FAK promotes recruitment of talin to nascent adhesions to control cell motility

Christine Lawson, Ssang-Taek Lim, Sean Uryu, Xiao Lei Chen, David A. Calderwood, and David D. Schlaepfer

Vol. 196 No. 2, January 23, 2012. Pages 223–232.

In the supplemental material originally provided with this paper, incorrect panels appeared in Fig. S3 B. The supplemental PDF has been corrected, and the new panels appear below. The figure legend text also has been revised.

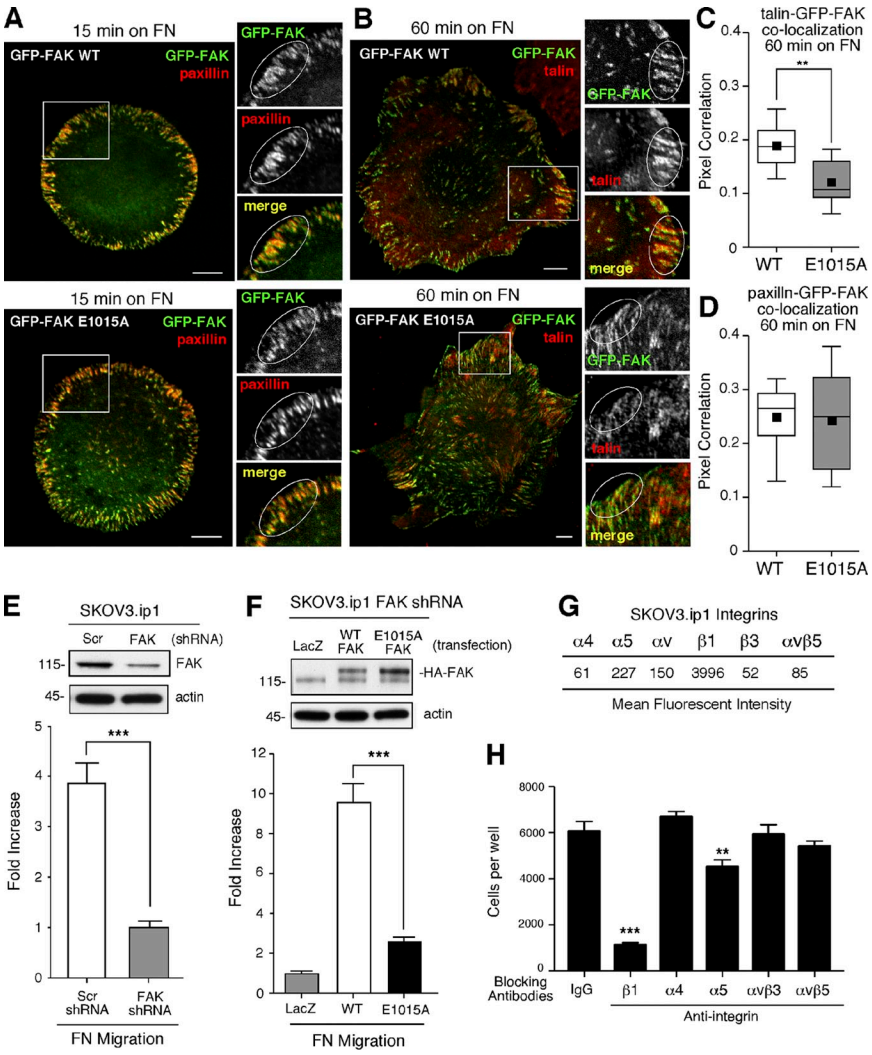

Figure S3. **FAK E1015A localizes to nascent adhesions but does not promote β1 integrin–mediated cell motility.** (A–D) FAK$^{-/-}$ MEFs stably expressing GFP-FAK WT or GFP-FAK E1015A were plated onto FN-coated coverslips for 15 min (A) or 60 min (B), then stained for paxillin (red) and imaged for GFP-FAK (green) fluorescence (A). At 60 min on FN, cells were analyzed for GFP-FAK (green) and talin (red) colocalization (B). The merged image shows colocalization (yellow). Inset, enlarged area of peripheral adhesion staining (circled). Bar, 10 µm. (C and D) The degree of association exhibited by patterns of GFP-FAK and talin (C) or GFP-FAK and paxillin (D) staining was measured on a pixel-by-pixel basis within all adhesions in at least 10 cells per experimental group encompassing at least three independent experiments. Box and whisker plots show the distribution of the data: black square, mean; bottom line, 25th percentile; middle line, median; top line, 75th percentile; whiskers, fifth and 95th percentiles (**, $P < 0.01$). (E) Ovarian SKOV3.ip1 carcinoma cells expressing lentiviral FAK shRNA (or scrambled as control; Scr) were immunoblotted (top) for FAK and actin. (E, bottom) The indicated cells were evaluated for FN-stimulated (10 µg/ml) cell migration (3 h). Data represent the mean ± SEM of three independent experiments (error bars) where FAK shRNA-expressing cell motility was normalized to 1. Significance was determined using an unpaired two-tailed Student's *t* test (***, $P < 0.001$). (F) SKOV3.ip1 FAK shRNA-expressing cells were transiently cotransfected with pCDNA3.1-lacZ and either control vector, HA-tagged FAK-WT, or HA-tagged FAK-E1015A and were immunoblotted (top) for FAK and actin. (F, bottom) The indicated cells were evaluated for FN-stimulated (10 µg/ml) cell migration (3 h). Migratory cells were identified by β-gal staining and counted. Data represent the mean ± SEM of three independent experiments (error bars) where LacZ-transfected cell motility was normalized to 1. Significance was determined using a one-way ANOVA followed by a Tukey multiple comparison test (***, $P < 0.001$). (G) Flow cytometry was used to determine integrin α4, α5, αv, β1, β3, and αvβ5 surface expression, and values represent mean fluorescent intensity using mouse IgG as a control. (H) SKOV3.ip1 cell adhesion to FN-coated (2 µg/ml) dishes was evaluated in the presence of the indicated blocking antibodies to integrins or control IgG. After 15 min, adherent cells were enumerated, and the data represent the mean ± SEM from two independent experiments (error bars). Significance was determined using a one-way ANOVA followed by a Dunnett's multiple comparison test and compared with mouse IgG control group (**, $P < 0.01$; ***, $P < 0.001$).