de Jesus Perez et al., http://www.jcb.org/cgi/content/full/jcb.200806049/DC1

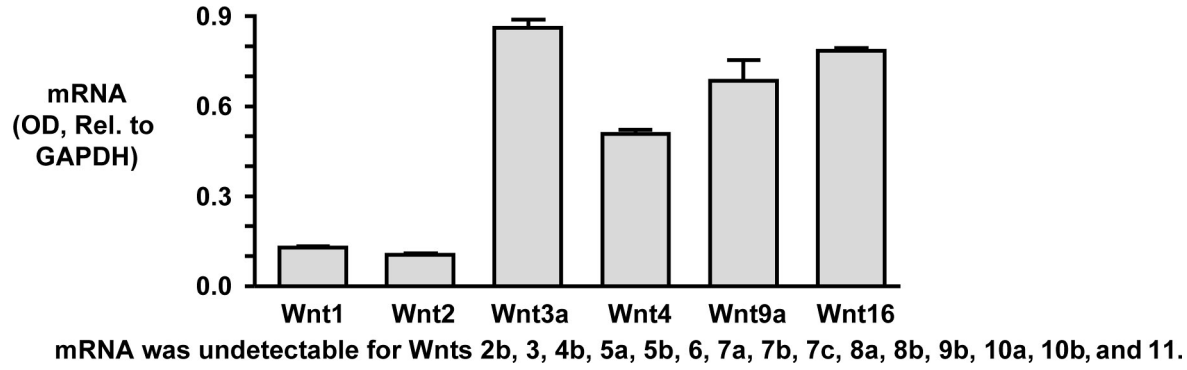

Figure S1. **Semiquantitative RT-PCR profile of Wnt genes in hPAECs.** Total RNA from unstimulated hPAECs was extracted and transcribed into cDNA followed by PCR reaction using primers against all known human Wnt genes. Values were normalized to levels of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Error bars denote mean ± SEM for three different experiments with triplicate assessments.

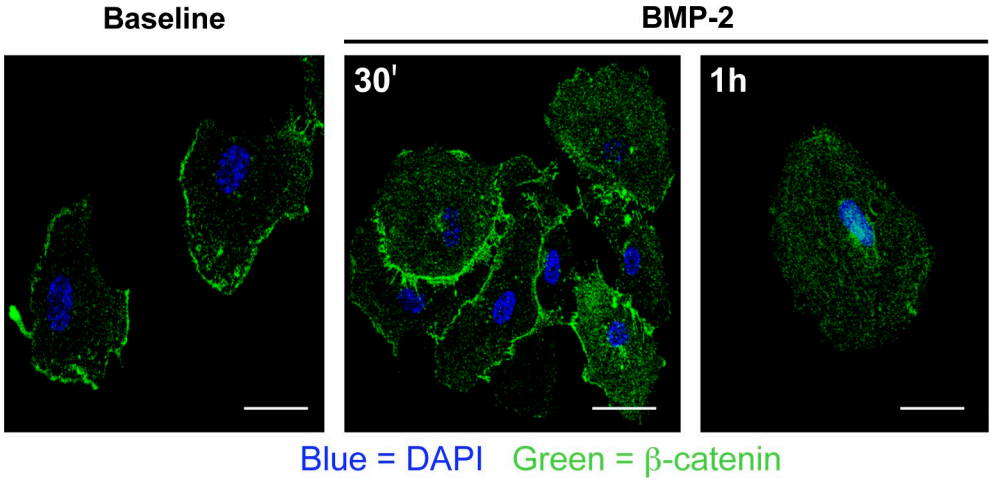

Figure S2. **BMP-2 induces nuclear accumulation of β-C in hPAECs.** Confocal images of hPAECs stimulated with 10 ng/ml BMP-2 for 1 h. After fixation and permeabilization, samples were incubated with primary antibody specific for β-C followed by the addition of Alexa Fluor 488–labeled secondary antibody (green) and DAPI (blue). Bars, 10 µm.

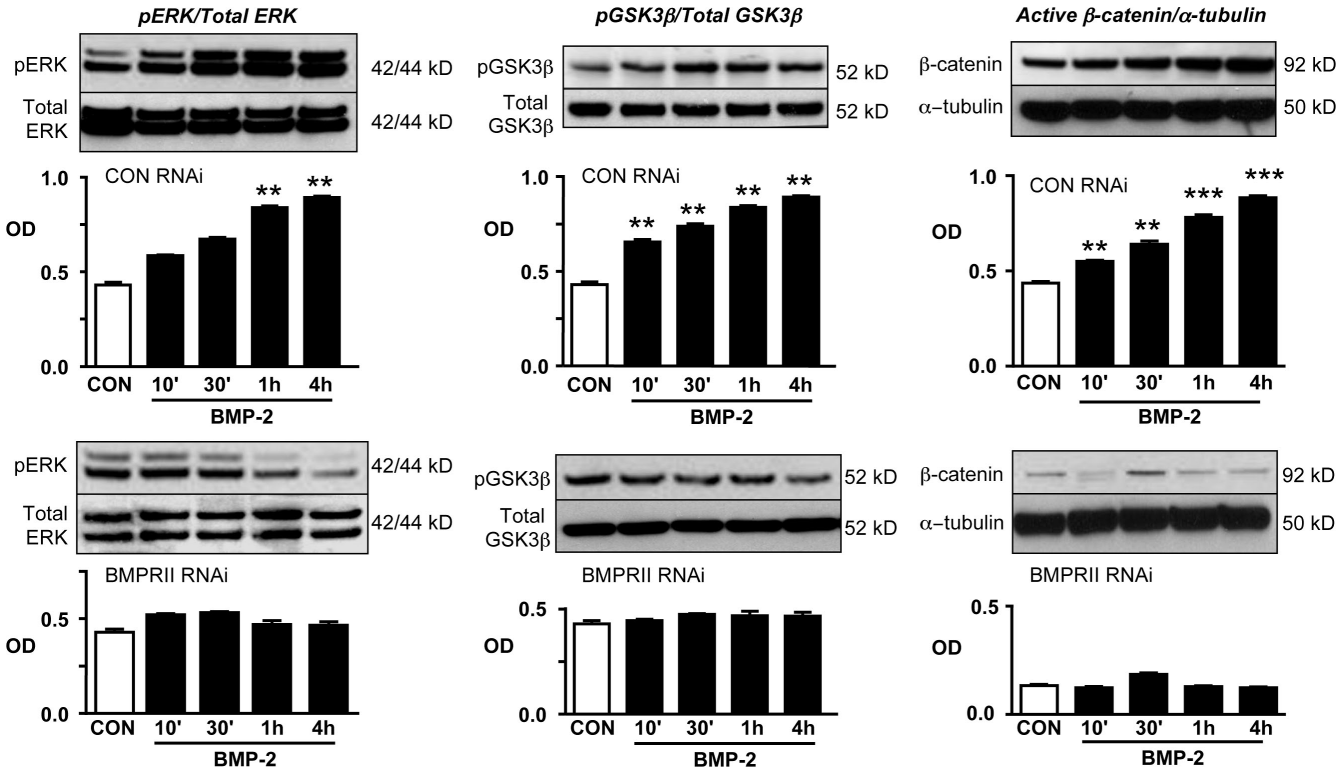

Figure S3. **BMPRII knockdown abrogates the ERK1/2-mediated response in BMP-2–stimulated hPAECs.** Western immunoblots of hPAECs nucleofected with nontargeting and BMPR2 siRNA. Membranes were probed for pERK1/2, total ERK1/2, phosphorylated and total GSK3-β, β-C, and α-tubulin. Error bars denote mean ± SEM for three different experiments performed in triplicate. **, $P < 0.001$ and ***, $P < 0.0001$ (vs. control [CON]).

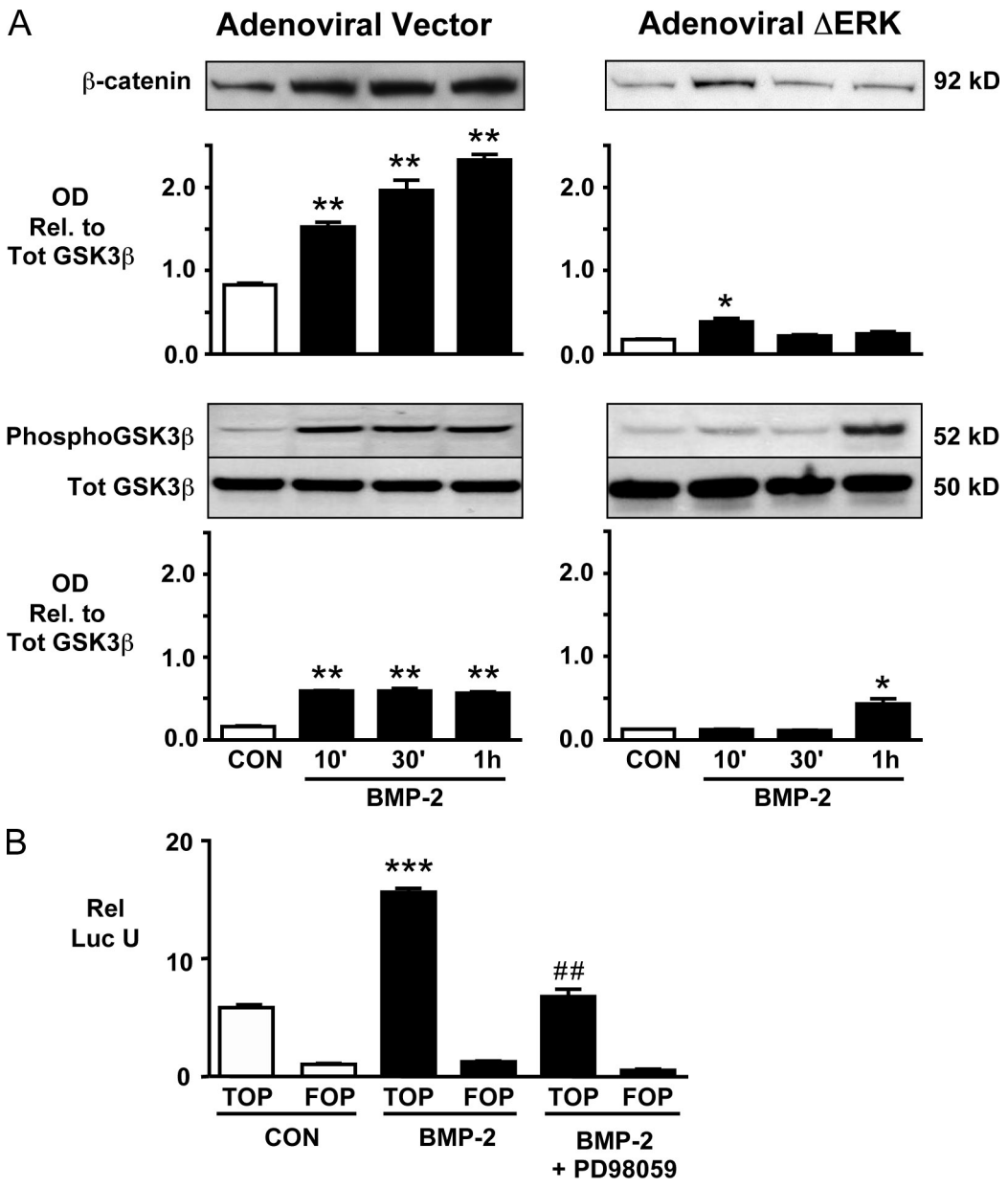

Figure S4. **Loss of BMP-2–mediated β-C accumulation and transcriptional activity in the presence of dominant-negative ERK.** (A) Transfection of dominant-negative ERK (ΔERK) prevents BMP-2–induced accumulation of β-C. Western Immunoblots of hPAECs transfected with adenovirus carrying either an empty plasmid or ΔERK construct followed by stimulation with 10 ng/ml BMP-2 for a period of 1 h. Immunoblots were probed for β-C and phosphorylated and total GSK3-β. Values were normalized to total GSK3-β. *, $P < 0.01$ and **, $P < 0.001$ (vs. control [CON]). (B) BMP-2–mediated TOPflash activity is reduced in the presence of PD98059. TOPflash activity as described in Fig. 1, with FOPflash as a negative control. Cells were starved for 24 h and stimulated with BMP-2 in the presence or absence of PD98059 as described in Fig. 4 A. ***, $P < 0.0001$ (vs. control TOPflash); ##, $P < 0.001$ (vs. BMP-2/TOPflash). Error bars denote mean ± SEM for three different experiments with triplicate assessments.

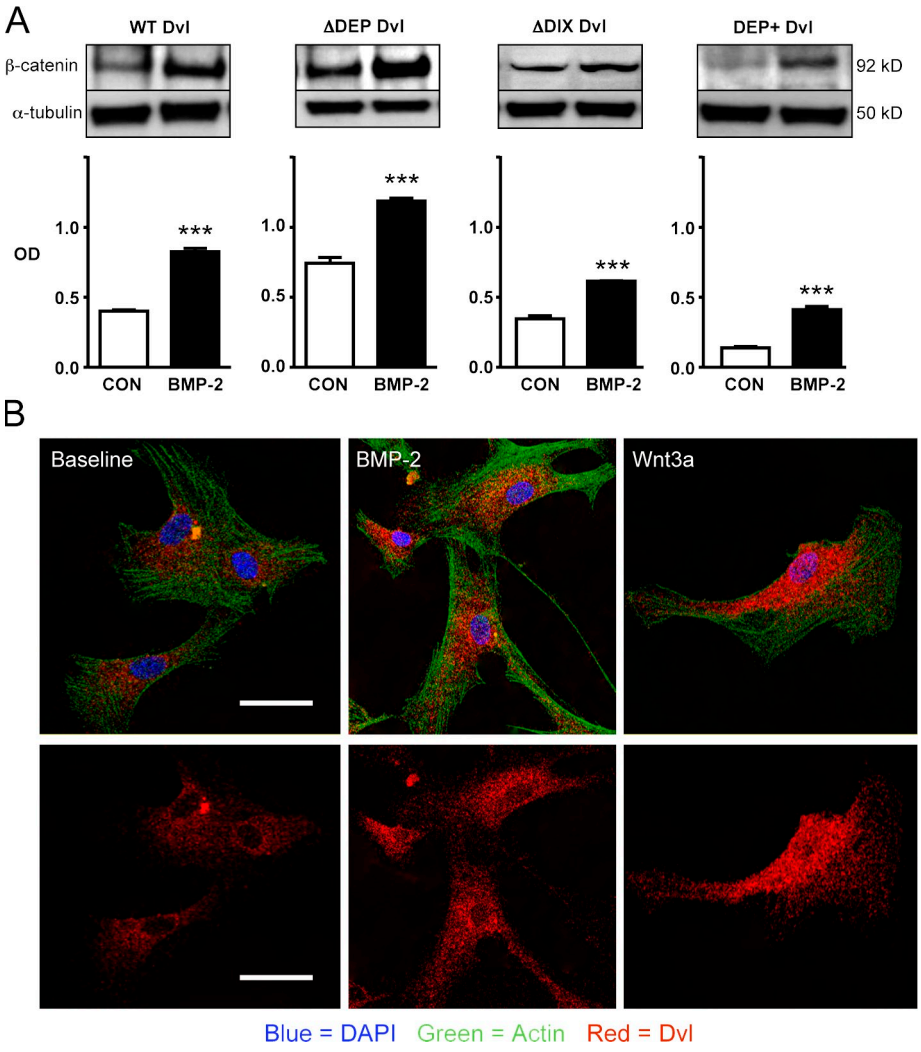

Figure S5. **The lack of impact of the Dvl mutant constructs on BMP-mediated β-C accumulation and the peripheral redistribution of Dvl in BMP-2– or Wnt3a-stimulated PAECs.** (A) Transfection of the various Dvl constructs does not interfere with BMP-2–induced accumulation of β-C in hPAECs. Immunoblots were probed for β-C and α-tubulin. Values were normalized to tubulin. Error bars denote mean ± SEM for three different experiments with triplicate assessments. ***, $P < 0.0001$ (vs. control [CON]). (B) Endogenous Dvl is redistributed to the cell periphery under BMP-2 stimulation. Confocal images of hPAECs stimulated with 10 ng/ml BMP-2 or 100 ng/ml Wnt3a for 4 h. Dvl is labeled red, actin is labeled green, and nuclei are labeled blue. A combined (top) and single channel (bottom) collection of images illustrating the distribution of Dvl (red) is shown. Bars, 10 µm.