Supplementary Table 1 Clinicopathologic Data of Patients with RCC

Patient	Age	Sex	Diagnosis	Tumor Stage	Metastasis
1	50-59	m	Metastatic clear cell renal cell carcinoma	pT3bN2Mx	liver, lung, lymph node
2	70-79	f	Metastatic clear cell renal cell carcinoma	pT3bN0M1	lung
3	80-89	m	Metastatic clear cell renal cell carcinoma	pT3bNxM1	bone, adrenal gland
4	40-49	m	Localized clear cell renal cell carcinoma	pT1aNxMx	none
5	40-49	m	Localized clear cell renal cell carcinoma	pT1aNxMx	none
6	60-69	m	Localized clear cell renal cell carcinoma	pT1aNxMx	none

Supplementary Movie 1 and 2 Live cell imaging (phase contrast, 10x) of 786-0 (movie #1) and MDA-MB-231 cells (movie #2) over 72 hours after embedding in fibrin.

Supplementary Fig. S1 (A), 48 hour fibrin embedded 786-0 and MDA-MB-231 cells were analyzed by confocal microscopy after incubation with TRITC phalloidin (red). Nuclei are visualized with draq5 (blue). Scale bar, 20 μm. (B), cell surface expression of integrin αvβ3 (Mean Fluorescence Intensity, MFI) analyzed by flow cytometry using LM609 antibody (EMD Millipore) compared to invadopodia formation in clotted plasma.

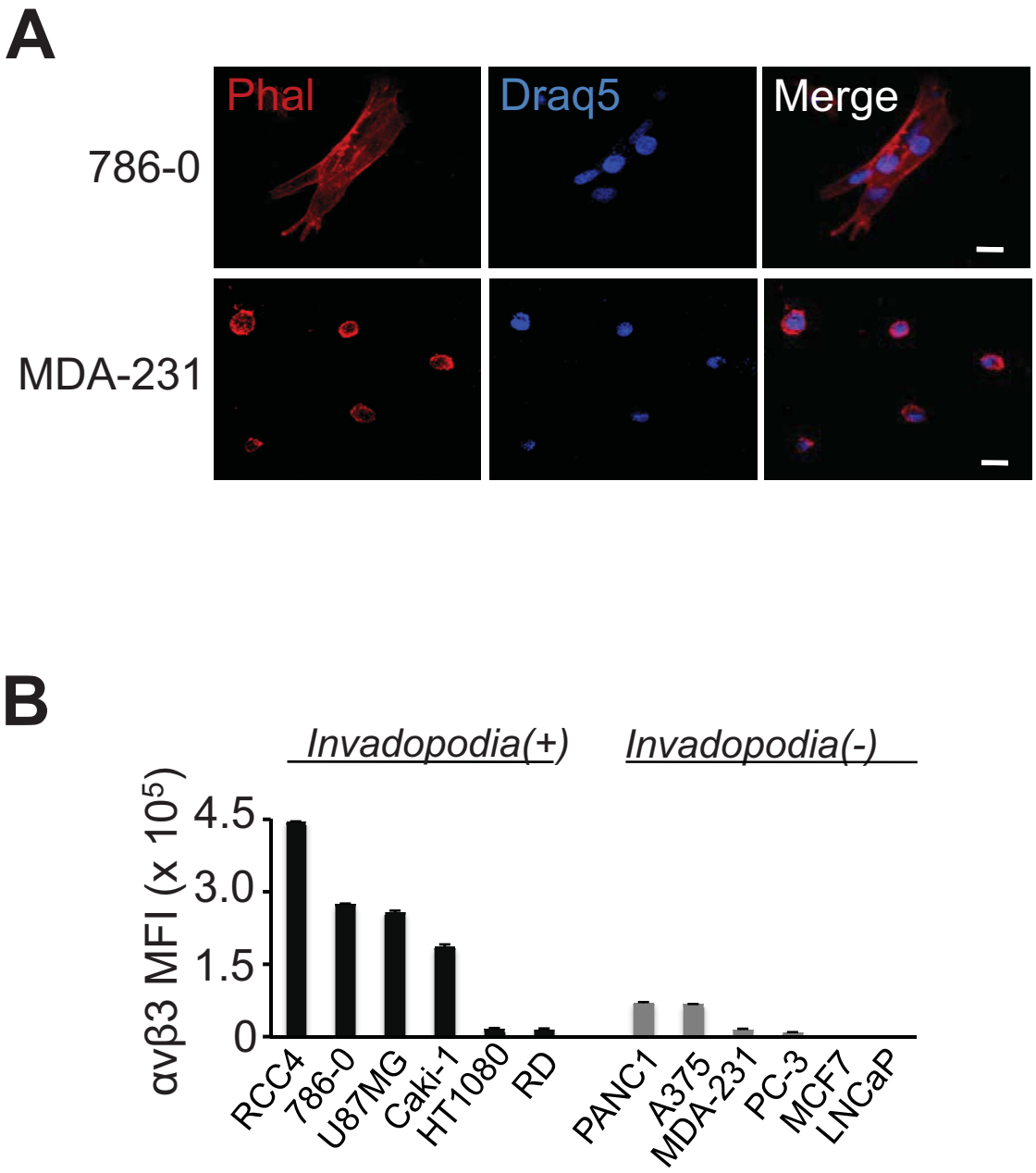
Supplementary Fig. S2 (A), protein expression of integrins β1 and β3 was analyzed by western blot from 786-0 and HT1080 cell extracts 48 hours after transfection with integrin β1 or integrin β3 siRNA compared to non-targeted control siRNA or after stable transformation with two types of β3 shRNA (target sequence #1, CCTTAGCCTTTGTCCCAGAAT; #2 GATGCAGTGAATTGTACCTAT) compared to scrambled shRNA. Ponceau S (PS) staining shows protein loading. (B-C), 786-0 cells transformed with scrambled control compared to two different integrin β3 shRNA clones were analyzed for invadopodia formation (B) and proliferation (C) 24 hours after embedding in fibrin. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus Control. (D), representative micrographs of fibrin clots containing 786-0 cells 0, 48 and 96 hours after embedding. Cells were treated with non-silencing control or β3 integrin siRNA prior to embedding. Scale bar, 200 μm.

Transformation with shRNA. Lentiviral constructs containing puromycin resistance genes were purchased from the University of Pittsburgh Cancer Institute Vector Core Facility (Pittsburgh, PA). 786-0 cells were transduced at 80% confluency using lentiviral suspensions containing 8 µg/mL polybrene and incubated 19 hours at 32°C and 5% CO2. Cells were allowed to recover for 24 hours at 37°C in complete medium and then placed under puromycin selection (4 µg/ml) for 2 weeks. After two weeks, protein was harvested and knockdown was confirmed by western blotting as previously described.

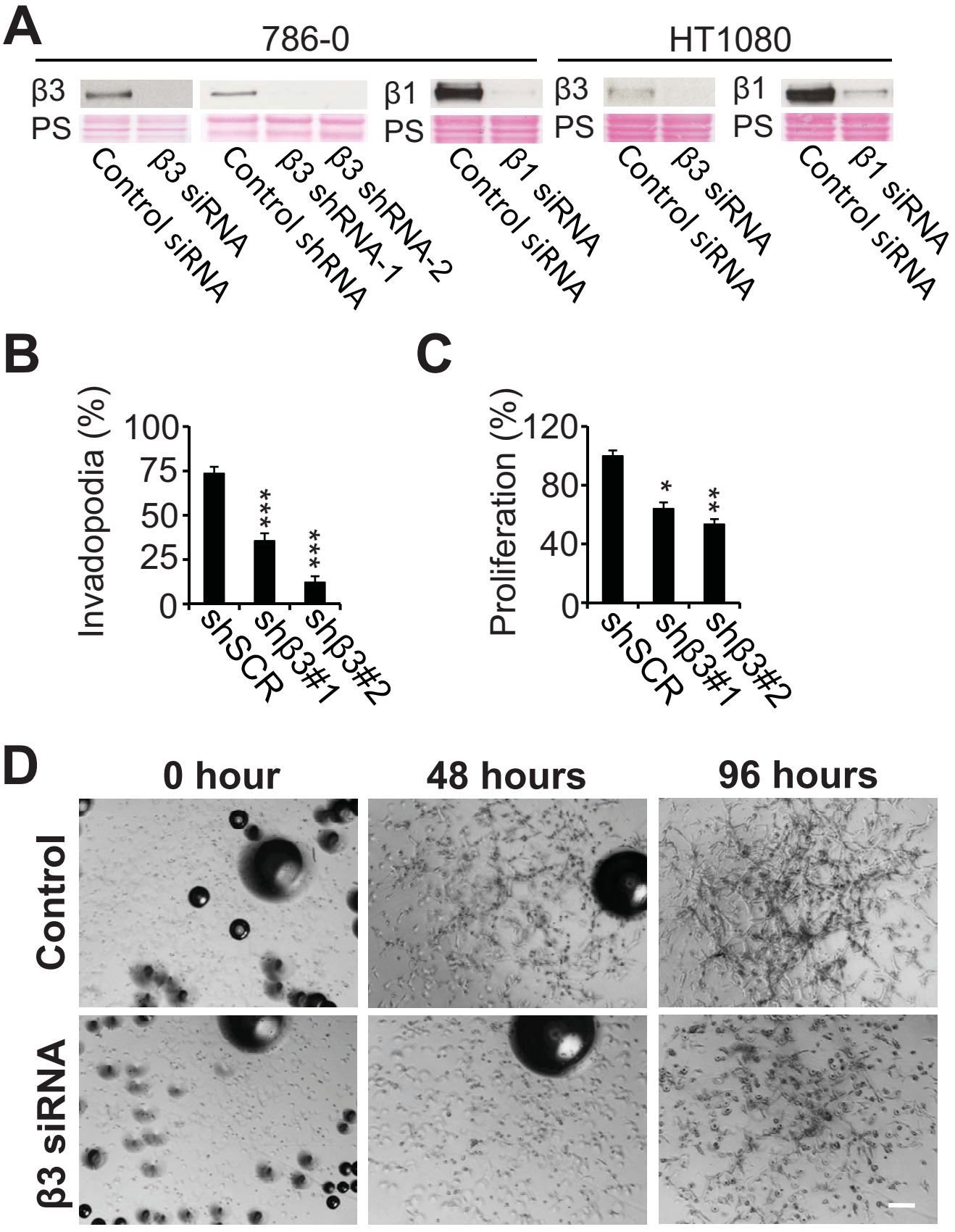
Supplementary Fig. S3 Phase contrast images showing spreading of invadopodia-positive tumor cell lines (786-0, RD, U87MG and HT1080) within 1 hour of attachment to plates coated with fibrinogen compared to invadopodia-negative A375 cells. Scale bar, 50 µm.

Supplementary Fig. S4 (A), protein expression of fibronectin (FN) was analyzed by western blot from HT1080 and 786-0 cell extracts 48 hours after transfection with FN siRNA compared to control siRNA or after stable transformation with two types of FN shRNA (target sequence #1, GCCTGCTCCAAGAATTGGTTT; #2, GCCTGCTCCAAGAATTGGTTT) compared to scrambled shRNA. Ponceau S (PS) staining shows protein loading. (B-C), HT1080 cells transformed with scrambled control compared to two different FN shRNA clones were analyzed for invadopodia formation (B; 24 hours after embedding in fibrin) and proliferation (C; 48 hours after embedding in fibrin). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus Control.

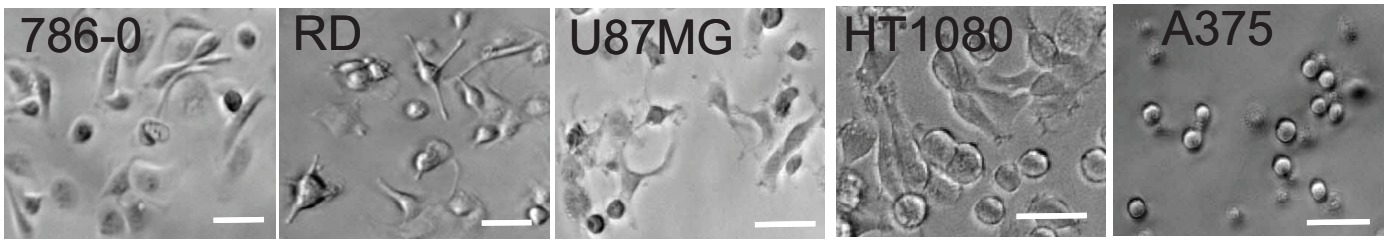
Supplementary Fig. S5 (A), total cell extracts from B16F1 mouse melanoma cells after transfection with non-silencing siRNA or siRNA against slug (Dharmacon On-TARGETplus SMARTpool L-042291-00; Lafayette, CO) were probed for slug protein expression. Ponceau S (PS) staining shows protein loading. (B), B16F1 mouse melanoma cells embedded in fibrin-fibronectin for 48 hours were analyzed for invadopodia formation after transfection with siRNA against slug or non-silencing control siRNA. *** $p < 0.001$ versus Control. (C), total cell extracts from clot invasive or non-clot invasive tumor cell lines were immunoblotted for phospho-Smad2 (Ser465/467) (Cell Signaling Technology). (D), extracts from 786-0 and HT1080 cells treated with increasing concentrations of the TGF-βRII inhibitor SB431542 (EMD Millipore) were immunoblotted for phospho-Smad2 (Ser465/467) and slug. Ponceau S (PS) staining shows protein loading.



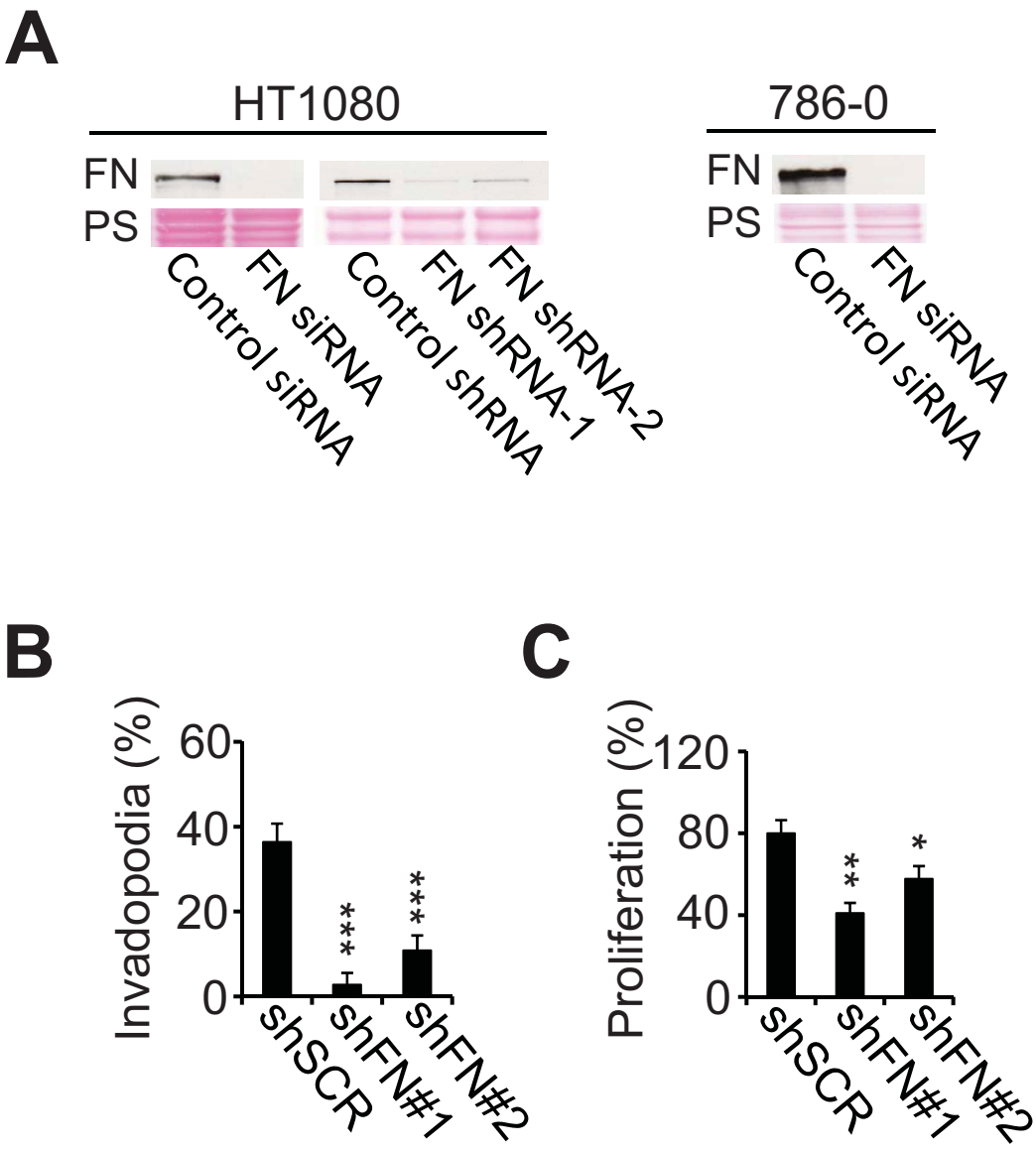
Knowles et al. Supplementary Fig. S1



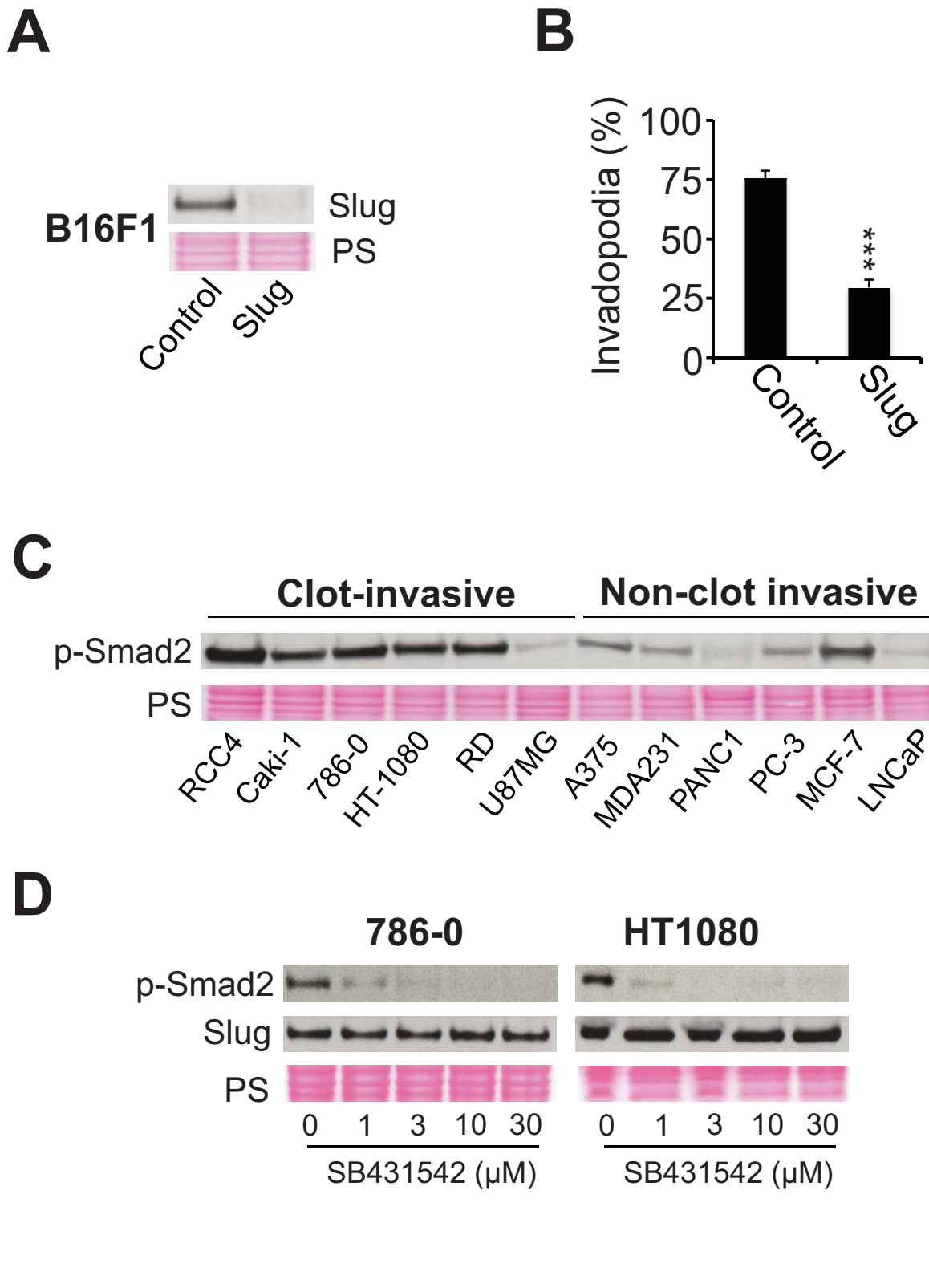
Knowles et al. Supplementary Fig. S2



Knowles et al. Supplementary Fig. S3



Knowles et al. Supplementary Fig. S4



Knowles et al. Supplementary Fig. S5