SUPPLEMENTAL INFORMATION

Materials and Methods

Patients and cells

Tissue samples were obtained from 33 CRC patients undergoing therapeutic intervention at the Unit of Oncological Surgery, IRCCS-AOU San Martino-IST, Genoa, provided informed consent (the study was approved by the institutional and regional ethical committee, PR163REG2014). All patients gave consent in according to the Declaration of Helsinki. Samples were used for preparation of cell suspensions and immunohistochemistry. Cells suspensions were obtained as previously described [1]. Lymphocytes were obtained by density Percoll (Sigma-Aldrich) gradient centrifugation.

Flow cytometry analyses and monoclonal antibodies

Cells were stained with the following mAbs: CD294 (CRTH2), CD117, CD123 and purchased from Miltenyi; CD19 and CD45 purchased from BD, CD94-FITC; CD127-BrilliantViolet421; NKp44, CD117, CD103 and CD127 purchased from BioLegend: CD56, CD34, CD14, CD19, CD3 and NKp44 purchased from IL-Beckman Coulter; and Live/dead fixable aqua dead purchased from Invitrogen/Molecular Probes. All samples were analyzed on Gallios (Beckman Coulter). Data analysis was done using FlowJo software (TreeStar Inc.) and Cytexpert (Beckman Coulter).

Immunohistochemistry

Paraffin-embedded samples from 20 CRC patients were analyzed. Immunohistochemistry (IHC) was performed on 4-µm-thin sections, deparaffinized in xylene, and treated with Peroxo-Block (Thermo Fisher Scientific) to quench endogenous peroxidase. An anti CD3 (SP7) rabbit polyclonal antibody (Thermoscientific) and anti RORγt mAb (Millipore) were used as primary antibodies on a Leica Bond RX immunostainer and detected with ChromoPlex 1 Dual Detection for BOND (Leica). Images of slides were acquired using Aperio AT2 scan (Leica).

Statistical analyses

The data obtained in multiple experiments are reported as absolute number or percentages ± SEM (standard error of the mean). Statistical analyses were performed with GraphPad Prism 7 software

(La Jolla, CA). Wilcoxon paired non-parametric test was used. We considered significant p-values of less than 0.05 (*), less than 0.01 (**), less than 0.001 (***) or less than 0,0001 (****). Where not indicated, data were not statistically significant.

Supplementary Table 1. Clinical and pathological data of CRC patients

Clinical information about CRC cohort and surgical sampling. TNM and Dukes staging, tumor grade, microsatellite instability (MSI), anatomical localization of the tumor (DX: right or ascending; TR: transverse; SG: sigmoid; RT: rectum) and sex of patients are shown.

Supplementary Figure 1

Gating strategy to identify different ILC subsets in normal (A) and tumor (B) biopsies. NK cells are in purple, ILC1 in blue, ILC2 in green and ILC3 in red. Lin1 includes CD3 and CD14. Lin2 includes CD34, CD123 and CD19. Numbers indicate the frequency of different subsets in parent population.

Supplemental Reference

1 Costa D, Vene R, Benelli R, Romairone E, Scabini S, Catellani S*, et al.* Targeting the Epidermal Growth Factor Receptor Can Counteract the Inhibition of Natural Killer Cell Function Exerted by Colorectal Tumor-Associated Fibroblasts. Frontiers in immunology 2018;**9**:1150.