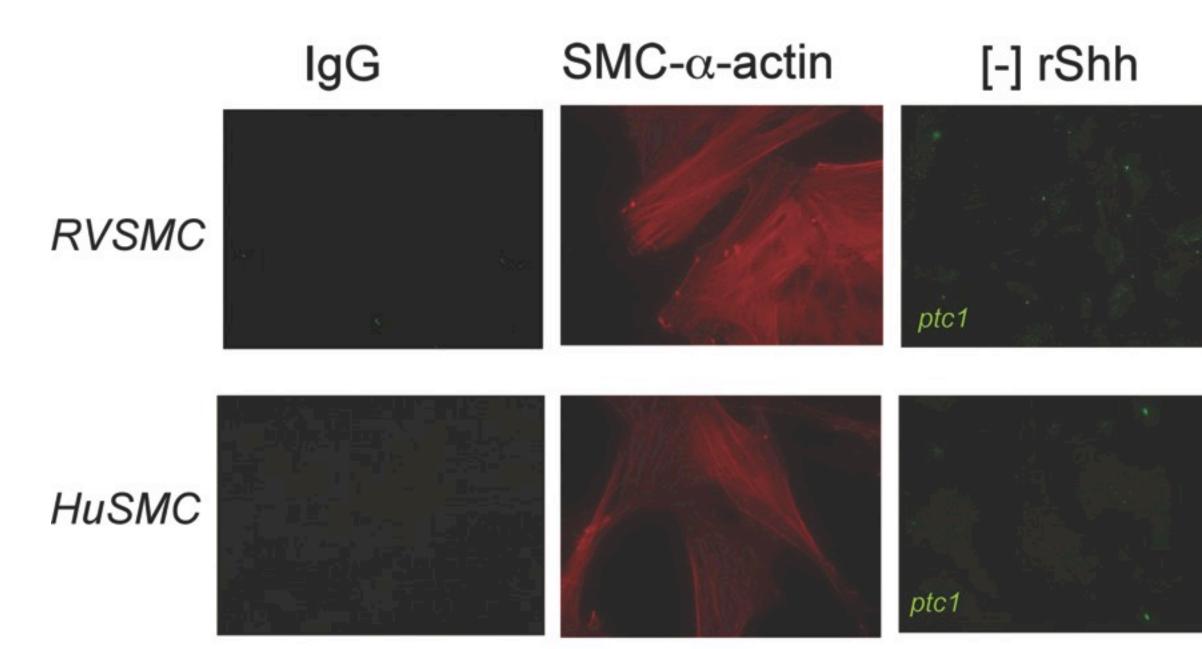
SUPPLEMENTAL FIGURES AND LEGENDS

Figure I: Shh stimulation of ptc1 expression in SMC *in vitro*. Representative immunocytochemical staining of Ptc1 expression in rat and human aortic SMC. Cells were treated with or without recombinant Shh (3.5 μ g/ml) for 24 h before ptc1 expression was examined. The immunoglobin control and smooth muscle cell α -actin is also presented and magnification is 40 x for all panels.

Figure II: Shh stimulates Notch target gene expression in rat SMC *in vitro*. (a) The effect of Hh activation with recombinant Shh (3.5 µg/ml) and Hh inhibition with cyclopamine (40µM) on baseline Hrt protein expression. The effect of (b) Hh activation with recombinant Shh (3.5 µg/ml) and (c) Hh inhibition with cyclopamine (40µM) on baseline Hrt mRNA levels in SMC (d) The effect of cyclopamine on rShh stimulated Hrt-3 mRNA levels (mean \pm SEM n=3, normalized to GAPDH) and protein expression (representative blot shown). *p≤0.05 vs control, **p≤0.05 vs Shh alone.

Figure III. Shh stimulates Notch Target gene expression in a CBF-1/RBP-J κ dependent manner. (a) Shh protein levels in SMC following ectopic expression of full-length mouse Shh cDNA and the subsequent effect of ectopic expression of Shh on *Hrt-1, 2* and *3* mRNA levels in SMC following inhibition of CBF-1/RBP-Jkdependent Notch signaling by co-transfection with RPMS-1, * p≤0.05 vs Mock Control, ** p≤0.05 vs Shh. (b) The temporal effects of serum stimulation on transactivation of CBF-1/RBP-J κ regulated promoter activity following cotransfection with 0.5 $\mu g/10$ cm² RPMS-1 and 0.5 $\mu g/10$ cm² R218H-RBP-J κ . Cells were transfected with CBF-1/RBP-Jk dependent reporter plasmid, pGa981-6, or control vector pGa50-7, lacking the CBF-1 enhancer, prior to serum stimulation over time, * p≤0.05 vs control and (c) The effect of the dominant negative R218H-RBP-J κ on rShh (3.5 $\mu g/m$) stimulation of Hrt-3 mRNA levels in SMC following transfection of cells with R218H-RBP-J κ . The cumulative data represents the mean values from three independent experiments ± SEM, *p≤0.05 vs control, **p≤0.05 vs Shh alone.

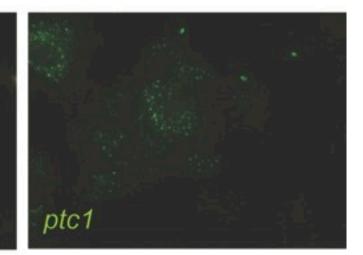


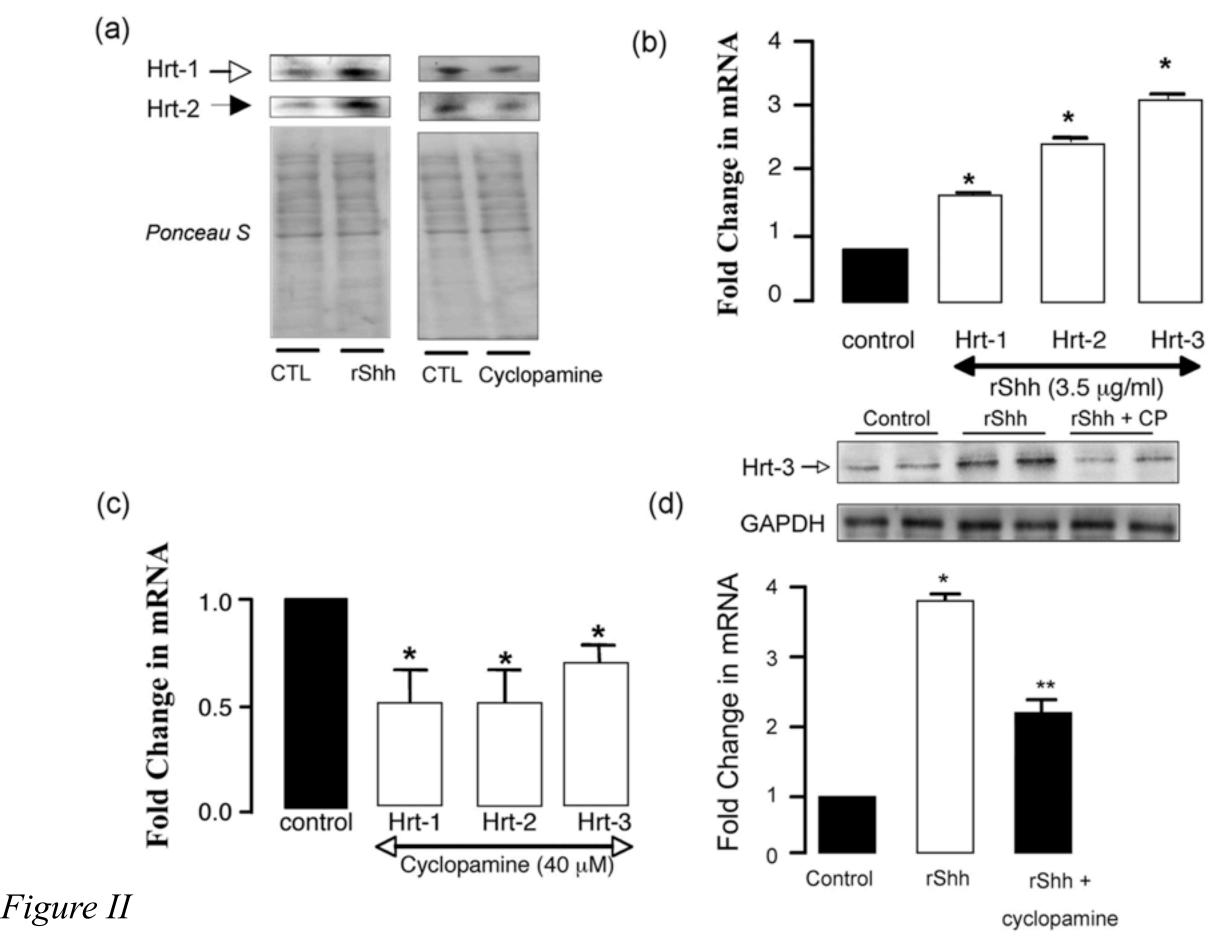
Supplemental Figure I

Tuesday 21 April 2009









Supplemental Figure II

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(a)

