Supplementary Material

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

Maria Bogdanova1\*, Aleksandra Kostina2,3, Katarina Zihlavnikova Enayati1, Arsenii Zabirnyk1, Anna Malashicheva2,3,4, Kåre-Olav Stensløkken1,Gareth John Sullivan1,5,6,7, Mari-Liis Kaljusto8, John-Peder Escobar Kvitting8, Anna Kostareva2,12, Jarle Vaage9,10#, Arkady Rutkovskiy1,9,11#\*

1Department of Molecular Medicine, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway; 2Almazov National Medical Research Centre; 3ITMO University and 4Faculty of Biology, St. Petersburg State University, St. Petersburg, Russia; 5Norwegian Center for Stem Cell Research, Oslo University Hospital and University of Oslo, Oslo, Norway; 6Institute of Immunology, Oslo University Hospital, Oslo, Norway, 7Hybrid Technology Hub - Centre of Excellence, Institute of Basic Medical Sciences, University of Oslo, Oslo, Norway; 8Department of Cardiothoracic Surgery and 9Department of Emergency Medicine and Intensive Care, Oslo University Hospital, Oslo, Norway; 10Institute of Clinical Medicine, University of Oslo, Oslo, Norway; 11Department of Cardiology, Akershus University Hospital, Oslo, Norway, 12Department of Woman and Children Health, Karolinska Institutet, Stockholm, Sweden

# Authors contributed equally to this manuscript

**\***Corresponding authors:

1. Arkady Rutkovskiy, [arkady.rutkovskiy@medisin.uio.no](file:///%5C%5Clagringshotell%5Cimb-pkfguro%5C01.%20Lab%20members%5CAortic%20valve%20calcification%20project%5CPAPERS%5CMethod%20paper%5Cpaper%20preparatiom%20for%20Life%20Science%20journal%5Carkady.rutkovskiy%40medisin.uio.no).

2. Maria Bogdanova, mariia.bogdanova@medisin.uio.no

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

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | Description | Forward primer (5’-3’) | Reverse primer (5’-3’) |
| *ICAM1* | intracellular adhesionmolecule 1 | AGACAGTGACCATCTACAGCTTTCC | CACCTCGGTCCCTTCTGAGA |
| *BMP2* | bone morphogeneticprotein 2 | GCCAGCCGAGCCAACAC | CCCACTCGTTTCTGGTAGTTCTTC |
| *RUNX2* | runt-related transcriptionfactor 2 | GGCACTAAACAGCCTCCTCAG | GTGCTCGGATCCCAAAAGAA |
| *POSTN* | periostin | CCCAGCAGTTTTGCCCATT | TGTGGTGGCTCCCACGAT |
| *THBSP1* | thrombospondin 1 | 5'‐TCCGCAAAGTGACTGAAGAGAA‐3' | 5'‐GAACTCCGTTGTGATAGCATAGG‐3' |
| *ACTA2* | alpha -smooth muscleactin | 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 |

****

**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.