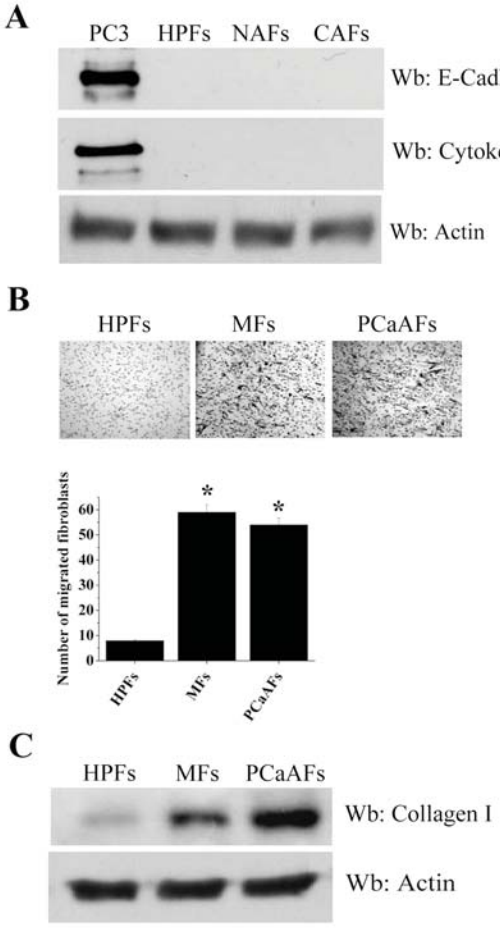
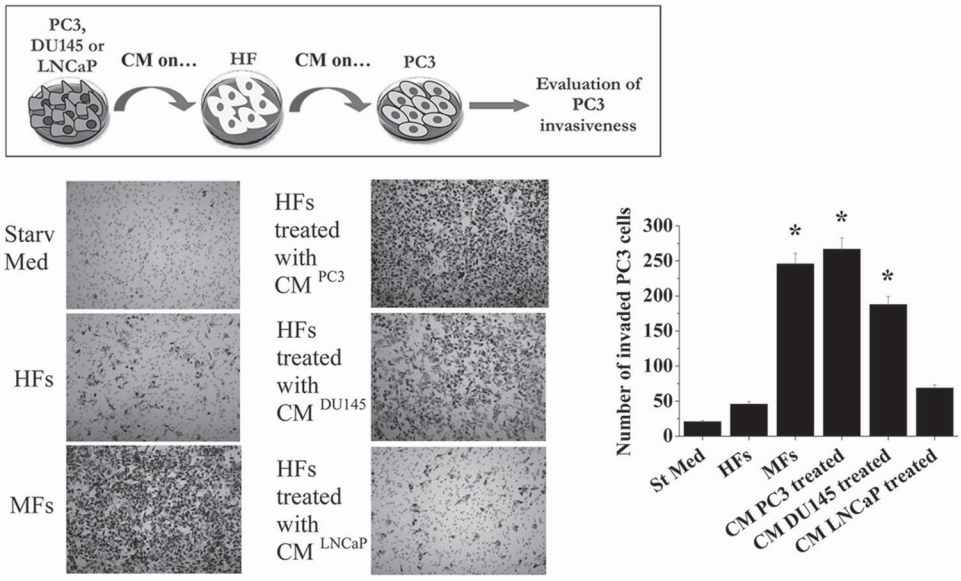
Giannoni E. et al. Supplementary Figure 1



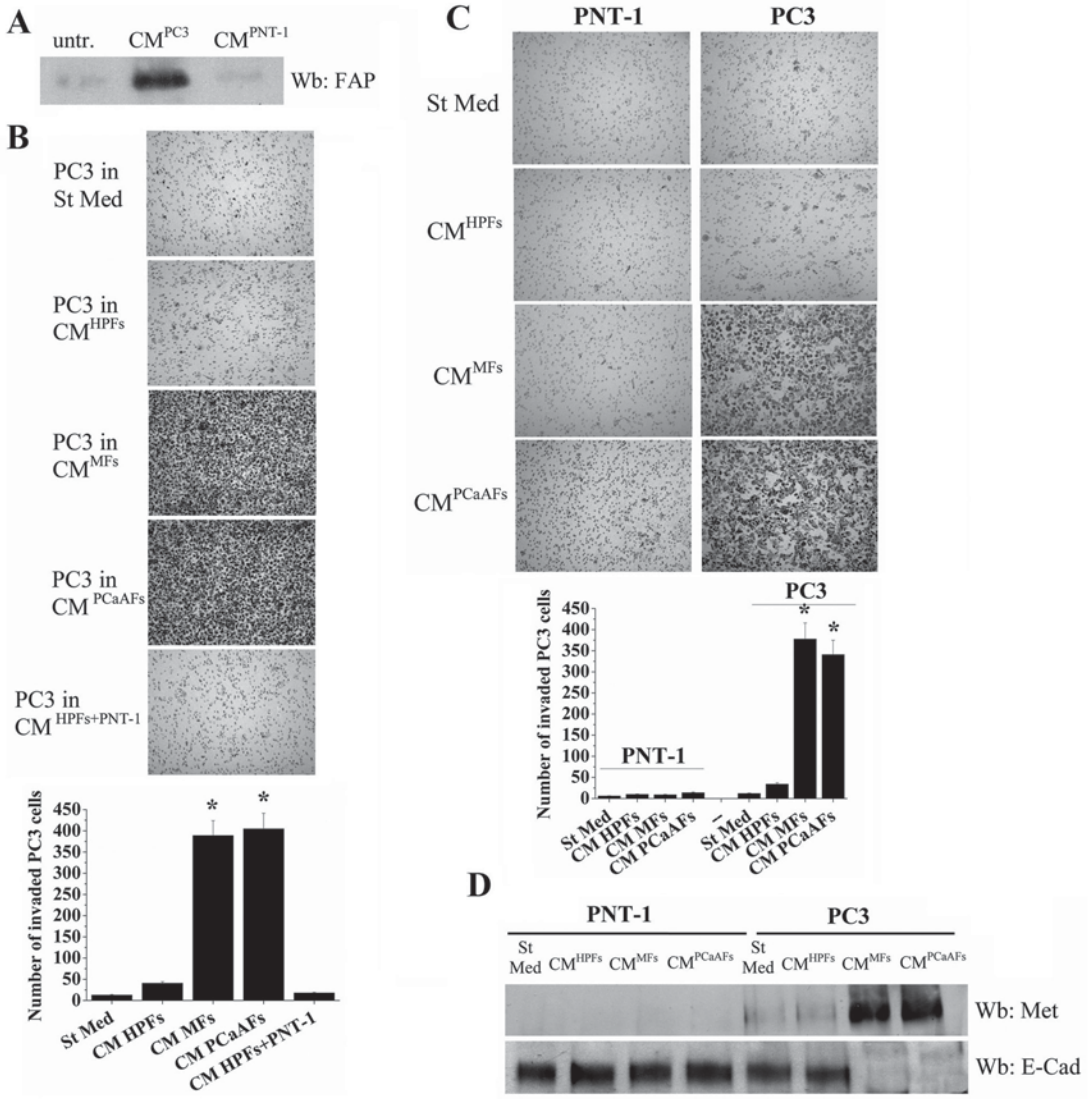
Supplementary FIG.1. Characterization of organ-specific human prostate fibroblasts. A. Fibroblasts isolated from patients affected by benign prostatic hyperplasia (HPFs) or from healthy and cancer regions of PCa-bearing patients (NAFs and CAFs, respectively) were analyzed by immunoblotting for the expression of the epithelial markers E-cadherin and cytokeratin. **B.** Sub-confluent HPFs were activated for 24 h with 10 ng/ml TGF-β1 (MFs) or CM^{PC3} (PCaAFs) and then serum-starved for additional 24 h. 1 x 10^4 cells were plated in 200 µl serum-free medium in the upper chamber (without Matrigel coating) and complete growth medium was used as chemoattractant in the lower reservoir. Photographs were taken and a representative image of each sample was shown. Photographs and bar graph, representative of six randomly chosen fields, are shown. *$p < 0.001$ vs. HPFs. **C.** HPFs were activated as in B and analyzed for the expression of Collagen I. Results are representative of three others with similar results.

Giannoni E. et al. Supplementary Figure 2



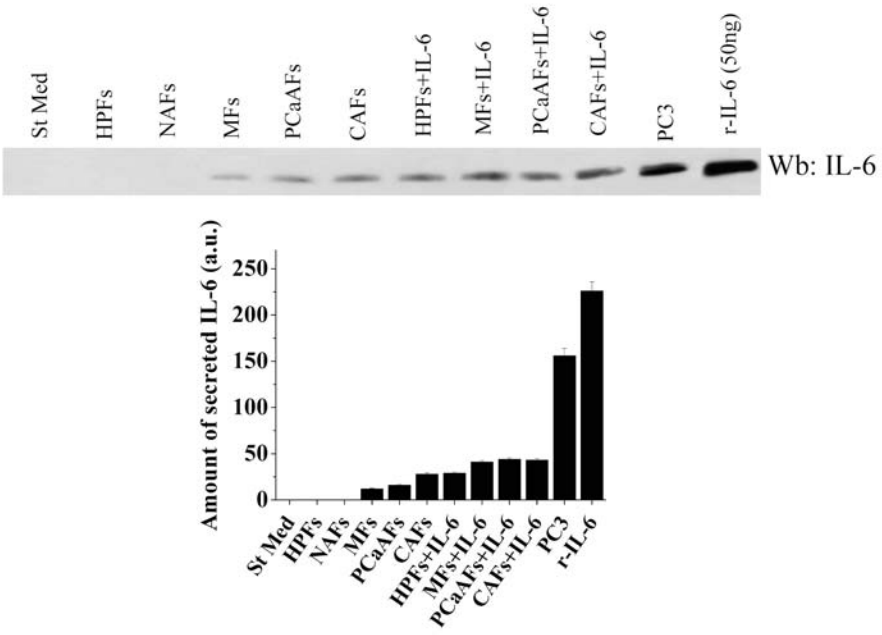
Supplementary Fig. 2. PC3 and DU145, but not LNCaP, have the ability to stimulate fibroblast activation and to increase PC3 invasiveness. HFs were incubated with CM obtained from PC3, DU145 or LNCaP for 24 h and then with serum-free medium for additional 24 h. CM from the differently treated-fibroblasts were collected and used to treat PC3 cells in a Boyden invasion assay. After 24 h, $5x10^4$ PC3 cells for each treatment were allowed to invade for 24 h as described in E. Photographs are representative of randomly chosen fields. The bar graph represents the number of invaded cells shown in five different fields of triplicate experiments. *$p < 0.001$ vs. St Med. Results are representative of three experiments with similar results.

Giannoni E. et al. Supplementary Figure 3



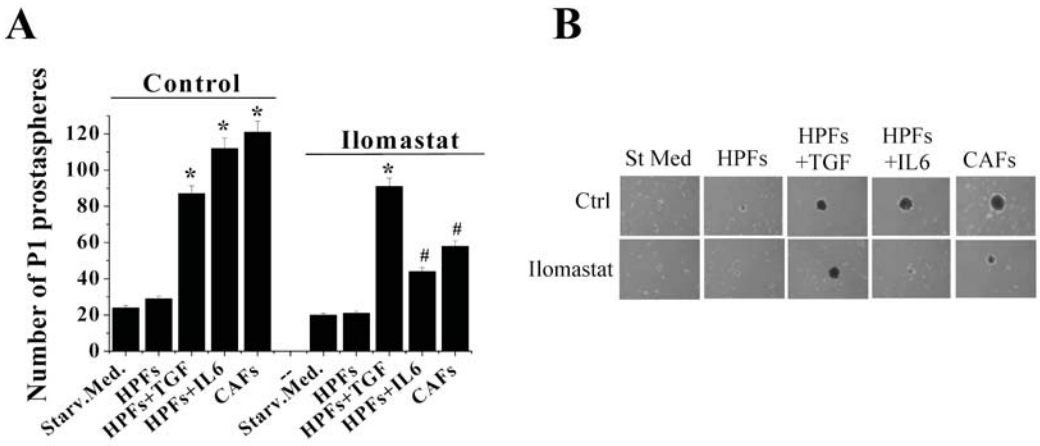
Supplementary Fig. 3. Non-transformed prostate epithelial cells are unable to promote fibroblast activation and are insensitive to CAFs. A. Sub-confluent HPFs were left untreated or activated for 24 h with CM^{PC3} (PCaAF) or CM^{PNT-1} and then serum-starved for additional 24h. Expression of FAP protein was assessed by immunoblot. **B.** PC3 cells were incubated with CM from HPFs, MFs, PCaAFs and HPFs treated with CM^{PNT-1} or serum-starved as a control. After 24 h, CM were replaced with serum-free medium and incubate for additional 24 h. $5x10^4$ PC3 cells for each treatment were allowed to migrate for 24 h (in the presence of Matrigel coating) toward complete growth medium as chemoattractant. **C.** PC3 and PNT-1 cells were incubated with CM from HPFs, MFs and PCaAFs or serum-starved as a control. After 24 h, CM were replaced with serum-free medium and incubate for additional 24 h. An invasion assay was performed as described in B. In both B and C, photographs are representative of several randomly chosen fields and the histograms represent the number of invaded cells shown in five different fields of triplicate experiments. *$p < 0.001$ vs. St Med. **D.** $5x10^5$ PC3 or PNT-1 cells were incubated for 48 h with CM from HPFs, MFs and PCaAFs or serum-starved as a control. CM were then removed and serum-free medium was added for additional 24 h. After the 72 h-total incubation period, cells were lysed and an anti-Met and an anti-E-Cad immunoblots were performed. All the experiments are representative of three others with similar results.

Giannoni E. et al. Supplementary Figure 4



Supplementary FIG.4. Evaluation of the amount of IL-6 in CM derived from *in vitro* and *in vivo* activated fibroblasts and PC3 cells. IL-6 was evaluated by immunoblotting on CM of PC3 cells, of resting fibroblasts (HPFs and NAFs), as well as on CM of HPFs activated for 24 h with 10 ng/ml TGF-β1 (MFs), CM^{PC3} (PCa-AFs) or 50 ng/ml IL-6 (HPFs+IL-6). In addition, MFs, PCaAFs and CAFs were further treated with 50 ng/ml IL-6 to analyze a further increase in the levels of secreted IL-6. In all the treatments, fresh serum-free medium was added for additional 24 h before collection of CM. Results are representative of three others with similar results.

Giannoni E. et al. Supplementary Figure 5



Supplementary FIG.5. MMPs are strumental for CAF-induced cancer cell stemness. PC3 cells were incubated with CM from HPFs, HPFs stimulated with 10 ng/ml TGF-β1 or 50 ng/ml IL-6, CAFs or serum-starved as control. After 24 h, CM were replaced with serum-free medium and incubated for additional 24 h. PC3 cells were allowed to form P0 spheres and individual spheres were dissociated and diluted in 96-well plate (1 cell/well). After 15-20 days the new P1 spheres were counted and plotted. *$p < 0.001$ vs. starv. med. #$p < 0.001$ vs. control HPFs+IL-6 or CAFs. **B.** Prostaspheres obtained in P1 were photographed and a representative image of each treatments is shown. Results are representative of three others with similar results.