Supplementary Material

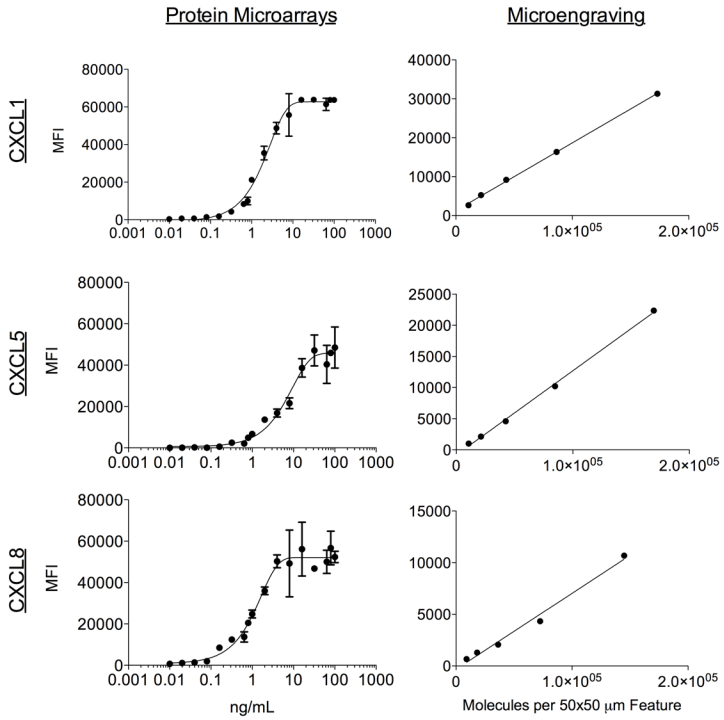

Fig. S1. Standard curves. Representative calibration curves for protein microarrays and microengraving used to calculate concentrations and secretion rates, respectively, for bulk and single-cell assays. Fluorescence intensity units were extracted using Genepix software.

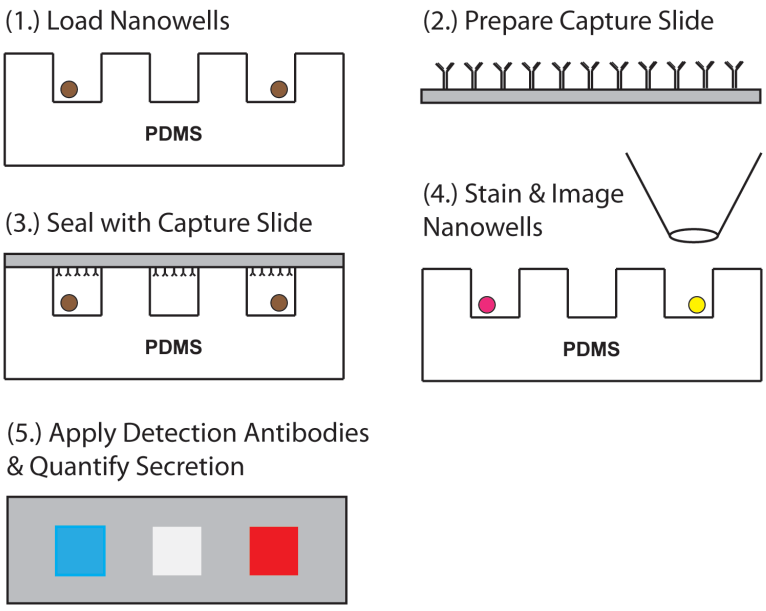

Fig. S2. Schematic of microengraving. Single cells are loaded non-deterministically in the nanowells from a suspension of cells and a poly-l-lysine slide is coated with a collection of capture antibodies, yielding the capture slide. The nanowells are then sealed with the capture slide and incubated for a set duration of time. Then, the capture slide is removed and processed separately with fluorescent detection antibodies; meanwhile, cells in the nanowells are stained and imaged via epifluorescence microscopy for immunophenotyping.

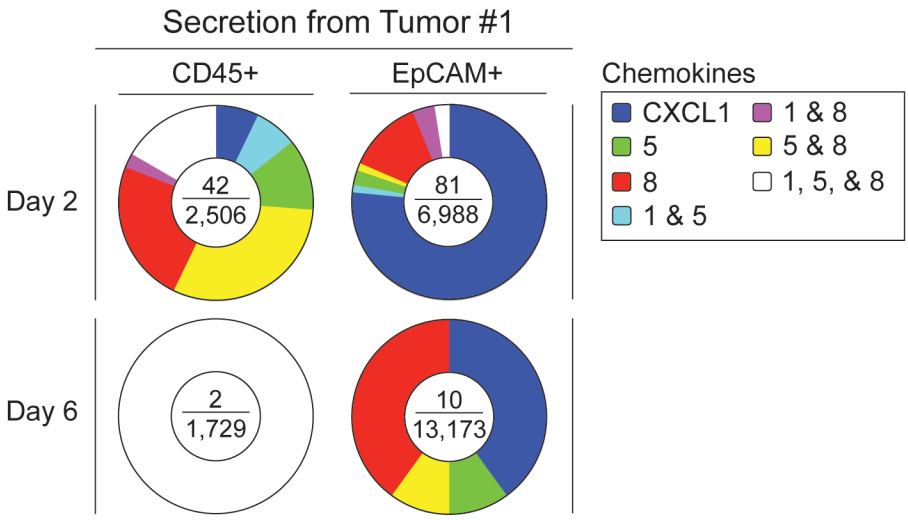

Fig. S3. Secretory data from live single cells from a human colorectal tumor cultured for six days. Pie charts of the distributions of secretion states observed from the primary tumor #1 assayed at two and six days post surgery. The center of each pie chart presents the number of single-cell secretion events over the total number of live single cells.

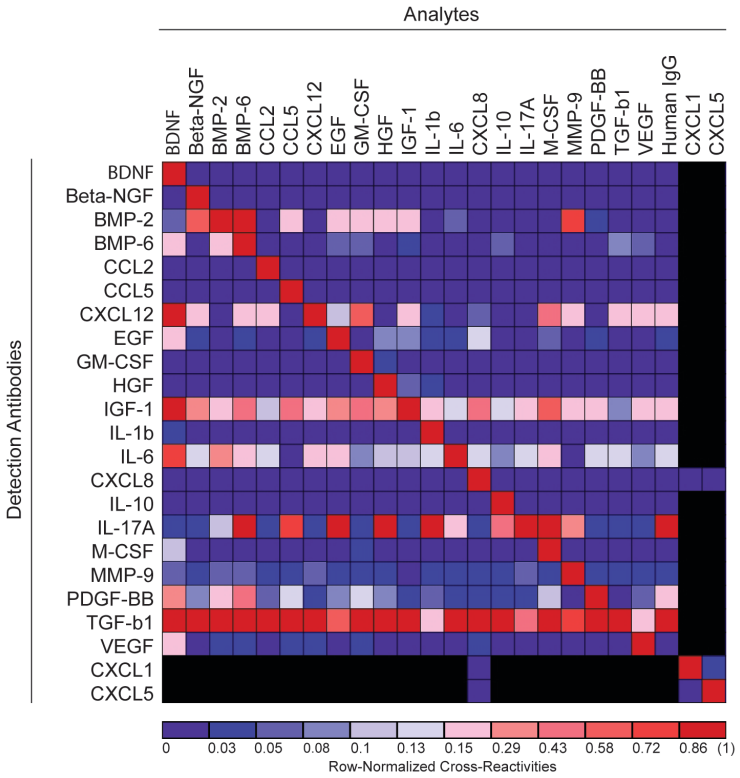

Fig. S4. Validation of antibody pairs. Heatmap of the signals from various detection antibodies used in sandwich assays with the respective combinations of capture antibodies and analyte (22 ng/mL). The median fluorescence intensities in each row are normalized to the median fluorescence intensity for the target analyte. Shades of red indicate considerable cross-reactivities of 30% or greater of the median signal of the target analyte at the same concentration. Black boxes indicate data not available.

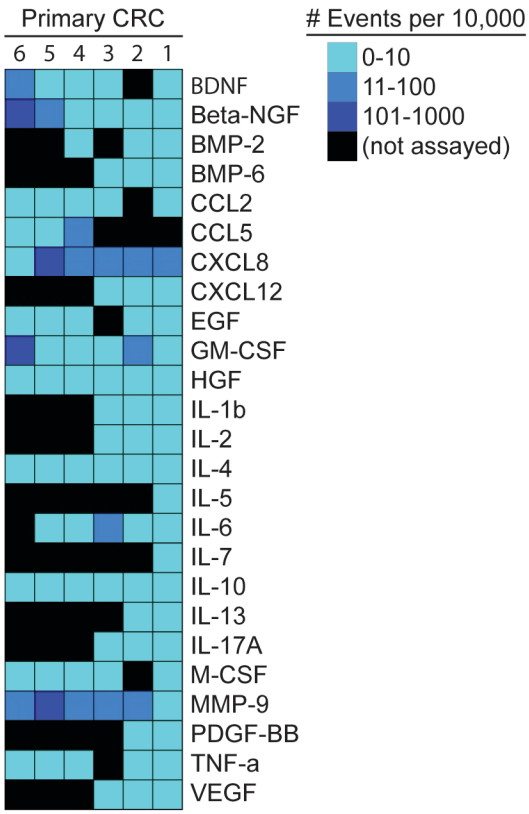

Fig. S5. Secretory profiles of single human primary colorectal tumor and stromal cells. Six human primary colorectal tumors (from CRC patients #1-6) were disaggregated, loaded in the nanowells, and assayed for protein secretion. Heatmap shows the fractions of single cells secreting each given protein. The minimum number of single cells evaluated per sample was 16,337.

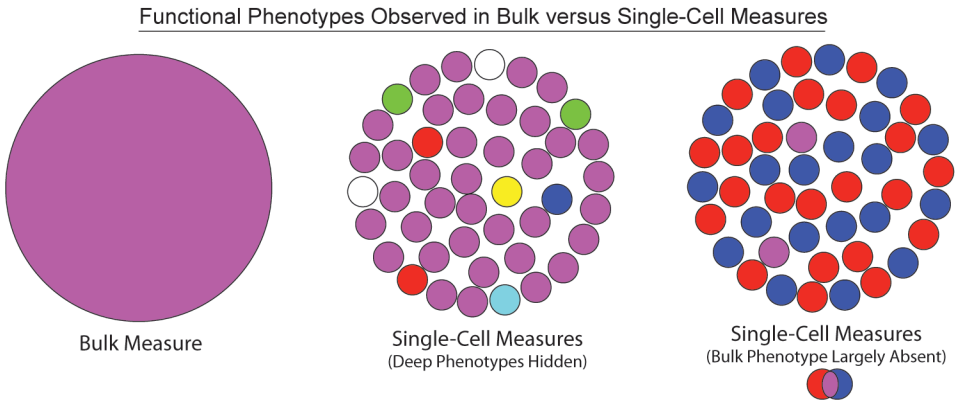

Fig. S6. Model of functional phenotypes observed in bulk versus single-cell measures. The functional phenotype suggested by a bulk measure may either hide complex phenotypes or be largely absent among single cells. The measurements in this study cannot infer the spatial positioning of the cells in the tumor and positioning shown is schematic.