SUPPLEMENTAL MATERIAL 1

Integrated analysis of the tumor microenvironment using a reconfigurable microfluidic cell culture platform

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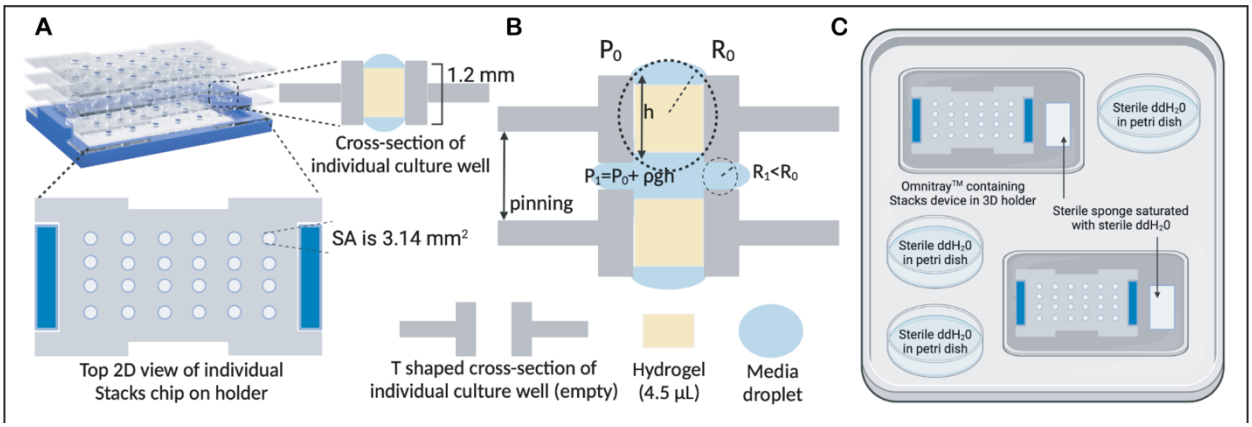

Figure S1. Stacks Wells Topography. A) Schematic representation of the Stacks device, including the dimensions of an individual well shown in cross-section and in a top 2D view. The height of the microwells is 1.2 mm with a surface area (SA) of 3.14 mm^2. Each microwell can hold 4.5 µl volume of hydrogel matrix. Microwells are arranged in a 4 by 6 configuration (customizable feature). Once polymerized, the hydrogel plug may hold a ~10 µl volume droplet on each surface in an open concept. **B)** Cross-section view of a 2-layer Stack assembly shows ~4.5 µl volume hydrogel droplets that are kept separate by system fluid and matrix concavity and communicate by the media droplets in between. Layers are easily separable and reconfigurable for subsequent procedures. **C)** Humidification chamber assembly, holding Stacks devices within a double set of bioassay trays, under sterile culture conditions.

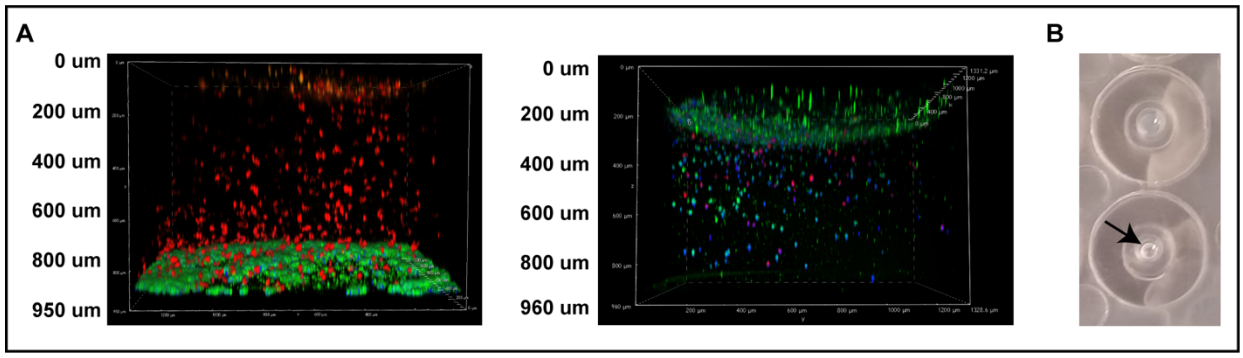

Figure S2. Technical Analysis of Stacks Wells. A) Images of migrating cells demonstrating retention of hydrogel thickness over long-term culture. Left: 14-day old matrix with macrophages (CD14 in red) migrating from top of well towards 22rv1 tumor cells (EpCAM in green) on bottom of well. Right: 2-day old matrix with T cells (CD4 in green, CD8 in red) migrating from top to bottom of well towards tumor cells in layer below (not pictured). **B)** Picture of 2 wells on a Stacks plate in a 2-layer assembly. Black Arrow points to a bubble between the two layers, demonstrating that air bubbles can be easily detected visually if they occur. Air bubbles occur in <1% of stacked wells and are excluded for culture/analysis, if detected.

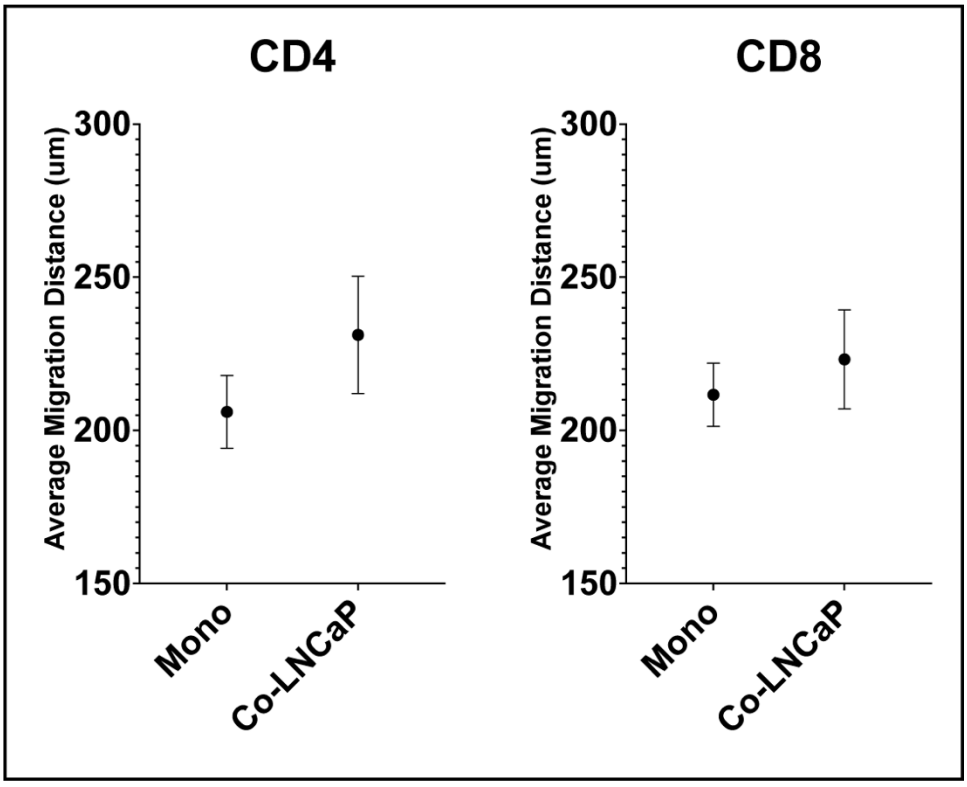

Figure S3. Directional Migration of T cells with and without Tumor Cells. Data show average migration distance of activated human primary $CD4^+$ (left) and $CD8^+$ (right) T cells in two-layer Stacks. For the monoculture (Mono) condition T cells were seeded onto a top layer Stack with a blank bottom layer. For the co-culture condition (Co-LNCaP) T cells were seeded onto the top layer Stack with a LNCaP tumor cell monolayer on the bottom. T cells were allowed to migrate for 24 hours prior to fixation and quantification of average migration distance using confocal microscopy. Data represent mean migration distance of total CD4+ or CD8+ T cells from individual donor specimens (n=3).

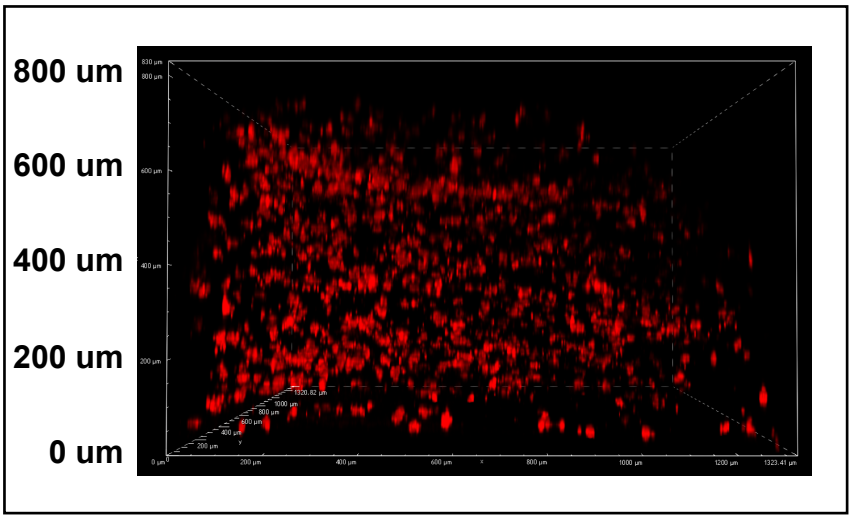

Figure S4. Upward Migration of Cells in Stacks Image of a Stacks well containing primary human macrophages stained for CD14 in red. Macrophages were seeded on the bottom of the Stacks well and allowed to migrate for 24h towards tumor cells in separate Stacks layer above (not shown). Distance measurements on left indicate distance of migration in the upward direction.