SUPPLEMENTARY FIGURES



Supplementary Figure S1: Fibroblast-induced elongation of cancer cells requires direct contact with living fibroblasts. A. Representative images of HT29-GFP cultured in the presence or absence of fibroblasts, fibroblasts and co-culture (cc) conditioned media. Bar graphs represent quantification of HT29 elongation and motility during 48 hours. **B.** Representative images of HT29-GFP cultured on ECM deposited by fibroblasts and on a fixed fibroblast layer. Bar graphs represent quantification of HT29 cell elongation and motility during 48 hours. **C.** Representative image and quantification of elongation and motility of HT29-GFP culture after separation from established co-culture. **D.** Representative images and quantification of elongation and motility of HT29-GFP cultured with HUVECs and fibroblasts for 48 h. All data are represented as mean +/– SD.



Supplementary Figure S2: Validation of isolation technics. A. Validation of fibroblasts-mediated ECM deposition by ELISA-mediated detection of fibronectin following fibroblasts removal. Absorption was normalized on fibronectin coating and represented +/– SD. **B.** FACS Analysis of SW620-GFP cultured with fibroblasts for 48 hours +/– MACS isolation of cancer cells. **C.** FACS Analysis of HT29-GFP cultured with fibroblasts for 48 hours +/– MACS isolation of cancer cells.



Supplementary Figure S3: Cancer cell elongation, motility and invasion abilities are not mediated by soluble factors. A. Representative images of SW620 and HT29 cancer cells cultured with various cytokines: TGF β , EGF and FGF-2. B. Quantification of cancer cell elongation and motility during 48 hours, represented as mean +/– SD. C. Representative images of SW620-GFP spheroid invasion on a fibroblast layer, with co-culture (cc) medium, with gelatin coating and on HUVECs layer after 4 days.



Supplementary Figure S4: Fibroblasts-mediated effects on cancer cells are mediated by FGFR. A. Quantification of SW620 and HT29 elongation in the presence of various inhibitors: Y-27632 (ROCK inhibitor), Wortmannin and Ly-294002 (PI3K/Akt inhibitors), AG-1478 (EGFR inhibitor), SU-11274 (c-Met inhibitor), PD-98059 (MAPK/ERK inhibitor), PF-562271 and PF-573228 (FAK inhibitors), PD-161570 and PD-173074 (FGFR inhibitors). B. Representative images of cancer cells expressing LifeAct-mCherry in co-culture with fibroblasts in presence or not of FGFR inhibitors. **C.** Quantification of HT29 motility with FGFR inhibitors in presence of fibroblasts for 48 hours. **D.** Quantification of FGF-2 mRNA variation in fibroblasts after co-culture. All data are represented as mean +/– SD.



Supplementary Figure S5: Fibroblast cell surface FGF-2, FGFR and $\alpha_{\nu}\beta_{5}$ integrin are required for HT29 cell adhesion to fibroblasts. A. Adhesion of HT29 on a fibroblast layer in presence or absence of FGFR inhibitors and FGF-2 specific blocking antibody, represented as mean +/- SD. B. Adhesion of HT29 on fibroblasts in presence of EMD-221975, $\alpha_{\nu}\beta_{6}$, $\alpha_{\nu}\beta_{5}$ and β_{1} anti-integrin specific blocking antibodies as indicated, represented as mean +/- SD. C. FACS histogram analysis of SW620, HT29 and fibroblast cell surface integrin expression.



Supplementary Figure S6: SRC in cancer cells mediate cell elongation, migration and invasion induced by fibroblasts. A. Representative images of HT29 SRC activation (green) in the presence or absence of fibroblasts, stained with DAPI (blue) and Phalloidin (red). **B.** Western Blot quantification of SRC activity in SW620 in presence of FGFR inhibitors. **C.** Western Blot validation of SRC activity down-regulation with PP-3 (neg. ctrl), PP-2 and CGP-77675 SRC inhibitors. **D.** Adhesion of HT29 on fibroblasts in presence or absence of SRC inhibitors, represented as mean +/– SD. **E.** Quantification of HT29 elongation and motility with PP-3, PP-2 and CGP-77675 in presence of fibroblasts for 48 hours represented as mean +/– SD. **F.** Representatives images of HT29-GFP cultured in the same conditions at 20× magnification. **G.** Viability staining using FACS for SW620, HT29 and fibroblasts cultured with DMSO, PP-3, PP-2 and CGP-77675 SRC inhibitors.



Supplementary Figure S7: SRC knockdown using shRNA constructs demonstrates that effects on cancer cells during co-culture are SRC dependent. A. Western Blot validation of the SRC knockdown for two different clones shRNA for SRC (shSRC 1 and shSRC 2). B. Elongation quantification of cancer cells expressing SRC shRNA in presence of fibroblasts (FB). C. Motility quantification of SW620 expressing SRC shRNA during 48 hours. D. Representatives images of SW620 and HT29 cancer cells expressing LifeAct-mCherry in co-culture with fibroblasts, expressing a non-silencing control (NS) and shRNA for SRC. E. Elongation quantification of cancer cells in presence of fibroblasts expressing SRC shRNA. F. Representative images of cancer cells expressing LifeAct-mCherry in presence of fibroblasts expressing SRC shRNA. All data are represented as mean +/– SD.



Supplementary Figure S8: Lack of evidence for a direct association between FGFR1 and β_5 integrin by co-immunoprecipitation. Western blotting analysis of FGFR1 and β_5 integrin co-immunoprecipitation in SW620 cultured in presence or absence of fibroblasts and fibroblasts alone under normal condition, of immunoprecipitated β_5 (IP Beta5) and FGFR1 immunoprecipitated (IP FGFR1) material. Western blotting conditions are given on the side.



Supplementary Figure S9: Representatives images of FGF-2 and FGFR1 expression in human colon cancer. Representatives images of consecutive sections of non-invasive (Patient A) and invasive (Patient B) lesion of human colorectal cancer stained for the indicated markers.