Supplementary Legends

Table S1. Primers used in the T7EI assay for each target region.

Table S2. Predicated off-target sites for gRNA-S4 in the human genome.

Table S3. Primers used in the off-target assay for each possible off-target region.

Fig S1. Homologous sequence analysis of gRNA target region in different HBV serotypes.

Fig S2. Efficiency of transfection in HepG2.A64 72 h after puromycin screening was routinely ~90%. Scale bars represent 200 μm.

Fig S3. Representative target S4 region insertion in gRNA-S4–treated cells (A) and HBV-Tg mice (B), generated using the Integrative Genomics Viewer.

Fig S4. Suppression of HBV expression and replication in T11. **(A, B)** Titers of HBsAg and HBV DNA in cell culture supernatants during 9 consecutive days' cultivation. The HBsAg test results were always negative (<0.05 IU/ml), and the amount of HBV DNA was near or below the negative critical value in T11 (n = 3). **(E)** The amount of cccDNA in T11 and N1on the 9thday after consecutive cultivation. **(F)** The amount of HBV DNA inside viral nucleocapsid in T11 was 10,000-fold lower than that in N1 on the 9th day after culture consecutive.

Fig S5. Primers that amplify the A1AT gene and HBS-gene were used to detect genomic DNA contamination. DNA was amplified from genomic DNA by A1AT gene specific primers, but not from HBV cccDNA.

Table S1. Primers used in the T7EI assay for each target region.

Primers	Sequence (5'-3')
S4-F	ACCCTGCGCTGAACATGGAG
S4-R	AAGCCCTACGAACCACTGAACAA
S5-F	TCAACTACCAGCACGGGACC
S5-R	GCCCAAAAGACCCACGATTC
XP-F	GCAGGCTTTCACTTTCTCGC
XP-R	GGAACGGCAGATGAAGAA
URR-F	CGCTCCGAAAGTTTCCTT
URR-R	GAGTGCGAATCCACACTCC
BCP-F	CCTTCTTCATCTGCCGTTCC
BCP-R	ATGTCCATGCCCCAAAGCC

Table S2. Predicated off-target sites for gRNA-S4 in the human genome.

No.	Sequence	Score	Mismatches	UCSC gene	Locus
1	GCCCTCTCTGGATGTGTCTGAGG	1.6	3MMs [3:4:7]		chr11:+75047287
2	GCTGTTGCTAGATGTGTCTGAAG	1.4	3MMs [4:6:10]		chr8:+47864962
3	TGTTTCGTTGGATGTGTCTGAGG	1.4	4MMs [1:2:4:8]		chr17:-74488431
4	AATTTCGTTGGATGTGTCTGTGG	1.4	4MMs [1:2:4:8]		chr10:-36941610
5	CCTAGCGCTGGATGTGACTGTGG	1.3	3MMs [1:5:17]		chr22:+49133545
6	CCTGTCGCTGGAGGTGTCTGTGG	1.2	3MMs [1:4:13]	NR_024527	chr11:-118015895
7	TCTAGCATTGGATGTGTCTGTGG	0.9	4MMs [1:5:7:8]		chr7:-20747255
8	ACTTTCCCTTGATGTGTCTGAAG	0.9	4MMs [1:4:7:10]		chr5:-125646588
9	CTAATGGCTGGATGTGTCTGGGG	0.8	4MMs [1:2:3:6]		chr5:+178213889
10	TCCGTGGCTGGATGTGTCTGAGG	0.8	4MMs [1:3:4:6]		chr18:-10706739
11	GCAGGTGCTGGATGTGTCTGGGG	0.8	4MMs [3:4:5:6]	NM_020676	chr3:+58279310
12	GCATTTGCTTGATGTGTCTGGGG	0.8	4MMs [3:4:6:10]		chr1:+194264939
13	GGTGTCGCTCGCTGTGTCTGTAG	0.7	4MMs [2:4:10:12]		chr2:+69959464
14	GCGGACGCTGGATGTGGCTGCAG	0.6	4MMs [3:4:5:17]		chr2:+129349323
15	CCTCTCCCCGGATGTGTCTGGGG	0.6	4MMs [1:4:7:9]		chr8:+99952106
16	CCTAAAACTGGATGTGTCTGTGG	0.6	4MMs [1:5:6:7]		chr7:-57842027
17	GAAATAACTGGATGTGTCTGCAG	0.5	4MMs [2:3:6:7]		chr3:+2365959
18	GATAACCCTGCATGTGTCTGAGG	0.5	4MMs [2:5:7:11]		chr8:-115773972
19	GCTGCACCTGGATGTGTCTGGGG	0.5	4MMs [4:5:6:7]		chr10:+50257134
20	CCTGTTGCAGGATGTGTCTGCAG	0.5	4MMs [1:4:6:9]		chr14:+99788041

Table S3. Primers used in the off-target assay for each possible off-target region.

Primers	Sequence (5'-3')
off-target1F	GGAAACCCCTCAGCTCACTC
off-target1R	CTGGCATGGACAGCTCAGAA
off-target2F	CTAGGGCAGATTAGGGGCCA
off-target2R	CATGACAAGTCCACCCGTCC
off-target3F	CCTGCCCCATAAAGCCCATA
off-target3R	GATGCCAAAGAGACCTGGGG

Figure S1

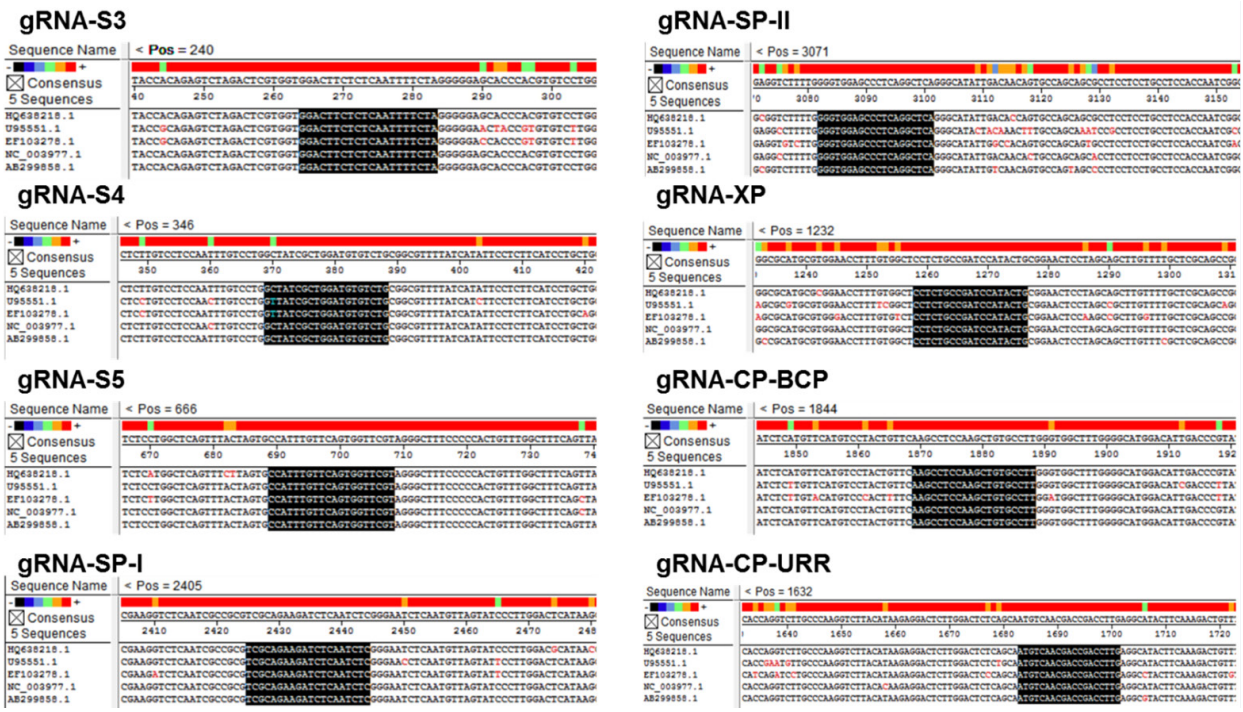


Fig S1.Homologous sequence analysis of gRNA target region in different HBV serotypes.

Figure S2

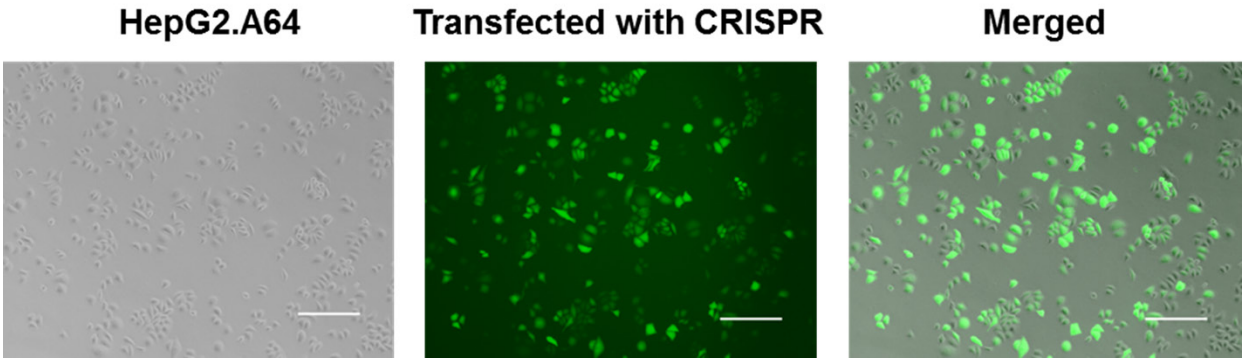


Fig S2. Efficiency of transfection in HepG2.A64 72 h after puromycin screening was routinely ~90%. Scale bars represent 200 μm.

Figure S3

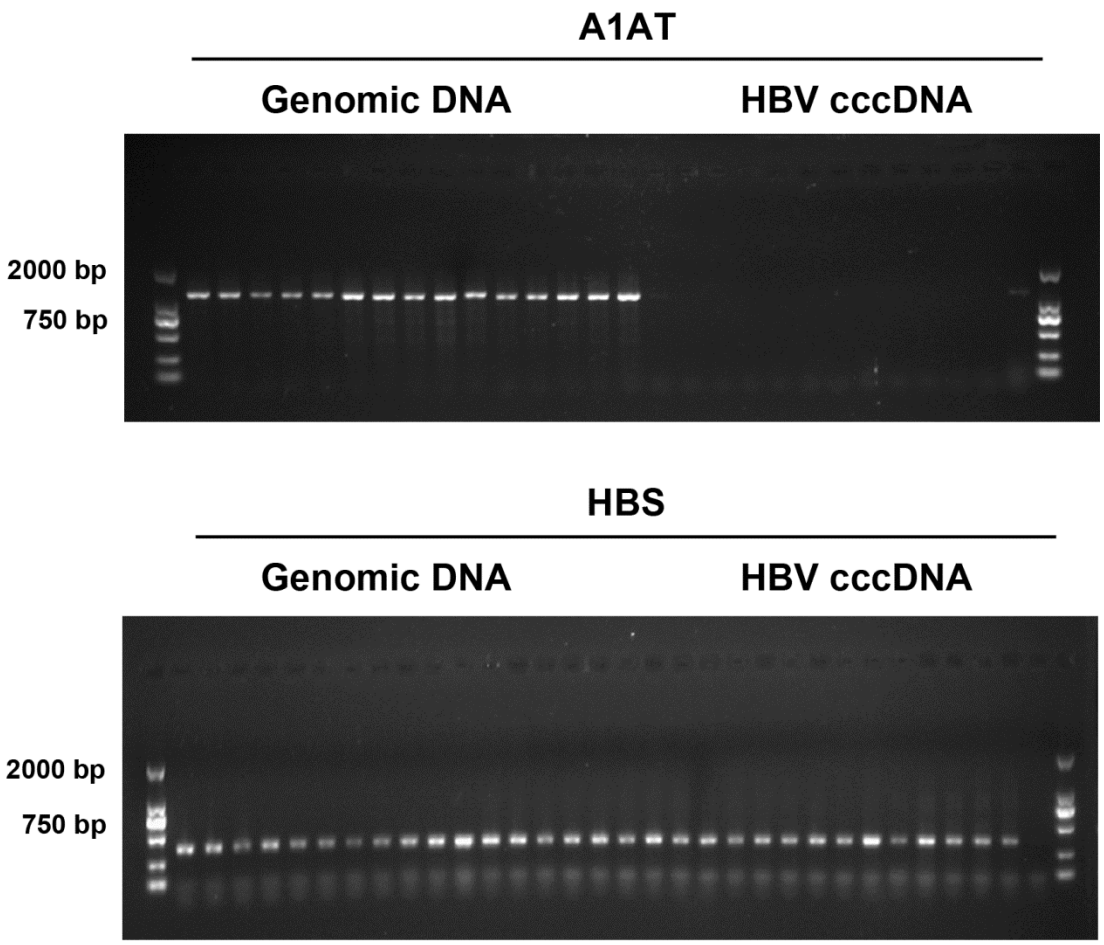


Fig S3. Primers that amplify the A1AT gene and HBS-gene were used to detect genomic DNA contamination. DNA was amplified from genomic DNA by A1AT gene specific primers, but not from HBV cccDNA.

Figure S4

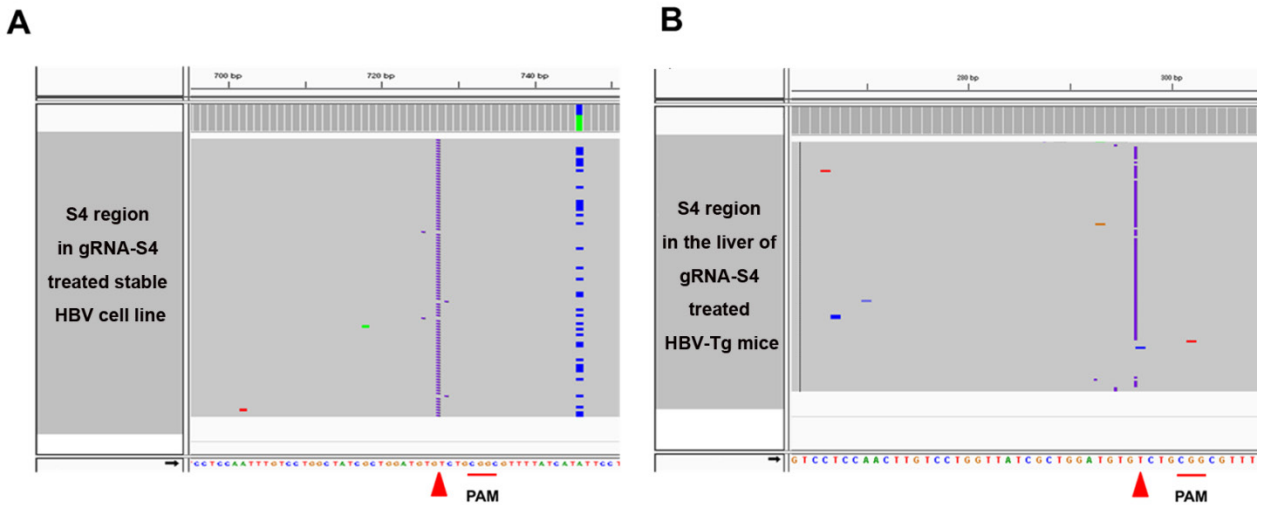
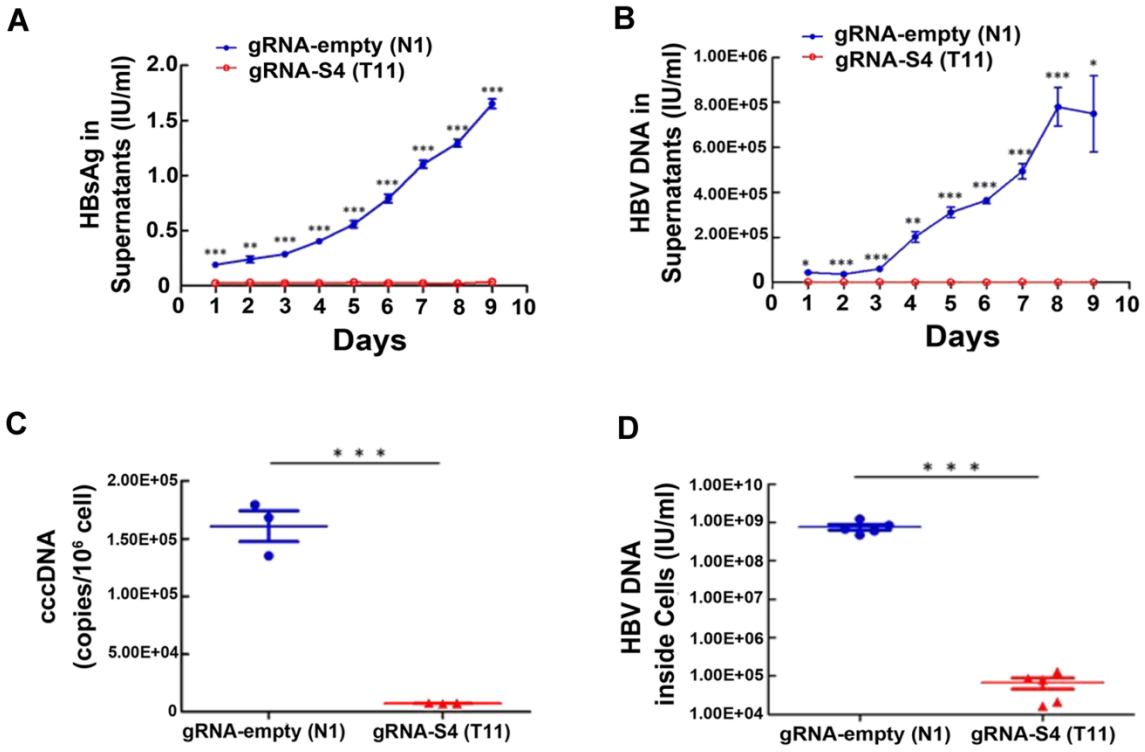


Fig S4. Representative target S4 region insertion in gRNA-S4–treated cells (A) and HBV-Tg mice (B), generated using the Integrative Genomics Viewer.

Figure S5



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