SUPPLEMENTAL FIGURE LEGENDS

Supplemental figure 1. **Knock down of syntaxin 6 in endothelial cells does not degrade α1β1, α2β1, αvβ3, αvβ5 and α4β1 integrins.** Uninfected (Control), si*STX6*- or si*STX16*-expressing HUVECs were cultured on fibronectin-coated surface in complete medium. After 48 h, a fraction of cells was taken out and cell lysates were prepared and western blotting was performed to assess extent of knockdown of syntaxin 6 and syntaxin 16 (**A**). Rest of the samples were harvested and cultured on collagen- (in **B**), laminin- (in **C**), vitronectin- (in **D**) and fibronectin-coated (in **E**) surfaces for additional 24 h. Proteins were subjected to SDS-PAGE and immunoblotting was performed with α1 (MAB1973, Millipore), α2 (AB1936, Millipore), αv (611012, BD Biosciences), β3 (4702, Cell Signaling), β5 (3629, Cell Signaling) and α4 (04-11311, Millipore), β1 and tubulin Abs. A representative blot is shown. (**F**) Integrin-band densities from "**B-E**" were quantified; values represent relative levels of α and β integrins after normalization to arbitrary value of 100 for uninfected (Control). (mean ± SD; n=3; $p \leq 0.003$).

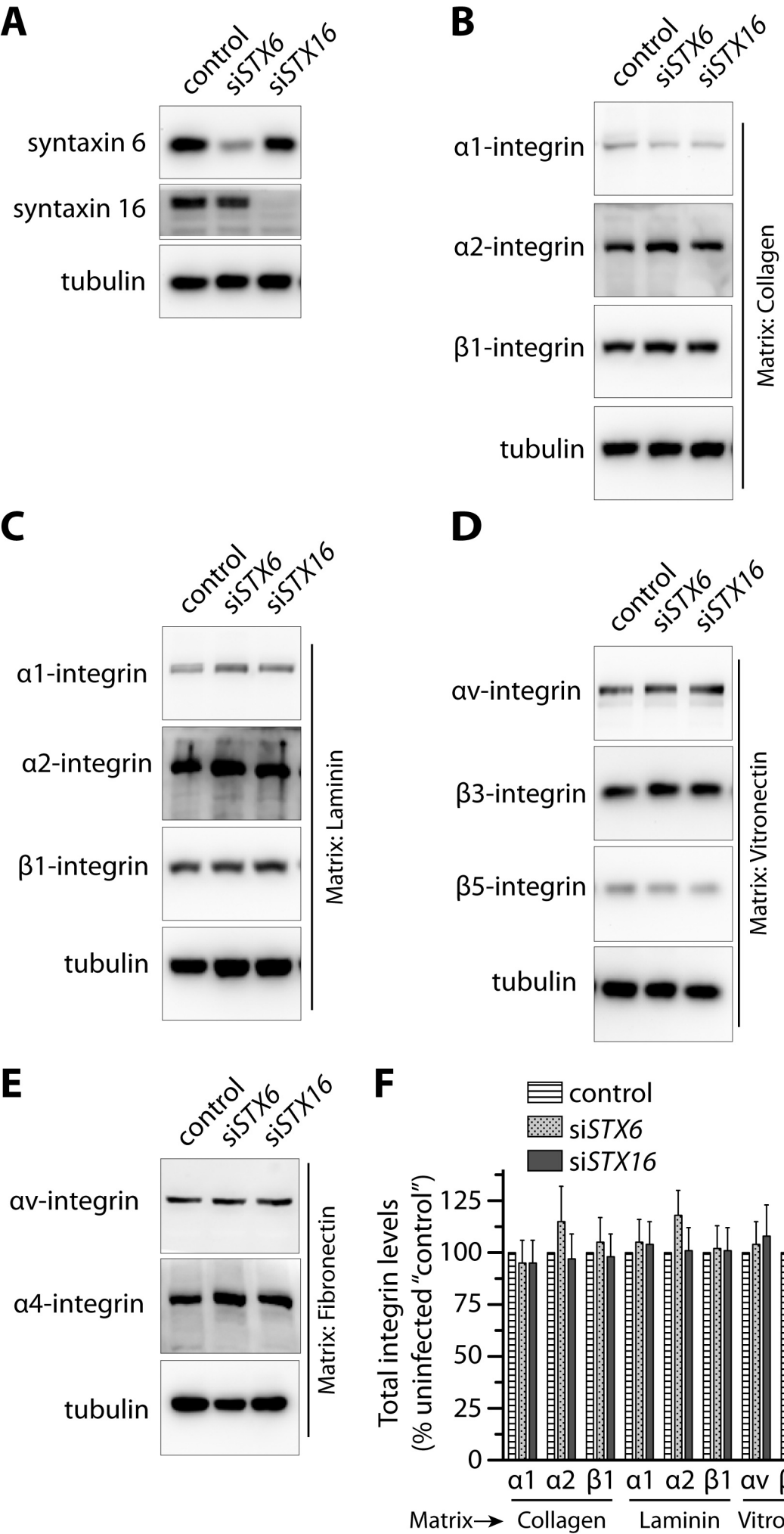
Supplemental figure 2. **Inhibition of syntaxin 6 does not affect endothelial cell spreading on collagen (type I), laminin-1 and, vitronectin**. Uninfected (Control), syntaxin 6-cyto- and, syntaxin 16-cyto-expressing HUVECs were cultured on fibronectin-coated surfaces in complete medium. (**A**) Cells were trypsinized and then allowed to spread on a collagen-, laminin- and, vitronectin-coated glass surface at 37ºC for the indicated times. Following fixation, permeabilization and staining with Alexa488-labeled phalloidin, the cells were imaged by epifluorescence microscopy. Representative images are shown. (**B**) Quantitation of the cell-surface areas of individual cells, based on epifluorescence images of cells described in **A**. Values represent mean ± SD (cells=200, from 3 separate experiments; $p \leq 0.005$). Scale bar represent 5 μm.

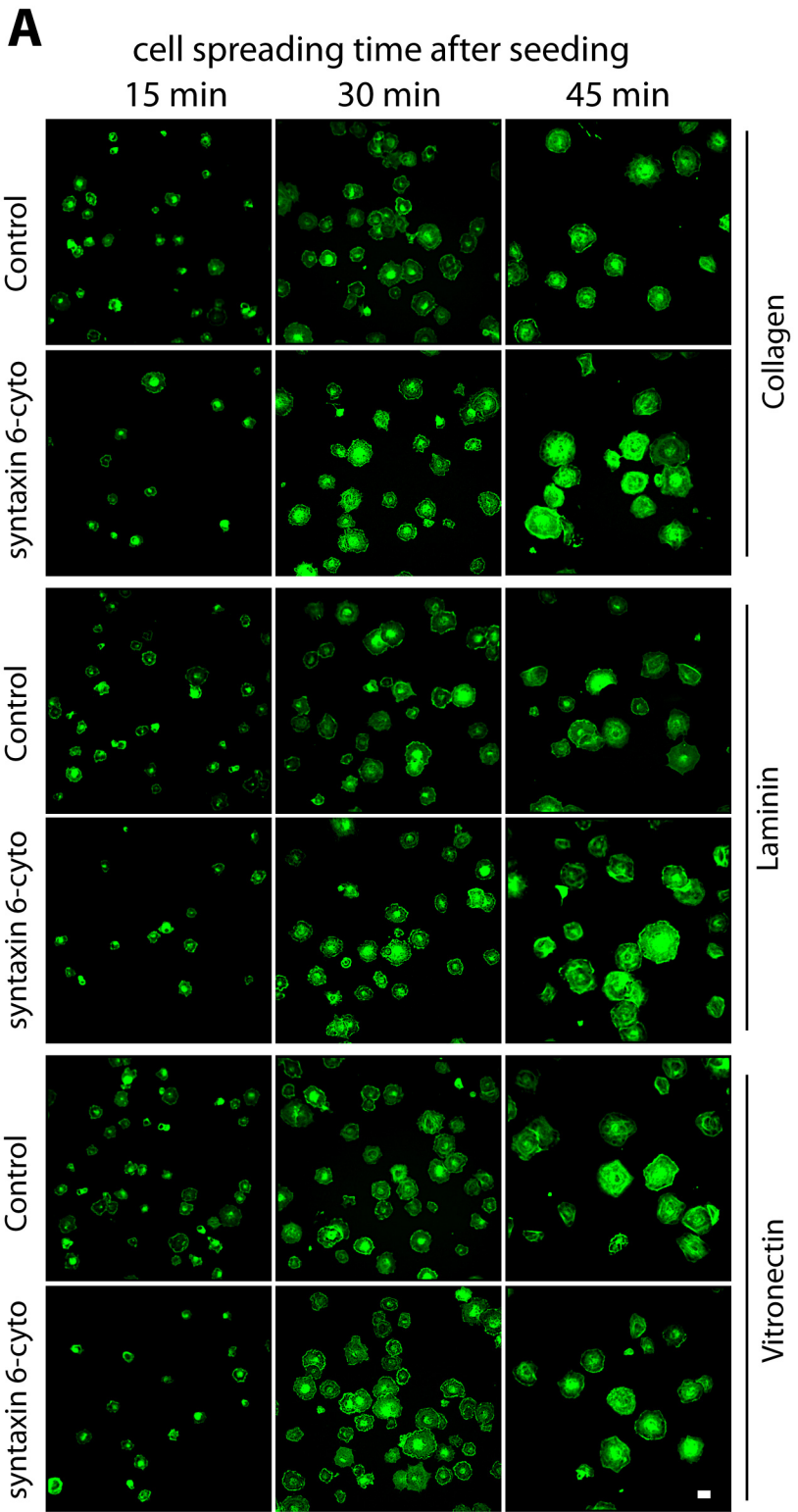
Supplemental figure 3. **Lysosomal proteases inhibitor treatment does not rescue syntaxin 6-inhibition-induced reduction in FAK phosphorylation, Rac1 activation and, cell spreading on fibronectin**. Uninfected (Control) and syntaxin 6-cyto- or syntaxin 16-cyto-infected HUVECs were grown on fibronectin-coated surfaces for 24 h and were further cultured in the presence of 300 μM leupeptin for 12 h. (**A**) Cells were serum starved for 2–3 h and then stimulated with serum-containing medium for 10 min and stained with Ab against pY397-FAK. Scale bars represent 5 μm. (**B**) Lysates were prepared and subjected to GST-PBD pull-down assay, followed by blotting with Rac1 Ab to detect active Rac1. The relative levels of total Rac1 and tubulin in cell lysates (10% input) were detected by immunoblotting with Abs. (**C**) The intensity of the band of active Rac1 was assessed after GST-PBD pull down assay and was quantitated and normalized to that of the control. Values represent mean ± SD (n=3; $p \leq 0.005$). (**D**) Cells were trypsinized and then allowed to spread on a fibronectin-coated glass surface at 37ºC for the indicated times in complete medium containing leupeptin (300 μM). Following fixation, permeabilization and staining with Alexa488-labeled phalloidin, the cells were imaged by epifluorescence microscopy. Representative images are shown. (**E**) Quantitation of the cell-surface areas of individual cells, based on epifluorescence images of cells described in **D**. Values represent mean ± SD (cells=150, from 3 separate experiments; $p \leq 0.005$). Scale bars represent 5 μm.

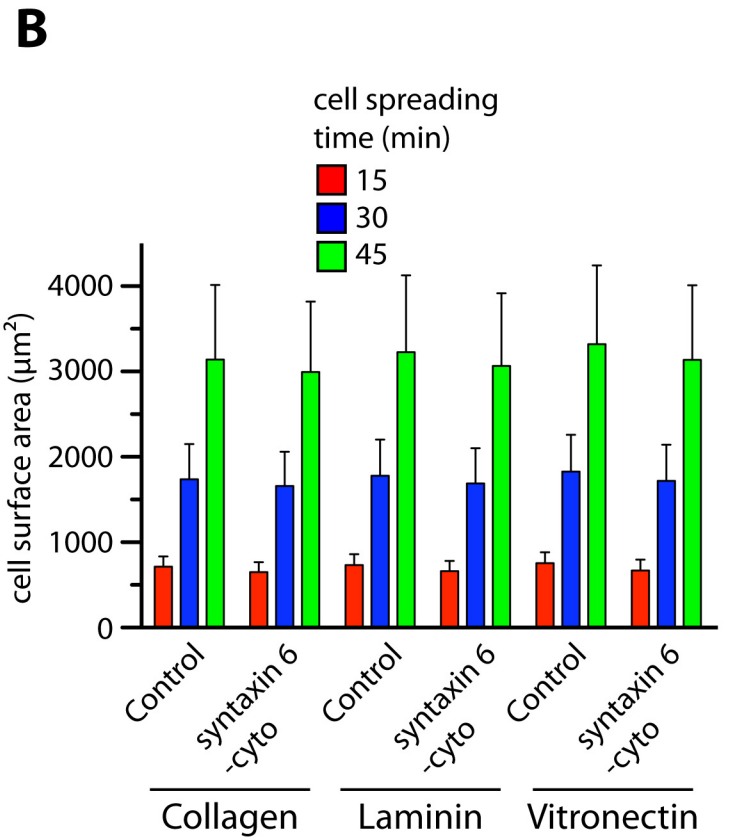
Supplemental figure 4. **Syntaxin 6 knockdown blocks spreading of endothelial cells on fibronectin**. Uninfected (Control), si*STX6*- or si*STX16*-expressing HUVECs were cultured on fibronectin-coated surface in complete medium for 72 h. Cells were trypsinized and then allowed to spread on a fibronectin-coated glass surface at 37ºC for the indicated times. Following fixation, permeabilization and staining with Alexa488-labeled phalloidin, the cells were imaged by epifluorescence microscopy. Representative images are shown. Scale bar represent 5 μm.

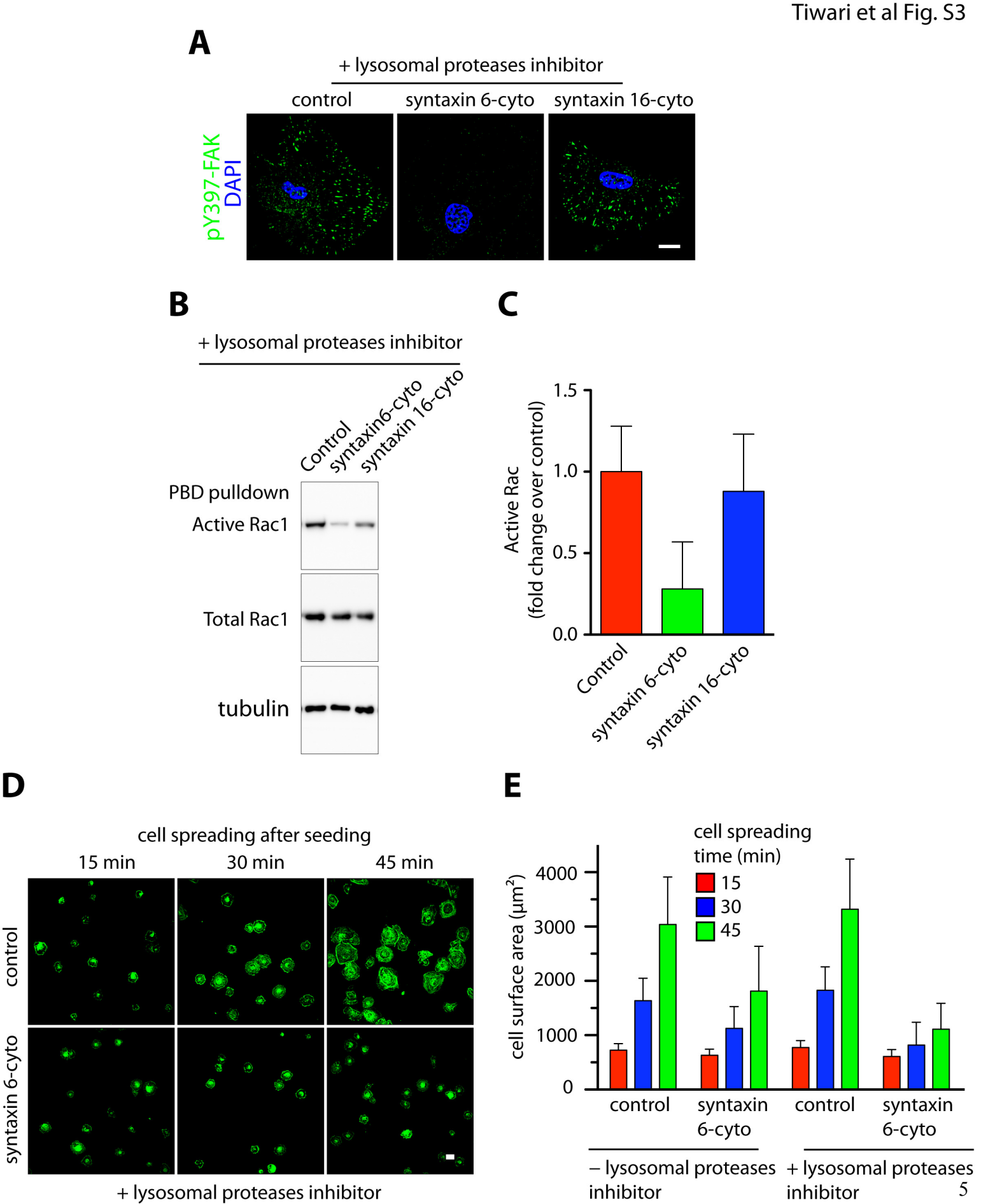
Supplemental figure 5. **Original linescans generated from images shown in figure 7B.** Epifluorescence images were processed using Metamorph image analysis software and, line was drawn from left edge to

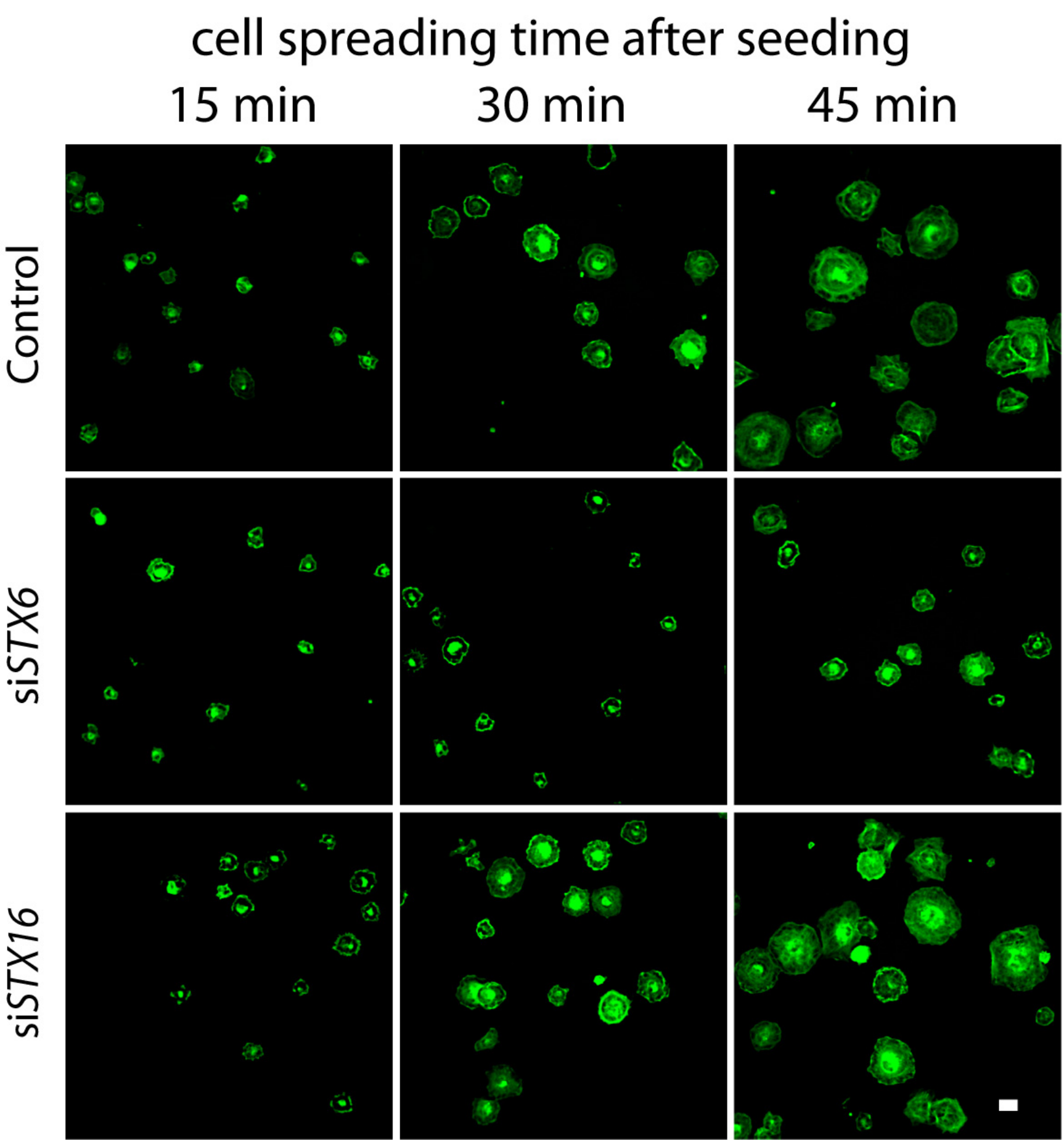
the right edge of the cells as shown in the image using linescan function of the software. Graphs show average pixel intensity changes from left edge of a cell to the right along the line marked on the image shown above.

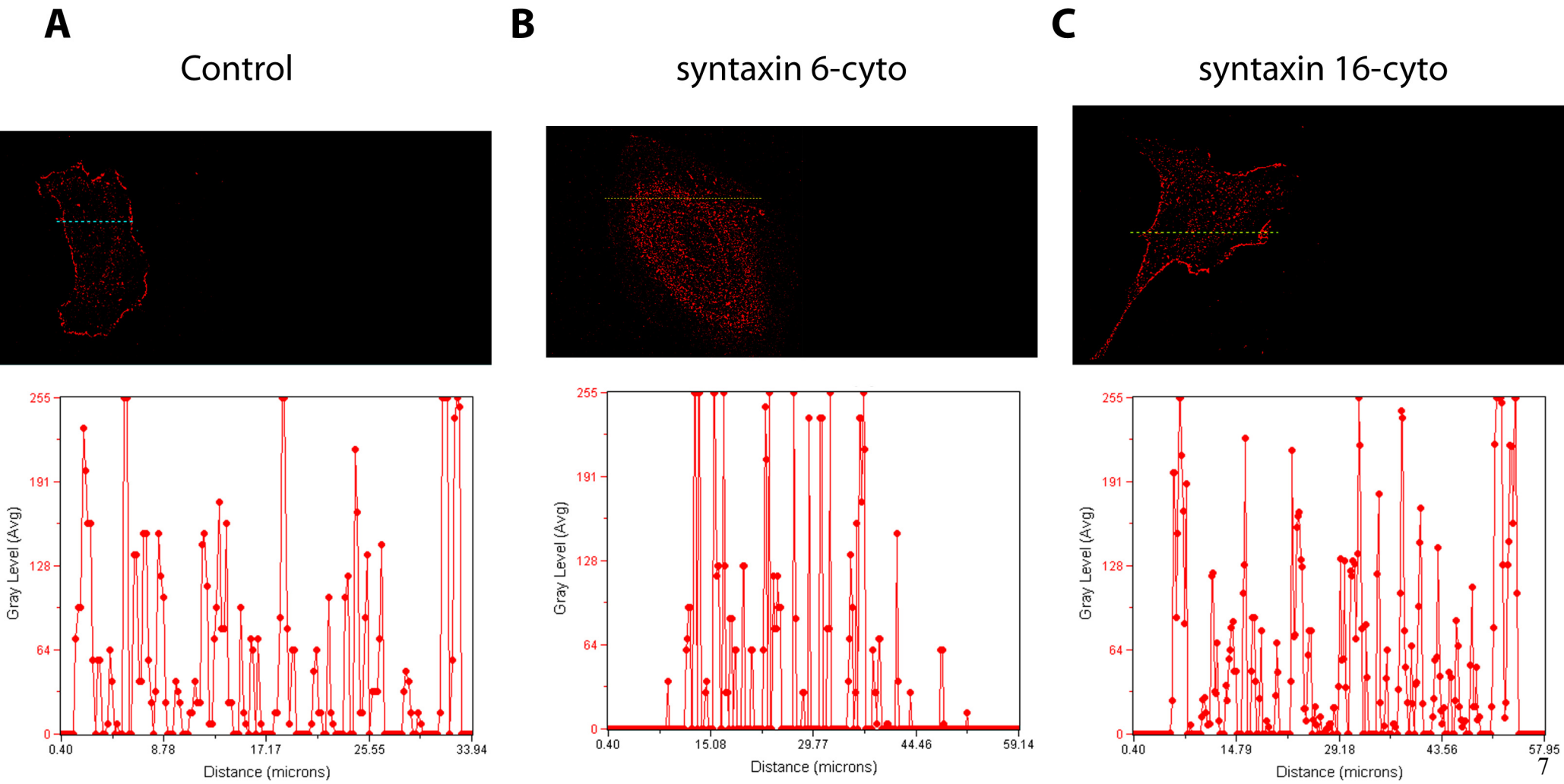

A
control
siSTX6
siSTX16
syntaxin 6
syntaxin 16
tubulin
B
control
siSTX6
siSTX16
α1-integrin
α2-integrin
β1-integrin
tubulin
Matrix: Collagen
C
control
siSTX6
siSTX16
α1-integrin
α2-integrin
β1-integrin
tubulin
Matrix: Laminin
D
control
siSTX6
siSTX16
αv-integrin
β3-integrin
β5-integrin
tubulin
Matrix: Vitronectin
E
control
siSTX6
siSTX16
αv-integrin
α4-integrin
tubulin
Matrix: Fibronectin
F
control
siSTX6
siSTX16
Total integrin levels
(% uninfected "control")
125
100
75
50
25
0
α1 α2 β1 α1 α2 β1 αv β3 β5 αv α4
Matrix→ Collagen Laminin Vitronectin Fibronectin


A
cell spreading time after seeding
15 min
30 min
45 min
Control
syntaxin 6-cyto
Collagen
Control
syntaxin 6-cyto
Laminin
Control
syntaxin 6-cyto
Vitronectin


B
cell spreading time (min)
15
30
45
cell surface area (μm²)
4000
3000
2000
1000
0
Control
syntaxin 6 -cyto
Control
syntaxin 6 -cyto
Control
syntaxin 6 -cyto
Collagen
Laminin
Vitronectin


A
+ lysosomal proteases inhibitor
control
syntaxin 6-cyto
syntaxin 16-cyto
pY397-FAK
DAPI
B
+ lysosomal proteases inhibitor
Control
syntaxin6-cyto
syntaxin 16-cyto
PBD pulldown
Active Rac1
Total Rac1
tubulin
C
Active Rac
(fold change over control)
1.5
1.0
0.5
0.0
Control
syntaxin 6-cyto
syntaxin 16-cyto
D
cell spreading after seeding
15 min
30 min
45 min
control
syntaxin 6-cyto
+ lysosomal proteases inhibitor
E
cell spreading
time (min)
15
30
45
cell surface area (μm²)
4000
3000
2000
1000
0
control
syntaxin
6-cyto
control
syntaxin
6-cyto
– lysosomal proteases inhibitor
+ lysosomal proteases inhibitor


cell spreading time after seeding
15 min
30 min
45 min
Control
siSTX6
siSTX16


A
Control
B
syntaxin 6-cyto
C
syntaxin 16-cyto
Gray Level (Avg)
255
191
128
64
0
0.40
8.78
17.17
25.55
33.94
Distance (microns)
Gray Level (Avg)
255
191
128
64
0
0.40
15.08
29.77
44.46
59.14
Distance (microns)
Gray Level (Avg)
255
191
128
64
0
0.40
14.79
29.18
43.56
57.95
Distance (microns)