Attieh et al., https://doi.org/10.1083/jcb.201702033

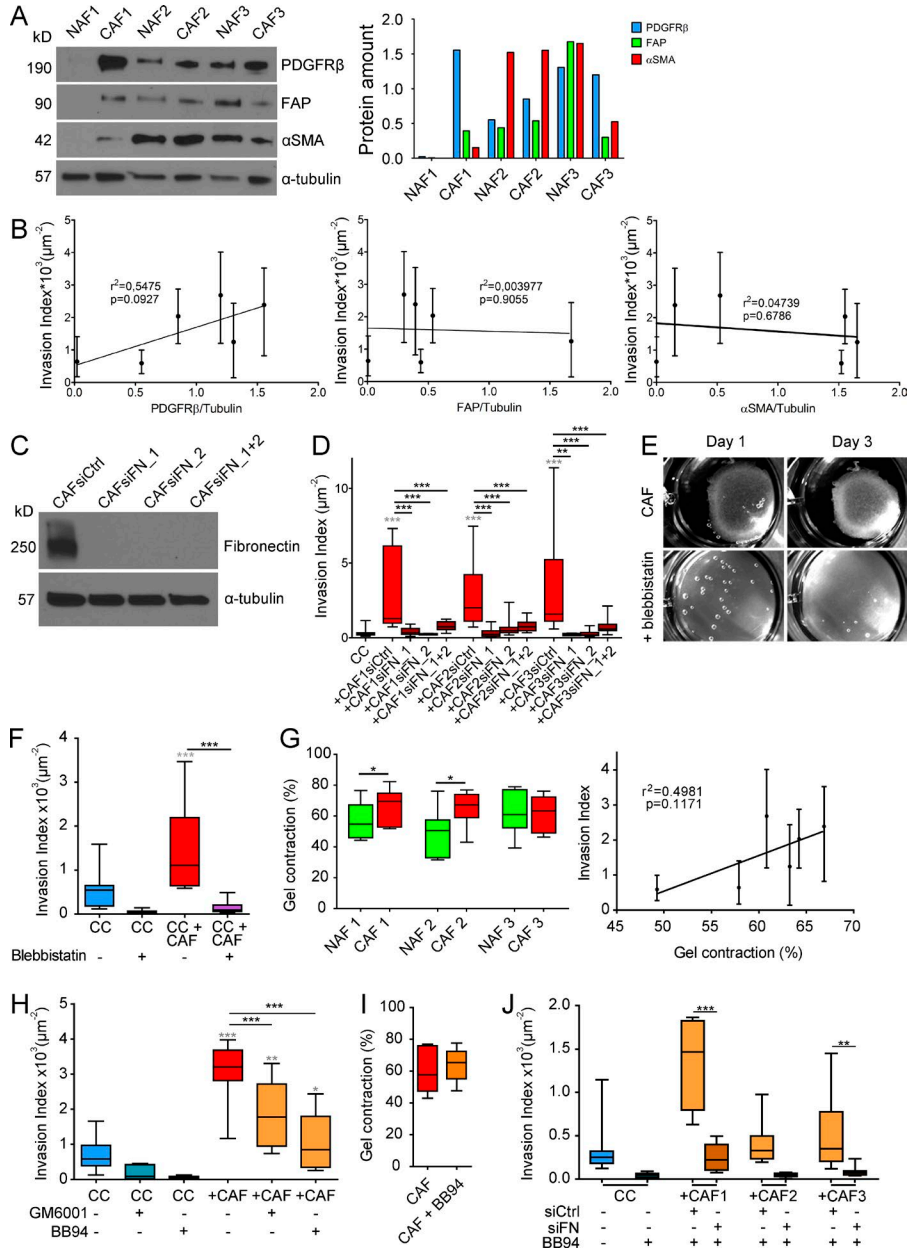


Figure S1. **CAFs remodel the ECM to induce cancer cell invasion.** (A) Immunoblot analysis. Lysates prepared from NAFs and CAFs were probed against α-SMA, FAP, and PDGFR-β antibodies. α-Tubulin served as a loading control. The protein amount was calculated by normalizing α-SMA, FAP, and PDGFR-β amounts to the α-tubulin amount. Results are represented as column bars. (B) Scatter dot graphs correlating the invasion index of cancer cells in the presence of NAFs and CAFs with the amount of α-SMA, FAP, and PDGFR-β in fibroblasts. Quality of linear regression is represented by the values of p and r^2. (C) Immunoblot analysis. Lysates prepared from CAFs treated with siRNA scrambled control and CAFs treated with two different siRNAs against FN, alone or together, were probed with FN antibody. α-Tubulin served as a loading control. (D) Extended data from Fig. 2 A. Quantification of cancer cell invasion alone or in the presence of CAFs from all patients, depleted or not for FN. (E) CAFs embedded in collagen plugs for 1 d and 3 d, with or without blebbistatin treatment. (F) Quantification of cancer cell invasion in the presence of CAFs from patient 1, with or without blebbistatin treatment. (G, left) percentage of gel contraction between all fibroblast couples calculated using the formula 100 × [gel area (T0) – gel area(T1)]/gel area (T0). (Right) Scatter dot graph correlating the invasion index of cancer cells in the presence of fibroblasts with the contractility of fibroblasts. Quality of linear regression is represented by the value of p and r^2. (H) Quantification of cancer cell invasion alone or in the presence of CAFs from patient 1 without treatment, with GM6001 treatment, or with BB94 treatment. (I) Percentage of gel contraction between control CAFs and BB94-treated CAFs from patient 1 calculated using the formula 100 × [gel area (T0) – gel area(T1)]/gel area (T0). (G and I) P-value was calculated using a paired *t* test for $n = 3$ over $n = 6$ separate experiments. (J) Extended data from Fig. 2 H. Quantification of cancer cell invasion alone or in the presence of control or FN-depleted CAFs from all patients and treated with BB94. (D, F, H, and J) Invasion index is defined as the ratio between the number of invading nuclei and the area of the spheroid contour. Results are expressed as box and whiskers (minimum to maximum) of at least $n = 3$ separate experiments. P-values are compared with cancer cells alone (in gray) and to cancer cells with CAFs (in black) using Newman-Keuls multiple comparisons test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

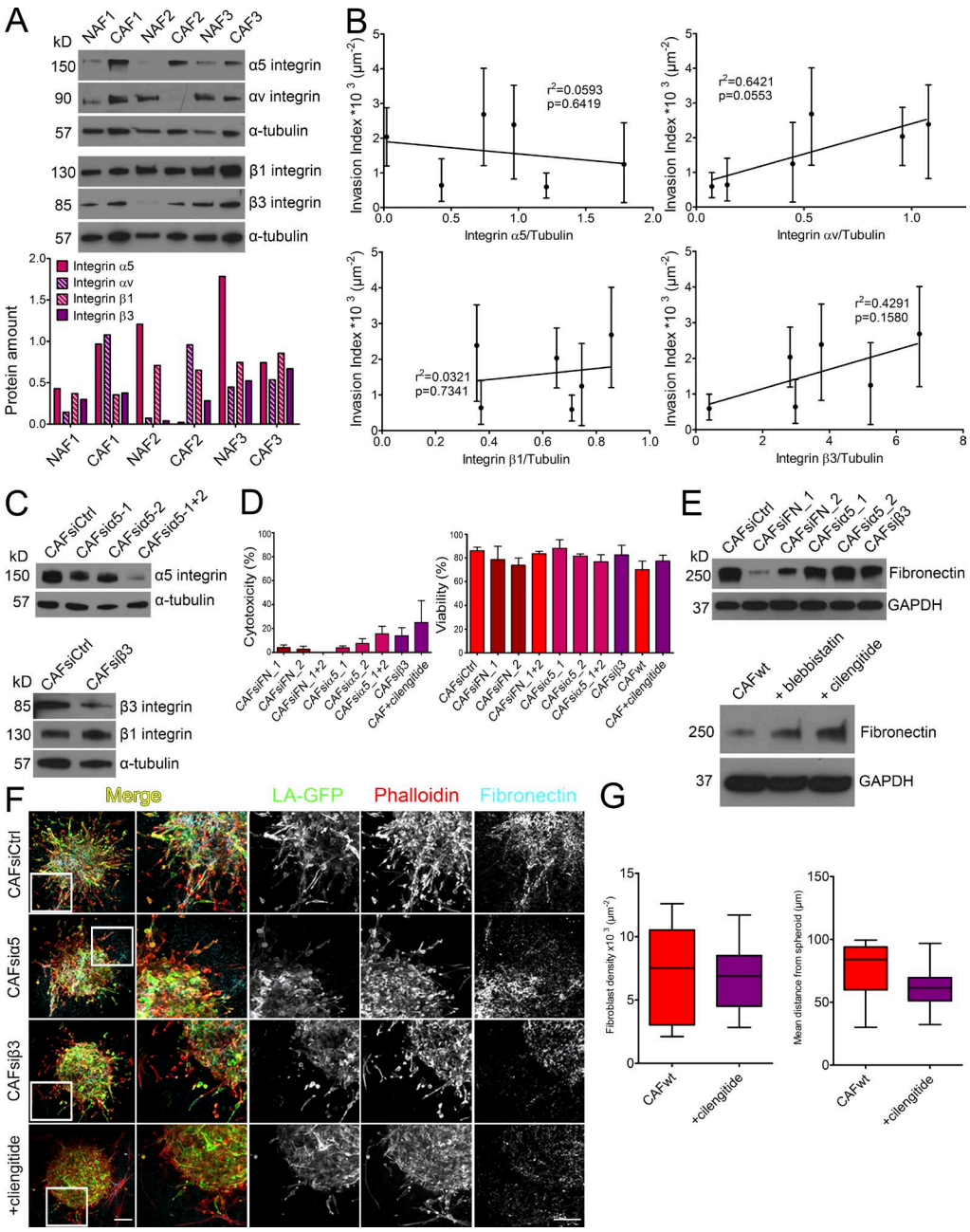


Figure S2. **CAFs induce invasion through FN assembly by integrins.** (A) Immunoblot analysis. Lysates from Fig. S1 A prepared from NAFs and CAFs were probed against integrin-α5, -αv, -β1, and -β3 antibodies. α-Tubulin served as a loading control. Blots on integrin-α5 and -αv were done together with blots of Fig. S1 A. Protein amount is calculated by normalizing each integrin amount to the α-tubulin amount. Results are represented as column bars. (B) Scatter dot graphs correlating the invasion index of cancer cells in the presence of fibroblasts with the amounts of integrin-α5, -αv, -β1, and -β3 in fibroblasts calculated by normalizing integrin amounts to the α-tubulin amount. Quality of linear regression is represented by the value of p and r^2. (C) Immunoblot analysis. (Top) Lysates prepared from CAFs treated with siRNA scrambled control and CAFs treated with two different siRNAs against integrin-α5 together or alone were probed with integrin-α5 antibody. (Bottom) Lysates prepared from CAFs treated with siRNA scrambled control and CAFs treated with siRNA against integrin-β3 were probed with integrin-β1 and integrin-β3 antibodies. α-Tubulin served as a loading control. (D) Cytotoxicity and viability of CAFs in the presence of siRNAs against FN, integrin-α5, and integrin-β3 and in the presence of cilengitide were evaluated. Results are represented as column bars with mean ± SEM. (E) Immunoblot analysis. (Top) Conditioned media and lysates prepared from CAFs treated with siRNA scrambled control and CAFs treated with siRNAs against FN, integrin-α5, or integrin-β3 were probed with FN antibody. (Bottom) Conditioned media and lysates prepared from WT CAFs or CAFs treated with blebbistatin or cilengitide were probed with FN antibody. GAPDH served as a loading control. (F) Maximum intensity projections of cancer cell spheroids in collagen I gels with CAFs treated with siRNA scrambled control, with siRNA targeting integrin-α5 (CAFsi-α5) or integrin-β3 (CAFsi-β3), or with cilengitide at day 3. Bar, 100 µm. The magnified region is represented by the white square. CT26 cancer cells express LifeAct-GFP (green), F-actin is stained with phalloidin-rhodamine (red), and FN is immunostained (cyan). Bar, 50 µm. (G, left) Quantification of the density of WT CAFs and CAFs treated with cilengitide from patient 2 around the spheroid. Fibroblast density was defined as the number of nuclei of non-GFP cells normalized to the surface area of the spheroid contour in 3D. (Right) Quantification of the mean distance of WT CAFs and CAFs treated with cilengitide from patient 2 from the spheroid. The mean distance from the spheroid was defined as the distance from the nuclei of non-GFP cells to the closest point along the cancer cell spheroid contour. Quantification results are expressed as box and whiskers (minimum to maximum), and p-value was calculated using Mann–Whitney test for $n = 3$ separate experiments.

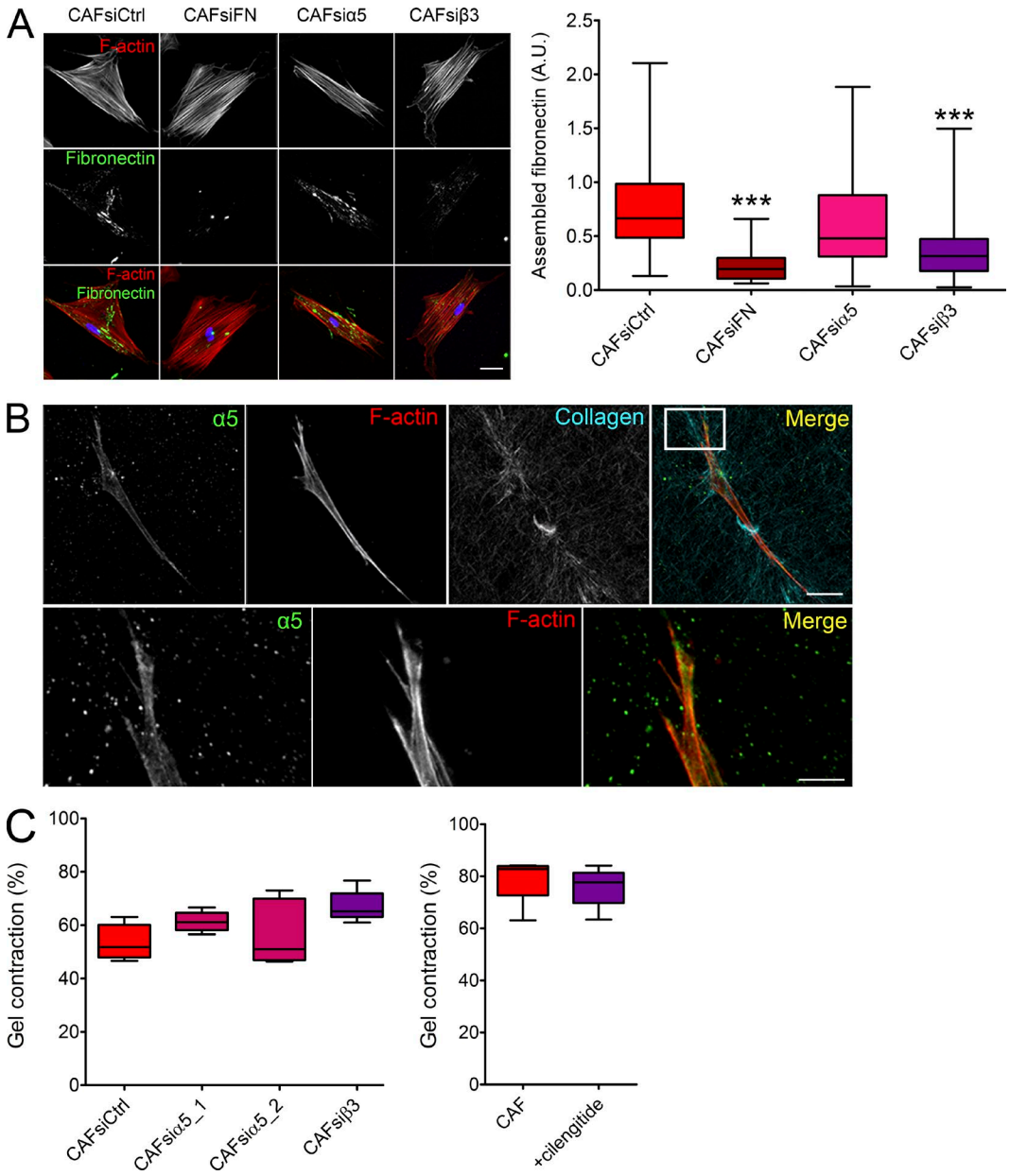
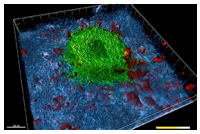
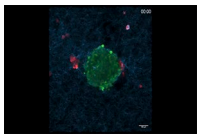


Figure S3. **Function of integrin-α5 and -αvβ3 in FN fibrillogenesis.** (A, left) Immunostaining of FN (green) in control CAFs, FN-depleted CAFs, α5-depleted CAFs, and β3-depleted CAFs 1 d after plating. F-actin was stained with phalloidin-rhodamine (red), and DNA was stained with DAPI (blue). Bar, 40 µm. (Right) Graph represents the amount of assembled FN per cell, defined as the amount of fluorescence in a cell (integrated density) normalized to the area of the cell and the background fluorescence. Depleted CAFs were compared with control CAFs for $n = 20$ cells over $n = 2$ separate experiments. P-values were calculated using Newman-Keuls multiple comparisons test (***, $P < 0.001$). (B) Maximum intensity projections of CAFs in 3D collagen matrices immunostained for integrin-α5 (green). F-actin was stained with phalloidin-rhodamine (red), and collagen was acquired using second harmonic generation (cyan). Bar, 40 µm. The magnified region is represented by the white square. Bar, 10 µm. (C, left) Percentage of gel contraction of control, integrin-α5–, and integrin-β3–depleted CAFs from patient 1. (Right) Percentage of gel contraction of CAFs with or without cilengitide treatment from patient 3. Gel contraction was calculated using the formula 100 × [gel area (T0) – gel area (T1)]/gel area (T0). Quantification results are expressed as box and whiskers (minimum to maximum). P-values are calculated using a paired *t* test for $n = 3$ over $n = 3$ separate experiments. A.U., arbitrary units.



Video 1. **Maximum intensity projection of CT26 cancer cells and CAFs from patient 1 in collagen.** CT26 cancer cells express LifeAct-GFP (green), CAFs are stained with a lyophilic carbocyanine dye (red), and collagen is acquired by reflection (blue). Images were recorded every 10 min for 48 h. 3D stacks were obtained at a step size of 2-µm intervals. Bar, 100 µm. The video was processed on IMARIS software using Normal Shading mode.



Video 2. **Maximum intensity projection of CT26 cancer cells and CAFs from patient 1 in collagen.** CT26 cancer cells express LifeAct-GFP (green), CAFs are stained with a lyophilic carbocyanine dye (red), and collagen is acquired by reflection (blue). Images were recorded every 10 min for 72 h. 3D stacks were obtained at a step size of 2-µm intervals. Bar, 100 µm. The video was processed on IMARIS software using Blend mode.

Table S1. **List of antibodies and working dilutions.**

Antibody	Company	Catalog No.	Clonal	Dilution		
				2D IF	3D IF	WB
α-SMA	Sigma-Aldrich	A2547	Monoclonal	x	x	1/1,000
FAP	R&D Systems	AF3715	Polyclonal	x	x	1/500
PDGFR-β	Cell Signaling	2.80E+02	Monoclonal	x	x	1/1,000
α-Tubulin	Sigma-Aldrich	T9026	Monoclonal	x	x	1/5,000
GAPDH	Sigma-Aldrich	G9545	Polyclonal	x	x	1/10,000
FN (rabbit)	Sigma-Aldrich	F3648	Polyclonal	1/500	1/300	1/5,000
FN (mouse)	AbCam	Ab6328	Monoclonal	1/100	x	x
Integrin-α5	AbCam	ab150361	Monoclonal	1/100	1/50	1/1,000
Integrin-αv	AbCam	ab179475	Monoclonal	x	x	1/2,500
Integrin-β1	Santa Cruz	sc-53711	Monoclonal	x	x	1/1,000
Integrin-β3	EMD Millipore	AB2984	Polyclonal	x	x	1/500
Integrin-αvβ3	AbCam	Ab190147	Monoclonal	1/100	1/50	x

IF, immunofluorescence; WB, Western blot; x, not used.

Table S2. **List of silencing RNAs and sequences**

siRNA	Company	Catalog No.	Sequence (5′–3′)	Species
Negative control	Qiagen	1027280	Proprietary	Human/mouse
FN oligo1	Qiagen	SI02664004	CCCGGTTGTTATGACAATGGA	Human
FN oligo2	Qiagen	SI02663997	CCGGTTGTTATGACAATGGAA	Human
Integrin-α5 oligo1	Qiagen	SI00034202	CCCATTGAATTTGACAGCAAA	Human
Integrin-α5 oligo2	Qiagen	SI02654841	AATCCTTAATGGCTCAGACAT	Human
Integrin-β3 oligo1	Qiagen	SI00004585	CCGCTTCAATGAGGAAGTGAA	Human
Integrin-β3 oligo2	Qiagen	SI00004599	CTCTCCTGATGTTAGCACTTAA	Human
Integrin-β3 oligo3	Qiagen	SI00004606	CAAGCTGAACCTAATAGCCAT	Human