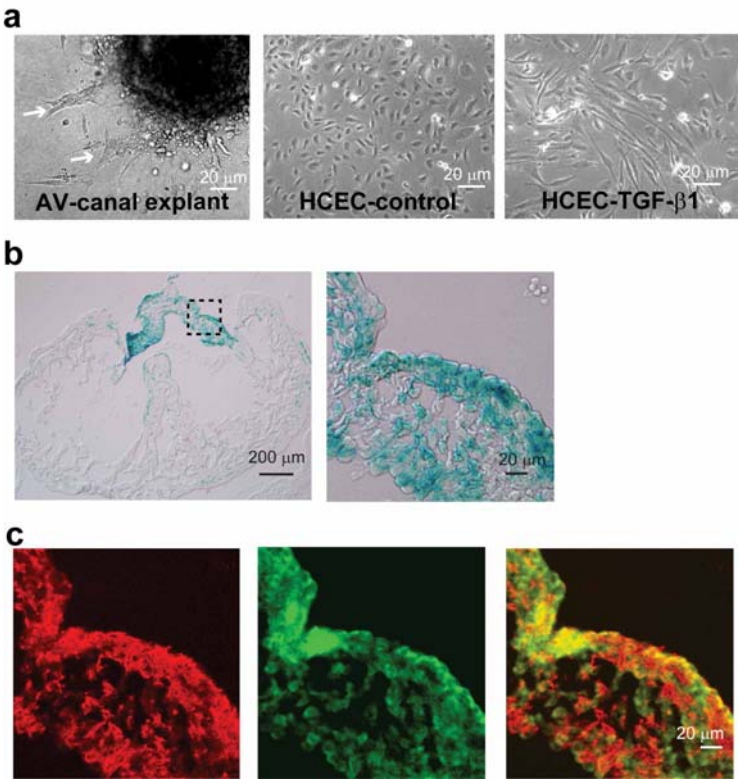
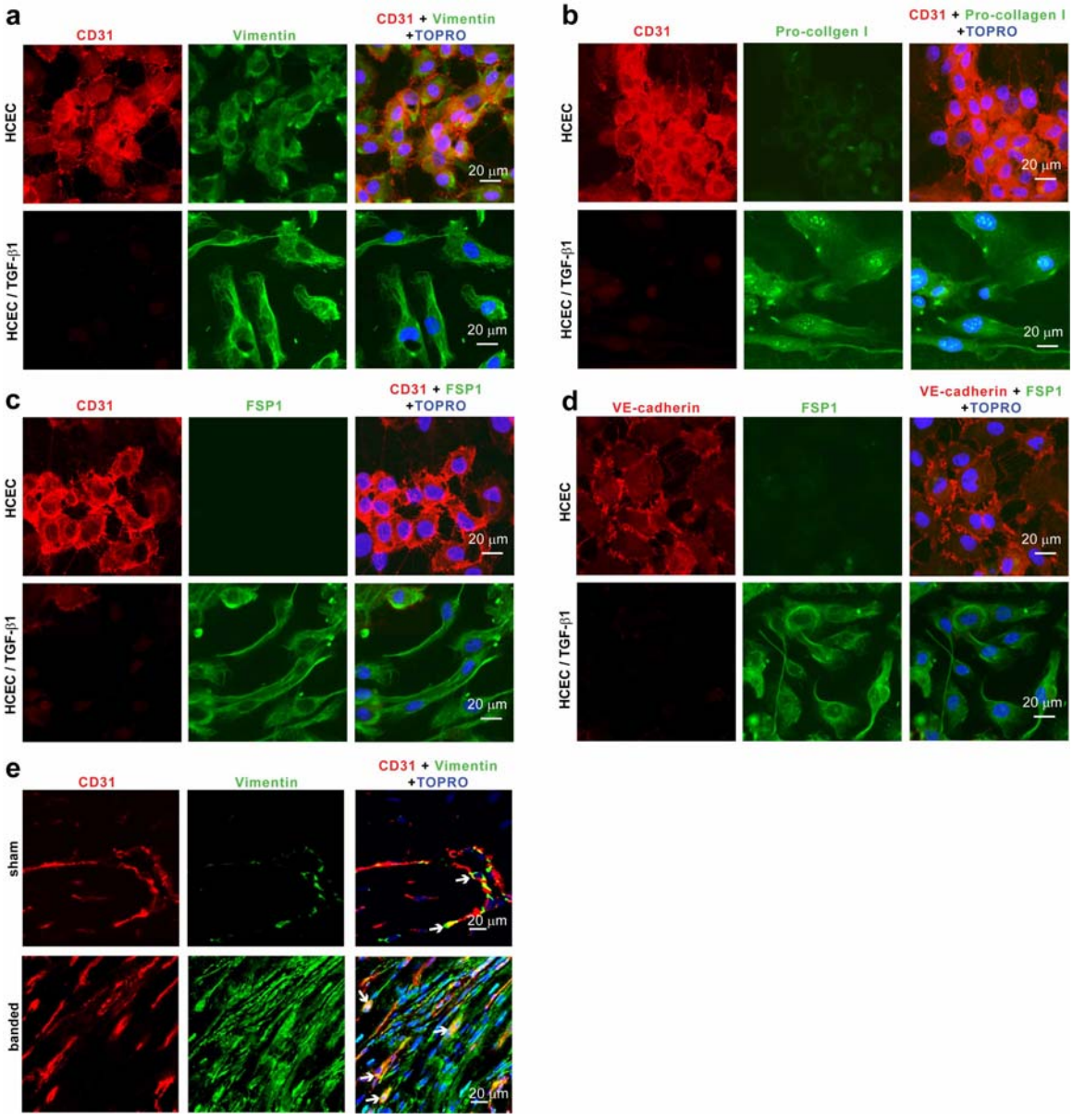
Supplementary Figure 1



EndMT in the embryonic heart. (**a**) The picture shows an AV canal explant of a 9.5 day old embryo, 24 hours after explantation. Endocardial cells migrate away from the explant and form spindle-shaped, mesenchymal cells (arrows). Phenotypic changes of adult human coronary endothelial cells (HCEC) treated with TGF-β1 resemble that of the embryonic cells. (**b**) A transverse section containing the heart with the AV cushion of a 12.5 day old *Tie1Cre;* R26Rstop*lacZ* embryo was analyzed by LacZ staining. The AV cushion expresses LacZ (blue), indicating its endothelial origin. The right picture shows a higher magnification of the boxed region. **(c)** We performed FSP1 and LacZ double-labeling to visualize EndMT. The left panel is equivalent to the right panel in **b**, except that the blue staining is presented as red pseudocolor. Simultaneous FSP1 immunofluorescence labeling (green) shows colocalization of LacZ and FSP1 (yellow), demonstrating that cells of endothelial origin have acquired a mesenchymal phenotype.

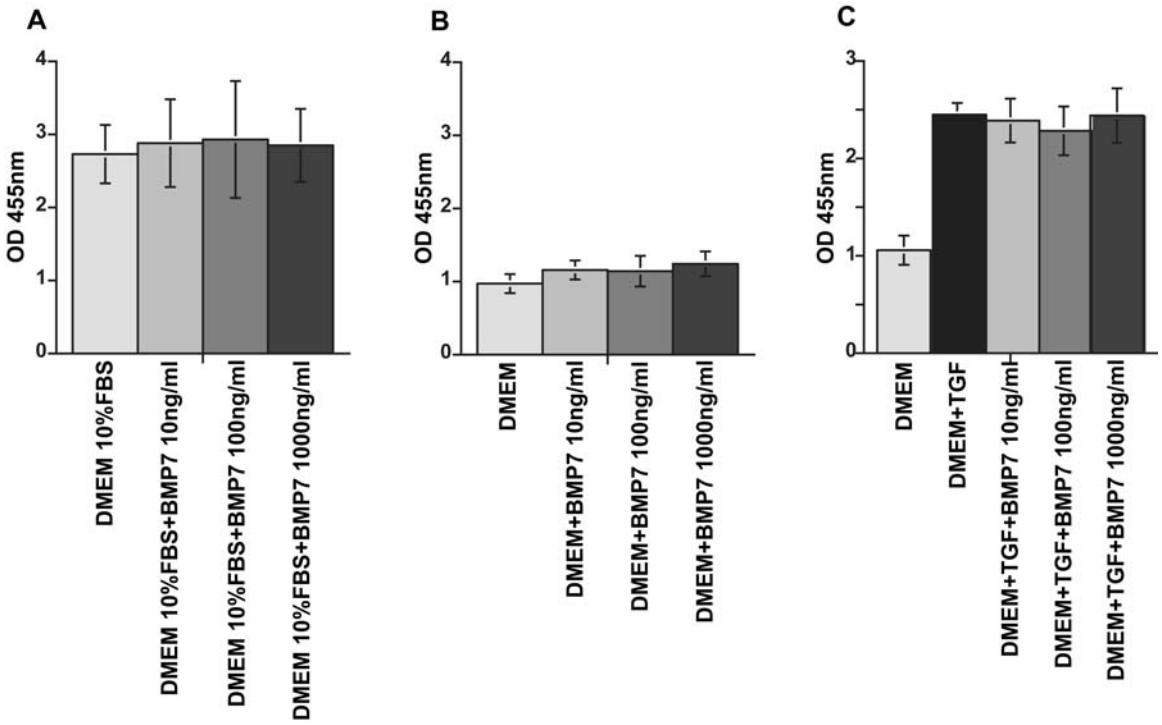
Supplementary Figure 2



Expression of proteins which are typically associated with fibroblasts. The pictures show confocal microscopy of immunofluorescence double labeling for CD31 (red), VE-cadherin (red), vimentin (green), FSP1 (green) and pro-collagen type I (green). The right picture in each panel shows the respective merged images of red, green and TOPRO blue nuclear staining. Basal expression of vimentin was detected in untreated HCEC (**a**), but was stronger in the spindle-shaped fibroblasts in TGF-β1-treated HCEC (**a**). FSP1 and pro-collagen type I were only expressed after treatment of HCEC with TGF-β1 (**b,c**), and were absent in untreated HCEC. (**d**) VE-cadherin expression (red) was lost in TGF-β1 treated

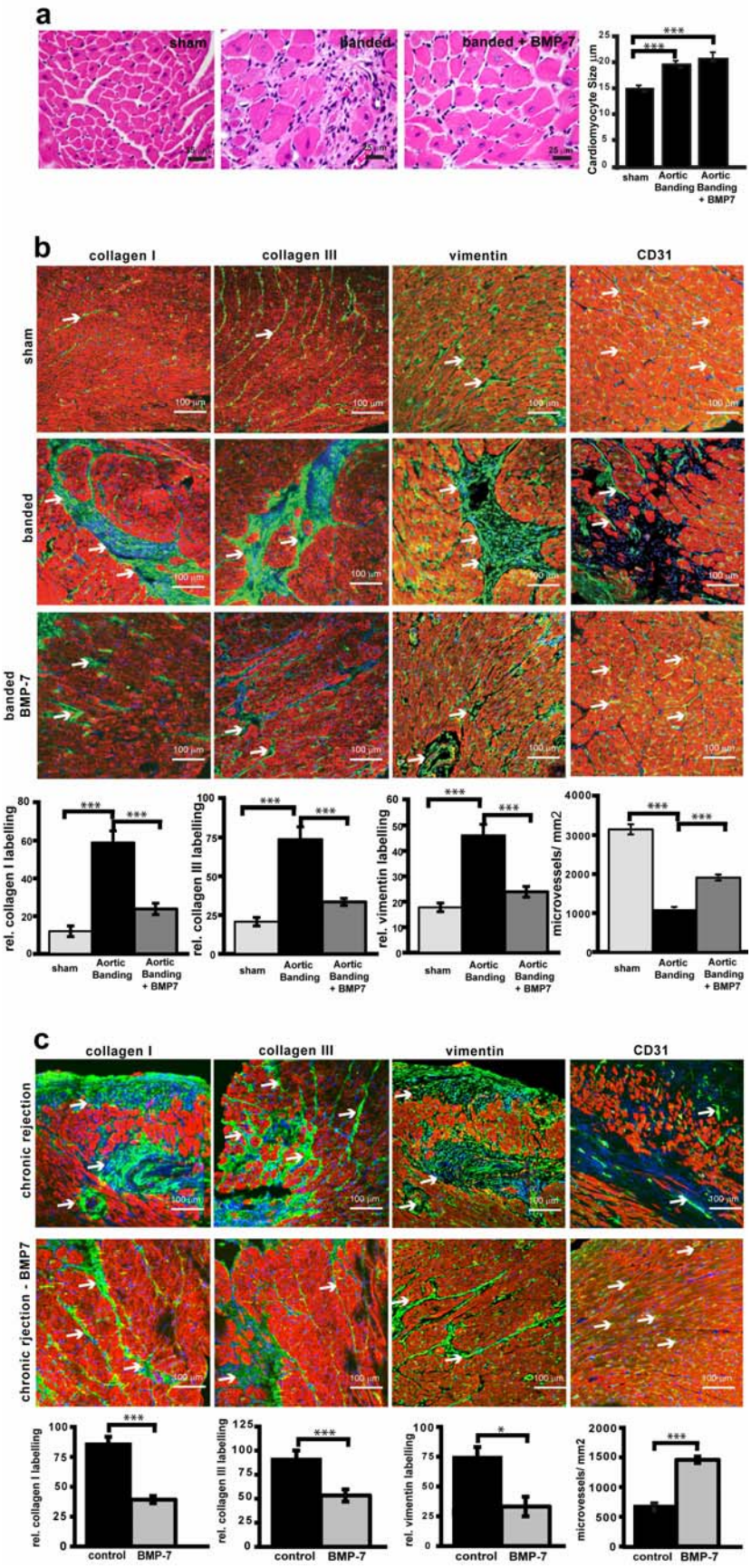
HCEC. (**e**) We performed double labelling experiments of hearts from sham-operated mice and from mice with aortic banding using antibodies for CD31 and vimentin. Vimentin and CD31 double-stained cells are presented in sham-operated control hearts, but are significantly increased in fibrotic hearts.

Supplementary Figure 3



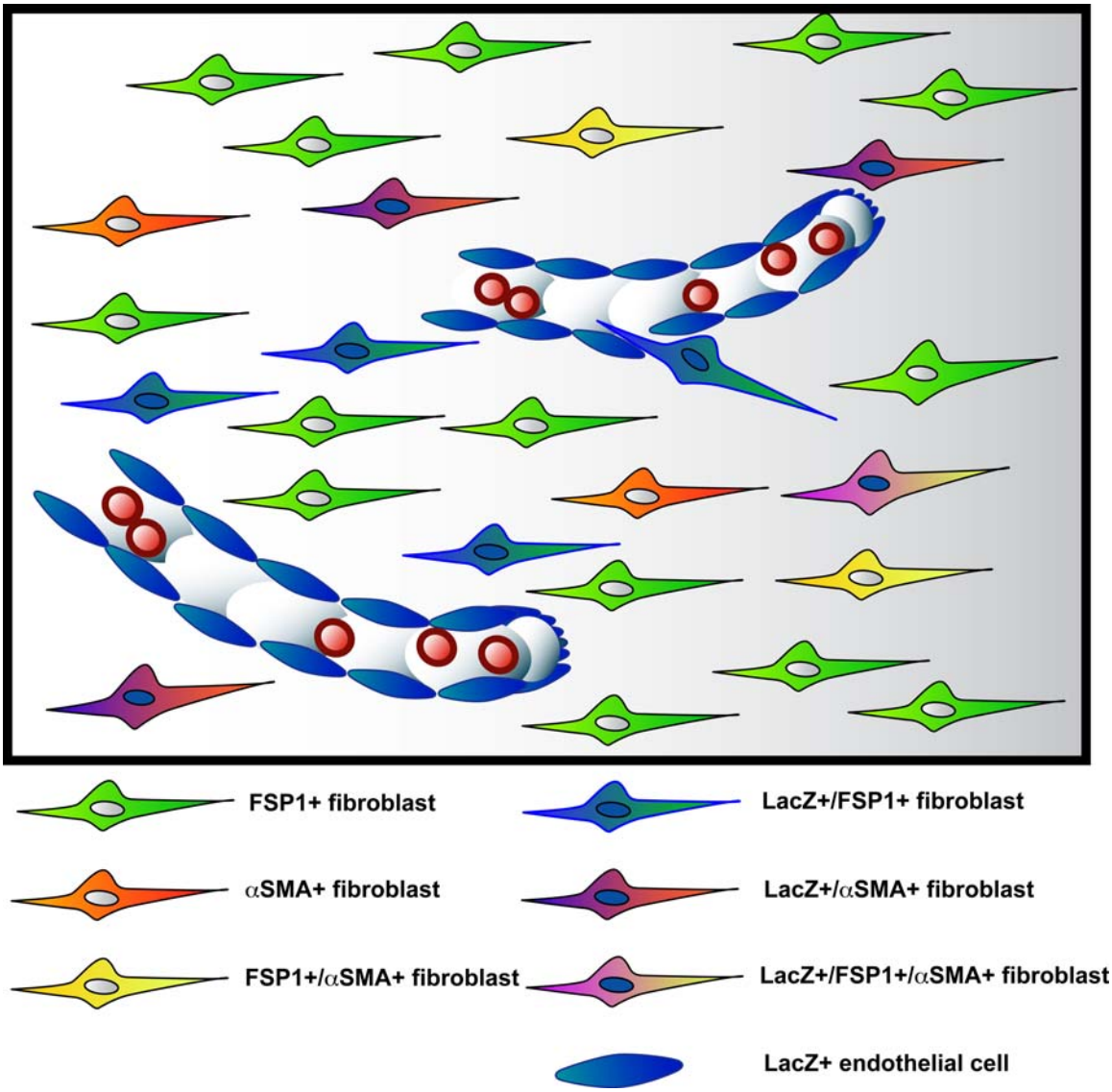
rhBMP-7 does not affect proliferation of cardiac fibroblasts. Primary fibroblasts were isolated from mouse hearts. **(a)** Fibroblasts were cultured in DMEM which contained 10% FBS. The media contained BMP-7 at concentrations of 0, 10, 100 and 1000 ng/ml. Cell proliferation was assessed by the WST assay as performed in our previous publications. BMP-7 did not significantly inhibit fibroblast proliferation. (**b)** Primary cardiac fibroblasts were maintained for 48 hrs in serum-free DMEM, before media was replaced with fresh serum-free DMEM containing BMP-7 at concentrations from 0-1000 ng/ml. BMP-7 did not significantly induce fibroblast proliferation. (**c)** BMP-7 was added to media which also contained 10 ng/ml TGF-β1.Presence of TGF-β1 induced fibroblast proliferation. Presence of BMP-7 had no significant impact on TGF-β1 induced fibroblast proliferation. Experiments were performed in quadruplicate and were repeated two times. The bar graphs summarize average optical density reading of representative experiments.

Supplementary Figure 4



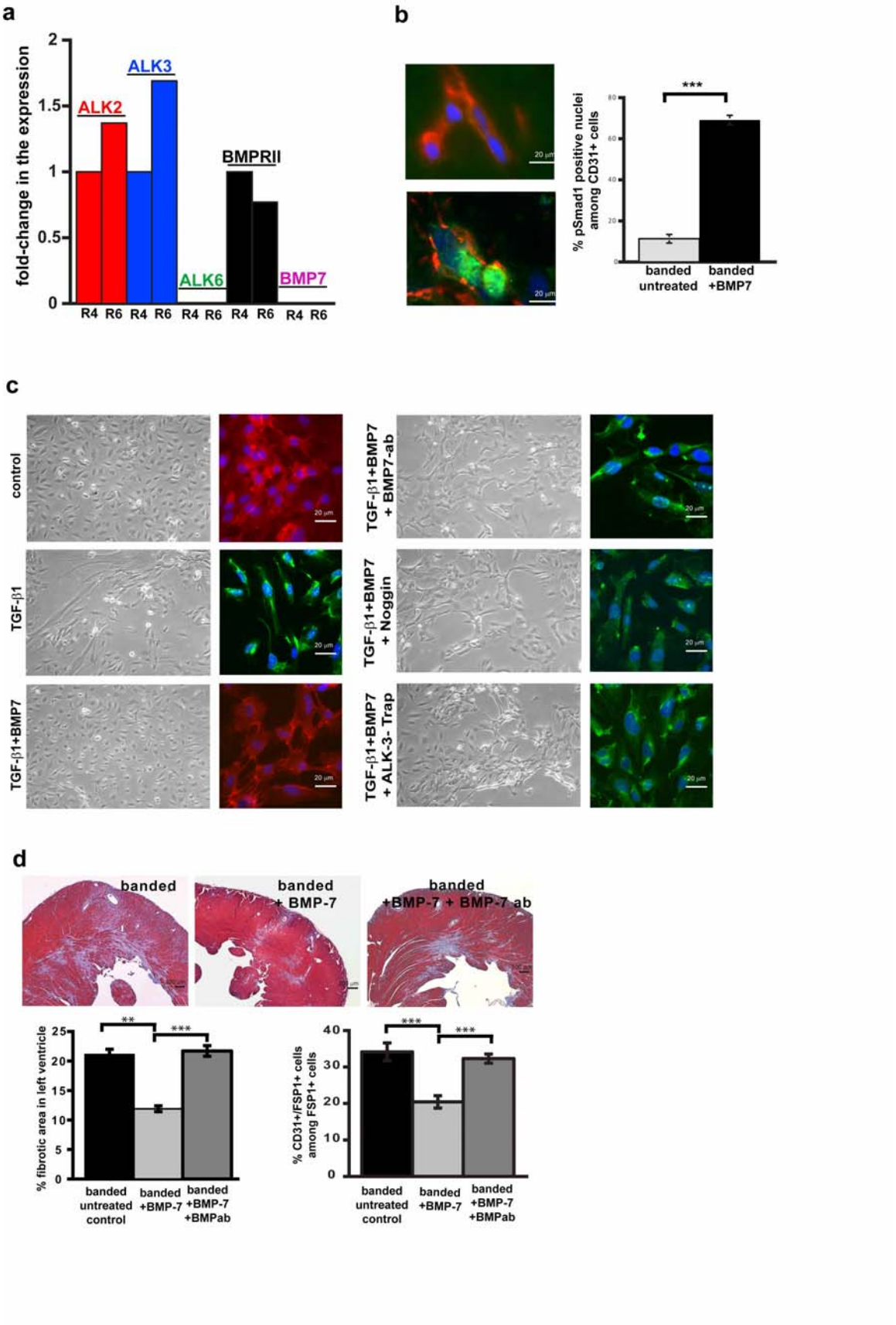
rhBMP-7 attenuates fibrosis in two mouse models of cardiac fibrosis. (a) Cardiomyocyte size was assessed by measuring the cell diameter on cross sections of hematoxylin and eosin stained hearts from sham operated, banded vehicle-administered, and banded BMP-7-administered mice. Quantitative analysis revealed a significant increase in cell size between the sham and both banded groups (vehicle-treated and BMP-7-treated) with negligible difference between BMP-7 and vehicle-treated mice. The bar graph summarizes average cardiomyocyte size of each group. (**b**) Tissue sections of sham-operated hearts, banded vehicle-treated hearts and banded hearts of mice treated with rhBMP-7 were stained with antibodies to desmin (red) and antibodies to either collagen type I, collagen type III, vimentin or CD31 (each in green). The pictures display representative photomicrographs of each group. The labeling for Type I collagen, type III collagen and vimentin was quantified using ImageJ software. Treatment with rhBMP-7 led to a significant reduction of staining for collagen type I, collagen type III and vimentin. We assessed the microvascular density in sham-operated hearts, banded, vehicle-treated hearts and BMP-7 treated banded hearts. The microvascular density was significantly reduced in banded hearts as compared to sham-operated hearts. Microvascular density was significantly improved in rhBMP-7 treated mice compared to vehicle-treated banded mice. The bar graphs display average values for each group. (**c**) The pictures display representative photomicrographs of heart sections from vehicle-treated transplanted mice and transplanted mice treated with rhBMP-7, stained with antibodies to desmin (red) and antibodies to either collagen I, collagen type III, vimentin and for CD31 (each in green). Digital imaging analysis demonstrates significant decrease in collagen type I staining, collagen type III staining and vimentin staining in mice which were administered with rhBMP-7 as displayed in the bar graphs. Morphometric analysis of vascular density revealed increased microvascular density in the rhBMP-7-administered mice compared to the transplanted vehicle-treated mice. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Supplementary Figure 5



Schematic illustrating EndMT. This schematic is based on the results of double stainings for FSP1/ α-SMA, FSP1/ LacZ and α-SMA/ LacZ in fibrotic hearts of *Tie1Cre;* R26Rstop*lacZ* mice. The schematic reflects that there are more FSP1-positive (green) than α-SMA-positive (red) fibroblasts (77 % vs. 31 %), with only a minor overlap between the markers (yellow). Endothelial cells (LacZ+, here shown in blue) contribute to both FSP1- and α-SMA-positive fibroblasts. EndMT-derived FSP1-positive fibroblasts are illustrated in green and blue, EndMT-derived α-SMA-positive fibroblasts are illustrated in red and blue. About 75 % of all α-SMA-positive fibroblasts are EndMT-derived, and 15 % of all FSP1-positive fibroblasts are of endothelial origin. Based on these data we estimate that 27-35 % of all (α-SMA- or FSP1-positive) fibroblasts are of endothelial origin (see also supplementary note 1).

Supplementary Figure 6



BMP-7 acts on endothelial cells undergoing EndMT. (a) LacZ/CD31 double positive cells (sorted from gate R4 in Figure 1f) and LacZ-positive/ CD31-negative cells (sorted from gate R6 in Figure 1f) were analyzed by quantitative real-time PCR for expression of BMP-receptors ALK2, ALK3, ALK6 and BMPR-II as well as BMP-7. **(b)** The pictures display immunofluorescence double staining for CD31 (red) and phosphorylated Smad1 (green) of a vehicle-treated (upper picture) and a BMP-7 treated heart (bottom picture). Nuclei are stained with TOPRO-3. Co-localization of CD31 and pSmad1 suggests that BMP-7 acts via Smad1 signalling in endothelial cells. The bar graph summarizes the percentage of pSmad1-postive nuclei within CD31-positive cells in each group. **(c)** HCEC were treated with TGF-β1 for 7 days. Cellular phenotype was assessed by light microscopy and immunofluorescent double-labeling using antibodies to CD31 (red) and FSP1 (green). Control HCEC display robust CD31 staining in absence of FSP1 staining. TGF-β1 induces acquisition of a spindle-shaped morphology associated with loss of CD31 staining and *de novo* FSP1 expression. Addition of rhBMP-7 neutralizes the effect of TGF-β1. HCEC containing TGF-β1, BMP-7 plus BMP-7 antibodies display a spindle-shaped morphology associated with loss of CD31 expression and increased FSP1 expression. Addition of recombinant Noggin to media containing both TGF-β1 and BMP-7 is associated with loss of CD31 staining and gain of FSP1 staining. Addition of soluble Alk3-Fc chimera to media containing TGF-β1 and BMP-7 neutralizes BMP-7 activity. **(d)**. Following aortic banding, mice either received vehicle buffer (banded vehicle-treated), rhBMP-7 (banded BMP-7) or both rhBMP-7 and BMP-7 neutralizing antibodies (banded rhBMP-7 plus BMP-7 ab). The pictures display representative Masson trichrome- stained sections of each group. The bar graphs summarize the morphometric analysis of the fibrotic area in each group and the effect of rhBMP-7 treatment and its neutralization with BMP-7 antibodies on cells that stain positive for both CD31 and FSP1. ** $p \leq 0.01$, *** $p \leq 0.001$.

Supplementary Table 1

	Sham	(n=5)	Banded Control	(n=8)	Banded rhBMP-7	(n=8)
	1 week	**4 weeks**	**1 week**	**4 weeks**	**1 week**	**4 weeks**
EDD, mm	3.09±0.14	3.11±0.16	2.79±0.13	2.89±0.09	2.72±0.14	3.21±0.15
ESD, mm	1.65±0.10	1.63±0.16	1.45±0.11	1.52±0.10	1.43±0.18	1.80±0.04
FS, %	47±2	48±3	48±2	48±2	48±4	44±2
LVPW, mm	0.79±0.04	0.79±0.04	0.95±0.05*	1.08±0.03*	1.00±0.07*	0.96±0.06
HR (bpm)	548±37	505±44	501±19	511±12	516±16	520±27
Aortic pressure gradient, mmHg	6±1 (n=3)	n.a.	23±3*	n.a.	20±3*	n.a.

Echocardiography Data. Results are presented as means ± SEM. EDD left ventricular end-diastolic diameter; ESD left ventricular end-systolic diameter; FS fractional shortening; LVPW, left ventricular posterior wall thickness; HR, heart rate; bpm, beats per minute; * $P<0.05$ versus sham.

Supplementary Table 2

	Forward primer	Reverse Primer	TaqMan probe
FSP1	TCAGGCAAAGAGGGTGACAAG	AGGCAGCTCCCTGGTCAGT	TCAAGCTGAACAAGACAGAGCTCAAGGAGC
DDR2	TTACTACCGGATCCAGGGCC	GCAAGATGCTTTCCCAGGAC	CGGTGCTCCCCATTCGCTGG
αSMA	CACTATTGGCAACGAGCGC	CCAATGAAGGAAGGCTGGAA	TCCGCTGCCCGGAGACCCT
Col1A1	ATGGATTCCCGTTCGAGTACG	TCAGCTGGATAGCGACATCG	AGCGAGGGCTCCGACCCCG
CD31	GCGCAGGACCACGTGTTAGT	CCTGCAATTTGAATCCGGAC	TTTCGCTGCCAAGCTGGGATCC
VE-Cad	GGTGGCCAAAGACCCTGAC	ACTGGTCTTGCGGATGGAGT	AGGCTCAGCGCAGCATCGGG
ALK-2	TGGCCTGACTGGTTGTCAGA	TTCCGTCAAAGCAGCCACT	CAGAATCCATCTGACCCCCTTCCCG
ALK-3	GGACATGCGTGAGGTTGTGT	CGCTGTTCCAGCGGTTAGAC	TGAAACGCTTGCGGCCAATCG
ALK-6	GCGGCCTATGCCATTTACAC	AGTCTCGATGGGCGATTGC	CGGAAATCTTTAGCACTCAAGGCAAGCC
BMPR-II	TCCACCTGGGTCATCTCCA	CCCTGTCACTGCCATTGTTG	CGAGCCGCTGGACTGTGAGGTCA
BMP-7	CCTCTGTTCTTGCTGCGCTC	AAGCTGGAGTGCACCTCGTT	CCCTGGCCGATTTCAGCCTGGA

Abbreviations:
FSP1 – fibroblast specific protein 1
DDR2 – discoidin domain receptor 2
αSMA – alpha smooth muscle actin
Col1A1 – type I collagen alpha1 chain
VE-cad – vascular endothelial cadherin
ALK-2 – activin-like kinase 2
ALK-3 – activin-like kinase 3
ALK-6 – activin-like kinase 6
BMPR-II – bone morphogenic protein receptor II
BMP-7 – bone morphogenic protein-7

Supplementary Note 1

Quantitation of EndMT-derived fibroblasts

Number of **FSP1**-positive cells: A
Number of **α-SMA**-positive cells: B
Number of **FSP1/ α-SMA** double positive cells: C
Number of **LacZ/ FSP1** double positive cells: D
Number of **LacZ/ α-SMA** double positive cells: E
Number of **LacZ/ FSP1/ α-SMA** triple positive cells: F

FSP1/ α-SMA double staining yields three populations of cells: the FSP1-positive cells (A), the α-SMA-positive cells (B) and some of these cells which are FSP1/ SMA double positive (C). Among the combined populations of FSP1- and α-SMA-positive cells (A+B-C), 77% are FSP1-positive (A)…

I. $\frac{A}{A+B-C} = 0.77 \Rightarrow$ A+B-C=$\frac{A}{0.77}$

…and 31% are α-SMA-positive (B)…

II. $\frac{B}{A+B-C} = 0.31 \Rightarrow$ B=0.31x(A+B-C) $\Rightarrow$ (replace A+B-C=$\frac{A}{0.77}$ from equation I)

B=0.31 x $\frac{A}{0.77} \Rightarrow$ B= 0.40xA

…with an overlap of FSP1/ α-SMA double positive cells (C) of 8% among the combined populations of FSP1- and α-SMA-positive cells.

III. $\frac{C}{A+B-C} = 0.08 \Rightarrow$ C=0.08 x (A+B-C) $\Rightarrow$ C=0.08xA+0.08xB-0.08xC $\Rightarrow$

1.08xC=0.08xA+0.08xB $\Rightarrow$ (Replace B=0.40xA from equation II)
1.08xC=0.08xA+0.08x0.40xA $\Rightarrow$ 1.08xC=0.112A $\Rightarrow$ C=0.104xA

In the LacZ/FSP1 double staining 15% among all FSP1-positive cells (A) are double positive for FSP1 and LacZ (D)

IV. $\frac{D}{A} = 0.15 \Rightarrow$ D= 0.15xA

In the LacZ/ α-SMA double staining 75% among all α-SMA -positive cells (B) are double positive for α-SMA and LacZ (E)

V. $\frac{E}{B} = 0.75 \Rightarrow$ E= 0.75xB $\Rightarrow$ (replace B=0.40xA from II) E= 0.75x0.40xA $\Rightarrow$ E=0.3xA

The number of LacZ/ FSP1/ α-SMA triple positive cells (F) is unknown; however the population must be smaller than any of the following double positive populations: LacZ/ FSP1 (D=0.15xA), LacZ/ α-SMA (E=0.30xA) or FSP1/ α-SMA (C=0.104xA). Since the FSP1/ α-SMA double-positive population (C) is the smallest, we can follow that

VI. $\frac{F}{A+B-C} \leq \frac{C}{A+B-C}$

Replace $\frac{C}{A+B-C}$ by 0.08 (from equation III)

$\frac{F}{A+B-C} \leq 0.08$

We are interested in the number of LacZ/ FSP1double positive cells (D) plus the number of LacZ/ α-SMA (E) double positive cells out of both the FSP1- and the α-SMA-positive populations (A+B-C), but we need to reduce this number by the LacZ/ FSP1/ α-SMA triple positive cells (F) to avoid counting these cells in duplicate.

X= $\frac{D}{A+B-C} + \frac{E}{A+B-C} - \frac{F}{A+B-C}$

Replace A+B-C= $\frac{A}{0.77}$ (from equation I):

X= $\frac{0.77xD}{A} + \frac{0.77xE}{A} - \frac{F}{A+B-C}$

Replace D= 0.15xA (from equation IV) and E=0.3xA (from equation V):

X= $\frac{0.77x0.15xA}{A} + \frac{0.77x0.3A}{A} - \frac{F}{A+B-C}$

X=0.12+0.23- $\frac{F}{A+B-C}$

X= 0.35- $\frac{F}{A+B-C}$

From equation VI we know that $0 \leq \frac{F}{A+B-C} \leq 0.08$

$\Rightarrow$X≥ 0.35 – 0.08 and X≤ 0.35

X≥0.27 and X≤ 0.35

This means that at least 27% but less than 35% of both the combined FSP1- and the α-SMA-positive fibroblast populations are also LacZ-positive.