Supporting Information

Induction of Fibrogenic Phenotype in Human Mesenchymal Stem Cells by Connective Tissue Growth Factor in a Hydrogel Model of Soft Connective Tissue

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Supporting information:
Eight supplementary pages
Six supplementary figures
One supplementary table

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Synthesis of HA-SH.

HA (430 kDa, 1.25 mmol), dissolved in deionized (DI) water at 10 mg/mL, was reacted with 3,3'-dithiobis-propanoic dihydrazide (DTP, 2.5 mmol), utilizing 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride (EDC, 1.88 mmol) activation. The reaction was allowed to proceed at pH 4.75 for 1 h under mechanical stirring. Upon adjusting the solution pH to 8.0, dithiothreitol (DTT, 16.21 mmol) was added to reduce disulfide bonds. After 48-h dialysis (MWCO: 10 kDa) against 0.1 M NaCl at pH 3.5, followed by 24-h dialysis against DI water at pH 3.5, the solution was sterilized by filtration through a 0.22 μm filter and lyophilized to yield the dry product.

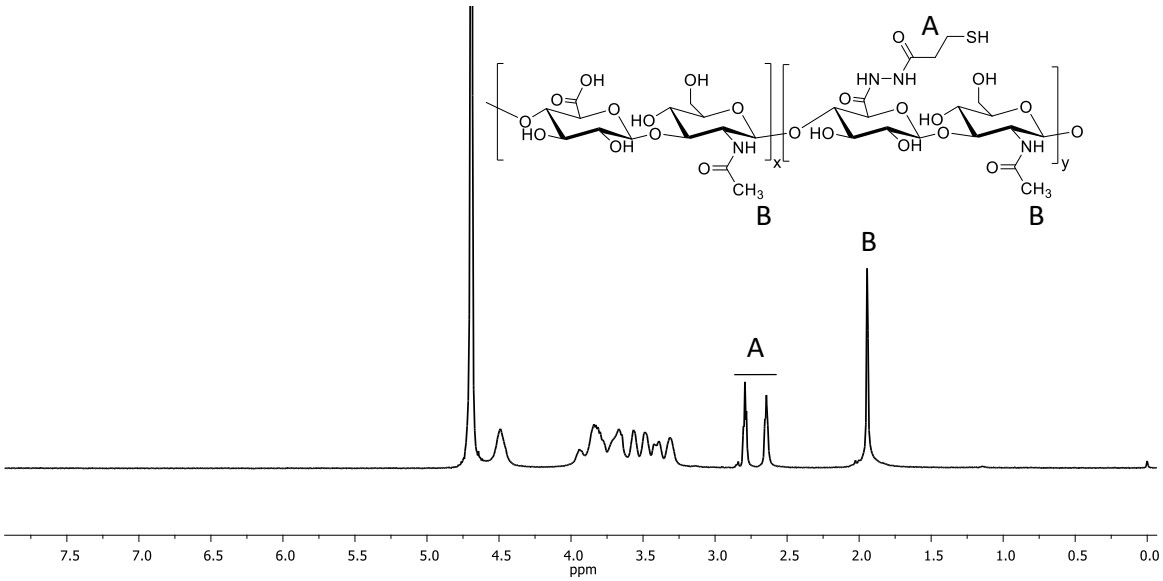

Figure S1. ^{1}H-NMR spectrum of HA-SH in D_2O. The degree of modification was determined by comparing the integrations for methylene protons from DTP (A) and the acetamide group (B) in HA.

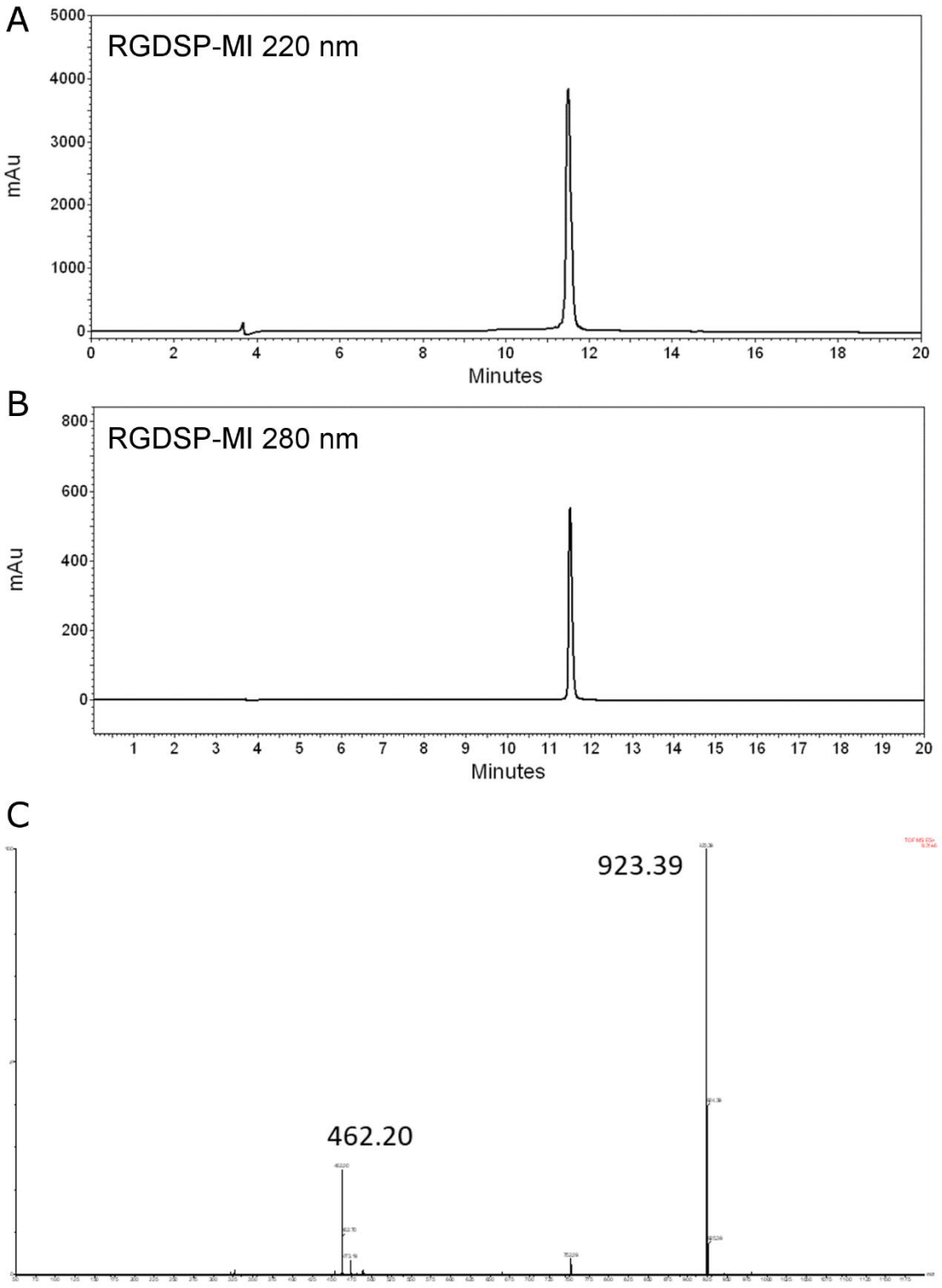

Figure S2. Characterization of RGDSP-MI. (A-B): Analytical HPLC trace of RGDSP-MI at 200 nm (A) and 280 nm (B). (C): ESI-MS spectrum confirming the mass of the peptide with a sequence MI-GGGRGDSPG. Exact Mass= 922.4; Observed: $[M+H]^{+}$ = 923.39, $[M+2H]^{2+}$= 462.20

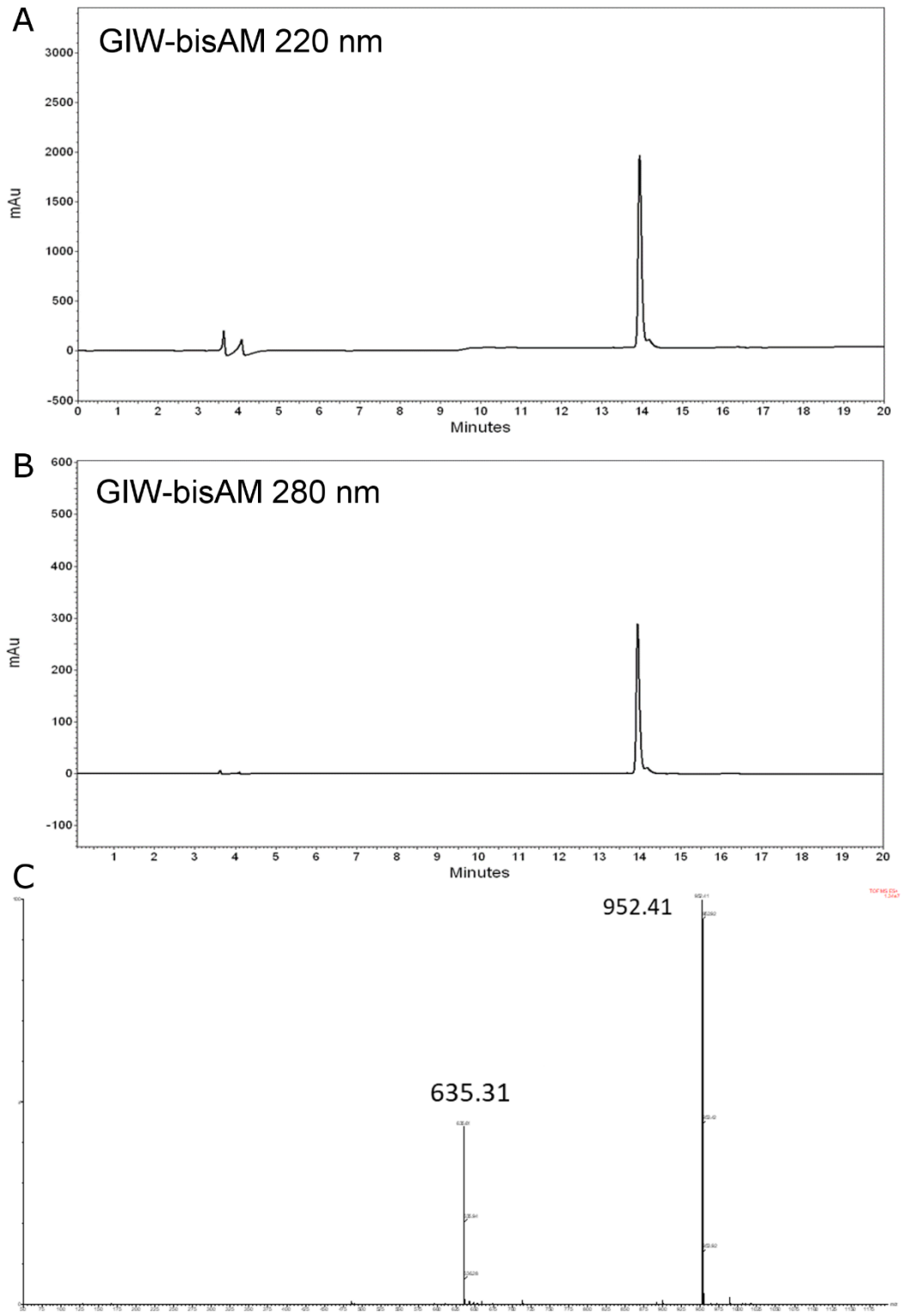

Figure S3. Characterization of GIW-bisAM. (A-B): Analytical HPLC trace of GIW-bisAM at 200 nm (A) and 280 nm (B). (C): ESI-MS spectrum confirming the mass of the peptide with a sequence of GK'RDGPQGIWGQDRK'G (K': acrylamide functionalized lysine). Exact Mass= 1902.94; Observed: $[M+2H]^{+}$ = 952.41, $[M+3H]^{3+}$= 635.31

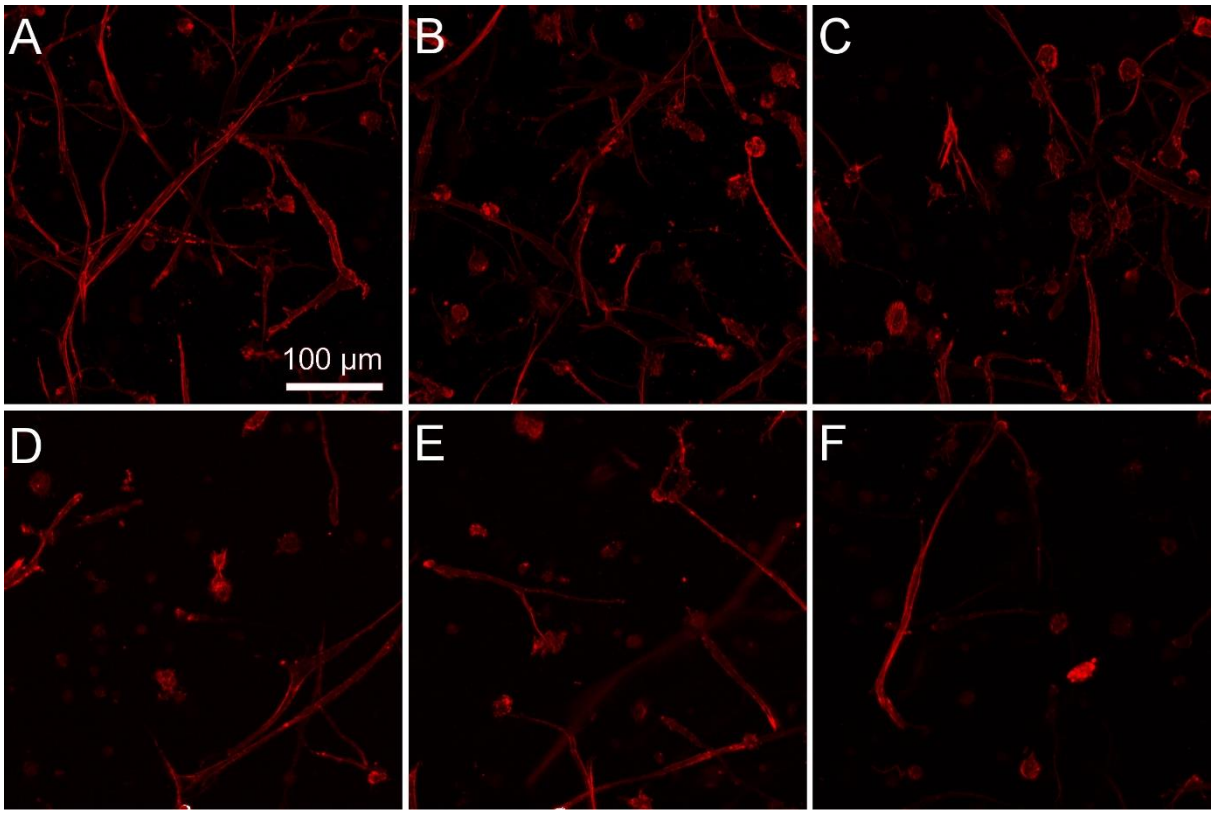

Figure S4. Representative confocal images of hMSCs cultured in HA gels for 21 days with (A-C) and without (D-F) CTGF. F-actin was staining by phalloidin-Alexa Fluor 568.

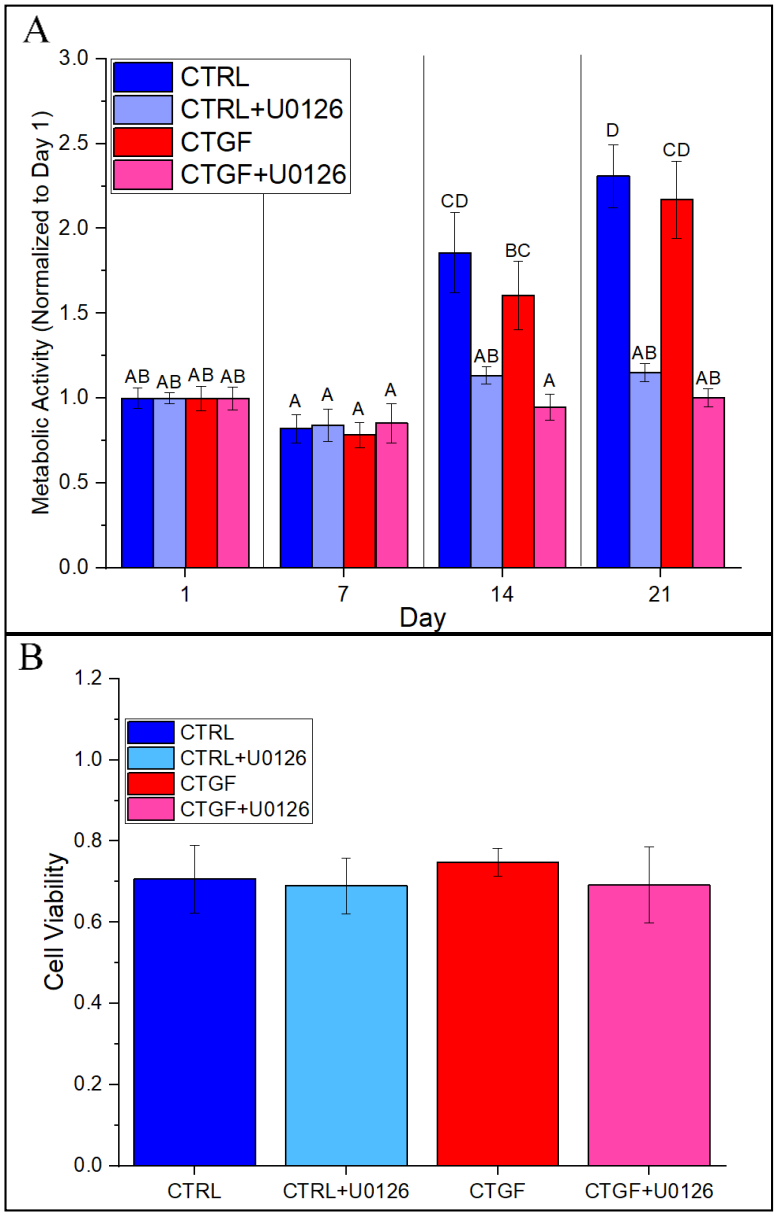

Figure S5. Effects of U0126 (20 mM) on cell metabolic activity (A) and viability (B). Metabolic activity was analyzed via PrestoBlue assay on day 1, 7, 14, and 21. Groups without matching letters are considered significant. Cell viability was determined via ImageJ quantification of confocal images after live/dead staining.

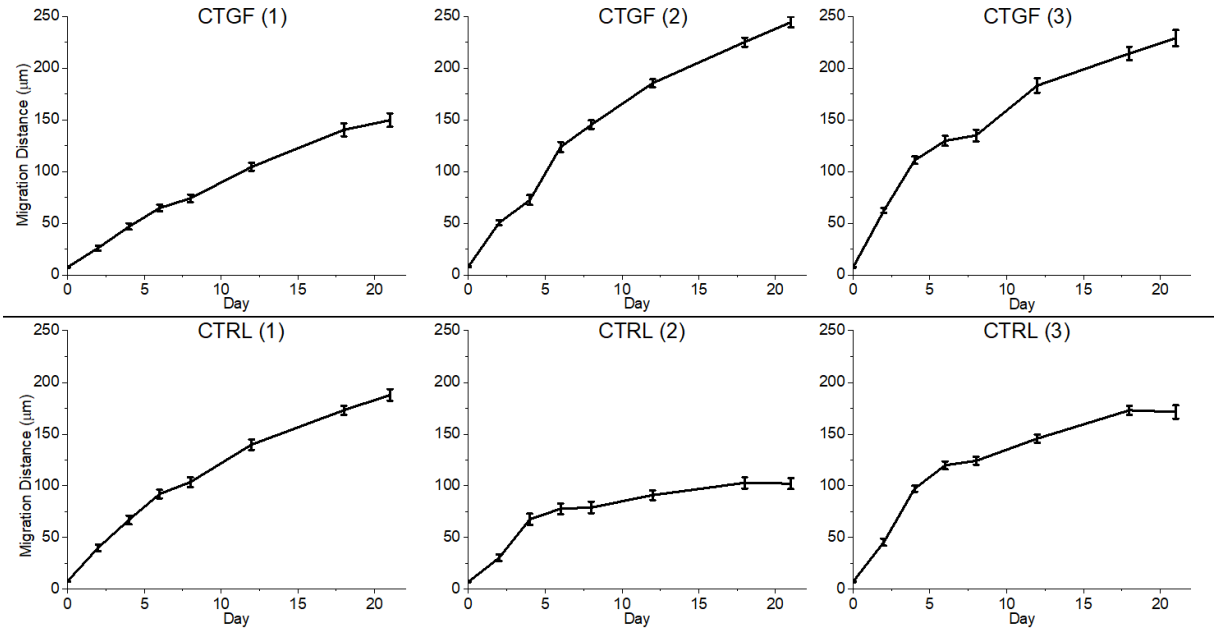

Figure S6. Individual cell outgrowth distance per aggregate. The average measured distance and standard error of the mean (SEM) were plotted at each timepoint for 3 CTGF-treated (top) and 3 CTRL samples (bottom).

Table S1. Summary of qPCR primers.

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')	GeneBank#	Efficiency	Product Size (bp)
GAPDH	GAAATCCCATCACCATCTTCCAGG	GAGCCCCAGCCTTCTCCATG	NM_001289746	2.08	120
FN	ACCTACGGATGACTCGTGCTTTGA	CAAAGCCTAAGCACTGGCACAACA	NM_001306132	2.10	116
ITGβ1	CTACCAACACGCCCTTCATT	ATGTGAATGCCAAAGCGAAG	XM_005252448	2.03	105
MMP1	GGGAGATCATCGGGACAACTC	GGGCCTGGTTGAAAAGCAT	NM_001145938	2.03	72
MMP2	GCCAATGGAGACTGTCTCAAGA	TTCTAAGGCAGCCAGCAGTGAA	NM_001302510	1.87	122
TIMP1	TTTCTTGGTTCCCCAGAATG	CAGAGCTGCAGAGCAACAAG	NG_012533	1.90	99
COL3α1	TGGTGCCCCTGGTCCTTGCT	TACGGGGCAAAACCGCCAGC	NM_000090	2.03	87
COL1α1	AATGGTGCTCCTGGTATTGCTGGT	ACCAGTGTCTCCTTTGCTGCCA	XM_005257058	2.10	141
HAS1	GTGAGTGGCTGTACAACGCG	AGAGGGACGTAGTTAGCGGC	NM_001523	1.91	355
CTGF	AGGAGTGGGTGTGTGACGA	CCAGGCAGTTGGCTCTAATC	NM_001901	1.90	117
HGF	TCCAGAGGTACGCTACGAAGTCT	CCCATTGCAGGTCATGCAT	NM_001010932	1.95	70
TGFβ1	GCAGAAGTTGGCATGGTAGC	CCCTGGACACCAACTATTGC	XM_011527242	1.99	131
VEGFA	GACAAGAAAATCCCTGTGGGC	AACGCGAGTCTGTGTTTTTGC	NM_001287044	1.91	102
TNC	GGGCTGGTTGTATTGATGCTTT	AGGGACCACTGGGTGAGAGA	XM_005251975	2.00	76
αSMA	CCAAGCACTGTCAGGAAT	AGGCAGTGCTGTCCTCTT	NM_001613	1.99	60
FAP	GGTGGATGGTCGAGGAACAGC	TCCTCCATAGGACCAGCCCCA	XM_011510796	2.01	169
FNEDA	CCCTAAAGGACTGGCATTCA	CATCCTCAGGGCTCGAGTAG	XM_017003692	1.99	113
ELN1	CCGCTAAGGCAGCCAAGTATGGA	AGCTCCAACCCCGTAAGTAGGAAT	XM_017011814	2.04	275
FSP1	AGCTTCTTGGGGAAAAGGAC	CCCCAACCACATCAGAGG	NM_019554	1.98	200
MCAM	GGGTACCCCATTCCTCAAGT	CAGTCTGGGACGACTGAATG	NM_006500	1.88	91
VCAM	AGTTGAAGGATGCGGGAGTAT	GGATGCAAAATAGAGCACGAG	NM_001078	1.99	143

Gene abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FN, fibronectin; ITGβ1: integrin beta 1; MMP1, matrix metalloproteinase 1; MMP2, matrix metalloproteinase 2; TIMP1, tissue inhibitor of metalloproteinase 1; COL3α1, collagen type III alpha-1; COL1α1, collagen type I alpha-1; HAS1, hyaluronic acid synthase 1; CTGF, connective tissue growth factor; HGF, hepatocyte growth factor; TGFβ1, transforming growth factor beta-1; VEGFA, vascular endothelial growth factor-A; TNC, tenascin C; αSMA, alpha-smooth muscle actin; FAP, fibroblast activation protein; FNEDA, fibronectin extra domain-A; ELN, elastin; FSP1, fibroblast specific protein-1; MCAM, melanoma cell adhesion molecule; VCAM, vascular cell adhesion molecule.