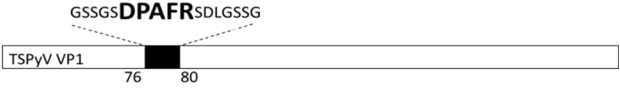
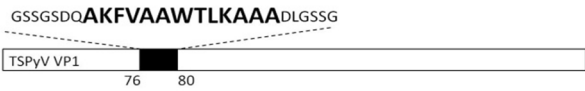
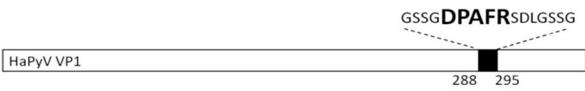
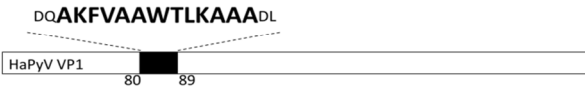


Supplementary Information

Table S1. The list of recombinant chimeric proteins with inserted HBV preS1 (PS1) and PADRE peptides.

	Construct Insert position Insert Target peptide Charge Attribute	TSVP1-PS1-1 #1 GSSGS DPAFR SDLGSSG DPAFR -1 acidic
	Construct Insert position Insert Target peptide Charge Attribute	TSVP1-PS1-4 #4 GSG DPAFR SDLGSG DPAFR -1 acidic
	Construct Insert position Insert Target peptide Charge Attribute	TSVP1-PADRE-1 #1 GSSGS DQAKFVAAWTLKAAAD LGSSG AKFVAAWTLKAAA 0 neutral
	Construct Insert position Insert Target peptide Charge Attribute	TSVP1-PADRE-4 #4 GSG DQAKFVAAWTLKAAAD LGSG AKFVAAWTLKAAA 0 neutral
	Construct Insert position Insert Target peptide Charge Attribute	HaVP1-PS1-1 #1 DPAFRSDL DPAFR -1 acidic
	Construct Insert position Insert Target peptide Charge Attribute	HaVP1-PS1-4 #4 GSSG DPAFRSDL GSSG DPAFR -1 acidic
	Construct Insert position Insert Target peptide Charge Attribute	HaVP1-PADRE-1 #1 DQAKFVAAWTLKAAADL AKFVAAWTLKAAA 0 neutral
	Construct Insert position Insert Target peptide Charge Attribute	HaVP1-PADRE-4 #4 GSSG DQAKFVAAWTLKAAAD LGSSG AKFVAAWTLKAAA 0 neutral

* Charge index was determined using GenScript Peptide Property Calculator (GeneScript). Hydrophobic uncharged aa residues are indicated in green, basic aa residues in blue, acidic aa residues in red and other aa residues in black.

Table S2. Isotypes and antigen-binding activity of MAbs raised against TSPyV VP1 VLPs as determined by an indirect ELISA and Western blot.

Clone	Isotype	MAb Reactivity with:					
		TSPyV VP1 (Western Blot)	TSPyV VP1 (ELISA)	TSVP1-PS1-1 (ELISA)	TSVP1-PADRE-1 (ELISA)	TSVP1-PS1-4 (ELISA)	TSVP1-PADRE-4 (ELISA)
2B4	IgG2a	-	+	-	-	+	+
7A2	IgG1	-	+	+	+	+	+
9F2	IgG1	-	+	+	+	+	+
9D3	IgG1	-	+	+	+	+	+
17G11	IgG2a	-	+	+	+	+	+
18A7	IgG2a	-	+	+	+	-	-
19E4	IgG1	-	+	+	+	+	+
20D3	IgG1	-	+	+	+	+	+
5E6	IgG2a	-	+	-	-	-	-
16H6	IgG2a	-	+	+	+	+	+
3C5	IgG1	+	+	+	+	+	+
4A1	IgG1	+	+	+	+	+	+
8C4	IgG1	+	+	+	+	+	+
11F12	IgG1	+	+	+	+	+	+
16A5	IgG1	+	+	+	+	+	+
17A5	IgG1	+	+	+	+	+	+
19A5	IgG2a	+	+	+	+	+	+

Western blot reactivity: (+) positive, stained protein band of expected molecular weight; (−) negative, no protein band stained; The reactivity in ELISA: (+) positive, OD > 1.0; (−) negative, OD < 0.1; For the ELISA test, optimal dilution (1:20) of hybridoma supernatants was used at which the highest specific immunostaining and no background staining was observed.

Table S3. Titers of VLP-specific (rows 1–10) and insert-specific (rows 11–12) IgG antibodies determined by an indirect ELISA after a primary immunization in groups of BALB/c mice (n = 3) immunized with different chimeric VLPs emulsified in a complete Freund adjuvant.

No.	Antigen in ELISA:	Antigen Used for Immunization:							
		TSVP1-PS1-1	TSVP1-PS1-4	TSVP1-PADRE-1	TSVP1-PADRE-4	HaVP1-PS1-1	HaVP1-PS1-4	HaVP1-PADRE-1	HaVP1-PADRE-4
1.	TSVP1-PS1-1	108,200	82,600						
2.	TSVP1-PS1-4	87,500	104,600						
3.	TSVP1-PADRE-1			31,100	3600				
4.	TSVP1-PADRE-4			4900	950				
5.	HaVP1-PS1-1					47,257	7100		
6.	HaVP1-PS1-4					14,900	16,400		
7.	HaVP1-PADRE-1							98,400	104,200
8.	HaVP1-PADRE-4							7500	31,200
9.	TSPyV VP1	21,500	30,900	14,300	900				
10.	HaPyV VP1					4100	3600	29,000	15,600
11.	PS1 peptide	2600	3900			1200	400		
12.	PADRE peptide			1300	300			4700	14,000

Antibody titer is defined as the reciprocal of the highest antiserum dilution giving OD₄₅₀ value greater than three times the background that corresponds to the mean OD₄₅₀ + 3SD of a the preimmune serum diluted 1:100.