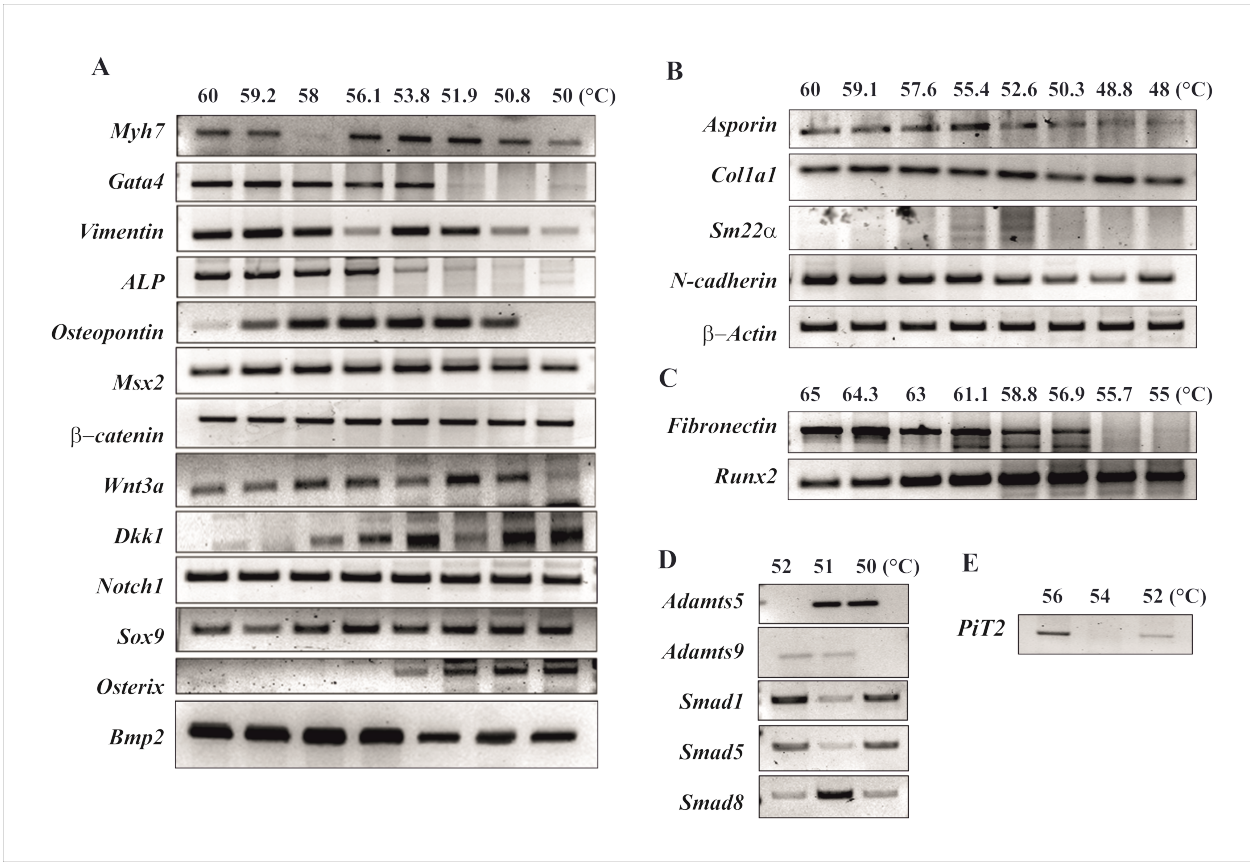
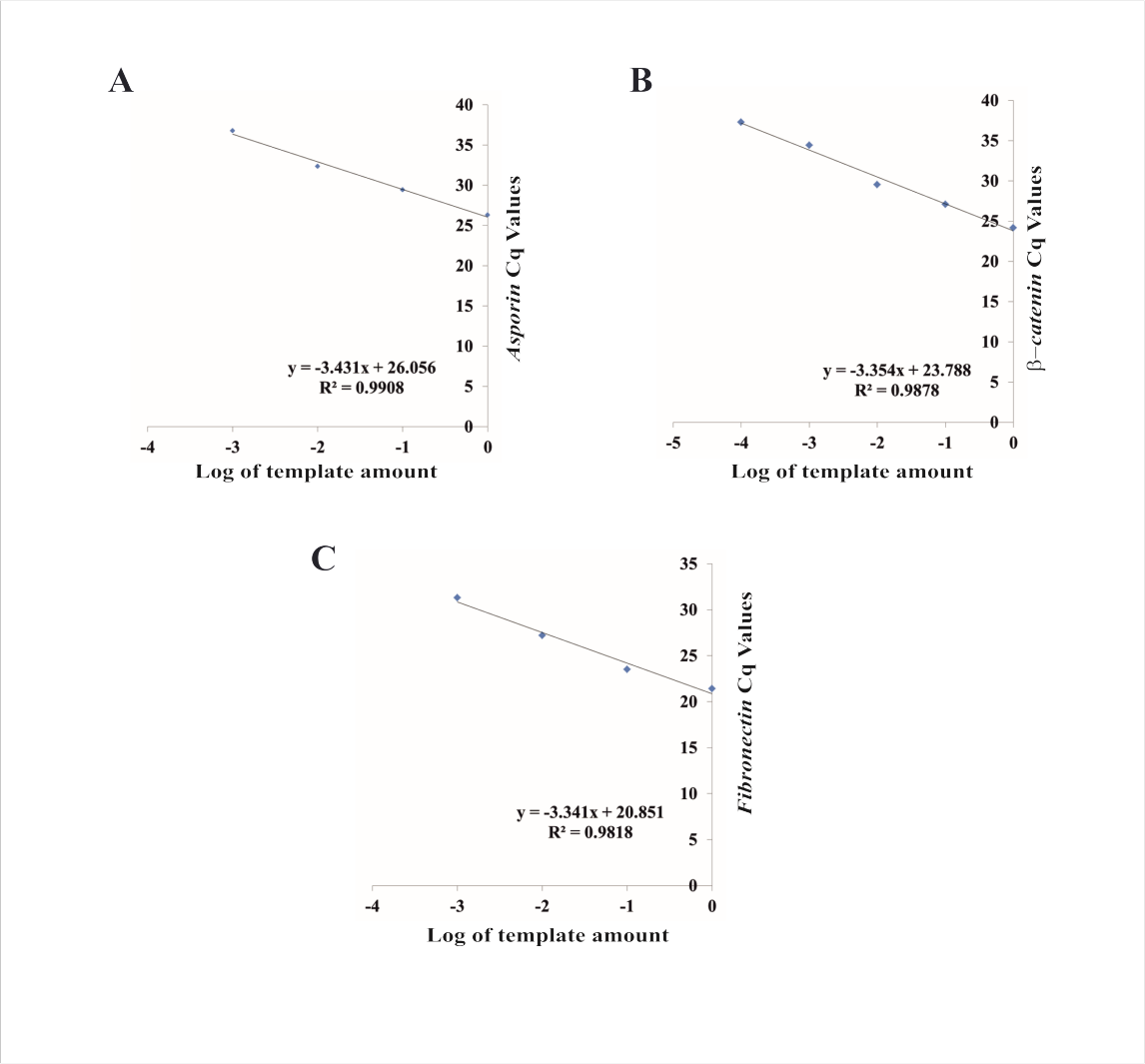


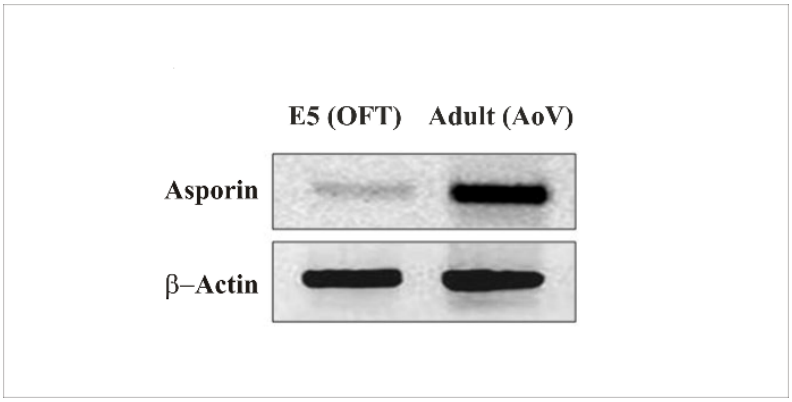
Supplementary figure I: **Optimization of the annealing temperatures of all the primers used for Reverse Transcriptase PCR assays by temperature gradient PCR. A:** 60°-50°C temperature gradient has been used for the *Myh7, Gata4, Vimentin, ALP, Osteopontin, Msx2, β-catenin, Wnt3a, Dkk1, Notch1, Sox9, Osterix* and *Bmp2*. **B:** 60°-48°C temperature gradient has been used for the *Asporin, Col1a1, Sm22α, N-cadherin* and *β-actin*. **C:** 65°-55°C temperature gradient has been used for the *Fibronectin* and *Runx2*. **D:** 52°-50°C temperature gradient has been used for the *Adamts5, Adamts9, Smad1, Smad5* and *Smad8*. **E:** 52°-56°C temperature

gradient has been used for the *PiT2*. Specific annealing temperature and product size for each set of primer is provided in Table 1.

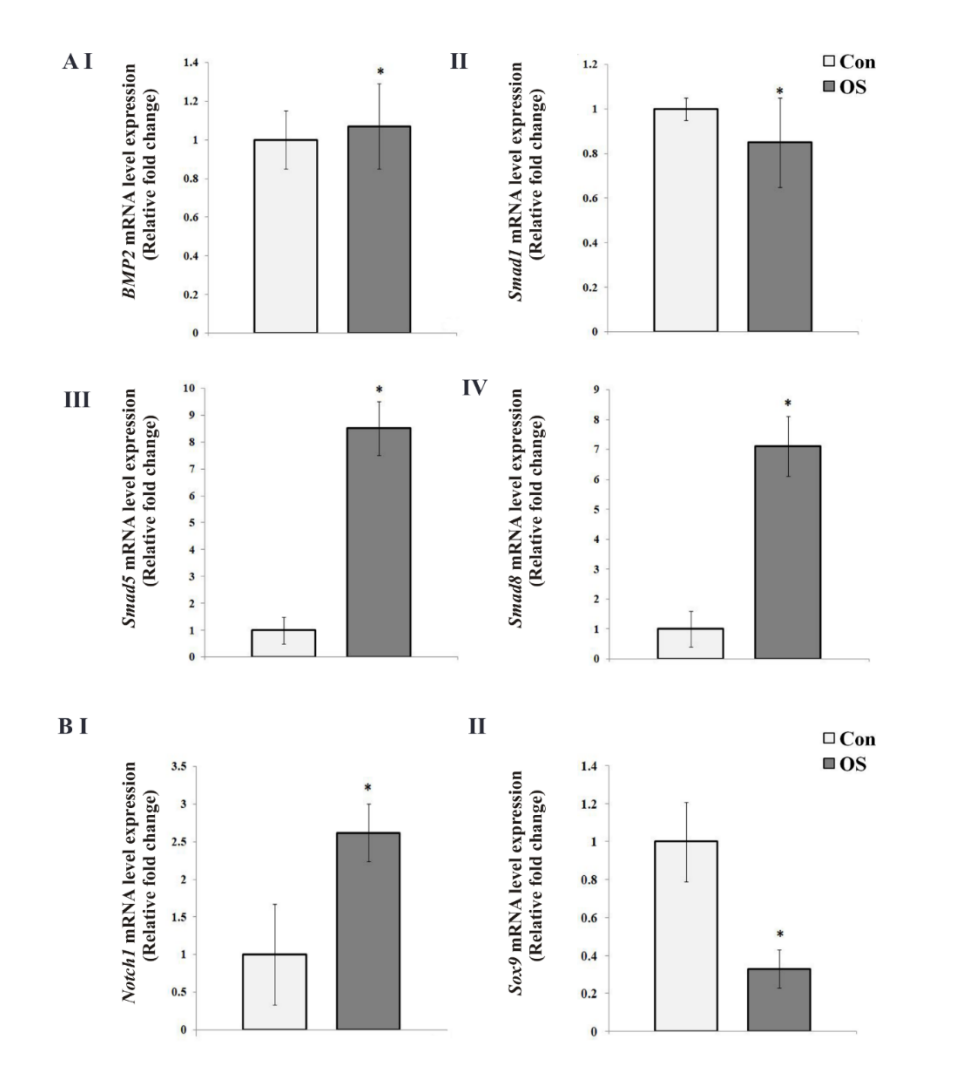


Supplementary figure II: **Derivation of the standard curves of the gene primers by quantitative Real Time PCR (A-C).** To determine the primer efficiencies threshold values (Cq) have been generated using template cDNA with serial dilution. Log of template cDNA has been plotted in X-axis and Cq values are plotted in Y-axis. **A:** Standard Curve of the *Asporin* gene primer (Calculated % efficiency = 95.68). **B:** Standard Curve of the *β-catenin* gene primer

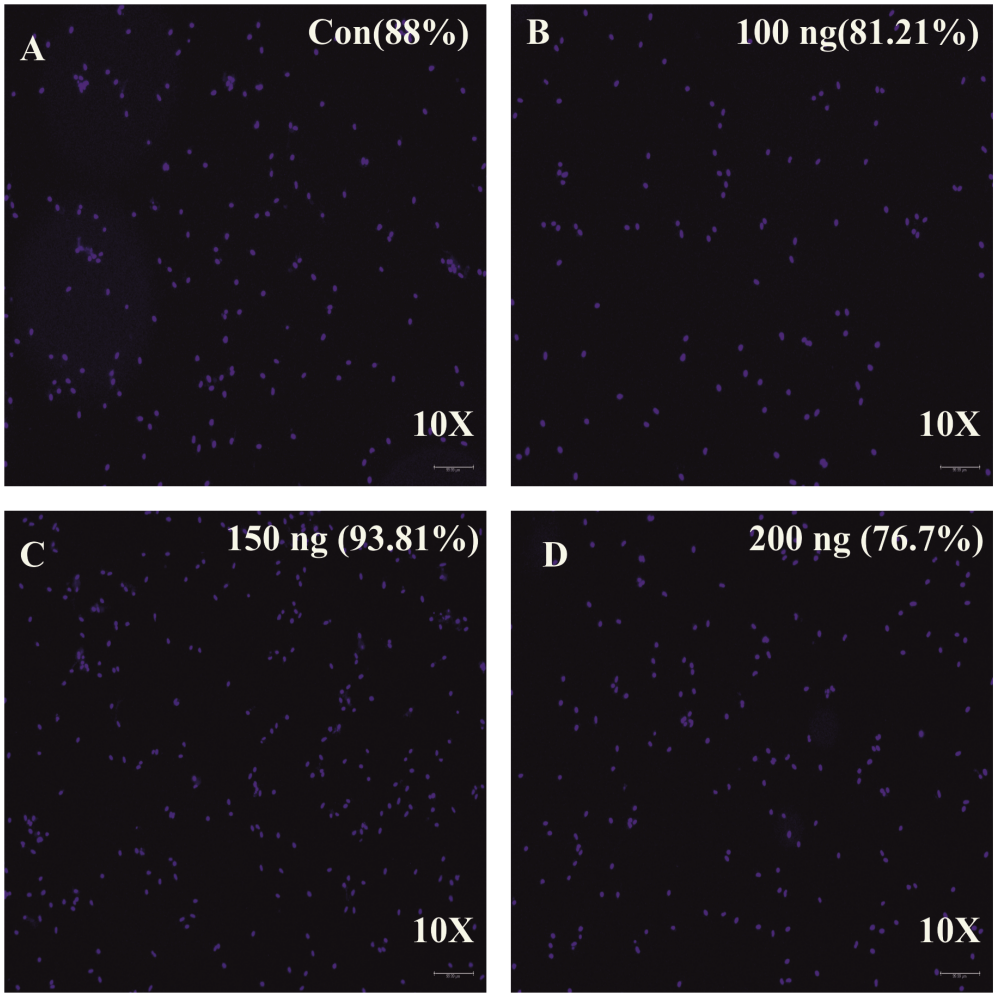
(Calculated % efficiency = 98.84). **C:** Standard Curve of the *Fibronectin* gene primer (Calculated % efficiency = 99.21).

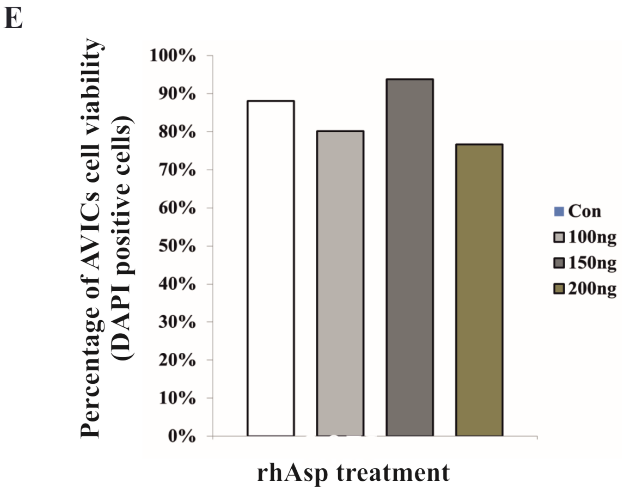


Supplementary figure III: **Detection of *Asporin* mRNA expression in embryonic outflow tract cushion and in adult aortic cusp.** Gene expression analysis by RT-PCR shows increased level of Asporin mRNA in isolated adult aortic valve tissue compared to embryonic day 5 (E5) outflow (OFT) tract cushion as detected by agarose gel band intensity. *β-actin* was used as loading control. (n=3)



Supplementary figure IV: **Quantitative gene expression analyses of other osteogenic inducing pathways involved in osteogenesis after OS induction in adult AVICs in culture.** qRT-PCR data show that BMP/Smad signaling specific markers, *BMP2* is increased by 0.07 fold, *Smad1* is decreased by 0.15 fold, *Smad5* and *Smad8* are increased by 7.51 and 6.11 folds, respectively in OS treated AVICs compared to uninduced control AVICs. Expression of *Notch1* mRNA is increased by 1.62 fold and *Sox9* mRNA is decreased by 0.67 fold in OS treated AVICs compared to uninduced control AVICs. Statistical significance was determined by Student's *t*-test, where * denotes $p \leq 0.05$ and n=3.


A
Con(88%)
10X
B
100 ng(81.21%)
10X
C
150 ng (93.81%)
10X
D
200 ng (76.7%)
10X


E
Percentage of AVICs cell viability (DAPI positive cells)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Con
100ng
150ng
200ng
rhAsp treatment

Supplementary figure V: **Cell viability after AVICs treatment with human recombinant Asporin (rhAsp) in culture:** Dose dependent concentrations (100ng/ml, 150ng/ml and 200ng/ml) of rhAsp were applied to normal untreated AVICs to check the viability of cells. To mark viable cells, nuclei were stained with DAPI (blue). **A-D**: Microscopic images show that in all the conditions [control (A) 88%; in 100 ng/ml (B) 81.21%; in 150 ng/ml (C) 93.81%; and in 200 ng/ml (D) 76.7%], there are more than 75% DAPI positive distinct nuclei indicating viable AVICs after rhAsp treatment in culture. **E**: Statistical data also show insignificant changes in cell viability ($DAPI^+$ nuclei) of rhAsp treated AVICs compared to untreated controls in culture, where $p > 0.05$ and $n = 3$.