Supplemental Methods

RNA isolation and QPCR-

Chick Embryonic Fibroblasts (CEFs) were used to confirm chicken specific knockdown of the genes we targeted with siRNA. Briefly, cells were reverse transfected using siPORT NeoFX (Ambion) into six well dishes at the indicated concentrations according to the manufacturers protocol. Next 1 ml of RNA-STAT-60 (Tel-Test) was added to each well. The mixture was mixed thoroughly and incubated for 10 minutes at RT. Next, 200 µl of chloroform was added, samples were mixed and incubated 2-3 minutes at RT, followed by a 15 minute centrifugation at 12,000 g at 4ºC. The upper aqueous phase was removed and saved in a separate tube to which 500 µl isopropanol was added and incubated 8-10 minutes at RT. The solution was then centrifuged for 10 minutes at 12,000 g at 4ºC, followed by aspiration. An ethanol wash using 1 ml of 75% ethanol was then performed. Samples were either stored at this step at -80ºC or directly centrifuged at 7,500 g at 4ºC for 5 minutes, followed by aspiration and reconstitution of RNA pellet. Following reconstitution of the pellet, RNeasy Mini Kit (Qiagen) was used for cleanup of RNA following the manufacturers protocol. Next, Turbo DNA-free (Ambion) was used for removal of genomic DNA. cDNA was then synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's established protocol. Additionally chick GAPDH was used to normalize. The forward primer was 5'(GGGCACGCCATCACTATCTTCC)3' and the reverse was 5'(GAGGGGCCATCCACCG TCTT)3' as previously described [1]. RT-PCR was preformed using Lightcycler Faststart DNA Master PLUS SYBG Green I (Roche) using the Lightcycler2 (Roche) according to the manufacturers instructions. The RT-PCR data was analyzed using the $2^{-\Delta\Delta C}{}_{T}$ method [2]. Confirmation of knockdown via all siRNA constructs is depicted in Figure S2.

Alkaline phosphatase and Luciferase assays- Performed as described [3].

1. Bushdid, P.B., et al., *NF-kappaB mediates FGF signal regulation of msx-1 expression.* Dev Biol, 2001. **237**(1): p. 107-15.
2. Livak, K.J. and T.D. Schmittgen, *Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method.* Methods, 2001. **25**(4): p. 402-8.
3. Desgrosellier, J.S., et al., *Activin receptor-like kinase 2 and Smad6 regulate epithelial-mesenchymal transformation during cardiac valve formation.* Dev Biol, 2005. **280**(1): p. 201-10.