Biglycan expression and its function in human ligamentum flavum

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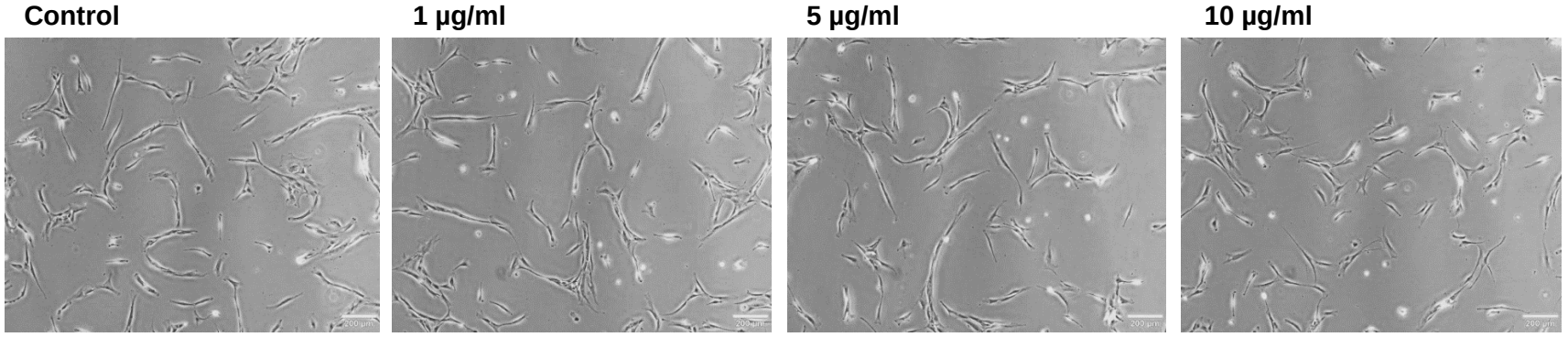
Supplementary Information

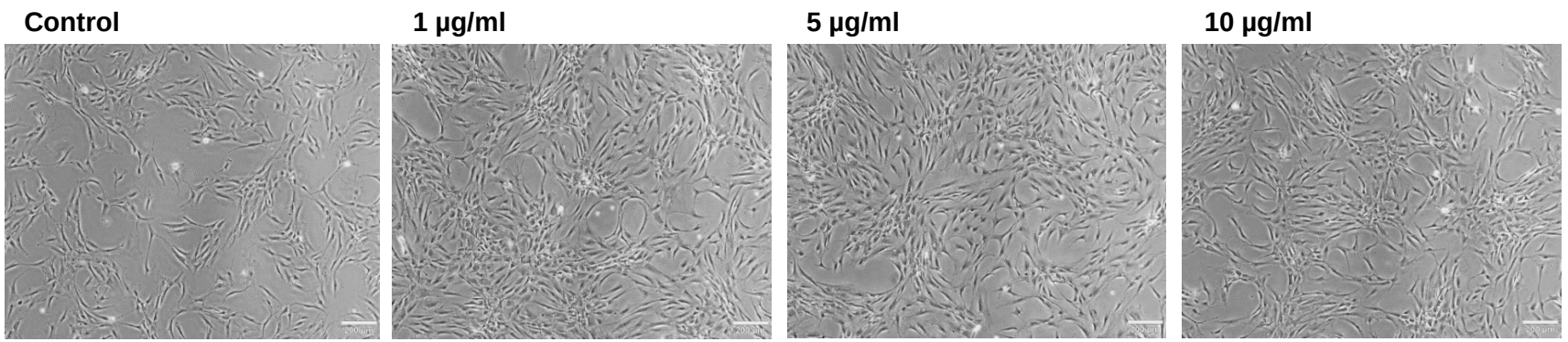
Figure Legends

Supplementary Figure S1. Effect of BGN on the proliferation of cells from non-hypertrophied (control) LF. Cell numbers before (a) and 48 h after (b) incubation with different concentrations of BGN, and c) total cell number at 48 h for each concentration of BGN.

a)


Control
1 µg/ml
5 µg/ml
10 µg/ml
Baseline

b)


Control
1 µg/ml
5 µg/ml
10 µg/ml
After 48 hours

c)

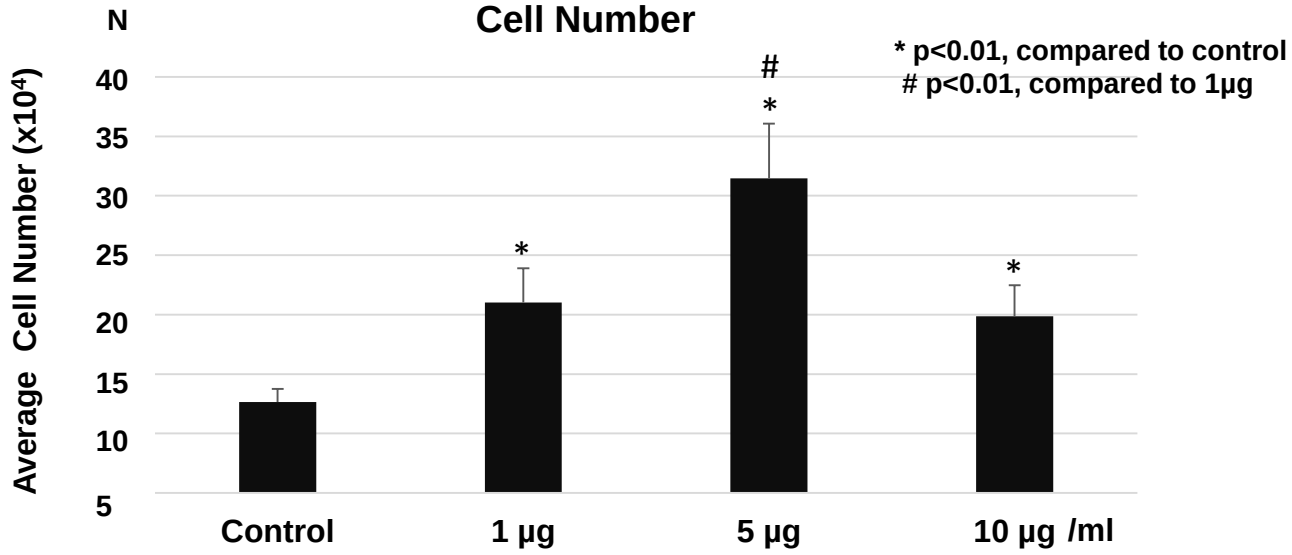
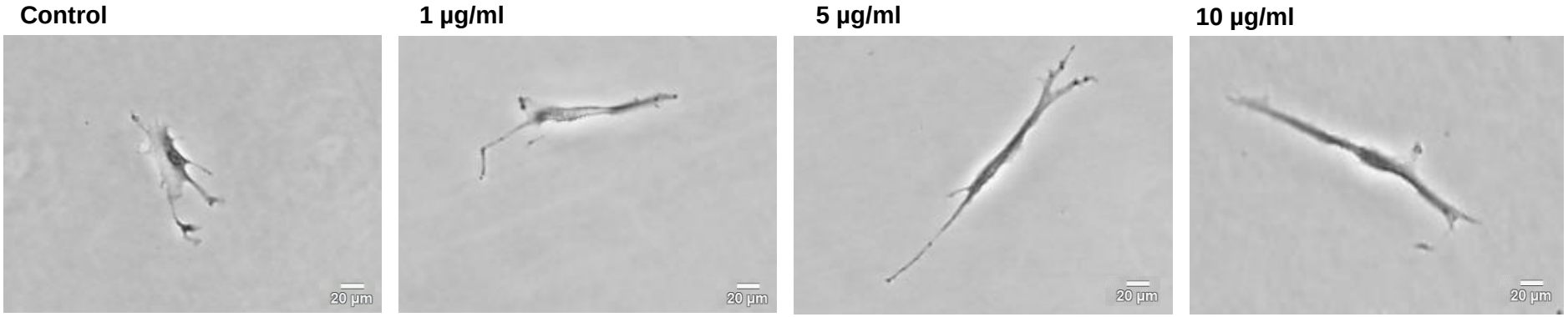

Cell Number
N
Average Cell Number (x10⁴)
40
35
30
25
20
15
10
5
* p<0.01, compared to control
# p<0.01, compared to 1µg
*
*
#
*
Control
1 µg
5 µg
10 µg /ml

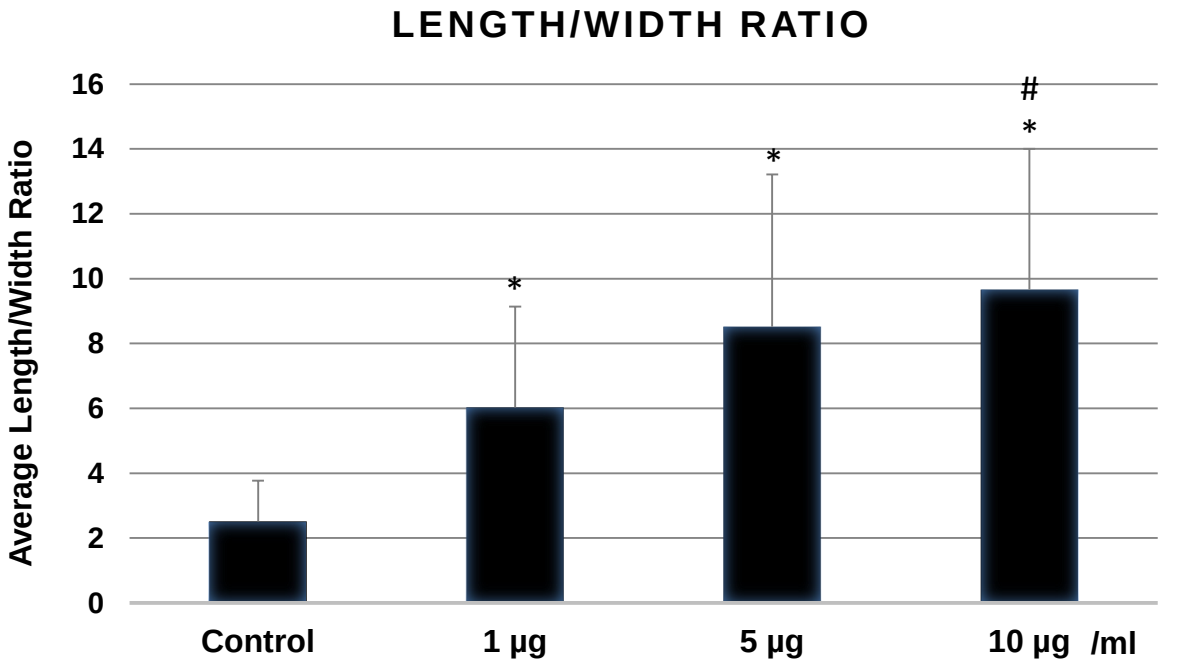
Figure Legends

Supplementary Figure S2. a) Cell morphology, and b) average length per width ratio of cells from non-hypertrophied (control) LF upon stimulation with different BGN concentrations.

a)



b)

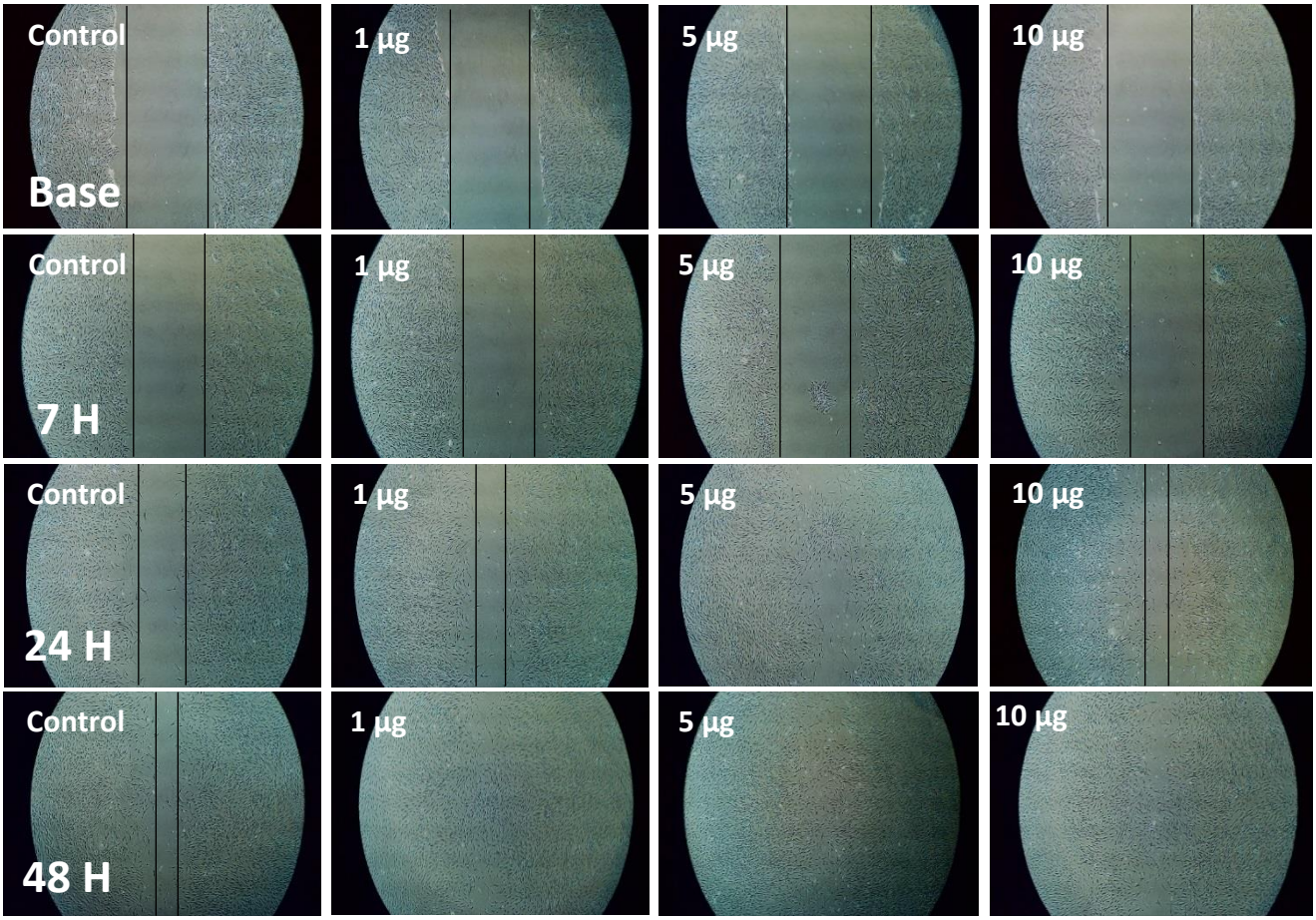


* p<0.01, compared to control
\# p<0.01, compared to 1µg

Figure Legends

Supplementary Figure S3. a) Immunocytochemistry for vimentin and α-SMA in the cells from non-hypertrophied (control) LF with the treatment of TGF-β, BGN, and the combination, b) the average percentage of α-SMA positive cells in each group.

a)


Control
1 µg
5 µg
10 µg
Base
7 H
24 H
48 H

b)

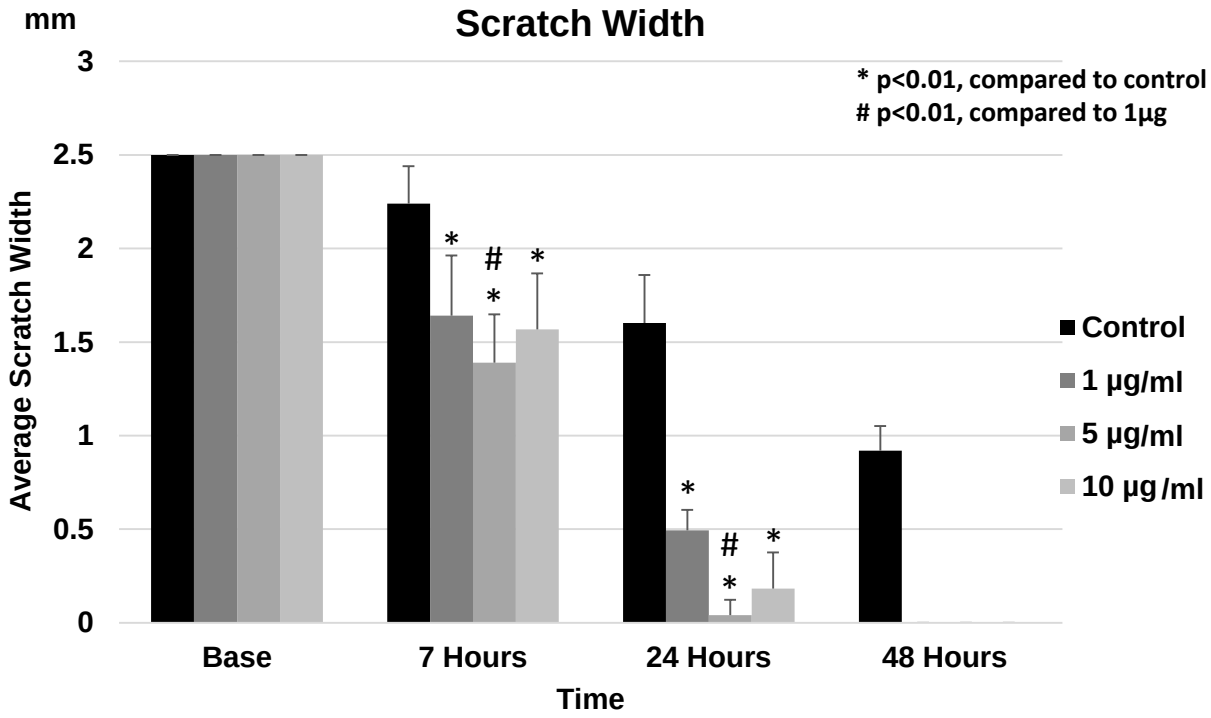
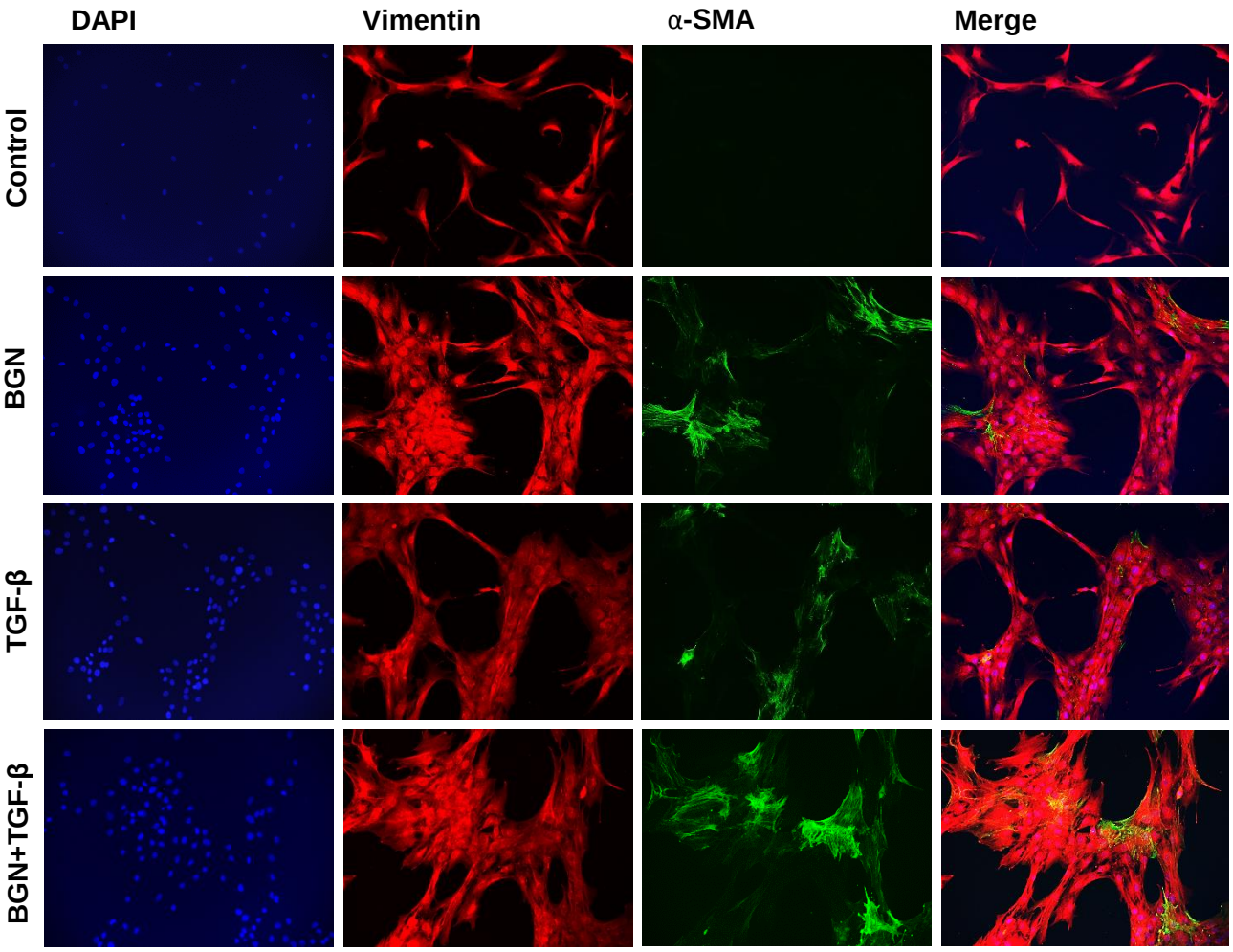

Scratch Width
mm
* p<0.01, compared to control
# p<0.01, compared to 1µg
Average Scratch Width
3
2.5
2
1.5
1
0.5
0
Base
7 Hours
24 Hours
48 Hours
Time
Control
1 µg/ml
5 µg/ml
10 µg/ml

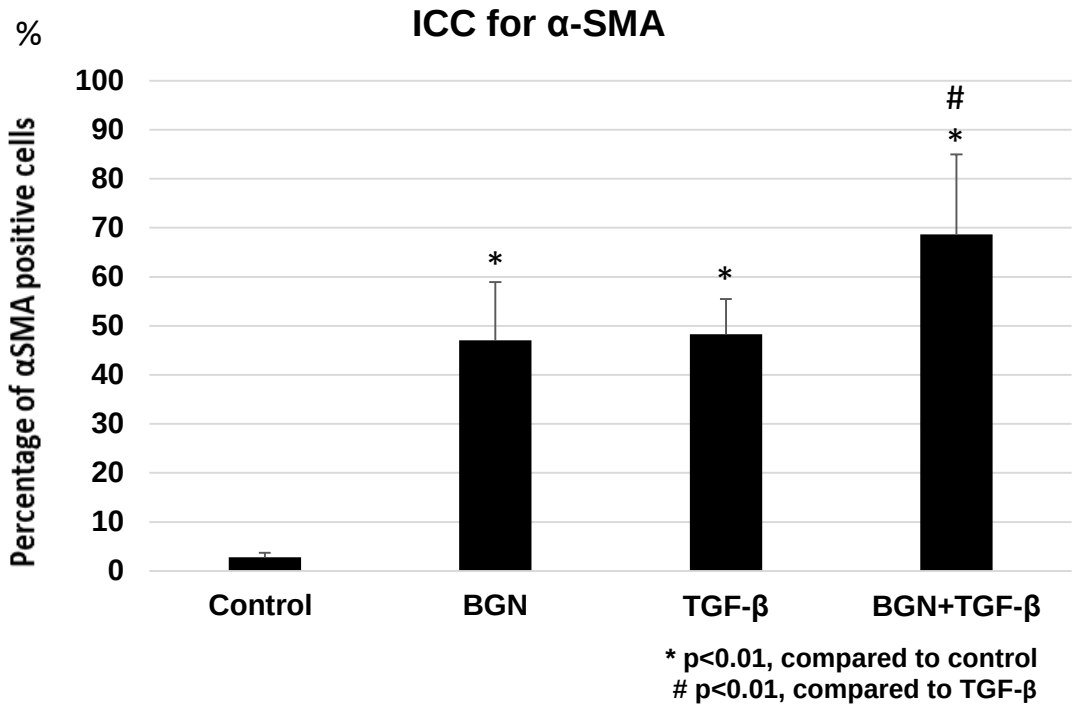
Figure Legends

Supplementary Figure S4. Effect of BGN on the migration of cells from non-hypertrophied (control) LF. (a) Scratch healing assay of LF cells at each time point after incubation with different concentrations of BGN. The black line indicates the wound borders. (b) Distance between the two wound borders for each BGN concentration at each time point.

a)


DAPI
Vimentin
α-SMA
Merge
Control
BGN
TGF-β
BGN+TGF-β

b)


ICC for α-SMA
%
Percentage of αSMA positive cells
100
90
80
70
60
50
40
30
20
10
0
*
*
#
*
Control
BGN
TGF-β
BGN+TGF-β
* p<0.01, compared to control
# p<0.01, compared to TGF-β