**Supplemental Methods**

*Tissue descriptions:*

*1. Multistage TMA (MDACC, TX).* Tumor microarrays (TMAs) containing tumor core samples from 394 patients with all clinical stages of ccRCC with matched clinical data were prepared for the initial study. Data from 14 patients were removed due to non-clear cell histology (2), sarcomatoid component (4) in clear cell, or prior intervention for ipsilateral RCC with embolization (6) or any cryoablation (2) resulting in a total 380 patients available for this study. The TMA comprised 4 paraffin blocks containing tumor sections arranged according to tumor stage (RCC1 through 4 contained cores from ccRCCs of clinical T stages 1 through 4 respectively).

*2.* Tissue sections *Stage 1 (MDACC, TX).* Large tissue sections contain tumor and uninvolved normal kidney from 12 patients with T Stage 1 disease (6 patients with Grade 2 tumors and 6 with grade 3 tumors). Regions of normal (when clearly identifiable) and tumor were designated using Aperio.

3. *Low stage TMA (Mayo Clinic, AZ).* TMAs containing tumor core samples with paired normal from 25 patients with ccRCC pathological grades 2-3 (19 samples with clinical T stage 1 (T1), 1 with T2, and 5 with T3).

*4. High-grade/Low-grade sections (Mayo Clinic, AZ).* This dataset comprised large tissue sections from 24 patients with low or high grade CCRCC (11 patients with T stage 1 tumors and 13 patients with T stage 3 tumors). Where available, regions of uninvolved adjacent normal tissue and tumor were quantitated as performed for the large sections from ccRCC T1 sections.

*5.* *High-grade/low-grade sections* (*HCI, UT*). Large tissue sections containing tumor from pathological grades 2 (10 patients), 3 (10 patients) and 4 (3 patients). Uninvolved tissue was obtained from non-adjacent blocks for 15 patients (9 uninvolved associated with Grade 2 tumors and 6 associated with grade 3 tumors).

*6.* *Baseline tissue from patients with advanced or metastatic RCC treated with anti-angiogenic therapy (HCI, UT)*. TMAs were constructed from formalin fixed paraffin embedded tumor tissue of 57 cases of known advanced metastatic RCC. Clinical parameters of patients are shown in in **Figure S12A**. Progression free survival was defined as time of therapy initiation until radiographic or clinical disease progression or death. Overall survival was defined as time from initiation of therapy until death. All specimens were reviewed by pathologists and representative tumor areas identified. Three spatially separated 2 mm cores from archival FFPE tissue were included for each case.

For tissue set 1, slides were scanned with the Vectra image scanning system, and HAF, HIF-1α or HIF-2α nuclear positivity was determined using the inform software (Caliper Life Sciences, Waltham, MA). For tissue sets 2-4, slides were scanned using the Aperio AT2 digital scanning system and nuclear staining intensity quantitation was performed using Aperio digital imaging software (Leica Biosystems, Buffalo Grove IL). Pathology analysis (NK, DS, AA, LE) was performed for tissue set 5. For tissue set 6, slides were scanned at 20X using a Leica Aperio AT2 scanner. Images were analyzed using Indica Labs HALO v2.3 TissueMicroArray module and CytoNuclear v1.6 algorithm customized for each stain type.

*Immunohistochemistry.* Antibodies used were HAF monoclonal antibody, HIF-2α (NB100-122, Novus Biologicals, Centennial, CO) and HIF-1α (EP1215Y Abcam, Cambridge MA) were commercially available. Alternative HIF-2α antibodies, MAB3472 (EMD Millipore, Burlington, MA) and ab109616 (Abcam) were used in tissue sets 4 and 5 respectively (**S7A, C**). Staining conditions for each antibody was optimized using staining controls of formalin-fixed paraffin embedded normoxic and hypoxic ACHN cell pellets. Tissues were stained using the Leica Bond III (TMAs) or Ventana Benchmark Ultra automated slide stainers.

The immunohistochemical single sequential double-staining was performed on 4-micron thick sections of formalin-fixed, paraffin-embedded tissues. Sections were air-dried and then melted in a 60°C oven for 30 minutes. Slides were loaded onto the Ventana BenchMark® Ultra automated staining instrument (Ventana Medical Systems, Tucson, AZ). The sections were de-paraffinized with the EZ Prep solution. Antigen retrieval was performed with CC1 (Cell Conditioning 1, pH 8.5), and the primary antibody was applied (see Table for specific vendor-clones/pretreatment times/antibody dilutions (1-2)).  The sections were detected using the UltraView Universal DAB Detection Kit, which is a cocktail of horseradish peroxidase (HRP) labeled secondary antibodies (goat anti-mouse IgG, goat anti-mouse IgM, and goat anti-rabbit) utilizing a hydrogen peroxide substrate and 3, 3’-diaminobenzidine tetra hydrochloride (DAB) as the chromogen, which produces a brown precipitate. A denaturation step was performed for 4 minutes at 90°C to remove any unbound reagent. The primary antibody was applied for sequential staining (see Table for specific vendor-clones/pretreatment times/antibody dilutions (3-8)).  The UltraView Universal Alkaline Phosphatase Red Detection Kit was then applied and utilizes a cocktail of enzyme labeled secondary antibodies (goat anti-mouse IgG, goat anti-mouse IgM, and goat anti-rabbit) that is visualized with a Naphthol and Fast Red chromogen, which produces a red precipitate. A blocker was applied for 20 minutes (Levamisole, L9756, Sigma). The sections were counterstained with hematoxylin for 12 minutes. The sections were removed from the immunostainer and placed in dH2O/DAWN™ mixture. The sections were gently washed in the dH2O/DAWN™ mixture to remove any coverslip oil applied by the automated instrument. The sections were dehydrated in graded alcohols (70%, 95% x2, & 100% x2), cleared in xylene then coverslipped.

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| VENDOR/ANTIBODY/CLONE | PRETREATMENT | DILUTION | ANTIBODY INCUBATION | DETECTION KIT |
| 1. Abcam: HIF1a/Rb EP1215Y | CC1 64 minutes at 95°C | 1:500 | 1 hour at 37°C, Amplification kit | UltraView Universal DAB |
| 2.Abcam: HIF2a/Rb ab109616 | CC1 64 minutes at 95°C | 1:500 | 1 hour at 37°C, Amplification kit | UltraView Universal DAB |
| 3. Dako/Agilent: CD31/Mo JC70A | Denature 4 minutes at 90°C | 1:50 | 1 hour at 37°C, levamisole 20 minutes | UltraView Universal Alkaline Phosphatase Red |
| 4. Leica: CD163/Mo 10D6 | Denature 4 minutes at 90°C | 1:100 | 1 hour at 37°C, levamisole 20 minutes | UltraView Universal Alkaline Phosphatase Red |
| 5. Dako/Agilent: C1q/Rb polyclonal A0136 | Denature 4 minutes at 90°C | 1:3000 | 24 minutes at 35°C, levamisole 20 minutes | UltraView Universal Alkaline Phosphatase Red |
| 6. Dako/Agilent: CD68/Mo K19 | Denature 4 minutes at 90°C | 1:1500 | 1 hour at 37°C, levamisole 20 minutes | UltraView Universal Alkaline Phosphatase Red |
| 7. Ventana/Roche: CD3/Rb 2GV6 | Denature 4 minutes at 90°C | Ready to use | 1 hour at 37°C, levamisole 20 minutes | UltraView Universal Alkaline Phosphatase Red |
| 8. Leica: CD14/Mo 7 | Denature 4 minutes at 90°C | 1:50 | 1 hour at 37°C, levamisole 20 minutes | UltraView Universal Alkaline Phosphatase Red |

*Statistical analysis of tissue stains*. For the MDACC multistage TMA, clinical measures were tabulated overall and by TMA slide. Each marker was reported descriptively and compared within clinical subgroups using ANOVA or Kruskal-Wallis test if ANOVA assumptions were violated. Kaplan-Meier curves were created in STATA 14.2 (StataCorp LLC, College Station, TX) and Recursive partitioning analysis (RPA) was performed using its classification and regression tree (CART) package for survival outcomes with univariable and multivariable models for each marker. If simple and multiple regression models produced the same cut-off, then that cut-off was reported as clinically meaningful. Univariable and multivariable Cox regression models for markers along with patient and tumor measures were performed including age, gender, race/ethnicity, ECOG status, T, N, and M stage, tumor size, grade, necrosis, and LVI. Multiple validated scores exist such as the Mayo SSIGN or Sorbellini, et al’s nomogram, so we included tumor measures important to these (1-4). Models and a likelihood ratio test were calculated in SAS 9.4 (SAS Institute, Inc., Cary, NC).

For tissue sets 2-5, significance was determined using paired or unpaired Student’s T-tests (**Fig. 2A** and **2B** respectively) or Mann Whitney U-tests (**Fig. 2B-C**) with p < 0.05 considered significant.

For tissue set 6, differences in HIF-1α or HIF-2α levels between the groups that showed benefit versus no clinical benefit were analyzed on the log scale with an unpaired t-test. The log transformed HIF-1α and HIF-2α values from both groups passed Shapiro-Wilk normality tests confirming normal distribution after log transformation.

Data for tissue sets 2-6 were plotted using GraphPad Prism software 7.03 (GraphPad Software, San Diego CA).

*Single cell sequencing and analysis.*

*Tumor dissociation and sequencing*: Tumors were dissociated using the Human Tumor Dissociation Kit (Macs Milteny Biotech, Cambridge, MA) according to the manufacturer’s guidelines. Red blood cells were removed from cell suspensions, by adding equal volume of human red blood cell lysis buffer (Alfa Aesar, Haverhill, MA), and incubating at room temperature for 10 minutes. Cell numbers and viability was determined using trypan blue exclusion and a Countess automated counter (Thermo Fisher Scientific, Waltham, MA). After counting, cell suspensions were frozen in 90% DMEM, 20% fetal bovine serum, 10% DMSO. For sequencing, samples were defrosted at 37oC, followed by several washed in 0.04% BSA in PBS. Three ccRCC and uninvolved kidney samples showing >40% cell viability were selected for sequencing. Assessment of gene expression in single cells was achieved using the 10X Genomics Chromium single-cell gene expression solution (10x Genomics, Pleasanton, CA). Library prep was performed according to the 10X Genomics Single Cell 3’ Gene expression Library prep protocol V3. Barcoded libraries were analyzed using Agilent D1000 ScreenTape on an Agilent Technology 2200 TapeStation system (Agilent Technologies, Santa Clara, CA), then quantified by PCR using KAPA Biosystems Library Quantification Kit for Illumina Platforms (KAPA Biosystems, Roche, Branchburg NJ). Libraries were normalized and sequences on a NovaSeq 6000 (Illumina, San Diego, CA) with 2x150bp paired-end reads.

*Data processing and quality control:* Sequencing data was processed using the 10X Genomics Cell Ranger pipeline, which uses the Spliced Transcripts Alignment to a Reference (STAR) aligner for genome and transcriptome alignment. The Cell Ranger pipeline produces matrices of counts and cell barcodes. Further processing was conducted using the Seurat (3.0.0) package for R (3.5.3), where genes detected in less than 3 cells were removed, and only cells with a minimum of 200 detected genes were taken for downstream analysis (5, 6). Additionally, cells with >10% mitochondrial gene expression were discarded from the dataset, as this signifies stressed or dying cells. For cell numbers and gene counts after processing and quality control procedures see **Supplemental Figure 14A**.

*Dimension reduction and clustering:* All downstream analysis was conducted using the Seurat package (3.0.0), detailed explanations of the functions and methods can be found at https://satijalab.org/seurat/. Initially datasets were normalized using the Log Normalize function, whereby counts were divided by the total counts of that cell, and multiplied by a scale factor of 10000, followed by natural log transformation. After normalization, the data from the three sequenced ccRCC samples and uninvolved samples were integrated and scaled. Batch correction was performed as part of the IntegrateData function in the Seurat package as described (6). To reduce dimensionality, Principal Component Analysis was performed using 15 components, determined as suitable using an elbow plot (**Supplemental Figure** **13**). To cluster the data, a graph-based clustering approach was employed using a resolution of 0.5. T-SNE was used for non-linear dimensional reduction and for data visualization.

*Differential Expression Analysis:* To identify differentially expressed genes between clusters, differential gene expression analysis was performed using Seurat, employing the ‘Wilcox’ test which identified differences in gene expression using a Wilcoxon Rank Sum Test. Results from differential gene expression analysis were used to aid in determining cell type based on previously described signatures shown in **Supplemental Figure** **14B** (7-13).

*Gene Set Enrichment Analysis*: Upregulated genes were ranked by fold change, and filtered using 10% FDR. GSEA was performed on the ranked gene list using FGSEA (1.2.0) package in R (14). The hallmarks gene set from MSigDB was used as the gene set input (15).

*Immunoblotting.* Western blotting was performed as described (16). Membranes were probed with primary antibodies for HIF-1α (BD Biosciences, 610959), HIF-2α (Cell Signalling Technologies, 70862), CD206 (Cell Signaling #91992), HLA-DR (Abcam #ab92511) and HAF described in (17). Images were acquired using Fluorochem M imaging system (Protein Simple, San Jose, CA). Blot quantification was performed using ImageJ (18).

*TaqMan Quantitative RT-PCR.* Polarized THP-1 cells were incubated in 1% O2 or 21% O2 for 4 hours followed by cell lysis and RNA extraction using RNeasy RNA extraction kit (Qiagen Inc, Germantown, MD). 1 µg of total RNA was reverse transcribed with the High capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA). For RT-PCR, cDNA was combined with TaqMan Gene Expression Mastermix (Applied Biosystems), and TaqMan primers for *B2M* (ThermoFisher, 4331182) or *HIF1A* (ThermoFisher, 4331182). RT-PCR experiments were run on a QuantStudio 6 Flex System (Applied Biosystems). mRNA levels were calculated relative to the housekeeping gene, B2M.

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