Supporting Information

Application of Small Organic Molecules Reveals Cooperative TGFβ and BMP Regulation of Mesothelial Cell Behaviors

Emily E. Cross[1], Rebecca T. Thomason[1], Mitchell Martinez[1], Corey R. Hopkins[2], Charles C. Hong[1,3,4,5*], and David M. Bader[1,3, 5*]

[1] Department of Cell and Developmental Biology

[2]Department of Pharmacology, Chemistry and Program in Drug Discovery

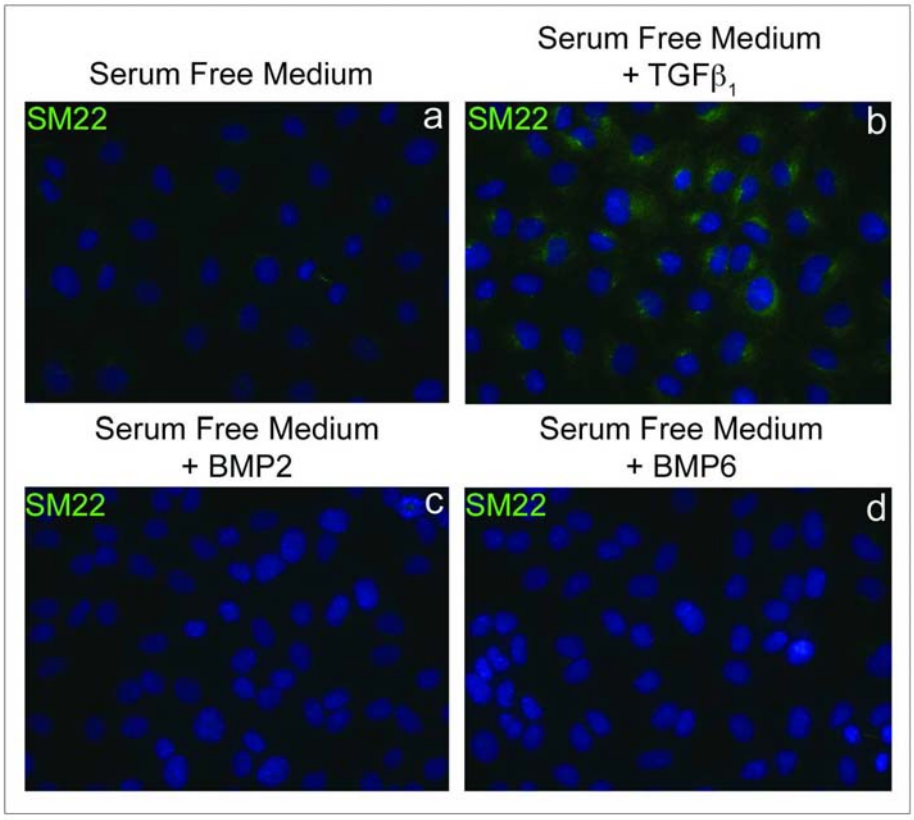
[3]Department of Medicine, Division of Cardiovascular Medicine

[4]Department of Pharmacology, Research Medicine, Veterans Affairs TVHS, Nashville TN 37212

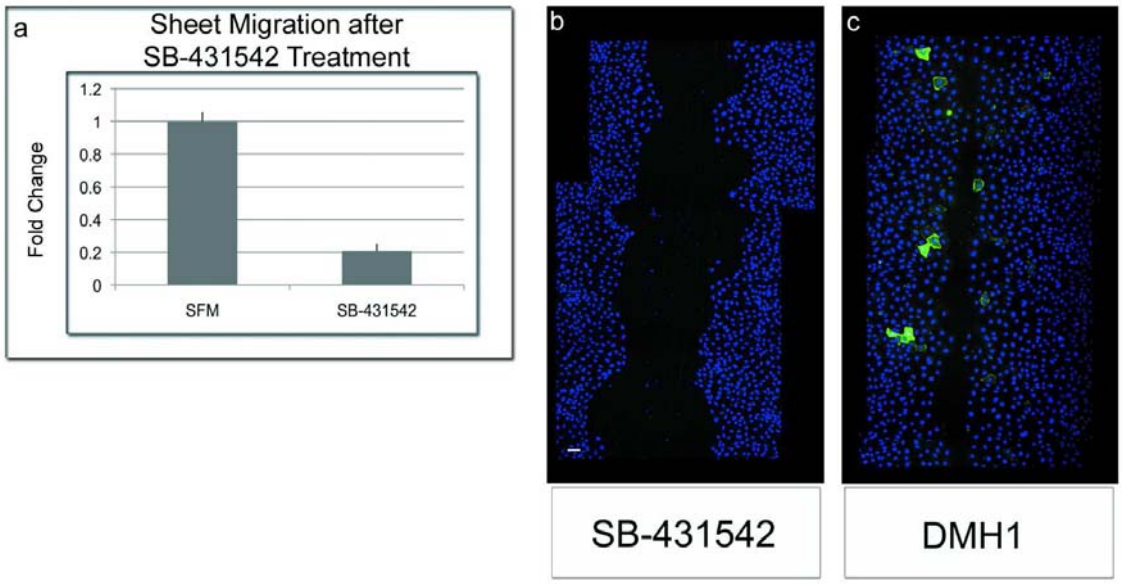
Vanderbilt University

School of Medicine

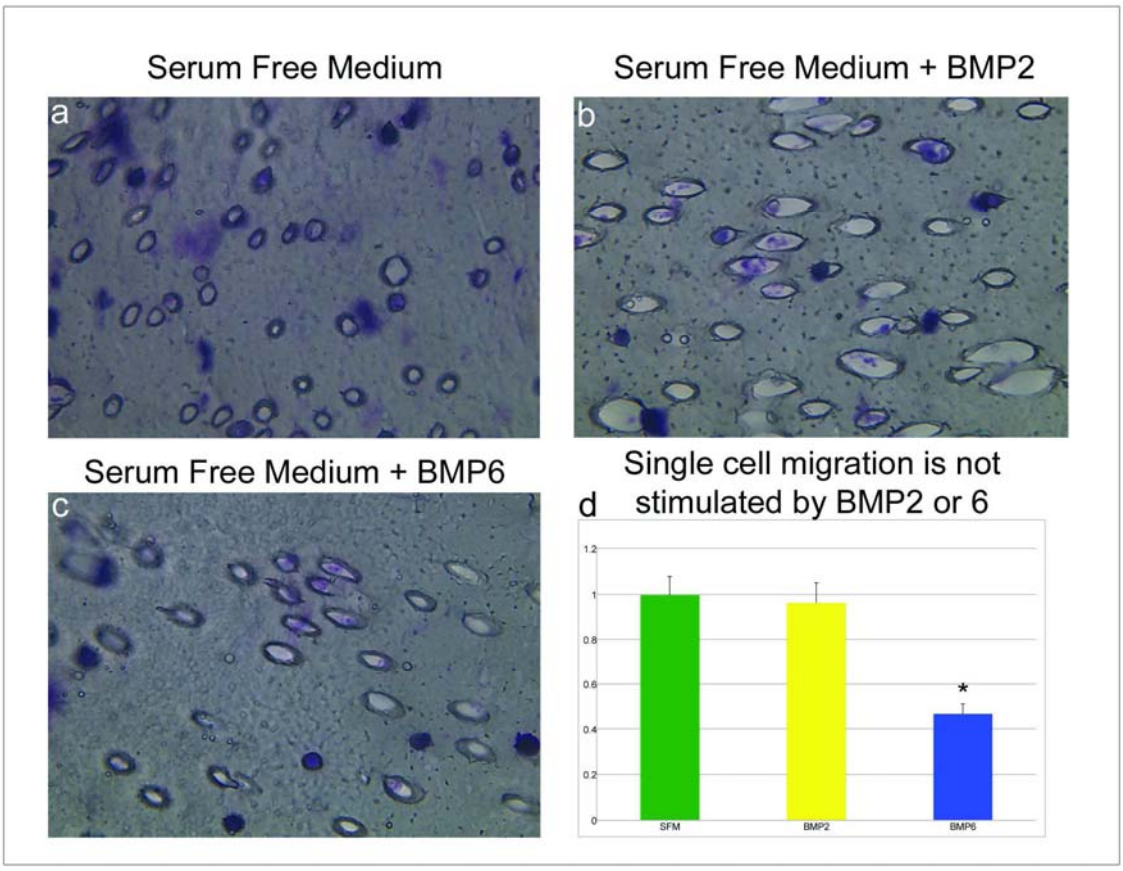
Nashville, TN 37232



Supplementary Figure S1. **BMP2 and 6 do not stimulate SM22 expression.** a) Epicardial cell cultures maintained in serum free medium did not express SM22 protein, b) while those treated with $TGF\beta_1$ had a strong induction in SM22 expression. Conversely, 20ng/mL of (c) BMP2 or (d) BMP6 did not induce SM22 expression in epicardial cells.



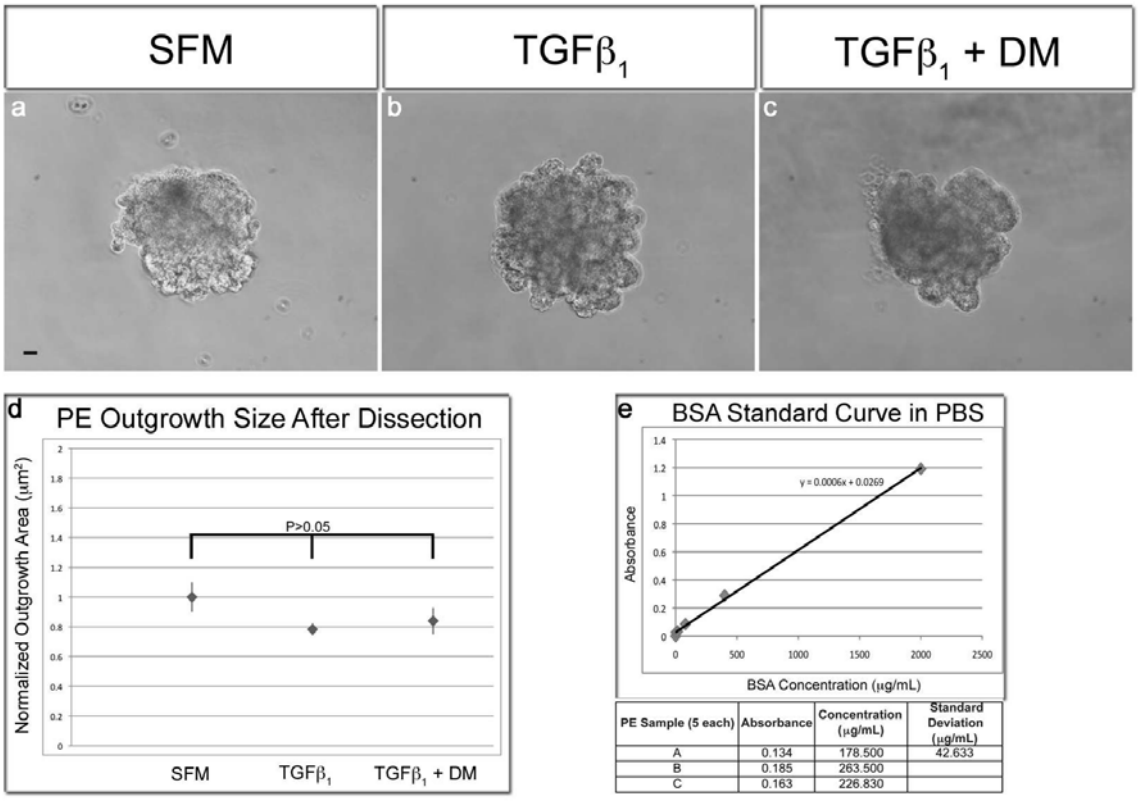
Supplementary Figure S2. SB-431542 treatment strongly inhibits epicardial sheet migration and SMA expression at the leading edge, while DMH1 inhibits only sheet migration. a) Treatment of epicardial sheets with SB-431542 strongly inhibited wound closure rate when compared to the SFM control. b) Additionally, all wounds failed to close after 18 hours in culture and did not exhibit expression of SMA at the leading edge with SB-431542 treatment. c) Similarly, sheets treated with DMH1 frequently failed to close, but maintained SMA expression at the leading edge, illustrating a similarity in ALK2/3 and TGFβ regulation of sheet migration, but a divergence in regulatory responsibilities of these cascades on SMA expression. [(*p≤0.001 from SFM); n=4 wounds.]



Supplementary Figure S3. BMP2 and 6 do not stimulate epicardial cell single cell migration. a) Epicardial cells maintained in serum free medium did not undergo significant single cell, random migration through filter membranes. Addition of (b) BMP2 or (c) BMP6 was insufficient to stimulate epicardial single cell migration. d) Single cell migration of BMP2 and 6 treated epicardial cells was quantified and normalized to the negative control. BMP2 treatment was statistically similar to untreated epicardial cultures ($p=0.777$). BMP6 treatment significantly decreased single cell migration rate compared to the negative control [(*$p<0.0001$); n=45 images from 3 experiments.]

Primers Used for RT-PCR		
Primer Name	**Sequence 5' → 3'**	**Product Size (bp)**
BMP1 Fwd	TCACCTACCGACCCTGCGGG	842
BMP1 Rvs	GCTGGGCCGGTAATCGTCGG	
BMP2 Fwd	TCACCCCGGCTGTGATGCGA	558
BMP2 Rvs	ACCCGCAACCCTCCACAACC	
BMP3 Fwd	CCCATAGCGGCCGGGTGTTC	441
BMP3 Rvs	ACCACCTGTCATGCGGTCGC	
BMP4 Fwd	GGAGGCGCGAGCCATGCTAG	814
BMP4 Rvs	CCCTCCGGCGGGTCAAGGTA	
BMP6 Fwd	CGGCGTCGACAGCAGAGTCG	305
BMP6 Rvs	GGTGCGCAGCATGGTTTGGG	
BMP7 Fwd	TCCCCAGCTGGAAGCGTGCA	468
BMP7 Rvs	GCCCTGCAGCCTCAGGAGAAT	
GAPDH Fwd	AGTGGTGAAGGTCGGTGTGAAC	1000
GAPDH Rvs	TTACTCCTTGGAGGCCA TGTAGG	

Supplementary Table S4. A list of the primers used in this study for qualitative RTPCR. Primer design and ABI primers for Quantitative RT-PCR are listed in the Methods.



PE Sample (5 each)	Absorbance	Concentration (μg/mL)	Standard Deviation (μg/mL)
A	0.134	178.500	42.633
B	0.185	263.500	
C	0.163	226.830	

Supplementary Figure S5. PE explants are comparable in size. Two methods were used to ensure that equally sized PE explants were used for analysis. First, the size of selected isolates was measured after two hours incubation from phase images taken at Vanderbilt CISR. Next, total protein in PE isolates was determined as a measure of explant size. Selected PEs were excised into 100DL ice cold PBS and spun for 10 minutes in a tabletop centrifuge at 1500 RPM after which the supernatant was removed. A BCA assay was performed on total protein content of three groups of five PEs. Total protein was calculated from a BSA standard curve performed using a colorimetric BCA protein assay kit (Pierce 23225). PEs were cultured in SFM, $TGF\beta_1$, or $TGF\beta_1$/DM mediums. a-d) PEs were imaged and measured for total area after 2 hours incubation to allow the PEs to settle on the slides. PEs were not statistically different in total area after culturing ($p>0.05$). e) Total protein content of unincubated PEs was also calculated and 3 groups of PEs were found to have similar concentration, with a standard deviation of 42.5 Dg/mL. For PE imaging and total protein content, 5 PEs were measured for each condition or group, respectively.

Supplementary Information. Antibodies and dilutions used in this study. Commercially available antibodies included: anti-α-SMA, (Sigma 2547, 1:200), anti-ZO- 1 (Zymed 61-7300, 1:200), anti-SM22, (Abcam, ab28811, 1:200), anti-cytokeratin, (Abcam, ab9377, 1:200), anti-WT-1, (Abcam, ab52933, 1:100), anti-vimentin, (Sigma, V 6630, 1:50) and anti-SM-MHC (Biomedical Technologies BT-562, 1:200) anti-Phospho- β-catenin (Cell Signaling, 9566, 1:500), Phospho-Smad1/5, Smad5, Smad3, Phospho- Smad3 (Cell Signaling, 9516, 9517, 1:1000 WB, 1:50 IF), anti-β-Catenin (BD Transduction Laboratories, 610154, 1:500),