

**Supplementary Table 1. Expression plasmids for the expression of wt- and vtHBsAg**

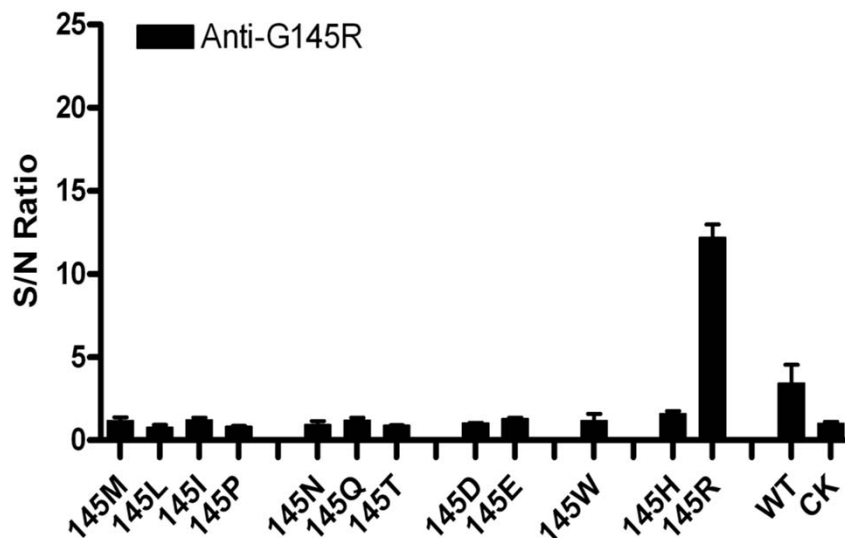
Plasmid	Subtype	Mutation(s) at 145/122 sites	Vector
pHBsAg-WT	<i>Adw2</i>	Wild-type sequence	pXF3H
pHBsAg-G145M	<i>Adw2</i>	G145M	pXF3H
pHBsAg-G145L	<i>Adw2</i>	G145L	pXF3H
pHBsAg-G145I	<i>Adw2</i>	G145I	pXF3H
pHBsAg-G145P	<i>Adw2</i>	G145P	pXF3H
pHBsAg-G145N	<i>Adw2</i>	G145N	pXF3H
pHBsAg-G145Q	<i>Adw2</i>	G145Q	pXF3H
pHBsAg-G145T	<i>Adw2</i>	G145T	pXF3H
pHBsAg-G145D	<i>Adw2</i>	G145D	pXF3H
pHBsAg-G145E	<i>Adw2</i>	G145E	pXF3H
pHBsAg-G145W	<i>Adw2</i>	G145W	pXF3H
pHBsAg-G145H	<i>Adw2</i>	G145H	pXF3H
pHBsAg-G145R	<i>Adw2</i>	G145R	pXF3H
pHBsAg-K122G	<i>Adw2</i>	K122G	pXF3H
pHBsAg-K122M	<i>Adw2</i>	K122M	pXF3H
pHBsAg-K122L	<i>Adw2</i>	K122L	pXF3H
pHBsAg-K122I	<i>Adw2</i>	K122I	pXF3H
pHBsAg-K122P	<i>Adw2</i>	K122P	pXF3H
pHBsAg-K122N	<i>Adw2</i>	K122N	pXF3H
pHBsAg-K122Q	<i>Adw2</i>	K122Q	pXF3H
pHBsAg-K122T	<i>Adw2</i>	K122T	pXF3H
pHBsAg-K122D	<i>Adw2</i>	K122D	pXF3H
pHBsAg-K122E	<i>Adw2</i>	K122E	pXF3H
pHBsAg-K122W	<i>Adw2</i>	K122W	pXF3H
pHBsAg-K122H	<i>Adw2</i>	K122H	pXF3H

**Supplementary Table 2. Primers used for cloning or real-time PCR analysis in the present study**

Designation	Position (nt)	Sequence	Polarity
SP1	160-176	5'-ACG <u>GAAITTC</u> GAGAACATCGCATCAGG-3'	sense
SP2	841-824	5'-GCAC <u>TGCAGC</u> GGTTTAAATGTATACCC-3'	antisense
T145	587-563	5'-TCCGTAGGTTTTGTACAGCAACATG-3'	antisense
G145M-U	570-601	5'-CTGTACAAAACCTACGGAC <u>ATG</u> AACTGCACCT-3'	sense
G145L-U	570-601	5'-CTGTACAAAACCTACGGAC <u>TTA</u> AACTGCACCT-3'	sense
G145I-U	570-601	5'-CTGTACAAAACCTACGGAC <u>ATA</u> AACTGCACCT-3'	sense
G145P-U	570-601	5'-CTGTACAAAACCTACGGAC <u>CCA</u> AACTGCACCT-3'	sense
G145N-U	570-601	5'-CTGTACAAAACCTACGGAC <u>AACA</u> ACTGCACCT-3'	sense
G145Q-U	570-601	5'-CTGTACAAAACCTACGGAC <u>CAA</u> AACTGCACCT-3'	sense
G145T-U	570-601	5'-CTGTACAAAACCTACGGAC <u>ACA</u> AACTGCACCT-3'	sense
G145D-U	570-601	5'-CTGTACAAAACCTACGGAC <u>GACA</u> ACTGCACCT-3'	sense
G145E-U	570-601	5'-CTGTACAAAACCTACGGAC <u>GAAA</u> ACTGCACCT-3'	sense
G145W-U	570-601	5'-CTGTACAAAACCTACGGAC <u>TGGA</u> ACTGCACCT-3'	sense
G145H-U	570-601	5'-CTGTACAAAACCTACGGAC <u>CACA</u> ACTGCACCT-3'	sense
G145R-U	570-601	5'-CTGTACAAAACCTACGGAC <u>CGAA</u> ACTGCACCT-3'	sense
T122	517-493	5'-ATGGTCCGGTGCTGGTTGTTGATGA-3'	antisense
K122G-U	500-531	5'-CAACCAGCACCGGACCATGCG <u>GAA</u> ACCTGCACG-3'	sense
K122M-U	500-531	5'-CAACCAGCACCGGACCATGCA <u>TG</u> ACCTGCACG-3'	sense
K122L-U	500-531	5'-CAACCAGCACCGGACCATGCT <u>TAA</u> ACCTGCACG-3'	sense
K122P-U	500-531	5'-CAACCAGCACCGGACCATGCC <u>CAA</u> ACCTGCACG-3'	sense
K122N-U	500-531	5'-CAACCAGCACCGGACCATGCA <u>AT</u> ACCTGCACG-3'	sense
K122Q-U	500-531	5'-CAACCAGCACCGGACCATGCC <u>CAA</u> ACCTGCACG-3'	sense
K122T-U	500-531	5'-CAACCAGCACCGGACCATGC <u>ACA</u> ACCTGCACG-3'	sense
K122D-U	500-531	5'-CAACCAGCACCGGACCATGCG <u>AT</u> ACCTGCACG-3'	sense
K122E-U	500-531	5'-CAACCAGCACCGGACCATGCG <u>AAA</u> ACCTGCACG-3'	sense
K122W-U	500-531	5'-CAACCAGCACCGGACCATGCT <u>GGA</u> ACCTGCACG-3'	sense
K122H-U	500-531	5'-CAACCAGCACCGGACCATGCC <u>CAT</u> ACCTGCACG-3'	sense
RC-FW		5'-GTTGCCCGTTTGTCTCTAATTC-3'	
RC-REV		5'-GGAGGGATACATAGAGGTTTCCTT-3'	

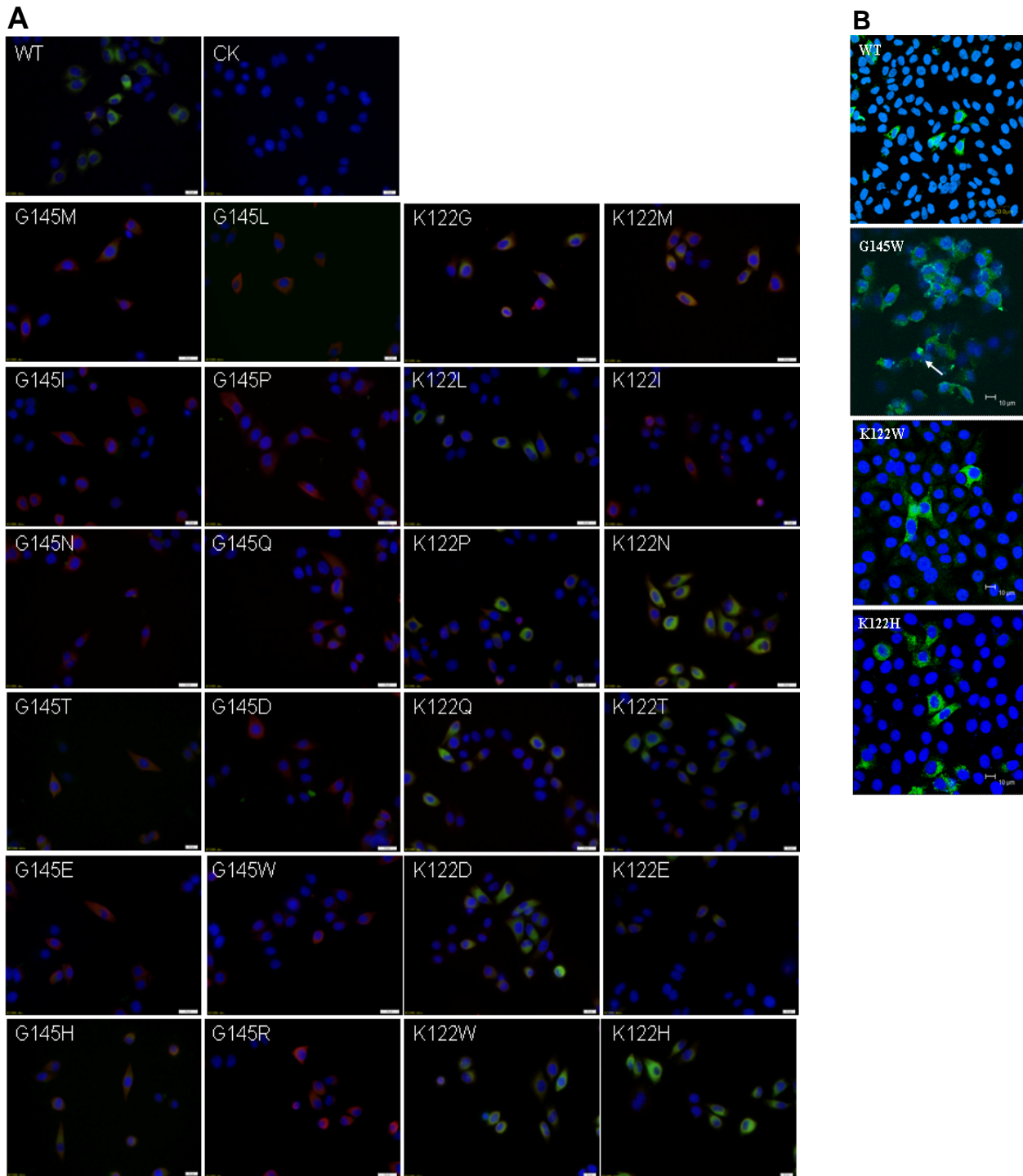
The nt position is given according to the reference sequence with the Genbank number of AF282918.

## Supplementary Fig. 1



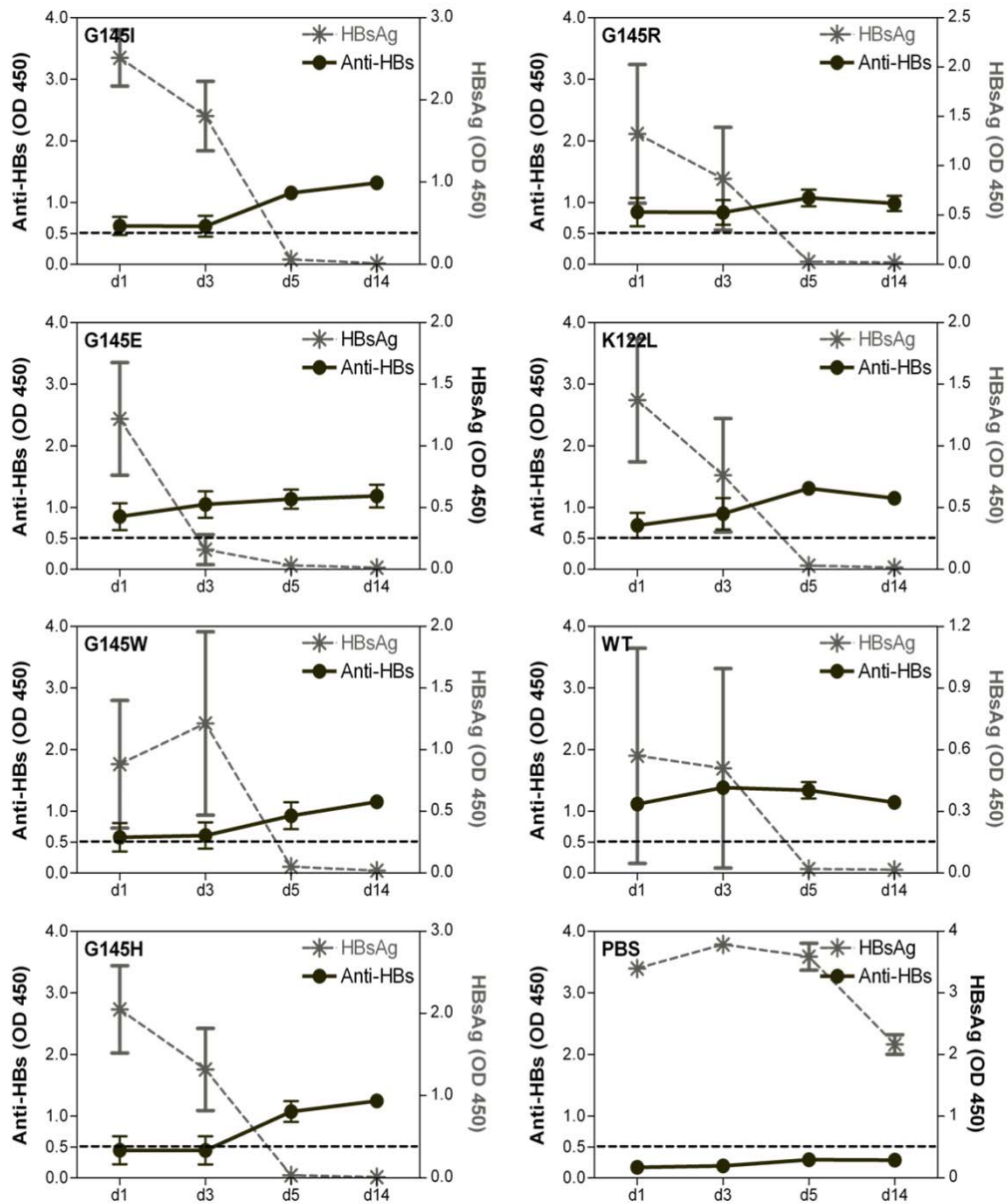
**Supplementary Fig. 1. Reactivity of vtHBsAg to monoclonal anti-HBs antibody specific to HBsAg with G145R substitution.** The culture supernatants of transiently transfected cells expressing wt- and vtHBsAgs were collected at 48 h. Wt- and vtHBsAgs were detected by ELISAs based on the monoclonal anti-HBs antibody anti-G145R, which is specific to HBsAg with G145R substitution. The reactivity of vtHBsAgs with the aa substitutions at the position of 145 was expressed as the ratio of samples to the negative control (S/N ratio) with  $S/N \geq 2.1$  as cut off value. The average value of 4 replicates was calculated and given as the final reactivity of wt or vtHBsAgs. CK: cells transfected with empty plasmid as a negative control.

## Supplementary Fig. 2



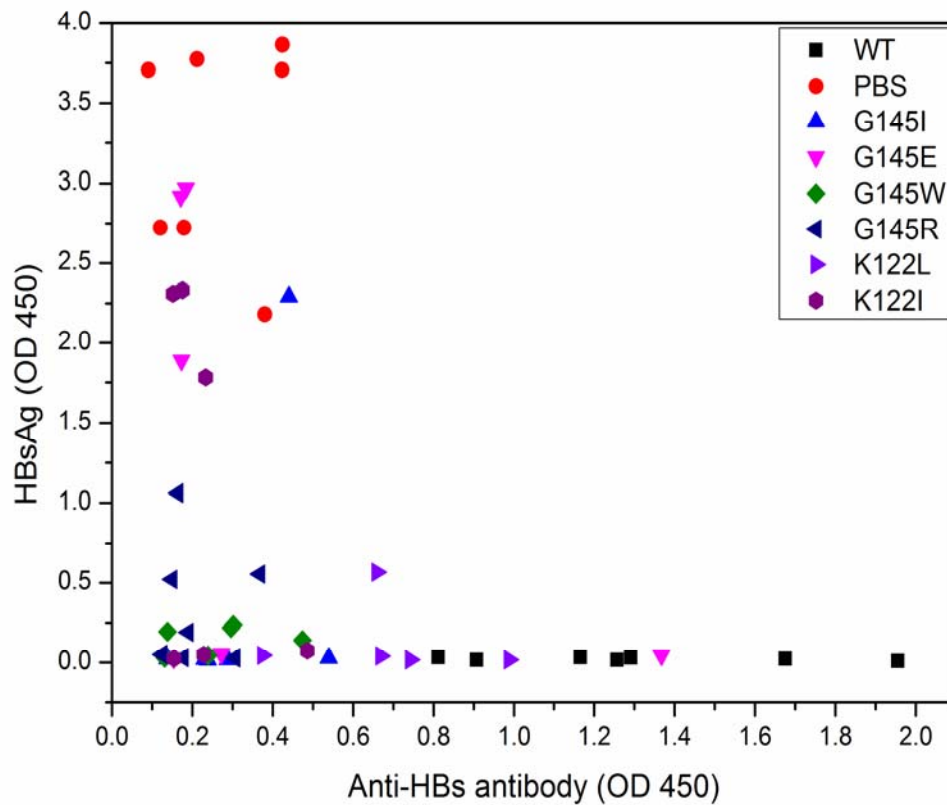
**Supplementary Fig. 2. The subcellular localization of wt- and vtHBsAg with the aa substitutions at the position of 145 or 122.** HeLa cells were transfected with the expression plasmids pHBsAgWT, pHBsAgG145X, or pHBsAgK122X, respectively. Cells were fixed at 48 h after transfection and stained with a monoclonal antibody to HA tag and an anti-HBs monoclonal antibody S1 as primary antibodies and fluoresceine isothiocyanate (FITC)- and Rhodamine-labeled rabbit antisera to mouse IgG (Novagen and Sigma-Aldrich) as secondary antibodies, respectively. The stained cells were analyzed by System Microscope (Olympus BX53, Japan) (A). Cell were stained with an anti-HBs monoclonal antibody S1 as primary antibodies and fluoresceine isothiocyanate (FITC)-labeled rabbit antisera to mouse IgG (Novagen and Sigma-Aldrich) as secondary antibodies for lasal confocal scanning microscopy (B). The nuclei were stained with Hoechst 33258. Magnification 400 $\times$ . ck: cells transfected with empty plasmid as a negative control. The arrow denoted dot-like distribution.

## Supplementary Fig.3



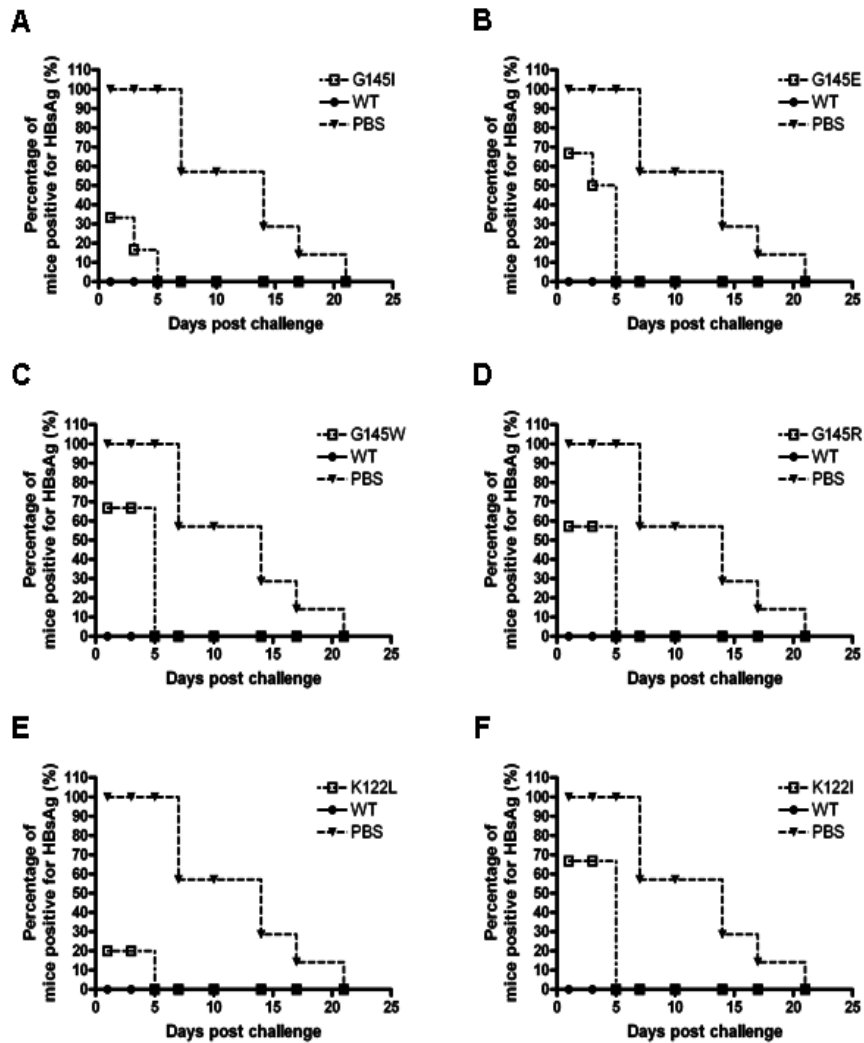
**Supplementary Fig. 3. Kinetics of HBsAg and anti-HBs antibody response in pre-immunized mice after HI of a different genotype/serotype.** The hydrodynamic injection with pAAV-HBV-GA1.3 containing 1.3-fold over-length HBV genotype A genome was carried out with immunized mice at day 14 after the last immunization. Sera were collected at 1 day post infection (d.p.i.), 3 d.p.i., 5 d.p.i. and 14 d.p.i.. HBsAg or anti-HBs were detected by ELISAs. The HBsAg level was presented by optical density value (OD 450). The anti-HBs antibody level was presented by optical density value (OD 450) with cut off value, which was assumed to be 2.1-fold the mean value of the negative samples. The hydrodynamic injection with pAAV-HBV-GA1.3 was performed with six mice per group.

## Supplementary Fig. 4



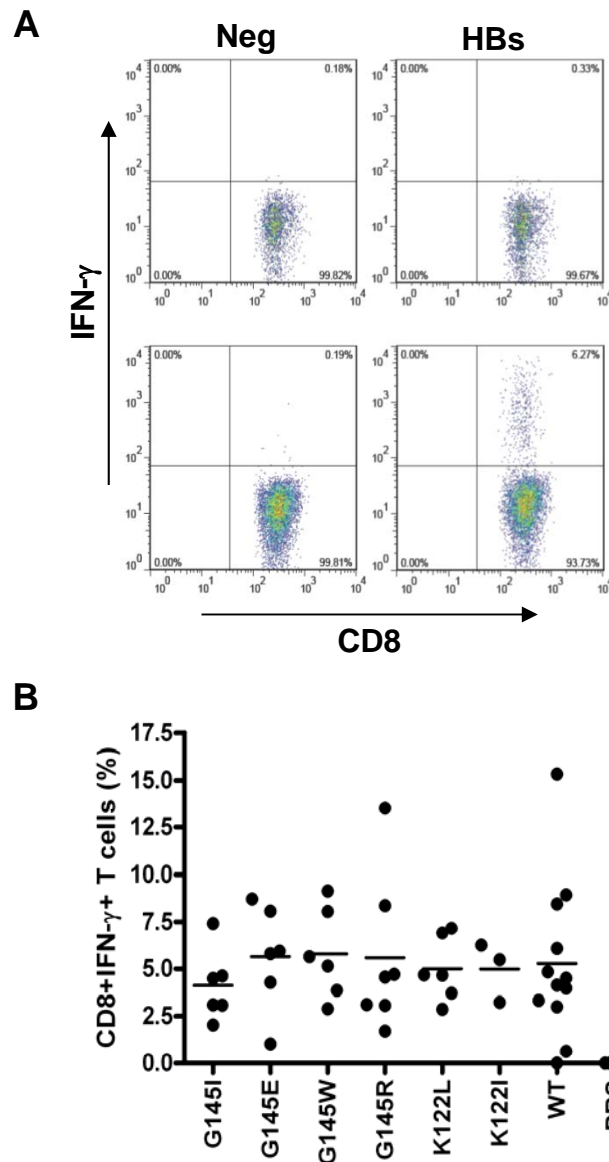
**Supplementary Fig. 4. The correlation of HBsAg and anti-HBs antibody in mouse sera.** The anti-HBs antibody levels in mouse sera before challenge with pAAV-HBV-GB1.3 are presented on the X-axis. The HBsAg levels in mouse sera at day 3 post infection with pAAV-HBV-GB1.3 are presented on the Y-axis.

# Supplementary Fig. 5



**Supplementary Fig. 5. The percentage of mice positive for HBsAg after challenge with pAAV-HBV-GB1.3. The percentage of mice positive for HBsAg immunized with vtHBsAgs including G145I (A), G145E (B), G145W (C), G145R (D), K122L (E) and K122I (F) was given at indicated time points after HI with pAAV-HBV-GB1.3.**

## Supplementary Fig.6



**Supplementary Fig. 6. VtHBsAgS with aa substitutions at the position 145 or 122 retained the ability to induce HBsAg-specific CTL responses.** BALB/c (H-2L<sup>d</sup>) mice were immunized with 30  $\mu$ g of the expression plasmids pHBsAgWT (n=12), pHBsAgG145I (G145I) (n=6), pHBsAgG145E (G145E) (n=6), pHBsAgG145W (G145W) (n=6), pHBsAgG145R (G145R) (n=7), pHBsAgK122L (K122L) (n=6), pHBsAgK122I (K122I) (n=3) or with 30  $\mu$ l of PBS (n=8) as control, respectively. The immunizations were given three times within 3-week intervals. PBMC were isolated from mice on day 7 after the last immunization. Cells from naïve (upper panel) or immunized mice (lower panel) were stimulated with or without peptide derived from HBs for 5 h, which is H-2L<sup>d</sup> restricted CTL epitope aa 29-38 (Ipqslswwtsl) (2  $\mu$ g/ml), and IFN- $\gamma$  secreting cells were analyzed by flow cytometry and presented in the upper part A. The percentage of IFN- $\gamma$  secreting cells to CD8+ T cells in each group was presented in the lower part B.