Supplemental Material

Patient characteristics

HBeAg, serum HBV DNA and AST/ALT values were assessed as part of the clinical diagnostics at the University Hospital Freiburg, Germany. Confirmation of HLA-A*02:01-, HLA-A*01:01 and HLA-A*11:01 was performed by four-digit HLA-typing by next generation sequencing.

SI Table 1: Study cohort of acute HBV infection.

	Patient ID	Sex	Age (years)	Viral Load [IU/ml]	AST [U/L]	ALT [U/L]	HBsAg Status	HBcAg Status	Therapy	Genotype	Sequences		detectable CD8+ T-cell response	
											$core_{18}$	pol_{455}	$core_{18}$	pol_{455}
HLA-A*02:01	aHBV #1	m	33	4722	nd	3400	pos	pos	naive	nd	----------	---------	$core_{18}$	pol_{455}
	aHBV #2	m	43	1411742	nd	3675	pos	pos	naive	nd	----------	---------	$core_{18}$	pol_{455}
	aHBV #3	f	42	6111	nd	3366	pos	pos	naive	nd	----------	---------	$core_{18}$	pol_{455}

Abbreviations: ALT: alanine aminotransaminase, AST: aspartate aminotransferase, f: female, HBsAg: Hepatitis B virus surface antigen, m: male, pos: positive

SI Table 2: Study cohort of HBeAg+ chronic hepatitis B (HBeAg+ CHB).

	Patient ID	Sex	Age (years)	Viral Load [IU/ml]	AST [U/L]	ALT [U/L]	HBeAg Status	Therapy	Genotype	Sequences		detectable CD8+ T-cell response	
										$core_{18}$	pol_{455}	$core_{18}$	pol_{455}
HLA-A*02:01	HBV #1*	m	78	155000000	118	159	pos	Entecavir	D	----------	---------	/	pol_{455}
	HBV #2	f	31	168344275	75	166	pos	naive	D	----------	---------	$core_{18}$	/
	HBV #3	m	61	32	nd	nd	pos	Tenofovir	A	nd	---------		pol_{455}
	HBV #4	m	59	88959230	160	322	pos	naive	nd	----------	---------	/	pol_{455}
	HBV #5	f	52	90.4	38	86	pos	Tenofovir	nd	----------	---------	/	pol_{455}

Abbreviations: ALT: alanine aminotransaminase, AST: aspartate aminotransferase, f: female, HBeAg: Hepatitis B virus envelope antigen, m: male, nd: not done, pos: positive

* recently started therapy

SI Table 3: Study cohort of HBeAg- chronic hepatitis B (HBeAg- CHB).

	Patient ID	Sex	Age (years)	Viral Load [IU/ml]	AST [U/L]	ALT [U/L]	HBeAg Status	Therapy	Genotype	Sequences		detectable CD8+ T-cell response	
										$core_{18}$	pol_{455}	$core_{18}$	pol_{455}
HLA-A*02:01	HBV #6*	m	54	20209	34	28	neg	naive	D	---------I	---------		pol_{455}
	HBV #7*	m	43	35590	152	387	neg	naive	D	---T----N-	---------		pol_{455}
	HBV #8*	f	54	7762471	92	130	neg	naive	A	------Y---	---------		pol_{455}
	HBV #9*	f	61	92090	48	72	neg	naive	nd	---------I	---------		pol_{455}
	HBV #10	m	35	3185613	35	47	neg	naive	A	----------	---------	$core_{18}$	nd
	HBV #11	m	41	11144	40	107	neg	naive	D	---Q----NL	---------		pol_{455}
	HBV #6*	m	54	neg	64	42	neg	Tenofovir	nd	---------I	---------		pol_{455}
	HBV #7*	m	43	10	25	27	neg	Tenofovir	nd	---T----N-	---------		pol_{455}
	HBV #8*	f	54	neg	31	31	neg	Entecavir	A	------Y---	---------		pol_{455}
	HBV #9*	f	61	neg	30	36	neg	Tenofovir	nd	---------I	---------		pol_{455}
	HBV #12**	m	76	neg	17	14	neg	Tenofovir	A	----------	---------	$core_{18}$	pol_{455}
	HBV #13	f	60	neg	28	29	neg	Tenofovir	nd	----------	---------	$core_{18}$	nd
	HBV #14	m	55	10	27	37	neg	Tenofovir	nd	----------	---------	$core_{18}$	pol_{455}
	HBV #15	m	52	10	29	32	neg	Entecavir	D	----------	---------	$core_{18}$	nd
	HBV #16	f	37	neg	29	37	neg	Tenofovir	nd	----------	---------	$core_{18}$	pol_{455}
	HBV #17	f	65	neg	27	23	neg	Entecavir	nd	---P-----I	---------		pol_{455}
	HBV #18	f	65	neg	23	22	neg	Tenofovir	nd	----------	---------	$core_{18}$	nd
	HBV #19	m	45	17	28	29	neg	Tenofovir	nd	nd	---------		pol_{455}
	HBV #20	m	36	52	21	35	neg	Entecavir	nd	----------	---------	nd	pol_{455}
	HBV #21	m	38	neg	25	34	neg	Tenofovir	nd	----------	---------	$core_{18}$	nd
	HBV #22	m	59	neg	27	28	neg	Telbivudine	nd	--------A-	---------		pol_{455}
	HBV #23	f	58	neg	nd	21	neg	Tenofovir	nd	----------	---------	nd	pol_{455}
	HBV #24	m	77	1455	27	23	neg	Tenofovir	nd	----------	---------	$core_{18}$	/
	HBV #25	m	63	neg	nd	52	neg	Tenofovir	nd	----------	---------	$core_{18}$	nd
	HBV #26	f	69	25	44	67	neg	Entecavir	D	----------	---G-----	$core_{18}$	
	HBV #27	m	30	10	36	22	neg	Entecavir	D	----------	nd	$core_{18}$	
	HBV #28	m	79	neg	25	24	neg	Entecavir	nd	----------	nd	$core_{18}$	

Abbreviations: ALT: alanine aminotransaminase, AST: aspartate aminotransferase, f: female, HBeAg: Hepatitis B virus envelope antigen, m: male, neg: negative, nd: not done

*included in longitudinal study shown in Fig. 1E.

** included in longitudinal study shown in Fig. 1D

SI Table 4: Study cohort of HBeAg-chronic HBV infection (HBeAg- cHBV).

	Patient ID	Sex	Age (years)	Viral Load [IU/ml]	AST [U/L]	ALT [U/L]	HBeAg Status	Therapy	Genotype	Sequences		detectable CD8+ T-cell response	
										$core_{18}$	pol_{455}	$core_{18}$	pol_{455}
HLA-A*02:01	HBV #29	f	48	1458	22	27	neg	naive	D	----------	---------	$core_{18}$	pol_{455}
	HBV #30	f	55	660	29	30	neg	naive	D	----------	--PG-----	$core_{18}$	
	HBV #31	f	59	27	23	22	neg	naive	nd	----------	nd	$core_{18}$	
	HBV #32	m	34	5456	25	36	neg	naive	D	----------	---------	$core_{18}$	/
	HBV #33	m	55	29	nd	30	neg	naive	nd	----------	---------	$core_{18}$	*nd*
	HBV #34	f	36	1003	19	17	neg	naive	nd	----------	---------	$core_{18}$	/
	HBV #35	f	45	26	nd	13	neg	naive	nd	----------	---------	$core_{18}$	/
	HBV #36	m	33	2095	25	27	neg	naive	nd	----------	---------	nd	pol_{455}
	HBV #37	f	37	902	25	20	neg	naive	D	----------	---------	$core_{18}$	nd
	HBV #38	m	54	789	35	41	neg	naive	nd	----------	---------	$core_{18}$	nd
	HBV #39	m	64	1480	nd	30	neg	naive	nd	---H--Y---	---------		pol_{455}
	HBV #40	f	30	31	23	19	neg	naive	nd	----------	nd	$core_{18}$	
	HBV #41	f	37	322	23	20	neg	naive	nd	----------	---------	$core_{18}$	pol_{455}
	HBV #42	m	37	6998	18	33	neg	naive	nd	---------I	---------		pol_{455}
	HBV #43	f	35	59	25	24	neg	naive	nd	----------	nd	$core_{18}$	
	HBV #44	f	32	134	24	23	neg	naive	nd	----------	nd	$core_{18}$	
	HBV #45	f	39	1585	nd	13	neg	naive	nd	nd	---------		pol_{455}
	HBV #46	f	40	82	24	23	neg	naive	D	----------	nd	$core_{18}$	

Abbreviations: ALT: alanine aminotransaminase, AST: aspartate aminotransferase, f: female, HBeAg: Hepatitis B virus envelope antigen, m: male, neg: negative, nd: not done

SI Table 5: Study cohort of HLA-A*02 negative patients with chronic HBV infection.

	Patient ID	Sex	Age (years)	Viral Load [IU/ml]	AST [U/L]	ALT [U/L]	HBeAg Status	Therapy	Genotype	Sequences		detectable CD8+ T-cell response	
										$core_{30}$	$core_{141}$	$core_{30}$	$core_{141}$
HLA-A*11:01	HBV #47	f	34	neg	nd	nd	neg	naive	D		----------		$core_{141}$
	HBV #48	m	60	neg	22	25	neg	Tenofovir	D		----------		$core_{141}$
	HBV #49	f	45	47	nd	25	neg	Tenofovir	B		----------		$core_{141}$
HLA-A*01:01	HBV #50	m	48	neg	23	25	neg	naive	nd	---------		$core_{30}$	
	HBV #51	m	47	2402	22	10	neg	naive	D	---------		$core_{30}$	
	HBV #52	f	38	neg	nd	37	neg	Tenofovir	D	---------		$core_{30}$	
	HBV #53	m	46	neg	14	19	neg	Tenofovir	nd	---------		$core_{30}$	

Abbreviations: ALT: alanine aminotransaminase, AST: aspartate aminotransferase, f: female, HBeAg: Hepatitis B virus envelope antigen, m: male, neg: negative

SI Table 6: Study cohort of acute resolved HBV infection.

	Patient ID	Sex	Age (years)	Viral Load [IU/ml]	AST [U/L]	ALT [U/L]	HBcAg	HBsAg	Anti-HBs	Therapy	detectable CD8+ T-cell response	
											$core_{18}$	pol_{455}
HLA-A*02:01	arHBV #1	m	58	neg	25	21	pos	neg	pos	naive	$core_{18}$	pol_{455}
	arHBV #2	m	42	neg	120	246	pos	neg	pos	naive	$core_{18}$	pol_{455}
	arHBV #3	m	58	neg	37	40	pos	neg	pos	naive	$core_{18}$	pol_{455}
	arHBV #4	f	71	neg	19	11	pos	neg	pos	naive	/	pol_{455}

Abbreviations: ALT: alanine aminotransaminase, AST: aspartate aminotransferase, f: female, HBcAg: Hepatitis B virus core antigen, HBsAg: Hepatitis B virus surface antigen, m: male, neg: negative, pos: positive

SI Table 7: Study cohort of chronic resolved HBV infection.

	Patient ID	Sex	Age (years)	Viral Load [IU/ml]	AST [U/L]	ALT [U/L]	HBcAg	HBsAg	Anti-HBs	Therapy	detectable CD8+ T-cell response	
											$core_{18}$	pol_{455}
HLA-A*02:01	crHBV #1*	f	34	neg	20	20	pos	neg	pos	naive	$core_{18}$	pol_{455}
	crHBV #2	m	76	neg	24	23	pos	neg	pos	Tenofovir	$core_{18}$	pol_{455}

Abbreviations: ALT: alanine aminotransaminase, AST: aspartate aminotransferase, f: female, HBcAg: Hepatitis B virus core antigen, HBsAg: Hepatitis B virus surface antigen, m: male, neg: negative, pos: positive

** is equallent to HBV #12 and is included in longitudinal study shown in Fig. 1D

SI Table 8: Study cohort of chronic HCV infection.

	Patient ID	Sex	Age (years)	Viral Load [IU/ml]	AST [U/L]	ALT [U/L]	Therapy	Genotype	Sequences		detectable CD8+ T-cell response	
									$NS3_{1073}$	$NS3_{1406}$	$NS3_{1073}$	$NS3_{1406}$
HLA-A*02:01	HCV #1	m	56	91000000	67	73	naive	1a	---------	nd	$NS3_{1073}$	
	HCV #2	f	57	73000000	26	26	naive	1b	---------	nd	$NS3_{1073}$	
	HCV #3	m	38	329000000	88	208	naive	1b	---------	nd	$NS3_{1073}$	
	HCV #4	m	62	1082000000	50	73	naive	1a	nd	----------		$NS3_{1406}$
	HCV #5	m	45	195000000	62	116	naive	1a	---------	nd	$NS3_{1073}$	
	HCV #6	f	22	294000000	80	94	naive	1a	nd	----------		$NS3_{1406}$
	HCV #7	f	55	127000000	40	48	naive	1a	---------	nd	$NS3_{1073}$	
	HCV #8	m	57	3090000	nd	70	naive	1a	---------	nd	$NS3_{1073}$	
	HCV #9	m	41	4010000	nd	nd	naive	1a	---------	nd	$NS3_{1073}$	
	HCV #10	m	49	840000	65	104	naive	1a	nd	----------		$NS3_{1406}$
	HCV #11	m	55	2030000	107	151	naive	1a	---------	nd	$NS3_{1073}$	
	HCV #12	f	73	6870000	44	47	naive	1b	---------	nd	$NS3_{1073}$	

Abbreviations: ALT: alanine aminotransaminase, AST: aspartate aminotransferase, f: female, m: male

SI Table 9: Study cohort of healthy controls.

	Patient ID	Sex	Age (years)	Detectable CD8+ T-cell response		
				CMV	EBV	FLU
HLA-A*02:01	HD #1	m	54	*nd*	EBV	FLU
	HD #2	m	31	*nd*	*nd*	FLU
	HD #3	m	30	*nd*	EBV	FLU
	HD #4	f	37	CMV	EBV	FLU
	HD #5	f	30	*nd*	EBV	FLU
	HD #6	f	34	*nd*	nd	FLU
	HD #7	f	31	*nd*	EBV	FLU
	HD #8	m	37	*nd*	EBV	*nd*
	HD #9	f	33	*nd*	*nd*	FLU
	HD #10	f	26	*nd*	*nd*	FLU
	HD #11	m	68	CMV	EBV	*nd*
	HD #12	m	40	CMV	EBV	FLU
	HD #13	m	81	CMV	*nd*	*nd*
	HD #14	f	26	*nd*	*nd*	FLU
	HD #15	f	36	CMV	*nd*	*nd*
	HD #16	f	29	*nd*	EBV	FLU
	HD #17	f	25	*nd*	EBV	FLU
	HD #18	f	25	CMV	/	*nd*
	HD #19	f	25	CMV	EBV	FLU
	HD #20	f	29	CMV	*nd*	*nd*
	HD #21	f	44	CMV	*nd*	*nd*
	HD #22	f	36	FLU	*nd*	*nd*
	HD #23	m	25	/	EBV	FLU
	HD #24	m	61	CMV	*nd*	nd
	HD #25	f	26	*nd*	*nd*	FLU
	HD #26	f	29	*nd*	EBV	nd
	HD #27	m	26	CMV	*nd*	*nd*
	HD #28	f	53	CMV	*nd*	*nd*
	HD #29	m	31	CMV	*nd*	*nd*
	HD #30	m	19	*nd*	EBV	FLU
	HD #31	m	26	EBV	EBV	FLU

Abbreviations: f: female, m: male, nd: not done

Supplemental experimental procedures

PBMC isolation. Peripheral blood mononuclear cells (PBMCs) were isolated from EDTA anticoagulated blood samples using Pancoll (Pan-Biotech, Germany) density gradient centrifugation. Frozen PBMCs were thawed in complete medium (RPMI 1640 with 10% fetal bovine serum, 1% penicillin/streptomycin and 1.5% 1M HEPES (all Thermo Fisher, Germany) and incubated for 15 min at 37 °C in complete medium containing 50 U/mL benzonase (Sigma, Germany) before further processing.

Viral sequencing (HBV). Viral DNA was extracted from 1 ml patient's plasma at the used bleed-date or latest available HBV DNA positive time point using QIAamp UltraSens technology (Qiagen, Germany) according to the manufacturer's protocol. A two-step nested polymerase chain reaction (PCR) approach was used to amplify HBV DNA fragments for viral sequence analyses of HBV$core_{18}$- and HBVpol_{455}-epitopes. Specific primers used for amplifications are listed in table 9 below. Purified PCR products were sequenced via Sanger sequencing (Eurofins, Germany). Sequences and chromatograms were evaluated with the program Geneious (Biomatters, New Zealand), allowing comparison with wildtype reference sequences (NCBI accession codes: genotype A X02763, genotype D X02496, genotype E X75657).

SI Table 9: Oligonucleotides used for viral sequencing (HBV).

Epitope	PCR	Forward Primer	Reverse Primer
$core_{18}$	**1st**	CACCTCTGCCTAATCATCTC	CCGGAAGTGTTGATAAGATAGG
	2nd	ACTGTTCAAGCCTCCAAGCTG	GAGGAGTGCGAATCCACACTC
pol_{455}	**1st**	ACCAAACTCTGCARGATCCCAG	TGGTGGCTCCAGTTCAGGAAC
	2nd	TGGTGGCTCCAGTTCAGGAAC	ATCAATAGGCCTGTTAACAGGAAG

Viral sequencing (HCV). RNA was extracted from patient's plasma using the QIAmp viral RNA minikit (Qiagen, Germany). Reverse transcription was performed according to the manufacturer instructions (RT; SuperScript III First-Strand Kit, Invitrogen, Germany) followed by DNA amplification via nested PCR. Specific primers used for amplifications are listed in table 10 below. Purified PCR products were sequenced via Sanger sequencing (Eurofins, Germany).

SI Table 10: Oligonucleotides used for viral sequencing (HCV).

	Epitope	PCR	Forward Primer	Reverse Primer
Genotype 1a	$NS3_{1073}$	1st	CGTCTGCTCCTGCTTGTGG	ATCCGTGGARTGGCACTCR
		2nd	ATGTGGCCTCTCCTCCTGC	GCCACCTGGAAGCTCTGGG
	$NS3_{1406}$	1st	GACAAAAACCARGYGGAGGG	GAGGACCTTCCCCAGYCC
		2nd	ATAGCAGGGGYAGCCTGC	AGCACAGCCYGCGTCATAGC
Genotype 1b	$NS3_{1073}$	1st	GCCGCGATGCCATCATCC	CATTAGAGCGTCTGTTGC
		2nd	TTGCGGTGGCAGHAGAGC	CGCCCGTGGTGATGGTCC
	$NS3_{1406}$	1st	ACAAGAACCAGGTCGAGGG	TCTGCTTGAAYTGCTCGG
		2nd	CCTACYTGAAGGGCTCYTCGGG	GGTGTATTTAGGTAAGCCCG

Multiparametric flow cytometry. The following reagents were used for multi-parametric flow cytometry: anti-CD57 (NK-1, 1:20) (Beckman Coulter, USA), anti-CD14 (61D3, 1:100), anti-CD19 (HIB19, 1:100), anti-Eomes (WD1928, 1:50), anti-KLRG1 (13F12F2, 1:50), anti-Tbet (4B10, 1:200), anti-TOX (TRX10, 1:100) (all eBioscience, US), anti-CCR7 (150503, 1:50), anti-CD8 (RPA-T8, 1:100), anti-CD8 (SK1, 1:100), anti-CD28 (CD28.2, 1:100), anti-CD107a (H4A3, 1:100), anti-IFNγ (4S.B3, 1:8), anti-TNF (MAb11, 1:50) (all BD Bioscience, Germany), anti-BCL2 (Bcl-2/100, 1:50), anti-CCR7 (G043H7, 1:33), anti-CD45RA (HI100, 1:200), anti-CD127 (A019D5, 1:33), anti-Helios (22F6, 1:20), anti-PD1 (EH12.2H7, 1:33), anti-Rabbit IgG (Poly4064, 1:200) (all BioLegend, USA), anti-TCF1 (C63D9, 1:100) (Cell signaling, USA). Fixable Viability Dyes eFluor780 (1:200) (eBioscience, Germany) and 7-AAD (1:33) (BD Biosciences, Germany) were used for live/dead discrimination. Fixation/Permeabilization Solution Kit (BD Biosciences, Germany) and FoxP3/Transcription Factor Staining Buffer Set (eBioscience, Germany) were applied according to the manufacturer's instructions to stain for cytoplasmic and intranuclear molecules, respectively. Cells were fixed with 2% paraformaldehyde (PFA, Sigma, Germany). Analyses were performed using FACSCanto II or LSRFortessa (BD, Germany). We performed a machine and measurement standardization procedure applying the CS&T system (BD Biosciences) to optimize and standardize cytometer setup and to receive reproducible data. Data were evaluated with FlowJo 10 (Treestar, USA). For reliable data interpretation, further

characterization of virus-specific CD8+ T-cell populations only included patients harboring >80% antigen-experienced cells, being either CD45RA+CCR7-, CD45RA-CCR7-, or CD45RA-CCR7+. Moreover, only those antigen-experienced populations with more than 10 cells after pHLA-A*02:01 tetramer enrichment were further considered. Moreover, cut-off for data analysis and interpretation was a minimum of 10 cells per respective subpopulation.

Dimensionality reduction of multiparametric flow cytometry data. The visualization of multiparametric flow cytometry data was done with R using the Bioconductor (CATALYST package (Crowell H, Zanotelli V, Chevrier S, Robinson M (2020). CATALYST: Cytometry dATa anALYSis Tools. R package version 1.12.2, https://github.com/HelenaLC/CATALYST). The analyses were performed on gated TOX+ and TOX- HBV-specific CD8+ T cells as well as TOX+ HBV- versus CMV- and EBV-specific CD8+ T cells and included the markers PD1, CD127, KLRG1, CD57, CD39, CD38, TOX, TCF1, Tbet and Eomes. Down sampling of cells to 1000 was performed prior to dimensionality reduction in order to facilitate the visualization of different samples. Marker intensities were transformed by arcsinh (inverse hyperbolic sine) with a cofactor of 150. Dimensionality reduction on the transformed data was achieved by t-distributed stochastic neighbor embedding (t-SNE) and multidimensional scaling (MDS) using the CATALYST package functions runDR and pbMDS, respectively with default parameters. Importantly, default perplexity value (set to 30) was used for the dimensionality reduction of the datasets using t-SNE.

Expansion of virus-specific CD8+ T cells and assessment of CD8+ T-cell function. PBMCs ($2x10^6$) were stimulated with epitope-specific peptides (10 µg/mL) and anti-CD28 monoclonal antibody (0.5 µg/mL, BD Bioscience, Germany) and expanded at 37 °C for 14 days in complete culture medium containing rIL-2 (20 IU/mL, Stemcell Technologies, Canada). Tetramer staining was performed at day 14. The expansion index (EI) was calculated as previously described [1]. Functional analyses of virus-specific CD8+ T cells were performed after 14 days of *in vitro* expansion. Cells were re-stimulated with epitope-specific peptides (10 µg/mL) in the presence of anti-CD107a (H4A3, 1:100) (BD Bioscience, Germany) for 1 h at 37 °C. Afterwards, brefeldin A (GolgiPlug, 0.5 µL/mL) and monensin (GolgiStop, 0.5 µL/mL) (all BD Biosciences, Germany) were added for additional 5 h. Stimulation with PMA (50 ng/mL) and Ionomycin (1 µg/mL) (all Sigma, Germany) was performed as positive control. Unstimulated controls were used for background subtraction. After incubation, surface and intracellular staining were performed.

1 Wieland D, Kemming J, Schuch A, Emmerich F, Knolle P, Neumann-Haefelin C*, et al.* TCF1(+) hepatitis C virus-specific CD8(+) T cells are maintained after cessation of chronic antigen stimulation. Nature communications 2017;**8**:15050.