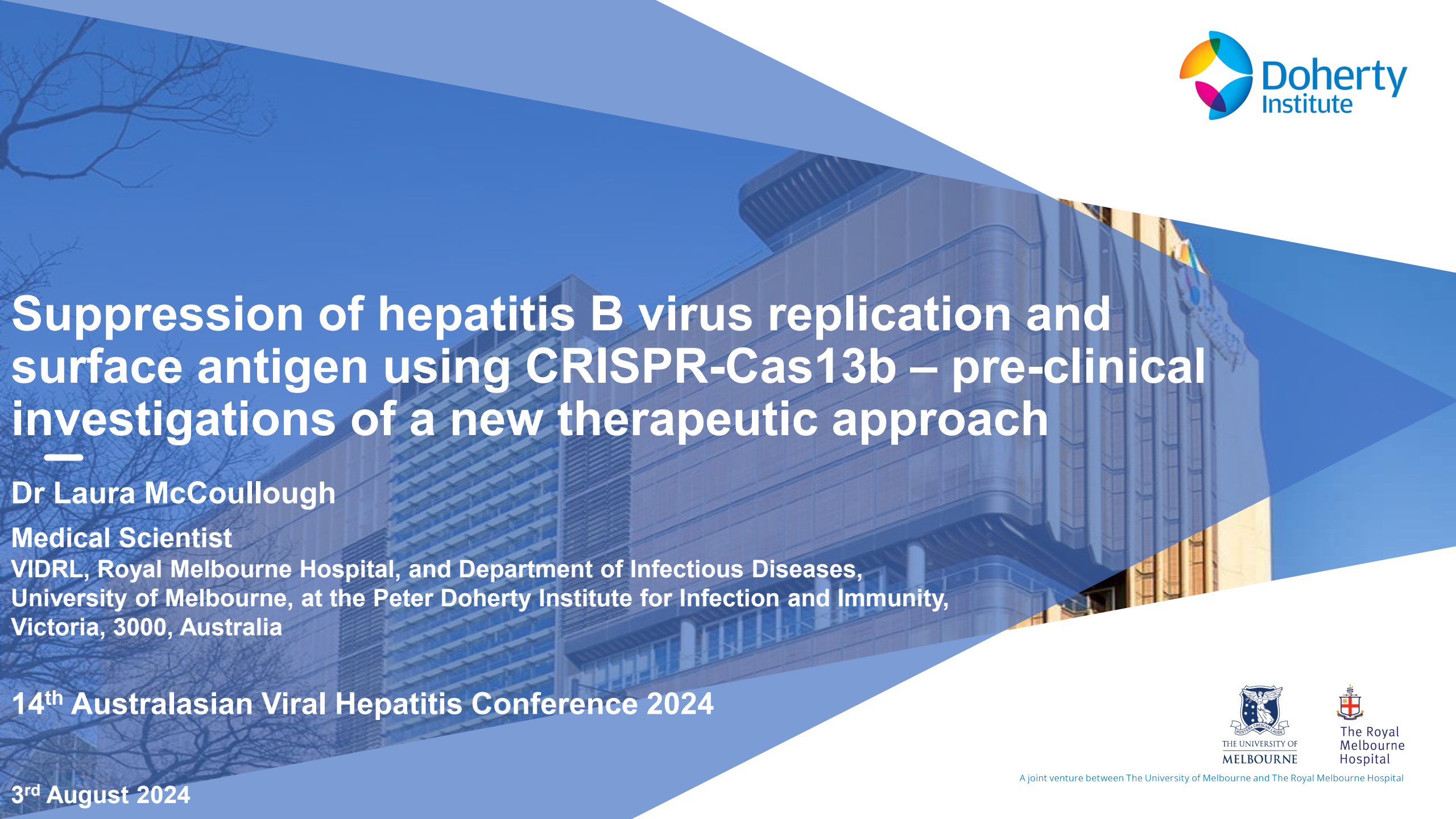

Suppression of hepatitis B virus replication and surface antigen using CRISPR-Cas13b – pre-clinical investigations of a new therapeutic approach

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14th Australasian Viral Hepatitis Conference 2024

3rd August 2024

Doherty Institute

THE UNIVERSITY OF MELBOURNE

The Royal Melbourne Hospital

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Hepatitis B Virus

- DNA virus that replicates through RNA
- No cure for chronic HBV infection

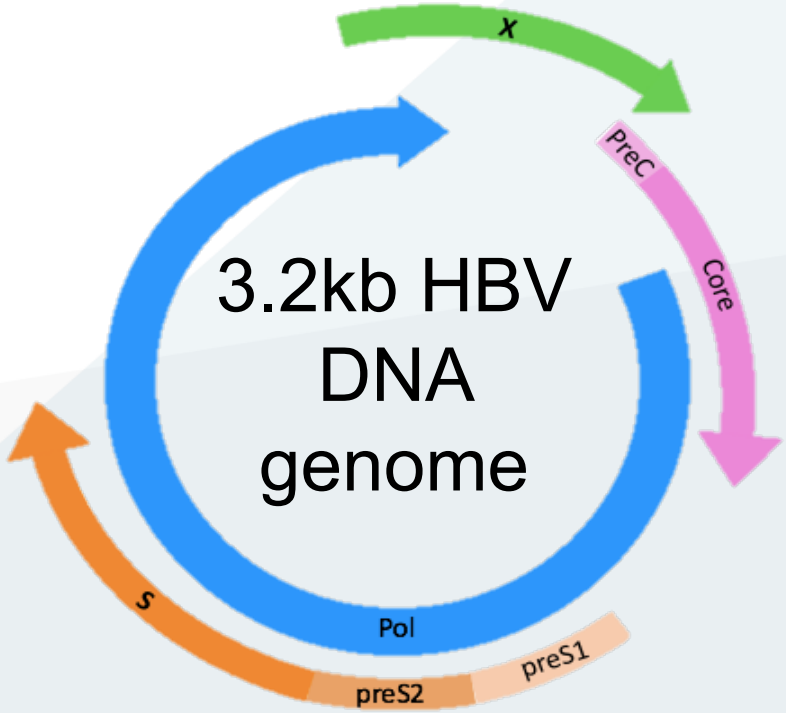


Fig 1. Hepatitis B genome

254 million
people are living with chronic HBV infection

>1 million
Deaths annually

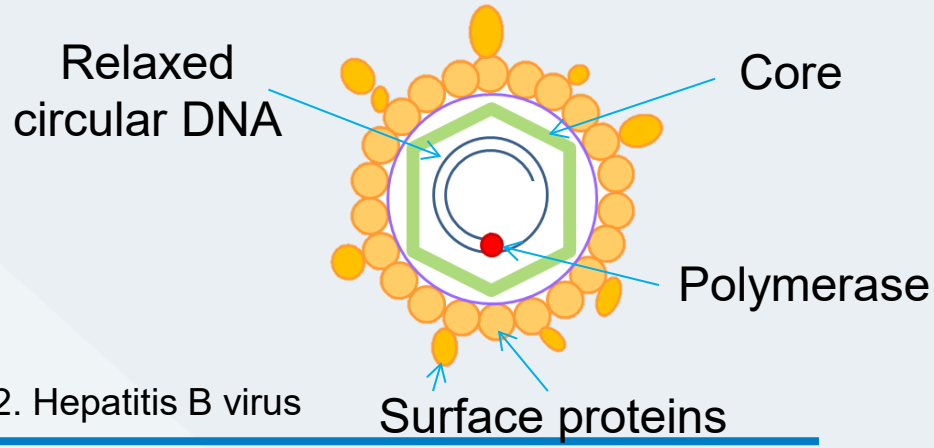


Fig 2. Hepatitis B virus

HBV replication cycle

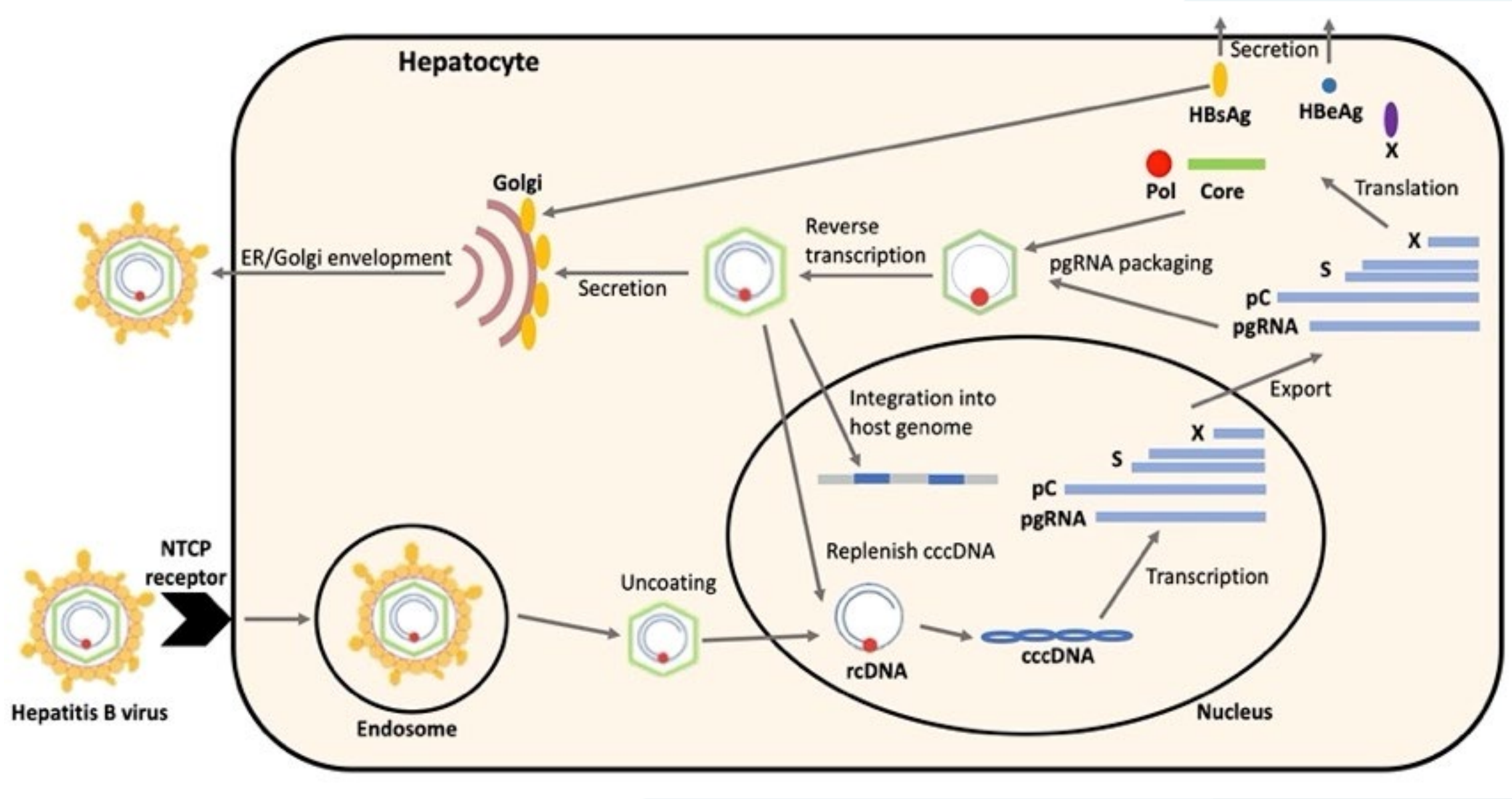


Fig 3. HBV replication cycle

HBV treatments

- Current direct-acting antivirals only target the reverse transcription step
- Current treatments rarely achieve functional cure: loss of HBsAg
- HBV RNAs are a potential target for new novel therapies

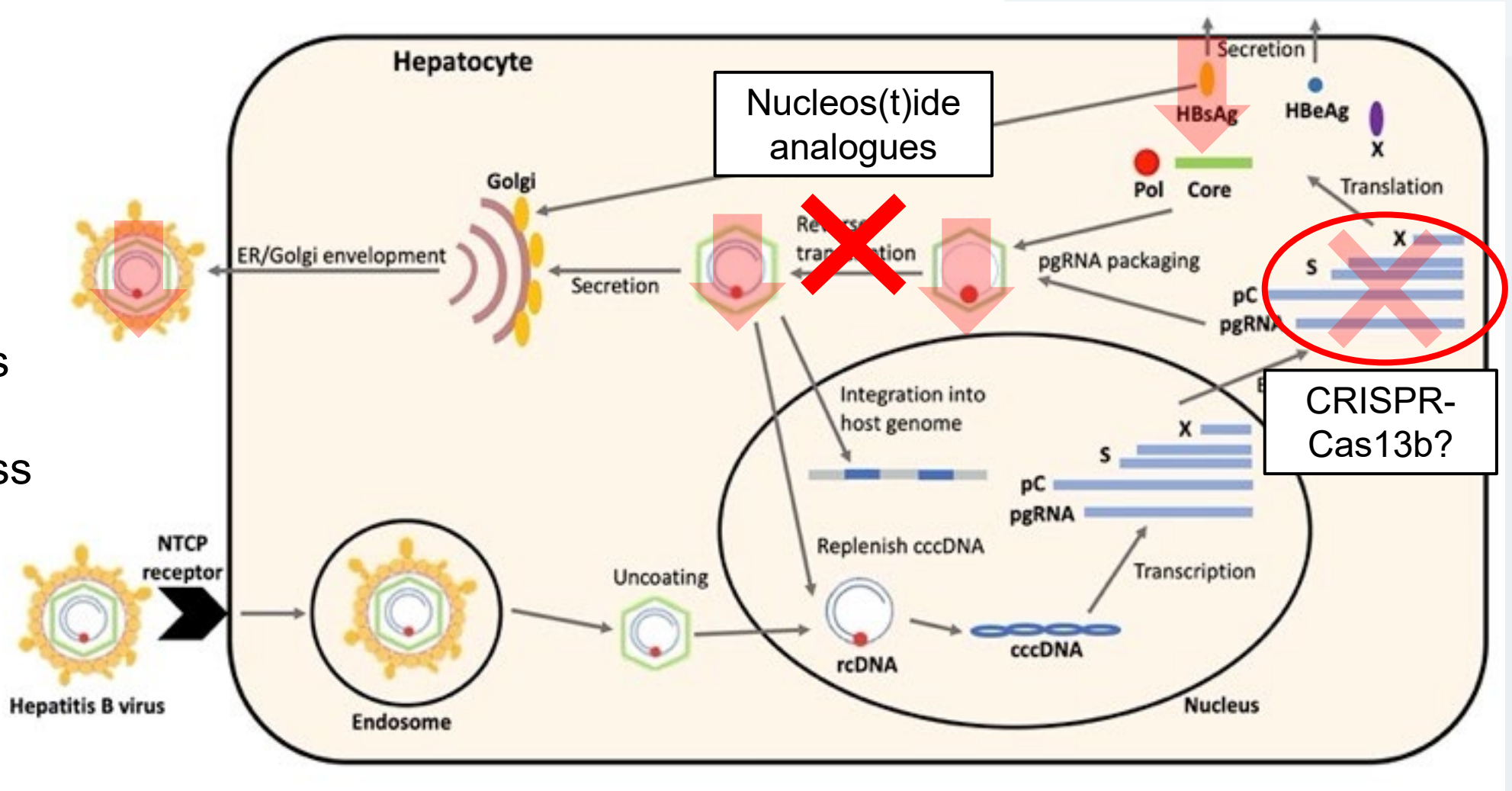


Fig 3. HBV replication cycle

CRISPR-Cas13

- CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
- Adaptive immune system in prokaryotes
- Targets RNA
- Can be repurposed to target RNA in mammalian cells
- High specificity due to 30 nucleotide CRISPR RNA (crRNA) (Abudayyeh et al. *Nature* 2017)

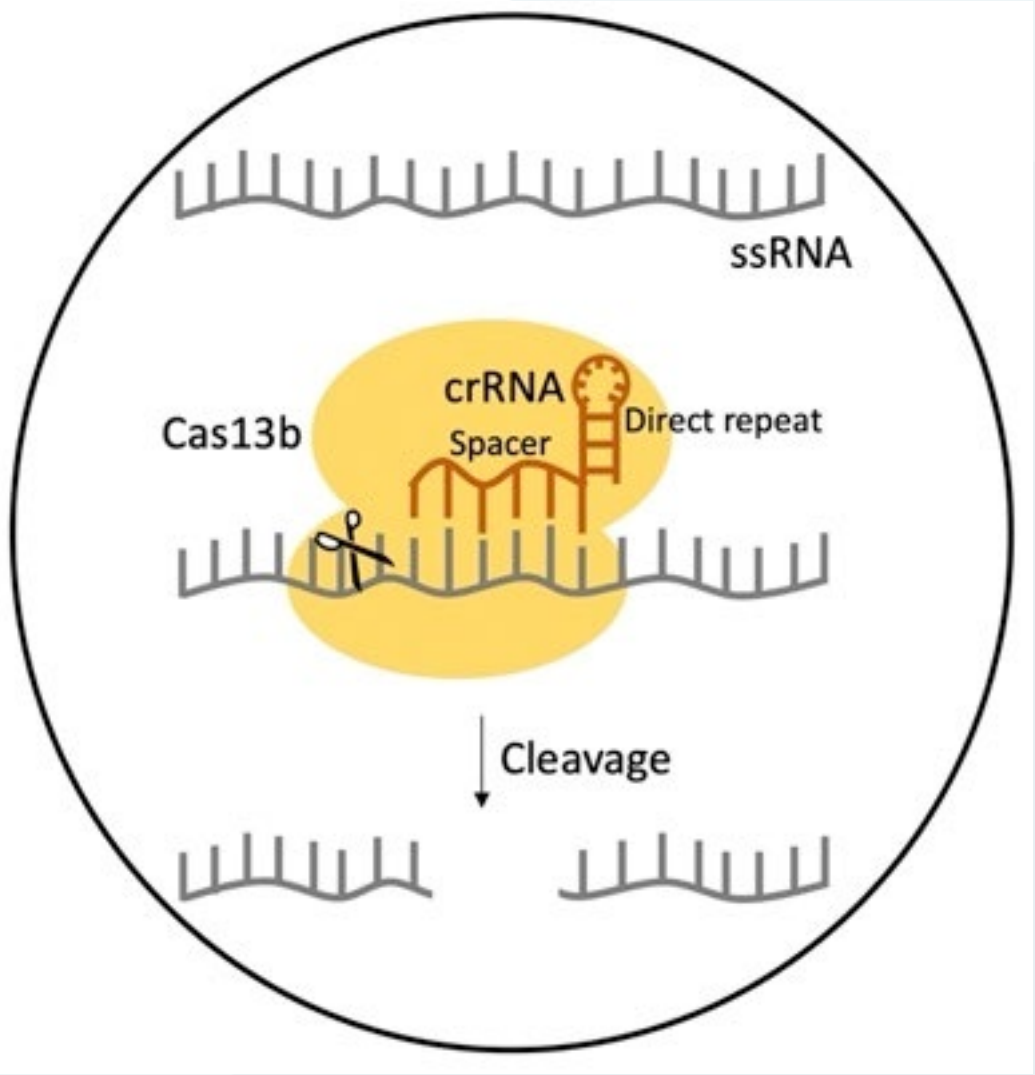


Fig 4. Cas13b mechanism

CRISPR-Cas13 targeting viral RNAs

Programmable inhibition and detection of RNA viruses using Cas13

Catherine A. Freije[1,2,12,*], Cameron Myhrvold[1,3,12,13,*], Chloe K. Boehm[1], Aaron E. Lin[1,2], Nicole L. Welch[1,2], Amber Carter[1], Hayden C. Metsky[1,4], Cynthia Y. Luo[1,3], Omar O. Abudayyeh[1,5,6,7,8], Jonathan S. Gootenberg[1,5,6,7,9], Nathan L. Yozwiak[1,3], Feng Zhang[1,5,6,7,10], Pardis C. Sabeti[1,2,3,10,11,*]

Reprogrammed CRISPR-Cas13a targeting the HPV16/18 E6 gene inhibits proliferation and induces apoptosis in E6-transformed keratinocytes

CHUNJING LI[1*], LIWEN GUO[2*], GUOQING LIU[1], MINGJUAN GUO[1], HUILING WEI[1], QIQIONG YANG[1], JIANFENG WANG[1] and HUIHUA CHEN[2]

CRISPR-Cas13a mediated targeting of hepatitis C virus internal-ribosomal entry site (IRES) as an effective antiviral strategy

Muhammad Usman Ashraf[a,c,1], Hafiz Muhammad Salman[a,1], Muhammad Farhan Khalid[a], Muhammad Haider Farooq Khan[a], Saima Anwar[b], Samia Afzal[c], Muhammad Idrees[c], Safee Ullah Chaudhary[a,*]

Reprogrammed CRISPR-Cas13b suppresses SARS-CoV-2 replication and circumvents its mutational escape through mismatch tolerance

Mohamed Fareh[1,2✉], Wei Zhao[3], Wenxin Hu[1,2], Joshua M. L. Casan[1,2], Amit Kumar[1,2], Jori Symons[3], Jennifer M. Zerbato[3], Danielle Fong[3], Ilia Voskoboinik[1,2], Paul G. Ekert[1,2,4,5], Rajeev Rudraraju[3,6,7], Damian F. J. Purcell[7], Sharon R. Lewin[3,8,9,10✉] & Joseph A. Trapani[1,2,10]

HYPOTHESES

- Cas13b may be reprogrammed to target HBV RNAs to reduce viral replication and antigen expression

AIM

- Optimise the CRISPR-Cas13b system to target the HBV pregenomic RNA and viral mRNAs in pre-clinical models

Designing Cas13b crRNAs

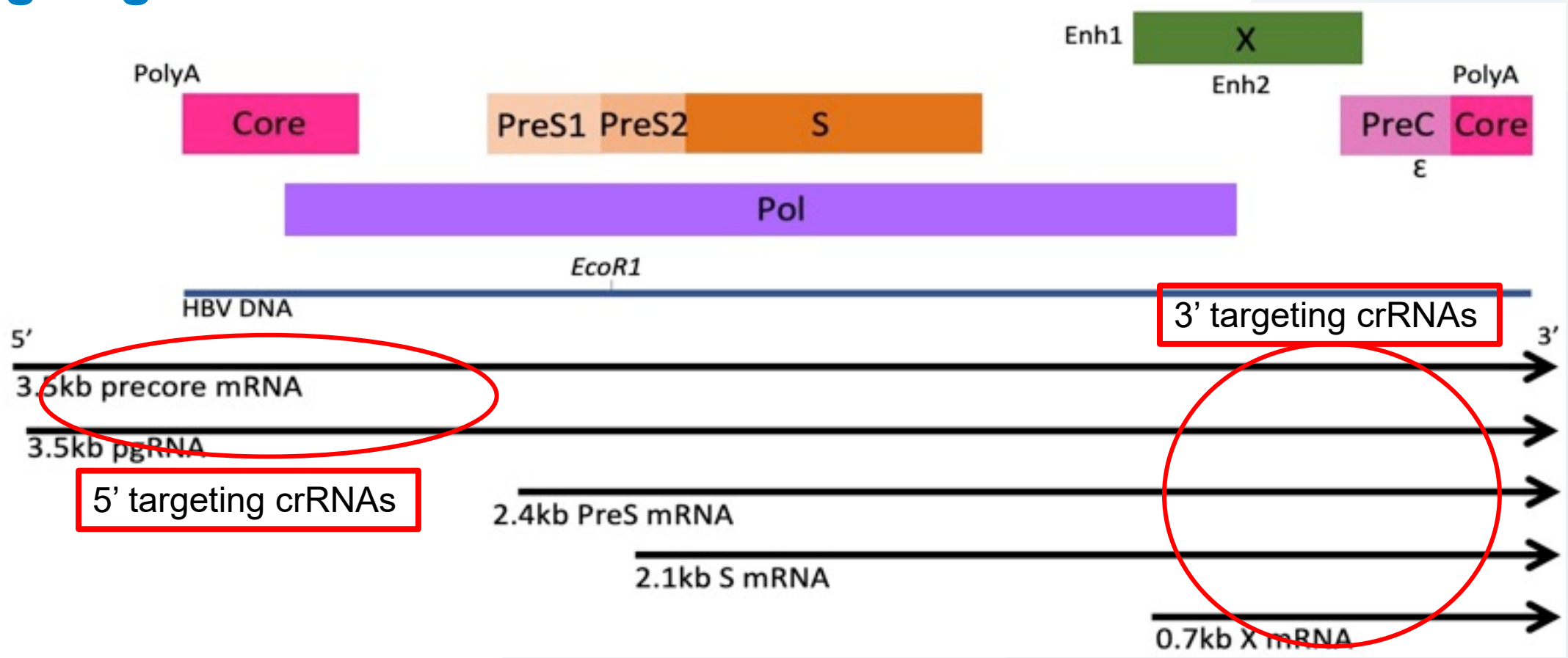
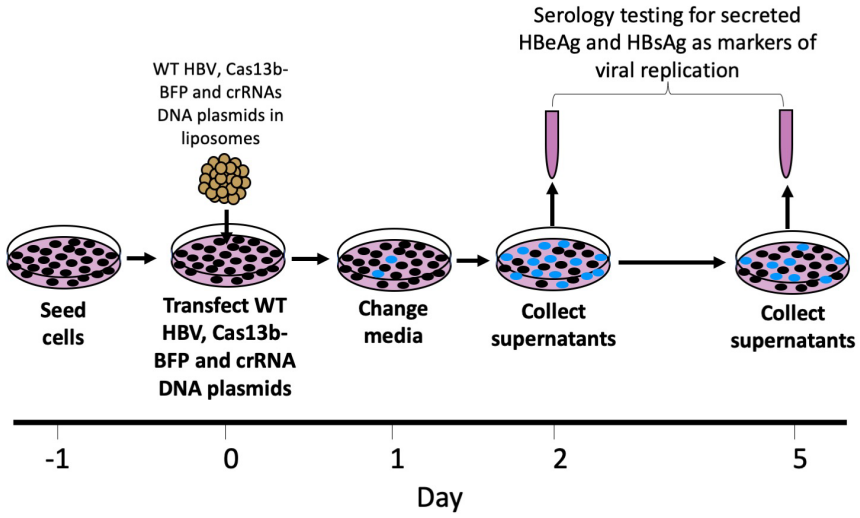


Fig 5. crRNA locations

- 5' targeting crRNAs – ↓ HBeAg, core, polymerase and viral replication
- 3' targeting crRNAs – ↓ HBeAg, core, polymerase, viral replication, HBx and HBsAg

Cas13b significantly reduced HBV proteins produced in cells



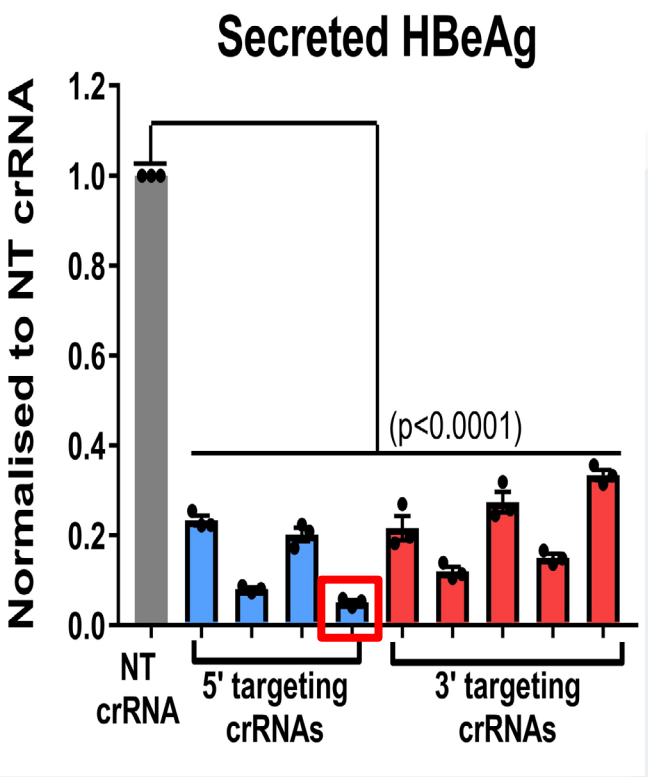


Fig 6. Secreted HBeAg five days post-transfection of HepG2 cells with WT HBV, Cas13b-BFP and crRNAs using the optimised molar ratio. N=3.

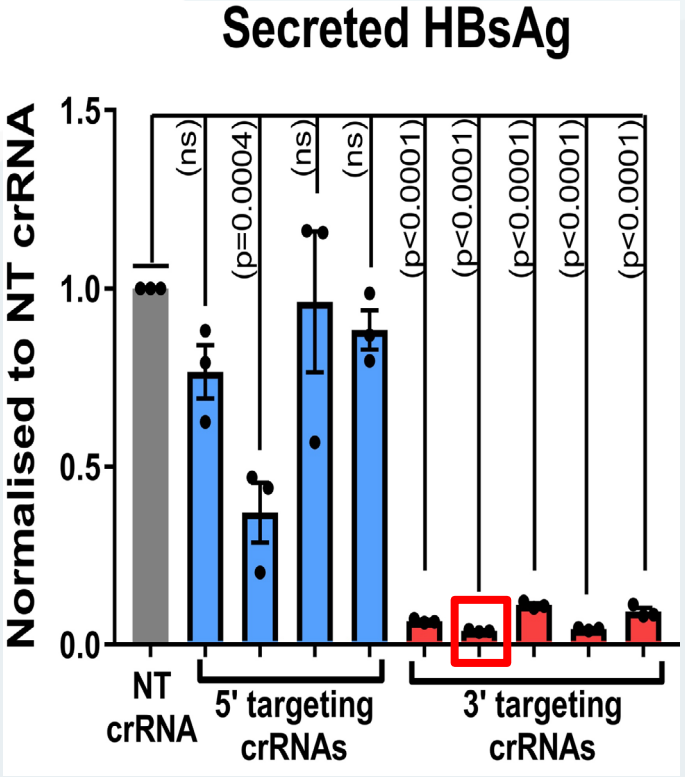
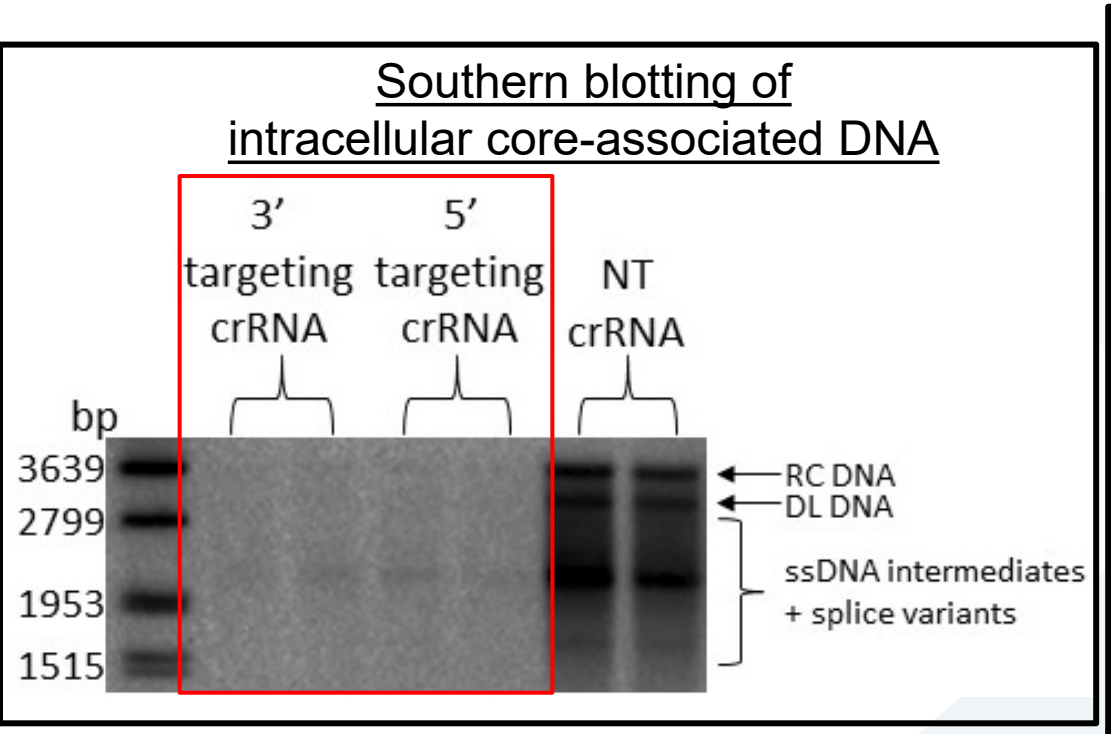


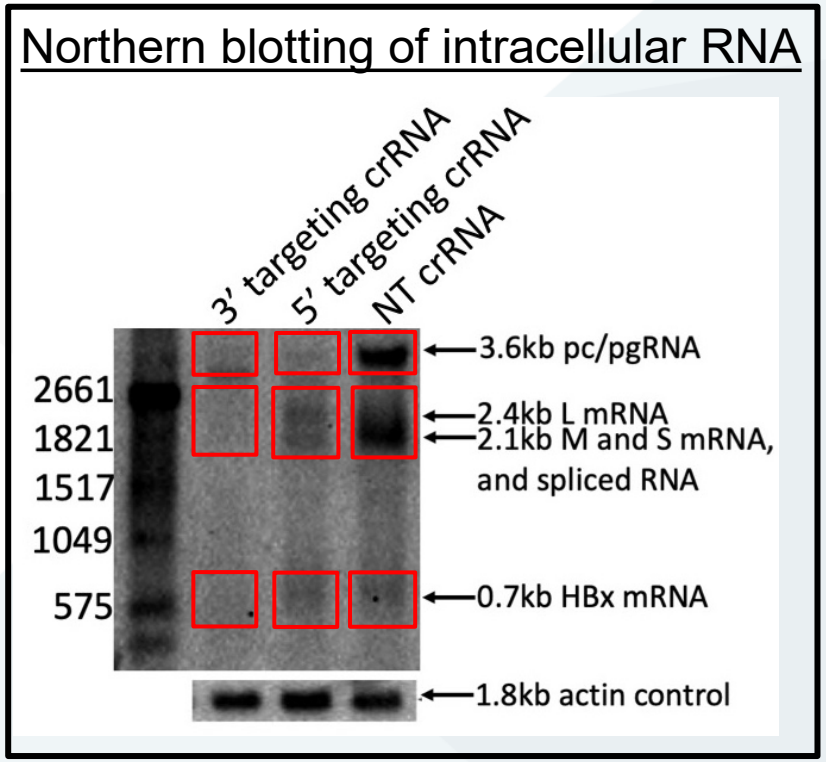
Fig 7. Secreted HBsAg five days post-transfection of HepG2 cells with WT HBV, Cas13b-BFP and crRNAs using the optimised molar ratio. N=3.

95% reduction of secreted HBeAg by 5' targeting crRNA

96% reduction of secreted HBsAg by 3' targeting crRNA

Cas13b strongly reduced HBV replication in cells





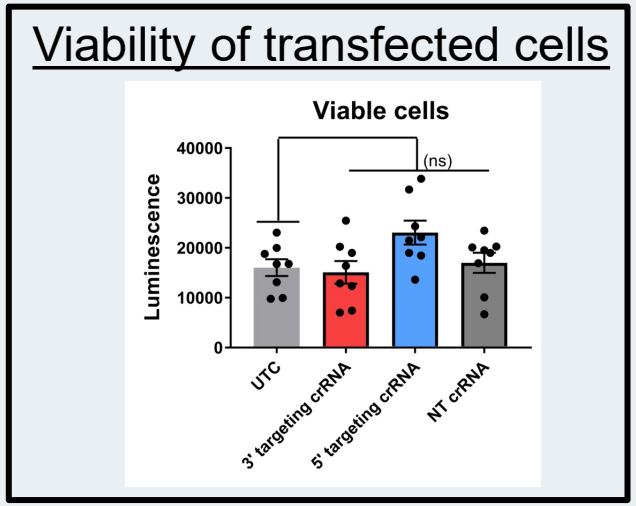


Fig 8. Analysis of WT HBV replication five days post-transfection of HepG2 cells with WT HBV, Cas13b-BFP and crRNAs using the optimised molar ratio.

Knockdown of HBV proteins by Cas13b crRNAs was pan-genotypic

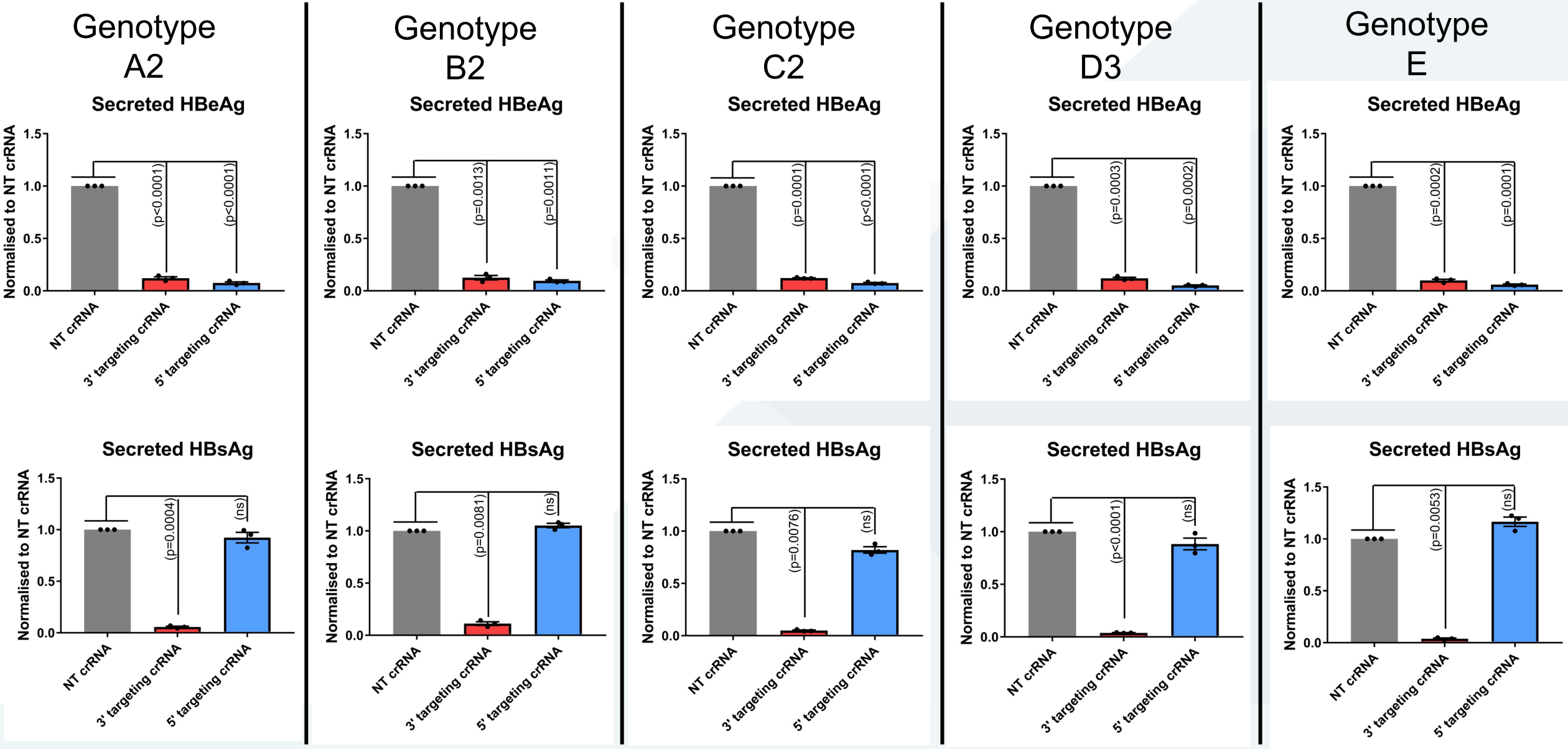


Fig 9. Secreted HBeAg and HBsAg from HepG2 cells five days post-transfection with WT HBV genotypes A2, B2, C2, D3 and E, Cas13b-BFP and crRNAs using the optimal molar ratio. N=3.

Cas13b reduced HBV proteins in a HBV-stable cell line

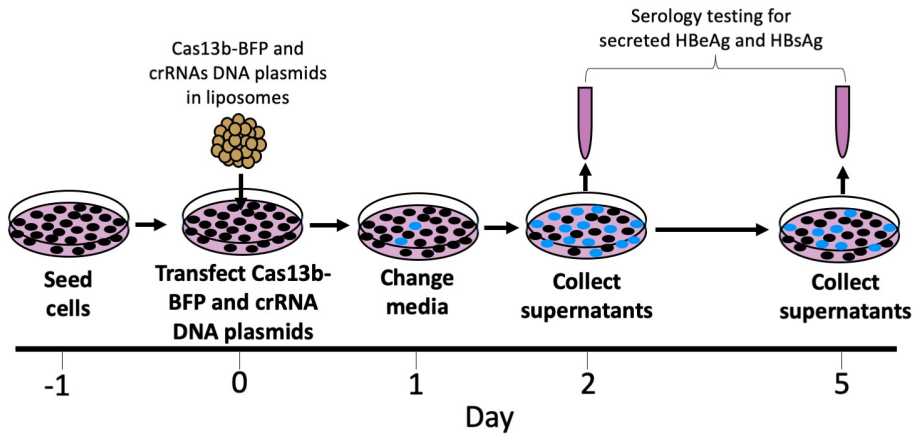


Fig 10. Cas13b and crRNA transfection in HepAD38 cells.

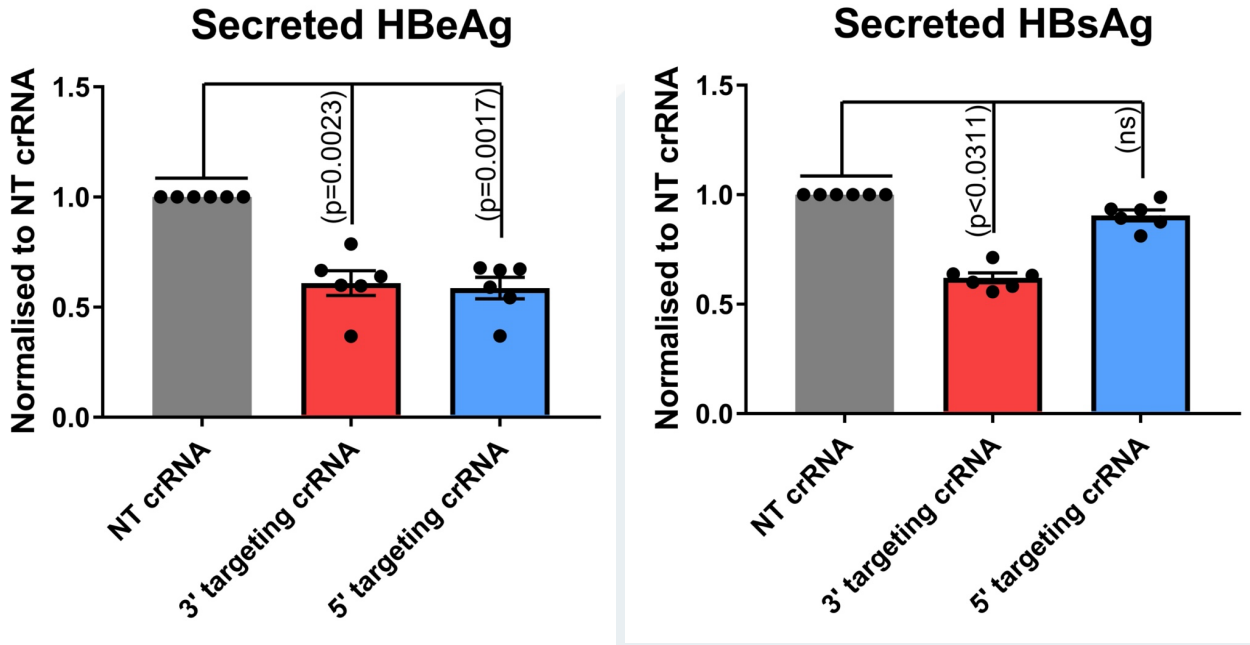


Fig 11. Secreted HBeAg and HBsAg five days post-transfection of HepAD38 cells with Cas13b-BFP and crRNAs. N=3.

40% reduction of secreted HBeAg by 5' and 3' targeting crRNA

36% reduction of secreted HBsAg by 3' targeting crRNA

Cas13b reduced HBV proteins in a HBV-infection model

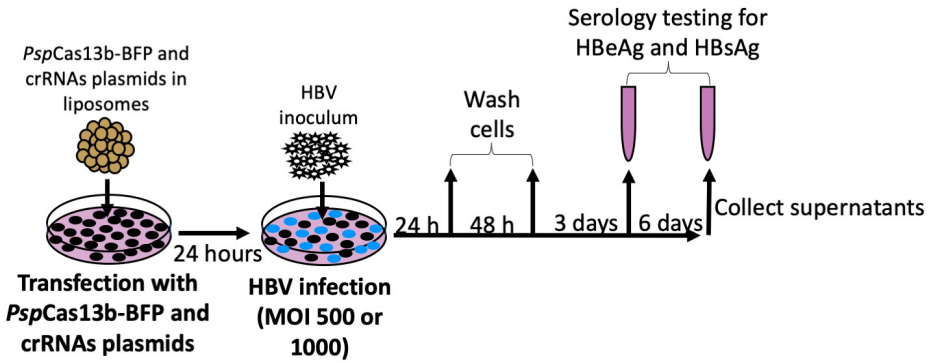


Fig 12. Cas13b and crRNA transfection in NTCP-HepG2 cells.

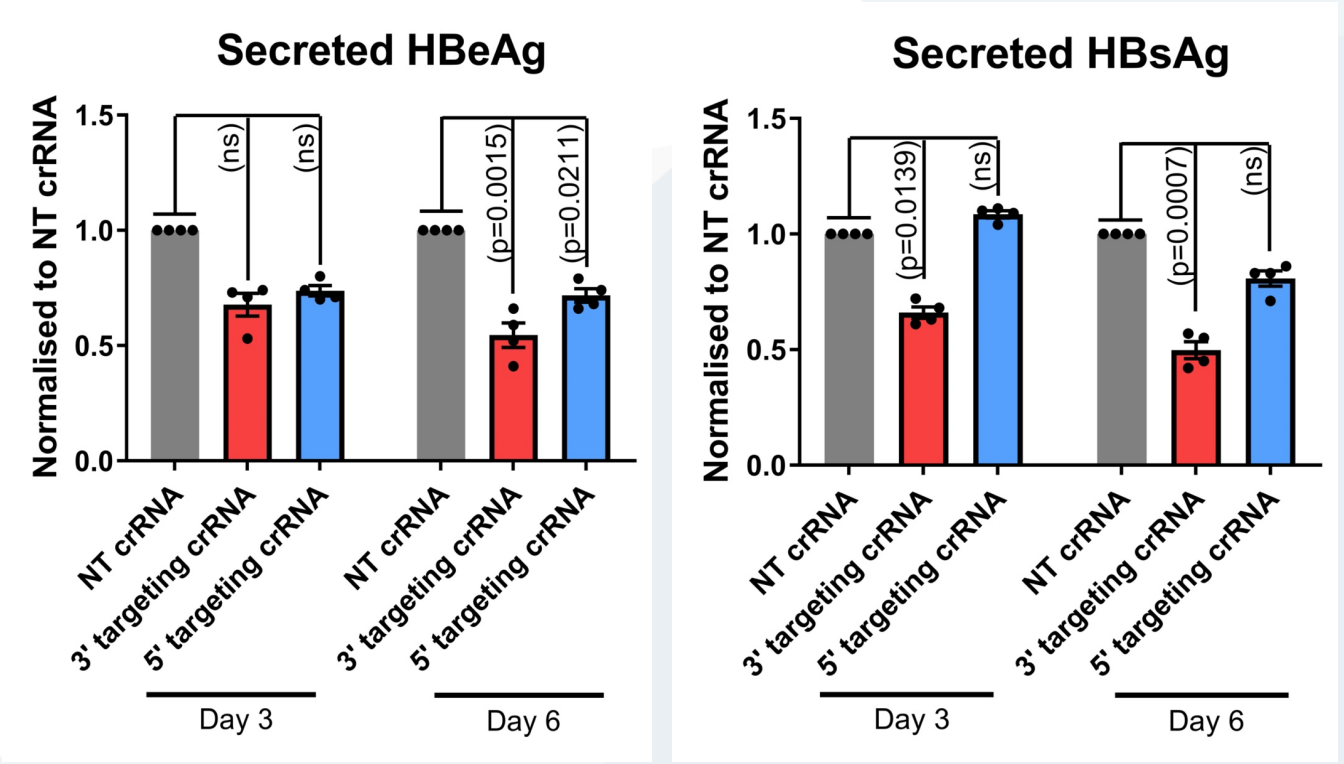


Fig 13. Secreted HBeAg and HBsAg three and six days post infection of WT HBV in Cas13b-BFP and crRNA transfected NTCP-HepG2 cells. N=3.

46% reduction of secreted HBeAg by 3' targeting crRNA

50% reduction of secreted HBsAg by 3' targeting crRNA

Testing the efficacy of Cas13b in reducing sera HBsAg *in vivo*

- Hydrodynamic injection of a greater than genome length WT HBV plasmid into CBA mice

Huang LR, Wu HL, Chen PJ, Chen DS (2006) An immunocompetent mouse model for the tolerance of human chronic hepatitis B virus infection. Proc Natl Acad Sci USA 103(47):17862–17867.

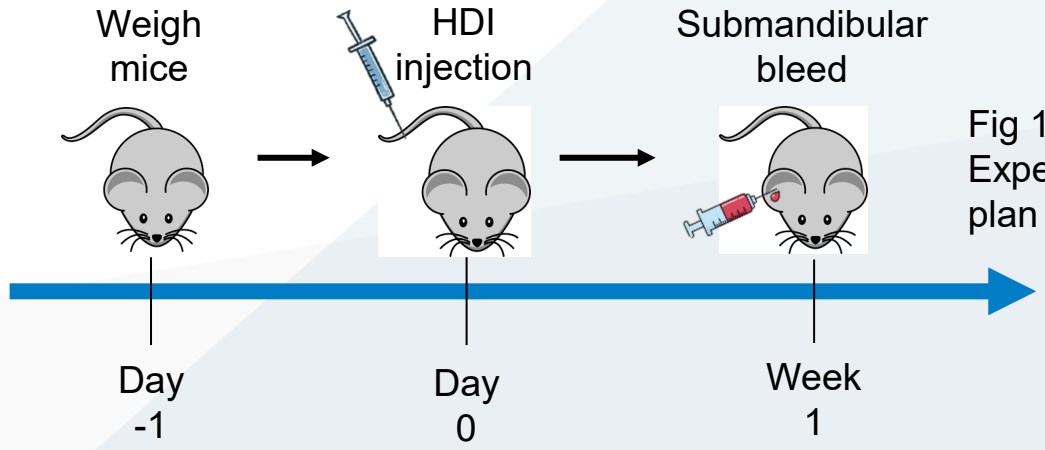
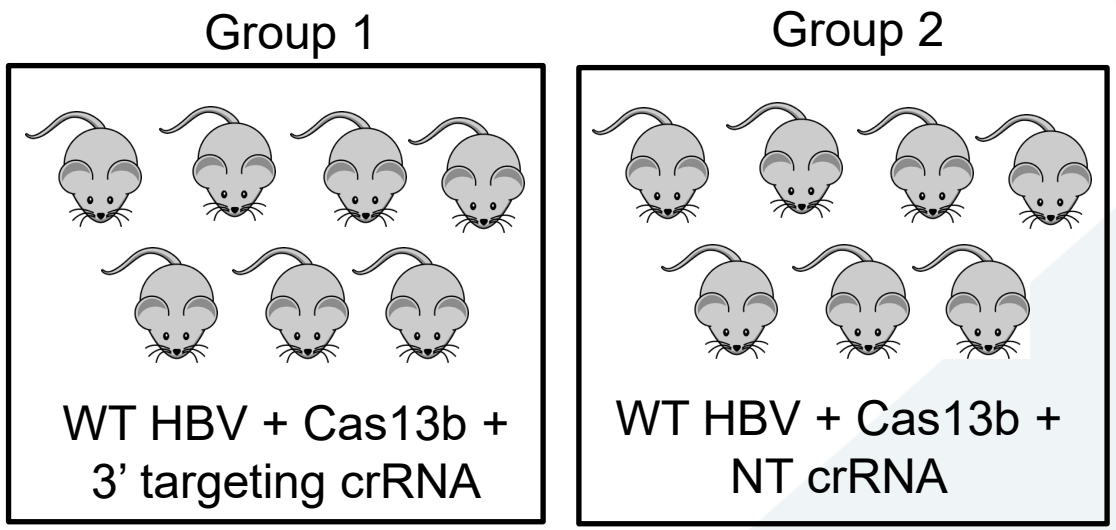
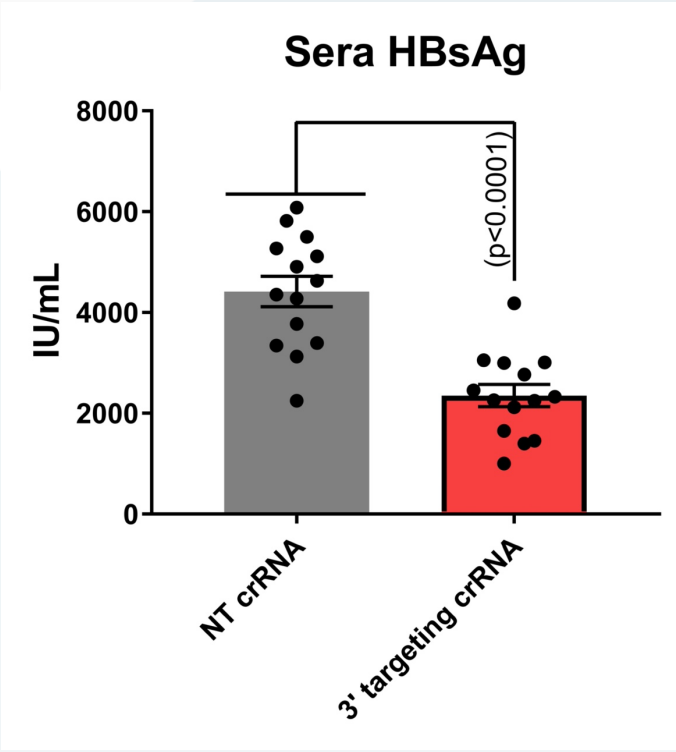


Fig 14. Experimental plan





50%
Reduction in sera HBsAg one week post-HDI

Fig 15. Sera HBsAg from CBA mice one week post HDI of WT HBV, Cas13b-BFP and crRNA DNA plasmids. n=2

Lipid nanoparticle delivery of Cas13b mRNA

- Lipid nanoparticles (LNPs) are a potential delivery mechanism for Cas13b if it was to be used as a treatment for HBV
- LNP/mRNA has been approved for the SARS-CoV-2 vaccines, and LNPs have been approved to deliver siRNA in humans
- LNPs can specifically deliver material to hepatocytes – the site of HBV infection

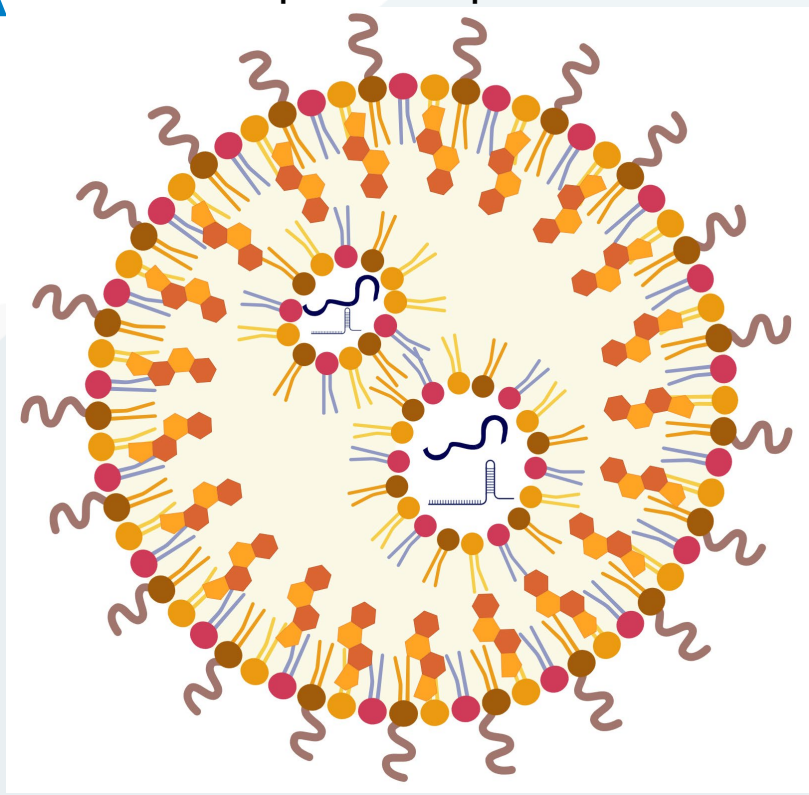


Fig 16. Lipid nanoparticle

LNP-encapsulated Cas13b mRNA reduced secreted HBsAg from a HBsAg-expressing stable cell line

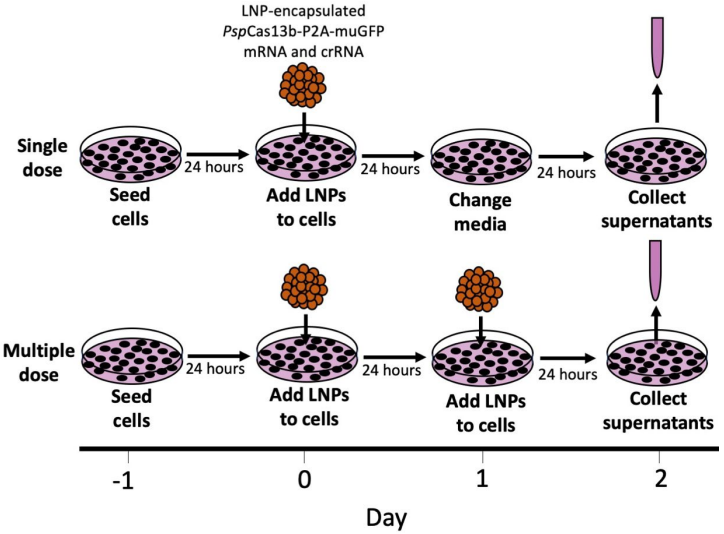


Fig 17. Method of LNP/Cas13b mRNA delivery in PLC/PRF/5 cells

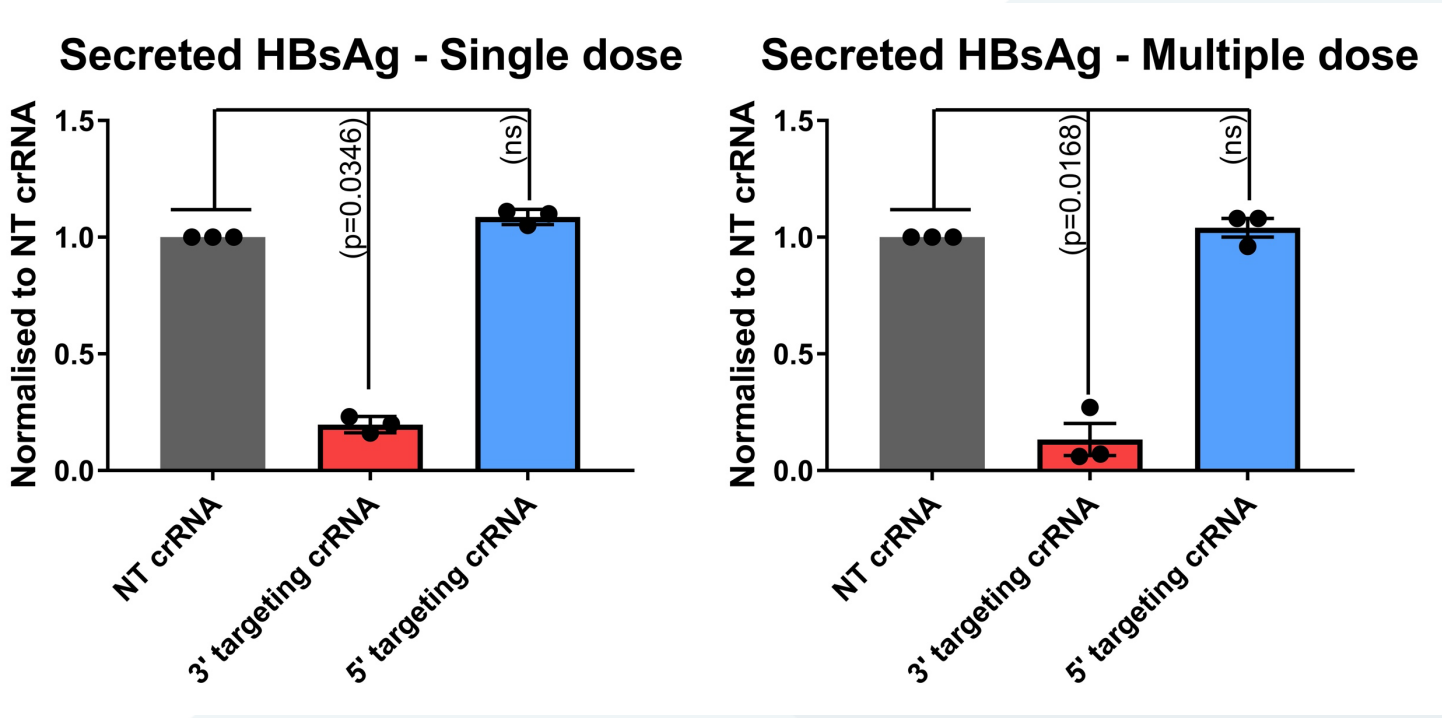


Fig 18. Secreted HBsAg from PLC/PRF/5 cells after one or two doses of LNP-encapsulated Cas13b mRNA and crRNA. N=3.

80% reduction of secreted HBsAg by 3' targeting crRNA after one dose

87% reduction of secreted HBsAg by 3' targeting crRNA after two doses

Summary of findings

- Cas13b strongly reduced HBV replication and protein expression in cell culture models
- crRNAs were pan-genotypic
- Cas13b reduced sera HBsAg by 50% in a mouse model
- Cas13b mRNA was effectively delivered by lipid nanoparticles to reduce secreted HBsAg in a HBsAg-expressing stable cell line

Future studies

- Deliver LNP-encapsulated Cas13b mRNA and crRNA into mice with persistent HBV replication

Acknowledgements

Revill/Littlejohn Lab

Peter Revill

Margaret Littlejohn

Yianni Droungas

Mina Takawy

Jacinta Holmes

Mohamed Fareh

Wenxin Hu

Joseph Trapani

Stanislav Kan

Paula Cevaal

Sharon Lewin

Damian Purcell

Bioresources Facility Staff

Vitina Sozzi

Hans Netter

Chee Leng Lee

Christina Mahklouf

Marcela Toro

Thomas Payne

Stewart Fabb

Colin Pouton