Supplemental Materials

Molecular Biology of the Cell

Hoskin et al.

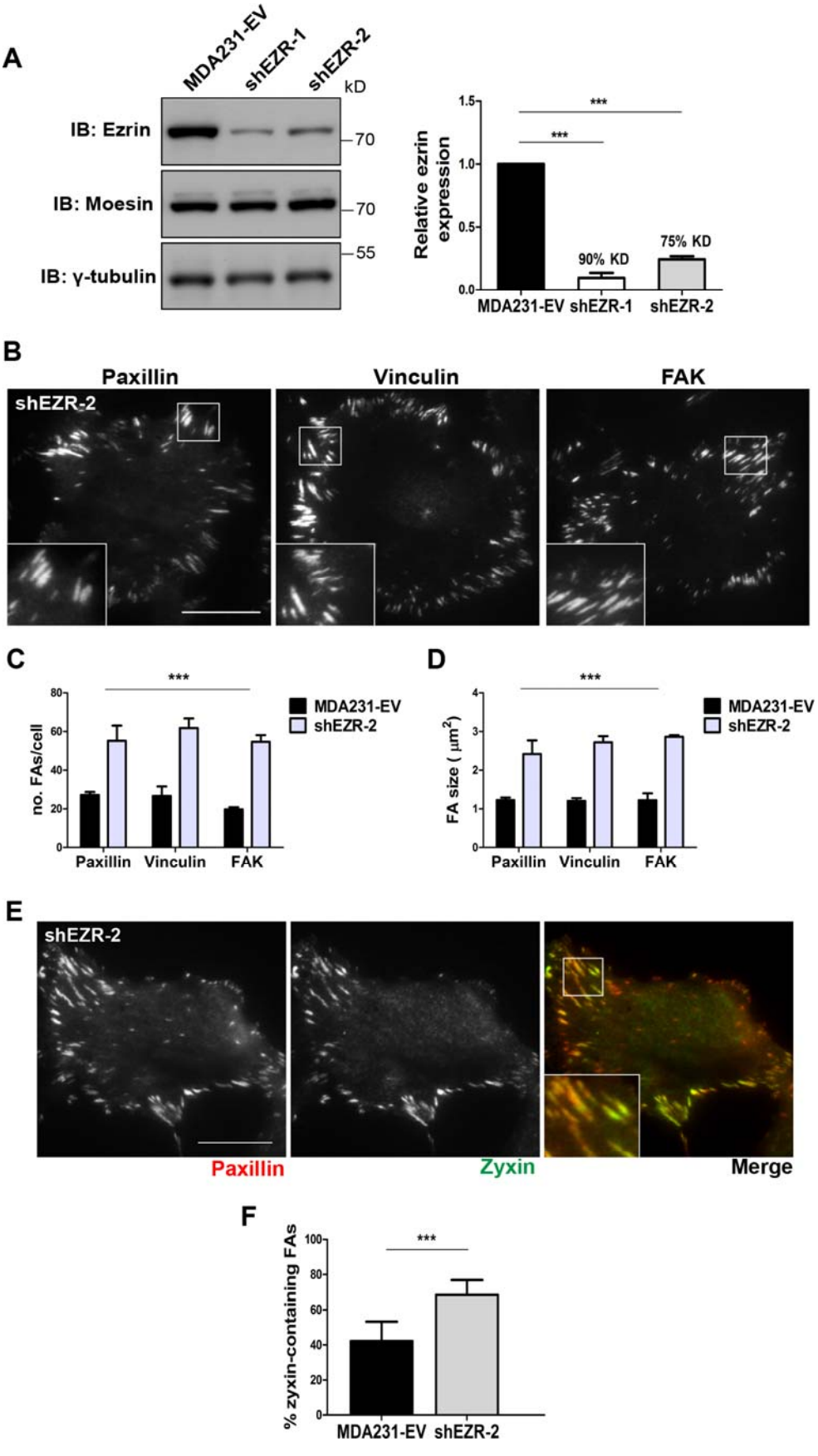
A
MDA231-EV
shEZR-1
shEZR-2
kD
IB: Ezrin
IB: Moesin
IB: γ-tubulin
70
70
55
Relative ezrin expression
1.5
1.0
0.5
0.0
***
***
90% KD
75% KD
MDA231-EV
shEZR-1
shEZR-2
B
Paxillin
Vinculin
FAK
shEZR-2
C
no. FAs/cell
80
60
40
20
0
***
MDA231-EV
shEZR-2
Paxillin
Vinculin
FAK
D
FA size (μm2)
4
3
2
1
0
***
MDA231-EV
shEZR-2
Paxillin
Vinculin
FAK
E
shEZR-2
Paxillin
Zyxin
Merge
F
% zyxin-containing FAs
100
80
60
40
20
0
***
MDA231-EV
shEZR-2

Figure S1. Ezrin-depletion construct shEZR-2 produces similar phenotypes as shEZR-1

(A) Cell lysates from control MDA231-EV and ezrin-depleted (shEZR-1 and shEZR-2) cells were analysed by immunoblotting using anti-ezrin, anti-moesin or anti-γ-tubulin antibodies. The percent reduction in endogenous ezrin protein expression by each shRNA construct was determined by densitometry. (B) Immunofluorescence staining of anti-paxillin, anti-vinculin or anti-FAK was performed on MDA231 cells expressing an independent shRNA construct, shEZR-2, with similar effects as shEZR-1. Quantification of the number (C) and size (D) of FAs in shEZR-2 cells was calculated and compared to control MDA231-EV cells. (E) Immunofluorescence staining of anti-paxillin and anti-zyxin was performed on shEZR-2 cells. The percentage of zyxin-containing FAs was quantified by assessing the number of FAs where zyxin and paxillin colocalized, compared to the total number of paxillin-containing FAs counted. Data shown represent means + SD of at least 3 independent experiments. **, $p<0.01$; ***, $p< 0.001$ by two-way ANOVA (C, D) or unpaired *t* test (A, E). Scale bars, 15μm.

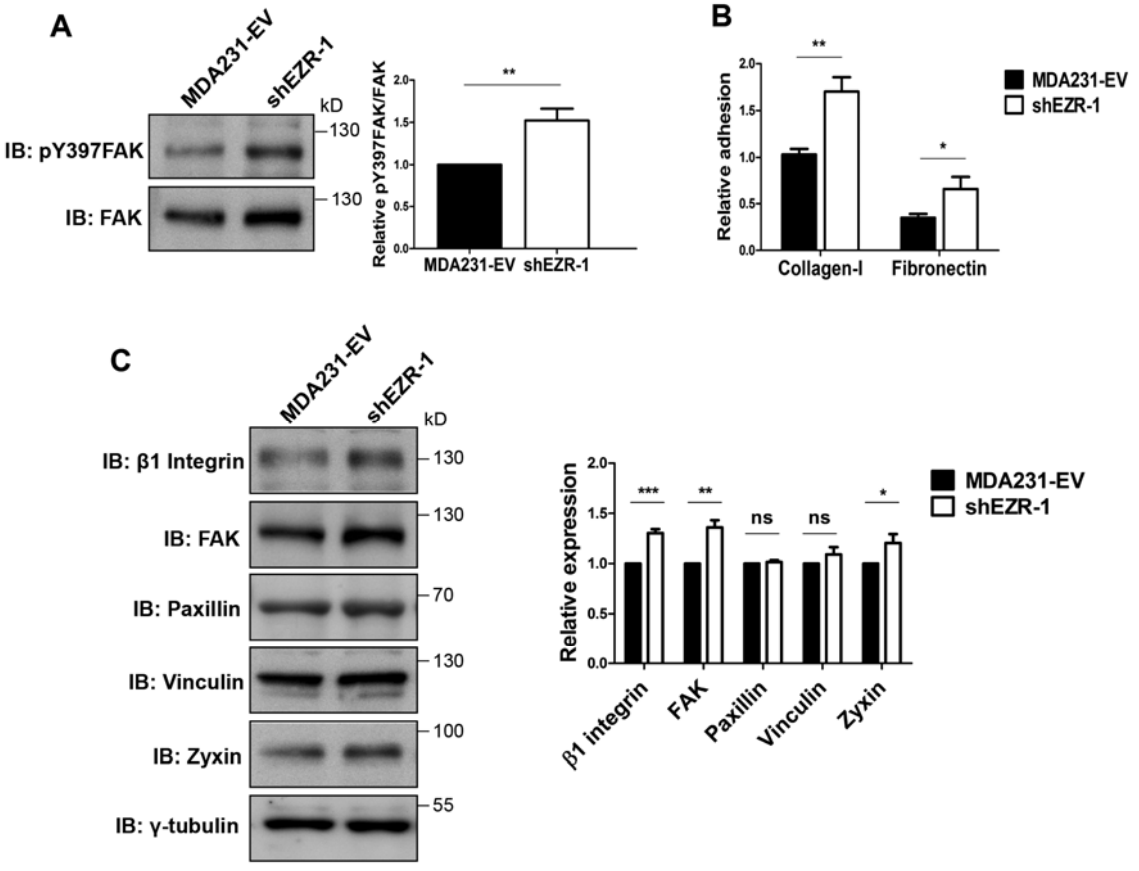

Figure S2. Ezrin regulates cell adhesion

(A) MDA231-EV and ezrin-depleted (shEZR-1) cell lysates were analyzed by immunoblotting using phospho-specific anti-tyrosine 397FAK (pY397FAK) or anti-FAK antibodies. Densitometry was performed to assess relative changes in pY397FAK to total FAK protein. (B) MDA231-EV and ezrin-depleted cells were plated on collagen-I or fibronectin-coated 96 well plates and allowed to adhere for 30 min prior to fixation and

staining. Quantification of data is shown relative to MDA231 cells on collagen-I. (C) Cell lysates from MDA231 and ezrin depleted cell were analyzed by immunoblotting using anti-β1 Integrin, anti-paxillin, anti-vinculin, anti-zxin and anti-γ-tubulin antibodies. Quantification of immunoblots was performed using densitometry. Expression levels were calculated relative to the levels in control MDA231-EV cells. Data shown represent means + SD of three independent experiments. *, p< 0.05; ** p<0.01; ***, p< 0.001 by one sample *t* test (A, C) or unpaired *t* test (B); ns, not significant.

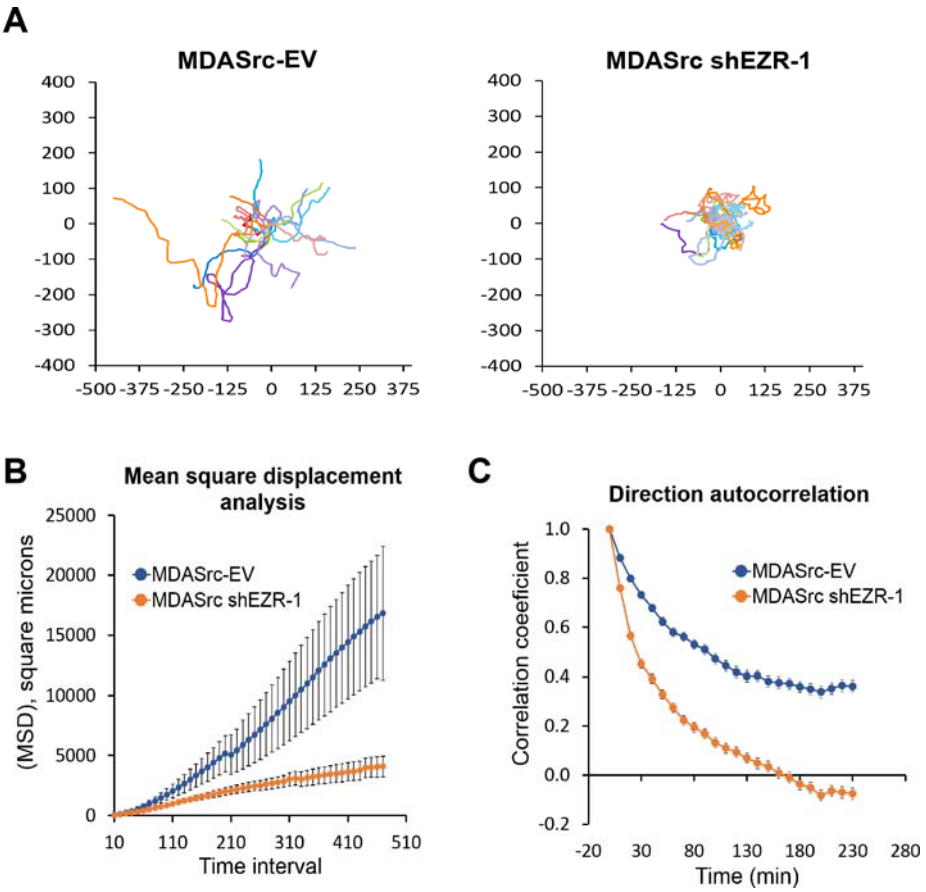

Figure S3. Ezrin depletion in MDASrc cells reduces cell directionality

(A) MDASrc-EV and MDASrc ezrin depleted cells were seeded sparsely onto collagen-I-coated 8 well µ-Slides and analyzed by time-lapse microscopy for 18 h. Cell trajectory data were generated using Metamorph Software and plotted using Graphpad Prism software. MSD (B) and direction autocorrelation (C) analyses were performed using the open source program DiPer. Data shown represent means ± SE of 3 independent experiments, with a minimum of 40 cells analyzed. **, p< 0.001 by linear regression analysis (B); ***, p< 0.0001 (C) by unpaired *t* test.

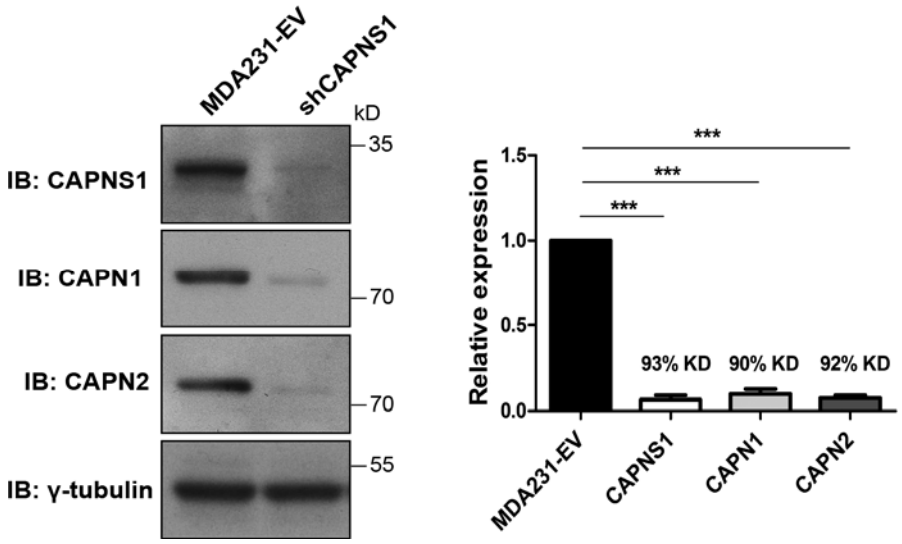

Figure S4. CAPNS1 depletion in MDA231 cells
MDA231-EV and shCAPNS1-depleted cell lysates were analyzed by immunoblotting using anti-CAPN1, anti-calpain-2 and anti-γ-tubulin antibodies. Densitometry was performed to assess the efficiency of CAPN1, CAPN2 and CAPNS1 knockdown, relative to control MDA231 cells. Data shown represent means + SD of three independent experiments. ***, $p<0.001$ by one-way ANOVA with Tukey's Multiple Comparison Test.

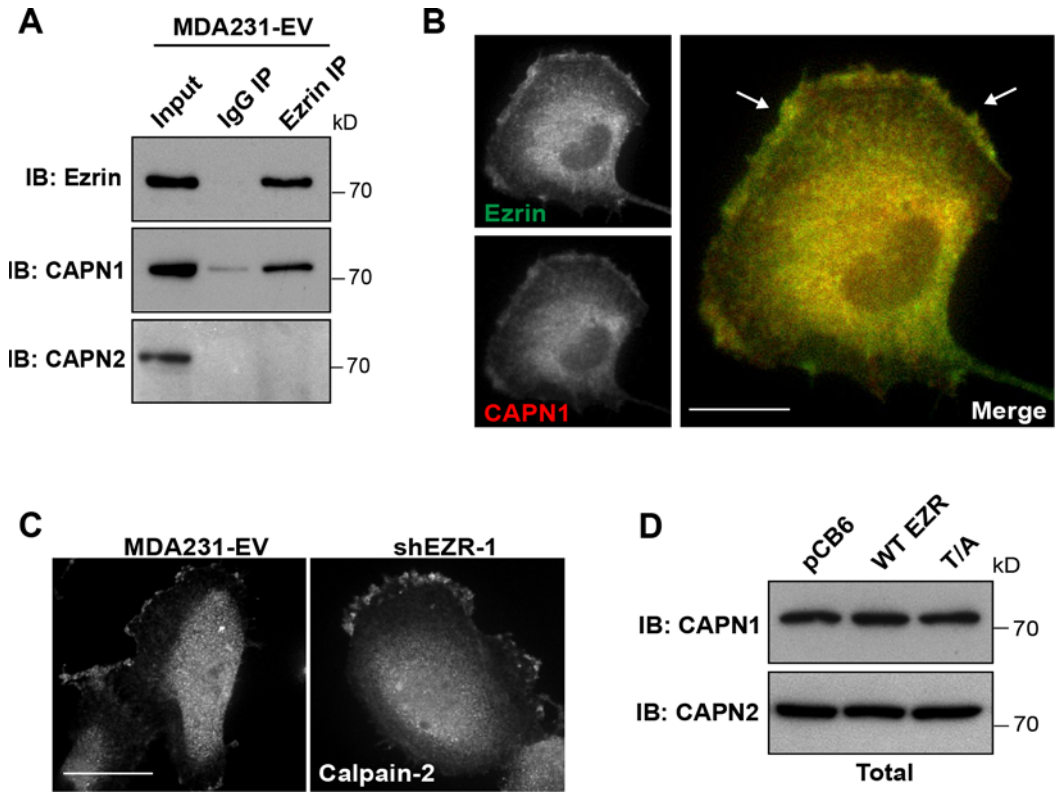

Figure S5. Co-localization of ezrin and calpain-1 in MDA231-EV cells
(A) MDA231-EV cell lysates were immunoprecipitated (IP) using anti-ezrin or IgG antibodies and were then immunoblotted (IB) using anti-ezrin, anti-CAPN1 or anti-calpain-2 antibodies. (B) MDA231-EV cells were stained by immunofluorescence using anti-ezrin and anti-CAPN1 antibodies. White arrows indicate areas of co-localization at the membrane. At least 30 cells were analyzed.(C) Immunofluorescence staining for calpain-2 in MDA231-EV and shEZR-1 cells. (D) Whole cell lysates from MDA231-EV cells overexpression pCB6, wild-type ezrin (WT EZR) or T/A ezrin were analyzed by immunoblotting using anti-CAPN1 and anti-calpain-2 antibodies. Data shown are representative of 3 independent experiments. Scale bar, 15 μm.

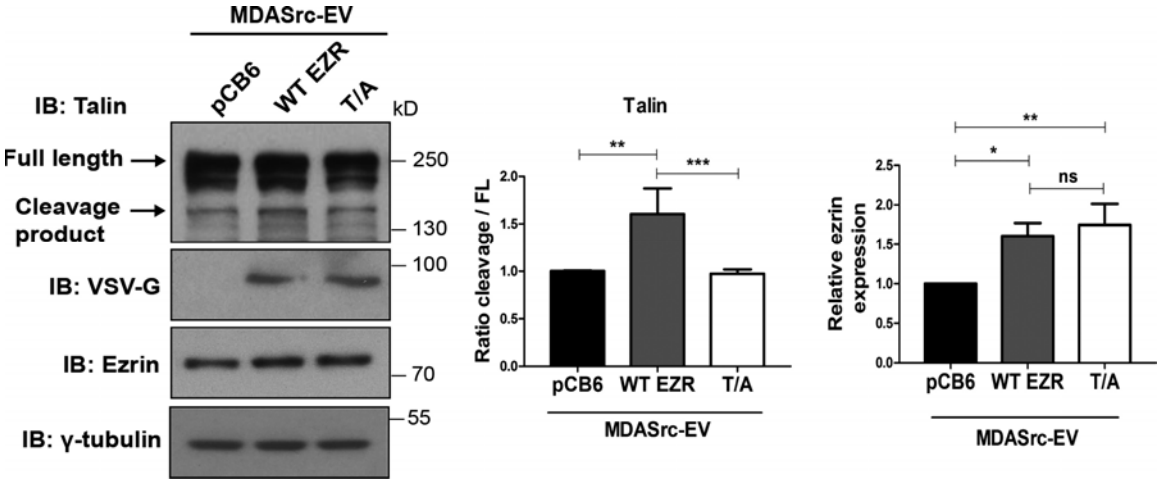

Figure S6. Expression of WT ezrin induces talin cleavage by calpain in MDASrc-EV cells
(A) pCB6 (empty vector control), Wild-type ezrin (WT-EZR) or T/A ezrin were transiently over-expressed in MDASrc cells. Corresponding lysates were obtained and analyzed by Western blotting and probed for anti-ezrin, anti-talin, anti-VSV-G or anti-γ-tubulin antibodies. Densitometry was performed to assess changes in talin cleavage relative to pCB6 control. Data shown represent means $\pm$ SD of three independent experiments. **, $p < 0.01$; ***, $p< 0.001$ by one-way ANOVA with Tukey's Multiple Comparison Test.