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Supplemental Information

Type-I-IFN-Stimulated Gene TRIM5γ Inhibits HBV Replication by Promoting HBx Degradation

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Supplementary figures and figure legends

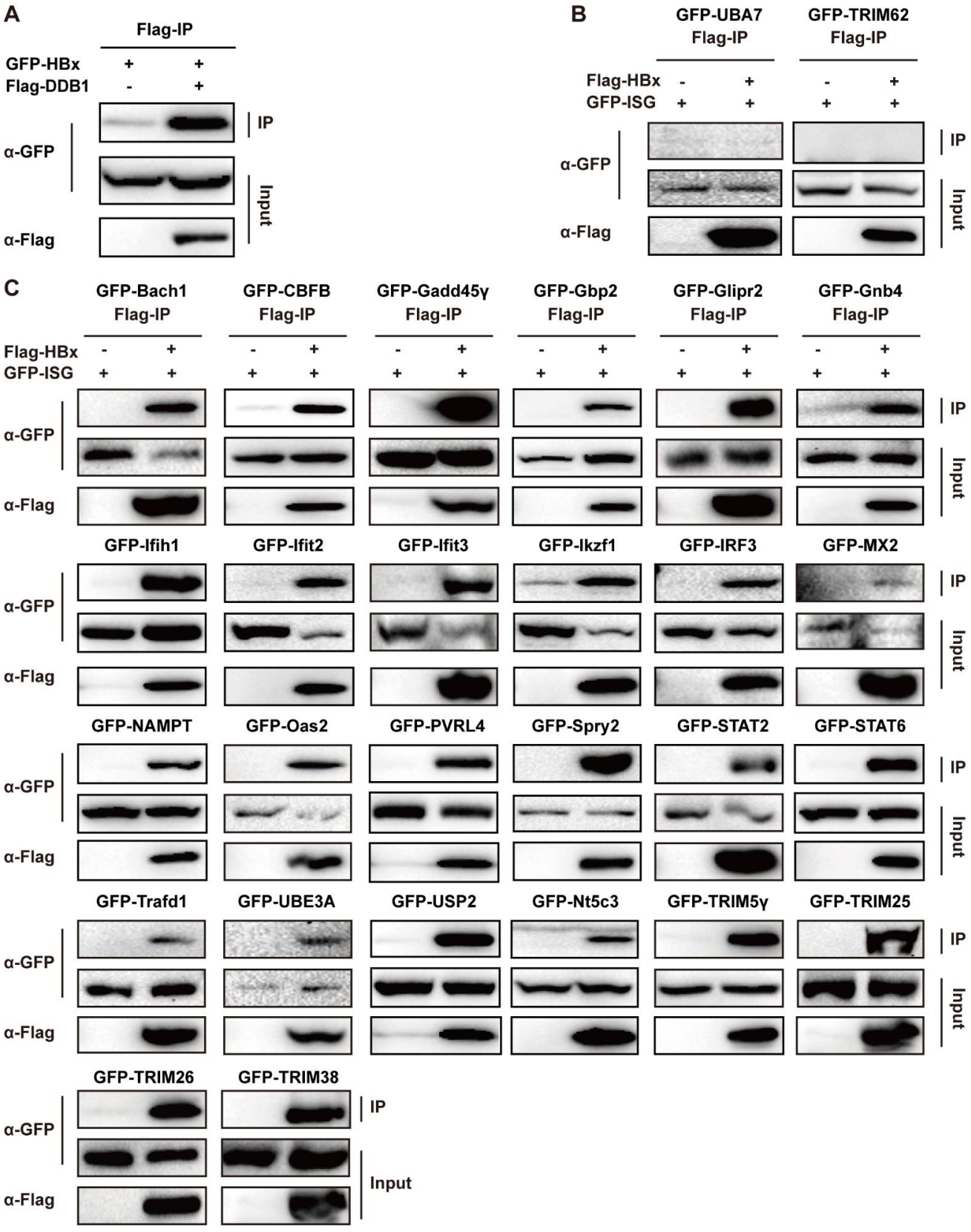
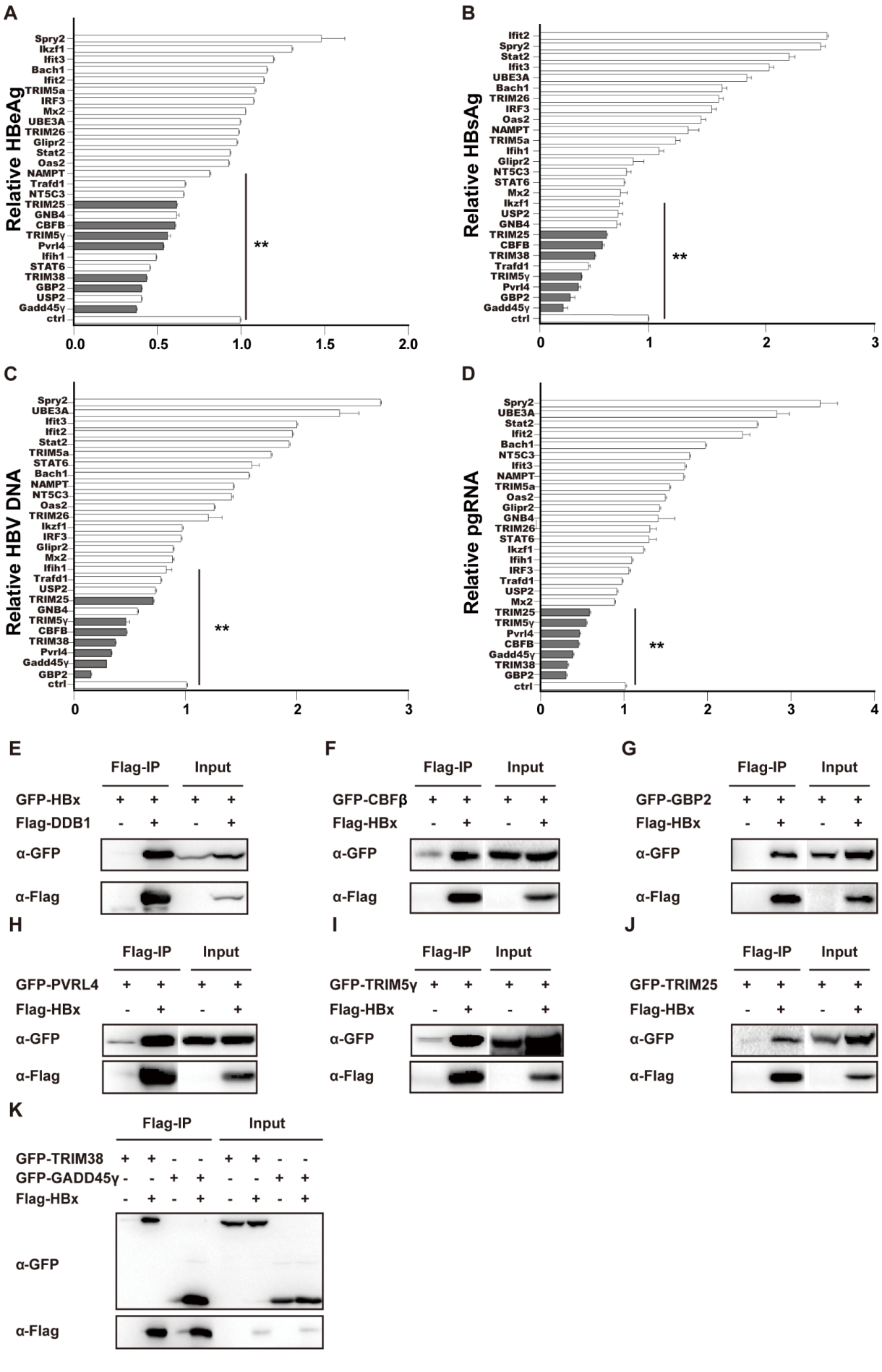


Figure S1. Confirming the ISGs-HBx interaction by Co-immunoprecipitation assay in 293T cells, Related to Figure 1.

(A) The GFP-HBx expression plasmid was co-transfected with Flag-DDB1 or an empty Flag reporter plasmid (Flag-EV) into 293T cells as indicated; 28 h later, cells were harvested and lysed. 1/20 of the whole cell lysates were used as input, and the rest were incubated overnight with Flag-tagged beads. After washing, the beads were boiled with SDS loading buffer and subjected to immunoblotting using anti-GFP or anti-Flag antibodies. (**B)** Plasmids expressing two ISGs (UBA7 or TRIM62) negative for HBx interaction in the BIFC assay were co-transfected with Flag-HBx or Flag-EV plasmids into 293T cells as indicated, and analyzed as in **A. (C)** Plasmids expressing ISGs positive for HBx interaction by BIFC assay were co-transfected with Flag-HBx or Flag-EV plasmids into 293T cells as indicated, and analyzed as in **A**.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Bach1
Ifit2
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Stat2
Oas2
NAMPT
Trafd1
NT5C3
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α-GFP
α-Flag

Figure S2. Evaluation of anti-HBV replication and HBx interaction activity of 7 ISG products in HepG2 cells, Related to Figure 1.

(A,B) pHBV1.3 plasmid DNA was co-transfected into HepG2 cells with either an empty GFP reporter plasmid (GFP-EV) or GFP reporter plasmids encoding ISG products positive for interaction with HBx by both BiFC and Co-IP assays. After 72 hours, culture media was collected and screened for HBeAg, HBsAg expression by ELISA. **(C,D)** The supernatant and cell lysates were subjected to HBV DNA and RNA isolation; HBV DNA and pgRNA were analyzed by Q-PCR. Mean (±SD) values from three independent experiments are shown. $^{**}p<0.01$. **(E)** The GFP-HBx expression plasmid was co-transfected with Flag-DDB1 or an empty Flag reporter plasmid (Flag-EV) plasmids into HepG2 cells as indicated; 24 hours later, cells were harvested and lysed. 1/20 of the whole cell lysates were used as input, and the rest were incubated overnight with Flag-tagged beads. After washing, the beads were boiled with SDS loading buffer and subjected to immunoblotting using anti-GFP or -Flag antibodies. **(F-K)** 7 GFP-tagged ISGs expression plasmids were co-transfected with Flag-HBx or an Flag-EV plasmids as indicated, and cells were collected after 24 hours and analyzed as in **E.**

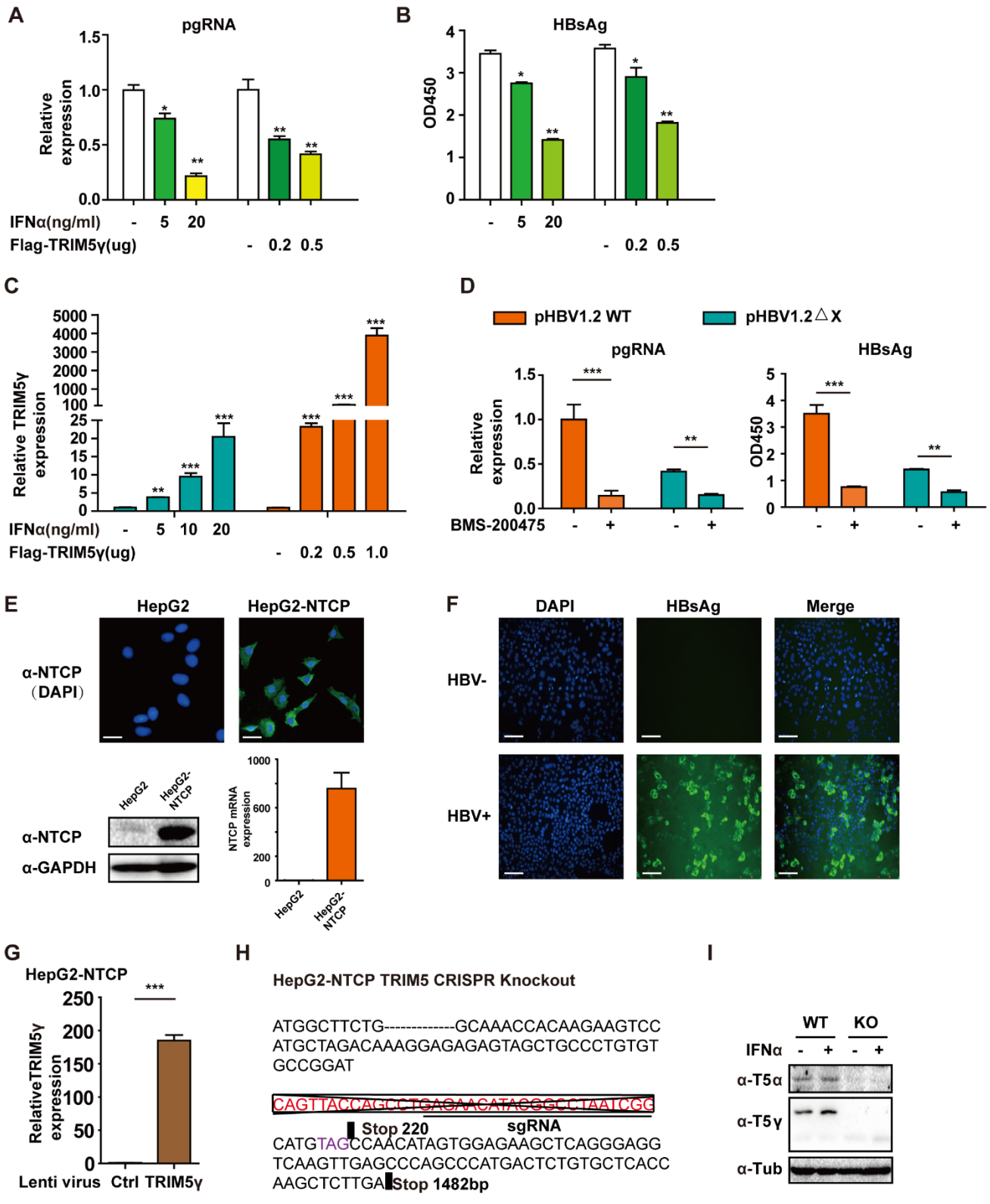

Figure S3. Related to Figure 3. (A, B) pHBV1.3 plasmid DNA was transfected into HepG2 cells alone or co-transfected with constructs expressing TRIM5γ proteins. 24 h later, cells transfected with pHBV1.3 plasmid alone were treated with IFN-α as indicated, after 48 h, cells and supernatant were harvested and subjected to Q-PCR or ELISA. **(C)** HepG2 cell were transfected and treated as in A, and subjected to Q-PCR to analyze TRIM5γ mRNA expression. **(D)** HepG2 cells were transfected

with constructs expressing pHBV1.2 WT or ΔX as indicated. 24 h later, cells were treated with BMS-200475(1μM) for 24 h or left untreated, cells and supernatant were harvested and subjected to Q-PCR or ELISA. **(E)** Expression of NTCP in HepG2 or HepG2-NTCP cells were analyzed by Immunofluorescence, Western Blot or Q-PCR. Scale bar represents 20μm. Mean (±SD) values from three independent experiments are shown. *$p<0.05$,**$p<0.01$, ***$p<0.001$. **(F)** HBsAg expression in HepG2-NTCP cells with or without HBV infection were analyzed by Immunofluorescence**.** Scale bar represents 100μm. **(G)** Samples from Figure 3C were subjected to Q-PCR to analyse the expression of TRIM5γ mRNA. Mean (±SD) values from three independent experiments are shown. ***$p<0.001$. **(H)** Diagram of HepG2-NTCP cells TRIM5α/γ KO results, bases from 180 to 213 were cut off in the coding region of TRIM5 and make a shorter transcript stopped at 220 bp. **(I)** Samples from Figure 3D were analyzed by immunoblotting using antibodies as indicated.

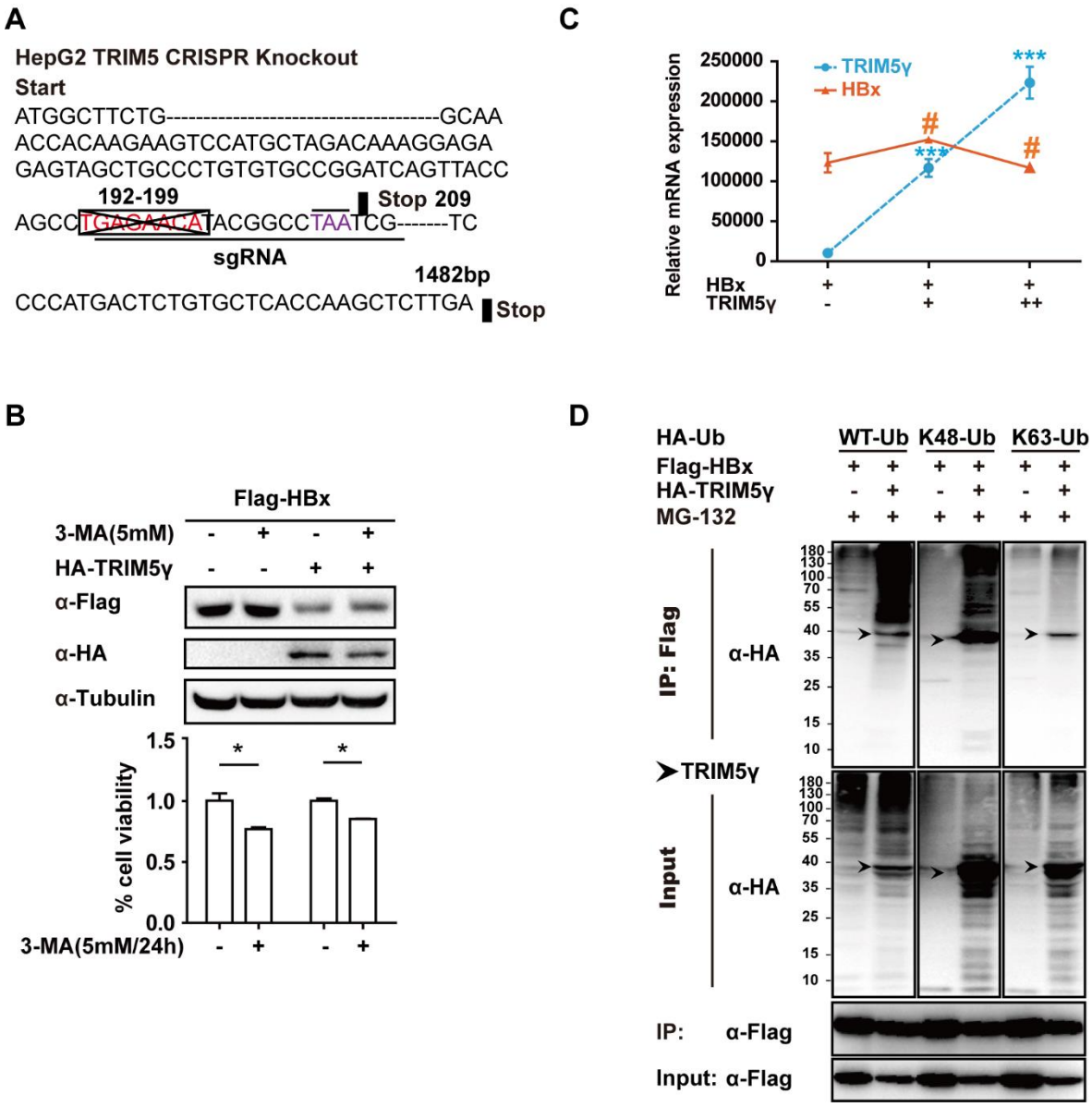

Figure S4. Related to Figure 4. (A) Diagram of HepG2 cells TRIM5α/γ KO results; bases from 192 to 199 were cut off in the coding region of TRIM5 and make a shorter transcript stopped at 209bp. **(**B**)** HepG2 cells were co-transfected with HA-TRIM5γ and Flag-HBx as indicated; 24 h later, cells were treated with 3-MA (5mM, autophagy inhibitor) for another 24 h or left untreated, cell viabillity was mesured by a CCK8 kit, and whole cell lysates were subjected to immunoblotting analysis using the indicated antibodies. **(**C**)** 293T cells were co-transfected with HA-TRIM5γ and Flag-HBx as indicated; 36 h later, cells were subjected to RNA extraction and Q-PCR was performed to evaluate the expression levels of TRIM5γ and HBx transcripts. Mean (±SD) values from three independent experiments are shown. *p<0.05,**p<0.01, #p>0.05. **(D)** HepG2 cells were co-transfected with HA-Ub and Flag-HBx, with or without HA-TRIM5γ expression plasmids; 24 h later, cells were treated with MG-132 as indicated for 6 h, and subjected to Co-IP analysis. Cell lysates and co-IP samples were subjected to immunoblotting analysis using the indicated antibodies.

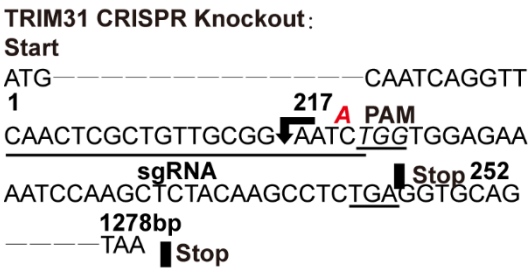

Figure S5. Related to Figure 7. Diagrams of TRIM31 KO results. An ‘A’ was inserted before the 217bp ‘A’ in the coding region of TRIM31, which make a shorter transcript stopped at 252bp.

Supplementary Table 1. HBV infected patients with IFN treatment, Related to Figure 2.

			before treatment				17 weeks after treatment				Gene induction	
NO	Sex	Age	AST 0	ALT 0	HBeAG 0	HBV DNA 0	AST 17	ALT 17	HBeAG 17	HBV DNA 17	TRIM5γ fold	TRIM22 fold
1	M	28	188	280	963.762	170000000	63	64	13.754	119000	10.29	5.86
2	F	22	60	106	861.489	170000000	59	96	782.74	170000000	1.82	1.23
3	F	22	130	162	903.773	124000000	104	141	657.992	14500000	0.91	2.54
5	F	36	78	152	1368.318	170000000	21	17	35.267	165000	6.63	0.71
6	M	55	111	293	1240	170000000	55	117	667.605	170000000	0.47	1.4
7	M	43	95	219	675.52	170000000	73	135	434.09	170000000	1.11	10.56
8	F	24	54	108	0.455	2560000	44	65	0.349	296	10.18	17.1
9	M	38	89	145	1028.726	170000000	80	82	20.289	1310000	0.78	0.99
10	M	39	89	91	0.433	25400000	193	273	0.415	224	1.67	1.35
11	M	24	86	227	12.351	498000	39	68	4.455	1120	2.4	20.13
12	M	26	64	188	1134.673	255000000	42	73	1071.894	142000000	2.03	15.03
13	M	31	52	89	1293.93	165000000	45	54	803.594	5120000	2.73	2.33
14	M	49	88	206	1045.839	170000000	83	154	686.078	56200000	0.96	1.95
15	F	31	62	172	840.371	170000000	30	36	2.837	1390	12.71	2.1
16	M	23	52	89	9.171	2080000	40	51	2.771	3170	3.36	1.71
17	M	42	62	128	1256.277	38000000	29	36	1.202	1130	10.34	7.37
18	M	22	101	360	117.151	1440000	18	15	81.417	196000	3.21	3.11
19	F	34	144	337	202.293	31300000	49	54	23.13	42800000	6.52	3.48
20	M	32	255	247	684.086	9570000	74	69	15.661	2720	10.18	1.16
21	M	22	92	108	1011.575	84600000	52	45	559.859	2330000	3.74	1.86
22	M	31	58	125	5.325	9950000	29	36	0.469	306	44.28	15.26
23	M	25	54	84	1145.428	45000000	38	31	582.172	3120000	5.89	1.23
24	M	35	79	117	732.383	170000000	24	18	742.352	20600000	7.64	3.01
25	F	33	81	164	776.892	170000000	29	43	318.496	31800000	4.78	0.23
26	M	41	73	206	824.12	35100000	49	87	12.839	45600	31.03	3.88
27	F	31	64	101	0.345	65900000	41	44	0.41	6690000	3.96	5.86
28	M	56	72	177	0.399	198000	129	194	0.408	23.1	2.78	44.23
29	F	26	52	93	20.685	170000000	39	52	14.56	102000000	1.79	60.15
30	M	20	60	86	344.962	10100000	52	72	69.589	1740000	4.88	0.26
31	F	33	94	205	1589.529	170000000	88	171	1639.966	170000000	4.40	1.98
32	F	33	41	101	158.344	767000	33	36	713.4	4040000	1	3.12

Supplementary Table 2. SgRNAs and Primers for infusion cloning, Related to STAR Methods

TRIM5 primes

GENE	PRIMER
T5a cloning F	GAAGAATTCATGGGTTACCCTTATGATGTGCCAGATTATGCCATG GCTTCTGGAATC
T5a cloning R	GAACTCGAGTCA AGA GCTTGGTGAG
T5γ cloning F	GAAGAATTCATGGGTTACCCTTATGATGTGCCAGATTATGCCATGGCTTCTGGA ATC
T5γ cloning R	GAACTCGAGTTATAAGGAGGGGTAAG
ΔRB F	GAATCTAGAATGGGTTACCCTTATGATGTGCCAGATT ATGCCATGACAGAGGAGGTTGCC
ΔR F	GAA TCTAGA ATGGGTTACCCTTATGATGTGCCAGATTATGCC ATGATCAGTTACCAGCCT G
RB R	GAA GGATCC TTA GAG GAA CGT GTG GTG
BB F	GAA GAATTC ATG CAT TGT GCA CGC CAT G
BB R	GAA GTCGAC TTA GAG GAA CGT GTG GTG

HBx primers

GENE	PRIMER
HBx FORWARD	TCGCGGCCGCTCTAGA ATGGCTGCTCGGGTGTGCTGC
HBx REVERSE	AGGCGCCTGGTCTAGA TTAGGCAGAGGTGAAAAAG

TRIM31 primers

GENE	PRIMER
TRIM31C31H33A F	GTCACCATCGACGCTGGGGCCAATTTCTGCCTC
TRIM31C31H33A R	GAGGCAGAAATTGGCCCCAGCGTCGATGGTGAC
TRIM31cloning F	TCGCGGCCGCTCTAGAATGGCCAGTGGGCAGTTT
TRIM31cloning R	AGGCGCCTGGTCTAGATTAGCTTGAAGGAACCTCACAA

sgRNAs

GENE	PRIMER
TRIM5α sgRNA1F	CACCgTGGTAACTGATCCGGCACAC
TRIM5α sgRNA1R	AAACGTGTGCCGGATCAGTTACCAc
TRIM5α sgRNA2F	CACCgCGATTAGGCCGTATGTTCTC
TRIM5α sgRNA2R	AAACGAGAACATACGGCCTAATCGc
TRIM31 sgRNAF	CACC CAA CTC GCT GTT GCG GAA TC
TRIM31 sgRNAR	AAAC GA TTC CGC AAC AGC GAG TTG

Supplementary Table 3. Q-PCR primers, Related to STAR Methods

Gene	Forward	Reverse
GAPDH	CGGATTTGGTCGTATTGGG	TCTCGCTCCTGGAAGATGG
HBVDNA	GAGTGTGGATTCGCACTCC	GAGGCGAGGGAGTTCTTCT
pgRNA	TCTTGCCTTACTTTTGGAAG	AGTTCTTCTTCTAGGGGACC
HBx	ACGTCCTTTGTTTACGTCCCGT	CCCAACTCCTCCCAGTCCTTAA
TRIM5γ	TATCATAAGCCACCCTGCGG	TGTGTGTCTTGGAAGGAGAATCA
CBFB	AGTTTGATGAGGAGCGAGCC	TCTTCTTGCCTCCATTTCCTCC
GBP2	CATCCGAAAGTTCTTCCCCAA	CTCTAGGTGAGCAAGGTACTTCT
GADD45G	CAGATCCATTTTACGCTGATCCA	TCCTCGCAAAACAGGCTGAG
PVRL4	AGGACGCAAAACTGCCCTG	TGAAGCCCGTATTTGGAGTGC
TRIM25	AGGGATGAGTTCGAGTTTCTGG	GTTTTTGAGGTCTATGGTGCTCT
TRIM38	ACACGGAGAGCAGTTCCAC	GGCATACGTCTTCAACAAGAGC