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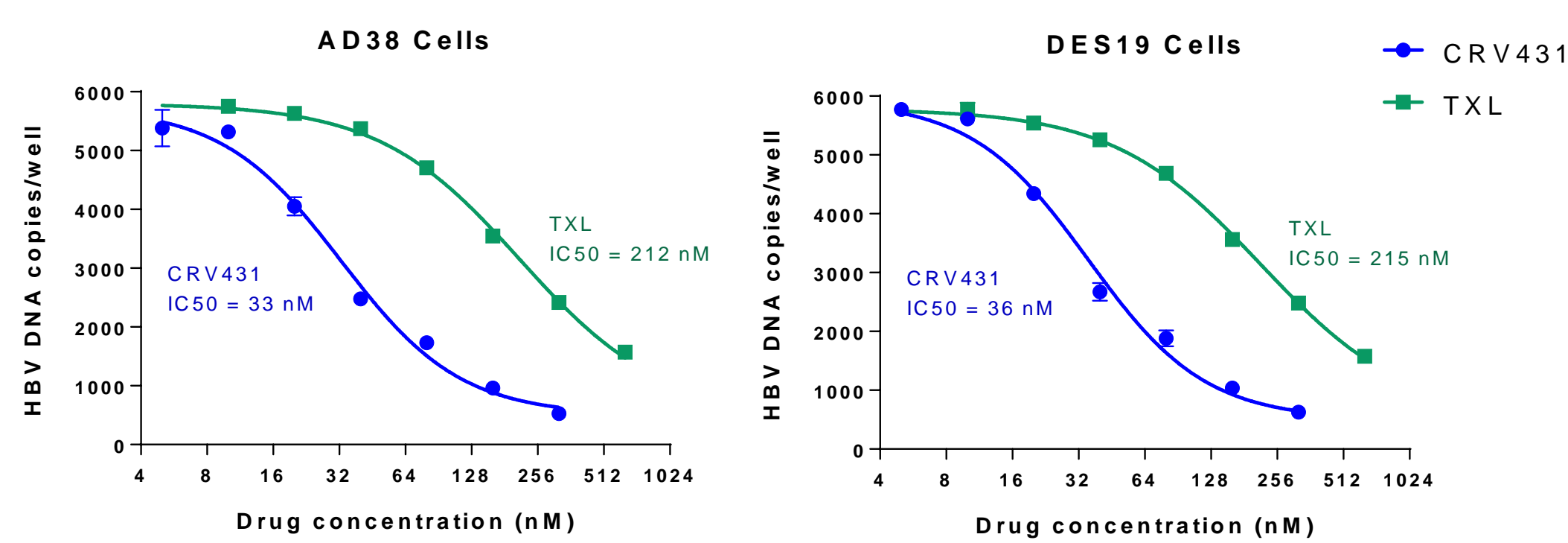
INTRODUCTION

CRV431 is a novel non-immunosuppressive analog of cyclosporine A (CsA) targeting cyclophilins. Cyclophilins are cellular host proteins that participate in the HBV life cycle, and their inhibition has been proposed as a treatment for chronic HBV. The molecular mechanism(s) of action of cyclophilin inhibitors in HBV infection are not well defined. Herein, we describe actions of CRV431 both *in vitro* and *in vivo* aimed at elucidating possible mechanisms of action.

CRV431 Reduces HBV DNA, HBsAg, and HBeAg in Cellular Models

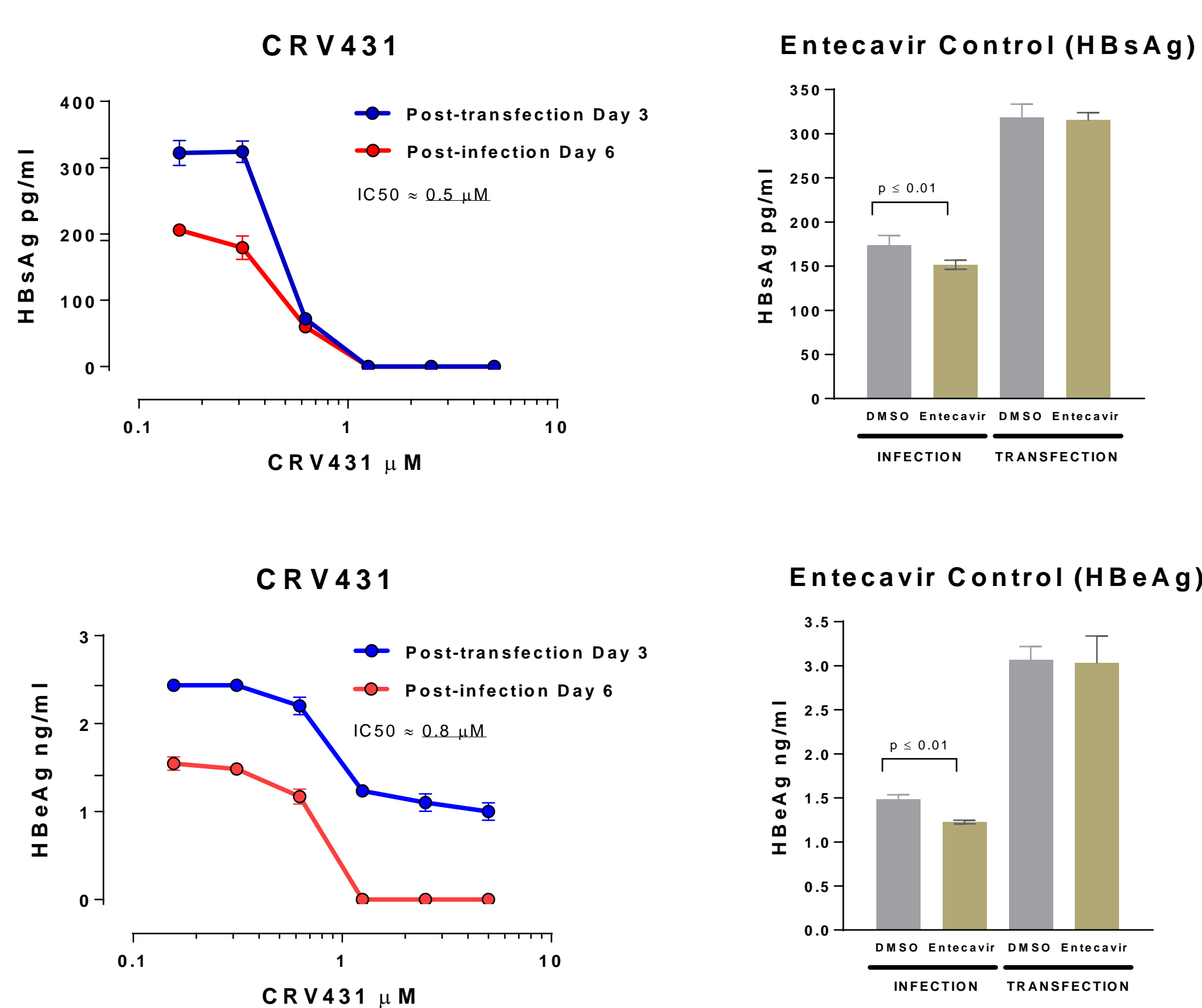
HBV-integrated cell lines: Reduction in HBV DNA

MODEL: AD38 and DES19 cell lines were induced (tetracycline removed) and treated with CRV431 or the tenofovir pro-drug, TXL, for 6 days

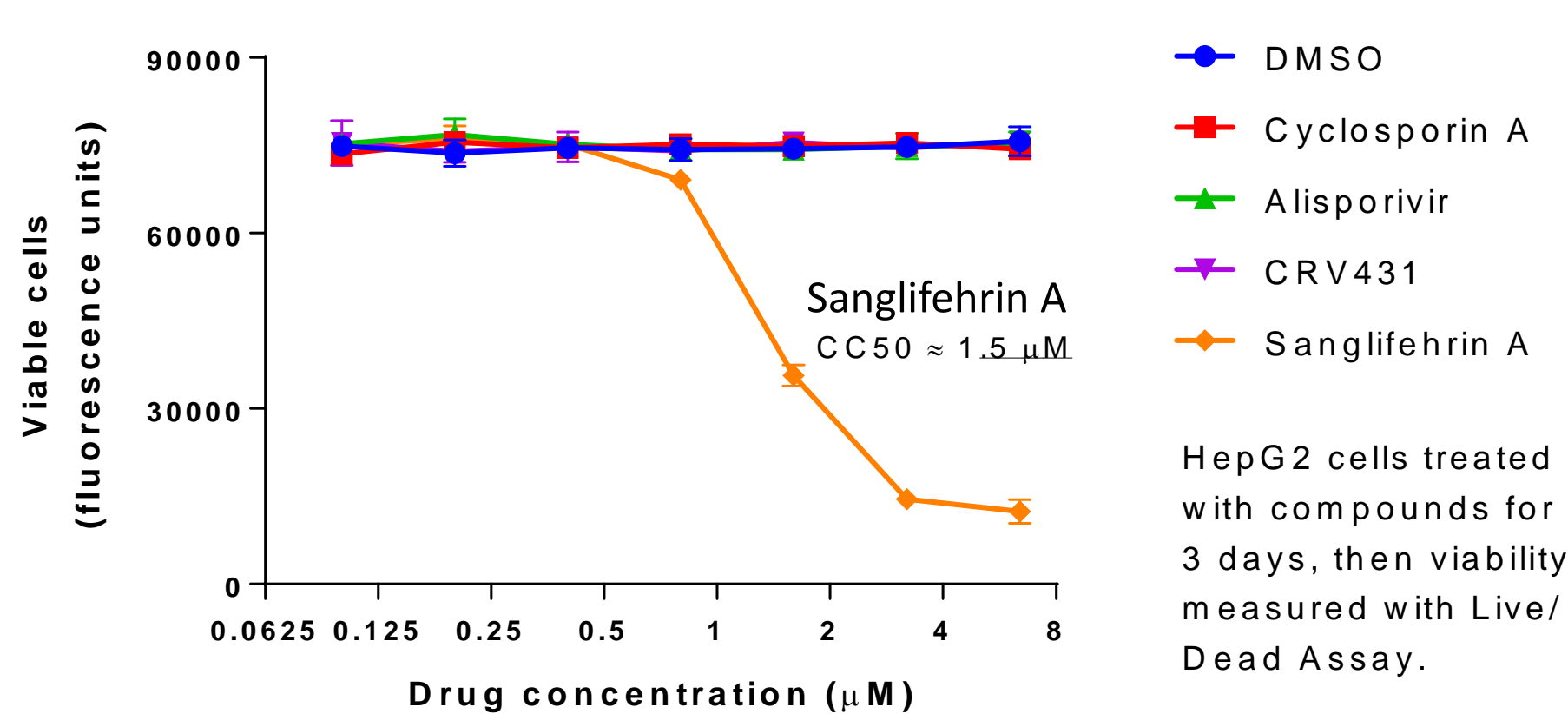


HBV infection and transfection: Reductions in HBsAg + HBeAg

MODEL: CRV431 or entecavir was applied for up to 6 days to NTCP-Huh7 cells infected with HBV or Huh7 cells transfected with HBV plasmid



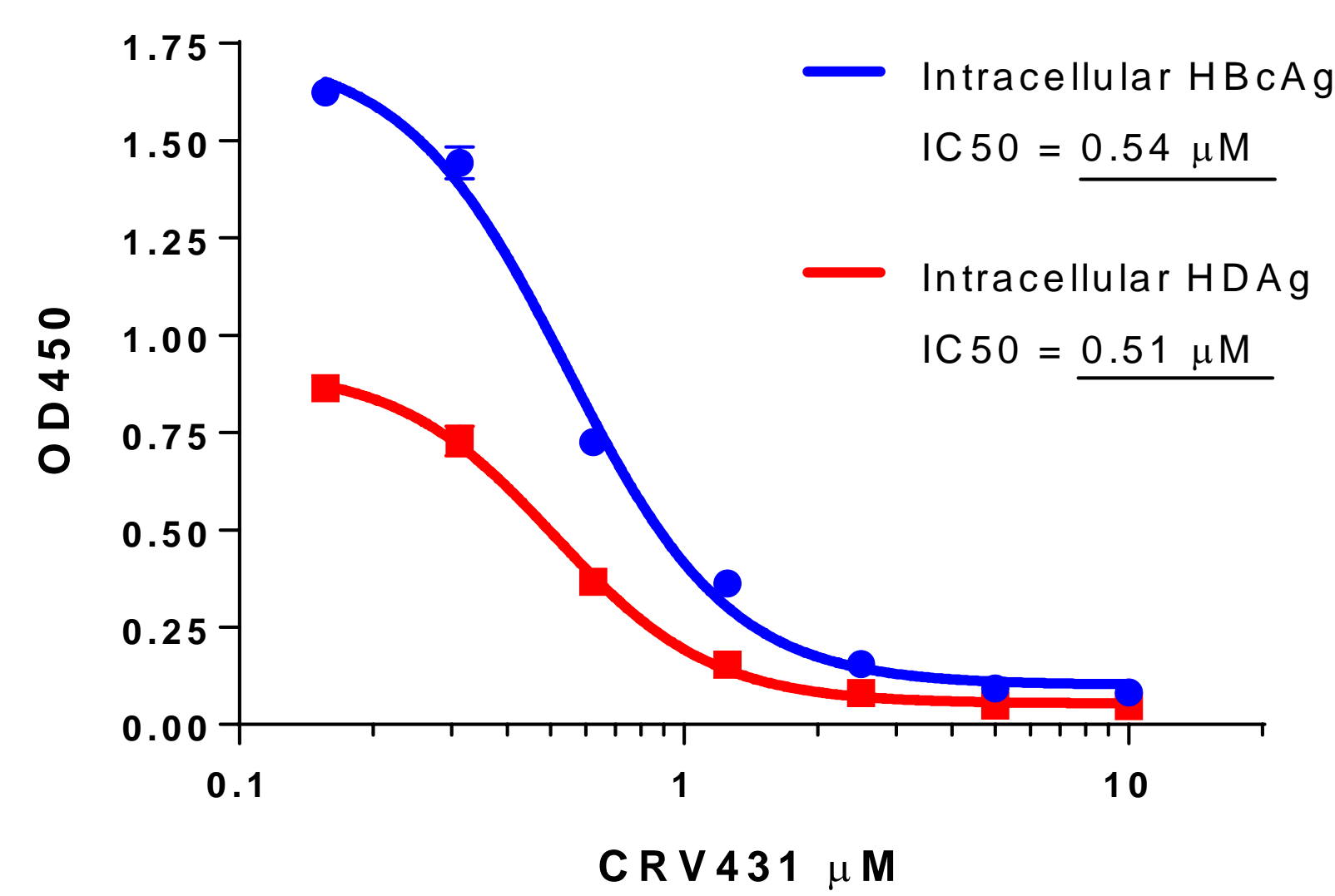
CRV431 Has a High Selective Index



CRV431 Inhibits NTCP in HBV + HDV Co-infection

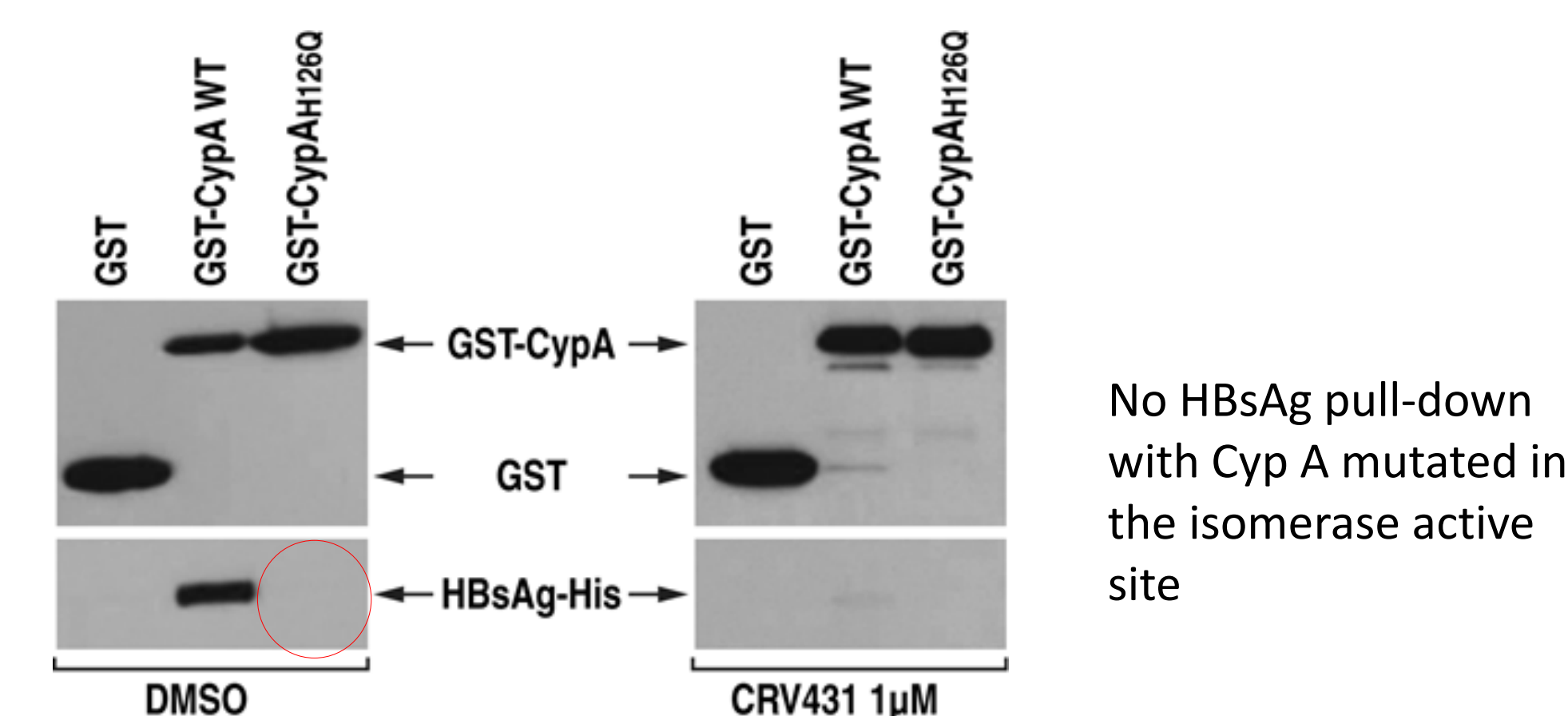
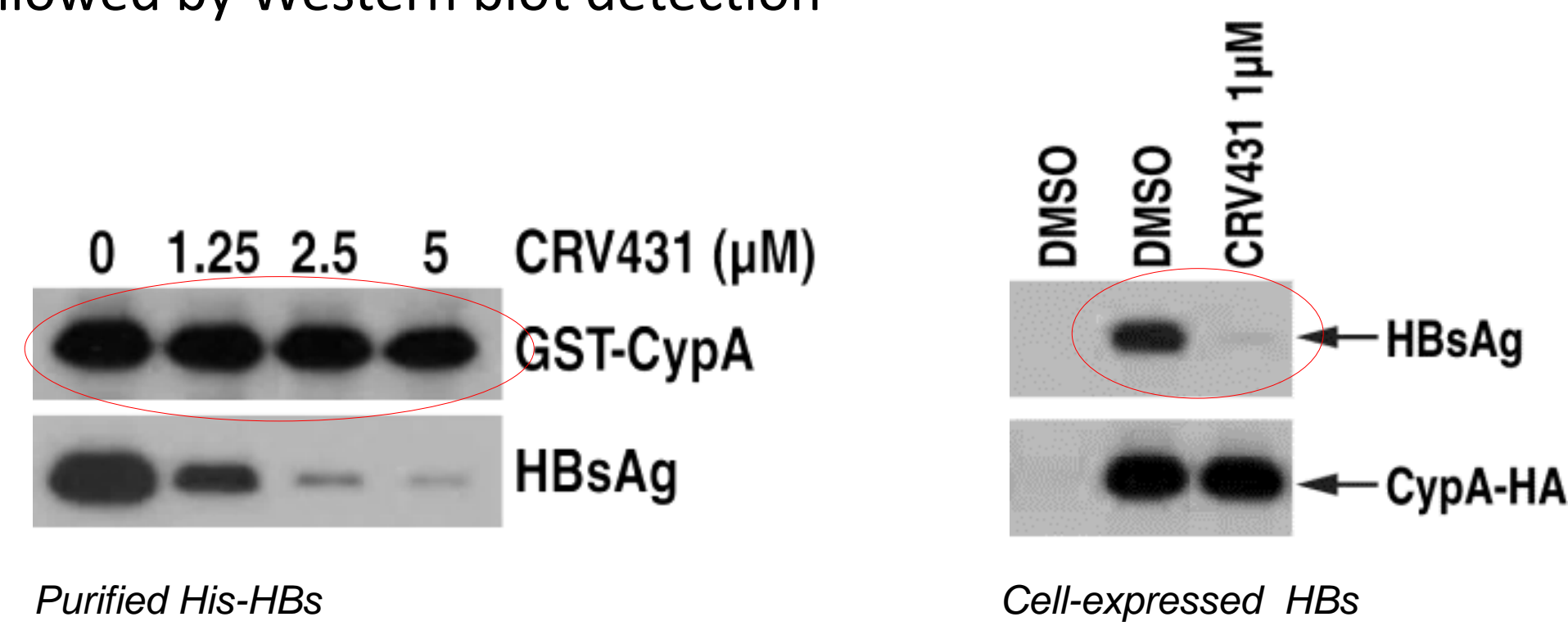
CRV431 inhibited HBV and HDV coinfection *in vitro*, consistent with blocking NTCP-mediated virus uptake

MODEL: HBV and HDV co-infection of stably transfected NTCP-HepG2 cells. CRV431 applied during 4-hr virus inoculation and for 6 days post-inoculation



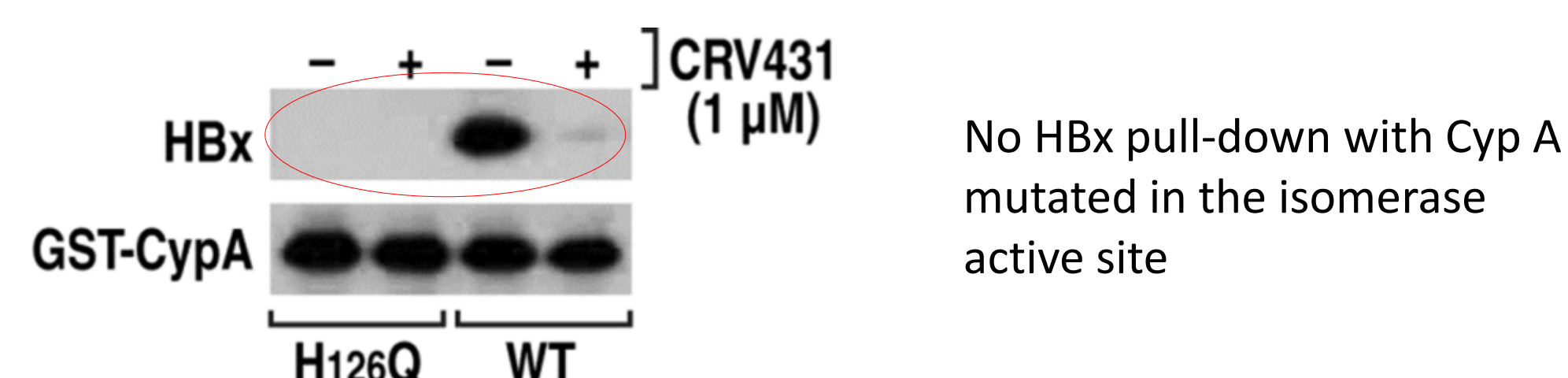
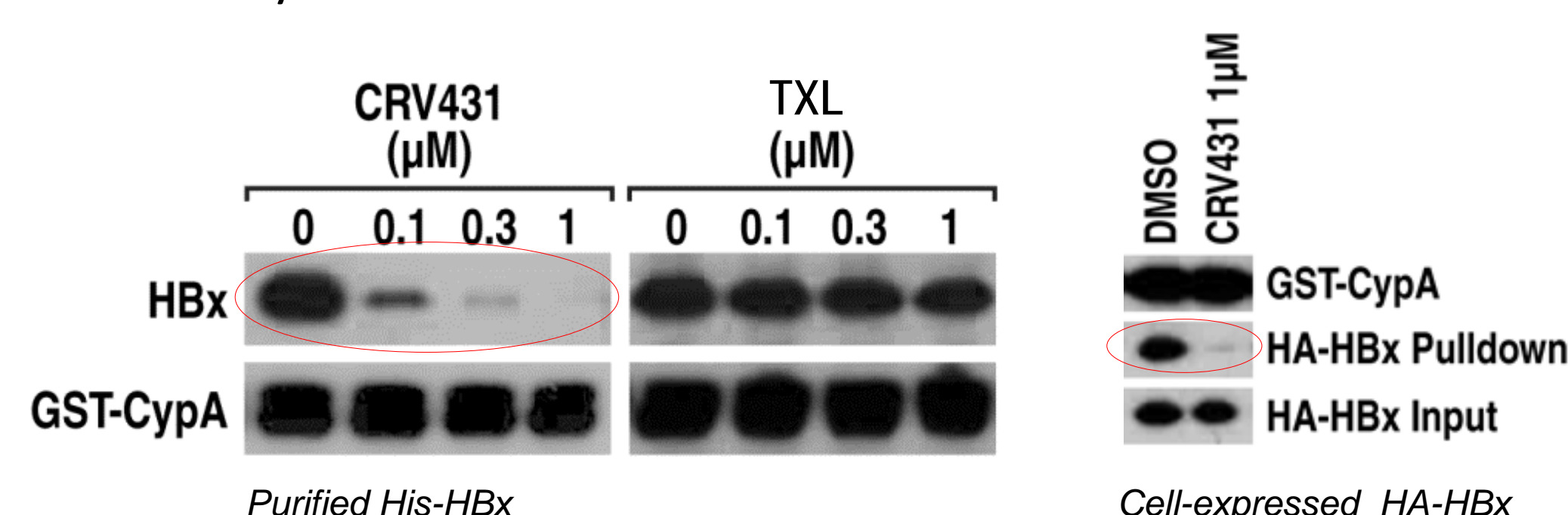
CRV431 Blocks HBsAg-Cyclophilin Binding

Pull-Down Assays: Tagged wild-type or isomerase-dead (H126Q) cyclophilin A (CypA) was used to capture purified His-HBsAg or native HBsAg in lysates from HBV plasmid-transfected cells, followed by Western blot detection



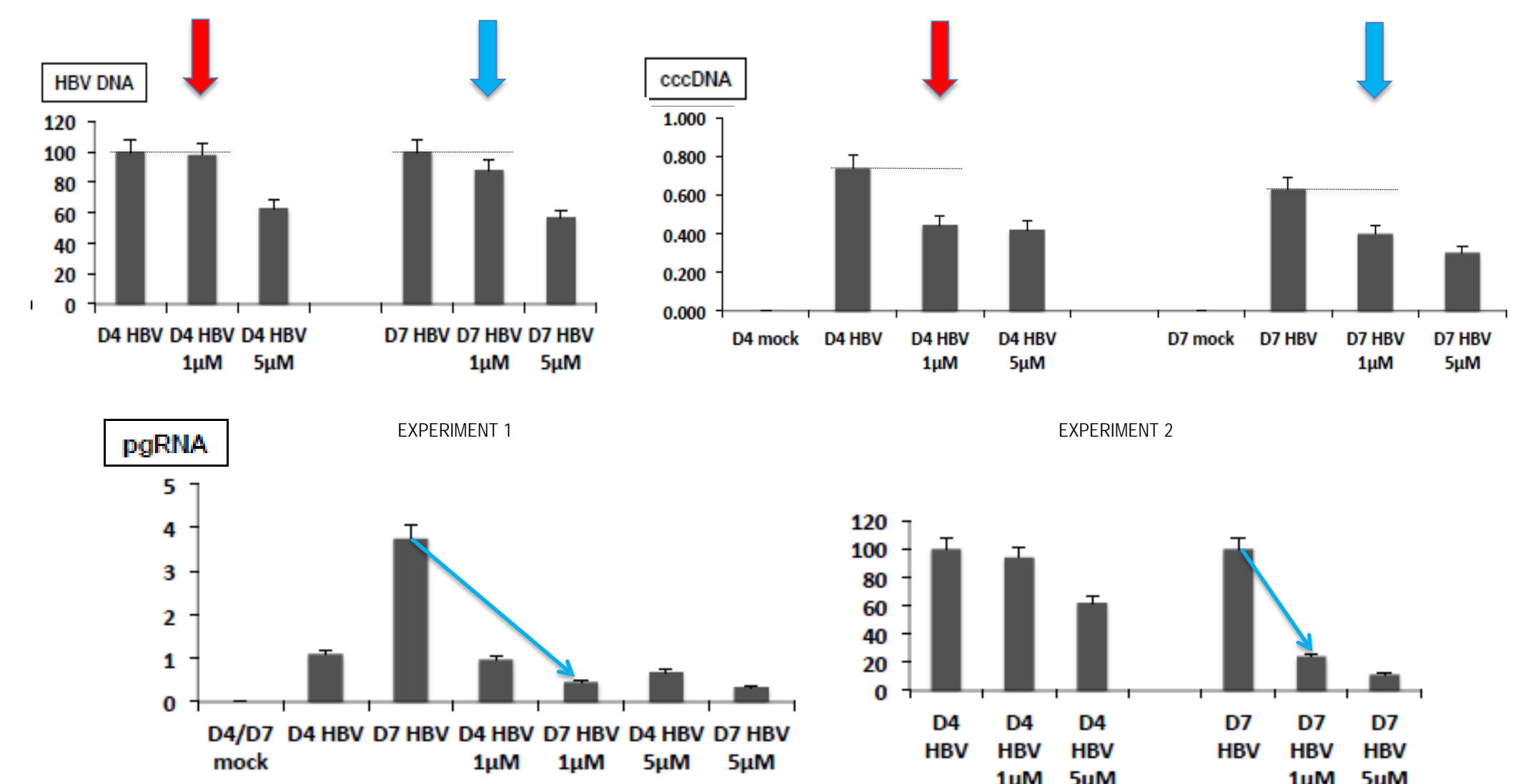
CRV431 Blocks HBx-Cyclophilin Binding

Pull-Down Assays: GST-CypA was used to capture purified His-HBx or HA-HBx in lysates from HA-HBx plasmid-transfected cells, followed by Western blot detection.



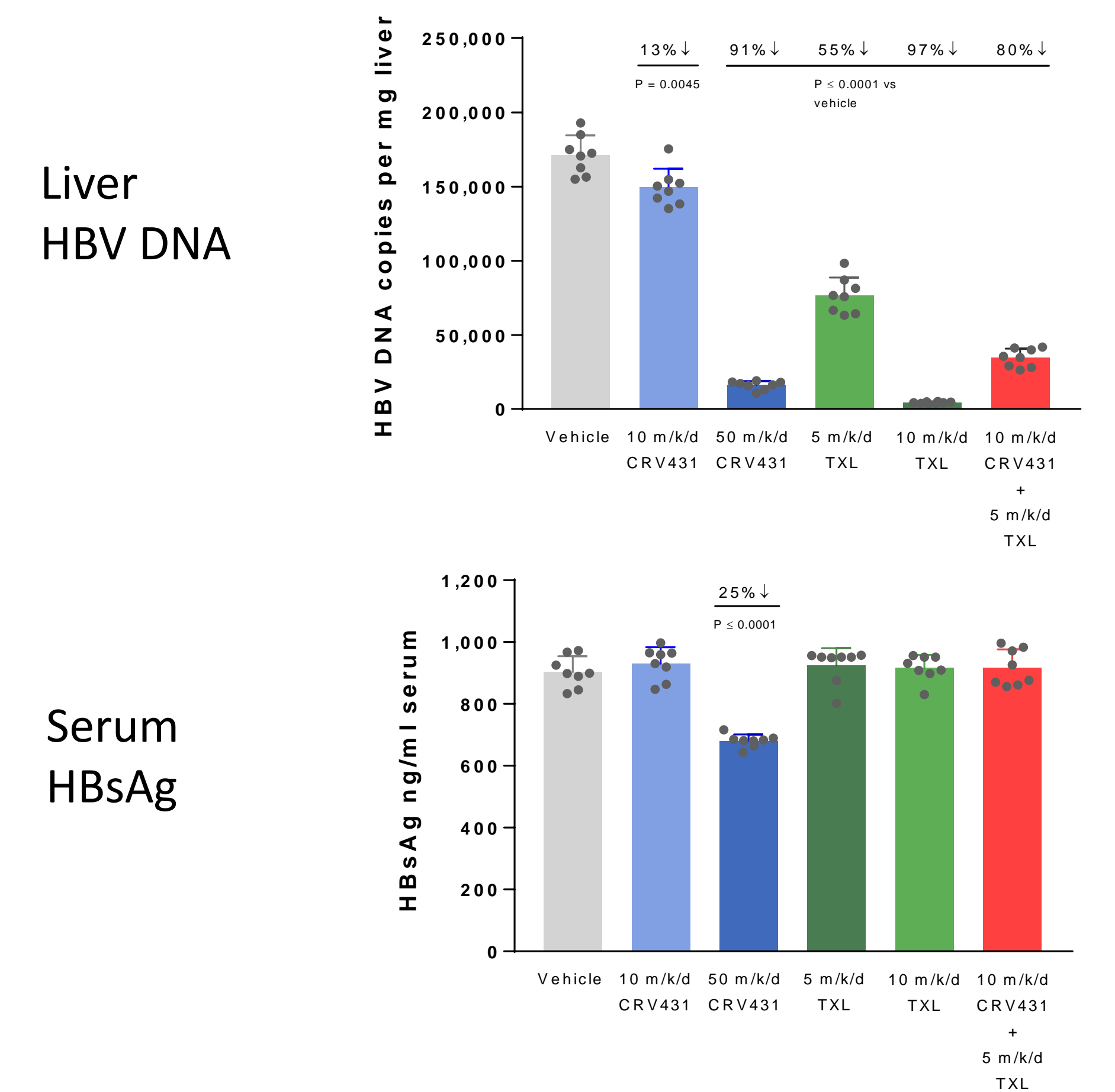
CRV431 Reduces cccDNA and pgRNA in Primary Human Hepatocytes

MODEL: PHH treated with CRV431 starting 1 day after the start of HBV infection. Assay HBV DNA, cccDNA, and pgRNA at Days 4 and 7.



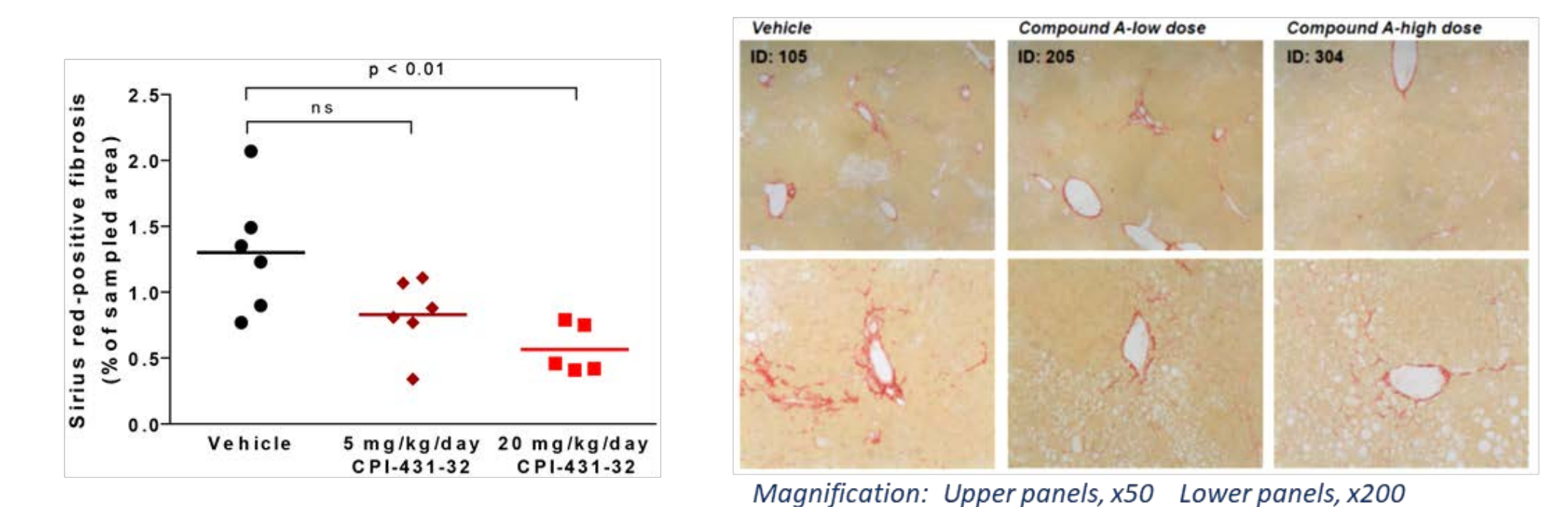
CRV431 Reduces HBV DNA and HBsAg in HBV Transgenic Mice

MODEL: CRV431 and/or the tenofovir pro-drug, TXL, were administered orally to HBV transgenic mice for 16 days, followed by measurement of HBV in livers.



CRV431 Reduces Liver Fibrosis in a NASH Model

MODEL: CRV431 was administered orally to "NASH" mice for 21 days (Weeks 6-9), followed by histological quantitation of fibrosis.



CONCLUSIONS

- ✓ CRV431 reduces HBV DNA, cccDNA, pgRNA, HBsAg, and HBeAg in a variety of cellular models
- ✓ CRV431 blocks cyclophilin A binding of to HBsAg and HBx, which may be part of the mechanism (s) of action
- ✓ CRV431 has anti-HBV activity and anti-fibrotic activity in mouse models
- ✓ CRV431 has the potential for multiple, therapeutic effects in hepatitis B patients with a favorable toxicity profile