Early phase of specific cellular immune status associates with HCV infection outcomes in marmosets

Bochao Liu[1,2*], Enhui Zhang[1*], Xiaorui Ma[1], Shengxue Luo[1], Chong Wang[1], Ling Zhang[1], Wenjing Wang[1], Yongshui Fu[2], Jean-Pierre Allain[3], Chengyao Li[#1], Tingting Li[#1]

[1] Department of Transfusion Medicine, School of Laboratory Medicine and Biotechnology, Southern Medical University, Guangzhou, China;

[2] Guangzhou blood center, Guangzhou, China;

[3] Emeritus professor of transfusion medicine, University of Cambridge, UK.

* These authors contributed equally to this work.

[#] **Corresponding author:** Tingting Li or Chengyao Li, Department of Transfusion Medicine, School of Laboratory Medicine and Biotechnology, Southern Medical University, No. 1838 North Guangzhou Avenue, Guangzhou, China.

Email: apple-ting-007@163.com (T Li); or chengyaoli@hotmail.com (C Li). Phone: 0086-20-61649360

Table S1. HCV and GBV-B peptides used in ELISpot assay

No.	Sequence	Location
HCV		
Core		
1	RGPRLGVRA	40-48
2	AQPGYPWPL	77-85
3	GYPWPLYGN	80-88
4	GSRPSWGPS	102-110
5	APLGGVARA	142-150
6	ARALAHGVR	148-156
7	GVRVLEDSV	154-162
E1		
8	DSVNYATGN	160-168
9	NLPGCSFSI	168-176
10	LTIPASAYE	185-193
11	TIPASAYEV	186-194
12	LTPTLAARN	242-250
13	ARNSSVPTK	248-256
14	PTKTIRRHV	254-262
15	SQLFTFSPR	288-296
16	FSPRRHETV	293-301
17	ETVQDCNCS	299-307
18	NCSLYPGHV	305-313
19	GHVSGHRMA	311-319
20	RMAWDMMMN	317-325
21	MNWSPTAAL	324-332
22	RIPQAVVDM	339-347
E2p7		
23	FSLGPTQRI	403-411
24	GPTQRIQLV	406-414
25	ASCRPIDKF	457-465
26	SSDQRPYCW	479-487
27	TTDRSGVPT	518-526
28	TRPPQGNWF	542-550
29	RPPQGNWFG	543-551
30	GPPCNIGGV	566-574
31	SGPWLTPRC	599-607
32	MVDYPYRLW	608-616
33	TTLPALSTG	680-688
34	HLHQNIVDV	691-699
GBV-B		
CoreE1E2p13		

1	ISTQTSPVP	4-12
2	TSPVPAPRT	8-16
3	QTQASYPVS	21-29
4	KSRNLGILL	83-91
5	LLDYPLGWI	90-98
6	DVTTHTPLV	100-108
7	HTPLVGPLV	104-112
8	VGPLVAGAV	108-116
9	ATGWFGVHL	134-142
10	TGWFGVHLF	135-143
11	CSPSTCLHE	181-189
12	YSPKWTRPI	387-395
13	VTPWLTTAW	457-465
14	DTPIVYFYD	501-509
15	RLPGTPPVV	523-531
16	VGPWOLVAL	712-720
17	GPWPLVALL	713-721
NS2-5B		
18	KSDDPYWCV	850-858
19	HATDATTVL	1023-1031
20	VIPTPHANI	1058-1066
21	PTPHANITE	1060-1068
22	LTDEGTIPF	1071-1079
23	CTPSGMVPE	1209-1217
24	RTQPGLPAI	1246-1254
25	RTADNYVLL	1280-1288
26	STITTTSPF	1633-1641
27	ITTPLPHKI	1690-1698
28	LTDARGALA	1713-1721
29	HTPGVRMQL	2060-2068
30	TTKLPAPSI	2117-2125
31	TATTASSYV	2232-2240
32	IVPKEEVFV	2412-2420
33	ITPEDIMVE	2499-2507
34	LSDQHRAGI	2516-2522
35	CVPQPKYSL	2623-2631
36	KSGKPYYFL	2647-2655
37	LTRDPRIPL	2655-2663
38	YSPEGDVFI	2825-2833

Note: these peptides were derived from the amino acid sequences of HCV-CE1E2p7 chimera (GenBank: KF285483.1) and GBV-B (GenBank: NC_001655.1) structural and non-structural proteins.

Table S2. Histopathological scoring of liver tissues from HCV chimera and GBV-B infected marmosets

Virus	Marmoset	Time point (week)	Necroinflammatory Grade			
			Periportal +/- bridging necrosis	Intralobular degeneration and focal necrosis	Portal inflammation	Total
HCV chimera infected marmosets	M37	W43	0	3	1	4
	M38	W43	1	3	1	5
	M43	W43	0	3	1	4
	M45	W17	0	4	1	5
GBV-B infected marmosets	M40	W15	0	1	1	2
	M41	W43	0	1	0	1
	M44	W43	0	2	1	3
	M46	W43	0	1	0	1

Note: Necroinflammatory grade was scored by the modified HAI system. The histological status was determined by the modified HAI system (Kondell score), which grades necrosis and inflammation on a scale of 0 to 18 (periportal inflammation and necrosis, 0 to 10; lobular inflammation and necrosis, 0 to 4; portal inflammation, 0 to 4).

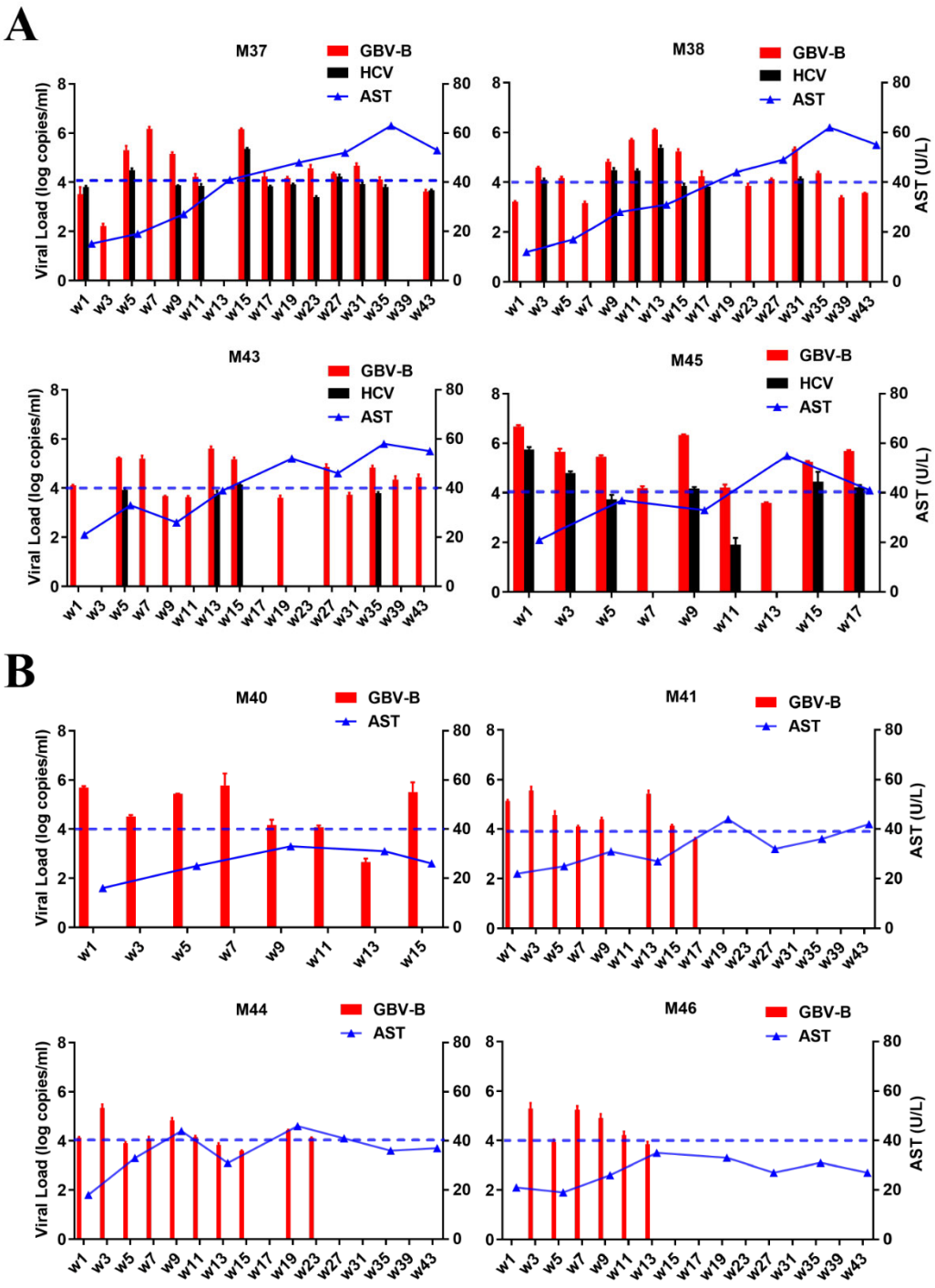

Figure S1. Detection of viremia or AST in serum from HCV chimera (A) and GBV-B infected marmosets (B). Viral RNA was measured by RT-qPCR with primers targeting the GBV-B 5' NCR (red) or HCV core (black).

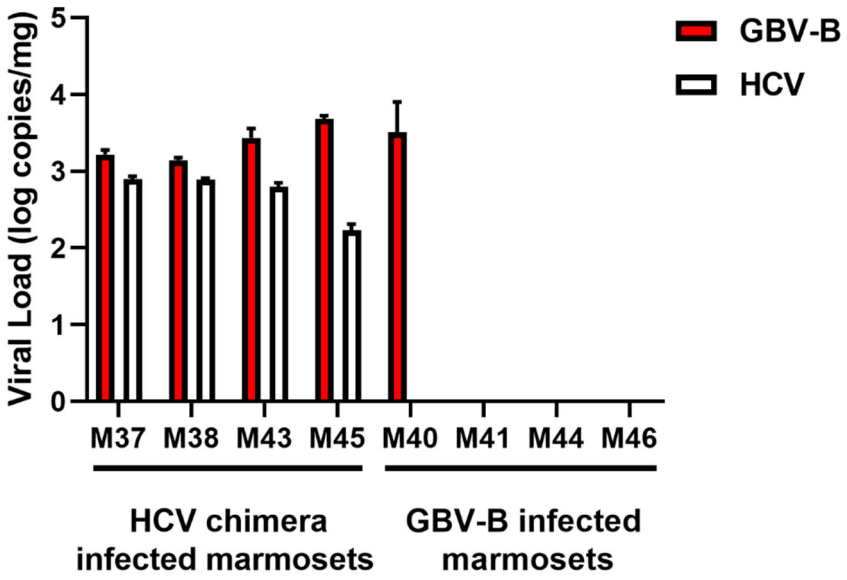

Figure S2. The quantification of viral load in liver tissues from the endpoint of HCV chimera or GBV-B infected marmosets. Hepatocyte viral load (log10 copies/mg) was quantified by RT-qPCR with primers targeting the GBV-B 5' NCR (red) or HCV core (white).

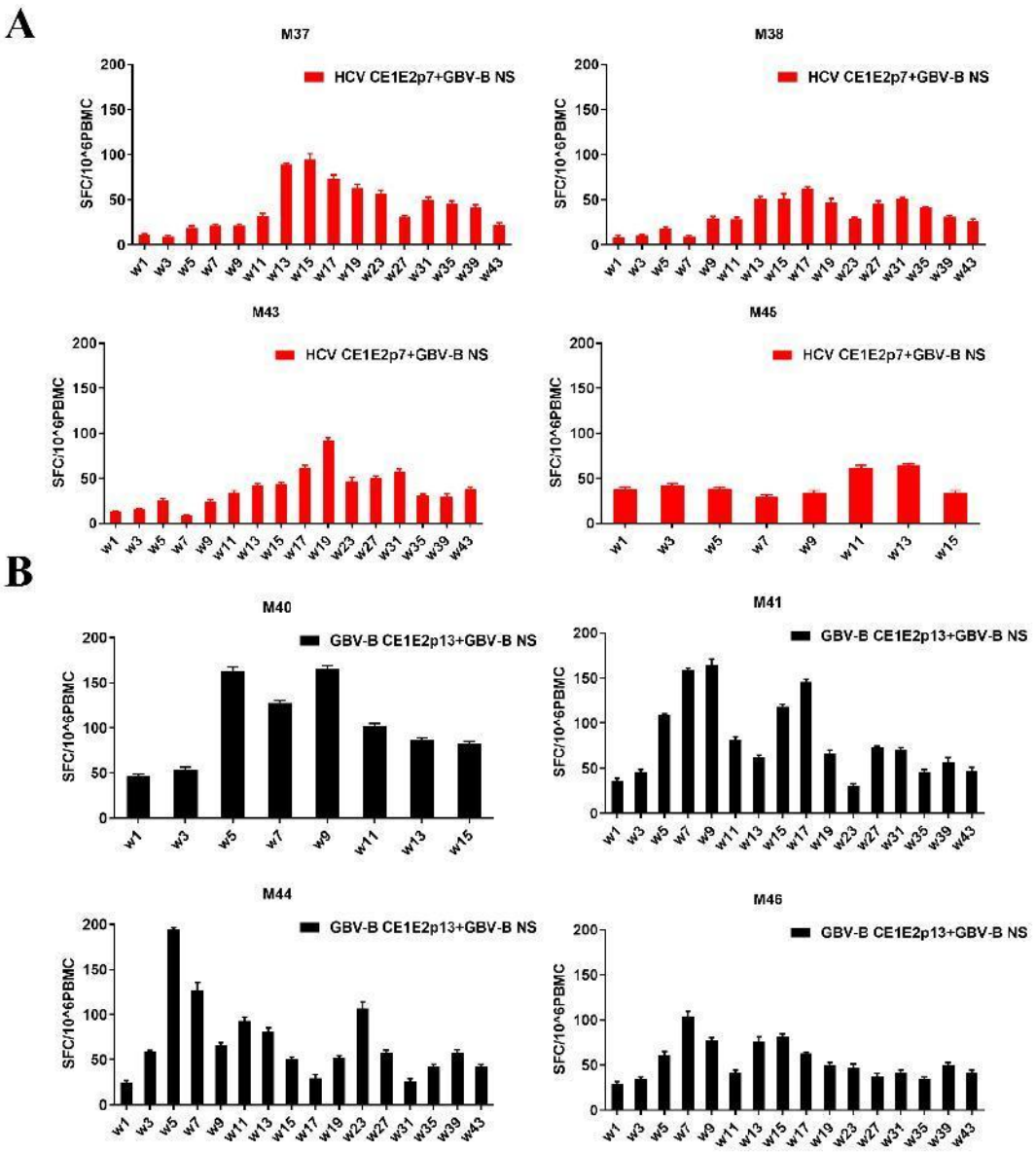

Figure S3. The specific IFN-γ secreting T-cell response of PBMCs (SFC/10^6 cells) detected from HCV chimera (A) and GBV-B infected marmosets (B).

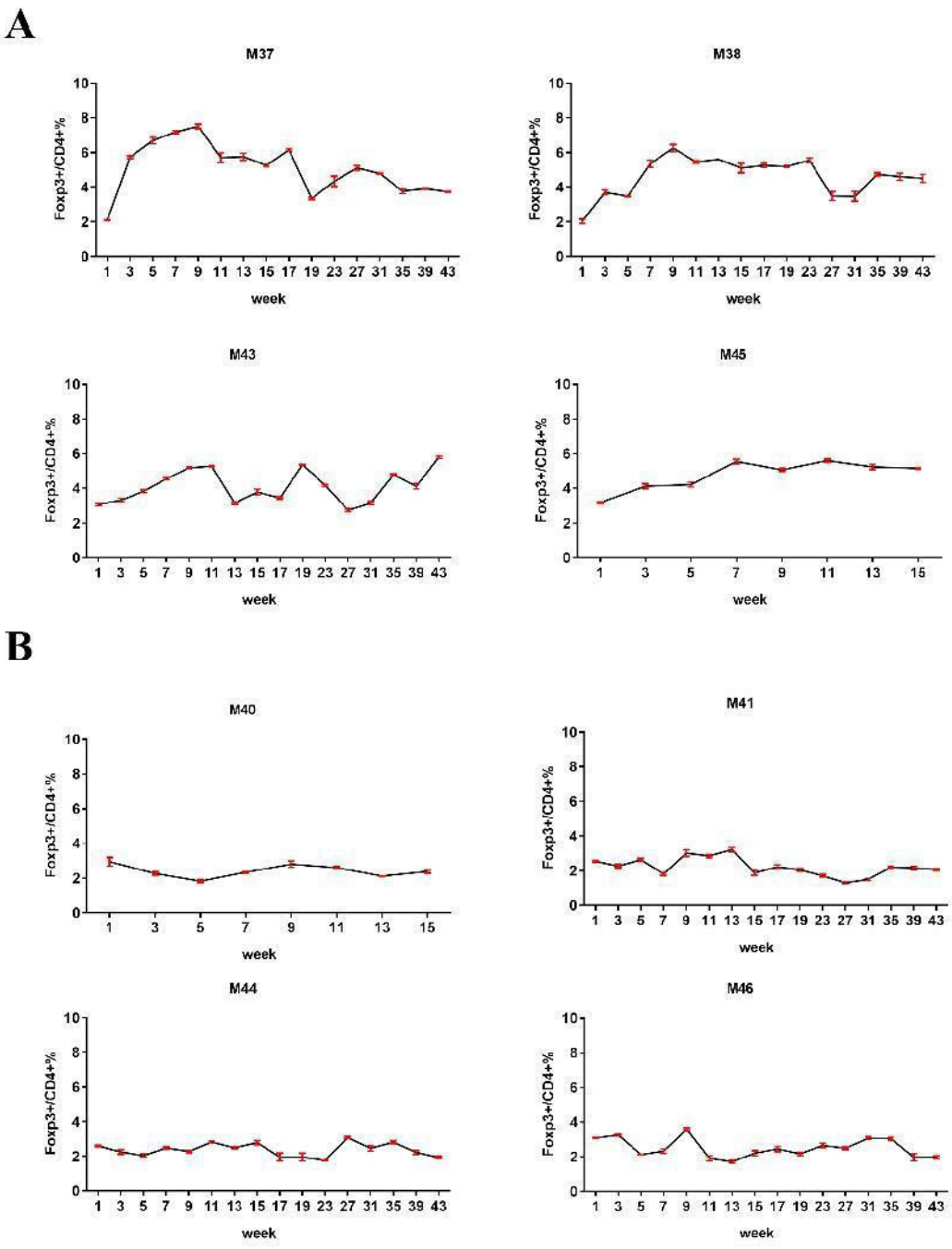

Figure S4. The percentage of Treg cells among lymphocytes from HCV chimera (A) and GBV-B infected marmosets (B).

.

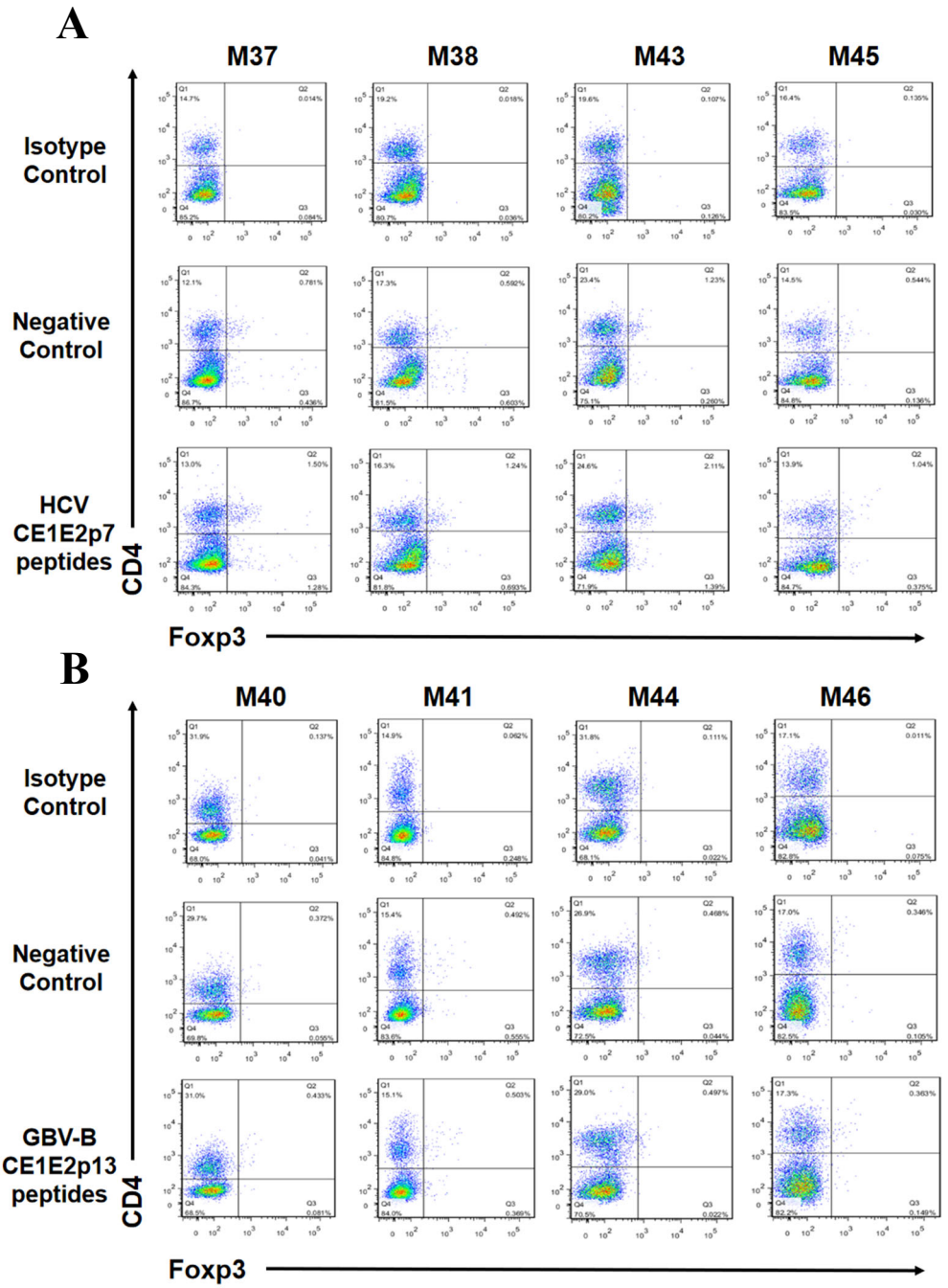

Figure S5. Frequency of Treg cells in PBMCS from HCV chimera or GBV-B infected marmosets was measured by flow cytometry. (A) Treg cells in PBMCs stimulated *in vitro* with HCV structural protein peptide pool from HCV chimera infected marmosets. (B) Treg cells in PBMCs stimulated *in vitro* with GBV-B structural protein peptide pool from GBV-B infected marmosets.

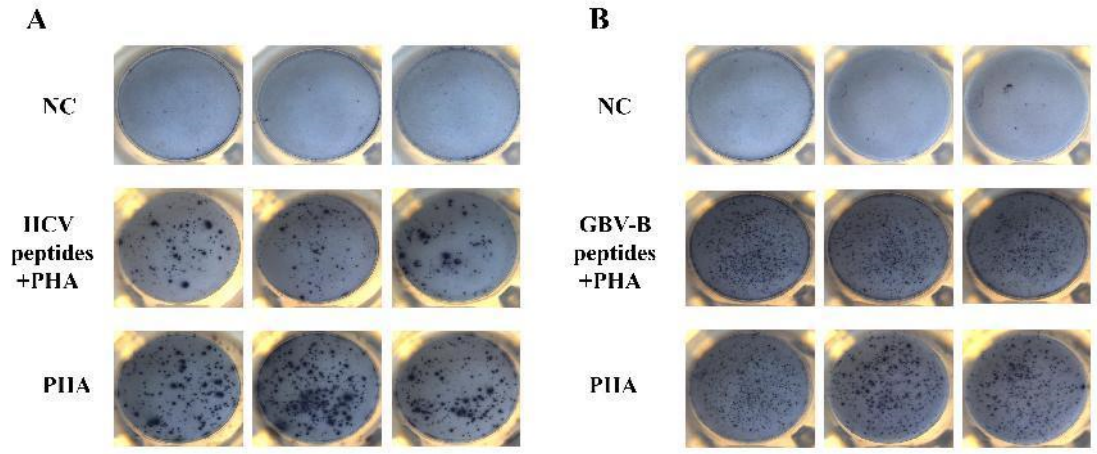

Figure S6. IFN- γ secretion T cell response was measured in PBMCs stimulated with HCV or GBV-B structural protein peptides plus PHA or PHA only. (A) Testing of IFN- γ secretion T cell response of PBMCs containing Treg cells from HCV chimera infected marmosets. (B) Testing of IFN- γ secretion T cell response of PBMCs containing Treg cells from GBV-B infected marmosets.