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Supplementary Table 1.

Flow Cytometry antibodies

Antigen	Clone	Provider	Ref/Catalog #
mouse Ly6G/Ly6C (Gr-1)	RB6-8C5	Biolegend (San Diego, USA)	108409
mouse B220	RA3-6B2	Biolegend (San Diego, USA)	103247
mouse B220	RA3-6B2	eBiosience (San Diego, USA)	RM2601
mouse CD11c	N418	Biolegend (San Diego, USA)	117313
mouse CD115	AFS98	eBiosience (San Diego, USA)	117-1152-82
mouse CD117 (c-kit)	2B8	eBiosience (San Diego, USA)	47-1171-82
mouse CD150	TC15-12F12.2	Biolegend (San Diego, USA)	115927
mouse CD16/32	93	eBiosience (San Diego, USA)	45-0161-80
mouse CD4	GK1.5	Biolegend (San Diego, USA)	100425
mouse CD40	3/23	Biolegend (San Diego, USA)	124613
mouse F4/80	BM8	Biolegend (San Diego, USA)	123119
mouse Ly6A/E (Sca-1)	D7	eBiosience (San Diego, USA)	25-5981-81
mouse Ly6C	HK1.4	Biolegend (San Diego, USA)	128024
mouse Ly6G	1A8	BD Bioscience (New Jersey, USA)	551461
mouse MHC-II	M5/114.15.2	Biolegend (San Diego, USA)	107617
mouse Ter119	TER-119	eBiosience (San Diego, USA)	11-5921-85
mouse/human CD11b	M1/70	eBiosience (San Diego, USA)	563168
mouse/human IRF8	REA516	Miltenyi Biotec (Bergisch Gladbach, Germany)	130-108-196
mouse/human PU.1	7C2C34	BioLegend (San Diego, USA)	681305
human CD11b	ICRF44	Biolegend (San Diego, USA)	311348
human CD14	61D3	eBioscience (San Diego, USA)	12-0149-42
human CD16	CB16	Invitrogen (Waltham, USA)	56-0168-42
human CD66b	G10F5	BD Pharmingen (Oxford, UK)	555724
human CD8	53-6.7	Biolegend (San Diego, USA)	100742
human CD8	RPA-T8	eBioscience (San Diego, USA)	557086
human HLA-DR	L243	Biolegend (San Diego, USA)	307616

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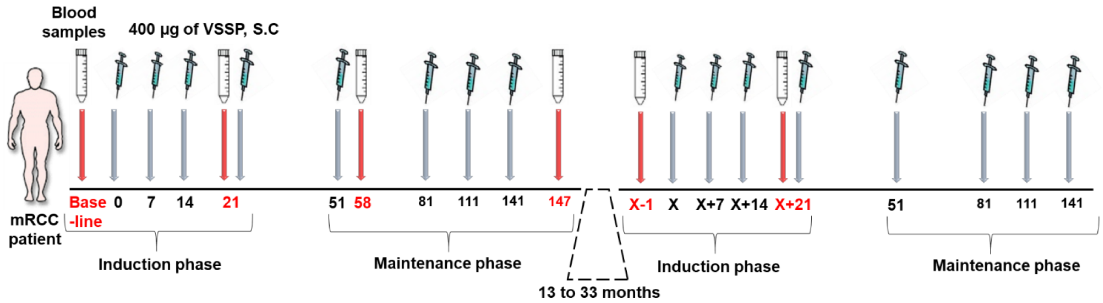
Figure S1.



Supplementary Figure S1. Treatment schedule and peripheral blood analyses in a clinical trial of patients receiving VSSP as a monotherapy. Briefly, fifteen mRCC patients were treated with VSSP. Eight doses consisting in 400µg of VSSP were administrated subcutaneously during an Induction Phase and a Maintenance Phase. Seven patients after a period of 13 to 33 months received a second cycle of treatment using the same schedule. Blood samples were taken on days -7 (D0) as baseline, 21, 58 and 147 (first cycle), and on baseline D(X) and day 21 D(X+21) (second cycle).

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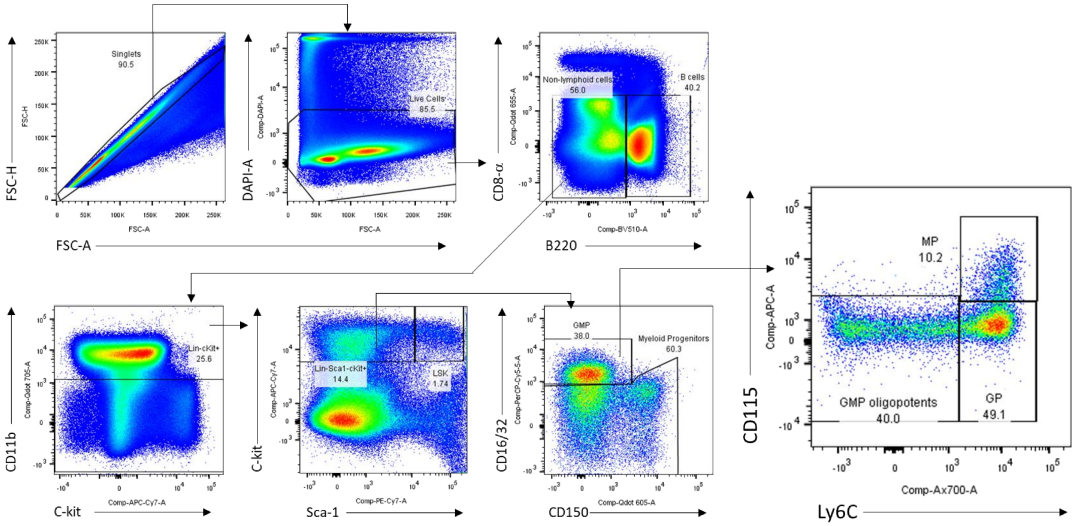
Figure S2.



Supplementary Figure S2. Evaluation of myeloid progenitor populations in a model of emergency myelopoiesis. Figure shows a representative flow cytometry gating strategy used for the evaluation of myeloid progenitor populations in the BM. Arrows indicate the gated population analyzed in the pseudo color dot plot.upper plot by the one below

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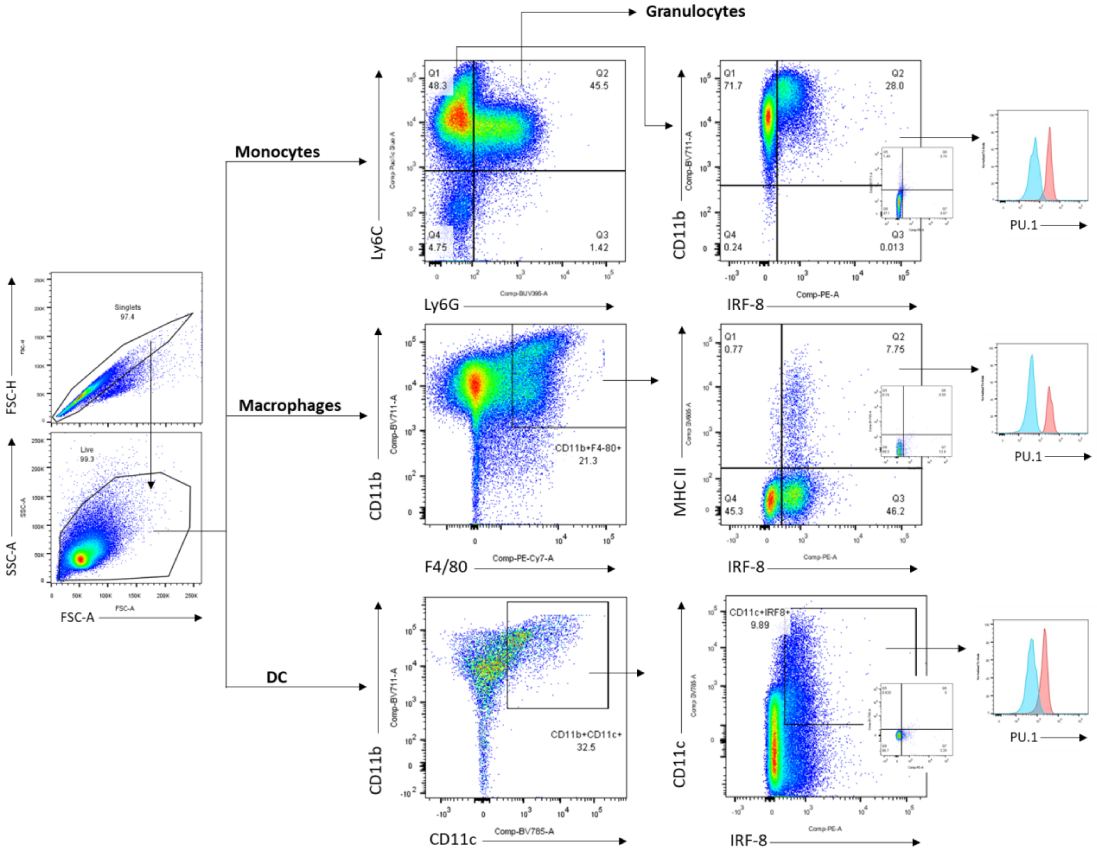
Figure S3.



Supplementary Figure S3. Representative flow cytometry gating strategy for the evaluation of the differentiated cells and the expression of IRF8 and PU.1. Arrows indicate the gated population analyzed in the pseudo-color dot plot. Monocytes were defined as $CD11b^{+}Ly6C^{hi}Ly6G^{-}$ population, while granulocytic cells were defined by $Ly6G^{+}$ expression. Frequencies of macrophages were calculated from the intersection of the$CD11b^{+}$, $F4/80^{+}$ and $MHC\text{-}II^{+}$ markers and DCs from the $CD11b^{+}$, $CD11c^{+}$ and $MHC\text{-}II^{+}$ markers. Percentages of $IRF8^{+}$ cells and IRF8 and PU.1-double positive cells were calculated from these defined monocytes, macrophage, and DCs populations. The small inserted dot plots correspond to staining with the appropriate isotype control Ab for IRF8, and the histograms represent those cells positive for PU.1 (red) relative to the isotype control (blue).

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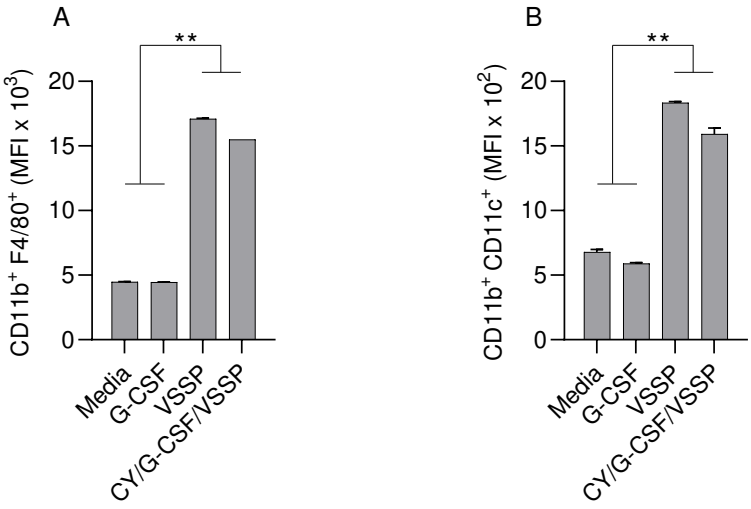
Figure S4.



Supplementary Figure S4. VSSP enhances surface expression of APC markers on MPs. Total MPs were sorted from the BM of CY-treated mice (200 mg/kg body weight, i.p.) on day 2 (≥ 90% purity) and cultured *in vitro* with SCF (50 ng/ml), IL-3 (10 ng/ml), G-CSF (10 ng/ml); or VSSP (10 µg/ml); or both VSSP and G-CSF for 4 days. The expression of prototypic APC markers was measured on macrophages ($CD11b^+F4/80^+$) **(A)** and DCs ($CD11b^+CD11c^+$) **(B)**, after the indicated treatments by flow cytometry and represented by MFI values. Statistically significant differences were detected by the Tukey test. Data are expressed as mean ± SEM and are representative of two experiments with similar results.

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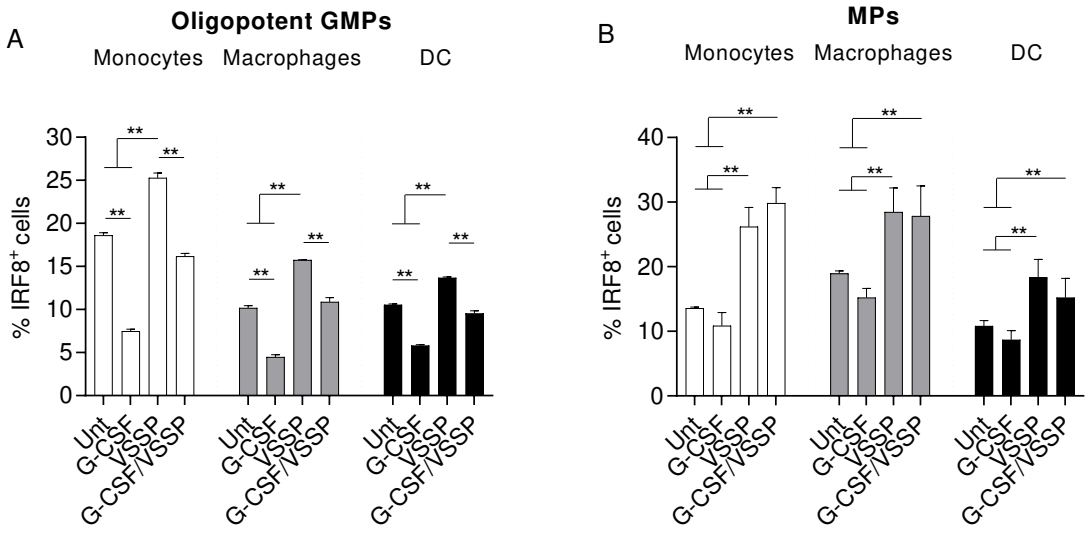
Figure S5.



Supplementary Figure S5. VSSP enhances the expression of IRF8 on APC differentiated from GMP populations during emergency myelopoiesis. Oligopotent GMPs **(A)** and MPs **(B)** were sorted from the BM and treated *in vitro* as described. The percentage of monocytes ($CD11b^+Ly6C^{hi}Ly6G^-$) (white bars), macrophages ($CD11b^+F4/80^+MHCII^+$) (grey bars), and DCs ($CD11b^+CD11c^+MHCII^+$), (black bars) positive for IRF8 was measured after the indicated treatments by flow analysis. Data represent the mean ± SEM from multiple replicates of one of two representative experiments. Statistical analyses were performed using the Tukey test.

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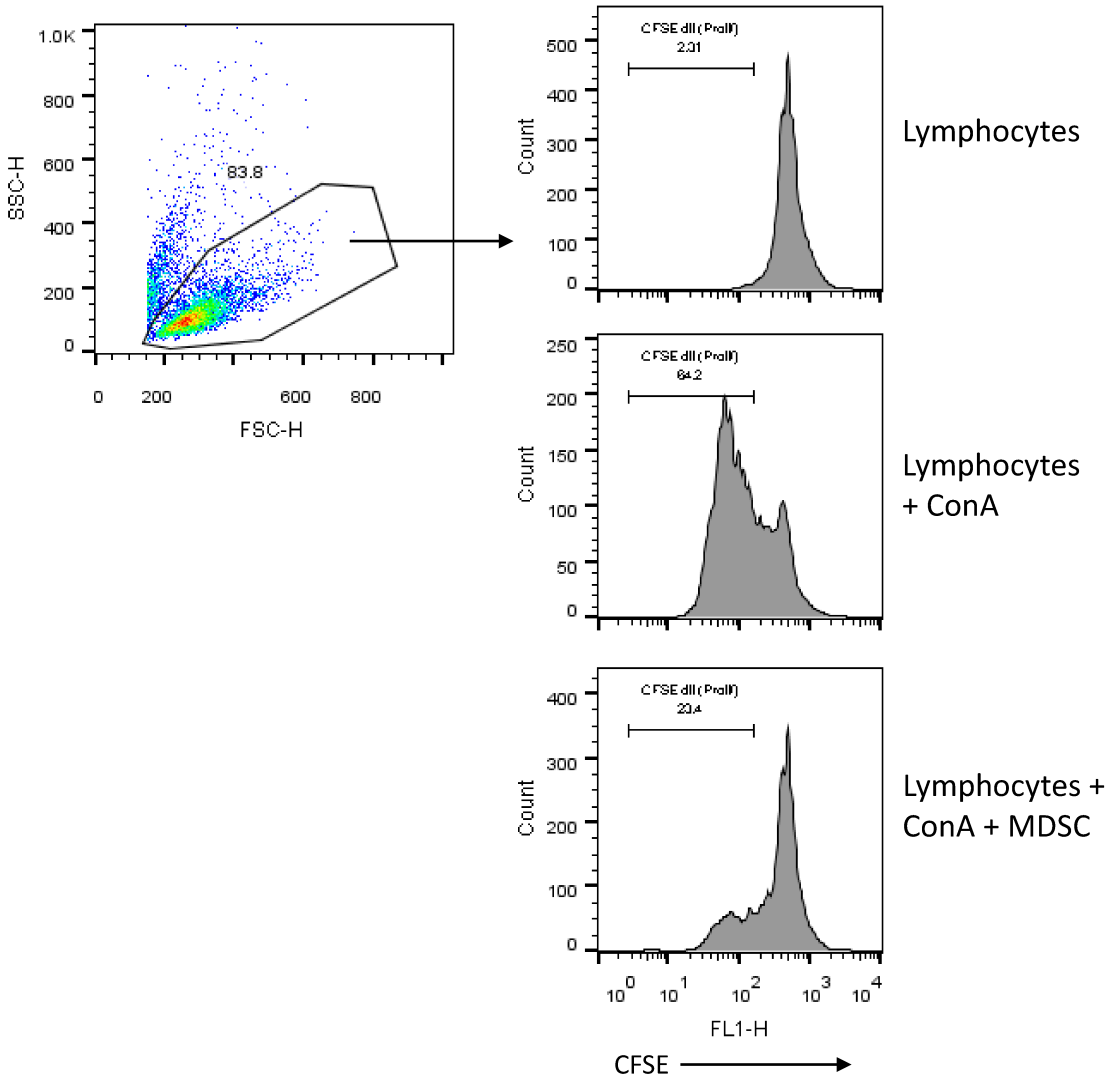
Figure S6.



Supplement Figure S6. Representative flow cytometry analysis for the evaluation of the suppressive capacity of murine CD11b⁺ cells. Naive splenocytes were stimulated with Con A for 96 hours and inhibition of lymphocytes proliferation by the presence of either *in vivo*-isolated or *in vitro*-treated CD11b⁺ cells was assessed by CFSE dye dilution.

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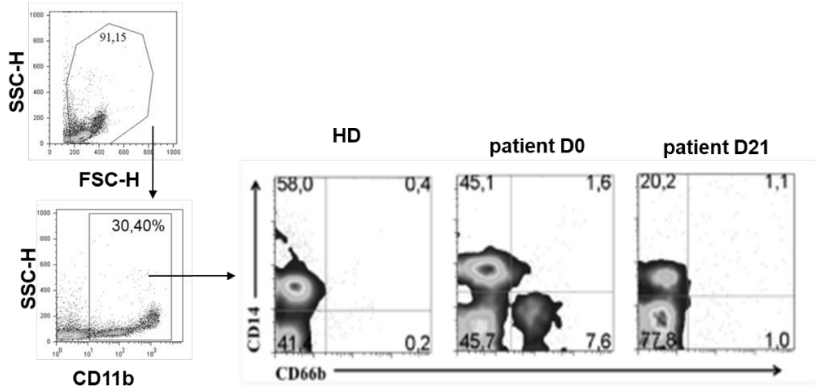
Figure S7.



Supplementary Figure S7. PMN-MDSC phenotype analysis by flow. Flow cytometry analysis of PMN-MDSCs ($CD11b^{+}CD66b^{+}CD14^{-}$) from a representative mRCC patient and a healthy donor, at baseline and day 21 after treatment.

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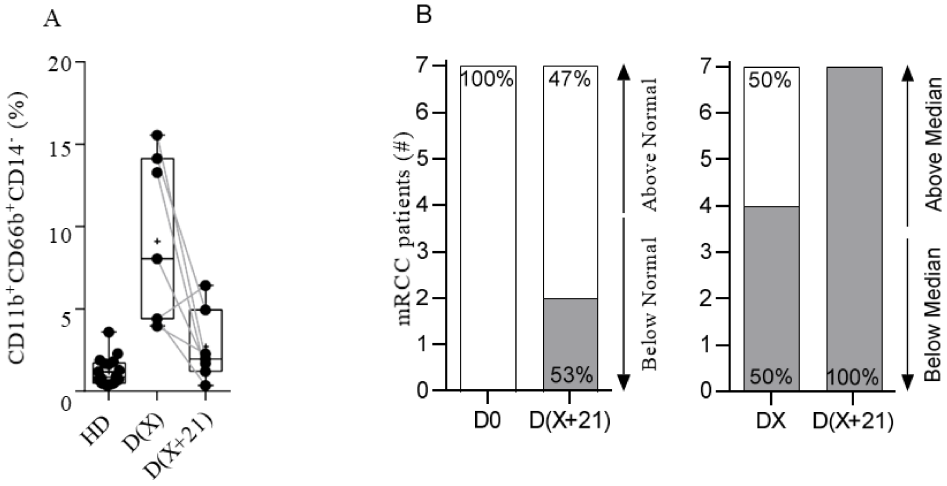
Figure S8.



Supplementary Figure S8. **Metastatic RCC patients treated with VSSP have reduced frequencies of circulating PMN-MDSCs after a second cycle of treatment.** Freshly isolated PBMCs of mRCC patients enrolled for the second treatment were analyzed by flow cytometry to determine the frequency of PMN-MDSCs ($CD11b^+$ $CD66b^+$ $CD14^-$) (**A**). Data indicate the number of mRCC patients with PMN-MDSCs above and below the normal value (D0) or median values corresponding to baseline at the time of second treatment (DX) on the days after second cycle, D(X+21) (**B**). Normal values were considered those under the upper 95% confidence interval of healthy donors reflecting the same immunophenotype used to define PMN-MDSCs in patients. Statistical analyses were performed via Kruskal-Wallis and Dunn's tests ($p<0.05$).

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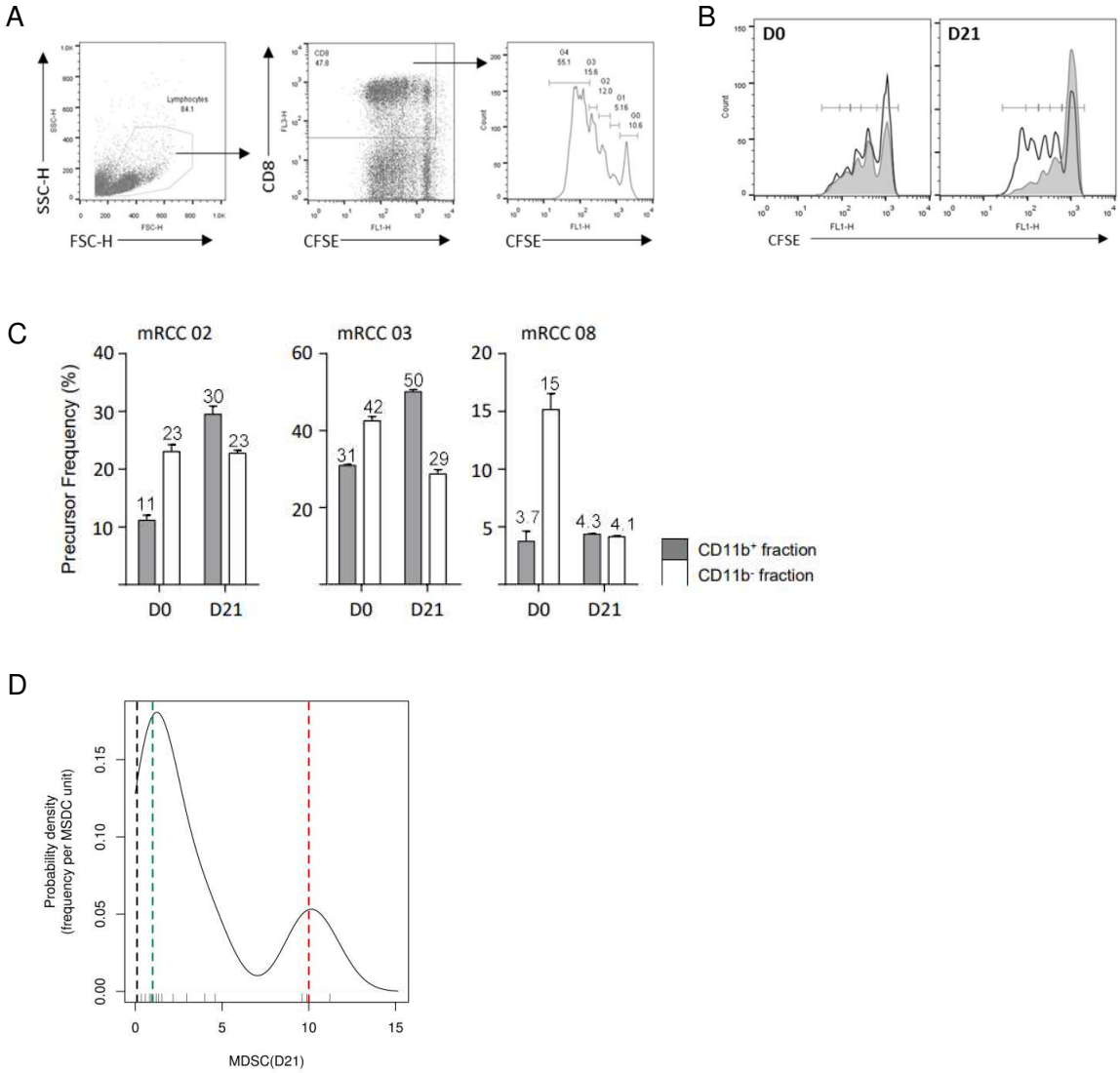
Figure S9.



Supplementary Figure S9. PMN-MDSC functional analysis. PBMCs from mRCC patients were first depleted or not of CD11b$^+$ cells, then pre-strained with CFSE followed by stimulation with anti-CD3 antibody for 96 hours. Proliferation of CD8$^+$ T cells was evaluated based on precursor frequency. Panel **A** represents the gating strategy and CFSE dilution analysis as a measure of the proliferation rounds of CD8$^+$ T lymphocytes. In addition, Panel **B** shows representative histograms of CD8$^+$ T cells proliferation from the CD11b$^+$ (grey filled) or CD11b$^-$ (open) fractions from PBMCs of a representative patient corresponding to baseline (D0) and day 21 (D21) of treatment. Precursor frequency of the proliferative CD8 T cells of three individual patients are also represented in bars **(C)**. In order to associate predicted survival and PMN-MDSC levels following VSSP treatment a Density plot of the observed MDSC levels at day 21 was performed. The levels of 0.1, 1 and 10 are indicated with dashed lines **(D).**