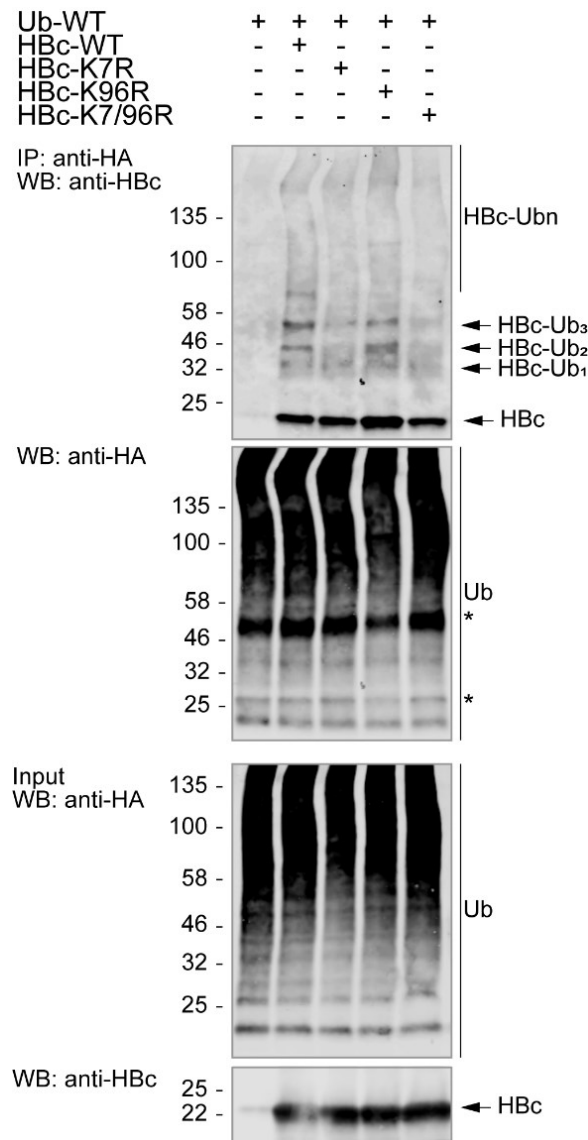
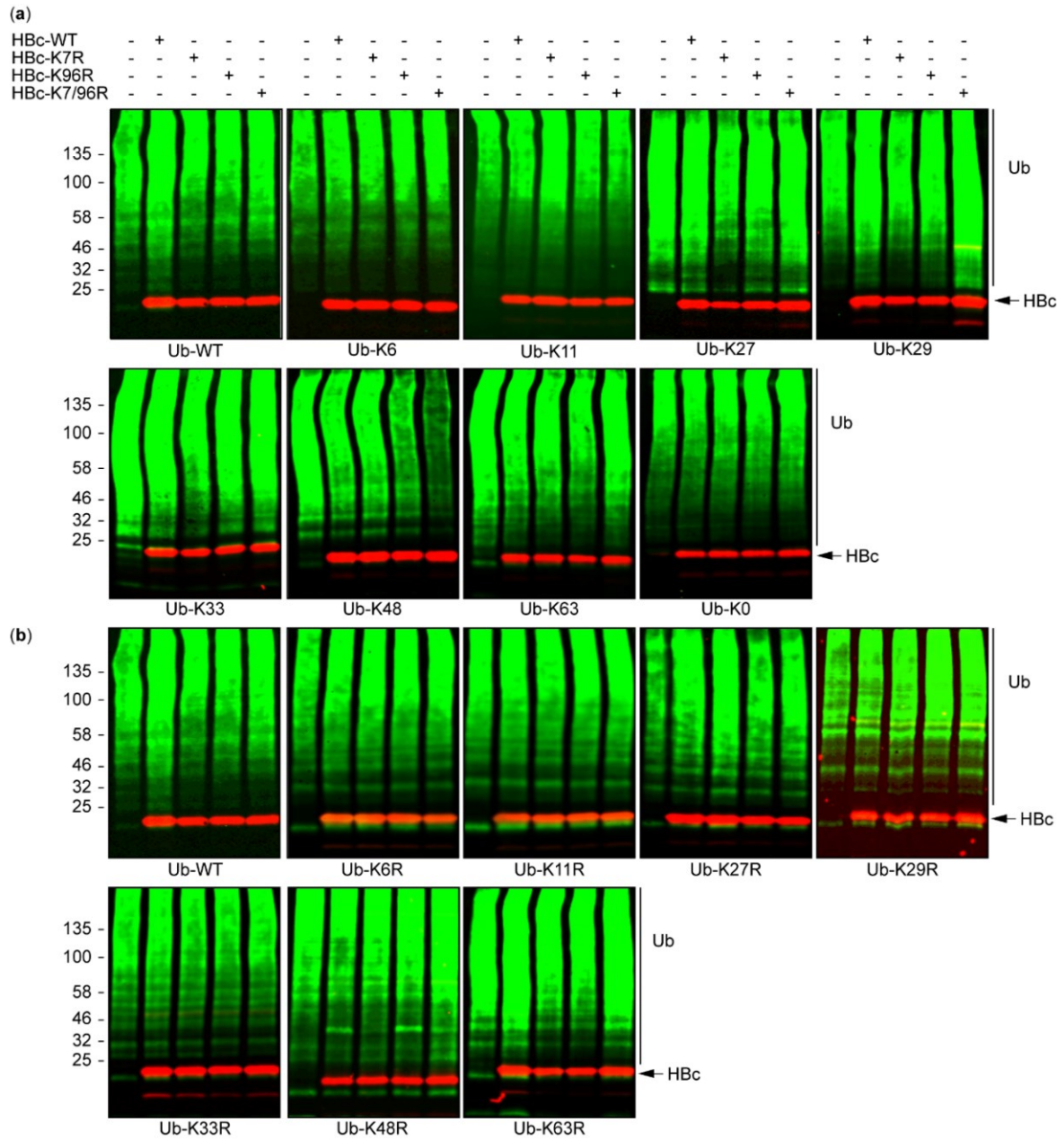


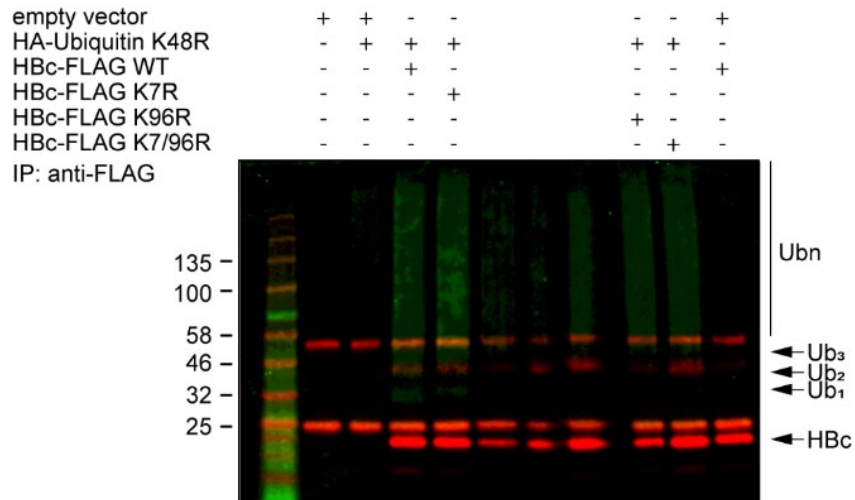
# Supplementary Materials



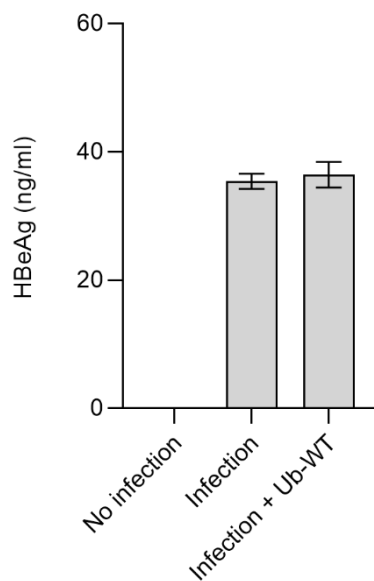
**Figure S1.** HBc without tag is ubiquitinated in the same manner as FLAG-tagged HBc. For transient co-transfection, HBc without tag and its mutants (HBc-K7R, HBc-K96R, HBc-K7/96R) and HA-Ub WT were used. Forty-eight hours post-transfection, ubiquitin was precipitated from precleared cell lysates using anti-HA magnetic beads. Immunoprecipitated complexes were analyzed by Western blot using an LI-COR Odyssey CLx system and Image Studio Lite Software.



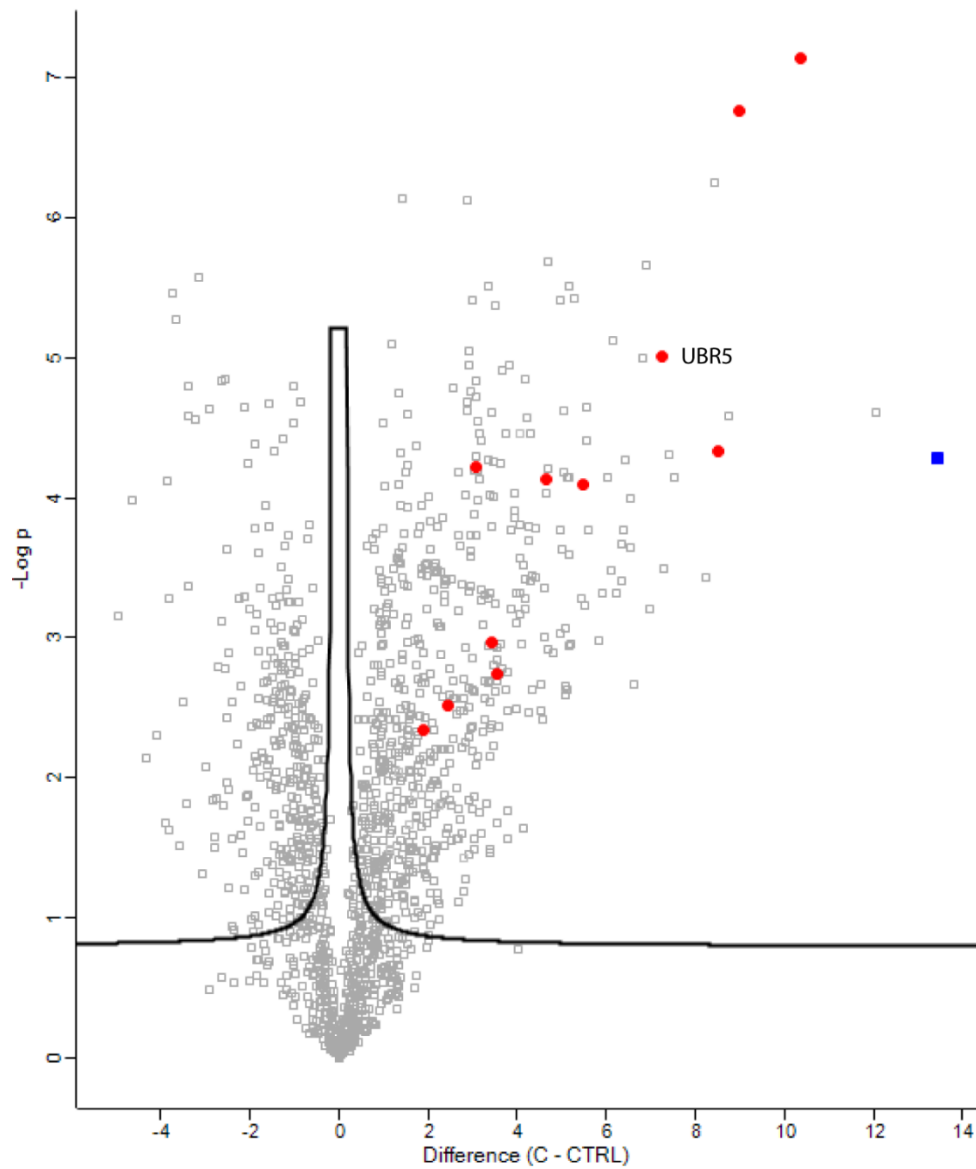
**Figure S2.** Expression of Hbc variants and different ubiquitin variants: (a) Ubiquitin single K mutants and lysine-free ubiquitin K0; and (b) ubiquitin K-to-R mutants (WB: Input 10  $\mu$ g of total protein/track). Huh7 cells were transiently co-transfected with one of ubiquitin variants and one of the Hbc variants. Protein expression was analyzed by Western blot. The rabbit antibody (IRDye 800CW) against HA-tag was used for ubiquitin detection and the mouse antibody (IRDye 680RD) against FLAG-tag was used for Hbc detection.



**Figure S3.** Ubiquitination of Hbc by the K48 ubiquitin variant. Original figure of Western blot. Immunoprecipitated complexes were analyzed by Western blot. The samples were loaded into lanes 6, 7, and 8 unevenly. The rabbit antibody (IRDye 800CW) against HA-tag was used for ubiquitin detection and the mouse antibody (IRDye 680RD) against FLAG-tag was used for Hbc detection.



**Figure S4.** Ubiquitin overexpression in HBV-infected cells does not influence an early marker HBeAg of viral replication. Forty-eight hours post-transfection of HBV-infected cells, the medium was harvested from three biological replicates and the quantity of secreted precore antigen (HBeAg) was determined using the ELISA technique. Data is shown as mean  $\pm$  standard deviation.



**Figure 5.** Volcano plot of the shotgun LC-MS/MS analysis of proteins co immunoprecipitated with HbC-HA expressed in HEPG2-hNTCP. Differential expression patterns between the HbC-HA sample and negative control sample (CTRL, empty vector) performed in biological triplicates. Red points in the plot represent the E3 ubiquitin-protein ligases with 1.5-fold upregulation with statistical significance  $p < 0.01$  (for a detailed list, see Table 2). Blue square represents HbC. E3 ubiquitin-protein ligase, UBR5, known to catalyze K29-linked ubiquitination is marked.

**Table S1.** E3 ubiquitin-protein ligases identified by shotgun LC-MS/MS analysis of proteins precipitated with empty vector, Ub-WT, Ub-K29, and d in Huh7 cells.

E3 Ubiquitin-Protein Ligase	Ubi-WT/K29/K29R/CTRL # Peptides	Ubi-WT/K29/K29R/CTRL # PSMs
E3 ubiquitin-protein ligase hectd1	32/32/11/0	48/52/17/0
E3 ubiquitin-protein ligase Itchy homolog	14/22/1/0	19/33/1/0
E3 ubiquitin-protein ligase UBR5	8/2/0/0	10/3/0/0
E3 ubiquitin-protein ligase HUWE1	5/2/1/0	6/3/4/0
E3 ubiquitin-protein ligase RBBP6	5/2/7/5	6/2/8/7
E3 ubiquitin-protein ligase NEDD4-like	4/2/0/0	5/2/0/0
E3 ubiquitin-protein ligase HECW2	3/2/0/0	4/2/0/0
E3 ubiquitin-protein ligase NEDD4	2/1/0/0	2/1/0/0
E3 ubiquitin-protein ligase RNF220	2/2/0/0	2/2/0/0
NEDD4-like E3 ubiquitin-protein ligase WWP1	1/2/0/0	2/4/0/0
E3 ubiquitin-protein ligase TRIP12	1/2/1/0	2/2/1/0
E3 ubiquitin-protein ligase ARIH1	1/6/0/0	1/7/0/0
E3 ubiquitin-protein ligase TRIM4	1/1/1/0	1/1/1/0
E3 ubiquitin-protein ligase RNF114	1/1/0/0	1/1/0/0
E3 ubiquitin-protein ligase RNF123	1/1/1/1	1/1/1/1
E3 ubiquitin-protein ligase RNF25	0/9/0/0	0/13/0/0
E3 ubiquitin-protein ligase RNF19A	0/8/0/0	0/12/0/0
E3 ubiquitin-protein ligase RNF5	0/1/0/0	0/2/0/0
E3 ubiquitin-protein ligase RAD18	0/2/0/0	0/2/0/0
E3 ubiquitin-protein ligase rififylin	0/1/0/0	0/1/0/0
E3 ubiquitin-protein ligase KCMF1	0/2/0/0	0/2/0/0
E3 ubiquitin-protein ligase RNF103	0/1/0/0	0/1/0/0
E3 ubiquitin-protein ligase RNF31	0/1/0/0	0/2/0/0
E3 ubiquitin-protein ligase mib1	0/1/0/0	0/1/0/0
NEDD4-like E3 ubiquitin-protein ligase WWP2	0/1/0/0	0/2/0/0
E3 ubiquitin-protein ligase UBR4	0/2/0/0	0/2/0/0

(Ubi-WT, pRK5-HA-Ubiquitin WT; Ubi-K29, pRK5-HA-Ubiquitin K29; Ubi-K29R, pRK5-HA-Ubiquitin K29R; CTRL, empty vector; # peptides, the number of detected peptide sequences unique to a protein; # PSM, the total number of identified peptide spectra matched for the protein).

**Table S2.** E3 ubiquitin-protein ligases identified by shotgun LC-MS/MS analysis of proteins co-immunoprecipitated with HBc-HA expressed in HEK293T cells.

E3 Ubiquitin-Protein Ligase	HBc # Peptides Exp1/Exp2	HBc # PSMs Exp1/Exp2	CTRL# Peptides Exp1/Exp2	CTRL # PSMs Exp1/Exp2
E3 ubiquitin-protein ligase MYCBP2	78/122	133/202	16/68	23/102
E3 ubiquitin-protein ligase HERC2	14/25	22/37	0/6	0/7
E3 ubiquitin-protein ligase TRIM21	19/21	35/49	24/29	60/81
E3 ubiquitin-protein ligase Midline-1	11/13	19/32	6/11	10/19
Probable E3 ubiquitin-protein ligase HECTD4	6/12	7/19	0/1	0/1
E3 ubiquitin-protein ligase RNF213	16/10	21/14	0/4	0/5
Probable E3 ubiquitin-protein ligase makorin-2	2/9	3/14	2/7	3/11
E3 ubiquitin-protein ligase listerin	13/9	18/14	2/5	3/9
E3 ubiquitin-protein ligase UBR5	6/8	10/9	3/3	3/4
E3 ubiquitin-protein ligase TRIM4	7/8	7/13	6/9	7/13
E3 ubiquitin-protein ligase BRE1B	7/5	11/9	3/3	3/4
E3 ubiquitin-protein ligase HECTD1	3/5	6/8	1/4	1/5
E3 ubiquitin-protein ligase RFWD3	2/5	2/6	0/4	0/6
E3 ubiquitin-protein ligase CBL OS	4/4	7/7	3/4	5/9
E3 ubiquitin-protein ligase RNF138	4/4	6/5	2/5	3/8
E3 ubiquitin-protein ligase RNF114	2/4	2/7	2/9	3/14

E3 ubiquitin-protein ligase RNF187	1/4	2/6	1/4	2/6
E3 ubiquitin-protein ligase LRSAM1	2/4	2/7	0/4	0/6
E3 ubiquitin-protein ligase TRIM33	1/3	1/4	1/2	1/3
E3 ubiquitin-protein ligase TRIM32	1/3	2/6	0/1	0/2
E3 ubiquitin-protein ligase makorin-1	0/3	0/3	0/0	0/0
E3 ubiquitin-protein ligase TRIM9	7/3	8/4	5/1	6/1
E3 ubiquitin-protein ligase TRIM71	2/3	3/4	0/1	0/2
E3 ubiquitin-protein ligase TRIP12	14/3	15/5	2/0	3/0
E3 ubiquitin-protein ligase MIB1	1/3	2/4	0/2	0/3
E3 ubiquitin-protein ligase CBL-B	2/2	4/4	0/2	0/2
E3 ubiquitin-protein ligase TRIM56	0/2	0/2	0/0	0/0
E3 ubiquitin-protein ligase BRE1A	4/2	7/3	2/0	3/0
E3 ubiquitin-protein ligase RNF31	0/2	0/2	0/0	0/0
RNA-binding E3 ubiquitin-protein ligase MEX3C	0/2	0/3	0/1	0/2
E3 ubiquitin-protein ligase UHRF1	0/2	0/3	0/0	0/0
Probable E3 ubiquitin-protein ligase MID2	1/2	1/4	0/2	0/3
E3 ubiquitin-protein ligase Praja-2	1/2	1/3	1/1	1/2
E3 ubiquitin-protein ligase SHPRH	1/2	2/3	0/0	0/0
E3 ubiquitin-protein ligase Praja-1	1/1	1/1	0/0	0/0
E3 ubiquitin-protein ligase RNF126	0/1	0/1	0/2	0/3
E3 ubiquitin-protein ligase TRIM23	0/1	0/1	0/0	0/0
E3 ubiquitin-protein ligase RLIM	1/1	1/1	0/0	0/0
E3 ubiquitin-protein ligase RING2	1/1	2/2	0/0	0/0
E3 ubiquitin-protein ligase ARIH1	0/1	0/2	0/4	0/5
NEDD4-like E3 ubiquitin-protein ligase WWP1	1/1	1/1	1/1	2/1
E3 ubiquitin-protein ligase NRDP1	0/1	0/2	0/2	0/2
E3 ubiquitin-protein ligase RBX1	0/1	0/2	0/1	0/1
<b>E3 Ubiquitin-Protein Ligase</b>	<b>HBc # Peptides Exp1/Exp2</b>	<b>HBc # PSMs Exp1/E xp2</b>	<b>CTRL# Peptides Exp1/Exp2</b>	<b>CTRL # PSMs Exp1/Exp2</b>
E3 ubiquitin-protein ligase XIAP	0/1	0/1	0/0	0/0
E3 ubiquitin-protein ligase TTC3	1/1	2/1	0/0	0/0
E3 ubiquitin-protein ligase RNF31	0/1	0/1	0/0	0/0
E3 ubiquitin-protein ligase RNF220	0/1	0/1	0/1	0/1
E3 ubiquitin-protein ligase HUWE1	2/1	2/1	0/1	0/1
Ubiquitin-protein ligase E3C	0/1	0/4	0/0	0/0
E3 ubiquitin-protein ligase RBBP6	2/0	2/0	0/0	0/0
NEDD4-like E3 ubiquitin-protein ligase WWP2	2/0	2/0	1/0	2/0
E3 ubiquitin-protein ligase Itchy homolog	2/0	3/0	1/0	2/0
E3 ubiquitin-protein ligase LNX	1/0	1/0	0/0	0/0
E3 ubiquitin-protein ligase pellino homolog 3	1/0	1/0	0/0	0/0
E3 ubiquitin-protein ligase ZFP91	1/0	1/0	0/0	0/0
E3 ubiquitin-protein ligase RING1	1/0	2/0	0/0	0/0

(HBc, HBc-HA sample; CTRL, negative control (no HBc) sample; # peptides, the number of detected peptide sequences unique to a protein; # PSM, the total number of identified peptide spectra matched for the protein).