



Supplement Figure 3: ALK5 inhibition does not alter BMP9-mediated Smad activation in endothelial cells.

(A) HMEC-1 were transfected with siALK5 or siCP (10nM each) using Dharmafect1 (DH1). After 28h, cells were serum-restricted for 16h, followed by treatment with BMP9 (1ng/ml) or TGFβ₁ (5ng/ml) in 0.1% for 1h. Immunoblotting was performed with antibodies against phospho-Smad1/5, phospho-Smad2 and phospho-Smad1/3.

Serum-restricted HMEC-1 (B) or HPAECs (C) were treated with SD208 (50 or 500nM) or DMSO vehicle in M199/0.1% FBS (0.1%) for 30 min. These were removed and the cells treated with BMP9 (1ng/ml) or TGFβ₁ (5ng/ml) in 0.1% for 1h. Immunoblotting was performed with antibodies against phospho-Smad1/5, phospho-Smad2 or phospho-Smad1/3. Blots are representative of 3 separate experiments. All blots were reprobed for β-actin to ensure equal loading.