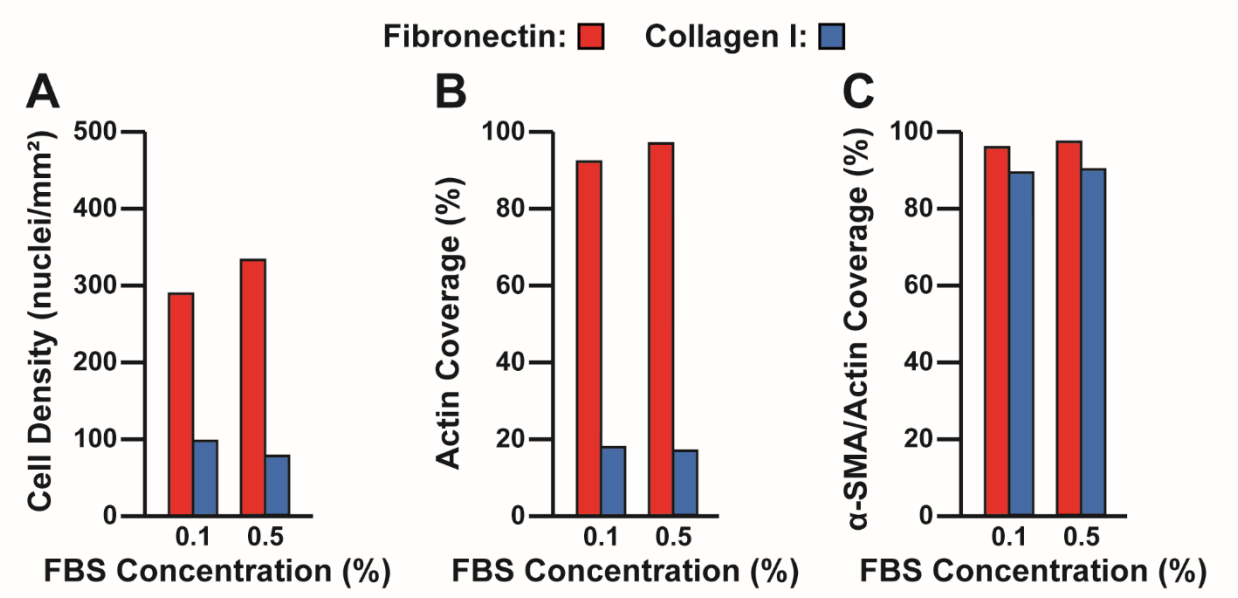
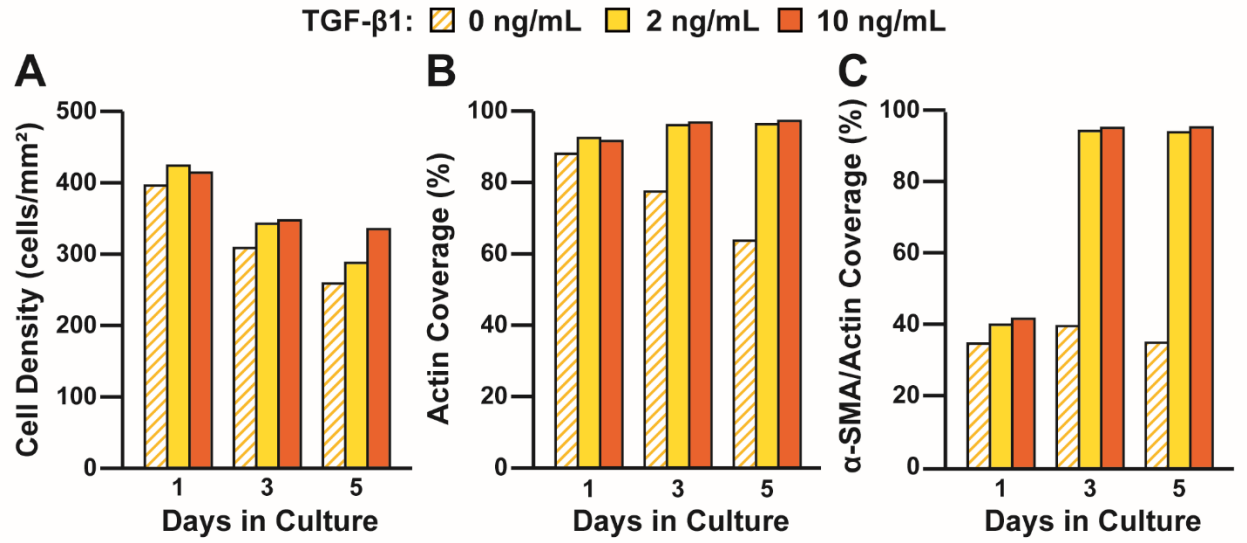


Supplementary Figure S1. Optimization of ECM ligand and serum concentration for human cardiac fibroblast culture. Human cardiac fibroblasts were seeded onto high PDMS-coated coverslips coated with the indicated ECM ligand. After one day, serum levels were reduced to the indicated levels and cells were treated with 10 ng/mL TGF-β1 for five days, followed by staining and imaging. Cell density (A), actin coverage (B), and α-SMA/actin coverage was quantified (n=1).



Supplementary Figure S2. Optimization of TGF-β1 concentration. Human cardiac fibroblasts were seeded onto high PDMS-coated coverslips coated fibronectin. After one day, serum was reduced to 0.1% and cells were treated with 0, 2, or 10 ng/mL TGF-β1 for one, three, and five days, following by staining and imaging. Cell density (A), actin coverage (B), and α-SMA/actin coverage was quantified (n=1).

Supplementary Table S1. Primers used for RT-PCR. All primers were purchased from BioRad.

Gene	Encoded Protein	Protein Function	Unique Assay ID
ACTA2	α-SMA	Contractile microfilament	qHsaCID0013300
POSTN	Periostin	ECM remodeling	qHsaCID0023310
FAP	Fibroblast activation protein	Wound healing	qHsaCID0018575
FSP1	Fibroblast specific protein-1	Cell motility	qHsaCID0013749
GJA1	Connexin 43	Gap junction electrical communication	qHsaCID0012977

Supplementary Table S2. Statistical analysis for cell density. All data was normally distributed, as determined by the Kolmogorov-Smirnov test, and compared using one-way ANOVA, with *p*-values indicated below.

Comparison	Test	*p*-value
Subset: Day 1	One-way ANOVA	NS (0.84)
Subset: Day 3	One-way ANOVA	NS (0.42)
Subset: Day 5	One-way ANOVA	NS (0.7)

Supplementary Table S3. Statistical analysis for actin coverage. All data was normally distributed, as determined by the Kolmogorov-Smirnov test, and compared using one-way ANOVA, with *p*-values indicated below. In timepoints with ANOVA *p*-values <0.05, multiple comparisons were performed with Tukey's test, with *p*-values indicated below.

Comparison	Test	*p*-value
Subset: Day 1	One-way ANOVA	NS (0.41)
Subset: Day 3	One-way ANOVA	6.60*10e-4
Low, -TGFB vs. Moderate, -TGFB	Tukey's test	NS
Low, -TGFB vs. High, -TGFB	Tukey's test	NS
Low, -TGFB vs. Low, +TGFB	Tukey's test	<0.01
Low, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.05
Low, -TGFB vs. High, +TGFB	Tukey's test	<0.01
Moderate, -TGFB vs. High, -TGFB	Tukey's test	NS
Moderate, -TGFB vs. Low, +TGFB	Tukey's test	<0.05
Moderate, -TGFB vs. Moderate, +TGFB	Tukey's test	NS (0.06)
Moderate, -TGFB vs. High, +TGFB	Tukey's test	<0.05
High, -TGFB vs. Low, +TGFB	Tukey's test	NS
High, -TGFB vs. Moderate, +TGFB	Tukey's test	NS
High, -TGFB vs. High, +TGFB	Tukey's test	NS (0.07)
Low, +TGFB vs. Moderate, +TGFB	Tukey's test	NS

Low, +TGFB vs. High, +TGFB	Tukey's test	NS
Moderate, +TGFB vs. High, +TGFB	Tukey's test	NS
Subset: Day 5	One-way ANOVA	3.1*10e-3
Low, -TGFB vs. Moderate, -TGFB	Tukey's test	NS
Low, -TGFB vs. High, -TGFB	Tukey's test	NS
Low, -TGFB vs. Low, +TGFB	Tukey's test	NS
Low, -TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, -TGFB vs. High, +TGFB	Tukey's test	NS (0.06)
Moderate, -TGFB vs. High, -TGFB	Tukey's test	NS
Moderate, -TGFB vs. Low, +TGFB	Tukey's test	<0.05
Moderate, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.05
Moderate, -TGFB vs. High, +TGFB	Tukey's test	<0.05
High, -TGFB vs. Low, +TGFB	Tukey's test	NS
High, -TGFB vs. Moderate, +TGFB	Tukey's test	NS
High, -TGFB vs. High, +TGFB	Tukey's test	NS
Low, +TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, +TGFB vs. High, +TGFB	Tukey's test	NS
Moderate, +TGFB vs. High, +TGFB	Tukey's test	NS

Supplementary Table S4. Statistical analysis for αSMA/actin coverage. All data was normally distributed, as determined by the Kolmogorov-Smirnov test, and compared using one-way ANOVA, with *p*-values indicated below. In timepoints with ANOVA *p*-values <0.05, multiple comparisons were performed with Tukey's test, with *p*-values indicated below.

Comparison	**Test**	***p*-value**
Subset: Day 1	One-way ANOVA	5.6*10e-3
Low, -TGFB vs. Moderate, -TGFB	Tukey's test	NS
Low, -TGFB vs. High, -TGFB	Tukey's test	NS
Low, -TGFB vs. Low, +TGFB	Tukey's test	NS
Low, -TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, -TGFB vs. High, +TGFB	Tukey's test	<0.01
Moderate, -TGFB vs. High, -TGFB	Tukey's test	NS
Moderate, -TGFB vs. Low, +TGFB	Tukey's test	NS
Moderate, -TGFB vs. Moderate, +TGFB	Tukey's test	NS
Moderate, -TGFB vs. High, +TGFB	Tukey's test	<0.01
High, -TGFB vs. Low, +TGFB	Tukey's test	NS
High, -TGFB vs. Moderate, +TGFB	Tukey's test	NS
High, -TGFB vs. High, +TGFB	Tukey's test	<0.05
Low, +TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, +TGFB vs. High, +TGFB	Tukey's test	NS
Moderate, +TGFB vs. High, +TGFB	Tukey's test	NS
Subset: Day 3	One-way ANOVA	2.35*10e-19
Low, -TGFB vs. Moderate, -TGFB	Tukey's test	NS
Low, -TGFB vs. High, -TGFB	Tukey's test	NS
Low, -TGFB vs. Low, +TGFB	Tukey's test	<0.00000003
Low, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.00000003
Low, -TGFB vs. High, +TGFB	Tukey's test	<0.00000003
Moderate, -TGFB vs. High, -TGFB	Tukey's test	<0.05
Moderate, -TGFB vs. Low, +TGFB	Tukey's test	<0.00000003

Moderate, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.00000003
Moderate, -TGFB vs. High, +TGFB	Tukey's test	<0.00000003
High, -TGFB vs. Low, +TGFB	Tukey's test	<0.00000003
High, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.00000003
High, -TGFB vs. High, +TGFB	Tukey's test	<0.00000003
Low, +TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, +TGFB vs. High, +TGFB	Tukey's test	NS
Moderate, +TGFB vs. High, +TGFB	Tukey's test	NS
Subset: Day 5	One-way ANOVA	8.36*10e-20
Low, -TGFB vs. Moderate, -TGFB	Tukey's test	NS
Low, -TGFB vs. High, -TGFB	Tukey's test	NS
Low, -TGFB vs. Low, +TGFB	Tukey's test	<0.00000003
Low, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.00000003
Low, -TGFB vs. High, +TGFB	Tukey's test	<0.00000003
Moderate, -TGFB vs. High, -TGFB	Tukey's test	NS
Moderate, -TGFB vs. Low, +TGFB	Tukey's test	<0.00000003
Moderate, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.00000003
Moderate, -TGFB vs. High, +TGFB	Tukey's test	<0.00000003
High, -TGFB vs. Low, +TGFB	Tukey's test	<0.00000003
High, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.00000003
High, -TGFB vs. High, +TGFB	Tukey's test	<0.00000003
Low, +TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, +TGFB vs. High, +TGFB	Tukey's test	NS
Moderate, +TGFB vs. High, +TGFB	Tukey's test	NS

Supplementary Table S5. Statistical analysis for *ACTA2* relative gene expression. All data was normally distributed, as determined by the Kolmogorov-Smirnov test, and compared using one-way ANOVA, with *p*-values indicated below. In timepoints with ANOVA *p*-values <0.05, multiple comparisons were performed with Tukey's test, with *p*-values indicated below.

Comparison	**Test**	***p*-value**
Subset: Day 1	One-way ANOVA	1.84*10e-7
Low, -TGFB vs. Moderate, -TGFB	Tukey's test	NS
Low, -TGFB vs. High, -TGFB	Tukey's test	NS
Low, -TGFB vs. Low, +TGFB	Tukey's test	<0.00003
Low, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.001
Low, -TGFB vs. High, +TGFB	Tukey's test	<0.00005
Moderate, -TGFB vs. High, -TGFB	Tukey's test	NS
Moderate, -TGFB vs. Low, +TGFB	Tukey's test	<0.00003
Moderate, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.0006
Moderate, -TGFB vs. High, +TGFB	Tukey's test	<0.00003
High, -TGFB vs. Low, +TGFB	Tukey's test	<0.00003
High, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.001
High, -TGFB vs. High, +TGFB	Tukey's test	<0.00006
Low, +TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, +TGFB vs. High, +TGFB	Tukey's test	NS
Moderate, +TGFB vs. High, +TGFB	Tukey's test	NS
Subset: Day 3	One-way ANOVA	1.26*10e-4
Low, -TGFB vs. Moderate, -TGFB	Tukey's test	NS

Low, -TGFB vs. High, -TGFB	Tukey's test	NS
Low, -TGFB vs. Low, +TGFB	Tukey's test	<0.005
Low, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.05
Low, -TGFB vs. High, +TGFB	Tukey's test	<0.005
Moderate, -TGFB vs. High, -TGFB	Tukey's test	NS
Moderate, -TGFB vs. Low, +TGFB	Tukey's test	<0.005
Moderate, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.05
Moderate, -TGFB vs. High, +TGFB	Tukey's test	<0.005
High, -TGFB vs. Low, +TGFB	Tukey's test	<0.01
High, -TGFB vs. Moderate, +TGFB	Tukey's test	NS (0.06)
High, -TGFB vs. High, +TGFB	Tukey's test	<0.01
Low, +TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, +TGFB vs. High, +TGFB	Tukey's test	NS
Moderate, +TGFB vs. High, +TGFB	Tukey's test	NS

Supplementary Table S6. Statistical analysis for *POSTN* relative gene expression. All data was normally distributed, as determined by the Kolmogorov-Smirnov test, and compared using one-way ANOVA, with *p*-values indicated below. In timepoints with ANOVA *p*-values <0.05, multiple comparisons were performed with Tukey's test, with *p*-values indicated below.

Comparison	**Test**	***p*-value**
Subset: Day 1	One-way ANOVA	9.8*10e-3
Low, -TGFB vs. Moderate, -TGFB	Tukey's test	NS
Low, -TGFB vs. High, -TGFB	Tukey's test	NS
Low, -TGFB vs. Low, +TGFB	Tukey's test	NS
Low, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.05
Low, -TGFB vs. High, +TGFB	Tukey's test	NS
Moderate, -TGFB vs. High, -TGFB	Tukey's test	NS
Moderate, -TGFB vs. Low, +TGFB	Tukey's test	NS (0.08)
Moderate, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.05
Moderate, -TGFB vs. High, +TGFB	Tukey's test	NS
High, -TGFB vs. Low, +TGFB	Tukey's test	NS
High, -TGFB vs. Moderate, +TGFB	Tukey's test	NS
High, -TGFB vs. High, +TGFB	Tukey's test	NS
Low, +TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, +TGFB vs. High, +TGFB	Tukey's test	NS
Moderate, +TGFB vs. High, +TGFB	Tukey's test	NS
Subset: Day 3	One-way ANOVA	NS (0.51)

Supplementary Table S7. Statistical analysis for *FAP* relative gene expression. All data was normally distributed, as determined by the Kolmogorov-Smirnov test, and compared using one-way ANOVA, with *p*-values indicated below. In timepoints with ANOVA *p*-values <0.05, multiple comparisons were performed with Tukey's test, with *p*-values indicated below.

Comparison	**Test**	***p*-value**
Subset: Day 1	One-way ANOVA	NS (0.051)
Subset: Day 3	One-way ANOVA	9.0*10e-3
Low, -TGFB vs. Moderate, -TGFB	Tukey's test	NS
Low, -TGFB vs. High, -TGFB	Tukey's test	NS

Low, -TGFB vs. Low, +TGFB	Tukey's test	NS (0.06)
Low, -TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, -TGFB vs. High, +TGFB	Tukey's test	NS
Moderate, -TGFB vs. High, -TGFB	Tukey's test	NS
Moderate, -TGFB vs. Low, +TGFB	Tukey's test	<0.05
Moderate, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.05
Moderate, -TGFB vs. High, +TGFB	Tukey's test	NS
High, -TGFB vs. Low, +TGFB	Tukey's test	NS
High, -TGFB vs. Moderate, +TGFB	Tukey's test	NS
High, -TGFB vs. High, +TGFB	Tukey's test	NS
Low, +TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, +TGFB vs. High, +TGFB	Tukey's test	NS
Moderate, +TGFB vs. High, +TGFB	Tukey's test	NS

Supplementary Table S8. Statistical analysis for *FSP1* relative gene expression. All data was normally distributed, as determined by the Kolmogorov-Smirnov test, and compared using one-way ANOVA, with *p*-values indicated below. In timepoints with ANOVA *p*-values <0.05, multiple comparisons were performed with Tukey's test, with *p*-values indicated below.

Comparison	**Test**	***p*-value**
Subset: Day 1	One-way ANOVA	8.71*10e-4
Low, -TGFB vs. Moderate, -TGFB	Tukey's test	NS
Low, -TGFB vs. High, -TGFB	Tukey's test	NS
Low, -TGFB vs. Low, +TGFB	Tukey's test	<0.05
Low, -TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, -TGFB vs. High, +TGFB	Tukey's test	<0.05
Moderate, -TGFB vs. High, -TGFB	Tukey's test	NS
Moderate, -TGFB vs. Low, +TGFB	Tukey's test	NS (0.08)
Moderate, -TGFB vs. Moderate, +TGFB	Tukey's test	NS
Moderate, -TGFB vs. High, +TGFB	Tukey's test	NS
High, -TGFB vs. Low, +TGFB	Tukey's test	<0.005
High, -TGFB vs. Moderate, +TGFB	Tukey's test	<0.05
High, -TGFB vs. High, +TGFB	Tukey's test	<0.01
Low, +TGFB vs. Moderate, +TGFB	Tukey's test	NS
Low, +TGFB vs. High, +TGFB	Tukey's test	NS
Moderate, +TGFB vs. High, +TGFB	Tukey's test	NS
Subset: Day 3	One-way ANOVA	NS (0.14)

Supplementary Table S9. Statistical analysis for *GJA1* relative gene expression. All data was normally distributed, as determined by the Kolmogorov-Smirnov test, and compared using one-way ANOVA, with *p*-values indicated below.

Comparison	**Test**	***p*-value**
Subset: Day 1	One-way ANOVA	NS (0.60)
Subset: Day 3	One-way ANOVA	NS (0.079)

Supplementary Table S10. Statistical analysis for cell density after re-plating. All data was normally distributed, as determined by the Kolmogorov-Smirnov test, and compared using one-

way ANOVA, with *p*-value of 4.06*10e-1. Multiple comparisons were performed with Tukey's test, with *p*-values indicated below.

Comparison	Test	*p*-value
Day 2, -TGFB vs. Day 2, +TGFB	Tukey's test	NS
Day 2, -TGFB vs. Day 7, -TGFB	Tukey's test	NS
Day 2, -TGFB vs. Day 7, +TGFB	Tukey's test	NS
Day 2, +TGFB vs. Day 7, -TGFB	Tukey's test	NS
Day 2, +TGFB vs. Day 7, +TGFB	Tukey's test	NS
Day 7, -TGFB vs. Day 7, +TGFB	Tukey's test	NS

Supplementary Table S11. Statistical analysis for actin coverage after re-plating. All data was normally distributed, as determined by the Kolmogorov-Smirnov test, and compared using one-way ANOVA, with *p*-value of 2.5*10e-4. Multiple comparisons were performed with Tukey's test, with *p*-values indicated below.

Comparison	Test	*p*-value
Day 2, -TGFB vs. Day 2, +TGFB	Tukey's test	<0.05
Day 2, -TGFB vs. Day 7, -TGFB	Tukey's test	NS
Day 2, -TGFB vs. Day 7, +TGFB	Tukey's test	NS
Day 2, +TGFB vs. Day 7, -TGFB	Tukey's test	<0.0003
Day 2, +TGFB vs. Day 7, +TGFB	Tukey's test	NS (0.085)
Day 7, -TGFB vs. Day 7, +TGFB	Tukey's test	<0.05

Supplementary Table S12. Statistical analysis for αSMA/actin coverage after re-plating. All data was normally distributed, as determined by the Kolmogorov-Smirnov test, and compared using one-way ANOVA, with *p*-value of 2.19*10e-5. Multiple comparisons were performed with Tukey's test, with *p*-values indicated below.

Comparison	Test	*p*-value
Day 2, -TGFB vs. Day 2, +TGFB	Tukey's test	<0.0003
Day 2, -TGFB vs. Day 7, -TGFB	Tukey's test	NS
Day 2, -TGFB vs. Day 7, +TGFB	Tukey's test	<0.0006
Day 2, +TGFB vs. Day 7, -TGFB	Tukey's test	<0.001
Day 2, +TGFB vs. Day 7, +TGFB	Tukey's test	NS
Day 7, -TGFB vs. Day 7, +TGFB	Tukey's test	<0.0019