**Supplementary Methods**

***Opal Multiplexed IHC staining and multispectral imaging***

The slides were baked at 60ºC for one hour followed by deparaffinization by Xylene for 10 minutes in triplicate. The TMA was then rehydrated with 100%, 90%, and 70% ethanol for 10 minutes each. Slides were then washed in deionized water for 2 minutes followed neutral buffered formalin for 30 minutes. The Opal 7 manual kit (PerkinElmer) was used according to manufacturer instructions. Multiple antigen retrieval steps using either AR6 (Perkin Elmer) or AR9 (Perkin Elmer) antigen retrieval buffer was used in between each staining set. See supplementary table for antibodies and stains used (Table S1). Images of the TMA cores were taken using the MantraTM Quantitative Pathology Work Station (Perkin Elmer). One image per core was captured at 20x magnification. All cube filters were used for each image capture (DAPI, CY3, CY5, CY7, Texas Red, Qdot) and the saturation protection feature was utilized. After all images were acquired, images were analyzed using inForm® Cell AnalysisTM software (Perkin Elmer). All images were batch analyzed and basic phenotypes were created using the inForm training software. Basic phenotypes were composed of the following: APC (CD163+), epithelial cells (Pan-cytokeratin+), T cells (CD3+) The scoring feature was used to determine the appropriate range of the mean signal intensity of each stain within the cytoplasm for CD8 and nucleus for FoxP3. These scoring ranges were used to make secondary phenotypes in R: CD8 positive T cell and Tregs. See supplementary table S2. Final phenotypes including primary phenotypes (APCs and epithelial cells) as well as the secondary phenotypes made in R (Tregs and CD8 positive T cells) were quantitatively analyzed using a program designed in R. The percentage of cell phenotypes was calculated as % of total cells.

***Cytometry by Time-of-Flight (CyTOF)***

Up to 1×107 cells was stained with Cell-ID Cisplatin (1.67µM) for 5 minutes at room temperature, and then a Combining Fix and Perm Sensitive Surface Epitopes and Nuclear Antigen Staining protocol was followed according to manufacturer’s instructions (Fluidigm). Briefly, after quenching Cisplatin reaction with 5X volume of MaxPar® Cell Staining Buffer, cells were centrifuged at 300 × g for 5 minutes. Then up to 3 million cells per sample were stained with cell surface antibody cocktail (see Supplementary Materials Table S1 for antibody panel and dilution) in 100µl volume of MaxPar® Cell Staining Buffer for 30 minutes at room temperature. After being washed twice in 1ml MaxPar® Cell Staining Buffer cells were fixed in 1.6% freshly made formaldehyde solution for 10 minutes at room temperature. After being washed once in 1ml MaxPar® Cell Staining Buffer cells were permeabilized in 1ml Nuclear Antigen Staining Buffer working solution for 20 minutes at room temperature. Then, cells were washed twice by centrifuge at 800 × g for 5 minutes with 1ml Nuclear Antigen Staining Perm and cells were stained with intracellular antibody cocktail (see Supplementary Materials Table S1 for antibody panel and dilution) in 50µl volume of Nuclear Antigen Staining Perm for 45 minutes at room temperature. After being washed once with 2 ml Nuclear Antigen Staining Perm and once with 2 ml MaxPar® Cell Staining Buffer, cells were re-suspended in 2 ml cell intercalation solution (125 nM Cell-ID Intercalator-Ir in Maxpar Fix and Perm Buffer) and shipped to the Flow Cytometry core at the University of Rochester Medical Center where the samples preparation were finalized and CyTOF2 Mass Cytometer analysis were performed. Human samples were similarly stained with Maxpar Cell Surface Staining with fixation protocol and shipped to Indiana University Simon Cancer Center Flow Cytometry for CyTOF2 Mass Cytometer analysis.

***CyTOF data analysis***

Raw FCS files were analyzed using the Premium CytoBank Software (cytobank.org). Data were checked for quality of staining and normalized by the use of internal bead standards. Live singlet cells were identified using the combination of Ir191 DNA Intercalator and Pt195 Cisplatin stain intensity. Filtered live single cells were saved as new FCS files for downstream analysis. Unbiased identification of cellular subpopulations was performed in parallel using two approaches – visualization through either FlowSOM-viSNE (29) or viSNE within CytoBank (28) and through the SPADE (ver 3.0, standalone package, Peng Qiu, Georgia Institute of Technology) (67). The relevant parameters for the SPADE analysis included ArcSinh transformation with a factor of 5, use of 100 nodes for the minimum spanning tree graph, downsampling to 20,000 events for the initial graph generation, and K-means clustering as the node generation. After the definition of the 100 nodes, the transformed median marker intensities for each node were exported from SPADE into the R statistical environment (ver 3.3.3). Unsupervised clustering through the heatmap2 package was used to generate an initial dendrogram of the 100 nodes, which was then reviewed and annotated based on known marker combinations to define cellular subpopulations. Nodes with similar marker expression profiles were condensed together into the final subpopulation list, which was then reanalyzed within SPADE to generate median marker expressions in each subpopulation. These expression profiles were reexported and a repeat unsupervised clustering was performed to generate the final heatmap and annotations in Fig. 5. SPADE also automatically calculates the actual cell numbers within each defined subpopulation/group of nodes within each analyzed sample. This data was also separately exported into R and forms the basis for Fig. S3D.