Supplementary Materials

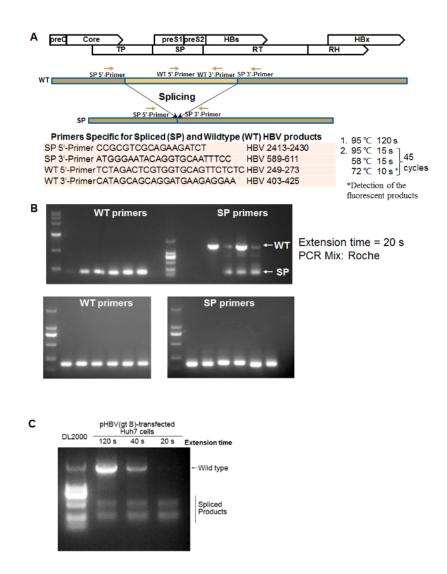
Hepatitis B virus spliced variants are associated with an impaired response to interferon therapy

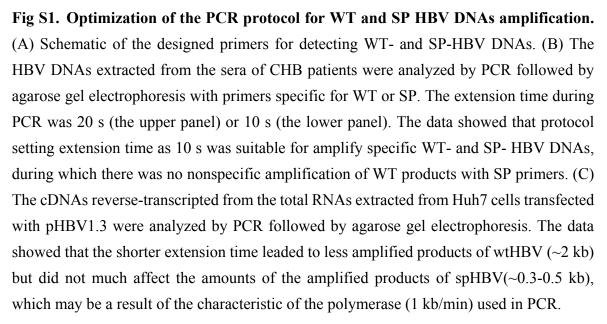
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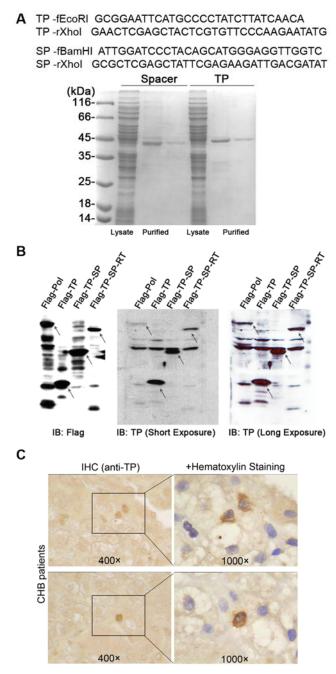


Fig S2. Preparation of the antibodies against TP and Spacer regions of viral polymerase. (A) Detection of the purified GST-Spacer and GST-TP proteins expressed in the E. coli BL21 by SDS-PAGE followed by coomassie staining. (B) Huh7 cells transfected with the indicated plasmids were extracted to detect the expression of full length- and truncated-Pol by western blotting with anti-Flag Abs or anti-TP Abs. (C) Detection of HBV proteins expression in liver biopsies from CHB patients by immunohistochemistry with anti-TP antibodies.

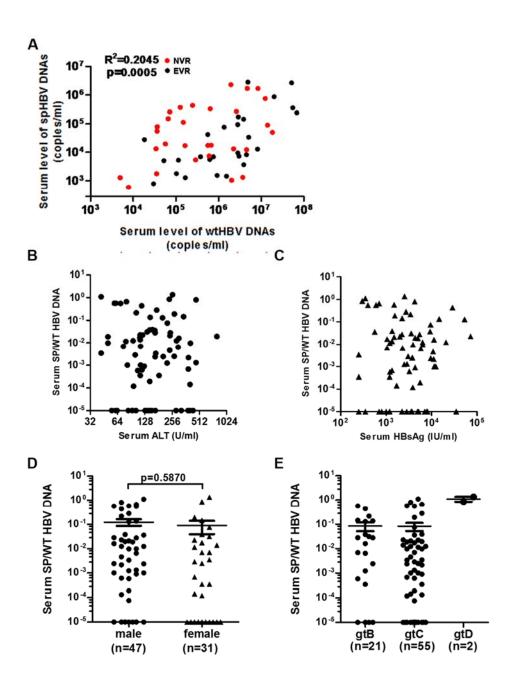
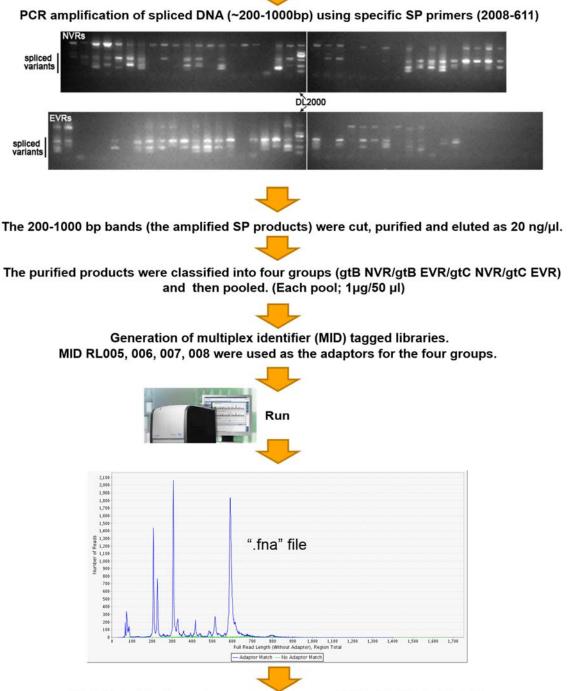


Fig S3. The correlation between spHBV variants and HBsAg, ALTs, genders or viral genotypes. (A) The level of spHBV and wtHBV DNAs in sera from the NVRs and EVRs before IFN therapy were quantified by qPCR. The relationship between spHBV DNAs and wtHBV DNAs in samples with spHBV>100 copies/ml was analyzed using Spearman's correlation. The correlation between the proportions of spHBV and the level of ALT (B) or HBsAg (C) in the sera from the 82 patients before IFN therapy. The ratio of spHBV to wtHBV DNAs in the serum samples was compared between males and females (D) (Student's t-tests) or among different genotypes (E).

Collection of serum samples from CHB patients (n=78)



BLAST with the reference sequence (5' HBV 2008- 611 3')

Fig S4. Work Flow of the 454 sequencing analysis of the spHBV variants in the sera of CHB patients.

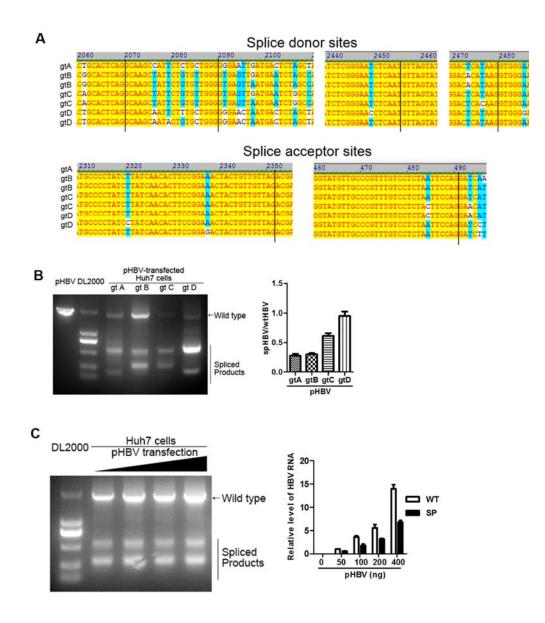


Fig S5. HBV splicing is associated with the viral genotypes. (A) The sequence alignment of the donor and acceptor sites among different HBV genotypes. (B) The cDNAs reverse-transcripted from the total RNAs extracted from Huh7 cells transfected with HBV constructs with different genotypes were analyzed by PCR followed by agarose gel electrophoresis (the left panel) and realtime PCR (the right panel). (C) The cDNAs reverse-transcripted from the total RNAs extracted from Huh7 cells transfected with different amounts of pHBV1.3 were analyzed by PCR followed by agarose gel electrophoresis (the left panel) and realtime PCR followed by agarose gel electrophoresis (the left panel) and realtime PCR followed by agarose gel electrophoresis (the left panel) and realtime PCR (the right panel). The data showed that the intracellular level of both the wt- and sp-HBV RNAs closely associated with the amounts of transfected plasmids, but there was no significant difference on the ratio of spHBV to wtHBV RNAs among cells transfected with different amounts of pHBV1.3.

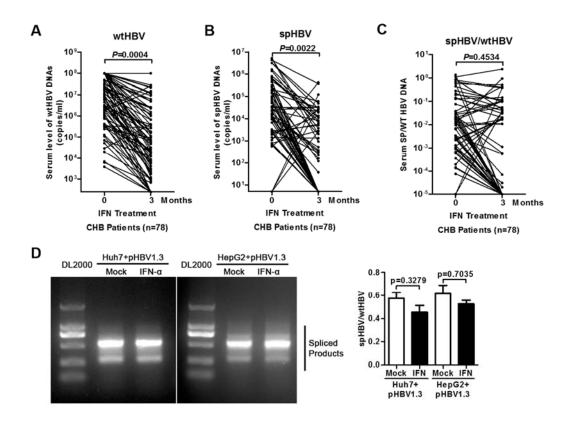


Fig S6. Effect of IFN treatment on the expression of the spHBV variants. Comparison of the absolute amount of wtHBV(A), spHBV(B), and the ratios of spHBV/wtHBV(C) DNA between paired samples from CHB patients before and after 3 months of IFN therapy. The cDNAs reverse-transcripted from the total RNAs extracted from mock- or IFN-α treated Huh7 or HepG2 cells transfected with pHBV1.3 were analyzed by PCR followed by agarose gel electrophoresis (the upper panel) and realtime PCR (the lower panel). The data, which represents results of three independent experiments performed in triplicates, was analyzed using Student's t-tests (D). To determine whether IFN treatment could affect HBV splicing, we compared the change of the amounts of spHBV and wtHBV in CHB patients following IFN therapy. Twelve weeks of IFN therapy resulted in varying degrees of reduction of both spHBV DNA level and wtHBV DNA level, and decreased the total detection rate of the serum spHBV, particularly in EVRs. Although the absolute amounts of spHBV and wtHBV decreased, the spHBV/wtHBV ratio was not much affected by IFN treatments, in both the NVRs and EVRs group. Similar results were obtained in replication-competent HBV constructs-transfected Huh7 and HepG2 cells, suggesting that IFN could globally reduce the viral DNA, including the spHBV DNA and wtHBV DNA, but did not much affect the proportions of spHBV variants among the total detected HBV genomes.

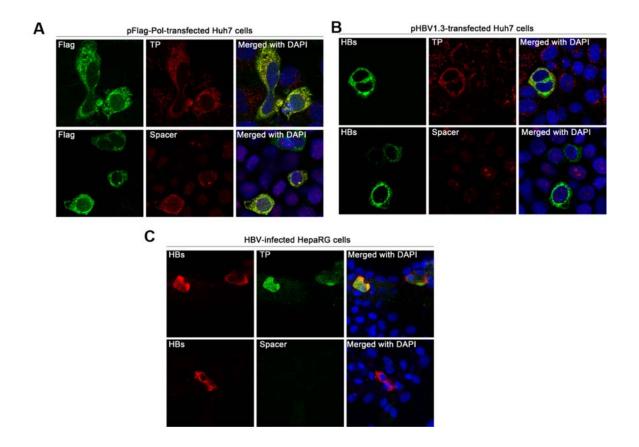


Fig S7. Detection of viral proteins expression using immunofluorescence with anti-TP and anti-SP antibodies. (A, B) Huh7 cells transfected with pFlag-Pol or pHBV1.3 were fixed, immunofluorescence stained with indicated antibodies, and analyzed using a confocal laser scanning microscope. (C) Ten days post HBV infection of the HepaRG cells, immunofluorescence assay was performed with indicated antibodies.

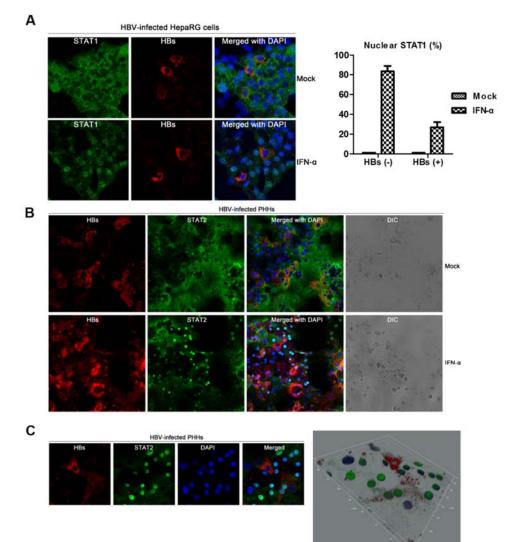


Fig S8. Impaired IFN-induced STAT1/2 nuclear accumulation in HepaRG cells and HBV-infected PHHs. (A) HepaRG cells plated in the chamber coverglass slides were treated with pooled sera from CHB patients overnight. 10 days post HBV infection, the cells were mock-treated or treated with IFN-α for 0.5 h, followed by the immunofluorescence assay with antibodies against HBs and STAT1 (the left panel). The percentage of cells with nuclear STAT1 from three independent fields covering approximately 100 HBs negative and 30 HBs positive cells was calculated (mean±SD) (the right panel). The experiments were performed three independent times in triplicates. (B) PHH cells were plated in the collagen I-pre-coated chamber coverglass slides. 10 days post HBV infection, the cells were treated with or without IFN-α for 0.5 hours, followed by the immunofluorescence assay with antibodies against HBs and STAT2. (C) The PHH cells with HBV viral protein expression showed an impaired STAT2 nuclear-translocation upon IFN treatment compared to those cells without HBV. The three-dimensional view of one of the representative field of IFN-treated PHHs were obtained by reconstruction of z-series sectioned fluorescent images using Leica Confocal Software.

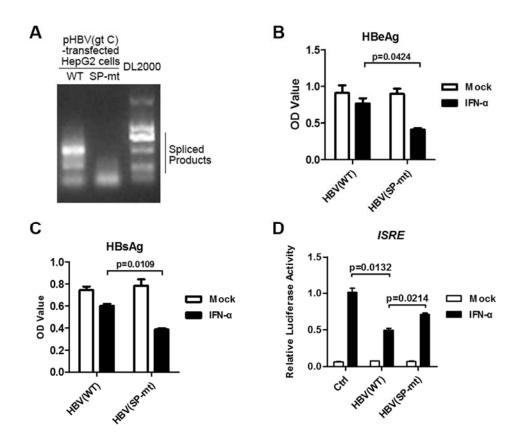


Fig S9. Mutation of the common splice sites of HBV impaired its anti-IFN activities in HepG2 cells.

The cDNAs reverse-transcripted from the total RNAs extracted from pHBV1.3- or pHBV1.3-SP-mt-transfected HepG2 cells were analyzed by PCR followed by agarose gel electrophoresis (A). The level of HBeAg and HBsAg in culture supernatants 48 h post transfection was analyzed by ELISA (B, C). HepG2 cells transfected with the indicated plasmids were mock- or IFN- α -treated for 12 hours and then harvested for luciferase reporter assay (D). The data was analyzed using Student's t-tests.

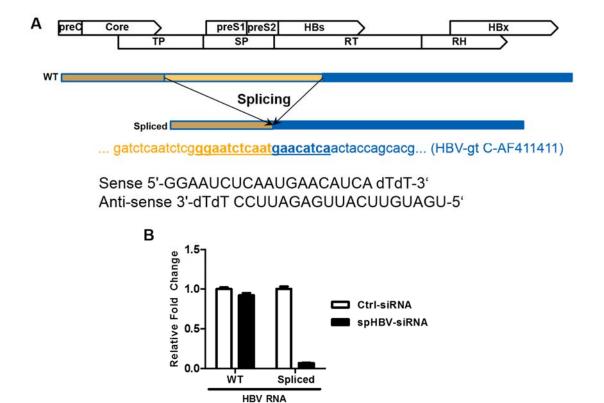


Fig S10. siRNA specifically targeting the major spHBV RNAs

(A) Schematic of the designed siRNA that specifically targets the major spliced HBV RNAs.(B) Huh7 cells were co-transfected with pHBV and control siRNAs or siRNAs targeting spHBV RNAs. 48 h post transfection, the RNAs were extracted for real-time PCR using indicated primers to determine the level of wildtype and spliced HBV RNAs.

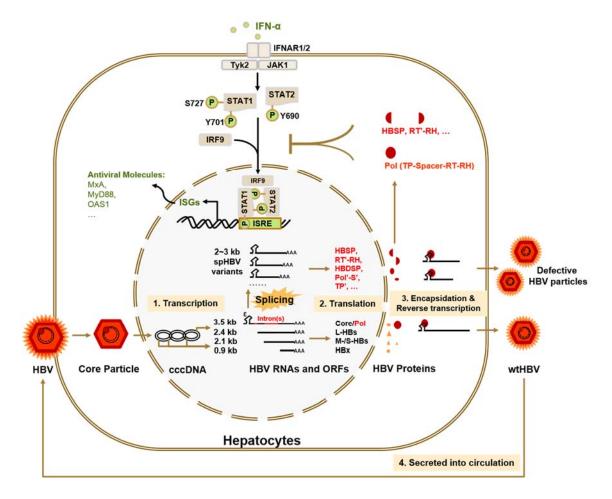


Fig S11. Model of HBV splicing-generated products-mediated inhibition on IFN signaling.

Types of HBV	Exon			Frequ	encies o varian		pliced
spliced	Counts	3' splice acceptor sites	5' splice donor sites	NV	′Rs	EVRs	
variants				gtB	gtC	gtB	gtC
SP1	2	488/489	2447/2448	43.59	48.60	40.96	40.25
SP2	3	2349/2350, 488/489	2067/2068, 2447/2448	17.18	17.62	23.13	23.70
SP3	2	488/489	2067/2068	9.95	7.61	9.79	9.90
SP4	3	2349/2350, 488/489	2087/2088, 2447/2448	7.99	7.52	7.69	7.15
SP5	2	488/489	2087/2088	9.84	5.42	5.66	4.68
SP6	2	488/489	2471/2472	0.42	0.26	0.11	0.25
SP7	3	2936/2937, 488/489	2447/2448, 3020/3021	0.03	0.02	0.01	0.02
SP8	4	2349/2350,2936/2937, 488/489	2067/2068, 2447/2448, 3020/3021	6.13	6.51	7.30	7.73
SP9	2	281/282	2447/2448	0.00	0.00	0.00	0.00
SP10	3	2349/2350, 281/282	2067/2068, 2447/2448	0.72	0.92	0.80	0.46
SP11	2	281/282	2471/2472	0.00	0.00	0.00	0.00
SP12	3	2235/2236, 281/282	2067/2068, 2447/2448	0.02	0.05	0.02	0.03
SP13	2	488/489	3020/3021	0.00	0.00	0.00	0.00
SP14	2	2936/2937	2447/2448	0.00	0.00	0.00	0.00
SP15*	3	2235/2236, 488/489	2067/2068, 2447/2448	1.28	1.07	1.19	1.39
SP16*	3	2349/2350, 488/489	2067/2068, 2471/2472	1.38	2.61	2.01	3.19
SP17*	2	281/282	2067/2068	1.47	1.80	1.33	1.25

Table S1. Species and frequency of HBV spliced variants detected in the sera from CHB patients.

Table	a. The c.	linear			e level o	spinov			1B patients en		
Patient No.	Gender			treatment		Genotype		n HBV DNA			T HBV DNA
1	Female	Age 40	4900	ALT(IU/m 55	HBeAg +	с	0 week * 2.31E+07	2.36E+06	Fold Reduction 9.788135593	0 week *	12 weeks **
1 2	Female	28	1350	155	+ +	c	1.54E+07	1.25E+06	9./88135593	0.038938355	<0.00001 <0.00001
3	Male	21	5450	166	+	B	3.36E+07	1.55E+06	21.67741935	0.027930749	0.055616892
4	Male	32	5000	50	-	C	4.72E+07	6.16E+06	7.662337662	0.018689008	0.000595133
5	Male	38	2850	141	+	С	4.99E+07	4.98E+06	10.02008032	< 0.00001	0.036406297
6	Male	36	20802	95	+	С	4.86E+07	9.11E+06	5.334796926	0.012340135	0.0110562
7	Male	29	9230	145	+	В	1.07E+07	6.58E+05	16.26139818	0.037873477	0.433619142
8	Male	26	700	80	+	C	4.44E+06	4.75E+04	93.47368421	0.667265235	0.375789702
9	Female	28	3550	136	+	B	2.00E+07	2.60E+06	7.692307692	0.031206732	0.103377863
10	Male	30 28	1000	93 472	-	B	3.19E+06	2.93E+05	10.88737201	<0.00001	< 0.00001
11 12	Male Female	28	4250 1550	272	+ +	C C	2.25E+06 2.56E+06	3.86E+05 1.59E+05	5.829015544 16.10062893	0.803573363 0.192109425	0.403320496 0.118448576
12	Female	24	1050	115	+	c	7.75E+06	1.77E+05	43.78531073	0.006963361	0.017247128
14	Female	29	2250	343	-	B	1.75E+06	1.46E+07	0.119863014	0.144987527	0.030416512
15	Male	25	2750	173	+	C	2.01E+06	2.66E+07	0.07556391	0.282632277	0.113126028
16	Female	39	2550	259	+	D	3.13E+05	7.41E+03	42.24021592	1.350975885	< 0.00001
17	Male	38	250	63	+	В	7.51E+05	7.43E+04	10.1076716	<0.00001	< 0.00001
18	Female	37	2250	61	+	С	4.20E+05	2.61E+04	16.09195402	< 0.00001	< 0.00001
19	Female	21	9500	227	+	С	3.40E+05	1.96E+04	17.34693878	0.07576458	0.020827958
20	Female	28	1200	79	+	C	3.57E+05	6.56E+04	5.442073171	0.011509732	0.929161323
21	Male	46	53122	201	+	B	2.44E+05	9.51E+04	2.565720294	0.129049604	0.147931381
22	Female	28	250	152	+	С	3.43E+05	2.68E+05	1.279850746	<0.00001	< 0.00001
23	Male	36	3700 300	102 239	+	C	2.01E+04	1.03E+03 1.09E+03	19.51456311	0.000132566	< 0.00001
24 25	Female Male	28 28	400	69	+ +	C	7.17E+04 7.13E+04	1.09E+03 1.22E+04	65.77981651 5.844262295	0.855595931 0.559547509	0.046049603 0.50417659
25	Male	20	350	42	+	c	2.94E+04	4.06E+05	0.072413793	1.10190465	2.36034609
20	Male	36	1500	61	+	c	6.17E+03	6.90E+02	8.942028986	0.573157051	< 0.00001
28	Male	38	5600	145	+	C	1.46E+07	2.38E+06	6.134453782	0.000193417	0.034078367
29	Male	29	14700	147	+	В	1.00E+08	2.27E+06	44.05286344	0.135246876	0.041569433
30	Male	39	9552	166	+	С	1.00E+08	1.11E+07	9.009009009	0.002387971	< 0.00001
31	Male	28	11150	389	+	С	1.00E+08	4.84E+06	20.66115702	0.002174653	0.016839571
32	Male	22	28850	118	+	В	1.00E+08	2.15E+07	4.651162791	0.440433932	0.09654411
33	Male	27	6350	50	+	С	1.00E+08	2.28E+07	4.385964912	0.009834045	2.0039E-05
34	Male	32	10800	241	+	С	1.00E+08	1.23E+07	8.130081301	0.004626072	0.000651476
35	Male	26	12400	115 114	+	C	1.00E+08 1.00E+08	2.82E+07	3.546099291	0.001013808	3.08829E-05
36	Male Male	28 26	72281 11450	82	+++++	C C	1.00E+08	1.00E+08 2.18E+07	4 597155062	0.02279193	0.001120481
37 38	Male	20	11450	305	+	B	1.00E+08	6.74E+05	4.587155963 148.3679525	0.001018033 0.016090284	<0.00001 0.101320541
39	Female	55	3550	403	+	c	1.00E+08	1.00E+06	148.5079525	< 0.00001	< 0.00001
40	Female	18	5212	385	+	č	3.42E+05	1.01E+03	338.6138614	<0.00001	< 0.00001
41	Male	40	561	165	+	С	8.53E+05	7.22E+03	118.1440443	0.036088069	< 0.00001
42	Female	28	7725	152	+	С	2.22E+06	2.04E+04	108.8235294	< 0.00001	< 0.00001
43	Female	32	250	184	+	С	2.93E+06	1.26E+04	232.5396825	0.000352685	< 0.00001
44	Male	27	250	169	+	С	2.12E+07	1.15E+05	184.3478261	0.003353	0.000552911
45	Female	34	250	42	+	С	2.74E+07	1.84E+05	148.9130435	0.003452043	< 0.00001
46	Male	39	5800	300	+	B	2.58E+07	250	103200	0.006443214	<0.00001
47	Female Female	33	1350 2000	387 121	+ +	C C	1.00E+08 3.03E+07	5.53E+03 6.72E+03	18083.18264	0.000681578 0.022436593	< 0.00001
48	Male	32	1216	107	+	B	1.00E+08	1.23E+03	4508.928571 81300.81301	0.000586872	<0.00001
50	Female	31	34800	129	+	C	4.39E+07	2.03E+03	21625.61576	<0.00001	< 0.00001
51	Male	25	3450	103	+	В	1.81E+07	4.20E+03	4309.52381	0.217939567	0.053598421
52	Male	30	3900	408	+	С	1.00E+08	3.23E+04	3095.975232	0.009691938	0.010964611
53	Female	28	5000	284	+	С	2.70E+07	5.40E+04	500	<0.00001	< 0.00001
54	Female	26	3600	143	+	С	1.18E+07	7.05E+04	167.3758865	<0.00001	0.374749805
55	Male	29	4350	126	+	C	1.00E+08	2.59E+04	3861.003861	0.000909272	< 0.00001
56	Male	42	2900	471	+	C	1.00E+08	1.96E+04	5102.040816	0.00131657	< 0.00001
57	Male Male	25	3750	204	+	B	1.00E+08 1.00E+08	3.71E+05 1.69E+05	269.541779	0.002439272	0.017361064
58	Female	36	4350 3800	803 271	+ +	C	4.77E+07	1.69E+05 1.09E+05	591.7159763 437.6146789	0.019419709	0.110913071
60	Male	33	3250	119	-	c	1.24E+06	7.88E+02	1573.604061	0.0002484204	
61	Male	25	6350	287	+	c	6.83E+06	1.77E+03	3858.757062	0.012410181	6.97071E-05
62	Male	49	2500	226	+	B	8.12E+06	1.71E+03	4748.538012	0.00124728	0.017762782
63	Female	29	1200	114	+	В	1.34E+06	250	5360	0.000352359	< 0.00001
64	Female	31	3850	96	+	С	9.53E+06	8.62E+03	1105.568445	0.000118842	< 0.00001
65	Female	38	1350	114	+	С	7.29E+06	3.22E+03	2263.975155	0.013888166	
66	Female	20	2300	74	+	B	5.74E+06	250	22960	0.006904083	< 0.00001
67	Male	33	1500	243	+	C	2.13E+06	2.17E+03	981.5668203	0.02095344	< 0.00001
68	Male Male	32	2250 8100	245 84	-	B	1.03E+06	250 5.59E+03	4120	0.028001874	<0.00001
69	Male	28	1200	84 436	+ +	C	1.60E+06 3.28E+06	5.59E+03 1.84E+04	286.2254025	<0.00001	<0.00001
70	Female	28	1200	405	+ +	c	3.28E+06	1.64E+04	178.2608696 200	<0.00001 0.000142574	<0.00001
72	Female	24	800	167	+	B	1.30E+05	9.65E+02	134.7150259	< 0.000142374	0.038473243
73	Male	26	350	397	+	B	4.18E+05	250	1672	<0.00001	< 0.00001
74	Female	21	1050	113	+	c	1.11E+05	250	444	0.017305012	< 0.00001
75	Female	25	3050	100.00	+	C	9.51E+05	2.71E+03	350.9225092	0.002878105	< 0.00001
76	Male	31	7040	324	-	С	5.18E+04	250	207.2	<0.00001	< 0.00001
77	Male	25	700	136	-	С	6.84E+04	250	273.6	<0.00001	< 0.00001
78	Male	46	600	59	•	В	3.82E+04	250	152.8	0.568407525	<0.00001
		1			-						

Table S2. The clinical information and the level of spHBV/wtHBV of the 82 CHB patients enrolled in this study

0 week	SP dtection rate (positive/total)	AVG of SP/WT	Two-tailed t-test
NVRs (<2 log)	31/37	0.203832576	p=0.00215
EVRs (>2 log)	29/41	0.024805674	p=0.00215
12 weeks	SP dtection rate (positive/total)	AVG of SP/WT	
NVRs (<2 log)	26/37	0.159418305	n = 0.02611
EVRs (>2 log)	11/41	0.018208319	p=0.03611

* Before IFN treatment; ** After 12 weeks of IFN treatment;

"SP-positive" was defined as spHBV positive and spHBV/wtHBV >0.00001; The viral load was determined using an HBV DNA quantitative real-time PCR kit (Qiagen) and HBV genotyping was performed as previously reported (1).

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HBs (U/ml)	SP/WT DNA	*Fold Reduction	Gender	Age	ALT(IU/ml	HBs(Log)	SP/WT DNA (Log)	Fold Reduction (Log)
4900	0.00001	9.788135593	2	40	55	3.69019608	-5	0.990699977
1350	0.038938355	12.32	2	28	155	3.130333768	-1.409622394	1.090610708
5450	0.027930749	21.67741935	1	21	166	3.736396502	-1.553917418	1.336007579
5000	0.018689008	7.662337662	1	32	50	3.698970004	-1.728413739	0.884361286
2850	0.00001	10.02008032	1	38	141	3.45484486	-5	1.000871203
20802	0.012340135	5.334796926	1	36	95	4.318105092	-1.908680077	0.727117892
9230	0.037873477	16.26139818	1	29	145	3.965201701	-1.421664827	1.211157884
700	0.667265235	93.47368421	1	26	80	2.84509804	-0.175701502	1.97068936
3550	0.031206732	7.692307692	2	28	136	3.550228353	-1.505751708	0.886056648
1000	0.00001	10.88737201	1	30	93	3	-5	1.036923063
4250	0.803573363	5.829015544	1	28	472	3.62838893	-0.094974468	0.765595213
1550	0.192109425	16.10062893	2	27	272	3.190331698	-0.716451328	1.206842841
1050	0.006963361	43.78531073	2	24	115	3.021189299	-2.157181059	1.641328436
2250	0.144987527	0.119863014	2	29	343	3.352182518	-0.838669357	-0.921314807
2750	0.282632277	0.07556391	1	25	173	3.439332694	-0.548778242	-1.121685579
2550	1.350975885	42.24021592	2	39	259	3.40654018	0.130647597	1.62572613
250	0.00001	10.1076716	1	38	63	2.397940009	-5	1.004651123
2250	0.00001	16.09195402	2	37	61	3.352182518	-5	1.206608783
9500	0.07576458	17.34693878	2	21	227	3.977723605	-1.120533777	1.239222846
1200	0.011509732	5.442073171	2	28	79	3.079181246	-1.938934772	0.735764377
53122	0.129049604	2.565720294	1	46	201	4.725274418	-0.889243324	0.409209309
250	0.00001	1.279850746	2	28	152	2.397940009	-5	0.107159326
1500	7.57706E-05	19.51456311	1	36	102	3.568201724	-3.87756753	1.290358833
3700	0.00001	65.77981651	2	28	239	2.477121255	-0.06773129	1.818092658
1150	0.00001	5.844262295	1	28	69	2.602059991	-0.252163033	0.766729699
300	0.855595931	0.072413793	1	22	42	2.544068044	0.042144016	-1.140178703
400	0.559547509	8.942028986	1	36	61	3.176091259	-0.241726361	0.951436073
350	1.10190465	6.134453782	1	38	145	3.748188027	-3.71350616	0.787775899
1500	0.573157051	44.05286344	1	29	147	4.167317335	-0.868872759	1.643974143
5600	0.000193417	9.009009009	1	39	166	3.980094314	-2.621970987	0.954677021
15150	0.000132566	20.66115702	1	28	389	4.047274867	-2.662610105	1.315154638
14700	0.135246876	4.651162791	1	22	118	4.460145817	-0.356119229	0.66756154
9552	0.002387971	4.385964912	1	27	50	3.802773725	-2.007267791	0.642065153
11150	0.002174653	8.130081301	1	32	241	4.033423755	-2.334787654	0.910094889
28850	0.440433932	3.546099291	1	26	115	4.093421685	-2.994044286	0.549750892
6350	0.009834045	1	1	28	114	4.859024152	-1.642218892	0
10800	0.004626072	4.587155963	1	26	82	4.058805487	-2.99223795	0.661543506
12400	0.001013808	148.3679525	1	24	305	4.054995862	-1.79343629	2.171340103
72281	0.02279193	100	2	55	403	3.550228353	-5	2
11450	0.001018033	338.6138614	2	18	385	3.717004407	-5	2.529704732
11350	0.016090284	118.1440443	1	40	165	2.748962861	-1.442636355	2.072411834
3550	0.00001	108.8235294	2	28	152	3.887898488	-5	2.036722807
5212	0.00001	232.5396825	2	32	184	2.397940009	-3.452613076	2.366497075
561	0.036088069	184.3478261	1	27	169	2.397940009	-2.474566454	2.265638021
7725	0.00001	148.9130435	2	34	42	2.397940009	-2.461923824	2.17293274
250	0.000352685	103200	1	39	300	3.763427994	-2.19089742	5.013679697
250	0.003353	18083.18264	2	33	387	3.130333768	-3.166484492	4.257274869
250	0.003452043	4508.928571	2	22	121	3.301029996	-1.649043083	3.654073355
5800	0.006443214	81300.81301	1	32	107	3.084933575	-3.231456325	4.910094889
1350	0.000681578	21625.61576	2	31	129	4.541579244	-5	4.334968482
2000	0.022436593	4309.52381	1	25	103	3.537819095	-0.661663917	3.634429284
1216	0.000586872	3095.975232	1	30	408	3.591064607	-2.013589393	3.490797478

Table S3. Regression	analysis of the factor	rs related to the prognosis	s of IFN responses

Regression Analysis

OVERALL FIT Multiple R 0.3421102 R Square 0.1170394 Adjusted R Squ 0.0557227 Standard Errc 1.2941637 Observation: 78

ANOVA				Alpha	0.05	
	df	SS	MS	F	p-value	sig
Regression	5	15.98459	3.196918	1.9087678	0.1034007	no
Residual	72	120.58989	1.6748596			
Total	77	136.57448				
	coeff	std err	t stat	p-value	lower	upper
Intercept	1.3641042	1.2830054	1.06321	0.2912397	-1.19352	3.9217288
Gender	0.1692558	0.3134792	0.5399265	0.5909138	-0.455654	0.7941652
Age	0.0084977	0.0200945	0.4228839	0.6736404	-0.03156	0.0485554
ALT(IU/ml)	0.0021851	0.0011098	1.9689618	0.0528068	-2.72E-05	0.0043975
HBs(Log)	-0.217254	0.2719143	-0.79898	0.4269301	-0.759305	0.3247972
SP/WT DNA (L	-0.167781	0.0923704	-1.816397	0.073472	-0.351918	0.0163557

34800	0.00001	500	2	28	284	3.698970004	-5	2.698970004	2
3450	0.217939567	167.3758865	2	26	143	3.556302501	-5	2.22369289	2
3900	0.009691938	3861.003861	1	29	126	3.638489257	-3.041306239	3.586700236	2
5000	0.00001	5102.040816	1	42	471	3.462397998	-2.880556102	3.707743929	2
3600	0.00001	269.541779	1	25	204	3.574031268	-2.612739686	2.43062609	2
4350	0.000909272	591.7159763	1	36	803	3.638489257	-1.711757283	2.772113295	2
2900	0.00131657	437.6146789	2	55	271	3.579783597	-2.604812707	2.641091881	2
3750	0.002439272	1573.604061	1	33	119	3.511883361	-3.208779131	3.196895468	2
4350	0.019419709	3858.757062	1	25	287	3.802773725	-1.906221867	3.586447437	2
3800	0.002484204	4748.538012	1	49	226	3.397940009	-2.904036175	3.676559919	2
3250	0.000618331	5360	2	29	114	3.079181246	-3.453014229	3.72916479	2
6350	0.012410181	1105.568445	2	31	96	3.58546073	-3.925029501	3.043585635	2
2500	0.00124728	2263.975155	2	38	114	3.130333768	-1.857355096	3.354871657	2
1200	0.000352359	22960	2	20	74	3.361727836	-2.16089402	4.360971884	2
3850	0.000118842	981.5668203	1	33	243	3.176091259	-1.678744662	2.99191987	2
1350	0.013888166	4120	1	32	245	3.352182518	-1.552812908	3.614897216	2
2300	0.006904083	286.2254025	1	31	84	3.908485019	-5	2.456708175	2
1500	0.02095344	178.2608696	1	28	436	3.079181246	-5	2.251056021	2
2250	0.028001874	200	2	26	405	3.230448921	-3.845959519	2.301029996	2
8100	0.00001	134.7150259	2	24	167	2.903089987	-5	2.129416039	2
1200	0.00001	1672	1	26	397	2.544068044	-5	3.223236273	2
1700	0.000142574	444	2	21	113	3.021189299	-1.761828087	2.64738297	2
800	0.00001	350.9225092	2	25	100.00	3.484299839	-2.540893326	2.545211226	2
350	0.00001	207.2	1	31	324	3.847572659	-5	2.316389751	2
1050	0.017305012	273.6	1	25	136	2.84509804	-5	2.437116093	2
3050	0.002878105	152.8	1	46	59	2.77815125	-0.245340181	2.184123354	2
7040	0.00001								

2: Female 1: Male

700

600

0.00001

0.568407525

* Fold Reduction of viral DNA after 3 months therapy

We have applied the multiple linear regression models (http://www.realstatistics.com/multiple-regression/) to determine whether independent variables including gender, age, ALT, HBsAg (Log), spHBV/wtHBV (Log) are making a significant contribution to the fold of viral DNA reduction after IFN therapy. Results showed that ALT and the ratio of sp/wt HBV tend to be associated with the responses to IFN treatment. The ratio of sp/wt HBV was statistically associated with the responses to IFN treatment when the patients with undetectable spHBV DNAs were excluded.

1 25 173 3.4393327 -0.548778242 -1.121685579 2 39 259 3.4065402 0.130647597 1.62572613 2 21 227 3.9777236 -1.120533777 1.239222846 2 28 79 3.0791812 -1.938934772 0.735764377 1 46 201 4.7252744 -0.889243324 0.409209309 2 28 239 2.4771213 -0.06773129 1.818092658 1 28 69 2.60206 -0.252163033 0.766729699 1 22 42 2.544068 0.042144016 -1.140178703 1 36 61 3.1760913 -0.241726361 0.951436073	Gender	Age				old Reduction of viral DNA (Log)			
1 32 90 3.69897 -1.72841379 0.884361286 OVERLETT 1 36 95 4.31801 -1.90880077 0.72117822 Nulliple 10.5466 2 28 106 3.550228 -1.20571708 0.28645648 Sandad Ei 1.28 1.28 472 3.633805 -0.04974643 0.165059231 -0.165059231 -0.165059231 -0.0497464328 -1.2065979 Nulliple 10.5466 P.Sandad Ei 1.28 0.08665648 Sandad Ei 1.28 0.08675703 -0.02771718 -0.02771718 -0.02771718 -0.0277613 NULL -0.0277613 -0.0277613 -0.0277613 -0.0277613 -0.0277613 -0.0277613 -0.0277613 -0.0277613 -0.0277613 -0.0277613 -0.0277613 -0.0277613 -0.0277613 -0.02776799 -0.04707912 -1.180970268 -0.0450 0.3786 -0.0450 0.3786 -0.048 0.07 -0.0430 0.3786 -0.0450 0.3786 -0.048 0.07 -0.0431 0.02 0.057799 -0.0451 0.3280 -0.04700140 -0.0470791 -0.0470791 -0.0470791 -0.0470791 -0.0170791 <td< th=""><th>2</th><th>28</th><th>155</th><th>3.1303338</th><th>-1.409622394</th><th>1.090610708</th><th>Regression Analysis</th><th></th><th></th></td<>	2	28	155	3.1303338	-1.409622394	1.090610708	Regression Analysis		
1 26 95 4.3181051 -1.00680077 0.72711792 1 26 80 2.850907 1.21157841 Agusta Astronomic Astreconomic Astreconomic Astronomic Astronomic Astreconomic Astronom	1	21	166	3.7363965	-1.553917418	1.336007579			
1 29 145 3 9652017 1.42165437 1.211157844 Aginato 2987 2 28 136 3 550228 1.90757108 0.886656648 Standard F: 1.258 Observatio Standard F: 1.258 Standard F: 1.258 Standard F: 1.258 Standard F: 1.258 Observatio Standard F: 1.258 Standard F: 1.258 Standard F: 1.258 Observatio Standard F: 1.258 Standard F: 1.258 Observatio Standard F: 1.258 Observatio Standard F: 1.258 Standard F: 1.258 Standard F: 1.258 Observatio Standard F: 1.258 Standard F: 1.258 Observatio Standard F: 1.258 Observatio Standard F: 1.258	1	32	50	3.69897	-1.728413739	0.884361286	OV <u>ERALL FIT</u>		
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1331193.5118834-3.2087791313.1968954681252873.8027737-1.9062218673.5864474371492263.39794-2.9040361753.6765599192291143.0791812-3.4530142293.72916479231963.5854607-3.9250295013.0435856352381143.130338-1.8573550963.354871657220743.3617278-2.160894024.3609718841332433.1760913-1.6787446622.991919871322453.3521825-1.5528129083.614897216	1	36	803	3.6384893	-1.711757283	2.772113295			
1252873.8027737-1.9062218673.5864474371492263.39794-2.9040361753.6765599192291143.0791812-3.4530142293.72916479231963.5854607-3.9250295013.0435856352381143.130338-1.8573550963.354871657220743.3617278-2.160894024.3609718841332433.1760913-1.6787446622.991919871322453.3521825-1.5528129083.614897216	2	55	271	3.5797836	-2.604812707	2.641091881			
1492263.39794-2.9040361753.6765599192291143.0791812-3.4530142293.72916479231963.5854607-3.9250295013.0435856352381143.130338-1.8573550963.354871657220743.3617278-2.160894024.3609718841332433.1760913-1.6787446622.991919871322453.3521825-1.5528129083.614897216	1	33	119	3.5118834	-3.208779131	3.196895468			
2291143.0791812-3.4530142293.72916479231963.5854607-3.9250295013.0435856352381143.1303338-1.8573550963.354871657220743.3617278-2.160894024.3609718841332433.1760913-1.6787446622.991919871322453.3521825-1.5528129083.614897216	1	25	287	3.8027737	-1.906221867	3.586447437			
231963.5854607-3.9250295013.0435856352381143.130338-1.8573550963.354871657220743.3617278-2.160894024.3609718841332433.1760913-1.6787446622.991919871322453.3521825-1.5528129083.614897216	1	49	226	3.39794	-2.904036175	3.676559919			
2381143.1303338-1.8573550963.354871657220743.3617278-2.160894024.3609718841332433.1760913-1.6787446622.991919871322453.3521825-1.5528129083.614897216	2	29	114	3.0791812	-3.453014229	3.72916479			
220743.3617278-2.160894024.3609718841332433.1760913-1.6787446622.991919871322453.3521825-1.5528129083.614897216	2	31	96	3.5854607	-3.925029501	3.043585635			
1 33 243 3.1760913 -1.678744662 2.99191987 1 32 245 3.3521825 -1.552812908 3.614897216	2	38	114	3.1303338	-1.857355096	3.354871657			
1 32 245 3.3521825 -1.552812908 3.614897216	2	20	74	3.3617278	-2.16089402	4.360971884			
	1	33	243	3.1760913	-1.678744662	2.99191987			
2 26 405 3.2304489 -3.845959519 2.301029996	1	32	245	3.3521825	-1.552812908	3.614897216			
	2	26	405	3.2304489	-3.845959519	2.301029996			

Regression	Ana	VSIS
Cogression	1 mu	19010

OV <u>ERALL FIT</u>
Multiple 1 0.5466
R Square 0.2987
djusted R Sc 0.2326
Standard Er 1.258
Observatio 59

ANOVA				Alpha	0.05	
	df	SS	MS	F	p-value	sig
Regressio	5	35.727	7.1454	4.5152	0.0017	yes
Residual	53	83.873	1.5825			
Total	58	119.6				
	coeff	std err	t stat	p-value	lower	upper
Intercept	2.5351	1.6067	1.5779	0.1205	-0.687	5.7577

	coejj	siu err	เ รเนเ	p-vaiue	lower	иррег
Interce	ept 2.5351	1.6067	1.5779	0.1205	-0.687	5.7577
Gende	er -0.045	0.3786	-0.12	0.9049	-0.805	0.7139
Age	0.0177	0.0234	0.7562	0.4529	-0.029	0.0646
ALT(IU	/m 0.0019	0.0012	1.5321	0.1315	-6E-04	0.0044
HBsAg(1	Lo -0.714	0.3269	-2.183	0.0335	-1.37	-0.058
SP/WT DNA	(-0.578	0.1567	-3.689	0.0005	-0.892	-0.264

2	21	113	3.0211893	-1.761828087	2.64738297
2	25	100	3.4842998	-2.540893326	2.545211226
1	46	59	2.7781513	-0.245340181	2.184123354

SP/WT HBV DNA>0.00001

Table S4. Primers for qPCR detection of OAS-1, GAPDH, mMx1, mSTAT1 and mGAPDH

Gene	Primer Sequences(5'→3')	Length	
OAS-1	F-TGCCAGACACGTGTTTCCGC	195 hp	
UAS-1	R- GAGGAAGACAACCAGGTCAGCG	185 bp	
GAPDH	F- GGTATCGTGGAAGGACTCATGA	188 bp	
GAPDH	R-ATGCCAGTGAGCTTCCCGTTCAGC		
mMx1	F- GGATGTGATGCGGAACCT	156 bp	
IIIIVIX I	R-AGAAGAATGCTGAAGTATGAGTGA		
mSTAT1	F- CTTCCACGACCTCCTCTC	167 bp	
IIISTATT	R- CTTCCTTCAGACAGTTGTAGATG		
mGAPDH	F- AGTATGTCGTGGAGTCTA	162 hr	
IIIUAPDH	R- CAATCTTGAGTGAGTTGTC	162 bp	

References

1. Naito H, Hayashi S, Abe K. Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by PCR using type-specific primers. J Clin Microbiol 2001;39:362-364.