de Jesus Perez et al., http://www.jcb.org/cgi/content/full/jcb.201008060/DC1

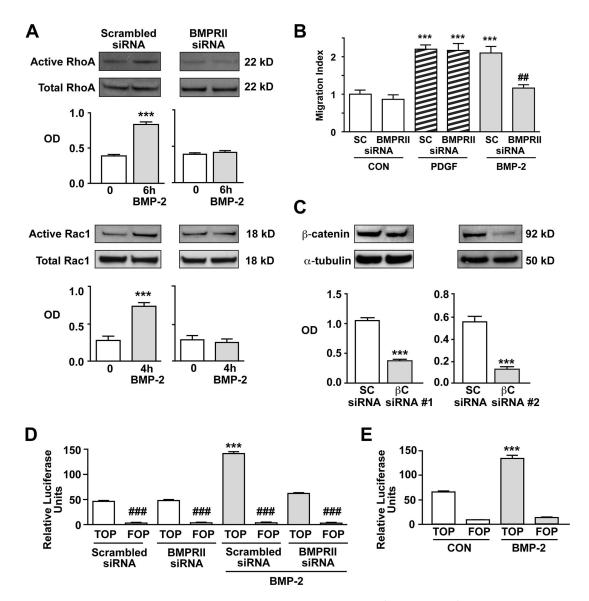


Figure S1. BMPRII knockdown reduces BMP-2-mediated motility and RhoA-Rac1 activation of hPASMCs transfected with either scrambled or BMPRII-specific siRNAs. (A and B) BMPRII knockdown reduces BMP-2-mediated RhoA (top) and Rac1 (bottom) activation (A) and motility in VSMCs (B). Densitometry values are shown relative to total RhoA and Rac1 in whole cell lysates run in different gels. In B, ***, P < 0.0001 by one-way ANOVA with Bonferroni's. ***, P < 0.001 versus respective control as indicated in B. (C) The siRNA-mediated β C knockdown in hPASMCs using two independent β C siRNAs was measured by Western immunoblot analysis and compared with scrambled (SC) siRNA-transfected PASMCs. ***, P < 0.0001 as demonstrated by unpaired t test. (D and E) Topflash (TOP) activity in scrambled or BMPRII siRNA-transfected (D) or untransfected (E) hPASMCs treated with BMP-2. Measurements were expressed as relative luciferase units. Bars represent means \pm SEM from n = 3. ***, P < 0.0001 as demonstrated by unpaired t test in A and C. ***, P < 0.0001 versus baseline; and ***, P < 0.0001 versus control as indicated in B, D, and E using one-way ANOVA with Bonferroni's. CON, control. FOP, fopflash.

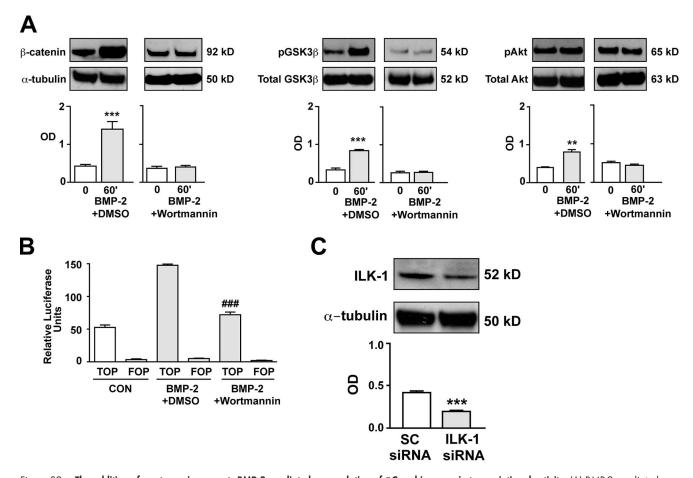


Figure S2. The addition of wortmannin prevents BMP-2-mediated accumulation of βC and increase in transcriptional activity. (A) BMP-2-mediated accumulation of BC, phosphorylation of GSK3B, and Akt are shown by Western immunoblotting and densitometry in the presence of 10 nM DMSO or the pAkt inhibitor wortmannin. (B) Transcriptional activity of βC in the presence of DMSO or wortmannin was measured in BMP-2-stimulated cells using the topflash [TOP] reporter assay. (C) The siRNA-mediated ILK-1 knockdown measured by Western immunoblotting and compared with scrambled (SC) siRNA-transfected hPASMCs. Bars represent means \pm SEM from n=3. **, P<0.001; and ***, P<0.001 in A and C using the unpaired t test. *##, P<0.0001 versus BMP-2 control (CON) as indicated in B using one-way ANOVA with Bonferroni's posttest. FOP, fopflash.

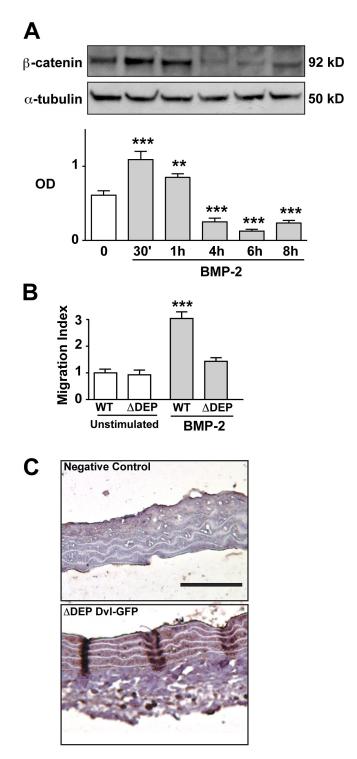
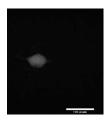


Figure S3. **BMP-2 requires** β C activation and Dvl to induce hAoSMC motility. (A) The β C levels were visualized by Western immunoblotting and quantified by densitometry. Densitometry values were expressed as the ratio of OD of β C relative to α -tubulin. (B) Motility studies using cells transfected with WT and Δ DEP Dvl were performed using the Boyden chambers. (C) Transfection of stented aortic grafts using sonoporation shows homogenous distribution of GFP-tagged construct. Representative images of grafts transfected either with vector only (top) or GFP-tagged Δ DEP (bottom). Paraffin-embedded tissue sections were incubated with an anti-GFP antibody followed by a biotin-labeled secondary antibody. Staining was obtained after addition of DAB for 3 min. Bar, 50 µm. Bars represent means \pm SEM from n = 3. **, P < 0.001; and ***, P < 0.0001 versus time 0 or unstimulated values using one-way ANOVA with Dunnett's (A) or Bonferroni's (B).



Video 1. hPASMCs transfected with WT DvI-GFP. QuickTime video showing hPASMCs transfected with WT DvI-GFP at baseline for 10 h (compare with cell treated with BMP-2 shown in Video 2). The WT DvI-GFP signal is shown in white. Frames were collected every 10 min (display rate is seven frames per hour). Bar, 10 µm.



Video 2. hPASMCs transfected with WT Dvl-GFP exhibit increased motility when exposed to BMP-2. QuickTime video showing hPASMCs transfected with Dvl-GFP in the presence of 10 ng/ml BMP-2 for 10 h (compare with untreated cells shown in Video 1). The WT Dvl-GFP signal is shown in white. Frames were collected every 10 min (display rate is seven frames per hour). Bar, 10 µm.



Video 3. **hPASMCs transfected with** Δ **DEP Dvl-GFP.** QuickTime video showing hPASMCs transfected with Δ DEP Dvl-GFP at baseline for 10 h (compare with cells treated with BMP-2 shown in Video 4). The Δ DEP Dvl-GFP signal is shown in white. Frames were collected every 10 min (display rate is seven frames per hour). Bar, 10 μ m.



Video 4. **hPASMCs transfected with** Δ **DEP fail to exhibit a motility response to BMP-2.** QuickTime video showing hPASMCs transfected with Δ DEP DvI-GFP in the presence of 10 ng/ml BMP-2 for 10 h (compare with untreated cells shown in Video 3). The Δ DEP DvI-GFP signal is shown in white. Frames were collected every 10 min (display rate is seven frames per hour). Bar, 10 μ m.