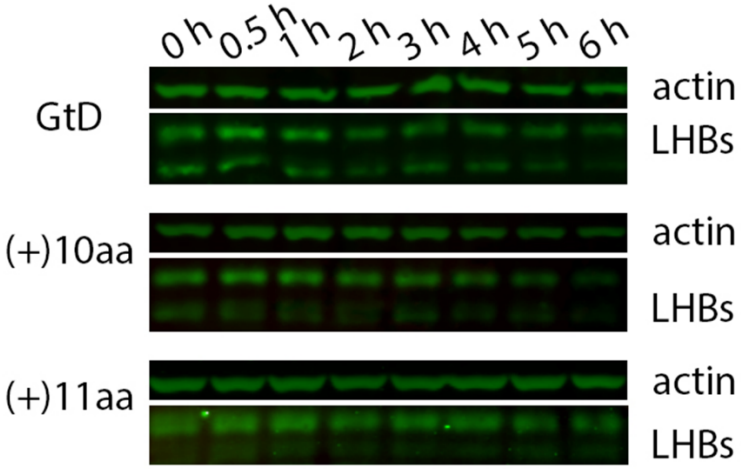
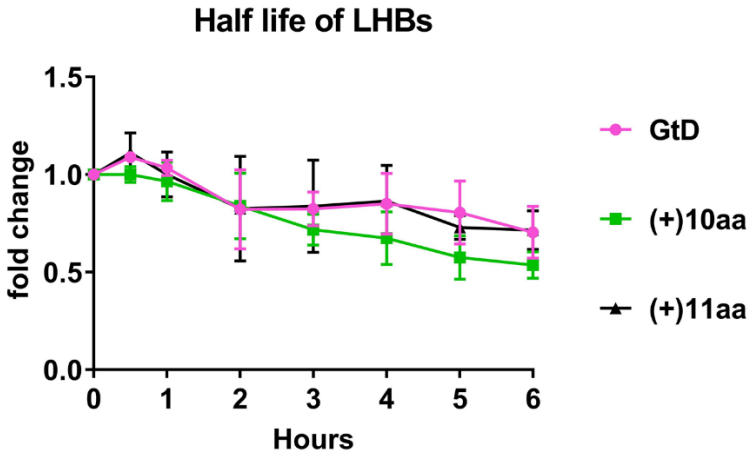
Table S1: Primers used for mutagenesis PCR.

Name	Primer Sequences	Remarks
Δ11aa_fw	CCATATTCTTGGGAACAAGAGCTACAGCTAGGGAGG TTGG	The first ATG of PreS1 was mutated to TAG.
Δ11aa_rev	GCGAGGTTTTGATGACCAACCTCCCTAGCTGTAGC	
(+)11aa(GtA) _fw	TCACCATATTCTTGGGAACAAGATCTACAGCATGGGA GGTTGGTCATCAAAACCTCGC	
(+)11aa(GtA) _rev	GCTGGTGGAAAGATTCTGCCCCATGCCTTTGCGAGGT TTTGATGACCAAC	
(+)10aa(GtE)_ fw	ACCATATTCTTGGGAACAAGATCTACAGCATGGGGCT TTCTTGGACGGTCCCTCTCG	
(+)10aa(GtE)_ rev	TTGCTGGTGGAAAGATTCTGCCCCCATTCGAGAGGGA CCGTCCAAG	
Δaa2-7_fw	GGTCACCATATTCTTGGGAACAAGAGCTACAGCATGC CTCGCAAAG	
Δaa2-7_rev	GAACAGAAAGATTCGTCCCCATGCCTTTGCGAGGCAT GCTGTA	
Δaa3-8_fw	GGTCACCATATTCTTGGGAACAAGAGCTACAGCATG GGACGCAAAG	
Δaa3-8_rev	GAACAGAAAGATTCGTCCCCATGCCTTTGCGTCCCAT GCTGTA	
Δaa4-9_fw	GGTCACCATATTCTTGGGAACAAGAGCTACAGCATG GGAGGAAAAGG	
Δaa4-9_rev	GAACAGAAAGATTCGTCCCCATGCCTTTTCCTCCCAT GCTGTAG	
Δaa5-10_fw	GGTCACCATATTCTTGGGAACAAGAGCTACAGCATG GGAGGTTGGG	
Δaa5-10_rev	GAACAGAAAGATTCGTCCCCATGCCCCAACCTCCCAT GCTGTA	
Δaa6-11_fw	GGTCACCATATTCTTGGGAACAAGAGCTACAGCATG GGAGGTTGGT	
Δaa6-11_rev	GGGAACAGAAAGATTCGTCCCCATTGACCAACCTCC CATGCTGTA	
GtA_aa3-8(GtE)_fw	GGGAACAAGAGCTACAGCATGGGACTTTCTTGGACG GTCCCTC	
GtA_aa3-8(GtE)_rev	GATTCGTCCCCATGCCTTTGCGAGGGACCGTCCAAGA AAG	
GtA_aa7-12(GtE)_fw	ACAGCATGGGAGGTTGGTCATCAACGGTCCCTCTCGA GTGGG	
GtA_aa7-12(GtE)_rev	GGTTGGGAACAGAAAGATTCGTCCCCCACTCGAGAG GGACCGT	
Δaa2-5_fw	TTTGCGGGTCACCATATTCTTGGGAACAAGAGCTACA GCATGTCAAAACCTCG	
Δaa2-5_rev	TGGGAACAGAAAGATTCGTCCCCATGCCTTTGCGAGG TTTTGACATGCTGTAG	
Δaa6-8_fw	GTCACCATATTCTTGGGAACAAGAGCTACAGCATGG GAGGTTGGTCACGCAAAGGC	
Δaa6-8_rev	CCCAGAGGGTTGGGAACAGAAAGATTCGTCCCCATG CCTTTGCGTGACCAAC	
Δaa9-11_fw	GGGAACAAGAGCTACAGCATGGGAGGTTGGTCATCA AAACCTATGGGGACGAA	
Δaa9-11_rev	GAAAGAATCCCAGAGGGTTGGGAACAGAAAGATTCG TCCCCATAGGTTTTGAT	

K7M_fw	CGGGTCACCATATTCTTGGGAACAAGAGCTACAGCA CGGGAGGTTGGTCATCAAT	The first ATG of PreS1 was mutated to ACG.The lysine at position 7 was mutated to methionine.
K7M_rev	ACAGAAAGATTCGTCCCCATGCCTTTGCGAGGCATTG ATGACCAACCTCCCG	
GtA_aa2-6(GtE)_fw	GGGAACAAGAGCTACAGCATGGGACTTTCTTGGAAA CCTC	
GtA_aa2-6(GtE)_rev	TCCCCATGCCTTTGCGAGGTTTCCAAGAAAGTCCCAT	
GtA_aa2-12(GtE)_fw	GGGAACAAGAGCTACAGCATGGGGCTTTCTTGGACG GTCCCTCTCG	
GtA_aa2-12(GtE)_rev	GTTGGGAACAGAAAGATTCGTCCCCCATTCGAGAGG GACCGTCCAAG	
W5M_fw	ACCTTATTCTTGGGAACAAGAGCTACATCACGGGGCT TTCTATGAC	The first ATG of PreS1 was mutated to ACG.The tryptophan at position 5 was mutated to methionine.
W5M_rev	TCCCCCATTCGAGAGGGACCGTCATAGAAAGCCCCG TG	
P8M_fw	CACCTTATTCTTGGGAACAAGAGCTACATCACGGGGC TTTCTTGGACGGTCA	The first ATG of PreS1 was mutated to ACG.The proline at position 8 was mutated to methionine.
P8M_rev	GGTGGAATGATTCTTCCCCCATTCGAGCATGACCGTC CAAGAAAGCC	
W11M_fw	CACCTTATTCTTGGGAACAAGAGCTACATCACGGGGC TTTCTTGGACGGTCCCTCT	The first ATG of PreS1 was mutated to ACG.The trypophan at position 11 was mutated to methionine.
W11M_rev	AGGATTGGTGGTGGAATGATTCTTCCCCATTTCGAGA GGGACCGTCCAAGAA	





	GtD	(+)10aa	(+)11aa
Half Life	11.1	6.178	10.39

Figure S1. 11 aa-associated higher amount of LHBs does not correlate with longer protein half life. Huh7.5 cells transfected with genomes GtD and the derivatives were treated with 100 μg/ml cycloheximide (CHX) up to 6 hours. The lysates were analyzed by Western blot using a PreS1-specific serum (MA18/7). β-actin was used as loading control. The signals of LHBs and β-actin in the Western blot were quantified by Image studio lite from LI-COR Biosciences. The signal from untreated sample was standardized as 1. These quantitative data are mean values from three independent experiments.

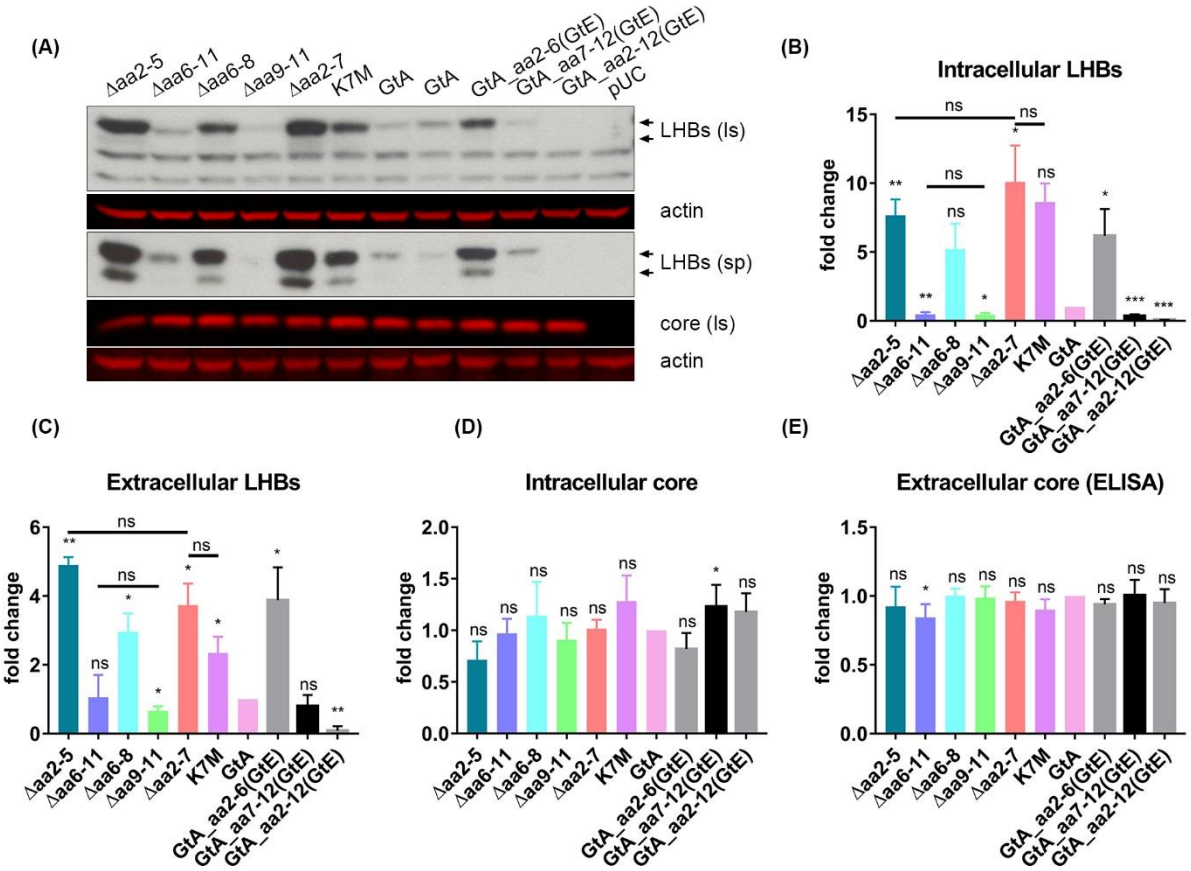


Figure S2. Deletion of only 3 aa in the GtA-derived 11 aa alters dramatically the amount of LHBs. (A) Supernatants and lysates from Huh7.5 cells transfected with genomes GtA and the derivatives were analyzed by Western blot using the PreS1-specific serum (MA18/7) and the core-specific serum (K46). β-actin was used as loading control. (B-D) The signals of LHBs and core in the Western blot were quantified by Image studio lite from LI-COR Biosciences. (E) Supernatants from Huh7.5 cells transfected with genomes GtA and the derivatives were analyzed by core ELISA. The signal from GtA was standardized as 1. Ls, lysate; sp, supernatant. These quantitative data are mean values from three independent experiments**.**

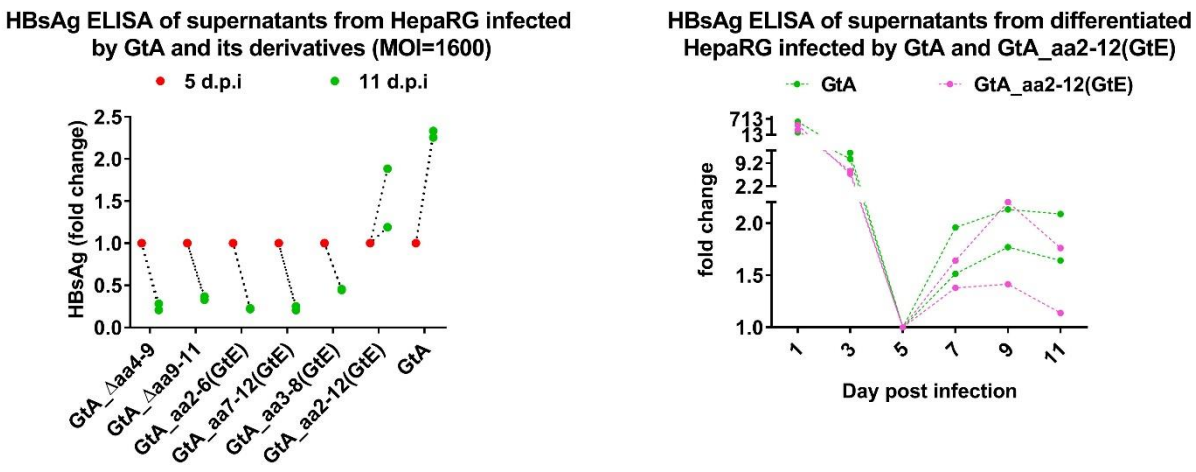


Figure S3. Substitution the N-terminal 11 aa in PreS1 domain of GtA with N-terminal 10 aa in the PreS1 domain of GtE does not impair the viral infectivity. Differentiated HepaRG were infected with HBV from GtA and its derivatives at MOI 1600. Culture supernatants collected at every two days post infection and samples from 5 d.p.i and 11 d.p.i (days post infection) were analyzed by HBsAg ELISA (left panel). To further confirm the successful infection established by virus from GtA_aa2-12(GtE), culture supernatants collected at indicated time points were analyzed by HBsAg ELISA. These data are from two independent experiments.