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Supplemental information

Fibroblast activation protein drives tumor metastasis via a protease-independent role in invadopodia stabilization

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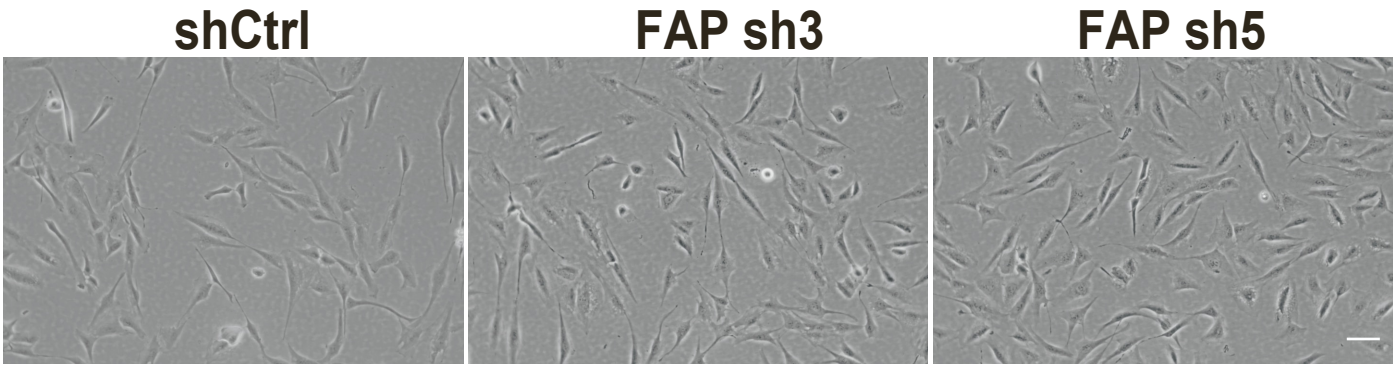
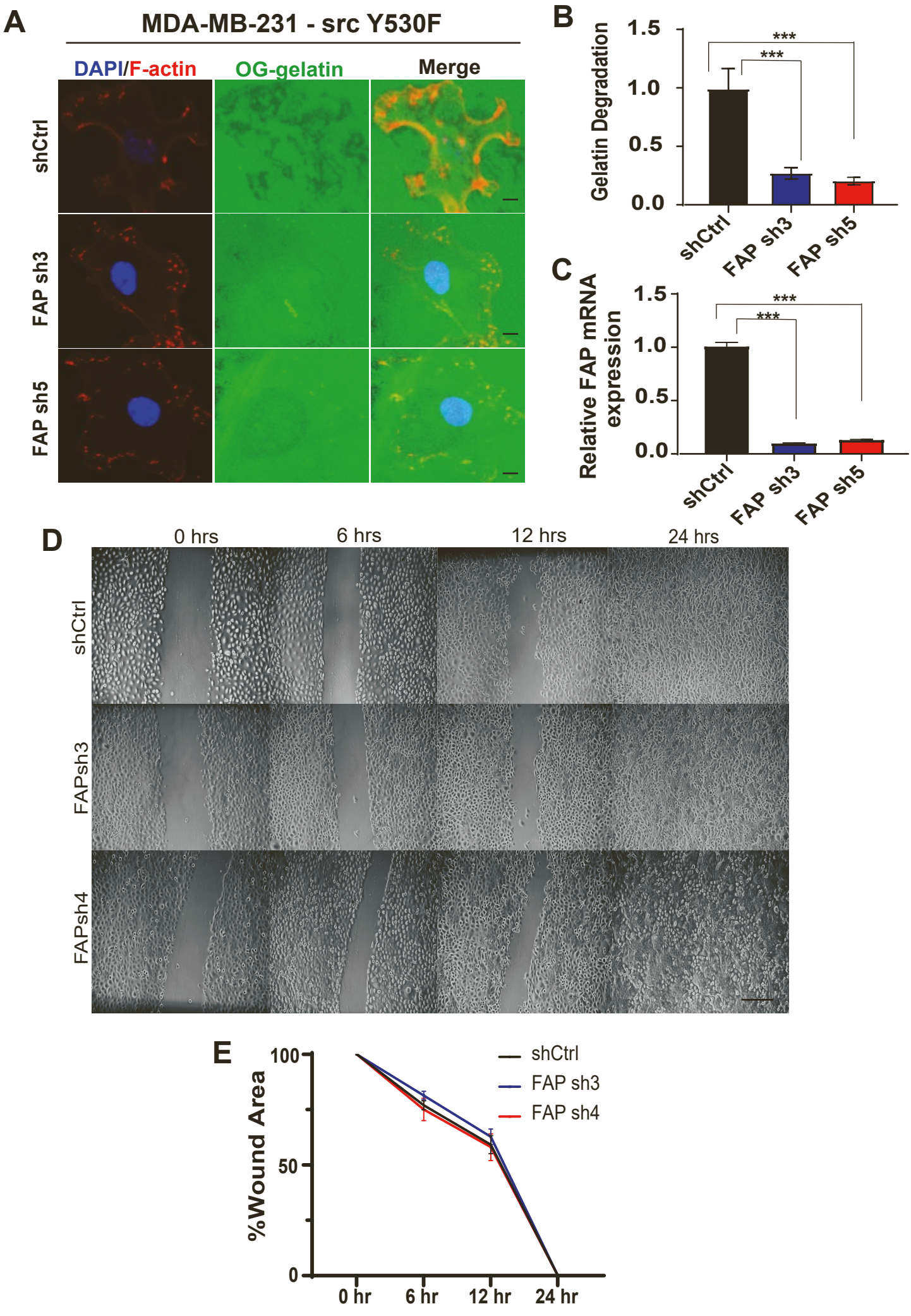
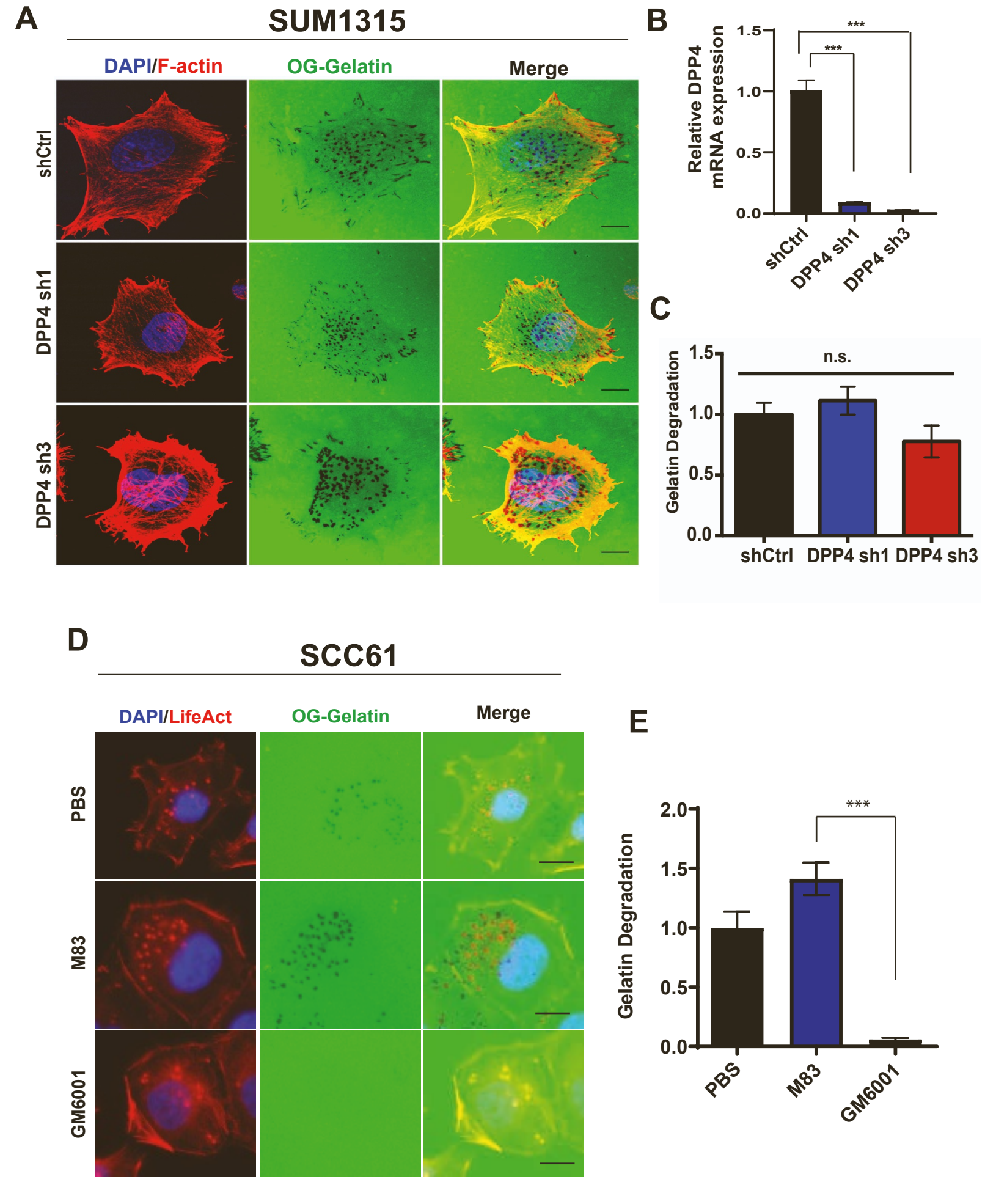
HMLE-TWIST1



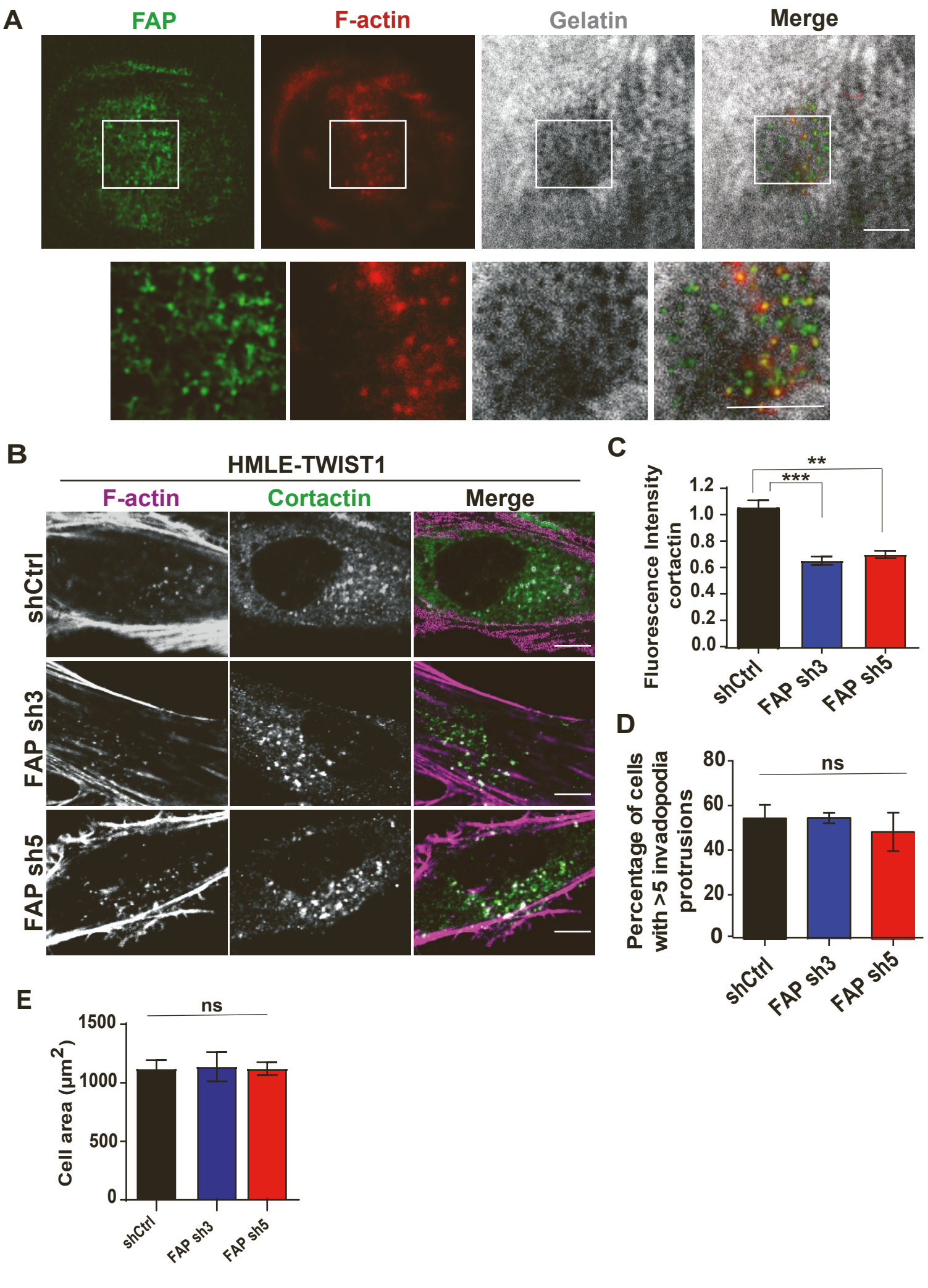
Supplemental Figure 1. FAP is not required for TWIST1-induced EMT, Related to Figure 2. TWIST1 was exogenously expressed in HMLE cells expressing the indicated shRNAs. All cells underwent EMT within 15 days after introduction of TWIST1, as shown in bright-field images. Scale bar represents 100μm.


A
MDA-MB-231 - src Y530F
DAPI/F-actin
OG-gelatin
Merge
shCtrl
FAP sh3
FAP sh5
B
Gelatin Degradation
1.5
1.0
0.5
0.0
***
***
shCtrl
FAP sh3
FAP sh5
C
Relative FAP mRNA expression
1.5
1.0
0.5
0.0
***
***
shCtrl
FAP sh3
FAP sh5
D
0 hrs
6 hrs
12 hrs
24 hrs
shCtrl
FAPsh3
FAPsh4
E
%Wound Area
100
50
0
0 hr
6 hr
12 hr
24 hr
shCtrl
FAP sh3
FAP sh4

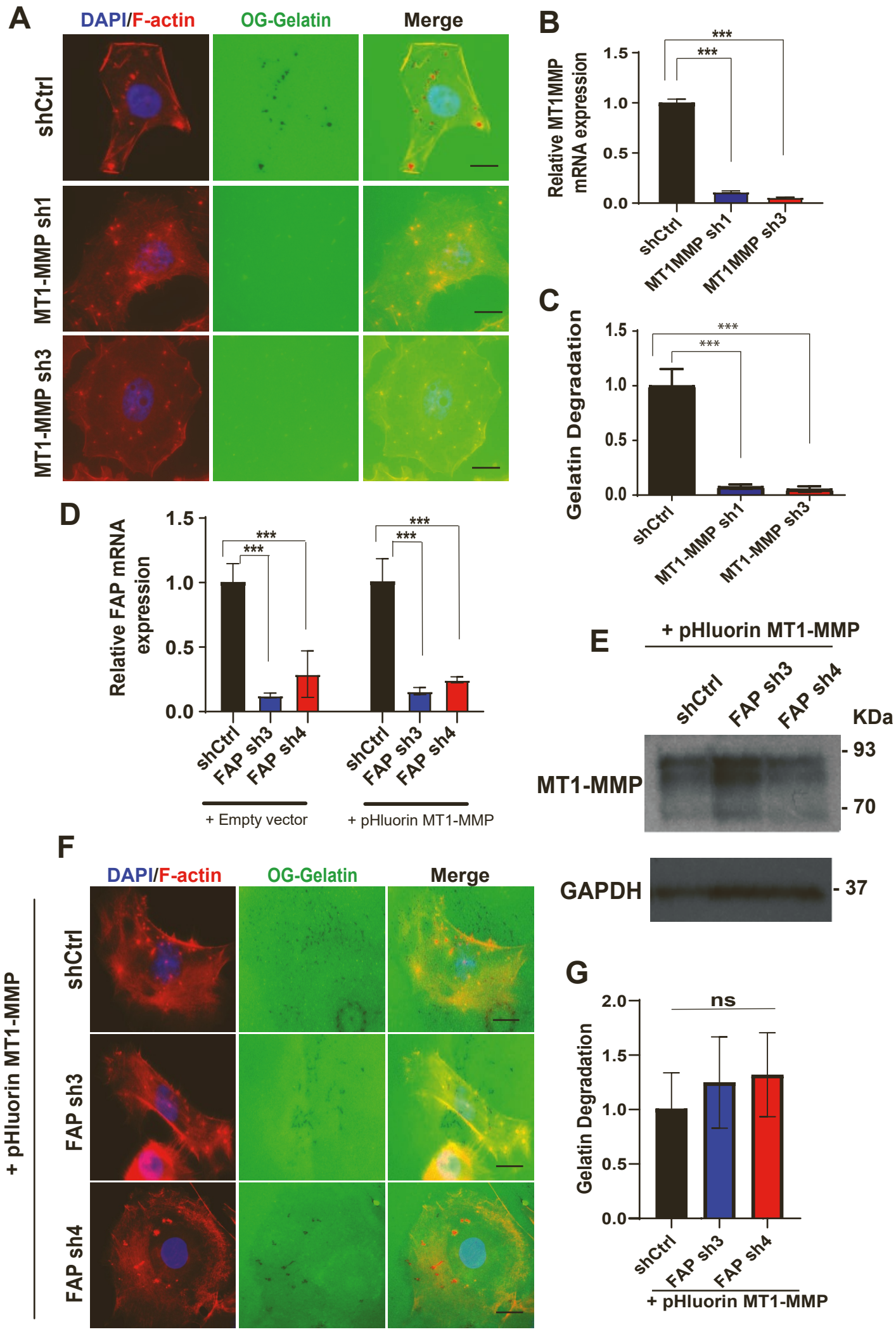
Supplemental Figure 2. FAP plays an essential role in promoting ECM degradation, but not cell migration, **Related to Figure 3.** A) MDA-MB-231SRCY530F cells expressing FAP shRNAs were plated on Oregon green conjugated gelatin for 4 hours and subsequently fixed and stained for F-actin (red) and nuclei (blue). Scale bar represents 10μm. B) Quantification of gelatin degradation from (A) normalized to control shRNA. Error bars represent SEM. N = 150 cells per group. **$p < 0.01$, ***$p < 0.001$. Student's t-test. C) qRT-PCR measurement of FAP mRNA levels in MDA-MB-231 SRCY530F cells expressing shRNAs targeting FAP. Error bars represent standard deviation (SD). D) SCC61 cells expressing the indicated FAP shRNAs were grown to confluency, and then a p1000 pipette tip was dragged through the confluent monolayer, creating a "wound." The cells were monitored over the next 24 hours. Scale bar represents 100μm. E) Quantification of wound area closure in triplicate experiments. Error bars represent SEM.


A
SUM1315
DAPI/F-actin
OG-Gelatin
Merge
shCtrl
DPP4 sh1
DPP4 sh3
B
Relative DPP4 mRNA expression
1.5
1.0
0.5
0.0
***
***
shCtrl
DPP4 sh1
DPP4 sh3
C
Gelatin Degradation
1.5
1.0
0.5
0.0
n.s.
shCtrl
DPP4 sh1
DPP4 sh3
D
SCC61
DAPI/LifeAct
OG-Gelatin
Merge
PBS
M83
GM6001
E
Gelatin Degradation
2.0
1.5
1.0
0.5
0.0
***
PBS
M83
GM6001

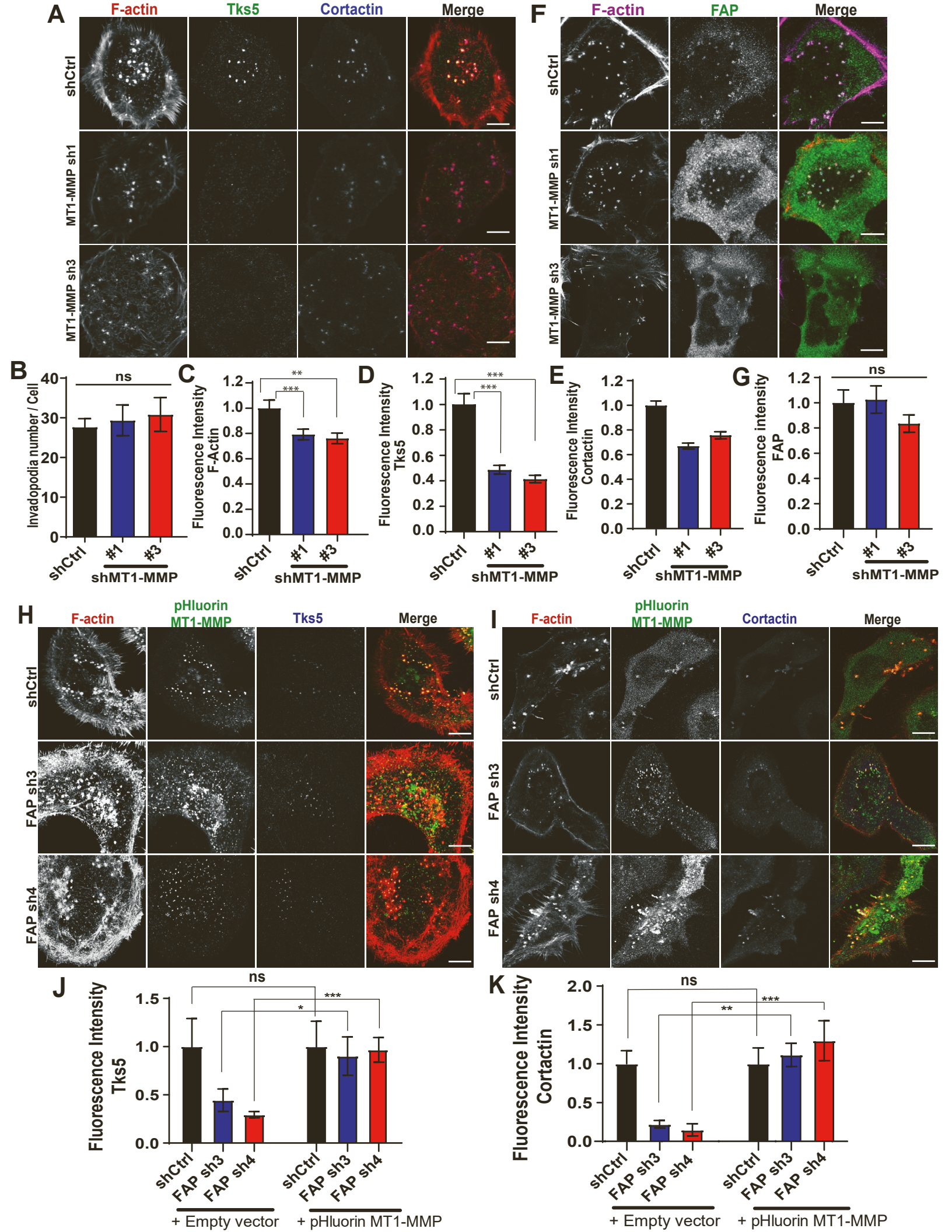
Supplemental Figure 3. FAP is required for ECM degradation independent of its proteolytic activity, Related to Figure 4. A) SUM1315 cells expressing the DPP4 shRNAs were seeded onto Oregon Green-labeled gelatin for 6 hours. Cells were then fixed and stained for F-actin (red), and nuclei were stained with DAPI (blue). B) Quantification of DPP4 mRNA in SUM1315 cells expressing either a control shRNA, or shRNAs targeting DPP4. C) Quantification of gelatin degradation from (A) and normalized to control shRNA. N = 150 cells per group. Error bars represent SEM. $^{**}p < 0.001$, ns: not significant ($p > 0.05$). Student's t-test. D) SCC61 cells were seeded on Oregon green labelled gelatin for 16 hours in the presence of PBS, M83 (10μM) or GM6001 (10μM) and subsequently fixed and stained for F-actin (red) and nuclei (blue). E) Quantification of gelatin degradation from (D) normalized to PBS treatment. Error bars represent SEM. N = 150 cells per group. $^{**}p < 0.01$, $^{***}p < 0.001$. Student's t-test. Scale bar represents 10μm.


A
FAP
F-actin
Gelatin
Merge
B
HMLE-TWIST1
F-actin
Cortactin
Merge
shCtrl
FAP sh3
FAP sh5
C
Fluorescence Intensity cortactin
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
***
shCtrl
FAP sh3
FAP sh5
D
Percentage of cells with >5 invadopodia protrusions
80
60
40
20
0
ns
shCtrl
FAP sh3
FAP sh5
E
Cell area (μm2)
1500
1000
500
0
ns
shCtrl
FAP sh3
FAP sh5

Supplemental Figure 4. FAP knockdown affects invadopodia precursor stabilization, Related to Figure 5 and 6. A) SCC61 cells expressing CFP-FAP were seeded on Oregon Green labelled gelatin for 16 hours. Cells were then fixed and stained for F-actin (red). TIRF microscopy was used at penetration depth of 100nm to visualize FAP localization at mature invadopodia as denoted by degradation spots in gelatin. FAP image was clarified and 2D deconvolved using NIS-Elements software to improve signal to noise ratio. Insets show zoomed in view of degradation area. Scale bar represents 10μm. B) HMLE-TWIST1 cells expressing the indicated FAP shRNAs were seeded onto gelatin-coated coverslips for 48 hours, fixed, and stained for F-actin (magenta), cortactin (green), and nuclei (blue). Scale bar represents 10μm. C) Quantification of cortactin signal per invadopodia in HMLE-TWIST1 cells relative to the control shRNA. D) The percentage of cells containing >5 invadopodia was quantified. N = 300 cells per group. Error bars in C, D and E represent SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, ns: not significant ($p > 0.05$). Student's t-test. E) Quantification of cell area in SCC61 cells expressing different FAP shRNAs.


A
DAPI/F-actin
OG-Gelatin
Merge
shCtrl
MT1-MMP sh1
MT1-MMP sh3
B
Relative MT1MMP mRNA expression
1.5
1.0
0.5
0.0
***
***
shCtrl
MT1MMP sh1
MT1MMP sh3
C
Gelatin Degradation
1.5
1.0
0.5
0.0
***
***
shCtrl
MT1-MMP sh1
MT1-MMP sh3
D
Relative FAP mRNA expression
1.5
1.0
0.5
0.0
***
***
***
***
shCtrl
FAP sh3
FAP sh4
shCtrl
FAP sh3
FAP sh4
+ Empty vector
+ pHluorin MT1-MMP
E
+ pHluorin MT1-MMP
shCtrl
FAP sh3
FAP sh4
KDa
MT1-MMP
- 93
- 70
GAPDH
- 37
F
DAPI/F-actin
OG-Gelatin
Merge
+ pHluorin MT1-MMP
shCtrl
FAP sh3
FAP sh4
G
Gelatin Degradation
2.0
1.5
1.0
0.5
0.0
ns
shCtrl
FAP sh3
FAP sh4
+ pHluorin MT1-MMP

Supplemental Figure 5. MT1-MMP overexpression rescues the invadopodia-mediated matrix degradation defects caused by FAP knockdown, Related to Figure 7. A) SCC61 cells expressing MT1-MMP shRNAs were seeded onto Oregon green gelatin for 16 hours and then fixed and stained for F-actin (red) and nuclei (blue). B) qPCR analysis of MT1-MMP mRNA level in SCC61 cells expressing MT1-MMP shRNAs and normalized to the control shRNA. Error bars represent SD. C) Quantification of gelatin degradation from (A) normalized to the control shRNA. Error bars represent SEM. N = 150 cells per group. ***$p < 0.001$. Student's t-test. D) qPCR analysis of FAP mRNA level in SCC61 cells expressing FAP shRNAs alongside either empty vector or MT1-MMP pHluorin. Error bars represent SD. E) Protein lysates from SCC61 cells expressing MT1-MMP pHluorin and FAP shRNAs were probed for MT1-MMP. GAPDH was used as loading control. F) SCC61 cells expressing MT1-MMP pHluorin and FAP shRNAs were seeded onto Oregon green gelatin for 16 hours in presence of GM6001 (10μM), GM6001 was then removed, and cells were incubated in culturing media for 1 hour followed by fixation and staining for F-actin (red) and nuclei (blue). G) Quantification of gelatin degradation from (F) and normalized to control shRNA. N=150 cells per group. Error bars represent SEM. **$p < 0.01$, ***$p < 0.001$, ns: not significant ($p > 0.05$). Student's t-test. Scale bar represents 10μm.


A
F-actin
Tks5
Cortactin
Merge
shCtrl
MT1-MMP sh1
MT1-MMP sh3
F
F-actin
FAP
Merge
B
Invadopodia number / Cell
ns
C
Fluorescence Intensity F-Actin
D
Fluorescence Intensity Tks5
E
Fluorescence Intensity Cortactin
G
Fluorescence intensity FAP
shCtrl
#1
#3
shMT1-MMP
H
F-actin
pHluorin MT1-MMP
Tks5
Merge
FAP sh3
FAP sh4
I
F-actin
pHluorin MT1-MMP
Cortactin
Merge
J
Fluorescence Intensity Tks5
+ Empty vector
+ pHluorin MT1-MMP
K
Fluorescence Intensity Cortactin

Supplemental Figure 6. MT1-MMP overexpression rescues the invadopodia structural defects caused by FAP knockdown, Related to Figure 7. A) SCC61 cells expressing MT1-MMP shRNAs were seeded onto gelatin-coated coverslips for 48 hours and labelled for F-actin (red), Tks5 (green), cortactin (blue). Quantification of Tks5 (D) and cortactin (E) signal intensity per invadopodia normalized to the control shRNA. Quantification of invadopodia precursor number per cell (B), and F-actin signal intensity per invadopodia normalized to the control shRNA (C) in SCC61 cells expressing MT1-MMP shRNAs. F) SCC61 cells expressing MT1-MMP shRNAs were seeded onto gelatin-coated coverslips for 48hr and labelled for F-actin (magenta) and FAP (green). G) Quantification of FAP fluorescence intensity at invadopodia relative to the control shRNA. MT1-MMP-pHluorin (green) was overexpressed in SCC61 cells expressing FAP shRNAs and seeded onto gelatin-coated coverslips for 48 hours and labelled for TKS5 (blue) (H) and cortactin (blue) (I), and F-actin (red). Quantification of Tks5 (J) and cortactin (K) at invadopodia in pHluorin MT1-MMP expressing cells relative to corresponding SCC61 FAP knockdown cells overexpressing empty vector shows rescue in Tks5 and cortactin recruitment at invadopodia in pHluorin MT1-MMP expressing cells. Error bars in all the graphs represent SEM. ***p<.001, **p<.01, *p<.05, n.s.: not significant (p>.05). N= >20 cells per group. Student's t-test. Scale bar represents 10μm.

Supplementary Table 1, Primers used in this study, Related to STAR Methods and the Key Resources Table.

Name	Sequence
hHPRT_qPCR_fwd	ACGTCTTGCTCGAGATGTGA
hHPRT_qPCR_rev	TCCCCTGTTGACTGGTCATT
hFAP_qPCR_fwd	AATGAGAGCACTCACACTGAAG
hFAP_qPCR_rev	CCGATCAGGTGATAAGCCGTAAT
hDPP4_qPCR_fwd	TCCTGATGGGCAGTTTATTCTCT
hDPP4_qPCR_rev	CATGTGACCCACTGTGTGTTG
hMMP14_qPCR_fwd	GAAGCCTGGCTACAGCAATATG
hMMP14_qPCR_rev	TGCAAGCCGTAAAACTTCTGC
FAP sh3-resistant WT FAP_fwd	GTGCATTGTCTTAAGACCAAGTAGAGTTCATAACTCT
FAP sh3-resistant WT FAP_rev	AGAGTTATGAACTCTACTTGGTCTTAAGACAATGCAC
FAP sh5-resistant WT FAP_fwd	GGATGATAATCTTGAACATTACAAGAATTCAACTGTG
FAP sh5-resistant WT FAP_rev	CACAGTTGAATTCTTGTAATGTTCAAGATTATCATCC
FAP S624A_fwd	CATATGGGGCTGGGCCTATGGAGGATAC
FAP S624A_rev	GTATCCTCCATAGGCCCAGCCCCATATG
FAP A657S_fwd	CCAGCTGGGAATATTACTCGTCTGTCTACACAGAG
FAP A657S_rev	CTCTGTGTAGACAGACGAGTAATATTCCCAGCTGG