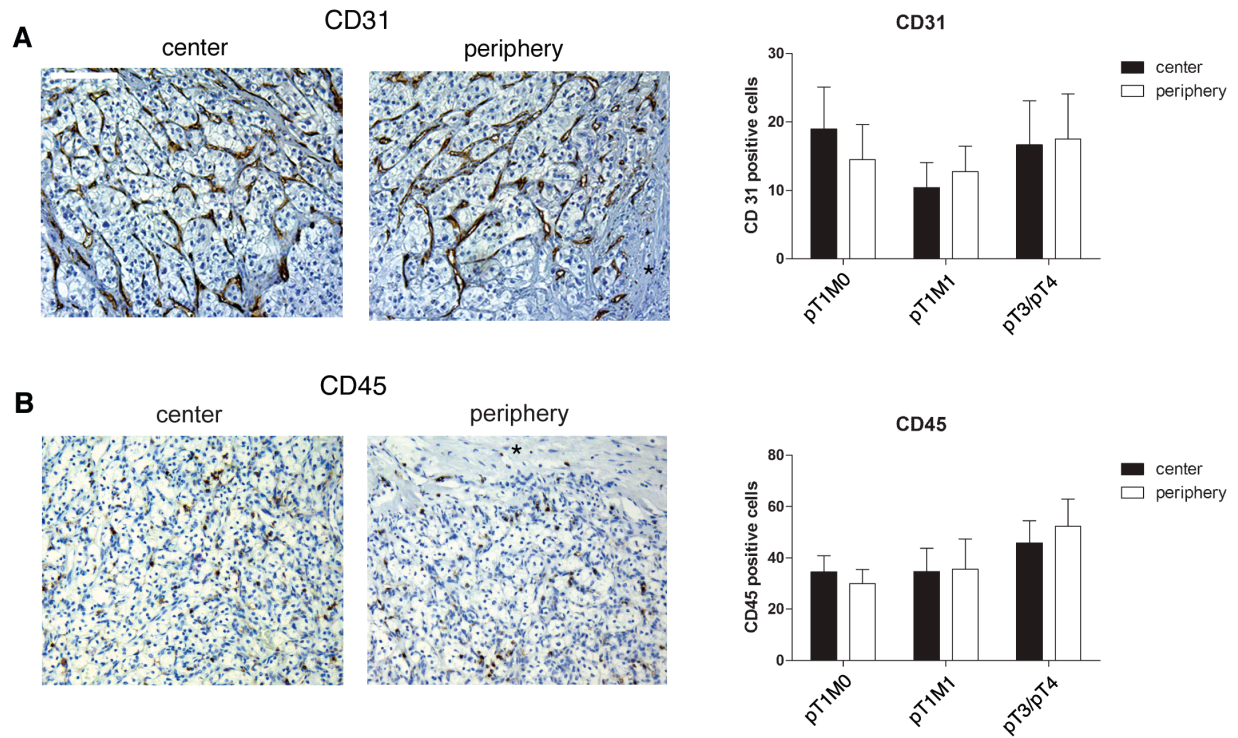


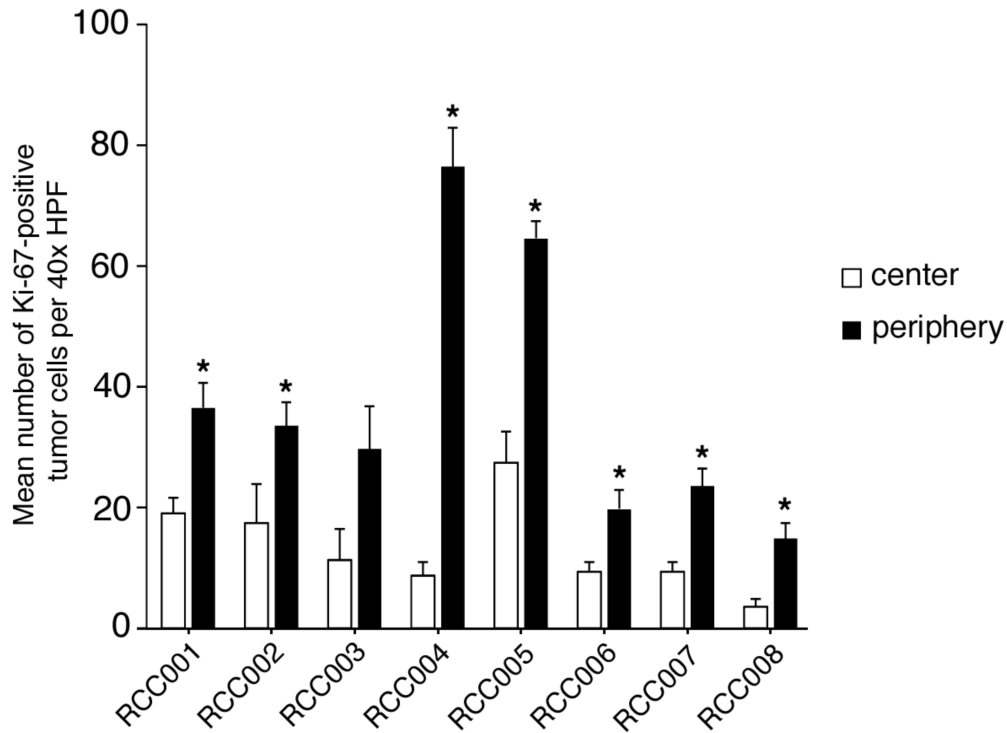
**Supplementary Figure 1. Overview of changes in signaling cascades inferred from correlative marker analysis.**

The correlation coefficient  $r$  demonstrates a positive (blue) or negative (green) correlation. Original data are shown in Supplementary Table 4.



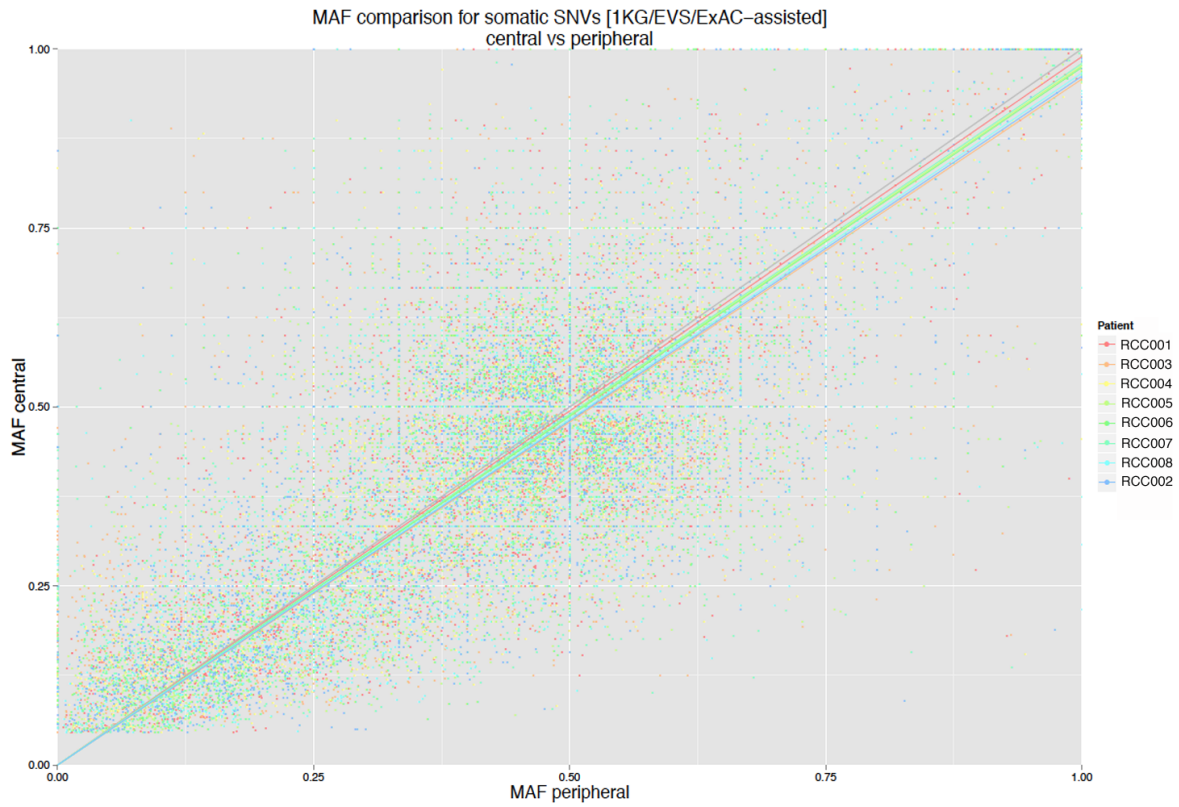
**Supplementary Figure 2. Microvessel density and leukocyte admixture are similar in the tumor center and periphery.**

(A,B) Representative immunohistochemical stainings for CD31 and CD45 to visualize blood vessels and leukocytes, respectively. Asterisks denote the adjacent stroma. Scale bar indicates 100  $\mu$ m. Bar graphs show mean + s.e.m. of positive cells per 40x high power field (HPF) for vital (>90%) tumor areas of the center (black) and periphery (white) for tumor subgroups as indicated. At least five HPFs were counted per region and per tumor.



**Supplementary Figure 3. Zonal pattern of functional heterogeneity in eight ccRCCs analyzed by regional whole exome sequencing.**

Bar graph shows mean + s.e.m. of Ki-67-positive cells per 40x high power field (HPF) for vital (>90%) tumor areas of the center (white) and periphery (black) for the tumors indicated. At least five HPFs were counted per area and per tumor. Asterisks indicate statistically significant differences ( $p < 0.05$ ; Mann-Whitney U test).



**Supplementary Figure 4. Mutant allele frequencies (MAFs) in the tumor center and periphery.**

Note the similar distribution of MAFs in the center and periphery indicating that results are not caused by an unequal admixture of non-tumorous cells.

**Supplementary Table 1. Patient characteristics (n=30, all ccRCCs)**

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Parameter	n=	%
Sex		
Male	20	66.7%
Female	10	33.3%
Age at diagnosis (yrs)		
<65	20	66.7%
≥65	10	33.3%
Tumor stage		
pT1	19	63.3%
pT3/4	11	36.7%
Lymph node metastasis		
N0/pN0	28	93.3%
N1/pN1	2	6.7%
Distant metastasis		
M0	12	40%
M1	18	60%
pTNM		
pT1 N0 M0	9	30%
pT1 N0 M1	10	33.3%
pT3/4 N0 M0	3	10%
pT3/4 N0 M1	6	20%
pT3/4 N1 M1	2	6.7%
Fuhrman Grade		
G1/2	22	73.3%
G3/4	8	26.7%

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## Supplementary Table 2. Overview of test results

D'Agostino-Pearson Omnibus Test	pT1M0	pT1M1	pT3/pT4	all*
<b>negative samples</b>	35	30	40	105
<b>significant (<math>p \leq 0.05</math>)</b>	35	30	40	105
<b>non-significant (<math>p &gt; 0.05</math>)</b>	0	0	0	0
<b>%</b>	78%	67%	73%	72%
<b>positive samples</b>	10	15	15	40
<b>significant (<math>p \leq 0.05</math>)</b>	0	0	0	0
<b>non-significant (<math>p &gt; 0.05</math>)</b>	10	15	15	40
<b>%</b>	22%	33%	27%	28%

\*one tumor omitted due to insufficient number of data points

**Supplementary Table 3. Correlation of tumor heterogeneity with Fuhrman grade**

	STDEV (median)			MAX- $\mu$ (median)		
	G1/2	G3/4	<i>p</i> -value*	G1/2	G3/4	<i>p</i> -value*
HIF-1 $\alpha$	10.8	6.6	0.38	24.7	15.3	0.27
HIF-2 $\alpha$	14.1	10.5	0.47	29.7	18.8	0.33
p-mTOR	2.2	0.3	0.08	8.8	3.5	0.21
p-S6RP	1.7	3.3	0.12	5.5	13.4	0.09
Ki-67	1.8	3.2	0.1	6.7	15.8	0.08

\*Mann Whitney U test

## Supplementary Table 4. Correlation between markers

<b>pT1M0</b>					
coefficient (r)*	p-mTOR	p-S6RP	HIF-1 $\alpha$	HIF-2 $\alpha$	Ki-67
p-mTOR		0.51	-0.51	-0.07	-0.11
p-S6RP	0.51		-0.25	-0.05	-0.38
HIF-1 $\alpha$	-0.51	-0.25		0.42	-0.07
HIF-2 $\alpha$	-0.07	-0.05	0.42		-0.27
Ki-67	-0.11	-0.38	-0.07	-0.27	
p-value	p-mTOR	p-S6RP	HIF-1 $\alpha$	HIF-2 $\alpha$	Ki-67
p-mTOR		<0.01	<0.01	0.10	0.01
p-S6RP	<0.01		<0.01	0.30	<0.01
HIF-1 $\alpha$	<0.01	<0.01		<0.01	0.11
HIF-2 $\alpha$	0.10	0.30	<0.01		<0.01
Ki-67	0.01	<0.01	0.11	<0.01	
<b>pT1M1</b>					
coefficient (r)*	p-mTOR	p-S6RP	HIF-1 $\alpha$	HIF-2 $\alpha$	Ki-67
p-mTOR		0.48	0.10	-0.10	0.24
p-S6RP	0.48		0.51	0.21	0.28
HIF-1 $\alpha$	0.10	0.51		0.43	0.19
HIF-2 $\alpha$	-0.10	0.21	0.43		-0.18
Ki-67	0.24	0.28	0.19	-0.18	
p-value	p-mTOR	p-S6 RP	HIF-1 $\alpha$	HIF-2 $\alpha$	Ki-67
p-mTOR		<0.01	<0.01	0.01	<0.01
p-S6RP	<0.01		<0.01	<0.01	<0.01
HIF-1 $\alpha$	<0.01	<0.01		<0.01	<0.01
HIF-2 $\alpha$	0.01	<0.01	<0.01		<0.01
Ki-67	<0.01	<0.01	<0.01	<0.01	
<b>pT3/T4</b>					
coefficient (r)*	p-mTOR	p-S6RP	HIF-1 $\alpha$	HIF-2 $\alpha$	Ki-67
p-mTOR		-0.09	0.35	0.16	-0.01
p-S6RP	-0.09		0.12	0.14	0.08
HIF-1 $\alpha$	0.35	0.12		0.24	0.11
HIF-2 $\alpha$	0.16	0.14	0.24		-0.46
Ki-67	-0.01	0.08	0.11	-0.46	
p-value	p-mTOR	p-S6RP	HIF-1 $\alpha$	HIF-2 $\alpha$	Ki-67
p-mTOR		0.03	<0.01	<0.01	0.84
p-S6RP	0.03		<0.01	<0.01	0.04
HIF-1 $\alpha$	<0.01	<0.01		<0.01	0.01
HIF-2 $\alpha$	<0.01	<0.01	<0.01		<0.01
Ki-67	0.84	0.04	0.01	<0.01	
<b>all</b>					
coefficient (r)*	p-mTOR	p-S6RP	HIF-1 $\alpha$	HIF-2 $\alpha$	Ki-67
p-mTOR		0.25	-0.10	-0.02	-0.01
p-S6RP	0.25		0.18	0.13	0.05
HIF-1 $\alpha$	-0.10	0.18		0.38	0.07
HIF-2 $\alpha$	-0.02	0.13	0.38		-0.29
Ki-67	-0.01	0.05	0.07	-0.29	
p-value	p-mTOR	p-S6RP	HIF-1 $\alpha$	HIF-2 $\alpha$	Ki-67
p-mTOR		<0.01	<0.01	0.52	0.76
p-S6RP	<0.01		<0.01	<0.01	0.05
HIF-1 $\alpha$	<0.01	<0.01		<0.01	<0.01
HIF-2 $\alpha$	0.52	<0.01	<0.01		<0.01
Ki-67	0.76	0.05	<0.01	<0.01	

\*Spearman correlation for non-parametric variables.

Blue indicates a positive correlation, green indicates a negative correlation.



**Supplementary Table 5. Patient characteristics and distribution of functional SNVs and Indels**

Patient	Sex/Age	pT Stage	Grade*	Center-specific functional SNVs and Indels (n)	Periphery-specific functional SNVs (n)
RCC001	M/75	pT1a	2	4	10
RCC002	M/63	pT1a	2	3	0
RCC003	M/75	pT1a	2	24	1
RCC004	M/44	pT2a	3	7	3
RCC005	M/82	pT4	3	3	0
RCC006	F/35	pT1b	2	6	0
RCC007	F/63	pT2a	2	15	4
RCC008	F/69	pT1a	1	6	2
total				68	20

\*All RCCs with clear cell histology.