Supplementary Material

**Inflammation and Mechanical Stress Stimulate Osteogenic Differentiation of Human Aortic Valve Interstitial Cells**

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**Supplementary Table 1.** Primers used for RT-qPCR.

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | Description | Forward primer (5’-3’) | Reverse primer (5’-3’) |
| *ICAM1* | intracellular adhesion  molecule 1 | AGACAGTGACCATCTACAGCTTTCC | CACCTCGGTCCCTTCTGAGA |
| *BMP2* | bone morphogenetic  protein 2 | GCCAGCCGAGCCAACAC | CCCACTCGTTTCTGGTAGTTCTTC |
| *RUNX2* | runt-related transcription  factor 2 | GGCACTAAACAGCCTCCTCAG | GTGCTCGGATCCCAAAAGAA |
| *POSTN* | periostin | CCCAGCAGTTTTGCCCATT | TGTGGTGGCTCCCACGAT |
| *THBSP1* | thrombospondin 1 | 5'‐TCCGCAAAGTGACTGAAGAGAA‐3' | 5'‐GAACTCCGTTGTGATAGCATAGG‐3' |
| *ACTA2* | alpha -smooth muscle  actin | CCGACCGAATGCAGAAG | ACAGAGTATTTGCGCTCCGAA |
| *CNN1* | calponin | GCATGTCCTCTGCTCACTTCAA | GGGCCAGCTTGTTCTTAACCT |
| *18 S* | 18S ribosomal RNA | Catalog number: qA-01-0106S (tataabiocenter) | |

**Supplementary Table 2.** Contribution of each gene expression (%) in principal component analysis. Each principal component (PC1 and PC2) combine expression data of all analyzed genes in cells from calcified valves cultured on collagen and elastin coatings and stimulated by LPS (Flex-LPS+) or stretch (Flex+LPS-) alone, or in combination (Flex+LPS+).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Flex-LPS+** | | **Flex+LPS-** | | **Flex+LPS+** | |
| **Gene** | **PC1** | **PC2** | **PC1** | **PC2** | **PC1** | **PC2** |
| *ICAM1* | 14,812 | 5,243 | 10,408 | 21,834 | 16,019 | 0,778 |
| *BMP2* | 22,281 | 12,758 | 23,068 | 0,217 | 19,849 | 2,201 |
| *SMA* | 8,872 | 27,633 | 22,790 | 0,444 | 16,721 | 2,307 |
| *RUNX2* | 12,113 | 5,089 | 7,743 | 25,220 | 26,186 | 2,400 |
| *THBS1* | 3,265 | 20,351 | 19,258 | 12,406 | 17,211 | 1,443 |
| *POSTN* | 13,234 | 26,919 | 8,642 | 7,454 | 0,039 | 62,908 |
| *CNN1* | 25,423 | 2,007 | 8,092 | 32,425 | 3,975 | 27,963 |

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**Supplementary Figure 1**

Relative gene expression of intracellular adhesion molecule 1 *(ICAM1)* **(A)** and bone morphogenetic protein 2 *(BMP2)* **(B)** in valve interstitial cells isolated from healthy (n=3), valves cultured on collagen I coating and stimulated by 0.01 µl/ml, 0.1 µl/ml or 1 µl/ml LPS for 24 hours. The cells without LPS stimulation were used as control. Data were analyzed using one-way ANOVA with Dunnett’s multiple comparisons post-test.\* indicates 0.01<p≤0.05, \*\* indicates 0.001< p≤0.01, \*\*\* indicates 0.0001< p≤0.001. Overall p-values from ANOVA is shown in bold. Data are presented as mean±SD. p-values less than 0.05 were considered statistically significant.