

Supplementary Information for

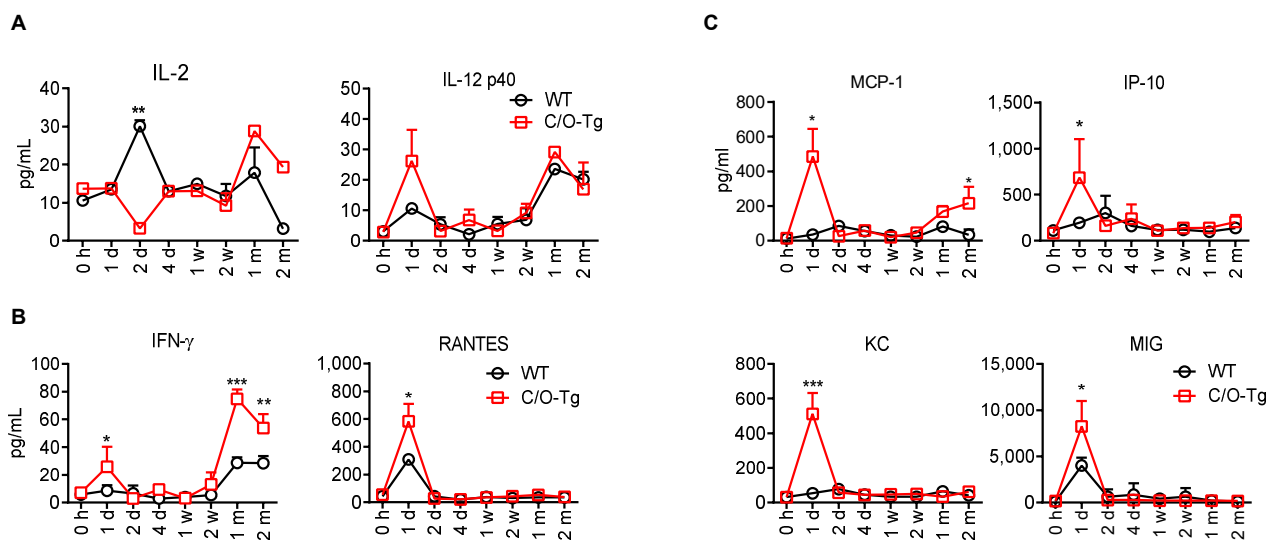
NKG2A is a NK cell exhaustion checkpoint for HCV persistence

Zhang et al.

This PDF file includes:

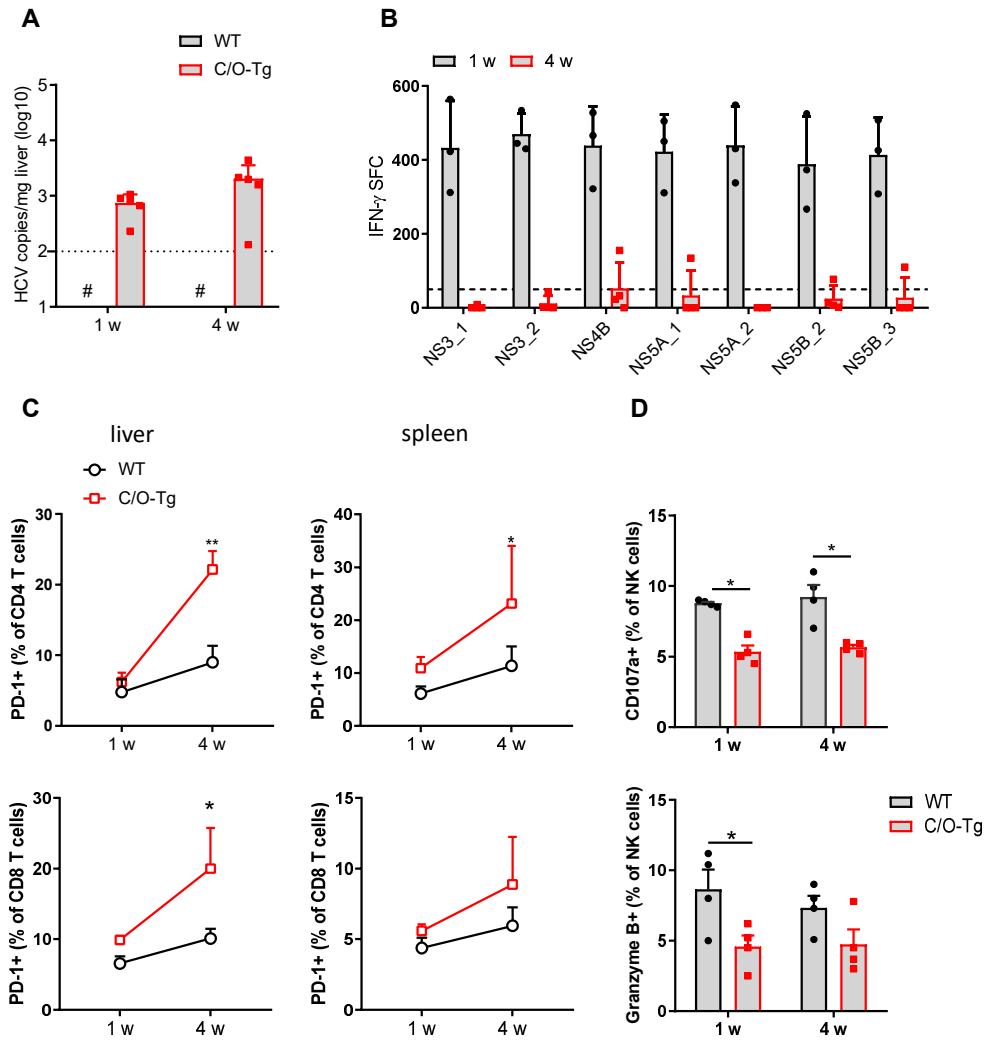
Supplementary Figure 1 to 10 and Legends

Supplementary Table 1 and 2

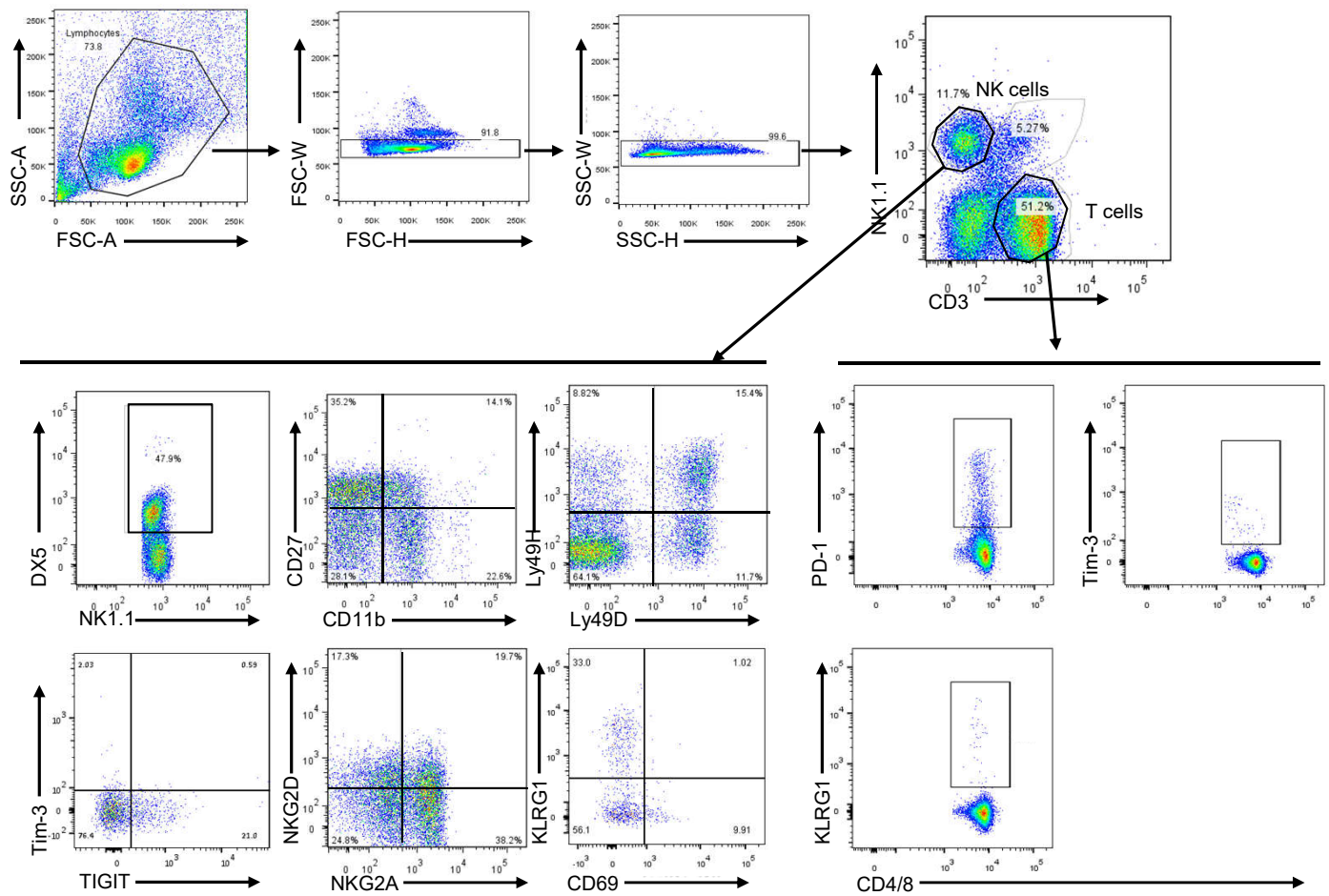


Supplementary Figure 1. Dynamics of serum chemokines and cytokines after J339EM infection.

Serum levels of Th1 cytokines (**A**), proinflammatory cytokines (**B**) and chemokines (**C**) were detected by LUMINEX assay. Other cytokines and chemokines (IL-3, IL-4 IL-5, IL-15, IL-12p70, IL-21, IL-23, IL-33, G-CSF, Eotaxin, TNF- α) without any difference between wt and transgenic mice were not shown. Data were mean $\pm$ SD, ANOVA test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Source data are provided as a Source Data file.

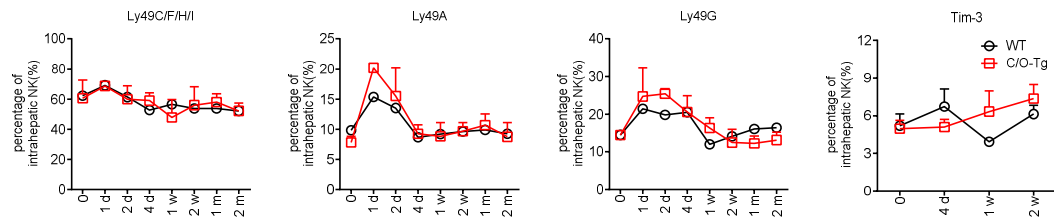


Supplementary Figure 2. Mice infected with patient sera positive for HCV1b. C/O-Tg or wt mice (n = 5 for each time point) were *i.v.* infected with 200 μ L HCV1b patient sera (3.93×10^6 copies/mL). **(A)** HCV genome copies in liver. **(B)** HCV specific CD8⁺ T cells response, and **(C)** PD-1 expression in CD4⁺ and CD8⁺ T cells isolated from spleens or livers. **(D)** NK cells were evaluated by FACS analysis. Data were mean $\pm$ SD, student *t* test. *, *P* < 0.05; **, *P* < 0.01. Source data are provided as a Source Data file.



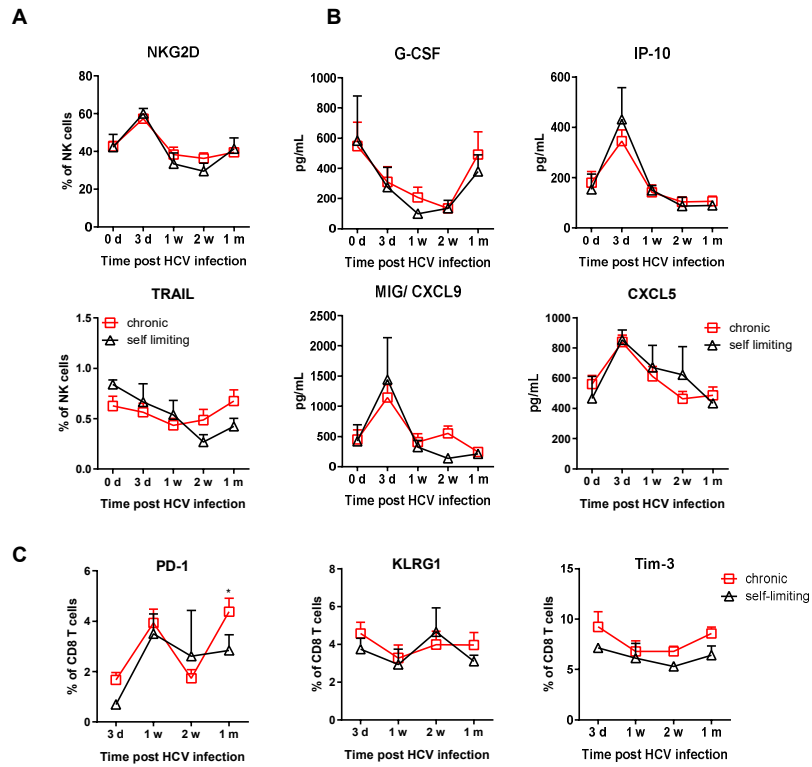
Supplementary Figure 3. Gating strategies for flow cytometric analysis.

NK cells were gated as CD3⁺ NK1.1⁺ and analyzed for the expression of indicated markers. CD4⁺ T and CD8⁺ T cells were gated and analyzed for the expression of inhibitory markers.



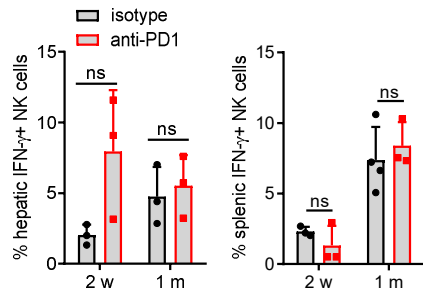
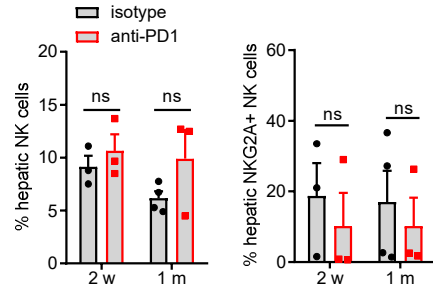
Supplementary Figure 4. Expression of inhibitory Ly49 family receptors and Tim-3 on hepatic NK cells in HCV infected mice.

Mice were treated as in Figure 1A. Analysis of indicated NK cell surface markers by FACS. Data were mean $\pm$ SD. Source data are provided as a Source Data file.



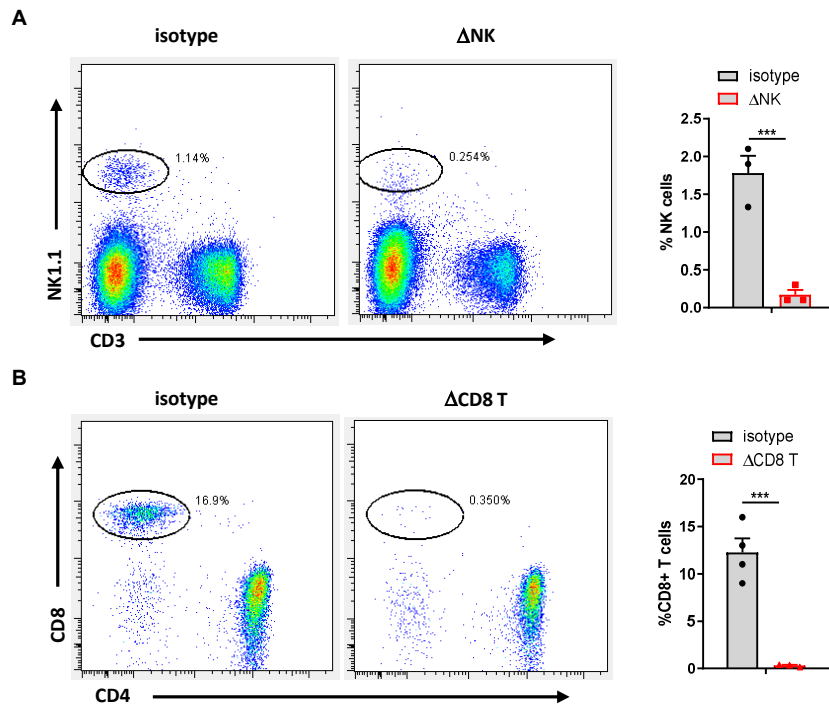
Supplementary Figure 5. Candidate factors to discriminate self-limiting from chronic infections in HCV infected mice.

Mice were treated as in Figure 3A. **(A)** Analysis of NK cell surface markers by FACS. **(B)** Analysis of serum cytokines and chemokines by LUMINEX assay. **(C)** Analysis of T cell inhibitory markers by FACS. Data were mean $\pm$ SD. ANOVA test. *, $P < 0.05$. Source data are provided as a Source Data file.

A**B**

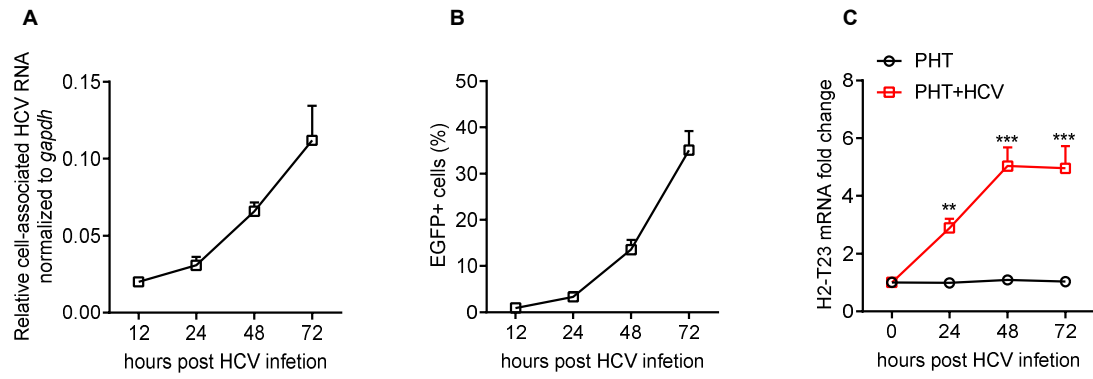
Supplementary Figure 6. PD-1 blockade alone was not able to restore NK cell function.

(A-B) Mice were treated as in Figure 1E, NK percentage, NKG2A+ and IFN- γ + cells among NK cells were detected by FACS. Data were mean $\pm$ SD, ns, not significant. Source data are provided as a Source Data file.



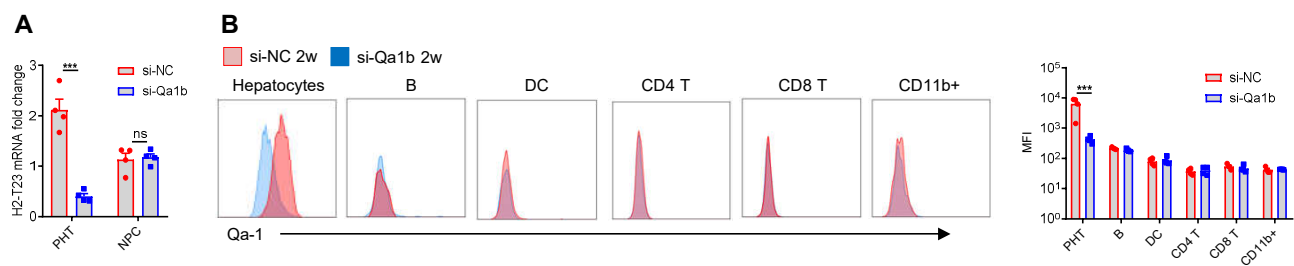
Supplementary Figure 7. Depletion efficiency of NK cells and CD8⁺ T cells.

Mice were treated as in Figure 4C. **(A)** Depletion efficiency of NK cells by anti-AGM1 examined by FACS in blood. **(B)** Depletion efficiency of CD8⁺ T cells by antibodies (TIB210) examined by FACS in blood. Data were mean $\pm$ SD, student *t* test. ***, *P* < 0.001. Source data are provided as a Source Data file.



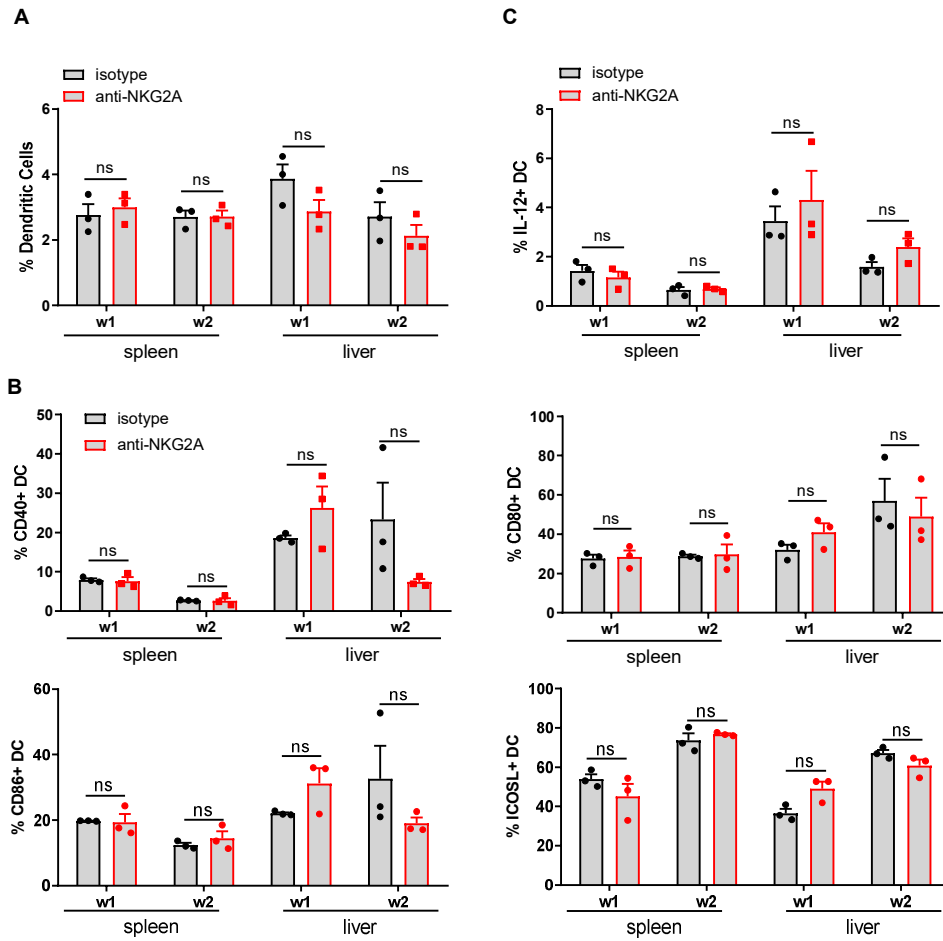
Supplementary Figure 8. HCV replication in C/O^{Tg}PHT and induction of Qa-1 upon infection.

C/O^{Tg} PHT were infected with HCV (J399EM) as in Figure 5A. **(A)** Dynamics of cell associated HCV RNA. **(B)** Percentage of EGFP positive cells after J399EM infection. **(C)** Qa-1 mRNA level after J399EM infection. Data were mean $\pm$ SD, student *t* test. **, $P < 0.01$. ***, $P < 0.001$. Source data are provided as a Source Data file.



Supplementary Figure 9. Delivery of cholesterol conjugated siRNA by tail vein injection to knockdown Qa-1 expression in hepatocytes.

Mice were treated as in Figure 6G. Measurement of Qa-1 mRNA (**A**) and protein (**B**) level on hepatocytes or NPC subsets as indicated 2 weeks post HCV infection. Data were mean $\pm$ SD, student *t* test. ***, $P < 0.001$. ns, not significant. Source data are provided as a Source Data file.



Supplementary Figure 10. Restored NK function by anti-NKG2A did not elevate T cell response via DC activation.

Mice were treated as in Figure 3D. **(A)** FACS analysis of DC percentage. **(B-C)** FACS analysis of DC cells for expression of **(B)** CD40, CD80, CD86, ICOSL and **(C)** intracellular IL-12 in spleen and liver. Data were mean $\pm$ SD, ns, not significant. Source data are provided as a Source Data file.

Supplementary Table 1. J399EM (HCV 2a) peptide information

peptide simble	HCV region	peptide sequence	H2 haplotype
KV8	NS3	KSIDFIPV	H-2-Kb
YL9	NS3	YYRGLDVS	H-2-Kd
QL9	NS4B	QYLAGLSTL	H-2-Kd
SI9	NS5A	SEALQQLAI	H-2-Kk
CL9	NS5B	CEKMALYDI	H-2-Kk
RL8	NS5B	RSLLFGLL	H-2-Kb
SL8	E2	VLLFLLL	H-2-Kb
WL9	E2	WEWVLLFL	H-2-Kk
VL8	Core	SIFLLALL	H-2-Kb
RA10	Core	VNYATGNL	H-2-Kb

Supplementary Table 2. HCV 1b peptide pool information

Pool Name	Sequence	Pool Name	Sequence	Pool Name	Sequence	Pool Name	Sequence		
NS3_1	LLAPITAYSQQTGRLLGC SQQTRGLLGCITSLTGR GCITSLTGRDKNQVEGE GRDKNQVEGEVQVVSAT GEVQVVSATQSFATCV ATQSFATCVNGVCWTVY CVNGVCWTVYHGAGSKTL VYHGAGSKTLAGPKGPT TLAGPKGPTOMYTNVDQ ITQMYTNVDQDLVGWPAP DQDLVGWPAPPGARSLTP APPGARSLTPCTCGSSDL TPCTCGSSDLVLRHAD DLVLRHADVIVRRRG ADVIVRRRGDSRGLLS RGDSRGLLSRPRVSYLK LSRPRVSYLKSSGGPLL LKSSGGPLLCPGSHAVG LCPGSHAVGIFRAAVCT VGIFRAAVCTRGVAKAVD CTRGVAKAVDFVPVESME VDFVPVESMETMRSPVF METMRSPVFTDNSSPPA VFTDNSSPPAVPQTFQVA PAVPQTFQVAHLHAPTGS VAHLHAPTGSKGSTKVP	NS4B	FDEMEECASHLPYIEQGM SHLPYIEQGMQLAEQFKQ GMQLAEQFKQKALGLLQT KQKALGLLQTATQAEAA QTATQAEAAAPVVESKW AAAPVVESKWRALETFWA KWRALETFWAKHWMNFIS WAKHWMNFISGIQYLAGL ISGIQYLAGLSTLPGNPA GLSTLPGNPAIASLMAFT PAIASLMAFTASITSPLT FTASITSPLTQSTLLFN LTTQSTLLFNILGGWVAA FNILGGWVAAQLAPPSAA AAQLAPPSAAAFVAGSI AASAFVAGIAGIAAVGSI GIAAGIAVSGISGLKVLVD	NS5A_2	GSPPSLASSASQLSAPS SSASQLSAPSLKATCTCTH PSLKATCTCTHHDSPADL THHDSPADLIEANLLWR DIEANLLWRQEMGGNIT WROEMGGNITRVESENKV ITRVESENKVILDSFDP KVILDSFDPRLAEEDER DPLRAEEDEREVSVAEI EREVSVAEILRTRKFP EILRTRKFPTRAMPPIAR FPPAMPPIARPPDPNPL ARPDNPLLESWKDPDY LLESWKDPDYVPVHVHGC DYVPVHVHGCPLPPTKAP GCPLPPTKAPPPIPPRRK APPIPPRRKRTVLTES RKRTVLTESVTSALAE ESTVTSALAEATKTFGS AELATKTFGSSGSAIDS GSSGSAIDSGTATAPPD DSGTATAPPDQASDDGDK PDQASDDGDKSDVESYS DKGSDVESYSMPPLEGE YSSMPPLEGEPDPLSD GEPDPLSDSGSWSTVSE SDGSWSTVSEASEDDVC SEASEDDVCCSMSYTWI	NS5B_2	GQRVEFLVNAWKSCKCPM NAWKSCKCPMGFSYDTRC PMGFSYDTRCFDSTVTE RCFDSTVTESDIRVEESI ESDIRVEESIQCQDLAP SIYQCQDLAPAEARQAIRS APEARQAIRSLTERLYG RSLTERLYGGLPLNSKG IGGLPLNSKGNGCYRRRC KGQNGCYRRRCASGVLTT RCRASGVLTTSCGNLTTC TTSCGNLTTCYLKASAAAC TCYLKASAAACRAAKLQDC ACRAAKLQDCMLVNGDD DCTMLVNGDDLVCESAA DDLVCESAGTQEDAAAS SAGTQEDAAASRVTEAM ASRVTEAMTRYAPP AMTRYAPPQDPPQPEYD PGDPPQPEYDELITSCS YDELITSCSSNVSAHD CSSNVSAHDSAGKRVY HDASAGKRVYLTTRDPTT VLTTRDPTTPLARAARHT TPLARAARHTWETARPVNS		
	NS3_2		GSQSKTKVPAAYAAQGYK PAAYAAQGYKVLVLPNSV YKVLVLPNSVAATLGFGA SVAATLGFGAYMSKAHGT GAYMSKAHGTDPNIRTVG GTDPNIRTVGRTITTTGAP GVRTITTTGAPITYSTYK APITYSTYKGFADGGG GKFLADGGCGSGAYDIIC CSGSGAYDIICDECHSTD IICDECHSTDSTILIGIG TDSSTILIGITVLQDAET IGTVLQDAETAGARLVLE ETAGARLVLELATTPPGS VLATATPPGSVTPVPHNI GSVTPVPHNIEVALSNT NIEVALSNTGEIPFYGK NTEGIPFYGKAIPIETIK GKAIPETIKGGRHLIFC IKGGRHLIFCHSKKKKDE FCHSKKKDELAALKSLG DELAALKSLGLNNAVAY GLGLNNAVAYRGLDVSVI YRGLDVSVIPTSGDVVW VPTSGDVVWVATDALMT VVATDALMTGFGDGFDS		NS5A_1		STPCSGSWLRDWDWICT LRDWDWICTVLTDFKTW CTVLTDFKTWLOSKLLPR TWLOSKLLPRLPVPFLS PRLPVPFLSCQRGYKGV LSCQRGYKGVWRGDGIMQ GVWRGDGIMQTCPCGAQ MQTCPCGAQITGHVKN AQITGHVKNMSRIVGPR NGSMRIVGPRCSNTWHG PRTCSNTWHGTFPINAYT HGTTFPINAYTGCTPSP YTTGCTPSPAPNYSRAL SPAPNYSRALRVAAEY ALRVAAEYEVTRVGD EYEVTRVGDHYFVTGMT GDHYFVTGMTDNVKKPC MTDNVKKPCQVPAPEFF PCQVPAPEFFTEVDGVR FFTEVDGVRHRYAPACK RLHRYAPACKPLRDEVA CKPLLRDEVAQVGLNQY VAFQVGLNQYVGSQQLPC QYLVGSQQLPCEPEPDVA PCEPEPDVAVLTSMILTD AVLTSMILTDPSHITAETA DPSHITAETAKRRLARGS TAKRRLARGSPSPSLASSS	NS5B_3	ETARHTPVNSWLGNIIMY NSWLGNIIMYAPTLWARM MYAPTLWARMILMTHFFS RMILMTHFFSILLAEQOL FSILLAEQOLEKALDCQI QLEKALDCQIYACYSIE QIYACYSIEPLDLQII IEPLDLQIIERLHGLSAA IERLHGLSAFSLHSYSP SAFSLHSYSPGEINRVAS SPGEINRVASCLRLKGV ASCLRLKGVPLRVWRHR VPLRVWRHRARSRAKL HRARSRAKLSSQGGRAA KLLSQGGRAATCGKYLFN AATCGKYLFNNAVARTKLK FNNAVARTKLKLTPIPAAS LKLTPIPAASRLDSGWGF ASRLDSGSWFVAGYSGDD WVFAVAGYSGDDIYHLSRA GDIYHLSRARPRWFMLC RARPRWFMLCLLLSVGV LCLLLSVGVGYLLPNR SVGYLLPNR