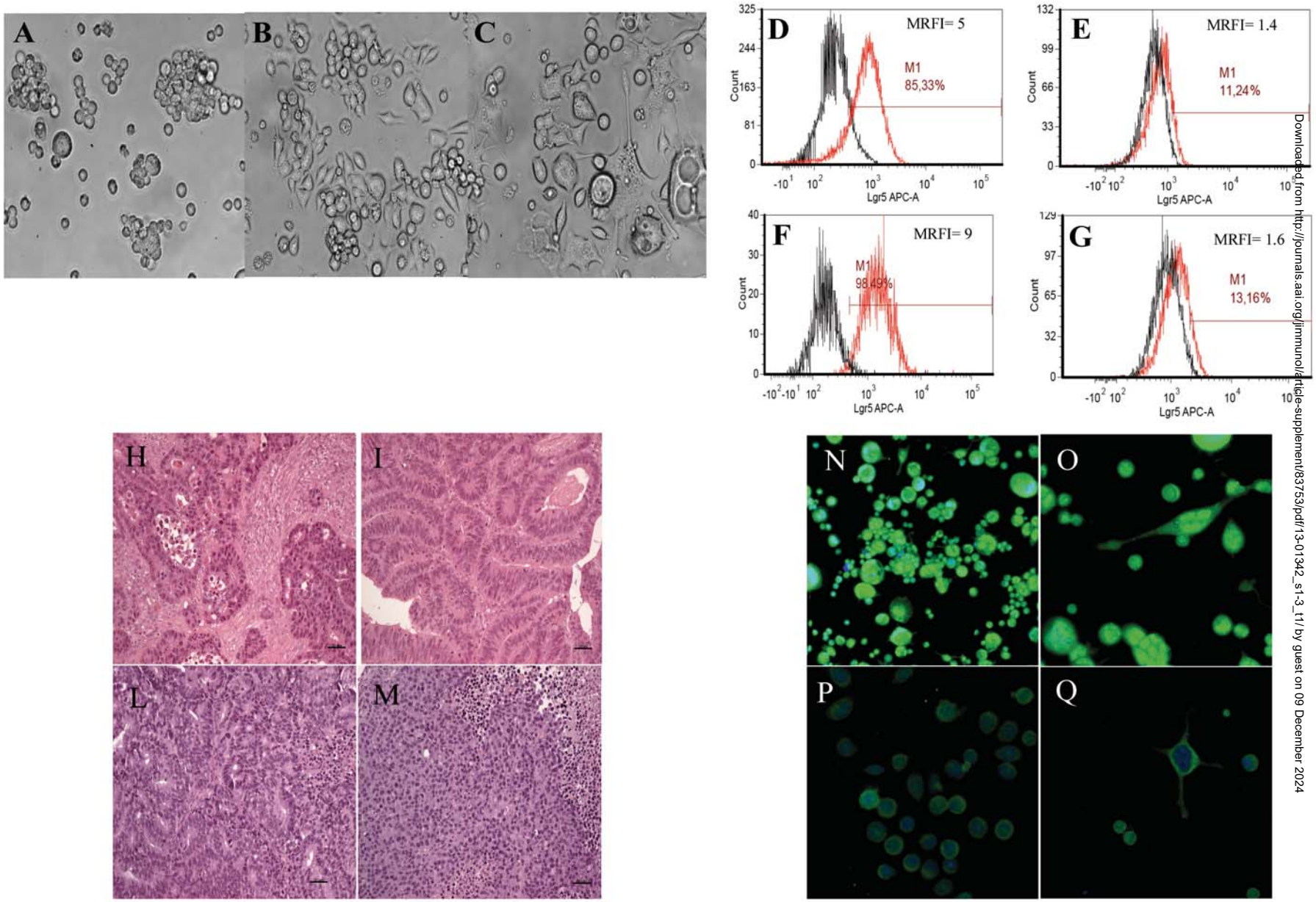
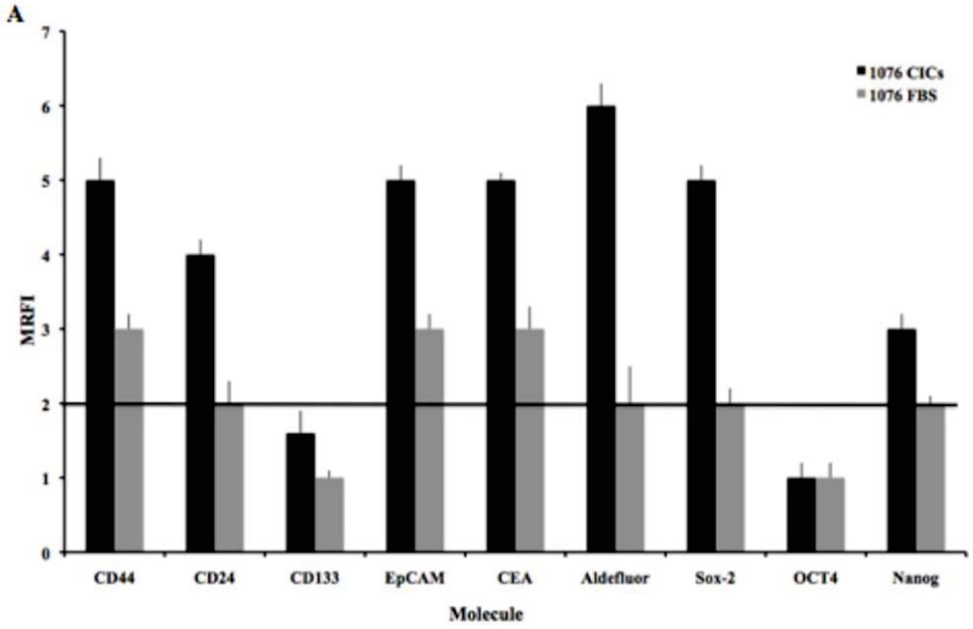
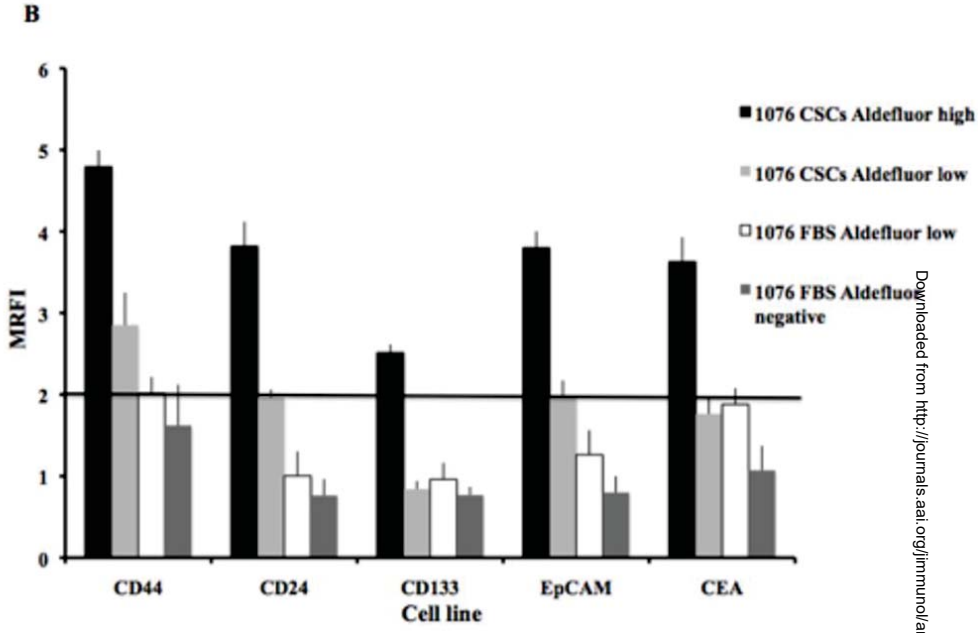
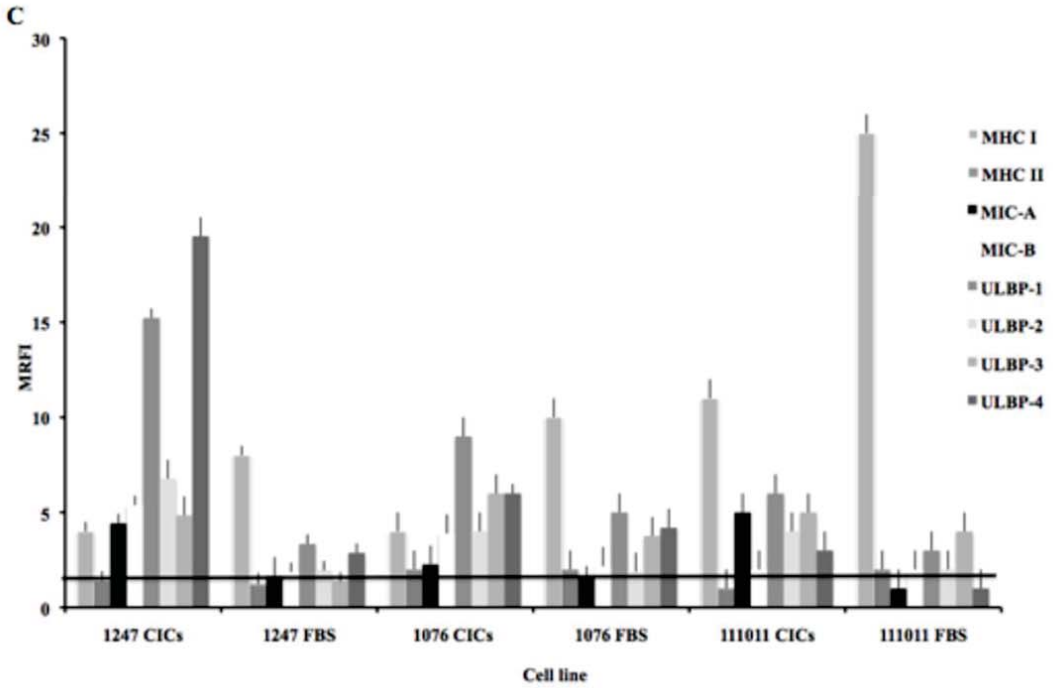
Supplemental Fig.1 Volontè et al.

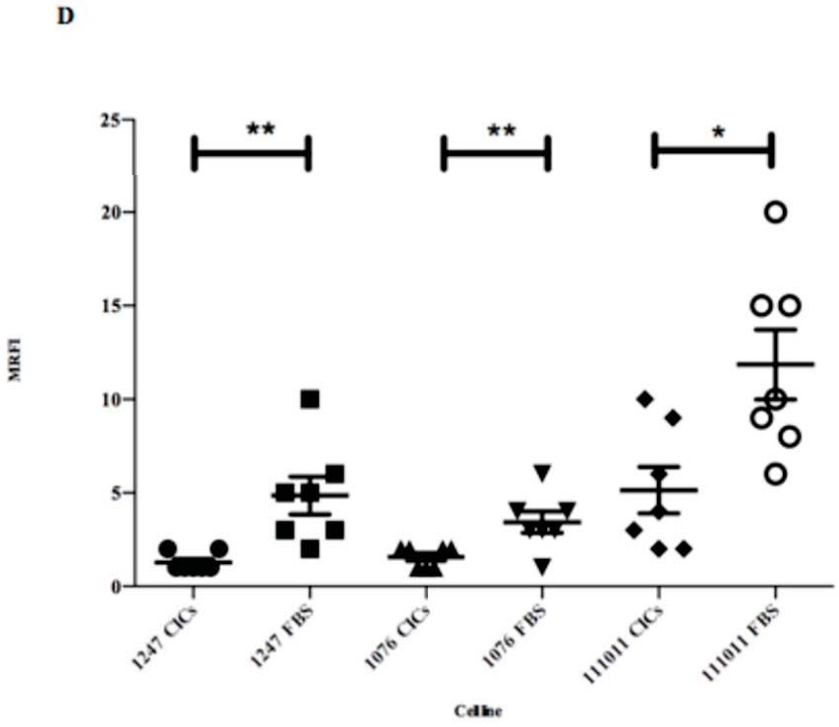


Supplemental Fig. 2 Volontè et al.

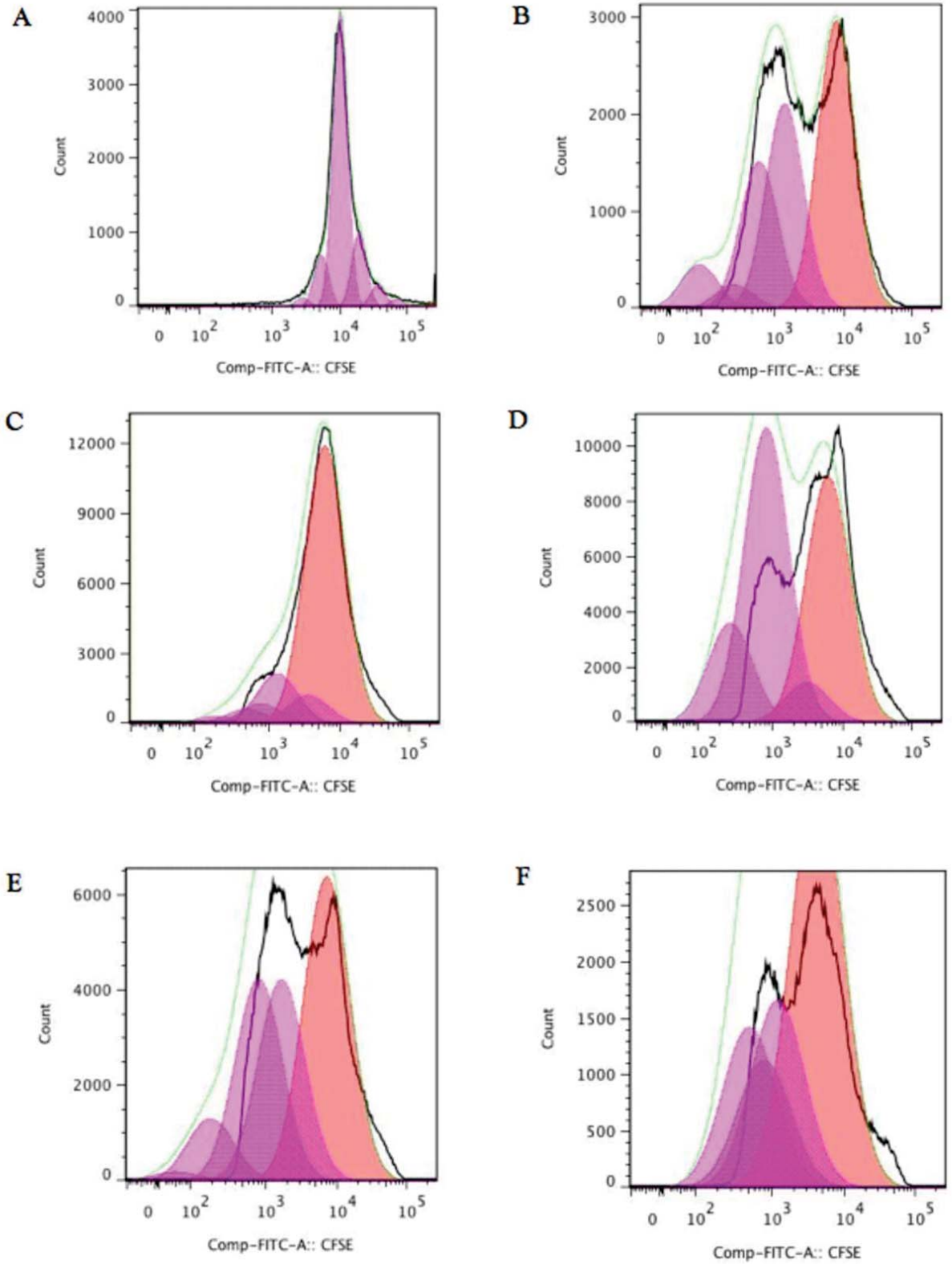








Supplemental Fig. 3 Volontè et al.



Supplemental results

Figure legends

Supplemental Figure 1. CIC properties of CRC sphere forming cells and their TAA expression.

Panels A-C: CICs growing *in vitro* in the form of spheres (Panels A) were cultured for 10 days in the presence of FBS representing the differentiating agent. These cells acquired adherence growth ability and polygonal morphology (Panel B) similarly to the non-CIC counterpart of the tumor whose cells were cultured since the origin with FBS (Panel C). 40x magnifications.

Panels D-G: CICs (#1076 and 1247 Panels D and F) and FBS tumor cells (#1076 and 1247 Panels E and G) were stained with the Lgr5 specific mAb and then cytofluorimetric analysis was performed. Negative control: black line; Lgr5 stained cells: red line. The percentage of positive cells and the MRFI are indicated in each histogram.

Panels H-M: the morphological analysis on tumor tissues has been performed by H&E staining. Panels H and I: human CRC tissues from the patient 1076. Panel L: xenograft derived tumor from 1076 CIC ($1x10^5$) injection in NOD/SCID mice. Panel M: xenograft derived tumor from 1076 FBS tumor cell ($1x10^5$) injection in NOD/SCID mice. 20X magnification. Scale bar: 100 μm.

Panels N-Q: the expression of COA-1 and SVV-1 was detected by intracellular staining with the specific Abs (*see* Material and Methods) and confocal microscopy analysis in CICs (Panels N and P) and FBS tumor cells (Panels O and Q) isolated from CRC patients (results from #1076 are shown). COA-1 staining (Panels N and O) and SVV-1 expression (Panels P and Q) at 40x magnification are shown in the figure. FITC-conjugated goat anti-rmouse (green) secondary antibodies were used for COA-1 or survivin staining. DAPI staining for nuclei was used (blue).

Supplemental Figure 2. Characterization of CRC CICs and their immunological profile.
The expression of the indicated CIC and CRC CICs molecules was evaluated by membrane or intracellular immunofluorescence with specific mAbs and cytofluorimetric analysis (*see* Material and Methods). Representative results from patient #1076 are shown in Panels A and B. Cell sorting of CICs and FBS tumor cells was performed to select ALDH1 expressing cells (Representing data from patient #1076 are shown in Panel B). The expression of CRC and CICs-associated markers was evaluated onto ALH1 positive or negative-sorted cells (Panel B).
The expression of MHC I and II molecules and of NKG2DLs (representative results of #1076, #1247, and #111011 Panel C) and of APM molecules (representative results for LMP2, LMP7, LMP10, TAP1, TAP2, B2M, ERp57 of patients #1076, #1247, and #111011, Panel D) was determined by immunofluorescence and cytofluorimetric analysis.
APM was determined by intracellular staining on permeabilized cells. For the mAbs used *see* Material and Methods. Cytofluorimetric analysis was performed by the usage of Canto HTS (Becton Dickinson). Data are represented as MRFI that is the ratio between the mean of fluorescence intensity of cells stained with the selected mAb and that of the negative Ab control; significant value are MRFI ≥ 2. The results are the mean of three independent experiments (SD < 5).

Supplemental Figure 3: CFSE staining of PBMC co-cultured or not with CICs.
PBMCs ($1x10^7$) from CRC patients (pt.# 1076, 1247, 14583) or healthy donors (for pt.#1, 2 and 3) were stained with 1 μM CSFE and stimulated with PHA/ConA in the presence or not of 3-day cultured CICs or FBS tumor cell lines. The co-culture of tumor cells and autologous or allogeneic PBMCs was performed in the presence or not of either anti-IL-4 mAb, anti-IL-4R mAb or both the mAbs. Then, cells were harvested and the CFSE profile was assessed by flow cytometry. Data are referred to $CD3^+$ gated cells from patient # 1247. Panel A: PBMCs not stimulated; Panel B: PBMCs + PHA/ConA; Panel C: PBMC + CICs; Panel D: PBMC + CICs + anti-IL-4 mAb; Panel E: PBMCs + FBS tumor cells; Panel F: PBMCs + FBS tumor cells + anti-IL-4 mAb.
Data represent the proliferation index tare shown in Figure 3.

Supplemental Table I. Clinical information of CRC patients

Patient	Diagnosis	Colonic localization	Stage TNM	Pre-treatment
#1076	Poorly differentiated colon adenocarcinoma	Sigmoid	pT3, pN0, pM0, G3	No
#1247	Poorly differentiated colon adenocarcinoma	Right colon	pT3, pN0, pM0, G3	No
#111011	Poorly differentiated colon adenocarcinoma	Right colon	pT4, pN0, pM0, G3	No
#14583	Moderately differentiated colon adenocarcinoma	Recto-sigmoid	pT2, pN0, pM0, G2	No
#1	Poorly differentiated colon adenocarcinoma	Right colon	pT2, pN0, pM0, G2	No
#2	Poorly differentiated colon adenocarcinoma	Caecum	pT3, pN2, pM1, G3	No
#3	Poorly differentiated colon adenocarcinoma	Recto-sigmoid	pT2, pN0, pM0, G2	No