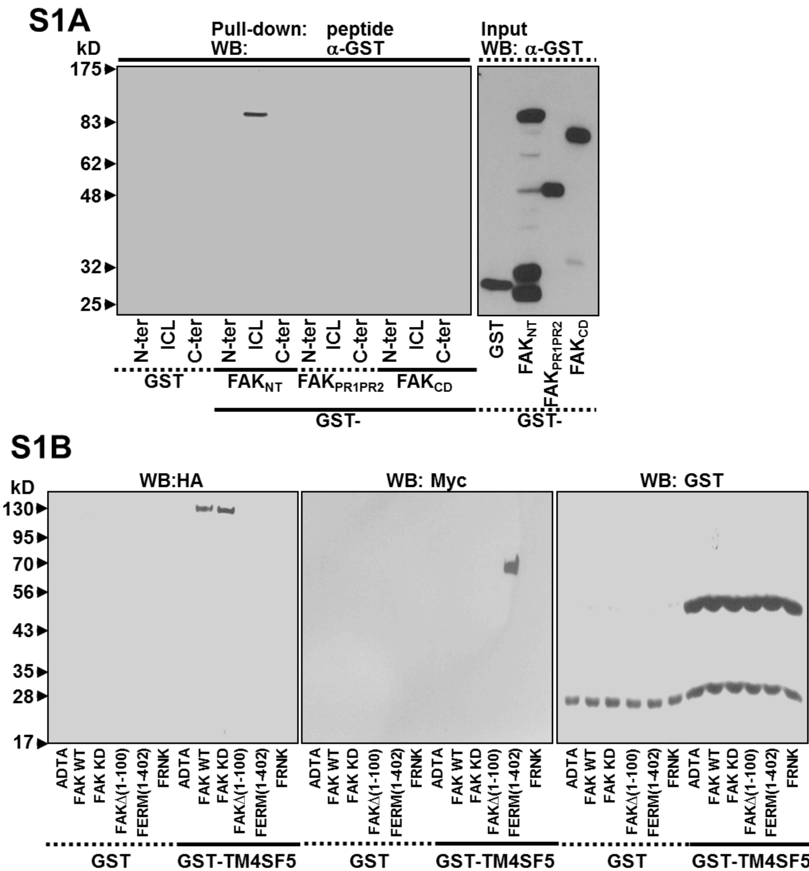


Fig. S1. Recombinant GST-FAK fusion proteins were prepared from bacterial cultures induced by IPTG (1 mM, 4 h) overnight at 4ºC. (A) In vitro pull-down using peptides of TM4SF5 cytosolic regions (1 μM) shows binding between the intracellular loop (ICL) of TM4SF5, FAK, and others. In vitro peptide pull-down using peptides for the cytosolic regions of TM4SF5 and either recombinant GST, GST-FAK_{NT}, GST-FAK_{PR1PR2}, or GST-FAK_{CD}. The GST-FAK fragments bound to Glutathione-Sepharose 4 Fast Flow (Amersham Bioscience, Piscataway, NJ) were assumed to be functionally folded because these have successfully been used for in vitro binding to a tyrosine kinase Fer (Oh et al., 2009). ICL, intracellular loop; C-ter, COOH-terminus; N-ter, NH_2-terminus. (B) In vitro pull-down using recombinant GST or GST-TM4SF5 proteins and extracts from cells infected with adenovirus for control (Ad-TA), $(HA)_3$-FAK WT, KD, N-terminal deletion [Δ(1-100)], FRNK, or myc-FERM (1-402 aa) was performed prior to immunoblottings for HA tag, myc tag, or GST. Data shown represent 3 independent experiments.

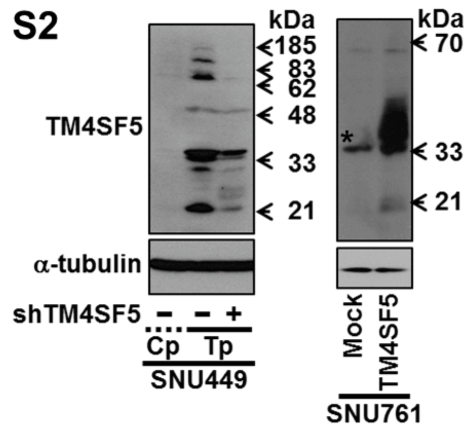


Fig. S2. SNU449Cp (Cp, control without TM4SF5 expression) or SNU449Tp (Tp, with TM4SF5 expression) cells that were transiently transfected with shRNA for a control scrambled sequence (-) or TM4SF5 (+), or SNU761 cells stable transfected with pFLAG-mock or pFLAG-TM4SF5 were harvested for the standard Western blots, using anti-TM4SF5 polyclonal antibody (homemade, left) or anti-FLAG antibody (Cell Signaling Tech., right). Data represent three independent experiments.

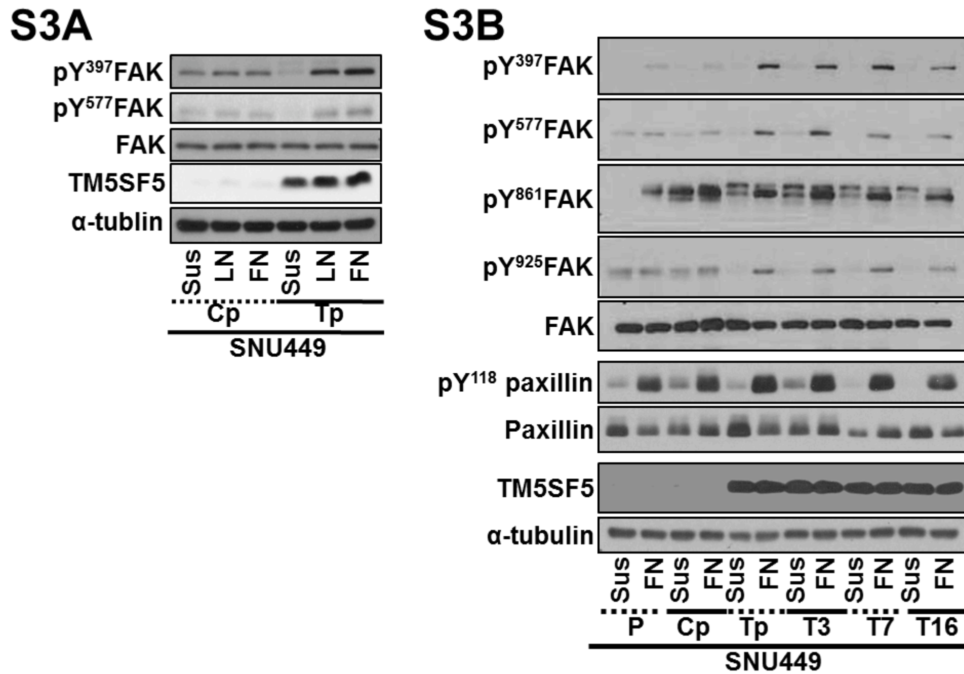


Fig. S3. (A) SNU449Cp (Cp, control without TM4SF5 expression) or SNU449Tp (Tp, with TM4SF5 expression) cells were kept in suspension (Sus) or replated onto laminin (LN, 10 μg/ml) or fibronectin (FN, 10 μg/ml) -precoated dishes for 2 h, prior to harvests of whole cell extracts for the standard Western blots. (B) Different SNU449 stable clones (P, SNU449 parental; Cp, TM4SF5-nonexpressing pooled clone; Tp, TM4SF5-expressing polled clone; T3, T7, and T16, single cell-derived TM4SF5-expressing stable clones) were kept in suspension (Sus) or reseeded on FN for 2 h, as in Fig S1a, prior to harvests of whole cell extracts for the standard Western blots. Data represent three independent experiments.

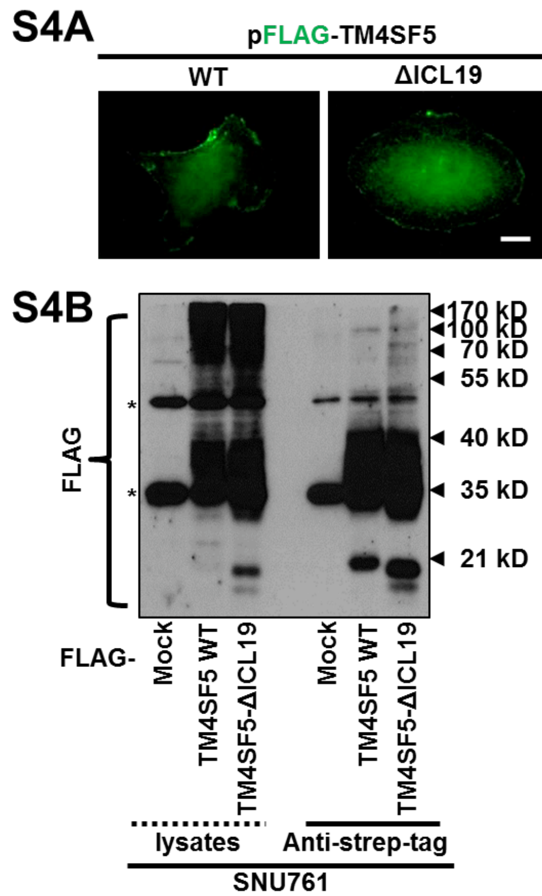
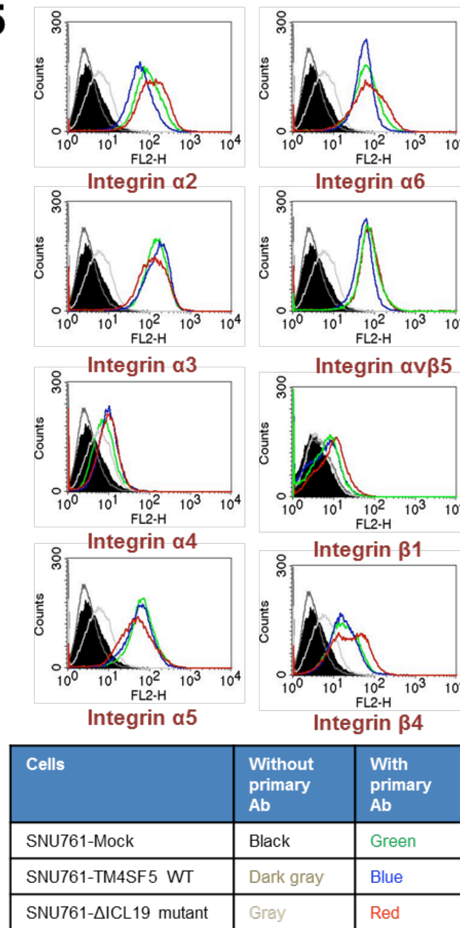


Fig. S4. (A) TM4SF5-null SNU449 cells were transiently transfected with either pFLAG-TM4SF5 WT or -TM4SF5ΔICL19 for 48 h, before immunostaining using anti-FLAG antibody and FITC-conjugated secondary antibody. Scale bar; 5 μm. (B) SNU761 cells stably-expressing pFLAG-Mock, -TM4SF5 WT, or -TM4SF5ΔICL19 were biotinylated using Pierce® Cell surface protein isolation kit (Thermo Scientific, Rockford, IL), following the manufacture's protocol. Biotinylated membrane proteins were immunoblotted using anti-FLAG in parallel with the lysate input. * depicts nonspecific bands of anti-FLAG antibody.



Cells	Without primary Ab	With primary Ab
SNU761-Mock	Black	Green
SNU761-TM4SF5 WT	Dark gray	Blue
SNU761-ΔICL19 mutant	Gray	Red

Fig. S5. Stable SNU761 cells expressing mock, FLAG-TM4SF5 WT, or △ICL19 mutant in subconfluent conditions were analyzed for expression profile of integrin on cell surface by flow cytometry. Note that among the cell clones, there was no specifically significant difference in integrin chain expression levels. Data represent three different experiments.

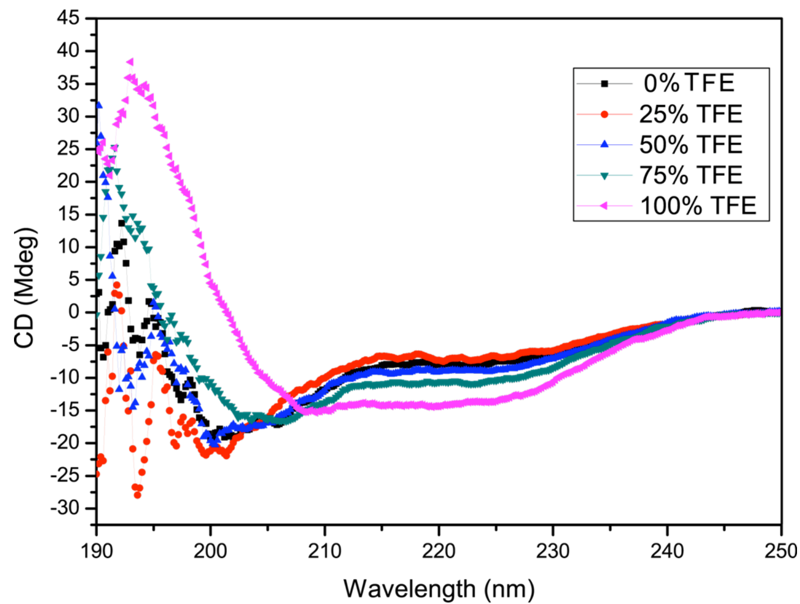


Fig. S6. The ICL peptide in trifluroethanol (TFE) solution was analyzed for CD. Note that as TFE concentration increased, peaks with more negative values around 208/220 nm (to indicate the secondary structure) and a less negative peak at 200 nm (to indicate random coil) were obtained. This suggested a possible adaptation of the secondary structure by the ICL peptide, although it has many Gly residues that increase the possibility of helix breakage.

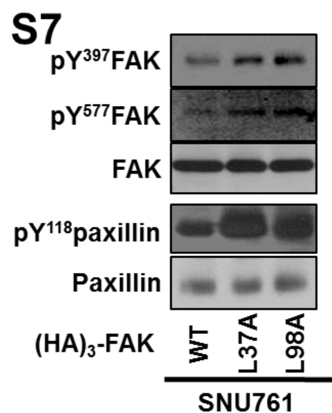


Fig. S7. $(HA)_3$-FAK WT or point mutant (L37A or L98A) plasmids were transfected into parental SNU761 cells for 48 h, before harvests of whole cells lysates for standard Western blots using antibodies against the indicated molecules. Data represent three independent experiments.

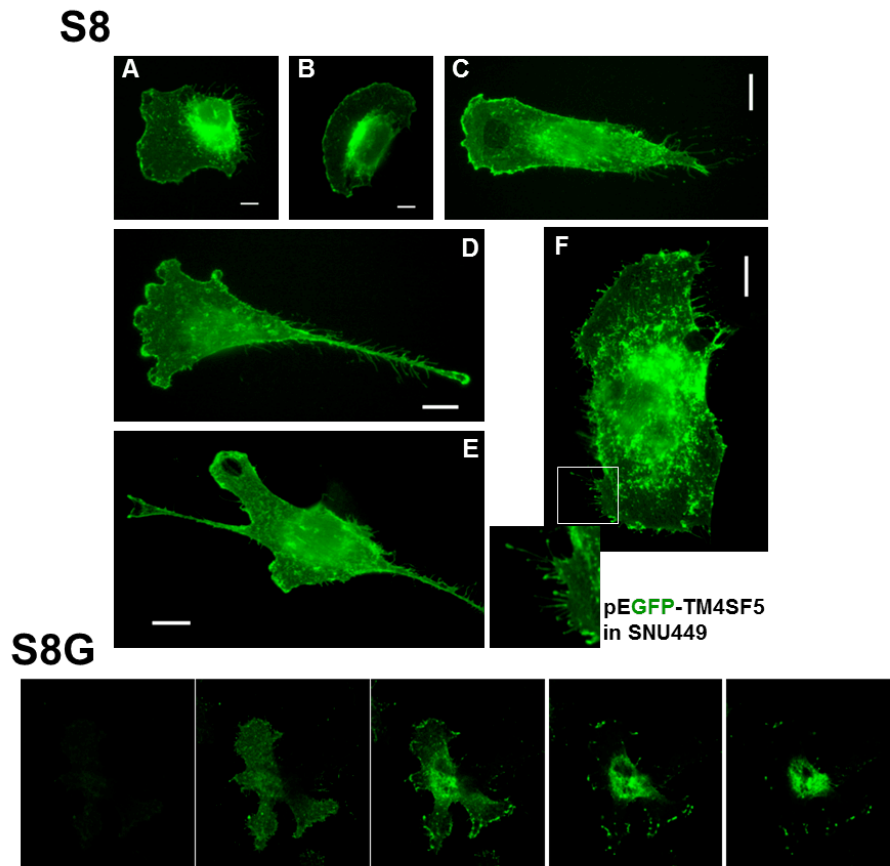


Fig. S8. (A to F) Transient transfection of pEGFP-TM4SF5 into SNU449 parental cells revealed its locations at cellular peripheries toward migration, such as lamellipodia (A, B, C, and D), filopodia (F), and protrusive (E) morphological features. (G) SNU449 cells were transiently transfected with pEGFP-TM4SF5 for 48 h, before mounting onto cover glasses in the presence of 10% serum and visualization along Z-dimension for the TM4SF5 localization at cellular peripheries using a confocal microscopy. Data represent three isolated experiments.

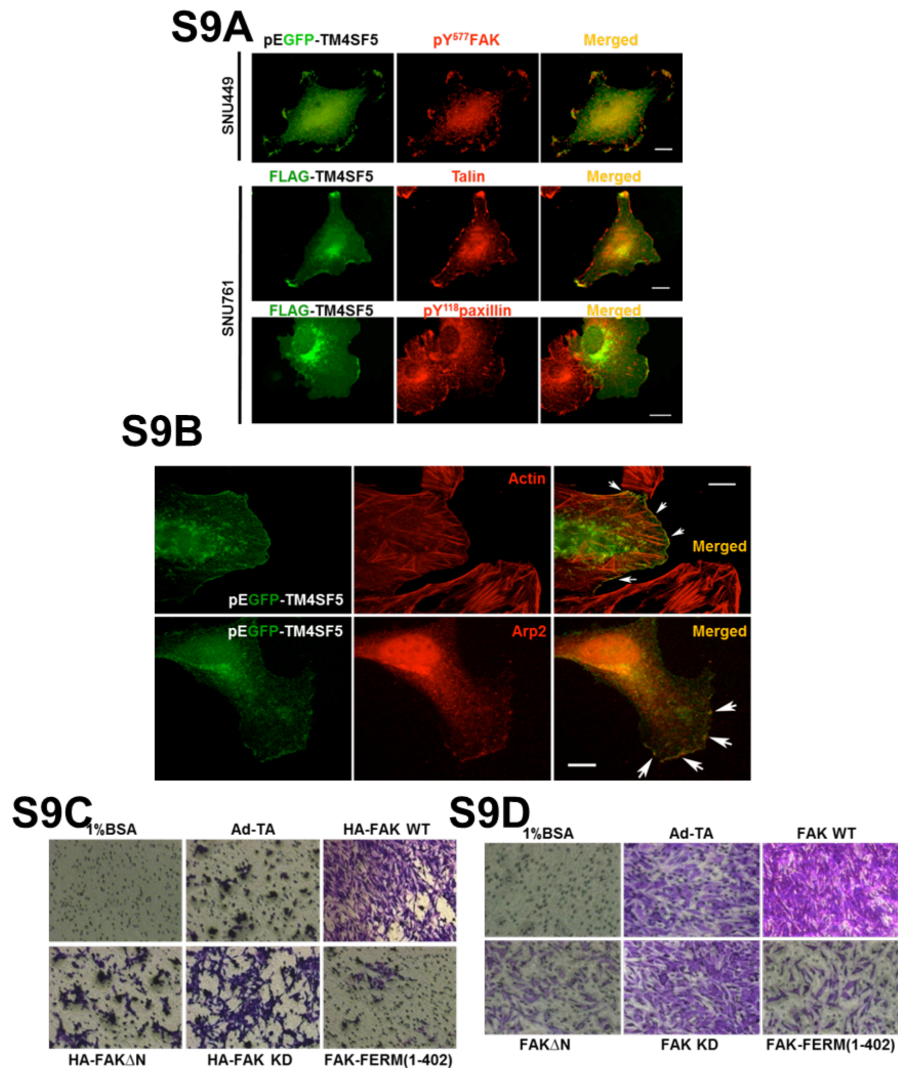


Fig. S9. (A) TM4SF5-null SNU449 or SNU761 parental cells were transfected with pEGFP-TM4SF5 or FLAG-TM4SF5 for 48 h, prior to indirect immunofluorescence for pY^{577}FAK, talin, or pY^{118}paxillin using fluorescence microscopy. Scale bar: 5 µm. (B) SNU449 cells transfected with pEGFP-TM4SF5 were stained with phalloidin. Arrows indicate stains positive for both TM4SF5 and Arp2. Scale bars: 10 µm. (c and d) Transwell migration analysis for SNU449 cells infected with adenovirus for diverse FAK forms toward 10% FBS (C) or fibronectin (D) for 12 h. Data represent three isolated experiments.

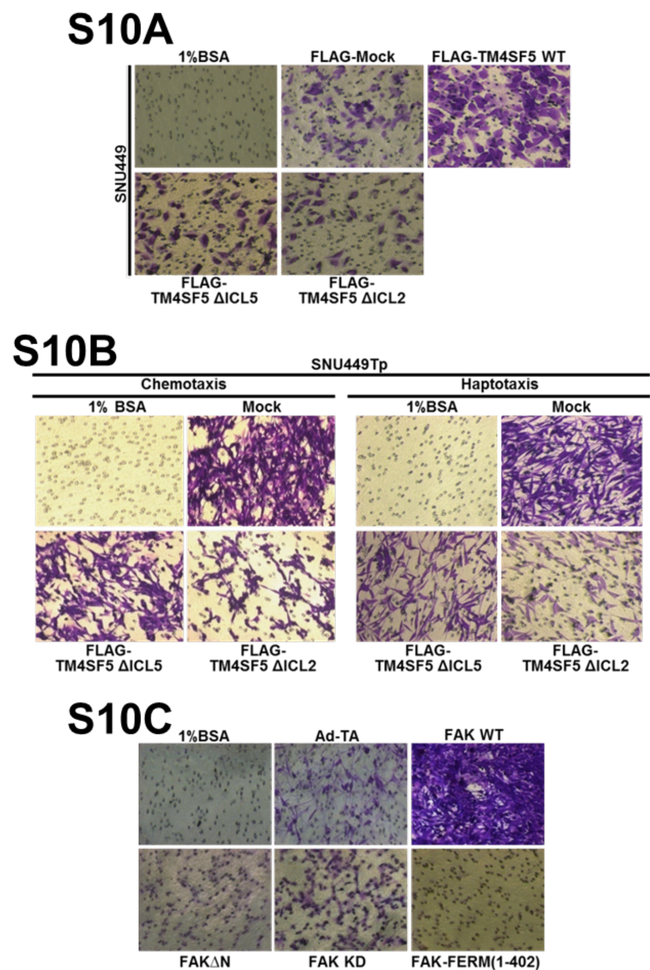


Fig. S10. (A to C) Staining of cells migrated (A and B) or invaded through matrigel (C). Representative images among 5 random images for each experimental condition are shown. Migration toward 10% FBS (chemotaxis, A and left B) or fibronectin (haptotaxis, right B) were analyzed using transwell chambers. Each panel is quantitatively graphed in the text; A for Fig. 5C (12 h), B for 5E (chemotaxis for 12 h and haptotaxis for 12 h), and C for 6B (24 h). Data represent three different experiments.

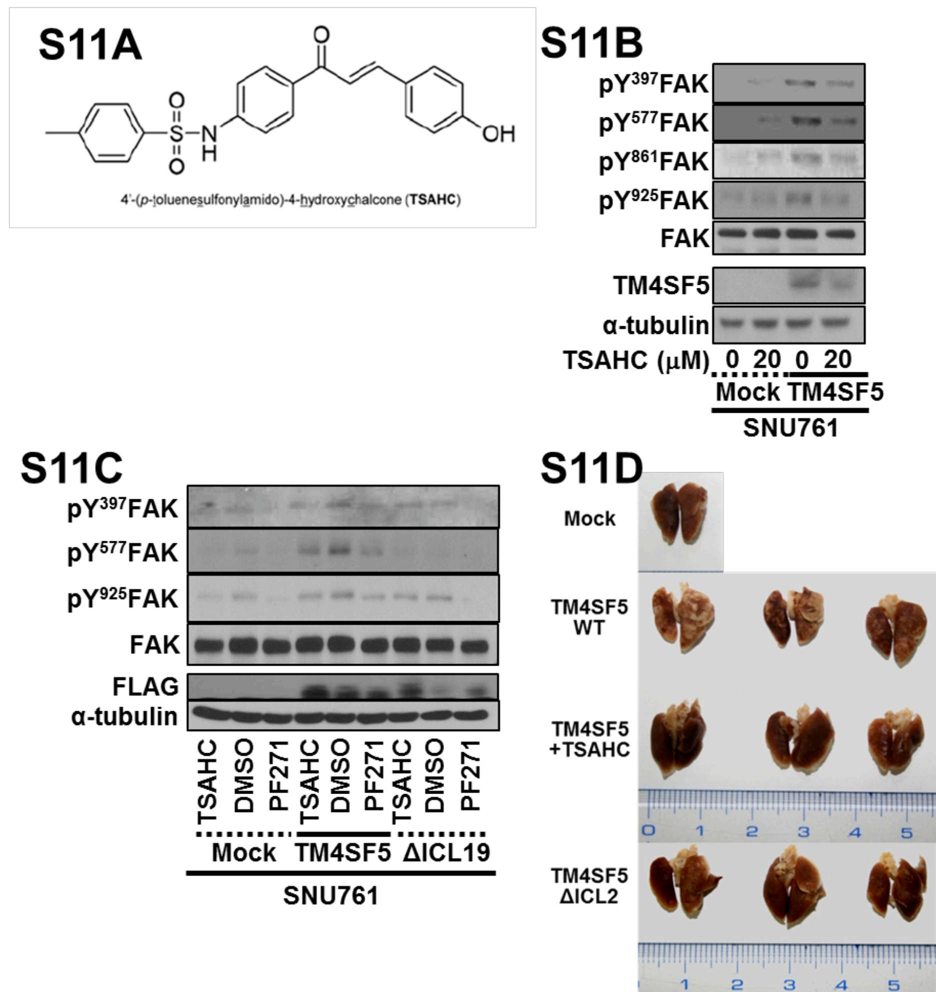


Fig. S11. (A) Chemical structure of TSAHC, [4'-(*p*-**t**oluene**s**ulfonyl**a**mido)-4-**h**ydroxy**c**halcone]. (B) Stable SNU761-mock or -FLAG-TM4SF5 WT cells in subconfluent conditions were treated with TSAHC at the indicated concentrations for 24 h, before whole cell extract preparation and standard Western blots. (C) Stable SNU761-mock, -FLAG-TM4SF5 WT, or ∆ICL19 mutant cells in subconfluent conditions were treated with vehicle DMSO or pharmacological inhibitors (20 μM TSAHC against TM4SF5, or 1.0 μM PF271 against FAK) for 24 h, before whole cell extract preparation and standard Western blots. (D) Representative lung images from mice that SNU761-mock, FLAG-TM4SF5 WT without or with TSAHC administration (TM4SF5 WT or TM4SF5 + TSAHC, respectively), or FLAG-TM4SF5∆ICL19 cells were injected through tail vein. The lungs were dissected out and stained with Bouin's solution, as explained in Materials and Methods.

Table S1. Antibodies used in the study.

Immunoblotting					
Antibody	Clone	Company	Antibody	Clone	Company
FAK	pAb, (C-20)	Santa Cruz	Cortactin	mAb	BD
FAK	mAb (77)	BD	Myc	mAb (9E10)	Genescript
FAK	mAb (4.47)	Millipore	p130Cas	mAb (21)	BD
Flag	mAb (M2)	Cell signal.	CyclophilinA	pAb	Abcam
pY397FAK	pAb	Abcam	Jab1	mAb (B-7)	Santa Cruz
pY397FAK	pAb	Santa Cruz	c-Src	mAb (B-12)	Santa Cruz
pY577FAK	pAb	Invitrogen	Arp2	pAb (H-84)	Santa Cruz
pY577FAK	pAb	Santa Cruz	GST	mAb	BD
pY861FAK	pAb	Santa Cruz	HA	mAb (F-7)	Santa Cruz
pY925FAK	pAb	Santa Cruz	pY486Cortactin	pAb	Chemicon
Paxillin	mAb (349)	BD	WASP	pAb (B-9)	Santa Cruz
pY118Paxillin	pAb	Santa Cruz	pY416c-Src	pAb	Cell signaling
pY	mAb (pY20)	Transd. Lab	HIS-probe	pAb (H-15)	Santa Cruz
a-tubulin	mAb	Sigma			

FACS					
Antibody	Clone	Company	Antibody	Clone	Company
a2	mAb (P1E6)	Chemicon	a6	mAb (4F10)	Chemicon
a3	mAb (P1B5)	Chemicon	ava5	mAb (P1F6)	Chemicon
a4	mAb (P1H4)	Chemicon	b1	mAb (TS2/16)	Chemicon
a5	mAb (P1D6)	Chemicon	b4	mAb (3E-1)	Homemade

Immunofluorescence					
Antibody	Clone	Company	Antibody	Clone	Company
Flag	mAb (M2)	Sigma	pY397FAK	pAb	Abcam
Talin	mAb (8D4)	Sigma	pY118Paxillin	pAb	Santa Cruz
pY577FAK	pAb	Santa Cruz	Arp2	pAb (H-84)	Santa Cruz

Immunoprecipitation	
Antibody	Company
High Capacity Streptavidin Agarose	Thermo Scientific
EZview™Red Anti-HA Affinity Gel	Sigma
Anti-FlagM2 Affinity Gel	Sigma