Supplementary Figure Legends

Supplementary Figure 1. Characterization of anti-MFAP5 monoclonal antibodies. ELISA based epitope mapping result showed that **(A)** anti-MFAP5 mAb clone 64A and 117B recognized the same human MFAP5 protein sequence (DETVLAVLA), while **(B)** clone 130A is specific to a consensus peptide sequence (LCRQMAGLPPRR) common for both human and murine MFAP5 proteins. Kinetic assays showed that the dissociation constant (K_d) of clone **(C)** 64A and **(D)** 117B for human MFAP5 protein were at 0.48nM and 6.7nM respectively, suggesting a high binding affinity to human MFAP5 protein.

Supplementary Figure 2. Characterization of MFAP5 overexpression ovarian fibroblast line NOF151 LvMFAP5. Quantitative real-time PCR analysis on NOF151, NOF151 LvMFAP5 and seven primary CAF cultures showed that MFAP5 expression level of NOF151 LvMFAP5 is approximately 40 times higher than its parental line NOF151 and is comparable to the primary CAF cultures tested (Range= 12.44-55.99 times higher than NOF151; Mean= 29.78 times higher than NOF151; Standard deviation=17.30).

Supplementary Figure 3. Prognostic significance of stromal MFAP5 expression in pancreatic ductal adenocarcinoma. (A) Immunolocalization of stromal MFAP5 on tumor tissue samples and the corresponding normal adjacent tissue samples from 64 PDAC patients showed that while the majority of normal adjacent tissue is negative for MFAP5 expression, expression levels of MFAP5 was significantly higher in pancreatic CAFs ($P<0.001$). **(B)** Kaplan-Meier survival analysis and log-rank test showed that high stromal MFAP5 expression in patients with PDAC is significantly associated to the reduction of overall survival duration. Using median expression level as the cut off, pancreatic cancer patients expressing high level of stromal MFAP5 had a median survival duration of 8 months (95%CI = 6.0 – 10.0 months), whereas patients expressing

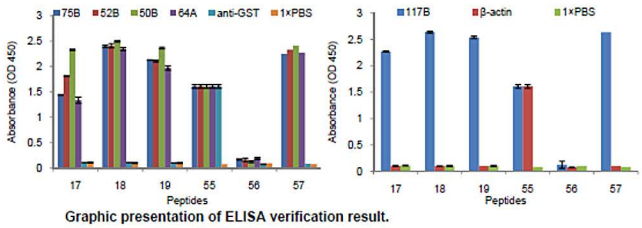
low level of stromal MFAP5 had a median survival duration of 31 months (95%CI = 11.3 – 50.8 months) (N=91, P=0.001)

Supplementary Figure 4. MFAP5-targeting monoclonal antibody clones 64A and 117B suppress ovarian cancer progression in mice. To evaluate the therapeutic efficacy of antibody clones 64A and 117B, which target MFAP5 of human origin. OVCA3, a MFAP5 expressing human ovarian cancer cell line, was used. Luciferase labeled OVCA3 cancer cells were intraperitoneally injected into nude mice and animals were subsequently treated with either 64A, 117B or control normal mouse IgG at the dosage of 15mg/kg. Animal study results showed that treatment of anti-MFAP5 monoclonal antibody clones 64A and 117B significantly suppressed OVCA3 ovarian tumor growth in mice ($P<0.001$ and $P=0.0029$ respectively).

Supplementary Figure 5. MFAP5-targeting monoclonal antibody reduces cancer fibrosis in pancreatic cancer-bearing mice. Picrosirius red staining of collagen on tumor tissues obtained from PDAC PDX tumor-bearing mice treated with 130A antibody or the control IgG showed that tumors in mice treated with 130A had significantly lower collagen coverage and intensity in cancer associated stromal tissue than in those treated with IgG.

Supplementary Figure 6. Presence of fibrosis at the time of MFAP5-targeting monoclonal antibody treatment initiation. Picrosirius red staining of collagen on tumor tissues obtained from OVCA432 tumor-bearing mice at 2 weeks after initial ovarian cancer cell injection confirmed the presence of collagen I positive stroma within the tumor tissue, suggested that antibody treatment was likely started after the onset of fibrosis.

A



Graphic presentation of ELISA verification result.

No.	Peptide
17	VNDPATDETVLAVLA
18	PATDETVLAVLADIA
19	DETVLAVLADIAPST

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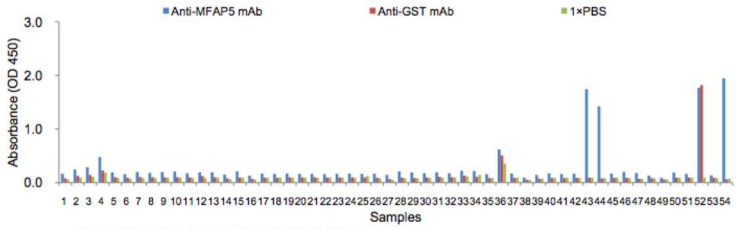
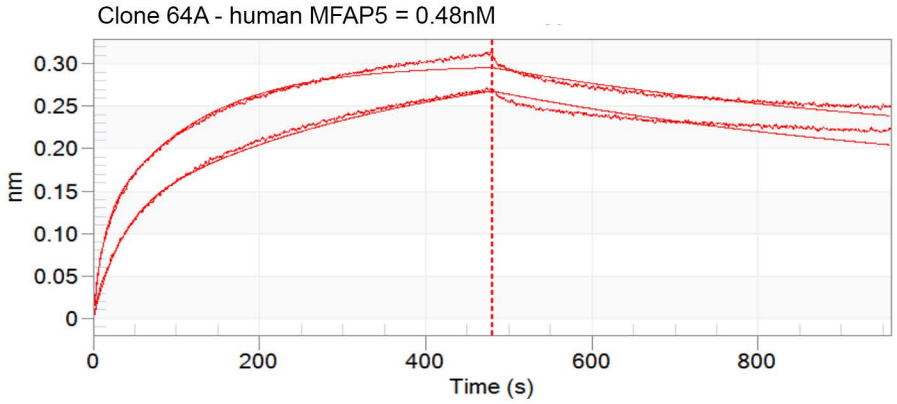


Graphic presentation of ELISA results

Epitope of anti-MFAP5 antibody

No.	Peptide
43	KDELSRQMAGLPPRR
44	LSRQMAGLPPRRLRR

C



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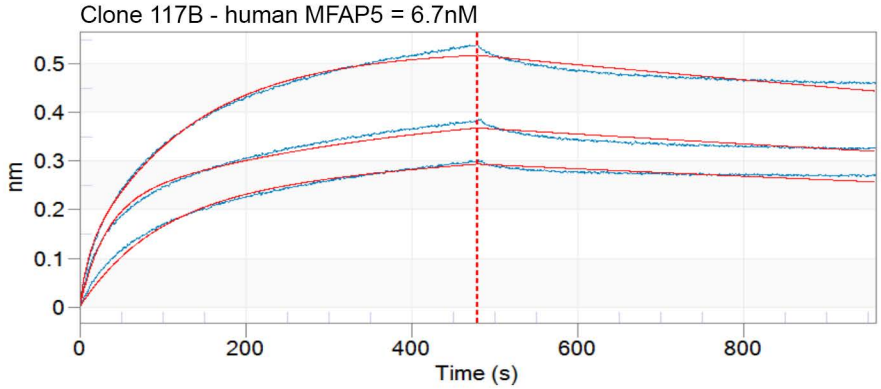
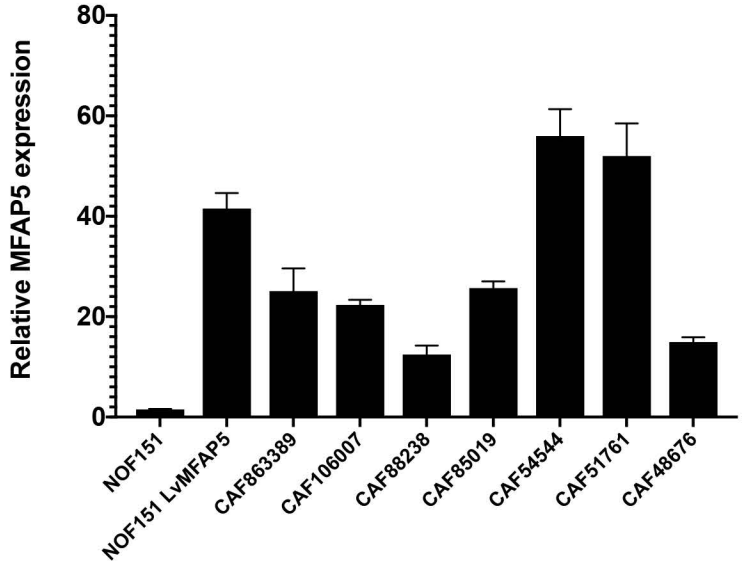


Supplementary figure 1



Supplementary Figure 2

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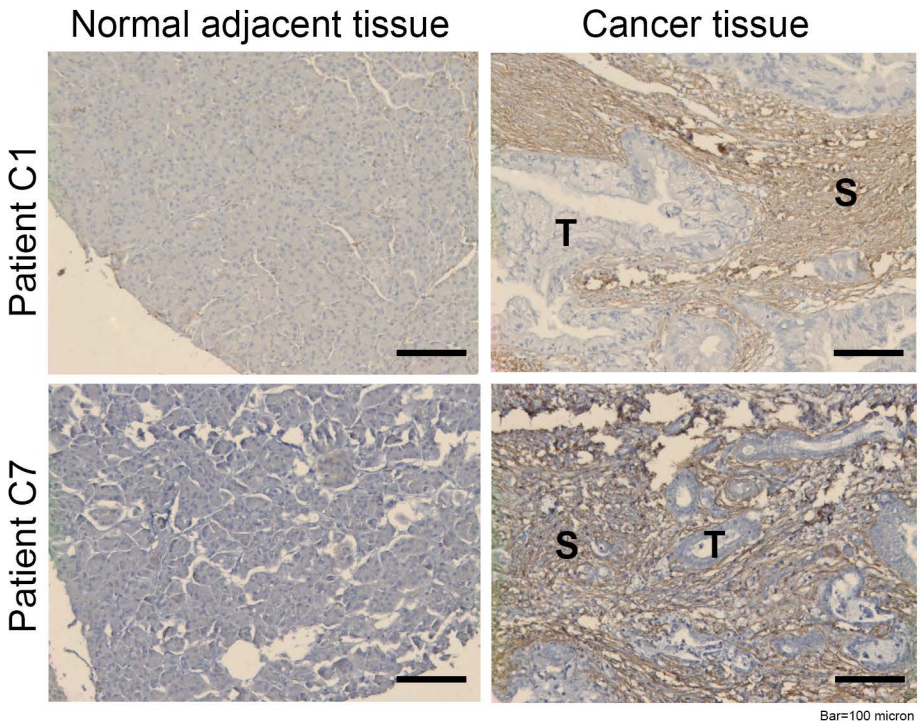

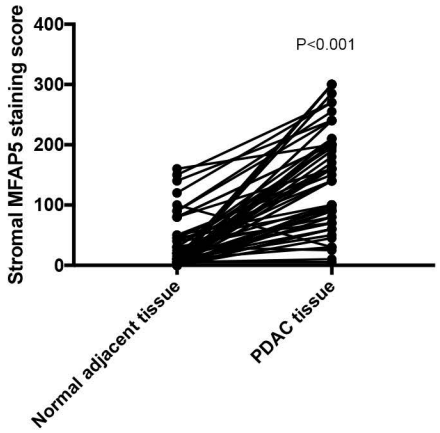

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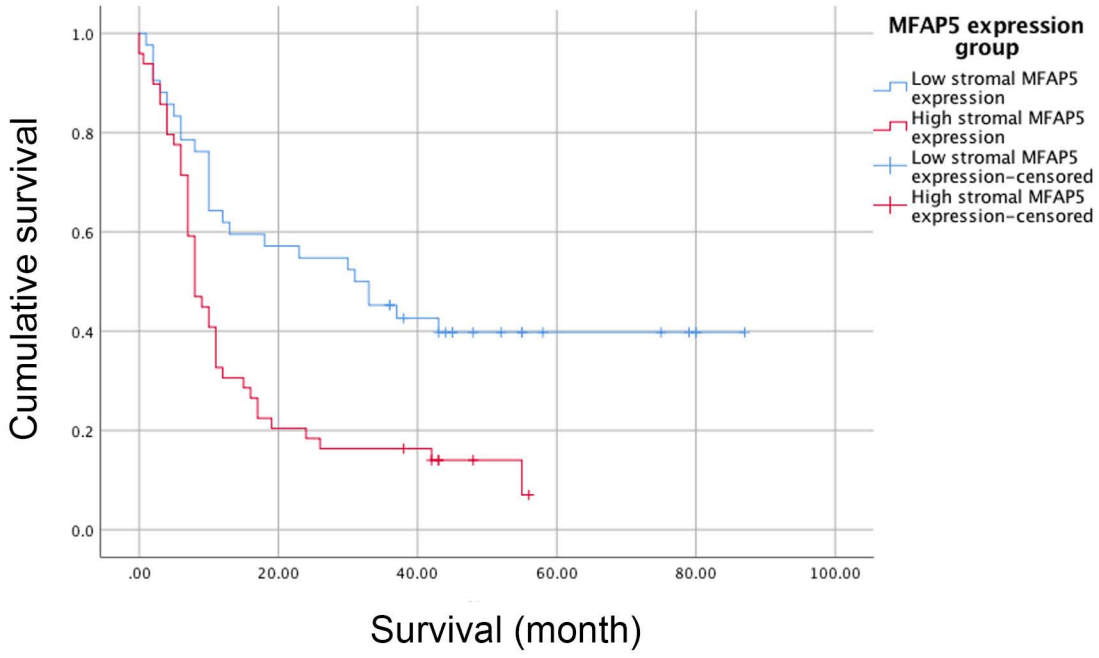

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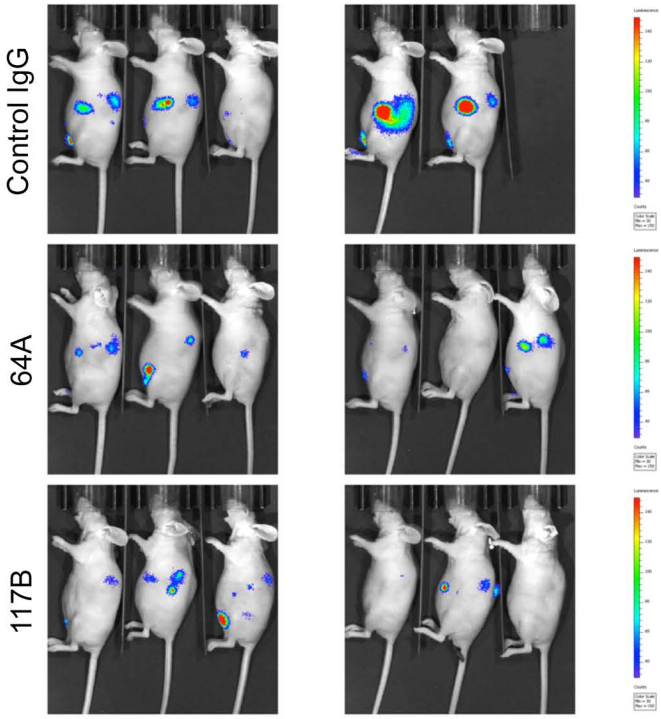

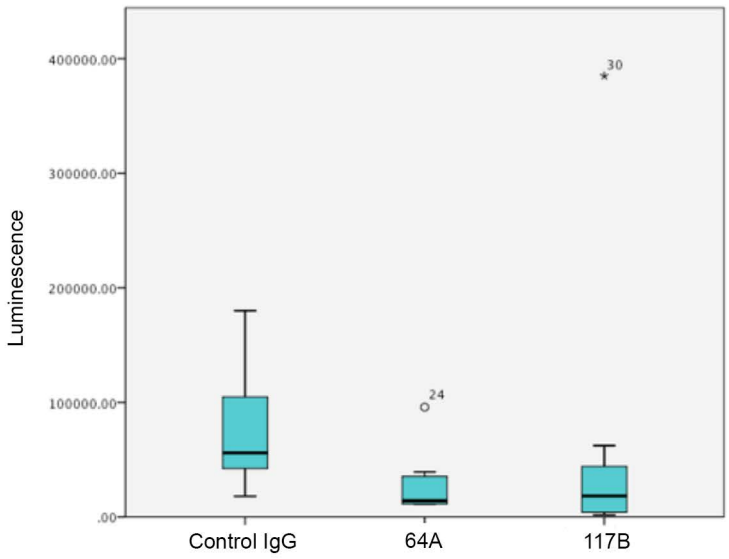

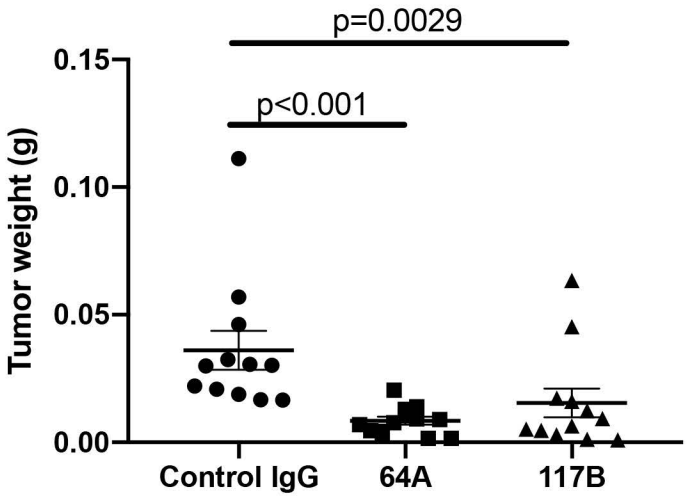

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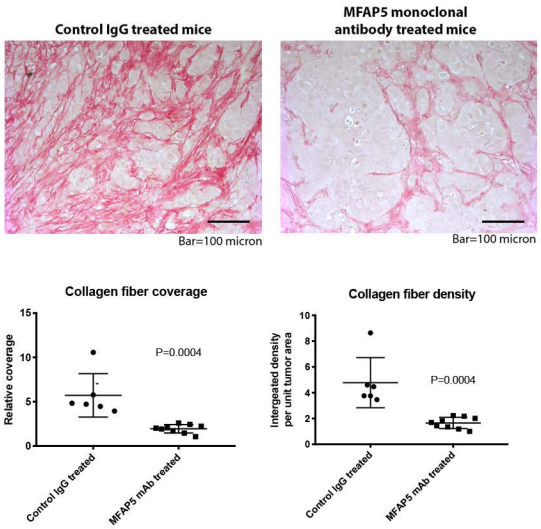
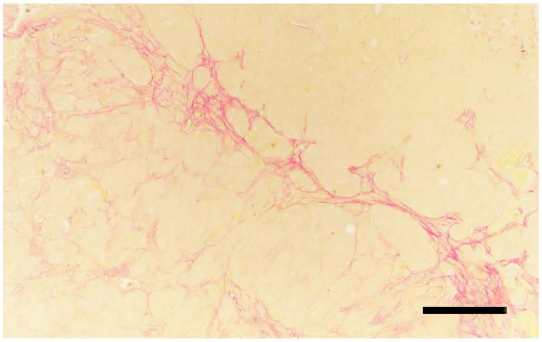


Supplementary figure 5



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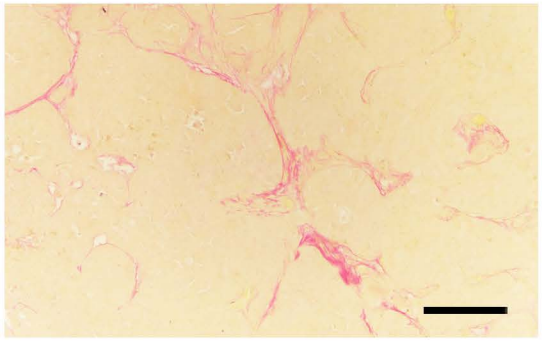

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Supplementary Figure 6

Supplementary Materials and Methods

Cell lines and culture conditions. OVCA432, A224 and ALST ovarian cancer cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. hMEC-1 cells were cultured in MCDB131 medium supplemented with 10% fetal bovine serum, 10 mM L-glutamine, 10 ng/mL epidermal growth factor, 1 μg/mL hydrocortisone, and 1% penicillin/streptomycin. TIME cells were cultured in EGM-2 medium (Lonza). SVEC4-10 and PANC1 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin.

Generation of luciferase expression OVCA432 cell line. OVCA432 ovarian cancer cell line was a gift from Dr. Robert Bast from the Department of Experimental Therapeutics at MD Anderson Cancer Center. The luciferase labeled clone was generated by introducing a constitutively expressed luciferase construct via lentiviral transduction. Both the parental cell line and the luciferase expressing clone were authenticated by the MD Anderson Cell Line Characterization Core.

Mice for antibody development. BALB/c mice for antibody development were purchased from Charles River. Mice were housed in a pathogen-free animal facility according to institutional guidelines. All animal studies were conducted under an approved protocol by the Institutional Animal Care and Use Committee (IACUC).

ELISA Screening of monoclonal antibodies. Costar EIA/RIA plates (Fisher Scientific, Hampton, NH) were coated with 0.1ug/ml of protein or negative control and allowed to dry overnight. Wells were blocked by incubation in PBST containing 2% bovine serum albumin for 1 hour at room temperature. Culture supernatant from hybridoma plates (100 µl) was then added, incubated for 1 hour at room temperature and then washed with PBST 3 times. Goat anti-mouse immunoglobulin G (IgG) Fc, horseradish peroxidase (HRP) conjugate (100 µl; Jackson Immunoresearch) was then added, incubated at room temperature for 1 hour and washed 5 times with PBST before the substrate was added. Absorbance was read at 450nm.

Epitope mapping of anti-MFAP5 antibodies. An overlapping peptide library, which was synthesized based on the sequence of the human and murine MFAP5 protein, and ELISA were used to identify epitope(s) recognized by anti-MFAP5 antibody clones 64A, 117B and 130. The peptide library was designed in the way that each peptide is 15 amino acids long with 12 amino acids overlapping its adjacent peptides. All cysteines in the peptides were replaced with serines. All peptides were synthesized with N-terminal biotinylation and were dissolved in DMSO to a final concentration of 10 mg/mL. The biotinylated peptides were captured in 96-well microtiter plates, which were pre-coated with streptavidin. The plates were then incubated with blocking buffer at 37°C for 2 hours. After washing the plates with PBST, 100 µL of anti-MFAP5 antibodies (1 µg/mL) or control antibodies (1 µg/mL) were added to the plates and incubated for 2 more hours at 37°C. The plates were washed with PBST and incubated with 100 µL of secondary antibody (0.1 µg/mL goat HRP-conjugated anti-mouse IgG) at 37°C. After 1 hour incubation, the plates were washed with PBST. The color was developed with TMB substrate for 10 minutes and the reaction was stopped by adding 100 µL of 1 M hydrochloric acid. The absorbance at 450 nm was measured using a spectrometer. The peptide epitope, which can be detected by a known antibody, was used as the positive control. An uncoated well was used as the blank control; and the peptide library incubated with the secondary antibody only was used as a negative control. Peptides that

exhibited positive binding (signal to noise ratio >2) were considered as the epitopes of the antibodies.

Kinetics of Antibody binding affinity using an Octet® platform (bio-layer interferometry). All bio-layer interferometry (BLI) measurements were performed at UT-MDACC-MAF on a FortéBio Octet RED 384 instrument using anti-mouse Fc capture (AMC) biosensors that immobilize and subsequently characterize the binding kinetics of the antibodies. Opaque 96-well plates were purchased from Pall-FortéBio. Samples or buffer were dispensed into 96-wells at a volume of 100 μl per well. The assay buffer (PBS + 0.1% BSA + 0.02 % Tween 20) and regeneration solution (10 mM glycine, pH 2.0) were used. Operation was maintained at room temperature. Antibodies were diluted to 20 ug/mL and captured on each of the eight AMC biosensors. The binding affinities of the antibodies were measured using the following concentration range of antigens or a monoclonal murine IgG isotype control: 0, 13, 26, 52.1, 104.2, 208.3, 416.7, and 833 nM. Dissociation was monitored for around 7 min. Between measurements, the biosensor surfaces were regenerated. Each data set was fitted globally to a 1:1 or 1:2 interaction models, to determine the kinetic parameters. Data are representative of two independent experiments. They were generated automatically by the Octet® User Software (version 8.1) and was subsequently analyzed.

Monoclonal anti-MFAP5 antibody attenuation of ovarian cancer cell motility. Immortalized normal human ovarian fibroblasts (NOF151) or MFAP5-overexpressing fibroblasts (NOF151 LvMFAP5) were seeded onto each well of a 24-well plate (1×10^5 cells/well) in serum-free media and incubated at 37°C for 3 days. Subsequently, they were incubated with 10 μg/mL control mouse IgG, anti-MFAP5 MAb clone 64A, or anti-MFAP5 MAb clone 117B for 1 h. After incubation, 5×10^4 A224 or ALST ovarian cancer cells in 8-μm porous cell culture inserts were incubated with fibroblasts for 15 hours. To visualize cell migration, cancer cells were stained with calcein AM.

Nonmigrated cells in the cell culture inserts were removed, and the cells that migrated through the porous cell culture membrane were quantified by obtaining images of the stained cells in nine random fields of view per membrane using fluorescent microscopy and the Image-Pro Plus software program (version 7.0).

Monoclonal anti-MFAP5 antibody attenuation of microvascular endothelial cell motility. Immortalized normal human ovarian fibroblasts (NOF151) or MFAP5-overexpressing fibroblasts (NOF151 LvMFAP5) were seeded onto each well of a 24-well plate (1 x 10^5 cells/well) in serum-free media and incubated at 37 °C for 3 days. Subsequently, they were incubated with 10 μg/mL control mouse IgG, anti-MFAP5 MAb clone 64A, or anti-MFAP5 MAb clone 117B for 1 h. After incubation, 5 x 10^4 hMEC-1 or TIME cells were seeded onto each 8-μm porous cell culture insert (BD Biosciences) in serum-free media and incubated with control fibroblasts or MFAP5-overexpressing fibroblasts in the presence of IgG or an anti-MFAP5 antibody for 4 hours followed by quantitation of cell migration.

To evaluate the inhibitory effect of anti-MFAP5 antibody clone 130A on mouse endothelial cell motility, motility assays were performed on a SV40-transformed mouse endothelial cell line SVEC4-10 (American Type Culture Collection; #CRL-2181) using Boyden chambers. Serum-free medium with or without 100 ng/mL recombinant murine recMFAP5 was preincubated with 10 μg/mL control IgG or clone 130A for 1 hour in the wells of a 24-well plate. After antibody preincubation, 8-μm porous cell culture inserts (BD Biosciences) were placed in each of the wells, and 5 x 10^4 SVEC4-10 cells were seeded onto the inserts in serum-free media and incubated for 4 hours followed by quantitation of cell migration.

Monoclonal anti-MFAP5 antibody attenuation of pancreatic cancer cell motility. To evaluate the inhibitory effect of anti-MFAP5 antibodies pancreatic cancer cell motility, motility assays were

performed on PANC1 human pancreatic cancer cells using Boyden chambers. Serum-free medium with or without 100 ng/mL recombinant murine recMFAP5 was preincubated with 10 μg/mL control IgG, clone 64A, clone 117B or clone 130A for 1 hour in the wells of a 24-well plate. After antibody preincubation, 8-μm porous cell culture inserts (BD Biosciences) were placed in each of the wells, and 5 x 10^4 PANC1 cells were seeded onto the inserts in serum-free media and incubated for 12 hours followed by quantitation of cell migration.

Toxicity testing of anti-MFAP5 antibodies. Nude mice were treated with anti-MFAP5 antibody clone 64A, 117B, or 130A (experimental groups) or PBS (control group; three mice/group) twice weekly for 2 weeks. Complete blood counts and chemistry panels were performed to measure the levels of albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, globulin, and total proteins in serum samples collected from the mice. In addition, hematoxylin- and eosin-stained tissue sections of major organs such as liver, kidney and spleen from the mice were examined to evaluate and identify any possible organ or tissue toxicity owing to the anti-MFAP5 antibody treatment.

Improved efficacy of ovarian cancer therapy with the combination of an anti-MFAP5 antibody and paclitaxel. Athymic nude mice were intraperitoneally injected with 3 x 10^6 luciferase-labeled OVCA432 cells 1 week before randomization into three treatment groups: 1) paclitaxel, 2) control IgG plus paclitaxel, and 3) anti-MFAP5 antibody clone 130A plus paclitaxel. In the following 2 weeks, mice in groups 2 and 3 were intraperitoneally injected with 15 mg/kg of control IgG or clone 130A twice a week for 2 weeks to normalize the structurally and functionally abnormal tumor vasculatures. Subsequently, weekly intraperitoneal injection of 3 mg/kg paclitaxel was added to the treatment schedule, and the combination therapy was administered for 4 weeks before tumor volumes were quantified using an IVIS 200 bioluminescence and fluorescence imaging system. In parallel with the 4-week combination study, weekly single-agent treatment

with paclitaxel (3 mg/kg) was administered via intraperitoneal injection to group 1 for 4 weeks. At week 8, the animals were sacrificed, and their tumor weights and *ex vivo* tumor luminescence signals were recorded. One hour prior to necropsy, five mice in each group were injected with either FITC-dextran or Oregon Green 488-conjugated paclitaxel for the evaluation of tumor vessel leakiness and paclitaxel bioavailability. Tumors collected were snap frozen or fixed in formalin, and processed for histological evaluation.

Picrosirius red staining of collagen in tissue samples. Coverage and density of collagen in tumor tissue samples harvested from animal studies were visualized by picrosirius red staining. Deparaffinized and rehydrated FFPE tissue sections were first stained with Weigert's haematoxylin for 8 minutes, followed by washing in running distill water for 10 minutes. Subsequently, tissue sections were stained in picrosirius red for one hour. Stained slides were washed in two changes of acidified water (0.5% acetic acid) and then dehydrated in three changes of 100% ethanol prior to mounting. Coverage and density of collagen in tumor tissue samples was quantified using the Image-Pro Plus software program (version 7.0).

Anti-MFAP5 antibody clone 130A abrogated MFAP5-induced collagen expression in ovarian fibroblast. To evaluate the effect of MFAP5 on collagen expression in CAFs, qRT-PCR analyses on two of the key fibrosis-related genes, COL1A1 and COL11A1 were performed on fibroblasts treated with 200ng/mL exogenous MFAP5 or control diluent. To demonstrate the inhibitory effect of the 130A antibody on MFAP5-induced collagen expression, CAFs were treated with 200ng/mL exogenous MFAP5 protein in the presence of 130A anti-MFAP5 antibody or in the presence of control IgG, followed by expression analysis of COL1A1 and COL11A1by qRT-PCR.