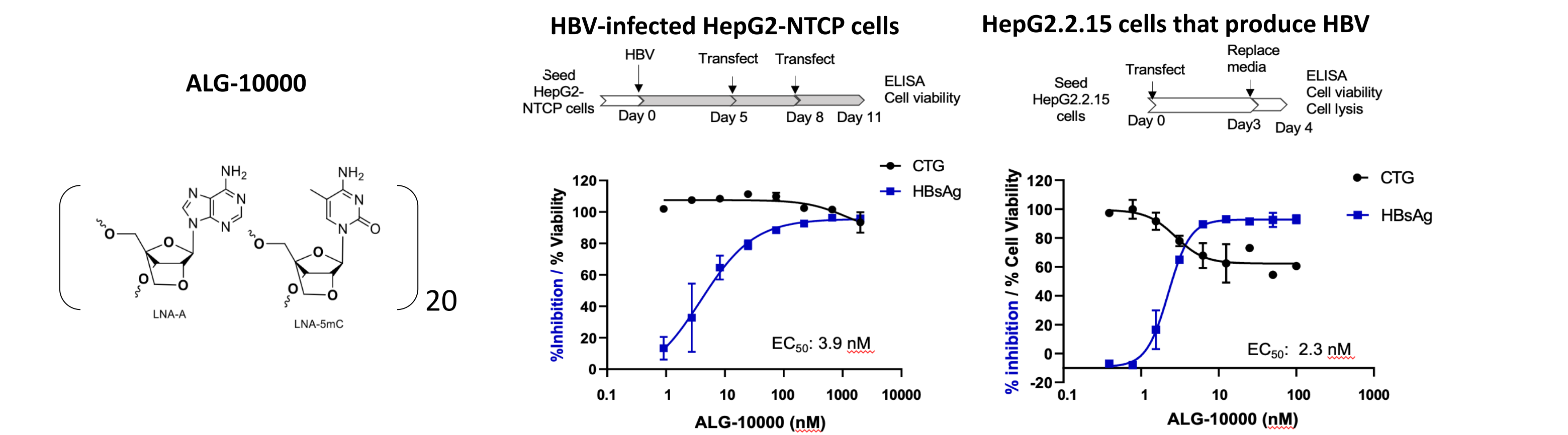


Abstract

Functional cure of chronic hepatitis B requires the elimination of serum hepatitis B virus surface antigen (HBsAg).¹ Nucleic acid polymers (NAPs), single-stranded phosphorothioated oligonucleotides, can reduce HBsAg in cultured cells and in patients.²⁻⁴ STOPS, phosphorothioated oligonucleotides that contain novel chemistry, have enhanced inhibition of HBsAg by HBV-infected cells in vitro. This project aims to elucidate how STOPS cause the reduction of HBsAg.

The STOPS molecule ALG-10000 functions only when transfected into cells, where it localized to the cytoplasm. ALG-10000 reduced both intracellular and extracellular HBsAg. In addition, ALG-10000 reduced the level of HBV E-antigen, HBV polymerase, the core protein, total HBV RNA, and secreted rcDNA in HepG2.2.15 cells. ALG-10000 did not significantly co-localize with or bind to the HBsAg, indicating that STOPS acts through host-encoded factors. Five host proteins that function in RNA processing, translation, and protein folding/degradation were identified to bind ALG-10000: SRSF1, HNRNPA2B1, GRP78, RPLP1, and RPLP2. Notably, all of these factors have been previously described to function in viral replication and infection.⁵⁻⁸ Silencing RNAs targeting these host factors reduced the levels of HBsAg and other HBV molecules known to be affected by STOPS. Interaction with multiple host factors is required to account for the inhibition of HBV infection. Finally, the introduction of ALG-10000, or the knockdown of GRP78, RPLP1, and RPLP2 in cells, increased the ubiquitination of HBsAg and proteasome-mediated degradation of HBsAg.



Methods

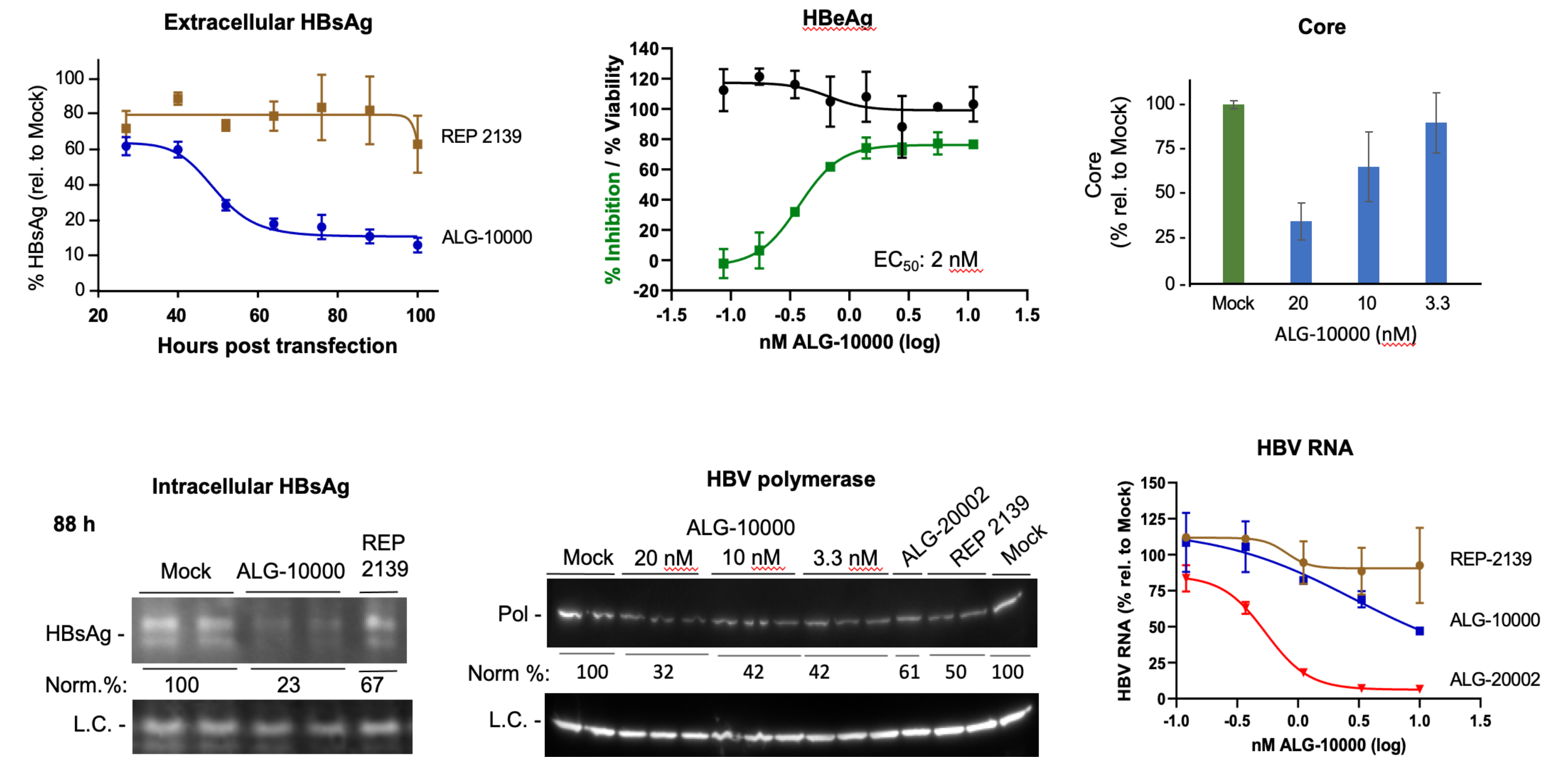
STOPS and siRNAs were transfected into HepG2.2.15 cells that contain integrated HBV genomes using Lipofectamine RNAiMax. Proteins from HepG2.2.15 cell lysates that bind STOPS were identified using affinity chromatography, tandem mass spectrometry, and searches of the human proteome database. Fluorophore-labeled ALG-10000 in cells was visualized using a Leica SP6 microscope. HBsAg and other HBV proteins were quantified using ELISA and Western blotting. HBV nucleic acids were quantified using a Quantigene assay. Ubiquitination was detected by ELISA using a monoclonal antibody that was conjugated to horseradish peroxidase.

References and Disclosure

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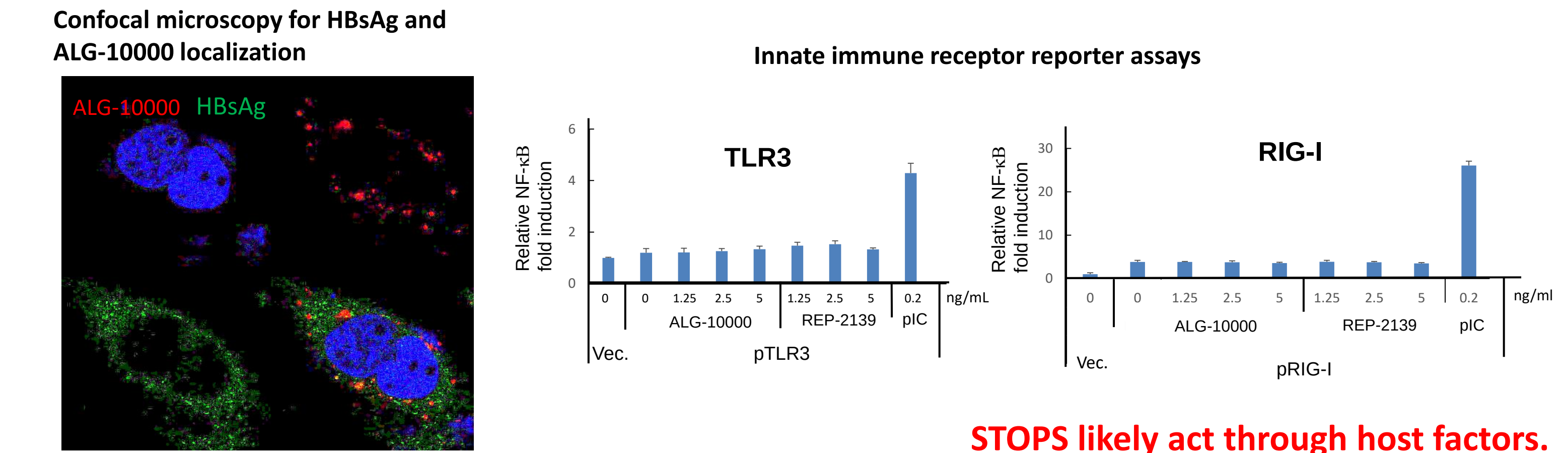
Disclosure: All authors are employees or consultants of Aligos Therapeutics, Inc.

ALG-10000 inhibits multiple HBV molecules but to different extents



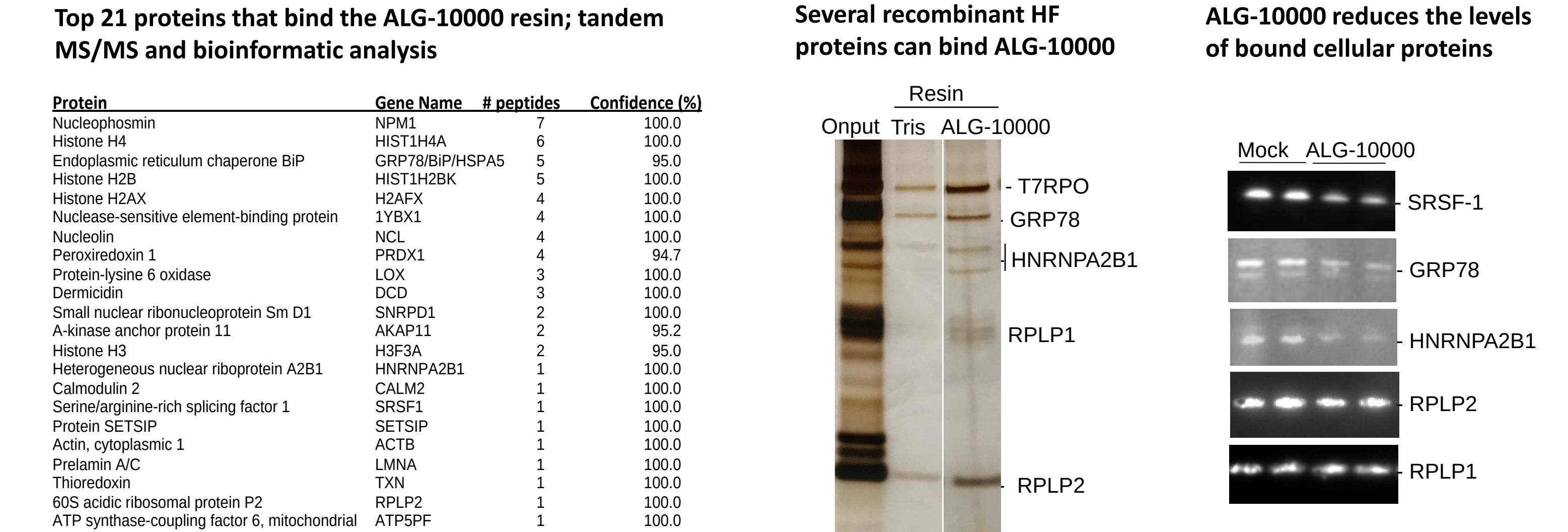
STOPS have a complex mechanism of inhibition.
Inhibition of intracellular HBsAg suggests that inhibition precedes HBsAg trafficking and secretion.

ALG-10000 does not co-localize with HBsAg or activate nucleic acid-sensing immune receptors

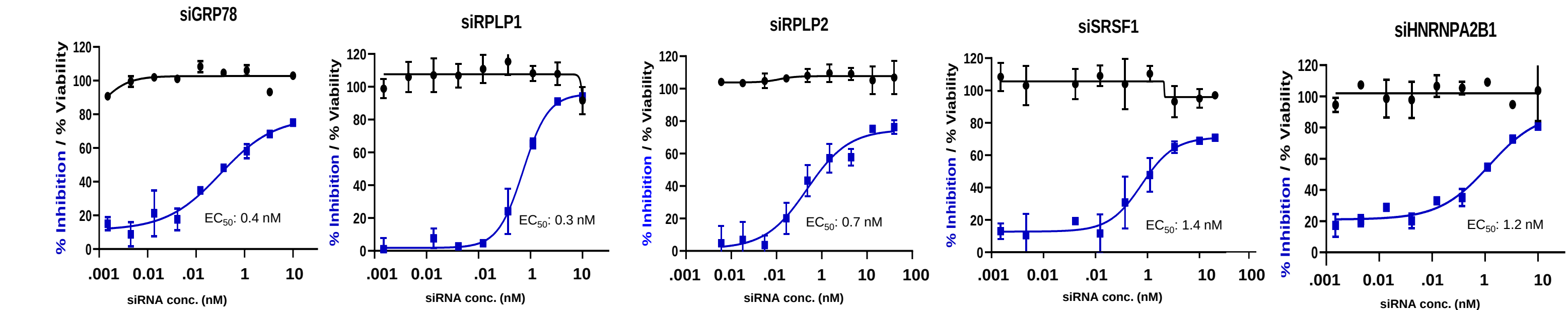


STOPS likely act through host factors.

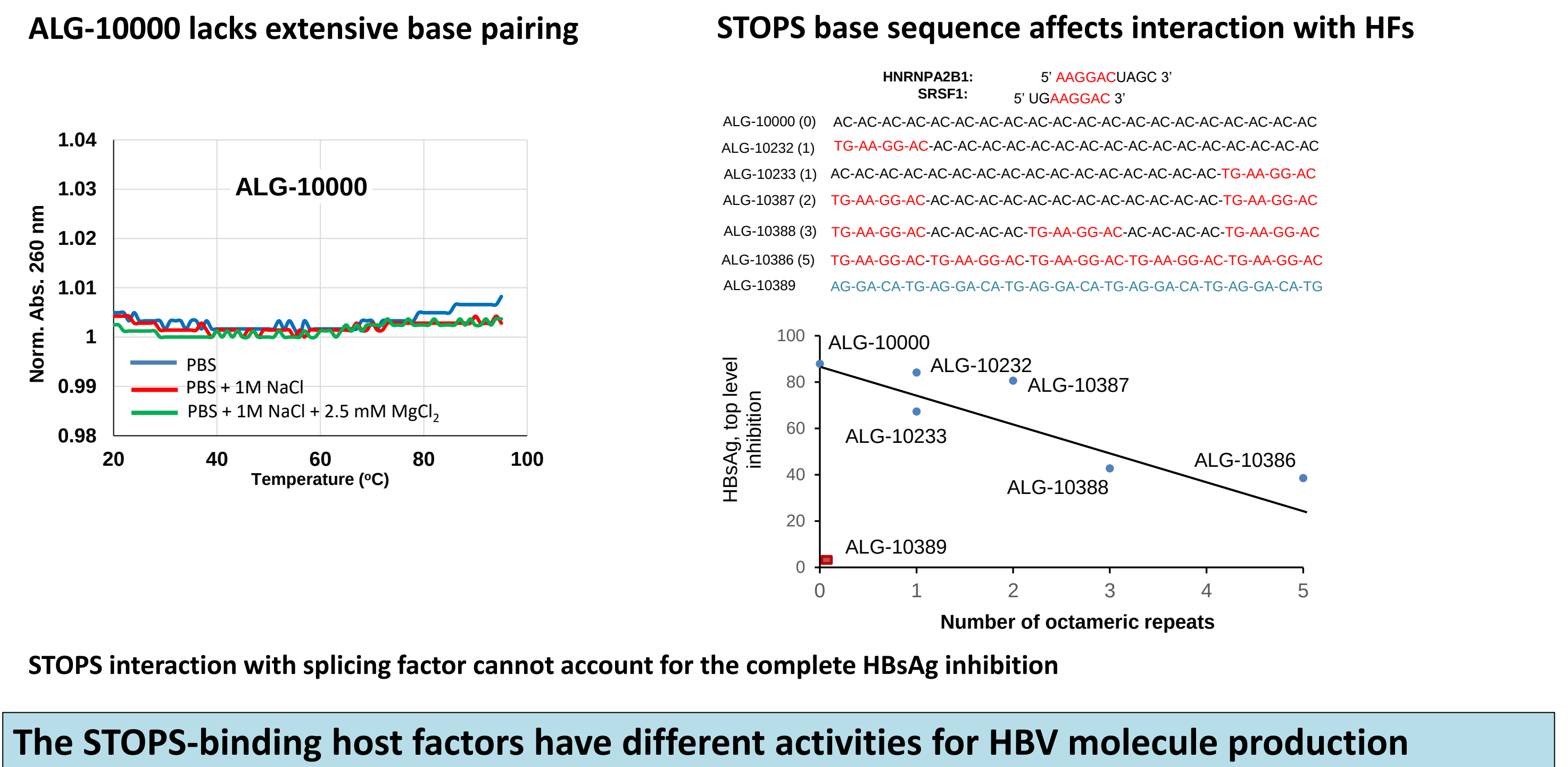
ALG-10000 binds cellular proteins that function in gene expression



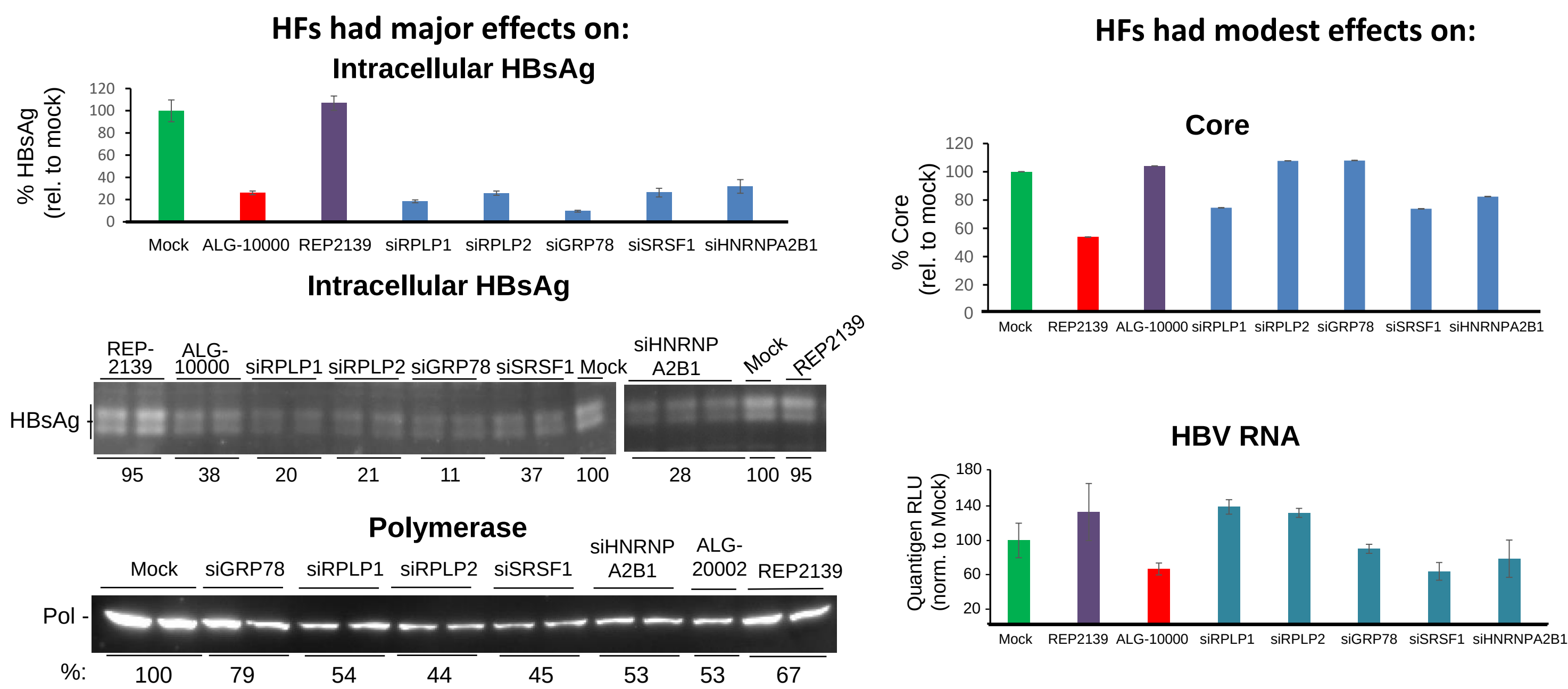
Five of the cellular proteins that bind ALG-10000 are required for optimal HBsAg production. All 5 proteins have previously been reported to function in viral infections⁵⁻⁸.



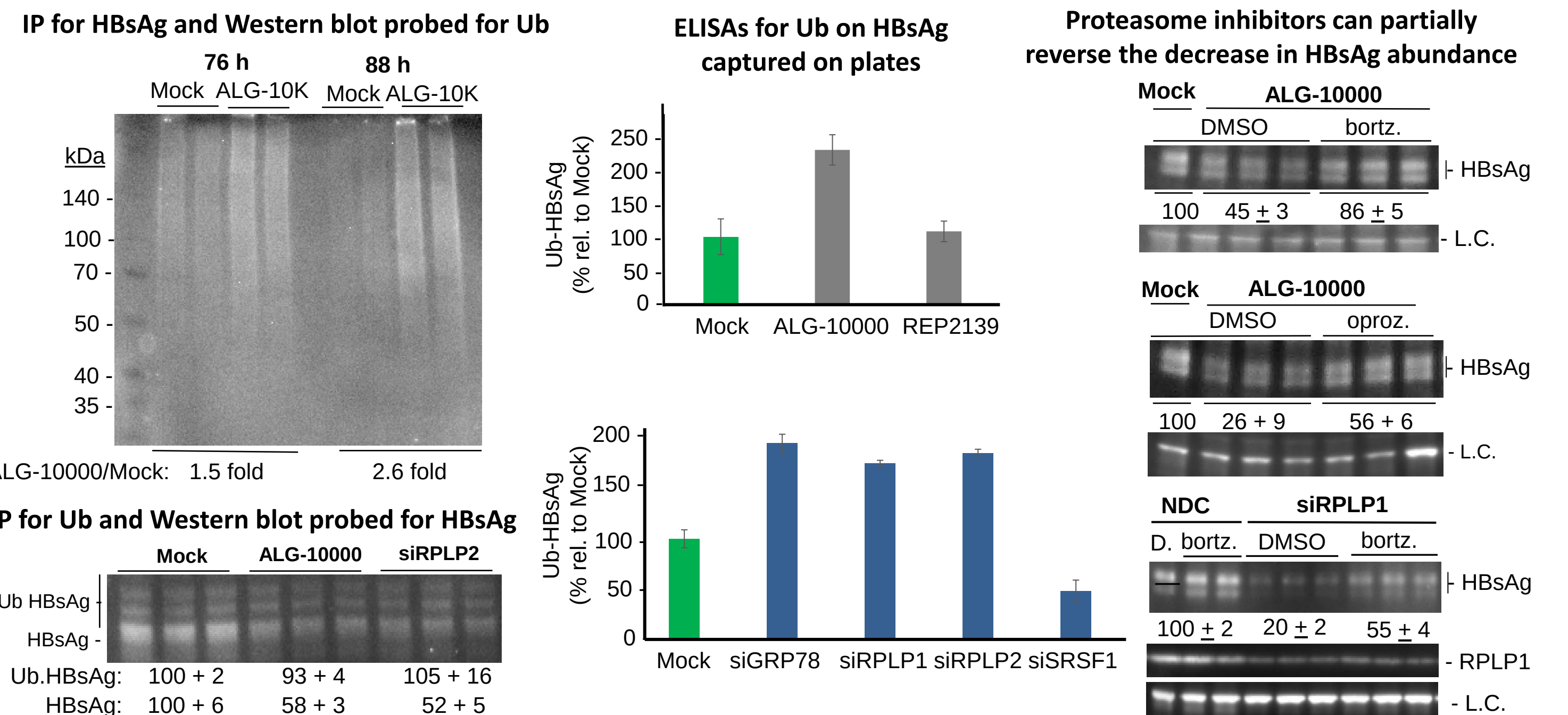
STOPS function optimally by interacting with multiple host factors



The STOPS-binding host factors have different activities for HBV molecule production



STOPS and knockdowns of RPLP1/2 and GRP78 increase ubiquitination and proteasome degradation of HBsAg



Conclusions and Discussion

STOPS have a complex mechanism of action to inhibit HBV infection and HBsAg production. Through sequestration of cellular factors involved in RNA processing, STOPS decrease the expression of HBV molecules. Through the sequestration of cellular factors needed to translate and properly fold the HBV S-antigen, increase their ubiquitination and intracellular degradation.