Supplementary Figure 1 Integrin $\alpha v \beta 3$ is highly expressed in a sub-population of human pancreatic cancer cells and correlates with lymph node invasion. (a,b) Representative images of immunohistochemical staining for the integrin $\beta 3$ subunit in human pancreatic carcinoma sections from 18 patient samples. (a) (top left) $\beta 3$ is absent or minimally expressed in acinar cells (arrow) and ductal epithelial cells (arrowhead) in areas of normal human pancreas adjacent to tumor. (top right & bottom left) A significant sub-population of carcinoma cells display enhanced levels of $\beta 3$ staining. (bottom right) Other carcinoma cells in the same tumor lack $\beta 3$ expression. (b) Despite heterogeneous expression of $\beta 3$ in the primary tumor (top), tumor cells invading a pancreatic lymph node in the same section were primarily $\beta 3$-positive (bottom). Scale bars, 50 $\mu$m.
**Supplementary Figure 2** Integrin \( \alpha \nu \beta 3 \) is expressed on the surface of FG-\( \beta 3 \) cells. FACS analysis of FG cell lines with the \( \alpha \nu \beta 3 \)-specific monoclonal antibody LM609. FG cells lack surface expression of integrin \( \alpha \nu \beta 3 \) (left panel) while FG-\( \beta 3 \) cells express surface \( \alpha \nu \beta 3 \) (right panel) relative to an isotype control.

**Supplementary Figure 3** Metastasis to the liver hilar lymph nodes was confirmed by detection of GFP-expressing tumor cells and histologic evaluation. (a) An example of GFP-labeled FG-\( \beta 3 \) cells present in excised tissue including liver hilar lymph nodes. Scale Bar, 1mm and 200 \( \mu \)m (inset). (b) A representative H&E stained section from excised tissue showing FG-\( \beta 3 \) tumor cells present within a liver hilar lymph node. The distinctive outer lymph node wall (inset; arrow) is nearly all that remains of the normal lymph node morphology. Scale Bar, 200 \( \mu \)m & 50 \( \mu \)m (inset).

**Supplementary Figure 4** Knock-down of \( \beta 3 \) in Panc-1 cells modestly reduced primary tumor mass. (a) Stable expression of a human-specific \( \beta 3 \) shRNA (\( \beta 3 \) sh) significantly knocked-down total \( \beta 3 \) protein levels compared to Panc-1 cells stably expressing a non-silencing (n-s) shRNA. (b) Knock-down of \( \beta 3 \) decreased primary tumor mass, but was not statistically significant.
Supplementary Figure 5 VEGF secretion and tumor angiogenesis are unaffected by αvβ3 expression in FG pancreatic tumor cells. (a) Analysis of FG and FG-β3 48 h conditioned media for VEGF secretion by ELISA showed no difference in VEGF levels between the two cell lines. (b) Vessel staining in 8 week primary orthotopic tumors showed no difference in vascular density between FG and FG-β3 cells. Vessels were stained with a cocktail of VE-cadherin, CD-31, Flk, and endoglin antibodies and vascular density was determined by quantifying the number of vessels per 20x field (4 fields per section) using metamorph software. FG, n=8, FG-β3, n=8. Bars represent the mean±SEM. (c) Representative images of vascular staining in FG compared to FG-β3 8 week primary tumors. Scale bar, 100 μm.
Supplementary Figure 6 Integrin αvβ3 co-localizes with active SFK’s in response to FG-β3 cell adhesion to fibronectin. (a) A representative adhesion assay comparing FG and FG-β3 cells on 5 μg mL\(^{-1}\) fibronectin in the presence or absence of blocking antibodies against αvβ3 (LM609), β1 (P4C10) and α5β1 (P1D6). Results show that α5β1 is the predominant integrin required for FG cell adhesion to fibronectin. In FG-β3 cells, αvβ3 and α5β1 are both utilized for fibronectin adhesion. (b) Confocal analysis of pY416 SFK recruitment to αvβ3 focal adhesions in fibronectin adherent FG-β3 cells (arrows; top panels). These adhesions are not podosomes/invadopodia as judged by the absence of Tks/Fish localization. In both FG-β3 and FG cells α5β1 does not localize well to focal adhesions, but instead clusters along one edge of the cell where it overlaps with pY416 SFK in only a few locations (middle and lower panels). Scale Bar, 10μm.
**Supplementary Figure 7** Integrin αvβ3 activates FAK and CAS in response to fibronectin adhesion. (a) Relative to suspension controls (S), FG-β3 cells displayed increased levels of pY861 FAK (top) and pY165 CAS (bottom) in response to adhesion compared to FG cells. Note that pY861 FAK was not observed in suspended FG-β3 cells (asterisk). (b) Confocal analysis of FG-β3 cells allowed to spread on fibronectin-coated glass coverslips. Strong co-localization of pY861 FAK (top) and p-Cas (bottom) was observed with αvβ3 in focal adhesions (arrows).

**Supplementary Figure 8** (a) Formation of an αvβ3/c-src complex requires the last four amino acids of the β3 C-terminal tail. Immunoprecipitation of β3 from FG, FG-β3 and FG-759x whole cell lysates followed by immunoblotting for c-src. In FG-β3 cells, c-src is observed to co-immunoprecipitate with β3, whereas this complex is not found in FG cells expressing the β3 759x mutant, which lacks the last four amino acids of the β3 C-terminal tail and was previously characterized to be deficient in the ability to recruit c-src. (b,c) Integrin αvβ3 activates c-src independent of adhesion. (b) (top) FG-β3 cells in suspension (S) displayed increased levels of pY416 SFK compared to FG cells (asterisk). (bottom) Knock-down of β3 in Panc-1 cells reduced suspension levels of p-SFK (asterisk). (c) Timecourse analysis of the adhesion-dependent phosphorylation of pY397 FAK. Despite increased suspension levels of active SFK in FG-β3 cells, phosphorylation of FAK is still highly adhesion-dependent relative to cells in suspension (S).
**Supplementary Figure 9** (a) Adherent FG-β3 cells exhibit no difference in proliferation compared to FG cells. FG and FG-β3 cells were assessed for differences in proliferation over time by measuring BrdU incorporation or cell number. In both experiments cells were seeded onto tissue culture plastic in the presence of 10% FBS. No differences in proliferation were observed. Each panel depicts a representative experiment and each dose or time-point represents the Mean±SEM of triplicate wells. (b) Integrin αvβ3 substrate ligation does not appear required for anchorage-independent growth. Blockade of integrin αvβ3 substrate binding with LM609 does not affect the enhanced colony formation observed in FG-β3 cell treated with IgG1 isotype control. FG-β3 cells were pre-incubated in 50 μg mL−1 IgG1 or LM609 for 30 min prior to embedding in soft agar. After 3 days the media was replaced with media supplemented with 30 μg mL−1 IgG1 or LM609 and the experiment continued until colonies were counted after 7 days.

**Supplementary Figure 10** Integrin αvβ3 ligand binding is dispensable for its effect in enhancing anchorage-independent growth. (a) FG cells expressing either the wild-type β3 or the D119A mutant have similar levels of surface αvβ3 as determined by FACS analysis. (b) FG cells expressing the D119A mutant are incapable of adhering to the αvβ3 substrate vitronectin (10 μg mL−1), but maintain the ability to adhere to non-αvβ3 substrates such as collagen (10 μg mL−1) after 45 min. (c) FG cells expressing the β3 D119A mutant retain their ability to form increased numbers of soft agar colonies compared to FG control cells. Representative experiments are shown. Bars represent the mean±SEM of triplicate wells.
Supplementary Figure 11 (a,b) Integrin αvβ3-induces anchorage-independent growth in the MiaPaca-2 (MP-2) human pancreatic carcinoma cell line. (a) Stable expression of αvβ3 in MP-2 cells enhanced colony number in soft agar compared to MP-2 cells expressing vector alone. (b) Treatment with the SFK inhibitor dasatinib specifically reduced colony number in the αvβ3-expressing MP-2 cells. Representative experiments are shown. Bars represent the mean±SEM of triplicate wells. (c) Proliferation in adhesion-independent conditions is unaffected by αvβ3. To directly assay for anchorage-independent survival and proliferation FG & FG-β3 cells were cultured in suspension on 1% agar-coated wells for 24 and 48 hr prior to staining with trypan blue and counting viable and non-viable cells on a hemocytometer. Total cell number was equivalent at both time-points, suggesting no difference in proliferation between the two cell types.

Supplementary Figure 12 Integrin αvβ3 enhances anchorage-independent growth of breast and cervical cancer cells. (a,b) Transient knock-down of β3 in MDA-MB-231 breast cancer cells using a β3 shRNA plasmid inhibited colony growth in soft agar. Misexpression of β3 in the MCF-7 breast cancer (c,d) or the HeLa cervical cancer (e,f) cell lines resulted in increased soft agar colony formation relative to cells transfected with the vector control. Representative experiments are shown. Bars represent the mean±SEM of triplicate wells.
**Supplementary Figure 13** Dasatinib inhibits pY416 SFK in FG and FG-β3 cells. Suspension cultured FG and FG-β3 cells were treated with vehicle or varying doses of dasatinib for 8 h prior to lysis. Immunoblotting for pY416 showed that dasatinib effectively inhibits SFK activation to a similar extent in both cell types.

![Supplementary Figure 13](image)

**Supplementary Figure 14** FAK is not required for αvβ3-mediated growth in soft agar. (a) Inhibition of FAK with 500 nM of a FAK specific inhibitor (PF-228) or a dual FAK/Pyk2 inhibitor (PF-271) as determined by immunoblotting for the FAK pY397 autophosphorylation site in adherent cells. (b) Treatment with 500 nM of either one of these inhibitors had no effect on the growth of FG or FG-β3 cells in soft agar. A representative experiment is shown. Bars represent the mean±SEM of triplicate wells.
Supplementary Figure 15 c-src is knocked-down to similar levels in FG versus FG-β3 cells. In both FG and FG-β3 cells c-src is knocked-down to similar extent by stable expression of a specific shRNA (c-src sh) compared to cells expressing a non-silencing control (n-s). The specificity of c-src knockdown is shown by comparison to the homologous src family kinase member fyn.

Supplementary Figure 16 The c-src binding domain on the β3 tail is required for αvβ3-mediated anchorage-independent growth. As described previously, FG-β3 cells exhibit enhanced colony number in soft agar relative to the FG vector control. FG cells expressing the β3 759x mutant, which lacks the c-src binding domain, failed to enhance colony formation compared to wild-type β3. n=3 independent experiments. *P<0.05.

Supplementary Figure 17 The CAS Y1-15F mutant is expressed at similar levels in FG and FG-β3 cells. Immunoblot analysis for total CAS in whole cell lysates from FG and FG-β3 vector control and CAS Y1-15F expressing cells. The lower bands correspond to endogenous CAS whereas the upper bands represent the CAS Y1-15F-GFP fusion protein.
Supplementary Figure 18 The c-src binding domain on the β3 tail is required for αvβ3-mediated tumor progression. (a,b) GFP-labeled FG-β3, FG-759x and FG vector control cells were injected into the pancreas of nude mice and primary tumor mass was assessed after 8 weeks. As predicted, expression of wild-type β3 enhanced primary tumor mass compared to FG vector control cells. However, FG cells expressing the β3 759x mutant failed to increase tumor mass and in two examples, failed to form primary tumors. (b) FG, n=12, FG-β3, n=12, FG-759x, n=10. *P<0.05, **P<0.001.

Supplementary Figure 19 c-src is not required for αvβ3-mediated cell migration. (a) Transwell migration assays comparing the affect of c-src knock-down on FG and FG-β3 cell haptotaxis toward the αvβ3 substrates vitronectin (VN) and fibronectin (FN). Integrin αvβ3 mediates enhanced migration on both vitronectin and fibronectin, however c-src was not required for this effect. (b,c) SFK kinase activity is not required for αvβ3-mediated cell migration. (b) The SFK inhibitor SKI-606 significantly inhibits pY416 SFK in adherent FG or FG-β3 cells relative to cells treated with vehicle alone. (c) Treatment with SKI-606 failed to inhibit αvβ3-mediated cell migration on vitronectin. n=5 independent experiments. Representative experiments are shown. Bars represent the mean±SEM of triplicate wells.
Supplementary Methods

Cell lines. Stable transfectants of FG cells$^{50}$ with either human β3/pcDNA3.1 (FG-β3), β3 759x/pcDNA3.1 (FG-759x) or empty vector (FG) were selected in 500 μg mL$^{-1}$ G418 and the FG-β3 and FG-759x were FACS sorted for surface expression of αvβ3. FG, FG-β3 and FG-759x cells were subsequently made to stably express GFP using an empty pGIPZ plasmid (Open Biosystems), selecting in 2 μg mL$^{-1}$ puromycin and FACS sorting for a homogeneous population of GFP-expressing cells. Stable knock-down of c-src was achieved by transfecting FG and FG-β3 cells with a human-specific c-src shRNA/pGIPZ or a non-silencing/pGIPZ (Open Biosystems) and pooling three puromycin-resistant colonies. Similar methods were employed to generate the Panc-1 non-silencing/pGIPZ and human β3 shRNA/pGIPZ (Open Biosystems) cell lines. The FG CAS Y1-15F was generated as previously described$^{51}$ and stable expression of αvβ3 was achieved by 5 rounds of enrichment on LM609-coated dishes.

Adhesion timecourse lysates. All cells were serum-starved overnight prior to resuspending in serum-free DMEM/0.1% BSA and incubating in suspension for 3 h at 37 °C. We coated 6-well dishes with 5 μg mL$^{-1}$ fibronectin/PBS overnight before blocking with 5% BSA/PBS for at least 2 h and seeding with 2×10$^6$ cells per well of the suspended cells. The rest of the suspended cells were spun down and lysed for suspension controls. We allowed cells plated on fibronectin to adhere for various amounts of time prior to performing two gentle PBS washes to remove non-adherent cells and lysing with scraping in RIPA lysis buffer.
**Immunoprecipitations**

The integrin β3 subunit was pulled-down from 60 μg of triton insoluble lysate from FG-β3 cells plated for 2 hr on 10 μg mL⁻¹ vitronectin, or from 3.5 mg of whole cell lysates lysed with triton lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, and 1% Triton X-100, 50 mM NaF, Protease inhibitor cocktail (Roche), 2 mM PMSF, 2mM sodium orthovanadate) from serum-starved adherent FG, FG-β3 and FG-759x cells. Lysates were incubated for 2 h with 6 μg of AP3 prior to adding protein A/G beads (Pierce) and incubating overnight at 4 ºC. The beads were washed 3x with lysis buffer prior to eluting proteins with 2x sample buffer.

**Immunoblotting.** Standard Western blotting procedures were performed. The following antibodies were used: phosphotyrosine (4G10; Millipore), β3 (AP3), c-src (GD11; Millipore), β1 rabbit polyclonal (Chemicon), pY416 SFK (Cell Signaling Technologies), Yes (BD transduction labs), Fyn (Santa Cruz), β-actin (Sigma), pY397 FAK (Invitrogen), FAK (BD transduction labs), pY165 CAS (Cell Signaling Technologies), CAS (BD transduction labs), pY861 FAK (Invitrogen), pY473 Akt rabbit mAb (Cell Signaling Technologies), Akt (BD transduction labs), phospho-Thr202/Tyr204 MAPK mAb (Cell Signaling Technologies) and ERK2 (Santa Cruz).

**Adherent cell survival assays.** 96-well non-TC plates were coated overnight with 5 μg mL⁻¹ fibronectin and blocked with 5% BSA/PBS prior to seeding with
20,000 serum-starved FG or FG-β3 cells in 100 μL of serum-free DMEM/0.1% BSA. We allowed cells to adhere for 4 h prior to washing once with PBS and adding various doses of gemcitabine or anti-Fas antibody (CH11; Millipore) diluted in serum-free DMEM/0.1% BSA. After 24 h (CH11) or 48 h (gemcitabine) we added XTT substrate (Sigma) and measured the A450 nm. We determined specific adhesion by subtracting away the absorbance from wells coated with BSA alone. Cell viability was assessed for each dose as a percent of the untreated control wells for each cell type.

**Migration assays.** We performed haptotaxis migration assays as previously described\(^{27}\). Briefly, the underside of 6.5 mm diameter 8 μm pore size transwell inserts was coated overnight with either 2 μg mL\(^{-1}\) vitronectin or 5 μg mL\(^{-1}\) fibronectin diluted in PBS. Cells were serum-starved overnight prior to trypsinization and seeding 200,000 cells per well. We allowed cells to migrate 12–16 h prior to staining with 0.1% crystal violet/MeOH in PBS and counting three 20× fields per transwell.