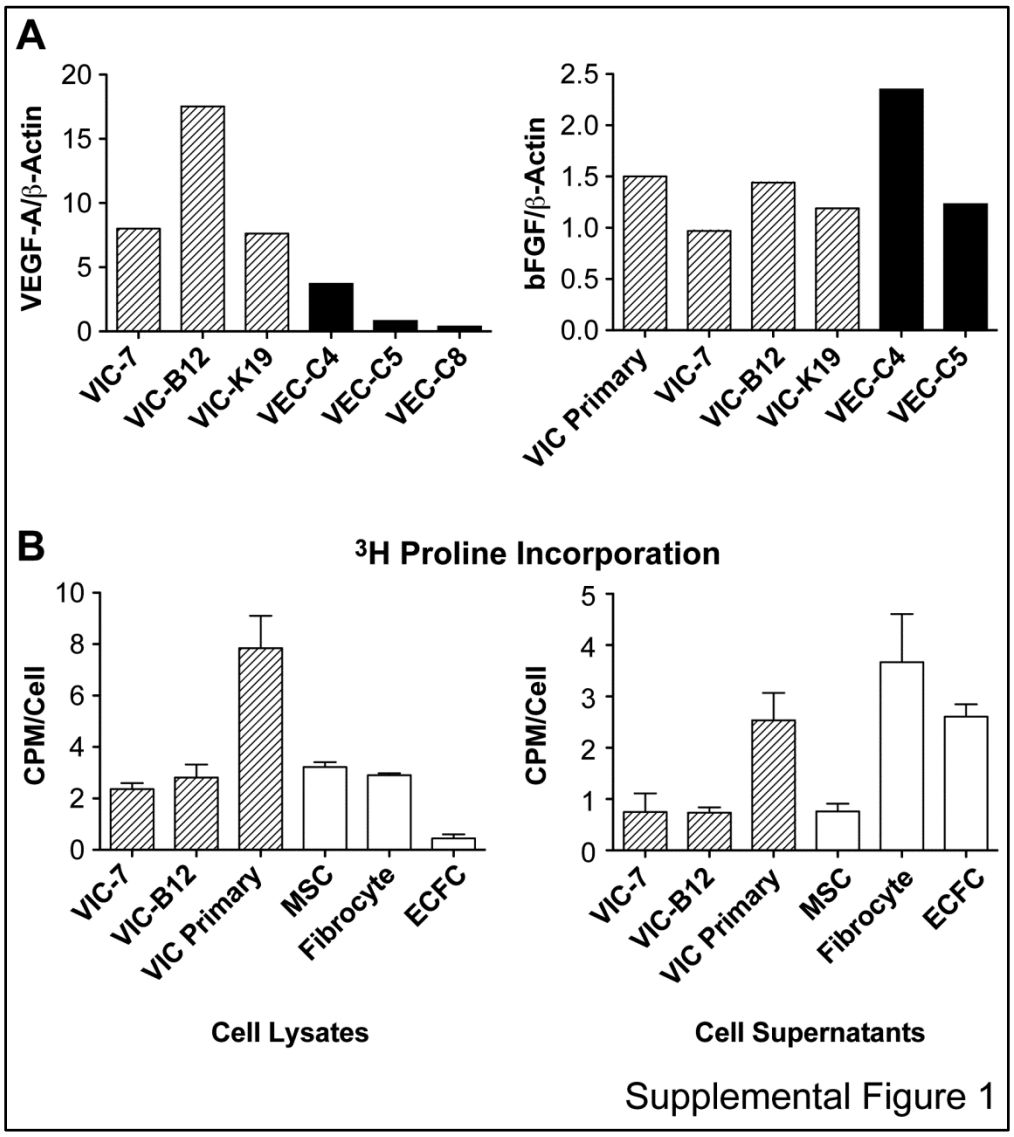
Supplemental Table and Figures

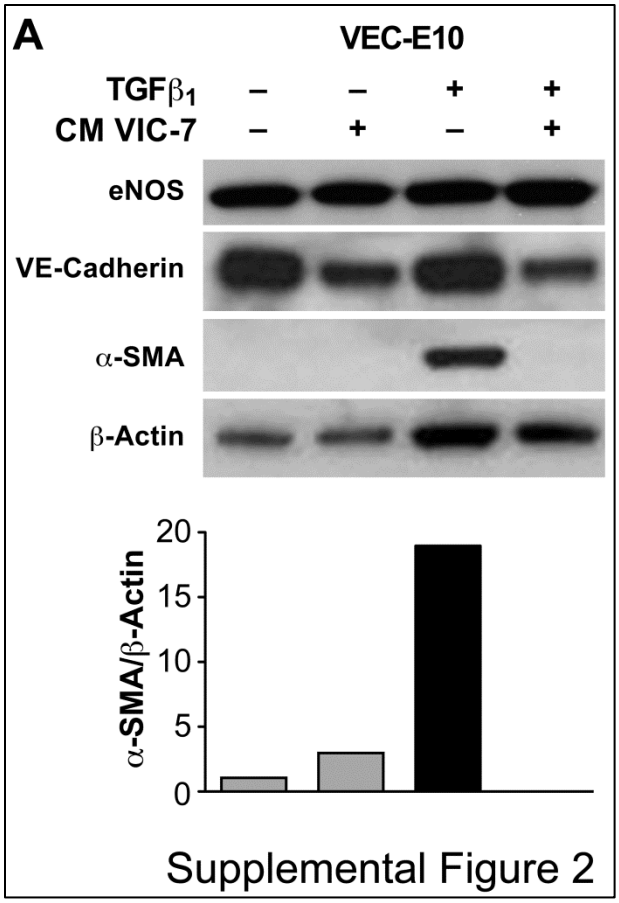
Supplemental Table 1 – Oligonucleotide primers used for RT-qPCR

Gene	Oligonucleotide Primers (forward and reverse)
Slug	5'- GGACGCACACCTTACCTTGT -'3
	5'- CGAGAAGGTTTTGGAGCAAC -'3
Snai1	5'- ATCAGCTGGGCTTCTCTCCT -'3
	5'- TGAAGGCTTTGCGAGACTG -'3
MMP-2	5'- GAGACTCCCACTTCGACGAC -'3
	5'- AACACCAGAGGAAACCATCG -'3
NFATc1	5'- CTTTCTGCAGGACTCCAAGG -'3
	5'- AGGGCTGGTTATCCTCTGGT -'3
αSMA	5'- TGCCATGTATGTGGCTATTCA -'3
	5'- ACCAGTTGTACGTCCAGAAGC -'3
Collagen	5'- CAGGAAGAAGGCCAAGAAGA -'3
	5'- AGGGACCCAAAGGAGACACT -'3
Chondromodulin1	5'- CACCAGCAGGAAGGAGAAAG -'3
	5'- TTATAGGGCCATGGGTGGTA -'3
Basic FGF	5'- GTGCAAACCGTTACCTTGCT -'3
	5'- TCGTTTCAGTGCCACATACC -'3
VEGF-A	5'- TTGCCTTGCTGCTCTACCTT -'3
	5'- ACACAGGACGGCTTGAAAAT -'3
RPS9	5'- GGAGACCCTTCGAGAAGTCC -'3
	5'- GGGCATTACCTTCGAACAGA -'3
β Actin	5'- CTCTTCCAGCCTTCCTTCCT -'3
	5'- GGGCAGTGATCTCTTTCTGC -'3

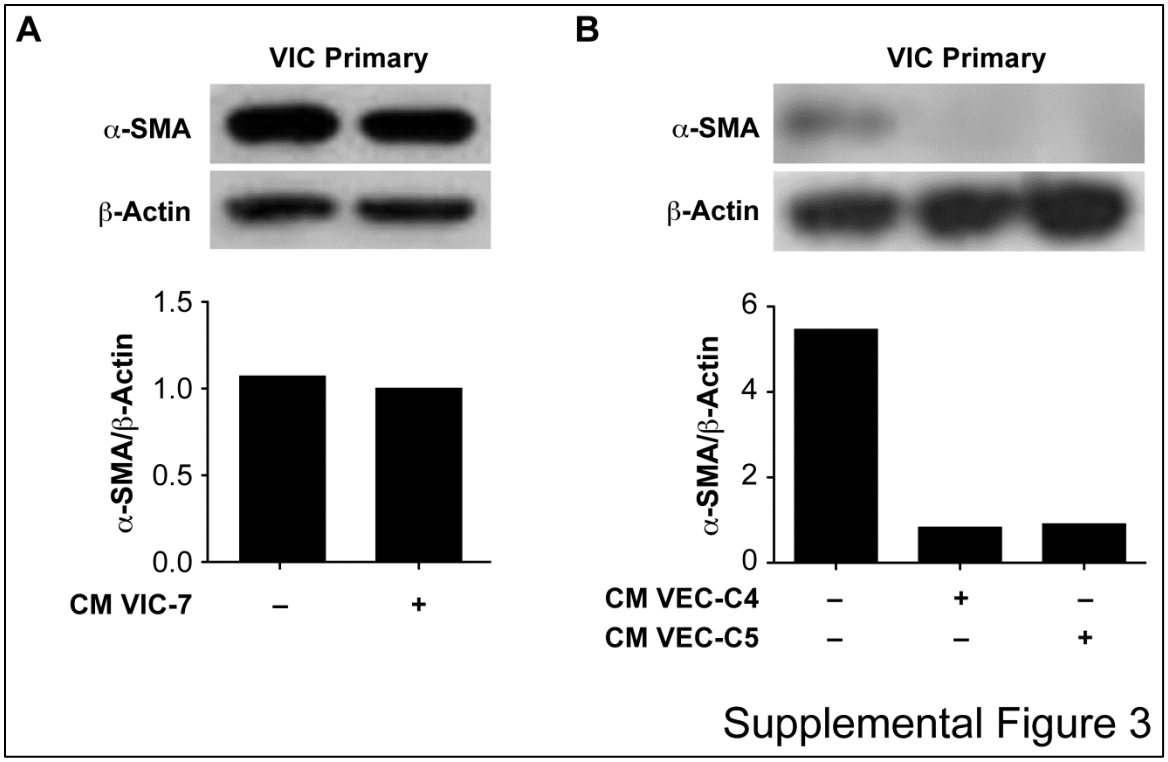
Supplemental Figures 1-4



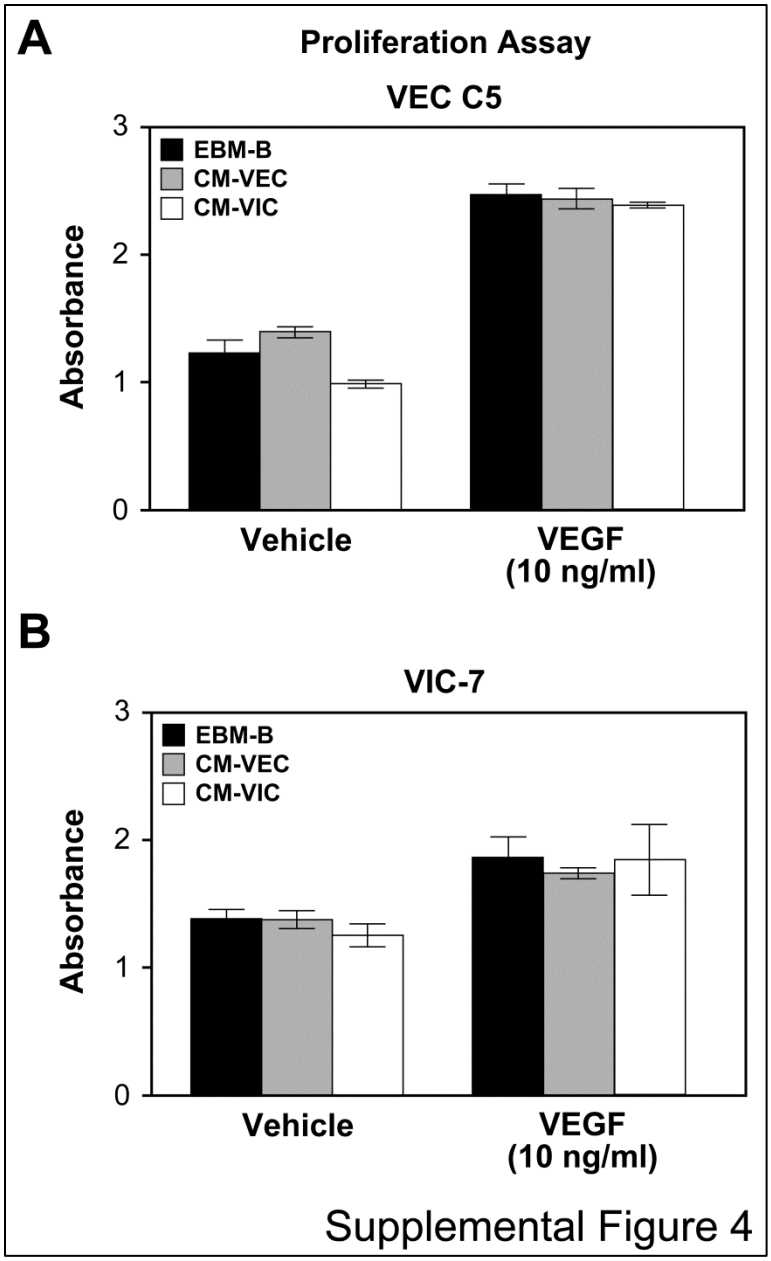
Supplemental Figure 1 (A) Ovine specific primers for VEGF-A (left panel) and basic FGF (right panel) were used to measure mRNA transcript levels in ovine VIC clones and primary cultures (hatched bars) and VEC clones (black bars). (B) Collagen biosynthesis was measured by ^{3}H-proline incorporation into protein in cell lysates (left panel) and cell supernatants (right panel). Cells were labeled ^{3}H-proline for 4 hours and chased for 24 hours before protein precipitation. Three ovine VIC cultures (hatched bars) and three non-valvular control cells (white bars) were analyzed.



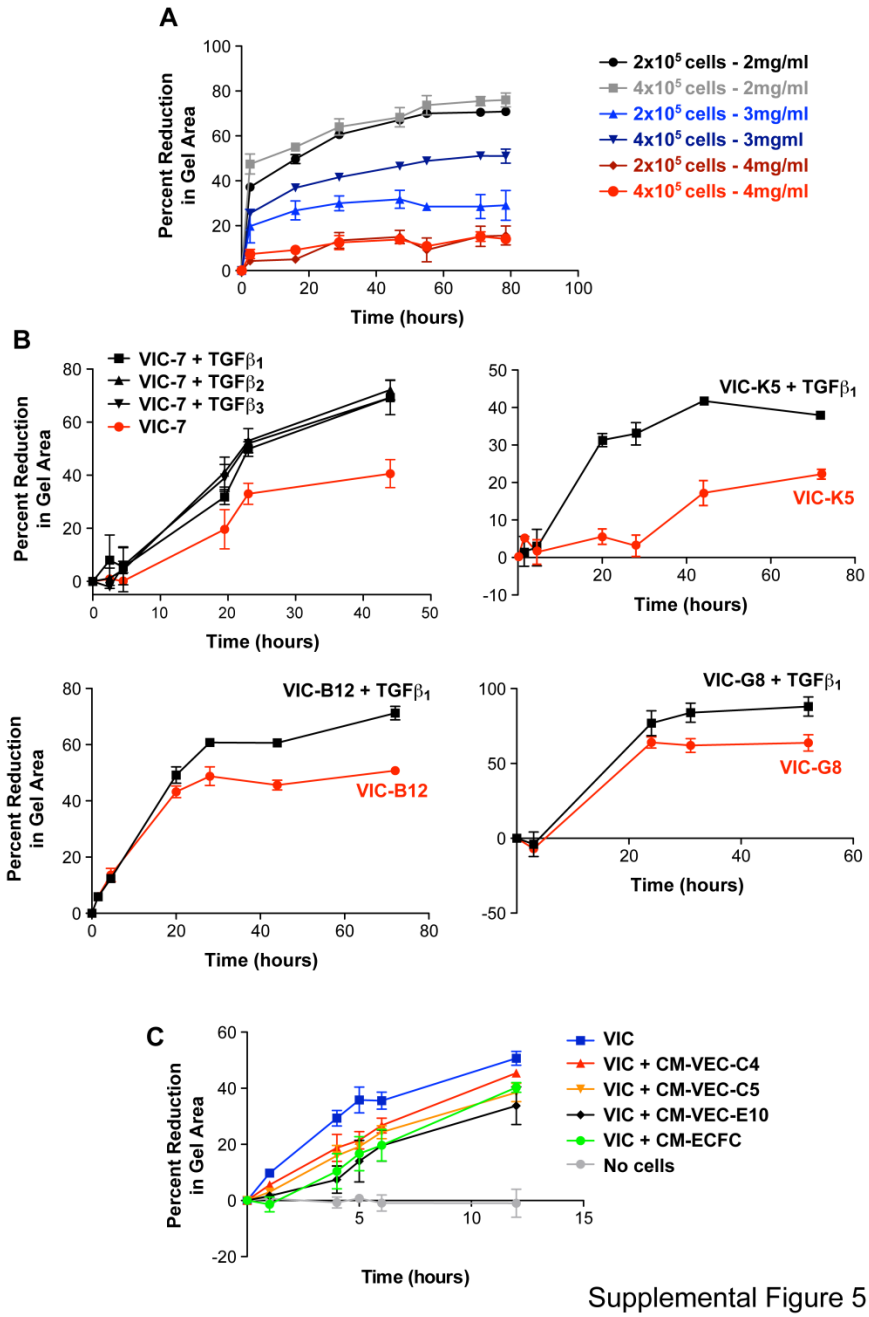
Supplemental Figure 2 (A) Conditioned media (CM) from VIC-7 was tested for ability to block TGFβ1-induced α-SMA expression. VEC clone E10 cells were treated ± 2ng/ml TGFβ1 and ± CM for 4 days. Cell lysates were analyzed by Western blot for eNOS, VE-Cadherin, α-SMA and β-actin. The bar graph shows quantification of the α-SMA band intensities normalized to β-actin.



Supplemental Figure 3 (A) Conditioned media (CM) from VIC-7 was tested for ability to suppress α-SMA in VIC primary, as a negative control. The bar graph shows quantification of the α-SMA band intensity normalized to β-actin. (B) CM from VEC-C4 and VEC-C5 was incubated with VIC primary for 5 days. Cell lysates were analyzed for α-SMA and β-actin by Western blot. The bar graph shows quantification of the α-SMA band intensity normalized to β-actin.



Supplemental Figure 4 Cellular Proliferation Assays (A) Mitral VEC C5 were assayed for proliferation over 72 hours, +/- VEGF-A, in normal growth media (EBM-B) or in CM from VEC (gray bars) or CM from VIC (white bars) to determine if CM from VIC had an effect on VEC proliferation. (B) Mitral VIC-7 were assayed for proliferation over 72 hours, +/- VEGF-A, in normal growth media (EBM-B) or in CM from VEC (gray bars) or CM from VIC (white bars) to determine if CM from VEC had an effect on VIC proliferation. The results in both panels show that VEC and VIC proliferation was not significantly affected by CM from VEC or VIC.



Supplemental Figure 5

Supplemental Figure 5 Collagen gel contraction assays (A) Assay optimized for cell number and collagen concentration. Here after 2 x 10^5 cells seeded in 3mg/ml collagen gels. (B) TGFβ increased contractility of VIC. VIC-7 pretreated with 2ng/ml of TGFβ isoforms 1, 2 and 3 for 5 days and seeded in collagen gels. All three isoforms increased contractility of VIC-7. Individual clones VIC-K5, VIC-B12 and VIC-G8 pretreated with 2ng/ml TGFβ1 for 5 days and seeded in collagen gels. TGFβ1 increased contractility of all clones tested. (C) Contractility of VIC treated with CM from three different VEC clones and from ECFC over a 12 hour time course.