OCTOBER 11-12, 2024 MILAN, ITALY
DeltaCure 3rd International Meeting

ORAL PRESENTATION

Integrative transcriptomics and epigenomics reveals a viral footprint of chronic HDV infection in HBV co-infected chimeric livers

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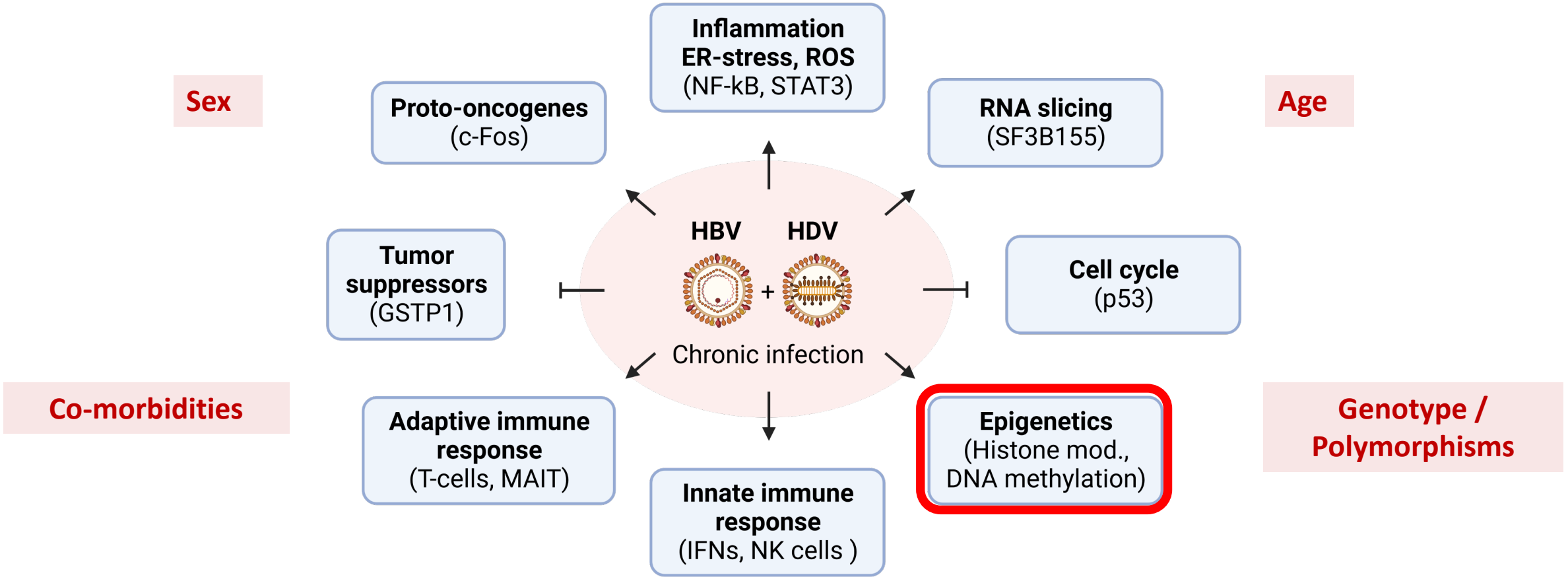
Disclosures

I have no disclosures/conflicts of interest

Chronic HDV infection is a major HCC risk factor



- **Chronic HDV infection contributes to liver complications and HCC by multiple direct and indirect factors promoting liver inflammation, fibrosis and stress**

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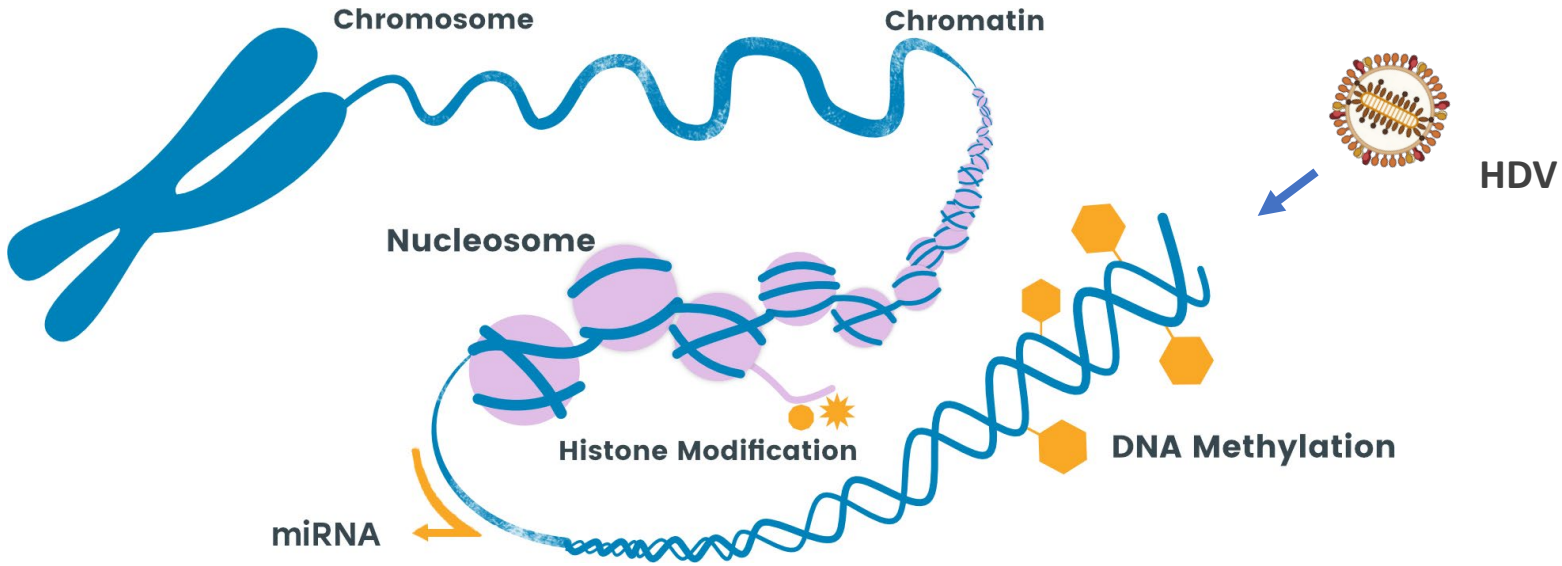
HDV-associated epigenetic dysregulation of the host genome

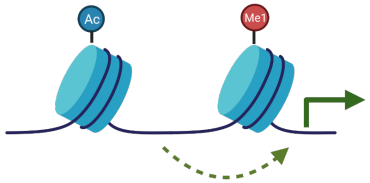


- **DNA methylation: Large delta antigen of HDV dysregulates DNA methylases DNMT1 and DNMT3b, causing hypermethylation of cell cycle regulator genes (e.g., E2F1)** (Benegiano et al., *FEBS Lett.* 2014)
- **miRNAs: miR-222 is upregulated in several cancers and in livers of patients with chronic HDV infection** (Sokhanvar et al., *Avicenna J Med Biotechnol.* 2021)
- **Histone modifications: HDV-induced Histone 3 (H3) acetylation promotes clusterin expression *in vitro*, an chaperon and oxidative stress response gene** (Liao et al., *J Gen Virol.* 2009)

Image modified from labclinics.com

Histone modifications determine transcriptional accessibility

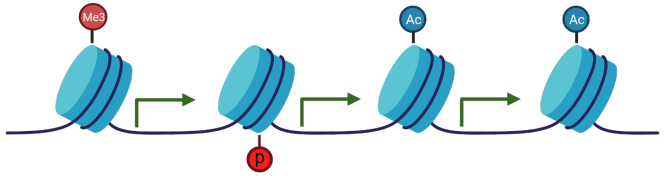
Active Enhancer Marks



Histone 3 (**H3**)

- **K4 me**thylation (me1)
- **K27 ac**etylation

Euchromatin Marks



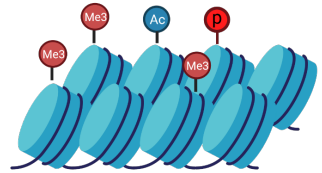
Histone 3 (**H3**)

- **K4 me**thylation (me3)
- **K9 ac**etylation
- **K10/S10** phosphorylation
- **K14 ac**etylation
- **K36 me**thylation
- **K79 me**thylation

Histone 4 (**H4**)

- **R3 me**thylation
- **K5 ac**etylation
-

Heterochromatin Marks



Histone 3 (**H3**)

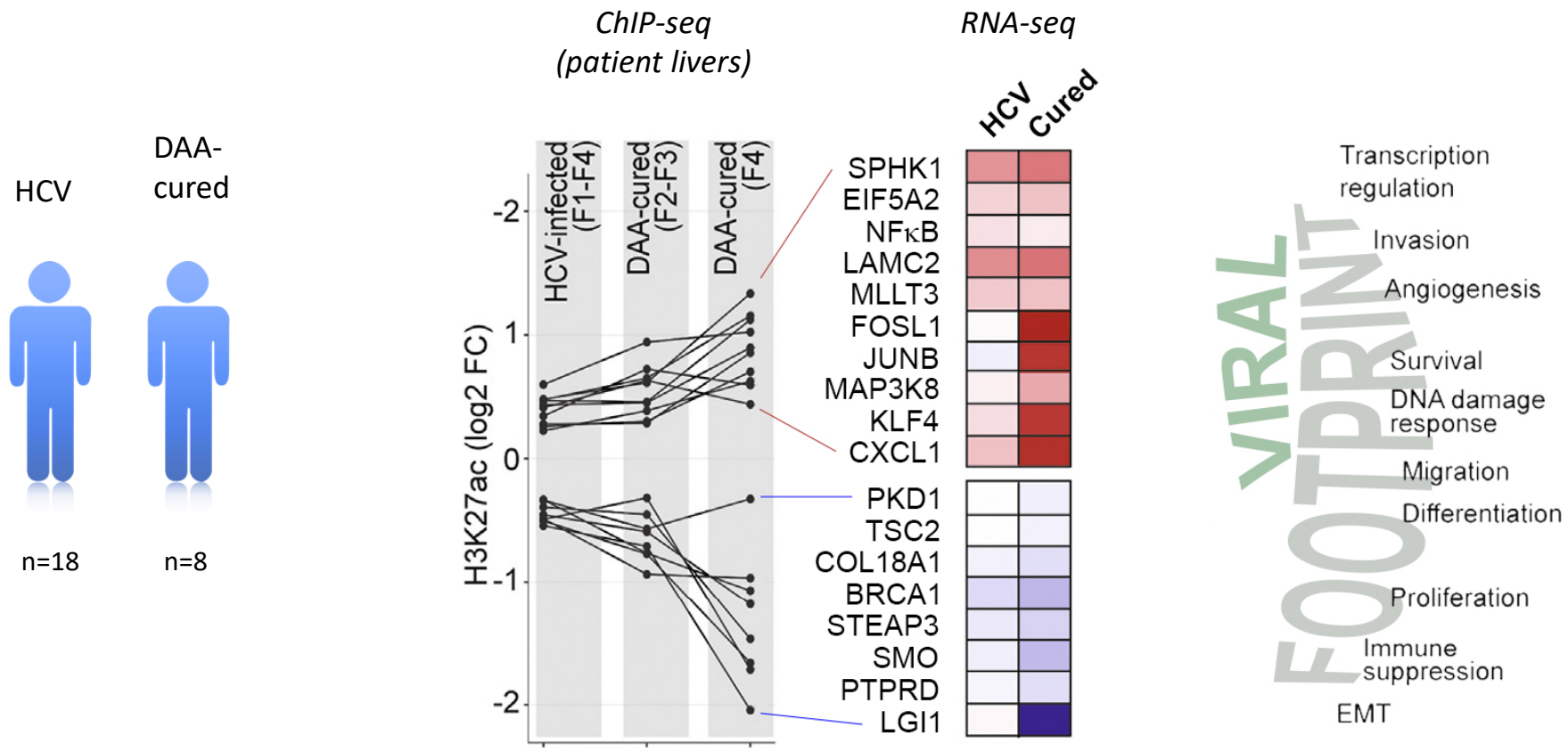
- **K9 me**thylation
- **S7** phosphorylation
- **S10** phosphorylation
- **K14 ac**etylation
- **K20 me**thlyation
- **K27 me**thylation

Histone 4 (**H4**)

- **K12 ac**etylation
- **K20 me**thylation
-

- **Epigenetic modifications of histones lead to chromatin opening or compaction** (Rivera and Ren. *Cell* 2013)
- **Histone modifications on H3 and H4 (acetylation, methylation, phosphorylation of Lys, Arg, Ser) are major determinants of gene expression** (Karsli-Ceppioglu et al. *Epigenomics* 2015)
- **Alteration of the epigenetic program has a functional impact in pathogenesis of disease biology** (Polak et al. *Nature* 2015; Gjoneska et al. *Nature* 2015)

Chronic HCV infection leaves an epigenetic viral footprint in patient livers after DAA-cure



➢ **Persistently dysregulated gene expression after HCV cure involves cancer-risk genes**

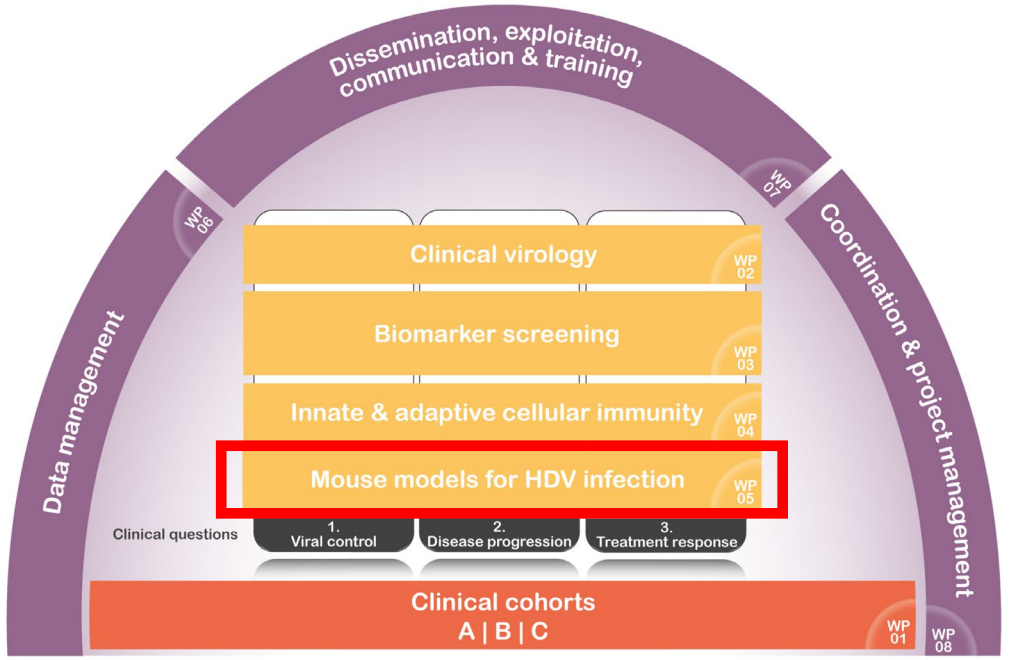
Hamdane et al., Gastroenterology 2019

D-SOLVE – biomarkers for antiviral treatment response and disease

HORIZON-HLTH-2021-DISEASE (***D-SOLVE***),
PI Heiner Wedemeyer

Work package 5 (Inserm)**:**

- **Identification of epigenetic HDV footprint in the liver associating with disease and HCC risk**
- **Studying the impact of BLV treatment on such an imprint**
- **Identification of minimal-invasive biomarker candidates for treatment response and HCC risk**



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Human liver chimeric mice - a model for chronic liver injury

FRG-NOD mice engrafted with primary human hepatocytes

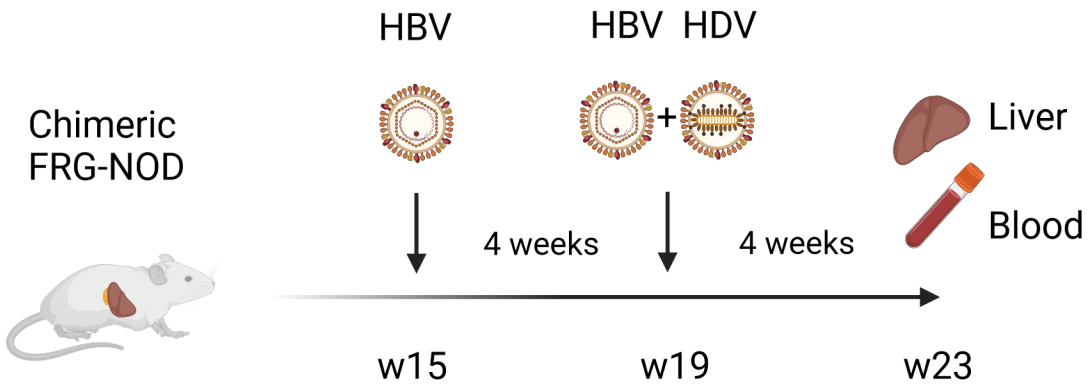
- **FRG-NOD mice degrade mouse hepatocytes due to metabolic stress.**
- **Chimeric livers repopulated** (50-70%) **with primary human hepatocytes** (PHH)
- **Immunodeficient mouse model** (T, B, NK deficiency) **with functional macrophages and stellate cells**
- **Model develops liver disease** (steatosis, fibrosis, HCC) **in response to chronic injury** (diet, carcinogen, virus)
- **Chimeric mice are permissive for human viral hepatitis viruses** (HCV, HBV, HDV)

FRG-NOD
(Fah-/-, Rag2-/-, IL2Rg-/-)

Transplanted PHH

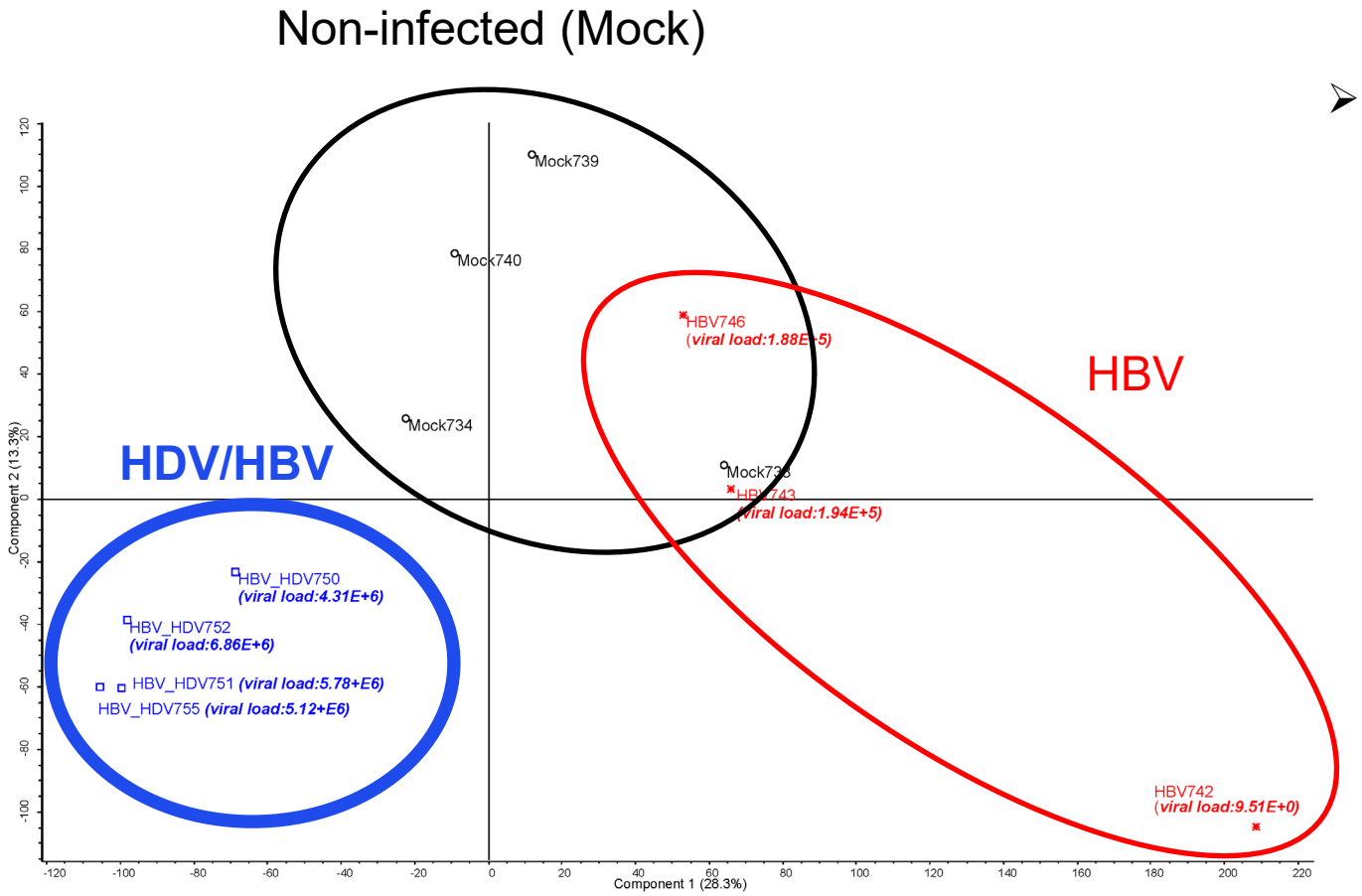
Chimeric FRG-NOD mice are efficiently infected with HDV/HBV

HBV/HDV superinfection



- **Mice were super-infected for four weeks with HBV or HDV/HBV**
- **Livers and blood were harvested 4 weeks post infection with HDV**
- **Endpoint analysis of livers and blood confirmed an efficient infection of the chimeric mice with HDV**

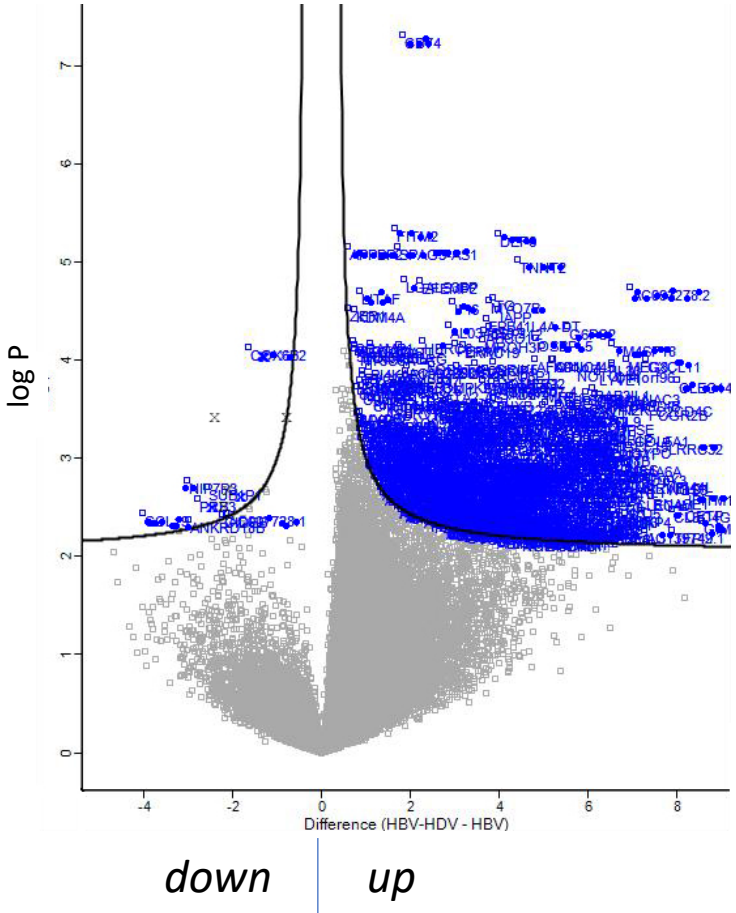
HDV causes a distinct pattern of hepatic gene transcription in chimeric mice

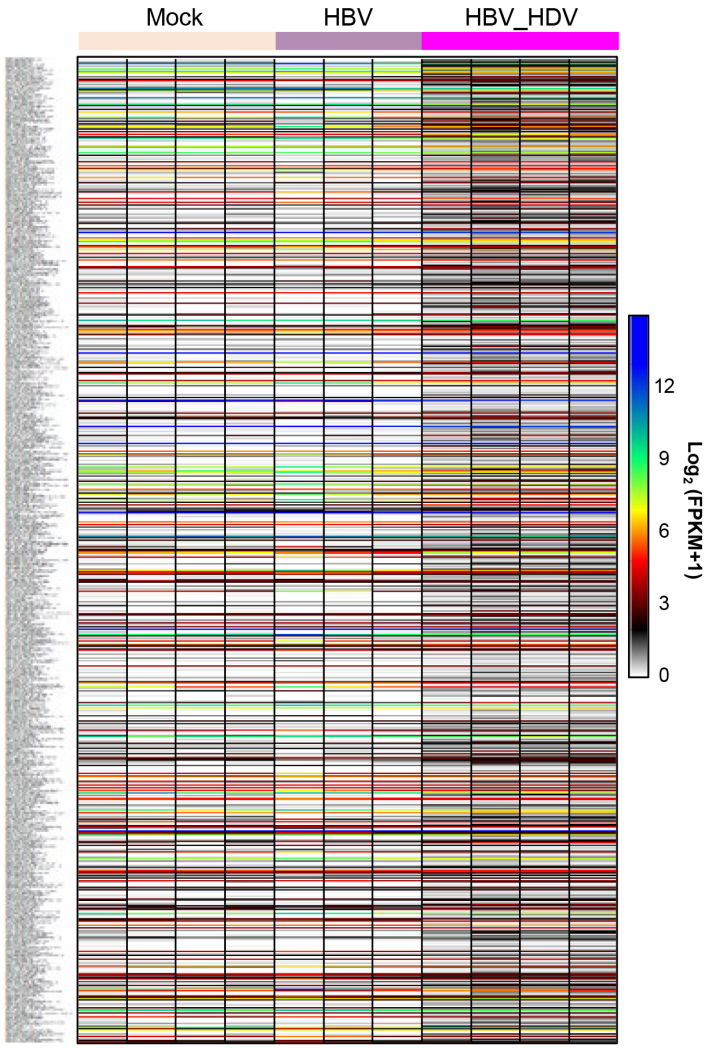


- **Liver transcriptomics (human) of HDV/HBV-infected mice clustered well apart from mock- or HBV mono-infected animals**

The majority of HDV-specific transcripts are upregulated in the liver

748-gene HDV signature in chimeric mice





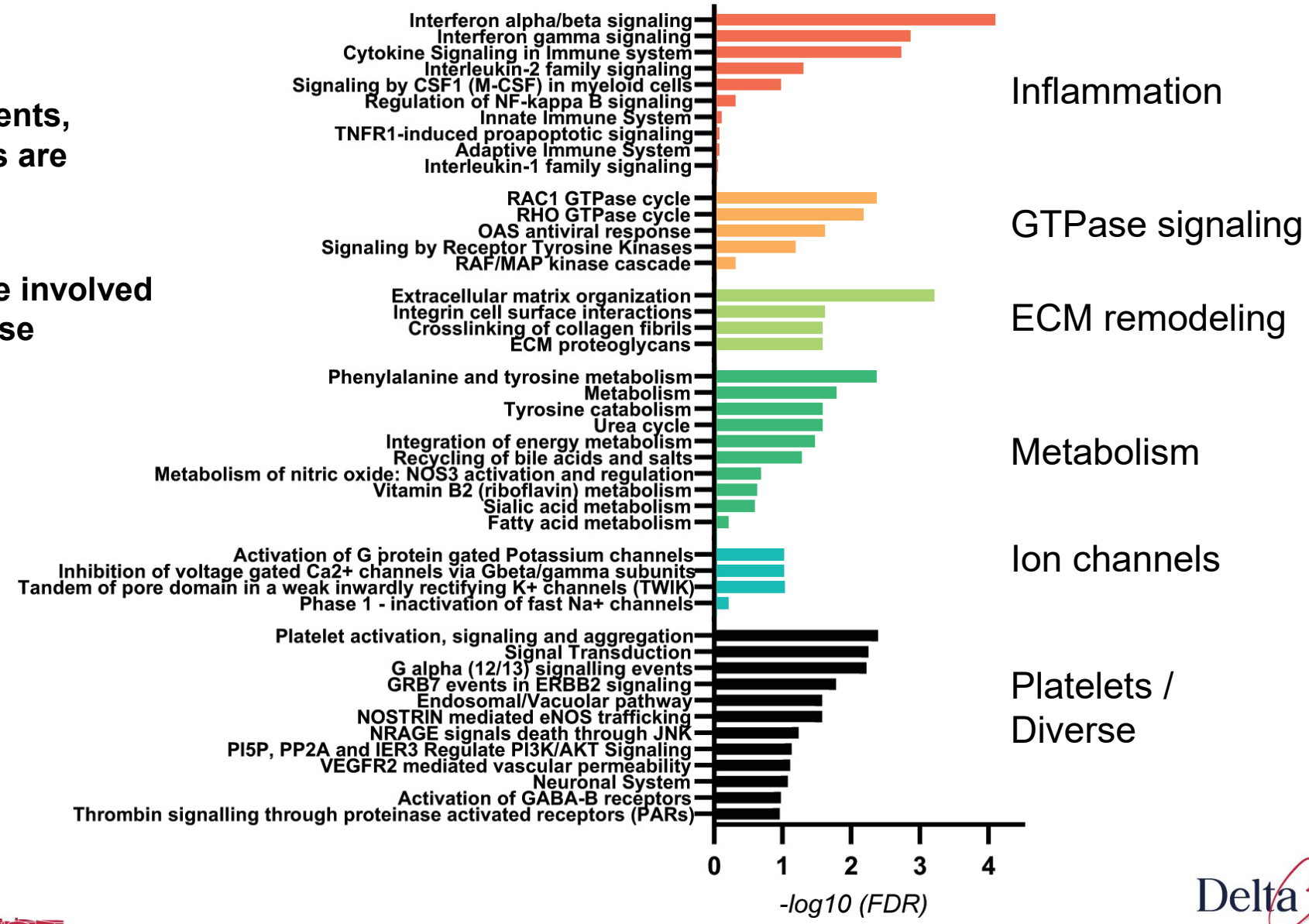
FPKM - Fragments Per Kilobase of transcript per Million mapped reads

- **The majority of transcripts are upregulated in HDV animals**
- **The identified 748-gene HDV signature in chimeric liver mice are potential biomarkers for liver disease and antiviral treatment response**

HDV-induces liver disease-relevant signaling pathways

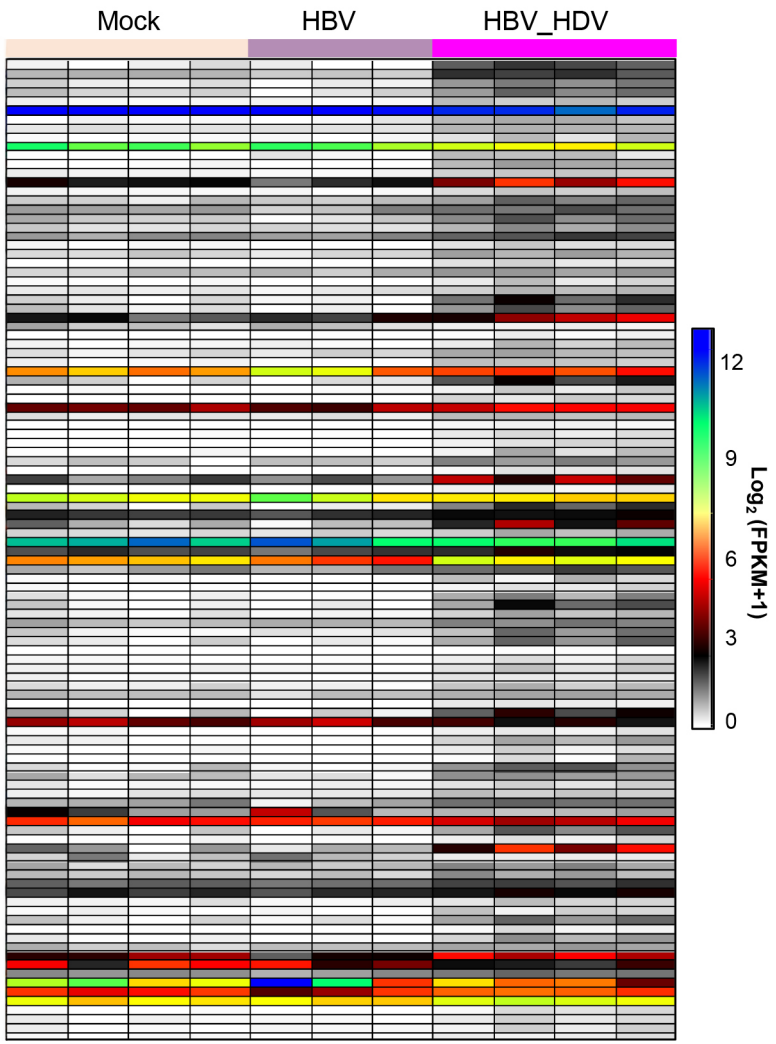
GO enrichment of the HDV 748-gene signature

- **Consistent with HDV patients, innate immune responses are among the top pathways**
- **All indicated pathways are involved in liver damage and disease progression**



HDV signature components are blood biomarker candidates

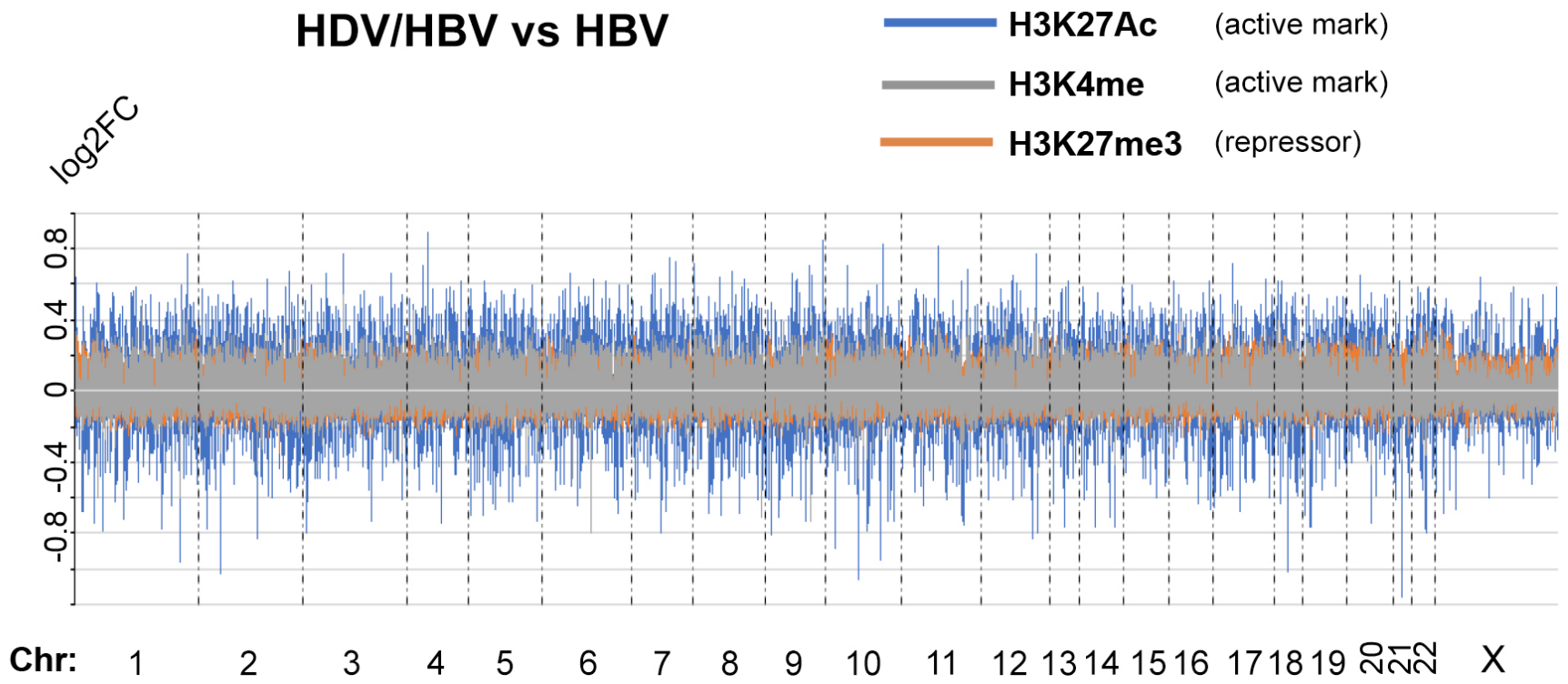
109 transcripts produce a potentially secreted protein



- **mRNAs of the 748-gene HDV signature were examined for the presence of a secretory signaling peptide sequence**
- **109 (~15%) of the signature components are predicted to be secreted to the extracellular space**

FPKM - Fragments Per Kilobase of transcript per Million mapped reads

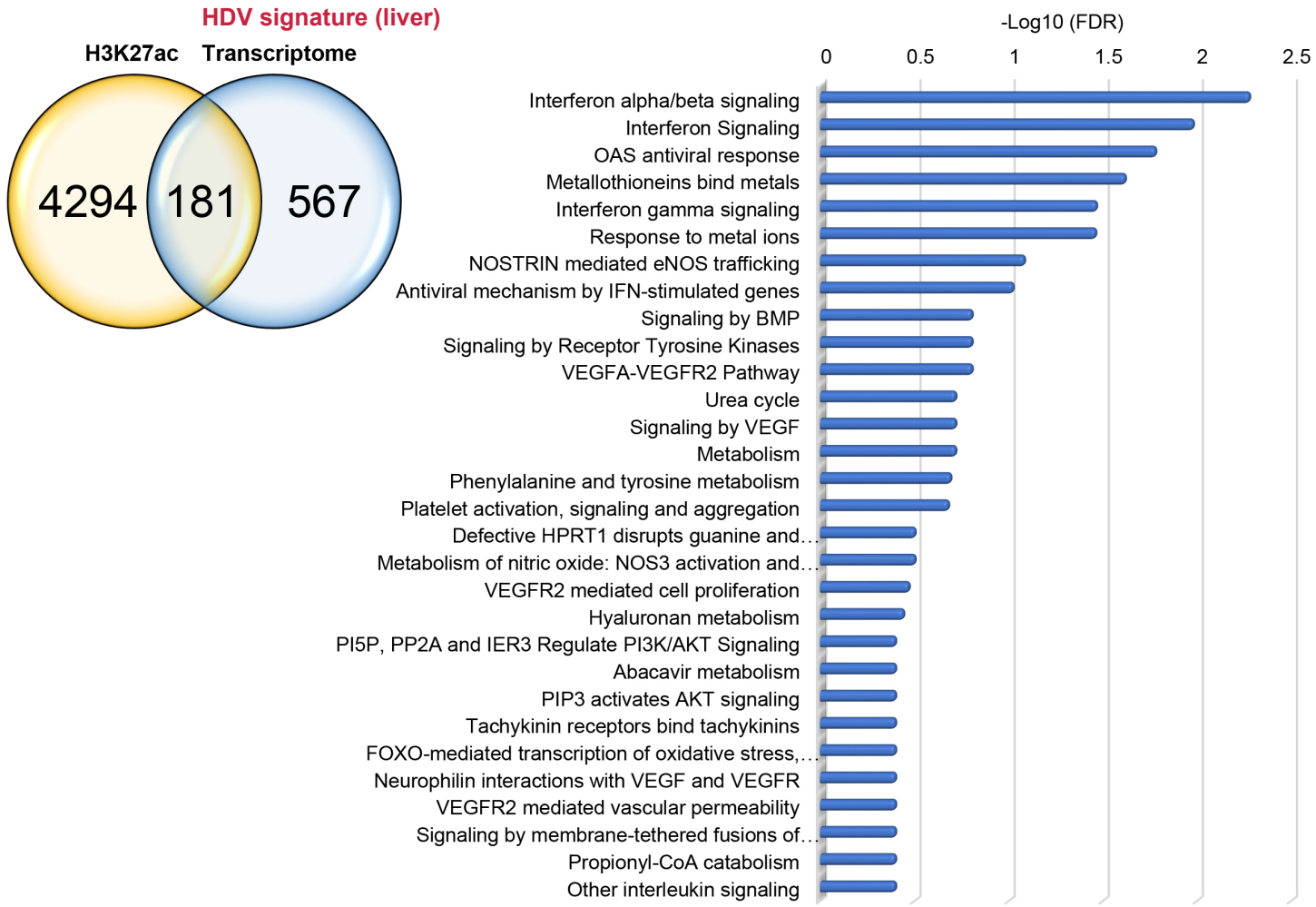
Epigenetic profiling of mouse livers highlights a role of the active mark H3K27ac in HDV infection



- **Of the three epigenetic marks profiled acetylation of histone 3 at lysine 27 (H3K27ac), which is an active mark was the most differential histone modification observed**

HDV signature is partially epigenetically coded

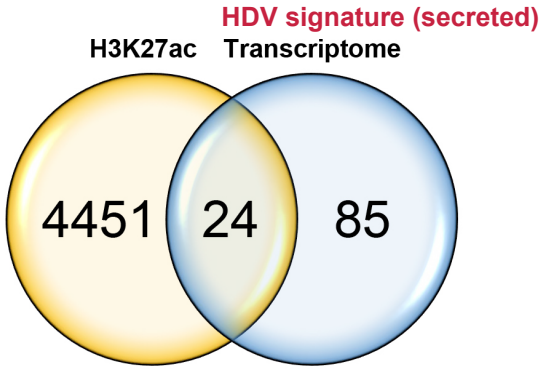
181-gene HDV footprint in the liver

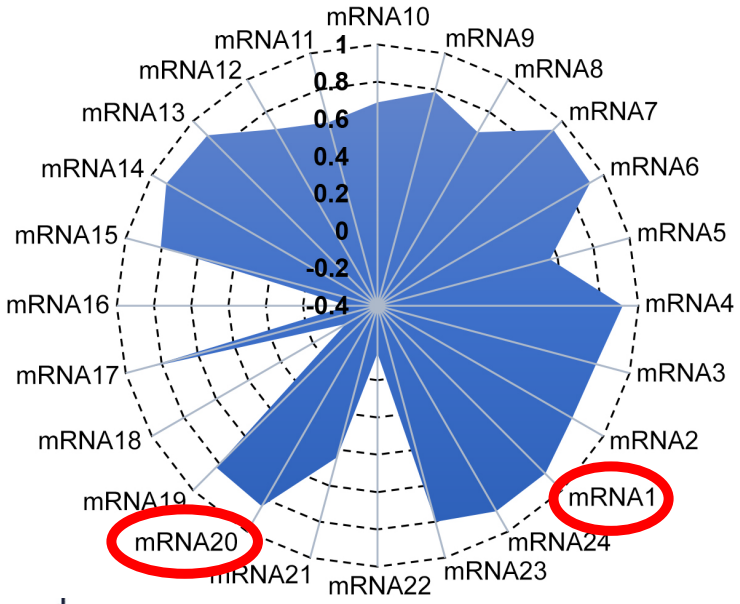


- **181 genes (~25%) of the HDV signature is associated with the active mark H3K27ac**
- **These include predominantly transcripts enriched in innate immune pathways**

Epigenetic HDV footprint is partially secreted to the blood

24-gene HDV footprint as candidate blood biomarkers





Summary and next steps

- **We identified a 748-gene transcriptional signature is specific for HDV in HDV/HBV-infected mice**
- **The HDV signature is associated with liver disease relevant pathways (inflammation, fibrosis, ..)**
- **The HDV signature is partially epigenetically regulated (H3K27ac)**
- **We identified 24 candidate blood biomarkers associated with a putative HDV epigenetic footprint and pathways linked to fibrosis and liver disease**

Next steps

- **HDV signature in chimeric animals treated with Bulevirtide – treatment response biomarkers?**
- **Proteomic analysis of blood plasma from the chimeric mice**
- **Validation of results in HDV patient livers and blood**

Acknowledgements

Inserm Unit 1110, U Strasbourg, France

Yogy Simanjuntak
Laura Meiss-Heydmann
Charlotte Bach
Sarah Durand
Marine Oudot
Valerio Taverniti
Romain Desert
Frank Juhling
Julien Moehlin
Lea Girard
Zakaria Boulahtouf
Emilie Crouchet
Laurent Mailly
Romain Martin
Patrick Pessaux
François Habersetzer
Catherine Schuster
Eloi Verrier
Thomas Baumert

The Chronobiotron, Strasbourg, France

Sophie Reibel-Fosset
Christian Galmiche

The *D-SOLVE* consortium

***Heiner Wedemeyer**, MHH, Germany*
***Markus Cornberg**, MHH, Germany*
***Pietro Lampertico,** Policlinico di Milano, Italy*
***Yang Li**, HZI, Germany*
***Niklas Bjorkstrom**, Karolinska Institutet, Sweden*
***Soo Aleman,** Karolinska University Hospital*
***Florin Caruntu,** INBIMD, Romania*
***Yang Zhang,** CISPA, Germany*
***Thomas Baumert**, Inserm U1110, France*

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