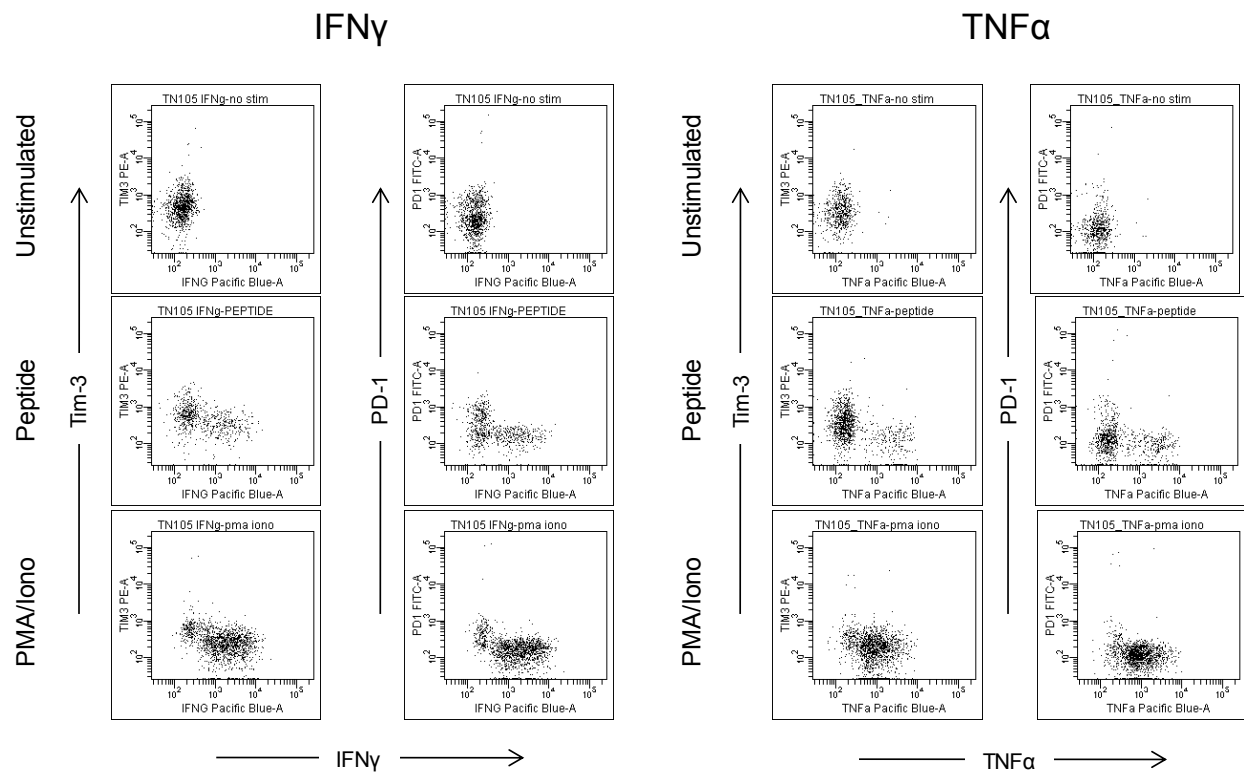
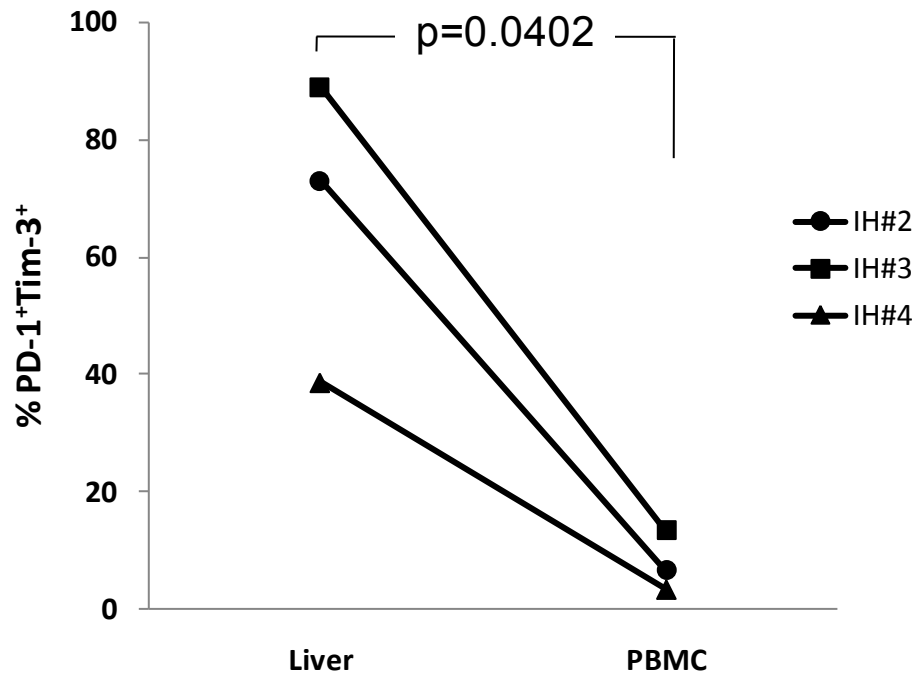


Supplemental Figure 1. Expression of Tim-3 on HCV-specific CTLs is lower in patients demonstrating viral variants. PBMC from HCV genotype 1a patients were stained with anti-CD8, anti-Tim3 and HCV-specific pentamers (HLA-A2₁₄₀₆, HLA-A2₂₅₉₄ and HLA-A1₁₄₃₆) and assessed by flow cytometry for the percentage of HCV-specific T cells expressing Tim-3. Autologous viral sequences of the CTL epitopes were compared to the consensus H77 genotype 1a sequence. The percentage of HCV-specific T cells expressing Tim-3 is higher if the virus contains the HCV consensus sequence for that epitope ($p=0.0235$).

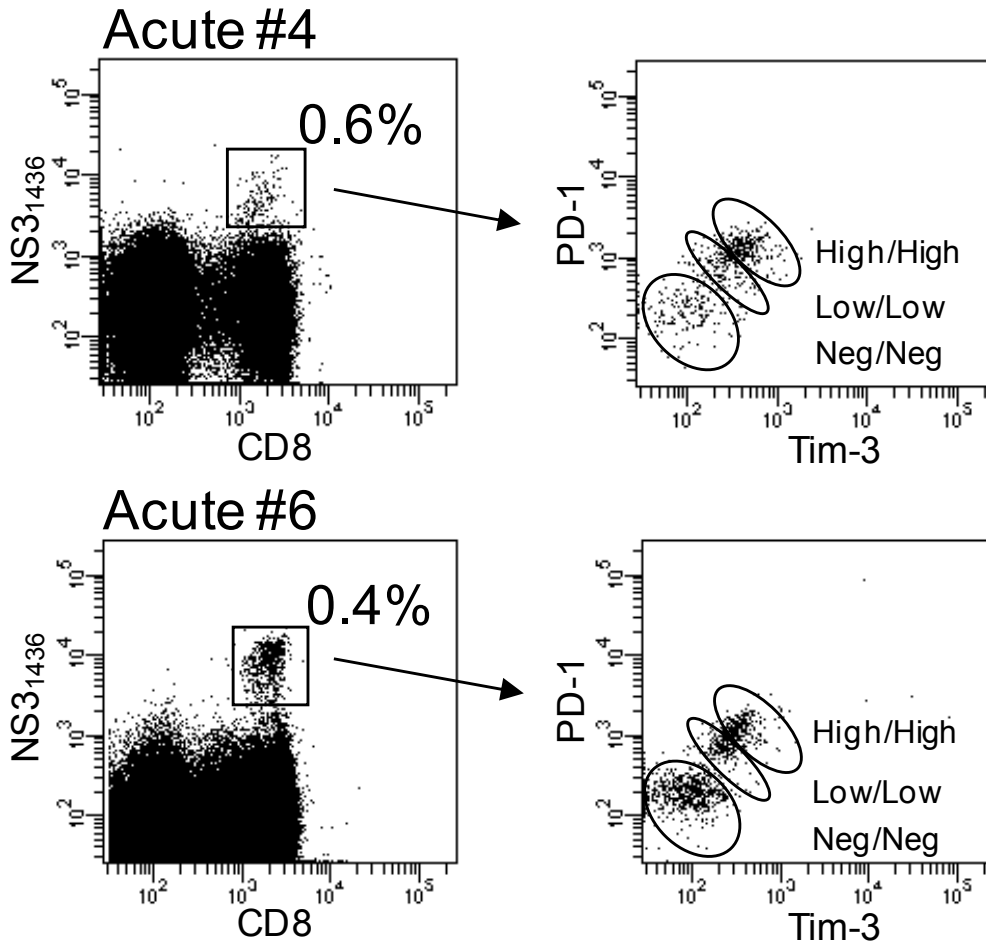


Supplemental Figure 2. PD-1⁺ and Tim-3⁺ T cells are functionally impaired.

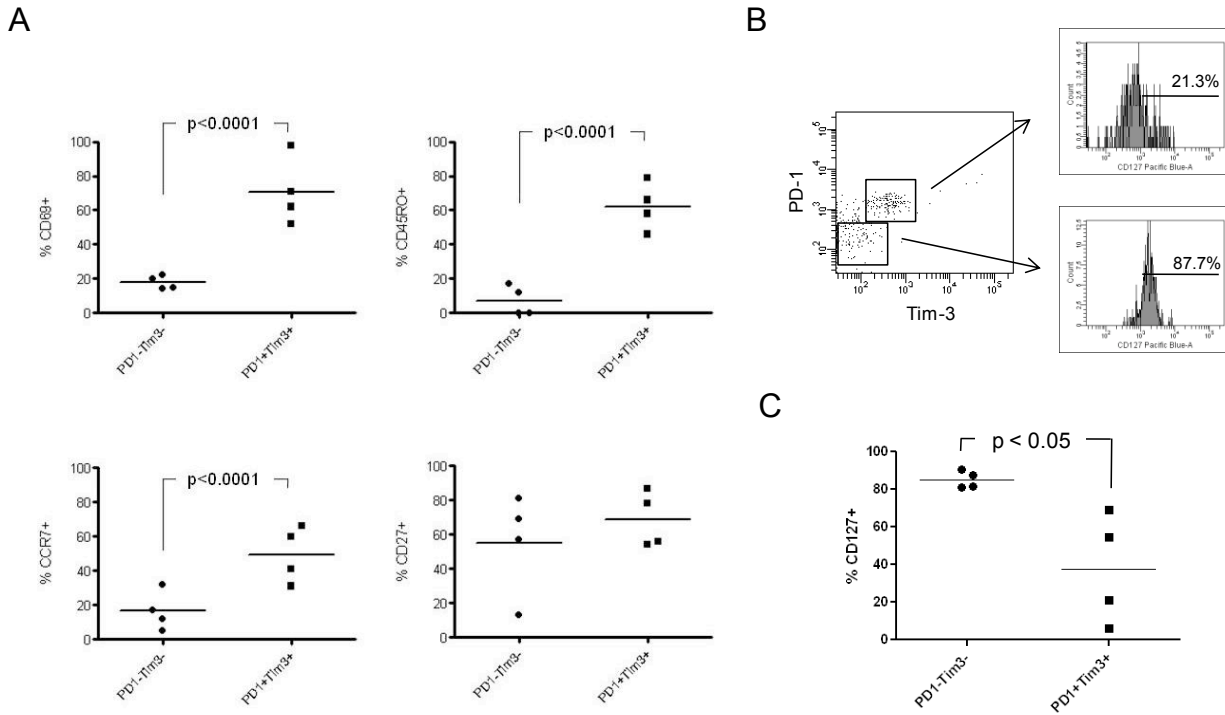
PBMCs from an acutely infected HCV patient who became chronic were stimulated with the HLA-A1 restricted NS3₁₄₃₆ epitope or PMA and Ionomycin for 5 hours in the presence of CD107a antibody. Cell surface staining for CD3, CD8, HLA-A1₁₄₃₆ pentamer, Tim-3 and PD-1 was carried out followed by intracellular cytokine staining for IFN γ and TNF α . Flow cytometric analysis was used to determine the proportions of the Tim-3-positive and PD-1-positive cells producing IFN γ and TNF α after stimulation.



Supplemental Figure 3. HCV-specific T cells in the intrahepatic compartment express higher levels of Tim-3 than paired peripheral blood. Intrahepatic lymphocytes and matched PBMC from 3 patients were stained with antibodies against CD3, CD8 and Tim-3. A higher percentage of CD8 T cells in the liver expressed Tim-3 compared to peripheral CD8 T cells ($p=0.0402$).



Supplemental Figure 4. Pentamer frequencies and PD-1/Tim-3 expression for Acutes #4 and #6. PBMC from two acutely infected patients who became chronically infected (Acute #4 and Acute #6) were stained with antibodies against CD3, CD8, PD-1 Tim-3 and the A1₁₄₃₆ pentamer and the percentage of CD8 T cells binding pentamer was determined. Gating of pentamer-positive T cells on Tim-3^{high}/PD-1^{high}, Tim-3^{low}/PD-1^{low} and Tim-3^{neg}/PD-1^{neg} is shown.



Supplemental Figure 5. Phenotypic analysis of CD4, CD8 and HCV-specific T

cells from chronically infected HCV patients. PD-1⁺ and Tim-3⁺ HCV-specific T

cells from acutely infected patients who became chronic were identified by staining with

anti-CD8 and the A1₁₄₃₆ pentamer. (A) The phenotype of the cells was determined by

staining with antibodies against PD-1, Tim-3, CCR7, CD27 CD69 and CD45RO and the

expression of the activation/memory markers on the dual PD-1⁺TIM3⁺ positive cells was

compared to cells negative for PD-1 and Tim-3. A significant portion of the dual positive

T cells expressed CCR7, CD69 and CD45RO relative to PD-1⁻Tim-3⁻ cells (* p <

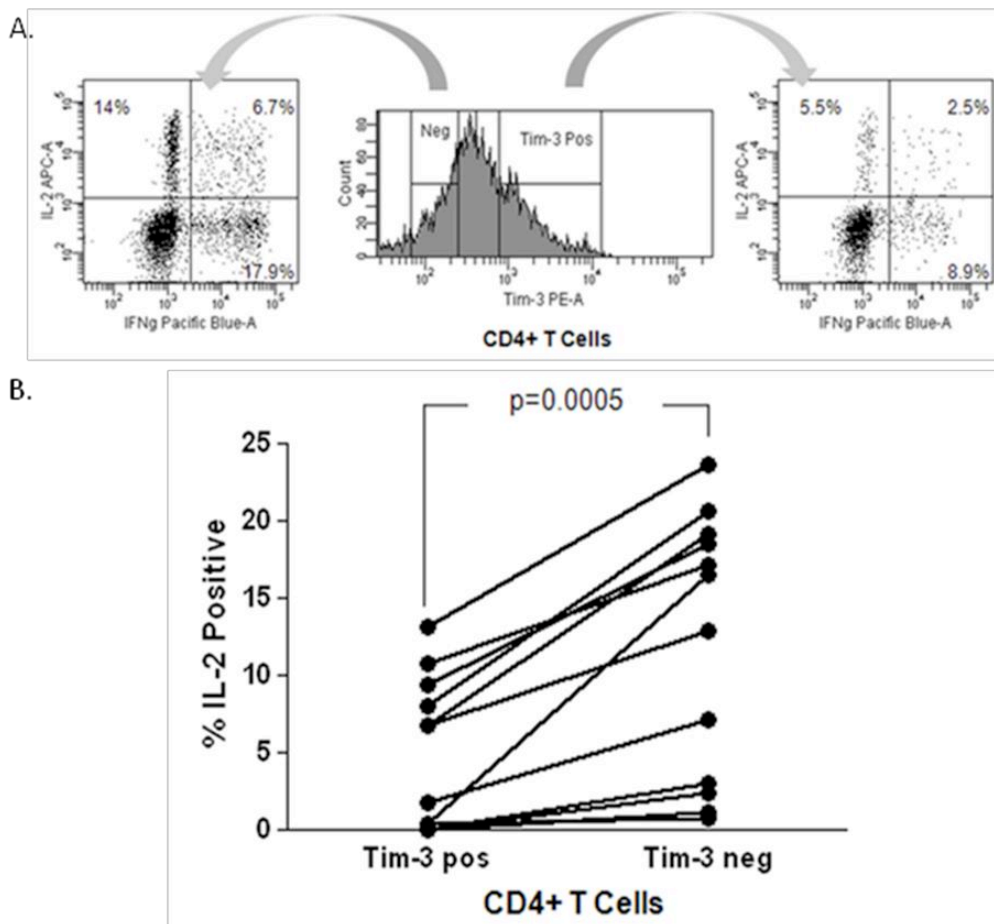
0.0001), The majority of HCV-specific T cells were CD45RA- (data not shown) . (B and

C) HCV-specific T cells were further analysed for CD127 expression on PD-1⁺Tim-3⁺

and PD-1⁻Tim-3⁻ cells. Representative plots from one patient are shown in (B). PD-

1⁺Tim-3⁺ HCV-specific T cells had lower levels of CD127 compared to PD-1⁺Tim-3⁺

(p=.0465). Horizontal bars represent the mean.



Supplementary Figure 6. Tim-3 positive helper T cells produce less IL-2. PBMCs from twelve subjects, six uninfected controls and six acute to chronic HCV infection patients (12-18 months post-infection), were stimulated for four hours with PMA and Ionomycin in the presence of brefeldin A to inhibit cytokine secretion. Cell surface staining was carried out for CD3, CD4 and Tim-3, followed by intracellular staining for IL-2. (A) Flow cytometric analysis was used to determine the proportions of the Tim-3-positive and –negative subsets of CD4 T cells producing IL-2 after brief stimulation. (B) Tim-3-positive helper T-cells produce less IL-2 than their Tim-3-negative counterparts. We did not find that in vitro stimulation for 6 hrs affected Tim-3 expression on CD4+ T cells (data not shown).

Supplemental Table 1: MHC Class I Pentamers

Protein	HLA Restriction	Amino Acids	Sequence
HCV:NS3-5H	A2	1406-1415	KLVALGINAV
HCV:NS3-5H	A1	1436-1444	ATDALMTGY
HCV:NS3-1P	A2	1073-1081	CINGVCWTV
HCV:NS5-B2	A2	2594-2602	ALYDVVTKL
CMV:pp65	A2	495-503	NLVPMVATV