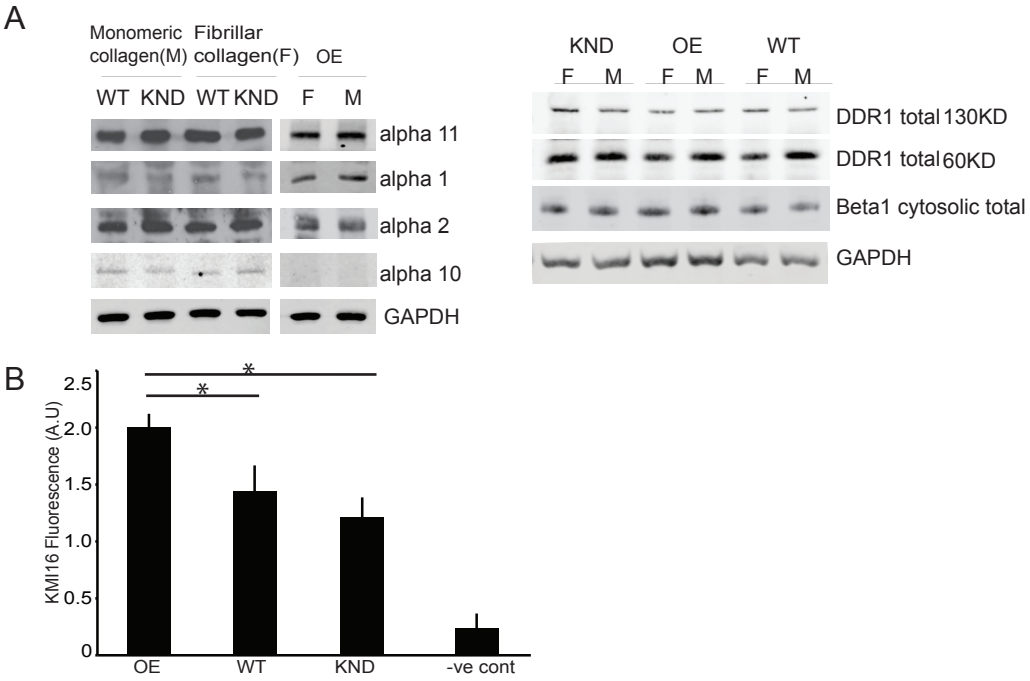
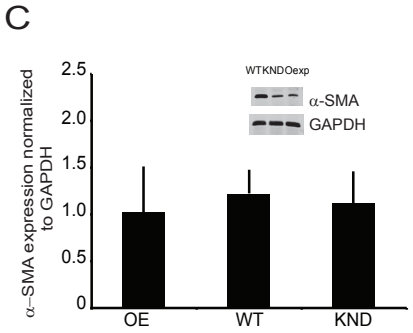
Supplemental Materials

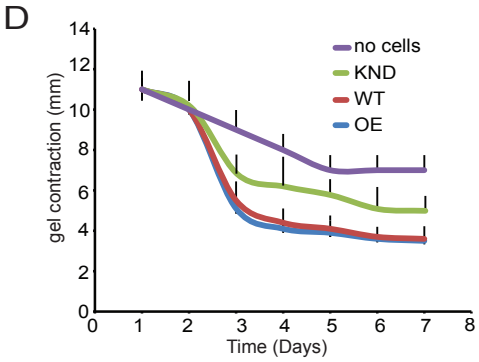
Molecular Biology of the Cell

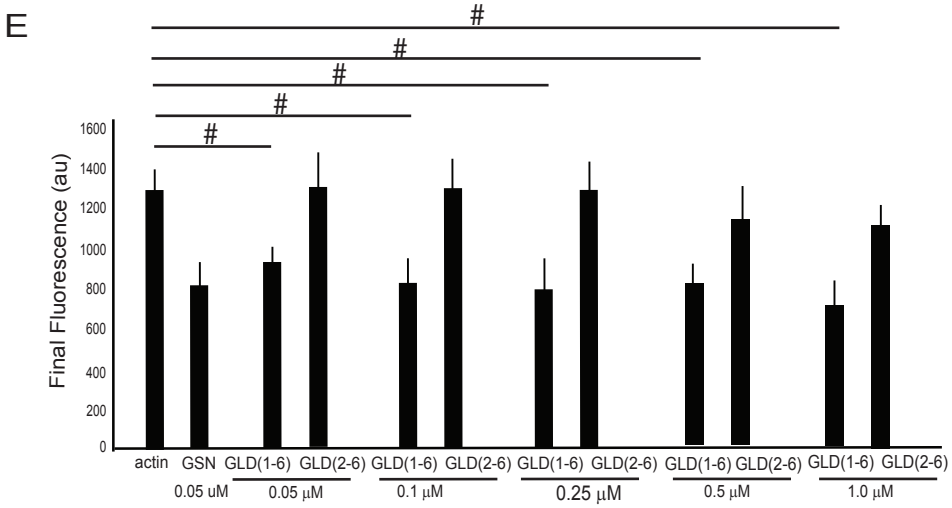
Arora et al.

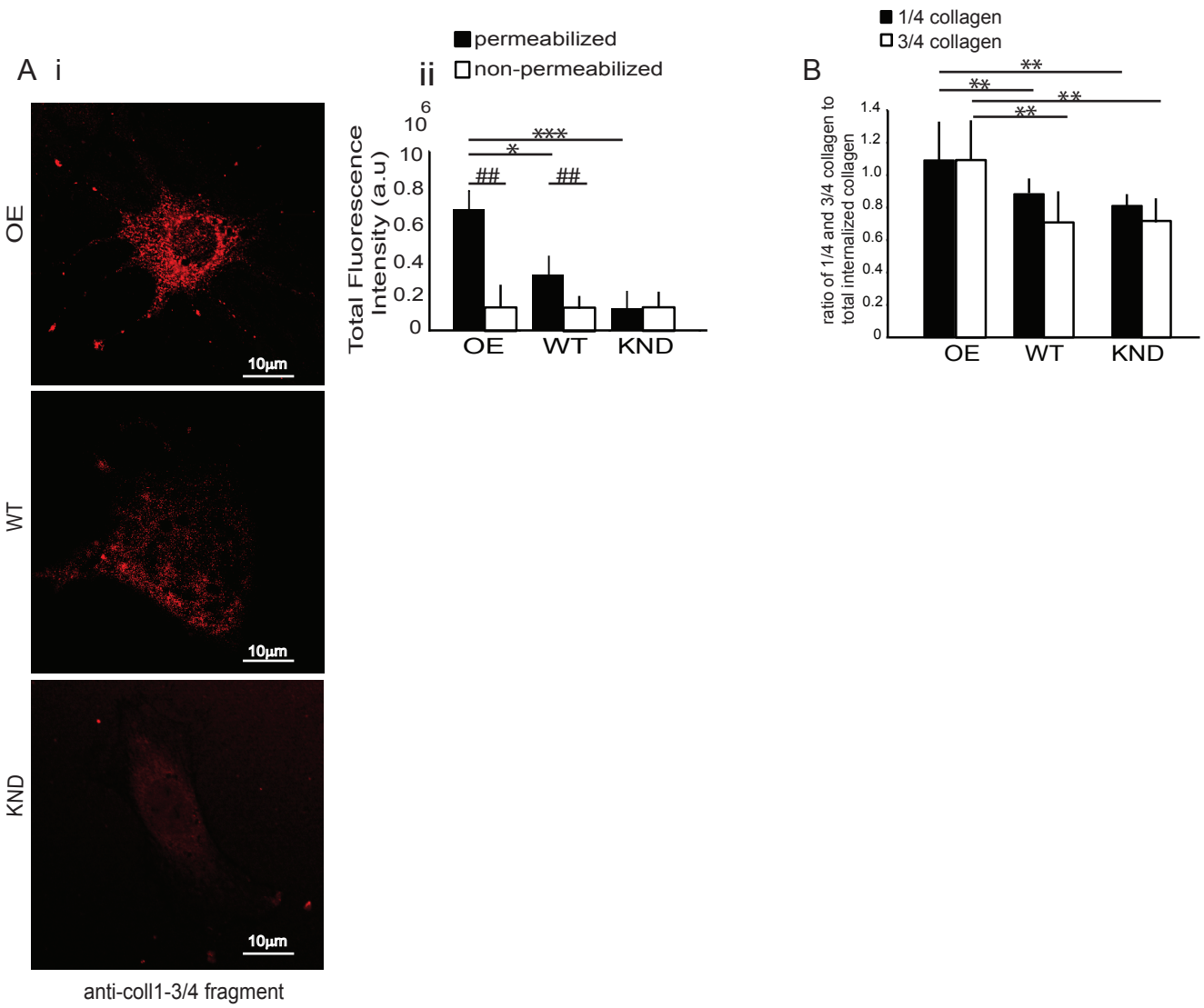

A
Monomeric collagen(M)
Fibrillar collagen(F)
OE
WT KND WT KND F M
alpha 11
alpha 1
alpha 2
alpha 10
GAPDH
KND OE WT
F M F M F M
DDR1 total 130KD
DDR1 total 60KD
Beta1 cytosolic total
GAPDH

B
KMI16 Fluorescence (A.U)
2.5
2.0
1.5
1.0
0.5
0
*
*
OE
WT
KND
-ve cont


C
α-SMA expression normalized to GAPDH
2.5
2.0
1.5
1.0
0.5
0
WTKNDOexp
α-SMA
GAPDH
OE
WT
KND


D
gel contraction (mm)
14
12
10
8
6
4
2
0
no cells
KND
WT
OE
0 1 2 3 4 5 6 7 8
Time (Days)


E
Final Fluorescence (au)
1600
1400
1200
1000
800
600
400
200
0
#
#
#
#
#
actin
GSN
GLD(1-6)
GLD(2-6)
GLD(1-6)
GLD(2-6)
GLD(1-6)
GLD(2-6)
GLD(1-6)
GLD(2-6)
GLD(1-6)
GLD(2-6)
0.05 uM
0.05 μM
0.1 μM
0.25 μM
0.5 μM
1.0 μM


A i
OE
WT
KND
10μm
10μm
10μm
anti-coll1-3/4 fragment
ii
permeabilized
non-permeabilized
Total Fluorescence Intensity (a.u)
6
10
0.8
0.6
0.4
0.2
0
***
*
##
##
OE
WT
KND
B
1/4 collagen
3/4 collagen
ratio of 1/4 and 3/4 collagen to total internalized collagen
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
**
**
**
**
OE
WT
KND

S**upplementary Fig. 1 A** Expression levels of α1, 2, 10, 11 integrins, β1 integrin (total cytosolic), and DDR1 in FliI OE, WT and KND cells plated on fibrillar or monomeric collagen for 30 mins. **B-**Non-permeabilized cells stained with KMI16 antibody and analyzed by flow cytometry show cell surface expression levels of β1 integrin in different cell types. Histogram showing data reported as mean ± SD, n=3; analysis by ANOVA. $^{*}p<0.05$ comparison of WT and FliI KND cells with FliI OE cells. **C-** α-smooth muscle actin expression levels in FliI OE, WT and KND cells. **D-** Measurement of collagen gel contraction in different cell types shows reduced contraction in FliI KND cells compared with WT or FLiI OE cells. **E-** End-point polymerization (measured after overnight incubation) due to capping of pyrene-labeled actin with 0.05 μM gelsolin or with various concentrations of FliI GLD 1-6 or FliI GLD 2-6 (0.05 μM, 0.1 μM, 0.25 μM, 0.5 μM, 1.0 μM). Data in histogram are mean ± SD, n=3; analyzed by ANOVA; $^{\#}p<0.05$ comparison between purified GLD 2-6 to GLD 1-6 at different concentrations.

S**upplementary Fig.2 A i,ii** Fixed and permeabilized cells showing fluorescence of ¾ collagen fragment immunostained cells. Note increased fluorescence in FliI OE cells compared with WT or FliI KND cells. ii- Data presented in histogram are mean ± SD, n=3; analyzed with ANOVA; 25 cells from each group.$^{*}p<0.05$ comparison between WT and FliI OE cells; *** $p<0.001$ comparison between FliI KND and FliI OE cells. ii- Quantification of ¾ collagen immunostaining in non-permeabilized cells show minimal fluorescence in all cell types but significantly different from permeabilized OE and WT cells compared to respective non-permeabilized cells, $^{\#\#}$ $p<0.01$. Data are reported as mean ± SD, n=3. Analysis by ANOVA. **B**. Cells plated for four hours were trypsinized and lysates incubated with high capacity streptavidin resin. Washed and boiled samples run on SDS PAGE and blots probed for ¾ and ¼ collagen fragment. Histogram shows enhanced intracellular degraded collagen for ¼ and ¾ fragments Data are reported as mean ± SD, n=3 analyzed by ANOVA. $^{**}p<0.01$ for ¼ fragment; ** $p<0.01$ for ¾ fragment comparison of WT and FliI KND cells to FliI OE cells.