

## Supplemental Table Legends

**Supplemental Table 1. Summary of RCC Patient Characteristics.** “\*” Indicates the 6 samples used for comparing autologous TIL expansion with the Panning, PreREP, and FTD + beads protocols. RCC55 was excluded from this table because TIL expansion was not attempted by any method due to less than 0.2 grams of tissue being acquired for research purposes. RCC73 was utilized for a different study after validation had been completed.

**Supplemental Table 2. Mutation profile of adherent RCC TCLs.** Pathogenic, likely pathogenic, and selected mutations with uncertain significance detected in primary adherent cell lines established from clear cell RCC using a 275 oncology NGS gene panel.

## Supplemental Figures

**Supplemental Figure 1.** Representative gating scheme for flow cytometry data analyzed in FlowJo.

**Supplemental Figure 2.** Interferon gamma (IFN $\gamma$ ) Enzyme-Linked Immunosorbent Assay (ELISA) results from RCC TIL conditioned media (CM) of autologous TILs left unstimulated or stimulated with IL-2 and anti-CD3/CD28 Dynabeads.

**Supplemental Figure 3.** HLA-A/B/C expression on adherent RCC cells cultured in fresh media or autologous TIL CM for 24 hours.

**Supplemental Figure 4.** Optimization of MDSC detection by flow cytometry.

**Supplemental Figure 5.** MDSCs characterization in whole FTDs and the corresponding adherent and non-adherent populations after overnight adherent cell depletion.

**Supplemental Figure 6.** Percent expression of CD28, CD27, KLRG1, CD62L, IL-7Ra, and CCR7 on the T-cells in the final TIL product.

**Supplemental Figures 7-12.** 11-parameter tSNE plots of the autologous TIL products which have been concatenated and displayed as heatmaps as well as density plots showing the clustering of autologous TIL products.

**Supplemental Figure 13.** (A) Representative intracellular Granzyme B staining. (B) Percent of GZMB+ TIL in the final products expanded by each method.

**Supplemental Figure 14.** (A) Representative characterization of the CD8- TIL responding in co-culture. (B) Summary of the CD8- TIL responding in co-culture.

**Supplemental Figure 15.** Repeat co-culture experiment with the addition of PD-1 to the lymphocyte function panel. \*Note, detection of PD-1 expression was reduced in all conditions compared to the initial PD-1 staining of live TIL at the 14-day mark (potentially a result of resting prior to co-culture or fixation/permeabilization required for simultaneous surface and intracellular staining).

### **Supplemental Methods.**

The following flow cytometry panels were used (all antibodies were purchased from BioLegend®):

*Pre-Expansion TIL Characterization:* CD3-FITC, CD4-PE/Cy5, CD8a-Alexa 647, CD45RA-PE Dazzle 594, CD62L-BV650 and Ghost Dye 780.

*Post-Expansion Memory Panel:* CD3-FITC, CD4-BV510, CD8-BV785, CD45RA-BV605, CD62L-BV650, CCR7-PE-Cy7, CD27-PE, CD28-PE-Cy5, IL-7Ra-APC, KLRG1-BV421, CD103-BV711 and Ghost Dye 780.

*Post-Expansion Checkpoint Panel:* CD3-FITC, CD4-BV510, CD8-BV785, TIM-3-PE, PD-1-BV711, LAG-3-BV421, TIGIT-PE-Cy7 and Ghost Dye 780.

*Post-Expansion Treg Panel:* CD3-FITC, CD4-BV510, CD25-BV785, FOXP3-PE and Ghost Dye 780.

*Post-Expansion Lymphocyte Function Panel:* CD3-FITC, CD4-BV510, CD8-BV785, CD56-PE-Cy7, IFN $\gamma$ -PE, GzmB-APC, CD107a-BV421 and Ghost Dye 780.

*Myeloid Derived Suppressor Cell (MDSC) Panel:* CD33-PE, HLA-DR-BV421, CD14-AF 647, CD15-PE-Cy7 and Ghost Dye 780.

*Tumor cell staining:* PD-L1-PE, Galectin-9-PE, HLA-ABC-PE and HLA-G-PE were used separately for staining tumor cells in the indicated conditions.