**Cytometry by time of flight (CyTOF)**

**Sample and Processing Mouse Samples**

Isolated cells (3-4 million) derived from tumors or spleen are resuspended in 1 mL of Maxpar / Fluidigm Cell Staining Buffer (CSB) (catalog number: 201068). Each sample was incubated for 5 minutes with 0.5 m Cisplatin solution(catalog number 201064, Fluidigm/Cell-ID Cisplatin reagent) in PBS. Samples were then washed twice with CSB. An antibody cocktail of the entire phenotyping or function panel was prepared as a master mix and filtered to remove aggregates prior to adding 50 μL of cocktail to samples resuspended in 50 μ L of CSB. Samples were then incubated at room temperature for 45 minutes. After washing twice with CSB, samples were fixed with 2% PFA in PBS.

**Preparation and Processing of Human Samples**

Tumor-, spleen-, and PBMC-derived cells were processed using standard CyTOF staining protocols, including viability staining, antibody labeling, and fixation. Human PBMCs were isolated by Ficoll density centrifugation, cryopreserved, thawed, Fc-blocked, and stained with CyTOF antibody panels.

Samples were acquired on a Helios CyTOF system (Fluidigm). Data were normalized using CytoNorm (1,2), cleaned using FlowJo, and analyzed with Cytofkit and Rphenograph (3–5). Dimensionality reduction was performed using tSNE, and cluster abundance and marker expression were visualized by heatmaps and density plots.

**Data Analysis**

Cleanup of cell debris, removal of doublets and dead cells was performed using FloJo software version 10.5.3 (Ashland, OR). To minimize batch effects, all human samples were normalized against a reference standard using the CytoNorm algorithm (1, 2). Cleaned fcs files were analyzed by the R-based tool Cytofkit version 3.8 (3-5). Clustering and dimensionality reduction to 10,000 events per file was performed using the Rphenograph algorithm. Visualization of clusters was mapped onto a tSNE map. Relative marker intensities and cluster abundances per sample were visualized by a heatmap or density maps.

**Migration and invasion assay**

Migration assays were performed using 24-mm Transwell inserts with 8.0-μm polycarbonate membranes according to the manufacturer's instructions. Cells were serum-starved for 24 h, seeded into Transwell inserts, fixed with methanol, and stained with 5% crystal violet after the indicated incubation period.

For collagen invasion assays, cells were seeded onto collagen gels and cultured under serum-free conditions for 3 days, then fixed with 3% paraformaldehyde, permeabilized with 0.5% Triton X-100, stained with DAPI, and assessed for invasion depth.

For microchannel assays, cells were seeded into the right port of UVO microchannels (5250), while 3.5 mg/mL collagen was loaded into the left port. Invasion was quantified by the distance of cell migration into collagen.