LEGENDS TO SUPPLEMENTARY FIGURES

Supplementary Table S1. Characteristics of the patient included in the study (see Figure 1).

Supplementary Table S2. Correlation between plasmatic levels of sCD146 and clinical parameters. (**A**) Univariate analysis by the Fisher or QuiSquare test. (**B**) Multivariate analysis of sCD146, the MSKCC score and PFS. (see Figure 2)

Supplementary Table S3. Characteristics of patients included in the study (see Figure 4).

Supplementary Figure S1. A general schema of the management of the patients enrolled in the different clinical trials. The material (mRNA, plasma) and the related Figures or Tables are mentioned.

Supplementary Figure S2. CD146 staining in M0 RCC tumor.

Supplementary Figure S3. Relative CD146 mRNA levels in different cancers. An analysis performed with the cbioportal database.

Supplementary Figure S4. PFS and OS of cohorts (sunitinib or bevacizumab treatment). (**A** and **B**) The Kaplan–Meier analysis of PFS (**A**) or OS (**B**) of patients from the prospective cohort – sunitinib group. (**C** and **D**) The Kaplan–Meier analysis of PFS (**C**) or OS (**D**) of patients from the prospective cohort – bevacizumab group.

Supplementary Figure S5. Plasmatic levels of sCD146 of cohort (sunitinib treatment). (**A** and **B**) Plasmatic levels of sCD146 before and after sunitinib treatment on "sCD146 < 120 % group" (**A**) or on "sCD146 ≥ 120 % group" (**B**).

Supplementary Figure S6. 786R cells overexpressed CD146. (**A**) 786 and 786R cells were stained with CD146-PE antibody. CD146 expression was evaluated by flow cytometry. (**B**) 786 and 786R cells were treated with 5µM sunitinib for 48 h. Histograms show quantification of CD146 expression evaluated by flow cytometry (mean of three independent experiments). (**C**) 786 and 786R cells were evaluated for CD146 expression by immunoblotting after treatment with or without 2.5 and 5 □M sunitinib. The graphs show the level of CD146 evaluated by immunoblotting (mean of five independent experiments). (**D**) Soluble CD146 was examined on the supernatants of 786R and 786 cells by western-blot (mean of three independent experiments). * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplementary Figure S7. JNK pathway was responsible of CD146 overexpression in 786R. 786 and 786R cells were treated with SP600125 (SP, JNK inhibitor). (**A**) p-JNK, p-Jun were evaluated by immunoblotting. Panel is representative of at least 3 independent experiments. (**B** to **D**) The levels of CD146 total (short + long form, **B**), CD146 long (**C**) and CD146 short (**D**) mRNA were determined by qPCR. Results are represented as the mean of 3 independent experiments ± SEM.. (**E**) Histograms show quantification of CD146 expression by flow cytometry. Results are represented as the mean of 3 independent experiments ± SEM. (**F**) The sCD146 protein in cell supernatants was evaluated by ELISA. Results are represented as the mean of 3 independent experiments ± SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplementary Figure S8. Sunitinib but not bevacizumab induced CD146 expression in ccRCC cells. (**A**) 786 and 786R cells were treated with 10µg/ml bevacizumab for 48 h. sCD146 protein in cell supernatants were evaluated by ELISA. Results are represented as the mean of three independent experiments ± SEM. (**B**) 786 cells were treated with 10µg/ml bevacizumab, in the presence of recombinant sCD146 (sCD146) for 48 h. The cell viability was measured with the XTT assay. Results are represented as the mean of three independent experiments ± SEM. (**C**) RCC10 cells were treated with 2.5 or 5µM sunitinib, in the presence of recombinant sCD146 (sCD146) for 48 h. Cell viability was measured with the XTT assay. Results are represented as the mean of three independent experiments ± SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

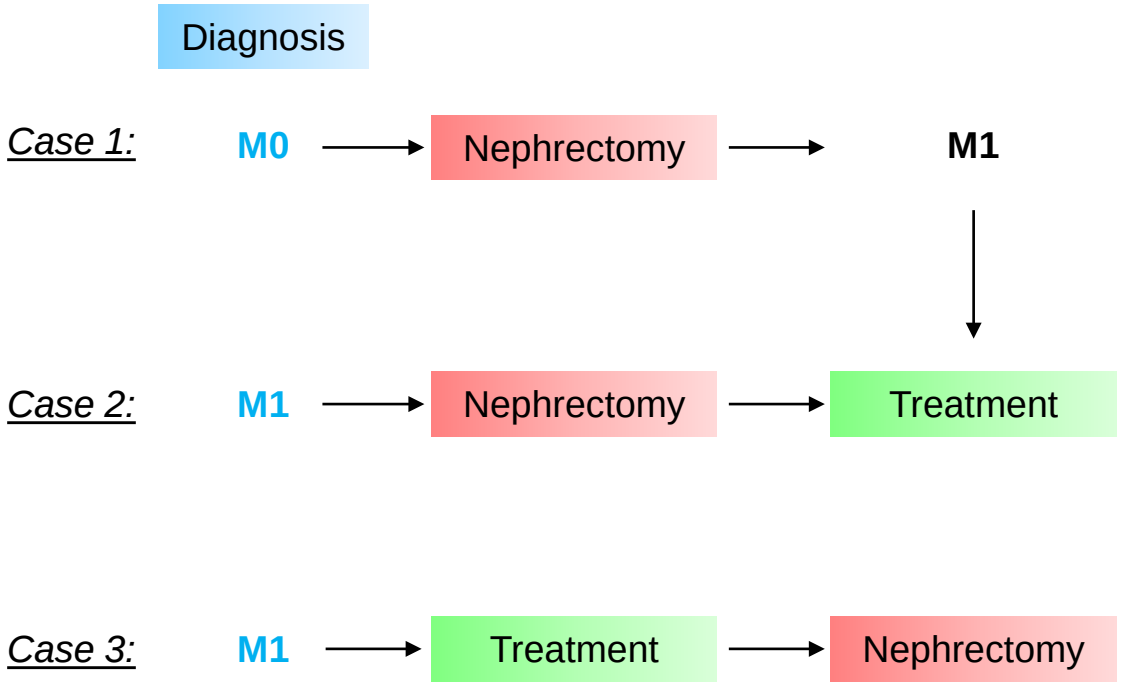
Supplementary Figure S9. The expression of CD146 and angiomotin is differently regulated in endothelial cells. (**A** and **B**) HUVEC were treated with 2 µM sunitinib for 48 h. CD146 (**A**) and angiomotin (**B**) were analyzed by qPCR. Results are represented as the mean of three independent experiments ± SEM. (**C**) HUVEC were treated with 62.5ng/ml bevacizumab, 1 or 2µM sunitinib for 48 h. The sCD146 protein in cell supernatants were evaluated by ELISA. Results are represented as the mean of three independent experiments ± SEM. (**D**) HUVEC were treated with the indicated concentrations of sunitinib. Cell proliferation was measured with the BrdU cell proliferation assay kit. Results are represented as the mean of three independent experiments ± SEM. (**E**) HUVEC were treated with the indicated concentrations of sunitinib or bevacizumab (62.5ng/ml), in the presence of recombinant sCD146 (50 or 100 ng/ml) for 48 h. Cell proliferation was measured with the BrdU cell proliferation assay kit. Results are represented as the mean of three independent experiments ± SEM. * or # $p<0.05$, ** $p<0.01$, *** or ### $p<0.001$.

Supplementary Figure S10. Sunitinib induced CD146 expression in primary ccRCC cells.

Primary cells were treated with sunitinib for 48h. (**A**) Cell viability was measured by XTT assays. Results are represented as the mean of three independent experiments ± SEM. (**B** to **D**) The levels of CD146 total (short + long form, **B**), CD146 long (**C**) and CD146 short (**D**) mRNA were determined by qPCR. Results are represented as the mean of three independent experiments ± SEM. (**E**) Histograms show quantification of CD146 expression by flow cytometry. Results are represented as the mean of three independent experiments ± SEM. (**F**) sCD146 in cell supernatants was evaluated by ELISA. Results are represented as the mean of three independent experiments ± SEM. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplementary Figure S11. Anti soluble CD146 antibodies decreased 786-R cells proliferation. 786R cells were treated for 48h with the anti-soluble CD146 M2J-1 mAb 1 □g/ml (sCD146 Ab) or with the control IgG1 1 □g/ml (CT Ab), as compared to control cells (CT). Cell proliferation was determined using a BrDu incorporation kit. Results are represented as the mean of three independent experiments ± SEM.* $p<0.05$

Supplementary Figure S12. **Recapitulative schema. CD146 is highly expressed on ccRCC cells the proliferation of which depends on CSF1/CSF1R and VEGF/NRP1-mediated autocrine loops.** Cells rendered resistant to sunitinib by chronic exposure over-expressed CD146. Shedding of the extracellular part leads to the release of sCD146. The multiple functions of this extracellular form comprise the autocrine effects of cells that produce it via the action of angiomotin and paracrine effects on cells of the microenvironment, especially endothelial cells. Autocrine effects mediate cell proliferation and resistance to apoptosis and paracrine effects modulate tumor angiogenesis.



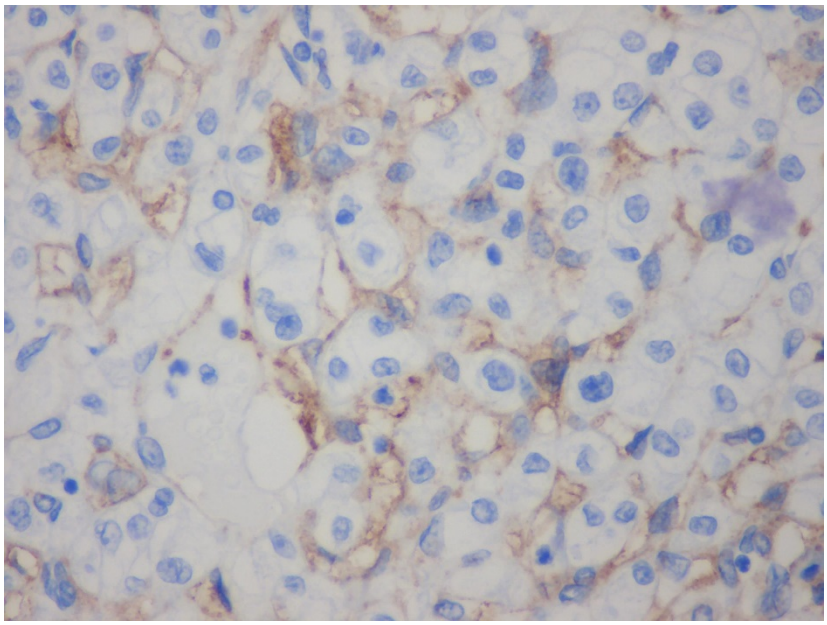
Goal
Materials
Type of study and trials

Figure 1 / Supplementary Table S1
To find a pronostic marker
Tumor mRNA
Retrospective study

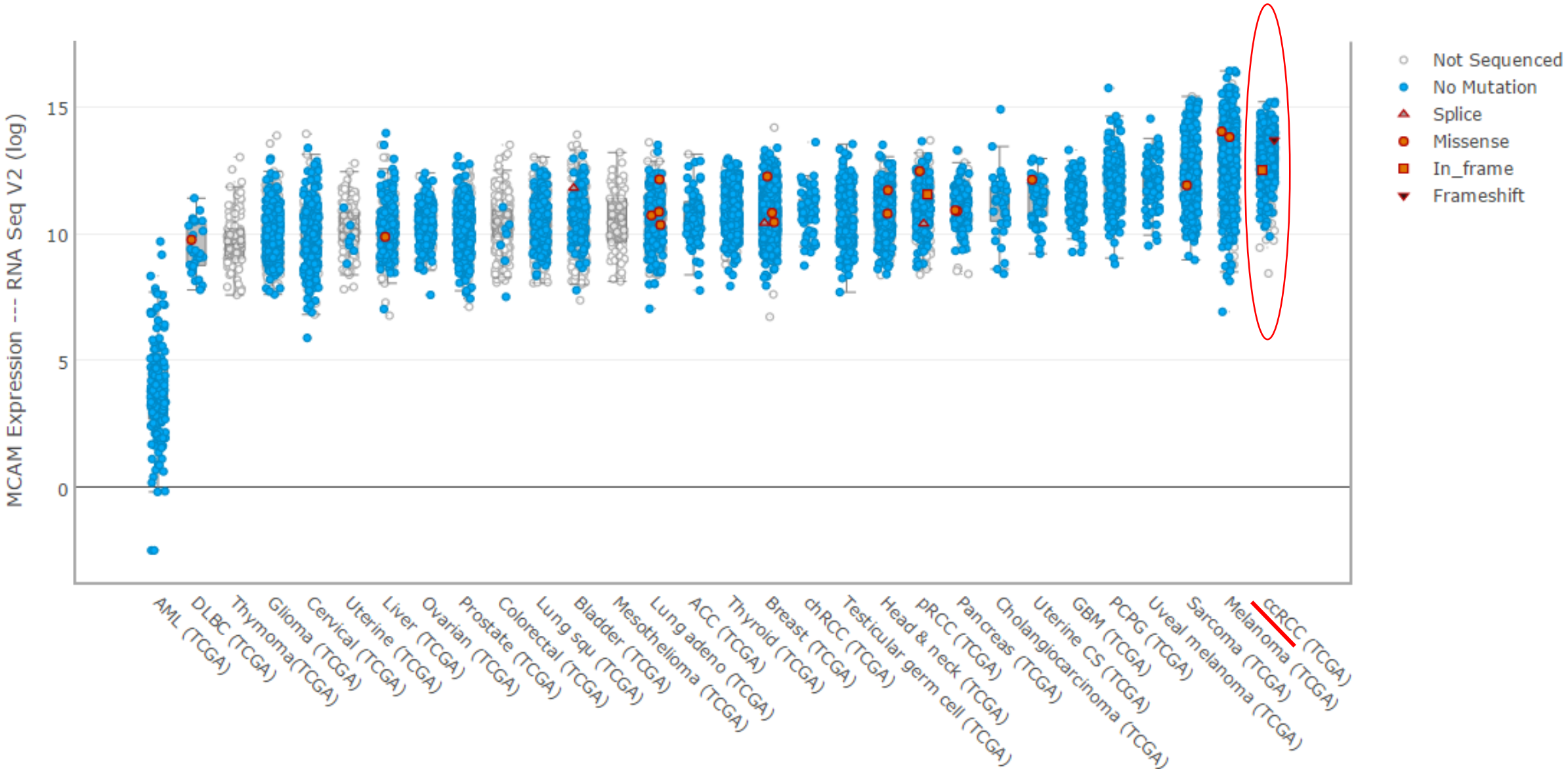
Figures 2 and 3 / Table 1 and Supplementary Table S2
To find a predictive marker of treatment efficacy
Plasma
Prospective study (SUVEGIL, TORAVA and PREINSUT Trials)

Figure 4 / Supplementary Table S3
To define the effects of treatment on tumors
Tumor mRNA
Retrospective study

Supplementary Figure S1

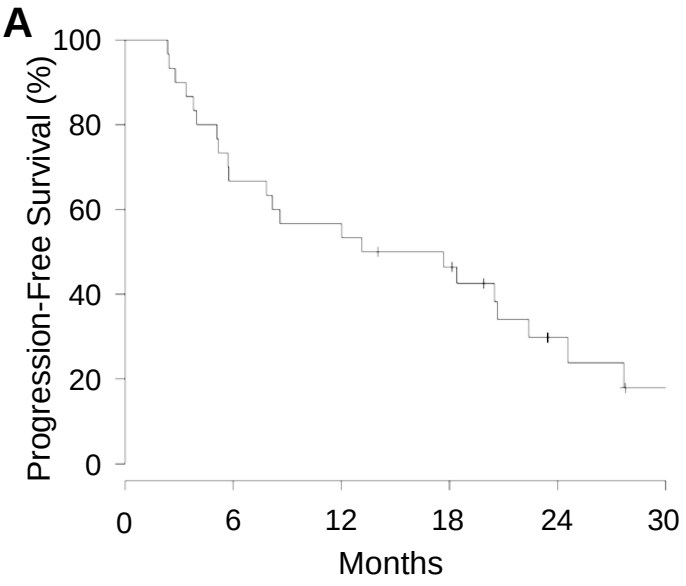


Supplementary Figure S2

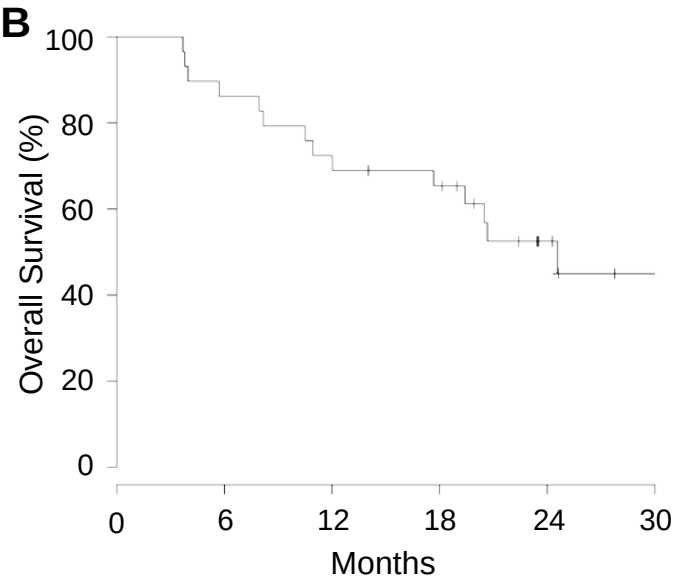


Supplementary Figure S3

Prospective cohort - Sunitinib group

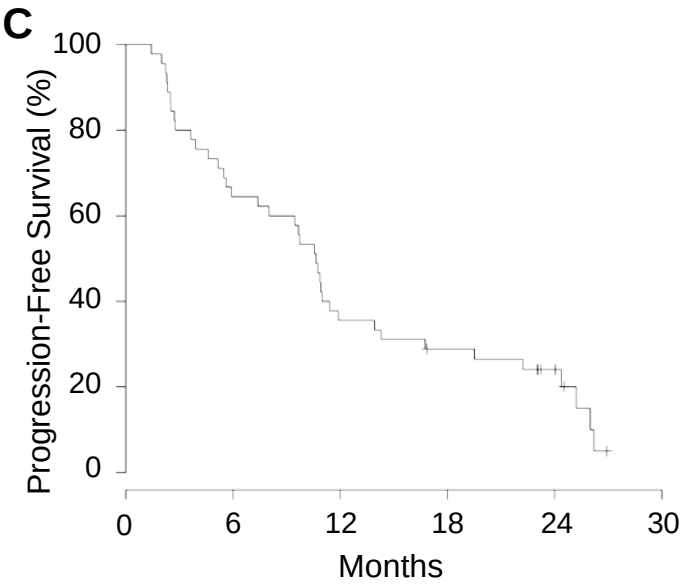


Median PFS (months)
15.4 (7.8-24.6)

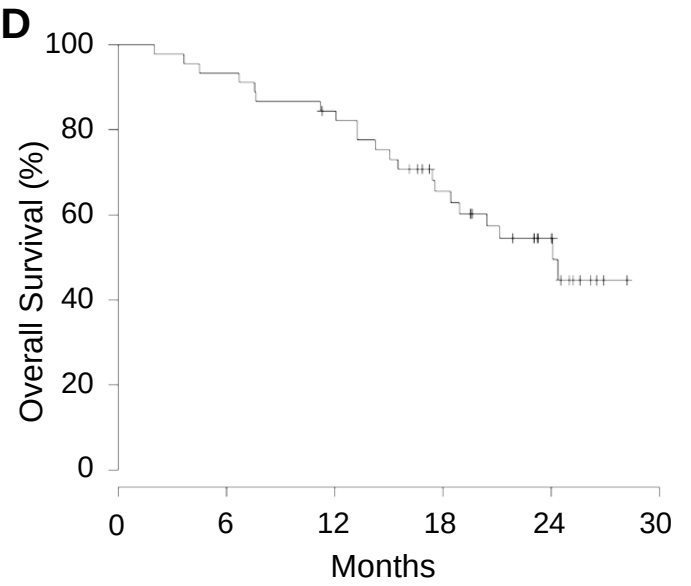


Median OS (months)
24.6 (19.4-NA)

Prospective cohort - Bevacizumab group

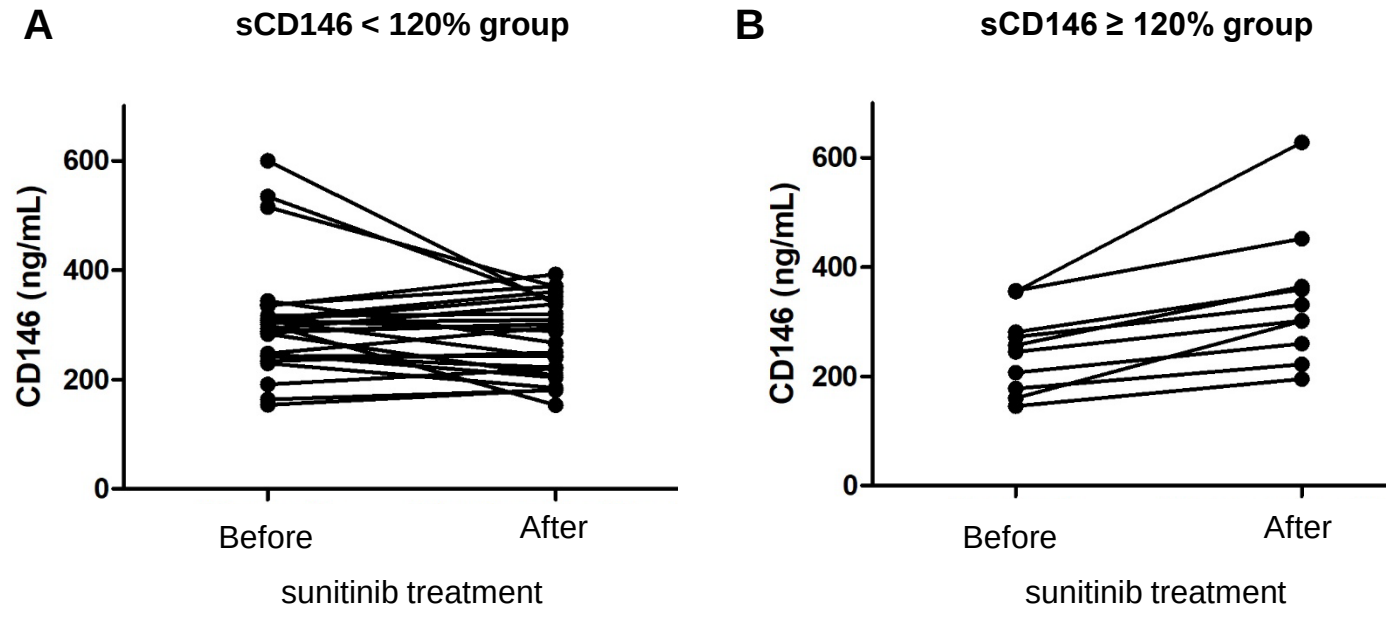


Median PFS (months)
10.6 (7.4-14.3)

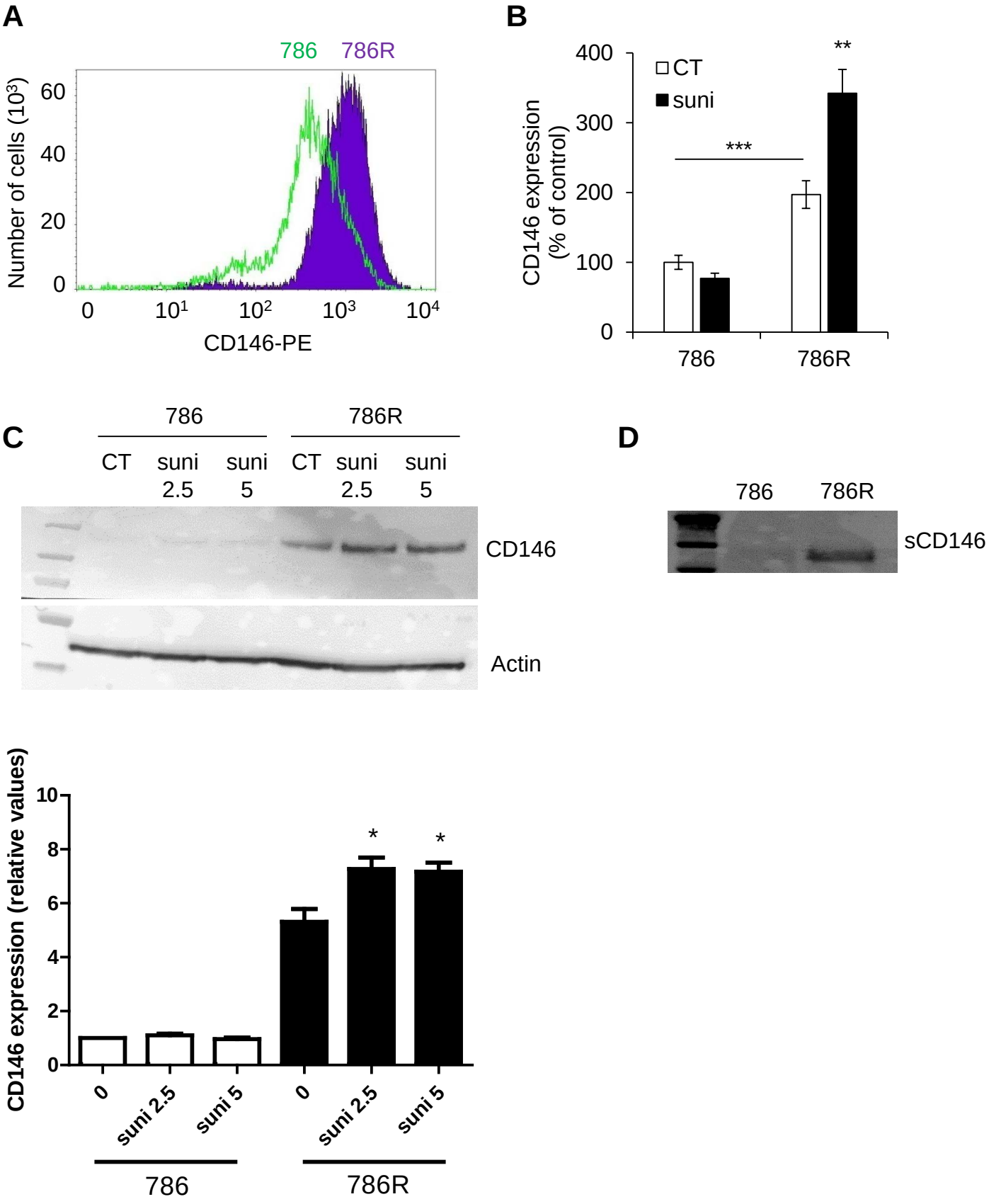


Median OS (months)
24.1 (18.4-NA)

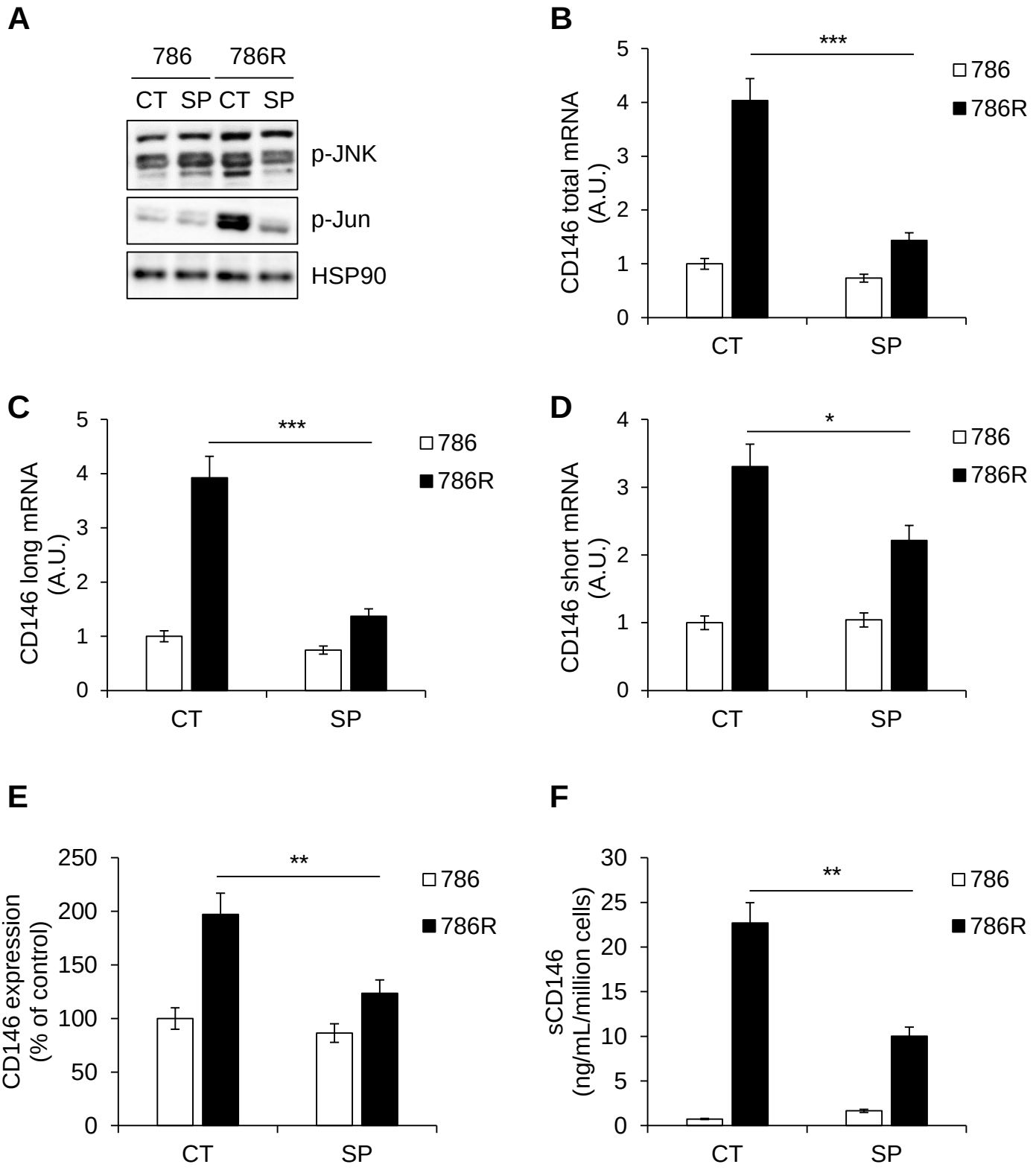
Supplementary Figure S4



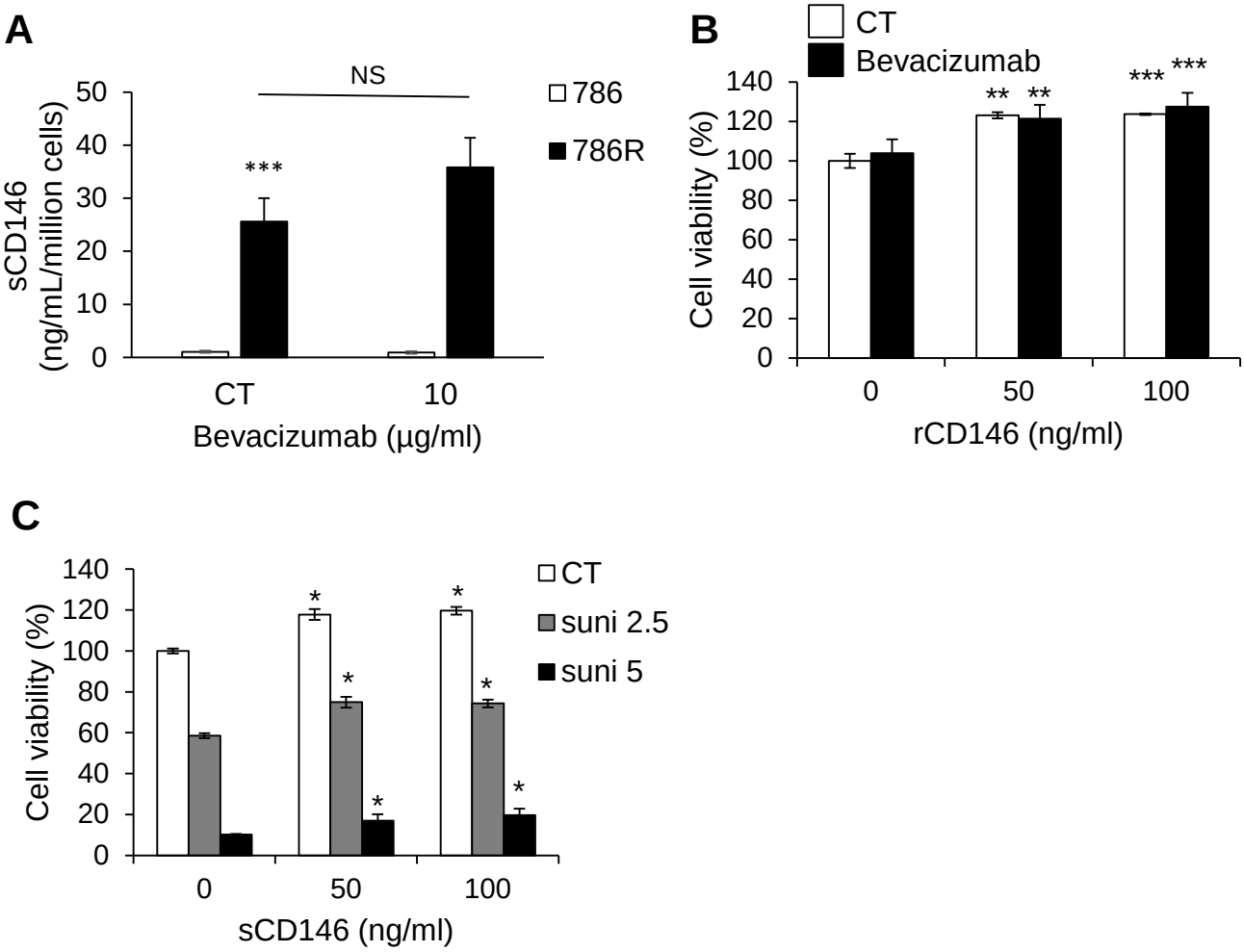
Supplementary Figure S5



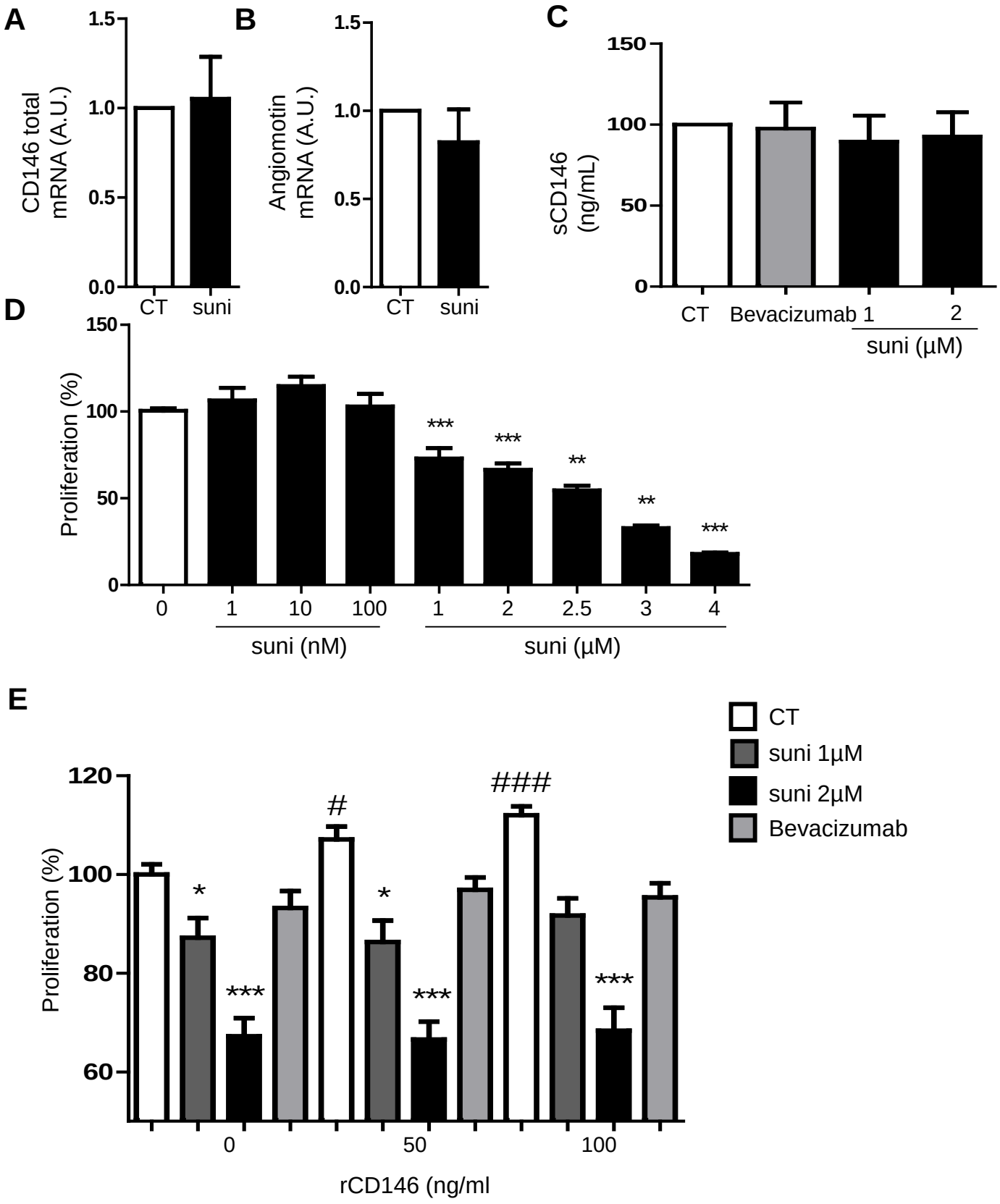
Supplementary Figure S6



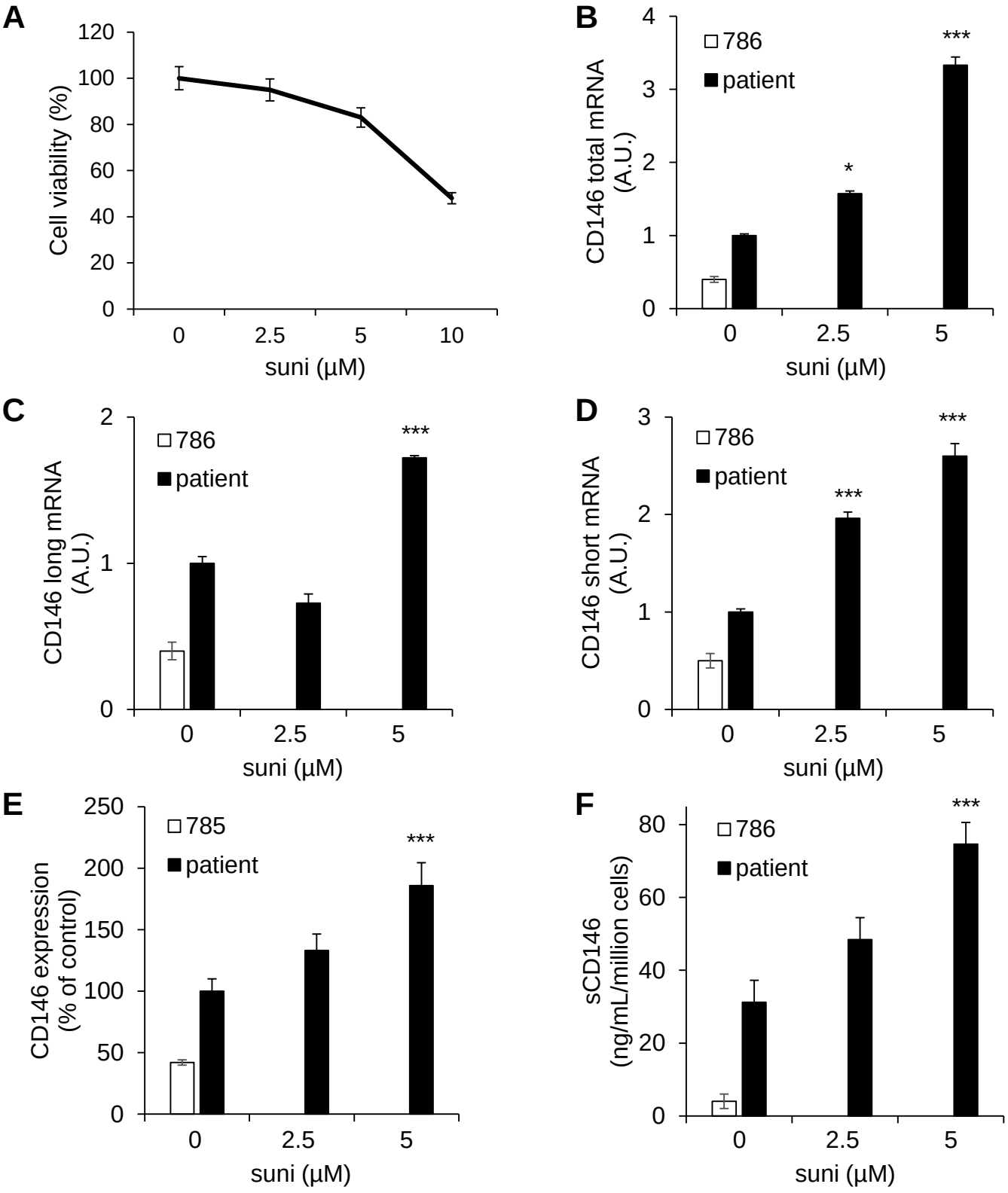
Supplementary Figure S7



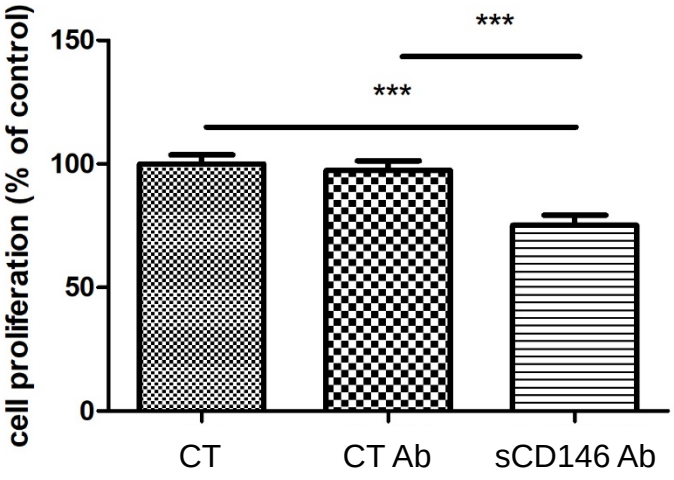
Supplementary Figure S8



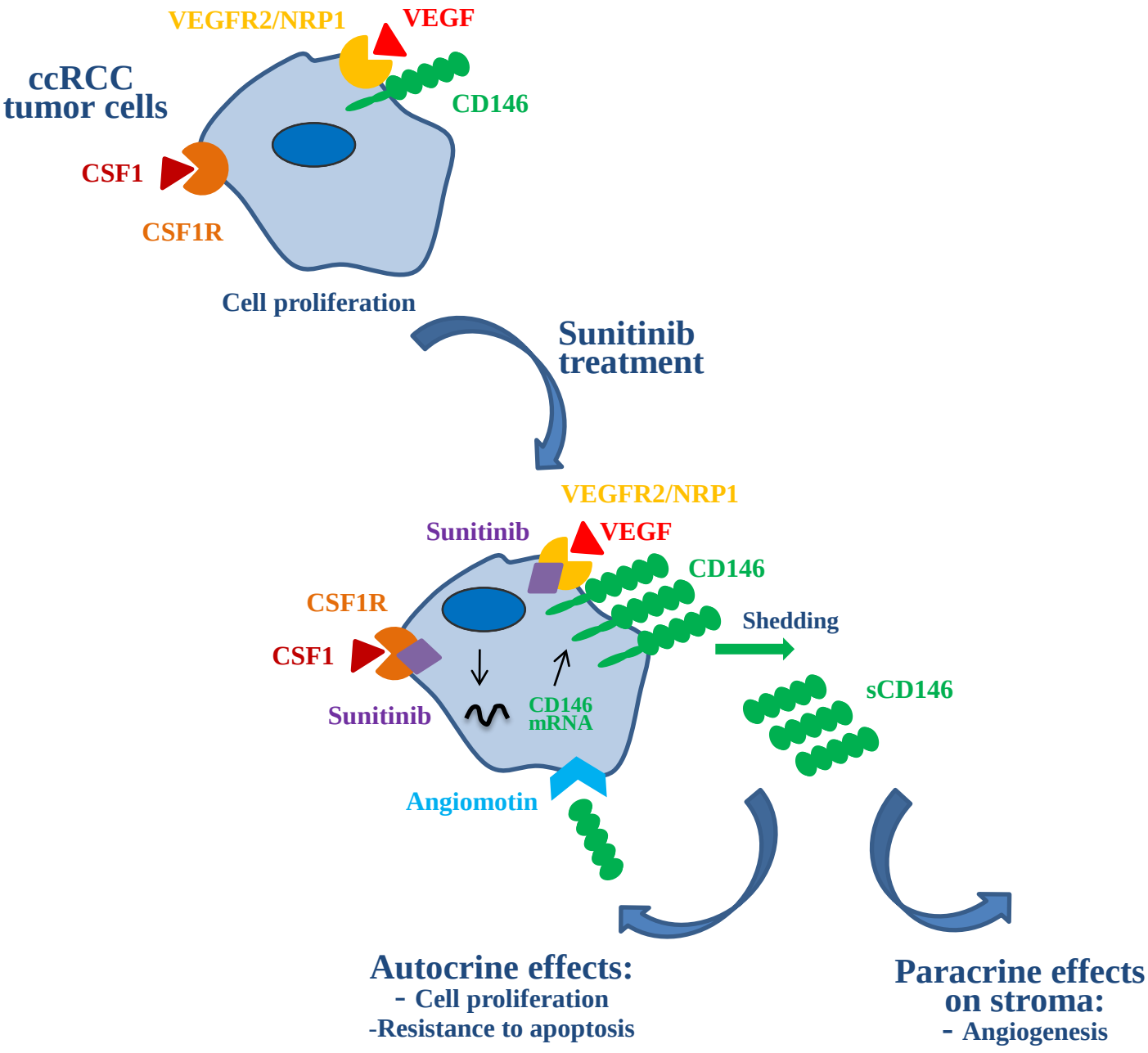
Supplementary Figure S9



Supplementary Figure S10



Supplementary Figure S11



Supplementary Figure S12

GROUP	
Number of patients	38
Sex - Woman - Man	 14 (36.8%) 24 (63.2%)
Age	62 (42-82)
Furhman grade - 2 - 3 - 4	 17 (44.7%) 16 (42.1%) 5 (13.2%)
Metastatic status pM - M0	 38 (100%)
Lymph node status pN - N0 - N1	 34 (89.5%) 4 (10.5%)
Size status pT - 1 - 2 - 3	 17 (44.7%) 6 (15.8%) 15 (39.5%)

Supplementary Table S1

A

	sCD146 < 120%	sCD146 ≥ 120%	P value
Age			0.749
Mean (SD)	60.9 (10.7)	59.7 (8)	
Gender			1
Female	4 (18.2%)	2 (18.2)	
Male	18 (81.8%)	9 (81.8)	
Fuhrman grade			0.257
1	1 (5.3%)	0 (0)	
2	6 (31.6%)	2 (20)	
3	6 (31.6%)	7 (70)	
4	6 (31.6%)	1 (10)	
pT			0.16
1	7 (35%)	1 (11.1%)	
2	6 (30%)	1 (11.1%)	
≥ 3	7 (35%)	7 (77.8%)	
pN			1
0	8 (88.9%)	1 (100%)	
1	1 (11.1%)	0 (0%)	
pM			0.206
0	13 (68.4%)	3 (37.5%)	
1	6 (31.6%)	5 (62.5%)	
Risk factor (MSKCC)			0.777
Good	6 (35.7%)	3 (30%)	
Intermediate	6 (35.7%)	3 (30%)	
Bad	4 (25%)	4 (40%)	

B

Variable	Description	Risk ratio [IC95% OR]	P value
Biological parameter **sCD146**	**≥ 120%** **< 120%**	1 8.298 [2.221 – 31]	0.001
Clinical parameters **MSKCC score**	**Good** **Intermediate** **Bad**	1 0.542 [0.122 – 2.399] 0.955 [0.256 – 3.561]	0.41 0.94

Supplementary Table S2

GROUP	CT	Sunitinib neoadjuvant
Number of patients	8	8
ccRCC	8 (100%)	8 (100%)
Sex - Woman - Man	 2 (25%) 6 (75%)	 1 (12.5%) 7 (87.5%)
AT DIAGNOSIS		
Age	57 (42-86)	55 (32-79)
Furhman grade - 3 - 4	 5 (62.5%) 3 (37.5%)	 5 (62.5%) 3 (37.5%)
Metastatic status - M0 - M1	 1 (12.5%) 7 (87.5%)	 1 (12.5%) 7 (87.5%)
Lymph node status - N0 - N1	 6 (75%) 2 (25%)	 5 (62.5%) 3 (37.5%)
Number of metastasis - 0 - 1 - 2	 1 (12.5%) 5 (62.5%) 2 (25%)	 1 (12.5%) 4 (50%) 3 (37.5%)
TREATMENT (NEOADJUVANT)		
Duration of treatment (months)	NA	4.4 (2-9)

Supplementary Table S3