Supplementary figure 1: A) Inclusion details of patients in the study. Flow chart showing details of patients recruited for and included in this study **B) Patient characteristics for cohort evaluated for peripheral response to immunotherapy** (n=36).

Supplementary figure 2: Flow cytometry characterization of T-cells after immunotherapy: A & B) CD4 and CD8 T-cells in blood after PD1 blockade. FACS plots shows cells gated on CD3+, live, singlets. The percent of total PBMCs that were CD4+ or CD8+ was determined from this gate at baseline and all timepoints during treatment. Shown in B is the fold change over baseline for matched patient samples throughout the treatment course. **C) Canonical CD8 memory subsets over treatment course.** Cells were gated as in panel A on CD8 and analyzed for expression of CD45RA and CCR7. Subsets were mostly stable over the course of treatment with no significant changes at any time points. **D & E) CD8 expression of Gzmb & Ki67.** CD8 cells as in panel A were gated and expression of GzmB and Ki67 analyzed. Adjacent plot shows summary data from patients over their treatment time course. **F) Gating Strategy to identify HLA-DR+CD38+ CD8 T cells. G & H) Expression of HLA-DR and CD38 by CD4 and CD8 T cells following checkpoint blockade.** Plots in F show HLA-DR and CD38 expression by CD4 and CD8 cells in a representative single patient over a treatment time course. The fold change in these cells from baseline is in all patients is shown in H. **I) Correlation of CD4 and CD8 response.** Plot shows the fold change in the HLA-DR+CD38+ cells for CD4 T cells on the x-axis and CD8 T cells on the y-axis. **J & K) HLA-DR+CD38+ T cells express Ki67.** Flow plots show CD8 and CD4 T cells gated as in panel A and their expression of HLA-DR and CD38. Overlayed in blue are CD8 and CD4 T cells that were positive for Ki67 (See E) to show their relative position in the HLA-DR x CD38 plots. Summary data shows the correlation in expansion of the HLA-DR+CD38+ cells and increased expression of Ki67 after checkpoint blockade.

Supplementary figure 3: DR+CD38+ CD8 T cells resemble effector CD8 T cell populations: A) Identification of genes expressed in HLA-DR+CD38+ CD8 T cells compared to naïve CD8 T-cells: RNA collected from naïve cells in Akondy et al[41] were compared to HLA-DR+CD38+ sorted from patient blood prior to receiving treatment. Genes highlighted are canonical genes upregulated (red) and downregulated (blue) in effector CD8 T-cells. **B) HLA-DR+CD38+ CD8 T cells enrich with effector CD8 T cell signatures:** Gene set enrichment analysis (GSEA) was performed comparing naïve cells to HLA-DR+CD38+ CD8 T cells isolated before receiving immunotherapy. Cells were compared to gene signatures of genes upregulated in CD8 T cells at the peak of the response to yellow fever vaccination, or at the peak of LCMV infection (in mice). **C) Pathways enriched in HLA-DR+CD38+ CD8 T cells:** GSEA was performed comparing naïve CD8 T cells to HLA-DR+CD38+ T cells for REACTOME pathways. Figure shows the top 10 most enriched pathways in the HLA-DR+CD38+ T cells. **D) Canonical CD8 T cell activation genes are unchanged compared to naïve cells over the time course of treatment:** Expression of key markers across the treatment time-course associated with effector function were compared to previously collected naïve CD8 T-cells[41].

Supplementary figure 4: Extended RNAseq analysis of CD8+ HLA-DR+ CD38+ T cells. **A) Genes differently expressed between patients with and without an objective response.** Differentially expressed genes between patients with a RECIST defined objective response (complete or partial response) were compared to patients with either stable or progressive disease. Volcano plots show the fold change between responders and other patients vs.

significance value. Data points highlighted in orange have an adjusted p-value less than 0.05. **B) Type I Interferon genes significantly upregulated in responding patients.** Heatmap shows z-scored expression values for each of the genes noted. Genes selected are all significantly higher in the HLA-DR+CD38+ CD8 T cells from patients with an objective response. **C) Correlation of genes with burst size.** Plot shows genes in order of correlation with the size of the HLA-DR+CD38+ T cell response to checkpoint blockade. Top genes were analyzed for what pathways they belong to. Top pathways included RNA Processing, Histone Modification, and Interferon Signaling. Example genes are highlighted. **D) Example genes that correlate with the size of HLA-DR+CD38+ CD8 T cell burst.** Plots show size of burst vs change in expression for genes listed. **E) Different treatment regimens have minimal effect on phenotype of HLA-DR+CD38+ cells:** Plots show comparison of pathway enrichment between treatment groups. Those highlighted in color are significantly different between treatment conditions. **F) Pathways significantly enriched in Nivolumab vs. Nivolumab + Ipilimumab treatment regimens:** Normalized enrichment scores for pathways found significantly different between treatments in E) are shown across all treatment.

Supplementary figure 5: Illustrations of TCR dynamics during treatment and response to therapy: A) Top 50% clonotype distribution. Number of TCR clonotypes that account for 50% of the repertoire for each patient at baseline and after checkpoint therapy. Mean +/- standard deviation are shown for each timepoint. **B) Treatment regimen has no significant effect on top clonotype frequency across treatment time course:** Plots show frequency of the top clonotype and are colored by treatment regime patients received. By ANOVA, there is no significant difference between any treatments at any of the timepoints. **C) Shannon's Entropy index of TCR repertoire** is shown for HLADR+CD38+ CD8 T cells from each patient before and after immunotherapy. **D) Representative TCR overlap between blood HLADR+CD38+ CD8 T cells and tumor infiltrating CD8 TILs**. The proportion of detected TCR repertoire in each cycle that is unique (gray) or shared (orange) with the tumor is shown. **E**) **Overall TCR diversity is unchanged over treatment time course in patients with and without clinical benefit**: Plots show the Simpson's D index for TCRs collected at each timepoint separated by if patients received clinical benefit (CB) or not (No CB). No significant difference in diversity is noted across timepoints in either the CB or No CB groups, or between the entire CB or No CB groups. **F**) **Morisita-Horn index comparing baseline to treatment timepoints is not altered by treatment type.** Morisita-Horn index was used to quantify the similarity between the different therapy cycles within each individual patient. Samples are separated here by what treatment condition patients received and if they received clinical benefit (CB) or not (No CB). **G) Sorting strategy for tumor infiltrating PD1+ CD45RA- CD8 TILs from a representative patient at the time of resection prior to undergoing checkpoint therapy.** The frequency (%) of PD1+ CD45RA- CD8 TILs for all three patients is represented in the bar graph.

Supplementary figure 6: Quantitative Imaging for a representative patient with an intermediate anti-tumor immune status (A-C). **A.** Hematoxylin and eosin whole slide for a representative patient with an intermediate anti-tumor immune status. Yellow outlines highlight the location of tumor tissue within the whole slide scan. **B.** Immunofluorescence whole slide scan for the patient shown in A, with DAPI (nuclei) shown in blue, CD8 in red, and MHC-II in green. Below, an area of interest niches from the whole slide scan shown above, illustrating strong immune cell infiltration and the presence of antigen presenting niches, with DAPI (nuclei) shown in blue, CD8 in red, MHC-II in green, and TCF1 in cyan. **C.** Immunomap quantifications of the whole slide immunofluorescence shown in B, illustrating from top to bottom, (1) the xy location of

all CD8 T cells in red and of TCF1+ CD8 T cells in cyan, (2) the xy location of all MHC-II+ cells in green, and (3) the location of immune niches, defined as those 100μm x 100μm areas containing ≥1 TCF1+ CD8 T cells and ≥1 MHC-II+ cells, in orange. **(D-G). Summary data for quantitative analysis of whole slide immunofluorescence imaging. D.** CD8 T cell infiltration (CD8+ cells per mm^2) correlates with MHC-II+ cell infiltration (MHC-II+ cells per mm^2). **E.** CD8 T cell infiltration (CD8+ cells per mm^2) correlates with the proportion of stem-like CD8 T cell infiltration (TCF1+ CD8 T cells as a proportion of all DAPI+ cells). **F.** MHC-II+ cell infiltration (MHC-II+ cells per mm^2) correlates with the proportion of tissue containing immune niches (as defined in **C**). **G.** Stem-like CD8 T cell infiltration (TCF1+ CD8 T cells as a proportion of all DAPI+ cells) correlates with the proportion of tissue containing immune niches (as defined in **C**). **H)** Summary data for quantitative immunofluorescence comparing the proportion of TCF1+ cells (as % of all DAPI+ cells) in patients with and without clinical benefit following therapy (CB, no CB, respectively). **Relationship of the intra-tumoral immune status at the time of surgery with the later, CD8 T cell response in the peripheral blood to immunotherapy (I-J). I.** Correlation of the burst in HLA-DR+CD38+ CD8 T cells in the blood following immunotherapy with the average MHC-II+ cellular density in tumor tissue at the time of surgery as measured by quantitative immunofluorescence. **J.** Correlation of the burst in HLA-DR+CD38+ CD8 T cells in the blood following immunotherapy with the proportion of tumor tissue occupied by immune niches (as defined in **C**). **K) Flow cytometric analysis of tumor samples:** Comparison of selected cohort with previously published unselected RCC patient cohort (Jansen *et al* Nature 2019). Proportion of CD8 T cells in kidney tumors by flow cytometry at the time of surgery shown as percent of total cells in patients from Jansen *et al* (black, n=68), patients with clinical benefit (CB, orange, n=9), and patients without clinical benefit (no CB, gray, n=28). **L) Sample gating strategy** for a representative patient to examine terminally differentiated (PD1+CD39+) and stem-like (PD1+CD39-CD28+) CD8 T cells. Leftmost plot is gated on live, single cells and shows selection of CD3+ cells. Moving rightward, CD8 (CD4-) T cells are selected for further examination, and terminally differentiated and stem-like cells are identified based on PD1, CD39 and CD28 expression.