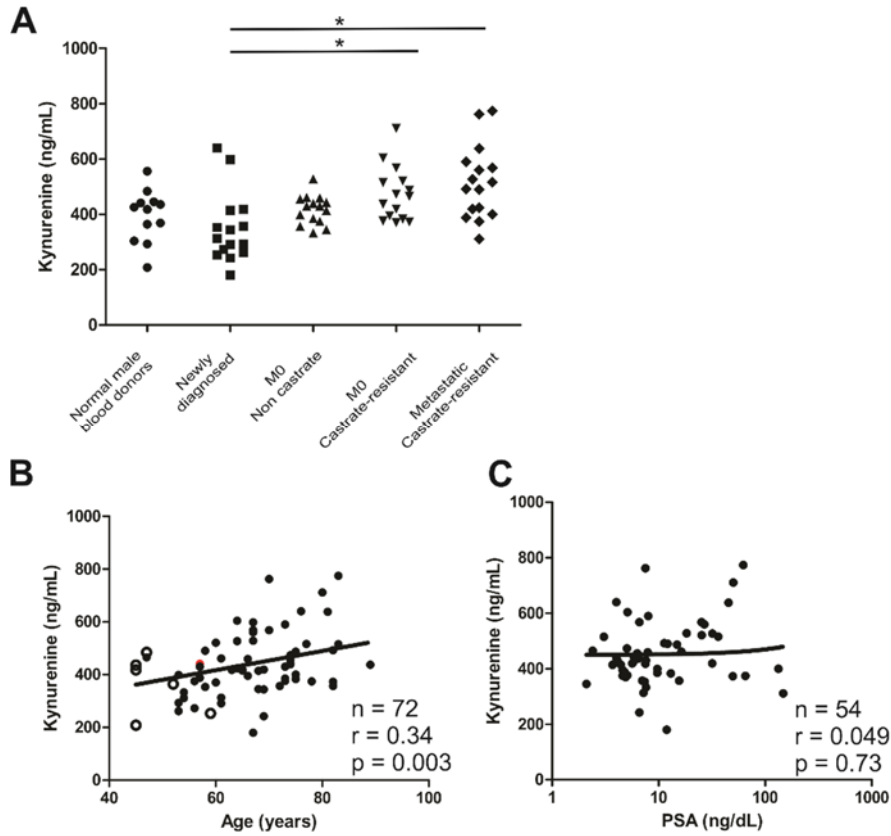
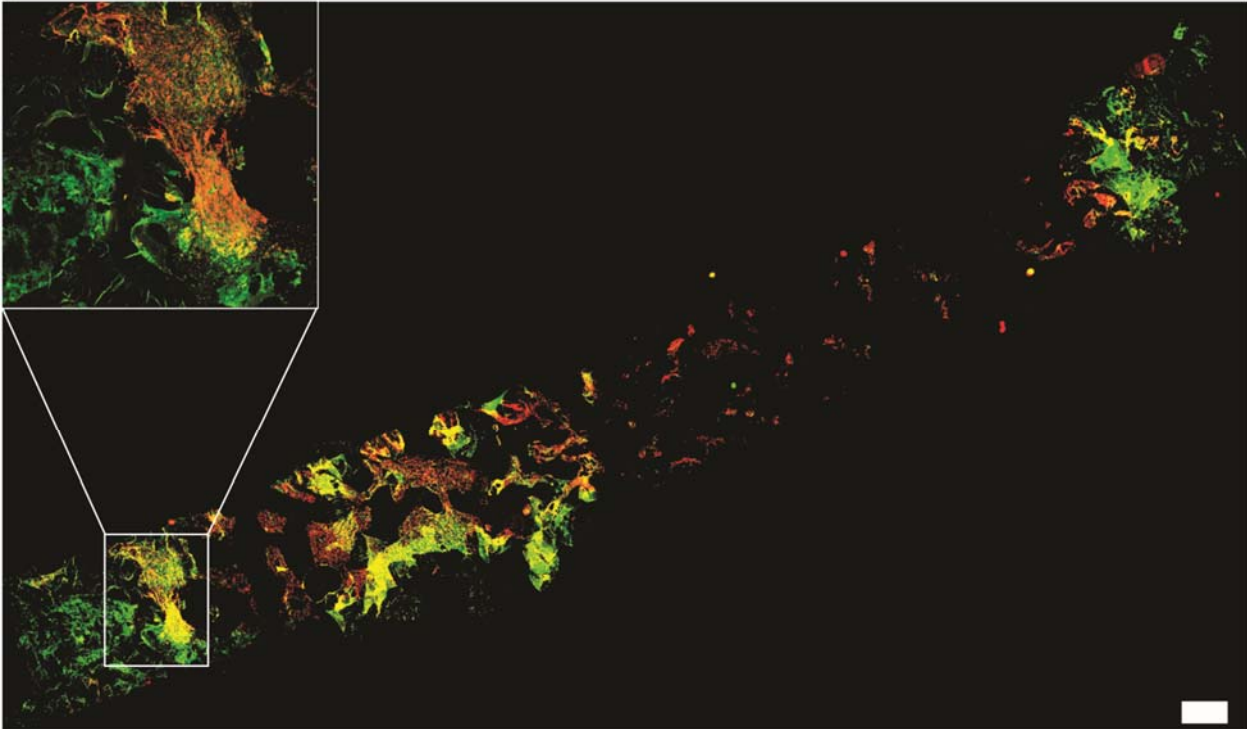
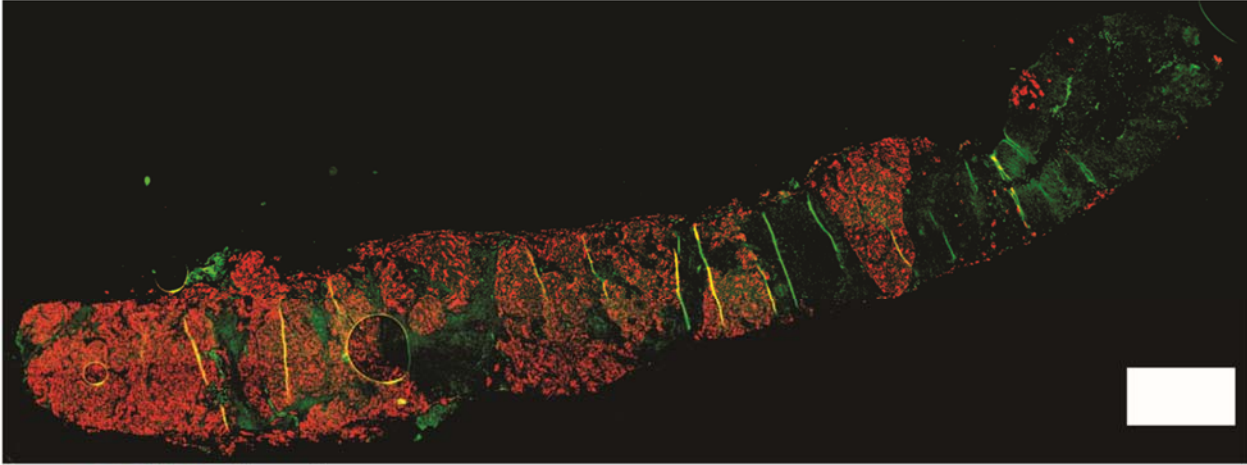
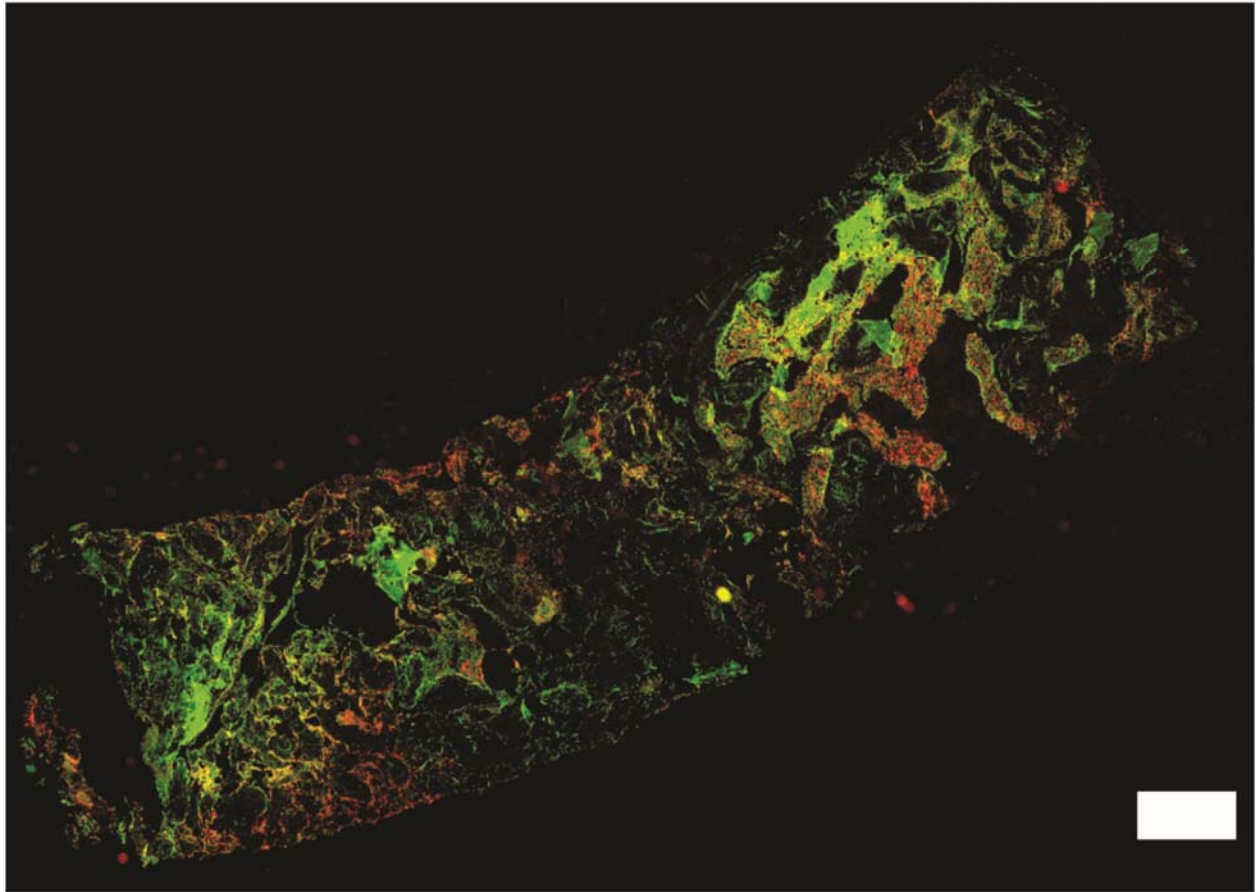
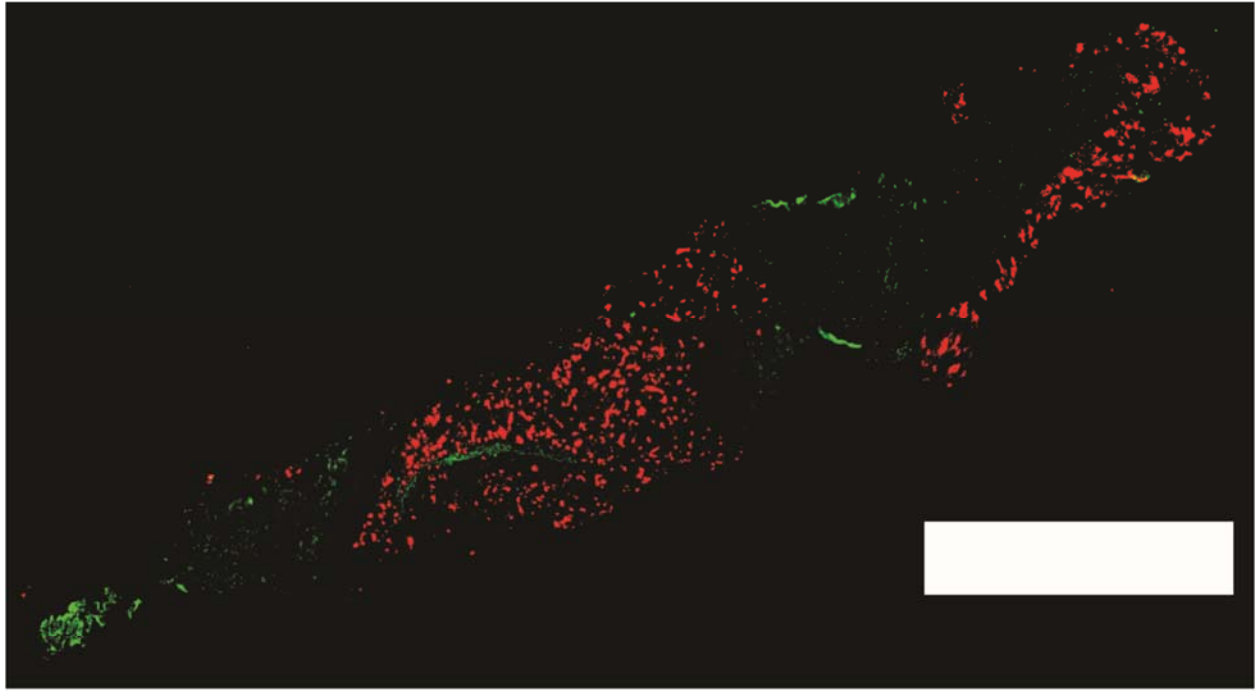


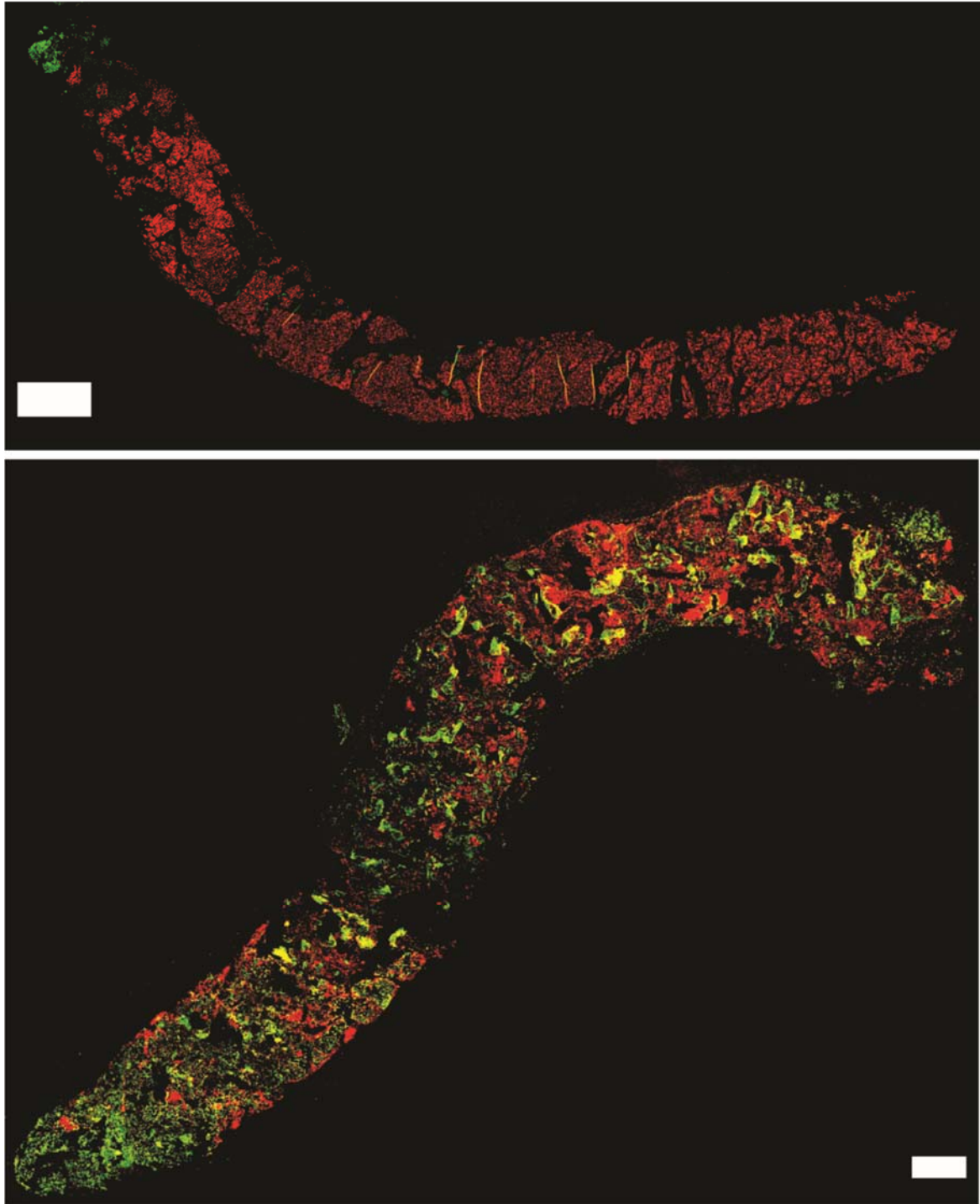
Supplemental Figures and Legends:



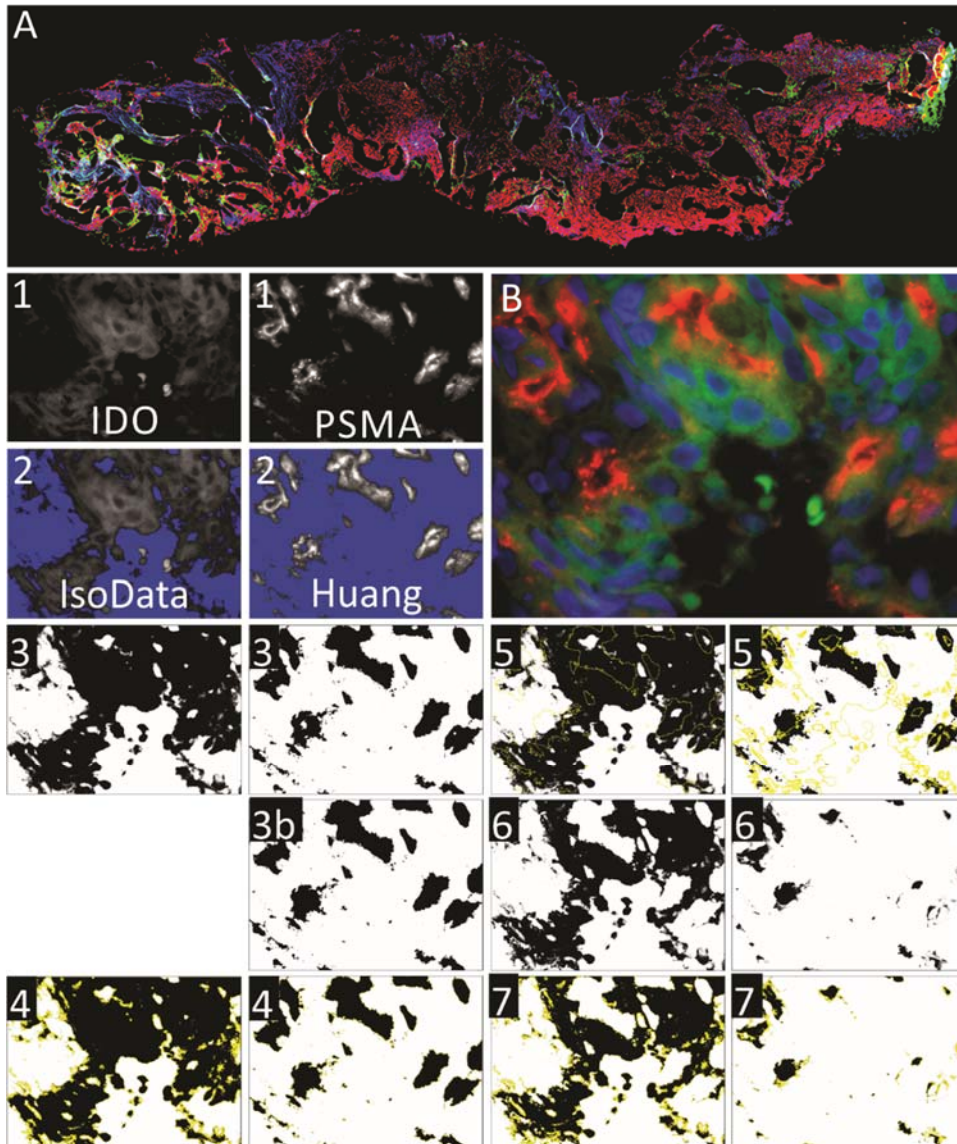
Supplemental figure S1: Kynurenine concentration are higher in sera of patients with advanced prostate cancer. Sera or plasma samples were evaluated for kynurenine concentrations from normal male volunteer blood donors (n=12), patients with newly diagnosed prostate cancer pre-treatment (n=14), patients with non-castrate, PSA-recurrent non-metastatic (M0) prostate cancer (n=15), castration-resistant, M0 prostate cancer (n=15), and castration-resistant, metastatic prostate cancer (n=16). Shown are the concentrations of kynurenine for each group (panel A), and overall with respect to subject age (panel B) or serum PSA for individuals with prostate cancer (panel C). Open circles in panel B are normal male blood donors. For panel A, * = $p < 0.05$ (Kruskal-Wallis test with Dunn's correction for multiple comparisons). Tests of correlation with age and PSA (panels B and C) were made by Spearman test.



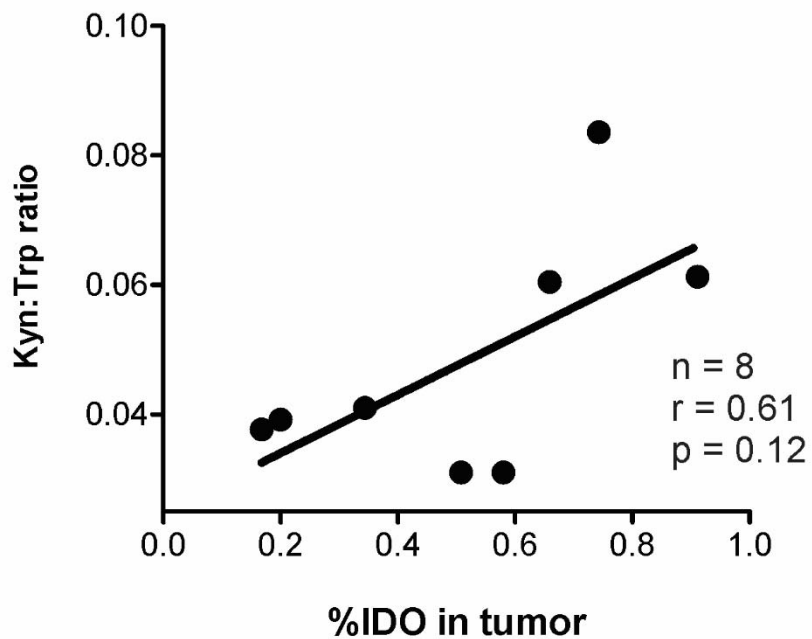




Supplemental figure S2: IDO, PSMA, and DAPI staining of metastatic prostate cancer tissues pre- and post-treatment. Shown are examples of IF staining from three additional patients separate from that shown in Figure 3. Top panels in each are from biopsies obtained pre-treatment, and bottom panels are from biopsies from the same metastatic site biopsied after immunotherapy (vaccine +/- anti-PD1) treatment. For each panel stains are DAPI (blue), PSMA (red), and IDO (green). Bars in the corners indicate size = 1000 μm .



Supplemental figure S3: Methods for quantification of IDO staining within tumor regions. Whole FFPE tumor biopsies were stained with IDO, PSMA, and DAPI as described in the Methods section. Whole section mosaic images were obtained on the Leica DMi8 at 10x (Panels A-B). Using the image in panel B, steps 1-7 depict the image processing steps taken to quantify the area of IDO expression as a percent of area of PSMA. First, a threshold was determined for the original greyscale images using the ImageJ built-in IsoData algorithm for IDO and Huang algorithm for PSMA (Steps 1-2). The threshold was applied, and the image converted into a binary mask. Because PSMA is a membrane stain, for that protein the conversion was followed by the “fills holes” ImageJ function (Steps 3-3b). Once the binary images were created a selection was made using the ImageJ built-in function (edit/selection/create selection) and the area quantified using the measure function (process/measure) to give total tumor (A_T) and IDO-expressing (A_I) areas (Step 4). To calculate the percentage of A_T that overlapped with A_I , the selection of IDO was applied to the mask of PSMA, filled white, and the remaining tissue area measured as described above. The process was repeated for PSMA over IDO (Steps 5-7).



Supplemental figure S4: Association of tumor expression of IDO and serum kyn:trp ratio. Quantitative imaging performed as in Figure 3 was used to determine the % IDO staining within tumor regions. These values are shown in relation to serum kyn:trp ratios obtained from the same individuals at the same time points (n=8). Test of correlation was made by Spearman test.