Supplementary Material: Senescent stroma induces nuclear deformations in cancer cells via the inhibition of RhoA/ROCK/myosin II-based cytoskeletal tension

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Figure S1

(a-b) Fluorescent confocal micrographs of round (a) and elongated (b) MCF7 cells stained for F-actin (green), Lamin A (red) and nuclear DNA (blue) after exposure to Sen CM for 48h. Left and right panels display xy and yz equatorial cross-sections respectively. Whole cell images are displayed as insets per image. Scale bar, 10 μm. **(c)** Averaged nuclear size for cells exposed to Sen CM after 48h. **(d)** Nuclear height of cells having round and elongated cell morphology using consequential z-slices; n=8 and n=10 respectively. **(e)** Binned correlation between cellular area and nuclear area. Blue triangle indicates an increase in cell area from left to right (elongated to round).

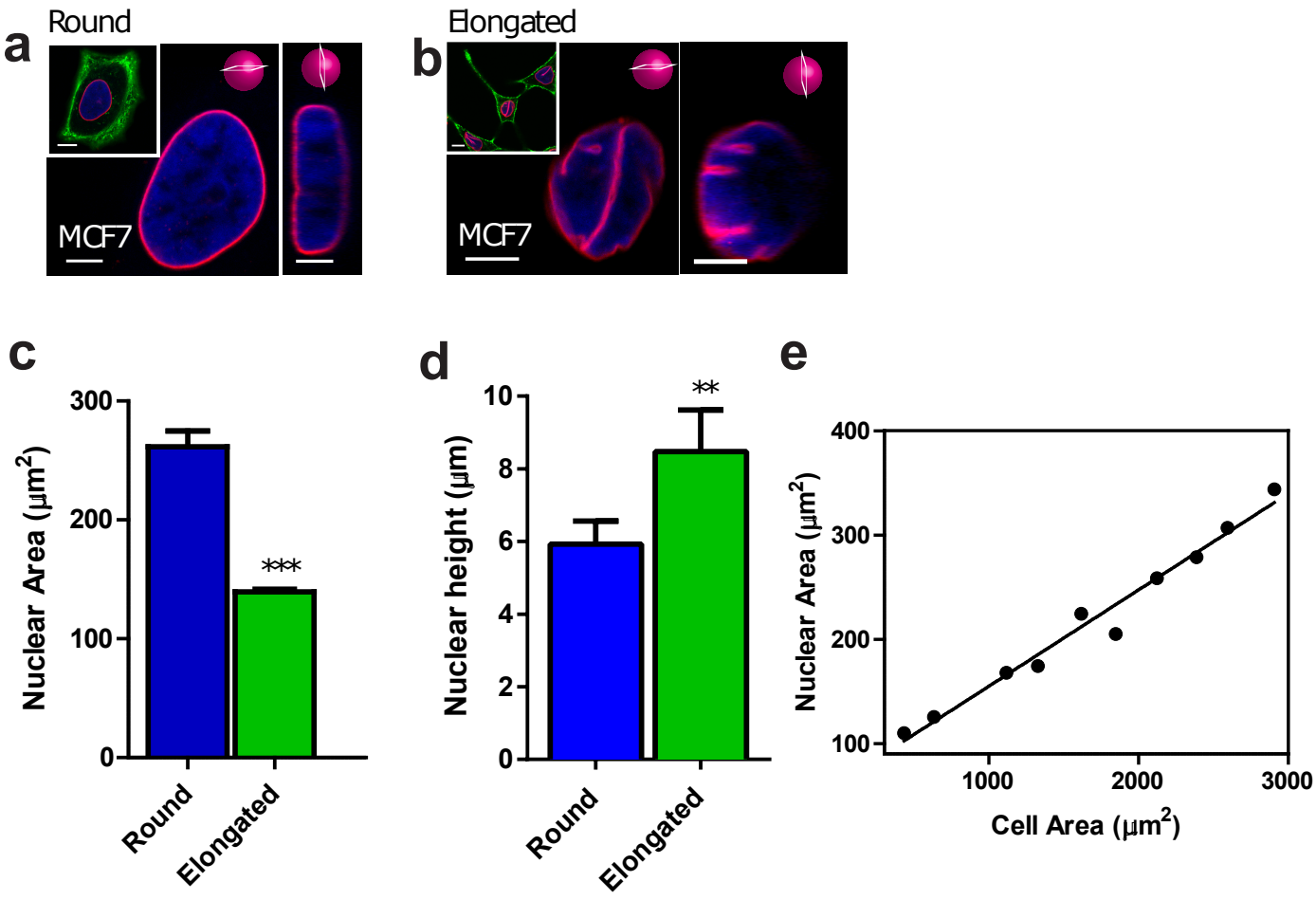

a
Round
MCF7
b
Elongated
MCF7
c
Nuclear Area (µm²)
300
200
100
0
***
Round
Elongated
d
Nuclear height (µm)
10
8
6
4
2
0
**
Round
Elongated
e
Nuclear Area (µm²)
400
300
200
100
1000
2000
3000
Cell Area (µm²)

Figure S1

Figure S2

Localization of nuclear lamina protein lamin A, nuclear envelope protein emerin, and LINC complex components Sun2, Nesprin2-giant, and Nesprin 3 in round and elongated T47D cells. Scale bar, 10 μm **(b)** Western blot of T47D cells exposed to FM and Sen CM. The bands correspond to lamin A, Lamin C, Nesprin 2, and β-actin from top to bottom.

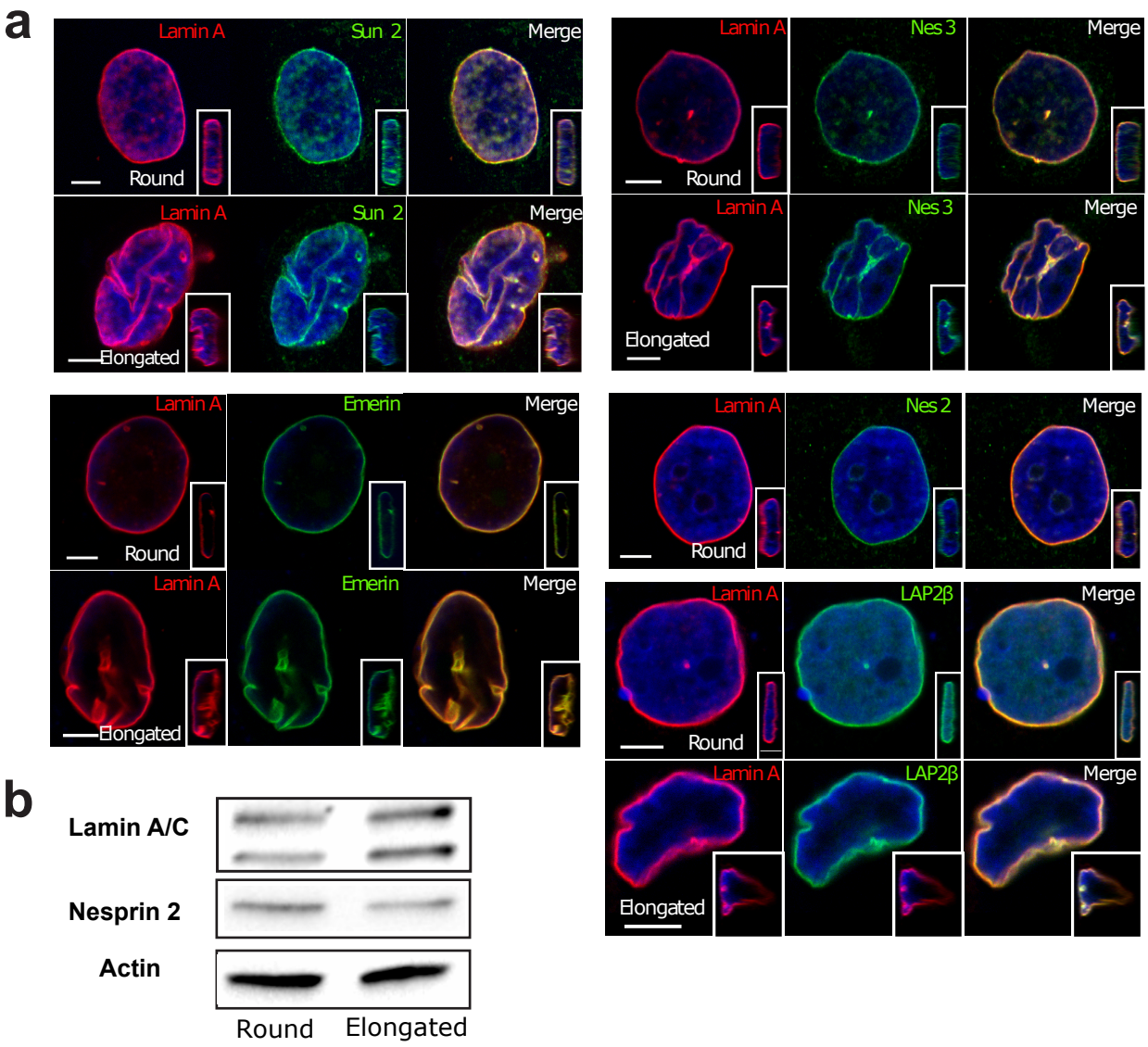

a
Lamin A
Sun 2
Merge
Round
Elongated
Nes 3
Emerin
Nes 2
LAP2β
b
Lamin A/C
Nesprin 2
Actin
Round
Elongated

Figure S2

Figure S3

(a-b) Fluorescence confocal micrographs of control and lamin A/C knockdown (262765) cells after stimulation with Sen CM (a) and FM (b) for 48h. Cells were stained with phalliodin, Lap2β, and Hoechst 33342 DNA stain. The following panels are zoomed in xy and yz cross-sections of the left panel. Scale bar 5 μm. These include the xy and yz equatorial cross-sections respectively. **(c-e)** Percentage of control and lamin A/C knockdown cells having lobulated nuclei 48h after exposure to Sen CM and FM.

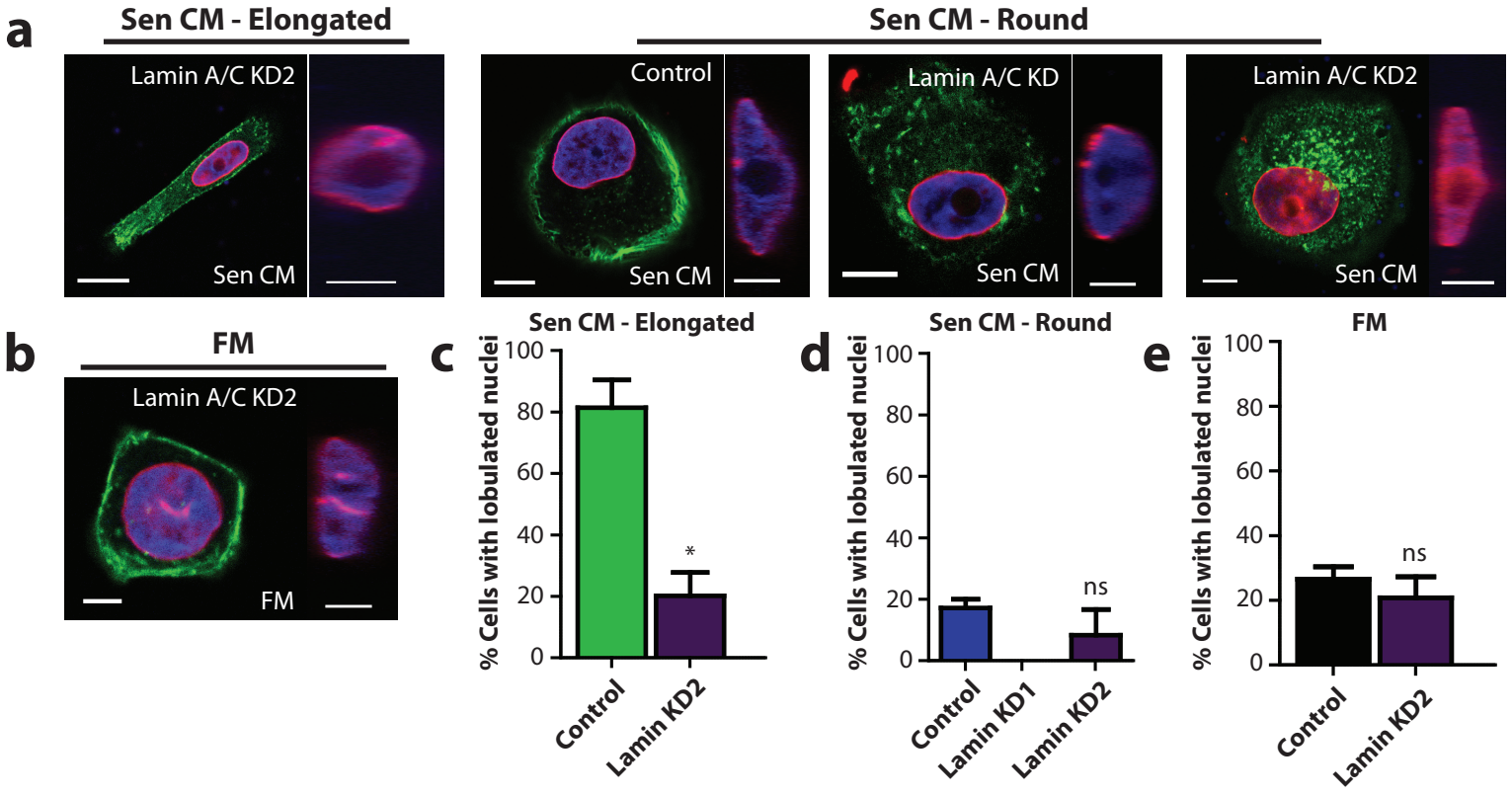
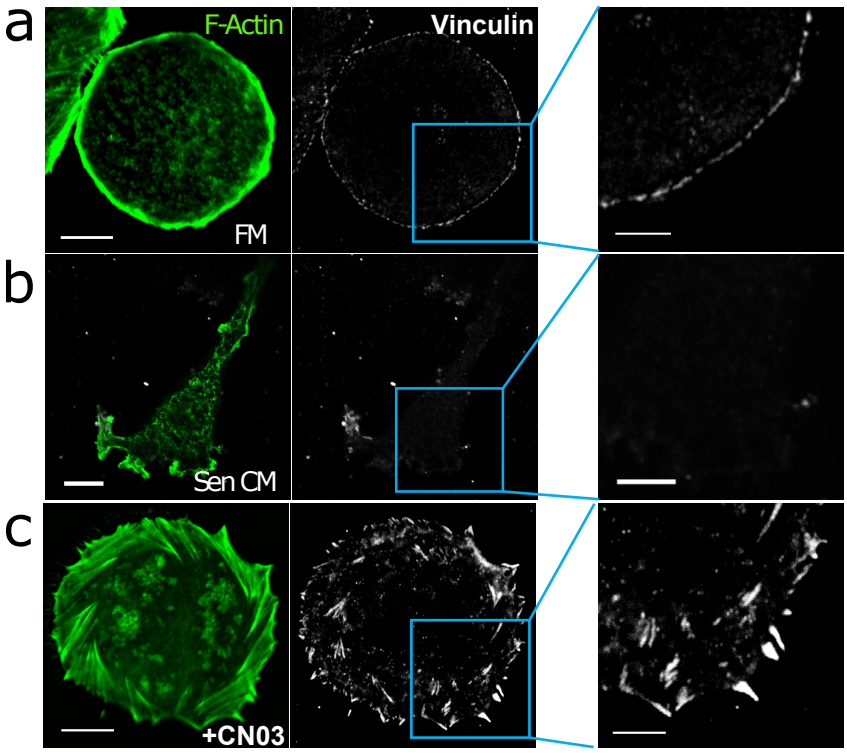

a
Sen CM - Elongated
Sen CM - Round
Lamin A/C KD2
Sen CM
Control
Sen CM
Lamin A/C KD
Sen CM
Lamin A/C KD2
Sen CM
b
FM
Lamin A/C KD2
FM
c
Sen CM - Elongated
% Cells with lobulated nuclei
100
80
60
40
20
0
*
Control
Lamin KD2
d
Sen CM - Round
% Cells with lobulated nuclei
100
80
60
40
20
0
ns
Control
Lamin KD1
Lamin KD2
e
FM
% Cells with lobulated nuclei
100
80
60
40
20
0
ns
Control
Lamin KD2

Figure S3

Figure S4

(a-c) Fluorescent confocal micrographs of T47D cells exposed to FM (a), Sen CM (b) and Sen CM +CN03 (c) then stained for vinculin, where blue boxes indicate zoomed region of T47D cells. Scale bar, 10 μm in original image and 5 μm in zoomed image. (**d**) distribution plot of cell circularity in each condition, 1 indicates a perfect circle on TGT surfaces.


a
F-Actin
Vinculin
FM
b
Sen CM
c
+CN03

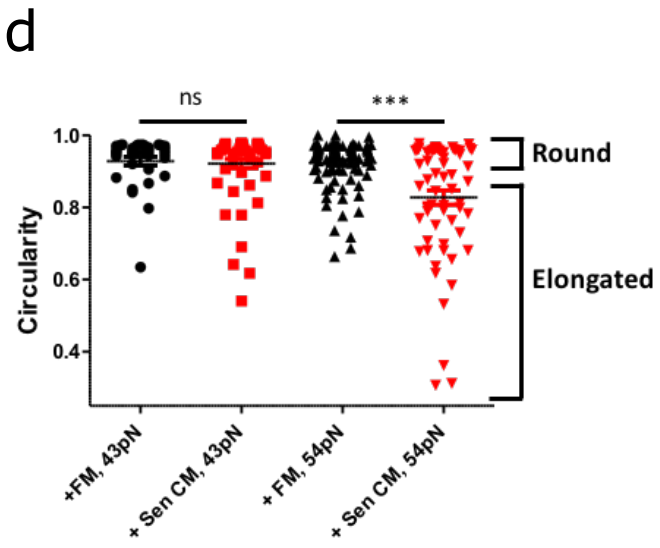
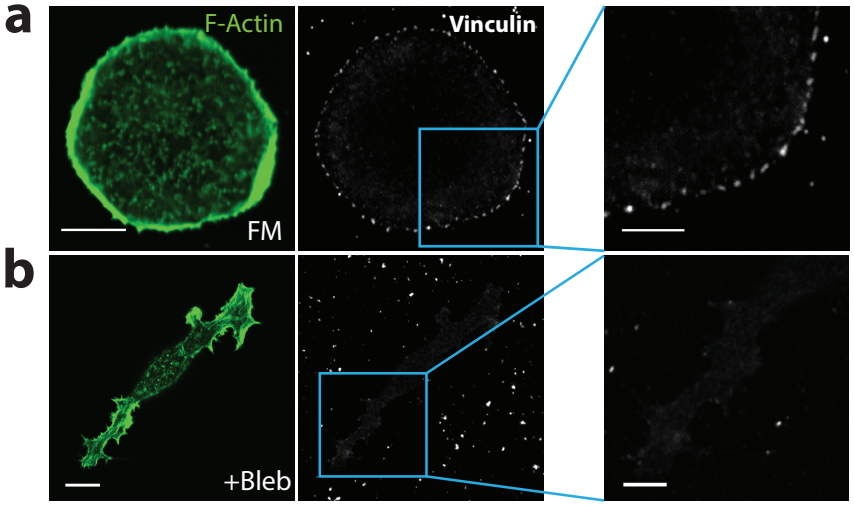
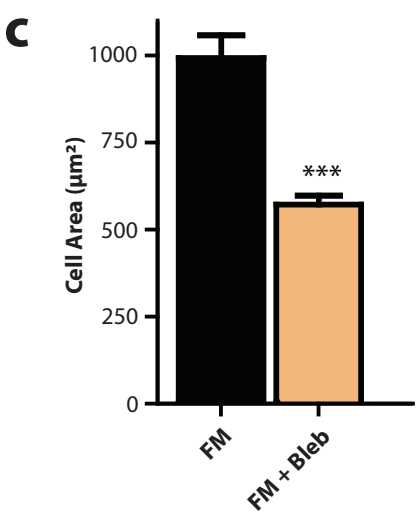

d
ns
***
1.0
0.8
0.6
0.4
Circularity
Round
Elongated
+FM, 43pN
+ Sen CM, 43pN
+ FM, 54pN
+ Sen CM, 54pN

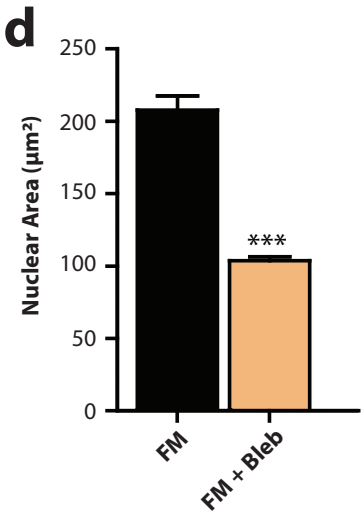
Figure S4

Figure S5

(a-b) Fluorescent confocal micrographs of T47D cells exposed to FM (**a**) and FM +Bleb (**b**) then stained for vinculin, where blue boxes indicate zoomed region of T47D cells. Scale bar, 10 μm in original image and 5 μm in zoomed image. **(c)** Average cell area, (**d**) average nuclear area of cells exposed to FM and FM+Bleb after 48h. **(e)** Nuclear height of cells having round and elongated cell morphology using consequential z-slices; n=13 and n=15 respectively.


a
F-Actin
Vinculin
FM
b
+Bleb


c
Cell Area (µm²)
1000
750
500
250
0
***
FM
FM + Bleb


d
Nuclear Area (µm²)
250
200
150
100
50
0
***
FM
FM + Bleb

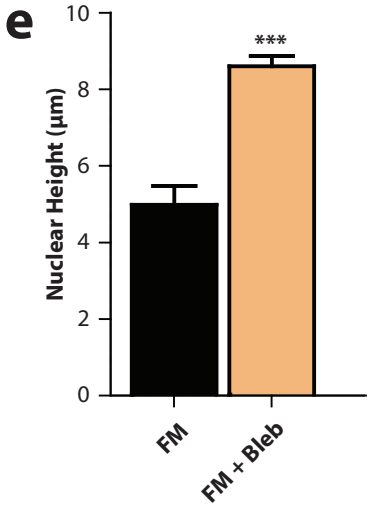

e
Nuclear Height (µm)
10
8
6
4
2
0
***
FM
FM + Bleb

Figure S5

Figure S6.

Fraction of cells with nuclear lobulation after exposure to Sen CM. (**a-c**) Bar plots showing the fraction of cells with lobulated nuclei for T47D cells treated with FM (n=109 cells), WI-38 CM (n=36 cells), and WI-38 veh (n=45 cells) (non-senescent WI-38 media) (a); T47D cells treated with GT125 FM=53 cells, GT125 CM=76 cells, WI-38 FM=181 cells, WI-38 CM=242 cells (b); T47D cells seeded on collagen-coated glass or collagen-coated 25kPa polyacrylamide hydrogel surfaces, Glass FM=109 cells, Glass CM=36 cells, 25 kPa FM=20 cells, and 25 kPa CM=21 cells (c). (**d**) Representative microscopy images of T47D cells treated with cytoskeletal modulators, FM, or non-senescent veh media, or seeded of glass or polyacrylamide surfaces. (**e**) Fraction of lobulated nuclei treated FM (60 cells), CM (181 cells), CM+JASP (85 cells), and CM+NOCO (91 cells). (**f**) Representative microscopy images of cancer cell lines (T47D, A375, and PANC-1) treated with non-senescent GT125 media (GT125 veh) or senescent GT125 CM. (**g-h**) Fraction of cells with lobulated nuclei treated with GT125 veh or GT125 CM, A375 (g), PANC-1 (h). A375 (GT125 FM=166 cells, GT125 CM=172 cells), and PANC-1 (GT125 FM=60 cells, GT125 CM=72 cells) (**i**) Bar plot showing the concentration of select interleukins in pg/ml. Secretions were collected from non-senescent GT125 and senescent GT125 fibroblasts.

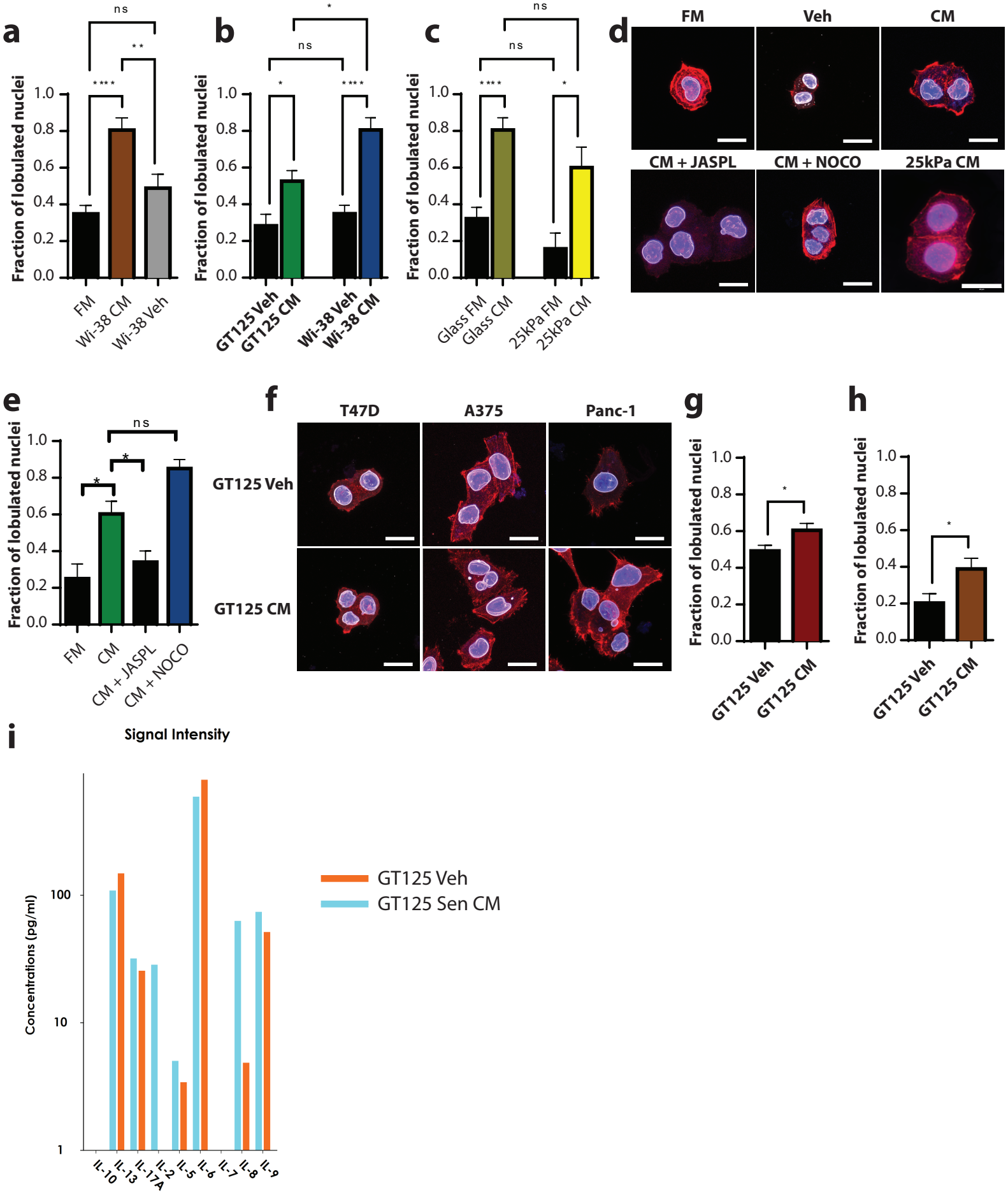

a
b
c
d
e
f
g
h
i
Fraction of lobulated nuclei
FM
Wi-38 CM
Wi-38 Veh
GT125 Veh
GT125 CM
Wi-38 Veh
Wi-38 CM
Glass FM
Glass CM
25kPa FM
25kPa CM
Veh
CM
CM + JASPL
CM + NOCO
25kPa CM
T47D
A375
Panc-1
GT125 Veh
GT125 CM
CM
CM + JASPL
CM + NOCO
Signal Intensity
Concentrations (pg/ml)
GT125 Veh
GT125 Sen CM
IL-10
IL-13
IL-17A
IL-2
IL-5
IL-6
IL-7
IL-8
IL-9

Figure S6