



Insight on HDV – host cell interactions for drug discovery

Eloi R. Verrier, PhD

Institute for Translational Medicine and Liver Disease, Strasbourg, France



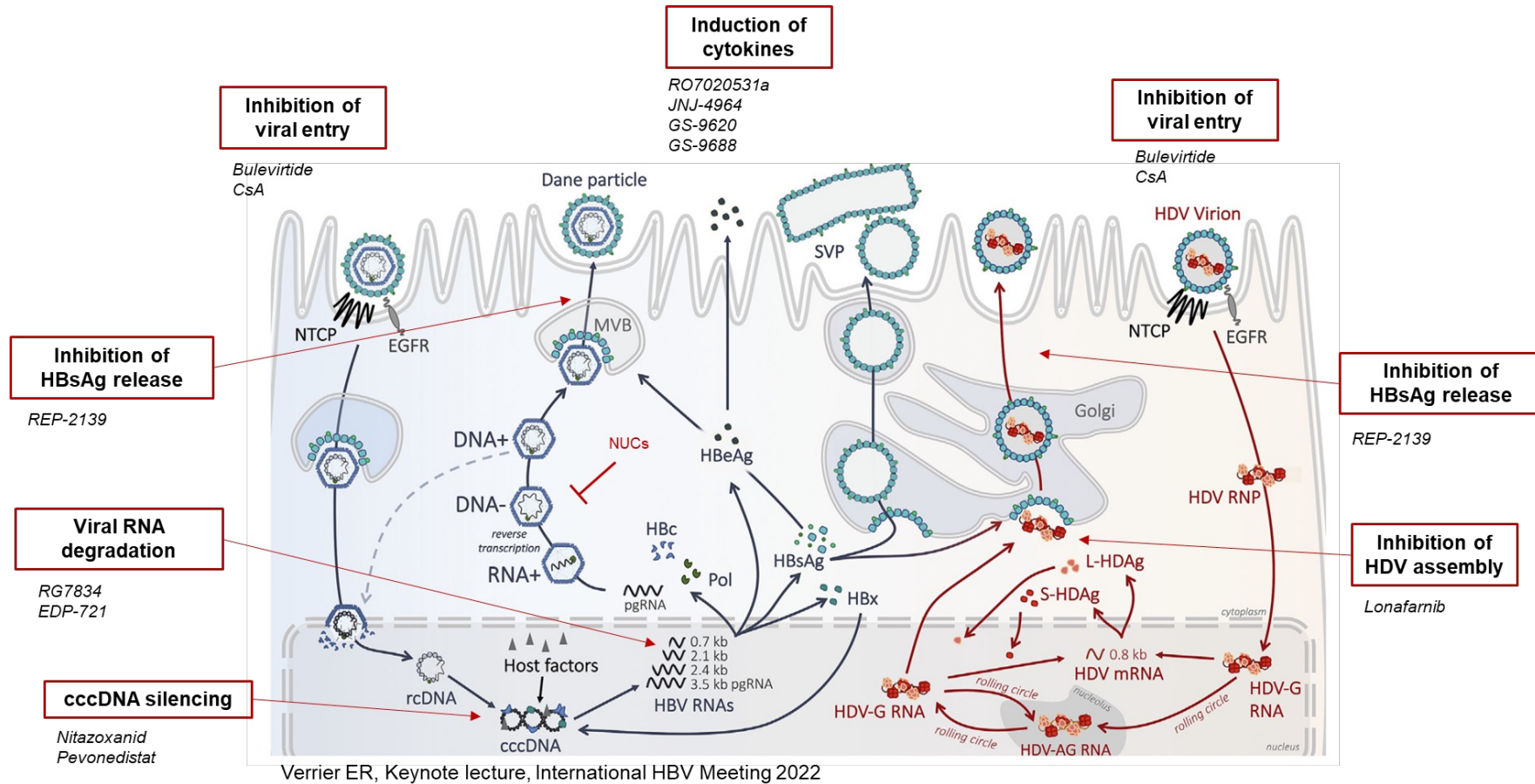
11th ABRS-MIE HBV Cure Workshop, Lyon

July 3, 2024



Anti-HDV therapies

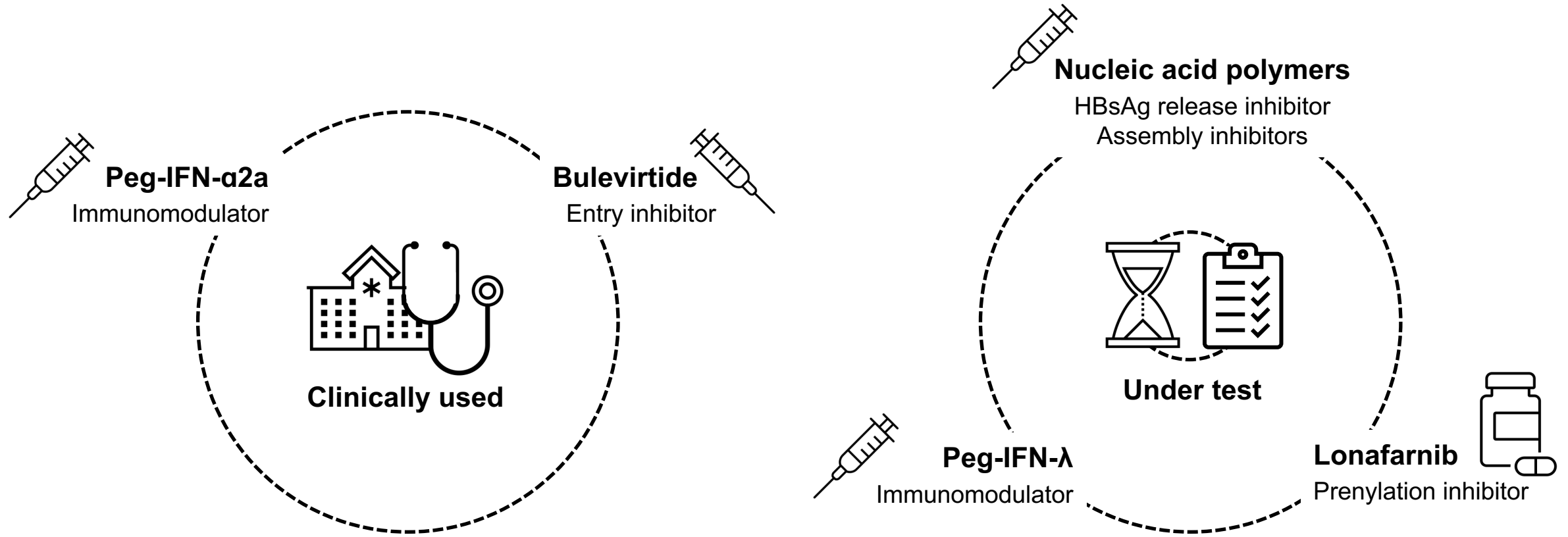
The need for innovative treatments



- Chronic hepatitis B virus infection is the leading cause of liver cancer worldwide
- Chronic hepatitis D is the most severe form of chronic viral hepatitis
- To date, no easy-to-use treatment allows viral clearance
- Host targeting agents (HTA) is a rising concept for the development of new antivirals

Anti-HDV therapies

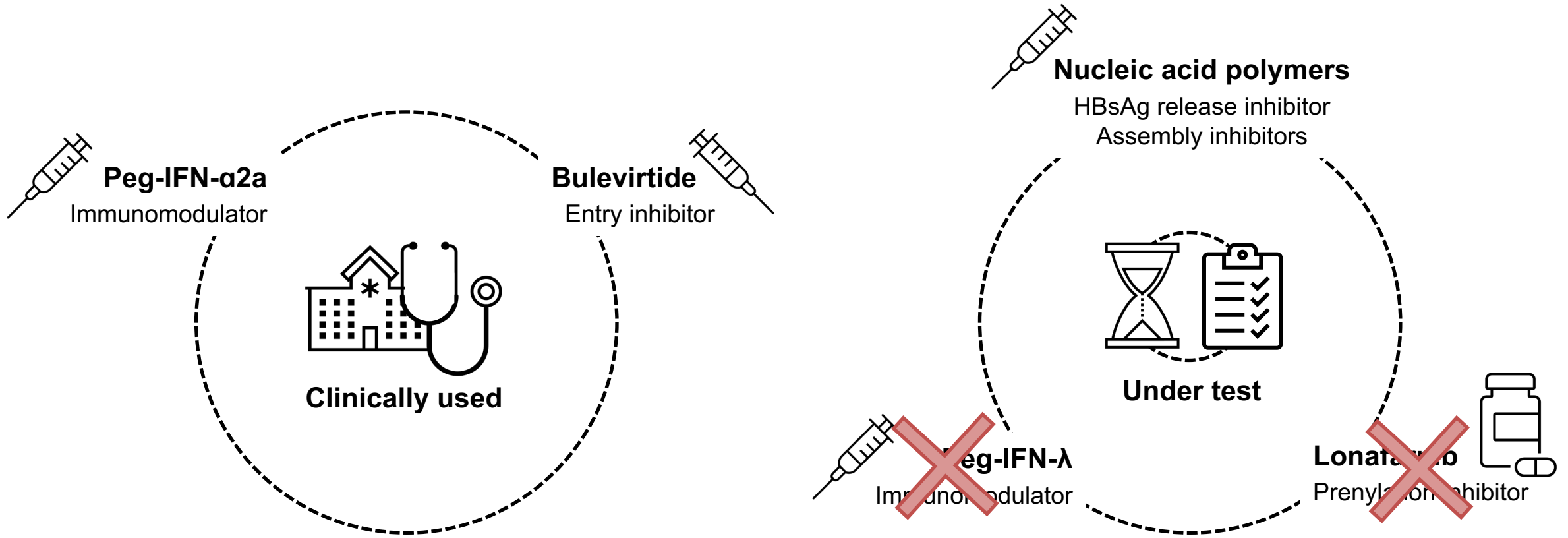
The need for innovative treatments



No FDA-approved treatment
Urgent need to optimize treatments

Anti-HDV therapies

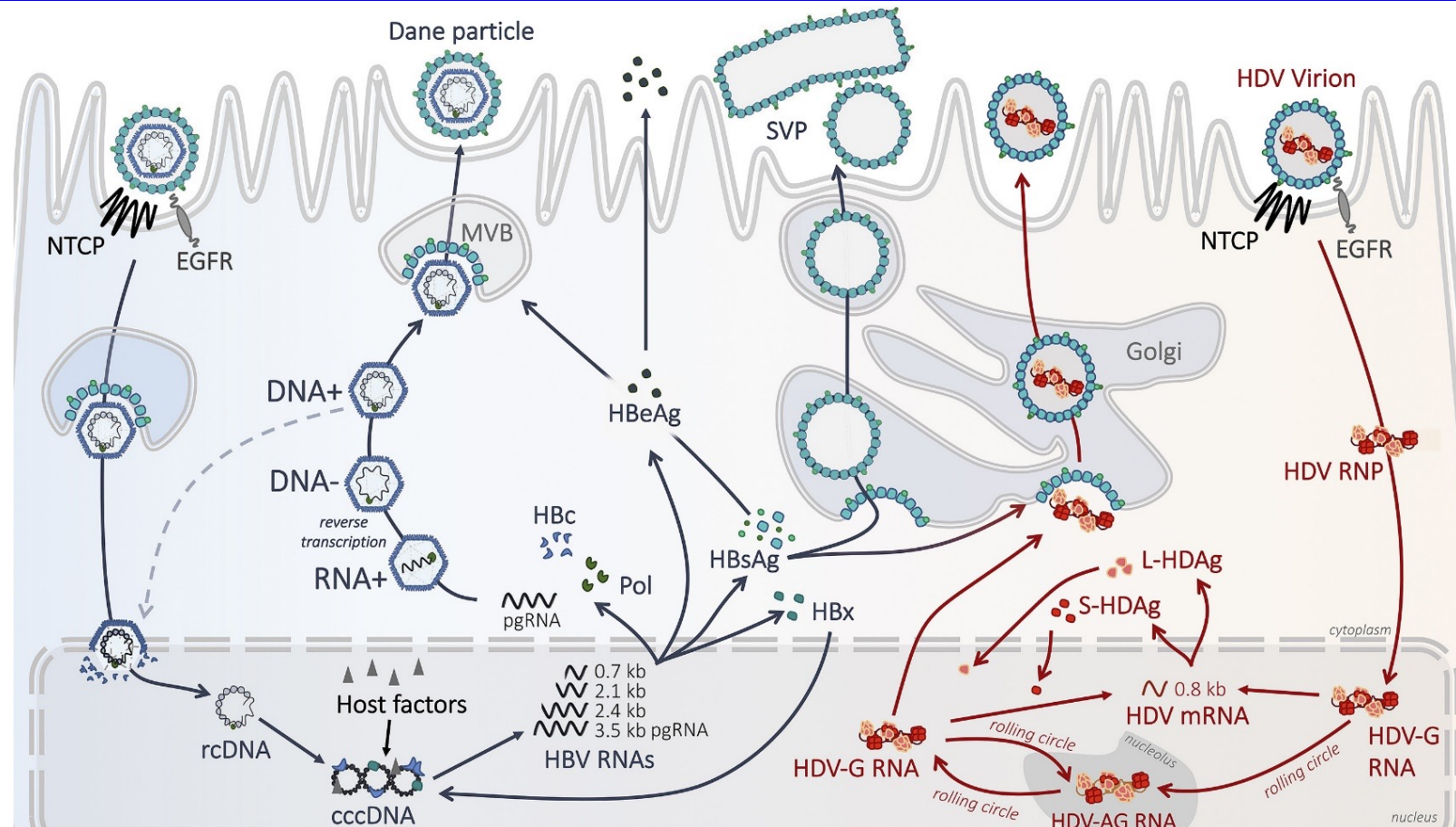
The need for innovative treatments



No FDA-approved treatment
Urgent need to optimize treatments

The HDV life cycle

The importance of cellular factors



Lucifora and Delphin, Antiviral Res 2020

- ✓ Until recently, viral entry was poorly understood
- ✓ RNP transport to the nucleus is poorly described
- ✓ The cellular regulators of HDV replication are not known
- ✓ Viral assembly is poorly described
- ✓ Lack of knowledge on the molecular interactions between HDV and the hepatocyte host factors

Objectives

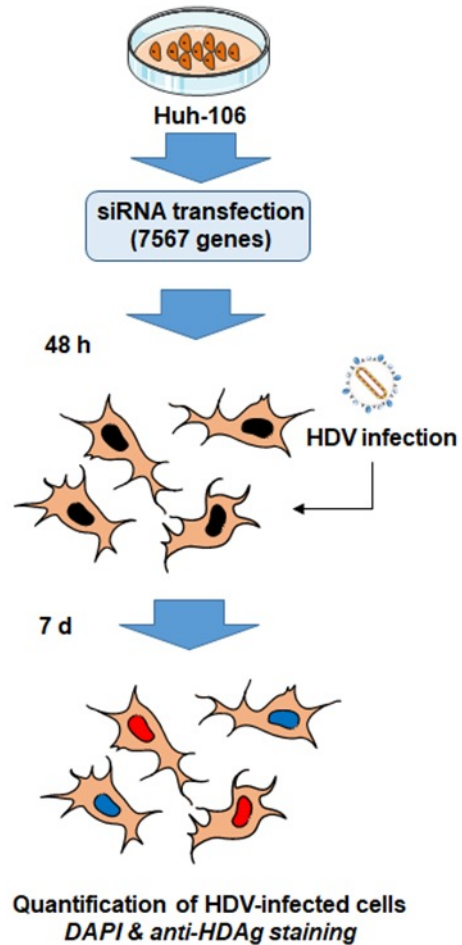
Identification of HDV-related host factors through high-throughput RNAi screening

Loss-of-function screen

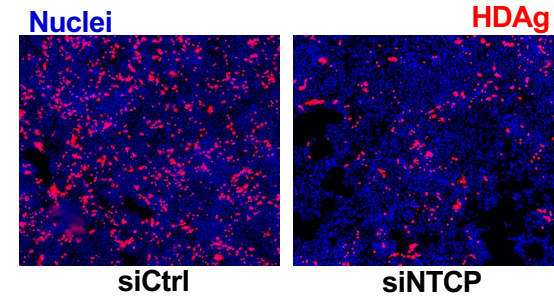
siRNA druggable genome library

Loss-of-function genetic screen

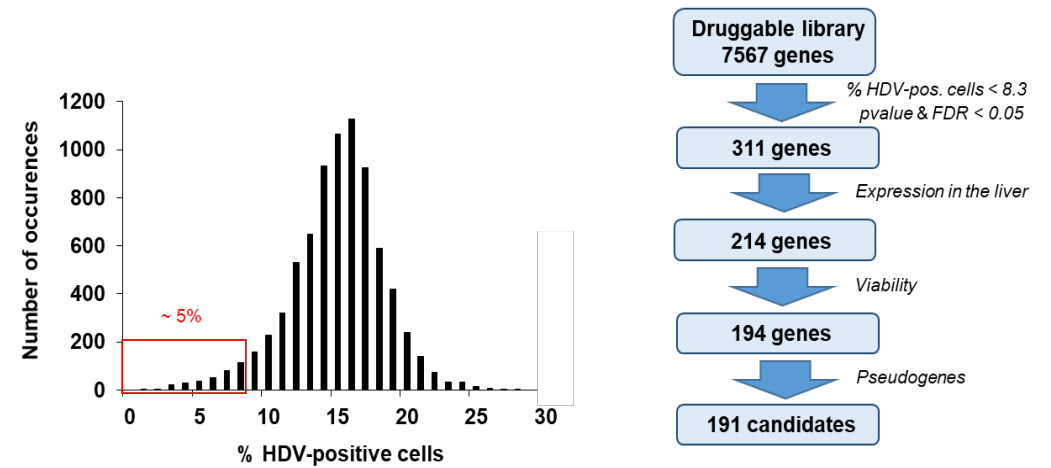
« Druggable genome » siRNA library- 7567 genes



B. Infection



C. Candidate selection



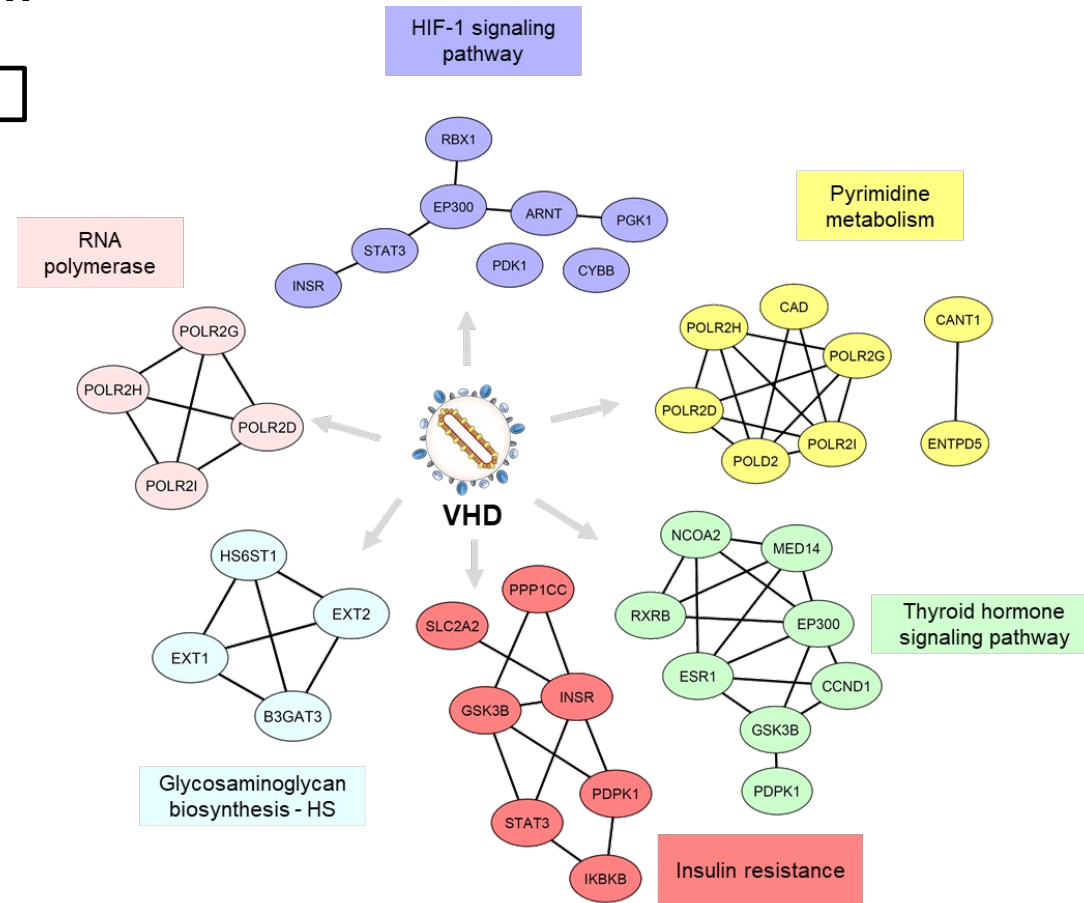
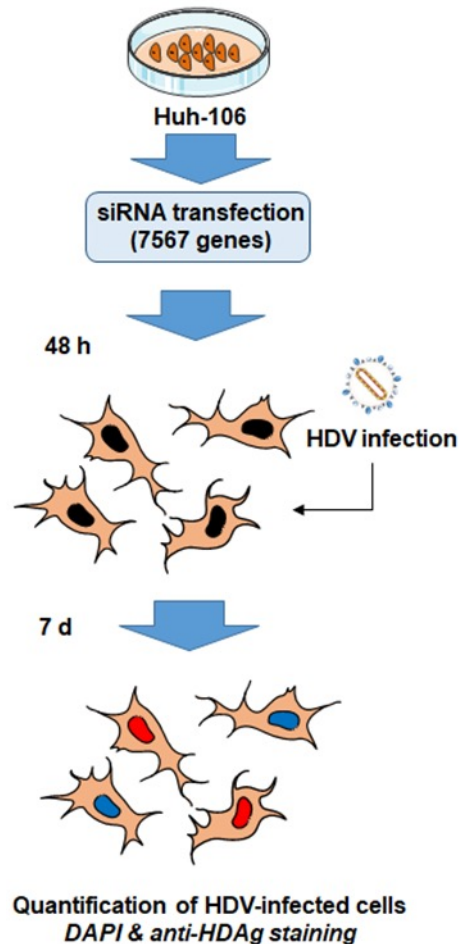
Identification of 191 candidate host factors for HDV infection

Loss-of-function screen

siRNA druggable genome library

Loss-of-function genetic screen

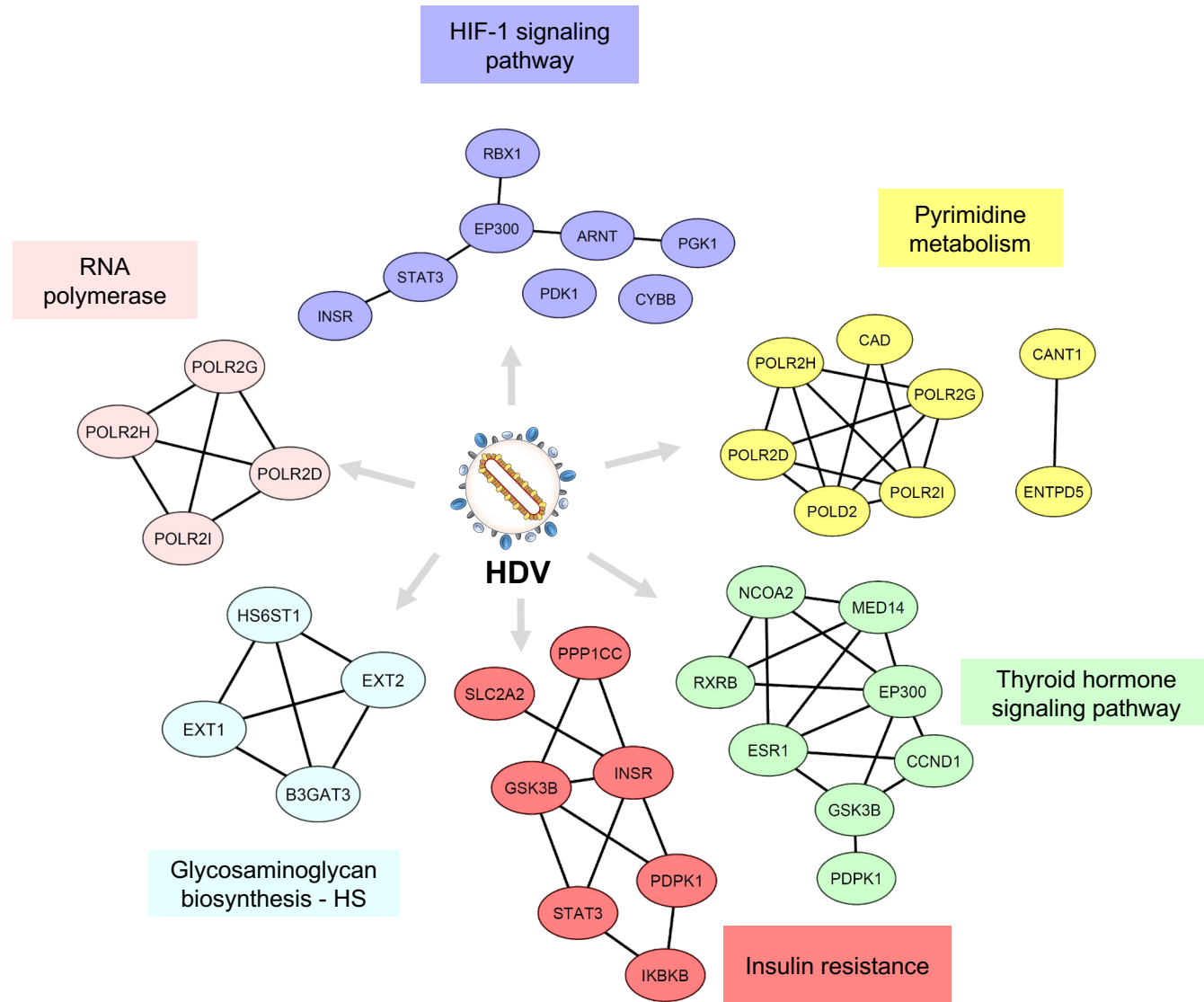
« Druggable genome » siRNA library- 7567 genes



Identification of 191 candidate host factors for HDV infection

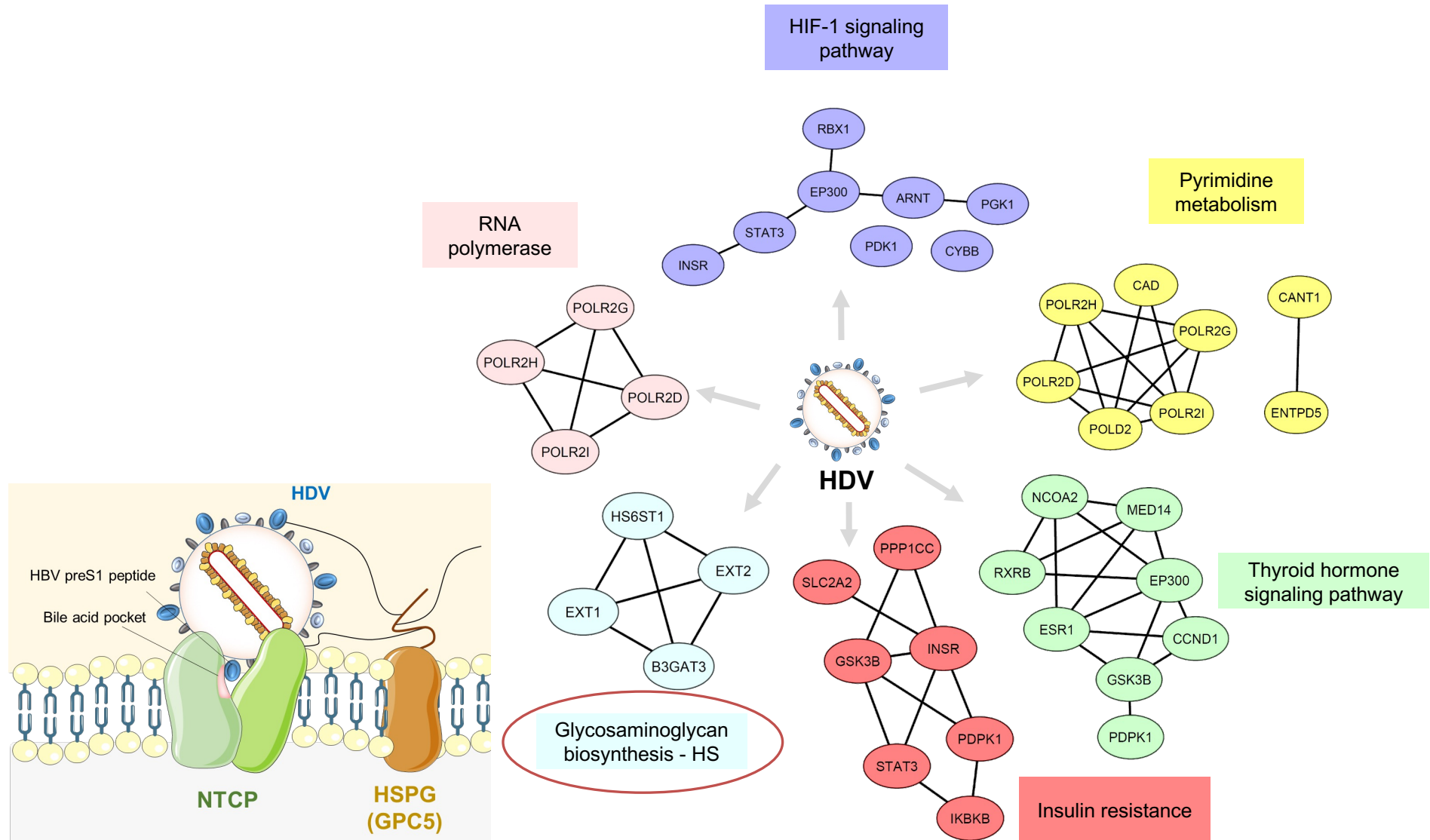
Loss-of-function screen

Pathways involved in HDV infection



Loss-of-function screen

siRNA druggable genome library

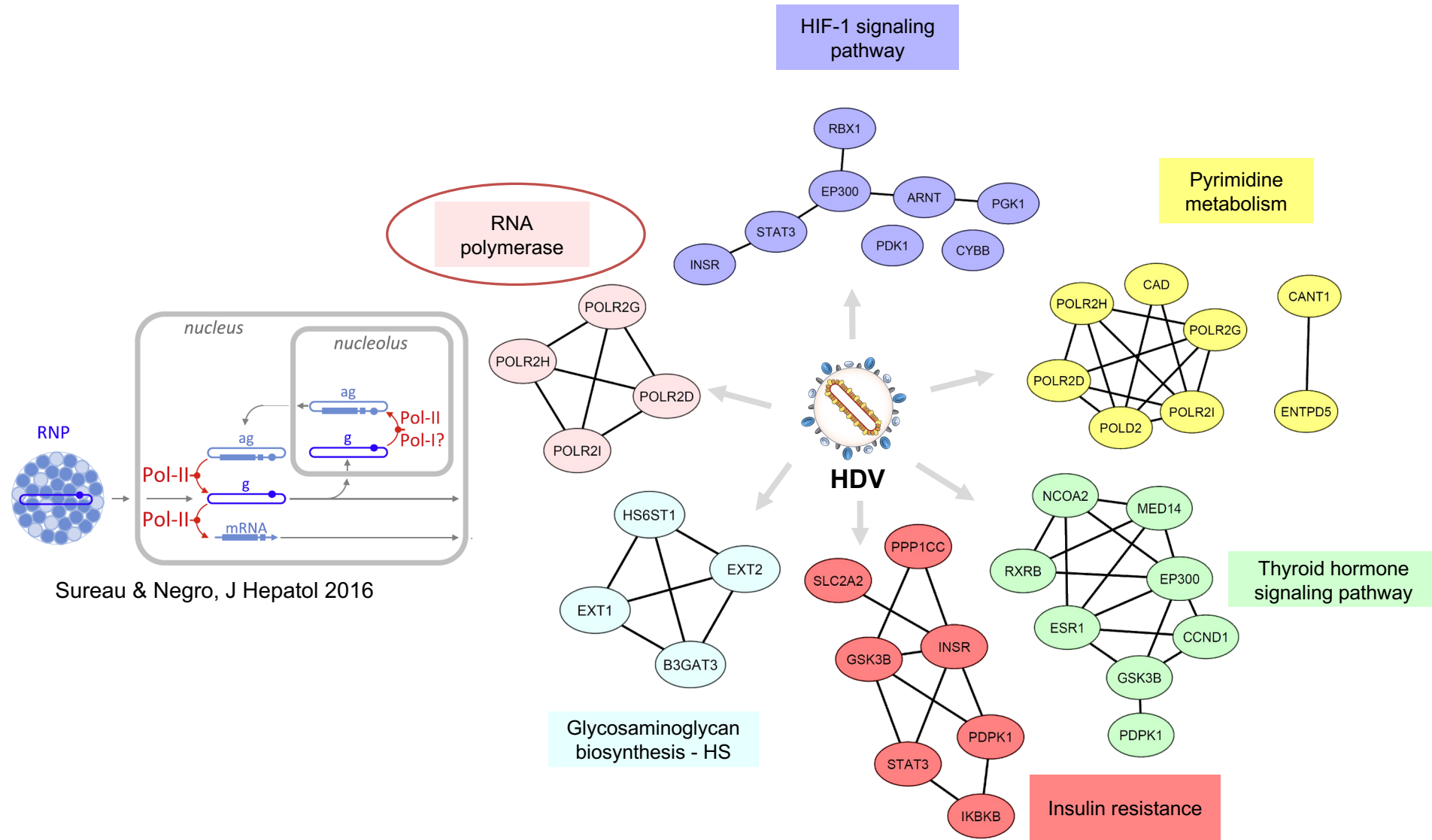


Adapted from Verrier et al., Virologie 2018

Verrier et al., Gut 2020

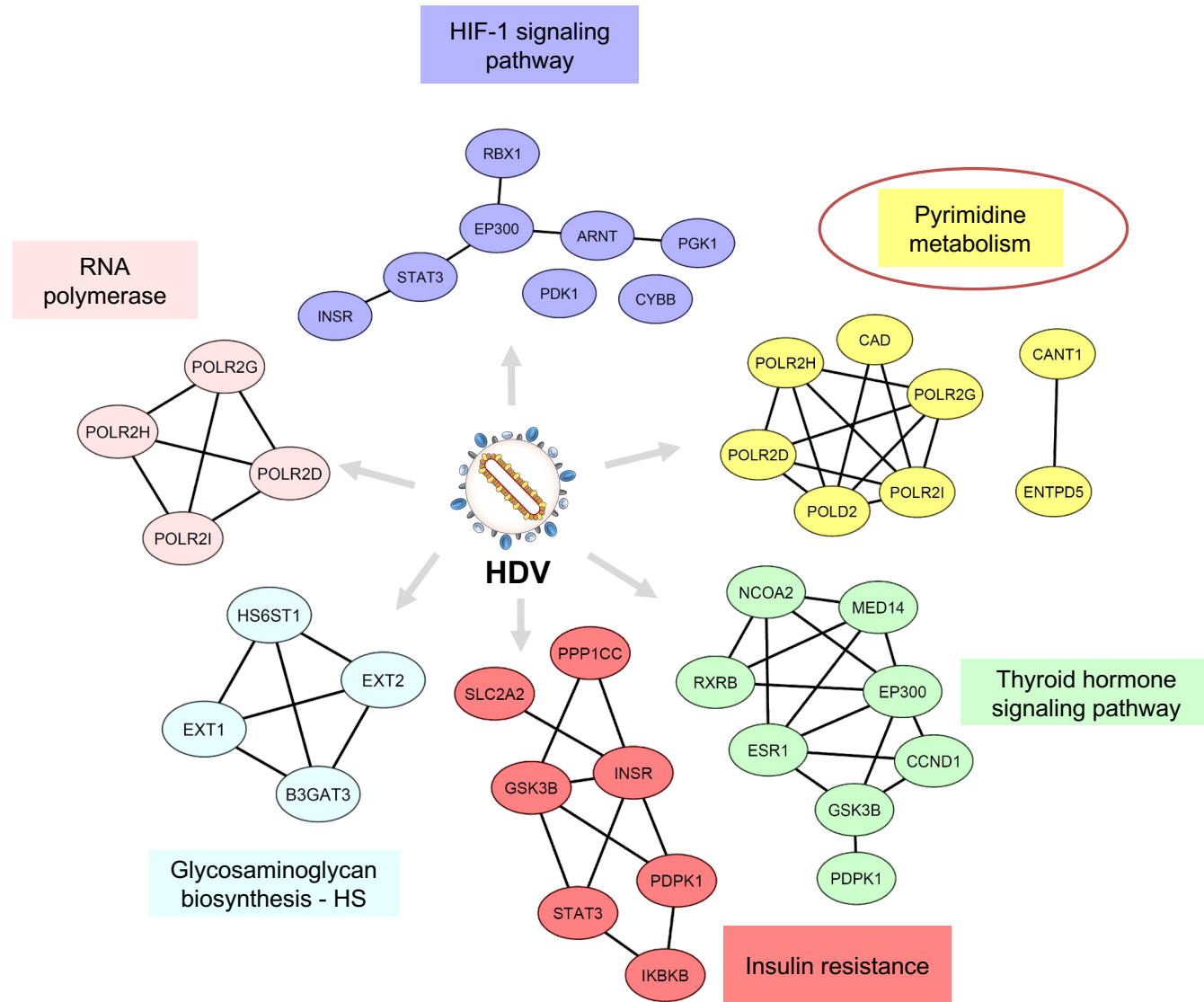
Loss-of-function screen

siRNA druggable genome library



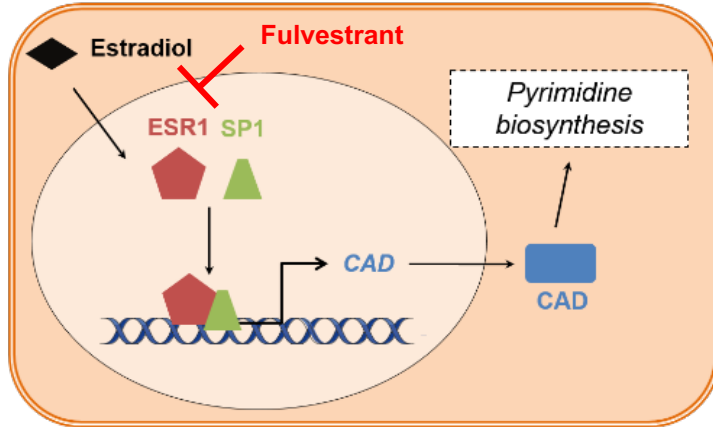
Loss-of-function screen

siRNA druggable genome library

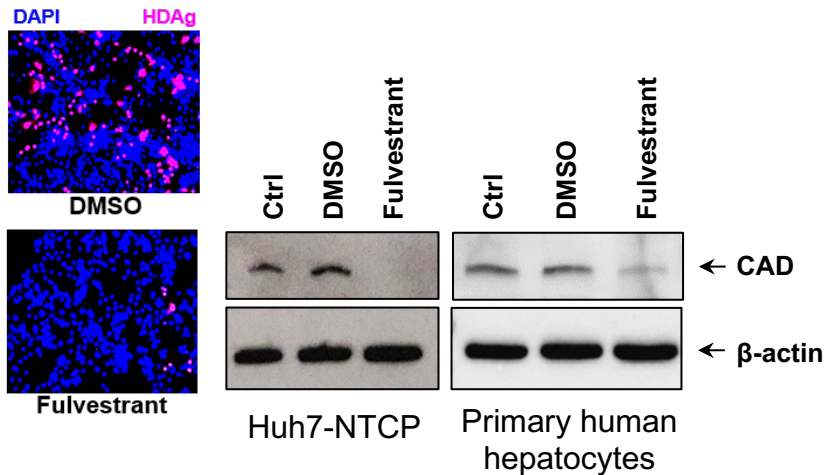
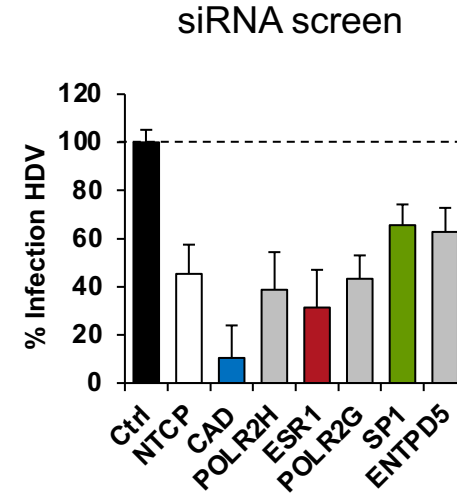


Pyrimidine synthesis and HDV infection

ESR1 and CAD are HDV host factors



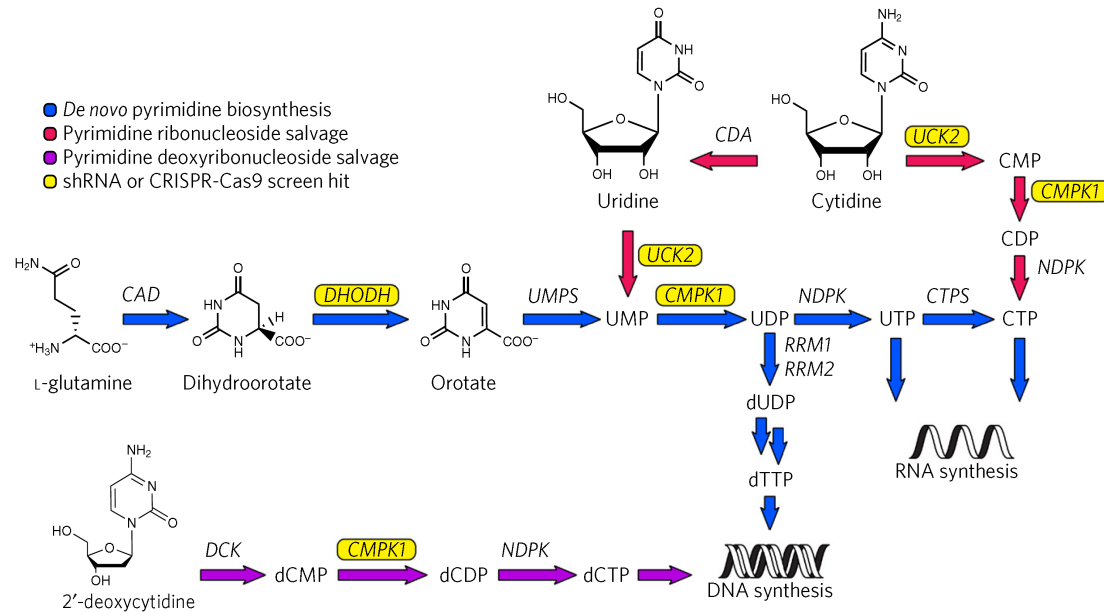
Model from Khan et al., Endocrinology 2003



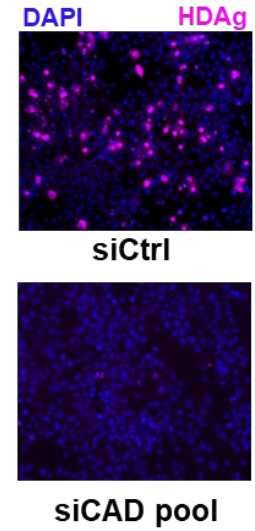
ESR1/CAD pathway is involved in HDV infection

Pyrimidine synthesis and HDV infection

Targeting CAD to inhibit HDV infection

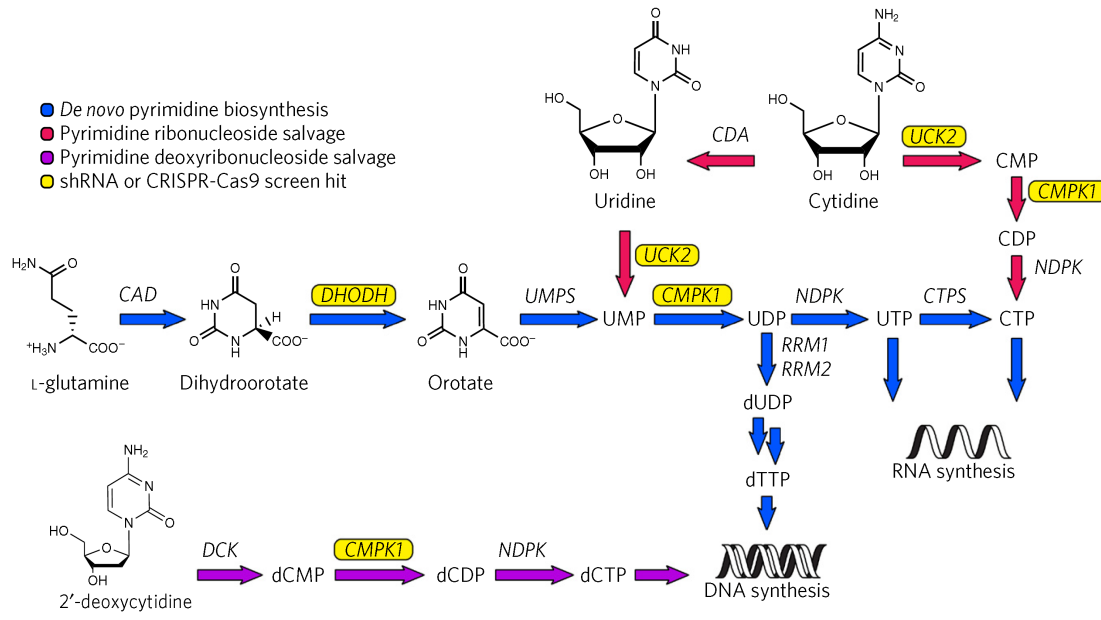


Deans et al., Nat Chem Biol 2016

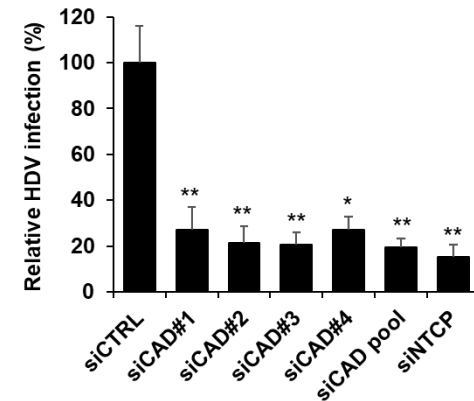
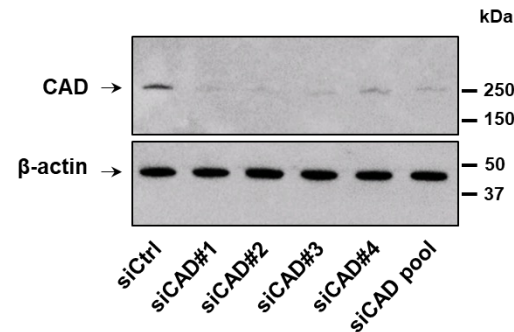
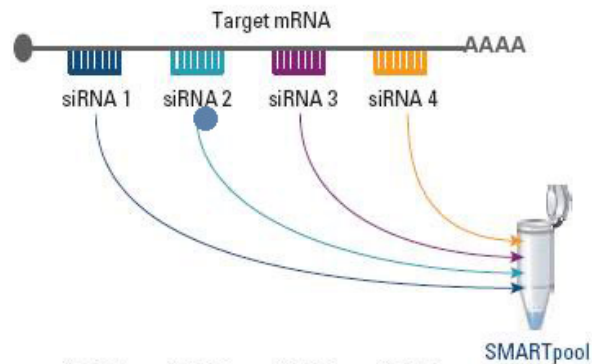
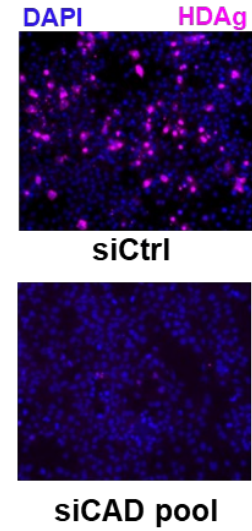


Pyrimidine synthesis and HDV infection

Targeting CAD to inhibit HDV infection

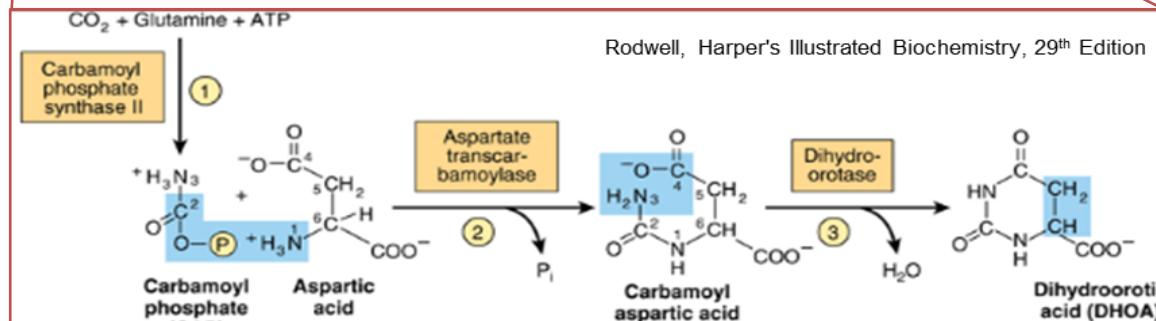
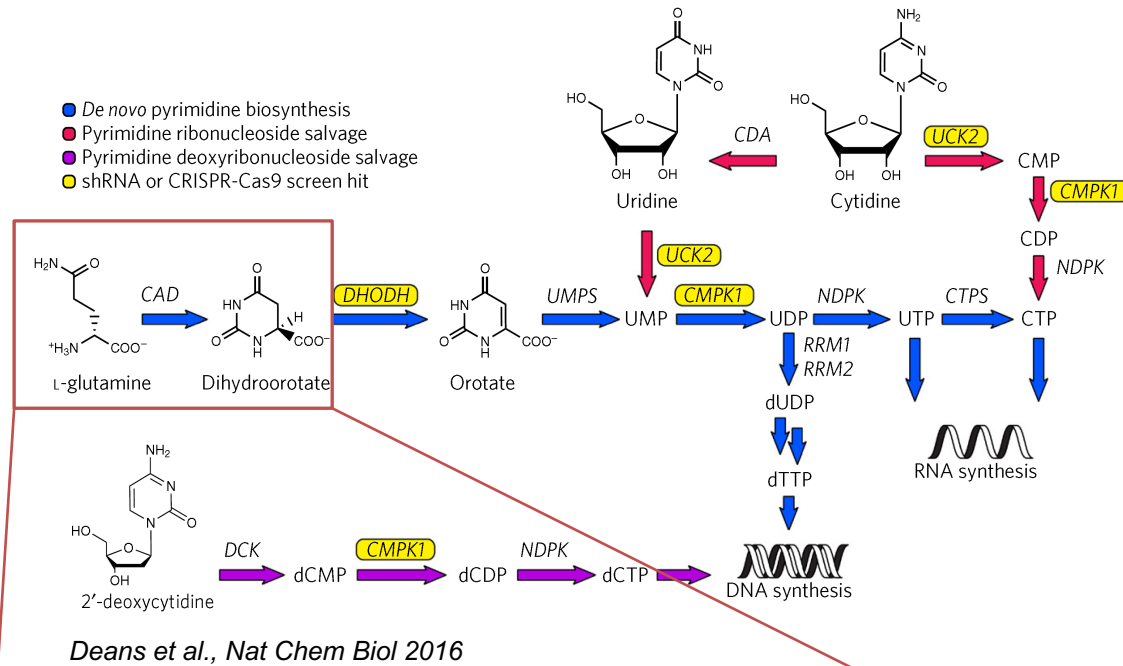


Deans et al., Nat Chem Biol 2016



Pyrimidine synthesis and HDV infection

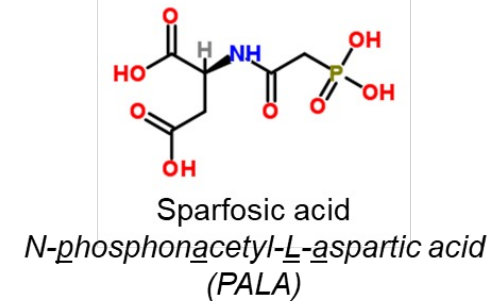
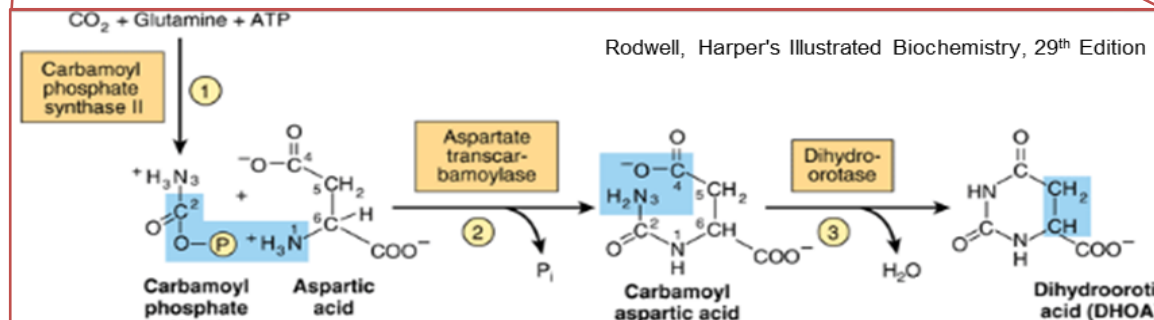
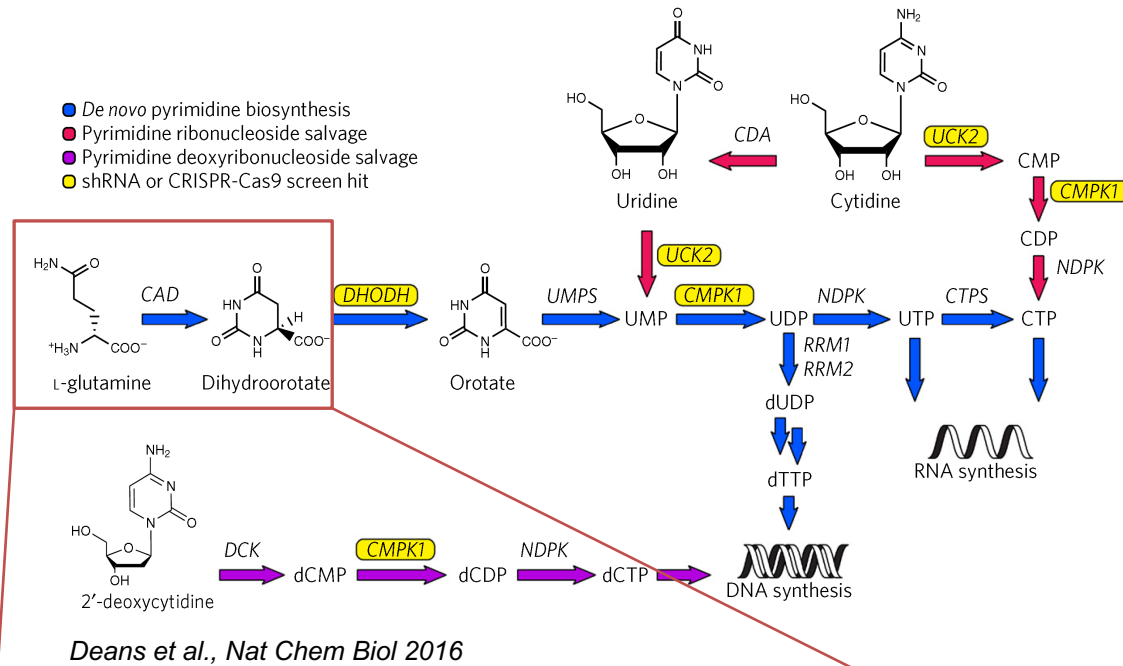
Targeting CAD to inhibit HDV infection



CAD : Carbamoyl-Phosphatase 2, Aspartate Transcarbamylase, And Dihydroorotase

Pyrimidine synthesis and HDV infection

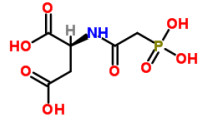
Targeting CAD to inhibit HDV infection



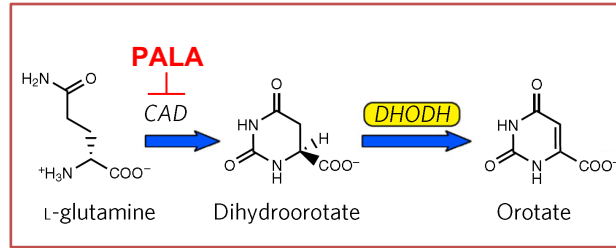
CAD : Carbamoyl-Phosphate Synthetase 2, Aspartate Transcarbamylase, And Dihydroorotase

Pyrimidine synthesis and HDV infection

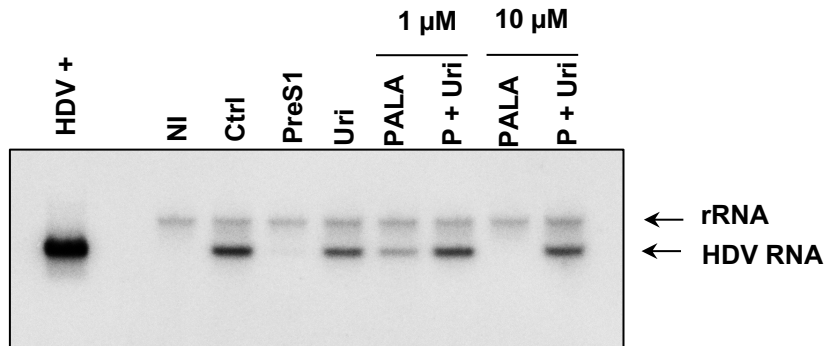
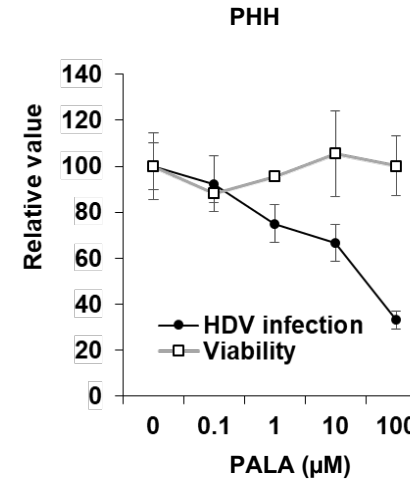
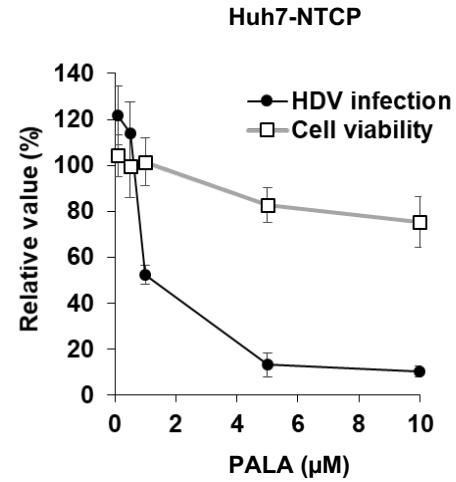
Targeting CAD to inhibit HDV infection



PALA – Sparfosic acid
N-phosphonacetyl-L-aspartic acid



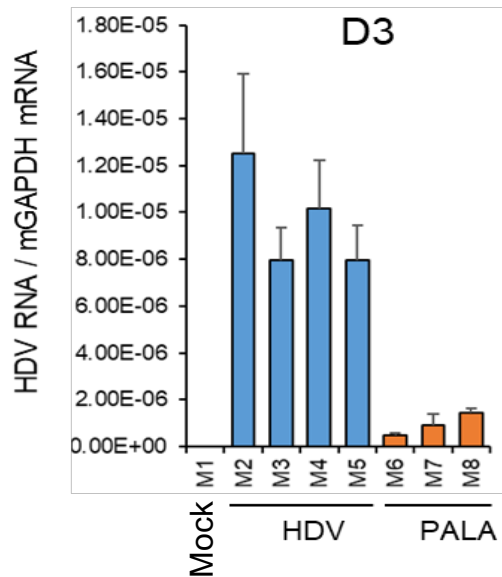
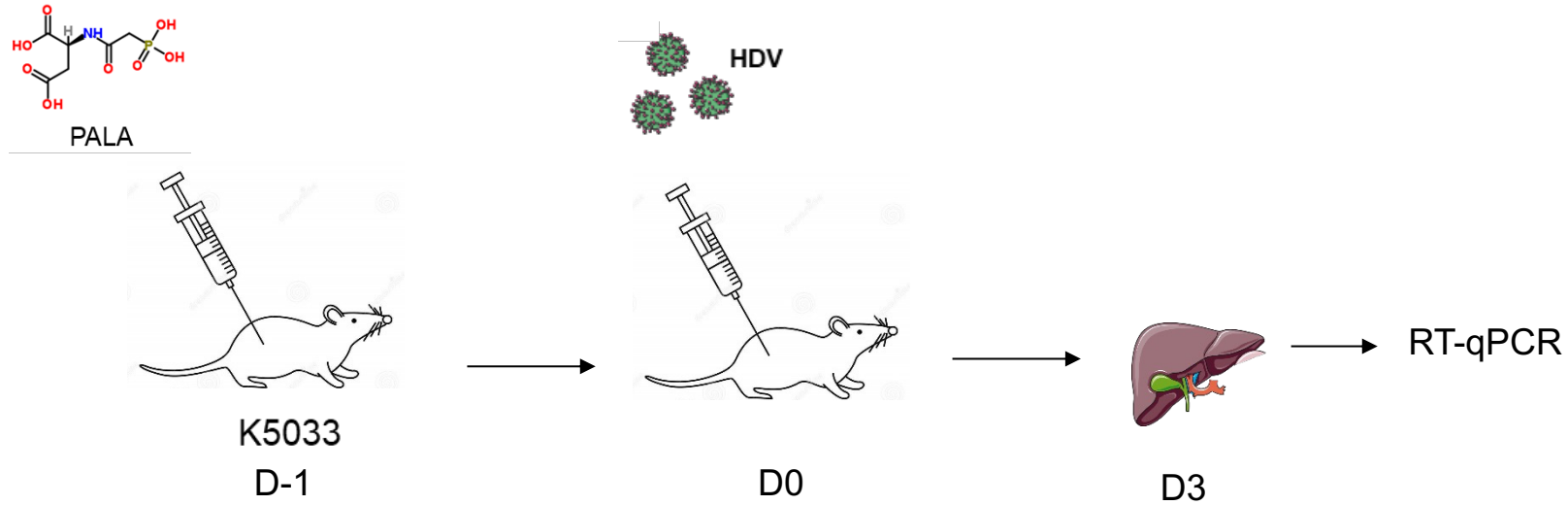
Adapted from Deans et al., Nat Chem Biol 2016



PALA does-dependently inhibits HDV infection in hepatocytes

Pyrimidine synthesis and HDV infection

PALA is a candidate molecule for preclinical studies



✓ **A preliminary study in an HDV-susceptible animal model suggests the anti-HDV activity of PALA in vivo**

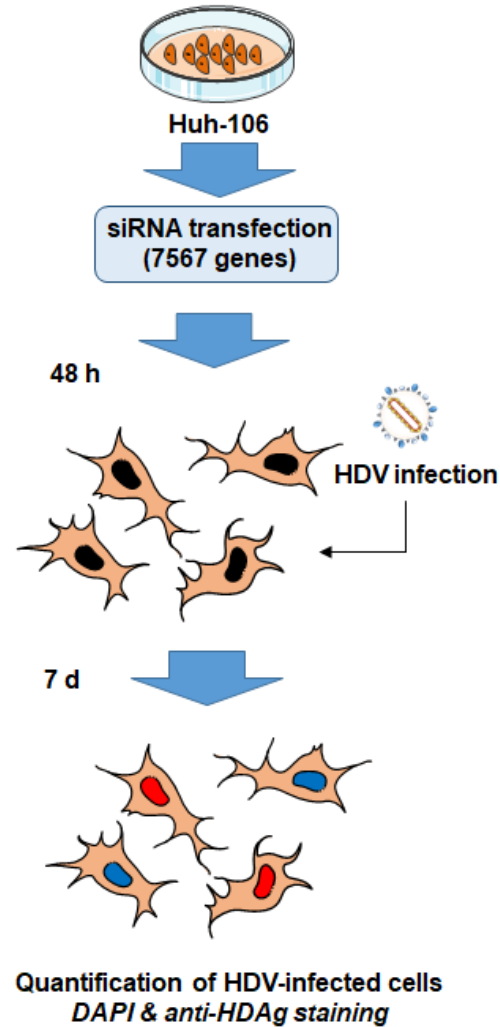
✓ **PALA is a candidate for preclinical studies**

In collaboration with Patrick Pale (University of Strasbourg)

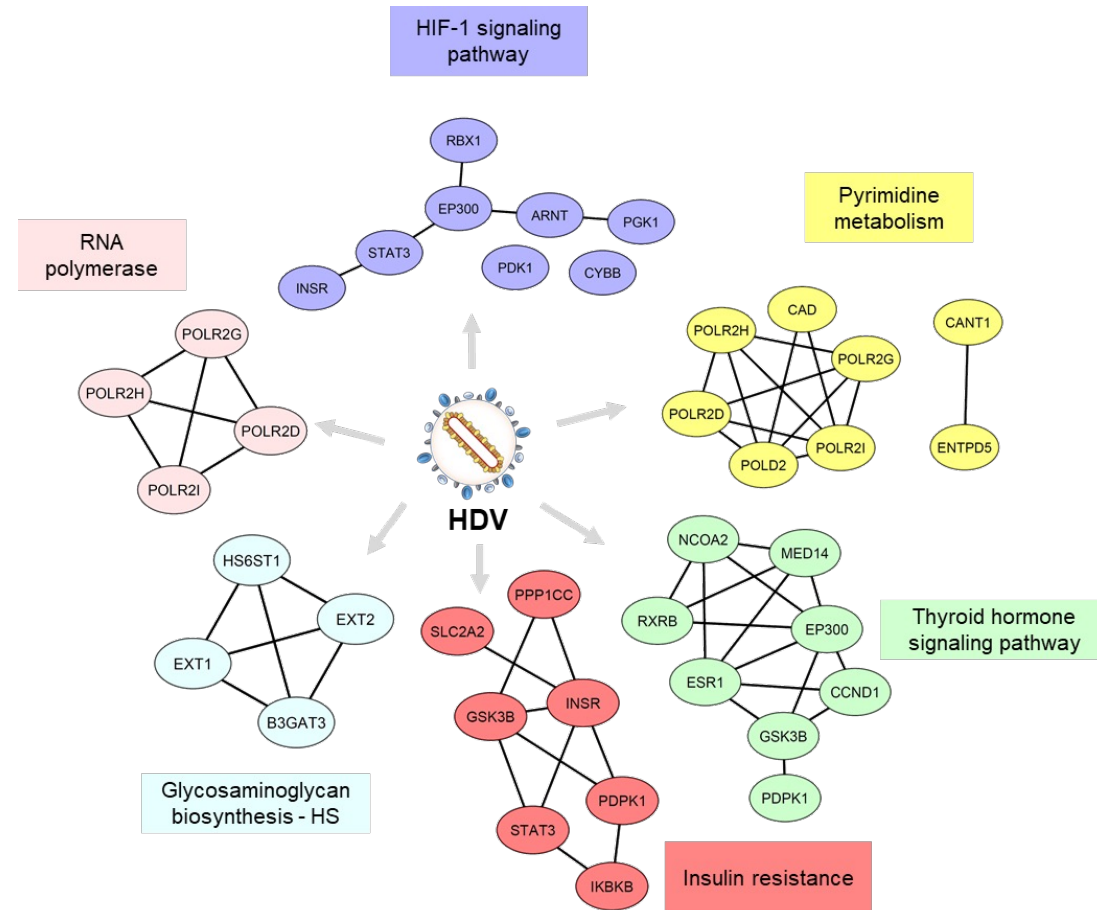
Loss-of-function screen

Validation of additional candidates

« Druggable Genome » siRNA screen

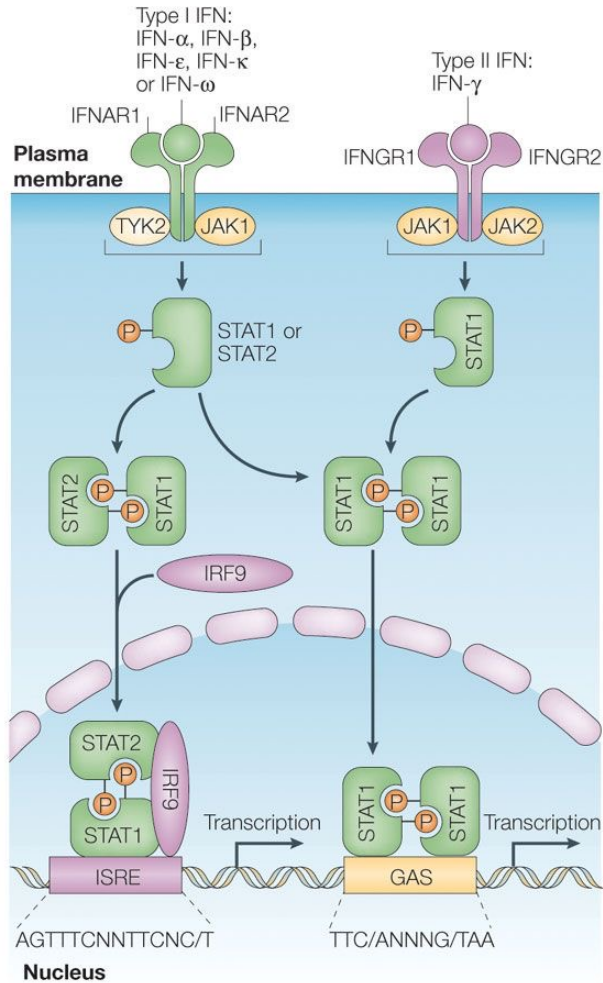


191 candidates
(CAD, ESR1, SLC10A1...)

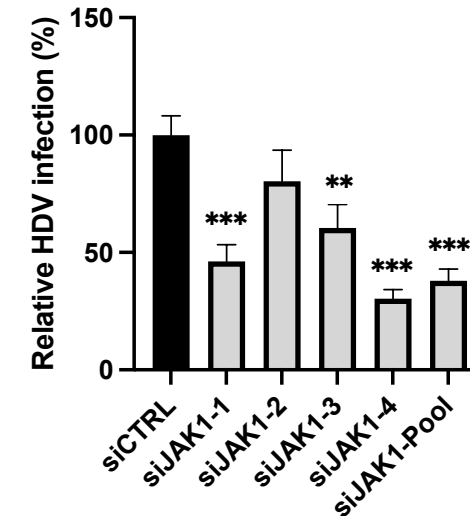
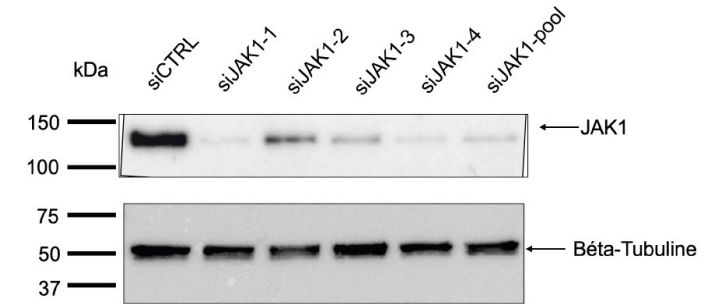
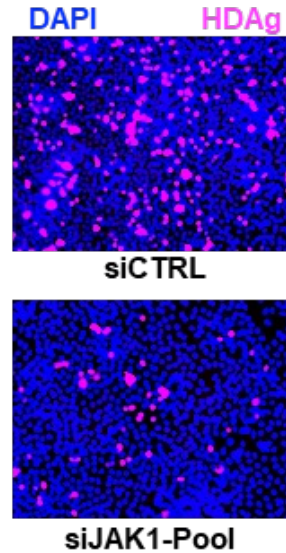


JAK1 is a candidate host factor for HDV infection

Validation of the primary screen result



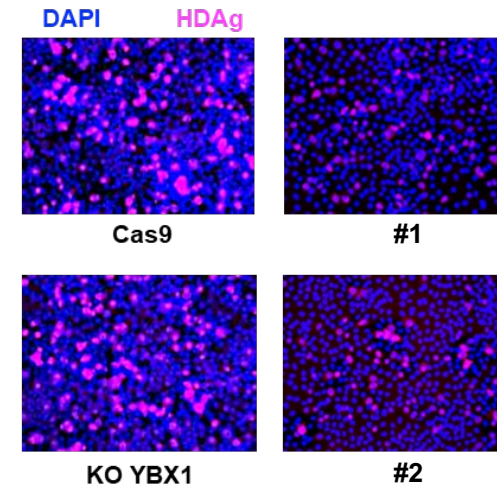
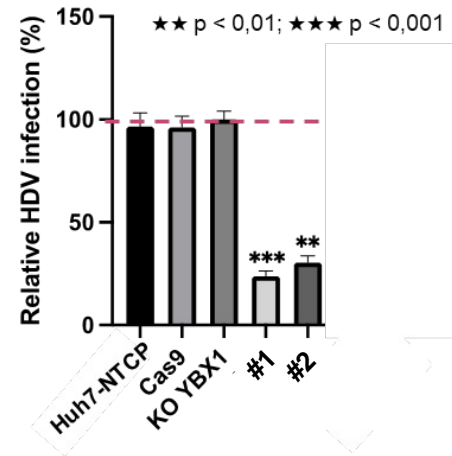
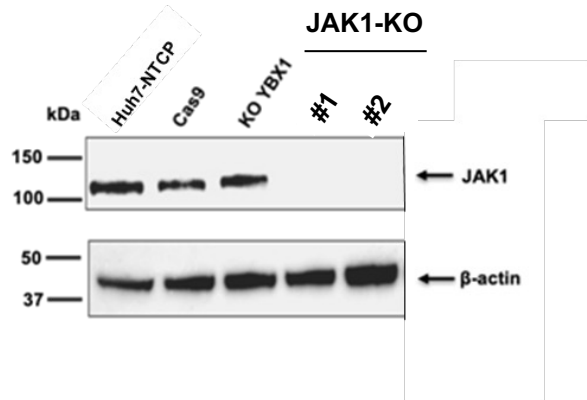
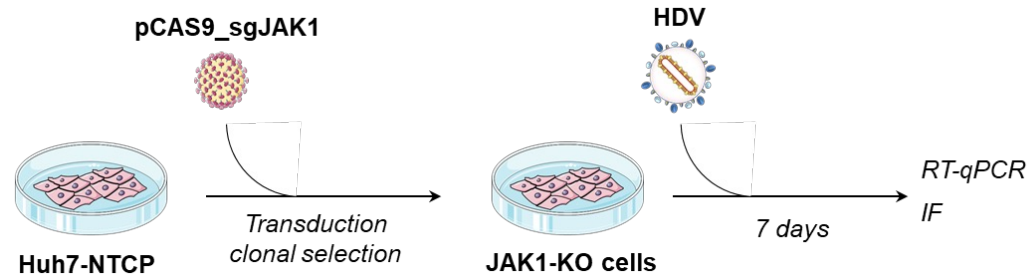
Platanias, Nat Rev Immunol 2005



JAK1 is a primary screen candidate factor and a decreased JAK1 expression is associated to an inhibition of HDV infection

JAK1 is a candidate host factor for HDV infection

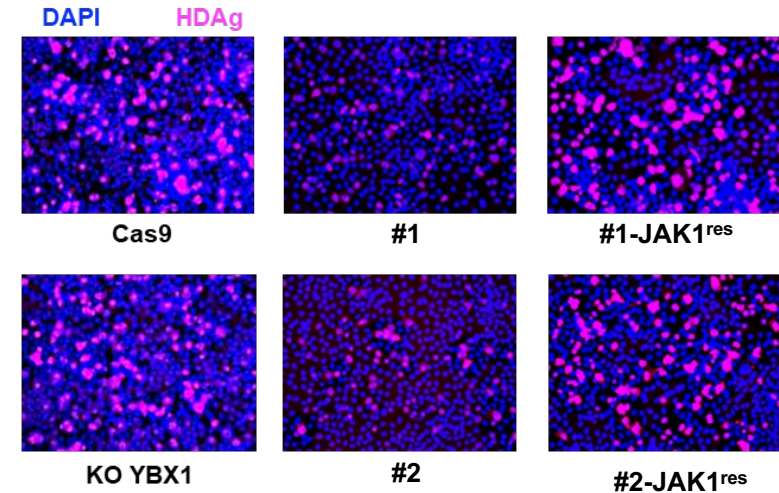
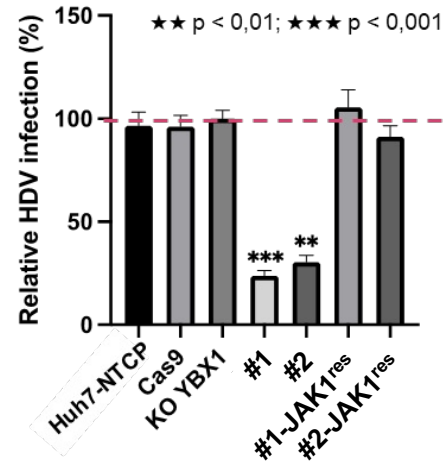
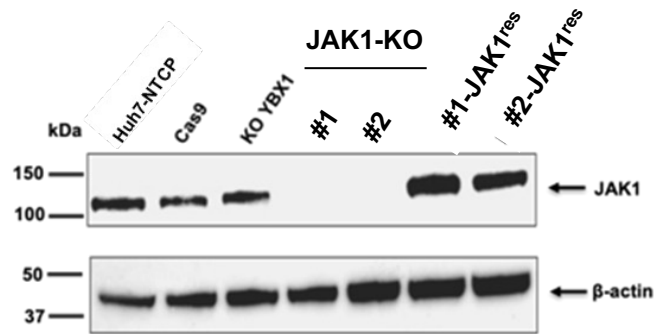
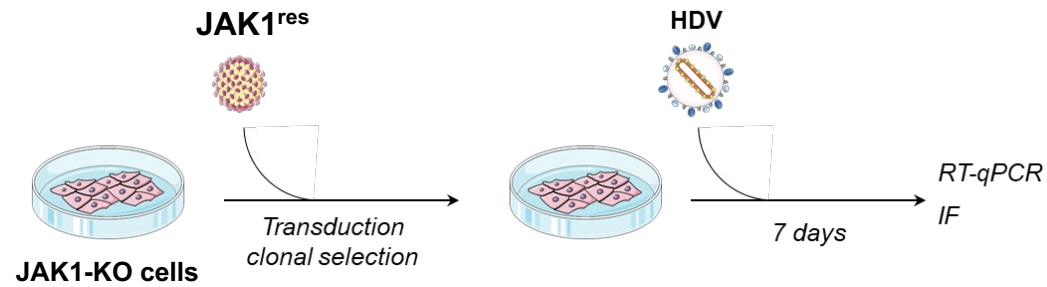
Validation of the primary screen result



✓ Decreased HDV infection in JAK1-KO cells

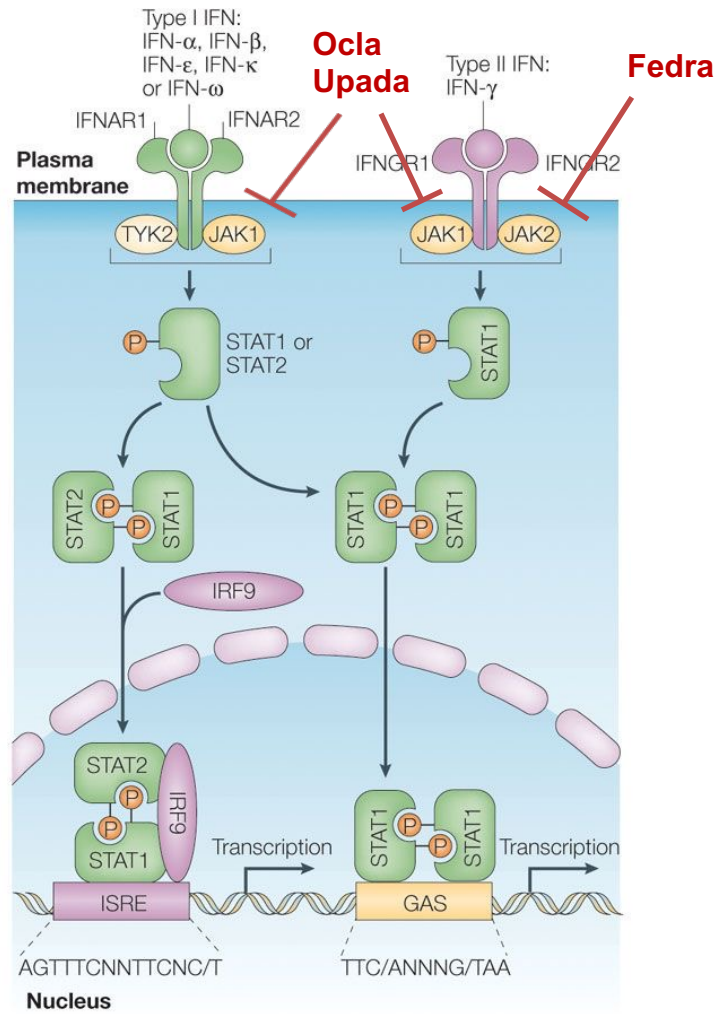
JAK1 is a candidate host factor for HDV infection

Validation of the primary screen result

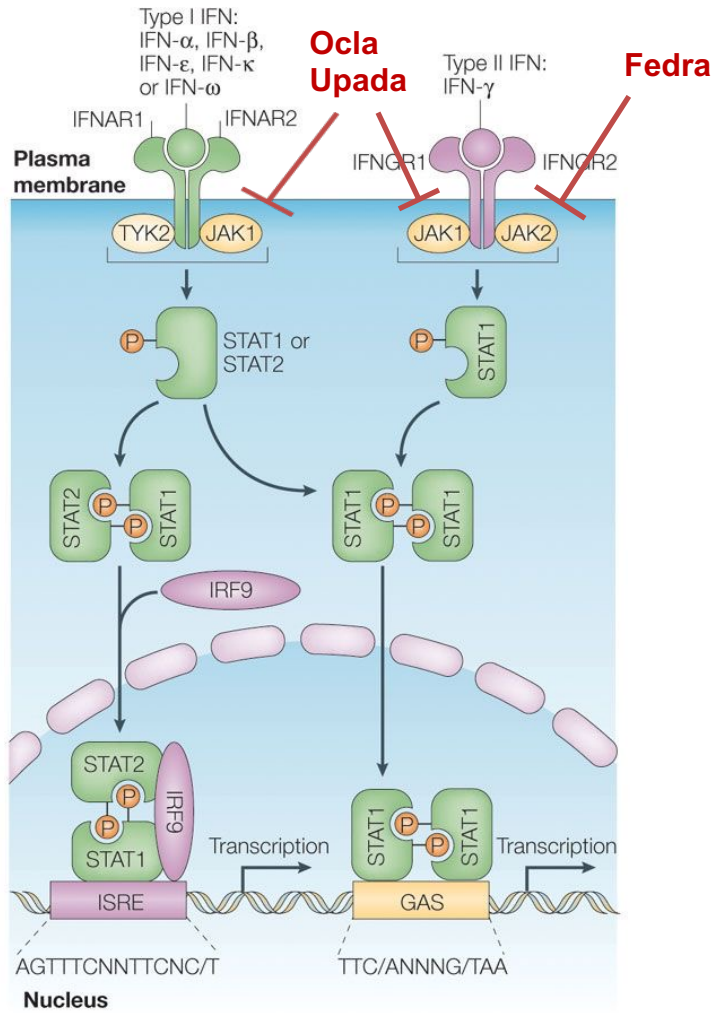


- ✓ Decreased HDV infection in JAK1-KO cells
- ✓ Ectopic expression of JAK1 restores HDV infection in JAK1-KO cells

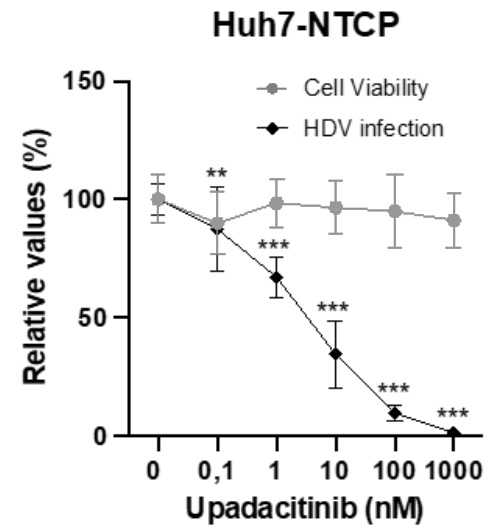
JAK1 inhibitors and HDV infection



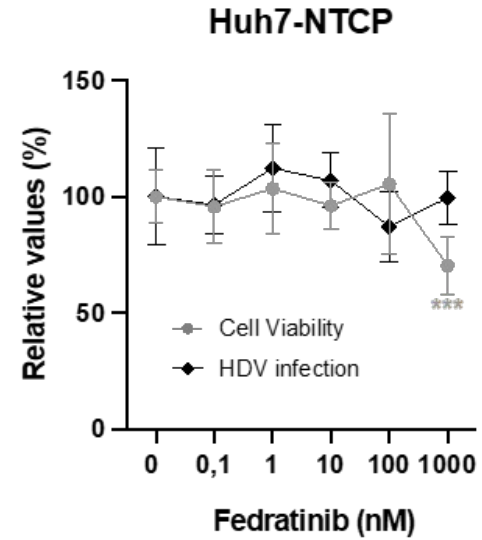
JAK1 inhibitors and HDV infection



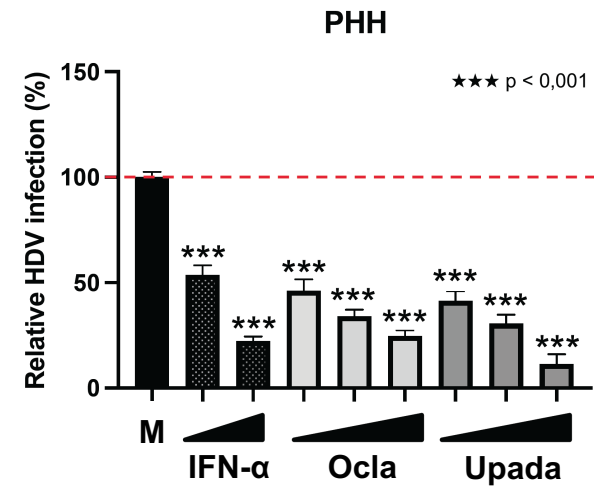
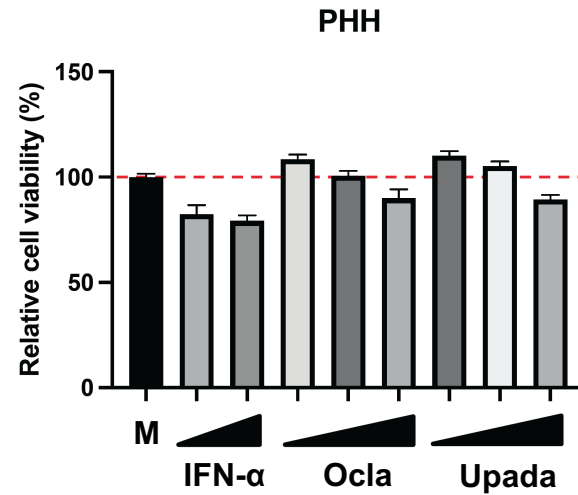
B



C



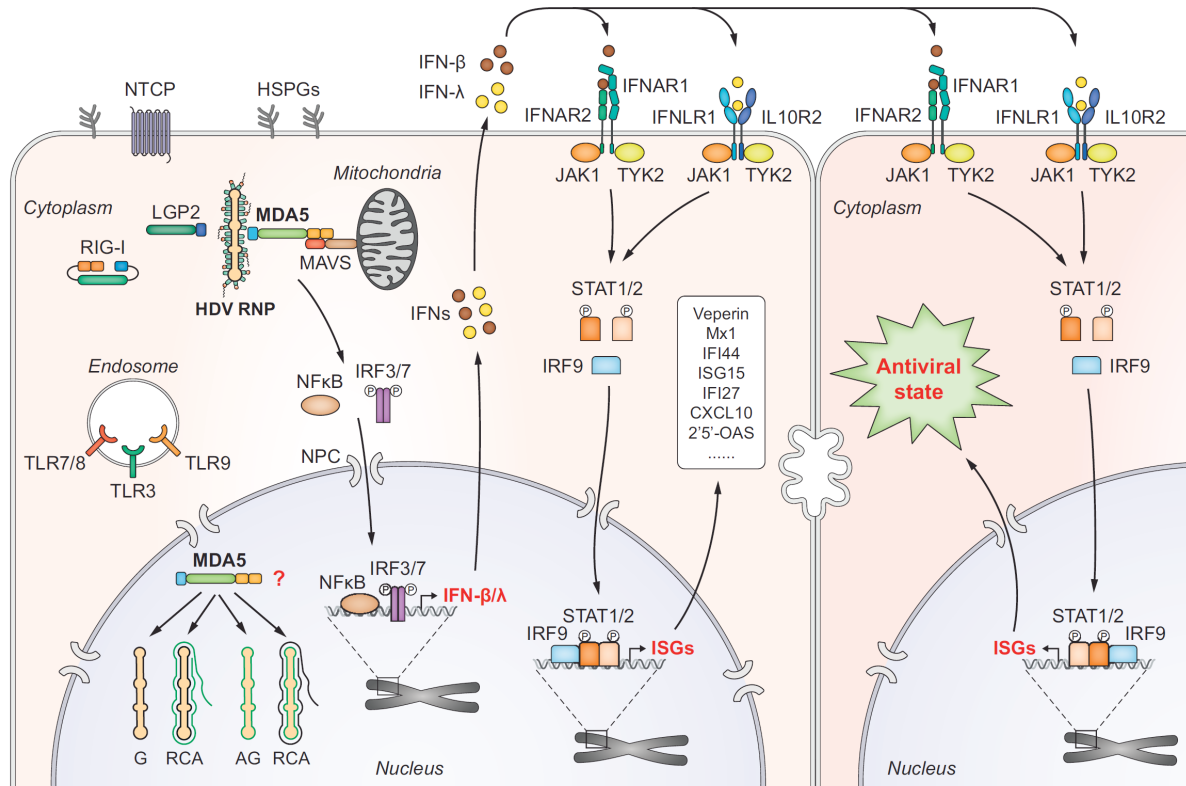
Validation in primary hepatocytes



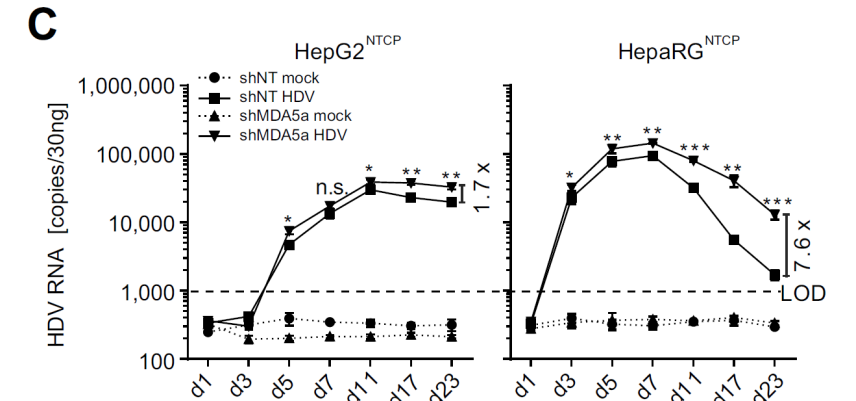
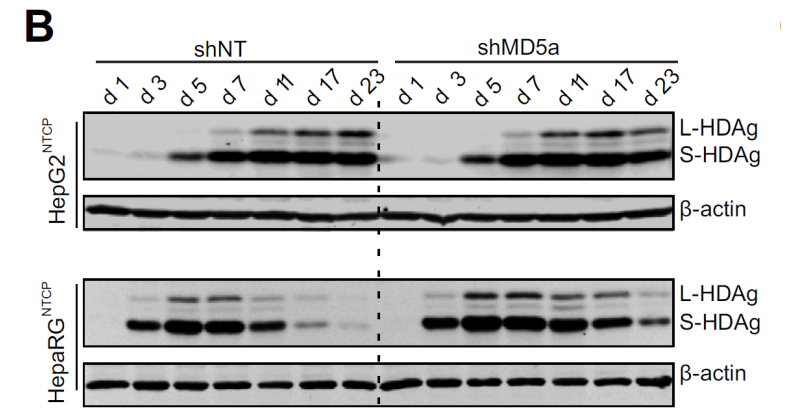
❖ **Dose-dependent inhibition** of HDV infection after treatment with Oclacitinib or Upadacitinib

JAK1 is a candidate host factor for HDV infection

MDA5 is a cytosolic sensor of HDV infection



Zhang & Urban, J Hepatol 2021



- ✓ **HDV RNA sensing by MDA5 triggers a type I and III IFN-mediated response and the expression of ISG**

Zhang et al., J Hepatol 2018

Gillish et al., J Hepatol 2023

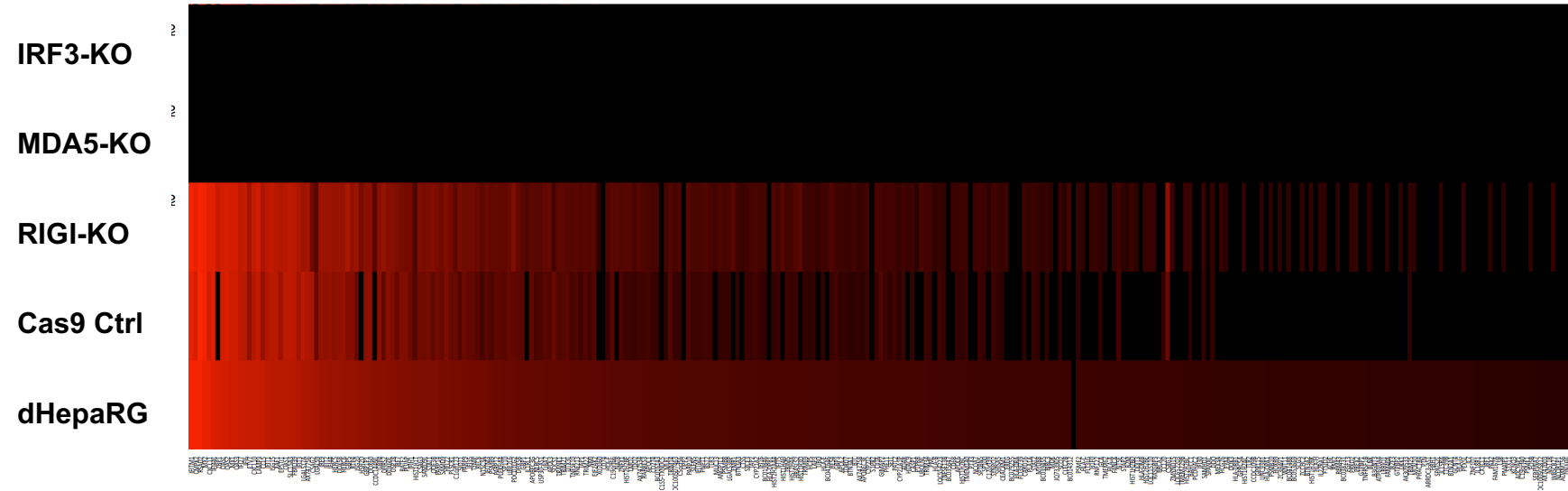
Lucifora et al., J Hepatol 2023

- ✓ **HDV replication is weakly sensitive to this innate immune response**

Zhang et al., J Hepatol 2018

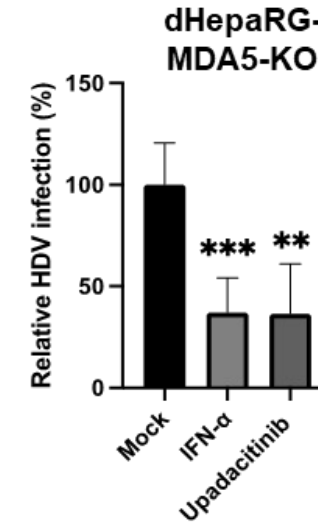
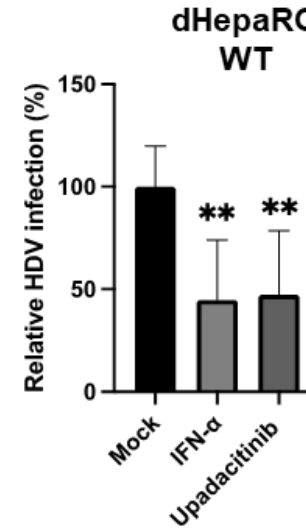
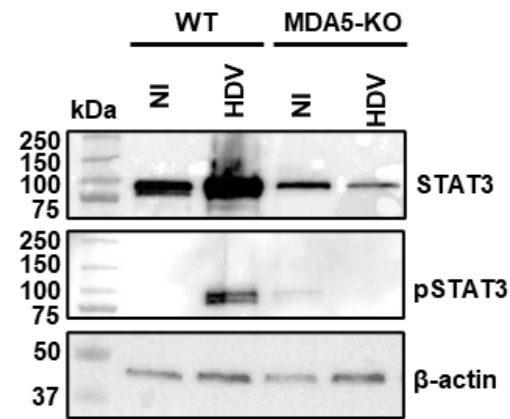
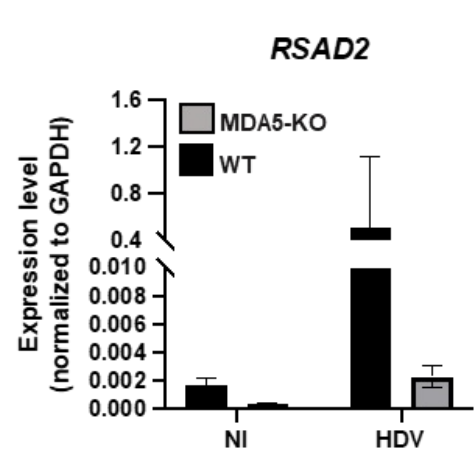
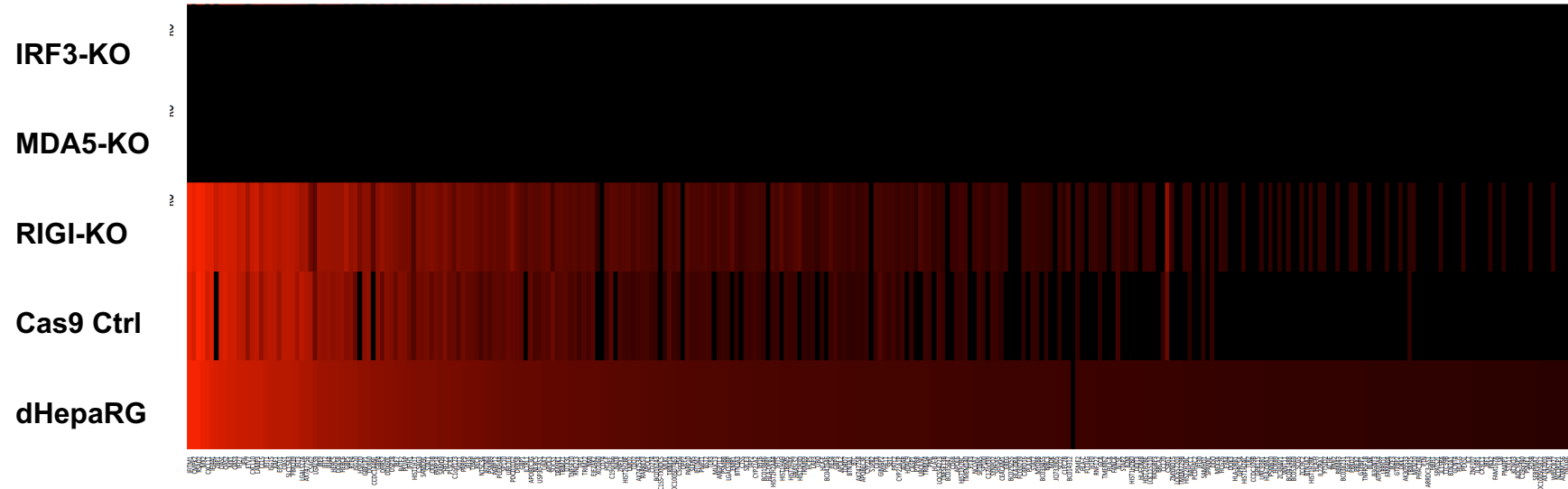
Validation in dHepaRG cells

ISG expression in HDV-infected dHepaRG cells



Validation in dHepaRG cells

ISG expression in HDV-infected dHepaRG cells



HDAg phosphorylation and HDV infection

> *J Virol.* 2008 Oct;82(19):9345-58. doi: 10.1128/JVI.00656-08. Epub 2008 Jul 16.

ERK1/2-mediated phosphorylation of small hepatitis delta antigen at serine 177 enhances hepatitis delta virus antigenomic RNA replication

Yen-Shun Chen ¹, Wen-Hung Huang, Shiao-Ya Hong, Yeou-Guang Tsay, Pei-Jer Chen

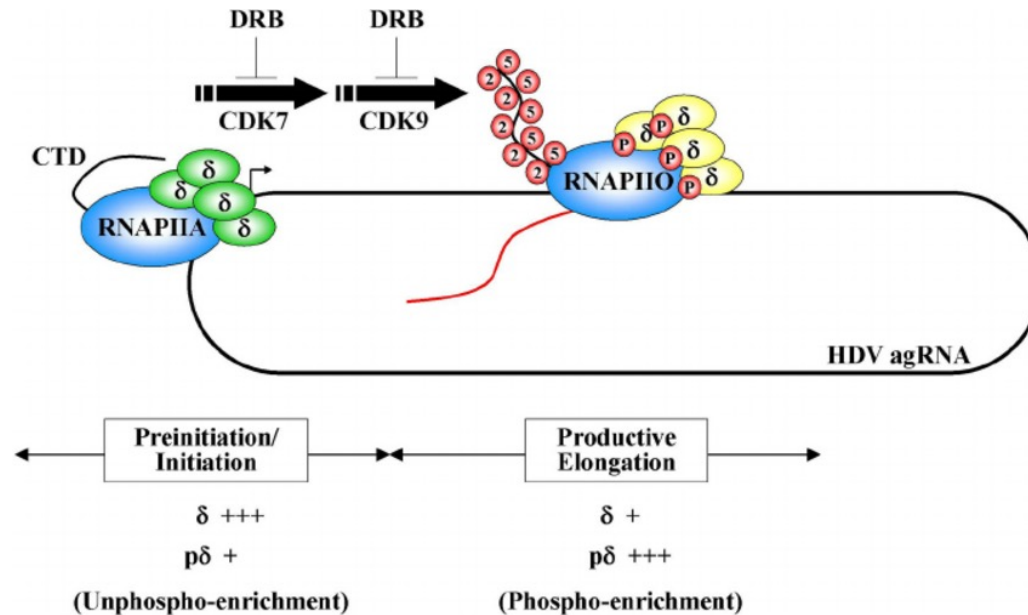
Affiliations + expand

PMID: 18632853 PMID: PMC2546944 DOI: 10.1128/JVI.00656-08

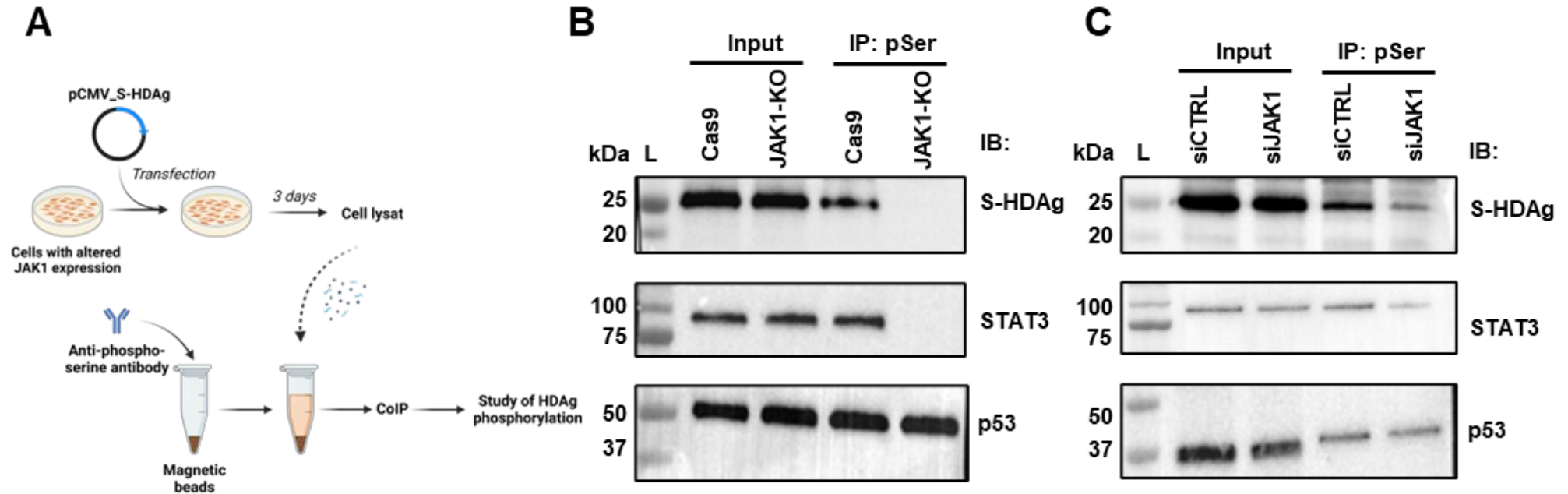
> *J Virol.* 2010 Feb;84(3):1430-8. doi: 10.1128/JVI.02083-09. Epub 2009 Nov 18.

Phosphorylation of serine 177 of the small hepatitis delta antigen regulates viral antigenomic RNA replication by interacting with the processive RNA polymerase II

Shiao-Ya Hong ¹, Pei-Jer Chen

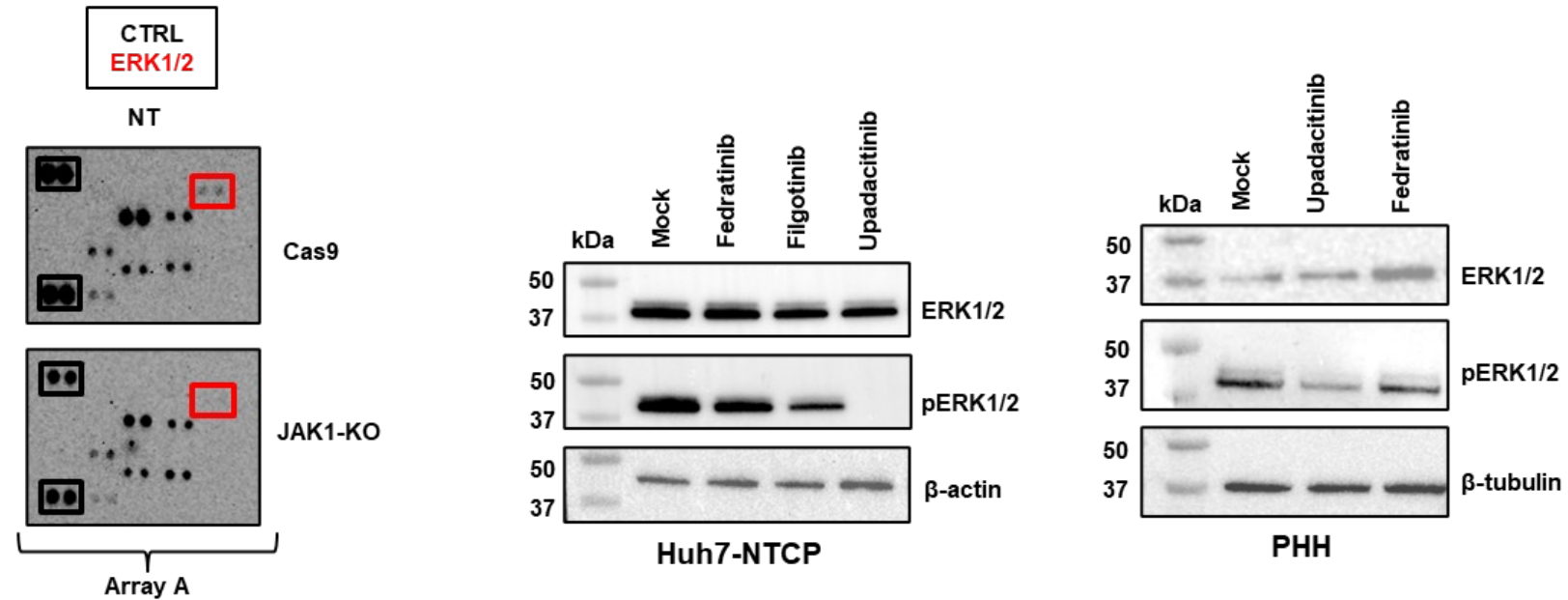


HDAg phosphorylation and JAK1 activity



Decreased HDAg phosphorylation levels in JAK1-KD or JAK1-KO cells

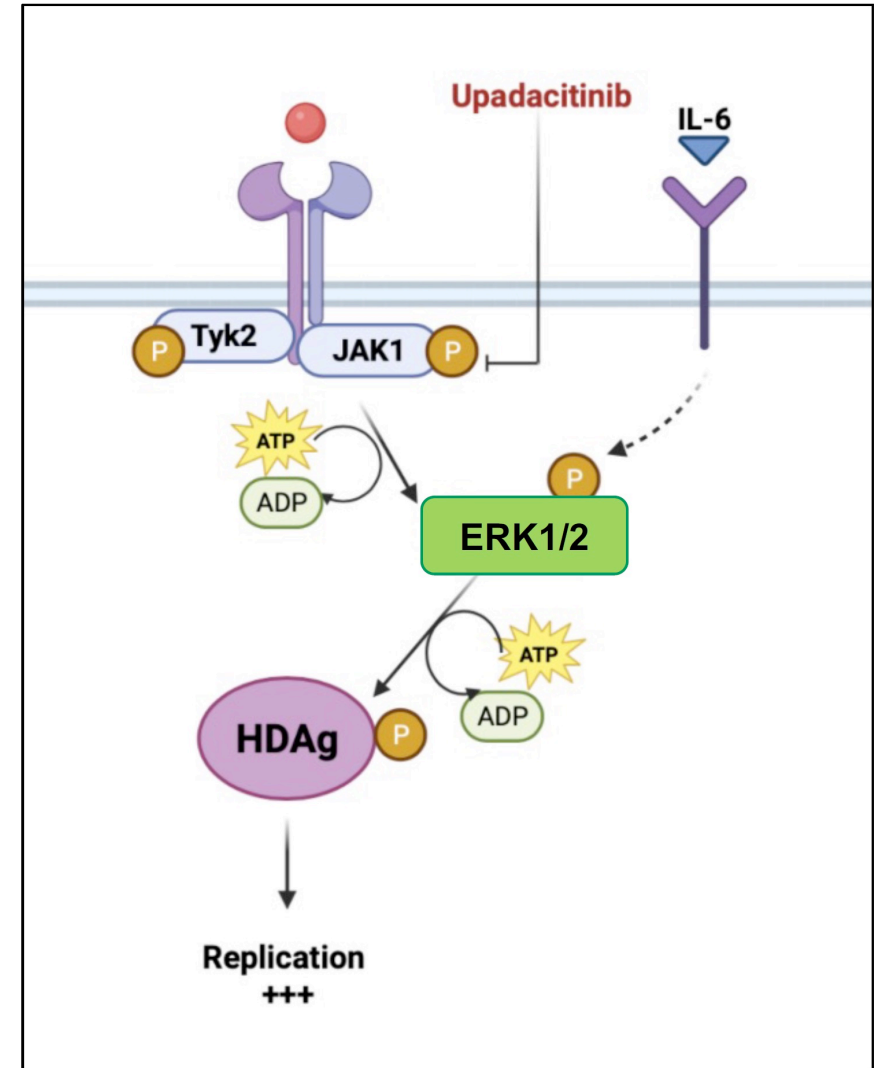
ERK1/2 phosphorylation in JAK1-KO cells



ERK1/2 phosphorylation is inhibited when JAK1 activity is altered

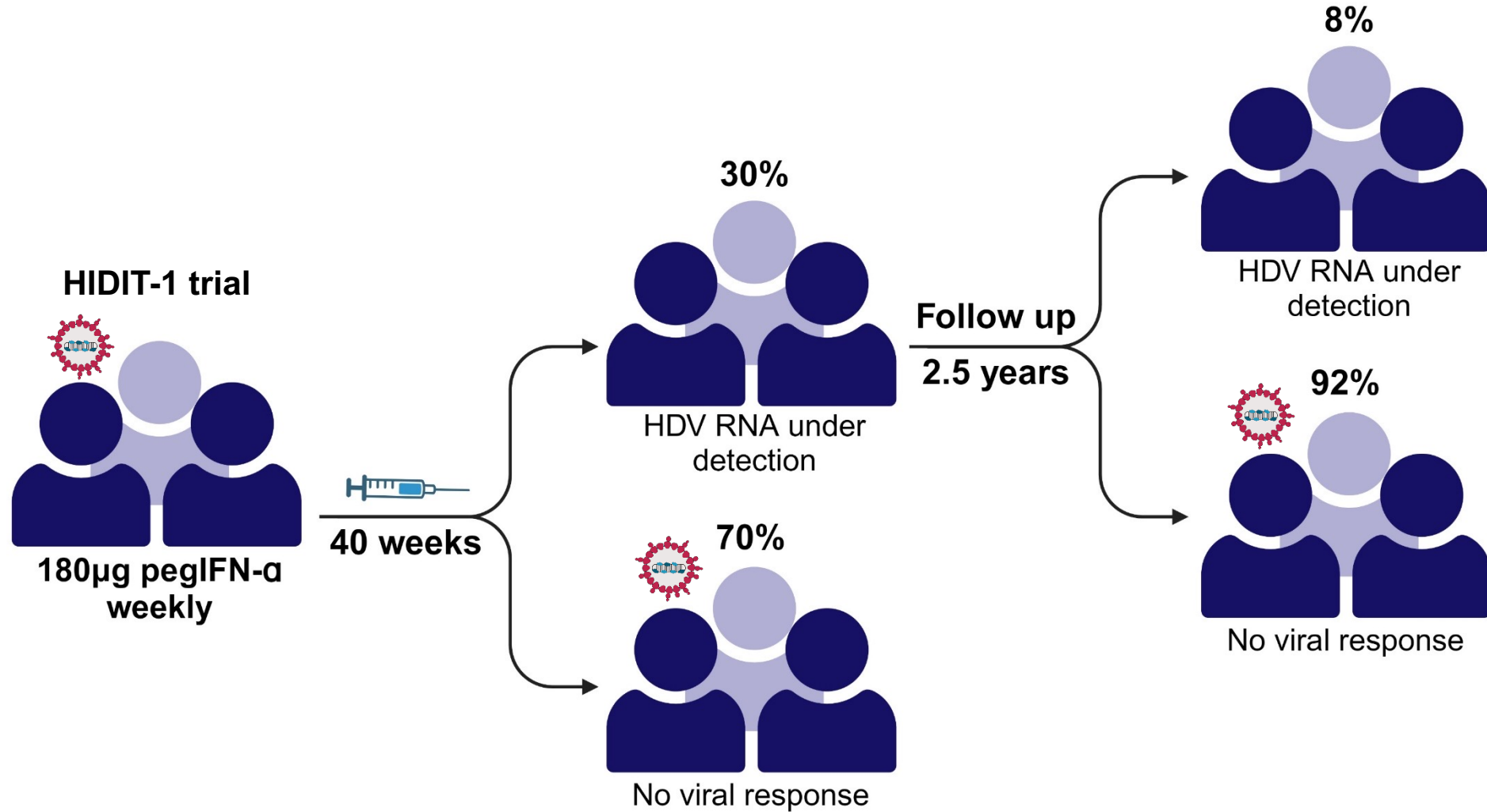
Summary

- ✓ A high-throughput screening approach identified key cellular factors involved in HDV infection, including CAD and JAK1
- ✓ JAK1 exhibits a proviral effect on HDV replication and is a target for antiviral therapy



Anti-HDV therapies

The moderate antiviral effect of pegIFN- α -2a



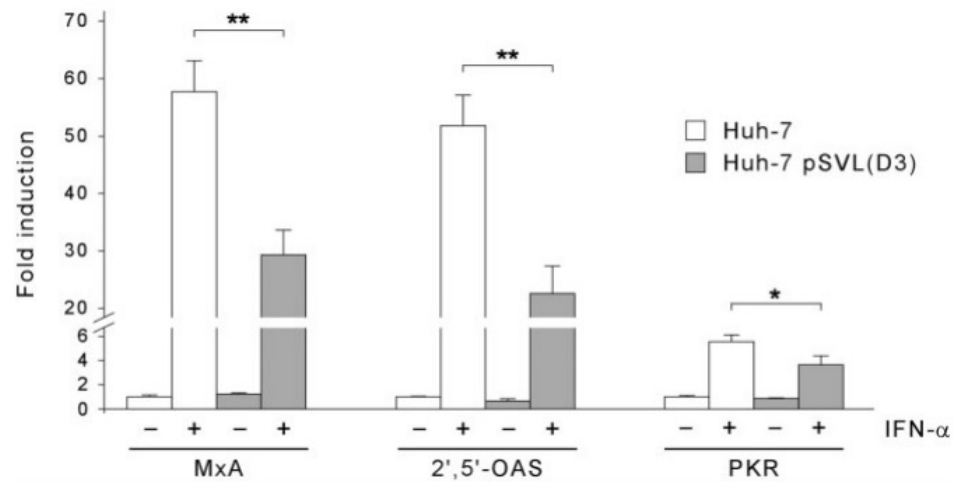
Importance to understand interactions between HDV and the IFN response in patients

Understanding the moderate response to IFN α in HDV-infected cells

Hepatitis Delta Virus Inhibits Alpha Interferon Signaling

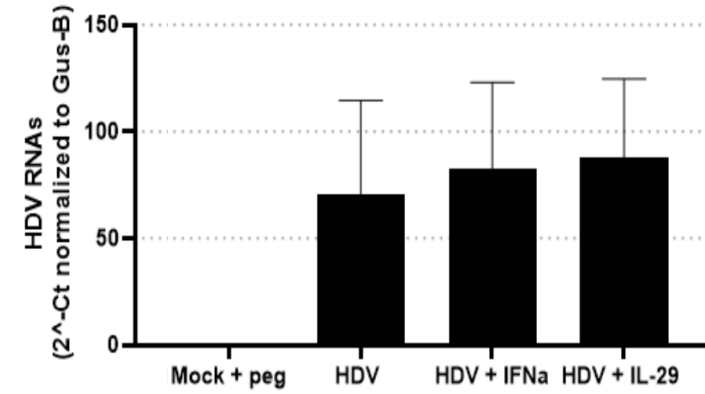
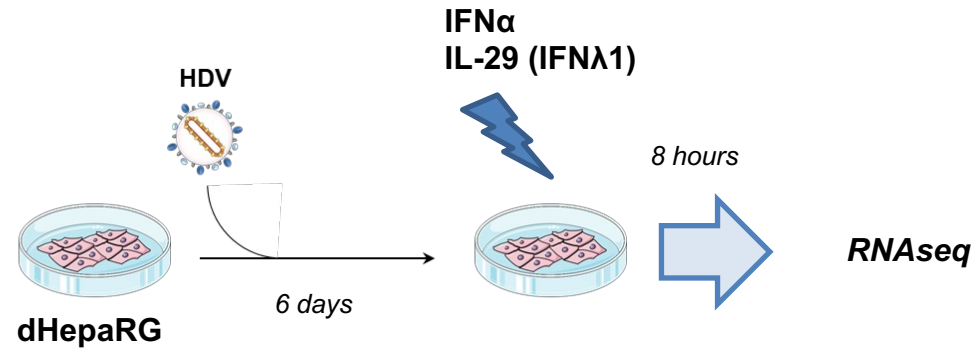
Paolo Pugnale,¹ Valerio Paziienza,¹ Kévin Guilloux,¹ and Francesco Negro^{1,2}

Hepatology 2009

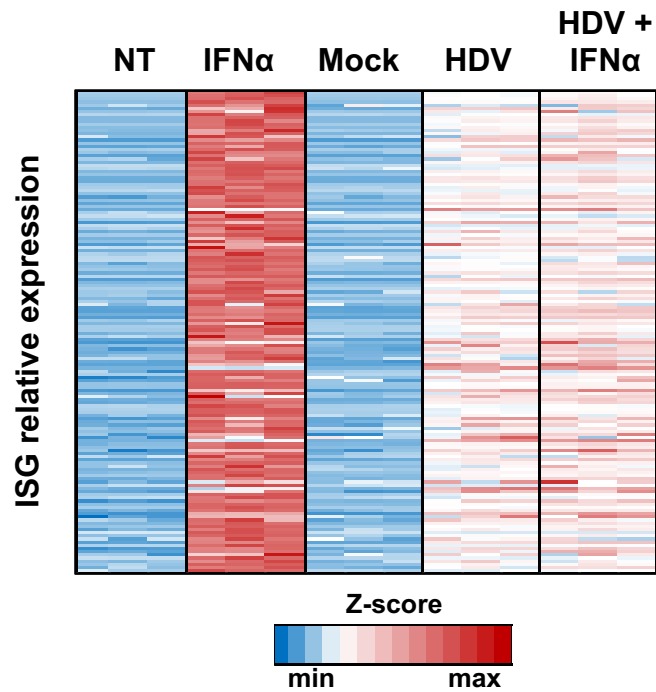
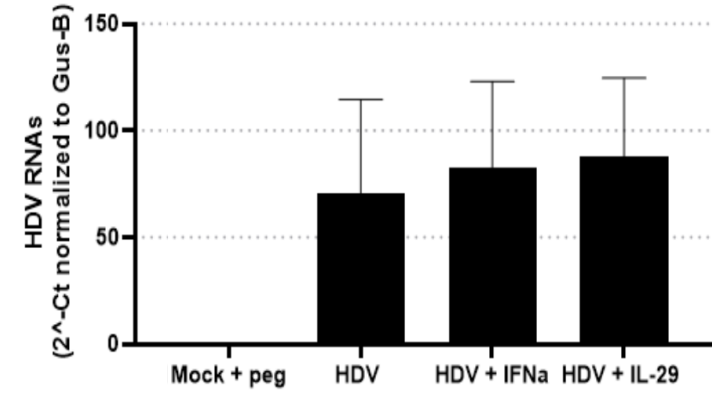
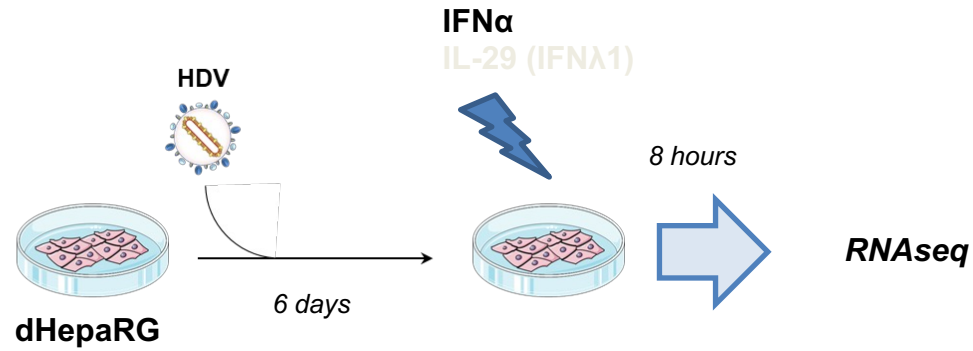


HDV replication is associated to a weaker induction of ISG upon IFN α treatment in a transfection model

Understanding the moderate response to IFN α in HDV-infected cells

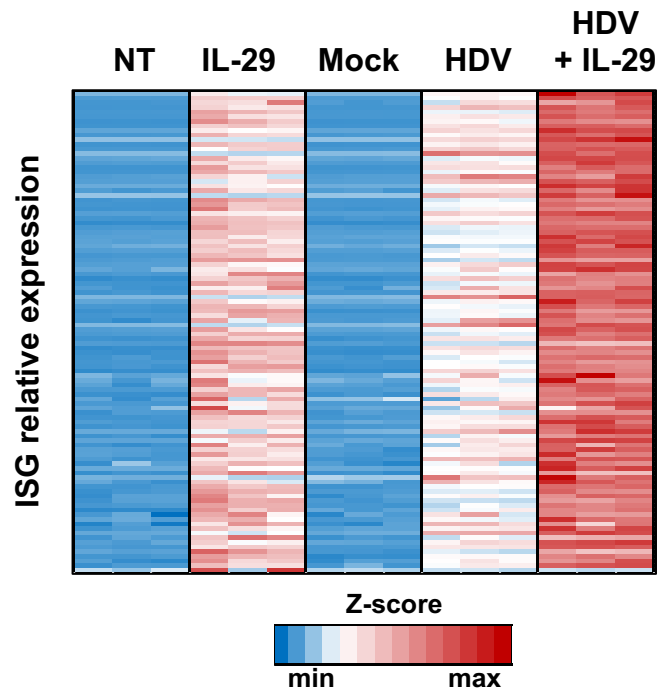
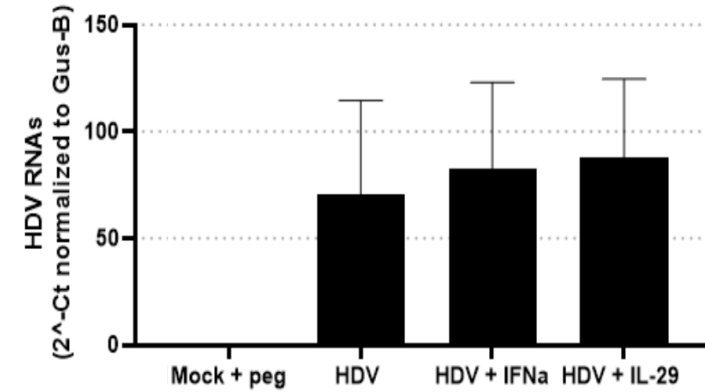
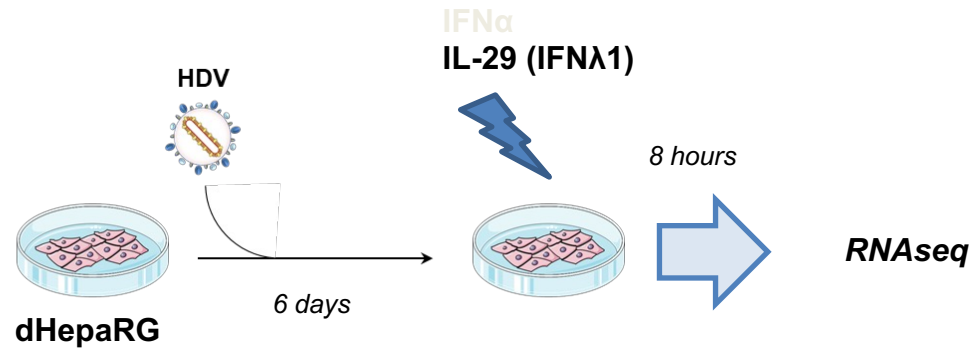


Understanding the moderate response to IFN α in HDV-infected cells



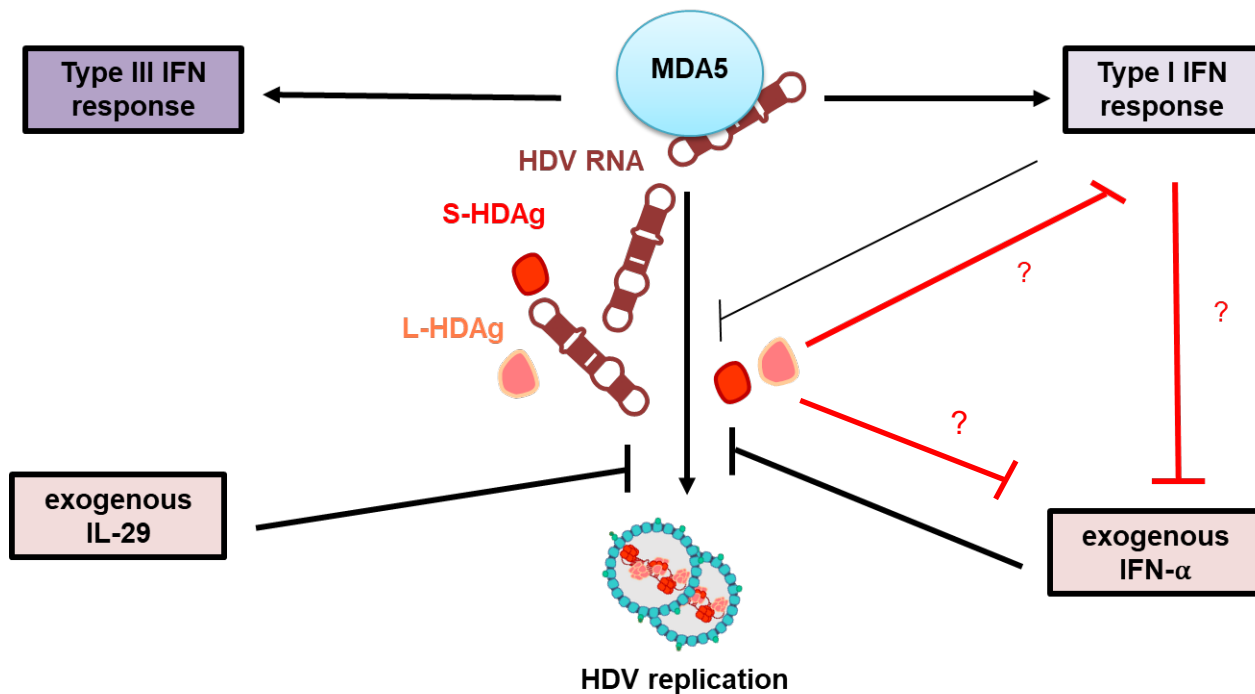
HDV-infected dHepaRG cells are unable to upregulate ISG expression upon IFN α treatment

Understanding the modest response to IFN α in HDV-infected cells



Additive ISG expression upon IL-29 treatment of HDV-infected dHepaRG cells

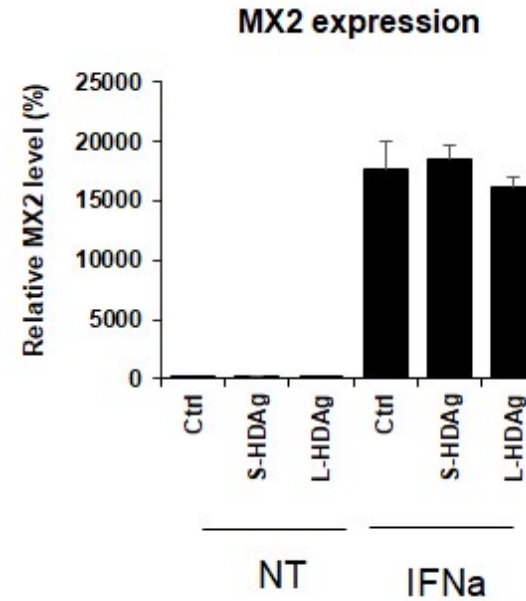
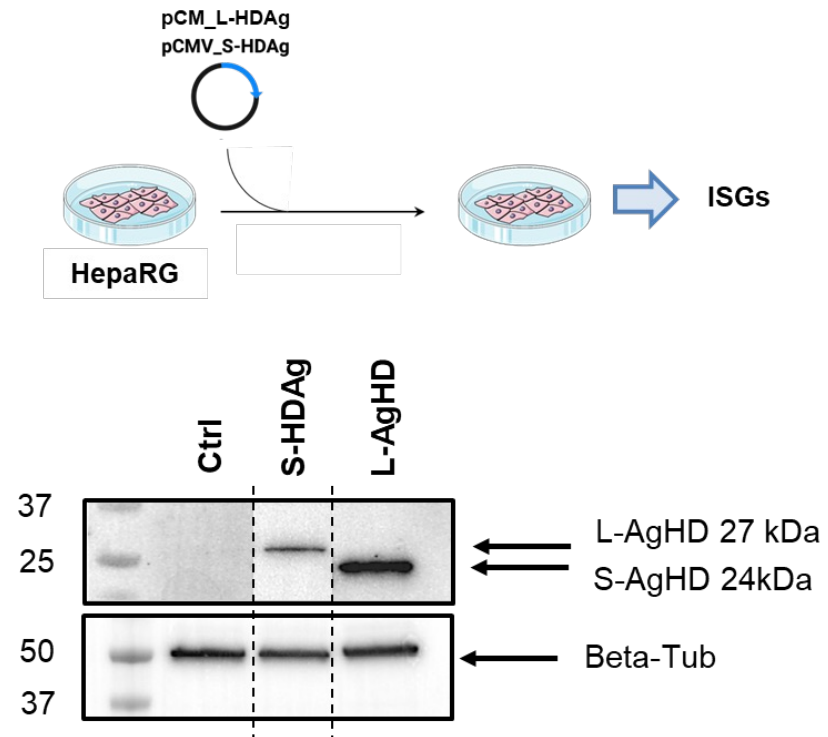
Inhibition of transcriptomic response to IFN α treatment in HDV-infected cells



HDV inhibition of type I IFN pathway only:

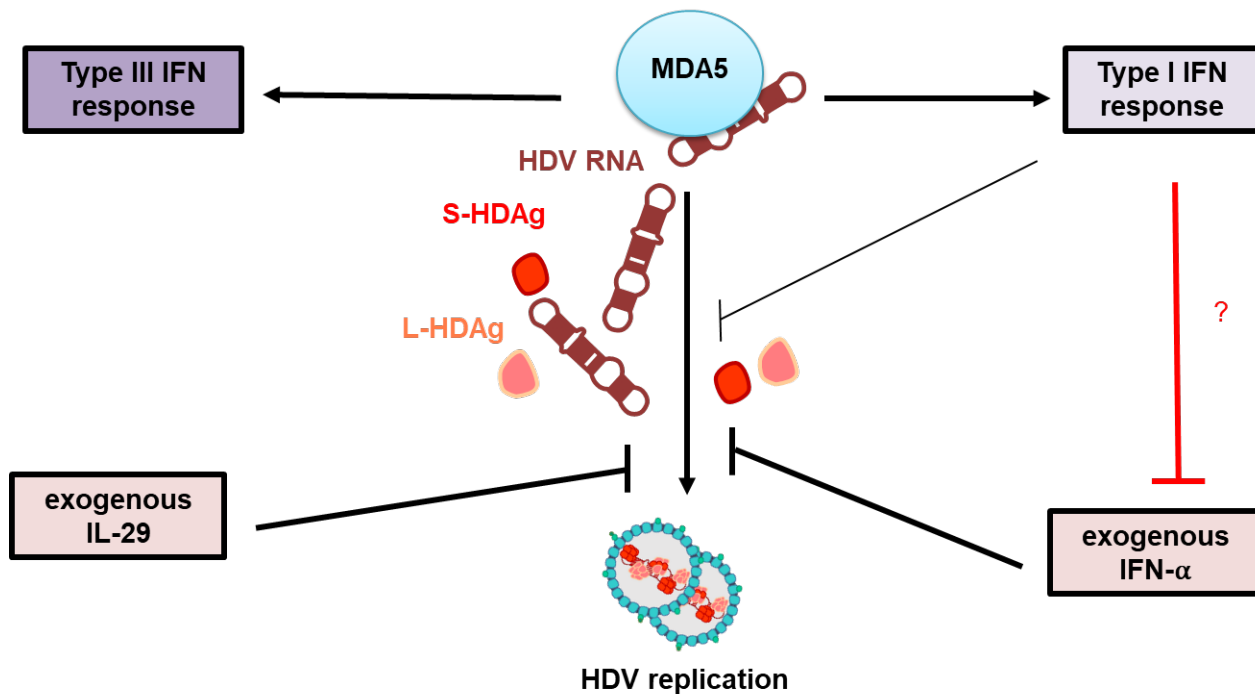
- Active mechanism via HDV antigens?
- Pre-activation and refractoriness?
- Active mechanism via modulation of host proteins?

Understanding the moderate response to IFN α in HDV-infected cells



Apparent absence of inhibitory effect by viral proteins

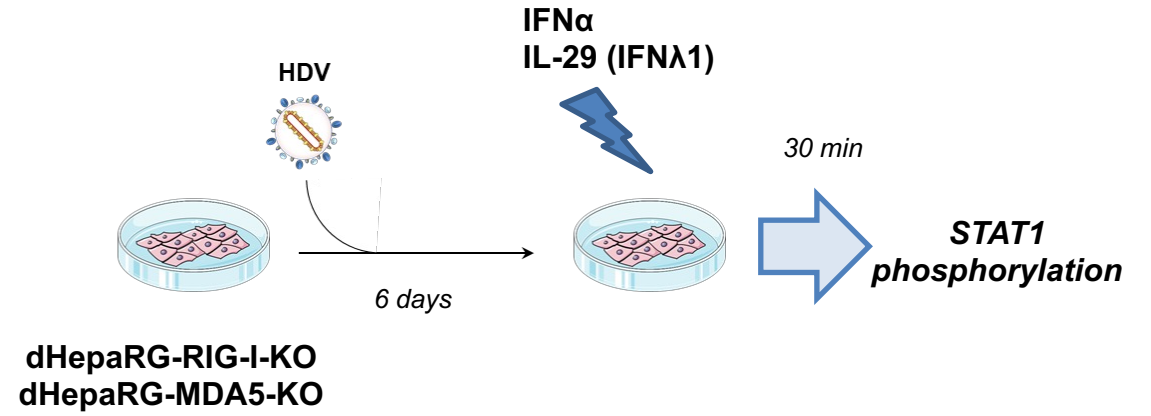
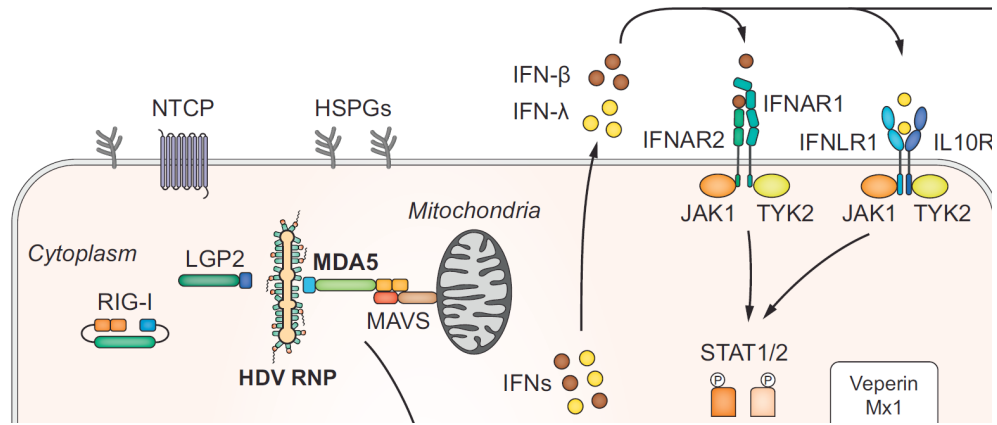
Inhibition of transcriptomic response to IFN α treatment in HDV-infected cells



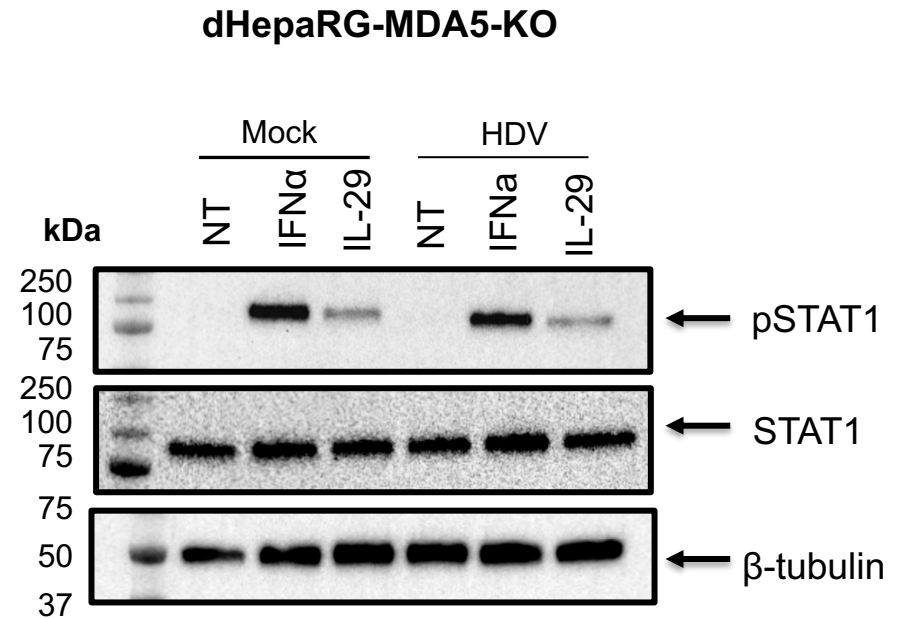
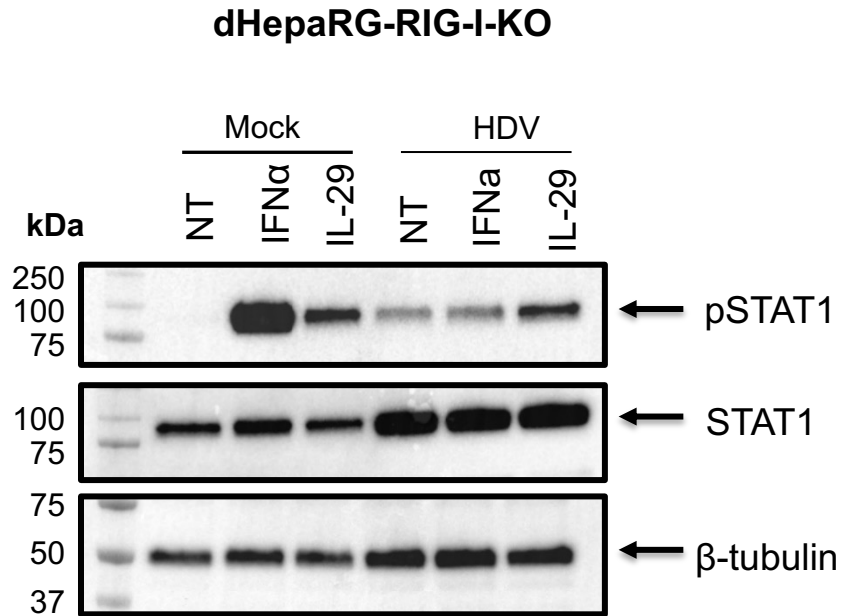
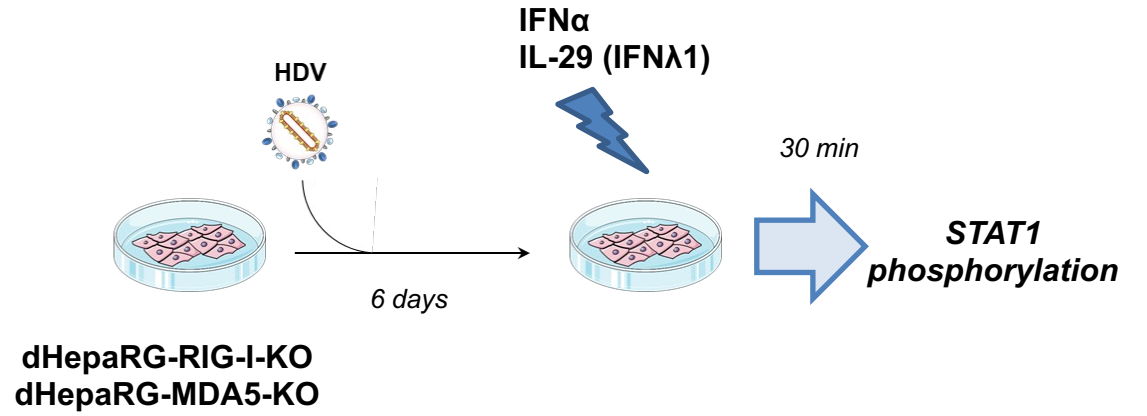
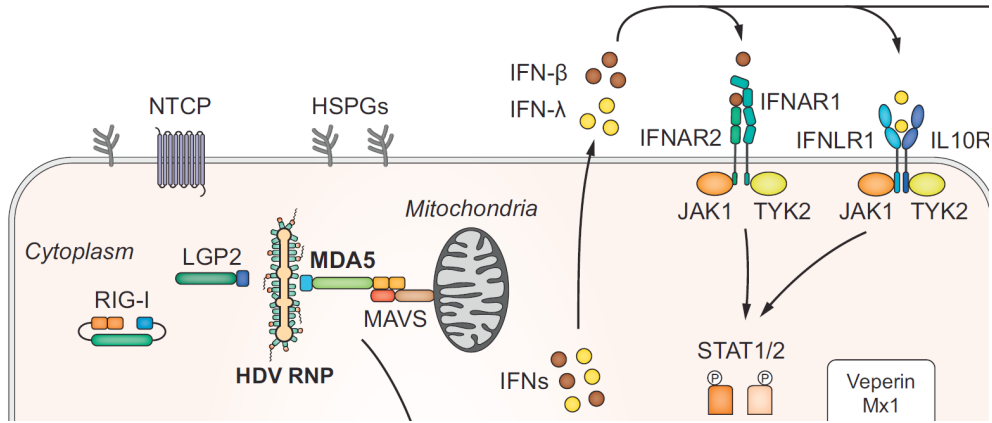
HDV inhibition of type I IFN pathway:

- ~~Active mechanism via HDV antigens?~~
- Pre-activation and refractoriness?
- Active mechanism via modulation of host proteins?

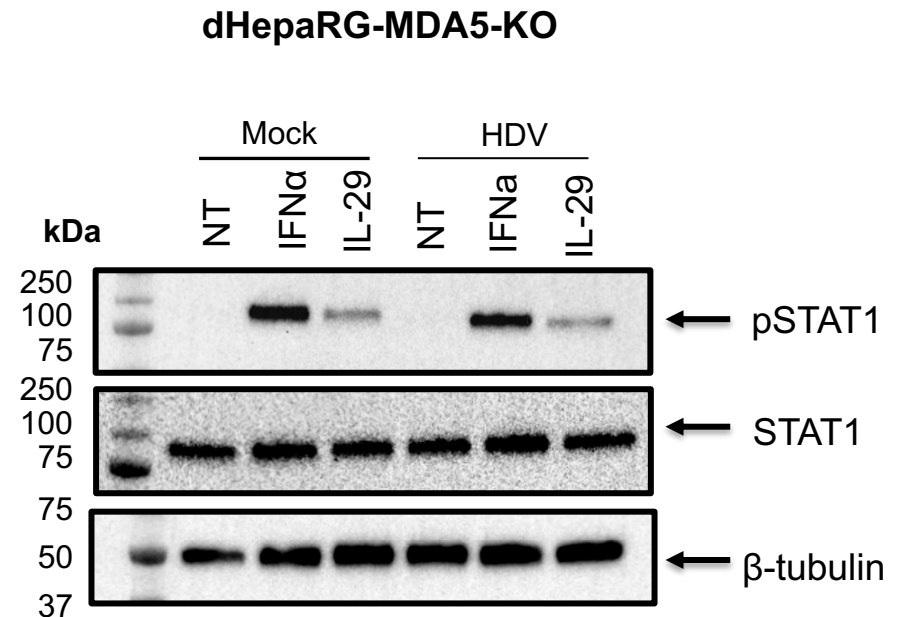
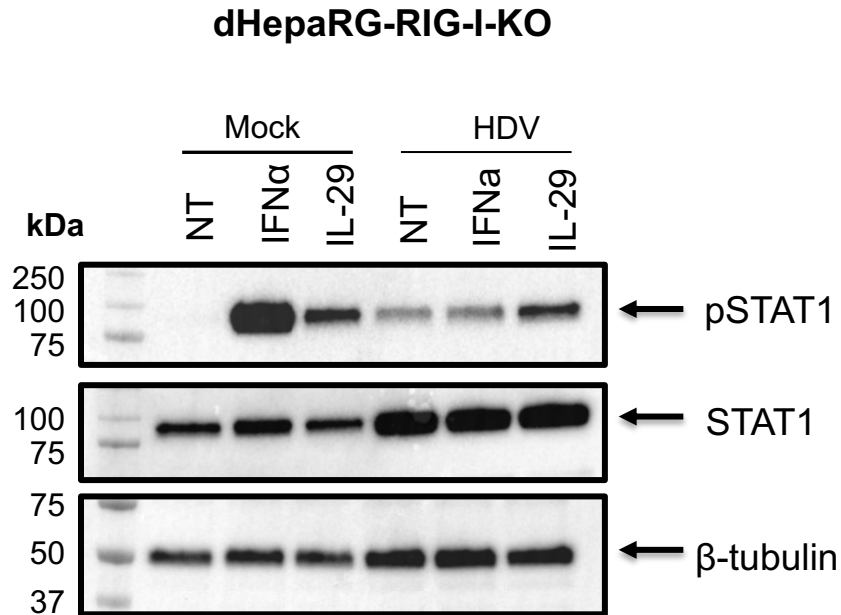
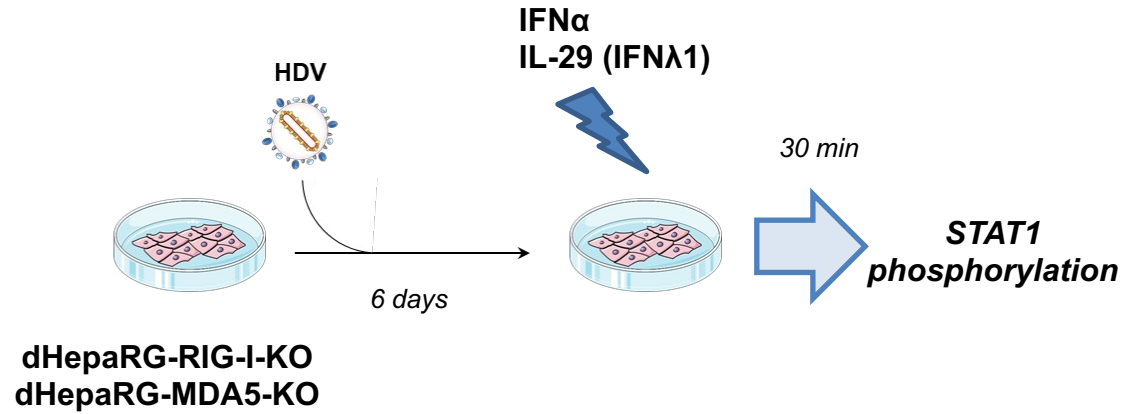
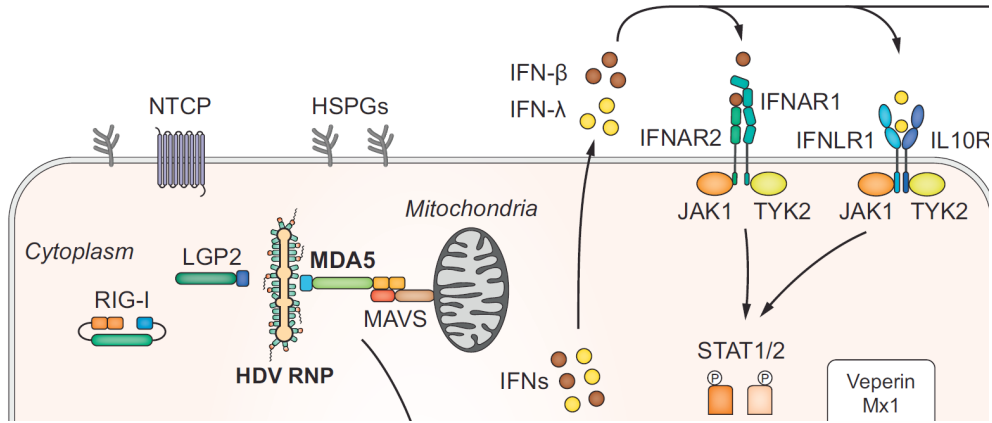
IFN α -induced STAT1 phosphorylation and HDV infection



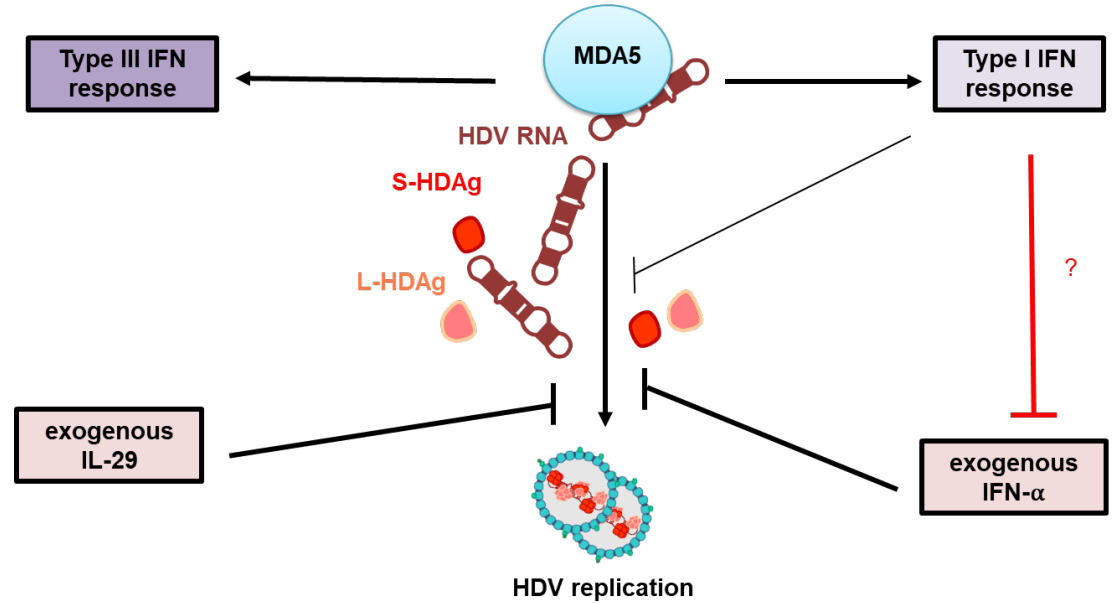
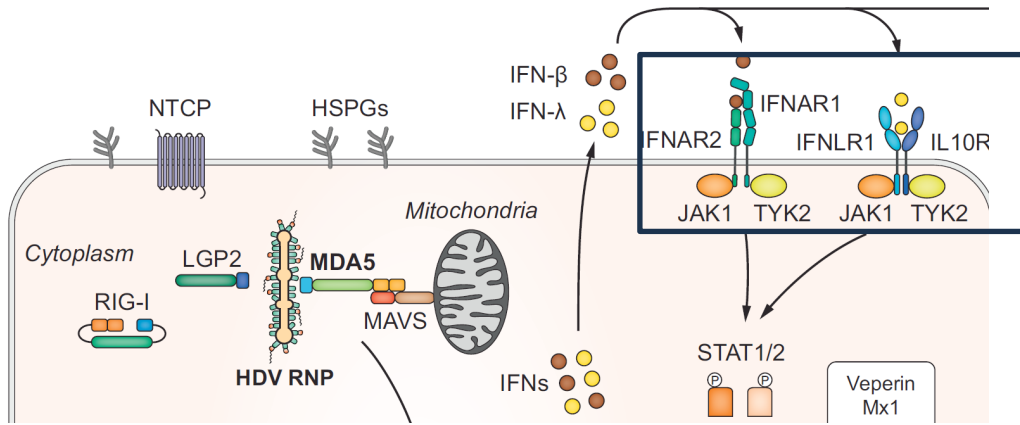
IFN α -induced STAT1 phosphorylation and HDV infection



IFN α -induced STAT1 phosphorylation and HDV infection



Summary



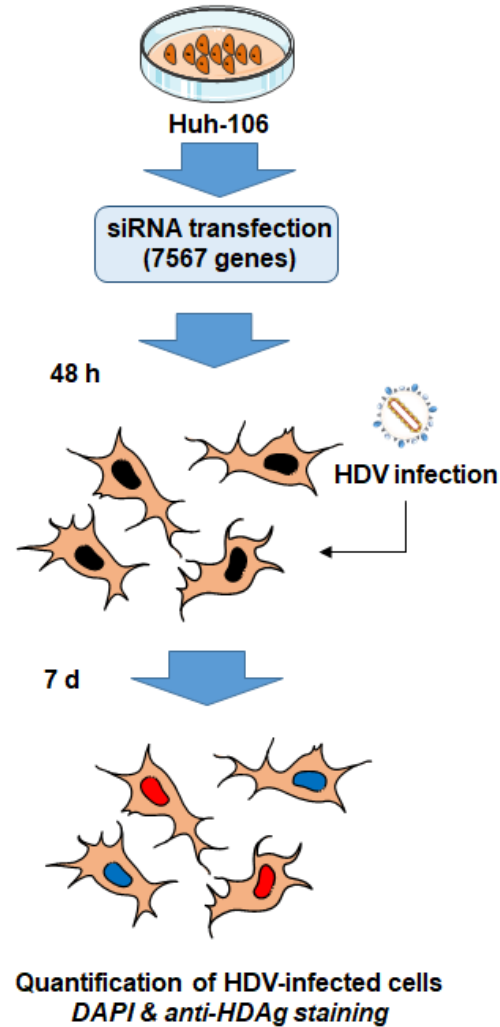
✓ HDV-induced innate immune response inhibits the cellular response to IFN α treatment

✓ This refractoriness does not affect the response to IL-29

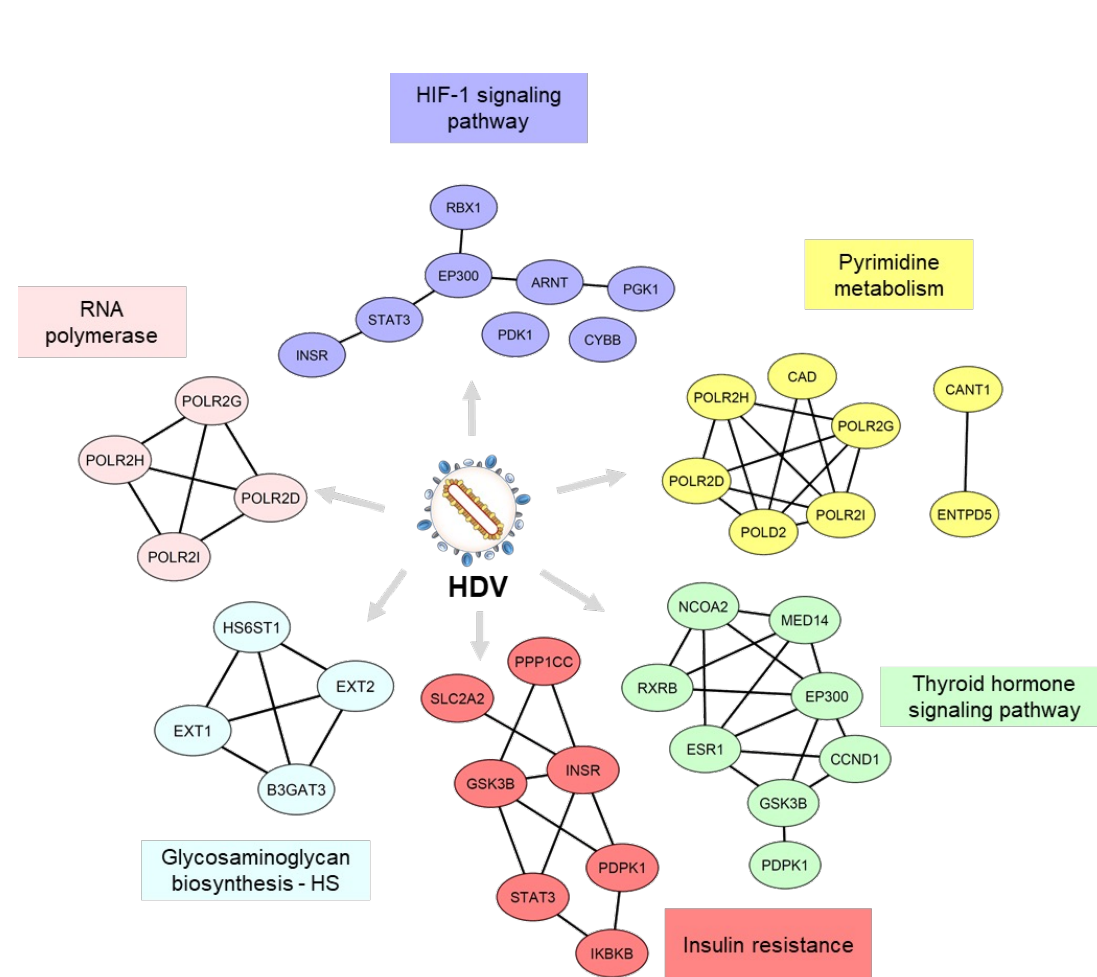
Loss-of-function screen

Validation of additional candidates

« Druggable Genome » siRNA screen



191 candidates
(CAD, ESR1, SLC10A1...)



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**AGENTSCHAP
INNOVEREN &
ONDERNEMEN**

Charlotte Bach
Laura Heydmann
Emma Gerges

CIRI – Lyon
Julie Lucifora
Roxanne Fouillé
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Sarah Durand
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