Supplementary Materials: Materials and Methods

Immunocytochemistry

Following fixation, constructs were washed with PBS and stored at 4°C until staining. Constructs were immersed in 0.2% (v/v) Triton X-100 for 5 min at room temperature to permeabilize cells, then blocked in 1 mL of 3% (v/v) BSA and 0.2% Triton X-100 in PBS at 4°C overnight on a rocking platform shaker. Primary and secondary antibodies were diluted in 3% BSA and 0.2% Triton X-100. Anti-EpCAM antibody (2929S, Cell Signaling Technology Inc., Danvers, MA), anti-FGFR-1 antibody (9740P, Cell Signaling Technology Inc.) and anti-Ki-67 antibody (NB110-89717, Novus Biologicals Inc., Littleton, CO) were used at 1:200. Anti-vimentin antibody (ab92547, Abcam Inc., Cambridge, MA) was used at 1:500, and anti-FGF9 (ABN41, EMD Millipore Corp., Billerica, MA) was used at 1:100. Hydrogel constructs were incubated in 1 mL of the diluted antibody constructs at 4°C overnight on a rocking platform shaker. Samples were washed three times with 3% (v/v) BSA in 0.2% (v/v) Triton X-100 before the addition of fluorophore-labeled secondary antibodies (AlexaFluor series, Life Technologies) directed against the appropriate host. Then 1 mL of secondary antibodies diluted 1:500 was added to each construct, and samples were incubated at 4°C overnight on a rocking platform shaker. Samples were then washed with 3% (v/v) BSA in 0.2% (v/v) Triton X-100 at least three times before the addition of DAPI (5 μg/mL) in PBS. All immunofluorescence images were captured using a Nikon A1-Rsi confocal microscope. Tissue sections of MDA PCa 118b PDX in bone were processed and immunostained for FGFR1 and FGF9 as previously described (*25, 17*). Images were obtained with a Zeiss Axio Imager Z2 microscope.

Quantification of Tumoroid Size

Mono- and co-culture constructs (n=2) collected at D1, 3, 6 and 9 were fixed and immunostained for EpCAM. The major axes of at least 60 tumoroids (between 60-380 tumoroids) were measured, and the distributions of tumoroid size were plotted.

Cell viability and growth

Cell-hydrogel constructs (n = 4) were collected at the designated time points and stored at -80°C until all samples were collected. Frozen samples were thawed at room temperature, immersed in 1 mL of Milli-Q water, and mechanically dissociated using an 18-gauge needle and 1-mL syringe. Samples were subjected to three cycles of freeze-thaw and ultrasonicated. The liquid supernatant was then assayed using the Quant-iT PicoGreen dsDNA quantification assay (Life Technologies) per the manufacturer's instructions. Acellular hydrogel constructs served as blank controls. Fluorescence at excitation and emission wavelengths of 485 and 528 nm, respectively, was measured using a FLx800 fluorescence microplate reader (BioTek Instruments, Winooski, VT). DNA content of each sample was obtained by comparison with a calibration standard curve of lambda DNA.

Quantitative PCR

Complementary (cDNA) was prepared using the qScriptTM cDNA SuperMix (Quanta BioSciences, Gaithersburg, MD). Real-time RT-PCR with SYBR Green dye (Life Technologies) and species-specific primers (*17*) were used for cDNA amplification. Primer sequences for components of the FGF axis can be found in our previously reported study (*17*). For mouse-specific osteoblast markers, the primers used were as follows: OC (Forward: GCT CTG TCT CTC TGA CCT CA; Reverse: TGG ACA TGA AGG CTT TGT CA), ALP (Forward: AGC TCA ACA CCA ATG TAG CC; Reverse: GTA

GCT GGC CCT TAA GGA TT), and BSP (Forward: CCT ACT TTT ATC CTC CTC TG; Reverse: CTC CTC TTC GGA ACT ATC GC). Relative transcript levels were determined using the $2^{-\Delta CT}$ method, using GAPDH as internal gene control. The thermal cycling program was as follows: 95°C for 10 min, 40 cycles of 95°C for 15 s followed by 60°C for 1 min, then 1 cycle of 95°C for 15 s followed by 60°C for 15 s.

Supplementary Materials: Figures

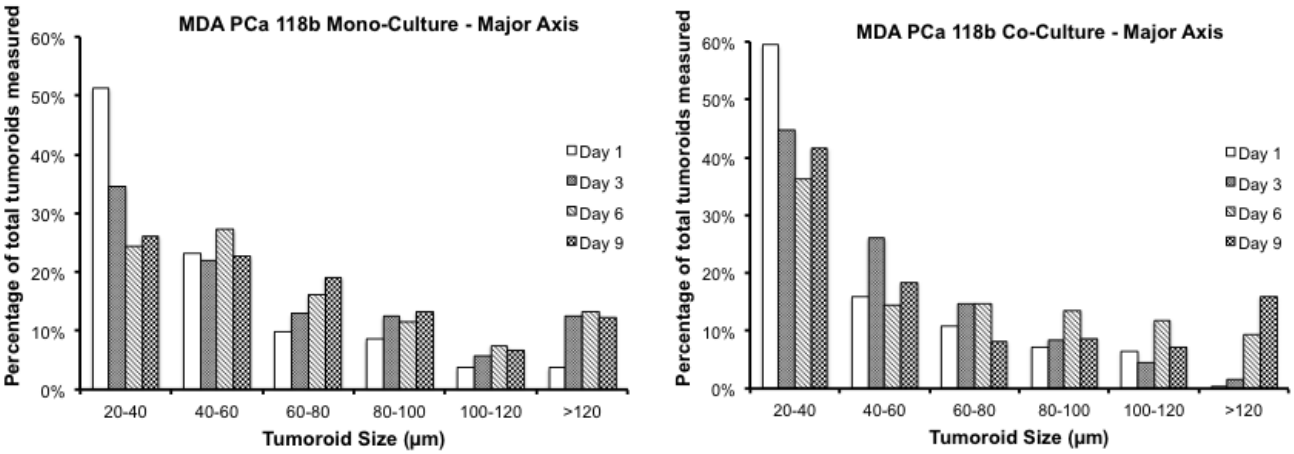

Fig. S1. Tumoroid size distribution of mono- and co-cultures over time.

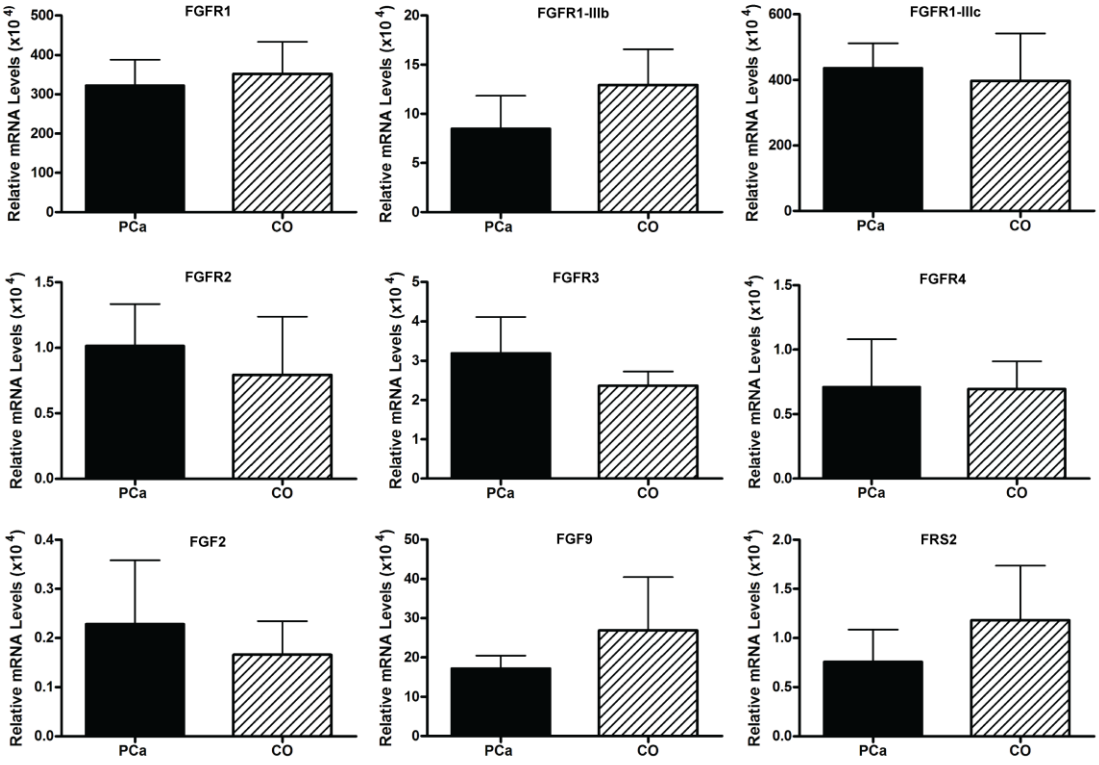

Fig. S2. Transcripts encoding FGF signaling components in the MDA PCa 118b cells in mono-culture (PCa) or co-culture (CO), relative to GAPDH.

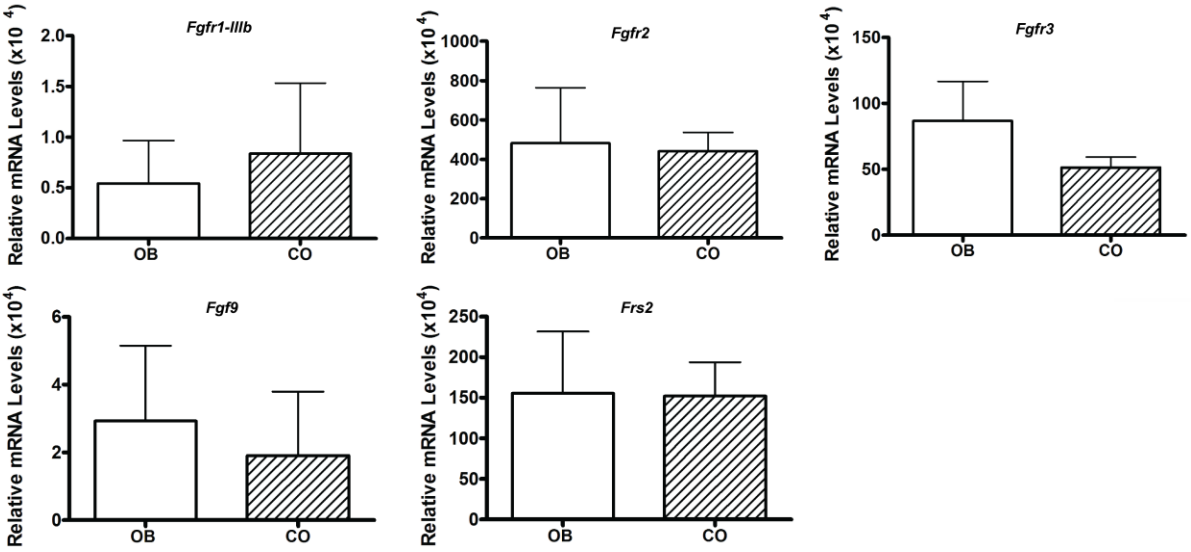

Fig. S3. Transcripts encoding FGF signaling components in the MC 3T3-E1 osteoblastic cells in mono-culture (OB) or co-culture (CO), relative to GAPDH.