

# Identification of DNAJB12 as a chaperone involved in the secretion of HBsAg and a target of the NAPs REP 2139.

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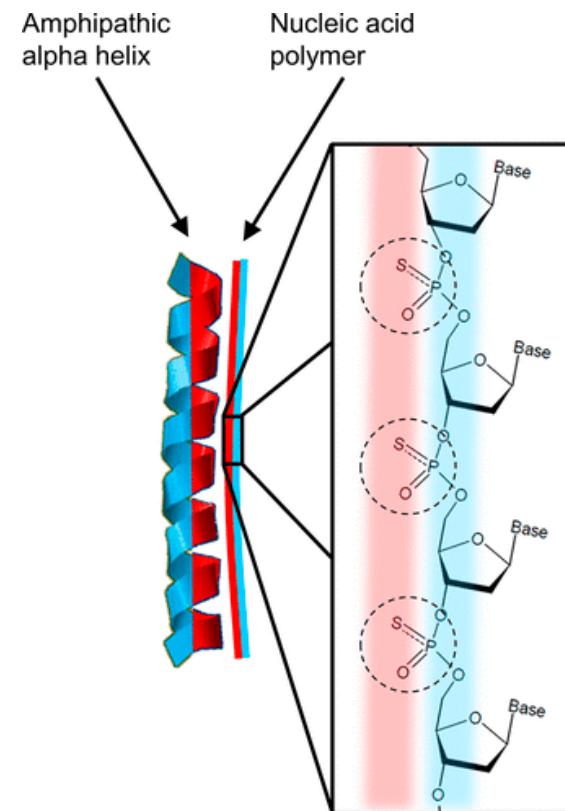
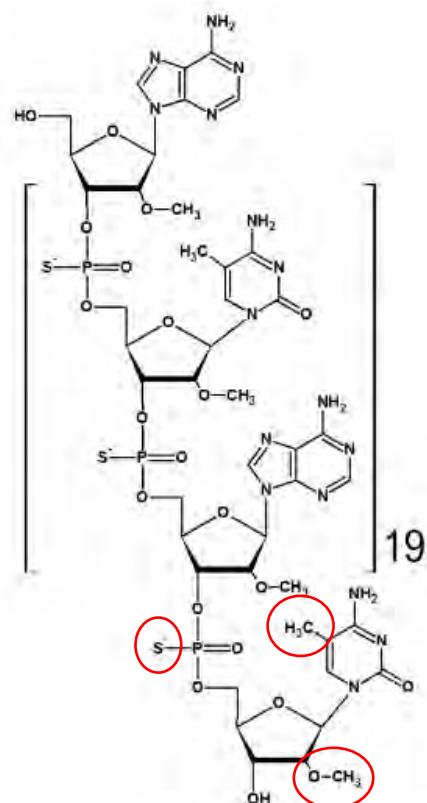


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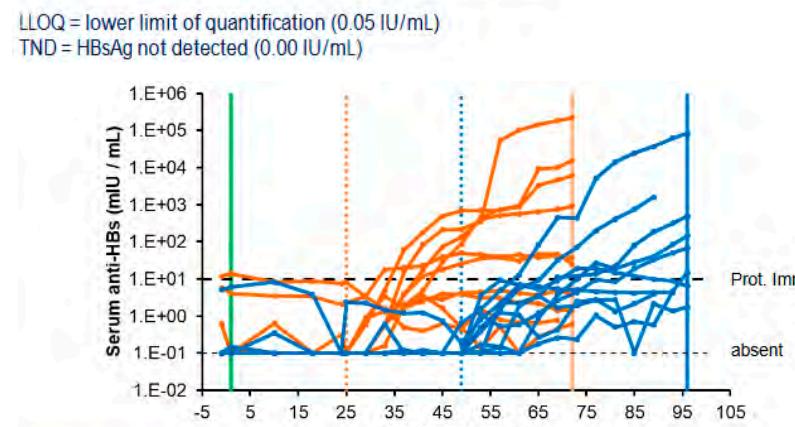
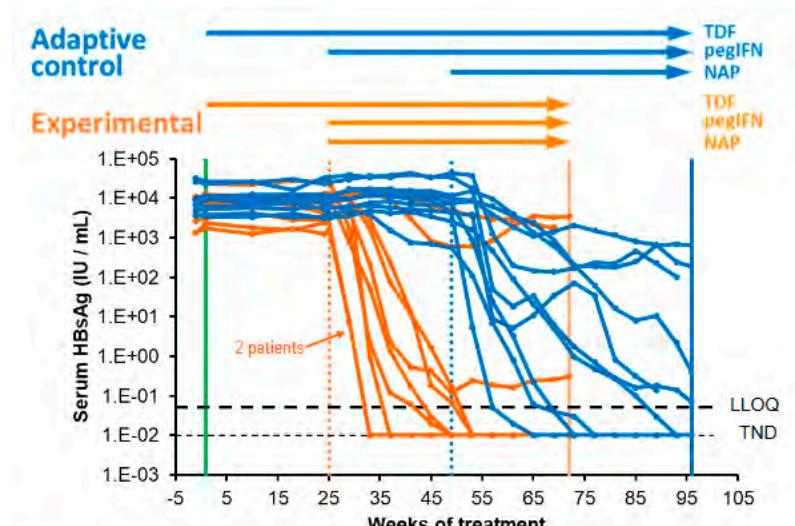
## NAPs : Nucleic acid polymers

**REP 2139**

$(2'\text{OMeA}, 2'\text{OMe}-5'\text{MeC})_{20}$   
Phosphorothioate oligonucleotide

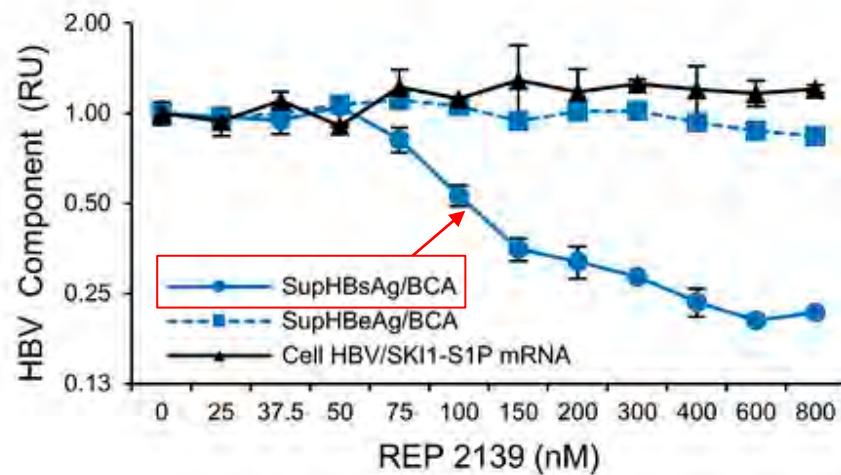


# Effect of REP 2139: inhibition of HBsAg secretion



**Clinical trials: main effect =  
loss of HBsAg secretion**

## In vitro model: loss of HBsAg secretion

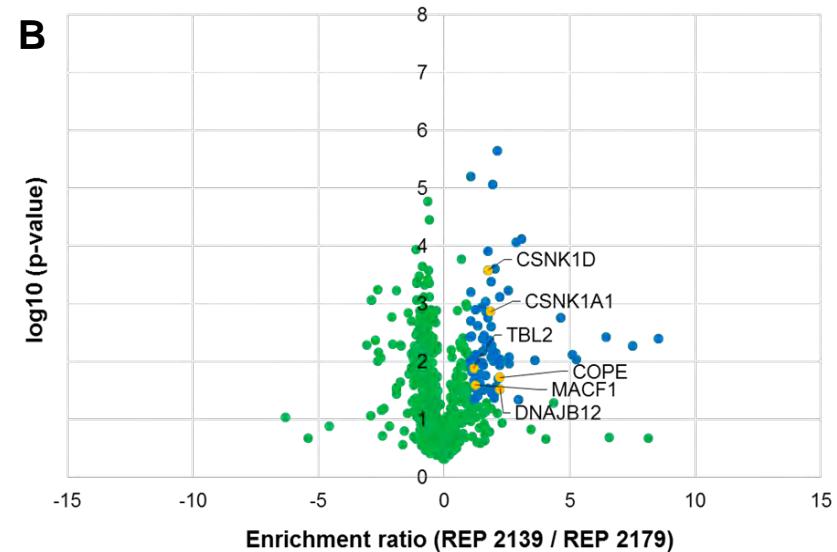
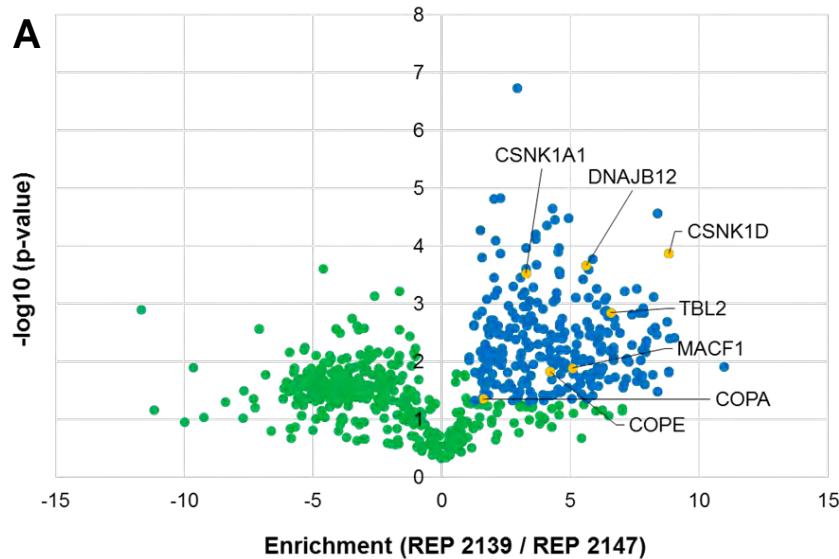


Identification of the mechanism-of-action ?

- Bazinet et al., Gastroenterol 2020
- Bazinet et al., J Viral Hep 2021
- Bazinet et al., Hepatol Comm 2021
- Blanchet et al, AntiViral Research 2019

# Proteins interacting with REP2139

## Mass spectrometry results:

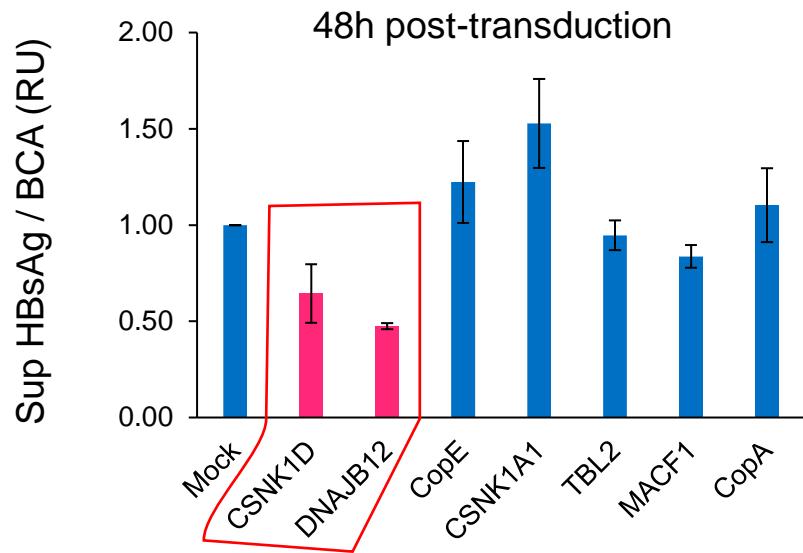


7 potential targeted proteins : CSNK1D ; DNAJB12 ; CopE ; CSNK1A1 ; TBL2 ; MACF1 ; CopA

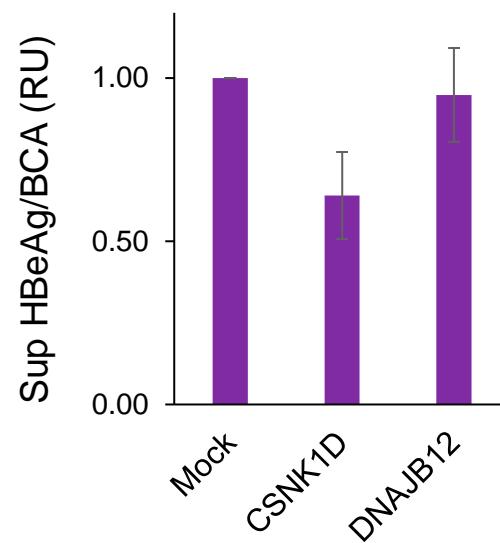
No interaction found with viral proteins

# Effects on HBsAg and HBeAg secretion

## Effect on HBsAg secretion :



## Effect on HBeAg secretion :

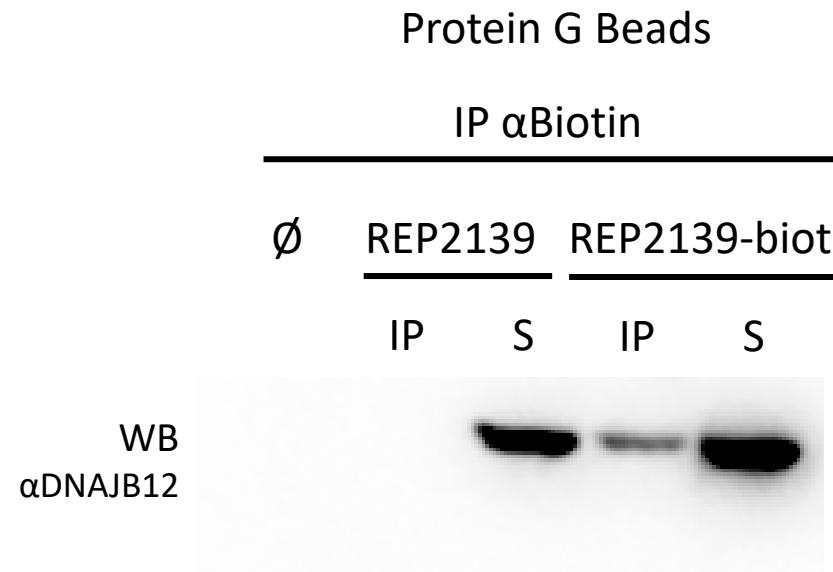


### DNAJB12 :

↓ HBsAg secretion  
= HBeAg secretion } Possible REP 2139 target

# Interaction with DNAJB12

## Pull-down of REP 2139–biotinylated :

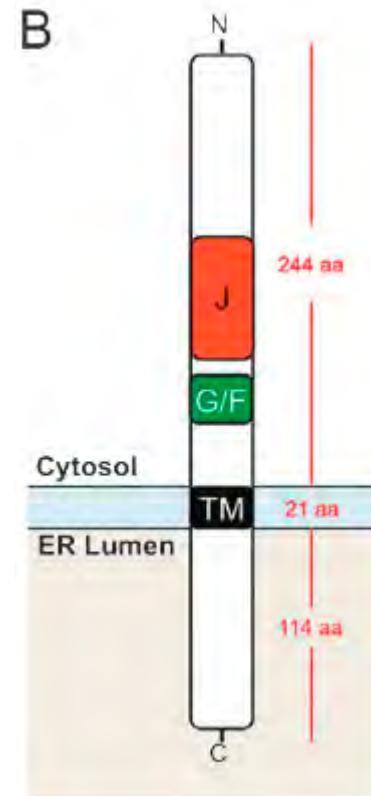
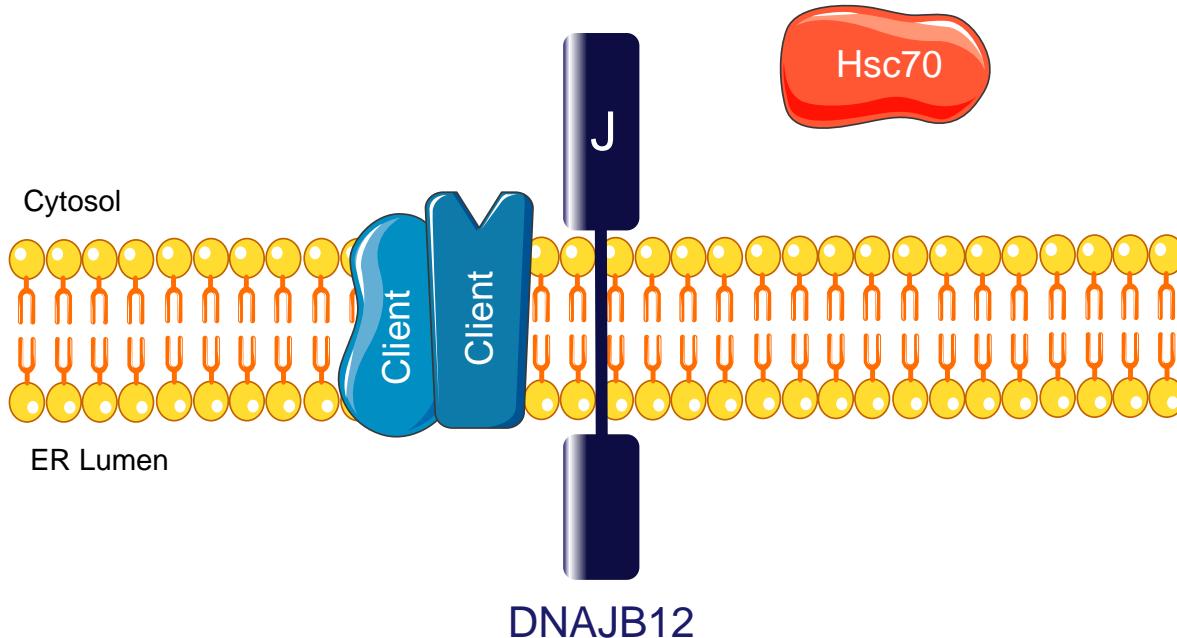


DNAJB12 is pull-downed by REP 2139-biot, confirming their interaction.

# What is DNAJB12 ?

## DNAJB12

- Hsp40/J-protein family
- Endoplasmic reticulum
- Co-chaperone with Hsc70/Hsp70  
→ folding and degradation

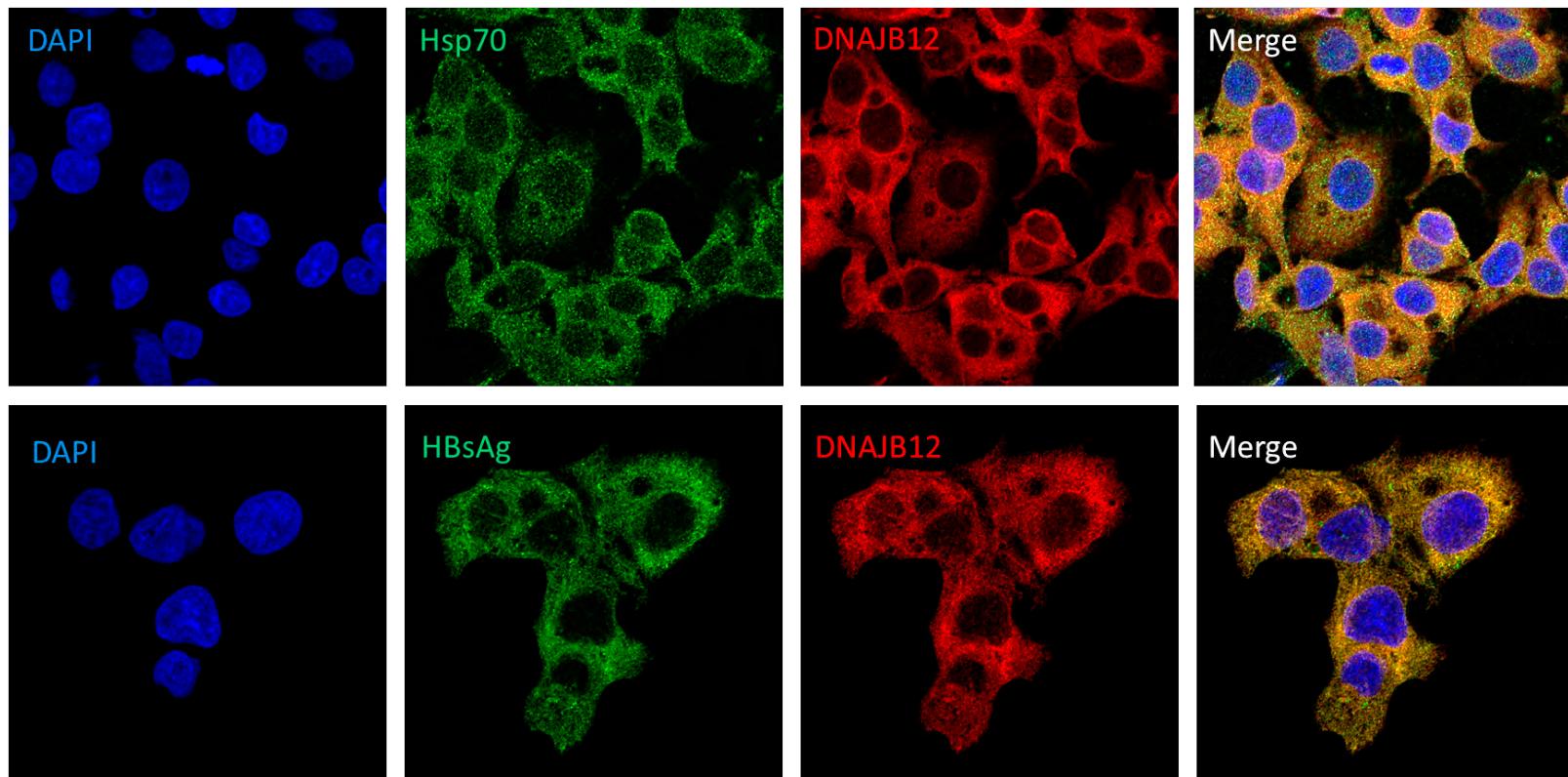


Sophia P. et al, 2012

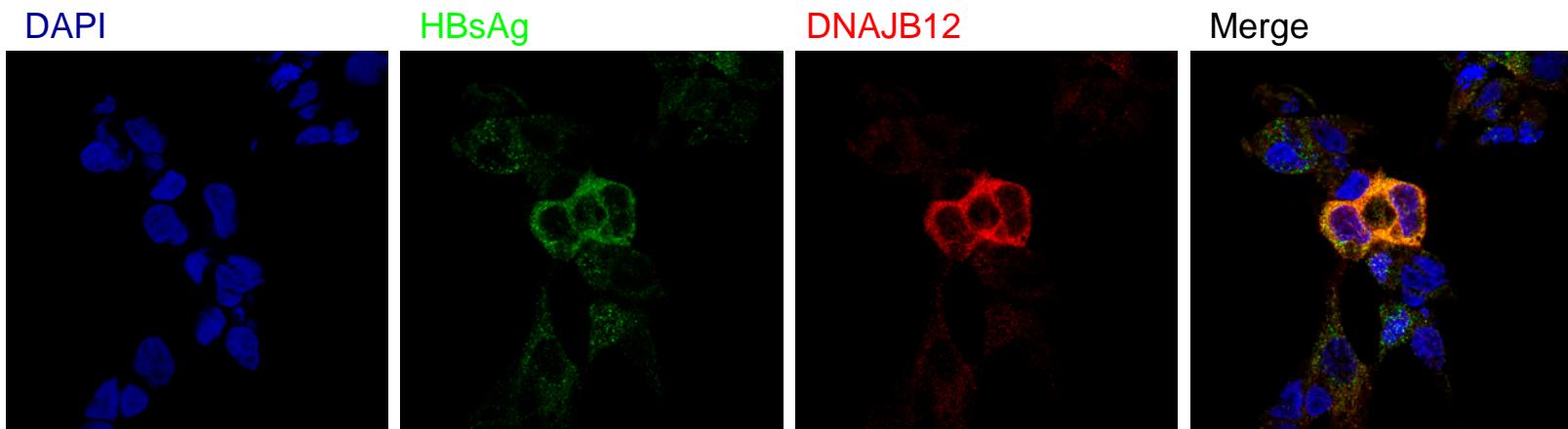
Proteasomal  
degradation

# Colocalization of DNAJB12, HBsAg and Hsp70

HepG2.2.15

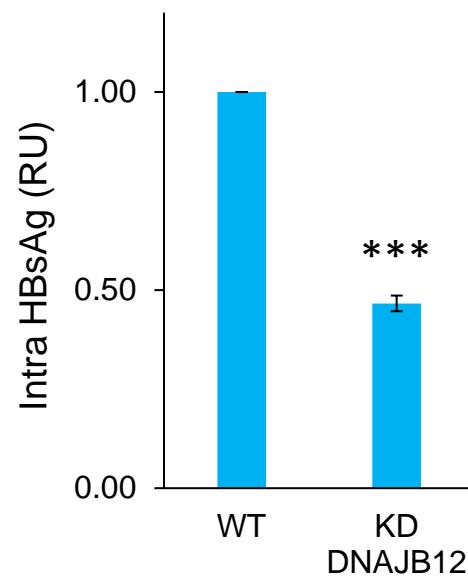
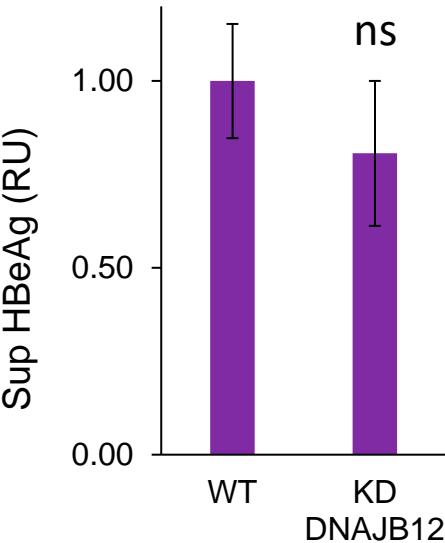
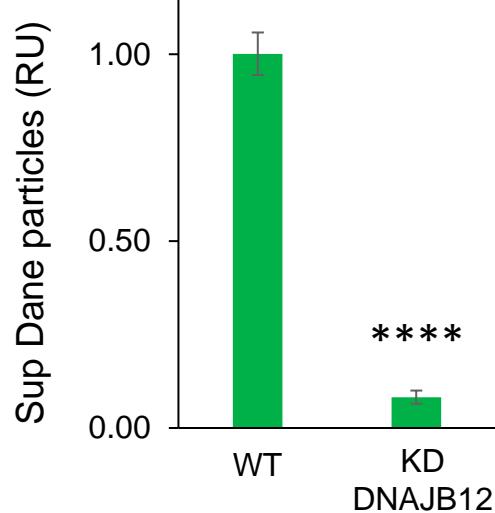
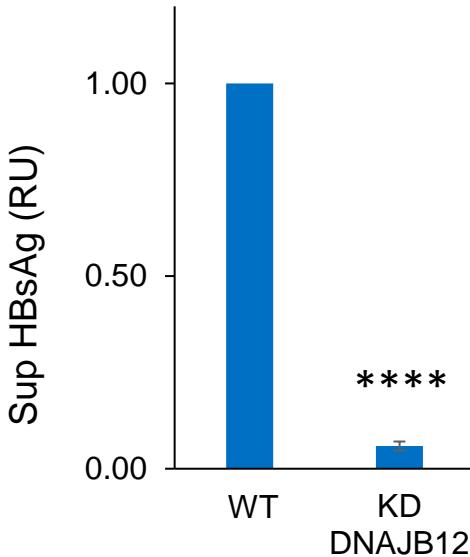
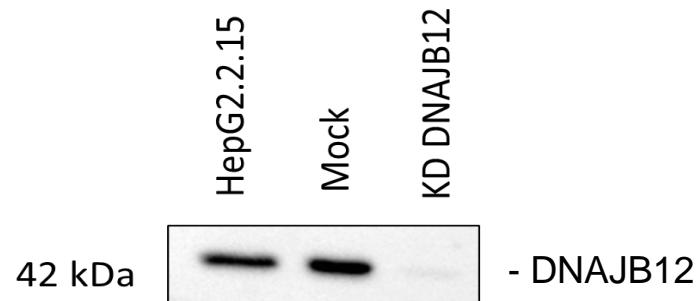


DNAJB12 KD

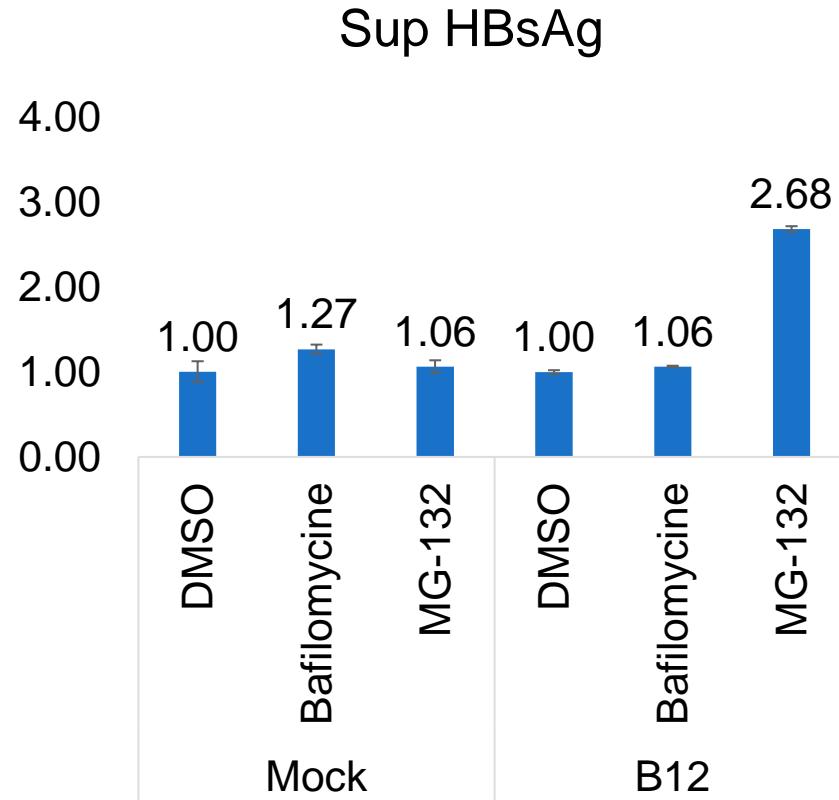
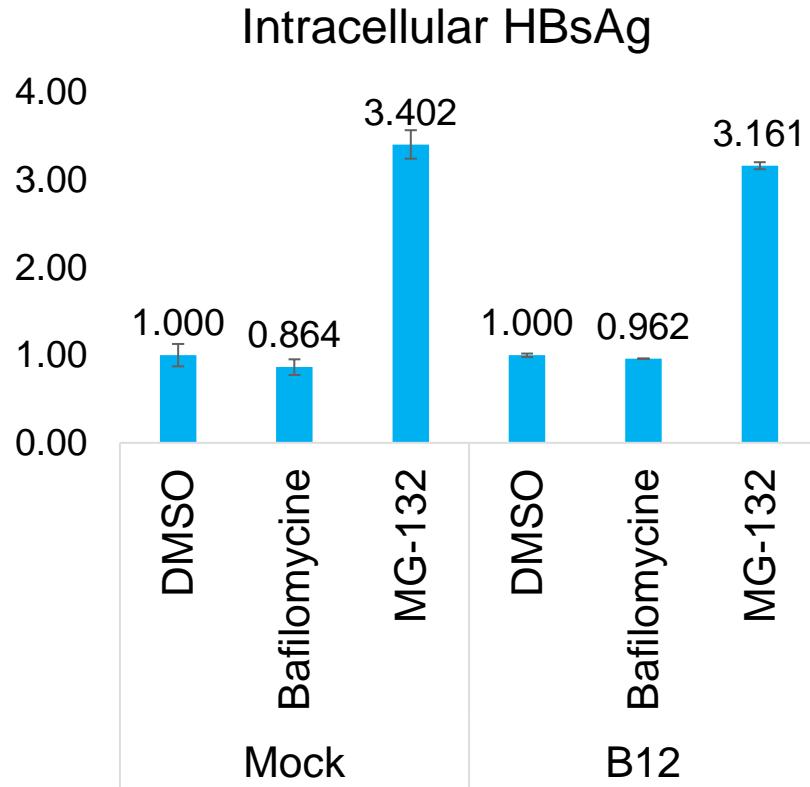


# Knock-down of DNAJB12

At 6d post-transduction :



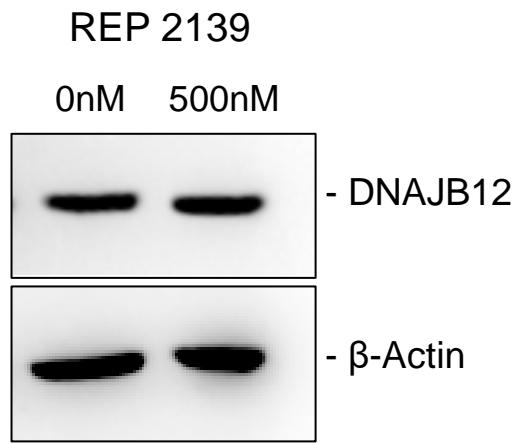
# DNAJB12 KD lead HBsAg to ERAD



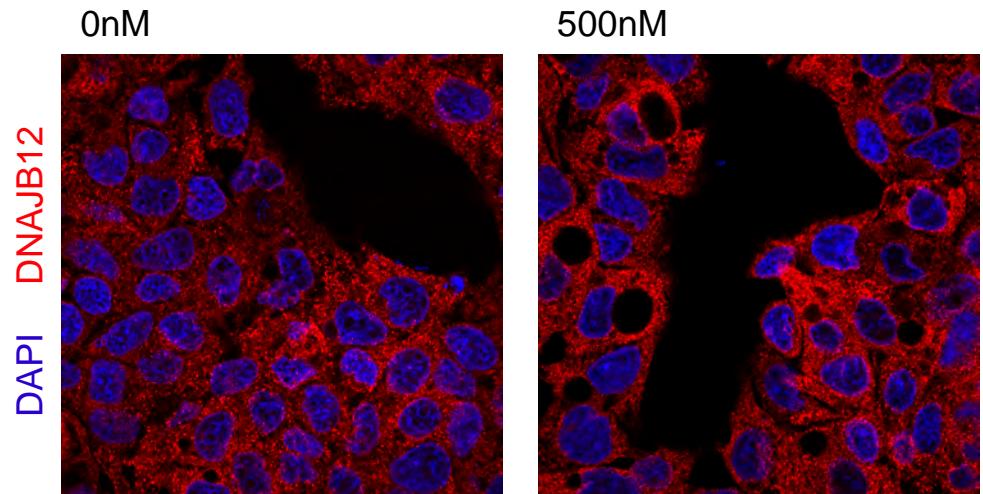
Inhibition of proteasome leads to intracellular accumulation of HBsAg.

# Does REP 2139 affects DNAJB12 ?

## Stability of DNAJB12:

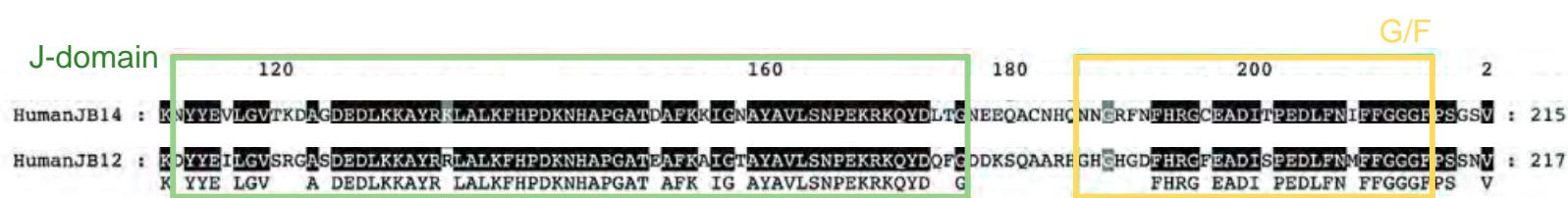
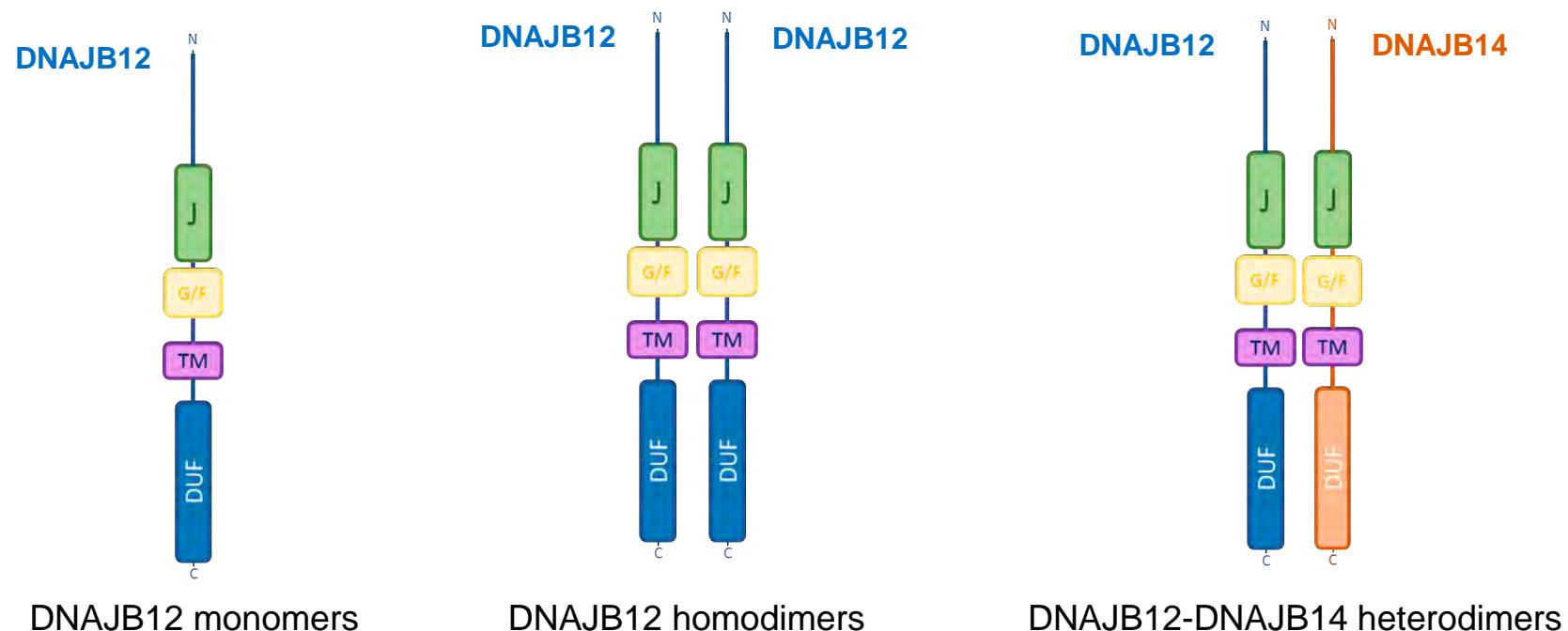


## Cellular localization of DNAJB12:



REP 2139 does not alter the stability of DNAJB12 nor its cellular localization

# What about DNAJB14 ?

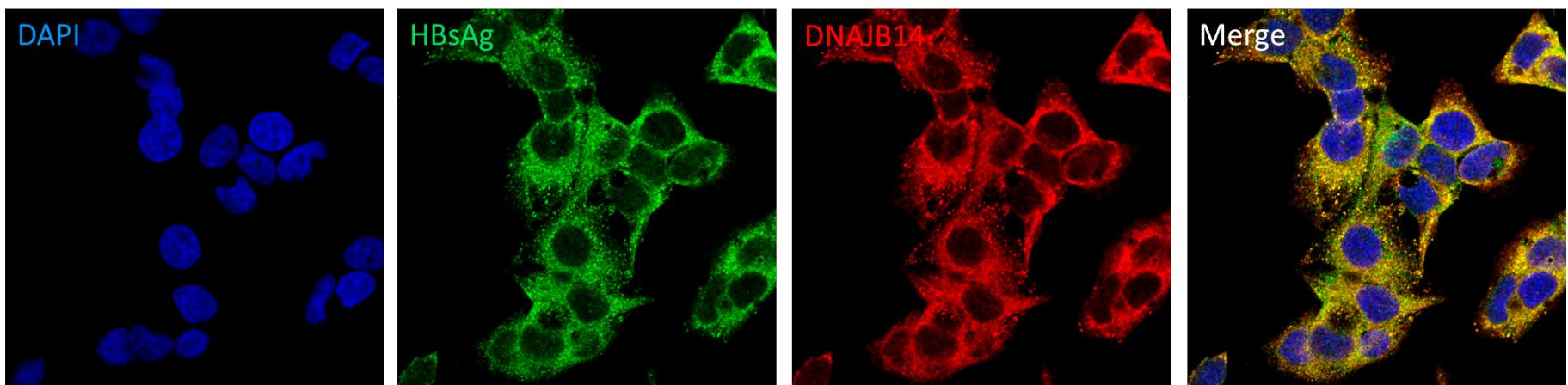


## DNAJB14 :

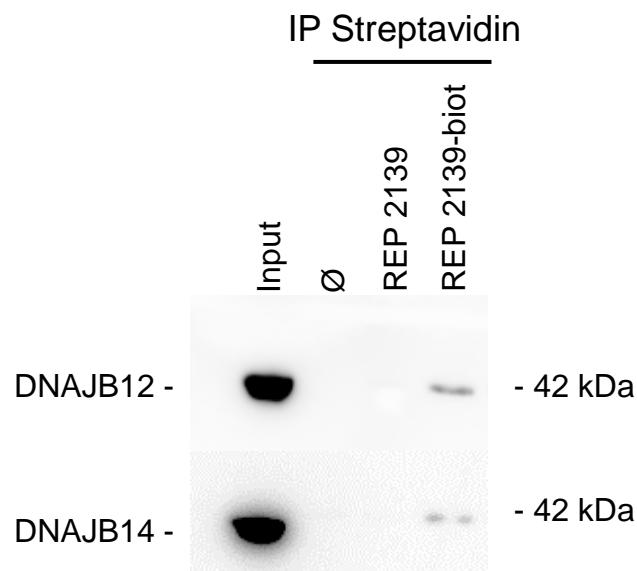
- Share some protein clients as heterodimer with DNAJB12

Sopha P. et al., 2012  
 Walczak C. et al., 2014  
 Rozales K. et al., 2022  
 Li K. et al., 2017

# What about DNAJB14 ?

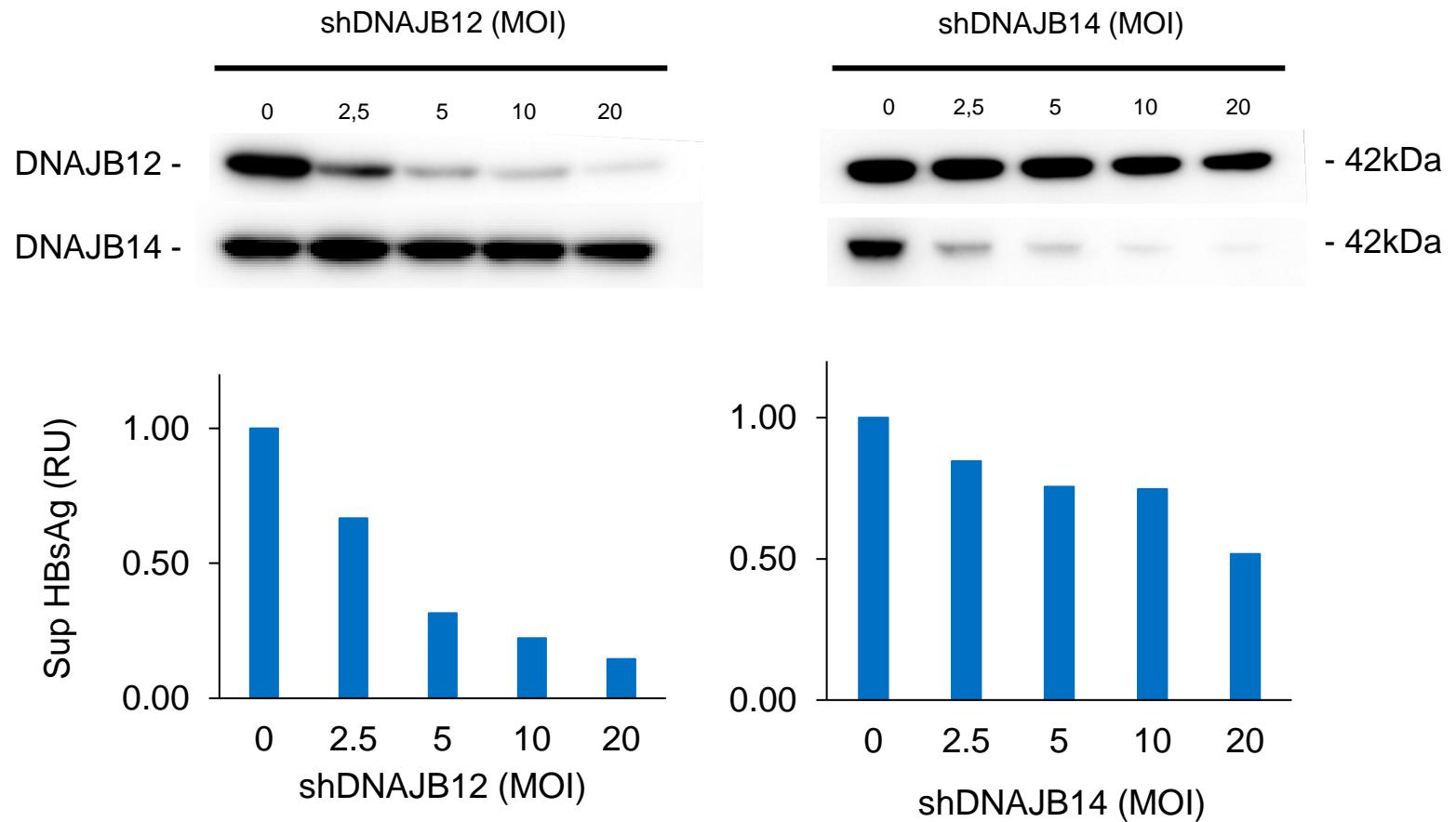


DNAJB14 colocalizes with HBsAg.



DNAJB14 is detected, along with DNAJB12, via REP 2139-biot pulldown.

# DNAJB14 KD effect on HBsAg secretion

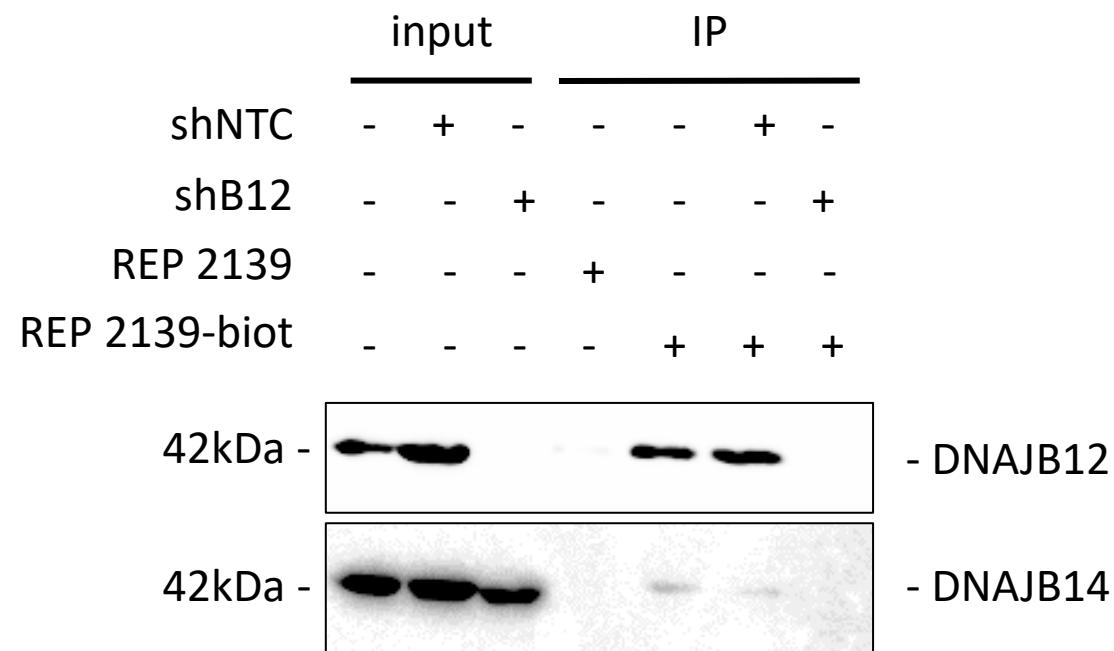


DNAJB14 only has a moderate effect on HBsAg secretion compared to DNAJB12, suggesting that DNAJB12 is the major chaperone involved.

# REP 2139 interaction with DNAJB14

REP 2139 does not interact with DNAJB14 :

HepG2.2.15



REP 2139 only interacts with DNAJB12.

# Conclusion

## Take-home message:

- REP 2139 interacts with DNAJB12.
- KD of DNJAB12 leads to intracellular HBsAg degradation via proteasomes.
- KD of DNJAB12 inhibits >90% inhibition of SVP and Dane particle secretion.
- Although DNAJB14 can be pulled down as heterodimer with DNAJB12 by REP 2139, it seems to have only a moderate effect on HBsAg secretion.

## In conclusion :

- DNAJB12 is a major chaperone involved in all HBV particle morphogenesis.
- We suggest that DNAJB12 is a cellular target of REP 2139.
- REP 2139 appears to exert its antiviral effect in HBV infection by preventing DNAJB12 functions required for HBV particle morphogenesis.

# Acknowledgments

## Labonté lab:

P. Labonté  
M. Blanchet  
R. Boulon  
Y. Tétreault



## Replicor:

A. Vaillant  
M. Blanchet

## Fundings:



Alliance Grant



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