Supplementary table 1:
Relative distribution of PD-1/PD-L1 positive immune cells

	PD-1 positive Immune Cells			PD-L1 positive Immune Cells		
HCC patient	Percent total cells	Percent Macrophages	Percent $CD8^+$ T cells	Percent total cells	Percent Macrophages	Percent $CD8^+$ T cells
#3	0.5	all	none	negative	none	none
#7	negative	none	none	1	all	none
#13	20	16	4	0.5	all	none
#16	10	1	8	1	all	none
#19	negative	none	none	0.6	0.3	0.3
#20	10	9.5	0.5	3	all	none
#22	0.5	all	none	negative	none	none
#29	negative	none	none	10	9.5	0.5
#33	negative	none	none	1	0.8	0.2
#42	negative	none	none	1	all	none
#46	10	0.5	9.5	negative	none	none

Supplementary table 2:
Checkpoint molecules on T cell subsets

	Healthy controls	Disease Controls without HCC	HCC
a) T effector cells			
Percent $CD4^+$ T cells (mean ± SD)	51.7 ± 6.9	49.9 ± 12.0	51.2 ± 11.6
Percent $CD8^+$ T cells (mean ± SD)	26.9 ± 6.6	26.2 ± 9.3	25.7 ± 10.9
Percent CTLA-4^+ $CD4^+$ T cells (mean ± SD)	5.0 ± 1.2	5.8 ± 2.0	5.8 ± 1.3
Percent CTLA-4^+ $CD8^+$ T cells (mean ± SD)	3.8 ± 1.4	4.9 ± 2.2	4.2 ± 1.4
Percent $GITR^+$ $CD4^+$ T cells (mean ± SD)	4.3 ± 1.2	5.0 ± 1.6	4.3 ± 1.3
Percent $GITR^+$ $CD8^+$ T cells (mean ± SD)	4.4 ± 1.4	5.1 ± 2.2	4.3 ± 1.7
Percent $Tim3^+$ $CD4^+$ T cells (mean ± SD)	4.6 ± 1.7	4.8 ± 1.8	4.5 ± 2.0
Percent $Tim3^+$ $CD8^+$ T cells (mean ± SD)	4.6 ± 1.9	6.1 ± 3.5	4.8 ± 2.1
b) Regulatory T cells			
Percent CTLA-4^+ cells among Tregs (mean ± SD)	4.8 ± 1.4 [a)]	6.5 ± 1.5	7.0 ± 1.7
Percent $GITR^+$ cells among Tregs (mean ± SD)	3.8 ± 1.3 [a)]	5.4 ± 2.0	4.9 ± 2.1
Percent PD-1^+ cells among Tregs (mean ± SD)	7.6 ± 1.9 [a)]	10.9 ± 1.9	10.9 ± 2.7
Percent Tim-3^+ cells among Tregs (mean ± SD)	3.1 ± 1.4 [a)]	6.1 ± 2.0	5.6 ± 1.7
Percent galectin-9^+ cells among Tregs (mean ± SD)	4.4 ± 1.2 [a)]	5.9 ± 1.6	6.1 ± 1.6
Percent TGF-$beta^+$ cells among Tregs (mean ± SD)	3.2 ± 1.0 [a)]	5.6 ± 1.5	5.1 ± 1.7

a) Significant differences between healthy controls and both the disease controls and HCC patients.

Supplementary table 3:
Genetic profile of P815 variants

Marker Analysis		**Allele Size**				
Locus	Chromosomal Location	**P815_Langhans et al.**	P815_ Almeida et al. [a)]	P815_Miller et al. [b)]	P815_ATCC	P815_DBA/2N
18-3	Chr 18	**162/166**	158	164, 173	156	151
4-2	Chr 04	**240**	240	237	237	237
6-7	Chr 06	**335/340**	335	331, 335	335	335
9-2	Chr 09	**225/230**	N/A	221, 233	221, 225	221
15-3	Chr 15	**201/205**	197/201	197, 217	193, 197	197
6-4	Chr 06	**297/301**	297/201	291, 303	295, 299	299
12-1	Chr 12	**231**	235	226, 230	226, 229	225
5-5	Chr 05	**335/343**	335	330	335	229
x-1	Chr X	**401**	409	422	409	413

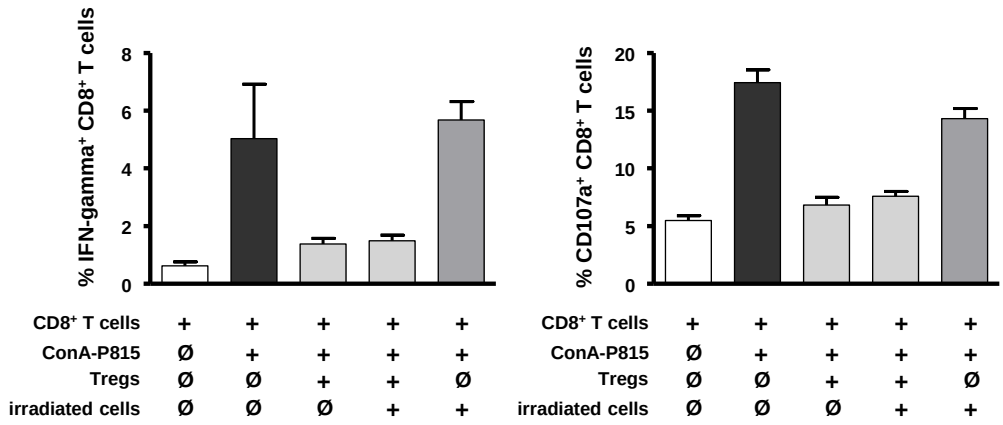
Authentification of murine mastocytoma cells is currently still difficult due to the lack of a standardized reference data base and there is considerable variation between P815 cells reported in different data bases. Thus, we could not find a perfect match to any of the reported data bases and the closest correspondance was to the recent report by Almeida and colleagues.

[a)] Almeida et al. Mouse cell line authentication. Cytotechnology 2014; 66: 133-147

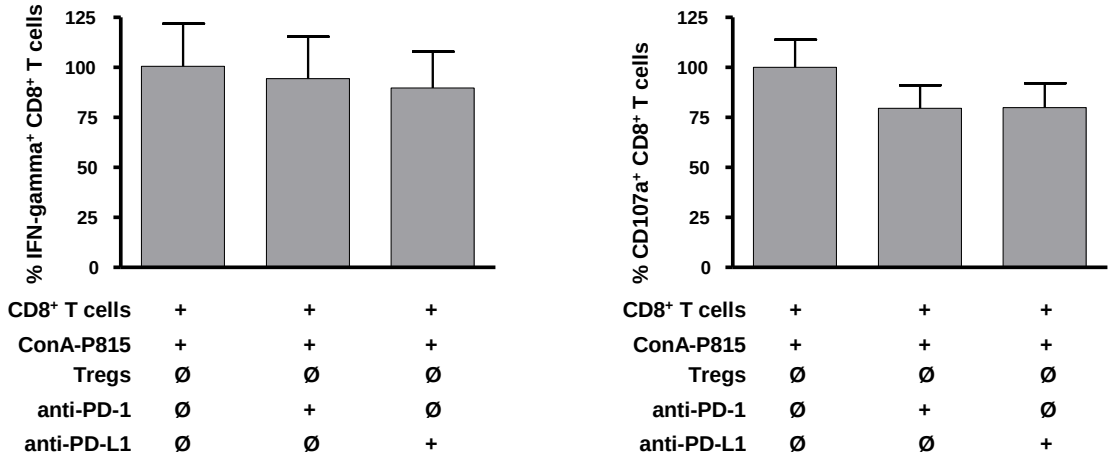
[b)] Miller et al. Splenectomy promotes indirect elimination of intraocular tumorsby CD8+ Tcells that is associated with IFNg- and Fas/FasL-dependent activation. Cancer Immunol Res 2014; 1175-1185

Supplementary figure 1

a)



b)

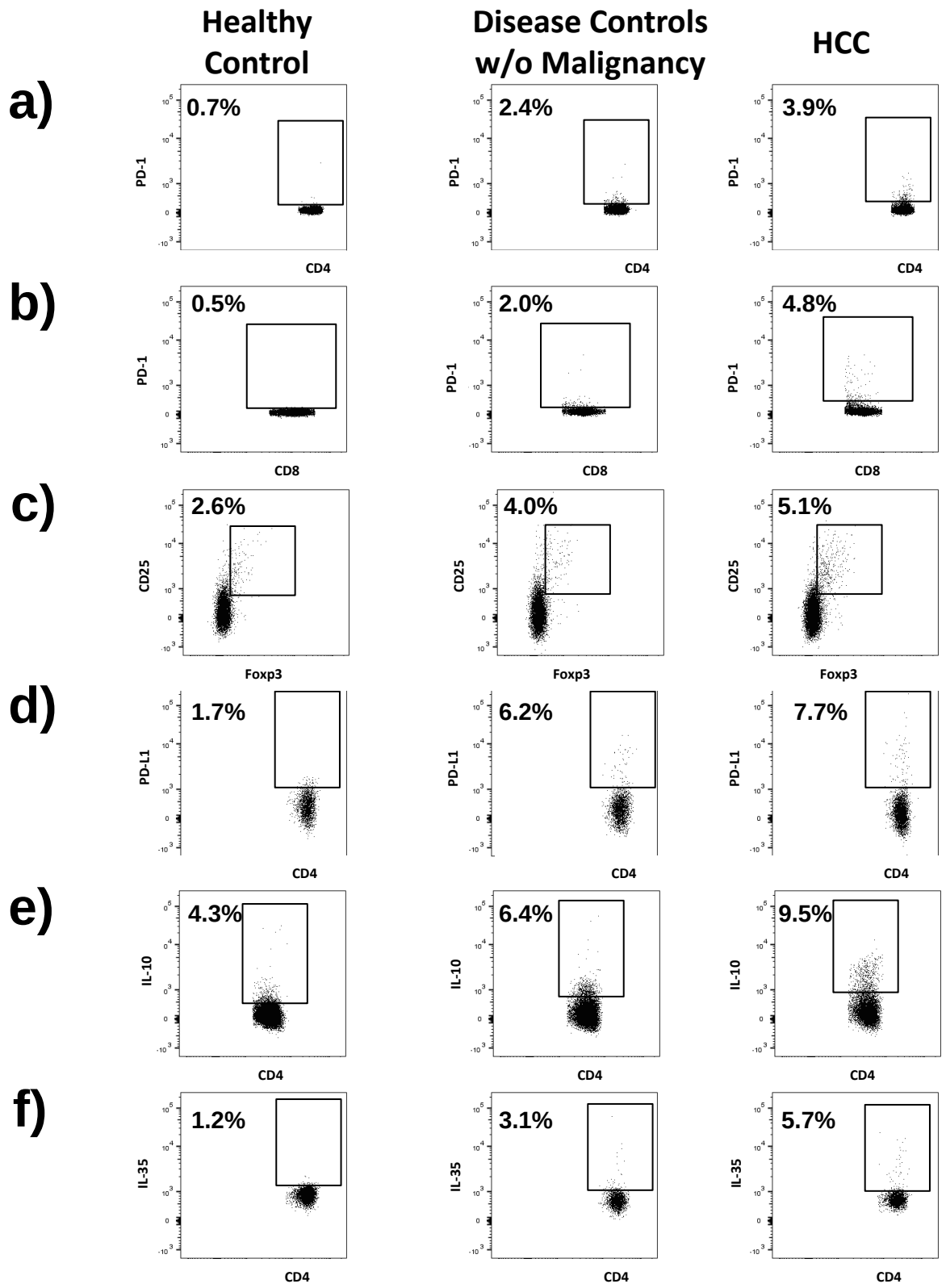


Supplementary figure 1: Control experiments to demonstrate specificity of Treg mediated inhibition (a) and lack of direct inhibition of T effector cells by neuralizing PD-1 and PD-L1 antibodies (b) in the lectin-dependent cellular cytotoxicity (LDCC) assays

To exclude bias resulting from dilutional effects in the co-culture experiments we repeated the LDCC experiments in the presence of irradiated autologous PBMC (3,000 rad) shown in **figure a**. Experiments were performed with lymphocytes isolated from the blood of HCV-infected patients (n=3).

Figure b illustrates that control LDCC experiments with neutralizing anti-PD-1 and anti-PD-L1 in the absence of autologous Tregs did not reveal any effect on IFN-gamma production and T cell degranulation in patients with HCC.

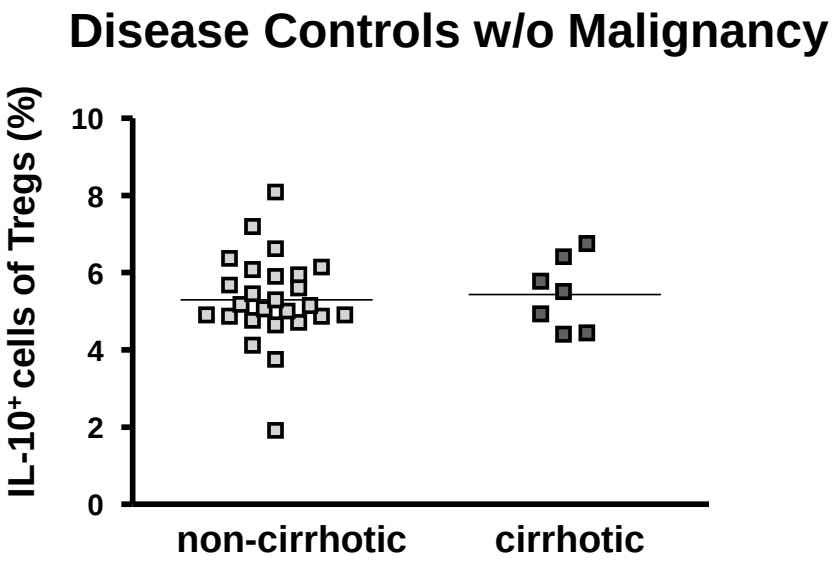
Supplementary figure 2



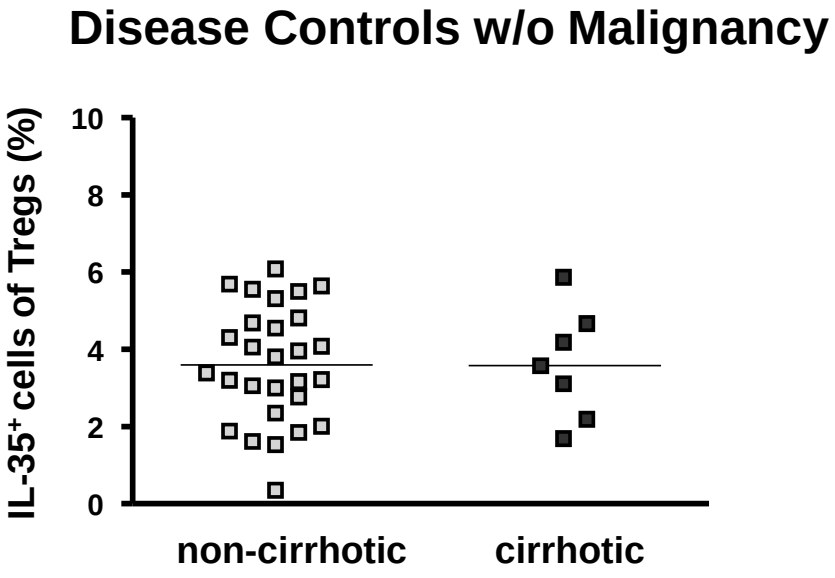
Supplementary figure 2: Representative analysis of frequency/phenotype of T cell subsets
These figures show representative dot plots of PD-1$^+$ cells among CD4$^+$ (**a**) and CD8$^+$ T cells (**b**) in healthy controls (left), disease controls without HCC (middle) and patients with HCC (right). In addition, overall frequencies of CD4$^+$CD25$^+$Foxp3$^+$ Tregs (**c**) and frequencies of PD-L1$^+$ (**d**), IL-10$^+$ (**e**) and IL-35$^+$ cells among Tregs are illustrated (**f**).

Supplementary figure 3

a)

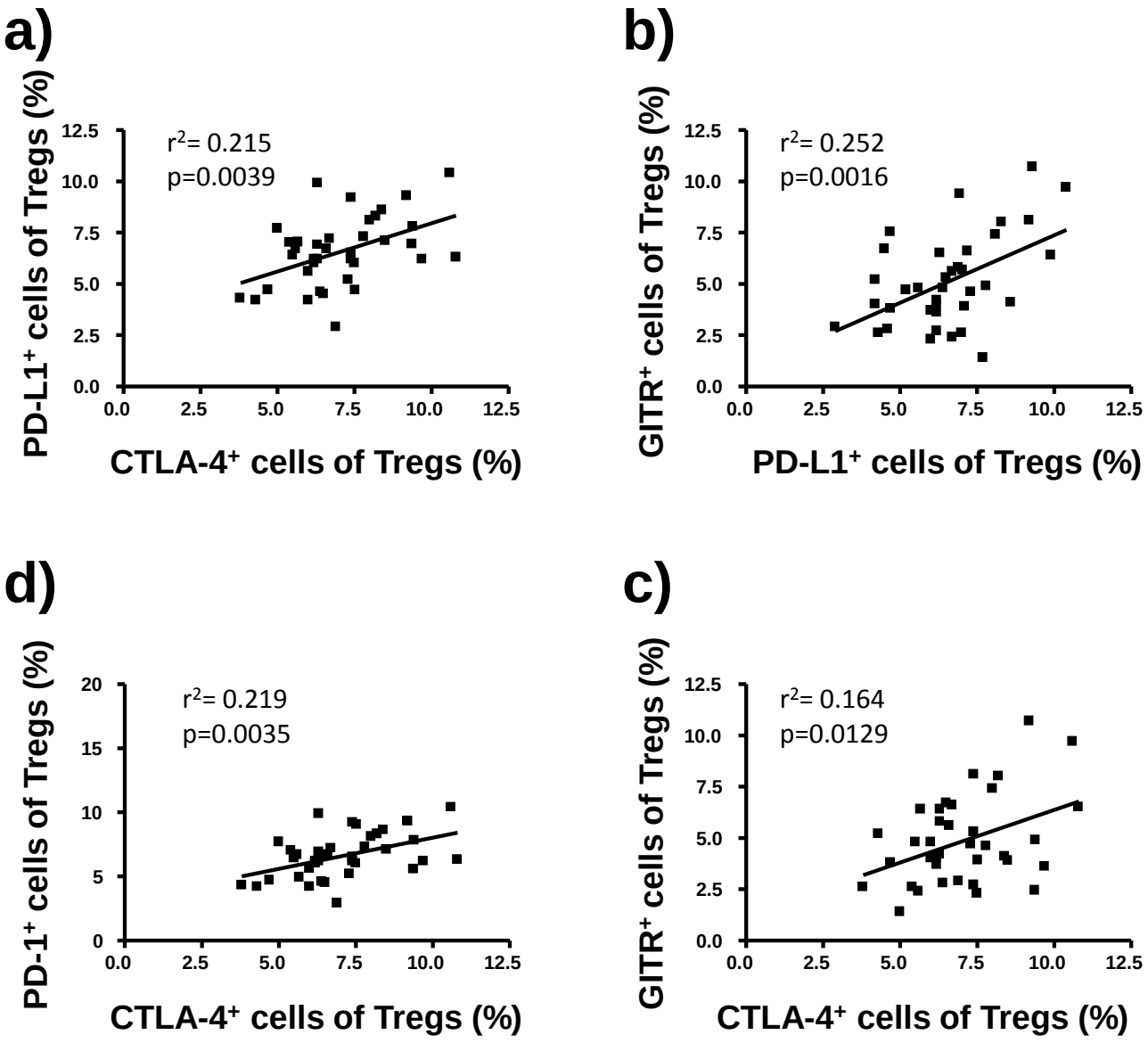


b)



Supplementary figure 3: Comparison of the frequency of IL-10$^+$ and IL-35$^+$ Treg subsets between cirrhotic and non-cirrhotic disease controls without HCC
To check whether the reported differences in IL-10$^+$ and IL-35$^+$ Tregs were attributed to an inbalance in the distribution of cirrhotic patients in the group with HCC and the disease controls we compared their frequency between cirrhotic and non-cirrhotic disease controls and found identical numbers of IL-10-positive (**a**) and IL-35-positive Tregs in the two subgroups (**b**).

Supplementary figure 4



Supplementary figure 4: **Correllation of Treg subset markers to each other in patients with HCC**

These figures demonstrate that frequencies of Tregs expressing CTLA-4, GITR, PD-1, and PD-L1 were signifcantly related to each other suggesting that these markers seem to stain closely related T cell subsets. Statistical parameters refer correlation coefficients and significances obtained by linear regression analysis.