DETAILED FIGURE LEGENDS

Figure 1: Notch Inhibition attenuates Shh-stimulated rat SMC growth *in vitro*. The effect of enforced ectopic expression of Shh alone or co-transfection with RPMS-1 in puromycin-pooled cells on (a) serum-stimulated SMC growth and pCNA expression, (b) Bcl-X_L and (c) Bax expression. Blots are representative of three independent experiments with similar results. Equal protein loading was confirmed by Ponceau S stain. Data are mean ± SEM, n=3. *p≤0.05 vs Shh, **p≤0.05 vs mock control.

Figure 2: VEGF mediates Shh activation of Notch target gene expression (a) The effect of Hh activation with recombinant Shh (3.5 μg/ml) and Hh inhibition with cyclopamine (40 μM) for 24 h on VEGF-A protein and mRNA expression, respectively. Data were corrected for total protein using a ponceau stain for each blot and GAPDH from mRNA levels, * p≤0.05 vs Control, ** p≤0.05 vs Shh. (b) QRT-PCR analysis of rVEGF-A (25 ng/ml) stimulation of *Hrt-1, 2 a*nd *3* mRNA levels after 24 h. * p≤0.05 vs control. (c) QRT-PCR analysis of Shh-stimulated VEGF-A mRNA levels following transfection with scrambled siRNA (control) and siRNA targeted against VEGF-A, ** p≤0.05 vs Control, * p≤0.05 vs scrambled Shh. (d) QRT-PCR analysis of Shh-stimulated *Hrt-3* mRNA levels following transfection with scrambled siRNA (control) or siRNA targeted against VEGF-A following re-addition of rVEGF (25 ng/ml). Data represent the mean values from at least six individual wells ± SEM, **p≤0.05 vs scambled control, *p≤0.05 vs scambled rShh treated group, ¶ p≤0.05 vs siRNA treated rShh group.

Figure 3. VEGF mediates Shh activation of SMC growth (a) The effect of recombinant VEGF-A (25 ng/ml) on 5% FCS stimulated SMC proliferation over 9 d. (b) Representative blot of the effect of VEGF-A knockdown on Shh stimulated PCNA and Notch 1 IC expression. Equal loading was confirmed with GAPDH (c) The effect of Hrt-3 knockdown on Hrt-3 protein expression and SMC growth (PCNA, cell counts). Representative blots are shown. Cell count data are mean ± SEM of three

wells, *p≤0.05 vs scambled (d) The effect of Hrt-3 knockdown on SMC apoptosis (mean ± SEM, *p≤0.05 vs scambled n=3).

Figure 4: Hedgehog components in murine SMC *in vivo.* (a) Hemotoxylin and eosin staining of representative sections of a carotid artery (CA) from C57Bl6/J mice 14 d post sham-operation or ligation injury. 40x magnification. Note the medial thickening and neointima formation in ligated vessel compared to the sham. Verhoeff-Van Gieson staining of ligated carotid artery is also shown (right panel) with elastic fibers: blue-black, nuclei: blue-black, collagen: pink. Scale bars=50 μm (b) Photomicrographs of smooth muscle cell α-actin, Notch 1 IC, Ptc1, Hrt-3 and Gli_2 immunohistochemical staining in sham-operated and ligated carotids, after 14 days. Control IgG staining also shown. Magnification 40x. Scale bars=50 μm.

Figure 5. **Temporal regulation of Hedgehog component expression following injury *in vivo*** (a) Quantitative analysis of intimal and medial volumes in histological sections from sham and ligated carotid arteries after 14 days. Values are mean ± SEM, n=6 vessels. (b) QRTPCR of temporal hedgehog and Notch component mRNA levels in the CA following ligation and (c) VEGF-A mRNA levels after 3 d post sham-operation or ligation. (d) Representative immunoblots and cumulative data for Notch 1 IC, Hrt-3, VEGF-A, and Ptc1 protein levels 14 d post ligation. Data were normalized to GAPDH protein or mRNA levels and expressed as fold change over sham controls. The cumulative data represents the mean values from six vessels in each group with the QRT-PCR performed in triplicate ± SEM, *p≤0.05 vs sham.