Supplemental Information

Heterogeneous cancer associated fibroblast population potentiates neuroendocrine differentiation and castrate resistance in a CD105-dependent manner

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Supplementary Figures

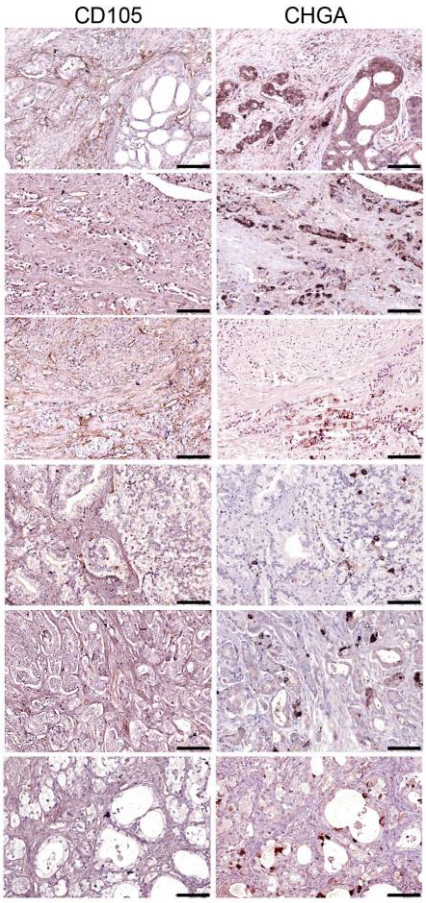

Figure S1 Stromal CD105 expression association with neuroendocrine differentiation of the adjacent epithelia. See also Figure 1. Representative paired serial sections from tissue array cores stained by immunohistochemistry for CD105 or chromogranin A (CHGA) counterstained with hematoxylin. Scale bar represents 100 μm.

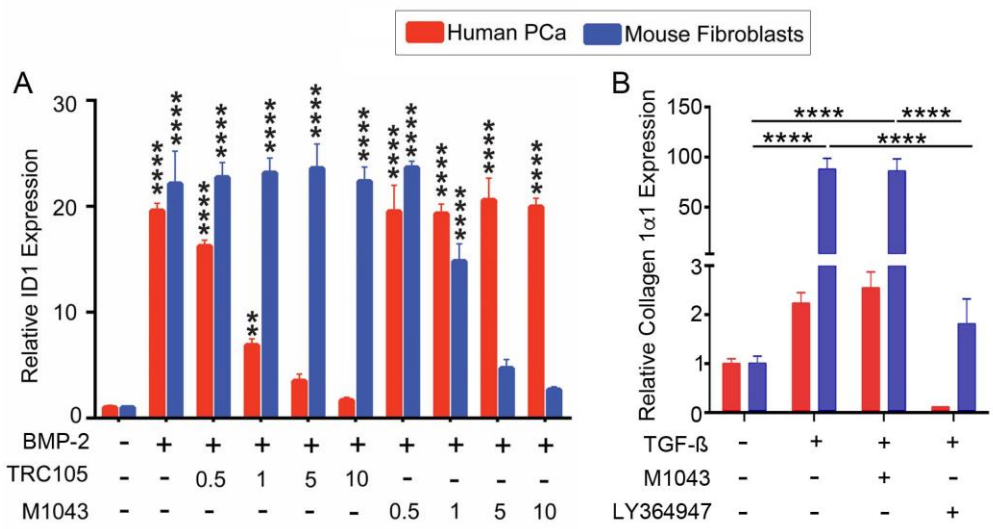

Figure S2 Species specific CD105 antagonists. See also Figure 4.

(A) Bar graph shows relative ID1 mRNA expression of 22Rv1 (Human PCa, red) and mouse wild-type fibroblasts (Mouse Fibroblasts, blue) normalized to control. All cells were pre-treated overnight in serum free (SF) media. The next day, in SF media, an overnight treatment with increasing concentrations of TRC105 or M1043 were given and then treated the following day with 50 ng/mL BMP-2 for 6 hours. Mean +/- SD is shown, n=2. (B) Bar graph shows relative collagen 1α1 mRNA expression of 22Rv1 (Human PCa, red) and mouse wild-type fibroblasts (Mouse Fibroblasts, blue) normalized to control. All cells were pre-treated overnight in serum free (SF) media. The next day, in SF media, an overnight treatment with 10µg/ml TRC105 or 10µg/ml M1043, or LY364947 (10 µM, Sigma Aldrich) were given and then treated the following day with 5 ng/ml TGF-β for 6 hours. Mean +/- SD is shown, n=2. p values of less than 0.05 were considered statistically significant (**P<0.01, and ****P<0.0001). The asterisks indicate significance compared to control, unless otherwise noted by a line.

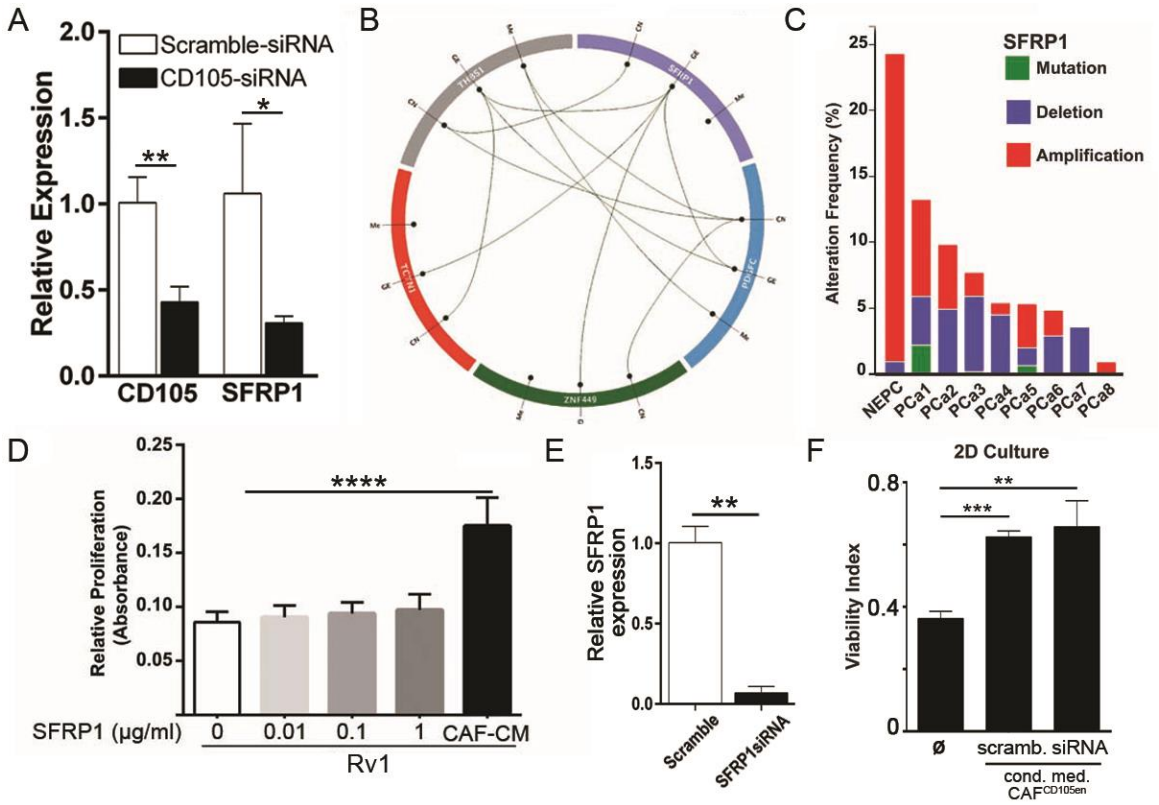

Figure S3 SFRP1 is associated with neuroendocrine differentiation. See also Figure 4.

(A) The siRNA mediated knockdown of CD105 in CAF result in downregulation of SFRP1 expression by quantiative rtPCR. (B) Circus plot, generated using Zodiac (http://www.compgenome.org/ZODIAC), shows the relationship among the genes shown (Zhu et al., 2015). Associations between copy number (CN), gene expression (GE), and methylation (Me) are denoted by lines from one node to another ($p \leq 0.01$). (C) Bar graph shows the alteration frequency of SFRP1 mutations (green), deletions (blue), and amplifications (red) for the indicated TCGA Research Network data sets: NEPC (Trento/Cornell/Broad 2016), PCa1 (FHCRC 2016), PCa2 (MICH), PCa3 (TCGA), PCa4 (TCGA 2015), PCa5 (SU2C), PCa6 (MSKCC 2010), PCa7 (Broad/Cornell 2013), and PCa8 (Broad/Cornell 2012). (D) Bar graph shows relative proliferation of 22Rv1 cells normalized to control when treated with the indicated concentrations of human recombinant SFRP1 or CAF conditioned media (CM) for 72 hr. Mean +/- SD is shown, n=2. (E) Bar graph shows relative SFRP1 expression of $CAF^{CD105en}$ cells. Mean +/- SD is shown. SFRP1-siRNA or scramble control was nucleofected into cells and SFRP1 mRNA expression was analyzed after 72 hours. (F) The knockdown of SFRP1 in $CAF^{CD105en}$ did not affect the affect the viability of 22Rv1 compared to scrambled siRNA. However, $CAF^{CD105en}$-conditioned media elevated the viability of 22Rv1 cells compared to 22Rv1 conditioned media (ø), as determined by MTT assay. P values of less than 0.05 were considered statistically significant (**P<0.01, and ****P<0.0001).

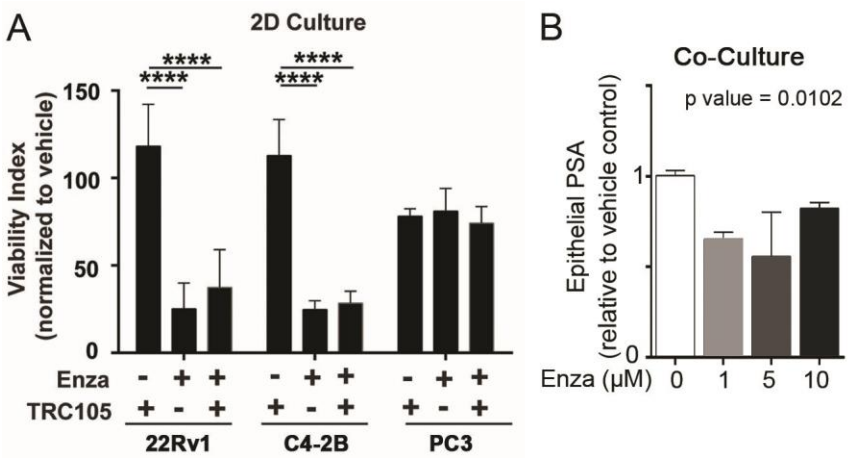

Figure S4 Viability of prostate cancer cell lines. See also Figure 5.
(A) Bar graph shows the relative viability index of 22Rv1, C4-2B, and PC3 cells normalized to vehicle control, mean +/- SD, as determined by MTT assay. All cells were treated as indicated with enzalutamide (5uM) or TRC105 (1 µg/mL) for 72 hours, n=3. (B) Bar graph shows the relative PSA expression by C4-2B cells in a co culture with wild type fibroblasts after 16 hours treatment with enzalutamide (significance calculated by two way ANOVA). p values of less than 0.05 were considered statistically significant (**P<0.01, ***P<0.001, and ****P<0.0001).

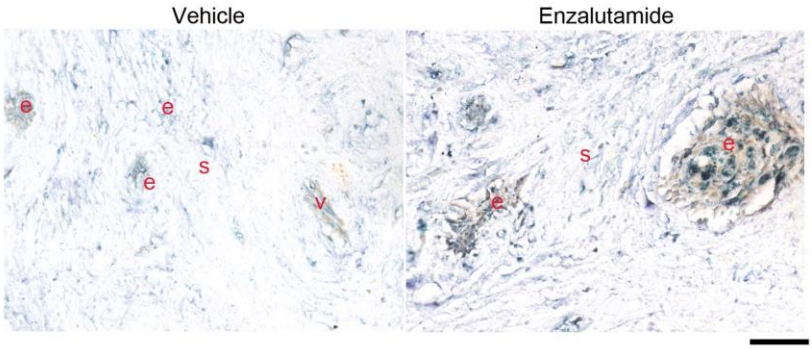

Figure S5 CD105+ stromal cells are adjacent to Chromogranin A+ epithelia. See also Figure 5.
Immunohistochemistry for representative PDX tumor sections. Chromogranin A immune-localization (brown) double stained with CD105 (blue) is shown (scale bar represents 32 μm), n > 10. The epithelia (e), vasculature (v), and stroma (s) are indicated.

Supplemental Experimental Procedures

Cell lines and culture

22Rv1, C4-2B, and PC3 cells were purchased from ATCC and expanded as directed. C4-2 were given as a gift from Dr. Leland Chung. Cell lines were authenticated by STR profiling and routinely checked for mycoplasma. TRC105 and M1043 were provided by TRACON Pharmaceuticals, Inc. (San Diego, CA). Cells were treated with TRC105 or M1043 (1 µg/mL, TRACON Pharmaceuticals, Inc., San Diego, CA) and enzalutamide (Xtandi, 5 µM, Medivation, San Francisco, CA), for 72 hours unless otherwise specified. SFRP1 siRNA pool (sc-39998, Santa Cuz Biotechnology), CD105 siRNA pool (sc-35302, Santa Cruz Biotechnology) and scrambled (sc-36869, Santa Cruz Biotechnology) were used at 100 nM and electroporated in primary CAF.

CAF conditioned media

CAF were plated at a density to reach confluence at the end of 72 hours in normal culture media as previously reported (Franco et al., 2011; Qi et al., 2013). After 72 hours the media was collected, centrifuged to remove cell debris, and supernatant was used fresh or stored at -80^{o}C. Target cells were treated with 50% conditioned media in combination with 50% control media.

Histopathology and immunohistochemistry

Paraffin embedded tissues were sectioned (5 µm thick) were subjected to hematoxylin and eosin (H&E) staining and immunohistochemistry as previously reported (Placencio et al., 2008). Serial sectioned tissue arrays of prostate cancer were purchased from US Biomax, Inc. Anti-phosphorylated histone H3 (06-570, Millipore), anti-CD105 (NCL-CD105, Leica Microsystems), anti-Ki67 (ab16667, Abcam), and anti-chromogranin A (sc-13090, Santa Cruz Biotechnology), anti-SFRP1 (601-401-475S, Rockland Immunochemicals), and anti-survivin (2808, Cell Signaling) antibodies were incubated at 4°C overnight. Secondary antibody development was performed with Dako Cytomation mouse or rabbit kits and visualized using 3,3'-diaminobenzidine tetrahydrochloride substrate. For double immunohistochemistry, the second primary antibody was visualized using TrueBlue peroxidase substrate (71-00-64, KPL). TUNEL staining was performed per manufacturer's protocol (S7100, Millipore). Slides were scanned with a Leica Biosystems Aperio AT. Up to five fields per tissue were quantitated with Fiji (ImageJ) using a custom written macro. Mitotic and death index were quantitated by taking the total number of positively stained nuclei divided by the total number of nuclei. Chromogranin A staining index was quantitated by taking the total number of positively stained pixels divided by the total tissue area.

siRNA knockdown

SFRP1 siRNA pool (sc-39998, Santa Cuz Biotechnology) and scramble (sc-36869, Santa Cruz Biotechnology) were used at 100 nM and electroporated in primary CAF. Media was changed the following day to remove any cell debris.

RNA analysis

Total RNA was extracted using the RNeasy kit (Qiagen). 1 µg RNA was used for cDNA synthesis using iSCRIPT cDNA synthesis kit (1708891, Bio-Rad). Quantitative RT-PCR was performed with 3 replicates using the Step One Real-Time PCR system (Applied Biosystems). Gene mRNA expression was normalized to GAPDH. Primer sequences can be found in the Table S1. For RNA-sequencing, Ion Proton AmpliSeq Transcriptome RNA Sequencing was performed achieving an average of 3M reads. We mapped an average of 88% of the reads to the human genome with Torrent Suite version 4.4.2. Gene enrichment analysis was performed with the PANTHER gene ontology (GO) package for secreted genes.

Immunoblot Assay

Primary antibodies against phosphorylated mTOR (S2448, Cell Signaling), and b-actin (sc-47778, Santa Cruz Biotechnology) were used to assess expression by protein gel electrophoresis using a 10% gel. Gels were transferred to a nitrocellulose membrane and incubated with the indicated primary antibodies. Proteins were detected using alkaline phosphatase.

MTT proliferation assay

3000 cells per 96-well were treated for 72 hours using 5 wells per treatment. MTT reagent (M6494, Life Technologies) was prepared as directed, incubated for one hour at 37 ^{o}C, and analyzed using manufacturer's recommendations.

Supplementary References

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