

## Supplementary information

# Association of AXL and PD-L1 expression with clinical outcomes in Patients with advanced Renal Cell Carcinomas treated with PD-1 blockade

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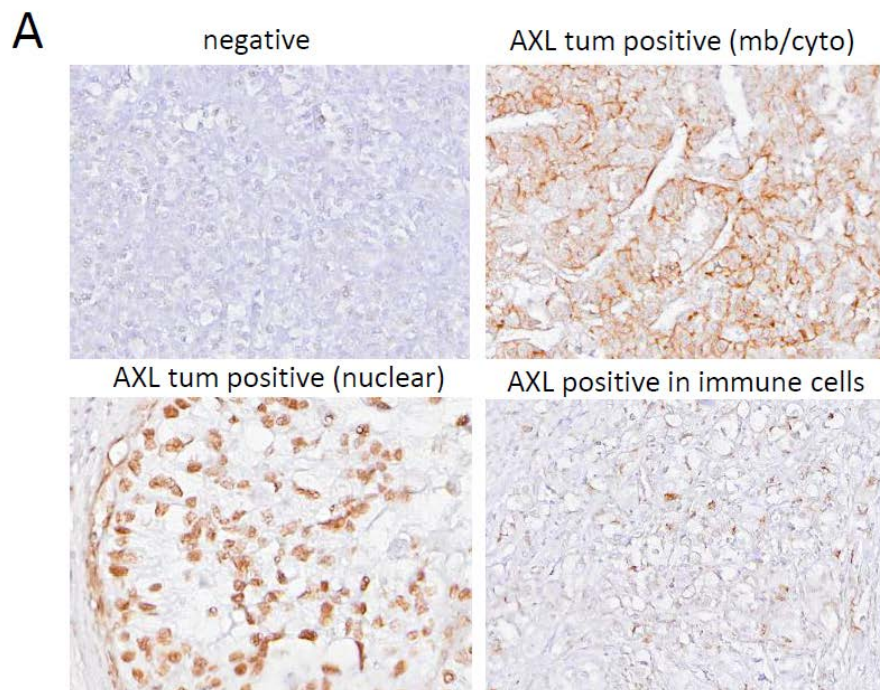
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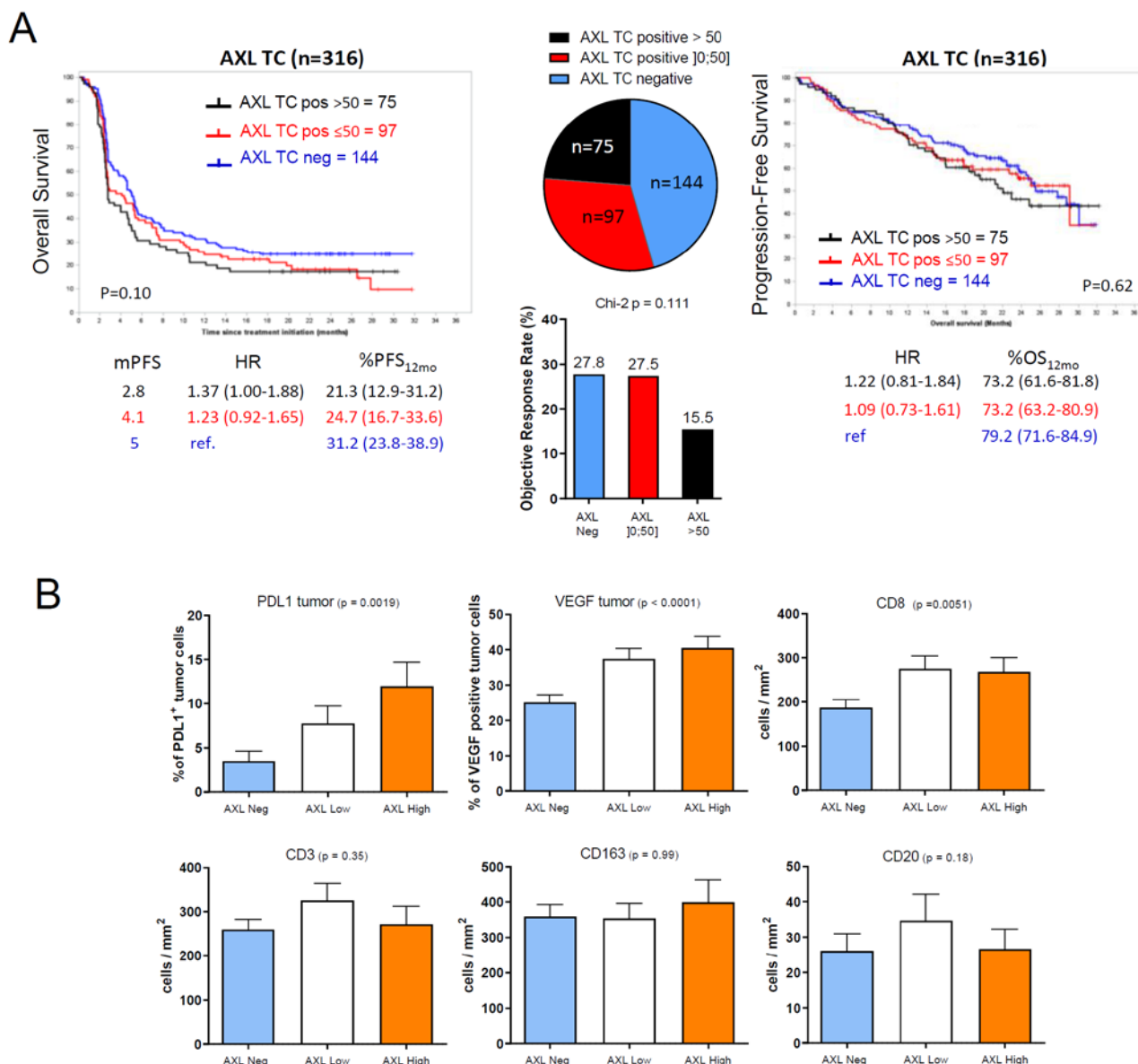
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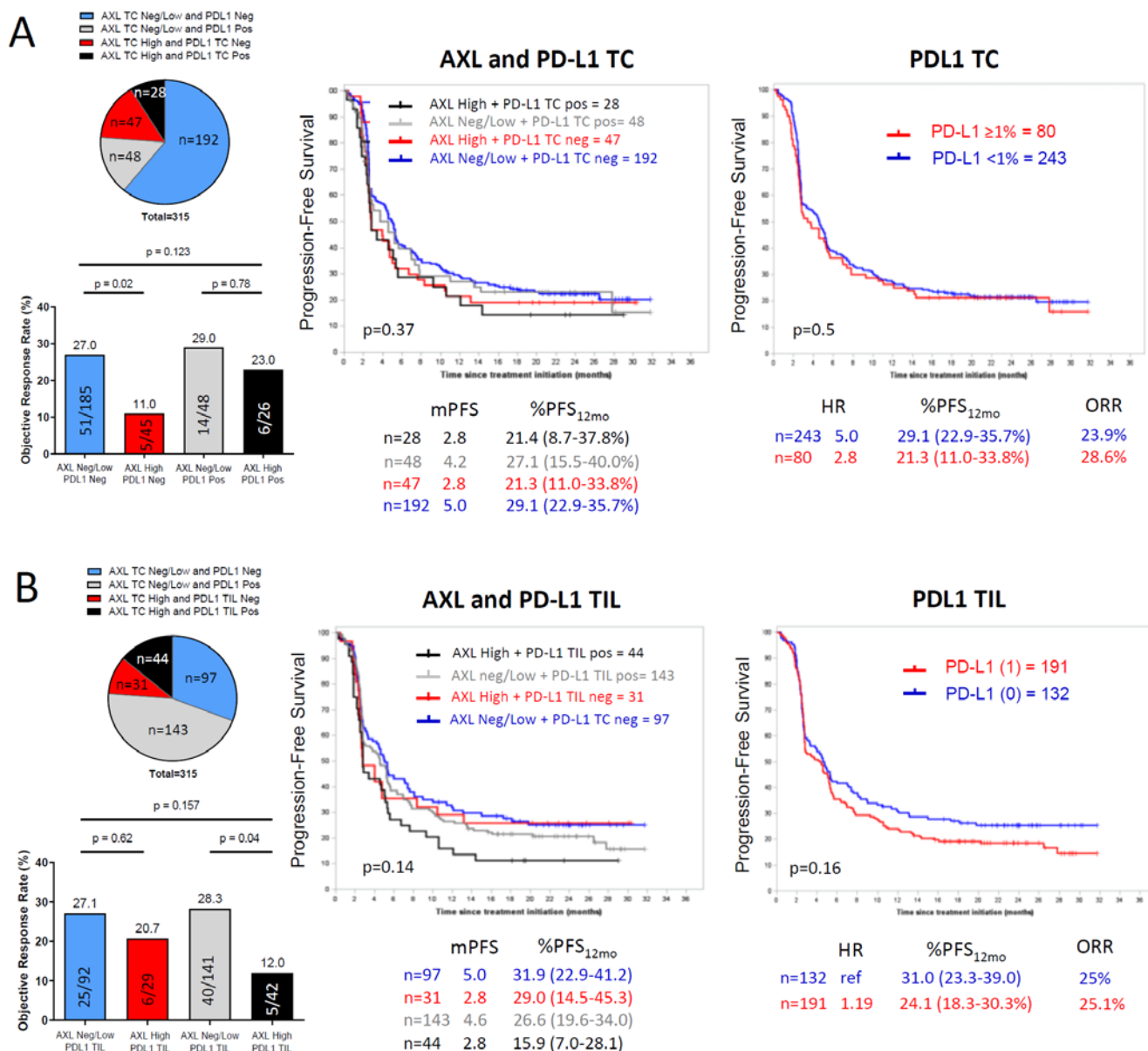
**Supplementary Figure S1 - Protein expression pattern of AXL in ccRCC specimens.**

Representative micrographs of tumor specimens with no expression (**A**) or AXL expression in RCC (**B**) with membranous/cytoplasmic staining, (**C**) nuclear staining of carcinoma cells, or in myeloid-like immune cells (**D**). Microphotographs were taken at 20x objective.



## Supplementary Figure S2 - Response rates, Survival and Biomarker analyses according to AXL<sup>neg</sup>, AXL<sup>low</sup> and AXL<sup>high</sup> expression

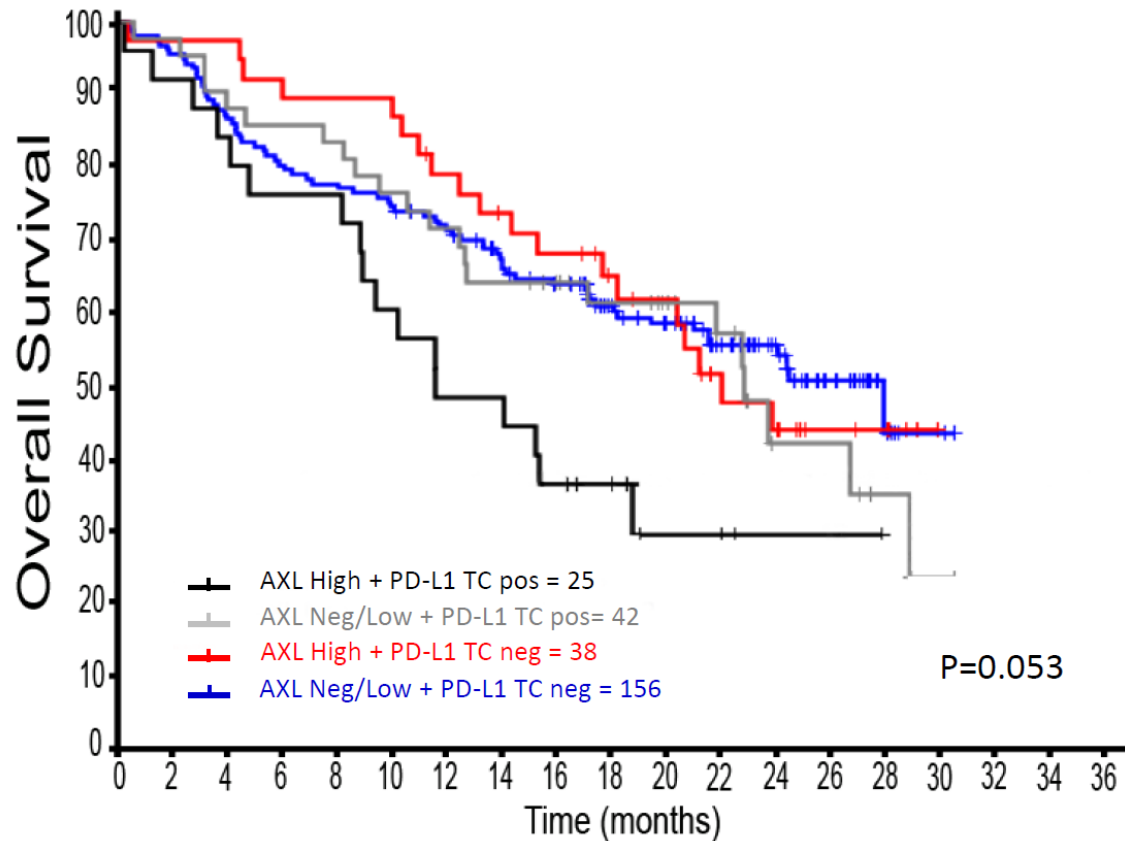
**A**, Kaplan–Meier survival curves for PFS and OS when RCC patients (n = 316) are stratified according to AXL expression score into 3 groups (negative, Low [0;50] and High >50). Corresponding Objective Response rates, hazard ratios, median PFS (mPFS), and survival OS and PFS estimates at 12 months post-treatment are shown. Pie charts show the proportion of patients in each group. **B**, Distribution of the biomarkers PD-L1, VEGF, CD8, CD3, CD163 and CD20 expression in the respective AXL groups. Data are presented as means. Errors bars are s.e.m. Kruskal-Wallis test was applied.



**Supplementary Figure S3 - ORR and Progression-Free Survival according to PD-L1 Status alone or by grouping AXL expression plus PD-L1 status**

**A**, Objective response rates and Kaplan-Meier estimates for PFS according to PD-L1 TC status (pos/neg) or by AXL expression and PD-L1 TC status (four biomarker subgroups). **B**, Objective response rates (Fisher's exact test) and Kaplan-Meier estimates for PFS according to PD-L1 TIL status (pos/neg) or by AXL expression and PD-L1 TIL status (four biomarker subgroups).

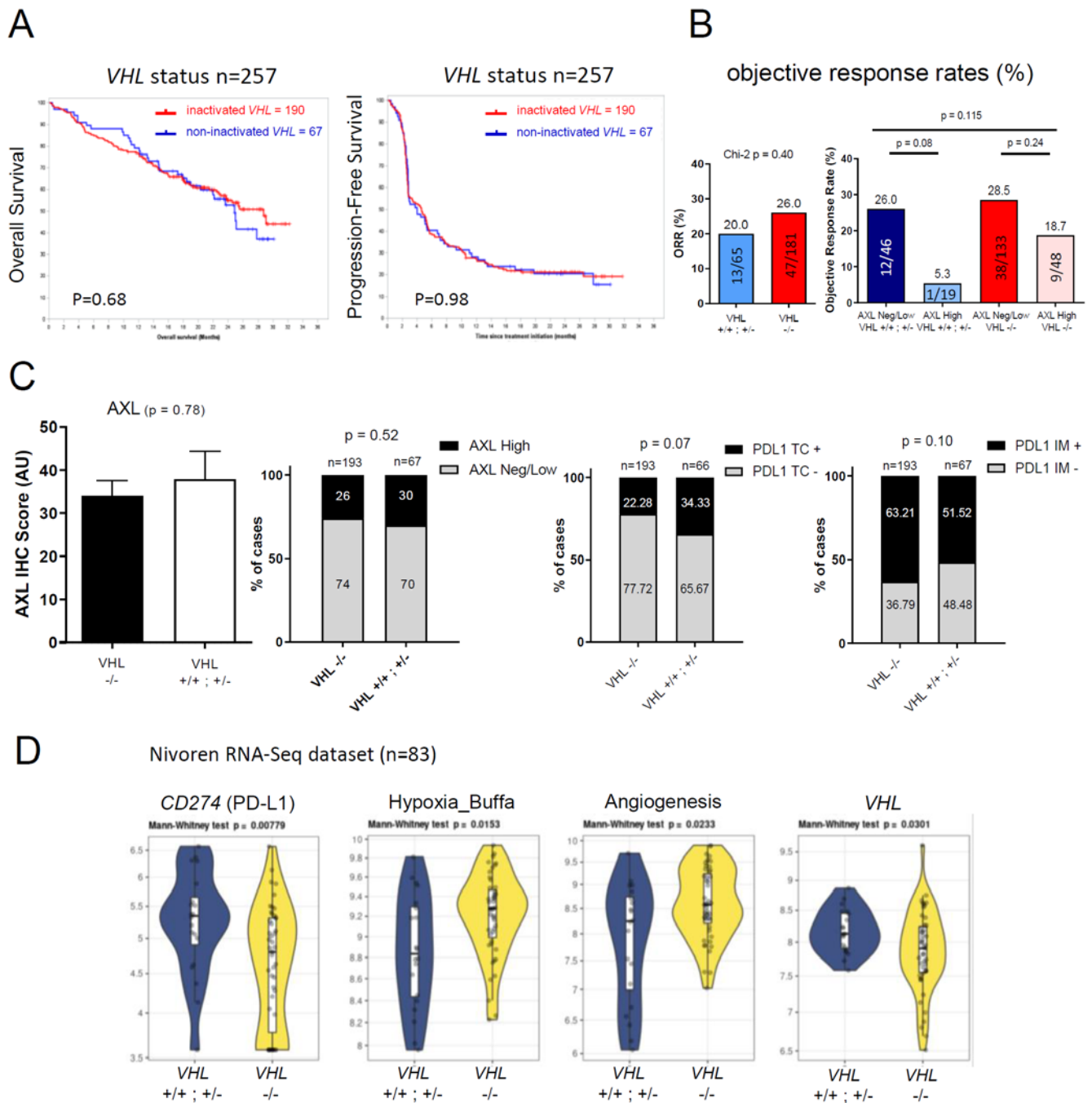
## AXL and PD-L1 TC in IMDC intermediate-risk/poor-risk patients



	HR	%OS <sub>12mo</sub>
n=25	2.05 (1.21-3.50)	56.0 (34.8-72.7%)
n=42	1.15 (0.71-1.86)	71.3 (55.1-82.6%)
n=38	0.99 (0.59-1.64)	78.9 (62.1-88.8%)
n=156 ref		73.1 (65.4-79.3%)

### Supplementary Figure S4 - High AXL expression plus PD-L1 TC positivity is associated with worse OS in Nivolumab-treated patients in IMDC intermediate-risk/poor-risk group

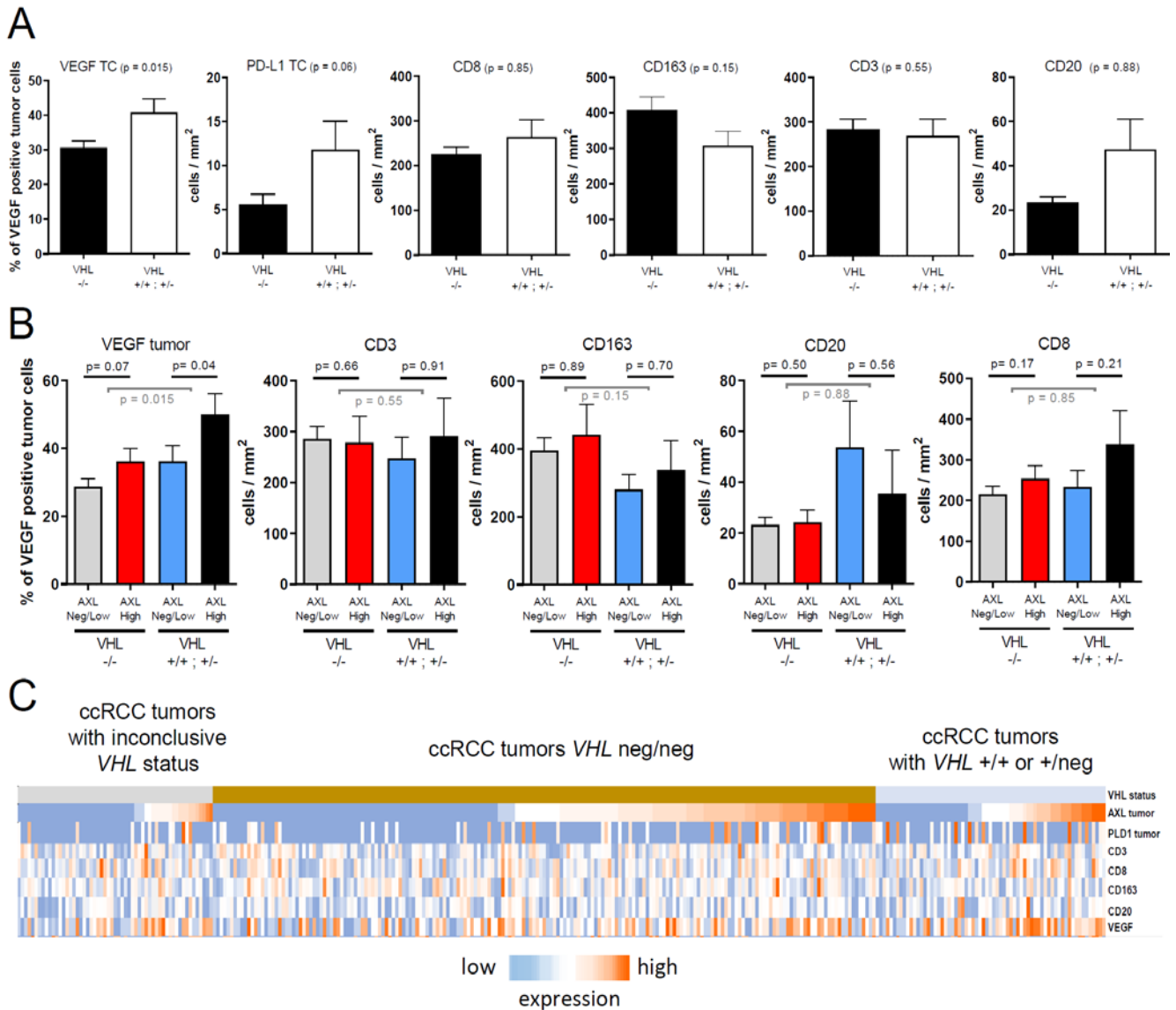
Kaplan-Meier estimates for OS according to AXL expression combined with PD-L1 TC status (four biomarker subgroups). Hazard ratios, and survival estimates at 12 months post-treatment, as well as P value from Log rank test is shown.



### Supplementary Figure S5 - VHL status does not interfere with outcomes or AXL expression upon treatment with Nivolumab

**A**, Kaplan–Meier survival curves for PFS, OS as well as ORR (**B**) of 257 RCC patients stratified according to VHL status ( $VHL^{neg/neg}$  vs  $VHL^{neg/neg}/VHL^{+/neg}$ ). ORR for patients according to AXL and VHL status are also shown. **C**, Graphs showing the distribution of cases of AXL and PD-L1 (neg/pos) staining in tumor cells or TILs in the groups according to either  $VHL^{neg/neg}$  or  $VHL^{neg/neg}/VHL^{+/neg}$  status, **D**, violin plots comparing gene expression of CD274, VHL and scores for hypoxia and angiogenesis signatures according to VHL status in 83 cases analyzed by RNA-Seq.





### Supplementary Figure S6 - Biomarker distribution across AXL groups stratified based on *VHL* status

**A**, PD-L1, VEGF, CD8; CD3, CD163, CD20 expression bases on *VHL* status of the tumors. Data are presented as means. Errors bars are s.e.m (Mann-Whitney test). **B**, same as **A** for VEGF, CD8; CD3, CD163, CD20 expression, but dichotomizing by AXL<sup>Neg/Low</sup> versus AXL<sup>High</sup> cases. Corresponding results for PD-L1 TC are shown in Figure 5D. **C**, A heatmap illustrating the biomarker composition according to AXL<sup>Neg/Low</sup> to AXL<sup>High</sup> expression and *VHL* status.



## Supplementary Table S1

### Multivariate analysis of prognostic factors for PFS in the Nivoren cohort

#### *Multivariate COX analysis model*

Variable	Hazard ratio	95% CI	P value
AXL H score >50 vs [0;50]	1.28	0.96-1.71	0.0976
Sex (F/M)	1.19	0.87-1.62	0.2742
Age [65;75[ vs <65; >=75 vs <65	0.97; 0.90	0.74-1.28; 0.60-1.34	0.8737
IMDC (intermediate; poor vs favorable)	1.19; 1.39	0.84-1.70; 0.92-2.09	0.2864
Number of lines > 2	1.15	0.84-1.58	0.3785

CI = confidence interval; IMDC = International Metastatic Renal Cell Carcinoma Database Consortium prognostic risk

## Supplementary Table S2

### Multivariate analysis of prognostic factors for OS in the Nivoren cohort

#### *Multivariate COX analysis model*

Variable	Hazard ratio	95% CI	P value
AXL H score >50 vs [0;50]	1.30	0.88-1.90	0.18
Sex (F/M)	0.79	0.51-1.22	0.2791
Age [65;75[vs <65 ; >=75 vs <65	1.09; 1.66	0.76-1.58; 1.01-2.72	0.1322
IMDC (intermediate; poor vs favorable)	2.19; 4.48	1.19-4.02; 2.36-8.53	<0.0001*
Number of lines > 2	1.25	0.84-1.85	0.2667

CI = confidence interval; IMDC = International Metastatic Renal Cell Carcinoma Database Consortium prognostic risk

**Supplementary Table S3 : Patient characteristics when categorized by 0, Low or High AXL H-scores**

Variables	AXL Tumor cell score			All Patients n=316	Tests
	0	]0;50]	>50		
	n=144	n=97	n=75		
<b>Age (years)</b>					
N	144	97	75	316	T-test
Mean (Std)	63.4 (9.9)	61.5 (12.1)	61.9 (10.4)	62.4 (10.7)	P = 0.365
Median (min; max)	64.5 (31.0; 87.0)	62.0 (22.0; 85.0)	62.0 (41.0; 86.0)	64.0 (22.0; 87.0)	
<b>Gender</b>					
Male	118 (81.9%)	76 (78.4%)	63 (84.0%)	257 (81.3%)	Chi-2
Female	26 (18.1%)	21 (21.6%)	12 (16.0%)	59 (18.7%)	P = 0.620
<b>IMDC group</b>					
Favorable	26 (18.1%)	16 (16.5%)	12 (16.0%)	54 (17.1%)	Chi-2
Intermediate	82 (56.9%)	60 (61.9%)	49 (65.3%)	191 (60.4%)	P = 0.792
Poor	36 (25.0%)	21 (21.6%)	14 (18.7%)	71 (22.5%)	
<b>ECOG</b>					
Missing	6	2	3	11	
0 or 1	118 (85.5%)	81 (85.3%)	63 (87.5%)	262 (85.9%)	Chi-2
2 or 3	20 (14.5%)	14 (14.7%)	9 (12.5%)	43 (14.1%)	P = 0.904
<b>M Stage</b>					
Missing	8	6	5	19	
0	60 (44.1%)	23 (25.3%)	28 (40.0%)	111 (37.4%)	Chi-2
1	33 (24.3%)	26 (28.6%)	20 (28.6%)	79 (26.6%)	P = 0.048
X	43 (31.6%)	42 (46.2%)	22 (31.4%)	107 (36.0%)	
<b>Furhman</b>					
Missing data	4	3	2	9	
I, II	49 (35.0%)	20 (21.3%)	13 (17.8%)	82 (26.7%)	Chi-2
III, IV	91 (65.0%)	74 (78.7%)	60 (82.2%)	225 (73.3%)	P = 0.010
<b>Brain Metastasis</b>					
Missing	8	8	3	19	
NO	116 (85.3%)	81 (91.0%)	65 (90.3%)	262 (88.2%)	Chi-2
YES	20 (14.7%)	8 (9.0%)	7 (9.7%)	35 (11.8%)	P = 0.353
<b>Sugery</b>	144 (100.0%)	97 (100.0%)	75 (100%)	316 (100%)	
<b>Nephrectomy</b>					
NO	5 (3.5%)	4 (4.1%)	3 (4.0%)	12 (3.8%)	Chi-2
YES	139 (96.5%)	93 (95.9%)	72 (96.0%)	304 (96.2%)	P = 1.000
<b>Radiotherapy</b>					
NO	80 (55.6%)	68 (70.1%)	47 (62.7%)	195 (61.7%)	Chi-2
YES	64 (44.4%)	29 (29.9%)	28 (37.3%)	121 (38.3%)	P = 0.073

**Supplementary Table S4 : gene signatures**

Immotion_angio	Immotion_Teff	inflammation	immunosuppression	Buffa_hypoxia
VEGFA	CD8A	TNF	CXCL12	ACOT7
KDR	EOMES	LTA	TGFB1	ADM
ESM1	PRF1	IL6	TGFB3	ALDOA
PECAM1	IFNG	IL6R	LGALS1	ANKRD37
ANGPTL4	CD274	CD40LG	IL10	ANLN
CD34		CD70		BNIP3
		FASLG		CA9
		TNFSF8		CDKN3
		TNFSF10		CHCHD2
		TNFSF13B		CORO1C
		IRF1		DDIT4
				ENO1
				ESRP1
				GAPDH
				GPI
				HK2
				KIF20A
				KIF4A
				LDHA
				LRRC42
				MAD2L2
				MAP7D1
				MCTS1
				MIF
				MRPL13
				MRPL15
				MRPS17
				NDRG1
				P4HA1
				PFKP
				PGAM1
				PGK1
				PSMA7
				PSRC1
				SEC61G
				SHCBP1
				SLC16A1
				SLC25A32

				SLC2A1
				TPI1
				TUBA1B
				TUBA1C
				TUBB6
				UTP11L
				VEGFA
				YKT6

**Supplementary Table S5 : Multivariate analysis of prognostic factors for OS in the Nivoreen cohort**

*Multivariate COX analysis model*

<b>Variable</b>	<b>Hazard ratio</b>	<b>95% CI</b>	<b>P value</b>
<b>AXL H score and PDL1 TC</b>			
AXL >50 and PDL1 TC≥1	2.01	1.18-3.44	0.0847
AXL [0;50] and PDL1 TC≥1	1.17	0.74-1.85	
AXL>50 and PDL1 TC=0;0.5	1.06	0.64-1.74	
AXL [0;50] and PDL1 TC=0;0.5	1		
<b>Sex (F/M)</b>	0.75	0.48-1.17	0.2100
<b>Age</b> [65;75 vs <65; ≥75 vs <65	1.10; 1.62	0.76-1.59; 0.99-2.67	0.1582
<b>IMDC</b> (intermediate; poor vs favorable)	2.16; 4.26	1.17-3.97; 2.23-8.15	<0.0001*
<b>Number of lines</b> > 2	1.29	0.87-1.92	0.2070

CI = confidence interval; IMDC = International Metastatic Renal Cell Carcinoma Database Consortium prognostic risk