

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 | HBeAg 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 |
| 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 |
| 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.

| HLA-A*02:01 | 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 |
| | 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.

| HLA-A*02:01 | 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 |
| | 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 equivalent 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 | <i>nd</i> | EBV | FLU |
| | HD #2 | m | 31 | <i>nd</i> | <i>nd</i> | FLU |
| | HD #3 | m | 30 | <i>nd</i> | EBV | FLU |
| | HD #4 | f | 37 | CMV | EBV | FLU |
| | HD #5 | f | 30 | <i>nd</i> | EBV | FLU |
| | HD #6 | f | 34 | <i>nd</i> | <i>nd</i> | FLU |
| | HD #7 | f | 31 | <i>nd</i> | EBV | FLU |
| | HD #8 | m | 37 | <i>nd</i> | EBV | <i>nd</i> |
| | HD #9 | f | 33 | <i>nd</i> | <i>nd</i> | FLU |
| | HD #10 | f | 26 | <i>nd</i> | <i>nd</i> | FLU |
| | HD #11 | m | 68 | CMV | EBV | <i>nd</i> |
| | HD #12 | m | 40 | CMV | EBV | FLU |
| | HD #13 | m | 81 | CMV | <i>nd</i> | <i>nd</i> |
| | HD #14 | f | 26 | <i>nd</i> | <i>nd</i> | FLU |
| | HD #15 | f | 36 | CMV | <i>nd</i> | <i>nd</i> |
| | HD #16 | f | 29 | <i>nd</i> | EBV | FLU |
| | HD #17 | f | 25 | <i>nd</i> | EBV | FLU |
| | HD #18 | f | 25 | CMV | / | <i>nd</i> |
| | HD #19 | f | 25 | CMV | EBV | FLU |
| | HD #20 | f | 29 | CMV | <i>nd</i> | <i>nd</i> |
| | HD #21 | f | 44 | CMV | <i>nd</i> | <i>nd</i> |
| | HD #22 | f | 36 | FLU | <i>nd</i> | <i>nd</i> |
| | HD #23 | m | 25 | / | EBV | FLU |
| | HD #24 | m | 61 | CMV | <i>nd</i> | <i>nd</i> |
| | HD #25 | f | 26 | <i>nd</i> | <i>nd</i> | FLU |
| | HD #26 | f | 29 | <i>nd</i> | EBV | <i>nd</i> |
| | HD #27 | m | 26 | CMV | <i>nd</i> | <i>nd</i> |
| | HD #28 | f | 53 | CMV | <i>nd</i> | <i>nd</i> |
| | HD #29 | m | 31 | CMV | <i>nd</i> | <i>nd</i> |
| | HD #30 | m | 19 | <i>nd</i> | 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 HBVcore₁₈- and HBVpol₄₅₅-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 |
|--------------------------|-----------------|-------------------------|--------------------------|
| core₁₈ | 1 st | CACCTCTGCCTAATCATCTC | CCGGAAGTGTTGATAAGATAGG |
| | 2 nd | ACTGTTCAAGCCTCCAAGCTG | GAGGAGTGCGAATCCCACTC |
| pol₄₅₅ | 1 st | ACCAAACCTCTGCARGATCCCAG | TGGTGGCTCCAGTTCAGGAAC |
| | 2 nd | 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 | 1 st | CGTCTGCTCCTGCTTGTGG | ATCCGTGGARTGGCACTCR |
| | | 2 nd | ATGTGGCCTCTCCTCCTGC | GCCACCTGGAAGCTCTGGG |
| | NS3 ₁₄₀₆ | 1 st | GACAAAAACCARGYGGAGGG | GAGGACCTTCCCCAGYCC |
| | | 2 nd | ATAGCAGGGGYAGCCTGC | AGCACAGCCYGCGTCATAGC |
| Genotype 1b | NS3 ₁₀₇₃ | 1 st | GCCGCGATGCCATCATCC | CATTAGAGCGTCTGTTGC |
| | | 2 nd | TTGCGGTGGCAGHAGAGC | CGCCCGTGGTGATGGTCC |
| | NS3 ₁₄₀₆ | 1 st | ACAAGAACCAGGTTCGAGGG | TCTGCTTGAAYTGCTCGG |
| | | 2 nd | 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-IFN γ (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 (2×10^6) 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.