

## Contact

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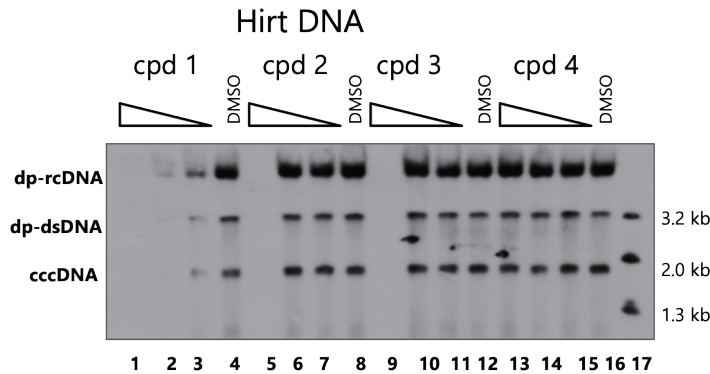
# A Platform Supporting HBV Drug Discovery

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A broad assay platform for the discovery of agents for the treatment of chronic HBV infection.

Services for assay establishment and validation, compound screening and profiling, and support of preclinical studies and clinical trials.

## HepDES19 cells

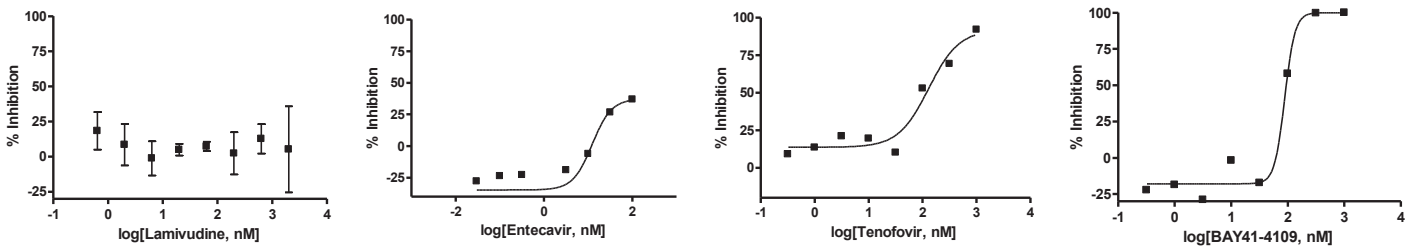


HepDES19 cells were treated with compounds for 8 days. HBV DNA was assessed by Sourthrn blot.

## HBV: Key Service Capabilities

- ◆ Drug screening using HBV producing cell lines
  - HepG2.2.15, DE19, and DES19 cells with qPCR, Southern blot, Northern blot, ELISA, cccDNA, encapsidated pgRNA and DNA
- ◆ HBV infectious assays
  - *Ex vivo* primary human hepatocyte (PHH) culture system
- ◆ Reporter assays: hTLR, THP1-Blue™ ISG, TNF-Induced NF-κB-luciferase cell lines
- ◆ Core protein expression and capsid assembly quenching system
- ◆ Compound profiling
  - Drug combination and serum shift
  - Clinical isolates and genotypes (up to 5 constructs for each of A to H)
- ◆ Drug-resistant mutants
  - Nuc (16 constructs) and CpAM (28 constructs) resistant mutants by transient transfection (qPCR)
  - Construction of drug-resistant mutants, assessment of fitness and drug susceptibility
- ◆ Clinical virology
  - Full-length HBV genome sequencing (Sanger and Deep Sequencing), genotyping, VL
  - Phenotyping (full-length, pol, capsid shuttle vectors)
  - New HBV markers: serum HBV RNA, HBcrAg, HBV RNA sequencing
- ◆ Animal models
  - Hydrodynamic injection (HDI) mouse model
  - AAV/HBV mouse model
  - Humanized FRG mouse model
  - Duck/DHBV model
  - Transgenic mouse model
  - WHV/woodchuck model
- ◆ Immunological assays supporting animal models
  - Isolation of immune cells: lymphocytes from mouse spleen, liver and lymph nodes, PBMC from blood
  - FACS: surface and intracellular markers
  - ELISPOT
  - Cytokines by Luminex, ELISA, MSD and RT-qPCR

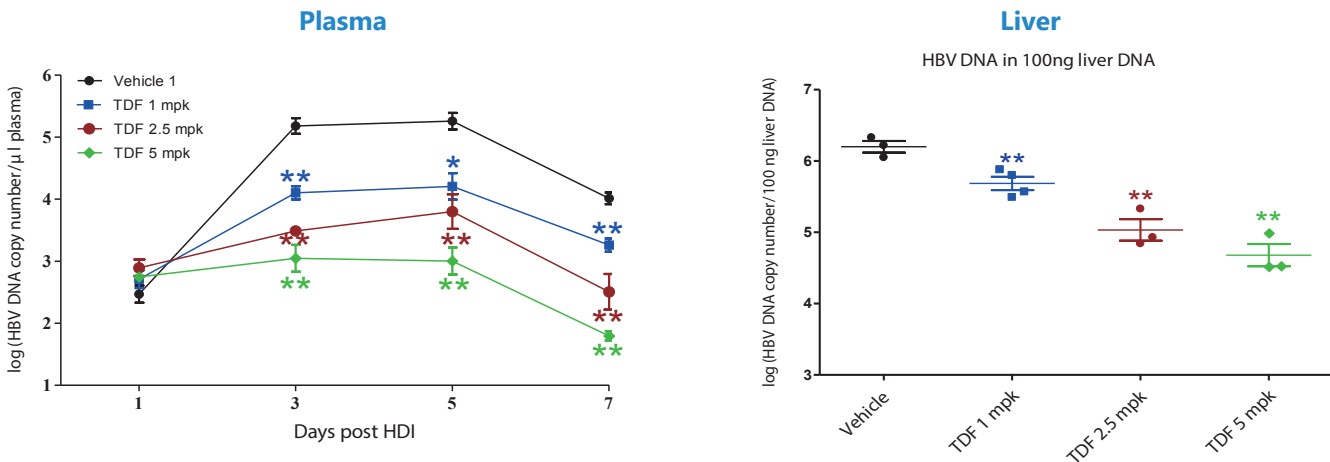
## Compound profiling in the transient transfection assay



	Lamivudine	Entecavir	Tenofovir	Bay41-4109
Clinical isolate A, EC <sub>50</sub> (nM)	>2000	>100	129.9	88.6

The activity of compounds against a clinical isolate in HepG2 cells was assessed by the transient transfection assay. Intracellular HBV DNA was quantified by qPCR.

## Hydrodynamic injection (HDI) mouse model: TDF shows dose dependent inhibition of HBV DNA replication

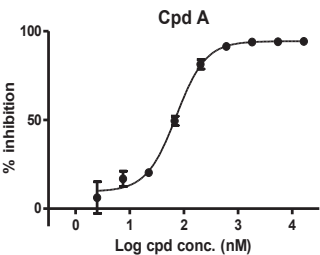


Mice were injected with HBV plasmid DNA by HDI and treated with TDF or vehicle for 7 days. HBV DNA was quantified by qPCR.

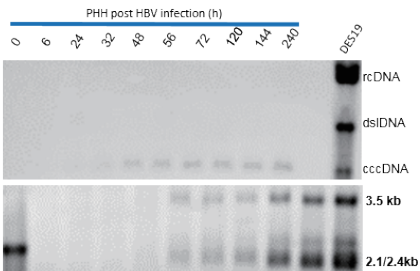
### Endpoints

- HBV DNA, HBsAg and HBeAg in plasma and liver
- HBV RNA
- HE, IHC and TUNEL of liver
- Cytokines and ISGs

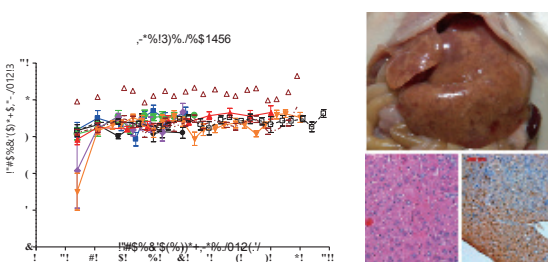
### HepG2.2.15 (qPCR)



### PHH/cccDNA/RNA

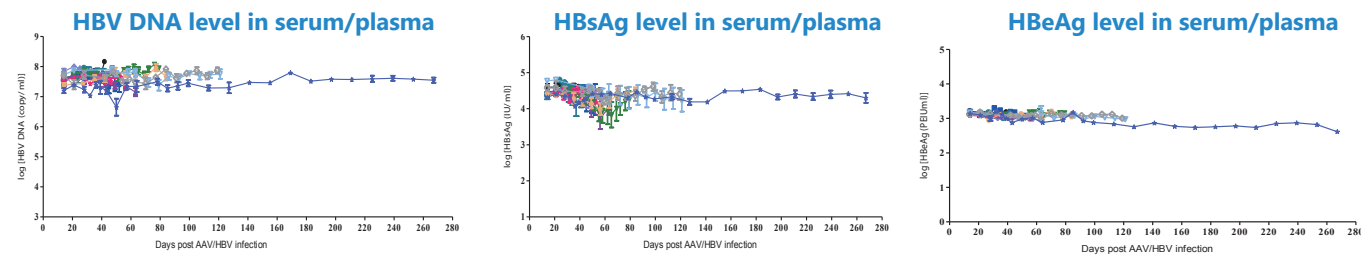


### AAV/HBV & humanized FRG mouse



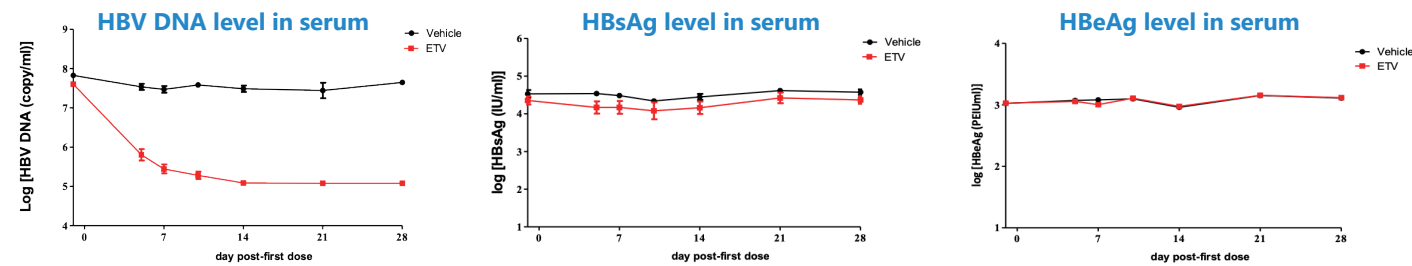


## AAV/HBV mouse model: high reproducibility

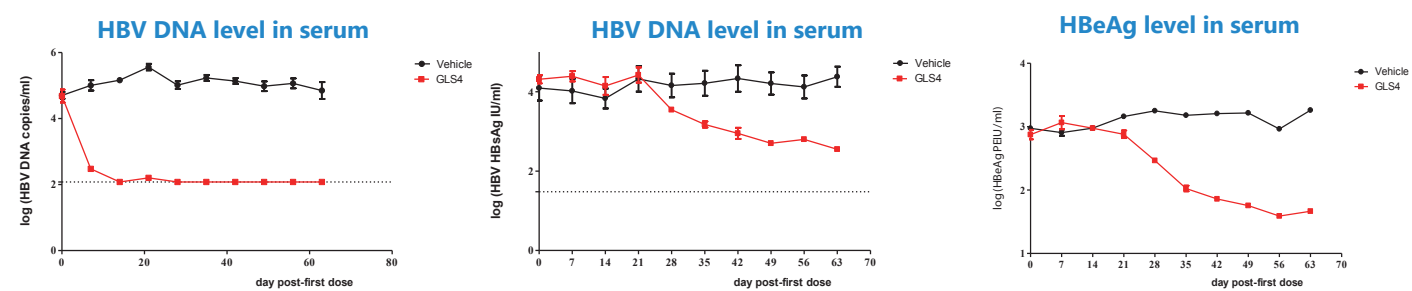


Data from 11 different experiments

## AAV/HBV mouse model: entecavir inhibits HBV replication

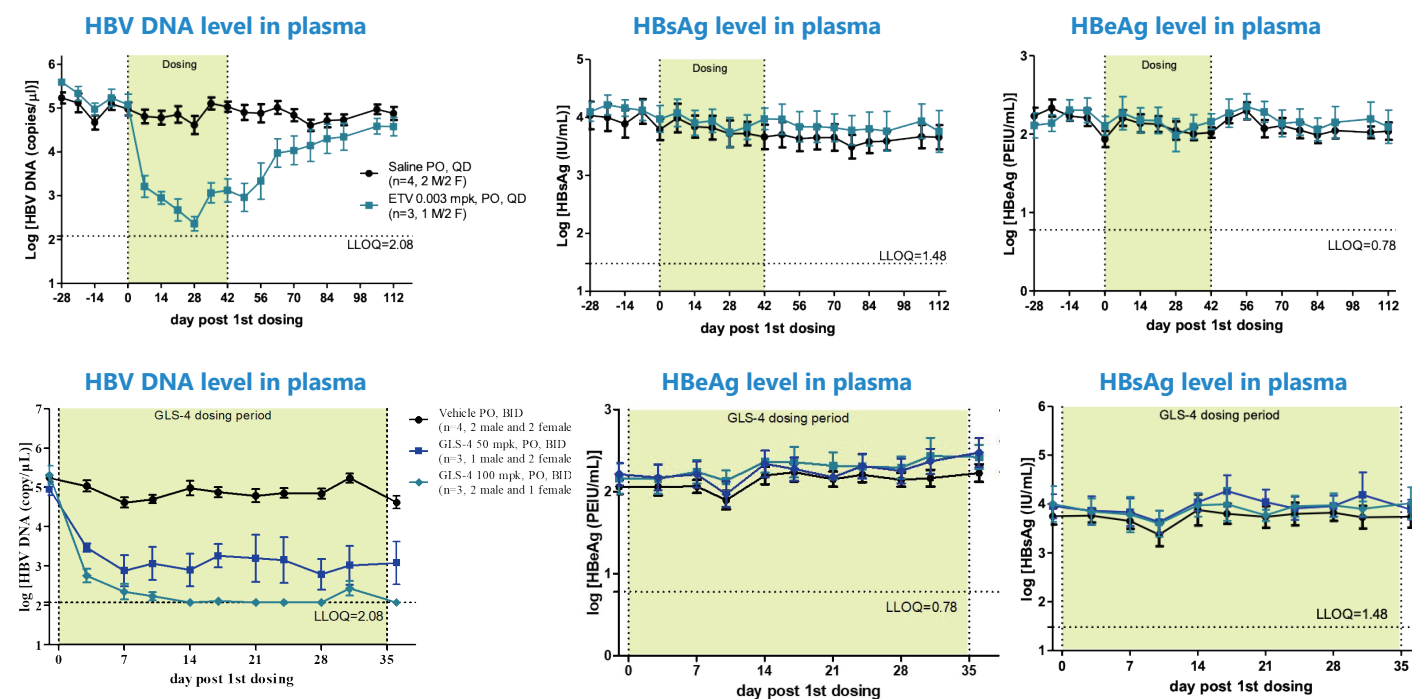


## AAV/HBV mouse model: GLS4 inhibits HBV replication



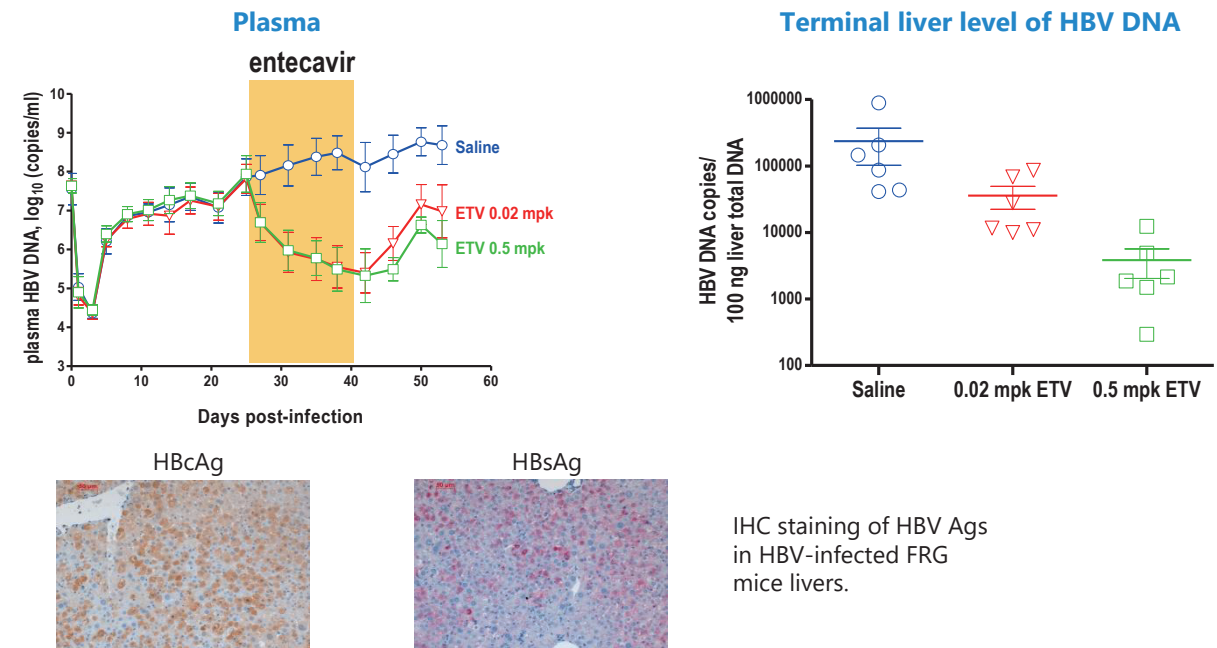
GLS4:100 mpk, bid, p.o. Samples: serum

## HBV Transgenic (Tg) mouse model: evaluation of efficacy of DAAs

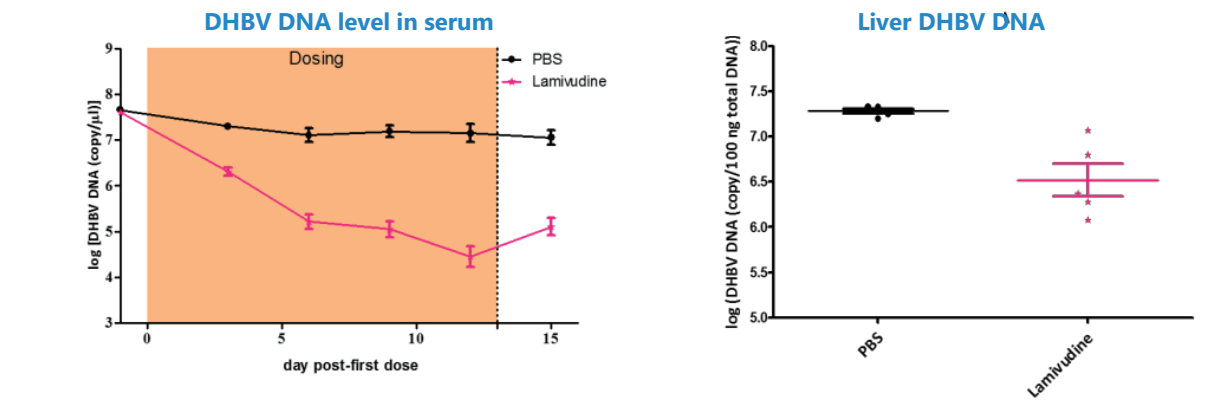


## FRG mouse model: entecavir inhibits HBV replication

- Humanized mice were infected with HBV and treated with entecavir for 2 weeks.
- Plasma and terminal liver HBV DNA was determined by qPCR.



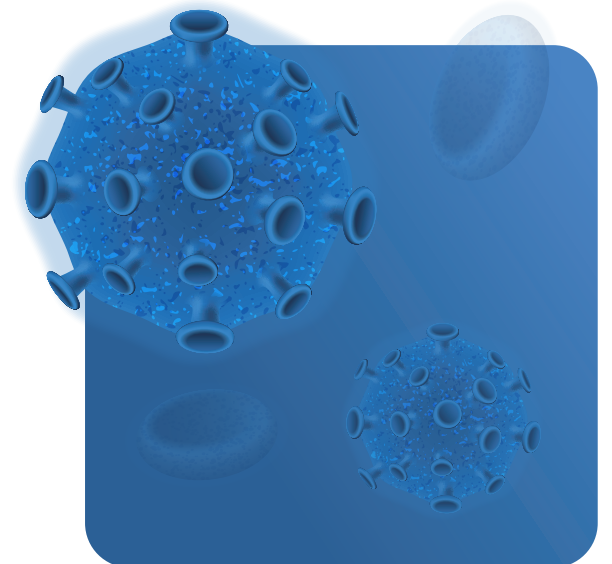
## Duck/DHBV model



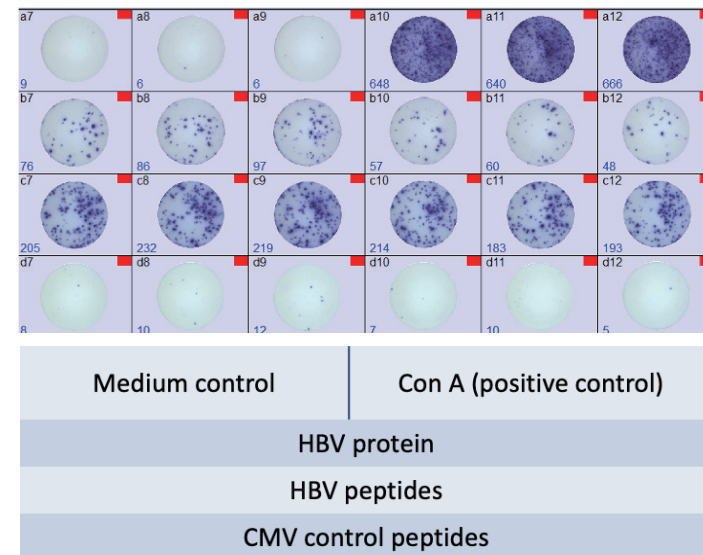
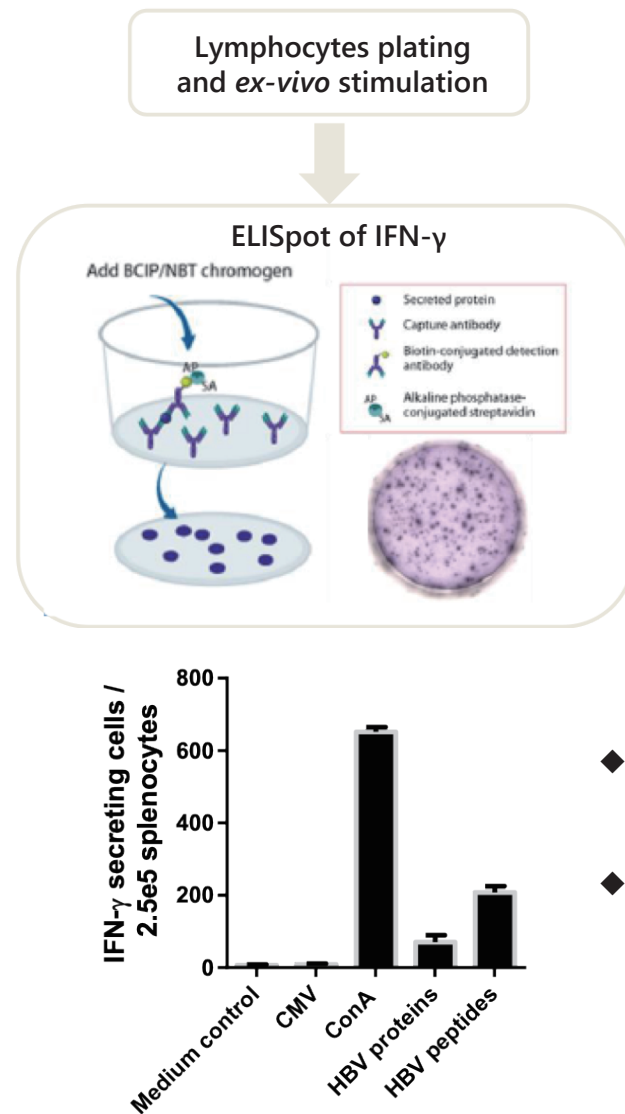
Pekin Aylesbury ducks were infected with DHBV at day -7

## Platform for HBV Immunological Assays

- Isolation of lymphocytes from mouse spleen, liver and lymph nodes
- Isolation of PBMC from mouse or human blood
- FCM platform
  - Intracellular cytokine staining (ICS)
  - Surface makers staining
  - Tetramer staining
- T cell and B cell ELISpot
- Cytokines by Luminex, ELISA and RT-qPCR
- ISG analysis by RT-qPCR
- ex vivo PBMC conditional media against HBV in PHHs

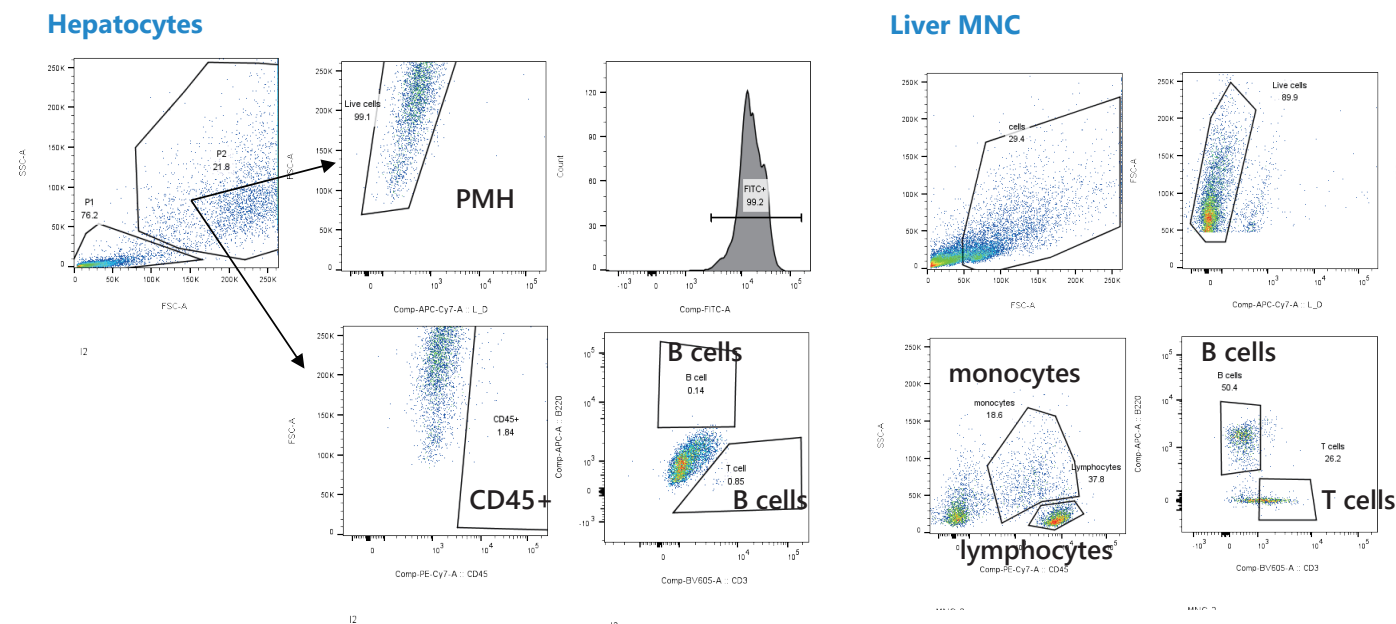


## ELISPOT: activation of HBV specific T cells in mouse



- ◆ Splenic lymphocytes were isolated from vaccinated C57BL/6 mice, and stimulated with HBV protein or peptides.
- ◆ HBV specific T cells were assessed by detecting IFN- $\gamma$  secreting cells using a mouse IFN- $\gamma$  ELISPOT kit.

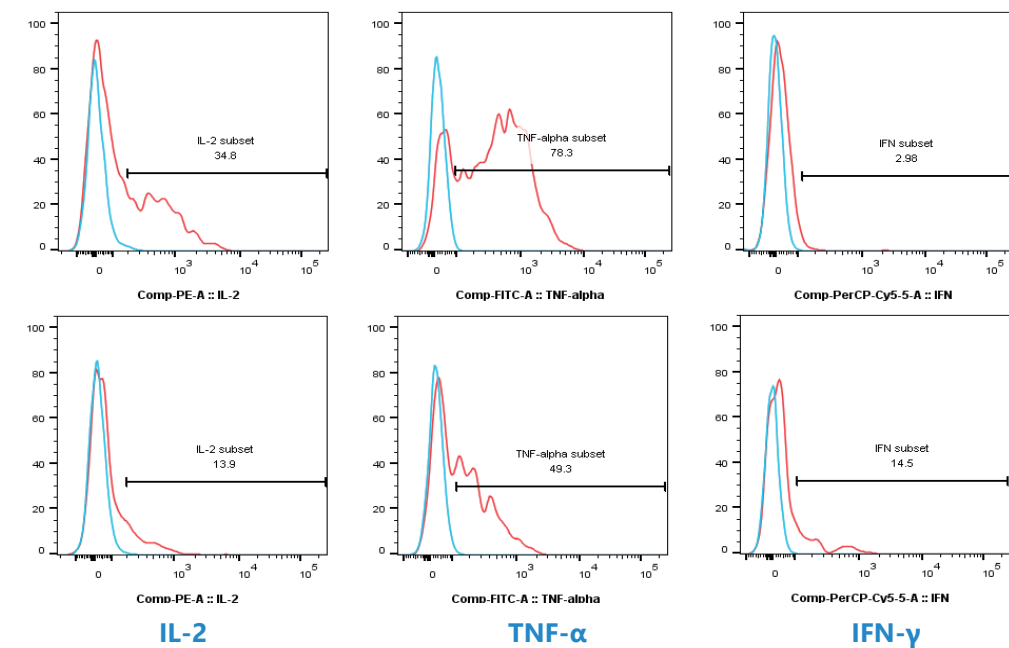
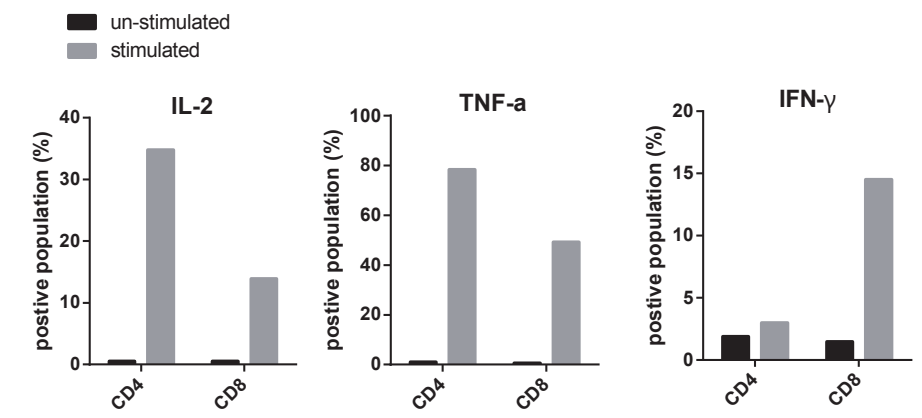
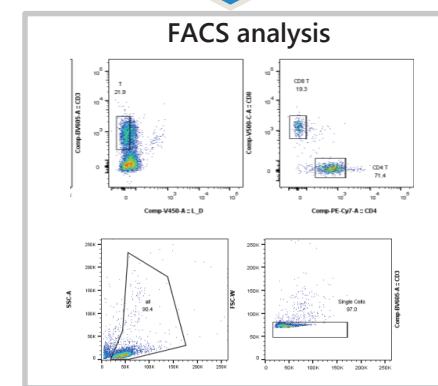
## FACS: Analysis of hepatocytes and liver MNC



PMH and liver MNC were purified by in vivo digestion, followed by density gradient centrifugation and then analyzed by FCM.

## ICS: analysis of splenocytes of AAV/HBV mouse

### Ex-vivo stimulation of lymphocytes



- ◆ Splenic lymphocytes were isolated from AAV/HBV mice and stimulated with an unspecific stimulator
- ◆ IL-2, TNF- $\alpha$  and IFN- $\gamma$  secreting CD4+ or CD8+ T cells were detected by flow cytometry

## Clinical Virology

- ◆ CAP (College of American Pathologists)-certified clinical virology support
  - Viral load: HBV DNA and RNA
  - DNA and RNA sequencing
  - Genotyping and ISG gene expressions
  - HBV serological markers: HBsAg, HBsAb, HBeAg, HBeAb and HBcAb, HBcrAg, cytokines
- ◆ Phenotyping
  - HBV full-length, pol and capsid shuttle vectors
  - Fitness
  - Drug susceptibility
- ◆ HBV antigens and antibodies
  - Antigens and antibodies
  - Cellular immunological markers: ELISPOT and FACS



AID ELISPOT Reader



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