Supplemental Materials

Molecular Biology of the Cell

Gu et al.

Supplemental Figure 1. ECM softness prevents AJs formation, upregulates MMP secretion, promotes MMP activity and induces ILP formation. (A) Primary human fibroblasts (1 x 10^4 cells per well) were seeded onto various stiffness gels in 12-well plates and cultured for 16 hrs. Cell surface cadherin-11 were live stained by monoclonal anti-cadherin-11 (3H10) antibody for 1hr at room temperature followed by fixation and secondary antibody staining. Plasma membranes were then permeablized and intracellular F-actins were stained by Phalloidin. Cell nuclei were stained by DRAQ5. Arrows point to cadherin-11 adherens junctions. Scale bar is 100 μm. **(B)** Primary human fibroblasts (2 x 10^4 cells per well) were seeded onto various stiffness gels in 12-well plates and cultured for 4 hrs. Cell culture medium from each condition was collected and subject to the RayBio® Human MMP Array to determine the total secreted MMP protein quantity in each media. **(C)** Primary human fibroblasts were seeded onto fibronectin-coated various stiffness gels and cultured for 4 hrs. MMP-14 and cortactin were stained by primary antibodies and followed by fluorescent secondary antibodies. F-actin was stained by phalloidin-Alexa 647. All 2D images were projected from relevant confocal 3D stacks by maximum projection method. Scale bar is 100 μm. Scale bar in zoom panels is 10 μm. Arrows point to ILPs.

Supplemental Figure 2. Time frame of ILP formation on 0.2 kPa stiffness gels. (A) Primary human fibroblasts were seeded onto 0.2 kPa stiffness gels and cultured for the time as indicated. MMP-14, and cortactin were stained by primary antibodies and followed by fluorescent secondary antibodies. F-actin was stained by phalloidin-Alexa 647. Scale bar is 100 μm. **(B)** Three independent experiments (n = 3, ±SD) as in (A) were performed and percentage of cells forming ILPs at various time points on 0.2 kPa

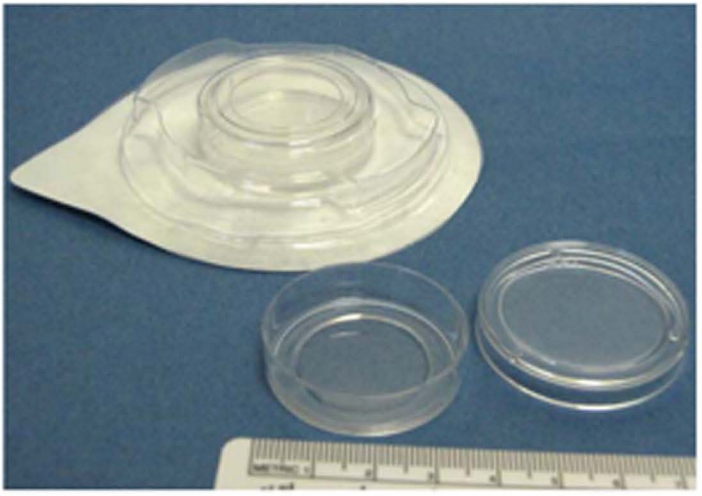
stiffness gels was quantified. In each experiment, 200 cells in total were counted. **(C)** Primary human fibroblasts were seeded onto various stiffness gels and cultured for 4 hrs with or without SFK inhibitor Dasatinib. Arrowheads in top panels point to cells unable to form ILPs upon SFK inhibition on 0.2 kPa stiffness gels. Arrows in bottom panels point to cells unable to form stress fibers upon SFK inhibition on 6.4 kPa stiffness gels. Scale bar is 100 μm.

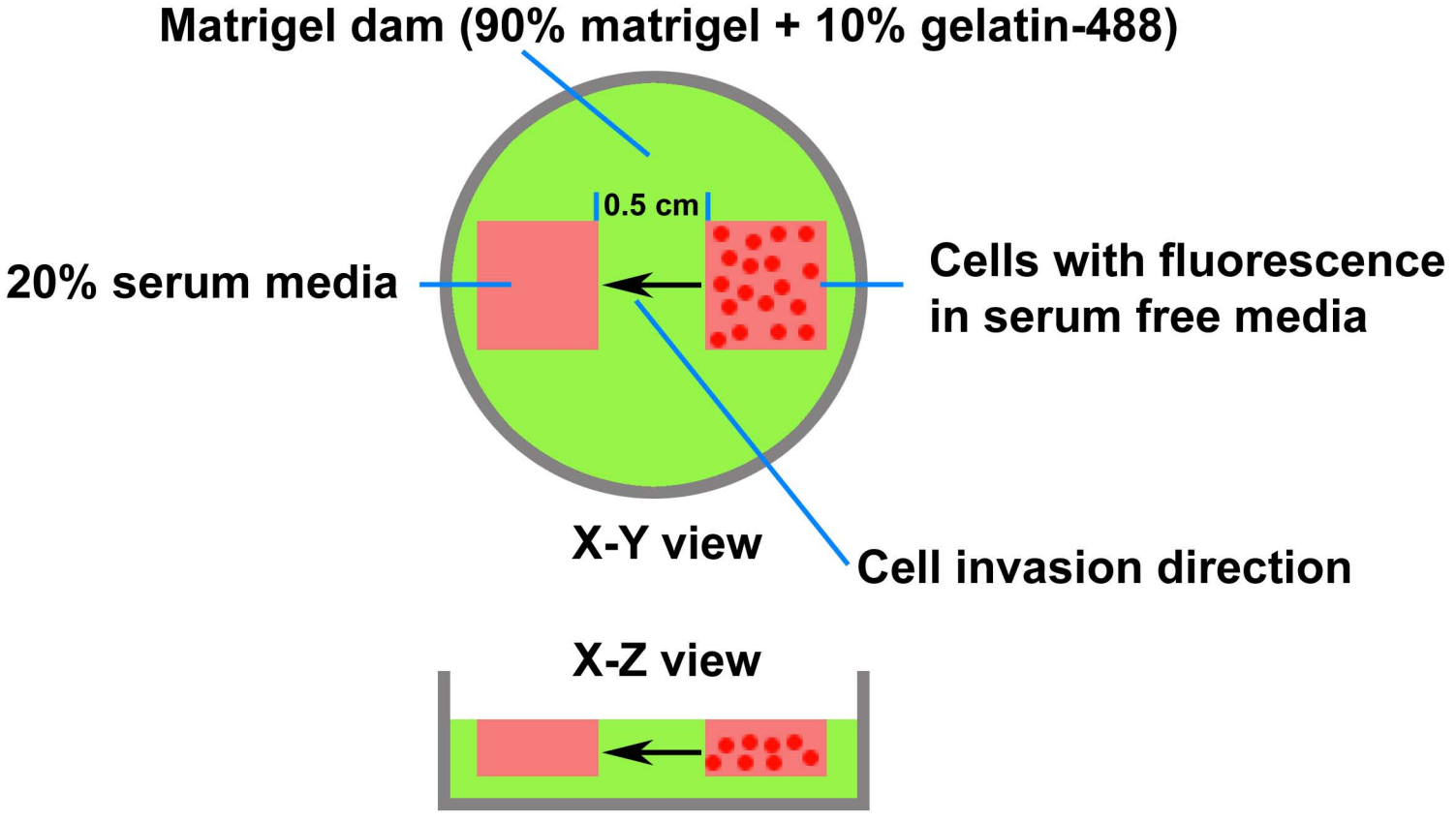
Supplemental Figure 3. Phospho-kinase antibody array. (A-E) Primary human fibroblasts were seeded onto various stiffness gels and cultured for the time as indicated. Whole cell lysates were subject to the phospho-kinase antibody array by following the manufacturer's protocols.

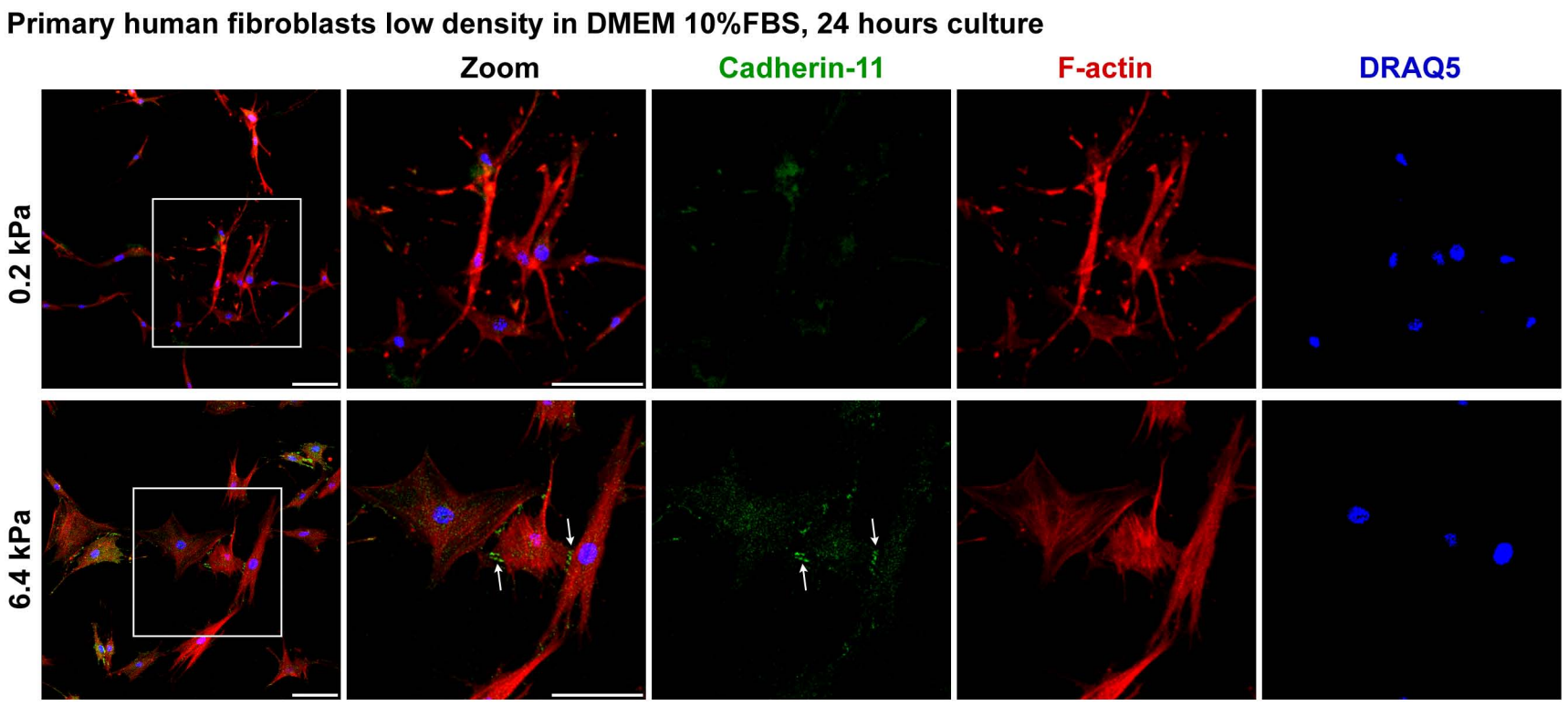
Supplemental Figure 4. ECM softness differentially induces spontaneous ILP formation in primary cells, cancer cells and fibrosarcoma cells. BT549 human breast cancer cells **(A)** and HS 913T human fibrosarcoma cells **(B)** were seeded onto various stiffness gels and cultured for 4 hrs. Invadosome marker proteins MMP-14 and cortactin were stained by primary antibodies and followed by fluorescent secondary antibodies. Arrowheads point to cells forming ILPs. Scale bar is 50 μm (A); 100 μm (B).

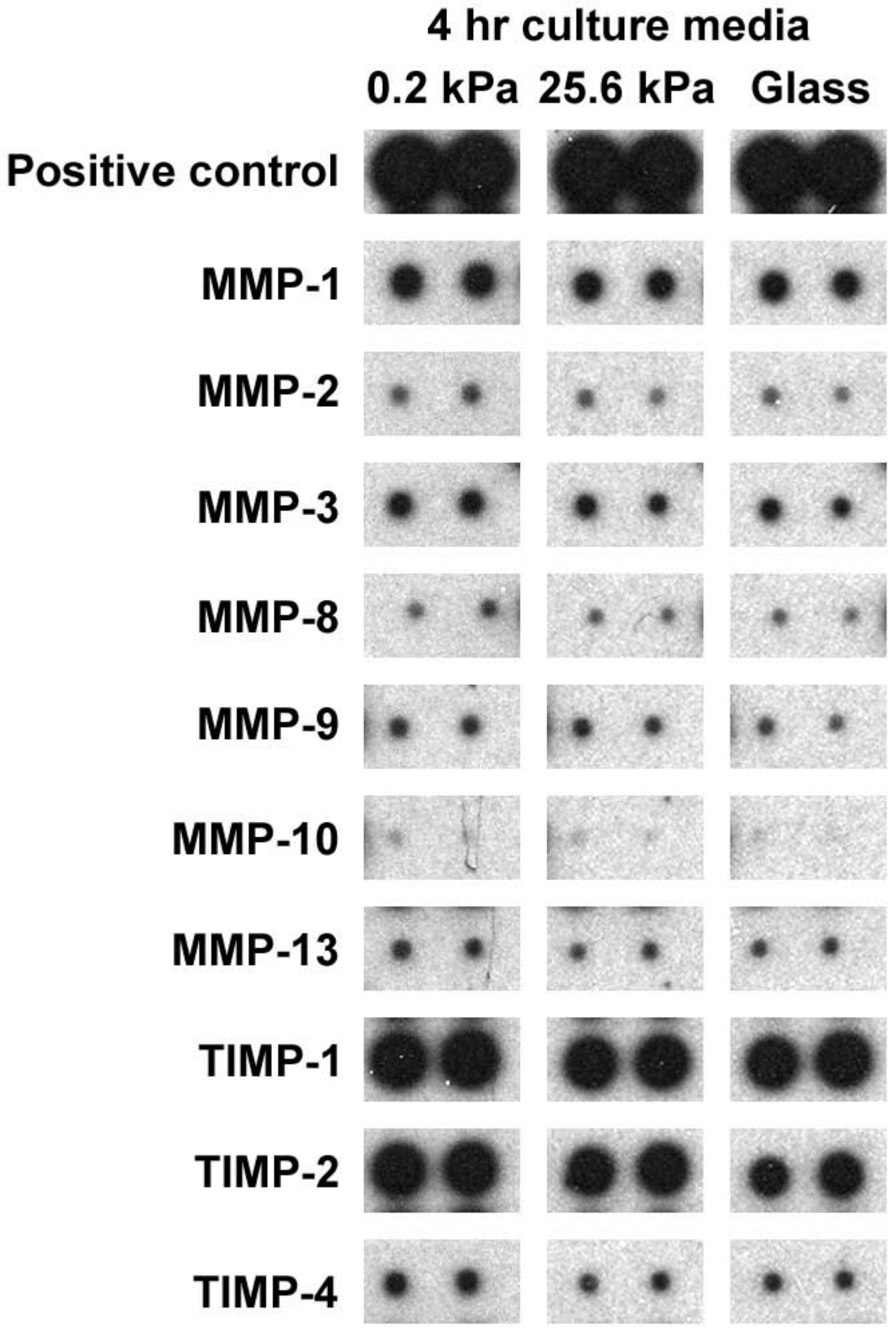
Supplemental Figure 5. Schematics of the 3D directional invasion assay.

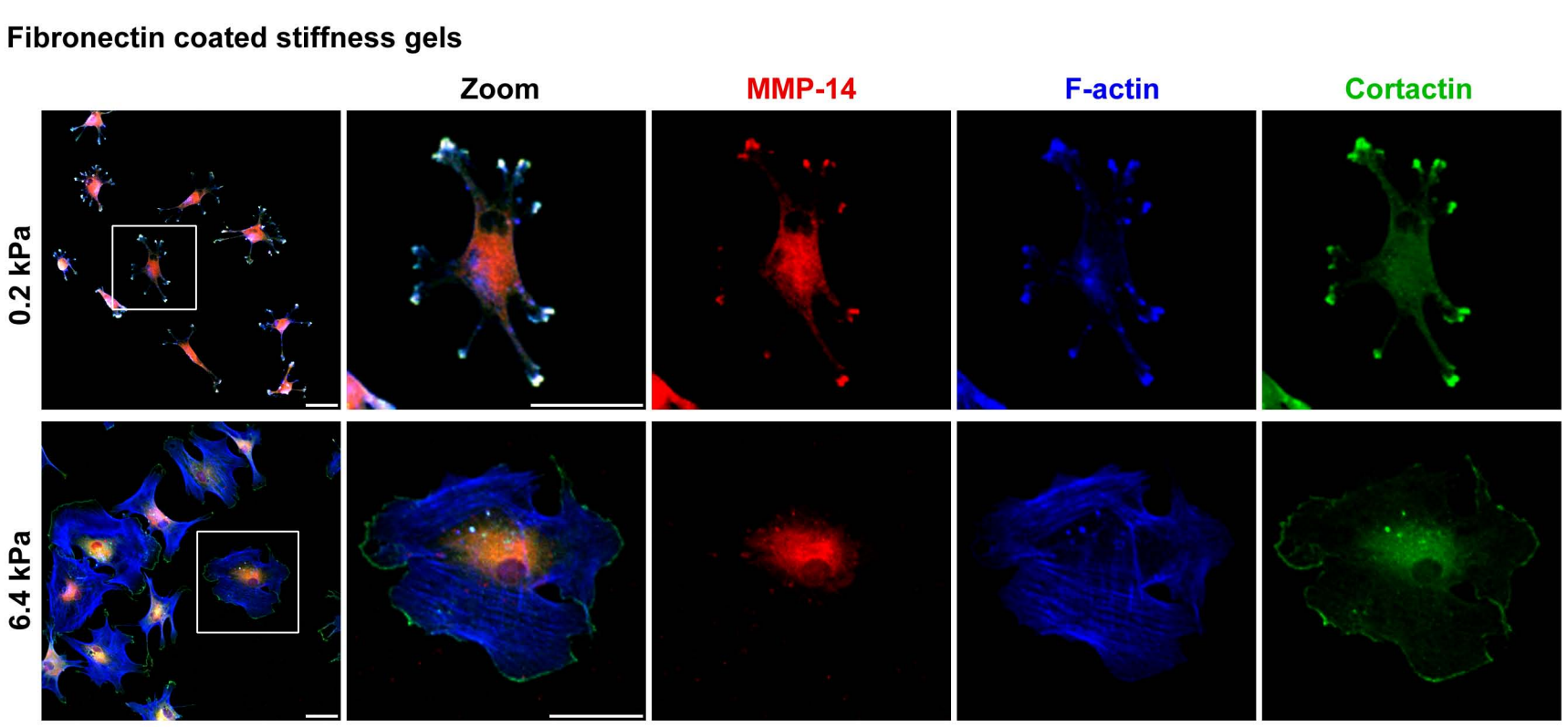
Video - ILP formation is detected in cells invading through soft 3D matrices Primary human fibroblasts were live stained by DiI and then seeded into the 3D cell invasion assay as in Supplemental Figure 5.

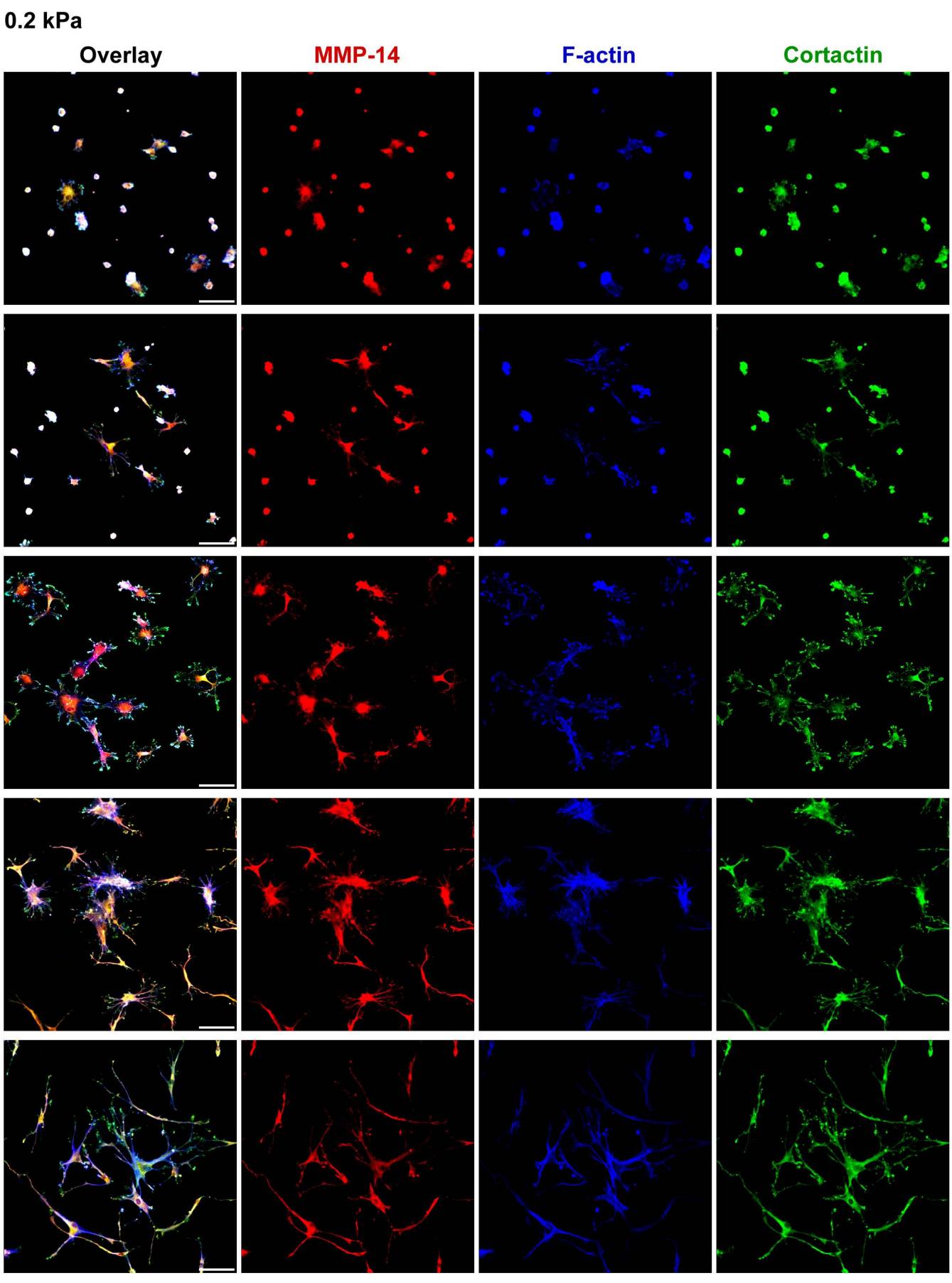


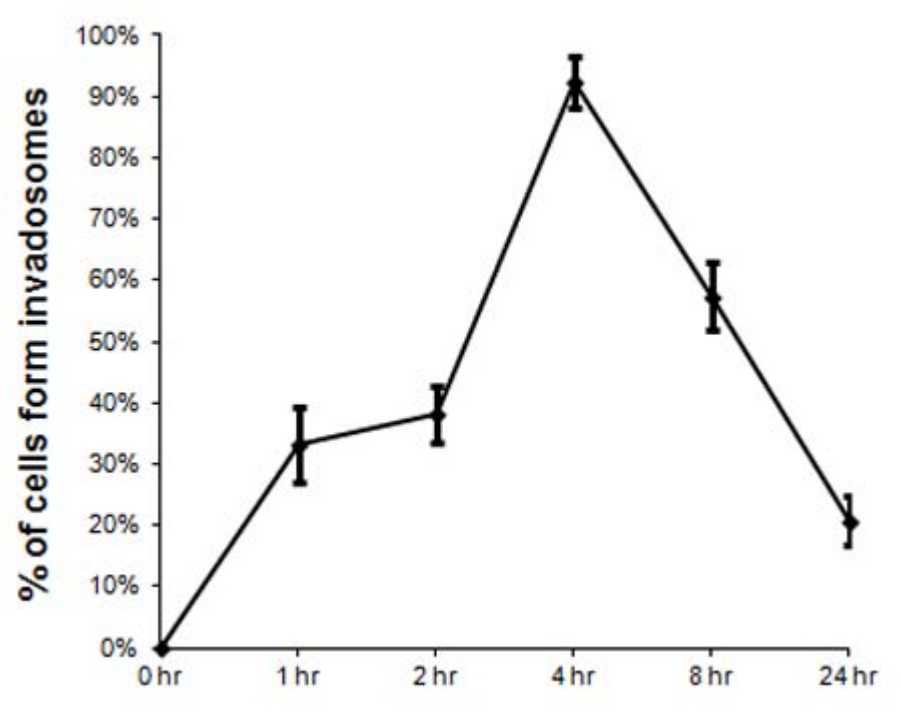

Matrigel dam (90% matrigel + 10% gelatin-488)
0.5 cm
20% serum media
Cells with fluorescence in serum free media
X-Y view
Cell invasion direction
X-Z view

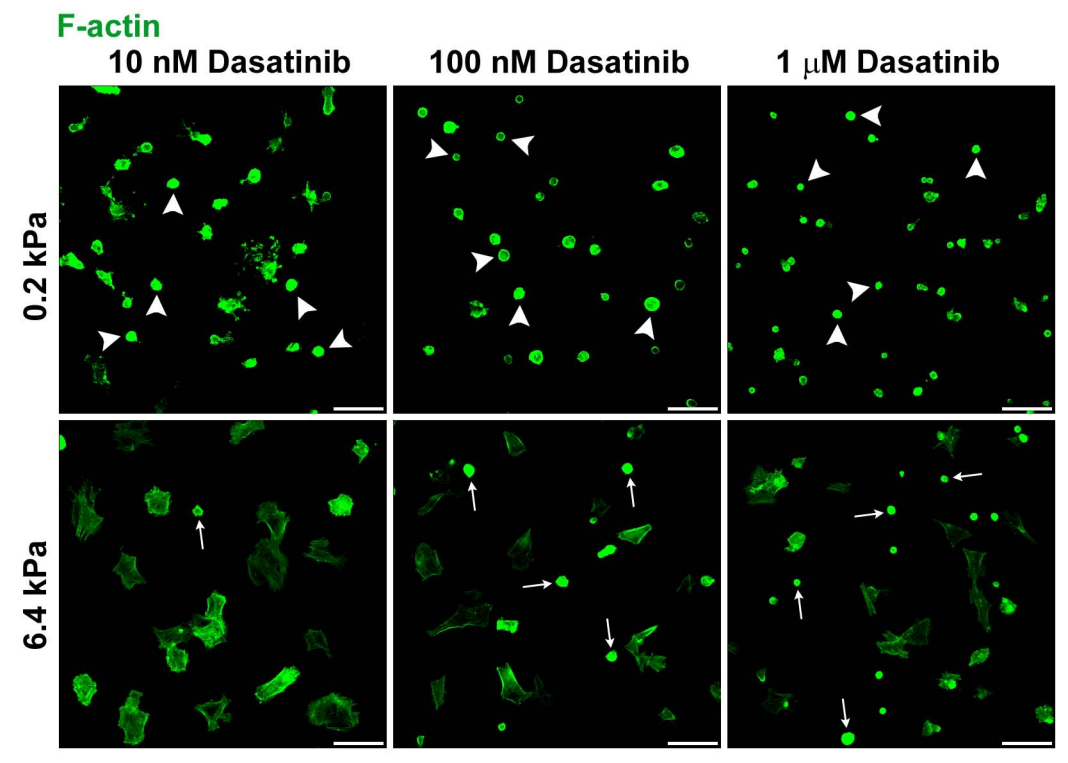

Primary human fibroblasts low density in DMEM 10%FBS, 24 hours culture
Zoom
Cadherin-11
F-actin
DRAQ5
0.2 kPa
6.4 kPa

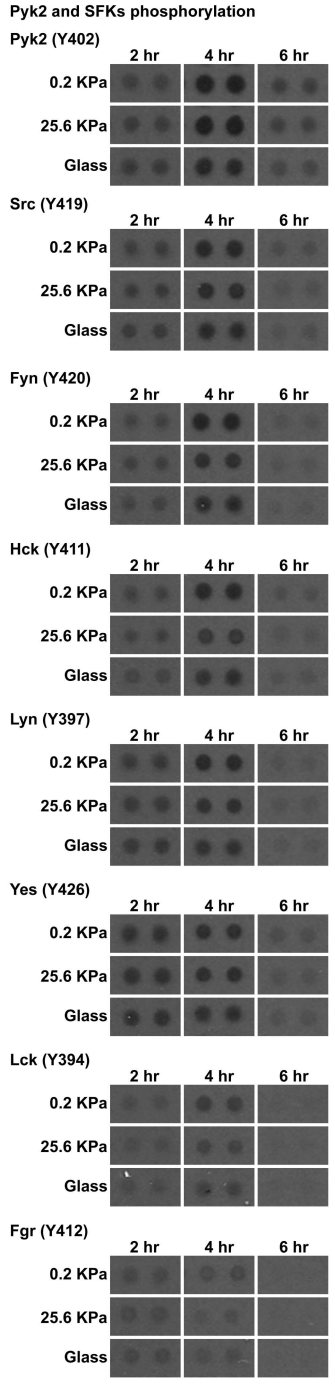

4 hr culture media
0.2 kPa
25.6 kPa
Glass
Positive control
MMP-1
MMP-2
MMP-3
MMP-8
MMP-9
MMP-10
MMP-13
TIMP-1
TIMP-2
TIMP-4


Fibronectin coated stiffness gels
Zoom
MMP-14
F-actin
Cortactin
0.2 kPa
6.4 kPa


0.2 kPa
Overlay
MMP-14
F-actin
Cortactin
1 hr
2 hrs
4 hrs
8 hrs
24 hrs

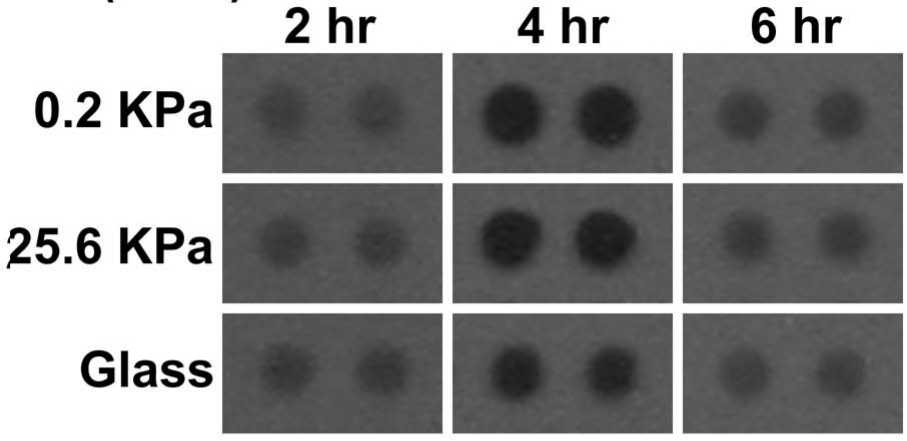

% of cells form invadosomes
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
0 hr
1 hr
2 hr
4 hr
8 hr
24 hr


F-actin
10 nM Dasatinib
100 nM Dasatinib
1 μM Dasatinib
0.2 kPa
6.4 kPa

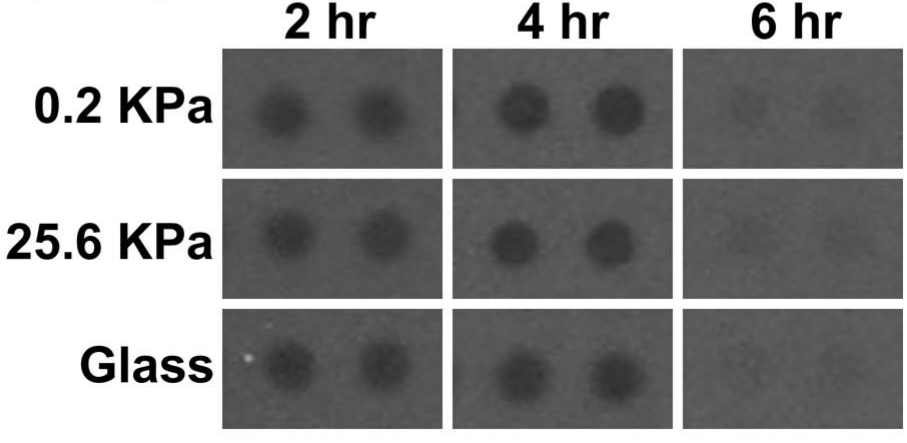

Pyk2 and SFKs phosphorylation
Pyk2 (Y402)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass
Src (Y419)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass
Fyn (Y420)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass
Hck (Y411)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass
Lyn (Y397)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass
Yes (Y426)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass
Lck (Y394)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass
Fgr (Y412)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass

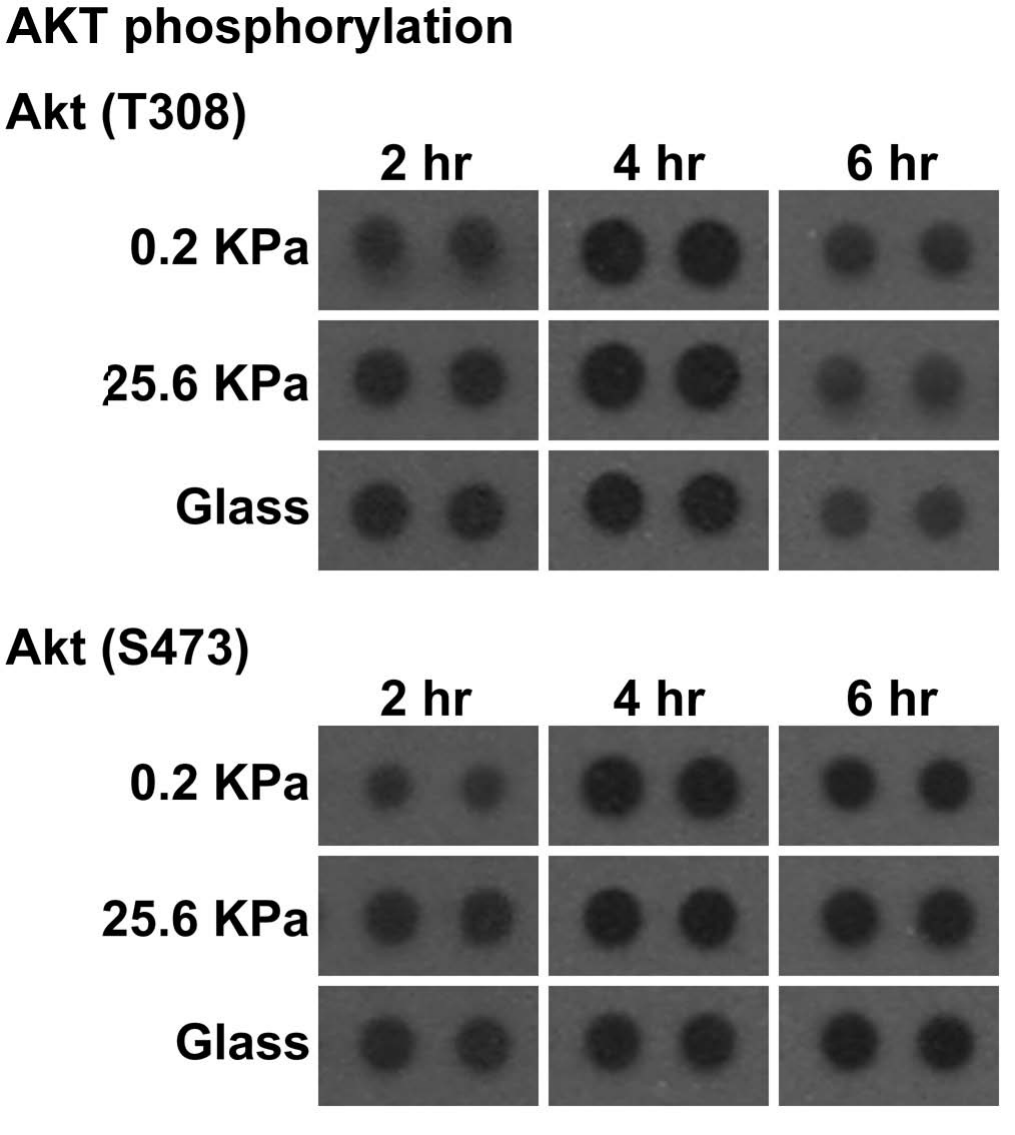
Paxillin and FAK phosphorylation

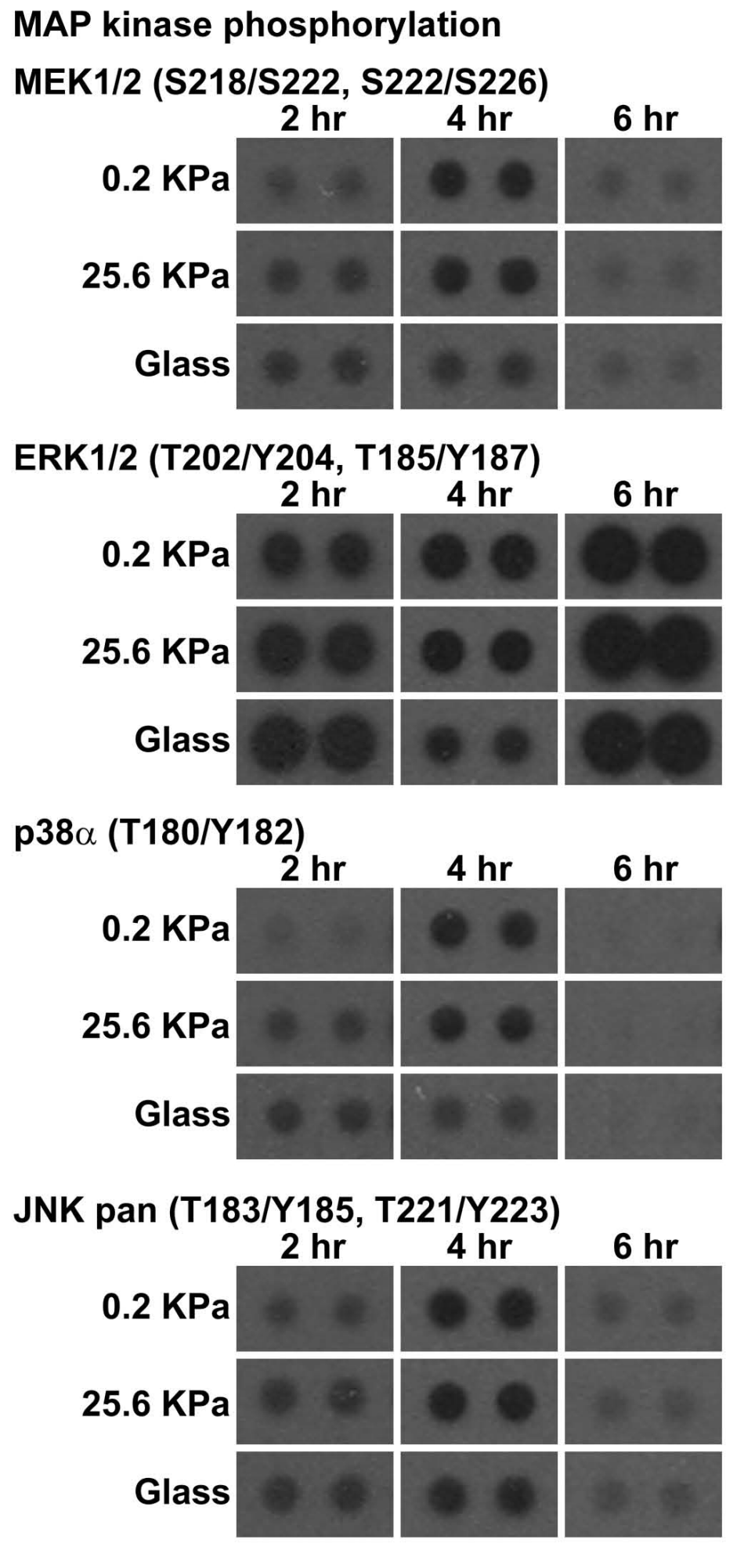
Paxillin (Y118)

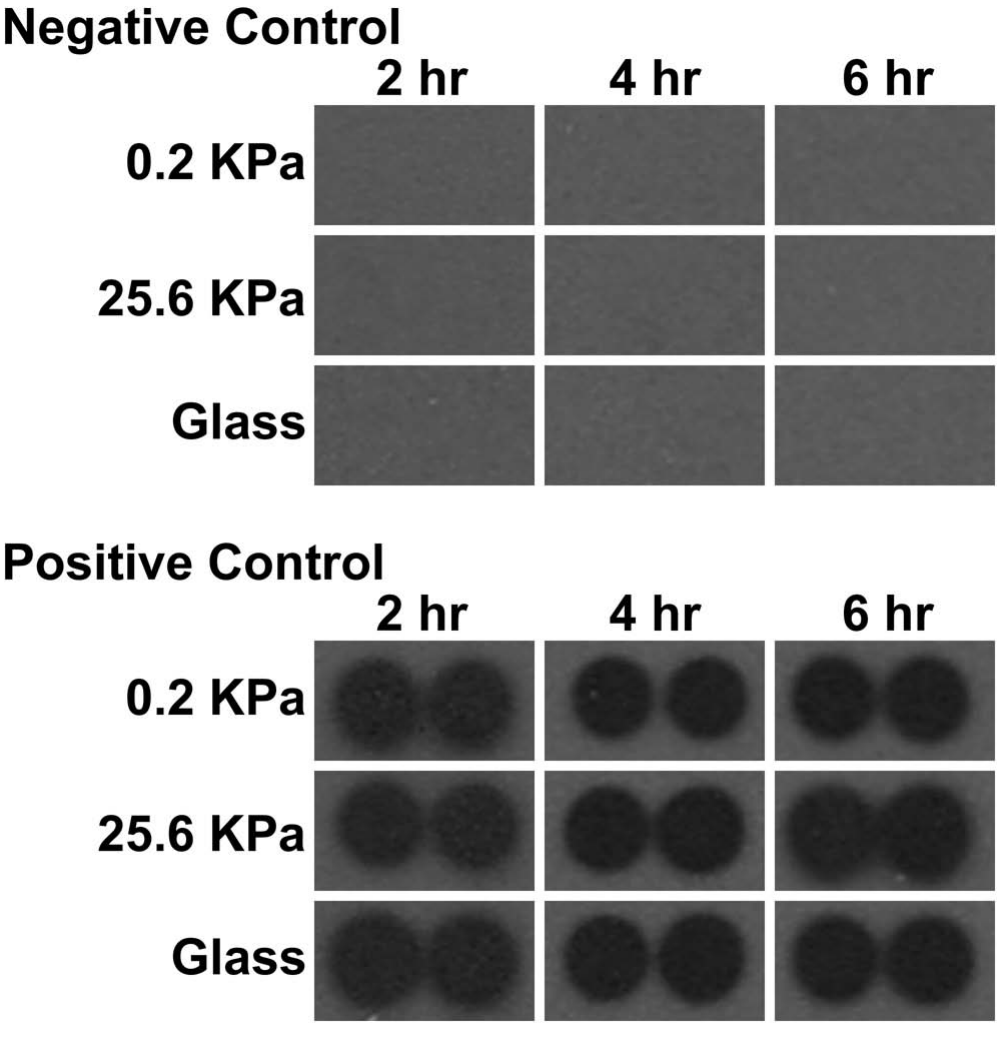


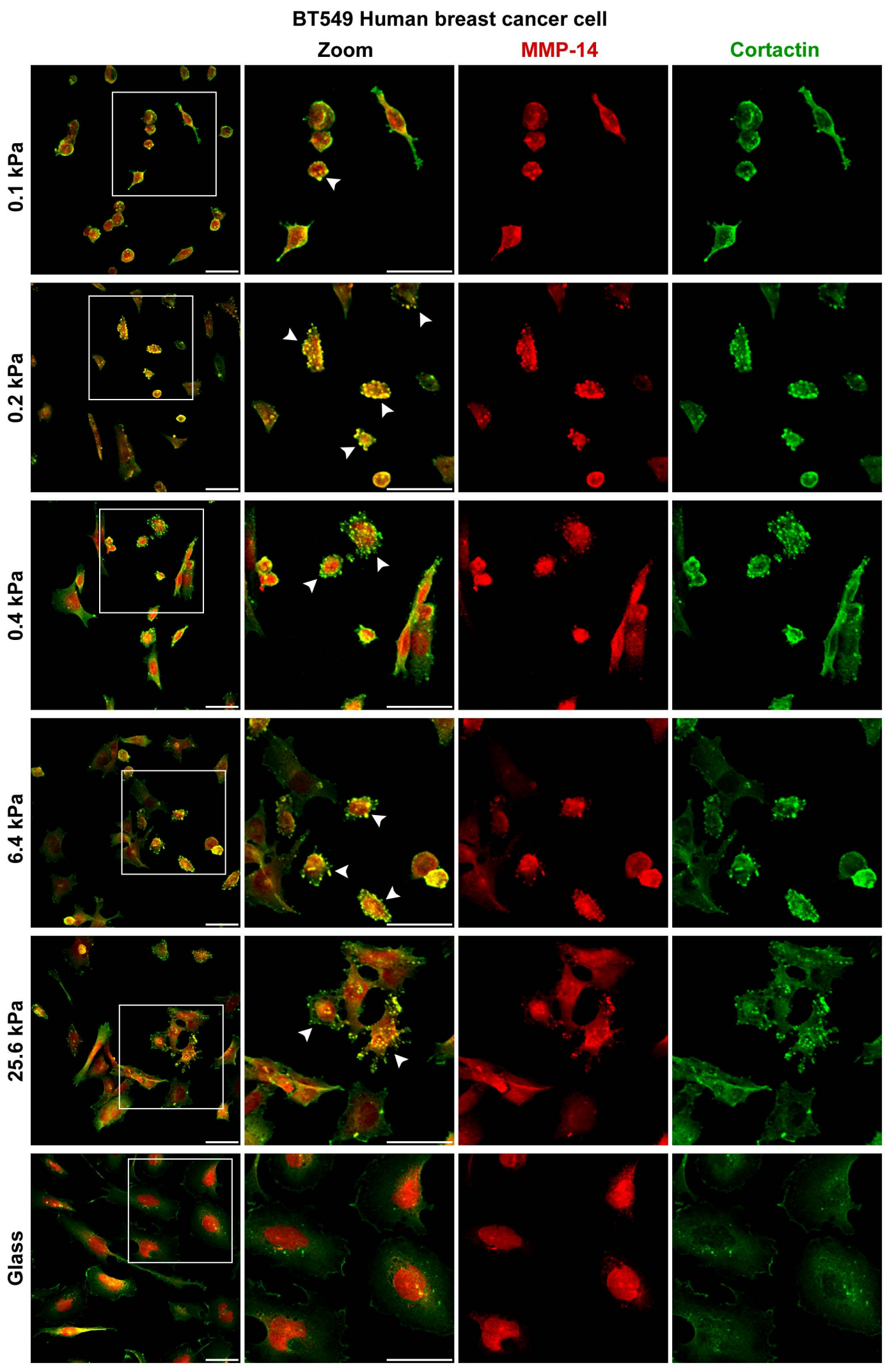
FAK (Y397)

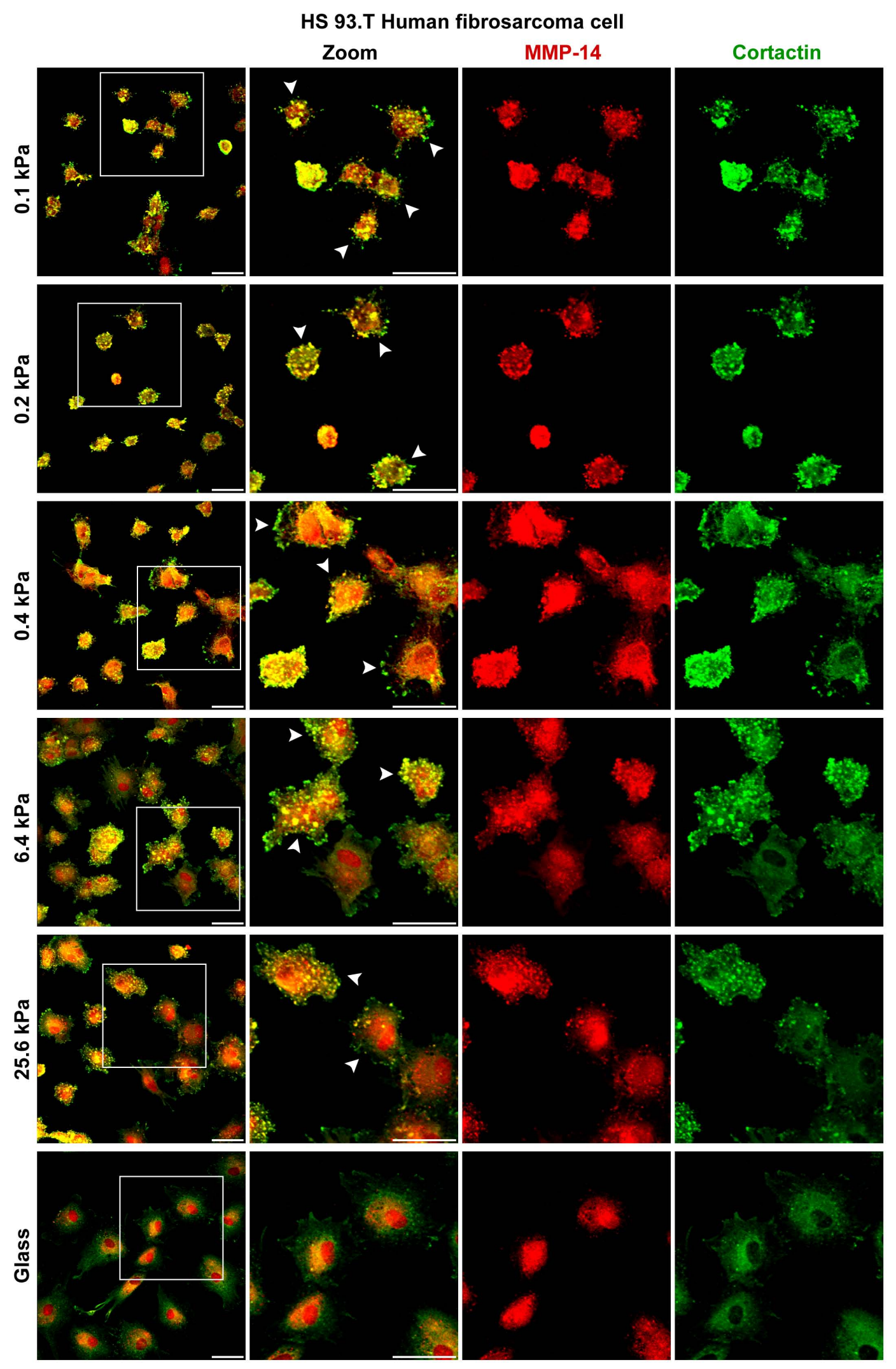



AKT phosphorylation
Akt (T308)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass
Akt (S473)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass


MAP kinase phosphorylation
MEK1/2 (S218/S222, S222/S226)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass
ERK1/2 (T202/Y204, T185/Y187)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass
p38α (T180/Y182)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass
JNK pan (T183/Y185, T221/Y223)
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass


Negative Control
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass
Positive Control
2 hr
4 hr
6 hr
0.2 KPa
25.6 KPa
Glass


BT549 Human breast cancer cell
Zoom
MMP-14
Cortactin
0.1 kPa
0.2 kPa
0.4 kPa
6.4 kPa
25.6 kPa
Glass


HS 93.T Human fibrosarcoma cell
Zoom
MMP-14
Cortactin
0.1 kPa
0.2 kPa
0.4 kPa
6.4 kPa
25.6 kPa
Glass