Supplementary Materials for

Vaccination to prevent T cell subversion can protect against persistent hepacivirus infection

Hartlage, et al. 2019

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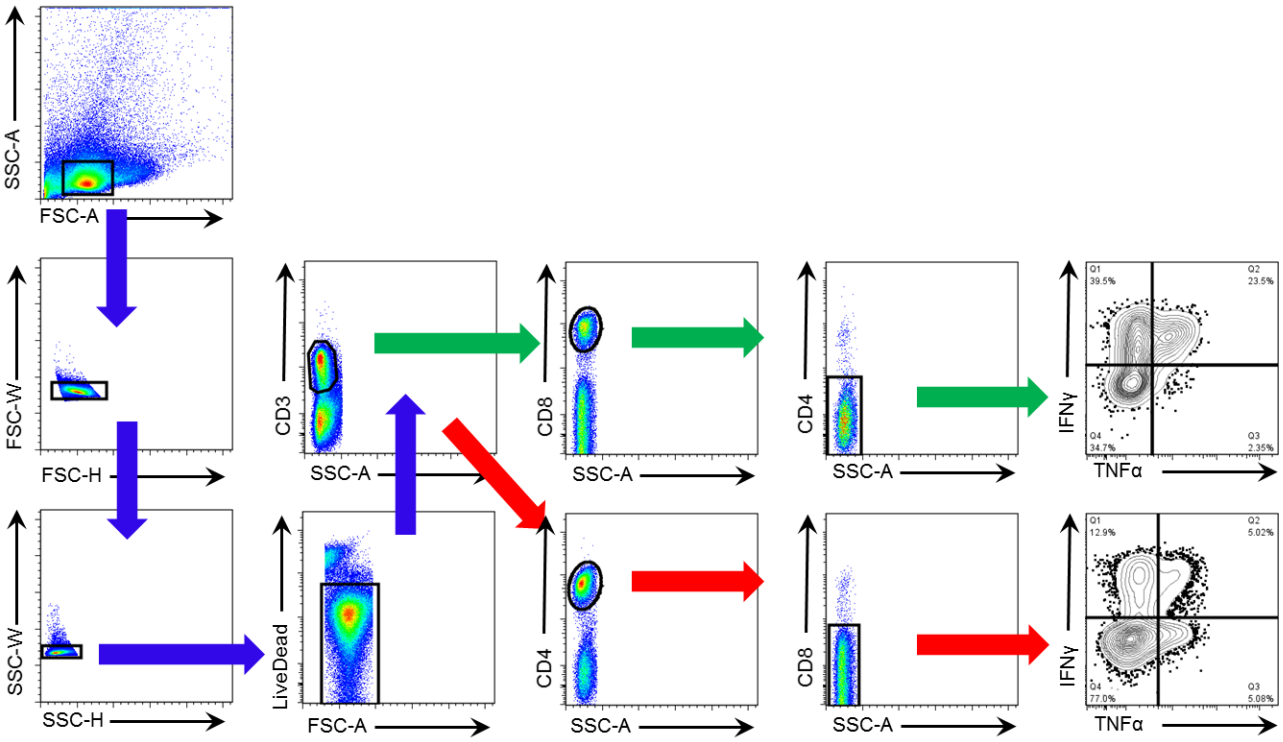


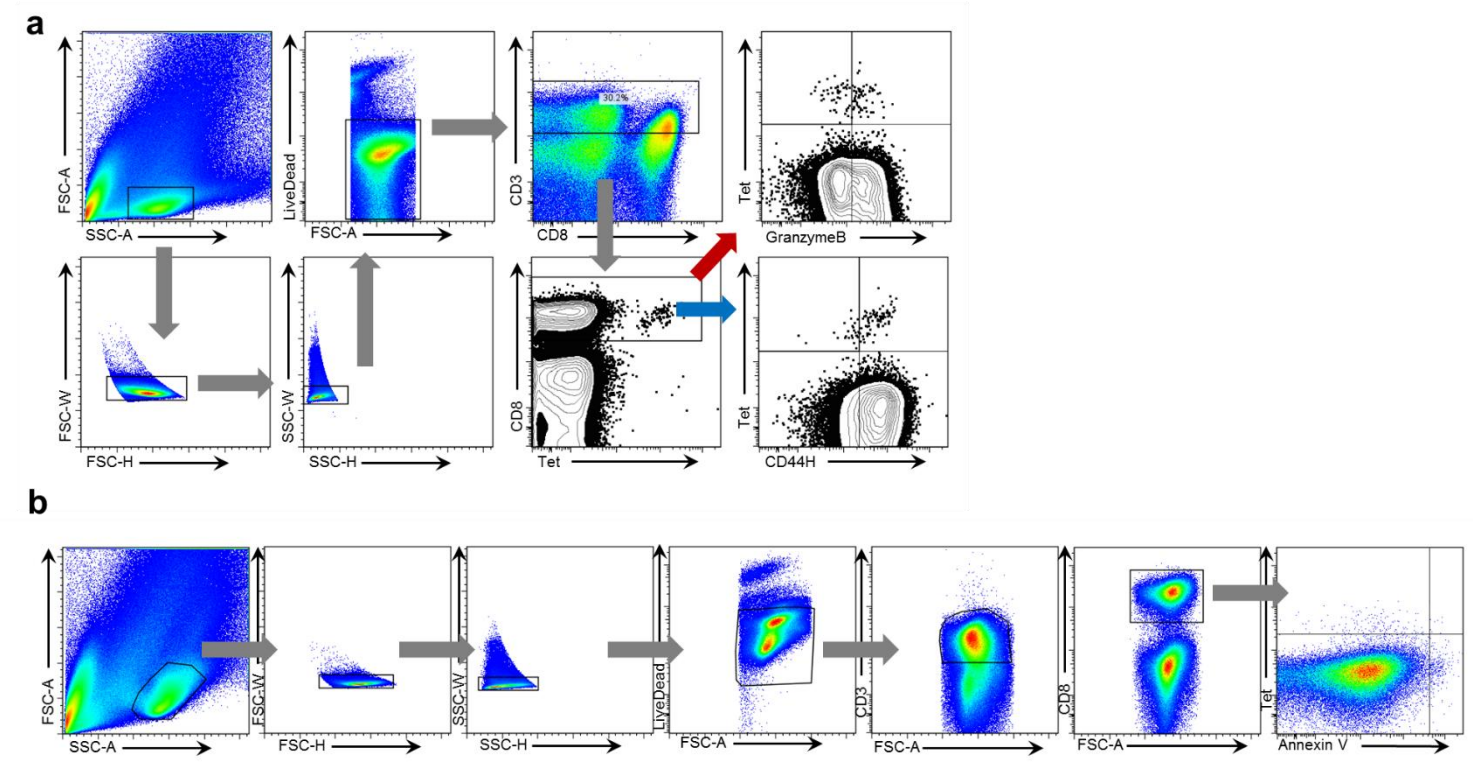
Supplemental Figure 1. Gating strategy for quantification of intracellular cytokines. Shown are intrahepatic mononuclear cells after 5-hr PMA/Ionomycin stimulation in the presence of brefeldin A.

Supplemental Table 1. Core-specific MHC class II epitopes.

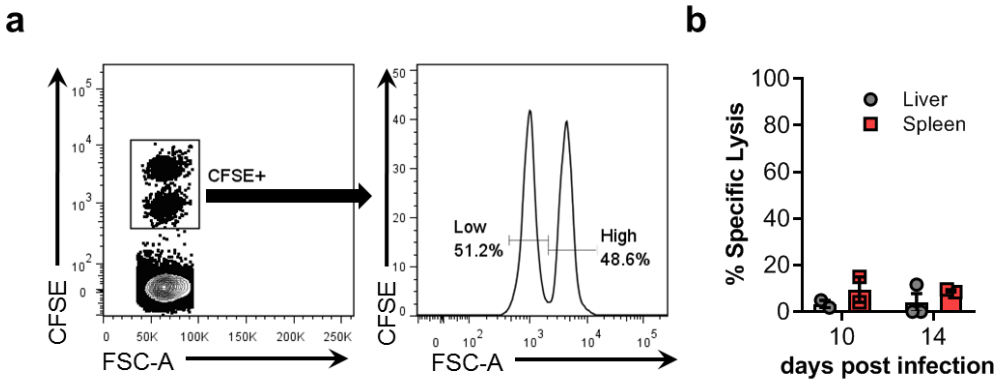
Peptide	Position	Sequence
P9	$Core_{57}$	AKRRRRHRRDQGGWRRSP
P12	$Core_{78}$	VDPYVRQGLQILLPSAAY
P13	$Core_{85}$	GLQILLPSAAYPVRDPRR
P17	$Core_{113}$	GTLGWTADLLHHVPLVGP
P18	$Core_{120}$	DLLHHVPLVGPLVGHPAR
P20	$Core_{134}$	HPARLICRAVRACEDGIN
P22	$Core_{148}$	DGINSFTGIAGVHLFLIC

Supplemental Table 2. List of PCR primers used in study.

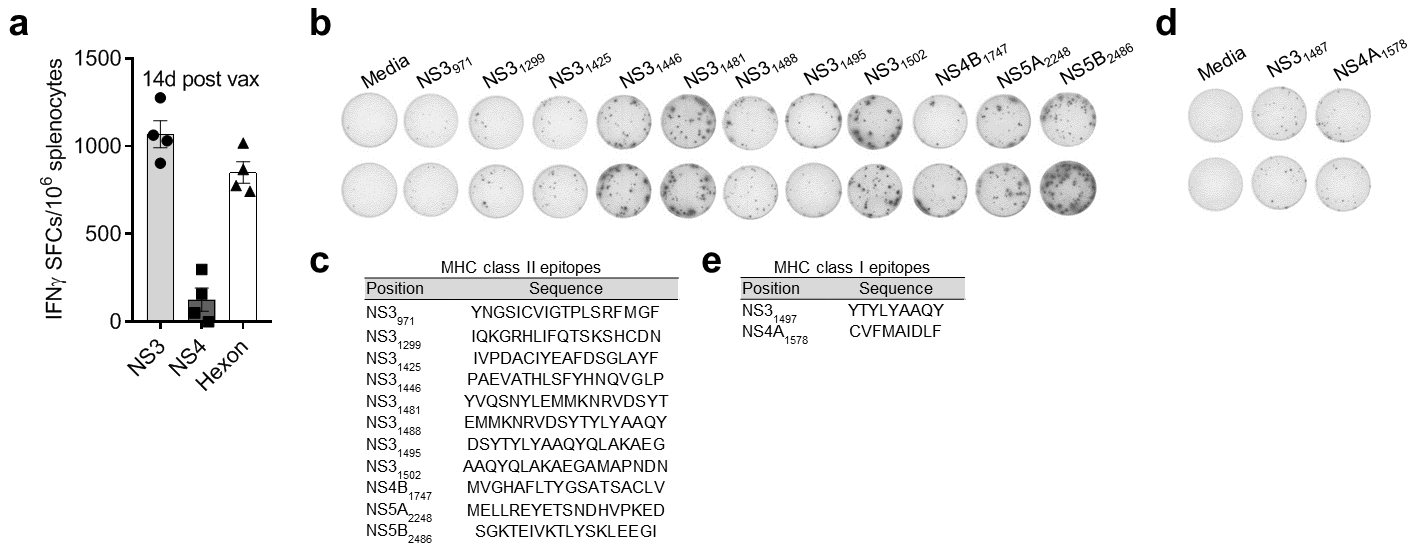
Name	Sequence (5'→3')
GNN-F1	GATTTGTGGAAATAACACGGTGTGCATATTTG
GNN-R1	AACCAGCTGGGTGACGCG
5'KpnI_NS35B_F	GATGGGGTACCATGGCCCCCTTCAACTTAAG
3'EcoRV_NS35B_R	GGTGGTAAGATATCGAGGTTGAGGGATTTACC
RHV_NS3amino-F	CCGCTGAGGACACTGGTTTCA
RHV_NS3amino-R	CGGCATGGCACTCGTCGC
RHV_NS3carboxy-F	CCATGATAACGGCTCAAGGCTC
RHV_NS3carboxy-R	GTCGCCCGTCAAGTCCCAGG
RHV_NS3amino-F2	CCGCTGAGGACACTGGTTTCA
RHV_NS3amino-R2	CGTGGCCGTCGCAAGAAGC
RHV_NS4B-F	GCTTCCAGCATCCAGGGGTG
RHV_NS4B-R	CGGGTCGGCAGGCGTAGAGT
RHV_NS5A-F	CAAGCCACCACACTCAGACAAAC
RHV_NS5A-R	TGGGATTACGCATCAAGCCG
RHV_NS5Bamino-F	GCCCACAGACCCAGACACTTTCA
RHV_NS5Bamino-R	GCTTCATGGCCACTGCGAAT
RHV_NS5Bcarboxy-F	TTACATGACAACCTGTATGCTGG
RHV_NS5Bcarboxy-R	ACATCAGGAGAAGATGAAGGCTAT
NS3aseq-F1	CCCTAAAGGGCTTACCAGTG
NS3aseq-F2	TCTTTCTAAGGTGCAGGAGC
NS3cseq-F1	CATGTTCCTAGCGAAGAACC
NS3cseq-F2	TGATGCCAACATTCAAGCTG
NS4Bseq-F1	GAAGAATACTTTGCTGAGACGG
NS4Bseq-F2	CTACAAAGCTAACAACACAGACG
NS5Aseq-F1	AAGTGGCTGGAAACAGCCTC
NS5Aseq-F2	CTGTAGGCTGGACTAGTATGGC
NS5Aseq-F3	TGCACACATGAAAGACATCTCACG
NS5Aseq-F4	GTGCATCCTGTTCCAGAATC
NS5Baseq-F1	AGTCGTCTGGATCATGGACC
NS5Baseq-F2	TGGTGGAGAAGATGGTGCTC
NS5Bcseq-F1	CATGGTGATGCAAGGTGCTG
NS5Bcseq-F2	GTGTGTGCAATTCTTACAGCAG



Supplemental Figure 2. Gating strategies for analysis of tetramer positive cells. Analysis of tetramer positive cells after staining with markers for CD44H and granzyme B (a) or Annexin V (b).



Supplemental Figure 3. Absence of in vivo killing during acute RHV infection. (a) Representative FACS plots of $CFSE^{+}$ cells in infected liver at 10 days post infection. **(b)** Summary of percent specific lysis of RHV-rn1 peptide-loaded cells at days (d) 10 and 14 post infection in livers (grey circles) and spleens (red squares).



c

MHC class II epitopes

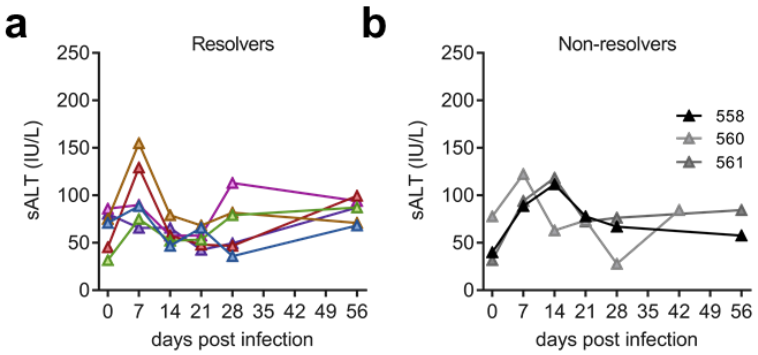
Position	Sequence
$NS3_{971}$	YNGSICVIGTPLSRFMGF
$NS3_{1299}$	IQKGRHLIFQTSKSHCDN
$NS3_{1425}$	IVPDACIYEAFDSGLAYF
$NS3_{1446}$	PAEVATHLSFYHNQVGLP
$NS3_{1481}$	YVQSNYLEMMKNRVDSYT
$NS3_{1488}$	EMMKNRVDSYTYLYAAQY
$NS3_{1495}$	DSYTYLYAAQYQLAKAEG
$NS3_{1502}$	AAQYQLAKAEGAMAPNDN
$NS4B_{1747}$	MVGHAFLTYGSATSACLV
$NS5A_{2248}$	MELLREYETSNDHVPKED
$NS5B_{2486}$	SGKTEIVKTLYSKLEEGI

e

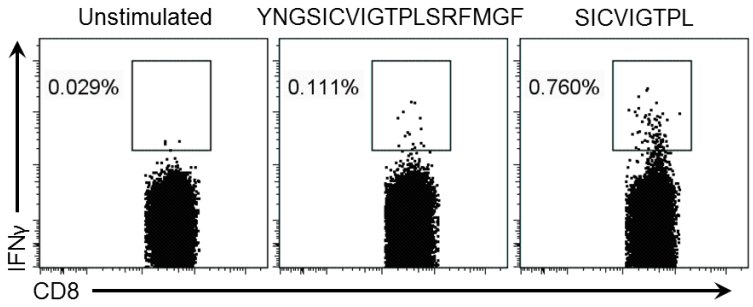
MHC class I epitopes

Position	Sequence
$NS3_{1497}$	YTYLYAAQY
$NS4A_{1578}$	CVFMAIDLF

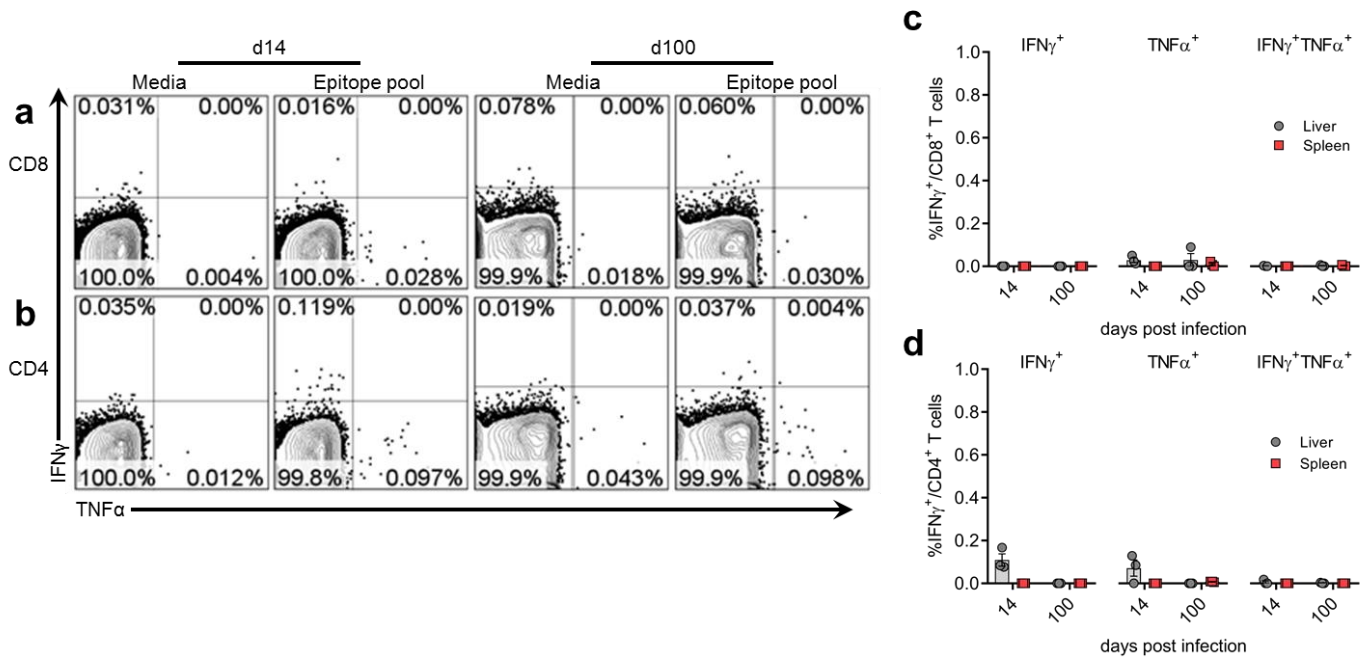
Supplemental Figure 4. Immunogenicity of Ad-NSmut and characterization of vaccine-elicited T cell repertoire. Lewis rats were vaccinated intramuscularly with 5 x 10^8 ifu Ad-NSmut. Two weeks after vaccination, splenocytes were harvested for analysis of cellular immunity. **(a)** IFNγ ELISpot responses against vaccine insert (NS3, NS4; 2 µg/mL) and backbone (Hexon; 0.6 nmol/mL) peptides. Data from n=3 rats per group is shown (mean ± SEM). **(b-e)** Splenocytes from four rats were pooled together at equal ratios, magnetically enriched for $CD8^{pos}$ and $CD8^{neg}$ cells, and used to map MHC class I and II epitopes in the NS3-5B insert by IFNγ ELISpot assay. MHC class II epitopes (b and c) were identified by testing individual NS3-4B peptides and NS5B matrix pools. MHC class I epitopes (d and e) for the RT1-A^l protein of the Lewis rat were screened and confirmed using the SYFPEITHI prediction algorithm. Panels b and d show representative ELISpot responses of identified epitopes in duplicate.



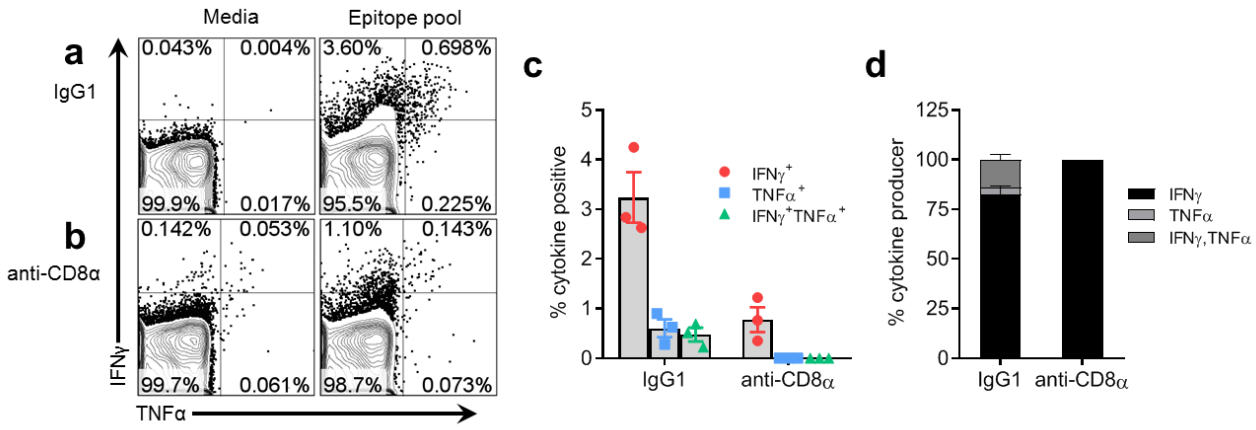
Supplemental Fig 5. Serum ALT values in vaccinated rats. Levels of serum ALT (sALT) in (a) resolvers and (b) non-resolvers after RHV infection.



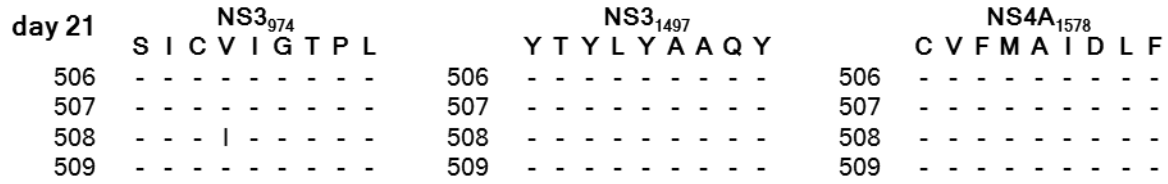
Supplemental Fig. 6. Mapping of $NS3_{974}$ class I epitope. Flow cytometric analysis of intracellular IFNγ production by $CD8^+$ T cells in immune splenocytes following 5-hr stimulation with untruncated or truncated $NS3_{974}$ peptide.



Extended Figure 7. RHV-specific T cell response in Ad-null vaccinated rats after virus challenge. Lewis rats were vaccinated intramuscularly with 5 x 10^8 ifu Ad-null. Three weeks after vaccination, rats were challenged intravenously with 10^6 genomes RHV. At days 14 and 100 post infection, virus-specific T cell responses were quantified in spleens and livers by flow cytometric analysis following 5-hr stimulation with a pool of MHC class I and II epitopes (5 µg/mL). **(a,b)** Representative flow plots of intracellular IFNγ and TNFα cytokine production in $CD8^+$ (a) and $CD4^+$ (B) T cells from livers of vaccinated rats. **(c,d)** Calculated frequencies of total and dual positive cytokine producers in $CD8^+$ (c) and $CD4^+$ (d) T cell compartments following RHV-specific peptide stimulation. Liver, grey circles; spleen, red squares. Data from n=3 rats per group are shown (mean ±SEM).



Supplemental Figure 8. RHV-specific CD4⁺ T cell responses in depleted rats after virus challenge. Lewis rats were vaccinated intramuscularly with 5 x 10^8 ifu Ad-NSmut. Three weeks after vaccination, rats were challenged intravenously with 10^6 genomes RHV. At -2 and 5 days post infection, rats were depleted of CD8α⁺ cells by antibody or isotype control. At day 9 post infection, CD4⁺ T cell responses were quantified in liver by flow cytometric analysis following 5-hr stimulation with a pool of MHC class I and II epitopes (5 µg/mL). **(a,b)** Representative flow plots of intracellular IFNγ and TNFα cytokine production by intrahepatic CD4⁺ T cells in (a) mock and (b) CD8α⁺ cell-depleted rats. **(c)** Calculated frequencies of total and dual positive cytokine producing cells in mock and CD8α⁺ cell-depleted rats. IFNγ, red circles; TNFα, blue squares; IFNγ,TNFα, green triangles. Data from n=3 rats per group are shown (mean ±SEM). **(d)** Relative frequency of intrahepatic CD4⁺ T cells producing dual or single IFNγ or TNFα cytokines. Data from n=3 rats per group are shown (mean ±SEM).



Supplemental Figure 9. Class I epitope evolution in CD4-depleted rats. Serum viral RNA in CD4-depleted rats at 21 days post infection was analyzed for presence of mutations in class I-restricted epitopes by consensus PCR sequencing. A single non-synonymous V→I mutation was identified at position 4 in the $NS3_{974}$ epitope of serum virus recovered from rat 508.