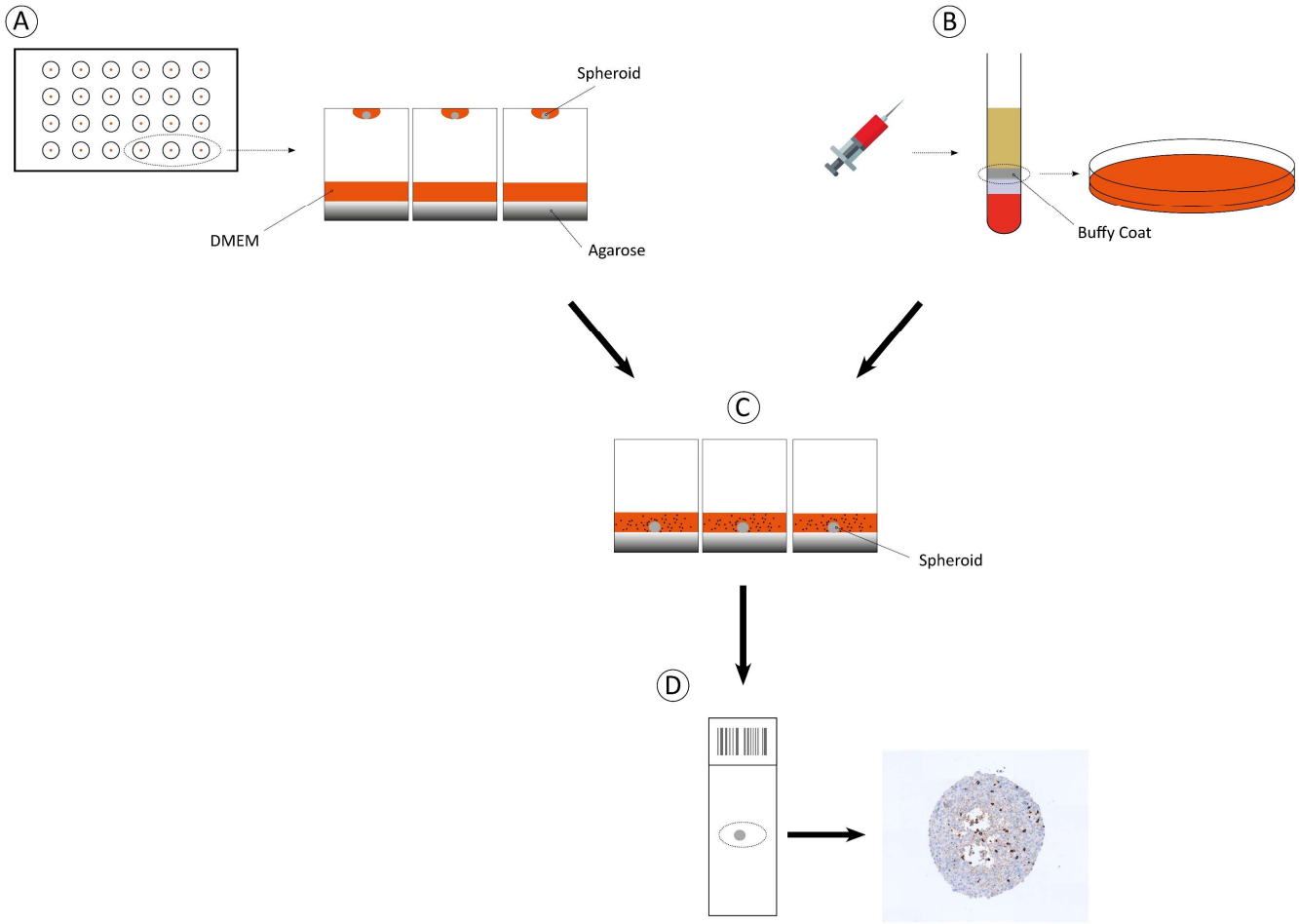
Supplementary Figure 2: *In vitro* spheroid immune cell co-cultures



(A) Spheroids are created either from wildtype FaDu cells (FaDu/WT), Cisplatin-resistant FaDu cells (FaDu/CR) or radiotherapy-resistant FaDu cells (FaDu/RR) by culturing 20000 cells in 20µl Dulbecco's Modified Eagle Medium (DMEM) as hanging drops on the lid of a 24-well-plate for 5 days. **(B)** Peripheral blood mononuclear cells (PBMC) are isolated from fresh heparinized blood of healthy donors using Ficoll-Paque Plus (GE Healthcare, Chicago, IL) density gradient centrifugation. PBMCs are maintained in RPMI-1640 medium (Biochrom, Berlin, Deutschland) for 72 hours. For activation of PBMCs, culture plates are coated with 1 µg/ml anti-CD3-antibody (BioLegend, San Diego, CA) and 5 µg/ml anti-CD28-antibody (BioLegend, San Diego, CA) and tumor cell lysate of approximately $5x10^5$ FaDU cells per 3 ml medium is added. Tumor cell lysates are prepared by repeating 5 cycles of freezing and thawing (liquid nitrogen and 60°C water bath) **(C)** Five days after start of the experiment, spheroids are transferred from the lid to agarose coated wells of the 24-well-plate containing 1ml DMEM. Activated PBMCs are washed and added to each well. Spheroids and PBMCs are then coincubated for 72 hours. **(D)** At the end of the experiment, coincubated spheroids are harvested, washed, fixed in formaldehyde and embedded in paraffine. Histological specimens are then analyzed regarding differences of the infiltration of FaDu/WT and FaDu/CR or FaDu/RR spheroids by various immune cell subtypes.