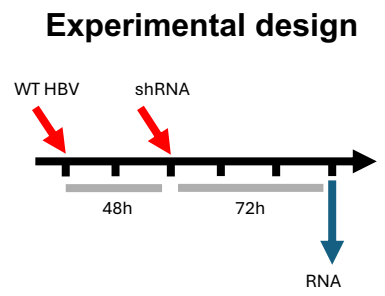
# Role of H2AZ.1 and H2AZ.2 on HBV transcription in primary human hepatocytes infected by HBV

## Experimental design



A decrease in H2A.Z.2 but not H2A.Z.1 leads to a decrease of both pre genomic and total viral RNA in primary human hepatocytes

# Role of SRCAP on HBV transcription in primary human hepatocytes infected by HBV



➔ Knocking-down SRCAP leads to a decrease in the amount of viral RNAs

# Conclusion and perspectives

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- H2A.Z is recruited on the viral chromatin
- Knocking-down H2A.Z.1 and/or H2A.Z.2 leads to a decrease in viral RNA while only the H2A.Z.1 knock-down seems to affect the cccDNA levels at later timepoints
- Knocking-down SRCAP but not ep400 leads to a sharp decrease in amounts of viral RNAs
- H2A.Z.1 or SRCAP silencing correlates with a decrease in H2A.Z recruitment, active histone marks deposition and RNA polymerase II recruitment
- In primary human hepatocytes the SRCAP and H2A.Z.2 KD phenotypes are replicated but not the H2A.Z.1 KD one

# Ongoin

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- Evaluating chromatin accessibility in knock-down conditions with ATACseq
- Assessing whether SRCAP activity is only dependant on H2A.Z deposition using SRCAP ATPase mutants
- Furthering the analysis of chromatin marks in knock-down conditions
- Studying the early loading of H2A.Z on the viral chromatin and the effect on cccDNA establishment
- Testing whether HIRA and SRCAP relative activities affect eachother
- Validating the results in primary human hepatocytes

# Acknowledgments



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