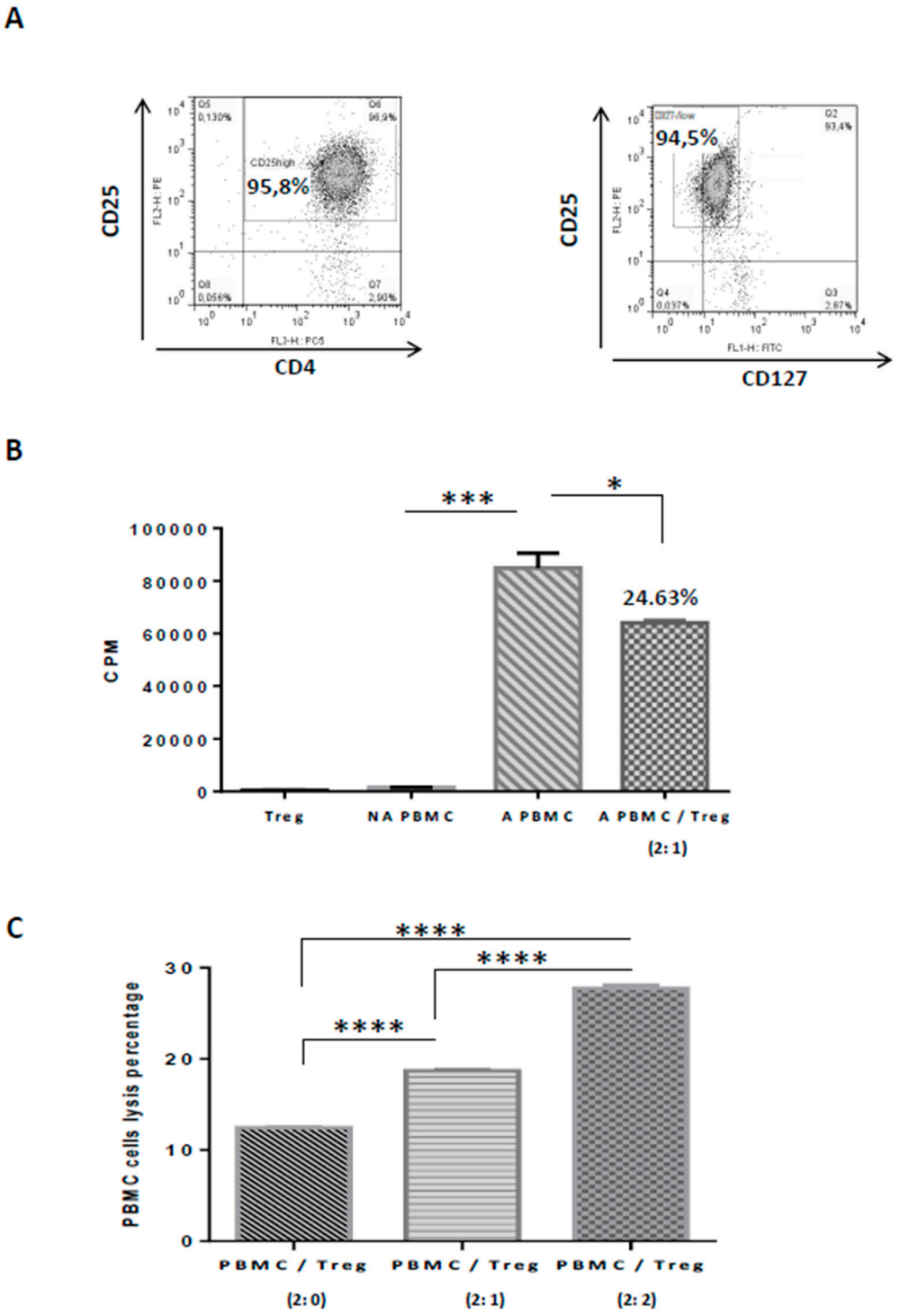

Figure S1. Phenotypic and functional characterization of freshly isolated natural regulatory T cells. Representative dot plot of triple stained CD4+CD25+/highCD127-/low Tregs after magnetic bead-isolation of 10 independent experiments (**A**). Based on CD4 and CD25 protein expression, the number in the upper-right quadrant indicates the percentage of CD4+CD25high Tregs which is superior to 95%. More than 94% of these freshly isolated Tregs are CD25highCD127-/low (upper-left quadrant). The suppressive activity of Tregs was addressed by using a model of lymphocyte reaction (MLR) by co-culture of autologous PBMC and Tregs at a 2:1 ratio in activated condition in the presence of plate-bound anti-human CD3 mAb (1µg/mL) and soluble mouse anti-human CD28 mAb (100ng/mL). Proliferation was

measured using [3H]-thymidin incorporation assays during the last 18 hours and values were obtained as counts per minute (cpm). Assays were performed after 48h and results revealed that isolated Tregs possess immunosuppressive capacity and significantly decrease of PBMC proliferation for around 24% (**B**). These representative results of 10 independent experiments are expressed as mean of cpm values of triplicate ± standard error of the mean (SEM) bars. The ability of Tregs to induce PBMC cells lysis was assessed by using a metabolic assay by co-culture of autologous PBMC and Tregs at 2:0, 2:1 and 2:2 ratios in activated condition (**C**). PBMC lysis percentage was measured by luminescence and normalized with the plate background value. Assays were performed after 48h of PBMC culture with Tregs. Tests were performed in duplicate in 3 independent experiments and results were expressed as percentage of PBMC cell lysis ± SEM bars.

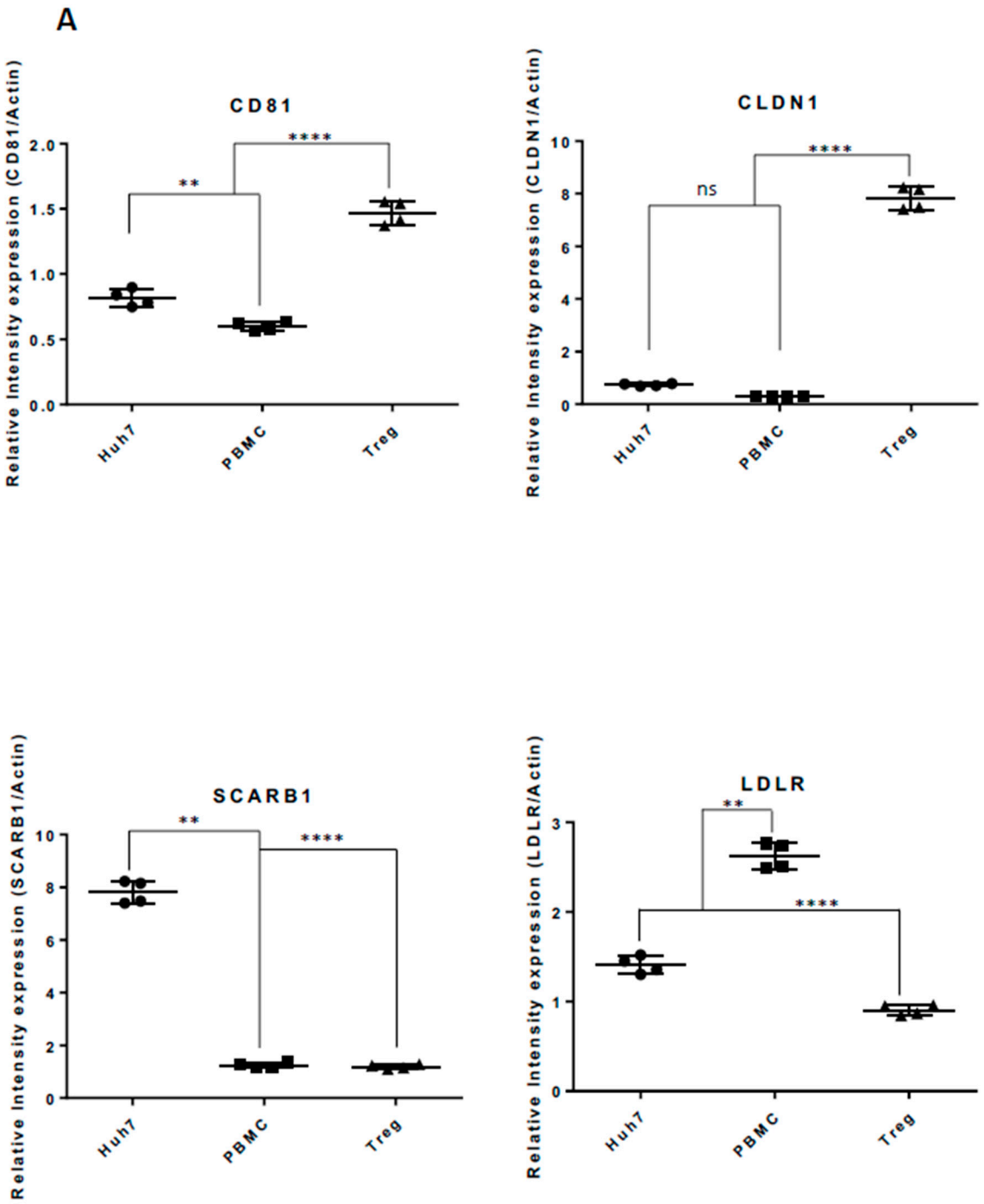

Figure S2. Tregs possess the classical HCV entry receptors: CD81, CLDN1 and LDLR. Relative total protein expression of HCV receptors detected by western-blot (**A**) and relative membranous protein expression of HCV receptors detected by FACS (**B**) on Huh7, PBMCs and Tregs. Results are the mean of four independent experiments ± SEM, statistical analysis represent the comparison in between Huh7 and either PBMCs or Tregs. ns = non-significant.

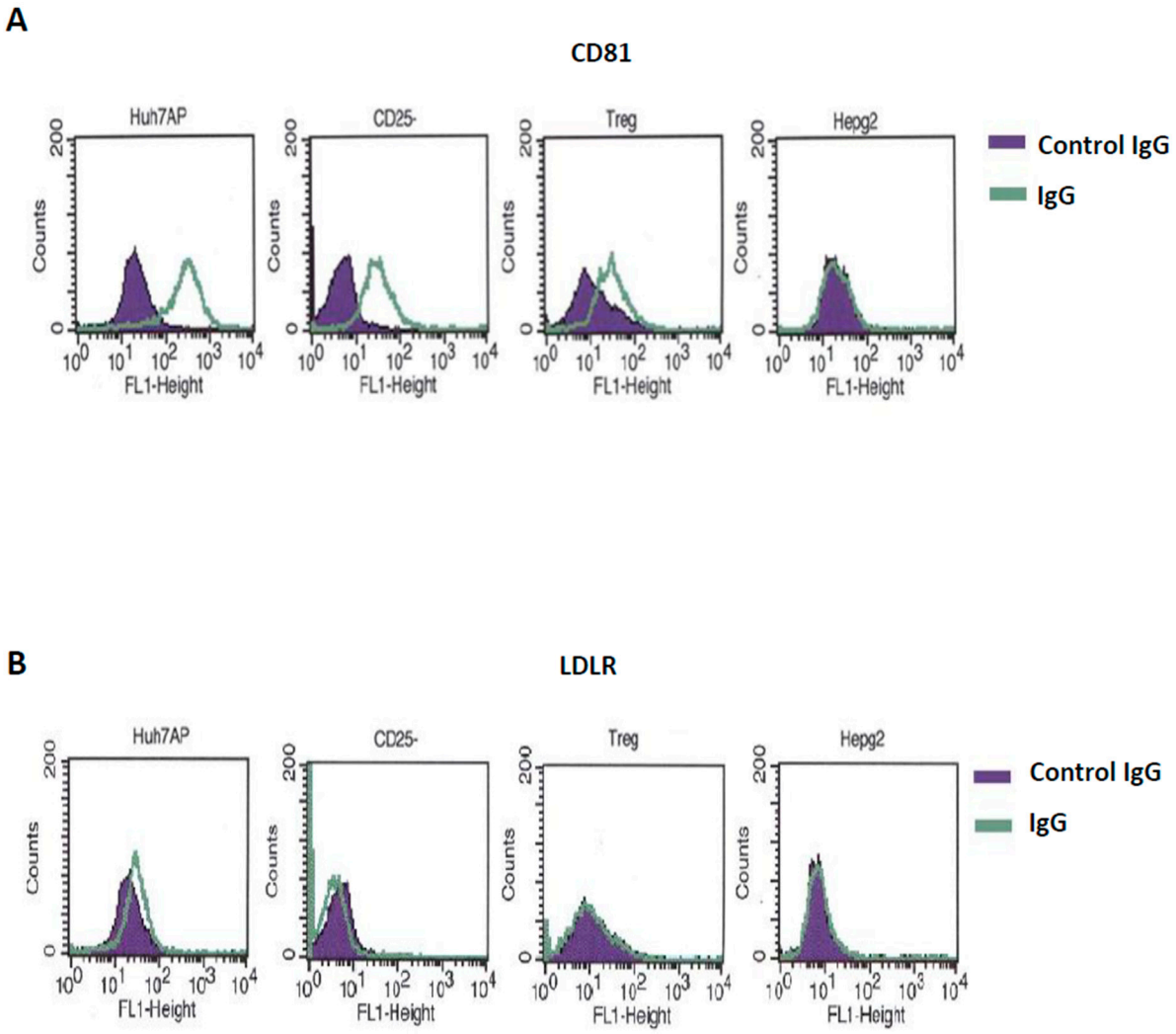

Figure S3. HepG2 cells do not express the HCV receptors CD81 and LDLR. Facs analyses were performed to evaluate the expression of HCV receptors CD81 (**A**) and LDLR (**B**) on Huh7 (HuhAP), Tconv (CD25-), Treg and HepG2 cells used as negative controls. Cells were labeled with either a specific antibody (IgG) or a relative isotype control (Control IgG). Results are representative of at least 5 independent experiments and are expressed in histograms displaying the percentage of cells positive for protein labeling compared to isotype control.

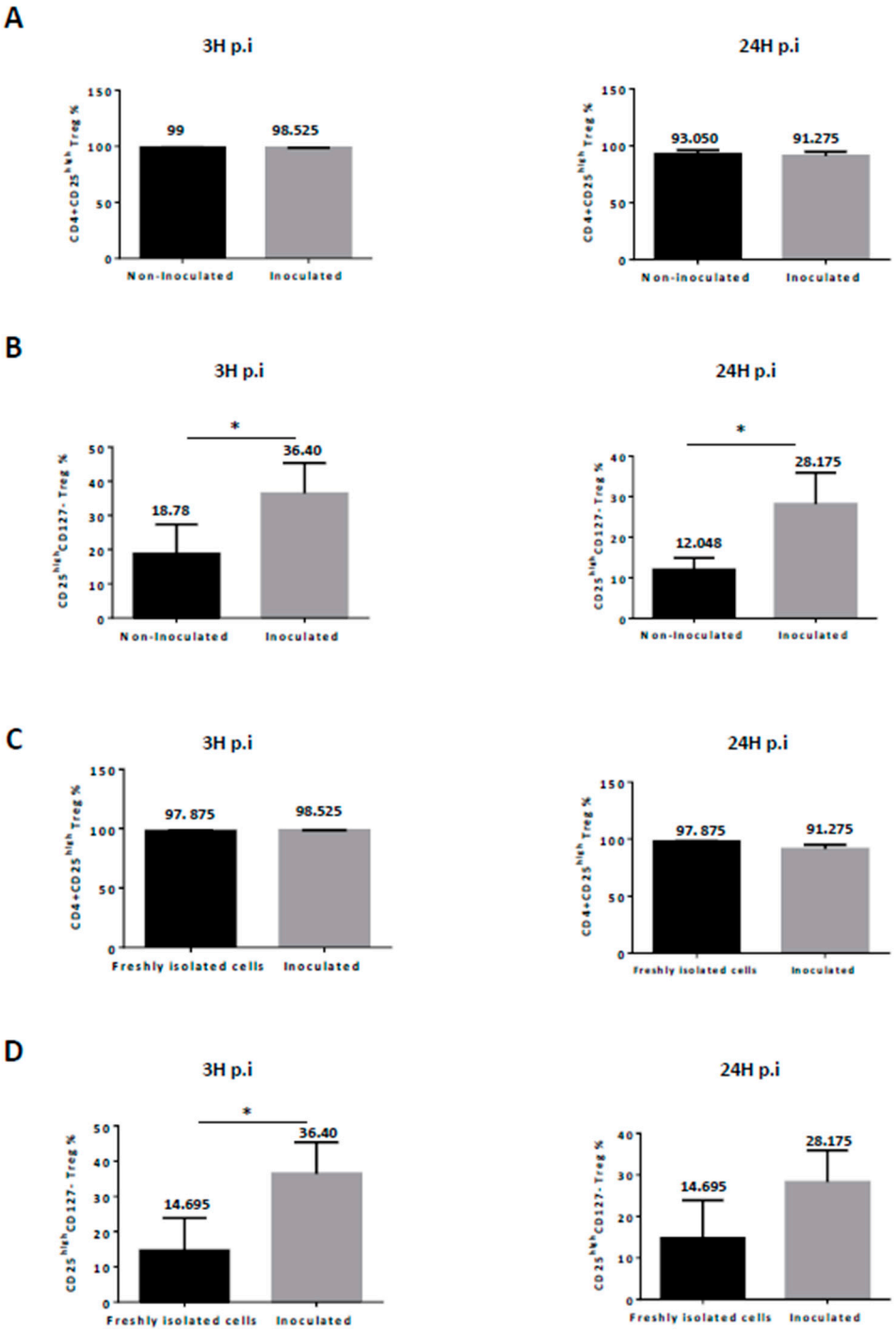

Figure S4. Impact of HCV inoculation on the frequency of suppressive Treg phenotype. FACS analyses were performed to evaluate the impact of HCV inoculation on the frequency of suppressive Tregs. CD4 + CD25high and CD25highCD127− T cells subsets were quantify between inoculated Tregs (light grey bars) vs non-inoculated Tregs (black bars) at both 3H p.i and 24H p.i (**A**,**B**). We also quantify these subsets between inoculated Tregs (light grey bars) versus freshly isolated cells (black bars) at both 3H p.i and 24H p.i (**C**,**D**). Results are expressed as means of percentage of the double stained population of 4 independent experiments ± SEM bars.

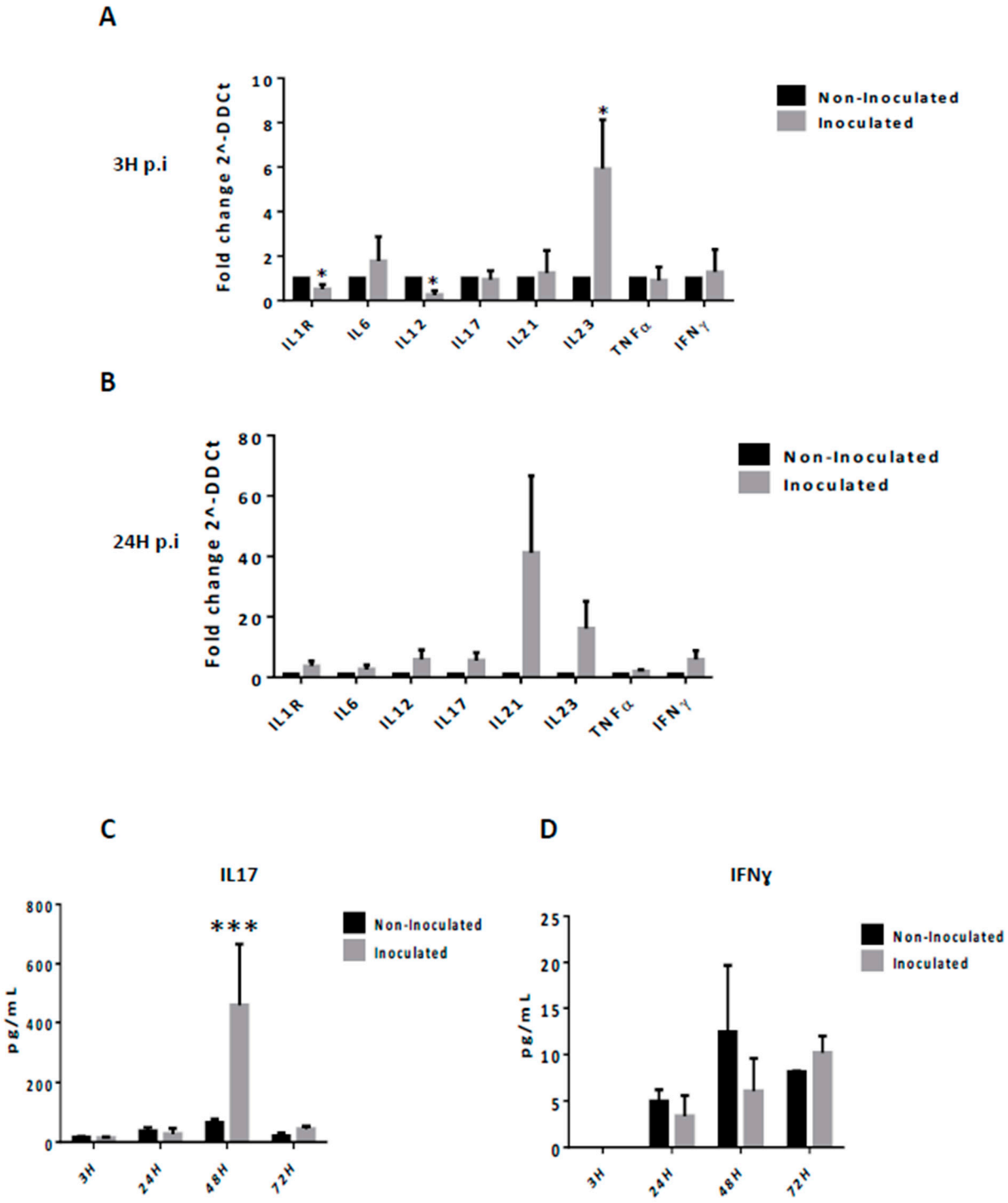

Figure S5. Impact of Tregs on the secretion of inflammatory cytokines afetr HCVcc inoculation. Tregs were handled in activated condition and were cultured in the presence or in the absence of HCV particles. Gene expression of inflammatory factors IL-1R, IL-6, IL-12, IL-16, IL-21, IL-23, TNFα and IFNɣ are expressed as means of 3 and 5 independent experiments (respectively 3H p.i and 24H p.i) (**A**,**B**). These gene expressions are normalized by using GADPH, β-actin, 18s and HPRT mRNA as housekeeping-gene before being reported to control and results are presented as fold change ($2^{-\Delta\Delta Ct}$) ± SEM bars comparing inoculated Tregs (light grey bars) versus non-inoculated Tregs (dark bars). Secretion of inflammatory cytokine IL-17 (**C**) and IFNɣ (D) were investigated by ELISA assays. Results are expressed as

mean of 3 independent experiment and presented in pg/mL ± SEM bars comparing secretion by inoculated Tregs (light grey bars) versus non-inoculated Tregs (dark bars).

Table 1. RT-Q-PCR Primers sequences.

Genes	primer sequences	
	Forward	**Reverse**
CD81	TGTATCTGGAGCTGGGAGACAAG	CCAGGAAGCCAACGAACATC
SCARB1	ATGGAACTTCTGGGCAAAG	CTTCAAACACCCCTGACTCC
CLDN1	GGTCAGGCTCTCTTCATCGG	GTTTTGGATAGGGCCTTGGT
LDLR	ACTGGTGTCAGAGGACCACC	CAAAGGAAGACGAGGAGCAC
OCLN	GGCCTCTTGAAAGTCCACCTC	CGAACATGCATCTCTCCACCA
EGFR	AGCTCTTCGGGGAGCAGCGA	ACTCGTGCCTTGGCAAACTTTCT
CD5	GAGCTCAATCATCTGCTACGGA	TTGTCGTTGGAGGTGTTGTCTT
CD4	GGGAAATCAGGGCTCCTTCTTA	TGGTCCCAAAGGCTTCTTCTT
IL2RA (CD25)	GGGACTGCTCACGTTCATCA	TTCAACATGGTTCCTTCCTTGTAG
IL7R (CD127)	GCAAGATACGTTTCCTCAGCAAC	TCCAAAGCTTTCTGGAGTGATGA
FOXP3	TCACCTACGCCACGGTCA	CACAAAGCACTTGTGCAG
CTLA4	TTCTTCTCTTCATCCCTGTCTTCT	GAGATGCATACTCACACACAAAGCT
LAG3	TGGCTTCAACGTCTCCATCA	CCCACCCTGGAACCTGCT
IL2	ACCAGGATGCTCACATTTAAGTTTTAC	TCCAGAGGTTTGAGTTCTTCTTCTAGA
IL4	CACAAGCAGCTGATCCGATTC	TTCCAAGAAGTTTTCCAACGTACTC
PRDM1 (BLIMP1)	GACGGGGGTACTTCTGTTCA	GGCATTCTTGGGAACTGTGT
BCL6	CTGCAGATGGAGCATGTTGT	CACCCGGGAGTATTTCTCAG
IL15	TTTCCATCCAGTGCTACTTGTGTT	CATTCACCCAGTTGGCTTCTGT
IL10	GAGAACCAAGACCCAGACATCAA	CCACGGCCTTGCTCTTGTT
IL24	AAGCCTTCTGGGCTGTGAAA	TGTGGACAAGGTAACAGCTCTCA
IL12A (p35)	CCTTCACCACTCCCAAAACCT	TGGTAAACAGGCCTCCACTGT
EBI3	CCCCGCCACTGCCACAATGA	GCCCTCCAACAGGTGTCCCG
GZMB	CGCCCCTACATGGCTTATCTT	CCCCCAAGGTGACATTTATGG
TGFB1	CGAGCCTGAGGCCGACTAC	CGGAGCTCTGATGTGTTGAAGA
IL1R(1)	CCACAAGGCCTGTGATTGTG	TCAACTGGCCGGTGACATTA
IL6	ATGTAGCCGCCCCACACA	CCAGTGCCTCTTTGCTGCTT
IL12B (p40)	CTTTCTAAGATGCGAGGCCAAG	AGAGGTGTAGCACTCCGCAC
IL17(A)	TCCTGGGAAGACCTCATTGG	AGAATTTGGGCATCCTGGATT
IL21	GATCGCCACATGATTAGAATGC	AGGAAAAAGCTGACCACTCACAGT
IL23(A)	GTGGGACACATGGATCTAAGAGAA	AAATCAGACCCTGGTGGATCCT
IFNG	ATGTAGCGGATAATGGAACTC	GACATTCAAGTCAGTTACC
TNFA	ATCTTCTCGAACCCCGAGTGA	GGAGCTGCCCCTCAGCTT
CCL2	GCTCATAGCAGCCACCTTCATT	ACTTGCTGCTGGTGATTCTTCTATA
CCL3	ATGGCTCTCTGCAACCAGTTCT	CGTCTCAAAGTAGTCAGCTATGAAATTC
CCL 4	GACTGTCCTGTCTCTCCTCATGCTA	AAGCTTCCTCGCGGTGTAAGA
CCL7	ATGGCTCTCTGCAACCAGTTGT	CGTCTCAAAGTAGTCAGCTATGA
CCL17	GGGCTTCTCTGCAGCACATC	GGTACCACGTCTTCAGCTTTCTAA
CCL20	GGGCTTCTCCTGGCTGCTTTG	GAATACGGTCTGTGTATCCAAGACA
CCL22	TTGCTGTGGCGCTTCAAG	CAGACGGTAACGGACGTAATCAC
CXCL9	GGCATCATCTTGCTGGTTCTG	GGTGGATAGTCCCTTGGTTGGT
CXCL11	TTGGCTGTGATATTGTGTGCTACA	TGCCACTTTCACTGCTTTTACC
CXCL16	ACACGAGGTTCCAGCTCCTTT	CAATCCCCGAGTAAGCATGTC
CCR2	GATCTGCTTTTTCTTATTACTCTCCCA	TCCGCCAAAATAACCGATGT
CCR3	GGTACCACATCCTACTATGATGACGT	CCACAGTGAACACCAGGGAGT
CCR4	CCACCCTCGATGAAAGCATATAC	TGCCTTGATGCCTTCTTTGG
CCR5	GTCAAGTCCAATCTATGACATCAATTATT	CGGGCTGCGATTTGCTT
CCR6	GTCAAGTCCAATCTATGACATCAATTATT	-CGGGCTGCGATTTGCTT
CXCR3	TCTTCCTATGACTATGGAGAAAACGA	CGGTCGAAGTTCAGGCTGAA
CXCR4	TCATGGGTTACCAGAAGAAACTGA	GAAGTTCCCAAAGTACCAGTTTGC

CXCR6	ACTATGGGTTCAGCAGTTTCAATG	CAGGTACATGCAGGGCAGAA
ACTB	CACGGCATCGTCACCAACT	GCCTGCTTCACCACCTTCTTGATGTC
GAPDH	GCCAAGGTCATCCATGACAACTTTGG	GCCTGCTTCACCACCTTCTTGATGTC
HRPT	CCCTGGCGTCGTGATTAG	ATGGCCTCCCATCTCCTT
UFD1 (ubiquitin)	CCGACCACAGTGGCTATGC	CCTCTTTTAATATCTCCAGGCTTGA
RNA 18S	TCAAGAACGAAAGTCGGAGG	GGACATCTAAGGGCATCACA

Table S2. Western Blot Density Analysis (A) and protein expression of HCV receptor in Huh7 to the expression within PBMC and Treg (B) (*n* = 4 donors).

A. Ratio = Net loading x protein/Net loaded Actin.					
		RUN 1*	**RUN 2**	**RUN 3**	**RUN 4**
CD81	Huh7	0,7810	0,8408	0,7498	0,8982
	PBMC	0,5748	0,6257	0,5690	0,6380
	Treg	1,4130	1,5402	1,3706	1,5543
CLDN1	Huh7	0,0318	0,0350	0,0309	0,0353
	PBMC	0,7146	0,7718	0,6860	0,7860
	Treg	0,2823	0,2992	0,2794	0,3246
SCARB1	Huh7	7,4816	8,1549	7,4068	8,2297
	PBMC	1,1819	1,3000	1,1465	1,3592
	Treg	1,1422	1,2222	1,0965	1,2678
LDLR	Huh7	1,3569	1,4519	1,3026	1,5198
	PBMC	2,5115	2,7375	2,4864	2,7627
	Treg	0,8690	0,9559	0,8430	0,9472

*data used as the representative one for figure1.

	B. Statistical Analysis ONE WAY ANOVA, Tukey's multiple comparisons test				
	Mean Diff	**95% CI of diff,**	**Significant Status**	**Summary**	**COMPARISON**
CD81	0,2156	0,1464 to 0,2848	**Yes**	**	*Huh7 vs. PBMC*
	−0,6521	−0,7248 to −0,5793	**Yes**	****	*Huh7 vs. Treg*
	-0,8677	−0,9870 to −0,7483	**Yes**	****	*PBMC vs. Treg*
CLDN1	0,4432	−0,05502 to 0,9415	**No**	**ns**	*Huh7 vs. PBMC*
	−7,079	−7,577 to −6,580	**Yes**	****	*Huh7 vs. Treg*
	−7,522	−8,020 to −7,024	**Yes**	****	*PBMC vs. Treg*
SCARB1	6,571	6,056 to 7,087	**Yes**	****	*Huh7 vs. PBMC*
	6,636	6,121 to 7,151	**Yes**	****	*Huh7 vs. Treg*
	0,06472	−0,4505 to 0,5799	**No**	**ns**	*PBMC vs. Treg*
LDLR	−1,217	−1,426 to −1,007	**Yes**	****	*Huh7 vs. PBMC*
	0,504	0,2945 to 0,7135	**Yes**	***	*Huh7 vs. Treg*
	1,721	1,511 to 1,930	**Yes**	****	*PBMC vs. Treg*

Diff: difference; CI: confident interval; ns: non-significant, * indicate $p \leq 0.05$, ** indicate $p \leq 0.01$***, indicate $p \leq 0.001$, **** indicate $p \leq 0.0001$.

Table S3. FACS Analysis of the expression of HCV entry receptors (**A**), statistical analysis of protein expression in Huh7 vs. expression within PBMC and Treg (**B**) (n = 4 donors).

A. Percentage of Marked Cells within population					
		RUN 1*	**RUN 2**	**RUN 3**	**RUN 4**
CD81	***Huh7***	57,60	63,36	55,30	66,24
	PBMC	28,00	29,96	27,72	31,08
	Treg	47,60	51,88	46,17	52,36
CLDN1	***Huh7***	54,20	59,62	52,57	60,16
	PBMC	65,50	70,74	64,85	72,05
	Treg	89,70	95,08	88,80	103,16
SCARB1	***Huh7***	1,50	1,64	1,49	1,65
	PBMC	4,20	4,62	4,07	4,83
	Treg	2,33	2,49	2,24	2,59
LDLR	***Huh7***	2,08	2,23	2,00	2,33
	PBMC	2,74	2,99	2,66	3,01
	Treg	2,16	2,38	2,14	2,35

*data used as the representative one for figure 1.

B. Statistical Analysis ONE WAY ANOVA , Tukey's multiple comparisons test						
		Mean Diff,	***95% CI of diff,***	***Significant Status***	***Summary***	**COMPARISON**
CD81	***Huh7***	0,4432	−0,05502 to 0,9415	**No**	ns	*Huh7 vs. PBMC*
	PBMC	−7,079	−7,577 to −6,580	**Yes**	****	*Huh7 vs. Treg*
	Treg	−7,522	−8,020 to −7,024	**Yes**	****	*PBMC vs. Treg*
CLDN1	***Huh7***	−11,64	−21,27 to −2,020	**Yes**	*	*Huh7 vs. PBMC*
	PBMC	−37,55	−47,17 to −27,92	**Yes**	****	*Huh7 vs. Treg*
	Treg	−25,9	−35,53 to −16,28	**Yes**	****	*PBMC vs. Treg*
SCARB1	***Huh7***	−2,864	−3,316 to −2,411	**Yes**	****	*Huh7 vs. PBMC*
	PBMC	−0,844	−1,297 to −0,3915	**Yes**	**	*Huh7 vs. Treg*
	Treg	2,019	1,567 to 2,472	**Yes**	****	*PBMC vs. Treg*
LDLR	***Huh7***	−0,692	−0,9916 to -0,3916	**Yes**	***	*Huh7 vs. PBMC*
	PBMC	−0,099	−0,3992 to 0,2008	**No**	ns	*Huh7 vs. Treg*
	Treg	0,5924	0,2924 to 0,8924	**Yes**	***	*PBMC vs. Treg*

Diff: difference; CI: confident interval; ns: non-significant. * indicate $p \leq 0.05$, ** indicate $p \leq 0.01$***, indicate $p \leq 0.001$, **** indicate $p \leq 0.0001$.

Table S4. Analysis of the ratio comparing expression levels of Tregs either to PBMCs or Huh7 for each employed technique of detection.

	Ratio Treg/PBMC		**Ratio Treg/Huh7**		
	FACS	**WB**	**FACS**	**WB**	**RT-QPCR**
CD81	1,70	2,44	0,82	1,80	2,09
CLDN1	1,38	0,40	1,66	8,91	1,02
SCARB1	0,54	0,95	1,54	0,15	0,08
LDLR	0,79	0,34	1,05	0,64	3,19

Table 5. Ranking levels of expression in Huh7, PBMC and Treg. (1 is the strongest and 3 the lowest).

	CD81		**CLDN1**		**SCARB1**		**LDLR**	
	WB	***FACS***	***WB***	***FACS***	***WB***	***FACS***	***WB***	***FACS***
Huh7	2	1	3	3	1	3	2	3
PBMC	3	3	1	2	2	2	1	1
Treg	1	2	2	1	3	1	3	3

WB: werstern-blot, FACS: flow cytometry, Treg: regulatrory T cells, PBMC: peripheral blood mononuclear cells.