

Supplemental Table 1. Characteristics of patients and healthy donors

Clinical Diagnosis	No. of Patients	Sex M/F	ALT (IU/L)	Etiology (HCV/HBV/others)	AFP (ng/ml)	Child-Pugh (A/B/C)	Tumor Size (large/small)	Tumor Multiplicity (multiple/solitary)	TNM Stage (I/II/III/IV)
HCC patients	123	83/40	52 ± 36	82/22/19	1051 ± 4391	84/33/6	69/54	69/54	32/45/35/11
CLD patients	26	14/12	46 ± 27	22/4/0	11 ± 7	26/0/0	ND	ND	ND
Healthy donors	13	8/5	17 ± 5	ND	ND	ND	ND	ND	ND

Abbreviations: ALT, alanine aminotransferase; HCV, hepatitis C virus; HBV hepatitis B virus; AFP, Alpha-fetoprotein; HCC, hepatocellular carcinoma; CLD, chronic liver disease;

SD, standard deviation

All continuous variables were expressed as mean ± SD.

Supplemental Table 2. Serum cytokines and CD4⁺ CD25⁺ CD127^{-/low} Tregs

Cytokine	Healthy donor (mean) (n=13)	Treg ≥7 % (mean) (n=29)	Range	Treg <7% (mean) (n=23)	Range	p value
IL-1ra	34.2	54.2	(11.0-430)	79.7	(5.9-600)	0.383
IL-2	10.5	26.0	(2.1-54.3)	16.0	(0.9-50.6)	0.167
IL-4	2.6	6.17	(1.47-11.9)	4.54	(0.71-10.9)	0.128
IL-6	9.9	14.6	(0.2-97.2)	15.1	(1.6-130)	0.872
IL-8	24.5	49.5	(6.2-291)	40.1	(10.9-256)	0.563
IL-10	2.76	4.99	(0.6-12.1)	2.97	(0.1-10.4)	0.030
IL-12(p70)	14.6	25.8	(0.6-140)	23.2	(2.8-78)	0.715
IL-13	7.6	11.6	(2.7-33.6)	8.8	(1.2-22.9)	0.167
IL-17	15.7	20.1	(4.2-70)	25.4	(2.1-119)	0.340
Eotaxin	104	132	(59.0-493)	119	(26.3-245)	0.906
G-CSF	7.9	11.2	(2.7-41.3)	10.1	(0.5-33.7)	0.424
IFN-γ	52.6	85.9	(23.1-417)	78.6	(2.5-343)	0.319
MCP-1	20.2	27.2	(3.5-114)	21.9	(8.4-71)	0.375
MIP-1b	97.6	123	(56.6-490)	103	(39.7-229)	0.696
PDGF	4012	4179	(1271-10136)	4305	(831-13557)	0.815
RANTES	2978	3041	(1040-4714)	3097	(599-6165)	0.598
TNF-α	10.5	29.1	(0.1-175)	32.7	(2.9-155)	0.747
VEGF	34.6	83.4	(24.5-371)	71.9	(9.3-224)	0.538

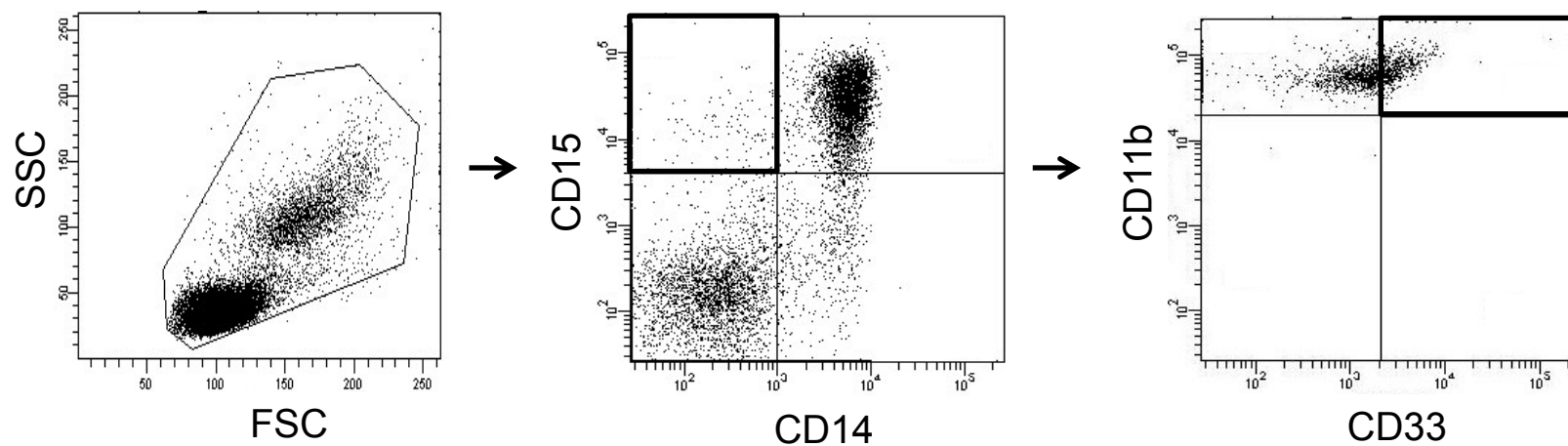
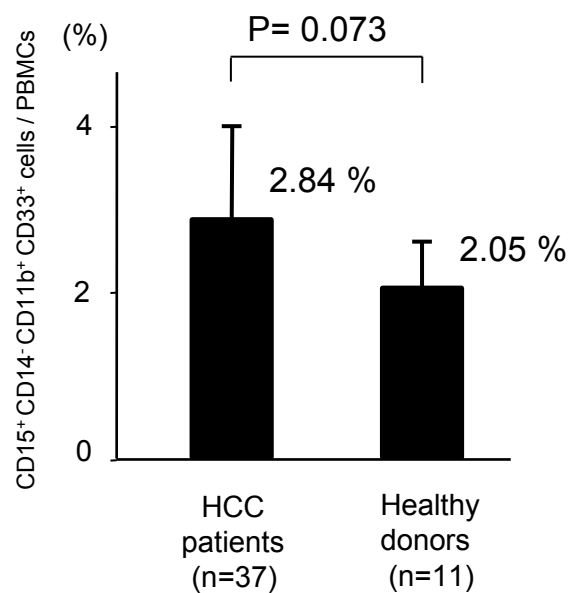
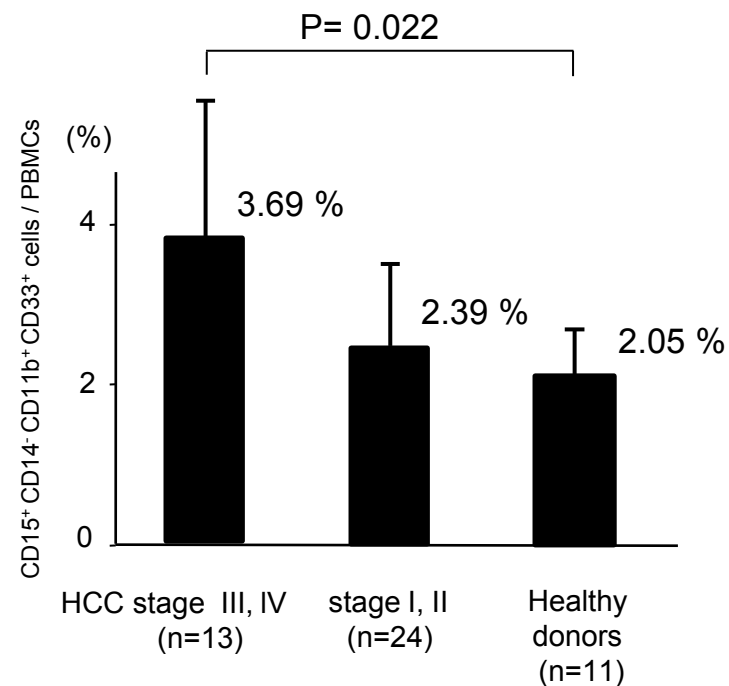
Abbreviations: IL, interleukin; G-CSF, granulocytic colony stimulating factor; IFN, interferon; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated upon activation, normal T cell-expressed and secreted; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor

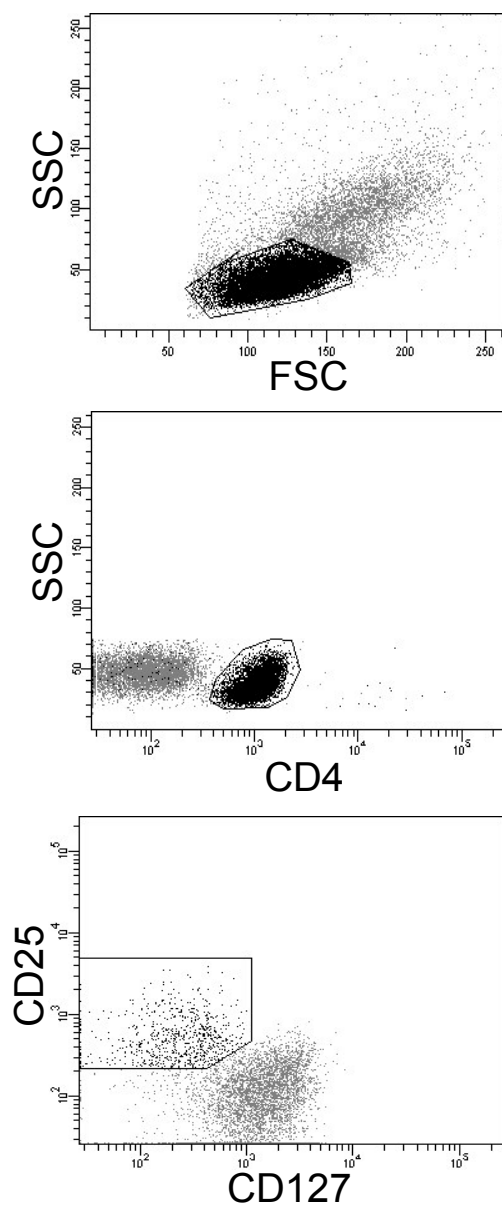
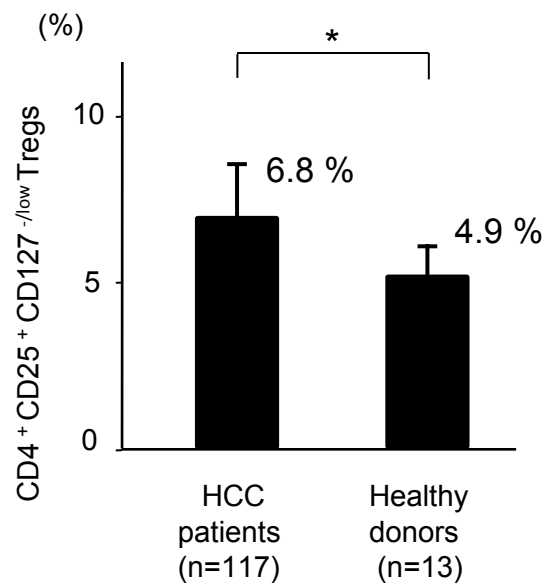
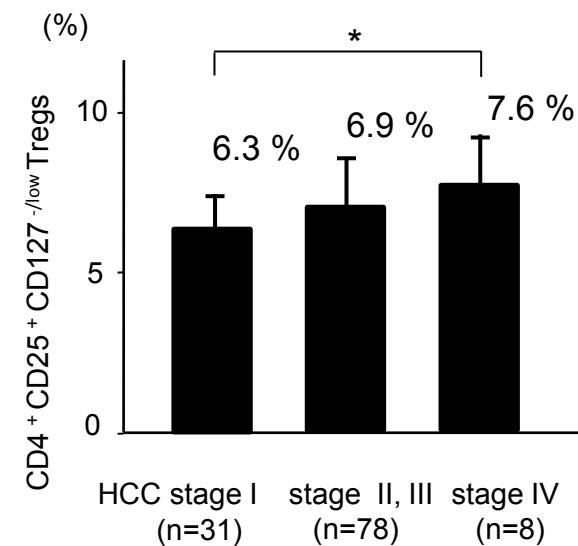
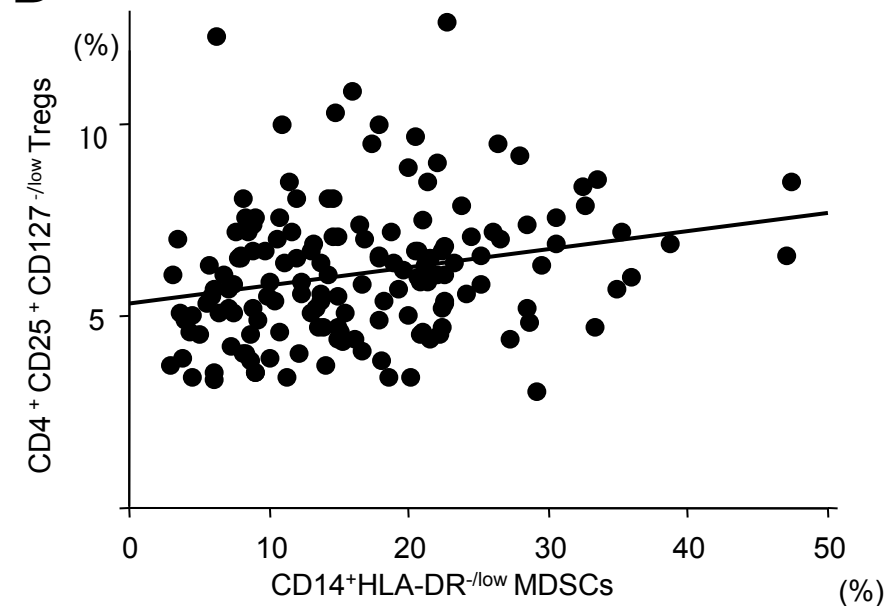
Mann-Whitney's U test was used for univariate analysis of two groups that were classified according to the frequency of MDSCs.

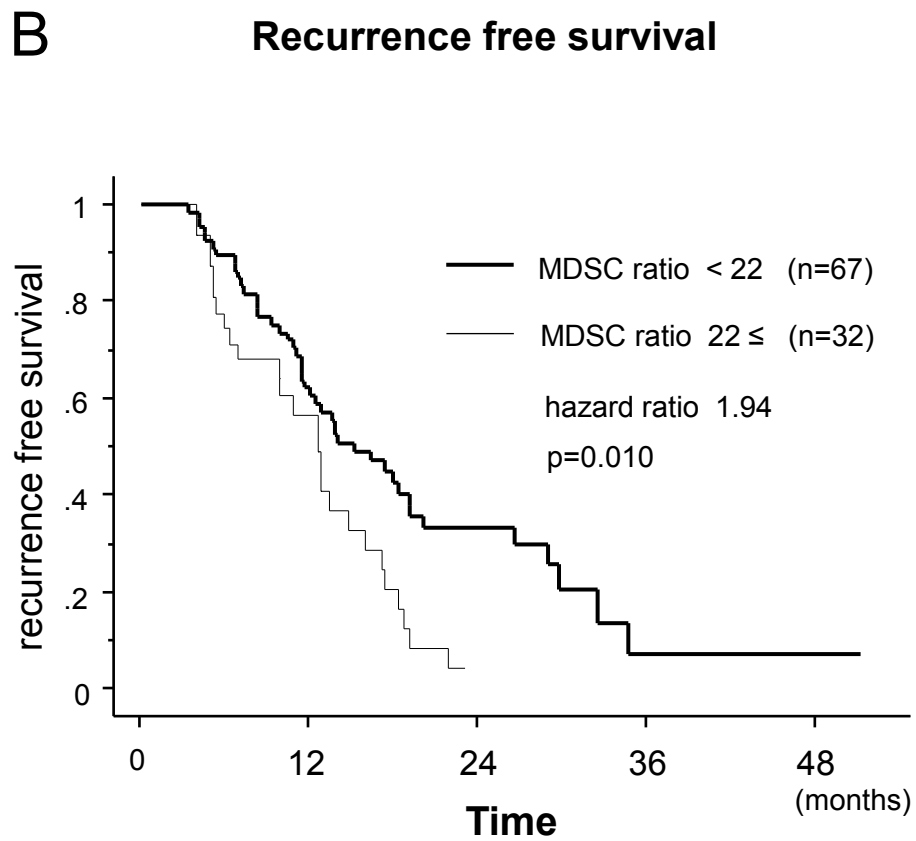
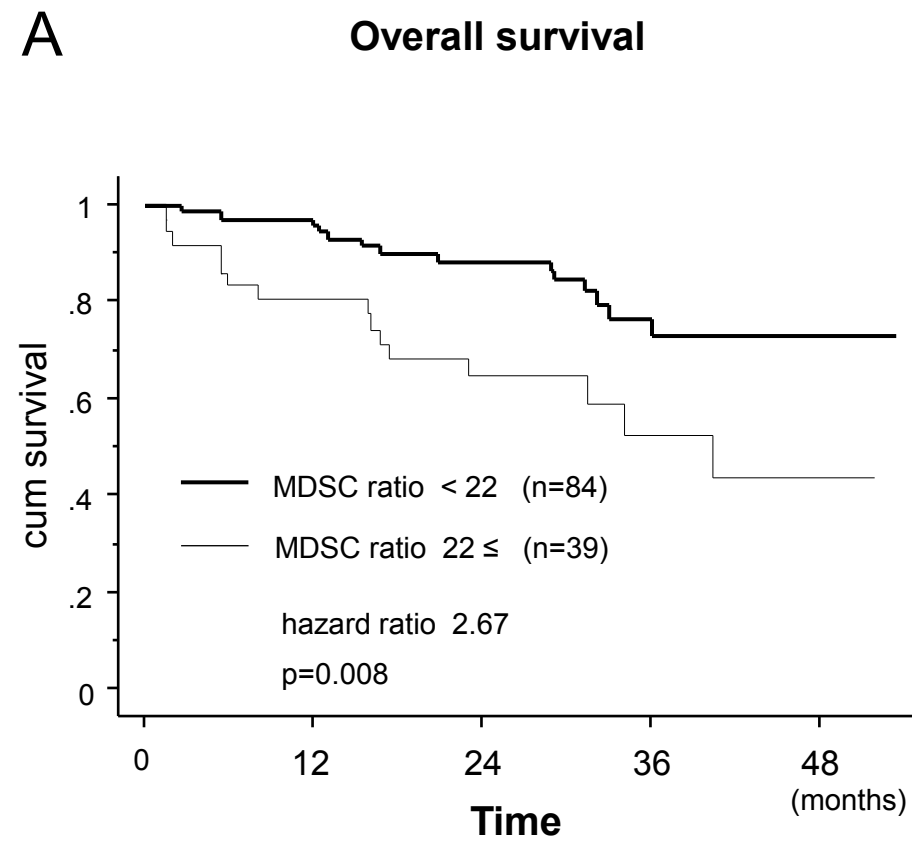
Supplemental table 3. The clinical parameters before RFA

Clinical characteristics	MDSC ratio ≥ 22	MDSC ratio < 22	p value
	(n=8)	(n=25)	
Pre MDSC ratio (%)	28.7	14.8	0.004
Post MDSC ratio (%)	30.2	10.8	<0.001
Sex (M/F)	6/2	13/12	0.252
Prothrombin time (%)	82.8	79.9	0.663
Serum albmin (g/dl)	3.70	3.67	0.794
Total bilirubin	0.83	1.03	0.408
Platelet ($10^4/\mu\text{l}$)	8.6	9.5	0.473
Type IV collagen 7S (ng/ml)	7.5	7.6	0.924
AFP (ng/ml)	146	41.7	0.214
Pre neutrophil/lymphocyte	1.98	2.19	0.486
Post neutrophil/lymphocyte	2.70	2.16	0.097
Pre ALT (IU/l)	45.0	55.3	0.421
Post ALT (IU/l)	42.6	52.2	0.584
Child-Pugh (A/B/C)	7/1/0	20/5/0	0.632
Tumor Size (mm)	21.8	18.2	0.313
Tumor Multiplicity (multiple/solitary)	6/2	13/12	0.252

Abbreviations: AFP, Alpha-fetoprotein; ALT, alanine aminotransferase

A CD15⁺ CD14⁻ CD11b⁺ CD33⁺ cells**B** CD15⁺ CD14⁻ CD11b⁺ CD33⁺ cells / PBMCs**C** CD15⁺ CD14⁻ CD11b⁺ CD33⁺ cells / PBMCs

A CD4⁺ CD25⁺ CD127^{-/low} Tregs**B** Tregs / CD4⁺ cells**C** Tregs / CD4⁺ cells**D**



Suppl. figure 1: (A) Gating strategy of CD15⁺CD14⁻CD11b⁺CD33⁺ cells (B) The frequency of CD15⁺CD14⁻CD11b⁺CD33⁺ cells in PBMCs was higher in HCC patients than healthy donors (2.84% vs. 2.06%, $p = 0.073$). (C) The frequency of CD15⁺CD14⁻CD11b⁺CD33⁺ cells was correlated with tumor progression (stage III and IV: 3.69% (n=13) vs. stage I and II: 2.39% (n=24), $p = 0.022$).

Suppl. Figure 2: (A) Gating strategy of CD4⁺ CD25⁺ CD127^{-/low} Tregs (B) The frequency of CD4⁺ CD25⁺ CD127^{-/low} Tregs was significantly increased in HCC patients than healthy donors (*, $p < 0.01$). (C) The frequency of Tregs was correlated with tumor progression. (D) There was only a weak correlation between the frequencies of CD14⁺HLA-DR^{-/low} MDSCs and CD4⁺ CD25⁺ CD127^{-/low} Tregs ($r = 0.24$).

Suppl. figure 3: (A) Kaplan-Meier curve for overall survival in our cohort. (B) Kaplan-Meier curve for recurrence free survival in our cohort.