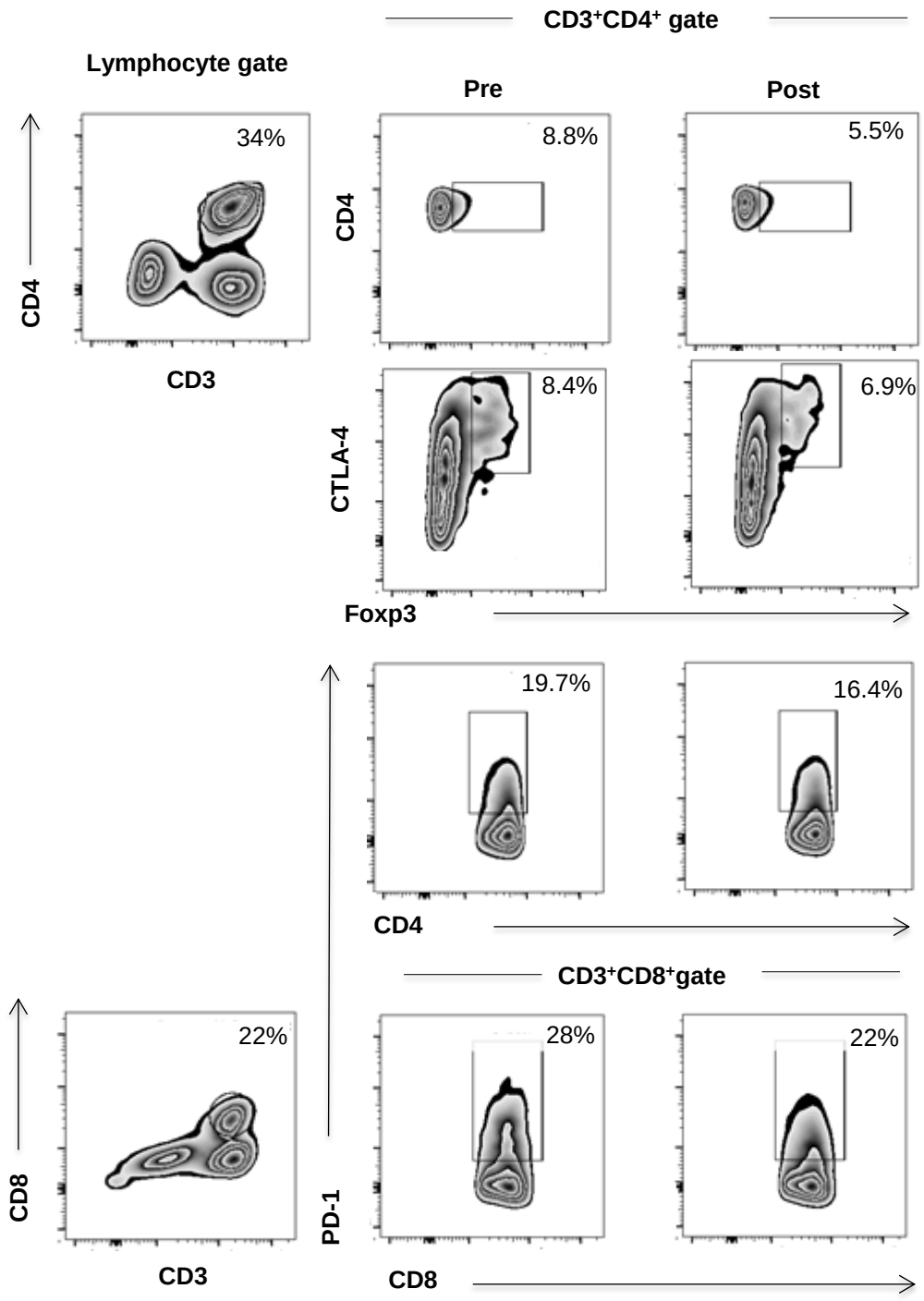
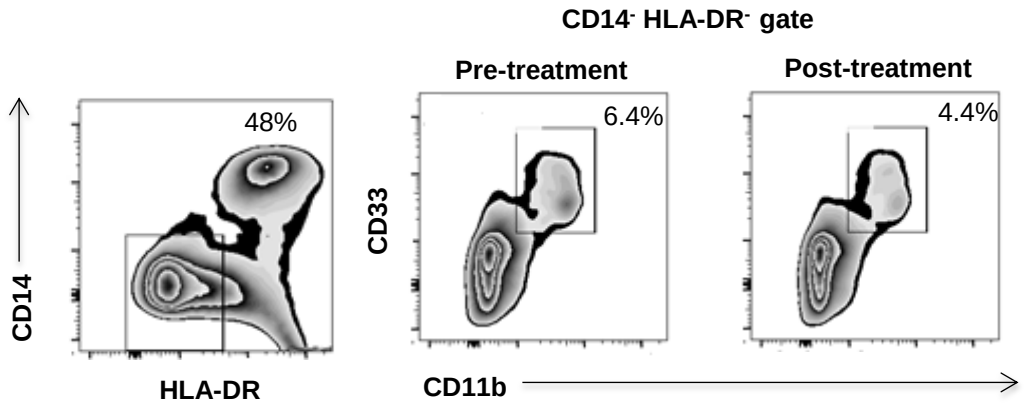


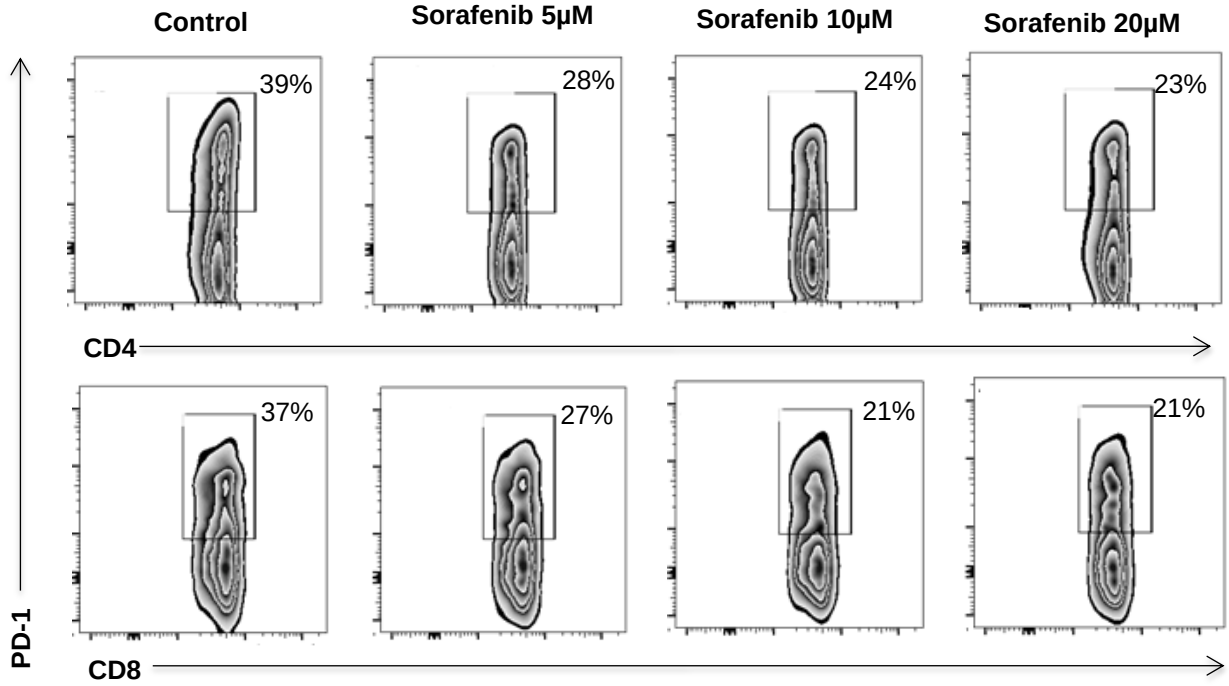
Supplementary Figure 1A.

Figure S1A. Representative flow cytometry analysis of PBMC from HCC patients showing the gating strategies utilized. Zebra plot analyses were gated on FSC/SSC and subsequently gated on $CD3^+CD4^+$ / $CD3^+CD8^+$cells and the percentages of $CD4^+Foxp3^+$ T cells, $Foxp3^+$ $CTLA\text{-}4^+$ T cells, $CD4^+PD\text{-}1^+$ T cells and $CD8^+PD\text{-}1^+$ T cells before and after treatment with sorafenib are shown. The percentage of cells is indicated in the upper right corner of each plot.



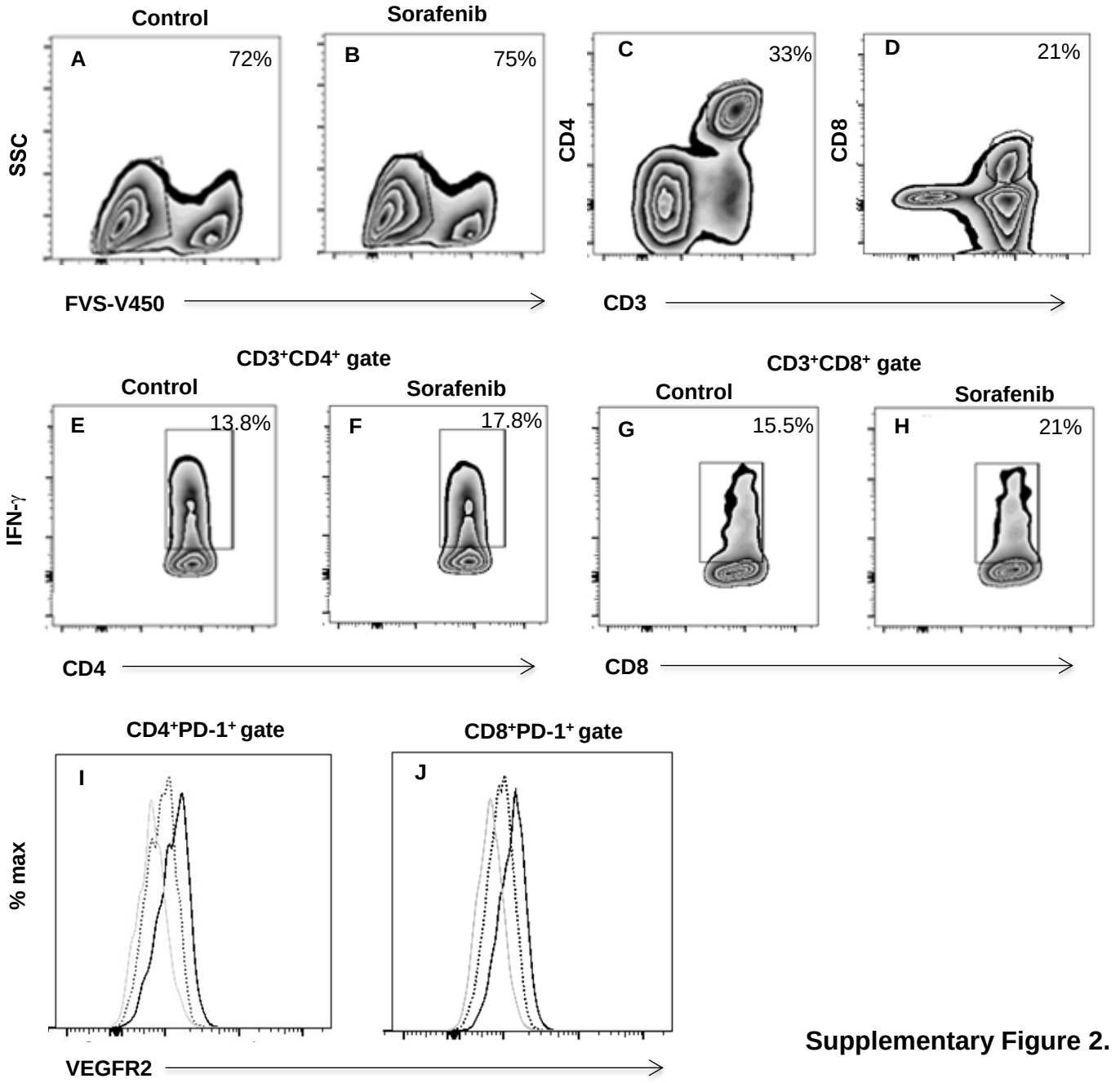
Supplementary Figure 1B.

Figure S1B. Representative flow cytometry analysis of PBMC from HCC patients showing the gating strategies applied for MDSC analysis. Zebra plot analyses were gated on FSC/SSC and subsequently gated on $CD14^-/HLA\text{-}DR^-$cells and the percentages of $CD11b^+CDD3^+$ MDSCs pre and post treatment with sorafenib are shown. The percentage of cells is indicated in the upper right corner of each plot.



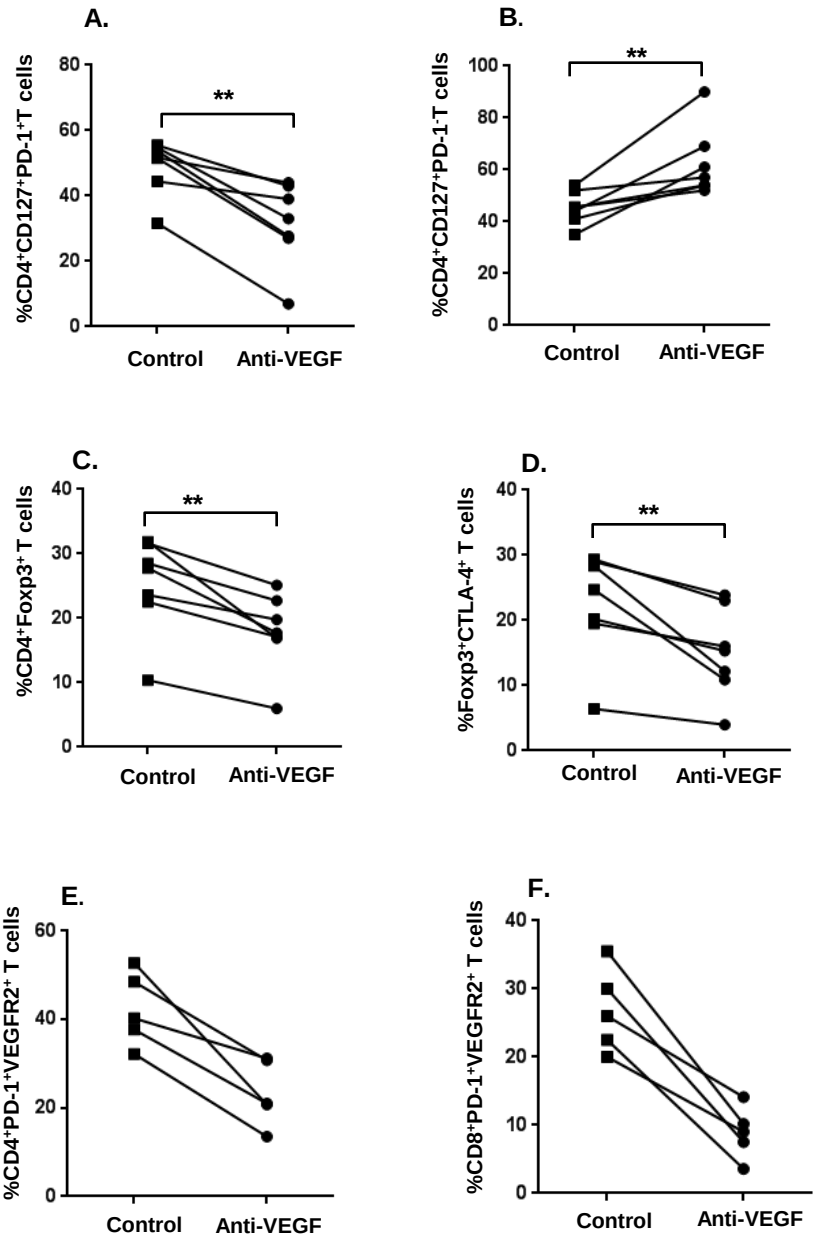
Supplementary Figure 1C.

Figure S1C. In vitro dose response experiments of sorafenib. Unsorted T cells from PBMC were activated via the TCR in the presence or absence of varying concentrations of sorafenib 5μM, 10μM and 20μM in vitro for 48 hours and PD-1 expression on gated $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells were determined by flow cytometry. The percentage of cells is indicated in the upper right corner of each plot.



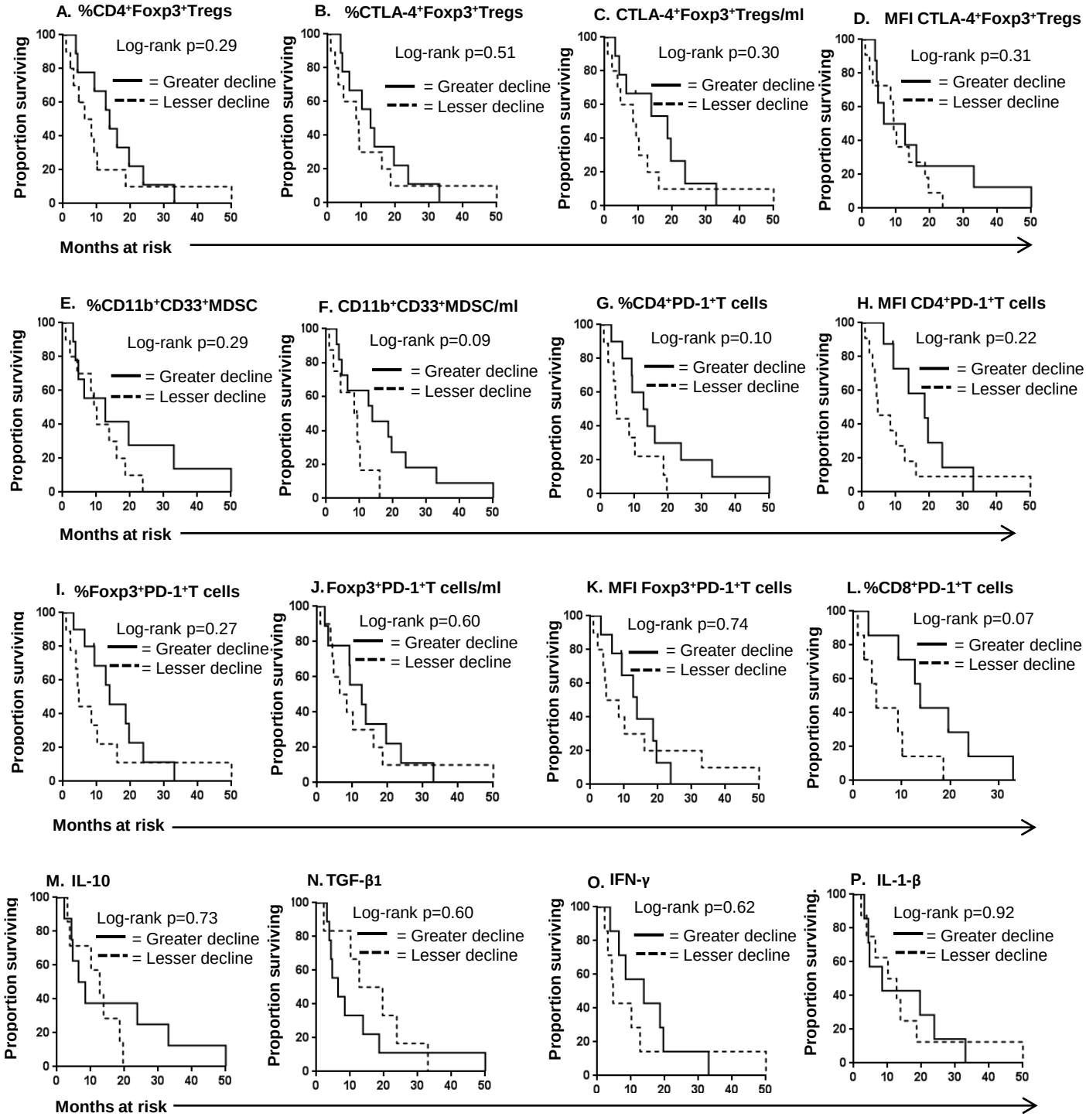
Supplementary Figure 2.

Figure S2. Representative flow cytometry analysis of in vitro experiments using PBMC from HCC patients. Pre-treatment samples from HCC patients were stimulated with anti-CD3/CD28 in the presence or absence of 10 µM concentration of sorafenib or 10µg/ml anti-VEGF antibody in vitro for 48 hrs as described in supplementary methods. Multi-color flow cytometry was done using viability exclusion dye FVS-V50 along with other fluorophores. Zebra plot analyses were gated on FSC/SSC, SSC/ FVS-V450 (**A, B**) and subsequently gated on $CD3^+CD4^+$ / $CD3^+$ $CD8^+$ T cells (**C, D**). The frequencies of $CD4^+IFN\text{-}g^+$ (**E, F**) and $CD8^+IFN\text{-}g^+$ (**G, H**) T cells in anti-CD3/CD28 treated (control) and anti-CD3/CD28 + sorafenib treated PBMC are shown. The percentage of cells is indicated in the upper right corner of each plot. Same experimental setting as in **A-H** but showing representative staining for VEGF-R2 are histogram overlays **I** and **J** gated on $CD4^+PD\text{-}1^+$ and $CD8^+PD\text{-}1^+$ T cells respectively. Reduced VEGF-R2 expression observed in $CD4^+PD\text{-}1^+$/$CD8^+PD\text{-}1^+$ T cells treated with sorafenib (dotted line) and anti-VEGF antibody (grey line) as compared to untreated control (black line).



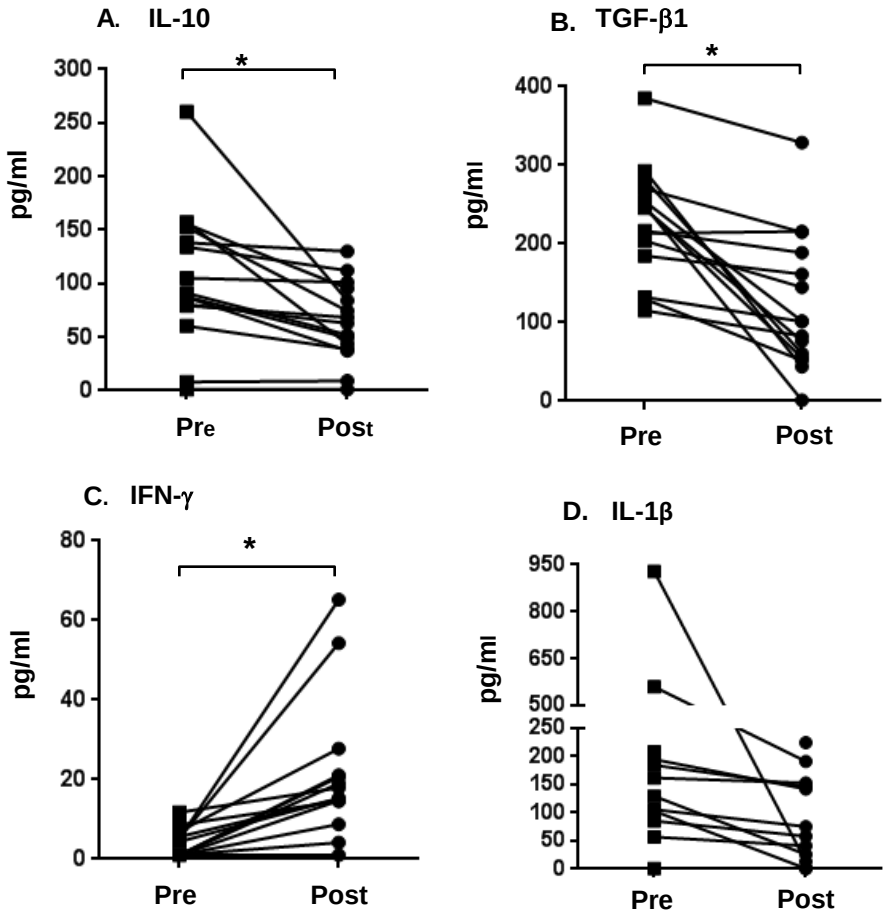
Supplementary Figure 3.

Figure S3. Effect of in vitro treatment with anti-VEGF antibody on T effector cells and Tregs. Pre-treatment samples of PBMCs from HCC patients were stimulated with anti-CD3/CD28 in the presence or absence of 10µg concentration of anti-VEGF antibody *in vitro* for 48 hrs and the frequencies of (**A**) $CD4^+CD127^+PD\text{-}1^+$ T effector cells (**B**) $CD4^+CD127^+PD\text{-}1^-$ T cells (**C**) $CD4^+Foxp3^+$ Tregs (**D**) $Foxp3^+CTLA\text{-}4^+$ Tregs (**E**) $CD4^+PD\text{-}1^+VEGFR2^+$ T cells (**F**) $CD8^+PD\text{-}1^+VEGFR2^+$ T cells were determined by flow cytometry as described in methods. Each symbol represents an individual HCC patient anti-CD3/CD28 treated control (■) or anti-VEGF anti-body treated PBMC (●). $^{**}P < 0.005$, paired *t*- test, (n=7 for **A-D**, n=5 for **E** and **F**).



Supplemental Figure 4.

Figure S4. **Kaplan-Meier plots showing the association of decrease in immnosuppressive cells and patient outcome after sorafenib treatment**. The association between overall survival (OS) and immune parameters of HCC patients was calculated based on the ratio of post vs pre- treatment measurements of immune markers for each patient as described in methods. (**A**) frequency of $CD4^+Foxp3^+$ Tregs (**B**) frequency of $CTLA\text{-}4^+Foxp3^+$ Tregs (**C**) absolute number of $CTLA\text{-}4^+Foxp3^+$ Tregs/ml (**D**) expression levels of CTLA-4 (MFI) on $Foxp3^+$ Tregs (**E**) frequency of $CD11b^+CD33^+$ MDSC (**F**) absolute number of $CD11b^+CD33^+$ MDSC/ml (**G**) frequency of $CD4^+PD\text{-}1^+$ T cells (**H**) expression levels of PD-1 (MFI) on $CD4^+$ T cells (**I**) frequency of $Foxp3^+PD\text{-}1^+$ T cells (**J**) absolute number of $Foxp3^+PD\text{-}1^+$ T cells/ml (**K**) expression levels of PD-1 (MFI) on $Foxp3^+CD4^+$ T cells (**L**) frequency of $CD8^+PD\text{-}1^+$ T cells (**M**) IL-10 (**N**) TGF-β1 (**O**) IFN-γ (**P**) IL-1β.



Supplementary Figure 5.

Figure S5. Changes in the plasma levels of cytokines in HCC patients after sorafenib treatment. (**A**) IL-10, **(B**) TGF-β1, (**C**) IFN-γ and (**D**) IL-1β. Each symbol represents an individual HCC patient pre (■) or post sorafenib treatment (●). * $P < 0.05$, Permutation *t*- test.

Supplementary Figure S1A. Representative flow cytometry analysis of PBMC from HCC patients showing the gating strategies utilized. Zebra plot analyses were gated on FSC/SSC and subsequently gated on $CD3^+CD4^+$ / $CD3^+CD8^+$cells and the percentages of $CD4^+Foxp3^+$ T cells, $Foxp3^+$ CTLA-4^+ T cells, $CD4^+$PD-1^+ T cells and $CD8^+$PD-1^+ T cells before after treatment with sorafenib are shown. The percentage of cells is indicated in the upper right corner of each plot.

Supplementary Figure S1B. Representative flow cytometry analysis of PBMC from HCC patients showing the gating strategies applied for MDSC analysis. Zebra plot analyses were gated on FSC/SSC and subsequently gated on $CD14^-$/HLA-DR^-cells and the percentages of $CD11b^+CDD3^+$ MDSCs before and after treatment with sorafenib are shown. The percentage of cells is indicated in the upper right corner of each plot.

Supplementary Figure S1C. In vitro dose response of sorafenib. Unsorted T cells from PBMC were activated via the TCR in the presence or absence of varying concentrations of sorafenib 5µM, 10µM and 20µM in vitro for 48 hours and PD-1 expression on gated $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells were determined by flow cytometry. The percentage of cells is indicated in the upper right corner of each plot.

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on FSC/SSC, SSC/FVS-V450 (**A, B**) and subsequently gated on $CD3^+CD4^+$ /$CD3^+$ $CD8^+$ T cells (**C, D**). The frequencies of $CD4^+$IFN-γ^+ (**E, F**) and $CD8^+$IFN-γ^+ (**G, H**) T cells in anti-CD3/CD28 treated (control) and anti-CD3/CD28 + sorafenib treated PBMC are shown. The percentages of cells is indicated in the upper right corner of respective plots. Same experimental setting as in **A-H** but showing representative staining for VEGF-R2 are histogram overlays **I** and **J** gated on $CD4^+PD\text{-}1^+$ and $CD8^+PD\text{-}1^+$ T cells respectively. Reduced VEGF-R2 expression observed in $CD4^+PD\text{-}1^+$/$CD8^+PD\text{-}1^+$ T cells treated with sorafenib (dotted line) and anti-VEGF antibody (grey line) as compared to untreated control (black line).

Supplementary Figure S3. Effect of in vitro treatment of anti-VEGF antibody on T effector cells and Tregs. Pre-treatment samples of PBMCs from HCC patients were stimulated with anti-CD3/CD28 in the presence or absence of 10µg concentration of anti-VEGF antibody in vitro for 48 hrs and the frequencies of (**A**) $CD4^+CD127^+PD\text{-}1^+$ T effector cells (**B**) $CD4^+CD127^+PD\text{-}1^-$ T cells (**C**) $CD4^+Foxp3^+$ Tregs (**D**) $Foxp3^+CTLA\text{-}4^+$ Tregs (**E**) $CD4^+PD\text{-}1^+VEGFR2^+$ T cells (**F**) $CD8^+PD\text{-}1^+VEGFR2^+$ T cells were determined by flow cytometry as described in methods. Each symbol represents an individual HCC patient; anti-CD3/CD28 treated control (■) or anti-VEGF anti-body treated PBMC (●). $^{**}P < 0.005$, paired *t*- test, (n=7 for **A-D**, n=5 for **E** and **F**).

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Supplementary methods 1.

Flow cytometry analysis

FACS analysis was performed to measure peripheral blood Treg frequency using APC-H7 anti-CD3, V450 anti-CD4, V500 anti-CD8, PE anti-CD127, PE-Cy5 anti-CTLA4 (BD Biosciences) and PE-Cy7 anti-PD-1, Alexa488 anti-Foxp3 (Biolegend). Intracellular analysis for IFN-γ, Foxp3 and CTLA-4 was performed after fixation and permeabilization of cells using intracellular staining kit (eBioscience) according to manufacturer's instructions. All samples were acquired on LSRII flow cytometer (BD Biosciences) and analyzed using Flowjo (Tree Star).

MDSCs were detected using FITC anti-CD11b (eBiosciences), PE-Cy5 anti-CD33, APC anti-CD14, V450 anti-HLA-DR (BD Biosciences).

Cytokine ELISA

Plasma isolated during PBMC separation was assayed to quantify the level of IFN-γ, IL-10, TGF-β1 and IL-1β using specific ELISA kits according to the manufacturer's instructions (eBioscience).

Supplementary methods 2.

Cell culture

PBMC (2.5×10^5 cells/well) were stimulated with anti-CD3 antibody (1µg/ml)/anti-CD28 (0.5/µg/ml) in 96-well microtiter plates. Cells were treated with 10µM concentration of sorafenib or anti-VEGF antibody (10µg/ml). Control wells included

unstimulated cells and anti-CD3 stimulated cells without sorafenib or anti-VEGF antibody treatment. Plates were incubated at 37°C in a humidified incubator containing 5% CO_2 for 48 hrs. After incubation, cells were washed and stained for flow cytometry analysis.

Statistical analysis software

All data analyses were generated using SAS/STAT software, Version 9.4. Copyright 2012, SAS Institute Inc. SAS is a registered trademark of SAS Institute Inc., Cary, NC, USA.

Supplemental Table 1. Nominal and multiplicity-adjusted p values

		P Values	
Figure	**Marker**	**Nominal**	**Hommel Adjusted**
Outcome: Mean Difference. Comparison: In vivo Post vs Pre			
1A	%$CD4^+$ $PD\text{-}1^+$ T cells	0.015	
1B	$CD4^+PD\text{-}1^+$ T cells/ml	0.098	0.167
1C	$CD4^+$PD-1 MFI	0.021	
1D	%$Foxp3^+PD\text{-}1^+$ T cells	0.065	
1E	$Foxp3^+PD\text{-}1^+$ T cells/ml	0.041	0.164
1F	$Foxp3^+$ PD-1 MFI	0.058	
1G	%$CD4^+$ $CD127^+$ T effector	0.167	0.167
1H	$CD4^+$ $CD127^+$ $PD\text{-}1^-$ T cells : $CD4^+$ $Foxp3^+PD\text{-}1^+$ T cells ratio	0.009	0.081
1I	% $CD8^+$ $PD\text{-}1^+$ T cells	0.208	
1J	$CD8^+PD\text{-}1^+$ T cells/ml	0.144	0.167
1K	$CD8^+$PD-1 MFI	0.303	
2A	% $Foxp3^+$ Tregs	0.018	
2B	$Foxp3^+$ Tregs/ml	0.050	0.167
2C	$CD4^+CD127^+$ T cells : $CD4^+Foxp3^+$ T cells ratio	0.018	0.108
2D	% $CD4^+CTLA\text{-}4^+$ Tregs	0.015	
2E	$CD4^+CTLA\text{-}4^+$ Tregs/ml	0.050	0.167
2F	% $CD11b^+CD33^+$ MDSC	0.616	
2G	$CD11b^+CD33^+$ MDSC/ml	0.910	
Outcome: Mean Difference. Comparison: In vitro Post vs Pre			
3A	%$CD4^+CD127^+PD\text{-}1^+$ T cells	0.002	0.032
3B	%$CD4^+CD127^+PD\text{-}1^-$ T cells	0.003	0.048
3C	%$CD4^+CD127^-Foxp3^+$ T cells	0.001	0.027
3D	%$CD127^-Foxp3^+CTLA\text{-}4^+$ T cells	0.007	0.073
3E	%$CD4^+$IFN-γ^+ T cells	0.002	0.041
3F	%$CD8^+$IFN-γ^+ T cells	0.003	0.044
3G	%$CD4^+PD\text{-}1^+VEGFR\text{-}2^+$ T cells	0.009	0.078
3H	%CD8+$PD\text{-}1^+VEGFR\text{-}2^+$ T cells	0.004	0.054
Outcome: Overall Survival. Comparison: In vivo Post vs Pre			
4A	$CD4^+PD\text{-}1^+$T cells/ml	0.037	0.148
4B	$CD8^+PD\text{-}1^+$T cells/ml	0.039	0.155
4C	MFI $CD8^+PD\text{-}1^+$T cells	0.035	0.140
4D	$CD4^+Foxp3^+$Tregs/m	0.005	0.067
4E	%$CD4^+CD127^+$T cells	0.002	0.041

Outcome: Overall Survival. Comparison: In vivo Pre (Predictive Markers)			
5A	$CD4^+PD\text{-}1^+$ T cells/ml	0.055	0.167
5B	$CD8^+$ $PD\text{-}1^+$ T cells/ml	0.048	0.167
5C	$\%CD4^+Foxp3^+$ T cells	0.013	0.094
5D	$Foxp3^+PD\text{-}1^+$ T cells/ml	0.010	0.082
5E	T eff : $Foxp3^+$ Treg ratio	0.012	0.088

Nominal p values for 36 comparisons were indicated in the manuscript. Of these, 26 comparisons were considered important to the central premise of the manuscript. The p values for these comparisons were adjusted for multiplicity using Hommel's method to maintain a family-wise type I error rate of 0.05. This adjustment identified six comparisons with nominal p values less than 0.003 as statistically significant.