Beaty et al., http://www.jcb.org/cgi/content/full/jcb.201312046/DC1

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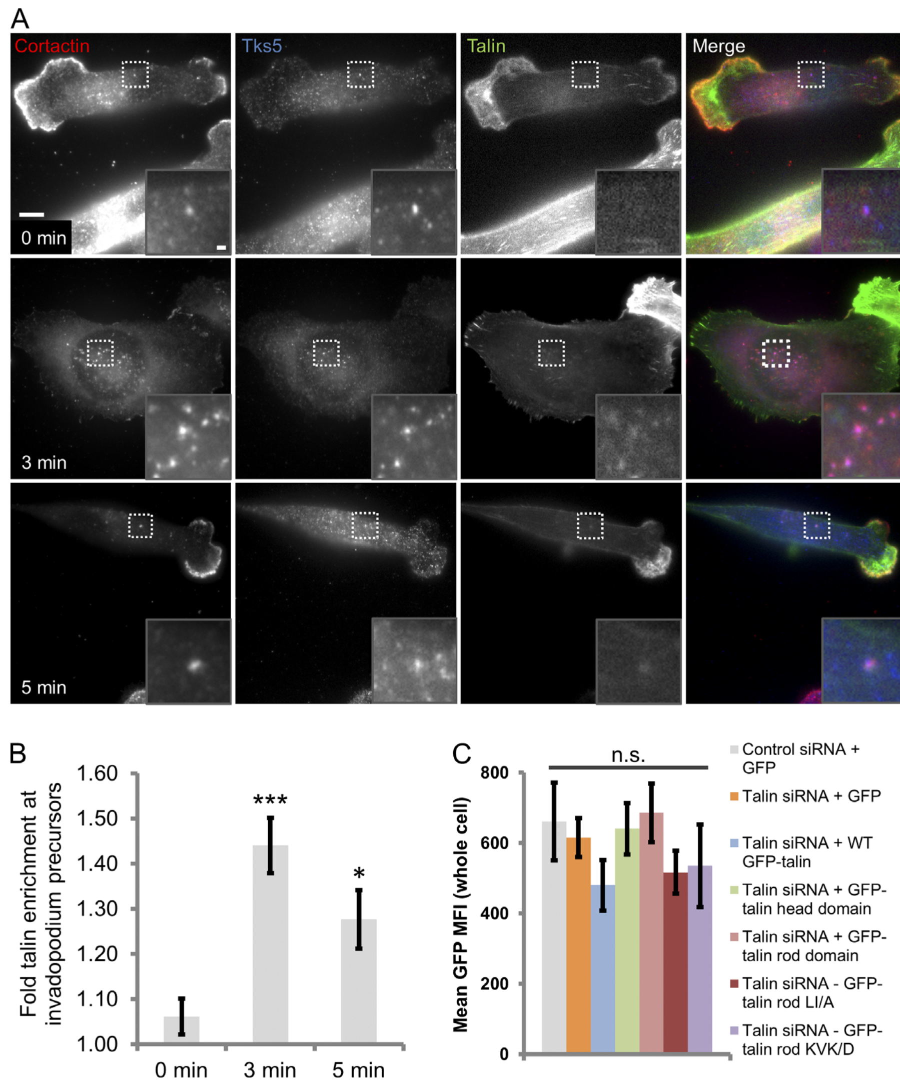


Figure S1. **Talin localizes to invadopodium precursors in response to EGF stimulation.** (A and B) Invadopodium precursor formation assay. (A) Representative image of MDA-MB-231 cell expressing GFP-talin and then stimulated with 2.5 nM EGF for 0 (unstimulated), 3 or 5 min. Cells were then stained for cortactin and Tks5 to identify invadopodium precursors. Inset shows magnified image of invadopodia in the box. Bars: (main panel) 10 µm; (inset) 1 µm. (B) Talin enrichment at invadopodium precursors was quantified at 0, 3, and 5 min after EGF stimulation. *n* > 20 invadopodia; two independent experiments. ***, P = 0.00055; *, P = 0.036; 3 vs. 5 min, not significant. (C) Quantification of the mean fluorescent intensity (MFI) of GFP and GFP-tagged talin constructs in cells transfected with control or talin siRNA, showing that the constructs are expressed to a similar level. *n* > 30 cells; three independent experiments. n.s., not significant (P > 0.05). Error bars represent the SEM.

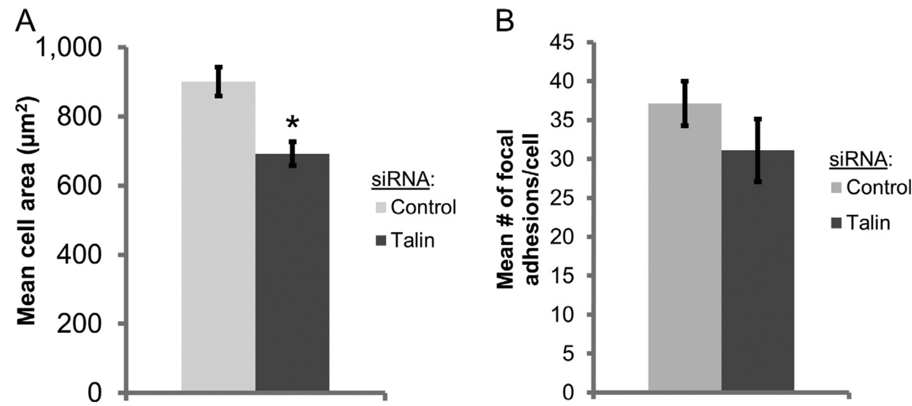


Figure S2. **Talin knockdown reduces cell area but does not affect focal adhesion formation in MDA-MB-231 cells plated on 405-labeled gelatin.** (A) Quantification of cell area in MDA-MB-231 cells plated on 405-labeled gelatin. $n > 31$ cells; two independent experiments. ***, P = 0.00039. (B) Quantification of focal adhesion number in MDA-MB-231 cells plated on 405-labeled gelatin. $n > 24$ cells; two independent experiments. n.s., not significant (P > 0.05). Error bars represent the SEM.

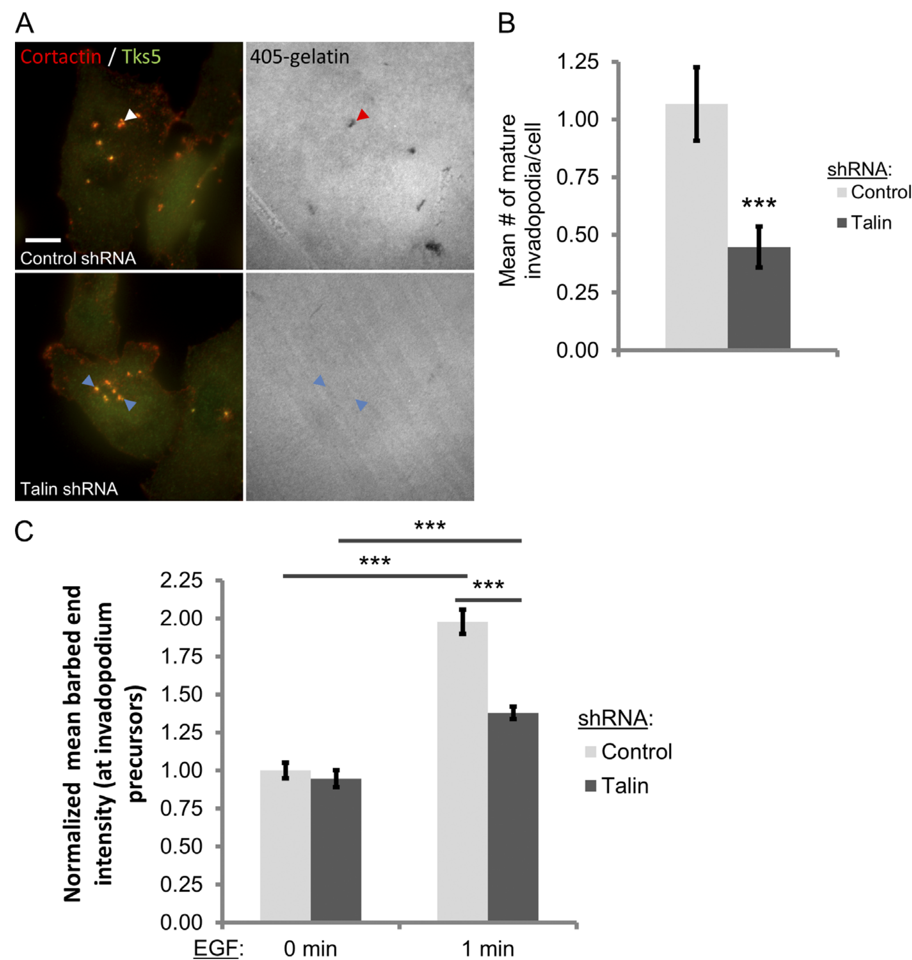


Figure S3. **Invadopodium maturation requires talin in MTLn3 cells.** (A and B) Steady-state invadopodial matrix degradation assay. (A) Representative images of MTLn3 cells stained for cortactin and Tks5. White/red arrowheads denote mature invadopodia; blue arrowheads denote invadopodium precursors. Bar, 10 µm. (B) Quantification of the number of mature invadopodia per MTLn3 control or talin shRNA cell. Mature invadopodia are defined as cortactin-/Tks5-rich punctate structures colocalized with a matrix degradation hole. $n > 89$ cells; three independent experiments. ***, $P = 5.5 \times 10^{-4}$. (C) Quantification of barbed end intensity within invadopodium precursors after stimulation with 5 nM EGF for 0 (unstimulated) or 1 min. $n > 107$ invadopodia; two independent experiments. ***, $P = 1.95 \times 10^{-9}$. Error bars represent the SEM.

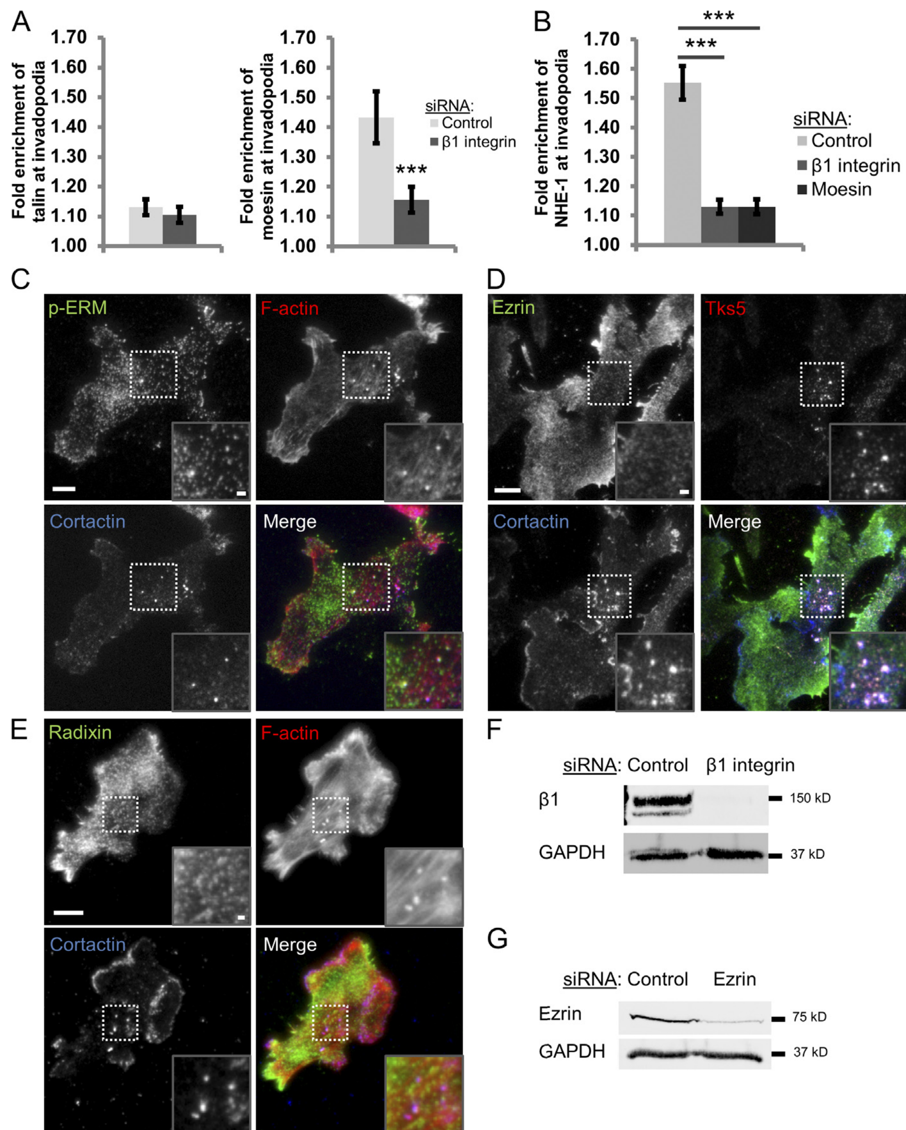


Figure S4. **β1 integrin is not required for talin localization to invadopodia, but is necessary for moesin and NHE-1 recruitment to invadopodia and characterization of ERM protein localization at invadopodia.** (A) Quantification of talin (left) and moesin (right) enrichment at invadopodia relative to cytosolic levels in control and β1 integrin siRNA-treated cells. Talin enrichment, $n > 94$ invadopodia; three independent experiments. Moesin enrichment, $n > 57$ invadopodia; two independent experiments. ***, $P < 0.0072$. (B) Quantification of NHE-1 enrichment at invadopodia in control, β1 integrin, and moesin siRNA-treated cells. $n > 85$ invadopodia; two independent experiments. ***, $P < 3.8 \times 10^{-7}$. Error bars represent the SEM. (C) Representative TIRF image of MDA-MB-231 cells stained for pan-phospho-ERM, F-actin (phalloidin), and cortactin, showing that phosphorylated ERM proteins are enriched at invadopodia above levels in the surrounding cytoplasm. Inset shows magnified image of invadopodia in the box. Bars: (main panel) 10 µm; (inset) 2 µm. (D) Representative TIRF image of MDA-MB-231 cells stained for ezrin, cortactin, and Tks5, showing that ezrin is not enriched above the surrounding cytoplasm. Inset shows magnified image of invadopodia in the box. Bars: (main panel) 10 µm; (inset) 2 µm. (E) Representative TIRF image of MDA-MB-231 cells stained for radixin, F-actin (phalloidin), and cortactin, showing that radixin localizes poorly to invadopodia. Inset shows magnified image of invadopodia in the box. Bars: (main panel) 10 µm; (inset) 2 µm. (F) Western blot analysis of MDA-MB-231 cells expressing control or β1 integrin siRNA for 96 h. Blots were stained for β1 integrin and GAPDH (loading control). (G) Western blot analysis of MDA-MB-231 cells expressing control or ezrin siRNA for 72 h. Blots were stained for ezrin and GAPDH (loading control).

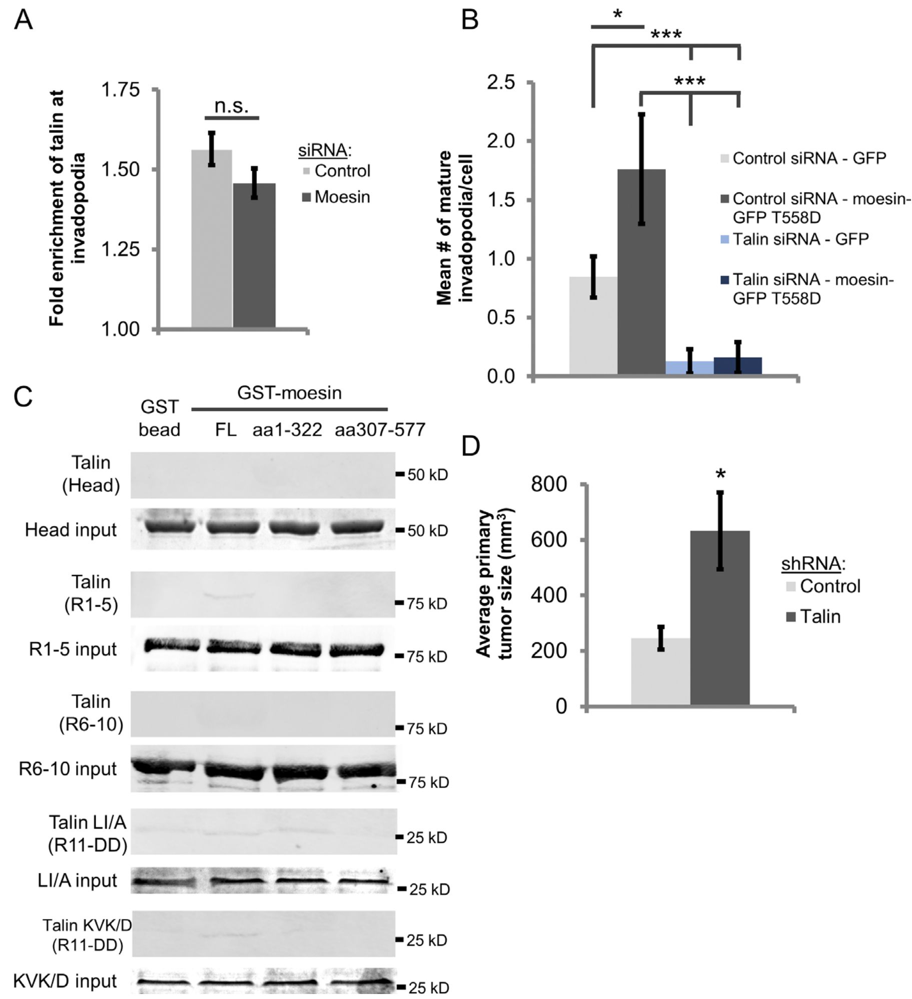
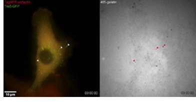
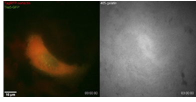


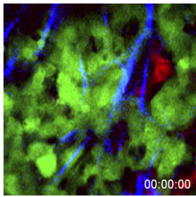
Figure S5. **Talin–moesin interaction in vitro and in vivo.** (A) Moesin knockdown does not affect talin recruitment to invadopodia. Quantification of talin enrichment at invadopodia relative to cytosolic levels in control and moesin siRNA-treated cells. $n > 44$ cells; two independent experiments. (B) Talin is required for moesin-dependent invadopodium maturation. Quantification of the number of mature invadopodia in control and talin siRNA-treated cells expressing either GFP or moesin-GFP T558D and plated on 405-labeled gelatin. $n > 31$ cells; two independent experiments. *, $P = 0.047$; ***, $P < 0.01$. (C) In vitro direct binding pulldown assay with His-tagged talin rod constructs and GST-tagged moesin fused to agarose beads. Western blots are stained for talin (antibody clone TA205, head; 8d4, R1-5; goat polyclonal, R6-10; R11-DD LI/A and KVK/D, anti-His tag). Three independent experiments. (D) Effect of talin knockdown on primary tumor growth. Quantification of mammary MTLn3 tumor size in control or talin shRNA mice. $n = 5$ mice; *, $P = 0.043$. Error bars represent the SEM.



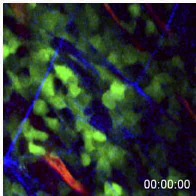
Video 1. **Control siRNA-treated MDA-MB-231 cells form long-lived, stable invadopodia that mature to degrade the ECM.** Control cells were transfected with TagRFP-cortactin (red) and GFP-Tks5 (green) and plated on 405-labeled gelatin. Cells were imaged using the DeltaVision Core Microscope (Applied Precision). Movie shows time-lapse images acquired every 2 min; 4.2 frames/s. Arrowheads denote mature invadopodia (white) and their corresponding matrix degradation holes (red). Invadopodia formed in these cells are very stable, remaining in a single location often for hours, and degrade the ECM.



Video 2. **Talin siRNA-treated MDA-MB-231 cells form short-lived, unstable invadopodia that fail to mature and degrade the ECM.** Talin-depleted cells were transfected with TagRFP-cortactin (red) and GFP-Tks5 (green) and plated on 405-labeled gelatin. Cells were imaged using the DeltaVision Core Microscope (Applied Precision). Movie shows time-lapse images acquired every 2 min; 4.2 frames/s. Arrowheads denote invadopodium precursors (white) and their lack of corresponding matrix degradation holes (red). Invadopodia formed in these cells are unstable, remaining in a single location for an average of only 10–20 min, and do not degrade the ECM.



Video 3. **Control shRNA tumor cell motility in vivo**. MTLn3 cells stably expressing control shRNA and Dendra2 migrating in vivo. Tumors were imaged using the custom multiphoton microscope (Entenberg et al., 2011). Movie shows time-lapse images of an area of the tumor near blood vessels (images acquired every 3 min for 1.5 h; 10 frames/s). White arrowheads denote motile cells.



Video 4. **Talin shRNA tumor cells have reduced motility in vivo**. MTLn3 cells stably expressing talin shRNA and Dendra2 migrating in vivo. Tumors were imaged using the custom multiphoton microscope (Entenberg et al., 2011). Movie shows time-lapse images of an area of the tumor near blood vessels (images acquired every 3 min for 1.5 h; 10 frames/s). Note that these cells are considerably less motile than control tumor cells in similar microenvironments in vivo.

Reference

Entenberg, D., J. Wyckoff, B. Gligorijevic, E.T. Roussos, V.V. Verkhusha, J.W. Pollard, and J. Condeelis. 2011. Setup and use of a two-laser multiphoton microscope for multichannel intravital fluorescence imaging. *Nat. Protoc.* 6:1500–1520. http://dx.doi.org/10.1038/nprot.2011.376