Supplementary Materials

Characterization and Application of Precore/Core-Related Antigens in Animal Models of Hepatitis B Virus Infection

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Supplementary Materials and Methods

Immunoblot analysis

Serum samples, concentrated cell culture supernatant, and cell lysates were separated using 12.5% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) at heights of 35 cm or 12.5 cm gels and immunoblotting (1). The mouse monoclonal antibodies (mAb) clone T2221 (Cat no. 2AHC24) and clone 19C18 (Cat no. 2Z19C18) specific for the HBc NTD were purchased from Tokyo Future Style (2). The mouse monoclonal anti-HBc NTD clone 10E11 was purchased from Abcam (Cat no. ab8639). The anti-HBc mouse mAb A701, selective for the non-phosphorylated CTD, and the HBc CTD-specific rabbit mAbs 366-2 (relatively non-selective for the CTD state of phosphorylation), 25-7 (selective for non-phosphorylated CTD), and 14-2 (selective for phosphorylated CTD) were custom-made (Abcam) using CTD peptides as an immunogen and have been described before (3-5). The polyclonal rabbit antibody against HBsAg (Cat no. 1811) (Virostat, Portland, ME, USA) was used for the detection of surface proteins. The mouse anti-precore mAbs, 7E9 and 1A11 (VIDRL, Australia), were used for specific detection of precore-related proteins (5, 6).

Plasmid, Cell cultures, and transient transfection

WHV DNA sequence containing the WHV precore or core ORF was amplified from pucCMVWHV by PCR (7). The plasmid for expressing WHc (pCI-WHc) and WHV precore protein (full-length WHV precore) (pCI-WPC) were made in the pCI vector (Promega, WI) background. The woodchuck hepatoma cell line, WC3 (a gift from Dr. Haitao Guo, University of Pittsburgh) (8), was cultured in the Dulbecco's modified Eagle's medium

(DMEM)-F12 (Gibco) supplemented with 10% fetal bovine serum (FBS) (Hyclone). For transient transfection, WC3 cells seeded in 60-mm dishes were transfected with 4 μg of plasmid using X-tremeGENE™ HP DNA transfection reagent (Roche). The cell culture supernatants were collected at 3 days post-transfection for further analysis.

Deglycosylation of woodchuck serum

The woodchuck serum samples or concentrated WC3 cell culture supernatants were treated with PNGase F (New England Biology, MA) following the manufacturer's instruction with a slight modification. 5 μl of serum was combined with 2 μl of 10X Glycoprotein Denaturing Buffer (New England Biology, MA) and water to make up a 20 μl total reaction volume. The mixture was denatured by boiling at 100^{o}C for 10 min and chilled on ice, and 4 μl of 10X GlycoBuffer 2 (New England Biology, MA), 4 μl of 10% Nonidet P-40, and 12 μl of water were then added to make up a 40 μl total reaction volume. The denatured mixture was incubated at 37^{o}C with or without 1 μl of PNGase F for 1 hr unless indicated otherwise. For PNGase A treatment, we followed the same procedure as PNGase F treatment, except using 4 μl of 10X GlycoBuffer 3 (New England Biology, MA), instead of 10X GlycoBuffer 2.

Native agarose gel electrophoresis (NAGE) for analyzing viral particles. Fractionated or unfractionated serum samples from WHV-infected woodchucks and HBV-infected chimpanzees were resolved by NAGE as described previously (1). Following the transfer of viral particles from the gel to nitrocellulose membrane, a ^{32}P-labelled HBV or WHV DNA probe was used to detect encapsidated DNA in HBV or WHV particles.

Subsequently, the HBc or surface proteins were detected by the indicated HBc- or surface-specific antibody as described previously (1, 4, 9).

Measurements of WHV parameters.

Serum WHV DNA was quantified by dot blot hybridization or real-time PCR, as described previously (10). Serum WHsAg levels were measured by WHV-specific quantitative enzyme immunoassays as described (11). Liver WHV cccDNA was extracted and quantified by Southern blot analysis or real-time PCR (10, 12, 13).

Analysis of HBV and WHV virion secretion by CsCl density gradient centrifugation

Virions, subviral particles, and soluble viral antigens in the serum of HBV-infected chimpanzees and WHV-infected woodchucks were purified by CsCl density gradient ultracentrifugation (14, 15). Fractions were analyzed by NAGE for virion particles and by SDS-PAGE for viral antigens/proteins.

Analysis of WHV antigens by sucrose gradient ultracentrifugation

Serum samples from WHV-infected woodchucks were fractionated by rate-zonal sucrose gradient ultracentrifugation as described (16), with minor modifications. 1.5 ml sera were layered on the top of the 15%-30% linear sucrose gradient containing TNE buffer [10mM Tris-HCl (pH 8)/100mM NaCl/1mM EDTA] in a 12 ml ultracentrifugation tube, and ultracentrifugation was performed at 27,000 rpm for 4hr at 4^{o}C in a Beckman SW40Ti rotor. Fractions were collected from the top to bottom and were analyzed by SDS-PAGE for analysis of viral antigens/proteins.

Mass spectrometry-based proteomic analysis

The WPreC1 protein was prepared from pooled low-density WPreC1 peak fractions from a CsCl density gradient used for fractionation of sera from woodchucks with chronic WHV infection. WPreC1 was resolved by 10% SDS-PAGE and gel pieces containing the glycosylated form of WPreC1 (g-WPreC1) were cut out and processed by in-gel trypsin digestion according to established protocols (17). LC-MS was performed on a ThermoFisher Scientific QExactive HF-X hyphenated to an Ultimate 3000 RSLCnano system, utilizing a 50cm uPAC PharmaFluidics analytical column and a uPAC Trapping Column. LC-MS was achieved over a 180 min gradient of 2%-27% buffer B (solvent A: 100% water with 0.1% formic acid; solvent B: 100% acetonitrile with 0.1% formic acid) at 200 nl/min with the instrument in DDA mode. Full-scan MS1 spectra were acquired at a resolution setting of 120K over the range 400-1800 *m/z,* and HCD MS/MS spectra were achieved for the top 10 most abundant ions at a resolution setting of 7.5K. Raw data was matched against the Eastern woodchuck and WHV proteomes using ProteomeDiscoverer 2.4 (ThermoFisher Scientific) equipped with the Mascot 2.5 search engine (Matrix Science), using a 1% FDR peptide- and protein-level cutoff. Spectral matches to the WPreC1 protein were evaluated manually. Sample processing and data acquisition was performed at the Harvard Center for Mass Spectrometry (Cambridge, MA).

Quantitative analysis of HBV cccDNA.

Hirt DNA was extracted from liver needle biopsies of chimpanzees as previously described (18) with some modifications. Liver biopsies (ca. 100 mg) were homogenized

in a bead blender with 500 µl TNE buffer on ice. 250 µl of cell homogenate were lysed by adding 25 µl 10% SDS and incubated at room temperature for 30 min with occasional mixing. After adding 62.5 µl 2.5M KCl, the homogenate mix was incubated at 4^{o}C overnight with rotation. After being centrifugated at 14,000 rpm for 30 min at 4^{o}C, the supernatant was extracted three times with phenol and once with chloroform. The DNA was precipitated with ethanol and washed with 70% ethanol three times, vacuum dried, and resuspended in 25 µl TE [10mM Tris-HCl/1mM EDTA] (pH 8.0). HBV cccDNA quantification was performed using the One Step Real-Time PCR system (ABI) in a 20 µl volume consisting of SYBR Green PCR Master Mix (Life Technologies), 300 nM each primer and serially diluted DNA samples. Primers specific for cccDNA were: forward: 5'-GTCTGTGCCTTCTCATCTGC-3', reverse: 5'-ACAAGAGATGATTAGGCAGAGG-3' (19). Serial dilutions of a plasmid containing a HBV monomer served as quantification standards. Mitochondrial DNA was measured simultaneously from the same samples for normalization of cccDNA signals (18). Mitochondrial DNA PCR primer sequences were: forward: 5'-CCCTCTCGGCCCTCCTAATAACCT-3', reverse: 5'-GCCTTCTCGTATAACATCGCGTCA-3' (18). The cycling condition was 95^{o}C for 10 min, and 40 cycles of 95^{o}C for 15 s and 60^{o}C for 1 min (19). Protein-free (PF) rcDNA in our Hirt DNA extract was less than, or at most equal to, cccDNA from the chimpanzee liver biopsies (Fig. S9F). As reported before, our cccDNA-specific qPCR conditions has a selectivity of ca. 100-fold in amplifying cccDNA over rcDNA (19). Our cccDNA qPCR assay would thus not be affected by the small amount of PF-rcDNA in the Hirt DNA extract from the chimpanzee liver biopsies. Indeed, we repeated the qPCR analysis following

Exonuclease I and III digestion, which eliminates rcDNA and other non-covalently closed circular DNA (19), and obtained the same results as without the prior nuclease digestion.

Supplementary Results

Lack of CTD sequence in WHeAg and WPreC

To further verify the lack of CTD sequence in WHeAg and WPreC, we employed two mAbs developed against the HBc CTD, A701 and 14-2, that cross-react with WHc CTD (**Fig. 1A**). In CsCl gradient fractions containing serum WHc (virion) or WHeAg/WPreC, both mAb A701 and 14-2 detected WHc, but neither detected g-WHe1 or g-WPreC1 (**Fig. S3C**, lanes 15, 16, 21, 22). In contrast, mAb 1A11 and 19C18 detected both g-WHe1 and g-WPreC1 (**Fig. S3C**, lanes 3, 4, 9, 10). These results thus indicated that WPreC lacked the CTD sequence, but most likely retained the N-terminal signal peptide accounting for its slower mobility on SDS-PAGE, as reported for HBV PreC (5, 20). Compared to the NTD mAb 19C18, which detected all the HBc and WHc proteins irrespective of their CTD phosphorylation state (**Fig. S3C**, lanes 7, 9, 10, 12), mAb A701, but not 14-2, detected the (non-phosphorylated) HBc expressed in E. coli (**Fig. S3C**, lanes 13, 19), verifying the phosphor-selectivity of the CTD mAbs. Furthermore, mAb 14-2 detected the WHc strongly in the woodchuck liver lysate but only weakly the WHc in the serum WHV virions (**Fig. S3C**, lanes 21, 22, 24); in contrast, mAb A701 detected strongly the WHc in the serum WHV virions but only weakly the WHc in the woodchuck liver lysate (**Fig. S3C**, lanes 15, 16, 18). These results indicated that whereas the intracellular WHc in the woodchuck liver lysate contained mostly phosphorylated CTD, the serum virion WHc contained mostly dephosphorylated CTD. This was also consistent with the results that no significant amounts of WHV empty virions present in WHV-infected woodchucks (**Table S2**).

WHeAg and WPreC showed heterogeneous characteristics

We employed sucrose gradient ultracentrifugation to fractionate the serum from a chronically-infected woodchuck, F6006, and observed serum g-WHe1 and WPreC1 co-sedimented on the top fractions and peaked in fraction 2 (**Fig. S7**), suggesting g-WHe1 and the WPreC1 proteins had similarly small sizes. However, the fact that a portion of g-WHe1 and the WPreC1 proteins sedimented to the lower fractions (from fraction 3 to 7) suggested that some of these proteins might be associated with other serum factors in chronically-infected animals, in line with our buoyant density analysis (**Fig.3**, **S6**).

Supplementary References

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Supplementary Figures

Figure S1. Sensitivity of anti-HBc NTD mAb T2221 for WHc vs. HBc. WHc from the liver lysate of a chronically WHV-infected woodchuck and recombinant HBc expressed in *E. coli* were titrated and resolved by SDS-PAGE, followed by western blotting analysis using the anti-HBc NTD mAbs 10E11 and T2221. Based on 10E11, WHc amount was ca. 120 ng/μL; however, based on T2221, WHc amount was ca. 25 ng/μL. As 10E11 should detect WHc and HBc equally well due to the complete conservation of its epitope (**Fig. 1A**), T2221 was estimated to detect WHc at ca. 20% sensitivity of HBc. However, mAb 10E11 was not nearly as sensitive as T2221 for HBc and thus T2221 was chosen here as the HBc or WHc NTD mAb.

Figure S2. Analysis of WHeAg N-glycosylation. (A) Serum samples from two chronically (F6005 and F6006, *left panel*) and two acutely (F7386 and M7392, *right panel*) WHV-infected woodchucks, with or without PNGase F treatment, at the indicated duration, were resolved by SDS-PAGE and followed by immunoblotting using the anti-precore mAb 7E9 and anti-NTD mAb T2221. M7386 (Pre, lanes 15 and 28) was the serum collected before infection, which served as a negative control. **(B)** Serum samples from different time points in the chronically (F1018) and acutely WHV-infected (M7392) woodchucks were treated with or without the PNGase F or PNGase A and resolved by SDS-PAGE, followed by immunoblotting using the anti-precore mAb 7E9 and anti-NTD mAb T2221. M7392 (Pre, lanes 16-18, 34-36) was the serum collected before infection, which served as a negative control. **(C)** Culture supernatants from WHV precore or core-transfected

255 WC3 cells were concentrated by ultrafiltration. Concentrated supernatants were treated
256 with PNGase F or not and resolved by regular SDS-PAGE, followed by immunoblotting
257 with mAb 1A11 and 19C18 sequentially on the same membrane. WHV core-transfected
258 cell culture supernatant served as the control for the background bands. *, cross-reactive
259 background bands.
260
261 **Figure S3. Heterogeneous patterns of WHcrAg after deglycosylation.** Serum
262 samples from chronically (F6005) and acutely WHV-infected (M7392) woodchucks were
263 treated with or without PNGase F and detected by immunoblotting using the indicated
264 mAbs following resolution by SDS-PAGE. A short exposure **(A)** and a long exposure **(B)**
265 are shown. M7392 (Pre, lanes 8-10) was the serum collected before infection, which
266 served as a negative control. **(C)** Fractions 16 (F16, low density fraction of g-WHe1 and
267 g-WPreC1) and 17 (F17, WHV virion fraction) from CsCl gradient fractionation of sera
268 from chronically WHV-infected woodchucks were resolved by SDS-PAGE, followed by
269 immunoblotting using mAbs (**Fig. 1A**) 1A11 (eAg- and PreC-specific), 19C18 (NTD-
270 specific), A701 (selective for non-phosphorylated CTD), and 14-2 (selective for
271 phosphorylated CTD). HBc expressed from *E. coli* served as the non-phosphorylated HBc
272 protein control, and was loaded after mixing into the HepG2 lysate, which helped the
273 purified HBc to migrate better on SDS-PAGE. HepG2 lysate alone was also loaded as a
274 negative control. The liver lysate from the WHV-negative woodchuck (-) and from WHV-
275 infected woodchuck (+) served as the negative and positive controls for WHc respectively.
276 *, background bands in the woodchuck serum or HepG2 cell lysate that were cross-

277 reactive with A701 or 14-2. The diagram depicts the various WHe, WHc, and WPreC
278 proteins and the locations of the mAb epitopes.
279
280 **Figure S4. Mass spectrometry analysis of WPreC1.** The g-WPreC1 protein band was
281 cut out following resolution by SDS-PAGE, digested with trypsin, and analyzed by LC-
282 MS/MS analysis. Peptide spectral match for MS/MS of [M+3H]3+ ion at m/z 847.47116
283 to WPreC1 peptide 128-150 (**Fig. 1A**), with a Mascot ion score of 53 and E value of
284 1.6e^6 . Indicated in the spectrum are the prominent b-ion (red) and y-ion (blue) fragment
285 assignments, and inset above is a fragmentation flag diagram displaying the positions of
286 the detected b-type (red) and y-type (blue) fragments.
287
288 **Figure S5. Analysis of serum WHeAg and WHV DNA kinetics in woodchucks.**
289 Changes in serum WHV DNA, WHeAg, WHsAg, and intrahepatic WHV cccDNA from an
290 acutely infected woodchuck (M7392) **(A)** and a chronic carrier treated with wIFN-α (F1018)
291 **(B)** relative to pre-infection or pre-treatment baseline. Serum WHV DNA and intrahepatic
292 cccDNA were measured by Southern blotting or real-time PCR, and WHeAg was
293 measured by SDS-PAGE followed by immunoblots of anti-NTD mAb T2221. The dashed
294 horizontal line indicates the lower limit of detection. Dashed vertical boxes indicate the
295 different phases of viral clearance: red, viremia drop alone; purple, drop of viremia and
296 antigenemia.
297
298 **Figure S6. Analysis of WHV virions and antigens by CsCl density gradient**
299 **fractionation.** Serum samples from one chronically (M6004) and one acutely (F7394)

WHV-infected woodchucks were fractionated by CsCl density gradient ultracentrifugation. Fractions were loaded 15 µl each and analyzed by SDS-PAGE **(A)**, or 25 µl each (Fractions 12-17) and 5 µl (Fractions 18-24) on NAGE **(B)**. WHeAg was detected by immunoblotting using the indicated mAbs, and viral DNA and envelope proteins were detected by Southern blotting using a ^{32}P-labeled WHV DNA probe followed by the anti-HBs antibody on the same membrane sequentially. Fraction 18 is the peak of WHV virions, Fraction 16 is the peak of WHsAg, and Fraction 21 is the peak of WHeAg. V, virions, containing WHV rcDNA; S, WHsAg.

Figure S7. Analysis of WHV virions and antigens by sucrose gradient fractionation. Serum from chronic WHV-infected woodchuck F6006 was fractionated by sucrose density gradient centrifugation. The fractions were resolved by SDS-PAGE followed by immunoblotting using the anti-precore mAb 1A11 (top) and anti-NTD mAb T2221 (middle), sequentially on the same membrane. The gel was stained by Coomassie blue after transfer (bottom).

Figure S8. HBcrAg in the serum of HBV-infected chimpanzees. (A) The concentration of HBc, HBeAg, PreC, and total HBcrAg in the chimpanzee sera. The different proteins were quantified from the immunoblot using mAb T2221, with recombinant HBc of known concentrations as standards (means ± SDs, n=14). **(B)-(E)** Correlations of HBeAg, HBc, and PreC with total HBcrAg, and correlation of HBeAg with PreC. The correlation coefficient was calculated by Spearman's correlation test. Two-tailed *p*-value was calculated for a 95% confidence interval.

Figure S9. Kinetics of viremia, antigenemia, and intrahepatic cccDNA in HBV-infected chimpanzees during acute infections. HBV DNA in the sera of infected chimpanzees was quantified by qPCR in a previous study (**Table S3**) (14), and intrahepatic cccDNA was quantified from chimpanzee liver biopsies collected from that study by qPCR as described in the Materials and Methods; HBeAg, HBc, and HBcrAg were quantified by immunoblotting in **Fig. 6A**. The relative changes in viremia, antigenemia, and intrahepatic cccDNA of 1603 **(A)**, 1616 **(B)**, 1618 **(C)**, A2A007 **(D)**, and AOA006 **(E)** during the time course of infection were normalized to the first time-point in each animal. cccDNA extracted from the AOA006 and A2A007 liver biopsies at the indicated time points was detected by Southern blotting **(F)**.

Supplementary Tables

Table S1. Mass spectrometry analysis of WPreC

The g-WPreC1 protein band was cut out following resolution by SDS-PAGE, digested with trypsin, and analyzed by LC-MS/MS analysis. The results were analyzed against WHV strain 7 precore protein sequence with consideration of probable post-translational modifications. Three precore/core peptides were detected.

Peptide	Sequence	Modification	Theo. MH+ [Da]
1	$_{128}$TPAPYRPPNAPILSTLPEHTVIR$_{150}$	Deamidated [N9]	2541.38238
2	$_{128}$TPAPYRPPNAPILSTLPEHTVIRR$_{151}$		2696.49948
3	$_{128}$TPAPYRPPNAPILSTLPEHTVIR$_{150}$		2540.39837

343 **Table S2. WHc concentration in the sera of WHV-infected woodchucks**

Woodchuck	Week	Viremia (GE/ml)	WHc predicted from complete virions (µg/ml)	WHc detected (µg/ml)	Ratio of WHc detected to predicted
7979	Wk-2	2.19E+11	1.92	2.41	1.25
	Wk0	2.96E+11	2.60	2.23	0.86
7994	Wk-2	1.08E+11	0.95	2.17	2.29
	Wk0	1.28E+11	1.12	2.18	1.94
1018	Wk-3	9.51E+10	0.83	0.56	0.67
6005		1.54E+11	1.35	1.60	1.18

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345

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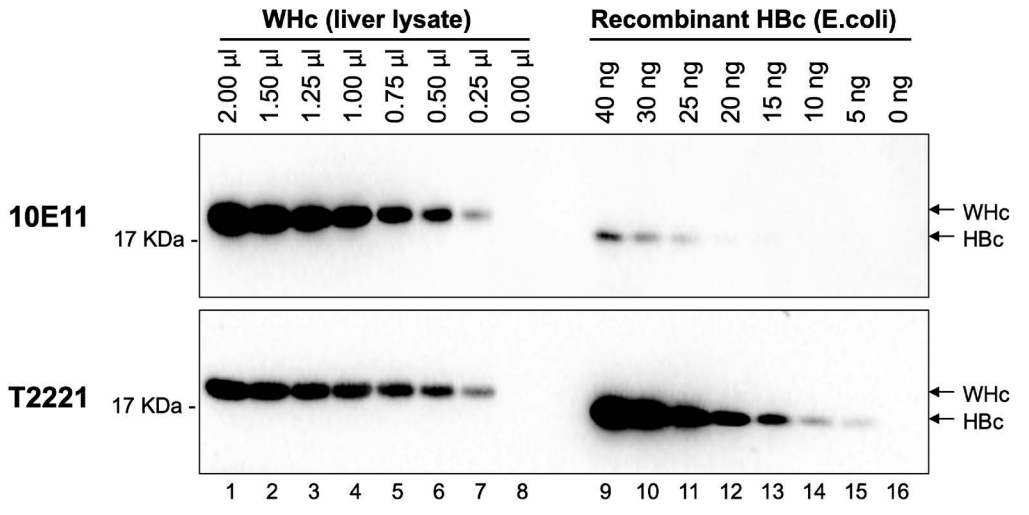
Table S3. HBV Viremia and Antigenemia in Infected Chimpanzees

Chimpanzee	week	Viremia* (GE/ml)	HBsAg* (µg/ml)	HBeAg* (µg/ml)
1603	8	4.28E+04	0.0012	#pos
	15	2.00E+10		
	16	1.90E+10		
	22	4.03E+09	204	0.58992
1616	10	6.73E+09	29.06	$nd
	20	1.20E+09		
	22	1.69E+09	192	~ 3
	23	3.30E+09		
	38	4.38E+08	13	~ 0.0002
1618	9	1.51E+07	0.09	#pos
	12	6.33E+09	24	> 0.3
	14	1.20E+10		
	17	1.40E+09		
	18	2.45E+08	124	< 0.5
A2A007	9	2.31E+08	0.21	0.919
	13	8.53E+09	125	2.86
	21	2.02E+09	223	1.08
AOA006	4	1.60E+09		
	5	1.81E+10	34	1.29741
	7	2.40E+10		
	9	1.05E+08	4.82	0.1171

* Viremia was quantified by qPCR, HBsAg and HBeAg by ELISA

pos, below the quantification limit (<0.0001 µg/ml)

$ nd, not done


WHc (liver lysate)
Recombinant HBc (E.coli)
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1.50 µl
1.25 µl
1.00 µl
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0.25 µl
0.00 µl
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30 ng
25 ng
20 ng
15 ng
10 ng
5 ng
0 ng
10E11
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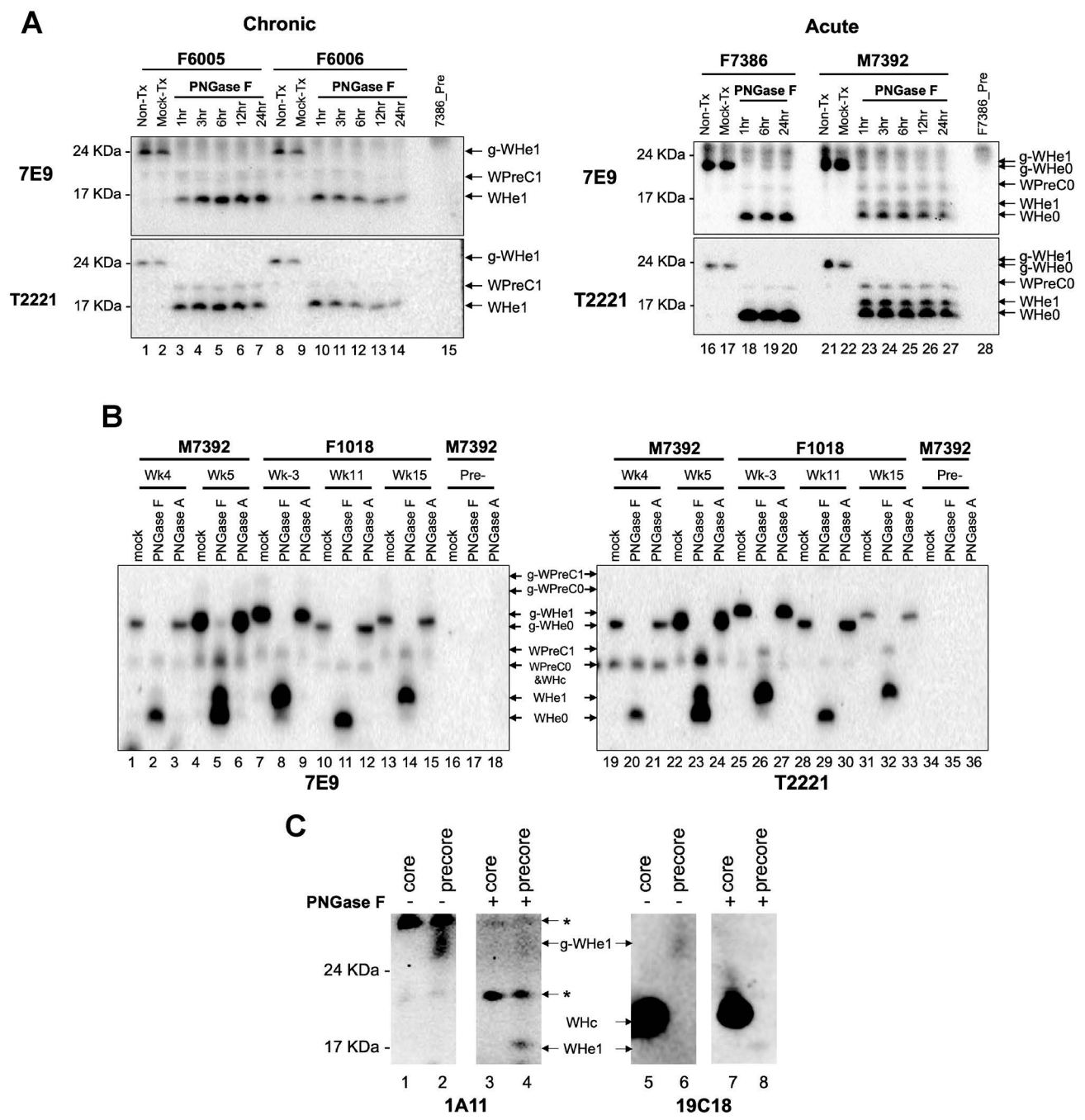
WHc
HBc
T2221
17 KDa -
WHc
HBc
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16


A
Chronic
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F6005
F6006
F7386
M7392
PNGase F
Non-Tx
Mock-Tx
1hr
3hr
6hr
12hr
24hr
7386_Pre
F7386_Pre
7E9
T2221
24 KDa
17 KDa
g-WHe1
g-WHe0
WPreC1
WPreC0
WHe1
WHe0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
16 17 18 19 20 21 22 23 24 25 26 27 28
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M7392
F1018
Wk4
Wk5
Wk-3
Wk11
Wk15
Pre-
mock
PNGase F
PNGase A
g-WPreC1
g-WPreC0
WPreC0 &WHc
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
C
PNGase F
- core
- precore
+ core
+ precore
24 KDa
17 KDa
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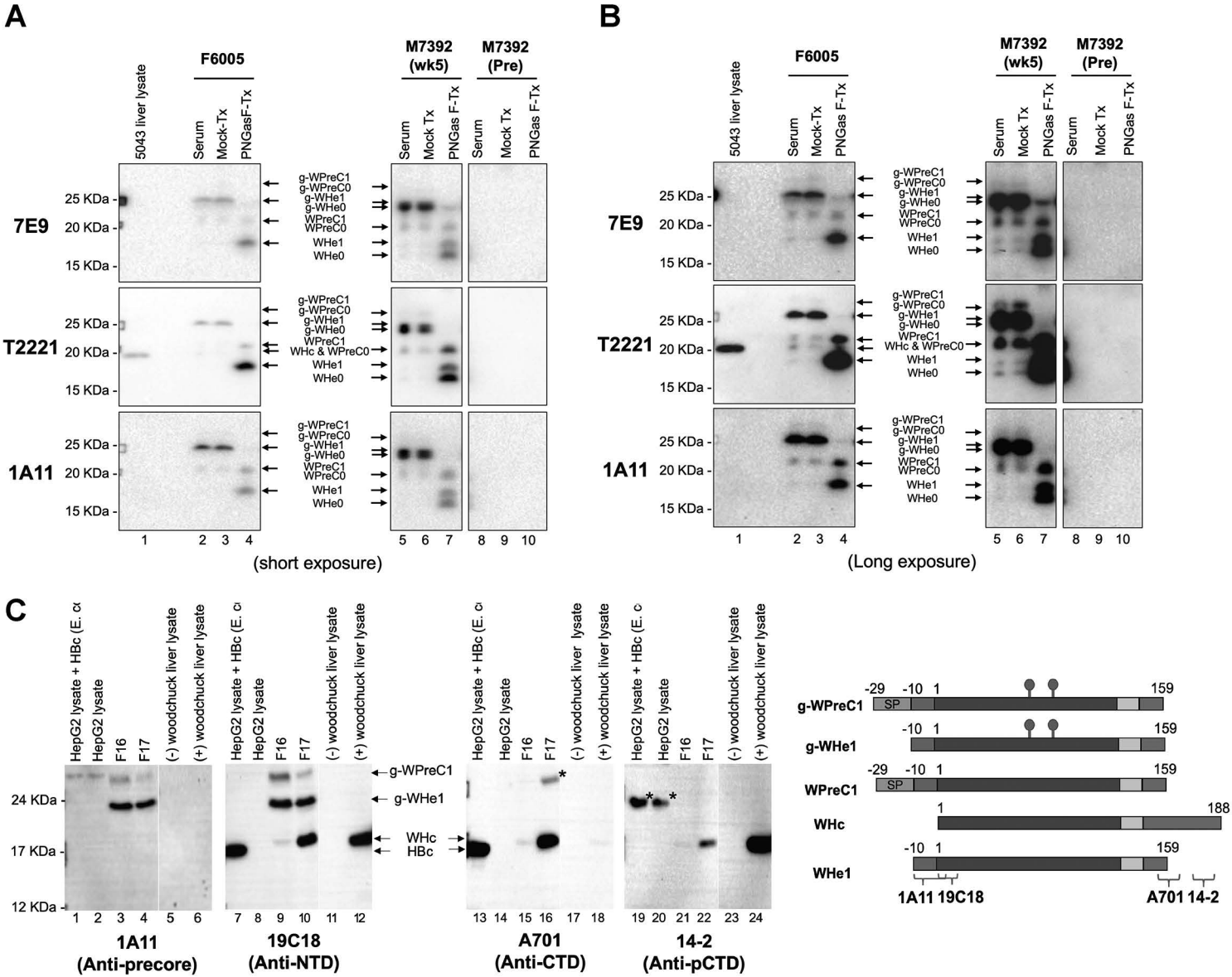
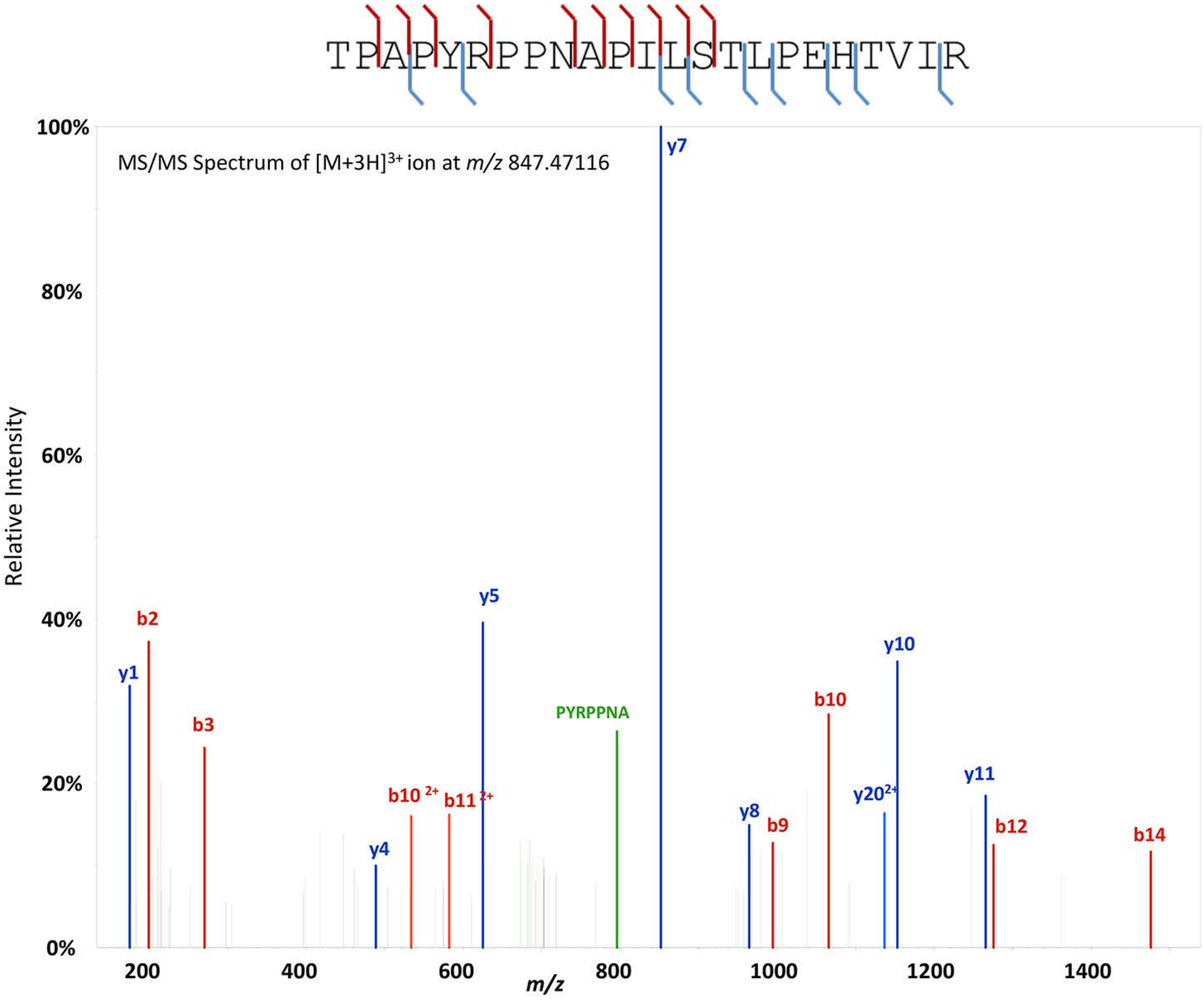
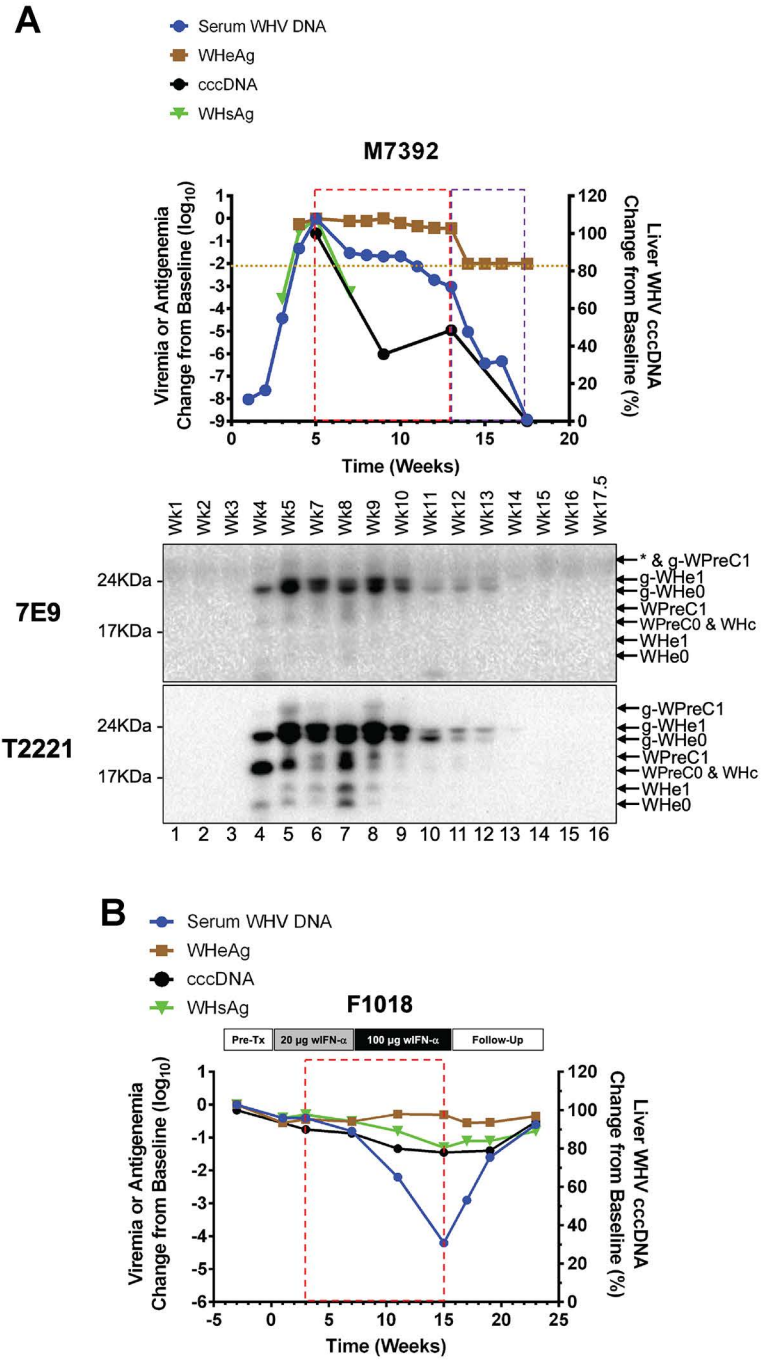
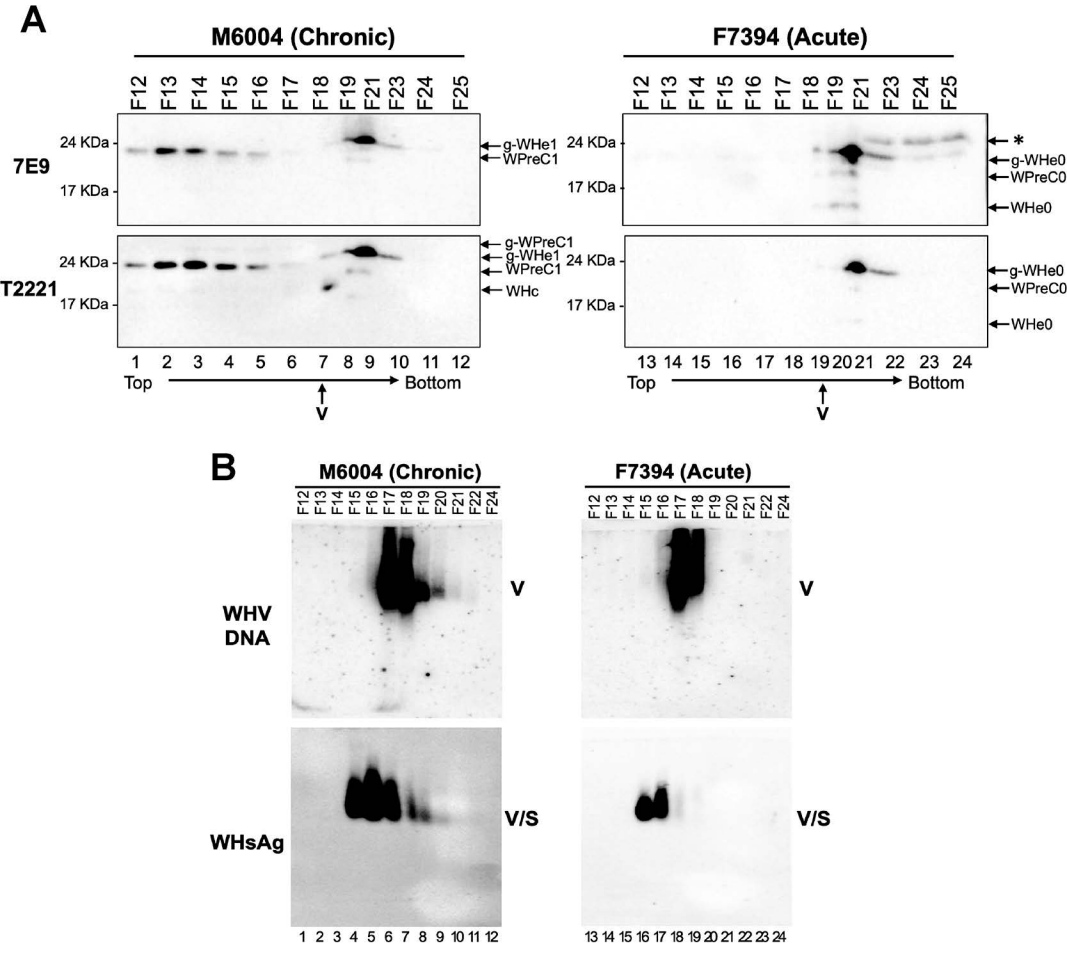
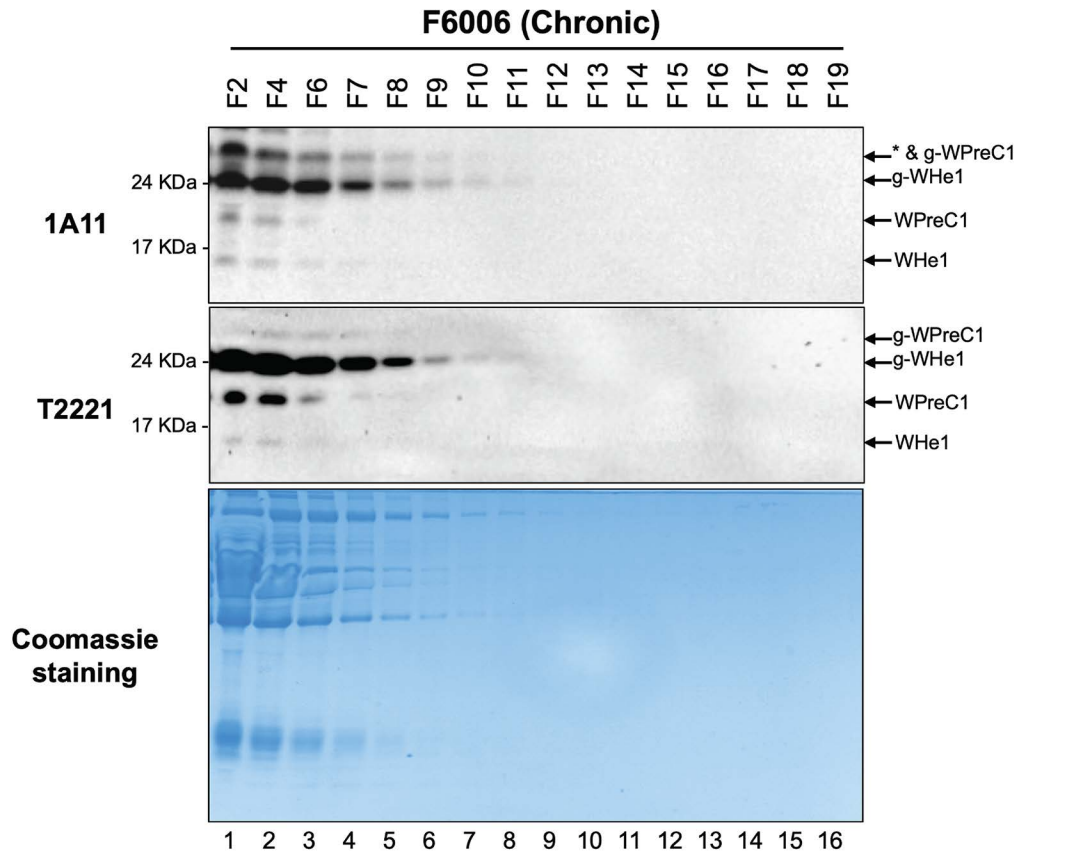
g-WHe1
WHc
WHe1
1 2 3 4 5 6 7 8
1A11
19C18

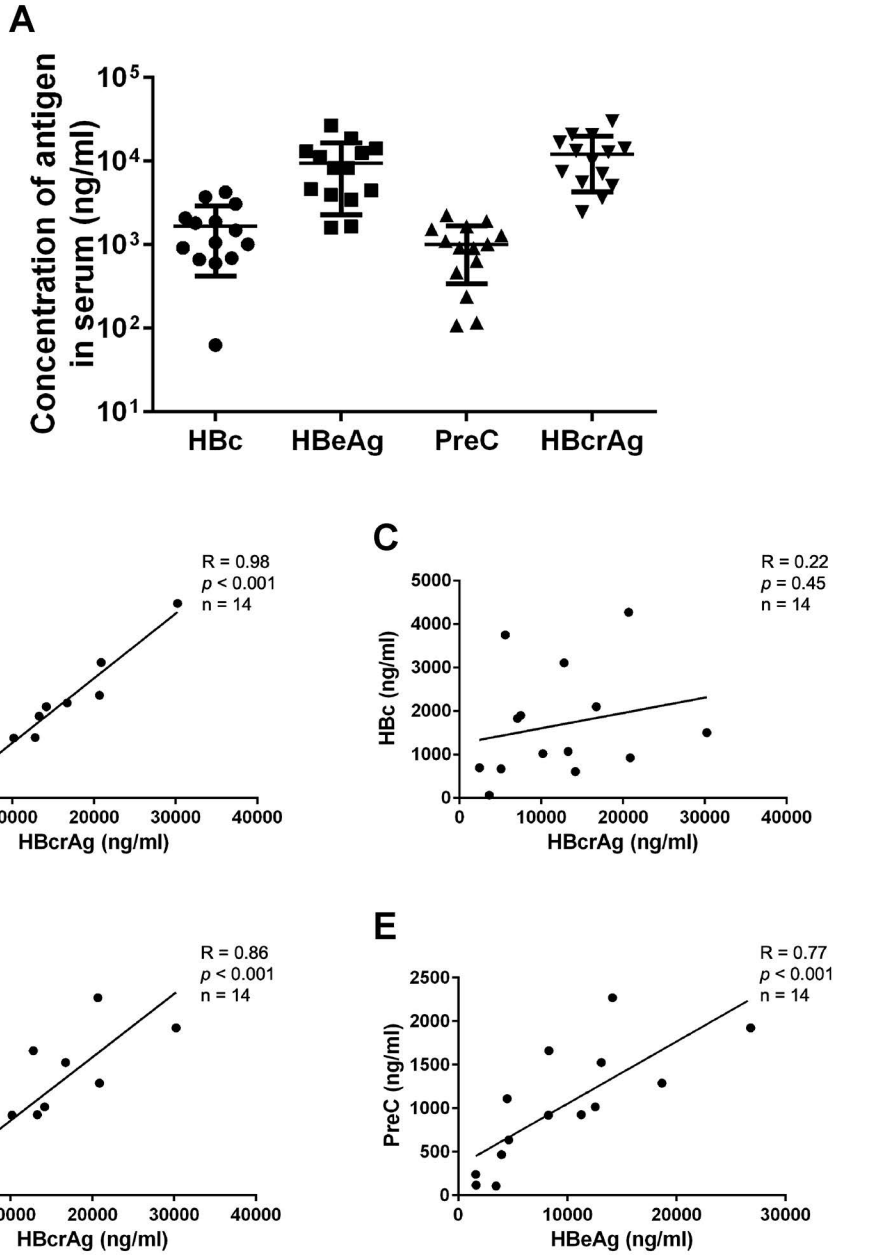

A
B
C
5043 liver lysate
F6005
Serum
Mock-Tx
PNGasF-Tx
M7392 (wk5)
M7392 (Pre)
Mock Tx
PNGas F-Tx
7E9
T2221
1A11
25 KDa
20 KDa
15 KDa
g-WPreC1
g-WPreC0
g-WHe1
g-WHe0
WPreC1
WPreC0
WHe1
WHe0
WHc & WPreC0
(short exposure)
(Long exposure)
HepG2 lysate + HBc (E. coli)
HepG2 lysate
F16
F17
(-) woodchuck liver lysate
(+) woodchuck liver lysate
24 KDa
17 KDa
12 KDa
WHc
HBc
1A11 (Anti-precore)
19C18 (Anti-NTD)
A701 (Anti-CTD)
14-2 (Anti-pCTD)
-29
-10
1
159
188
SP
WHc
WHe1
1A11 19C18
A701 14-2

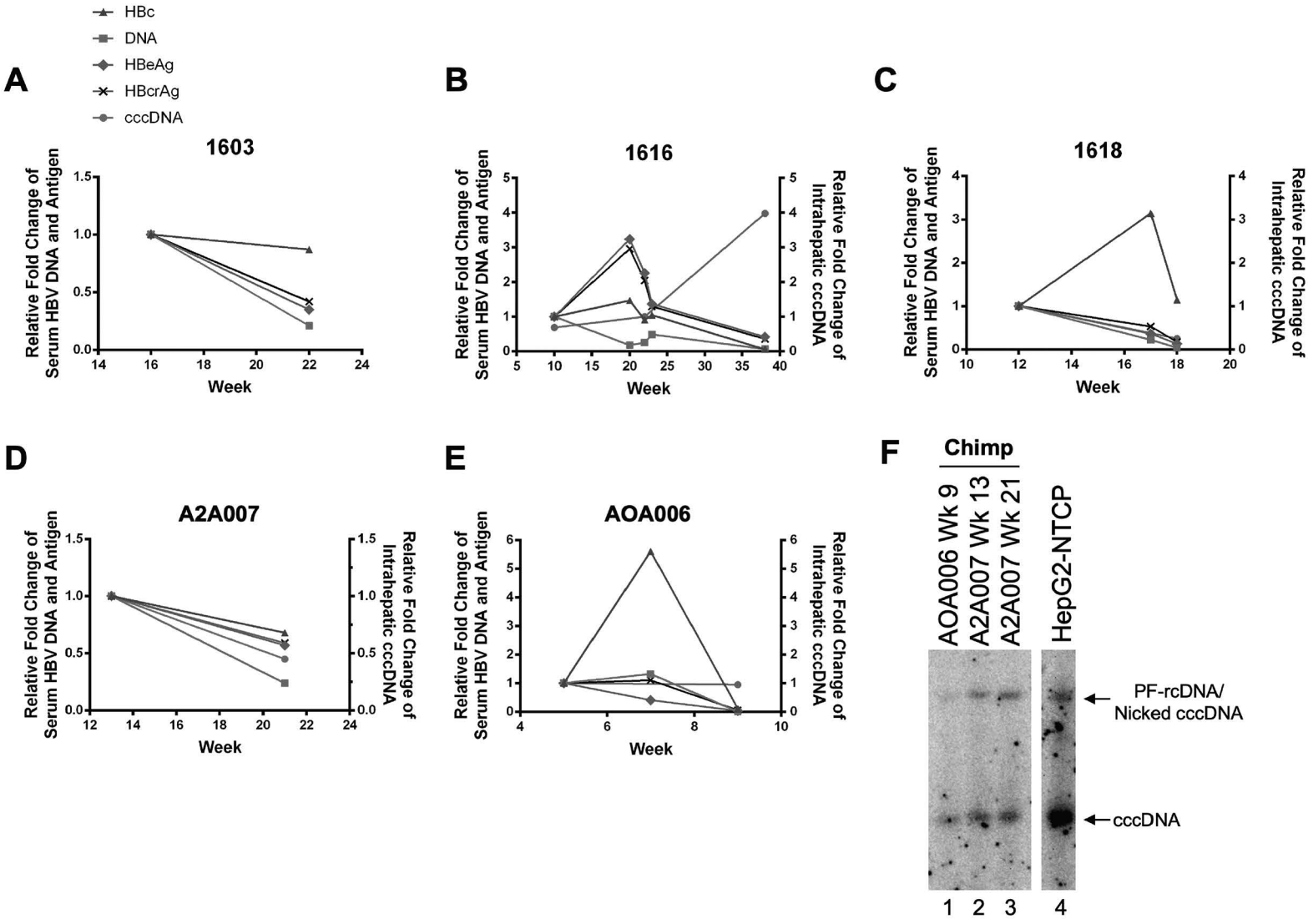

TPAPYRPPNAPILSTLPEHTVIR
MS/MS Spectrum of [M+3H]3+ ion at m/z 847.47116
Relative Intensity
100%
80%
60%
40%
20%
0%
200
400
600
800
1000
1200
1400
m/z
y7
y5
b2
y1
b3
PYRPPNA
y10
b10
b10 2+
b11 2+
y4
y8
b9
y20 2+
y11
b12
b14


A
Serum WHV DNA
WHeAg
cccDNA
WHsAg
M7392
Viremia or Antigenemia Change from Baseline (log10)
Liver WHV cccDNA Change from Baseline (%)
Time (Weeks)
Wk1 Wk2 Wk3 Wk4 Wk5 Wk7 Wk8 Wk9 Wk10 Wk11 Wk12 Wk13 Wk14 Wk15 Wk16 Wk17.5
7E9
24KDa
17KDa
* & g-WPreC1
g-WHe1
g-WHe0
WPreC1
WPreC0 & WHc
WHe1
WHe0
T2221
24KDa
17KDa
g-WPreC1
g-WHe1
g-WHe0
WPreC1
WPreC0 & WHc
WHe1
WHe0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
B
Serum WHV DNA
WHeAg
cccDNA
WHsAg
F1018
Pre-Tx
20 μg wIFN-α
100 μg wIFN-α
Follow-Up
Viremia or Antigenemia Change from Baseline (log10)
Liver WHV cccDNA Change from Baseline (%)
Time (Weeks)


A
M6004 (Chronic)
F7394 (Acute)
F12 F13 F14 F15 F16 F17 F18 F19 F21 F23 F24 F25
7E9
T2221
24 KDa
17 KDa
g-WHe1
WPreC1
g-WPreC1
WHc
*
g-WHe0
WPreC0
WHe0
1 2 3 4 5 6 7 8 9 10 11 12
13 14 15 16 17 18 19 20 21 22 23 24
Top
Bottom
V
B
F12 F13 F14 F15 F16 F17 F18 F19 F20 F21 F22 F24
WHV DNA
WHsAg
V
V/S
1 2 3 4 5 6 7 8 9 10 11 12
13 14 15 16 17 18 19 20 21 22 23 24


F6006 (Chronic)
F2
F4
F6
F7
F8
F9
F10
F11
F12
F13
F14
F15
F16
F17
F18
F19
1A11
24 KDa
17 KDa
* & g-WPreC1
g-WHe1
WPreC1
WHe1
T2221
24 KDa
17 KDa
g-WPreC1
g-WHe1
WPreC1
WHe1
Coomassie staining
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16


A
Concentration of antigen in serum (ng/ml)
10^5
10^4
10^3
10^2
10^1
HBc
HBeAg
PreC
HBcrAg
B
R = 0.98
p < 0.001
n = 14
HBeAg (ng/ml)
HBcrAg (ng/ml)
C
R = 0.22
p = 0.45
n = 14
HBc (ng/ml)
HBcrAg (ng/ml)
D
R = 0.86
p < 0.001
n = 14
PreC (ng/ml)
HBcrAg (ng/ml)
E
R = 0.77
p < 0.001
n = 14
PreC (ng/ml)
HBeAg (ng/ml)


HBc
DNA
HBeAg
HBcrAg
cccDNA
A
1603
Relative Fold Change of Serum HBV DNA and Antigen
Week
B
1616
Relative Fold Change of Serum HBV DNA and Antigen
Relative Fold Change of Intrahepatic cccDNA
Week
C
1618
Relative Fold Change of Serum HBV DNA and Antigen
Relative Fold Change of Intrahepatic cccDNA
Week
D
A2A007
Relative Fold Change of Serum HBV DNA and Antigen
Relative Fold Change of Intrahepatic cccDNA
Week
E
AOA006
Relative Fold Change of Serum HBV DNA and Antigen
Relative Fold Change of Intrahepatic cccDNA
Week
F
Chimp
AOA006 Wk 9
A2A007 Wk 13
A2A007 Wk 21
HepG2-NTCP
PF-rcDNA/ Nicked cccDNA
cccDNA
1 2 3 4