

Figure S1

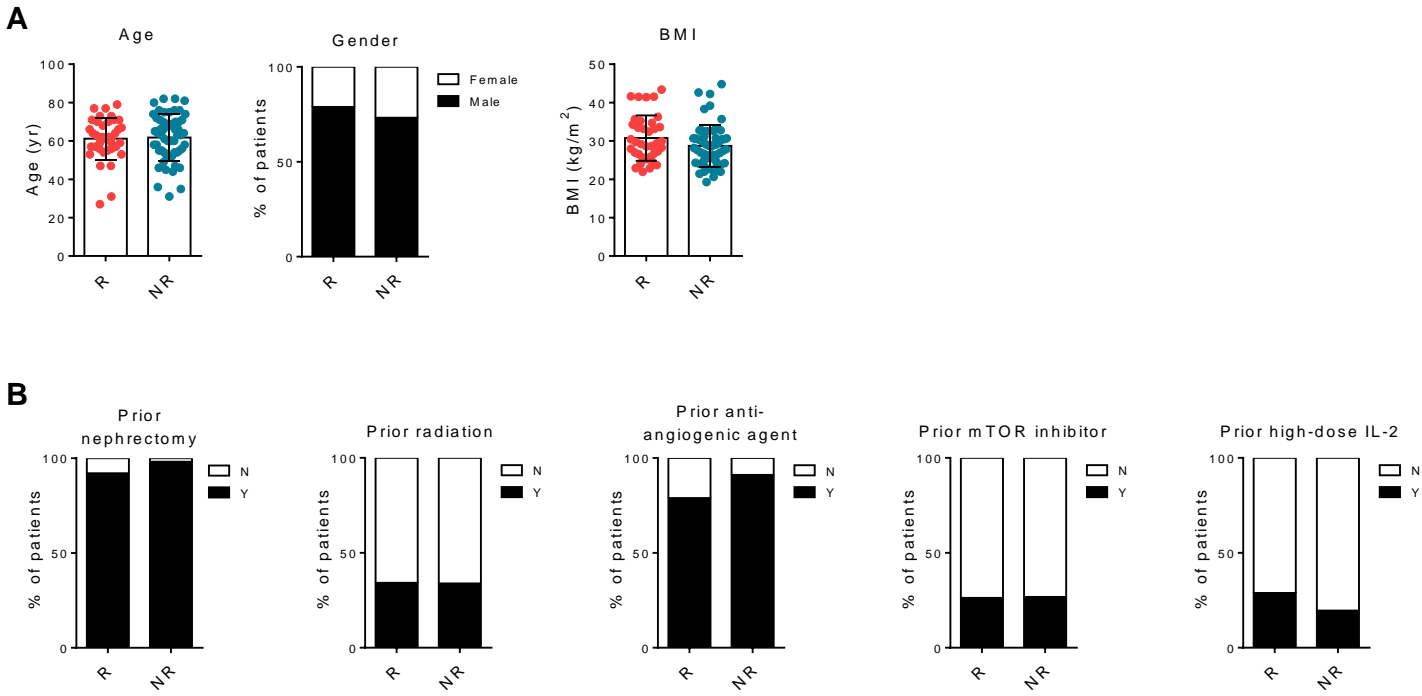


Figure S2

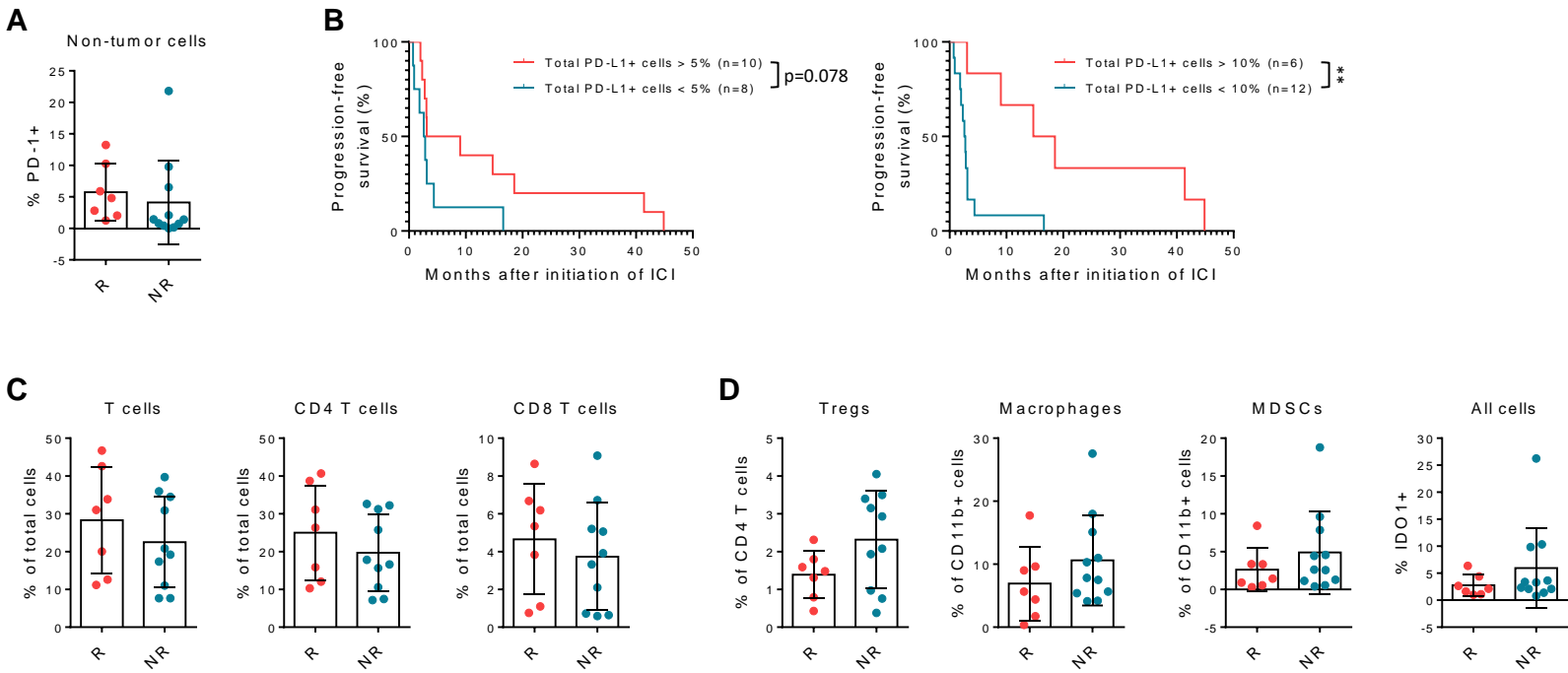
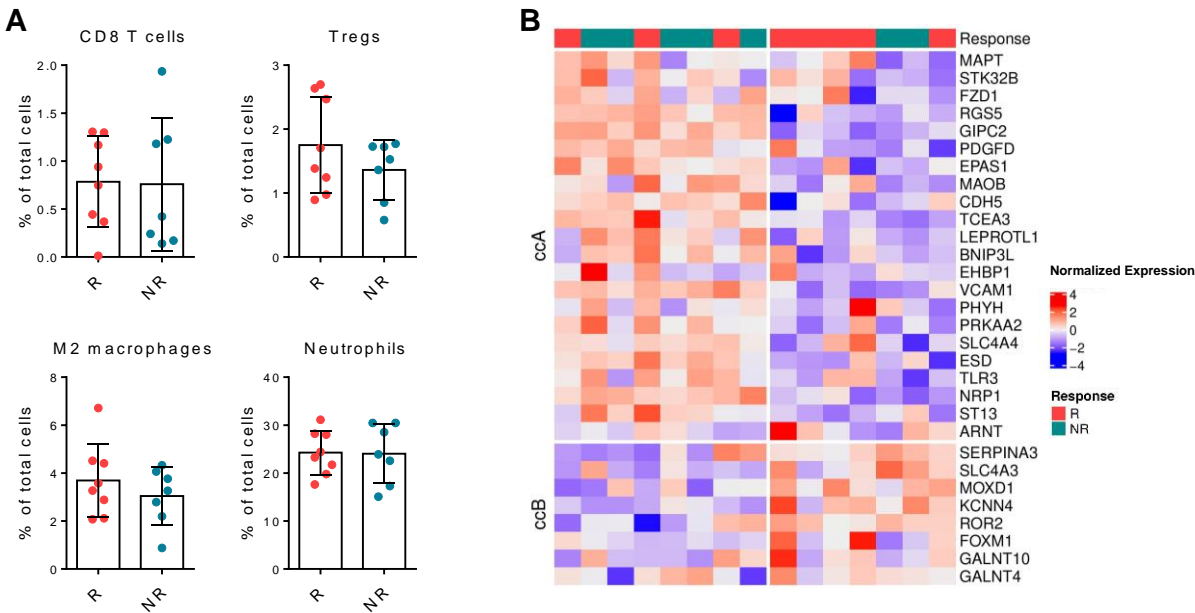


Figure S3



**Table S1.** Antibody panels used in multiplexed immunofluorescence assay

Panel	Antibody	Clone	Secondary	Fluorophore
PD-1/PD-L1/CK	PD-1	NAT105	Mouse EnVision+ HRP	TSA+Cy3.5
	PD-L1	E1L3N	Rabbit EnVision+ HRP	TSA+Cy5
CD11b/HLA-DR/IDO-1/CK	CD11b	EP1345Y	Rabbit EnVision+ HRP	Opal520
	HLA-DR	TAL.1B5	Mouse EnVision+ HRP	TSA+Cy3
	IDO-1	SP260	Rabbit EnVision+ HRP	TSA+Cy5
	Pan-Cytokeratin	AE1/AE3	N/A	Alexa488
CD4/CD8/CD25/FoxP3/Ki67/CK	CD4	4B12	Mouse EnVision+ HRP	Opal520
	CD8	C8/144B	Mouse EnVision+ HRP	Opal620
	CD25	SP176	Rabbit EnVision+ HRP	Opal570
	FoxP3	D2W8E	Rabbit EnVision+ HRP	Opal540
	Ki67	MIB-1	Mouse EnVision+ HRP	Opal650
	Pan-Cytokeratin	AE1/AE3	Mouse EnVision+ HRP	Opal690

**Table S2.** Clinical characteristics of biomarker cohort of patients with RCC

	Biomarker cohort, N = 26	Responders (CR, PR, mixed), N = 13	Non-responders (PD), N = 13
<b>Best response to ICI therapy (%)</b>			
CR	1 (3.8)	1 (7.7)	0 (0.0)
PR	10 (38.5)	11 (76.9)	0 (0.0)
PD	13 (50.0)	0 (0.0)	13 (100.0)
Mixed	2 (7.7)	2 (15.4)	0 (0.0)
<b>Median age at initiation of ICI (range), yr</b>	61 (44-85)	62 (47-79)	60 (44-85)
<b>Sex (%)</b>			
Male	20 (76.9)	9 (69.2)	11 (84.6)
Female	6 (23.1)	4 (30.8)	2 (15.4)
<b>Stage at diagnosis (%)†</b>			
I	3 (16.7)	2 (22.2)	1 (11.1)
II	1 (5.6)	1 (11.1)	0 (0.0)
III	5 (27.8)	2 (22.2)	3 (33.3)
IV	9 (50.0)	4 (44.4)	5 (55.6)
<b>Pathology†</b>			
Clear cell	17 (88.9)	8 (88.9)	8 (88.9)
Papillary	1 (5.6)	0 (0.0)	1 (11.1)
Sarcomatoid	1 (5.6)	1 (11.1)	0 (0.0)
<b>IMDC risk group (%)†</b>			
Favorable	3 (16.7)	2 (22.2)	1 (11.1)
Intermediate	9 (50.0)	6 (66.7)	3 (33.3)
Poor	6 (33.3)	1 (11.1)	5 (55.6)
<b>Previous therapies (%)†</b>			
Nephrectomy	17 (94.4)	8 (88.9)	9 (100.0)
Radiation	10 (55.6)	5 (55.6)	5 (55.6)
Anti-angiogenic agent	16 (88.9)	8 (88.9)	8 (88.9)
mTOR inhibitor	7 (38.9)	4 (44.4)	4 (44.4)
High-dose IL-2	6 (33.3)	2 (22.2)	3 (33.3)
<b>ICI agent (%)</b>			
Nivolumab	25 (96.2)	12 (92.3)	13 (100.0)
Atezolizumab	1 (3.8)	1 (7.7)	0 (0.0)
<b>ICI line of therapy (%)†</b>			
First-line	1 (5.6)	1 (11.1)	0 (0.0)
Second-line	3 (16.7)	1 (11.1)	2 (22.2)
Third-line	8 (44.4)	4 (44.4)	4 (44.4)
Fourth-line+	6 (33.3)	3 (33.3)	3 (33.3)
<b>Median duration of ICI therapy (range), days</b>	123 (27-1637)	448 (27-1637)***	73 (28-238)***
<b>Median survival (95% CI), months</b>			
PFS	4.03 (2.86-13.4)	13.4 (9.01-not reached)###	2.76 (2.33-not reached)###
OS†	22.4 (12.4-not reached)	Not reached (25.1-not reached)#	10.6 (5.95-not reached)#
<b>irAE while on ICI therapy (%)†</b>	5 (27.8)	5 (55.6)	0 (0.0)

†Clinical characteristic available only for subset of patients

\*\*\*p&lt;0.001, two-tailed Mann-Whitney U test

#p&lt;0.05, ###p&lt;0.001, log-rank test

Abbreviations: CR, complete response; PR, partial response; PD, progression of disease; ICI, immune checkpoint inhibitor; IMDC: International Metastatic RCC Database Consortium; irAE, immune-related adverse event; CI, confidence interval; PFS, progression-free survival

**Table S3.** irAEs during ICI therapy in primary patient cohort

	Primary cohort, N = 94	Responders, N = 38	Non-responders, N = 56
<b>irAE type (%)</b>			
All	35 (37.2)	19 (50.0)*	16 (28.6)*
Thyroid dysfunction	12 (12.8)	6 (15.8)	6 (10.7)
Dermatologic	11 (11.7)	7 (18.4)	4 (7.1)
Pneumonitis	7 (7.4)	3 (7.9)	4 (7.1)
Arthritis	5 (5.3)	4 (10.5)	1 (1.8)
Hepatitis	5 (5.3)	5 (13.2)	0 (0.0)
Colitis	4 (4.3)	2 (5.3)	2 (3.6)
Encephalitis	3 (3.2)	2 (5.3)	1 (1.8)
Nephritis	2 (2.1)	2 (5.3)	0 (0.0)
Hematologic	2 (2.1)	1 (2.6)	1 (1.8)
Myalgia	2 (2.1)	1 (2.6)	1 (1.8)
Hypophysitis	1 (1.1)	1 (2.6)	0 (0.0)
Diabetes mellitus	1 (1.1)	1 (2.6)	0 (0.0)
Adrenal insufficiency	1 (1.1)	1 (2.6)	0 (0.0)
Neuropathy	1 (1.1)	1 (2.6)	0 (0.0)
Arrhythmia	1 (1.1)	1 (2.6)	0 (0.0)
Other	1 (1.1)	0 (0.0)	1 (1.8)
<b>Patients with irAE</b>	<b>n = 35</b>	<b>n = 19</b>	<b>n = 16</b>
<b>irAE management (%)</b>			
Immunosuppression	18 (51.4)	10 (52.6)	8 (50.0)
Oral steroids	17 (48.6)	10 (52.6)	7 (43.8)
Intravenous steroids	6 (17.1)	2 (10.5)	4 (25.0)
<b>Highest grade of irAE (%)</b>			
Grade 1	11 (31.4)	6 (31.6)	5 (31.3)
Grade 2	16 (45.7)	8 (42.1)	8 (50.0)
Grade 3	7 (20.0)	4 (21.1)	3 (18.8)
Grade 4	1 (2.9)	1 (5.3)	0 (0.0)

\*p<0.05, OR = 2.50 (95% CI = 1.057 – 5.911), chi-square test

Abbreviations: irAE, immune-related adverse event

**Table S4.** Sites of metastasis prior to initiation of ICI in primary patient cohort

	Primary cohort, N = 94	Responders, N = 38	Non-responders, N = 56
<b>Sites of metastasis (%)</b>			
<b>All</b>	90 (95.7)	36 (94.7)	54 (96.4)
<b>Visceral</b>	84 (89.4)	33 (86.8)	51 (91.1)
<b>Lung</b>	67 (71.3)	29 (76.3)	38 (67.9)
<b>Lymph node</b>	57 (60.1)	23 (60.5)	34 (60.7)
<b>Bone</b>	36 (38.3)	14 (36.8)	22 (39.3)
<b>Adrenal</b>	30 (31.9)	12 (31.6)	18 (32.1)
<b>Liver</b>	28 (29.8)	8 (21.1)	20 (35.7)
<b>Soft tissue</b>	22 (23.4)	7 (18.4)	15 (26.8)
<b>Pancreas</b>	17 (18.1)	3 (7.9)*	14 (25.0)*
<b>Brain</b>	12 (12.8)	4 (10.5)	8 (14.3)
<b>Gastrointestinal</b>	5 (5.3)	2 (5.3)	3 (5.4)
<b>Other</b>	6 (6.4)	1 (2.6)	5 (8.9)

\*p&lt;0.05, OR = 0.257 (95% CI = 0.0683 – 0.968), chi-square test

**Table S5.** Selected differentially expressed pathways from gene set variation analysis

Pathway	Pathway type	Log <sub>e</sub> (ratio)	P-value
GO_KETOSTEROID_MONOOXYGENASE_ACTIVITY	Metabolism	-0.03933	0.00019
GO_BILE_ACID_BINDING	Metabolism	0.007758	0.000194
GSE6674_UNSTIM_VS_CPG_STIM_BCELL_DN	Immune	-0.04345	0.001921
GSE15659_CD45RA_NEG_CD4_TCELL_VS_ACTIVATED_TREG_UP	Immune	0.007549	0.001928
GO_COLLAGEN_BINDING_INVOLVED_IN_CELL_MATRIX_ADHESION	Metabolism	-0.02938	0.002564
GSE32423_CTRL_VS_IL4_MEMORY_CD8_TCELL_UP	Immune	0.003767	0.003148
GO_NEGATIVE_REGULATION_OF_SYNAPTIC_VESICLE_TRANSPORT	Metabolism	0.032617	0.003375
REACTOME_GLYCOGEN_BREAKDOWN_GLYCOGENOLYSIS	Metabolism	-0.06214	0.003438
TONKS_TARGETS_OF_RUNX1_RUNX1T1_FUSION_GRANULOCYTE_UP	Immune	0.000645	0.003607
MORF_IFNA1	Immune	0.018098	0.003761
GO_MEDIUM_CHAIN_FATTY_ACID_COA_LIGASE_ACTIVITY	Metabolism	-0.00155	0.004061
GSE21380_NON_TFH_VS_GERMINAL_CENTER_TFH_CD4_TCELL_UP	Immune	0.011311	0.004099
GSE3982_CTRL_VS_LPS_1H_NEUTROPHIL_DN	Immune	-0.01864	0.004152
GO_PYRUVATE_TRANSMEMBRANE_TRANSPORT	Metabolism	-0.01757	0.004259
GSE17974_IL4_AND_ANTI_IL12_VS_UNTREATED_4H_ACT_CD4_TCELL_UP	Immune	-0.03967	0.004449
GSE5589_LPS_VS_LPS_AND_IL10_STIM_MACROPHAGE_180MIN_UP	Immune	-0.03487	0.005379
GO_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_LIPID_ANTIGEN_VIA_MHC_CLASS_II	Immune	0.043893	0.00661
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS_VIA_24_HYDROXYCHOLESTEROL	Metabolism	-0.00361	0.006839
GO_REGULATION_OF_DEFENSE_RESPONSE_TO_VIRUS	Immune	-0.03198	0.008726
GSE26928_EFF_MEM_VS_CENTR_MEM_CD4_TCELL_DN	Immune	-0.01357	0.00939
GO_POSITIVE_REGULATION_OF_DEFENSE_RESPONSE_TO_VIRUS_BY_HOST	Immune	-0.02478	0.009429
GSE15659_NONSUPPRESSIVE_TCELL_VS_ACTIVATED_TREG_UP	Immune	0.017029	0.009708