Supplementary Methods

Cell Culture

MCF10A, MCF10CA1a, MCF10CA1a.cl1 and MCF10CA1d.cl1 cells were cultured in DMEM/F12 supplemented with 10% horse serum, 20 ng/ml EGF, 10 μg/ml insulin, 0.5 μg/ml hydrocortisone, 100 ng/ml cholera toxin, 100 IU/ml penicillin, and 100 μg/ml streptomycin. MCF-7, T47D, MDA-MB-453, CAMA-1, SKBR3, BT-549 and MDA-MB-231 cells were cultured in DMEM, MDA-MB-436 and MDA-MB-468 in DMEM/F12, and ZR-75-30 in RPMI all containing 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. SUMPE44 cells were cultured in serum-free Ham's F-12 supplemented with 5 μg/ml insulin and 1μg/ml hydrocortisone. SUM149PT were cultured in the same medium as SUMPE44 cells supplemented with 5% FCS.

Plasmids

The pUHG10-3(FER) vector (kindly provided by N. Heisterkamp) was used as a template to amplify the full-length human FER cDNA by PCR and subclone it into the pcDNA5/FRT vector (Invitrogen), modified to contain the EF1α instead of CMV promoter. The EF1α-FER cassette was amplified by PCR thereby adding a C-terminal V5 epitope tag to the wild-type FER cDNA and flanking *BstB*I/*Xho*I restriction sites. EF1α-FER-V5 was then cloned in the *Cla*I/*Xho*I sites of pLV/CMV to generate pLV/EF1α-FER-V5 (WT FER). The QuikChange II XL Site-Directed Mutagenesis Kit (200521; Stratagene) was used to generate kinase dead (D742R) FER (D742D FER).

Control and FER-targeting oligonucleotides were annealed and cloned into the FH1tUTG vector. Oligonucleotide sequences were as follows (targeting sequences are capitalized):

control sense:

5’-tcccTTCTCCGAACGTGTCACGTttcaagagaACGTGACACGTTCGGAGAAtttttc-3’

control anti-sense:

5’-tcgagaaaaaTTCTCCGAACGTGTCACGTtctcttgaaACGTGACACGTTCGGAGAA-3’

FER[680-698] sense:

5’-tcccGGCTCACCATGATGATTAAttcaagagaTTAATCATCATGGTGAGCCtttttc-3’

FER[680-698] anti-sense:

5’-tcgagaaaaaGGCTCACCATGATGATTAAtctcttgaaTTAATCATCATGGTGAGCC-3’

FER[978-998] sense:

5’-tcccGTATTATGATATCACACTTCCttcaagagaGGAAGTGTGATATCATAATACtttttc-3’

FER[978-998] anti-sense:

5’-tcgagaaaaa GTATTATGATATCACACTTCCtctcttgaaGGAAGTGTGATATCATAATAC-3’

Immunofluorescence analysis

Primary antibodies used were: rat anti-human CD49f (555734, BD Pharmingen) 1:300, rat anti-integrin β_1 (AIIB2, Developmental Studies Hybridoma Bank, The University of Iowa, Iowa City, USA) 1:200, rabbit anti-paxillin pY^{118} (44-722; Invitrogen) 1:500. Secondary antibodies were: Alexa Fluor 488 goat anti-rat IgG (A11006) and Alexa Fluor 555 goat anti-rabbit IgG (A21429, Invitrogen) 1:1000. F-actin was visualized with Alexa Fluor 633-conjugated phalloidin (A22284; Invitrogen) 1:200.

Integrin expression measurement

The following primary antibodies and dilutions were used: mouse anti-α_2 integrin (clone 10G11; 1:100), mouse anti-α_3 integrin (clone J143; 1:2), mouse anti-α_V integrin (clone 13C2; 1:10), rat anti-β_1 integrin (clone A11B2; 1:200), rat anti-α_5 integrin (clone M16; 1:1000), rat anti-α_6 integrin (555734, BD Pharmingen; 1:100). The secondary antibodies and dilutions used were: Alexa Fluor 488 goat anti-mouse IgG (A11001, Invitrogen; 1:500), Alexa Fluor 488 goat anti-rat IgG (A11006, Invitrogen, 1:500).